Design and Synthesis of Pyrrolidine-5,5-*trans*-lactams (5-Oxo-hexahydro-pyrrolo[3,2-*b*]pyrroles) as Novel Mechanism-Based Inhibitors of Human Cytomegalovirus Protease. 1. The α-Methyl-*trans*-lactam Template

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Mechanism-based inhibitors of human cytomegalovirus (HCMV) protease have been designed based on the pyrrolidine-5,5-*trans*-lactam ring system. New routes to the β-methyl-, desmethyl-, and α-methyl-pyrrolidine-5,5-*trans*-lactam templates have been developed from 2,4-diaminobutyric acid. ESI/MS studies have shown that these inhibitors can bind covalently and reversibly to the viral enzyme in a time-dependent manner by a mechanism which is consistent with acylation of HCMV δAla protease at the active site nucleophile Ser 132. SAR in this series of pyrrolidine-5,5-*trans*-lactams has defined the relative stereochemisty of the methyl substituent adjacent to the lactam carbonyl, the functionality on the lactam nitrogen, and the mechanism of action of this novel series of serine protease inhibitors against the HCMV δAla protease. Activity decreases on moving from the α-methyl to the desmethyl to the β-methyl series. This selectivity is the opposite of that observed for these templates against the elastase and thrombin enzymes. The activity against HCMV δAla protease is the greatest with inhibitors based on the Cbz-protected α-methyl-5,5-*trans*-lactam template which have low micromolar activity against the viral enzyme.

Introduction

Human herpes viruses cause a range of diseases: HSV-1 (cold sores), HSV-2 (genital herpes), VZV (chicken pox, shingles), and HCMV (retinitis, pneumonitis). The current treatment of these diseases uses nucleoside (acyclovir, ganciclovir) and phosphonate (foscarnet) substrate analogues. Because of the toxicity associated with foscarnet and ganciclovir, together with the emergence of mutants resistant to acyclovir, there is a need for a new class of antiherpes compounds based on a novel mechanism.

Human herpes viruses encode a serine protease which is essential for viral replication.[1] The protease is part of the scaffold around which the capsid of the virus is built. The protease then cleaves the scaffold, which enables viral DNA to be packaged into the capsid. Inhibition of the protease blocks capsid formation and hence viral replication. Mutation studies, reaction with protease inhibitors, and recent X-ray structures indicate that the herpes virus proteases are members of the serine protease superfamily with little homology to the human (chymotrypsin) and bacterial (subtilisin) serine protease classes.[1,2] The recent crystal structures of HCMV protease[3−6] show it to be a 7-stranded β-barrel stabilized by surrounding α-helices; it exists as a dimer of two of these units in the crystal structure. Moreover it has a novel catalytic strategy for peptide cleavage, where the serine nucleophile at position 132 is activated by two juxtapositioned histidine residues at positions 63 and 157. The substrate cleavage sites across all the herpes virus family are unique and highly conserved: [1,2] the cleavage occurs between Ala/Ser and the consensus sequence for HCMV is Val-Xxx-Ala ↓ Ser. These enzymes have become attractive molecular targets for the design of novel antiviral drugs.[2] The pyrrolidine-5,5-*trans*-lactam serine protease inhibitors developed for our elastase[7,8] and thrombin[9] projects are ideal starting points for a chemical program aimed at developing inhibitors of herpes protease, as they have the potential to access all three specificity sites (S1′, S1, and S3). These proven serine protease inhibitors also have other advantages: they are novel and stable in plasma, their activity in vivo has been demonstrated indicating that these systems can have a favorable pharmacokinetic profile, and the synthesis of the ring systems has been established.

Our design of inhibitors was based on the *trans*-lactam ring incorporating the natural substrate requirements of the consensus sequence of HCMV protease. Hence the pyrrolidine-5,5-*trans*-lactam template A (Scheme 1) with a methyl substituent alpha to the lactam carbonyl (for access of the S1 site) became our initial target, where variation of substituents on the nitrogen atoms would allow us to access the S1′ and S3 sites. The initial exercise described in this publication was to determine the stereochemistry of this substituent for access of the S1 site, which is the major determinant of specificity in serine proteases, the SAR of the functionality on the lactam nitrogen, and the mechanism of action of this novel series of serine protease inhibitors against the HCMV protease.

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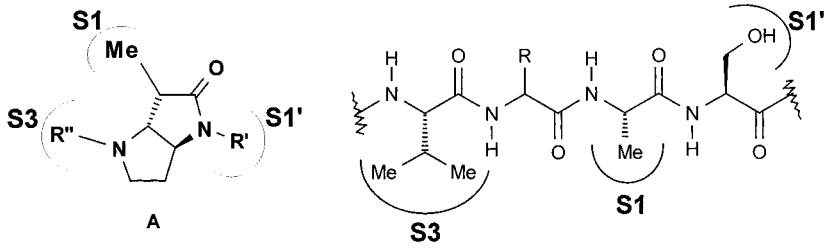
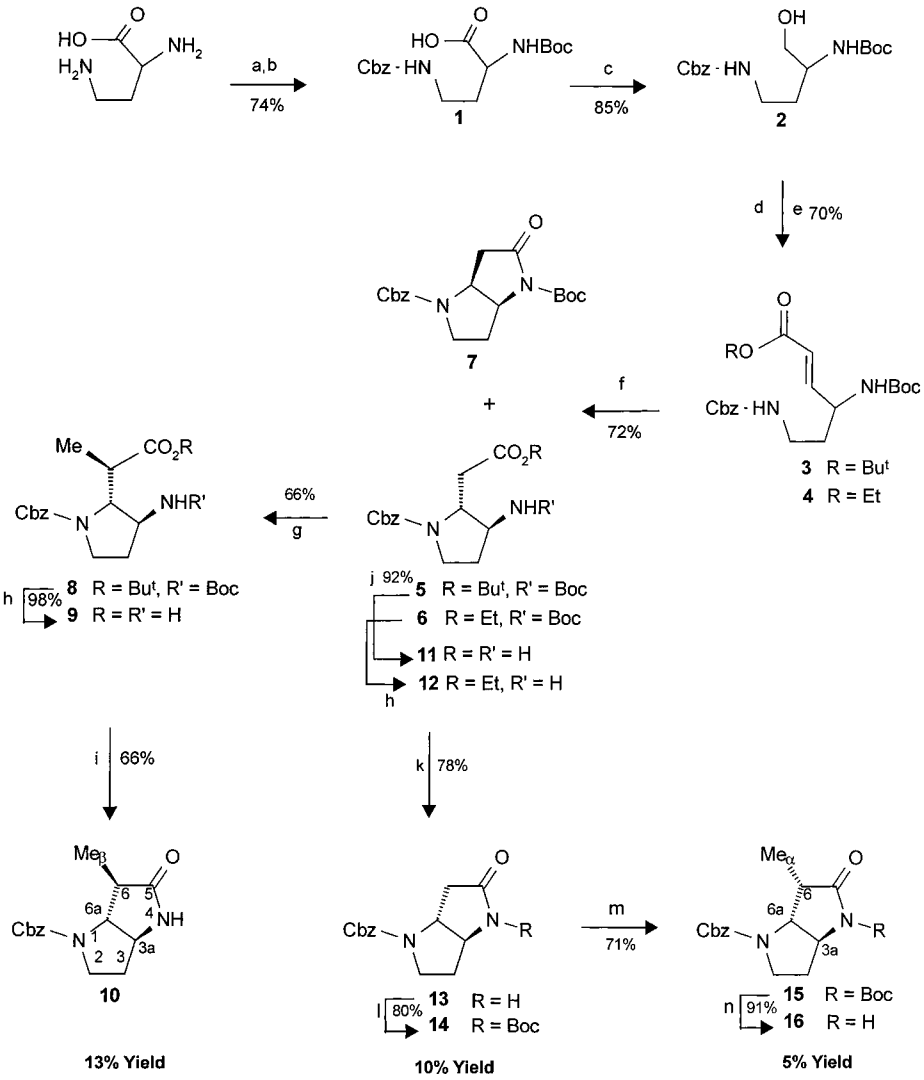
Scheme 1. *trans*-Lactam Template and Natural Substrate for HCMV Protease



Scheme 2[a]



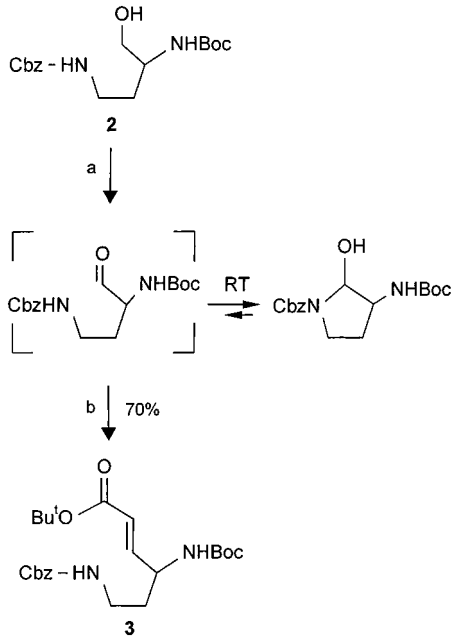
[a] Reagents and conditions: (a) Cu $(OAc)_2$, aq NaOH, 70 °C, 1−2 h, rt, pH→7−8, then CbzCl/dioxan/aq NaOH, 0 °C, then 1 h at rt; (b) EDTA, aq HCl, 1.5 h, pH→4−5, then $(Boc)_2O$/dioxan, pH→8, 40 h; (c) $ClCO_2Et$, *N*-methylmorpholine, THF, −10 °C, then $NaBH_4/H_2O$; (d) ClCOCOCl, DMSO, CH_2Cl_2, −70 °C, then Et_3N at −50 °C; (e) $(Ph)_3P{=}CHCO_2Bu^t$ added in situ, −50 °C; (f) LHMDS (0.25 equiv)/hexane, TMEDA/toluene, rt; (g) LHMDS (4 equiv)/THF/HMPA, −78 °C, 30 min, then MeI titrated in; (h) aq TFA, 2 h, rt; (i) $(PhO)_2PON_3$, Et_3N, DMF, 23 h, rt; (j) aq TFA, 75 min, rt; (k) Bu^tMgCl (3 equiv)/THF, <1 °C, 1 h, then 0 °C to rt, 45 min; (l) LHMDS (1.2 equiv)/THF, −78 °C, 30 min, then $(Boc)_2O$ (2.8 equiv)/THF, −78 °C; (m) LHMDS (1.3 equiv), THF/DMPU (4:1), MeI (2 equiv), −20 °C, or LHMDS (1.3 equiv), THF, MeI (10 equiv), −78 °C; (n) TFA, 35 min, rt.

Chemistry

The three templates to explore this stereochemistry were synthesized from 2,4-diaminobutyric acid via the protected 3-aminopyrrolidine-2-acetic acid ester[7] **(5)** (Scheme 2).

This is shorter than the previous route,[7] which contained some difficult chromatographic purification steps, and the starting material is available in both racemic and chiral forms. Bis-protection of D,L-2,4-diaminobutyric acid with CbzCl in the presence of Cu^{2+} followed by Boc-anhydride in the presence of EDTA (ethylenediaminetetraacetic acid) to remove copper from the first complex gave *N*-α-Boc-*N*-γ-Cbz-2,4-diaminobutyric acid[11] **(1)** in 74% overall yield. Attempts to reduce the acid to the alcohol using diborane in THF failed. However reduction of the mixed anhydride[12] (formed from the acid and ethyl chloroformate) with $NaBH_4/H_2O$ gave the alcohol **2** in 85% yield. Swern oxidation to form

Scheme 3[a]

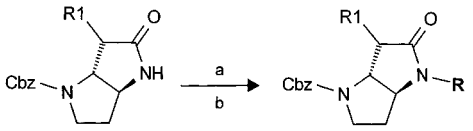


[a] Reagents and conditions: (a) ClCOCOCl, DMSO, CH_2Cl_2, −70 °C, then Et_3N at −50 °C; (b) $(Ph)_3P{=}CHCO_2Bu^t$ added in situ, −50 °C.

the aldehyde followed by Horner−Emmons reaction of *tert*-butyl diethylphosphonoacetate ($(EtO)_2P(O)CH_2CO_2Bu^t$) on the isolated aldehyde resulted in a poor yield of **3**. Attempts to form the aldehyde from the *N*-methoxy-*N*-methylcarboxamide derivative of the carboxylic acid followed by lithium aluminum hydride[13] reduction also resulted in poor yields. It was shown by NMR that the aldehyde exists as the aminal at room temperature (Scheme 3) and slowly decomposes in solution.

To avoid formation of the aminal, Swern conditions[14] were used that generated the reactive aldehyde at −78 °C which then was reacted in situ at that temperature with the milder Wittig reagent. These conditions resulted in a high yield (70−85%) of exclusively the (*E*)-α,*β*-unsaturated ester **3** in a one-pot sequence for the two steps. Addition of the Wittig reagent ($Ph_3P{=}CHCO_2Bu^t$) at low temperature (−50 °C) to the Swern oxidation mixture enables the aldehyde that is formed to be captured immediately before it cyclizes to the aminal. Similarly the phosphorane ethyl ester gave the unsaturated ester **4**. Cyclization of the α,*β*-unsaturated ester **3** with 0.25 equiv of LHMDS (lithium hexamethyldisilazide) in the presence of TMEDA (tetramethylethylenediamine) gave the major *trans*-substituted pyrrolidine **5** in 62% yield together with the 5,5-*cis*-lactam **7** in 13% yield, which resulted from the internal cyclization of the minor *cis*-substituted pyrrolidine product. Similarly **4** gave **6** in 75% yield. Stereoselective methylation[10] of the *tert*-butyl ester **5** with methyl iodide afforded predominantly the *β*-methyl isomer **8** (plus 10% of α-methyl isomer) in 66% yield. The one-pot deprotection of the *tert*-butyl ester and the Boc-amine in **8** occurs in 98% yield to give the amino acid **9**. Cyclization of **9** with diphenyl phosphorazidate was achieved in 66% yield to give the *β*-methyl-5,5-*trans*-lactam **10** plus 10% of the α-methyl isomer **16** (Scheme 2) which were readily separated by preparative HPLC. Deprotection of **5** with TFA was quantitative; however, cyclization of

Scheme 4[a]



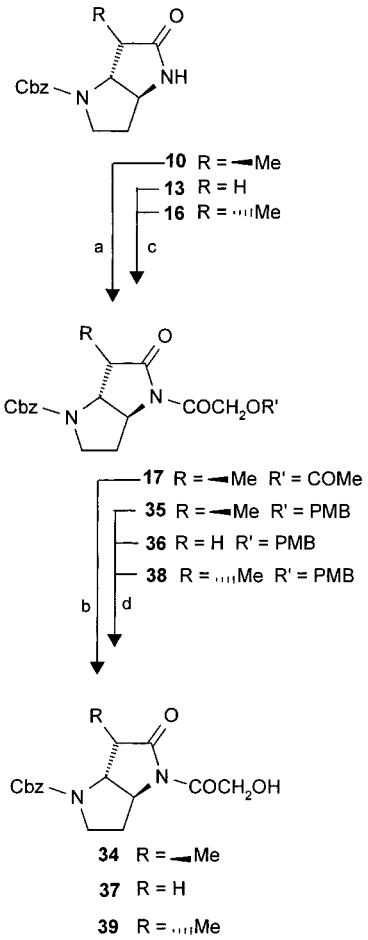
R	R1 = β Me		R1 = H		R1 = α Me	
	Yield		Yield		Yield	
$COCH_2OCOMe$	**17**	64%	**22**	59%	**29**	61%
COMe	**18**	44%*	**23**	89%	**30**	89%
$CO_2CH{=}CH_2$		-	**24**	52%	**31**	78%
CO_2Me	**19**	77%	**25**	95%		-
SO_2Me	**20**	68%	**26**	67%	**32**	80%
$SO_2CH{=}CHPh$	**21**	67%	**27**	10%	**33**	14%
$COCMe_2OCOMe$	-		**28**	34%	-	
CONHMe	-		**40**	69%		-
$CONHCHMe_2$	-			-	**41**	31%
COCHCl	-			-	**42**	38%
$SO_2PhNHCOMe$	-			-	**43**	5%

[a] Reagents and conditions: (a) LHMDS (1.2 equiv)/THF, −78 °C, 30 min at 0 °C; (b) electrophile, −78 °C. *This reaction was carried out using acetic anhydride and NaH.

the amino acid **11** with diphenyl phosphorazidate gave only 15% of the cyclized unsubstituted *trans*-lactam **13** under a variety of conditions, in contrast to the *β*-methyl series above. However an optimization exercise[15] showed that, after deprotection of **6** with TFA in 80% yield, lactamization of ethyl ester **12** with Bu^tMgCl gave the unsubstituted *trans*-lactam **13** in 78% yield. Protection of the desmethyl-5,5-*trans*-lactam **13** with Boc-anhydride to give the *tert*-butyl ester **14** followed by stereospecific methylation[10] with methyl iodide (2 equiv) in the presence of DMPU (1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone) at −20 °C gave **15,** which on deprotection with TFA gave the α-methyl-5,5-*trans*-lactam **16** in 51% yield for the three steps.

The base of choice for introducing substituents onto the lactam nitrogen was found to be LHMDS (1.2 equiv in THF for 30 min at 0 °C and then adding the electrophile at −78 °C) which was far superior in terms of yield, ease of manipulation, and time required compared to sodium hydride, which had been used previously, and is compatible with a range of electrophiles including acid chlorides, anhydrides, chloroformates, and especially sulfonyl chlorides (Scheme 4). Surprisingly with *β*-styrenesulfonyl chloride low yields were seen with the desmethyl-5,5-*trans*-lactam and α-methyl-5,5-*trans*-lactam ring systems compared to the *β*-methyl-5,5-*trans*-lactam ring system. In part, this may be due to the instability of any anion, generated under equilibrium conditions, next to the lactam carbonyl in the α-methyl-5,5-*trans* series relative to the *β*-methyl-5,5-*trans* series, and the bulk of the electrophile. The

Scheme 5[a]



[a] Reagents and conditions: (a) LHMDS (3 equiv)/THF, −78 °C, then $ClCOCH_2OAc$; (b) H_2SO_4 or enzymes; (c) LHMDS (3 equiv)/THF, −78 °C, then $ClCOCH_2OPMB$ or LHMDS (1.2 equiv)/THF, −78 °C, then $(Me)_3CCO_2COCH_2OPMB$; (d) DDQ in H_2O/CH_2Cl_2, rt.

more hindered acetoxydimethylacetyl derivative **28** was also formed in low yield. Also amides **40** and **41** were prepared in 69% and 31% yields, respectively, by reacting the anion of the lactam with the required isocyanate. Reacting the anion of **10** with chloroacetyl chloride and with 4-acetylaminophenylsulfonyl chloride gave **42** and **43** in 38% and 5% yields, respectively.

The hydroxymethylacetyl derivative **34** was envisaged as a crucial intermediate for attempts to mimic the conserved serine at S1′ (Scheme 5). Attempted hydrolysis of the acetoxy group in **17** with base ($KHCO_3$ or K_2CO_3) caused loss of the acetoxyacetyl group giving only the unsubstituted lactam, while hydrolysis with acid or a range of enzymes gave varying amounts of alcohol **34** (the best yield being 26%), unsubstituted lactam, and starting material.

Other methods were investigated to prepare this alcohol. The *para*-methoxybenzyl (PMB) analogue was chosen as this group can be removed oxidatively avoiding basic and acidic conditions. *para*-Methoxybenzyloxyacetyl chloride was prepared[16] and used crude with lactam **10** to give the *β*-methyl-5,5-*trans*-lactam derivative **35** in 29% yield and then deprotected with DDQ to give the required alcohol **34** in 86% yield. Attempts to purify the crude *para*-methoxybenzyloxyacetyl chloride by distillation resulted in decomposition of the acid chloride. A higher yield in the acylation reaction was achieved using pivaloyl mixed anhydride[17] (prepared from *para*-methoxybenzyloxyacetic acid and pivaloyl chloride) which in the desmethyl-5,5-*trans*-lactam series gave the protected alcohol **36** from **13** as a white solid in 65% yield. Deprotection of this product with DDQ gave **37** in 75% yield (Scheme 5). Similarly prepared in the α-methyl-5,5-*trans*-lactam series was the protected alcohol **38** from **16** in 67% yield. Oxidative deprotection of **38** with DDQ gave the alcohol **39** in 71% yield.

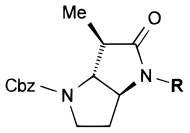
Results and Discussions

HCMV *δ*Ala protease formed by removing the internal cleavage site (Ala 142/Ala 143) from the wild-type 28-kb HCMV protease has been cloned and expressed in *E. coli*.[18] We have shown that this HCMV *δ*Ala protease cleaves synthetic peptides (14-mers) based on the natural substrates,[18] and this forms the basis of an HPLC-based assay that has been developed and which allows evaluation of potential inhibitors.[19] The percent inhibition and IC_{50} values for the pyrrolidine-5,5-*trans*-lactams (Tables 1−3) were determined using this HPLC assay. Compounds were routinely assayed by determining percent inhibition after various periods of preincubation. The results obtained give an idea of the 'on' rate (acylation) and 'off' rate (deacylation) and the duration of action of each compound. Determination of percent inhibition using a range of inhibitor concentrations enabled values for IC_{50} (the inhibitor concentration giving 50% inhibition) to be determined.

β-Methyl-pyrrolidine-5,5-*trans*-lactams with Cbz protecting the pyrrolidine nitrogen and various electron-withdrawing groups on the lactam nitrogen were only weakly active. The best was the acetoxyacetyl derivative **17** (IC_{50} = 805 *μ*M; Table 1). Compounds in the desmethyl-5,5-*trans*-lactam series (Table 2) are more active than the corresponding *β*-methyl-5,5-*trans*-lactams. However the effects of various lactam nitrogen substituents on protease activity are similar in the two series. The acetoxyacetyl derivative **22** was best (IC_{50} = 102 *μ*M), but the acyl compounds **23** and **37** and the protected analogue **36** were also highly active. However, bulk adjacent to the carbonyl in **28** or the ester in **14** is not tolerated. Uniquely, the alkenes **24** and **27** retained good activity after 24 h of preincubation. This may indicate that the electron-deficient double bond is acting as a Michael acceptor or picking up additional binding. The lipophilic styrene and the PMB ether needed lengthy preincubations to achieve maximum effect, possibly due to insolubility.

With α-methyl-5,5-*trans*-lactams, a pattern similar to the other two series is seen with a range of lactam nitrogen substitutions (Table 3): the general trend in activity against HCMV *δ*Ala protease has been found to be acyl > ester > sulfonyl > carbamate. This is in line with the electron-withdrawing ability of the trigonal (sp^2) substituents (which activates the lactam carbonyl required for acylation of the active site serine) but not the tetrahedral (sp^3) sulfone, which indicates that the latter's stereochemistry is not so favored at this position probably due to steric reasons. The acetoxyacetyl analogue **29** is the most potent compound in any series (IC_{50} = 13 *μ*M) and achieves maximum inhibition during 30 min, while the lipophilic styrene analogue **33** achieves

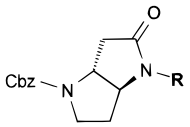
Table 1. *β*-Methyl-pyrrolidine-5,5-*trans*-lactams[a]



compd	R	inhib at 15 min	max % inhib (h)	inhib at 24 h	protease IC_{50} (μM)
17	$COCH_2OCOMe$	46.0	61.2 (1)	24.9	805
21	$SO_2CH{=}CHPh$	27.9	34.9 (2)	27.7	
18	COMe	15.4	39.8 (2)	36.5	1800
34	$COCH_2OH$	11.4	21.4 (24)	21.4	
35	$COCH_2OCH_2PhOMe$	nt	nt		
19	CO_2Me	7.9	17.8 (0.5)	0	
20	SO_2Me	15	19.4 (24)	19.4	

[a] [Compound] = 500 μM for all tests except IC_{50} determinations; nt, not tested.

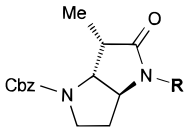
Table 2. Desmethyl-pyrrolidine-5,5-*trans*-lactams[a]



compd	R	inhib at 15 min	max % inhib (h)	inhib at 24 h	protease IC_{50} (μM)
22	$COCH_2OCOMe$	85.3	85.3 (0.08)	28.1	102
23	COMe	81.9	82.3 (1)	0	204
37	$COCH_2OH$	81.9	81.9 (0.25)	21.8	
36	$COCH_2OCH_2PhOMe$	69	81.7 (1)	19.1	
24	$CO_2CH{=}CH_2$	49.3	62 (0.5)	41	
27	$SO_2CH{=}CHPh$	46	77 (2)	60	
25	CO_2Me	35.9	54.6 (0.5)	10.8	
26	SO_2Me	29.6	48.4 (1)	8.3	
40	CONHMe	8.8	38 (2)	12.3	
28	$COCMe_2OCOMe$	7.4	14.9 (4)	0	
14	CO_2CMe_3	6.3	19.3 (4)	16.9	

[a] [Compound] = 500 μM for all tests except IC_{50} determinations.

Table 3. α-Methyl-pyrrolidine-5,5-*trans*-lactams[a]



compd	R	inhib at 15 min	max % inhib (h)	inhib at 24 h	protease IC_{50} (μM)
29	$COCH_2OCOMe$	96.6	96.6 (0.5)	0	13
30	COMe	97.7	97.9 (0.25)	8.2	110
31	$CO_2CH{=}CH_2$	73.8	96.5 (2)	48.9	
32	SO_2Me	48.8	59.7 (2)	22.1	
33	$SO_2CH{=}CHPh$	43.9	90.7 (24)	90.7	
39	$COCH_2OH$	96.1	97.0 (0.5)	0	200
38	$COCH_2OCH_2PhOMe$	90.3	97.3 (0.5)	17.9	32
43	$SO_2PhNHCOMe$	48.9	74.6 (4)	71.0	
42	$COCH_2Cl$	10.0	63.4 (1)	60.9	
41	$CONHCH(CH_3)_2$	3.2	9.2 (1)	0	

[a] [Compound] = 500 μM for all tests except IC_{50} determinations.

maximum inhibition only after 24 h of preincubation. Comparing the three templates (Table 4), potency is seen to increase in the order *β*-methyl-5,5-*trans*-lactam < desmethyl-5,5-*trans*-lactam < α-methyl-5,5-*trans*-lactam for acetoxyacetyl, acetyl, and mesyl substituents on the lactam nitrogen.

Selectivity in Comparison to the Mammalian Serine Proteases. The best compounds from each series were tested against thrombin and elastase (Table 5). While the α-methyl diastereomer is more active than the *β*-methyl diastereomer for the viral enzyme, the converse is true for the mammalian enzymes, elastase and thrombin, suggesting that selectivity may not be too difficult to achieve.

Long Duration of Action. Substitution on the lactam nitrogen of our α-methyl-lactam template has revealed three classes of inhibitors (Figure 1). These include the acyl compounds **29** and **30** which reach their maximum inhibition within ≤30 min and are turned over by 24 h. The majority of our acyl-*trans*-lactams in Tables 2 and 3 follow this pattern. The second group **33** (R = $SO_2CH{=}CHPh$; see Figure 1) is slower to reach a maximum (1−24 h) and is turned over more slowly (24−50 h). The third group includes **43** and **42** which

Table 4. Comparison of *trans*-Lactam Templates

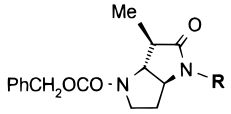
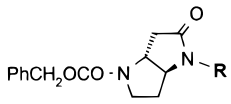
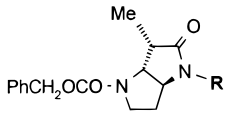
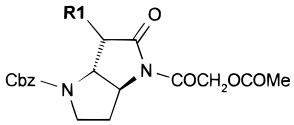
	HCMV δAla protease inhib				% inhib @ 500 μM, IC (μM)					
R	$COCH_2OCOMe$		COMe		$CO_2CH{=}CH_2$		$SO_2CH{=}CHPh$		SO_2Me	
	46%	805	15%	1800	–	–	28%	–	15%	–
	85%	131	82%	204	49%	–	46%	–	30%	–
	97%	13	98%	148	74%	418	44%	–	49%	–

Table 5. Protease Inhibitor Selectivity



	IC_{50} (μM)		
compd	HCMV	thrombin	elastase
17, R1 = β-Me	805	24.2	0.71
22, R1 = H	131	6.2	7.58
29, R1 = α-Me	13	>100	9.08

reach a maximum inhibition in 4 and 1 h, respectively, and both maintain that for 48 h. It is noteworthy that both of the long-duration lactams **33** and **43** are more sterically hindered than the carbonyl derivatives in the first group because of the bulky tetrahedral sulfur on the lactam nitrogen present in these sulfonyl derivatives. This hinders approach of the catalytic serine, which is shown by the longer time they take to reach maximum inhibition (24 and 4 h, respectively) and likewise hinders hydrolysis of the acylserine product, which accounts for their longer duration of action. In contrast the long duration of action of the halocarbonyl derivative **42** is probably due to the alkylation of the protease by this reactive functionality.

MS Characterization of the Acylation of δAla HCMV Protease by Pyrrolidine α-Methyl-5,5-*trans*-lactams. To investigate the mechanism of action of these *trans*-lactams, the interaction of HCMV δAla protease and inhibitors **29** and **31** has been studied by liquid chromatography (LC) and electrospray ionization mass spectroscopy (ESI/MS). Analysis of complexes formed between HCMV δAla protease and the inhibitors was conducted over a time course, enabling an assessment of the speed and duration of the covalent modification of the enzyme. Mass spectra were recorded after incubations of 3 min and 0.5, 3, and 21 h (Chart 1). The average molecular weight (M_{av}) of the unmodified HCMV δAla protease was determined as 27 826 ± 2 Da. The M_{av}'s of the complexes formed were consistent with the addition of the complete inhibitor molecule to the enzyme.

This study indicates the maximum formation of a covalent HCMV δAla protease/**29** complex during a period of 3 min, with an M_{av} of 28 200 Da, a difference of +374 Da with respect to the native protease (M_{av} = 27 826 Da). While for **31** the maximum formation of a covalent HCMV δAla protease/**31** complex occurs during a period of 30 min, with an M_{av} of 28 170 Da, a difference of +344 Da with respect to the native protease. This is consistent with the acylation of the enzyme by these *trans*-lactams. The enzyme gradually turns over the compounds within a 21-h period. Thus **29** and **31** have been shown by ESI/MS studies to acylate HCMV δAla protease in a reversible and covalent fashion.

Conclusions

Mechanism-based inhibitors of HCMV protease have been designed based on the novel pyrrolidine-5,5-*trans*-lactam ring system. New synthetic routes to three of these pyrrolidine-5,5-*trans*-lactam templates have been developed from 2,4-diaminobutyric acid. The β-methyl template has been synthesized in 8 stages in 13% yield, the desmethyl template in 7 stages in 10% yield, and the α-methyl template in 10 stages in 5% yield. The key intermediate pyrrolidine **5** for these templates has been synthesized in 32% yield in 5 stages from 2,4-diaminobutyric acid.

The activity against HCMV δAla protease is the greatest in the α-methyl series, and the best compound is the acetoxyacetyl derivative **29** with an IC_{50} = 13 μM. Activity decreases on moving from the α-methyl to the desmethyl to the β-methyl series. This selectivity is the opposite of that observed for the elastase and thrombin enzymes. For a range of lactam nitrogen substituents, the general trend in activity against HCMV δAla protease has been found to be acyl > ester > sulfonyl > carbamate. This is in line with the electron-withdrawing ability of the trigonal (sp^2) substituents but not the tetrahedral (sp^3) sulfone, which indicates that the latter's stereochemistry is less favored at this position.

ESI/MS studies of the interaction of HCMV δAla protease with the *trans*-lactams **29** and **31** have shown that these inhibitors bind covalently and reversibly and acylate HCMV δAla protease in a similar way, which is consistent with acylation of HCMV δAla protease at the active site nucleophile Ser 132.

Experimental Section

General Procedures. Melting points were obtained using an Electrothermal digital melting point apparatus and are uncorrected. All purifications by flash chromatography were performed using Kieselgel 60, Merck 9385 silica gel. Monitoring of reactions by TLC used Merck 60 F_{254} silica gel glass backed plates (5 × 10 cm), eluted with mixtures of ethyl

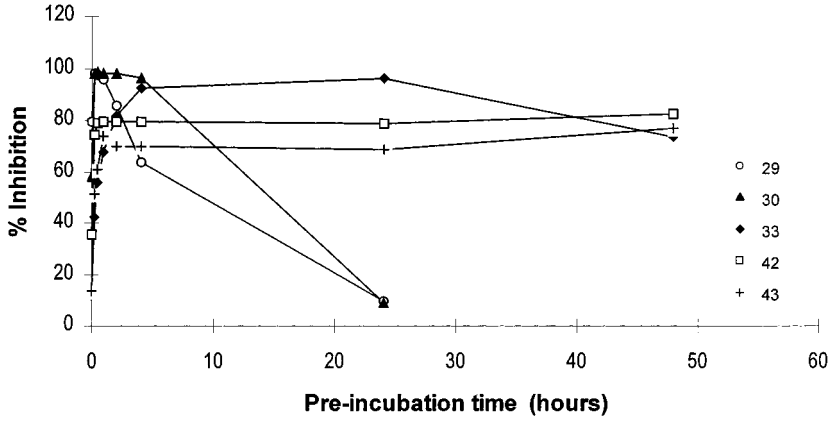
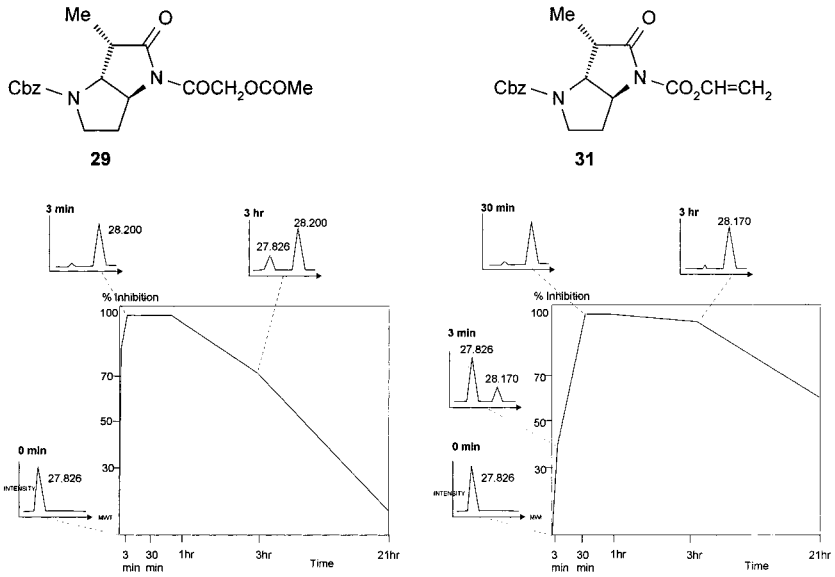


Figure 1. Protease inhibition with respect to preincubation duration.

Chart 1



acetate and cyclohexane, and visualized by UV light, followed by heating with aqueous phosphomolybdic acid. Analytical HPLC was run on a Hewlett-Packard 1090 HPLC instrument, equipped with an Intersil M column ODS2. Standard conditions were eluent system A (H_2O, 0.1% H_3PO_4) and system B (95% MeCN/H_2O, 0.1% H_3PO_4): gradient 0% B 2 min, 0−100% B 40 min, 100% B 10 min; flow rate = 1 mL/min, λ = 215 nm. Retention times (t_R) are given in minutes. LCMS was run on a Hewlett-Packard 1050 instrument coupled with a Micromass Platform II equipped with a Supelco ABZplus column. Standard conditions were eluent system A (H_2O, 0.1% formic acid, 10 mmol ammonium acetate) and system B (MeCN, 0.05% formic acid): gradient 100% A 0.7 min, 100% A−100% B 3.5 min, 100% B 3.5 min, 100−0% B 0.3 min; flow rate = 1 mL/min. All NMR spectra were run on a Bruker 250-MHz instrument generally as solutions in $CDCl_3$ unless otherwise stated. IR spectra were recorded on a Bio-rad FTS7 spectrometer from thin films on NaCl plates, a KBr mix or solutions in the solvent specified. Mass spectra were run on an Hewlett-Packard electrospray 5989B instrument. Final organic solutions were dried over $MgSO_4$ before filtration and evaporation using a Buchi rotavapor. Ambient temperature was 20 °C and all compounds were produced as racemic mixtures. All solvents used were Fisons analytical reagents except for pentane (Aldrich Chemical Co.) and anhydrous THF (Fluka sureseal). All other reagents were usually obtained from Aldrich, Fluka or Lancaster. Elemental microanalyses were determined by the Microanalytical Laboratory, GlaxoWellcome, Stevenage.

4-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-butyric Acid (1). d,l-2,4-Diaminobutyric acid dihydrochloride (20.78 g, 108.0 mmol) was dissolved in 1 M aqueous sodium hydroxide solution (218 mL, 218 mmol) and a suspension of copper(II) acetate monohydrate (11.95 g, 59.83 mmol) in water (135 mL) was added. The deep blue solution was stirred at ca. 70 °C for 1.75 h before being allowed to cool to room temperature. Then solid potassium hydrogen carbonate was added to the solution to pH 7.4 and the mixture filtered. The blue solution was cooled in an ice/water bath and diluted with dioxan (400 mL). Benzyl chloroformate (18.65 mL, 130.6 mmol) was added to the stirred, cooled solution immediately followed by 1 M aqueous sodium hydroxide (110 mL). The mixture was stirred in an ice/water bath for 20 min and then stirred at room temperature for 1 h before the precipitate was collected by filtration, and then washed sequentially with water, ethanol and diethyl ether and dried in vacuo to afford the copper complex as a blue solid. Ethylenediaminetetraacetic acid (12.58 g, 43.04 mmol) was suspended in 0.33 M hydrochloric acid (522 mL, 172 mmol) at room temperature. The copper complex (24.4 g, 43.02 mmol) was added portionwise to the vigorously stirred mixture over 30 min. After stirring for another 1.5 h, 4 M aqueous sodium hydroxide was added portionwise (over a period of 1 h) to the stirred mixture until the mixture was at pH 4−5. Then after a further 30 min the solid was collected by filtration, washed with water and dried, in vacuo, for approximately 1 h at room temperature to afford γ-Cbz-diaminobutyric acid, which was used without further purification. The γ-Cbz-diaminobutyric acid was suspended in 1 M aqueous sodium hydroxide (120 mL, 120 mmol) and a solution of di-*tert*-butyl dicarbonate (26.19 g, 120 mmol) in dioxan (200 mL) was added. The pH of the reaction mixture was maintained at pH 8 with the addition of 1 M aqueous sodium hydroxide. The mixture was stirred at room temper-

ature for 24 h and the reaction monitored by TLC (*n*-butanol−acetic acid−water, 4:1:1); if the reaction was incomplete additional di-*tert*-butyl dicarbonate (3.76 g, 17.23 mmol) in dioxan (16 mL) was added and the mixture stirred at room temperature for a further 24 h. Then the reaction mixture was mixed with ethyl acetate (250 mL) and acidified with 2 M hydrochloric acid to pH 2. The phases were separated and the aqueous one extracted with ethyl acetate (3 × 200 mL). The organic phases were combined, washed with saturated brine (2 × 150 mL) and the solvents then removed by evaporation. The residue was treated with 1 M aqueous sodium hydroxide solution (120 mL, 120 mmol), diluted with water (400 mL) and washed with diethyl ether (3×, 300 mL). Then the aqueous phase was mixed with ethyl acetate (200 mL) and acidified to pH 1 using 2 M hydrochloric acid, the organic layer was separated and the aqueous layer extracted with ethyl acetate (2 × 200 mL). The combined ethyl acetate phase was dried, filtered, evaporated and dried in vacuo at room temperature to afford **1** as a pale orange foam: ^{1}H NMR ($CDCl_3$) δ 7.35 (s, 5H, C_6H_5), 6.2−6.4,5.6,5.4 (m, 2H, 2NH), 5.0−5.2 (m, 2H, OCH_2), 4.3−4.5 (m, 1H, $NCHCO_2H$), 3.4−3.6, 3.0−3.4 (m, 2H, CH_2N), 2.0−2.2, 1.8−2.0 (m, 2H, $CHCH_2CH_2$), 1.45 (s, 9H, *t*-BuO); MS (thermospray) *m*/*z* 353 (MH^+); HPLC 99% (t_R 23.7 min). Anal. ($C_{17}H_{24}N_2O_6$) C, H, N.

(3-Benzyloxycarbonylamino-1-hydroxymethyl-propyl)carbamic Acid *tert*-Butyl Ester (2). A solution of acid **1** (28.03 g, 79.54 mmol) in tetrahydrofuran (250 mL) was cooled to −10 °C under nitrogen and 4-methylmorpholine (8.8 mL, 80.04 mmol) was added followed by the addition of ethyl chloroformate (7.65 mL, 80.01 mmol) over 2 min. After stirring for 8 min, a freshly prepared solution of sodium borohydride (9.03 g, 241.6 mmol) in water (70 mL) was added dropwise over a period of 12 min at −15 °C. After a further 5 min the mixture was poured into water (1.5 L) and stirred for another 10 min at room temperature. The mixture was extracted with ethyl acetate (3 × 500 mL) and the combined organic extracts washed sequentially with 1 M hydrochloric acid (500 mL), water (500 mL), saturated solution of sodium hydrogen carbonate (500 mL) and saturated brine (500 mL), then dried, filtered and evaporated. The crude residue, a colorless syrup, was purified by flash chromatography using cyclohexane−ethyl acetate (3:2) as eluent, to give **2** as a white solid (21.15 g, 78%): IR ($CHBr_3$) ν_{max} 3431, 2973, 1704 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.35 (s, 5H, C_6H_5), 5.55, 4.95 (m, 2H, 2NH), 5.0−5.2 (m, 2H, OCH_2), 3.5−3.8 (m, 3H, $HOCH_2CH$), 3.4−3.6, 2.9−3.2 (m, 2H, CH_2N), 2.5 (t, 1H, HO), 1.5−1.8 (m, 2H, $CHCH_2CH_2$), 1.45 (s, 9H, *t*-BuO); HPLC 98.9% (t_R 23.25 min). Anal. ($C_{17}H_{26}N_2O_5$) C, H, N.

(*E*)-6-Benzyloxycarbonylamino-4-*tert*-butoxycarbonylamino-hex-2-enoic Acid *tert*-Butyl Ester (3). A solution of dimethyl sulfoxide (12.83 mL, 0.18 mol) in dichloromethane (240 mL) was cooled to −70 °C under nitrogen. Oxalyl chloride (13.3 mL, 0.15 mol) was added dropwise (care: evolution of gas) and the solution stirred for 15 min while maintaining the temperature at −60 to −70 °C. A solution of the alcohol **2** (23.7 g, 0.07 mol) in dichloromethane (200 mL) was added over 25 min at −65 °C. The reaction mixture was then stirred for 20 min during which time the temperature rose to −52 °C. The reaction was recooled to −70 °C prior to the addition of triethylamine (102 mL, 0.7 mol) at a rate which maintained the temperature below −45 °C (∼10 min, exothermic), followed by the addition of (*tert*-butoxycarbonylmethylene)triphenylphosphorane (184 mg, 0.49 mmol), in one portion. The reaction mixture was allowed to warm to 0 °C and then poured into a mixture of saturated brine (1 L) and diethyl ether (1 L). The organic phase was separated, dried ($MgSO_4$) and then concentrated under reduced pressure. The crude residue was purified by flash chromatography using (4:1→2:1), cyclohexane−ethyl acetate as eluent to give **3** (27.3 g, 90%) as a white solid: mp 113−116 °C; IR ($CHBr_3$) ν_{max} 3432, 1707, 1506 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.35 (s, 5H, C_6H_5), 6.75 (dd, J = 15 Hz, 5 Hz, 1H, $CHCHCH$), 5.85 (dd, J = 15 Hz, 1.2 Hz, 1H, O_2CCHCH), 5.4,4.6 (br, 2H, 2NH), 5.0−5.2 (m, 2H, OCH_2), 4.4 (m, 1H, $CHCHNH$), 3.4−3.6, 2.9−3.1 (m, 2H, CH_2N), 1.8−2.0, 1.4−1.6 (m, 2H, $CHCH_2CH_2$), 1.5,1.45 (2s, 18H, 2*t*-BuO); HPLC 99.3% (t_R 32.3 min). Anal. ($C_{23}H_{34}N_2O_6$) C, H, N.

(*E*)-6-Benzyloxycarbonylamino-4-*tert*-butoxycarbonylamino-hex-2-enoic Acid Ethyl Ester (4). Compound **2** (12.6 g) was reacted with oxalyl chloride/dimethyl sulfoxide followed by (ethoxycarbonylmethylene)triphenylphosphorane as described for **3** to give **4** (10.71 g, 71%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.40−7.30 (m, 5H), 6.86 (dd, 1H), 5.93 (dd, 1H), 5.42−5.28 (br, 1H), 5.12 (ABq, 2H), 4.72−4.60 (m, 1H), 4.50−4.32 (m, 1H), 4.19 (q, 2H), 3.60−3.30 (m, 1H), 3.15−2.98 (m, 1H), 2.00−1.80 (m, 1H), 1.65−1.50 (m, 1H), 1.45 (s, 9H) and 1.28 (t, 3H); TLC R_f 0.45 (2:3 ethyl acetate−cyclohexane); HPLC 98.7% (t_R 11.25 min). Anal. ($C_{21}H_{30}N_2O_6$) C, H, N.

***cis*-2-Oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1,4-dicarboxylic Acid 4-Benzyl Ester 1-*tert*-Butyl Ester (7) and *trans*-3-*tert*-Butoxycarbonylamino-2-*tert*-butoxycarbonylmethyl-pyrrolidine-1-carboxylic Acid Benzyl Ester (5).** To a solution of **3** (902 mg, 2.07 mmol) in anhydrous toluene (12 mL), stirred under nitrogen at 21 °C, was slowly added 1 M LHMDS solution in hexane (1.03 mL, 1.03 mmol). The yellow solution was stirred for 1.5 h, poured into saturated aqueous ammonium chloride solution (50 mL) and the mixture extracted twice with ethyl acetate (50 mL and 25 mL). The combined organic phase was washed with saturated brine (50 mL), dried and evaporated to give a yellow oil (859 mg). The crude oil was purified by flash chromatography eluting with cyclohexane−ethyl acetate (4:1) to give **5** (557 mg, 62%) as a white solid: mp 125−126 °C; IR (Nujol) ν_{max} 3303, 1703, 1463 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.28 (m, 5H, C_6H_5), 5.18−5.10 (m, 2H, $PhCH_2$), 4.76−4.61 (broad signal, 1H, broad signal, $BocNH$), 4.15−4.01 (m, 1H, $CHNHBoc$), 4.00−3.94 (m, 1H, $CbzNCHCH_2$), 3.60−3.42 (m, 2H, $CbzNCH_2$), 2.88 and 2.69 (2d, 1H, J = 15.5 Hz, $CHHCO_2Bu^t$), 2.40−2.30 (dd, 1H, J = 15.5, 9.5 Hz, $CHHCO_2Bu^t$), 2.23−2.11 (m, 1H, $CbzNCH_2CHH$), 1.94−1.82 (broad signal, 1H, $CbzNCH_2CHH$), 1.44 (s, 18H, 2 × $C(CH_3)_2$); TLC R_f 0.38 (2:1 cyclohexane-EtOAc). Anal. ($C_{23}H_{34}N_2O_6$) C, H, N.

Further elution with cyclohexane−ethyl acetate (2:1) gave the *cis*-lactam **7** (97.7 mg, 13%): IR ($CHBr_2$) ν_{max} 1783, 1743, 1698 and 1419 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.36 (s, 5H, C_6H_5), 5.08 (ABq, 2H, J = 12.4 Hz, $PhCH_2$), 4.58 (m, 1H, H-3a), 4.27 (m, 1H, H-6a), and 3.54 (m, 1H, H-2), 3.32 (m, 1H, H-2), 2.84 (m, 1H, H-6) and 2.45 (1H, m, H-6), 2.12 (m, 2H, H-3) 1.45 (s, 9H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 172.6, 154.4, 249.7, 136.4, 128.6, 128.3, 128.2, 83.6, 67.4, 61.5, 53.0, 44.1, 39.1, 31.2, 28.2, 28.1, 27.9; MS (thermospray) *m*/*z* 261 (MH^+ − Boc); TLC R_f 0.15 (2:1 cyclohexane−EtOAc). Anal. ($C_{19}H_{24}N_2O_5$) C, H, N.

***trans*-3-*tert*-Butoxycarbonylamino-2-ethoxycarbonylmethyl-pyrrolidine-1-carboxylic Acid Benzyl Ester (6).** Compound **4** (12.2 g) was reacted with tetramethylethylenediamine followed by LHMDS as described for **3** to give **6** (8.49 g, 70%) as a white solid: mp 99−100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.28 (m, 5H, C_6H_5), 5.17−5.10 (m, 2H, $PhCH_2$), 4.75−4.60 (broad signal, 1H, $BocNH$), 4.18−3.97 (m, 4H, $CHNHBoc$ plus $CbzNCHCH_2$ plus CO_2CH_2Me), 3.64−3.43 (m, 2H, $CbzNCH_2$), 2.88 and 2.72 (2d, 1H, J = 15.3 Hz, $CHHCO_2Bu^t$), 2.55−2.41 (m, 1H, $CHHCO_2Et$), 2.23−2.11 (m, 1H, $CbzNCH_2CHH$), 1.91−1.81 (broad signal, 1H, $CbzNCH_2CHH$), 1.43 (s, 9H, $C(CH_3)_3$), 1.27−1.18 (m, 3H, CH_2CH_3); MS (thermospray) *m*/*z* 407.4 (MH^+); TLC R_f 0.8 (1:1 cyclohexane−EtOAc). Anal. ($C_{21}H_{30}N_2O_6$) C, H, N.

***rel*-(2*R*,3*S*)-3-*tert*-Butoxycarbonylamino-2-((1*R*)-*tert*-butoxycarbonyl-ethyl)pyrrolidine-1-carboxylic Acid Benzyl Ester (8).** To a solution of ester **5** (2.00 g, 4.60 mmol) in dry tetrahydrofuran (8 mL), stirred at −78 °C under nitrogen was added 1 M LHMDS solution in tetrahydrofuran (20.0 mL, 20.0 mmol). The reaction mixture was stirred at −78 °C for 25 min, and then hexamethylphosphoric triamide (5 mL) was added. After 10 min, iodomethane (0.33 mL, 5.3 mmol) was added. Additional iodomethane (0.16 mL, 2.57 mmol) was added after a further 28 min. The reaction mixture was stirred at −78 °C for a further 25 min, quenched with saturated aqueous ammonium chloride (68 mL), warmed to room temperature and extracted with ethyl acetate (4×, 35 mL). The

combined organic phase was dried, filtered and evaporated. The crude residue was purified by flash column chromatography eluting with 6:1 cyclohexane−EtOAc to yield **8** (1.42 g; 68%): mp 115−117 °C; IR ($CHBr_3$) 3430, 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5H, C_6H_5), 5.16 (s, 2H, PhCH_2), 4.54 (bs, 1H, N*H*Boc), 4.17 (m, 1H, C*H*N), 3.88 (d, J = 7.0 Hz, 1H, C*H*N), 3.60 (m, 1H, CH*H*NCbz), 3.39 (m, 1H, CH*H*NCbz), 2.73 (bd, 1H, COC*H*Me), 2.16 (m, 1H, CH_2CH_2CHNBoc), 1.83 (bs, 1H, CH_2CH_2CHNBoc), 1.42 (s, 18H, 2 x C(CH_3)$_2$), 1.17 (bs, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.9, 155.6, 155.1, 128.9, 128.4, 67.3, 67.1, 66.5, 54.2, 53.3, 45.0, 43.6, 42.9, 30.9, 30.1, 28.7, 28.3, 14.4; MS (thermospray) *m*/*z* 449 (MH^+). Anal. ($C_{24}H_{36}N_2O_6$) C, H, N. Also eluted was the reverse Michael product (0.35 g, 17%). Spectral and chromatographic properties were identical to those for **3**.

***rel*-(2*R*,3*S*)-3-Amino-2-((1*R*)-carboxyethyl)pyrrolidine-1-carboxylic Acid Benzyl Ester (9).** To **8** (159 mg, 0.35 mmol) were added trifluoroacetic acid (1 mL) and water (0.04 mL), and the resulting solution stirred at room temperature under nitrogen for 2 h. The reaction mixture was poured into diisopropyl ether (50 mL) and a white solid was precipitated. The supernatant liquid was decanted off and the solid dissolved in ethyl acetate (3 mL) and evaporated under reduced pressure to give **9** as a white, hygroscopic foam (110 mg; 71%): ^1H NMR ($CDCl_3$) δ 8.13 (bs, 2H, NH_2), 7.27 (s, 5H, C_6H_5), 5.15 (m, 1H, PhCH_2), 4.92 (m, 1H, PhCH_2), 4.44 (m, 1H, C*H*NH_2), 3.80 (m, 2H, CH*H*NCbz and C*H*NCbz), 3.37 (m, 1H CH*H*NCbz), 2.45 (m, 1H) and 2.20 (m, 2H) (CH_2CH_2CHNBoc and COC*H*Me), 1.12 (m, 3H, CH_3); MS (thermospray) *m*/*z* 293 (MH^+). Anal. ($C_{24}H_{36}N_2O_6$·0.1EtOAc·1.1TFA·0.6H_2O) C, H, N, F.

***rel*-(3a*S*,6*R*,6a*R*)-6-Methyl-5-oxo-hexahydro-pyrrolo-[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (10).** To a solution of the amino acid **9** (302 mg, 0.714 mmol) in anhydrous DMF (73 mL), stirred at room temperature under nitrogen, were added triethylamine (0.54 mL, 3.874 mmol) and then diphenyl phosphorazidate (0.330 mL, 1.53 mmol). The reaction mixture was stirred for 23 h, and then evaporated. The crude residue was purified by flash column chromatography eluting with ethyl acetate to yield **10** (128 mg, 65%) as a white solid: mp 163−165 °C; IR (KBr) ν_{max} 3200, 1704, 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3−7.4 (s, 5H, C_6H_5), 6.08 (br, 1H, N*H*), 5.25 (m, 1H, C*H*CH$_3$), 5.14 (m, 2H; PhCH_2), 3.92−3.68 (m, 2H, NC*H*HCH$_2$ and NC*H*CH$_2$), 3.28 (m, 1H, NCH*H*CH$_2$), 3.03 (2d, J = 10 Hz, 1H, NC*H*CHMe), 2.52 (m, 1H, C*H*Me), 2.21 (2t, J = 6 Hz, 1H, NCH$_2$C*H*H) and 1.80 (2t, J = 10 Hz, 1H, NCH$_2$CH*H*), 1.55−1.24 (br, 3H, Me); NOEs detected from NCH$_2$C*H*H to NCH$_2$C*H*H, NC*H*, NCH*H*; NCH$_2$C*H*H to NCH$_2$C*H*H, NC*H*, NC*H*, N*H*; C*H*MeCO to NC*H*; NC*H* to NCH$_2$C*H*H; NC*H* to N*H*, NCH$_2$C*H*H, C*H*MeCO; NCH*H* to NCH*H*, NC*H*; NCH*H* to NCH*H*, NCH$_2$C*H*H; N*H* to NC*H*, NC*H*; COSY H^1 NMR 2D spectra as expected; ^{13}C NMR ($CDCl_3$) δ 154.6, 128.1, 127.8, 127.7, 70.1, 66.7, 58.1, 49.1, 44.5, 26.9, 13.4; MS (thermospray) *m*/*z* 292 (MNH_4^+), 275 (MH^+). Anal. ($C_{15}H_{18}N_2O_3$) C, H, N.

***trans*-3-Amino-2-carboxymethyl-pyrrolidine-1-carboxylic Acid Benzyl Ester (11).** A solution of **5** (4.34 g, 10 mmol) in 24/1 trifluoroacetic acid/water (25 mL) was stirred at room temperature for 75 min and then poured into diisopropyl ether (500 mL). The mixture was stirred at room temperature for a further 45 min and the precipitate filtered, washed with diisopropyl ether (150 mL) dissolved in methanol (100 mL) and evaporated. The residue was dried in vacuo at room temperature overnight to give a pale yellow foam (3.51 g, 89%): IR (KBr) ν_{max} 1712, 1671 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.98 (bm, 6H, C_6H_5 and O*H*), 5.02 (bm, 2H, PhCH_2), 4.28 (m, 1H, C*H*NH$_2$), 3.79 (m, 1H, C*H*CH$_2$), 3.50 (m, 1H, NC*H*HCH$_2$), 3.33 (m, 1H, NC*H*HCH$_2$), 2.98 (bd, 1H) and 2.18 (m, 3H) (NCH$_2$C*H*$_2$ and C*H*$_2$CO); MS (thermospray) *m*/*z* 279 (MH^+); HPLC 95.9% (t_R 11.40 min), 2.6% (t_R 17.75 min). Anal. ($C_{14}H_{18}N_2O_4$·1.0TFA·0.1MeOH·0.1H_2O) C, H, N, F.

***trans*-3-Amino-2-ethoxycarbonylmethyl-pyrrolidine-1-carboxylic Acid Benzyl Ester (12).** To the Boc-protected compound **6** (60 g, 1 equiv, 0.148 mol) was added trifluoroacetic acid (557 g, 376 mL, 33 equiv, 4.88 mol) at room temperature. After stirring for 35 min the solution was evaporated and the resulting oil was dissolved in ethyl acetate (400 mL) and washed with 2 M sodium hydroxide (3 × 200 mL), water (250 mL), brine (200 mL), dried ($MgSO_4$) and evaporated and then left under high vacuum for 24 h to give **12** as a golden oil (41.68 g, 92%): IR (KBr) ν_{max} 3370, 1729, 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (m, 5H, C_6H_5), 5.14 (s, 2H, PhCH_2), 4.12 (m, 2H, CH_2-CH$_3$), 3.90 (br d, J = 9.4 Hz, 1H), 3.65 (m, 1H) and 3.48 (m, 2H), (CH$_2$C*H*NH$_2$, CH_2NCbz and C*H*NCbz), 2.98 (d, J = 16.3 Hz, 0.5H) and 2.77 (d, J = 16.3 Hz, 0.5H) (EtOCOCH*H*), 2.31 (dd, J = 10.0, 15.7 Hz, 1H, EtOCOCH*H*) 2.08 (m, 1H, $CH_2CH_2CHN_2$), 1.68 (m, 1H, $CH_2CH_2CHNH_2$), 1.41 (s, 2H, NH_2), 1.26 (m, 3H, CH_3); MS (Thermospray), *m*/*z* 307 (MH^+), 613 (2M + H^+); HPLC 89% (t_R 14.27 min). Anal. ($C_{16}H_{22}N_2O_4$) C, H, N.

***trans*-5-Oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (13).** To the amino ester **12** (1.25 g, 4.1 mmol) in dry tetrahydrofuran (32 mL) under nitrogen in an ice−salt bath was added dropwise 1 M Bu^{t}MgCl solution in tetrahydrofuran (13.1 mL, 13.1 mmol) over 15 min, keeping the temperature <1 °C. The mixture was stirred at 0 °C for 1 h and warmed to room temperature over 45 min, then quenched with saturated aqueous ammonium chloride (30 mL) while cooling in an ice bath. The phases were separated and the aqueous phase diluted with water (30 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic phase was washed with water (30 mL) and brine (30 mL), dried and evaporated to give the title compound as a crude, white solid. This residue was purified by flash column chromatography eluting with ethyl acetate to give the lactam **13** (833 mg, 78%) as a white solid: mp 157−159 °C; IR (KBr) ν_{max} 3277, 1699, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5H, C_6H_5), 5.99 (s, 1H, N*H*), 5.12 (ABq, J = 12.5 Hz, 2H, PhCH_2), 3.87 (dd, 1H, J = 10.4 Hz, J = 12.4 Hz, NC*H*HCH$_2$), 3.71 (m, 1H, NCH*H*CH$_2$), 3.34 (m, 2H, NC*H*CH$_2$ and NC*H*CH$_2$CO), 2.81 (m, 1H, C*H*HCO), 2.45 (m, 1H, CH*H*CO), 2.23 (m, 1H, NCH$_2$C*H*H), 1.87 (m, 1H, NCH$_2$CH*H*); ^{13}C NMR ($CDCl_3$) δ 179.5, 155.8, 136.2, 128.6, 128.2, 128.0, 67.2, 63.4, 60.5, 49.3, 39.6, 27.7; MS (thermospray) *m*/*z* 261 (MH^+), 278 (MNH_4^+); HPLC 99.5% (t_R 18.56 min). Anal. ($C_{14}H_{16}N_2O_3$·0.05CH_2Cl_2) C, H, N.

Alternative Preparation of 13. To a solution of the amino acid **11** (35 mg, 0.088 mmol) in dichloromethane (12 mL), stirred under nitrogen at room temperature, were added triethylamine (65 μL, 0.44 mmol) and then 2-chloro-1-methylpyridinium iodide (79 mg, 0.308 mmol). The reaction mixture was stirred for 66 h and then reduced in vacuo. The resulting yellow oil was purified by flash column chromatography eluting with ethyl acetate to yield **13** (9 mg, 41%), identical in all respects with compound **13** prepared above.

***trans*-2-Oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1,4-dicarboxylic Acid 4-Benzyl Ester 1-*tert*-Butyl Ester (14).** To a solution of **13** (509 mg, 1.96 mmol) in tetrahydrofuran (26 mL), stirred at −78 °C under nitrogen, was added 1 M LHMDS solution in tetrahydrofuran (2.35 mL, 2.35 mmol). The reaction mixture was stirred at −78 °C for 6 min and then warmed rapidly to 0 °C. After a further 15 min, the reaction mixture was cooled −78 °C and a solution of di-*tert*-butyl dicarbonate (1.22 g, 5.11 mmol) in tetrahydrofuran (7 mL) added. After 20 min, the reaction was quenched with saturated aqueous ammonium chloride (85 mL), warmed to room temperature and extracted with ethyl acetate (2 × 150 mL). The combined organic phase was dried, filtered and evaporated. The crude residue was purified by flash column chromatography eluting with ethyl acetate−cyclohexane (3:7) to yield **14** (563 mg, 80%) as a white solid: mp 109−111 °C; IR ($CDCl_3$) nmax 1794, 1766, 1713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (s, 5H, C_6H_5), 5.12 (ABq, J = 12.5 Hz, PhCH_2), 3.85 (m, 1H, NC*H*HCH$_2$), 3.73 (m, 1H, NCH*H*CH$_2$), 3.47 (m, 1H, NC*H*CH$_2$), 3.32 (m, 1H, NC*H*CHMe), 2.93 (m, 1H, C*H*HCO), 2.53 (m, 2H, NCH$_2$C*H*H and C*H*HCO), 2.02 (m, 1H, NCH$_2$CH*H*), 1.53 (s, 9H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) δ 173.2, 134.7, 128.0, 127.7, 127.5, 82.8, 66.8, 62.8, 59.5, 48.1, 40.1, 28.2, 27.4; MS (thermospray) *m*/*z* 378 (MNH_4^+), 361 (MH^+); HPLC 99.74% (t_R 27.65 min). Anal. ($C_{19}H_{24}N_2O_5$) C, H, N.

***rel*-(3*S*,3a*R*,6a*S*)-3-Methyl-2-oxo-hexahydro-pyrrolo-[3,2-*b*]pyrrole-1,4-dicarboxylic Acid 4-Benzyl Ester 1-*tert*-Butyl Ester (15).** To a solution of **14** (40.0 g, 110 mmol) in tetrahydrofuran (500 mL), stirred at −78 °C under nitrogen, was added 1 M LHMDS solution in tetrahydrofuran (143 mL, 143 mmol) keeping the temperature below −68 °C. The reaction mixture was stirred at −78 °C for 15 min, and then iodomethane (69 mL, 1.1 mol) was added dropwise. After 45 min, the reaction was quenched with saturated aqueous ammonium chloride (500 mL) and warmed to room temperature. Water (300 mL) was added and then the aqueous phase was extracted with ethyl acetate and the combined organic phase was washed with water, brine, dried ($MgSO_4$) and evaporated to give a red-brown oil. The crude residue was purified by flash column chromatography eluting with ethyl acetate−cyclohexane (3:7) to yield **15** (28.6 g, 69%) as a yellow foam: mp 89−91 °C; IR (KBr diffuse reflectance) ν_{max} 1790, 1763, 1717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 5H, C_6H_5), 5.13 (m, 2H, PhCH_2), 3.88 (m, 1H, NCHHCH$_2$), 3.70 (m, 2H, NCHHCH$_2$ and NCHCH$_2$) and 3.45 (dd, $J_{3a,6a}$ = 11 Hz, $J_{6,6a}$ = 6.6 Hz, 1H, NCHCHMe), 3.07 (br m, 1H, CHMe), 2.51 (m, 1H, NCH$_2$CHH), 2.01 (m, 1H, NCH$_2$CHH), 1.54 (s, 9H, C(CH$_3$)$_3$), 1.12 (bs, 3H, CH$_3$); NOE Me → H6 (6%), Me → H3a (6%); ^{13}C NMR ($CDCl_3$) δ 178.0, 156.1, 153.7, 129.1, 128.8, 128.6, 83.8, 68.0, 63.9, 59.2, 49.5, 43.7, 29.3, 28.4, 8.4; MS (thermospray) *m*/*z* 375 (MH^+); HPLC 97% (t_R 28.75 min). Anal. ($C_{20}H_{26}N_2O_5$) C, H, N.

Alternative Conditions at Higher Temperature in the Presence of DMPU. To a solution of **14** (180 mg, 0.5 mmol) in tetrahydrofuran (2 mL) were added DMPU (400 μL) followed by iodomethane (67 μL, 1 mmol). The mixture was stirred under nitrogen, cooled to −20 °C and 1 M LHMDS solution in tetrahydrofuran (0.55 μL, 0.55 μmol) was added. The mixture was stirred at −20 °C for 10 min, then quenched at this temperature by the addition of saturated aqueous ammonium chloride (5 mL) and allowed to warm to room temperature. The resulting mixture was partitioned between ethyl acetate and water, the organic phase separated, dried ($MgSO_4$) and evaporated. The residue was purified by flash column chromatography eluting with ethyl acetate−cyclohexane (3:7) to give a yellow foam (133 mg, 71%) identical in all respects with **15** prepared above.

***rel*-(3a*S*,6*S*,6a*R*)-6-Methyl-5-oxo-hexahydro-pyrrolo-[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (16).** To **15** (475 mg, 0.127 mmol) was added trifluoroacetic acid (1.6 mL), and the resulting solution stirred under nitrogen at room temperature for 35 min. The reaction mixture was then diluted with toluene (60 mL) and evaporated. The crude residue was dissolved in dichloromethane−diethyl ether (1:1) (60 mL) and evaporated and the entire procedure repeated. The crude residue was purified by flash column chromatography eluting with ethyl acetate to yield **16** (316 mg, 91%): mp 169−170 °C; IR ($CDCl_3$) ν_{max} 3275, 1710, 1698, 1683 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 5H, C_6H_5), 6.99 (bs, 1H, NH), 5.12 (ABq, *J* = 12.5 Hz, 2H, PhCH_2); 3.78 (br m, 1H, NCHHCH$_2$), 3.60 (m, 3H, NCHHCH$_2$, NCHCH$_2$ and NCHCHMe), 2.88 (br m, 1H, CHMe), 2.21 (m, 1H, NCH$_2$CHH), 1.85 (m, 1H, NCH$_2$CHH), 1.08 (br s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 183.1, 155.4, 135.9, 128.3, 127.9, 127.7,67.0, 66.4, 55.8, 49.3, 41.8, 27.6, 7.2; MS (thermospray) *m*/*z* 549 (2M + H^+), 275 (MH^+); HPLC 98.13% (t_R 19.82 min), 1.2% (t_R 18.51 min). Anal. ($C_{15}H_{18}N_2O_3$·0.01EtOAc) C, H, N.

***rel*-(3a*S*,6*R*,6a*R*)-4-Acetoxyacetyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (17).** To a solution of the lactam **10** (548 mg, 2 mmol) in anhydrous tetrahydrofuran (20 mL) cooled to −78 °C under nitrogen was added 1 M LHMDS solution in tetrahydrofuran (3 mL, 3 mmol) over ca. 2 min. The solution was stirred at −78 °C for 8 min, then at 0 °C for 30 min and then recooled to −78 °C. Acetoxyacetyl chloride (0.54 mL, 5 mmol) was added dropwise keeping the temperature below −70 °C and the solution was stirred at −78 °C for 1 h. Saturated ammonium chloride solution (20 mL) was added and the cooling bath was removed. The mixture was extracted with ethyl acetate (50 mL), the organic phase was washed with saturated ammonium chloride solution (20 mL) and the combined aqueous phase was extracted with ethyl acetate (25 mL). The combined organic phase was washed with water (25 mL) and then with saturated brine (25 mL), dried and evaporated to give an oil which was purified by flash column chromatography eluting with cyclohexane−ethyl acetate (4:1) to give the title compound **17** (429 mg, 57%) as a solid: mp 107−109 °C. A sample was crystallized from 2-propanol to give needles: mp 110−111 °C; IR ($CDCl_3$) ν_{max} 1755, 1747, 1721, 1713, 1698 cm^{-1}; ^{1} NMR ($CDCl_3$) δ 7.37 (s, 5H, C_6H_5), 5.15 (s, 2H, CH_2OAc), 5.09 (q, *J* = 18 Hz, 2H, PhCH_2), 3.7−3.9 (m, 2H, NCH_2CH$_2$), 3.48 (m, 1H, NCHCH$_2$), 3.07 (t, *J* = 11 Hz, 1H, NCHCHMe), 2.70 (m, 2H, NCH$_2$CHH and CHMe), 1.95 (2t, *J* = 11 Hz, 1H, NCH$_2$CHH), 2.19 (s, 3H, OAc), 1.46 (br, 3H, CH_3); TLC R_f 0.24 (cyclohexane−ethyl acetate, 2:1). Anal. ($C_{19}H_{22}N_2O_6$) C, H, N.

Preparation of 18−33, 35, 36, 38, 42, and 43. Using essentially the same procedure as for the preparation of **17**, the following compounds listed in Scheme 5 were prepared from **10, 13,** or **16**.

***rel*-(3a*S*,6*R*,6a*R*)-4-Acetyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (18):** derived from **10** and acetyl chloride to give **18** as a white amorphous solid (68%); IR ($CHBr_3$) ν_{max} 1756, 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (s, 5H, C_6H_5); 5.15 (s, 2H, PhCH_2), 3.80 (m, 2H, NCH_2CH$_2$), 3.45 (m, 1H, NCHCH$_2$), 3.00 (t, *J* = 11 Hz, 1H, NCHCHMe), 2.70 (m, 2H, NCH$_2$CHH and CHMe), 2.47 (s, 3H, COCH_3), 1.90 (2t, *J* = 11 Hz, 1H, NCH$_2$CHH), 1.42 (br, 3H, CH_3); MS (thermospray) *m*/*z* 317 (MH^+); TLC R_f 0.35, (cyclohexane−ethyl acetate, 7:3). Anal. ($C_{17}H_{20}N_2O_4$·0.25H_2O) C, H, N.

***rel*-(3*R*,3a*R*,6a*S*)-3-Methyl-2-oxo-hexahydro-pyrrolo-[3,2-*b*]pyrrole-1,4-dicarboxylic acid 4-benzyl ester 1-methyl ester (19):** derived from **10** and methyl chloroformate to give **19** (77%) as a white foam; IR (KBr) ν_{max} 1800, 1766, 1718, 1708, 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 5H, C_6H_5), 5.1 (s, 2H, PhCH_2), 3.9 (s, 3H, CO_2Me), 3.9−3.7 (m, 2H, NCH_2-CH$_2$), 3.5 (m, 1H, NCHCH$_2$), 3.0 (t, *J* = 11 Hz, 1H, NCH-CHMe**)**, 2.6 (m, 2H, NCH$_2$CHH and CHMe), 2.0 (m, 1H, NCH$_2$CHH); MS(thermospray) *m*/*z* 350 (MNH_4^+), 333 (MH^+); HRMS calcd for $C_{19}H_{23}N_2O_6$ (MH^+) 375.155612, found 375.155523; HPLC 98.5% (t_R 24.68 min).

***rel*-(3a*S*,6*R*,6a*R*)-4-Methanesulfonyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (20):** derived from **10** and methanesulfonyl chloride to yield **20** (68%) as a white foam; IR (KBr) ν_{max} 1756, 1702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5H, C_6H_5), 5.1 (s, 2H, PhCH_2), 3.9−3.7 (m, 2H, NCH_2CH$_2$), 3.6 (m, 1H, NCHCH$_2$), 3.3 (s, 3H, SO_2CH_3), 3.1 (t, *J* = 11 Hz,1H, NCHCHMe**)**, 2.8 (m, 1H, CHMe), 2.6 (m, 1H, NCH$_2$CHH), 2.0 (m, 1H, NCH$_2$CHH), 1.5 (bs, 3H, CH_3); MS (thermospray) *m*/*z* 370 (MNH_4^+), 353 (MH^+); HRMS calcd for $C_{16}H_{21}N_2O_5S$ (MH^+) 353.117119, found 353.117038; HPLC 94.9% (t_R 25.13 min).

***rel*-(3a*S*,6*R*,6a*R*)-6-Methyl-5-oxo-4-(2-phenylethenesulfonyl)-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (21):** derived from **10** and β-styrenesulfonyl chloride to give **21** (64%) as a white solid; mp 199−201 °C; IR (KBr) ν_{max} 1755, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (m, 10H, 2 × C_6H_5), 7.70, 7.00 (2d, *J* = 15.0 Hz, *J* = 15.0 Hz, 2H, HC=CH), 5.12 (s, 2H, CH_2Ph), 3.80 (m, 2H, NCH_2CH$_2$), 3.52 (m, 1H, NCHCH$_2$), 3.14 (t, *J* = 10 Hz,1H, NCHCHMe**)**, 2.68 (bm, 1H, CHMe), 2.59 (m, 1H, NCH$_2$CHH), 2.09 (m, 1H, NCH$_2$-CHH), 1.42 (b, 3H, CH$_3$); MS (thermospray) *m*/*z* 458 (MNH_4^+); HPLC 96.52% (t_R 31.65 min). Anal. ($C_{23}H_{24}N_2O_5S$·0.05EtOAc) C, H, N, S.

***rel*-(3a*S*,6*R*,6a*R*)-4-[(4-Methoxybenzyloxy)acetyl]-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (35):** derived from **10** and 4-methoxybenzyloxyacetyl chloride to give **35** (53 mg, 29%) as a solid; mp 100−101 °C; IR (KBr) ν_{max} 1751, 1710, 1698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.37 (s, 5H, C_6H_5) 7.30 (d, 2H, *J* = 8.0 Hz, 2,6-ArH), 6.87 (d, 2H, *J* = 8.0 Hz, 3,5-ArH), 5.13 (s, 2H, PhCH_2), 4.56 (q, 2H, *J* = 12 Hz) and 4.60 (s, 2H) (CH_2OCH_2), 3.8 (s and m, 5H, OCH_3, NCHHCH$_2$ and NCHCH$_2$), 3.45 (m, 1H, NCH-

*H*CH$_2$), 3.02 (t, J = 11 Hz, 1H, NC*H*CHMe), 2.75 (m, 1H, CH$_2$C*H*H), 2.66 (m,1H, C*H*Me), 1.92 (2t, J = 11 Hz, 1H, NCH$_2$-CH*H*), 1.42 (br, 3H, CH$_3$); TLC R_f 0.13 (cyclohexane−ethyl acetate, 1:1); MS *m/z* 470 (MNH$_4^+$). Anal. (C$_{25}$H$_{28}$N$_2$O$_6$·0.25H$_2$O) C, H, N.

***trans*-4-Acetoxyacetyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]-pyrrole-1-carboxylic acid benzyl ester (22):** derived from **13** and acetoxyacetyl chloride to yield **22** (66%); IR (KBr) ν_{max} 1754, 1712 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.3 (s, 5H, C$_6$H$_5$), 5.2 (dm, 1H, COC*H*$_2$O), 5.1 (s, 2H, PhC*H*$_2$), 5.0 (dm, 1H, COC*H*$_2$O), 3.9 (m, 1H, NC*H*HCH$_2$), 3.8 (m, 1H, NCH*H*CH$_2$)**,** 3.6−3.4 (m, 2H, NC*H*CH$_2$ and NC*H*CH$_2$CO), 3.2−2.9 (bs, 1H, C*H*HCO), 2.8−2.6 (m, 2H, NCH$_2$C*H*H, CH*H*CO), 2.2 (s, 3H, OCOC*H*$_3$), 2.1 (m, 1H, NCH$_2$CH*H*); MS (thermospray) *m/z* 378 (MNH$_4^+$), 361 (MH$^+$); HRMS calcd for C$_{18}$H$_{21}$N$_2$O$_6$ (MH$^+$) 361.139962, found 361.141020; HPLC 95.4% (t_R 24.41 min).

***trans*-4-Acetyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (23):** derived from **13** and acetyl chloride to afford **23** (89%) as a white solid; mp 128−132 °C; IR (KBr) ν_{max} 2897, 1755, cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.35 (m, 5H, C$_6$H$_5$), 5.15 (m, 2H, PhC*H*$_2$), 3.9−3.6 (m, 2H, NC*H*CH$_2$ and NC*H*CHMe), 3.6−3.2 (m, 2H, NC*H*$_2$CH$_2$), 3.2−2.8, 2.8−2.5 (m, 2H, C*H*$_2$CO), 2.8−2.6, 2.1−1.7 (2m, 2H, NCH$_2$C*H*$_2$), 2.5 (s, 3H, CH$_3$); HPLC 99% (t_R 23.4 min). Anal. (C$_{16}$H$_{18}$N$_2$O$_4$) C, H, N.

***trans*-2-Oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1,4-dicarboxylic acid 4-benzyl ester 1-vinyl ester (24):** derived from **13** and vinyl chloroformate to give **24** (34%) as a white solid; mp 120−121 °C; IR (KBr) ν_{max} 1809, 1733, 1715, 1702 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.36 (s, 5H, C$_6$H$_5$), 7.21 (t, J = 7.5 Hz, 1H, C*H*═CH$_2$), 5.12 (q, J = 11 Hz, 2H, PhC*H*$_2$), 4.68 (dd,, J = 7.5, 2 Hz, 1H and 4.99 dd, J = 18, 2 Hz, 1H) (CH═C*H*$_2$), 3.95−3.3 (m, 4H, NC*H*$_2$CH$_2$, NC*H*CH$_2$ and NC*H*CH$_2$CO), 3.00 and 2.64 (2m, 2H, C*H*$_2$CO), 2.64 (m, 1H, NCH$_2$C*H*H) and 2.09 (m, 1H, NCH$_2$CH*H*); TLC R_f 0.33 (toluene−ethyl acetate−acetic acid, 20:10:0.5). Anal. (C$_{17}$H$_{18}$N$_2$O$_5$) C, H, N.

***trans*-2-Oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1,4-dicarboxylic acid 4-benzyl ester 1-methyl ester (25):** derived from **13** and methyl chloroformate to yield **25** (95%) as a white foam; IR (KBr) ν_{max} 1800, 1771, 1715, 1706 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.4 (s, 5H, C$_6$H$_5$), 5.2 (s, 2H, PhC*H*$_2$), 3.9 (s, 3H, CO$_2$*Me*), 3.9−3.8 (m, 1H, NC*H*HCH$_2$), 3.8−3.7 (m, 1H, NCH-*H*CH$_2$)**,** 3.6 (m, 1H, NC*H*CH$_2$), 3.4 (m, 1H, NC*H*CH$_2$CO), 3.2−2.9 (bs, 1H, C*H*HCO), 2.7−2.6 (m, 2H, CH*H*CO, NCH$_2$C*H*H), 2.10 (m, 1H, NCH$_2$CH*H*); MS (thermospray) *m/z* 336 (MNH$_4^+$), 319 (MH$^+$); HRMS calcd for C$_{16}$H$_{18}$N$_2$O$_5$Na (MH$^+$) 341.111342, found 341.111572; HPLC 95.4% (t_R 22.12 min).

***trans*-4-Methanesulfonyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (26):** derived from **13** and methanesulfonyl chloride to give **26** (67%) as a white solid; mp 155−157 °C; IR (KBr) ν_{max} 1750, 1712, 1702; ^{1}H NMR (CDCl$_3$) δ 7.35 (m, 5H, C$_6$H$_5$), 5.01 (ABq, 2H, J = 12.2 Hz, C*H*$_2$Ph), 3.88, 3.73, 3.58, 3.44 (4m, 4H, NC*H*$_2$CH$_2$**,** NC*H*CH$_2$ and NC*H*CH$_2$CO), 3.25 (s, 3H, CH$_3$), 3.00 (m, 1H, C*H*HCO), 2.60 (m, 2H, CH*H*CO and NCH$_2$C*H*H), 2.10 (m, 1H, NCH$_2$-CH*H*); MS (thermospray) *m/z* 339 (MH$^+$), 356 (MNH$_4^+$); HPLC 99.7% (t_R 22.91 min). Anal. (C$_{15}$H$_{18}$N$_2$O$_5$S·0.05C$_6$H$_{12}$) C, H, N, S.

***trans*-5-Oxo-4-(2-phenyl-(*E*)-ethenesulfonyl)-hexahydro-pyrrolo[3,2-*b*]pyrrole carboxylic acid benzyl ester (27):** derived from **13** and β-styrenesulfonyl chloride to give **27** (7%) as a white foam; IR (KBr) ν_{max} 1755, 1712 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.42 (m, 10H, 2 × C$_6$H$_5$), 6.99, 7.70 (2d, 2H, J = 18.7 Hz, HC═CH), 5.11 (AB q, 2H, J = 12 Hz, C*H*$_2$Ph), 3.89, 3.72, 3.50 (3m, 4H, NC*H*$_2$CH$_2$**,** NC*H*CH$_2$ and NC*H*CH$_2$CO), 2.93, 2.61 (2m, 2H, C*H*$_2$CO), 2.61, 2.16 (2m, 2H, NCH$_2$C*H*$_2$); MS (thermospray) *m/z* 444 (MNH$_4^+$); HPLC 86.68% (t_R 29.66 min) and 12.34% (t_R 31.65 min). Anal. (C$_{22}$H$_{22}$N$_2$O$_5$S·0.3CH$_2$-Cl$_2$) C, H, N, S.

***trans*-4-Acetoxyisobutyryl-5-oxo-hexahydro-pyrrolo-[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (28):** derived from **13** and 2-acetoxyisobutyryl chloride to yield **28** (35%); ^{1}H NMR (CDCl$_3$) δ 7.4 (s, 5H, C$_6$H$_5$), 5.1 (d, 2H, PhC*H*$_2$), 3.9−3.7 (m, 2H, NC*H*$_2$CH$_2$)**,** 3.55 (m, 1H, NC*H*CH$_2$), 3.35 (m, 1H, NC*H*CH$_2$CO), 3.2−2.8 (bs, 1H, C*H*HCO), 2.7−2.5 (m, 2H, NCH$_2$C*H*H, CH*H*CO), 2.0 (m, 4H, OCOC*H*$_3$, NCH$_2$CH*H*), 1.7−1.5 (m, 6H, C*Me*$_2$); MS (thermospray) *m/z* 406 (MNH$_4^+$), 389 (MH$^+$); HRMS calcd for C$_{20}$H$_{24}$N$_2$O$_6$ (MH$^+$) 389.139961, found 389.141010; HPLC 89.52% (t_R 27.48 min).

***trans*-4-[(4-Methoxybenzyloxy)acetyl]-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (36):** derived from **13** and 4-methoxyphenoxyacetylpivaloyl anhydride[17] to give **36** (65%) as a white solid; mp 114−115 °C; IR (KBr) ν_{max} 1756, 1722, 1712 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.39−7.25 (m, 2H, 3,5-ArH, and m, 5H C$_6$H$_5$), 6.88 (d, 2H, J = 8.0 Hz, 2,6-ArH) 5.12 (q, 2H, J = 12.0 Hz, C*H*$_2$Ph), 4.59 (q, 2H, J = 11.7 Hz, OC*H*$_2$Ar), 4.54 (q, 2H, J = 17.5 Hz, O═CC*H*$_2$OCH$_2$-Ar), 3.81 (s, 3H, OC*H*$_3$), 3.78 (m, 2H, NC*H*$_2$CH$_2$), 3.50 (m, 1H, NC*H*CH$_2$), 3.35 (m, 1H, NC*H*CH$_2$CO), 2.92 (bm, 1H, C*H*HCO), 2.78 (m, 1H, NCH$_2$C*H*H), 2.63 (m, 1H, CH*H*CO), 2.00 (m, 1H, NCH$_2$CH*H*); MS (electrospray) *m/z* 453 (MH$^+$); HPLC 97.77% (t_R 28.90 min). Anal. (C$_{24}$H$_{26}$N$_2$O$_6$) C, H, N.

***rel*-(3a*S*,6*S*,6a*R*)-4-Acetoxyacetyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (29):** derived from **16** and acetoxyacetyl chloride to give **29** (74%) as a white solid; mp 108−109 °C; IR (CDCl$_3$) ν_{max} 1750, 1716, 1700 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.40 (s, 5H, C$_6$H$_5$), 4.9−5.3 (m, 4H, PhC*H*$_2$ and C*H*$_2$OAc), 3.90 (m, 1H, NC*H*HCH$_2$), 3.78 (m, 1H, NC*H*CH$_2$), 3.70 (m, 1H, NCH*H*CH$_2$), 3.50 (m, 1H, NC*H*CHMe), 3.1 (bm, 1H, C*H*Me), 2.77 (m, 1H, NCH$_2$C*H*H), 2.20 (s, 3H, OAc), 2.00 (m, 1H, NCH$_2$CH*H*), 1.15 (br, d J = 21.3 Hz, 3H, C*H*$_3$CH); TLC R$_f$ 0.20 (cyclohexane−EtOAc, 2:1). Anal. (C$_{19}$H$_{22}$N$_2$O$_6$) C, H, N.

***rel*-(3a*S*,6*S*,6a*R*)-4-Acetyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (30):** derived from **16** and acetyl chloride to give **30** (89%) as a gum; IR (KBr) ν_{max} 1747, 1712, 1697 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.37 (s, 5H, C$_6$H$_5$), 5.14 (q, J = 11 Hz, 2H, PhC*H*$_2$), 3.7 and 3.86 (m, 3H, NC*H*$_2$CH$_2$ and NC*H*CH$_2$), 3.45 (dd, J = 12 Hz, J = 7.5 Hz, 1H; NC*H*CHMe), 3.1 (bm, 1H, C*H*Me), 2.74 (dt, J = 6 Hz, 1H, NCH$_2$C*H*H), 2.46 (s, 3H, COC*H*$_3$), 1.97 (2t, J = 11 Hz, 1H, NCH$_2$CH*H*), 1.14 (br, 3H, C*H*$_3$CH); TLC R_f 0.32 (cyclohexane−ethyl acetate, 1:1). Anal. (C$_{17}$H$_{20}$N$_2$O$_4$·0.25Et$_2$O) C, H, N.

***rel*-(3*S*,3a*R*,6a*S*)-3-Methyl-2-oxo-hexahydro-pyrrolo-[3,2-*b*]pyrrole-1,4-dicarboxylic acid 4-benzyl ester 1-vinyl ester (31):** derived from **16** and vinyl chloroformate to give **31** (78%) as an oil; IR (KBr) ν_{max} 1805, 1735, 1710, 1697 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.35(s, 5H, C$_6$H$_5$), 7.23 (dd, 1H, J = 15, 7.5 Hz, OC*H*═CH$_2$), 5.13 (q, J = 12 Hz, 2H, PhC*H*$_2$), 5.00 (dd, J = 15, 2.5 Hz, 1H) and 4.68 (dd, J = 7.5, 2.5 Hz, 1H, OCH═C*H*$_2$), 4.0−3.64 (m, 3H, NC*H*$_2$CH$_2$ and NC*H*CH$_2$), 3.52 (dd, J = 11 Hz, J = 7.5 Hz, 1H, NC*H*CHMe), 3.2 and 3.02 (br d, 1H, C*H*Me), 2.62 (2t, J = 6 Hz, 1H, NCH$_2$C*H*H), 2.05 (2t, J = 12 Hz, 1H, NCH$_2$CH*H*), 1.15 (br, 3H, C*H*$_3$CH); TLC R_f 0.54 (cyclohexane−ethyl acetate, 1:1). Anal. (C$_{18}$H$_{20}$N$_2$O$_5$·0.25Et$_2$O) C, H, N.

***rel*-(3a*S*,6*S*,6a*R*)-4-Methanesulfonyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (32):** derived from **16** and methanesulfonyl chloride to give **32** (80%) as a white solid; IR (KBr) ν_{max} 1745, 1712, 1705 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.35 (s, 5H, C$_6$H$_5$), 5.33 (q, J = 12 Hz, 2H, PhC*H*$_2$), 4.0−3.75 (m, 2H, NC*H*$_2$CH$_2$), 3.67 (m, 1H, NC*H*CH$_2$), 3.57 (dd, J = 10 Hz, J = 6 Hz, 1H, NC*H*CHMe), 3.25 (s, 3H, OC*H*$_3$), 3.0 (br, 1H, C*H*Me), 2.58 (dt, J = 11 Hz, 1H, NCH$_2$C*H*H), 2.08 (2t, J = 6 Hz, 1H, NCH$_2$CH*H*), 1.15 (br, 3H, C*H*$_3$CH); TLC R_f 0.25 (cyclohexane−ethyl acetate, 1:1). Anal. (C$_{16}$H$_{20}$N$_2$O$_5$S·0.2CH$_2$Cl$_2$) C, H, N, S.

***rel*-(3a*S*,6*S*,6a*R*)-6-Methyl-5-oxo-4-(2-phenyl-(*E*)-ethenesulfonyl)-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (33):** derived from **16** and β-styrenesulfonyl chloride to give **33** (22%) as a light brown solid; mp 128−132 °C; IR (KBr) ν_{max} 1753, 1709, 1450, cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.6−7.3 (m, 10H, 2 × C$_6$H$_5$), 7.7, 7.0 (d, J = 15 Hz, 2H, SO$_2$C*H*═C*H*), 5.2 (m, 2H, PhC*H*$_2$), 4.0−3.5 (m, 4H, NC*H*$_2$CH$_2$, NC*H*CH$_2$ and NC*H*CHMe), 3.3−2.9 (m, 1H, C*H*Me), 2.7−2.5, 2.3−2.0 (m, 2H, NCH$_2$C*H*$_2$), 1.1 (br s, 3H, *CH*$_3$); HPLC 95% (t_R 30.55 min). Anal. (C$_{23}$H$_{25}$N$_2$O$_5$S·0.3EtOAc) C, H, N.

***rel*-(3a*S*,6*S*,6a*R*)-4-Chloroacetyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (42):** derived from **16** and chloroacetyl chloride to give **42** (38%) as a yellow gum; IR (KBr) ν_{max} 2874, 1751, 1712 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.35 (s, 5H, C_6H_5), 5.17 (d, J = 13 Hz, 1H, PhCH_2), 5.09 (d, J = 13 Hz, 1H, PhCH_2), 4.67 (d, J = 16 Hz, 1H, CH_2-Cl), 4.56 (d, J = 16 Hz, 1H, CH_2Cl), 4.0−3.4 (m, 4H, NCH_2-CH_2, NC*H*CH$_2$ and NC*H*CHMe), 3.4−2.9 (m, 1H, C*H*Me), 2.85−2.7, 2.1−1.9 (m, 2H, NCH$_2$CH_2), 1.4−1.0 (br, 3H, CH_3); MS (thermospray) *m*/*z* 368/369 (Cl, MNH_4^+); HPLC 94% (t_R 27.43 min). Anal. ($C_{17}H_{17}ClN_2O_4$·0.1$CHCl_3$) C, H, N.

***rel*-(3a*S*,6*S*,6a*R*)-4-(4-Acetylaminobenzenesulfonyl)-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (43):** derived from **16** and *N*-acetylsulfanilyl chloride to afford **43** (5%) as a white solid; IR (KBr) ν_{max} 1703, 1590, 1529 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.95, 7.7 (d, J = 9 Hz, 2H, Ar*H*), 7.5 (s, 1H, N*H*), 7.3 (s, 5H, C_6H_5), 5.17 (d, J = 13 Hz, 1H, PhCH_2), 5.08 (d, J = 13 Hz, 1H, PhCH_2), 4.0−3.4 (m, 4H, NCH_2CH$_2$, NC*H*CH$_2$ and NC*H*CHMe), 3.2−2.7 (m, 1H, C*H*Me), 2.75−2.55, 2.3−2.0 (m, 2H, NCH$_2$CH_2), 2.25 (s, 3H, COCH_3), 1.0−0.8 (br, 3H, CH_3); MS (thermospray) *m*/*z* 472 (MH^+), 489 (MNH_4^+); HRMS (ESP) calcd for $C_{23}H_{26}N_3O_6S$ (MH^+) 472.154233, found 472.152869; HPLC 99% (t_R 28.12 min).

***rel*-(3a*S*,6*S*,6a*R*)-4-[(4-Methoxybenzyloxy)acetyl]-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic acid benzyl ester (38):** derived from **16** and 4-methoxyphenoxyacetylpivaloyl anhydride[17] to give **38** (67%) as a white wax; mp 87−89 °C; IR (KBr) ν_{max} 1746, 1720, 1711 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36 (m, 5H C_6H_5), 7.32 (d, 2H, J = 8 Hz, 3,5-ArH), 6.88 (d, 2H, J = 8 Hz, 2,6-ArH), 5.13 (q, 2H, J = 11.5 Hz, CH_2Ph), 4.59, 4.53 (m, 4H, O=CCH_2OCH_2Ar), 3.81 (s, 3H, OCH_3), 3.95−3.63 (m, 3H, NCH_2CH$_2$ and NC*H*CH$_2$), 3.47 (dd, 1H J = 7, 12 Hz, NC*H*CHMe), 3.07 (bm, 1H, C*H*Me), 2.78, 2.00 (2m, 2H, NCH$_2$CH_2), 1.13 (bs, 3H, CH_3); MS (electrospray) *m*/*z* 453 (MH^+); HPLC 98.86% (t_R 30.06 min). Anal. ($C_{25}H_{28}N_2O_6$) C, H, N.

***rel*-(3a*S*,6*R*,6a*R*)-4-Hydroxyacetyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (34).** To a solution of the 4-methoxybenzyloxy ether **35** (42 mg, 0.093 mmol) in dichloromethane (2 mL) were added water (0.1 mL) and DDQ (25 mg, 0.11 mmol). The resulting mixture was stirred for 2 h, more DDQ (12 mg, 0.05 mmol) was added and stirring continued for a further 1.25 h when another portion of DDQ (10 mg, 0.045 mmol) and more dichloromethane (1 mL) were added. The reaction mixture was stirred for a total of 23 h before the pink solid was filtered off and washed with dichloromethane. The filtrate was concentrated and purified by flash chromatography eluting with cyclohexane−ethyl acetate (2:1) to give the title compound **34** (26.5 mg, 86%) as a white solid: mp 124−125 °C; IR (KBr) ν_{max} 3500, 1754, 1746, 1731, 1711, 1692 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37 (s, 5H, C_6H_5), 5.15 (s, 2H, PhCH_2), 4.01 (m, 2H, CH_2-OH), 3.83 (m, 2H, NC*H*HCH$_2$ and NC*H*CH$_2$), 3.08 and 3.5 (s 1H, OH and m, 2H, NC*H*CHMe and NCH*H*CH$_2$), 2.77 (m, 2H, NCH$_2$C*H*H and C*H*Me), 1.97 (m, 1H, NCH$_2$CH*H*), 1.45 (br, 3H, CH$_3$); TLC R_f 0.17 (cyclohexane−ethyl acetate, 1:1). Anal. ($C_{17}H_{20}N_2O_5$) C, H, N.

***trans*-4-Hydroxyacetyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (37).** Compound **36** was reacted with DDQ as described for **34** to give **37** (63%) as a white gum: IR (KBr) ν_{max} 3480 (OH), 1758, 1712, 1695 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H, C_6H_5), 5.13 (ABq, 2H, J = 12.0 Hz, CH_2Ph), 4.61 (dm, 2H, CH_2OH), 3.82, 3.54, 3.40 (3m, 4H, NCH_2CH$_2$, NC*H*CH$_2$ and NC*H*CH$_2$CO), 3.05 (t, 1H, J = 5 Hz, O*H*), 2.99, 2.67 (2bm, 2H, CH_2CO), 2.77, 2.06 (2m, 2H, NCH$_2$CH_2); MS (thermospray) *m*/*z* 319 (MH^+), 336 (MNH_4^+); HPLC 96.44% (t_R 19.81 min). Anal. ($C_{16}H_{18}N_2O_5$·0.2C_6H_{12}·0.1EtOAc) C, H, N.

***rel*-(3a*S*,6*S*,6a*R*)-4-Hydroxyacetyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (39).** Compound **38** was reacted with DDQ as described for **34** to give **39** (71%) as a white wax: mp 130−132 °C; IR (KBr) ν_{max} 3495 (broad) (OH), 1748, 1712, 1695 cm^{-1}; ^{1}H NMR ($CHCl_3$) δ 7.36 (m, 5H, C_6H_5), 5.14 (ABq, 2H, J = 12.0 Hz, CH_2Ph), 4.60 (dABq, 2H, J = 4.0 Hz, J = 19.0 Hz, CH_2OH), 3.98−3.65, 3.53 (2m, 4H, NCH_2CH$_2$, NC*H*CH$_2$ and NC*H*CHMe), 3.10 (bm, 1H, C*H*Me), 3.05 (t, 1H, J = 5 Hz, O*H*), 2.77, 2.06 (2m, 2H, NCH$_2$CH_2), 1.17 (bs, 3H, CH$_3$); MS (thermospray) *m*/*z* 333 (MH^+); HPLC 97.78% (t_R 21.59 min). Anal. ($C_{17}H_{20}N_2O_5$·0.15C_6H_{12}) C, H, N.

***trans*-4-Methylcarbamoyl-5-oxo-hexahydro-pyrrolo-[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (40).** To a solution of compound **13** (50 mg, 0.192 mmol) in tetrahydrofuran (10 mL) under nitrogen was added sodium hydride (11 mg, 60% dispersion in mineral oil, 0.288 mmol). The mixture was stirred at room temperature for 5 min and then reacted with methyl isocyanate (0.017 mL, 0.288 mmol). The mixture was stirred for 1.5 h, quenched with saturated aqueous ammonium chloride solution (20 mL) and the mixture extracted with ethyl acetate (4 × 10 mL). The combined organic layer was separated and washed with brine (30 mL), dried, filtered and evaporated. The crude residue, a clear syrup was purified by flash column chromatography eluting with cyclohexane−ethyl acetate (1:2) to yield **40** (42 mg, 69%) as a white solid: IR (KBr) ν_{max} 1728, 1698, 1548 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.7 (br s, 1H, N*H*), 7.4 (s, 5H, C_6H_5), 5.15 (d, J = 13 Hz, 1H, PhCH_2), 5.10 (d, J = 13 Hz, 1H, PhCH_2), 4.0−3.0 (m, 4H, NCH_2CH$_2$, NC*H*CH$_2$ and NC*H*CH$_2$CO), 2.89 (d, J = 5 Hz, 3H, CH_3N), 3.2−2.8, 2.8−2.6 (br m, 2H, CH_2CO), 2.8−2.6, 2.2−2.0 (m, 2H, NCH$_2$CH_2); MS (thermospray) *m*/*z* 318 (MH^+); HPLC 97% (t_R 21.98 min). Anal. ($C_{16}H_{18}N_3O_4$) C, H, N.

***rel*-(3a*S*,6*S*,6a*R*)-4-Isopropylcarbamoyl-6-methyl-5-oxo-hexahydro-pyrrolo[3,2-*b*]pyrrole-1-carboxylic Acid Benzyl Ester (41).** Compound **16** was reacted with isopropyl isocyanate as described for **40** to give **41** (31%) as a white solid: IR (KBr) ν_{max} 3330, 1728, 1714, 1694 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.7 (br s, 1H, NH), 7.36 (s, 5H, C_6H_5), 5.15 (q, J = 12 Hz 2H, PhCH_2), 3.45 (dd, J = 12 Hz, J = 7.5 Hz, 1H, NC*H*CHMe) and 4.06−3.6 (m, 4H, (Me)$_2$C*H*, NCH_2CH$_2$ and NC*H*CH$_2$), 2.95 and 3.2 (br d, 1H, C*H*Me), 2.72 (2t, J = 5.5 Hz 1H, NCH$_2$C*H*H), 2.05 (2t, J = 11 Hz, 1H, NCH$_2$CH*H*), 1.05−1.3 (br s and d, J = 7 Hz 9H, CH_3CH and (CH_3)$_2$CH); TLC R_f 0.31 (cyclohexane−ethyl acetate, 1:1); MS (thermospray) *m*/*z* 360 (MH^+). Anal. ($C_{19}H_{25}N_3O_4$·0.2C_6H_{12}) C, H, N.

Biochemical Assay. Compounds were dissolved in DMSO and added at a concentration of 500 μM to reaction mixtures containing 6.65 μM HCMV δAla protease in 85 mM HEPES/NaOH buffer, pH 7.5, containing 0.17 mM EDTA, 8.5 mM NaCl, 1.7 mM dithiothreitol and 25.5% (v/v) glycerol. The final concentration of DMSO present in assays was 2% (v/v). Routinely, mixtures were incubated at 37 °C for 15 min, prior to addition of 1 mM substrate (RESYVKASVSPEAA), and then incubated for a further 15 min. For time-course experiments, incubation times of 0, 0.25, 0.5, 1, 2, 3, 6, 24 and 48 h were used prior to substrate addition. Reactions were stopped by the addition of TFA (1% v/v final concentration), and the extent of peptide substrate cleavage was determined by reverse-phase HPLC using a C_8 column and an acetonitrile gradient in 0.1% (v/v) TFA. Products were quantified by comparison of peak areas with calibration plots generated using peptide solutions of known concentration.

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