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Azetidin-2-one Derivatives as Inhibitors of Thrombin

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Abstract—A series of 3-(3-guanidinopropyl)-azetidin-2-one derivatives was prepared and evaluated as inhibitors of cleavage of synthetic substrates *in vitro* by the serine proteases thrombin, trypsin and plasmin. The N-unsubstituted, 4-phenethyl derivative **9a** demonstrated weak inhibition of these enzymes but acetylation of the β-lactam N atom afforded **9b**, an effective, time-dependent inhibitor of thrombin and a potent inhibitor of plasmin. Variation of the 4-position of the β-lactam ring was examined in conjunction with different N-substituents to provide a series of potent, time-dependent inhibitors of thrombin. A C-4 substituent was essential for good inhibitory properties and, in general, polar C-4 substituents enhanced the selectivity of inhibition for thrombin compared to plasmin. A *trans* relationship between the C-4 and C-3 substituents was found to be superior to a *cis* disposition whilst homologation of the guanidinopropyl side chain to that of a guanidinobutyl moiety reduced activity. Several compounds were effective inhibitors of thrombin-induced clot formation in human plasma *in vitro* but activity in this assay did not correlate well with inhibition of thrombin-induced cleavage of a synthetic substrate, presumably a consequence of inherent chemical instability and degradation in plasma.

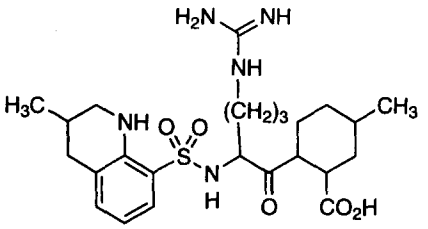
Introduction

Thrombin (Factor IIa, EC 3.4.21.5) is a serine protease activated by factor Xa-mediated cleavage of prothrombin and is the final product of the coagulation cascade.[1-3] Although thrombin is most prominently associated with blood coagulation, it plays a central and complex role in the regulation of hemostasis since it is also involved in clot dissolution.[4] While early investigations defined thrombin as a key mediator of venous thrombosis, more recent studies have clarified its role in the etiology of arterial thrombosis and restenosis injury. As a consequence, inhibitors of thrombin are of potential clinical value for the prevention and treatment of both venous and arterial thrombotic episodes.[5] Thrombin activates blood coagulation and clot formation by cleaving several peptidic substrates, including fibrinogen, factor V, factor VIII and factor IX, that promote clot development and factor XIII, which contributes to clot stabilization. Thrombin also cleaves and activates a receptor on the surface of platelets, smooth muscle and endothelial cells.[6-8] At the molecular level, thrombin preferentially cleaves its substrates at the carboxamide moiety of the basic amino acids arginine and lysine. Potent and selective inhibitors of thrombin directed to the active site of the enzyme have been synthesized that take advantage of this recognition element and are frequently arginine or lysine derivatives.[9-13] Several of these agents have been evaluated in animal models of thrombosis in an effort to determine their potential as anti-thrombotic and anti-coagulant agents.[9-13] MD 805 (argatroban, **1**) is a competitive inhibitor of thrombin that has emerged from this approach and has been advanced into clinical trials.[14,15] In addition, several classes of inhibitor have been synthesized that take advantage of mechanistic aspects of thrombin action[16] to produce enzyme–inhibitor complexes that are either transiently or permanently covalently associated. Included in this class are boronic acids,[17] α-halo ketones,[18-24] cyclic anhydrides,[25] cyclic lactones,[26-28] activated ketone derivatives[29] and the aldehydes **2a**,[30] **2b**[30,31] and **2c**.[32]

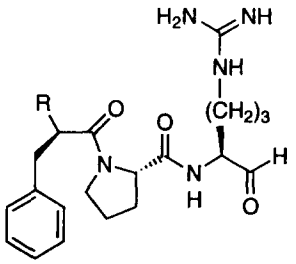
β-Lactam derivatives have been described as potent, mechanism-based inhibitors of serine proteases with the emphasis of these studies directed towards identifying inhibitors of human leucocyte elastase.[33-48] Examples of this structural class of enzyme inhibitor are the cephalosporin **3**[34] and the mono-bactam derivative **4**.[36] In this article, we describe the preparation and biological evaluation of a series of β-lactam derivatives[49-51] that incorporate structural elements designed to be recognized by thrombin and related serine proteases. Many of these compounds are highly potent and time-dependent inhibitors of thrombin and establish a foundation for the further development of this pharmacophore.

Chemistry

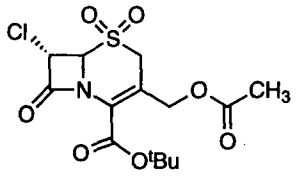
The 3,4-disubstituted azetidin-2-one **7** was identified as a versatile synthetic intermediate and was prepared by the route depicted in Scheme 1. Heating a mixture of *N,N'*-dicarbobenzyloxy-S-methylisothiourea[52] (**5**) and 5-



1 (MD 805, Argatroban)



2a R = NH_2, GYKI 14166
2b R = $NHCH_3$, GYKI 14766
2c R = H, BMY 44621



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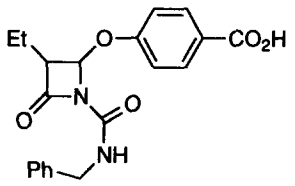

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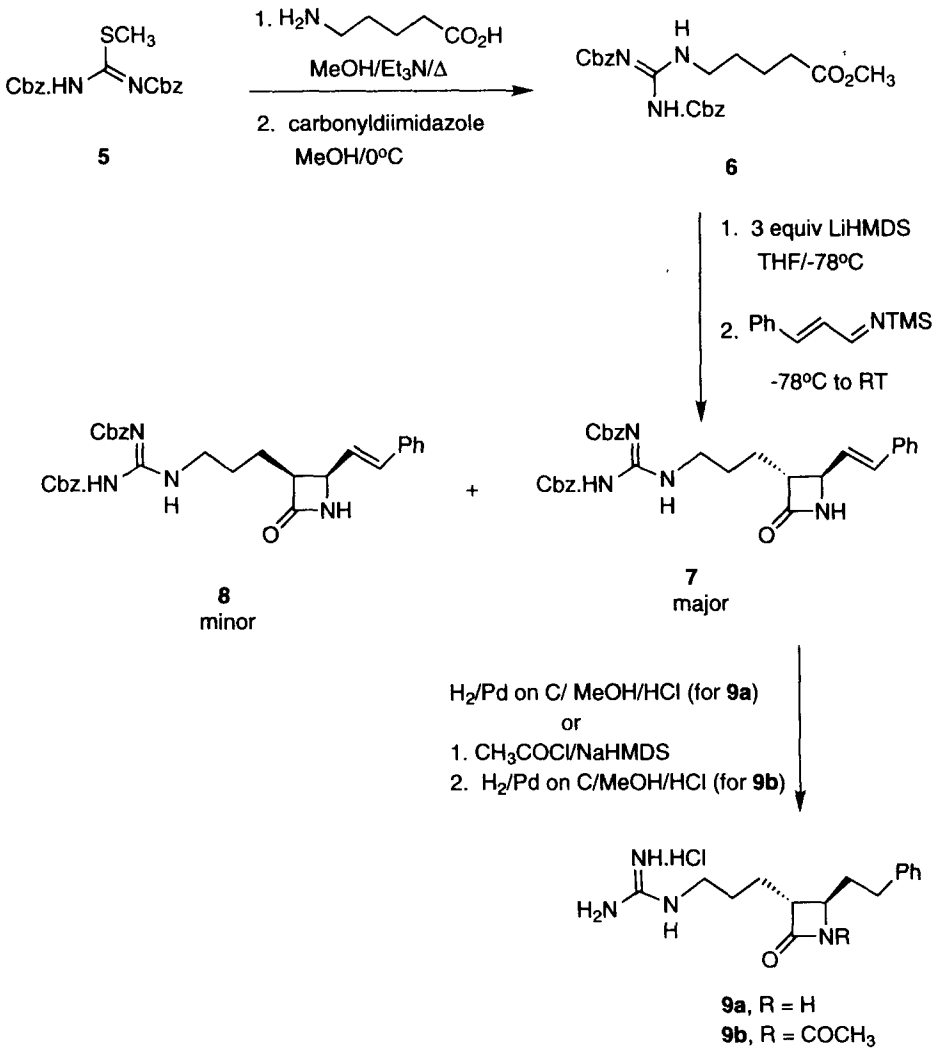
aminopentanoic acid in methanol at reflux[53] provided 5-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]pentanoic acid which was esterified, using carbonyldiimidazole[54] and MeOH, to give the methyl ester **6**. Construction of the β-lactam ring followed previously described synthetic protocols[55–59] that comprised treating a solution of **6** in THF at –78 °C with a base followed by the addition of 1,1,1-trimethylsilyl-*N*-(3-phenyl-2-propen-1-ylidene)-silanamine[60] to afford a mixture of the azetidin-2-ones **7** and **8**. The use of less than three equivalents of lithium bis(trimethylsilyl)amide in this reaction failed to provide appreciable quantities of the cyclized products, suggesting that the essential reacting species was a trianion. The azetidin-2-ones **7** and **8** were produced in a ratio of 4:1 as judged from the ^{1}H NMR spectrum and the two compounds were separated by careful column chromatography. The relative stereochemical relationships between the ring substituents of **7** and **8** were assigned after examining the ^{1}H NMR spectra.[61,62] The ring proton α to the amide carbonyl of the chromatographically more mobile isomer, which constituted the major component, resonated at δ 3.97, upfield of the corresponding proton in the isomeric material which resonated at δ 4.32, consistent with the structural assignments in Scheme 1.[61,62] In addition, the coupling constant between the azetidinone ring protons was larger for the minor component **8** than the major isomer **7**.[61,62]

The guanidino protecting groups of **7** were removed by catalytic hydrogenation which, concomitantly, saturated the olefin moiety to afford the target compound **9a**. Acetylation of the β-lactam nitrogen atom of **7**, using NaHDMS as the base, prior to hydrogenation furnished the activated azetidinone **9b**.

The β-lactam **7** was converted into the carboxylic acid derivatives **10** and **11**, useful synthetic precursors to several target compounds, by the methods outlined in Scheme 2. For the preparation of **10**, the azetidin-2-one nitrogen atom was protected as its TBDMS derivative and the olefin cleaved by treating with O_3 in CH_2Cl_2 at –78 °C. The intermediate aldehyde was oxidized using Jones reagent in acetone, conditions under which the sensitive N–silicon bond was unstable, to afford the acid **10**. Esterification of **10**, using diazomethane in Et_2O, followed by derivatization of the β-lactam nitrogen (except for the preparation of **12a**) and deprotection of the guanidino moiety, afforded the series of esters **12a–e**. Alternatively, coupling of the acid **10** with an amine, mediated by carbonyl-diimidazole,[54] was followed by functionalization of the β-lactam nitrogen atom and deprotection to furnish the amides **14a,c,d**. The piperidinamide **14b**, in which the azetidinone ring substituents are *cis*-disposed, was obtained from the *cis*-substituted β-lactam **8** using an identical synthetic protocol. A complementary strategy, in which the β-lactam nitrogen was acetylated prior to ozonolysis of the olefin and Jones oxidation, was adopted to prepare the carboxylic acid **11**. Amide bond formation, using either a mixed anhydride or diphenylphosphoryl azide,[63] was followed by hydrogenolysis of the Cbz protecting groups to afford the amides **14e–g**. Alternatively, hydrogenolytic deprotection of **11** provided the acid **13**.

As depicted in Scheme 3, oxidative decarboxylation[56,61] of **10** afforded the acetate **15**, isolated as an inseparable mixture of *cis* and *trans* isomers. Functionalization of the azetidinone nitrogen atom and subsequent catalytic hydrogenation provided the target compounds **16a–c**.



Scheme 1.

The acetoxy moiety of **15** was readily displaced by thiol nucleophiles,[64,65] which provided the sulfides **17**. Acetylation of **17**, using LiHMDS or NaHMDS as the base, gave the sulfides **18**. Catalytic hydrogenation of the ethyl sulfide provided the target compound **19** whilst oxidation of the sulfur atom followed by deprotection afforded the sulfones **20a** and **20b**. The thioether **17a** (R = Et) was desulfurized by treatment with Raney nickel, the β-lactam N atom derivatized with acetyl chloride or dansyl chloride and the Cbz protecting groups removed by hydrogenolysis, to furnish target compounds **21a** and **21b**, respectively.

Compound **23**, the immediate homologue of **12c**, was prepared by an identical synthetic approach with the exception that 6-aminohexanoic acid was employed as a starting material (Scheme 4). The β-lactam derivatives that constitute this study are compiled in Table 1.

Biological Evaluation

The target compounds were evaluated as inhibitors of thrombin cleavage of the synthetic substrate s-2238 (D-Phe-Pip-Arg-*p*-nitroanilide).[66] Since many of these β-lactam derivatives demonstrated time-dependent inhibitory activity, the IC_{50} reported in Table 1 represents the concentration of drug required to inhibit thrombin-induced substrate cleavage by 50% following a three minute incubation period of the drug with the enzyme. For those compounds demonstrating time-dependent inhibition of thrombin, the second-order rate constant k_2/K_i (M^{-1} min^{-1}) for inactivation of the enzyme was determined.[67] The IC_{50}s reported for the inhibition of trypsin and plasmin were determined in a fashion analogous to that described for thrombin.

The effect of drugs on the clotting time of human plasma was determined by adding human thrombin to human plasma in the presence of various concentrations of the drug. The concentration of drug required to double the time to clot formation, compared to drug-free control, was calculated and is presented in Table 1.

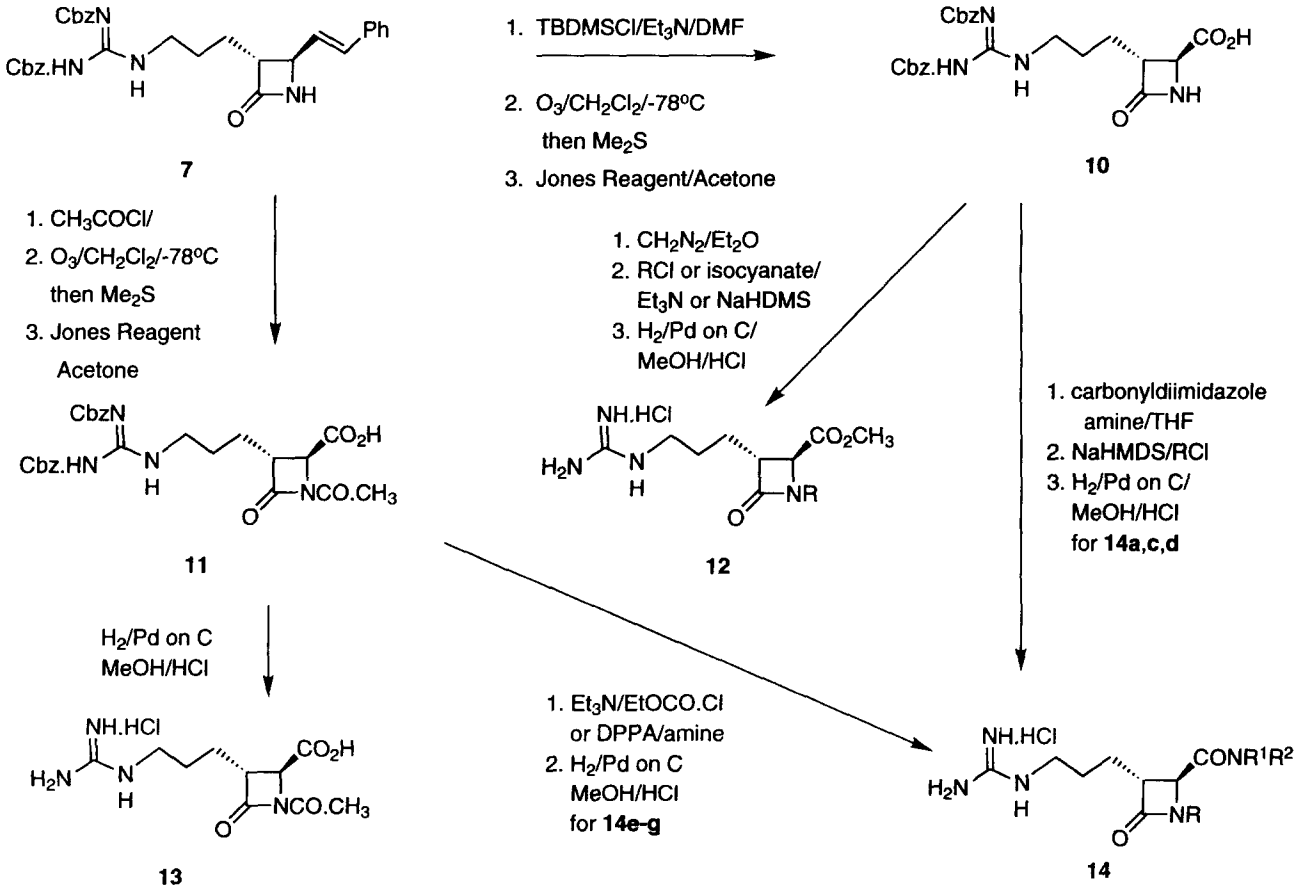
Results and Discussion

In an effort to identify potential inhibitors of thrombin and other trypsin-like serine proteases, the effect of combining an arginine-like side chain with an electro-

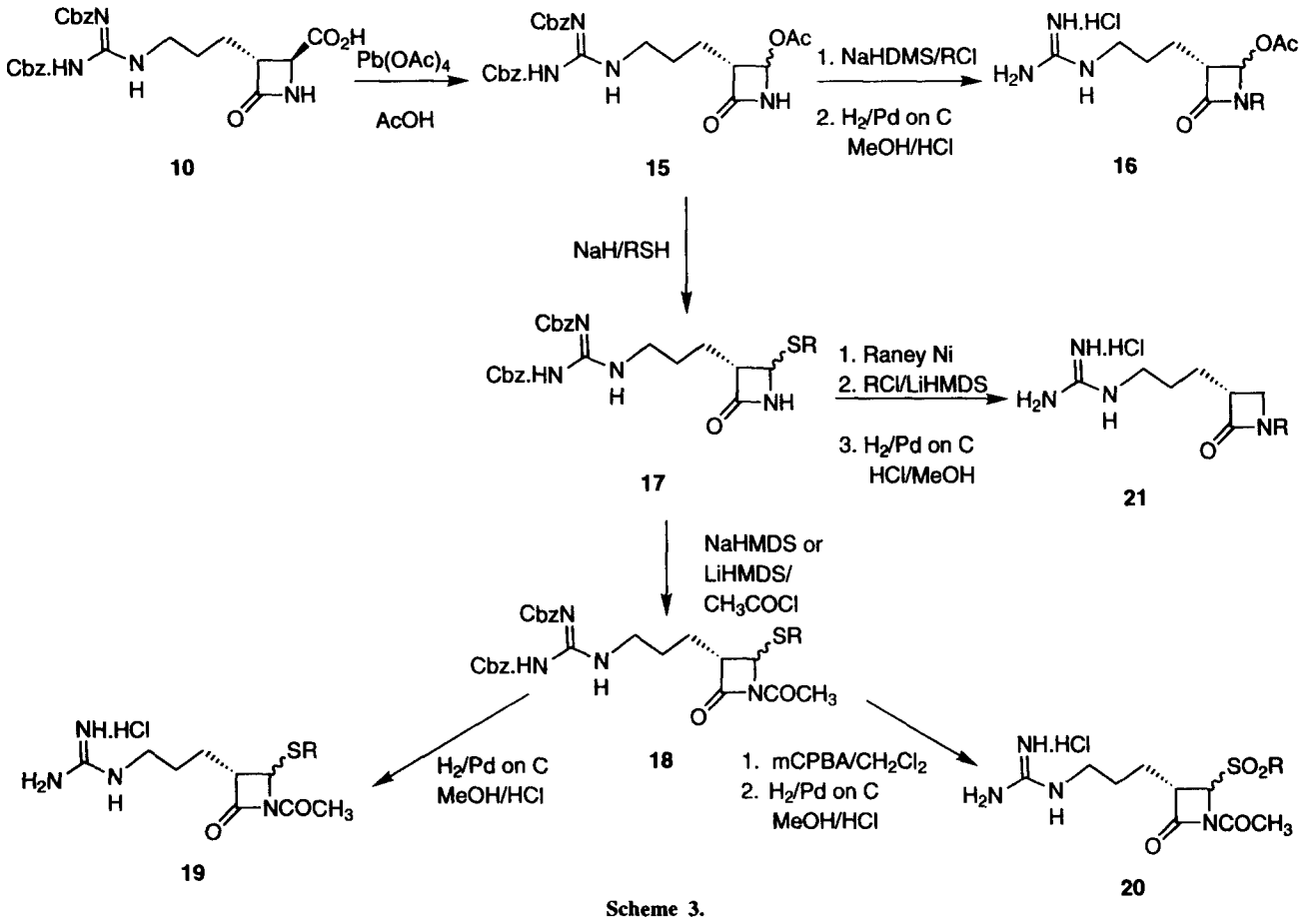
philic azetidin-2-one ring was examined. This combination was designed to take advantage of structural elements associated with both the primary recognition pocket of the enzyme and the amino acid residues intimately involved in catalytic function.

The synthetic compounds were evaluated for their ability to inhibit thrombin, trypsin and plasmin and the results are presented in Table 1. In general, these compounds are potent and effective inhibitors of thrombin- and trypsin-like serine proteases. The data presented in Table 1 provide insight into the funda-mental structure–activity relationships for this structural class of enzyme inhibitor. The structure–activity study summarized in Table 1 focuses primarily on the effect of variation of the azetidin-2-one nitrogen activating group and modification of the 4-substituent of the β-lactam ring. The 4-phenethyl derivative **9a**, in which the β-lactam nitrogen is unsubstituted, is a relatively weak inhibitor of thrombin with a K_i estimated to be about 25 μM. This figure is an estimate because the inhibition of thrombin by **9a** shows some time-dependence. However, the bimolecular rate constant, k_2/K_i, of 1760 is small compared to more reactive serine protease inhibitors of this structural type.[36–40] This result suggested that activation of the azetidin-2-one ring may be necessary in order to increase its reactivity towards the serine hydroxyl of the catalytic triad and enhance enzymatic inactivation. This indeed proved to be the case since the N-acetyl derivative **9b** is a considerably more effective, time-dependent inhibitor of thrombin. The bimolecular rate constant, k_2/K_i, for **9b** is substantially greater than for the unactivated β-lactam **9a**, reflecting the increased electrophilicity of the azetidinone ring. In a more physiologically relevant situation, **9b** is a reasonably effective inhibitor of thrombin-induced clot generation in plasma since it doubles clotting time at a concentration of 3 μM. Although **9b** is a similarly effective inhibitor of trypsin, it is 50-fold more potent as an inhibitor of the clot dissolving enzyme plasmin. From the perspective of identifying and developing an effective anti-thrombotic agent, this specificity profile is clearly less than desirable but provided a clear goal for the further development of structure–activity relationships.

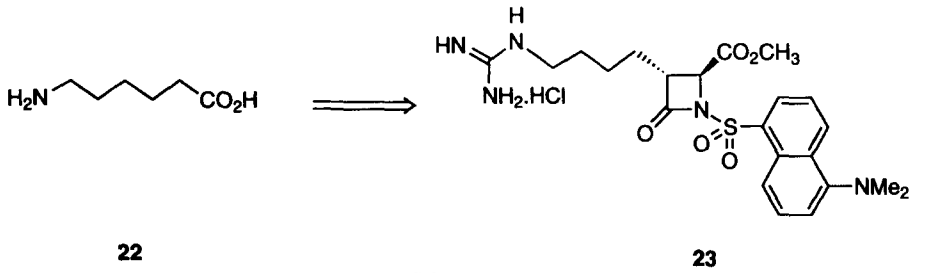
Replacing the phenethyl moiety of **9a** with a more polar carbomethoxy group led to a compound **12a** that is a much less effective inhibitor of thrombin. However, acetylation of the β-lactam N atom not only markedly increased potency but also provided a compound, **12b**, that more selectively inhibited thrombin. Although **12b** effectively inhibits trypsin at similar concentrations, this β-lactam derivative is approximately 1500-fold selective for thrombin over plasmin. As a consequence, the focus of further study was directed towards the elaboration of polar groups as the C-4 substituent. A series of analogues, **12c–e**, was prepared and evaluated



Scheme 2.



Scheme 3.



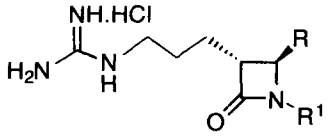
Scheme 4.

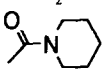
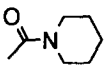
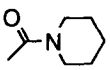
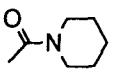
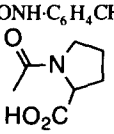
in order to explore the role of the β-lactam activating functionality of **12b** on enzyme inhibitory potency and selectivity. Within this series, both tosyl (**12c**) and dansyl (**12d**) N-substituents provide potent inhibitors of thrombin and trypsin but at the expense of selectivity for these enzymes when compared to plasmin. The phenylurea **12e** is a considerably weaker inhibitor of thrombin, an observation that presumably reflects the reduced electron-withdrawing capacity of this substituent and the associated diminished electrophilicity of the β-lactam ring.[36–38]

The carboxy ester of **12b** provided a convenient functionality for further structural elaboration and facilitated a structure–activity study of this region of the pharmacophore. The free carboxylic acid **13** is a significantly less potent inhibitor of thrombin than its methyl ester **12b** but retains the potent inhibitory activity towards trypsin that characterizes **12b**. An amide substituent at C-4 generally afforded potent inhibitors of both thrombin and trypsin that are weak inhibitors of plasmin, providing a series of compounds **14a–g** with good to excellent enzyme inhibitory selectivity. In the single example where a direct comparison can be made, compound **14a**, a *trans* disposition of the functionality at C-3 and C-4 of the β-lactam ring is approximately 10-fold superior to its *cis*-configured isomer **14b**. Within the series of amides **14a–g**, N-sulfonyl substituted β-lactam derivatives appear to be the more effective inhibitors of thrombin cleavage of s-2231 synthetic substrate than N-acetyl derivatives. However, this increased activity does not translate to the clotting assay where the N-sulfonyl compounds **14c** and **14d** are inferior to the N-acetyl analogue **14a**, possibly reflecting reduced stability of **14c** and **14d** in plasma.

The C-4 acetoxy-substituted β-lactam **16a** is a potent

Table 1. Structure of azetidin-2-one derivatives and their associated enzyme inhibitory activity and effects on blood clotting



Compd #*	R	R¹	IC_{50} versus thrombin cleavage of s-2231 (μM)	k_2/K_i (M^{-1} min^{-1})	Concentration of compound to double clotting time (μM)	IC_{50} versus trypsin (μM)	IC_{50} versus plasmin (μM)
9a	CH_2CH_2Ph	H	K_i ~ 25	1760		> 100	72
9b	CH_2CH_2Ph	$COCH_3$	0.21	1.5×10^6	3.0	0.47	0.0042
12a	CO_2CH_3	H	K_i > 100	330		NT	NT
12b	CO_2CH_3	$COCH_3$	0.002	1.9×10^7	1.25	0.04	3
12c	CO_2CH_3	$SO_2C_6H_4CH_3$	0.002–0.009	1.2×10^8	65	0.008	0.09
12d	CO_2CH_3	dansyl	0.016	2.1×10^7	50	0.72	1.2
12e	CO_2CH_3	CONH·Ph	1.7	8.3×10^4	30	0.005	NT
13	CO_2H	$COCH_3$	0.39	NT	12	0.012	
14a		$COCH_3$†	0.09	4.2×10^5	0.5	0.009	3.13
14b		$COCH_3$‡	NT	4.3×10^4	20	NT	NT
14c		$SO_2C_6H_4CH_3$	0.003	4.5×10^7	80	0.12	0.75
14d		dansyl	0.01	2.8×10^6	30	0.014	12
14e	$CONH{\cdot}C_6H_4CH_3$	$COCH_3$	0.025	1.3×10^6	0.5	0.12	7
14f		$COCH_3$	0.049	4.3×10^6	1.25	NT	NT
14g	$CONH{\cdot}CH_2CO_2H$	$COCH_3$	0.012	2×10^7	0.43	NT	NT
16a	$OCO{\cdot}CH_3$	$COCH_3$§	0.012	4.2×10^7	0.34	0.012	0.39
16b	$OCO{\cdot}CH_3$	COPh‖	0.035	¶	10.0	NT	NT
16c	$OCO{\cdot}CH_3$	Boc**	0.074	2.4×10^6	2	NT	NT
19	SCH_2CH_3	$COCH_3$	0.0125	5.6×10^6	0.75	0.036	1.56
20a	$SO_2CH_2CH_3$	$COCH_3$	0.0028	3.0×10^6	1.2	NT	NT
20b	SO_2Ph	$COCH_3$	0.0032	3×10^6	15	0.12	0.75
21a	H	$COCH_3$	0.1	2.5×10^5	4.0	NT	NT
21b	H	dansyl	1		30	NT	
23	CO_2CH_3††	dansyl		3.7×10^5	50	NT	NT
1(MD 805)			0.038		0.042		
2 (BMY 44621)			0.39		0.46		

*All compounds are racemic. A single enantiomer is shown for purposes of clarity. NT = not tested.
†Evaluated as the *trans* isomer.
‡Evaluated as the *cis* isomer.
§Evaluated as a 2:1 mixture of *trans:cis* isomers.
‖Evaluated as a 3:1 mixture of *trans:cis* isomers.
¶Inhibition of thrombin rapidly reversed.
**Evaluated as a 3:2 mixture of *trans:cis* isomers.
††Guanidinobutyl side chain rather than guanidinopropyl.

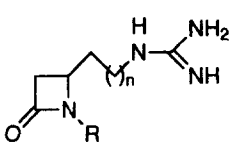
inhibitor of thrombin that exhibits a profile comparable to its isomer **12b**. The inhibitory activity of **16a** is not highly sensitive to the identity of the azetidinone N-substituent since **16b** and **16c** are also potent inhibitors of thrombin-mediated cleavage of the tripeptide synthetic substrate. However, in human plasma, the N-acetyl derivative is the superior inhibitor of thrombin-induced clot formation. It should be emphasized that the β-lactams **16a–c** were evaluated as mixtures of *cis* and *trans* isomers, with the *trans* isomer predominant based on an analysis of ^1H NMR spectral data. If the inhibitory activity in this series demonstrates a similar dependence on the stereochemical relationship of the β-lactam C-3 and C-4 substituents as the amides **14a** and **14b**, then the activity of the more effective isomer is underestimated.

The short series of C-4 sulfur-substituted β-lactams **19**, **20a** and **20b**, demonstrated potent inhibitory activity towards thrombin and, where comparisons can be made, reasonable enzyme selectivity. Inhibition of thrombin-induced clot formation by this series of compounds showed some dependency on the identity of the 4-substituent with the alkyl derivatives **19** and **20a** superior to the phenylsulfone **20b**.

Two additional aspects of the structure–activity relationships associated with this series of thombin inhibitors are evident from the potency of compounds **21a**, **21b** and **23**. The relatively weak inhibitory activity of the structurally simpler azetidinones **21a** and **21b** further underlines the importance of a 4-substituent on the β-lactam ring. The 10-fold weaker activity of **23** compared to its immediate homologue **12d** indicates a dependence on the relationship between the guanidino moiety and the β-lactam carbonyl, which appears to be optimal with the propyl spacer.

The correlation between inhibition of thrombin cleavage of a synthetic substrate by this series of β-lactam derivatives and the increase in thrombin-induced clotting time is limited. Although many of these azetidinones are highly potent inhibitors of thrombin cleavage of the tripeptide substrate s-2231, comparable to both MD 805 (**1**) and BMY 44621 (**2**), many are considerably weaker inhibitors in the clotting assay where the efficacy of **1** and **2** is readily apparent. This observation presumably reflects the stabilities of the individual compounds in human plasma since several of these β-lactam derivatives were sensitive to exposure to plasma where they appeared to be easily deactivated. Despite these potential limitations, ip administration of **14g** to rats at doses of 10 and 30 mg Kg^{-1} resulted in a doubling of blood clotting time measured *ex vivo* 30 minutes after drug delivery.

It was established early on in this study that attachment of the arginine-like chain at C-3 of the β-lactam ring was critical since representative examples of the 4-substituted isomers **24**, with $n = 2$ or 3, were ineffective inhibitors of thrombin. This finding is consistent with the topological relationships established for a series of β-lactam-based inhibitors of human leucocyte elastase.[39,44] Those studies concluded that the C-3 substituent of β-lactam serine protease inhibitors is accommodated in the S_1 pocket, the primary recognition site of the enzyme.[39,44] As further support for this contention, synthetic intermediates in which the guanidine moiety of these β-lactam derivatives was protected with Cbz or modified with other functionality, were either inactive or only poorly active.



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Conclusion

In summary, we have developed synthetic approaches to a series of β-lactam derivatives that incorporate an arginine-like side chain, a structural element recognized by trypsin-like serine proteases. Many of these compounds are potent and time-dependent inhibitors of thrombin-induced cleavage of a synthetic substrate *in vitro*, extending the versatility of β-lactam derivatives as inhibitors of serine protease inhibitors. In human plasma, all of the compounds inhibited thrombin-induced clot formation but only at concentrations considerably higher than those required to inhibit thrombin in the isolated enzyme assay. These observations are presumably reflective of the inherently high chemical reactivity of the activated β-lactam ring, essential for the expression of potent and effective enzyme inhibitory activity but also a possible source of instability in plasma. Nevertheless, ip administration of **14g** to rats resulted in a significant increase in clotting time measured *ex vivo*, suggesting that this class of thrombin inhibitor can express activity *in vivo*. Whilst the chemical and biological properties of the specific β-lactam derivatives compiled in Table 1 may limit their therapeutic potential, this investigation nevertheless provides a useful foundation for further structural modification and development. This study constitutes the first description of β-lactam derivatives as rationally designed inhibitors of thrombin and firmly establishes the viability of this approach to the generation of potential anti-thrombotic agents. Careful structural elaboration of these azetidinone derivatives is required with the objective of tempering the chemical reactivity of the β-lactam ring while simultaneously enhancing the affinity of these compounds for thrombin. This can be accomplished by incorporating recognition elements that selectively and effectively complement functionality present both in the active site of thrombin and the immediate vicinity. The implementation of this strategy will ultimately lead to compounds with enhanced plasma stability and increased specificity for thrombin.[68]

Experimental

Melting points were recorded on a Thomas–Hoover capillary apparatus and are uncorrected. Proton (^1H NMR) magnetic resonance spectra were recorded on either a Bruker AM or a Varian Gemini FT instrument operating at 300 MHz. All spectra were recorded using tetramethylsilane as an internal standard and signal multiplicity was designated according to the following abbreviations: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, *bs* = broad singlet. Infrared (IR) spectra were obtained using a Perkin–Elmer 1800 FT IR, scanning from 4000 to 400 cm^{-1} and calibrated to the 1601 cm^{-1} absorption of a polystyrene film. Mass spectral data were obtained on a Finnigan Model 4500 GC–MS using electrical or chemical ionization (*iso*-butane) procedures. Fast atom bombardment (FAB) mass spectra were obtained on a Kratos MS 25

spectrometer using *meta*-nitrobenzyl alcohol (NOBA) as the matrix. Analytical samples were dried *in vacuo* at 78 °C or in the presence of P_2O_5 at room temperature for at least 12 h. Elemental analyses were provided by the Analytical Chemistry Department, Bristol-Myers Squibb or Oneida Research Services, Whitesboro, NY. Unless otherwise stated, an extractive work-up procedure comprised extraction of the aqueous layer with solvent (three times), washing the combined extracts with H_2O (usually a single time except where DMF or AcOH was present when the organic phase was washed three times) and drying over Na_2SO_4 or $MgSO_4$ prior to evaporation of the solvent *in vacuo*.

5-[N',N"-bis(Carbobenzyloxy)guanidino]pentanoic acid

5-Aminovaleric acid (86.9 g, 0.74 mol) was added portionwise to a solution of **5** (323 g, 0.9 mol) and Et_3N (75.1 g, 0.74 mol) in MeOH (1 L). The resultant suspension was heated at reflux for 1.5 h, concentrated and the residue partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The aqueous layer was acidified to pH 2.0 with HCl and extracted with EtOAc to give a solid. Recrystallization from a mixture of EtOAc and hexane afforded the title compound (135.1 g, 54% based on 5-aminovaleric acid), mp 102–103 °C. IR (KBr) 1735, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.80 (4H, *m*), 2.40 (2H, *bt*, *J* = 4.8 Hz), 3.45 (2H, *bq*, *J* = 6.5Hz), 5.13 (2H, *s*), 5.18 (2H, *s*), 7.25–7.43 (10H, *m*), 8.35 (1H, *bs*). Anal. calcd for $C_{22}H_{25}N_3O_6$: C, 61.81; H, 5.89; N, 9.83; found: C, 61.39; H, 6.29; N, 9.92.

5-[N',N"-bis(Carbobenzyloxy)guanidino]pentanoic acid, methyl ester (6)

Carbonyldiimidazole (55.3 g, 0.34 mol) was added portionwise to a solution of 5-[*N'*,*N"*-bis(carbobenzyloxy)guanidino]pentanoic acid (133 g, 0.3 mol) in THF (700 mL) maintained at 0 °C under an atmosphere of N_2. After the addition was complete, the mixture was warmed to room temperature and stirred for 45 min before adding anhydrous MeOH (100 mL). The solution was stirred for 16 h, concentrated *in vacuo* and the residue triturated with Et_2O (400 mL). The supernatant was separated and the process repeated twice using 200 mL portions of Et_2O. The Et_2O phases were combined, washed with H_2O, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel using a mixture of Et_2O and hexane (1:1) as eluant to afford **6** (130 g, 95%), mp 36–39 °C. IR (KBr) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24–1.50 (4H, *m*), 2.32 (2H, *bt*, *J* = 4.9 Hz), 3.42 (2H, *bq*, *J* = 4.9 Hz), 3.64 (3H, *s*), 5.09 (2H, *s*), 5.15 (2H, *s*), 7.46–7.21 (10H, *m*), 8.36–8.28 (1H, *bs*). Anal. calcd for $C_{23}H_{27}N_3O_6$: C, 62.54; H, 6.15; N, 9.51; found: C, 62.64; H, 6.03; N, 9.37.

trans-*4-(2-Phenylethenyl)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (7) and* cis-*4-(2-phenylethenyl)-3[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (8)*

n-Butyllithium in hexane (272 mL of a 2.5 M solution, 0.69 mol) was added to a solution of 1,1,1,3,3,3-hexamethyldisilazane (110.0 g, 0.69 mol) in THF (800 mL) maintained at –40 °C under an atmosphere of N_2. The mixture was cooled to –78 °C and a solution of **6** (106.0 g, 0.24 mol) in THF (300 mL) added dropwise. After the addition was complete, the mixture was stirred at –30 °C for 1 h, cooled to –78 °C and a solution of 1,1,1-trimethyl-*N*-(3-phenyl-2-propen-1-ylidene)silanamine (51.50 g, 0.25 mol) in THF (100 mL) added dropwise. The mixture was stirred at –20 °C for 16 h, concentrated to half of its original volume and diluted with Et_2O and 5 N HCl solution. Additional 5 N HCl solution was added to the stirred mixture as necessary to maintain the pH of the aqueous phase at 2.0. The organic phase was separated, washed with H_2O and brine, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel to afford **7** (50.0 g, 38%), mp 101 °C. IR (film) 1734 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70–1.85 (4H, *m*), 3.01 (1H, *m*), 3.45–3.56 (2H, *m*), 3.97 (1H, *bd*, *J* = 8.0 Hz), 5.15 (2H, *s*), 5.22 (2H, *s*), 6.27 (1H, *dd*, *J* = 14.5 Hz, *J'* = 8.0 Hz), 6.58 (1H, *s*), 6.66 (1H, *d*, *J* = 14.5 Hz), 7.24–7.50 (15H, *m*), 8.43 (1H, *bt*, *J* = 4.0 Hz). Anal. calcd for $C_{31}H_{32}N_4O_5$: C, 68.86; H, 5.96; N, 10.36; found: C, 68.91; H, 5.94; N, 10.29.

Further elution gave a mixed fraction (26.5 g, 20%) followed by **8** (5.70 g, 4.4%). IR (film) 1760, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50–1.78 (4H, *m*), 3.25–3.48 (3H, *m*), 4.32 (1H, *t*, *J* = 7.2 Hz), 5.05 (2H, *s*), 5.07 (2H, *s*), 5.95 (1H, *bs*), 6.12 (1H, *dd*, *J* = 14.5, 7.2 Hz), 7.14–7.37 (15H, *m*), 8.27 (1H, *bt*, *J* = 5.6 Hz). High resolution FABMS m/z calcd for $C_{31}H_{33}N_4O_5$: 541.2451 (MH^+); found: 541.2441.

trans-*4-(2-Phenylethyl)-3-(3-guanidinopropyl)-2-azetidinone hydrochloride salt (9a)*

A mixture of **7** (350 mg, 0.65 mmol), MeOH (20 mL), 1 N HCl (0.65 mL) and 10% Pd/C was stirred under an atmosphere of H_2 at 50 psi. After 16 h, the mixture was filtered through a pad of Celite and solvent evaporated to afford **9a** as a colorless foam. IR (film) 1735 cm^{-1}; ^1H NMR (D_2O) δ 1.35–1.51 (4H, *m*), 1.61–1.79 (2H, *m*), 2.37–2.60 (3H, *m*), 2.98 (2H, *bt*, *J* = 5.40 Hz), 3.17 (1H, *bt*, *J* = 6.65 Hz), 7.06–7.20 (5H, *m*).

trans-*4-(2-Phenylethenyl)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 7.64 mL) was added dropwise to a solution of **7** (4.1 g, 7.64 mmol) in THF (20 mL) stirred at –78 °C under N_2. After 15 min acetyl chloride (600 mg, 7.6 mmol) was added, the solution warmed to room temperature and stirred for 75 min. The mixture was diluted with aqueous buffer (pH 4.0), extracted with Et_2O and the residue chromatographed on a column of silica gel. Elution with a mixture of Et_2O and hexane (3:1) afforded the title compound (2.30 g, 52%). IR (film) 1780, 1735, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.65–1.90 (4H, *m*), 2.38 (3H, *s*), 3.00–3.10 (1H, *m*), 3.40–3.53 (2H, *m*), 6.20 (1H, *dd*, *J* = 15.3 Hz, *J'* = 7.7 Hz), 6.68

(1H, *d*, *J* = 15.3 Hz), 7.20–7.43 (15H, *m*), 8.36 (1H, *bs*). Anal. calcd for $C_{33}H_{34}N_4O_6$: C, 68.03; H, 5.88; N, 9.62; found: C, 68.05; H, 5.99; N, 9.30. High resolution FABMS m/z calcd for $C_{33}H_{34}N_4O_6$: 583.2556 (MH^+); found: 583.2543.

trans-*4-(2-Phenylethyl)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (**9b**)*

A mixture of *trans*-4-(2-phenylethenyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (470 mg, 0.82 mmol), MeOH (3 mL), EtOAc (1 mL), 1 N HCl (0.9 mL) and a catalytic quantity of 10% Pd/C was stirred under an atmosphere of H_2 until TLC indicated the disappearance of starting material (about 30 min). The mixture was filtered through a pad of Celite and concentrated to afford **9b** (220 mg, 76%). IR (film) 1770, 1710 cm^{-1}; ^1H NMR (CD_3OD, selected peaks) δ 2.30 (3H, *s*), 3.72–3.87 (1H, *m*), 7.10–7.50 (5H, *m*). High resolution FABMS m/z calcd for $C_{17}H_{25}N_4O_2$: 317.1977 (MH^+); found: 317.1971.

trans-*4-(2-Phenylethenyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-1-(*tert*-butyldimethylsilyl)-2-azetidinone*

tert-Butyldimethylsilyl chloride (3.12 g, 21 mmol) was added to a stirred solution of **7** (7.80 g, 14.4 mmol) and Et_3N (2.10 g, 21 mmol) in DMF (40 mL). The mixture was stirred at room temperature for 15 h, diluted with H_2O and extracted with Et_2O. The residual oil was chromatographed on a column of silica gel using a mixture of Et_2O and hexane (1:1) as eluant to afford the title compound (8.30 g, 88%). ^1H NMR ($CDCl_3$) δ 0.10 (3H, *s*), 0.16 (3H, *s*), 0.88 (9H, *s*), 1.65–1.90 (4H, *m*), 2.95–3.04 (1H, *m*), 3.41–3.45 (2H, *m*), 3.86 (1H, *dd*, *J* = 9.9 Hz, *J'* =1.5 Hz), 5.13 (2H, *s*), 5.60 (2H, *s*), 6.17 (1H, *dd*, *J* = 16 Hz, *J'* = 9.9 Hz), 6.58 (1H, *d*, *J* = 16 Hz), 7.23–7.45 (15H, *m*), 8.37 (1H, *t*, *J* = 4.5 Hz). Anal. calcd for $C_{37}H_{46}N_4O_5Si$: C, 67.86; H, 7.08; N, 8.56; found: C, 67.12; H, 7.06; N, 8.21. High resolution FABMS m/z calcd for $C_{37}H_{46}N_4O_5Si$: 655.3316 (MH^+); found: 655.1295.

trans-*4-Carboxy-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (**10**)*

Ozone was bubbled through a stirred solution of *trans*-4-(2-phenylethenyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)-guanidino]propyl]-1-(*tert*-butyldimethylsilyl)-2-azetidinone (55.0 g, 84 mmol) in CH_2Cl_2 (650 mL) maintained at –78 °C until a blue color persisted. Excess ozone was purged with N_2, Me_2S (60 mL) was added and the solution allowed to warm to room temperature. The mixture was allowed to stand for 48 h before being concentrated to afford crude *trans*-4-formyl-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]prop-yl]-1-(*tert*-butyldimethylsilyl)-2-azetidinone which was used directly. ^1H NMR ($CDCl_3$) δ 0.13 (3H, *s*), 0.20 (3H, *s*), 0.97 (9H, *s*), 1.65–1.93 (4H, *m*), 3.21–3.28 (1H, *m*), 3.40–3.53 (2H, *m*), 3.67 (1H, *m*), 5.13 (2H, *s*), 7.25–7.60 (10H, *m*), 5.17 (2H, *s*), 8.40 (1H, *bt*, *J* = 5.3 Hz), 9.63 (1H, *d*, *J* = 5.3 Hz).

The aldehyde obtained was dissolved in acetone (75 mL) and freshly prepared Jones reagent was added dropwise, while maintaining the temperature between 20 and 25 °C, until TLC indicated the disappearance of starting material. The suspension was concentrated to half of its original volume and partitioned between EtOAc and H_2O. The organic phase was separated, dried over Na_2SO_4 and concentrated. The residual solid was recrystallized from EtOAc/hexane to afford **10** (8.80 g, 54% overall from **7**), mp 172–173 °C. ^1H NMR ($CDCl_3$) δ 1.50–1.70 (4H, *m*), 3.74 (1H, *d*, *J* = 1.8 Hz), 4.99 (2H, *s*), 5.17 (2H, *s*), 7.20–7.45 (10H, *m*), 8.24 (1H, *s*), 8.43 (1H, *bs*). High resolution FABMS m/z calcd for $C_{24}H_{27}N_4O_7$: 483.1890 (MH^+); found: 483.1880.

trans-*4-(Methoxycarbonyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone*

A solution of CH_2N_2 in Et_2O was added to a solution of **10** (4.69 g, 9.72 mmol) in CH_2Cl_2 (50 mL) until the yellow color was maintained. Excess CH_2N_2 was destroyed by adding CH_3CO_2H, the solution was concentrated and the residue chromatographed on a column of silica gel. Elution with a mixture of EtOAc and Et_2O (9:1) afforded the title product (3.41 g, 71%). ^1H NMR ($CDCl_3$) δ 1.70–1.95 (4H, *m*), 3.22–3.31 (1H, *m*), 3.42–3.54 (2H, *m*), 3.77 (3H, *s*), 3.88 (1H, *d*, *J* = 2.4 Hz), 5.13 (2H, *s*), 5.18 (2H, *s*), 6.27 (1H, *bs*), 7.25–7.45 (10H, *m*), 8.38 (1H, *t*, *J* = 5.3 Hz). Anal. calcd for $C_{25}H_{28}N_4O_7$: C, 60.47; H, 5.68; N, 11.28; found: C, 60.36; H, 5.76; N, 11.26.

trans-*4-(Methoxycarbonyl)-3-(3-guanidinopropyl)-2-azetidinone hydrochloride salt (**12a**)*

A mixture of *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N''*-bis-(carbobenzyloxy)guanidino]propyl]-2-azetidinone (135 mg, 0.27 mmol), MeOH, 1 N HCl (0.5 mL) and 10% Pd/C was stirred under an atmosphere of H_2 at 40 psi. After 16 h the mixture was filtered through a pad of Celite and concentrated to afford **12a** (30 mg, 42%). IR (film) 1760, 1740 cm^{-1}; ^1H NMR (D_2O) δ 1.69–1.88 (4H, *m*), 3.22 (2H, *bt*, *J* = 6.3 Hz), 3.32–3.40 (1H, *m*), 3.77 (3H, *s*), 4.12 (1H, *d*, *J* = 1.95 Hz). FABMS m/z 229 (MH^+).

trans-*4-(Methoxycarbonyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

Et_3N (12.30 g, 0.12 mol) was added dropwise to a vigorously stirred solution of acetyl chloride (9.50 g, 0.12 mol) and *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (2.00 g, 4.0 mmol) in CH_2Cl_2 (50 mL) maintained at –10 °C. After the addition was complete, the mixture was stirred at room temperature for 2 h before being diluted with H_2O. The organic phase was separated, washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated. The residue was subjected to chromatography on a column of silica gel using a mixture of CH_2Cl_2 and MeOH (99.5:0.5) as eluant to furnish the title compound (700 mg, 36%). IR (film)

1800, 1740, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.60–1.93 (4H, *m*), 2.34 (3H, *s*), 3.15–3.26 (1H, *m*), 3.38–3.47 (2H, *m*), 3.37 (3H, *s*), 4.08 (1H, *d*, *J* = 2.3 Hz), 5.07 (2H, *s*), 5.14 (2H, *s*), 7.20–7.37 (10H, *m*), 8.32 (1H, *bt*, *J* = 5 Hz);. High resolution FABMS m/z calcd for $C_{27}H_{31}N_4O_8$: 539.2125 (MH^+); found: 539.2142.

trans-*4-(Methoxycarbonyl)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (12a)*

A solution of *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)guanidino] propyl] -1-acetyl-2-azetidinone (313 mg, 0.58 mmol) in MeOH (2.5 mL), EtOAc (2.5 mL) and 1 N HCl (0.58 mL) was stirred under a H_2 atmosphere over 10% Pd/C catalyst. When the starting material had disappeared as determined by TLC, the reaction mixture was filtered through a pad of Celite and concentrated to afford **12a** (150 mg, 84%) as a yellow foam. IR (film) 1800 cm^{-1}; ^1H NMR (CD_3OD) δ 1.60–1.90 (4H, *m*), 2.35 (3H, *s*), 3.10–3.50 (3H, *m*), 3.78 (3H, *s*), 4.27 (1H, *d*, *J* = 3.1 Hz). High resolution FABMS m/z calcd for $C_{11}H_{18}O_4$: 271.1406 (MH^+); found: 271.1404.

trans-*4-(Methoxycarbonyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-(*p*-toluenesulfonyl)-2-azetidinone*

Sodium bis(trimethylsilyl)amide (0.67 mL, 0.67 mmol) in THF was added to a stirred solution of *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)-guanidino]propyl]-2-azetidinone (436 mg, 0.67 mmol) in THF (5 mL) maintained at –78 °C under an atmosphere of N_2. After 15 min, *p*-toluenesulfonyl chloride (134 mg, 0.70 mmol) was added, the mixture stirred at –78 °C for 4 h and then at –20 °C for 2.5 days. The mixture was partitioned between EtOAc and H_2O and the organic layer separated and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by chromatography on a column of silica gel. Elution with a mixture of Et_2O and hexane (3:2) afforded the title compound (210 mg, 48%). IR (film) 1805, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55–1.85 (4H, *m*), 2.42 (3H, *s*), 3.13–3.23 (1H, *m*), 3.35–3.45 (2H, *m*), 3.72 (3H, *s*), 4.28 (1H, *d*, *J* = 1.5 Hz), 4.98 (1H, *bs*), 5.06 (2H, *s*), 5.10 (2H, *s*), 7.20–7.40 (14H, *m*), 7.88 (1H, *d*, *J* = 8.4 Hz), 8.32 (1H, *bt*, *J* = 6.1 Hz). Anal. calcd for $C_{32}H_{34}N_4O_9S$: C, 58.77; H, 5.14; N, 8.14; found: C, 59.06; H, 5.26; N, 8.61.

trans-*4-(Methoxycarbonyl)-3-(3-guanidinopropyl)-1-(p-toluenesulfonyl)-2-azetidinone hydrochloride salt (12b)*

A solution of *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)guanidino]propyl]-1-(*p*-toluenesulfonyl)-2-azetidinone (197 mg, 0.3 mmol) in EtOAc (2.5 mL), MeOH (2.5 mL) and 1 N HCl (0.6 mL) was stirred under an atmosphere of H_2 over 10% Pd/C catalyst until TLC indicated the disappearance of the starting material. The suspension was filtered through a pad of Celite and concentrated to afford **12b** (30 mg, 24%) as a foam. IR (film) 1800, 1755 cm^{-1}; ^1H NMR (CD_3OD) δ 1.56–1.86 (4H, *m*), 2.46 (3H, *s*), 3.16 (2H, *t*, *J* = 6.6 Hz), 3.42 (1H, *dt*, *J* = 7.2 Hz, *J'* = 3.6 Hz), 3.74 (3H, *s*), 4.47 (1H, *d*, *J* = 3.6 Hz), 7.44 (2H, *d*, *J* = 8.5 Hz), 7.88 (2H, *d*, *J* = 8.5 Hz). Anal. calcd for $C_{16}H_{24}N_4O_5Cl$: C, 44.18; H, 5.33; N, 12.88; Cl, 8.15; found: C, 43.79; H, 5.62; N,12.52; Cl, 8.01. High resolution FABMS m/z calcd for $C_{16}H_{24}N_4O_5Cl$: 383.1389 (MH^+); found: 383.1376.

trans-*4-(Methoxycarbonyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone*

A solution of sodium bis(trimethylsilyl)amide (1 N in THF, 2.08 mL, 2.08 mmol) was added dropwise to a solution of *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N"*-bis-(carbobenzyloxy)guanidino]propyl]-2-azetidinone (1.03 g, 2.08 mmol) in THF (5 mL) stirred at –78 °C under an atmosphere of N_2. After 20 min dansyl chloride (0.50 g, 2.08 mmol) was added, the mixture was allowed to warm to room temperature and stirred for 1 h. Aqueous buffer, pH 7.0, was added, the mixture extracted with EtOAc and the residue subjected to chromatography on a column of silica gel. Elution with Et_2O afforded the title compound (0.45 g, 30%). IR (KBr) 1810, 1755 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.53–1.86 (4H, *m*), 2.84 (6H, *s*), 3.24–3.31 (1H, *m*), 3.33–3.42 (2H, *m*), 3.46 (3H, *s*), 4.22 (1H, *d*, *J* = 2.3 Hz), 5.08 (2H, *s*), 5.16 (2H, *s*), 7.15–7.45 (11H, *m*), 7.49–7.63 (2H, *m*), 8.28 (1H, *d*, *J* = 6.9 Hz), 8.47 (1H, *d*, *J* = 9.2 Hz), 8.61 (1H, *d*, *J* = 8.4 Hz). FABMS m/z 730 (MH^+).

trans-*4-(Methoxycarbonyl)-3-(3-guanidinopropyl)-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone hydrochloride salt (12c)*

A mixture of *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N"*-bis-(carbobenzyloxy)guanidino]-propyl]-1-[5-(dimethyl-amino)-1-naphthalenesulfonyl]-2-azetidinone (0.45 g, 0.62 mmol), MeOH (5 mL), EtOAc (2 mL), 1 N HCl (0.7 mL) and 10% Pd/C catalyst was stirred under an atmosphere of H_2. After 25 min, the mixture was filtered through a pad of Celite and concentrated to afford **12c** (276 mg, 89%). IR (KBr) 1805, 1755 cm^{-1}; ^1H NMR (CD_3OD) δ 1.30–1.60 (4H, *m*), 2.77 (6H, *s*), 1.84–3.09 (3H, *m*), 3.19 (3H, *s*), 4.20 (1H, *d*, *J* = 2.3 Hz), 7.28 (1H, *bd*, *J* = 6.9 Hz), 7.40-7.53 (2H, *m*), 8.08 (1H, *d*, *J* = 7.7 Hz), 8.38 (1H, *d*, *J* = 9.2 Hz), 8.46 (1H, *d*, *J* = 8.4 Hz). High resolution FABMS m/z calcd for $C_{21}H_{28}N_5O_5S$: (MH^+) 462.1811; found: 462.1801.

trans-*1-(Methoxycarbonyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-[(phenylamino)carbonyl]-2-azetidinone*

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 0.149 mL, 0.149 mmol) was added dropwise to a solution of *trans*-4-(methoxycarbonyl)-3-[3-[*N'*,*N"*-bis-(carbobenzyloxy)guanidino]propyl]-2-azetidinone (690 mg, 1.38 mmol) in THF (10 mL) stirred at –78 °C under an atmosphere of N_2. After 10 min, phenyliso-cyanate (0.149 mL, 1.38 mmol) was added, the mixture

warmed to room temperature and stirred for 1 h. The solution was concentrated, the residue was diluted with aqueous buffer (pH 4.0) and extracted with EtOAc to give an oil. Chromatography on a column of silica gel using a mixture of Et_2O and hexane (3:1) as eluant afforded the title compound (450 mg, 53%) as a white foam. IR (KBr) 1780, 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.68–2.00 (4H, *m*), 3.26–3.36 (1H, *m*), 3.40–3.53 (2H, *m*), 3.78 (3H, *s*), 4.23 (1H, *d*, *J* = 2.3 Hz), 5.05 (2H, *s*), 5.17 (2H, *s*), 7.03–7.53 (15H, *m*). High resolution FABMS m/z calcd for $C_{32}H_{34}H_5O_8$: (MH^+) 616.2407; found: 616.2413.

trans-*1-(Methoxycarbonyl)-3-(3-guanidinopropyl)-1-[(phenylamino)carbonyl]-2-azetidinone hydrochloride salt (12d)*

A mixture of *trans*-1-(methoxycarbonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-[(phenylamino)carbonyl]-2-azetidinone (421 mg, 0.68 mmol), MeOH (2.5 mL), EtOAc (2.5 mL), 1 N HCl (0.68 mL) and 10% Pd/C was stirred under an atmosphere of H_2 for 1 h. The mixture was filtered through a pad of Celite and concentrated to afford the title compound (270 mg, 100%) as a yellow foam. IR (KBr) 1780, 1740 cm^{-1}; ^1H NMR (CD_3OD) δ 1.73–1.98 (4H, *m*), 3.21–3.51 (3H, *m*), 3.82 (3H, *s*), 4.38 (1H, *d*, *J* = 2.8 Hz), 7.09–7.49 (5H, *m*). High resolution FABMS m/z calcd for $C_{16}H_{22}O_4N_5$: (MH^+) 348.1672; found: 348.1679.

trans-*4-(1-Piperidinylcarbonyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone*

Carbonyldiimidazole (400 mg, 2.5 mmol) was added to a stirred solution of **10** (1.00 g, 2.1 mmol) in THF (20 mL). After 30 min piperidine (0.21 g, 2.5 mmol) was added, the mixture stirred for 1 h and concentrated. The residue was diluted with 1 N HCl solution and extracted with EtOAc to give an oil. Chromatography on a column of silica gel using EtOAc as the eluant afforded the title compound (720 mg, 62%). ^1H NMR ($CDCl_3$) δ 1.30–1.85 (10H, *m*), 3.05 (7H, *m*), 3.05–3.55 (7H, *m*), 3.93 (1H, *s*), 5.01 (2H, *s*), 5.07 (2H, *s*), 7.10–7.32 (10H, *m*), 8.32 (1H, *bt*, *J* = 5.1 Hz). High resolution FABMS m/z calcd for $C_{29}H_{36}H_5O_6$: (MH^+) 550.2666; found: 550.2656.

trans-*4-(1-Piperidinylcarbonyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 0.65 mL, 0.65 mmol) was added to a solution of *trans*-4-(1-piperidinylcarbonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (334 mg, 0.61 mmol) in THF (10 mL) stirred at –78 °C under N_2. After 15 min acetyl chloride (47.4 mg, 0.6 mmol) was added, the mixture warmed to room temperature and stirred for 30 min. The solvent was evaporated, the residue diluted with aqueous buffer (pH 4.0) and extracted with EtOAc to give an oil. Chromatography on a column of silica gel using a mixture of Et_2O and EtOAc (3:1) afforded the title compound (155 mg, 78%). IR (film) 1795, 1735, 1720 cm^{-1}; ^1H NMR δ 1.30–1.85 (10H, *m*), 2.33 (3H, *s*), 3.18–3.28 (1H, *m*), 3.30–3.62 (6H, *m*), 4.43 (1H, *bs*), 5.04 (2H, *s*), 5.13 (2H, *s*), 7.18–7.36 (10H, *m*), 8.33 (1H, *bt*, *J* = 5.3 Hz). High resolution FABMS m/z calcd for $C_{31}H_{38}N_5O_7$: (MH^+) 592.2771; found: 592.2765.

trans-*4-(1-Piperidinylcarbonyl)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (14a)*

A mixture of *trans*-4-(1-piperidinylcarbonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (150 mg, 0.27 mmol), MeOH (3 mL), 1 N HCl (0.27 mL) and 10% Pd/C was stirred under an atmosphere of H_2 for 5 min. The mixture was filtered through a pad of Celite and concentrated to afford **14a** (66 mg, 68%). IR (film) 1795, 1710 cm^{-1}; ^1H NMR (D_2O) δ 1.23–1.97 (10H, *m*), 2.38 (3H, *s*), 3.22–3.76 (7H, *m*), 4.93 (1H, *bs*). High resolution FABMS m/z calcd for $C_{15}H_{26}O_3N_5$: 324.2035; found: 324.2030.

trans-*4-(1-Piperidinylcarbonyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-1-(*p*-toluenesulfonyl)-2-azetidinone*

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 0.7 mL) was added dropwise to a stirred solution of *trans*-4-(1-piperidinylcarbonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (379 mg, 0.69 mmol) in THF (10 mL) maintained at –78 °C under an atmosphere of N_2. After 20 min *p*-toluenesulfonyl chloride (131 mg, 0.69 mmol) was added and the mixture warmed to room temperature. The solvent was evaporated, the residue diluted with aqueous buffer (pH 4.0) and extracted with EtOAc to give an oil. Chromatography on a column of silica gel gave the title compound (160 mg, 33%). IR (KBr) 1802, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40–1.85 (10H, *m*), 2.37 (3H, *s*), 3.15–3.23 (1H, *m*), 3.32–3.54 (6H, *m*), 4.70 (1H, *d*, *J* = 1.5 Hz), 5.07 (2H, *s*), 5.14 (2H, *s*), 7.22–7.40 (10H, *m*), 7.28 (2H, *d*, *J* = 8.4 Hz), 7.87 (2H, *d*, *J* = 8.4 Hz). High resolution FABMS m/z calcd for $C_{36}H_{42}N_5O_8S$: (MH^+) 704.2754; found: 704.2767.

trans-*4-(1-Piperidinylcarbonyl)-3-(3-guanidinopropyl)-1-(p-toluenesulfonyl)-2-azetidinone hydrochloride salt (14c)*

A mixture of *trans*-4-(1-piperidinylcarbonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-(*p*-toluenesulfonyl)-2-azetidinone (157 mg, 0.22 mmol), EtOAc (2.5 mL), MeOH (2.5 mL), 1 N HCl (0.22 mL) and 10% Pd/C was stirred under an atmosphere of H_2 for 10 min. The mixture was filtered through a pad of Celite and concentrated to afford **14c** (70 mg, 67%). IR (KBr) 1800, 1740 cm^{-1}; ^1H NMR (CD_3OD) δ 1.50–1.90 (10H, *m*), 2.44 (3H, *s*), 3.10–3.74, (7H, *m*), 5.06 (1H, *d*, *J* = 2.6 Hz), 7.42 (2H, *d*, *J* = 8.3 Hz), 7.86 (2H, *d*, *J* = 8.3 Hz). High resolution FABMS m/z calcd for $C_{20}H_{30}N_5O_4S$: (MH^+) 436.2018; found: 436.2011.

trans-*4-(1-Piperidinylcarbonyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone*

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 1.25 mL, 1.25 mmol), was added dropwise to a stirred solution of *trans*-4-(1-piperidinylcarbonyl)-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (640 mg, 1.16 mmol) in THF (12 mL) maintained at –78 °C under N_2. After 20 min a solution of dansyl chloride (320 mg, 1.17 mmol) in THF (20 mL) was added and the mixture stirred at room temperature for 45 min. The solvent was evaporated, the residue diluted with aqueous buffer (pH 4.0) and extracted with EtOAc to give an oil. Chromatography on a column of silica gel using Et_2O as eluant furnished the title compound (380 mg, 42%). IR (KBr) 1800, 1737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35–1.87 (10H, *m*), 2.84 (6H, *s*), 3.27–3.55 (7H, *m*), 4.68 (1H, *d*, *J* = 1.6 Hz), 5.09 (2H, *s*), 5.16 (2H, *s*), 7.15 (1H, *d*, *J* = 6.3 Hz), 7.26–7.35 (10H, *m*), 7.51 (1H, *t*, *J* = 6.7 Hz), 7.56 (1H, *t*, *J* = 6.7 Hz), 8.26 (1H, *d*, *J* = 6.0 Hz), 8.45 (1H, *d*, *J* = 7.2 Hz), 8.57 (1H, *d*, *J* = 7.1 Hz). High resolution FABMS m/z calcd for $C_{41}H_{47}N_6O_8S$: (MH^+) 783.3176; found: 783.3162.

trans-*4-(1-Piperidinocarbonyl)-3-(3-guanidinopropyl)-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone hydrochloride salt (**14d**)*

A mixture of *trans*-4-(1-piperidinylcarbonyl)-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)guanidino]propyl]-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone (360 mg, 0.48 mmol), MeOH (3 mL), EtOAc (1.5 mL), 1 N HCl (0.55 mL), and 10% Pd/C was stirred under an atmosphere of H_2 for 10 min. The mixture was filtered through a pad of Celite and concentrated to afford **14d** (196 mg, 73%). IR (KBr) 1800, 1735 cm^{-1}; ^1H NMR (CD_3OD) δ 1.43–1.92 (10H, *m*), 3.22 (6H, *s*), 3.06–3.42 (4H, *m*), 3.50–3.72 (3H, *m*), 5.07 (1H, *d*, *J* = 2.1 Hz), 7.76–7.81 (3H, *m*), 8.36 (1H, *d*, *J* = 6.1 Hz), 8.66 (1H, *d*, *J* = 7.1 Hz), 8.82 (1H, *bd*, *J* = 7.0 Hz). High resolution FABMS m/z calcd for $C_{25}H_{35}N_6O_4$: (MH^+) 515.2441; found: 515.2438.

trans-*1-Formyl-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

Ozone was bubbled through a solution of *trans*-4-(2-phenylethenyl)-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (0.70 g, 1.2 mmol) in CH_2Cl_2 (20 mL) stirred at –78 °C until a blue color was maintained. Excess ozone was removed by purging the solution with N_2, and Me_2S (1 mL) was added. The mixture was warmed to room temperature, allowed to stand for 72 h, and the solvent evaporated. The residue was chromatographed on a column of silica gel using a mixture of Et_2O and EtOAc (3:1) as eluant to afford the title product (230 mg, 38%). IR (film) 1797, 1734, 1703 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50–1.94 (4H, *m*), 2.40 (3H, *s*), 3.20–3.30 (1H, *m*), 3.30–3.56 (2H, *m*), 4.17 (1H, *bs*), 5.10 (2H, *s*), 5.17 (2H, *s*), 7.50–7.20 (10H, *m*), 9.70 (1H, *s*). High resolution FABMS m/z calcd for $C_{26}H_{29}N_4O_7$ (MH^+): 509.2036; found: 509.2031.

trans-*4-Carboxy-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (**11**)*

Freshly prepared Jones reagent was added dropwise to a solution of *trans*-4-formyl-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (3.50g, 6.8 mmol) in acetone (70 mL) until TLC indicated the disappearance of the starting material. The acetone layer was decanted, the residual solid washed with acetone and organic phases combined and concentrated. The residue was chromatographed on a column of silica gel using a mixture of EtOAc and MeOH (99:1) to afford **11** (2.40 g, 66%). ^1H NMR ($CDCl_3$) δ 1.65–1.96 (4H, *m*), 2.40 (3H, *s*), 3.17–3.26 (1H, *m*), 3.39–3.50 (2H, *m*), 4.13 (1H, *d*, *J* = 2.3 Hz), 5.11 (2H, *s*), 5.17 (2H, *s*), 7.11–7.40 (10H, *m*). High resolution FABMS m/z calcd for $C_{26}H_{29}N_4O_8$ (MH^+): 525.1985; found: 525.1991.

trans-*4-[[(4-Methylphenyl)amino]carbonyl]-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

Diphenylphosphoryl azide (DPPA)[63] (550 mg, 2 mmol) was added to a solution of **11** (700 mg, 1.34 mmol) and *p*-toluidine (210 mg, 2 mmol) in DMF (3.5 mL) stirred at –5 °C. The mixture was stirred for 30 min, warmed to room temperature and stirred for 1.5 h before being diluted with H_2O and extracted with Et_2O. The residual oil was chromato-graphed on a column of silica gel using a mixture of Et_2O and hexane (2:1) as eluant to afford the title compound (150 mg, 18%). ^1H NMR ($CDCl_3$) δ 1.62–2.00 (4H, *m*), 2.28 (3H, *s*), 2.42 (3H, *s*), 3.27–3.70 (3H, *m*), 4.43 (1H, *d*, *J* = 2.3 Hz), 5.07 (2H, AB quartet), 5.17 (2H, *s*), 7.03 (2H, *d*, *J* = 8.4 Hz), 7.20–7.40 (10H, *m*), 7.24 (2H, *d*, *J* = 8.4 Hz). High resolution FABMS m/z calcd for $C_{33}H_{36}N_5O_7$: 614.2615 (MH^+); found: 614.2602.

trans-*4-Carboxy-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (**13**)*

A solution of compound **11** (200 mg, 0.38 mmol) in MeOH (3 mL), EtOAc (0.5 mL) and 1 N HCl (0.5 mL) was hydrogenated over 10% Pd/C until TLC indicated the disappearance of the starting material (15 min). The mixture was filtered through a pad of Celite and the filtrate concentrated to afford **13** (37 mg, 33%). IR (KBr) 1800, 1709 cm^{-1}; ^1H NMR(CD_3OD) δ 2.02–1.74 (4H, *m*), 2.35 (3H, *s*), 3.20–3.37 (3H, *m*), 4.18 (1H, *d*, *J* = 3.3 Hz). High resolution FABMS m/z calcd for $C_{10}H_{19}N_4O_4$: 257.1250 (MH^+); found: 257.1244.

trans-*4-[[(4-Methylphenyl)amino]carbonyl]-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (**14e**)*

A mixture of *trans*-4-[[(4-methylphenyl)amino]carbonyl]-3-[3-[*N'*,*N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (150 mg, 0.25 mmol), MeOH (2 mL), EtOAc (0.5 mL), 1 N HCl (0.28 mL) and a catalytic quantity of 10% Pd/C was stirred under H_2

until TLC indicated the disappearance of the starting material (10 min). The mixture was filtered through a pad of Celite and the solvent evaporated to leave **14e** (70 mg, 73%). IR (KBr) 1802 cm^{-1}; ^1H NMR (CD_3OD) δ 1.59–1.91 (4H, *m*), 2.29 (3H, *s*), 2.37 (3H, *s*), 3.20–3.39 (3H, *m*), 4.32 (1H, *d*, *J* = 3.2 Hz), 7.12 (2H, *d*, *J* = 7.2 Hz), 7.45 (2H, *d*, *J* = 7.2 Hz). High resolution FABMS m/z calcd for $C_{17}H_{24}N_5O_3$: 346.1879 (MH^+); found: 346.1875.

trans-*4-[[2-[(Phenylmethoxy)carbonyl]-1-pyrrolidinyl]carbonyl]-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

Et_3N (0.112 g, 1.1 mmol) was added to a stirred solution of ethyl chloroformate (0.12 g, 1.1 mmol) and **11** (0.35 g, 0.7 mmol) in CH_2Cl_2 (5 mL) maintained at –5 °C. The mixture was stirred for 1 h at –5 °C to +5 °C before adding Et_3N (0.11 g, 1.1 mmol) and proline benzyl ester hydrochloride (260 mg, 1.1 mmol). After 15 min the mixture was warmed to room temperature and stirred for 16 h. The mixture was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. The residue was subjected to chromatography on a column of silica gel using a mixture of Et_2O and EtOAc (8:2) as eluant to afford the title compound (70 mg, 14%). ^1H NMR ($CDCl_3$) δ 1.50–2.24 (8H, *m*), 2.29 (3/2H, *s*), 2.34 (3/2H, *s*), 3.17–3.92 (6H, *m*), 4.20–4.26 (1/2H, *m*), 4.43–4.60 (1/2H, *m*), 5.03–5.18 (6H, *m*), 7.13–7.40 (15H, *m*). High resolution FABMS m/z calcd for $C_{38}H_{42}N_5O_9$: 712.2990 (MH^+); found: 712.2982.

trans-*4-[(2-Carboxy-1-pyrrolidinyl)carbonyl]-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (**14f**)*

A mixture of *trans*-4-[[2-[(phenylmethoxy)carbonyl]-1-pyrrolidinyl]carbonyl]-3-[3-[*N',N"*-bis(carbobenzyloxy)-guanidino]propyl]-1-acetyl-2-azetidinone (150 mg, 0.2 mmol), MeOH (2 mL), EtOAc (0.5 mL), 1 N HCl (0.2 mL) and a catalytic quantity of 10% Pd/C was stirred under H_2 for 1.5 h. The mixture was filtered through a pad of Celite and concentrated to afford **14f** (47 mg, 60%). IR (KBr) 1800, 1712 cm^{-1}; ^1H NMR (CD_3OD) δ 1.63–2.13 (8H, *m*), 2.32 (1.5H, *s*), 2.33 (1.5H, *s*), 3.15–3.42 (5H, *m*), 3.50–3.58 (0.5 H, *m*), 3.92–4.04 (0.5H, *m*), 4.33 (0.25H, *d*, *J* = 2.7 Hz), 4.40–4.45 (0.25H, *m*), 4.49–4.52 (0.25H, *m*), 4.59 (0.25H, *d*, *J* = 2.9 Hz).

trans-*4-[[[2-Oxo-2-(phenylmethoxy)ethyl]amino]carbonyl]-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

Et_3N (530 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) was added to a solution of **11** (220 mg, 0.44 mmol) and ethyl chloroformate (57 mg, 0.53 mmol) in CH_2Cl_2 (2 mL) stirred at –10 °C. The mixture was stirred between –5 °C and +5 °C for 1 h before adding glycine benzyl ester hydrochloride (0.11 g, 0.64 mmol) and Et_3N (0.53 g, 13 mmol). The mixture was stirred at room temperature for 21 h, filtered and the solid washed with CH_2Cl_2. The combined organic phase was washed with 1 N HCl, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel using a mixture of Et_2O and EtOAc (9:1) as eluant to afford the title compound (140 mg, 47%). IR (film) 1798, 1738, 1715 cm^{-1}; ^1H NMR (CD_3OD) δ 1.50–2.00 (4H, *m*), 2.39 (3H, *s*), 3.17–3.27 (1H, *m*), 3.28–3.38 (1H, *m*), 3.57 (1H, *dd*, *J* = 18.1 Hz, *J'* = 4.8 Hz), 3.63–3.82 (1H, *m*), 4.13 (1H, *dd*, *J* = 18.1 Hz, *J'* = 6.6 Hz), 4.26 (1H, *d*, *J* = 3.2 Hz), 5.00–5.30 (6H, *m*), 7.18–7.43 (15H, *m*).

*4-[[(2-Hydroxy-2-oxoethyl)amino]carbonyl]-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (**14g**)*

A mixture of *trans*-4-[[[2-oxo-2-(phenylmethoxy)ethyl]-amino]carbonyl]-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (240 mg, 0.36 mmol), MeOH , EtOAc , 1 N HCl (0.4 mL) and a catalytic quantity of 10% Pd/C was stirred under H_2 for 1 h. The mixture was filtered through a pad of Celite and concentrated to afford **14g** (55 mg, 44%). IR (KBr) 1802, 1758, 1720, 1670 cm^{-1}; ^1H NMR (CD_3OD) δ 1.58–1.97 (4H, *m*), 2.35 (3H, *s*), 3.13–3.38 (3H, *m*), 3.85–4.08 (2H, *m*), 4.24 (1H, *d*, *J* = 3.3 Hz). High resolution FABMS m/z calcd for $C_{12}H_{20}N_5O_5$: 314.1469 (MH^+); found: 314.1460.

cis-*4-(2-Phenylethenyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)-guanidino]propyl]-1-(*tert*-butyldimethylsilyl)-2-azetidinone*

Et_3N (1.73 g 17 mmol) and *tert*-butyldimethylsilyl chloride (1.86 g, 12 mmol) were added to a stirred solution of **8** (5.60 g, 10.4 mmol) in DMF (30 mL). After 1 h the mixture was diluted with H_2O and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated, and the residue subjected to chromatography on a column of silica gel. Elution with a mixture of Et_2O and hexane (3:2) afforded the title compound (4.73 g, 78%). ^1H NMR ($CDCl_3$) δ 0.14 (3H, *s*), 0.23 (3H, *s*), 0.94 (9H, *s*), 1.51–1.84 (4H, *m*), 3.26–3.50 (3H, *m*), 4.11 (2H, *s*), 4.22 (1H, *dd*, *J* = 8.4 Hz, *J'* = 6.1 Hz), 5.08 (2H, *s*), 6.02 (1H, *dd*, *J* = 15.2 Hz, *J'* = 8.4 Hz), 6.57 (1H, *d*, *J* = 15.2 Hz), 7.21–7.39 (15H, *m*), 8.30 (1H, *bt*, *J* = 5.4 Hz). High resolution FABMS m/z calcd for $C_{37}H_{47}N_4O_5Si$: 655.3316 (MH^+); found: 655.3323.

cis-*4-Carboxy-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone*

Ozone was bubbled through a solution of *cis*-4-(2-phenylethenyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]-propyl]-1-(*tert*-butyldimethylsilyl)-2-azetidinone (4.73 g, 7.22 mmol) in CH_2Cl_2 (100 mL) maintained at –78 °C until a blue color persisted. Excess ozone was purged with N_2, Me_2S (10 mL) was added and the solution allowed to stand for 16 h. Concentration afforded *cis*-4-formyl-3-[3-[*N',N"*-bis(carbobenzyloxy)-guanidino]propyl]-1-(*tert*-butyldimethysilyl)-2-azetidinone which was used without further purification.

The aldehyde was dissolved in acetone (50 mL) and Jones reagent added dropwise until an orange color persisted. The reaction mixture was stirred for 15 min, concentrated and the residue partitioned between H_2O and EtOAc. The organic layer was separated, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel using a mixture of Et_2O, EtOAc and AcOH (4:1:0.05) as eluant to afford the title compound (1.80 g, 51%). ^1H NMR ($CDCl_3$) δ 1.80–1.50 (4H, *m*), 3.10–3.50 (3H, *m*), 4.19 (1H, *d*, *J* = 6.9 Hz), 5.07 (2H, *s*), 5.17 (2H, *s*), 6.70 (1H, *bs*), 7.10–7.45 (10H, *m*), 8.4 (1H, *bs*). High resolution FABMS m/z calcd for $C_{24}H_{27}N_4O_7$: 483.1880 (MH^+); found: 483.1878.

cis-4-(1-Piperidinylcarbonyl)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone

Carbonyldiimidazole (0.53 g, 3.24 mmol) was added to a solution of *cis*-4-carboxyl-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (1.30 g, 2.7 mmol) in THF (12 mL). The mixture was stirred for 35 min, piperidine (230 mg, 2.7 mmol) added, and the mixture stirred for an additional 1 h. The solvent was evaporated, the residue partitioned between EtOAc and 0.5 N HCl and extracted. The organic phase was dried over $MgSO_4$, concentrated and the residue chromatographed on a column of silica gel to afford the title compound (360 mg, 24%). ^1H NMR ($CDCl_3$) δ 1.40–1.90 (10H, *m*), 3.02–3.52 (7H, *m*), 4.33 (1H, *d*, *J* = 5.4 Hz), 5.03 (2H, *s*), 5.12 (2H, *s*), 7.18–7.38 (10H, *m*).

cis-4-(1-Piperidinylcarbonyl)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone

Sodium bis(trimethylsilyl)amide in THF (0.65 mL of a 1 N solution) was added to a stirred solution of *cis*-4-(1-piperidinylcarbonyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)-guanidino]propyl]-2-azetidinone (360 mg, 0.65 mmol) in THF (2 mL) maintained at –78 °C under an atmosphere of N_2. The mixture was stirred for 20 min, acetyl chloride (0.05 mL, 0.7 mmol) added, and the solution warmed to room temperature. After stirring for 1 h the mixture was diluted with a mixture of Et_2O and EtOAc and pH 4.0 aqueous buffer solution. The organic phase was separated, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel using EtOAc as eluant to afford the title compound (240 mg, 62%). IR (KBr) 1798, 1740, 1722 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34–1.86 (10H, *m*), 2.36 (3H, *s*), 3.12–3.58 (7H, *m*), 4.76 (1H, *d*, *J* = 6.1 Hz), 5.06 (2H, *s*), 5.13 (2H, *s*), 7.20–7.40 (10H, *m*). High resolution FABMS calcd for $C_{31}H_{38}N_5O_7$: 592.2711 (MH^+); found: 592.2769.

*cis-4-(1-Piperidinylcarbonyl)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (**14b**)*

A solution of *cis*-4-(1-piperidinylcarbonyl)-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (240 mg, 0.4 mmol) in MeOH (2 mL) and 1 N HCl (0.5 mL) was hydrogenated over 10% Pd/C for 15 min. The mixture was filtered through a pad of Celite and the filtrate concentrated to afford **14b** (124 mg, 86%). IR (film) 1798, 1715 cm^{-1}; ^1H NMR (CD_3OD) δ 1.50–1.92 (10H, *m*), 2.34 (3H, *s*), 3.17–3.83 (7H, *m*), 5.04 (1H, *d*, *J* = 6.7 Hz). High resolution FABMS calcd for $C_{15}H_{26}N_5O_3$: 324.2035 (MH^+); found: 324.2032.

*4-(Acetyloxy)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (**15**)*

$Pb(OAc)_4$ (71.50 g, 160 mmol) was added in small portions to a solution of **10** (26.10 g, 54 mmol) in CH_3CO_2H (105 mL) and DMF (15 mL) at room temperature. After completing the addition the suspension was stirred at 60 °C for 5 h and then concentrated. The residue was diluted with CH_2Cl_2, filtered through a pad of Celite and the filtrate concentrated. The residue was chromatographed on a column of silica gel using a mixture of Et_2O and hexane (3:2) as eluant to give **15** (15.5 g, 57%) as a 2:1 mixture of *trans* and *cis* isomers. ^1H NMR ($CDCl_3$) δ 2.05 (2H, *s*), 2.07 (1H, *s*), 3.08–3.15 (1H, *m*), 3.36–3.45 (2H, *m*), 5.07 (2H, *s*), 5.13 (2H, *s*), 5.46 (2/3H, *bs*), 5.78 (1/3H, *d*, *J* = 3 Hz), 7.18–7.37 (10H, *m*). High resolution FABMS m/z calcd for $C_{25}H_{29}N_4O_7$: 497.2036; found: 497.2031.

4-(Acetyloxy)-3-[3-[N',N"-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 1.36 mL, 1.36 mmol) was added dropwise to a stirred solution of **15** (580 mg, 1.16 mmol; *trans:cis* ratio, 2:1) in THF (20 mL) maintained at –78 °C under N_2. After 10 min acetyl chloride (0.96 mL, 1.36 mmol) was added and the mixture warmed to room temperature. After 30 min the solvent was evaporated, the residue was diluted with H_2O and extracted with Et_2O to leave an oil. Chromatography on a column of silica gel using a mixture of Et_2O and hexane (4:1) afforded the title compound (420 mg, 57%) as a 2:1 mixture of *trans* and *cis* isomers. IR (film) 1803, 1758, 1723 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57–1.90 (4H, *m*), 2.05 (1H, *s*), 2.08 (2H, *s*), 2.32 (1H, *s*), 2.36 (2H, *s*), 3.07–3.17 (1H, *m*), 3.40–3.52 (2H, *m*), 5.08 (2H, *s*), 5.14 (2H, *s*), 6.04 (2/3H, *d*, *J* = 1.5 Hz), 6.52 (1/3H, *d*, *J* = 5.4 Hz), 7.20–7.42 (10H, *m*). High resolution FABMS m/z calcd for $C_{11}H_{20}N_4O_4$: 271.1406 (MH^+); found: 271.1404.

*4-(Acetyloxy)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (**16a**)*

A mixture of 4-(acetyloxy)-3-[3-[*N',N"*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (420 mg, 0.78 mmol), MeOH (1.20 mL), EtOAc (5 mL), 1 N HCl (0.76 mL) and a catalytic quantity of 10% Pd/C was shaken in a Parr hydrogenator at 45 psi. After 2 h the mixture was filtered through a pad of Celite and concentrated to afford **16a** (170 mg, 71%) as a 2:1 mixture of *trans* and *cis* isomers. IR (film) 1805, 1760, 1720 cm^{-1}; ^1H NMR (CD_3OD) δ 1.63–1.95 (4H, *m*), 2.09 (2H, *s*), 2.11 (1H, *s*), 2.31 (1H, *s*), 2.33 (2H, *s*), 3.13–3.34 (3H, *m*), 6.11 (2/3H, *d*, *J* = 1.7 Hz), 6.55 (1/3H, *d*,

J = 5.0 Hz). High resolution FABMS m/z calcd for $C_{11}H_{19}N_4O_4$: 271.1406 (MH^+); found: 271.1404.

4-(Acetyloxy)-3-[3-[N',N''-bis(carbobenzyloxy)guanidino]propyl]-1-benzoyl-2-azetidinone

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 1.0 mL, 1.0 mmol) was added dropwise to a stirred solution of **15** (500 mg, 1 mmol) in THF (4 mL) maintained at –78 °C. After 15 min benzoyl chloride (140 mg, 1 mmol) was added, the mixture was stirred at –78 °C for 30 min at –78 °C and at room temperature for 90 min. The mixture was diluted with aqueous buffer (pH 4) and extracted with Et_2O. The residue was chromatographed on a column of silica gel using a mixture of Et_2O and hexane (2:1) as eluant to afford the title compound (200 mg, 33%) as a 3:1 mixture of *trans* and *cis* isomers. IR (film) 1866, 1749 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.63–1.93 (4H, *m*), 2.09 (0.75H, *s*), 2.11 (2.25H, *s*), 3.16–3.30 (1H, *m*), 3.35–3.56 (2H, *m*), 5.10 (2H, *s*), 5.15 (2H, *s*), 6.30 (0.75H, *d*, J = 1.9 Hz), 6.75 (0.25H, *d*, J = 5.1 Hz), 7.16–7.63 (15H, *m*). High resolution FABMS m/z calcd for $C_{32}H_{33}N_4O_8$: 601.2298 (MH^+); found: 601.2290.

*4-(Acetyloxy)-3-(3-guanidinopropyl)-1-benzoyl-2-azetidinone hydrochloride salt (**16b**)*

A mixture of 4-(acetyloxy)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-benzoyl-2-azetidinone (200 mg, 0.33 mmol), MeOH, EtOAc, 1 N HCl (0.33 mL) and a catalytic amount of 10% Pd/C was stirred under an atmosphere of H_2 for 30 min. The mixture was filtered through a pad of Celite and concentrated to afford **16b** (100 mg, 82%) as a 3:1 mixture of *trans* and *cis* isomers. IR (film) 1806, 1749 cm^{-1}; ^1H NMR (CD_3OD) δ 1.58–2.06 (4H, *m*), 2.12 (2.25H, *s*), 2.14 (0.75H, *s*), 3.10–3.44 (3H, *m*), 6.32 (0.75H, *d*, J = 1.9 Hz), 6.76 (0.25H, *d*, J = 5.2 Hz), 7.42–7.68 (5H, *m*). High resolution FABMS m/z calcd for $C_{16}H_{21}N_4O_4$: 333.1563 (MH^+); found: 333.1565.

4-(Acetyloxy)-3-[3-[N',N''-bis(carbobenzyloxy)guanidino]propyl]-1-(tert-butylcarbonyl)-2-azetidinone

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 1.20 mL, 1.2 mmol) was added dropwise to a stirred solution of **15** (600 mg, 1.2 mmol; 2:1 mixture of *trans*:*cis* isomers) in THF (6 mL) maintained at –78 °C under N_2. After 15 min, pivaloyl chloride (150 mg, 1.24 mmol) was added, the solution was stirred for 30 min at –78 °C and then at room temperature for 1.5 h. The mixture was diluted with aqueous buffer (pH 4.0), extracted with Et_2O and the residual oil subjected to chromatography on a column of silica gel. Elution with a mixture of Et_2O and hexane (1:1) afforded the title compound (300 mg, 43%) as a 3:2 mixture of *trans* and *cis* isomers. IR (film) 1802, 1734, 1703 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (3.6H, *s*), 1.28 (5.4H, *s*), 1.60–1.84 (4H, *m*), 2.04 (1.2H, *s*), 2.06 (1.8H, *s*), 2.97–3.03 (1H, *m*), 3.38–3.47 (2H, *m*), 5.08 (2H, *s*), 5.14 (2H, *s*), 6.07 (0.6H, *d*, J = 1.5 Hz), 6.53 (0.4H, *d*, J = 5.3 Hz), 7.20–7.41 (10H, *m*). High resolution FABMS m/z calcd for $C_{30}H_{37}N_4O_8$: 581.2611; found: 581.2603.

*1-(Acetyloxy)-3-(3-guanidinopropyl)-1-(tert-butylcarbonyl)-2-azetidinone hydrochloride salt (**16c**)*

A mixture of 4-(acetyloxy)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy) guanidino]propyl]-1-(*tert*-butylcarbonyl)-2-azetidinone (300 mg, 0.5 mmol) MeOH, EtOAc, 1 N HCl (0.52 mL) and a catalytic quantity of 10% Pd/C was stirred under an atmosphere of H_2 for 30 min. The mixture was filtered through Celite and concentrated to afford **16c** (0.10 g, 64%) as a 3:2 mixture of *trans* and *cis* isomers. IR (KBr) 1804, 1751, 1705 cm^{-1}; ^1H NMR (CD_3OD) δ 1.26 (3.6H, *s*), 1.27 (5.4H, *s*), 1.52–1.97 (4H, *m*), 2.08 (1.8H, *s*), 2.10 (1.2H, *s*), 3.10–3.35 (3H, *m*), 6.11 (0.6H, *d*, J = 1.6 Hz), 6.56 (0.4H, *d*, J = 5.1 Hz). High resolution FABMS m/z calcd for $C_{14}H_{25}N_4O_4$: 313.1876 (MH^+); found: 313.1872.

trans-*4-(Ethylthio)-3-[3-[N',N''-bis(carbobenzyloxy)-guanidino]propyl]-2-azetidinone (**17a**)*

EtSH (0.37 mL, 4.98 mmol) was added to a stirred suspension of NaH (200 mg of a 60% dispersion in mineral oil, 498 mmol) in THF (20 mL) cooled in an ice–water bath. After 10 min, the mixture was cooled to –20 °C and **15** (1.90 g, 3.83 mmol) added. The solution was warmed to room temperature, stirred for 30 min and concentrated. The residue was diluted with an aqueous buffer solution (pH 7.0) and extracted with EtOAc to leave an oil. Chromatography on a column of silica gel using Et_2O as eluant afforded **17a** (718 mg, 36%). IR (film) 1764, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (3H, *t*, J = 7.7 Hz), 1.60–1.85 (4H, *m*), 2.57 (2H, *q*, J = 7.7 Hz), 3.05–3.13 (1H, *m*), 3.40–3.50 (2H, *m*), 4.43 (1H, *d*, J = 1.5 Hz), 5.07 (2H, *s*), 5.14 (2H, *s*), 7.20–7.40 (10H, *m*). High resolution FABMS m/z calcd for $C_{25}H_{31}N_4O_5S$: 499.2015 (MH^+); found: 499.2010.

trans-*4-(Ethylthio)-3-[3-[N',N''-bis(carbobenzyloxy)-guanidino]propyl]-1-acetyl-2-azetidinone (**18a**)*

A solution of lithium bis(trimethylsilyl)amide (1 M in THF, 1.5 mmol) was added dropwise to a stirred solution of **17a** (718 mg, 1.26 mmol) in THF (10 mL) maintained at –78 °C. After 10 min acetyl chloride (0.10 mL, 1.5 mmol) was added and the solution warmed to room temperature. After 1 h the mixture was concentrated, the residue diluted with an aqueous buffer solution (pH 7.0) and extracted with EtOAc. The residual oil was chromatographed on a column of silica gel using a mixture of Et_2O and hexane (1:1) as eluant to afford the title compound (615 mg, 90%). ^1H NMR ($CDCl_3$) δ 1.65–1.90 (4H, *m*), 2.33 (3H, *s*), 2.84–3.07 (3H, *m*), 3.40–3.52 (2H, *m*), 4.75 (1H, *d*, J = 2.3 Hz), 5.08 (2H, *s*), 5.16 (2H, *s*), 7.22–7.42 (10H, *m*). High resolution FABMS m/z calcd for $C_{27}H_{33}N_4O_6S$: 541.2121 (MH^+); found: 541.2114.

trans-*4-(Ethylthio)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (19)*

A mixture of *trans*-4-(ethylthio)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (117 mg, 0.19 mmol), MeOH (1.5 mL), EtOAc (1.5 mL), 1 N HCl (0.19 mL) and a catalytic quantity of 10% Pd/C was stirred under H_2 until TLC indicated the disappearance of the starting material (about 1 h). The mixture was filtered through a pad of Celite and concentrated to afford **19** (40 mg, 68%) as a foam. IR (film) 1795, 1735, 1713 cm^{-1}; ^1H NMR (CD_3OD) δ 1.27 (3H, *t*, *J* = 7.4 Hz), 1.63–1.95 (4H, *m*), 2.32 (3H, *s*), 2.82–3.40 (5H, *m*), 4.93 (1H, *d*, *J* = 3.6 Hz). High resolution FABMS m/z calcd for $C_{11}H_{21}N_4O_2S$: 273.1385 (MH^+); found: 273.1391.

trans-*4-(Ethylsulfonyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

mCPBA (85% pure, 500 mg, 1.93 mmol) was added in one portion to a solution of **18a** (469 mg, 0.77 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 30 min, diluted with CH_2Cl_2 and saturated $NaHCO_3$ solution and the organic phase separated and dried over Na_2SO_4. The solvent was evaporated and the residue chromatographed on a column of silica gel to afford the title compound (386 mg, 88%). IR (KBr) 1780, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (3H, *t*, *J* = 8.4 Hz), 1.67–1.98 (4H, *m*), 2.40 (3H, *s*), 3.18–3.62 (4H, *m*), 3.70–3.80 (1H, *m*), 4.93 (1H, *d*, *J* = 2.3 Hz), 5.10 (2H, AB quartet), 5.16 (2H, *s*), 7.30–7.42 (10H, *m*). High resolution FABMS m/z calcd for $C_{11}H_{21}N_4O_4S$: 305.1284 (MH^+); found: 305.1276.

trans-*4-(Ethylsulfonyl)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (20a)*

A mixture of *trans*-4-(ethylsulfonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (380 mg, 0.66 mmol), MeOH (2.5 mL), EtOAc (2.5 mL), 1 N HCl (0.66 mL) and a catalytic amount of 10% Pd/C was stirred under H_2 until TLC indicated the disappearance of the starting material. The mixture was filtered through a pad of Celite and concentrated to afford **20a** (140 mg, 62%) as a yellow foam. IR (film) 1810, 1720 cm^{-1}; ^1H NMR (CD_3OD) δ 1.42 (3H, *t*, *J* = 7.4 Hz), 1.73–2.02 (4H, *m*), 2.39 (3H, *s*), 3.19–3.49 (4H, *m*), 3.70–3.76 (1H, *m*), 5.27 (1H, *d*, *J* = 3.1 Hz). High resolution FABMS m/z calcd for $C_{11}H_{21}N_4O_4S$: 305.1284 (MH^+); found: 305.1276.

trans-*4-(Phenylthio)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (17b)*

PhSH (0.54 g, 4.9 mmol) was added to a suspension of NaH (190 mg of a 60% dispersion in mineral oil, 4.90 mmol) in THF (20 mL). After H_2 evolution had ceased, **15** (1.22 g, 2.45 mmol) was added and the mixture stirred for 30 min. The solvent was removed, the residue diluted with H_2O and extracted with EtOAc to give an oil which was chromatographed on a column of silica gel. Elution with a mixture of Et_2O and hexane (3:1) afforded **17b** (450 mg, 34%). IR (film) 1770, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55–1.85 (4H, *m*), 2.96–3.07 (1H, *m*), 3.36–3.52 (2H, *m*), 4.64 (1H, *bs*), 5.10 (2H, *s*), 5.17 (2H, *s*), 6.47 (1H, *bs*), 7.20–7.48 (15H, *m*), 8.26–8.37 (1H, *s*).

trans-*4-(Phenylthio)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (18b)*

A solution of sodium bis(trimethylsilyl)amide (1 M in THF, 0.8 mL) was added dropwise to a stirred solution of **17b** (450 mg, 0.82 mmol) in THF (7 mL) maintained at –78 °C under N_2. After 10 min acetyl chloride (0.054 mL, 0.76 mmol) was added and the mixture was warmed to room temperature. After 30 min the solvent was evaporated, the residue diluted with H_2O and extracted with EtOAc to leave **18b** (450 mg, 93%) which was used directly. IR (film) 1800, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52–1.80 (4H, *m*), 2.32 (3H, *s*), 3.03 (1H, *dt*, *J* = 8.4 Hz, *J'* = 2.3 Hz), 3.38 (2H, *q*, *J* = 6.9 Hz), 4.82 (1H, *d*, *J* = 2.3 Hz), 5.08 (2H, *s*), 5.12 (2H, *s*), 7.20–7.53 (15H, *m*), 8.27 (1H, *bt*, *J* = 5.3 Hz). High resolution FABMS m/z calcd for $C_{31}H_{33}N_4O_6S$: 589.2121; found: 589.2111.

trans-*4-(Phenylsulfonyl)-3-[3-[*N',N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone*

mCPBA (327 mg, 1.52 mmol, 80% purity) was added to a stirred solution of **18b** (450 mg, 0.76 mmol) in CH_2Cl_2 (10 mL). After 20 min the mixture was concentrated, the residue diluted with saturated $NaHCO_3$ solution and extracted with EtOAc. The residual oil was chromatographed on a column of silica gel using a mixture of Et_2O and hexane (3:1) as eluant to give the title compound (210 mg, 44%). IR (KBr) 1815, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.65–1.95 (4H, *m*), 2.28 (3H, *s*), 3.40–3.55 (2H, *m*), 3.80–3.89 (1H, *m*), 4.90 (1H, *d*, *J* = 2.3 Hz), 5.08 (2H, *s*), 5.15 (2H, *s*), 7.20–7.92 (15H, *m*), 8.40–8.30 (1H, *bt*). High resolution FABMS m/z calcd for $C_{15}H_{21}N_4O_4S$: 353.1284; found: 353.1281.

trans-*4-(Phenylsulfonyl)-3-(3-guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (20b)*

A mixture of *trans*-4-(phenylsulfonyl)-3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (210 mg, 0.34 mmol), MeOH (12.5 mL), EtOAc (12.5 mL), 1 N HCl (0.34 mL) and a catalytic quantity of 10% Pd/C was stirred under H_2. After the disappearance of the starting material as determined by TLC, the mixture was filtered through a pad of Celite and concentrated to afford **20b** (100 mg, 76%) as a colorless foam. IR (KBr) 1815, 1730 cm^{-1}; ^1H NMR (CD_3OD) δ 1.70–2.01 (4H, *m*), 2.26 (3H, *s*), 3.22 (2H, *t*, *J* = 6.9 Hz), 3.76–3.83 (1H, *m*), 5.29 (1H, *d*, *J* = 2.9 Hz), 7.60–8.03 (5H, *m*). High resolution FABMS m/z calcd for $C_{15}H_{21}N_4O_4S$: 353.1284 (MH^+); found: 353.1281.

3-[3-[N',N''-bis(Carbobenzyloxy)guanidino]propyl]-2-azetidinone

A mixture of **17a** (1.80 g, 3.5 mmol), Raney-Nickel (Aldrich) and dioxane (100 mL) was stirred at 60 °C for 30 min. The mixture was filtered to remove the Raney-Nickel and the filtrate concentrated. The residue was chromatographed on a column of silica gel to afford the title product (830 mg, 45%). IR (film) 1755 (shoulder), 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57–1.80 (4H, *m*), 3.14–3.23 (1H, *m*), 3.33 (1H, *t*, *J* = 5.4 Hz), 3.35–3.47 (2H, *m*), 3.88–3.94 (1H, *m*), 5.05 (2H, *s*), 5.13 (2H, *s*), 7.17–7.38 (10H, *m*). High resolution FABMS m/z calcd for $C_{23}H_{27}N_4O_5$: 439.1981 (MH^+); found: 439.1976.

3-[3-[N',N''-bis(Carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone

A 1 N solution of lithium bis(trimethylsilyl)amide in THF (1.0 mL, 1 mmol) was added to a stirred solution of 3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (366 mg, 0.84 mmol) in THF (6 mL) maintained at –78 °C under an atmosphere of N_2. After 10 min acetyl chloride (0.071 mL, 1 mmol) was added, the solution was warmed to room temperature and stirred for 1 h. The mixture was diluted with pH 7.0 aqueous buffer and extracted with Et_2O. The organic phase was dried over Na_2SO_4, concentrated and the residue chromatographed on a column of silica gel. Elution with a mixture of Et_2O and hexane (9:1) afforded the title product (255 mg, 63%). IR (film) 1792, 1740, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.63–1.96 (4H, *m*), 2.32 (3/4H, *s*), 2.33 (9/4H, *s*), 3.15–3.30 (2H, *m*), 3.35–3.48 (2H, *m*), 3.60–3.70 (1H, *m*), 5.05 (0.5H, *s*), 5.07 (1.5H, *s*), 5.12 (0.5H, *s*), 5.14 (1.5H, *s*), 7.18–7.40 (10H, *m*). High resolution FABMS m/z calcd for $C_{25}H_{29}N_4O_6$: 481.2087 (MH^+); found: 481.2076.

3-(3-Guanidinopropyl)-1-acetyl-2-azetidinone hydrochloride salt (21a)

A solution of 3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-acetyl-2-azetidinone (255 mg, 0.58 mmol) in MeOH (2.5 mL), EtOAc (2.5 mL) and 1 N HCl (0.58 mL), was hydrogenated over 10% Pd/C until TLC indicated the disappearance of the starting material (about 10 min). The suspension was filtered through a pad of Celite and the filtrate concentrated to afford **21a** (99 mg, 69%) as a yellow foam. IR (film) 1790, 1700 (shoulder), 1670 cm^{-1}; ^1H NMR (CD_3OD) δ 1.65–1.87 (4H, *m*), 2.31 (3H, *s*), 3.16–3.40 (4H, *m*), 3.70 (1H, *t*, *J* = 6.7 Hz). High resolution FABMS m/z calcd for $C_8H_{17}N_4O_2$: 213.1352 (MH^+); found: 213.1349.

3-[3-[N',N''-bis(Carbobenzyloxy)guanidino]propyl]-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone

A 1 N solution of lithium bis(trimethylsilyl)amide (1.00 mL, 1 mmol) was added to a stirred solution of 3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-2-azetidinone (650 mg, 1.48 mmol) in THF (5 mL) maintained at –78 °C under an atmosphere of N_2. After 10 min dansyl chloride (399 mg, 1.48 mmol) was added and the solution warmed to room temperature. After stirring for 1 h the solvent was evaporated and the residue partitioned between EtOAc and pH 7.0 aqueous buffer. The organic phase was washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on a column of silica gel using a mixture of Et_2O and hexane (4:1) as eluant to afford the title compound (186 mg, 19%). IR (KBr) 1798, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47–1.70 (4H, *m*) 2.80 (6H, *s*), 3.16–3.34 (3H, *m*), 3.59–3.67 (1H, *m*), 5.05 (2H, *s*), 5.13 (2H, *s*), 7.20–7.35 (10H, *m*), 7.47–7.57 (2H, *m*), 8.28 (1H, *d*, *J* = 6.9 Hz), 8.33 (1H, *d*, *J* = 9.2 Hz), 8.56 (1H, *d*, *J* = 9.2 Hz). High resolution FABMS m/z calcd for $C_{37}H_{38}N_5O_7S$: 672.2492 (MH^+); found: 672.2479.

3-(3-Guanidinopropyl)-1-[5-(dimethylamino)-1-naphthalenesulfonyl)]-2-azetidinone hydrochloride salt (21b)

A solution of 3-[3-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]propyl]-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone (186 mg, 0.28 mmol) in MeOH (2.5 mL), EtOAc (2.5 mL) and 1 N HCl (0.28 mL) was hydrogenated over 10% Pd/C until TLC indicated the disappearance of the starting material. The suspension was filtered through a pad of Celite and the filtrate concentrated to leave **21b** (90 mg, 73%). IR (film) 1795 cm^{-1}; ^1H NMR (CD_3OD) δ 1.43–1.61 (4H, *m*), 2.93 (6H, *s*), 2.99–3.31 (3H, *m*), 3.64 (1H, *t*, *J* = 5.9 Hz), 7.41 (1H, *d*, *J* = 7.5 Hz), 7.57–7.68 (2H, *m*), 8.27 (1H, *d*, *J* = 7.4 Hz), 8.45 (1H, *d*, *J* = 7.6 Hz), 8.59 (1H, *d*, *J* = 7.7 Hz). High resolution FABMS m/z calcd for $C_{19}H_{26}N_5O_5S$: 404.1756 (MH^+); found: 404.1752.

6-[N',N''-bis(Carbobenzyloxy)guanidino]hexanoic acid

A mixture of *N,N'*-bis(carbobenzyloxy)-S-methyl-isothiourea (60.00 g, 0.18 mol), Et_3N (18.20 g, 0.18 mol) and 6-aminocaproic acid (23.30 g, 0.18 mol) in MeOH (350 mL) was stirred at reflux for 30 min and at room temperature for 4 h. The solution was concentrated, the residue diluted with H_2O and extracted with EtOAc. The organic phase was separated, dried over $MgSO_4$ and concentrated to leave a solid which was recrystallized from EtOAc and hexane to afford the title compound (52.30 g, 66%), mp 93 °C. IR (KBr) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30–1.45 (2H, *m*), 1.50–1.71 (4H, *m*), 2.33 (2H, *d*, *J* = 6.9 Hz), 3.34–3.47 (2H, *m*), 5.10 (2H, *s*), 5.17 (2H, *s*), 7.20–7.43 (10H, *m*). Anal. calcd for $C_{23}H_{27}N_3O_6$: C, 61.81; H, 5.89; N, 9.52; found: C, 61.39; H, 6.29; N, 9.65.

6-[N',N''-bis(Carbobenzyloxy)guanidino)hexanoic acid methyl ester

Carbonyldiimidazole (21.40 g, 0.132 mol) was added to solution of 6-[*N'*,*N''*-bis(carbobenzyloxy)guanidino]hexanoic acid (50.8 g, 0.12 mol) in THF (300 mL) and the mixture stirred at room temperature for 1 h. MeOH (75 mL) was added and the mixture stirred for 19 h before being concentrated. The residue was partitioned between H_2O and Et_2O, the organic phase separated and

dried over $MgSO_4$. The solvent was evaporated and the residue chromatographed on a column of silica gel using a mixture of Et_2O and hexane (1:1) as eluant to afford the title compound (22.80 g, 42%). IR (film): 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28–1.42 (2H, *m*), 1.50–1.68 (4H, *m*), 2.27 (2H, *t*, *J* = 6.9 Hz), 3.38 (2H, *q*, *J* = 6.1 Hz), 3.64 (3H, *s*), 5.12 (2H, *s*), 5.16 (2H, *s*), 7.20–7.42 (10H, *m*). Anal. calcd for $C_{24}H_{29}N_3O_6$: C, 63.28; H, 6.42; N, 9.23; found: C, 63.30; H, 6.51; N, 9.26.

trans-*4-(2-Phenylethenyl)-3-[4-[*N',N"*-bis(carbobenzyloxy)guanidino]butyl]-2-azetidinone*

n-BuLi in THF (60 mL of a 2.5 M solution, 0.15 mol) was added to a stirred solution of hexamethyldisilazane (24.15 g, 0.15 mol) in THF (15 mL) maintained at –40 °C under N_2. After 1 h the mixture was cooled to –78 °C and a solution of 6-[*N',N"*-bis(carbobenzyloxy)guanidino)hexanoic acid methyl ester (22.20 g, 0.05 mol) in THF (30 mL) was added. After stirring at –78 °C for 1 h the mixture was stirred at –30 °C for 1 h before being cooled to –78 °C. A solution of 1,1,1-trimethyl-*N*-(3-phenyl-2-propen-1-ylidene)silanamine (10.20 g, 0.05 mol) in THF (30 mL) was added, the mixture stirred at –30 °C for 30 min and then stood at –20 °C for 16 h. The mixture was concentrated to half of its original volume, diluted with Et_2O and sufficient 1 N HCl added to maintain the pH of the aqueous phase below 2.0. The organic phase was separated, washed with H_2O and brine, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel using a mixture of Et_2O and EtOAc (9:1) as eluant to afford the title compound (6.80 g, 36%). IR (KBr) 1760, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40–1.92 (6H, *m*), 2.95 (1H, *bt*, *J* = 6.6 Hz), 3.37–3.48 (2H, *q*, *J* = 6.6 Hz), 3.93 (1H, *dd*, *J* = 8.4 Hz, *J'* = 2.0 Hz), 5.10 (2H, *s*), 5.17 (2H, *s*), 6.18 (1H, *dd*, *J* = 15.3 Hz, *J'* = 8.4 Hz), 6.58 (1H, *d*, *J* = 15.3 Hz), 7.20–7.43 (15H, *m*). High resolution FABMS m/z calcd for $C_{32}H_{35}N_4O_5$: 555.2607 (MH^+); found: 555.2594.

trans-*4-(2-Phenylethenyl)-3-[4-[*N',N"*-bis(carbobenzyloxy)guanidino]butyl]-1-(*tert*-butyldimethylsilyl)-2-azetidinone*

Et_3N (2.05 g, 20 mmol) and *tert*-butyldimethylsilyl chloride (3.05 g, 20 mmol) were added to a stirred solution of *trans*-4-(2-phenylethenyl)-3-[4-[*N',N"*-bis-(carbobenzyloxy)guanidino]butyl]-2-azetidinone (6.80 g, 12.3 mmol) in DMF (25 mL) maintained at 0 °C. The mixture was stirred at room temperature for 1.5 h, diluted with pH 7.0 aqueous buffer and extracted with Et_2O. The organic extracts were dried over $MgSO_4$ and concentrated and the residue chromatographed on a column of silica gel to afford the title compound (8.40 g, 100%). IR (film) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.1 (3H, *s*), 0.2 (3H, *s*), 0.9 (9H, *s*), 1.35–1.85 (6H, *m*), 2.87–2.95 (1H, *m*), 3.37 (2H, *q*, *J* = 6.1 Hz), 3.77 (1H, *dd*, *J* = 8.4 Hz, *J'* = 2.0 Hz), 5.05 (2H, *s*), 5.1 (2H, *s*), 6.10 (1H, *dd*, *J* = 16.8 Hz, *J'* = 8.4 Hz), 6.52 (1H, *d*, *J* = 16.8 Hz), 7.15–7.40 (15H, *m*). High resolution FABMS m/z calcd for $C_{38}H_{49}N_4O_5Si$: 669.3472 (MH^+); found: 669.3466.

trans-*4-(Methoxycarbonyl)-3-[4-[*N',N"*-bis(carbobenzyloxy)guanidino]butyl]-2-azetidinone*

Ozone was bubbled through solution of *trans*-4-(2-phenylethenyl)-3-[4-[*N',N"*-bis(carbobenzyloxy)guanidino]butyl] -1-(*tert*-butyldimethylsilyl)-2-azetidinone (8.20 g, 12 mmol) in CH_2Cl_2 (100 mL) stirred at –78 °C. When the solution retained a blue color, Me_2S (10 mL) was added and the mixture warmed to room temperature. After standing for 16 h, the solvent was evaporated to leave *trans*-1-formyl-3-[4-[*N',N"*-bis(carbobenzyloxy)guanidino]butyl]-1-(*tert*-butyldimethylsilyl)-2-azetidinone which was used directly.

The aldehyde was dissolved in acetone (100 mL) and Jones reagent added dropwise with stirring until TLC indicated the disappearance of starting material. The mixture was stirred for 2 h, concentrated to one third of its original volume and diluted with H_2O. The mixture was extracted with EtOAc, the organic phase dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel using a mixture of EtOAc and AcOH (99:1) as eluant to afford a dark oil (0.60 g) which was dissolved in CH_2Cl_2. An ethereal solution of diazomethane was added dropwise until a yellow color persisted. The excess diazomethane was destroyed by adding AcOH, the solution concentrated and the residue chromatographed on a column of silica gel. Elution with Et_2O afforded the title compound (250 mg, 24% overall yield). ^1H NMR ($CDCl_3$) δ 1.37–1.89 (6H, *m*), 3.17 (1H, *bt*, *J* = 9.0 Hz), 3.38 (2H, *q*, *J* = 6.1 Hz), 5.07 (2H, *s*), 5.13 (2H, *s*), 7.20–7.38 (10H, *m*). High resolution FABMS m/z calcd for $C_{26}H_{31}N_4O_7$: 511.2193 (MH^+); found: 511.2182.

trans-*4-(Methoxycarbonyl)-3-[4-[*N',N"*-bis(carbobenzyloxy)guanidino]butyl]-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone*

Sodium bis(trimethylsilyl)amide in THF (0.55 mL of a 1 N solution) was added to a solution of *trans*-4-(methoxycarbonyl)-3-[4-[*N',N"*-bis(carbobenzyloxy)-guanidino]butyl]-2-azetidinone (250 mg, 0.49 mmol) in THF (3 mL) stirred at –78 °C under N_2. The mixture was stirred for 20 min and a solution of dansyl chloride (0.135 g, 0.5 mmol) in THF (1 mL) added. The mixture was warmed to room temperature and concentrated. The residue was partitioned between EtOAc and aqueous pH 7.0 buffer, the organic layer separated, dried over $MgSO_4$ and concentrated. The residue was subjected to chromatography on a column of silica gel to furnish the title compound (141 mg, 39%). ^1H NMR ($CDCl_3$) δ 1.36–1.81 (6H, *m*), 2.85 (6H, *s*), 3.22–3.34 (3H, *m*), 3.45 (3H, *s*), 4.21 (1H, *d*, *J* = 3.1 Hz), 5.09 (2H, *s*), 5.15 (2H, *s*), 7.17–7.37 (11H, *m*), 7.53 (1H, *t*, *J* = 7.5 Hz), 7.60 (1H, *t*, *J* = 7.6 Hz), 8.27 (1H, *d*, *J* = 6.2 Hz), 8.47 (1H, *d*, *J* = 8.7 Hz), 8.59 (1H, *d*, *J* = 8.5 Hz). FABMS m/z: 744 (MH^+).

trans-*4-(Methoxycarbonyl)-3-(4-guanidinobutyl)-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone hydrochloride salt (23)*

A solution of *trans*-4-(methoxycarbonyl)-3-[4-[*N',N"*-

bis(carbobenzyloxy)guanidino]butyl]-1-[5-(dimethylamino)-1-naphthalenesulfonyl]-2-azetidinone (130 mg, 0.18 mmol) in MeOH (3 mL), EtOAc (1 mL) and 1 N HCl was hydrogenated over 10% Pd/C for 16 h. The mixture was filtered through a pad of Celite and the filtrate concentrated to afford **23** (45 mg, 50%). IR (film) 1807, 1753 cm^{-1}; ^1H NMR (CD_3OD) δ 1.20–1.88 (6H, *m*), 3.03–3.33 (3H, *m*), 3.44 (6H, *s*), 3.49 (3H, *s*), 4.52 (1H, *s*), 7.89–7.94 (2H, *m*), 8.10 (1H, *d*, *J* = 6.9 Hz), 8.43 (1H, *d*, *J* = 7.1 Hz), 8.86 (1H, *d*, *J* = 8.2 Hz), 8.99 (1H, *d*, *J* = 8.5 Hz). High resolution FABMS m/z calcd for $C_{22}H_{30}N_5O_5S$: 467.1968 (MH^+); found: 476.1978.

Biological evaluation

Enzyme assays for the inhibition of thrombin. For evaluation of thrombin enzymatic activity, 3 mM D-Phe-Pip-Arg-*p*-nitroanilide (s-2238) dissolved in H_2O was used as the substrate for 3 U mL^{-1} of purified human α-thrombin, which was dissolved in a buffer. The assay buffer comprised 145 mM NaCl, 5 mM KCl, 30 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid, pH 7.4 and 1 mg mL^{-1} polyethylene glycol (PEG-8000). Test compounds were dissolved in H_2O, methanol or DMSO immediately prior to use.

270 μL of assay buffer was added to each well in a 96-well microtiter plate followed by human α-thrombin (10 μL of 3 U mL^{-1}) and 10 μL of the inhibitor. The samples were incubated at room temperature for a defined period of time (3 min for initial IC_{50} determinations). The enzymic reaction was initiated by adding 10 μL of 3 mM s-2238 substrate and the reaction allowed to proceed at room temperature. A kinetic microplate reader (Molecular Devices Corporation Vmax) was used to measure the change in optical density, measured at 405 nm, over time.

Enzyme assays for the inhibition of trypsin. 3 mM Z-Val-Gly-Arg-*p*NA (Chromzyme TRY) dissolved in H_2O was used as the substrate for 6 μg mL^{-1} of purified bovine pancreatic trypsin dissolved in an assay buffer. The trypsin assay buffer comprised 2 mM $CaCl_2$, 50 mM Tris/Cl pH 8.0. Test compounds were dissolved in H_2O, methanol or DMSO immediately prior to use.

270 μL of assay buffer was added to each well in a 96-well microtiter plate followed by bovine trypsin (10 μL of a 6 μg mL^{-1} solution) and the test compound (10 μL). The mixture was incubated at room temperature for 3 min before initiating the enzymic reaction by introducing 10 μL of a 3 mM solution of the substrate, Z-Val-Gly-Arg-*p*NA. A kinetic microplate reader (Molecular Devices Corporation Vmax) was used to measure the change in optical density at 405 nm over time.

Procedure for determining the concentration of drug required to double thrombin-induced clotting time of human plasma. Preparation of human citrated plasma: blood from human volunteers was drawn into vacutainer tubes containing one tenth final volume of 0.129 M (3.8%) buffered citrate (16 mg $Na_3citrate{\cdot}2H_2O$ and 2.1 mg citric acid per mL of H_2O). The blood was centrifuged at 3500 rpm (480 *g*) for 15 min at room temperature using a Sorvall RT 6000B centrifuge. The plasma was removed, pooled, and aliquoted into small tubes which were stored frozen for later use.

Clotting times were determined by pipetting 0.1 mL of Owren's buffer (125 mM NaCl, 28.4 mM sodium barbital, pH 7.35), pre-warmed to 37 °C, and 0.1 mL of human plasma into yellow sample cuvettes. 10 U mL^{-1} human thrombin in the thrombin buffer described above (10 mL) was placed in the reservoir assembly station of a Medical Laboratory Automation, Electra 700 Reservoir Assembly (MLA 700). The cuvettes were vortexed and then placed on the MLA 700 sample wheel. The coagulation timer (MLA 700) automatically dispenses 0.1 mL human thrombin into the sample in each cuvette. Detection of the fibrin clot was determined optically by the MLA 700.

Studies were performed to determine the concentration of drug which caused a doubling of the clotting time in human plasma. From standard curves of thrombin activity added to the sample versus the clotting time, the concentration of drug which caused a doubling of the thrombin clotting time corresponded to inhibition of approximately 1/2 of the added thrombin clotting activity.

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68. Many of the β-lactam derivatives prepared as part of this study were evaluated as potential antibacterial agents against a broad spectrum of representative organisms. None showed significant anti-bacterial properties.

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