# Lactam-Conformationally Restricted Analogs of $N^{\alpha}$-Arylsulfonyl Arginine Amide: Design, Synthesis and Inhibitory Activity toward Thrombin and Related Enzymes

Toru OKAYAMA, Sumie SEKI, Hajime ITO, Tomoko TAKESHIMA, Masaki HAGIWARA, and Tadanori MORIKAWA*

*Research Institute, Fuji Chemical Industries, Ltd., 530 Chokeiji, Takaoka, Toyama 933, Japan.*
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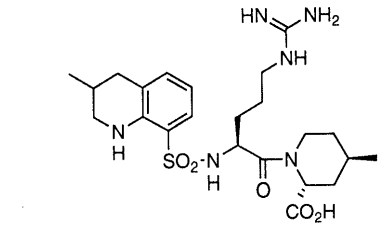
**Three new lactam-conformationally restricted arginine derivatives, 1-butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)-3-(3-guanidinopropyl)-substituted γ-, δ-, and ε-lactams (2—4), were synthesized on the basis of backbone modification of the lead structure, 6,7-dimethoxy-2-naphthylsulfonylarginine *n*-butylmethylamide (1). We tested these compounds for inhibitory activity toward thrombin and other trypsin-like enzymes (trypsin, factor Xa, plasmin, and kallikrein). All the compounds synthesized (1—4) potently inhibited thrombin with $IC_{50}$ values of 0.75, 0.70, 0.92, and 3.2 μM, respectively; they inhibited thrombin over 40-fold more effectively than the other enzymes tested. The γ-lactam (2) with the most profound inhibitory activity toward thrombin was a reversible inhibitor with a $K_i$ of 0.26 μM. Compound 2 also showed better thrombin selectivity than the lead compound (1). The lactam-conformational restriction of arylsulfonylarginine amides, especially γ-lactam, has thus proved to be a useful device for the improvement of antithrombotic activity.**

**Key words** thrombin inhibitor; lactam; arginine; $IC_{50}$; antithrombotic

Thrombin, a trypsin-like serine protease, plays a central role in hemostasis and thrombosis in that it cleaves fibrinogen to form fibrin and can activate factors V, VIII and XIII, and protein C, which are essential for the control of the blood coagulation system.[1] Thrombin also activates platelets and endothelial cells *via* a unique proteolytic cleavage reaction at a cell surface receptor.[2] Because of these actions, the proteolytic activity of thrombin plays a critical role in thrombosis; therefore, thrombin inhibitors are considered as promising antithrombotics.[3]

Many synthetic thrombin inhibitors such as tripeptides,[4] *p*-amidinophenylalanine derivatives[5] and *N*-arylsulfonylarginine amides,[6] have been reported. Among them, only MQPA (Chart 1),[7] an optimized *N*-arylsulfonylarginine amide,[6c] has been brought into clinical use for various thrombotic indications, but it is very short-acting and only suitable for parenteral administration. X-Ray crystallographic analysis has been used to determine the mode of binding of these inhibitors to trypsin[8] or thrombin,[9] and this has facilitated the understanding of structure–activity relationships and the design of new thrombin inhibitors.

In this paper, we report the design and synthesis of potent and selective thrombin inhibitors with new non-peptidic structures. We have designed a new basic structure that is expected to lead to the development of an advanced antithrombotic, with the aid of computer modeling. As the lead structure, we selected newly synthesized 6,7-dimethoxy-2-naphthylsulfonyl-L-arginine *n*-butylmethylamide (**1**), a member of the arylsulfonylarginine amide family. The arylsulfonylarginine amide inhibitors bind to thrombin in the following manner: they form antiparallel β hydrogen bonds with $Gly_{216}$,[10] the guanidino group interacts with the carboxylate group of $Asp_{189}$ in the thrombin specificity pocket, and the two terminal bulky hydrophobic inhibitor groups are a good sterical fit with the hydrophobic aryl binding site and the $S_2$-cavity, respectively, so that binding to the thrombin active site is tight. On the basis of MQPA–thrombin complex structure,[9a] we planned derivatives of **1** possessing lactam-conformationally restricted structure (**2**—**4**, Chart 2).[11] The newly introduced lactam methylenes




**MQPA**

Chart 1

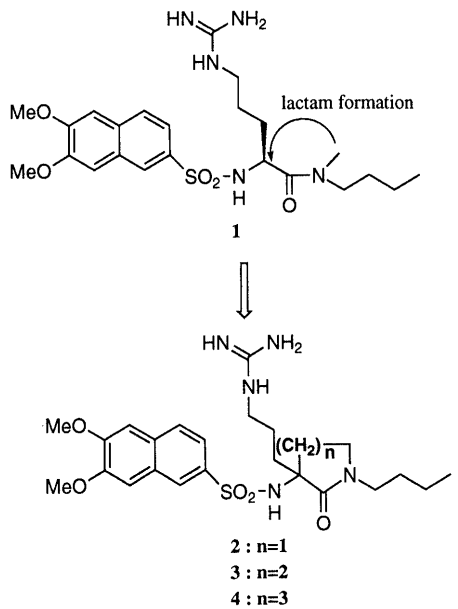



Chart 2. Design of Lactam-Conformationally Restricted Arginine Derivatives

* To whom correspondence should be addressed.

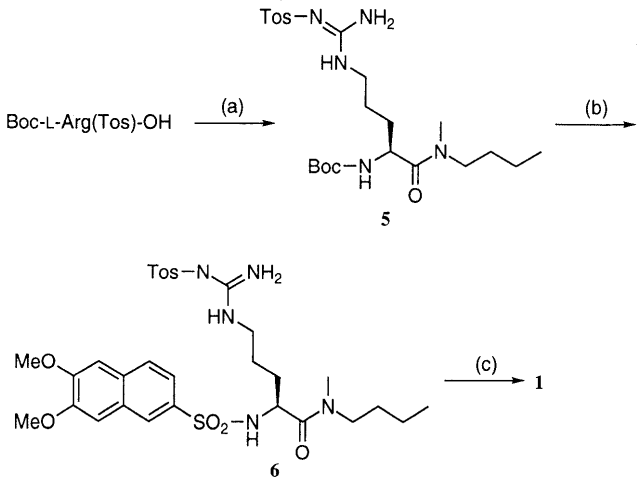
should not affect the affinity of the inhibitor–enzyme interaction, since they are located on the opposite side in the inhibitor molecule to the region interacting with the enzyme primary specificity pocket.

## Chemistry

The lead arginine compound (**1**) has been synthesized as the L-form since only the L-arginine isomer of aryl-sulfonylarginine amides potently inhibits thrombin.[12] On the other hand, the unnatural *p*-amidino phenylalanine derivatives, NAPAP and TAPAP, bind to thrombin in a quite similar manner to MQPA, but it has been reported that the D-isomer of NAPAP forms the complex with thrombin, while TAPAP requires the L-isomer.[9a] Therefore, we chose initially to perform racemic synthesis so that both lactam enantiomers would be available for testing.

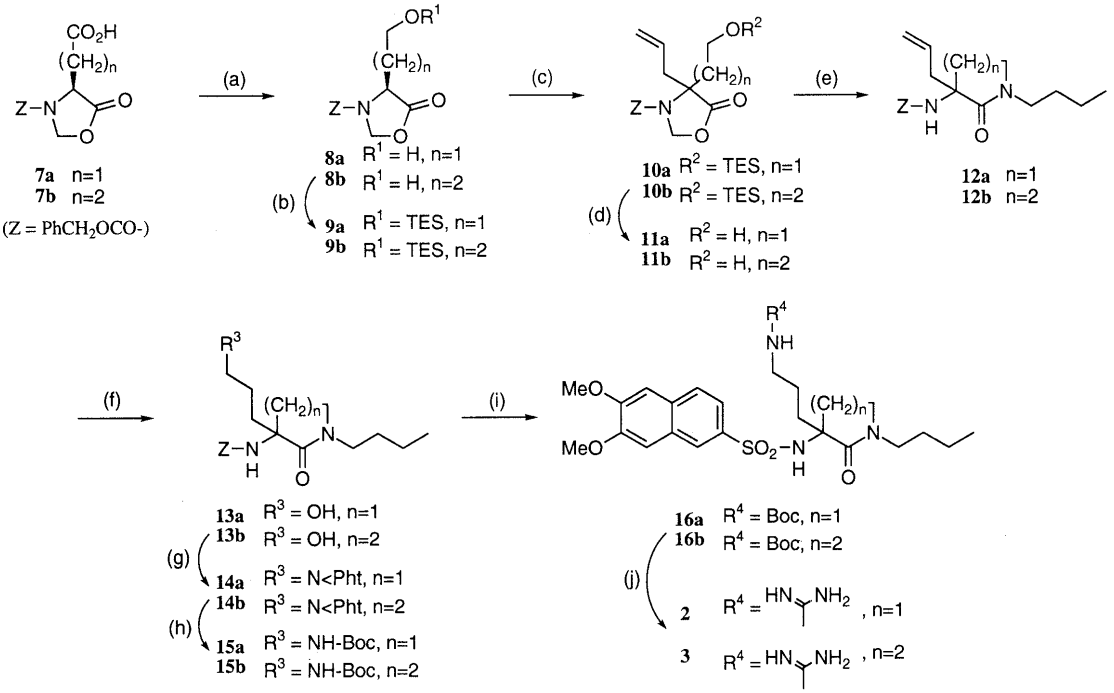
Chart 3 shows the synthetic scheme for the lead compound (**1**). Boc–L-Arg(Tos)–OH and *n*-butylmethylamine were first condensed with water-soluble carbodiimide hydrochloride (WSCI·HCl) in the presence of 1-hydroxybenzotriazole (HOBt) to give the amide (**5**). After removal of the Boc group of **5** by HCl/EtOAc, the α-amino group was sulfonylated with 6,7-dimethoxy-2-naphthylsulfonyl chloride in the presence of $Et_3N$ in $CH_2Cl_2$. Finally, the tosyl group protecting the guanidino function was removed by HF/anisole treatment to afford the desired lead compound (**1**).

The γ- and δ-lactams (**2** and **3**) were synthesized basically through a common synthetic route as outlined in Chart 4. The known oxazolidinones **7a** and **7b** were prepared from Z–Asp–OH or Z–Glu–OH, respectively,[13] in the presence of paraformaldehyde. After borane reduction of the carboxylic acid followed by protection of the resulting hydroxy group *via* the procedure of Zydowsky *et al.*,[14] **9a** or **9b** was converted to the corresponding potassium enolate and alkylated with allyl bromide to give a racemic α,α-dialkylated amino acid derivative, **10a** or **10b**. Removal of the triethylsilyl (TES) group, iodination, and subsequent treatment with excess *n*-butylamine converted **10a** or **10b** to the lactam **12a** or **12b**. Regio-specific hydroboration of **12a** or **12b** with 9-borabicyclo[3.3.1]nonane (9-BBN) and subsequent conversion of the resulting hydroxy group to an amino group by Mitsunobu reaction provided the phthalimide **14a** or **14b**. After conversion of the phthalimide protecting group to an alkali-stable Boc




(a) n-butylmethylamine, WSCI·HCl, HOBt/DMF; (b) 2,3-dimethoxynaphthalene-6-sulfonyl chloride, $Et_3N$/ DMF; (c) HF/anisole

Chart 3. Scheme for the Synthesis of the Lead Compound (**1**)

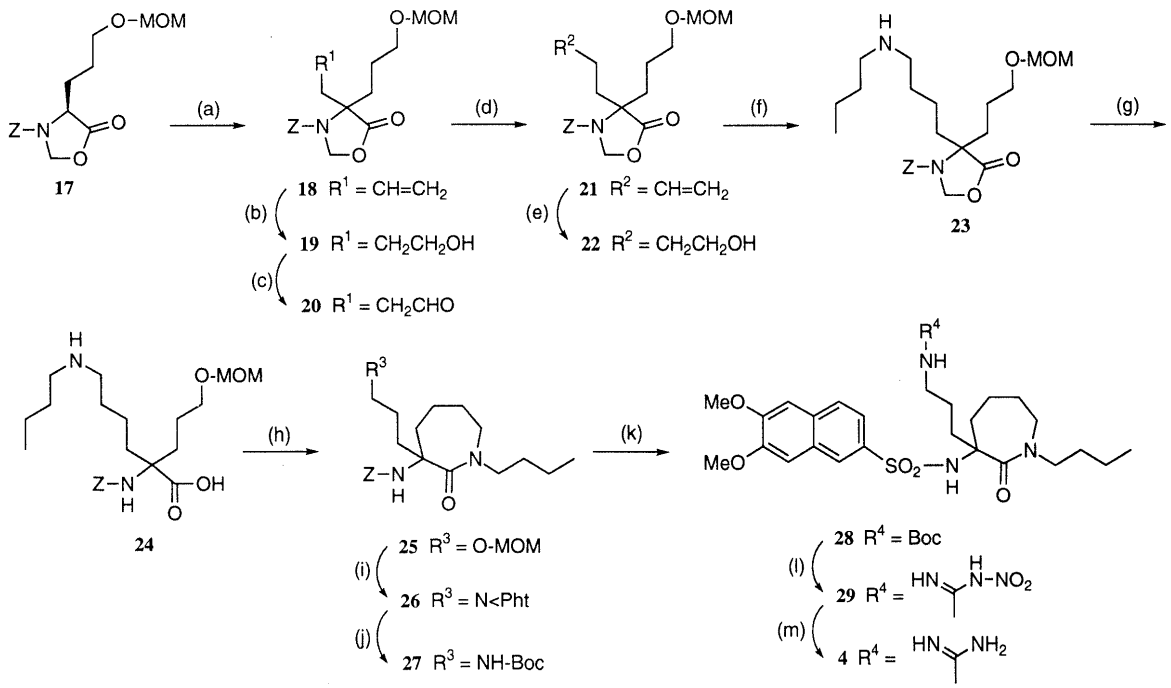



(a) $B_2H_6$-THF/THF; (b) TES-Cl, imidazole/DMF; (c) KHMDS, allyl bromide/THF; (d) AcOH/THF/$H_2O$; (e) $I_2$, $Ph_3P$, imidazole/benzene, then n-butylamine; (f) 9-BBN, then $H_2O_2$, NaOH; (g) phthalimide, $Ph_3P$, diethyl azodicarboxylate/THF; (h) hydrazine hydrate/EtOH, then $Boc_2O$/$CH_2Cl_2$-10% aqueous $Na_2CO_3$; (I)Pd-C, $H_2$/MeOH, then 2,3-dimethoxynaphthalene-6-sulfonyl chloride/$CH_2Cl_2$-10% aqueous $Na_2CO_3$; and (j) HCl/EtOH, then 3,5-dimethylpyrazole-1-carboxamidine nitrate, $Et_3N$/DMF

Chart 4

group, the Z group was removed by hydrogenation. The resulting free amino group on the lactam quaternary carbon was then sulfonylated to furnish the precursor **16a** or **16b**. Submission of **16a** or **16b** to deprotection of the Boc group by HCl/EtOAc treatment followed by guanidination with 3,5-dimethylpyrazole-1-carboxamidine nitrate[15] afforded the desired arginine derivative **2** or **3** as a colorless solid after purification by chromatography on a column of HP-20, a highly porous polymer-type synthetic adsorbent.

The ε-lactam **4** was synthesized as outlined in Chart 5. The common intermediate **8b** was treated with methoxymethyl chloride in the presence of diisopropylethylamine (DIEA) to give the methoxymethyl (MOM) ether (**17**). Alkylation and hydroboration converted **17** to the alcohol (**19**). Swern oxidation of the alcohol to give the aldehyde (**20**), and Wittig reaction of **20** furnished the C1-elongated olefin (**21**). Compound **21** was then treated with 9-BBN to afford **22**, a homologous alcohol of **19**. Iodination followed by treatment with excess *n*-butylamine isolated the secondary amine (**23**), but sequential conversion to the lactam ring was unsuccessful, in contrast with the γ- or δ-lactam synthesis. Our attempts to close **23** to the corresponding ε-lactam under the reported conditions (NaOAc, MeOH, reflux or isopropylamine, MeOH, reflux)[14] also failed. Further experimentation revealed that basic hydrolysis to give the amino acid (**24**) and subsequent lactam closure with WSCI and HOBt provided the desired ε-lactam (**25**) in a 90% yield. After removal of the MOM group, the sequence **26** to **28** was performed according to the procedure described for the synthesis of γ- and δ-lactam. In our preliminary experiment, the ε-lactam (**4**) was isolated as an amorphous solid and found to be difficult to purify with HP-20 on account of its greater hydrophobicity. In order to prepare a fair amount (> 100 mg) of **4**, we changed the sequence, beginning with deprotection of **28** to give the free amine, followed by treatment with 1-methyl-3-nitro-1-nitrosoguanidine,[16] and purification by silica gel column chromatography to give the pure $N^g$-nitro protected compound (**29**). Finally, removal of the nitro group by HF treatment furnished the desired ε-lactam containing an arginine derivative (**4**).




(a) KHMDS, allyl bromide/THF; (b) 9-BBN, then $H_2O_2$, NaOH; (c) PCC/$CH_2Cl_2$; (d) $MePPh_3Br$, LHMDS/DMSO; (e) 9-BBN, then $H_2O_2$, NaOH; (f) $I_2$, $Ph_3P$, imidazole/benzene, then n-butylamine; (g) NaOH/MeOH; (h) WSCI·HCl, $Et_3N$/DMF; (i) conc. HCl/MeOH, then phthalimide, $Ph_3P$, diethyl azodicarboxylate/THF; (j) hydrazine hydrate/EtOH, then $Boc_2O$/$CH_2Cl_2$-10% aqueous $Na_2CO_3$; (k)Pd-C, $H_2$/MeOH, then 2,3-dimethoxynaphthalene-6-sulfonyl chloride/$CH_2Cl_2$-10% aqueous $Na_2CO_3$; (l) HCl/EtOH, then 1-methyl-3-nitro-1-nitrosoguanidine, $Et_3N$/DMF; and (m) HF/anisole

Chart 5

Table 1. Comparison of *in Vitro* Enzyme-Inhibitory Activities of the Arginine Compound (**1**) and the Lactam Derivatives (**2**—**4**)

| Compound | $IC_{50}$ (μM)[a] | | | | |
|---|---|---|---|---|---|
| | Thrombin[b] | Trypsin[b] | Factor Xa[b] | Plasmin[b] | Kallikrein[b] |
| **1** | 0.75 | > 100 [15][c] | 68 [64][c] | > 100 [ 0][c] | 71 [61][c] |
| **2** | 0.70 ($K_i$ = 0.27 μM) | > 100 [46][c] | > 100 [15][c] | > 100 [28][c] | > 100 [39][c] |
| **3** | 0.92 | 42 [72][c] | > 100 [35][c] | > 100 [18][c] | 71 [58][c] |
| **4** | 3.2 | > 100 [32][c] | > 100 [29][c] | > 100 [ 2][c] | > 100 [ 3][c] |

*a*) $IC_{50}$: concentration required to inhibit the hydrolysis rate of the indicated substrate by 50% was determined by measuring the rate in the presence of various concentrations of test compound. *b*) Human α-thrombin, bovine trypsin, bovine factor Xa, bovine plasmin, and porcine plasma kallikrein were used. *c*) [Inhibition (%) at 100 μM].

**Inhibitory Activity toward Thrombin and Related Enzymes** The inhibitory activities of the synthesized compounds, **1**—**4**, toward thrombin and other trypsin-like serine proteases, trypsin, factor Xa, plasmin, and plasma kallikrein, were measured using synthetic peptide-MCA substrates,[17] and the results are shown in Table 1.

All the compounds synthesized in this paper potently inhibited thrombin and showed a high selectivity for thrombin, more than 40-fold greater than that for the other enzymes tested. The lead L-arginine compound (**1**) and the $\gamma$-lactam compound (**2**) showed the strongest inhibition with $IC_{50}$'s of 0.75 and 0.70 $\mu$M, respectively. The $\delta$-lactam compound (**3**) also exhibited potent thrombin-inhibitory activity with an $IC_{50}$ of 0.9 $\mu$M, followed by 3.2 $\mu$M for $\varepsilon$-lactam (**4**). Thus, among the three lactam-containing derivatives, the inhibitory potency varied with the size of the lactam ring; the smaller the ring, the stronger the thrombin inhibition. The $\gamma$-lactam derivative (**2**), which proved to be the most potent among the four compounds synthesized here, was subjected to measurement of the inhibition constant ($K_i$) for thrombin with respect to the synthetic substrate Boc–Val–Pro–Arg–MCA; it showed competitive inhibition with a $K_i$ value of 0.27 $\mu$M.

Thus, lactam formation elevated the potency of trypsin inhibition and reduced that of factor Xa inhibition, compared with the original compound (**1**). With plasmin, none of the four compounds synthesized exhibited any noticeable inhibitory activity even at a concentration of 100 $\mu$M. The $\gamma$- and $\varepsilon$-lactam restriction reduced the kallikrein inhibition whereas the $\delta$-lactam retained profound inhibitory potency, compared to **1**.

Table 2. Reaction System for Enzyme Activity Measurement

| Enzyme[a] (U/ml) | Substrate[b] ($\mu$M) | Buffer[c] |
|---|---|---|
| Thrombin 0.05 | Boc Val–Pro–Arg MCA 50 (100, 70, 50, 33, 25, and 20 for $K_i$) | A |
| Trypsin 0.5 | Boc–Ile Glu–Gly–Arg MCA 50 | A |
| Factor Xa 0.04 | Boc–Ile–Glu Gly–Arg MCA 200 | A |
| Plasmin 0.01 | Boc–Val–Ler–Lys–MCA 100 | B |
| Kallikrein 0.1 | Boc–Phe–Arg–MCA 50 | A |

*a*) Human $\alpha$-thrombin (Green Cross, 500 units/vial), bovine trypsin (Sigma, 12000 units/mg), bovine factor Xa (Sigma, 0.4 units/vial), bovine plasmin (Sigma, 0.15 units/mg), and porcine plasma kallikrein (Sigma, 33 units/mg) were used. *b*) Peptide-MCA substrates were purchased from Peptide Institute (Osaka, Japan). *c*) Buffer systems used were as follows: A, 50 mM Tris–HCl (pH 8.0), 100 mM NaCl and 10 mM $CaCl_2$; B, 50 mM Tris–HCl (pH 7.4) and 150 mM NaCl.

## Discussion

The mode of interaction of trypsin-like serine proteases with their inhibitors has been studied in detail and is well understood.[18] Thrombin inhibitors are among the most extensively studied targets on account of their expected therapeutic potential in thrombosis.

In order to acquire a basic structure for a potent and selective thrombin inhibitor, we designed and synthesized several new lactam-conformationally constrained structures (**2**—**4**) derived from the known crystal structure of the active site-directed thrombin inhibitor MQPA–thrombin complex, using computer modeling. Since a docking study[19] showed good binding of each lactam to thrombin and the $\gamma$-lactam derivative (**2**) exhibited reversible inhibition ($K_i = 0.27$ $\mu$M), as MQPA ($K_i = 0.02$ $\mu$M) did, we believe the binding mode is the same as that in the MQPA–thrombin complex, *i.e.*, the "inhibitor-binding mode".[9b] The energy-minimized conformation of **2** docked onto the thrombin active site can be superimposed well on the X-ray structure of MQPA bound to human thrombin (Fig. 1).

Among the three lactam derivatives, the $\gamma$-lactam derivative (**2**) proved to be the best inhibitor for thrombin. The $IC_{50}$ value of **2** to thrombin is essentially the same as that of the original L-arginine amide compound. It has been established from X-ray analyses that only one stereo isomer of arginine- or *p*-amidinophenylalanine-derived inhibitors possesses a potent binding ability to proteases such as trypsin or thrombin. Since the lactam derivatives

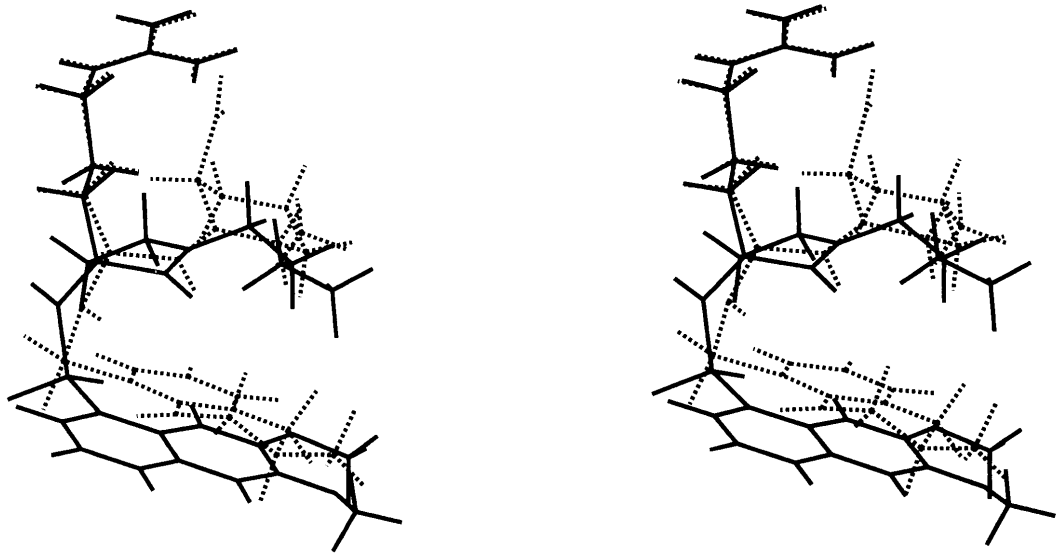


Fig. 1. Overlay of the Minimized Conformation of **2** (Unbroken Lines) onto MQPA (Dotted Lines)

(**2**—**4**) described here were prepared in racemic form, the optically pure isomer of **2** is expected to inhibit thrombin with an $IC_{50}$ value about 2-fold smaller than **1**. The thrombin-inhibitory activity of lactams (**2**—**4**) was noted to depend on the size of the lactam ring. Thus, with the addition of methylenes into the lactam ring, the thrombin inhibition was reduced, although good binding of each derivative to thrombin was anticipated from the computer modeling. The apparent discrepancy should be due to differences in the hydrophobicity of the compounds, because an increase in hydrophobicity with lactam ring enlargement was observed through both reverse-phase HPLC and the solubility in water. The methylenes in the lactam derivatives (**2**—**4**), accounting for the increase in hydrophobicity of the molecule, will be exposed upon formation of the complex with thrombin, eventually affecting the stability of the complex.

The lactam formation resulted in reduction of the factor Xa-inhibitory activity, compared with **1**, in contrast to the retained thrombin-inhibitory activity. It is known that 3-amino-substituted lactam derivatives have the most stable conformations with $\psi$ values confined within the range from 130° to 170°.[19] Therefore, our results suggest that conformational restriction of the lactam is consistent with the inhibitor–thrombin binding through hydrogen bonding at $Gly^{216}$, whilst factor Xa requires a different $\psi$ torsion angle in order to form the complex.

The $\gamma$- and $\delta$-lactams (**2** and **3**) showed satisfactory thrombin-inhibitory activity, relative to the original arginine compound (**1**), but only a slight difference was observed between them in the selectivity for thrombin. The $\gamma$-lactam (**2**) had better thrombin selectivity than **1**, because **2** inhibited other enzymes with $IC_{50}$ values of $>100\,\mu M$. On the other hand, the $\delta$-lactam (**3**) inhibited trypsin and kallikrein with $IC_{50}$'s of 42 and 71 $\mu M$, respectively. Thus, the $\gamma$-lactam (**2**) proved to be the most thrombin-specific inhibitor among the four compounds tested here.

## Conclusion

In this paper, we have described the design, synthesis, and inhibitory activity toward thrombin and related enzymes of three lactam-conformationally restricted arginine inhibitors derived from 6,7-dimethoxy-2-naphthylsulfonylarginine *n*-butylmethyl amide (**1**). Of the three derivatives, the $\gamma$-lactam derivative (**2**) exhibited potent and highly selective thrombin-inhibitory activity, being superior to the lead compound (**1**). Lactam-conformational restriction has thus proven to be an efficient method for the development of a new thrombin inhibitor. We chose the $\gamma$-lactam (**2**) as the most promising lead compound for further structure–activity relationship studies, which are in progress.

### Experimental

Melting points were determined on a Yanagimoto melting point apparatus without correction. Column chromatography was performed on Silica gel BW-200 (Fuji Silisia Chemical Ltd.) and on Diaion HP-20 (Mitsubishi Chemical Industries). The purity of all new compounds was monitored by analytical TLC on Merck Silica gel plates 60 $F_{254}$. $^1H$- and $^{13}C$-NMR spectra were recorded on a JEOL FX90A spectrometer, and chemical shifts are given in ppm ($\delta$) from tetramethylsilane (TMS), which was used as the internal standard. Mass spectra were obtained using a JEOL JMS-HX110 or a JEOL JMS-DX303 spectrometer. IR spectra were recorded on a JASCO IR-810 spectrometer. Optical rotations were measured in a JASCO DIP-140 apparatus.

**Molecular Modeling and Docking Study** The energy-minimizations were performed using the CHARMm program[20] and the utilities of QUANTA in the Molecular Simulation Incorporation Software. The coordinates of $\alpha$-thrombin were taken from the atomic coordinates of MQPA–$\alpha$-thrombin[9b] registered in the Protein Data Bank (Brookhaven National Laboratory, Upton, N. Y.) under the identification code 1DWC.

The basic three-dimensional structures of the inhibitors (**1**—**4**) were constructed using standard bond angles and lengths, and were manually superposed on the thrombin-bound MQPA structure. The resultant conformations were respectively docked into the active site model of thrombin, which was cut off within 17 Å from the inhibitors. The minimization was performed by 500 steps of adopted basis Newton–Raphson method without moving all the atoms of thrombin (15.0 Å-cut off; distance-dependent dielectric constant ($\varepsilon = 4_{rij}$)). The calculations were performed on an IRIS computer (Silicon Graphics Incorporation).

***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-*N*$^{\omega}$-tosyl-L-arginine *n*-Butylmethylamide (5)** 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.25 g, 6.50 mmol) was added to a solution of *N*$^{\alpha}$-(*tert*-butoxycarbonyl)-*N*$^{\omega}$-tosyl-L-arginine (2.14 g, 5.00 mmol), *n*-butylmethylamine (0.89 ml, 7.5 mmol) and 1-hydroxybenzotriazole (811 mg, 6.00 mmol) in *N*,*N*-dimethylformamide (DMF) (20 ml) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and further stirred at 5 °C for 14 h. The DMF solution was concentrated *in vacuo*, and the residue was partitioned between AcOEt and 1 N HCl. The organic layer was washed (saturated aqueous $NaHCO_3$, brine) and dried ($MgSO_4$). The EtOAc solution was concentrated to give a crude oil, which was chromatographed on a silica gel column using $CHCl_3$–MeOH (40 : 1) as the eluting solvent. The product was isolated as a viscous oil (2.08 g, 83.5%), $[\alpha]_D^{23}$ −5.1° ($c$ = 1.0, EtOH). $^1H$-NMR ($CDCl_3$, TMS) $\delta$: 7.72 (d, $J$ = 7.8 Hz, 2H), 7.20 (d, $J$ = 7.8 Hz, 2H), 6.56 (br s, 2H), 5.70 (m, 1H), 4.48 (m, 1H), 3.24 (m, 4H), 2.92 (d, $J$ = 6.0 Hz, 3H), 2.39 (s, 3H), 1.70-1.05 (m, 8H), 1.40 (s, 9H), 0.90 (t, $J$ = 6.1 Hz, 3H). IR (KBr): 3430, 3330, 1720, 1640, 1555, 1260, 1180 $cm^{-1}$. MS (FAB) $m/z$: 498 $(M+H)^+$.

***N*$^{\alpha}$-(6,7-Dimethoxy-2-naphthylsulfonyl)-*N*$^{\omega}$-tosyl-L-arginine *n*-Butylmethylamide (6)** Compound **5** (710 mg, 1.43 mmol) was dissolved in 4 N HCl/EtOAc (20 ml) at room temperature for 1 h and the solution was concentrated *in vacuo*. The residue was solidified with ether, collected by filtration, and dried in a vacuum desiccator with NaOH. This product was dissolved in $CH_2Cl_2$ (15 ml), and to this solution, 6,7-dimethoxy-2-naphthylsulfonyl chloride (460 mg, 1.72 mmol) and $Et_3N$ (0.49 ml, 3.5 mmol) were added at 4 °C. The mixture was stirred at 4 °C for 1.5 h. The $CH_2Cl_2$ solution was concentrated *in vacuo*, and the residue was partitioned between AcOEt and 1 N HCl. The organic layer was washed (brine) and dried ($MgSO_4$), then concentrated *in vacuo*. The residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (35 : 1) as the eluting solvent. After removal of the solvent, the residue was solidified from hexane to afford amorphous solid (900 g, 100%), $[\alpha]_D^{23}$ +32.9° ($c$ = 1.0, EtOH). $^1H$-NMR ($CDCl_3$, TMS) $\delta$: 8.23 (s, 1H), 7.76 (d, $J$ = 10.5 Hz, 1H), 7.73 (d, $J$ = 7.0 Hz, 2H), 7.68 (s, 1H), 7.23 (s, 1H), 7.18 (d, $J$ = 7.0 Hz, 2H), 7.14 (d, $J$ = 10.5 Hz, 1H), 6.48 (m, 3H), 4.20 (m, 1H), 4.00 (s, 3H), 3.95 (s, 3H), 3.28 (m, 2H), 2.93 (m, 2H), 2.63 (s, 3H), 2.38 (s, 3H), 1.62 (m, 4H), 1.20—0.70 (m, 4H), 0.63 (t, $J$ = 5.5 Hz, 3H). IR (KBr): 3440, 3350, 1640, 1560, 1270, 1260, 1160 $cm^{-1}$. MS(FAB) $m/z$: 648 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{39}N_5O_5S$: C, 55.62; H, 6.38; N, 10.81. Found: C, 55.85; H, 6.30; N, 10.55.

***N*$^{\alpha}$-(6,7-Dimethoxy-2-naphthylsulfonyl)-L-arginine *n*-Butylmethylamide Hydrochloride (1)** Compound **5** (840 mg, 1.30 mmol) was dissolved in anhydrous HF (9 ml) and anisole (1 ml) at 0 °C. After 30 min, HF was evaporated *in vacuo* below 5 °C. The residue was partitioned between $CHCl_3$ and 0.1 N NaOH. The $CHCl_3$ phase was dried ($MgSO_4$), 3 N HCl/EtOAc (1 ml) was added at 4 °C, and the solution was concentrated *in vacuo*. The residual colorless oil was recrystallized from ether to afford a colorless powder (510 mg, 74.3%), mp 150—151 °C (dec.), $[\alpha]_D^{23}$ +22.0° ($c$ = 1.02, MeOH). $^1H$-NMR (DMSO-$d_6$, TMS) $\delta$: 8.23 (s, 1H), 7.95 (s, 1H), 7.87 (d, $J$ = 10.8 Hz, 1H), 7.63 (br s, 1H), 7.46 (d, $J$ = 10.8 Hz, 2H), 7.30 (m, 4H), 4.12 (m, 1H), 3.91 (s, 6H), 3.14 (m, 4H), 2.82 (s, 3H), 1.51 (m, 4H), 1.30—0.70 (m, 4H), 0.65 (t, $J$ = 5.3 Hz, 3H). IR (KBr): 3410, 3320, 1670, 1660, 1635, 1520, 1260, 1160 $cm^{-1}$. MS (FAB) $m/z$: 694 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{36}ClN_5O_5S$: C, 52.15; H, 6.86; N, 13.23. Found: C, 51.90; H, 7.01; N, 12.95.

**(4*S*)-3-(Benzyloxycarbonyl)-4-(2-hydroxyethyl)-1,3-oxazolidin-5-one (8a)** A solution of the acid **7a** (14.0 g, 50 mmol) in dry tetrahydrofuran (THF) (20 ml) under $N_2$ was cooled to $-15$ °C and treated dropwise with a 1 M $BH_3$·THF solution in THF (95 ml) over a 20-min period. Then the solution was stirred at 4 °C for 20 h. The reaction mixture was poured into $H_2O$ and extracted with $Et_2O$. The organic extract was washed (1 M $KHSO_4$, saturated aqueous $NaHCO_3$, brine), dried ($MgSO_4$), and concentrated to give a crude oil, which was chromatographed on a silica gel column using benzene–hexane (1 : 2) as the eluting solvent. The product was isolated as a colorless oil (8.74 g, 65.9%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.36 (s, 5H), 5.18 (s, 2H), 5.08—4.76 (m, 2H), 4.76—3.97 (m, 3H), 3.44 (m, 1H), 2.50 (m, 2H). IR (neat): 3440, 1780, 1710 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{13}H_{15}NO_5$: 265.0950. Found: 265.0938.

**(4*S*)-3-(Benzyloxycarbonyl)-4-(3-hydroxypropyl)-1,3-oxazolidin-5-one (8b)**[21] The acid **7b** (15.5 g, 53.0 mmol) and 1 M $BH_3$·THF solution in THF (56 ml) were reacted according to the procedure described for the preparation of **8a** to give a colorless oil (12.4 g, 83.8%) after purification by silica gel column chromatography using EtOAc–benzene (1 : 2) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$ 7.35 (s, 5H), 5.50 (d, $J$=4.6 Hz, 1H), 5.20 (d, $J$=4.6 Hz, 1H), 4.34 (t, $J$=5.6 Hz, 2H), 3.59 (t, $J$=6.2 Hz, 2H), 2.65 (br s, 1H), 2.38—1.30 (m, 4H). IR (neat): 3445, 1780, 1710 cm$^{-1}$.

**(4*S*)-3-(Benzyloxycarbonyl)-4-(2-triethylsiloxyethyl)-1,3-oxazolidin-5-one (9a)** Imidazole (3.17 g, 46.5 mmol) and triethylsilylchloride (TES-Cl) (3.75 ml, 22.3 mmol) were added to a solution of **8a** (4.94 g, 18.6 mmol) in DMF (20 ml). The mixture was stirred for 2 h with ice-bath cooling and then poured into $Et_2O$ (50 ml) and $H_2O$ (300 ml). The aqueous layer was extracted with $Et_2O$ (2 × 50 ml), and the combined $Et_2O$ phases were washed (brine), dried ($MgSO_4$), and concentrated to give a pale yellow oil, which was chromatographed on a silica gel column using EtOAc–hexane (1 : 5) as the eluting solvent. The product was isolated as a colorless oil (6.31 g, 89.4%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$ 7.34 (s, 5H), 5.17 (br s, 2H), 5.01 (d, $J$=8.4 Hz, 1H), 4.89 (d, $J$=8.4 Hz, 1H), 4.38 (t, $J$=9.5 Hz, 2H), 4.20 (t, $J$=7.2 Hz,1H), 2.66 (dt, $J$=7.2, 9.5 Hz, 2H), 0.86 (t, $J$=7.7 Hz, 9H), 0.60 (q, $J$=7.7 Hz, 6H): IR (neat): 1790, 1720, 1080 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{19}H_{29}NO_5Si$: 379.1815. Found: 379.1821.

**(4*S*)-3-(Benzyloxycarbonyl)-4-(3-triethylsiloxypropyl)-1,3-oxazolidin-5-one (9b)** The alcohol **8b** (2.00 g, 7.16 mmol), imidazole (1.22 g, 17.9 mmol) and TES-Cl (1.44 ml, 8.59 mmol) were reacted according to the procedure described for the preparation of **9a** to give a colorless oil (1.93 g, 68.4%) after purification by silica gel column chromatography using EtOAc–hexane (1 : 5) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.37 (s, 5H), 5.54 (d, $J$=5.1 Hz, 1H), 5.22 (d, $J$=5.1 Hz, 1H), 5.20 (s, 2H), 4.36 (t, $J$=5.9 Hz, 1H), 3.61 (t, $J$=6.1 Hz, 2H), 2.20—1.14 (m, 4H), 0.95 (m, 9H), 0.56 (m, 6H). IR (neat): 1780, 1720, 1080 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{20}H_{31}NO_5Si$: 393.1972. Found: 393.1978.

**3-(Benzyloxycarbonyl)-4-(2-propenyl)-4-(2-triethylsiloxyethyl)-1,3-oxazolidin-5-one (10a)** A solution of **9a** (6.30 g, 16.6 mmol) in dry THF (60 ml) under $N_2$ was cooled to $-78$ °C, and a 0.5 M potassium bis(trimethylsilyl)amide solution in toluene (34 ml) was added dropwise over a 26-min period. After 3 min, allyl bromide (2.85 ml, 33.2 mmol) was added. The solution was stirred at $-78$ °C for 20 min, then allowed to warm to room temperature, and partitioned between $Et_2O$ and 10% citric acid. The organic phase was washed (saturated aqueous $NaHCO_3$, brine), dried ($MgSO_4$), and concentrated to give a yellow oil, which was chromatographed on a silica gel column using EtOAc–hexane (1 : 8) as the eluting solvent. The product was isolated as a colorless oil (4.81 g, 69.0%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.34 (s, 5H), 5.72 (m, 1H), 5.53—4.95 (m, 3H), 5.15 (s, 2H), 4.79 (d, $J$=10.8 Hz, 1H), 4.20 (m, 2H), 2.73 (d, $J$=6.9 Hz, 2H), 2.40 (m, 2H), 0.89 (t, $J$=6.8 Hz, 9H), 0.53 (q, $J$=6.8 Hz, 6H). IR (neat): 1785, 1715, 1065 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{22}H_{33}NO_5Si$: 419.2128. Found: 419.2126.

**3-(Benzyloxycarbonyl)-4-(2-propenyl)-4-(3-triethylsiloxypropyl)-1,3-oxazolidin-5-one (10b)** Compound **9b** (39.8 g, 101 mmol), 0.5 M potassium bis(trimethylsilyl)amide solution in toluene (222 ml) and allyl bromide (17.5 ml, 150 mmol) were reacted according to the procedure described for the preparation of **10a** to give a colorless oil (30.4 g, 70.0%) after purification by silica gel column chromatography using benzene as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.36 (s, 5H), 5.83—4.76 (m, 7H), 3.52 (dt, $J$=7.0, 7.0 Hz, 2H), 3.20—1.12 (m, 6H), 0.92 (m, 9H), 0.53 (m, 6H). IR (neat): 1780, 1720, 1635, 1070 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{23}H_{35}NO_5Si$: 433.2285. Found: 433.2311.

**3-(Benzyloxycarbonyl)-4-(2-hydroxyethyl)-4-(2-propenyl)-1,3-oxazolidin-5-one (11a)** A solution of **10a** (3.95 g, 9.41 mmol) in THF–AcOH–$H_2O$ (1 : 3 : 2, 60 ml) was stirred at 50 °C for 50 min. The solvent was removed *in vacuo*, and the residue was dissolved in $Et_2O$. The $Et_2O$ solution was washed, dried, and evaporated to dryness. The residue was chromatographed on a silica gel column using EtOAc–benzene (1 : 4) as the eluting solvent. The product was isolated as a colorless oil (2.66 g, 92.7%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.33 (m, 5H), 5.84—4.80 (m, 7H), 3.88—3.23 (m, 2H), 3.20—1.90 (m, 5H). IR (neat): 3500, 1800, 1720 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{16}H_{19}NO_5$: 305.1263. Found: 305.1250.

**3-(Benzyloxycarbonyl)-4-(3-hydroxypropyl)-4-(2-propenyl)-1,3-oxazolidin-5-one (11b)** The silyl ether **10b** (3.95 g, 9.41 mmol) was deprotected according to the procedure described for the preparation of **11a** to give a colorless oil 2.66 g (92.7%) after purification by silica gel column chromatography using EtOAc–benzene (1 : 4) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$ 7.33 (m, 5H), 5.85—4.78 (m, 7H), 3.50 (dt, $J$=6.9, 7.0 Hz, 2H), 3.20—1.05 (m, 7H). IR (neat): 3490, 1790, 1715 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{17}H_{21}NO_5$: 319.1420. Found: 319.1438.

**3-Benzyloxycarbonylamino-1-butyl-3-(2-propenyl)-2-pyrrolidinone (12a)** Imidazole (1.94 g, 28.5 mmol), triphenylphosphine (5.98 g, 22.8 mmol) and $I_2$ (5.79 g, 22.8 mmol) were added to a solution of **11a** (3.48 g, 11.4 mmol) in benzene (55 ml). The reaction mixture was stirred at room temperature for 1 h. The precipitate was removed by filtration and the filtrate was washed (saturated aqueous $Na_2SO_3$, brine), dried ($MgSO_4$) and concentrated to give a yellow oil, which was chromatographed on a silica gel column with benzene as the eluting solvent. The iodide, which was obtained as a colorless oil (4.85 g), was dissolved in benzene (20 ml). This solution was added dropwise to *n*-butylamine (22.5 ml, 228 mmol) over a 6-h period at 60 °C and then stirred for 30 min at the same temperature. The *n*-butylamine and benzene were removed *in vacuo* and the residue was diluted with EtOAc and 1 N HCl. The layers were separated, and the organic phase was washed (saturated aqueous $NaHCO_3$, brine) and dried ($MgSO_4$). The EtOAc was removed *in vacuo* and the residue was chromatographed on a silica gel column with EtOAc–benzene (1 : 5) as the eluting solvent. The product was isolated as a colorless oil (2.63 g, 69.7%): $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.32 (s, 5H), 5.93—5.15 (m, 3H), 5.06 (s, 2H), 3.53—3.06 (m, 4H), 2.62—2.22 (m, 4H), 1.70 (s, 1H), 1.70—1.10 (m, 4H), 0.93 (t, $J$=6.1 Hz, 3H). IR (neat): 3410, 3300, 1730, 1700, 1650, 1530 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{19}H_{26}N_2O_3$: 330.1943. Found: 330.1942.

**3-Benzyloxycarbonylamino-1-butyl-3-(2-propenyl)-2-piperidone (12b)** The alcohol **11b** (4.47 g, 14.0 mmol), imidazole (2.38 g, 35.0 mmol), triphenylphosphine (7.34 g, 28.0 mmol) and $I_2$ (7.11 g, 28.0 mmol) were reacted in the same manner as described above for compound **12a** to give 5.14 g (85.5%) of the corresponding iodide as a colorless oil. This iodide (4.72 g, 11.0 mmol) was treated with *n*-butylamine (21.7 ml, 220 mmol) according to the procedure described for the preparation of **12a** to give white crystals (2.89 g, 76.3%) after recrystallization from pentane: mp 74—75 °C. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.31 (br s, 5H), 6.03—5.52 (m, 2H), 5.18 (br s, 1H), 5.04 (s, 2H), 3.64—2.98 (m, 4H), 2.58 (br d, $J$=7.2 Hz, 2H), 2.44—2.10 (m, 2H), 2.05—1.70 (m, 2H), 1.70—1.07 (m, 5H), 0.91 (t, $J$=6.7 Hz, 3H). IR (KBr): 3270, 1720, 1655, 1550 cm$^{-1}$. MS (FAB): $m/z$ 345 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{28}N_2O_3$: C, 69.74; H, 8.19; N, 8.13. Found: C, 69.81; H, 8.03; N, 8.08.

**3-Benzyloxycarbonylamino-1-butyl-3-(3-hydroxypropyl)-2-pyrrolidinone (13a)** The alkene **12a** (1.21 g, 3.66 mmol) was dissolved in dry THF (11 ml) and then treated with a 0.5 M 9-BBN in THF (11 ml). The reaction mixture was stirred for 14 h, after which time excess 9-BBN was quenched by the addition of $H_2O$ (1 ml). The mixture was then stirred for 30 min at room temperature followed by the concurrent dropwise addition of 2 N NaOH (18 ml) and 30% $H_2O_2$ (18 ml) in an ice-bath. Stirring was continued for 1 h after the addition was completed, then the solution was extracted twice with $Et_2O$. The $Et_2O$ extract was washed (brine) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give a residue, which was chromatographed on a silica gel column with $CHCl_3$–MeOH (50 : 1) as the eluting solvent to give **13a** as a colorless oil (1.05 g, 82.7%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.30 (s, 5H), 6.22 (br s, 1H), 5.03 (s, 2H), 3.90—2.10 (m, 2H), 2.00—1.05 (m, 9H), 0.92 (t, $J$=5.9 Hz, 3H). IR (KBr): 3380, 3270, 3250, 1720, 1690, 1560 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{19}H_{28}N_2O_4$: 348.2049. Found: 348.2049.

**3-Benzyloxycarbonylamino-1-butyl-3-(3-hydroxypropyl)-2-piperidone (13b)** The alkene **12b** (2.76 g, 8.00 mmol) and 0.5 M 9-BBN in THF (24 ml) were reacted according to the procedure described for the

preparation of **13a** to give a colorless oil (2.59 g, 89.3%) after purification by silica gel column chromatography using EtOAc–benzene (1 : 2) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.34 (s, 5H), 6.18 (br s, 1H), 5.05 (s, 2H), 3.57 (t, $J$=5.9 Hz, 2H), 3.44—3.00 (m, 4H), 2.78 (br s, 1H), 2.56—1.07 (m, 12H), 0.90 (t, $J$=6.2 Hz, 3H). IR (neat): 3400, 1720, 1640, 1540 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{20}H_{30}N_2O_4$: 362.2206. Found: 362.2231.

**3-Benzyloxycarbonylamino-1-butyl-3-(3-phthalimidopropyl)-2-pyrrolidinone (14a)** Diethyl azodicarboxylate (272 ml, 1.73 mmol) was added to a stirred solution of the alcohol **13a** (500 mg, 1.43 mmol), triphenylphosphine (453 mg, 1.73 mmol) and phthalimide (233 mg, 1.58 mmol) in dry THF (10 ml) at room temperature. The resultant solution was stirred for 14 h. After removal of the solvent, the residue was purified by silica gel column chromatography using EtOAc–hexane (1 : 1) as the eluting solvent, providing a colorless oil (825 mg, 100%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.95—7.43 (m, 4H) , 7.32 (s, 5H), 5.50 (br s, 1H), 5.03 (s, 2H), 3.65 (t, $J$=5.6 Hz, 2H), 3.30 (t, $J$=7.2 Hz, 2H), 3.23 (t, $J$=7.0 Hz, 2H), 2.39 (t, $J$=7.2 Hz, 2H), 2.00—1.61 (m, 4H), 1.55—1.05 (m, 4H), 0.91 (t, $J$=6.7 Hz, 3H). IR (KBr): 3300, 1780, 1710, 1635, 1550 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{27}H_{31}N_3O_5$: 477.2264. Found: 477.2256.

**3-Benzyloxycarbonylamino-1-butyl-3-(3-phthalimidopropyl)-2-piperidone (14b)** The alcohol **13b** (2.36 g, 6.51 mmol), triphenylphosphine (2.05 g, 7.81 mmol), phthalimide (1.15 g, 7.81 mmol) and diethyl azodicarboxylate (1.12 ml, 7.81 mmol) were reacted according to the procedure described for the preparation of **14a** to give a colorless oil (3.07 g, 96%) after purification by silica gel column chromatography using EtOAc–benzene (1 : 5) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.90—7.63 (m, 4H) , 7.33 (s, 5H), 5.81 (br s, 1H), 5.03 (s, 2H), 3.65 (t, $J$=5.9 Hz, 2H), 3.48—3.03 (m, 4H), 2.55—1.05 (m, 12H), 0.89 (t, $J$=6.4 Hz, 3H). IR (KBr): 3280, 1780, 1720, 1640, 1540 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{28}H_{33}N_3O_5$: 491.2420. Found: 491.2438.

**3-Benzyloxycarbonylamino-1-butyl-3-(3-*tert*-butoxycarbonylaminopropyl)-2-pyrrolidinone (15a)** The phthalimide **14a** (667 mg, 1.49 mmol) was dissolved in anhydrous EtOH (30 ml), and this solution was treated with hydrazine hydrate (335 $\mu$l, 6.9 mmol). The reaction mixture was brought to reflux for 30 min, then allowed to cool. Concentrated HCl was carefully added to adjust the pH to 2 in an ice-bath. The solid that precipitated was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was partitioned between $CH_2Cl_2$ and 10% aqueous $Na_2CO_3$. Then $(Boc)_2O$ (488 mg, 2.23 mmol) and 10% aqueous $Na_2CO_3$ (2 ml) were added to the $CH_2Cl_2$ phase. The mixture was vigorously stirred at room temperature for 1 h. The organic phase was washed (brine), dried ($MgSO_4$), and concentrated to give a residue, which was chromatographed on a silica gel column using EtOAc–hexane (1 : 3) as the eluting solvent. The product was isolated as a colorless oil (570 mg, 89.0%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.32 (s, 5H), 5.47 (br s, 1H), 5.08 (s, 2H), 4.51 (m, 1H), 3.36 (t, $J$=6.9 Hz, 2H), 3.12 (t, $J$=7.0 Hz, 2H), 3.05 (t, $J$=7.0 Hz, 2H), 2.38 (t, $J$=7.0 Hz, 2H), 2.00—1.10 (m, 8H), 1.41 (s, 9H), 0.95 (t, $J$=6.7 Hz, 3H). IR (neat): 3400, 1725, 1625, 1550 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{24}H_{37}N_3O_5$: 447.2733. Found: 447.2754.

**3-Benzyloxycarbonylamino-1-butyl-3-(3-*tert*-butoxycarbonylaminopropyl)-2-piperidone (15b)** The phthalimide **14b** (20.8 g, 40.0 mmol) was deprotected with hydrazine hydrate (4.85 ml, 100 mmol) and then treated with $(Boc)_2O$ (11.3 g, 52.0 mmol) and saturated aqueous $NaHCO_3$ (100 ml) according to the procedure described for the preparation of **15a** to give white crystals (14.4 g, 78.0%) after recrystallization from a $CHCl_3$/$Et_2O$ mixture, mp 87—88 °C. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.31 (s, 5H), 5.95 (br s, 1H), 5.03 (s, 2H), 4.90 (t, $J$=6.4 Hz, 1H), 3.58—2.85 (m, 6H), 2.50—2.13 (m, 2H), 2.00—1.05 (m, 10H), 1.40 (s, 9H), 0.90 (t, $J$=6.4 Hz, 3H). IR (KBr): 3340, 3300, 1725, 1710, 1625, 1540, 1525 cm$^{-1}$. MS (FAB) $m/z$: 462 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{39}N_3O_5$: C, 65.05; H, 8.52; N, 9.10. Found: C, 65.00; H, 8.48; N, 9.20.

**3-(3-*tert*-Butoxycarbonylaminopropyl)-1-butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)amino-2-pyrrolidinone (16a)** The *N*-protected amino lactam **15a** (570 mg, 1.30 mmol) was dissolved in MeOH (20 ml) and deprotected *via* catalytic hydrogenation with 5% Pd–C at room temperature for 2 h. The catalyst was filtered off, and the solvent was removed *in vacuo* to give an oil. This material was dissolved in $CH_2Cl_2$. To this solution, 6,7-dimethoxy-2-naphthylsulfonyl chloride (704 mg, 2.60 mmol) and 10% aqueous $Na_2CO_3$ (5 ml) were added. The mixture was refluxed with vigorous stirring for 30 h, then allowed to cool. The organic phase was washed (1 N HCl, brine), dried ($MgSO_4$) and evaporated to dryness. The residue was chromatographed on a silica gel column using EtOAc–hexane (1 : 1) as the eluting solvent to give **16a** as a colorless oil (450 mg, 63.0%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 8.28 (br s, 1H), 7.72 (d, $J$=1.0 Hz, 2H), 7.13 (d, $J$=5.1 Hz, 2H), 5.71 (s, 1H), 4.53 (t, $J$=5.6 Hz, 1H), 4.03 (s, 3H), 4.00 (s, 3H), 3.45—2.76 (m, 6H), 2.68—2.00 (m, 2H), 1.98—1.00 (m, 8H), 1.38 (s, 9H), 0.87 (t, $J$=6.4 Hz, 3H). IR (KBr): 3410, 1720, 1640, 1520, 1275, 1165 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{28}H_{41}N_3O_7S$: 563.2665. Found: 563.2648.

**3-(3-*tert*-Butoxycarbonylaminopropyl)-1-butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)amino-2-piperidone (16b)** Compound **15b** was hydrogenated and then treated with 6,7-dimethoxy-2-naphthylsulfonyl chloride (26.7 g, 93.0 mmol) and 10% aqueous $Na_2CO_3$ (50 ml) according to the procedure described for the preparation of **16a** to give a colorless amorphous solid (16.7 g, 93.3%) after purification by silica gel column chromatography using EtOAc–benzene (2 : 3) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 8.25 (br s, 1H), 7.72 (d, $J$=1.2 Hz, 2H), 7.17 (d, $J$=5.6 Hz, 2H), 6.00 (br s, 1H), 4.60 (t, $J$=5.9 Hz, 1H), 4.01 (s, 6H), 3.25—2.70 (m, 6H), 2.25—2.00 (m, 2H), 1.92—1.00 (m, 10H), 1.42 (s, 9H), 0.87 (br t, $J$=6.4 Hz, 3H). IR (KBr): 3400, 3330, 1715, 1640, 1515, 1270, 1160 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{29}H_{43}N_3O_7S$: 577.2822. Found: 577.2841.

**1-Butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)amino-3-(3-guanidinopropyl)-2-pyrrolidinone Hydrochloride (2)** Compound **16a** (450 mg, 0.80 mmol) was dissolved in 4 M HCl/EtOAc (10 ml) at room temperature for 1 h and the solution was concentrated *in vacuo*. The residue was solidified with ether, collected by filtration, and dried in a vacuum desiccator with NaOH. This product was dissolved in DMF (20 ml) and treated at room temperature with 3,5-dimethylpyrazole-1-carboxamidine nitrate (644 mg, 3.20 mmol) and $Et_3N$ (0.56 ml, 4.00 mmol). After 15 h, the DMF was removed *in vacuo*, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with 2 N NaOH, dried ($MgSO_4$), and evaporated to dryness. The obtained residue was dissolved in 0.2 N HCl (10 ml) and purified by HP-20 column chromatography with MeOH–$H_2O$ (1 : 9—8 : 2) as the eluting solvent. After removal of the solvent, the residue was recrystallized from a $CHCl_3$–$Et_2O$ mixture to afford a white solid (300 mg, 74%), mp 126—131. $^1$H-NMR ($CD_3OD$, TMS) $\delta$: 8.16 (s, 1H), 7.60 (s, 2H), 7.52 (m, 2H), 7.15 (m, 2H), 7.03 (d, $J$=6.7 Hz, 2H), 3.93 (s, 6H), 3.40—2.65 (m, 6H), 2.60—1.45 (m, 6H), 1.45—0.90 (m, 5H), 0.72 (t, $J$=4.6 Hz, 3H). $^{13}$C-NMR ($CD_3OD$, TMS) $\delta$: 173.3 (s), 157.2 (s), 151.8 (s), 150.7 (s), 137.5 (s), 131.0 (s), 127.9 (s), 127.5 (d), 125.8 (d), 120.9 (d), 107.3 (d), 106.1 (d), 63.7 (s), 56.5 (q), 56.5 (q), 44.0 (t), 43.2 (t), 41.6 (t), 34.2 (t), 29.0 (t), 23.2 (t), 20.0 (t), 20.0 (t), 13.9 (q). IR (KBr): 3350, 3280, 3150, 1700, 1660, 1515, 1265, 1155 cm$^{-1}$. MS (FAB) $m/z$: 506 $(M+H)^+$. *Anal.* Calcd for $C_{24}H_{36}ClN_5O_5S$: C, 53.18; H, 6.69; N, 12.92. Found: C, 52.94; H, 6.51; N, 13.07.

**1-Butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)amino-3-(3-guanidinopropyl)-2-piperidone Hydrochloride (3)** Compound **16b** (16.0 g, 27.7 mmol) was deprotected with 5 N HCl/EtOAc (120 ml) and treated with 3,5-dimethylpyrazole-1-carboxamidine nitrate (644 mg, 3.20 mmol) and $Et_3N$ (560 ml) according to the procedure used for the preparation of **2**, to give a white powder (12.3 g, 79.6%) after purification by HP-20 column chromatography (MeOH–$H_2O$, 1 : 4—4 : 1) followed by recrystallization from a $CHCl_3$–$Et_2O$ mixture, mp 199—210. $^1$H-NMR ($CD_3OD$, TMS) $\delta$: 8.23 (s, 1H), 7.72 (s, 2H), 7.60 (m, 1H), 7.17 (d, $J$=6.4 Hz, 2H), 7.00 (m, 3H), 3.98 (s, 6H), 3.45—2.80 (m, 6H), 2.40—1.50 (m, 8H), 1.50—1.00 (m, 5H), 0.82 (br t, $J$=5.1 Hz, 3H). $^{13}$C-NMR ($CD_3OD$, TMS) $\delta$: 171.3 (s), 157.0 (s), 151.6 (s), 150.3 (s), 137.8 (s), 131.2 (s), 128.0 (s), 127.3 (d), 126.0 (d), 121.1 (d), 107.4 (d), 106.5 (d), 61.4 (s), 56.5 (q), 56.5 (q), 47.8 (t), 47.3 (t), 41.6 (t), 35.1 (t), 23.8 (t), 22.8 (t), 20.4 (t), 20.4 (t), 19.3 (t), 14.0 (q). IR (KBr): 3350, 3280, 3150, 1660, 1635, 1515, 1265, 1160 cm$^{-1}$. MS (FAB) $m/z$: 520 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{38}ClN_5O_5S$: C, 54.00; H, 6.89; N, 12.59. Found: C, 53.78; H, 6.97; N, 12.52.

**(4*S*)-3-(Benzyloxycarbonyl)-4-(3-methoxymethoxypropyl)-1,3-oxazolidin-5-one (17)** MOM-Cl (36.6 ml, 482 mmol) was added to a cooled (0 °C) solution of the alcohol **8b** (44.9 g, 161 mmol) and DIEA (112 ml, 643 mmol) in $CH_2Cl_2$ (112 ml) dropwise over a 20-min period. The reaction mixture was stirred for 2 h and then poured into 10% citric acid. The aqueous phase was extracted with $CH_2Cl_2$. The combined $CH_2Cl_2$ extracts were washed (saturated aqueous $NaHCO_3$, brine), dried ($MgSO_4$) and evaporated to dryness. The residue was chromatographed on a silica gel column using EtOAc–hexane (1 : 2) as the eluting solvent. The product was isolated as a colorless oil (27.4 g, 52.7%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.36 (s, 5H), 5.53 (d, $J$=5.5 Hz, 1H), 5.26 (d, $J$=5.5 Hz, 1H), 5.19 (s, 2H), 4.57 (s, 2H), 4.36 (t, $J$=5.1 Hz, 1H), 3.51 (t, $J$=6.4 Hz,

2H), 3.33 (s, 3H), 2.25—1.40 (m, 4H). IR (neat):1780, 1710 $cm^{-1}$: HRMS ($M^+$): Calcd for $C_{16}H_{21}NO_6$: 323.1369. Found: 323.1369.

**3-(Benzyloxycarbonyl)-4-(3-methoxymethoxypropyl)-4-(2-propenyl)-1,3-oxazolidin-5-one (18)** Compound **17** (93.7 g, 290 mmol), 0.5 M potassium bis(trimethylsilyl)amide solution in toluene (683 ml) and allyl bromide (50.2 ml, 580 mmol) were reacted according to the procedure described for the preparation of **10a** to give a colorless oil (66.7 g, 63.3%) after purification by silica gel column chromatography using EtOAc–hexane (1 : 5—1 : 2) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.33 (s, 5H), 5.88—5.33 (m, 1H), 5.33—4.80 (m, 6H), 4.55 (d, $J$=2.0 Hz, 2H), 3.49 (t, $J$=7.4 Hz, 2H), 3.31 (s, 3H), 3.22—1.15 (m, 6H). IR (neat): 1805, 1715, 1645, 1505 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{19}H_{25}NO_6$: 363.1682. Found: 363.1685.

**3-(Benzyloxycarbonyl)-4-(3-hydroxypropyl)-4-(3-methoxymethoxypropyl)-1,3-oxazolidin-5-one (19)** The alkene **18** (81.8 g, 225 mmol) and 0.5 M 9-BBN in THF (675 ml) were reacted according to the procedure described for the preparation of **13a** to give a colorless oil (67.4 g, 78.6%) after purification by silica gel column chromatography using $CHCl_3$–MeOH (20 : 1) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.36 (s, 5H), 5.29 (S, 2H), 5.19 (d, $J$=4.8 Hz, 2H), 4.54 (d, $J$=2.2 Hz, 2H), 3.69—3.35 (m, 4H), 3.32 (s, 3H), 2.58—1.13 (m, 9H). IR (neat): 3480, 1800, 1720, 1595, 1505 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{19}H_{27}NO_7$: 381.1788. Found: 381.1794.

**3-[Benzyloxycarbonyl-4-(3-methoxymethoxypropyl)-5-oxo-1,3-oxazolidin-4-yl]propanal (20)** Dimethyl sulfoxide (37.7 ml, 530 mmol) was added dropwise to a solution of oxalyl chloride (23.1 ml, 265 mmol) in $CH_2Cl_2$ (450 ml) at −60 °C with stirring over a 20-min period. After 15 min, a solution of the alcohol **19** (67.4 g, 177 mmol) in $CH_2Cl_2$ (200 ml) was added dropwise over 20 min, and this mixture was stirred for 15 min at −60°C. The reaction was quenched by addition of $Et_3N$ (123 ml, 884 mmol) and the mixture was stirred with warming to room temperature. To this white, heterogeneous mixture was added $H_2O$ and the aqueous phase was extracted with $CH_2Cl_2$ (400 ml). The combined $CH_2Cl_2$ extracts were washed (1 N HCl, saturated aqueous $NaHCO_3$, brine), dried ($MgSO_4$) and evaporated to dryness. The residue was chromatographed on a silica gel column using EtOAc–hexane (1 : 2) as the eluting solvent. The product was isolated as a colorless oil (65.3 g, 97.5%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 9.61 (br d, $J$=2.8 Hz, 1H), 7.36 (s, 5H), 5.35—5.10 (m, 4H), 4.56 (d, $J$=1.9 Hz, 2H), 3.47 (m, 2H), 3.32 (s, 3H), 2.77—1.10 (m, 8H). IR (neat): 1800, 1725 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{19}H_{25}NO_7$: 379.1631. Found: 379.1633.

**3-(Benzyloxycarbonyl)-4-(3-butenyl)-4-(3-methoxymethoxypropyl)-1,3-oxazolidin-5-one (21)** A solution of methyltriphenylphosphonium bromide (98.4 g, 276 mmol) in dry THF (520 ml) under $N_2$ was cooled to −10 °C. A 0.5 M potassium bis(trimethylsilyl)amide solution in toluene (517 ml) was added dropwise to it over a 1-h period. After a further 30min, this solution was cooled to −76 °C and a solution of the alcohol **20** (67.4 g, 177 mmol) in THF (250 ml) was added dropwise over a 30-min period. The whole was stirred for 15 min at −60 °C, and then allowed to warm to room temperature over a 2-h period. The reaction was quenched by addition of Rochelle salt solution (1.5 l) and the mixture was extracted with $Et_2O$ (2 × 500 ml). The combined organic extracts were washed (brine), dried ($MgSO_4$), and concentrated to give an oil, which was chromatographed on a silica gel column using EtOAc–hexane (1 : 5—1 : 2) as the eluting solvent. The product was isolated as a colorless oil (49.7 g, 76.5%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.36 (s, 5H), 5.88—5.36 (m, 1H), 5.32—5.12 (m, 4H), 5.10—4.80 (m, 2H), 4.56 (d, $J$=2.3 Hz, 2H), 3.48 (t, $J$=6.7 Hz, 2H), 3.32 (d, $J$=1.5 Hz, 3H), 2.58—1.17 (m, 8H). IR (neat): 1810, 1720, 1645, 1510 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{20}H_{27}NO_6$: 377.1838. Found: 377.1838.

**3-(Benzyloxycarbonyl)-4-(4-hydroxybutyl)-4-(3-methoxymethoxypropyl)-1,3-oxazolidin-5-one (22)** The alkene **21** (38.3 g, 102 mmol) and 0.5 M 9-BBN in THF (575 ml) were reacted according to the procedure described for the preparation of **13a** to give a colorless oil (36.6 g, 91.3%) after purification by silica gel column chromatography using $CHCl_3$–MeOH (20 : 1) as the eluting solvent. $^1$H NMR ($CDCl_3$, TMS) $\delta$: 7.36 (s, 5H), 5.28 (s, 2H), 5.19 (d, $J$=4.6 Hz, 2H), 4.53 (d, $J$=1.8 Hz, 2H), 3.67—3.33 (m, 4H), 3.31 (s, 3H), 2.55—0.95 (m, 11H). IR (neat): 3450, 1805, 1715 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{20}H_{29}NO_7$: 395.1944. Found: 395.1952.

**3-Benzyloxycarbonyl-4-(4-butylaminobutyl)-4-(3-methoxymethoxypropyl)-1,3-oxazolidin-5-one (23)** The alcohol **22** (5.00 g, 12.6 mmol), imidazole (2.15 g, 31.6 mmol), triphenylphosphine (8.30 g, 31.6 mmol) and $I_2$ (6.42 g, 25.3 mmol) were reacted according to the procedure described for the preparation of **12a** to give 5.14 g (85.5%) of the corresponding iodide as a colorless oil after purification by silica gel column chromatography using EtOAc–hexane (1 : 5—1 : 1) as the eluting solvent. The iodide was dissolved in benzene (20 ml), and this solution was added dropwise to *n*-butylamine (23.3 ml, 235 mmol) over 15 min at room temperature. The whole was stirred for 90 min at the same temperature. The *n*-butylamine and benzene were removed *in vacuo* and the residue was diluted with EtOAc and 1 N HCl. The organic phase was washed (saturated aqueous $NaHCO_3$, brine) and dried ($MgSO_4$). The EtOAc was removed under vacuum and the residue was chromatographed on a silica gel column with $CHCl_3$→$CHCl_3$–MeOH (10 : 1) as the eluting solvent. The product was isolated as a colorless oil (4.53 g, 85.4%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.36 (s, 5H), 5.28 (s, 2H), 5.19 (d, $J$=4.4 Hz, 2H), 4.54 (d, $J$=2.3 Hz, 2H), 3.62—3.33 (m, 4H), 3.30 (s, 3H), 2.78—2.50 (m, 4H), 2.50—1.03 (m, 14H), 0.96 (t, $J$=6.5 Hz, 3H). IR (neat): 3400, 1800, 1715 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{24}H_{38}N_2O_6$: 450.2730. Found: 450.2746.

**2-Benzyloxycarbonylamino-6-butylamino-2-(3-methoxymethoxypropyl)hexanoic Acid (24)** A solution of **23** (4.53 g, 10.1 mmol) in a mixture of EtOH (45 ml) and 1 N NaOH (10.1 ml) was heated at reflux for 10 min. The solvent was removed *in vacuo* and the residue was dissolved in $H_2O$ (10 ml). The pH of the solution was adjusted to 7 with 2 N HCl and the resulting crystals were collected (3.26 g, 74%), mp 234—235 °C (dec.). IR (neat): 3500, 3380, 1720, 1645, 1510 $cm^{-1}$. MS (FAB) *m/z*: 439 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{38}N_2O_6$: C, 62.99; H, 8.73; N, 6.39. Found: C, 62.71; H, 8.99; N, 6.21.

**3-Benzyloxycarbonylamino-1-butyl-3-(3-methoxymethoxypropyl)-hexahydro-2*H*-azepin-2-one (25)** 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (656 mg, 3.42 mmol) was added to a cold (−20 °C) solution of the amino acid **24** (1.00 g, 2.28 mmol) and HOBt (308 mg, 2.28 mmol) in $CH_2Cl_2$ (100 ml). The reaction mixture was stirred at 4 °C for 15 h and then washed (1 N HCl, saturated aqueous $NaHCO_3$, brine). The organic phase was dried ($MgSO_4$) and concentrated to give a residue, which was chromatographed on a silica gel column using EtOAc–hexane (1 : 2) as the eluting solvent. The product was obtained as a colorless oil (901 mg, 94.0%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.32 (s, 5H), 6.93 (s, 1H), 5.04 (s, 2H), 4.57 (s, 2H), 3.88—3.02 (m, 6H), 3.32 (s, 3H), 2.65—2.00 (m, 4H), 2.00—1.05 (m, 10H), 0.92 (t, $J$=6.4 Hz, 3H). IR (neat): 3370, 1725, 1635, 1520 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{23}H_{36}N_2O_5$: 420.2624. Found: 420.2635.

**3-Benzyloxycarbonylamino-1-butylhexahydro-3-(3-phthalimidopropyl)-2*H*-azepin-2-one (26)** A solution of **25** (865 mg, 2.06 mmol) in a mixture of MeOH (20 ml) and concentrated HCl (0.5 ml) was heated at reflux for 15 min. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc (50 ml). The EtOAc solution was washed (saturated aqueous $NaHCO_3$, brine) and dried ($MgSO_4$) to give a colorless oil, which was used without further purification (901 mg, 94.0%). The alcohol thus obtained (1.51g, 4.00 mmol), triphenylphosphine (1.26 g, 4.80 mmol), phthalimide (0.71 g, 4.80 mmol) and diethyl azodicarboxylate (0.76 ml, 4.80 mmol) were reacted according to the procedure described for the preparation of **14a** to give a colorless oil (1.93 g, 95%) after purification by silica gel column chromatography using EtOAc–benzene (2 : 25) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.89—7.56 (m, 4H) , 7.42—7.18 (m, 5H), 6.84 (br s, 1H), 4.97 (s, 2H), 3.65 (t, $J$=5.9 Hz, 2H), 3.55—3.00 (m, 4H), 2.66—2.20 (m, 2H), 2.20—1.05 (m, 12H), 0.89 (br t, $J$=6.5 Hz, 3H). IR (neat): 3370, 1775, 1720, 1630, 1520 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{29}H_{35}N_3O_5$: 505.2577. Found: 505.2555.

**3-Benzyloxycarbonylamino-3-(3-*tert*-butoxycarbonylaminopropyl)-1-butylhexahydro-2*H*-azepin-2-one (27)** The phthalimide **26** (1.80 g, 3.55 mmol) was deprotected with hydrazine hydrate (0.53 ml, 11 mmol) and then treated with $(Boc)_2O$ (1.09 g, 5.00 mmol) and saturated aqueous $NaHCO_3$ (30 ml) according to the procedure described for the preparation of **15a** to give a colorless oil (1.69 g, 100%) after purification by silica gel column chromatography using EtOAc–benzene (1 : 5) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 7.35 (br s, 5H), 6.87 (br s, 1H), 5.06 (s, 2H), 4.44 (m, 1H), 3.80—2.88 (m, 6H), 2.58—1.05 (m, 14H), 1.43 (s, 9H), 0.91 (br t, $J$=6.1 Hz, 3H). IR (neat): 3360, 1720, 1630, 1540 $cm^{-1}$. HRMS ($M^+$): Calcd for $C_{26}H_{41}N_3O_5$: 475.3046. Found: 475.3061.

**3-(3-*tert*-Butoxycarbonylaminopropyl)-1-butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)aminohexahydro-2*H*-azepin-2-one (28)** Compound **27** (550 mg, 1.16 mmol) was hydrogenated and then treated with 6,7-dimethoxy-2-naphthylsulfonyl chloride (1.08 g, 4.00 mmol) and 10%

aqueous $Na_2CO_3$ (10 ml) according to the procedure described for the preparation of **16a** to give a colorless amorphous solid (540 mg, 78.3%) after purification by silica gel column chromatography using EtOAc–hexane (1 : 3) as the eluting solvent. $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 8.24 (br s, 1H), 7.74 (d, $J$=1.3 Hz, 2H), 7.18 (d, $J$=4.6 Hz, 2H), 6.99 (br s, 1H), 4.42 (t, $J$=5.6 Hz, 1H), 4.02 (s, 6H), 3.70—2.50 (m, 6H), 2.40—1.00 (m, 14H), 1.42 (s, 9H), 0.89 (br t, $J$=6.4 Hz, 3H). IR (KBr): 3380, 3180, 1720, 1630, 1520, 1270, 1260, 1160 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{30}H_{45}N_3O_7S$: 591.2978. Found: 591.2988.

**1-Butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)aminohexahydro-3-[3-(3-nitro)guanidino]propyl-2*H*-azepin-2-one (29)** The sulfonamide **28** (178 mg, 0.30 mmol) was dissolved in 3.3 M HCl/EtOAc (20 ml) at room temperature for 30 min and the solution was concentrated *in vacuo*. The residue was solidified with ether, collected by filtration, and dried in a vacuum desiccator with NaOH. This product was dissolved in DMF (4 ml) and treated at 4 °C with *N*-methyl-*N*-nitronitrosoguanidine (88 mg, 0.60 mmol) and $Et_3N$ (0.14 ml, 1.00 mmol). The mixture were stirred for 24 h at room temperature and then dissolved in EtOAc. The EtOAc solution was washed (2 N NaOH), dried ($MgSO_4$), and evaporated to dryness. The residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (40 : 1) as the eluting solvent. The product was obtained as a colorless amorphous solid (155 mg, 89.1%). $^1$H-NMR ($CDCl_3$, TMS) $\delta$: 8.26 (s, 2H), 8.02 (s, 1H), 7.73 (br s, 2H), 7.60 (m, 1H), 7.18 (d, $J$=7.7 Hz, 2H), 6.96 (br s, 1H), 4.01 (s, 6H), 3.60—3.03 (m, 6H), 2.30—1.03 (m, 14H), 0.87 (br t, $J$=5.6 Hz, 3H). IR (KBr): 3420, 3300, 1630, 1610, 1515, 1265, 1160 cm$^{-1}$. HRMS ($M^+$): Calcd for $C_{26}H_{38}N_6O_7S$: 578.2523. Found: 578.2546.

**1-Butyl-3-(6,7-dimethoxy-2-naphthylsulfonyl)amino-3-(3-guanidino)-propylhexahydro-2*H*-azepin-2-one Hydrofluoride (4)** Compound **29** (800 mg, 1.38 mmol) was dissolved in anhydrous HF (10 ml) and anisole (1 ml) at 0 °C. After 30 min, HF was evaporated *in vacuo* below 5 °C. The residue was washed with dry $Et_2O$ (2 × 30 ml) by successive centrifugation and decantation. The product was recrystallized from MeOH–$Et_2O$ mixture to afford a colorless amorphous solid (580 mg, 75.9%). $^1$H-NMR ($CD_3OD$, TMS) $\delta$: 8.18 (s, 1H), 7.71 (s, 2H), 7.40 (m, 2H), 7.18 (br s, 2H), 7.12 (d, $J$=6.5 Hz, 2H), 3.95 (s, 6H), 3.45—2.60 (m, 6H), 2.40—1.00 (m, 15H), 0.79 (t, $J$=5.0 Hz, 3H). $^{13}$C-NMR ($CD_3OD$, TMS) $\delta$: 172.3 (s), 157.4 (s), 151.5 (s), 150.7 (s), 138.8 (s), 130.9 (s), 128.0 (s), 127.2 (d), 125.6 (d), 121.0 (d), 107.2 (d), 106.1 (d), 66.7 (s), 56.2 (q), 56.2 (q), 51.3 (t), 48.2 (t), 41.7 (t), 33.6 (t), 29.7 (t), 29.7 (t), 28.1 (t), 23.3 (t), 20.2 (t), 20.2 (t), 13.8 (q). IR (KBr): 3400, 3360, 3220, 1675, 1630, 1515, 1270, 1160 cm$^{-1}$. MS (FAB) $m/z$: 534 $(M+H)^+$. *Anal.* Calcd for $C_{26}H_{40}FN_5O_5S$: C, 56.40; H, 7.28; N, 12.65. Found: C, 56.35; H, 7.24; N, 12.48.

**Enzyme Assay and Measurement of Kinetic Constants** The fluorescence of 7-amino-4-methylcoumarin formed by hydrolysis of peptide-MCA substrates was measured in the presence and absence of inhibitors with a Shimadzu RF-5000 fluorescence spectrophotometer. The measurements were carried out with excitation at 306 nm and emission at 470 nm. Table 2 summarizes the reaction system used for enzyme activity measurements. The enzyme assay (final volume, 0.5 ml) was started by the addition of substrate after pre-incubation of the enzyme with an inhibitor for 10 min at 37 °C. The inhibition constant ($K_i$) was estimated from a Lineweaver–Burk analysis of the inhibition of thrombin by compound **2**. The $K_m$ value was 21 $\mu$M.

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