Peptide Based Interleukin-1β Converting Enzyme (ICE) Inhibitors: Synthesis, Structure Activity Relationships and Crystallographic Study of the ICE-inhibitor Complex

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Based on the X-ray structure of the complex of Ac–Tyr–Val–Ala–Asp–H (L-709049) and interleukin-1β converting enzyme (ICE), we synthesized compounds which were derived from 2-NapCO–Val–Pro–Asp–CH_2OPh (1) to obtain a potent inhibitor in the cell assay. Among these compounds, (3*S*)-*N*-methanesulfonyl-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]-4-oxobutanamide (27c) showed high potency not only in the enzyme assay but also cell assay with IC_{50} values of 38 nM and 0.23 μM, respectively. Compound 27c, with a c log P value of 1.76, had a more hydrophilic character compared with 1. Compound 27c also dose dependently inhibited LPS-primed ATP-induced IL-1β release in mice. The crystal structure of the complex of compound 27c and ICE revealed that compound 27c had further interactions with ICE in the naphthoyl group at the P4 position and in the methyl group of the methanesulfonamidecarbonyl group at the P1 position, compared with L-709049. To our knowledge, compound 27c is the first example that shows a strong inhibitory activity without the carboxyl group at the P1 position.

Key words X-ray structure; interleukin-1β converting enzyme inhibitor; interleukin-1β; structure–activity relationship; complex; methanesulfonamidecarbonyl group

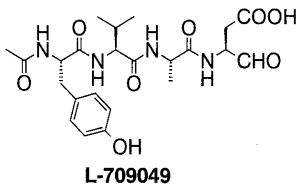
Interleukin-1β converting enzyme (ICE, caspase-1) is a cysteine protease, which cleaves the 31 kDa inactive precursor of interleukin-1β (IL-1β) between residues Asp27 and Gly28 and between residues Asp116 and Ala117 sites to release the 17.5 kDa active IL-1β.[1—3] It has been reported that ICE may play a major role in the inflammation caused by the cowpox virus[4] and in cell death induced by deprivation of nerve growth factor.[5] In addition, IL-1β is a potent mediator in the pathogenesis of chronic and acute inflammatory diseases such as rheumatoid arthritis.[6] A specific inhibitor of ICE, therefore, may have potential as a therapeutic agent for the treatment of inflammatory diseases.

Several peptide-based ICE inhibitors have been reported.[7—9] Among these peptide-based ICE inhibitors, tetrapeptide aldehyde ICE inhibitor Ac–Tyr–Val–Ala–Asp–H (L-709049) (Fig. 1) was reported to show potent ICE inhibitory activity with a K_i value of 0.76 nM.[7] The three-dimensional structure of the complex with ICE and L-709049 has already been determined by X-ray crystallographic study.[10] L-709049 interacts with ICE through a covalent bond between the sulfhydryl group of Cys285 of ICE and the P1 carbonyl group, and through three hydrogen bonds between P1-NH, P3-NH, P3-C=O and the enzyme backbone.

We have already found 2-NapCO–Val–Pro–Asp–CH_2OPh (**1**) (Fig. 2) which showed ICE inhibitory activity with an IC_{50} value of 13 nM.[11] However, compound **1** showed poor inhibitory activity in the cell assay of IL-1β release from lipopolysaccharide (LPS)-stimulated human monocytic cells, with an IC_{50} value of 0.90 μM. To obtain a more potent inhibitor in the cell assay, we designed some hydrophilic analogues of compound **1**. Based on the binding mode of L-709049 with ICE, we speculate that the P4, P2 and P1 positions may be exchangeable to other groups and that these transformations may lead to the improvement of the hydrophilicity and cell permeability. In this paper, we describe the synthesis and structure activity relationships (SARs) of the P4, P2 and P1 site analogues of **1** and also describe the X-ray crystallographic analysis of the complex of ICE and the selected inhibitor.

Chemistry

The synthesis of the P4 site analogues (**8a—d**) is outlined in Chart 1. Cbz–Val–Pro (**2**) was coupled with aminoalcohol (**3**)[11] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenztriazole (HOBT) and *N*-methylmorpholine (NMM). The Cbz group



L-709049

Fig. 1. Chemical Structure of L-709049

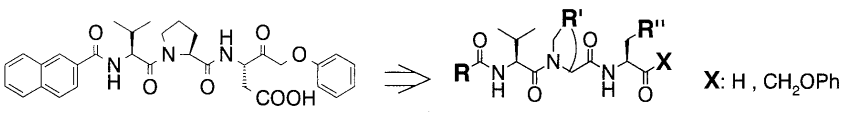


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Fig. 2. Modification of Compound **1**

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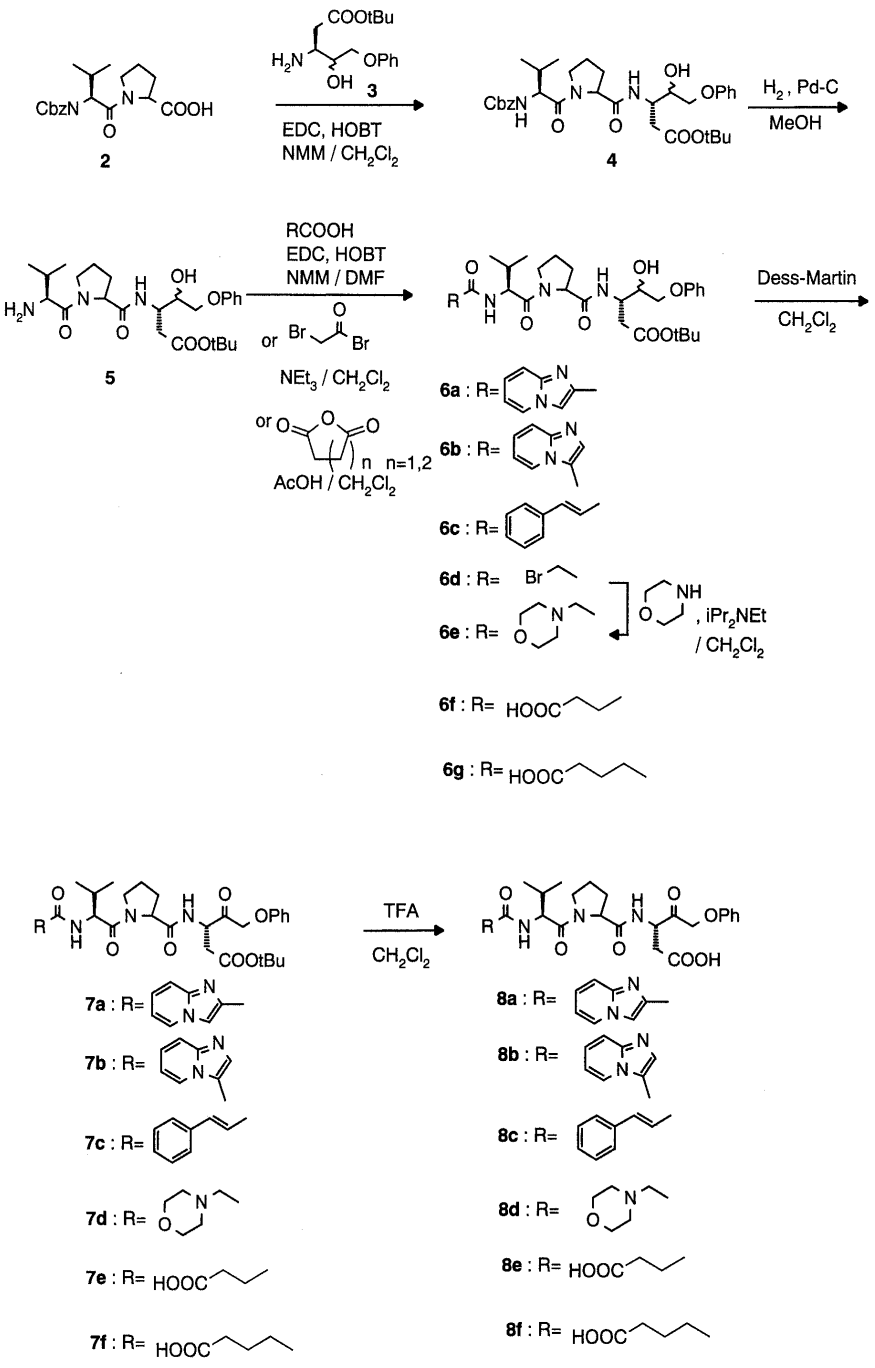


Chart 1

of compound **4** was removed by catalytic hydrogenation to give the aminoalcohol (**5**) followed by condensation with the corresponding carboxylic acid derivative, yielding **6a**—**d**, **f**, **g**. Compound **6e** was provided by the treatment of **6d** with morpholine. Oxidation of the hydroxyl group in **6a**—**c**, **e**—**g** to a carbonyl group was carried out with Dess–Martin periodinane,[12] and subsequent removal of the *tert*-butyl group with trifluoroacetic acid provided **8a**—**f**.

The synthesis of the P2 site analogues (**17a**—**d**) is shown in Chart 2. *N*-Butyl (**11a**) and the cyanopropyl glycine ethyl ester (**11b**) was prepared by alkylation of the glycine ethyl ester (**9**) with iodobutane or 4-bromobutyronitrile. *N*-[3-(1*H*-Imidazol-1-yl)propyl]glycine ethyl ester (**11c**) was prepared by the reaction of ethyl bromoacetate (**10**) with 3-(1*H*-imidazol-1-yl)propylamine. *N*-Alkylglycine ethyl ester derivatives (**11a**—**c**) were coupled with Boc-L-valine to afford **12a**—**c**. Removal of the *tert*-butoxycarbonyl group of **12a**—**c** and subsequent condensation with 2-naphthoyl chloride afforded **13a**—**c**. The *N*-tetrazolylpropylglycine derivative (**13e**) was prepared by the reaction of **13b** with sodium azide, followed by trityl chloride. The ester groups of **13a**—**c** and **13e** were hydrolyzed to afford **14a**—**d**, and these were then condensed with the aminoalcohol (**3**) to obtain compounds **15a**—**d**. Compounds **15a**—**d** were oxidized with Dess–Martin periodinane, then deprotected under acidic conditions to provide **17a**—**d**.

The synthesis of the P1 site analogues (**27a**—**c**) is outlined in Chart 3. 1-(L-Valyl)-L-proline methyl ester monohydrochloride (**18**) was condensed with 2-naphthoyl chloride, followed by saponification to afford **20**. The alcohol **21** was

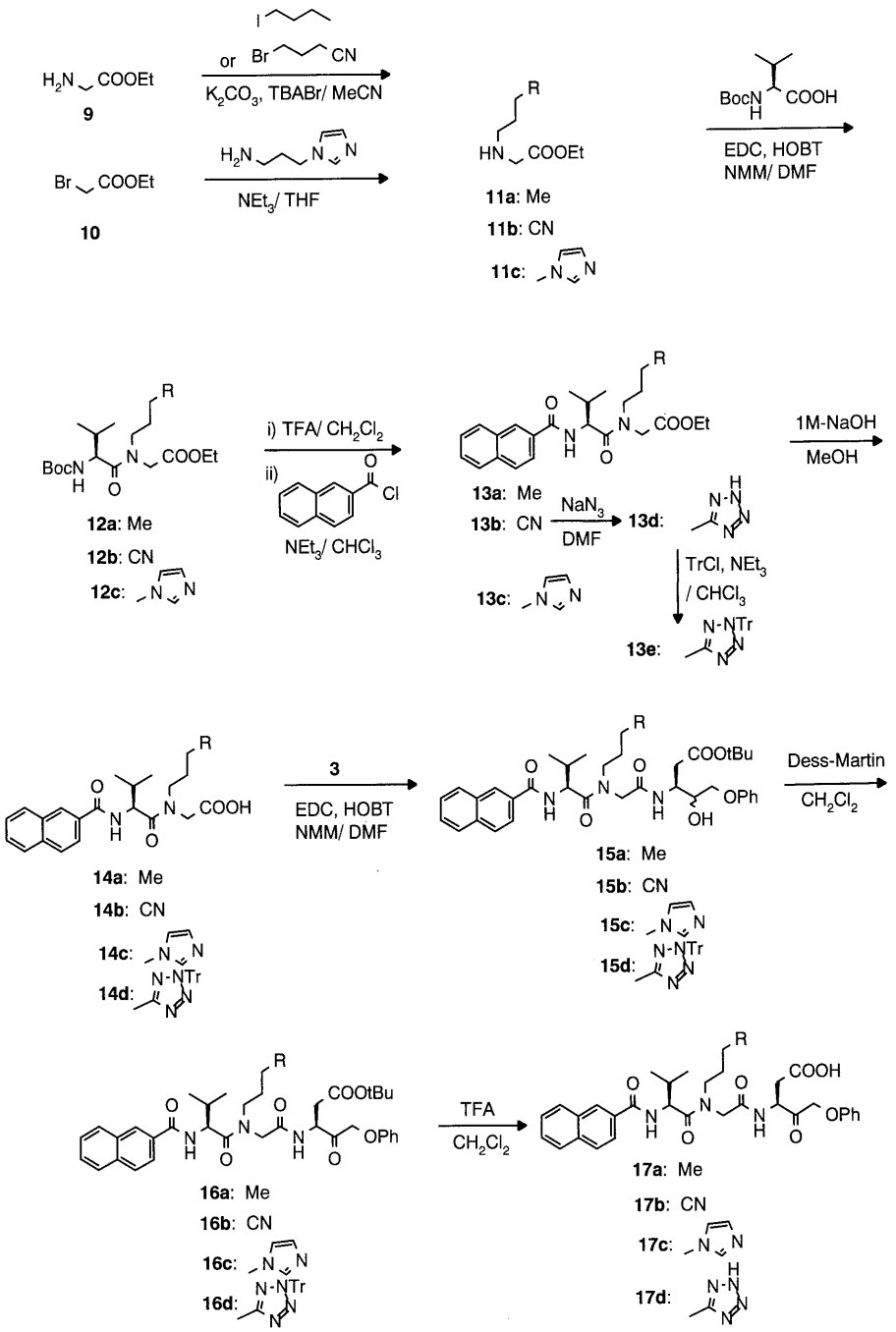


Chart 2

reacted with *tert*-butyldimethylsilyl chloride to afford **22**. The *tert*-butoxycarbonyl group of **22** was removed under acidic conditions, and the resulting amine was then condensed with **20** to afford **23**. The benzyl ester of **23** was hydrolyzed to afford **24**. Carboxylic acid **24** was condensed with *O*-benzylhydroxylamine in the presence of EDC, HOBT and NMM, or with methanesulfonamide in the presence of 1,1′-carbonylbis-1*H*-imidazole and 1,8-diazabicyclo[5.4.0]-undec-7-ene to give **25a** and **25b**, respectively. Compounds **25a** and **25b** were desilylated, then oxidized with Dess–Martin periodinane to afford **27a** and **27c**, respectively. The *O*-benzyl group of **27a** was removed by hydrogenation in the presence of Pd–C to afford the hydroxamic acid derivative (**27b**).

Results and Discussion

The new synthetic compounds were evaluated for their inhibitory activities against human recombinant ICE using Ac–Tyr–Val–Ala–Asp–AFC (AFC : 7-amino-4-trifluoromethylcoumarin) as a substrate.

The P4 residue of L-709049 was known to interact with ICE through hydrophobic interaction.[10] Therefore, first we introduced aromatic or non-aromatic substituents at the P4 position to examine the influence on the ICE inhibitory activity. The results are summarized in Table 1. Replacement of the naphthyl group of **1** with imidazo[1,2-*a*]pyridin-2-yl (**8a**), imidazo[1,2-*a*]pyridin-3-yl (**8b**) and stylyl (**8c**) groups resulted in almost the same potency as **1**. However, the 1-morpholinomethyl (**8d**), 4-oxobutylic acid (**8e**) and 5-oxopentanoic acid (**8f**) derivatives showed much less potent activity

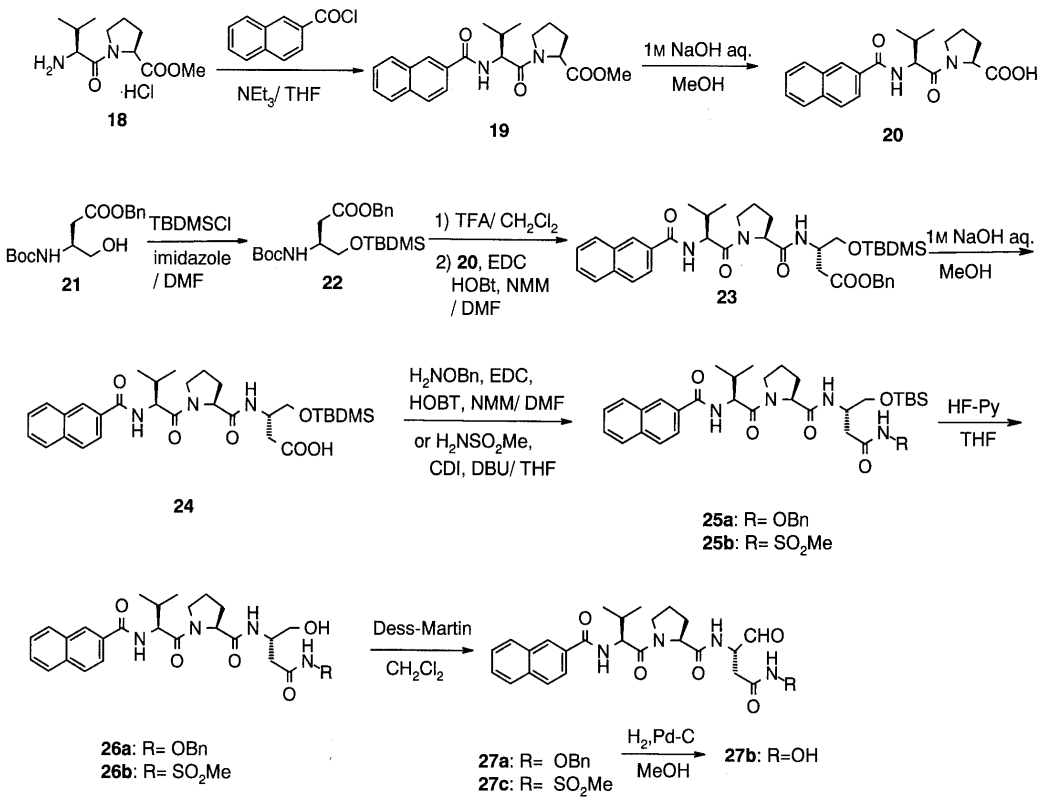
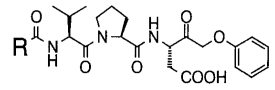


Chart 3

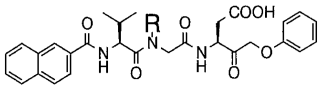
Table 1. Inhibitory Effects of the P4 Analogues on Human ICE



Compound	R	IC_{50} (nM)[a] Enzyme
8a		43
8b		23
8c		13
8d		1200
8e	HOOC	260
8f	HOOC	180
1		13

a) The method of measurement is described in Experimental.

Table 2. Inhibitory Effects of the P2 Analogues on Human ICE



Compound	R	IC_{50} (nM)[a] Enzyme
17a		22
17b		27
17c		18
17d		13
1		13

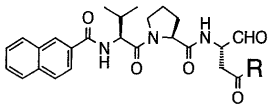
a) The method of measurement is described in Experimental.

compared with **1**. From these results, it was speculated that an aromatic ring at the P4 position may be essential to show inhibitory activity and that a hydrophilic group may be unsuitable at this position.

The NH group at the P2 position of L-709049 was known to have no interaction with the ICE backbone,[10] and the P2 pocket of ICE would be expected to tolerate the bulkiness of the substituent and to have the possibility of accepting various substituents. Therefore, it may be possible to improve the cell permeability by examining the structure activity relationships (SARs) at this position. These facts led us to examine the influence of the residue at the P2 position on the ICE inhibitory activity. The results are summarized in Table 2. Replacement of the proline residue of **1** with *N*-butyl (**17a**), *N*-cyanobutyl (**17b**), *N*-3-(5-tetrazolyl)propyl (**17c**) and *N*-3-(1-imidazolyl)propylglycine (**17d**) resulted in almost the same inhibitory activity against ICE as that of **1**. These results suggest that the P2 position of L-709049 was exchangeable with *N*-alkyl glycine, and the bulkiness of the alkyl substituents did not influence ICE potency.

At last, the possibility of the replacement of the carboxyl group at the P1 position was examined. The results are summarized in Table 3. The acidic hydrogen atom of the carboxyl group at the P1 position of L-709049 was known to interact with the ICE backbone through hydrogen bonding.[10] Ator and Dolle[13] reported that P1 aldehyde-type compounds showed almost the same inhibitory activity against ICE as the respective phenoxyacetyl-type compounds. We examined,

Table 3. Inhibitory Effects of the P1 Analogues on Human ICE



Compound	R	IC_{50} (nM)[a] Enzyme
27a		600
27b		1300
27c		38
1		13

a) The method of measurement is described in Experimental.

Table 4. Inhibitory Effects on IL-1β Release from LPS-Stimulated Human Monocytic Cells

Compound	IC_{50} (μM)[a]	Ratio of IC_{50} Cell/enzyme	c log P
8c	0.34	26	3.97
17a	0.89	40	5.40
17d	0.77	23	3.64
27c	0.23	6.1	1.76
1	0.90	69	4.35

a) The method of measurement is described in Experimental.

therefore, the SARs of the compounds having a group with an acidic hydrogen atom and an aldehyde group at the P1 position instead of carboxyl and phenoxyacetyl groups, respectively. Replacement of the P1 carboxyl group with benzyloxyaminocarbonyl (**27a**) and hydroxyaminocarbonyl groups (**27b**) resulted in a drastic decrease in ICE inhibitory activity. Replacement with a methanesulfonylaminocarbonyl group (**27c**), however, resulted in almost the same potency as **1**. These results suggest that the carboxyl group in the P1 position is not necessary to show ICE inhibitory activity. The carboxyl group in the P1 position is known to play an important role in ICE inhibitory activity, and it has been reported that the replacement of the carboxyl group with a tetrazole group or methyl substitution of the β-carbon of the carboxyl group led to a great loss of the inhibitory activity.[14] To our knowledge, compound **27c** is the first example that shows potent inhibitory activity against ICE without the carboxyl group at the P1 position.

Selected compounds (**8c**, **17a**, **d** and **27c**), which showed potent ICE inhibitory activity, were examined in the cell assay, and their c log P values were calculated.[15] The results are summarized in Table 4. In the P4 (**8c**) and P2 (**17a** and **d**) analogues, compound **8c** showed 2.6 times more potent inhibitory activity and showed better cell permeability (the ratio of IC_{50} values of cell to enzyme) than **1**. The P1 analogue, compound **27c**, showed about 4 times more potent inhibitory activity and showed 11 times better permeability than **1**. These results indicated that the compounds with low c log P values tended to show good cell permeability in this series. Namely, the hydrophilicity of these compounds may play an important role in penetrating into the cells. It was also speculated that serum protein-binding may be influenced in cell assay in which fetal bovine serum was added. In genaral, hydrophilic compounds show low serum protein-binding and high concentration as a nonconjugated form.[16] Indeed, compound **27c**, which is the most hydrophilic with a c log P value of 1.76, exhibited the highest potency and cell permeability with an IC_{50} value of 0.23 μM and a ratio of IC_{50} values of 6.1, respectively.

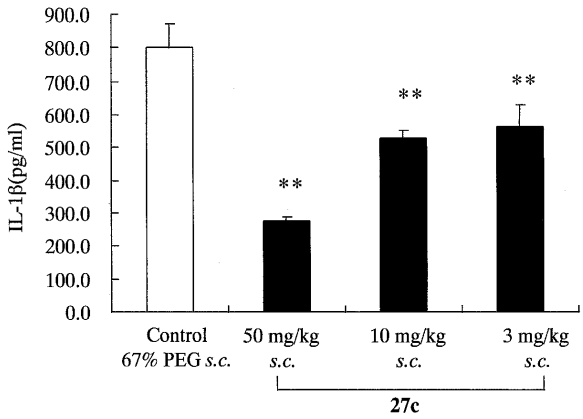


Fig. 3. Inhibitory Activity of **27c** on LPS-primed ATP-induced IL-1β Release in Mice

The Method of Measurement is Described in Experimental. $^{**}p<0.01$ *vs.* control (Dunnett's multiple range test). $n=7$

Table 5. Crystallographic Analysis Data of the Complex of **27c** and ICE

Data collection		
Space group	$P4_32_12$	
Cell constants (a, b, c) (Å)	64.7, 64.7, 161.4	
Resolution range (Å)	161—2.7	
No. of observed reflections	53897	
No. of unique reflections	9838	
Completeness (%)	97.3	
R_{merge}[a] (%)	9.0	
Refinement		
No. of atoms and average temperature factor (Å^2)		
Protein	2032	26.1
Inhibitor	38	32.4
All	2070	26.1
Resolution range (Å)	8.0—2.7	
No. of used reflections ($>2\sigma$)	8742	
R factor	0.231	
Free *R* factor	0.321	
Standard deviation from ideal geometry		
Bond length (Å)	0.006	
Bond angle (°)	1.138	

a) $R_{merge}=\Sigma|I_{obs.}-\langle I\rangle|/\Sigma I_{obs.}$.

Furthermore, compound **27c** was evaluated for *in vivo* ICE inhibitory activity, as described in Experimental. When subcutanously administered in mice, compound **27c** was also found to show inhibitory activity on LPS-primed ATP-induced IL-1β release dose-dependently (Fig. 3). From these results, compound **27c** showed ICE potency not only in the *in vitro* assay, but also in the *in vivo* assay.

Crystallographic Study The size of the P1 residue of compound **27c** is bigger than that of an original carboxyl group; thus, the P1 residue does not appear to fit in the S1 pocket of the enzyme in investigating the crystal structure of ICE complexed with L-709049 (PDB code: 1ICE).[10] To understand the interaction of the inhibitor with the enzyme, a crystallographic study on ICE complexed with compound **27c** was carried out.

Figure 4 shows the interaction between compound **27c** and

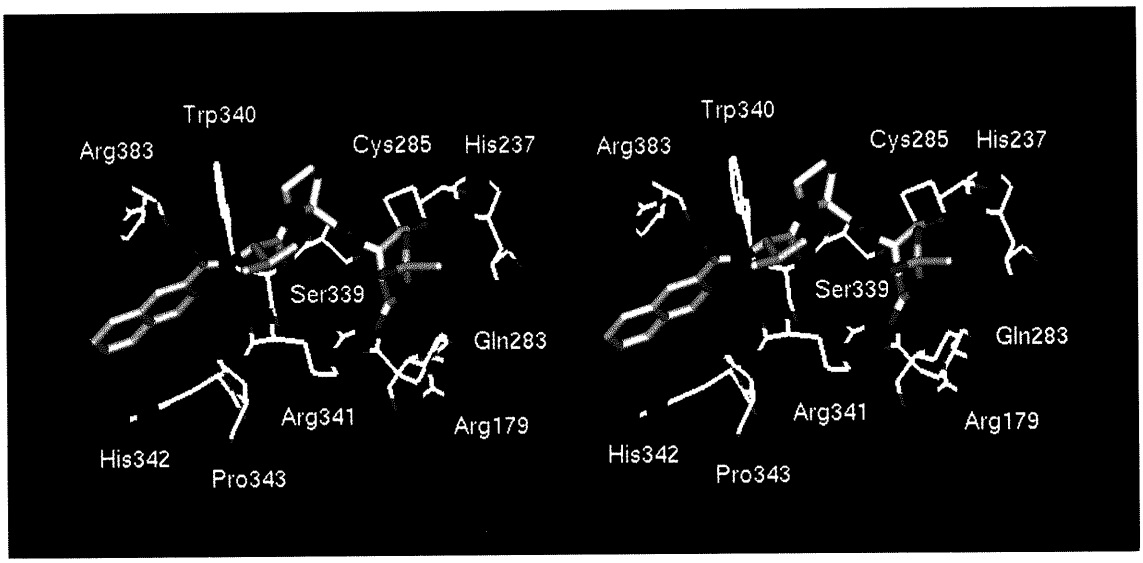


Fig. 4. Stereoview of the Inhibitor Binding Site

Compound **27c** is drawn as a thick line. The inhibitor is covalently linked to the Sγ atom of Cys285.

ICE. The overall structure of ICE, including a covalent bond formed between Cys285 and the aldehyde group of compound **27c**, is basically the same as the previously reported structure.[10] The interactions observed between ICE and the P2 or P3 position, especially the main chain hydrogen bonds with Ser339 and Arg341, are also similar with those of other inhibitors. The proline residue at the P2 position and the valine residue at the P3 are stabilized by hydrophobic interactions with Trp340 and Pro343, respectively. The proline residue is partly exposed to the solvent region, which is consistent with the fact that a series of *N*-alkylglycine analogues (**17a—d**) showed similar inhibitory activity in spite of various functional groups attached to the nitrogen atom.

On the other hand, the P4 naphthyl group and the P1 methanesulfonaminocarbonyl group have a unique binding mode. The naphthyl group in the P4 position, which corresponds to the tyrosine residue of L-709049, has stacking interactions with the side chain of His342 and Arg383. This stacking was not observed with the tyrosine residue[10] and probably contributed to the enhancement of the binding affinity of compound **27c**. When a non-aromatic group was introduced in the P4 position, as in compounds **8d—f**, the stacking interaction was no longer preserved in the structure, which resulted in a drastic loss of binding affinity.

A methanesulfonaminocarbonyl group in the P1 position is tightly accommodated in the S1 pocket, being somewhat less deeply buried in the binding pocket compared with the carboxyl group of the aspartic acid residue of L-709049. The carbonyl oxygen atom forms hydrogen bonds with the side chains of Arg179 and Arg341. The nitrogen atom of the amide group seems to be deprotonated and to have a salt bridge with the above arginine residues. In the case of the aspartic acid residue at the P1 position, there are also hydrogen bonds between the carboxylate of aspartic acid and the side chain of Gln283. In the structure of the complex of compound **27c** and ICE, however, the distance between the oxygen atom of the methanesulfonamide group and the nitrogen atom of Gln283 became longer, approximately 3.6 Å. This distortion of the hydrogen bonding network was caused by

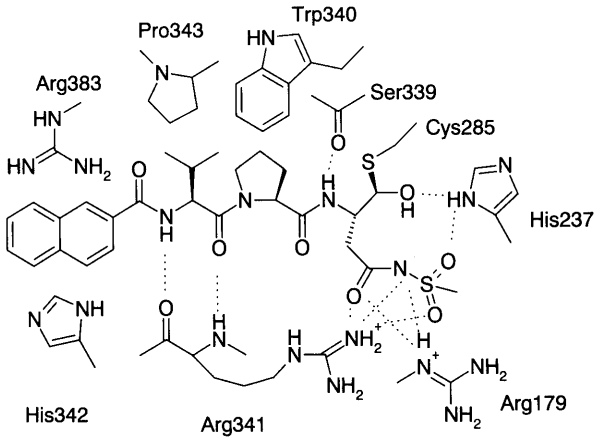


Fig. 5. Schematic Drawing of Interaction of **27c** and ICE

the accommodation of the bigger group in the S1 pocket. The methyl moiety in the methanesulfonaminocarbonyl group is positioned at the outer area of the pocket, in contact with the side chain of residues such as Arg179, His237 and Arg341. The position of the side chain of His237 was dramatically changed from those in other structures in order to avoid steric clash. As mentioned above, a simple modeling study using the crystal structure of ICE indicated that it would be difficult for compound **27c** to bind to ICE without conformational change in the structure of the enzyme. Approximately 95° rotation of the χ 1 angle of His237 enables the binding of compound **27c**. It should also be noted that the methanesulfonamidocarbonyl group had the highest temperature factors in the groups of compound **27c**, suggesting that this group would be disordered in the crystalline environment. The schematic drawing of the interaction of compound **27c** and ICE is shown in Fig. 5.

In conclusion, we synthesized the P4, P2 and P1 site analogues of ICE inhibitor **1** to improve the inhibitory activity in the cell assay. Replacement of the carboxyl group at the P1 position with the methanesulfonylaminocarbonyl group (**27c**) resulted in almost the same inhibitory potency against ICE as

that of **1**. Compound **27c** showed more potent inhibitory activity in the cell assay than did **1**. The c log P value indicated that compound **27c** had a hydrophilic character. The crystal structure of the complex of **27c** and ICE revealed that the overall structure is basically the same as those previously reported. On the other hand, the naphthyl group in the P4 position interacted through a stacking interaction. In the methanesulfonaminocarbonyl group of the P1 position, the carbonyl group and nitrogen atom interact with ICE in the same binding mode as the carboxyl group in L-709049, though the methyl moiety is positioned at the outer area of the pocket. To our knowledge, compound **27c** is the first example of strong ICE inhibitory activity without a carboxyl group at the P1 position. Compound **27c** was also found to show inhibitory activity on LPS-primed ATP-induced IL-1β release in mice. Further pharmacological evaluations of **27c** are in progress.

Experimental

^{1}H-NMR spectra were obtained on a JEOL JNM-EX90 or JNM-A500 spectrometer and chemical shifts are expressed in δ (ppm) values with tetramethylsilane as the internal standard. Abbreviations of the ^{1}H-NMR signal patterns are as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet: br, broad. Mass spectra were obtained on a JEOL JMS-DX300 or Hitachi M-80 spectrometer. High-resolution mass spectra were recorded on VG ZAB-VSE mass spectrometers. Column chromatography on silica gel was performed with Wakogel C-200. Preparative thin-layer chromatography was performed with Merck PLC plate Silica gel 60 F_{254}.

(3*S*,4*RS*)-3-[[*N*-(*N*-Benzyloxycarbonyl-L-valyl)-L-prolyl]amino]-4-hydroxy-5-phenoxypentanoic Acid *tert*-Butyl Ester (4) A mixture of *N*-(*N*-benzyloxycarbonyl-L-valyl)-L-proline (**2**, 9.28 g, 26.6 mmol), (3*S*,4*RS*)-3-amino-4-hydroxy-5-phenoxypentanoic acid *tert*-butyl ester (**3**, 7.14 g, 25.4 mmol), 1-hydroxybenzotriazole (3.60 g, 26.6 mmol), *N*-methylmorpholine (3.4 ml, 30.9 mmol), and 1-ethyl-3-dimethylaminopropylcarbodiimide monohydrochloride (5.11 g, 26.7 mmol) in dichloromethane (200 ml) was stirred at room temperature for 12 h. To the mixture was added saturated aqueous $NaHCO_3$ solution, followed by extraction with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, saturated aqueous NH_4Cl solution and brine, then dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel (AcOEt : hexane=65 : 35) gave **4** (12.0 g, 77%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.86—1.02 (6H, m), 1.41—1.44 (9H, m), 1.96—2.14 (5H, m), 2.50—2.80 (2H, m), 3.40—4.50 (9H, m), 5.04—5.12 (2H, m), 5.40—5.53 (1H, m), 6.88—7.37 (10H, m). FAB-MS *m/z*: 612 $[M+H]^+$.

(3*S*,4*RS*)-3-[*N*-(L-Valyl-L-prolyl)amino]-4-hydroxy-5-phenoxypentanoic Acid *tert*-Butyl Ester (5) A solution of **4** (11.9 g, 19.5 mmol) in MeOH (200 ml) was stirred at room temperature in the presence of 10% Pd–C (3.20 g) under a hydrogen atmosphere. When hydrogen consumption ceased, Pd–C was filtered off. The filtrate was evaporated *in vacuo* to give **5** (9.14 g, 98%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.75—1.02 (6H, m), 1.43—1.44 (9H, m), 1.87—2.63 (6H, m), 2.80—3.20 (3H, m), 3.41—3.58 (3H, m), 3.91—4.10 (3H, m), 4.30—4.70 (2H, m), 6.97—6.97 (3H, m), 7.22—7.31 (2H, m). FAB-MS *m/z*: 478 $[M+H]^+$.

(3*S*,4*RS*)-3-[[*N*-(*N*-Imidazo[1,2-*a*]pyridin-2-yl-carbonyl-L-valyl)-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (7a) A mixture of imidazo[1,2-*a*]pyridine-2-carboxylic acid (89 mg, 0.55 mmol), 1-hydroxybenzotriazole (78 mg, 0.58 mmol), *N*-methylmorpholine (63 μl, 0.58 mmol), 1-ethyl-3-dimethylaminopropylcarbodiimide monohydrochloride (111 mg, 0.58 mmol) and **5** (250 mg, 0.52 mmol) in *N*,*N*-dimethylformamide (DMF, 3 ml) was stirred at room temperature for 8 h. The mixture was added to saturated aqueous $NaHCO_3$ solution and extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, saturated aqueous NH_4Cl solution and brine, then dried over Na_2SO_4 and evaporated *in vacuo* to give the crude product of (3*S*,4*RS*)-4-hydroxy-3-[[*N*-(*N*-imidazo[1,2-*a*]pyridin-2-yl-carbonyl-L-valyl)-L-prolyl]amino]-5-phenoxypentanoic acid *tert*-butyl ester (**6a**) as a pale yellow oil. To an ice-cooled solution of crude **6a** in dichloromethane (5 ml) was added Dess–Martin periodinane (699 mg, 1.65 mmol), and the mixture was stirred for 1 h. To the mixture was added saturated aqueous $NaHCO_3$ solution, followed by extraction with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. Purification with preparative thin layer chromatography of the residue (20% MeOH–$CHCl_3$) gave **7a** (270 mg, 79%) as a pale yellow amorphous substance. ^{1}H-NMR ($CDCl_3$): 1.02—1.09 (6H, m), 1.44—1.45 (9H, m), 1.95—2.32 (5H, m), 2.72—2.78 (1H, m), 2.99—3.05 (1H, m), 3.68—3.77 (1H, m), 3.86—3.96 (1H, m), 4.48—4.60 (1H, m), 4.78—4.85 (1H, m), 4.84—4.86 (1H, m), 4.88—4.93 (1H, m), 5.00—5.08 (1H, m), 6.81—6.85 (1H, m), 6.90—7.03 (3H, m), 7.20—7.36 (2H, m), 7.47—7.64 (2H, m), 7.93—7.99 (1H, m), 8.11 (1H, s), 8.11—8.14 (1H, m). FAB-MS *m/z*: 620 $[M+H]^+$.

(3*S*,4*RS*)-3-[[*N*-(*N*-Imidazo[1,2-*a*]pyridin-3-yl-carbonyl-L-valyl)-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (7b) The title compound was prepared from imidazo[1,2-*a*]pyridine-3-carboxylic acid in the same manner as described above, and was also a yellow amorphous substance (88%). ^{1}H-NMR ($CDCl_3$): 1.00—1.11 (6H, m), 1.44—1.45 (9H, m), 2.00—2.30 (5H, m), 2.73—2.82 (1H, m), 2.98—3.05 (1H, m), 3.70—3.78 (1H, m), 3.85—3.93 (1H, m), 4.50—4.59 (1H, m), 4.83—4.84 (1H, m), 4.83—4.96 (2H, m), 4.96—5.03 (1H, m), 6.75—6.80 (1H, m), 6.89—7.01 (4H, m), 7.25—7.30 (1H, m), 7.34—7.39 (1H, m), 7.52—7.58 (1H, m), 7.68—7.71 (1H, m), 8.13 (1H, s), 9.42—9.44 (1H, m). FAB-MS *m/z*: 620 $[M+H]^+$.

(3*S*,4*RS*)-3-[[*N*-(*N*-Cinnamoyl-L-valyl)-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (7c) The title compound was prepared from cinnamic acid in the same manner as the yellow amorphous substance described above (78%). ^{1}H-NMR (DMSO-d_6): 0.85—1.00 (6H, m), 1.38—1.41 (9H, m), 1.70—2.16 (5H, m), 2.48—2.60 (1H, m), 2.71—2.85 (1H, m), 3.58—3.72 (1H, m), 3.82—3.93 (1H, m), 4.29—4.75 (3H, m), 4.90—4.98 (1H, m), 5.08—5.18 (1H, m), 6.76—6.95 (4H, m), 7.19—7.29 (2H, m), 7.34—7.45 (5H, m), 7.50—7.56 (2H, m), 8.28—8.34 (1H, m). FAB-MS *m/z*: 606 $[M+H]^+$.

(3*S*,4*RS*)-3-[[*N*-(*N*-Bromomethylcarbonyl-L-valyl)-L-prolyl]amino]-4-hydroxy-5-phenoxypentanoic Acid *tert*-Butyl Ester (6d) To an ice-cooled mixture of **5** (2.50 g, 5.23 mmol) and triethylamine (0.73 ml, 5.23 mmol) in dichloromethane (100 ml) was added bromoacetylbromide (0.46 ml, 5.23 mmol), and the reaction mixture was stirred for 1 h. The mixture was diluted with water and extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. Column chromatography of the residue on silica gel (2% MeOH–$CHCl_3$) gave **6d** (2.75 g, 88%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.90—1.03 (6H, m), 1.42—1.46 (9H, m), 1.99—2.17 (5H, m), 2.50—2.80 (m, 2H), 3.42—4.70 (10H, m), 6.89—7.30 (5H, m). FAB-MS *m/z*: 598 $[M+H]^+$.

(3*S*,4*RS*)-4-Hydroxy-3-[[*N*-[*N*-(1-morpholinomethylcarbonyl)-L-valyl]-L-prolyl]amino]-5-phenoxypentanoic Acid *tert*-Butyl Ester (6e) To an ice-cooled mixture of **6d** (300 mg, 0.50 mmol) and diisopropylethylamine (0.11 ml, 0.60 mmol) in dichloromethane (20 ml) was added morpholine (0.06 ml, 0.60 mmol); then the mixture was stirred overnight. The mixture was diluted with water and extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated *in vacuo*. Column chromatography of the residue on silica gel (2% MeOH–$CHCl_3$) gave **6e** (313 mg, 100%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.88—1.02 (6H, m), 1.42—1.46 (9H, m), 1.99—2.15 (5H, m), 2.49—2.75 (6H, m), 2.90—3.02 (2H, m), 3.38—4.70 (12H, m), 6.90—6.96 (3H, m), 7.24—7.30 (2H, m). FAB-MS *m/z*: 605 $[M+H]^+$.

(3*S*,4*RS*)-3-[[*N*-[*N*-(1-Morpholinomethylcarbonyl)-L-valyl]-L-prolyl]-amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (7d) To an ice-cooled solution of **6e** (275 mg, 0.46 mmol) in dichloromethane (20 ml) was added Dess–Martin periodinane (386 mg, 0.91 mmol) and the mixture was stirred for 3 h. To the mixture was added saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. Preparative thin-layer chromatography of the residue (20% MeOH–$CHCl_3$) gave **7d** (311 mg, quant.) as a pale yellow amorphous substance. ^{1}H-NMR ($CDCl_3$): 0.92—1.03 (6H, m), 1.41—1.44 (9H, m), 2.01—2.26 (5H, m), 2.54—2.55 (4H, m), 2.70—2.80 (1H, m), 2.98—3.04 (3H, m), 3.70—3.96 (6H, m), 4.28—4.30 (1H, m), 4.45—4.80 (2H, m), 4.79—5.05 (3H, m), 6.89—6.99 (3H, m), 7.25—7.29 (2H, m), 7.65—7.71 (1H, m). FAB-MS *m/z*: 603 $[M+H]^+$.

(3*S*,4*RS*)-3-[[*N*-[*N*-(4-Carboxypropionyl)-L-valyl]-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (7e) To a mixture of **5** (273 mg, 0.57 mmol) and succinic anhydride (57 mg, 0.57 mmol) in dichloromethane (3 ml) was added acetic acid (2 ml), and the mixture was stirred at room temperature for 1 h. The mixture was evaporated *in vacuo*. To the residue was added toluene (10 ml), then the solution was evaporated *in vacuo* again to give the crude product of (3*S*,4*RS*)-4-hydroxy-3-[[*N*-[*N*-(4-carboxypropionyl)-L-valyl]-L-prolyl]amino]-5-phenoxypentanoic acid *tert*-

butyl ester (**6f**) as a pale yellow oil. To an ice-cooled solution of crude **6f** in dichloromethane (5 ml) was added Dess–Martin periodinane (607 mg, 1.43 mmol), and the mixture was stirred for 2 h. To the mixture was added saturated aqueous $NaHCO_3$ solution, followed by extraction with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. Purification with preparative thin layer chromatography of the residue (20% MeOH–$CHCl_3$) gave **7e** (230 mg, 70%) as a pale yellow amorphous substance. ^{1}H-NMR (DMSO-d_6): 0.81—0.93 (6H, m), 1.38—1.41 (9H, m), 1.70—2.12 (5H, m), 2.24—2.41 (4H, m), 2.50—2.60 (1H, m), 2.70—2.84 (1H, m), 3.53—3.65(1H, m), 3.73—3.83 (1H, m), 4.23—4.34 (2H, m), 4.37—4.72 (1H, m), 4.88—4.95 (1H, m), 5.08—5.16 (1H, m), 6.82—6.95 (3H, m), 7.19—7.28 (2H, m), 7.66—8.17 (2H, m), 8.62—8.86 (1H, m). FAB-MS *m/z*: 598 $[M+Na]^+$.

(3*S*,4*RS*)-3-[[*N*-[*N*-(4-Carboxybutyryl)-L-valyl]-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (7f) The title compound was prepared from glutaric anhydride in the same manner described above, and was a yellow amorphous substance (65%). ^{1}H-NMR (DMSO-d_6): 0.81—0.94 (6H, m), 1.38—1.41 (9H, m), 1.62—2.20 (11H, m), 2.50—2.60 (1H, m), 2.70—2.84 (1H, m), 3.53—3.65(1H, m), 3.76—3.87 (1H, m), 4.23—4.34 (2H, m), 4.37—4.72 (1H, m), 4.89—4.98 (1H, m), 5.08—5.16 (1H, m), 6.82—6.95 (3H, m), 7.19—7.31 (2H, m), 7.66—8.06 (2H, m), 8.62—8.93 (1H, m). FAB-MS *m/z*: 612 $[M+Na]^+$.

(3*S*,4*RS*)-3-[[*N*-(*N*-Imidazo[1,2-*a*]pyridin-2-yl-carbonyl-L-valyl)-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid (8a) To a solution of **7a** in dichloromethane (5 ml) was added trifluoroacetic acid (1.0 ml), and the mixture was stirred for 2 h at room temperature. The mixture was diluted with benzene (5 ml) and the solvent was evaporated *in vacuo*. To the residue was added benzene (5 ml) again, followed by evaporation *in vacuo*. Preparative thin-layer chromatography of the residue (20% MeOH–$CHCl_3$) gave **8a** (210 mg, 89%) as a pale yellow solid. ^{1}H-NMR ($CDCl_3$): 0.97—1.03 (6H, m), 1.85—2.30 (5H, m), 2.75—3.19 (2H, m), 3.70—3.78 (1H, m), 3.88—3.97 (1H, m), 4.50—4.90 (5H, m), 6.80—6.95 (4H, m), 7.20—7.28 (3H, m), 7.53—7.68 (1H, m), 8.08—8.18 (3H, m). FAB-MS *m/z*: 562 $[M-H]^-$. HRMS *m/z* Calcd for $C_{29}H_{34}N_5O_7$ $[M+H]^+$: 564.2458. Found: 564.2437.

(3*S*,4*RS*)-3-[[*N*-(*N*-Imidazo[1,2-*a*]pyridin-3-yl-carbonyl-L-valyl)-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid (8b) The title compound was prepared from **7b** in the same manner as described above as a yellow amorphous substance (70%). ^{1}H-NMR ($CDCl_3$): 0.95—1.20 (6H, m), 1.90—2.30 (5H, m), 2.50—3.25 (2H, m), 3.70—5.20 (7H, m), 6.75—8.40 (10H, m). FAB-MS *m/z*: 562 $[M-H]^-$. HRMS *m/z* Calcd for $C_{29}H_{34}N_5O_7$ $[M+H]^+$: 564.2458. Found: 564.2444.

(3*S*,4*RS*)-3-[[*N*-(*N*-Cinnamoyl-L-valyl)-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid (8c) The title compound was prepared from **7c** in the same manner as described above as a yellow amorphous substance (56%). ^{1}H-NMR (DMSO-d_6): 0.85—1.05 (6H, m), 1.90—2.35 (5H, m), 2.60—2.85 (1H, m), 3.05—3.25 (1H, m), 3.60—3.75 (1H, m), 3.95—4.10 (1H, m), 4.30—5.00 (5H, m), 6.45—6.60 (1H, m), 6.75—7.00 (3H, m), 7.15—8.00 (10H, m). FAB-MS *m/z*: 550 $[M+H]^+$. HRMS *m/z* Calcd for $C_{30}H_{36}N_3O_7$ $[M+H]^+$: 550.2553. Found: 550.2530.

(3*S*,4*RS*)-3-[[*N*-[*N*-(1-Morpholinomethylcarbonyl)-L-valyl]-L-prolyl]-amino]-4-oxo-5-phenoxypentanoic Acid (8d) The title compound was prepared from **7d** in the same manner as described above as a yellow amorphous substance (87%). ^{1}H-NMR (DMSO-d_6): 0.86—0.98 (6H, m), 1.80—2.20 (5H, m), 2.50—3.45 (8H, m), 3.55—3.90 (7H, m), 4.40—5.00 (5H, m), 6.85—6.99 (3H, m), 7.23—7.33 (2H, m), 7.70—8.00 (2H, m). FAB-MS *m/z*: 547 $[M+H]^+$. HRMS *m/z* Calcd for $C_{27}H_{39}N_4O_8$ $[M+H]^+$: 547.2768. Found: 547.2781.

(3*S*,4*RS*)-3-[[*N*-[*N*-(4-Carboxypropionyl)-L-valyl]-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid (8e) The title compound was prepared from **7e** in the same manner as described above as a yellow oil (84%). ^{1}H-NMR (DMSO-d_6): 0.82—0.93 (6H, m), 1.70—2.12 (5H, m), 2.28—2.44 (4H, m), 2.54—2.80 (2H, m), 3.54—3.64(1H, m), 3.71—3.81 (1H, m), 4.25—4.35 (2H, m), 4.42—4.50 (1H, m), 4.83—4.94 (1H, m), 5.02—5.11 (1H, m), 6.84—6.95 (3H, m), 7.20—7.28 (2H, m), 7.35—8.08 (2H, m), 8.56—8.73 (1H, m).FAB-MS *m/z*: 564 $[M+2Na-H]^+$. HRMS *m/z* Calcd for $C_{25}H_{32}N_3O_9$ $[M-H]^+$: 518.2138. Found: 518.2130.

(3*S*,4*RS*)-3-[[*N*-[*N*-(4-Carboxybutyryl)-L-valyl]-L-prolyl]amino]-4-oxo-5-phenoxypentanoic Acid (8f) The title compound was prepared from **7f** in the same manner as described above as a yellow oil (75%). ^{1}H-NMR (DMSO-d_6): 0.81—0.94 (6H, m), 1.62—2.20 (11H, m), 2.50—2.76 (2H, m), 3.53—3.65 (1H, m), 3.70—3.80 (1H, m), 4.23—4.34 (2H, m), 4.37—4.72 (1H, m), 4.89—4.98 (1H, m), 5.00—5.20 (1H, m), 6.82—6.95 (3H, m), 7.19—7.31 (2H, m), 7.40—8.06 (2H, m), 8.55—8.80 (1H, m). FAB-MS *m/z*: 578 $[M+2Na-H]^+$. HRMS *m/z* Calcd for $C_{26}H_{34}N_3O_9$ $[M-H]^+$: 532.2295. Found: 532.2291.

***N*-Butylglycine Ethyl Ester (11a)** A mixture of glycine ethyl ester **monohydrochloride** (**9**, 12.4 g, 0.10 mol), iodobutane (3.68 g, 0.020 mol) and potassium carbonate (17.3 g, 0.13 mol) in acetonitrile (200 ml) was stirred at room temperature for 18 h. The mixture was diluted with water (100 ml) and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. Column chromatography of the residue on silica gel ($CHCl_3$) gave **11a** (1.58 g, 50%) as a yellow oil. ^{1}H-NMR ($CDCl_3$): 0.92 (3H, t, *J*=7.4 Hz), 1.28 (3H, t , *J*=7.4 Hz), 1.32—1.53 (4H, m), 2.61 (1H, t, *J*=7.0 Hz), 3.41 (2H, d, *J*=8.1 Hz), 4.20 (2H, q, *J*=7.4 Hz). FAB-MS *m/z*: 160 $[M+H]^+$.

***N*-(3-Cyanopropyl)glycine Ethyl Ester (11b)** The title compound was prepared from 4-bromobutyronitrile in the same manner as described above as a pale yellow oil (91%). ^{1}H-NMR ($CDCl_3$): 1.29 (3H, t, *J*=7.4 Hz), 1.82 (2H, tt, *J*=7.0, 6.6 Hz), 2.48 (2H, t, *J*=7.0 Hz), 2.76 (2H, t, *J*=6.6 Hz), 3.39 (2H, s), 4.20 (2H, q, *J*=7.4 Hz). FAB-MS *m/z*: 171 $[M+H]^+$.

***N*-[3-(1*H*-Imidazol-1-yl)propyl]glycine Ethyl Ester (11c)** A mixture of ethyl bromoacetate (**10**, 3.34 g, 20 mmol), triethylamine (2.43 g, 24 mmol) and 3-(1*H*-imidazol-1-yl)propylamine (2.50 g, 20 mmol) in tetrahydrofuran (THF, 50 ml) was stirred at room temperature for 15 h. The mixture was diluted with water (300 ml) and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel (2% MeOH–$CHCl_3$) gave **11c** (1.59 g, 38%) as a yellow oil. ^{1}H-NMR ($CDCl_3$): 1.28 (3H, t, *J*=6.9 Hz), 1.88—1.97 (2H, m), 2.59 (2H, t, *J*=6.5 Hz), 3.64 (2H, s), 4.06 (2H, t, *J*=6.9 Hz), 4.19 (2H, q, *J*=6.9 Hz), 6.92 (1H, t, *J*=1.2 Hz), 7.06 (1H, t, *J*=1.2 Hz), 7.48 (1H, s). FAB-MS *m/z*: 212 $[M+H]^+$.

***N*-(*N*-*tert*-Butoxycarbonyl-L-valyl)-*N*-butylglycine Ethyl Ester (12a)** A mixture of **11a** (318 mg, 2.0 mmol), *N*-*tert*-butoxycarbonyl-L-valine (437 mg, 2.0 mmol), 1-hydroxybenzotriazole (270 mg, 2.0 mmol), *N*-methylmorpholine (0.22 ml, 2.0 mmol), and 1-ethyl-3-dimethylaminopropylcarbodiimide monohydrochloride (384 mg, 2.0 mmol) in DMF (10 ml) was stirred at room temperature for 4 h. To the mixture was added a saturated aqueous $NaHCO_3$ solution, followed by extraction with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, saturated aqueous NH_4Cl solution and brine, then dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel ($CHCl_3$) gave **12a** (595 mg, 83%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.89—1.02 (9H, m) 1.26 (3H, t, *J*=7.2 Hz), 1.31—1.59 (4H, m), 1.43 (9H, s), 1.93—2.04 (1H, m), 3.30—3.35 (1H, m), 3.45—3.53 (1H, m), 3.70 (1H, d, *J*=17.1 Hz), 4.18 (2H, q, *J*=7.2 Hz), 4.16—4.23 (1H, m), 4.41 (1H, d, *J*=17.1 Hz), 4.38—4.51 (1H, m), 5.19 (1H, t, *J*=9.3 Hz). FAB-MS *m/z*: 359 $[M+H]^+$.

***N*-(*N*-*tert*-Butoxycarbonyl-L-valyl)-*N*-(3-cyanopropyl)glycine Ethyl Ester (12b)** The title compound was prepared from **11b** (6.90 g, 40 mmol) in the same manner as described above, as a colorless oil (10.1 g, 68%). ^{1}H-NMR ($CDCl_3$): 0.85—1.08 (6H, m), 1.23—1.33 (3H, m), 1.43 (9H, s), 1.80—2.18 (3H, m), 2.37—2.52 (2H, m), 3.42—3.85 (3H, m), 3.73—4.55 (4H, m), 5.00—5.20 (1H, m). FAB-MS *m/z*: 370 $[M+H]^+$.

***N*-(*N*-*tert*-Butoxycarbonyl-L-valyl)-*N*-[3-(1*H*-imidazol-1-yl)propyl]-glycine Ethyl Ester (12c)** The title compound was prepared from **11c** (760 mg, 3.6 mmol) in the same manner as described above to give a colorless oil (1.11 g, 75 %). ^{1}H-NMR ($CDCl_3$): 0.92—0.95 (6H, m) 1.26 (3H, t, *J*=7.2 Hz), 1.43 (9H, s), 1.91—2.23 (3H, m), 3.23—3.72 (3H, m), 3.95—4.44 (6H, m), 5.10—5.13 (1H, m), 6.95—7.01 (1H, m), 7.05—7.08 (1H, m), 7.49—7.51 (1H, m). FAB-MS *m/z*: 411 $[M+H]^+$.

***N*-Butyl-*N*-(*N*-(2-naphthoyl)-L-valyl)glycine Ethyl Ester (13a)** To a solution of **12a** (570 mg, 1.6 mmol) in dichloromethane (10 ml) was added trifluoroacetic acid (2 ml), and the mixture was stirred at room temperature for 2 h. To the mixture was added benzene (5 ml) and the solvent was evaporated *in vacuo*. To the residue was added benzene (5 ml), followed by evaporation *in vacuo*. The residue was dissolved in $CHCl_3$ (10 ml), and 2-naphthoyl chloride (303 mg, 1.6 mmol) was added to the solution. The mixture was cooled to 4 °C, then triethylamine (322 mg, 3.2 mmol) was added and stirred for 1 h. To the mixture was added brine, and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. Column chromatography of the residue on silica gel (2% MeOH–$CHCl_3$) gave **13a** (340 mg, 93%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.99 (3H, t, *J*=7.3 Hz), 1.05 (3H, d, *J*=6.6 Hz), 1.13 (3H, d, *J*=6.6 Hz), 1.27 (3H, t, *J*=7.2 Hz), 1.37—1.69 (4H, m), 2.20—2.26 (1H, m), 3.36—3.61 (2H, m), 3.75 (1H, d, *J*=16.8 Hz), 4.20 (2H, q, *J*=7.3 Hz), 4.43 (1H, d, *J*=16.8 Hz), 5.16 (1H, m), 7.03 (1H, d, *J*=8.8 Hz), 7.51—7.59 (2H, m), 7.86—7.95 (4H, m), 8.33 (1H, s). FAB-MS *m/z*: 413 $[M+H]^+$.

***N*-(3-Cyanopropyl)-*N*-[*N*-(2-naphthoyl)-L-valyl]glycine Ethyl Ester

(13b) The title compound was prepared from **12b** (5.00 g, 14 mmol) in the same manner as described above as a colorless amorphous substance (5.17 g, 87%). ^{1}H-NMR ($CDCl_3$): 0.90—1.42 (9H, m), 1.80—2.65 (5H, m), 3.40—4.55 (6H, m), 4.68—5.18 (1H, m), 6.85—7.10 (1H, m), 7.45—7.68 (2H, m), 7.75—8.05 (4H, m), 8.31 (1H, s). FAB-MS *m/z*: 424 $[M+H]^+$.

***N*-[3-(1*H*-Imidazol-1-yl)propyl]-*N*-[*N*-(2-naphthoyl)-L-valyl]glycine Ethyl Ester (13c)** The title compound was prepared from **12c** (1.10 g, 2.7 mmol) in the same manner as described above as a colorless oil (1.11 g, 89%). ^{1}H-NMR (DMSO-d_6): 0.81—0.95 (6H, m), 1.17—1.21 (3H, m), 1.88—2.20 (3H, m), 3.23—3.72 (3H, m), 3.90—4.76 (5H, m), 5.10—5.13 (1H, m), 6.85—6.89 (1H, m), 7.14—7.21 (1H, m), 7.59—7.64 (2H, m), 7.95—8.02 (3H, m), 8.29—8.30 (1H, m), 8.51—8.75 (2H, m). FAB-MS *m/z*: 465 $[M+H]^+$.

***N*-[*N*-(2-Naphthoyl)-L-valyl]-*N*-[3-(2*H*-tetrazol-5-yl)propyl]glycine Ethyl Ester (13d)** To a solution of **13b** (2.50 g, 5.9 mmol) in DMF (15 ml) was added ammonium chloride (376 mg, 7.0 mmol) and sodium azide (460 mg, 7.1 mmol), and the mixture was then stirred at 100 °C for 24 h. The mixture was cooled to room temperature, diluted with ethyl acetate (100 ml), washed with 1 M HCl and brine, dried over $MgSO_4$ and evaporated *in vacuo*. Column chromatography of the residue on silica gel (2% MeOH–$CHCl_3$) gave **13d** (1.26 g, 46%) as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$): 1.00—1.08 (6H, m), 1.24—1.34 (3H, m), 1.94—2.42 (3H, m), 2.99—3.70 (4H, m), 3.79—4.30 (3H, m), 4.36—4.81 (1H, m), 4.83—4.90 (1H, m), 7.53—7.64 (2H, m), 7.84—7.97 (4H, m), 8.33—8.38 (1H, m). FAB-MS *m/z*: 465 $[M-H]^-$.

***N*-[*N*-(2-Naphthoyl)-L-valyl]-*N*-[3-(2-triphenylmethyltetrazol-5-yl)-propyl]glycine Ethyl Ester (13e)** To a solution of **13d** (1.05 g, 2.3 mmol) in $CHCl_3$ (20 ml) was added triethylamine (0.35 ml, 2.5 mmol) and chlorotriphenylmethane (0.69 g, 2.5 mmol), and the mixture was stirred at room temperature for 2 h. The mixture was diluted with $CHCl_3$ (100 ml), washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue, washed with ether, gave **13e** (1.81 g, quant.) as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$): 0.99—1.10 (6H, m), 1.21—1.32 (3H, m), 2.00—2.25 (3H, m), 2.89—3.76 (5H, m), 4.11—4.20 (2H, m), 4.30—4.42 (1H, m), 4.84—5.14 (1H, m), 7.07—7.37 (15H, m), 7.50—7.58 (2H, m), 7.82—7.94 (4H, m), 8.28—8.31 (1H, m). FAB-MS *m/z*: 707 $[M-H]^-$.

(3*S*)-3-[[*N*-Butyl-*N*-[*N*-(2-naphthoyl)-L-valyl]glycyl]amino]-4-hydroxy-5-phenoxypentanoic Acid *tert*-Butyl Ester (15a) To a solution of **13a** (570 mg, 1.4 mmol) in methanol (5 ml) was added aqueous 1 M NaOH solution (1.5 ml), and the mixture was stirred at room temperature for 2 h. To the mixture was added 5% aqueous citric acid solution (5 ml) and water (10 ml). The mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give crude *N*-butyl-*N*-[*N*-(2-naphtoyl)-L-valyl]glycine (**14a**, 540 mg, quant.). Using the crude **14a** (105 mg, 0.37 mmol), the title compound was prepared in the same manner as described in the synthesis of **4** as a colorless amorphous substance (151 mg, 63%). ^{1}H-NMR ($CDCl_3$): 0.86—1.00 (3H, m), 1.06—1.12 (6H, m), 1.33—1.80 (4H, m), 1.41 (9H, s), 2.10—2.25 (1H, m), 2.55—2.67 (4H, m), 3.52—3.57 (2H, m), 3.89—4.09 (4H, m), 4.30—4.50 (1H, m), 4.90—5.00 (1H, m), 6.79—6.94 (4H, m), 7.17—7.26 (3H, m), 7.49—7.59 (2H, m), 7.82—7.90 (4H, m), 8.22—8.31 (1H, m). FAB-MS *m/z*: 648 $[M+H]^+$.

(3*S*)-3-[[*N*-(3-Cyanopropyl)-*N*-[*N*-(2-naphthoyl)-L-valyl]glycyl]-amino]-4-hydroxy-5-phenoxypentanoic Acid *tert*-Butyl Ester (15b) The title compound was prepared from **13b** (2.00 g, 4.7 mmol) in the same manner as described above as a colorless amorphous substance (683 mg, 69%). ^{1}H-NMR ($CDCl_3$): 1.04—1.13 (6H, m), 1.37—1.43 (9H, m), 1.89—2.74 (7H, m), 3.35—4.92 (9H, m), 6.82—6.96 (3H, m), 7.17—7.26 (2H, m), 7.50—7.64 (2H, m), 7.75—7.93 (4H, m), 8.23—8.31 (1H, m). FAB-MS *m/z*: 659 $[M+H]^+$.

(3*S*)-3-[[*N*-[3-(1*H*-Imidazol-1-yl)propyl]-*N*-[*N*-(2-naphthoyl)-L-valyl]-glycyl]amino]-4-hydroxy-5-phenoxypentanoic Acid *tert*-Butyl Ester (15c) The title compound was prepared from **13c** in the same manner as described above as a colorless amorphous substance (63%). ^{1}H-NMR (DMSO-d_6): 0.90—1.09 (6H, m), 1.36—1.45 (9H, m), 1.50—2.75 (5H, m), 3.00—4.41 (10H, m), 4.68—4.78 (1H, m), 6.84—6.92 (3H, m), 7.06—7.26 (8H, m), 7.54—7.61 (1H, m), 7.83—7.88 (3H, m), 8.24—8.30 (1H, m). FAB-MS *m/z*: 700 $[M+H]^+$.

(3*S*)-3-[[*N*-(*N*-(2-Naphthoyl)-L-valyl)-*N*-[3-(2-triphenylmethyltetrazol-5-yl)propyl]glycyl]amino]-4-hydroxy-5-phenoxypentanoic Acid *tert*-Butyl Ester (15d) The title compound was prepared from **13e** in the same manner as described above as a pale yellow oil (54%). ^{1}H-NMR (DMSO-d_6): 0.99—1.10 (6H, m), 1.21—1.32 (3H, m), 2.00—2.25 (3H, m), 2.89—3.76 (5H, m), 4.11—4.20 (2H, m), 4.30—4.42 (1H, m), 4.84—5.14 (1H, m), 7.07—7.37 (15H, m), 7.50—7.58 (2H, m), 7.82—7.94 (4H, m), 8.28—8.31 (1H, m). FAB-MS *m/z*: 707 $[M-H]^-$.

(3*S*)-3-[*N*-Butyl-*N*-[*N*-(2-naphthoyl)-L-valyl]glycyl]amino-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (16a) The title compound was prepared from **15a** in the same manner as described for the synthesis of **7a** as a colorless oil (61%). ^{1}H-NMR ($CDCl_3$): 0.91—1.03 (3H, m), 1.06—1.11 (6H, m), 1.35 (3H, s), 1.39 (6H, s), 1.57—1.75 (4H, m), 2.20—2.30 (1H, m), 2.76—3.03 (2H, m), 3.54—3.58 (2H, m), 4.07 (2H, s), 4.70—5.06 (4H, m), 6.83—6.96 (4H, m), 7.20—7.38 (3H, m), 7.51—7.59 (2H, m), 7.80—7.93 (4H, m), 8.29—8.33 (1H, m). FAB-MS *m/z*: 646 $[M+H]^+$.

(3*S*)-3-[[*N*-(3-Cyanopropyl)-*N*-[*N*-(2-naphthoyl)-L-valyl]glycyl]-amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (16b) The title compound was prepared from **15b** in the same manner as described in the synthesis of **7a** as a colorless oil (45%). ^{1}H-NMR ($CDCl_3$): 1.01—1.13 (6H, m), 1.37—1.41 (9H, m), 1.90—2.30 (3H, m), 2.34—2.56 (2H, m), 2.80—3.04 (2H, m), 3.60—4.20 (4H, m), 4.70—5.00 (4H, m), 6.75—7.00 (3H, m), 7.20—7.28 (2H, m), 7.50—7.61 (2H, m), 7.78—7.94 (4H, m), 8.22—8.31 (1H, m). FAB-MS *m/z*: 657 $[M+H]^+$.

(3*S*)-3-[[*N*-[3-(1*H*-imidazol-1-yl)propyl]-*N*-[*N*-(2-naphthoyl)-L-valyl]-glycyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (16c) The title compound was prepared from **15c** in the same manner as described for the synthesis of **7a** as a colorless oil (48%). ^{1}H-NMR (DMSO-d_6): 0.91—1.08 (6H, m), 1.32—1.41 (9H, m), 2.03—2.45 (2H, m), 2.75—3.00 (2H, m), 3.25—3.70 (2H, m), 3.84—4.35 (5H, m), 4.71—4.95 (4H, m), 6.84—6.92 (3H, m), 7.06—7.27 (7H, m), 7.45—7.59 (3H, m), 7.84—7.91 (3H, m), 8.31 (1H, s). FAB-MS *m/z*: 698 $[M+H]^+$.

(3*S*)-3-[[*N*-(*N*-(2-Naphthoyl)-L-valyl)-*N*-[3-(2-triphenylmethyltetrazol-5-yl)propyl]glycyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (16d) The title compound was prepared from **15d** in the same manner as described for the synthesis of **7a** as a pale yellow oil (62%). ^{1}H-NMR ($CDCl_3$): 1.00—1.06 (6H, m), 1.33—1.37 (9H, m), 2.00—2.30 (3H, m), 2.70—3.07 (4H, m), 3.65—3.80 (2H, m), 3.94—4.35 (2H, m), 4.70—5.00 (4H, m), 6.82—6.93 (3H, m), 7.06—7.12 (5H, m), 7.15—7.25 (2H, m), 7.25—7.34 (10H, m), 7.48—7.58 (2H, m), 7.76—7.90 (4H, m), 8.25—8.31 (1H, m). FAB-MS *m/z*: 942 $[M+H]^+$.

(3*S*)-3-[*N*-Butyl-*N*-[*N*-(2-naphthoyl)-L-valyl]glycyl]amino-4-oxo-5-phenoxypentanoic Acid (17a) The title compound was prepared from **16a** in the same manner as described for the synthesis of **8a** as a pale yellow amorphous substance (37%). ^{1}H-NMR ($CDCl_3$): 0.83—0.99 (9H, m), 1.20—1.70 (4H, m), 2.20—2.30 (1H, m), 2.60—2.80 (2H, m), 3.20—3.84 (3H, m), 4.11—4.21 (1H, m), 4.50—5.11 (3H, m), 6.85—6.90 (3H, m), 7.19—7.23 (2H, m), 7.58—7.63 (2H, m), 7.90—8.01 (4H, m), 8.52 (1H, s), 8.52—8.75 (1H, m), 12.5 (1H, br s). FAB-MS *m/z*: 588 $[M+H]^+$. HRMS *m/z* Calcd for $C_{33}H_{38}N_3O_7$ $[M+H]^+$: 588.2710. Found: 588.2723.

(3*S*)-3-[[*N*-(3-Cyanopropyl)-*N*-[*N*-(2-naphthoyl)-L-valyl]glycyl]-amino]-4-oxo-5-phenoxypentanoic Acid (17b) The title compound was prepared from **16b** in the same manner as described for the synthesis of **8a** as a pale yellow amorphous substance (72%). ^{1}H-NMR (DMSO-d_6): 0.87—1.04 (6H, m), 1.75—2.78 (7H, m), 3.25—3.70 (2H, m), 3.70—4.25 (2H, m), 4.52—5.10 (4H, m), 6.83—6.95 (3H, m), 7.18—7.27 (2H, m), 7.53—7.65 (2H, m), 7.93—8.05 (4H, m), 8.49—8.72 (1H, m). FAB-MS *m/z*: 599 $[M-H]^-$. HRMS *m/z* Calcd for $C_{33}H_{37}N_4O_7$ $[M+H]^+$: 601.2662. Found: 601.2673.

(3*S*)-3-[[*N*-[3-(1*H*-Imidazol-1-yl)propyl]-*N*-[*N*-(2-naphthoyl)-L-valyl]-glycyl]amino]-4-oxo-5-phenoxypentanoic Acid (17c) The title compound was prepared from **16c** in the same manner as described for the synthesis of **8a** as a colorless amorphous substance (65%). ^{1}H-NMR (DMSO-d_6): 0.90—0.96 (6H, m), 1.90—2.35 (2H, m), 2.60—2.85 (2H, m), 3.20—3.60 (2H, m), 3.77—3.79 (1H, m), 4.00—4.20 (4H, m), 4.50—5.20 (4H, m), 6.84—6.91 (3H, m), 7.15—7.41 (3H, m), 7.52—7.70 (3H, m), 7.96—8.14 (4H, m), 8.51 (1H, s), 8.60—8.80 (1H, m). FAB-MS *m/z*: 642 $[M+H]^+$. HRMS *m/z* Calcd for $C_{35}H_{40}N_5O_7$ $[M+H]^+$: 642.2928. Found: 642.2916.

(3*S*)-3-[[*N*-(*N*-(2-Naphthoyl)-L-valyl)-*N*-[3-(2*H*-tetrazol-5-yl)propyl]-glycyl]amino]-4-oxo-5-phenoxypentanoic Acid *tert*-Butyl Ester (17d) The title compound was prepared from **16e** using the same manner as described for the synthesis of **8a** to give a pale yellow amorphous substance (82%). ^{1}H-NMR ($CDCl_3$): 0.88—1.03 (6H, m), 1.75—2.80 (7H, m), 3.60—3.70 (2H, m), 3.90—4.25 (2H, m), 4.50—5.20 (4H, m), 6.80—6.97 (3H, m), 7.15—7.28 (2H, m), 7.52—7.63 (2H, m), 7.92—8.03 (4H, m), 8.38—8.77 (1H, m). FAB-MS *m/z*: 640 $[M-H]^-$. HRMS *m/z* Calcd for $C_{33}H_{36}N_7O_7$ $[M+H]^+$: 642.2676. Found: 642.2690.

(*S*)-3-(*tert*-Butoxycarbonylamino)-4-(*tert*-butyldimethylsiloxy)butyric Acid Benzyl Ester (22) To a solution of (*S*)-3-(*tert*-butoxycarbonylamino)-4-hydoxybutyric acid benzyl ester (**21**) (2.46 g , 8.0 mmol) in DMF (25 ml) was added *tert*-butyldimethylchlorosilane (1.44 g, 11 mmol) and imi-

dazole (1.35 g, 20 mmol), and the mixture was stirred at room temperature for 6 h. The mixture was added to water and extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel (10% hexane–AcOEt) gave **22** (3.03 g, 93%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.02 (6H, s), 0.87 (9H, s), 1.44 (9H, s), 2.63 (2H, m), 3.65 (2H, s), 4.05 (1H, br s), 5.06 (1H, br s), 5.11 (2H, s), 7.35 (5H, s). FAB-MS *m/z*: 409 $[M+H]^+$.

1-[*N*-(2-Naphthoyl)-L-valyl]-L-proline Methyl Ester (19) To an ice-cooled solution of 1-(L-valyl)-L-proline methyl ester monohydrochloride (**18**, 3.20 g, 14.0 mmol) in THF (64 ml) was added 2-naphthoyl chloride (2.67 g, 14.0 mmol) and triethylamine (4.29 ml, 30.8 mmol), and the mixture was stirred for 0.5 h. The mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel (20% hexane–AcOEt) gave **19** (4.60 g, 86%) as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$): 1.06 (3H, d, J=6.6 Hz), 1.15 (3H, d, J=6.6 Hz), 1.97—2.15 (3H, m), 2.21—2.30 (2H, m), 3.72—3.81 (1H, m), 3.76 (3H, s), 3.90—3.98 (1H, m), 4.54 (1H, dd, J=5.1, 8.8 Hz), 7.06 (1H, d, J=8.5 Hz), 7.51—7.59 (2H, m), 7.85—7.93 (4H, m), 8.32 (1H, s). FAB-MS *m/z*: 383 $[M+H]^+$.

1-[*N*-(2-Naphthoyl)-L-valyl]-L-proline (20) To a solution of 1-[*N*-(2-naphthoyl)-L-valyl]-L-proline methyl ester (**19**, 4.65 g, 12.2 mmol) in MeOH (93 ml) was added 1 M aqueous NaOH solution (18.3 ml), and the mixture was stirred at room temperature for 20 h. The mixture was evaporated *in vacuo*, diluted with Et_2O and extracted with water. The aqueous layer was acidified with 10% aqueous citric acid solution and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give **20** (4.64 g, quant.) as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$): 1.03 (3H, d, J=6.6 Hz), 1.08 (3H, d, J=6.6 Hz), 1.98—2.29 (5H, m), 3.72—3.78 (1H, m), 4.00—4.08 (1H, m), 4.56 (1H, dd, J=4.4, 8.4 Hz), 4.89 (1H, t, J=8.3 Hz), 7.50—7.57 (2H, m), 7.66 (1H, d, J=8.8 Hz), 7.84—7.92 (4H, m), 8.36 (1H, s). FAB-MS *m/z*: 369$[M+H]^+$.

(3*S*)-4-(*tert*-Butyldimethylsiloxy)-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]butyric Acid Benzyl Ester (23) To a solution of (3*S*)-3-(*tert*-butoxycarbonylamino)-4-(*tert*-butyldimethylsiloxy)butyric acid benzyl ester (**22**, 1.20 g, 2.83 mmol) in dichloromethane (12 ml) was added trifluoroacetic acid (6 ml); then the mixture was stirred at room temperature for 0.25 h. The mixture was evaporated *in vacuo*; benzene was then added to the residue, followed by evaporation in *vacuo* again to give crude (3*S*)-3-amino-4-(*tert*-butyldimethylsiloxy)butyric acid benzyl ester monotrifluoroacetate. To an ice-cooled solution of 1-[*N*-(2-naphthoyl)-L-valyl]-L-proline (**20**, 1.00 g, 2.71 mmol) in THF (20 ml) was added 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide monohydrochloride (569 mg, 2.97 mmol), *N*-methylmorpholine (0.33 ml, 3.0 mmol) and 1-hydroxybenzotriazole (401 mg, 2.97 mmol), and the mixture was stirred for 0.25 h. The mixture was added to a solution of the above (3*S*)-3-amino-4-(*tert*-butyldimethylsiloxy)butyric acid benzyl ester monotrifluoroacetate in THF (5 ml). The mixture was stirred at room temperature for 14 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel (33% AcOEt–hexane) gave **23** (1.05 g, 57%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.04 (3H, s), 0.05 (3H, s), 0.90 (9H, s), 1.02 (3H, d, J=6.6 Hz), 1.09 (3H, d, J=6.9 Hz), 1.75—2.35 (5H, m), 2.62—2.66 (2H, m), 3.60—3.82 (4H, m), 4.30—4.40 (1H, m), 4.54 (1H, dd, J=3.3, 8.4 Hz), 4.92 (1H, dd, J=6.3, 8.7 Hz), 5.11 (2H, s), 7.00 (1H, d, J=9.3 Hz), 7.18 (1H, d, J=8.7 Hz), 7.33—7.37 (5H, m), 7.54—7.60 (2H, m), 7.84—7.95 (4H, m), 8.32 (1H, s). FAB-MS *m/z*: 674 $[M+H]^+$.

(3*S*)-4-(*tert*-Butyldimethylsiloxy)-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]butylic Acid (24) To a solution of (3*S*)-4-(*tert*-butyldimethylsiloxy)-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]butyric acid benzyl ester (**23**) (1.03 g, 1.53 mmol) in methanol (20 ml) was added 1 M aqueous NaOH solution (3.1 ml). The mixture was stirred at room temperature for 20 h and then evaporated *in vacuo*. The residue was diluted with ether and extracted with water. The aqueous layer was acidified with 10% aqueous citric acid solution, extracted with AcOEt, washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to give **24** (820 mg, 92%) as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$): 0.03 (3H, s), 0.85 (9H, s), 1.05 (3H, d, J=6.9 Hz), 1.09 (3H, d, J=6.6 Hz), 1.92—2.05 (2H, m), 2.10—2.35 (3H, m), 2.60—2.64 (2H, m), 3.62—3.68 (3H, m), 3.97—4.02 (1H, m), 4.27—4.33 (1H, m), 4.52 (1H, dd, J=3.3, 8.1 Hz), 4.88 (1H, dd, J=7.8, 8.7 Hz), 7.19 (1H, d, J=8.4 Hz), 7.51—7.57 (3H, m), 7.85—7.94 (4H, m), 8.36 (1H, s). FAB-MS *m/z*: 584 $[M+H]^+$.

(3*S*)-*N*-Benzyloxy-4-(*tert*-butyldimethylsiloxy)-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]butanamide (25a) To a solution of **24** (300 mg, 0.51 mmol) in THF (4 ml) and DMF (4 ml) was added 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide monohydrochloride (108 mg, 0.57 mmol), *N*-methylmorpholine (0.12 ml, 1.13 mmol), and 1-hydroxy-1*H*-benzotriazole (76 mg, 0.57 mmol), and the mixture was stirred for 0.25 h. To the mixture was added *O*-benzylhydroxylamine monohydrochloride (90 mg, 0.57 mmol), and the mixture was stirred at room temperature for 18 h. The mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel (10% MeOH–$CHCl_3$) gave **25a** (295 mg, quant.) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 0.03 (6H, s), 0.85 (9H, s), 0.98 (3H, d, J=7.0 Hz), 1.04 (3H, d, J=7.0 Hz), 1.80—2.30 (5H, m), 2.70—2.90 (2H, m), 3.61—3.71 (3H, m), 3.80—3.90 (1H, m), 4.00—4.25 (1H, m), 4.78—4.86 (4H, m), 7.20—7.40 (4H, m), 7.45—7.52 (3H, m), 7.85—7.94 (5H, m), 8.29 (1H, s). FAB-MS *m/z*: 689 $[M+H]^+$.

(3*S*)-4-(*tert*-Butyldimethylsiloxy)-*N*-methanesulfonyl-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]butanamide (25b) To a solution of **24** (220 mg, 0.38 mmol) in THF (6 ml) was added 1,1′-carbonylbis-1*H*-imidazole (122 mg, 0.75 mmol). The mixture was stirred at room temperature for 3 h, then cooled in an ice bath. To the mixture, methanesulfonamide (72 mg, 0.75 mmol) and 1,8-diazabicyclo[5.4.0]-7-undecene (0.11 ml, 0.75 mmol) was added. The mixture was stirred for 3 h at room temperature and evaporated *in vacuo*. Column chromatography of the residue on silica gel (2% MeOH–$CHCl_3$) gave **25b** (162 mg, 65%) as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$): 0.05 (3H, s), 0.07 (3H, s), 0.89 (9H, s), 1.04 (3H, d, J=6.6 Hz), 1.11 (3H, d, J=6.6 Hz), 1.85—2.32 (5H, m), 2.45—2.80 (2H, m), 3.23 (3H, s), 3.60—3.80 (3H, m), 3.95—4.05 (1H, m), 4.15—4.26 (1H, m), 4.30—4.45 (1H, m), 4.85—5.00 (1H, m), 7.18 (1H, m), 7.37—7.39 (1H, m), 7.52—7.58 (2H, m), 7.84—7.97 (4H, m), 8.39 (1H, s). FAB-MS *m/z*: 661 $[M+H]^+$.

(3*S*)-*N*-Benzyloxy-4-hydroxy-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]-amino]butanamide (26a) To an ice-cooled solution of **25a** (295 mg, 0.51 mmol) in THF (6 ml) was added HF–pyridine (0.30 ml), and the mixture was stirred for 22 h at room temperature. This mixture was added to phosphate buffer (pH 7), extracted with $CHCl_3$, washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography of the residue on silica gel (2% MeOH–$CHCl_3$) gave **26a** (160 mg, 55%) as a colorless amorphous substance. ^{1}H-NMR ($CDCl_3$): 1.01—1.09 (6H, m), 1.80—2.20 (4H, m), 2.35—2.60 (4H, m), 2.65—2.75 (1H, m), 3.40—4.45 (6H, m), 4.78—4.97 (3H, m), 7.19—7.53 (5H, m), 7.53—7.61 (2H, m), 7.86—7.96 (4H, m), 8.34 (1H, s). FAB-MS *m/z*: 575 $[M+H]^+$.

(3*S*)-4-Hydroxy-*N*-methanesulfonyl-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]butanamide (26b) The title compound was prepared from **25b** in the same manner as described above as a colorless amorphous substance (100%). ^{1}H-NMR ($CDCl_3$): 1.05 (3H, d, J=6.6 Hz), 1.09 (3H, d, J=6.6 Hz), 1.86—2.26 (5H, m), 2.50—2.80 (2H, m), 3.21 (3H, s), 3.60—3.90 (3H, m), 3.98—4.08 (1H, m), 4.17—4.32 (2H, m), 4.54—4.66 (2H, m), 4.84 (1H, t, J=8.1 Hz), 7.01 (1H, d, J=8.4 Hz), 7.35—7.58 (3H, m), 7.84—8.01 (4H, m), 8.40 (1H, s). FAB-MS *m/z*: 547 $[M+H]^+$.

(3*S*)-*N*-Benzyloxy-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]-4-oxobutanamide (27a) The title compound was prepared from **26a** in the same manner as described for the synthesis of **7a** as a yellow amorphous substance (81%). ^{1}H-NMR ($CDCl_3$): 0.99—1.06 (6H, m), 1.80—2.26 (6H, m), 2.65—2.90 (1H, m), 3.60—4.00 (2H, m), 4.20—4.50 (2H, m), 4.82—5.06 (3H, m), 7.33—7.60 (7H, m), 7.80—7.95 (4H, m), 8.33—8.35 (1H, m). FAB-MS *m/z*: 571 $[M+H]^+$. HRMS *m/z* Calcd for $C_{32}H_{35}N_4O_6$ $[M+H]^+$: 571.2557. Found: 571.2568.

(3*S*)-*N*-Methanesulfonyl-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]-amino]-4-oxobutanamide (27c) The title compound was prepared from **26b** in the same manner as described for the synthesis of **7a** as a colorless amorphous substance (22%). ^{1}H-NMR ($CDCl_3$): 0.98—1.06 (6H, m), 1.98—2.45 (5H, m), 2.52—3.02 (2H, m), 3.26 (3H, s), 3.62—3.75 (1H, m), 3.98—4.17 (2H, m), 4.48—4.60 (2H, m), 4.63—4.76 (1H, m), 5.55 (1H, m), 7.20—7.59 (4H, m), 7.80—7.95 (4H,m), 8.31—8.39 (1H, s). FAB-MS *m/z*: 545 $[M+H]^+$. HRMS *m/z* Calcd for $C_{26}H_{33}N_4O_7S$ $[M+H]^+$: 545.2070. Found: 545.2068.

(3*S*)-*N*-Hydroxy-3-[[1-[*N*-(2-naphthoyl)-L-valyl]-L-prolyl]amino]-4-oxobutanamide (27b) A solution of **27a** (120 mg, 0.21 mmol) in EtOH (4 ml) was stirred at room temperature in the presence of 10% Pd–C (100 mg) under a hydrogen atmosphere. When hydrogen consumption ceased, Pd–C was filtered off. The filtrate was evaporated *in vacuo* to give **27b** (26 mg, 26%) as a yellow powder. ^{1}H-NMR ($CDCl_3$): 0.90—1.00 (6H, m), 1.80—2.30 (5H, m), 2.60—2.80 (1H, m), 3.55—3.70 (1H, m), 3.90—4.15 (2H, m), 4.30—4.70 (3H, m), 7.46—8.20 (6H, m), 8.33—8.41 (1H, m).

FAB-MS *m/z*: 483 $[M+H]^+$. HRMS *m/z* Calcd for $C_{25}H_{31}N_4O_6$ $[M+H]^+$: 483.2244. Found: 483.2259.

Assay of ICE Activity ICE activities were essentially measured as described by Thornberry *et al.*[1] with the following modifications. As the fluorogenic substrate, Ac–Tyr–Val–Ala–Asp–AFC (AFC: 7-amino-4-trifluoromethylcoumarin) was used in place of Ac–Tyr–Val–Ala–Asp–AMC (AMC: 7-amino-4-methylcoumarin). The release of AFC was monitored using a CORONA ELECTRIC MTP-32 fluorimeter capable of reading 96-well microtiter plates with excitation and emission filters of 395 and 515 nm, respectively.

Measurement of Interleukin-1β Secretion The human mononuclear cell fraction was prepared as described by Boyum.[17] The cells were suspended in RPMI1640 medium supplemented with penicillin/streptomycin and 10% fetal bovine serum, and were then distributed into 24-well plates as inocula of 1×10^6 cells/well. The cells were pretreated for 15 min with or without ICE inhibitors and stimulated by LPS (20 μg/ml) for 24 h at 37 °C. The culture media were harvested and mature IL-1β was measured by an ELISA method using an IL-1β assay kit (CAYMAN).

Animals Male Balb/c mice weighing 20—30 g were used. Animals were purchased from Charles River Japan, Inc. The animals were housed at least 1 week before use in a temperature-controlled room with a 12-h light-dark cycle and were allowed free access to standard laboratory chow and water.

Induction of IL-1β Production *in Vivo*[18] Mice were given an i.p. injection of 1 μg of LPS in 0.5 ml of phosphate-buffered-salts (PBS). **27c** was administered s.c. and a second i.p. injection of adenosine 5′-triphosphate (ATP) disodium salt in 0.5 ml PBS (10 mM) was given 60 and 120 min after the LPS injection, respectively. The pH of these solutions was adjusted to 7.3 before injection. At 180 min after the LPS injection, the mice were killed and the peritoneal cavity was lavaged with 1 ml of ice-cold PBS containing 10 U/ml heparin sodium salt, 0.25 mM phenylmethylsulfonylfluoride (PMSF) and 1 mM EDTA. The samples were kept on ice until centrifugation. The supernatants were stored at −20 °C.

IL-1β was measured by an ELISA method using an IL-1β assay kit (GENZYME). Results are shown as the mean±S.E.M. of 7 mice for each datum point. Statistical analysis of data for multiple comparisons was performed by Dunnett's multiple range test (SAS). Probabilities of <5% ($p<0.05$) were considered significant.

Crystallization and X-Ray Analysis Subunits p20 and p10 of recombinant human ICE were separately expressed in *E. coli* with a pET vector system. Both subunits were present in the insoluble fraction, and approximately an equal amount of each protein solubilized with 6 M guanidine hydrochloride was added. Following a refolding step in the presence of an inhibitor, the solution was exchanged with 10 mM Tris–HCl pH 8.0, 10 mM dithiothreitol (DTT), 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 10 μM compound **27c**, then concentrated to 20 mg/ml using a Centricon 30 concentrator (Amicon). Purification using chromatography was skipped because the p10 subunit was easily degraded during this step. After concentration, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis confirmed that there were few protein contaminants in the solution.

Crystals of the complex were obtained by a vapor diffusion method in the hanging drop mode when mixing the protein solution with the precipitating solution, 100 mM HEPES pH 7.0 and 10—15% PEG6000 (Hampton Research). Crystals of tetragonal bipyramids, whose cell constants and space group were almost the same as those previously reported,[10,19] appeared within one day at 4 °C. A set of diffraction data was collected using an R-AXIS IIc diffractometer with a rotating-anode X-ray source (Rigaku). The completeness of the data up to 2.7 Å resolution and in the last shell (2.8—2.7 Å resolution) was 97.3% and 93.1%, respectively.

Crystallographic refinement with simulated annealing using an X-PLOR package[20] was carried out after rigid body refinement. The inhibitor molecule was built in the binding pocket based on the difference electron density map. The model was adjusted manually using program O[21] during the refinement. Statistics of the crystallographic study are summarized in Table 5.

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