Novel Non-Peptide GPIIb/IIIa Antagonists: Synthesis and Biological Activities of 2-[4-[2-(4-Amidinobenzoylamino)-2-(substituted)acetyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acids

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To improve the *in vitro* and *in vivo* potency of our first low molecular weight GPIIb/IIIa antagonist 1 (TAK-029), a series of 2-[4-[2-(4-amidinobenzoylamino)-2-(substituted)acetyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic acids were synthesized through modification of the glycine moiety of 1 and evaluated for their ability to inhibit *in vitro* adenosine 5′-diphosphate (ADP)-induced platelet aggregation of guinea pig platelet rich plasma (PRP). Among the compounds examined, the (3*S*,2*S*)-4-methoxyphenylalanine derivative 4h showed the most potent antagonistic activity with an IC_{50} value of 13 nM. Dose-dependent inhibition of *ex vivo* platelet aggregation was achieved with oral administration of 4h (0.3—1.0 mg/kg) to guinea pigs. Complete inhibition was observed for up to 8 h, and 43% inhibition could still be observed 24 h after oral administration of 1.0 mg/kg. The long-lasting antiplatelet effect of 4h suggests that 4h would be suitable for once-a-day dosing. Structure–activity relationships (SAR) were examined in the series of the phenylalanine derivatives. An increase in the electron density around the 4-position of the phenyl ring of the phenylalanine moiety led to an increase in the antiplatelet activity, suggesting the existence of a hydrophobic and electrostatic interaction site in addition to the ionic binding sites in the GPIIb/IIIa.

Key words GPIIb/IIIa antagonist; antiplatelet effect; 2-oxopiperazine derivative; 4-methoxyphenylalanine derivative

Platelets play an important role in the pathogenesis of thrombotic diseases such as acute myocardial infarction, unstable angina and cerebral thrombosis.[1—4] The binding of the plasma protein fibrinogen to platelet glycoprotein IIb/IIIa (GPIIb/IIIa) *via* the Arg-Gly-Asp (RGD) recognition sequence, is the final obligatory step in the platelet aggregation cascade stimulated by various mediators such as adenosine 5′-diphosphate (ADP) and collagen.[5,6] Therefore, GPIIb/IIIa antagonists are expected to inhibit platelet aggregation more extensively than the various inhibitors of the individual mediators.[7—10] In the last decade, intensive effort has been devoted to the development of GPIIb/IIIa inhibitors as an attractive new type of antithrombotic agent.[11—18] The clinical efficacy of GPIIb/IIIa antagonists, including some monoclonal antibodies, cyclic peptides and low molecular weight non-peptides, has been validated. For example, use of the anti-GPIIb/IIIa c7E3 Fab antibody (ReoPro) in high-risk patients undergoing percutaneous transluminal coronary angioplasty (PTCA), has been shown to reduce the composite incidence of major ischemic events, including the need for revascularization.[19,20]

Previous studies on the structure–activity relationships (SAR) of GPIIb/IIIa antagonists demonstrated that appropriately spaced guanidino and carboxylate equivalents were a critical prerequisite for inhibitory activity, and that a Gly residue was an essential part for high affinity for GPIIb/IIIa in a series of RGD-containing peptides and peptide mimetics.[13] However, Alig *et al*. demonstrated that the Gly residue could be replaced with other amino acids or linkers without loss of activity.[21]

Recently, we reported the design and synthesis of the potent and orally active GPIIb/IIIa antagonist **1** (TAK-029),[22] which was designed from tetrapeptides such as RGDS, RGDF and RGDV using conformational constraint by incorporation of cyclic structures (Chart 1). Since the effect of the Gly moiety of **1** was not previously examined, we have performed detailed modification of this part in an effort to find compounds with greater activity and better pharmacological profiles than **1**. In this paper, we describe the design, synthesis and biological activities of 2-oxopiperazine derivatives containing various amino acids in place of the Gly residue of **1**. We also discuss the SAR of our new compounds, which imply the existence of an additional binding site in GPIIb/IIIa besides the known ionic interaction sites. Although there are some reports on modification of the Gly residue of RGD-containing peptides and peptide mimetics, there have been few reports on detailed SAR focused on the substituent at the Gly position.

Chemistry

The general synthetic route for the target compounds **4a**—**w** is outlined in Chart 2. Condensation of **2**[22] with various *N*-protected α-amino acids using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in methylene dichloride provided the intermediates **3a**—**w**. Conversion of **3a**—**w** to **4a**—**w** was achieved by one of two routes depending on the nitrogen-protecting group utilized. In the case where the protective group was a benzyloxycarbonyl (*Z*) group, hydrogenolysis of **3a**—**l** in the presence of 10% Pd–C followed by acylation with 4-amidinobenzoylchloride hydrochloride and subsequent acid hydrolysis of the *tert*-butyl ester group afforded **4a**—**l** (method A). When a *tert*-butoxycarbonyl (Boc) group was used, simultaneous acid hydrolysis of the Boc group and *tert*-butyl ester group of **3m**—**w** followed by acylation with 4-amidinobenzoylchloride hydrochloride gave **4m**—**w** (method B).

Conversion of the vinyl intermediate **3v** to the 4-ethyl derivative **10**, formyl derivative **11**, hydroxymethyl derivative

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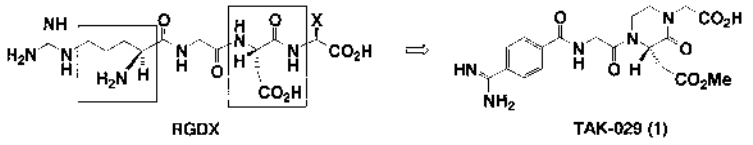
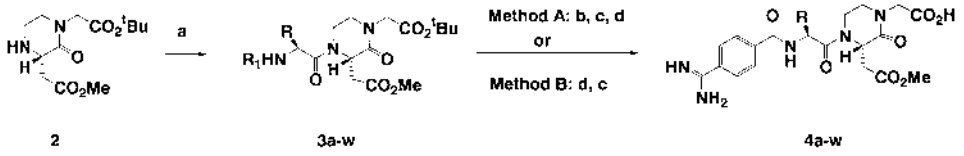
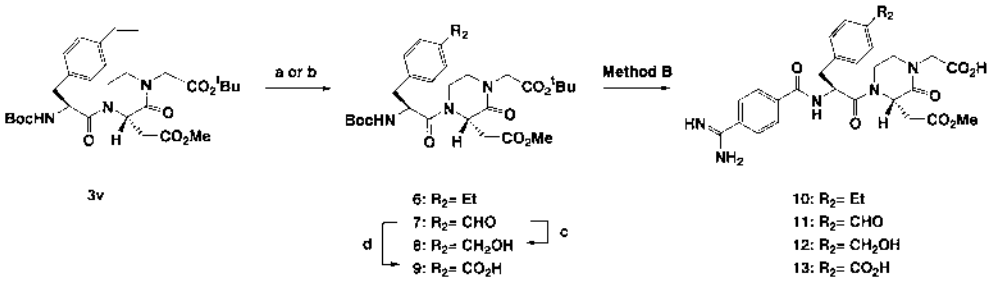


Chart 1



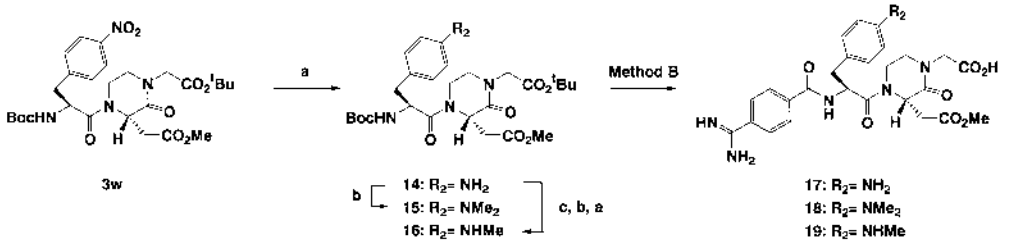
Reagents: (a) *N*-Z- or *N*-Boc-amino acid derivatives, EDC, CH_2Cl_2; (b) 10%Pd-C, H_2, MeOH; (c) $NaHCO_3$, 4-Amidinobenzoylchloride hydrochloride, 1,4-dioxane, H_2O; (d) CF_3CO_2H, toluene.

Chart 2. Synthetic Route for 2-Oxopiperazine Derivatives



Reagents: (a) H_2, 10%Pd-C, MeOH; (b) $NaIO_4$, $RuCl_3 \cdot nH_2O$, CCl_4, MeCN, H_2O; (c) $NaBH_4$, MeOH, 0°C; (d) $KMnO_4$, AcOH, acetone.

Chart 3



Reagents: (a) H_2, 10%Pd-C, MeOH; (b) 37%HCHO, $NaBH_3CN$, AcOH, MeCN; (c) PhCHO, $NaBH_3CN$, AcOH, MeCN.

Chart 4

12 and carboxyl derivative **13** is depicted in Chart 3. Hydrogenation or oxidative cleavage of the double bond of **3v** gave the 4-ethyl or 4-formyl intermediate (**6**, **7**), respectively. Reduction of **7** with $NaBH_4$, or oxidation of **7** with $KMnO_4$, afforded the 4-hydroxymethyl or 4-carboxyl intermediate (**8**, **9**), respectively. These intermediates **6**—**9** were converted to the corresponding target compounds **10**—**13** using method B described in Chart 2.

Amino derivatives were synthesized as shown in Chart 4. Hydrogenation of **3w** afforded the aniline **14**. Reductive methylation of **14** using excess 37% aqueous HCHO and $NaBH_3CN$, afforded the dimethylamino intermediate **15**. The sequential benzylation, methylation and de-benzylation of **14** gave the methylamino intermediate **16**. These amino intermediates **14**—**16** were also converted to the corresponding target compounds **17**—**19** using method B.

Compounds **5a**—**c** (Table 2), stereoisomers of **4a**, were prepared in a manner similar to that described for **4a** using *Z*-D-Asp(OMe)-OH and *Z*-D-Tyr-OH.

The phenylalanine derivatives used in the preparation of **4** were synthesized as follows (Chart 5). Simultaneous alkylation and esterification of **20a**—**c** followed by hydrolysis of the ester groups gave **21a**—**h**.[23] Treatment of **23**, which was obtained from *Z*-L-Tyr-O*t*Bu (**22**) with trifluoromethanesulfonic anhydride (Tf_2O) and 2,6-lutidine, with CO gas in the presence of palladium (II) acetate ($Pd(OAc)_2$)[24] in MeOH followed by acid hydrolysis, provided the 4-methoxycarbonylphenylalanine **25**. Treatment of **27** with ethynyltributyltin or vinyltributyltin[25] in the presence of dichlorobis(triphenylphosphine)palladium(II) ($PdCl_2(PPh_3)_2$) and LiCl followed by hydrolysis, gave the corresponding 4-ethynyl- and 4-vinylphenylalanine (**30**, **31**), respectively.

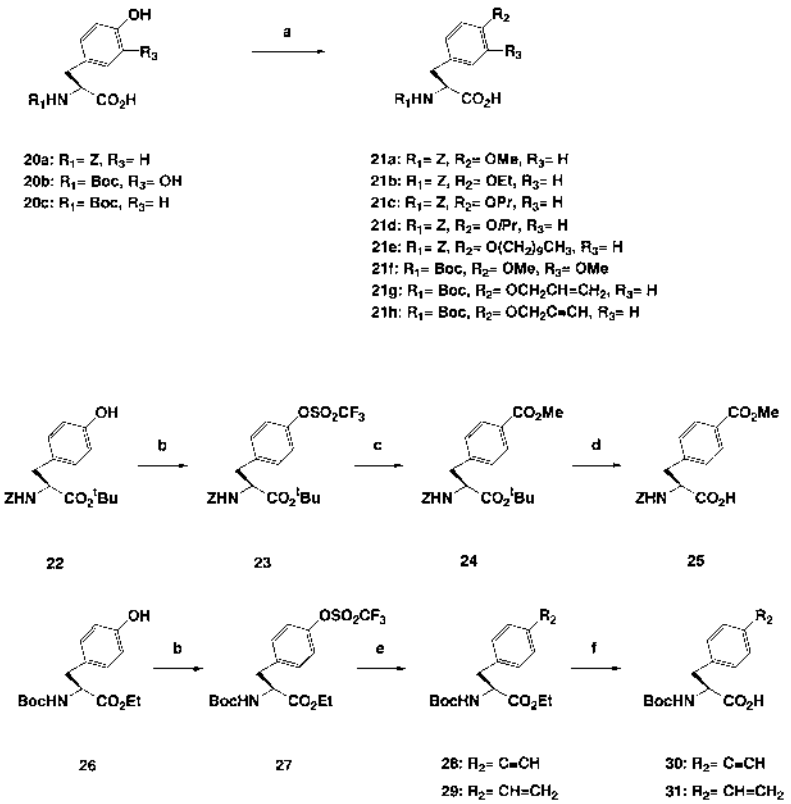


Chart 5. Preparation of Phenylalanine Derivatives

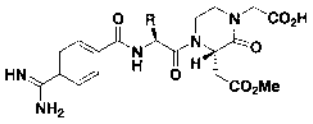
Results and Discussion

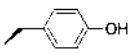
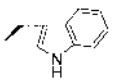
The compounds synthesized in this study were evaluated for their ability to inhibit *in vitro* ADP-induced platelet aggregation of guinea pig platelet rich plasma (PRP) (Tables 1—3).

First of all, replacement of the Gly residue of **1** with typical α-amino acids was examined (Table 1). Among the compounds synthesized, the Tyr derivative **4a** showed a 2.4-fold increase in inhibitory activity as compared to **1**, while the other derivatives, **4b**—**g**, had inhibitory activity comparable to or less potent than that of **1**. The aromatic group-possessing amino acid derivatives (**4a**—**c**) other than **4d** seem to have much higher affinity for the GPIIb/IIIa receptor than the non-aromatic group-possessing amino acid derivatives (**4e**—**g**). In the case of **4d**, in which the phenyl ring is directly attached to the α-carbon, steric hindrance of the phenyl ring might have resulted in a change in the spatial arrangement of the *C*-terminal carboxylate and the *N*-terminal amidinophenyl group, and the significant decrease in potency that was observed. It is worth noting that the introduction of a hydroxyl group onto the 4-position of the phenyl ring of **4c** made a 13-fold increase in potency (**4a** *vs.* **4c**). These results indicated that the substituent on the phenyl ring might affect the activity, and prompted us to investigate phenylalanine derivatives further.

We also examined the effect of the stereochemistry in phenylalanine derivatives on the inhibitory potency by comparison of **4a** and its stereoisomers (**5a**—**c**) (Table 2). Among the four stereoisomers, the (3*S*,2*S*)[26]-isomer **4a** was found to exert the most potent inhibitory activity, and the potency was decreased in the order of the (3*S*,2*S*)-isomer **4a**>(3*S*, 2*R*)-isomer **5a**>(3*R*,2*S*)-isomer **5b**>(3*R*,2*R*)-iso-

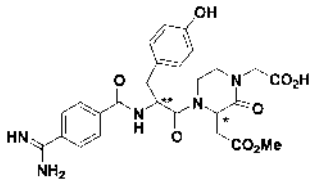
Table 1. Effects of Various Amino Acid Derivatives on ADP-Induced *in Vitro* Platelet Aggregation in Guinea Pig PRP



Compound	R	Inhibition of platelet aggregation[a] (IC_{50}: nM)
1 (TAK-029)	H	43
4a (Tyr)		18
4b (Trp)		52
4c (Phe)		230
4d (Phg)		5000
4e (Leu)		2000
4f (Glu)		3500
4g (Asp(OMe))		3400

a) See the Experimental section for details.

Table 2. Effects of Stereoisomers of **4a** on ADP-Induced *in Vitro* Platelet Aggregation in Guinea Pig PRP



Compound	Configuration at C-3 position of piperazinone scaffold (*)	Configuration at the Tyr moiety (**)	Inhibition of platelet aggregation[a] (IC_{50}: nM)
4a	*S*	*S*	18
5a	*S*	*R*	750
5b	*R*	*S*	1100
5c	*R*	*R*	30000

a) See the Experimental section for details.

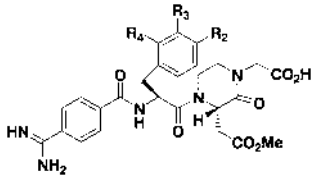
mer **5c**. The chiral centers in the phenylalanine derivatives are considered to play a significant role in GPIIb/IIIa recognition.

Table 3 shows the influence of the substituents on the phenyl group in the (3*S*,2*S*)-phenylalanine derivatives. Among the compounds examined, the 4-methoxyphenylalanine derivative **4h** showed the most potent inhibitory activity (IC_{50}: 13 nM), and the other compounds inhibited platelet aggregation with IC_{50} values of 22 to 11000 nM.

Interesting SAR were observed in these phenylalanine derivatives. At the beginning, we thought that the remarkably enhanced potency of **4a**, as compared to that of **4c**, could be ascribed to hydrogen bond formation between the phenolic hydroxyl group (hydrogen bond donor) and the GPIIb/IIIa (hydrogen bond acceptor), or coordination of the hydroxyl group with the Ca^{2+} ions existing around GPIIb.[27] However, hydrogen bonding was found not to be an important factor, since the 4-methoxy derivative **4h**, which lost the nature of the phenolic hydroxyl group, showed potent inhibitory activity. Moreover, coordination of the hydroxyl group with the Ca^{2+} ions was also found not to be the reason, because the 3,4-dihydroxyphenyl derivative **4m**, which was expected to have enhanced coordination with the Ca^{2+} ions, showed slightly less potent activity than **4a**.

We then changed our focus to the hydrophobic and electrostatic interaction between the ligand and the GPIIb/IIIa, since the fluoro derivative **4s** showed greater activity than **4c**. We assumed that an electron negative cloud around the 4-position of the phenyl ring of the phenylalanine moiety, caused by such things as a lone pair of electrons of the oxygen atom or enhancement of the electron density resulting from the high electronegativity of the fluoro atom on the phenyl ring, interacts with an electron positive site on the receptor. This hypothesis is supported by the results as follows. Conversion of the methylene or methyl group in ethyl derivative **10** into an oxygen atom (the methoxy derivative **4h** and hydroxymethyl derivative **12**) resulted in a 41- or 17-fold increase in inhibitory activity, respectively. Enhancement of the electron density by introduction of an unsaturated bond, led to an increase in potency (**4o**, **4p** *vs.* **4j**; **4u**, **4v** *vs.* **10**). The presence of a methoxycarbonyl group or formyl group at the 4-position of the phenyl ring increased the potency (**4t**, **11** *vs.* **4c**).

Table 3. Effects of Phenylalanine Derivatives on ADP-induced *in Vitro* Platelet Aggregation in Guinea Pig PRP



Compound	R_2	R_3	R_4	Inhibition of platelet aggregation[a] (IC_{50}: nM)
4h	OMe	H	H	13
4i	OEt	H	H	24
4j	OPr	H	H	34
4k	O-iso-Pr	H	H	100
4l	$O(CH_2)_9CH_3$	H	H	1900
4m[b]	OH	OH	H	46
4n	OMe	OMe	H	120
4o	$OCH_2CH{=}CH_2$	H	H	26
4p	$OCH_2C{\equiv}CH$	H	H	22
4q	OH	F	H	47
4r	OH	H	F	38
4s	F	H	H	38
4t	CO_2Me	H	H	72
4u	$C{\equiv}CH$	H	H	36
4v	$CH{=}CH_2$	H	H	93
10	Et	H	H	530
11	CHO	H	H	50
12	CH_2OH	H	H	31
13	CO_2H	H	H	1100
4w	NO_2	H	H	180
17	NH_2	H	H	700
18	NMe_2	H	H	11000
19	NMe_2	H	H	1200

a) See the Experimental section for details. *b*) Boc-DL-Phe(3,4-di-OH)-OH was used for the preparation of **4m**.

On the other hand, lowering the electron density of the oxygen atom by introducing a fluoro atom at the 3- or 2-position of the phenyl ring resulted in a decrease in the potency (**4q**, **4r** *vs.* **4a**). The amino derivatives **17**, **18** and **19**, and the carboxyl derivative **13**, showed remarkably decreased activity. The high hydrophilicity of these ionic functional groups might negatively interact with the hydrophobic site on the receptor. The size of the functional group was also an important factor; an increase in the bulkiness of the functional group decreased the potency. Lengthening or branching of the alkyl chain led to a decrease in potency (**4h**—**n**).

From these results, we propose the existence of a binding site, other than the site of ionic interaction in the GPIIb/IIIa, which is dependent on hydrophobic and electrostatic interaction (Fig. 1). The possible existence of a hydrophobic binding site in the GPIIb/IIIa has been previously suggested by a couple of research groups[15,28]; however, the hydrophobic binding site we discovered in the present study is characterized by its electrostatic feature.

A comparison of the pharmacological profiles of **4h** with **1** is shown in Table 4 and Figs. 2 and 3. Derivative **4h** inhibited the binding of biotin-labeled human fibrinogen to immobilized human GPIIb/IIIa and *in vitro* ADP-induced aggregation of human and monkey platelets more potently than **1** (Table 4). Dose-dependent inhibition of *ex vivo* platelet ag-

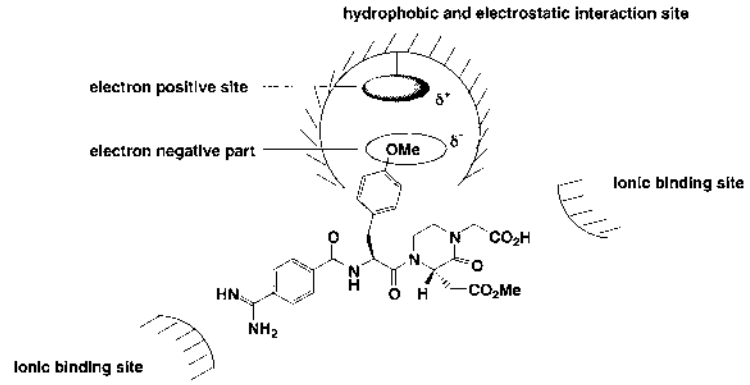


Fig. 1. Proposed Pharmacophore Model of the Fibrinogen Receptor for the Binding of Phenylalanine Derivatives

Table 4. Effects of **4h** and **1** on Fibrinogen and on *in Vitro* Platelet Aggregation Induced by ADP in Human and Monkey PRP

Compound	GPIIb/IIIa–fibrinogen binding[a] (IC_{50}: nM)	Inhibition of platelet aggregation[b] (IC_{50}: nM)	
		Human	Monkey
4h	0.20	12	15
1	1.0	31	46

a,*b*) See the Experimental section for details.

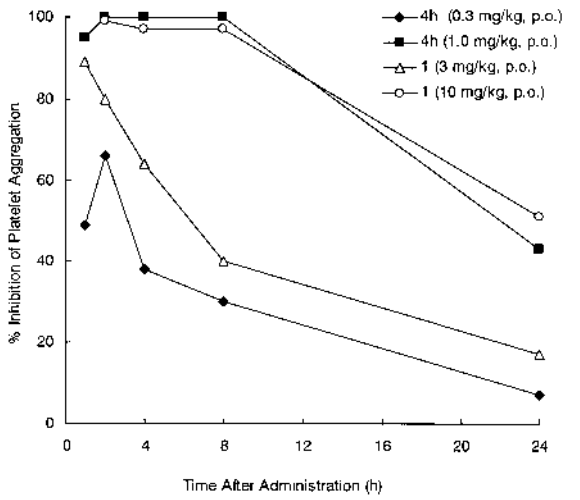


Fig. 2. *Ex Vivo* Antiplatelet Effects of **4h** and **1** in Guinea Pigs

gregation induced by ADP was achieved with oral administration of 0.3—1.0 mg/kg of **4h** to guinea pigs, and complete inhibition was observed for up to 8 h, and 43% inhibition could still be observed 24 h after oral administration of 1.0 mg/kg (Fig. 2). Intravenous administration of **4h** (0.003—0.01 mg/kg) to guinea pigs also inhibited platelet aggregation dose dependently (Fig. 3). Oral and intravenous administration of **4h** showed approximately 10-fold greater antiplatelet activity than **1**. In our previous pharmacokinetics study of **1** in rats, the dicarboxyl derivative was not observed in plasma after oral administration of **1**. The ester group at the 3-position of the 2-oxopiperazine scaffold was assumed to be stable in plasma, and the ester form (**1**) was regarded as the biological active form by itself (unpublished data). Therefore, in the case of **4h**, we considered that the ester form (**4h**) is the biological active form by itself as well as **1**.

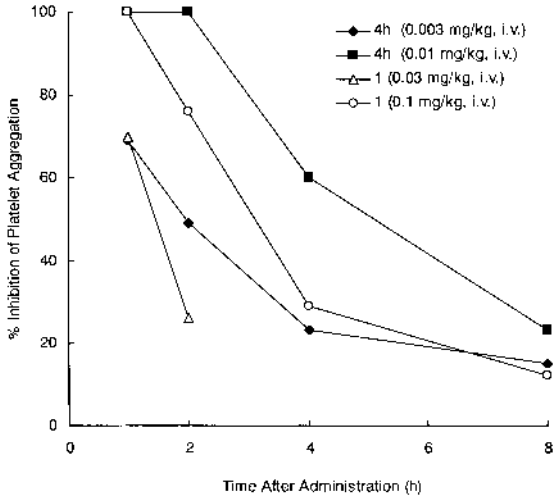


Fig. 3. *Ex Vivo* Antiplatelet Effects of **4h** and **1** in Guinea Pigs

Conclusion

Modification of the Gly moiety of **1** has led to the potent, orally active and long-acting GPIIb/IIIa antagonist **4h**, which showed approximately 3- and 10-fold more potent *in vitro* and *ex vivo* antiplatelet effects, respectively, than **1**. Derivative **4h** exhibited increased potency as compared to **1** in the human and monkey platelet aggregation assays as well as in the guinea pig assay. The SAR obtained in this study revealed the existence of a hydrophobic and electrostatic interaction site, in addition to the ionic binding sites in the GPIIb/IIIa. A hydrophobic electron negative cloud around the 4-position of the phenyl ring of the phenylalanine moiety may enhance the interaction between the fibrinogen receptor and the antagonists.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were obtained on a Hitachi IR-215 spectrometer. ^{1}H-NMR spectra were taken on a Varian Gemini 200 (200 MHz) spectrometer. Chemical shifts are given in ppm with tetramethylsilane (TMS) as the internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet, dd=double doublet, dt=double triplet. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-methoxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (3h) To a mixture of methyl 2-[(2*S*)-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**2**) (0.53 g, 1.85 mmol), *Z*-L-Tyr(Me)-OH (**21a**) (0.61 g, 1.85 mmol) and CH_2Cl_2 (10.6 ml) was added EDC (0.38 g, 1.98 mmol) at room temperature. After being stirred for 2 h, the reaction mixture

was poured into 5% aqueous $KHSO_4$ and extracted with ethyl acetate (EtOAc). The EtOAc extract was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 3 : 7) to give **3h** (0.84 g, 76%) as a colorless amorphous powder. IR (KBr) cm^{-1}: 3287, 2978, 1738, 1651, 1514, 1248, 1157. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.40—2.60 (1H, m), 2.66—3.22 (5H, m), 3.63 (3H, s), 3.78 (3H, s), 3.54—4.04 (4H, m), 4.70—4.90 (1H, m), 4.97 (1H, t, J=5.4 Hz), 5.10 (2H, s), 5.58 (1H, d, J=8.8 Hz), 6.82 (2H, d, J=8.8 Hz), 7.10 (2H, d, J=8.8 Hz), 7.24—7.40 (5H, m).

Compounds **3a**—**g** and **3i**—**w** were prepared in a manner similar to that described for **3h**. Compounds **3b**—**g**, **3m**, **3q**—**3r** and **3u** were used for a next reaction without purification.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-hydroxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3a**): A colorless amorphous powder (76%). IR (KBr) cm^{-1}: 3300, 1738, 1651, 1516, 1447, 1370, 1229, 1155. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.30—2.52 (1H, m), 2.68—3.24 (5H, m), 3.62 (3H, s), 3.36—3.84 (2H, m), 3.84 (1H, d, J=17.2 Hz), 4.03 (1H, d, J=17.2 Hz), 4.78 (1H, q, J=7.6 Hz), 4.96 (1H, t, J=5.4 Hz), 5.10 (2H, s), 5.60 (1H, d, J=9.2 Hz), 5.74—5.94 (1H, m), 6.75 (2H, d, J=8.6 Hz), 7.02 (2H, d, J=8.6 Hz), 7.20—7.50 (5H, m).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-ethoxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3i**): A colorless oil (65%). IR (KBr) cm^{-1}: 3424, 2980, 1739, 1652, 1508, 1441, 1367, 1242, 1152. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.0 Hz), 1.45 (9H, s), 2.36—2.58 (1H, m), 2.66—3.24 (5H, m), 3.56—3.86 (5H, m), 3.90 (2H, d, J=6.4 Hz), 3.99 (2H, q, J=7.0 Hz), 4.70—4.90 (1H, m), 4.98 (1H, t, J=5.4 Hz), 5.10 (2H, s), 5.59 (1H, d, J=8.8 Hz), 6.80 (2H, d, J=8.8 Hz), 7.08 (2H, d, J=8.8 Hz), 7.27—7.40 (5H, m).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-propoxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3j**): A colorless oil (63%). IR (KBr) cm^{-1}: 3304, 2970, 1737, 1655, 1510, 1440, 1244, 1153. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, t, J=7.4 Hz), 1.45 (9H, s), 1.67—1.89 (2H, m), 2.36—2.58 (1H, m), 2.66—3.20 (5H, m), 3.56—3.79 (5H, m), 3.86 (2H, d, J=6.2 Hz), 3.86 (1H, d, J=17.2 Hz), 3.99 (1H, d, J=17.2 Hz), 4.70—4.90 (1H, m), 5.02 (1H, t, J=5.3 Hz), 5.09 (2H, s), 5.68 (1H, d, J=8.8 Hz), 6.80 (2H, d, J=8.8 Hz), 7.08 (2H, d, J=8.8 Hz), 7.22—7.43 (5H, m).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-isopropoxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3k**): A colorless oil (53%). IR (KBr) cm^{-1}: 3304, 2980, 1740, 1657, 1508, 1445, 1368, 1242, 1154. ^{1}H-NMR ($CDCl_3$) δ: 1.26—1.35 (6H, m), 1.45 (9H, s), 2.32—2.54 (1H, m), 2.66—3.20 (5H, m), 3.54—3.84 (6H, m), 4.04 (1H, d, J=17.4 Hz), 4.40—4.58 (1H, m), 4.72—4.88 (1H, m), 5.01 (1H, t, J=5.4 Hz), 5.09 (2H, s), 5.74 (1H, d, J=8.8 Hz), 6.79 (2H, d, J=8.8 Hz), 7.08 (2H, d, J=8.8 Hz), 7.30—7.40 (5H, m).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-decyloxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3l**): A colorless oil (54%). IR (KBr) cm^{-1}: 2926, 1740, 1657, 1510, 1436, 1367, 1244, 1154. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.4 Hz), 1.09—1.54 (23H, m), 1.65—1.85 (2H, m), 2.37—2.58 (1H, m), 2.65—3.35 (5H, m), 3.63 (3H, s), 3.54—4.08 (6H, m), 4.68—4.89 (1H, m), 4.99 (1H, t, J=5.2 Hz), 5.09 (2H, s), 5.58 (1H, d, J=8.4 Hz), 6.80 (2H, d, J=8.4 Hz), 7.08 (2H, d, J=8.4 Hz), 7.30—7.40 (5H, m).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(3,4-dimethoxyphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3n**): A colorless oil (79%). IR (KBr) cm^{-1}: 3426, 2978, 1741, 1706, 1655, 1515, 1443, 1366, 1260, 1237, 1156, 1026. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.45 (9H, s), 2.40—2.60 (1H, m), 2.66—3.10 (5H, m), 3.65 (3H, s), 3.84 (3H, s), 3.85 (3H, s), 3.62—4.04 (4H, m), 4.64—4.88 (1H, m), 5.02 (1H, t, J=5.2 Hz), 5.34 (1H, d, J=8.4 Hz), 6.62—6.95 (3H, m).

Methyl 2-[(2*S*)-1-[(2*S*)-3-(4-Allyloxyphenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3o**): A colorless oil (72%). IR (KBr) cm^{-1}: 2978, 2934, 1741, 1707, 1654, 1510, 1437, 1366, 1242, 1157. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.45 (9H, s), 2.38—2.63 (1H, m), 2.65—3.30 (5H, m), 3.65 (3H, s), 3.45—3.82 (2H, m), 3.86 (1H, d, J=18.0 Hz), 4.00 (1H, d, J=18.0 Hz), 4.51 (2H, dt, J=5.2, 1.5 Hz), 4.63-4.83 (1H, m), 4.99 (1H, t, J=5.2 Hz), 5.22—5.48 (3H, m), 5.90—6.18 (1H, m), 6.84 (2H, d, J=8.6 Hz), 7.11 (2H, d, J=8.6 Hz).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[4-(2-propynyloxy)phenyl]propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3p**): A colorless oil (75%). IR (KBr) cm^{-1}: 2980, 2932, 1741, 1708, 1651, 1510, 1442, 1368, 1220, 1156. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.45 (9H, s), 2.30—2.56 (2H, m), 2.70—3.30 (5H, m), 3.65 (3H, s), 3.50—3.81 (2H, m), 3.93 (2H, s), 4.55—4.82 (3H, m), 4.85 (1H, t, J=5.0 Hz), 5.31 (1H, d, J=8.4 Hz), 6.90 (2H, d, J=8.4 Hz), 7.14 (2H, d, J=8.4 Hz).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-fluorophenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3s**): A colorless oil (42%). IR (KBr) cm^{-1}: 3310, 2978, 1741, 1640, 1507, 1438, 1366, 1220, 1154. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.50—2.73 (1H, m), 2.64—3.24 (5H, m), 3.55—3.77 (2H, m), 3.63 (3H, s), 3.85 (1H, d, J=17.4 Hz), 4.06 (1H, d, J=17.4 Hz), 4.72—4.90 (1H, m), 4.96—5.02 (1H, m), 5.09 (2H, d, J=1.8 Hz), 5.58 (1H, d, J=8.4 Hz), 6.86—7.04 (2H, m), 7.08—7.20 (2H, m), 7.28—7.42 (5H, m).

Methyl 4-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[(2*S*)-4-[2-(*tert*-butoxy)-2-oxoethyl]-2-(2-methoxy-2-oxoethyl)-3-oxopiperazinyl]-3-oxopropyl]benzoate (**3t**): A colorless oil (51%). IR (KBr) cm^{-1}: 3322, 2980, 1719, 1649, 1525, 1435, 1367, 1281, 1152. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.61—2.80 (1H, m), 2.80—3.25 (5H, m), 3.63 (3H, s), 3.40—4.05 (4H, m), 3.90 (3H, s), 4.78—5.00 (1H, m), 4.98 (1H, t, J=5.2 Hz), 5.08 (2H, d, J=1.4 Hz), 5.61 (1H, d, J=8.0 Hz), 7.16—7.46 (7H, m), 7.95 (2H, d, J=8.0 Hz).

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-vinylphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3v**): A colorless crystalline powder (76%, EtOAc–petroleum ether), mp 148—152 °C. $[\alpha]_D^{20}$ +94.3° (c=0.47, $CHCl_3$). IR (KBr) cm^{-1}: 3446, 1746, 1699, 1653, 1624, 1507, 1366, 1232, 1174, 1156. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.44 (9H, s), 2.25—2.45 (1H, m), 2.70—3.25 (5H, m), 3.50—3.80 (2H, m), 3.65 (3H, s), 3.78 (1H, d, J=17.2 Hz), 3.96 (1H, d, J=17.2 Hz), 4.64—4.83 (1H, m), 4.99 (1H, t, J=5.2 Hz), 5.22 (1H, d, J=10.8 Hz), 5.32 (1H, d, J=8.6 Hz), 5.71 (1H, d, J=17.6 Hz), 6.66 (1H, dd, J=10.8, 17.6 Hz), 7.15 (2H, d, J=8.2 Hz), 7.34 (2H, d, J=8.2 Hz). *Anal.*. Calcd for $C_{29}H_{41}N_3O_8$: C, 62.24; H, 7.38; N, 7.51. Found: C, 62.01; H, 7.51; N, 7.47.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-nitrophenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (**3w**): A colorless crystalline powder (70%, CH_2Cl_2–hexane), mp 167—170 °C. $[\alpha]_D^{20}$ +51.2° (c=0.60, $CHCl_3$). IR (KBr) cm^{-1}: 1736, 1700, 1654, 1518, 1347, 1232, 1158. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (9H, s), 1.45 (9H, s), 2.70—3.30 (6H, m), 3.40—3.97 (3H, m), 3.66 (3H, s), 4.15—4.35 (1H, m), 4.73—4.90 (1H, m), 5.00 (1H, t, J=5.4 Hz), 5.32 (1H, d, J=9.0 Hz), 7.30—7.60 (2H, m), 8.16 (2H, d, J=8.8 Hz). *Anal.* Calcd for $C_{27}H_{38}N_4O_{10}$: C, 56.05; H, 6.62; N, 9.68. Found: C, 55.85; H, 6.36; N, 9.67.

General Synthetic Procedure (Method A). 2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-methoxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (4h) Under an H_2 atmosphere, a suspension of **3h** (0.84 g, 1.41 mmol) and 10% Pd–C (0.14 g) in MeOH (4.2 ml) was stirred at room temperature for 40 min. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to give a colorless syrup, which was dissolved in H_2O (11.7 ml) and 1,4-dioxane (5.9 ml). To this solution were added $NaHCO_3$ (0.24 g, 2.81 mmol) and 4-amidinobenzoylchloride hydrochloride (0.37 g, 1.69 mmol) at room temperature. After being stirred for 1.5 h, the mixture was adjusted to pH 7 with 1 N HCl and concentrated *in vacuo*. Then, a mixture of the residue and toluene (5.9 ml) was treated with trifluoroacetic acid (5.9 ml) and the mixture was stirred for 1 h at room temperature. The mixture was concentrated *in vacuo*, and the residue was chromatographed on MCI GEL CHP-20 (Mitsubishi Chemical Industry, gradient elution: H_2O to 15% aqueous MeCN) to give **4h** (0.52 g, 60%) as a colorless crystalline powder (H_2O–EtOH), mp 208—212 °C. $[\alpha]_D^{20}$ −76.7° (c=1.04, dimethyl sulfoxide (DMSO)). IR (KBr) cm^{-1}: 1727, 1631, 1484, 1452, 1383. ^{1}H-NMR (D_2O) δ: 2.36—2.56 (1H, m), 2.76—3.02 (2H, m), 3.02—3.24 (3H, m), 3.59 (3H, s), 3.82 (3H, s), 3.44—4.20 (4H, m), 4.80—5.20 (2H, m), 6.95 (2H, d, J=8.6 Hz), 7.24 (2H, d, J=8.6 Hz), 7.80—8.00 (4H, m). *Anal.* Calcd for $C_{27}H_{31}N_5O_8 \cdot 3H_2O$: C, 53.37; H, 6.14; N, 11.53. Found: C, 53.15; H, 6.14; N, 11.36.

General Synthetic Procedure (Method B). 2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-nitrophenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (4w) To a solution of **3w** (1.60 g, 2.77 mmol) in toluene (8.0 ml) was added trifluoroacetic acid (8.0 ml) at room temperature. After being stirred for 1 h, the mixture was concentrated *in vacuo*, and the resulting oil was dissolved in H_2O (30 ml) and 1,4-dioxane (15 ml). To the solution were added $NaHCO_3$ (0.93 g, 11.1 mmol) and 4-amidinobenzoylchloride hydrochloride (0.79 g, 3.60 mmol) at room temperature, and the mixture was stirred for 30 min. Then, the mixture was adjusted to pH 2 with 1 N HCl and concentrated *in vacuo*. The residue was purified by means of CHP-20 column chromatography (gradient elution: H_2O to 25% aqueous MeCN) to give **4w** (1.12 g, 68%) as a colorless crystalline powder

(H_2O–EtOH), mp 218—222 °C. $[\alpha]_D^{20}$ −146.7° (c=0.96, DMSO). IR (KBr) cm^{-1}: 1715, 1634, 1604, 1517, 1345. ^{1}H-NMR (D_2O) δ: 2.80—3.08 (2H, m), 3.10—3.50 (4H, m), 3.61 (3H, m), 3.50—4.60 (4H, m), 5.13 (1H, t, J=6.0 Hz), 5.32 (1H, t, J=7.2 Hz), 7.54 (2H, d, J=8.4 Hz), 7.68—7.95 (4H, m), 8.16 (2H, d, J=8.4 Hz). *Anal.* Calcd for $C_{26}H_{28}N_6O_9\cdot 4H_2O$: C, 48.75; H, 5.66; N, 13.12. Found: C, 48.75; H, 5.29; N, 13.12.

Compounds **4a**—**g**, **4i**—**v** and **5a**—**c** were prepared in a manner similar to that described for **4h** (method A) or **4x** (method B).

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-hydroxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4a**): Method A (46% from **3a**), a colorless crystalline powder (H_2O–EtOH), mp 229—235 °C. $[\alpha]_D^{20}$ +82.09° (c=1.03, H_2O). IR (KBr) cm^{-1}: 1734, 1634, 1513, 1445, 1382. ^{1}H-NMR (D_2O) δ: 2.25—2.50 (1H, m), 2.83—3.24 (5H, m), 3.59 (3H, s), 3.40—4.20 (4H, m), 4.60—5.20 (2H, m), 6.86 (2H, d, J=8.4 Hz), 7.18 (2H, d, J=8.4 Hz), 7.80—8.00 (4H, m). *Anal.* Calcd for $C_{26}H_{29}N_5O_8\cdot 1.5H_2O$: C, 55.12; H, 5.69; N, 12.36. Found: C, 55.38; H, 5.66; N, 12.40.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(1H-indol-2-yl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4b**): Method A (32% from **2**), a colorless crystalline powder, mp 230—232 °C. $[\alpha]_D^{20}$ −64.56° (c=0.25, DMSO). IR (KBr) cm^{-1}: 3292, 1739, 1631, 1451, 1286. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.30—4.60 (13H, m), 4.82—4.98 (1H, m), 5.03—5.35 (1H, m), 6.92—7.18 (2H, m), 7.21 (1H, s), 7.30—7.42 (1H, m), 7.52—7.72 (1H, m), 7.78—8.08 (4H, m). *Anal.* Calcd for $C_{28}H_{30}N_6O_7\cdot H_2O$: C, 57.92; H, 5.56; N, 14.47. Found: C, 57.69; H, 5.81; N, 14.25.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-phenylpropanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4c**): Method A (43% from **2**), a colorless crystalline powder, mp 206—209 °C. $[\alpha]_D^{20}$ +76.21° (c=0.28, H_2O). IR (KBr) cm^{-1}: 1735, 1649, 1438, 1383, 1296. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.55—4.55 (13H, m), 4.80—4.95 (1H, m), 5.00—5.30 (1H, m), 7.10—7.40 (5H, m), 7.70—8.05 (4H, m). *Anal.* Calcd for $C_{26}H_{29}N_5O_7\cdot 1.5H_2O$: C, 56.72; H, 5.86; N, 12.72. Found: C, 56.50; H, 5.99; N, 12.57.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)phenylethanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4d**): Method A (39% from **2**), a colorless amorphous powder. $[\alpha]_D^{20}$ +22.8° (c=0.28, MeOH). IR (KBr) cm^{-1}: 1730, 1630, 1540, 1438, 1382, 1282, 1185. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.60—4.60 (11H, m), 4.85—5.08 (1H, m), 6.00—6.30 (1H, m), 7.20—7.56 (5H, m), 7.78—7.96 (2H, m), 7.96—8.16 (2H, m). *Anal.* Calcd for $C_{25}H_{27}N_5O_7\cdot 3.5H_2O$: C, 52.44; H, 5.99; N, 12.23. Found: C, 54.46; H, 5.73; N, 12.00.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-4-methylpentanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4e**): Method A (41% from **2**), a colorless amorphous powder. $[\alpha]_D^{20}$ +44.0° (c=0.30, MeOH). IR (KBr) cm^{-1}: 3300, 1730, 1638, 1550, 1445, 1385, 1300. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 0.93 (6H, d, J=5.4 Hz), 1.42—1.75 (3H, m), 2.70—4.45 (8H, m), 3.65 (3H, s), 4.80—5.00 (1H, m), 5.05—5.18 (1H, m), 7.80 (2H, d, J=8.4 Hz), 8.08 (2H, d, J=8.4 Hz),. *Anal.* Calcd for $C_{23}H_{31}N_5O_7\cdot H_2O$: C, 54.41; H, 6.55; N, 13.80. Found: C, 54.25; H, 6.71; N, 13.87.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-4-carboxybutanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4f**): *Z*-L-Glu(O^{t}Bu)-OH was used as the amino acid moiety. Method A (35% from **2**), a colorless amorphous powder. $[\alpha]_D^{20}$ +52.50° (c=0.25, H_2O). IR (KBr) cm^{-1}: 1733, 1634, 1436, 1384, 1294. ^{1}H-NMR (D_2O) δ: 1.95—2.30 (2H, m), 2.30—2.65 (2H, m), 2.80—3.20 (2H, m), 3.40—4.50 (2H, m), 3.63 (3H, s), 3.93 (1H, d, J=17.0 Hz), 4.12 (1H, d, J=17.0 Hz), 4.96—5.10 (1H, m), 5,17 (1H, t, J=6.2 Hz), 7.85—8.05 (4H, m). *Anal.* Calcd for $C_{21}H_{25}N_5O_9\cdot 2H_2O$: C, 47.82; H, 5.54; N, 13.28. Found: C, 47.81; H, 5.44; N, 13.25.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-4-methoxy-4-oxobutanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4g**): Method A (40% from **2**), a colorless amorphous powder. $[\alpha]_D^{20}$ +18.7° (c=0.74, H_2O). ^{1}H-NMR (D_2O) δ: 2.80—4.55 (10H, m), 3.52 (3H, s), 3.69 (3H, s), 5.02—5.20 (1H, m), 5.25—5.55 (1H, m), 7.75—8.00 (4H, m). *Anal.* Calcd for $C_{22}H_{27}N_5O_9\cdot 2.5H_2O$: C, 48.00; H, 5.86; N, 12.72. Found: C, 47.84; H, 5.89; N, 12.75.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-ethoxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4i**): Method A (64% from **3i**), a colorless crystalline powder (H_2O–EtOH), mp 212—215 °C. $[\alpha]_D^{20}$ −78.7° (c=1.00, DMSO). IR (KBr) cm^{-1}: 1737, 1634, 1509. ^{1}H-NMR (D_2O+DCl) δ: 1.22 (3H, t, J=7.0 Hz), 2.10—2.30 (1H, m), 2.73 (2H, d, J=5.6 Hz), 2.86—3.20 (3H, m), 3.45 (3H, s), 3.32—4.10 (6H, m), 4.60—5.10 (2H, m), 6.78 (2H, d, J=8.4 Hz), 7.07 (2H, d, J=8.4 Hz), 7.60—7.90 (4H, m). *Anal.* Calcd for $C_{28}H_{33}N_5O_8\cdot 3H_2O$: C, 54.10; H, 6.32; N, 11.27. Found: C, 53.85; H, 6.22; N, 11.09.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-propoxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4j**): Method A (72% from **3j**), a colorless crystalline powder (H_2O), mp 204—208 °C. $[\alpha]_D^{20}$ −78.7° (c=1.01, DMSO). ^{1}H-NMR (DMSO-d_6+D_2O) δ: 0.88—1.02 (3H, m), 1.58—1.78 (2H, m), 2.65—4.50 (12H, m), 3.61 (3H, s), 4.80—5.45 (2H, m), 6.79 (2H, d, J=8.6 Hz), 7.20 (2H, d, J=8.6 Hz), 7.70—8.05 (4H, m). *Anal.* Calcd for $C_{29}H_{35}N_5O_8\cdot 3H_2O$: C, 54.80; H, 6.50; N, 11.02. Found: C, 55.06; H, 6.66; N, 11.17.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-isopropoxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4k**): Method A (65% from **3k**), a colorless crystalline powder (H_2O–EtOH), mp 217—220 °C. $[\alpha]_D^{20}$ −80.2° (c=1.00, DMSO). ^{1}H-NMR (DMSO-d_6+D_2O) δ: 1.15—1.30 (6H, m), 2.60—4.65 (11H, m), 3.60 (3H, s), 4.80—5.43 (2H, m), 6.77 (2H, d, J=8.4 Hz), 7.19 (2H, d, J=8.4 Hz), 7.70—8.05 (4H, m). *Anal.* Calcd for $C_{29}H_{35}N_5O_8\cdot 2H_2O$: C, 56.39; H, 6.36; N, 11.34. Found: C, 56.45; H, 6.24; N, 11.43.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-decyloxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Hydrochloride (**4l**): Method A (53% from **3l**), a colorless amorphous powder. $[\alpha]_D^{20}$ +41.1° (c=0.92, H_2O). IR (KBr) cm^{-1}: 1734, 1635, 1509, 1244. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 0.85 (3H, t, J=6.4 Hz), 1.10—1.50 (14H, m), 1.44—1.78 (2H, m), 2.54—2.84 (2H, m), 2.84—4.54 (13H, m), 4.76—4.96 (1H, m), 4.96—5.18 (1H, m), 6.78 (2H, d, J=7.0 Hz), 7.12—7.32 (2H, m), 7.80—8.06 (4H, m). *Anal.* Calcd for $C_{36}H_{49}N_5O_8\cdot HCl\cdot 3H_2O$: C, 56.13; H, 7.33; N, 9.09. Found: C, 56.36; H, 7.07; N, 9.20.

2-[(3*S*)-4-[2-(4-Amidinobenzoylamino)-3-(3,4-dihydroxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4m**): Method B (42% from **2**), a colorless amorphous powder. ^{1}H-NMR (D_2O) δ: 2.17—3.85 (11H, m), 4.00—4.60 (2H, m), 5.00—5.20 (2H, m), 6.60—6.90 (3H, m), 7.80—8.00 (4H, m). *Anal.* Calcd for $C_{26}H_{29}N_5O_9\cdot 2H_2O$: C, 52.79; H, 5.62; N, 11.84. Found: C, 52.83; H, 5.75; N, 11.66.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(3,4-dimethoxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4n**): Method B (66% from **3n**), a colorless amorphous powder. $[\alpha]_D^{20}$ −51.2° (c=1.01, DMSO). ^{1}H-NMR (D_2O) δ: 2.18—2.40 (1H, m), 2.76—3.04 (2H, m), 3.04—3.26 (3H, m), 3.38—4.25 (4H, m), 3.60 (3H, s), 3.76 (3H, s), 3.85 (3H, s), 4.90—5.25 (2H, m), 6.75—7.10 (3H, m), 7.80—8.05 (4H, m). *Anal.* Calcd for $C_{28}H_{33}N_5O_9\cdot 1.5H_2O$: C, 55.08; H, 5.94; N, 11.47. Found: C, 54.92; H, 6.10; N, 11.46.

2-[(3*S*)-4-[(2*S*)-3-(4-Allyloxypheny)-2-(4-amidinobenzoylamino)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4o**): Method B (57% from **3o**), a colorless crystalline powder (H_2O), mp 200—205 °C. $[\alpha]_D^{20}$ −84.1° (c=0.81, DMSO). IR (KBr) cm^{-1}: 1733, 1636, 1507; ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.60—4.60 (12H, m), 3.61 (3H, s), 4.80—5.45 (4H, m), 5.88—6.15 (1H, m), 6.81 (2H, d, J=8.4 Hz), 7.21 (2H, d, J=8.4 Hz), 7.70—8.05 (4H, m). *Anal.* Calcd for $C_{29}H_{33}N_5O_8\cdot 1.5H_2O$: C, 57.42; H, 5.98; N, 11.54. Found: C, 57.52; H, 5.87; N, 11.56.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-[4-(2-propynyloxy)-phenyl]propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Hydrochloride (**4p**): Method B (55% from **3p**), a colorless amorphous powder. $[\alpha]_D^{20}$ +78.0° (c=1.00, H_2O). IR (KBr) cm^{-1}: 1732, 1639, 1507, 1444, 1216. ^{1}H-NMR (D_2O) δ: 2.12—2.33 (1H, m), 2.70—3.20 (6H, m), 3.53 (3H, s), 3.40—4.15 (6H, m), 4.85—5.15 (2H, m), 6.94 (2H, d, J=8.4Hz), 7.18 (2H, d, J=8.4 Hz), 7.70—7.95 (4H, m). *Anal.* Calcd for $C_{29}H_{31}N_5O_8\cdot HCl\cdot 2H_2O$: C, 53.58; H, 5.58; N, 10.77. Found: C, 53.35; H, 5.37; N, 10.77.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(3-fluoro-4-hydroxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4q**): Method B (18% from **2**), a colorless amorphous powder. $[\alpha]_D^{20}$ −112.0° (c=0.40, DMSO). ^{1}H-NMR (D_2O+DCl) δ: 2.44—2.64 (1H, m), 2.90 (2H, d, J=6.0 Hz), 3.04—3.38 (3H, m), 3.56—3.82 (1H, m), 3.59 (3H, s), 3.94—4.24 (3H, m), 5.00—5.25 (2H, m), 6.85—7.15 (3H, m), 7.80—7.98 (4H, m). *Anal.* Calcd for $C_{26}H_{28}FN_5O_8\cdot 3H_2O$: C, 51.06; H, 5.60; N, 11.45. Found: C, 51.30; H, 5.56; N, 11.43.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(2-fluoro-4-hydroxyphenyl)propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4r**): Method B (22% from **2**), a colorless amorphous powder. $[\alpha]_D^{20}$ −116.0° (c=0.40, DMSO). ^{1}H-NMR (D_2O+DCl) δ: 2.72—2.98 (3H, m), 3.14 (2H, d, J=7.2Hz), 3.34 (1H, d, J=11.0Hz), 3.60 (3H, s), 3.64—3.86 (1H, m), 4.00—4.26 (3H, m), 5.00—5.25 (2H, m), 6.58—6.73 (2H, m), 7.14 (1H, t, J=8.4 Hz), 7.75—7.93 (4H, m). *Anal.* Calcd for $C_{26}H_{28}FN_5O_8\cdot 1.5H_2O$: C, 53.42; H, 5.35; N, 11.98. Found: C, 53.49; H, 5.57; N, 12.05.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-fluorophenyl)-

propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**4s**): Method B (63% from **3s**), a colorless amorphous powder. $[\alpha]_D^{20}$ +55.2° (c=0.40, H_2O). ^{1}H-NMR (D_2O) δ: 2.68—3.04 (3H, m), 3.06—3.42 (3H, m), 3.60 (3H, s), 3.64—3.86 (1H, m), 3.78 (1H, d, J=16.8 Hz), 3.96 (1H, d, J=16.8 Hz), 4.04—4.22 (1H, m), 5.09 (1H, t, J=6.2 Hz), 5.20 (1H, t, J=7.6 Hz), 7.03—7.18 (2H, m), 7.24—7.40 (2H, m), 7.76—8.00 (4H, m). *Anal.* Calcd for $C_{26}H_{28}FN_5O_7\cdot 2H_2O$: C, 54.07; H, 5.58; N, 12.13. Found: C, 54.40; H, 5.60; N, 12.33.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-[4-(methoxycarbonyl)-phenyl]propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Hydrochloride (**4t**): Method A (53% from **3t**), a colorless amorphous powder. $[\alpha]_D^{20}$ +57.1° (c=0.99, H_2O). ^{1}H-NMR (D_2O) δ: 2.30—2.50 (1H, m), 2.82 (2H, d, J=5.6 Hz), 3.00—3.38 (3H, m), 3.52 (3H, s), 3.85 (3H, s), 3.40—4.10 (4H, m), 4.90—5.30 (2H, m), 7.34 (2H, d, J=8.4 Hz), 7.65—7.95 (6H, m). *Anal.* Calcd for $C_{28}H_{31}N_5O_9\cdot HCl\cdot 2.5H_2O$: C, 50.72; H, 5.62; N, 10.56. Found: C, 50.68; H, 5.50; N, 10.48.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-ethynylphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Hydrochloride (**4u**): Method B (3% from **27**), a colorless amorphous powder. $[\alpha]_D^{20}$ +57.2° (c=0.96, H_2O). ^{1}H-NMR (D_2O) δ: 2.15—2.40 (1H, m), 2.60—2.85 (2H, m), 2.85—4.10 (8H, m), 3.47 (3H, s), 4.60—5.10 (2H, m), 7.06—7.48 (4H, m), 7.64—7.88 (4H, m). *Anal.* Calcd for $C_{28}H_{29}N_5O_7\cdot HCl\cdot 3H_2O$: C, 52.71; H, 5.69; N, 10.98. Found: C, 52.61; H, 5.51; N, 11.06.

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-vinylphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Hydrochloride (**4v**): Method B (58% from **3v**), a colorless amorphous powder. $[\alpha]_D^{20}$ +84.9° (c=0.97, H_2O). ^{1}H-NMR (D_2O) δ: 2.05—2.30 (1H, m), 2.83 (2H, d, J=5.4 Hz), 2.95—3.30 (3H, m), 3.40—4.20 (4H, m), 3.55 (3H, s), 4.99 (1H, t, J=6.6 Hz), 4.90—5.20 (1H, m), 5.28 (1H, d, J=11.0 Hz), 5.79 (1H, d, J=17.8 Hz), 6.72 (1H, dd, J=17.8, 11.0 Hz), 7.10—7.50 (4H, m), 7.70—8.00 (4H, m). *Anal.* Calcd for $C_{28}H_{31}N_5O_7\cdot HCl\cdot 2H_2O$: C, 54.06; H, 5.83; N, 11.26. Found: C, 53.82; H, 5.52; N, 11.11.

2-[(3*S*)-4-[(2*R*)-2-(4-Amidinobenzoylamino)-3-(4-hydroxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**5a**): Method A (29%), a colorless needles (H_2O), mp 231—232 °C. $[\alpha]_D^{20}$ +259.2° (c=0.92, DMSO). *Anal.* Calcd for $C_{26}H_{29}N_5O_8\cdot 4.5H_2O$: C, 50.32; H, 6.17; N, 11.28. Found: C, 50.02; H, 5.93; N, 11.33.

2-[(3*R*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-hydroxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**5b**): Method A, a colorless crystalline powder (H_2O), mp 223—232 °C. $[\alpha]_D^{20}$ −37.02° (c=0.60, 0.1 N HCl). *Anal.* Calcd for $C_{26}H_{29}N_5O_8\cdot 4.5H_2O$: C, 50.32; H, 6.17; N, 11.28. Found: C, 50.34; H, 6.13; N, 11.06.

2-[(3*R*)-4-[(2*R*)-2-(4-Amidinobenzoylamino)-3-(4-hydroxyphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (**5c**): Method A, a colorless crystalline powder (H_2O–EtOH), mp 221—226 °C. $[\alpha]_D^{20}$ −63.2° (c=1.06, DMSO). *Anal.* Calcd for $C_{26}H_{29}N_5O_8\cdot 2H_2O$: C, 54.26; H, 5.78; N, 12.17. Found: C, 53.88; H, 5.66; N, 12.23.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-ethylphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (6) Under an H_2 atmosphere, a suspension of **3v** (0.60 g, 1.07 mmol) and 10% Pd–C (0.24 g) in MeOH (6.0 ml) was stirred for 1.5 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to give **6** (0.53 g, 88%) as a colorless amorphous powder. IR (KBr) cm^{-1}: 3288, 2970, 2940, 1746, 1698, 1655, 1625, 1507, 1436, 1365, 1231, 1174, 1157. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=7.6 Hz), 1.42 (9H, s), 1.45 (9H, s), 2.23—2.43 (1H, m), 2.60 (2H, q, J=7.6 Hz), 2.74—3.36 (5H, m), 3.40—4.00 (7H, m), 4.62—4.84 (1H, m), 4.97 (1H, t, J=5.0 Hz), 5.30 (1H, d, J=9.0 Hz), 7.00—7.25 (4H, m).

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-ethylphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Hydrochloride (10) Compound **10** was prepared from **6** using method B (69%), a colorless amorphous powder. $[\alpha]_D^{20}$ +75.0° (c=1.00, H_2O). IR (KBr) cm^{-1}: 1732, 1659, 1632, 1485. ^{1}H-NMR (D_2O) δ: 1.15 (3H, t, J=7.4 Hz), 2.04—2.28 (1H, m), 2.44—2.74 (2H, m), 2.74—3.42 (5H, m), 3.44—4.28 (4H, m), 3.57 (3H, s), 4.90—5.23 (2H, m), 7.10—7.40 (4H, m), 7.75—8.05 (4H, m). *Anal.* Calcd for $C_{28}H_{33}N_5O_7\cdot HCl\cdot 2H_2O$: C, 53.89; H, 6.14; N, 11.22. Found: C, 53.91; H, 5.87; N, 11.28.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-formylphenyl)propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (7) To a mixture of **3v** (2.21 g, 3.95 mmol), $NaIO_4$ (3.46 g, 16.2 mmol), CCl_4 (22.1 ml), MeCN (22.1 ml) and H_2O (33.2 ml) was added $RuCl_3\cdot nH_2O$ (16.4 mg, 0.079mmol) at room temperature. After being stirred for 1 h at room temperature, the mixture was extracted with EtOAc. The EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 1 : 2) to give **7** (1.14 g, 51%) as a colorless syrup. IR (KBr) cm^{-1}: 2978, 2934, 1740, 1702, 1654, 1436, 1366, 1157. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (9H, s), 1.45 (9H, s), 2.66—3.27 (6H, m), 3.42—3.93 (6H, m), 4.05—4.36 (1H, m), 4.72—4.90 (1H, m), 4.98 (1H, t, J=5.0 Hz), 5.35 (1H, d, J=9.0 Hz), 7.34—7.60 (2H, m), 7.83 (2H, d, J=8.0 Hz), 9.98 (1H, s).

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-formylphenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (11) Compound **11** was prepared from **7** using method B (65%), a colorless amorphous powder. $[\alpha]_D^{20}$ +28.6° (c=0.97, H_2O). IR (KBr) cm^{-1}: 1731, 1690, 1637, 1603, 1539, 1437, 1384, 1302. ^{1}H-NMR (D_2O) δ: 2.30—2.90 (3H, m), 2.95—3.30 (3H, m), 3.45 (3H, s), 3.35—4.10 (4H, m), 4.85—5.00 (1H, m), 5.03—5.20 (1H, m), 7.34 (2H, d, J=8.0 Hz), 7.55—7.85 (6H, m), 9.70 (1H, s). *Anal.* Calcd for $C_{27}H_{29}N_5O_8\cdot 3H_2O$: C, 53.55; H, 5.83; N, 11.56. Found: C, 53.61; H, 5.74; N, 11.68.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[4-(hydroxymethyl)phenyl]propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (8) To a solution of **7** (0.65 g, 1.16 mmol) in MeOH (13.0 ml) was added $NaBH_4$ (21.9 mg, 0.58 mmol) at 0 °C. After being stirred for 1 h at room temperature, the mixture was poured into H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (CH_2Cl_2–EtOAc, 2 : 3) to give **8** (0.56 g, 86%) as a colorless syrup. IR (KBr) cm^{-1}: 3428, 2978, 1740, 1708, 1647, 1437, 1366, 1248, 1156. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.45 (9H, s), 2.18—2.40 (1H, m), 2.52—3.25 (6H, m), 3.63 (3H, s), 3.40—4.02 (3H, m), 4.30 (1H, d, J=16.0 Hz), 4.63 (2H, s), 4.68—4.94 (2H, m), 5.23—5.50 (1H, m), 7.10—7.40 (4H, m).

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-[4-(hydroxymethyl)-phenyl]propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (12) Compound **12** was prepared from **8** using method B (61%), a colorless amorphous powder. $[\alpha]_D^{20}$ +62.5° (c=1.01, H_2O). IR (KBr) cm^{-1}: 1730, 1631, 1541, 1446, 1385. ^{1}H-NMR (D_2O+DCl) δ: 2.17—2.40 (1H, m), 2.60—3.32 (5H, m), 3.54 (3H, m), 3.40—4.20 (4H, m), 4.56 (2H, s), 4.99 (1H, t, J=6.0 Hz), 5.07—5.25 (1H, m), 7.10—7.40 (4H, m), 7.70—7.98 (4H, m). *Anal.* Calcd for $C_{27}H_{31}N_5O_8\cdot 3H_2O$: C, 53.37; H, 6.14; N, 11.53. Found: C, 53.50; H, 5.94; N, 11.46.

4-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[(2*S*)-4-[2-(*tert*-butoxy)-2-oxoethyl]-2-(2-methoxy-2-oxoethyl)-3-oxopiperazinyl]-3-oxopropyl]benzoic Acid (9) To a mixture of **7** (0.48 g, 0.86 mmol), acetic acid (AcOH) (0.24 ml) and acetone (4.8 ml) was added $KMnO_4$ (0.14 g, 0.86 mmol) at 0 °C. After being stirred for 40 min at 0 °C and for 1 h at room temperature, the mixture was poured into H_2O and extracted with EtOAc. The EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (CH_2Cl_2–MeOH, 10:1) to give **9** (0.48 g, 97%) as a colorless syrup. IR (KBr) cm^{-1}: 3426, 2978, 1736, 1707, 1650, 1438, 1366, 1247, 1159. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (9H, s), 1.43 (9H, s), 2.60—3.30 (6H, m), 3.40—4.16 (4H, m), 3.65 (3H, s), 4.74—4.94 (1H, m), 5.01 (1H, t, J=5.4 Hz), 5.57 (1H, d, J=7.8 Hz), 7.20—7.50 (2H, m), 8.02 (2H, d, J=8.2 Hz).

4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-[(2*S*)-4-(carboxymethyl)-2-(2-methoxy-2-oxoethyl)-3-oxopiperazinyl]-3-oxopropyl]benzoic Acid Hydrochloride (13) Compound **13** was prepared from **9** using method B (55%), a colorless amorphous powder. $[\alpha]_D^{20}$ +59.3° (c=1.02, H_2O). IR (KBr) cm^{-1}: 1645, 1541, 1485, 1441, 1223, 1181. ^{1}H-NMR (D_2O) δ: 2.26—2.54 (1H, m), 2.70—2.98 (2H, m), 3.02—3.42 (3H, m), 3.42—4.18 (4H, m), 3.54 (3H, s), 4.99 (1H, t, J=6.0Hz), 5.10—5.30 (1H, m), 7.37 (2H, d, J=8.0 Hz), 7.65—8.05 (4H, m), 7.91 (2H, d, J=8.0Hz). *Anal.* Calcd for $C_{27}H_{29}N_5O_9\cdot HCl\cdot 3.5H_2O$: C, 48.62; H, 5.59; N, 10.50. Found: C, 48.56; H, 5.34; N, 10.61.

Methyl 2-[(2*S*)-1-[(2*S*)-3-(4-Aminophenyl)-2-[(*tert*-butoxycarbonyl)-amino]propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (14) Under an H_2 atmosphere, a suspension of **3w** (5.0 g, 8.64 mmol) and 10% Pd–C (2.0 g) in MeOH (100 ml) was stirred for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was chromatographed on SiO_2 (EtOAc) to give **14** (4.74 g, quant.) as a colorless syrup. ^{1}H-NMR ($CDCl_3$+D_2O) δ: 1.43 (9H, s), 1.45 (9H, s), 2.30—2.50 (1H, m), 2.70—3.10 (5H, m), 3.65 (3H, s), 3.40—3.80 (2H, m), 3.86 (1H, d, J=17.2 Hz), 4.00 (1H, d, J=17.2 Hz), 4.55—4.80 (1H, m), 5.00 (1H, t, J=5.4 Hz), 6.61 (2H, d, J=8.4 Hz), 6.96 (2H, d, J=8.4 Hz).

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-(4-aminophenyl)-propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (17) Compound **17** was prepared from **14** using method B (77%), a colorless crystalline powder (H_2O–EtOH), mp 232—237 °C. $[\alpha]_D^{20}$ −48.8° (c=0.94, DMSO). IR (KBr) cm^{-1}: 3301, 1723, 1626, 1518, 1385, 1292. ^{1}H-NMR

(DMSO-d_6+D_2O) δ: 2.54—4.50 (13H, m), 4.87 (1H, t, J=6.2 Hz), 4.70—5.10 (1H, m), 6.43 (2H, d, J=8.2 Hz), 6.94 (2H, d, J=8.2 Hz), 7.70—8.10 (4H, m). *Anal.* Calcd for $C_{26}H_{30}N_6O_7\cdot 2.5H_2O$: C, 53.51; H, 6.04; N, 14.40. Found: C, 53.71; H, 5.74; N, 14.61.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[4-(dimethylamino)phenyl]propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (15) To a mixture of **14** (0.66 g, 1.21 mmol), 37% aqueous HCHO (0.34 ml, 12.1 mmol), AcOH (0.69 ml, 1.21 mmol) and MeCN (13.3 ml) was added $NaBH_3CN$ (0.12 g, 1.94 mmol) at room temperature. After being stirred for 2 h, the mixture was poured into H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 1 : 2) to give **15** (0.49 g, 70%) as a colorless syrup. IR (KBr) cm^{-1}: 2932, 1744, 1697, 1652, 1624, 1510, 1444, 1366, 1231, 1156. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.45 (9H, s), 2.41—2.52 (1H, s), 2.90 (6H, s), 2.60—3.30 (5H, m), 3.65 (3H, s), 3.40—3.90 (3H, m), 4.01 (1H, d, J=17.2 Hz), 4.55—4.80 (1H, m), 4.99 (1H, t, J=5.2 Hz), 5.31 (1H, d, J=8.4 Hz), 6.64 (2H, d, J=8.8 Hz), 7.04 (2H, d, J=8.8 Hz).

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-[4-(dimethylamino)phenyl]propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid (18) Compound **18** was prepared from **15** using method B (60%), a pale yellow crystalline powder (H_2O–EtOH), mp 217—220 °C. $[\alpha]_D^{20}$ −58.7° (c=0.92, DMSO). IR (KBr) cm^{-1}: 1732, 1637, 1521, 1446, 1381, 1295. ^{1}H-NMR (DMSO-d_6+D_2O) δ: 2.56—4.56 (13H, m), 2.82 (6H, s), 4.86 (1H, t, J=6.0 Hz), 4.84—5.10 (1H, m), 6.61 (2H, d, J=8.4 Hz), 7.02—7.22 (2H, m), 7.70—8.10 (4H, m). *Anal.* Calcd for $C_{28}H_{34}N_6O_7\cdot 2.5H_2O$: C, 54.98; H, 6.43; N, 13.74. Found: C, 54.84; H, 6.20; N, 13.86.

Methyl 2-[(2*S*)-1-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[4-(methylamino)phenyl]propanoyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxopiperazinyl]acetate (16) To a mixture of **14** (3.06 g, 5.58 mmol), benzaldehyde (0.85 ml, 8.37 mmol), AcOH (0.32 ml, 5.58 mmol) and MeCN (31 ml) was added $NaBH_3CN$ (0.53 g, 8.37 mmol) at room temperature. After being stirred for 2 h, the mixture was poured into H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 2 : 3) to give the benzylamino intermediate (1.10 g, 31%) as a colorless syrup. To a mixture of this intermediate, 37% aqueous HCHO (1.29 ml, 17.2 mmol), AcOH (0.1 ml, 1.72 mmol) and MeCN (11.0 ml) was added $NaBH_3CN$ (0.17 g, 2.75 mmol) at room temperature. After being stirred for 2 h, the mixture was purified by the procedure described above to give the *N*-benzyl-*N*-methylamino intermediate (1.10 g, 98%) as a colorless syrup. Then, a suspension of this intermediate and 10% Pd–C (0.44 g) in MeOH (11.0 ml) was stirred for 2 h at room temperature under an H_2 atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 1:2) to give **16** (0.95 g, 100%) as a colorless syrup. IR (KBr) cm^{-1}: 2978, 1741, 1652, 1522, 1444, 1366, 1156. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.45 (9H, s), 2.29—2.50 (1H, m), 2.79 (3H, s), 2.62—3.37 (5H, m), 3.65 (3H, s), 3.50—3.80 (3H, m), 3.79 (1H, d, J=17.3 Hz), 4.01 (1H, d, J=17.3 Hz), 4.55—4.78 (1H, m), 4.99 (1H, t, J=5.2 Hz), 5.29 (1H, d, J=8.8 Hz), 6.52 (2H, d, J=8.4 Hz), 7.00 (2H, d, J=8.4 Hz).

2-[(3*S*)-4-[(2*S*)-2-(4-Amidinobenzoylamino)-3-[4-(methylamino)phenyl]propanoyl]-3-(2-methoxy-2-oxoethyl)-2-oxopiperazinyl]acetic Acid Trifluoroacetate (19) Compound **19** was prepared from **16** using method B (60%), a colorless amorphous powder. $[\alpha]_D^{20}$ +4.9° (c=0.81, H_2O). IR (KBr) cm^{-1}: 1638, 1541, 1440, 1385, 1296. ^{1}H-NMR (D_2O) δ: 2.40—4.20 (13H, m), 3.55 (3H, s), 4.90—5.30 (2H, m), 7.05—8.10 (8H, m). *Anal.* Calcd for $C_{27}H_{32}N_6O_7\cdot CF_3CO_2H\cdot 1.5H_2O$: C, 50.22; H, 5.23; N, 12.12. Found: C, 50.48; H, 5.16; N, 12.52.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-methoxyphenyl)propanoic Acid (21a) To a solution of *Z*-L-Tyr-OH (2.0 g, 6.34 mmol) and potassium carbonate (2.1 g, 15.22 mmol) in *N*,*N*-dimethylformamide (DMF) and water (10 : 1, v : v; 20 ml) was added methyl iodide (8.2 ml, 25.36 mmol) at 0 °C. After being stirred for 2 h at room temperature, the mixture was diluted with EtOAc, washed with water and 5% aqueous $KHSO_4$, dried over $MgSO_4$ and concentrated under reduced pressure to give *Z*-L-Tyr(Me)-OMe (1.94 g, 5.65 mmol) as a yellow oil. Then, 1 N NaOH (5.7 ml) was added to a solution of *Z*-L-Tyr(Me)-OMe in 1,4-dioxane (5.7 ml) at room temperature. After being stirred for 1 h at room temperature, the mixture was acidified with 5% aqueous $KHSO_4$ and extracted with EtOAc. The EtOAc extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on SiO_2 (hexane–EtOAc, 1 : 4) to give **21a** (1.6 g, 79%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 2.90—3.23 (2H, m), 3.77 (3H, s), 4.55—4.75 (1H, m), 5.09 (2H, s), 5.10—5.32 (1H, m), 6.81 (2H, d, J=8.8 Hz), 7.06 (2H, d, J=8.8 Hz), 7.20—7.50 (5H, m).

Compounds **21b**—**h** were prepared in a manner similar to that described for **21a**.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-ethoxyphenyl)propanoic Acid (**21b**): A colorless solid (78%). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.0 Hz), 2.80—3.22 (2H, m), 3.99 (2H, q, J=7.0 Hz), 4.55—4.75 (1H, m), 5.09 (2H, s), 5.10—5.35 (1H, m), 6.79 (2H, d, J=8.6 Hz), 7.04 (2H, d, J=8.6 Hz), 7.20—7.50 (5H, m).

(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-propoxyphenyl)propanoic Acid (**21c**): A colorless solid (85%). ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, t, J=7.2 Hz), 1.67—1.90 (2H, m), 2.90—3.22 (2H, m), 3.87 (2H, t, J=6.4 Hz), 4.55—4.70 (1H, m), 5.09 (2H, s), 5.18—5.37 (1H, m), 6.78 (2H, d, J=8.8 Hz), 7.05 (2H, d, J=8.8 Hz), 7.20—7.45 (5H, m).

(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-isopropoxyphenyl)propanoic Acid (**21d**): A colorless solid (53%). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, s), 1.32 (3H, s), 2.90—3.22 (2H, m), 4.36—4.72 (2H, m), 5.06 (1H, d, J=12.4 Hz), 5.13 (1H, d, J=12.4 Hz), 5.22—5.44 (1H, m), 6.77 (2H, d, J=8.8 Hz), 7.05 (2H, d, J=8.8 Hz), 7.20—7.40 (5H, m).

(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-decyloxyphenyl)propanoic Acid (**21e**): A colorless solid (32%). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.6 Hz), 1.10—1.50 (14H, m), 1.65—1.85 (2H, m), 2.90—3.20 (2H, m), 3.90 (2H, t, J=6.6 Hz), 4.53—4.70 (1H, m), 5.09 (2H, s), 5.20—5.42 (1H, m), 6.71 (2H, d, J=8.4 Hz), 6.97 (2H, d, J=8.4 Hz), 7.20—7.40 (5H, m).

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(3,4-dimethoxyphenyl)propanoic Acid (**21f**): A beige solid (60%). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 2.80—3.24 (2H, m), 3.85 (3H, s), 3.86 (3H, s), 4.42—4.66 (1H, m), 4.82—5.08 (1H, m), 6.60—6.87 (3H, m).

(2*S*)-3-(4-Allyloxyphenyl)-2-[(*tert*-Butoxycarbonyl)amino]propanoic Acid (**21g**): A pale yellow syrup (70%). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 2.90—3.22 (2H, m), 4.30—4.65 (3H, m), 4.80—5.05 (1H, m), 5.20—5.50 (2H, m), 5.92—6.18 (1H, m), 6.86 (2H, d, J=8.8 Hz), 7.09 (2H, d, J=8.8 Hz).

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[4-(2-propynyloxy)phenyl]-propanoic Acid (**21h**): A colorless syrup (65%). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 2.52 (1H, t, J=2.2 Hz), 2.90—3.22 (2H, m), 4.46—4.66 (1H, m), 4.68 (2H, d, J=2.2 Hz), 4.84—5.00 (1H, m), 6.92 (2H, d, J=8.8 Hz), 7.12 (2H, d, J=8.8 Hz).

***tert*-Butyl (2*S*)-2-[(Benzyloxycarbonyl)amino]-3-[4-[(trifluoromethylsulfonyl)oxy]phenyl]propanoate (23)** To a mixture of **22** (15.6 g, 42.1 mmol), 2,6-lutidine (7.36 ml, 63.2 mmol), 4-dimethylaminopyridine (1.03 g, 8.42 mmol) and CH_2Cl_2 (210 ml) was added dropwise Tf_2O (10.6 ml, 63.2 mmol) at −30 °C. After being stirred for 1 h at room temperature, the mixture was poured into H_2O and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 4 : 1) to give **23** (11.39 g, 54%) as a colorless powder. IR (KBr) cm^{-1}: 3380, 1741, 1697, 1530, 1501, 1419, 1348, 1249, 1226, 1143, 1058, 1016, 892, 712, 696, 608, 498. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (9H, s), 3.10 (2H, d, J=6.2 Hz), 4.42—4.60 (1H, m), 5.05 (1H, d, J=14.2 Hz), 5.13 (1H, d, J=14.2 Hz), 5.31 (1H, d, J=7.8 Hz), 7.10—7.28 (4H, m), 7.28—7.48 (5H, m).

Methyl 4-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(*tert*-butoxy)-3-oxopropyl]benzoate (24) CO gas was bubbled through a mixture of **23** (2.0 g, 3.97 mmol), 1,3-bis(diphenylphosphino)propane (0.16 g, 0.40 mmol), $Pd(OAc)_2$ (89.1 mg, 0.40 mmol), triethylamine (1.11 ml, 7.94 mmol), MeOH (15.0 ml) and DMSO (20.0 ml at room temperature for 5 min, and the mixture was heated at 80 °C for 2 h under a CO atmosphere. The mixture was cooled to room temperature, poured into H_2O and extracted with EtOAc. The EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 3 : 1) to give **24** (1.52 g, 93%) as a colorless syrup. IR (KBr) cm^{-1}: 3346, 2978, 1720, 1610, 1515, 1367, 1279, 1220, 1154, 1107, 1056. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (9H, s), 3.00—3.25 (2H, m), 3.90 (3H, s), 4.48—4.65 (1H, m), 5.00—5.18 (2H, m), 5.32 (1H, d, J=8.0 Hz), 7.23 (2H, d, J=8.0 Hz), 7.20—7.50 (5H, m), 7.94 (2H, d, J=8.0 Hz).

(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-[4-(methoxycarbonyl)phenyl]-propanoic Acid (25) To a solution of **24** (0.55 g, 1.33 mmol) in toluene (2.75 ml) was added trifluoroacetic acid (2.75 ml) at room temperature. After being stirred for 1 h, the mixture was concentrated *in vacuo* to give **25** (0.47 g, 99%) as a colorless crystalline powder (EtOAc–petroleum ether), mp 102—106 °C. $[\alpha]_D^{20}$ +58.6° (c=0.06, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.90—3.36 (2H, m), 3.90 (3H, s), 4.60—4.80 (1H, m), 4.94—5.16 (2H, m), 5.18—5.30 (1H, m), 7.22 (2H, d, J=8.2 Hz), 7.10—7.50 (5H, m), 7.94 (2H, d, J=8.2 Hz). *Anal.* Calcd for $C_{19}H_{19}NO_6$: C, 63.89; H, 5.36; N, 3.92. Found: C, 63.70; H, 5.47; N, 3.95.

Ethyl (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[4-[(trifluoromethylsulfonyl)oxy]phenyl]propanoate (27) Compounds **27** was prepared in a manner similar to that described for **23**, a pale reddish crystalline powder (EtOAc), mp 49—50 °C. $[\alpha]_D^{20}$ +32.3° (c=0.98, $CHCl_3$). IR (KBr) cm^{-1}: 2984, 1716, 1501, 1424, 1213, 1141. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=7.0 Hz), 1.41 (9H, s), 2.95—3.25 (2H, m), 4.16 (2H, q, J=7.0 Hz), 4.48—4.64 (1H, m), 4.97—5.14 (1H, m), 7.15—7.28 (4H, m). *Anal.* Calcd for $C_{17}H_{22}F_3NO_7S$: C, 46.25; H, 5.02; N, 3.17. Found: C, 46.52; H, 5.07; N, 3.13.

Ethyl (2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-vinylphenyl)propanoate (29) A mixture of **27** (2.89 g, 6.56 mmol), LiCl (0.83 g, 19.68 mmol), $PdCl_2(PPh_3)_2$ (92.1 mg, 0.13 mmol), vinyltributyltin (1.99 ml, 6.82 mmol) and DMF (49.1 ml) was stirred at 90 °C under an N_2 atmosphere for 2 h. After being cooled, the mixture was poured into H_2O and extracted with EtOAc. The EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (hexane–EtOAc, 4 : 1) to give **29** (1.65 g, 79%) as a pale yellow oil. IR (KBr) cm^{-1}: 2978, 1713, 1510, 1366, 1249, 1168. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7.2 Hz), 1.42 (9H, s), 2.90—3.21 (2H, m), 4.17 (2H, q, J=7.2 Hz), 4.47—4.63 (1H, m), 4.90—5.08 (1H, m), 5.23 (1H, d, J=10.8 Hz), 5.72 (1H, d, J=17.6 Hz), 6.69 (1H, dd, J=10.8, 17.6 Hz), 7.10 (2H, d, J=8.0 Hz), 7.34 (2H, d, J=8.0 Hz).

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-vinylphenyl)propanoic Acid (31) To a solution of **29** (1.65 g, 5.17 mmol) in MeOH (4.7 ml) and H_2O (0.47 ml) was added $LiOH \cdot H_2O$ (0.24 g, 5.69 mmol) at room temperature. After being stirred for 20 min, the mixture was adjusted to pH 2 with 1 N HCl and extracted with EtOAc. The EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give **31** (1.40 g, 93%) as a colorless oil. IR (KBr) cm^{-1}: 3428, 2978, 1714, 1511, 1366, 1164. ^{1}H-NMR ($CDCl_3$+D_2O) δ: 1.42 (9H, s), 2.80—3.28 (2H, m), 4.53—4.70 (1H, m), 4.83—5.04 (1H, m), 5.23 (1H, d, J=10.8 Hz), 5.73 (1H, d, J=17.6 Hz), 6.69 (1H, dd, J=10.8, 17.6 Hz), 7.14 (2H, d, J=8.2 Hz), 7.35 (2H, d, J=8.2 Hz).

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-ethynylphenylphenyl)-propanoic Acid (30) Compound **30** was prepared in a manner similar to that described for **31** and used for a next reaction without purification.

Fibrinogen Binding Studies Human platelet GPIIb/IIIa was purified from a human erythroleukemia cell line and used for the binding assay. In brief, 100 μl of GPIIb/IIIa complex (1.0 μg/ml) in a buffer containing 20 mM Tris–HCl, 150 mM NaCl, 1 mM $CaCl_2$ and 0.02% NaN_3 (pH 7.4) was added to 96-well microtiter plates and incubated for 48 h at 4 °C. After blocking non-specific binding sites with a blocking agent (Block Ace, Dainippon Pharmaceutical Co., Osaka, Japan) for 3 h at room temperature, biotinated-fibrinogen (1 μg/ml) with various concentrations of test compounds was added to the receptor-coated wells and incubated for 18 h at room temperature. Bound biotinated-fibrinogen was measured by an ELISA using anti-biotin rabbit antibody-conjugated alkaline phosphatase and *p*-nitrophenyl phosphate as the substrate.

Platelet Aggregation Studies *In Vitro*: Blood was collected by venipuncture from healthy human volunteers and Cynomolgus monkeys. Guinea pigs were anesthetized with sodium pentobarbital and blood was collected by aortic puncture. Blood was withdrawn into a plastic syringe containing 3.8% (human and monkey) or 3.15% (guinea pig) sodium citrate (1 : 10 citrate/blood, v/v). PRP and platelet-poor plasma (PPP) were obtained by centrifugation at 1000×***g*** for 3—5 s and 1000×***g*** for 20 min at room temperature, respectively. The platelet count in PRP was adjusted to 3×10^5/ml (human, monkey) and 4×10^5/ml (guinea pig) using an automatic blood cell counter (Sysmex E2500, Toaiyoudenshi Co., Tokyo, Japan). Platelet aggregation was measured using an 8 channel aggregometer (Hematracer VI, Niko Bioscience, Tokyo, Japan). PRP (250 ml), in a cuvette stirred at 1000 rpm, was prewarmed for 2 min at 37 °C with various concentrations of test compounds (25 μl). The change in light transmittance was measured after the addition of aggregating agents (25 μl) to the cuvette. Submaximal concentrations of aggregating agents were used in each experiment.

Ex Vivo: Male Hartley guinea pigs (300—400 g) were used. At various times after i.v. or *p.o.* administration of test compounds, blood was collected, and PRP and PPP were prepared as described for the *in vitro* study. ADP (20 μl) was added to the cuvette containing the prewarmed PRP (220 μl). In order to eliminate the possible influence of the sensitivity differences of guinea pig platelets to ADP, which is dependent on the animal lot and experimental conditions including drug administration protocol, two or three vehicle-treated animals were used as a control at each measuring point. The percentage inhibition of platelet aggregation in drug-treated animals was determined by comparison with the aggregation in the controls at each point.

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