# Potent Dibasic GPIIb/IIIa Antagonists with Reduced Prolongation of Bleeding Time: Synthesis and Pharmacological Evaluation of 2-Oxopiperazine Derivatives

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*Received October 4, 2000*

A series of 2-oxopiperazine derivatives, possessing basic moieties at the 3- and the 4-positions, were synthesized and evaluated for their abilities to inhibit platelet aggregation and for their effects on bleeding time. Among the compounds, 2-[(3*S*)-4-[2-[(4-guanidinobenzoyl)amino]acetyl]-3-[3-[(4-guanidinobenzoyl)amino]propyl]-2-oxopiperazinyl]acetic acid (**12c**) showed a potent inhibitory effect on platelet aggregation and good dissociation between the efficacy and the bleeding side effect. Intravenous infusion of compound **12c** at 1.6 $\mu$g/mL/min completely prevented arterial thrombus formation induced by endothelial injury in guinea pigs. The dose of **12c** that prolonged the bleeding time to three times the control value was 5.8 $\mu$g/mL/min. These results suggest that compound **12c** might be useful in the clinical treatment of thrombotic diseases, and we selected **12c** (TAK-024) as a candidate for the clinical trials.

## Introduction

Platelet-mediated thrombus formation in the coronary artery is a primary factor in the development of thrombotic disorders such as unstable angina, myocardial infarction, and reocclusion after angioplasty.[1,2] The activation, adhesion, and aggregation of platelets are important processes in the initiation of thrombus formation at sites showing high-grade stenosis, ruptured atheromatous plaque, and endothelial damage within arteries. Recent studies on the biochemical mechanisms of platelet activation indicate that the final obligatory step in platelet aggregation, regardless of the activating signal, is the cross-linking of plasma protein fibrinogen and platelet membrane glycoprotein IIb/IIIa (GPIIb/IIIa) exposed on activated platelets.[3−5] The inhibition of GPIIb/IIIa receptor binding therefore has been thought of as an attractive target in the development of more effective antithrombotic agents. In the past decade, clinical efficacy of GPIIb/IIIa antagonists including some monoclonal antibodies, cyclic peptides, and low molecular weight nonpeptides has been validated. For example, 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.[6,7] These GPIIb/IIIa antagonists have demonstrated useful therapeutic potential as antithrombotic agents; however, severe bleeding complications have occasionally occurred.[8,9]

In our previous report, we described the synthesis and pharmacological activities of the potent GPIIb/IIIa antagonists **1** (TAK-029) and **2** (Figure 1).[10,11] Although these compounds likewise showed useful therapeutic potential as antithrombotic agents, they prolonged the bleeding time at doses showing more than 50−60% inhibition of platelet aggregation. Recently, a cyclic nonapeptide, **4** (TP-9201), which potently inhibits the platelet-mediated thrombus formation without prolongation of template bleeding time was reported by Cheng and co-workers.[12] Compound **4** possesses an R-G-D-Ar-R (Ar = hydrophobic residue) sequence, and the arginine residue adjacent to the carboxyl terminal of the R-G-D-Ar sequence is considered to be a key biological effector element that could function to dissociate the antithrombotic effect from the prolongation of bleeding time (Figure 1).[12] This prompted us to reexamine one of our other GPIIb/IIIa antagonists, **3**, which has an additional basic moiety at the 3-position of the 2-oxopiperazine ring, for its effects on the bleeding time, and we found that **3** has a better dissociation profile than **1**. The $ID_{50}$ values for ex vivo platelet aggregation in guinea pigs of **3** and **1** are 0.25 and 0.16 $\mu$g/kg/min, respectively. The doses of **3** and **1** that prolong bleeding time to three times the basal value, BT(×3), are 1.4 and 0.35 $\mu$g/kg/min, respectively. The dose ratios for BT(×3) to $ID_{50}$ of **3** and **1** are 5.6 and 2.2, respectively. While a number of studies on GPIIb/IIIa antagonists have appeared, the structure−activity relationships (SARs) for the dissociation of the efficacy from the bleeding side effect have not been studied. We were interested in the effect of the additional basic moiety which may contribute to reduce the bleeding side effect and decided to investigate analogues of **3** to clarify the SARs and to find new GPIIb/IIIa antagonists with good dissociation profiles (Figure 2). On comparison of the structure of **3** with that of **4**, the aminobutyl side chain in **3** seemed to be short

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10.1021/jm0004345 CCC: $20.00 
Published on Web 06/21/2001

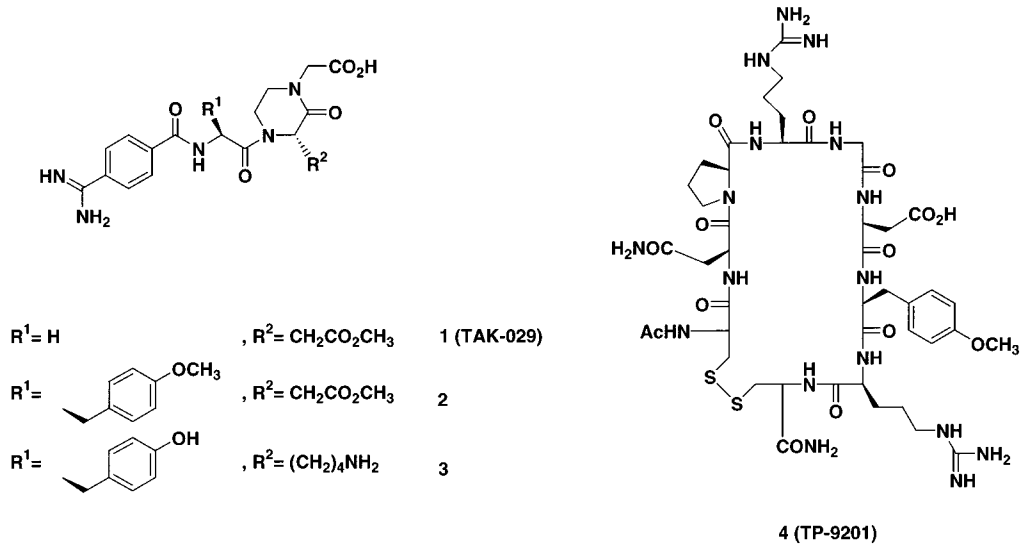



**Figure 1.**

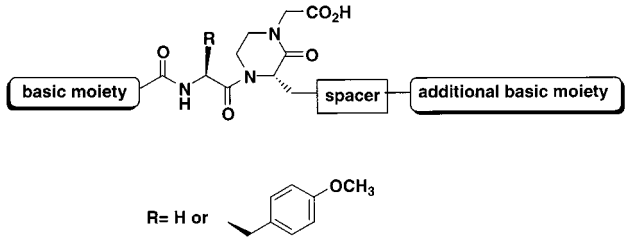
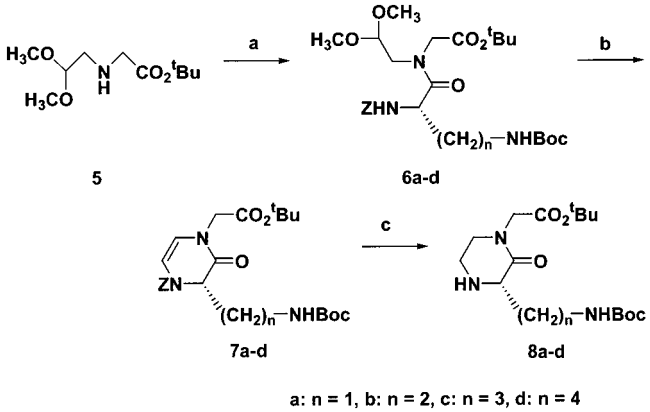



**Figure 2.** Synthetic design of dibasic compounds.

**Scheme 1**[a]




[a] Reagents: (a) *ω*-Boc-amino-α-Z-aminoalkanoic acid, EDC, $CH_2Cl_2$; (b) cat. *p*-toluenesulfonic acid monohydrate, toluene, 70 °C; (c) $H_2$, 10% Pd−C, EtOAc.

and insufficient to interact with the GPIIb/IIIa receptor. We planned to extend this side chain with varying methylene lengths and a terminal amino group with reference to the structure of **4**. In this paper we describe the synthesis and the pharmacological profiles of these dibasic compounds.

## Chemistry

The chiral 1,3-disubstituted 2-oxopiperazine derivatives **8a**−**d** as conformationally restricted C-terminal dipeptide mimetics[10] were prepared by Dimaio's method with slight modification (Scheme 1). Condensation of the secondary amine **5** with *ω*-Boc-amino-α-Z-aminoalkanoic acids[13] using 1-ethyl-3-[3-(*N*,*N*-dimethylamino)-propyl]carbodiimide hydrochloride (EDC) in methylene chloride ($CH_2Cl_2$) provided the acetal amides **6a**−**d**. Acid-catalyzed intramolecular cyclization of **6a**−**d** in toluene at 70 °C followed by hydrogenation in the presence of 10% Pd−C in ethyl acetate (EtOAc) afforded the 1,3-disubstituted 2-oxopiperazine derivatives **8a**−**d**.

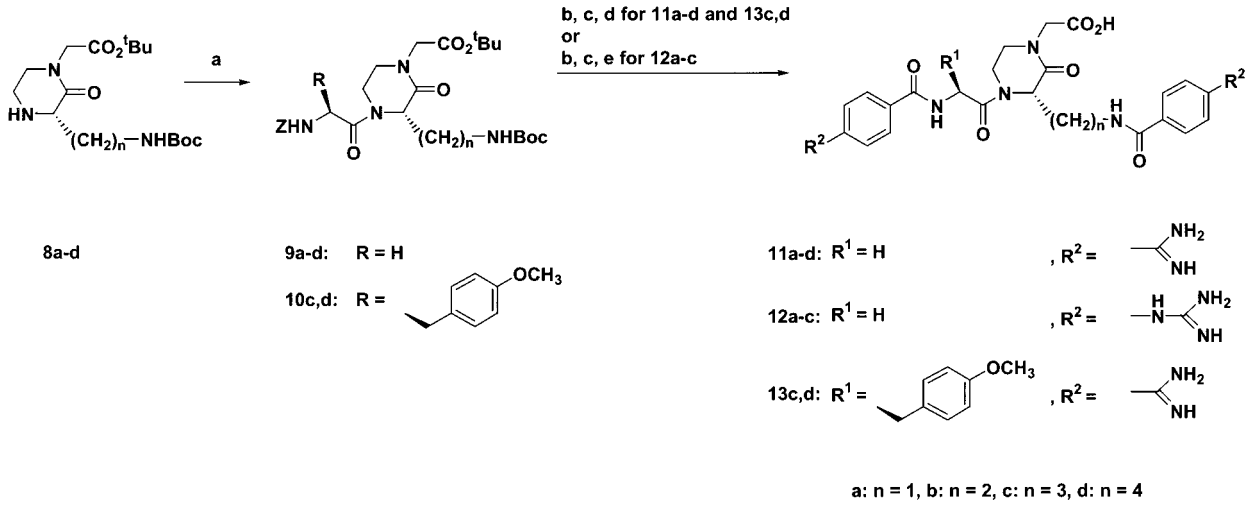
Preparation of the bis(4-amidinophenyl)derivatives **11a**−**d**, **13c**,**d**, and bis(4-guanidinophenyl)derivatives **12a**−**c** was accomplished as outlined in Scheme 2. Condensation of **8a**−**d** with Z-Gly-OH or Z-L-Tyr(Me)-OH gave the key intermediates **9a**−**d** and **10c**,**d**. Simultaneous diacylation of the two amino groups arising from successive Z and Boc group removal of **9a**−**d** and **10c**,**d** furnished **11a**−**d**, **12a**−**c**, and **13c**,**d**. The enantiomer of **12c**, **(*R*)-12c**, was prepared by the same procedure as that for the preparation of **12c** but using Z-D-Orn(Boc)-OH instead of Z-L-Orn(Boc)-OH.

The 4-[2-[(4-amidinobenzoyl)amino]acetyl]-2-oxopiperazine derivatives **20a**−**c**, **21a**,**c**, **22c**−**24c**, and **26** were synthesized from **9a**−**c** (Scheme 3). Treatment of **9a**−**c** with trifluoroacetic acid (TFA) followed by condensation with various carboxylic acids gave the amides **14**−**19**. Removal of the Z group in **14** and **15** and acylation with 4-amidinobenzoyl chloride hydrochloride yielded **20a**−**c** and **21a**,**c**, respectively. In the case of **15a**,**c**, simultaneous hydrogenolysis of the oxadiazolone rings was used to generate the amidino group. The compounds **22c**−**24c** were synthesized as described for the synthesis of **20** and **21** followed by Boc group removal. Guanidination of the terminal amine resulting from Boc group removal of **16c** followed by Z group removal and acylation with 4-amidinobenzoyl chloride hydrochloride gave the guanidinobutyramide **26**.

The carbamoyl derivative **28** was also obtained from **9c** by treatment with TFA, followed by acylation, Z group removal, and finally condensation with 4-carbamoylbenzoic acid (Scheme 4).

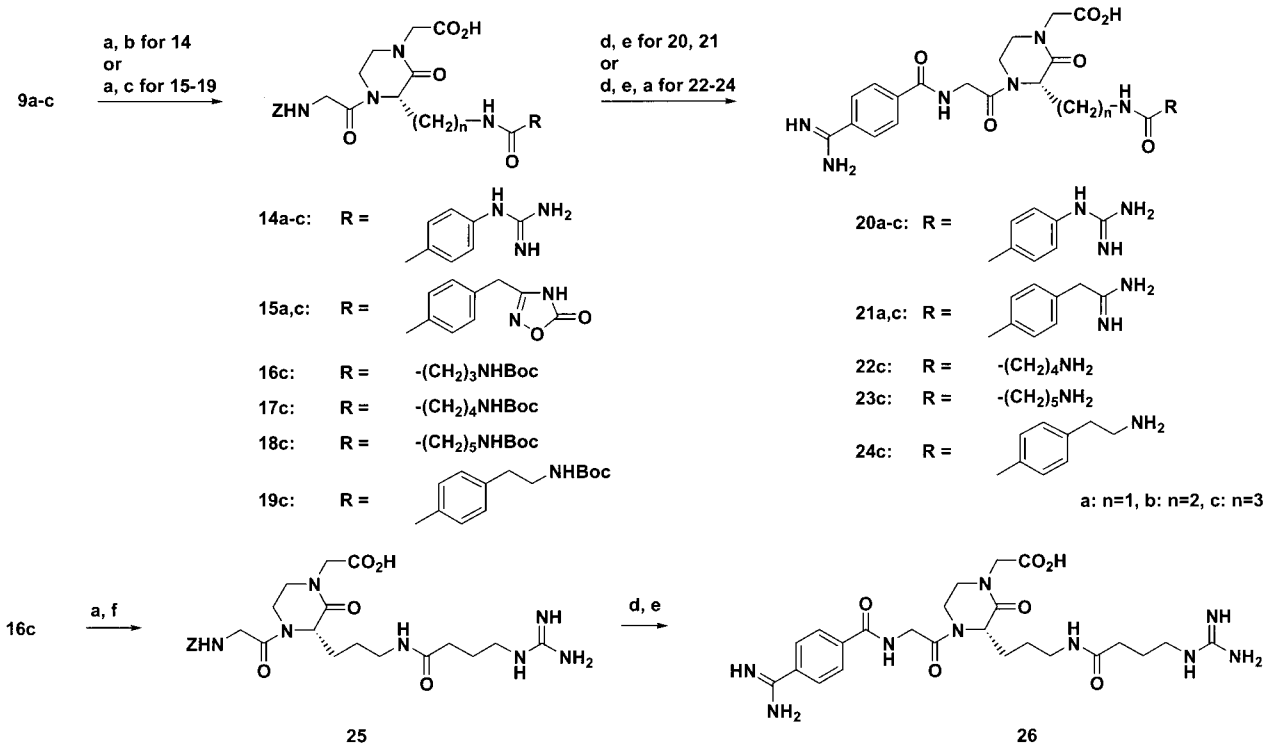
The synthesis of (4-oxadiazolylmethyl)benzoic acid **32**, which was used for the preparation of **15a**,**c**, is depicted in Scheme 5. Methyl (4-chloromethyl)benzoate **29** was converted into the nitrile **30** using NaCN. Transformation of the cyano group to amidoxime with hydroxylamine hydrochloride, subsequent reaction with carbonyl

**Scheme 2**[a]




[a] Reagents: (a) Z-Gly-OH or Z−L-Tyr(Me)-OH, EDC, $CH_2Cl_2$; (b) $H_2$, 10% Pd−C, MeOH; (c) $CF_3CO_2H$, toluene; (d) 4-amidinobenzoyl chloride hydrochloride, $NaHCO_3$, 1,4-dioxane, $H_2O$; (e) *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride, $NaHCO_3$, $H_2O$, 1,4-dioxane.

**Scheme 3**[a]




[a] Reagents: (a) $CF_3CO_2H$, toluene; (b) *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride, $NaHCO_3$, $H_2O$, 1,4-dioxane; (c) $RCO_2H$, *N*-hydroxy-5-norbornene-2,3-dicarboxyimide (HONB) or *N*-hydroxysuccinimide (HOSU), $Et_3N$, EDC or 1,3-dicyclohexylcarbodiimide (DCC), *N*,*N*-dimethylformamide (DMF); (d) $H_2$, 10% Pd−C, MeOH; (e) 4-amidinobenzoyl chloride hydrochloride, $NaHCO_3$, 1,4-dioxane, $H_2O$; (f) *S*-methylisothiourea sulfate, NaOH, $H_2O$.

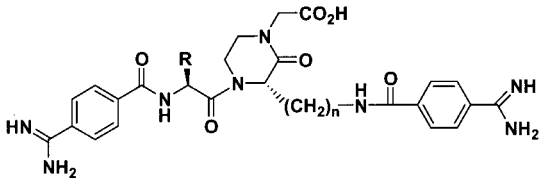
diimidazole, and saponification gave the oxadiazolone **32**.

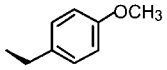
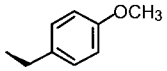
## Results and Discussion

**Structure−Activity Relationships of 2-Oxopiperazine Derivatives.** The compounds synthesized in this study were evaluated for their abilities to inhibit ADP-induced platelet aggregation in guinea pig platelet rich plasma (in vitro and ex vivo) and for their effects on bleeding time. The dose ratio of BT(×3) to the $ID_{50}$ was used as a parameter to represent the dissociation ratio.

First, we examined the influence of the 4-methoxybenzyl group, which enhanced the inhibitory effect on platelet aggregation in a series of 2-oxopiperazine derivatives,[11] upon the dissociation profile by comparing **11c**,**d** with **13c**,**d**. The 4-amidinophenyl group was employed for the additional basic moiety, because it is known to exert a strong interaction by reinforced ionic mode of binding with the negatively charged receptor.[14] As shown in Table 1, nonsubstituted compounds (R = H, **11c**,**d**) showed more favorable dissociation profiles than 4-methoxybenzyl substituted compounds (R = $CH_2$-Ph(4-OMe), **13c**,**d**), although **13c**,**d** exhibited greater

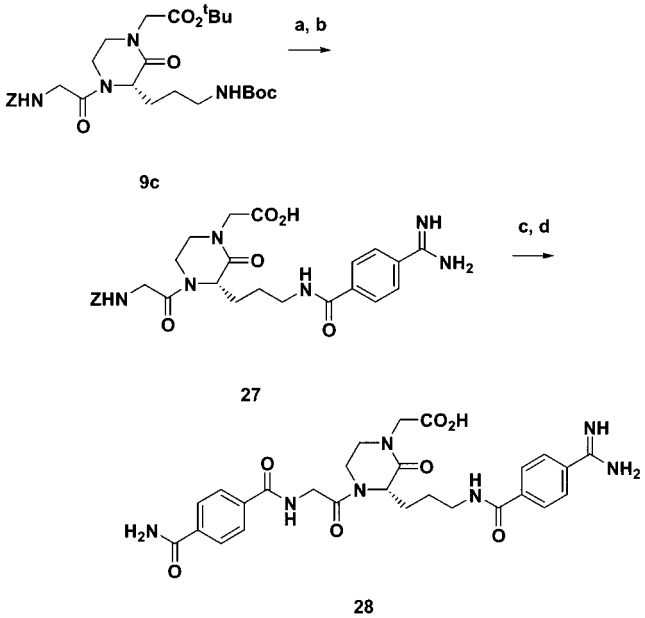
**Table 1.** Effects of the Bis(4-amidinophenyl) Derivatives on ADP-Induced in Vitro and ex Vivo Platelet Aggregation and Bleeding Time in Guinea Pigs



| | | | | inhibition of platelet aggregation[b] | | effect on bleeding time (BT) | | dissociation ratio[e] |
|---|---|---|---|---|---|---|---|---|
| compound | n | R | atom numbers[a] | *in vitro* $IC_{50}$ (nM) | *ex vivo* $ID_{50}$ (μg/kg/min) | dose (μg/kg/min) | BT[c] | BT (×3)/$ID_{50}$ |
| **11c** | 3 | H | 16 | 37 (32-42) | 0.19 (0.15-0.23) | 2.2 | 660±110 | 12 |
| **11d** | 4 | H | 17 | 150 (55-240) | 0.79 (0.70-0.87) | 5.0 | 620±191 | 6 |
| **13c** | 3 |  | 16 | 23 (13-39) | 0.021 (0.019-0.023) | 0.20 | 660±69 | 10 |
| **13d** | 4 |  | 17 | 12 (5-21) | 0.041 (0.032-0.047) | 0.20 | >900[d] | <5 |

[a] Atom numbers between the C-terminal carbon atom and the N-terminal nitrogen atom at the 3-position of the 2-oxopiperazine ring. [b] The concentrations of each compound inhibiting the in vitro or ex vivo ADP-induced aggregation of guinea pig platelets by 50%. The 95% confidence intervals are shown in parentheses, $n = 2-5$. [c] Bleeding time at the denoted dose, mean ± SE, $n = 3$. [d] All guinea pigs prolonged the bleeding time over 900 s. [e] Dose ratio for dose causing prolongation of bleeding time to 3 times the basel value (BT(×3)) vs $ID_{50}$ value of ex vivo ADP-induced platelet aggregation. Baseline template bleeding time in guinea pigs was ca. 220 s.[15]

**Scheme 4**[a]

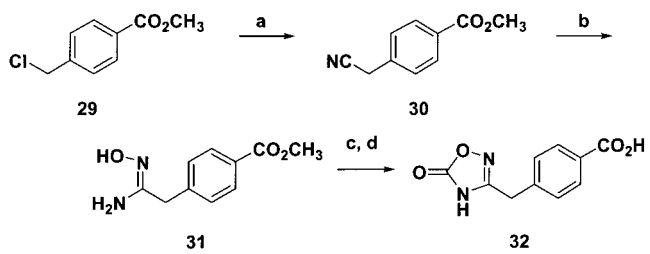


[a] Reagents: (a) $CF_3CO_2H$, toluene; (b) 4-amidinobenzoyl chloride hydrochloride, $NaHCO_3$, 1,4-dioxane, $H_2O$; (c) $H_2$, 10% Pd−C, MeOH; (d) 4-carbamoylbenzoic acid, HOSU, EDC, DMF.

inhibitory effects on platelet aggregation than **11c**,**d**. Consequently, we chose **11c**,**d** as lead compounds and investigated the dibasic compounds analogous to **11c**,**d**.

In the course of study on SARs of our GPIIb/IIIa antagonists,[10,11] the basic moiety at the 4-position of the 2-oxopiperazine ring was considered to be an essential element for the inhibitory effect on platelet aggregation. The dibasic compounds possess an additional basic moiety at the 3-position which could work as the essential element for the antiplatelet effect instead of the basic moiety at the 4-position, and therefore, we

**Scheme 5**[a]

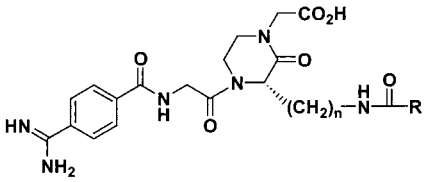


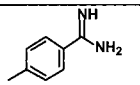
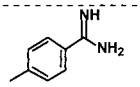
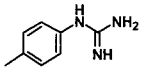
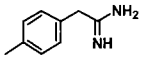
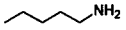
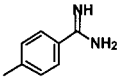
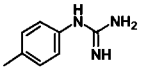
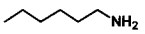
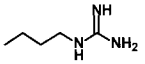
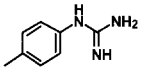
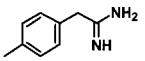
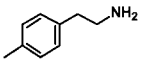
[a] Reagents: (a) NaCN, dimethyl sulfoxide; (b) $NH_2OH{\cdot}HCl$, $NaHCO_3$, MeOH; (c) 1,1′-carbonyldiimidazole, 1,8-diazabicyclo[5,4,0]-undec-7-ene, 1,4-dioxane; (d) NaOH, $H_2O$, MeOH.

attempted to determine which amino group is important for the inhibitory activity. Replacing the 4-amidinophenyl group at the 4-position in **11c** with a 4-carbamoylphenyl group, which resulted in a loss of basic character, resulted in a remarkable decrease in inhibitory activity (**28**, in vitro, $IC_{50}$: 3000 nM). This result suggests that the basic terminal at the 4-position in the dibasic antagonists plays a crucial role in the inhibition of platelet aggregation.

Table 2 summarizes the biological activities of the 4-[2-[(4-amidinobenzoyl)amino]acetyl]-2-oxopiperazine derivatives **11**, **20**−**24**, and **26** bearing different types of additional basic moieties of 14−17 atoms in length between the C-terminal carbon atom and the additional N-terminal nitrogen atom. Among these compounds, **20c** showed the most potent inhibitory activities of in vitro and ex vivo platelet aggregation with $IC_{50}$ values of 19 nM and $ID_{50}$ values of 0.087 μg/kg/min, respectively, and inhibitory activities of in vitro and ex vivo platelet aggregation of other compounds were 37 to 210 nM ($IC_{50}$) and 0.12 to 0.94 μg/kg/min ($ID_{50}$), respectively. From these results, the substituent group at the 3-position was also found to influence the antagonistic activity

**Table 2.** Effects of the 4-[2-[(4-Amidinobenzoyl)amino]acetyl]-2-oxopiperazine Derivatives on ADP-Induced in Vitro and ex Vivo Platelet Aggregation and Bleeding Time in Guinea Pigs



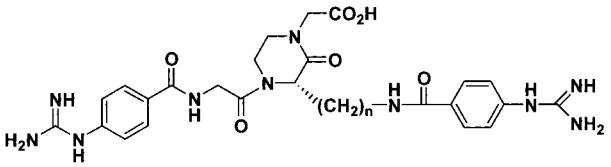
| compound | n | R | atom numbers[a] | inhibition of platelet aggregation[b] *in vitro* $IC_{50}$ (nM) | inhibition of platelet aggregation[b] *ex vivo* $ID_{50}$ (μg/kg/min) | effect on bleeding time (BT) dose (μg/kg/min) | effect on bleeding time (BT) BT[c] | dissociation ratio[e] BT (×3)/$ID_{50}$ |
|---|---|---|---|---|---|---|---|---|
| **11a** | 1 |  | 14 | 120 (0-270) | 0.72 (0.56-0.86) | 4.0 | >900[d] | <6 |
| **11b** | 2 |  | 15 | 190 (82-330) | 0.91 (0.79-1.4) | 8.0 | 800±100 | <9 |
| **20a** | 1 |  | 15 | 85 (72-100) | 0.66 (0.62-0.70) | 6.5 | 660 (n = 2) | 10 |
| **21a** | 1 |  | 15 | 57 (41-74) | 0.42 (0.40-0.44) | 4.0 | 760±140 | <10 |
| **22c** | 3 |  | 15 | 210 (150-290) | 0.94 (0.78-1.1) | 5 | 490±175 | >5 |
| **11c** | 3 |  | 16 | 37 (32-42) | 0.19 (0.15-0.23) | 2.2 | 660±110 | 12 |
| **20b** | 2 |  | 16 | 37 (24-55) | 0.12 (0.098-0.13) | 2.0<br>2.5 | 410±78<br>820±53 | 17-21 |
| **23c** | 3 |  | 16 | 160 (120-250) | 0.86 (0.61-0.97) | 10 | 780±120 | <12 |
| **26** | 3 |  | 16 | 88 (76-100) | 0.47 (0.42-0.52) | 10 | 620±56 | 21 |
| **20c** | 3 |  | 17 | 19 (11-29) | 0.087 (0.076-0.098) | 0.9<br>1.4 | 490±56<br>>900[d] | 10-16 |
| **21c** | 3 |  | 17 | 67 (54-84) | 0.41 (0.35-0.46) | 4.0 | 620±156 | 10 |
| **24c** | 3 |  | 17 | 81 (68-96) | 0.56 (0.51-0.61) | 3.0<br>6.0 | 270±17<br>860±40 | 5-11 |

[a–e] See corresponding footnotes in Table 1.

slightly. These effects on the potency of the additional basic moiety seem to arise from an alteration of spatial arrangement of the *C*-terminal carboxylate and the *N*-terminal amidinophenyl group at the 4-position caused by a conformational change dependent on the substituent at the 3-position. A $\pi$-stacking and lipophilic interaction of the phenyl ring in the additional basic moiety with the receptor may also have some influence on the potency. As a comparison of the compounds with the same atom numbers, the derivative with the benzene ring in the additional basic moiety showed greater activity than the compounds with a linear alkyl chain (**11b**, **20a**, **21a** vs **22c**; **11c**, **20b** vs **23c**, **26**).

These dibasic compounds displayed good dissociation profiles as we expected. Particularly, high dissociation ratios (BT(×3)/$ID_{50}$ > 10) were observed in a series with atom numbers of 16. In the beginning, we presumed that a strong ionic interaction between the additional basic moiety and the GPIIb/IIIa receptor is important for the dissociation profile as well as for the inhibitory activity and that amidinophenyl derivatives should provide the highest dissociation ratio. However, dissociation ratios of the amidinophenyl derivatives that are lower than those of the guanidinophenyl derivatives (**11c** vs **20b**) revealed that a strong ionic interaction is not responsible for the dissociation. Although the basicity of the additional basic moiety was another possible factor, it does not appear to be correlated with the dissociation ratio. Upon comparison of **11c**, **20b**, and **23c**, the guanidinophenyl derivative **20b** showed the highest dissociation ratio, even though the basicity of the guanidinophenyl group is as weak as that of a

**Table 3.** Effects of the Bis(4-guanidinophenyl) Derivatives on ADP-Induced in Vitro and ex Vivo Platelet Aggregation and Bleeding Time in Guinea Pigs



| compound | n | atom numbers[a] | inhibition of platelet aggregation[b] *in vitro* $IC_{50}$ (nM) | inhibition of platelet aggregation[b] *ex vivo* $ID_{50}$ (μg/kg/min) | effect on bleeding time (BT) dose (μg/kg/min) | effect on bleeding time (BT) BT[c] | dissociation ratio[e] BT (×3)/$ID_{50}$ |
|---|---|---|---|---|---|---|---|
| **12a** | 1 | 15 | 140 (100-200) | 1.0 (0.82-1.2) | 10.0 | 720 (n = 2) | <10 |
| **12b** | 2 | 16 | 97 (56-160) | 0.88 (0.69-1.1) | 9.0 | 800±100 | <10 |
| **12c** | 3 | 17 | 51 (48-54) | 0.18 (0.16-0.19) | 6.0 | 510±79 | >33 |

[a−c,e] See corresponding footnote in Table 1.

primary amine. Furthermore, comparable dissociation ratios were observed with the weakly basic guanidinophenyl derivative **20b** and the highly basic guanidinopropyl derivative **26**.

We turned our attention to the relationship between the hydrogen bonding ability of the additional basic moiety and the dissociation profile and found parallelism between the hydrogen bonding ability and the dissociation ratio, i.e., guanidino group > amidino group > primary amino group. Guanidinophenyl derivative **20b** and guanidinopropyl derivative **26** showed higher dissociation ratios than amidinophenyl derivative **11c** which was superior to primary amine derivative **23c**. This tendency is observed in a series with atom numbers of 15 and 17 (**11b**, **20a**, **21a**, and **22c**; **20c**, **21c**, and **24c**). These results suggest that the hydrogen bonding ability of the additional basic moiety is one of the important factors affecting the dissociation profile.

The orientation of the additional basic moiety seems to be another important factor for the dissociation profile. Due to the conformational mobility of the guanidino group in comparison to the amidino group, the guanidinophenyl derivatives could favorably interact with the receptor as compared with the amidinophenyl derivatives (**11b** and **20a**, **11c** and **20b**, **11d** (see Table 1) and **20c**). Similarly, since the 4-amidinomethylphenyl group is able to rotate more flexibly than the 4-amidinophenyl group, 4-amidinomethylphenyl derivatives **21a**,**c** might interact better with the receptor than 4-amidinophenyl derivatives **11b**,**d**. Thus, the orientation of the additional basic moiety may also be another factor affecting the dissociation profile.

So far, the amidinophenyl group was employed as the basic moiety at the 4-position since this group enhanced the inhibitory activity in a series of 2-oxopiperazine derivatives.[10] We examined the effects of the guanidinophenyl group as a basic moiety at the 4-position on the inhibitory activity and the dissociation profile (Table 3). These bis(guanidinophenyl) derivatives **12a**−**c** showed less potent in vitro and ex vivo inhibitory activity than the corresponding amidinophenyl derivatives **20a**−**c**. However, we found that **12c** showed the highest dissociation ratio in the series of dibasic compounds in this study. The reason **12c** exhibited such a good dissociation ratio is not clear, but we suggest that the intensity of the interaction of the guanidinophenyl group at the 3-position and the conformational fitness of this compound for the receptor might be responsible for this result.

Finally, to confirm the chirality requirement of **12c**, we evaluated the enantiomer **(*R*)-12c** (in vitro, $IC_{50}$: 11000 nM) and found that it has 200 times less potent inhibitory activity than **12c**.

## Pharmacological Activities of 12c

A comparison of the pharmacological profiles of **12c**, **1**, and **4** is shown in Figures 3 and 4 and Tables 4 and 5.

Figure 3A shows the effect of **12c**, **1**, and **4** on bleeding time in guinea pigs. The doses which prolong bleeding time to 3 times the basal value for **12c**, **1**, and **4** were 5.8, 0.35 and 12 μg/kg/min, respectively. Since the $ID_{50}$ values of **12c**, **1**, and **4** on ex vivo ADP-induced platelet aggregation in guinea pigs were 0.18, 0.16, and 0.50 μg/kg/min, respectively, the dissociation ratios of these compounds were found to be 32, 2.2, and 24, respectively. Moreover, the dissociation ratios of **12c** and Aggrastat, a marketed GPIIb/IIIa antagonist, in monkeys were 10 and 3.5, respectively ($n = 3$).[16] Compound **12c** exhibited a much greater dissociation ratio as compared with **1**, **4**, and Aggrastat. On the other hand, it has been reported that the antiplatelet effect of certain classes of GPIIb/IIIa antagonist, such as **4**, which demonstrate an antithrombotic effect with only a small increased risk of bleeding, is overestimated in the citrated blood sample as compared to that in the noncitrated blood sample.[17−19] In a preliminary experiment, the $IC_{50}$ value of **12c** in the heparinized blood sample was 230 nM, 4.5-fold less potent than that in the citrated physiological blood sample.[16] The compound **12c** was found to have a similar pharmacological profile

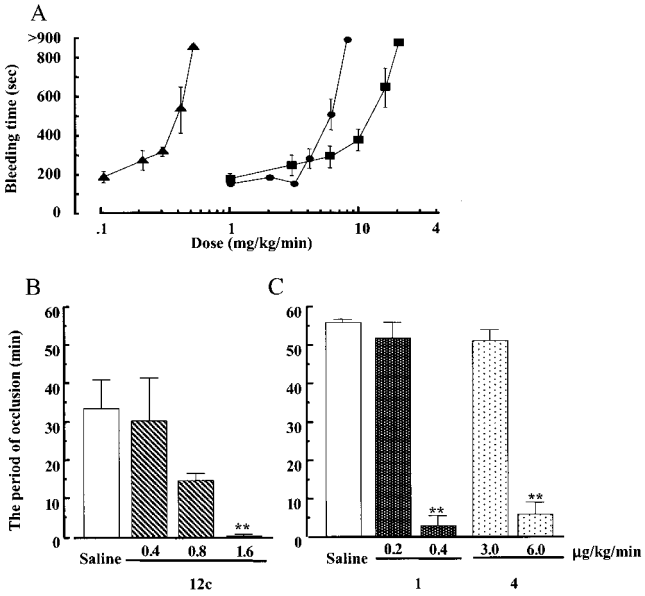



**Figure 3.** Effects of **12c**, **1**, and **4** on bleeding time and thrombus formation in guinea pigs. Template bleeding time in guinea pigs dosed with **12c** (●), **1** (▲), and **4** (■) (A). Baseline value of bleeding time was 183 s ($n = 8$). Antithrombotic effects of **12c** (B), **1**, and **4** (C) on balloon injury-induced carotid thrombosis model in guinea pigs. Data are expressed as mean ± SE, $n = 3-8$. $^{**}p < 0.01$ vs saline.

as **4**, and the reduction seems to affect the dissociation ratio between antithrombotic and bleeding time prolonging effect.

Figure 3B,C also shows the inhibitory effects of **12c**, **1**, and **4** on the carotid thrombosis induced by balloon injury in guinea pigs ($ID_{50} = 0.73$, 0.28, and 4.3 μg/kg/min, respectively). Intravenous infusion of **12c** at 0.8 μg/kg/min or 1.6 μg/kg/min showed ca. 60% inhibition and complete inhibition of thrombus formation, respectively, without prolongation of bleeding time. While **1** at 0.4 μg/kg/min showed complete anthithrombotic effects, the bleeding time was prolonged to over 3 times the control value.

The time course of the inhibitory effects of **12c** and **1** on ex vivo ADP-induced platelet aggregation in guinea pigs is shown in Figure 4. A single dose of **12c** at 100 μg/kg iv produced almost complete inhibition for 120 min, and about 40% inhibition was observed after 240 min. Dose-dependent inhibition of platelet aggregation was achieved with a single iv dose of 30−100 μg/kg of **12c**, and the potency and the duration of action of **12c** were found to be similar to those of **1**.

The in vitro inhibitory effects of **12c** and **1** on ADP-induced platelet aggregation were examined in human, monkey, and guinea pig platelet rich plasma (Table 4). Compound **12c** inhibited ADP-induced aggregation of human, monkey, and guinea pig platelets with $IC_{50}$ values of 31, 79, and 51 nM, respectively. There were slight species differences in the antiplatelet effect of **12c** similar to those seen with **1**.

Finally, the inhibitory effects of **12c** and **1** on human platelet aggregation induced by various agonists including ADP, collagen, platelet-activating factor (PAF), and thrombin receptor agonist peptide (TRAP) were examined and are summarized in Table 5. Platelet aggregation induced by ADP and PAF, relatively weak agonists, was inhibited by **12c** as strongly as by **1**. However, the inhibitory effects of **12c** on platelet aggregation induced by collagen and TRAP, strong agonists, were 2- to 3-fold less potent than those of **1**.

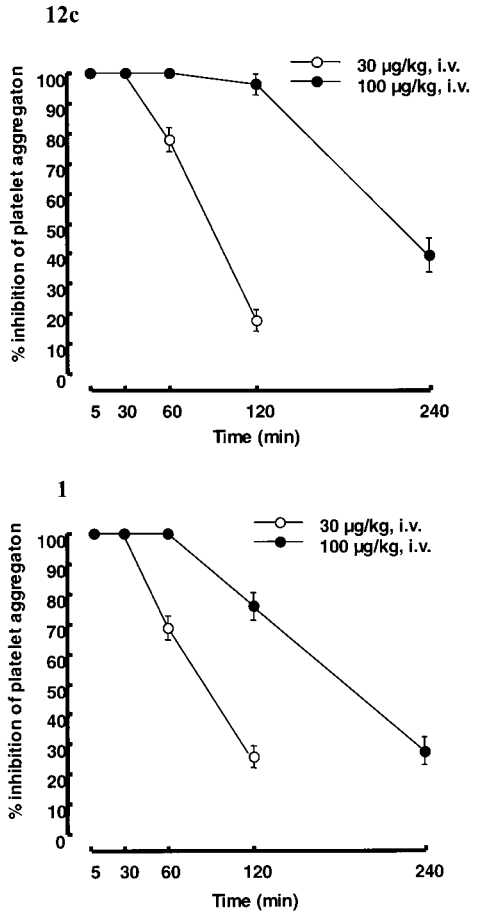



**Figure 4.** Time course of the inhibitory effects of **12c** and **1** on ex vivo platelet aggregation in guinea pig. At various time intervals after intravenous injection of drugs, ex vivo platelet aggregation induced by ADP was examined. Each point represents the mean ± SE of 3−7 animals.

**Table 4.** Effects of **12c** and **1** on in Vitro Platelet Aggregation Induced by ADP in Human, Monkey, and Guinea Pig Platelet Rich Plasma

| | $IC_{50}$ (nM)[a] | |
|---|---|---|
| species | **12c** | **1**[b] |
| human | 31 (21−41, $n = 4$) | 31 (27−35, $n = 8$) |
| monkey | 79 (59−100, $n = 4$) | 46 (40−52, $n = 9$) |
| guinea pig | 51 (48−54, $n = 4$) | 43 (23−50, $n = 6$) |

[a] The 95% confidence intervals are shown in parentheses. The follwing ADP concentrations were used: human, 1−10 μM; monkey, 30−40 μM; guinea pig, 0.8−1 μM. [b] Data obtained from ref 15 for comparison purposes.

**Table 5.** Effects of **12c** and **1** on in Vitro Human Platelet Aggregation Induced by ADP, Collagen, Platelet-Activating Factor (PAF), and Thrombin Receptor Agonist Peptide (TRAP)

| | $IC_{50}$ (nM)[a] | |
|---|---|---|
| aggregating agent | **12c** | **1** |
| ADP | 31 (21−41, $n = 4$) | 31 (27−35, $n = 8$)[b] |
| collagen | 59 (38−98, $n = 4$) | 31 (25−39, $n = 5$)[b] |
| PAF | 30 (25−35, $n = 4$) | 29 (20−45, $n = 4$)[b] |
| TRAP | 91 (67−130, $n = 4$) | 32 (14−58, $n = 3$) |

[a] The 95% confidence intervals are shown in parentheses. The following concentrations of inducers were used: ADP, 1−10 μM; collagen, 1−3 μg/mL; PAF, 1−10 μM; TRAP, 10−30 μM. [b] Data obtained from ref 15 for comparison purposes.

It is considered that thrombin plays a crucial role in thrombus formation at the hemostatic site. In fact, it is known that thrombin inhibitors such as warfarin and heparin cause hemorrhagic complications frequently in clinical situations. Since thrombin inhibitors are ineffective in several arterial thrombosis models, platelet stimulating mediators other than thrombin seem to contribute to thrombus formation.[20,21] Therefore, the compound, which inhibits more potently platelet aggregation induced by mediators other than thrombin might show the dissociation of efficacy and bleeding risk. TRAP, which is the *N*-terminal fragment of thrombin receptor produced by thrombin, stimulates platelets strongly like thrombin. The inhibitory effect of **12c** on the platelet aggregation induced by TRAP was weaker than that on the aggregation induced by other aggregating inducers. The in vitro antiplatelet profile may be related to the in vivo pharmacological profile showing the dissociation of efficacy and bleeding risk.

Platelet aggregation is classified into "microaggregation" and "macroaggregation". Especially in the large vessel, antithrombotic effects of GPIIb/IIIa antagonists may be mainly dependent on their inhibitory potency of platelet macroaggregation. On the other hand, the differential effects of GPIIb/IIIa antagonists on these platelet aggregations have been implicated in the varying "bleeding risk/antithrombotic efficacy" ratio. It has been reported that eptifibatide potently inhibited the platelet macroaggregation more than platelet microaggregation, and this difference may be related to a benefit of the dissociation between the antithrombotic efficacy and bleeding side effect.[22] The inhibitory ratio "micro-/macro-aggregation" may relate to the pharmacological profiles of **12c** and remains to be clarified.

## Conclusion

In this study, we discovered a new type of antithrombotic agent, **12c**, having potent antiplatelet activity and antithrombotic efficacy with reduced bleeding time prolongation. The bleeding complication is one of the most severe side effects of antithorombotic agents. Therefore, 2-[(3*S*)-4-[2-[(4-guanidinobenzoyl)amino]acetyl]-3-[3-[(4-guanidinobenzoyl)amino]propyl]-2-oxopiperazinyl]acetic acid (**12c**) should be a clinically useful GPIIb/IIIa antagonist, and **12c** was selected as a candidate for clinical trials.

## Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Hitachi IR-215 spectrometer. $^1$H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer. Chemical shifts are given in $\delta$ value (ppm) with tetramethylsilane (TMS) as the internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, dd = double doublet, m = multiplet, br = broad peak, brs = broad singlet. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. Elemental analyses were within ±0.4% of the theoretical values for the elements indicated unless otherwise noted.

***tert*-Butyl 2-[[(2*S*)-2-[(Benzyloxycarbonyl)amino]-5-[(*tert*-butoxycarbonyl)amino]pentanoyl](2,2-dimethoxyethyl)amino]acetate (6c).** To a mixture of *tert*-butyl *N*-[(2,2-dimethoxyethyl)amino]acetate (**5**) (6.0 g, 27.3 mmol), Z-L-Orn(Boc)-OH (9.8 g, 27.3 mmol), and $CH_2Cl_2$ (55 mL) was added 1-ethyl-3-[3-(*N*,*N*-dimethylamino)propyl]carbodiimide hydrochloride (EDC) (5.6 g, 29.2 mmol) at room temperature. After being stirred for 2 h at room temperature, the mixture was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography ($SiO_2$, hexane:EtOAc = 1:1) to give **6c** (14.6 g, 94%) as a colorless oil: IR (neat) cm$^{-1}$ 2970, 2930, 1740, 1718, 1650, 1520, 1455, 1362; $^1$H NMR ($CDCl_3$) $\delta$ 1.43 (9H, s), 1.45 (9H, s), 1.10−1.90 (4H, m), 3.00−4.58 (14H, m), 4.60−4.87 (1H, m), 5.05−5.20 (2H, m), 5.52−5.68 (1H, m), 7.25−7.45 (5H, m).

**Benzyl (2*S*)-2-[3-[(*tert*-Butoxycarbonyl)amino]propyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxo-3,4-dihydro-1(2*H*)-pyrazinecarboxylate (7c).** To a solution of **6c** (14.3 g, 25.2 mmol) in toluene (146 mL) was added *p*-toluenesulfonic acid monohydrate (0.48 g, 2.52 mmol) at room temperature, and the reaction mixture was stirred at 75 °C for 4 h. After being cooled, the mixture was washed with water, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography ($SiO_2$, hexane:EtOAc = 2:1) to give **7c** (8.3 g, 65%) as a colorless oil: IR (neat) cm$^{-1}$ 1740, 1713, 1685, 1155; $^1$H NMR ($CDCl_3$) $\delta$ 1.43 (9H, s), 1.46 (9H, s), 1.10−1.90 (4H, m), 2.15−4.95 (5H, m), 5.06−5.32 (2H, m), 5.40−5.66 (1H, m), 5.98−6.10 (1H, m), 6.20−6.44 (1H, m), 7.25−7.50 (5H, m).

***tert*-Butyl 2-[(3*S*)-3-[3-[(*tert*-Butoxycarbonyl)amino]propyl]-2-oxopiperazinyl]acetate Oxalate (8c).** A solution of **7c** (8.3 g, 16.5 mmol) in EtOAc (150 mL) was treated with 10% Pd−C (1.6 g) and hydrogenated under balloon pressure for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give **8c** (4.1 g, 67%) as a colorless oil, which was converted into an oxalate and recrystallized from MeOH−EtOAc to afford colorless crystals: mp 181 °C; $[\alpha]^{20}_D$ −29.3° ($c$ = 0.73, $H_2O$); IR (KBr) cm$^{-1}$ 3400, 2970, 1740, 1660, 1520, 1365; $^1$H NMR ($CD_3OD$) $\delta$ 1.43 (9H, s), 1.48 (9H, s), 1.30−2.30 (4H, m), 3.09 (2H, t, $J$ = 6.6 Hz), 3.36−3.86 (4H, m), 3.94−4.10 (1H, m), 4.01 (1H, d, $J$ = 17.4 Hz), 4.16 (1H, d, $J$ = 17.4 Hz). Anal. ($C_{18}H_{33}N_3O_5 \cdot C_2H_2O_4$) C, H, N.

**Benzyl (2*S*)-2-[[(*tert*-Butoxycarbonyl)amino]methyl]-4-[2-(*tert*-butoxy)-2-oxoethyl]-3-oxo-3,4-dihydro-1(2*H*)-pyrazinecarboxylate (7a).** Compound **7a** was synthesized by the same procedure as that described for the synthesis of **7c** using (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(*tert*-butoxycarbonyl)amino]propanoic acid instead of Z-L-Orn(Boc)-OH: colorless oil (yield 67% from (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[(*tert*-butoxycarbonyl)amino]propanoic acid); $^1$H NMR ($CDCl_3$) $\delta$ 1.42 (9H, s), 1.47 (9H, s), 3.30−4.55 (4H, m), 4.76−5.00 (1H, m), 5.10−5.30 (3H, m), 5.36−5.58 (1H, m), 6.30−6.54 (1H, m), 7.38 (5H, s).

***tert*-Butyl 2-[(3*S*)-3-[2-[(*tert*-Butoxycarbonyl)amino]-ethyl]-2-oxopiperazinyl]acetate (8b).** Compound **8b** was synthesized by the same procedure as that described for the synthesis of **8c** using (2*S*)-2-[(benzyloxycarbonyl)amino]-4-[(*tert*-butoxycarbonyl)amino]butanoic acid instead of Z-L-Orn-(Boc)-OH: colorless crystals (yield 29% from (2*S*)-2-[(benzyloxycarbonyl)amino]-4-[(*tert*-butoxycarbonyl)amino]butanoic acid); mp 165−169 °C. Anal. ($C_{17}H_{31}N_3O_5 \cdot C_2H_2O_4$) C, H, N.

***tert*-Butyl 2-[(3*S*)-3-[4-[(*tert*-Butoxycarbonyl)amino]-butyl]-2-oxopiperazinyl]acetate (8d).** Compound **8d** was synthesized by the same procedure as that described for the synthesis of **8c** using Z-L-Lys(Boc)-OH instead of Z-L-Orn(Boc)-OH: colorless crystals (yield 48% from Z-L-Lys(Boc)-OH); mp 170−172 °C; $[\alpha]^{20}_D$ −29.0° ($c$ = 1.02, DMSO); IR (KBr) cm$^{-1}$ 2978, 1706, 1650, 1365, 1163; $^1$H NMR ($D_2O$) $\delta$ 1.42 (9H, s), 1.47 (9H, s), 1.10−2.30 (6H, m), 2.98−3.18 (2H, m), 3.40−3.94 (4H, m), 4.03 (1H, d, $J$ = 17.4 Hz), 4.02−4.22 (1H, m), 4.22 (1H, d, $J$ = 17.4 Hz). Anal. ($C_{19}H_{35}N_3O_5 \cdot C_2H_2O_4$) C, H, N.

***tert*-Butyl 2-[(3*S*)-4-(2-[(Benzyloxycarbonyl)amino]-acetyl)-3-[3-[(*tert*-butoxycarbonyl)amino]propyl]-2-oxopiperazinyl]acetate (9c).** To a mixture of **8c** (1.29 g, 3.47 mmol), Z-Gly-OH (0.87 g, 4.16 mmol), and $CH_2Cl_2$ (13.0 mL) was added EDC (0.87 g, 4.51 mmol) at room temperature. After being stirred for 1 h at room temperature, the mixture was washed successively with 5% aqueous $KHSO_4$ and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography ($SiO_2$, EtOAc) to give **9c** (1.95 g, quant.) as a colorless

amorphous powder: IR (neat) $cm^{-1}$ 3360, 2970, 1713, 1650, 1513, 1448, 1363, 1246, 1158; $^1H$ NMR ($CDCl_3$) $\delta$ 1.43 (9H, s), 1.46 (9H, s), 1.30−2.20 (4H, m), 3.02−4.80 (11H, m), 4.94−5.08 (1H, m), 5.13 (2H, s), 5.64−5.86 (1H, m), 7.30−7.45 (5H, m).

Compounds **9a**,**b** and **9d** were prepared by the same procedure as that described for the synthesis of **9c**.

***tert*-Butyl 2-[(3*S*)-4-(2-[(Benzyloxycarbonyl)amino]-acetyl)-3-[[(*tert*-butoxycarbonyl)amino]methyl]-2-oxopiperazinyl]acetate (9a):** colorless oil (yield 77% from **7a**); $^1H$ NMR ($CDCl_3$) $\delta$ 1.38 (9H, s), 1.47 (9H, s), 3.20−4.40 (10H, m), 4.90−5.10 (2H, m), 5.13 (2H, s), 5.60−5.90 (1H, m), 7.36 (5H, s).

***tert*-Butyl 2-[(3*S*)-4-(2-[(Benzyloxycarbonyl)amino]-acetyl)-3-[2-[(*tert*-butoxycarbonyl)amino]ethyl]-2-oxopiperazinyl]acetate (9b):** colorless oil (yield 95% from **8b**); IR (neat) $cm^{-1}$ 3450, 1705, 1655, 1640, 1500, 1450, 1360, 1240, 1160; $^1H$ NMR ($CDCl_3$) $\delta$ 1.43 (9H, s), 1.46 (9H, s), 2.05−2.33 (1H, m), 2.73−2.95 (1H, m), 3.15−4.20 (10H, m), 5.05 (1H, dd, $J$ = 3.0 Hz), 5.13 (2H, s), 5.30 (1H, brs), 5.83 (1H, brs), 7.36 (5H, s).

***tert*-Butyl 2-[(3*S*)-4-(2-[(Benzyloxycarbonyl)amino]-acetyl)-3-[4-[(*tert*-butoxycarbonyl)amino]butyl]-2-oxopiperazinyl]acetate (9d):** colorless oil (yield 99% from **8d**); IR (KBr) $cm^{-1}$ 3400, 2990, 2945, 1713, 1657, 1520, 1458, 1368, 1253, 1166; $^1H$ NMR ($CDCl_3$) $\delta$ 1.42 (9H, s), 1.46 (9H, s), 1.18−2.12 (6H, m), 2.92−4.28 (10H, m), 4.48−4.84 (1H, m), 4.94−5.10 (1H, m), 5.13 (2H, s), 5.60−5.88 (1H, m), 7.22−7.45 (5H, m).

***tert*-Butyl 2-[(3*S*)-4-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-methoxyphenyl)propanoyl]-3-[3-[(*tert*-butoxycarbonyl)amino]propyl]-2-oxopiperazinyl]acetate (10c).** Compound **10c** was synthesized by the same procedure as that described for the synthesis of **9c** using Z-L-Tyr(Me)-OH instead of Z-Gly-OH: colorless oil (yield 92% from **8c**); IR (KBr) $cm^{-1}$ 3360, 2975, 1710, 1643, 1512, 1448, 1360, 1245, 1152, 1033; $^1H$ NMR ($CDCl_3$) $\delta$ 1.41 (9H, s), 1.44 (9H, s), 1.30−2.10 (4H, m), 2.20−2.44 (1H, m), 2.80−3.84 (8H, m), 3.77 (3H, s), 4.23 (1H, d, $J$ = 17.2 Hz), 4.50−4.85 (2H, m), 4.85−5.00 (1H, m), 5.05 (1H, d, $J$ = 12.0 Hz), 5.14 (1H, d, $J$ = 12.0 Hz), 5.67 (1H, d, $J$ = 8.8 Hz), 6.80 (2H, d, $J$ = 8.8 Hz), 7.09 (2H, d, $J$ = 8.8 Hz), 7.22−7.50 (5H, m).

***tert*-Butyl 2-[(3*S*)-4-[(2*S*)-2-[(Benzyloxycarbonyl)amino]-3-(4-methoxyphenyl)propanoyl]-3-[4-[(*tert*-butoxycarbonyl)amino]butyl]-2-oxopiperazinyl]acetate (10d).** Compound **10d** was synthesized by the same procedure as that described for the synthesis of **9c** using Z-L-Tyr(Me)-OH instead of Z-Gly-OH: colorless oil (yield 99% from **8d**); IR (KBr) $cm^{-1}$ 2975, 1712, 1646, 1512, 1447, 1364, 1244, 1155; $^1H$ NMR ($CDCl_3$) $\delta$ 1.43 (9H, s), 1.44 (9H, s), 1.00−2.45 (7H, m), 2.80−3.90 (8H, m), 3.78 (3H, s), 4.23 (1H, d, $J$ = 17.4 Hz), 4.70−5.10 (5H, m), 5.74 (1H, d, $J$ = 8.8 Hz), 6.81 (2H, d, $J$ = 8.6 Hz), 7.10 (2H, d, $J$ = 8.6 Hz), 7.25−7.50 (5H, m).

***N*-(4-Guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide Hydrochloride.** To a mixture of 4-guanidinobenzoic acid hydrochloride (5.0 g, 23 mmol), *N*-hydroxy-5-norbornene-2,3-dicarboxyimide (5.0 g, 28 mmol), and DMF (46 mL) was added 1,3-dicyclohexylcarbodiimide (6.2 g, 30 mmol) at room temperature. After being stirred for 2 h at room temperature, the mixture was filtered, and the filtrate was concentrated in vacuo to give the title compound (7.6 g, 87%) as a colorless powder. This compound was used for a condensation reaction without purification.

**2-[(3*S*)-4-[2-[(4-Guanidinobenzoyl)amino]acetyl]-3-[3-[(4-guanidinobenzoyl)amino]propyl]-2-oxopiperazinyl]-acetic Acid (12c).** Under an $H_2$ atmosphere, a suspension of **9c** (0.70 g, 1.24 mmol) and 10% Pd−C (0.07 g) in MeOH (7.0 mL) was stirred for 30 min at room temperature. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. To a solution of the residual oil in toluene (7.0 mL) was added TFA (7.0 mL) at 0 °C, and the mixture was stirred for 2 h at room temperature and concentrated in vacuo. Then, to a mixture of the residual oil, 1,4-dioxane (7.0 mL), and $H_2O$ (14 mL) were added successively $NaHCO_3$ (0.52 g, 6.2 mmol) and *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride (1.12 g, 3.0 mmol) at room temperature. After being stirred for 1 h, the mixtute was adjusted to pH 3 with 1 N HCl and concentrated in vacuo. The residue was purified by column chromatography (MCI GEL CHP-20 (Mitsubishi Chemical Industry), gradient elution: $H_2O$ to 5% aqueous $CH_3CN$) to give **12c** (0.48 g, 55%) as a colorless amorphous powder, which was converted into a dihydrochloride and recrystallized from $H_2O$−EtOH to afford a colorless crystalline powder: mp 245−251.5 °C; $[\alpha]^{20}_D$ +61.8° ($c$ = 1.00, $H_2O$, on the anhydrous basis); IR (KBr) $cm^{-1}$ 3287, 1730, 1634, 1557, 1505, 1449, 1323, 1262, 1179; $^1H$ NMR ($D_2O$) $\delta$ 1.60−2.25 (4H, m), 3.30−5.20 (11H, m), 7.30−7.48 (4H, m), 7.72−7.94 (4H, m). Anal. ($C_{27}H_{34}N_{10}O_6 \cdot 2HCl \cdot 2H_2O$) C, H, N.

**2-[(3*R*)-4-[2-[(4-Guanidinobenzoyl)amino]acetyl]-3-[3-[(4-guanidinobenzoyl)amino]propyl]-2-oxopiperazinyl]-acetic Acid (*R*-12c).** Compound ***R*-12c** was synthesized by the same procedure as that described for the synthesis of **12c** using Z-D-Orn(Boc)-OH instead of Z-L-Orn(Boc)-OH: colorless amorphous powder; $[\alpha]^{20}_D$ −45.1° ($c$ = 0.40, MeOH). Anal. ($C_{27}H_{34}N_{10}O_6 \cdot HCl \cdot 3H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[[(4-amidinobenzoyl)amino]methyl]-2-oxopiperazinyl]acetic Acid (11a).** Compound **11a** was synthesized by the same procedure as that described for the synthesis of **12c** using 4-amidinobenzoyl chloride hydrochloride instead of *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride: colorless amorphous powder (yield 11% from **9a**); $[\alpha]^{20}_D$ +60.2° ($c$ = 0.54, MeOH); IR (KBr) $cm^{-1}$ 1640, 1550, 1490; $^1H$ NMR ($D_2O$) $\delta$ 3.30−4.55 (10H, m), 5.19 (1H, t, $J$ = 6.8 Hz), 7.68 (2H, d, $J$ = 8.0 Hz), 7.78 (2H, d, $J$ = 8.0 Hz), 7.90 (4H, s). Anal. ($C_{25}H_{28}N_8O_6 \cdot HCl \cdot 3H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[2-[(4-amidinobenzoyl)amino]ethyl]-2-oxopiperazinyl]acetic Acid (11b).** Compound **11b** was synthesized by the same procedure as that described for the synthesis of **12c** using 4-amidinobenzoyl chloride hydrochloride instead of *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride: colorless amorphous powder (yield 30% from **9b**); $[\alpha]^{20}_D$ +30.3° ($c$ = 0.47, $H_2O$); $^1H$ NMR ($CD_3OD$) $\delta$ 2.00−2.50 (2H, m), 3.20−3.50 (2H, m), 3.50−4.25 (6H, m), 4.25−4.70 (2H, m), 4.80−5.10 (1H, m), 7.82 (2H, d, $J$ = 8.4 Hz), 7.88 (2H, d, $J$ = 8.4 Hz), 7.98 (2H, d, $J$ = 8.4 Hz), 8.04 (2H, d, $J$ = 8.4 Hz). Anal. ($C_{26}H_{30}N_8O_6 \cdot CF_3CO_2H \cdot 3H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[3-[(4-amidinobenzoyl)amino]propyl]-2-oxopiperazinyl]acetic Acid (11c).** Compound **11c** was synthesized by the same procedure as that described for the synthesis of **12c** using 4-amidinobenzoyl chloride hydrochloride instead of *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride: colorless amorphous powder (yield 32% from **9c**); $[\alpha]^{20}_D$ +41.9° ($c$ = 0.73, MeOH); IR (KBr) $cm^{-1}$ 3280, 1630, 1545, 1480, 1380, 1290; $^1H$ NMR ($D_2O$) $\delta$ 1.50−2.20 (4H, m), 3.20−5.00 (11H, m), 7.70−8.00 (8H, m). Anal. ($C_{27}H_{32}N_8O_6 \cdot CF_3CO_2H \cdot 2H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[4-[(4-amidinobenzoyl)amino]butyl]-2-oxopiperazinyl]acetic Acid (11d).** Compound **11d** was synthesized by the same procedure as that described for the synthesis of **12c** using 4-amidinobenzoyl chloride hydrochloride instead of *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride: colorless amorphous powder (yield 44% from **9d**); $[\alpha]^{20}_D$ +44.3° ($c$ = 1.01, $H_2O$); $^1H$ NMR ($CD_3OD$) $\delta$ 1.34−2.20 (6H, m), 3.26−4.62 (10H, m), 4.76−5.04 (1H, m), 7.87 (2H, d, $J$ = 8.6 Hz), 7.91 (2H, d, $J$ = 8.6 Hz), 8.02 (2H, d, $J$ = 8.4 Hz), 8.09 (2H, d, $J$ = 8.4 Hz). Anal. ($C_{28}H_{34}N_8O_6 \cdot CF_3CO_2H \cdot HCl \cdot 3H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Guanidinobenzoyl)amino]acetyl]-3-[[(4-guanidinobenzoyl)amino]methyl]-2-oxopiperazinyl]acetic Acid (12a).** Compound **12a** was synthesized by the same procedure as that described for the synthesis of **12c**: colorless amorphous powder (yield 16% from **9a**); $[\alpha]^{20}_D$ +44.2° ($c$ = 1.03, $H_2O$); IR (KBr) $cm^{-1}$ 1650, 1540, 1500; $^1H$ NMR ($D_2O$) $\delta$ 3.35−4.55 (8H, m), 3.92 (2H, d, $J$ = 7.0 Hz), 5.20 (1H, t, $J$ = 7.0

Hz), 7.27 (2H, d, $J$ = 8.6 Hz), 7.39 (2H, d, $J$ = 8.2 Hz), 7.71 (2H, d, $J$ = 8.6 Hz), 7.76 (2H, d, $J$ = 8.2 Hz). Anal. ($C_{25}H_{30}N_{10}O_6 \cdot HCl \cdot 4.5H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Guanidinobenzoyl)amino]acetyl]-3-[2-[(4-guanidinobenzoyl)amino]ethyl]-2-oxopiperazinyl]acetic Acid (12b).** Compound **12b** was synthesized by the same procedure as that described for the synthesis of **12c**: colorless amorphous powder; $[\alpha]^{20}_D$ +35.2° ($c$ = 0.65, $H_2O$); $^1$H NMR ($CD_3OD$) $\delta$ 1.96−2.48 (2H, m), 3.12−4.60 (10H, m), 4.90−5.20 (1H, m), 7.32 (2H, d, $J$ = 8.4 Hz), 7.38 (2H, d, $J$ = 8.4 Hz), 7.90 (2H, d, $J$ = 8.4 Hz), 7.97 (2H, d, $J$ = 8.4 Hz). Anal. ($C_{26}H_{32}N_{10}O_6 \cdot CF_3CO_2H \cdot 3H_2O$) C, H, N.

**2-[(3*S*)-4-[(2*S*)-2-[(4-Amidinobenzoyl)amino]-3-(4-methoxyphenyl)propanoyl]-3-[3-[(4-amidinobenzoyl)amino]propyl]-2-oxopiperazinyl]acetic Acid (13c).** Compound **13c** was synthesized by the same procedure as that described for the synthesis of **12c** using 4-amidinobenzoyl chloride hydrochloride instead of *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride: colorless amorphous powder (yield 58% from **10c**); $[\alpha]^{20}_D$ +52.8° ($c$ = 0.76, MeOH); IR (KBr) cm$^{-1}$ 3067, 1634, 1539, 1387, 1302, 1248; $^1$H NMR ($CD_3OD$) $\delta$ 1.45−2.20 (4H, m), 2.30−2.55 (1H, m), 2.90−3.52 (7H, m), 3.60−4.55 (2H, m), 3.77 (3H, s), 4.80−5.20 (2H, m), 6.84 (2H, d, $J$ = 8.4 Hz), 7.18 (2H, d, $J$ = 8.4 Hz), 7.76−8.08 (8H, m). Anal. ($C_{35}H_{40}N_8O_7 \cdot CF_3CO_2H \cdot 3H_2O$) C, H, N.

**2-[(3*S*)-3-[4-[(4-Amidinobenzoyl)amino]butyl]-4-[(2*S*)-2-[(4-amidinobenzoyl)amino]-3-(4-methoxyphenyl)propanoyl]-2-oxopiperazinyl]acetic Acid (13d).** Compound **13d** was synthesized by the same procedure as that described for the synthesis of **12c** using 4-amidinobenzoyl chloride hydrochloride instead of *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride: colorless amorphous powder (yield 61% from **10d**); $[\alpha]^{20}_D$ +54.5° ($c$ = 0.88, $H_2O$); IR (KBr) cm$^{-1}$ 3094, 1634, 1549, 1385, 1302, 1248; $^1$H NMR ($CD_3OD$) $\delta$ 1.20−2.10 (6H, m), 2.35−2.60 (1H, m), 2.90−3.60 (7H, m), 3.60−4.55 (2H, m), 3.77 (3H, s), 4.80−5.20 (2H, m), 6.84 (2H, d, $J$ = 8.6 Hz), 7.19 (2H, d, $J$ = 8.6 Hz), 7.76−8.08 (8H, m). Anal. ($C_{36}H_{42}N_8O_7 \cdot HCl \cdot 6H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(Benzyloxycarbonyl)amino]acetyl]-3-[3-[(4-guanidinobenzoyl)amino]propyl]-2-oxopiperazinyl]acetic Acid (14c).** A mixture of **9c** (0.70 g, 1.24 mmol), toluene (3.5 mL), and TFA (3.5 mL) was stirred for 1 h at room temperature and concentrated in vacuo. To a mixture of the residual oil, $H_2O$ (7.0 mL), and 1,4-dioxane (3.5 mL) were added successively $NaHCO_3$ (0.31 g, 3.7 mmol) and *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride (0.56 g, 1.50 mmol) at room temperature. After being stirred for 1 h, the mixture was adjusted to pH 3 with 1 N HCl and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, gradient elution: $H_2O$ to 25% aqueous $CH_3CN$) to give **14c** (0.60 g, 85%) as a colorless amorphous powder: IR (KBr) cm$^{-1}$ 1640, 1502, 1455, 1382, 1260, 1050; $^1$H NMR ($CD_3OD$) $\delta$ 1.50−2.20 (4H, m), 3.20−4.20 (10H, m), 4.70−5.00 (1H, m), 5.09 (2H, s), 7.20−7.45 (7H, m), 7.90 (2H, d, $J$ = 8.4 Hz).

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[3-[(4-guanidinobenzoyl)amino]propyl]-2-oxopiperazinyl]acetic Acid (20c).** A solution of **14c** (0.60 g, 1.06 mmol) in MeOH (6.0 mL) was treated with 10% Pd−C (0.06 g) and hydrogenated under balloon pressure for 30 min. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. To a mixture of the residual oil, 1,4-dioxane (6.0 mL), and $H_2O$ (12 mL) were added successively $NaHCO_3$ (0.45 g, 5.30 mmol) and 4-amidinobenzoyl chloride hydrochloride (0.28 g, 1.27 mmol) at room temperature. After being stirred for 1 h, the mixture was adjusted to pH 3 with 1 N HCl and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, gradient elution: $H_2O$ to 5% aqueous $CH_3CN$) to give **20c** (0.19 g, 24%) as a colorless amorphous powder: $[\alpha]^{20}_D$ +48.6° ($c$ = 1.02, $H_2O$); $^1$H NMR ($CD_3OD$) $\delta$ 1.55−2.20 (4H, m), 3.20−4.60 (10H, m), 4.75−5.05 (1H, m), 7.32 (2H, d, $J$ = 8.4 Hz), 7.80−7.96 (4H, m), 7.98−8.10 (2H, m). Anal. ($C_{27}H_{33}N_9O_6 \cdot 1.1CF_3CO_2H \cdot 1.5H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[[(4-guanidinobenzoyl)amino]methyl]-2-oxopiperazinyl]acetic Acid (20a).** Compound **20a** was synthesized by the same procedure as that described for the synthesis of **20c**: colorless amorphous powder (yield 8% from **9a**); $[\alpha]^{20}_D$ +46.0° ($c$ = 1.01, $H_2O$); IR (KBr) cm$^{-1}$ 1640, 1560, 1500; $^1$H NMR ($D_2O$) $\delta$ 3.30−4.60 (10H, m), 5.20 (1H, t, $J$ = 6.6 Hz), 7.26 (2H, d, $J$ = 8.8 Hz), 7.72 (2H, d, $J$ = 8.8 Hz), 7.87 (4H, s). Anal. ($C_{25}H_{29}N_9O_6 \cdot 2HCl \cdot 3H_2O$) C, H; N: calcd, 18.58; found, 17.56.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[2-[(4-guanidinobenzoyl)amino]ethyl]-2-oxopiperazinyl]acetic Acid (20b).** Compound **20b** was synthesized by the same procedure as that described for the synthesis of **20c** as a colorless amorphous powder (yield 36% from **9b**); $[\alpha]^{20}_D$ +26.1° ($c$ = 0.45, MeOH); IR (KBr) cm$^{-1}$ 3287, 1634, 1559, 1505, 1387, 1304; $^1$H NMR ($D_2O$) $\delta$ 2.05−2.50 (2H, m), 3.25−5.20 (11H, m), 7.33 (2H, d, $J$ = 8.8 Hz), 7.81 (2H, d, $J$ = 8.8 Hz), 7.90 (2H, d, $J$ = 8.8 Hz), 7.99 (2H, d, $J$ = 8.8 Hz). Anal. ($C_{26}H_{31}N_9O_6 \cdot HCl \cdot 5H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[[[4-(amidinomethyl)benzoyl]amino]methyl]-2-oxopiperazinyl]acetic Acid (21a).** A mixture of **9a** (0.53 g, 1.0 mmol), toluene (2.0 mL), and TFA (2.0 mL) was stirred for 1 h at room temperature and concentrated in vacuo to give an oil. A mixture of 4-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-ylmethyl)-benzoic acid (**32**) (0.22 g, 1.0 mmol), 1,3-dicyclohexylcarbodiimide (0.22 g, 1.1 mmol), *N*-hydroxy-5-norbornene-2,3-dicarboxyimide (0.18 g, 1.0 mmol), and DMF (10 mL) was stirred for 1 h at room temperature. To the mixture was added a mixture of the above oil, triethylamine (0.45 mL), and $H_2O$ (10 mL) at room temperature. After being stirred for 3 h at room temperature, the mixture was diluted with EtOAc, washed with water, dried over $MgSO_4$, and concentrated in vacuo. Then, a mixture of the residual oil, 3 N HCl (0.5 mL), and MeOH (20 mL) was treated with 10% Pd−C (0.1 g) and hydrogenated under balloon pressure at room temperature for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. To a solution of the residual oil, $H_2O$ (10 mL), and 1,4-dioxane (5.0 mL) were added successively $NaHCO_3$ (0.4 g, 4.8 mmol) and 4-amidinobenzoyl chloride hydrochloride (0.39 g, 1.8 mmol) at room temperature. After being stirred for 1 h, the mixture was adjusted to pH 2.5 with 2 N HCl and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, gradient elution: $H_2O$ to 10% aqueous $CH_3CN$) to give **21a** (0.12 g, 14%) as a colorless amorphous powder: $[\alpha]^{20}_D$ +48.4° ($c$ = 0.67, MeOH); IR (KBr) cm$^{-1}$ 1640, 1545, 1490; $^1$H NMR ($D_2O$) $\delta$ 3.30−4.50 (10H, m), 4.13 (2H, s), 5.12 (1H, t, $J$ = 6.6 Hz), 7.25 (2H, d, $J$ = 8.4 Hz), 7.55 (2H, d, $J$ = 8.4 Hz), 7.71 (4H, s). Anal. ($C_{26}H_{30}N_8O_6 \cdot 2HCl \cdot 4.5H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[3-[[4-(amidinomethyl)benzoyl]amino]propyl]-2-oxopiperazinyl]acetic Acid (21c).** Compound **21c** was synthesized by the same procedure as that described for the synthesis of **21a**: colorless amorphous powder (yield 18% from **9c**); $[\alpha]^{20}_D$ +44.1° ($c$ = 1.03, $H_2O$); IR (KBr) cm$^{-1}$ 1640, 1550, 1495; $^1$H NMR ($D_2O$) $\delta$ 1.70−2.10 (4H, m), 3.20−4.60 (12H, m), 4.80−5.00 (1H, m), 7.41 (2H, d, $J$ = 8.4 Hz), 7.71 (2H, d, $J$ = 8.4 Hz), 7.83 (2H, d, $J$ = 8.6 Hz), 7.93 (2H, d, $J$ = 8.6 Hz). Anal. ($C_{28}H_{34}N_8O_6 \cdot HCl \cdot 2H_2O$) C, H; N: calcd, 17.21; found, 16.20.

**2-[(3*S*)-4-[2-[(Benzyloxycarbonyl)amino]acetyl]-3-[3-[[6-[(*tert*-butoxycarbonyl)amino]hexanoyl]amino]propyl]-2-oxopiperazinyl]acetic Acid (18c).** A mixture of **9c** (0.60 g, 1.07 mmol), toluene (3.0 mL), and TFA (3.0 mL) was stirred for 1 h at room temperature and concentrated in vacuo to give an oil. A mixture of 6-[(*tert*-butoxycarbonyl)amino]capronic acid (0.26 g, 1.12 mmol), EDC (0.22 g, 1.14 mmol), *N*-hydroxysuccinimide (0.13 g, 1.12 mmol), and DMF (2.0 mL) was stirred for 1 h at room temperature. To the mixture was added a mixture of the above oil, triethylamine (0.30 mL), and DMF (2.1 mL) at room temperature. After being stirred for 4 h at room temperature, the mixture was diluted with EtOAc, washed with 5% aqueous $KHSO_4$ and saturated aqueous $NaHCO_3$ successively, dried over $MgSO_4$, and concentrated in

vacuo. The residue was purified by column chromatography ($SiO_2$, EtOAc:MeOH:AcOH = 20:10:0.6) to give **18c** (0.42 g, 63%) as a colorless amorphous powder: IR (KBr) $cm^{-1}$ 3320, 2930, 1643, 1533, 1448, 1203, 1173, 1046; $^1H$ NMR ($CD_3OD$) $\delta$ 1.42 (9H, s), 1.20−2.09 (10H, m), 2.17 (2H, t, $J$ = 7.4 Hz), 2.80−4.20 (12H, m), 4.80−4.98 (1H, m), 5.11 (2H, s), 7.22−7.44 (5H, m).

**2-[(3*S*)-4-[2-[(Benzyloxycarbonyl)amino]acetyl]-3-[3-[[4-[(*tert*-butoxycarbonyl)amino]butanoyl]amino]propyl]-2-oxopiperazinyl]acetic Acid (16c).** Compound **16c** was synthesized by the same procedure as that described for the synthesis of **18c** using 4-[(*tert*-butoxycarbonyl)amino]butyric acid instead of 6-[(*tert*-butoxycarbonyl)amino]capronic acid: colorless amorphous powder (yield 56% from **9c**); IR (KBr) $cm^{-1}$ 3350, 2930, 1642, 1530, 1452, 1252, 1170, 1050; $^1H$ NMR ($CD_3OD$) $\delta$ 1.42 (9H, s), 1.30−2.10 (6H, m), 2.18 (2H, t, $J$ = 7.6 Hz), 3.04 (2H, t, $J$ = 6.8 Hz), 3.10−4.20 (10H, m), 4.83−4.97 (1H, m), 5.11 (2H, s), 7.22−7.50 (5H, m).

**2-[(3*S*)-4-[2-[(Benzyloxycarbonyl)amino]acetyl]-3-[3-[[5-[(*tert*-butoxycarbonyl)amino]pentanoyl]amino]propyl]-2-oxopiperazinyl]acetic Acid (17c).** Compound **17c** was synthesized by the same procedure as that described for the synthesis of **18c** using 5-[(*tert*-butoxycarbonyl)amino]valeric acid instead of 6-[(*tert*-butoxycarbonyl)amino]capronic acid: colorless amorphous powder (yield 69% from **9c**); IR (KBr) $cm^{-1}$ 3370, 2940, 1650, 1533, 1455, 1254, 1170, 1050; $^1H$ NMR ($CD_3OD$) $\delta$ 1.42 (9H, s), 1.28−2.08 (8H, m), 2.18 (2H, t, $J$ = 7.0 Hz), 3.03 (2H, t, $J$ = 6.8 Hz), 3.10−4.20 (10H, m), 4.82−5.00 (1H, m), 5.11 (2H, s), 7.22−7.52 (5H, m).

**2-[(3*S*)-4-[2-[(Benzyloxycarbonyl)amino]acetyl]-3-[3-[[4-[2-[(*tert*-butoxycarbonyl)amino]ethyl]benzoyl]amino]propyl]-2-oxopiperazinyl]acetic Acid (19c).** Compound **19c** was synthesized by the same procedure as that described for the synthesis of **18c** using 4-[2-[(*tert*-butoxycarbonyl)amino]ethyl]benzoic acid instead of 6-[(*tert*-butoxycarbonyl)amino]capronic acid: colorless amorphous powder (yield 64% from **9c**); IR (KBr) $cm^{-1}$ 3410, 1640, 1540, 1455, 1205, 1180; $^1H$ NMR ($CD_3OD$) $\delta$ 1.41 (9H, s), 1.50−2.20 (4H, m), 2.80 (2H, t, $J$ = 7.2 Hz), 3.10−4.20 (12H, m), 4.80−5.05 (1H, m), 5.09 (2H, s), 7.06−7.42 (5H, m), 7.76 (2H, d, $J$ = 8.0 Hz).

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[3-[(6-aminohexanoyl)amino]propyl]-2-oxopiperazinyl]acetic Acid (23c).** A solution of **18c** (0.42 g, 0.68 mmol) in MeOH (8.4 mL) was treated with 10% Pd−C (0.17 g) and hydrogenated under balloon pressure for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. To a mixture of the residual oil, 1,4-dioxane (4.2 mL), and $H_2O$ (8.4 mL) were added successively $NaHCO_3$ (0.18 g, 2.1 mmol) and 4-amidinobenzoyl chloride hydrochloride (0.20 g, 0.93 mmol) at room temperature. After being stirred for 1 h, the mixture was adjusted to pH 4 with 1 N HCl and concentrated in vacuo. To a suspension of the residual powder in toluene (4.3 mL) was added TFA (4.3 mL) at 0 °C, and the mixture was stirred for 1 h at room temperature and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, gradient elution; $H_2O$ to 5% aqueous $CH_3CN$) to give **23c** (0.26 g, 55%) as a colorless amorphous powder: $[\alpha]^{20}_D$ +42.7° ($c$ = 0.99, MeOH); $^1H$ NMR ($CD_3OD$) $\delta$ 1.25−2.20 (10H, m), 2.21(2H, t, $J$ = 6.8 Hz), 2.92 (2H, t, $J$ = 7.6 Hz), 3.05−5.20 (11H, m), 7.90 (2H, d, $J$ = 8.6 Hz), 8.05 (2H, d, $J$ = 8.6 Hz). Anal. ($C_{25}H_{37}N_7O_6 \cdot 1.1CF_3CO_2H \cdot 2H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[3-[(5-aminopentanoyl)amino]propyl]-2-oxopiperazinyl]acetic Acid (22c).** Compound **22c** was synthesized by the same procedure as that described for the synthesis of **23c**: colorless amorphous powder (yield 70% from **17c**); $[\alpha]^{20}_D$ +46.0° ($c$ = 1.01, MeOH); $^1H$ NMR ($CD_3OD$) $\delta$ 1.45−2.15 (8H, m), 2.15−2.35 (2H, m), 2.80−3.00 (2H, m), 3.10−4.60 (10H, m), 4.70−5.05 (1H, m), 7.90 (2H, d, $J$ = 8.6 Hz), 8.07 (2H, d, $J$ = 8.6 Hz). Anal. ($C_{24}H_{35}N_7O_6 \cdot CF_3CO_2H \cdot 2.5H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[3-[[4-(2-aminoethyl)benzoyl]amino]propyl]-2-oxopiperazinyl]acetic Acid (24c).** Compound **24c** was synthesized by the same procedure as that described for the synthesis of **23c**: colorless amorphous powder (yield 49% from **19c**); $[\alpha]^{20}_D$ +42.2° ($c$ = 1.00, MeOH); $^1H$ NMR ($CD_3OD$) $\delta$ 1.55−2.20 (4H, m), 2.90−3.10 (2H, m), 3.10−4.55 (12H, m), 4.75−5.05 (1H, m), 7.36 (2H, d, $J$ = 8.4 Hz), 7.81 (2H, d, $J$ = 8.4 Hz), 7.88 (2H, d, $J$ = 8.4 Hz), 8.05 (2H, d, $J$ = 8.4 Hz). Anal. ($C_{28}H_{35}N_7O_6 \cdot CF_3CO_2H \cdot 3.5H_2O$) C, H, N.

**2-[(3*S*)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[3-[[4-(guanidino)butanoyl]amino]propyl]-2-oxopiperazinyl]acetic Acid (26).** A mixture of **16c** (0.33 g, 0.56 mmol), toluene (6.6 mL), and TFA (6.6 mL) was stirred for 1 h at room temperature and concentrated in vacuo. A mixture of the residual oil, $NaHCO_3$ (0.14 g, 1.68 mmol), and $H_2O$ (3.3 mL) was added to a solution of *S*-methylisothiourea sulfate (0.93 g, 3.35 mmol) in 1 N NaOH (3.4 mL) at room temperature. The mixture was stirred for 14 h at room temperature and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, gradient elution; $H_2O$ to 15% aqueous $CH_3CN$) to give **25** as a colorless powder. Under an $H_2$ atmosphere, a suspension of **25** and 10% Pd−C (0.3 g) in MeOH was stirred for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. To a mixture of the residual oil, $H_2O$ (6.0 mL), and 1,4-dioxane (3.0 mL) were added successively $NaHCO_3$ (0.19 g, 2.2 mmol) and 4-amidinobenzoyl chloride hydrochloride (0.16 g, 0.73 mmol) at room temperature. After being stirred for 1 h, the mixture was adjusted to pH 4 with 1 N HCl and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, $H_2O$) to give **26** (0.09 g, 25%) as a colorless amorphous powder: $[\alpha]^{20}_D$ +48.4° ($c$ = 0.96, $H_2O$); $^1H$ NMR ($CD_3OD$) $\delta$ 1.95−2.15 (6H, m), 2.28 (2H, t, $J$ = 6.8 Hz), 3.10−3.85 (7H, m), 3.95−4.65 (5H, m), 4.80−5.05 (1H, m), 7.91 (2H, d, $J$ = 8.4 Hz), 8.10 (2H, d, $J$ = 8.4 Hz). Anal. ($C_{24}H_{35}N_9O_6 \cdot 2HCl \cdot 3.5H_2O$) C, H, N.

**2-[(3*S*)-3-[3-[(4-Amidinobenzoyl)amino]propyl]-4-[2-[(benzyloxycarbonyl)amino]acetyl]-2-oxopiperazinyl]acetic Acid (27).** A mixture of **9c** (1.35 g, 2.40 mmol), toluene (6.8 mL), and TFA (6.8 mL) was stirred for 1 h at room temperature and concentrated in vacuo. To a mixture of the residual oil, $H_2O$ (27 mL), and 1,4-dioxane (14 mL) were added successively $NaHCO_3$ (0.81 g, 9.6 mmol) and 4-amidinobenzoyl chloride hydrochloride (0.68 g, 3.1 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was adjusted to pH 4 with 1 N HCl and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, gradient elution; $H_2O$ to 20% aqueous $CH_3CN$) to give **27** (1.0 g, 71%) as a colorless amorphous powder: $[\alpha]^{20}_D$ +106.6° ($c$ = 0.48, 0.1 N HCl); $^1H$ NMR ($D_2O$+DCl) $\delta$ 1.45−2.20 (4H, m), 3.15−3.82 (5H, m), 3.85−5.20 (8H, m), 7.12−7.50 (5H, m), 7.74−7.96 (4H, m). Anal. ($C_{27}H_{32}N_6O_7 \cdot 2H_2O$) C, H, N.

**2-[(3*S*)-3-[3-[(4-Amidinobenzoyl)amino]propyl]-4-[2-[[4-(aminocarbonyl)benzoyl]amino]acetyl]-2-oxopiperazinyl]acetic Acid (28).** A solution of **27** (0.5 g, 0.85 mmol) in MeOH (10 mL) was treated with 10% Pd−C (0.05 g) and hydrogenated under balloon pressure for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give an oil. A mixture of 4-carbamoylbenzoic acid (0.17 g, 1.02 mmol), EDC (0.20 g, 1.02 mmol), *N*-hydroxysuccinimide (0.12 g, 1.02 mmol), and DMF (2.0 mL) was stirred for 1 h at room temperature. To the mixture was added a mixture of the above oil, triethylamine (0.27 mL), and DMF (2.0 mL) at room temperature. After being stirred for 4 h at room temperature, the mixture was adjusted to pH 2 with 1 N HCl and concentrated in vacuo. The residue was purified by column chromatography (CHP-20, gradient elution; $H_2O$ to 15% aqueous $CH_3CN$) to give **28** (0.38 g, 70%) as a colorless amorphous powder: IR (KBr) $cm^{-1}$ 3252, 1645, 1634, 1559, 1385, 1296; $^1H$ NMR ($D_2O$) $\delta$ 1.60−2.30 (4H, m), 3.30−5.20 (11H, m), 7.66−7.94 (8H, m). Anal. ($C_{27}H_{31}N_7O_7 \cdot 4H_2O$) C, H, N.

**Methyl 4-(Cyanomethyl)benzoate (30).** A solution of methyl 4-chloromethylbenzoate (**29**) (25.5 g, 138 mmol) in dimethyl sulfoxide (100 mL) was treated with NaCN (6.86 g, 140 mmol), and the mixture was stirred for 3 h at room temperature. Cold water was added to the mixture, and

precipitated powder was collected by filtration. The powder was dissolved in EtOAc, and the solution was washed with water, dried over $MgSO_4$, and concentrated in vacuo to give **30** (23.2 g, 79%) as colorless powder: $^1$H NMR ($CDCl_3$) $\delta$ 3.83 (2H, s), 3.93 (3H, s), 7.42 (2H, d, $J$ = 8.4 Hz), 8.06 (2H, d, $J$ = 8.4 Hz).

**Methyl 4-[2-Amino-2-(hydroxyimino)ethyl]benzoate (31).** To a solution of **30** (23.2 g, 110 mmol) in MeOH (200 mL) were added $NaHCO_3$ (9.7 g, 115 mmol) and $NH_2OH$·HCl (8.0 g, 115 mmol) at room temperature. The mixture was refluxed for 4 h and concentrated in vacuo. Water and EtOAc were added to the residue, and the precipitated powder was collected by filtration, washed with $H_2O$ and MeOH, and dried under reduced pressure to give **31** (16 g, 70%) as colorless powder: $^1$H NMR (DMSO-$d_6$) $\delta$ 3.34 (2H, s), 3.84 (3H, s), 5.47 (2H, s), 7.42 (2H, d, $J$ = 8.2 Hz), 7.89 (2H, d, $J$ = 8.2 Hz), 8.96 (1H, s).

**4-[(5-Oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)methyl]benzoic Acid (32).** To a solution of **31** (3.1 g, 14.9 mmol) in 1,4-dioxane (15 mL) were added 1,1′-carbonyldiimidazole (3.0 g, 18.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (2.4 mL, 16.0 mmol) at room temperature, and the mixture was stirred for 30 min at 105 °C. After being cooled, the mixture was diluted with water, washed with EtOAc, and adjusted to pH 2 with 3 N HCl and extracted with EtOAc. The later EtOAc extract was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Then, a mixture of the residue and 2 N NaOH (25 mL) in MeOH (50 mL) was refluxed for 2 h. After being cooled, the mixture was concentrated in vacuo, and the residue was dissolved in water and adjusted to pH 2 with 3 N HCl. The precipitated crystals were collected by filtration and dried under reduced pressure to give **32** (2.4 g, 73%) as colorless crystalline powder: mp 260−263 °C; IR (KBr) $cm^{-1}$ 1810, 1740, 1690; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.99 (2H, s), 7.44 (2H, d, $J$ = 8.4 Hz), 7.94 (2H, d, $J$ = 8.4 Hz), 11.0−12.8 (1H, br). Anal. ($C_{10}H_8N_2O_4$·0.1$H_2O$) C, H, N.

**In Vitro Platelet Aggregation Studies.** Blood was collected from healthy human volunteers and cynomolgus monkeys by venipuncture. 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). Platelet rich plasma (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 × $10^5/\mu$L (human and monkey) and 4 × $10^5/\mu$L (guinea pig) using an automatic blood cell counter (Sysmex E2500, Toaiyoudenshi Co., Tokyo, Japan). Platelet aggregation was measured using an eight-channel aggregometer (Hematracer VI, Niko Bioscience, Tokyo, Japan). PRP (250 $\mu$L), in a cuvette stirred at 1000 rpm, was prewarmed for 2 min at 37 °C with various concentrations of test compounds (25 $\mu$L). The change in light transmittance was measured after the addition of aggregating agents (25 $\mu$L) to the cuvette. Submaximal concentration of aggregating agents were used in each experiment.

**Ex Vivo Platelet Aggregation Studies.** Male guinea pigs (250−400 g) were used. Compounds were dissolved in 0.9% physiological saline and given as continuous iv infusions, and the vehicle was given to the control animals. Ninety minutes after starting the infusion, citrated blood was collected from the abdominal aorta under anesthesia, and PRP was prepared. As the aggregation inducer, ADP (20 $\mu$L, submaximal concentration) was used. The bleeding time (BT) was also examined 90 min after starting the infusion according to the template method.

**Template BT.** The hind limbs of the guinea pigs were shaved the day before the experiment. Each animal was anesthetized with sodium pentobarbital (25 mg/kg, ip) and a circular pressure cuff placed on the hind limb proximal to the bleeding site. The size of the cuff was changed to fit the hind limb of each guinea pig. The cuff was connected to a pressure calibrator (XCaliber, Bio-Tek Instruments Inc., VT) and inflated to 40−50 mmHg. Two cuts were made on the ventral aspect of the thigh with a Simplate IIR device (Organon Teknika Co., Durham, NC), and these were blotted every 30 s with filter paper until either bleeding had stopped or 30 min had elapsed. Data were recorded as the mean BT of two cuts in each animal.

**Balloon Catheterization Induced-Carotid Thrombosis Model.** The guinea pigs were anesthetized with sodium pentobarbital (25 mg/kg, ip) and maintained with supplemental anesthetic as necessary during each experiment. The left jugular vein was cannulated for administration of drugs. A fogarty 2F balloon catheter (Baxter Healthcare, McGraw Park, IL) was introduced from the left iliac artery to the right common carotid artery to injure the endothelium. After denudation of the endothelium by five passes of the inflated balloon catheter, an electromagnetic flow probe (Skalar, Delft, The Netherlands), with a lumen diameter of 0.7 mm, was immediately attached around the carotid to measure the carotid blood flow. In each case, the flow fell to zero within 5 min of balloon injury. The flow probe was removed after the observation of thrombotic occlusion. The balloon catheter was reintroduced, inflated, and withdrawn in order to remove the thrombus. The electromagnetic flow probe was then attached, and carotid blood flow measured over 60 min. Just before the thromboembolectomy, 25% of the total dose of drug was given as a bolus injection (1 mL/kg), and then the remaining 75% was infused over 1 h at a rate of 50 $\mu$L/kg/min. In preliminary experiments, we observed that thrombotic occlusion did not occur when platelet depletion (<2 × $10^5/\mu$L) was produced with using anti guinea pig platelet antibody. At the end of the experiment, an injured carotid segment was removed, fixed with 10% neutral buffered formalin solution, and embedded in paraffin for light microscopy. Thin cross-sections were cut and stained with hematoxylin-eosin.

## References


(1) Davies, M. J.; Thomas, A. C. Plaque Fissuring − the Cause of Acute Myocardial Infarction, Sudden Ischaemic Death, and Crescendo Angina. *Br. Heart J.* **1985**, *53*, 363−373.

(2) Fuster, V.; Badimon, L.; Badimon, J. J.; Chesebro, J. H. The Pathogenesis of Coronary Artery Disease and the Acute Coronary Syndromes (1). *N. Engl. J. Med.* **1992**, *326*, 242−250.

(3) Plow, E. F.; Pierschbacher, M. D.; Ruoslahti, E.; Marguerie, G. A.; Ginsberg, M. H. The Effect of Arg-Gly-Asp-containing Peptides on Fibrinogen and von Willebrand Factor Binding to Platelets. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 8057−8061.

(4) Gartner, T. K.; Bennett, J. S. The Tetrapeptide Analogue of the Cell Attachment Site of Fibronectin Inhibits Platelet Aggregation and Fibrinogen Binding to Activated Platelets. *J. Biol. Chem.* **1985**, *260*, 11891−11894.

(5) Phillips, D. R.; Charo, I. F.; Parise, L. V.; Fitzgerald, L. A. The Platelet Membrane Glycoprotein IIb/IIIa Complex. *Blood* **1988**, *71*, 831−843.

(6) The EPIC Investigators. Use of a Monoclonal Antibody Directed Against the Platelet Glycoprotein IIb/IIIa Receptor in High-Risk Coronary Angioplasty. *N. Engl. J. Med.* **1994**, *330*, 956−961.

(7) Topol, E. J.; Califf, R. M.; Weisman, H. F.; Ellis, S. G.; Tcheng, J. E.; Worley, S.; Ivanhoe, R.; George, B. S.; Fintel, D.; Weston, M.; Sigmon, K.; Anderson, K. M.; Lee, K. L.; Willerson, J. T.; The EPIC Investigators. Randomised Trial of Coronary Intervention with Antibody against Platelet IIb/IIIa Integrin for Reduction of Clinical Restenosis: Results at Six Months. *Lancet* **1994**, *343*, 881−886.

(8) Aguirre, F. V.; Topol, E. J.; Ferguson, J. J.; Anderson, K.; Blankenship, J. C.; Heuser, R. R.; Sigmon, K.; Taylor, M.; Gottlieb, R.; Hanovich, G.; Rosenberg, M.; Donohue, T. J.; Weisman, H. F.; Califf, R. M.; for the EPIC Investigators. Bleeding Complications with the Chimeric Antibody to Platelet Glycoprotein IIb/IIIa Integrin in Patients Undergoing Percutaneous Coronary Intervention. *Circulation* **1995**, *91*, 2882−2890.

(9) Simpfendorfer, C.; Kottke-Marchant, K.; Lowrie, M.; Andres, R. J.; Burns, D. M.; Miller, D. P.; Cove, C. S.; DeFranco, A. C.; Ellis, S. G.; Moliterno, D. J.; Raymond, R. E.; Sutton, J. M.; Topol, E. J. First Chronic Platelet Glycoprotein IIb/IIIa Integrin Blockade. A Randomized, Placebo-Controlled Pilot Study of Xemilofiban in Unstable Angina with Percutaneous Coronary Interventions. *Circulation* **1997**, *96*, 76−81.

(10) Sugihara, H.; Fukushi, H.; Miyawaki, T.; Imai, Y.; Terashita, Z.; Kawamura, M.; Fujisawa, Y.; Kita, S. Novel Non-Peptide Fibrinogen Receptor Antagonists. 1. Synthesis and Glycoprotein IIb-IIIa Antagonistic Activities of 1,3,4-Trisubstituted 2-Oxopiperazine Derivatives Incorporating Side-Chain Functions of the RGDF Peptide. *J. Med. Chem.* **1998**, *41*, 489−502.

(11) Kitamura, S.; Fukushi, H.; Miyawaki, T.; Kawamura, M.; Terashita, Z.; Sugihara, H.; Naka, T. 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. *Chem. Pharm. Bull.* **2001**, *49*, 258−267.

(12) Cheng, S.; Craig, W. S.; Mullen, D.; Tschopp, J. F.; Dixon, D.; Pierschbacher, M. D. Design and Synthesis of Novel Cyclic RGD-Containing Peptides as Highly Potent and Selective Integrin $\alpha_{IIb}\beta_3$ Antagonists. *J. Med. Chem.* **1994**, *37*, 1−8.

(13) Waki, M.; Kitajima, Y.; Izumiya, N. A Facile Synthesis of $N^2$-Protected L-2,3- Diaminopropanoic Acid. *Synthesis* **1981**, 266−268.

(14) Zablocki, J. A.; Miyano, M.; Garland, R. B.; Pireh, D.; Schretzman, L.; Rao, S. N.; Lindmark, R. J.; Panzer-Knodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. Potent in Vitro and in Vivo Inhibitors of Platelet Aggregation Based upon the Arg-Gly-Asp-Phe Sequence of Fibrinogen. A Proposal on the Nature of the Binding Interaction between the Arg- guanidine of RGDX Mimetics and the Platelet GP IIb-IIIa Receptor. *J. Med. Chem.* **1993**, *36*, 1811−1819.

(15) Kawamura, M.; Imura, Y.; Moriya, N.; Kita, S.; Fukushi, H.; Sugihara, H.; Nishikawa, K.; Terashita, Z. Antithrombotic Effects of TAK-029, a Novel GPIIb/IIIa Antagonist, in Guinea Pigs: Comparative Studies with Ticlopidine, Clopidogrel, Aspirin, Prostaglandin $E_1$ and Argatroban. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 502−510.

(16) We are now preparing another paper concerning the pharmacological evaluation of **12c** in monkeys and human.

(17) Phillips, D. R.; Teng, W.; Arfsten, A.; Nannizzi-Alaimo, L.; White, M. M.; Longhurst, C.; Shattil, S. J.; Randolph, A.; Jakubowski, J. A.; Jennings, L. K.; Scarborough, R. M. Effect of $Ca^{2+}$ on GP IIb-IIIa Interactions With Integrilin. Enhanced GP IIb-IIIa Binding and Inhibition of Platelet Aggregation by Reductions in the Concentration of Ionized Calcium in Plasma Anticogulated With Citrate. *Circulation*. **1997**, *96*, 1488−1494.

(18) Collen, D.; Lu, H. R.; Stassen, J.-M.; Vreys, I.; Yasuda, T.; Bunting, S.; Gold, H. K. Antithrombotic Effects and Bleeding Time Prolongation with Synthetic Platelet GPIIb/IIIa Inhibitors in Animal Models of Platelet-Mediated Thrombosis. *Thromb. Haemostasis* **1994**, *71*, 95−102.

(19) Mousa, S. A.; Bozarth, J. M.; Forsythe, M. S.; Slee, A. Differential Antiplatelet Efficacy for Various GPIIb/IIIa Antagonists: Role of Plasma Calcium Levels. *Cardiovasc. Res.* **2000**, *47*, 819−826.

(20) Golino, P.; Ashton, J. H.; Glas-Greenwalt, P.; McNatt, J.; Buja, L. M.; Willerson, J. T. Mediation of Reocculusion by Thromboxane $A_2$ and Serotonin after Thrombolysis with Tissue-Type Plasminogen Activator in a Canine Preparation of Coronary Thrombosis. *Circulation* **1988**, *77*, 678−684.

(21) Yasuda, T.; Gold, H. K.; Fallon, J. T.; Leinbach, R. C.; Guerrero, J. L.; Scudder, L. E.; Kanke, M.; Shealy, D.; Ross, M. J.; Collen, D.; Coller, B. S. Monoclonal Antibody against the Platelet Glycoprotein (GP) IIb/IIIa Receptor Prevents. Coronary Artery Reocculusion after Reperfusion with Recombinant Tissue-Type Plasminogen Activator in Dogs. *J. Clin. Invest.* **1988**, *81*, 1284−1291.

(22) Storey, R. F.; Wilcox, R. G.; Heptinstall, S. Differential Effects of Glycoprotein IIb/IIIa Antagonists on Platelet Microaggragtion and Macroaggregation Formation and Effect of Anticoagulant on Antagonist Potency. Implications for Assay Methodology and Comparison of Different Antagonists. *Circulation* **1998**, *98*, 1616−1621.

JM0004345