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

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Based on the lead tetrapeptide RGDF, two possible non-peptide glycoprotein (GP) IIb-IIIa antagonists possessing an *(S)*-2-oxopiperazine-3-acetic acid moiety as a scaffold incorporating the indispensable Asp fragment were prepared, and *(S)*-4-[[*trans*-[4-(guanidinomethyl)-cyclohexyl]carbonyl]glycyl]-2-oxopiperazine-1,3-diacetic acid, **1a**, was identified as a potential lead. A series of 3-substituted 2-oxopiperazine-1-acetic acids bearing the Arg-Gly equivalent at the 4-position were prepared and evaluated for their ability to prevent platelet aggregation and for their binding affinity for the GP IIb-IIIa receptor purified from human HEL cells. (*S*)-4-[(4-Amidinobenzoyl)glycyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid, **9** (TAK-029), inhibited in vitro human platelet aggregation with an IC_{50} value of 0.03 μM and GP IIb-IIIa−fibrinogen binding with an IC_{50} value of 0.49 nM. The [4-(2-aminoethyl)benzoyl]glycyl derivative **26** showed activity comparable to that of **9** (IC_{50} = 0.093 μM, guinea pig platelet aggregation assay). Compound **9** dose-dependently inhibited ex vivo platelet aggregation in guinea pigs (0.03 and 0.1 mg/kg, iv), and long-lasting inhibition of platelet aggregation was observed upon oral administration of **9** (3 mg/kg) to guinea pigs. On the other hand, the activity of **26** disappeared within 1 h after a dose of 1 mg/kg (iv). Compound **9** may therefore be useful in the clinical treatment of arterial thrombotic diseases.

Introduction

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 aggregation, regardless of the initiating stimulus, is the cross-linking of the dimeric plasma protein fibrinogen between membrane glycoprotein IIb-IIIa (GP IIb-IIIa) receptor complexes exposed on adjacent activated platelets.[1−4] Antagonism of the fibrinogen−GP IIb-IIIa interaction therefore represents an attractive therapeutic target with potential utility in the treatment and prevention of acute myocardial infarction, unstable angina, or transient ischemic attacks (TIA).[5−7]

Fibrinogen interacts with binding sites on GP IIb-IIIa through peptide domains present on the α- and γ-chains: RGDF (α95−98), RGDS (α572−575), and HHLGGAKQAGDV (γ400−411).[8−10] Most of the molecules that produce antithrombotic activity via the inhibition of the GP IIb-IIIa−fibrinogen interaction fall into four categories: RGD-based peptides (small linear and cyclic peptides containing the RGD sequence or its equivalent),[1,2,8,11−14] snake venom peptides,[15−19] monoclonal antibodies raised against GP IIb-IIIa,[20,21] and non-peptide GP IIb-IIIa receptor antagonists that mimic the RGD tripeptide sequence.[22−33]

Previous structure−activity studies (SARs) on fibrinogen receptor antagonists have suggested that the sole prerequisite for receptor recognition is a molecule which contains critical guanidino (A) and carboxylate (B) equivalents spaced appropriately.[7,22−33] In the structural and stereochemical elucidation of RGD peptides in cell adhesion, it has been demonstrated that substitution of aspartic acid with D-Asp or Glu or of glycine with Ala or Val resulted in a complete loss of activity and that the tetrapeptide RGDF (Fgα95−98) has 3−5-fold more potent antiplatelet activity than RGDS (Fgα572−575).[8,34,35] Besides the indispensable carboxylate of aspartic acid, the presence of the C-terminal negative charge appears to contribute to the effective interaction with the receptor and to the antithrombic activity.[7,12,25]

One approach to designing non-peptide and peptide mimetics of bioactive peptides has been the use of conformational constraints by various types of cyclization.[36−38] The resulting increase in the structural rigidity of peptides has been found to provide some useful properties including enhancement of affinity, specificity, and enzymatic stability. We decided to use this approach in our compounds, and in addition, we postulated on the basis of the three-ligand concept[39] that the hydrophobic phenyl ring (C) or the carboxylate (D)

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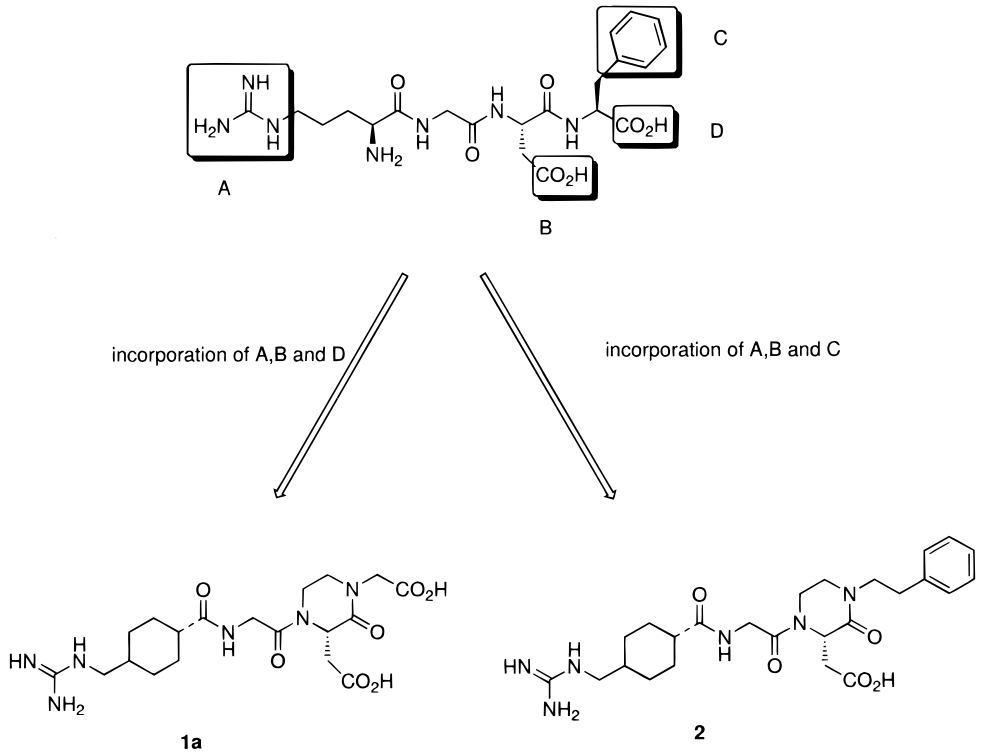


Figure 1. Two hypothetical candidates, **1a** and **2**, incorporating the functions of the RGDF peptide into a 2-oxopiperazine scaffold as a dipeptide mimic.

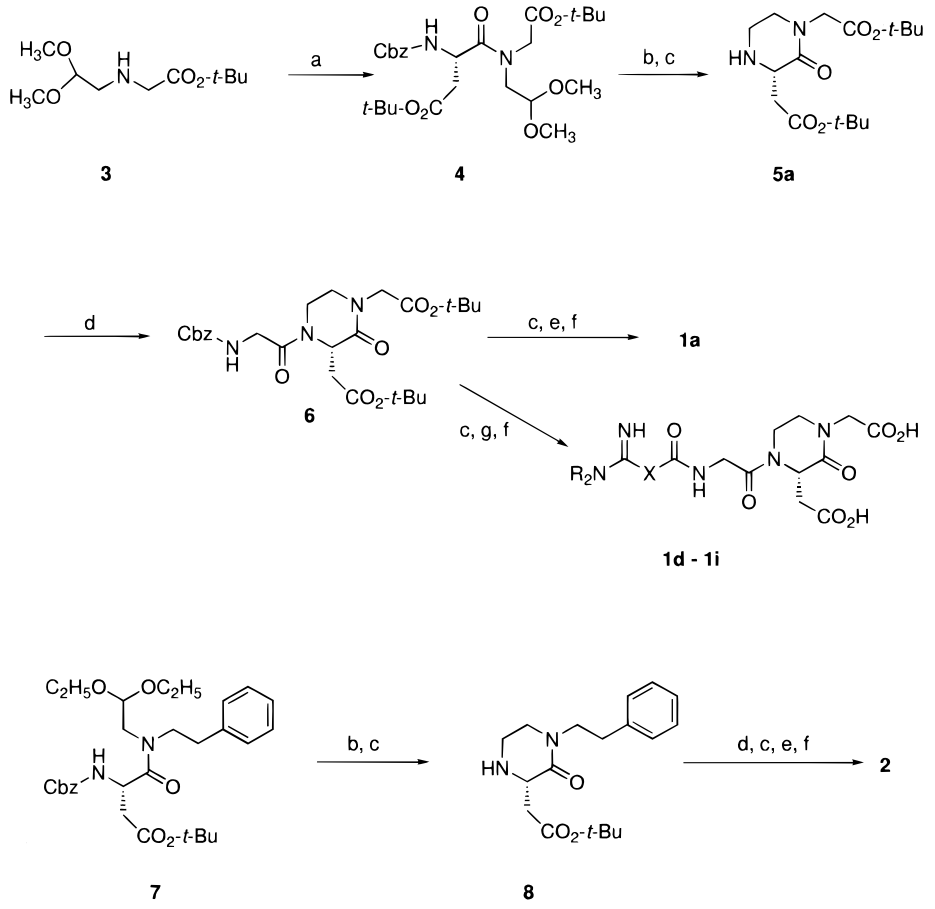
of the C-terminal phenylalanine in the prototypical RGDF molecule may be the third functional group participating in ligand−receptor interaction. On trial we designed two possible pharmacophores, (*S*)-1-(carboxymethyl)- and (*S*)-1-phenethyl-2-oxopiperazine-3-acetic acids, as the Asp-Phe mimic and synthesized two compounds (**1a** and **2**, Figure 1) incorporating a [*trans*-[4-(guanidinomethyl)cyclohexyl]carbonyl]glycyl group as an Arg-Gly equivalent. Compound **1a** exhibited significant antiaggregatory activity and binding affinity for GP IIb-IIIa, but **2** did not show any activity in either biological test. Therefore, we chose **1a** as a lead compound and investigated optimization of the tentative Arg-Gly moiety and modification of the substituents at the 1- and/or 3-position on the 2-oxopiperazine ring. In this paper, we describe the synthesis of novel 2-oxopiperazine derivatives and biological activities of compounds thus obtained. Molecular modeling studies are also described.

Chemistry

Synthesis of the chiral 1,3-disubstituted 2-oxopiperazine derivatives **5a**−**g** and **6** as conformationally restricted dipeptide mimetics was achieved in two steps starting from N-substituted *N*-(2,2-dimethoxyethyl)-amides of *N*-Cbz-protected α-amino acids by modifying the method developed by DiMaio et al.[40] (Schemes 1−3). The synthesis of **1a** and **2** is illustrated in Scheme 1. Condensation of *tert*-butyl *N*-(2,2-dimethoxyethyl)glycine, **3**, and *N*-Cbz-Asp(*O*-*t*-Bu)-OH using 1-ethyl-3-[3-(*N*,*N*-dimethylamino)propyl]carbodiimide hydrochloride (EDC) in methylene chloride provided the intermediary acetal amide **4**. Intramolecular cyclization of **4** using a catalytic amount of *p*-toluenesulfonic acid in toluene at 70 °C proceeded smoothly with an acetal exchange and the following elimination of methanol to yield a 2-oxotetrahydropyrazine intermediate, which was hydrogenated in the presence of 10% Pd/C in MeOH to afford crystalline *tert*-butyl (*S*)-[(3-*tert*-butyloxycarbonyl)methyl]-2-oxopiperazine-1-acetate, **5a**, as an oxalate. A similar procedure using *N*-(2,2-dimethoxyethyl)phenethylamine instead of **3** was used to prepare *tert*-butyl (*S*)-1-phenethyl-2-oxopiperazine-3-acetate, **8**, as a key intermediate (Scheme 1). Acylation of **5a** and **8** with *N*-Cbz-glycine using EDC in methylene chloride followed by removal of the Cbz group, subsequent coupling of the resulting glycyl derivative with the *N*-hydroxysuccinimide ester of *trans*-4-guanidinocyclohexanecarboxylic acid activated by utilizing hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in DMF, and finally deprotection of the *tert*-butyl ester using trifluoroacetic acid (TFA) in methylene chloride gave **1a** and **2**, respectively. Compounds **1b**−**i** were prepared in three steps in a similar fashion by hydrogenolysis of di-*tert*-butyl (*S*)-4-(*N*-Cbz-glycyl)-2-oxopiperazine-1,3-diacetate, **6**, subsequent condensation with various kinds of activated *N*-hydroxysuccinimide esters of carboxylic acids bearing a guanidino or amidino moiety, and finally deprotection (Scheme 1, Table 1).

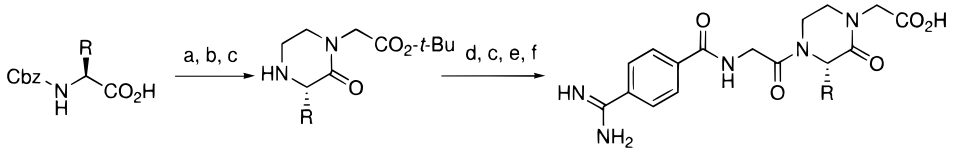
The synthesis of the 3-substituted 4-[(4-amidinobenzoyl)glycyl]-2-oxopiperazine-1-acetic acids **9**−**14** is illustrated in Scheme 2. The *tert*-butyl 3-substituted 2-oxopiperazine-1-acetates **5b**−**g** were afforded by applying the method described for the preparation of **5a**. Condensation of **3** with α-*N*-Cbz-protected α-amino acids, acid-catalyzed annulation, and catalytic hydrogenation furnished **5b**−**g**. Standard acylation of **5b**−**g** with *N*-Cbz-glycine, subsequent condensation of amines arising from deprotection of the *N*-Cbz group with *p*-amidinobenzoyl chloride in the presence of sodium bicarbonate in a 1:1 mixture of H_2O and dioxane, and finally treatment with TFA afforded **9**−**14**. Enantio-

Scheme 1[a]



[a] Reagents: (a) *N*-Cbz-Asp(*O*-*t*-Bu)-OH, EDC; (b) *p*-TsOH in toluene; (c) H_2, Pd/C in MeOH; (d) *N*-Cbz-Gly-OH, EDC; (e) *trans*-(guanidinomethyl)cyclohexanecarboxylic acid, HOSu, DCC in DMF; (f) TFA; (g) $R_2N(HN{=})C\text{-}X\text{-}CO_2H$, HOSu, DCC.

Scheme 2[a]



5b: R = $CH_2CO_2CH_3$
5c: R = H
5d: R = $CH_2CONH\text{-}CH(p\text{-}CH_3O\text{-}Ph)_2$
5e: R = CH_2Ph
5f: R = $(CH_2)_2CO_2\text{-}t\text{-}Bu$
5g: R = $(CH_2)_2CO_2CH_3$

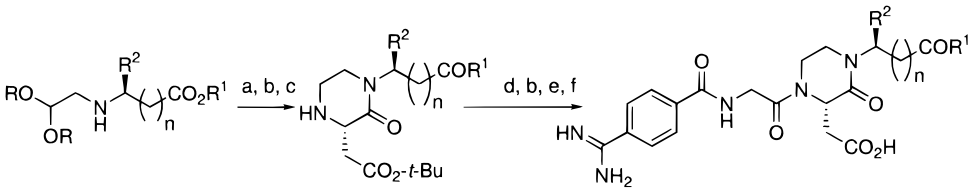
[a] Reagents (a) **3**, EDC; (b) *p*-TsOH in toluene; (c) H_2, Pd/C in MeOH; (d) *N*-Cbz-Gly-OH, EDC; (e) 4-amidinobenzoyl chloride, $NaHCO_3$ in dioxane/H_2O; (f) TFA.

meric (*R*)-**9** of **9** was prepared in the same manner as that described for **9** except that *N*-Cbz-D-Asp(*O*-*t*-Bu)-OH was used as the starting material. Optical purities of **9** and (*R*)-**9** were determined by chiral HPLC and estimated to be over 99.3% for **9** and 98.3% for (*R*)-**9** (Scheme 2, Table 2).

Modification of the substituent at the 1-position of the (*S*)-3-(carboxymethyl)-2-oxopiperazine ring (**17**−**21**) was carried out as shown in Scheme 3. The *tert*-butyl (*S*)-1-substituted 2-oxopiperazine-3-acetates **16a**−**e** as key intermediates were similarly prepared in three steps using the acetal amines **15a**−**e** and *N*-Cbz-Asp(*O*-*t*-Bu)-OH as starting materials. Acylation of **16a**−**e** with *N*-Cbz-glycine followed by removal of the Cbz group, subsequent condensation with 4-amidinobenzoyl chloride using sodium bicarbonate as a base, and finally treatment with TFA gave compounds **17**−**21** (Scheme 3, Table 2).

Acylation of **5a**−**c**,**f**,**g** with *N*-Cbz-glycine followed by removal of the Cbz group, condensation with 4-[(*N*-Cbz-amino)alkyl]benzoic acid using diethylphosphoryl cyanide (DEPC) as a coupling agent in DMF, stepwise deprotection by treatment with TFA, and subsequent hydrogenolysis (Pd/C in MeOH) afforded the 4-(ω-aminoalkyl)benzoyl derivatives **23**−**28**. The 4-[2-(*N*,*N*-dimethylamino)ethyl]benzoyl derivatives **29** and **30** were obtained by condensation of **5f**,**g** with 4-[2-(*N*,*N*-

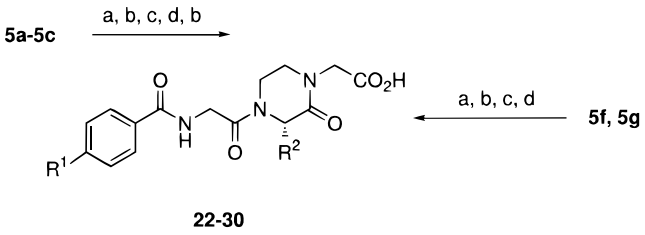
Scheme 3[a]



15a; 16a: R = C_2H_5, R^1 = O-*t*-Bu, R^2 = H, n = 1
15b; 16b: R = CH_3, R^1 = OCH_3, R^2 = H, n = 0
15c; 16c: R = C_2H_5, R^1 = NH_2, R^2 = H, n = 0
15d; 16d: R = C_2H_5, R^1 = O-*t*-Bu, R^2 = CH_3, n = 0
15e; 16e: R = C_2H_5, R1 = O-*t*-Bu, R^2 = CH_2Ph, n = 0

[a] Reagents: (a) *N*-Cbz-Asp(*O*-*t*-Bu)-OH, EDC; (b) *p*-TsOH in toluene; (c) H_2, Pd/C in MeOH; (d) *N*-Cbz-Gly-OH, EDC; (e) 4-amidinobenzoyl chloride, $NaHCO_3$ in dioxane/H_2O; (f) TFA.

Scheme 4[a]



[a] Reagents: (a) *N*-Cbz-Gly-OH, EDC; (b) H_2, Pd/C in MeOH; (c) (*N*-Cbz-aminoalkyl)benzoic acid, DEPC, Et_3N in DMF; (d) TFA.

dimethylamino)ethyl]benzoic acid using DEPC and finally treatment with TFA (Scheme 4, Table 3).

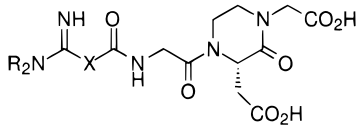
Results and Discussion

The compounds synthesized in this study were evaluated in vitro for their ability to inhibit ADP-induced aggregation of human and guinea pig platelet-rich plasma (PRP) and for their ability to inhibit the binding of biotin-labeled human fibrinogen to immobilized GP IIb-IIIa receptors purified from human erythroleukemia (HEL) cells.[41] In general, a good correlation was observed between the order of potency in the platelet aggregation assay and that in the binding assay. No species differences between humans and guinea pigs were observed in the platelet aggregation assay (Tables 1−3).

Compound **1a** exhibited a significant inhibitory effect on platelet aggregation (IC_{50} = 1.1 and 4.2 μM for human and guinea pig PRP, respectively) and sufficient affinity for the GP IIb-IIIa receptor (IC_{50} = 20 nM). On the other hand, compound **2** did not show any activity in the platelet aggregation assay even at a concentration of 30 μM and was 130-fold less potent than **1a** in the binding affinity assay. In comparison with the prototypical peptide RGDF, compound **1a** showed a 5.5-fold increase in binding affinity for human GP IIb-IIIa. These biological results indicate that, of the two compounds we designed based on our hypothesis, compound **1a** incorporating the dipeptide mimic lacking the phenethyl side chain of the terminal phenylalanine in the RGDF sequence might be promising. In the initial trial, the use of the cyclohexylmethyl group (**1a**) as a substitute for the flexible *n*-butyl moiety (**1b**) of the Arg side chain resulted in a 2.3-fold increase in the binding affinity. We then examined further optimization of a side-chain mimic of arginine. Aromatization of the cyclohexane ring of **1a** had little effect on affinity in the binding assay (**1f**, IC_{50} = 18 nM). This implies the π orbital of the phenyl ring contributes little to the interaction with the receptor in this case. Deletion of a single methylene of the *p*-(guanidinomethyl)phenyl group of **1f** provided the *p*-guanidinophenyl derivative **1e** which showed a 4-fold improvement in binding affinity (IC_{50} = 4.8 nM). Further replacement of the guanidino moiety of **1e** with an amidino group (**1d**) caused an additional 4.8-fold increase in affinity for the GP IIb-IIIa receptor (IC_{50} = 1.0 nM). After optimization at the *N*-terminus of compound **1a** (**1a**−**g**), the *p*-amidinophenyl moiety of **1d** was found to be the most favorable surrogate for the arginine side chain, as has been reported for known GP IIb-IIIa antagonists.[23,27−29,31−33] On the other hand, replacement of the phenyl ring of the *p*-amidinophenyl group of **1d** with a piperidine ring gave the *N*-amidinopiperidin-4-yl derivative **1c** which showed 21 times less binding affinity. Transposition of amidino moiety to the *m*-position from the *p*-position on the phenyl ring (**1d** → **1g**) resulted in a marked decrease in both activities. These results suggest that a strict spatial arrangement of the positively charged site and the anionic carboxylate is required for interaction with the GP IIb-IIIa receptor.

Next, to determine which carboxyl group of the two carboxylic acids in **1d** mimics the side-chain carboxylic acid of Asp in the RGDF sequence, two monoesters (**9** and **18**) and two monoamides (**11** and **19**) were prepared. Compounds possessing an acetate moiety at the 1-position, **9** and **11**, exhibited full activities, but compounds in which the 1-acetate was modified, **18** and **19**, had markedly reduced activities. These SARs indicate that the side-chain carboxylic acid of Asp in the RGDF sequence does not correspond to the 3-acetate of the 2-oxopiperazine ring in the compound we designed but to the acetic acid at the 1-position. Further confirmation was accomplished as follows. Introduction of an alanyl or phenylalanyl side chain into the α-carbon of the acetic acid at the 1-position resulted in more than a 20-fold decrease in the inhibitory potency in the platelet aggregation assay (**20** and **21**). Homologation of the 1-acetic acid by one methylene unit (**17**) caused marked reduction of the activity. All these data demonstrated that the acetic acid at the 1-position is a crucial Asp function for manifestation of GP IIb-IIIa

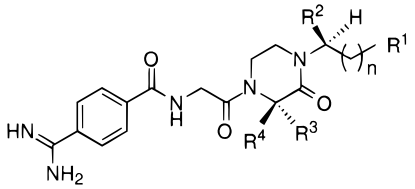
Table 1. Modification of an *n*-Butyl Surrogate of the Arg Side Chain in the Lead Compound **1a**



compd			platelet aggregation IC$_{50}$ (μM)[a]		GP IIb-IIIa–Fg binding
no.	X	R	human	guinea pig	IC$_{50}$ (nM)[b]
1a	-HNCH$_2$-C$_6$H$_4$-	H	1.1	4.2	20
1b	-NH(CH$_2$)$_4$-	H	3.6	5.3	46
1c	-N(C$_5$H$_{10}$)-	H	0.8	10	21
1d	-C$_6$H$_4$-	H	0.11	0.15	1.0
1e	-HN-C$_6$H$_4$-	H	0.35	0.34	4.8
1f	-HNH$_2$C-C$_6$H$_4$-	H	0.56	2.6	18
1g	-C$_6$H$_4$-	H	13	16	130
1h	-C$_6$H$_4$-CH$_2$-	H	1.2	1.6	8.1
1i	HN-C$_6$H$_4$-	CH$_3$	11	11	210
2			nt[c]	> 1000	2630
RGDF			48	nt[c]	110
RGDS			62	370	180

[a] The concentrations of each compound inhibiting the ADP-induced aggregation of human and guinea pig platelets by 50%. [b] The concentrations of each compound inhibiting the binding of biotinated fibrinogen to immobilized human GP IIb-IIIa by 50%. IC$_{50}$ values were calculated using linear regression analysis, plotting the percent inhibition versus the logarithm of the concentration of the compound; $n = 2-8$. [c] nt, not tested.

Table 2. Modification of the Substituent at the 3- and/or 1-Position of the 2-Oxopiperazine Derivatives **9**–**21** and Elucidation of the Asp Carboxylate Equivalent

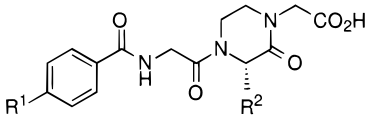


compd no.	R^1	R^2	R^3	R^4	*n*	platelet aggregation IC$_{50}$ (μM)[a] human	platelet aggregation IC$_{50}$ (μM)[a] guinea pig	GP IIb-IIIa–Fg binding IC$_{50}$ (nM)[b]
9	CO$_2$H	H	CH$_2$CO$_2$CH$_3$	H	0	0.03	0.053	0.49
(*R*)-**9**	CO$_2$H	H	H	CH$_2$CO$_2$CH$_3$	0	0.58	2.81	16.4
10	CO$_2$H	H	H	H	0	0.094	0.21	0.42
11	CO$_2$H	H	CH$_2$CONH$_2$	H	0	0.74	0.11	0.92
12	CO$_2$H	H	CH$_2$Ph	H	0	0.11	0.16	1.95
13	CO$_2$H	H	CH$_2$CH$_2$CO$_2$H	H	0	0.052	0.066	0.21
14	CO$_2$H	H	(CH$_2$)$_2$CO$_2$CH$_3$	H	0	0.09	0.11	0.29
17	CO$_2$H	H	CH$_2$CO$_2$H	H	1	30	nt[c]	63
18	CO$_2$CH$_3$	H	CH$_2$CO$_2$H	H	0	2.6	1.8	20
19	CONH$_2$	H	CH$_2$CO$_2$H	H	0	30	nt[c]	330
20	CO$_2$H	CH$_3$	CH$_2$CO$_2$H	H	0	1.5	1.5	nt[c]
21	CO$_2$H	CH$_2$Ph	CH$_2$CO$_2$H	H	0	4.1	4.1	6.1

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

antagonistic activity. Alig et al. showed that the C-terminal dipeptide of RGDX could be replaceable with a simple (piperidinyloxy)acetic acid moiety.[23] They also described compounds with two carboxyl termini and found that one carboxyl group was necessary but the other not. It is interesting that the two different approaches reached similar simplifications; the two carboxylate groups of the catechol-*O*,*O*′-diacetate part or of the Asp and Phe fragments in RGDF were replaceable with achiral oxyacetate or acetate moieties

Table 3. Modification of the Amidino Moiety



compd no.	R^1	R^2	platelet aggregation IC_{50} (μM)[a] human	platelet aggregation IC_{50} (μM)[a] guinea pig	GP IIb-IIIa−Fg binding IC_{50} (nM)[b]
22	H_2NCH_2	CH_2CO_2H	2.8	2.9	18
23	$H_2N(CH_2)_2$	CH_2CO_2H	0.13	0.23	2.5
24	$H_2N(CH_2)_3$	CH_2CO_2H	1.03	1.1	15
25	$H_2N(CH_2)_2$	H	0.15	0.18	1.6
26	$H_2N(CH_2)_2$	$CH_2CO_2CH_3$	0.078	0.093	0.91
27	$H_2N(CH_2)_2$	$(CH_2)_2CO_2H$	0.11	0.15	1.0
28	$H_2N(CH_2)_2$	$(CH_2)_2CO_2CH_3$	0.12	0.11	0.89
29	$(CH_3)_2N(CH_2)_2$	$(CH_2)_2CO_2H$	2.5	4.2	45
30	$(CH_3)_2N(CH_2)_2$	$(CH_2)_2CO_2CH_3$	2.3	7.3	37

[a,b] See corresponding footnotes in Table 1.

attached to the six-membered heterocyclic ring. Modification of the substituent at the 3-position on the 2-oxopiperazine ring of **1d** was then explored, while the (*p*-amidinobenzoyl)glycyl moiety was used as the N-terminus to serve as an Arg-Gly fragment surrogate (Table 2). Replacement of the 3-acetic acid group with nonacidic functions such as esters (**9** and **14**), an amide (**11**), a benzyl group (**12**), and even a hydrogen atom (**10**) did not affect the activities of **1d** in the platelet aggregation or binding assay. Slight discrepancies between potency of physiological response and binding affinity were observed for compounds **10**, **13**, and **14**, which showed rather higher affinity for the GP IIb-IIIa receptor in comparison with the inhibitory effect on platelet aggregation. Among our compounds (**9**−**14**), 3-(methoxycarbonyl)methyl derivatives (**9**) showed the most potent activity in the human platelet aggregation assay ($IC_{50} = 0.03\ \mu M$). To confirm the requirement for chirality, we synthesized the enantiomeric compound (*R*)-**9**, which exhibited 33 times less binding affinity and 19 times less activity in the human platelet aggregation assay.

Finally, modification of the amidino moiety was examined (Table 3). Primary [*p*-(*ω*-aminoalkyl)benzoyl]-glycyl derivatives (**22**−**24**) were prepared to optimize the methylene length between the terminal amino group and the phenyl ring as a bioisostere of the *p*-amidinophenyl group. A *p*-(2-aminoethyl) substituent on the phenyl ring was optimum with regard to the methylene length. From the SARs of *p*-(2-aminoethyl)benzoyl derivatives (**23**, **25**−**28**), the (methoxycarbonyl)methyl moiety (**26**) proved to be the most preferable substituent at the 3-position on the 2-oxopiperazine ring as well as in the series of *p*-amidinobenzoyl derivatives (IC_{50} = 0.91 nM for binding affinity). However, N,N-dimethylation of the primary amine caused a ca. 40-fold decrease in binding affinity for the GP IIb-IIIa receptor. The *N*,*N*-dimethylguanidino derivative (**1i**) was 210-fold less potent than the corresponding guanidino derivative (**1d**). Thus, susceptibility of the N-terminal cationic site to removal of a proton-donating hydrogen along with a decrease in the basicity and an increase in steric hindrance on interacting with the GP IIb-IIIa receptor may indicate that the reinforced ionic mode of the ligand binding function to the GP IIb-IIIa receptor[27] is implicated in the binding interaction between the N-terminal cationic site of RGDX mimetics and the platelet GP IIb-IIIa receptor.

From the SARs, it has been suggested that the distance between Arg and Asp surrogates plays an important role in the GP IIb-IIIa antagonistic activity. We therefore analyzed this distance for RGDF, **1a**, **9**, and **18**. As shown in Figure 2, RGDF adopted a variety of conformations in which the distance was dispersed in the range of 3−15 Å and the relative energy was dispersed in a range of over 15 kcal/mol. In the case of **1a**, the distance was distributed in the range of 3−18 Å, but a tendency toward clustering in a longer-distance range than in RGDF was observed. Almost all conformations of **1a** were within 10 kcal/mol of the most stable conformer. The conformers generated were less diverse than those of RGDF, suggesting that the conformation of **1a** is more restricted than that of RGDF. In the case of **9**, the most potent antagonist we have obtained, the conformations were localized in a narrower range in terms of both the distance and relative energy. This indicates that **9** adopts a small number of favorable conformations. The conformation of **18** was also localized, but the distribution pattern was different from that of **9**, 6−13 Å as compared with 8−15 Å. Figure 3 shows the population of conformers plotted against the distance. RGDF had three peaks with similar height at 4, 7, and 11 Å. There was a single peak at 14 Å for **1a** and one at 11−13 Å for **9**. With these compounds, the peak positions were consistent with the region where the compounds adopted stable conformations. On the contrary, **18** showed a different profile from **1a** and **9** with peaks at 10 and 12 Å, which is inconsistent with the stable region of 6−8 Å. There have been some reports that analyzed the distance between the surrogates theoretically or experimentally.[33,42−44] Zablocki et al. reported that the interatomic distance between the central carbon atoms of the carboxylate and amidine moieties calculated by a theoretical method is greater than 10 Å, and especially in the global minimum conformation, the central carbon atoms of the key pharmacophores were separated by 14 Å in the (aminobenzamidino)succinyl series of fibrinogen receptor antagonists.[33] This value is not identical with but is very similar to our value of 11−13 Å. As other reports are concerned with the distances between C_α or C_β atoms of the Arg and Asp residues in cyclic peptides including RGD,[43,44] the results cannot be compared with our results directly. As mentioned before, Alig et al. also described non-peptidic antagonists with two carboxyl termini and found out that just one carboxyl group

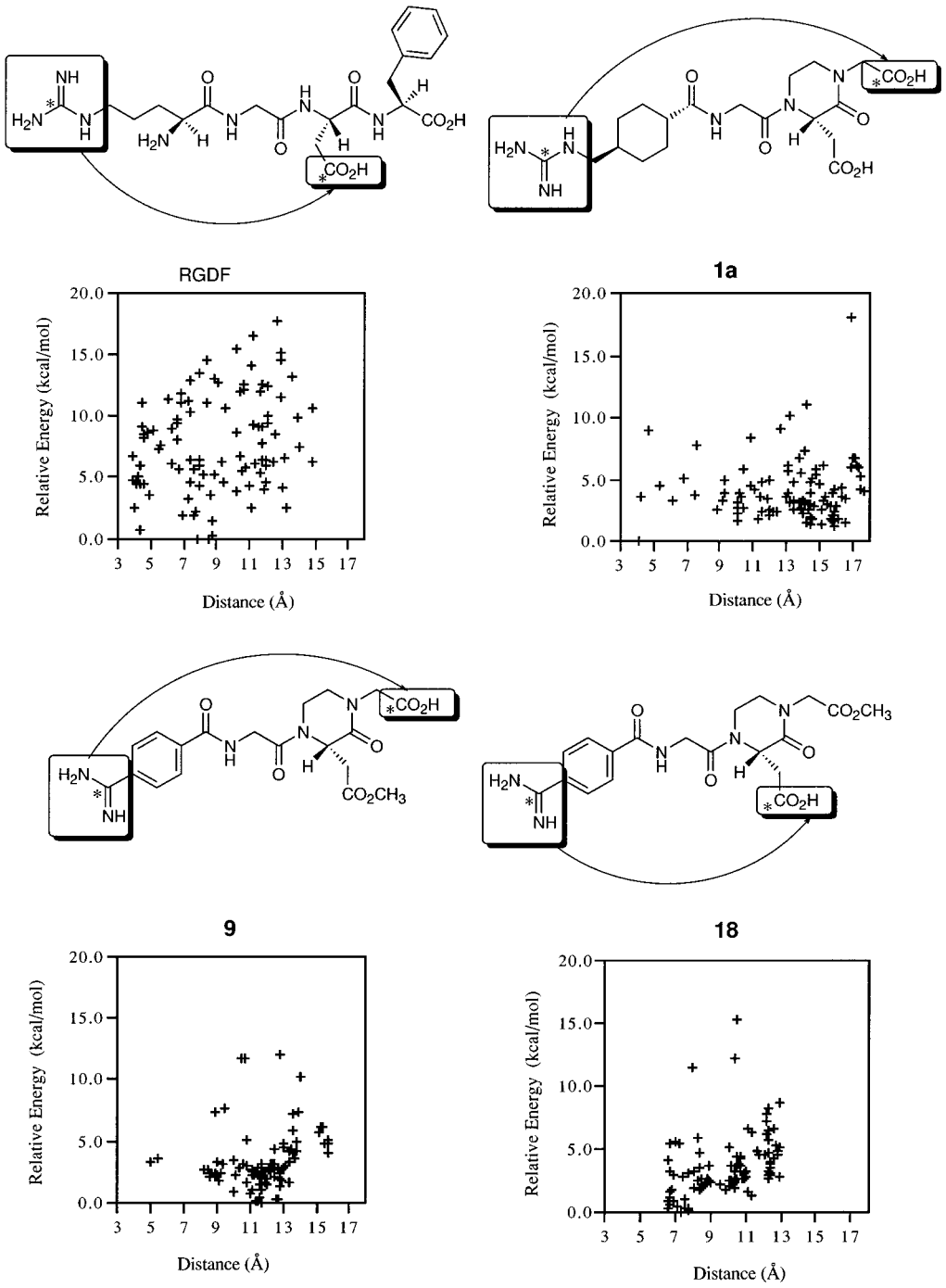


Figure 2. Relative energy of the model compounds plotted against the distance between the Arg and Asp surrogates.

was necessary for the activity.[23] We had an interest in that they reached the result similar to ours while the two approaches are very different from each other in modifying the C-terminus moiety. Comparison of our compounds with their (piperidinyloxy)acetic acid derivatives demonstrates that the distance distributions between the surrogates can be overlapped (data not shown). With this result, we speculate that their compounds may interact in a mode similar to that of **9**. In conclusion, the following can be said about the relationship between the distance and the activity: (1) RGDF, a linear peptide, is very flexible and can assume a variety of conformations. RGDF would have the lowest probability of adopting the active conformation, and this is consistent with the fact that it has the weakest activity of the four compounds described here. (2) In the most potent antagonist, **9**, the distance between the surrogates in the major conformations was within the range of 11−13 Å. This suggests that a distance of 11−13 Å is important for GP IIb-IIIa antagonistic activity. This assumption is supported by the results that weaker antagonists such as RGDF and **1a** have a significantly different peak distribution. (3) As for **18**, it was interesting to determine whether the carboxymethyl group at the 3-position could be substituted for that of **9** at the 1-position. As shown in Figure 3, **18** showed a distance profile different from that of **9**. This indicates that its carboxymethyl group cannot play the same role as in **9**, being consistent with its weak activity.

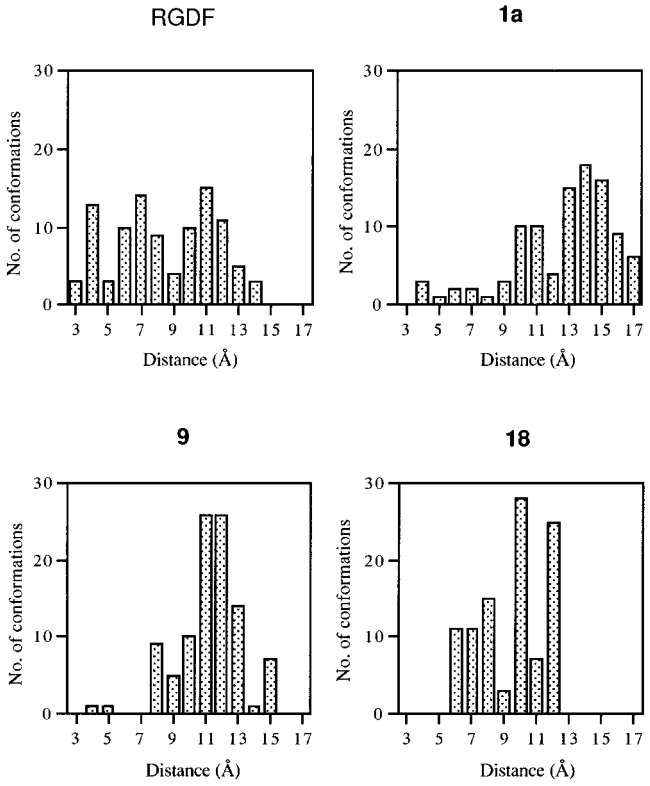


Figure 3. Conformers population of the model compounds plotted against the distance between the Arg and Asp surrogates.

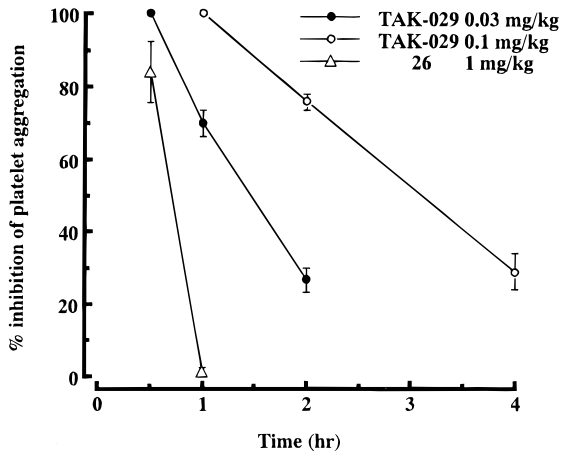


Figure 4. Time course of the inhibitory effect of TAK-029 and **26** on ex vivo platelet aggregation in guinea pigs. At various time intervals after intravenous administration of drugs, ex vivo platelet aggregation induced by ADP was examined. Each point represents the mean for 3−6 animals.

There were marked species differences in the antiplatelet effect of **9**; its relative potencies on ADP-induced platelet aggregation were as follows: human 1, guinea pig 1/1.4, monkey 1/1.5, dog 1/8, mouse 1/21, hamster 1/280, rabbit 1/1100, and rat <1/13 000.[45] Similar relative potencies were obtained for **26** (human 1 and guinea pig 1/1.2). Therefore, the in vivo antiplatelet activities of **9** and **26** were evaluated by determining the extent of inhibition of ex vivo aggregation after iv administration to guinea pigs (Figure 4). Intravenous administration of **9** resulted in dose-dependent inhibition of platelet aggregation at doses of 0.03 and 0.1 mg/kg, and the activity lasted for 4 h at 0.1 mg/kg. On the other hand, the antiplatelet activity of compound **26** (1 mg/kg, iv) disappeared rapidly, and no activity was observed 1 h after administration. The antiplatelet effect of **9** over the first 24 h after oral administration was examined at doses of 3 and 10 mg/kg. Compound **9** showed an antiplatelet effect for up to 8 h at a dose of 3 mg/kg. At 10 mg/kg, **9** caused more than 50% inhibition even 24 h after administration (Figure 5). Compound **9** (TAK-029) was selected as a candidate for further detailed pharmacological evaluation. Compound **9** inhibited the aggregation of human PRP induced by ADP, collagen, arachidonic acid, and platelet-activating factor (PAF) in vitro with IC_{50} values of 29−38 nM and showed potent in vivo antithrombic effects in guinea pigs without severely prolonging the bleeding time.[45] It was found that **9** is a specific antagonist for interaction at GP IIb-IIIa from a comparison of the inhibitory effects on the attachment of human umbilical vein endotherial cells (HUVEC) to immobilized peptide ligands containing the RGD sequence, fibrinogen, von Willebrand factor, fibronectin, and vitronectin.[46]

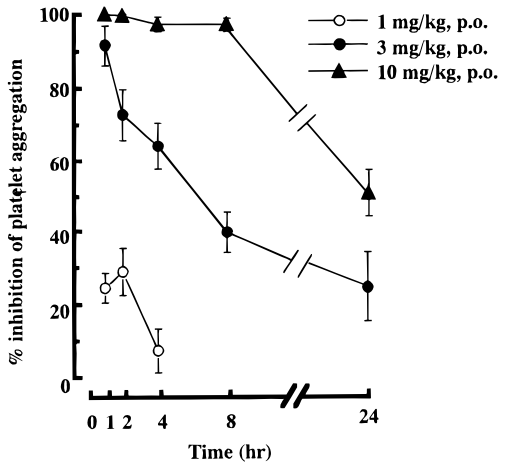


Figure 5. Time course of the inhibitory effect of TAK-029 on ex vivo platelet aggregation in guinea pigs. At various time intervals after oral administration of TAK-029, ex vivo platelet aggregation induced by ADP (final concentration: 0.5−1.0 μM) was examined. Each point represents the mean for 6−13 animals. From ref 45 with permission.

Conclusions

Starting with the lead tetrapeptide RGDF, (*S*)-4-[[*trans*-[4-(guanidinomethyl)cyclohexyl]carbonyl]glycyl]-2-oxopiperazine-1,3-diacetic acid, **1a**, was chosen as a potential lead. Optimization of **1a** has led to the potent and orally active GP IIb-IIIa antagonist **9**. The in vivo antiplatelet activity of the primary 4-(2-aminoethyl)-benzoyl derivative **26** disappeared rapidly, despite the compound's high in vitro potency comparable to that of **9**. The SAR study indicated the importance of the spatial arrangement of the indispensable cationic site and anionic site for receptor binding and of the *p*-amidino moiety susceptible to modifications on the antiplatelet effect. Further pharmacological studies on **9** are in progress, and the results will be reported in due course.[45−47]

Experimental Section

Melting points were determined on a Yanagimoto micromelting apparatus and are uncorrected. Infrared (IR) spectra were recorded with a JASCO IR-800 spectrophotom-

eter. ^{1}H NMR spectra were taken on a Varian XRD-200 spectrometer in $CDCl_3$ unless otherwise noted. Chemical shifts are given in ppm with tetramethylsilane (TMS) as an internal standard, and coupling constants (*J*) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, q = quartet, t = triplet, dd = double doublet, m = multiplet, b = broad peak. Mass spectra (MS) were recorded with a JEOL JMS-AX505W apparatus. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. The yields obtained are not optimized. The following abbreviations are used for reagents: EDC, 1-ethyl-3-[3-(*N*,*N*-dimethylamino)-propyl]carbodiimide hydrochloride; TFA, trifluoroacetic acid; DCC, dicyclohexylcarbodiimide; DEPC, diethylphosphoryl cyanide.

***tert*-Butyl *N*-(2,2-Dimethoxyethyl)glycine (3).** *tert*-Butyl chloroacetate (200 g, 1.45 mol) was added dropwise to a mixture of 2,2-dimethoxyethylamine (150 g, 1.43 mol), anhydrous K_2CO_3 (200 g, 1.45 mol), and DMF (1.4 L) over 1 h with stirring at room temperature. The solution was stirred for another 7 h, poured into ice−water, and extracted with EtOAc. The organic layer was washed with saturated NaCl solution, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual oil was distilled under reduced pressure to give **3** (162.7 g, 51.9%) as a colorless oil: bp 80−85 °C, 0.4 mmHg; IR (neat) cm^{-1} 2970, 2900, 1735, 1455, 1240, 1045, 860; ^{1}H NMR 1.49 (9H, s), 2.75 (2H, d, *J* = 5 Hz), 3.33 (2H, s), 3.39 (6H, s), 4.47 (1H, t, *J* = 5.4 Hz).

***tert*-Butyl *N*-[*N*-(Benzyloxycarbonyl)-*O*-*tert*-butyl-L-aspartyl]-*N*-(2-dimethoxyethyl)aminoacetate (4).** EDC (5.7 g, 30 mmol) was added to a mixture of **3** (6.8 g, 28 mmol), *N*-Cbz-Asp(*O*-*t*-Bu)-OH (8.1 g, 25 mmol), and CH_3CN (100 mL) with stirring at room temperature. After stirring for another 2 h, the reaction mixture was evaporated in vacuo. The resulting oil was dissolved in EtOAc, washed successively with 5% $KHSO_4$ and saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was subjected to column chromatography (SiO_2, 1:4 EtOAc/*n*-hexane) to give **4** (6.9 g, 50%) as a colorless oil: IR (neat) cm^{-1} 3300, 2975, 2930, 1720, 1655, 1525, 1450, 1360, 1160, 640, 745, 690; ^{1}H NMR 1.43 (9H, s), 1.45 (9H, s), 2.60−2.94 (2H, m), 3.40−4.05 (4H, m), 3.66 (6H, s), 4.58 (1H, t, *J* = 5.4 Hz), 5.12 (2H, s), 5.81 (1H, t, *J* = 3 Hz), 7.34 (5H, s).

Di-*tert*-butyl (*S*)-2-Oxopiperazine-1,3-diacetate (5a). A mixture of **4** (20 g, 36.2 mmol), *p*-toluenesulfonic acid (680 mg, 3.5 mmol), and toluene (750 mL) was stirred at 70 °C for 2 h. After cooling, the solution was washed with saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual oil was dissolved in methanol (500 mL), treated with 10% Pd/C (10 g), and hydrogenated under atmospheric pressure at room temperature for 12 h. The reaction mixture was filtered, and the filtrate was condensed in vacuo to give **5a** (12.7 g, 75%) as a colorless oil, which was converted into an oxalate and recrystallized from MeOH−EtOAc−hexane to afford colorless crystals: mp 158−159 °C; IR (neat) cm^{-1} : 3450, 3300, 2830, 2810, 1740, 1645, 1500, 1450, 1370, 1240, 1160, 990; ^{1}H NMR 1.48 (9H, s), 1.51 (9H, s), 2.75−3.95 (8H, m), 4.03 (1H, t, *J* = 4.5 Hz). Anal. ($C_{16}H_{28}N_2O_5 \cdot C_2H_2O_4$) C, H, N.

Di-*tert*-butyl (*S*)-4-[*N*-(Benzyloxycarbonyl)glycyl]-2-oxopiperazine-1,3-diacetate (6). A mixture of **5a** (3.7 g, 9 mmol), *N*-Cbz-Gly-OH (2.1 g, 9 mmol), EDC (2.5 g, 13 mmol), and CH_3CN (20 mL) was stirred at room temperature for 2 h and then concentrated in vacuo. The residual oil was dissolved in EtOAc, washed with aqueous 5% $KHSO_4$ and then saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was subjected to column chromatography (SiO_2, 1:1 EtOAc/*n*-hexane) to give **6** (3.7 g, 79%) as a colorless oil: IR (neat) cm^{-1} : 3420, 2975, 2900, 1720, 1650, 1495, 1450, 1360, 1240, 1205, 1155, 1040; ^{1}H NMR 1.42 (9H, s), 1.44 (9H, s), 2.50−3.68 (4H, m), 3.68−3.97 (4H, m), 4.85 (1H, t, *J* = 5.2 Hz), 5.13 (2H, s), 5.66 (1H, b s), 7.35 (5H, s) Anal. ($C_{26}H_{37}N_3O_8$) Calcd: 519.2581. Found: 519.2584..

(*S*)-4-[[*trans*-[4-(Guanidinomethyl)cyclohexyl]carbonyl]-glycyl-2-oxopiperazine-1,3-diacetic Acid (1a). A mixture of *trans*-4-(guanidinomethyl)cyclohexanecarboxylic acid (300 mg, 1.5 mmol), *N*-hydroxysuccinimide (174 mg, 1.5 mmol), DCC (320 mg, 2 mmol), and DMF (5 mL) was stirred at room temperature for 1 h and concentrated in vacuo. The residue was dissolved in 1,4-dioxane (1 mL) (solution A). A solution of **6** (520 mg, 1.0 mmol) in MeOH (10 mL) was treated with 10% Pd/C (50 mg) and hydrogenated under atmospheric pressure at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. To a mixture of the residual oil and $NaHCO_3$ (420 mg, 5 mmol) in H_2O (5 mL) and 1,4-dioxane (5 mL) was added solution A dropwise at room temperature. The resulting mixture was stirred for 2 h and then concentrated in vacuo. The residue was subjected to column chromatography (ODS, 1:9 CH_3CN/H_2O) to give 1a (260 mg, 43%) as a colorless powder: $[\alpha]^{20}_D$ +65.8° (*c* = 0.25, MeOH); IR (KBr) cm^{-1} 3390, 2930, 1730, 1645, 1550, 1445, 1210, 970; ^{1}H NMR (DMSO-d_6) 0.82−1.08 (2H, m), 1.20−1.52 (3H, m), 1.68−1.88 (3H, m), 2.08−2.26 (1H, m), 2.69 (2H, d, *J* = 5.4 Hz), 2.97 (2H, dd, *J* = 5.4, 5.6 Hz), 3.58−3.71 (1H, m), 3.81−4.35 (4H, m), 4.89 (1H, t, *J* = 5.4 Hz), 7.64−8.06 (4H, m). Anal. ($C_{19}H_{30}N_6O_7 \cdot HCl$) C, H, N.

***tert*-Butyl (*S*)-3-[*N*-(Benzyloxycarbonyl)amino]-4-[*N*-(2,2-diethoxyethyl)-*N*-phenethylamino]-4-oxobutylate (7).** EDC (2.4 g, 12.5 mmol) was added to a solution of (2,2-diethoxyethyl)phenethylamine (2.96 g, 12.5 mmol) and *N*-Cbz-Asp(*O*-*t*-Bu)-OH (3.42 g, 10 mmol) in CH_3CN (50 mL). The mixture was stirred at room temperature for 1.5 h and evaporated in vacuo. The residual oil was dissolved in EtOAc, washed with 5% aqueous $KHSO_4$ and then saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography (SiO_2, 1:1 *n*-hexane/EtOAc) to give **7** (4.6 g, 78%) as a colorless oil: $[\alpha]^{20}_D$ −8.7° (*c* = 0.63, MeOH); IR (neat) cm^{-1} 2975, 1720, 1640, 1530, 1450, 1365, 1290, 970; ^{1}H NMR 1.16 (3H, t, *J* = 7 Hz), 1.19 (3H, t, *J* = 7 Hz), 1.26 (3/2H, t, *J* = 7.0 Hz), 1.42 (9H, s), 2.05 (3/2H, s), 2.30−3.05 (4H, m), 3.20−3.85 (8H, m), 4.12 (2/2H, q, *J* = 7.0 Hz), 4.61 (1H, t, *J* = 5.3 Hz), 5.01 (1H, m), 5.10 (2H, s), 5.50 (1H, t, *J* = 8.8 Hz), 7.15−7.40 (10H, m). Anal. ($C_{30}H_{41}N_2O_7 \cdot {}^1/_2EtOAc$) C, H, N.

***tert*-Butyl (*S*)-1-Phenethyl-2-oxopiperazine-3-acetate (8).** A solution of **7** (3.93 g, 7.25 mmol) and *p*-toluenesulfonic acid monohydrate (0.14 g) in toluene (200 mL) was stirred at 80 °C for 4 h. After cooling, the mixture was washed with saturated aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography (3:1 hexane/EtOAc) to give a tetrahydropyrazine intermediate as a colorless oil (1.5 g, 46%): IR (neat) cm^{-1} 1715, 1680, 1400, 1310, 1145, 1115, 695; ^{1}H NMR 1.14 (9H, s), 2.55 (2H, d, *J* = 6.4 Hz), 2.88 (2H, m), 3.72 (2H, t, *J* = 6.4 Hz), 5.00−5.55 (2H, m), 5.19 (2H, s), 6.10−6.35 (1H, m), 7.15−7.35 (5H, m), 7.37 (5H, s).

The oil thus obtained (1.5 g, 3.2 mmol) was dissolved in MeOH (10 mL), treated with 10% Pd/C (1.5 g), and then hydrogenated under atmospheric pressure at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was subjected to column chromatography (SiO_2, 9:1 EtOAc/MeOH) to give **8** (0.76 g, 69%) as a colorless oil: $[\alpha]^{20}_D$ −57.7° (*c* = 0.583, MeOH); IR (neat) cm^{-1} 2975, 1720, 1640, 1490, 1450, 1360, 1150; ^{1}H NMR 1.45 (9H, s), 2.50−3.74 (12H, m), 7.15−7.37 (5H, m). Anal. ($C_{18}H_{26}N_2O_3 \cdot {}^1/_2H_2O$) C, H, N.

(*S*)-4-[[*trans*-[4-(Guanidinomethyl)cyclohexyl]carbonyl]-glycyl]-3-phenethyl-2-oxopiperazine-1-acetic Acid (2). A mixture of *trans*-4-(guanidinomethyl)cyclohexanecarboxylic acid (600 mg, 3 mmol), *N*-hydroxysuccinimide (348 mg, 3 mmol), and DCC (830 mg, 4 mmol) in DMF (8 mL) was stirred at room temperature for 1 h. The solution was filtered to give a solution of an activated ester (solution A). EDC (0.48 g, 2.5 mmol) was added to a solution of **8** (0.69 g, 2.1 mmol) and *N*-Cbz-Gly-OH (0.44 g, 2.1 mmol) in CH_3CN (15 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was evaporated in vacuo. The residue was dissolved in EtOAc, washed with 5% aqueous $KHSO_4$ and then saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated in

vacuo. A solution of the residual oil in MeOH (20 mL) was treated with 10% Pd/C (500 mg) and hydrogenated under atmospheric pressure at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. To a mixture of the residual oil and $NaHCO_3$ (840 mg, 10 mmol) in H_2O (5 mL) and dioxane (5 mL) was added solution A dropwise followed by stirring at room temperature for 2 h. The mixture was evaporated in vacuo, and the residue was treated with CH_2Cl_2 (10 mL)−TFA (10 mL) at room temperature for 1 h. The mixture was evaporated in vacuo, and the residue was then dissolved in 0.5 N HCl and subjected to column chromatography (ODS, 1:4 CH_3CN/H_2O) to give **2** (520 mg, 45%) as a colorless powder: $[\alpha]^{20}_D$ +87.4° (c = 0.57, MeOH); IR (KBr) cm^{-1} 3350, 2940, 1740, 1650, 1540, 1495, 1455, 1360, 1280, 1210, 1165, 750, 700; ^1H NMR (DMSO-d_6) 0.85−1.05 (2H, m), 1.20−1.50 (3H, m), 1.69−1.87 (3H, m), 2.10−2.27 (1H, m), 2.69−2.83 (3H, m), 2.97 (2H, t, J = 5.8 Hz), 3.05−4.40 (11H, m), 4.77 (1H, t, J = 5.6 Hz), 7.20−7.34 (5H, m). Anal. ($C_{25}H_{36}N_6O_5 \cdot HCl \cdot {}^1/_2H_2O$) C, H, N.

Compounds **1b**−**i** were synthesized by the same procedure as that described for the synthesis of **2**.

(*S*)-4-[(4-Guanidinobutyl)glycyl]-2-oxopiperazine-1,3-diacetic acid (1b): amorphous powder (yield 34%); $[\alpha]^{20}_D$ +83.0° (c = 0.28, MeOH). Anal. ($C_{17}H_{26}O_7 \cdot HCl \cdot H_2O$) C, H, N.

(*S*)-4-[(*N*-Amidinoisonipecotyl)glycyl]-2-oxopiperazine-1,3-diacetic acid (1c): amorphous powder (yield 59%); $[\alpha]^{20}_D$ +57.5° (c = 0.50, MeOH). Anal. ($C_{17}H_{26}N_6O_7 \cdot H_2O$) C, H, N.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-2-oxopiperazine-1,3-diacetic acid (1d): recrystallization from H_2O−methanol gave colorless crystals (yield 53%); mp 254−258 °C; $[\alpha]^{20}_D$ +90.1° (c = 1.0, H_2O). Anal. ($C_{18}H_{21}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

(*S*)-4-[(4-Guanidinobenzoyl)glycyl]-2-oxopiperazine-1,3-diacetic acid (1e): amorphous powder (yield 64%); $[\alpha]^{20}_D$ +78.6° (c = 0.30, MeOH). Anal. ($C_{18}H_{22}N_6O_7 \cdot HCl$) C, H, N.

(*S*)-4-[[4-(Guanidinomethyl)benzoyl]glycyl]-2-oxopiperazine-1,3-diacetic acid (1f): amorphous powder (yield 46%); $[\alpha]^{20}_D$ +58.5° (c = 0.18, MeOH). Anal. ($C_{19}H_{24}N_6O_7 \cdot HCl$) C, H, N.

(*S*)-4-[(3-Amidinobenzoyl)glycyl]-2-oxopiperazine-1,3-diacetic acid (1g): amorphous powder (yield 29%); $[\alpha]^{23}_D$ +92.6° (c = 0.30, H_2O). Anal. ($C_{19}H_{23}N_5O_7 \cdot HCl \cdot H_2O$) C, H, N.

(*S*)-4-[(3-Amidinobenzoyl)glycyl]-2-oxopiperazine-1,3-diacetic acid (1h): amorphous powder (yield 71%); $[\alpha]^{20}_D$ +73.7° (c = 0.15, H_2O). Anal. ($C_{18}H_{21}N_5O_7 \cdot HCl$) C, H, N.

(*S*)-4-[[4-*N*,*N*-Dimethylguanidino)benzoyl]glycyl]-2-oxopiperazine-1,3-diacetic acid (1i): amorphous powder (yield 58%); $[\alpha]^{23}_D$ +95.6° (c = 0.15, H_2O). Anal. ($C_{20}H_{26}N_6O_7 \cdot HCl \cdot H_2O$) C, H, N.

***tert*-Butyl (*S*)-3-[(Methoxycarbonyl)methyl]-2-oxopiperazine-1-acetate (5b).** A mixture of **3** (7.6 g, 34.7 mmol), *N*-Cbz-Asp(*O*-*t*-Bu)-OH (8.8 g, 31.6 mmol), and EDC (7.87 g, 41 mmol) in CH_2Cl_2 (100 mL) was stirred at room temperature for 2 h. The mixture was concentrated in vacuo. The residual oil was dissolved in EtOAc, washed with aqueous 5% $KHSO_4$ and then saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue and *p*-toluenesulfonic acid (665 mg, 3.5 mmol) were dissolved in toluene (350 mL), and the mixture was stirred at 70 °C for 1.5 h. After cooling, the reaction mixture was washed with saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual oil was dissolved in MeOH (500 mL), treated with 10% Pd/C (5 g), and hydrogenated under atmospheric pressure at room temperature for 20 h. The reaction mixture was filtered, and the filtrate was evaporated in vacuo to give **5b** (7.9 g, 72%) as a colorless oil, which was converted into an oxalate and recrystallized from MeOH−EtOAc−hexane to give colorless crystals: mp 146−147 °C; $[\alpha]^{20}_D$ −26.0° (c = 0.60, MeOH); IR (KBr) cm^{-1} 3450, 2975, 1730, 1660, 1490, 1445, 1365, 1240, 1150; ^1H NMR (DMSO-d_6) 1.42 (9H, s), 2.71 (1H, dd, J = 16, 6.6 Hz), 2.84 (1H, dd, J = 16, 5.6 Hz), 3.09−3.91 (7H, m), 3.63 (3H, s). Anal. ($C_{13}H_{22}N_2O_5 \cdot C_2H_2O_4$) C, H, N.

Piperazine derivatives **5c**−**g** were prepared by the same procedure as that described for the synthesis of **5b**.

***tert*-Butyl 2-oxopiperazine-1-acetate (5c):** conversion into a hydrochloride and recrystallization from MeOH−Et_2O gave colorless crystals (yield 65%); mp 156 °C. Anal. ($C_{10}H_{18}N_2O_3 \cdot HCl$) C, H, N.

***tert*-Butyl (*S*)-3-[[*N*-(4,4′-dimethoxybenzhydryl)carbamoyl]methyl]-2-oxopiperazine-1-acetate (5d):** conversion into a hydrochloride and recrystallization from EtOH−Et_2O gave colorless crystals (yield 60%); mp 120−123 °C; $[\alpha]^{20}_D$ −93.2° (c = 0.91, MeOH). Anal. ($C_{27}H_{35}N_3O_6 \cdot HCl$) C, H, N.

***tert*-Butyl (*S*)-3-benzyl-2-oxopiperazine-1-acetate (5e):** conversion into a hydrochloride and recrystallization from MeOH−Et_2O gave colorless crystals (yield 83%); mp 206−208 °C; $[\alpha]^{23}_D$ −93.2° (c = 0.95, MeOH). Anal. ($C_{17}H_{24}N_2O_3 \cdot HCl$) C, H, N.

***tert*-Butyl (*S*)-3-[2-(*tert*-butoxycarbonyl)ethyl]-2-oxopiperazine-1-acetate (5f):** conversion into an oxalate and recrystallization from MeOH−EtOAc−hexane gave colorless crystals (yield 70%); mp 153−155 °C; $[\alpha]^{20}_D$ −18.7° (c = 1.0, MeOH). Anal. ($C_{17}H_{30}N_2O_5 \cdot C_2H_2O_4$) C, H, N.

***tert*-Butyl (*S*)-3-[2-(methoxycarbonyl)ethyl]-2-oxopiperazine-1-acetate (5g):** conversion into an oxalate and recrystallization from MeOH−Et_2O gave colorless crystals (yield 65%); mp 139−141 °C; $[\alpha]^{20}_D$ −21° (c = 0.75, MeOH). Anal. ($C_{14}H_{24}N_2O_5 \cdot C_2H_2O_4$) C, H, N.

***tert*-Butyl (*R*)-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetate ((*R*)-5b):** conversion into an oxalate and recrystallization from MeOH−Et_2O gave colorless crystals: mp 140−141 °C; $[\alpha]^{20}_D$ +25.4° (c = 0.60, MeOH). Anal. ($C_{13}H_{22}N_2O_5 \cdot C_2H_2O_4$) C, H, N.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic Acid (9). A mixture of **5b** (39 g, 136 mmol), *N*-Cbz-Gly-OH (28.4 g, 136 mmol), EDC (27.8 g, 146 mmol), and DMF (200 mL) was stirred at room temperature for 2 h and evaporated in vacuo. The residual oil was dissolved in EtOAc, and the solution was washed with aqueous 5% $KHSO_4$ and saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated in vacuo. A solution of the residual oil in MeOH (15 mL) was treated with 10% Pd/C (300 mg) and hydrogenated under atmospheric pressure at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was evaporated in vacuo. To a mixture of the residual oil and $NaHCO_3$ (1.05 g, 12.5 mmol) in H_2O (20 mL) and 1,4-dioxane (20 mL) was added 4-amidinobenzoyl chloride hydrochloride (1.67 g, 7.64 mmol) portionwise over 10 min with stirring at room temperature. After stirring for 2 h, the reaction mixture was neutralized with 1 N HCl and concentrated in vacuo. To a suspension of the residual powder in CH_2Cl_2 (20 mL) was added TFA (20 mL) at 0 °C, and the resulting mixture was gradually warmed to room temperature, stirred for 1 h, and evaporated in vacuo. The crude product was purified by column chromatography (CHP-20P, 5:95 CH_3CN/H_2O) to give **9** (1.9 g, 70%). Recrystallization from H_2O−EtOH gave colorless crystals: mp 278 °C dec; $[\alpha]^{20}_D$ +82.0° (c = 1.0, H_2O); IR (KBr) cm^{-1} 3350, 3045, 1735, 1690, 1630, 1590, 1450, 1380, 1340; ^1H NMR (D_2O) 2.84−2.89 (2H, m), 3.05−3.44 (2H, m), 3.60 (3H, s), 3.98−4.96 (6H, m), 5.08 (1H, t, J = 6.2 Hz), 7.20 (2H, d, J = 8.4 Hz), 7.80 (2H, d, J = 8.4 Hz). Anal. ($C_{19}H_{23}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

Compounds **10**−**14** and (*R*)-**9** were prepared by the same procedure as that described for the synthesis of **9**.

4-[(4-Amidinobenzoyl)glycyl]-2-oxopiperazine-1-acetic acid (10): recrystallization from H_2O gave colorless prisms (yield 69%); mp 255 °C dec. Anal. ($C_{16}H_{19}N_5O_5 \cdot {}^3/_2H_2O$) C, H, N.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-3-(carboxymethyl)-2-oxopiperazine-1-acetic acid (11): recrystallization from 50% MeOH gave colorless crystals (yield 57%); mp 226−228 °C; $[\alpha]^{20}_D$ +70.3° (c = 0.4, H_2O). Anal. ($C_{18}H_{22}N_6O_6 \cdot 2H_2O$) C, H, N.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-3-benzyl-2-oxopiperazine-1-acetic acid (12): conversion into a hydrochloride and recrystallization from H_2O gave colorless crystals (yield 61%);

mp 290−296 °C; $[\alpha]^{23}_D$ +96.9° (*c* = 0.1, 1 N HCl). Anal. ($C_{23}H_{25}N_5O_3$·HCl) C, H, N.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-3-(2-carboxyethyl)-2-oxopiperazine-1-acetic acid (13): recrystallization from H_2O−MeOH gave colorless prisms (yield 80%); mp 235−241 °C; $[\alpha]^{23}_D$ +66.6° (*c* = 0.3, H_2O). Anal. ($C_{19}H_{23}N_5O_7$·$^1/_2H_2O$) C, H, N.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-3-[2-(methoxycarbonyl)ethyl]-2-oxopiperazine-1-acetic acid (14): amorphous powder (72%); $[\alpha]^{20}_D$ +57.8° (*c* = 0.5, H_2O). Anal. ($C_{20}H_{25}N_5O_7$·H_2O) C, H, N.

(*R*)-4-[(4-Amidinobenzoyl)glycyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid ((*R*)-9): recrystallization from H_2O−MeOH gave colorless crystals; mp 283 °C dec; $[\alpha]^{20}_D$ −81.0° (*c* = 1.0, H_2O). Anal. ($C_{19}H_{23}N_5O_7$·$^1/_2H_2O$) C, H, N.

The optical purities of **9** and (*R*)-**9** were elucidated by HPLC analysis on a chiral column to be 100% and 98.3%, respectively; column, SUMICHIRAL OA-5000 (150 × 4.6 mm); temperature, room temperature; eluant, a methanol solution containing 4% 3 mM aqueous $CuSO_4$; flow rate, 1.0 mL/min; detector, 237 nm; retention time, 33.07 min for **9** and 29.28 min for (*R*)-**9**.

***tert*-Butyl 3-[*N*-(2-Diethoxyethyl)amino]propionate (15a).** A solution of 2,2-diethoxyethylamine (2.7 g, 20.3 mmol) and *tert*-butyl acrylate (2.6 g, 20.3 mmol) in toluene (50 mL) was heated under reflux for 3 h and evaporated in vacuo. The residue was subjected to column chromatography (SiO_2, 1:1 EtOAc/*n*-hexane) to give **15a** (2.4 g, 45%) as a colorless oil: IR (neat) cm^{-1} 2974, 1728, 1367, 1157, 1129, 1062; ^1H NMR 1.22 (6H, t, *J* = 7.4 Hz), 1.45 (9H, s), 1.57 (1H, b s), 2.42 (2H, t, *J* = 6.6 Hz), 2.74 (2H, d, *J* = 5.6 Hz), 2.87 (2H, t, *J* = 6.6 Hz), 3.45−3.80 (4H, m), 4.59 (1H, t, *J* = 5.6 Hz).

Methyl *N*-(2-Dimethoxyethyl)glycinate (15b). Methyl chloroacetate (15 g, 0.14 mol) was added to a solution of 2,2-dimethoxyethylamine (15 g, 0.14 mol) and K_2CO_3 (20 g, 0.15 mol) in DMF (150 mL), and the resulting mixture was stirred at room temperature for 7 h. The reaction mixture was poured into ice−water and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was subjected to column chromatography (SiO_2, 1:1 EtOAc/*n*-hexane) to give **15b** (15.2 g, 51.9%) as a colorless oil: IR (neat) cm^{-1} 2975, 2900, 1735, 1460, 1240, 1050, 855; ^1H NMR 2.73 (2H, d, *J* = 5.0 Hz), 3.39 (6H, s), 3.45 (2H, s), 3.73 (3H, s), 4.46 (1H, t, *J* = 5.0 Hz).

***N*-(2-Diethoxyethyl)glycinamide (15c).** Chloroacetamide (6.5 g, 0.14 mmol) was added to a solution of 2,2-diethoxyethylamine (7.5 g, 70 mmol) and K_2CO_3 (10 g, 75 mmol) in DMF (50 mL), and the resulting solution was stirred at room temperature for 5 h. The reaction solution was poured into ice−water and extracted with EtOAc. The extract was washed with saturated NaCl, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residue was subjected to column chromatography (SiO_2, EtOAc) to give **15c** (7.0 g, 54%) as a colorless oil: ^1H NMR 1.22 (6H, t, *J* = 7 Hz), 2.72 (2H, d, *J* = 5.2 Hz), 3.30 (2H, s), 3.45−3.78 (4H, m), 4.54 (1H, t, *J* = 5.2 Hz), 5.75 (2H, b s).

***tert*-Butyl *N*-(2-Diethoxyethyl)-L-alaninate (15d).** A suspension of H-Ala-*O*-*t*-Bu hydrochloride (5 g, 33.4 mmol), K_2CO_3 (5.52 g, 40 mmol), sodium iodide (0.3 g), and 2-bromo-1,1-diethoxyethane (7.9 g, 40 mmol) in DMF was heated at 100 °C for 24 h. After cooling, the mixture was poured into ice−water and extracted with *n*-hexane. The extract was washed with saturated NaCl, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was subjected to column chromatography (SiO_2, 1:1 EtOAc/*n*-hexane) to give **15d** (48%) as a colorless oil: $[\alpha]^{20}_D$ −11.0° (*c* = 0.5, MeOH); ^1H NMR 1.17−1.46 (9H, m), 2.60 (1H, dd, *J* = 11.6, 4.8 Hz), 2.76 (1H, dd, *J* = 11.6, 6.4 Hz), 3.25 (1H, dd, *J* = 14, 7.0 Hz), 3.56−3.80 (2H, m), 4.60 (1H, t, *J* = 4.8 Hz).

***tert*-Butyl *N*-(2-Diethoxyethyl)-L-phenylalaninate (15e).** A suspension of H-Phe-*O*-*t*-Bu hydrochloride (2.5 g, 10 mmol), K_2CO_3 (2.76 g, 20 mmol), sodium iodide (0.1 g), and 2-bromo-1,1-diethoxyethane (2.2 g, 11 mmol) in DMF was heated at 100 °C for 24 h. After cooling, the mixture was poured into ice−water and extracted with *n*-hexane. The extract was then dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residual oil was subjected to column chromatography (Si_2O, 1:1 EtOAc/*n*-hexane) to give **15e** (2.2 g, 65.3%) as a colorless oil: $[\alpha]^{20}_D$ +10.0° (*c* = 1.045, MeOH); ^1H NMR 1.16 (3H, t, *J* = 6.0 Hz), 1.20 (3H, t, *J* = 6.0 Hz), 2.59 (1H, dd, *J* = 11.8, 5.0 Hz), 2.75 (1H, dd, *J* = 11.8, 6.2 Hz), 2.89 (2H, dd, *J* = 6.6, 3.4 Hz), 3.32−3.70 (5H, m), 7.13−7.38 (5H, m).

***tert*-Butyl (*S*)-3-[(Methoxycarbonyl)methyl]-2-oxopiperazine-1-propionate (16a).** EDC (5.75 g, 30 mmol) was added to a mixture of **15a** (7.0 g, 27 mmol), *N*-Cbz-Asp(*O*-*t*-Bu)-OH (9.2 g, 27 mmol), and CH_2Cl_2 (100 mL), and the resulting solution was stirred at room temperature for 5 h. The solution was evaporated in vacuo, and the residue was dissolved in EtOAc, washed with aqueous 5% $KHSO_4$, dried over anhydrous $MgSO_4$, and concentrated in vacuo. A mixture of the residual oil and *p*-toluenesulfonic acid (500 mg, 2.6 mmol) in toluene (500 mL) was heated at 70 °C for 1 h. After cooling, the solution was washed with saturated $NaHCO_3$, dried over anhydrous $MgSO_4$, and evaporated in vacuo to give a pale-yellow oil. The residual oil was dissolved in MeOH (200 mL), treated with 10% Pd/C, and hydrogenated under atmospheric pressure at room temperature for 2 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was subjected to column chromatography (SiO2, 1:4 → 1:2 EtOAc/*n*-hexane) to give **16a** (7.2 g, 78%) as a colorless oil: IR (neat) cm^{-1} 2932, 2742, 1726, 1646, 1366, 1251, 1153; ^1H NMR 1.40 (9H, s), 2.27 (1H, b s), 2.53 (2H, t, *J* = 7.0 Hz), 2.64 (1H, dd, *J* = 17.2, 8.6 Hz), 2.91−3.32 (4H, m), 2.96 (1H, dd, *J* = 17.2, 3.2 Hz), 3.58 (2H, t, *J* = 7.0 Hz), 3.66 (1H, dd, *J* = 8.6, 3.2 Hz).

2-Oxopiperazine derivatives **16b**−**e** were synthesized by the same procedure as that described for the synthesis of **16a**.

Methyl (*S*)-3-[(*tert*-butoxycarbonyl)methyl]-2-oxopiperazine-1-acetate (16b): conversion into an oxalate and recrystallization from MeOH−Et_2O gave colorless crystals (yield 81%); mp 140−141 °C; $[\alpha]^{20}_D$ −29.0° (*c* = 0.8, MeOH). Anal. ($C_{13}H_{22}N_2O_5$·$C_2H_2O_4$) C, H, N.

***tert*-Butyl (*S*)-1-(carbamoylmethyl)-2-oxopiperazine-3-acetate (16c):** conversion into an oxalate and recrystallization from MeOH−EtOAc gave colorless crystals (yield 79%); mp 160−163 °C dec; $[\alpha]^{20}_D$ −16.6° (*c* = 0.53, MeOH). Anal. ($C_{12}H_{21}N_3O_4$·$C_2H_2O_4$) C, H, N.

***tert*-Butyl (*S*)-2-[3-[(*tert*-butoxycarbonyl)methyl]-2-oxopiperazin-1-yl]propionate (16d):** conversion into an oxalate and recrystallization from MeOH−EtOAc-*n*-hexane gave colorless crystals (yield 50%); mp 175−177 °C dec; $[\alpha]^{20}_D$ −34.4° (*c* = 0.45, MeOH). Anal. ($C_{17}H_{30}N_2O_5$·$C_2H_2O_4$) C, H, N.

***tert*-Butyl (*S*)-2-[3-[(*tert*-butoxycarbonyl)methyl]-2-oxopiperazin-1-yl]phenylacetate (16e):** conversion into an oxalate and recrystallization from MeOH−EtOAc gave colorless crystals (yield 71%); mp 180−181 °C dec; $[\alpha]^{20}_D$ −68.3° (*c* = 0.21, MeOH). Anal. ($C_{23}H_{28}N_2O_5$·$C_2H_2O_4$) C, H, N.

(*S*)-4-(4-Amidinobenzoyl)-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-propionic Acid (17). A mixture of **16a** (2.0 g, 5.85 mmol), *N*-Cbz-Gly-OH (1.2 g, 5.85 mmol), EDC (1.4 g, 7.0 mmol), and CH_2Cl_2 (20 mL) was stirred at room temperature for 1 h. The mixture was evaporated in vacuo, and the residual oil was dissolved in EtOAc, washed with aqueous 5% $KHSO_4$, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in MeOH (30 mL), treated with 10% Pd/C (100 mg), and hydrogenated under atmospheric pressure at room temperature for 30 min. The reaction mixture was filtered, and the filtrate was evaporated in vacuo. To a mixture of the residue and $NaHCO_3$ (420 mg, 5 mmol) in H_2O (5 mL) and 1,4-dioxane (5 mL) was added 4-amidinobenzoyl chloride hydrochloride (283 mg, 1.3 mmol) portionwise with stirring, and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in TFA (20 mL) followed by stirring for 1 h. The reaction mixture was concentrated in vacuo. After addition of 1 N HCl (5 mL), the residue was subjected to

column chromatography (CHP-20P, 5:95 CH_3CN/H_2O) to give **17** (110 mg, 23%) as a colorless powder: $[\alpha]^{20}_D$ +83.5° (*c* = 1.0, H_2O); IR (KBr) cm^{-1} 3390, 1639, 1549, 1448, 1302, 1201; ^1H NMR (D_2O) 2.44−2.54 (2H, m), 2.75−2.86 (2H, m), 3.20−5.12 (10H, m), 7.90 (2H, d, *J* = 8.0 Hz), 7.99 (2H, d, *J* = 8.0 Hz). Anal. ($C_{19}H_{23}N_5O_7$·HCl) C, H, N.

Compounds **18**−**21** were prepared by the same procedure as that described for the synthesis of **17**.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-1-[(methoxycarbonyl)methyl]-2-oxopiperazine-3-acetic acid (18): amorphous powder (yield 41%); $[\alpha]^{20}_D$ +91.4° (*c* = 0.2, H_2O). Anal. ($C_{19}H_{23}N_5O_7$·HCl·$^1/_2H_2O$) C, H, N.

(*S*)-4-[(4-Amidinobenzoyl)glycyl]-1-(carbamoylmethyl)-2-oxopiperazine-3-acetic acid (19): amorphous powder (yield 58%); $[\alpha]^{20}_D$ +115.9° (*c* = 1.0, H_2O). Anal. ($C_{18}H_{21}N_6O_6$-Na·2.3H_2O) C, H, N.

(*S*)-2-[4-[(4-Amidinobenzoyl)glycyl]-3-(carboxymethyl)-2-oxopiperazin-1-yl]propionic acid (20): amorphous powder (yield 64%); $[\alpha]^{20}_D$ +52.7° (*c* = 1.0, MeOH). Anal. ($C_{19}H_{23}N_5O_7$·HCl·H_2O) C, H, N.

(*S*)-2-[4-[(4-Amidinobenzoyl)glycyl]-3-(carboxymethyl)-2-oxopiperazin-1-yl]phenylacetic acid (21): amorphous powder (yield 39%); $[\alpha]^{20}_D$ −30.9° (*c* = 0.3, H_2O). Anal. ($C_{25}H_{27}N_5O_7$·HCl) C, H, N.

(*S*)-4-[(4-(Aminomethyl)benzoyl]glycyl]-2-oxopiperazine-1,3-diacetic Acid (22). A solution of **5a** (410 mg, 1 mmol), *N*-Cbz-Gly-OH (210 mg, 1 mmol), and EDC (285 mg, 1.5 mmol) in CH_3CN (3 mL) was stirred at room temperature for 2 h and then evaporated in vacuo. The residue was dissolved in EtOAc, washed with aqueous 5% $KHSO_4$, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was dissolved in MeOH (10 mL), treated with 10% Pd/C (50 mg), and hydrogenated under atmospheric pressure at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was concentrated to dryness in vacuo. To a solution of the residual oil in DMF (5 mL) were added 4-[(*N*-carbobenzoxyamino)methyl]benzoic acid (285 mg, 1 mmol), DEPC (245 mg, 1.5 mmol), and triethylamine (0.3 mL) at room temperature followed by stirring for 1 h. The reaction mixture was diluted with EtOAc, and the resulting solution was washed with 5% aqueous $KHSO_4$, dried over anhydrous $MgSO_4$, and evaporated in vacuo. The residual oil was dissolved in TFA (3 mL), and the solution was stirred for 1 h at room temperature and then evaporated in vacuo. The residue was dissolved in MeOH (10 mL), treated with 10% Pd/C (100 mg), and hydrogenated under atmospheric pressure at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was subjected to column chromatography (CHP-20P, 10% CH_3CN in H_2O) to give **22** (520 mg, 65%) as a colorless powder: $[\alpha]^{20}_D$ +90.4° (*c* = 0.25, H_2O); IR (KBr) cm^{-1} 3400, 3050, 2925, 1730, 1645, 1540, 1505, 1450, 1340, 1200, 1185, 1135, 970, 835; ^1H NMR (DMSO-d_6) 2.68−2.95 (2H, m), 3.15−3.50 (2H, m), 3.55−4.50 (10H, m), 4.91 (1H, t, *J* = 6.0 Hz), 7.56 (2H, d, *J* = 8.0 Hz), 7.93 (2H, d, *J* = 8.0 Hz). Anal. ($C_{18}H_{22}N_4O_7$·CF_3CO_2H) C, H, N.

Compounds **23**−**30** were synthesized from **5a**−**h** by the same procedure as that described for the synthesis of **22**.

(*S*)-4-[[4-(2-Aminoethyl)benzoyl]glycyl]-2-oxopiperazine-1,3-diacetic acid (23): amorphous powder (49%); $[\alpha]^{20}_D$ +88.7° (*c* = 0.20, H_2O). Anal. ($C_{19}H_{24}N_4O_7$·CF_3CO_2H) C, H, N.

(*S*)-4-[[4-(3-Aminopropyl)benzoyl]glycyl]-2-oxopiperazine-1,3-diacetic acid (24): amorphous powder (yield 49%); $[\alpha]^{20}_D$ +99.9° (*c* = 0.20, H_2O). Anal. ($C_{20}H_{26}N_4O_7$·HCl) C, H, N.

4-[[4-(2-Aminoethyl)benzoyl]glycyl]-2-oxopiperazine-1-acetic acid (25): amorphous powder (yield 67%). Anal. ($C_{17}H_{22}N_4O_5$·HCl) C, H, N.

(*S*)-4-[[4-(2-Aminoethyl)benzoyl]glycyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid (27): amorphous powder (yield 72%); $[\alpha]_D^{20}$ +62.7° (*c* = 0.26, H_2O). Anal. ($C_{21}H_{26}N_4O_7$·HCl) C, H, N.

(*S*)-3-(2-Carboxyethyl)-4-[[4-[2-(*N*,*N*-dimethylamino)-ethyl]benzoyl]glycyl]-2-oxopiperazine-1-acetic acid (29): amorphous powder (yield 69%); $[\alpha]^{20}_D$ +61.5° (*c* = 0.24, H_2O). Anal. ($C_{22}H_{30}N_4O_7$·HCl) C, H, N.

(*S*)-4-[[4-[2-(*N*,*N*-Dimethylamino)ethyl]benzoyl]glycyl]-2-oxopiperazine-1-acetic acid (30): isolated as a mixture of free base and hydrochloride (1:1); amorphous powder (yield 75%); $[\alpha]^{20}_D$ +55.7° (*c* = 0.2, H_2O). Anal. ($C_{23}H_{32}N_4O_7$·$^1/_2$HCl) C, H, N.

Molecular Modeling Studies. We chose four compounds for molecular modeling: RGDF, **1a**, **9**, and **18**. One hundred stable conformers were generated for each model compound by 500-ps molecular dynamics calculation at 900 K, subsequent annealing to 300 K, and energy minimization using the CVFF force field of Discover (Molecular Simulation Inc., San Diego, CA). To avoid overestimation of the electrostatic effect, we adopted a distance-dependent dielectric constant 4**R* and assumed that the Asp and Arg surrogates were not charged. The distance between the central carbon atoms on the amidine (or guanidine) and carboxylate groups was plotted versus relative energy and conformer population. The SARs of compounds **9**−**14** indicate that the carboxymethyl group at the 1-position on the 2-oxopiperazine ring is necessary for GP IIb-IIIa antagonistic activity, while that at the 3-position is not. We assumed that this relationship also holds true for **1a**, which has two carboxymethyl groups, and chose the carboxymethyl group at the 1-position on the 2-oxopiperazine ring as the Asp surrogate. All molecular modeling and calculations were performed on a Silicon Graphics INDIGO^2 computer using InsightII/Discover (Molecular Simulation Inc.).

In Vitro Platelet Aggregation Studies. Blood was collected from healthy human volunteers 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) 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 at 1000*g* for 20 min at room temperature, respectively. The platelet count in PRP was adjusted to 3 × 10^5/mL (human) and 4 × 10^5/mL (guinea pig) using an automatic blood cell counter (Sysmex E2500, Touaiyoudenshi Co., Tokyo, Japan). Platelet aggregation was measured using an 8-channel aggregometer (Hematracer VI, Niko Bioscience, Tokyo, Japan). PRP (250 μL), 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 reagents (25 μL). Submaximal concentrations of aggregating agents were used in each experiment.

Fibrinogen Binding Studies. The fibrinogen binding assay was performed by the method of Yoshimura et al.[41] In brief, 100 μL of purified GP IIb-IIIa (1 μg/mL) in buffer A (20 mmol Tris-HCl, 150 mmol NaCl, 1 mmol $CaCl_2$, and 0.02% NaN_3, pH 7.4) was added to the wells of a 96-well plate (MaxiSorp, Nunc, Denmark) followed by incubation overnight at 4 °C. The plate was blocked with buffer B (3.5% BSA, 50 mmol Tris-HCl, 100 mmol NaCl, 2 mmol $CaCl_2$, and 0.02% NaN_3, pH 7.4) for 2 h at 30 °C. One hundred microliters of 1 nM biotinylated fibrinogen solutions containing various concentrations of test compounds was added to the wells followed by incubation at 30 °C for 3 h. After the plate was washed with buffer C (1 mg/mL BSA, 50 mM Tris-HCl, 100 mM NaCl, 2 mM $CaCl_2$, pH 7.4), 100 μL of antibiotin-alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO; 1:2000 dilution) was added followed by incubation at 30 °C for 1 h. One hundred microliters of *p*-nitrophenyl phosphate (Bio-Rad Laboratories, Richmond, CA) was added to each well followed by incubation at room temperature. When the absorbance of the control wells at 405 nm reached 1.0−1.2 (ca. 2 h later), 100 μL of 0.4 N NaOH was added to stop the reaction, and the absorbance at 405 nm was measured again. IC_{50} values were determined from the dose−response curves.

Ex Vivo Studies. Male Hartley guinea pigs (300−400 g) were used. At various times after iv or po 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). To eliminate the possible influence of the sensitivity differences of guinea pig platelets to ADP, which is dependent on the animal lot and on 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 aggregation in the controls at each point.

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References

(1) 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.

(2) 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.

(3) Plow, E. F.; Pierschbacher, M. D.; Ruoslahti, E.; Marguerie, G.; Ginsberg, M. H. Arginyl-Glycyl-Aspartic Acid Sequences and Fibrinogen Binding to Platelets. *Blood* **1987**, *70*, 110−115.

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

(5) Cahill, M.; Mistry, R.; Barnet, D. B. The Human Platelet Fibrinogen Receptor: Clinical and Therapeutic Significance. *Br. J. Clin. Pharmacol.* **1992**, *33*, 3−9.

(6) Nichols, A. J.; Ruffolo, R. R.; Huffman, W. F., Jr.; Poste, G.; Samanen, J. Development of GP IIb-IIIa Antagonists as Antithrombotic Drugs. *Trends Pharm. Sci.* **1992**, *13*, 413−417.

(7) Blackburn, B. K.; Gadek, T. R. Glycoprotein IIb-IIIa Antagonists. *Annu. Rep. Med. Chem.* **1993**, *28*, 79−88.

(8) Hawiger, J.; Kloczewiak, M.; Bednarek, M. A.; Timmons, S. Platelet Receptor Recognition Domains on the α-Chain of Human Fibrinogen: Structure−Function Analysis. *Biochemistry* **1989**, *28*, 2909−2914.

(9) Bennett, J. S.; Shattil, S. J.; Power, J. W.; Gartner, T. K. Interaction of Fibrinogen with Its Platelet Receptor. *J. Biol. Chem.* **1988**, *263*, 12948−12953.

(10) Andrieux, A.; Hudry-Clergeon, G.; Ryckewaert, J.-J.; Chapel, A.; Ginsberg, M. H.; Plow, E. F.; Marguerie, G. Amino Acid Sequences in Fibrinogen Mediating Its Interaction with Its Platelet Receptor GP IIb-IIIa. *J. Biol. Chem.* **1989**, *264*, 9258−9265.

(11) Samanen, J.; Ali, F.; Romoff, T.; Calvo, R.; Sorenson, E.; Vasko, J.; Storer, B.; Berry, D.; Bennett, D.; Strohsacker, M.; Powers, D.; Stadel, J.; Nichols, A. Development of a Small RGD Peptide Fibrinogen Receptor Antagonist with Potent Antiaggregatory Activity in Vitro. *J. Med. Chem.* **1991**, *34*, 3114−3125.

(12) Barker, P. L.; Bullens, S.; Bunting, S.; Burdick, D. J.; Chang, K. S.; Deisher, T.; Eigenbrot, C.; Gadek, T. R.; Gantzos, R.; Lipari, M. T.; Muir, C. D.; Napier, M. A.; Pitti, R. M.; Padua, A.; Quan, C.; Stanley, M.; Struble, M.; Tom, J. Y. K.; Burnier, J. P. Cyclic RGD Peptide Analogues as Antiplatelet Antithrombotics. *J. Med. Chem.* **1992**, *35*, 2040−2048.

(13) Scarborough, R. M.; Naughton, M. A.; Teng, W.; Rose, J. W.; Phillips, D. R.; Nannizzi, L.; Arfsten, A.; Campbell, A. M.; Charo, I. F. Design of Potent and Specific Integrin Antagonists. *J. Biol. Chem.* **1993**, *268*, 1066−1073.

(14) 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 αIIbβ3 Antagonists. *J. Med. Chem.* **1994**, *37*, 1−8.

(15) Gan, Z.-R.; Gould, R. J.; Jacobs, J. W.; Friedman, P. A.; Polokoff, M. A. Eichistatin. A Potent Platelet Aggregation Inhibitor from the Venom of the Viper, *Echis carinatus*. *J. Biol. Chem.* **1988**, *263*, 19827−19832.

(16) Gould, R. J.; Polokoff, M. A.; Friedman, P. A.; Huang, T.-F.; Holt, J. C.; Cook, J. J.; Niewiarowski, S. Disintegrins: A Family of Integrin Inhibitory Proteins from Viper Venoms. *Proc. Soc. Exp. Biol. Med.* **1990**, *195*, 168−171.

(17) Huang, T.-F.; Holt, J. C.; Lukasiewicz, H.; Niewiarowski, S.; Trigramin. A Low Molecular Weight Peptide Inhibiting Fibrinogen Interaction with Platelet Receptors Expressed on Glycoprotein IIb-IIIa Complex. *J. Biol. Chem.* **1987**, *262*, 16157−16163.

(18) Shebuski, R. J.; Ramjit, D. R.; Bencen, G. H.; Polokoff, M. A. Characterization and Platelet Inhibitory Activity of Bitistatin, a Potent Arginine-Glycine-Aspartic Acid-containing Peptide from the Venom of the Viper *Bitis arietans*. *J. Biol. Chem.* **1989**, *264*, 21550−21556.

(19) Dennis, M. S.; Henzel, W. J.; Pitti, R. M.; Lipari, M. T.; Napier, M. A.; Deisher, T. A.; Bunting, S.; Lazarus, R. A. Platelet Glycoprotein IIb-IIIa Protein Antagonists from Snake Venoms: Evidence for a Family of Platelet-Aggregation Inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *87*, 2471−2475.

(20) Yasuda, T.; Gold, H. K.; Fallon, J. T.; Leinbach, R. C.; Guerrero, J. L.; Schudder, L. E.; Kunke, M.; Shelly, D.; Ross, M. J.; Colleen, D.; Coller, B. S. Monoclonal Antibody against the Platelet (GP) IIb-IIIa Receptor Prevents Coronary Artery Reocclusion after Repercussion with Recombinant Tissue-type Plasminogen Activator in Dogs. *J. Clin. Invest.* **1988**, *81*, 1284−1291.

(21) Gold, H. K.; Gimple, L.; Yasuda, T.; Leinbach, R. S.; Jordan, R.; Iuliucci, J.; Coller, B. S. Phase I Human Trial of the Potent Antiplatelet Agent 7E3-(ab′)$_2$, a Monoclonal Antibody to the GP IIb-IIIa Receptor. *Circulation* **1989**, *80* (Suppl. II), 267.

(22) Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.; Shakespeare, W. C.; Smith, A. B., III. The First Design and Synthesis of a Steroidal Peptidemimetic. The Potential Value of Peptidomimetics in Elucidating the Bioactive Conformation of Peptide Ligands. *J. Am. Chem. Soc.* **1992**, *114*, 9699−9701.

(23) Alig, L.; Edenhofer, A.; Hadváry, P.; Hürzeler, M.; Knopp, D.; Müller, M.; Steiner, B.; Trzeciak, A.; Weller, T. Low Molecular Weight, Non-Peptide Fibrinogen Receptor Antagonists. *J. Med. Chem.* **1992**, *35*, 4393−4407.

(24) Hartman, G. D.; Egbertson, M. S.; Halczenko, W.; Laswell, W. L.; Duggan, M. E.; Smith, R. L.; Nayler, A. M.; Manno, P. D.; Lynch, R. J.; Zhang, G.; Chan, C. T.-C.; Gould, R. J. Non-Peptide Fibrinogen Receptor Antagonists. 1. Discovery and Design of Exosite Inhibitors. *J. Med. Chem.* **1992**, *35*, 4640−4642.

(25) Zablocki, J. A.; Miyano, M.; Rao, S. N.; Panzer-Knodle, S.; Nicholson, N.; Feigen, L. Potent 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 Asp-Carboxylate of RGDX Mimetics and the Platelet GP IIb-IIIa Receptor. *J. Med. Chem.* **1992**, *35*, 4914−4917.

(26) Callahan, J. F.; Bean, J. W.; Burgess, J. L.; Eggleston, D. S.; Hwang, S. M.; Kopple, K. D.; Koster, P. F.; Nichols, A.; Peishoff, C. E.; Samanen, J. M.; Vasko, J. A.; Wong, A.; Huffman, W. F. Design and Synthesis of a C_7 Mimetic for the Predicted γ-Turn Conformation Found in Several Constrained RGD Antagonists. *J. Med. Chem.* **1992**, *35*, 3970−3972.

(27) 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.

(28) Ku, T. W.; Ali, F. E.; Barton, L. S.; Bean, J. W.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, L.; Eggleston, D. S.; Gleason, J. G.; Huffman, W. F.; Hwang, S. M.; Jakas, D. R.; Karash, C. B.; Keenan, R. M.; Kopple, K. D.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M. F.; Peishoff, C. E.; Samanen, J. M.; Uzinskas, I.; Venslavsky, J. W. Direct Design of a Potent Non-Peptide Fibrinogen Receptor Antagonist Based on the Structure and Conformation of a Highly Constrained Cyclic RGD Peptide. *J. Am. Chem. Soc.* **1993**, *115*, 8861−8862.

(29) Ku, T. W.; Miller, W. H.; Bondinell, W. E.; Erhard, K. F.; Keenan, R. M.; Nichols, A. J.; Peishoff, C. E.; Samanen, J. M.; Wong, A. S.; Huffman, W. F. Potent Non-Peptide Fibrinogen Receptor Antagonists Which Present an Alternative Pharmacophore. *J. Med. Chem.* **1995**, *38*, 9−12.

(30) Egbertson, M. S.; Chang, C. T.-C.; Duggan, M. E.; Gould, R. J.; Halczenko, W.; Hartman, G. D.; Laswell, W. L.; Lynch, J. J.; Lynch, R. J. Jr.; Manno. P. D.; Nayler, A. M.; Prugh, J. D.; Ramjit, D. R.; Sitko, G. R.; Smith, R. S.; Truchi, L. M.; Zhang, G. Non-Peptide Fibrinogen Receptor Antagonists. 2. Optimization of a Tyrosine Template as a Mimic for Arg-Gly-Asp. *J. Med. Chem.* **1994**, *37*, 2537−2551.

(31) McDowell, R. S.; Blackburn, B. K.; Gadek, T. R.; McGee, L. R.; Rawson, T.; Reynolds, M. E.; Robarge, K. D.; Somers, T. C.; Thorsett, E. D.; Tischler, M.; Webb, R. R., II; Venuti, M. C. From Peptide to Non-Peptide. 2. The *de Novo* Design of Potent, Non-Peptidal Inhibitors of Platelet Aggregation Based on a Benzodiazepinedione Scaffold. *J. Am. Chem. Soc.* **1994,** *116*, 5077−5083.

(32) Eldred, C. D.; Evans, B.; Hindley, S.; Judkins, B. D.; Kelly, H. A.; Kitchin, J.; Lumley, P.; Porter, B.; Ross, B. C.; Smith, K. J.; Taylor, N. R.; Wheatcroft, J. R. Orally Active Non-Peptide

Fibrinogen Receptor (GP IIb-IIIa) Antagonists: Identification of 4-[4-[4-(Aminoiminomethyl)phenyl]-1-piperazinyl]-1-piperidineacetic Acid as a Long-Acting, Broad-Spectrum Antithrombic Agent. *J. Med. Chem.* **1994**, *37*, 3882−3885.

(33) Zablocki, J. A.; Rico, J. G.; Garland, R. B.; Rogers, T. E.; Williams, K.; Schretzman, L. A.; Rao, S. A.; Bovy, P. R.; Tjoeng, F. S.; Lindmark, R. J.; Toth, M. V.; Zupec, M. E.; McMackins, D. E.; Adams, S. P.; Miyano, M.; Markos, C. S.; Milton, M. N.; Paulson, S.; Herin, M.; Jacqmin, P.; Nicholson, N. S.; Panzer-Knodle, S. G.; Haas, N. F.; Page, J. D.; Szalony, J. A.; 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 Sequence of Fibrinogen. (Aminobenzamidino)succinyl (ABAS) Series of Orally Active Fibrinogen Receptor Antagonists. *J. Med. Chem.* **1995**, *38*, 2378−2394.

(34) Pierschbacher, M. D.; Ruoslahti, E. Influence of Stereochemistry of the Sequence Arg-Gly-Asp-Xaa on Binding Specificity in Cell Adhesion. *J. Biol. Chem.* **1987**, *262*, 17294−17298.

(35) Timmons, S.; Bednarek, M. A.; Kloczewiak, M.; Hawiger, J. Antiplatelet "Hybrid" Peptides Analogous to Receptor Recognition Domains on γ and α Chains of Human Fibrinogen. *Biochemistry* **1989**, *28*, 2919−2923.

(36) Hurby, V. J. Conformational Restrictions of Biologically Active Peptides via Amino Acid Side Chain Groups. *Life Sci.* **1982**, *31*, 189−199.

(37) Hurby, V. J. Design of Peptide Hormone and Neurotransmitter analogues. *Trends Pharm. Sci.* **1985**, *6*, 259−262.

(38) Toniolo, C. Conformationally Restricted Peptides through Short-Range Cyclizations. *Int. J. Pept. Protein Res.* **1990**, *35*, 287−300.

(39) Farmer, P. S. Bridging the Gap between Bioactive Peptides and Nonpeptides: Some Perspectives in Design. In *Drug Design*; Ariëns, E. J., Ed.; Academic Press: New York, 1980; Vol. X, pp 119−143.

(40) DiMaio, J.; Belleau, B. Synthesis of Chiral Piperazin-2-ones as Model Peptidomimetics. *J. Chem. Soc., Perkin Trans. I* **1989**, 1687−1688.

(41) Yoshimura, K.; Miyazaki, T.; Furuyama, N.; Terashita, Z.-i.; Fujisawa, Y. Binding Properties of Fibrinogen Receptor GP IIb-IIIa Purified from Human Erythroleukemia Cells. *Biochem. Mol. Med.* **1995**, *56*, 166−171.

(42) Naylor, A. M.; Egbertson, M. S.; Vassallo, L. M.; Birchenough, L. A.; Zhang, G. X.; Gould, R. J.; Hartman, G. D. Non-Peptide Fibrinogen Receptor Antagonists. 4. Proposed Three-Dimensional Requirements in Centrally Constrained Inhibitors. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1841−1846.

(43) Peishoff, C. E.; Ali, F. E.; Bean, J. W.; Calvo, R.; D'Ambrosio, C. A.; Eggleston, D. S.; Hwang, S. M.; Kline, T. P.; Koster, P. F.; Nichols, A.; Powers, D.; Romoff, T.; Samanen, J. M.; Stadel, J.; Vasko, J. A.; Kopple, K. D. Investigation of Conformational Specificity at GP IIb-IIIa: Evaluation of Conformationally Constrained RGD Peptides. *J. Med. Chem.* **1992**, *35*, 3962−3969.

(44) Bogusky, M. J.; Naylor, A. M.; Pitzenberger, S. M.; Nutt, R. F.; Brady, S. F.; Colton, C. D.; Sisko, J. T.; Anderson, P. S.; Veber, D. F. NMR and Molecular Modeling Characterization of RGD containing Peptides. *Int. J. Pept. Protein Res.* **1992**, *39*, 63−76.

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

(46) S. Kita, et al., manuscript in preparation.

(47) Kawamura, M.; Tsuji, N.; Moriya, N.; Terashita, Z.-i.; Effect of TAK-029, A Novel GP IIb-IIIa Antagonist, on Arterial Thrombosis in Guinea Pigs, Dogs and Monkeys. *Thromb. Res.* **1997**, *86*, 275−285.

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