

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 14-137450- -3	FECHA: 2015-05-07 14:37:10
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 7
ACT: 519 TRASLADOINFORME	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
MARGARITA CASTELLANOS ABONDANO

Asunto: Radicación: 14-137450- -3
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 4610
Folios: 7

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2012/066938				
Fecha de Presentación Internacional	29/11/2012				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado No. 14-137450-00000-0000	26/junio/2014
Reivindicaciones:	Radicado No. 14-137450-00000-0000	26/junio/2014
	Radicado No. 14-137450-00001-0000	17/diciembre/2014
Prioridad:	US 61/564559	29/noviembre/2011
	US 61/614292	22/marzo/2012
	US 61/641698	2/mayo/2012
	US 61/666291	29/junio/2012

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Modificaciones aceptadas

Se acepta el nuevo capítulo reivindicatorio (Radicado No. 14-137450-00001-0000) porque no se amplía el objeto de la solicitud.

4. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso, se tiene en cuenta la totalidad del nuevo capítulo reivindicatorio (Radicado No. 14-137450-00001-0000) para el examen de patentabilidad.

Título de la solicitud: "AGENTES DE REVERSIÓN ANTICOAGULANTE".

El problema planteado en esta solicitud consiste en la necesidad de suministrar agentes de reversión de la anticoagulación.

Para resolver este problema técnico la solicitud en estudio proporciona compuestos derivados de la fórmula (I) o una sal farmacéuticamente aceptable:



El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K31/495; A61P7/00; C07D241/04; C07D295/13.

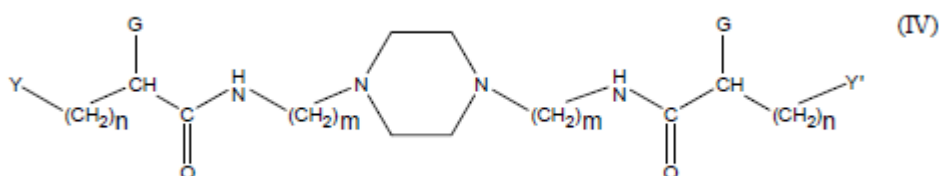
5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de la reivindicación 22 no es patentable

6. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 a 7 carecen de concisión y sustento descriptivo debido a que abarcan un ilimitado número de agentes ya que no se define de manera clara ninguno de los sustituyentes de la fórmula (I), no existe una estructura o grupo farmacofórico fijo que permita realizar una búsqueda de la totalidad de la materia reclamada. Según lo definido en la descripción la materia realmente obtenida por la solicitante corresponde a los compuestos que comprenden piperazina como anillo central disustituida representados por la fórmula (IV):



Esto se fundamenta en el hecho de que los esquemas de reacción están relacionados con la obtención de estos compuestos y que los compuestos ejemplificados y ensayados corresponden a esta fórmula genérica. Se sugiere a la solicitante realizar una generalización racional de la materia reclamada con fundamento en la divulgación realizada en la descripción, en los ejemplos específicos y ensayos que evidencian la solución al problema técnico.

De acuerdo con lo definido en la reivindicación 1 la fórmula general (I) puede reducirse a la siguiente fórmula:

Y-A-Y'.

Existen innumerables compuestos que poseen un anillo central o una molécula lineal unida a dos grupos que pueden convertirse en cationes bajo condiciones fisiológicas, los cuales son ampliamente conocidos en el campo de la química. Por ejemplo el documento Sigma-Aldrich Chemie GmbH¹, revela el 1,4-diaminobutano, 1,2-diaminociclohexano, 1,4-diaminociclohexano, 1,8-diaminooctano, 1,5-diaminopentano, 1,3-diaminopropano, clorhidrato de 1,4-diaminopiperazina, o-fenilendiamina, m-fenilendiamina, p-fenilendiamina, entre muchos otros. En consecuencia esta inmensa cantidad de compuestos reivindicados es conocida tal y como se encuentran reivindicados y en consecuencia no serían patentables por pertenecer al estado de la técnica. Estas apreciaciones fueron establecidas en el international preliminary report on patentability y son igualmente aceptadas por esta Oficina.

La reivindicación 21 reclama un kit diagnóstico que comprende el compuesto de cualquiera de las reivindicaciones 1 a 15. Un kit de partes se debe caracterizar por:

- Una preparación farmacéutica o medicamento A en una cantidad o proporción definida y
- Una preparación farmacéutica o medicamento B en una cantidad o proporción definida

De igual forma este kit carece de claridad y sustento debido a que se fundamenta en una fórmula Markush que no se encuentra claramente definida. Tampoco existen ejemplos específicos y ensayos de dicho kit ni información en la solicitud que evidencie su uso para resolver un problema técnico específico, en donde el conjunto de elementos que conforman el kit interactúen como una unidad funcional para dar solución al problema planteado.

Descripción (Art 28 D 486)

La descripción no divulga la invención de manera suficientemente completa para que el la persona del oficio normalmente versada en la materia pueda ejecutarla o reproducirla, debido a que abarca una cantidad ilimitada de materia, no existen ejemplos que permitan evidenciar la posesión real de la solicitante de todos los agentes reclamados ni tampoco hay sustento de la utilidad de toda la materia reivindicada a pesar de la variabilidad estructural propuesta.

Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el Requisito de Unidad de Invención porque se refiere a un número indeterminado de Grupos Inventivos debido a que no se establece un grupo central definido.

Los Grupos Inventivos generales están definidos por las siguientes características **esenciales**:

- 1° Compuestos en los que A es un anillo carbocíclico.
- 2° Compuestos en los que A es un anillo heterocíclico.

1 <http://www.sigmaaldrich.com/catalog/product/aldrich/z273163?lang=en®ion=CO>. 1996

- 3° Compuestos en los que A es un anillo aromático.
- 4° Compuestos en los que A es un anillo no aromático.
- 5° Compuestos en los que X y X' están ausentes
- 6° Compuestos en los que X y X' son un grupo funcional que une el enlace L a M y el enlace L' a M' respectivamente.
- 7° Compuestos en los que M y M' están ausentes
- 8° Compuestos en los que M y M' son un enlace que une X a Y y X' a Y' respectivamente.
- 9° Compuestos en los que Y e Y' son iguales o diferentes y son un resto que contiene uno o más átomos catiónicos.
- 10° Compuestos en los que Y e Y' son iguales o diferentes y son uno o más grupos que se vuelven catiónicos en condiciones fisiológicas.

No se guarda un concepto común inventivo porque los agentes reclamados no comparten una estructura esencial para la actividad común. De igual forma, dentro de estos grupos existen más subgrupos inventivos debido al uso de términos funcionales generales tales como “anillo carbocíclico”, “heterocíclico”, “aromático”, “no aromático”, “un grupo funcional”, “un resto que contiene uno o más átomos catiónicos” y “grupos que se vuelven catiónicos”, estas expresiones abarcan una innumerable cantidad de agentes que se encuentran contenidos en clasificaciones diferentes.

Se sugiere presentar las Solicitudes Divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 29 de noviembre de 2011, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	Catálogo	Sigma-Aldrich Chemie GmbH Catalogue	1996
D2	Bischoff, J. Biol. Chem. 80, pp 345-355	“Preparation of some substituted guanidines”	1928
D3	Mull, J. Med.Chem 5(5), pp 944-949	“Guanidines with Anthihypertensive Activity. II”	1962
D4	Kitamura, J Med Chem. 2001 Jul 19;44(15):2438-50.	“Potent dibasic GPIIb/IIIa antagonists with reduced prolongation of bleeding time: synthesis and pharmacological evaluation of 2-oxopiperazine derivatives”.	2001

D1² divulga compuestos comúnmente utilizados en la industria química (Págs. 497, 502, 1305).

D2³ divulga compuestos derivados de guanidinas (Págs. 345-355).

2http://worldwide.espacenet.com/publicationDetails/biblio?CC=WO&NR=2007134876&KC=&FT=E&locale=en_EP

3<http://www.jbc.org/content/80/2/345.full.pdf+html?sid=7bc4fdcd-b347->

D3⁴ Enseña derivados de etil-guanidina (Tabla III)

D4⁵ da a conocer una serie de 2-oxopiperazinas las cuales son útiles como antagonistas GPb/IIIa (Págs. 2438-50)

8. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en Y-M-X-L-A-L'-X'-M'-Y' (I)

Solicitud	Características esenciales
	Y—M—X—L—A—L'—X'—M'—Y' (I)
D1	Revela el 1,4 diaminobutano, 1,2-diaminociclohexano, 1,4-diaminociclohexano, 1,8-diaminooctano, 1,5-diaminopentano, 1,3-diaminopropano, clorhidrato de 1,4-diaminopiperazina, o-fenilendiamina, m-fenilendiamina, p-fenilendiamina, entre muchos otros. (Págs. 497, 502, 1305)
D2	Da a conocer la diguanilpiperazina <i>Diguanilpiperazine</i> $H_2N-C(=NH)-N(CH_2-CH_2)_4-N-C(=NH)-NH_2$ (Pág. 347)
D3	Enseña los compuestos: $\left[R-N(CH_2)_m-N(CH_2)_n-N(CH_2)_x-NHC(=NH)NH_2 \right]_{1-2} \cdot H_2SO_4$ m=2, n=2 (Compuesto 8)
D4	12 a-c: R ¹ = H , R ² = , R ² = , R ² =

Las características esenciales de la reivindicación 1 habían sido divulgadas previamente en los documentos D1 a D4 que divulgan compuestos específicos comprendidos en la definición establecida para la fórmula (I) de la presente solicitud.

En consecuencia, la reivindicación 1 y sus dependientes no son nuevas según lo divulgado en los documentos D1 a D4.

4be7-b3ca-dd2cdedcc2e9

4 <http://pubs.acs.org/doi/abs/10.1021/jm01240a007>

5 <http://www.ncbi.nlm.nih.gov/pubmed/11448226>

9. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Catálogo	1 – 11 y 16 a 22	Novedad
D2	Bischoff, J. Biol. Chem. 80, pp 345-355	1 – 11 y 16 a 22	Novedad
D3	Mull, J. Med.Chem 5(5), pp 944-949	1 – 11 y 16 a 22	Novedad
D4	Kitamura, J Med Chem. 2001 Jul 19;44(15):2438-50.	1 – 11 y 16 a 22	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JESÚS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-05-12

Elaboró: Emilse Cano Urrego

Revisó: Gloria Jacqueline Alfonso Rozo

PREPARATION OF SOME SUBSTITUTED GUANIDINES.

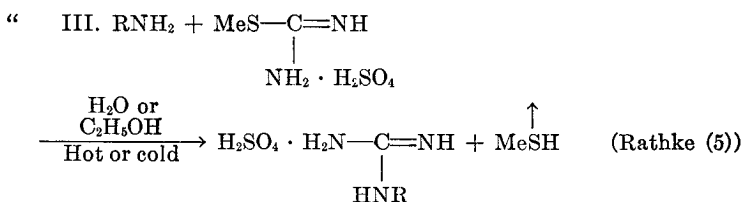
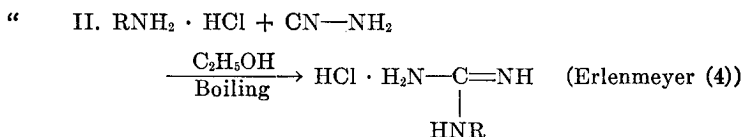
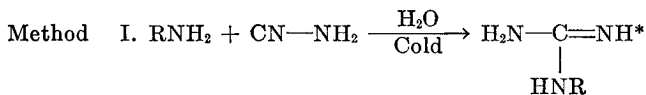
BY FRITZ BISCHOFF.

(From the Chemical Laboratory of the Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara.)

(Received for publication, August 28, 1928.)

INTRODUCTION.

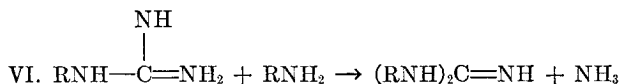
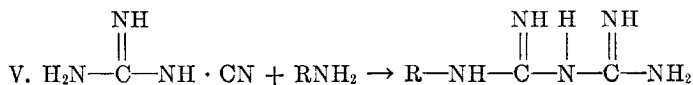
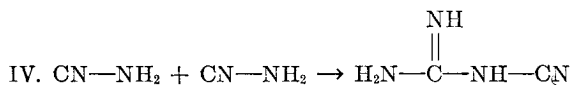
In studying the nature of the hypoglycemic action of guanidine compounds, it was found necessary to prepare a number of guanidine derivatives not obtainable and in many instances not described in the literature. This paper concerns itself with syntheses in which the original material was an amine or imine. The preparation of diguanylpiperazine, guanylpiperidine, guanyl-*p*-aminodimethylaniline, diguanidinopentamethylene, and diguanidinoctamethylene is recorded. No new principle has been developed, the general methods of guanidine preparation having been adapted. These may be summarized as follows:



* We do not know whose name is associated with Method I. It was used in converting amino acids to guanidino acids by Engeland and Kutscher (1). Ripke (2) and Kiesel (3) prepared diguanidinopentamethylene and agmatine in this manner.

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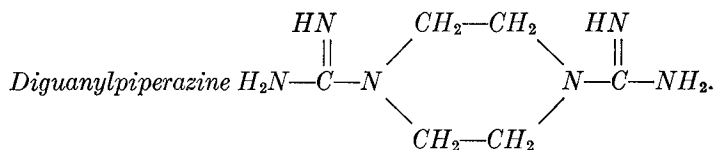
Side reactions are possible in each instance. The three most common side reactions and the ones encountered in these preparations are the polymerization of cyanamide to dicyanodiamide, the condensation of this latter compound with the parent amine to give a diguanide, and the reaction of the desired mono-substituted guanidine with unchanged parent amino base to give a di-substituted product. These may be outlined as follows:



Side reaction (IV) takes place readily in alkaline solution and is consequently met with in Method I. It would appear that Method I becomes practical when the rate of condensation for the guanidine compound exceeds the dicyanodiamide polymerization. According to Werner and Bell (6) dicyanodiamide acts potentially as cyanamide, methylguanidine being obtained when dicyanodiamide is heated with methylamine hydrochloride at 180°. No instance of this reaction was encountered by us at room temperature or 100°. The conditions under which reaction (V) takes place were observed by Cohn (7) and Bamberger and Seeberger (8). The mechanism of reaction (VI) is not clearly understood and is apparently not as simple as represented by equation (VI). In some instances this reaction may be induced to take place almost quantitatively (9). It has been studied at elevated temperatures by Klinger (10). An excellent discussion is given by Schotte *et al.* (11).

A survey of the recent guanidine literature indicates the popularity of methylisothiurea sulfate, Method III, over the older cyanamide condensations, Methods I and II. In our experience, the methylisothiurea sulfate method is practical when the desired guanidine sulfate is sufficiently insoluble as to crystallize out from

the reaction mixture. It is impractical when an alkaline silver separation is required for purification.



Four modifications of conditions were tried out in attempting to prepare diguanylpiperazine. When piperazine hydrochloride was refluxed in absolute alcohol with cyanamide, the hydrochloride of diguanylpiperazine was isolated (Method II). Piperazine hydrate was found to react with methylisothiurea sulfate in aqueous solution at the boiling point of water and even at room temperature, but the isolation of the pure sulfate was unsuccessful. In crystallizing from water, the salt was contaminated with methylisothiurea sulfate. An alkaline silver separation, with subsequent conversion to the hydrochloride and chloroplatinate, yielded a precipitate which analyzed too high for platinum and melted 40° below the chloroplatinate obtained by Method I described above. When piperazine hydrate was permitted to react with cyanamide in aqueous solution at room temperature, 40 per cent of the cyanamide crystallized out as dicyanodiamide in 10 days standing (m. p. $204\text{--}206^\circ$). When the solution was concentrated, more dicyanodiamide crystallized out, and when alcohol was added, unchanged piperazine hydrate was precipitated.

Preparation.—15.0 gm. of piperazine hydrochloride, made by evaporation of the Eastman hydrate with hydrochloric acid and 11.0 gm. of cyanamide, were refluxed in 250 cc. of absolute alcohol for 24 hours. After the mixture was cooled, the insoluble residue was filtered off, 9.5 gm. This analyzed 38.9 per cent HCl. The alcoholic filtrate was evaporated to dryness, and the semi-crystalline mass obtained, crystallized from water, 6.5 gm. The crystals analyzed 24.1 per cent HCl. Both of these products were recrystallized from 20 cc. of hot water. In the case of the alcohol-insoluble residue, solution was complete, while in the alcohol-insoluble fraction an amorphous precipitate was filtered off. The filtrate of this product yielded large crystalline clusters, 2.7 gm., and a fine amorphous precipitate upon cooling. The amorphous

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precipitate was decanted off. The crystals (5.5 gm.) obtained from both the hot water solutions, crumpled to a white powder on drying, and did not blacken or melt up to 310°.

Analysis.— $(\text{CN}_3\text{H}_3)_2(\text{CH}_2)_4\text{HCl}$.

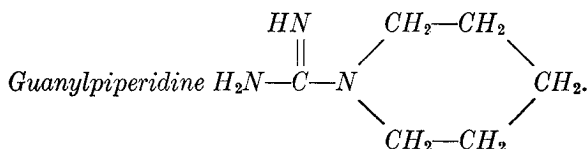
Calculated.	HCl	30.0.
Found.	" (Volhard)	30.3, 30.1.

The two products were converted to the chloroplatinates by precipitation with PtCl_4 from aqueous solution. The very insoluble precipitates were washed several times with water and dried at 110°. There were blackening and frothing at 279–280°.

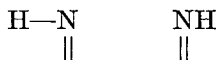
Analysis.— $(\text{CN}_3\text{H}_3)_2(\text{CH}_2)_4\text{H}_2\text{PtCl}_6$.

Calculated.	N	14.5, Pt 33.6.
Found.	" (Dumas)	14.6, " 33.7.

The yield of diguanylpiperazine hydrochloride was only 22 per cent of theory.



Guanylpiperidine was readily prepared in aqueous solution at room temperature from piperidine and methylisothiurea sulfate (Method III). By concentration of the reaction mixture, pure crystals of the desired compound crystallized out. The reaction may be accelerated by raising the temperature. The reaction of cyanamide with piperidine in aqueous solution, with subsequent removal of the excess piperidine by steam distillation, yielded a resinous product from which the pure guanylpiperidine sulfate was not isolated. It is highly probable that



much piperylbisguanide, $\text{NH}_2-\text{C}-\text{NH}-\text{C}-\text{NC}_5\text{H}_{10}$, is formed under these conditions. Bamberger and Seeberger (8) prepared piperylbisguanide by heating dicyanodiamide with piperidine in aqueous solution. They showed further that the reaction takes place at room temperature. Since cyanamide condenses to dicy-

anodiamide in alkaline solution, the conditions of our condensation were similar to those of Bamberger. We were unable to isolate pure piperylbiquanide, but two constants of crystals, obtained by fractional crystallization from alcohol, indicated its presence; *viz.*, a sulfate which melted below 180° and analyzed 31.6 per cent H_2SO_4 .

Preparation.—Eastman crude piperidine was used in the trial condensation. Kahlbaum's highly purified piperidine was then substituted in duplicating the successful synthesis. Because of the unusual physiological properties revealed by the first sample of guanylpiperidine obtained, it was considered expedient to prepare the compound from another starting material, in order to minimize the possibility that traces of an impurity carried the physiologically active group.

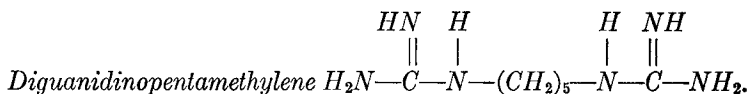
5 gm. of piperidine and 13 gm. of methylisothiurea sulfate were dissolved in 50 cc. of water. After standing 3 days, the volume of the solution was taken down to 30 cc. when crystals formed, 3.7 gm. The reaction of the solution was alkaline. In several trials dilute sulfuric acid was added at this point, with the result that the crystals which formed were contaminated with methylisothiurea sulfate, and recrystallization from water was necessary for purification. In the cases where the reaction mixture was left alkaline, recrystallization was unnecessary. It is to be expected that in the distribution of the sulfuric acid between the guanylpiperidine and the unchanged methylisothiurea, the stronger base should take up the preponderance of available acid. The crystals which formed were filtered off and washed with ice water. By concentration of the mother liquor to 20 cc., another crop of crystals formed. Yield 40 per cent.

Analysis.— $(\text{CN}_3\text{H}_3\text{C}_6\text{H}_{10})_2\text{H}_2\text{SO}_4$.

Calculated. N 23.9, H_2SO_4 27.9.

Found. " (Dumas) 24.2, " 27.9.

The guanylpiperidine sulfate did not melt below 270° , where it commenced to char. The picrate is very insoluble, forming in a dilution of 1:2000. It decomposes at $242\text{--}243^{\circ}$.



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Ripke (2) obtained this compound by the reaction of cyanamide with pentamethylenediamine in aqueous solution at room temperature for 17 days. The impure sulfate was isolated from the reaction mixture by the conventional alkaline silver salt separation. By conversion of the sulfate to the chloride, a gold salt which gave the correct analytical values was obtained.

We have applied the Erlenmeyer synthesis (Method II (4)) to the preparation of the compound. The whole procedure may be carried out in 2 days with an increased yield over the Ripke method and the elimination of the tedious alkaline silver salt separation. As did Ripke, we obtained a sulfate which analyzed high for sulfur, but which gave excellent values when converted to the chloroaurate.

Preparation.—Pentamethylenediamine hydrochloride was prepared by the method of Ladenburg (12) with Eastman dicyanotrimethylene. 10.0 gm. of pentamethylenediamine hydrochloride, 9.0 gm. of cyanamide, and 150 cc. of absolute alcohol were refluxed for 12 hours. The alcohol was then evaporated off, an oily syrup remaining. This was dissolved in the minimum amount of water, 6.0 gm., Na_2CO_3 added, and the solution evaporated on the water bath to dryness. The residue was extracted with absolute alcohol, the insoluble material being discarded. A cold mixture of concentrated sulfuric acid and absolute alcohol was then added until no more precipitate formed. The crystals were filtered off, recrystallized from water, and dried at 105° . Yield 8.0 gm. (47 per cent).

Analysis.— $(\text{CN}_2\text{H}_4)_2(\text{CH}_2)_6\text{H}_2\text{SO}_4$.

Calculated.	N	29.6,	H_2SO_4	34.5.
Found.	"	(Dumas) 27.0,	" by BaSO_4	35.4.

By concentration of the mother liquor, an additional crop of crystals was obtained. These upon analysis gave 27.8 per cent N and 37.1 per cent H_2SO_4 . The impure sulfate decomposed at 285° . The chloroaurate was prepared by dissolving 1 part of the sulfate in 8 parts of water and adding 105 per cent of the theoretical amount of BaCl_2 to precipitate the H_2SO_4 . The BaSO_4 was centrifuged off and AuCl_3 added to the filtrate. The double gold salt precipitated immediately as microscopic crystals. It was washed with water and dried at 100° .

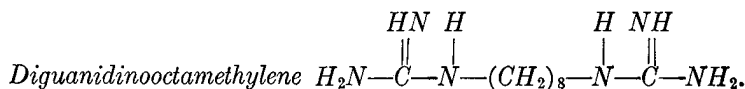
Analysis.— $(\text{CN}_3\text{H}_4)_2(\text{CH}_2)_8(\text{HAuCl}_4)_2$.

Calculated. N 9.7, Au 45.6

Found. " (Dumas) 9.5, " 45.7.

The diguanidinopentamethylene chloraurate shrinks at 139.5° , melting at 151° .

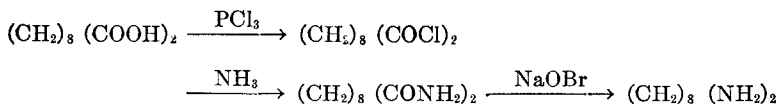
Ripke's synthesis and alkaline silver separation were duplicated with the same pentamethylenediamine used in the method described above. 6 gm. of sulfate were obtained from 10.0 gm. of pentamethylenediamine hydrochloride, equivalent to a yield of 35 per cent. The melting point of the chloraurate obtained by this procedure was 147° . One crystallization from alcohol-water raised the melting point to 151° , the same value obtained by our method. Ripke gives the melting point of the chloraurate as 161° . We were unable to raise the melting point of our chloraurates by three crystallizations. Since our analytical values are in closer agreement with the theoretical values than those obtained by Ripke, we are at a loss to explain this difference in melting points. It should be noted, however, that the lowering of the melting point of the chloraurates of the guanidine bases by impurities is more marked than it is for the chloroplatinates or the picrates. We found this to be true in the isolation of the guanidine base synthalin in the commercial preparation, glukhorment (13). In this instance, the first chloraurate melted 8° below the melting point for the pure compound, the chloroplatinate and picrate only 2° lower.



This compound has probably been prepared by the synthalin workers but no details have appeared in the literature. We used diaminooctamethylene hydrochloride as the initial material. An attempt to convert this compound to the diguanidino compound by refluxing in absolute alcohol with cyanamide was unsuccessful, the cyanamide going over to dicyanodiamide (recovery of 80 per cent of the cyanamide added as dicyanodiamide, m. p. 205°). The desired compound was readily prepared in aqueous solution and at room temperature from the free amine base and methylisothiurea sulfate.

352 Preparation of Substituted Guanidines

Preparation.—Diaminooctamethylene was prepared from Eastman sebacic acid, by the following scheme, outlined in Beilstein.



The diaminooctamethylene was separated from the hypobromite reaction mixture by steam distillation and converted to the hydrochloride. This formed a platinum salt which upon analysis gave 35.2 per cent Pt (theory 35.3 per cent Pt). 3.1 gm. of diaminooctamethylene hydrochloride and 1.0 gm. of caustic were dissolved in 11 cc. of water. To this solution were added 6.0 gm. of methylisothiurea sulfate in 42 cc. of water. After it had stood at room temperature for 7 days, 1.4 gm. of crystals were filtered off. After concentration of the solution, another 1.0 gm. of crystals was obtained. The combined crystals were dried at 110°. Yield 60 per cent.

Analysis.— $(\text{N}_3\text{CH}_4)_2(\text{CH}_2)_8\text{H}_2\text{SO}_4$.

Calculated.	N	25.0, H_2SO_4	30.0.
Found.	"	(Dumas) 25.0, "	by BaSO_4 29.6.

The sulfate was converted to the chloride by the addition of the theoretical amount of BaCl_2 . The platinum salt was precipitated from the chloride in aqueous solution. Upon analysis this gave 30.5 per cent Pt (theory 30.6 per cent Pt), m. p. 214–216°. The picrate was precipitated from an aqueous solution of the sulfate, m. p. 205–206.5°. The gold salt was thrown down in aqueous solution, m. p. 143–144°. Fractional crystallization of the gold salt from water gave products with identical melting points.

p-Guanidinodimethylaniline.

The condensation of *p*-aminodimethylaniline with cyanamide takes place readily, either in boiling alcohol as the hydrochloride or in water as the free base. The reaction product is contaminated with highly colored impurities. These may be separated by fractional crystallization of the sulfate from water. The base is very soluble in water, alcohol, or ether. The hydrochloride, nitrate, and sulfate are very water-soluble.

Preparation.

Condensation in Alcohol.—17 gm. of *p*-amino-dimethylaniline hydrochloride, 300 cc. of alcohol, and 4.5 gm. of cyanamide were refluxed 5 hours. A clear solution resulted. The alcohol was then distilled off, 50 cc. of water added, and concentrated NaOH to make strongly alkaline. The solution was then extracted three times with a total volume of 500 cc. of ether. The ether was evaporated off under a vacuum, leaving a dark brown viscous oil which crystallized within several days. The crystals were separated from the oil by being washed by decantation with a little water and alcohol. M. p. 117–122°.

Analysis.— $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{N}_3\text{CH}_4 \cdot \text{H}_2\text{O}$.

Calculated. N 28.6.

Found. " (Kjeldahl) 28.6, 27.9.

Larger yields of the purified compound are obtained by converting the crude ether extract to the nitrate or sulfate and crystallizing fractionally from water. The nitrate was obtained as prisms, m. p. 185°. The sulfate crystallizes in large rhombohedral aggregates, m. p. 112–112.5°.

Analysis.— $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{N}_3\text{CH}_4\text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$.

Calculated. N 17.9, H_2SO_4 31.4.

Found. " (Kjeldahl) 17.7, 17.3, " by BaSO_4 31.3, 31.1.

The chloroplatinate was obtained from the crude ether extract by fractional precipitation in water-alcohol. Upon addition of PtCl_4 to a 70 per cent solution of the crude hydrochloride, purple crystals precipitated immediately. These gave upon analysis 34.2 per cent Pt and 7.0 per cent N. When the filtrate was evaporated to two-thirds the original volume, yellow crystals containing the required amount of Pt, 33.2 per cent, were obtained. The purple chloroplatinate, which was isolated several times from different water-alcohol concentrations, always had the same Pt content; viz., 34.2 per cent Pt. This approximates the theoretical value for a compound containing 1 cyanamide molecule for 2 dimethylaminoaniline molecules.

Condensation in Water.—17 gm. of hydrochloride, 5.2 gm. of Na_2CO_3 , 5.0 gm. of cyanamide, and 140 cc. of water were autoclaved 5 hours at 110°. The purification was the same as for the alcohol condensation.

Diguanylbenzidine.

Diguanylbenzidine is not described in the literature, though the isomeric biguanide compound and the dibiguanide have been prepared (7). We were unable to isolate diguanylbenzidine in a pure state by refluxing benzidine hydrochloride and cyanamide in absolute alcohol. Much unchanged benzidine was always recovered even though an excess of cyanamide was used. The fraction which reacted with the cyanamide was relatively cold water-soluble, and by conversion to the sulfates, a cold water-soluble sulfate and hot water-soluble sulfate fractionation was made. The nitrogen values for these fractions indicated that the cold water-soluble sulfate consisted mainly of diguanylbenzidine sulfate and the hot water-soluble sulfate of monoguanylbenzidine sulfate. Fractional crystallization of these products showed, however, that they contained more than one compound. Attempts to isolate the pure derivatives were unsuccessful.

A summary of the constants of some of these basic fractions is included. The calculated nitrogen content of monoguanylbenzidine is 24.9 per cent N; and for diguanylbenzidine 31.4 per cent N.

Fraction A, relatively water-insoluble, dilute HCl-soluble, alcohol-insoluble, 23.6 per cent N. Not melting below 230°. Fraction B, relatively water-insoluble, dilute HCl-soluble, alcohol-soluble, 24.5 per cent N. Melting with decomposition at 205°. Chloroplatinate analyzed 27.5 per cent. Fraction C, dilute HCl-soluble, dilute H₂SO₄-soluble, hot, 26.6 per cent N. Charring at 215°. Does not melt up to 240°. Fraction D, relatively water-, and dilute H₂SO₄-soluble, cold. M. p. 187°. 28 to 32 per cent N. Sulfate analyzed 18 per cent N.

It should be noted that certain of these fractions had a physiological action similar to synthalin. Benzidine was found to be unreactive with methylisothiurea sulfate in aqueous alcohol solution in the cold.

Negatively Substituted Aromatic Amines.

Kampf (14) makes the statement that cyanamide will not react with aromatic amines in alcohol solution, it being necessary to use salts of the bases. He further points out that negatively substituted amines will not react under either condition. We confirmed his observation in the case of *p*-nitroaniline, pure un-

changed *p*-nitroaniline HCl being recovered from an absolute alcohol solution which had been refluxed for several days with cyanamide. The effect of the negative group was found to extend even to a compound such as *p*-aminoacetanilide. The hydrochloride of this base remained unchanged when refluxed with cyanamide in alcohol solution, and the free base unchanged when boiled with cyanamide in water or when permitted to stand as long as a month in aqueous solution with methylisothiurea sulfate or when heated with this compound. (The unchanged base was identified by the melting point, 162°, and the platinum analysis of the chloroplatinate.)

We are indebted to Miss Elsie Hill for some of the analyses.

SUMMARY.

The preparation of diguanylpiperazine, guanylpiperidine, guanyl-*p*-aminodimethylaniline, and diguanidinooctamethylene is described. A new method for preparing diguanidinopentamethylene is included.

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ARTICLE:
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Guanidines with Antihypertensive Activity. II¹

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A number of substituted piperazines, hexahydro-1,4-diazepines and octahydro-1,5-diazocines possessing the ethyl guanidine side chain were prepared and found to have cardiovascular properties.

The disclosure that certain amidoximes and guanidines, particularly when associated with large membered heterocyclic systems, possess antihypertensive properties,^{2,3} has resulted in a study of other ring systems in the hope that they might give biologically active compounds.⁴

Early in the study it was found that [2-(4-methyl-1-piperazinyl)-ethyl]-guanidine sulfate also manifests interesting cardiovascular properties. This compound suppresses sympathetic nerve impulses, presumably at the efferent nerve terminals; it interferes with powerful vasoconstrictor reflexes to the limb while apparently reducing or eliminating their neurogenic tone. Since it has only mild hypotensive activity in normotensive animals and produces a minimum of early sympathetic-like actions following intravenous administration, it seemed a potential drug against those peripheral vascular diseases where sympathetic hyperactivity is suspected to play a part. As a result of these findings a representative array of related piperazines and higher homologs of piperazine was prepared for pharmacological testing.

In addition to the 4-methyl derivative, several other 4-alkyl and 4-phenyl piperazines proved to have cardiovascular properties. With

(1) For the preceding paper in this series, see R. P. Mull, M. E. Egbert, and M. R. Dapero, *J. Org. Chem.*, **25**, 1953 (1960).

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(4) The compounds were most easily tested by administering 15 to 30 mg./kg. of the test substance to dogs. Active compounds produced the following effects which were graded for their intensity: (a) relaxation of the nictitating membranes, (b) bradycardia, (c) blockade of carotid occlusion reflex pressor responses and (d) suppression of the pressor response to intravenously administered amphetamine. For a more detailed description of the pharmacological test methods see R. A. Maxwell, S. D. Ross, and A. J. Plummer, *J. Pharmacol. Exptl. Therap.*, **123**, 128 (1958), and R. A. Maxwell, A. J. Plummer, F. Schneider, H. Povalski, and A. I. Daniels, *ibid.*, **128**, 22 (1960).

the latter, introduction of methylene groups between the phenyl and piperazine ring caused a decrease of activity. Modifications at the 1-position of piperazine produced variations in activity similar to those noted with the hexahydro-1-azepine and octahydro-1-azocine compounds,^{1,5} *i.e.*, optimal activity was associated with the ethyl guanidine side chain. Unlike the postulated factor controlling the activity of pempidine, *i.e.*, the shielding effect of the methyl groups around the basic nitrogen rather than ring size,⁶ [2-(4-methyl-*cis*-2,5-dimethyl-1-piperazinyl)-ethyl]-guanidine sulfate was less active than the corresponding unsubstituted ring compound in which the 2 and 5 positions were not substituted. In this regard too it is interesting to note that quaternization of both hexahydro-1-azepinylpropionamidoxime and [2-(octahydro-1-azocinyl)-ethyl]-guanidine hydriodide resulted in a decrease of activity.⁷

To examine the influence of large ring systems, a few hexahydro-1,4-diazepines and octahydro-1,5-diazocines were prepared. Although cardiovascular activity was noted, their pharmacological properties were not greater than the corresponding piperazine compounds. This is in contrast to the remarkable increase in activity obtained with the previously described hexahydro-1-azepine and octahydro-1-azocine series.

Acknowledgment.—The authors wish to express their appreciation to Mr. Louis Dorfman and his associates for the microanalyses.

Experimental⁸

1-Substituted Piperazines.—These intermediates were obtained from commercial sources or prepared by known methods as demonstrated by the examples given for the hexahydro-1,4-diazepines.

Hexahydro-1-methyl-1,4-diazepine.—The formyl compound, which was obtained from commercially available hexahydro-1,4-diazepine according to the method of Horrom and co-workers,⁹ was reduced with lithium aluminum hydride¹⁰ to give the desired compound in 42% yield, b.p. 70–73° (32 mm.) [lit.¹¹ 74–75° (35 mm.)].

1-Benzyl-hexahydro-1,4-diazepine.—Prepared in 56% yield from equal molar

(5) R. P. Mull, P. Schmidt, M. R. Dapero, J. Higgins, and M. J. Weisbach, *J. Am. Chem. Soc.*, **80**, 3769 (1958).

(6) L. Bretherick, G. E. Lee, E. Lunt, and W. R. Wragg, *Nature*, **184**, 1707 (1959).

(7) Previously unpublished results from these laboratories. The methiodide of the former was recrystallized from ethanol, m.p. 115–117°. *Anal.* Calcd. for $C_{10}H_{12}IN_2O$: C, 36.73; H, 6.78; N, 12.85; I, 38.81. Found: C, 36.57; H, 6.80; N, 13.02; I, 38.59. The methiodide of the latter was recrystallized from ethanol, m.p. 167–170°. *Anal.* Calcd. for $C_{11}H_{12}I_2N_4$: C, 28.23; H, 5.60; N, 11.97; I, 54.20. Found: C, 28.46; H, 5.69; N, 12.27; I, 54.52.

(8) Boiling points and melting points are uncorrected.

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TABLE I

PHYSICAL PROPERTIES OF THE NITRILES

R	m	n	x	Yield %	M.p. or b.p., °C. mm.	n _D ²⁰	t	Molecular formula	R—N $\left[\begin{array}{c} \text{---}(\text{CH}_2)_m\text{---} \\ \text{---}(\text{CH}_2)_n\text{---} \end{array} \right]$ N $\left[\begin{array}{c} \text{---}(\text{CH}_2)_x\text{---} \\ \text{---}(\text{CH}_2)_y\text{---} \end{array} \right]$ CN		Carbon, %		Hydrogen, %		Nitrogen, %	
									Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
CH ₃ ^a	2	2	1	80	120–125 ^b	12		C ₇ H ₁₃ N ₃	60.48	60.52	9.43	9.50	30.23	30.34		
CH ₃	2	2	2	67	88–92 ^c	0.7		C ₈ H ₁₆ N ₃	62.80	62.62	9.88	9.78				
C ₂ H ₅	2	2	1	73	117–118	13	1.4732	C ₈ H ₁₅ N ₃	62.80	62.55	9.88	9.92				
(C ₂ H ₅) ₂ NCH ₂ CH ₂	2	2	1	69	115–116	0.5	1.4788	C ₁₂ H ₂₄ N ₄	64.34	64.74	10.80	10.94	25.01	25.28		
NOCH ₃	2	2	1	90	165–166 ^{d,e}			C ₈ H ₁₇ N ₄	58.59	58.40	6.57	6.48	34.17	33.95		
C ₆ H ₅	2	2	1	95	65–66 ^f			C ₁₂ H ₁₆ N ₃	71.70	71.53	7.52	7.36	20.91	20.72		
o-ClC ₆ H ₄	2	2	1	70	109–111 ^d			C ₁₂ H ₁₄ ClN ₃ ^g					17.85	17.70		
p-ClC ₆ H ₄	2	2	1	67	120–125 ^h			C ₁₂ H ₁₄ ClN ₃	61.20	61.24	6.00	5.94	17.85	17.84		
o-CH ₃ OC ₆ H ₄	2	2	1	66	68–70 ⁱ			C ₁₃ H ₁₇ N ₃ O	67.50	67.88	7.41	7.78				
m-CH ₃ OC ₆ H ₄	2	2	1	68	89–92 ^d			C ₁₃ H ₁₇ N ₃ O	67.50	67.25	7.41	7.38				
o-CH ₃ C ₆ H ₄	2	2	1	78	114–117 ^d			C ₁₃ H ₁₇ N ₃	72.62	72.31	7.97	7.90				
C ₆ H ₅ CH ₂	2	2	1	73	152–155	0.2	1.5422	C ₁₃ H ₁₇ N ₃	72.62	72.80	7.97	8.10	19.55	19.33		
C ₆ H ₅ CH ₂ CH ₂	2	2	1	70	170–172	13	1.5319	C ₁₄ H ₁₉ N ₃	73.42	73.92	8.36	8.43	18.35	18.69		
C ₆ H ₅ N ⁱ	2	2	1	77	120–122	0.5		C ₁₄ H ₁₉ N ₄	65.40	65.81	6.99	6.71	27.74	27.69		
C ₆ H ₅ N ⁱ	2	2	3	72	141–143	1		C ₁₃ H ₁₈ N ₄	67.88	67.52	7.89	7.79	24.36	24.56		
CH ₃	2	3	1	40	68–72	0.2	1.4831	C ₁₄ H ₂₀ N ₃	62.80	62.78	9.88	9.70	27.47	27.32		
C ₆ H ₅	2	3	1	88	156–158	1.2	1.5365	C ₁₄ H ₁₉ N ₃	73.32	73.67	8.55	8.57	18.33	17.96		
CH ₃	3	3	1	49	83–85	0.4	1.4837	C ₉ H ₁₇ N ₃	67.72	67.32	10.26	10.28	25.16	24.79		
C ₆ H ₅	3	3	1	57	147–149	0.25	1.5350	C ₁₃ H ₂₁ N ₃	74.14	74.04	8.71	9.09	17.29	16.96		

^a The corresponding *cis*-2,5-dimethylpiperazine was obtained in 53% yield, b.p. 125–126° (13 mm.), *n*_D²⁰ 1.4711. *Anal.* Calcd. for C₉H₁₇N₃: C, 64.72; H, 10.26; N, 25.16. Found: C, 64.58; H, 10.55; N, 24.77. ^b Solidified on cooling, m.p. 53–56°. ^c Rice, L. M. and Grogan, C. H., *J. Org. Chem.*, **20**, 1687 (1955), recorded b.p. 68–72° (0.3 mm.). ^d Recrystallized from ethanol. ^e Adelson, D. E. and Pollard, C. B., *J. Am. Chem. Soc.*, **57**, 1280 (1935), recorded m.p. 165°. ^f Recrystallized from hexane. ^g *Anal.* Calcd.: Cl, 15.04. Found: Cl, 14.91. ^h Recrystallized from heptane. ⁱ 2-Pyridyl.

TABLE II

PHYSICAL PROPERTIES OF THE AMINES															
$\begin{array}{c} \text{R}-\text{N} \begin{array}{c} \text{---} (\text{CH}_2)_m \text{---} \\ \text{---} (\text{CH}_2)_n \text{---} \end{array} \text{N}-(\text{CH}_2)_x \text{NH}_2 \end{array}$															
R	m	n	x	Yield %	B.p.		Molecular formula		Carbon, %		Hydrogen, %		Nitrogen, %		
					°C.	mm.	<i>t</i>		Calcd.	Found	Calcd.	Found	Calcd.	Found	
CH ₃ ^a	2	2	2	47	87-89	16	1.4779	26	C ₇ H ₁₇ N ₃	58.79	58.50	11.98	11.86	29.39	29.68
CH ₃	2	2	3	43	107-109 ^b	14			C ₈ H ₁₉ N ₃	61.20	61.18	12.20	12.00	26.77	26.68
C ₂ H ₅	2	2	2	56	97-98	13	1.4777	26	C ₈ H ₁₉ N ₃	61.20	61.07	12.20	11.96	26.77	26.54
(C ₂ H ₅) ₂ CHCH ₂ CH ₂	2	2	2	68	95-96	0.3	1.4814	26	C ₁₂ H ₂₃ N ₄	63.21	63.12	12.38	12.26	24.58	24.19
H ₂ NCH ₂ CH ₂	2	2	2	38	140-141	15	1.4997	26	C ₈ H ₂₀ N ₄	55.86	55.39	11.72	11.35	32.58	32.50
C ₄ H ₉	2	2	2	37	170-175	14			C ₁₂ H ₁₉ N ₃	70.30	70.15	10.34	10.18	20.50	20.38
<i>o</i> -ClC ₆ H ₄	2	2	2	77	130-134	0.1	1.5694	20	C ₁₃ H ₁₈ ClN ₃	60.11	60.60	7.58	7.58		
<i>p</i> -ClC ₆ H ₄	2	2	2	72	146-158 ^c	0.3			C ₁₃ H ₁₈ ClN ₃	60.11	60.89	7.58	7.21	17.51	17.18
<i>o</i> -CH ₂ OC ₆ H ₄	2	2	2	78	133-136	0.05			C ₁₃ H ₂₁ N ₃ O	66.35	65.97	9.00	9.17		
<i>m</i> -CH ₂ OC ₆ H ₄	2	2	2	71	152-157	0.05	1.5704	20	C ₁₃ H ₂₁ N ₃ O	66.35	66.31	9.00	8.90		
<i>o</i> -CH ₃ C ₆ H ₄	2	2	2	86	104-107	0.05	1.5539	20	C ₁₃ H ₂₁ N ₃					19.19	19.07
C ₆ H ₅ CH ₂	2	2	2	80	180-187	13	1.5361	26	C ₁₃ H ₂₁ N ₃	71.29	71.85	9.67	9.76	19.19	18.83
C ₆ H ₅ CH ₂ CH ₂	2	2	2	62	157-158	14	1.5362	27	C ₁₄ H ₂₃ N ₃	72.15	72.14	9.95	9.78	18.04	18.22
C ₆ H ₅ N ^d	2	2	2	32	112-115	0.05			C ₁₁ H ₁₈ N ₄	64.13	64.15	8.81	8.65	27.20	27.41
C ₃ H ₇ N ^d	2	2	2	82	113-115	0.05			C ₁₃ H ₂₂ N ₄	66.72	66.93	9.48	9.43	23.95	24.14
CH ₃	2	3	2	76	104-105	13	1.4886	23	C ₉ H ₁₉ N ₃	61.20	60.77	12.20	12.23	26.77	27.41
C ₆ H ₅	2	3	2	67	125-126	0.3	1.5381	27	C ₁₄ H ₂₃ N ₃	72.16	71.44	9.95	10.12	18.04	17.89
CH ₃	3	3	2	56	112-117	13	1.4889	28	C ₉ H ₂₁ N ₃	63.21	62.91	12.38	12.41	24.58	25.14
C ₆ H ₅	3	3	2	61	134-137	0.3	1.5347	26	C ₁₃ H ₂₃ N ₃	72.94	72.81	10.20	10.44	17.01	17.11

^a The corresponding *cis*-2,5-dimethylpiperazine was obtained in 56% yield, b.p. 102-103° (13 mm.), *n*_D²⁰ 1.4741. *Anal.* Calcd. for C₅H₁₃N₂: C, 63.21; H, 12.38; N, 24.58. Found: C, 63.52; H, 12.42; N, 24.70. ^b Rice, L. M. and Grogan, C. H., *J. Org. Chem.*, 20, 1887 (1955), recorded b.p. 52° (0.3 mm.). ^c Solidified on cooling, m.p. 97-101°. ^d 2-Pyridyl.

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TABLE III

PHYSICAL PROPERTIES OF THE GUANIDINES

R	m	n	x	Yield %	M. p., °C. dec.	Molecular formula	Carbon, %		Hydrogen, %		Nitrogen, %	
							Calcd.	Found	Calcd.	Found	Calcd.	Found
H	2	2	2	60	274-340	C ₇ H ₁₉ N ₅ O ₄ S ^c	31.25	31.39	7.06	6.92	26.04	26.12
CH ₃ ^d	2	2	2	51	182-185	C ₈ H ₂₀ N ₅ O ₄ S ^e	38.13	38.53	8.40	8.14	27.79	27.49
CH ₃	2	2	3	80	99-100	C ₉ H ₂₁ N ₅ O ₄ S ^e	36.38	35.97	7.80	7.78	23.56	23.24
C ₂ H ₅	2	2	2	90	201-203	C ₁₀ H ₂₄ N ₅ O ₄ S ^f	43.58	43.42	8.94	9.14	28.24	28.48
HOCH ₂ CH ₂	2	2	0	66	260-290	C ₁₄ H ₃₄ N ₅ O ₄ S	35.10	35.37	8.00	8.04	23.40	23.78
(C ₂ H ₅) ₂ NCH ₂ CH ₂	2	2	2	89	208-212	C ₂₀ H ₄₂ N ₅ O ₄ S ^f	48.87	48.79	9.78	10.09	26.31	26.34
H ₂ N-C=NH	2	2	2	75	253-300	C ₈ H ₂₁ N ₇ O ₄ S ^f	30.84	30.65	6.80	6.71	31.48	30.99
H ₂ NC(=NH)NHCH ₂ CH ₂	2	2	2	68	225-295	C ₁₀ H ₂₄ N ₈ O ₄ S ^g	26.57	26.39	6.24	6.85	24.79	25.02
C ₆ H ₅	2	2	2	78	256-258	C ₂₆ H ₄₄ N ₅ O ₄ S ^c	52.66	51.45	7.48	7.29	23.64	23.96
o-ClC ₆ H ₄	2	2	2	87	258-259	C ₂₈ H ₄₂ Cl ₂ N ₅ O ₄ S ^c	47.17	46.81	6.40	6.72	21.15	21.01
p-ClC ₆ H ₄	2	2	2	70	250-265	C ₂₈ H ₄₀ Cl ₂ N ₅ O ₄ S					21.15	20.69
o-CH ₃ OC ₆ H ₄	2	2	2	84	263-265	C ₂₈ H ₄₀ N ₅ O ₆ S ^h					20.48	20.16
m-CH ₃ OC ₆ H ₄	2	2	2	84	249-251	C ₂₈ H ₄₀ N ₅ O ₆ S ⁱ					20.48	20.21
o-CH ₃ C ₆ H ₄	2	2	2	79	264-267	C ₂₈ H ₄₀ N ₅ O ₆ S ^j					22.59	22.72
C ₆ H ₅ CH ₂	2	2	2	81	185-190	C ₂₃ H ₄₃ N ₅ O ₄ S	54.24	53.84	7.80	8.08	22.59	22.29
C ₆ H ₅ CH ₂ CH ₂	2	2	2	68	266-271	C ₂₉ H ₅₂ N ₅ O ₄ S	55.56	55.65	8.02	8.18	21.60	21.30
C ₆ H ₅ N [*]	2	2	2	65	272-274	C ₂₄ H ₃₂ N ₅ O ₄ S	48.53	48.47	7.13	6.87	28.28	28.61
C ₆ H ₅ N [*]	2	2	4	63	280-283	C ₂₃ H ₅₀ N ₅ O ₄ S	51.74	51.45	7.75	7.78	25.84	25.92
CH ₃	2	3	2	75	142-145	C ₁₈ H ₄₄ N ₅ O ₄ S	43.98	44.02	8.94	9.27	28.24	28.39
C ₆ H ₅	2	3	2	51	205-207	C ₂₀ H ₃₂ N ₅ O ₄ S ^j	55.60	55.28	8.09	8.27	21.62	21.60
CH ₃	3	3	2	54	198-215	C ₂₀ H ₄₆ N ₅ O ₄ S ^e	45.84	45.62	9.23	9.17	26.73	26.56
C ₆ H ₅	3	3	2	56	188-191	C ₂₂ H ₄₆ N ₅ O ₄ S	56.77	56.86	8.34	8.37	20.73	20.41

FOOTNOTES TO TABLE III

^a The recrystallizations were from ethanol-water unless otherwise noted. ^b Obtained as Base·H₂SO₄, all others are (Base)₂·H₂SO₄. ^c Recrystallized from water. ^d The corresponding *cis*-2,5-dimethylpiperazine was obtained in 70% yield, recrystallized from ethanol-ether, m.p. 225–231°. *Anal.* Calcd. for C₂₀H₂₈N₂O₂S: C, 45.84; H, 9.23; N, 26.73. Found: C, 45.56; H, 9.04; N, 26.82. ^e Recrystallized from methanol-ether. ^f Recrystallized from ethanol-ether. ^g *Anal.* Calcd.: Cl, 10.74. Found: Cl, 10.41. ^h *Anal.* Calcd.: S, 4.91. Found: S, 4.96. ⁱ *Anal.* Calcd.: S, 4.91. Found: S, 5.19. ^j *Anal.* Calcd.: S, 5.17. Found: S, 4.83. ^k 2-Pyridyl.

quantities of hexahydro-1,4-diazepine and benzyl chloride in ethanol according to the procedure of Baltzly and co-workers,¹² b.p. 91–101° (0.2 mm.), *n*_D²⁰ 1.5484.

Anal. Calcd. for C₁₂H₁₈N₂: C, 75.79; H, 9.47; N, 14.74. Found: C, 75.62; H, 9.16; N, 15.04.

Octahydro-1-methyl-1,5-diazocine.—Prepared in 46% yield from octahydro-1,5-diazocine¹³ in the manner described for the corresponding hexahydro-1-methyl-1,4-diazepine, b.p. 72–75° (12 mm.), [lit.¹⁴ 76–79° (18 mm.)].

1-Benzyl-octahydro-1,5-diazocine.—Prepared in 36% yield from octahydro-1,5-diazocine in the manner described for the corresponding 1-benzyl-hexahydro-1,4-diazepine, b.p. 97–100° (0.25 mm.), *n*_D²⁵ 1.5394.

Anal. Calcd. for C₁₈H₂₆N₂: C, 76.53; H, 9.88; N, 13.78. Found: C, 76.09; H, 9.77; N, 13.58.

Preparation of the Nitriles of Table I.—The nitriles were prepared by refluxing equimolecular quantities of the 1-substituted piperazine and a halonitrile in benzene containing an excess of anhydrous sodium carbonate in suspension. Vigorous stirring and heating was continued for 6 hr. The reaction mixture was filtered hot, concentrated *in vacuo* and fractionated.

Preparation of the Amines of Table II.—In a typical example, 0.08 mole of the nitrile in 50 ml. of ether was added slowly to a cooled solution of 0.12 mole of lithium aluminum hydride in 200 ml. of ether. The solution was then refluxed for 3 hr. and stirred at room temperature overnight. The reaction mixture was cooled and decomposed by carefully adding water and 20% sodium hydroxide. After filtration and concentration of the ether solution, the residual oil was fractionated *in vacuo*.

Preparation of the Guanidines of Table III.—A mixture of 0.01 mole of 2-methyl-2-thiopseudourea sulfate and 0.02 mole of the amine in a minimum amount of water was refluxed for 4 hr. The solid which separated upon cooling or concentration was purified by recrystallization.

(12) R. Baltzly, J. S. Buck, E. Lorz, and W. Schön, *ibid.*, **66**, 263 (1944).

(13) E. L. Buhle, A. M. Moore and F. Y. Wiselogle, *ibid.*, **65**, 29 (1943).

(14) J. Hernandez-Mora, Dissertation, University of Michigan, Ann Arbor, 1959.

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

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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-[(3S)-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 $\mu\text{g/mL/min}$ 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 $\mu\text{g/mL/min}$. 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 $\mu\text{g/kg/min}$, respectively. The doses of **3** and **1** that prolong bleeding time to three times the basal value, $\text{BT}(\times 3)$, are 1.4 and 0.35 $\mu\text{g/kg/min}$, respectively. The dose ratios for $\text{BT}(\times 3)$ 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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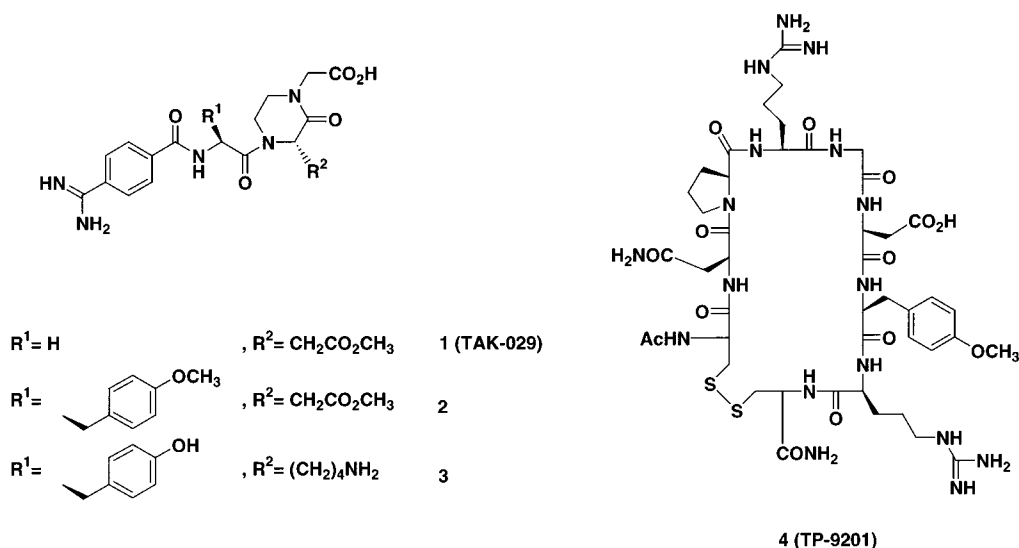


Figure 1.

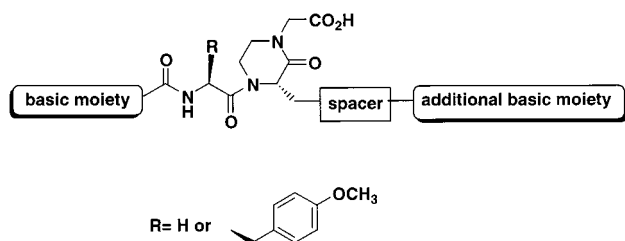
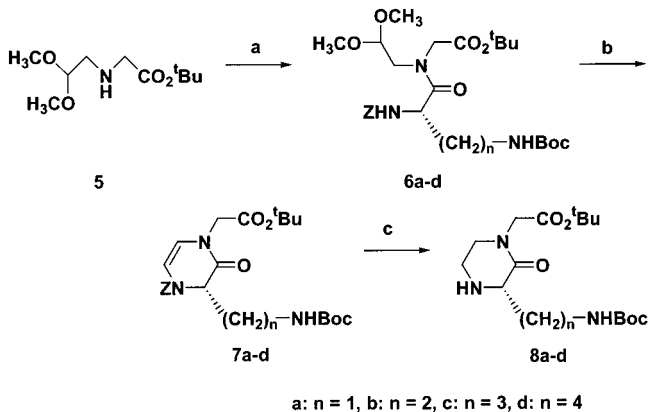


Figure 2. Synthetic design of dibasic compounds.

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 mimetics¹⁰ were prepared by Dimaio's method with slight modification (Scheme 1). Condensation of the secondary amine **5** with ω -Boc-amino- α -Z-aminoalkanoic acids¹³ using 1-ethyl-3-[3-(*N,N*-dimethylamino)propyl]carbodiimide hydrochloride (EDC) in methylene

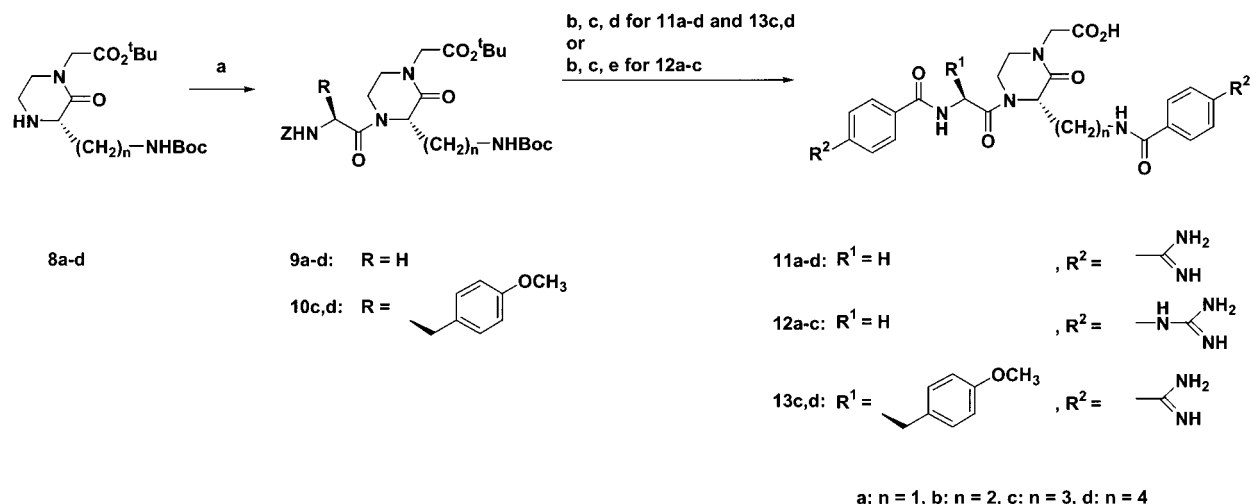
chloride (CH_2Cl_2) provided the acetal amides **6a–d**. Acid-catalyzed intramolecular cyclization of **6a–d** in toluene at 70 °C followed by hydrogenation in the presence of 10% Pd-C in ethyl acetate (EtOAc) afforded the 1,3-disubstituted 2-oxopiperazine derivatives **8a–d**.

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.

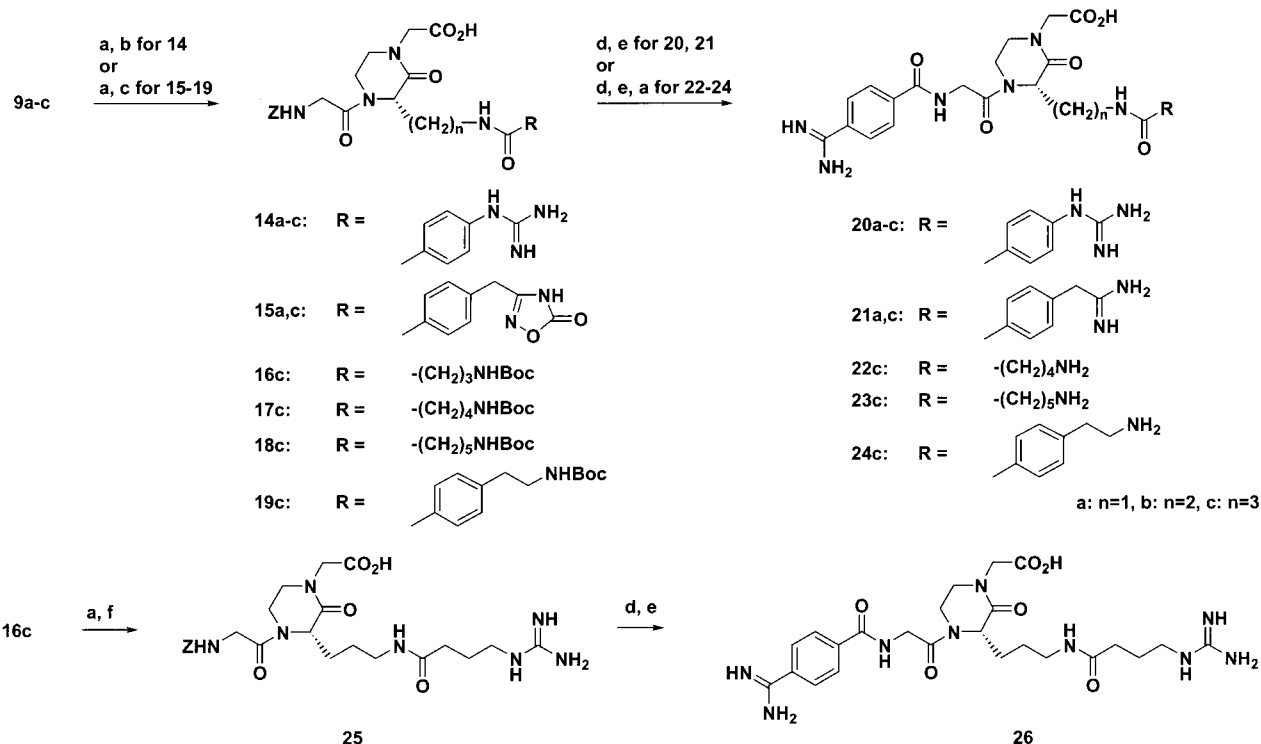
The 4-[2-[(4-amidinobenzoyl)amino]acetyl]-2-oxopiperazine derivatives **20a–c**, **21a,c**, **22c–24c**, and **26** were synthesized from **9a–c** (Scheme 3). Treatment of **9a–c** with trifluoroacetic acid (TFA) followed by condensation with various carboxylic acids gave the amides **14–19**. Removal 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) $\text{CF}_3\text{CO}_2\text{H}$, 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

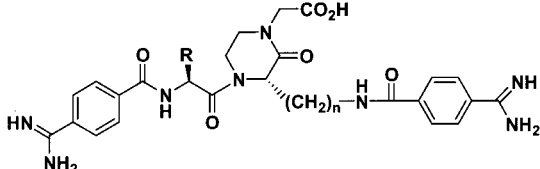
^a Reagents: (a) $\text{CF}_3\text{CO}_2\text{H}$, toluene; (b) *N*-(4-guanidinobenzoyloxy)-5-norbornene-2,3-dicarboxyimide hydrochloride, NaHCO_3 , H_2O , 1,4-dioxane; (c) RCO_2H , *N*-hydroxy-5-norbornene-2,3-dicarboxyimide (HONB) or *N*-hydroxysuccinimide (HOSU), Et_3N , EDC or 1,3-dicyclohexylcarbodiimide (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*-methylisothiurea sulfate, NaOH , H_2O .

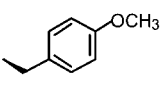
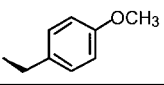
diimidazole, and saponification gave the oxadiazolone 32.

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 $\text{BT}(\times 3)$ to the ID_{50} was used as a parameter to represent the dissociation ratio.

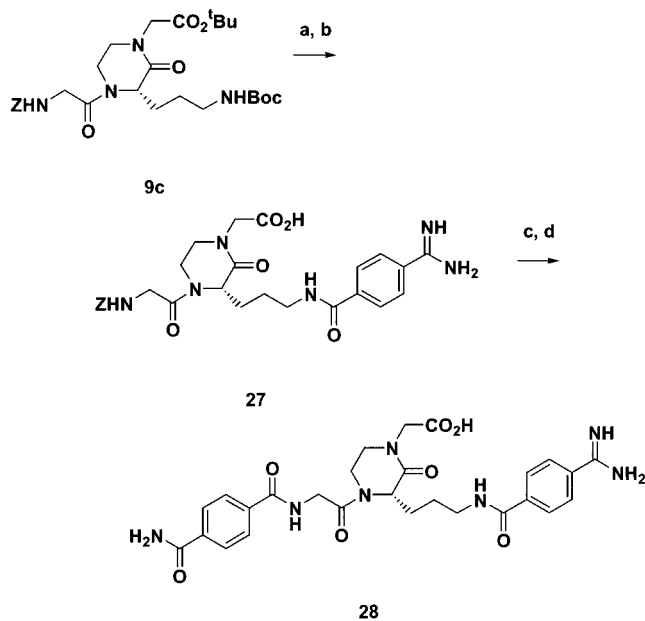
First, we examined the influence of the 4-methoxybenzyl group, which enhanced the inhibitory effect on platelet aggregation in a series of 2-oxopiperazine derivatives,¹¹ upon the dissociation profile by comparing 11c,d with 13c,d. The 4-amidinophenyl group was employed for the additional basic moiety, 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 ($\text{R} = \text{H}$, 11c,d) showed more favorable dissociation profiles than 4-methoxybenzyl substituted compounds ($\text{R} = \text{CH}_2\text{-Ph(4-OMe)}$, 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


compound	n	R	atom numbers ^a	inhibition of platelet aggregation ^b		effect on bleeding time (BT)		dissociation ratio ^c
				in vitro IC ₅₀ (nM)	ex vivo ID ₅₀ (μg/kg/min)	dose (μg/kg/min)	BT ^e	
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.

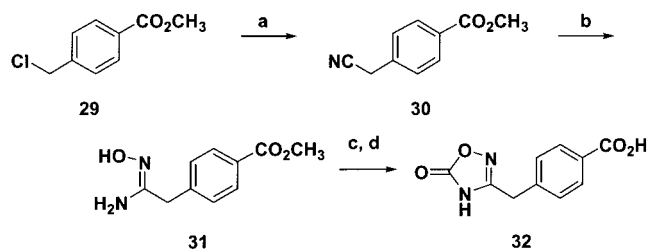
^b The concentrations of each compound inhibiting the in vitro or ex vivo ADP-induced aggregation of guinea pig platelets by 50%. The 95% confidence intervals are shown in parentheses, $n = 2-5$. ^c Bleeding time at the denoted dose, mean $\pm$ SE, $n = 3$. ^d All guinea pigs prolonged the bleeding time over 900 s. ^e Dose ratio for dose causing prolongation of bleeding time to 3 times the basal value (BT($\times 3$)) vs ID₅₀ 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) CF₃CO₂H, toluene; (b) 4-amidinobenzoyl chloride hydrochloride, NaHCO₃, 1,4-dioxane, H₂O; (c) H₂, 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) NH₂OH·HCl, NaHCO₃, MeOH; (c) 1,1'-carbonyldiimidazole, 1,8-diazabicyclo[5.4.0]-undec-7-ene, 1,4-dioxane; (d) NaOH, H₂O, 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, IC₅₀: 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 IC₅₀ values of 19 nM and ID₅₀ 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 (IC₅₀) and 0.12 to 0.94 μg/kg/min (ID₅₀), 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		effect on bleeding time (BT)		dissociation ratio ^c
				in vitro IC ₅₀ (nM)	ex vivo ID ₅₀ (μg/kg/min)	dose (μg/kg/min)	BT ^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 2.5	410±78 820±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 1.4	490±56 >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 6.0	270±17 860±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 π -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)/ID₅₀ > 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		effect on bleeding time (BT)		dissociation ratio ^c
			<i>in vitro</i>	<i>ex vivo</i>	dose	BT ^c	
			IC ₅₀ (nM)	ID ₅₀ (μg/kg/min)	(μg/kg/min)		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, IC₅₀: 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 ID₅₀ 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 IC₅₀ 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

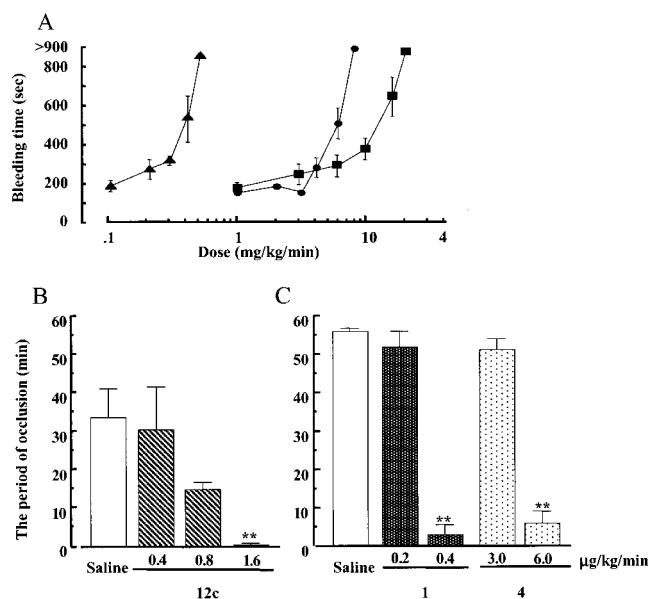


Figure 3. Effects of **12c**, **1**, and **4** on bleeding time and thrombus formation in guinea pigs. Template bleeding time in guinea pigs dosed with **12c** (●), **1** (▲), and **4** (■) (A). Baseline value of bleeding time was 183 s ($n = 8$). Antithrombotic effects of **12c** (B), **1**, and **4** (C) on balloon injury-induced carotid thrombosis model in guinea pigs. Data are expressed as mean $\pm$ 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 $4.3 \mu\text{g/kg/min}$, respectively). Intravenous infusion of **12c** at $0.8 \mu\text{g/kg/min}$ or $1.6 \mu\text{g/kg/min}$ showed ca. 60% inhibition and complete inhibition of thrombus formation, respectively, without prolongation of bleeding time. While **1** at $0.4 \mu\text{g/kg/min}$ showed complete antithrombotic 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 $100 \mu\text{g/kg}$ 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 $30-100 \mu\text{g/kg}$ 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

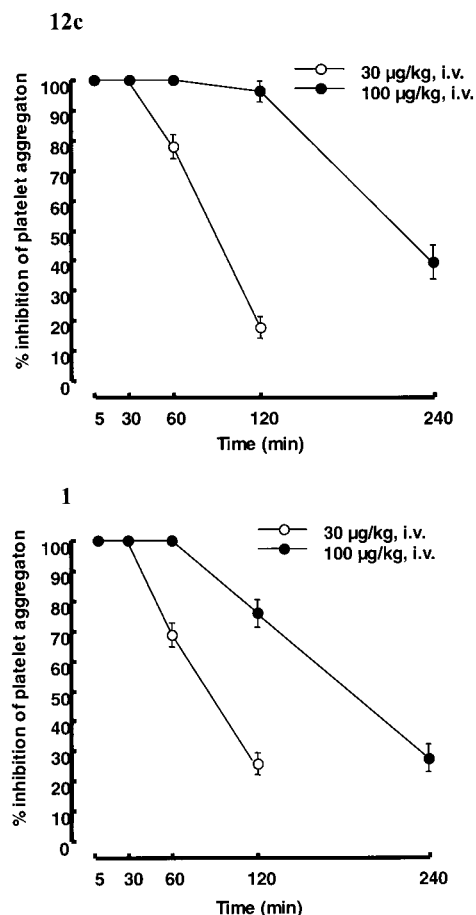


Figure 4. Time course of the inhibitory effects of **12c** and **1** on ex vivo platelet aggregation in guinea pig. At various time intervals after intravenous injection of drugs, ex vivo platelet aggregation induced by ADP was examined. Each point represents the mean $\pm$ SE of 3-7 animals.

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

species	IC_{50} (nM) ^a	
	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 following ADP concentrations were used: human, $1-10 \mu\text{M}$; monkey, $30-40 \mu\text{M}$; guinea pig, $0.8-1 \mu\text{M}$. ^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)

aggregating agent	IC_{50} (nM) ^a	
	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, $1-10 \mu\text{M}$; collagen, $1-3 \mu\text{g/mL}$; PAF, $1-10 \mu\text{M}$; TRAP, $10-30 \mu\text{M}$. ^b Data obtained from ref 15 for comparison purposes.

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**.

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 antithrombotic 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. ¹H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer. Chemical shifts are given in δ 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 $\pm 0.4\%$ 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 CH₂Cl₂ (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 MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 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; ¹H NMR (CDCl₃) δ 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 MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, hexane:EtOAc = 2:1) to give **7c** (8.3 g, 65%) as a colorless oil: IR (neat) cm⁻¹ 1740, 1713, 1685, 1155; ¹H NMR (CDCl₃) δ 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; [α]_D²⁰ –29.3° (*c* = 0.73, H₂O); IR (KBr) cm⁻¹ 3400, 2970, 1740, 1660, 1520, 1365; ¹H NMR (CD₃OD) δ 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. (C₁₈H₃₃N₃O₅·C₂H₂O₄) 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); ¹H NMR (CDCl₃) δ 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. (C₁₇H₃₁N₃O₅·C₂H₂O₄) 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; [α]_D²⁰ –29.0° (*c* = 1.02, DMSO); IR (KBr) cm⁻¹ 2978, 1706, 1650, 1365, 1163; ¹H NMR (D₂O) δ 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. (C₁₉H₃₅N₃O₅·C₂H₂O₄) 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 CH₂Cl₂ (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 KHSO₄ and saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by column chromatography (SiO₂, 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) δ 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-[(3S)-4-(2-[(Benzyloxycarbonyl)amino]acetyl)-3-[[*tert*-butoxycarbonyl]amino]methyl]-2-oxopiperazinyl]acetate (9a**):** colorless oil (yield 77% from **7a**); ^1H NMR (CDCl_3) δ 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-[(3S)-4-(2-[(Benzyloxycarbonyl)amino]acetyl)-3-[[*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) δ 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-[(3S)-4-(2-[(Benzyloxycarbonyl)amino]acetyl)-3-[[*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) δ 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-[(3S)-4-[(2S)-2-[(Benzyloxycarbonyl)amino]-3-(4-methoxyphenyl)propanoyl]-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) δ 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-[(3S)-4-[(2S)-2-[(Benzyloxycarbonyl)amino]-3-(4-methoxyphenyl)propanoyl]-3-[[*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) δ 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-guanidinobenzoyloxy 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-[(3S)-4-[2-[(4-Guanidinobenzoyl)amino]acetyl]-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 $^\circ\text{C}$, 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 mixture 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 $^\circ\text{C}$; $[\alpha]_D^{20} +61.8^\circ$ ($c = 1.00$, H_2O , on the anhydrous basis); IR (KBr) cm^{-1} 3287, 1730, 1634, 1557, 1505, 1449, 1323, 1262, 1179; ^1H NMR (D_2O) δ 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. ($\text{C}_{27}\text{H}_{34}\text{N}_{10}\text{O}_6 \cdot 2\text{HCl} \cdot 2\text{H}_2\text{O}$) C, H, N.

2-[(3R)-4-[2-[(4-Guanidinobenzoyl)amino]acetyl]-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; $[\alpha]_D^{20} -45.1^\circ$ ($c = 0.40$, MeOH). Anal. ($\text{C}_{27}\text{H}_{34}\text{N}_{10}\text{O}_6 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$) C, H, N.

2-[(3S)-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**); $[\alpha]_D^{20} +60.2^\circ$ ($c = 0.54$, MeOH); IR (KBr) cm^{-1} 1640, 1550, 1490; ^1H NMR (D_2O) δ 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. ($\text{C}_{25}\text{H}_{28}\text{N}_8\text{O}_6 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$) C, H, N.

2-[(3S)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[[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**); $[\alpha]_D^{20} +30.3^\circ$ ($c = 0.47$, H_2O); ^1H NMR (CD_3OD) δ 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. ($\text{C}_{26}\text{H}_{30}\text{N}_8\text{O}_6 \cdot \text{CF}_3\text{CO}_2\text{H} \cdot 3\text{H}_2\text{O}$) C, H, N.

2-[(3S)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-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**); $[\alpha]_D^{20} +41.9^\circ$ ($c = 0.73$, MeOH); IR (KBr) cm^{-1} 3280, 1630, 1545, 1480, 1380, 1290; ^1H NMR (D_2O) δ 1.50–2.20 (4H, m), 3.20–5.00 (11H, m), 7.70–8.00 (8H, m). Anal. ($\text{C}_{27}\text{H}_{32}\text{N}_8\text{O}_6 \cdot \text{CF}_3\text{CO}_2\text{H} \cdot 2\text{H}_2\text{O}$) C, H, N.

2-[(3S)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[[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**); $[\alpha]_D^{20} +44.3^\circ$ ($c = 1.01$, H_2O); ^1H NMR (CD_3OD) δ 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. ($\text{C}_{28}\text{H}_{34}\text{N}_8\text{O}_6 \cdot \text{CF}_3\text{CO}_2\text{H} \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$) C, H, N.

2-[(3S)-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**); $[\alpha]_D^{20} +44.2^\circ$ ($c = 1.03$, H_2O); IR (KBr) cm^{-1} 1650, 1540, 1500; ^1H NMR (D_2O) δ 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-[(3S)-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; $[\alpha]^{20}_D +35.2^\circ$ ($c = 0.65$, H_2O); 1H NMR (CD_3OD) δ 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-[(3S)-4-[2-[(4-Amidinobenzoyl)amino]-3-(4-methoxyphenyl)propionyl]-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**); $[\alpha]^{20}_D +52.8^\circ$ ($c = 0.76$, MeOH); IR (KBr) cm^{-1} 3067, 1634, 1539, 1387, 1302, 1248; 1H NMR (CD_3OD) δ 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-[(3S)-3-[4-[(4-Amidinobenzoyl)amino]butyl]-4-[(2S)-2-[(4-amidinobenzoyl)amino]-3-(4-methoxyphenyl)propionyl]-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**); $[\alpha]^{20}_D +54.5^\circ$ ($c = 0.88$, H_2O); IR (KBr) cm^{-1} 3094, 1634, 1549, 1385, 1302, 1248; 1H NMR (CD_3OD) δ 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-[(3S)-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; 1H NMR (CD_3OD) δ 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-[(3S)-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: $[\alpha]^{20}_D +48.6^\circ$ ($c = 1.02$, H_2O); 1H NMR (CD_3OD) δ 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-[(3S)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[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**); $[\alpha]^{20}_D +46.0^\circ$ ($c = 1.01$, H_2O); IR (KBr) cm^{-1} 1640, 1560, 1500; 1H NMR (D_2O) δ 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-[(3S)-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**); $[\alpha]^{20}_D +26.1^\circ$ ($c = 0.45$, MeOH); IR (KBr) cm^{-1} 3287, 1634, 1559, 1505, 1387, 1304; 1H NMR (D_2O) δ 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-[(3S)-4-[2-[(4-Amidinobenzoyl)amino]acetyl]-3-[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: $[\alpha]^{20}_D +48.4^\circ$ ($c = 0.67$, MeOH); IR (KBr) cm^{-1} 1640, 1545, 1490; 1H NMR (D_2O) δ 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-[(3S)-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**); $[\alpha]^{20}_D +44.1^\circ$ ($c = 1.03$, H_2O); IR (KBr) cm^{-1} 1640, 1550, 1495; 1H NMR (D_2O) δ 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-[(3S)-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 (SiO₂, 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 (CD₃OD) δ 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-[(3S)-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 (CD₃OD) δ 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-[(3S)-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 (CD₃OD) δ 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-[(3S)-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 (CD₃OD) δ 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-[(3S)-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 H₂O (8.4 mL) were added successively NaHCO₃ (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; H₂O to 5% aqueous CH₃CN) to give **23c** (0.26 g, 55%) as a colorless amorphous powder: [α]_D²⁰ +42.7° (*c* = 0.99, MeOH); ¹H NMR (CD₃OD) δ 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. (C₂₅H₃₇N₇O₆·1.1CF₃CO₂H·2H₂O) C, H, N.

2-[(3S)-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**); [α]_D²⁰ +46.0° (*c* = 1.01, MeOH); ¹H NMR (CD₃OD) δ 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. (C₂₄H₃₅N₇O₆·CF₃CO₂H·2.5H₂O) C, H, N.

2-[(3S)-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**); [α]_D²⁰ +42.2° (*c* = 1.00, MeOH); ¹H NMR (CD₃OD) δ 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. (C₂₈H₃₅N₇O₆·CF₃CO₂H·3.5H₂O) C, H, N.

2-[(3S)-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, NaHCO₃ (0.14 g, 1.68 mmol), and H₂O (3.3 mL) was added to a solution of *S*-methylisothiurea 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; H₂O to 15% aqueous CH₃CN) to give **25** as a colorless powder. Under an H₂ 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, H₂O (6.0 mL), and 1,4-dioxane (3.0 mL) were added successively NaHCO₃ (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, H₂O) to give **26** (0.09 g, 25%) as a colorless amorphous powder: [α]_D²⁰ +48.4° (*c* = 0.96, H₂O); ¹H NMR (CD₃OD) δ 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. (C₂₄H₃₅N₉O₆·2HCl·3.5H₂O) C, H, N.

2-[(3S)-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, H₂O (27 mL), and 1,4-dioxane (14 mL) were added successively NaHCO₃ (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; H₂O to 20% aqueous CH₃CN) to give **27** (1.0 g, 71%) as a colorless amorphous powder: [α]_D²⁰ +106.6° (*c* = 0.48, 0.1 N HCl); ¹H NMR (D₂O+DCl) δ 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. (C₂₇H₃₂N₆O₇·2H₂O) C, H, N.

2-[(3S)-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; H₂O to 15% aqueous CH₃CN) to give **28** (0.38 g, 70%) as a colorless amorphous powder: IR (KBr) cm⁻¹ 3252, 1645, 1634, 1559, 1385, 1296; ¹H NMR (D₂O) δ 1.60–2.30 (4H, m), 3.30–5.20 (11H, m), 7.66–7.94 (8H, m). Anal. (C₂₇H₃₁N₇O₇·4H₂O) 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: ^1H NMR (CDCl_3) δ 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 $\text{NH}_2\text{OH}\cdot\text{HCl}$ (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: ^1H NMR ($\text{DMSO}-d_6$) δ 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; ^1H NMR ($\text{DMSO}-d_6$) δ 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. ($\text{C}_{10}\text{H}_6\text{N}_2\text{O}_4\cdot 0.1\text{H}_2\text{O}$) 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 1000g for 3–5 s and 1000g for 20 min at room temperature, respectively. The platelet count in PRP was adjusted to $3 \times 10^5/\mu\text{L}$ (human and monkey) and $4 \times 10^5/\mu\text{L}$ (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 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 agents (25 μL) 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 μL , 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 thromboembolism, 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 $\mu\text{L}/\text{kg}/\text{min}$. In preliminary experiments, we observed that thrombotic occlusion did not occur when platelet depletion ($<2 \times 10^5/\mu\text{L}$) 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.

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