New Platelet Fibrinogen Receptor Glycoprotein IIb-IIIa Antagonists: Orally Active Series of *N*-Alkylated Amidines with a 6,6-Bicyclic Template

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The design, synthesis, and pharmacological evaluation of (*S*)-(−)-ethyl [6-[4-(morpholino-formimidoyl)benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate hydrochloride ((*S*)-**4**·HCl, MS-180), an orally active glycoprotein IIb-IIIa (GPIIb-IIIa) antagonist, are reported. Pharmacophore mapping of amidino and carboxyl groups of already known GPIIb-IIIa antagonists led to the synthesis of nine amidino acids containing 6,6-bicyclic ring skeletons (**10a**−**i**). Among them, the compounds **10a**,**c**,**e** having an amide bond and 1,2,3,4-tetrahydronaphthalene or 3,4-dihydro-2*H*-1-benzopyran skeleton showed marked inhibitions with IC_{50} values of 46−57 nM in human platelet aggregation assay in vitro, but low oral activities. *N*-Alkylation of the amidino group coupled with the ester prodrug approach afforded MS-180 ((*S*)-**4**·HCl), which generates in vivo the corresponding carboxylic acid (*S*)-**3** as an active species. In vitro, (*S*)-**3** inhibited ADP-induced aggregation of guinea pig, dog, and human platelets (IC_{50} = 110, 253, and 35 nM, respectively) and inhibited the binding of fibrinogen to immobilized GPIIb-IIIa of human platelets (IC_{50} = 0.12 nM). After oral administration of MS-180 ((*S*)-**4**·HCl) to fasted beagle dog, ex vivo inhibition of platelet aggregation was observed. The maximal inhibitions were observed 2−4 h after dosing with dose dependency (60% inhibition at a dose of 1 mg/kg, 85% at 3 mg/kg, and 100% at 10 mg/kg, respectively) and the extent of the inhibitions paralleled the plasma concentration of the active species (*S*)-**3**. On the basis of these studies, we selected MS-180 ((*S*)-**4**·HCl) as a candidate for clinical evaluation as a drug for the treatment and prevention of thrombosis in patients.

Introduction

While platelets play a vital role in homeostasis, uncontrolled platelet aggregation leads to thrombus formation and causes thrombotic disorders such as myocardial infarction, transient ischemic attacks, unstable angina, stroke, and peripheral arterial disease.[1,2] Platelets are activated by a variety of agonists including adenosine diphosphate (ADP), collagen, epinephrine, platelet-activating factor (PAF), thrombin, and thromboxane A_2, and the final common stage resulting in platelet aggregation is the binding of fibrinogen to GPIIb-IIIa on the surface of activated platelets.[3−6] Inhibition of platelet aggregation at the activation stage has proven difficult due to the many activation pathways available to platelets. Therefore, inhibition of the binding of fibrinogen to GPIIb-IIIa on the surface of activated platelets, the final stage of platelet aggregation, has become an attractive strategy. In fact, GPIIb-IIIa monoclonal antibody Reo-Pro[7] is now available for use in patients undergoing percutaneous transluminal coronary angioplasty (PTCA) who are at high risk for abrupt artery closure. In addition, a number of GPIIb-IIIa antagonists such as Integrelin,[8] Tirofiban,[9] and Lamifiban[10] are now under clinical trials for intravenous treatment of acute thrombus disorders. The latest tendency has focused on orally active GPIIb-IIIa antagonists useful for chronic thrombotic disorders. A number of the orally active antagonists represented by Xemilofiban,[11] Orbofiban,[12] and Ro 48-3657[13] are now under clinical trials.[14]

Herein, we describe our approach for an orally active GPIIb-IIIa antagonist, designed with the aid of pharmacophore mapping of known GPIIb-IIIa antagonists, synthesis, and quantitative structure-activity relationships (QSAR) using comparative molecular field analysis (CoMFA).[15] From these studies, we obtained an orally active series of *N*-alkylated amidines with a 6,6-bicyclic ring template, culminating with the discovery of MS-180 ((*S*)-**4**·HCl) with a morpholinoformimidoyl group.

Drug Design

Pharmacophore Mapping of Known GPIIb-IIIa Antagonists. Since the three-dimensional structure of GPIIb-IIIa is unknown, we designed several lead compounds from the structures of already known GPIIb-

Chart 1

(S)-**3**: R = H
(S)-**4**: R = C$_2$H$_5$ (MS-180 as HCl salt)
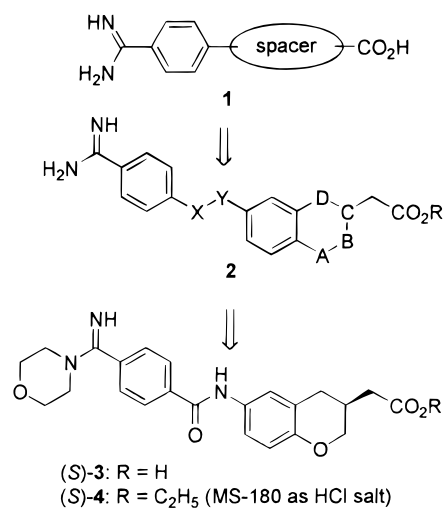
IIIa antagonists using computed pharmacophore mapping. Chart 1 illustrates our approach to MS-180 ((*S*)-**4**·HCl). The structural features of known GPIIb-IIIa antagonists are (1) two functional groups (amidino or guanidino group and carboxyl group) essential for its activity and (2) a spacer which correctly positions the two functional groups. Then, to search the spatial parameters of the two functional groups vital for the activity, we performed pharmacophore mapping using five compounds (Chart 2).[16−20] Alignments of all compounds were achieved by using the DISCO[21] module of SYBYL version 6.3,[22] and the ring structure of DMP 728 (**7**)[18] obtained from the Cambridge Structure Database was used as a template structure.[23] The superimposed structures of known GPIIb-IIIa antagonists obtained from DISCO analysis are shown in Figure 1.

Parameter definitions and pharmacophoric features of the known GPIIb-IIIa antagonists extracted from alignments are summarized in Table 1. The distance between carbon of cationic amidino or guanidino group (C^1) and carbon of anionic carboxyl group (C^2) ranged from 13.5 to 14.5 Å (distance *a*). The distance between amidino group-substituted atom (A) and carboxyl group-substituted atom (B) ranged from 11.5 to 13.0 Å (distance *b*). The α defined as angle C^1−A−B ranged from 150° to 170°, and the β defined as C^2−B−A ranged from 85° to 110°, respectively. We designed compounds
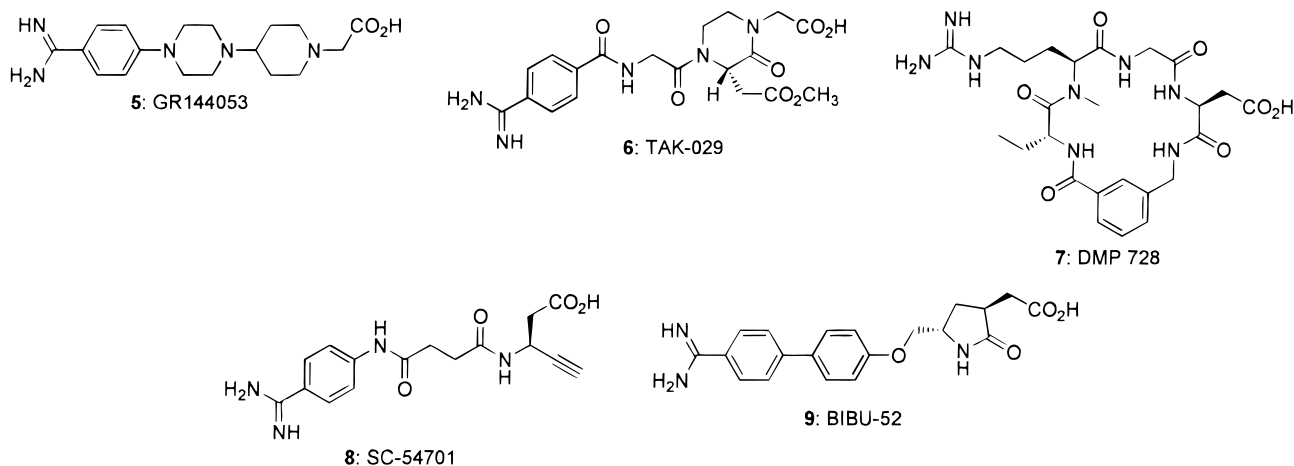
to match the extracted parameters and synthesized nine compounds that have different bond forms and 6,6-bicyclic templates as spacers (Figure 2).

Chemistry

The newly designed GPIIb-IIIa antagonists **10a**−**i** were prepared following the general procedure depicted in Scheme 1. Cyano compound **11** was treated with ethanolic HCl and reacted with ammonium acetate to give the corresponding amidine **12**, which was hydrolyzed to afford amidino acid **10**.

The synthesis of (2-naphthalenyl)acetate and (1,2,3,4-tetrahydro-2-naphthalenyl)acetate intermediates of **11** is depicted in Scheme 2. 2-Oxo-1,2,3,4-tetrahydronaphthalene intermediate **15** was prepared from 1-oxo-1,2,3,4-tetrahydronaphthalene derivative **13** (**13a**,**c** were commercially available, **13b** was synthesized following the method of Gerlach et al.[24]) following a modification of the ketone transposition procedure described by Nichols et al.[25] Reduction of the carbonyl group of **13** with NaBH$_4$, followed by dehydration in the presence of strongly acidic ion-exchange resin (Amberlyst 15) or pyridinium *p*-toluenesulfonate (PPTS), gave olefin **14**. Epoxidation of **14** with 3-chloroperbenzoic acid followed by treatment with ZnI$_2$ gave **15**, which was converted to **17** following a modification of the procedure described by Strasser et al.[26] Horner−Emmons reaction with appropriate phosphonates gave (3,4-dihydro-2-naphthalenyl)acetate **16** with a small amount of olefin isomer α,β-unsaturated ester. This mixture was taken on directly without separation. Hydrogenation of **16** catalyzed by Pd/C gave (1,2,3,4-tetrahydro-2-naphthalenyl)-acetate **17**. The nitro group of **16a** was also converted to the amino group in this hydrogenation condition. Compound **17** was converted to several types of 6,6-bicyclic ring intermediates of **11**. According to the method of Xue et al.,[27] the amino group of **17a** was protected by Boc and *N*-methylated with NaH/MeI in DMF to afford **18**. Cleavage of the Boc protecting group of **18** gave the *N*-methyl derivative **19**. The methyl ester of **17b** was selectively hydrolyzed to afford monoacid **20**. The methyl ether of **17c** was cleaved to afford phenol derivative **21**, which was converted to triflate **22**. Naphthol derivative **23** was synthesized from **16c** via dehydrogenation with *p*-chloranil[26] and cleavage of the methyl ether.
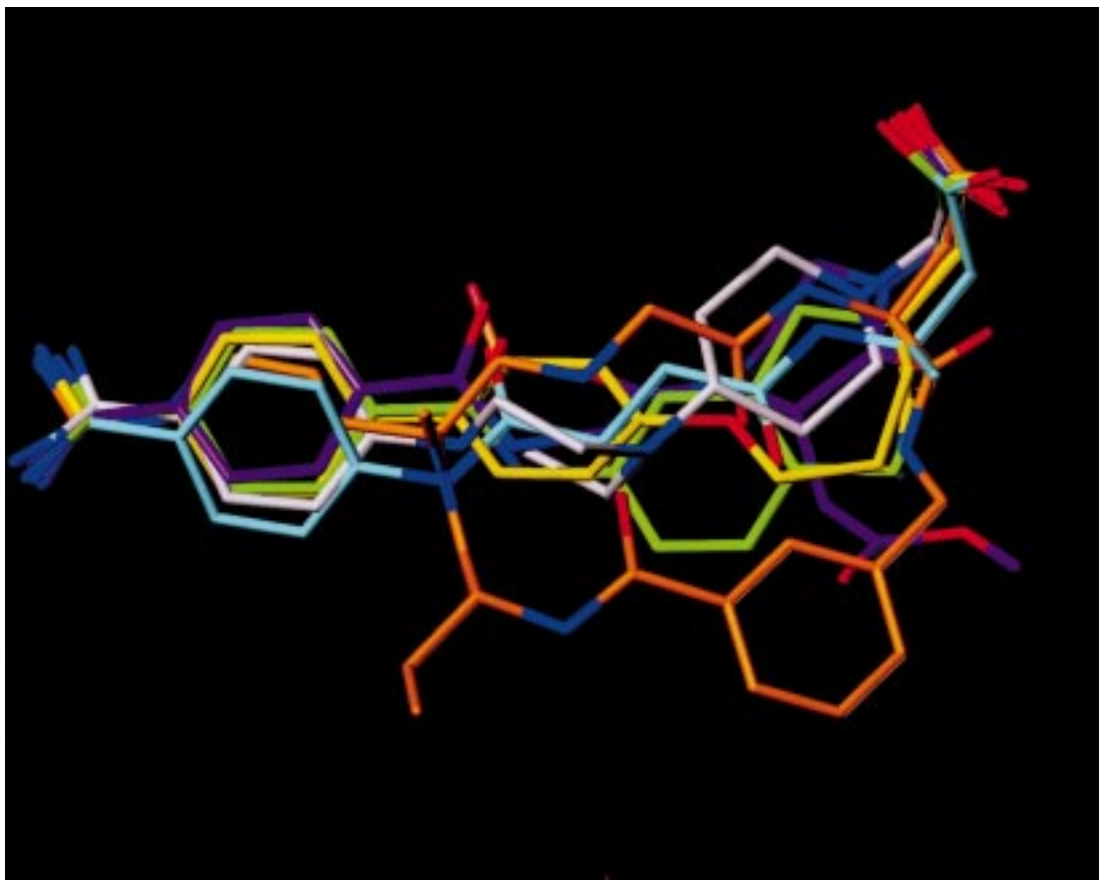
Chart 2. Known GPIIb-IIIa Antagonists Used in Pharmacophore Mapping

5: GR144053

6: TAK-029

7: DMP 728

8: SC-54701

9: BIBU-52

Figure 1. Alignment of the known GPIIb-IIIa antagonists using hydrogen-devoid backbone-type representation. The carbons of compounds **5**−**9** are shown in white, violet, orange, cyan, and yellow, respectively. Compound **10a**, whose carbons are shown in green, was also aligned with these antagonists.
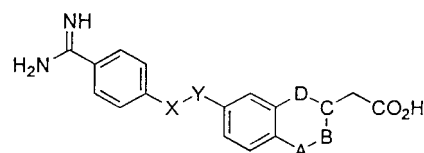
Table 1. Parameter Definition and Extracted Parameters of Known GPIIb-IIIa Antagonists

	distance, Å		angle, deg	
compd	a	b	α	β
5[a]	13.66	11.78	156.84	105.75
6[b]	13.90	12.35	166.32	88.96
7[c]	14.19	12.44	165.50	106.28
8[d]	13.85	12.46	156.02	87.82
9[e]	13.89	12.31	166.81	91.00

[a] See ref 16. [b] See ref 17. [c] See ref 18. [d] See ref 19. [e] See ref 20.

10a: A = B = D = CH₂, C = CH, X-Y = C(=O)-NH
10b: A = B = D = CH₂, C = CH, X-Y = C(=O)-N(CH₃)
10c: A = O, B = D = CH₂, C = CH, X-Y = C(=O)-NH
10d: A = B = D = CH₂, C = N, X-Y = C(=O)-NH
10e: A = B = D = CH₂, C = CH, X-Y = NH-C(=O)
10f: A = B = D = CH₂, C = CH, X-Y = CH₂-O
10g: A-B = CH=CH, C-D = C=CH, X-Y = CH₂-O
10h: A = B = D = CH₂, C = CH, X-Y = CH=CH
10i: A = B = D = CH₂, C = CH, X-Y = CH₂CH₂

Figure 2. Newly designed compounds to match extracted parameters.

To prepare **17a** as a key intermediate, an improved synthetic method was developed (Scheme 3). Compound **13a** was converted to the α,β-unsaturated acid **24** by aldol condensation with glyoxylic acid in the presence of H_2SO_4. Hydrogenation of nitro, keto, and olefin groups of **24** catalyzed by Pd/C in the presence of H_2-SO_4 accomplished the one-step conversion to **17a**. According to this procedure, ethyl (6-amino-3,4-dihydro-$2H$-1-benzopyran-3-yl)acetate (**27**) was also synthesized in two steps from 6-nitro-4-oxo-3,4-dihydro-$2H$-1-benzopyran (**25**).

Ethyl (7-amino-1,2,3,4-tetrahydro-2-isoquinolinyl)acetate (**30**), an intermediate of **11d**, was synthesized as shown in Scheme 4. Nitration at the 7-position of 1,2,3,4-tetrahydroisoquinoline (**28**) with KNO_3/H_2SO_4-
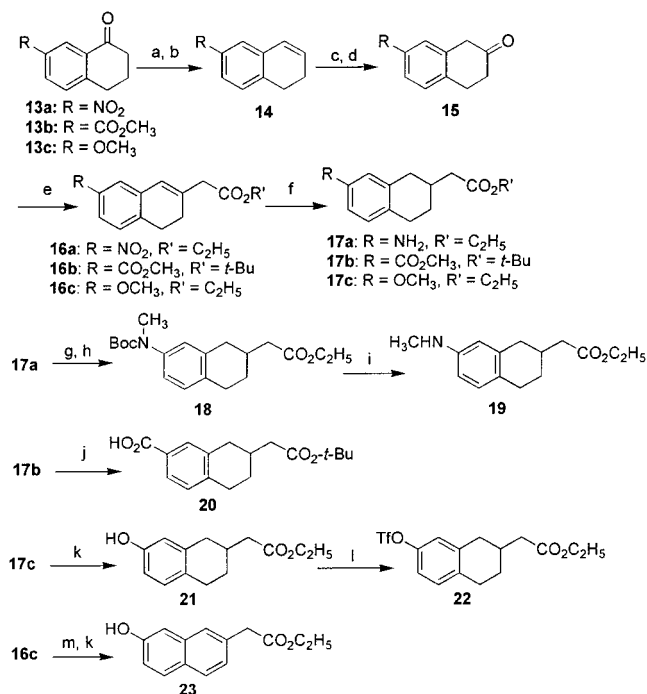
28 gave **29**. N-Alkylation of **29** with ethyl bromoacetate, followed by hydrogenation catalyzed by Pd/C, afforded **30**.

Cyano compounds **11a**−**i** were synthesized as shown in Scheme 5. Amide compounds **11a**−**d** were prepared by acylation of amino compounds **17a**, **19**, **27**, and **30**, respectively, with 4-cyanobenzoyl chloride. Compound **11e** was prepared from **20**. Treatment of **20** with $(COCl)_2$ in the presence of a catalytic amount of DMF, followed by the reaction with 4-aminobenzonitrile in the presence of Et_3N and 4-(dimethylamino)pyridine, afforded **11e**. Ether type compounds **11f,g** were synthesized from **21** and **23**, respectively, by reaction with p-cyanobenzyl bromide. Compounds **11h,i** were synthesized via palladium catalyzed coupling with orga-

Scheme 1. General Procedure for the Preparation of Amidino Acid **10**

11a-i

1) HCl, EtOH
2) NH$_4$OAc

12a-i

NaOH

10a-i
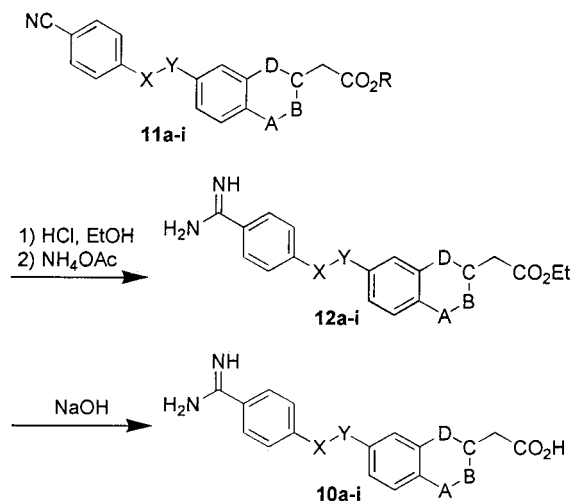
10a-12a: A = B = D = CH$_2$, C = CH, X-Y = C(=O)-NH
10b-12b: A = B = D = CH$_2$, C = CH, X-Y = C(=O)-N(CH$_3$)
10c-12c: A = O, B = D = CH$_2$, C = CH, X-Y = C(=O)-NH
10d-12d: A = B = D = CH$_2$, C = N, X-Y = C(=O)-NH
10e-12e: A = B = D = CH$_2$, C = CH, X-Y = NH-C(=O)
10f-12f: A = B = D = CH$_2$, C = CH, X-Y = CH$_2$-O
10g-12g: A-B = CH=CH, C-D = C=CH, X-Y =CH$_2$-O
10h-12h: A = B = D = CH$_2$, C = CH, X-Y = CH=CH
**10i-12i**: A = B = D = CH$_2$, C = CH, X-Y = CH$_2$CH$_2$

Scheme 2. Synthesis of 6,6-Bicyclic Intermediates (Step 1)[a]

13a: R = NO$_2$
13b: R = CO$_2$CH$_3$
13c: R = OCH$_3$

16a: R = NO$_2$, R' = C$_2$H$_5$
16b: R = CO$_2$CH$_3$, R' = t-Bu
16c: R = OCH$_3$, R' = C$_2$H$_5$

17a: R = NH$_2$, R' = C$_2$H$_5$
17b: R = CO$_2$CH$_3$, R' = t-Bu
17c: R = OCH$_3$, R' = C$_2$H$_5$

[a] Reagents: (a) NaBH$_4$, MeOH; (b) Amberlyst 15 or PPTS, azeotrope; (c) m-CPBA; (d) ZnI$_2$; (e) ethyl (diethylphosphoryl)acetate or t-butyl P,P-dimethylphophonoacetate, NaH; (f) H$_2$, Pd/C; (g) Boc$_2$O; (h) NaH, MeI; (i) HCl; (j) NaOH; (k) TMSCl, NaI; (l) Tf$_2$O, pyridine; (m) p-chloranil.

nostannane.[29] 4-Ethynylbenzonitrile (**32**) was synthesized from p-cyanophenol (**31**) in three steps: (1) conversion to the triflate, (2) Pd(0)−Cu(I)-catalyzed coupling with (trimethylsilyl)acetylene,[30] and (3) desilylation with potassium fluoride. Hydrostannation of

Scheme 3. Synthesis of 6,6-Bicyclic Intermediates (Step 2): Convergent Synthesis of **17a** and Ethyl (6-Amino-2H-1-benzopyran-3-yl)acetate (**27**)[a]

13a: X = CH$_2$
25: X = O

24: X = CH$_2$
26: X = O

17a: X = CH$_2$
27: X = O
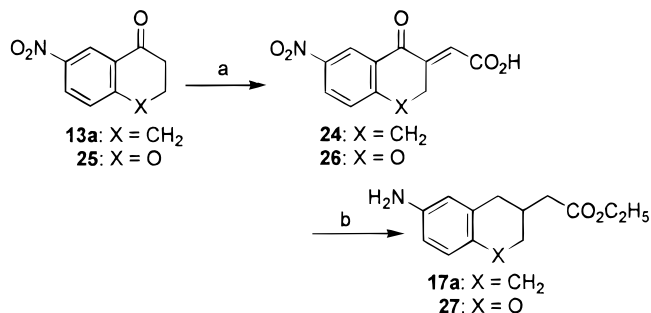
[a] Reagents: (a) glyoxylic acid, H$_2$SO$_4$; (b) H$_2$, Pd/C, H$_2$SO$_4$, EtOH.

Scheme 4. Synthesis of 6,6-Bicyclic Intermediates (Step 3): Ethyl (7-Amino-1,2,3,4-tetrahydro-2-isoquinolinyl)acetate (**30**)[a]

28   **29**   **30**
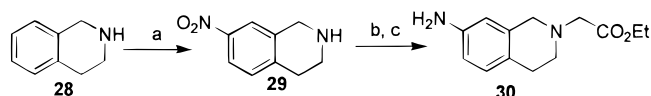
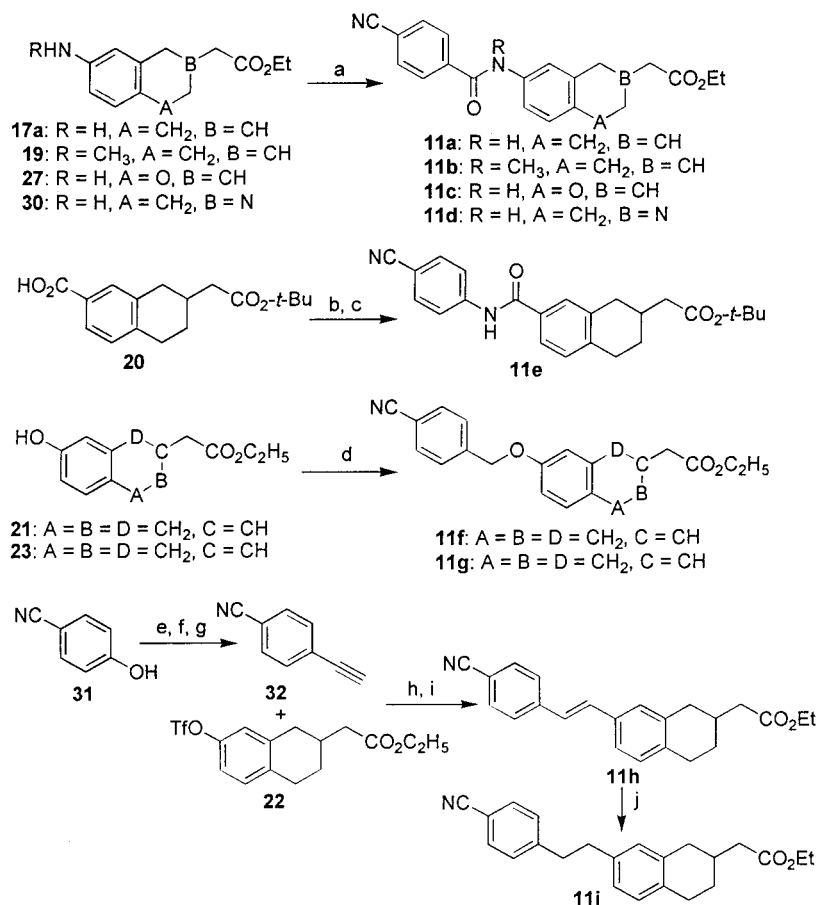[a] Reagents: (a) KNO$_3$, H$_2$SO$_4$; (b) ethyl bromoacetate, Et$_3$N; (c) H$_2$, Pd/C.

32 with tri-n-butyltin hydride, followed by palladium-catalyzed coupling[29] with triflate **22** gave (E)-olefin **11h**. Compound **11i** was obtained by hydrogenation of **11h**.

N-Alkylated amidino acids **34a−e**, **36**, and (RS)-**3** were synthesized from **11a**,**c** (Scheme 6). Cyano compound **11** was converted to the corresponding imidate, which was treated with appropriate amines to afford a series of N-alkylated amidino esters **33a−e**, **35**, and (RS)-**4**. Hydrolysis of each ester gave corresponding N-alkylated amidino acids **34a−e**, **36**, and (RS)-**3**.
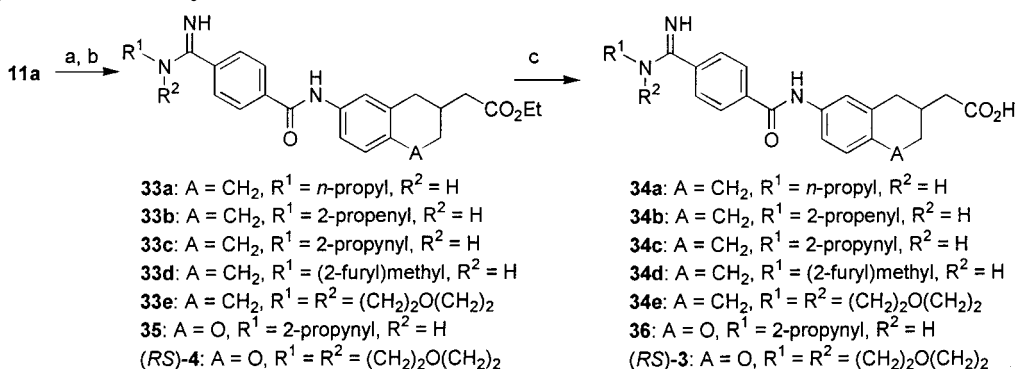
MS-180 ((S)-**4**·HCl) and corresponding carboxylic acid (S)-**3** were synthesized as enantiomerically pure forms via optical resolution using chiral HPLC (Scheme 7). Methyl [6-(N-Boc-amino)-3,4-dihydro-2H-1-benzopyran-3-yl]acetate ((RS)-**37**), which was prepared from **26** in two steps: (1) hydrogenation in the presence of Pd/C and H$_2$SO$_4$ and (2) protection as the Boc derivative, was resolved on large scale using CHIRALCEL-OD (Daicel Chemical Industries, Ltd.). The absolute configuration of the enantiomer that has the longer retention time was determined to be the (S)-form by single-crystal X-ray crystallographic analysis of the amide **39** obtained from N-(4-nitrophenylsulfonyl)-L-phenylalanine. Treatment of (S)-**37** with ethanolic HCl afforded (S)-**27**, which was converted to MS-180 ((S)-**4**·HCl) and its acid (S)-**3**. Enantiomeric purities of (S)-**4** and (S)-**3** were determined as greater than 99% ee by chiral HPLC analysis. According to the same procedure, (R)-**4** and (R)-**3** were synthesized from (R)-**37**.

Results and Discussion

Amidino Acid Derivatives with 6,6-Bicyclic Template. The synthesized compounds **10a−i** were evaluated for their abilities to inhibit in vitro ADP-induced platelet aggregation of guinea pig and human platelet-rich plasma (PRP). The results are given as IC$_{50}$ values and are shown in Table 2. These compounds showed IC$_{50}$ values of 230−18000 nM in guinea pig PRP and 46−1500 nM in human PRP with the exception of

Scheme 5. Synthesis of Key Intermediates **11a**−**i**[a]

17a: R = H, A = CH₂, B = CH
19: R = CH₃, A = CH₂, B = CH
27: R = H, A = O, B = CH
30: R = H, A = CH₂, B = N

11a: R = H, A = CH₂, B = CH
11b: R = CH₃, A = CH₂, B = CH
11c: R = H, A = O, B = CH
11d: R = H, A = CH₂, B = N

20

11e

21: A = B = D = CH₂, C = CH
23: A = B = D = CH₂, C = CH

11f: A = B = D = CH₂, C = CH
11g: A = B = D = CH₂, C = CH

31

32

22

11h

11i

[a] Reagents: (a) 4-cyanobenzoyl chloride, Et₃N; (b) (COCl)₂, DMF; (c) 4-aminobenzonitrile, Et₃N; (d) 4-cyanobenzyl bromide, K₂CO₃; (e) Tf₂O, pyridine, (f) (trimethylsilyl)acetylene, Pd(PPh₃)₄, CuI, n-PrNH₂; (g) KF; (h) n-Bu₃SnH, AIBN; (i) **22**, Pd(PPH₃)₄, LiCl, DMF; (j) H₂, Pd/C.

Scheme 6. Synthesis of *N*-Alkylated Amidines[a]

33a: A = CH₂, R¹ = n-propyl, R² = H
33b: A = CH₂, R¹ = 2-propenyl, R² = H
33c: A = CH₂, R¹ = 2-propynyl, R² = H
33d: A = CH₂, R¹ = (2-furyl)methyl, R² = H
33e: A = CH₂, R¹ = R² = (CH₂)₂O(CH₂)₂
35: A = O, R¹ = 2-propynyl, R² = H
(*RS*)-**4**: A = O, R¹ = R² = (CH₂)₂O(CH₂)₂

34a: A = CH₂, R¹ = n-propyl, R² = H
34b: A = CH₂, R¹ = 2-propenyl, R² = H
34c: A = CH₂, R¹ = 2-propynyl, R² = H
34d: A = CH₂, R¹ = (2-furyl)methyl, R² = H
34e: A = CH₂, R¹ = R² = (CH₂)₂O(CH₂)₂
36: A = O, R¹ = 2-propynyl, R² = H
(*RS*)-**3**: A = O, R¹ = R² = (CH₂)₂O(CH₂)₂

[a] Reagents: (a) saturated HCl/EtOH; (b) appropriate amine, EtOH; (c) NaOH.

compound **10g**. Compounds **10a,c,e** showed especially marked inhibitions of platelet aggregation in human PRP.

Then, to elucidate the quantitative structure-activity relationships (QSAR) with the present compounds, comparative molecular field analysis (CoMFA)[15] was performed. The inhibitory potencies of platelet aggregation in guinea pig were used for the CoMFA study. For the convenience of the CoMFA study, an IC$_{50}$ value of 2×10^6 nM was substituted for the inhibitory potency of compound **10g**, for which the experimental IC$_{50}$ value was greater than 10^6 nM. Molecular alignments were achieved by using the alignment rule above-defined for

known GPIIb-IIIa antagonists. Using the default CoMFA settings, which included both steric and electrostatic fields, we observed the cross-validated r^2 (q^2) of 0.398 with five principal components. The choice of CoMFA options was tried to minimize the standard error of prediction rather than simply maximizing the q^2 value. This resulted in using a smaller number of partial least-squares (PLS) principal components, and that was appropriate for our small data set. The final model, which includes both steric and electrostatic fields, with three principal components was obtained using grid box translation from SYBYL's default position 1.0 Å in the Z direction relative to the orientation of our aligned

Scheme 7. Synthesis of Optically Active **3** and **4**[a]
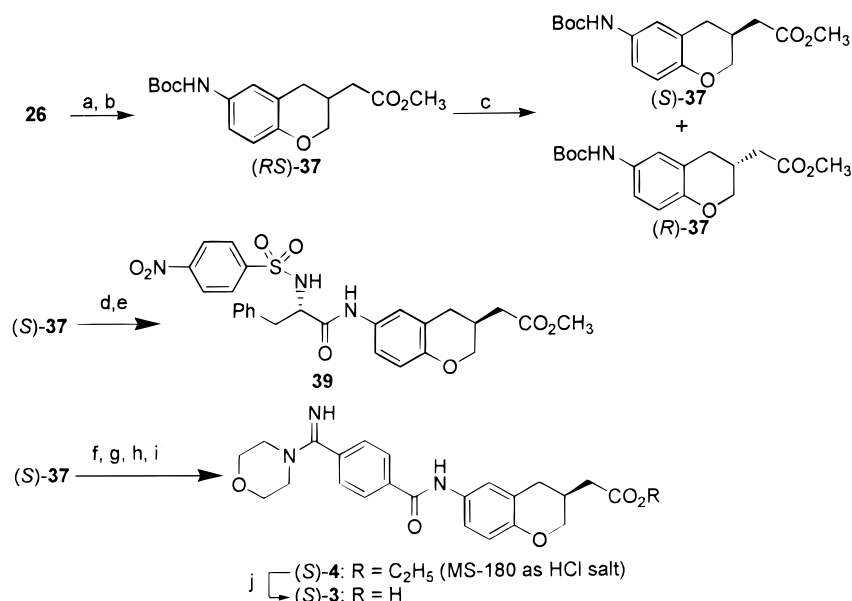
[a] Reagents: (a) H$_2$, Pd/C, H$_2$SO$_4$, MeOH; (b) Boc$_2$O; (c) separation by chiral HPLC, CHIRALCEL-OD; (d) HCl, MeOH; (e) *N*-(*p*-nitrophenylsulfonyl)-L-phenylalanyl chloride, Et$_3$N, DMAP; (f) HCl, EtOH; (g) 4-cyanobenzoyl chloride, Et$_3$N; (h) saturated HCl/EtOH; (i) morpholine; (j) NaOH.

Table 2. In Vitro Inhibition of ADP-Induced Platelet Aggregation with **10a**−**i**[a]

compd	IC$_{50}$,[b] nM	
	guinea pig[c]	human[d]
10a	370	57
10b	1800	200
10c	480	49
10d	6600	290
10e	230	46
10f	18000	1500
10g	>10^6	nt[e]
10h	5000	nt[e]
10i	2500	370

[a] Expressed as the average of at least two determinations. [b] Concentration required to inhibit by 50%. [c] Inhibition of guinea pig PRP aggregation induced by 5 μM ADP. [d] Inhibition of human PRP aggregation induced by 5 μM ADP. [e] nt, not tested.

structure. This model had the cross-validated q^2 values of 0.637 and r^2 values of 0.993. The contour map of the CoMFA model is shown in Figure 3. Because the molecules used in this study have the same benzamidine and carboxylic acid units, we could not get any information around these functional groups. The blue contour located around one side on the 6,6-bicyclic ring indicates an area in which electron-deficient groups increase the affinity. This result suggested that **10g**, which has the electron-rich naphthalene ring, is unfavorable to bind with the receptor site. The red contours located around the oxygen atom of the amide bond and the blue contours located around the hydrogen of the amide bond indicate the electrostatic interaction with the amide bond and the receptor. When compounds **10a**,**c**,**e** containing an amide bond reach the receptor site, the electrostatic interaction between the amide bond and

the receptor will increase their activity relative to the non-amide compounds **10f**,**h**,**i**. The yellow contour located around the *N*-methyl group of **10b** indicates the area in which the *N*-methyl group is detrimental to the affinity at the receptor. This result suggests compound **10b** is more unfavorable to interact with the receptor than amide compounds **10a**,**c**,**e**. Among them, compound **10e**, which showed marked inhibitions of platelet aggregation in vitro, was not selected for further investigation due to its short half-life (<30 min) in guinea pig after intravenous administration.

The oral profiles of the representative compound **10a** and ester prodrugs **12a**,**c** were assessed by platelet aggregation assay in guinea pig (Table 3). The inhibition of ex vivo ADP-induced platelet aggregation was measured at 1 h after oral administration of these compounds to guinea pigs (dose: 37 μmol/kg). Though amidino acid **10a** showed only a weak inhibition (8%), ester prodrugs **12a**,**c** showed moderate enhancement of inhibitory potency (41% and 40% inhibitions, respectively). Consequently, we selected **12a**,**c** as lead compounds and attempted to improve the oral profiles focused on the modification of the amidino group.

N-Alkylated Amidine Derivatives. We modified the amidino groups of **10a**,**c** to the *N*-alkylated amidino groups. The in vitro activities of the *N*-alkylated amidino acids **34a**−**e**, **36**, and (*RS*)-**3** were assessed by the inhibition of the ADP-induced platelet aggregation of guinea pig and human PRP and the inhibition of the fibrinogen binding to human immobilized GPIIb-IIIa (Table 4). The *N*-alkylated amidino acids possessed almost the same inhibitory potencies of platelet aggregation and of fibrinogen binding compared with the parent amidino acids **10a**,**c**. The results of the fibrinogen binding assay clearly showed that *N*-alkylated amidino acids themselves bind to the GPIIb-IIIa. We next assessed oral profiles of *N*-alkylated amidino esters **33a**−**e**, **35**, and (*RS*)-**4**. The inhibition of ex vivo ADP-induced platelet aggregation in guinea pig was mea-

Figure 3. Steric and electrostatic CoMFA field from the analysis of ADP-induced platelet aggregation of guinea pig without cross-validation. Compounds **10a−i** are shown inside the field. Favoring activity: yellow, less bulky; blue, positive charge; red, negative charge (contribution level 80%).

Table 3. Ex Vivo Inhibition of ADP-Induced Platelet Aggregation by Oral Administration of **10a** and **12a,c**[a] and Solubility in Saline
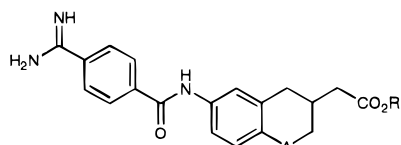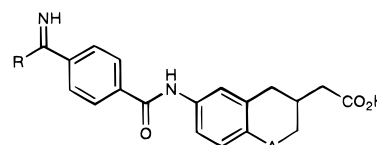

compd	R	A	inhibition,[b] %	sol,[c] mg/mL
10a	H	CH$_2$	8	nd[d]
12a	C$_2$H$_5$	CH$_2$	41	0.26
12c	C$_2$H$_5$	O	40	0.41

[a] Data are expressed as the average of three animals. [b] Percent inhibition of ex vivo platelet aggregation induced by 5 μM ADP 1 h after oral administration (dose: 37 μmol/kg). [c] The solubility of the compounds in saline was determined by UV spectrophotometry. [d] nd, not determined.

sured at 1 h after oral administration (37 μmol/kg) of these compounds (Table 5). Excepting **33a,b**, these *N*-alkylated amidines showed stronger inhibition (>90%) than the parent amidines **12a,c**.

Next, we estimated plasma concentrations of the corresponding carboxylic acids **34a−e**, **36**, and (*RS*)-**3** at 1 h after oral administration of *N*-alkylated amidino esters **33a−e**, **35**, and (*RS*)-**4** in guinea pig (Table 5). Compounds **33e**, **35**, and (*RS*)-**4** showed high plasma concentrations of the corresponding *N*-alkylated acids compared with **33a−d**. Furthermore, since we could not find amidino acid **10a** or **10c** in plasma, the active species in vivo must be the *N*-alkylated amidino acids themselves. Although the mechanism underlying the

Table 4. In Vitro Inhibition of ADP-Induced Platelet Aggregation and Fibrinogen Binding by **10a,c**, **34a−e**, **36**, and (*RS*)-**3**[a]


compd	R	A	IC$_{50}$,[b] nM guinea pig[c]	human[d]	fibrinogen binding[e]
10a	NH$_2$	CH$_2$	370	57	0.12
10c	NH$_2$	O	480	49	0.073
34a	CH$_3$(CH$_2$)$_2$NH	CH$_2$	170	68	0.13
34b	H$_2$C=CHCH$_2$NH	CH$_2$	490	nt[f]	0.26
34c	HC≡CCH$_2$NH	CH$_2$	300	100	0.16
34d	(2-furyl)CH$_2$NH	CH$_2$	620	nt[f]	0.20
34e	morpholino	CH$_2$	190	80	0.15
36	HC≡CCH$_2$NH	O	350	78	0.13
(*RS*)-**3**	morpholino	O	230	66	0.098

[a−d] See footnotes a−d in Table 2. [e] Inhibition of fibrinogen binding to immobilized human GPIIb-IIIa. [f] nt, not tested.

improvement of the oral profile by *N*-alkylated amidine is not clear, we assumed that the lack of oral activity of **10a**, **12a,c**, **33a,b** was due to poor oral absorption. Features of these compounds which we considered to contribute to the lack of oral absorption included poor aqueous solubility. Compounds **33e**, **35**, and (*RS*)-**4** showed good aqueous solubility (Table 5). These compounds also showed good oral activity, supporting the importance of good aqueous solubility for oral absorption

Table 5. Ex Vivo Inhibition of ADP-Induced Platelet Aggregation, Plasma Concentration of the Active Species by Oral Administration of *N*-Alkylated Amidino Esters,[a] and Solubility in Saline

R' = Et: tested compounds
R' = H: active species
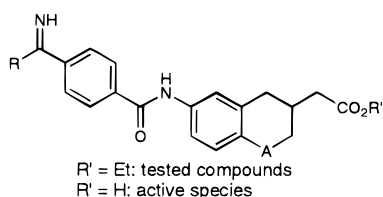
compd	R	A	inhibition,[b] %	plasma level,[c] μM	sol,[d] mg/mL
33a	$CH_3(CH_2)_2NH$	CH_2	3	<0.18	nd[e]
33b	$H_2C{=}CHCH_2NH$	CH_2	21	0.38	nd[e]
33c	$HC{\equiv}CCH_2NH$	CH_2	99	1.0	1.50
33d	(2-furyl)CH_2NH	CH_2	91	0.85	1.06
33e	morpholino	CH_2	98	7.3	>200
35	$HC{\equiv}CCH_2NH$	O	99	3.8	106
(*RS*)-**4**	morpholino	O	99	8.4	>200

[a,b] See footnotes a and b in Table 3. [c] Concentration of active species in plasma 1 h after oral administration (dose: 37 μmol/kg). [d,e] See footnotes c and d in Table 3.

Table 6. Comparison of the Enantiomers of **3** by in Vitro Profile[a]

3
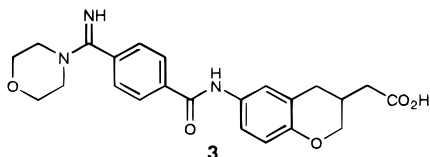
	IC$_{50}$,[b] nM		
compd	guinea pig[c]	human[d]	fibrinogen binding[e]
(*S*)-**3**	110	35	0.12
(*R*)-**3**	4400	360	2.6

[a–d] See footnotes a–d in Table 2. [e] See footnote e in Table 4.

in these series. Finally, we selected (*RS*)-**4** as a promising compound, because it had the highest plasma concentration.

The in vitro antiplatelet activity of each enantiomer of (*RS*)-**3**, the active species of (*RS*)-**4**, was compared by determining the ability to inhibit ADP-induced platelet aggregation of guinea pig and human PRP and the ability to inhibit binding of fibrinogen to immobilized human GPIIb-IIIa (Table 6). (*S*)-**3** was found to be more active than (*R*)-**3** (10.3-fold in human PRP, 44-fold in guinea pig PRP, and 21.7-fold in binding assay) and considered to be the active enantiomer. (*S*)-**3** also showed in vitro inhibition of ADP-induced platelet aggregation in beagle dog (IC$_{50}$ = 253.3 nM), and the oral profile of the prodrug (S)-**4** was evaluated in dog.

Pharmacology of MS-180 ((*S*)-4·HCl). The antiplatelet activity of (*S*)-**4**·HCl (MS-180) was assessed by oral administration of the solid compound in gelatin capsules at a dose of 1, 3, or 5 mg/kg to fasted conscious male dogs (*n* = 4). After administration, plasma samples were drawn at specified time points, and the derived PRP was evaluated for the extent of ADP-induced platelet aggregation and plasma concentrations of (*S*)-**3** (Figure 4). Maximal inhibitions and highest concentrations of (*S*)-**3** were observed within 2−4 h after administration. The maximal inhibitions of ADP-induced platelet aggregation have dose dependency (60.2% inhibition at a dose of 1 mg/kg, 84.9% at 3 mg/kg, and 101.8% at 10 mg/kg, respectively), and the
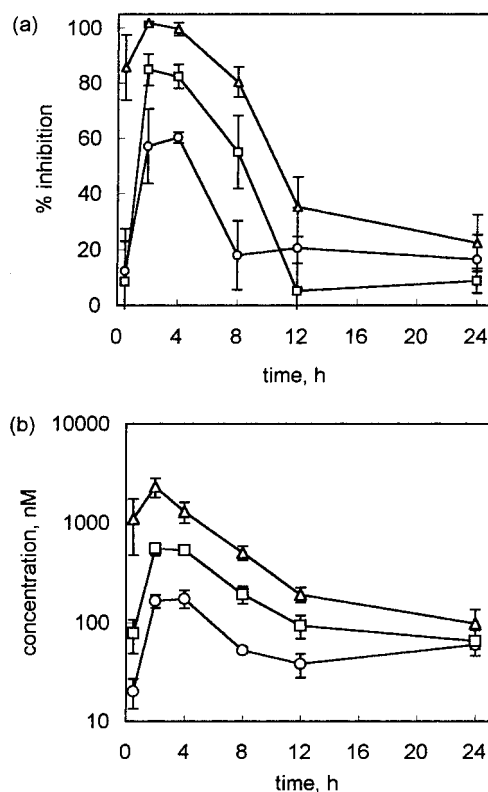

Figure 4. Effect of MS-180 ((*S*)-**4**·HCl) upon oral administration in conscious dogs (*n* = 4). Data are expressed as mean ± SE: (a) inhibition of ADP-induced ex vivo platelet aggregation; (b) plasma concentration of the active species ((*S*)-**3** determined by bioassay. Legend: (○) 1 mg/kg, (□) 3 mg/kg, (△) 10 mg/kg.

extent of the inhibitions declined in parallel with the plasma concentration of (*S*)-**3**.

Conclusion

In this paper, we have reported our approach to orally active GPIIb-IIIa antagonists: namely, design with the aid of pharmacophore mapping of amidino and carboxyl groups of known GPIIb-IIIa antagonists, synthesis, and quantitative structure-activity relationships using CoM-FA. We obtained compounds **10a,c,e** containing amide bonds and the 1,2,3,4-tetrahydronaphthalene or 3,4-dihydro-2*H*-1-benzopyran skeleton that showed potent inhibition of platelet aggregation in vitro but had weak oral activities. In an attempt to improve the oral profiles of these compounds, *N*-alkylated amidino esters (**33a−e**, **35**, and (*RS*)-**4**) were prepared. These compounds showed good oral activities with regard to the inhibition of ex vivo platelet aggregation in guinea pig or dog, with the in vivo hydrolysis to the corresponding acids. *N*-Alkylated amidino acids (**34a−e**, **36**, and (*RS*)-**3**), the active species of the corresponding ester, showed in vitro antiplatelet activities similar to those of the parent amidino acids **10a,c**. Among these *N*-alkylated amidino esters, (*RS*)-**4** was selected as the most promising compound by virtue of it having the highest plasma concentration of the active species at 1 h after oral administration in guinea pig (8.4 μM of (*RS*)-**3** at a dose of 37 μmol/kg). The two enantiomers of (*RS*)-**3** were compared with regards to their in vitro profile, and the (*S*)-form was found to be the active enantiomer. On the basis of these studies, optically active (*S*)-**4**·HCl (MS-180) was selected as a candidate for clinical evaluation

as a drug for the treatment and prevention of thrombosis in patients.

Experimental Section

Chemistry. Melting points were determined on a Büchi 535 melting point apparatus. Proton magnetic resonance spectra (^{1}H NMR) were obtained on a JEOL EX-270 (270 MHz) or GX-400 (400 MHz) spectrometer using either deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform (CDCl$_3$) as the solvent, and chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Carbon magnetic resonance spectra (^{13}C NMR) were obtained on a JEOL EX-90 (22.40 MHz) or GX-400 (100.4 MHz) spectrometer using either DMSO-d_6 or CDCl$_3$ as the solvent, and chemical shifts are reported in ppm downfield from TMS. Infrared spectra (IR) were recorded on a JASCO IR 7300 spectrometer. Mass (MS) spectra were recorded on a HITACHI M-80B (CI) or a Micromass QUATTRO-II (ESI) spectrometer. Elemental analyses were determined on a Perkin-Elmer CHN2400 elemental analyzer. The optical rotations were recorded on a JASCO DIP-730 polarimeter. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10A or LC-6A HPLC system.

All reactions requiring anhydrous conditions and/or inert atmosphere were carried out under a nitrogen atmosphere. The reactions were monitored by thin-layer chromatography (TLC) analysis by using Merck Kiesel gel 60$_{F254}$ TLC plates. Silica gel column chromatographies were carried out on silica gel 60 purchased from E. Merck. Reverse-phase column chromatographies were carried out on Chromatorex ODS silica gel purchased from Fuji-Silysia Chemical Ltd.

Ethyl [7-(4-Cyanobenzamido)-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (11a). To an ice-cooled solution of **17a** (15.26 g, 65.4 mmol) and Et$_3$N (7.62 g, 75.3 mmol) in CHCl$_3$ (200 mL) was added 4-cyanobenzoyl chloride (11.8 g, 71.2 mmol) by portions. The mixture was stirred for 2 h at 0 °C and washed with 5% aqueous NaOH solution (2 × 100 mL), water (50 mL), 0.6 N HCl (150 mL), and brine (50 mL) successively. The organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. Purification by column chromatography (SiO$_2$, CHCl$_3$/EtOAc = 20/1) afforded 20.7 g (88%) of **11a** as a white solid: mp 140−142 °C. Anal. (C$_{22}$H$_{22}$N$_2$O$_3$) C, H, N.

Ethyl [7-(4-Cyano-N-methylbenzamido)-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (11b). Synthesized from **19** in 66% yield using a similar procedure described for the preparation of **11a** as a colorless oil. Anal. (C$_{23}$H$_{24}$N$_2$O$_3$) H; C: calcd, 73.38; found, 72.97. N: calcd, 7.44; found, 6.65.

Ethyl [6-(4-Cyanobenzamido)-3,4-dihydro-2H-1-benzopyran-3-yl]acetate (11c). Synthesized from **27** in 98% yield using a similar procedure described for the preparation of **11a** as a pale-yellow solid: mp 166−168 °C. Anal. (C$_{21}$H$_{20}$N$_2$O$_4$) C, H, N.

Ethyl [7-(4-Cyanobenzamido)-1,2,3,4-tetrahydro-2-isoquinolinyl]acetate Hydrochloride (11d·HCl). A free amine of the title compound was prepared from **30·**2HCl using a similar procedure described for the preparation of **11a**. Treatment of the free amine with 4 N HCl in 1,4-dioxane afforded the **11d·**HCl salt (60% yield from **30·**2HCl): mp 205−207 °C. Anal. (C$_{21}$H$_{21}$N$_3$O$_3$ and HCl) C, H, N.

$tert$-Butyl [7-[N-(4-Cyanophenyl)carbamoyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (11e). To a mixture of **20** (0.70 g, 2.4 mmol) and (COCl)$_2$ (2.18 g, 17.2 mmol) in toluene (5.0 mL) was added DMF (20 μL). The mixture was stirred for 1 h and concentrated in vacuo to afford a pale-yellow solid, which was dissolved in THF (5.0 mL). To this were added 4-aminobenzonitrile (350 mg, 2.94 mmol), pyridine (1.0 g, 12.6 mmol), and 4-(dimethylamino)pyridine (50 mg, 0.4 mmol). The mixture was stirred for 12 h at room temperature. After addition of CHCl$_3$ (100 mL), the mixture was washed with 1 N HCl, saturated NaHCO$_3$ solution, and brine successively. The organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. Purification of the residue by column chromatography (SiO$_2$, n-hexane/EtOAc = 3/1) afforded 473

mg (48%) of **11e** as a white solid: mp 176−177 °C. Anal. (C$_{24}$H$_{26}$N$_2$O$_3$) C, H, N.

Ethyl [7-[(4-Cyanophenyl)methoxy]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (11f). A mixture of **21** (2.20 g, 9.4 mmol), 4-cyanobenzyl bromide (3.7 g, 18.8 mmol), and K$_2$CO$_3$ (3.0 g, 21.7 mmol) in 2-butanone (20 mL) was refluxed for 3 h. After concentration in vacuo, the mixture was diluted with EtOAc and washed with brine three times. The organic phase was dried over MgSO$_4$, filtered, and concentrated in vacuo to afford an oil, which was purified by column chromatography (SiO$_2$, n-hexane/EtOAc = 3/1) to afford 2.83 g (95%) of **11f** as a white solid: mp 71−72 °C. Anal. (C$_{22}$H$_{23}$NO$_3$) C, H, N.

Ethyl [7-[(4-Cyanophenyl)methoxy]-2-naphthalenyl]acetate (11g). Synthesized from **23** in 48% yield following a similar procedure described for the preparation of **11f**: mp 100−101 °C. Anal. (C$_{22}$H$_{19}$NO$_3$) C, H, N: calcd, 4.05; found, 3.63.

Ethyl (E)-[7-[2-(4-Cyanophenyl)ethenyl-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (11h). To a mixture of acetylene **32** (3.0 g, 23.5 mmol), tri-n-butyltin hydride (7.4 g, 25.4 mmol), and toluene (10 mL) was added 2,2′-azobis-(isobutyronitrile) (2.0 mg, 0.012 mmol) at room temperature. The mixture was stirred for 1 h at 100 °C and concentrated in vacuo to afford vinylstannane, which was dissolved in DMF (80 mL). To this were added triflate **22** (6.6 g, 18.0 mmol), LiCl (2.56 g, 60.4 mmol), and Pd(PPh$_3$)$_4$ (0.52 g, 0.45 mmol). The mixture was stirred for 3 h at 70 °C and concentrated in vacuo to afford an oily residue, which was dissolved in diisopropyl ether and washed with aqueous 5% potassium fluoride solution twice. The organic phase was dried over MgSO$_4$ and concentrated in vacuo. Purification by column chromatography (SiO$_2$, n-hexane/EtOAc = 7/1) gave 4.23 g (68%) of **11h** as a pale-yellow solid: mp 99−100 °C. Anal. (C$_{23}$H$_{23}$NO$_2$) C, H, N.

Ethyl [7-[2-(4-Cyanophenyl)ethyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (11i). A mixture of **11h** (610 mg, 1.76 mmol) and 10% Pd/C (400 mg) in EtOH (20 mL) was stirred under H$_2$ atmosphere at room temperature. After completion of the hydrogenation, the catalyst was removed by filtration. The filtrate was concentrated in vacuo and purified by column chromatography (SiO$_2$, n-hexane/EtOAc = 5/1) to afford 540 mg (88%) of **11i** as a white solid: mp 30−32 °C. Anal. (C$_{23}$H$_{25}$NO$_2$) C, H, N.

Ethyl [7-(4-Amidinobenzamido)-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (12a·HCl). Anhydrous hydrogen chloride was bubbled into an ice-cooled suspension of **11a** (1.79 g, 4.93 mmol) in EtOH (20 mL) for 30 min. The mixture was stirred for 4 h at room temperature and concentrated in vacuo. The residual solid was rinsed with isopropyl ether, dried under reduced pressure, and dissolved in 30 mL of EtOH. To this solution was added ammonium acetate (1.04 g, 13.5 mmol), and the mixture was stirred for 20 h. The resulting precipitate was collected by filtration and treated with ethanolic HCl to afford 1.31 g (64%) of **12a·**HCl as a white solid: mp 207−210 °C; IR (KBr) 2924, 1733, 1671, 1614, 1596, 1542, 1506, 1413, 1334, 1155 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.38 (s, 1H), 9.54 (s, 2H), 9.30 (s, 2H), 8.16 (d, 2H, J = 8.6 Hz), 7.97 (d, 2H, J = 8.6 Hz), 7.53−7.50 (m, 2H), 7.06 (d, 1H, J = 8.9 Hz), 4.10 (q, 2H, J = 7.3 Hz), 2.86−2.75 (m, 3H), 2.52−2.35 (m, 3H), 2.15−2.05 (m, 1H), 1.91−1.87 (m, 1H), 1.49−1.34 (m, 1H), 1.22 (t, 3H, J = 7.3 Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 172.0, 165.1, 163.9, 139.3, 136.3, 135.6, 131.6, 130.3, 128.6, 128.1 (2C), 128.0 (2C), 120.7, 118.4, 59.7, 40.0, 35.0, 30.9, 28.5, 27.6, 14.1; CI-MS m/e 380 (M + 1)$^+$. Anal. (C$_{22}$H$_{25}$N$_3$O$_3$ and 0.95HCl plus H$_2$O) C, H, N, Cl.

Ethyl [7-(4-Amidino-N-methylbenzamido)-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (12b·HCl). Following the procedure described for the preparation of **12a·**HCl, the title compound was prepared in 53% yield from the nitrile **11b** as a white solid: mp 187−189 °C; IR (KBr) 2977, 1732, 1683, 1622, 1503, 1437, 1394, 1313, 1286, 1250, 1155, 1102, 1028, 861, 770, 722 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 9.42 (br s, 2H), 9.26 (br s, 2H), 7.73 (d, 2H, J = 8.2 Hz), 7.49 (d, 2H, J = 8.2 Hz), 6.96−6.85 (m, 3H), 4.08 (q,

2H, $J = 7.1$ Hz), 3.34 (s, 3H), 2.76–2.68 (m, 3H), 2.88–2.23 (m, 3H), 2.10–1.95 (m, 1H), 1.86–1.81 (m, 1H), 1.41–1.26 (m, 1H), 1.20 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.8, 168.1, 165.0, 141.6, 141.2, 136.7, 134.5, 129.3, 128.2 (2C), 128.1, 127.7 (2C), 127.0, 124.5, 60.0, 39.9, 38.0, 34.6, 30.6, 28.1, 27.6, 14.1. Anal. ($C_{23}H_{27}N_3O_3$ and 1.1HCl) C, H, N, Cl.

Ethyl [6-(4-Amidinobenzamido)-3,4-dihydro-2H-1-benzopyran-3-yl]acetate Hydrochloride (12c·HCl). Following the procedure described for the preparation of **12a**·HCl, the title compound was prepared in 63% yield from the nitrile **11c** as a pale-yellow solid: mp 238–240 °C; IR (KBr) 3290, 3077, 1706, 1687, 1651, 1560, 1500, 1426, 1299, 1260, 1176, 1094, 1028, 856, 829, 691 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.33 (s, 1H), 9.53 (br s, 2H), 9.28 (br s, 2H), 8.16 (d, 2H, $J = 8.2$ Hz), 7.96 (d, 2H, $J = 8.2$ Hz), 7.52 (d, 1H, $J = 2.3$ Hz), 7.47 (dd, 1H, $J = 2.3, 8.6$ Hz), 6.75 (d, 1H, $J = 8.6$ Hz), 4.18 (d, 1H, $J = 10.9$ Hz), 4.11 (q, 2H, $J = 7.1$ Hz), 3.85–3.78 (m, 1H), 2.93–2.86 (m, 1H), 2.52–2.33 (m, 4H), 1.21 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.4, 165.1, 163.6, 150.4, 139.3, 131.6, 130.2, 128.1 (2C), 127.9 (2C), 122.2, 120.7, 120.3, 115.9, 68.8, 59.9, 35.3, 30.2, 28.7, 14.0; ESI-MS m/e 382 (M + 1)$^+$. Anal. ($C_{21}H_{23}N_3O_4$ and 1.12HCl) C, H, N, Cl.

Ethyl [7-(4-Amidinobenzamido)-1,2,3,4-tetrahydro-2-isoquinolinyl]acetate Dihydrochloride (12d·2HCl). Following the procedure described for the preparation of **12a**·HCl, the title compound was prepared in 60% yield from the nitrile **11d**·HCl as a white solid: mp 172–173 °C; IR (KBr) 1869, 1748, 1676, 1617, 1601, 1542, 1508, 1489, 1421, 1303, 1216, 1072, 1018, 862, 700 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.70 (s, 1H), 9.61 (br s, 2H), 9.38 (br s, 2H), 8.21 (d, 2H, $J = 8.2$ Hz), 8.00 (d, 2H, $J = 8.2$ Hz), 7.74 (s, 1H), 7.71 (d, 1H, $J = 8.4$ Hz), 7.24 (d, 1H, $J = 8.4$ Hz), 4.52 (br s, 2H), 4.36 (br s, 2H), 4.27 (q, 2H, $J = 7.1$ Hz), 3.75–3.25 (broad peak, 3H), 3.20–2.90 (broad peak, 2H), 1.28 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 165.6, 165.0, 164.1, 138.9, 137.4, 130.3, 128.7, 128.1 (5C), 126.7, 120.2, 118.0, 61.7, 54.2, 52.5, 49.6, 24.4, 13.8. Anal. ($C_{21}H_{24}N_4O_3$ and 2HCl plus H$_2$O) C, H, N, Cl.

Ethyl [7-[N-(4-Amidinophenyl)carbamoyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (12e·HCl). Following the procedure described for the preparation of **12a**·HCl, the title compound was prepared in 75% yield from the nitrile **11e** as a white solid: mp 219–220 °C; IR (KBr) 3294, 3056, 1732, 1674, 1655, 1609, 1518, 1499, 1476, 1320, 1245, 1186, 1167, 1028, 934, 856, 754 cm^{-1}; ^{1}H NMR (270 MHz, CDCl$_3$) δ 10.57 (s, 1H), 9.27 (br s, 2H), 8.99 (br s, 2H), 8.03 (d, 2H, $J = 8.9$ Hz), 7.86 (d, 2H, $J = 8.9$ Hz), 7.74–7.71 (m, 2H), 7.25 (d, 1H, $J = 8.6$ Hz), 4.10 (q, 2H, $J = 7.1$ Hz), 2.92–2.82, (m, 3H), 2.58–2.48 (m, 1H), 2.40 (d, 2H, $J = 6.9$ Hz), 2.20–2.05 (m, 1H), 1.94–1.90 (m, 1H), 1.53–1.42 (m, 1H), 1.21 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.8, 165.9, 164.8, 144.4, 140.4, 135.7, 131.4, 128.9 (2C), 128.7, 128.5, 125.2, 121,7, 119.6 (2C), 59.7, 39.9, 34.7, 30.7, 28.1 (2C), 14.1. Anal. ($C_{22}H_{25}N_3O_3$ and 1.05HCl) C, H, N, Cl.

Ethyl [7-[(4-Amidinophenyl)methoxy]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (12f·HCl). Following the procedure described for the preparation of **12a**·HCl, the title compound was prepared in 68% yield from the nitrile **11f** as a white solid: mp 161–162 °C; IR (KBr) 3152, 2916, 1722, 1671, 1615, 1502, 1260, 1160, 1068, 1031, 834, 726 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 9.40 (br s, 2H), 9.19 (br s, 2H), 7.84 (d, 2H, $J = 8.6$ Hz), 7.64 (d, 2H, $J = 8.6$ Hz), 6.97 (d, 1H, $J = 8.2$ Hz), 6.75 (dd, 1H, $J = 8.2, 2.6$ Hz), 6.71 (d, 1H, $J = 2.6$ Hz), 5.19 (s, 2H), 4.08 (q, 2H, $J = 7.1$ Hz), 2.79 (dd, 1H, $J = 16.5, 4.6$ Hz), 2.70–2.64 (m, 2H), 2.45–2.32 (m, 3H), 2.12–1.98 (m, 1H), 1.90–1.80 (m, 1H), 1.44–1.29 (m, 1H), 1.20 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.9, 165.4, 155.8, 143.6, 136.7, 129.5, 128.2 (3C), 127.4 (2C), 127.0, 114.5, 112.7, 68.1, 59.7, 40.0, 35.1, 30.9, 28.6, 27.3, 14.1. Anal. ($C_{22}H_{26}N_2O_3$ and 1.1HCl) C, H, N, Cl.

Ethyl [7-[(4-Amidinophenyl)methoxy]-2-naphthalenyl]acetate Hydrochloride (12g·HCl). Following the procedure described for the preparation of **12a**·HCl, the title compound was prepared in 89% yield from the nitrile **11g** as

a white solid: mp 209–210 °C; IR (KBr) 1732, 1671, 1195, 1163, 1128, 837 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 9.45 (s, 2H), 9.27 (s, 2H), 7.91–7.71 (m, 6H), 7.64 (s, 1H), 7.36 (d, 1H, $J = 2.3$ Hz), 7.27 (t, 1H, $J = 2.1$ Hz), 7.24 (t, 1H, $J = 2.5$ Hz), 5.38 (s, 2H), 4.09 (q, 2H, $J = 7.1$ Hz), 3.79 (s, 2H), 1.19 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.0, 165.5, 156.0, 143.1, 134.1, 132.6, 129.2 (2C), 128.3 (2C), 127.6 (2C), 127.5, 127.3, 126.6, 125.5, 118.4, 107.3, 68.3, 60.2, 40.5, 14.0. Anal. ($C_{22}H_{22}N_2O_3$ and HCl) C, H, N, Cl.

Ethyl (E)-[7-[2-(4-Amidinophenyl)ethenyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (12h·HCl). Following the procedure described for the preparation of **12a**·HCl, the title compound was prepared in 77% yield from the nitrile **11h** as an yellow solid: mp 234–236 °C; IR (KBr) 3124, 2920, 1724, 1671, 1604, 1540, 1491, 1293, 1265, 1155, 1066, 1029, 972, 873, 838, 705 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 9.42 (br s, 2H), 9.23 (br s, 2H), 7.88 (d, 2H, $J = 8.2$ Hz), 7.80 (d, 2H, $J = 8.2$ Hz), 7.43 (d, 1H, $J = 16.5$ Hz), 7.37–7.34 (m, 2H), 7.29 (d, 1H, $J = 16.5$ Hz), 7.11 (d, 1H, $J = 7.9$ Hz), 4.10 (q, 2H, $J = 7.1$ Hz), 2.91–2.78 (m, 3H), 2.52–2.36 (m, 3H), 2.20–2.05 (m, 1H), 1.95–1.83 (m, 1H), 1.50–1.39 (m, 1H), 1.21 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.9, 165.0, 142.7, 136.5, 135.9, 133.8, 131.9, 129.1, 128.6 (2C), 127.5, 126.4 (2C), 125.7 (2C), 124.2, 59.7, 40.0, 34.8, 30.9, 28.3, 28.1, 14.1. Anal. ($C_{23}H_{26}N_2O_2$ and 1.2HCl plus 0.2H$_2$O) C, H, N, Cl.

Ethyl [7-[2-(4-Amidinophenyl)ethyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (12i·HCl). Following the procedure described for the preparation of **12a**·HCl, the title compound was prepared in 68% yield from the nitrile **11i** as a white solid: mp 149–151 °C; IR (KBr) 3124, 2925, 1728, 1671, 1614, 1541, 1490, 1187, 1155, 1019, 834, 705 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 9.33 (br s, 2H), 9.14 (br s, 2H), 7.77 (d, 2H, $J = 8.2$ Hz), 7.48 (d, 2H, $J = 8.2$ Hz), 6.96–6.93 (m, 3H), 4.09 (q, 2H, $J = 7.1$ Hz), 2.99–2.71 (m, 7H), 2.45–2.33 (m, 3H), 2.14–2.03 (m, 1H), 1.95–1.80 (m, 1H), 1.45–1.30 (m, 1H), 1.20 (t, 3H, $J = 7.1$ Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.9, 165.4, 148.3, 138.0, 135.3, 133.3, 128.9 (2C), 128.7, 128.5, 128.0 (2C), 125.7, 125.3, 59.7, 40.1, 36.7, 36.0, 34.9, 31.0, 28.5, 27.8, 14.1. Anal. ($C_{23}H_{28}N_2O_2$ and 1.05HCl) C, H, N, Cl.

[7-(4-Amidinobenzamido)-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (10a·HCl). To a suspension of **12a**·HCl (1.04 g, 2.5 mmol) in EtOH (10 mL) was added 2 N NaOH (4.0 mL) at room temperature. The mixture was stirred for 20 h at room temperature and concentrated in vacuo. The resulting residue was suspended in H$_2$O, and the mixture was acidified with 2 N HCl. Purification by reverse-phase column chromatography (Chromatorex-ODS, 0.1 N HCl/MeCN = 30/70) afforded 0.63 g (70%) of **10a**·HCl as a white solid: mp 236–238 °C; IR (KBr) 3397, 3250, 1721, 1661, 1545, 1506, 1486, 1335, 1315, 1019, 864, 829, 778, 703 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.11 (br s, 1H), 10.37 (s, 1H), 9.54 (br s, 2H), 9.29 (br s, 2H), 8.17 (d, 2H, $J = 8.6$ Hz), 7.97 (d, 2H, $J = 8.6$ Hz), 7.53–7.50 (m, 2H), 7.05 (d, 1H, $J = 8.9$ Hz), 2.84 (dd, 1H, $J = 4.6, 16.5$ Hz), 2.78–2.75 (m, 2H), 2.45 (dd, 1H, $J = 10.2, 16.5$ Hz), 2.30–2.27 (m, 2H), 2.20–2.10 (m, 1H), 1.95–1.85 (m, 1H), 1.50–1.34 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 165.0, 163.8, 139.3, 136.3, 135.7, 131.7, 130.2, 128.6, 128.1 (2C), 128.0 (2C), 120.7, 118.3, 40.2, 35.1, 30.7, 28.5, 27.6; ESI-MS m/e 352 (M + 1)$^+$. Anal. ($C_{20}H_{21}N_3O_3$ and 1.05HCl plus H$_2$O) C, H, N, Cl.

[7-(4-Amidino-N-methylbenzamido)-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (10b·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 40% yield from the ester **12b**·HCl as a white solid: mp 175–177 °C; IR (KBr) 3055, 1683, 1624, 1506, 1437, 1387, 1290, 1159, 1101, 1017, 856, 716 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.01 (br s, 1H), 9.37 (br s, 2H), 9.18 (br s, 2H), 7.70 (d, 2H, $J = 8.2$ Hz), 7.49 (d, 2H, $J = 8.2$ Hz), 6.97 (s, 1H), 6.93 (d, 1H, $J = 7.9$ Hz), 6.86 (d, 1H, $J = 7.9$ Hz), 3.34 (s, 3H), 2.79–2.67 (m, 3H), 2.37–2.22 (m, 3H), 2.10–1.90 (m, 1H), 1.88–1.82 (m, 1H), 1.37–1.28 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.4, 168.1,

165.0, 141.6, 141.2, 136.8, 134.6, 129.3, 128.2 (2C), 128.1, 127.7 (2C), 127.0, 124.5, 40.3, 38.0, 34.7, 30.5, 28.3, 27.7. Anal. ($C_{21}H_{23}N_3O_3$ and 1.05HCl plus H_2O) C, H, N Cl.

[6-(4-Amidinobenzamido)-3,4-dihydro-2*H***-1-benzopyran-3-yl]acetic Acid Hydrochloride (10c·HCl).** Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 55% yield from the ester **12c**·HCl as a pale-yellow solid: mp >250 °C; IR (KBr) 3375, 3280, 1704, 1676, 1652, 1538, 1504, 1321, 1256, 1220, 1032, 860, 810, 717 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.28 (br s, 1H), 10.34 (s, 1H), 9.54 (br s, 2H), 9.30 (br s, 2H), 8.16 (d, 2H, J = 8.6 Hz), 7.97 (d, 2H, J = 8.6 Hz), 7.51 (d, 1H, J = 2.3 Hz), 7.47 (dd, 1H, J = 2.3, 8.6 Hz), 6.75 (d, 1H, J = 8.6 Hz), 4.18 (d, 1H, J = 10.6 Hz), 3.82 (dd, 1H, J = 6.9, 10.6 Hz), 2.92–2.85 (m, 1H), 2.58–2.49 (m, 1H), 2.38–2.23 (m, 3H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 172.9, 165.0, 163.6, 150.4, 139.3, 131.4, 130.1, 128.1 (2C), 127.9 (2C), 122.2, 120.9, 120.2, 115.8, 68.9, 35.5, 30.2, 28.5; ESI-MS *m/e* 354 (M + 1)$^+$. Anal. ($C_{19}H_{19}N_3O_4$ and 1.2HCl plus 1.3H_2O) C, H, N, Cl.

[7-(4-Amidinobenzamido)-1,2,3,4-tetrahydro-2-isoquinolinyl]acetic Acid Dihydrochloride (10d·2HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 60% yield from the ester **12d**·2HCl as a white solid: mp 241–243 °C; IR (KBr) 3336, 3090, 1671, 1653, 1617, 1542, 1508, 1398, 1318, 910, 865, 837, 696 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.54 (br s, 2H), 9.47 (br s, 2H), 8.18 (d, 2H, J = 8.4 Hz), 7.98 (d, 2H, J = 8.4 Hz), 7.62–7.59 (m, 2H), 7.14 (d, 1H, J = 8.9 Hz), 5.30–3.20 (broad peak, 1H), 4.02 (s, 2H), 3.58 (s, 2H), 3.13–3.10 (m, 2H), 2.95–2.92 (m, 2H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 170.0, 165.1, 164.0, 139.1, 136.6, 132.7, 130.5, 128.7, 128.1 (4C), 127.9, 119.2, 118.1, 57.7, 53.8, 49.8, 26.9. Anal. ($C_{19}H_{20}N_4O_3$ and 1.85HCl plus H_2O) C, H, N, Cl.

[7-[*N***-(4-Amidinophenyl)carbamoyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (10e·HCl).** Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 79% yield from the ester **12e**·HCl as a white solid: mp >250 °C; IR (KBr) 3397, 3135, 2925, 1716, 1671, 1663, 1654, 1608, 1521, 1496, 1490, 1409, 1331, 1255, 1190, 846, 750 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.15 (br s, 1H), 10.58 (s, 1H), 9.30 (br s, 2H), 9.05 (br s, 2H), 8.04 (d, 2H, J = 8.9 Hz), 7.87 (d, 2H, J = 8.9 Hz), 7.74–7.71 (m, 2H), 7.23 (d, 1H, J = 8.6 Hz), 2.97 (dd, 1H, J = 10.3, 4.1 Hz), 2.90–2.83 (m, 2H), 2.58–2.48 (m, 1H), 2.34–2.30 (m, 2H), 2.15–2.05 (m, 1H), 1.98–1.92 (m, 1H), 1.54–1.39 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 165.9, 164.8, 144.4, 140.5, 135.8, 131.4, 128.9 (2C), 128.7, 128.4, 125.1, 121.7, 119.6 (2C), 40.2, 34.9, 30.6, 28.2 (2C). Anal. ($C_{20}H_{21}N_3O_3$ and 1.1HCl plus 1.6H_2O) C, H, N, Cl.

[7-[(4-Amidinophenyl)methoxy]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (10f·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 79% yield from the ester **12f**·HCl as a white solid: mp 249–250 °C; IR (KBr) 3379, 3263, 3105, 2925, 1718, 1669, 1502, 1261, 1161, 1025, 867, 832, 802 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.14 (s, 1H), 9.40 (br s, 2H), 9.20 (br s, 2H), 7.84 (d, 2H, J = 8.6 Hz), 7.64 (d, 2H, J = 8.6 Hz), 6.97 (d, 1H, J = 8.2 Hz), 6.75 (dd, 1H, J = 8.2, 2.6 Hz), 6.71 (d, 1H, J = 2.6 Hz), 5.19 (s, 2H), 2.78 (dd, 1H, J = 4.3, 16.5 Hz), 2.70–2.66 (m, 2H), 2.39 (dd, 1H, J = 10.4, 16.5 Hz), 2.28–2.25 (m, 2H), 2.12–1.98 (m, 1H), 1.95–1.80 (m, 1H), 1.43–1.28 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 165.4, 155.7, 143.6, 136.8, 129.5, 128.2 (3C), 127.3 (2C), 126.9, 114.4, 112.7, 68.0, 40.3, 35.2, 30.7, 28.7, 27.4. Anal. ($C_{20}H_{22}N_2O_3$ and HCl plus H_2O) C, H, N, Cl.

[7-[(4-Amidinophenyl)methoxy]-2-naphthalenyl]acetic Acid Hydrochloride (10g·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 68% yield from the ester **12g**·HCl as a white solid: mp >260 °C; IR (KBr) 3362, 3219, 3096, 2921, 1710, 1691, 1674, 1632, 1616, 1512, 1486, 1392, 1265, 1222, 1202, 1167, 1124, 1039, 1016, 841, 726, 670 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.40 (s, 1H), 9.42 (s, 2H), 9.25 (s, 2H), 7.88–7.70 (m, 6H), 7.62 (s, 1H), 7.35 (d, 1H, J = 2.5 Hz), 7.26 (dd,

1H, J = 2.0, 5.0 Hz), 7.23 (dd, 1H, J = 2.2, 5.7 Hz), 5.38 (s, 2H), 3.71 (s, 2H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 172.5, 165.5, 155.9, 143.1, 134.1, 133.2, 129.1, 128.3 (2C), 127.6 (2C), 127.4 (2C), 127.2, 126.6, 125.7, 118.3, 107.3, 68.2, 40.9. Anal. ($C_{20}H_{18}N_2O_3$ and HCl plus 0.2H_2O) C, H, N, Cl.

(*E***)-[7-[2-(4-Amidinophenyl)ethenyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (10h·HCl).** Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 66% yield from the ester **12h**·HCl as a pale-yellow solid: mp >260 °C; IR (KBr) 3326, 3179, 3071, 1710, 1676, 1605, 1541, 1508, 1475, 1436, 1389, 1234, 1149, 967, 947, 836, 718 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.17 (s, 1H), 9.40 (br s, 2H), 9.21 (br s, 2H), 7.87 (d, 2H, J = 8.6 Hz), 7.80 (d, 2H, J = 8.6 Hz), 7.43 (d, 1H, J = 16.5 Hz), 7.40–7.34 (m, 2H), 7.29 (d, 1H, J = 16.5 Hz), 7.11 (d, 1H, J = 7.9 Hz), 2.93–2.78 (m, 3H), 2.52–2.42 (m, 1H), 2.30 (d, 2H, J = 7.3 Hz), 2.18–2.02 (m, 1H), 1.98–1.83 (m, 1H), 1.49–1.34 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 165.0, 142.7, 136.6, 136.0, 133.7, 131.9, 129.0, 128.5 (2C), 127.4, 126.4 (3C), 125.7, 124.2, 40.3, 34.9, 30.7, 28.4, 28.1. Anal. ($C_{21}H_{22}N_2O_2$ and HCl) C, H, N, Cl.

[7-[2-(4-Amidinophenyl)ethyl]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (10i·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 88% yield from the ester **12i**·HCl as a white solid: mp 219–221 °C; IR (KBr) 3104, 2913, 1708, 1685, 1614, 1489, 1242, 1157, 835, 765, 669 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.14 (s, 1H), 9.34 (br s, 2H), 9.16 (br s, 2H), 7.77 (d, 2H, J = 8.2 Hz), 7.49 (d, 2H, J = 8.2 Hz), 6.96–6.93 (m, 3H), 3.00–2.71 (m, 7H), 2.39 (dd, 1H, J = 10.2, 16.5 Hz), 2.29–2.25 (m, 2H), 2.12–1.98 (m, 1H), 1.95–1.80 (m, 1H), 1.45–1.30 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 165.4, 148.2, 138.0, 135.4, 133.4, 128.9 (2C), 128.6, 128.5, 128.0 (2C), 125.6, 125.3, 40.3, 36.6, 35.9, 35.0, 30.8, 28.6, 27.8. Anal. ($C_{21}H_{24}N_2O_2$ and 1.05HCl) C, H, N, Cl.

Methyl 5,6-Dihydro-2-naphthoate (14b). To an ice-cooled solution of **13b**[24] (15.7 g, 77 mmol) in a mixture of methanol (20 mL) and THF (40 mL) was added NaBH$_4$ (3.1 g, 42.0 mmol) by portions. The mixture was stirred for 1 h at room temperature, neutralized with 1 N HCl, and extracted with ether. The organic phase was washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. Purification of the residue by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 3/1) afforded 15.1 g (95%) of methyl 8-hydroxy-5,6,7,8-tetrahydro-2-naphthoate.

The preceding alcohol (14.47 g, 70.1 mmol) was dissolved in benzene (200 mL), and Amberlyst 15 (5.0 g) was added. The mixture was refluxed for 3 h, filtered, and concentrated in vacuo to afford an oily residue. Purification by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 7/1) afforded 11.7 g (89%) of **14b** as a colorless oil. Anal. ($C_{12}H_{12}O_2$) C, H.

Methyl 7-Oxo-5,6,7,8-tetrahydro-2-naphthoate (15b). To a solution of **14b** (10.2 g, 54.1 mmol) in benzene (140 mL) was added 3-chloroperbenzoic acid (12.0 g, 69.5 mmol) by portions. The mixture was stirred for 2 h at room temperature. The reaction was quenched with aqueous Na$_2$SO$_3$ solution, and the mixture was extracted with benzene. The organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. Purification by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 4/1) afforded 10.5 g (95%) of methyl 7,8-epoxy-5,6,7,8-tetrahydro-2-naphthoate.

A mixture of the preceding epoxide (10.4 g, 50.9 mmol) and ZnI$_2$ (19.2 g, 60.2 mmol) in benzene (200 mL) was stirred for 1 h at room temperature. After addition of water, the mixture was extracted with benzene. The organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. Purification of the residue by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 5/1) gave 9.55 g (92%) of **15b** as a white solid: mp 56–58 °C.

Methyl 7-[(*tert***-Butoxycarbonyl)methyl]-5,6-dihydro-2-naphthoate (16b).** To an ice-cooled solution of *tert*-butyl *P*,*P*-dimethylphosphonoacetate (12.4 g, 55.3 mmol) in benzene (100 mL) was added NaH (1.26 g, 52.5 mmol) by portions, and the mixture was stirred for 30 min at room temperature. To

this was added a solution of **15b** (9.4 g, 46.0 mmol) in THF (30 mL). The mixture was stirred for an additional 2 h at room temperature. After addition of 1 N HCl (100 mL), the mixture was extracted with toluene. The organic phase was washed with saturated aqueous NaHCO$_3$ and brine, dried over Na$_2$-SO$_4$, filtered, and concentrated in vacuo. Purification by column chromatography (SiO$_2$, *n*-hexane/isopropyl ether = 1/6) gave 12.16 g (87%) of a mixture of **16b** and its olefin isomer, which was used for the next reaction without further purification. An analytical pure **16b** was afforded by flash chromatography (SiO$_2$, *n*-hexane/isopropyl ether = 8/1) as a colorless solid: mp 60−61 °C. Anal. (C$_{18}$H$_{22}$O$_4$) C, H.

Methyl 7-[(*tert*-Butoxycarbonyl)methyl]-5,6,7,8-tetrahydro-2-naphthoate (17b). A mixture of **16b** (9.1 g, 30.0 mmol) and 10% Pd/C (0.70 g) in MeOH (50 mL) was stirred at 0.1 MPa of H$_2$ atmosphere for 2 h. Filtration and evaporation of the filtrate gave 8.88 g (29.2 mmol, 97%) of **17b** as a white solid: mp 54−55 °C. Anal. (C$_{18}$H$_{24}$O$_4$) C, H.

6-Methoxy-1,2-dihydronaphthalene (14c). To an ice-cooled solution of 50.3 g (0.285 mol) of **13c** in a mixture of MeOH (50 mL) and THF (250 mL) was added NaBH$_4$ (10.6 g, 0.280 mol). The mixture was stirred for 2 h at room temperature. The reaction mixture was neutralized with 3 N HCl solution, concentrated in vacuo, and extracted with ether twice. The combined organic phases were dried over MgSO$_4$, filtered, and concentrated in vacuo to afford an oil, which was passed through a pad of silica gel. Concentration in vacuo gave a crude alcohol (51.1 g).

A mixture of the preceding alcohol (51.1 g) and pyridinium *p*-toluenesulfonate (3.60 g, 14.3 mmol) in toluene (500 mL) was refluxed for 3 h. The mixture was washed with aqueous NaHCO$_3$, aqueous NaHSO$_4$, and brine successively. The organic phase was dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford an oily residue. Distillation (82−85 °C at 30 Pa) gave 39.0 g (0.243 mol, 85% from **13c**) of **14c** as an oil. Anal. (C$_{11}$H$_{12}$O) H; C: calcd, 82.46; found, 82.01.

7-Methoxy-2-oxo-1,2,3,4-tetrahydronaphthalene (15c). To an ice-cooled mixture of **14c** (35.0 g, 218 mmol), 10% aqueous NaHCO$_3$ (200 mL), and toluene (300 mL) was added of 3-chloroperbenzoic acid (50.0 g, 289 mmol). The mixture was vigorously stirred for 2 h at room temperature. The reaction was quenched with 10% aqueous Na$_2$SO$_3$ solution. The organic phase was separated, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. Purification by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 3/1) afforded 36.5 g (207 mmol, 95%) of 1,2-epoxy-7-methoxy-1,2,3,4-tetrahydronaphthalene as a colorless oil.

To a suspension of ZnI$_2$ (7.9 g, 24.8 mmol) in benzene (300 mL) was dropwise added the above epoxide (13.0 g, 73.9 mmol). The mixture was refluxed for 3 h and washed with H$_2$O. The organic phase was dried over MgSO$_4$, filtered, and concentrated in vacuo to afford a residual oil. Purification of column chromatography (SiO$_2$, CHCl$_3$) afforded 9.6 g (74%) of **15c** as a colorless oil.

Ethyl (7-Methoxy-3,4-dihydro-2-naphthalenyl)acetate (16c). To a solution of ethyl (diethylphosphoryl)acetate (13.5 g, 60.1 mmol) in 1,2-dimethoxyethane (200 mL) was added NaH (1.44 g, 60 mmol) by portions at room temperature, and the mixture was stirred for 1 h at room temperature. After addition of **15c** (9.6 g, 54.5 mmol), the mixture was stirred for 3 h at room temperature, poured onto ice−water, and extracted with EtOAc twice. The combined organic phases were dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford an oil, which was purified by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 10/1) to give 9.6 g (60%) of **16c** with a small amount of olefin isomer, ethyl (7-methoxy-1,2,3,4-tetrahydro-2-naphthalenylidene)acetate. An analytical pure **16c** was afforded by flash chromatography (SiO$_2$, *n*-hexane/isopropyl ether = 10/1) as a colorless oil.

Ethyl (7-Methoxy-1,2,3,4-tetrahydro-2-naphthalenyl)acetate (17c). According to the procedure described for the preparation of **17b**, the title compound was prepared from **16c** in 95% yield as a colorless oil.

Ethyl [7-(*N*-Methyl-*tert*-butoxycarbamoyl)-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (18). A mixture of **17a** (5.83 g, 25.0 mmol) and di-*tert*-butyl dicarbonate (6.0 g, 27.5 mmol) in 1,4-dioxane (30 mL) was stirred for 20 h at room temperature and concentrated in vacuo to afford an oily residue, which was crystallized by addition of *n*-hexane. Recrystallization from a mixture of *n*-hexane and EtOAc (5:1) afforded 5.74 g (69%) of Boc-aniline derivative as a white solid: mp 112−114 °C.

To a solution of the preceding Boc-aniline derivative (0.67 g, 2.0 mmol) in DMF (10 mL) was added NaH (57 mg, 2.4 mmol), and the mixture was stirred for 5 min. To this was added 0.57 g (4.0 mmol) of methyl iodide, and the mixture was stirred for 18 h at room temperature. After removal of the solvent, the mixture was diluted with H$_2$O and extracted with EtOAc twice. The combined organic phases were dried over MgSO$_4$, filtered, and concentrated in vacuo. The residual oil was purified by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 10/1) to afford 0.67 g (96%) of **18** as a colorless oil. Anal. (C$_{20}$H$_{29}$NO$_4$) C, H, N.

Ethyl [7-(*N*-Methylamino)-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (19·HCl). Into an ice-cooled solution of **18** (3.0 g, 8.63 mmol) in EtOH (30 mL) was bubbled HCl for 15 min. The mixture was stirred for 3 h at room temperature and concentrated in vacuo to afford 2.47 g (quantitative) of **19·HCl**, which was used for the next reaction without further purification: mp 138−140 °C.

7-[(*tert*-Butoxycarbonyl)methyl]-5,6,7,8-tetrahydro-2-naphthoic Acid (20). To a solution of **17b** (10.8 g, 35.5 mmol) in MeOH (100 mL) was added 2.5 N NaOH (20 mL). The mixture was stirred for 20 h at room temperature and concentrated in vacuo. The resulting residue was suspended with H$_2$O and acidified by 3 N HCl. The mixture was extracted with EtOAc. The organic phase was washed with brine, dried over MgSO$_4$, and concentrated in vacuo to afford a residue. Purification by column chromatography (SiO$_2$, CHCl$_3$/MeOH = 1/20) gave 6.62 g (64%) of **20** as a white solid: mp 125−126 °C. Anal. (C$_{17}$H$_{22}$O$_4$) C, H.

Ethyl (7-Hydroxy-1,2,3,4-tetrahydro-2-naphthalenyl)acetate (21). To a mixture of NaI (6.04 g, 40.0 mmol) and TMSCl (4.44 g, 40.0 mmol) in MeCN (100 mL) was added 7.70 g (31.0 mmol) of **17c**, and the mixture was refluxed for 6 h. The reaction was quenched by addition of 10% Na$_2$SO$_3$ solution. The mixture was extracted with ether twice, and the combined organic phases were dried over MgSO$_4$ and concentrated in vacuo. Purification by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 5/1) gave 3.96 g (55%) of **21** as a colorless oil.

Ethyl [7-[(Trifluoromethyl)sulfonyloxy]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate (22). To an ice-cooled solution of **21** (4.0 g, 17.5 mmol) and pyridine (20.0 g, 253 mmol) in CHCl$_3$ (50 mL) was slowly added trifluoromethanesulfonic anhydride (6.37 g, 22.5 mmol). The mixture was stirred for 2 h at 0 °C. After addition of 2 N HCl, the mixture was extracted with CHCl$_3$. The organic phase was washed with aqueous NaHCO$_3$ solution and brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. Purification of the residue by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 7/1) gave 6.6 g (quantitative) of **22** as a colorless oil.

Ethyl (7-Hydroxy-2-naphthalenyl)acetate (23). A mixture of **16c** (6.60 g, 26.8 mmol) and *p*-chloranil (8.57 g, 34.8 mmol) in xylene (50 mL) was refluxed for 1.5 h and concentrated in vacuo. Purification of the residue by column chromatography (SiO$_2$, *n*-hexane/EtOAc = 15/1) gave 3.11 g (48%) of ethyl (7-methoxy-2-naphthalenyl)acetate as a pale-yellow solid: mp 68−69 °C. Anal. (C$_{15}$H$_{16}$O$_3$) C, H.

Following the procedure described for the preparation of **21**, the preceding ether was converted to **23** in 49% yield as a white solid: mp 132−134 °C. Anal. (C$_{14}$H$_{14}$O$_3$) C, H.

(7-Nitro-1-oxo-1,2,3,4-tetrahydro-2-naphthylidene)acetic Acid (24). A mixture of **13a** (5.0 g, 26.5 mmol), 40% glyoxylic acid (15.0 g, 81.0 mmol), and H$_2$SO$_4$ (3.2 g, 32.6 mmol) in 1,4-dioxane (10 mL) was refluxed for 5 h. After addition of water (10 mL), the mixture was cooled to room temperature.

The resulting precipitate was collected by filtration and washed with water and MeOH successively. Recrystallization from 1,4-dioxane gave 4.1 g (63%) of **24** as a pale-yellow solid: mp 204−206 °C. Anal. ($C_{12}H_9NO_5$) C, H, N.

Ethyl (7-Amino-1,2,3,4-tetrahydro-2-naphthalenyl)acetate (17a). A mixture of **24** (5.0 g, 18.1 mmol), H_2SO_4 (2.13 g, 21.7 mmol), and 10% Pd/C (1.0 g) in EtOH (50 mL) was placed in an autoclave. The mixture was stirred under H_2 atmosphere (1.0 MPa) for 20 h at 60 °C and filtered. The filtrate was neutralized with 20% NaOH and concentrated in vacuo to afford an oily residue, which was dissolved in $CHCl_3$ (50 mL) and washed with H_2O. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography (SiO_2, *n*-hexane/EtOAc = 3/1) gave 2.10 g (50%) of **17a** as solid: mp 45−47 °C.

(*E*)-(6-Nitro-4-oxo-3,4-dihydro-2*H*-1-benzopyran-3-ylidene)acetic Acid (26). Following the procedure described for the preparation of **24**, the title compound was prepared from **25** in 61% yield as a yellow solid: mp 193−194 °C. Anal. ($C_{11}H_7NO_6$) C, H, N.

Ethyl (6-Amino-3,4-dihydro-2*H*-1-benzopyran-3-yl)acetate (27). Following the procedure described for the preparation of **17a**, the title compound was prepared from **26** in 74% yield: mp 57−58 °C. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

Ethyl (7-Amino-1,2,3,4-tetrahydro-2-isoquinolinyl)acetate Dihydrochloride (30·2HCl). A mixture of **29**[28] (2.89 g, 13.5 mmol), ethyl bromoacetate (2.25 g, 13.5 mmol), and Et_3N (2.83 g, 27.9 mmol) in EtOH (20 mL) was refluxed for 5 h and concentrated in vacuo. After addition of water, the mixture was extracted with EtOAc twice. The combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford a residue. Purification by column chromatography (SiO_2, *n*-hexane/EtOAc = 3/1) gave a free amine, which was treated with 4 N HCl in 1,4-dioxane to afford 2.14 g (57%) of ethyl (7-nitro-1,2,3,4-tetrahydro-2-isoquinolinyl)acetate hydrochloride as a hygroscopic solid: mp 174−177 °C.

A mixture of the preceding nitro compound (2.0 g, 6.65 mmol) and 10% Pd/C (1.0 g) in EtOH (40 mL) was vigorously stirred under H_2 atmosphere for 2 h at room temperature. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo to afford a residue. Treatment of the residue with 4 N HCl in 1,4-dioxane gave 2.0 g (100%) of **30·2HCl** as a hygroscopic solid, which was immediately used for the next reaction without purification.

4-Ethynylbenzonitrile (32). To an ice-cooled mixture of 11.9 g (0.10 mol) of 4-hydroxybenzonitrile (**31**) and Et_3N (12.1 g, 0.10 mol) in CH_2Cl_2 (100 mL) was dropwise added trifluoromethanesulfonic anhydride (30.9 g, 0.109 mol). The mixture was stirred at ambient temperature for 1 h, and aqueous $NaHCO_3$ solution was added. The mixture was extracted with CH_2Cl_2. The organic phase was washed with aqueous $NaHSO_4$ solution and brine successively, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, *n*-hexane/EtOAc = 10/1) gave 20.0 g (80%) of the 4-cyanophenyl trifluoromethanesulfonate as a colorless oil.

A mixture of the preceding triflate (18.3 g, 77.8 mmol), (trimethylsilyl)acetylene (15.2 g, 154.7 mmol), CuI (2.83 g, 14.9 mmol), Pd(PPh₃)₄ (5.0 g, 4.3 mmol), and *n*-propylamine (14.0 g, 0.236 mol) in benzene (200 mL) was stirred at room temperature for 3 h. The reaction mixture was washed with saturated aqueous NH_4Cl solution three times. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, *n*-hexane/CHCl₃ = 3/1) afforded 15.1 g (97%) of 4-[2-(trimethylsilyl)ethynyl]benzonitrile as a white solid: mp 107−108 °C. Anal. ($C_{12}H_{13}NSi$) C, H, N.

A mixture of 12.2 g (59.7 mmol) of the preceding silylacetylene and KF (15.0 g, 258 mmol) in acetonitrile (120 mL) was refluxed for 4 h. After addition of water, the mixture was extracted with ether twice. The combined organic phases were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, *n*-hexane/EtOAc =

7/1) gave 7.4 g (98%) of **32** as a white solid: mp 156−157 °C. Anal. (C_9H_5N) C, N; H: found, 4.52; calcd, 3.96.

Ethyl [7-[4-[*N*-(*n*-Propyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (33a·HCl). Anhydrous HCl was bubbled into an ice-cooled suspension of **11a** (2.45 g, 6.76 mmol) in EtOH (20 mL) for 30 min. The mixture was stirred for 4 h at room temperature and concentrated in vacuo. The residual solid was triturated with isopropyl ether, dried under reduced pressure, and dissolved in EtOH (18 mL). To this was added *n*-propylamine (0.81 g, 13.6 mmol), and the mixture was stirred for 20 h at room temperature. The resulting precipitate was collected by filtration and treated with ethanolic HCl to afford 2.8 g (89%) of **33a·HCl** as a white solid: mp 250 °C; IR (KBr) 3328, 3212, 3043, 2931, 1727, 1675, 1626, 1596, 1538, 1508, 1420, 1157, 1026, 831, 714 cm⁻¹; ¹H NMR (270 MHz, DMSO-d_6) δ 10.36 (s, 1H), 10.10−9.20 (broad peak, 3H), 8.16 (d, 2H, *J* = 8.4 Hz), 7.88 (d, 2H, *J* = 8.4 Hz), 7.53−7.50 (m, 2H), 7.06 (d, 1H, *J* = 8.6 Hz), 4.10 (q, 2H, *J* = 7.3 Hz), 3.40 (t, 2H, *J* = 7.2 Hz), 2.85−2.75 (m, 3H), 2.49−2.35 (m, 3H), 2.15−2.05 (m, 1H), 1.91−1.87 (m, 1H), 1.75−1.62 (m, 2H), 1.49−1.39 (m, 1H), 1.21 (t, 3H, *J* = 7.3 Hz), 0.98 (t, 3H, *J* = 7.3 Hz); ¹³C NMR (22.4 MHz, DMSO-d_6) δ 171.9, 163.9, 162.2, 138.7, 136.3, 135.6, 131.6, 131.3, 128.6, 128.2 (2C), 127.9 (2C), 120.8, 118.4, 59.7, 44.3, 40.0, 35.0, 30.9, 28.5, 27.6, 20.7, 14.1, 11.1. Anal. ($C_{25}H_{31}N_3O_3$ and HCl) C, H, N, Cl.

Ethyl [7-[4-[*N*-(2-Propenyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (33b·HCl). The title compound was prepared from **11a** and allylamine in 85% yield following the procedure described for the preparation of **33a·HCl**: mp 248−250 °C; IR (KBr) 3329, 3050, 2931, 1725, 1673, 1623, 1597, 1539, 1508, 1431, 1263, 1159, 1025, 830, 714 cm⁻¹; ¹H NMR (DMSO-d_6) δ 10.38 (s, 1H), 10.19 (br s, 1H), 9.72 (br s, 1H), 9.25 (br s, 1H), 8.17 (d, 2H, *J* = 8.6 Hz), 7.92 (d, 2H, *J* = 8.6 Hz), 7.53−7.50 (m, 2H), 7.05 (d, 1H, *J* = 8.9 Hz), 6.00−5.89 (m, 1H), 5.38−5.26 (m, 2H), 4.16−4.06 (m, 4H), 2.80−2.75 (m, 3H), 2.47−2.35 (m, 3H), 2.20−2.05 (m, 1H), 1.91−1.87 (m, 1H), 1.46−1.40 (m, 1H), 1.21 (t, 3H, *J* = 7.1 Hz); ¹³C NMR (22.4 MHz, DMSO-d_6) δ 171.9, 163.8, 162.1, 138.8, 136.4, 135.5, 131.5 (2C), 131.0, 128.6, 128.3 (2C), 127.9 (2C), 120.8, 118.4, 117.2, 59.7, 44.4, 40.0, 35.0, 30.9, 28.5, 27.6, 14.1; CI-MS *m/e* 420 (M + 1)⁺. Anal. ($C_{25}H_{29}N_3O_3$ and HCl) C, H, N, Cl.

Ethyl [7-[4-[*N*-(2-Propynyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (33c·HCl). The title compound was prepared from **11a** and propargylamine in 82% yield following the procedure described for the preparation of **33a·HCl**: mp 203−205 °C; IR (KBr) 3422, 3236, 3043, 1721, 1671, 1617, 1541, 1506, 1418, 1333, 1269, 1158, 1028, 858, 713 cm⁻¹; ¹H NMR (270 MHz, DMSO-d_6) δ 10.45 (br s, 1H), 10.36 (s, 1H), 9.93 (br s, 1H), 9.46 (br s, 1H), 8.16 (d, 2H, *J* = 8.2 Hz), 7.89 (d, 2H, *J* = 8.6 Hz), 7.52−7.49 (m, 2H), 7.05 (d, 1H, *J* = 8.9 Hz), 4.37−4.35 (m, 2H), 4.10 (q, 2H, *J* = 7.1 Hz), 3.50 (s, 1H), 2.86−2.75 (m, 3H), 2.45−2.35 (m, 3H), 2.20−2.13 (m, 1H), 1.91−1.87 (m, 1H), 1.46−1.42 (m, 1H), 1.22 (t, 3H, *J* = 7.1 Hz); ¹³C NMR (22.4 MHz, DMSO-d_6) δ 171.9, 163.8, 162.3, 139.0, 136.3, 135.6, 131.5, 130.7, 128.6, 128.3 (2C), 127.9 (2C), 120.7, 118.3, 77.2, 75.9, 59.6, 39.9, 35.0, 32.1, 30.9, 28.4, 27.6, 14.1; CI-MS *m/e* 418 (M + 1)⁺. Anal. ($C_{25}H_{27}N_3O_3$ and 1.03HCl plus 0.1H₂O) C, H, N, Cl.

Ethyl [7-[4-[*N*-(2-Furylmethyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (33d·HCl). The title compound was prepared from **11a** and furfurylamine in 55% yield following the procedure described for the preparation of **33a·HCl**: mp 240−242 °C; IR (KBr) 3316, 3032, 1732, 1678, 1622, 1596, 1539, 1507, 1420, 1337, 1258, 1205, 1149, 1018, 826, 744 cm⁻¹; ¹H NMR (270 MHz, DMSO-d_6) δ 10.48 (br s, 1H), 10.39 (s, 1H), 9.90 (br s, 1H), 9.61 (br s, 1H), 8.17 (d, 2H, *J* = 8.2 Hz), 7.90 (d, 2H, *J* = 8.2 Hz), 7.70 (s, 1H), 7.53−7.50 (m, 2H), 7.05 (d, 1H, *J* = 8.9 Hz), 6.48−6.46 (m, 2H), 4.77 (s, 2H), 4.10 (q, 2H, *J* = 7.1 Hz), 2.85−2.75 (m, 3H), 2.44−2.35 (m, 3H), 2.20−2.05 (m, 1H), 1.91−1.87 (m, 1H), 1.46−1.39 (m, 1H), 1.21 (t, 3H, *J* = 7.1

Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.9, 163.9, 162.3, 148.3, 143.2, 138.9, 136.4, 135.6, 131.5, 130.8, 128.6, 128.4 (2C), 128.0 (2C), 120.9, 118.5, 110.6, 109.2, 59.7, 40.0, 39.5, 35.1, 30.9, 28.5, 27.6, 14.1; ESI-MS *m/e* 460 (M + 1)$^+$. Anal. (C$_{27}$H$_{29}$N$_3$O$_4$ and HCl) C, H, N, Cl.

Ethyl [7-[4-(Morpholinoformimidoyl)benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetate Hydrochloride (33e·HCl). The title compound was prepared from **11a** and morpholine in 75% yield following the procedure described for the preparation of **33a**·HCl: mp 242–244 °C; IR (KBr) 3023, 1731, 1668, 1613, 1535, 1505, 1418, 1322, 1266, 1159, 1114, 1068, 1019, 869, 704 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.35 (s, 1H), 9.70 (br s, 2H), 8.18 (d, 2H, *J* = 8.2 Hz), 7.77 (d, 2H, *J* = 8.2 Hz), 7.51–7.49 (m, 2H), 7.05 (d, 1H, *J* = 8.9 Hz), 4.10 (q, 2H, *J* = 7.1 Hz), 3.76–3.31 (broad peak, 8H), 2.86–2.75 (m, 3H), 2.44–2.35 (m, 3H), 2.20–2.05 (m, 1H), 1.91–1.87 (m, 1H), 1.49–1.38 (m, 1H), 1.21 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 172.0, 164.0, 163.2, 137.8, 136.5, 135.7, 131.4 (2C), 128.4 (5C), 120.8, 118.5, 65.2 (2C), 59.7, 48.4 (2C), 40.0, 35.1, 30.9, 28.5, 27.6, 14.1; CI-MS *m/e* 450 (M + 1)$^+$. Anal. (C$_{26}$H$_{31}$N$_3$O$_4$ and 1.03HCl plus 0.2H$_2$O) C, H, N, Cl.

Ethyl [6-[4-[*N*-(2-Propynyl)amidino]benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate Hydrochloride (35·HCl). The title compound was prepared from **11c** and propargylamine in 65% yield following the procedure described for the preparation of **33a**·HCl: mp 247–249 °C; IR (KBr) 3226, 3044, 1729, 1667, 1621, 1551, 1501, 1327, 1255, 1217, 1158, 1031, 856, 822, 760 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.51 (br s, 1H), 10.35 (s, 1H), 9.96 (br s, 1H), 9.50 (br s, 1H), 8.16 (d, 2H, *J* = 8.2 Hz), 7.90 (d, 2H, *J* = 8.2 Hz), 7.51 (d, 1H, *J* = 2.3 Hz), 7.47 (dd, 1H, *J* = 2.3, 8.9 Hz), 6.75 (d, 1H, *J* = 8.9 Hz), 4.38–4.36 (m, 2H), 4.18 (d, 1H, *J* = 10.9 Hz) 4.11 (q, 2H, *J* = 7.1 Hz), 3.85–3.78 (m, 1H), 3.51 (s, 1H), 2.92–2.85 (m, 1H), 2.55–2.33 (m, 4H), 1.21 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 171.4, 163.6, 162.3, 150.4, 139.1, 131.6, 130.6, 128.4 (2C), 127.9 (2C), 122.2, 120.7, 120.3, 115.8, 77.3, 75.9, 68.8, 59.9, 35.3, 32.2, 30.2, 28.7, 14.0; CI-MS *m/e* 420 (M + 1)$^+$. Anal. (C$_{24}$H$_{25}$N$_3$O$_4$ and HCl) C, H, N, Cl.

Ethyl [6-[4-(Morpholinoformimidoyl)benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate Hydrochloride ((*RS*)-4·HCl). The title compound was prepared from **11c** and morpholine in 61% yield following the procedure described for the preparation of **33a**·HCl: mp 251–253 °C; IR (KBr) 3432, 3218, 3028, 1730, 1662, 1619, 1538, 1499, 1422, 1252, 1221, 1110, 1019, 702 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 9.82 (br s, 1H), 9.78 (s, 1H), 8.19 (d, 2H, *J* = 8.3 Hz), 7.76 (d, 2H, *J* = 8.3 Hz), 7.55 (d, 1H, *J* = 2.5 Hz), 7.49 (dd, 1H, *J* = 2.5, 8.8 Hz), 6.75 (d, 1H, *J* = 8.8 Hz), 4.18 (d, 1H, *J* = 10.7 Hz), 4.11 (q, 2H, *J* = 7.1 Hz), 3.95–3.20 (m, 9H), 2.92–2.86 (m, 1H), 2.56–2.35 (m, 4H), 1.21 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (100.4 MHz, DMSO-d_6) δ 171.6, 163.9, 163.5, 150.6, 138.1, 131.8, 131.5, 128.6 (2C), 128.4 (2C), 122.3, 120.9, 120.4, 116.0, 69.0, 65.7, 64.9, 60.1, 49.9, 47.2, 35.5, 30.4, 28.9, 14.2; CI-MS *m/e* 452 (M + 1)$^+$. Anal. (C$_{25}$H$_{29}$N$_3$O$_5$ and HCl) C, H, N, Cl.

[7-[4-[*N*-(*n*-Propyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (34a·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 85% yield from the ester **33a**·HCl: mp >250 °C; IR (KBr) 3340, 3142, 1706, 1673, 1623, 1593, 1538, 1508, 1418, 1336, 1251, 1167, 1016, 921, 860, 829, 710 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.30–11.80 (broad peak, 1H), 10.41 (s, 1H), 10.02 (br s, 1H), 9.65 (br s, 1H), 9.33 (br s, 1H), 8.18 (d, 2H, *J* = 8.2 Hz), 7.91 (d, 2H, *J* = 8.2 Hz), 7.54–7.52 (m, 2H), 7.05 (d, 1H, *J* = 8.2 Hz), 3.42 (t, 2H, *J* = 7.1 Hz), 2.87–2.75 (m, 3H), 2.46–2.28 (m, 3H), 2.15–2.05 (m, 1H), 1.93–1.89 (m, 1H), 1.76–1.63 (m, 2H), 1.46–1.33 (m, 1H), 0.98 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 163.9, 162.0, 138.7, 136.3, 135.7, 131.6, 131.2, 128.6, 128.3 (2C), 127.9 (2C), 120.8, 118.4, 44.2, 40.3, 35.1, 30.8, 28.6, 27.6, 20.8, 11.1. Anal. (C$_{23}$H$_{27}$N$_3$O$_3$ and 1.15HCl plus 0.5H$_2$O) C, H, N, Cl.

[7-[4-[*N*-(2-Propenyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (34b·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 49% yield from the ester **33b**·HCl: mp >250 °C; IR (KBr) 3263, 3089, 1693, 1653, 1538, 1501, 1439, 1389, 1318, 1236, 1155, 1063, 985, 939, 864, 823, 719, 692 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.38 (s, 1H), 10.20–9.20 (broad peak, 2H), 8.18 (d, 2H, *J* = 8.6 Hz), 7.93 (d, 2H, *J* = 8.6 Hz), 7.53–7.50 (m, 2H), 7.05 (d, 1H, *J* = 8.6 Hz), 6.00–5.90 (m, 1H), 5.38–5.25 (m, 2H), 4.15 (d, 2H, *J* = 5.3 Hz), 2.88–2.75 (m, 3H), 2.46–2.27 (m, 3H), 2.20–2.05 (m, 1H), 1.94–1.89 (m, 1H), 1.45–1.39 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 163.9, 162.1, 138.8, 136.3, 135.7, 131.6, 131.5, 131.0, 128.6, 128.3 (2C), 128.0 (2C), 120.8, 118.4, 117.2, 44.5, 40.3, 35.2, 30.8, 28.6, 27.6. Anal. (C$_{23}$H$_{25}$N$_3$O$_3$ and 1.1HCl plus 0.7H$_2$O) C, H, N, Cl.

[7-[4-[*N*-(2-Propynyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (34c·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 91% yield from the ester **33c**·HCl: mp 247–249 °C; IR (KBr) 3284, 3046, 1690, 1654, 1628, 1612, 1538, 1503, 1391, 1335, 1318, 1249, 1156, 863, 721, 692 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.48 (br s, 1H), 10.36 (s, 1H), 9.94 (br s, 1H), 9.46 (br s, 1H), 8.17 (d, 2H, *J* = 8.4 Hz), 7.90 (d, 2H, *J* = 8.4 Hz), 7.52–7.49 (m, 2H), 7.05 (d, 1H, *J* = 8.9 Hz), 4.36 (dd, 2H, *J* = 2.4, 5.4 Hz), 3.49 (t, 1H, *J* = 2.4 Hz), 2.86–2.75 (m, 3H), 2.44–2.30 (m, 3H), 2.20–2.05 (m, 1H), 1.91–1.87 (m, 1H), 1.46–1.39 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 163.8, 162.2, 139.0, 136.3, 135.7, 131.6, 130.6, 128.6, 128.4 (2C), 128.0 (2C), 120.8, 118.4, 77.3, 75.9, 40.3, 35.1, 32.2, 30.7, 28.5, 27.6; ESI-MS *m/e* 390 (M + 1)$^+$. Anal. (C$_{23}$H$_{23}$N$_3$O$_3$ and 1.05HCl plus 0.5H$_2$O) C, H, N, Cl.

[7-[4-[*N*-(2-Furylmethyl)amidino]benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (34d·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 81% yield from the ester **33d**·HCl: mp 172–174 °C; IR (KBr) 3035, 2924, 1702, 1672, 1624, 1596, 1541, 1507, 1419, 1339, 1151, 1018, 860, 815, 746, 705 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.10–11.90 (broad peak, 1H), 10.60–10.40 (broad peak, 1H), 10.36 (s, 1H), 1.00–9.40 (broad peak, 2H), 8.15 (d, 2H, *J* = 8.2 Hz), 7.88 (d, 2H, *J* = 8.2 Hz), 7.72–7.71 (m, 1H), 7.51–7.48 (m, 2H), 7.06 (d, 1H, *J* = 8.2 Hz), 6.59 (d, 1H, *J* = 2.9 Hz), 6.49 (dd, 1H, *J* = 1.7, 2.9 Hz), 4.74 (s, 2H), 2.87–2.75 (m, 3H), 2.46–2.27 (m, 3H), 2.20–2.05 (m, 1H), 1.93–1.89 (m, 1H), 1.48–1.37 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.5, 163.9, 162.3, 148.3, 143.2, 138.9, 136.3, 135.7, 131.6, 130.8, 128.6, 128.4 (2C), 128.0 (2C), 120.9, 118.5, 110.6, 109.2, 40.2, 39.3, 35.1, 30.8, 28.5, 27.7; ESI-MS *m/e* 432 (M + 1)$^+$. Anal. (C$_{25}$H$_{25}$N$_3$O$_4$ and HCl plus 0.2H$_2$O) C, H, N, Cl.

[7-[4-(Morpholinoformimidoyl)benzamido]-1,2,3,4-tetrahydro-2-naphthalenyl]acetic Acid Hydrochloride (34e·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 55% yield from the ester **33e**·HCl: mp 243–245 °C; IR (KBr) 3278, 3025, 1716, 1684, 1635, 1593, 1542, 1420, 1340, 1266, 1119, 1013, 872, 718 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.36 (s, 1H), 10.10–9.40 (broad peak, 2H), 8.18 (d, 2H, *J* = 8.6 Hz), 7.76 (d, 2H, *J* = 8.6 Hz), 7.52–7.50 (m, 2H), 7.05 (d, 1H, *J* = 8.9 Hz), 3.76–3.33 (m, 8H), 2.87–2.75 (m, 3H), 2.46–2.28 (m, 3H), 2.20–2.03 (m, 1H), 1.93–1.89 (m, 1H), 1.49–1.34 (m, 1H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 173.6, 164.0, 163.2, 137.8, 136.3, 135.7, 131.6, 131.4, 128.4 (5C), 120.8, 118.4, 65.2 (2C), 48.5 (br s, 2C), 40.2, 35.1, 30.8, 28.6, 27.6. Anal. (C$_{24}$H$_{27}$N$_3$O$_4$ and 1.05HCl plus 0.5H$_2$O) C, H, N, Cl.

[7-[4-[*N*-(2-Propynyl)amidino]benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetic Acid Hydrochloride (36·HCl). Following the procedure described for the preparation of **10a**, the title compound was prepared in 75% yield from the ester **35**·HCl: mp 173–175 °C; IR (KBr) 3493, 3425, 3365, 3257, 2126, 1717, 1680, 1647, 1619, 1549, 1497, 1425, 1251, 1218, 1155, 1129, 1031, 861, 827, 697 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 12.27 (s, 1H), 10.49 (br s, 1H), 10.33 (s, 1H), 9.95

(br s, 1H), 9.48 (br s, 1H), 8.16 (d, 2H, *J* = 8.2 Hz), 7.90 (d, 2H, *J* = 8.2 Hz), 7.50−7.44 (m, 2H), 6.75 (d, 1H, *J* = 8.6 Hz), 4.36 (s, 2H), 4.20−4.16 (m, 1H), 3.85−3.78 (m, 1H), 3.82 (s, 1H), 2.92−2.86 (m, 1H), 2.57−2.23 (m, 4H); ^{13}C NMR (22.4 MHz, DMSO-d_6) δ 172.9, 163.6, 162.3, 150.5, 139.0, 131.5, 130.6, 128.3 (2C), 127.9 (2C), 122.3, 120.9, 120.3, 115.8, 77.3, 75.9, 69.0, 35.5, 32.2, 30.3, 28.6; CI-MS *m/e* 392 (M + 1)$^+$. Anal. ($C_{22}H_{21}N_3O_4$ and HCl) C, H, N.

[6-[4-(Morpholinoformimidoyl)benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetic Acid Hydrochloride ((*RS*)-3·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 50% yield from the ester (*RS*)-**4**·HCl: mp 245−247 °C; IR (KBr) 3423, 3229, 3030, 1715, 1663, 1621, 1540, 1499, 1424, 1256, 1220, 1112 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.60−12.00 (broad peak, 1H), 10.30 (s, 1H), 9.79 (br s, 2H), 8.16 (d, 2H, *J* = 8.6 Hz), 7.76 (d, 2H, *J* = 8.2 Hz), 7.53−7.43 (m, 2H), 6.75 (d, 1H, *J* = 8.6 Hz), 4.18 (d, 1H, *J* = 9.8 Hz), 3.85−3.32 (m, 9H), 2.90−2.86 (m, 1H), 2.57−2.26 (m, 4H); ^{13}C NMR (100.4 MHz, DMSO-d_6) δ 173.2, 163.9, 163.5, 150.7, 138.1, 131.8, 131.6, 128.6 (2C), 128.4 (2C), 122.4, 121.1, 120.5, 116.1, 69.2, 65.6, 65.1, 49.9, 47.2, 35.8, 30.5, 28.8; ESI-MS *m/e* 424 (M + 1)$^+$. Anal. ($C_{23}H_{25}N_3O_5$ and HCl) C, H, N, Cl.

Methyl [6-(*tert*-Butoxycarboxamido)-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate ((*RS*)-37). According to a similar hydrogenation procedure in MeOH described for the preparation of **17a**, methyl (6-amino-3,4-dihydro-2*H*-1-benzopyran-3-yl)acetate was prepared from **26** in 62% yield as a white solid: mp 107−109 °C. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

To a solution of the preceding amino compound (21.6 g, 97.6 mmol) in EtOAc (100 mL) was added di-*tert*-butyl dicarbonate (22.7 g, 104.1 mmol). The mixture was stirred for 6 h at room temperature and washed with 10% citric acid (2 × 50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and concentrated in vacuo to afford an oily residue. Purification by column chromatography (SiO_2, *n*-hexane/EtOAc = 5/1) followed by trituration from *n*-hexane gave 28.1 g (90%) of (*RS*)-**37** as a white solid: mp 86−88 °C. Anal. ($C_{17}H_{23}NO_5$) C, H, N.

Optical Resolution of (*RS*)-37 by Chiral HPLC. Preparative HPLC was performed on a Shimadzu LC-8A system. (*RS*)-**37** (500 mg/injection) was submitted to semipreparative HPLC containing a CHIRALCEL-OD column (20 mm i.d. × 300 mm), eluted (20 mL/min) at 20 °C with a mixture of *n*-hexane/EtOH (5/1), and detected at 254 nm. The first peak at 10.2 min was collected to afford (*R*)-**37** (210−220 mg/injection) as a white solid: mp 84−85 °C; [α]^{20}$_D$ +22.6° (*c* 1.05, MeOH). Anal. ($C_{17}H_{23}NO_5$) C, H, N.

The second peak at 22.6 min was collected to afford (*S*)-**37** (205−214 mg/injection) as a white solid: mp 84−85 °C; [α]^{20}$_D$ −22.1° (*c* 0.98, MeOH). Anal. ($C_{17}H_{23}NO_5$) C, H, N.

Enantiomeric purities of (*R*)-**37** and (*S*)-**37** were found to be greater than 99.5% ee by analytical HPLC (CHIRALCEL-OD (4.6 mm i.d. × 250 mm), *n*-hexane/EtOH = 5/1, 0.7 mL/min, 35 °C, 254 nm).

Methyl [6-[2(*S*)-[(4-Nitrophenylsulfonyl)amino]-3-phenylpropanamido]-3,4-dihydro-2*H*-1-benzopyran-3(*S*)-yl]acetate (39). To a solution of HCl (22 g) in EtOH (180 mL) was added (*S*)-**37** (15.0 g, 48.5 mmol) at room temperature. The mixture was stirred for 5 h at room temperature and concentrated in vacuo to afford a white solid, which was dissolved in CHCl$_3$ (100 mL). The solution was washed with aqueous NaHCO$_3$ solution and extracted with CHCl$_3$. The organic phase was dried over MgSO$_4$ and concentrated in vacuo to afford 10.14 g (94%) of methyl (*S*)-(6-amino-3,4-dihydro-2*H*-1-benzopyran-3-yl)acetate.

To a solution of 0.70 g (3.16 mmol) of the preceding aniline, 0.9 mL (12.2 mmol) of triethylamine, and 60 mg (0.49 mmol) of 4-(dimethylamino)pyridine in 10 mL of CHCl$_3$ was added 1.4 g (3.8 mmol) of *N*-(4-nitrophenylsulfonyl)-L-phenylalanyl chloride (Tokyo Chemical Industry Co. Ltd.) at room temperature, and the mixture was stirred for 12 h at room temperature. After addition of aqueous NaHCO$_3$ solution, the mixture was extracted with CHCl$_3$. The organic phase was

dried over MgSO$_4$ and concentrated in vacuo to afford a crude amide. Purification by column chromatography (SiO$_2$, CHCl$_3$/EtOAc = 7/1) gave 1.12 g (64%) of **39**. Recrystallization from 2-propanol gave an analytically pure sample as a yellow solid: mp 141−143 °C. Anal. ($C_{27}H_{27}N_3O_8S$) C, H, N.

Ethyl (*S*)-(6-Amino-3,4-dihydro-2*H*-1-benzopyran-3-yl)-acetate ((*S*)-27). To a solution of HCl (approximately 50 g) in absolute EtOH (200 mL) was added (*S*)-**37** (20.0 g, 62.2 mmol) by portions. The mixture was stirred for 4 h at 60 °C and concentrated in vacuo. The residue was dissolved in absolute EtOH (350 mL), and H$_2$SO$_4$ (3.5 g) was added. The mixture was refluxed for 4 h, neutralized by 10% K$_2$CO$_3$ solution, and concentrated in vacuo. The residual solid was diluted with CHCl$_3$, and the organic phase was separated, dried over MgSO$_4$, filtered, and concentrated in vacuo. Purification of the residue by column chromatography (SiO$_2$, CHCl$_3$/MeOH = 100/3) afforded 11.5 g (79%) of (*S*)-**27** as a pale-yellow solid: mp 75−76 °C; [α]^{20}$_D$ −26.5° (*c* 1.02, MeOH). Anal. ($C_{13}H_{17}NO_2$) C, H, N.

Ethyl (*R*)-(6-amino-3,4-dihydro-2*H*-1-benzopyran-3-yl)-acetate ((*R*)-27): mp 75−76 °C; [α]^{20}$_D$ +26.1° (*c* 1.03, MeOH). Anal. ($C_{13}H_{17}NO_2$) C, H, N.

Ethyl (*S*)-[6-(4-Cyanobenzamido)-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate ((*S*)-11c). According to the procedure for the preparation of **11c**, the title compound was prepared from (*S*)-**27** in 93% yield as a pale-yellow solid: mp 148−149 °C; [α]^{20}$_D$ −25.9° (*c* 1.00, 1,4-dioxane). Anal. ($C_{21}H_{20}N_2O_4$) C, H, N.

Ethyl (*R*)-[6-(4-cyanobenzamido)-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate ((*R*)-11c): mp 148−149 °C; [α]^{20}$_D$ +25.5° (*c* 0.99, 1,4-dioxane). Anal. ($C_{21}H_{20}N_2O_4$) C, H, N.

Ethyl (*S*)-[6-[4-(Morpholinoformimidoyl)benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate Hydrochloride (MS-180, (*S*)-4·HCl). Following the procedure described for the preparation of **33a**·HCl, the title compound was prepared in 90% yield from the nitrile (*S*)-**11c** as a pale-yellow solid: mp 242−244 °C; [α]^{20}$_D$ −14.5° (*c* 1.0, MeOH). The enantiomeric purity was found to be greater than 99.5% ee by HPLC analysis on CHIRAL-AGP column (ChromTech AB, Hägelsten, Sweden; 4.0 mm i.d. × 100 mm) with a flow rate of 0.9 mL/min at 25 °C and a mobile phase of 0.01 M KH$_2$PO$_4$ (pH 7.0) and 1 mM *N*,*N*-dimethyloctylamine. Anal. ($C_{25}H_{29}$-N$_3$O$_5$ and HCl) C, H, N, Cl.

Ethyl (*R*)-[6-[4-(morpholinoformimidoyl)benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetate hydrochloride ((*R*)-4·HCl): mp 242−244 °C; [α]^{20}$_D$ +14.0° (*c* 1.0, MeOH). Anal. ($C_{25}H_{29}N_3O_5$ and HCl) C, H, N, Cl.

(*S*)-[6-[4-(Morpholinoformimidoyl)benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetic Acid Hydrochloride ((*S*)-3·HCl). Following the procedure described for the preparation of **10a**·HCl, the title compound was prepared in 92% yield from the ester (*S*)-**4**·HCl as a yellow solid: mp 242−244 °C; [α]^{25}$_D$ −9.3° (*c* 1.0, H$_2$O). Enantiomeric purity of (*S*)-**3**·HCl was found to be greater than 99.5% ee by HPLC analysis on CHIRAL-AGP column (4.0 mm i.d. × 100 mm) with a flow rate of 0.7 mL/min at 30 °C and a mobile phase of 0.01 M KH$_2$-PO$_4$ (pH 5.6). Anal. ($C_{23}H_{25}N_3O_5$ and HCl plus 1.05H$_2$O) C, H, N, Cl.

(*R*)-[6-[4-(Morpholinoformimidoyl)benzamido]-3,4-dihydro-2*H*-1-benzopyran-3-yl]acetic acid hydrochloride ((*R*)-3·HCl): mp 242−244 °C; [α]^{25}$_D$ +9.1° (*c* 1.0, H$_2$O). Anal. ($C_{23}H_{25}N_3O_5$ and HCl plus H$_2$O) C, H, N, Cl.

Computational Methods. Molecular Modeling and Pharmacophore Mapping. All molecular modeling studies were performed on a Silicon Graphics IMPACT R10000 graphic workstation using the molecular modeling software package SYBYL version 6.3 from TRIPOS Associates (St. Louis, MO). Geometry optimization was carried out using TRIPOS force field[31] with Gasteiger−Hückel charge.[32] Conformational databases were developed for each compound using the Random Search evaluation subroutine[33] of SYBYL 6.3. In each case, the energy cutoff for conformers was designated as 292.88 kJ/mol. Each search resulted in a molecular database that was representative of the conforma-

tional space that each analogue occupies within the specified energy window. The DISCO algorithm was used to find multipoint pharmacophoric models.

CoMFA and PLS Regression Analysis. CoMFA studies were carried out using the QSAR option of SYBYL version 6.3. The partial atomic charges used in CoMFA were computed using the semiempirical method PM3 implemented in MOPAC93.[34] Single-point calculations were performed on the geometries previously optimized with SYBYL/TRIPOS force field. The steric and electrostatic probe−ligand interaction energies (kJ/mol) were calculated with the Lennard−Jones and Coulomb potential functions of the TRIPOS force field using a carbon sp[3] probe atom, having a 1.0 charge. The steric and electrostatic energies were truncated at 125.52 kJ/mol. The dimensions of CoMFA lattice were determined through an automatic procedure, featured by the SYBYL/CoMFA routine, which extends the lattice walls beyond the dimensions of each structure by 4.0 Å in all directions. The lattice spacing was set to a value of 2.0 Å. The PLS regression analysis were performed with the SYBYL/QSAR routine at default setting.

Pharmacology. In Vitro Inhibition of Platelet Aggregation. The inhibition of platelet aggregation was determined as essentially described by Nicholsonl et al.[35] Venous blood was obtained from anesthetized male guinea pigs or male healthy human volunteers. Blood was anticoagulated by addition of 3.8% sodium citrate (1/9 = citrate/blood). Platelet-rich plasma (PRP) was prepared by centrifugation at 120g for 15 min. The remaining blood was centrifuged at 1200g for 15 min, and platelet-poor plasma (PPP) was removed. The platelet count in PRP was adjusted to $2.5-3.0 \times 10^8$ platelets/mL with PPP. After incubation of PRP (240 μL) at 37 °C for 2 min in an aggregometer (hema tracer 1, NKK Co., Japan), 30 μL of test compound (Table 2, 4, and 6) or vehicle was added to PRP. A further 2 min later, 30 μL of ADP (final concentration 5 μM) was added to induce platelet aggregation. The inhibition percentage was obtained by comparing the maximum aggregation of treated PRP with that of the control, and then a test compound concentration giving a 50% inhibition (IC$_{50}$) value was estimated.

Inhibition of Fibrinogen Binding to Immobilized Human GPIIb-IIIa. The binding of fibrinogen to immobilized GPIIb-IIIa was performed using a modification of the method of Charo et al.[36] GPIIb-IIIa was extracted from concentrated and lyophilized human platelets (Organon Teknika Co.) suspended in 10 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1 mM CaCl$_2$, 10 μM leupeptin, and 1 mM phenylmethanesulfonyl fluoride (pH 7.4). The suspension was incubated for 1 h at 4 °C, and centrifuged at 30000g for 15 min. GPIIb-IIIa was purified from the supernatant using concanavalin A-Sepharose, heparin-Sepharose, and Sephacryl S-300 according to the method of Phillips et al.[37] Human fibrinogen (glade L, KabiVitrum) was further purified by Sepharose CL-6B gel chromatography and lysine-Sepharose affinity chromatography. Purified fibrinogen was incubated with NHC-LC-biotin (Pierce Co.), and then the mixture was dialyzed against Tris-buffered saline (TBS; 20 mM Tris-HCl, 150 mM NaCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, pH 7.4) to obtain biotinylated fibrinogen. Protein concentrations of the GPIIb-IIIa and the biotinylated fibrinogen preparations were determined by Bradford's method (Bio-Rad).

A 96-well microtiter plate was coated with 100 μL/well of purified GPIIb-IIIa diluted to 1.5 μg/mL with TBS containing 0.0005% Triton X-100 for 24 h at 4 °C. After the plate was washed with a washing solution (TBS containing 0.01% Tween 20), TBS containing 3.5% bovine serum albumin (BSA; 200 μL/well) was added and incubated for 1 h at room temperature. The test compound (Tables 4 and 6) was dissolved in DMSO at 10 mM, further diluted with TBS, and mixed with an equal volume of biotinylated fibrinogen (2 μg/mL) in TBS containing 1% BSA. After the plate was washed, the mixture (100 μL/well) was added and incubated for 24 h at room temperature. The plate was thereafter washed, and peroxidase-labeled streptavidin (Amersham) diluted 3000 times with TBS (100 μL/well) was added. The mixture was incubated for 30 min

at room temperature. After the plate was washed, 200 μL of o-phenylenediamine (0.4 mg/mL in 0.05 M citrate-phosphate buffer (pH 5.0), containing 0.012% H$_2$O$_2$) was added to develop color for 20−40 min. The reaction was terminated by addition of 0.3 M H$_2$SO$_4$ (50 μL), and absorbance at 490 nm was measured. The difference between total (in the absence of the inhibitor) and nonspecific (in the presence of 500 μg/mL fibrinogen) binding was designated as specific binding. IC$_{50}$ values were determined from the dose−response curves of the inhibition percent plotted against the log-concentration of the test compounds.

Ex Vivo Inhibition Studies in Guinea Pig. Test compounds (Tables 3 and 5) suspended or dissolved in saline containing 0.5% Tween 80 were orally administered (37 μmol/kg) to male guinea pigs starved overnight. The guinea pigs were anesthetized with pentobarbital 1 h after the administration. Blood sample was collected by venipuncture, and PPP and PRP were obtained as described above. The platelet count of PRP was adjusted to 3.0×10^8 platelets/mL by the addition of PPP. The PRP (270 μL) was preincubated for 2 min in the aggregometer, and successively aggregation was measured by addition of 30 μL of ADP (final concentration 5 μM). Three vehicle-treated animals were used as a control group at each experiment. The percentage inhibition of platelet aggregation in drug-treated animals was determined by comparison with the aggregation in the control group.

The concentration of the active species in PPP was estimated by a bioassay as described by Salyers et al.[38] with modifications. PPP from treated guinea pigs was diluted with PPP from nontreated donor guinea pigs and mixed with PRP from nontreated donors. Platelet aggregation was determined as described above. The concentration of active species in PPP was estimated by comparing the aggregation observed with a standard curve prepared using PPP spiked with known amounts of the active species of administered prodrug.

Ex Vivo Studies in Dog. MS-180 (((S)-**4**·HCl) was orally administered by capsule to conscious beagle dogs starved overnight. Blood samples (4.5 mL) were withdrawn via venipuncture into plastic syringes containing 0.5 mL of 3.8% trisodium citrate at predetermined times before and after dosing. PRP and PPP were prepared by centrifuging blood samples. Aggregation was induced by the addition of 30 μL of ADP (final concentration 200 μM) to 270 μL of the PRP, and the percent inhibition for individual dogs was calculated by comparing the aggregation of predosing sample.

The concentration of the active species ((S)-**3**) was estimated from the inhibition of fibrinogen binding to GPIIb-IIIa. After 2-fold dilution of PPP with TBS, an equal volume of MeCN was added, and the mixture was centrifuged at 19000g for 5 min. The supernatant was removed and further diluted with TBS containing 0.5% BSA. The inhibitory activity of the supernatant to fibrinogen−GPIIb-IIIa binding was determined as described above. The concentration of (S)-**3** in PPP was estimated using the standard curve of inhibition established with PPP from untreated dogs that had been spiked with known amounts of (S)-**3**. Reliability of this assay method was confirmed by the result that concentrations of (S)-**3** measured both by this method and by HPLC were almost identical in plasma of four dogs to which MS-180 ((S)-**4**·HCl) had been orally administered at 5 mg/kg.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **11a−i**, **14b,c**, **15b,c**, **16b,c**, **17a−c**, **18−24**, **26**, **27**, **30**, **32**, (RS)-**37**, and **39**; X-ray crystallographic analysis data of compound **39** (18 pages). Ordering information is given on any current masthead page.

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