Dibasic (Amidinoaryl)propanoic Acid Derivatives as Novel Blood Coagulation Factor Xa Inhibitors

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Since activated factor X (FXa) is a coagulant enzyme that generates thrombin and participates in both intrinsic and extrinsic coagulation pathways, inhibition of FXa may be more effective than inactivation of thrombin for interrupting blood coagulation. To assess the possible effectiveness of FXa inhibition as an anticoagulant, we designed and synthesized 3-(amidinoaryl)-2-[4-[(3*S*)-3-pyrrolidinyloxy]phenyl]propanoic acid derivatives as low molecular weight, nonpeptidic, orally active FXa inhibitors. These derivatives exhibited potent and highly selective anti-FXa activity in vitro and anticoagulant activity on oral administration. The most promising compound, (2*S*)-2-[4-[[(3*S*)-1-acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic acid hydrochloride pentahydrate (4, DX-9065a), inhibited 50% of FXa activity (IC_{50}) at 0.07 μM, doubled plasma recalcification time (PRCT) at 0.5 μM, and significantly prolonged activated partial thromboplastin time (APTT) at a dose of 100 mg/kg on oral administration. In contrast with FXa inhibition, 4 showed no activity against thrombin (IC_{50} > 2000 μM).

Introduction

Fatal damage is often caused by thrombotic events. Therefore, numerous efforts have been made to synthesize antithrombotics such as antiplatelet, anticoagulant, and thrombolytic agents. Thrombin plays a critical role in thrombosis, since it not only converts fibrinogen to fibrin for clot formation but also strongly induces platelet aggregation. Synthesized[1,2] and naturally occurring[3,4] direct thrombin inhibitor could be considered as one of the most promising antithrombotics. Nevertheless, thrombin inhibitors have shown a tendency to prolong bleeding time at their effective doses in experimental thrombotic models.[5]

The activated factor Xa (FXa), whose major practical role is the generation of thrombin by the limited proteolysis of prothrombin, holds a central position that links the intrinsic and extrinsic activation mechanisms in the final common pathway of coagulation. Thrombin generation from its precursor is amplified by formation of prothrombinase complex (FXa, factor Va, Ca^{2+} and phospholipid). Since it was calculated that a molecule of FXa could generate 138 molecules of thrombin,[6] inhibition of FXa may be more efficient than inactivation of thrombin in interrupting the blood coagulation system.

The ideal anticoagulant should be free of certain limitations affecting clinical administration, such as hemostatic vulnerability, the need for monitoring of drug concentration, and adverse reactions.[7] Accordingly, we designed and synthesized a novel anticoagulant which could inhibit human FXa, according to the following principal requirements: (a) highly specific inhibition of human FXa, (b) activity on oral administration, and (c) non-peptide structure, with low molecular weight. It is known that the bis(amidinoaryl) compounds, represented by 1,2-bis(5-amidino-2-benzofuranyl)ethane (1), show selective and potent anti-FXa activity.[8] However, these bis(amidinoaryl) compounds are inactive on oral administration. The benzamidine derivative 2 (APPA) was shown to be absorbed in large amounts when taken orally.[9] It seems likely that the zwitterionic compound 2 was absorbed through a route of transportation for amino acids or low molecular weight peptides, and so we designed amidinoaryl derivatives such as those with general structure 3, which contains a carboxylic acid (Figure 1). Synthetic studies were carried out as follows. Initially, a basic structure for anti-FXa activity was constituted from 1. Next, the optimum position of carboxylic acid for manifesting selective activity against FXa was sought. Finally, the structure of the amidinoaryl moiety was optimized to increase affinity for the S1 site.

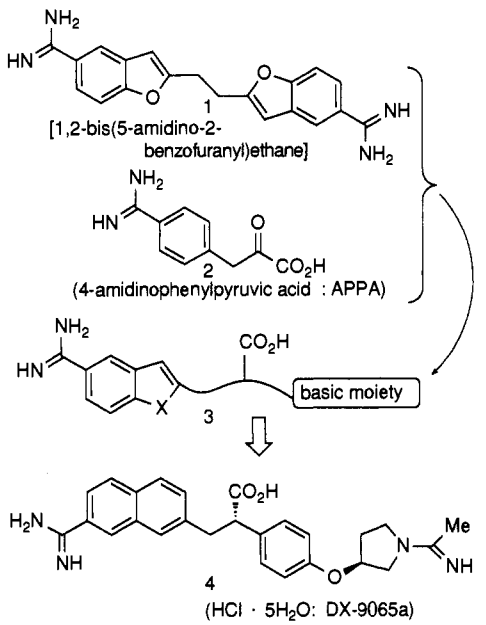

Figure 1. Design of amidinoaryl derivatives as oraly active FXa inhibitors.

In this study, we evaluated the anticoagulant properties of amidinoaryl derivatives and characterized an orally active FXa inhibitor, (2*S*)-2-[4-[((3*S*)-1-acetimidoyl-3-pyrrolidinyl)oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic acid hydrochloride pentahydrate (4, DX-9065a).

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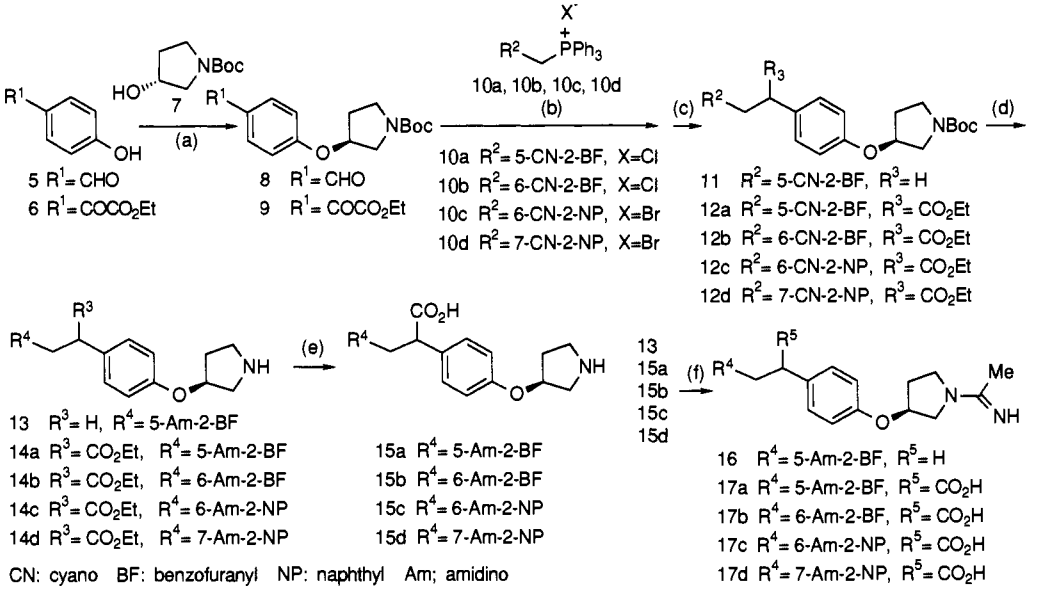
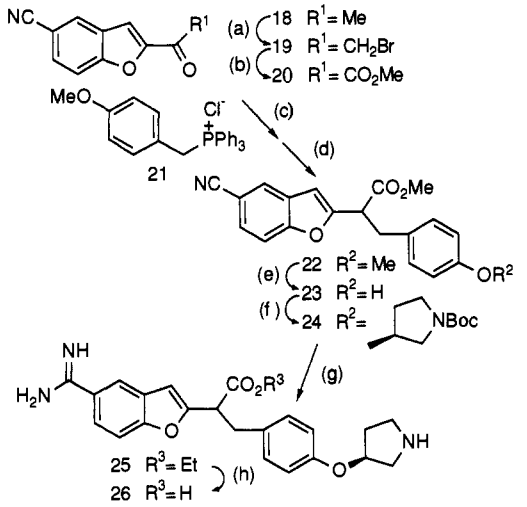
Scheme 1[a]



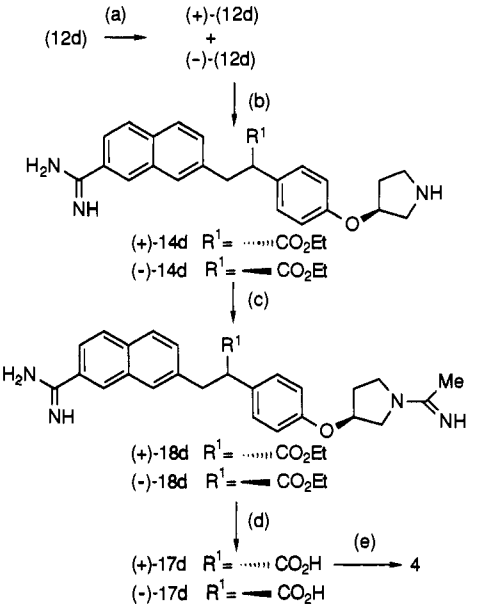
[a] (a) Diethyl azodicarboxylate, PPh_3/THF; (b) DBU/THF–EtOH; (c) Pd(0)·H_2O·$BaSO_4$, H_2/EtOH; (d) EtOH–HCl, then NH_3–EtOH; (e) 2 N HCl; (f) ethyl acetimidate hydrochloride–1 N–NaOH/H_2O.

Scheme 2[a]



[a] (a) Bromine/CH_2Cl_2; (b) SeO_2/MeOH; (c) DBU/THF–EtOH; (d) Pd(0)·H_2O·$BaSO_4$, H_2/EtOH; (e) BBr_3/CH_2Cl_2; (f) (3*R*)-1-(*tert*-butoxycarbonyl)-3-pyrrolidinol, diethyl azodicarboxylate, PPh_3/THF; (g) EtOH–HCl; then NH_3–EtOH; (h) 2 N HCl.

Scheme 3[a]



[a] (a) Recrystallization from EtOH; (b) EtOH–HCl, then NH_3–EtOH; (c) ethyl acetimidate hydrochloride/Et_3N; (d) concd HCl; (e) ion-exchange resin, then H_2O/EtOH.

Chemistry

While almost all compounds (**12a–d**, **14a–d**, **15a–d**, **17a–d**, and **22–26**) were synthesized as mixtures of two epimers at the carbon where the carboxylic acid or its ester group was substituted, the two epimers of the most promising compound, **17d**, were stereoselectively prepared.

Compounds **16** and **17a–d** were synthesized in a convergent synthesis as outlined in Scheme 1. The aldehyde **8** or the α-keto ester **9** was prepared from the phenol **5** or **6** and the alcohol **7**, respectively, by using a procedure described by Bitter.[10] Wittig reaction of **8** or **9** with **10a,b**[11] and newly synthesized phosphonium salts **10c,d** using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base, followed by catalytic hydrogenation, gave compounds **11** and **12a–d**. Treatment of **11** and **12a–d** with saturated HCl ethanol solution and then with ammonia in ethanol gave the corresponding amidine derivatives **13** and **14a–d**. Acid hydrolysis of **14a–d** gave the carboxylic acid derivatives **15a–d**, respectively. Acetimidation of the pyrrolidine moieties was accomplished by treating **13** or **15a,b** with ethyl acetimidate hydrochloride in an aqueous solution, maintaining the pH range between 7.5 and 8.5.[12]

Compound **26** was synthesized as outlined in Scheme 2. Bromination of the reported acetylbenzofuran **18**[11] gave the α-bromo ketone **19**. Treatment of **19** with selenium dioxide in anhydrous methanol gave the α-keto ester **20**.[13] Wittig reaction of **20** with the phosphonium salt **21** employing DBU as a base, followed by catalytic hydrogenation, gave the methoxy derivative **22**. After demethylation of **22** using boron tribromide in a dichloromethane, condensation of the obtained phenol **23** with the alcohol **7** afforded the nitrile **24**. Compound **24** was converted to **26** in the same manner as described for **15a–d**.

Scheme 3 demonstrates the sequences of reactions which led to the stereoselective synthesis of the two epimers of

Table 1. In Vitro Anticoagulant and Enzyme Inhibitory Activity

no.	PRCT[a] (CT2,[b] μM)	FXa (IC_{50},[c] μM)	thrombin (IC_{50},[c] μM)
1	1.6	0.10	5
4[d] (>99.5% de[e])	0.5	0.07	>2000
13	7.5	0.94	100
14a	20.0	1.00	21
14b	8.3	1.70	36
14c	12.4	4.20	38
14d	3.2	0.31	>600
15a	14.0	1.50	230
15b	16.0	1.70	>1000
15c	22.0	4.10	>100
15d	3.2	0.20	>1600
16	1.2	0.64	4
17a	6.0	0.50	15
17b	5.6	0.84	>400
17c	4.4	0.36	22
17d	1.3	0.16	>1200
(−)-17d (84.0% de[e])	6.0	0.50	>1000
25	31.0	52.00	20
26	14.0	45.00	9

[a] Plasma recalcification clotting time. [b] Concentration needed to double the human PRCT. [c] Concentration needed for inhibiting enzymes by 50%. Methods are described in the Experimental Section. [d] Crystalline form of (+)-17d. [e] Diastereomeric excess.

Table 2. Ex Vivo Anticoagulant Activity on Oral Administration

no. (dose, mg/kg)	test/control APTT[a] ratio 0.5 h	1 h	2 h	4 h
4 (100) $n = 5$	1.63 ± 0.09	1.51 ± 0.11	1.48 ± 0.04	1.28 ± 0.04
14a (300) $n = 4$	1.17 ± 0.09	1.17 ± 0.05	1.19 ± 0.07	1.21 ± 0.12
15a (300) $n = 4$	1.28 ± 0.12	1.40 ± 0.12	1.25 ± 0.04	1.20 ± 0.08
25 (300) $n = 4$	0.98 ± 0.04	1.30 ± 0.07	1.11 ± 0.06	1.05 ± 0.02
26 (300) $n = 4$	1.09 ± 0.04	1.33 ± 0.12	1.15 ± 0.09	1.16 ± 0.14

[a] Activated partial thromboplastin time. Methods are described in the Experimental Section. Values are mean ± SE.

compound 17d. Compound (+)-12d was crystallized selectively from a solution of 12d in ethanol [>77% diastereomeric excess (de)]. Concentration of the filtrate of the crystallization afforded (−)-12d (>87% de) as a viscous oil. Two recrystallizations of the obtained (+)-12d from ethanol gave pure (+)-12d (>99.5% de). The nitriles (+)-12d and (−)-12d were converted into the amidines (+)-14d and (−)-14d, respectively, by successive treatment with saturated HCl ethanol solution and then with ammonia in ethanol at a low temperature. The pyrrolidine moieties of (+)-14d and (−)-14d were modified with ethyl acetimidate hydrochloride in ethanol by using triethylamine as a base to give corresponding imidoyl derivatives (+)-18 and (−)-18. Acid hydrolysis of the esters of (+)-18 and (−)-18 gave the carboxylic acid derivatives (+)-17d and (−)-17d, in 95% de and 84% de, respectively. Biological assay revealed that the (+)-epimer, (+)-17d, was the active component of 17d. After pH adjustment of the solution of (+)-17d with an ion-exchange resin, crystallization from aqueous ethanol solution gave 4 as colorless prisms.

Results and Discussion

The plasma recalcification times (PRCT), anti-FXa, and antithrombin activities of synthesized compounds are shown in Table 1. The anticoagulant activities (activated partial thromboplastin time; APTT) of 14a, 15a, 25, 26, and 4 on oral administration are shown in Table 2.

Selective FXa inhibitor 1 is not suitable as a basic compound for determining the best position of the carboxylic group because of its symmetrical structure. Although the binding mode of FXa and 1 has not been reported, one 5-amidinobenzofuran moiety of 1 must bind to the S1 site of FXa, and the other 5-amidinobenzofuran is thought to fit a basic–hydrophobic moiety binding site. Not only an amidinoaryl structure binding to the S1 site but also a structure having steric extension and an appropriately positioned positive charge (basic part) such as a 5-amidinobenzofuran structure was thought to be essential for potent anti-FXa activity. Recently, the structure of FXa that lacks a Gla domain has been determined by X-ray crystallography, and a model of the commonly used dansylGluGlyArg methylene inhibitor-FXa interaction has been proposed by Tulinsky et al.[14] The aryl binding site (S2–S3) into which the dansyl group was judged to fit seemed to be identical with the basic–hydrophobic moiety binding site. At first, several (aminoalkyl)phenyl and (aminoalkoxy)phenyl structures were designed, modeled, and compared with 5-amidinobenzofuran. The stable conformation of one of them, positively charged 3-(phenyloxy)pyrrolidine, generated on 3-dimensional computer graphics, can be superimposed well on the structure of positively charged 5-amidinobenzofuran (Figure 2). Two structures had almost equal steric extensions, and about 80% of them could be overlaid. The nitrogen atom of the pyrrolidine ring was located in a position almost corresponding to that the nitrogen of 5-amidinobenzofuran. According to the above study, the unsymmetrical compound 13 which had a (pyrrolidinyloxy)phenyl group as a basic–hydrophobic moiety instead of 5-amidinobenzofuran was designed. Synthesized 13 showed selective anti-FXa activity, but its magnitude of inhibition was one-fifth of that of 1. The difference of basicity between the secondary cyclic amine of 13 and the arylamidine of 1 was thereby thought to affect the inhibitory activity. It was conceivable that increasing basicity, by modification of pyrrolidine to an amidine type moiety such as acetimidoyl, might intensify the anti-FXa and anticoagulant activity; actually, compound 16 showed very similar anticoagulant activity and anti-FXa selectivity to 1.

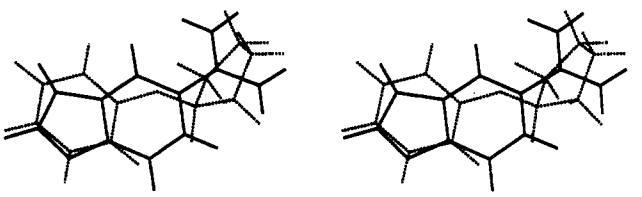


Figure 2. Overlay of the stable conformation of 3-(phenyloxy)-pyrrolidine (dotted lines) onto 5-amidinobenzofuran (unbroken lines).

Introduction of carboxylic aicd on the benzofuran ring or the pyrrolidine ring of 16 may not be favorable, since these alterations could interrupt the access of the compound to the S1 site and the basic–hydrophobic moiety binding site of FXa, respectively. An ethoxycarbonyl or carboxyl group was introduced onto one of the two benzyl positions of 13 to give 14a, 25, 15a, and 26. Although each of the compounds 14a, 25, 15a, and 26 showed a similar anticoagulant activity in vitro, there were many differences in selectivity to enzyme inhibition. While the (5-amidino-2-benzofuranyl)propanoic acid derivative 15a and its ethyl ester 14a exhibited selectivity for FXa, the (5-amidino-2-benzofuranyl)acetic acid derivative 26 and its ethyl ester 25 showed thrombin selectivity. On the other hand, while carboxylic acid type 15a exhibited significant activity on oral administration, compound 26 was less active. Only a slight change of the position of the carboxyl group

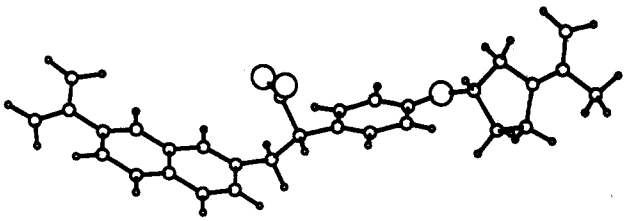


Figure 3. Relative stereochemistry of **4** as determined by X-ray crystallography.

affected oral activity. The [(amidinoaryl)propanoic acid] structure probably contributed to oral absorption.

Accordingly, the **15a**-related compounds **15b–d** and their acetimidoyl type modified derivatives **17a–d** were synthesized and evaluated. These compounds had similar anticoagulant profiles to **15a**, except for their degrees of anticoagulant activity. In general, the anticoagulant activities of the carboxylic acid derivatives were equal to those of their esters. However, the carboxylic acid derivatives were superior in their selectivity of FXa to their esters. This suggests that the FXa selectivity of **15a–d** and **17a–d** depends on an ionic repulsion between the carboxyl moiety and some negative charge of thrombin near the active site rather than a steric barrier. Three negatively charged amino acid residues, Glu-217, Glu-192, and Glu-146 (whose numbering follows the system of Bode et al.[15]), are present around the S1 site of thrombin. We considered that Glu-192 was probably responsible for the loss of inhibitory activity by **15a–d** and **17a–d**.[16]

The 7-amidino-2-naphthyl derivative **17d** was the most promising inhibitor, since it showed potent and selective anti-FXa activity. Since the stereoselectively synthesized (+)-**17d** showed 7 times the potency of (−)-**17d** in in vitro anti-FXa activity, the (+)-epimer was considered to be the active component. This epimer not only showed higher potency than **1**, but also selectively inhibited FXa without any effect on thrombin. Crystallization of (+)-**17d** gave a hydrochloride pentahydrate, **4**, whose X-ray crystallographic analysis demonstrated its relative configuration (2*S**,3′*S**) (Figure 3). Because the absolute configuration of the 3 position on the pyrrolidine ring of **4** is 3′*S* based on the configuration of the starting material **7** and the reaction mechanism,[17] it was considered that the absolute configuration of **4** must be 2*S*,3′*S*. Therefore, compound **4** was confirmed as (2*S*)-2-[4-[[(3*S*)-1-acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(amidino-2-naphthyl)propanoic acid hydrochloride pentahydrate.

Conclusion

In this paper, we have reported the design, synthesis, and anticoagulant activities of some arylamidine derivatives having an additional basic moiety along with a carboxyl or ester group. Of them, the (amidinoaryl)-propanoic acid derivatives represented by **4** exhibited potent and highly selective anti-FXa activity. We chose compound **4** (DX-9065a) as a candidate for a useful pharmacological tool for studies on the pathophysiological role of FXa and for a useful antithrombotic agent. Further studies of structure–activity relationships and pharmacological evaluation of **4** will be reported elsewhere.

Experimental Section

General. Melting points were determined on a Büchi 520 apparatus in glass capillary tubes and are uncorrected. Column chromatography was performed on Merck silica gel 60 (70–230-mesh ASTM) and on Diaion HP-20 (highly porous polymer type synthetic adsorbent: Mitsubishi Chemical Industries). Preparative HPLC was performed using a reverse-phase ODS column (Senshu Park ODS-H-5301 20 × 300 mm), a mobile phase of acetonitrile/water (7/93–1/9), and a flow rate of 10 mL/min. Thin-layer chromatography (TLC) was performed on Merck TLC aluminum sheets precoated with silica gel 60 F_{254} and detected by UV quenching at 254 nm or spraying with phosphomolybdic acid or ninhydrin. All analytical samples were found to be homogeneous on TLC. ^{1}H NMR spectra were recorded on a JEOL FX90Q or a JEOL JNM-EX400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane, which was used as the internal standard. Mass spectra were performed using a JEOL JMS-AX505W (EI) or a JEOL JMS-HX110 (FD, FAB) spectrometer. IR spectra were recorded on a Hitachi 270-30 spectrometer. Optical rotations were measured in a Horiba SEPA-200 polarimeter.

Computational Molecular Modeling. Positively charged conformations of 5-amidinobenzofuran and (aminoalkyl)phenyl and (aminoalkoxy)phenyl structures including 3-(phenyloxy)-pyrrolidine were generated by using the Quanta and CHARMm programs (Molecular Simulations Inc.), and energy was minimized using the semiempirical molecular orbital PM3 method as implemented in the MOPAC program on a Power Iris 400 (Silicon Graphics Inc.).

4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]benzaldehyde (8). To a stirred solution of *p*-hydroxybenzaldehyde (**5**) (1.31 g, 11 mmol), (3*R*)-1-(*tert*-butoxycarbonyl)-3-hydroxypyrrolidine (**7**)[18] (1.87 g, 10 mmol), and triphenylphosphine (2.88 g, 11 mmol) in anhydrous THF (40 mL) was added diethyl azodicarboxylate (1.91 g, 11 mmol) at room temperature. The resultant solution was stirred for 45 min. After removal of the solvent, the residue was purified by silica gel column chromatography using the solvent system toluene/AcOEt (17:3), providing a viscous oil (2.9 g, 99.7%). ^{1}H NMR ($CDCl_3$): δ 1.48 (9H, s), 2.00–2.40 (2H, m), 3.30–3.80 (4H, m), 4.90–5.10 (1H, m), 6.98 (2H, d, J = 9.0 Hz), 7.84 (2H, d, J = 9.0 Hz), 9.89 (1H, s). HRMS (M^+): calcd for $C_{16}H_{21}NO_4$ 291.1471, found 291.1477.

Ethyl 2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]-oxy]phenyl]-2-oxoacetate (9). Ethyl 2-(4-hydroxyphenyl)-2-oxoacetate (**6**) (1.80 g, 9.3 mmol), (3*R*)-1-(*tert*-butoxycarbonyl)-3-hydroxypyrrolidine (**7**) (1.74 g, 9.3 mmol), triphenylphosphine (2.92 g, 11 mmol), and diethyl azodicarboxylate (1.94 g, 11 mmol) were reacted according to the procedure described for the preparation of **8** to give a viscous yellow oil (2.53 g, 72.4%). ^{1}H NMR ($CDCl_3$): δ 1.41 (3H, t, J = 7.0 Hz), 1.46 (9H, s, *t*-Boc), 2.00–2.40 (2H, m), 3.00–3.75 (4H, m), 4.43 (2H, q, J = 7.0 Hz), 5.00 (1H, br), 6.93 (2H, d, J = 9.0 Hz), 8.00 (2H, d, J = 9.0 Hz). HRMS (M^+): calcd for $C_{19}H_{25}NO_6$ 363.1682, found 363.1693.

[(6-Cyano-2-naphthyl)methyl]triphenylphosphonium Bromide (10c). A solution of 6-(bromomethyl)-2-naphthalenecarbonitrile[19] (2.0 g, 8.1 mmol) and triphenylphosphine (2.77 g, 10.6 mmol) in xylene (50 mL) was refluxed for 18 h. After cooling, the precipitate was collected by filtration to give a colorless powder (3.31 g, 80.0%). MP: >270 °C. ^{1}H NMR ($CDCl_3$): δ 5.93 (2H, d, J = 15 Hz), 7.4–8.0 (21H, m). IR (KBr): 2230, 1629, 1587 cm^{-1}. MS (FAB): m/z 428 (508 − Br)$^+$. Anal. ($C_{30}H_{23}BrNP$) H, N; C: calcd, 70.88; found, 69.82.

[(7-Cyano-2-naphthyl)methyl]triphenylphosphonium Bromide (10d). 7-(Bromomethyl)-2-naphthalenecarbonitrile[19] (8.34 g, 33.9 mmol) and triphenylphosphine (11.6 g, 44.1 mmol) were reacted according to the procedure described for the preparation of **10c** to give a colorless powder (12.10 g, 70.2%). MP: >270 °C. Anal. ($C_{30}H_{23}BrNP$) C, H, Br, N.

General Procedure A. Preparation of 11 and 12a–d Using the Wittig Reaction of the Aldehyde or the α-Keto Ester 9 with Phosphonium Salts 10a–d, Followed by Catalytic Hydrogenation. 2-[2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]ethyl]-5-benzofurancarbonitrile (11). To a stirred solution of compound **8** (1.51 g, 5.2 mmol) and [(5-cyano-2-benzofuranyl)methyl]triphenylphosphonium chloride (1.87 g, 5.2 mmol) in dry THF (20 mL)–EtOH (20 mL) was added DBU (790 mg, 5.2 mmol) at room temperature. The mixture was stirred for 1 h at room temperature. After removal

of the solvent, the residue was purified by silica gel column chromatography using $CHCl_3$ as an eluant, providing a mixture of (*E*)- and (*Z*)-2-[2-[4-[[(3*S*)-1-(*tert*-butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]vinyl]-5-benzofurancarbonitrile (1.9 g) as a viscous yellowish oil (1.90 g). The mixture of the olefins *E* and *Z* (1.9 g) and $PdO{\cdot}H_2O{\cdot}BaSO_4$[20] (300 mg) in THF (100 mL)–EtOH (100 mL) was shaken at room temperature under a current of hydrogen (1 atm) for 6 h. After filtration of the catalyst, followed by evaporation of the filtrate, the residue was purified by silica gel column chromatography using $CHCl_3$ as an eluant, yielding a colorless viscous oil (1.80 g, 86.8%). ^1H NMR ($CDCl_3$): δ 1.46 (9H, s), 2.00–2.40 (2H, m), 2.95–3.10 (4H, m), 3.40–3.65 (4H, m), 4.83 (1H, br), 6.39 (1H, s), 6.78 (2H, d, *J* = 9.0 Hz), 7.05–7.15 (2H, m). HRMS (M^+): calcd for $C_{26}H_{28}N_2O_4$ 432.2049, found 432.2044.

Compounds **12a–d** were prepared according to procedure A.

Ethyl 2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]-3-(5-cyano-2-benzofuranyl)propanoate (12a). Colorless viscous oil (yield, 43.8%). ^1H NMR ($CDCl_3$): δ 1.16 (3H, t, *J* = 7.3 Hz), 1.46 (9H, s), 2.00–2.25 (2H, m), 3.16 (1H, dd, *J* = 15.4 and 6.5 Hz), 3.40–3.70 (5H, m), 4.00–4.25 (3H, m), 4.84 (1H, br), 6.40 (1H, s), 6.80 (2H, d, *J* = 8.7 Hz), 7.25 (2H, d, *J* = 8.7 Hz), 7.45 (2H, s), 7.74 (1H, s). HRMS (M^+): calcd for $C_{29}H_{32}N_2O_6$ 504.2260, found 504.2300.

Ethyl 2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]-3-(6-cyano-2-benzofuranyl)propanoate (12b). Colorless viscous oil (yield, 63.1%). HRMS (M^+): calcd for $C_{29}H_{32}N_2O_6$ 504.2260, found 504.2253.

Ethyl 2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]-3-(6-cyano-2-naphthyl)propanoate (12c). Colorless viscous oil (yield, 89.4%). HRMS (M^+): calcd for $C_{31}H_{34}N_2O_5$ 514.2468, found 514.2483.

Ethyl 2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]-3-(7-cyano-2-naphthyl)propanoate (12d). Colorless viscous oil (yield, 90.2%). HRMS (M^+): calcd for $C_{31}H_{34}N_2O_5$ 514.2468, found 514.2468.

General Procedure B. Pinner Synthesis of Amidines 13 and 14a–d. 2-[2-[4-[(3*S*)-3-Pyrrolidinyloxy]phenyl]ethyl]-5-benzofurancarboxamidine (13). A solution of 11 (1.66 g, 3.8 mmol) in dry CH_2Cl_2 (20 mL)–EtOH (40 mL) was saturated with HCl gas with ice cooling and left to stand for 18 h at 20 °C. After distilling off the solvents and HCl, the resulting residue was dissolved in ethanolic ammonia solution (11% w/v), and the whole was left to stand for 18 h at 20 °C. After removal of the solvent, the resulting residue was purified by preparative HPLC. After addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (800 mg, 46.3%). $[\alpha]^{24}_D$: +9.66 (*c* = 1.20, H_2O). ^1H NMR (DMSO-d_6): δ 1.90–2.30 (2H, m), 3.06 (4H, br), 3.00–3.80 (4H, br), 5.08 (1H, br), 6.73 (1H, s), 6.88 (2H, d, *J* = 8.3 Hz), 7.19 (2H, d, *J* = 8.3 Hz), 7.74 (2H, s), 8.11 (1H, s), 9.26 (2H, br), 9.47 (2H, br). IR (KBr): 1680, 1602 cm^{-1}. MS (FAB): *m/z* 350 $(M + H)^+$. Anal. ($C_{21}H_{23}N_3O_2{\cdot}1.9HCl{\cdot}1.7H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compounds **14a–d**. Purification was carried out using HP-20 column chromatography and/or HPLC.

Ethyl 3-(5-Amidino-2-benzofuranyl)-2-[4-[(3*S*)-3-pyrrolidinyloxy]phenyl]propanoate (14a). Colorless amorphous solid (yield, 30.1%). ^1H NMR (DMSO-d_6): δ 1.08 (3H, t, *J* = 7.0 Hz), 1.90–2.30 (2H, m), 3.00–3.80 (6H, m), 3.80–4.30 (3H, m), 5.08 (1H, br), 6.73 (1H, s), 6.93 (2H, d, *J* = 8.3 Hz), 7.33 (2H, d, *J* = 8.3 Hz), 8.08 (1H, s), 9.25 (2H, br), 9.40 (2H, br), 9.50–10.00 (2H, br). IR (KBr): 1735, 1677 cm^{-1}. MS (FAB): *m/z* 422 $(M + H)^+$. Anal. ($C_{24}H_{27}N_3O_4{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

Ethyl 3-(6-Amidino-2-benzofuranyl)-2-[4-[(3*S*)-3-pyrrolidinyloxy]phenyl]propanoate (14b). Colorless amorphous solid (yield, 25.6%). MS (FAB): *m/z* 422 $(M + H)^+$. Anal. ($C_{24}H_{27}N_3O_4{\cdot}1.7HCl{\cdot}2.1H_2O$) C, H, N, Cl.

Ethyl 3-(6-Amidino-2-naphthyl)-2-[4-[(3*S*)-3-pyrrolidinyloxy]phenyl]propanoate (14c). Colorless amorphous solid (yield, 42.6%). Anal. ($C_{28}H_{29}N_3O_3{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

Ethyl 3-(7-Amidino-2-naphthyl)-2-[4-[(3*S*)-3-pyrrolidinyloxy]phenyl]propanoate (14d). Colorless amorphous solid (yield, 56.1%). MS (FAB): *m/z* 432 $(M + H)^+$. Anal. ($C_{26}H_{29}N_3O_3{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N.

General Procedure C. Preparation of 15a–d by Acidic Hydrolysis. 3-(5-Amidino-2-benzofuranyl)-2-[4-[(3*S*)-pyrrolidinyloxy]phenyl]propanoic Acid (15a). A solution of **14a** (3.2 g, 6.1 mmol) in 2 N HCl was heated at reflux for 30 min. After evaporation of the solvent, the residue was purified using HP-20 column chromatography (acetonitrile/H_2O, 1/19–1/5). After addition of small amount of concentrated HCl to selected fractions, the solvents were removed to give **15a** as a colorless amorphous solid (1.25 g, 41.6%). ^1H NMR (DMSO-d_6): δ 2.00–2.30 (2H, m), 3.00–3.80 (6H, m), 4.10 (1H, t, *J* = 7.2 Hz), 5.10 (1H, br), 6.74 (1H, s), 6.94 (2H, d, *J* = 8.3 Hz), 7.40 (2H, d, *J* = 8.3 Hz), 7.74 (2H, s), 8.09 (1H, s), 9.22 (2H, br), 9.40 (2H, br), 9.10–10.00 (2H, br). IR (KBr): 1720, 1674 cm^{-1}. MS (FAB): *m/z* 394 $(M + H)^+$. Anal. ($C_{22}H_{23}N_3O_4{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compounds **15b–d**. Purification was carried out using HP-20 column chromatography and/or HPLC.

3-(6-Amidino-2-benzofuranyl)-2-[4-[(3*S*)-pyrrolidinyloxy]phenyl]propanoic Acid (15b). Colorless amorphous solid (yield, 34.6%). Anal. ($C_{22}H_{23}N_3O_4{\cdot}2HCl{\cdot}H_2O$) C, H, N.

3-(6-Amidino-2-naphthyl)-2-[4-[(3*S*)-pyrrolidinyloxy]phenyl]propanoic Acid (15c). Colorless amorphous solid (yield, 73.2%). MS (FAB): *m/z* 404 $(M + H)^+$. Anal. ($C_{24}H_{25}N_3O_3{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

3-(7-Amidino-2-naphthyl)-2-[4-[(3*S*)-pyrrolidinyloxy]phenyl]propanoic Acid (15d). Colorless amorphous solid (yield, 76.4%). MS (FAB): *m/z* 404 $(M + H)^+$. Anal. ($C_{24}H_{25}N_3O_3{\cdot}2HCl{\cdot}1.8H_2O$) C, H, N, Cl.

General Procedure D. Preparation of 16 and 17a–d. 2-[2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]ethyl]-5-benzofurancarboxamidine (16). To a stirred and ice-cooled solution of compound **13** (1.10 g, 2.6 mmol) in H_2O (10 mL) was added ethyl acetimidate hydrochloride (1.61 g, 13.0 mmol) in small portions while the reaction mixture was maintained at pH 7.5–8.5 with 1 N NaOH. After stirring for further 20 min with cooling on an ice bath, the reaction mixture was adjusted to pH 2.0 with dilute HCl and concentrated to dryness. The obtained residue was purified using HP-20 column chromatography (acetonitrile/H_2O, 3/97–8/92). After addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give **15a** as a colorless amorphous solid (890 mg, 69.2%). $[\alpha]^{24}_D$: +24.0° (*c* = 1.15, H_2O). ^1H NMR (DMSO-d_6): δ 2.15–2.40 (2H, m), 2.27 (1.5H, s), 2.31 (1.5H, s), 3.00 (2H, t, *J* = 7.3 Hz), 3.12 (2H, t, *J* = 7.3 Hz), 3.50–4.00 (4H, m), 5.12 (0.5H, br), 5.18 (0.5H, br), 6.76 (1H, s), 6.83–6.90 (2H, m), 7.15–7.25 (2H, m), 7.68–7.75 (2H, m), 8.08 (1H, s), 8.53 (0.5H, s), 8.61 (0.5H, s), 9.19 (2H, s), 9.30–9.45 (3H, m). IR (KBr): 1700, 1673 cm^{-1}. MS (FAB): *m/z* 391 $(M + H)^+$. Anal. ($C_{23}H_{26}N_4O_2{\cdot}2HCl{\cdot}2H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compounds **17a–d**. Purification was carried out using HP-20 column chromatography and/or HPLC.

2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(5-amidino-2-benzofuranyl)propanoic Acid (17a). Colorless amorphous solid (yield, 62.9%). MS (FAB): *m/z* 435 $(M + H)^+$. Anal. ($C_{24}H_{26}N_4O_4{\cdot}2.2HCl{\cdot}2.5H_2O$) C, H, N, Cl.

2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(6-amidino-2-benzofuranyl)propanoic Acid (17b). Colorless amorphous solid (yield, 26.8%). MS (FAB): *m/z* 435 $(M + H)^+$. Anal. ($C_{24}H_{26}N_4O_4{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(6-amidino-2-naphthyl)propanoic Acid (17c). Colorless amorphous solid (yield, 31.5%). Anal. ($C_{28}H_{28}N_4O_3{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic Acid (17d). Colorless amorphous solid (yield, 28.4%). MS (FAB): *m/z* 445 $(M + H)^+$. Anal. ($C_{28}H_{28}N_4O_3{\cdot}2HCl{\cdot}1.6H_2O$), C, H, N, Cl.

2-(2-Bromo-1-oxoethyl)-5-benzofurancarbonitrile (19). Compound **18** (21.0 g, 114 mmol) was dissolved in CH_2Cl_2 (300 mL) and cooled to −10 °C. To the stirred solution was added a solution of bromine (18.2 g, 114 mmol) in CH_2Cl_2 (30 mL) at such a rate as to maintain the temperature −10 °C. After gradual warming up to ice-cooled temperature, the reaction mixture was washed with 10% $Na_2S_2O_3$ and dried over $MgSO_4$. After removal of the solvent, the crystalline residue was recrystallized from a

benzene/*n*-hexane mixture to afford colorless crystals (21.0 g, 69.7%). MP: 156–158 °C. ^{1}H NMR ($CDCl_3$): δ 4.44 (2H, s), 7.60–7.90 (3H, m), 8.11 (1H, s). IR (KBr): 2228, 1696 cm^{-1}. HRMS (M^+): calcd for $C_{11}H_6{}^{81}BrNO_2$ 264.9521, $C_{11}H_6{}^{79}BrNO_2$ 262.9582, found 264.9541, 262.9569.

Methyl 2-(5-Cyano-2-benzofuranyl)-2-oxoacetate (20). To a solution of SeO_2 (444 mg, 4 mmol) in boiling MeOH (10 mL) was added compound **19** (1.06 g, 4 mmol). The mixture was refluxed for 12 h. After cooling, insoluble materials were removed, and the resulting filtrate was concentrated to dryness. Purification of the residue using silica gel column chromatography (toluene/AcOEt, 9/1) afforded colorless needles (129 mg, 14.0%). MP: 196–199 °C. ^{1}H NMR ($CDCl_3$): δ 4.03 (3H, s), 7.66–7.96 (2H, m), 8.17 (2H, m). IR (KBr): 2224, 1740 cm^{-1}. HRMS (M^+): calcd for $C_{12}H_7NO_4$ 229.0375, found 229.0353.

Methyl 2-(5-Cyano-2-benzofuranyl)-3-(4-methoxyphenyl)propanoate (22). Compound **20** (3.10 g, 13.5 mmol) and [(*p*-methoxyphenyl)methyl]triphenylphosphonium chloride (**21**) (6.20 g, 14.8 mmol) were reacted (DBU; 2.19 g, 14.4 mmol) and then hydrogenated ($PdO{\cdot}H_2O{\cdot}BaSO_4$; 1.10 g) according to procedure A to give **22** as a colorless viscous oil (4.20 g, 68.3%). ^{1}H NMR ($CDCl_3$): δ 3.24 (1H, J = 14.1 and 7.8 Hz), 3.37 (1H, dd, J = 14.1 and 7.8 Hz), 3.67 (3H, s), 3.76 (3H, s), 4.10 (1H, t, J = 7.8 Hz), 6.61 (1H, s, 1H), 6.78 (2H, d, J = 8.8 Hz), 7.07 (2H, d, J = 8.8 Hz), 7.53 (2H, s), 7.83 (1H, s). HRMS (M^+): calcd for $C_{20}H_{17}NO_4$ 335.1158, found 335.1157.

Methyl 2-(5-Cyano-2-benzofuranyl)-3-(4-hydroxyphenyl)propanoate (23). Compound **22** (4.20 g, 12.8 mmol) was dissolved in CH_2Cl_2 (150 mL), and the solution was cooled to −50 °C. To the stirred solution was added a solution of BBr_3 (9.97 g, 29.8 mmol) in CH_2Cl_2 (30 mL) at such a rate as to maintain a temperature of −50 °C. After gradual warming up to 15 °C, the reaction mixture was washed with dilute HCl and dried over $MgSO_4$. After removal of the solvent, the residue was subjected to silica gel column chromatography ($CHCl_3$/EtOH, 49/1) to give colorless crystals (3.1 g, 77.0%): Mp: 110–111 °C. ^{1}H NMR ($CDCl_3$): δ 3.18 (1H, dd, J = 14.4 and 7.8 Hz), 3.36 (1H, dd, J = 14.4 and 7.8 Hz), 3.69 (3H, s), 4.09 (1H, t, J = 7.8 Hz), 6.60 (1H, s), 6.69 (2H, d, J = 8.4 Hz), 7.00 (2H, d, J = 8.4 Hz), 7.53 (2H, s), 7.83 (1H, s). IR (KBr): 2228, 1722 cm^{-1}. Anal. ($C_{19}H_{15}NO_4$) C, H, N.

Methyl 3-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]-2-(5-cyano-2-benzofuranyl)propanoate (24). To a stirred solution of compound **23** (3.0 g, 9.3 mmol), (3*R*)-1-(*tert*-butoxycarbonyl)-3-hydroxypyrrolidine (**7**) (1.92 g, 10.3 mmol), and triphenylphosphine (2.69 g, 10.3 mmol) in dry THF (150 mL) was added diethyl azodicarboxylate (1.79 g, 10.3 mmol) at 20 °C. After removal of the solvent, the residue was purified by silica gel column chromatography using the solvent system toluene/AcOEt (9:1), providing a viscous oil (1.2 g, 25.5%). ^{1}H NMR ($CDCl_3$): δ 1.46 (9H, s), 1.88–2.24 (2H, m), 3.10–3.60 (6H, m), 4.10 (1H, t), 4.81 (1H, br), 6.61 (1H, s), 6.73 (2H, d), 7.04 (2H, d), 7.54 (2H, s), 7.83 (1H, s). MS (FD): m/z 504 (M^+).

Ethyl 2-(5-Amidino-2-benzofuranyl)-3-[4-[(3*S*)-pyrrolidinyl]oxy]phenyl]propanoate (25). Compound **24** (880 mg, 17.5 mmol) was treated with ethanolic HCl and then with ethanolic ammonia according to general procedure B to give **25** as a colorless amorphous solid (420 mg, 46.1%) after purification by HP-20 column chromatography (acetonitrile/H_2O, 1/49–1/19) and then preparative HPLC (acetonitrile/H_2O, 1/9). ^{1}H NMR (DMSO-d_6): δ 1.08 (3H, t, J = 7.0 Hz), 1.80–2.30 (2H, m), 2.70–3.70 (6H, m), 4.08 (2H, t, J = 7.0 Hz), 4.35 (1H, t, J = 7.9 Hz), 5.08 (1H, br), 6.84 (2H, d, J = 8.3 Hz), 6.96 (1H, s), 7.17 (2H, d, J = 9.0 Hz), 7.79 (2H, s), 8.12 (1H, s), 9.33 (2H, br), 9.51 (2H, br), 9.80 (2H, br). IR (KBr): 1732, 1674 cm^{-1}. MS (FAB): m/z 422 ($M + H)^+$. Anal. ($C_{24}H_{27}N_3O_4{\cdot}2.1HCl{\cdot}H_2O$) C, H, N, Cl.

2-(5-Amidino-2-benzofuranyl)-3-[4-[(3*S*)-pyrrolidinyloxy]phenyl]propanoic Acid (26). Compound **25** (780 mg, 1.5 mmol) was treated with 2 N HCl according to general procedure C to give **26** as a colorless amorphous solid (480 mg, 65.0%) after purification by preparative HPLC (acetonitrile/H_2O, 7/93). ^{1}H NMR (DMSO-d_6): δ 1.90–2.30 (2H, m), 2.90–3.70 (6H, m), 4.26 (1H, t, J = 7.9 Hz), 5.06 (1H, br), 6.83 (2H, d, J = 8.3 Hz), 6.93 (1H, s), 7.17 (2H, d, J = 9.0 Hz), 7.78 (2H, s), 8.14 (1H, s), 9.30 (2H, br), 9.47 (2H, br), 9.80 (2H, br). IR (KBr): 1674 cm^{-1}. MS (FAB): m/z 394 ($M + H)^+$. Anal. ($C_{22}H_{23}N_3O_4{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

Ethyl (2*S*)-2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]-3-(7-cyano-2-naphthyl)propanoate [(+)-12d] and Ethyl (2*R*)-2-[4-[[(3*S*)-1-(*tert*-Butoxycarbonyl)-3-pyrrolidinyl]oxy]phenyl]-3-(7-cyano-2-naphthyl)-propanoate [(−)-12d]. Compound **12d** (2.0 g, 3.9 mmol) was dissolved in EtOH (10 mL) under heating. After cooling to room temperature, precipitates were collected by filtration (77% de) and then recrystallized from EtOH twice to obtain (+)-**12d** (>99.5% de) as colorless crystals (640 mg, 32.0%). Mp: 132–133.2 °C. $[\alpha]^{24}_D$: +118.5° (c = 1.01, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.11 (3H, t, J = 7.3 Hz), 1.47 (9H, s), 2.00–2.30 (2H, m), 3.18 (1H, dd, J = 14.2 and 6.8 Hz), 3.40–3.70 (5H, m), 3.87 (1H, t, J = 7.6 Hz), 4.00–4.10 (2H, m), 4.85 (1H, br), 6.80 (2H, d, J = 8.8 Hz), 7.20–7.30 (2H, m), 7.42 (1H, d, J = 8.3 Hz), 7.55 (1H, d, J = 8.3 Hz), 7.63 (1H, s), 7.77 (1H, d, J = 8.3 Hz), 7.85 (1H, d, J = 8.3 Hz), 8.12 (1H, s). IR (KBr): 2228, 1724, 1688 cm^{-1}. Anal. ($C_{31}H_{34}N_2O_5$) C, H, N.

Concentration of the filtrate gave 87.0% de of (−)-**12d** (940 mg, 47.0%). A small portion was crystallized from an *n*-hexane/EtOH mixture. The crystals thus collected were recrystallized from the same mixed solvent system three times to give (−)-**12d** (91% de) as a colorless powder. Mp: 82.5–85.0 °C. $[\alpha]^{23}_D$: −80.0° (c 0.53, $CHCl_3$). HRMS (M^+): calcd for $C_{31}H_{34}N_2O_5$ 514.2468, found 514.2466.

HPLC Separation of the Epimers (+)-12d and (−)-12d. The determinations of the de of (+)-**12d** and (−)-**12d** were carried out by HPLC using an amylose-based column (Chiralpak AD, 4.6 i.d. × 250 mm, Daicel Chemical Industries, Ltd.) and detection of UV absorbance at 254 nm. For compounds (+)-**12d** and (−)-**12d**, the practical separation was performed using a mixture of *n*-hexane/2-PrOH (17:3) as an eluant (1 mL/min) at 25 °C. Under the above conditions, the retention times were 23.2 min for (−)-**12d** and 31.4 min for (+)-**12d**.

Ethyl (2*S*)-3-(7-Amidino-2-naphthyl)-2-[4-[(3*S*)-3-pyrrolidinyloxy]phenyl]propanoate [(+)-14d]. Compound (+)-**12d** (123.1 g, 239 mmol; >99.5% de) was treated according to general procedure B, except for the reaction temperature and the time, to give (+)-**14d** (107.0 g, 85.6%) as a colorless amorphous solid.

Reaction at 0 °C required 3 days for complete imino ester formation and a further 3 days for amidine formation. Purification was carried out using HP-20 column chromatography (acetonitrile/H_2O, 1/19–1/4). ^{1}H NMR (DMSO-d_6): δ 1.01 (3H, t, J = 7.2 Hz), 2.00–2.30 (2H, m), 3.10–3.60 (6H, m), 3.90–4.05 (2H, m), 4.05–4.15 (1H, m), 5.10 (1H, br), 6.93 (2H, d, J = 8.8 Hz), 7.32 (2H, d, J = 8.8 Hz), 7.60 (1H, d, J = 8.3 Hz), 7.78 (1H, d, J = 8.3 Hz), 7.85 (1H, s), 7.96 (1H, d, J = 8.3 Hz), 8.08 (1H, d, J = 8.3 Hz), 8.41 (1H, s), 9.20–9.30 (2H, br), 9.40–9.70 (4H, br). IR (KBr): 1728, 1678, 1608, 1506 cm^{-1}. MS (FAB): m/z 432 ($M + H)^+$. Anal. ($C_{26}H_{29}N_3O_3{\cdot}2HCl{\cdot}H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compound (−)-**14d**. Purification was carried out using HP-20.

Ethyl (2*R*)-3-(7-Amidino-2-naphthyl)-2-[4-[(3*S*)-3-pyrrolidinyloxy]phenyl]propanoate [(−)-14d]. Colorless amorphous solid. MS (FAB): m/z 432 ($M + H)^+$. Anal. ($C_{26}H_{29}N_3O_3{\cdot}2HCl{\cdot}1.5H_2O$) C, H, N, Cl.

Ethyl (2*S*)-2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoate [(+)-18]. To a stirred and cooled solution of compound (+)-**14d** (105.3 g, 201.5 mmol) and ethyl acetimidate hydrochloride (51.5 g, 416.8 mmol) was added Et_3N (89 mL, 640.0 mmol) at such a rate to maintain the temperature of the reaction mixture at 3–5 °C. After being stirred for 2.5 h at 5 °C, the reaction mixture was concentrated and adjusted to pH 4–5 with dilute HCl. The mixture was subjected to HP-20 column chromatography (acetonitrile/H_2O, 0/100–1/4) to give (+)-18 (115.1 g, 99.1%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.02 (3H, m), 2.10–2.35 (2H, m), 2.26 (1.5H, s), 2.31 (1.5H, s), 3.19 (1H, m), 3.40–3.80 (5H, m), 3.90–4.05 (2H, m), 4.05–4.15 (1H, m), 5.13 (0.5H, br), 5.20 (0.5H, s), 6.90–6.97 (2H, m), 7.32 (2H, m), 7.61 (1H, d, J = 8.3 Hz), 7.80 (1H, dd, J = 8.3 and 1.5 Hz), 7.85 (1H, s), 7.96 (1H, d, J = 8.3 Hz), 8.08 (1H, d, J = 8.3 Hz), 8.43 (1H, s), 8.52 (0.5H, br), 8.61 (0.5H, br), 9.28–9.40 (3H, br), 9.50–9.60

(4H, br). IR (KBr): 1728, 1674 cm^{-1}. MS (FAB): m/z 473 $(M + H)^+$. Anal. ($C_{28}H_{32}N_4O_3 \cdot 2.1HCl \cdot H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compound (-)-18. Purification was carried out using HP-20.

Ethyl (2*R*)-2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]-phenyl]-3-(7-amidino-2-naphthyl)propanoate [(-)-18]. Colorless amorphous solid. MS (FAB): m/z 473 $(M + H)^+$. Anal. ($C_{28}H_{32}N_4O_3 \cdot 2.1HCl \cdot 2H_2O$) C, H, N, Cl.

(2*S*)-2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic Acid [(+)-17d]. A solution of (+)-18 (110.1 g, 191.1 mmol) in concentrated HCl (3300 mL) was stirred for 232 h at 5 °C. After concentration (ca. 800 mL) under reduced pressure, the mixture was subjected to HP-20 column chromatography (acetonitrile/H_2O, 0/100–1/9) to give (+)-**17d** (96.0% de) as a colorless amorphous solid (103.6 g, 97.6%): ^1H NMR (DMSO-d_6): δ 2.10–2.40 (2H, m), 2.28 (1.5H, s), 2.31 (1.5H, s), 3.10–3.30 (1H, m), 3.40–4.10 (6H, m), 5.14 (0.5H, br), 5.20 (0.5H, s), 6.90–7.00 (2H, m), 7.35–7.40 (2H, m), 7.60 (1H, d, J = 8.3 Hz), 7.80 (1H, d, J = 8.3 Hz), 7.84 (1H, s), 7.94 (1H, d, J = 8.3 Hz), 8.06 (1H, d, J = 8.3 Hz), 8.42 (1H, s), 8.55 (0.5H, br), 8.65 (0.5H, br), 9.30–9.70 (5H, br). IR (KBr): 1674 cm^{-1}. MS (FAB): m/z 445 $(M + H)^+$. Anal. ($C_{26}H_{28}N_4O_3 \cdot 1.4HCl \cdot 2.2H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compound (-)-**17d**.

(2*R*)-2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic Acid [(-)-17d]. Colorless amorphous solid (84.0% de). MS (FAB): m/z 445 $(M + H)^+$. Anal. ($C_{26}H_{28}N_4O_3 \cdot 2.2HCl \cdot 1.5H_2O$) C, H, N, Cl.

HPLC Separation of the Epimers (+)-17d and (-)-17d. The determinations of the de of (+)-**17d** and (-)-**17d** were carried out by HPLC using a ligand-exchange type column with D-penicillamine as the optically active site (Sumichiral OA-5000, 4.6 i.d. × 150 mm, Sumika Analysis Center) and detection of UV absorbance at 254 nm. For compounds (+)-**17d** and (-)-**17d**, the practical separation was performed using a mixture of 2 mM $CuSO_4$(aq)/acetonitrile (17:3) as an eluant (1 mL/min) at 60 °C. Under the above conditions, the retention times were 38.1 min for (-)-**17d** and 43.6 min for (+)-**17d**.

(2*R*)-2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic Acid Hydrochloride Pentahydrate (4). An amorphous (+)-**17d** (102.6 g, 193.0 mmol) was dissolved in H_2O (1000 mL). With stirring, the prepared solution was adjusted to pH 4.8 by adding an OH type ion-exchange resin (Amberlite IRA-410). The resin was removed by filtration, and the resulting filtrate was concentrated to dryness. The residue (94.6 g) was dissolved in H_2O (142 mL), and the resulting solution was mixed with EtOH (1570 mL) and stirred at room temperature for 1 h. After removal of the precipitates, the filtrate was stirred at 8 °C for 40 h. The precipitated crystals were collected by filtration, washed with EtOH, and then dried under an air current (60–70% relative humidity) for 6.5 h at room temperature to give colorless crystals (>99.5% de; 70.3 g, 63.8%). $[\alpha]^{24}_D$: +57.4° (c = 1.000, H_2O) (measured after 30 min of heating at 40°C). ^1H NMR (DMSO-d_6): δ 2.20–2.35 (2H, m, CH_2), 2.29 (1.5H, s, $^1/_2$ CH_3), 2.32 (1.5H, s, $^1/_2$ CH_3), 2.80–2.95 (1H, m, $CHHCCO_2$), 3.30–4.00 (6H, m, $CHHCHCO_2$ and CH_2NCH_2), 5.16 (0.5H, br, $^1/_2$ OCH), 5.22 (0.5H, s, $^1/_2$ OCH), 6.90–7.00 (2H, m), 7.45–7.51 (2H, m), 7.57 (1H, d, J = 8.3 Hz), 7.66 (1H, d, J = 8.0 Hz), 7.93 (1H, d, J = 8.3 Hz), 7.97 (1H, d, J = 8.3 Hz), 8.11 (1H, s), 8.68 (1H, br), 8.70–9.30 (br), 11.50–12.20 (br). IR (KBr): 1682 cm^{-1}. MS (FAB): m/z 445 $(M + H)^+$. Anal. ($C_{26}H_{28}N_4O_3 \cdot HCl \cdot 5H_2O$) C, H, N, Cl.

X-ray Crystallographic Analysis of 4. A crystal of 4 enclosed in a capillary tube was used for X-ray diffraction study. The data for 4 was as follows: $C_{26}H_{26}N_4O_3 \cdot HCl \cdot 5H_2O$, M_r = 571.07, monoclinic space group $C2$, a = 18.750(7) Å, b = 20.402(9) Å, c = 8.284(3) Å, β = 97.95(3)°, V = 3147(2) Å^3, Z = 4, D_m (by flotation) = 1.200 g cm^{-3}, D_x = 1.205 g cm^{-3}. Data were collected using a Mac Science MXC18 diffractometer in the θ range 3–65° with a scan speed of 15 deg/min, using graphite monochromated Cu Kα radiation (λ = 1.541 78 Å). A total of 2401 unique reflections were used for calculation, and the structure was solved by the direct method of CRYSTAN. A full-matrix least-squares method was used for the refinement. All non-hydrogen atoms were refined anisotropically, and refinement converged at R 0.0914 and R_w 0.0899.

Anticoagulant Activity. Plasma Recalcification Time (PRCT). Plasma was mixed with saline (100 μL) or a saline solution of the inhibitor in a glass tube and incubated for 2 min at 37 °C. Coagulation was started with the addition of 20 mM $CaCl_2$ solution (100 μL). Anticoagulant activity was evaluated with the plasma clotting time doubling concentration (CT2).

Anti-FXa Activity. Anti-FXa activities were measured by using chromogenic substrate S-2222 (KabiVitrum) and human FXa. FXa was obtained with the activation of factor X (Enzyme Research Laboratories, Inc.) by Russell viper venom. Saline (20 μL) or a saline solution of the inhibitor and 1 mM S-2222 (100 μL) were mixed with 0.1 M Tris–0.2 M NaCl buffer pH 8.4 (360 μL). The reaction was started with the addition of 0.5 unit/mL human FXa solution (20 μL), and the mixture was incubated for 10 min at 37 °C. The reaction was terminated with the addition of 60% AcOH (100 μL), and the optical densities (OD) were measured (405 nm)

$$\text{anti-FXa activity (inhibition \%)} = 1 - (\text{OD of the inhibitor/OD of saline control}) \times 100$$

The IC_{50} value was obtained by plotting the inhibitor concentrations against the anti-FXa activity (inhibition %) on statistical probability paper.

Antithrombin Activity. Saline (100 μL; Tris-HCl buffered to pH 7.45) (TBS) containing fibrinogen (6 mg/mL; Type 1, Daiichi Pure Chemicals Co., Ltd.) was mixed with saline or a saline solution of the inhibitors (100 μL). After addition of a solution of thrombin (100 μL; 4 units/mL: Sankyo Co., Ltd.) to the above mixture, the clotting time was measured at 37 °C, and then a calibration curve was prepared. Antithrombin activities (inhibition %) were obtained by measuring clotting time using solutions of inhibitors in saline (100 μL). The IC_{50} value was obtained from the inhibition percentage.

Ex Vivo Anticoagulant Activity on Oral Administration. Male Wistar rats (200–250 g) were fasted overnight. Synthetic compounds were dissolved in water and administered orally to rats with a stomach tube. Fifteen minutes after administration, rats were anesthetized with sodium thiopental (100 mg/kg, ip). Blood samples were collected from the jugular vein (in the presence of trisodium citrate) at several time points. After these blood samples were centrifuged, the platelet poor plasma samples were used for measuring their activated thromboplastin times (APTT).

APTT. Plasma (20 μL) and saline (20 μL) or a saline solution of the inhibitors were mixed with Platerin Plus Activator (Enzyme Research Laboratories, Inc.) (20 μL) in the process tube, and the coagulation was started by addition of 20 mM $CaCl_2$ (20 μL).

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