4-(Pyrrolidinyl)methoxybenzoic Acid Derivatives as a Potent, Orally Active VLA-4 Antagonist

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A novel series of benzoic acid derivatives as VLA-4 antagonists were synthesized. Optimization, focusing on activity and lipophilicity needed for cell permeability, resulted in the identification of 15b and 15e with good activity (IC_{50}=1.6 nM each) and moderate lipophilicity (Log *D*=2.0, 1.8). Furthermore, 15e demonstrated efficacy in murine asthma model by an oral dose of 30 mg/kg.

Key words VLA-4; integrin; human serum albumin; asthma

VLA-4 (very late antigen 4, $\alpha_4\beta_1$ integrin, CD49d/CD29) is a key cell receptor expressed on most leukocytes.[1)] Natural ligands include VCAM-1 (vascular cell adhesion molecule-1) expressed on cytokine-stimulated endothelial cells, the alternatively spliced connecting segment-1 domain of fibronectin on the extracellular matrix,[2—4)] and junctional adhesion molecule 2 on endothelial cells.[5)] Through interaction with ligands, VLA-4 plays an important role in the process of adhesion, migration, and activation of inflammatory leukocytes at sites of inflammation. *Anti*-VLA-4 antibodies or small molecular VLA-4 antagonists[6—12)] have been reported to inhibit leukocyte infiltration into extravascular tissue and prevent tissue damage in models of inflammatory such as asthma,[13)] multiple sclerosis,[14,15)] rheumatoid arthritis,[16)] and inflammatory bowel disease.[17,18)] It has recently been reported that the humanized monoclonal *anti*-α_4 antibody, Tysabri (Elan Pharmaceuticals Inc. and Biogen Idec Inc.),[19—21)] demonstrated promising results in treating patients with multiple sclerosis, Crohn's disease, and rheumatoid arthritis in clinical trials. Therefore, the development of small molecular VLA-4 antagonists with acceptable oral pharmacokinetic profiles are viewed as a reasonable approach to a novel class of therapeutic agents.

We have recently reported the identification of a morpholinyl-piperidinylacetic acid **1** as a potent VLA-4 antagonist (IC_{50}=4.4 nM), which showed efficacy in a murine airway inflammation model by oral administration (Fig. 1).[22)] Unfortunately, **1** had a poor pharmacokinetic profiles such as low oral availability and high plasma clearance (F<1%, 69 ml/min/kg) in rats.[22)] To improve the poor pharmacokinetic property, structural modifications were made to this class of compounds. As a result of this study, 3-hydroxyprolyl-1-piperazinylacetic acid **2** (IC_{50}=1.1 nM) and 3,4-dihydroxyprolyl-1-piperazinylacetic acid **3** (IC_{50}=1.4 nM) were identified; both these compounds have moderate plasma clearance (**2**, 30 ml/min/kg; **3**, 21 ml/min/kg) in rats.[23)] We also have made it clear that the improvement of plasma clearance is caused by the change of the main excretion pathway, from bile for **1** to urine for **2** and **3**.[12)] Unfortunately, neither **2** nor **3** showed any oral availability. From result of permeability assay using Madin–Darby canine kidney (MDCK) cell monolayers, it was found that **1**, **2**, and **3** showed low permeability (P_{app} values, **1**: 0.02×10^{-6} cm/s; **2**: $<0.15\times10^{-6}$ cm/s, **3**: $<0.05\times10^{-6}$ cm/s). These results stand contrast to value for metoprolol ($17.3—20.3\times10^{-6}$ cm/s), which exhibits good oral availability in human. In addition, it was found that these compounds showed low Log *D* values (**1**, −0.7; **2**, **3**, −3), suggesting that their poor permeability is probably caused by their low lipophilicity. Further attempts to modify **2** and **3** to ethyl-ester, as a pro-drugs, were conducted as one way to increase the absorption across the intestinal epithelium. However, the strategy did not lead to a significant improvement of oral availability in rats (F<3%). Since the extremely low lipophilicity of **2** and **3** is probably responsible at least in part for the improvement in their plasma clearance, but, this physicochemical characteristic is at odds with good cell permeability. Thus, further development on these compounds were halted.

During the course of the study, we had also identified prolyl-benzoic acid derivative **6** (IC_{50}=1.6 nM, Fig. 1). This

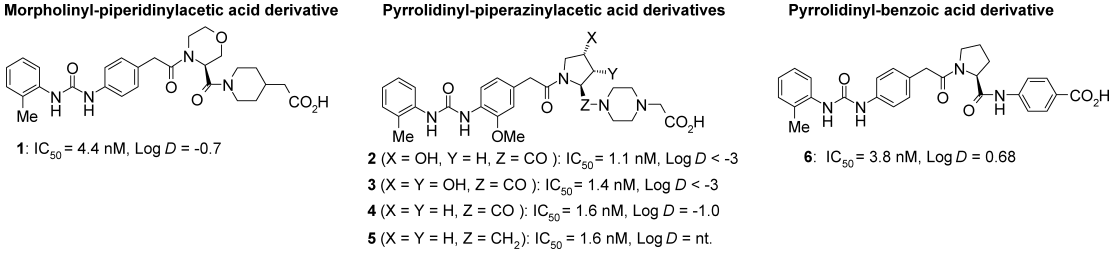

Fig. 1. VLA-4 Antagonists

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compound shows relatively higher lipophilicity (Log D= 0.68) than those of **2** and **3**. Additionally, with regard to plasma clearance, given that compounds with relatively high lipophilicity tend to bind to serum albumin,[24] this characteristic would improve the maintenance at a given blood concentration. Consequently, we thought that benzoic acid derivatives might be useful to achieve a desired compound with low plasma clearance and good oral bioavailability. Thus, compound **6** was chosen for further studies. In addition to the VLA-4/VCAM-1 binding assay used previous studies, we performed to evaluate the activities of compounds in the presence of 3% human serum albumin (HSA) to estimate their effectiveness *in vivo*.

The modification of the amide bond between the pyrrolidine ring and the benzoic acid is performed firstly, since it has been found that the removal of oxygen atom in the corresponding amide bond of prolyl-1-piperazinylacetic acid derivative **4** (IC_{50}=1.6 nM, Fig. 1) dose not affect on the activity (**5**, IC_{50}=1.6 nM, Fig. 1).[23] Next, appending a variety of substituents into pyrrolidine ring and benzene ring of benzoic acid portion of **6** was focused on.

In this paper, we wish to report the optimization efforts on the physicochemical properties of the benzoic acid derivatives that retain the activity. We also present the results of representative compounds in treating a murine model of asthma and the pharmacokinetic profiles.

Chemistry

The syntheses of benzoic acid derivatives **9a**—**g** (Synthetic route A), **12a**—**p** (Synthetic route B), and **15a**—**k** (Synthetic route C) are shown in Chart 1. Pyrrolidine derivatives **8a**—**c**, **8e**, **11a**—**n**, and **14a**—**g** were condensed with 3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetic acid (**7a**) or penetafluorophenyl 3-methoxy-4-[*N*-(2-methylphenyl)-ureido]phenylacetate (**7b**) using EDC and HOBt followed by basic hydrolysis to produce **9a**—**c**, **9e**, **12a**—**n**, and **15a**—**g**. After the hydrolysis, the hydrogenation of C=C double bond in **9b** afforded **9d**. Oxidation of sulfide **10**, which was obtained in the preparation on **9e**, by using *m*CPBA (1 eq or 2.2 eq) and basic hydrolysis resulted in **9f** and **9g**, respectively. In the case of **12o** and **12p**, each of bearing hydroxyl group in the pyrrolidine ring, deprotection of the *O*-protective groups was conducted before basic hydrolysis of intermediates **13m** and **13n**. Compound **15h** was prepared from **15g** by $SnCl_2$ reduction of the NO_2 group. The subsequent standard *N*-acylation and *N*-alkylation protocols provided **15i** and **15j**, respectively. Compound **15k**, with a *N*-methyl group at the 3-position of benzoic acid, was synthesized from intermediate **16**, which was obtained in the preparation on **15g**, *via* mono-*N*-methylation followed by basic hydrolysis.

Pyrrolidine derivatives **8a**—**c**, **8e**, **11a**—**n**, and **14a**—**g** were prepared from L-proline derivatives **17**, **22**, and **26**[23,25] as shown in Chart 2—6.

Compound **8a**, with a methylamino linker (CH_2NH) between the pyrrolidine ring and the benzoic acid moiety, was prepared from *N*-Boc-L-prolinal (**17**) with methyl 4-aminobenzoate by use of $NaBH_3CN$, followed by deprotection of the Boc group with TFA as shown in Chart 2.

Compound **8b** with an ethenyl linker (CH=CH) and **8c** with an ethynyl linker (C≡C) were prepared as shown in Chart 3. *N*-Boc-L-prolinal (**17**) was subjected to Wittig–Horner reaction with ethyl 4-(diethoxyphosphorylmethyl)benzoate,[26] followed by deprotection of the Boc group to give **8b**. On the other hand, **17** was converted to 2-ethynylpyrrolidine **20** according to the Trybulsk's protocol.[27] Following Pd catalyzed coupling of **20** with ethyl 4-iodobenzoate, deprotection of Boc group afforded **8c**.

The synthesis of **8e** containing a methylthio linker (CH_2S) was achieved in 4 steps as shown in Chart 4. Following tosylation[28] of *N*-Boc-L-prolinol (**22**), coupling of **23** with 4-iodothiophenol[29] in the presence of NaH yielded **24**. Subsequently, **24** was subjected to Pd catalyzed methoxycarbonylation reaction with CO and MeOH, followed by deprotection of the Boc group to afford **8e**.

Substituted pyrrolidines **11a**—**n**, which contain a methyloxy linker (CH_2O) at 2-position in the pyrrolidine ring, were prepared as shown in Chart 5. Substituted proline derivatives **26a**, **b**, **26d**, **26f**—**j**, and **26m**, **n**, which were commercially available or easily prepared from *N*-Boc-(4*R*)-hydroxy-L-proline,[23,25] were reduced with borane-dimethylsulfide complex, followed by coupling with methyl 4-hydroxybenzoate employing Mitsunobu reaction to afford **27a**, **b**, **27d**, **27f**—**j**, and **27m**, **n**. Additionally, **27c**, **27e**, **27k**, and **27l** were readily prepared from **27b**. Thus, the selective hydrolysis of the acetoxy group in **27b** with K_2CO_3/MeOH and Mitsunobu reaction of the resulting (4*R*)-hydroxypyrrolidine derivative with acetic acid provided **27c** with inversion of configuration at C-4. The acetoxy group in **27c** was also selectively hydrolyzed under the same condition to give the (4*S*)-hydroxypyrrolidine derivative, which was subjected to *O*-methylation using MeI and NaH to afford **27e**. The (4*S*)-hydroxypyrrolidine derivative was also converted to **27k** *via* triflation followed by S_N2 displacement with dimethylamine (Me_2NH). In an analogous manner, **27b** was readily converted to **27l**. The *N*-protective groups of **27a**—**n** were removed according to standard deprotection protocols to furnish **11a**—**n**.

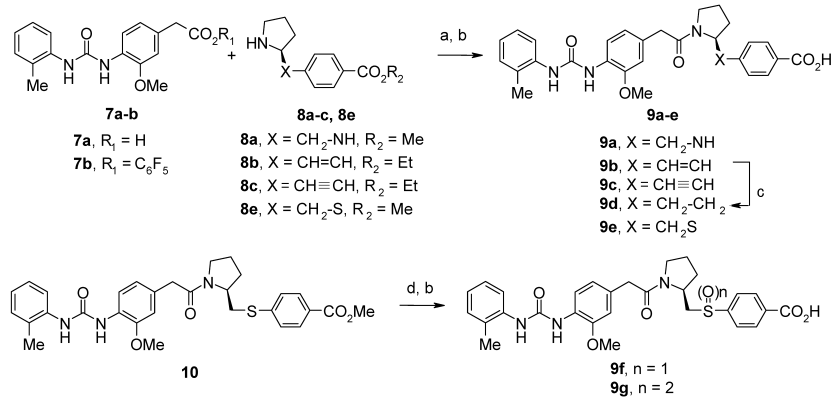
Compounds **14a**—**g**, which have substituents on the benzene ring of the benzoic acid portion, were synthesized as shown in Chart 6. Compound **22** was coupled with 4-bromo-3-methylphenol, 4-iodo-2-methylphenol, and 3,5-dimethyl-4-iodophenol by means of Mitsunobu reaction to give **28a**—**c**, respectively. The methoxycarbonylation was carried out by treatment of **28a**—**c** with *n*-BuLi and methyl chloroformate or $Pd(OAc)_2$ in MeOH under CO atmosphere to afford **29a**—**c**, which were followed by deprotection of the Boc group to afford **14a**—**c**, respectively. Additionally, *N*-Boc-L-prolinol (**22**) was also converted to **14d**—**g** using commercially available 3- or 3,5-substituted benzoic acid derivatives in a manner similar to that described for preparation of **11** in Chart 5.

Results and Discussion

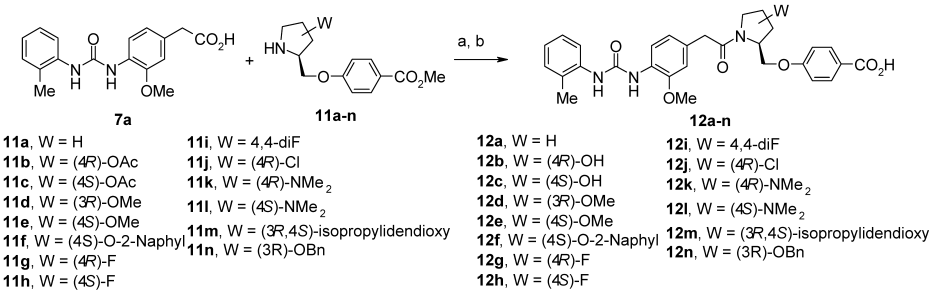
The compounds were evaluated for their VLA-4 inhibitory activities in the receptor binding assay, with or without the addition of 3% human serum albumin (HSA). Additionally, distribution coefficient [Log D, *n*-Octanol/PBS (pH 7.4)] was determined to assess the oral bioavailability. The results are summarized in Tables 1—3.

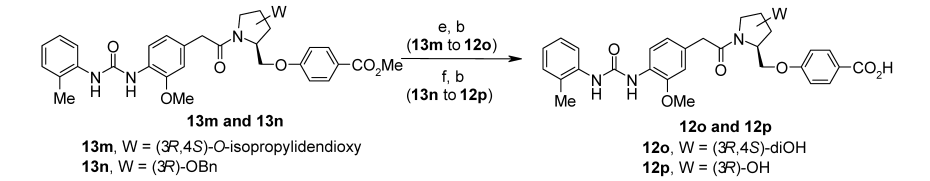
Table 1 shows the results in the modifications of the linker portion (CONH as in **6**) between the pyrrolidine ring and the benzoic acid portion. Almost all the linkers maintained the good activity with IC_{50} values of less than 10 nM. However, the CH_2S linker (**9e**) and the CH_2SO_2 linker (**9g**) slightly de-

Synthetic route A

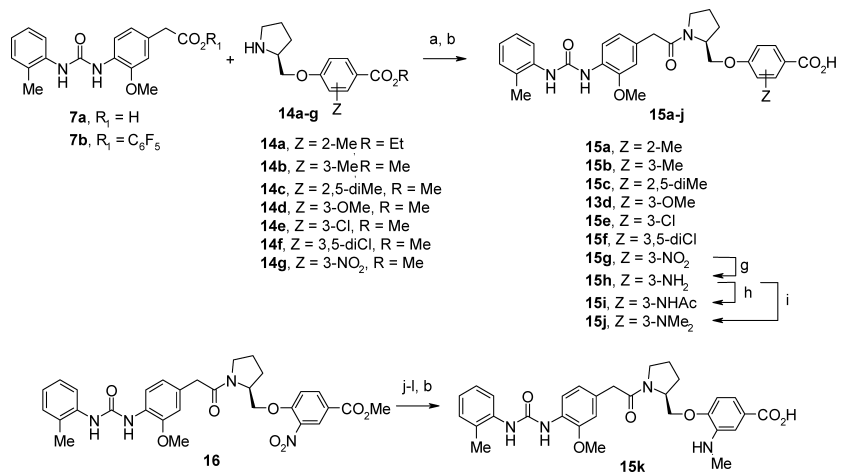


Synthetic route B



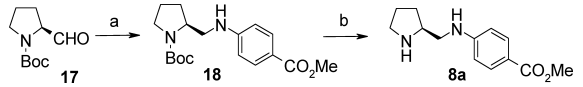


Synthetic route C



Reagents: (a) EDC·HCl, HOBt, DMAP or Et_3N, DMF or CH_2Cl_2; (b) aq. NaOH or LiOH, THF/MeOH; (c) H_2, Pd/C, MeOH; (d) *m*CPBA (1 eq for **9f**, 2.2 eq for **9g**), CH_2Cl_2; (e) g. HCl/MeOH; (f) H_2, Pd $(OH)_2$/C, THF; (g) H_2, Pd/C, MeOH; (h) $(CH_3CO)_2O$, DMAP, pyridine; (i), aq. HCHO, $NaBH_3CN$, HOAc, MeOH; (j) $SnCl_2$, EtOH; (k) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2; (l) MeI, K_2CO_3, DMF.

Chart 1

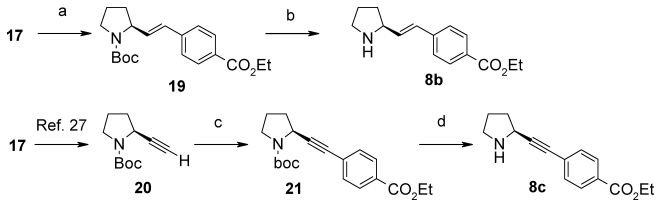


Reagents: (a) methyl 4-aminobenzoate, toluene then $NaBH_3CN$, MeOH, HOAc; (b) TFA, CH_2Cl_2.

Chart 2

creased the activity. These modifications suggest that the amide bond in the liker portion was not necessary to maintain an activity, which SAR was similar to that of piperazine analogue described above. As for the activity in the presence of HSA, the order was nearly the same with that of the activity in the absence of HSA. In addition, considering that the ratio of IC_{50} (−HSA) to IC_{50} (+HSA), it was found that

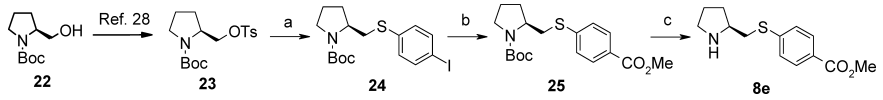
CH_2O linker (**12a**) would less likely to be affected negative by HSA. With regard to the lipophilicity, all compounds measured, except for **9f** with the CH_2SO linker (Log D= −0.4), showed Log D values of more than 1.0, indicating that modification of CONH linker (**6**, Log D=0.68) would increase of the lipophilicity without lack of the activity. On the other hand, **9a** with Log D value of 1.6 showed the ratio value of 2.8, whereas **9f** with Log D value of −0.4 showed the ratio value of 6.3, indicating that the apparent correlation between the ratio and the Log D value was not recognized. We consider that potent compound with IC_{50} value about 1 nM might not be affected in the binding activity in the presence of HSA. From these results, and the relative ease of synthesis, we considered that compounds contained CH_2O linker would be favorable for the further optimization. Thus, next we turned our attention to appending substituents on the pyrrolidine ring and the benzene ring of benzoic acid portion beginning with compound **12a**.



Reagents: (a) ethyl 4-(diethoxyphosphorylmethyl)benzoate, LiHMDS, THF; (b) TFA, CH_2Cl_2; (c) CO, $Pd(PPh_3)_4$, CuI, iPr_2NH; (d) TFA, CH_2Cl_2.

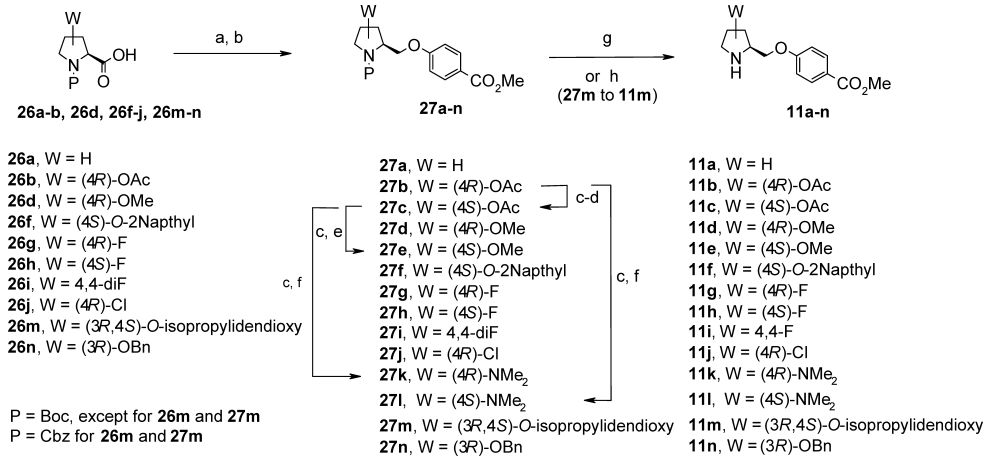
Chart 3

The result of introduction substitutions onto 3, 4, or both positions on the pyrrolidine ring is shown in Table 2. As for the configuration at the 4 position on the pyrrolidine ring, it was found that the S isomers (**12c**, W=OH; **12e**, W=OMe; **12h**, W=F; **12l**, W=NMe_2) bound the receptor about 6 to 7 fold more potent than the corresponding R isomers (**12b**, W=OH; **12d**, W=OMe; **12g**, W=F; **12k**, W=NMe_2). Despite the bulkiness (**12f**, W=2-*O*-naphtyl) of the substituents at the 4 position, there was almost no effect on activity. As for piperazine and piperidine acetic acid derivatives,[11] the configuration, bulkiness, and the nature of substituents at the



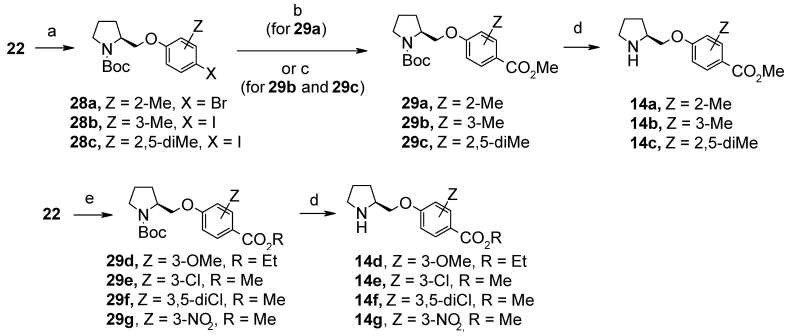
Reagents: (a) 4-iodothiophenol, NaH, THF; (b) CO, $Pd(OAc)_2$, dppp, Et_3N, DMSO/MeOH; (c) TFA, CH_2Cl_2.

Chart 4



Reagents and conditions: (a) $BH_3 \cdot Me_2S$, THF; (b) 4-HOPhCO_2Me, Ph_3P, diisopropyl azodicarboxylate (DIAD), THF; (c) K_2CO_3, MeOH; (d) HOAc, Ph_3P, DIAD, THF; (e) NaH, MeI, DMF; (f) Tf_2O, diisopropylethylamine (DIPEA), THF, then Me_2NH; (g) TFA, CH_2Cl_2; (h) H_2, 10% Pd/C, EtOH.

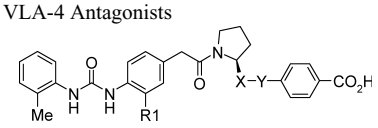
Chart 5



Reagents and conditions: (a) Ph(-X)(-Z)OH, DIAD, PPh_3, THF; (b) *n*-BuLi, TMEDA, methyl chloroformate; (c) $Pd(OAc)_2$, CO, 1,3-bis(diphenylphosphino)propane, Et_3N, DMSO/MeOH; (d) TFA, CH_2Cl_2; (e) 4-HOPh(-Z)CO_2R, DIAD, PPh_3, THF.

Chart 6

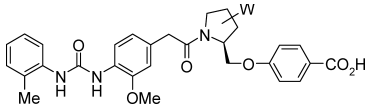
Table 1. Inhibitory Activity and Distribution Coefficient of VLA-4 Antagonists



Compounds	R_1	X–Y	IC_{50} (nM)	IC_{50} (+3% HSA) (nM)	Ratio[a]	Log D[b] (pH 7.4)
6	H	–CO–NH–	3.8	104	27.4	0.68
9a	MeO	$–CH_2–NH–$	1.5	4.2	2.8	1.6
9b	MeO	–CH=CH–	1.7	13	7.6	1.3
9c	MeO	–C≡C–	7.9	140	17.7	1.3
9d	MeO	$–CH_2–CH_2–$	3.7	62	16.7	1.5
9e	MeO	$–CH_2–S–$	13.1	>1000	nd.	nt.
9f	MeO	$–CH_2–SO–$	3.0	19	6.3	−0.4
9g	MeO	$–CH_2–SO_2–$	10.7	583	54.5	nt.
12a	MeO	$–CH_2–O–$	2.4	6.1	2.5	1.5

nd., not detected, nt., not tested. *a*) Ratio=IC_{50} (+3% HSA)/IC_{50}. *b*) Log *D*, *n*-octanol/PBS (pH 7.4).

Table 2. Inhibitory Activity and Distribution Coefficient of VLA-4 Antagonists



Compounds	W	IC_{50} (nM)	IC_{50} (+3% HSA) (nM)	Ratio[a]	Log D[b] (pH 7.4)
6	—	3.8	104	27.4	0.68
12a	H	2.4	6.1	2.5	1.5
12b	4*R*-OH	3.5	9.1	2.6	0.5
12c	4*S*-OH	0.62	2.9	4.7	0.7
12d	4*R*-OMe	9.8	14.0	1.4	1.0
12e	4*S*-OMe	1.4	4.6	3.3	1.2
12f	4*S*-*O*-2-Napthyl	4.2	118	28.1	4.1
12g	4*R*-F	7.9	28	3.5	1.1
12h	4*S*-F	1.2	3.7	3.1	1.1
12i	4,4-diF	2.0	9.1	4.6	1.6
12j	4*R*-Cl	8.2	33	4.0	1.8
12k	4*R*-NMe_2	5.4	4.0	0.74	0.4
12l	4*S*-NMe_2	0.83	1.9	2.3	0.4
12m	3*R*,4*S*-*O*-Isopropylidene	34	373	11.0	nt.
12n	3*S*-OBn	4.2	37	8.8	2.7
12o	3*R*,4*S*-diOH	1.1	3.5	3.2	0
12p	3*S*-OH	1.4	3.2	2.3	0.5

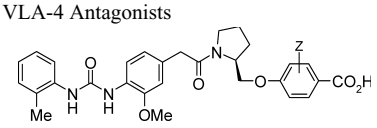
nd., not detected, nt., not tested. *a*) Ratio=IC_{50} (+3% HSA)/IC_{50}. *b*) Log *D*, *n*-octanol/PBS (pH 7.4).

same position on the pyrrolidine ring did not affect the activity. The fact suggests that the SAR of the benzoic acid derivatives would be subtly different from those of both piperazine and piperidine acetic acid derivatives. The substituents [**12p**, W=(3*R*)-OH; **12n**, W=(3*R*)-OBn] at the 3-position on the pyrrolidine ring also maintained activity. Although **12o** [W=(3*R*,4*S*)-dihydroxypyrrolidine] showed good activity, the shielding of the hydroxyl groups of **12m** by isopropylidene ketal was 30-fold loss of activity compared with **12o**. As for lipophilicity, **12f** [W=(4*S*)-*O*-2-naphtyl] had a Log *D* of 4.1, **12i** (W=4,4-difluoro) a Log *D* of 1.6, **12j** [W=(4*R*)-chloro] a Log *D* of 1.8, and **12n** [W=(3*S*)-OBn] a Log *D* of 2.7. However, the most lipophilic compound **12f** had significantly decreased activity in the presence of HSA (IC_{50}= 118 nM, ratio=28.1).

The results of introduction of several substitutes into the benzoic acid portion in **12a** are shown in Table 3. The substituents, including methyl (**15b**), methoxy (**15d**), chloro (**15e**), nitro (**15g**), amino (**15h**), methylamino (**15k**), dimethylamino (**15i**), and acetylamino (**15j**), were tolerated at 3 position on the benzene ring. However, 2-methyl (**15a**) and 3,5-dichloro (**15f**) substituents decreased binding affinity, with IC_{50} values of 6.0 nM for **15a** and 47 nM for **15f**. Unexpectedly, the introduction of 2,5-dimethyl substituents (**15c**) caused a loss of activity at 1 μM. Among these compounds, **15b** and **15e** showed higher Log *D* values (**15b**, 2.0; **15e**, 1.8) than **12a**, while the activities in the presence of HSA were relatively maintained.

On the basis of activity and Log *D* value, **15b** and **15e** were selected for *in vivo* testing of VLA-4 inhibitory activity. These compounds were evaluated for *Ascaris*-antigen sensitized murine airway inflammation model by an oral dose of 30 mg/kg/b.i.d. for 2 d (Fig. 2). The result showed that **15e** inhibited eosinophil infiltration into bronchial alveolar lavage (BAL) fluid by 72% compared with the vehicle alone. Compound **15b**, however, only weakly inhibited (30%) eosinophil

Table 3. Inhibitory Activity and Distribution Coefficient of VLA-4 Antagonists



Compounds	Z	IC_{50} (nM)	IC_{50} (+3% HSA) (nM)	Ratio[a]	Log D[b] (pH 7.4)
6	—	3.8	104	27.4	0.68
12a	H	2.4	6.1	2.5	1.5
15a	2-Me	6.0	118	19.7	1.8
15b	3-Me	1.6	16	10.0	2.0
15c	2,5-diMe	>1000	nd.	nt.	nt.
15d	3-MeO	1.3	nt.	nt.	1.1
15e	3-Cl	1.6	37	23.1	1.8
15f	3,5-diCl	47	nt.	nt.	nt.
15g	3-NO_2	1.4	nt.	nt.	0.7
15h	3-NH_2	1.9	3	1.6	0.5
15i	3-NMe_2	1.4	nt.	nt.	1.2
15j	3-NHAc	1.1	nt.	nt.	0
15k	3-NHMe	1.8	11	6.1	1.2

nd., not detected, nt., not tested. *a*) Ratio=IC_{50} (+3% HSA)/IC_{50}. *b*) Log *D*, *n*-octanol/PBS (pH 7.4).

Table 4. Pharmacokinetic Properties of **15e** in Rats

Compounds	i.v.					*p.o.*		
	$AUC_{(0—inf.)}$[c] (ng·h/ml)	CL (ml/min/kg)	MRT (min)	$t1/2\lambda1$ (min)	$t1/2\lambda2$ (min)	$AUC_{(0—inf.)}$[c] (ng·h/ml)	CL/F (ml/min/kg)	F[d] (%)
1[a]	398	69	15.5	6.8	42.2	nd.	nd.	<1
15e[b]	402	59	13.9	2.9	85.9	20	1670	5

nd., not detected. *a*) Male Sprague–Dawley rats. Dose: i.v. infusion (2 h) at 2.4 mg/kg; *p.o.* at 5 mg/kg. *b*) Male Sprague–Dawley rats. Dose: i.v. infusion (2 h) at 1.5 mg/kg; *p.o.* at 2 mg/kg. *c*) AUC were normalized to 1.5 mg/kg. *d*) F was calculated by dividing CL i.v. with CL/F.

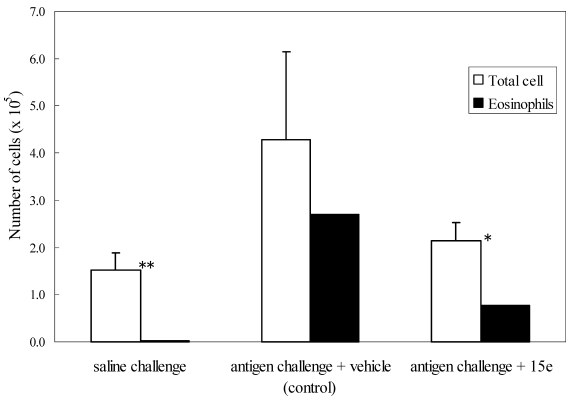


Fig. 2. Effect of VLA-4/VCAM-1 Antagonist **15e** on Leukocyte Infiltration into BAL Fluid 48 h after Allergen Challenge in *Ascaris suum* Sensitized Mice

The compound was administered at a dosage at 30 mg/kg/b.i.d. for 2 d. $*p<0.05$, $**p<0.01$ *vs.* control (Aspin–Welch test). The total number of cells in BAL fluid isolates were counted separately. Eosinophil numbers are expressed as the mean from each treatment group ($n=7$).

infiltration in mice (data not shown).

Next, the pharmacokinetic of **15e** in rats was evaluated to determine whether this compound attained any improvement over the initial lead compound **1**. Compound **15e** showed low bioavailability $F=5\%$, which indicated a slight improvement compared with **1** ($F<1\%$) (Table 4). In order to evaluate the causes of the difference in oral absorption, **15e** was assessed for the cell permeability using Madin–Darby canine kidney (MDCK) cell monolayers. As a result, **15e** demonstrated P_{aap} values of 0.78×10^{-6} cm/s, indicating that **15e** is about 40-fold more permeable than **1**. In comparison, the P_{app} value of metoprolol, which is known to show good oral availability in humans is $17.3—20.3\times10^{-6}$ cm/s. These results suggest that compound needs to enhance its permeability. As for the plasma clearance following i.v. administration, there was no difference between **15e** (Cl=59 ml/min/kg) and **1** (Cl=69 ml/min/kg), disappointingly.

Conclusion

A novel series of benzoic acid derivatives were explored to obtain VLA-4 antagonists with good pharmacokinetic profiles. We focused on increasing the lipophilicity of lead compounds to enhance the cell permeability while maintaining potent VLA-4 inhibitory activity. Thus, modification of the linker portion between the pyrrolidine ring and benzoic acid, and introduction of a variety of substituents into the pyrrolidine ring and benzene ring of benzoic acid as in **6** were conducted. These modifications yielded several compounds with potent activity even in the presence of HSA and moderate lipophilicity. Among them, compound **15e** demonstrated efficacy in an *in vivo* model of asthma by an oral dose of 30 mg/kg. However, this compound did not show a significant improvement in oral pharmacokinetic profile compared with the initial lead **1**. Further improvements and structural modifications of this series of compounds will be presented in forthcoming publications.

Experimental

Melting points were determined on a MP-J3 (YANACO) and were uncorrected. Column chromatography was performed with silica gel 60 (Merck, particle size 0.060—0.200 or 0.040—0.063). Flash column chromatography was performed using a Biotage Si (Biotage) or a Hi-Flash (YAMAZEN). Thin-layer chromatography (TLC) was performed on pre-coated TLC glass sheets with silica gel 60 F_{254} (Merck). ^{1}H-NMR spectra were recorded on a JNM-EX-400 spectrometer (JEOL), and chemical shifts are given in ppm (δ) from tetramethylsilane as the internal standard. IR spectra were recorded on a 270-30 spectrometer (HITACHI). ESI mass spectra were recorded on an API-150EX spectrometer (SCIEX). FAB mass spectra were recorded on a JMS-HX110 spectrometer (JEOL). HR-FAB mass spectra were recorded on a JMS-700 spectrometer (JEOL).

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylaminobenzoic Acid (9a) (General Procedure A) A mixture of methyl 4-[(2*S*)-pyrrolidinyl]methylaminobenzoate (**6a**) (398 mg, 1.69 mmol), 3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetic acid (**7a**) (587 mg, 1.87 mmol), EDC·HCl (486 mg, 2.54 mmol), HOBt (23 mg, 0.17 mmol), and DMAP (21 mg, 0.17 mmol) in DMF (4 ml) was stirred at room temperature for 12 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with water, brine, and then dried over Na_2SO_4. After the solvent was removed under reduced pressure, the residue was purified by chromatography on silica gel with $CHCl_3$/MeOH (50/1, v/v) as an eluent to give methyl 4-[1-[3-methoxy-4-[*N*-(2*S*)-methylphenyl]ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylaminobenzoate (882 mg, 1.66 mmol) as a brownish amorphous solid.

To a stirred solution of methyl 4-[1-[3-methoxy-4-[*N*-(2*S*)-methylphenyl]ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylaminobenzoate (882 mg, 1.66 mmol) in THF/MeOH (18/5 ml) was added 1 M NaOH (5.0 ml, 5.0 mmol), and refluxed for 3 d. After cooling to room temperature, the mixture was concentrated *in vacuo*. The residue was diluted with 1 M HCl and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated. The resulting solid was recrystallized from *n*-hexane/diisopropyl ether/$CHCl_3$/MeOH to afford compound **9a** (181 mg, 21%) as a pale yellow solid. IR (KBr) 1604, 1535, 1511, 1454, 1255, 1224, 1174 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.79—1.99 (4H, m), 2.25 (3H, s), 2.90—2.94 (1H, m), 3.35—3.62 (6H, m), 3.87 (3H, s), 4.12—4.15 (1H, s), 6.63—6.78 (4H, m), 6.89—7.17 (3H, m), 7.65 (2H, d, J=8.3 Hz), 7.80 (1H, d, J=8.3 Hz), 8.02 (1H, d, J=8.3 Hz), 8.47 (1H, s), 8.57 (1H, s), 12.0 (1H, s); FAB-MS *m/z*: 517 (M^++1); *Anal.* Calcd for $C_{29}H_{32}N_4O_5\cdot H_2O$: C, 65.15; H, 6.41; N, 10.48. Found: C, 65.45; H, 6.33; N, 10.02.

Compounds **9b**, **c**, **12a**—**n**, and **15g** were prepared according to general procedure A.

4-[2-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]ethenyl]benzoic Acid (9b) Colorless crystalline material (yield, 92%); IR (ATR) 3338, 2976, 2943, 2879, 1701, 1589, 1527, 1485 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.78—2.13 (4H, m), 2.50 (3H, s), 3.44—3.68 (4H, m), 3.75 and 3.82 (total 3H, each s, amide isomers), 4.71 (1H, m), 6.26—8.59 (15H, m); FAB-MS *m/z*: 514 (M^++1); FAB-MS (HR) *m/z*: 514.2308 (Calcd for $C_{30}H_{32}N_3O_5$: 514.2342)

4-[2-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]ethynyl]phenylacetic Acid (9c) Colorless crystalline powder (yield, 24%) recrystallized from $CHCl_3$/*n*-hexane; mp 161—164 °C; IR (ATR) 3346, 2979, 2939, 2877, 1691, 1603, 1529, 1485 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.96—2.10 (4H, m), 2.24 (3H, s), 3.32—3.81 (2H, m), 3.62 (2H, s), 3.81 (3H, s), 4.87—5.10 (1H, m), 6.76—8.58 (13H, m); FAB-MS *m/z*: 512 (M^++1); FAB-MS (HR) *m/z*: 512.2181 (Calcd for $C_{30}H_{30}N_3O_5$: 512.2185).

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacethyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12a) Colorless amorphous solid (yield, 40%); IR (KBr) 3348, 2945, 2879, 1680, 1603, 1529, 1485, 1452, 1415, 1300, 1248 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.83—2.14 (4H, m), 2.21 (3H, s), 2.46 (2H, s), 3.78 (3H, s), 3.95—4.02 (1H, m), 4.13—4.16 (1H, m), 4.24 (1H, s), 6.51—7.98 (11H, m), 8.43 (1H, s), 8.53 (1H, s), 12.57 (1H, s); FAB-MS *m/z*: 518 (M^++1); FAB-MS (HR) *m/z*: 518.2254 (Calcd for $C_{29}H_{32}N_3O_6$: 518.2291).

4-[(4*R*)-Hydroxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12b) Colorless crystalline powder (yield, 34%) recrystallized from $CHCl_3$/^{i}Pr_2O; mp 178—182 °C; IR (ATR) 3313, 1671, 1651, 1603, 1556, 1452, 1431, 1417 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.92—2.10 (2H, m), 2.23 (3H, s), 3.40—3.44 (1H, m), 3.56—3.67 (3H, m), 3.78 (3H, s), 4.05—4.39 (4H, m), 5.04—5.08 (1H, m), 6.71—7.01 (5H, m), 7.10—7.16 (2H, m), 7.77—7.79 (1H, m), 7.85—7.89 (2H, m), 7.98—8.00 (1H, m), 8.45 (1H, s), 8.54 (1H, s), 12.59 (1H, s); FAB-MS *m/z*: 534 (M^++1); *Anal.* Calcd for $C_{29}H_{31}N_3O_7\cdot 0.5H_2O$: C, 64.20; H, 5.94; N, 7.74. Found: C, 64.35; H, 5.83; N, 7.68.

4-[(4*S*)-Hydroxy-1-[4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12c) Colorless powder (yield, 11%) crystallized from $CHCl_3$/EtOH/ether; mp 148—152 °C (dec.); IR (KBr) 3356, 2939, 1687, 1604, 1533, 1454, 1255 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.95—2.09 (2H, m), 2.25 (3H, s), 3.59 (2H, d, J=5.9 Hz), 3.69—3.73 (1H, m), 3.81 and 3.85 (total 3H, each s, amide isomers), 4.13—4.47 (4H, m), 5.19 (1H, s), 6.70—7.21 (7H, m), 7.79 (1H, d, J=7.9 Hz), 7.86 (2H, d, J=8.8 Hz), 8.01 (1H, d, J=8.3 Hz), 8.47 (1H, s), 8.57 (1H, s); ESI-MS *m/z*: 533 (M^++1); *Anal.* Calcd for $C_{29}H_{31}N_3O_7\cdot H_2O$: C, 63.15; H, 6.03; N, 7.62. Found: C, 63.29; H, 5.76; N, 7.46.

4-[(4*R*)-Methoxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12d) Colorless crystalline powder (yield, 28%) crystallized from $CHCl_3$/MeOH/^{i}Pr_2O; mp 112—115 °C; IR (ATR) 3348, 2937, 1703, 1682, 1603, 1529, 1485, 1454 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.04—2.17 (2H, m), 2.25 (3H, s), 3.21 (3H, s), 3.56—3.75 (4H, m), 3.79 (3H, s), 4.04—4.35 (4H, m), 6.73—7.17 (7H, m), 7.79—7.81 (1H, m), 7.87—7.89 (2H, m), 7.99—8.01 (1H, m), 8.47 (1H, s), 8.55 (1H, s), 12.63 (1H, s); FAB-MS *m/z*: 548 (M^++1); *Anal.* Calcd for $C_{30}H_{33}N_3O_7\cdot 0.25H_2O$: C, 65.26; H, 6.12; N, 7.61. Found: C, 65.36; H, 6.45; N, 7.24.

4-[(4*S*)-Methoxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12e) Colorless amorphous solid (yield, 83%); IR (KBr) 3354, 2937, 1709, 1685, 1604, 1533, 1454 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.09—2.23 (2H, m), 2.25 (3H, s), 3.22 (3H, s), 3.49—3.78 (4H, m), 3.82 and 3.83 (total 3H, each s, amide isomers), 3.87—4.52 (3H, m), 6.71—7.17 (5H, m), 7.79 (1H, d, J=8.1 Hz), 7.86—8.03 (3H, m), 8.45—8.57 (2H, m), 12.64 (1H, s); FAB-MS *m/z*: 548 (M^++1); FAB-MS (HR) *m/z*: 548.2425 (Calcd for $C_{30}H_{34}N_3O_7$: 548.2397).

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12f) Colorless amorphous solid (yield, 32%); IR (KBr) 3354, 2937, 1685, 1601, 1533, 1255 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.24 (3H, s), 2.25—2.43 (2H, m), 3.65 (2H, s), 3.81 (3H, s), 3.81—3.85 (1H, m), 4.05—4.70 (4H, m), 5.21—5.33 (1H, s), 6.76 (1H, d, J=7.3 Hz), 6.86—7.35 (9H, m), 7.44 (1H, t, J=7.3 Hz), 7.76—7.89 (6H, m), 8.01 (1H, d, J=8.3 Hz), 8.48 (1H, s), 8.56 (1H, s); FAB-MS *m/z*: 660 (M^++1); FAB-MS (HR) *m/z*: 660.2715 (Calcd for $C_{39}H_{38}N_3O_7$: 660.2710).

4-[(4*R*)-Fluoro-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12g) Pale yellow amorphous solid (yield, 70%); IR (KBr) 3354, 3060, 2972, 2937, 2877, 1707, 1685, 1604, 1533 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.24—2.41 (5H, m), 3.42—4.66 (10H, m), 5.31 and 5.44 (total 1H, each s, amide isomers), 6.71—7.16 (7H, m), 7.79 (1H, d, J=8.1 Hz), 7.85—7.89 (2H, m), 7.98—8.00 (1H, m), 8.47 (1H, s), 8.55 (1H, s); FAB-MS *m/z*: 536 (M^++1); FAB-MS (HR) *m/z*: 536.2231 (Calcd for $C_{29}H_{31}FN_3O_6$: 536.2197).

4-[(4*S*)-Fluoro-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12h) Pale yellow amorphous solid (yield, 30%); IR (KBr) 3338, 3060, 2970, 2937, 2875, 1709, 1685, 1604, 1539, 1485, 1454 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.25—2.51 (5H, m), 3.33—4.41 (10H, m), 5.30—5.50 (1H, m), 6.75—7.17 (7H, m), 7.79 (1H, d, J=8.1 Hz), 7.87—8.04 (3H, m), 8.46—8.50 (1H, m), 8.56—8.60 (1H, m); FAB-MS *m/z*: 536 (M^++1); FAB-MS (HR) *m/z*: 536.2174 (Calcd for $C_{29}H_{31}FN_3O_6$: 536.2197).

4-[4,4-Difluoro-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoate (12i) Colorless crystalline powder (yield, 61%); mp 135—140 °C; IR (KBr) 3354, 2956, 2935, 1678, 1633, 1604, 1531, 1487, 1454 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.23 (3H, s), 2.49—2.73 (2H, m), 3.36—4.55 (10H, m), 6.73 (1H, d, J=8.3 Hz), 6.84 (1H, s), 6.93 (1H, t, J=7.3 Hz), 7.00 (2H, d, J=8.3 Hz), 7.10—7.16 (2H, m), 7.78 (1H, d, J=8.3 Hz), 7.86 (2H, d, J=8.3 Hz), 8.00 (1H, d, J=8.3 Hz), 8.47 (1H, s), 8.56 (1H, s); FAB-MS *m/z*: 554 (M^++1); *Anal.* Calcd for $C_{29}H_{29}F_2N_3O_6\cdot 0.75H_2O$: C, 61.42; H, 5.44; N, 7.06. Found: C, 61.30; H, 5.44; N, 7.06.

4-[(4*R*)-Chloro-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12j) Colorless amorphous solid (yield, 53%); mp 128—132 °C; IR (ATR) 3346, 1684, 1603, 1529, 1454, 1417, 1338 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.25 (3H, s), 2.29—2.46 (2H, m), 3.57—3.73 (2H, m), 3.78 (3H, s), 3.81—3.99 (2H, m), 4.11—4.31 (2H, m), 4.43—4.67 (1H, m), 4.83—4.85 (1H, m), 6.71—7.17 (7H, m), 7.78—7.80 (1H, m), 7.87—7.91 (2H, m), 7.99—8.01 (1H, m), 8.47 (1H, s), 8.56 (1H, s), 12.66 (1H, s); FAB-MS *m/z*: 552 (M^++1); *Anal.* Calcd for $C_{29}H_{30}ClN_3O_6\cdot 0.75H_2O$: C, 61.59; H, 5.61; Cl, 6.27; N, 7.43. Found: C,

61.56; H, 5.51; Cl, 6.68; N, 7.26.

4-[(4*R*)-Dimethylamino-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]-phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12k) Colorless crystalline solid (yield, 68%); mp 145—150 °C; IR (KBr) 3355, 2948, 1698, 1604, 1533, 1454, 1417, 1255, 1226, 1166, 1035, 755 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.82—1.98 (1H, m), 2.08—2.11 (1H, m), 2.20 (6H, s), 2.25 (3H, s), 3.40—3.60 (3H, m), 3.64 (2H, s), 3.82 (3H, s), 4.01—4.16 (2H, m), 4.34—4.38 (1H, m), 6.74—7.15 (7H, m), 7.77—8.02 (4H, m), 8.44 (1H, s), 8.54 (1H, s); FAB-MS *m/z*: 561 (M^++1); *Anal.* Calcd for $C_{31}H_{36}N_4O_6\cdot 1.2H_2O$: C, 63.95; H, 6.65; N, 9.62. Found: C, 63.82; H, 6.72; N, 9.44.

4-[(4*S*)-Dimethylamino-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]-phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12l) Colorless crystalline solid (yield, 65%); mp 147—150 °C; IR (KBr) 3353, 2952, 1700, 1604, 1533, 1454, 1415, 1255, 1166, 1035, 755 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.83—1.84 (1H, m), 2.08—2.10 (1H, m), 2.21 (6H, s), 2.24 (3H, s), 2.98—3.02 (2H, m), 3.60 (2H, s), 3.78 (3H, s), 3.85—4.29 (4H, m), 6.71—7.16 (7H, m), 7.77—8.01 (4H, m), 8.46 (1H, s), 8.54 (1H, s); FAB-MS *m/z*: 561 (M+H)$^+$; *Anal.* Calcd for $C_{31}H_{36}N_4O_6\cdot 2H_2O$: C, 62.40; H, 6.76; N, 9.39. Found: C, 62.51; H, 6.60; N, 9.36.

4-[(3*R*,4*S*)-Isopropylidenedioxy-1-[4-[*N*-(2-methylphenyl)ureido]-3-methoxyphenylacetyl]-(2*R*)-pyrrolidinyl]methoxybenzoic Acid (12m) Colorless amorphous solid (yield, 98%); IR (KBr) 3354, 2983, 2937, 1707, 1604, 1533 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.24 and 1.32 (total 3H, each s, amide isomers), 1.26 (3H, s), 2.24 (3H, s), 3.40 (1H, dd, *J*=14.0, 5.1 Hz), 3.58—3.62 (2H, m), 3.69—3.71 (1H, m), 3.76 and 3.82 (total 3H, each s, amide isomers), 3.92—4.96 (5H, m), 6.74 and 6.78 (total 1H, each d, *J*=8.3, 8.6 Hz respectively, amide isomers), 6.83—7.16 (6H, m), 7.79 (1H, d, *J*=8.3 Hz), 7.87 (2H, t, *J*=9.1 Hz), 7.99—8.03 (1H, m), 8.49 (1H, d, *J*=3.4 Hz), 8.57 (1H, s); FAB-MS *m/z*: 590 (M^++1); FAB-MS (HR) *m/z*: 590.2458 (Calcd for $C_{32}H_{36}N_3O_8$: 590.2502).

4-[(3*R*)-Benzyloxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12n) Colorless amorphous solid (yield, 42%); IR (ATR) 3344, 2947, 2875, 1684, 1603, 1529, 1454, 1417 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.11—2.25 (1H, m), 2.26—2.30 (1H, m), 2.30 (3H, s), 3.58 (3H, s), 3.62 (2H, s), 3.65—3.72 (2H, m), 4.13 (1H, dd, *J*=8.3, 3.9 Hz), 4.18 (1H, d, *J*=3.9 Hz), 4.25 (1H, d, *J*=8.3 Hz), 4.48 (1H, d, *J*=12.0 Hz), 4.54—4.57 (2H, m), 6.71 (1H, s), 6.75 (1H, d, *J*=7.8 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.15—7.33 (10H, m), 7.47 (1H, d, *J*=7.8 Hz), 7.99 (2H, d, *J*=8.8 Hz), 8.02 (1H, d, *J*=8.3 Hz); ESI-MS *m/z*: 624 (M^++1); *Anal.* Calcd for $C_{36}H_{38}N_3O_7\cdot 0.5H_2O$: C, 68.34; H, 6.05; N, 6.64. Found: C, 68.38; H, 6.31; N, 6.32.

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacethyl]-(2*S*)-pyrrolidinylmethoxy]-3-nitrobenzoic Acid (15g) Yellow crystalline solid (yield, 63%); IR (ATR) 3344, 2974, 2937, 2881, 1697, 1612, 1527, 1485, 1452 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.91—2.09 (4H, m), 2.28 (3H, s), 3.54—3.62 (4H, m), 3.64 (3H, s), 4.15 and 4.59 (total 1H, each d, each *J*=7.8 Hz, amide isomers), 4.41—4.51 (1H, m), 6.66 (1H, s,), 6.72 (1H, d, *J*=8.3 Hz), 7.11—7.28 (5H, m), 7.46 (1H, d, *J*=7.8 Hz), 7.74 (1H, d, *J*=7.8 Hz), 7.85 (1H, s), 8.17 (1H, dd, *J*=2.0, 8.8 Hz), 8.48 (1H, d, *J*=2.4 Hz); FAB-MS *m/z*: 563; FAB-MS (HR) Calcd for $C_{29}H_{31}N_4O_8$: 563.2142. Found: 563.2150.

Compounds **9e** and **15a**—**f** were prepared according to general procedure A, but with **5b** in place of **7a**.

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylthiobenzoic Acid (9e) Colorless crystalline powder (yield, 51%) recrystallized from *n*-hexane/EtOAc/MeOH; mp 161—164 °C; IR (KBr) 3318, 2952, 1596, 1536, 1299, 1155 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.82—2.05 (4H, m), 2.25 (3H, s), 2.91 (1H, dd, *J*=9.8, 13.2 Hz), 3.47—3.52 (3H, m), 3.57 (2H, s), 3.87 (3H, s), 4.14 (1H, m), 6.76 (1H, dd, *J*=1.5, 8.3 Hz), 6.89 (1H, d, *J*=1.5 Hz), 6.94 (1H, t, *J*=7.3 Hz), 7.11—7.19 (2H, m), 7.57 (2H, d, *J*=8.3 Hz), 7.80 (1H, d, *J*=8.3 Hz), 7.83 (2H, d, *J*=8.3 Hz), 8.02 (1H, d, *J*=8.3 Hz), 8.49 (1H, s), 8.58 (1H, s); FAB-MS *m/z*: 534 (M^++1); *Anal.* Calcd for $C_{29}H_{31}N_3O_5S\cdot 1.25H_2O$: C, 62.63; H, 6.07; N, 7.36; S, 5.77. Found: C, 62.62; H, 5.74; N, 7.36; S, 5.67.

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxy-2-methylbenzoic Acid (15a) Colorless crystalline powder (yield, 32%) recrystallized from EtOAc/$CHCl_3$/diisopropylether; mp 106—109 °C; IR (KBr) 1708, 1602, 1531, 1245 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.84—2.03 (4H, m), 2.22 (3H, s), 3.47—3.59 (7H, m), 3.75—3.79 (1H, m), 3.80 (3H, s), 3.94—3.98 (1H, m), 4.11—4.26 (1H, m), 6.72 (1H, d, *J*=8.3 Hz), 6.80—6.89 (3H, m), 6.91 (1H, t, *J*=7.8 Hz), 7.11 (1H, t, *J*=7.8 Hz), 7.14 (1H, d, *J*=7.8 Hz), 7.79 (2H, t, *J*=8.8 Hz), 7.98 (1H, dd, *J*=2.0, 7.8 Hz), 8.44 (1H, s), 8.54 (1H, s), 12.41 (1H, s); FAB-MS *m/z*: 514 (M^++1); *Anal.* Calcd for $C_{30}H_{33}N_3O_6\cdot 0.5H_2O$: C, 66.65; H, 6.34; N, 7.77. Found: C, 66.83; H, 6.47; N, 7.42.

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxy-3-methylbenzoic Acid (15b) Colorless crystalline powder (yield, 54%) recrystallized from *n*-hexane/EtOAc/$CHCl_3$/MeOH; IR (ATR) 3354, 2956, 2875, 1682, 1604, 1529, 1452 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.87—2.10 (4H, m), 2.12 (3H, s), 2.25 (3H, s), 3.51—3.71 (4H, m), 3.76 (3H, s), 4.08—4.18 (2H, m), 4.32—4.36 (1H, m), 6.74 (1H, dd, *J*=1.5, 9.8 Hz), 6.84 (1H, d, *J*=1.5 Hz), 6.94 (1H, t, *J*=6.8 Hz), 7.06 (1H, d, *J*=8.8 Hz), 7.12 (1H, d, *J*=7.8 Hz), 7.16 (1H, d, *J*=7.8 Hz), 7.72 (1H, s), 7.76 (1H, dd, *J*=2.0, 8.3 Hz), 7.79 (1H, d, *J*=7.8 Hz), 7.99 (1H, d, *J*=8.3 Hz), 8.46 (1H, s), 8.54 (1H, s); FAB-MS *m/z*: 532 (M^++1); FAB-MS (HR) *m/z*: 532.2433 (Calcd for $C_{30}H_{34}N_3O_6$: 532.2448).

2,5-Dimethyl-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxy]benzoic Acid (15c) Pale brown powder (yield, 18%) recrystallized from EtOAc/$CHCl_3$/diisopropylether; IR (ATR) 3354, 2966, 2877, 1705, 1682, 1599, 1529, 1485 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.86—2.09 (5H, m), 2.06 (3H, s), 2.25 (3H, s), 3.47—3.67 (6H, m), 3.76 (3H, s), 4.05—4.12 (2H, m), 4.30—4.31 (1H, m), 6.51 (1H, s), 6.55 (1H, s), 6.73—6.95 (2H, m), 7.11—7.17 (2H, m), 7.64 (1H, s), 7.79 (1H, d, *J*=7.8 Hz), 7.99 (1H, d, *J*=7.8 Hz), 8.47 (1H, s), 8.55 (1H, s); FAB-MS *m/z*: 546 (M^++1); FAB-MS (HR) *m/z*: 546.2567 (Calcd for $C_{31}H_{36}N_3O_6$: 546.2604).

3-Methoxy-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (15d) Yellow amorphous solid (yield, 56%); IR (KBr) 1707 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.84—2.18 (4H, m), 2.25 (3H, s), 2.49—2.51 (2H, m), 3.29—3.59 (4H, m), 3.80 (3H, s), 3.82 (3H, s), 4.00—4.05 (1H, m), 6.53—8.01 (10H, m), 8.45 (1H, s), 8.54 (1H, s), 12.63 (1H, s); FAB-MS *m/z*: 548 (M^++1); *Anal.* Calcd for $C_{30}H_{33}N_3O_7\cdot 0.75H_2O$: C, 64.22; H, 6.20; N, 7.59. Found: C, 64.14, H, 6.33, N, 6.99.

3-Chloro-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (15e) Colorless crystalline material (yield, 98%) recrystallized from *n*-hexane/EtOAc/$CHCl_3$; IR (ATR) 3346, 2952, 2873, 1685, 1597, 1529, 1500, 1452 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.82—2.24 (4H, m), 2.25 (3H, s), 3.48—3.60 (4H, m), 3.78 (3H, s), 4.16—4.20 (2H, m), 4.29—4.33 (1H, m), 6.74 (1H, dd, *J*=1.5, 8.3 Hz), 6.84 (1H, d, *J*=2.0 Hz), 6.91—6.95 (1H, m), 7.11—7.17 (3H, m), 7.79 (2H, dd, *J*=2.0, 8.3 Hz), 7.85 (1H, d, *J*=2.0 Hz), 7.98 (1H, d, *J*=8.3 Hz), 8.53 (1H, s), 8.58 (1H, s); FAB-MS *m/z*: 552 (M^++1); FAB-MS (HR) *m/z*: 552.1877 (Calcd for $C_{29}H_{31}ClN_3O_6$: 552.1901).

3,5-Dichloro-4-[1-[3-methoxy-4-(*N*-(2-methyl)phenylureido)phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (15f) Colorless crystalline material (yield, 91%) recrystallized from *n*-hexane/MeOH/$CHCl_3$; IR (ATR) 3344, 2958, 2879, 1697, 1612, 1589, 1529, 1452 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.83—2.24 (4H, m), 2.24 (3H, s), 3.50—3.58 (4H, m), 3.84 (3H, s), 3.98—4.05 (1H, m), 4.15 (1H, dd, *J*=2.9, 8.7 Hz), 4.27—4.31 (1H, m), 6.74 (1H, d, *J*=8.3 Hz), 6.87 (1H, s), 6.93 (1H, t, *J*=7.3 Hz), 7.11 (1H, d, *J*=7.8 Hz), 7.16 (1H, d, *J*=8.3 Hz), 7.79 (1H, d, *J*=8.3 Hz), 7.86 (1H, s), 7.87 (1H, d, *J*=9.8 Hz), 7.99 (1H, d, *J*=8.3 Hz), 8.49 (1H, s), 8.58 (1H, s); FAB-MS *m/z*: 586 (M^++1); FAB-MS (HR) *m/z*: 588.1511 (Calcd for $C_{29}H_{30}Cl_2N_3O_6$: 586.1512).

4-[2-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]ethyl]benzoic Acid (9d) A suspension of ethyl 4-[2-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]-ethenyl]benzoic acid (**9b**) (184 mg, 0.358 mmol) and 5% Pd/C (368 mg) in MeOH (5 ml) was hydrogenated under 1 atom hydrogen atmosphere at room temperature for 21 h. After the catalyst was removed by filtration, the filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (4/1 to 3/1, v/v) as an eluent to give the title compound **9d** (123 mg, 66%) as a colorless crystalline solid. IR (ATR) 3345, 2927, 1677, 1589, 1529, 1452, 1414, 1338 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.55—2.03 (6H, m), 2.24 (3H, s), 2.60 (2H, m), 3.17—3.59 (4H, m), 3.83 (3H, s), 3.95—3.99 (1H, m), 6.61—8.57 (13H, m); FAB-MS *m/z*: 516 (M^++1); FAB-MS (HR) *m/z*: 516.2490 (Calcd for $C_{30}H_{34}N_3O_5$: 516.2489).

Methyl 4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylthiobenzoate (10) as an Intermediate of 9e Colorless crystalline powder (yield, 82%). IR (KBr) 1785, 1224, 1216 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.88—1.99 (4H, m), 2.30 (3H, s), 2.75 (1H, dd, *J*=9.8, 13.2 Hz), 3.43—3.55 (3H, m), 3.56 (2H, s), 3.64 (1H, dd, *J*=1.1, 14.2 Hz), 3.73 (3H, s), 3.88 (3H, s), 4.31—4.35 (1H, m), 6.29 (1H, s), 6.78—6.81 (2H, m), 7.11—7.26 (5H, m), 7.50 (3H, d, *J*=8.3 Hz), 7.93 (2H, d, *J*=8.8 Hz), 8.07 (1H, d, *J*=7.8 Hz); FAB-MS *m/z*: 548 (M^++1); *Anal.* Calcd for $C_{30}H_{33}N_3O_5S\cdot 0.25H_2O$: C, 65.26; H, 6.12; N, 7.61. Found: C, 65.48; H, 6.20; N, 7.47.

4-[1-[3-Methoxy-4-[*N*-(2-Methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylsulfinylbenzoic Acid (9f) To a stirred solution of methyl 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylthiobenzoate (**10**) (264 mg, 0.482 mmol) in CH_2Cl_2 (5.2 ml) was added *m*CPBA (119 mg, 0.482 mmol) at 0 °C, and stirred at room temperature for 1 h. The mixture was diluted with $CHCl_3$, and quenched with sat. $Na_2S_2O_3$. The separated organic layer was washed with sat. $NaHCO_3$, brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford methyl 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylsulfinylbenzoate as a colorless amorphous solid.

To a stirred solution of methyl 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)-ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylsulfinylbenzoate in THF/H_2O (4/1 ml) was added LiOH (34.6 mg, 1.45 mmol). After 12 h stirring, the mixture was diluted with $CHCl_3$, washed with 1 M HCl, brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the obtained solid was recrystallized from *n*-hexane/$CHCl_3$/MeOH to afford compound (**9f**) (193 mg, 73%) as a colorless crystalline powder. IR (KBr) 3338, 2956, 1708, 1529, 1299, 1207, 1155 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.70—2.06 (4H, m), 2.24 (3H, s), 2.90 (1H, dd, J=8.3, 13.2 Hz), 3.02—3.08 (1H, m), 3.16—3.25 (1H, m), 3.41—3.60 (3H, m), 3.84 (3H, s), 4.40 (1H, s), 6.74 (1H, d, J=7.8 Hz), 6.87 (1H, s), 6.94 (1H, d, J=7.3 Hz), 7.11—7.17 (2H, m), 7.75—7.81 (3H, m), 7.98—8.05 (1H, m), 8.10 (2H, d, J=8.3 Hz), 8.46 (1H, s), 8.56 (1H, s); FAB-MS m/z: 550 (M^++1), 572 (M^++Na); *Anal.* Calcd for $C_{29}H_{31}N_3O_6S\cdot1.5H_2O$: C, 60.40; H, 5.94; N, 7.29. Found: C, 60.15; H, 5.82; N, 6.90.

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methylsulfonylbenzoic Acid (9g) By the procedure described for compound **9f** using 2.2 eq of *m*CPBA, compound **9g** was obtained as colorless powder (128 mg, 41%) recrystallized from *n*-hexane/EtOAc. IR (KBr) 3388, 2974, 1537, 1298, 1155 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.80—1.98 (4H, m), 2.24 (3H, s), 2.54 (1H, s), 3.20—3.70 (5H, m), 3.82 (3H, s), 4.14—4.18 (1H, m), 6.67 (1H, dd, J=1.5, 8.3 Hz), 6.80 (1H, d, J=1.5 Hz), 6.93 (1H, d, J=7.3 Hz), 7.10—7.16 (2H, m), 7.78 (1H, d, J=7.3 Hz), 7.95 (2H, d, J=8.3 Hz), 7.98 (1H, d, J=8.3 Hz), 8.14 (2H, d, J=8.3 Hz), 8.49 (1H, s), 8.57 (1H, s); FAB-MS m/z: 566 (M^++1); FAB-MS (HR) m/z: 566.1937 (Calcd for $C_{29}H_{32}N_3O_7S$: 566.1961).

Methyl 4-[(3*R*,4*S*)-Isopropylidenedioxy-1-[4-[*N*-(2-methylphenyl)ureido]-3-methoxyphenylacetyl]-(2*R*)-pyrrolidinyl]methoxybenzoate (13m) as an Intermediate of 12m Colorless amorphous solid (yield, 100%); IR (KBr) 3354, 2985, 2939, 1716, 1533, 1254 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, s), 1.42 (3H, s), 2.05 (3H, s), 3.50 (3H, s), 3.55—3.88 (4H, m), 3.89 (3H, s), 4.11—4.15 (1H, m), 4.67 (1H, s), 4.78 (1H, d, J=6.1 Hz), 4.88 (1H, t, J=5.6 Hz), 6.46 (1H, s), 6.62 (1H, d, J=1.5 Hz), 6.72—6.76 (3H, m), 7.05 (1H, s), 7.14 (1H, d, J=7.3 Hz), 7.23 (2H, m), 7.57 (1H, d, J=7.8 Hz), 7.91—8.08 (3H, m); ESI-MS m/z: 604 (M^++1); *Anal.* Calcd for $C_{33}H_{37}N_3O_8\cdot0.6H_2O$: C, 64.50; H, 6.27; N, 6.84. Found: C, 64.38; H, 6.18; N, 6.66.

4-[(3*R*,4*S*)-Dihydroxy-1-[4-[*N*-(2-methylphenyl)ureido]-3-methoxyphenylacetyl]-(2*R*)-pyrrolidinyl]methoxybenzoic Acid (12o) A mixture of methyl 4-[(3*R*,4*S*)-isopropylidenedioxy-1-[4-[*N*′-(2-methylphenyl)ureido]-3-methoxyphenylacetyl]-(2*R*)-pyrrolidinyl]methoxybenzoate (**13m**) (183 mg, 0.303 mmol) and gas.HCl–MeOH (6 ml) was stirred at room temperature for 17 h. The mixture was concentrated *in vacuo*. The residue was purified on TLC [$CHCl_3$/MeOH (10/1, v/v)] to afford methyl 4-[(3*R*,4*S*)-dihydroxy-1-[4-[*N*-(2-methylphenyl)ureido]-3-methoxyphenylacetyl]-(2*R*)-pyrrolidinyl]methoxybenzoate (162 mg, 95%) as a colorless amorphous solid. IR (KBr) 3342, 1716, 1604, 1535, 1255 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.25 (3H, s), 3.33— 3.75 (7H, m), 3.87 (3H, s), 4.10 (1H, d, J=8.3 Hz), 4.24 (2H, s), 4.35—4.39 (2H, m), 6.62—7.94 (13H, m); ESI-MS m/z: 564 (M^++1).

To a solution of methyl 4-[(3*R*,4*S*)-dihydroxy-1-[4-[*N*-(2-methylphenyl)ureido]-3-methoxyphenylacetyl]-(2*R*)-pyrrolidinyl]methoxybenzoate (63 mg, 0.112 mmol) in THF (0.89 ml) was added 0.25 M NaOH (0.89 ml). After stirring at room temperature for 3 d, the mixture was acidified with 1 M HCl and extracted with $CHCl_3$/MeOH (10/1, v/v). The combined extracts were dried over Na_2SO_4 and concentrated *in vacuo*, to give compound **12o** (54 mg, 88%) as a colorless amorphous solid. IR (KBr) 3356, 2958, 2927, 1685, 1604, 1535, 1255 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.24 (3H, s), 3.438—3.42 (1H, m), 3.58 (2H, s), 3.66 (1H, dd, J=9.8, 6.6 Hz), 3.80 (3H, s), 3.99—4.30 (5H, m), 5.10 (1H, s), 6.72 (1H, d, J=8.1 Hz), 6.85 (1H, s), 6.93 (1H, t, J=7.3 Hz), 7.03 (2H, d, J=8.8 Hz), 7.14 (2H, t, J=8.8 Hz), 7.79 (2H, d, J=8.1 Hz), 7.86 (2H, d, J=8.8 Hz), 7.99 (1H, d, J=8.3 Hz), 8.46 (1H, s), 8.56 (1H, s); FAB-MS m/z: 550 (M^++1); FAB-MS (HR) m/z: 550.2188 (Calcd for $C_{29}H_{32}N_3O_8$: 550.2189).

Methyl 4-[(3*R*)-Benzyloxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoate (13n) as an Intermediate of 12n Yellow amorphous solid (yield, 93%); ^{1}H-NMR ($CDCl_3$) δ: 2.13—2.17 (1H, m), 2.22—2.26 (1H, m), 2.29 (3H, s), 3.54—3.58 (1H, m), 3.58 (3H, s), 3.62 (2H, s), 3.70—3.74 (1H, m), 3.88 (3H, s), 4.02 (1H, dd, J=10.0, 7.1 Hz), 4.17 (1H, d, J=4.2 Hz), 4.28 (1H, dd, J=10.0, 3.2 Hz), 4.46 (1H, d, J=12.0 Hz), 4.51—4.55 (1H, m), 4.54 (1H, d, J=12.0 Hz), 6.48 (1H, s), 6.73 (1H, d, J=1.5 Hz), 6.78 (1H, dd, J=8.1, 1.5 Hz), 6.87 (1H, d, J=8.8 Hz), 7.13—7.16 (2H, m), 7.20—7.36 (7H, m), 7.52—7.56 (1H, m), 7.94 (1H, d, J=8.8 Hz), 8.05 (1H, d, J=8.1 Hz); ESI-MS m/z: 638 (M^++1).

4-[(3*R*)-Hydroxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (12p) A suspension of methyl 4-[(3*R*)-benzyloxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]-phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoate (**13n**) (1.85 g, 2.90 mmol) and $Pd(OH)_2$ (1.0 g) in THF (30 ml) was hydrogenated under hydrogen atmosphere at room temperature for 3 h. After removal of the catalyst by filtration, the filtrate was concentrated to afford methyl 4-[(3*R*)-hydroxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]-methoxybenzoate (1.56 g, 98%) as a colorless amorphous solid. ^{1}H-NMR (CD_3OD) δ: 2.07—2.11 (1H, m), 2.29 (3H, s), 2.31—2.35 (1H, m), 3.68 (2H, s), 3.78 (3H, s), 3.72—3.82 (2H, m), 3.84 (3H, s), 4.12 (1H, dd, J=7.4, 4.2 Hz), 4.24—4.30 (2H, m), 4.39—4.43 (1H, m), 6.79 (1H, dd, J=8.0, 2.0 Hz), 6.86 (1H, d, J=1.2 Hz), 6.98 (2H, d, J=8.8 Hz), 7.02 (1H, td, J=7.6, 1.2 Hz), 7.14—7.20 (2H, m), 7.57—7.61 (1H, m), 7.93 (2H, d, J=8.8 Hz), 7.96 (1H, d, J=8.0 Hz); ESI-MS m/z: 548 (M^++1).

To a solution of methyl 4-[(3*R*)-hydroxy-1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoate (214 mg, 0.39 mmol) in THF (4 ml) was added 0.25 M NaOH (2.00 ml, 0.50 mmol) at room temperature. After being stirred for 24 h, the reaction mixture was poured into 1 M HCl and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated to afford the title compound **12p** (160 mg, 77%) as a colorless amorphous solid. IR (KBr) 3345, 2954, 1685, 1604, 1536, 1454, 1417 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.83—1.87 (1H, m), 2.18—2.22 (1H, m), 2.24 (3H, s), 3.54—3.66 (4H, m), 3.82 (3H, s), 3.90 (1H, dd, J=9.8, 8.3 Hz), 4.08 (1H, dd, J=7.8, 1.9 Hz), 4.16 (1H, dd, J=10.0, 3.2 Hz), 4.21—4.25 (1H, m), 5.10 (1H, d, J=3.1 Hz), 6.74 (1H, dd, J=8.3, 1.7 Hz), 6.87 (1H, d, J=1.7 Hz), 6.92 (1H, t, J=7.4 Hz), 7.06 (2H, d, J=8.8 Hz), 7.10—7.16 (2H, m), 7.78 (1H, d, J=7.8 Hz), 7.86 (2H, d, J=8.8 Hz), 8.00 (1H, d, J=8.3 Hz), 8.46 (1H, s), 8.55 (1H, s), 12.60 (1H, s); ESI-MS m/z: 534 (M^++1); *Anal.* Calcd for $C_{29}H_{31}N_3O_7\cdot1.25H_2O$: C, 62.64; H, 6.07; N, 7.56. Found: C, 62.66; H, 5.93; N, 7.17.

3-Amino-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (15h) A suspension of 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacethyl]-(2*S*)-pyrrolidinyl-methoxy]-3-nitrobenzoic acid (**15g**) (101 mg, 0.190 mmol) and 5 % Pd/C (247 mg) in MeOH (5 ml) was hydrogenated under 1 atom hydrogen atmosphere at room temperature for 21 h. After the catalyst was removed by filtration, the filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel with $CHCl_3$/EtOH (1/1, v/v) as an eluent to give compound **15h** (61 mg, 60%) as pale brown crystalline material. IR (ATR) 3346, 2972, 2941, 2875, 1678, 1589, 1529, 1450 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.91—1.99 (4H, m), 2.23 (3H, s), 3.60—4.34 (5H, m), 3.81 (3H, s), 4.88 (2H, m), 6.74 (1H, d, J=8.3 Hz), 6.86—7.28 (5H, m), 7.78 (1H, d, J=7.8 Hz), 7.99 (1H, d, J=8.3 Hz), 8.30 (1H, s), 8.45 and 8.55 (total 1H, each s, amide isomers); FAB-MS m/z: 533; FAB-MS (HR) m/z: 533.3467 (Calcd for $C_{29}H_{33}N_4O_6$: 533.3400).

3-Acetylamino-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (15i) A solution of 3-amino-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic acid (**15h**) (130 mg, 0.244 mmol) and DMAP (2.9 mg, 0.024 mmol) in pyridine/acetic anhydride (5/5 ml) was stirred at room temperature for 2 h. The mixture was poured into water and extracted with $CHCl_3$. The combined extracts were dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by chromatography on silica gel with $CHCl_3$/MeOH (15/1 to 1/1, v/v) as an eluent to afford compound **15i** (29 mg, 21%) as white crystalline material. IR (ATR) 3346, 2924, 2852, 1685, 1589, 1529, 1485, 1450, 1415 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.30 (4H, m), 2.04 (3H, s), 2.26 (3H, s), 3.33 (3H, s), 3.40—4.80 (7H, m), 6.59 (1H, s), 6.74 (1H, d, J=8.3 Hz), 6.79 (1H, d, J=8.8 Hz), 7.07—7.57 (6H, m), 7.75 (1H, d, J=8.8 Hz), 8.07 (1H, d, J=8.3 Hz), 8.41 and 8.96 (total 1H, each s, amide isomers); FAB-MS m/z: 575; FAB-MS (HR) m/z: 575.2532 (Calcd for $C_{31}H_{35}N_4O_7$: 575.2506).

3-*N*,*N*-Dimethylamino-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic Acid (15j) To a solution of 3-amino-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoic acid (**15h**) (67 mg, 0.126 mmol) in MeOH (1.0 ml)/HOAc (0.5 ml), 37% aq. HCHO solution (1.0 ml) and $NaBH_3CN$ (118 mg, 1.88 mmol) was added at room temperature and stirred 4 d. The reaction mixture was diluted with water and extracted with $CHCl_3$/MeOH (10/1, v/v). The combined extracts were washed with sat. NaCl, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by TLC with $CHCl_3$/MeOH (5/1, v/v) to afford compound **15j** (57 mg, 81%) as pale yellow amorphous solid. IR (ATR) 3340, 2943, 2873, 2829, 2781, 1701, 1678, 1589, 1529, 1452 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.87—2.14 (4H, m), 2.24—2.31 (4H, m), 2.67 (6H, s), 3.45—3.67 (4H, m), 3.72—3.87 (4H, m), 3.98—4.52 (2H, m), 6.66—7.20 (6H, m), 7.42 (1H, d, J=2.0 Hz), 7.46—7.56 (1H, m), 7.79 (1H, d, J=7.4 Hz), 7.99 (1H, d, J=8.1 Hz), 8.46 (1H, s), 8.55 (1H, s); FAB-MS *m*/*z*: 561 (M^++1); FAB-MS (HR) *m*/*z*: 561.2740 (Calcd for $C_{31}H_{37}N_4O_6$: 561.2713).

4-[1-[3-Methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxy-3-methylaminobenzoic Acid (15k) A mixture of methyl 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxy-3-nitrobenzoate (**16**) (5.8 g, 10.1 mmol) and $SnCl\cdot 2H_2O$ (8.2 g, 36.3 mmol) in EtOH (80 ml) was refluxed for 1 h. After cooled to room temperature, the reaction mixture was concentrated. The residue was poured into 1 M NaOH at 0 °C, and extracted with $CHCl_3$. The combined extracts were washed with sat. NaCl and dried over Na_2SO_4. After the solvent was removed, the resulting residue was purified by chromatography on silica gel with *n*-hexane/EtOAc (1/1, v/v) as an eluent to afford methyl 3-amino-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoate (3.8 g, 69%) as colorless amorphous solid. IR (KBr) 3458, 3338, 2949, 2877, 2843, 1711, 1620, 1595, 1533, 1485 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.99—2.06 (4H, m), 2.30 (3H, s), 3.46—3.52 (3H, m), 3.55 (3H, s), 3.85 (3H, s), 4.14—4.17 (2H, m), 4.50—4.54 (1H, m), 6.63 (1H, s), 6.76 (1H, s), 6.79 (1H, d, J=2.0 Hz), 6.80 (1H, d, J=8.3 Hz), 7.13 (1H, t, J=7.8 Hz), 7.18—7.25 (3H, m), 7.33 (1H, d, J=2.0 Hz), 7.39 (1H, dd, J=8.3, 2.0 Hz), 7.56 (1H, d, J=8.8 Hz), 8.02 (1H, d, J=7.8 Hz); FAB-MS *m*/*z*: 547 (M^++1).

To a solution of 3-amino-4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]-phenylacetyl]-(2*S*)-pyrrolidinyl]methoxybenzoate (350 mg, 0.64 mmol) and Et_3N (0.134 ml, 0.96 mmol) in CH_2Cl_2 (15 ml) was added trifluoroacetic acid anhydride (0.109 ml, 0.77 ml) at 0 °C. After 1.5 h stirring, the reaction mixture was poured into water and extracted with $CHCl_3$. The combined extracts were washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel with MeOH/$CHCl_3$ (1/100, v/v) as an eluent to afford methyl 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinyl]methoxy-3-trifluoroacetylaminobenzoate (302 mg, 74%) as pale orange amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.97—2.17 (3H, m), 2.30 (3H, s), 3.46—3.60 (8H, m), 3.89 (3H, s), 4.17—4.28 (2H, m), 4.54—4.56 (1H, m), 6.41 (1H, s), 6.73—6.75 (2H, m), 7.00—7.02 (1H, m), 7.08 (1H, s), 7.12—7.16 (1H, m), 7.22—7.24 (2H, m), 8.50—8.52 (1H, m), 7.86—7.89 (1H, m), 7.97—7.99 (1H, m), 8.74 (1H, s), 8.80 (1H, s).

To a solution of methyl 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)-ureido]phenylacetyl]-(2*S*)-pyrrolidinylmethoxy]-3-trifluoroacetylaminobenzoate (289 mg, 0.45 mmol) and K_2CO_3 94 mg (0.68 mmol) in DMF (10 ml) was added MeI (34 μl, 0.78 mmol) at room temperature. After 12 h stirring, the reaction mixture was poured into 1 M HCl and resulting solid were collected the resulting solid, which were diluted with $CHCl_3$ and dried over $MgSO_4$. After the solvent was removed, the residue was purified by chromatography on silica gel with MeOH/$CHCl_3$ (1/100, v/v) as an eluent to afford methyl 4-[1-[3-methoxy-4-[*N*-(2-methylphenyl)ureido]-phenylacetyl]-(2*S*)-pyrrolidinylmethoxy]-3-*N*-trifluoroacetyl-*N*-methylaminobenzoate (229 mg, 78%) as pale brown amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.92—2.07 (4H, m), 2.29 (3H, s), 3.09—3.71 (10H, m), 3.90 (3H, s), 4.14—4.41 (3H, m), 6.36 (1H, s), 6.77—6.79 (2H, m), 7.06—7.25 (5H, m), 7.48—7.51 (1H, m), 7.84—7.90 (1H, m), 8.04—8.06 (2H, m).

To a stirred solution of methyl 4-[1-(3-methoxy-4-(*N*-(2-methylphenyl)-ureido)phenylacetyl)-(2*S*)-pyrrolidinylmethoxy]-3-*N*-trifluoroacetyl-*N*-methylaminobenzoate (223 mg, 0.34 mmol) in THF (5 ml) was added 0.25 M NaOH (4 ml). The resulting mixture was stirred at room temperature for 12 h. The mixture was acidified with 1 M HCl and collected the resulting solid. The resulting solid was diluted with $CHCl_3$ and dried over $MgSO_4$. After the solvent was removed, the residue was recrystallized with $CHCl_3$/*n*-hexane to afford compound **15k** (51 mg, 28%) as a colorless crystalline material. mp 136—140 °C; IR (ATR) 3346, 2947, 2877, 1701, 1678, 1597, 1525, 1483 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 1.90—5.06 (19H, m), 6.71—7.21 (9H, m), 7.78—7.80 (1H, m), 8.00—8.02 (1H, m), 8.46 (1H, s), 8.55 (1H, s), 12.36 (1H, s); FAB-MS *m*/*z*: 547 (M^++1); FAB-MS (HR) *m*/*z*: 547.2572 (Calcd for $C_{30}H_{35}N_4O_6$: 547.2557).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]methylaminobenzoate (18) A mixture of methyl 4-aminobenzoate (1.52 g, 10.04 mmol) and *N*-Boc-L-prolinal (**17**) (3.00 g, 15.1 mmol) in toluene (30 ml) was refluxed with stirring for 3 h. The reaction mixture was cooled to room temperature and dried over Na_2SO_4. After removing the solvent under reduced pressure, the resulting precipitate was diluted with MeOH/HOAc (27/3 ml), then $NaBH_3CN$ (1.33 g, 20.1 mmol) was added and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was poured into water and extracted with EtOAc. The combined extracts were washed with water and brine, dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel with *n*-hexane/EtOAc (3/1, v/v) as an eluent to afford compound **18** (2.17 g, 65%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.51—2.09 (4H, m), 3.05—3.07 and 3.43—3.48 (total 1H, m, amide isomers), 3.18 (1H, s), 3.36 (2H, s), 3.84 (3H, s), 4.06—4.24 (1H, m), 6.49—6.65 (2H, m), 7.84 (2H, d, J=8.3 Hz); FAB-MS *m*/*z*: 335 (M^++1).

Methyl 4-[(2*S*)-Pyrrolidinyl]methylaminobenzoate (8a) (General Procedure B, Boc Removal Procedure) To a stirred solution of methyl 4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinyl]methylamino- benzoate (**18**) (2.17 g, 6.49 mmol) in CH_2Cl_2 (44 ml) was added TFA (9 ml) at 0 °C and stirred at room temperature for 12 h. After the solvent was removed, the residue was diluted with 1 M NaOH and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to afford compound **8a** (1.34 g, 88%) as a brown oil. ^{1}H-NMR ($CDCl_3$) δ: 1.45—1.50 (1H, m), 1.70—1.94 (3H, m), 2.93 (2H, t, J=6.8 Hz), 2.96—3.02 (1H, m), 3.19—3.23 (1H, m), 3.37—3.41 (1H, m), 3.84 (3H, s), 4.73 (1H, s), 6.56 (2H, d, J=8.8 Hz), 7.84 (2H, d, J=8.8 Hz); FAB-MS *m*/*z*: 235 (M^++1).

Ethyl 4-[2-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]-(*E*)-ethenyl]-benzoate (19) To a solution of ethyl 4-(diethoxyphosphorylmethyl)benzoate[26] (904 mg, 3.01 mmol) in THF (20 ml) was added LiHMDS (1.0 M in THF, 3 ml, 3.00 mmol) at −78 °C. After 1 h stirring, *N*-Boc-L-prolinal (**17**) (500 mg, 2.51 mmol) in THF (10 ml) was added to the reaction mixture, and allowed to warm up to room temperature over 1 h. After being stirred for 2 h, the mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (8/1, v/v) as an eluent to give compound **19** (713 mg, 82%) as a colorless crystalline solid. mp 68—70 °C; IR (KBr) 1710, 1697, 1681 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.39 (12H, m), 1.77—1.93 (3H, m), 2.09—2.13 (1H, m), 3.45—3.49 (2H, m), 4.34—4.54 (3H, m), 6.20—6.24 (1H, m), 6.43 (1H, d, J=14.2 Hz), 7.39 (2H, d, J=8.3 Hz), 7.97 (2H, d, J=8.3 Hz); FAB-MS *m*/*z*: 346 (M^++1); *Anal.* Calcd for $C_{20}H_{27}NO_4$: C, 69.54; H, 7.88; N, 4.05. Found: C, 69.52; H, 8.08; N, 4.07.

Ethyl 4-[2-(2-Pyrrolidinyl)-(*E*)-ethenyl]benzoate (8b) According to the general procedure B, **8b** was obtained from **19** as a brown oil (yield, 87%). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.3 Hz), 1.52—2.06 (4H, m), 2.93—2.99 (1H, m), 3.07—3.13 (1H, m), 3.74 (1H, q, J=7.3 Hz), 4.37 (2H, q, J=7.3 Hz), 6.34 (1H, dd, J=15.6, 7.3 Hz), 6.54 (1H, d, J=15.6 Hz), 7.41 (2H, d, J=8.3 Hz), 7.97 (2H, d, J=8.3 Hz).

Ethyl 4-[2-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]ethynyl]phenylacetate (21) A stirred suspension of ethyl 4-iodobenzoate (1.7 ml, 10 mmol), $Pd(PPh_3)_4$ (578 mg, 0.5 mmol), and CuI (190 mmol, 1 mmol) in *i*-Pr_2NH (20 ml) was added a solution of 1-(*tert*-butoxycarbonyl)-(2*S*)-ethynylpyrrolidine (**20**)[27] (1.95 g, 10 mmol) in *i*-Pr_2NH (20 ml) over 10 min at room temperature under an atmosphere of nitrogen. After 3 h stirring, the mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified column chromatography on silica gel with *n*-hexane/EtOAc (10/1, v/v) as an eluent to give compound **21** (2.77 g, 81%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=6.8 Hz), 1.49 (9H, s), 1.85—2.12 (4H, m), 3.37—3.51 (2H, m), 4.37 (2H, q, J=6.8 Hz), 4.54—4.77 (1H, m), 7.44 (2H, d, J=7.8 Hz), 7.96 (2H, d, J=7.8 Hz).

Ethyl 4-[2-[(2*S*)-Pyrrolidinyl]ethynyl]phenylacetate (8c) According to the general procedure B, **8c** was obtained from **21** as a light yellow oil (yield, 100%). ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=6.8 Hz), 1.82—2.16 (4H, m), 3.01—3.48 (2H, m), 4.00—4.11 (1H, m), 4.37 (2H, q, J=6.8 Hz), 4.54—4.77 (1H, m), 7.44—7.46 (2H, m), 7.95—7.97 (2H, m).

1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinylmethyl 4-Iodophenyl Sulfide (24) To a stirred mixture of *p*-iodothiophenol[29] (1.75 g, 7.43 mmol) and 1-

(*tert*-butoxy)-(2*S*)-tosyloxymethylpyrrolidine (**21**)[28] (2.39 g, 6.75 mmol) in pyridine (12.7 ml) was added 8 N KOH (1.27 ml) at room temperature. After 4 h stirring, the reaction mixture was diluted with EtOAc. The solution was washed with H_2O, sat. NH_4Cl, brine, and dried over Na_2SO_4. The organic layer was concentrated under a reduced pressure. The residue was purified column chromatography on silica gel with *n*-hexane/EtOAc (5/1, v/v) as an eluent to afford compound **24** (1.49 g, 53%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.78—2.01 (4H, m), 2.69—2.73 (1H, m), 3.32—3.49 (3H, m), 3.90—4.02 (1H, m), 7.12 (1H, d, J=7.8 Hz), 7.18 (1H, d, J=7.8 Hz), 7.57 (2H, dd, J=2.0, 8.3 Hz); FAB-MS *m/z*: 420 (M^++1).

Methyl 4-[(2*S*)-Pyrrolidinyl]methylthiobenzoate (8e) To a stirred mixture of 1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethyl 4-iodophenyl sulfide (**24**) (1.49 g, 3.56 mmol), Et_3N (1.09 ml, 7.84 mmol), $Pd(OAc)_2$ (40 mg, 0.178 mmol), and 1,3-bis(diphenylphosphino)propane (73.4 mg, 0.178 mmol) in DMSO/MeOH (16/13 ml) was induced CO gas for 5 min, and stirred at 70 °C for 12 h. After cooling to room temperature, the mixture was concentrated to a small volume. The resulting residue was diluted with EtOAc, washed with brine, and then dried over Na_2SO_4. After the solvent was removed, the residue was purified column chromatography on silica gel with *n*-hexane/EtOAc (5/1, v/v) as an eluent to afford methyl 4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinyl]methylthiobenzoate (**25**) (1.16 g, 93%) as a yellow oil.

To a stirred solution of methyl 4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinyl]methylthiobenzoate (**25**) (1.16 g, 3.32 mmol) in CH_2Cl_2 (20 ml) was added TFA (4 ml), and the mixture was stirred at room temperature for 1.5 h. The solvent was removed under a reduced pressure and the residue was treated with 1 M NaOH. The mixture was extracted with $CHCl_3$. The extract was washed with brine, dried over KOH, and concentrated under reduced pressure to afford compound **8e** (767 mg, 92%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.69 (1H, dt, J=3.9, 12.7 Hz), 1.85—2.09 (2H, m) 2.11—2.15 (1H, m), 3.11—3.27 (3H, m), 3.40 (1H, dd, J=6.8, 13.2 Hz), 3.54 (1H, dd, J=7.3, 15.1 Hz), 3.89 (3H, s), 5.07 (1H, s), 7.38 (2H, d, J=8.3 Hz), 7.91 (2H, d, J=8.3 Hz); FAB-MS *m/z*: 252 (M^++1).

Preparation of Methyl 4-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]-methoxybenzoate (11a) (General procedure C). *N*-Boc-L-prolinol (22) To a stirred solution of *N*-Boc-L-proline (**26a**) (20.0 g, 92.9 mmol) in THF (500 ml) was added BH_3·DMS (9.7 ml, 102 mmol) and the mixture was refluxed for 1 h with stirring. After cooling to room temperature, the mixture was concentrated. The resulting residue was diluted with water and extracted with CH_2Cl_2. The combined extracts were washed with sat. $NaHCO_3$ and brine, which was dried over Na_2SO_4 and evaporated to afford compound **22** (15.6 g, 83%) as a colorless crystalline powder. *N*-Boc-L-prolinol (**22**) is commercially available. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.53—1.57 (1H, m), 1.79—1.83 (2H, m), 1.98—2.02 (1H, m), 3.30—3.34 (1H, m), 3.42—3.46 (1H, m), 3.51—3.63 (2H, m), 3.94—3.98 (1H, m), 4.72—4.76 (1H, m).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]methoxybenzoate (27a) To a stirred solution of methyl 4-hydroxybenzoate (1.96 g, 12.9 mmol), *N*-Boc-L-prolinol (**22**) (2.59 g, 12.9 mmol) and Ph_3P (4.06 g, 15.5 mmol) in THF (40 ml) was added diisopropyl azodicarboxylate (3.10 ml, 15.7 mmol) and the reaction mixture was heated under reflux for 14 h. After cooling to room temperature, the mixture was concentrated under reduced pressure. The resulting residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (6/1, v/v) as an eluent to afford compound (**27a**) (3.34 g, 77%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.67 (1H, d, J=9.3 Hz), 1.87—2.03 (3H, m), 3.36—3.43 (2H, m), 3.82—4.09 (4H, m), 4.13—4.20 (2H, m), 6.94 (2H, d, J=8.3 Hz), 7.98 (2H, d, J=8.3 Hz); ESI-MS *m/z*: 336 (M^++1).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]methoxybenzoate (11a) According to the general procedure B, **11a** was obtained from **27a** as yellow oil (yield, 73%). ^{1}H-NMR ($CDCl_3$) δ: 1.54—1.61 (1H, m), 1.77—1.86 (2H, m), 1.87—1.97 (1H, m), 2.00 (1H, s), 2.93—3.06 (2H, m), 3.52—3.57 (1H, m), 3.88 (3H, s), 3.90—3.99 (2H, m), 6.92 (2H, d, J=9.0 Hz), 7.98 (2H, d, J=9.0 Hz); ESI-MS *m/z*: 236 (M^++1)

The following compound **11b**, **11f**—**j**, and **11n** were prepared from **26b**, **26f**—**j**, and **26n**, respectively, according to general procedure C.

Methyl 4-(*N*-*tert*-Butoxycarbonyl-4-hydroxy-(2*S*)-pyrrolidinylmethoxy)-benzoate (27b) Colorless oil (yield, 39%); ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.06 (3H, s), 2.14—2.41 (3H, m), 3.41—3.75 (2H, m), 3.88 (3H, s), 4.08—4.36 (2H, m), 5.30—5.36 (1H, m), 6.88—6.95 (2H, m), 7.97 (2H, d, J=8.6 Hz); ESI–MS *m/z*: 394 (M^++1).

Methyl 4-[(4*R*)-Acetoxy-(2*S*)-pyrrolidinyl]methoxybenzoate (11b) Brown oil (yield, 95%); ^{1}H-NMR ($CDCl_3$) δ: 1.86—1.93 (1H, m), 2.00—2.12 (5H, m), 3.03—3.29 (1H, m), 3.73—3.80 (1H, m), 3.88 (3H, s), 3.93—4.01 (2H, m), 5.27—5.30 (1H, m), 6.91 (2H, d, J=9.0 Hz), 7.98 (2H, d, J=9.0 Hz); FAB-MS *m/z*: 294 (M^++1).

Methyl 4-[1-*tert*-Butoxycarbonyl-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinyl]methoxybenzoate (27f) Colorless oil (yield, 93%); ^{1}H-NMR ($CDCl_3$) δ: 1.49 and 1.51 (total 9H, each s, amide isomers), 2.32—2.36 (1H, m), 2.53 (1H, d, J=14.2 Hz), 3.72—3.85 (1H, m), 3.86 and 3.87 (total 3H, each s, amide isomers), 4.15—4.19 (1H, m), 4.26—4.52 (2H, m), 5.06 (1H, s), 6.87 (1H, d, J=8.8 Hz), 6.94 (2H, d, J=8.8 Hz), 7.04 (2H, s), 7.33 (1H, t, J=7.3 Hz), 7.42 (1H, t, J=7.3 Hz), 7.64—8.02 (5H, m).

Methyl 4-[(4*S*)-(2-Naphthyloxy)-(2*S*)-pyrrolidinyl]methoxybenzoate (11f) Black oil (yield, 100%); ^{1}H-NMR ($CDCl_3$) δ: 1.99 (1H, dd, J=14.2, 5.6 Hz), 2.46—2.50 (1H, m), 3.22 (1H, dd, J=12.2, 4.6 Hz), 3.43 (1H, d, J=12.5 Hz), 3.65—3.69 (1H, m), 3.86 and 3.87 (total 3H, each s, amide isomers), 4.09—4.13 (2H, m), 5.02—5.06 (1H, m), 6.83 (1H, d, J=8.5 Hz), 6.89 (2H, d, J=8.8 Hz), 7.07 (1H, d, J=2.0 Hz), 7.12 (1H, d, J=9.0 Hz), 7.33 (1H, dt, J=8.1, 1.2 Hz), 7.44 (1H, dt, J=6.8, 1.2 Hz), 7.70 (1H, d, J=8.1 Hz), 7.75 (2H, dd, J=9.0, 5.1 Hz), 7.90 (1H, d, J=8.5 Hz), 7.96 (2H, dd, J=6.8, 2.0 Hz); ESI-MS *m/z*: 377 (M^++1).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(4*R*)-fluoro-(2*S*)-pyrrolidinyl]methoxybenzoate (27g) Colorless crystalline powder (yield, 65%); mp 122—124 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.49 (9H, s), 2.21—2.44 (2H, m), 3.39—3.69 (1H, m), 3.88 (3H, s), 4.00—4.36 (4H, m), 5.16—5.29 (1H, m), 6.91 (2H, d, J=8.3 Hz), 7.97 (2H, d, J=8.3 Hz); FAB-MS *m/z*:, 354 (M^++1); *Anal.* Calcd for $C_{18}H_{24}FNO_5$: C, 61.18; H, 6.85; N, 3.96. Found: C, 60.90; H, 6.89; N, 3.98.

Methyl 4-[(4*R*)-Fluoro-(2*S*)-pyrrolidinyl]methoxybenzoate (11g) Pale yellow crystalline powder (yield, 67%); mp 78—80 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.71—1.88 (1H, m), 2.24—2.34 (1H, m), 3.06—3.32 (2H, m), 3.81—4.00 (6H, m), 5.20—5.33 (1H, m), 6.91 (2H, d, J=8.8 Hz), 7.98 (2H, d, J=8.8 Hz); FAB-MS *m/z*:, 254 (M^++1); *Anal.* Calcd for $C_{13}H_{16}FNO_3$: C, 61.65; H, 6.37; N, 5.53. Found: C, 61.49; H, 6.45; N, 5.65.

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinyl]methoxybenzoate (27h) Colorless oil (yield, 74%); ^{1}H-NMR ($CDCl_3$) δ: 1.49—1.59 (9H, m), 2.05—2.21 (1H, m), 2.45—2.53 (1H, m), 3.56—4.43 (8H, m), 5.19—5.32 (1H, m), 6.95—6.99 (2H, m), 7.98 (2H, d, J=8.5 Hz), ESI-MS *m/z*:, 354 (M^++1).

Methyl 4-[(4*S*)-Fluoro-(2*S*)-pyrrolidinyl]methoxybenzoate (11h) Yellow solid (yield, 98%); ^{1}H-NMR ($CDCl_3$) δ: 1.89—2.02 (1H, m), 2.16—2.31 (1H, m), 2.98 (1H, ddd, J=35.3, 13.0, 3.7 Hz), 3.35 (1H, dd, J=21.1, 13.0 Hz), 3.46—3.68 (1H, m), 3.86 (3H, s), 4.00—4.08 (2H, m), 5.16 and 5.29 (total 1H, each t, each J=4.7 Hz), 6.91 (2H, d, J=8.8 Hz), 7.96 (2H, d, J=8.8 Hz); ESI-MS *m/z*: 254 (M^++1).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-4,4-difluoro-(2*S*)-pyrrolidinyl]methoxybenzoate (27i) Colorless oil (yield, 88%); ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 2.53—2.61 (2H, m), 3.63—4.41 (8H, m), 6.94 (2H, d, J=8.8 Hz), 7.99 (2H, d, J=8.8 Hz); ESI-MS *m/z*: 389 (M^++18).

Methyl 4-(4,4-Difluoro-(2*S*)-pyrrolidinylmethoxy)benzoate (11i) Pale yellow solid (yield, 91%); ^{1}H-NMR ($CDCl_3$) δ: 2.17—2.22 (1H, m), 2.41—2.45 (1H, m), 3.19—3.41 (2H, m), 3.75—3.79 (1H, m), 3.89 (3H, s), 4.00—4.09 (2H, m), 6.92 (2H, d, J=9.0 Hz), 7.99 (2H, d, J=9.0 Hz); FAB-MS *m/z*:, 272 (M^++1); *Anal.* Calcd for $C_{13}H_{15}F_2NO_3$: C, 57.56; H, 5.57; N, 5.16. Found: C, 57.65; H, 5.67; N, 5.16.

1-(*tert*-Butoxycarbonyl)-(4*R*)-chloro-(2*S*)-pyrrolidinylcarboxylic Acid (26j) To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-(4*S*)-hydroxy-(2*S*)-pyrrolidinylcarboxylate (1.81 g, 7.34 mmol) in CCl_4/CH_2Cl_2 (10/10 ml) was added Ph_3P (3.87 mmol, 14.8 mmol) and the reaction mixture was stirred at room temperature for 2 h. To the mixture was added EtOH (5 ml) and the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (3/1, v/v) as an eluent to afford methyl 1-(*tert*-butoxycarbonyl)-(4*R*)-chloro-(2*S*)-pyrrolidinylcarboxylate (1.36 g, 70%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 2.32—2.39 (1H, m), 2.49—2.54 (1H, m), 3.66—3.92 (5H, m), 4.44—4.55 (2H, m); FAB-MS *m/z*: 264 (M^++1).

To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-(4*R*)-chloro-(2*S*)-pyrrolidinylcarboxylate (1.35 g, 5.12 mmol) in THF (10 ml) was added 0.5 M NaOH (10 ml) and heated under reflux for 1.5 h. After cooling to room temperature, the mixture was poured into 1 M HCl and the mixture was extracted with $CHCl_3$/MeOH (9/1, v/v). The combined extracts were washed with brine, dried over Na_2SO_4 and evaporated to afford compound (**26j**) (1.28 g, quant.) as colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 2.37—2.54 (2H, m), 3.68—3.88 (2H, m), 4.42—4.45 (2H, m).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(4*R*)-chloro-(2*S*)-pyrrolidinyl]methoxybenzoate (27j) Colorless solid (yield, 65%); mp 116—120 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 2.39—2.53 (2H, m), 3.69—4.17 (3H, m),

3.88 (3H, s), 4.30—4.41 (2H, m), 4.50—4.55 (1H, m), 6.90—6.92 (2H, m), 7.96—7.98 (2H, m); FAB-MS *m/z*: 370 (M^++1); *Anal.* Calcd for $C_{18}H_{24}ClNO_5$: C, 58.46; H, 6.54; Cl; 9.59; N, 3.79. Found: C, 58.35; H, 6.56; Cl, 9.75; N, 3.77.

Methyl 4-[(4*R*)-Chloro-(2*S*)-pyrrolidinyl]methoxybenzoate (11j) Colorless solid (yield, 95%); mp 61—64 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.85 (1H, s), 2.03—2.10 (1H, m), 2.29—2.35 (1H, m), 3.19—3.31 (2H, m), 3.88 (3H, s), 3.92—4.06 (3H, m), 4.53—4.56 (1H, m), 6.91 (2H, d, *J*=8.8 Hz), 7.98 (2H, d, *J*=8.8 Hz); FAB-MS *m/z*: 270 (M^++1).

Methyl 4-[3-Benzyloxy-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinyl]methoxybenzoate (27n) Reddish oil (yield, 85%); ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.95—2.17 (2H, m), 3.36—3.80 (2H, m), 3.89 (3H, s), 4.10—4.19 (3H, m), 4.25—4.29 (1H, m), 4.48—4.59 (2H, m), 6.85—6.95 (2H, m), 7.27—7.44 (5H, m), 7.92—7.80 (2H, m); ESI-MS *m/z*: 442 (M^++1).

Methyl 4-[3-Benzyloxy-(2*S*)-pyrrolidinyl]methoxybenzoate (11n) Brown oil (yield, 98%); ^{1}H-NMR ($CDCl_3$) δ: 1.92—2.05 (2H, m), 2.12 (1H, s), 3.05—3.09 (1H, m), 3.16—3.18 (1H, m), 3.55 (1H, td, *J*=5.9, 3.2 Hz), 3.88 (3H, s), 3.92—3.99 (2H, m), 4.00—4.04 (1H, m), 4.52 (1H, d, *J*=11.8 Hz), 4.58 (1H, d, *J*=11.8 Hz), 6.89 (1H, d, *J*=8.8 Hz), 7.27—7.36 (5H, m), 7.97 (2H, d, *J*=8.8 Hz); ESI-MS *m/z*: 342 (M^++1).

Methyl 4-[(4*S*)-Acetoxy-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinyl]methoxybenzoate (27c) To a stirred solution of methyl 4-[(4*R*)-acetoxy-1-*tert*-butoxycarbonyl-4-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate (**27b**) (3.31 g, 8.41 mmol) in MeOH (100 ml) was added K_2CO_3 (1.05 g, 7.57 mmol) at room temperature. After 1 h stirring, the mixture was diluted with H_2O and extracted $CHCl_3$. The combined extracts were washed with sat. NaCl. After dried over Na_2SO_4, the extracts were concentrated *in vacuo*. The resulting residue was purified by flush column chromatography with *n*-hexane/EtOAc (10—60%, v/v) as an eluent to afford the methyl 4-[(2*S*,4*R*)-1-*tert*-butoxycarbonyl-4-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate (2.84 g, 96%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.05—2.36 (3H, m), 3.51 (1H, dd, *J*=11.6, 4.8 Hz), 3.88 (3H, s), 4.07—4.37 (3H, m), 4.51—4.59 (1H, m), 6.92 (2H, d, *J*=8.3 Hz), 7.97 (2H, d, *J*=8.8 Hz); ESI-MS *m/z*: 352 (M^++Na).

To a solution of methyl 4-[(2*S*,4*R*)-1-*tert*-butoxycarbonyl-4-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate (1.00 g, 2.85 mmol), HOAc (0.48 ml, 8.54 mmol) and PPh_3 (0.90 g, 3.42 mmol) in THF (20 ml) was added diisopropyl azodicarboxylate (0.67 ml, 3.42 mmol) at room temperature. After 3 d, the reaction mixture was concentrated to dryness. The residue was purified by flush column chromatography with *n*-hexane/EtOAc (10—80%, v/v) as an eluent to afford compound **27c** (1.00 g, 93%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 2.03 (3H, s), 2.25—2.29 (2H, m), 3.44—3.48 (1H, m), 3.70—3.74 (1H, m), 3.88 (3H, s), 3.98 (1H, t, *J*=9.0 Hz), 4.21—4.47 (2H, m), 5.31 (1H, s), 6.96 (2H, s), 7.98 (2H, d, *J*=8.8 Hz); ESI-MS *m/z*: 416 (M^++Na).

Methyl 4-[(4*S*)-Acetoxy-(2*S*)-pyrrolidinyl]methoxybenzoate (11c) According to the general procedure B, **11c** was obtained from **27c** as pale purple solid (1.89 g, 100%); ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, s), 2.12—2.16 (1H, m), 2.63—2.67 (1H, m), 3.52—3.63 (2H, m), 3.89 (3H, s), 4.16—4.20 (1H, m), 4.28 (2H, d, *J*=5.9 Hz), 5.36—5.40 (1H, m), 6.93 (2H, d, *J*=8.8 Hz), 7.99 (2H, d, *J*=8.8 Hz); ESI-MS *m/z*: 294 (M^++1).

Methyl 4-[(4*R*)-Methoxy-(2*S*)-pyrrolidinyl]methoxybenzoate (11d) To a stirred solution of methyl 4-hydroxybenzoate (1.18 g, 7.76 mmol), 1-(*tert*-butoxycarbonyl)-(4*R*)-methoxy-(2*S*)-pyrrolidinylmethanol (1.79 g, 7.74 mmol) and Ph_3P (2.44 g, 9.30 mmol) in THF (30 ml) was added diisopropyl azodicarboxylate (1.83 ml, 9.29 mmol) and the reaction mixture was heated under reflux for 5 h. After cooling to room temperature, the mixture was evaporated. The residue was filtered on silica gel with toluene/acetone (5/1, v/v) as an eluent to afford methyl 4-[1-*tert*-butoxycarbonyl-(4*R*)-methoxy-(2*S*)-pyrrolidinyl]methoxybenzoate (**27d**) including inseparable material.

To a solution of methyl 4-[1-*tert*-butoxycarbonyl-(4*R*)-methoxy-(2*S*)-pyrrolidinyl]methoxybenzoate (**27d**) in CH_2Cl_2 (20 ml) was added TFA (20 ml) at room temperature. After 12 h stirring, the mixture was concentrated *in vacuo*. The residue was made basic by sat. $NaHCO_3$ and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over K_2CO_3, and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (30/1 to 30/2, v/v) as an eluent to afford compound **11d** (1.67 g, 81% for 2 steps) as a reddish brown oil. ^{1}H-NMR ($CDCl_3$) δ: 1.65—1.72 (1H, m), 1.89 (1H, s), 2.05—2.22 (1H, m), 2.95—3.15 (2H, m), 3.31 (3H, s), 3.69—3.76 (1H, m), 3.88 (3H, s), 3.91—4.06 (3H, m), 6.89—6.92 (2H, m), 7.96—7.98 (2H, m); FAB-MS *m/z*: 266 (M^++1).

Methyl 4-[1-*tert*-Butoxycarbonyl-(4*S*)-methoxy-(2*S*)-pyrrolidinyl]methoxybenzoate (27e) To a stirred solution of methyl 4-[(4*S*)-acetoxy-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinyl]methoxybenzoate (**27c**) (7.43 g, 18.9 mmol) in MeOH (150 ml) was added cat. K_2CO_3 at room temperature. After 1 d stirring, the mixture was concentrated *in vacuo*. The resulting residue was recrystallized with $CHCl_3$/*n*-hexane to afford methyl 4-[1-*tert*-butoxycarbonyl-(4*S*)-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate (5.76 g, 87%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.09—2.13 (1H, m), 2.35 (1H, s), 3.27—3.65 (2H, m), 3.89 (3H, s), 4.07—4.54 (4H, m), 6.96 (2H, d, *J*=6.9 Hz), 7.99 (2H, d, *J*=6.9 Hz); ESI-MS *m/z*: 352 (M^++1).

To a stirred solution of methyl 4-[1-*tert*-butoxycarbonyl-(4*S*)-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate (2.10 g, 5.98 mmol) in THF (60 ml) was added 60% oil NaH (359 mg, 8.97 mmol) at 0 °C. After 15 min stirring, MeI (1.20 ml, 8.97 mmol) was added to the mixture at same temperature, and the resulting mixture was allowed to room temperature for over 1 h. Then, 60% oil NaH (359 mg, 8.97 mmol) and MeI (1.20 ml, 8.97 mmol) were added to the reaction mixture at room temperature and stirred for 14 h. The reaction mixture was poured into ice water and extracted with $CHCl_3$. The combined extracts were washed with aq. $NaHCO_3$ and brine. After drying over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (4/1, v/v) as an eluent to give compound **27e** (1.32 g, 60%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 2.03—2.07 (1H, m), 2.29 (1H, d, *J*=14.2 Hz), 3.30 (3H, s), 3.36—3.68 (2H, m), 3.88 (3H, s), 3.95—4.38 (4H, m), 6.76 (2H, s), 7.97 (2H, d, *J*=8.8 Hz); ESI-MS *m/z*: 366 (M^++1).

Methyl 4-[(4*S*)-methoxy-(2*S*)-pyrrolidinyl]methoxybenzoate (11e) According to the general procedure B, **11e** was obtained from **27e** as a yellow oil (yield, 99%); ^{1}H-NMR ($CDCl_3$) δ: 2.16 (1H, t, *J*=5.3 Hz), 2.72 (1H, s), 2.95 (1H, d, *J*=6.8 Hz), 3.11 (1H, d, *J*=11.0 Hz), 3.26 (3H, t, *J*=1.9 Hz), 3.52 (1H, br), 3.84 (3H, d, *J*=1.7 Hz), 3.92 (1H, s), 4.00 (2H, d, *J*=4.1 Hz), 6.86—6.90 (2H, m), 7.92—7.96 (2H, m); ESI-MS *m/z*: 266 (M^++1).

Methyl 4-[1-*tert*-Butoxycarbonyl-(4*R*)-dimethylamino-(2*S*)-pyrrolidinyl]methoxybenzoate (27k) To a stirred solution of methyl 4-[1-*tert*-butoxycarbonyl-(4*S*)-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate (300 mg, 0.85 mmol) in CH_2Cl_2 (10 ml) was added DIPEA (2.08 ml, 12.0 mmol) at room temperature and cooled to −78 °C. After 30 min stirring, Tf_2O (0.56 ml, 2.42 mmol) was added to the mixture at same temperature and the solution was stirred for 30 min. Two mol/l Me_3NH in THF (2.13 ml, 4.26 mmol) was added to the reaction mixture at −78 °C. After 20 min stirring at −78 °C, the reaction mixture was raised to 5 °C and stirred for 18 h. The reaction mixture was poured into sat. $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined extracts were washed with brine. After drying over Na_2SO_4, the extracts were concentrated *in vacuo*. The residue was purified by flush column chromatography (Biotage 25S) with MeOH/EtOAc (0—40%, v/v) as an eluent, to afford compound **27k** (380 mg, over yields) including inseparable materials as a red oily solid. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.80—1.95 (1H, m), 2.20—2.23 (1H, m), 2.24 (6H, s), 2.90—2.95 (1H, m), 3.10—3.30 (1H, m), 3.50—3.65 (1H, m), 3.88 (3H, s), 3.95—4.35 (3H, m), 6.93—6.95 (2H, m), 7.96—7.98 (2H, m); ESI-MS *m/z*: 379 (M^++1).

Methyl 4-[(4*R*)-Dimethylamino-(2*S*)-pyrrolidinyl]methoxybenzoate (11k) According to the general procedure B, **11k** was obtained from **27k** as a pale yellow oil (yield 61%). ^{1}H-NMR ($CDCl_3$) δ: 1.77—2.05 (2H, m), 2.25—2.25 (1H, m), 2.30 (6H, s), 2.72—2.93 (1H, m), 3.26 (1H, dd, *J*=9.8, 6.4 Hz), 3.66—3.75 (1H, m), 3.88 (3H, s), 3.91 (1H, dd, *J*=9.1, 6.6Hz), 3.98 (1H, dd, *J*=9.1, 4.9 Hz), 6.91 (2H, d, *J*=8.8 Hz), 7.98 (2H, d, *J*=8.8 Hz); ESI-MS *m/z*: 279 (M^++1).

Methyl 4-[1-*tert*-Butoxycarbonyl-(4*S*)-dimethylamino-(2*S*)-pyrrolidinyl]methoxybenzoate (27l) By the procedure described for **27k**, but with methyl 4-[1-*tert*-butoxycarbonyl-(4*R*)-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate in place of methyl 4-[1-*tert*-butoxycarbonyl-(4*S*)-hydroxy-(2*S*)-pyrrolidinyl]methoxybenzoate, compound **27l** was obtained as a yellow oil (quant.). ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.70—1.90 (1H, m), 2.26 (6H, s), 2.33 (1H, m), 2.55—2.59 (1H, m), 3.04—3.08 (1H, m), 3.85—4.23 (4H, m), 3.88 (3H, s), 6.93 (2H, m), 7.95 (2H, m); ESI-MS *m/z*: 379 (M^++1).

Methyl 4-[1-*tert*-Butoxycarbonyl-(4*S*)-dimethylamino-(2*S*)-pyrrolidinyl]methoxybenzoate (11l) According to the general procedure B, **11l** was obtained from **27l** as a pale yellow oil (yield 79%). ^{1}H-NMR ($CDCl_3$) δ: 1.48—1.58 (1H, m), 2.17—2.29 (1H, m), 2.30 (6H, s), 2.72—2.82 (1H, m), 2.87—2.96 (1H, m), 3.17 (1H, dd, *J*=10.3, 6.6 Hz), 3.59—3.68 (1H, m), 3.88 (3H, s), 3.98 (2H, d, *J*=5.9 Hz), 6.91 (2H, d, *J*=8.8 Hz), 7.97 (2H, d, *J*=8.8 Hz); ESI-MS *m/z*: 279 (M^++1).

Methyl 4-[1-Benzyloxycarbonyl-(3*R*,4*S*)-isopropylidenedioxy-(2*R*)-pyrrolidinyl]methoxybenzoate (27m) According to the general procedure

C, **27m** was obtained from **26m** as a colorless oil (yield, 83%); ^{1}H-NMR ($CDCl_3$) δ: 1.01 (7H, s), 1.03 (3H, s), 2.21—2.25 (1H, m), 2.61—2.65 (1H, m), 3.61 (1H, d, J=12.5 Hz), 3.80—4.27 (4H, m), 4.84 (1H, s), 5.01 and 5.08 (total 1H, each ABq, each J=12.2 Hz, amide isomers), 6.75—6.87 (3H, m), 7.19—7.63 (5H, m).

Methyl 4-[(3*R*,4*S*)-Isopropylidenedioxy-(2*R*)-pyrrolidinyl]methoxybenzoate (11m) A suspension of methyl 4-[benzyloxycarbonyl-(3*R*,4*S*)-isopropylidenedioxy-(2*R*)-pyrrolidinyl]methoxybenzoate (**27m**) (2.37 g, 5.76 mmol) and 10% Pd/C (240 mg) in EtOH (170 ml) was stirred at room temperature under an atmosphere of hydrogen. After 1 d stirring, the catalyst and solvent were changed for 10% Pd/C (500 mg) and THF (50 ml). The suspension was stirred at room temperature under an atmosphere of hydrogen for 5 d. After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel [100 g, $CHCl_3$/Acetone (20/1)], to afford compound (**11m**) (930 mg, 53%) as a brown oil. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, s), 1.50 (3H, s), 3.02 (1H, dd, J=13.7, 4.1 Hz), 3.13 (1H, d, J=13.7 Hz), 3.58 (1H, t, J=6.3 Hz), 3.88 (3H, s), 3.90 (1H, dd, J=9.3, 6.6 Hz), 4.02 (1H, dd, J=9.5, 3.9 Hz), 4.74 (1H, d, J=5.6 Hz), 4.77—4.81 (1H, m), 6.90 (2H, d, J=9.0 Hz), 7.98 (2H, d, J=9.0 Hz); ESI-MS m/z: 308 (M^++1).

Methyl 2-Methyl-4-[(2*S*)-pyrrolidinyl]methoxybenzoate (14a) To a stirred solution of 4-bromo-3-methylphenol (500 mg, 2.67 mmol), *N*-Boc-L-prolinol (**22**) (538 mg, 2.67 mmol), and Ph_3P (911 mg, 3.48 mmol) in THF (5.0 ml) was added diisopropyl azodicarboxylate (690 μl, 3.48 mmol) at room temperature, and the mixture was stirred for 4 d at 70 °C. After cooling to room temperature, the reaction mixture was concentrated. The residue was purified by chromatography on silica gel with *n*-hexane/EtOAc (7/1, v/v) as an eluent to afford 1-bromo-4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-(2*S*)-methylbenzene (**28a**) (755 mg, 76%) as a colorless oil.

To a cooling stirred solution of 1-bromo-4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-(2*S*)-methylbenzene (**28a**) (546 mg, 1.48 mmol) in THF (6 ml) was added *n*-BuLi (283 μl, 2.04 mmol), and TMEDA (468 μl, 3.10 mmol) at −78 °C. The mixture was stirred for 1 h, methyl chloroformate (429 μl, 3.69 mmol) was added, and the resulting mixture was stirred for 12 h. The reaction mixture was poured into sat. NH_4Cl solution and extracted with EtOAc. The combined extracts were washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by chromatography on silica gel with *n*-hexane/EtOAc (5/1, v/v) as an eluent to afford methyl 4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-(2*S*)-methylbenzoate (29a) (108 mg, 21%) as a colorless oil, which was followed to the general procedure B to afford **14a** [75 mg, 97%, (3 steps 15%)] as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.54—1.62 (1H, m), 1.77—2.00 (3H, m), 2.59 (3H, s), 2.93—3.04 (2H, m), 3.51—3.54 (1H, m), 3.85 (3H, s), 3.87—3.98 (2H, m), 6.74 (1H, dd, J=3.0, 7.3 Hz), 6.75 (1H, s), 7.91 (1H, dd, J=1.5, 7.3 Hz); FAB-MS m/z: 250 (M^++1).

4-[1-*N*-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-1-iodo-3-methylbenzene (28b) To a stirred solution of 4-iodo-2-methylphenol (465 mg, 1.99 mmol), *N*-Boc-L-prolinol (**22**) (400 mg, 1.99 mmol), and Ph_3P (625 mg, 2.38 mmol) in THF (7 ml) was slowly added diisopropyl azodicarboxylate (0.5 ml, 2.40 mmol) at room temperature and the stirring was continued for 13 h at 70 °C. The reaction mixture was concentrated. The residue was purified by column chromatography on silica with *n*-hexane/EtOAc (9/1, v/v) as an eluent to give compound **28b** (645 mg, 78%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.83—1.89 (1H, m), 1.96—2.04 (3H, m), 2.16 (3H, s), 3.37—3.43 (2H, m), 3.79—3.96 (1H, m), 4.08—4.18 (2H, m), 6.62 (1H, s), 7.42 (2H, s); FAB-MS m/z: 418 (M^++1).

Methyl 4-[1-*N*-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-3-methylbenzoate (29b) To a solution of 4-[1-*N*-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-1-iodo-3-methylbenzene (**28b**) (645 mg, 1.55 mmol), Et_3N (0.47 ml, 3.401 mmol), $Pd(OAc)_2$ (17.4 mg, 0.077 mmol), and 1,3-bis(diphenylphosphino)propane (31.5 mg, 0.077 mmol) in DMSO/MeOH (7/6 ml) was babbled CO gas for 10 min. After being stirred at 70 °C for 2 d, the reaction mixture was concentrated. The resulting residue was diluted with EtOAc and washed with brine, dried over Na_2SO_4. After the solvent was removed, the residue was purified by chromatography on silica gel with *n*-hexane/EtOAc (5/1, v/v) as an eluent to afford compound **29b** (302 mg, 56%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.86—2.10 (4H, m), 2.33 (3H, s), 3.32—3.50 (2H, m), 3.88 (3H, s), 3.88—4.04 (1H, m), 4.13—4.20 (2H, m), 6.87—6.89 (1H, m), 7.82 (1H, s), 7.85 (1H, dd, J=2.0, 8.8 Hz); FAB-MS m/z: 350 (M^++1).

Methyl 3-Methyl-4-[(2*S*)-pyrrolidinyl]methyloxybenzoate (14b) According to the general procedure B, **14b** was obtained as a colorless oil (yield, 100%). ^{1}H-NMR ($CDCl_3$) δ: 1.58—1.65 (1H, m), 1.78—2.00 (3H, m), 2.24 (3H, s), 2.97 (1H, dt, J=6.8, 10.2 Hz), 3.05 (1H, dt, J=5.9, 6.8 Hz), 3.54—3.58 (1H, m), 3.87 (3H, s), 3.92 (1H, dd, J=6.3, 9.3 Hz), 3.99 (1H, dd, J=4.9, 9.3 Hz), 6.81 (1H, d, J=8.3 Hz), 7.83 (1H, s), 7.85 (1H, dd, J=2.0, 8.3 Hz); FAB-MS m/z: 250 (M^++1).

4-[1-*N*-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]methyloxy-3,5-dimethyl-1-iodobenzene (28c) The procedure described for **28b**, but with 2,5-dimethyl-4-iodophenol in place of 4-iodo-2-methylphenol, compound **28c** was obtained as a pale red oil (yield, 77%). ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.83—1.95 (2H, m), 1.97—2.09 (3H, s), 2.13 (3H, s), 2.36 (3H, s), 3.79—3.95 (2H, m), 4.05—4.19 (2H, m), 6.70 (1H, s), 7.51 (1H, s); FAB-MS m/z: 432 (M^++1).

Methyl 2,5-Dimethyl-4-[(2*S*)-pyrrolidinyl]methyloxybenzoate (14c) To a solution of 4-[1-*N*-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinyl]methyloxy-3,5-dimethyl-1-iodobenzene (**28c**) (400.1 mg, 0.927 mmol) in DMSO (5 ml) and MeOH (4 ml) was added Et_3N (0.283 ml, 2.04 mmol), $Pd(OAc)_2$ (10.4 mg, 0.046 mmol), and 1,3-bis(diphenylphosphino)propane (19.0 mg, 0.046mmol), then CO gas was babbled for 10 min, and the mixture was stirred at 70 °C for 1 d. The reaction mixture is concentrated *in vacuo*. The residue was diluted with EtOAc and washed with brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel eluting with *n*-hexane/EtOAc (5/1, v/v) to afford methyl 4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinyl]methyloxy-2,5-dimethylbenzoate **29c** (228 mg, 68%) as a yellow oil, which was followed to the general procedure B to afford **14c** (86.9 mg, 53%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.58—1.65 (1H, m), 1.76—1.87 (2H, m), 1.92—1.98 (1H, m), 2.19 (3H, s), 2.57 (3H, s), 2.93—2.99 (1H, m), 3.02—3.08 (1H, m), 3.54—3.57 (1H, m), 3.85 (3H, s), 3.89—3.99 (2H, m), 6.63 (1H, s), 7.75 (1H, s); FAB-MS m/z: 264 (M^++1).

Ethyl 3-Methoxy-4-[(2*S*)-pyrrolidinyl]methoxybenzoate (14d) To a solution of ethyl 4-hydroxy-3-methoxybenzoate (3.00 g, 15.29 mmol), *N*-Boc-L-prolinol (**22**) (3.08 g, 15.3 mmol), Ph_3P (4.81 g, 18.3 mmol) in THF (50 ml) was added diisopropyl azodicarboxylate (3.61 ml, 18.3 mmol) and the reaction mixture was heated under reflux for 6.5 h. After cooling to room temperature, the solution was evaporated and the residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (50/1, v/v) as eluent to give ethyl 3-methoxy-4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinyl]-methoxybenzoate (**29d**) containing inseparable compounds.

To a solution of ethyl 3-methoxy-4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinyl]methoxybenzoate (**29d**) in CH_2Cl_2 (50 ml) was added TFA (45 ml) at room temperature. After stirring for 2 d, the reaction mixture was concentrated *in vacuo*. The residue was made basic with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The combined extracts were washed with brine, and dried over $MgSO_4$. After the solvent was removed, the resulting residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (20/1, v/v) as an eluent to give compound **14d** [3.27 g, 77% (2 steps)] as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.1 Hz), 1.52—1.59 (1H, m), 1.76—1.88 (2H, m), 1.92—2.01 (1H, m), 2.92—3.06 (2H, m), 3.56—3.63 (1H, m), 3.90 (3H, s), 3.91—4.02 (2H, m), 4.35 (2H, q, J=7.1 Hz), 6.89 (1H, d, J=8.3 Hz), 7.54 (1H, d, J=2.0 Hz), 7.65 (1H, dd, J=2.0, 8.3 Hz).

The following compound **14e**—**g** were prepared from **22** according to general procedure C.

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-3-chlorobenzoate (29e) Yellow oil (yield, 97%); ^{1}H-NMR ($CDCl_3$) δ: 1.46 and 1.48 (total 9H, s each, amide isomers), 1.59—1.63 (1H, m), 1.88 (1H, s), 2.05 (1H, s), 2.05—2.21 (2H, m), 3.34—3.97 (5H, m), 4.21 (2 H, s), 7.05 (1H, d, J=8.8 Hz), 7.90 (1H, dd, J=2.0, 8.8 Hz), 8.04 (1H, d, J=2.0 Hz); FAB-MS m/z: 370 (M^++1).

Methyl 3-Chloro-4-[(2*S*)-pyrrolidinyl]methoxybenzoate (14e) Yellow oil (yield, 89%); ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.67 (1H, m), 1.78—2.02 (3H, m), 2.93—2.98 (1H, m), 3.03—3.09 (1H, m), 3.59 (1H, dt, J=2.0, 9.3 Hz), 3.89 (3H, s), 3.98 (1H, dd, J=6.3, 8.8 Hz), 4.05 (1H, dd, J=4.9, 9.3 Hz), 6.93 (1H, d, J=8.8 Hz), 7.90 (1H, dd, J=2.0, 8.8 Hz), 8.04 (1H, d, J=2.0 Hz); FAB-MS m/z: 270 (M^++1).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinylmethyloxy]-3,5-dichlorobenzoate (29f) Pale yellow oil (yield, 90%); ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.88—2.15 (3H, m), 2.34 (1H, s), 3.40—3.44 (2H, m), 3.92 (3H, s), 4.14 (1H, m), 4.18 (2H, s), 7.98 (2H, s); FAB-MS m/z: 404 (M^++1).

Methyl 3,5-Dichloro-4-[(2*S*)-pyrrolidinyl]methoxybenzoate (14f) Pale yellow oil (yield, 68%); ^{1}H-NMR ($CDCl_3$) δ: 1.62—1.69 (1H, m), 1.78—1.86 (2H, m), 1.89—1.99 (1H, m), 2.92—2.98 (1H, m), 3.04—3.09 (1H, m), 3.55—3.60 (1H, m), 3.91 (3H, s), 4.01 (1H, dd, J=6.8, 8.8 Hz), 4.08 (1H, dd, J=4.9, 8.8 Hz), 7.97 (2H, s); FAB-MS m/z: 304 (M^++1).

Methyl 4-[1-(*tert*-Butoxycarbonyl)-(2*S*)-pyrrolidinyl]methoxybenzoate (29g) Yellow oil (yield, 73%); ^{1}H-NMR ($CDCl_3$) δ: 1.58 (9H, s), 1.80—

2.15 (5H, m), 3.93 (3H, s), 4.03—4.22 (2H, m), 4.25—4.39 (2H, m), 7.27—7.11 (1H, m), 8.19 (1H, dd, J=8.8, 2.2 Hz), 8.47—8.52 (1H, m); ESI-MS m/z: 381 (M^++1).

Methyl 3-Nitro-4-[(2*S*)-pyrrolidinyl]methoxybenzoate (14g) Yellow oil (yield, 94%); ^{1}H-NMR ($CDCl_3$) δ: 1.60—1.96 (4H, m), 2.96—3.05 (2H, m), 3.53—3.62 (2H, m), 3.93 (3H, s), 4.06 (1H, dd, J=6.8, 8.8 Hz), 7.12 (1H, d, J=8.8 Hz), 8.19 (1H, dd, J=2.0, 8.8 Hz), 8.51 (1H, d, J=2.0 Hz); ESI-MS m/z: 281 (M^++1).

VLA-4/VCAM-1 Binding Assay A human VLA-4-expressing cell line, 4B4, was established at Pharmacopeia, by transfecting both the α4 gene and β1 gene of VLA-4 into CHO-K1 cells. The 4B4 cells were maintained in Ham's F-12 medium (Sigma) supplemented with 10% (v/v) fetal calf serum (REHATUIN Fetal Bovine Serum, Serologicals Corporation), 100 U/ml penicillin (Invitrogen Corporation), 100 μg/ml streptomycin (Invitrogen Corporation), and 2 mM L-glutamine (Invitrogen Corporation) and 1 mg/ml G-418 (Geneticin, Invitrogen Corporation). An Eu-labelling Reagent (PerkinElmer Inc.) was used to labeled to human VCAM-1/Fc chimeric antibody (R&D Systems Inc.). The Eu-labelled protein was purified using a PD-10 column (Amersham Biosciences KK.) and stored at −80 °C until use. All assays were performed in duplicate. In preparation for the assay, the 4B4 cells were suspended at 3×10^5 cells/ml in Ham's F-12 medium. One hundred microliters of the 4B4 cell suspension was into each well of a 96-well-culture plate (Costar). The plates were incubated at 37 °C in a 95% air/5% CO_2 atmosphere humidified incubator (Themo Forma, model 3120; Forma Scientific) for 2 d. Prior to the assay the medium was discarded, and each well was washed twice with 300 μl of chilled Wash Buffer (25 mM HEPES, pH 7.5; 150 mM NaCl; 1 mM $CaCl_2$; 1 mM $MgCl_2$; 4 mM $MnCl_2$). Then, 50 μl of compound solutions was added to a well, followed by 50 μl of 2 nM of Eu-labelled Human VCAM-1/Fc Chimera diluted with the Wash Buffer (final concentration: 1 nM). For assays conducted in the presence of human serum albumin, 50 μl of compound at various concentrations and an equal volume of 2 nM Eu-labeled human VCAM-1/Fc Chimera in a 3% (w/v) human serum albumin (Sigma) were distributed into each well (final concentration: 1 nM). The plates were incubated for 60 min at room temperature, and the wells were washed 4 times with 300 μl of chilled wash Buffer. Finally, 100 μl of the enhancement solution (PerkinElmer Inc.) was added to each well. The plates were placed on a shaker for 5 min. Eu fluorescence was then measured using a time-resolved fluorometer (DELFIA Research fluorometer, model 1234-001; PerkinElmer Inc.). The concentration of compound required for 50% inhibition in the assay was determined.

Inhibition of Airway Inflammation in Mice (*in Vivo* Evaluation) Female BALB/c AnNCrj mice (18—23 g, 8 weeks old, Charles River Japan, Inc.) were used. Animals were housed in disposable mouse cage [4 animals/cage, $220^W\times320^D\times130^H$ (mm)] with lids in unidirectional air flow rooms maintained controlled temperature (23±2 °C) and relative humidity (55±20%) on a 12 h light–dark cycle. Animals were provided food and tap water *ad libitum*. Norm-Based Group Building ver. 5.0.0 (Visions) was used to divide the animals into groups. Each group consisted of 7 animals. Mice received oral administration of cyclophosphamide (Endoxane, Shionogi & Co. Ltd.) dissolved in 5% (w/v) carboxymethylcellulose (CMC) at a dose of 150 mg/10 ml/kg (day 0). A solution containing 500 μg *Ascaris suum* extract (LSL Co., Ltd.) for every 0.2 ml saline wad prepared. The solution also contained 5 mg aluminum hydroxide as an adjuvant. On day 2, 0.2 ml of the antigen solution was injected intraperitoneally into each mouse. On day 14, the same antigen solution was injected intraperitoneally as a booster. Finally, on day 22, mice were anesthetized with intraperitoneal injection of 70 mg/10 ml/kg of pentobarbital sodium. After 10 min, mice were challenged intratracheally with 300 mg protein of *Ascaris suum* extract. In the negative control group, sensitized mice were challenged with saline instead of antigen. Test compound, which was dissolved in a 5% CMC suspension containing 0.03% Tween, was orally administered 1 h before antigen challenge, 8, 24 and 32 h after antigen challenge at a dose of 30 mg/kg (day 22, 23). Forty-eight hours later, the mice were sacrificed by cutting abdominal aorta while the animals were under pentobarbital anesthesia (1.95 mg/0.3 ml i.p. injection). Lungs were lavaged using tracheal polyethylene cannula (outside diameter 1.33 mm, Hibiki No. 4, Hibiki Co.) with 2×0.5 ml Hanks' balanced salt solution supplemented with 0.05 mM potassium EDTA. The BAL fluid was immediately centrifuged (5 min, 4 °C, 500× ***g***). After removing the supernatant, the cells in the BAL fluid were resuspended in 0.2 ml of fetal calf serum. The cells were counted in a particle analyzer CDA-500 (Sysmex). Cytocentrifuged preparations (Cytospin 2; Shandon) were stained with Wright's stain solution (Muto Chemicals) for differential counts, based on standard morphologic criteria. Taking the ratio for the number of eosinophils in cytocentrifuged preparations, eosinophils were estimated by multiplication of the ratio and pooled total cells as the mean from each treatment group.

Distribution Coefficient The distribution coefficients (Log D) were determined by the shake-flask method.[30] Milliliters of 400 μM solution of each compound in a 2 ml *n*-octanol/2 ml PBS solution was placed on a shaker for 30 min. After centrifuging each solution separately at for 10 min, an LC/MS method was used to assay each layer. The LC/MS system was consisted of an 1100 Series LC/MSD (Agilent) and X Terra® MSC18 3.5 μm, 3.0×30 mm column (Waters). The mobile phase was a 10 mM ammonium acetate buffer (pH 4.5)/0.05% (v/v) acetic acid mixture in acetonitrile; the gradient condition 95/5 to 10/90). Analyst software program (version 1.4, Applied Bio. Systems) was used to calculated the Log D.

Madin–Darby Canine Kidney Cell Permeability The cell permeability of selected compounds was determined using Madin–Darby canine kidney (MDCK, American Type Culture Collection) cells. MDCK cells were maintained in Minimum Essential Medium (GIBCO) containing 10% (v/v) fetal bovine serum (Bioproducts Inc.), a penicillin–streptomycin mixture (GIBCO), and L-glutamine. For the transport assay, cells were seeded on HTS 24 well transwells (Costar) at 3×10^5 cells/ml and grown for 6 d after seeding to allow formation of a cell monolayer. The Transport Buffer was prepared using $NaHCO_3$ (final 0.35 g/l), D-Glucose (final 3.5 g/l), HEPES (Sigma; final 10 mM), $CaCl_2$ (final 0.14 g/l) and $MgSO_4$ (final 0.098 g/l) in ×10 Hank's Balanced Salt Solution (GIBCO) and adjusted to pH 6.0 or 7.4 in 1 M HCl or 1 M NaOH. For each test compound a dosing solution containing one of the compounds at a concentration of 10 μM in Transport Buffer (pH 6.0) (100 μl) was added to the apical (A) side of a monolayer. A blank solution containing 4% (w/v) BSA in Transport Buffer (pH 7.4) (600 μl), which was re-adjusted to pH 7.4, was added to the basolateral (B) side of the monolayer. Metoprolol was used as a positive control. After 1 h of incubation at 37 °C, aliquots of the apical and basal solutions were separately analyzed on an LC/MS/MS system consisting an Alliance 2790 HPLC (Waters), Atlantis dC18, 2.1 mm I.D.×20 mm L, 3 μm particle size column (Waters), and TSQ7000 mass spectrometer (ThermoQuest). The mobile phase was consisted of a 10 mM HCO_2NH_4–acetonitrile step gradient 100/0 to 80/20 to 100/0. The concentration of each compound in the apical and the basolateral solution were determined from a peak area *versus* concentration standard curve. For each compound, Eq. 1 was used to calculate an apparent permeability coefficient (P_{app}) from the LC/MS/MS-determined concentration in the basolateral compartment (C_b, μM) and the initial 10 μM concentration in the donor compartment. In the following equation 3600 s is the total time for the measurement of compound flux and 0.33 cm^2 is the area of transwell filter.

$$P_{app}\,(10^{-6}\,\text{cm/s}) = (C_b\times600\,\mu\text{l})/(10\,\mu\text{M}\times3600\,\text{s}\times0.33\,\text{cm}^2) \quad (1)$$

Pharmacokinetic Studies on Rats Male Sprague–Dawley rats (280—360 g, Hilltop Laboratories and Charles River Laboratories) were used. Animals were surgically implanted with catheters in the right and left jugular veins. The jugular catheters were composed of silastic tubing (i.d. 0.02 inch and o.d. 0.037 inch). The lock solution for the catheter contained streptokinase (Kabikinase®, 18750 units/ml) and heparin (500 units/ml) in 25% (w/v) dextrose. Care was taken to apply only the catheter volume (approx 0.05—0.07 ml) of lock solution in order to minimize systemic exposure to this mixture. Animals were housed in clear PVC boxes with lids in unidirectional air flow rooms maintained controlled temperature (20±4 °C) and relative humidity (50%±20%) on a 12 h light–dark cycle. Animals were provided food and tap water *ad libitum*, except for the 12 h fasting (food withdrawn) period prior to test substance administration. For intravenous administration, each compound was dissolved in sterile 0.9% (w/v) saline (pH adjusted to 8.0 with sodium bicarbonate) for a target concentration of 1.0 or 0.75 mg of each compound/ml. Each compound was administered by 2 h intravenous infusion at a targeted dose of 2.4 or 1.5 mg/kg. For oral administration, compound **1** was dissolved in 10% (w/v) aqueous Encapsin® (hydroxypropyl-β-cyclodextrin) for a target concentration of 2 mg/ml and was administered by oral gavage at a targeted dose of 5 mg/kg. Compound **15e** was suspended in 0.5% (w/v) aqueous methylcellulose for a target concentration of 0.2 mg/ml and was administered by oral gavage at a targeted dose of 2 mg/kg. Plasma samples were analyzed on an LC/MS/MS method consisting HP 1100 Binary Pump HPLC (Hewlett-Packard), Columbus, C8, 2 mm×100 mm, 5 μm column (Phenomenex), and Micro Quattro II mass spectrometer (Micromass, Inc) or 2690 Separations Module (Waters), XDB-C18, 2.1×150 mm, 5 μm column (Zorbax), and TSQ 7000 Triple Stage Quadrupole (Finnigan). The mobile phase was consisted of 2% (v/v) formic acid in water/acetonitrile; the gradient condition 90/10 to 10/90) or 2% (v/v) acetic acid in water/acetonitrile; the gradient condition 76/24 to 55/45). Plasma concentra-

tions were analyzed using WinNolin software program (version 1.1, Scientific Consulting, Inc.).

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