Bioorganic & Medicinal Chemistry 21 (2013) 42–61

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

A novel, potent, and orally active VLA-4 antagonist with good aqueous solubility: *trans*-4-[1-[[2-(5-Fluoro-2-methylphenylamino)-7-fluoro-6-benzoxazolyl]acetyl]-(5*S*)-[methoxy(methyl)amino]methyl-(2*S*)-pyrrolidinyl-methoxy]cyclohexanecarboxylic acid

Masaki Setoguchi [a,*], Shin Iimura [a], Yuuichi Sugimoto [a], Yoshiyuki Yoneda [a], Jun Chiba [a], Toshiyuki Watanabe [a], Fumihito Muro [a], Yutaka Iigo [b], Gensuke Takayama [a], Mika Yokoyama [a], Tomoe Taira [a], Misato Aonuma [b], Tohru Takashi [b], Atsushi Nakayama [a], Nobuo Machinaga [a]

[a] *R&D Division, Daiichi Sankyo Co., Ltd, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*
[b] *Daiichi Sankyo RD Novare Co., Ltd, 1-16-13, Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan*

ARTICLE INFO

Article history:
Received 11 September 2012
Revised 31 October 2012
Accepted 2 November 2012
Available online 10 November 2012

Keywords:
VLA-4
Eosinophil infiltration
Asthma
Aqueous solubility

ABSTRACT

We have carried out the optimization of substituents at the C-3 or the C-5 position on the pyrrolidine ring of VLA-4 antagonist **3** with 2-(phenylamino)-7-fluorobenzoxazolyl moiety for the purpose of improving in vivo efficacy while maintaining good aqueous solubility. As a result, we successfully increased in vitro activity in the presence of 3% human serum albumin and achieved an exquisite lipophilic and hydrophilic balance of compounds suitable for oral administrative regimen. The modification resulted in the identification of zwitterionic compound **7n** with (5*S*)-[methoxy(methyl)amino]methylpyrrolidine, which significantly alleviated bronchial hyper-responsiveness to acetylcholine chloride at 12.5 mg/kg, p.o. in a murine asthma model and showed favorable aqueous solubility (JP1, 89 μg/mL; JP2, 462 μg/mL). Furthermore, this compound showed good oral bioavailability (F = 54%) in monkeys.

1. Introduction

Most of circulating monocytes, granulocytes, and lymphocytes express the very late antigen-4 (VLA-4, integrin $\alpha_4\beta_1$, CD49d/CD29) on their surface.[1] The VLA-4 binds to its natural ligands, the vascular cell adhesion molecule-1 (VCAM-1, CD106) expressed on cytokine-stimulated endothelial cells and the alternatively spliced portion of the type III connecting segment of fibronectin (FN).[2,3] It has been well-known that VLA-4 plays an important role in the process of adhesion, migration, invasion, and proliferation of inflammatory cells at the site of inflammation through its interactions. The inappropriate continuation of the cellular process causes tissue damage and dysfunction, resulting in the pathogenesis of inflammatory and autoimmune diseases such as asthma,[4] rheumatoid arthritis,[5] multiple sclerosis (MS)[6] and Crohn's disease (CD).[7] Furthermore, *anti*-α_4 antibodies and small-molecule VLA-4 antagonists have demonstrated efficacy in a wide variety of inflammatory animal models, validating the blockade of the VLA-4/VCAM-1 or FN interaction as a therapeutic target.[8]

Up to date, the humanized monoclonal *anti*-α_4 antibody natalizumab was approved for the treatment of relapsing forms of MS in the US and relapsing and remitting MS in the EU as well as moderately to severely active CD in the US.[9] However, none of the small molecules have reached the marketplace yet. Thus, from a safety and cost point of view, development of an oral small-molecule VLA-4 antagonist could be desired in this field.

We have recently reported that starting with *trans*-4-[(4*S*)-substituted-(2*S*)-pyrrolidinylmethyloxy]cyclohexanecarboxylic acid derivative **1**,[10] we worked on structural modification of the lipophilic moiety in order to improve the poor pharmacokinetic profile, resulting in the identification of 2-(1-methyl-1*H*-indol-3-yl)-1,3-benzoxazole derivative **2** as a potent VLA-4 antagonist.[11,12] Compound **2** demonstrates excellent oral efficacy in a bronchial inflammatory model,[12] however, it has been revealed that its extremely poor aqueous solubility (<0.1 and 8.2 μg/mL in JP1; Japanese Pharmacopeia First Fluid at pH 1.2 and JP2; Japanese Pharmacopeia Second Fluid at pH 6.8, respectively) is troublesome for further standard evaluation to see if it could have the potential as a clinical candidate. In addition, poor solubility of clinical candidates has been recognized as one of the major causes to hamper drug development.[13] Thus, our next objective was to obtain VLA-4 antagonists possessing potent oral efficacy as well as good

* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5740 3640.
E-mail address: setoguchi.masaki.d4@daiichisankyo.co.jp (M. Setoguchi).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.11.003

aqueous solubility. At this point, since other 2-(1-methyl-1*H*-indol-3-yl)-1,3-benzoxazole derivatives similarly showed poor aqueous solubility (data not shown), we concluded that it would be difficult to obviate the drawback without replacing the 2-(1-methyl-1*H*-indol-3-yl)-1,3-benzoxazole structure with another lipophilic structure. In our continuous work on VLA-4 antagonist, we have discovered 2-phenylaminobenzoxazole derivative **3**[11] with potent activity with an IC_{50} value of 2.8 nM and preferable solubility (11 and 489 μg/mL in JP1 and 2). However, compound **3** did not show a significant inhibition of eosinophil infiltration into bronchoalveolar lavage fluid (BALF) in the bronchial inflammatory model at 15 mg/kg, p.o., bid for 2 days. We assumed that the cause of the weak efficacy would be low in vitro activity with an IC_{50} value of 439 nM in the presence of 3% human serum albumin (HSA). Thus, to improve the weak activity, we attempted to optimize substituents on the pyrrolidine ring in compound **3** while fixing 2-(phenylamino)-7-fluorobenzoxazolyl group to keep good aqueous solubility. Regarding substituent at the C-4 position on the pyrrolidine ring in our study, a fluorine atom gave positive results leading to promising compounds which showed good in vivo efficacy in many cases,[10–12,14] however, the effect was unfortunately not observed in the case of compound **3**. Therefore we decided to explore introduction of substituents to the C-3 or the C-5 position (Fig. 1). In addition, we also sought for an appropriate lipophilic and hydrophilic balance of compounds at the same time, which balance would be instrumental to make aqueous solubility compatible with absorption from gastrointestinal tract.

Herein, we report the synthesis, the structure–activity relationships (SAR) and the physicochemical properties of a series of compounds that we synthesized as well as in vivo evaluation results of some of the representative compounds.

2. Chemistry

The general synthesis procedure of designed compounds is illustrated in Scheme 1. Thus, 2-(2-methylphenylamino)-7-fluorobenzoxazolyl acetic acids **4a**–**b**[11] were condensed with pyrrolidines **5a**–**n** using EDC·HCl and HOBt to obtain **6a**–**k** and **6m**–**q**. In the case of **6k**, the methylthiomethyl group in **6k** was converted to the methanesulfonylmethyl group by oxidation using *m*CPBA to give **6l**. The resulting amides **6a**–**q** were subjected to basic hydrolysis to afford the *trans*-4-substituted cyclohexanecarboxylic acids **7a**–**q**. The syntheses of intermediates **5a**–**n** are presented in Schemes 2–6.

The preparation of *trans*-4-[(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate **5a** is depicted in Scheme 2. Starting from commercially available (3*S*)-hydroxyproline (**8**), methyl esterification of the carboxylic acid and protection of the nitrogen atom with benzyloxycarbonyl (*Z*) group gave **9**. Ester **9** was converted to silylether **10** by reduction of the ester group using sodium borohydride and protection of the resulting primary alcohol with *tert*-butyldimethylsilyl (TBS) group. Intermediate **10** was subjected to methylation of the secondary alcohol and desilylation by treatment of tetrabutylammonium fluoride (TBAF) to afford **11**. Condensation of alcohol **11** with methyl 4-hydroxybenzoate by Mitsunobu reaction, followed by deprotection of the *Z* group by hydrogenation on Pd/C furnished benzoate **12**. Reduction of the benzene ring in **12** was achieved by hydrogenation (10 atm) over Rh/Al_2O_3 under acidic condition to afford a mixture of the *cis*- and *trans*-4-substituted cyclohexanecarboxylate (*cis*-rich). Following *N*-Boc protection, isomerization with sodium methoxide in refluxing methanol and subsequent esterification with (trimethylsilyl)diazomethane provided **13** (*cis*/*trans* = ca. 1/1).

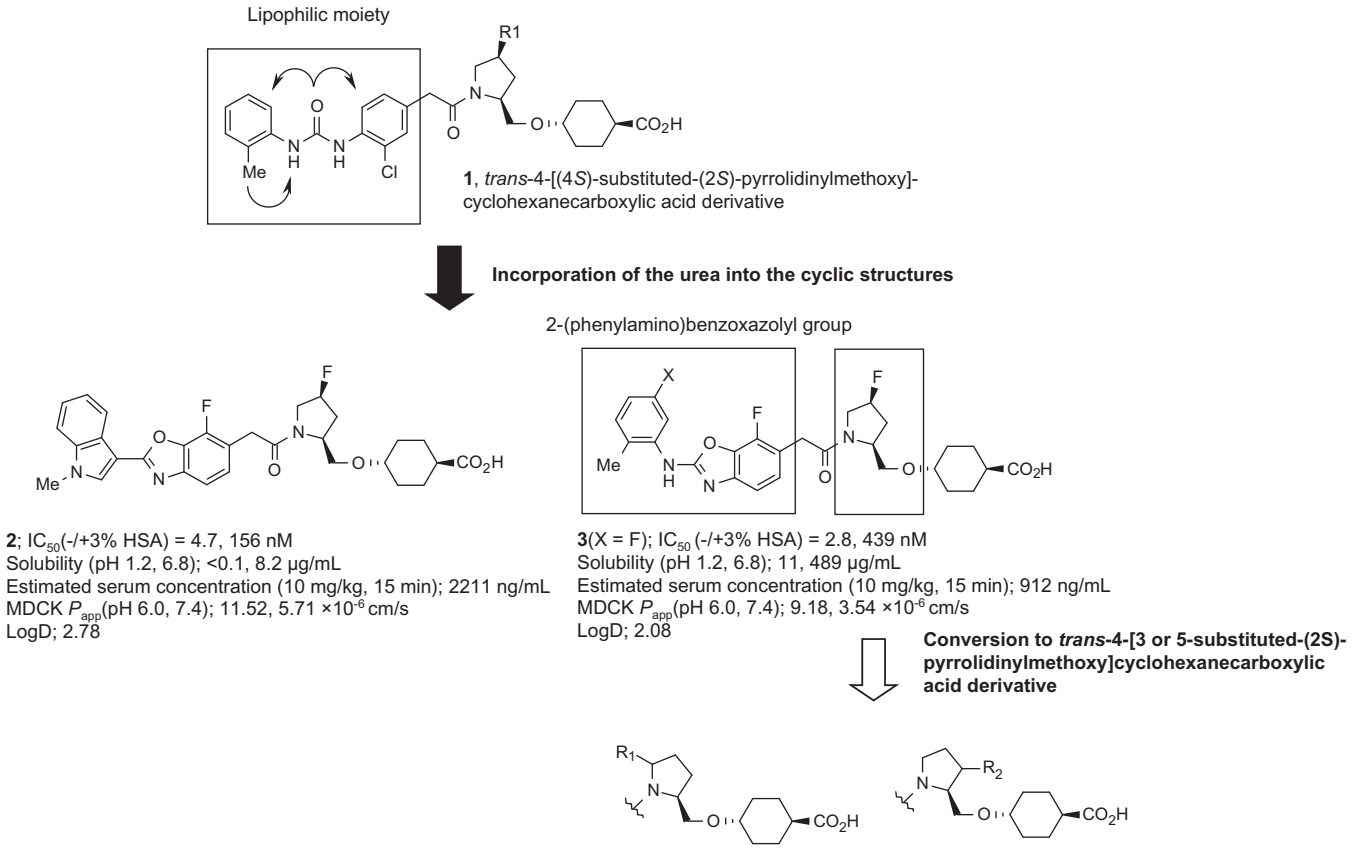
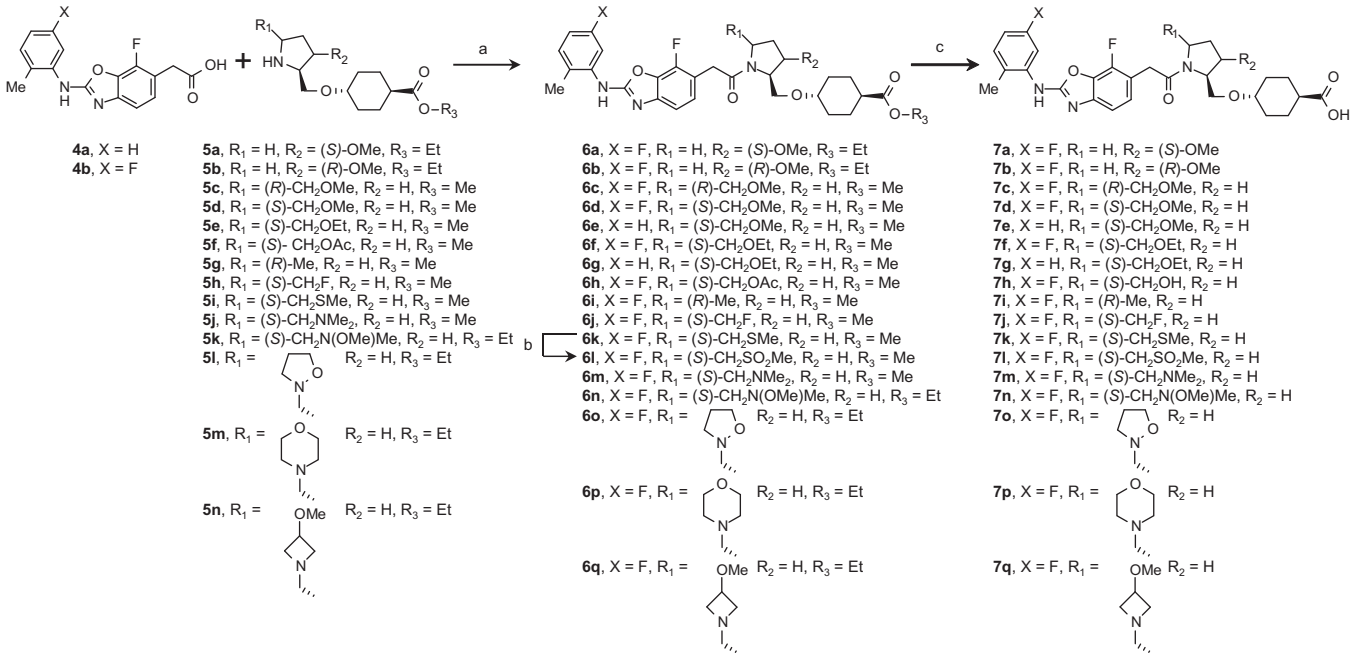
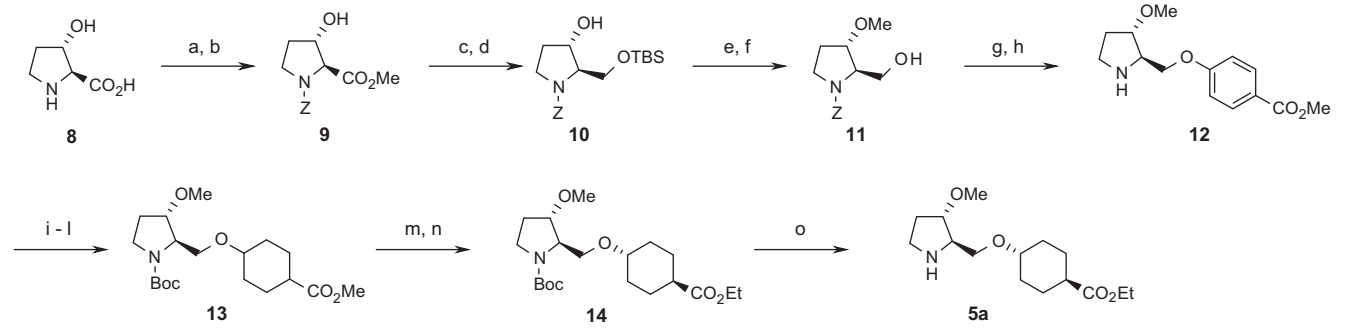


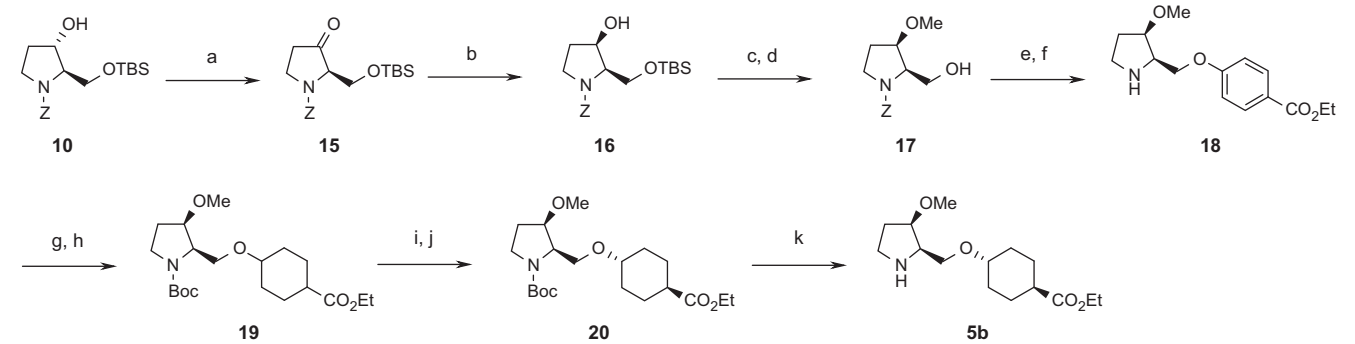
Figure 1. Drug designs of 2-(phenylamino)benzoxazole derivatives.



Scheme 1. Reagents and conditions: (a) EDC·HCl, HOBt, Et_3N, DMF; (b) *m*CPBA, CH_2Cl_2, 0 °C; (c) aq NaOH, THF.

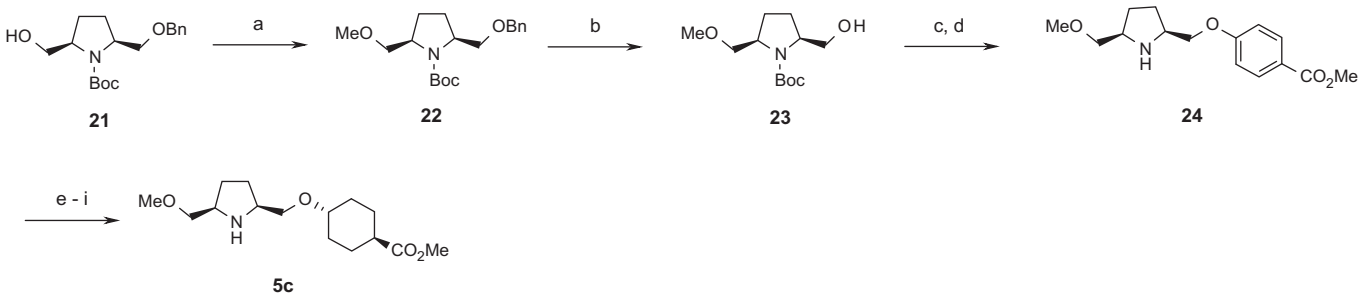


Scheme 2. Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C to rt; (b) ZCl, Et_3N, MeCN/H_2O; (c) $NaBH_4$, MeOH, toluene, 0 °C to rt; (d) TBSCl, Et_3N, CH_2Cl_2, 0 °C to rt; (e) MeI, NaH, DMF, 0 °C to rt; (f) TBAF, THF, 0 °C to rt; (g) methyl 4-hydroxybenzoate, DIAD, Ph_3P, THF, reflux; (h) 10% Pd/C, H_2, MeOH; (i) 5% Rh/Al_2O_3, H_2 (10 atm), MeOH/AcOH; (j) Boc_2O, DMAP, MeCN; (k) NaOMe, MeOH, reflux; (l) $TMSCHN_2$, benzene/MeOH; (m) 1 N NaOH, THF; (n) EtI, K_2CO_3, DMF, 70 °C, then separation; (o) TFA, CH_2Cl_2.

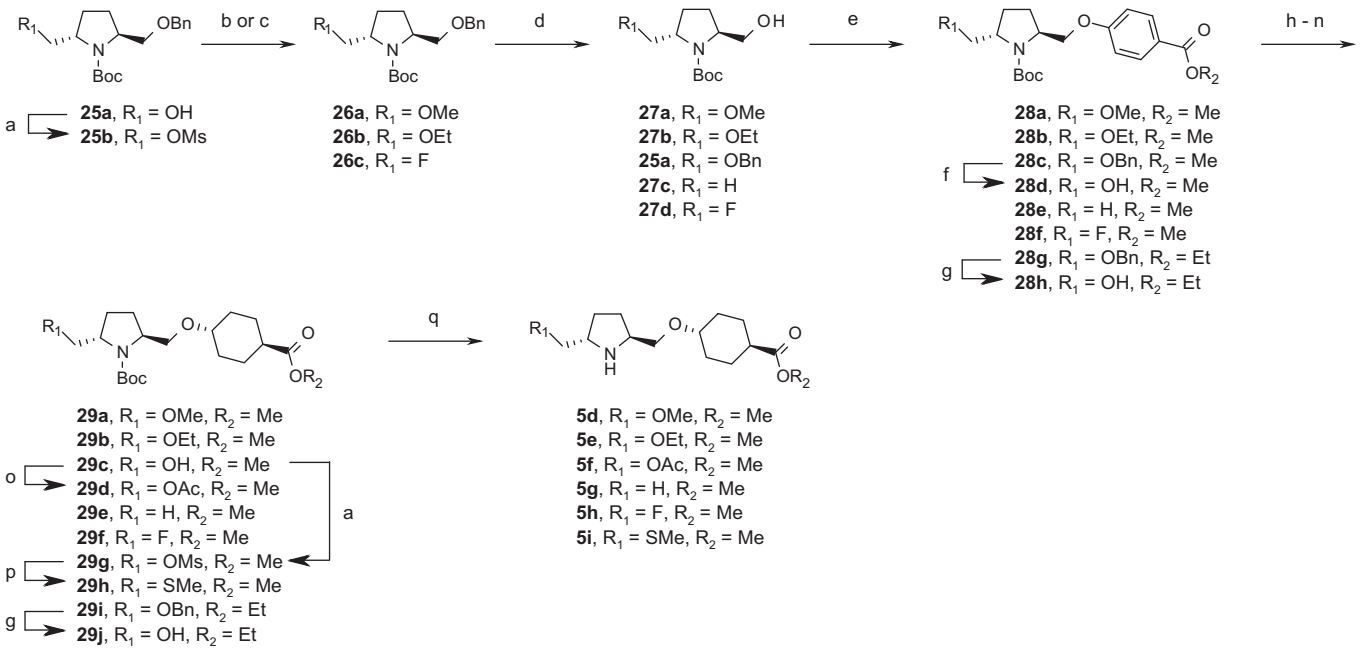


Scheme 3. Reagents and conditions: (a) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, −78 °C; (b) $NaBH_4$, MeOH, −10 °C to rt; (c) MeI, NaH, DMF, 0 °C to rt; (d) TBAF, THF, 0 °C to rt; (e) ethyl 4-hydroxybenzoate, DIAD, Ph_3P, THF, reflux; (f) $Pd(OH)_2$, H_2, MeOH; (g) 5% Rh/Al_2O_3, H_2 (7 atm), MeOH/TFA; (h) Boc_2O, MeCN, satd $NaHCO_3$; (i) NaOEt, EtOH, reflux; (j) EtI, K_2CO_3, DMF, 60 °C, then separation; (k) TFA, CH_2Cl_2.

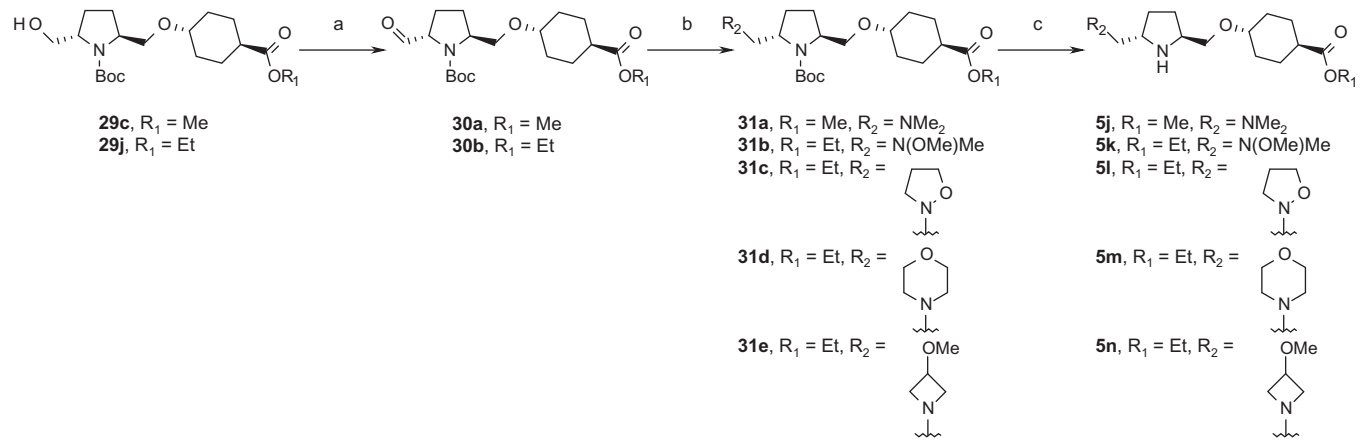
After the methyl ester group in **13** was transformed to an ethyl ester group via basic hydrolysis and esterification using EtI in order to easily isolate the *trans*-isomer, isolation of the *trans*-isomer was successfully performed by flash column chromatography to furnish compound **14**. Removal of the Boc group in **14** by TFA treatment produced the *trans*-4-substituted cyclohexanecarboxylate **5a**. *trans*-4-[(3*R*)-Methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate **5b** was prepared as shown in Scheme 3. Swern's oxidation



Scheme 4. Reagents and conditions: (a) MeI, NaH, DMF, 0 °C to rt; (b) 10% Pd/C, H_2, MeOH; (c) methyl 4-hydroxybenzoate, DIAD, Ph_3P, THF, reflux; (d) TFA, CH_2Cl_2; (e) Rh/Al_2O_3, H_2 (10 atm), MeOH/TFA; (f) Boc_2O, 1,4-dioxane, satd $NaHCO_3$; (g) NaOMe, MeOH, reflux; (h) $TMSCHN_2$, benzene /MeOH, then separation; (i) TFA, CH_2Cl_2.



Scheme 5. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, 0 °C; (b) MeI (for **26a**) or EtI (for **26b**), NaH, DMF, 0 °C to rt; (c) Bu_4NF, THF, reflux (for **26c**); (d) Pd/C, H_2, MeOH; (e) methyl 4-hydroxybenzoate (for **28a–c** and **28e–f**) or ethyl 4-hydroxybenzoate (for **28g**), DIAD, Ph_3P, THF, rt or heating; (f) Pd/C, H_2, EtOH; (g) $Pd(OH)_2$, H_2, EtOH/AcOH; (h) TFA, CH_2Cl_2 (for **29b**, **29e** and **29i**); (i) Rh/AlO_3, H_2 (3.5–10 atm), EtOH/AcOH (for **29a** and **29c**), MeOH/AcOH (for **29b** and **29f**), MeOH (for **29e**) or EtOH/AcOH/TFA (for **29i**); (j) Boc_2O, Et_3N, CH_2Cl_2 (for **29b** and **29i**) or MeCN/H_2O (for **29e**); (k) BnBr, NaH, DMF (only for **29i**); (l) NaOMe, MeOH, reflux (for **29a**, **29c**, **29e** and **29f**); (m) NaH, MeOH (for **29b**) or NaH, EtOH (for **29i**), DMF, 0 °C to rt; (n) $TMSCHN_2$, benzene or toluene/MeOH (for **29a–c**, **29e** and **29f**) or H_2O, K_2CO_3, EtI (for **29i**), then separation; (o) $(AcO)_2O$, pyridine; (p) NaSMe, DMF, 60 °C, then $TMSCHN_2$, benzene/MeOH, 0 °C to rt; (q) TFA, CH_2Cl_2 or 4 N HCl/dioxane.



Scheme 6. Reagents and conditions: (a) TEMPO, trichloroisocyanuric acid, CH_2Cl_2, 0 °C to rt; (b) Me_2NH (for **31a**), *N*-methoxymethanamine hydrochloride (for **31b**), isoxazolidine hydrochloride (for **31c**), morpholine (for **31d**), or 3-methoxyazetidine hydrochloride (for **31e**), $NaBH(OAc)_3$, THF; (c) 4 N HCl/dioxane or TFA, CH_2Cl_2.

of the hydroxyl group in intermediate **10**, followed by stereo specific reduction of the resulting ketone **15** using $NaBH_4$ afforded (3*R*)-hydroxypyrrolidine **16**. Alcohol **16** was subjected to methylation, desilylation, Mitsunobu reaction, and deprotection of the *Z* group to provide **18**. In a procedure similar to that described for the preparation of **5a**, benzoate **18** was converted to *trans*-4-substituted cyclohexanecarboxylate **5b**.

The preparation of methyl *trans*-4-[(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**5c**) is depicted in Scheme 4. Methylation of the hydroxyl group in compound **21**[15] and removal of the benzyl group provided alcohol **23**, which was subjected to Mitsunobu reaction with methyl 4-hydroxybenzoate and deprotection of the Boc group to furnish **24**. In a procedure similar to that described for the preparation of **5a**, benzoate **24** was converted to **5c**.

4-(2,5-*trans*-Substituted-pyrrolidinylmethoxy)cyclohexanecarboxylates **5d–i** were synthesized as shown in Scheme 5.

Alkylation or fluorination via mesylation of the hydroxyl group in **25a**[16] gave **26a–c**, in which the benzyl group was removed by hydrogenation to provide **27a–b** and **27d**. Condensation of **25a** and **27a–d**[17] with methyl or ethyl 4-hydroxybenzoate by Mitsunobu reaction gave **28a–c** and **28e–g**, in which **28c** and **28g** were subjected to hydrogenation to give the alcohols **28d** and **28h**. In a procedure similar to that described for the preparation of **5a**, those benzoates were converted to the corresponding *trans*-cyclohexanecarboxylates **29a–c**, **29e–f**, and **29i**. Compound **29c** was further converted to **29d** by acetylation of the hydroxyl group and **29h** by substitution with a methylthio group via mesylation. In addition, **29i** was hydrogenated to give alcohol **29j**. The resulting *N*-Boc derivatives were treated with TFA or hydrochloride to furnish the target intermediates **5d–i**.

The preparation of (5*S*)-acyclic or cyclicaminomethylpyrrolidine derivatives **5j–5n** was shown in Scheme 6. Oxidation of (5*S*)-hydroxymethylpyrrolidine derivatives **29c** and **29j** by treatment with 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) and trichloroisocyanuric acid, followed by reductive amination of the resulting aldehyde **30a–b** with dimethylamine, *N*-methoxymethanamine hydrochloride, isoxazolidine hydrochloride, morpholine, or 3-methoxyazetidine hydrochloride gave the corresponding (5*S*)-aminomethyl derivatives **31a–e**, in which the Boc group was removed under acidic conditions to furnish **5j–n**.

3. Results and discussion

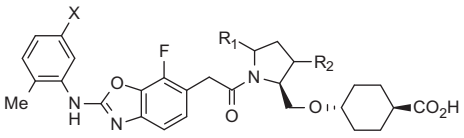
3.1. In vitro activity and physicochemical properties

The synthesized compounds (**7a–q**) were evaluated for their VLA-4 inhibitory activities in an established receptor binding assay (VLA-4 overexpressed Chinese hamster ovary cells/Human VCAM-1 labeled with Europium) with or without the addition of 3% HSA. In addition, we determined their solubility in the Japanese Pharmacopoeia fluids (JP1, at pH 1.2 and JP2, at pH 6.8) and distribution coefficient (Log*D* at pH 6.8) to predict the oral absorption in this study, because compound **3** exhibits acceptable oral exposure due to good membrane permeability (P_{app} value = 3.54×10^{-6} cm/s) in a permeability assay using Madin-Darby canine kidney (MDCK) cell monolayers,[18] which is likely based on its acceptable Log*D* value of 2.08.

The evaluation results are shown in Tables 1 and 2. At first, we examined the effect of a substituent at the C-3 position in the pyrrolidine ring on inhibitory activity. (3*S*)-Methoxypyrrolidine **7a** was less potent than (3*R*)-methoxypyrrolidne **7b** (**7a**, IC_{50} = 16 nM; **7b**, IC_{50} = 4.3 nM), however, in the presence of 3% HSA, both of them showed relatively low activity with IC_{50} values of more than 100 nM (Table 1).

Next, we examined the introduction of several substituents at the C-5 position in the pyrrolidine ring (Table 1). Compound **7c–7h** with an alkoxymethyl or hydroxymethyl group exhibited good potent activities with IC_{50} values of 1.1–3.7 nM and less than 100 nM (+3% HSA), finding that a fluorine atom at the C-5 position (X) in the terminal benzene ring was not critical for the activity. In comparison between compound **7c** and **7d**, it was shown that the absolute configuration at the C-5 position (R_1) did not affect the activity. Alternatively, introduction of a methyl (**7i**), a fluoromethyl (**7j**), a methylthiomethyl (**7k**) or a methylsulfonylmethyl group (**7l**) resulted in a slight loss of potency (**7i**, IC_{50} = 3.6 nM; **7j**, IC_{50} = 3.7 nM; **7k**, IC_{50} = 2.9 nM; **7l**, IC_{50} = 5.5 nM) as well as activities with IC_{50} values of more than 100 nM in the presence of 3%

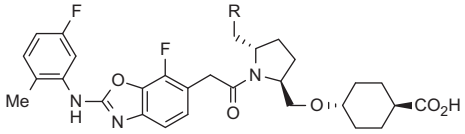
Table 1
Inhibitory activity, solubility and distribution coefficient of 2-(phenylamino)benzoxazole derivatives **7a–7l**

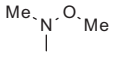
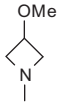


Compound	X	R1	R2	IC_{50} (nM) (±3% HSA)	Solubility (μg/mL)		Log*D* (pH 6.8)
					JP1[a]	JP2[b]	
2	—	—	—	4.7/156	<0.1	8.2	2.78
3	—	—	—	2.8/439	11	489	2.08
7a	F	H	(*S*)-OMe	16/132	31	1711	1.95
7b	F	H	(*R*)-OMe	4.3/144	28	865	2.04
7c	F	(*R*)-CH_2OMe	H	3.7/36	11	560	2.54
7d	F	(*S*)-CH_2OMe	H	1.1/42	11	833	2.49
7e	H	(*S*)-CH_2OMe	H	1.8/37	66	1200	2.17
7f	F	(*S*)-CH_2OEt	H	1.7/79	3.5	340	2.94
7g	H	(*S*)-CH_2OEt	H	1.7/90	35	440	2.57
7h	F	(*S*)-CH_2OH	H	1.7/31	49	1599	1.8
7i	F	(*R*)-Me	H	3.6/184	5	292	2.81
7j	F	(*S*)-CH_2F	H	3.7/137	3	194	2.6
7k	F	(*S*)-CH_2SMe	H	2.9/202	1	111	3.2
7l	F	(*S*)-CH_2SO_2Me	H	5.5/132	20	1109	1.5

[a] JP1; Japanese Pharmacopeia First Fluid at pH 1.2.
[b] JP2; Japanese Pharmacopeia Second Fluid at pH 6.8.

Table 2
Inhibitory activity, solubility and distribution coefficient of 2-(phenylamino)benzoxazole derivatives **7m**–**7q**

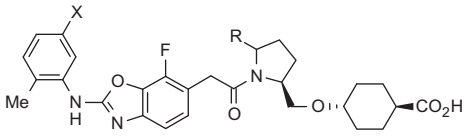


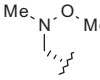
Compound	R	IC_{50} (nM) (±3% HSA)	Solubility (μg/mL)		Log*D* (pH 6.8)
			JP1	JP2	
7m		6.8/21	1945	1875	0.6
7n		1.7/86	89	462	2.57
7o		2.2/75	>2000	1225	2.26
7p		1.2/9	1900	1300	1.85
7q		4.2/35	>670	>670	1.1

HSA. Regarding aqueous solubility, all compounds exhibited preferable solubility in JP2, however, **7f** and **7i**–**7k** showed low solubility of less than 10 μg/mL in JP1. On the other hand, all the compounds except **7a**, **7h** and **7l** showed relatively appropriate Log*D* values of more than 2.0, suggesting that the compounds would have appropriate cell permeability leading to good oral exposure. In addition, it was found that compounds **7e** and **7g** without a fluorine atom at the C-5 position (X) in the terminal benzene ring showed a decrease in log*D* value by 0.32 and 0.37, respectively, in comparison with the corresponding compounds **7d** and **7f**, implying that the introduction of a fluorine atom at that position should have a positive effect to enhance lipophilicity.

Since introduction of the substituents into the C-5 position in the pyrrolidine ring gave relatively good results for both activity and physicochemical properties, we next investigated the introduction of basic substituents at this position as described in Table 2. In this modification, we fixed the fluorine atom at the 5-position on the terminal benzene ring in order to maintain the moderate lipophilicity. All the compounds exhibited potent activities with IC_{50} values of less than 100 nM even in the presence of 3% HSA, and showed preferable solubility in both JP1 and JP2 due to zwitterionic character of the compounds. On the other hand, **7n** and **7o** showed acceptable log*D* values of 2.57 and 2.26, respectively, however, the others showed likely insufficient log*D* values (**7m**, Log*D* = 0.6; **7p**, Log*D* = 1.85; **7q**, Log*D* = 1.1).

Table 3
Anti-inflammatory effect in an *Ascaris*-antigen-induced murine bronchial inflammatory model



Compound	X	R	Inhibition of the eosinophil infiltration (%)	
			5 mg/kg	15 mg/kg
7c	F	(*R*)-$MeOCH_2$	14	12
7f	F	(*S*)-$EtOCH_2$	24	23
7g	H	(*S*)-$EtOCH_2$	8	42
7n	F		44*	68[a,**]

[a] Compound **7n** was administered at 12.5 mg/kg.
* p <0.05, significant difference from the control (Dunnett's multiple comparison test).
** p <0.01; significant difference from the control (Dunnett's multiple comparison test).

3.2. In vivo efficacy

On the basis of the potency in the presence of 3% HSA (IC_{50} <100 nM) and lipophilicity (Log*D* >2.5), we selected compounds **7c**, **7f**, **7g** and **7n**, evaluating their anti-inflammatory effect in an *Ascaris*-antigen-induced murine bronchial inflammatory model by measuring the level of eosinophils in bronchoalveolar lavage fluid (BALF) at 48 h after the antigen challenge. The results are summarized in Table 3. Oral administration of **7n** significantly reduced eosinophil infiltration into BALF by 68% at 12.5 mg/kg and 44% at 5 mg/kg. On the other hand, compound **7c**, **7f** and **7g** didn't show significant efficacy in this model, implying that the concentration in blood and/or the duration might be less than that of **7n**.

Next, compound **7n** showing excellent efficacy in the inflammation model was evaluated in an actively sensitized murine asthma model, assessing its ability to reduce bronchial hyper-responsiveness (BHR) to acetylcholine chloride (ACh) at 48 h after the antigen challenge. As shown in Figure 2, intratracheal antigen challenge induced BHR. Compound **7n** reduced the response in a dose-dependent manner, and higher dose of the compound reduced the response to the level of the antigen non-challenged group.

3.3. Pharmacokinetic properties

Concerning compound **7n** exhibiting favorable efficacy in the murine asthma model, we investigated the pharmacokinetic profile in monkeys. As shown in Table 4, compound **7n** showed good

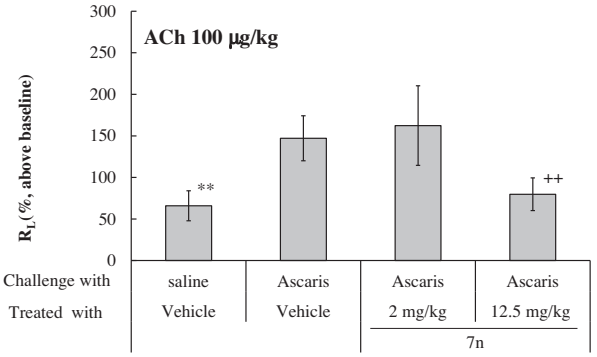


Figure 2. Effect of compound **7n** on antigen induced bronchial hyper-responsiveness (BHR) to acetylcholine chloride (ACh) in *Ascaris suum* sensitized mice. Female BALB/c mice sensitized with *Ascaris suum* extract were used. The mice were challenged intratracheally with saline or *Ascaris suum* extract. Vehicle or **7n** were given to the mice p.o. four times (0.25 h before and 8, 24 and 32 h after the challenge). Then BHR to ACh was measured 2 days after the antigen challenge. Each column represents mean ± S.D. of eight animals. $^{++}$ p <0.01; significant difference from the control (Dunnett's multiple comparison test), $^{**}p$ <0.01; significant difference from the control (student's t-test).

oral exposure (AUC_∞ = 1253 ng h/mL at 0.5 mg/kg) and bioavailability (F = 54%).

4. Conclusion

In this study, we explored the effects of substituents at the C-3 or the C-5 position in the pyrrolidine ring of 2-(phenylamino)-7-fluorobenzoxazolyl derivative **3** on in vitro activity and physicochemical properties. Consequently, we made it clear that introduction of substituents into the C-5 position was effective to maintain high activity in the presence of 3% HSA while adjusting lipophilic and hydrophilic balance of compound suitable for oral administration regimen, resulting in the identification of zwitterionic compound **7n** with (5*S*)-[methoxy(methyl)amino]methylpyrrolidine moiety, which demonstrated excellent efficacy at oral dosing (12.5 mg/kg) in a murine asthma model as well as favorable aqueous solubility (JP1, 89 μg/mL; JP2, 462 μg/mL). Furthermore, this compound showed good oral bioavailability (F = 54%) in monkeys.

5. Experimental

5.1. General

All starting materials and synthesis reagents were obtained commercially. Column chromatography was performed with a Merck silica gel 60 (particle size 0.060–0.200 or 0.040–0.063). Flash column chromatography was performed with Biotage FLASH Si or YAMAZEN Hi-Flash packed columns. Thin-layer chromatography (TLC) was performed on Merck precoated TLC glass sheets with silica gel 60 F254. Yields were of purified products and were not optimized. The ^{1}H NMR spectra were recorded on a JEOL JNM-EX-400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane as an internal standard. The special splitting patterns are designated as follows: s, singlet; d, doublet; dd, double of doublet; t, triplet; td, triple of doublet; tt, triple of triplet; q, quartet; m, multiple. The IR spectra were recorded on a HORIBA FT-720 spectrometer. The mass spectra were recorded on a SCIEX API-150EX spectrometer (ESI). The high-resolution mass (HRMS) spectra were recorded on a JEOL JMS-T100CS spectrometer. Elemental analysis was performed using a PerkinElmer CHNS/O 2400II, a Leco CHNS-932 and a YOKOKAWA analysis IC7000RS.

5.2. General procedure A: preparation of ethyl *trans*-4-[1-[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl] acetyl]-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6a)

A mixture of [7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetic acid (**4b**) (199 mg, 0.63 mmol), ethyl *trans*-4-[(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**5a**) (170 mg, 0.63 mmol), EDC·HCl (180 mg, 0.94 mmol), HOBt (127 mg, 0.94 mmol), and Et_3N (130 μl, 0.93 mmol) in DMF (5 mL) was stirred at room temperature for 2 h. The mixture was diluted with H_2O and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (30:1, v/v) as eluent to give the title compound (323 mg, 88%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.14–1.28 (m, 5H), 1.37–1.48 (m, 2H), 1.91–2.09 (m, 5H), 2.17–2.24 (m, 2H), 2.31 (s, 3H), 3.20–3.90 (series of m, total 11H, including s, 3H, at δ 3.33), 4.07–4.13 (m, 2H), 4.23–4.26 (m, 1H), 6.72–6.76 (m, 1H), 7.08–7.15 (m, 2H), 7.21–7.23 (m, 1H), 7.41 (broad s, 1H), 8.04–8.07 (m, 1H); MS (ESI), m/z 586 $[M+H]^+$.

Compound **6b–6i**, **6k**, and **6m–6n** were prepared according to general procedure A.

5.3. Ethyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(3*R*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6b)

Yield 100%. A brown viscous oil. ^{1}H NMR ($CDCl_3$) δ 1.19–1.27 (m, 5H), 1.39–1.49 (m, 2H), 1.82–2.28 (series of m, total 7H), 2.30 (s, 3H), 3.16–3.29 (m, 1H), 3.41 and 3.42 (each s, total 3H), 3.44–3.78 (series of m, total 5H), 3.90–4.00 (m, 2H), 4.07–4.15 (m, 2H), 4.28–4.32 (m, 1H), 6.72–6.77 (m, 1H), 7.05–7.24 (m, 4H), 8.07–8.09 (m, 1H); MS (ESI), m/z 586 $[M+H]^+$.

5.4. Methyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6c)

Yield 99%. A pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.33 (m, 2H), 1.40–1.53 (m, 2H), 1.86–2.11 (m, 8H), 2.23–2.27 (m, 1H), 2.30 (s, 3H), 3.19–3.28 (m, 1H), 3.34–3.71 (m, total 10H), 3.83–3.97

Table 4
Pharmacokinetic properties of **7n** in monkeys (n = 3)

F [a](%)	p.o. (0.5 mg/kg)			iv (0.5 mg/kg)			
	AUC_∞ [b] (ng h/mL)	C_{max} [c] (ng/mL)	$T_{1/2}$ [d] (h)	AUC_∞ (ng h/mL)	CL [e] (mL/min/kg)	V_{dss} [f] (L/kg)	$T_{1/2}$ (h)
54	1253	511	1.7	2324	3.8	0.125	6.5

[a] Oral bioavailability.
[b] Pharmacokinetic area under curve.
[c] Pharmacokinetic maximum concentration.
[d] Plasma half-life.
[e] Pharmacokinetic clearance.
[f] Volume of distribution.

(m, 2H), 4.19–4.25 (m, 2H), 6.72–6.76 (m, 1H), 7.05–7.35 (m, 4H), 8.05 (dd, J = 2.4, 10.7 Hz, 1 H); MS (ESI), m/z 586 $[M+H]^+$.

5.5. Methyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6d)

Yield 87%. A colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.08–2.30 (m, 13H), 2.31 (s, 3H), 3.09–3.48 (m, 6H, including each s, total 3H, at δ 3.30 and 3.38), 3.49–3.70 (m, 5H, including each s, total 3H, at δ 3.63 and 3.67), 3.73–3.85 (m, 1H), 3.87–3.96 (m, 1H), 4.08–4.29 (m, 2H), 6.70–6.79 (m, 1H), 7.09–7.17 (m, 2H), 7.20–7.25 (m, 1H), 8.04–8.12 (m, 1H); MS (ESI), m/z 586 $[M+H]^+$.

5.6. Methyl *trans*-4-[1-[[7-fluoro-2-(2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methoxymethyl-(2*S*)-pyrrolidinyl methoxy]cyclohexanecarboxylate (6e)

Yield 91%. A colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.06–1.54 (m, 4H), 1.80–2.10 (m, 7H), 2.11–2.42 (m, 5H, including each s, total 3H, at δ 2.35 and 2.36), 3.09–3.47 (m, 6H, including each s, total 3H, at δ 3.30 and 3.38), 3.48–3.70 (m, 5H, including each s, total 3H, at δ 3.63 and 3.66), 3.72–3.84 (m, 1H), 3.85–3.95 (m, 1H), 4.05–4.29 (m, 2H), 7.04–7.16 (m, 2H), 7.17–7.36 (m, 4H), 8.05–8.12 (m, 1H); MS (ESI), m/z 568 $[M+H]^+$.

5.7. Methyl *trans*-4-[(5*S*)-ethoxymethyl-1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6f)

Yield 80%. A colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.13 and 1.22 (each t, J = 6.8 Hz, total 3H), 1.11–1.53 (series of m, 4H), 1.85–2.08 (m, 7H), 2.15–2.32 (m, 2H), 2.32 (s, 3H), 3.11–3.64 (series of m, 7H), 3.63 and 3.67 (each s, total 3H), 3.77–3.96 (series of m, 2H), 4.11–4.25 (m, 2H), 6.73–6.77 (m, 1H), 7.13–7.16 (m, 2H), 7.22–7.25 (m, 2H), 8.09 and 8.12 (each t, J = 3.2 Hz, total 1H); MS (ESI), m/z 600 $[M+H]^+$.

5.8. Methyl *trans*-4-[(5*S*)-ethoxymethyl-1-[[7-fluoro-2-(2-methylphenylamino)-6-benzoxazolyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6g)

Yield 92%. A pale yellow solid. IR (ATR) 2941, 1732, 1635, 1576 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.13 and 1.22 (each t, J = 7.1 Hz, total 3H), 1.26 (m, 2H), 1.44 (m, 2H), 1.87–2.08 (series of m, total 7H), 2.22 (m, 2H), 2.35 (m, 3H), 3.18 (m, 1H), 3.38 (m, 2H), 3.51 (m, 3H), 3.63 and 3.66 (each s, total 3 H), 3.64 (m, 1H), 3.76–3.94 (m, 2H), 4.22 (m, 2H), 6.92 (m, 1H), 7.08 (t, J = 7.2 Hz, 1H), 7.12 (d, J = 7.2 Hz, 1H), 7.22 (m, 2H), 7.33 (d, J = 7.2 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H); MS (ESI), m/z 582 $[M+H]^+$; Anal. Calcd for $C_{32}H_{40}FN_3O_6$: C, 66.08; H, 6.93; N, 7.22. Found: C, 65.94; H, 6.92; N, 7.03.

5.9. Methyl *trans*-4-[(5*S*)-acetoxymethyl-1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6h)

Yield 67%. A pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.06–1.55 (series of m, total 4H), 1.57–2.41 (series of m, total 15H, including each s, each 3H, at δ 2.02 and 2.11), 3.06–4.53 (series of m, total 12H, including s, 3H, at δ 3.67), 6.75 (m, 1H), 7.02–7.39 (series of m, total 3H), 8.08 (m, 1H).

5.10. Methyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6i)

Yield 88%. A pale yellow solid. IR (ATR) 2941, 1730, 1637, 1610, 1575 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.11–1.63 (series of m, total 8H), 1.88–2.31 (series of m, total 11H) 3.12–3.26 (m, total 1H), 3.34–3.47 (m, total 1H), 3.62–3.92 (series of m, total 6H, including s, total 3 H, at δ 3.63 and 3.67), 4.16–4.26 (series of m, total 2H), 6.74 (dt, J = 2.8 and 8.0 Hz, 1H), 7.10–7.16 (series of m, total 2H), 7.21 and 7.23 (each d, J = 4.4 Hz, total 2H), 8.06–8.10 (m, 1H); MS (ESI), m/z 556 $[M+H]^+$; Anal. Calcd for $C_{30}H_{35}F_2N_3O_5{\cdot}0.75H_2O$: C, 63.31; H, 6.46; N, 7.38. Found: C, 63.40; H, 6.27; N, 7.22.

5.11. Methyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methylthiomethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6k)

Yield 100%. A pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.10–1.55 (series of m, total 4H), 1.89–2.17 (m, total 10H, including s, 3H, at δ 2.13), 2.18–2.35 (m, total 5H, including each s, total 3H, at δ 2.30 and 2.31), 2.53–3.45 (series of m, total 3H), 3.54 (m, 1H), 3.59–3.70 (m, 4H, including s, 3H, at δ 3.67), 3.80 (d, J = 16.0 Hz, 1H), 3.88 (d, J = 16.0 Hz, 1H), 4.10-4.29 (m, 2H), 6.74 (m, 1H), 7.06–7.29 (m, 4H), 8.07 (br d, J = 10.8 Hz, 1H); MS (ESI), m/z 602 $[M+H]^+$.

5.12. Methyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-dimethylanimomethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6m)

Yield 73%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.09–1.57 (m, 4H), 1.88–2.71 (series of m, total 20H, including each s, 3H at δ 2.28 and 6H at δ 2.31,), 3.09–3.31 (m, 1H), 3.35–3.96 (series of m, total 7H, including each s, total 3H, at δ 3.63 and 3.67), 4.01–4.28 (m, 2H), 6.70–6.79 (m, 1H), 7.06–7.32 (m, 4H), 8.03–8.13 (m, 1H); MS (ESI), m/z 599 $[M+H]^+$.

5.13. Ethyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-[methoxy(methyl)amino]methyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6n)

Yield 81%. A yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.13–1.52 (m, 7H), 1.83–2.27 (m, 5H), 2.31 (s, 3H), 2.53–2.61 (m, 1H), 2.62 (s, 3H), 2.75–2.84 (m, 1H), 3.06–3.31 (m, 1H), 3.34–3.51 (m, 3H), 3.52 (s, 3H), 3.64–4.00 (m, 2H), 4.03–4.32 (m, 3H), 4.04–4.04 (m, 4H), 6.74 (td, J = 2.5 and 8.2 Hz, 1H), 7.11–7.25 (m, 4H), 8.04–8.13 (m, 1H); MS (ESI), m/z 629 $[M+H]^+$.

5.14. General procedure B: preparation of methyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl] acetyl]-(5*S*)-fluoromethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6j)

Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-fluoromethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**29f**) (280 mg, 0.750 mmol) was taken up in 4 N HCl–dioxane (10 mL). The mixture was stirred for 3 h and concentrated under reduced pressure to give methyl *trans*-4-[(5*S*)-fluoromethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate hydrochloride (**5h**) as a colorless solid. Compound **5h** was taken up in DMF (10 mL) and **4b** (239 mg,

0.75 mmol), Et_3N (552 μl, 3.75 mmol), EDC·HCl (216 mg, 1.13 mmol), and HOBt (153 mg, 1.13 mmol) were added. After 72 h stirring, the mixture was diluted with EtOAc (300 mL), followed by washing with brine (2 × 100 mL), drying over $MgSO_4$, and concentration under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$–EtOAc (4:1, v/v) as eluent to give the title compound (370 mg, 86% for two steps) as a pale yellow foam. ^{1}H NMR ($CDCl_3$) δ 1.11–2.30 (series of m, total 16H), 3.09–4.75 (series of m, total 12H), 6.70–6.75 (m, 1H), 6.98 (m, 1H), 7.08–7.15 (m, 2H), 7.21–7.24 (m, 1H), 8.07–8.10 (m, 1H); MS (ESI), *m*/*z* 574 $[M+H]^+$.

Compound **6o**–**6q** were prepared from **31c**–**31e** according to general procedure B.

5.15. Ethyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methyl phenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-(1-isoxazolidinyl methyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6o)

For ethyl *trans*-4-[(5*S*)-(1-isoxazolidinylmethyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate dihydrochloride (**5l**); A brown oil. MS (ESI), *m*/*z* 341 $[M+H]^+$.

For **6o**; Yield 63% (two steps). A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.06–1.56 (m, 8H), 1.85–2.37 (series of m, total 14H), 2.88 and 2.96 (each s, total 3H), 3.09–3.60 (m, 2H), 3.66–4.02 (m, 4H), 4.04–4.40 (m, 4H), 6.74 (td, *J* = 2.5 and 8.1 Hz, 1H), 6.98–7.24 (m, 3H), 7.40 (s, 1H), 8.00–8.09 (m, 2H); MS (ESI), *m*/*z* 641 $[M+H]^+$.

5.16. Ethyl *trans*-4-[1-[[7-fluoro-2-[[5-fluoro-2-methyl phenyl]amino]-6-benzoxazolyl]acetyl]-(5*S*)-(4-morpholinyl) methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6p)

Yield 80% (two steps). A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.12–1.55 (m, 7H), 1.90–2.75 (series of m, total 16H), 3.15–4.22 (series of m, total 15H), 6.72–6.77 (m, 1H), 7.13–7.27 (m, 3H), 8.09–8.12 (m, 1H).

5.17. Ethyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenyl amino)-6-benzoxazolyl]acetyl]-(5*S*)-(3-methoxyazetidinyl methyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6q)

For ethyl *trans*-4-[(5*S*)-(3-methoxyazetidinylmethyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate dihydrochloride (**5n**); ^{1}H NMR (DMSO-d_6) δ 1.13–1.29 (m, 5H, including t, *J* = 7.0 Hz, 3H, at δ 1.17), 1.30–1.44 (m, 2H), 1.50–1.72 (m, 2H), 1.86–1.96 (m, 2H), 1.98–2.11 (m, 3H), 2.12–2.21 (m, 1H), 2.22–2.31 (m, 1H), 3.25 (s, 3H), 3.29–3.93 (m, 8H), 3.94–4.10 (m, 3H, including q, *J* = 7.0 Hz, 2H, at δ 4.04), 4.11–4.56 (m, 3H), 8.99–9.33 (m, 1H), 9.42–9.72 (m, 1H), 10.83–11.18 (m, 1H); MS (ESI), *m*/*z* 355 $[M+H]^+$.

For **6q**; Yield 56% (two steps). A yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.08–1.55 (m, 8H), 1.83–2.67 (series of m, total 10H), 2.77–4.40 (series of m, total 20H), 6.74 (t, *J* = 8.2 Hz, 1H), 7.06–7.16 (m, 2H), 7.20 and 7.21 (each t, *J* = 2.2 Hz, total 1H), 7.41 (s, 1H), 8.00–8.07 (m, 1H).

5.18. Methyl *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methanesulfonylmethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (6l)

To a stirred solution of **6k** (154.9 mg, 0.257 mmol) in CH_2Cl_2 (3.0 mL) was added *m*CPBA (133.3 mg, 0.772 mmol) at 0 °C. After stirring for 1.5 h at 0 °C, the reaction mixture was diluted with $CHCl_3$–MeOH (10:1, v/v), washed with satd $NaHCO_3$ aq, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (30:1, v/v) to give the title compound (193.0 mg, 100%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.18–1.35 (m, 2H), 1,39–1.69 (m, 2H), 1.90–2.40 (series of m, total 12H, including s, 3H, at δ 2.33), 2.78 (dd, *J* = 10.0 and 14.0 Hz, 1H), 3.01 (s, 3H), 3.26 (m, 1H), 3.47 (dd, *J* = 6.8 and 9.6 Hz, 1H), 3.54 (dd, *J* = 6.0 and 9.6 Hz, 1H), 3.59–3.77 (m, 4H, including s, 3H, at δ 3.67), 3.83 (d, *J* = 15.6 Hz, 1H), 3.92 (d, *J* = 15.6 Hz, 1H), 4.21 (dd, *J* = 6.4 and 12.4 Hz, 1H), 4.42 (m, 1H), 6.77 (m, 1H), 7.07 (dd, *J* = 6.8 and 6.8 Hz, 1H), 7.16 (dd, *J* = 6.8 and 6.8 Hz, 1H), 7.21–7.32 (m, 1H), 7.93–8.12 (m, 1H); MS (ESI), *m*/*z* 634 $[M+H]^+$.

5.19. General procedure C: preparation of *trans*-4-[1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7a)

To a stirred solution of **6a** (323 mg, 0.55 mmol) in THF (4 mL) was added 0.5 N NaOH (3.5 mL, 1.75 mmol) and the reaction mixture was stirred at room temperature for 20 h. The mixture was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography with $CHCl_3$–MeOH (9:1, v/v) as eluent to give the title compound (193 mg, 63%) as a colorless amorphous solid. IR (ATR) 2937, 2864, 1720, 1701, 1639, 1610, 1577, 1550, 1502, 1450, 1281, 1201, 1068, 802 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.10–1.40 (series of m, total 4H), 1.84–2.18 (series of m, total 7H), 2.30 (s, 3H), 3.17–3.65 (series of m, total 8H, including s, 3H, at δ 3.23), 3.71–4.15 (series of m, total 4H), 6.86–6.91 (m, 1H), 7.07–7.10 (m, 1H), 7.20–7.27 (m, 2H), 7.91–7.94 (m, 1H), 10.04 (broad s, 1H), 12.04 (broad s, 1H); MS (ESI), *m*/*z* 558 $[M+H]^+$; Anal. Calcd for $C_{29}H_{33}F_2N_3O_6$: C, 62.47; H, 5.97; N, 7.54; F, 6.81. Found: C, 62.27; H, 6.12; N, 7.30; F, 6.65.

Compound **7b**–**7q** were prepared according to general procedure C.

5.20. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(3*R*)-methoxy-(2*R*)-pyrrolidinyl methoxy]cyclohexanecarboxylic acid (7b)

Yield 69%. A colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 1.15–1.40 (m, 4H), 1.76–2.17 (series of m, total 7H), 2.29 (s, 3H), 3.12–4.39 (series of m, total 12H, including s, 3H, at δ 3.31), 6.87–6.91 (m, 1H), 7.00–7.10 (m, 1H), 7.19–7.28 (m, 2H), 7.91–7.94 (m, 1H), 10.01 (broad s, 1H), 12.02 (broad s, 1H); MS (ESI), *m*/*z* 558 $[M+H]^+$; Anal. Calcd for $C_{29}H_{33}F_2N_3O_6 \cdot 0.25H_2O$: C, 61.97; H, 6.01; N, 7.48; F, 6.76. Found: C, 62.11; H, 5.96; N, 7.44; F, 6.73.

5.21. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7c)

Yield 78%. A colorless solid. IR (ATR) 2935, 2861, 1724, 1701, 1639, 1610, 1577, 1502, 1450, 1348, 1280, 1201, 1105, 1068, 999, 802 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.13–1.36 (m, 4H), 1.75–1.94 (m, 8H), 2.14 (m, 1H), 2.28 (s, 3H), 3.15–3.53 (m, 8H), 3.86–3.88 (m, 2H), 3.90–4.02 (m, 1H), 4.21–4.23 (m, 1H), 6.86–6.89 (m, 1H), 7.02–7.04 (m, 1H), 7.18–7.26 (m, 2H), 7.90–7.93 (m, 1H); MS (ESI), *m*/*z* 572 $[M+H]^+$; Anal. Calcd for $C_{30}H_{35}F_2N_3O_6$: C, 63.04; H, 6.17; N, 7.35; F, 6.65. Found: C, 63.01; H, 6.35; N, 6.96; F, 6.28.

5.22. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methoxymethyl-(2*S*)-pyrrolidinyl methoxy]cyclohexanecarboxylic acid (7d)

Yield 94%. A colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.06–1.58 (m, 4H), 1.82–2.11 (m, 7H), 2.11–2.38 (m, 5H, including each s, total 3H, at δ 2.29 and 2.31), 3.06–3.28 (m, 1H), 3.28–3.48 (m, 5H, including each s, total 3H, at δ 3.30 and 3.39), 3.49–3.65 (m, 2H), 3.71–3.87 (m, 1H), 3.87–3.98 (m, 1H), 4.09–4.30 (m, 2H), 6.72–6.80 (m, 1H), 7.09–7.22 (m, 3H), 7.79–7.94 (m, 1H); MS (ESI), *m*/*z* 572 $[M+H]^+$; Anal. Calcd for $C_{30}H_{35}F_2N_3O_6$: C, 63.04; H, 6.17; N, 7.35. Found C, 63.29; H, 6.32; N, 7.35.

5.23. *trans*-4-[1-[[7-Fluoro-2-(2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7e)

Yield 93%. A colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.06–1.57 (m, 4H), 1.77–2.41 (series of m, total 12H, including each s, total 3H, at δ 2.34 and 2.35), 3.06–3.46 (m, 6H, including each s, total 3H, at δ 3.29 and 3.37), 3.47–3.63 (m, 2H), 3.69–3.83 (m, 1H), 3.85–3.97 (m, 1H), 4.07–4.29 (m, 2H), 7.05–7.17 (m, 3H), 7.18–7.32 (m, 2H), 7.82 and 7.87 (each d, *J* = 7.8 Hz, total 1H); MS (ESI), *m*/*z* 554 $[M+H]^+$; Anal. Calcd for $C_{30}H_{36}FN_3O_6{\cdot}0.5H_2O$: C, 64.56; H, 6.59; N, 7.53. Found: C, 64.64; H, 6.60; N, 7.35.

5.24. *trans*-4-[(5*S*)-Ethoxymethyl-1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7f)

Yield 100%. A colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.22 and 1.31 (each t, *J* = 6.8 Hz, total 3H), 1.18–1.69 (series of m, total 4H), 1.93–2.16 (m, 8H), 2.25–2.34 (m, 2H), 2.40 and 2.43 (each s, total 3H), 3.17–3.35 (series of m, total 1H), 3.44–3.73 (series of m, total 6H), 3.83–4.08 (series of m, total 2H), 4.19–4.34 (m, 2H), 6.84 (td, *J* = 2.8 and 8.4 Hz, 1H), 7.20–7.30 (series of m, total 3H), 7.92 and 8.01 (each dd, *J* = 2.8 and 10.8 Hz, total 1H); MS (ESI), *m*/*z* 586 $[M+H]^+$; Anal. Calcd for $C_{31}H_{37}F_2N_3O_6{\cdot}0.5H_2O$: C, 63.09; H, 6.40; N, 7.12. Found: C, 62.89; H, 6.38; N, 7.03.

5.25. *trans*-4-[(5*S*)-Ethoxymethyl-1-[[7-fluoro-2-(2-methylphenylamino)-6-benzoxazolyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7g)

Yield 89%. A colorless solid. IR (ATR) 1699, 1637, 1577 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 and 1.22 (each t, *J* = 6.8 Hz, total 3H), 1.25 (m, 2H), 1.42 (m, 2H), 1.84–2.07 (series of m, total 7H), 2.22 (m, 2H), 2.34 and 2.35 (each s, total 3H), 3.10–3.22 (m, 1H), 3.34–3.64 (series of m, total 6H), 3.85 (m, 2H), 4.18 (m, 2H), 7.09 (t, *J* = 7.2 Hz, 1H), 7.15 (m, 2H), 7.22 (m, 1H), 7.27 (s, 1H), 7.29 (m, 1H), 7.87 and 7.96 (each d, *J* = 8.0 Hz, total 1H); MS (ESI), *m*/*z* 568 $[M+H]^+$; Anal. Calcd for $C_{31}H_{38}FN_3O_6{\cdot}0.25H_2O$: C, 65.59; H, 6.75; N, 7.40. Found: C, 65.21; H, 6.78; N, 7.21.

5.26. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-hydroxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7h)

Yield 60%. A colorless amorphous solid. IR (ATR) 2939, 2864, 1703, 1639, 1610, 1577, 1502, 1452, 1280, 1203, 1068, 804 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.07–1.46 (m, 4H), 1.79–2.21 (m, 9H), 2.32 (s, 3H), 3.06–4.99 (series of m, total 9H), 6.78 (m, 1H), 7.06 and 7.08 (each d, *J* = 8.0 Hz, total 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.19 (d, *J* = 7.6 Hz, 1H), 7.89 and 7.92 (each s, total 1H), 8.11 (s, 1H), 9.87 (broad s, 1H); MS (ESI), *m*/*z* 558 $[M+H]^+$; Anal. Calcd for $C_{29}H_{33}F_2N_3O_6{\cdot}0.5H_2O$: C, 61.48; H, 6.05; F, 6.71; N, 7.42. Found: C, 61.59; H, 5.98; F, 6.68; N, 7.20.

5.27. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylic acid (7i)

Yield 89%. A colorless solid. IR (ATR) 2933, 1639, 1610, 1577 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15–1.61 (series of m, total 8H), 1.95–2.29 (series of m, total 11H), 3.14–3.23 (m, 1 H), 3.36–3.92 (series of m, total 4H), 4.14–4.26 (series of m, total 2H), 6.75 (td, *J* = 2.4 and 8.4 Hz, 1H), 7.10–7.16 (series of m, total 4H), 7.75 and 7.78 (each dd, *J* = 2.8 and 10.8 Hz, total 1 H); MS (ESI), *m*/*z* 542 $[M+H]^+$; HRMS (ESI) Calcd for $C_{29}H_{33}F_2N_3O_5$+H: 542.24665. Found: 542.24704.

5.28. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-fluoromethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7j)

Yield 53%. A colorless amorphous solid. IR (ATR) 2939, 2862, 1701, 1639, 1610, 1577, 1502, 1452, 1411, 1280, 1201, 1105, 1068, 1004, 802 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.10–1.39 (m, 4H), 1.78–2.18 (m, 9H), 2.30 (s, 3H), 3.08–4.57 (series of m, total 9H), 6.89 (dt, *J* = 2.7 and 8.3 Hz, 1 H), 7.10 (t, *J* = 7.8 Hz, 1H), 7.20–7.28 (m, 2H), 7.93 (dd, *J* = 2.2 and 11.5 Hz, 1H), 10.04 (broad s, 1H), 12.05 (broad s, 1H); MS (ESI), *m*/*z* 560 $[M+H]^+$; Anal. Calcd for $C_{29}H_{32}F_3N_3O_5$: C, 62.25; H, 5.76; N, 7.51. Found: C, 62.16; H, 5.97; N, 7.24.

5.29. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methylthiomethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7k)

Yield 90%. A colorless amorphous solid. IR (ATR) 2937, 2862, 1701, 1639, 1610, 1577, 1502, 1452, 1423, 1280, 1203, 1105, 1068, 802 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07–1.57 (m, 4H), 1.80–2.45 (series of m, total 15H, including s, 3H, at δ 2.14 and each s, total 3H, at δ 2.30 and 2.31), 2.50–3.95 (series of m, total 7H), 4.06–4.35 (m, 2H), 6.75 (m, 1H), 7.05–7.35 (m, 3H), 7.80–8.00 (m, 1H); MS (ESI), *m*/*z* 588 $[M+H]^+$; Anal. Calcd for $C_{30}H_{35}F_2N_3O_5S{\cdot}0.75H_2O$: C, 59.94; H, 6.12; F, 6.32; N, 6.99; S, 5.33. Found C, 59.83; H, 5.79; F, 6.39; N, 6.93, S, 5.62.

5.30. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-methanesulfonylmethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7l)

Yield 98%. A colorless amorphous solid. IR (ATR) 2933, 1722, 1639, 1610, 1577, 1504, 1452, 1408, 1298, 1201, 1128, 1105, 1068, 960 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18–1.38 (m, 2H), 1.40–1.60 (m, 2H), 1.95–2.18 (m, 6H), 2.18–2.40 (series of m, total 6H, including s, 3H, at δ 2.32), 2.77 (dd, *J* = 10.4 and 14.0 Hz, 1H), 3.01 (s, 3H), 3.27 (m, 1H), 3.48 (dd, *J* = 6.4 and 9.2 Hz, 1H), 3.54 (dd, *J* = 5.6 and 9.2 Hz, 1H), 3.68 (d, *J* = 14.0 Hz, 1H), 3.84 (d, *J* = 16.0 Hz, 1H), 3.90 (d, *J* = 16.0 Hz, 1H), 4.21 (dd, *J* = 6.4 and 12.4 Hz, 1H), 4.42 (m, 1H), 6.77 (m, 1H), 7.09 (dd, *J* = 7.6 and 7.6 Hz, 1H), 7.16 (dd, *J* = 7.6 and 7.6 Hz, 1H), 7.19–7.30 (m, 1H), 7.98 (m, 1H); MS (ESI), *m*/*z* 620 $[M+H]^+$; Anal. Calcd for $C_{30}H_{35}F_2N_3O_7S{\cdot}0.25H_2O$: C, 57.73; H, 5.73; F, 6.09; N, 6.73; S, 5.14. Found: C, 57.63; H, 5.64; F, 5.87; N, 6.51; S, 5.04.

5.31. *trans*-4-[(5*S*)-Dimethylanimomethyl-1-[[7-fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7m)

Yield 62%. A pale yellow amorphous solid. IR (ATR) 2937, 2864, 1712, 1637, 1577, 1452 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.04–1.45 (m, 4H), 1.79–2.26 (m, 8H), 2.30 (s, 3H), 2.43–2.87 (m, 9H), 2.91–3.66 (m, 5H), 3.86–4.06 (m, 1H), 4.17–4.36 (m, 1H), 6.85–6.98 (m, 1H), 7.06–7.18 (m, 1H), 7.18–7.36 (m, 2H), 7.86–7.99 (m, 1H), 10.05 (s, 1H), 12.02 (broad s, 1H); MS (ESI), *m*/*z* 585 $[M+H]^+$; HRMS (ESI) Calcd for $C_{31}H_{38}F_2N_4O_5$+H: 585.28885. Found: 585.28695.

5.32. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-[methoxy(methyl)amino]methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7n)

Yield 99%. A colorless amorphous solid. IR (ATR) 2937, 2862, 1701, 1639, 1610, 1577, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03–1.58 (m, 4H), 1.82–2.47 (m, 12H), 2.52–2.68 (m, 4H), 2.72–2.85 (m, 1H), 3.32–4.07 (m, 1H), 3.39–4.02 (m, 7H), 4.08–4.32 (m, 2H), 6.72–6.80 (m, 1H), 7.07–7.25 (m, 3H), 7.75–7.91 (m, 1H); MS (ESI), *m*/*z* 601 $[M+H]^+$; Anal. Calcd for $C_{31}H_{38}F_2N_4O_6{\cdot}0.5H_2O$: C, 61.07; H, 6.45; F, 6.23; N, 9.19. Found: C, 61.15; H, 6.46; F, 6.23; N, 8.99.

5.33. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-(1-isoxazolidinylmethyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7o)

Yield 67%. A colorless amorphous solid. ^1H NMR ($CDCl_3$) δ 1.03–1.60 (m, 4H), 1.87–2.36 (m, 17H), 2.89 and 2.96 (each s, total 3H), 3.33–3.71 (m, 2H), 3.74–4.02 (m, 4H), 4.08–4.39 (m, 2H), 6.76 (tt, *J* = 2.3 and 8.1 Hz, 1H), 7.18–7.08 (m, 3H), 7.84–7.74 (m, 1H); MS (ESI), *m*/*z* 613 $[M+H]^+$; HRMS (ESI) Calcd for $C_{32}H_{38}F_2N_4O_6$+H: 613.28377. Found: 613.28085.

5.34. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-(4-morpholinyl)methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7p)

Yield 69%. A colorless solid. IR (ATR) 3224, 1716, 1637, 1610, 1577, 1452 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.10–1.40 (m, 4H), 1.75–2.28 (m, 9H), 2.30 (s, 3H), 2.35–2.60 (m, 4H), 3.10–4.20 (m, 13H), 6.87–6.90 (m, 1H), 7.09–7.28 (m, 3H), 7.29 (dd, *J* = 2.7 and 11.3 Hz, 1H); MS (ESI), *m*/*z* 627 $[M+H]^+$; Anal. Calcd for $C_{33}H_{40}F_2N_4O_6{\cdot}0.7H_2O$: C, 62.00; H, 6.53; N, 8.76. Found: C, 61.79; H, 6.25; N, 8.72.

5.35. *trans*-4-[1-[[7-Fluoro-2-(5-fluoro-2-methylphenylamino)-6-benzoxazolyl]acetyl]-(5*S*)-(3-methoxyazetidinylmethyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (7q)

Yield 74%. A colorless amorphous solid. IR (ATR) 2933, 2858, 1712, 1637, 1610, 1576, 1552 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.09–1.34 (m, 2H), 1.34–1.55 (m, 2H), 1.81–2.14 (m, 8H), 2.15–2.35 (m, 5H, including s, total 3H, at δ 2.27 and 2.30), 2.54–2.74 (m, 1H), 2.95–3.29 (m, 6H, including s, total 3H, at δ 3.14 and 3.26), 3.33–3.69 (m, 3H), 3.76–3.96 (m, 3H), 3.96–4.21 (m, 3H), 6.70–6.78 (m, 1H), 7.03–7.23 (m, 3H), 7.85 and 7.95 (each dd, *J* = 2.6 and 10.8 Hz, total 1H); MS (ESI), *m*/*z* 627 $[M+H]^+$; Anal. Calcd for $C_{33}H_{40}F_2N_4O_6{\cdot}H_2O$: C, 61.48; H, 6.57; F, 5.89; N, 8.69. Found: C, 61.44; H, 6.53; F, 5.65; N, 8.30.

5.36. Methyl 1-benzyloxycarbonyl-(3*S*)-hydroxy-(2*S*)-pyrrolidinecarboxylate (9)

To a cooled (0 °C), stirred solution of $SOCl_2$ (27.0 mL, 0.37 mol) in MeOH (200 mL) was added *trans*-3-hydroxy-L-proline (**8**) (16.2 g, 0.12 mol) and the reaction mixture was stirred at 0 °C to room temperature for 2 h. After removal of the solvent, the crude solid was triturated with Et_2O to give methyl (3*S*)-hydroxy-(2*S*)-pyrrolidinecarboxylate hydrochloride (22.4 g) as a colorless solid.

To a stirred suspension of methyl (3*S*)-hydroxy-(2*S*)-pyrrolidinecarboxylate hydrochloride (22.4 g, 0.12 mol) and Et_3N (42.9 mL, 0.31 mol) in MeCN–H_2O (400 mL, 1:1, v/v) was added ZCl (22.9 mL, 0.16 mol) and the reaction mixture was stirred at room temperature for 20 h. The mixture was diluted with H_2O and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (4:1 to 1:1, v/v) as eluent to give the title compound (33.2 g, 96%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.91–1.99 (m, 1H), 2.08–2.24 (m, 2H), 3.65–3.73 (m, 2H), 3.76 (s, 3H), 4.29 and 4.37 (each m, total 1H), 4.48 (m, 1H), 5.03–5.21 (m, 2H), 7.28–7.37 (m, 5H); MS (ESI), *m*/*z* 280 $[M+H]^+$.

5.37. 1-Benzyloxycarbonyl-(2*R*)-*tert*-butyldimethylsilyl oxymethyl-(3*S*)-hydroxypyrrolidine (10)

To a cooled (0 °C), stirred solution of **9** (32.3 g, 0.116 mol) in toluene (330 mL) was added $NaBH_4$ (10.8 g, 0.289 mol) and MeOH (70.3 mL, 1.74 mol). After being stirred at room temperature for 4 h, the mixture was quenched by H_2O and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give 1-benzyloxycarbonyl-(3*S*)-hydroxy-(2*R*)-pyrrolidinemethanol (25.9 g, 89%) as a colorless viscous oil. MS (ESI), *m*/*z* 252 $[M+H]^+$.

To a cooled (0 °C), stirred solution of 1-benzyloxycarbonyl-(3*S*)-hydroxy-(2*R*)-pyrrolidinemethanol (26.6 g, 0.106 mol) and Et_3N (22.1 mL, 0.159 mol) in CH_2Cl_2 (400 mL) was added TBDMSCl (17.5 g, 0.116 mol) and the reaction mixture was stirred at room temperature for 2 days. The mixture was diluted with H_2O and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (4:1 to 2:1, v/v) as eluent to give the title compound (34.7 g, 90%) as a colorless solid. ^1H NMR ($CDCl_3$) δ −0.04 and −0.02 (each s, total 3H), 0.03 and 0.05 (each s, total 3H), 0.84 and 0.88 (each s, total 9H), 1.74–1.89 (m, 2H), 2.09–2.19 (m, 1H), 3.38–3.92 (series of m, total 5H), 4.40 (m, 1H), 5.06–5.20 (m, 2H), 7.28–7.36 (m, 5H); MS (ESI), *m*/*z* 366 $[M+H]^+$.

5.38. 1-Benzyloxycarbonyl-(3*S*)-methoxy-(2*R*)-pyrrolidine methanol (11)

To a cooled (0 °C), stirred solution of **10** (2.60 g, 7.11 mmol) and NaH (0.34 g, 14.2 mmol) in DMF (10 mL) was added MeI (0.53 mL, 8.51 mmol) and the reaction mixture was stirred at room temperature for 13 h. The mixture was quenched by H_2O and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (6:1, v/v) as eluent to give 1-benzyloxycarbonyl-(2*R*)-*tert*-butyldimethylsilyloxymethyl-(3*S*)-methoxypyrrolidine (2.73 g, 100%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.90 and 0.93 (each s, total 9H), 2.02–2.08 (m, 2H), 3.35–3.37 (each s, total 3H), 3.41–3.64 (m, 3H), 3.75–3.98 (m, 3H), 5.08–5.27 (m, 2H), 7.32–7.40 (m, 5H).

To a cooled (0 °C), stirred solution of *1*-benzyloxycarbonyl-(2*R*)-*tert*-butyldimethylsilyloxymethyl-(3*S*)-methoxypyrrolidine (2.73 g, 7.19 mmol) in THF (15 mL) was added tetrabutylammonium fluoride (TBAF) (1 M solution in THF, 14.4 mL, 14.4 mmol) and the reaction mixture was stirred at room temperature for 18 h. The mixture was concentrated to a small volume, diluted with 1 N HCl, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (20:1, v/v) as eluent to give the title compound (2.01 g, 100%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.98 (m, 2H), 3.34 (s, 3H), 3.41–3.74 (m, 5H), 3.88 and 4.01 (each m, total 1H), 5.14 (s, 2H), 7.30–7.36 (m, 5H); MS (ESI), *m*/*z* 266 $[M+H]^+$.

5.39. Methyl 4-[(3*S*)-methoxy-(2*R*)-pyrrolidinyl methoxy]benzoate (12)

To a stirred solution of **11** (1.90 g, 7.16 mmol), methyl 4-hydroxybenzoate (1.20 g, 7.89 mmol) and Ph_3P (2.25 g, 8.58 mmol) in THF (20 mL) was added diisopropyl azodicarboxylate (DIAD) (1.69 mL, 8.58 mmol) and the reaction mixture was refluxed for 12 h. After the mixture was cooled to the room temperature, the solvent was evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (1:1, v/v) as eluent to give methyl 4-[1-benzyloxycarbonyl-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]benzoate as a crude product. MS (ESI), *m*/*z* 400 $[M+H]^+$.

A stirred solution of the crude product in MeOH (50 mL) was hydrogenated over 10% Pd/C (1.50 g) for 20 h. The mixture was filtered to remove the catalyst, and the solvent was evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (30:1, v/v) as eluent to give the title compound (1.39 g, 73% for two steps) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.87–2.01 (m, 2H), 3.03–3.18 (m, 2H), 3.35 (s, 3H), 3.43–3.47 (m, 1H), 3.79–3.82 (m, 1H), 3.89 (s, 3H), 3.95–4.02 (m, 2H), 6.91–6.93 (m, 2H), 7.97–8.00 (m, 2H).

5.40. Methyl 4-[1-*tert*-butoxycarbonyl-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (13)

A stirred solution of **12** (1.39 g, 5.24 mmol) in MeOH/AcOH (22 mL, 10:1, v/v) was hydrogenated at 10 atm over Rh/Al_2O_3 (0.55 g) for 2 days. The mixture was filtered to remove the catalyst and the solvent was evaporated. The residue was dissolved in $CHCl_3$, washed with satd $NaHCO_3$ aq, brine, dried over Na_2SO_4, and evaporated to give methyl 4-[(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (1.42 g) as a crude product.

A mixture of the crude product (1.42 g), $(Boc)_2O$ (1.26 g, 5.77 mmol), and DMAP (0.06 g, 0.49 mmol) in MeCN (20 mL) was stirred at room temperature for 20 h. After removal of the solvent, the residue was purified by column chromatography on silica gel with hexane–EtOAc (4:1 to 3:1, v/v) as eluent to give methyl 4-[1-*tert*-butoxycarbonyl-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (*cis*-rich, 1.67 g, 86% for 2 steps) as a pale yellow oil. MS (ESI), *m*/*z* 372 $[M+H]^+$.

To a stirred solution of the *cis*-rich product (1.67 g, 4.50 mmol) in MeOH (15 mL) was added NaOMe (0.73 g, 13.5 mmol) and the reaction mixture was heated under reflux for 24 h. After being cooled to the room temperature, the mixture was concentrated to a small volume. The mixture was acidified with 1 N HCl, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give the crude product.

To a stirred solution of the crude product in MeOH–benzene (20 mL, 1:4, v/v) was added $TMSCHN_2$ (2.0 M solution in hexane, 1.10 mL, 2.20 mmol) and the reaction mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was purified by flash column chromatography with hexane–EtOAc (2:1, v/v) as eluent to give the title compound (*cis*/*trans* = ca. 1/1, 896.0 mg, 54%) as a colorless oil. MS (ESI), *m*/*z* 372 $[M+H]^+$.

5.41. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (14)

To a stirred solution of **13** (437 mg, 1.18 mmol) in THF (4 mL) was added 1 N NaOH (3.5 mL, 3.50 mmol) and the reaction mixture was stirred at room temperature for 4 days. The mixture was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give 4-[1-*tert*-butoxycarbonyl-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy] cyclohexanecarboxylic acid (413 mg, 98%) as a pale yellow oil.

A mixture of 4-[1-*tert*-butoxycarbonyl-(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (413 mg, 1.16 mmol), EtI (940 μl, 11.8 mmol), and K_2CO_3 (325 mg, 2.35 mmol) in DMF (10 mL) was stirred at 70 °C for 17 h. After being cooled to room temperature, the mixture was acidified with 1 N HCl, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography with hexane–EtOAc (5:1, v/v) as eluent to give the title compound (142 mg, 31%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.22–1.26 (m, total 5H, including t, *J* = 7.1 Hz, 3H, at δ 1.25), 1.40–1.50 (m, total 11H, including s, 9H, at δ 1.46), 1.98–2.07 (m, 6H), 2.24–2.28 (m, 1H), 3.20–3.26 (m, 1H), 3.32–3.50 (series of m, total 6H, including s, 3H, at δ 3.32), 3.60–3.68 (m, 1H), 3.76–3.92 (m, 2H), 4.11 (q, *J* = 7.1 Hz, 2H).

5.42. Ethyl *trans*-4-[(3*S*)-methoxy-(2*R*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (5a)

To a stirred solution of **14** (142 mg, 0.37 mmol) in CH_2Cl_2 was added TFA (3 mL) and the reaction mixture was stirred at room temperature for 1 h. The mixture was made basic with satd $NaHCO_3$ aq and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give the title compound (97 mg, 92%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.30 (m, total 5H, including t, *J* = 7.3 Hz, 3H, at δ 1.24), 1.41–1.51 (m, 2H), 1.78–1.92 (m, 2H), 1.98–2.08 (m, 4H), 2.20–2.28 (m, 1H), 2.99–3.05 (m, 2H), 3.13–3.27 (m, 2H), 3.31 (s, 3H), 3.41–3.50 (m, 2H), 3.64–3.67 (m, 1H), 4.11 (q, *J* = 7.3 Hz, 2H).

5.43. 1-Benzyloxycarbonyl-(2*R*)-*tert*-butyldimethylsilyl oxymethyl-3-pyrrolidinone (15)

To a cooled (−78 °C), stirred solution of $(COCl)_2$ (17.9 mL, 0.205 mol) and DMSO (21.8 mL, 0.307 mol) in CH_2Cl_2 (250 mL) was added **10** (37.5 g, 0.102 mol) in CH_2Cl_2 (100 mL). After the mixture was stirred at -78 °C for 1.5 h, Et_3N (71.4 mL, 0.512 mol) was added and the reaction mixture was stirred at −78 °C to room temperature for 13 h. The mixture was poured into ice-H_2O and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (10:1 to 5:1, v/v) as eluent to give the title compound (37.8 g, 100%) as a light reddish brown oil. ^{1}H NMR ($CDCl_3$) δ −0.05 and −0.02 (each s, total 6H), 0.82 (s, 9H), 2.45–2.62 (m, 2H), 3.67–3.74 (m, 1H), 3.88–4.19 (series of m, total 4H), 5.14–5.25 (m, 2H), 7.32–7.38 (m, 5H).

5.44. 1-Benzyloxycarbonyl-(2*R*)-*tert*-butyldimethylsilyl oxymethyl-(3*R*)-hydroxypyrrolidine (16)

To a cooled (−10 °C), stirred solution of **15** (37.8 g, 0.104 mol) in MeOH (300 mL) was added $NaBH_4$ (4.73 g, 0.125 mol) and the reaction mixture was stirred at −10 °C to room temperature for 7 h.

After being concentrated to a small volume, the mixture was quenched by H_2O and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (5:1 to 3:1, v/v) as eluent to give the title compound (39.0 g, 100%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ −0.02 (s, 3H), 0.05 and 0.08 (each s, total 3H), 0.85 and 0.88 (each s, total 9H), 1.94–2.08 (m, 2H), 3.32–3.34 and 3.48–3.52 (each m, total 3H), 3.85–4.13 (series of m, total 3H), 4.46–4.50 (m, 1H), 5.05–5.20 (m, 2H), 7.32–7.36 (m, 5H).

5.45. 1-Benzyloxycarbonyl-(3*R*)-methoxy-(2*R*)-pyrrolidinemethanol (17)

The title compound was prepared in a procedure similar to that described for **11**, starting from **16** via 1-benzyloxycarbonyl-(2*R*)-*tert*-butyldimethylsilyloxymethyl-(3*R*)-methoxypyrrolidine.

For 1-benzyloxycarbonyl-(2*R*)-*tert*-butyldimethylsilyloxymethyl-(3*R*)-methoxypyrrolidine; Yield 85%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ −0.02 to 0.08 (m, total 6H), 0.87 and 0.90 (each s, total 9H), 2.04 (m, 2H), 3.38–3.51 (m, total 5H, including s, 3H, at δ 3.39), 3.76 (m, 1H), 3.88–3.92 (m, 3H), 5.10–5.13 (m, 2H), 7.29–7.36 (m, 5H).

For **17**; Yield 100%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.65 (s, 1H), 1.96–2.14 (m, 2H), 3.37 and 3.41 (each s, total 3H), 3.50–3.53 (m, 2H), 3.80–4.08 (m, 4H), 5.11–5.18 (m, 2H), 7.31–7.37 (m, 5H).

5.46. Ethyl 4-[(3*R*)-methoxy-(2*R*)-pyrrolidinylmethoxy] benzoate (18)

To a stirred solution of **17** (2.17 g, 8.18 mmol), ethyl 4-hydroxybenzoate (1.36 g, 8.18 mmol) and Ph_3P (2.57 g, 9.80 mmol) in THF (50 mL) was added DIAD (2.42 mL, 12.3 mmol) and the reaction mixture was heated under reflux for 7 h. After being cooled to room temperature, the solvent was evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (3:1, v/v) as eluent to give ethyl 4-[1-benzyloxycarbonyl-(3*R*)-methoxy-(2*R*)-pyrrolidinylmethoxy]benzote (5.71 g) as a crude product. A stirred solution of the crude product (5.17 g) in MeOH (100 mL) was hydrogenated over 20% $Pd(OH)_2$/C (1.03 g) for 22 h. The mixture was filtered to remove the catalyst and the solvent was evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (60:1 to 10:1, v/v) as eluent to give the title compound (1.81 g, 79% for two steps) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.38 (t, J = 7.1 Hz, 3H), 1.93–1.98 (m, 2H), 2.18 (broad s, 1H), 2.92–2.99 (m, 1H), 3.14–3.18 (m, 1H), 3.30 (s, 3H), 3.38–3.45 (m, 1H), 3.93–3.96 (m, 1H), 4.05–4.09 (m, 1H), 4.21–4.25 (m, 1H), 4.34 (q, J = 7.1 Hz, 2H), 6.94–6.96 (m, 2H), 7.97–7.99 (m, 2H).

5.47. Ethyl 4-[1-(*tert*-butoxycarbonyl)-(3*R*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (19)

A mixture of **18** (1.81 g, 6.48 mmol) in MeOH–TFA (31 mL, 30:1, v/v) was hydrogenated over 5% Rh/Al_2O_3 (0.54 g) at 10 atm H_2 for 15 h. The mixture was filtered to remove the catalyst, and the solvent was evaporated. The residue was dissolved in MeCN–satd $NaHCO_3$ aq (20 mL, 1:1, v/v). $(Boc)_2O$ (1.70 g, 7.79 mmol) was added to the mixture, and the mixture was stirred at room temperature for 2 days. The mixture was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (60:1, v/v) as eluent to give the title compound (1.77 g, 71%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.21–1.29 (m, total 5H, including t, J = 7.1 Hz, 3H, at δ 1.24) 1.45–1.66 (m, total 13H, including s, 9H, at δ 1.46), 1.80–1.88 (m, 2H), 1.98–2.05 (m, 2H), 2.30 (m, 1H), 3.23–3.54 (series of m, total 8H, including s, 3H, at δ 3.41), 3.89–3.93 (m, 2H), 4.11 (q, J = 7.1 Hz, 2H).

5.48. Ethyl *trans*-4-[1-(*tert*-butoxycarbonyl)-(3*R*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (20)

A mixture of **19** (1.77 g, 4.59 mmol) and NaOEt (0.94 g, 13.8 mmol) in EtOH (15 mL) was refluxed under N_2 atmosphere for 16 h. After being cooled to room temperature, the mixture was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was dissolved in DMF (15 mL). To the solution was added K_2CO_3 (1.58 g, 11.4 mmol) and EtI (0.69 mL, 8.63 mmol), and the reaction mixture was stirred at 60 °C for 2 h. After being cooled to room temperature, the mixture was acidified with 1 N HCl and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography with hexane–EtOAc (5:1, v/v) as eluent to give the title compound (551 mg, 31%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.30 (series of m, total 5H, including t, J = 7.1 Hz, 3H, at δ 1.24), 1.40–1.50 (m, total 11H, including s, 9H, at δ 1.46), 1.97–2.08 (m, 6H), 2.21–2.27 (m, 1H), 3.20–3.42 (m, total 6H, including s, 3H, at δ 3.40), 3.57–3.69 (m, 2H), 3.89–3.95 (m, 2H), 4.11 (q, J = 7.1 Hz, 2H).

5.49. Ethyl *trans*-4-[(3*R*)-methoxy-(2*R*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5b)

The title compound was prepared in a procedure similar to that described for **5a**, starting from **20**. Yield 95%. A pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.30 (series of m, total 5H, including t, J = 7.1 Hz, 3H, at δ 1.24), 1.41–1.51 (m, 2H), 1.89–2.12 (m, 6H), 2.20–2.28 (m, 1H), 2.68 (broad s, 1H), 2.88–2.94 (m, 1H), 3.11–3.32 (m, total 6H, including s, 3H, at δ 3.29), 3.52–3.56 (m, 1H), 3.67–3.71 (m, 1H), 3.82–3.85 (m, 1H), 4.11 (q, J = 7.1 Hz, 2H).

5.50. (2*S*)-Benzyloxymethyl-1-*tert*-butoxycarbonyl-(5*R*)-methoxymethylpyrrolidine (22)

To a cooled (0 °C), stirred solution of (2*S*)-benzyloxymethyl-1-*tert*-butoxycarbonyl-(5*R*)-hydroxymethylpyrrolidine (**21**) (190 mg, 0.59 mmol) and NaH (28 mg, 1.17 mmol) in DMF (5 mL) was added MeI (44 μl, 0.71 mmol) and the reaction mixture was stirred at room temperature for two days. The mixture was quenched by H_2O, extracted with EtOAc, washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (5:1, v/v) as eluent to give the title compound (183 mg, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.87–1.99 (m, 4H), 3.28–3.34 (m, total 5H, including s, 3H, at δ 3.31), 3.49 (m, 1H), 3.51–3.61 (m, 1H), 3.96 (m, 2H), 4.52 (s, 2H), 7.25–7.33 (m, 5H).

5.51. 1-*tert*-Butoxycarbonyl-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinemethanol (23)

A stirred solution of **22** (183 mg, 0.55 mmol) in MeOH (10 mL) was hydrogenated over 10% Pd/C (40 mg) for 15 h. The mixture was filtered to remove the catalyst and the solvent was evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (2:1, v/v) as eluent to give the title compound (116 mg, 87%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.87–2.01 (m, 4H), 3.36 (s, 3H), 3.38–3.39 (m, 1H), 3.45–3.51 (m, 1H), 3.78–3.99 (m, 3H), 4.66–4.69 (m, 1H).

5.52. Methyl 4-[(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy] benzoate (24)

To a stirred solution of **23** (950 mg, 3.87 mmol), methyl 4-hydroxybenzoate (648 mg, 4.26 mmol) and Ph_3P (1.22 g, 4.65 mmol) in THF (20 mL) was added DIAD (1.14 mL, 5.79 mmol) and the reaction mixture was heated under reflux for 10 h. After being cooled to the room temperature, the solvent was evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (6:1, v/v) as eluent to give methyl 4-[1-*tert*-butoxycarbonyl-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy] benzoate (1.62 g) as a crude product.

To a stirred solution of the crude product (1.62 g) in CH_2Cl_2 (15 mL) was added TFA (15 mL) and the reaction mixture was stirred at room temperature for 17 h. The mixture was made basic with satd $NaHCO_3$ aq and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (20:1, v/v) as eluent to give the title compound (1.03 g, 95% for 2 steps) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.49–2.01 (m, 5H), 3.27–3.33 (m, 1H), 3.37–3.44 (m, total 5H, including s, 3H, at δ 3.37), 3.53–3.59 (m, 1H), 3.87–4.00 (m, total 5H, including s, 3 H, at δ 3.88), 6.09 (d, J = 8.8 Hz, 2 H), 7.97 (d, J = 8.8 Hz, 2 H).

5.53. Methyl *trans*-4-[(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5c)

A stirred solution of **24** (1.03 g, 3.69 mmol) in MeOH–TFA (21 mL, 20:1, v/v) was hydrogenated over Rh/Al_2O_3 (0.50 g) at 10 atm H_2 for 1 day. The mixture was filtered to remove the catalyst and the solvent was evaporated to give methyl 4-[(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate TFA salt (1.15 g) as a crude product.

To a stirred solution of the crude product (1.15 g) in 1,4-dioxane-satd $NaHCO_3$ aq (30 mL, 1:1, v/v) was added $(Boc)_2O$ (0.81 g, 3.71 mmol) and the reaction mixture was stirred at room temperature for 2 h. The mixture was acidified with 1 N HCl, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with hexane–EtOAc (5:1, v/v) as eluent to give methyl 4-[1-*tert*-butoxycarbonyl-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (1.32 g, 93% for 2 steps) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.64–1.67 (m, 2H), 1.80–1.98 (m, 6H), 2.10–2.34 (m, 5H), 3.32–3.39 (m, total 5H, including s, 3H, at δ 3.35), 3.46–3.63 (m, 3H), 3.67 (s, 3H), 3.91 (m, 2H).

To a stirred solution of methyl 4-[1-*tert*-butoxycarbonyl-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (1.32 g, 3.42 mmol) in MeOH (10 mL) was added NaOMe (0.55 g, 10.2 mmol) and the reaction mixture was heated under reflux for 20 h. After being cooled to the room temperature, the mixture was concentrated to a small volume. The mixture was diluted with H_2O, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give the crude product. To a stirred solution of the crude product in MeOH–benzene (10 mL, 1:4, v/v) was added $TMSCHN_2$ (2.0 M solution, 680 μl, 1.36 mmol) and the reaction mixture was stirred at room temperature for 3 h. After the mixture was evaporated, the residue was purified by flash column chromatography with hexane–EtOAc (7:1, v/v) as eluent to give methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (321 mg, 24%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.22–1.33 (m, 2H), 1.41–1.51 (m, total 11H, including s, 9H, at δ 1.46), 1.87–2.07 (m, 8H), 2.24–2.29 (m, 1H), 3.17–3.63 (m, total 8H, including s, 3H, at δ 3.35), 3.66 (s, 3H), 3.87–3.97 (m, 2H).

To a stirred solution of methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*R*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (321 mg, 0.83 mmol) in CH_2Cl_2 (3 mL) was added TFA (3 mL) and the reaction mixture was stirred at room temperature for 1 h. The mixture was made basic with satd $NaHCO_3$ aq and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4, and evaporated to give the title compound (240 mg, 100%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.21–1.33 (m, 2H), 1.41–1.50 (m, 2H), 1.80–1.86 (m, 3H), 1.99–2.09 (m, 5H), 2.23–2.30 (m, 1H), 3.18–3.26 (m, 1H), 3.29–3.44 (m, total 8H, including s, 3H, at δ 3.36), 3.49–3.52 (m, 1H), 3.66 (s, 3H).

5.54. (2*S*)-Benzyloxymethyl-1-*tert*-butoxycarbonyl-(5*S*)-methoxymethylpyrrolidine (26a)

The title compound was prepared in a procedure similar to that described for **22**, starting from (2*S*)-benzyloxymethyl-1-*tert*-butoxycarbonyl-(5*S*)-hydroxymethylpyrrolidine (**25a**). Yield 74%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.40 and 1.47 (s, total 9H), 1.86–2.02 (m, 4H), 3.20–3.68 (series of m, 4H), 3.34 (s, 3H), 3.86–4.00 (m, 2H), 4.46–4.58 (m, 2H), 7.27–7.34 (m, 5H).

5.55. 1-*tert*-Butoxycarbonyl-(2*S*)-hydroxymethyl-(5*S*)-methoxymethylpyrrolidine (27a)

The title compound was prepared in a procedure similar to that described for **23**, starting from **26a**. Yield 100%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 9H), 1.57–1.70 (m, 1H), 1.87–1.98 (m, 2H), 2.01–2.14 (m, 1H), 3.17–4.02 (series of m, 6H), 3.35 (s, 3H).

5.56. Methyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]benzoate (28a)

To a stirred solution of **27a** (865 mg, 3.53 mmol), triphenylphosphine (1.11 g, 4.23 mmol) and methyl 4-hydroxybenzoate (536 mg, 3.53 mmol) was added DIAD (803 μl, 3.88 mmol) at room temperature, and the resulting mixture was stirred at 60 °C for 2 h. The mixture was concentrated in vacuo, and the residue was chromatographed on silica gel with hexane–EtOAc (3:1, v/v) as eluent to give the title compound (1.13 g, 84%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.94–2.14 (m, 4H), 3.26–4.27 (series of m, 6H), 3.36 (s, 3H), 3.88 and 3.89 (each s, total 3H), 6.93 and 6.96 (each d, J = 8.8 Hz, total 2H), 7.96 and 7.99 (each d, J = 4.0 Hz, total 2H).

5.57. Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (29a)

A mixture of **28a** (1.02 g, 2.69 mmol) and 5% Rh/Al_2O_3 (500 mg) in EtOH–AcOH (10:1, v/v, 55 mL) was stirred under hydrogen atmosphere (8 atm) at room temperature for 2.5 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel with hexane–EtOAc (3:1, v/v) as eluent to give methyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (771 mg, 74%) as a colorless oil. A mixture of the oil (770 mg, 1.99 mmol) in MeOH was added NaOMe (324 mg, 5.99 mmol) at room temperature, and the resulting mixture was stirred under reflux overnight. After being cooled to room temperature, the mixture was added to 1 N HCl and concentrated in vacuo, then extracted with $CHCl_3$–MeOH (10:1). The extract was dried over Na_2SO_4, then concentrated in vacuo to give a crude colorless oil. To a stirred solution of the oil in benzene–MeOH (10:1, 22 mL) was added $TMSCHN_2$ (2.0 M in hexane, 0.50 mL, 0.25 mmol) at room temperature, and the resulting mixture was stirred for an

hour. The mixture was concentrated in vacuo, and the residue was purified by flash chromatography with hexanae–EtOAc (5:1, v/v) as eluent to give the title compound (178 mg, 23%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.15–1.33 (m, 2H), 1.36–1.54 (m, 11H, including s, 9H, at δ 1.47), 1.78–2.10 (m, 8H), 2.19–2.33 (m, 1H), 3.12–3.50 (m, 7H, including s, 3H, at δ 3.34), 3.51–3.62 (m, 1H), 3.65 and 3.66 (each s, total 3H), 3.75–3.97 (m, 2H); MS (ESI), *m*/*z* 386 $[M+H]^+$.

5.58. Methyl *trans*-4-[(5*S*)-methoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5d)

The title compound was prepared in a procedure similar to that described for **5a**, starting from **29a**. Yield 100%. A yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.18–1.31 (m, 2H), 1.38–1.52 (m, 2H), 1.81–1.95 (m, 4H), 1.96–2.12 (m, 4H), 2.21–2.32 (m, 1H), 3.17–3.47 (m, 10H), 3.66 (s, 3H); MS (ESI), *m*/*z* 286 $[M+H]^+$.

5.59. (2*S*)-Benzyloxymethyl-1-*tert*-butoxycarbonyl-(5*S*)-ethoxymethylpyrrolidine (26b)

The title compound was prepared in a procedure similar to that described for **22**, starting from **25a** using EtI. Yield 90%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.16 and 1.18 (each t, J = 7.3 Hz, total 3H), 1.40 and 1.47 (each s, total 9H), 1.84–2.12 (m, 4H), 3.16–3.73 (m, 6H), 3.80–4.05 (m, 2H), 4.42–4.62 (m, 2H), 7.23–7.38 (m, 5H); MS (ESI), *m*/*z* 350 $[M+H]^+$.

5.60. 1-*tert*-Butoxycarbonyl-(5*S*)-ethoxymethyl-(2*S*)-hydroxymethylpyrrolidine (27b)

The title compound was prepared in a procedure similar to that described for **23**, starting from **26b**. Yield 97%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 3H), 1.48 (s, 9H), 1.49–2.18 (m, 4H), 3.24–3.87 (m, 6H), 3.88–4.30 (m, 2H).

5.61. Methyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]benzoate (28b)

The title compound was prepared in a procedure similar to that described for **28a**, starting from **27b**. Yield 84%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.13–1.24 (m, 3H), 1.47 (s, 9H), 1.90–2.23 (m, 4H), 3.23–3.68 (m, 4H), 3.79–4.31 (m, 7H), 6.88–7.02 (m, 2H), 7.92–8.06 (m, 2H); MS (ESI), *m*/*z* 394 $[M+H]^+$.

5.62. Methyl *trans*-4-[1-*tert*-butoxycarbaonyl-(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (29b)

To a stirred solution of **28b** (9.10 g, 23.13 mmol) in CH_2Cl_2 (100 mL) was added TFA (50 mL) at room temperature, and the resulting mixture was stirred for 20 min. The mixture was concentrated in vacuo and poured into satd $NaHCO_3$ aq The mixture was extracted with $CHCl_3$–MeOH (10:1), and the extract was dried over Na_2SO_4, then concentrated in vacuo to give methyl 4-[(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]benzoate (6.24 g, 92%) as a pale yellow oil. A mixture of methyl 4-[(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]benzoate (6.24 g, 21.3 mmol) and 5% Rh/AlO_3 (1.41 g) in MeOH–AcOH (7:1, 80 mL) was stirred under hydrogen atmosphere (11 atm) at room temperature overnight. The mixture was filtered, and the filtrate was concentrated in vacuo to give methyl 4-[(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6.19 g, 97%) as a colorless oil. To a stirred mixture of methyl 4-[(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (6.19 g, 20.7 mmol) and di-*tert*-butyl dicarbonate (4.96 g, 22.7 mmol) in CH_2Cl_2 (100 mL) was added Et_3N (4.32 mL, 31.0 mmol) at room temperature, and the resulting mixture was stirred for 3.5 h. The mixture was concentrated in vacuo, and the residue was chromatographed on silica gel with hexane–EtOAc (3:1) as eluent to give methyl 4-[1-*tert*-butoxycarbaonyl-(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (7.45 g, 90%) as a colorless oil. To a mixture of methyl 4-[1-*tert*-butoxycarbaonyl-(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (7.45 g, 18.7 mmol) and NaH (60% in mineral oil, 895 mg, 22.4 mmol) in DMF (100 mL) was added MeOH (1.51 mL, 37.3 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 1 h. The mixture was poured into 1 N HCl, then extracted with Et_2O. The extract was washed with water and brine, dried over Na_2SO_4, then concentrated in vacuo to give a crude colorless oil. To a stirred solution of the oil in benzene–MeOH (6:1, 60 mL) was added trimethylsilyl diazomethane (2.0 M in hexane, 1.87 mL, 3.73 mmol) at room temperature, and the resulting mixture was stirred for 1 h. The mixture was concentrated in vacuo, and the residue was purified by flash chromatography with hexane–EtOAc (5:1) as eluent to give the title compound (1.37 g, 18%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.12–1.31 (m, 5H), 1.37–1.52 (m, 11H, including s, 9H, at δ 1.46), 1.81–2.10 (m, 8H), 2.19–2.31 (m, 1H), 3.14–3.69 (m, 10H, including each s, total 3H, at δ 3.65 and 3.66), 3.75–3.97 (m, 2H).

5.63. Methyl *trans*-4-[(5*S*)-ethoxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5e)

The title compound was prepared in a procedure similar to that described for **5a**, starting from **29b**. Yield 97%. A pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.15–1.32 (m, 5H, including t, J = 6.9 Hz, 3H, at δ 1.19), 1.37–1.54 (m, 2H), 1.83–2.15 (m, 8H), 2.20–2.32 (m, 1H), 3.17–3.27 (m, 1H), 3.27–3.44 (m, 6H), 3.49 (q, J = 6.9 Hz, 2H), 3.66 (s, 3H); MS (ESI), *m*/*z* 300 $[M+H]^+$.

5.64. Methyl 4-[(5*S*)-benzyloxymethyl-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinylmethoxy]benzoate (28c)

The title compound was prepared in a procedure similar to that described for **28a**, starting from **25a**. Yield 80%. A clear oil. ^{1}H NMR ($CDCl_3$) δ 1.40 and 1.48 (each s, total 9H), 1.92–2.23 (m, 4H), 3.31–3.71 (series of m, total 2H), 3.80–3.96 (m, total 4H, including each s, total 3H, at δ 3.88 and 3.89), 3.96–4.28 (series of m, total 3H), 4.45–4.60 (m, 2H), 6.95 (m, 2H), 7.23–7.39 (m, 5H), 7.97 (m, 2H).

5.65. Methyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-hydroxymethyl-(2*S*)-pyrrolidinylmethoxy]benzoate (28d)

The title compound was prepared in a procedure similar to that described for **23**, starting from **28c**. Yield 91%. A colorless foam. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.57–1.74 (m, 2H), 1.97–2.25 (m, 2H), 3.53–3.78 (m, 2H), 3.84–3.97 (m, 4H, including s, 3H, at δ 3.89), 4.01–4.28 (m, 3H), 6.93 (m, 2H), 7.98 (m, 2H); MS (ESI), *m*/*z* 366 $[M+H]^+$.

5.66. Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-hydroxymethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (29c)

The title compound was prepared in a procedure similar to that described for **29a**, starting from **28d**. Yield 18% (three steps). A pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.17–2.40 (series of m, total 22H, including s, 9H, at δ 1.48), 3.14–4.28 (series of m, total 10H, including each s, total 3H, at δ 3.66 and 3.67).

5.67. Methyl *trans*-4-[(5*S*)-acetoxymethyl-(2*S*)-pyrrolidinyl methoxy]cyclohexanecarboxylate (5f)

To a stirred solution of **29c** (280.6 mg, 0.755 mmol) in pyridine (6.0 mL) was added $(AcO)_2O$ (3.0 mL) at room temperature. After being stirred for 2.5 h, the reaction was quenched with EtOH at room temperature. The mixture was diluted with EtOAc, washed with 1 N HCl, dried over Na_2SO_4, and concentrated to give methyl *trans*-4-[(5*S*)-acetoxymethyl-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**29d**). To a stirred solution of **29d** in CH_2Cl_2 (6.0 mL) was added TFA (6.0 mL) at 0 °C. The mixture was stirred for 2 h at room temperature, concentrated, and neutralized with satd $NaHCO_3$ aq The mixture was extracted with $CHCl_3$–MeOH (10:1, v/v), dried over Na_2SO_4 and evaporated to give the title compound (100%) as a pale yellow oil, which was used to the subsequent reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.17–1.57 (series of m, total 5H), 1.61–2.21 (series of m, total 10H, including s, 3H, at δ 2.07), 2.26 (m, 1H), 3.16–3.40 (series of m, total 2H), 3.40–3.55 (m, 2H), 3.66 and 3.67 (each s, total 3H), 3.89 (dd, J = 7.6 and 10.8 Hz, 1H), 4.03 (dd, J = 4.8 and 10.8 Hz, 1H), 4.14 and 4.49 (each m, total 1H); MS (ESI), m/z 314 $[M+H]^+$.

5.68. Methyl 4-[1-*tert*-butoxycarbonyl-(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy]benzoate (28e)

The title compound was prepared in a procedure similar to that described for **28a**, starting from **27c**. Yield 81%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.16 and 1.20 (each d, J = 6.4 Hz, total 3H), 1.47 (s, 9H), 1.55 (m, 1H), 2.01 (m, 1H), 2.12 (m, 2H), 3.80–3.95 (series of m, total 2H), 3.87 (s, 3H), 4.11–4.26 (series of m, total 2H), 6.95 (m, 2H), 7.96 (d, J = 8.8 Hz, 2H).

5.69. Methyl *trans*-4-[(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5g)

To a stirred solution of **28e** (471 mg, 1.37 mmol) in CH_2Cl_2 (6 mL) was added TFA (1 mL) at room temperature. After being stirred for 3 h at room temperature, the reaction mixture was concentrated in vacuo to give methyl 4-[(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy]benzoate as a yellow oil. The oil was dissolved in MeOH (12 mL) and hydrogenated at 20 atm over Rh/Al_2O_3 (250 mg) for 5 h. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo to give methyl 4-[(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (550 mg, quant.) as a pale brown solid. To a solution of this solid in MeCN–H_2O (1:1, v/v, 12 mL) was added Boc_2O (299 mg, 1.37 mmol) and Et_3N (420 μl, 3.01 mmol), and the mixture was stirred at room temperature for 14 h. The reaction mixture was concentrated to a small volume. The residue was partitioned between 1 N HCl and EtOAc. The phases were separated and the aqueous phase was extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give methyl 4-[1-*tert*-butoxycarbonyl-(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (440 mg, 90%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.07–1.34 (m, 4H), 1.38–1.58 (m, 12H, including s, 9H, at δ 1.46), 1.58–1.74 (m, 2H), 1.74–2.23 (m, 6H), 2.25–2.45 (m, 1H), 3.14–3.62 (m, 3H), 3.67 (s, 3H), 3.77–4.06 (m, 2H).

The resulting oil was dissolved in MeOH (40 mL). To this solution was added NaOMe (200 mg, 3.70 mmol) under N_2, and the mixture was refluxed for 18 h. After being cooled to room temperature, the resulting mixture was concentrated, acidified with 1 N HCl at 0 °C, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give a yellow oil, which was dissolved in benzene–MeOH (4:1, 10 mL). To this solution was added $TMSCHN_2$ (2.0 M in hexane, 600 μl, 1.2 mmol), and the resulting mixture was stirred at room temperature for 16 h. To the reaction mixture was added AcOH (200 μl, 0.12 mmol) and concentrated in vacuo. The residue was purified by flash column chromatography with hexane–EtOAc (5:1, v/v) as eluent to give methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*R*)-methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**29e**) (120 mg, 27% for 2 steps) as a pale yellow oil.

To a stirred solution of **29e** (234 mg, 0.66 mmol) in CH_2Cl_2 (5 mL) was added TFA (1 mL), and the mixture was stirred at room temperature for 3 h. After the reaction mixture was concentrated in vacuo, the residue was treated with satd $NaHCO_3$ aq and extracted with $CHCl_3$–MeOH (5:1, v/v). The extract was dried over $MgSO_4$, and concentrated to give the title compound (168 mg, quant.) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.13 (d, J = 6.0 Hz, 3H), 1.27 (m, 3H), 1.39-1.48 (series of m, total 3H), 1.88–2.09 (series of m, total 7H), 2.27 (tt, J = 4.0 and 12.0 Hz, 1H), 3.23 (m, 2H), 3.32–3.42 (series of m, total 3H), 3.66 (s, 3H); MS (ESI), m/z 256 $[M+H]^+$.

5.70. (2*S*)-Benzyloxymethyl-1-*tert*-butoxycarbonyl-(5*S*)-methansulfonyloxymethylpyrrolidine (25b)

To a stirred solution of **25a** (2.0 g, 6.22 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (1.7 mL, 12.4 mmol) and MsCl (722 μl, 9.33 mmol) at 0 °C. After being stirred at the same temperature for 1 h, the mixture was quenched with H_2O (50 mL) and extracted with $CHCl_3$ (200 mL). The extract was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$–EtOAc (4:1) as eluent to give the title compound (2.54 g, quant.) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.40–1.48 (m, 9H), 1.87–2.21 (m, 4H), 2.99–3.02 (m, 3H), 3.36–4.57 (series of m, total 8H), 7.26–7.36 (m, 5H); MS (ESI), m/z 400 $[M+H]^+$.

5.71. (2*S*)-Benzyloxymethyl-1-*tert*-butoxycarobnyl-(5*S*)-fluoromethylpyrrolidine (26c)

To a stirred solution of **25b** (499 mg, 1.25 mmol) in THF (22 mL) was added TBAF (1.0 M in THF, 12.5 mL, 12.5 mmol). After being refluxed for 8 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on silica gel with hexane–EtOAc (4:1) as eluent to give the title compound (273 mg, 68%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.40 and 1.46 (each s, total 9H), 1.87–2.18 (m, 4H), 3.33–3.68 (m, 2H), 3.90–4.04 (m, 2H), 4.23–4.62 (m, 4H), 7.31 (m, 5H); MS (ESI), m/z 324 $[M+H]^+$.

5.72. 1-*tert*-Butoxycarbonyl-(5*S*)-fluoromethyl-(2*S*)-hydroxymethylpyrrolidine (27d)

The title compound was prepared in a procedure similar to that described for **23**, starting from **26c**. Yield 83%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.93–2.11 (m, 4H), 3.41–4.51 (series of m, 6H); MS (ESI), m/z 234 $[M+H]^+$.

5.73. Methyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-fluoromethyl-(2*S*)-pyrrolidnylmethyl]benzoate (28f)

The title compound was prepared in a procedure similar to that described for **28a**, starting from **27d**. Yield 92%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 and 1.48 (each s, total 9H), 1.99–2.26 (m, 4H), 3.88–4.72 (series of m, 9H), 6.91–6.97 (m, 2H), 7.96–7.99 (m, 2H); MS (ESI), m/z 368 $[M+H]^+$.

5.74. Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-fluoromethyl-(2*S*)-pyrrolidinylmethyl]cyclohexanecarboxylate (29f)

The title compound was prepared in a procedure similar to that described for **29a**, starting from **28f** via methyl 4-[1-(*tert*-butoxycarbonyl)-(5*S*)-fluoromethyl-(2*S*)-pyrrolidinylmethyl] cyclohexanecarboxylate.

For methyl 4-[1-(*tert*-butoxycarbonyl)-(5*S*)-fluoromethyl-(2*S*)-pyrrolidinylmethyl]cyclohexanecarboxylate; Yield 54%. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.11–2.41 (m, 22H), 3.13–3.73 (m, 6H), 3.79–4.07 (m, 2H), 4.21–4.44 (m, 1H), 4.45–4.66 (m, 1H); MS (ESI), *m*/*z* 374 $[M+H]^+$.

For **29f**; Yield 41% (two steps). A pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.10–1.30 (m, 2H), 1.34–1.52 (m, 11H, including s, 9H, at δ 1.44), 1.79–2.30 (m, 9H), 3.11–3.23 (m, 1H), 3.24–3.49 (m, 1H), 3.50–3.69 (m, 4H, including each s, total 3H, at δ 3.63 and 3.64), 3.78–4.03 (m, 2H), 4.17–4.40 (m, 1H), 4.42–4.62 (m, 1H); MS (ESI), *m*/*z* 374 $[M+H]^+$.

5.75. Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-methanesulfonyloxymethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (29g)

The title compound was prepared in a procedure similar to that described for **25b**, starting from **29c**. Yield 73%. A clear oil. ^{1}H NMR ($CDCl_3$) δ 1.12–1.34 (m, 2H), 1.36–1.55 (m, total 11H, including each s, total 9H, at δ 1.47 and 1.49), 1.60–2.38 (series of m, total 9H), 3.00 (s, 3H), 3.19 (m, 1H), 3.31–3.65 (series of m, total 2H), 3.66 (s, 3H), 3.81–4.38 (series of m, total 4H); MS (ESI), *m*/*z* 450 $[M+H]^+$.

5.76. Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-methylthiomethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (29h)

To a stirred solution of **29g** (270.5 mg, 0.602 mmol) in DMF (5.5 mL) was added sodium thiomethoxide (126.5 mg, 1.805 mmol). After being stirred for 4.5 h at 60 °C, the mixture was diluted with EtOAc, washed with 1 N HCl, dried over Na_2SO_4, and concentrated. The residue was dissolved in benzene–MeOH (10:1, v/v, 5.5 mL), and to the solution was added $TMSCHN_2$ (0.30 mL, 2.0 M in hexane, 0.602 mmol) at 0 °C. The mixture was stirred for 30 min at room temperature, and then the reaction was quenched with AcOH at 0 °C. The solution was concentrated and the residue was chromatographed on silica gel with hexane–EtOAc (2:1, v/v) to give the title compound (202.4 mg, 84%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ 1.15–1.33 (m, 2H), 1.37–1.55 (m, total 11H, including each s, total 9H, at δ 1.46 and 1.47), 1.85–2.10 (m, total 7H), 2.13 and 2.16 (each s, total 3H), 2.20–2.39 (m, 2H), 2.79 and 2.95 (each d, J = 12.4 Hz, total 1H), 3.15–3.70 (series of m, total 7H, including each s, total 3H, at δ 3.65 and 3.66), 3.84 (m, 1H), 3.93 (m, 1H); MS (ESI), *m*/*z* 402 $[M+H]^+$.

5.77. Methyl *trans*-4-[(5*S*)-methylthiomethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5i)

To a stirred solution of **29h** (202.4 mg, 0.504 mmol) in CH_2Cl_2 (4 mL) was added TFA (2 mL) at 0 °C. After being stirred at room temperature for 2 h, the mixture was concentrated, diluted with $CHCl_3$ and neutralized with sat $NaHCO_3$ aq The resulting mixture was extracted with $CHCl_3$–MeOH (10:1, v/v), and the organic layer was dried over Na_2SO_4, and concentrated to give the title compound (165.6 mg, quant.) as a pale yellow oil, which was used to the subsequent reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.18–1.33 (m, 2H), 1.37–1.56 (m, 4H), 1.87–2.31 (series of m, total 11H, including s, 3H, at δ 2.12), 2.54 and 2.55 (each s, total 2H), 3.22 (m, 1H), 3.26–3.48 (m, 4H), 3.66 (s, 3H); MS (ESI), *m*/*z* 302 $[M+H]^+$.

5.78. Ethyl 4-[(5*S*)-benzyloxymethyl-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinylmethoxy]benzoate (28g)

The title compound was prepared in a procedure similar to that described for **28a**, starting from **25a** using ethyl 4-hydroxybenzoate. Yield 74%. A colorless oil.

5.79. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-benzyloxymethyl-(2*S*)-pyrrolidinylmethoxy] cyclohexanecarboxylate (29i)

A mixture of **28g** (54 g, 115 mmol) and $Pd(OH)_2$ (7.0 g) in EtOH–AcOH (5:1, 600 mL) was stirred under hydrogen atmosphere (1 atm) at room temperature for 5 days. The mixture was filtered to remove the catalyst and the solvent was evaporated to give ethyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-hydroxymethyl-(2*S*)-pyrrolidinylmethoxy]benzoate (**28h**), which was used to the subsequent reaction without further purification. To a stirred solution of **28h** (43.6 g, 115 mmol) in CH_2Cl_2 (150 mL) was added TFA (200 mL) at 0 °C. After being stirred at room temperature for 40 min, the mixture was concentrated. A mixture of the residue and 5% Rh/Al_2O_3 (10 g) in EtOH–AcOH–TFA (25:5:1, v/v, 620 mL) was stirred under hydrogen atmosphere (10 atm) at room temperature overnight. The mixture was filtered, and the filtrate was concentrated in vacuo. To a stirred solution of the residue in CH_2Cl_2 (500 mL) was added Et_3N (64 mL, 460 mmol) and Boc_2O (27.6 g, 127 mmol) at 0 °C. After the mixture was stirred at room temperature for 75 min, Et_3N (100 mL, 719 mmol) was added to the mixture, which was stirred for 75 min. The solution was concentrated and the residue was chromatographed on silica gel with hexane–EtOAc (4:1 to 1:3, v/v) to give ethyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-hydroxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30 g, 68% for 4 steps) as a light blue oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.29 (m, 3H), 1.37–1.71 (m, 13H, including s, 9H, at δ 1.47), 1.73–2.55 (m, 9H), 3.12–3.37 (m, 1H), 3.38–3.48 (m, 1H), 3.48–4.08 (m, 5H), 4.08–4.17 (m, 2H), 4.19–4.40 (m, 1H).

To a stirred solution of ethyl 4-[1-*tert*-butoxycarbonyl-(5*S*)-hydroxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30 g, 77.9 mmol) and benzyl bromide (18.6 mL, 155.8 mmol) in DMF (300 mL) was added NaH (4.7 g, 116.9 mol) portionwise at 0 °C. After being stirred for 3 h, the mixture was diluted with H_2O, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with hexane–EtOAc (7:1 to 4:1, v/v) to give ethyl 4-[(5*S*)-benzyloxymethyl-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30 g, 81%) as a colorless oil. To a stirred solution of the oil (30 g, 63.2 mmol) in DMF (300 mL) was added NaH (5.56 g, 139.0 mmol) and EtOH (7.36 mL, 126.4 mmol) at 0 °C. After the mixture was stirred at 0 °C for 40 min, H_2O (1.36 mL, 75.8 mmol) was added to the mixture, which was stirred at room temperature overnight. To the mixture was added H_2O (1.36 mL, 63.2 mmol), K_2CO_3 (26.2 g, 189.6 mmol) and EtI (15.2 mL, 189.6 mmol), the resulting mixture was stirred at 70 °C for 30 min. The mixture was diluted with H_2O and EtOAc and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography with hexane–EtOAc (8:1, v/v) as eluent to give the title compound (16 g, 53% for two steps) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.16–1.30 (m, 5H), 1.33–1.54 (m, 11H), 1.84–2.11 (m, 8H), 2.18–2.30 (m, 1H), 3.14–3.32 (m, 2H), 3.35–3.50 (m, 1H), 3.54–3.71 (m, 2H), 3.76–4.03 (m, 2H), 4.06–4.16 (m, 2H), 4.44–4.60 (m, 2H), 7.22–7.37 (m, 5H).

5.80. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-hydroxymethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (29j)

A mixture of **29i** (16 g, 33.7 mmol) and $Pd(OH)_2$ (2.0 g) in EtOH–AcOH (4:1, 250 mL) was stirred under hydrogen atmosphere (1 atm) at room temperature overnight. The mixture was filtered to remove the catalyst and the filtrate was evaporated. The residue was purified by flash column chromatography with hexane–EtOAc (3:1, v/v) as eluent to give the title compound (13 g, 100%) as colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.17–1.31 (m, 5H, including t, J = 7.2 Hz, 3H, at δ 1.25,), 1.39–1.72 (m, 11H, including s, 9H, at δ 1.48), 1.75–2.19 (m, 8H), 2.19–2.31 (m, 1H), 3.13–3.25 (m, 1H), 3.28–3.76 (m, 4H), 3.77–4.36 (m, 4H, including q, J = 7.2 Hz, 2H, at δ 4.12).

5.81. General procedure D: preparation of methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-dimethylaminomethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (31a)

5.81.1. Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-formyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30a)

To a stirred solution of **29c** (3.56 g, 9.58 mmol) and trichloroisocyanuric acid (2.45 g, 10.5 mmol) in CH_2Cl_2 (50 mL) was added 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) (0.15 g, 0.96 mmol) at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was filtered through a Celite pad. The filtrate was made basic with satd $NaHCO_3$ aq and extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel with hexane–EtOAc (2:1) to give the title compound (2.88 g, 81%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.17–1.30 (m, 2H), 1.36–1.52 (m, 11H, including s, toral 9H, at δ 1.42 and 1.49), 1.83–2.09 (m, 8H), 2.20–2.33 (m, 1H), 3.16–3.27 (m, 1H), 3.36–3.74 (m, 5H, including s, total 3H, at δ 3.66 and 3.67), 3.98–4.31 (m, 2H), 9.52 and 9.59 (each d, J = 2.1 Hz, total 1H).

5.81.2. Methyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-dimethylaminomethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (31a)

To a stirred mixture of **30a** (545 mg, 1.48 mmol) and Me_2NH (2.0 M in THF, 0.74 mL, 1.48 mmol) in THF (30 mL) was added $NaBH(OAc)_3$ (941 mg, 4.44 mmol) at room temperature. After being stirred for 2.5 h, the reaction mixture was poured into satd $NaHCO_3$ aq and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4 and condensed in vacuo. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (20:1) to give the title compound (470 mg, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.18–1.32 (m, 3H), 1.39–1.42 (m, 1H), 1.46 and 1.47 (each s, total 9H), 1.58 (m, 1H), 1.83–2.19 (m, 10H), 2.25 (s, 3H), 2.28 (s, 3H), 3.17–3.24 (m, 1H), 3.32 and 3.36 (each t, J = 8.8 Hz, total 1H), 3.58 (dd, J = 2.7 and 8.6 Hz, 1H), 3.65 and 3.66 (each s, total 3H), 3.71–3.91 (m, 2H); MS (ESI), m/z 399 $[M+H]^+$.

Compound **31b**–**31e** were prepared according to general procedure D.

5.82. Methyl *trans*-4-[(5*S*)-dimethylaminomethyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate dihydrochloride (5j)

A solution of **31a** (470 mg, 1.18 mmol) in 4 N HCl–dioxane (20 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure. The residue was washed with Ether to give the title compound (373 mg, 85%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.12–1.29 (m, 2H), 1.30–1.45 (m, 2H), 1.48–1.77 (m, 2H), 1.81–2.11 (m, 5H), 2.12–2.36 (m, 2H), 2.84 (s, 6H), 3.22–3.44 (m, 2H), 3.47–3.72 (m, 6H, including s, 3H, at δ 3.59), 3.73–3.89 (m, 1H), 3.94–4.12 (m, 1H), 8.76–9.28 (m, 1H), 9.61–9.87 (m, 1H), 10.65–10.89 (m, 1H); MS (ESI), m/z 299 $[M+H]^+$.

5.83. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-[methoxy(methyl)amino]methyl-(2*S*)-pyrrolidinyl methoxy]cyclohexanecarboxylate (31b)

5.83.1. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-formyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (30b)

The title compound was prepared in a procedure similar to that described for **30a**, starting from **29j**. A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.15–1.30 (m, 5H), 1.36–1.52 (m, 11H, including s, total 9H, at δ 1.42 and 1.49), 1.80–2.11 (m, 8H), 2.15–2.32 (m, 1H), 3.15–3.28 (m, 1H), 3.35–3.66 (m, 2H), 3.95–4.31 (m, 4H), 9.52 and 9.58 (each d, J = 2.1 Hz, total 1H).

5.83.2. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-[methoxy(methyl)amino]methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (31b)

Yield 67% (two steps). A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.16–1.32 (m, 5H), 1.39–1.52 (m, 12H), 1.89–2.51 (m, 9H), 2.57 and 2.59 (each s, total 3H), 2.81–3.41 (m, 3H), 3.46 and 3.47 (each s, total 3H), 3.54–4.01 (m, 3H), 4.11 (q, J = 7.1 Hz, 2H); MS (ESI), m/z 429 $[M+H]^+$.

5.84. Ethyl *trans*-4-[(5*S*)-[methoxy(methyl)amino]methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5k)

The title compound was prepared in a procedure similar to that described for **5a**, starting from **31b**. Yield 99%. A brown oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.31 (m, 5H), 1.37–1.52 (m, 3H), 1.54–2.34 (m, 9H), 2.52–2.56 (m, 2H), 2.56 (s, 3H), 3.18–3.48 (m, 5H), 3.52 (s, 3H), 4.11 (q, J = 7.1 Hz, 2H); MS (ESI), m/z 329 $[M+H]^+$.

5.85. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-(1-isoxazolidinylmethyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (31c)

Yield 39% (two steps). A colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.11–1.34 (m, 6H), 1.35–1.62 (m, 12H), 1.75–2.14 (m, 8H), 2.15–2.32 (m, 3H), 2.79–3.13 (m, 2H), 3.14–3.42 (m, 2H), 3.49–3.72 (m, 1H), 3.73–4.06 (m, 4H), 4.11 (q, J = 7.1 Hz, 2H); MS (ESI), m/z 441 $[M+H]^+$.

5.86. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-(4-morpholinyl)methyl-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (31d)

Yield 77% (two steps). A yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.22–1.26 (m, 5H), 1.40–1.46 (m, 11H), 1.85–2.62 (m, 13H), 3.20–3.90 (m, 11H), 4.10–4.13 (m, 2H); MS (ESI), m/z 455 $[M+H]^+$.

5.87. Ethyl *trans*-4-[1-*tert*-butoxycarbonyl-(5*S*)-(3-methoxyazetidinylmethyl)-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (31e)

Yield 85%. A yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.09–1.31 (m, 6H), 1.42–1.51 (m, 11H), 1.74–2.30 (m, 9H), 2.35–2.78 (m, 1H), 2.88–3.27 (m, 5H), 3.28–3.93 (m, 7H), 3.97–4.06 (m, 1H), 4.06–4.16 (m, 2H); MS (ESI), m/z 455 $[M+H]^+$.

5.88. Determination of aqueous solution solubility

JP1 and JP2 were used as solvents for solubility measurements. Two milligrams of one of the compounds was accurately weighed into a disposable glass tube equipped with a fitted cap, and 1 mL of one of the solvents was added. The glass tube was capped, sonicated for 5 min, and shaken vigorously for 30 s at 5-min intervals for 30 min. The sample solutions were filtered through a 0.45 μm membrane filter (Millex-HV; Millipore Corp.). The filtrate was diluted if necessary, and the resulting solutions were assayed by High-performance liquid chromatography (HPLC) system (Waters Alliance 2690; Waters Corp.). The concentration of the solution was calculated from the calibration curve for the appropriate compound.

5.89. Distribution coefficient (log*D*)

The same volume of 1-octanol and JP2 (pH 6.8) were mixed and shaken for 30 min at room temperature. The resulting solution was allowed to stand for 24 h at room temperature; this process yielded 1-octanol-saturated JP2 solution layer (S_{JP2}) and a JP2-saturated 1-octanol layer (S_{oct}). Two milligrams of one of the compounds was accurately weighed into a disposable glass tube. Ten milliliters of the S_{oct} was added and the compound was dissolved. After filtering the sample solution (Millex-LH; Millipore Corp.), 3 mL of the resulting solution was mixed with 3 mL of S_{JP2}. This mixture was shaken for 30 min and then centrifuged for 10 min at 3000 rpm. The upper and lower fractions of solutions were collected separately as the S_{oct} phase and S_{JP2} phase. One part of the S_{oct} phase was diluted with 25 parts of an acetonitrile–water (1:1, v/v) solution, and then loaded onto HPLC system to determine the concentration of the compound. The S_{JP2} phase was diluted with 2 parts of the acetonitrile–water (1:1, v/v) solution, and then loaded onto HPLC system to determine the concentration of the compound. The octanol-JP2 partition coefficient (log *D*) was calculated from the following equation:

$$\log D\ (\text{pH } 6.8) = \log[(\text{peak area of } S_{oct} \text{ sample} \times 25)/(\text{peak area of } S_{JP2} \text{ sample} \times 2)]$$

5.90. VLA-4/VCAM-1 binding assay

A human VLA-4-expressing cell line, 4B4, was established at Pharmacopeia Inc., 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 Corp.) supplemented with 10% (v/v) fetal calf serum (REHATUIN Fetal Bovine Serum, Serologicals Corp.), 100 U/mL penicillin (Invitrogen Corp.), 100 μg/mL streptomycin (Invitrogen Corp.), 2 mM L-glutamine (Invitrogen Corp.) and 1 mg/mL G-418 (Geneticin, Invitrogen Corp.). A Eu-labeling Reagent (PerkinElmer Inc.) was used to label the human VCAM-1/Fc chimera (R&D Systems Inc.). The Eu-labeled protein was purified with 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 microliter of the 4B4 cell suspension was placed into each well of a 96-well-culture plate (Costar Inc.). The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 2 days. 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 solution was added to a well, followed by 50 μl of 2 nM of Eu-labeled 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 6% (w/v) human serum albumin (Sigma Corp.) 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.

5.91. Murine bronchial inflammatory model

Female BALB/c AnNCrj mice (8 weeks old, Charles River Japan, Inc.) received an oral administration of cyclophosphamide dissolved in water at a dose of 150 mg/kg (day 0). On day 2 and 14, 500 μg protein of *Ascaris suum* extract (LSL Co., Ltd) in 0.2 mL saline containing 4.5 mg aluminum hydroxide was injected intraperitoneally. On day 22, the mice were challenged intratracheally under anesthesia with 300 μg (30 μl) protein of *Ascaris suum* extract. In the negative control group, sensitized mice were challenged with saline instead of the antigen.

5.92. Effect on eosinophil infiltration

Test compounds, which were dissolved in 0.5% MC containing 0.03% Tween 80, were orally administered 15 min before and 8, 24, and 32 h after the antigen challenge at a dose of 5 or 15 mg/kg (for **7c**, **7f**, and **7g**) and 5 or 12.5 mg/kg (for **7n**). Forty-eight hours after antigen challenge, the mice were sacrificed and BALF was collected using tracheal polyethylene cannula with 2×0.5 mL Hanks' balanced salt solution. The cells in the BALF were counted in a particle analyzer CDA-500 (Sysmex Corp.). Cytocentrifuged preparations (Cytospin 2; Shandon) were stained with Wright's stain solution (Muto Chemical Co., Ltd) for differential counts, based on standard morphologic criteria. The number of eosinophils was calculated by multiplying the total cell number by the percentage of eosinophils in the cytocentrifuged preparations.

5.93. Effect on hyper-responsiveness

Compound **7n**, which was dissolved in 0.5% MC containing 0.03% Tween 80, was orally administered 15 min before and 8, 24, and 32 h after the antigen challenge at a dose of 2 or 12.5 mg/kg. The bronchial hyper-responsiveness in each mouse was estimated from the increase in lung resistance by acetylcholine chloride (ACh; Sigma Corp.) injection at 48 h after the antigen challenge. Ten minutes before the start of the measurement, the mice were anesthetized by an intraperitoneal injection of pentobarbital at a dose of 100 mg/kg. The trachea was cannulated and connected to a rodent ventilator (Mini Vent type 845; Hugo Sachs Electronik-Harvard Apparatus) with an in-line pressure transducer (TRD-4510; Buxco Electronics, Inc.) that was coupled to a pulmonary mechanics analyzer (Bio-System XA; Buxco Electronics, Inc.). The flows were determined by measuring the differential pressure (TRD-5100; Buxco Electronics, Inc.) across eight layers of 400-mesh wire cloth covering a 1.3-cm hole in a plethysmograph box (Plyan-M; Buxco Electronics, Inc.). The mice were placed in the plethysmograph box and then ventilated at 140 strokes/min with a stroke volume of 150 μl. After establishing a stable baseline of lung resis-

tance, ACh, dissolved in saline was cumulatively administered (25, 50, and 100 (μg/mL)/kg) via the tail vein, and the changes in lung resistance were monitored.

5.94. Pharmacokinetic studies on monkeys

Female cynomolgus monkeys (3.5–4 kg, HAMRI Co.) were used. The animals were fasted for 18 h prior to dosing. Each group consisted in three animals. Compound **7n** was suspended in 0.5% (w/v) MC for oral dosing (0.5 mg/kg) or dissolved in saline with 3 equiv NaOH for intravenous dosing (0.5 mg/kg). Blood samples (1 mL) were collected after 0.08, 0.25 (for iv), 0.25 (for p.o.), 0.5, 1, 2, 4, 8, and 10 (for p.o.) h. After the 4 h sampling, the animals were provided with food. These analytical samples were left to stand at room temperature, followed by centrifugation at 15,000 rpm for 10 min at 4 °C. The plasma fractions were subsequently stored in a −20 °C freezer until being analyzed. The concentrations of the compounds were determined by an LC/MS/MS method, comprised of an Alliance 2695 HPLC (Waters), Symmetry Shield RP8, 2.1 × 50 mm, 3.5 μm column (Waters), and TSQ-700 (Thermo Electron, Waltham, MA). The mobile phase consisted of 10 mM $HCOONH_4$ in water/methanol; the gradient condition was 90/10 to 10/90. The plasma concentrations versus the time data were analyzed by non-compartmental approaches using the WinNonlin software program (version 1.13.1 Pharsight, Mountain View, CA).

All the animal experiments were conducted with the approval of the Animal Experiment Ethics Committee of Daiichi Pharmaceutical Co., Ltd.

References and notes

1. Hynes, R. O. *Cell* **1987**, *48*, 549.
2. Elices, M. J.; Osborn, L.; Takeda, Y.; Crouse, C.; Luhowskyj, S.; Hemler, M. E.; Lobb, R. R. *Cell* **1990**, *60*, 577.
3. (a) Guan, J. L.; Hyens, R. O. *Cell* **1990**, *60*, 53; (b) Wayner, E. A.; Garcia-Pardo, A.; Humphries, M. J.; McDonald, J. A.; Carter, W. G. *J. Cell. Biol.* **1989**, *109*, 1321.
4. (a) Barthel, S. R.; Johansson, M. W.; McNamee, D. M.; Mosher, D. F. *J. Leukoc. Biol.* **2008**, *83*, 1; (b) Abonia, J. P.; Hallgren, J.; Jones, T.; Shi, T.; Xu, Y.; Koni, P.; Flavell, R. A.; Boyce, J. A.; Austen, K. F.; Gurish, M. F. *Blood* **2006**, *108*, 1588.
5. (a) Silverman, M. D.; Hass, C. S.; Rad, A. M.; Arbab, A. S.; Koch, A. E. *Arthritis Rheum.* **1817**, *2007*, 56; (b) Carter, R. A.; Wicks, I. P. *Arthritis Rheum.* **2001**, *44*, 985; (c) Seiffge, D. *J. Rheumatol.* **1996**, *23*, 2086.
6. (a) Rice, G. P. A.; Hartung, H. P.; Calabresi, P. A. *Neurology* **2005**, *64*, 1336; (b) Yednock, T. A.; Cannon, C.; Fritz, L. C.; Sanchez-Madrid, F.; Steinman, L.; Karin, N. *Nature* **1992**, *356*, 63; (c) Piraino, P. S.; Yednock, T. A.; Freedman, S. B.; Messersmith, E. K.; Pleiss, M. A.; Vandevert, C.; Thorsett, E. D.; Karlik, S. J. *J. Neuroimmunol.* **2002**, *131*, 147.
7. (a) Podolsky, D. K. *N. Engl. J. Med.* **1991**, *325*, 928; (b) Bischoff, S. C.; Wedemeyer, J.; Herrmann, A.; Meier, P. N.; Trautwein, C.; Cetin, Y.; Maschek, H.; Stolte, M.; Gebel, M.; Manns, M. P. *Histopathology* **1996**, *28*, 1; (c) Hogan, S. P.; Rothenberg, M. E. *Aliment. Pharmacol. Ther.* **2004**, *20*, 1231.
8. (a) Tilley, J. W. *Expert Opin. Ther. Pat.* **2008**, *18*, 841; (b) Yang, G. X.; Hagmann, W. K. *Med. Res. Rev.* **2003**, *23*, 369; (c) Jackson, D. Y. *Curr. Pharm. Des.* **2002**, *8*, 1229; (d) Vanderslice, P.; Biediger, R. J.; Woodside, D. G.; Berens, K. L.; Holland, G. W.; Dixon, R. A. F. *Pulm. Pharmacol. Ther.* **2004**, *17*, 1.
9. (a) Miller, D. H.; Khan, O. A.; Sheremata, W. A.; Blumhardt, L. D.; Rice, G. P. A.; Libonati, M. A.; Willmer-Hulme, A. J.; Dalton, C. M.; Miszkiel, K. A.; O'Connor, P. W. *N. Engl. J. Med.* **2003**, *348*, 15; (b) Ghosh, S.; Goldin, E.; Gordon, F. H.; Malchow, H. A.; Madsen, J. R.; Rutgeerts, P.; Vynálek, P.; Zádorová, Z.; Palmer, T.; Donoghue, S. *N. Engl. J. Med.* **2003**, *348*, 24; (c) Steinman, L. *Nat. Rev. Drug Disc.* **2005**, *4*, 510; (d) Elan-Biogen official home page: http://www.tysabri.com/.
10. Muro, F.; Iimura, S.; Yoneda, Y.; Chiba, J.; Watanabe, T.; Setoguchi, M.; Takayama, G.; Yokoyama, M.; Takashi, T.; Nakayama, A.; Machinaga, N. *Bioorg. Med. Chem.* **2009**, *17*, 1232.
11. Muro, F.; Iimura, S.; Sugimoto, Y.; Yoneda, Y.; Chiba, J.; Watanabe, T.; Setoguchi, M.; Iigou, Y.; Matsumoto, K.; Satoh, A.; Takayama, G.; Taira, T.; Yokoyama, M.; Takashi, T.; Nakayama, A.; Machinaga, N. *J. Med. Chem.* **2009**, *52*, 7974.
12. Setoguchi, M.; Iimura, S.; Sugimoto, Y.; Yoneda, Y.; Chiba, J.; Watanabe, T.; Muro, F.; Iigo, Y.; Takayama, G.; Yokoyama, M.; Taira, T.; Aonuma, M.; Takashi, T.; Nakayama, A.; Machinaga, N. *Bioorg. Med. Chem.* **2012**, *20*, 1201.
13. (a) Alelyunas, Y. W.; Empfield, J. R.; McCarthy, D.; Spreen, R. C.; Bui, K.; Pelosi-Kilby, L.; Shen, C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7312; (b) Ishikawa, M.; Hashimoto, Y. *J. Med. Chem.* **2011**, *54*, 1539.
14. (a) Chiba, J.; Takayama, G.; Takashi, T.; Yokoyama, M.; Nakayama, A.; Baldwin, J. J.; McDonald, E.; Moriarty, K. J.; Sarko, C. R.; Saionz, K. W.; Swanson, R.; Hussain, Z.; Wong, A.; Machinaga, N. *Bioorg. Med. Chem.* **2006**, *14*, 2725; (b) Chiba, J.; Iimura, S.; Yoneda, Y.; Sugimoto, Y.; Horiuchi, T.; Muro, F.; Ochiai, Y.; Ogasawara, T.; Tsubokawa, M.; Iigou, Y.; Takayama, G.; Taira, T.; Takata, Y.; Yokoyama, M.; Takashi, T.; Nakayama, A.; Machinaga, N. *Chem. Pharm. Bull.* **2006**, *54*, 1515.
15. (a) Katoh, T.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron Lett.* **1993**, *34*, 5743; (b) Katoh, T.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S. *Tetrahedron* **1994**, *50*, 6221.
16. (a) Takano, S.; Ogasawara, K., Iwabuchi, Y.; Moriya, M. JP03220175.; (b) Takano, S.; Moriya, M.; Iwabuchi, Y.; Ogasawara, K. *Tetrahedron Lett.* **1989**, *30*, 3805.
17. Siegrist, R.; Zürcher, M.; Baumgartner, C.; Seiler, P.; Diederich, F.; Daum, S.; Fischer, G.; Klein, C.; Dangl, M.; Schwaiger, M. *Helv. Chim. Acta* **2007**, *90*, 217.
18. Chiba, J.; Iimura, S.; Yoneda, Y.; Watanabe, T.; Muro, F.; Tsubokawa, M.; Iigou, Y.; Satoh, A.; Takayama, G.; Taira, T.; Yokoyama, M.; Takashi, T.; Nakayama, A.; Machinaga, N. *Bioorg. Med. Chem.* **2007**, *15*, 1679.