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Tricyclic pharmacophore-based molecules as novel integrin $\alpha_v\beta_3$ antagonists. Part IV: Preliminary control of $\alpha_v\beta_3$ selectivity by *meta*-oriented substitution

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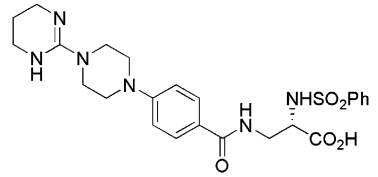
Abstract—To establish the in vivo efficacy of $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists possessing a tricyclic pharmacophore, a corresponding $\alpha_v\beta_3$-selective antagonist was required as a control. We initially took two synthetic approaches to obtain $\alpha_v\beta_3$-selective antagonists based on the RGD recognition pattern or on modification of the dihedral angle between the central benzene ring and the adjacent heterocycle, but both proved unsuccessful. However, synthesis of novel antagonists with *meta*-substitution of the central benzene ring generated weak selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$ for the first time in the family of compounds with the tricyclic pharmacophore. Optimization of *meta*-oriented antagonists furnished an $\alpha_v\beta_3$-selective antagonist exhibiting inhibitory activity not only in a receptor-binding assay, but also in a cell adhesion assay.

1. Introduction

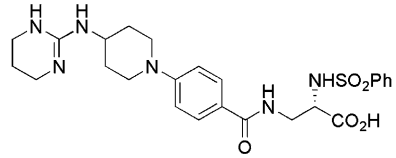
The vitronectin receptor, integrin $\alpha_v\beta_3$,[1,2] is involved in the pathogenesis of various diseases in which cell adhesion or migration plays a key role. The function of integrin $\alpha_v\beta_3$ in vascular smooth muscle cells and leukocytes led us to hypothesize that a potent $\alpha_v\beta_3$ antagonist with an $\alpha_{IIb}\beta_3$-antagonistic effect, that is, a dual antagonist, would be a useful candidate for treatment of acute ischemic diseases, such as myocardial infarction or stroke.[3] In order to confirm the superior in vivo efficacy of dual antagonists possessing a tricyclic pharmacophore, we synthesized a corresponding $\alpha_v\beta_3$-selective antagonist as a control molecule. In our previous report,[4] we described the synthesis of the piperazine-based compound **1**, exhibiting strong $\alpha_{IIb}\beta_3$ antagonism, and the piperidine-based compound **2**, showing $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual activity (Fig. 1). Here, we describe a successful approach to obtain selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$ in this class of compounds.

2. Synthetic approaches to $\alpha_v\beta_3$-selective antagonists. Part 1

Initially, we focused on the RGD recognition pattern to control selectivity. It is known that the simple existence



1: 160 nM/0.73 nM/0.0046



2: 1.3 nM/3.1 nM/2.4

Figure 1. Reported integrin antagonists in our previous research. (IC_{50}: $\alpha_v\beta_3/\alpha_{IIb}\beta_3/\alpha_v\beta_3$ selectivity over $\alpha_{IIb}\beta_3$).

Keywords: Integrin $\alpha_v\beta_3$-selective antagonist; Integrin $\alpha_{IIb}\beta_3$ antagonist; Acute ischemic disease; *meta*-Oriented substitution.
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of an RGD sequence is not necessarily sufficient for a molecule to serve as a ligand for $\alpha_v\beta_3$.[5] In fact, some molecules such as interstitial collagen type I have multiple RGD sequences for interaction with integrin $\alpha_v\beta_3$. Thus, we synthesized several dimeric molecules in an attempt to increase the interaction between integrin $\alpha_v\beta_3$ and the candidate molecules, and thereby improve the $\alpha_v\beta_3$ selectivity.

For construction of the dimers, two kinds of spacers were prepared (Scheme 1). The first dimer (**4**) with a C_6 spacer was directly synthesized using 2 equiv of compound **3**[4] with 1,6-dibromohexane in a low yield (less than 10%). The yield in the direct coupling of **3** with 1,12-dibromododecane, for synthesis of **5** with a longer spacer, was not improved. Then, a C_{12} spacer was first introduced into the monomer (**3**) in a moderate yield to afford the alcohol **6**. Unfortunately, direct coupling of an alcohol **6** with the sulfonamide **3** according to a modified Mitsunobu protocol[6] did not give the desired dimer **5**. Thus, the alcohol was transformed to its iodide **7** via two steps, and **7** was successfully coupled with the sulfonamide **3** to furnish the dimer **5**. Dimers **4** and **5** were deprotected to afford the dimeric antagonists **8** and **9**, respectively. Compound **9** was further converted to a tetrahydropyrimidine analogue **10** by hydrogenolysis.

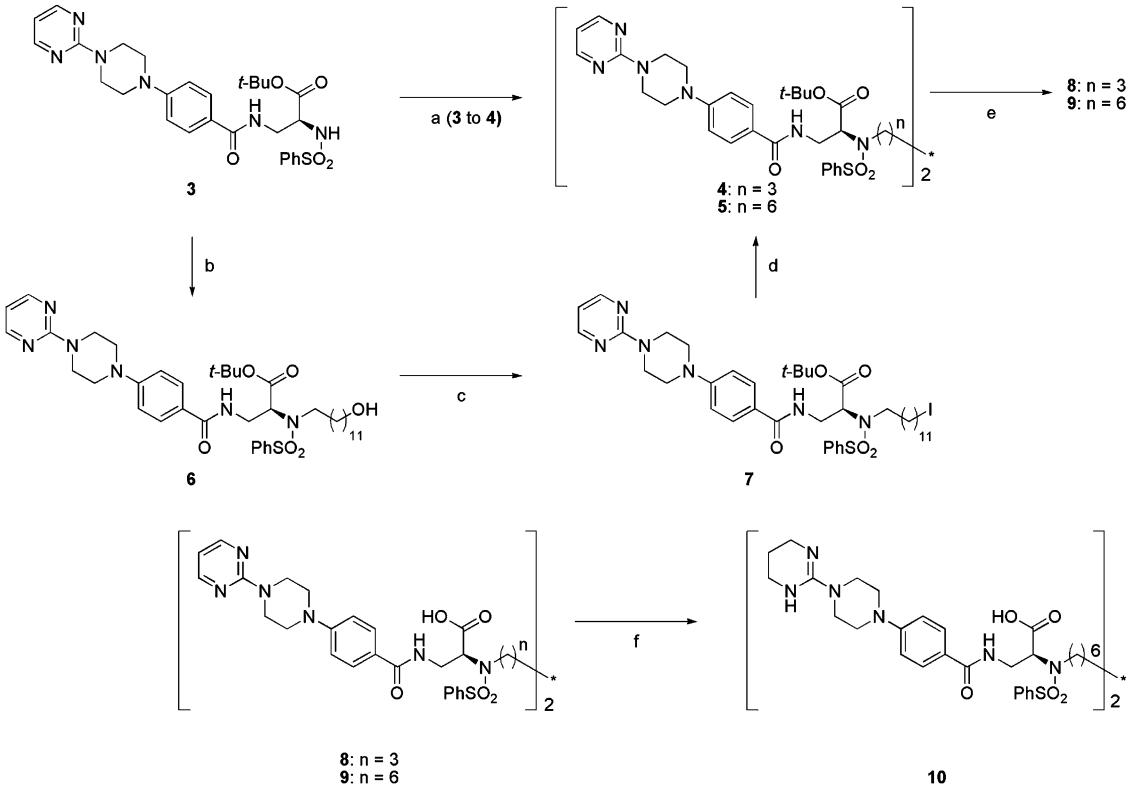
Although the $\alpha_{IIb}\beta_3$-antagonistic activity of some of these compounds was suppressed, none of them showed even weak $\alpha_v\beta_3$ selectivity (Table 1).

3. Synthetic approaches to $\alpha_v\beta_3$-selective antagonists. Part 2

Our preliminary SAR data[4] showed that introduction of a halogen atom into the C-3 position at the central aromatic ring improved the $\alpha_v\beta_3/\alpha_{IIb}\beta_3$-antagonistic activity balance in tricyclic pharmacophore-containing compounds (Table 2). As a matter of fact, the calculated dihedral angle between the central benzene ring and the piperazine ring had a qualitative correlation only with $\alpha_v\beta_3$-antagonistic activity inhibition, not with $\alpha_{IIb}\beta_3$-antagonistic activity. Therefore, we planned to introduce a substituent onto the hetero ring to increase the dihedral angle. However, the 4-(2-methylpiperazin-1-yl)benzoate could not be synthesized by nucleophilic substitution, although the 4-(3-methylpiperazin-1-yl)benzoate could be prepared in a reasonable yield.

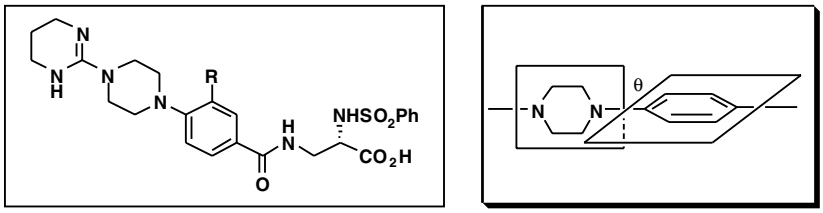
Table 1. Inhibitory activity of dimeric molecules in receptor-binding assay

Compound	Spacer (*n*)	IC_{50} (nM)		$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	
1	None	160	0.73	0.0046
8	6	70,000	43,000	0.61
9	12	29,000	3100	0.11
10	12	8800	11	0.0013



Scheme 1. Reagents and conditions: (a) 1,6-dibromohexane, DBU, DMF, rt, 12 days; (b) 12-bromododecanol, DBU, DMF, rt, 16 h; (c) i—MsCl, TEA, DMAP, CH_2Cl_2, rt, 16 h; ii—NaI, acetone, 40 °C, 40 h; (d) **3**, DBU, DMF, rt, 3 days; (e) TFA, anisole, CH_2Cl_2, 10 h, rt for **8**, 0–4 °C for **9**; (f) H_2, Pd/C, AcOH, HCl, 3 atm, rt, 3 h.

Table 2. Effect of substitution of the central aromatic ring on inhibitory activity in receptor-binding assay



Compound	R	IC_{50} (nM)		$\alpha_v\beta_3/\alpha_{IIb}\beta_3$	θ (deg)
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$		
1	H	160	0.73	0.0045	64.3
11	F	22	1.0	0.045	71.6
12	Cl	3.6	0.12	0.033	88.4

Modeling. All modeling experiments were done using the program package QUANTA/CHARMm (Accelrys Inc.) on SGI workstation.

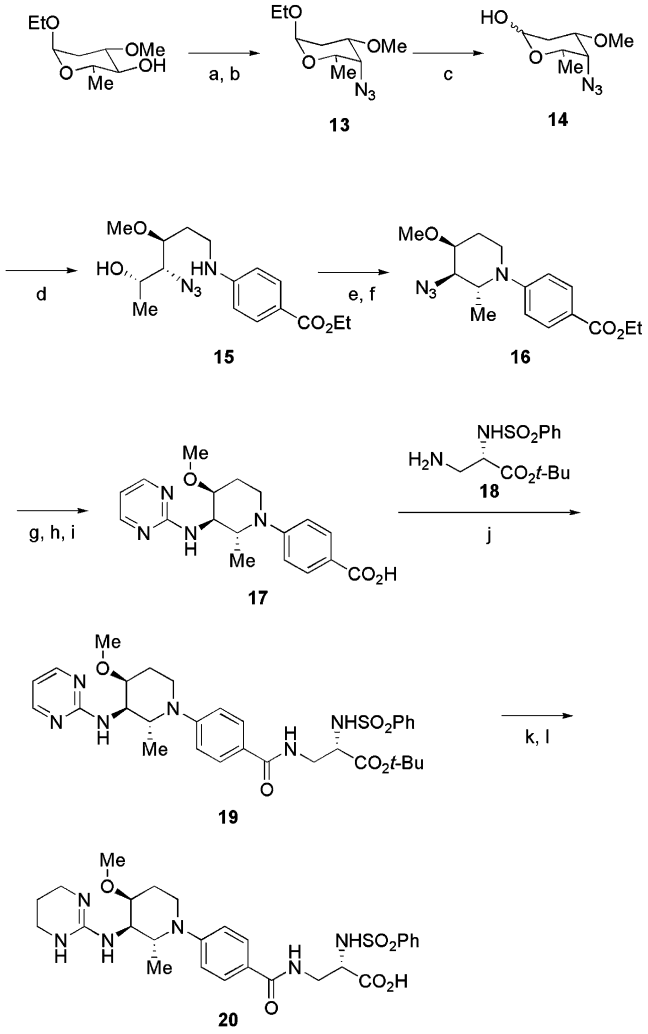
Then, we introduced a methyl group at the C-2 position of the hetero ring by utilizing a natural product (Scheme 2).

L-Oleandrose,[7] isolated as an α-ethyl glycoside, was transformed to its azide derivative (**13**). After acidic hydrolysis, the obtained azide lactol **14** was reacted with ethyl 4-aminobenzoate by reductive amination to afford the key intermediate **15** with a linear substituent. Intramolecular cyclization of **15** proceeded via the mesylate to furnish a 2-methyl-heterocycle framework. Successive transformations gave the desired antagonist **20**. Unfortunately, this molecule did not exhibit $\alpha_v\beta_3$ selectivity ($\alpha_v\beta_3$: 7.8 nM, $\alpha_{IIb}\beta_3$: 3.4 nM, and $\alpha_v\beta_3/\alpha_{IIb}\beta_3$: 0.44).

4. Synthesis of *meta*-oriented antagonists with $\alpha_v\beta_3$ selectivity

We next synthesized *meta*-oriented antagonists **21** and **22** as representative piperazine- and piperidine-based molecules, respectively (Fig. 2), based on the idea that the distance between the *N*-terminus and the *C*-terminus affects the selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$.[8]

Nucleophilic substitution of 3-fluorobenzoate with a secondary amine-containing heterocycle did not proceed even under heating, in contrast to the reaction using 4-fluorobenzoate. However, nucleophilic substitution of 3-fluorobenzonitrile with 4-hydroxypiperidine was achieved to afford a *meta*-oriented intermediate, compound **23** (Scheme 3). The 4-hydroxypiperidine moiety was transformed to 4-aminopiperidine in three steps and reacted with 2-bromopyrimidine to construct the tricyclic pharmacophore. Acid hydrolysis of the benzonitrile moiety of **25** afforded the tricyclic benzoic acid (**26**). On the other hand, palladium-mediated coupling reaction[9] of 3-bromobenzoate with 4-hydroxypiperidine gave the *meta*-oriented benzoate **27** in a low yield. Sequential transformation of the piperidinone moiety, followed by introduction of pyrimidine, gave a tricyclic molecule (**29**), which was then converted to the intermediate **26** by basic hydrolysis. Moreover, the *meta*-oriented



Scheme 2. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, 0 °C, 2.0 h; (b) NaN_3, DMF, 80 °C, 18 h; (c) HCl, 1,4-dioxane, 60 °C, 3.0 h; (d) $NaBCNH_3$, ethyl 4-aminobenzoate, AcOH, CH_2Cl_2/MeOH, rt, 42 h; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h; (f) DIPEA, toluene, reflux, 18 h; (g) H_2, Pd/C, rt, 18 h; (h) 2-bromopyrimidine, DIPEA, 120 °C, 18 h; (i) NaOH, MeOH/H_2O, 50 °C, 6 h; (j) BOP, DIPEA, DMF, rt, 18 h; (k) TFA, CH_2Cl_2, rt, 3 h; (l) H_2, Pd/C, 1,4-dioxane/H_2O, rt, 18 h.

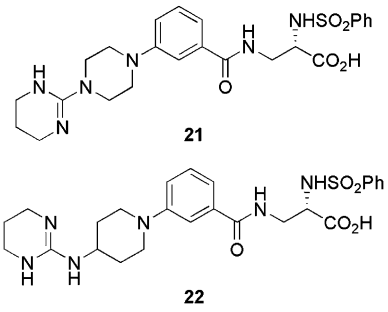
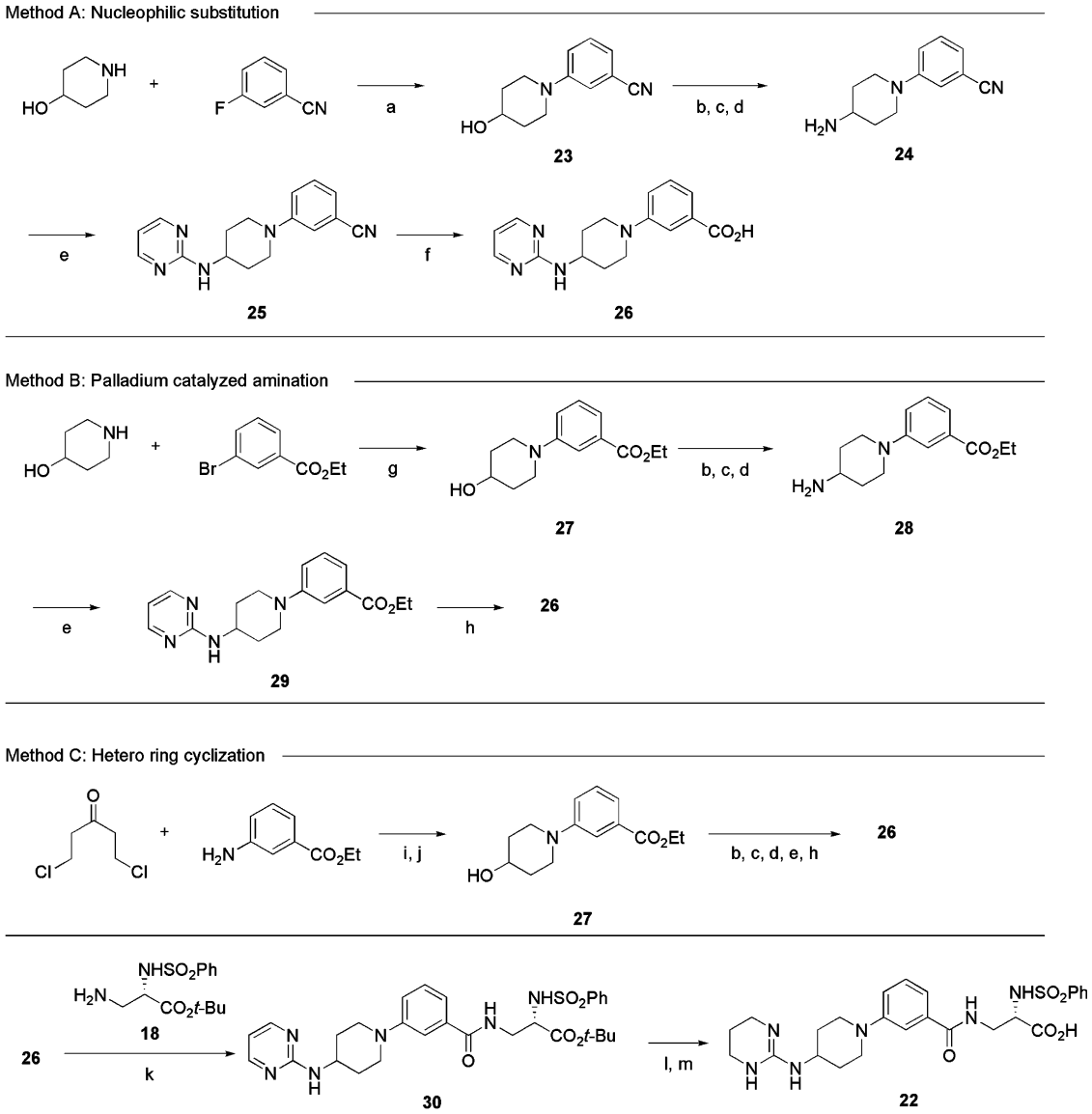


Figure 2. Prototype of *meta*-oriented antagonists.

benzoate **27** could be synthesized using 1,5-dichloropentan-3-one.[10,11] The tricyclic benzoic acid **26** was coupled with the amine **18** to construct the full framework of an antagonist, **30**, which was treated with trifluoroacetic acid and then finally hydrogenated to furnish the desired antagonist **22** (Scheme 3).

The selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$ of the *meta*-oriented antagonists **21** and **22** was improved in comparison with those of *para*-oriented **1** and **2** (Table 3) as expected. Compound **22** not only exhibited antagonistic activity in a receptor-binding assay, but also showed moderate inhibitory activity in a cell adhesion assay. Then, optimization of **22** by chemical modification was started.

First, the heterocyclic moiety of the lead compound (**22**) was altered (Table 4 and Scheme 4). A stereoisomer **34** possessing a 3-aminopiperidine[12,13] moiety and two substituted pyrrolidine derivatives (**36** and **37**) were as potent as **22** in an $\alpha_v\beta_3$-binding inhibition assay. We



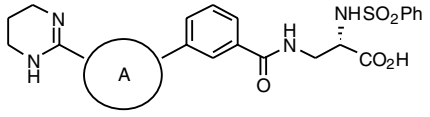
Scheme 3. Reagents and conditions: (a) $NaHCO_3$, NMP, 100 °C, 5 h; (b) MsCl, Et_3N, CH_2Cl_2, rt, 10 min; (c)NaN_3, DMF, 80 °C, 14 h; (d) H_2, Pd/C, 1,4-dioxane/H_2O, rt, 10 h; (e) 2-bromopyrimidine, DIPEA, DMSO, 120 °C, 6 h; (f) 50% H_2SO_4/H_2O, 80 °C, 4 h; (g) (*R*)-(+)-BINAP, $CsCO_3$, $Pd(OAc)_2$, toluene, 90 °C, 5 h then 100 °C, 2 h; (h) NaOH, THF/MeOH/H_2O, 45 °C, 16 h; (i) i—TsOH, MeOH, 65 °C, 7 h; ii—HCO_2H/H_2O, rt, 2 h; (j) $NaBH_4$, THF, rt, 3.5 h; (k) BOP, DIPEA, DMF, rt, 16 h; (l) TFA, CH_2Cl_2, rt, 8 h; (m) H_2, Pd/C, 1,4-dioxane/H_2O, rt, 6 h.

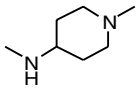
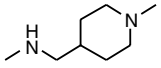
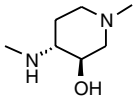
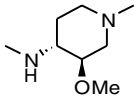
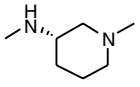
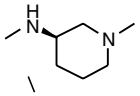
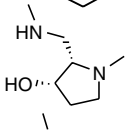
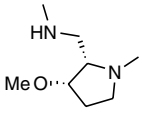
Table 3. Inhibitory activity of *meta*-oriented antagonists in receptor-binding assay and cell adhesion assay

Compound	IC_{50} (nM)			$\alpha_v\beta_3/\alpha_{IIb}\beta_3$
	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC[a]	
1	160	0.73	NT	0.0046
2	1.3	3.1	190	2.4
21	2.2	6.4	NT	2.9
22	6.6	70	1090	11

[a] $\alpha_v\beta_3$-Mediated cell adhesion assay: vascular smooth muscle cells—vitronectin.

Table 4. Effect of replacement of hetero ring on inhibitory activity in receptor-binding assay



Compound	A	IC_{50} (nM)		$\alpha_v\beta_3/\alpha_{IIb}\beta_3$	Synthetic method[a]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$		
22		6.6	70	11	A, B, C
31		1500	61	0.041	A
32		32	89	2.8	D
33		180	80	0.44	D
34		2.6	11	4.2	A
35		560	39	0.070	A
36		3.9	1.8	0.46	D
37		3.5	5.0	1.4	D

[a] Methods. A: nucleophilic substitution; B: palladium-catalyzed amination; C: hetero ring cyclization; and D: see Scheme 4.

selected 4-aminopiperidine as the heterocycle next to the central benzene ring for further study because of its relatively remarkable $\alpha_v\beta_3$ selectivity.

Next, modification of the *C*-terminal substituent was examined (Table 5). Unfortunately, modification of the benzenesulfonyl group decreased the $\alpha_v\beta_3$-binding activity. Exceptionally, the *p*-methoxybenzenesulfonyl derivative (**40**) retained its $\alpha_v\beta_3$ activity, but the $\alpha_v\beta_3$ selectivity disappeared.

Finally, optimization of the central benzene ring was performed, as shown in Table 6. When substitution effects were investigated using fluorine, substitution at the C-5 position was found to be very effective for improvement of the selectivity. Thus, a trifluoromethyl group was introduced at the C-5 position to obtain **47**. Compound **47** exhibited marked selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$ in a receptor-binding assay and showed moderate inhibitory activity in an $\alpha_v\beta_3$-mediated cell adhesion assay, without antiplatelet aggregation activity (hPRP: >10,000 nM). Thus, we obtained an $\alpha_v\beta_3$-selective antagonist containing our tricyclic pharmacophore. As noted in the introduction, this will be useful for control purposes in in vivo studies of dual antagonists, even though more potent and more $\alpha_v\beta_3$-selective antagonists have already been reported.[14]

5. Conclusion

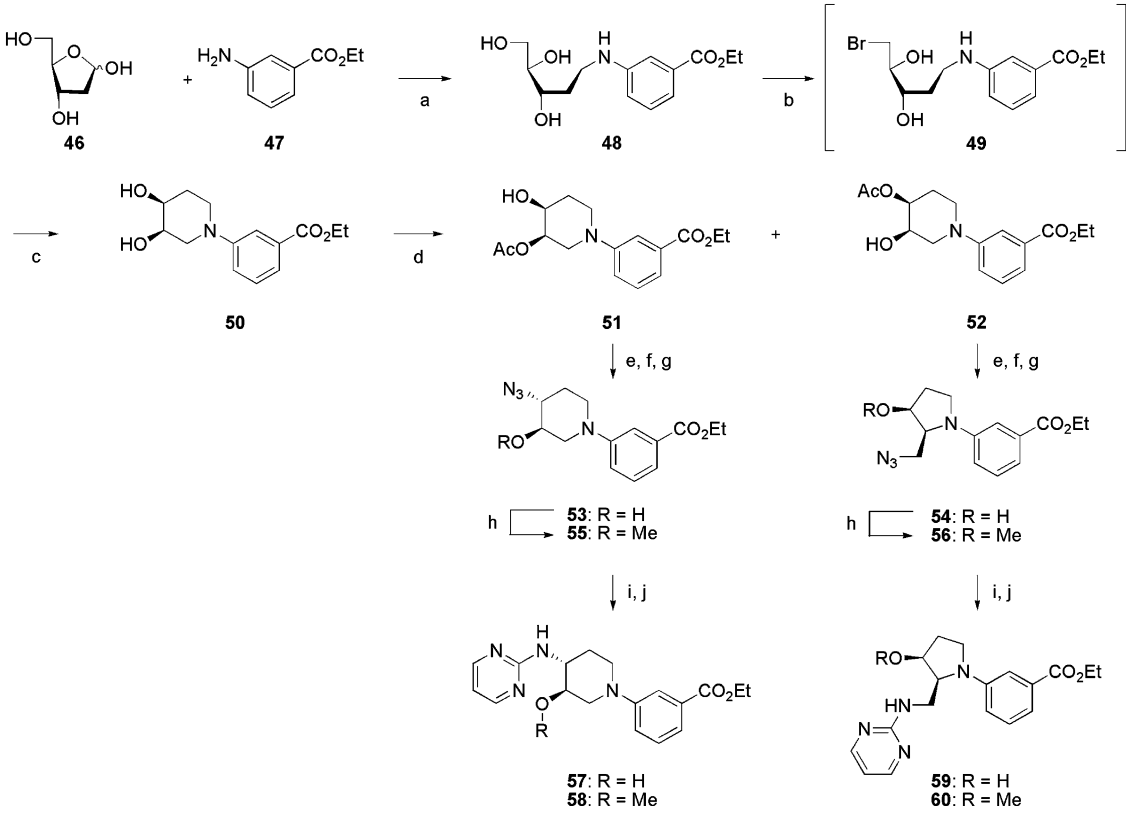
In order to prepare an $\alpha_v\beta_3$-selective antagonist possessing the tricyclic pharmacophore, we tried three approaches. Dimerization dramatically decreased the $\alpha_{IIb}\beta_3$-antagonistic activity, but also suppressed $\alpha_v\beta_3$ activity. Second, a novel heterocycle was introduced in place of piperazine or piperidine, in order to alter the dihedral angle between the central benzene ring and adjacent heterocycle. However, the antagonistic activity balance was not markedly altered. Finally, we altered the distance between the *N*-terminus and the *C*-terminus. Several *meta*-oriented molecules with a shorter inter-terminal distance were designed and synthesized. The prototype molecule **22** exhibited an acceptable $\alpha_v\beta_3$ activity and showed weak selectivity for $\alpha_v\beta_3$ over $\alpha_{IIb}\beta_3$. Further optimization afforded the selective antagonist, **47**, which was found to show inhibitory activity in an $\alpha_v\beta_3$-mediated cell adhesion assay without antiplatelet aggregation activity. This molecule should be useful as a control $\alpha_v\beta_3$-selective antagonist for in vivo studies of our dual antagonists possessing the tricyclic pharmacophore.[15]

6. Experimental

^{1}H NMR spectra were recorded on JNM-LA400 spectrometers with chemical shifts in parts per million with the internal tetramethylsilane as a standard. Electron ionization (EI) mass spectra were recorded on a Hitachi M-80B instrument. Fast-atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-700 instrument. Thermospray (TSP) mass spectra were recorded on a Hewlett-Packard 5989A instrument. Atmospheric pressure chemical ionization (APCI) mass spectra were recorded on a Hewlett-Packard 5989A instrument. High-resolution mass spectra (HRMS) were recorded under FAB conditions. Optical rotations were obtained on a JASCO DIP-370 polarimeter.

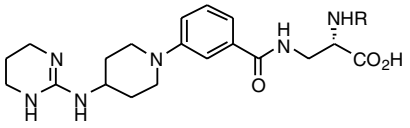
6.1. Preparation of compound 8

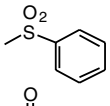
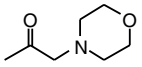
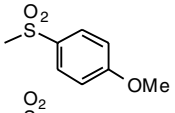
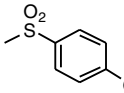
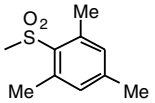
6.1.1. Compound 4. DMF (0.58 ml) was added to 1,6-dibromohexane (6.2 mg, 0.025 mmol) to prepare a solution and compound **3**[4] (29 mg, 0.050 mmol) and DBU



Scheme 4. Reagents and conditions: (a) MeOH, rt, 16 h then $NaBCNH_3$, AcOH, rt, 4 h; (b) CBr_4, PPh_3, THF, 0 °C to rt, 1 h; (c) rt, 2 h; (d) TsOH, $CH_3C(OCH_3)_3$, rt, 3 h; (e) MsCl, Et_3N, CH_2Cl_2, rt, 5 min; (f) NaN_3, DMF, 90 °C, 10 h; (g) NaOEt, THF, 30 °C, 3.5 h; (h) NaH, MeI, THF, rt, 4 h; (i) H_2, Pd/C, 1,4-dioxane/H_2O, rt, 3 h; (j) 2-bromopyrimidine, DIPEA, DMSO, 120 °C, 14 h.

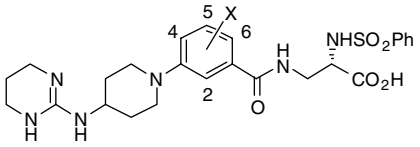
Table 5. Effect of replacement of the *C*-terminus on inhibitory activity in receptor-binding assay

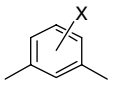


Compound	R	IC_{50} (nM)		$\alpha_v\beta_3/\alpha_{IIb}\beta_3$	Synthetic method[a]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$		
22		6.6	70	11	A, B, C
38		17,000	1700	0.10	A
39		17,000	1300	0.076	A
40		7.6	3.9	0.51	A
41		47	370	7.9	A
42		15	11	0.73	A

[a] Methods. A: nucleophilic substitution; B: palladium-catalyzed amination; and C: hetero ring cyclization.

Table 6. Effect of substitution of the central aromatic ring on inhibitory activity in receptor-binding assay and cell adhesion assay



Compound		IC$_{50}$ (nM)			$\alpha_v\beta_3/\alpha_{IIb}\beta_3$	Synthetic method[b]
		$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	VSMC[a]		
22	Unsubstitutional	6.6	70	1300	11	A, B, C
43	2-F	6.9	31	310	4.5	B
44	4-F	87	120	NT	1.4	B
45	5-F	14	710	1200	51	B
46	6-F	330	130	NT	0.39	B
47	5-CF_3	18	2000	500	110	C

[a] $\alpha_v\beta_3$-Mediated cell adhesion assay: vascular smooth muscle cells—vitronectin.
[b] Methods. A: nucleophilic substitution; B: palladium-catalyzed amination; C: hetero ring cyclization.

(15.5 mg, 0.10 mmol) were added to the solution. The mixture was stirred at room temperature for 12 days. After addition of ethyl acetate (12 ml), the organic layer was washed with water and brine. Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (ethyl acetate/*n*-hexane, 4:1) to prepare compound **4** (3.6 mg, 5.8%) as a colorless powder with accompanies with the recovered starting material (15 mg); ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, 9:1) δ: 1.18 (4H, m, CH_2), 1.35 (18H, s, *t*-Bu), 1.48–1.64 (4H, m, CH_2), 3.14, 3.68, 3.88 (6H, m, CH_2CHNCH_2), 3.39 (8H, br dd, piperazine), 3.95 (8H, br dd, piperazine), 6.61 (2H, t, pyrimidine), 6.96 (4H, br dd, C_6H_4), 7.48 (4H, m, C_6H_5), 7.57 (2H, m, C_6H_5), 7.71 (4H, d, C_6H_4), 7.87 (4H, m, C_6H_5), 8.34 (4H, d, pyrimidine); TSPMS *m*/*z* 1215 $(M+H)^+$.

6.1.2. Compound 8. CH_2Cl_2 (0.50 ml) was added to compound **4** (3.4 mg, 2.8 μmol) to prepare a solution. Trifluoroacetic acid (0.50 ml) and anisole (0.040 ml) were added to the solution, and stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced pressure to afford a residue, which was twice co-evaporated by toluene for azeotrope, and then dried in vacuo. This material was finally washed with isopropyl ether twice to prepare compound **8** (3.0 mg, 97%) as a colorless powder; FAB-HRMS $(M+H)^+$ calcd for $C_{54}H_{62}N_{12}O_{10}S_2$: 1103.4232. Found: 1103.4211.

6.2. Preparation of compound 10

6.2.1. Compound 6. DMF (1.6 ml) was added to 12-bromo-1-dodecanol (190 mg, 0.72 mmol) to prepare a solution, and compound **3** (80 mg, 0.14 mmol) and DBU (130 mg, 0.85 mmol) were added to the solution. The mixture was stirred at room temperature for 16 h and then evaporated. After addition of ethyl acetate, the organic layer was washed with water and brine. Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography twice (ethyl acetate/*n*-hexane, 9:1 and then benzene/MeOH/ethyl acetate, 10:1:2) to prepare compound **6** (61 mg, 56%) as a colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.15–1.25 (16H, m, CH_2), 1.35 (9H, s, *t*-Bu), 1.50–1.70 (4H, m, CH_2), 3.11, 3.33, 3.74, 3.95, 4.47 (5H, m, CH_2CHNCH_2), 3.39 (4H, br dd, piperazine), 3.63 (2H, t, CH_2OH), 3.99 (4H, br dd, piperazine), 6.54 (1H, t, pyrimidine), 6.95 (2H, d, C_6H_4), 7.51 (2H, m, C_6H_5), 7.58 (1H, m, C_6H_5), 7.76 (2H, d, C_6H_4), 7.91 (2H, m, C_6H_5), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 751 $(M+H)^+$.

6.2.2. Compound 7. CH_2Cl_2 (1.2 ml) was added to compound **6** (61 mg, 0.081 mmol) to prepare a solution. DMAP (0.50 mg, 0.0041 mmol), TEA (11 mg, 0.11 mmol) and methanesulfonyl chloride (11 mg, 0.096 mmol) were subsequently added to the solution. The mixture was stirred at room temperature for 16 h, and it was directly purified by preparative thin-layer chromatography (ethyl acetate/*n*-hexane, 3:2) to prepare the corresponding mesylate (47 mg, 64%). Acetone (4.6 ml) was added to the mesylate (47 mg, 0.057 mmol) to prepare a solution, and then NaI (42 mg, 0.28 mmol) was added thereto. The mixture was stirred at 40 °C for 40 h and then evaporated. After addition of ethyl acetate, the organic layer was washed with water, aqueous $Na_2S_2O_3$ solution, and brine. Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (ethyl acetate/*n*-hexane, 3:2) to prepare compound **7** (40 mg, 84%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.15–1.25 (16H, m, CH_2), 1.35 (9H, s, *t*-Bu), 1.45–1.65 (2H, m, CH_2), 1.81 (2H, ddd, CH_2), 3.10, 3.33, 3.75, 3.97, 4.47 (5H, m, CH_2CHNCH_2), 3.18 (2H, t, CH_2I), 3.39 (4H, br dd, piperazine), 3.99 (4H, br dd, piperazine), 6.54 (1H, t, pyrimidine), 6.95 (2H, d, C_6H_4), 7.51 (2H, m, C_6H_5), 7.58 (1H, m, C_6H_5), 7.76 (2H, d, C_6H_4), 7.91 (2H, m, C_6H_5), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 861 $(M+H)^+$.

6.2.3. Compound 5. DMF (0.58 ml) was added to compound **3** and compound **7** to prepare a solution, and DBU (9.4 mg, 0.062 mmol) was added to the solution. The mixture was stirred at room temperature for 3 days and then evaporated. After addition of ethyl acetate, the organic layer was washed with water, aqueous $Na_2S_2O_3$

solution, and brine. Then the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative thin-layer chromatography (benzene/MeOH/ethyl acetate, 10:1:2) to prepare compound **5** (24 mg, 60%) as a colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.14 (16H, br s, CH_2), 1.35 (18H, s, *t*-Bu), 1.45–1.65 (4H, m, CH_2), 3.10, 3.32, 3.75, 3.96, 4.47 (10H, m, CH_2CHNCH_2), 3.38 (8H, br dd, piperazine), 3.98 (8H, br dd, piperazine), 6.53 (2H, t, pyrimidine), 6.94 (4H, br dd, C_6H_4), 7.51 (4H, m, C_6H_5), 7.57 (2H, m, C_6H_5), 7.75 (4H, d, C_6H_4), 7.91 (4H, m, C_6H_5), 8.34 (4H, d, pyrimidine); TSPMS *m*/*z* 1299 $(M+H)^+$.

6.2.4. Compound 9. CH_2Cl_2 (0.50 ml) was added to compound **5** (45 mg, 0.035 mmol) to prepare a solution. Trifluoroacetic acid (0.50 ml) and anisole (0.040 ml) were added to the solution at 0 °C and stirred at 4 °C for 10 h. The reaction mixture was concentrated under reduced pressure to afford a residue, which was twice coevaporated by 1,4-dioxane and toluene for azeotrope, and then dried in vacuo to prepare a crude bis-carboxylic acid (53 mg). A part of this crude acid (12 mg) was finally purified by preparative thin-layer chromatography ($CHCl_3$/MeOH/concd NH_4OH, 90:20:1) to prepare compound **9** (5.6 mg) as a colorless powder; ^{1}H NMR (400 MHz, $CD_3OD/CDCl_3$, 1:1) δ: 1.16 (16H, br s, CH_2), 1.50–1.70 (4H, m, CH_2), 3.23, 3.33, 3.80, 4.54 (8H, m, CH_2CHNCH_2), 3.41 (8H, br dd, piperazine), 3.98 (8H, br dd, piperazine), 6.61 (2H, t, pyrimidine), 6.98 (4H, br dd, C_6H_4), 7.48 (4H, m, C_6H_5), 7.55 (2H, m, C_6H_5), 7.75 (4H, d, C_6H_4), 7.91 (4H, m, C_6H_5), 8.35 (4H, d, pyrimidine); FAB-HRMS $(M+H)^+$ calcd for $C_{60}H_{74}N_{12}O_{10}S_2$: 1187.5171. Found: 1187.5166.

6.2.5. Compound 10. Acetic acid (4.0 ml) and concentrated hydrochloric acid (0.36 ml) were added to crude compound **9** mentioned above (41 mg) to prepare a solution. 10% Pd/C (36 mg) was added to the solution, and the mixture was vigorously shaken at room temperature for 3.0 h under a hydrogen pressure of 3 atm. The insolubles were filtered, and then washed twice with water. The filtrate was combined with the washings, followed by concentration under reduced pressure. The residue was purified by preparative thin-layer chromatography ($CHCl_3$/EtOH/H_2O/concd NH_4OH, 15:10:1:1) and Sephadex LH-20 chromatography ($CHCl_3$/MeOH/concd NH_4OH, 2:10:1) to prepare compound **10** (20 mg, 48% (two steps)) as a colorless solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.91–1.04 (16H, m, 8× CH_2), 1.35–1.55 (4H, m, 2× CH_2), 3.13, 3.60, 4.28 (6H, m, CH_2CHNCH_2), 3.26 (8H, br dd, piperazine), 3.49 (8H, br dd, piperazine), 6.91 (4H, br dd, C_6H_4), 7.46 (4H, m, C_6H_5), 7.53 (2H, m, C_6H_5), 7.64 (4H, m, C_6H_5), 7.93 (4H, d, C_6H_4); FAB-HRMS $(M+H)^+$ calcd for $C_{60}H_{82}N_{12}O_{10}S_2$: 1195.5797. Found: 1195.5791.

6.3. Preparation of compound 20 (Experimental works were performed by Dr. Taku Yamada.)

6.3.1. Compound 15. CH_2Cl_2 (180 ml) was added to ethyl α-L-oleandroside[16] (3.8 g, 18 mmol) to prepare a solution, which was then ice cooled. TEA (4.00 ml, 28.9 mmol) and methanesulfonyl chloride (19 ml, 24 mmol) were added to the solution, and the mixture was stirred at 0 °C for 2 h. Ice was added to the reaction solution, and the mixture was extracted once with $CHCl_3$. The organic layer was washed with water, was dried over anhydrous $MgSO_4$, and was then concentrated under reduced pressure to give a methanesulfonyl compound (5.9 g, 100%).

DMF (100 ml) was added to the crude methanesulfonyl compound (18 mmol) to prepare a solution. Sodium azide (1.4 g, 22 mmol) was added to the solution, and the mixture was stirred at 80 °C for 18 h. Water was added to the reaction solution, and the mixture was extracted once with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 2:1) to give compound **13** (5.2 g, 99%).

1,4-Dioxane (50 ml) was added to the azide compound (2.1 g, 9.6 mmol) to prepare a solution, and 50 ml of 1 N hydrochloric acid was added to the solution. The mixture was stirred at 60 °C for 3 h and was then ice cooled. The mixture was adjusted to pH 8 by the addition of a 5 N NaOH and was then extracted three times with $CHCl_3$. The organic layers were combined, and the combined organic layers were dried over anhydrous Na_2SO_4 and were then concentrated under reduced pressure to give compound **14** (1.6 g, 86%).

CH_2Cl_2 (35 ml) and MeOH (35 ml) were added to the crude hemiacetal compound (1.4 g, 7.2 mmol) to prepare a solution. Ethyl 4-aminobenzoate (900 mg, 5.4 mmol), acetic acid (1.5 ml, 26 mmol), and sodium cyanoborohydride (990 mg, 16 mmol) were added to the solution, and the mixture was stirred at room temperature for 24 h. Ethyl 4-aminobenzoate (230 mg, 1.4 mmol), acetic acid (1.3 ml, 22 mmol), and sodium cyanoborohydride (850 mg, 14 mmol) were added again, and the mixture was stirred at room temperature for 18 h. Water was added to the reaction solution, and the mixture was extracted twice with $CHCl_3$. The extract was dried over anhydrous $MgSO_4$ and was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate/concd NH_4OH, 5:5:0.3) to prepare compound **15** (1.58 g, 65%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.27 (3H, d, H-6′), 1.36 (3H, t, Et), 1.93 (2H, m, H-2′), 3.35 (2H, m, H-1′ and H-4′), 3.48 (3H, s, OCH_3), 3.55 (1H, dt, H-3′), 3.94 (1H, br, H-5′), 4.32 (2H, q, Et), 6.56 (2H, m, C_6H_4), 7.88 (2H, m, C_6H_4); TSPMS *m*/*z* 337 $(M+H)^+$; $[\alpha]_D^{27}$ +0.40 (*c* 1.3, $CHCl_3$).

6.3.2. Compound 16. CH_2Cl_2 (50 ml) was added to compound **15** (1.6 g, 4.8 mmol) to prepare a solution, which was then ice cooled. TEA (2.0 ml, 14 mmol) and methanesulfonyl chloride (0.56 ml, 7.2 mmol) were added to the cooled solution. The temperature of the mixture was raised to room temperature, and the mixture was stirred for 2.0 h. Ice was added to the reaction solution, and the mixture was extracted once with $CHCl_3$. The organic layer was washed with water, was dried over

anhydrous $MgSO_4$, and was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate/concd NH_4OH, 5:5:0.3) to give ethyl 4-{(4*R*)-azido-(5*R*)-methanesulfonyloxy-(3*S*)-methoxyhexylamino}benzoate (1.68 g, 84%).

Toluene (45 ml) was added to ethyl 4-{(4*R*)-azido-(5*R*)-methanesulfonyloxy-(3*S*)-methoxyhexylamino}benzoate (1.9 g, 4.5 mmol) to prepare a solution. *N*,*N*-Diisopropylethylamine (1.6 ml, 9.2 mmol) was added to the solution, and the mixture was stirred under reflux for 18 h. The reaction solution was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate/concd NH_4OH, 5:5:0.3) to prepare compound **16** (1.16 g, 81%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.14 (3H, d, CH_3), 1.36 (3H, t, Et), 2.01 (2H, m, piperidine), 3.04 (1H, m, piperidine), 3.46 (3H, s, OCH_3), 3.70 (2H, m, piperidine), 3.92 (1H, m, piperidine), 4.32 (1H, m, piperidine), 4.32 (2H, q, Et), 6.85 (2H, m, C_6H_4), 7.91 (2H, m, C_6H_4); FABMS *m*/*z* 319 $(M+H)^+$; $[\alpha]_D^{28}$ +62 (*c* 1.2, $CHCl_3$).

6.3.3. Compound 17. EtOH (24 ml) was added to compound **16** (860 mg, 2.7 mmol) to prepare a solution. To the solution was added 10% Pd/C (77 mg). The mixture was vigorously stirred under a hydrogen pressure of 1 atm at room temperature for 18 h. The insolubles were filtered and were then washed with EtOH. The filtrate and the washings were combined, and the combined solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/concd NH_4OH, 10:0.3) to give ethyl 4-{(3*R*)-amino-(4*S*)-methoxy-(2*R*)-methylpiperidin-1-yl}-benzoate (850 mg, 100%).

N-Methylpyrrolidone (32 ml) was added to ethyl 4-{(3*R*)-amino-(4*S*)-methoxy-(2*R*)-methylpiperidin-1-yl}-benzoate (920 mg, 3.2 mmol) to prepare a solution. *N*,*N*-Diisopropylethylamine (2.8 ml, 16 mmol) and 2-bromopyrimidine (510 mg, 3.2 mmol) were added to the solution, and the mixture was stirred at 120 °C for 18 h. Water was added to the reaction solution, and the mixture was extracted twice with ethyl acetate. The organic layers were combined, and the combined organic layers were dried over anhydrous $MgSO_4$and were then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate) to give ethyl 4-{(4*S*)-methoxy-(2*R*)-methyl-(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (500 mg, 43%).

MeOH (4.0 ml) and water (2.8 ml) were added to ethyl 4-{(4*S*)-methoxy-(2*R*)-methyl-(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (490 mg, 1.3 mmol) to prepare a suspension, to which a 1 N NaOH (1.3 ml) was added. The mixture was stirred at 50 °C for 6 h and was then ice cooled. The reaction solution was adjusted to pH 4 by the addition of 5 N hydrochloric acid. The precipitated solid was collected by filtration, was washed twice with water, and was then dried to prepare compound **17** (280 mg, 61%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.25 (3H, d, CH_3), 2.03 (1H, m, piperidine), 2.14 (1H, dddd, piperidine), 3.18 (1H, ddd, piperidine), 3.42 (3H, s, OCH_3), 3.79 (1H, ddd, piperidine), 3.87 (1H, m, piperidine), 4.37 (1H, m, piperidine), 4.72 (1H, m, piperidine), 6.54 (1H, t, pyrimidine), 6.65 (2H, br d, C_6H_4), 7.32 (1H, br d, NH), 7.56 (2H, br d, C_6H_4), 8.27 (2H, br, pyrimidine); EIMS *m*/*z* 342 $(M)^+$; $[\alpha]_D^{26}$ +191 (*c* 1.1, $CHCl_3$).

6.3.4. Compound 19. DMF (4.0 ml) was added to compound **17** (150 mg, 440 mmol) to prepare a solution, and compound **18** (150 mg, 493 mmol) was added to the solution. Further, benzotriazol-1-yloxytri(dimethylamino)phosphonium hexafluorophosphate (BOP) (240 mg, 550 mmol) and *N*,*N*-diisopropylethylamine (0.092 ml, 0.53 mmol) were added thereto, and the mixture was stirred at room temperature for 18 h. Water and an aqueous $NaHCO_3$ solution were added to the reaction solution, and the mixture was extracted twice with ethyl acetate. The organic layers were combined, and the combined organic layers were washed with a mixed solution composed of brine and water, were dried over anhydrous Na_2SO_4, and were then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 7:1) to prepare compound **19** (250 mg, 91%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.18 (3H, d, CH_3), 1.26 (9H, s, *t*-Bu), 1.89 (1H, m, piperidine), 2.00 (1H, m, piperidine), 3.11 (1H, ddd, piperidine), 3.39 (3H, s, OCH_3), 3.58 (2H, m, piperidine), 3.78 (1H, ddd, $CONHCH_2CH$), 3.85 (1H, ddd, $CONHCH_2CH$), 3.92 (1H, ddd, $CONHCH_2CH$), 4.49 (2H, m, piperidine), 5.72 (1H, d, NH), 5.98 (1H, d, NH), 6.54 (1H, t, pyrimidine), 6.58 (1H, dd, NH), 6.81 (2H, m, C_6H_4), 7.46 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.62 (2H, m, C_6H_4), 7.85 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); FABMS *m*/*z* 625 $(M+H)^+$; $[\alpha]_D^{27}$ +46 (*c* 0.99, $CHCl_3$).

6.3.5. Compound 20. CH_2Cl_2 (3.0 ml) was added to compound **19** (100 mg, 0.17 mmol) to prepare a solution. Trifluoroacetic acid (3.0 ml) was added to the solution, and the mixture was stirred at room temperature for 3 h. The reaction solution was concentrated under reduced pressure to give a trifluoroacetate of (2*S*)-benzenesulfonylamino-3-[4-{(4*S*)-methoxy-(2*R*)-methyl-(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-propionic acid.

1,4-Dioxane (3.0 ml) and water (0.30 ml) were added to the trifluoroacetate of crude (2*S*)-benzenesulfonylamino-3-[4-{(4*S*)-methoxy-(2*R*)-methyl-(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (0.17 mmol) to prepare a solution. To the solution was added 10% Pd/C (18 mg). The mixture was vigorously stirred under a hydrogen pressure of 1 atm at room temperature for 18 h. The insolubles were filtered and were then washed with EtOH. The filtrate and the washings were combined, and the combined solution was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 9:3:0.3) and was then purified by Sephadex LH-20 (MeOH) to prepare compound **20** (63 mg, 66%); ^1H NMR (400 MHz, CD_3OD) δ: 1.18

(3H, d, CH_3), 1.26 (9H, s, *t*-Bu), 1.89 (1H, m, piperidine), 2.00 (1H, m, piperidine), 3.11 (1H, ddd, piperidine), 3.39 (3H, s, OCH_3), 3.58 (2H, m, piperidine), 3.78 (1H, ddd, $CONHCH_2CH$), 3.85 (1H, ddd, $CONHCH_2CH$), 3.92 (1H, ddd, $CONHCH_2CH$), 4.49 (2H, m, piperidine), 5.72 (1H, d, NH), 5.98 (1H, d, NH), 6.54 (1H, t, pyrimidine), 6.58 (1H, dd, NH), 6.81 (2H, m, C_6H_4), 7.46 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.62 (2H, m, C_6H_4), 7.85 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); FAB-HRMS $(M+H)^+$ calcd for $C_{27}H_{36}N_6O_6S$: 573.2495. Found: 573.2499; $[\alpha]_D^{25}$ +136 (*c* 0.15, MeOH).

6.4. Preparation of compound 21

6.4.1. Ethyl 3-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate. DMF (11 ml) was added to ethyl 3-(piperazin-1-yl)benzoate (WO2000061556) (264 mg, 1.1 mmol), and 2-bromopyrimidine (269 mg, 1.7 mmol) and *N*,*N*-diisopropylethylamine (1.0 ml) were then successively added thereto. The mixture was stirred at 120 °C for 12 h. The temperature of the reaction mixture was returned to room temperature, and the reaction mixture was then added dropwise to 250 ml of water followed by stirring at room temperature for 1 h. The insolubles were collected by filtration, and were then washed twice with water (20 ml). The solid was dried under reduced pressure in the presence of diphosphorus pentoxide at 50 °C, and was then purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:2) to prepare the title compound (317 mg, 92%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 (3H, t, Et), 3.30 (4H, m, piperazine), 4.00 (4H, m, piperazine), 4.38 (2H, q, Et), 6.53 (1H, t, pyrimidine), 7.15 (1H, br ddd, C_6H_4), 7.34 (1H, t, C_6H_4), 7.56 (1H, br ddd, C_6H_4), 7.64 (1H, br dd, C_6H_4), 8.34 (2H, d, pyrimidine); EIMS *m*/*z* 312.

6.4.2. 3-{4-(Pyrimidin-2-yl)piperazin-1-yl}benzoic acid. THF (27 ml) and MeOH (9.0 ml) were added to ethyl 3-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoate (300 mg, 0.96 mmol) to prepare a solution. NaOH, 1 N (9.0 ml) was added to the solution. The reaction mixture was stirred at 45 °C for 7 h. The reaction solution was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 90:10) to prepare the title compound (264 mg, 96%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 1:1) δ: 3.32 (4H, m, piperazine), 3.99 (4H, m, piperazine), 6.61 (1H, t, pyrimidine), 7.22 (1H, br dd, C_6H_4), 7.36 (1H, t, C_6H_4), 7.57 (1H, br ddd, C_6H_4), 7.67 (1H, br dd, C_6H_4), 8.35 (2H, d, pyrimidine); TSPMS *m*/*z* 285 $(M+H)^+$.

6.4.3. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate. DMF (6.5 ml) and CH_2Cl_2 (6.5 ml) were added to 3-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoic acid (256 mg, 0.90 mmol) and BOP (597 mg, 1.3 mmol) to prepare a solution. *N*,*N*-Diisopropylethylamine (0.24 ml) was added to the solution, and a reaction was allowed to proceed at room temperature for 2 h. Separately, CH_2Cl_2 (6.5 ml) was added to compound **18** (325 mg, 1.1 mmol) to prepare a solution. This solution was added to the above active ester solution at 0 °C. *N*,*N*-Diisopropylethylamine (0.12 ml) was added thereto, and a reaction was allowed to proceed at room temperature for 16 h. The reaction solution was concentrated under reduced pressure, and the residue was extracted with ethyl acetate, followed by washing with an aqueous $NaHCO_3$ and saturated brine in that order. The extract was then dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (acetone/*n*-hexane, 6:4) to prepare the title compound (482 mg, 94%) as a colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 3.33 (4H, m, piperazine), 3.57 (1H, ddd, $CONHCH_2$), 3.93 (2H, m, $CONHCH_2CH$), 3.99 (4H, m, piperazine), 6.53 (1H, t, pyrimidine), 7.10 (1H, br dd, C_6H_4), 7.22 (1H, br d, C_6H_4), 7.34 (1H, t, C_6H_4), 7.46 (1H, br dd, C_6H_4), 7.50 (2H, m, Ph), 7.58 (1H, m, Ph), 7.86 (2H, m, Ph), 8.34 (2H, d, pyrimidine); FABMS *m*/*z* 567 $(M+H)^+$; $[\alpha]_D^{25}$ +45 (*c* 1.0, $CHCl_3$).

6.4.4. (2*S*)-Benzenesulfonylamino-3-[3-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **8** as a colorless solid. Yield: (67 mg, 87%); ^{1}H NMR (400 MHz, CD_3OD) δ: 3.30 (4H, m, piperazine), 3.54 (1H, dd, $CONHCH_2$), 3.71 (1H, dd, $CONHCH_2$), 3.83 (1H, dd, $CONHCH_2CH$), 3.96 (4H, m, piperazine), 6.60 (1H, t, pyrimidine), 7.18 (1H, br dd, C_6H_4), 7.26 (1H, br d, C_6H_4), 7.33 (1H, t, C_6H_4), 7.46 (3H, m, 1H of C_6H_4 and 2H of Ph), 7.52 (1H, m, Ph), 7.86 (2H, m, Ph), 8.34 (2H, d, pyrimidine); TSPMS *m*/*z* 511 $(M+H)^+$; $[\alpha]_D^{25}$ +60 (*c* 1.0, MeOH).

6.4.5. Compound 21. The title compound was prepared from (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-yl)piperazin-1-yl}benzoylamino]propionic acid by the same procedure as employed for compound **10** as a colorless syrup. Yield: (31 mg, 50%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.94 (2H, quintet, tetrahydropyrimidine), 3.28 (4H, m, piperazine), 3.38 (4H, t, tetrahydropyrimidine), 3.51 (4H, m, piperazine), 3.54 (1H, dd, $CONHCH_2$), 3.69 (1H, dd, $CONHCH_2$), 3.76 (1H, dd, $CONHCH_2CH$), 7.10 (1H, br d, C_6H_4), 7.30 (2H, m, C_6H_4), 7.41 (1H, br s, C_6H_4), 7.47 (2H, m, Ph), 7.53 (1H, m, Ph), 7.85 (2H, m, Ph); FAB-HRMS $(M+H)^+$ calcd for $C_{24}H_{30}N_6O_5S$: 515.2077. Found: 515.2083; $[\alpha]_D^{25}$ +69 (*c* 1.0, MeOH).

6.5. General procedure for preparation of compound 26

6.5.1. Method A

6.5.1.1. Compound 23. DMSO (20 ml) was added to 3-fluorobenzonitrile (6.1 g, 60 mmol) and 4-hydroxypiperidine (6.1 g, 50 mmol). The mixture was heated at 100 °C for 5 h. The temperature of the reaction mixture was returned to room temperature, and the reaction mixture was then added dropwise to water (500 ml). The mixture was extracted twice with ethyl acetate (300 ml). The ethyl acetate layer was washed twice with water (200 ml) and brine (300 ml). The organic layer was extracted six times

with 1 N hydrochloric acid (150 ml), and then the aqueous layer was adjusted to pH 10 by the addition of $NaHCO_3$. The mixture was extracted twice with ethyl acetate (300 ml). The organic layer was dried over anhydrous Na_2SO_4 and was then concentrated under reduced pressure to prepare compound **23** (1.9 g, 19%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.62–1.73 (2H, m, piperidine), 1.97–2.04 (2H, m, piperidine), 2.97–3.05 (2H, dd, piperidine), 3.54–3.61 (2H, m, piperidine), 3.91 (1H, tt, piperidine), 7.04–7.08 (1H, m, C_6H_4), 7.10-7.14 (2H, m, C_6H_4), 7.27–7.33 (1H, m, C_6H_4); TSPMS *m*/*z* 203 $(M+H)^+$.

6.5.1.2. Compound 24. CH_2Cl_2 (40 ml) was added to compound **23** (1.9 g, 9.4 mmol) to prepare a solution. Methanesulfonyl chloride (0.94 ml, 12 mmol) and TEA (2.8 ml, 20 mmol) were added to the solution, and a reaction was allowed to proceed at room temperature for 10 min. H_2O (400 ml) was added to stop the reaction and extracted twice with CH_2Cl_2 (300 ml). The methylene chloride layer was dried over anhydrous Na_2SO_4 and was then concentrated under reduced pressure to give crude 3-{4-(methanesulfonyloxy)piperidin-1-yl}benzonitrile (2.6 g).

DMF (50 ml) was added to this crude 3-{4-(methanesulfonyloxy)piperidin-1-yl}benzonitrile to prepare a solution. Sodium azide (1.2 g, 19 mmol) was added to the solution, and the mixture was stirred with heating at 80 °C for 14 h. The temperature of the reaction mixture was returned to room temperature, and then poured into H_2O. The reaction mixture was extracted twice with ethyl acetate (300 ml), followed by washing twice with water (200 ml) and brine (200 ml). The washed organic layer was dried over anhydrous Na_2SO_4 and was then concentrated under reduced pressure to prepare 3-(4-azidopiperidin-1-yl) benzonitrile (2.0 g).

1,4-Dioxane (20 ml) and water (10 ml) were added to 3-(4-azidopiperidin-1-yl)benzonitrile to prepare a solution. To the solution was added 10% Pd/C (270 mg), and the mixture was stirred in a hydrogen atmosphere at room temperature for 10 h. The insolubles were filtered and washed twice with a solvent (4.0 ml) having the same composition as the mixed solvent used in the reaction. The filtrate and the washings were combined, followed by concentration under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 10:1:0.1) to prepare compound **24** (410 mg, 43%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41–1.53 (2H, m, piperidine), 1.90–1.98 (2H, m, piperidine), 2.80–2.96 (3H, m, piperidine), 3.63-3.72 (2H, m, piperidine), 7.06 (1H, dt, C_6H_4), 7.09–7.14 (2H, m, C_6H_4), 7.27–7.32 (1H, m, C_6H_4); EIMS *m*/*z* 201 $(M)^+$.

6.5.1.3. Compound 25. DMSO (10 ml) was added to compound **24** (410 mg, 2.0 mmol). Next, 2-bromopyrimidine (340 mg, 2.1 mmol) and *N*,*N*-diisopropylethylamine (2.0 ml, 11mmol) were added thereto, and the mixture was heated at 120 °C for 6 h. The temperature of the reaction mixture was returned to room temperature and the reaction mixture was then added dropwise to water (600 ml). The temperature of the reaction mixture was returned to room temperature and then poured into H_2O. The reaction mixture was extracted twice with ethyl acetate (300 ml), followed by washing twice with water (200 ml) and brine (300 ml). The washed organic layer was dried over anhydrous Na_2SO_4 and was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/*n*-hexane, 2:1) to prepare compound **25** (380 mg, 67%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.62–1.72 (2 H, m, piperidine), 2.15–2.23 (2H, m, piperidine), 2.96–3.05 (2H, m, piperidine), 3.65–3.72 (2H, m, piperidine), 4.00–4.11 (1H, m, piperidine), 6.58 (1H, t, pyrimidine), 7.07–7.10 (1H, m, C_6H_4), 7.12–7.15 (2H, m, C_6H_4), 7.29–7.34 (1H, m, C_6H_4), 8.30 (2H, d, pyrimidine); EIMS *m*/*z* 279 $(M)^+$.

6.5.1.4. Compound 26. 50% H_2SO_4 aqueous solution (20 ml) was added to compound **25** (380 mg, 1.4 mmol) to prepare a solution, and a reaction was allowed to proceed at 80 °C for 4 h. The temperature of the reaction solution was returned to room temperature, and the reaction solution was adjusted to pH 7 by the addition of $NaHCO_3$. The precipitated insolubles were then collected by filtration and were washed twice with water (6.0 ml). The solid was dried under reduced pressure to prepare compound **26** (250 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.58 (2H, br dq, piperidine), 1.94 (2H, br d, piperidine), 2.85 (2H, br t, piperidine), 3.74 (2H, br d, piperidine), 3.90 (1H, m, piperidine), 6.55 (1H, t, pyrimidine), 7.21 (1H, dt, C_6H_4), 7.31 (1H, t, C_6H_4), 7.33 (1H, m, C_6H_4), 7.47 (1H, br s, C_6H_4), 8.27 (2H, d, pyrimidine); TSPMS *m*/*z* 299 $(M+H)^+$.

6.5.2. Method B

6.5.2.1. Compound 27. Toluene (200 ml) was added to ethyl 3-bromobenzoate (5.0 g, 22 mmol) to prepare a solution. The solution was added to 4-hydroxypiperidine (2.7 g, 26 mmol). Further, anhydrous cesium carbonate (10 g, 31 mmol), palladium(II) acetate (74 mg, 0.33 mmol), and (*R*)-(+)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (200 mg, 0.32 mmol) were added thereto, and the mixture was stirred with heating at 90 °C for 5 h and then at 100 °C for 2 h. The temperature of the reaction mixture was returned to room temperature, and the reaction mixture was then added dropwise to an aqueous ammonium chloride solution (400 ml), followed by extraction with ethyl acetate (200 ml). The ethyl acetate layer was dried over anhydrous Na_2SO_4 and was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/*n*-hexane, 2:1) to prepare compound **27** (490 mg, 8.9%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.70 (2H, m, piperidine), 2.02 (2H, m, piperidine), 2.97 (2H, ddd, piperidine), 3.61 (2H, m, piperidine), 3.87 (1H, m, piperidine), 4.37 (2H, q, Et), 7.12 (1H, br ddd, C_6H_4), 7.30 (1H, t, C_6H_4), 7.50 (1H, br ddd, C_6H_4), 7.61 (1H, br dd, C_6H_4); TSPMS *m*/*z* 250 $(M+H)^+$.

6.5.2.2. Compound 28. The title compound was prepared from compound **27** by the same procedure as employed for compound **24**. Yield: (720 mg, 72%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.50 (2H, m, piperidine), 1.94 and 2.04 (2H, each br d, piperidine),

2.83 (3H, m, piperidine), 3.71 (2H, m, piperidine), 4.36 (2H, q, Et), 7.12 (1H, br dd, C_6H_4), 7.29 (1H, t, C_6H_4), 7.49 (1H, br ddd, C_6H_4), 7.61 (1H, br dd, C_6H_4); TSPMS *m*/*z* 249 $(M+H)^+$.

6.5.2.3. Compound 29. The title compound was prepared from compound **28** by the same procedure as employed for compound **25**. Yield: (530 mg, 56%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.66 (2H, br dq, piperidine), 2.19 (2H, br d, piperidine), 2.99 (2H, m, piperidine), 3.71 (2H, br d, piperidine), 4.02 (1H, m, piperidine), 4.37 (2H, q, Et), 6.54 (1H, t, pyrimidine), 7.13 (1H, br ddd, C_6H_4), 7.31 (1H, t, C_6H_4), 7.52 (1H, br ddd, C_6H_4), 7.63 (1H, br dd, C_6H_4), 8.28 (2H, d, pyrimidine); TSPMS *m*/*z* 327 $(M+H)^+$.

6.5.2.4. Compound 26. THF (45 ml), MeOH (15 ml), and 1 N NaOH (15 ml) were successively added to compound **29** (530 mg, 1.6 mmol) to prepare a solution, and a reaction was allowed to proceed at 45 °C for 16 h. The temperature of the reaction solution was returned to room temperature, and the reaction solution was then concentrated to dryness. The residue was dissolved in water (16 ml). The solution was adjusted to pH 3 by the addition of 5 N hydrochloric acid (2.5 ml) and 1 N hydrochloric acid (2.0 ml). The precipitated insolubles were then collected by filtration and were washed twice with water (6.0 ml). The solid was dried under reduced pressure in the presence of diphosphorus pentoxide at 60 °C for 3 h to prepare compound **26** (470 mg, 97%). Compound **26** synthesized by method B was identified with that prepared by method A with 400 MHz ^1H NMR and TSP mass spectrum.

6.5.3. Method C

6.5.3.1. Compound 27. Ethyl 3-aminobenzoate (3.3 g, 20 mmol) was added to 1,5-dichloropentan-3-one,[10] and the mixture was dissolved in 200 ml of MeOH. *p*-Toluenesulfonic acid monohydrate (4.6 g, 24 mmol) was added to the solution, and a reaction was allowed to proceed at 65 °C for 7 h, and the reaction mixture was then concentrated under reduced pressure. An aqueous $NaHCO_3$ solution (300 ml) was added to the residue, and the mixture was extracted twice with CH_2Cl_2 (200 ml). The combined organic layers were washed with an aqueous $NaHCO_3$ solution (300 ml), were dried over anhydrous $MgSO_4$, and were then concentrated under reduced pressure. Immediately, formic acid (70 ml) and water (7.0 ml) were added to the residue to prepare a solution. A reaction was allowed to proceed at room temperature for 2 h, and the reaction mixture was then concentrated under reduced pressure. An aqueous $NaHCO_3$ solution (200 ml) was added to the residue, and the mixture was extracted twice with ethyl acetate (200 ml). The combined organic layers were washed with an aqueous $NaHCO_3$ solution (200 ml), were dried over anhydrous $MgSO_4$, and were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 2:1) to prepare ethyl 3-(4-oxopiperidin-1-yl)benzoate.

THF (150 ml) was added to ethyl 3-(4-oxopiperidin-1-yl)benzoate to prepare a solution. Sodium borohydride (601 mg, 16 mmol) was added to the solution at room temperature, and the mixture was stirred for 3.5 h. Water (300 ml) was added thereto, and the mixture was extracted with ethyl acetate (300 ml), followed by washing with saturated brine (100 ml). The extract was dried over anhydrous $MgSO_4$ and was concentrated under reduced pressure to prepare compound **27** (3.6 g, 72%); compound **27** synthesized by method C was identified with that prepared by method B with 400 MHz ^1H NMR and TSP mass spectrum.

6.5.3.2. Compound 26. The title compound was prepared by the same procedure as method B.

6.6. Preparation of compound 22

6.6.1. Compound 30. DMF (5.4 ml) and CH_2Cl_2 (5.4 ml) were added to compound **26** (93 mg, 0.31 mmol) and 207 mg of benzotriazol-1-yloxytri(dimethylamino)phosphonium hexafluorophosphate (210 mg, 0.47 mmol) to prepare a solution. *N*,*N*-Diisopropylethylamine (0.082 ml, 0.47 mmol) was added to the solution, and a reaction was allowed to proceed at room temperature for 2 h. Separately, CH_2Cl_2 (5.4 ml) was added to *tert*-butyl (2*S*)-*N*-benzenesulfonyl-2,3-diaminopropionate (110 mg, 0.38 mmol) to prepare a solution. *N*,*N*-Diisopropylethylamine (0.041 ml, 0.24 mmol) was added to the solution. The above active ester solution was added to this mixture at 0 °C, and a reaction was allowed to proceed at room temperature for 16 h. The reaction solution was concentrated under reduced pressure, and the residue was extracted with ethyl acetate (40 ml), followed by washing with an aqueous $NaHCO_3$ solution and saturated brine. The extract was then dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 30:1:0.03) to prepare compound **30** (180 mg, 100%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.64 (2H, br q, piperidine), 2.17 (2H, m, piperidine), 2.99 (2H, br t, piperidine), 3.60 (1H, ddd, $CONHCH_2$), 3.73 (1H, br d, piperidine), 3.89 (1H, ddd, $CONHCH_2$), 3.93–4.05 (2H, m, $CONHCH_2CH$ and piperidine), 6.53 (1H, t, pyrimidine), 7.07 (1H, br dd, C_6H_4), 7.15 (1H, br d, C_6H_4), 7.29 (1H, t, C_6H_4), 7.42 (1H, br dd, C_6H_4), 7.49 (2H, m, C_6H_5), 7.57 (1H, m, C_6H_5), 7.86 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 581 $(M+H)^+$; $[\alpha]_D^{25}$ +46 (*c* 0.70, $CHCl_3$).

6.6.2. Compound 22. CH_2Cl_2 (4.0 ml) and anisole (0.20 ml) were added to compound **30** (170 mg, 0.29 mmol) to prepare a solution, and the solution was cooled to 0 °C. Trifluoroacetic acid (4.0 ml) was added thereto, and a reaction was allowed to proceed at room temperature for 8 h. The reaction solution was concentrated under reduced pressure, and the residue was subjected to azeotropic distillation twice with toluene (4.0 ml). The product obtained by the azeotropic distillation was then washed twice with isopropyl ether (4.0 ml), and the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 9:2:0.2) to prepare (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid.

1,4-Dioxane (10.5 ml), water (3.0 ml), and 1 N hydrochloric acid (1.5 ml) were successively added to (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (160 mg) to prepare a solution. To the solution was added 10% Pd/C (40 mg). The mixture was stirred in a hydrogen atmosphere at room temperature for 6 h. The insolubles were filtered and were washed twice with 4.0 ml of a solvent having the same composition as the mixed solvent used in the reaction. The filtrate and the washings were combined, followed by concentration under reduced pressure. The residue was purified by preparative thin-layer chromatography ($CHCl_3$:EtOH:H_2O:concd NH_4OH, 8:8:1:1) to prepare the title compound as crude. Finally, the crude compound was purified by Sephadex LH-20 (10% concd NH_4OH/MeOH) to prepare compound **22** (120 mg, 78%); ^{1}H NMR (400 MHz, 10% ND_4OD/CD_3OD) δ: 1.63 (2H, m, piperidine), 1.94 (2H, quintet, tetrahydropyrimidine), 2.00 (2H, br d, piperidine), 2.90 (2H, m, piperidine), 3.35 (4H, t, tetrahydropyrimidine), 3.49 (1H, m, piperidine), 3.52 (1H, dd, $CONHCH_2$), 3.70 (1H, dd, $CONHCH_2$), 3.72 (2H, br d, piperidine), 3.78 (1H, dd, $CONHCH_2CH$), 7.13 (1H, br dd, C_6H_4), 7.24 (1H, br d, C_6H_4), 7.32 (1H, t, C_6H_4), 7.42 (1H, br s, C_6H_4), 7.48 (2H, m, C_6H_5), 7.55 (1H, m, C_6H_5), 7.85 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_5S$: 529.2233. Found: 529.2223; $[\alpha]_D^{25}$ +65 (*c* 1.0, 10% concd NH_4OH/MeOH).

6.7. Preparation of the compounds displayed in Table 4

6.7.1. Preparation of compound 31 (method A)

6.7.1.1. 3-{4-(Aminomethyl)piperidin-1-yl}benzonitrile. The title compound was prepared from 4-aminopiperidine by the same procedure as employed for compound **23**. Yield: (40 mg, 38%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.33 (2H, ddd, piperidine), 1.43–1.55 (1H, m, piperidine), 1.85 (2H, br d, piperidine), 2.64 (2H, d, $NHCH_2$), 2.77 (2H, ddd, piperidine), 3.73 (2H, br d, piperidine), 7.05 (1H, ddd, C_6H_4), 7.09–7.14 (2H, m, C_6H_4), 7.29 (1H, dd, C_6H_4); TSPMS *m*/*z* 216 $(M+H)^+$.

6.7.1.2. 3-{4-(Pyrimidin-2-ylaminomethyl)piperidin-1-yl}benzonitrile. The title compound was prepared from 3-{4-(aminomethyl)piperidin-1-yl}benzonitrile by the same procedure as employed for compound **25**. Yield: (160 mg, 54%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (2H, dddd, piperidine), 1.76–1.87 (1H, m, piperidine), 1.86–1.94 (2H, m, piperidine), 2.78 (2H, ddd, piperidine), 3.38 (2H, dd, $NHCH_2$), 3.69–3.76 (2H, m, piperidine), 6.54 (1H, t, pyrimidine), 7.05 (1H, ddd, C_6H_4), 7.09–7.13 (2H, m, C_6H_4), 7.29 (1H, m, C_6H_4), 8.28 (1H, d, pyrimidine); TSPMS *m*/*z* 294 $(M+H)^+$.

6.7.1.3. 3-{4-(Pyrimidin-2-ylaminomethyl)piperidin-1-yl}benzoic acid. The title compound was prepared from 3-{4-(pyrimidin-2-ylaminomethyl)piperidin-1-yl}benzonitrile by the same procedure as employed for compound **26**. Yield: (44 mg, 80%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.20–1.30 (2H, m, piperidine), 1.74–1.83 (3H, m, piperidine), 2.67 (2H, br dd, piperidine), 3.38 (2H, m, $NHCH_2$), 3.72 (2H, br d, piperidine), 6.52 (1H, t, pyrimidine), 7.15–7.25 (2H, m, C_6H_4), 7.29 (1H, dd, C_6H_4), 7.44 (1H, br s, C_6H_4), 8.24 (1H, d, pyrimidine); TSPMS *m*/*z* 313 $(M+H)^+$.

6.7.1.4. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-ylaminomethyl)piperidin-1-yl}benzoylamino]-propionate. The title compound was prepared from 3-{4-(pyrimidin-2-ylaminomethyl)piperidin-1-yl}benzoic acid by the same procedure as employed for compound **30**. Yield: (43 mg, 52%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.37–1.50 (2H, m, piperidine), 1.73–1.85 (1H, m, piperidine), 1.90 (2H, br d, piperidine), 2.77 (2H, br dd, piperidine), 3.37 (2H, dd, $NHCH_2$), 3.53–3.62 (1H, m, $CONHCH_2$), 3.78 (2H, br d, piperidine), 3.85–3.95 (2H, m, $CONHCH_2CH$), 6.52 (1H, t, pyrimidine), 7.05 (1H, d, C_6H_4), 7.14 (1H, d, C_6H_4), 7.28 (1H, dd, C_6H_4), 7.40 (1H, br s, C_6H_4), 7.46–7.60 (3H, m, C_6H_5), 7.83–7.88 (2H, m, C_6H_5), 8.27 (2H, d, pyrimidine); TSPMS *m*/*z* 595 $(M+H)^+$.

6.7.1.5. Compound 31. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-ylaminomethyl)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (17 mg, 58%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.37 (2H, ddd, piperidine), 1.65–1.76 (1H, m, piperidine), 1.81 (2H, br d, piperidine), 1.94 (2H, dddd, tetrahydropyrimidine), 2.75 (2H, ddd, piperidine), 3.03 (2H, d, $NHCH_2$), 3.36 (4H, br t, tetrahydropyrimidine), 3.55 (1H, dd, $CONHCH_2$), 3.69 (1H, dd, $CONHCH_2$), 3.75 (1H, dd, $CONHCH_2CH$), 3.80 (2H, br d, piperidine), 7.11 (1H, ddd, C_6H_4), 7.22 (1H, ddd, C_6H_4), 7.29 (1H, dd, C_6H_4), 7.43 (1H, dd, C_6H_4), 7.46–7.52 (2H, m, C_6H_5), 7.52–7.58 (1H, m, C_6H_5), 7.84–7.89 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_5S$: 543.2390. Found: 543.2380; $[\alpha]_D^{25}$ +66 (*c* 0.32, MeOH).

6.7.2. Preparation of compound 32 (method D, Scheme 4)

6.7.2.1. Compound 48. MeOH (50 ml) was added to 2-deoxy-D-ribose (1.3 g, 10 mmol) to prepare a solution. Separately, CH_2Cl_2 (50 ml) was added to ethyl 3-aminobenzoate (1.6 g, 9.7 mmol) to prepare a solution which was then added to the above methanol solution. A reaction was allowed to proceed at room temperature for 16 h. Acetic acid (1.0 ml) and 500 mg of sodium cyanoborohydride (500 mg) were then added thereto, and a reaction was allowed to proceed at room temperature for 4 h. The reaction solution was concentrated under reduced pressure, and the residue was extracted with $CHCl_3$ (300 ml). The organic layer was washed with an aqueous $NaHCO_3$ solution (200 ml) containing a minor amount of sodium chloride. The aqueous layer was subjected to back extraction with $CHCl_3$ (100 ml). The chloroform layers were combined and were then dried over anhydrous Na_2SO_4, followed by concentration under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 10:1.3:0.1) to prepare compound **48** (2.2 g, 78%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.36 (3H, t, Et), 1.80 (2H, m, $NHCH_2CH_2$), 3.32 (2H, m, $NHCH_2$), 3.62 (1H, br s, CHOH), 3.77 (2H, br s, CH_2OH), 3.89 (1H, br s, CHOH), 4.33 (2H, q, Et),

6.78 (1H, br dd, C_6H_4), 7.20 (1H, t, C_6H_4), 7.29 (1H, br s, C_6H_4), 7.37 (1H, br d, C_6H_4); TSPMS *m/z* 284 $(M+H)^+$; $[\alpha]_D^{25}$ −17 (*c* 1.0, $CHCl_3$).

6.7.2.2. Compound 50. THF (15 ml) was added to compound **48** (370 mg, 1.3 mmol) to prepare a solution. Carbon tetrabromide (653 mg, 2.0 mmol) was added to the solution. The mixture was cooled to 0 °C, and triphenylphosphine (690 mg, 2.6 mmol) was then added thereto. The temperature of the mixture was gradually raised to room temperature over a period of 1 h. Moreover, this solution was kept at room temperature for two more hours. The reaction solution was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (40 g, $CHCl_3$/MeOH/concd NH_4OH, 20:1:0.05) to prepare the title compound as a crude compound. The crude compound was purified by preparative thin-layer chromatography ($CHCl_3$/MeOH/benzene/ethyl acetate, 9:1:6:4) to prepare compound **50** (160 mg, 46%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.94 (2H, m, NCH_2CH_2), 2.99 (1H, m, NCH_2CH_2), 3.16 (1H, dd, NCH_2CHOH), 3.42 (1H, m, NCH_2CH_2), 3.51 (1H, ddd, NCH_2CHOH), 3.84 (1H, br s, CHOH), 3.95 (1H, br s, CHOH), 4.37 (2H, q, Et), 7.14 (1H, br ddd, C_6H_4), 7.32 (1H, t, C_6H_4), 7.56 (1H, br ddd, C_6H_4), 7.63 (1H, br dd, C_6H_4); FABMS *m/z* 266 $(M+H)^+$; $[\alpha]_D^{25}$ +3.0 (*c* 1.0, $CHCl_3$).

6.7.2.3. Compounds 51 and 52. Trimethyl orthoacetate (0.50 ml) was added to compound **50** (134 mg, 0.51 mmol) to prepare a suspension. *p*-Toluenesulfonic acid monohydrate (15.4 mg, 0.082 mmol) was added to the suspension at room temperature, and a reaction was allowed to proceed for 3 h. The reaction solution was concentrated under reduced pressure. Acetic acid (1.0 ml) was then added to the residue at room temperature, and a reaction was allowed to proceed for 45 min. Water (100 ml) was then added thereto. The mixture was extracted twice with ethyl acetate (100 ml). The organic layers were combined, and the combined organic layers were washed with saturated brine (100 ml), were dried over anhydrous $MgSO_4$, and were then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH/benzene/ethyl acetate, 9:1:6:4) to prepare compound **51** (47 mg, 30%) and compound **52** (86 mg, 55%);

6.7.2.4. Compound 51. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.90–2.04 (2H, m, piperidine), 2.10 (3H, s, acetyl), 3.20–3.28 (1H, m, piperidine), 3.88 (1H, dd, piperidine), 3.44 (1H, ddd, piperidine), 3.52 (1H, dd, piperidine), 4.07 (1H, dddd, piperidine), 4.37 (2H, q, Et), 5.04 (1H, ddd, C*H*(OAc)), 7.12 (1H, ddd, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.51 (1H, ddd, C_6H_4), 7.60 (1H, dd, C_6H_4); EIMS *m/z* 307 $(M)^+$; $[\alpha]_D^{25}$ −25 (*c* 1.1, CH_2Cl_2).

6.7.2.5. Compound 52. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.94–1.99 (1H, m, piperidine), 2.07–2.16 (1H, m, piperidine), 2.15 (3H, s, acetyl), 3.07 (1H, ddd, piperidine), 3.23 (1H, dd, piperidine), 3.43 (1H, m, piperidine), 3.50 (1H, ddd, piperidine), 4.08 (1H, br s, piperidine), 4.38 (2H, q, Et), 5.00 (1H, ddd, C*H*(OAc)), 7.16 (1H, dd, C_6H_4), 7.33 (1H, dd, C_6H_4), 7.58 (1H, m, C_6H_4), 7.64 (1H, m, C_6H_4); EIMS *m/z* 307 $(M)^+$; $[\alpha]_D^{25}$ +4.9 (*c* 1.1, CH_2Cl_2).

The chemical structure of compound **52** was determined by ^1H NMR analysis and COSY spectrum.

6.7.2.6. Compound 53. CH_2Cl_2 (3.0 ml) was added to compound **51** (47 mg, 0.15 mmol) to prepare a solution. TEA (45 μl, 0.32 mmol) and methanesulfonyl chloride (15 μl, 0.20 mmol) were added to the solution at room temperature, and a reaction was allowed to proceed for 5 min. Water (100 ml) was added thereto, and the mixture was extracted twice with CH_2Cl_2 (50 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and were concentrated under reduced pressure to prepare ethyl 3-{(3*R*)-acetoxy-(4*S*)-methanesulfonyloxypiperidin-1-yl}benzoate (40 mg, 67%).

DMF (2.0 ml) was added to ethyl 3-{(3*R*)-acetoxy-(4*S*)-methanesulfonyloxypiperidin-1-yl}benzoate (39 mg, 0.10 mmol) to prepare a solution. Sodium azide (15 mg, 0.23 mmol) was added to the solution, and a reaction was allowed to proceed at 90 °C for 10 h. The reaction mixture was returned to room temperature, water (100 ml) was then added thereto, and the mixture was extracted twice with ethyl acetate (70 ml). The combined organic layers were washed twice with water (100 ml) and once with saturated brine (100 ml), were then dried over anhydrous $MgSO_4$, and were concentrated under reduced pressure. The residue was then purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 1:1) to give ethyl 3-{(3*R*)-acetoxy-(4*R*)-azidopiperidin-1-yl}benzoate (34 mg, 100%).

THF (11 ml) was added to ethyl 3-{(3*R*)-acetoxy-(4*R*)-azidopiperidin-1-yl}benzoate (390 mg, 1.2 mmol) to prepare a solution. Sodium ethoxide (99 mg, 1.4 mmol) was added to the solution, and a reaction was allowed to proceed at 30 °C for 3.5 h. The reaction solution was adjusted to pH 4 by the addition of 1 N hydrochloric acid, and water (100 ml) was added thereto. The mixture was extracted twice with ethyl acetate (150 ml). The combined organic layers were then washed with saturated brine (150 ml), were dried over anhydrous $MgSO_4$, and were concentrated under reduced pressure to prepare compound **53** (350 mg, 100%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.79 (1H, dddd, piperidine), 2.15 (1H, dddd, piperidine), 2.84 (1H, ddd, piperidine), 2.94 (1H, ddd, piperidine), 3.45 (1H, ddd, piperidine), 3.60 (1H, dddd, piperidine), 3.72 (1H, dd, piperidine), 3.76 (1H, ddd, piperidine), 4.37 (2H, q, Et), 7.11 (1H, dd, C_6H_4), 7.32 (1H, dd, C_6H_4), 7.56 (1H, ddd, C_6H_4), 7.60 (1H, dd, C_6H_4); EIMS *m/z* 290 $(M)^+$.

6.7.2.7. Compound 57. 1,4-Dioxane (1.0 ml) and water (0.50 ml) were successively added to compound **53** (11 mg, 0.039 mmol) to prepare a solution. 10% Pd/C (3.0 mg) was added to the solution, and the mixture was stirred in a hydrogen atmosphere at room temperature for 3 h. The insolubles were filtered and were

washed with solvent (20 ml) having the same composition as the mixed solvent used in the reaction. The filtrate and the washings were combined, followed by concentration under reduced pressure to give ethyl 3-{(4*R*)-amino-(3*R*)-hydroxypiperidin-1-yl}benzoate (11 mg, 100%).

DMSO (3.0 ml) was added to ethyl 3-{(4*R*)-amino-(3*R*)-hydroxypiperidin-1-yl}benzoate (87 mg, 0.33 mmol) to prepare a solution. 2-Bromopyrimidine (55 mg, 0.33 mmol) and *N*,*N*-diisopropylethylamine (320 μl, 1.9 mmol) were successively added to the solution, and a reaction was allowed to proceed at 120 °C for 14 h. The reaction mixture was returned to room temperature, and water (500 ml) was added thereto, followed by extraction three times with ethyl acetate (250 ml). The combined organic layers were washed twice with water (200 ml) and twice with saturated brine (200 ml), were then dried over anhydrous $MgSO_4$, and were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (benzene/ethyl acetate, 1:4) to prepare compound **57** (51 mg, 45%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.80 (1H, dddd, piperidine), 2.14 (1H, dddd, piperidine), 2.74 (1H, dd, piperidine), 2.89 (1H, ddd, piperidine), 3.72–3.86 (3H, m, piperidine), 3.97 (1H, ddd, piperidine), 4.37 (2H, q, Et), 6.64 (1H, t, pyrimidine), 7.14 (1H, dd, C_6H_4), 7.32 (1H, dd, C_6H_4), 7.53 (1H, ddd, C_6H_4), 7.63 (1H, dd, C_6H_4), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 343 $(M+H)^+$.

6.7.2.8. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*R*)-hydroxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. 3-{(3*R*)-Hydroxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid was prepared from compound **57** by the same procedure as employed for compound **26** from compound **29**.

The title compound was prepared from 3-{(3*R*)-hydroxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid by the same procedure as employed for compound **30**. Yield: (18 mg, 21%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.72–1.83 (1H, m, piperidine), 2.09–2.15 (1H, m, piperidine), 2.75 (1H, dd, piperidine), 2.90 (1H, ddd, piperidine), 3.54–3.63 (1H, m, piperidine), 3.70–3.83 (3H, m, piperidine and $CONHCH_2CH$), 3.86–3.95 (2H, m, piperidine and $CONHCH_2$), 3.98 (1H, ddd, piperidine), 6.62 (1H, t, pyrimidine), 7.10 (1H, dd, C_6H_4), 7.18 (1H, br d, C_6H_4), 7.31 (1H, dd, C_6H_4), 7.43 (1H, dd, C_6H_4), 7.47–7.60 (3H, m, C_6H_5), 7.84–7.88 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 597 $(M+H)^+$; $[\alpha]_D^{25}$ +75 (*c* 0.48, CH_2Cl_2).

6.7.2.9. Compound 32. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*R*)-hydroxy-(4*R*)-(pyrimidin-2- ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (3.1 mg, 21%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.68 (1H, dddd, piperidine), 1.95 (2H, dddd, tetrahydropyrimidine), 1.92–2.02 (1H, m, piperidine), 2.67 (1H, dd, piperidine), 2.83 (1H, ddd, piperidine), 3.26–3.32 (1H, m, piperidine), 3.36 (4H, br t, tetrahydropyrimidine), 3.50–3.57 (1H, m, piperidine), 3.56 (1H, dd, $CONHCH_2$), 3.67 (1H, dd, $CONHCH_2$), 3.74 (1H, dd, $CONHCH_2CH$), 3.77–3.85 (1H, m, piperidine), 3.91 (1H, m, piperidine), 7.11 (1H, ddd, C_6H_4), 7.24 (1H, ddd, C_6H_4), 7.31 (1H, dd, C_6H_4), 7.42 (1H, dd, C_6H_4), 7.46–7.52 (2H, m, C_6H_5), 7.52–7.58 (1H, m, C_6H_5), 7.85–7.89 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_6S$: 545.2182. Found: 545.2189; $[\alpha]_D^{25}$ +70 (*c* 0.14, MeOH).

6.7.3. Preparation of compound 33

6.7.3.1. Compound 55. THF (5.0 ml) was added to sodium hydride (60%, 35 mg, 0.87 mmol) in an argon atmosphere to prepare a suspension. Separately, compound **53** (208 mg, 0.72 mmol) was dissolved in THF (2.0 ml). This solution was added dropwise to the above suspension at room temperature, and the mixture was stirred for 30 min. A solution (1.0 ml) of methyl iodide (67 μl, 1.1 mmol) in THF was added dropwise thereto. The mixture was stirred for 4 h, an aqueous ammonium chloride solution was then added to stop the reaction, and water (100 ml) was added thereto. The mixture was extracted twice with ethyl acetate (100 ml). The combined organic layers were then dried over anhydrous $MgSO_4$ and were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) to prepare the title compound (77 mg, 35%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.70 (1H, m, piperidine), 2.06 (1H, m, piperidine), 2.68 (1H, dd, piperidine), 2.84 (1H, ddd, piperidine), 3.34 (1H, ddd, piperidine), 3.44 (1H, ddd, piperidine), 3.55 (3H, s, OMe), 3.63 (1H, br d, piperidine), 3.88 (1H, ddd, piperidine), 4.37 (2H, q, Et), 7.12 (1H, d, C_6H_4), 7.32 (1H, dd, C_6H_4), 7.55 (1H, d, C_6H_4), 7.60 (1H, br s, C_6H_4); TSPMS *m*/*z* 305 $(M+H)^+$.

6.7.3.2. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*R*)-methoxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}-benzoylamino]propionate. 1,4-Dioxane (4.0 ml) and water (2.0 ml) were successively added to compound **55** (69 mg, 0.23 mmol) to prepare a solution. 10% Pd/C (18 mg) was added to the solution, and the mixture was stirred in a hydrogen atmosphere at room temperature for 4 h. The insolubles were filtered and were washed with a solvent (90 ml) having the same composition as the mixed solvent used in the reaction. The filtrate and the washings were combined followed by concentration under reduced pressure to give ethyl 3-{(4*R*)-amino-(3*R*)-methoxypiperidin-1-yl}benzoate (66 mg, 100%).

DMSO (2.5 ml) was added to the ethyl 3-{(4*R*)-amino-(3*R*)-methoxypiperidin-1-yl}benzoate (66 mg, 0.23 mmol) thus obtained to prepare a solution. *N*,*N*-Diisopropylethylamine (230 μl, 1.3 mmol) and 2-bromopyrimidine (42 mg, 0.27 mmol) were added in that order to the solution. A reaction was allowed to proceed at 120 °C for 24 h, and the temperature of the reaction mixture was then returned to room temperature. Water (500 ml) was added thereto, and the mixture was extracted twice with ethyl acetate (500 ml). The combined organic layers were washed twice with water (500 ml) and once with saturated brine (500 ml), were

dried over anhydrous $MgSO_4$, and were then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate) to give ethyl 3-{(3*R*)-methoxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (**58**) (34 mg, 40%).

THF (1.5 ml) and MeOH (0.50 ml) were added to compound **58** (34 mg, 0.096 mmol) thus obtained to prepare a solution, and a 1 N NaOH (0.50 ml) was added to the solution. A reaction was allowed to proceed at 50 °C for 3 h. The temperature of the reaction mixture was then returned to room temperature, the reaction mixture was adjusted to pH 3 by the addition of 1 N hydrochloric acid, and water (50 ml) was added thereto. The mixture was extracted twice with ethyl acetate (50 ml), and the extract was then dried over anhydrous $MgSO_4$ and was concentrated under reduced pressure to give 3-{(3*R*)-methoxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (29 mg, 95%).

DMF (1.5 ml) was added to 3-{(3*R*)-methoxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid (28 mg, 0.086 mmol) to prepare a solution, and *tert*-butyl (2*S*)-*N*-benzenesulfonyl-2,3-diaminopropionate (32 mg, 0.10 mmol) was added to the solution. Further, 1-hydroxybenzotriazole (19 mg, 0.14 mmol), *N*-methylmorpholine (47 μl, 0.43 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (34 mg, 0.18 mmol) were added thereto, and a reaction was allowed to proceed at room temperature for 2.5 h. An aqueous $NaHCO_3$ solution (10 ml) was added to stop the reaction, and water (100 ml) was added thereto. The mixture was extracted twice with ethyl acetate (100 ml), and the combined organic layers were washed with saturated brine (100 ml) and were dried over anhydrous $MgSO_4$, followed by concentration under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) to prepare the title compound (36 mg, 26% (four steps)); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.65–1.70 (1H, m, piperidine), 2.41 (1H, m, piperidine), 2.85 (1H, dd, piperidine), 3.01 (1H, ddd, piperidine), 3.37 (1H, ddd, piperidine), 3.49 (3H, s, OMe), 3.49–3.58 (1H, m, $CONHCH_2$), 3.60–3.68 (1H, m, piperidine), 3.87–4.03 (4H, m, piperidine and $CONHCH_2CH$), 6.65 (1H, t, pyrimidine), 7.09 (1H, d, C_6H_4), 7.21 (1H, d, C_6H_4), 7.32 (1H, dd, C_6H_4), 7.46 (1H, br s, C_6H_4), 7.48–7.61 (3H, m, C_6H_5), 7.84–7.88 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 611 $(M+H)^+$; $[\alpha]_D^{25}$ +17 (*c* 0.54, CH_2Cl_2).

6.7.3.3. Compound 33. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*R*)-methoxy-(4*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (15 mg, 32% (two steps)); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.71 (1H, dddd, piperidine), 1.95 (2H, dddd, tetrahydropyrimidine), 1.95–2.03 (1H, m, piperidine), 2.59 (1H, dd, piperidine), 2.82 (1H, ddd, piperidine), 3.22 (1H, ddd, piperidine), 3.27–3.38 (1H, m, $CONHCH_2$), 3.36 (4H, br t, tetrahydropyrimidine), 3.50 (3H, s, OMe), 3.45–3.60 (1H, m, piperidine), 3.67–3.82 (3H, m, piperidine and $CONHCH_2CH$), 4.17 (1H, ddd, piperidine), 7.15 (1H, ddd, C_6H_4), 7.27 (1H, d, C_6H_4), 7.32 (1H, dd, C_6H_4), 7.46–7.52 (3H, m, C_6H_4 and C_6H_5), 7.53–7.59 (1H, m, C_6H_5), 7.84–7.89 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_6S$: 559.2339. Found: 559.2350; $[\alpha]_D^{25}$ +93 (*c* 0.44, MeOH).

6.7.4. Preparation of compound 34 (method A)

6.7.4.1. (3*S*)-Aminopiperidin-2-one. MeOH (770 ml) was added to L-ornithine hydrochloride (131 g, 0.78 mol) in an argon atmosphere to prepare a suspension, to which thionyl chloride (170 ml, 2.0 mol) was added dropwise at an internal temperature of −45 °C over a period of 30 min or longer, followed by stirring for 30 min. The temperature of the reaction mixture was then returned to room temperature, and the reaction mixture was vigorously stirred for 19 h and was concentrated under reduced pressure. Water (500 ml) was then added to the residue to prepare a solution. The solution was subjected to column chromatography using a column packed with an Amberlite IRA-400 (OH^-) anion exchange resin (1.1 kg), eluting with water to prepare the title compound as a crude product. MeOH (500 ml) was added to the crude product to prepare a solution, and the solution was slowly poured into $CHCl_3$ (5.0 l). The suspension thus obtained was then filtered, and the filtrate was concentrated under reduced pressure to prepare the title compound (81 g, 69%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.48 (1H, m, piperidine), 1.72 (2H, m, piperidine), 1.99 (1H, dddd, piperidine), 3.16 (2H, dd, piperidine), 3.24 (1H, dd, piperidine); EIMS *m*/*z* 114 $(M)^+$.

6.7.4.2. (3*S*)-Aminopiperidine. THF (100 ml) was added to aluminum lithium hydride (3.3 g, 87 mmol) to prepare a suspension, to which (3*S*)-aminopiperidin-2-one (4.1 g, 27 mmol) was added under ice cooling. The temperature of the reaction mixture was returned to room temperature, and the reaction mixture was then stirred for 5.5 h, and, while vigorously stirring, water (3.3 ml), 5 N NaOH (3.3 ml), and water (10 ml) were added in that order to stop the reaction, followed by filtration. The filtrate was then dried over anhydrous $MgSO_4$. A 4 N hydrogen chloride ethyl acetate solution (14 ml) was added thereto, and the mixture was concentrated under reduced pressure. The residue was subjected to azeotropic distillation with MeOH to prepare dihydrochloride of the title compound (5.1 g, 100%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.75 (1H, dddd, piperidine), 1.90 (1H, m, piperidine), 2.09 (1H, ddddd, piperidine), 2.23 (1H, br d, piperidine), 3.02 (1H, ddd, piperidine), 3.09 (1H, dd, piperidine), 3.41 (1H, br d, piperidine), 3.62 (2H, m, piperidine); EIMS *m*/*z* 100 $(M)^+$.

6.7.4.3. 3-{(3*S*)-Aminopiperidin-1-yl}benzonitrile. The title compound was prepared from (3*S*)-aminopiperidine by the same procedure as employed for compound **23**. Yield: (137 mg, 30%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.32 (1H, ddd, piperidine), 1.50–1.67 (1H, m, piperidine), 1.79–1.87 (1H, m, piperidine), 1.96 (1H, dddd, piperidine), 2.62 (1H, dd, piperidine), 2.82 (1H, ddd, piperidine), 2.88 (1H, dddd, piperidine), 3.53 (1H, ddd, piperidine), 3.65 (1H, dddd, piperidine), 7.05 (1H, ddd,

C_6H_4), 7.20–7.25 (2H, m, C_6H_4), 7.33 (1H, ddd, C_6H_4); EIMS *m*/*z* 201 $(M)^+$.

6.7.4.4. 3-{(3*S*)-(Pyrimidin-2-ylamino)piperidin-1-yl}benzonitrile. The title compound was prepared from 3-{(3*S*)-aminopiperidin-1-yl}benzonitrile by the same procedure as employed for compound **25**. Yield: (144 mg, 75%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.60–1.81 (2H, m, piperidine), 1.83–1.93 (1H, m, piperidine), 1.96–2.04 (1H, m, piperidine), 2.95 (1H, dd, piperidine), 3.11 (1H, dd, piperidine), 3.41 (1H, ddd, piperidine), 3.75 (1H, dd, piperidine), 4.15 (1H, ddddd, piperidine), 6.58 (1H, t, pyrimidine), 7.06 (1H, d, C_6H_4), 7.15 (1H, dd, C_6H_4), 7.24 (1H, br s, C_6H_4), 7.29 (1H, dd, C_6H_4), 8.32 (2H, d, pyrimidine); EIMS *m*/*z* 279 $(M)^+$; $[\alpha]_D^{25}$ +8.6 (*c* 0.67, CH_2Cl_2).

6.7.4.5. 3-{(3*S*)-(Pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid. The title compound was prepared from 3-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzonitrile by the same procedure as employed for compound **26** from compound **25**. Yield: (79 mg, 60%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.46–1.67 (2H, m, piperidine), 1.74–1.84 (1H, m, piperidine), 1.91–1.99 (1H, m, piperidine), 2.64 (1H, dd, piperidine), 2.77 (1H, br dd, piperidine), 3.67 (1H, br d, piperidine), 3.83 (1H, br d, piperidine), 3.86–3.97 (1H, m, piperidine), 6.58 (1H, t, pyrimidine), 7.13 (1H, d, C_6H_4), 7.19 (1H, ddd, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.53 (1H, br s, C_6H_4), 8.29 (2H, d, pyrimidine); EIMS *m*/*z* 298 $(M)^+$.

6.7.4.6. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl} benzoylamino]propionate. The title compound was prepared from 3-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid by the same procedure as employed for compound **30**. Yield: (130 mg, 85%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.62–1.71 (1H, m, piperidine), 1.71–1.81 (1H, m, piperidine), 1.85–2.00 (2H, m, piperidine), 3.02 (1H, dd, piperidine), 3.10–3.20 (1H, m, piperidine), 3.32–3.39 (1H, m, piperidine), 3.60 (1H, ddd, CONHCH$_2$), 3.67 (1H, dd, piperidine), 3.85–3.96 (2H, m, CONHCH$_2$CH), 4.16–4.25 (1H, m, piperidine), 6.53 (1H, t, pyrimidine), 7.10 (1H, dd, C_6H_4), 7.16 (1H, d, C_6H_4), 7.29 (1H, dd, C_6H_4), 7.44 (1H, dd, C_6H_4), 7.46–7.52 (2H, m, C_6H_5), 7.54–7.59 (1H, m, C_6H_5), 7.83–7.87 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 581 $(M+H)^+$; $[\alpha]_D^{25}$ +40 (*c* 0.54, CH_2Cl_2).

6.7.4.7. Compound 34. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*S*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]-propionate by the same procedure as employed for compound **22**. Yield: (59 mg, 59%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.60 (1H, ddd, piperidine), 1.70–1.81 (1H, m, piperidine), 1.85–1.93 (4H, m, piperidine and tetrahydropyrimidine), 3.06 (1H, dd, piperidine), 3.10–3.15 (1H, m, piperidine), 3.28 (1H, m, piperidine), 3.33 (4H, br t, tetrahydropyrimidine), 3.48 (1H, dd, piperidine), 3.52 (1H, dd, CONHCH$_2$), 3.65–3.72 (1H, m, piperidine), 3.71 (1H, dd, CONHCH$_2$), 3.77 (1H, dd, CONHCH$_2$CH), 7.13 (1H, ddd, C_6H_4), 7.26 (1H, ddd, C_6H_4), 7.31 (1H, dd, C_6H_4), 7.46 (1H, dd, C_6H_4), 7.47–7.53 (2H, m, C_6H_5), 7.53–7.59 (1H, m, C_6H_5), 7.85–7.90 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_5S$: 529.2233. Found: 529.2239; $[\alpha]_D^{25}$ +66 (*c* 0.57, MeOH).

6.7.5. Preparation of compound 35 (method A)

6.7.5.1. (3*R*)-Aminopiperidin-2-one. The title compound was prepared from D-ornithine hydrochloride by the same procedure as employed for (3*S*)-aminopiperidin-2-one. Yield: (1.7 g, 63%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.48 (1H, m, piperidine), 1.72 (2H, m, piperidine), 1.99 (1H, dddd, piperidine), 3.16 (2H, dd, piperidine), 3.24 (1H, dd, piperidine); EIMS *m*/*z* 114 $(M)^+$.

6.7.5.2. (3*R*)-Aminopiperidine. The title compound was prepared from (3*R*)-aminopiperidin-2-one by the same procedure as employed for (3*S*)-aminopiperidine. Yield: (1.0 g, 60%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.75 (1H, dddd, piperidine), 1.90 (1H, m, piperidine), 2.09 (1H, ddddd, piperidine), 2.23 (1H, br d, piperidine), 3.02 (1H, ddd, piperidine), 3.09 (1H, dd, piperidine), 3.41 (1H, br d, piperidine), 3.62 (2H, m, piperidine); EIMS *m*/*z* 100 $(M)^+$.

6.7.5.3. 3-{(3*R*)-(Pyrimidin-2-ylamino)piperidin-1-yl}benzonitrile. The title compound was prepared from (3*R*)-aminopiperidine by the same procedure as employed for compound **25**. Yield: (59 mg, 10% (two steps)); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.60–1.95 (3H, m, piperidine), 1.96–2.05 (1H, m, piperidine), 2.97 (1H, dd, piperidine), 3.11 (1H, ddd, piperidine), 3.37–3.45 (1H, m, piperidine), 3.74 (1H, dd, piperidine), 4.12–4.21 (1H, m, piperidine), 6.60 (1H, t, pyrimidine), 7.06 (1H, ddd, C_6H_4), 7.15 (1H, ddd, C_6H_4), 7.24 (1H, dd, C_6H_4), 7.29 (1H, dd, C_6H_4), 8.33 (2H, d, pyrimidine); TSPMS *m*/*z* 280 $(M+H)^+$; $[\alpha]_D^{25}$ −7.0 (*c* 0.65, CH_2Cl_2).

6.7.5.4. 3-{(3*R*)-(Pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid. The title compound was prepared from 3-{(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzonitrile by the same procedure as employed for compound **26** from compound **25**. Yield: (44 mg, 71%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.45–1.66 (2H, m, piperidine), 1.74–1.82 (1H, m, piperidine), 1.95 (1H, br d, piperidine), 2.63 (1H, dd, piperidine), 2.76 (1H, ddd, piperidine), 3.65 (1H, br d, piperidine), 3.81 (1H, br d, piperidine), 3.86–3.96 (1H, m, piperidine), 6.57 (1H, t, pyrimidine), 7.11 (1H, d, C_6H_4), 7.18 (1H, ddd, C_6H_4), 7.28 (1H, dd, C_6H_4), 7.52 (1H, br s, C_6H_4), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 299 $(M+H)^+$.

6.7.5.5. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl} benzoylamino]propionate. The title compound was prepared from 3-{(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid by the same procedure as employed for compound **30**. Yield: (80 mg, 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.63–1.72 (1H, m, piperidine), 1.73–1.82 (1H, m, piperidine), 1.86–2.00 (2H, m, piperidine), 3.03 (1H, dd, piperidine), 3.16 (1H, ddd, piperidine), 3.30–3.39 (1H, m, piperidine), 3.61 (1H, ddd, CONHCH$_2$), 3.67 (1H, dd, piperidine), 3.88 (1H, m,

$CONHCH_2$), 3.90–3.96 (1H, m, $CONHCH_2CH$), 4.10–4.25 (1H, m, piperidine), 6.53 (1H, t, pyrimidine), 7.10 (1H, dd, C_6H_4), 7.17 (1H, d, C_6H_4), 7.29 (1H, dd, C_6H_4), 7.44 (1H, dd, C_6H_4), 7.46–7.52 (2H, m, C_6H_5), 7.53–7.59 (1H, m, C_6H_5), 7.83–7.87 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m/z* 581 $(M+H)^+$; $[\alpha]_D^{25}$ +34 (*c* 0.64, CH_2Cl_2).

6.7.5.6. Compound 35. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[3-{(3*R*)-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (29 mg, 37% (two steps)); ^1H NMR (400 MHz, CD_3OD) δ: 1.55–1.65 (1H, m, piperidine), 1.71–1.81 (1H, m, piperidine), 1.84–1.95 (4H, m, piperidine and tetrahydropyrimidine), 3.05 (1H, dd, piperidine), 3.08–3.18 (1H, m, piperidine), 3.25–3.38 (5H, m, piperidine and tetrahydropyrimidine), 3.42–3.54 (1H, m, piperidine), 3.54 (1H, dd, $CONHCH_2$), 3.65–3.77 (3H, m, piperidine and $CONHCH_2CH$), 7.13 (1H, ddd, C_6H_4), 7.27 (1H, ddd, C_6H_4), 7.32 (1H, dd, C_6H_4), 7.47 (1H, dd, C_6H_4), 7.48–7.53 (2H, m, C_6H_5), 7.54–7.60 (1H, m, C_6H_5), 7.85–7.90 (2H, m, C_6H_5); TSPMS *m/z* 529 $(M+H)^+$; $[\alpha]_D^{25}$ +72 (*c* 0.55, MeOH).

6.7.6. Preparation of compound 36

6.7.6.1. Compound 36. The title compound was prepared from compound **52** via compounds **54** and **59** by the same procedure as employed for compounds **32** from compound **51** via compounds **53** and **57**. Yield: (66 mg, 20% (eight steps)); ^1H NMR (400 MHz, CD_3OD) δ: 1.64 (2H, ddd, tetrahydropyrimidine), 2.00–2.12 (1H, m, pyrrolidine), 2.21–2.31 (1H, m, pyrrolidine), 3.10–3.26 (6H, m, tetrahydropyrimidine and $NHCH_2$), 3.51 (1H, dd, $CONHCH_2$), 3.51–3.62 (2H, m, pyrrolidine), 3.76 (1H, dd, $CONHCH_2$), 3.86 (1H, dd, $CONHCH_2CH$), 4.01 (1H, ddd, pyrrolidine), 4.48 (1H, ddd, pyrrolidine), 6.74–6.78 (1H, m, C_6H_4), 7.04–7.10 (2H, m, C_6H_4), 7.27 (1H, dd, C_6H_4), 7.50–7.56 (2H, m, C_6H_5), 7.56–7.62 (1H, m, C_6H_5), 7.86–7.91 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_6S$: 545.2182. Found: 545.2189; $[\alpha]_D^{25}$ +89 (*c* 0.058, MeOH).

6.7.7. Preparation of compound 37

6.7.7.1. Compound 37. The title compound was prepared from compound **54** via compounds **56** and **60** by the same procedure as employed for compound **33** from compound **53** via compounds **55** and **58**. Yield: (3.1 mg, 5.3% (nine steps)); ^1H NMR (400 MHz, CD_3OD) δ: 1.56 (2H, ddd, tetrahydropyrimidine), 2.06 (1H, dddd, pyrrolidine), 2.37 (1H, dddd, pyrrolidine), 3.00–3.10 (4H, m, tetrahydropyrimidine), 3.20 (1H, ddd, pyrrolidine), 3.26 (1H, dd, $NHCH_2$), 3.45–3.58 (3H, m, pyrrolidine and $CONHCH_2$ and $NHCH_2$), 3.47 (3H, s, OMe), 3.78 (1H, dd, $CONHCH_2CH$), 3.84 (1H, dd, $CONHCH_2$), 4.08 (1H, ddd, pyrrolidine), 4.22 (1H, ddd, pyrrolidine), 6.74 (1H, dd, C_6H_4), 7.06 (1H, d, C_6H_4), 7.12 (1H, dd, C_6H_4), 7.27 (1H, dd, C_6H_4), 7.50–7.57 (2H, m, C_6H_5), 7.57–7.63 (1H, m, C_6H_5), 7.86–7.93 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_6S$: 559.2339. Found: 559.2343.

6.8. Preparation of the compounds displayed in Table 5

6.8.1. Preparation of *tert*-butyl (2*S*)-amino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate, which is the common intermediate for replacement of the *C*-terminus

6.8.1.1. *tert*-Butyl (2*S*)-(benzyloxycarbonyl)amino-3-[3-{4-(pyrimidin-2-ylamino)- piperidin-1-yl}benzoylamino]propionate. DMF (10 ml) was added to compound **26** (201 mg, 0.67 mmol) to prepare a solution, and *tert*-butyl (2*S*)-*N*-benzyloxycarbonyl-2,3-diaminopropionate (WO9938849) (212 mg, 0.74 mmol) was added to the solution. Further, 1-hydroxybenzotriazole (142 mg, 1.0 mmol), *N*-methylmorpholine (370 μl, 3.4 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (270 mg, 1.4 mmol) were added thereto, and a reaction was allowed to proceed at room temperature for 13 h. An aqueous $NaHCO_3$ solution (20 ml) was added to stop the reaction, and water (400 ml) was added thereto. The mixture was extracted twice with ethyl acetate (250 ml). The organic layers were combined, and the combined organic layers were washed with saturated brine (400 ml), were dried over anhydrous $MgSO_4$, and were then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 20:1) to prepare the title compound (370 mg, 95%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.47 (9H, s, *t*-Bu), 1.64 (2H, m, piperidine), 2.18 (2H, m, piperidine), 2.99 (2H, dd, piperidine), 3.72 (2H, br d, piperidine), 3.82 (2H, m, $CONHCH_2$), 4.01 (1H, m, piperidine), 4.46 (1H, m, $CONHCH_2CH$), 5.11 (2H, s, CO_2CH_2Ph), 6.54 (1H, t, pyrimidine), 7.07 (1H, dd, C_6H_4), 7.12 (1H, br d, C_6H_4), 7.28–7.35 (6H, m, C_6H_5 and C_6H_4), 7.42 (1H, br s, C_6H_4), 8.28 (2H, d, pyrimidine); EIMS *m/z* 574 $(M)^+$; $[\alpha]_D^{25}$ −2.4 (*c* 1.1, CH_2Cl_2).

6.8.1.2. *tert*-Butyl (2*S*)-amino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. THF (60 ml) was added to *tert*-butyl (2*S*)-(benzyloxycarbonyl)amino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}-benzoylamino]propionate (230 mg, 0.40 mmol) to prepare a solution. To the solution was added 10% Pd/C (230 mg). The mixture was stirred in a hydrogen atmosphere at room temperature for 4 h. The insolubles were filtered and were washed with THF (200 ml). The filtrate and the washings were combined followed by concentration under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) to prepare the title compound (110 mg, 63%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.48 (9H, s, *t*-Bu), 1.62–1.74 (2H, m, piperidine), 2.18 (2H, br d, piperidine), 2.99 (2H, br dd, piperidine), 3.48 (1H, ddd, $CONHCH_2$), 3.61 (1H, dd, $CONHCH_2CH$), 3.68–3.76 (2H, m, piperidine), 3.83 (1H, ddd, $CONHCH_2$), 3.96–4.06 (1H, m, piperidine), 6.54 (1H, t, pyrimidine), 7.07 (1H, dd, C_6H_4), 7.14 (1H, br d, C_6H_4), 7.29 (1H, dd, C_6H_4), 7.43 (1H, br s, C_6H_4), 8.28 (2H, d, pyrimidine); EIMS *m/z* 440 $(M)^+$; $[\alpha]_D^{25}$ +6.5 (*c* 1.0, CH_2Cl_2).

6.8.2. Preparation of compound 38. 6.8.2.1. *tert*-Butyl (2*S*)-acetamido-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. CH_2Cl_2 (10 ml) was added

to *tert*-butyl (2*S*)-amino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl} benzoylamino]propionate (101 mg, 0.25 mmol) to prepare a solution. TEA (70 μl, 0.50 mmol) and acetic anhydride (24 μl, 0.30 mmol) were added in that order to the solution at room temperature, and a reaction was allowed to proceed for 1 h. Water (50 ml) was added thereto, and the mixture was extracted twice with CH_2Cl_2 (100 ml). The extract was dried over anhydrous $MgSO_4$ and was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) to prepare the title compound (94 mg, 77%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.49 (9H, s, *t*-Bu), 1.60–1.72 (2H, m, piperidine), 2.05 (3H, s, Ac), 2.19 (2H, m, piperidine), 3.00 (2H, m, piperidine), 3.68–3.75 (3H, ddd, piperidine and $CONHCH_2$), 3.86 (1H, ddd, $CONHCH_2$), 4.01 (1H, m, piperidine), 4.67 (1H, ddd, $CONHCH_2CH$), 6.54 (1H, t, pyrimidine), 7.07 (1H, dd, C_6H_4), 7.08 (1H, m, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.42 (1H, dd, C_6H_4), 8.27 (2H, d, pyrimidine); EIMS *m*/*z* 482 $(M)^+$; $[\alpha]_D^{25}$ −6.8 (*c* 1.0, CH_2Cl_2).

6.8.2.2. Compound 38. The title compound was prepared from *tert*-butyl (2*S*)-acetamido-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (29 mg, 47% (two steps)); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.64 (2H, dddd, piperidine), 1.97 (3H, s, Ac), 1.92–2.05 (4H, m, piperidine and tetrahydropyrimidine), 2.90 (2H, br dd, piperidine), 3.37 (4H, br t, tetrahydropyrimidine), 3.48 (1H, m, piperidine), 3.67 (1H, dd, $CONHCH_2$), 3.70–3.78 (2H, m, piperidine), 3.74 (1H, dd, $CONHCH_2$), 4.48 (1H, dd, $CONHCH_2CH$), 7.12 (1H, m, C_6H_4), 7.23 (1H, br d, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.38 (1H, br s, C_6H_4); FAB-HRMS $(M+H)^+$ calcd for $C_{21}H_{30}N_6O_4$: 431.2407. Found: 431.2403; $[\alpha]_D^{25}$ +11 (*c* 0.32, MeOH).

6.8.3. Preparation of compound 39

6.8.3.1. *tert*-Butyl (2*S*)-{2-(morpholin-4-yl-acetyl)amino}-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. DMF (10 ml) was added to morpholin-4-ylacetic acid (36 mg, 0.25 mmol) and *tert*-butyl (2*S*)-amino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (110 mg, 0.25 mmol) to prepare a solution. 1-Hydroxybenzotriazole (53 mg, 0.37 mmol), *N*-methylmorpholine (140 μl, 1.3 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (97 mg, 0.50 mmol) were further added to the solution, and a reaction was allowed to proceed at room temperature for 19 h. An aqueous $NaHCO_3$ solution (5.0 ml) was added to stop the reaction, and water (100 ml) was added thereto. The mixture was extracted twice with ethyl acetate (100 ml). The combined organic layers were washed with saturated brine (100 ml), were dried over anhydrous $MgSO_4$, and were then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) to prepare the title compound (94 mg, 66%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.50 (9H, s, *t*-Bu), 1.65 (2H, m, piperidine), 2.18 (2H, m, piperidine), 2.55 (4H, m, piperidine and morpholine), 3.00 (2H, m, piperidine), 3.04 (2H, br d, $COCH_2N$), 3.74 (7H, m, morpholine and $CONHCH_2$), 3.91 (1H, ddd, $CONHCH_2$), 4.02 (1H, m, piperidine), 4.68 (1H, ddd, $CONHCH_2CH$), 6.55 (1H, t, pyrimidine), 7.07 (1H, m, C_6H_4), 7.15 (1H, br d, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.44 (1H, br s, C_6H_4), 8.28 (2H, d, pyrimidine); EIMS *m*/*z* 567 $(M)^+$; $[\alpha]_D^{25}$ −19 (*c* 1.1, CH_2Cl_2).

6.8.3.2. Compound 39. The title compound was prepared from *tert*-butyl (2*S*)-{2-(morpholin-4-yl-acetyl)amino}-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (42 mg, 68% (two steps)); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.48 (2H, dddd, piperidine), 1.77–1.92 (4H, m, piperidine and tetrahydropyrimidine), 2.39 (4H, m, morpholine), 2.79 (2H, br dd, piperidine), 2.88 (2H, d, $COCH_2N$), 3.24 (4H, br dd, tetrahydropyrimidine), 3.40–3.51 (3H, m, piperidine and $CONHCH_2$), 3.55 (4H, br s, morpholine), 3.68 (2H, br dd, piperidine), 4.03 (1H, dd, $CONHCH_2CH$), 7.01 (1H, dd, C_6H_4), 7.13 (1H, d, C_6H_4), 7.25 (1H, dd, C_6H_4), 7.32 (1H, br s, C_6H_4); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{37}N_7O_5$: 516.2934. Found: 516.2929; $[\alpha]_D^{25}$ +2.2 (*c* 0.75, MeOH).

6.8.4. Preparation of compound 40

6.8.4.1. *tert*-Butyl (2*S*)-{(4-methoxybenzenesulfonyl)amino}-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}-benzoylamino]propionate. DMF (3.0 ml) was added to *tert*-butyl (2*S*)-amino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (61 mg, 0.14 mmol) to prepare a solution. *N*,*N*-Diisopropylethylamine (48 μl, 0.28 mmol) and 4-methoxybenzenesulfonyl chloride (29 mg, 0.14 mmol) were added in that order to the solution at room temperature, and a reaction was allowed to proceed for 1 h. Piperazine was added to stop the reaction, and an aqueous $NaHCO_3$ solution (20 ml) and water (30 ml) were added thereto. The mixture was extracted twice with ethyl acetate (50 ml). The combined organic layers were washed twice with water (50 ml) and once with saturated brine (50 ml), were dried over anhydrous $MgSO_4$, and were concentrated under reduced pressure to prepare the title compound (79 mg, 94%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.32 (9H, s, *t*-Bu), 1.60–1.72 (2H, m, piperidine), 2.19 (2H, br d, piperidine), 3.00 (2H, br dd, piperidine), 3.51–3.61 (1H, m, $CONHCH_2$), 3.69–3.79 (2H, m, piperidine), 3.85 (3H, s, OMe), 3.86–3.96 (2H, m, $CONHCH_2CH$), 3.97–4.08 (1H, m, piperidine), 6.53 (1H, t, pyrimidine), 6.95 (2H, d, C_6H_4OMe), 7.07 (1H, dd, C_6H_4), 7.17 (1H, d, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.43 (1H, dd, C_6H_4), 7.78 (2H, d, C_6H_4OMe), 8.28 (2H, d, pyrimidine); TSPMS *m*/*z* 611 $(M+H)^+$; $[\alpha]_D^{25}$ +40 (*c* 1.6, CH_2Cl_2).

6.8.4.2. Compound 40. The title compound was prepared from *tert*-butyl (2*S*)-{(4-methoxybenzenesulfonyl)amino}-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (8.7 mg, 17% (two steps)); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.58–1.70 (2H, m, piperidine), 1.96 (2H, dddd, tetrahydropyrimidine), 2.01 (2H, br d, piperidine), 2.92 (2H, ddd, piperidine), 3.36 (4H, br t, tetrahydropyrimidine), 3.44–3.52 (1H, m, piperidine), 3.54 (1H, dd, $CONHCH_2$), 3.65–3.76

(2H, m, $CONHCH_2CH$), 3.76 (2H, br d, piperidine), 3.82 (3H, s, OMe), 6.95–7.00 (2H, m, C_6H_4OMe), 7.13 (1H, ddd, C_6H_4), 7.24 (1H, ddd, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.43 (1H, dd, C_6H_4), 7.76–7.80 (2H, m, C_6H_4OMe); FAB-HRMS $(M+H)^+$ calcd for $C_{26}H_{34}N_6O_6S$: 559.2339. Found: 559.2343; $[\alpha]_D^{25}$ +71 (*c* 0.30, MeOH).

6.8.5. Preparation of compound 41

6.8.5.1. Compound 41. 1,2-Dichloroethane (7.0 ml) was added to *tert*-butyl (2*S*)-{(4-methoxybenzenesulfonyl)amino}-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate (44 mg, 0.072 mmol) to prepare a solution, and a 1.0 M boron tribromide methylene chloride solution (0.40 ml) was added to the solution. A reaction was allowed to proceed at 40 °C for 3.5 h, and 1,4-dioxane (3.0 ml), water (1.0 ml), and TEA (1.0 ml) were then added thereto. The mixture was concentrated under reduced pressure. 1,4-Dioxane (20 ml) was added to the residue, followed by filtration. The filtrate was then concentrated under reduced pressure to give (2*S*)-{(4-hydroxybenzenesulfonyl)amino}-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid.

1,4-Dioxane (20 ml) and water (10 ml) were added to (2*S*)-{(4-hydroxybenzenesulfonyl)amino}-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid to prepare a solution, 10% Pd/C (24 mg) was added to the solution, and a reaction was allowed to proceed in a hydrogen atmosphere at room temperature for 6 h. The insolubles were filtered and were washed with a solvent (60 ml) having the same composition as the mixed solvent used in the reaction. The filtrate and the washings were combined followed by concentration under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOH/H_2O/concd NH_4OH, 8:8:1:1) and was then purified by Sephadex LH-20 (MeOH) to prepare compound **41** (11 mg, 26% (two steps)); ^1H NMR (400 MHz, CD_3OD) δ: 1.58–1.70 (2H, m, piperidine), 1.96 (2H, dddd, tetrahydropyrimidine), 2.01 (2H, br d, piperidine), 2.87–2.98 (2H, m, piperidine), 3.36 (4H, br t, tetrahydropyrimidine), 3.43–3.53 (1H, m, piperidine), 3.53 (1H, dd, $CONHCH_2$), 3.66–3.74 (2H, m, $CONHCH_2CH$), 3.76 (2H, br d, piperidine), 6.81–6.85 (2H, m, C_6H_4OH), 7.13 (1H, ddd, C_6H_4), 7.25 (1H, ddd, C_6H_4), 7.31 (1H, dd, C_6H_4), 7.44 (1H, dd, C_6H_4), 7.66–7.71 (2H, m, C_6H_4OH); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{32}N_6O_6S$: 545.2182. Found: 545.2187; $[\alpha]_D^{25}$ +76 (*c* 0.28, MeOH).

6.8.6. Preparation of compound 42

6.8.6.1. Compound 42. The title compound was prepared from *tert*-butyl (2*S*)-amino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **40**. Yield: (31 mg, 42% (three steps)); ^1H NMR (400 MHz, CD_3OD) δ: 1.64 (2H, ddd, piperidine), 1.96 (2H, dddd, tetrahydropyrimidine), 2.01 (2H, br d, piperidine), 2.23 (3H, s, Me), 2.64 (6H, s, Me), 2.91 (2H, br dd, piperidine), 3.36 (4H, br t, tetrahydropyrimidine), 3.44–3.52 (1H, m, piperidine), 3.54 (1H, dd, $CONHCH_2$), 3.63 (1H, dd, $CONHCH_2$), 3.69 (1H, dd, $CONHCH_2CH$), 3.76 (2H, br d, piperidine), 6.94 (2H, s, C_6H_2), 7.12 (1H, ddd, C_6H_4), 7.23 (1H, ddd, C_6H_4), 7.30 (1H, dd, C_6H_4), 7.43 (1H, dd, C_6H_4); FAB-HRMS $(M+H)^+$ calcd for $C_{28}H_{38}N_6O_5S$: 571.2703. Found: 571.2702; $[\alpha]_D^{25}$ +75 (*c* 0.32, MeOH).

6.9. Preparation of the compounds displayed in Table 6

6.9.1. Preparation of compound 43 (method B)

6.9.1.1. 3-Bromo-2-fluorobenzoic acid (*Tetrahedron Lett.* 1995, *36*, 881). THF (60 ml) was added to diisopropylamine (9.6 ml, 68 mmol) in an argon atmosphere. *n*-Butyllithium (hexane solution, 1.5 M, 38 ml, 57 mmol) was added dropwise thereto at −10 °C, and the mixture was stirred for 1 h. Separately, THF (55 ml) was added to 1-bromo-2-fluorobenzene (10 g, 57 mmol) to prepare a solution, which was then added dropwise to the lithium reagent solution at −78 °C. The mixture was stirred for 2 h and was then stirred for additional 30 min while blowing carbon dioxide thereinto. The temperature of the reaction mixture was returned to room temperature, and the reaction mixture was concentrated under reduced pressure. Water (200 ml) was added to the residue to prepare a solution, and the solution was washed twice with diethyl ether (100 ml). The aqueous layer was adjusted to pH 1 by the addition of 1 N hydrochloric acid, was extracted twice with CH_2Cl_2 (300 ml), was dried over anhydrous $MgSO_4$, and was concentrated under reduced pressure to prepare the title compound (7.1 g, 57%); ^1H NMR (400 MHz, $CDCl_3$) δ: 7.14 (1H, ddd, C_6H_3), 7.81 (1H, ddd, C_6H_3), 7.98 (1H, ddd, C_6H_3); EIMS *m*/*z* 218, 220 $(M)^+$.

6.9.1.2. Ethyl 3-bromo-2-fluorobenzoate. EtOH (30 ml) was added to 3-bromo-2-fluorobenzoic acid (3.0 g, 14 mmol) to prepare a solution. Concentrated sulfuric acid (0.30 ml) was added to the solution, and the mixture was heated under reflux for 8 h. The temperature of the reaction mixture was returned to room temperature, the reaction mixture was then slowly poured into an aqueous $NaHCO_3$ solution (500 ml), and the mixture was extracted twice with ethyl acetate (500 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 4:1) to prepare the title compound (2.7 g, 79%); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.40 (3H, t, Et), 4.41 (2H, q, Et), 7.10 (1H, ddd, C_6H_3), 7.73 (1H, ddd, C_6H_3), 7.87 (1H, ddd, C_6H_3); EIMS *m*/*z* 246, 248 $(M)^+$.

6.9.1.3. Ethyl 2-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate. Toluene (30 ml) was added to 4-(*tert*-butyldimethylsilyloxy)piperidine (4.2 g, 20 mmol) and ethyl 3-bromo-2-fluorobenzoate (3.6 g, 15 mmol) in an argon atmosphere to prepare a solution. Tri(*tert*-butyl)phosphine (350 mg, 1.7 mmol), sodium *tert*-butoxide (2.1 g, 22 mmol), and palladium acetate (340 mg, 1.5 mmol) were added in that order to the solution, and the mixture was stirred at 80 °C for 19 h. The temperature of the reaction mixture was returned to room temperature, the insolubles were then filtered, and water (400 ml) was added thereto. The mixture was extracted twice with

ethyl acetate (200 ml), and the extract was then dried over anhydrous $MgSO_4$ and was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 10:1) and was then purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 1:2) to prepare the title compound (82 mg, 2.1%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.76 (2H, dddd, piperidine), 2.00–2.09 (2H, m, piperidine), 2.87 (2H, ddd, piperidine), 3.35 (2H, m, piperidine), 3.87 (1H, dtt, piperidine), 4.38 (2H, q, Et), 7.08 (1H, dd, C_6H_3), 7.13 (1H, ddd, C_6H_3), 7.47 (1H, ddd, C_6H_3); EIMS *m*/*z* 267 $(M)^+$.

6.9.1.4. Ethyl 3-(4-aminopiperidin-1-yl)-2-fluorobenzoate. The title compound was prepared from ethyl 2-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate by the same procedure as employed for compound **28**. Yield: (85 mg, 85% (three steps)); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.50–1.63 (2H, m, piperidine), 1.90–1.98 (2H, m, piperidine), 2.75 (2H, ddd, piperidine), 2.81 (1H, tt, piperidine), 3.41 (2H, br d, piperidine), 4.38 (2H, q, Et), 7.07 (1H, dd, C_6H_3), 7.10 (1H, ddd, C_6H_3), 7.46 (1H, ddd, C_6H_3); EIMS *m*/*z* 266 $(M)^+$.

6.9.1.5. Ethyl 2-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate. The title compound was prepared from ethyl 3-(4-aminopiperidin-1-yl)-2-fluorobenzoate by the same procedure as employed for compound **29**. Yield: (39 mg, 38%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, Et), 1.74 (2H, dddd, piperidine), 2.15–2.23 (2H, m, piperidine), 2.91 (2H, ddd, piperidine), 3.49–3.47 (2H, m, piperidine), 3.95–4.06 (1H, m, piperidine), 4.39 (2H, q, Et), 6.54 (1H, t, pyrimidine), 7.09 (1H, dd, C_6H_3), 7.14 (1H, ddd, C_6H_3), 7.48 (1H, ddd, C_6H_3), 8.29 (2H, d, pyrimidine); EIMS *m*/*z* 344 $(M)^+$.

6.9.1.6. 2-Fluoro-3-{4-pyrimidin-2-ylamino}piperidin-1-yl}benzoic acid. The title compound was prepared from ethyl 2-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate by the same procedure as employed for compound **26** (method B). Yield: (18 mg, 53%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.67 (2H, m, piperidine), 1.93–2.01 (2H, m, piperidine), 2.74–2.82 (2H, m, piperidine), 3.24–3.41 (2H, m, piperidine), 3.82–3.90 (1H, m, piperidine), 6.55 (1H, t, pyrimidine), 7.16 (1H, dd, C_6H_3), 7.26 (1H, ddd, C_6H_3), 7.37 (1H, ddd, C_6H_3), 8.27 (2H, d, pyrimidine); TSPMS *m*/*z* 317 $(M+H)^+$.

6.9.1.7. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[2-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. The title compound was prepared from 2-fluoro-3-{4-pyrimidin-2-ylamino}piperidin-1-yl}benzoic acid by the same procedure as employed for compound **30**. Yield: (34 mg, 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (9H, s, *t*-Bu), 1.76 (2H, dddd, piperidine), 2.22 (2H, br d, piperidine), 2.92 (2H, dddd, piperidine), 3.36–3.44 (2H, m, piperidine), 3.80–3.97 (2H, t, CONHCH$_2$), 3.97–4.07 (2H, m, piperidine and CONHCH$_2$CH), 6.55 (1H, t, pyrimidine), 7.09–7.16 (2H, m, C_6H_3), 7.44–7.50 (2H, m, C_6H_5), 7.51–7.58 (2H, m, C_6H_3 and C_6H_5), 7.83–7.88 (2H, m, C_6H_5), 8.30 (2H, d, pyrimidine); TSPMS *m*/*z* 599 $(M+H)^+$.

6.9.1.8. Compound 43. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[2-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl} benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (16 mg, 78%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.76 (2H, br ddd, piperidine), 1.96 (2H, dddd, tetrahydropyrimidine), 2.02 (2H, br d, piperidine), 2.83 (2H, br dd, piperidine), 3.33–3.50 (7H, m, piperidine and tetrahydropyrimidine), 3.66 (1H, d, CONHCH$_2$), 3.68 (1H, d, CONHCH$_2$), 3.74 (1H, dd, CONHCH$_2$CH), 7.13–7.20 (2H, m, C_6H_3), 7.34–7.40 (1H, m, C_6H_3), 7.47–7.53 (2H, m, C_6H_5), 7.53–7.59 (1H, m, C_6H_5), 7.85–7.89 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd $C_{25}H_{31}N_6O_5SF$: 547.2139. Found: 547.2148; $[\alpha]_D^{25}$ +54 (*c* 0.27, MeOH).

6.9.2. Preparation of compound 44 (method B)

6.9.2.1. Methyl 4-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate. The title compound was prepared from methyl 3-bromo-4-fluorobenzoate and 4-hydroxypiperidine by the same procedure as employed for compound **27**. Yield: (420 mg, 12%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.77 (2H, m, piperidine), 2.05 (2H, m, piperidine), 2.89 (2H, ddd, piperidine), 3.39 (2H, m, piperidine), 3.87 (1H, m, piperidine), 3.90 (3H, s, Me), 7.06 (1H, dd, C_6H_3), 7.65 (1H, ddd, C_6H_3), 7.67 (1H, dd, C_6H_3); FABMS *m*/*z* 254 $(M+H)^+$.

6.9.2.2. Methyl 3-(4-aminopiperidin-1-yl)-4-fluorobenzoate. The title compound was prepared from methyl 4-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate by the same procedure as employed for compound **28**. Yield: (288 mg, 60% (three steps)); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.57 (2H, m, piperidine), 1.95 (2H, br d, piperidine), 2.78 (2H, br t, piperidine), 2.83 (1H, m, piperidine), 3.45 (2H, br d, piperidine), 3.90 (3H, s, Me), 7.05 (1H, dd, C_6H_3), 7.63 (1H, ddd, C_6H_3), 7.66 (1H, dd, C_6H_3); TSPMS *m*/*z* 253 $(M+H)^+$.

6.9.2.3. Methyl 4-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate. The title compound was prepared from methyl 3-(4-aminopiperidin-1-yl)-4-fluorobenzoate by the same procedure as employed for compound **29**. Yield: (250 mg, 67%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.74 (2H, m, piperidine), 2.21 (2H, m, piperidine), 2.93 (2H, br t, piperidine), 3.47 (2H, br d, piperidine), 3.90 (3H, s, Me), 4.01 (1H, m, piperidine), 6.54 (1H, t, pyrimidine), 7.06 (1H, dd, C_6H_3), 7.65 (1H, ddd, C_6H_3), 7.68 (1H, dd, C_6H_3), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 331 $(M+H)^+$.

6.9.2.4. 4-Fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid. The title compound was prepared methyl 4-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate by the same procedure as employed for compound **26** (method B). Yield: (230 mg, 94%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.67 (2H, br dq, piperidine), 1.98 (2H, br d, piperidine), 2.81 (2H, br t, piperidine), 3.39 (2H, br d, piperidine), 3.88 (1H, m, piperidine), 6.56 (1H, t, pyrimidine), 7.24 (1H, dd, C_6H_3), 7.56 (1H, ddd, C_6H_3), 7.59 (1H, dd, C_6H_3), 8.28 (2H, d, pyrimidine); TSPMS *m*/*z* 317 $(M+H)^+$.

6.9.2.5. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[4-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. The title compound was prepared from 4-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic acid by the same procedure as employed for compound **30**. Yield: (94 mg, 22%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (9H, s, *t*-Bu), 1.71 (2H, m, piperidine), 2.17 (2H, m, piperidine), 2.91 (2H, br t, piperidine), 3.46 (1H, br d, piperidine), 3.59 (1H, ddd, $CONHCH_2$), 3.89 (1H, ddd, $CONHCH_2$), 3.97 (2H, m, $CONHCH_2CH$ and piperidine), 6.53 (1H, t, pyrimidine), 7.03 (1H, dd, C_6H_3), 7.32 (1H, ddd, C_6H_3), 7.49 (3H, m, 2H of C_6H_5 and 1H of C_6H_3), 7.57 (1H, m, C_6H_5), 7.85 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); FABMS *m*/*z* 599 $(M+H)^+$; $[\alpha]_D^{25}$ +50 (*c* 1.0, $CHCl_3$).

6.9.2.6. Compound 44. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[4-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (52 mg, 61%); ^{1}H NMR (400 MHz, 10% concd ND_4OD/CD_3OD) δ: 1.64 (2H, m, piperidine), 1.89 (2H, quintet, tetrahydropyrimidine), 1.97 (2H, br d, piperidine), 2.81 (2H, br t, piperidine), 3.30 (4H, t, tetrahydropyrimidine), 3.38 (2H, br d, piperidine), 3.46 (1H, dd, $CONHCH_2$), 3.67 (1H, dd, $CONHCH_2$), 3.75 (1H, dd, $CONHCH_2CH$), 7.07 (1H, dd, C_6H_3), 7.38 (1H, ddd, C_6H_3), 7.42 (2H, m, Ph), 7.48 (2H, m, C_6H_5 and C_6H_3), 7.80 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5SF$: 547.2139. Found: 547.2148; $[\alpha]_D^{25}$ +63 (*c* 1.0, 10% concd NH_4OH/MeOH).

6.9.3. Preparation of compound 45 (method B)

6.9.3.1. Methyl 5-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate. The title compound was prepared from methyl 3-bromo-5-fluorobenzoate by the same procedure as employed for compound **27**. Yield: (160 mg, 4.6%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.67 (2H, dddd, piperidine), 2.01 (2H, m, piperidine), 3.02 (2H, ddd, piperidine), 3.61 (2H, ddd, piperidine), 3.90 (1H, m, piperidine), 3.90 (3H, s, CO_2Me), 6.77 (1H, ddd, C_6H_3), 7.13 (1H, ddd, C_6H_3), 7.39 (1H, dd, C_6H_3); EIMS *m*/*z* 253 $(M)^+$.

6.9.3.2. Methyl 3-(4-aminopiperidin-1-yl)-5-fluorobenzoate. The title compound was prepared from methyl 5-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate by the same procedure as employed for compound **28**. Yield: (54 mg, 35% (three steps)); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (2H, m, piperidine), 1.93 (2H, m, piperidine), 2.82–2.94 (3H, m, piperidine), 3.71 (2H, m, piperidine), 3.90 (3H, s, CO_2Me), 6.76 (1H, ddd, C_6H_3), 7.12 (1H, ddd, C_6H_3), 7.52 (1H, dd, C_6H_3); EIMS *m*/*z* 252 $(M)^+$.

6.9.3.3. Methyl 5-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate. The title compound was prepared from methyl 3-(4-aminopiperidin-1-yl)-5-fluorobenzoate by the same procedure as employed for compound **29**. Yield: (36 mg, 51%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.63 (2H, m, piperidine), 2.18 (2H, m, piperidine), 3.01 (2H, m, piperidine), 3.72 (2H, m, piperidine), 3.91 (3H, s, CO_2Me), 4.03 (1H, m, piperidine), 6.55 (1H, t, pyrimidine), 6.78 (1H, ddd, C_6H_3), 7.14 (1H, ddd, C_6H_3), 7.40 (1H, m, C_6H_3), 8.29 (2H, d, pyrimidine); EIMS *m*/*z* 330 $(M)^+$.

6.9.3.4. 5-Fluoro-3-{4-(pyrimidin-2-ylamino)-piperidin-1-yl}benzoic acid. The title compound was prepared from methyl 5-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate by the same procedure as employed for compound **26** (method B). Yield: (28 mg, 91%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.55 (2H, br ddd, piperidine), 1.93 (2H, br d, piperidine), 2.91 (2H, br dd, piperidine), 3.79 (2H, br d, piperidine), 3.88–4.00 (1H, m, piperidine), 6.55 (1H, t, pyrimidine), 6.96 (1H, br d, C_6H_3), 7.12 (1H, d, C_6H_3), 7.29 (1H, s, C_6H_3), 8.27 (2H, d, pyrimidine); EIMS *m*/*z* 316 $(M)^+$.

6.9.3.5. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[5-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. The title compound was prepared from 5-fluoro-3-{4-(pyrimidin-2-ylamino)-piperidin-1-yl}benzoic acid by the same procedure as employed for compound **30**. Yield: (52 mg, 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (9H, s, *t*-Bu), 1.60–1.64 (2H, m, piperidine), 2.18 (2H, br d, piperidine), 3.03 (2H, br dd, piperidine), 3.54 (1H, ddd, $CONHCH_2$), 3.75 (2H, br d, piperidine), 3.91 (1H, ddd, $CONHCH_2$), 3.91 (1H, m, $CONHCH_2CH$), 3.98–4.08 (1H, m, piperidine), 6.54 (1H, t, pyrimidine), 6.72 (1H, ddd, C_6H_3), 6.85 (1H, br d, C_6H_3), 7.18 (1H, br dd, C_6H_3), 7.48–7.54 (2H, m, C_6H_5), 7.56–7.61 (1H, m, C_6H_5), 7.84–7.87 (2H, m, C_6H_5), 8.28 (2H, d, pyrimidine); EIMS *m*/*z* 598 $(M)^+$; $[\alpha]_D^{25}$ +31 (*c* 2.2, CH_2Cl_2).

6.9.3.6. Compound 45. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[5-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (27 mg, 61%); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.55–1.67 (2H, m, piperidine), 1.96 (2H, dddd, tetrahydropyrimidine), 1.96–2.03 (2H, m, piperidine), 2.95 (2H, ddd, piperidine), 3.36 (4H, br t, tetrahydropyrimidine), 3.45–3.51 (1H, m, piperidine), 3.52 (1H, dd, $CONHCH_2$), 3.69 (1H, dd, $CONHCH_2$), 3.77 (1H, dd, $CONHCH_2CH$), 3.81 (2H, br d, piperidine), 6.82 (1H, ddd, C_6H_3), 6.91 (1H, ddd, C_6H_3), 7.24 (1H, dd, C_6H_3), 7.46–7.52 (2H, m, C_6H_5), 7.53–7.58 (1H, m, C_6H_5), 7.84–7.88 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5SF$: 547.2139, found 547.2148; $[\alpha]_D^{25}$ +64 (*c* 0.30, MeOH).

6.9.4. Preparation of compound 46 (method B)

6.9.4.1. Methyl 6-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate. The title compound was prepared from methyl 3-bromo-6-fluorobenzoate by the same procedure as employed for compound **27**. Yield: (47 mg, 16%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.66–1.76 (2H, m, piperidine), 1.98–2.08 (2H, m, piperidine), 2.87–2.97 (2H, m, piperidine), 3.45–3.53 (2H, m, piperidine), 3.83–3.91 (1H, m, piperidine), 3.93 (3H, s, CO_2Me), 7.03 (1H, br dd, C_6H_3), 7.08 (1H, br s, C_6H_3), 7.45 (1H, br s, C_6H_3); EIMS *m*/*z* 253 $(M)^+$.

6.9.4.2. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[4-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate. The title compound was prepared from methyl 6-fluoro-3-(4-hydroxypiperidin-1-yl)benzoate by the same procedure as employed for compound **30** from compound **27** via compounds **28** and **29**. Yield: (53 mg, 10% (six steps)); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (9H, s, *t*-Bu), 1.59–1.72 (2H, m, piperidine), 2.12–2.22 (2H, m, piperidine), 2.88–2.97 (2H, m, piperidine), 3.56–3.65 (2H, m, piperidine), 3.70–3.85 (2H, m, CONHCH_2), 3.90–4.00 (1H, m, piperidine), 4.13 (1H, ddd, CONHCH_2CH), 6.54 (1H, t, pyrimidine), 6.98–7.10 (2H, m, C_6H_3), 7.43–7.49 (2H, m, C_6H_5), 7.50–7.55 (1H, m, C_6H_5), 7.57 (1H, dd, C_6H_3), 7.82–7.88 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); EIMS *m*/*z* 598 $(M)^+$; $[\alpha]_D^{25}$ +30 (*c* 1.1, CH_2Cl_2).

6.9.4.3. Compound 46. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[4-fluoro-3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (8.3 mg, 14% (two steps)); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.65 (2H, dddd, piperidine), 1.96 (2H, dddd, tetrahydropyrimidine), 1.96–2.04 (2H, m, piperidine), 2.86 (2H, ddd, piperidine), 3.36 (4H, br t, tetrahydropyrimidine), 3.41 (1H, m, piperidine), 3.60–3.66 (2H, m, piperidine), 3.61 (1H, dd, CONHCH_2), 3.70 (1H, dd, CONHCH_2), 3.75 (1H, dd, CONHCH_2CH), 7.06 (1H, dd, C_6H_3), 7.11 (1H, ddd, C_6H_3), 7.41 (1H, m, C_6H_3), 7.46–7.52 (2H, m, C_6H_5), 7.52–7.58 (1H, m, C_6H_5), 7.84–7.88 (2H, m, C_6H_5); FAB-HRMS $(M+H)^+$ calcd for $C_{25}H_{31}N_6O_5SF$: 547.2139. Found: 547.2148; $[\alpha]_D^{25}$ +63 (*c* 0.20, MeOH).

6.9.5. Preparation of compound 47 (method C)

6.9.5.1. Methyl 3-nitro-5-(trifluoromethyl)benzoate. MeOH (20 ml) was added to 3-nitro-5-(trifluoromethyl)benzoic acid (5.1 g, 22 mmol) to prepare a solution, and concentrated sulfuric acid (2.0 ml) was added to the solution. The mixture was heated under reflux for 1.5 h. The temperature of the reaction mixture was returned to room temperature, and the reaction mixture was then slowly poured into $NaHCO_3$. The insolubles were filtered, water (300 ml) was then added thereto, and the mixture was extracted twice with ethyl acetate (200 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/ethyl acetate, 6:1) to prepare the title compound (5.0 g, 91%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.04 (3H, s, CO_2Me), 8.63 (1H, br s, C_6H_3), 8.68 (1H, br s, C_6H_3), 9.05 (1H, br s, C_6H_3); TSPMS *m*/*z* 249 $(M+H)^+$.

6.9.5.2. Methyl 3-amino-5-(trifluoromethyl)benzoate. MeOH (20 ml) was added to methyl 3-nitro-5-(trifluoromethyl)-benzoate (5.0 g, 20 mmol) to prepare a solution, and 10% Pd/C (3.0 g) was added to the solution. The mixture was stirred in a hydrogen atmosphere at room temperature for 23.5 h. The insolubles were filtered and were concentrated under reduced pressure to prepare the title compound (4.3 g, 100%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.92 (3H, s, CO_2Me), 7.05 (1H, br s, C_6H_3), 7.49 (1H, br s, C_6H_3), 7.65 (1H, br s, C_6H_3); EIMS *m*/*z* 219 $(M)^+$.

6.9.5.3. Methyl 3-(4-hydroxypiperidin-1-yl)-5-(trifluoromethyl)benzoate. The title compound was prepared from methyl 3-amino-5-(trifluoromethyl)benzoate by the same procedure as employed for compound **27** by method C. Yield: (4.3 g, 71% (two steps)); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70 (2H, dddd, piperidine), 1.99–2.07 (2H, m, piperidine), 3.07 (2H, ddd, piperidine), 3.66 (2H, ddd, piperidine), 3.89–3.96 (1H, m, piperidine), 3.94 (3H, s, CO_2Me), 7.28 (1H, br s, C_6H_3), 7.69 (1H, br s, C_6H_3), 7.74 (1H, br s, C_6H_3); TSPMS *m*/*z* 304 $(M+H)^+$.

6.9.5.4. *tert*-Butyl (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}-5-(trifluoromethyl)benzoylamino]propionate. The title compound was prepared from methyl 3-(4-hydroxypiperidin-1-yl)-5-(trifluoromethyl)benzoate by the same procedure as employed for compound **30** from compound **27** via compounds **28** and **29**. Yield: (97 mg, 11% (six steps)); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (9H, s, *t*-Bu), 1.49–1.74 (2H, m, piperidine), 2.20 (2H, br d, piperidine), 3.07 (2H, br t, piperidine), 3.57 (2H, ddd, CONHCH_2), 3.77–3.82 (2H, m, piperidine), 3.88–3.98 (2H, m, CONHCH_2CH), 4.00–4.09 (1H, m, piperidine), 6.55 (1H, t, pyrimidine), 7.22 (1H, br s, C_6H_3), 7.38 (1H, br s, C_6H_3), 7.46–7.52 (2H, m, C_6H_5), 7.54–7.60 (2H, m, C_6H_3 and C_6H_5), 7.83–7.87 (2H, m, C_6H_5), 8.29 (2H, d, pyrimidine); TSPMS *m*/*z* 649 $(M+H)^+$; $[\alpha]_D^{25}$ +45 (*c* 0.63, $CHCl_3$).

6.9.5.5. Compound 47. The title compound was prepared from *tert*-butyl (2*S*)-benzenesulfonylamino-3-[3-{4-(pyrimidin-2-ylamino)piperidin-1-yl}-5-(trifluoromethyl)benzoylamino]propionate by the same procedure as employed for compound **22**. Yield: (67 mg, 73% (two steps)); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.69 (2H, dq, J = 3.6, 10.5, piperidine), 1.96 (2H, quintet, J = 5.8, tetrahydropyrimidine), 2.01 (2H, br d, J = 11.2, piperidine), 2.98 (2H, br t, J = 11.2, piperidine), 3.36 (4H, br t, J = 5.8, tetrahydropyrimidine), 3.45–3.54 (1H, m, piperidine), 3.54 (1H, dd, J = 8.5, 13.2, CONHCH_2), 3.72 (1H, dd, J = 4.6, 13.2, CONHCH_2), 3.80 (1H, dd, J = 4.6, 8.5, CONHCH_2CH), 3.85 (2H, br d, J = 11.2, piperidine), 7.29 (1H, br s, C_6H_3), 7.44–7.50 (3H, br s, C_6H_5 and C_6H_3), 7.50–7.56 (1H, m, C_6H_5), 7.64 (1H, br s, C_6H_3), 7.83–7.87 (2H, m, C_6H_5); ^{13}C NMR (CD_3OD) δ 21.2, 32.4, 39.8, 44.4, 48.9 (d, J_{CF} = 335.2 Hz), 58.7, 115.1 (q, J_{CF} = 46 Hz), 115.6 (q, J_{CF} = 46 Hz), 119.1, 125.6 (d, J_{CF} = 1,080 Hz), 128.2, 130.1, 132.8 (q, J_{CF} = 118.4 Hz), 133.7, 137.8, 141.7, 152.7, 153.8, 169.3, 175.0; FAB-HRMS $(M+H)^+$ calcd for $C_{26}H_{31}N_6O_5SF_3$: 597.2107. Found: 597.2102; $[\alpha]_D^{25}$ +54 (*c* 0.50, MeOH).

6.10. Integrin-binding assays

Compounds were evaluated for their inhibitory activities in $\alpha_v\beta_3$- and $\alpha_{IIb}\beta_3$-ELISA (enzyme-linked immunosorbent assay). $\alpha_v\beta_3$[17] was purified from human placenta, using RGDSPK-Sepharose CL-4B chromatography,

followed by mono Q chromatography (Pharmacia). $\alpha_{IIb}\beta_3$[17] was purified from human platelet by RGDSPK-Sepharose CL-4B. $\alpha_v\beta_3$- and $\alpha_{IIb}\beta_3$-binding assays were performed according to the modified method of Kouns et al.[18] EIA plates (Nunc) were coated with $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ and blocked with bovine serum albumin. In each reaction, the reaction mixture (20 mM Tris–HCl, 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4, 100 μl) including vitronectin (Calbiochem) or fibrinogen, added to the receptor-coated plate, was incubated for 4 h at 25 °C. Thereafter the ligand binding was measured using anti-vitronectin rabbit antibody (Calbiochem) and peroxidase-conjugated anti-rabbit IgG antibody (Capell) for $\alpha_v\beta_3$, or peroxidase-conjugated anti-fibrinogen antibody (Capell) for $\alpha_{IIb}\beta_3$, and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) as the substrate of peroxidase. The IC_{50} values were determined from measurement of absorbance at 415 nm.

6.11. Adhesion of human aorta smooth muscle cells to vitronectin

The adhesion of human aorta smooth muscle cells to vitronectin was measured as described before.[19] Briefly EIA plates (Nunc) were coated with human vitronectin (Calbiochem) and blocked with bovine serum albumin. The cell suspension of human aorta smooth muscle cells (50,000 cells/100 μl, Clonetics) in Dulbecco's modified Eagle's basal medium containing 0.1% bovine serum albumin was added to the vitronectin-coated plates and incubated for 1.5 h at 37 °C in the presence or absence of the test compounds. The adherent cells were stained with toluidine blue and calculated by measuring of absorbance at 405 nm after the cytolysis by SDS. The IC_{50} values were determined graphically from two or more independent experiments.

6.12. Platelet aggregation assay

Platelet aggregation was determined according to the previous method.[18] Human platelet-rich plasma obtained from healthy volunteers was prepared and the aggregation was induced with 5 μM ADP. The IC_{50} values were determined from two independent experiments.

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16. MeCN (200 ml) and EtOH (50 ml) were added to oleandomycin phosphate (25 g, 32 mmol) to prepare a solution. *p*-Toluenesulfonic acid (12 g, 65 mmol) was added to the solution, and the mixture was stirred at room temperature for 3.0 h. An aqueous Na_2CO_3 solution was added to the reaction solution, and the mixture was extracted three times with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4 and was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate) to give ethyl α-L-oleandroside (3.75 g, 61%).
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