Table 1
Systemic hemodynamic parameters throughout the study (Protocol I)

Groups	Baseline	LF-5	D-5	D-10	D-20
Mean blood pressure (mmHg)					
1. Control group	102 ± 4	102 ± 4	102 ± 3	101 ± 3	101 ± 2
2. Famotidine group	99 ± 3	100 ± 2	99 ± 3	99 ± 3	98 ± 2
Heart rate (min^{-1})					
1. Control group	134 ± 5	134 ± 4	132 ± 4	131 ± 3	133 ± 4
2. Famotidine group	134 ± 4	134 ± 4	132 ± 4	131 ± 4	128 ± 4

Values are expressed as mean ± S.E.M. LF-5 = at 5 minutes of low flow, D-5, D-10 and 20 = at 5, 10 and 20 minutes after the onset of either famotidine or a solvent of famotidine (control) infusion, respectively. There were no significant changes of these parameters among the two groups. Statistical significance was tested by ANOVA.

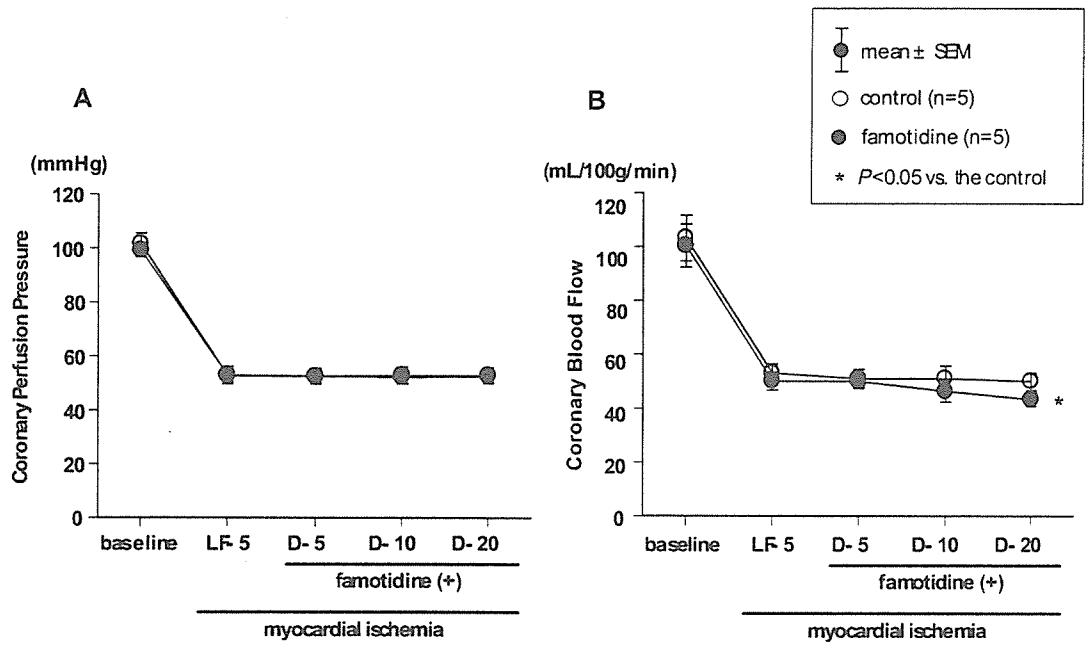

Fig. 1. Effects of famotidine on coronary hemodynamics in ischemic myocardium. A and B show the CPP and CBF, respectively. Abbreviations are the same as in Table 1. Data are mean ± S.E.M. *$P < 0.05$ vs. the control (a solvent of famotidine) group. Statistical analysis was performed by ANOVA, followed by Bonferroni's test.

measures. Results were expressed as the mean ± S.E.M., with $P < 0.05$ being considered significant.

4. Results

4.1. Effects of famotidine on myocardial anaerobic metabolism during coronary hypoperfusion

Before both CBF and CPP were reduced, there were no significant differences in the systemic hemodynamic and metabolic parameters (Table 1; Figs. 1 and 2). The reduction in both CPP and CBF increased the differences in histamine levels between coronary venous and arterial blood (dVA[histamine]) from 0.04 ± 0.03 to 0.28 ± 0.13 ng/ml ($P < 0.05$, $N = 5$), and cardiac NO levels prior to the administration of either famotidine or solvent in the famotidine and control groups (Fig. 3). The administration of a solvent did not affect CPP, FS, LER or $M\dot{V}O_2$. On the other hand, famotidine increased LER and decreased $M\dot{V}O_2$ and CBF and a slight decrease in FS, even in the constant low-CPP state (Figs. 1 and 2), suggesting that myocardial ischemia is improved by famotidine. The increment of cardiac NO levels was reduced by famotidine (Fig. 3).

Fig. 4 shows myocardial Gs protein levels and cAMP levels of the ischemic (the LAD artery-perfused area) and non-ischemic area (the left circumflex artery-perfused area) following 20 min of myocardial ischemia with and without an infusion of famotidine into the LAD. Although myocardial Gs protein levels were not modulated either by either famotidine or ischemia, the cAMP levels of the ischemic myocardium increased compared with those of the non-ischemic myocardium, and famotidine attenuated the ischemia-induced increases in myocardial cAMP levels.

4.2. Effects of either famotidine or cimetidine on infarct size

Nine out of 45 dogs were excluded from analysis because their subendocardial collateral flow was greater than 15 ml/100 g per minute, so 36 dogs completed the protocol satisfactorily. Of these 36 dogs, nine developed VF at least once, with VF that matched the exclusion criteria occurring in six dogs, so these animals were also excluded from analysis. The number of

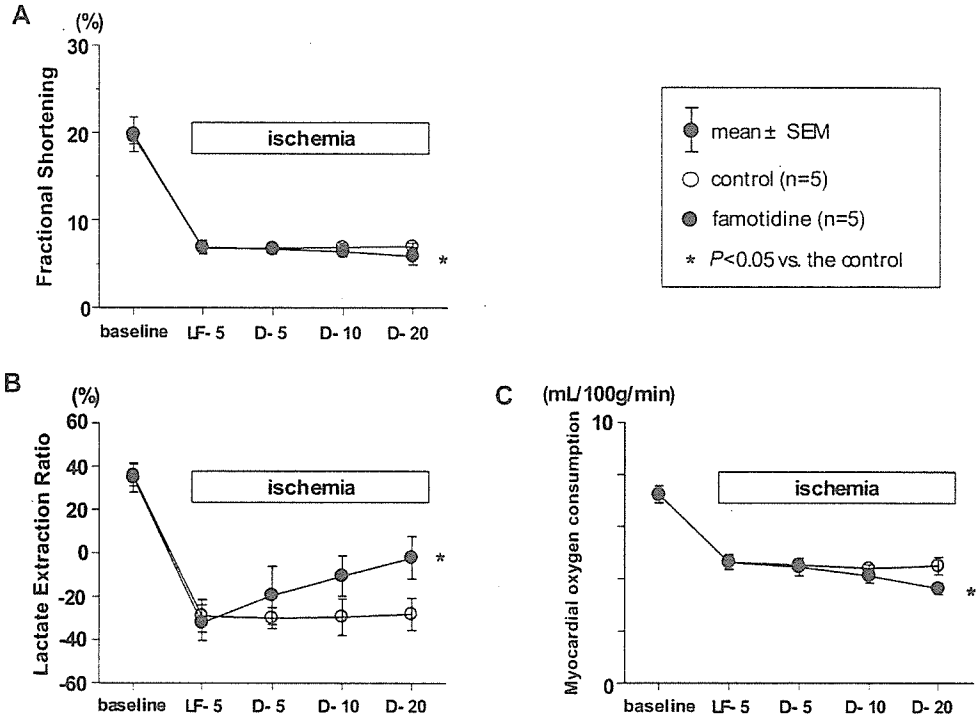

Fig. 2. Changes in fractional shortening (A), lactate extraction ratio (B) and myocardial oxygen consumption (C) in ischemic myocardium. Abbreviations are the same as in Table 1. Data are mean ± S.E.M. *$P < 0.05$ vs. the control (a solvent of famotidine) group. Statistical analysis was performed by ANOVA, followed by Bonferroni's test.

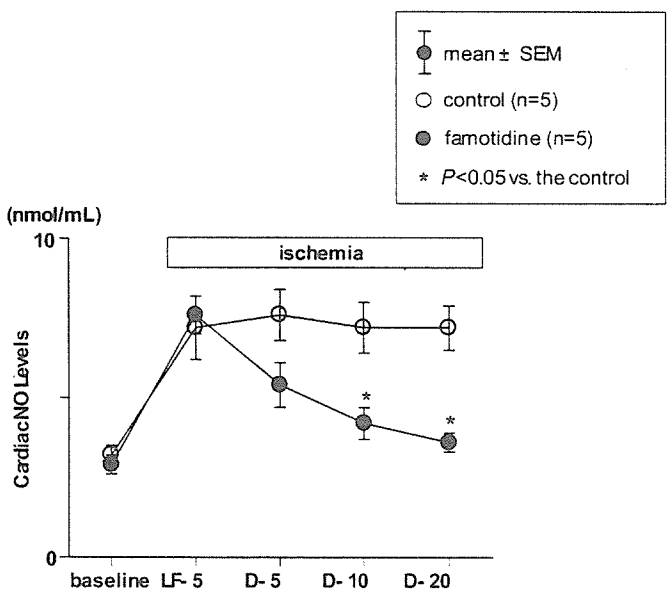

Fig. 3. Changes in cardiac NO levels during famotidine infusion in ischemic heart myocardium. Abbreviations are the same as in Table 1. Data are mean ± S.E.M. * $P < 0.05$ vs. the control (a solvent of famotidine) group. Statistical analysis was performed by ANOVA, followed by Bonferroni's test.

dogs that met the exclusion criteria of VF was 3, 1, 1, 1 and 0 in the control, famotidine, cimetidine, famotidine (Rep) or promethazine groups, respectively.

Neither aortic blood pressure nor heart rate showed any difference among the five groups throughout the protocol (Table 2). Table 3 shows the area at risk and the endocardial collateral blood flow in the LAD region during myocardial ischemia. There were no significant differences in the area at risk and collateral flow among the five groups during myocardial ischemia. Fig. 5 shows that either famotidine or cimetidine decreased infarct size compared with the control groups. Furthermore, famotidine administration during an early reperfusion period also limited infarct size. However, a histamine H_1 blocker, promethazine did not reduce infarct size.

5. Discussion

In the current study, we demonstrated that histamine release is increased from the ischemic myocardium, and the blockade of histamine H_2 receptors improves anaerobic myocardial metabolism in ischemic hearts with reduced myocardial oxygen consumption. We also showed that the blockade of histamine H_2 receptors limits infarct size but the blockade of histamine H_1 receptors does not. These findings demonstrate that the blockade of histamine H_2 receptors is beneficial against ischemia and reperfusion injury in canine hearts.

5.1. Histamine in ischemic hearts

We have shown that histamine release is augmented in the ischemic myocardium compared with the non-ischemic myocardium. Histamine is stored in mast cells, which, when stimulated, release histamine. There are reports that mast cells are found in the human heart [20], and have been implicated in cardiovascular diseases [21,22]. Indeed, an increase in mast cells has been observed in the hearts of hypertrophy [23], di-

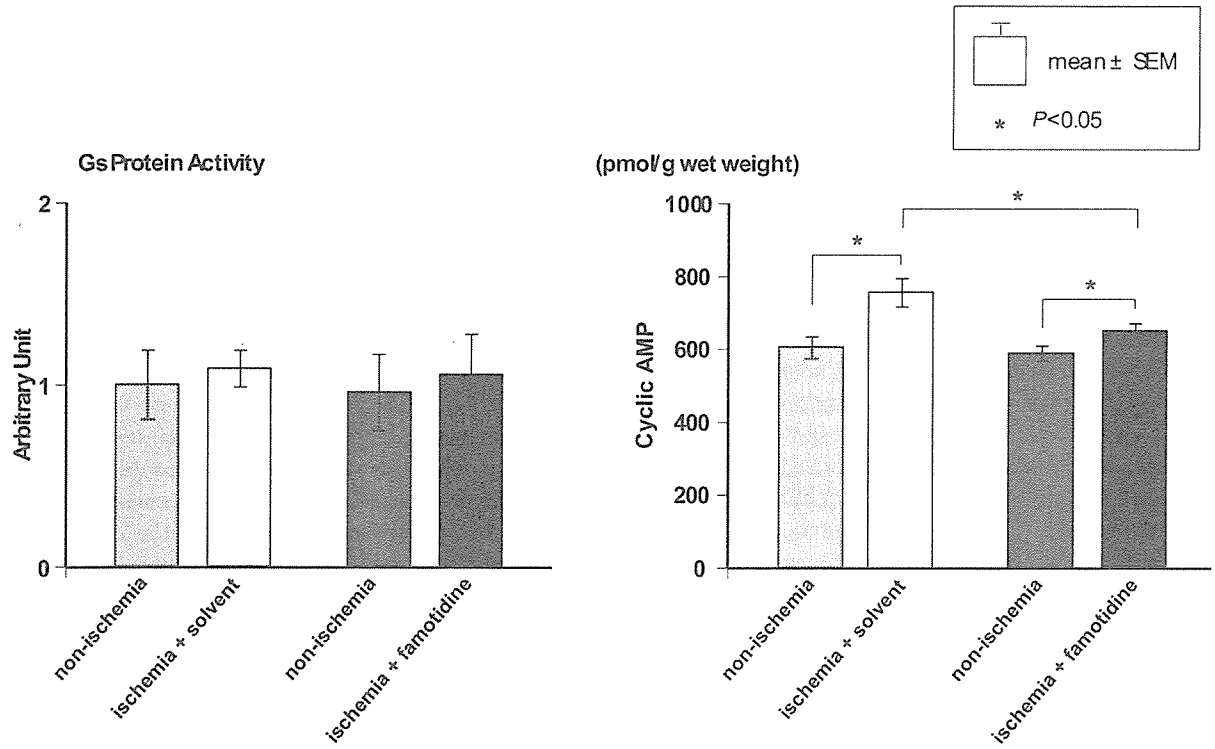

Fig. 4. Myocardial Gs protein levels and cAMP levels of the ischemic (the LAD artery-perfused area) and non-ischemic area (the left circumflex artery-perfused area) following 20 minutes of myocardial ischemia with and without famotidine.
Although myocardial Gs protein levels were not modulated by either famotidine or ischemia, the cAMP levels of the ischemic myocardium increase compared with those of the non-ischemic myocardium, and famotidine attenuated the ischemia-induced increases in myocardial cAMP levels. Statistical analysis was performed by ANOVA, followed by Bonferroni's test.

Table 2
Systemic hemodynamic parameters throughout the study (Protocol II)

Groups	Baseline	D-10	Isc-90	Rep-60	Rep-180	Rep-360
Mean blood pressure (mmHg)						
1. Control group	97 ± 3	102 ± 5	97 ± 4	102 ± 5	102 ± 7	102 ± 7
2. Famotidine group	102 ± 4	98 ± 6	99 ± 6	98 ± 7	100 ± 7	97 ± 7
3. Cimetidine group	100 ± 2	99 ± 2	98 ± 2	102 ± 4	102 ± 5	96 ± 5
4. Famotidine afterreperfusion group	102 ± 6	104 ± 6	100 ± 6	98 ± 4	98 ± 3	100 ± 4
5. Promethazine group	99 ± 4	101 ± 4	98 ± 5	99 ± 6	101 ± 6	98 ± 4
Heart rate (min^{-1})						
1. Control group	135 ± 5	135 ± 5	134 ± 4	131 ± 5	129 ± 6	128 ± 7
2. Famotidine group	135 ± 3	130 ± 2	131 ± 3	127 ± 2	126 ± 4	126 ± 3
3. Cimetidine group	134 ± 3	130 ± 4	129 ± 5	127 ± 8	125 ± 7	124 ± 4
4. Famotidine after reperfusion group	133 ± 5	131 ± 5	130 ± 4	128 ± 4	125 ± 5	125 ± 4
5. Promethazine group	132 ± 5	130 ± 5	131 ± 7	128 ± 6	128 ± 5	126 ± 6

Abbreviations are the same as in Table 1. Values are expressed as mean ± S.E.M. There were no significant changes of these parameters among the five groups. Statistical significance was tested by ANOVA.

Table 3
The area at risk and collateral blood flow during myocardial ischemia in each group

Groups	Risk area (%)	Collateral blood flow during myocardial ischemia (ml/100 g per minute)
1. Control group	39.8 ± 1.7	8.4 ± 1.3
2. Famotidine group	40.8 ± 3.4	7.5 ± 1.6
3. Cimetidine group	39.7 ± 2.1	7.9 ± 1.7
4. Famotidine after reperfusion group	40.5 ± 1.8	8.0 ± 1.6
5. Promethazine group	37.3 ± 2.8	7.5 ± 2.0

Values are expressed as mean ± S.E.M. There were no differences of the area at risk and collateral blood flow among all of the groups. Statistical significance was tested by ANOVA.

lated cardiomyopathy, ischemic cardiomyopathy [24], and ischemia–reperfusion [25], and infarction-related coronary arteries [26]. Furthermore, histamine is present in high concentrations in cardiac tissues in most animal species, including humans [6,27,28], and its release from cardiac stores and subsequent actions on the heart may be of importance in the pathophysiology of heart disease, which agrees with the current observation of enhanced histamine release from the ischemic myocardium. We did not clarify whether histamine is released from mast cells or histaminergic neurons in the hearts or the mechanisms by which mast cells or histaminergic neurons are stimulated; however, we showed that histamine release detected in the coronary vein is increased in response to ischemic stress. Furthermore, we showed that the blockade of histamine receptors in the heart has an impact on the pathophysiology of ischemic heart diseases. These lines of previous and current evidence strongly support the importance of histamine in ischemia and reperfusion injuries in hearts.

5.2. The role of histamine receptors in ischemic hearts

The currently known histamine receptors (H_1, H_2, H_3, and H_4) are all G protein-coupled molecules that transduce extracellular signals via Gq, Gs, and Gi/o, respectively [1,2,29]. Histamine H_2 receptors, in particular, are linked to Gs proteins that facilitate the production of cAMP, as do β-adrenoceptors [30]. Histamine H_2 receptor-stimulated cAMP accumulation or adenylyl cyclase activity has been demonstrated in a variety of tissues, including gastric cells [6,31], vascular smooth muscle cells [32], the brain [6,33], and cardiac tissue [6,34]. Indeed, in the present study, we found that the blockade of histamine H_2 receptors attenuates the ischemia-induced increases in myocardial cAMP accumulation.

β-Adrenoceptor blockers are known to protect ischemic or failing hearts because the accumulation of cAMP following the activation of β-adrenoceptors enhances myocardial contractility and oxygen consumption, and enhancements of myocardial contractility and oxygen consumption deteriorate the cardiac function [35,36]. The importance of β-adrenoceptors or β-adrenoceptor blockers in the pathophysiology of diseased hearts is attributable to the presence of catecholamine and β-adrenoceptors in the heart. Therefore, since both histamine H_2 receptors and histamine are located in ischemic hearts, it is very likely that blockers of histamine H_2 receptors protect against ischemia and reperfusion injury, as do β-adrenoceptor blockers. Indeed, we have shown that the blockade of the histamine H_2 receptors is beneficial in ischemia and reperfusion injuries. Therefore, the same scenario can be considered for cardioprotection attributable to the blockade of histamine H_2 receptors.

Histamine has been reported to cause NO release through histamine H_2 receptors in porcine endothelial cell [37]. Therefore, histamine H_2 blockers may decrease cardiac NO levels and increase coronary vascular resistance. Indeed, we observed that histamine H_2 blockers reduces cardiac NO levels. This may be attributable to the attenuation of histamine-induced NO production, however, may be due to the fact that histamine H_2 blockers attenuate the severity of myocardial ischemia, because the increased severity of myocardial ischemia increases NO production. Intriguingly, despite famotidine-induced decreases in NO release, we found that histamine H_2 blockers are cardioprotective against ischemia and reperfusion injury. This suggests that histamine is cardio-deleterious against ischemia and reperfusion injury via activation of Gs proteins even with enhancements of NO production, and the elimination of the effects of histamine H_2 receptors are protective as a whole.

Levi and coworkers have shown the presence of H_3 receptors in the heart and demonstrated that activation of H_3 receptors can inhibit norepinephrine release from cardiac nerve endings and this inhibition is closely related to the suppression of reperfusion-induced arrhythmia including VF [38,39]. The treatment with H_2 receptor blockers could result in an unhindered stimulation of H_3 receptors because cardiac histamine levels were shown to increase under the ischemic conditions. Then, the possibility that the effectiveness of H_2 receptor blockers on myocardial ischemia and reperfusion injury may be, at least in part, due to the activation of H_3 receptors. The stimulation of H_1 receptors with histamine is known to impair atrioventricular (AV) conduction [40,41]. This AV conduction slowing may contribute to the development of VF in myocardial ischemia and reperfusion injury. Thus, we tested whether H_1 receptor blocker, promethazine, shows some beneficial effects on myocardial ischemia and reperfusion injury in our model. Promethazine decreased VF because there were no dog that met the exclusion criteria of VF, but did not decrease infarct size (Fig. 5). In addition, it has been reported that histamine is a powerful vasoconstrictor in atherosclerotic coronary

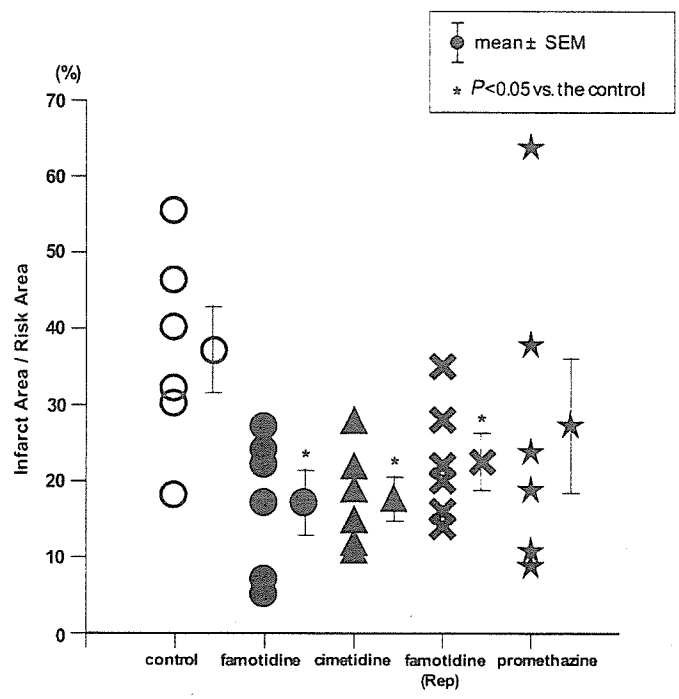

Fig. 5. Infarct size as a percentage of the area at risk. Infarct size was decreased in both the famotidine and cimetidine group compared with the control group. * $P < 0.05$ vs. the control (a solvent of famotidine) group. Statistical analysis was performed by ANOVA, followed by Bonferroni's test.

arteries [42], which may locally provoke coronary spasm and thus contribute to the onset of myocardial infarction [24].

5.3. Physiological and clinical relevance and the limitations of the present study

Since β-adrenoceptor blockers have been shown to be effective for the treatment of ischemic heart diseases and heart failure [43], and histamine receptor blockers are similar to β-adrenoceptor blockers, histamine plays an important role in the regulation and mal-regulation of cardiac and coronary functions. Furthermore, the histamine receptor blockers, such as famotidine and cimetidine, which are used to treat peptic ulcers all over the world, can be applied in ischemic heart diseases. Furthermore, β-adrenoceptor blockers ameliorate heart failure, especially ischemic heart failure, and the histamine receptor blockers may be beneficial for patients with CHF. This previous work hinted the present study. Therefore, we examined the effects of the histamine H_2 blocker on $M\dot{V}O_2$ and CBF in ischemic hearts in protocol I and the effects of the histamine H_2 blockers on infarct size following 90 min of ischemia in protocol II in the same condition, and found that the histamine H_2 blocker is cardioprotective against ischemia stress or reperfusion injury.

Fig. 2A and 2C show that both FS and $M\dot{V}O_2$ decreased after the administration of famotidine in ischemic conditions. However, it should be considered that the increases of both FS and $M\dot{V}O_2$ as index of the improvement of myocardial ischemia. The present results seem to be contradictory, but they are not. There are two strategies to reduce the ischemic burden of myocardium. One is to increase CBF, and another is to decrease myocardial workload. Ischemia per se decreases $M\dot{V}O_2$. First of all, when CBF is increased, $M\dot{V}O_2$ and thus FS are increased along with the reduction of reversible or irreversible ischemic injury such as myocardial stunning or necrosis. Secondly, when myocardial workload is attenuated, $M\dot{V}O_2$ is further attenuated and FS is decreased along with the reduction of reversible or irreversible ischemic injury. Our previous work [11] that showed the increases in both CBF and $M\dot{V}O_2$ are attributable to the former mechanism for the attenuation of ischemic injury, and the present results that showed the decreased CBF and $M\dot{V}O_2$ are attributable to the latter mechanisms. In both cases, if the severity of ischemia deceases, LER, an index of anaerobic myocardial metabolism and severity of myocardial ischemia, is to be increased. The increases in LER were shown in the previous [11] and present studies, which show the improvements of myocardial ischemia via different mechanisms, i.e. the increased CBF- or decreased $M\dot{V}O_2$-dependent mechanism.

On the other hand, Shen et al. [44] have shown that sodium pentobarbital severely inhibits myocardial mitochondrial function and greatly affects $M\dot{V}O_2$. Thus, there may be some differences in myocardial energy metabolism including $M\dot{V}O_2$ and glucose, free fatty acid and lactate metabolism between awake and anesthetized conditions by pentobarbital.

Despite these limitations, if this hypothesis in the present work is validated further, histamine-related drugs or substances may become candidates for the treatment of ischemic heart diseases or CHF.

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References

[1] Leurs R, Bakker RA, Timmerman H, de Esch IJ. The histamine H3 receptor: from gene cloning to H3 receptor drugs. Nat Rev Drug Discov 2005;4:107–20.
[2] Hough LB. Genomics meets histamine receptors: new subtypes, new receptors. Mol Pharmacol 2001;59:415–9.
[3] Gantz I, Schaffer M, DelValle J, Logsdon C, Campbell V, Uhler M, et al. Molecular cloning of a gene encoding the histamine H2 receptor. Proc Natl Acad Sci USA 1991;88:429–33.
[4] Kim J, Washio T, Yamagishi M, Yasumura Y, Nakatani S, Hashimura K, et al. A novel data mining approach to the identification of effective drugs or combinations for targeted endpoints—application to chronic heart failure as a new form of evidence-based medicine. Cardiovasc Drugs Ther 2004;18:483–9.
[5] Matsuda N, Jesmin S, Takahashi Y, Hatta E, Kobayashi M, Matsuyama K, et al. Histamine H1 and H2 receptor gene and protein levels are differentially expressed in the hearts of rodents and humans. J Pharmacol Exp Ther 2004;309:786–95.
[6] Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W, Levi R, Haas HL. International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev 1997;49:253–78.
[7] Eckel L, Gristwood RW, Nawrath H, Owen DA, Satter P. Inotropic and electrophysiological effects of histamine on human ventricular heart muscle. J Physiol 1982;330:111–23.
[8] Du XY, Schoemaker RG, X462P6, Bos E, Saxena PR. Effects of histamine on porcine isolated myocardium: differentiation from effects on human tissue. J Cardiovasc Pharmacol 1993;22:468–73.
[9] Hattori Y. Cardiac histamine receptors: their pharmacological consequences and signal transduction pathways. Methods Find Exp Clin Pharmacol 1999;21:123–31.
[10] Kirch W, Halabi A, Hinrichsen H. Hemodynamic effects of quinidine and famotidine in patients with congestive heart failure. Clin Pharmacol Ther 1992;51:325–33.
[11] Kitakaze M, Minamino T, Node K, et al. Beneficial effects of inhibition of angiotensin-converting enzyme on ischemic myocardium during coronary hypoperfusion in dogs. Circulation 1995;92:950–61.
[12] Yamatodani A, Fukuda H, Wada H. High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. J Chromatogr 1985;344:115–23.
[13] Bergmeyer HU. In: Methods of Enzymatic Analysis. 1st ed. New York: NY: Academic Press Inc; 1963. p. 266–70 (1994; 93: 2197-205).
[14] Green LC, Wagner DA, Glogowski J, et al. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. Anal Biochem 1982;126:131–8.
[15] Node K, Kitakaze M, Kosaka H, et al. Plasma nitric oxide end products are increased in the ischemic canine heart. Biochem Biophys Res Commun 1995;211:370–4.
[16] Hrbasova M, Novotny J, Hejnova L, Kolar F, Neckar J, Svoboda P. Altered myocardial Gs protein and adenylyl cyclase signaling in rats ex-

posed to chronic hypoxia and normoxic recovery. J Appl Physiol 2003; 94:2423–32.
[17] Mori H, Haruyama S, Shinozaki Y, Okino H, Iida A, Takanashi R, et al. New nonradioactive microspheres and more sensitive X-ray fluorescence to measure regional blood flow. Am J Physiol 1992;263:H1946–H1957.
[18] Snedecor GW, Cochran WG. In: Statistical Methods. Iowa: Iowa State University Press; 1972. p. 258–98.
[19] Steel RGD, Torrie JH. In: Principles and Procedures of Statistics. A Biomedical Approach. New York, NY: McGraw-Hill Publishing Co; 1980. p. 137–238.
[20] Dvorak AM. Mast-cell degranulation in human hearts. N Engl J Med 1986;315:969–70.
[21] Marone G, de Crescenzo G, Adt M, Patella V, Arbustini E, Genovese A. Immunological characterization and functional importance of human heart mast cells. Immunopharmacology 1995;31:1–18.
[22] Hara M, Ono K, Hwang MW, Iwasaki A, Okada M, Nakatani K, et al. Evidence for a role of mast cells in the evolution to congestive heart failure. J Exp Med 2002;195:375–81.
[23] Panizo A, Mindan FJ, Galindo MF, Cenarruzabeitia E, Hernandez M, Diez J. Are mast cells involved in hypertensive heart disease? J Hypertens 1995;13:1201–8.
[24] Patella V, Marino I, Arbustini E, Lamparter-Schummert B, Verga L, Adt M, et al. Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy. Circulation 1998;97:971–8.
[25] Frangogiannis NG, Perrard JL, Mendoza LH, Burns AR, Lindsey ML, Ballantyne CM, et al. Stem cell factor induction is associated with mast cell accumulation after canine myocardial ischemia and reperfusion. Circulation 1998;98:687–98.
[26] Laine P, Kaartinen M, Penttila A, Panula P, Paavonen T, Kovanen PT. Association between myocardial infarction and the mast cells in the adventitia of the infarct-related coronary artery. Circulation 1999;99:361–9.
[27] Wolff AA, Levi R. Histamine and cardiac arrhythmias. Circ Res 1986; 58:1–16.
[28] Bristow MR, Ginsburg R, Harrison DC. Histamine and the human heart: the other receptor system. Am J Cardiol 1982;49:249–51.
[29] Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. J Pharmacol Exp Ther 2003;305:1212–21.
[30] Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? Circ Res 2003;93:896–906.
[31] Gespach C, Bouhours D, Bouhours JF, Rosselin G. Histamine interaction on surface recognition sites of H2-type in parietal and non-parietal cells isolated from the guinea pig stomach. FEBS Lett 1982;149:85–90.
[32] Ottosson A, Jansen I, Edvinsson L. Pharmacological characterization of histamine receptors in the human temporal artery. Br J Clin Pharmacol 1989;27:139–45.
[33] Al-Gadi M, Hill SJ. The role of calcium in the cyclic AMP response to histamine in rabbit cerebral cortical slices. Br J Pharmacol 1987;91:213–22.
[34] Johnson CL, Weinstein H, Green JP. Studies on histamine H2 receptors coupled to cardiac adenylate cyclase. Blockade by H2 and H1 receptor antagonists. Mol Pharmacol 1979;16:417–28.
[35] Packer M, Coats AJ, Fowler MB, Katus HA, Krum H, Mohacsi P, et al. Effect of carvedilol on survival in severe chronic heart failure. N Engl J Med 2001;344:1651–8.
[36] Dargie HJ. Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. Lancet 2001;357:1385–90.
[37] Kishi F, Nakaya Y, Ito S. Histamine H2-receptor-mediated nitric oxide release from porcine endothelial cells. J Cardiovasc Pharmacol 1998;32: 177–82.
[38] Imamura M, Poli E, Omoniyi AT, Levi R. Unmasking of activated histamine H3-receptors in myocardial ischemia: their role as regulators of exocytotic norepinephrine release. J Pharmacol Exp Ther 1994;271: 1259–66.
[39] Hatta E, Yasuda K, Levi R. Activation of histamine H3 receptors inhibits carrier-mediated norepinephrine release in a human model of protracted myocardial ischemia. J Pharmacol Exp Ther 1997;283:494–500.
[40] Levi R, Kuye JO. Pharmacological characterization of cardiac histamine receptors: sensitivity to H1-receptor antagonists. Eur J Pharmacol 1974; 27:330–8.
[41] Hageman GR, Urthaler F, Isobe JH, James TN. Chronotropic and dromotropic effects of histamine on the canine heart. Chest 1979;75:597–604.
[42] Ginsburg R, Bristow MR, Davis K, Dibiase A, Billingham ME. Quantitative pharmacologic responses of normal and atherosclerotic isolated human epicardial coronary arteries. Circulation 1984;69:430–40.
[43] Metra M, Nardi M, Giubbini R, Dei Cas L. Effects of short- and long-term carvedilol administration on rest and exercise hemodynamic variables, exercise capacity and clinical conditions in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 1994;24:1678–87.
[44] Shen W, Xu X, Ochoa M, Zhao G, Wolin MS, Hintze TH. Role of nitric oxide in the regulation of oxygen consumption in conscious dogs. Circ Res 1994;75:1086–95.

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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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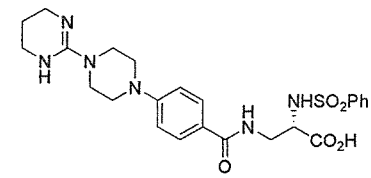
Abstract—To establish the in vivo efficacy of $\alpha_v\beta_3/\alpha_{IIb}\beta_3$ dual antagonists possessing a tricyclic pharmacophore, a corresponding $\alpha_v\beta_3$-selective antagonist was required as a control. We initially took two synthetic approaches to obtain $\alpha_v\beta_3$-selective 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

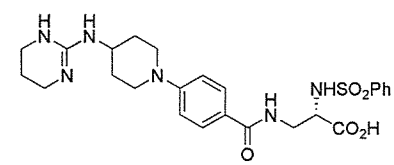
The vitronectin receptor, integrin $\alpha_v\beta_3$,[1,2] is involved in the pathogenesis of various diseases in which cell adhesion or migration plays a key role. The function of integrin $\alpha_v\beta_3$ in vascular smooth muscle cells and leukocytes led us to hypothesize that a potent $\alpha_v\beta_3$ 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.

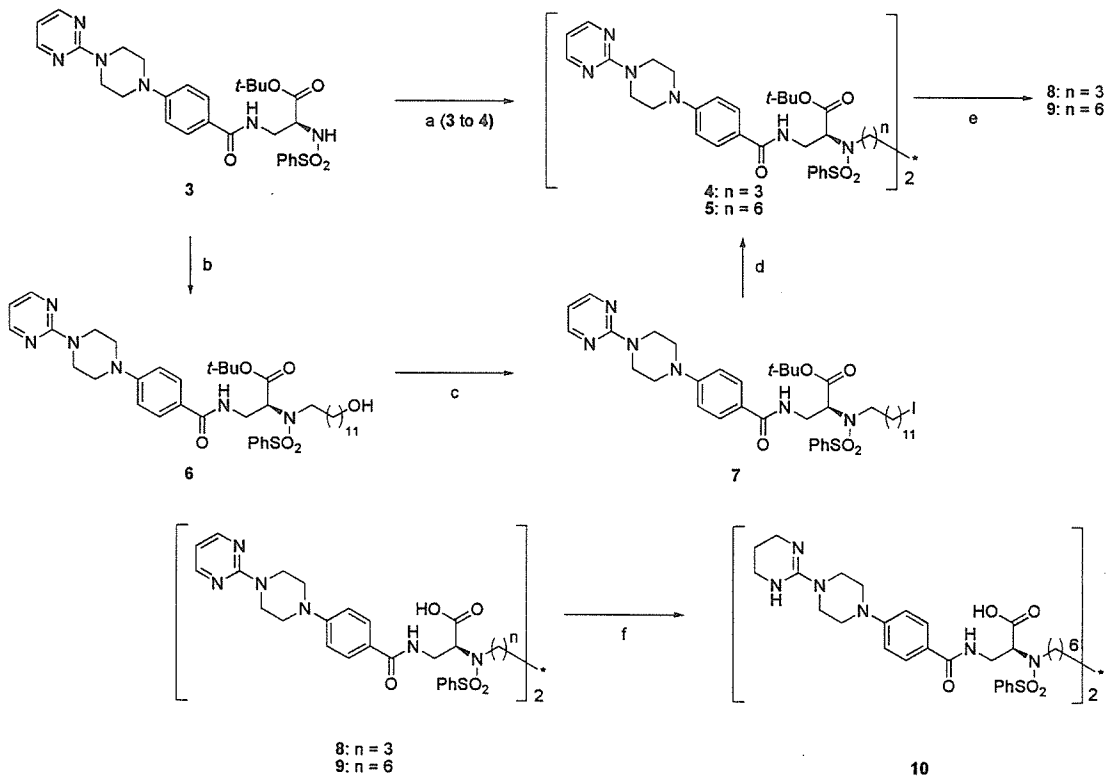
Although the $\alpha_{IIb}\beta_3$-antagonistic activity of some of these compounds was suppressed, none of them showed even weak $\alpha_v\beta_3$ selectivity (Table 1).

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.

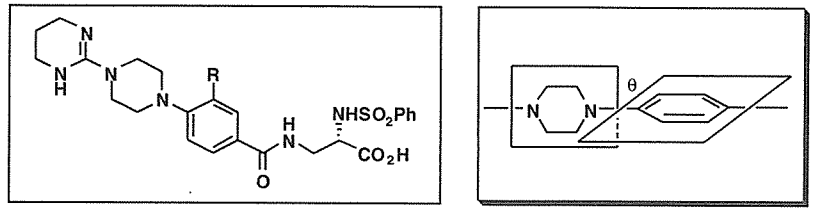
Table 1. Inhibitory activity of dimeric molecules in receptor-binding assay

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.

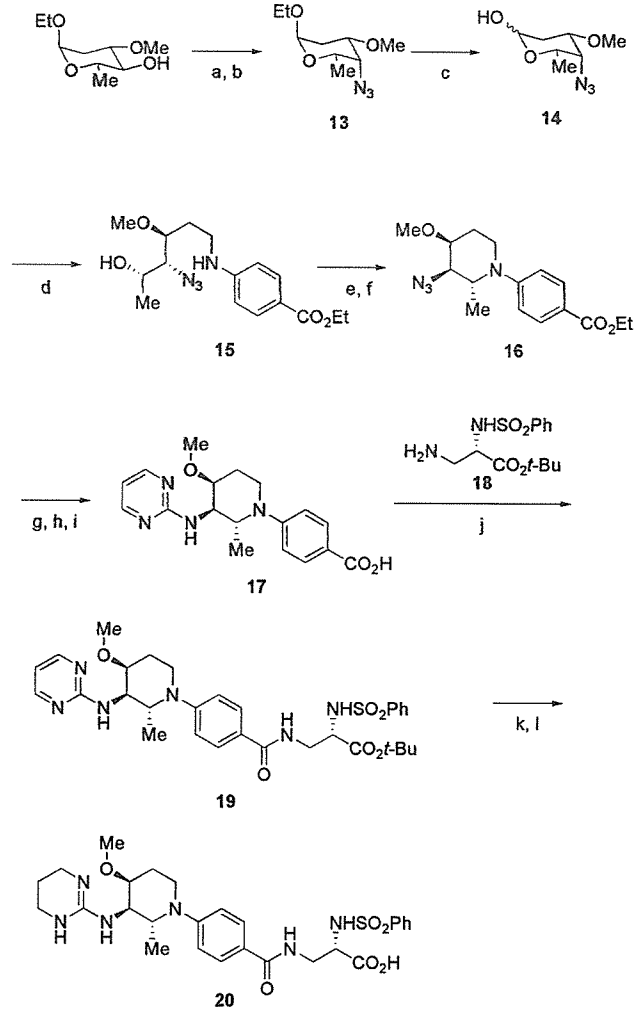
Then, we introduced a methyl group at the C-2 position of the hetero ring by utilizing a natural product (Scheme 2).

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.

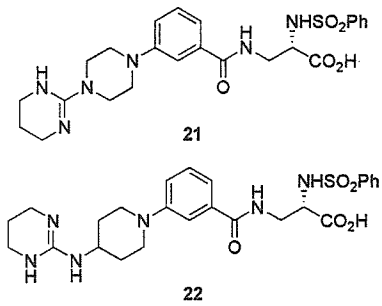
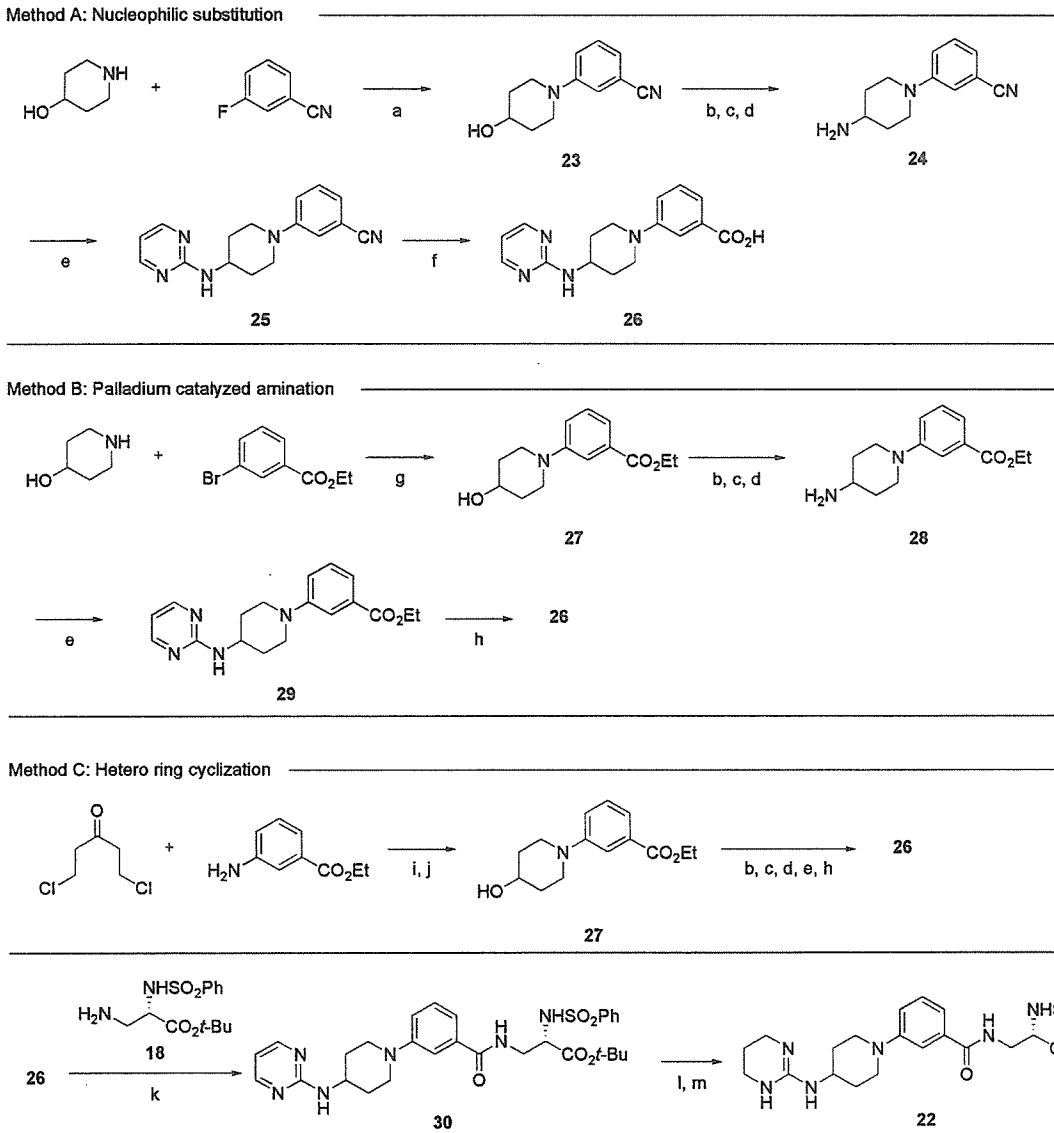


Figure 2. Prototype of *meta*-oriented antagonists.

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



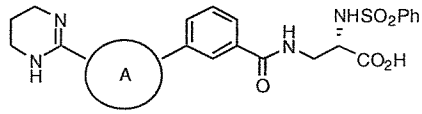
Scheme 3. Reagents and conditions: (a) $NaHCO_3$, NMP, 100 °C, 5 h; (b) MsCl, Et_3N, CH_2Cl_2, rt, 10 min; (c)NaN_3, DMF, 80 °C, 14 h; (d) H_2, Pd/C, 1,4-dioxane/H_2O, rt, 10 h; (e) 2-bromopyrimidine, DIPEA, DMSO, 120 °C, 6 h; (f) 50% H_2SO_4/H_2O, 80 °C, 4 h; (g) (*R*)-(+)-BINAP, $CsCO_3$, $Pd(OAc)_2$, 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.

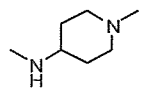
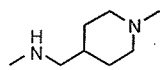
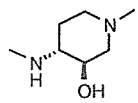
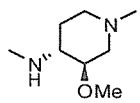
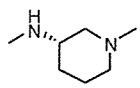
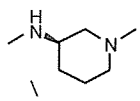
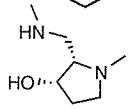
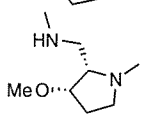
Table 3. Inhibitory activity of *meta*-oriented antagonists in receptor-binding assay and cell adhesion assay

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

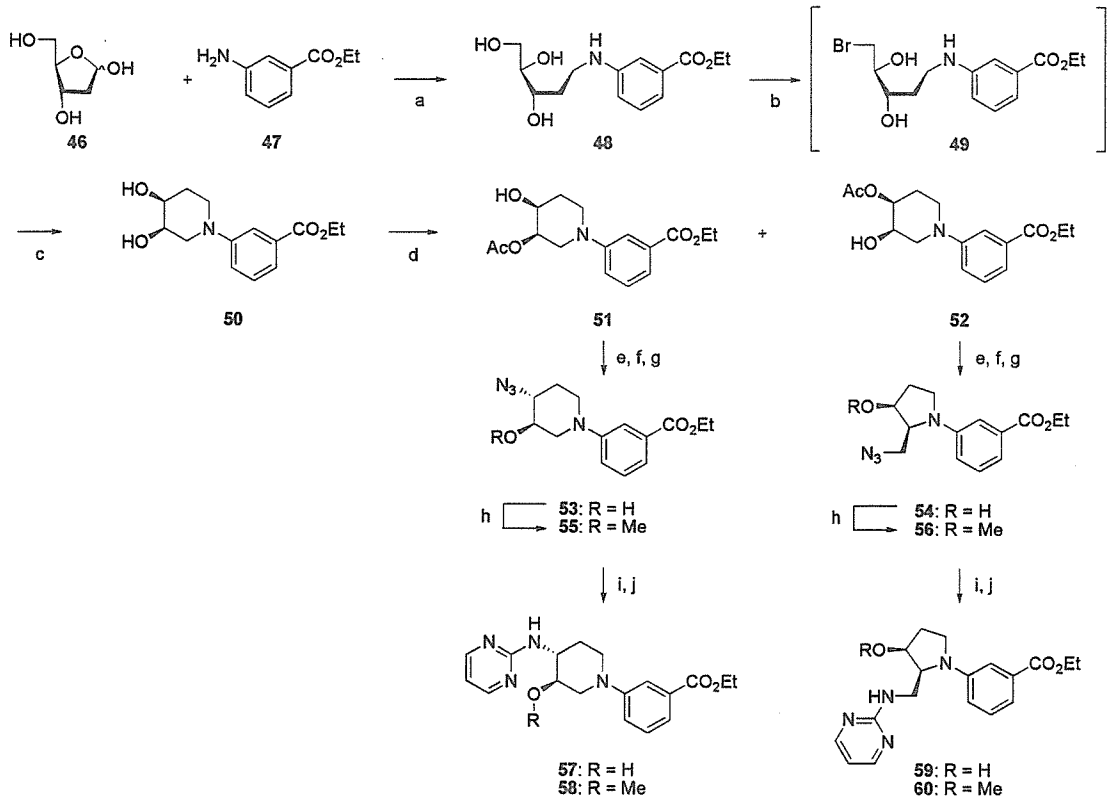
In order to prepare an $\alpha_v\beta_3$-selective antagonist possessing the tricyclic pharmacophore, we tried three approaches. Dimerization dramatically decreased the $\alpha_{IIb}\beta_3$-antagonistic activity, but also suppressed $\alpha_v\beta_3$ activity. Second, a novel heterocycle was introduced 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.

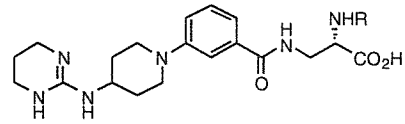
6.1. Preparation of compound 8

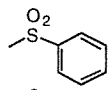
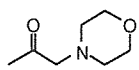
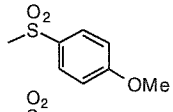
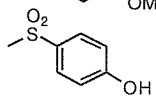
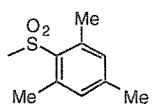
6.1.1. Compound 4. DMF (0.58 ml) was added to 1,6-dibromohexane (6.2 mg, 0.025 mmol) to prepare a solution and compound **3**[4] (29 mg, 0.050 mmol) and DBU



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.

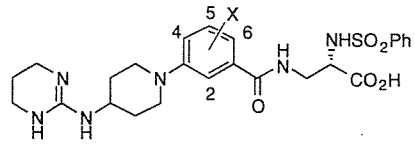
Table 5. Effect of replacement of the *C*-terminus on inhibitory activity in receptor-binding assay



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	X	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%); ^{1}H 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%); ^{1}H 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%); ^{1}H 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%); ^{1}H 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%); ^1H 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%); ^1H 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%); ^1H 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%). ^1H 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%); ^1H 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%); ^1H 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%); ^1H 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%); ^1H 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%); ^1H 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%); ^1H 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%); ^1H 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%); ^1H 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%); ^1H 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