Synthesis and Antimetastatic Activity of L-Iduronic Acid-Type 1-*N*-Iminosugars

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L-Iduronic acid-type 1-*N*-iminosugars, (3*R*,4*S*,5*R*,6*R*)- and (3*R*,4*S*,5*S*,6*R*)-6-acetamido-4-amino-5-hydroxypiperidine-3-carboxylic acid (**6** and **7**, respectively), (3*R*,4*S*,5*R*,6*R*)-6-acetamido-4-guanidino-5-hydroxypiperidine-3-carboxylic acid (**8**), and (3*R*,4*S*,5*R*,6*R*)-4-amino- and -guanidino-5-hydroxy-6-(trifluoroacetamido)piperidine-3-carboxylic acid (**9** and **10**, respectively), were synthesized from siastatin B (**1**), isolated from *Streptomyces* culture, by the intramolecular Michael addition of *O*-imidate to its α,β-unsaturated ester through *cis* oxiamination as a key step. Preincubation of B16 BL6 cells with these compounds inhibited invasion of the cells through reconstituted basement membranes. Pulmonary metastasis of B16 BL6 cells in mice was remarkably inhibited by pretreatment of the cells with these compounds in culture.

Introduction

Current biochemical researches[1−5] on the social behaviors of cells have shown that glycosidases and glycosyltransferases play an important role in controlling the metabolism of cell-surface carbohydrate, as glycoconjugates, which is involved in various biological function, such as tumor metastasis, immune response, viral infection, and so forth. Metastasis formation occurs *via* a complex multistage process which includes an important step of tumor cell penetration into endothelial basement membrane.[6a−c] Tumor invasion through the basement membrane involves cell adhesion to various basement membrane components, degradation of extracellular matrix and basement membranes, and cell migration of the target tissue.[6d,e] β-D-Glucuronidase and α-L-iduronidase are known to degrade the mammalian glycosaminoglycans (dermatan sulfate, heparan sulfate, and chondroitin sulfate), the major constituents of endothelial basement membranes.[7,8] Heparanase (*endo*-β-D-glucuronidase) activity in murine B16 melanoma cells correlates with lung colonization ability by degradation of heparan sulfate proteoglycan.[9a] Furthermore, heparanase inhibitors inhibit lung colonization of B16 melanoma cells in their syngeneic host.[9b,c]

A maltifunctional azasugar, siastatin B (**1**) was isolated as an inhibitor of β-glucuronidase as well as *N*-acetylneuraminidase from *Streptomyces* culture.[10] This discovery stimulated our interests in the synthesis of specific glucuronidase inhibitors for treatment of tumor metastasis and led to highly potent β-glucuronidase inhibitors (3*R*,4*R*,5*R*,6*R*)-6-(trifluoroacetamido)-3,4,5-trihydroxypiperidine-3-carboxylic acid (**2**) and (3*S*,4*S*,5*R*,6*R*)-6-(trifluoroacetamido)-4,5-dihydroxypiperidine-3-carboxylic acid (**3**). They showed the inhibition of invasion of highly metastatic B16 variant (B16 BL6) and Lewis lung carcinoma (3LL) cells through reconstituted basement membrane and the potent suppression of experimental and spontaneous pulmonary metastasis of B16 BL6 and/or 3LL cells in mice.[11] Compounds **2** and **3**, of which structure and shape are highly reminiscent of D-glucuronic acid (**4**) as a 1-*N*-iminosugar,[12] probably mimic **4** in binding to β-glucuronidase and strongly inhibit the enzymatic reaction. This observation was of particular relevance to our efforts to investigate new drugs for tumor metastasis by modification of metabolism of extracellular matrix and/or basement membranes with a new type of 1-*N*-iminosugar inhibitors of glycosidases.[12a] Moreover, the 6-epimer of **1** has recently been shown to be a heparanase inhibitor.[13] We speculated from above facts and results that if the metabolism of α-L-iduronide as well as β-D-glucuronide of basement membranes and/or extracellular matrix was responsible for tumor metastasis, L-iduronic acid (**5**)-type 1-*N*-iminosugars should inhibit tumor metastasis. We have recently communicated the synthesis of novel L-iduronic aicd-type 1-*N*-iminosugars, (3*R*,4*S*,5*R*,6*R*)- and (3*R*,4*S*,5*S*,6*R*)-6-acetamido-4-amino-5-hydroxypiperidine-3-carboxylic acid (**6** and **7**, respectively) and (3*R*,4*S*,5*R*,6*R*)-6-acetamido-4-guanidino-5-hydroxypiperidine-3-carboxylic acid (**8**).[14] We now report full details of the syntheses together with the evaluation of these candidates and their trifluoroacetamide analogues, (3*R*,4*S*,5*R*,6*R*)-4-amino- and -guanidino-5-hydroxy-6-(trifluoroacetamido)piperidine-3-carboxylic acid (**9** and **10**, respectively), as inhibitors of tumor metastasis.

Chemistry

The reason we chose **1** as a starting material is based on the structural similarity to 1-*N*-iminosugar corresponding to **5** by conformational change and on the easily obtainable source from *Streptomyces* culture (Scheme 1). In order to achieve the conformational change of **1**, an examine was made to inverse the configuration of carboxyl group. While we previously reported an epimerization of the carboxyl group by a conjugated Michael addition of benzyl alcohol to the α,β-unsaturated ester **12** with potassium carbonate, the yield and stereoselectivity were poor.[15] Several attempts of the direct epimerization of ester derivatives of **1** with base such as potassium and lithium bis(trimethylsilyl)amide, lithium diisopropylamide, and potassium *tert*-butoxide also failed. Attention was then directed to the intramolecular Michael addition of *O*-imidate to α,β-unsaturated ester **13** through the *cis*

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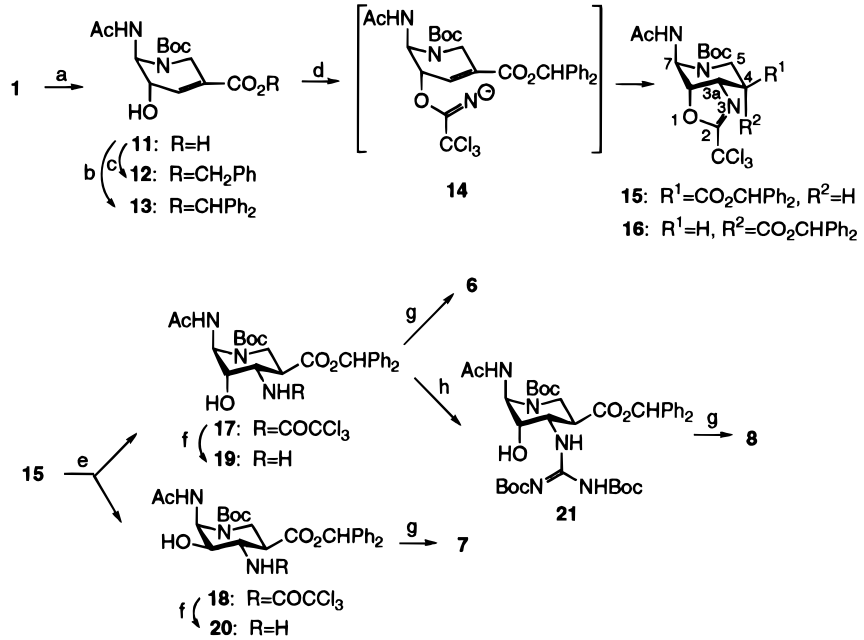
Scheme 1[a]



[a] (a) Reference 17; (b) Ph_2CN_2, CH_2Cl_2/CH_3OH; (c) $PhCH_2Cl$, *i*-Pr_2NEt, DMF; (d) CCl_3CN, DBU, CH_2Cl_2; (e) *p*-TsOH, Py/H_2O; (f) $NaBH_4$, EtOH; (g) 4 M HCl/dioxane; (h) $(BocNH)_2CS$, $HgCl_2$, Et_3N, DMF.

oxiamination.[16] The α,β-unsaturated ester **13** was prepared by esterification of the protected 3,4-didehydro-4-deoxysiastatin B (**11**)[17] readily derived from **1** with diphenyldiazomethane. Compound **13** smoothly underwent *cis* oxiamination to give the desired oxazoline **15** and its epimer **16** in yields of 76% and 3%, respectively. The intermediate imidate anion **14** from reaction with trichloroacetonitrile underwent efficient conjugate addition without the use of an electrophile to trigger oxazoline formation. Hydrolysis of the oxazoline ring of **15** was best achieved by treatment with *p*-toluenesulfonic acid in a mixture of pyridine and water[18] to afford **17** and **18** in yields of 77% and 9%, respectively. Reductive cleavage of the trichloroacetyl group with sodium borohydride[19] gave the amines **19** and **20** in good yields. Thus obtained compounds **19** and **20** were smoothly transformed into **6** and **7** by removal of the protecting groups with hydrochloric acid, respectively.

The reason we then introduced the guanidino function is based on the facts that the guanidine moiety is an important feature in many biologically active compounds, especially in binding to the enzyme such as influenza viral *N*-acetylneuraminidase inhibiting its infection *in vitro* and *in vivo*[20,21] and is also based on the molecular modeling study of α-L-iduronic acid (*vide infra*). The major isomer **19** can then be utilized for guanidine formation by use of *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea in the presence of mercuric chloride.[22] The reaction efficiently proceeded to afford the bis-Boc-protected guanidine **21** in 88% yield. Compound **21** was straightforwardly converted into **8** by treatment with acid. The $^1C_4$ conformations as well as stereochemistries of **6**, **7**, and **8** were established by ^1H NMR spectra in D_2O. The large coupling constants (11.0−11.5 Hz) between H-2 and H-3 and between H-3 and H-4, and small ones (2.7−3.2 Hz) between H-4 and H-5 and between H-5 and H-6 of **6** and **8** are clearly indicative of $^1C_4$ conformers in water solution. The spectrum of **7** also shows the large coupling constants (10.3−11.2 Hz) between H-2 and H-3, between H-3 and H-4, and between H-4 and H-5, and the small one (4.9 Hz) between H-5 and H-6, indicating the $^1C_4$ conformer of **7**.

On the other hand, the highly potent inhibition of **2** and **3** as 1-*N*-iminosugars for β-D-glucuronidase suggests that trifluoroacetamide function around the anomeric position corresponding to D-glucuronide may play an important role in the strong binding of 1-*N*-iminosugar to the enzyme. In order to improve the potency and examine the neighboring participation of trifluoroacetamide group of **6** and **8** for tumor metastasis, replacement of the acetamide function is next undertaken. The starting (2*S*,3*R*,4*S*,5R)-2-amino-1-*N*-(*tert*-butoxycarbonyl)-5-(hydroxymethyl)-3,4-*O*-isopropylidenepiperidine-3,4-diol (**22**) was easily obtained from **1** by the method developed by us (Scheme 2).[22,23] Protection of the amino group of **22** with benzyloxycarbonyl chloride gave **23** in 86% yield. Oxidation of the hydroxymethyl group of **23** to the carboxylic acid was best achieved by ruthenium tetraoxide-catalyzed oxidation in a solvent system of $CH_3CN/CCl_4/H_2O$ developed by Sharpless et al.[24] (80% yield). Esterification of **24** with diphenyldiazomethane followed by elimination with potassium *tert*-butoxide gave the α,β-unsaturated ester **26** in a good yield. Compound **26** was effectively converted to the key oxazolines **27** and **28** *via* a similar *cis* oxiamination described above in yields of 70% and 23%, respectively. Hydrolysis of the oxazoline ring of **27** and the subsequent removal of the trichloroacetyl group straightforwardly gave **30** which was transformed into **32** by protection of the amino group and exchange of the protecting group of carboxyl group. Hydrogenolysis of **32** followed by trifluoroacetylation with trifluoroacetic anhydride afforded **33** in a good yield. Compound **33** was smoothly converted into **9** by treatment with acid. On the other hand, the similar guanidine formation of **30** described above afforded the bis-Boc-protected guanidine **34** in 98% yield. Compound **34** was transformed into **10** by a similar sequences of reaction described above (**32** → **33** and **21** → **8**). The $^1C_4$

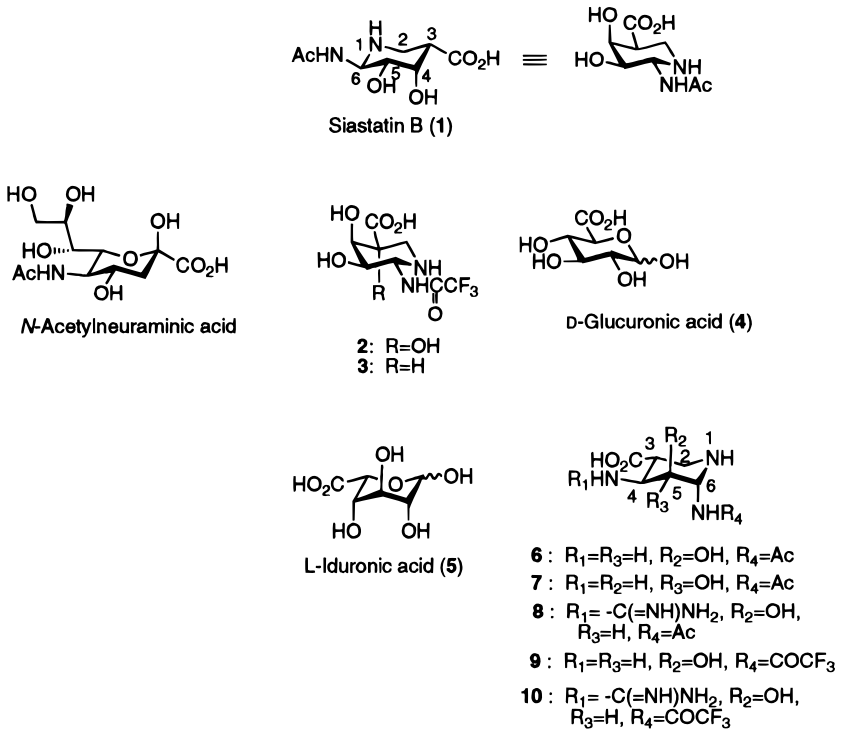
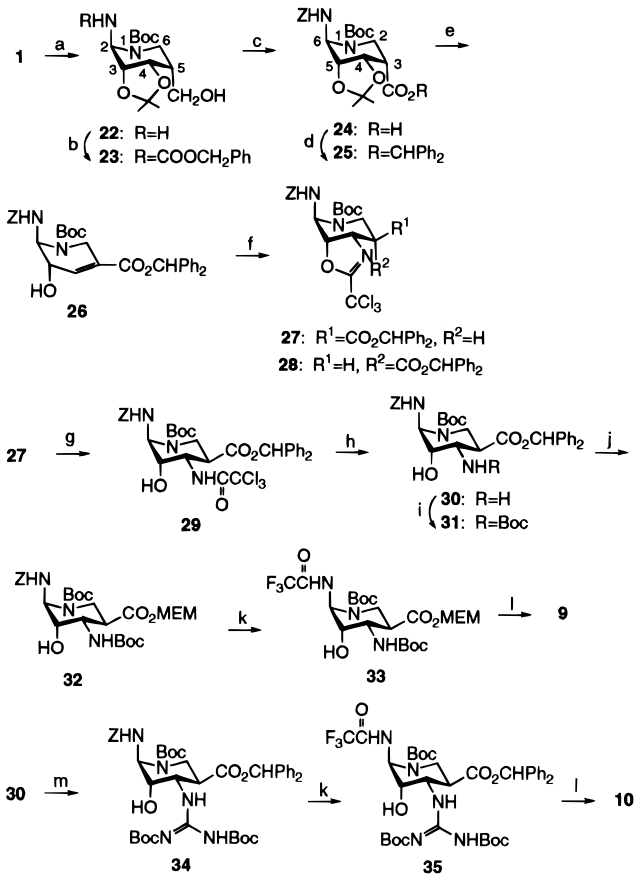


Figure 1.

Scheme 2[a]



[a] (a) Reference 23; (b) Ph_2CH_2COOCl, *i*-Pr_2NEt, CH_3OH; (c) RuO_2, $NaIO_4$, $CH_3CN/CCl_4/H_2O$; (d) Ph_2CN_2, CH_2Cl_2/CH_3OH; (e) *t*-BuOK, THF; (f) CCl_3CN, DBU, C_6H_6; (g) *p*-TsOH, Py/H_2O; (h) $NaBH_4$, EtOH; (i) $(t\text{-BuOCO})_2O$, *i*-Pr_2NEt, CH_3OH; (j) 1 M NaOH, CH_3OH; $CH_3OCH_2CH_2OCH_2Cl$, *i*-Pr_2NEt, CH_2Cl_2; (k) H_2/10% Pd–C, CH_3CN; $(CF_3CO)_2O$, Py; (l) 4 M HCl/dioxane; (m) $(BocNH)_2CS$, $HgCl_2$, Et_3N, DMF.

conformation as well as stereochemistries of **9** and **10** were also confirmed by ^1H NMR spectra. Unexpectedly, both **9** and **10** are unstable and decompose in an aqueous solution. The half-life of both compounds is about 3 and 24 h in water and hydrochloric acid (pH ~1.0), respectively.

Biological Results and Discussion

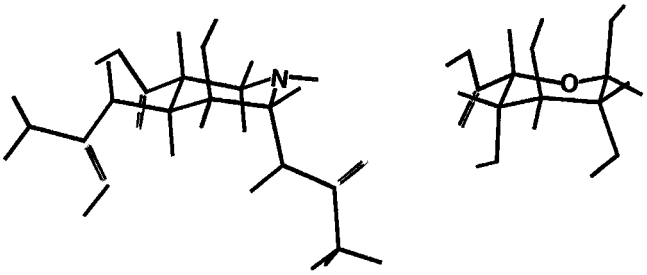
As expected, the analogues **6**, **7**, and **8** showed no inhibition against Baker's yeast α-glucosidase,[25a] almond *β*-glucosidase,[25b] Jack beans α-mannosidase,[25c] snail *β*-mannosidase,[25c] *Escherichia coli* α- and *β*-galactosidase,[25d] bovine liver *β*-glucuronidase,[25e] chicken liver 2-*N*-acetylgalactosaminidase,[25f] and bovine epididymis *β*-*N*-acetylglucosaminidase[25g] (IC_{50} > 0.39 mM). These results indicate that the analogues having the $^1C_4$ conformation are significantly distinct from the known analogues of **1** having the $^4C_1$ conformation on the inhibition of D-sugar hydrolases and that the $^4C_1$ conformation of analogues of **1** is important for specificity and potency of 1-*N*-iminosugar inhibitors against D-sugar hydrolases.[12a,26] While many analogues of **1** having the $^4C_1$ conformation inhibit bacterial *N*-acetylneuraminidases,[12a] **6**, **7**, and **8** also did not affect the enzymes from *Anthrobacter ureafaciencs*, *Streptococcus* sp., and *Clostridium perfringens* (IC_{50} > 0.5 mM). These results are also in accord with no structural similarity of **6**, **7**, and **8** to *N*-acetylneuraminic acid shown in Figure 1.

α-L-Iduronidase is now not available for us, and these analogues were then assayed for antiinvasive and antimetastatic activities of tumor cells. As shown in Table 1, **8** and **10** inhibited the invasiveness of B16 BL6 cells through reconstituted basement membranes. About 60% inhibition of invasion was consistently observed with **8** and **10** at 870 and 129 μM, respectively, in multiple experiments. Since the inhibitory activity of 1-*N*-iminosugar analogues modeled on siastatin B for experimental pulmonary metastasis is well proportional to that for spontaneous pulmonary metastasis,[11c] the experimental metastasis assay was employed for this

Table 1. Inhibitory Effect of **8** and **10** on B16 BL6 Cell Invasion *in Vitro*[a]

treatment	concn (μM)	incubation (h)	no. of invaded cells (mean ± SD)	inhibn of invasion (%)
none	0	3	43.0 ± 7.3	0
8	580	3	23.7 ± 14.8	44.8
	870	3	17.7 ± 5.0	58.9**
none	0	4	2.4 ± 0.2	0
10	129	4	0.9 ± 0.1	61.1***
	258	4	1.3 ± 0.8	44.4
	518	4	0.9 ± 0.6	63.9*

[a] The cells were cultured with **8** and **10** for 72 and 24 h, respectively, at 37 °C, and then used in an *in vitro* cell invasion assay. The results are expressed as the mean ± SD of three determinations. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.



8 α-L-Iduronic acid

Figure 2. PM3/MOPAC-optimized structures of **8** and α-L-iduronic acid.[34]

study. As shown in Table 2, when B16 BL6 cells were treated with **6**−**10** in cell culture, their metastatic activity was significantly decreased. Pulmonary colonization after intravenous transplantation of treated or untreated B16 BL6 cells into the lateral tail veins of BDF1 mice was significantly suppressed dose-dependently. Compounds **6** at 172 μM, **7** at 172 μM, and **8** at 150 μM inhibited the metastasis by 44.3, 30.9, and 97%, respectively. Compounds **9** at 145 μM and **10** at 129 μM also remarkably inhibited the metastasis by 75.5% and 81%, respectively, although they are unstable in an aqueous solution. As expected, the trifluoroacetamide functions of **9** and **10** assist in improving the inhibitory activity of tumor metastasis like those of **2** and **3**. These analogues, **6**−**10**, had no significant effects on cell growth at the concentration used in this study (third column in Table 2). It is possible that the metastatic effects of **8** and **10** are due to their antiinvasive activities.

On the other hand, molecular modeling was undertaken to understand the structural similarity between **8** and **5**. The structures were optimized first with molecular mechanics (MM2)[27] and then with PM3 in MOPAC.[28] Molecular modeling revealed that **8** superimposes well on **5** and has the hydroxyl and carboxyl groups lying in the same region of space as those of **5**, and that the acetamide and guanidino moieties of **8** are also topographically equivalent to the hydroxyl moieties of **5** (Figure 2). It is highly likely that in contrast with **2** and **3** for β-glucuronidase inhibitors, **8** mimics L-iduronic acid in metabolism of extracellular matrix and/or basement membranes.

The result in this study seems to indicate that the metabolism of α-L-iduronide of the basement membranes and/or extracellular matrix may participate in melanoma metastasis, and also that these analogues may modify the cell-surface glycoconjugates of tumor cells simultaneously, thereby altering cell properties involved in cellular recognition and adhesion.[29] The present study shows that the L-iduronic acid-type 1-*N*-iminosugars as well as D-glucuronic aicd-type ones may contribute to the study of the involvement of carbohydrates in malignant cell movement and are the promising candidates of new drugs for the cancer chemotherapy of metastasis.

Experimental Section

***In Vitro* Cell Invasion Assay.** The assay was carried out essentially by the method described by Albini *et al.*[30] and Saiki *et al.*[31] Nucleopore filters were coated with 5 μg of Matrigel (Collaborative Research Inc., MA) and 10 μg of laminin (Collaborative Research Inc., MA), in that order, and placed in a Transwell cell culture chamber (Coastar No. 3422, Cambridge, MA). RPMI 1640 medium (600 μL) containing 0.1% bovine serum albumin factor V (Nissui Seiyaku, Tokyo) was placed in the lower compartment of the Transwell chamber. The B16 BL6 cells (1×10^6) were removed from the bottom of flasks by incubation with 0.08% sodium citrate for 10 min at 37 °C, washed twice with RPMI 1640 medium containing 0.1% bovine serum albumin factor V, and dispersed in 1 mL of the same medium. The cell suspension (100 μL) was added to the upper chamber and incubated for 3 or 4 h at 37 °C in a humid 5% CO_2 atmosphere. Then, the cells on the upper surface of the filter were completely removed by wiping, and the filters were fixed with methanol and stained with Harris' hematoxylin. The cells that had penetrated through the filter were counted in five fields of 0.3 mm^2 area under a microscope (200×). All assays were done in triplicate.

Pulmonary Colonization Assay. Pulmonary colonization assay was carried out as described previously by Humphries *et al.*[32] and Filder.[33] B16 BL6 (3×10^5 cells) were cultured in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum under 5% CO_2 at 37 °C for 24 h. Cells were incubated with or without each test compound under the same condition for 72 or 24 h. After treatment with 0.05% trypsin and 0.02% EDTA solution, a cell suspension containing 1.3×10^{-6}−2.1×10^7 cells in 1 mL of divalent cation-free Dulbecco's phosphate-buffered saline was obtained. Cells (1×10^5) in 0.1 mL were collected and injected intravenously into the tail vein of each mouse (five female BDF1, 7 weeks old). Fourteen days later, after tumor cell implantation, the mice were autopsied. The number of pulmonary tumor nodules was counted. Inhibition (%) of metastasis was calculated from the ratio of tumor nodules in treated and control experiments.

(5*S*,6*S*)-6-Acetamido-1-*N*-(*tert*-butoxycarbonyl)-3,4-didehydro-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (13). To a solution of **11**[17] (1.87 g, 6.24 mmol) in a mixture of dichloromethane (30 mL) and methanol (30 mL) was added diphenyldiazomethane (3.64 g, 18.7 mmol), and the mixture was stirred at room temperature overnight. After the reaction was quenched with acetic acid (0.9 mL, 15.7 mmol), evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to a column chromatography on silica gel. Elution with CH_2Cl_2−CH_3OH (99:1) gave **13** (2.66 g, 92%) as a colorless foam: $[\alpha]^{24}_D$ +91° (*c* 0.86, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 1.49 (9H, s), 1.89 (3H, s), 3.86 (1H, br d, J = 19.1 Hz, H-2), 4.16 (1H, br m, H-5), 4.52 (1H, dd, J = 19.1 and 2.0 Hz, H-2), 6.10 (1H, br s, H-6), 6.97 (1H, s, Ph_2CH), 7.12 (1H, m, H-4), and 7.27−7.42 (10H, m). Anal. ($C_{26}H_{30}N_2O_6$) C, H, N.

(3a*S*,4*R*,7*S*,7a*S*)-7-Acetamido-6-*N*-(*tert*-butoxycarbonyl)-2-(trichloromethyl)-4-[(diphenylmethoxy)carbonyl]-3a,-7a-dihydrooxazolo[5,4-*c*]piperidine (15) and (3a*S*,4*S*,7*S*,-7a*S*)-Isomer 16. To a solution of **13** (233 mg, 0.5 mmol) in dichloromethane (5 mL) were added trichloroacetonitrile (65.2 μL) and DBU (15.0 μL), and the mixture was stirred at room temperature for 5 min. After addition of water and dilution with chloroform, the solution was washed with saturated

Table 2. Inhibitory Effect of **6**−**10** on the Experimental Metastasis of B16 BL6 Cells in Mice[a]

compd	concn (μM)	no. of cells (mL)	no. of lung metastasis: mean ± SD	no. of lung metastasis: range	inhibn of metastasis (%)
none		1.6×10^7	147.2 ± 29.5	181−110	0
6	34	1.4×10^7	147.2 ± 12.7	165−133	0
	103	1.4×10^7	129.4 ± 18.8	160−114	12.1
	172	1.8×10^7	82.0 ± 37.0	118−23	44.3*
7	34	1.6×10^7	145.2 ± 12.7	169−115	1.4
	103	2.1×10^7	108.8 ± 40.8	136−64	26.6
	172	1.6×10^7	101.6 ± 20.6	136−84	30.9*
8	30	1.9×10^7	88.2 ± 33.3	129−54	40.1*
	90	1.7×10^7	13.2 ± 5.3	20−7	91***
	150	1.9×10^7	4.4 ± 3.1	9−1	97***
none		1.2×10^6	255.0 ± 35.5	199−285	0
9	29	1.6×10^6	245.4 ± 29.6	200−270	3.8
	87	1.6×10^6	157.8 ± 48.3	122−237	38.1**
	145	1.4×10^6	62.4 ± 35.7	5−97	75.5***
10	26	1.9×10^6	219.0 ± 47.0	148−276	14.1
	78	1.3×10^6	105.6 ± 11.5	89−120	58.8***
	129	1.5×10^6	48.4 ± 27.8	13−87	81.0***

[a] The cells were cultured with 6, 7, and 8 for 72 h, and with 9 and 10 for 24 h; then the cells (1×10^5) were collected and injected into tail veins of mice. Results are expressed as the mean ± SD of five mice. *, **, and ***, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

aqueous NH_4Cl solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with toluene−acetone (6:1) gave **15** (234 mg, 76%) as a colorless foam and **16** (9 mg, 3%) as a colorless oil.

15: $[\alpha]^{24}_D$ +20.5° (*c* 0.86, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.47 (9H, s), 1.75 (3H, s), 2.77 (1H, dt, J = 8.3 and 5.0 Hz, H-3), 3.46 (1H, dd, J = 13.7 and 8.3 Hz, H_{ax}-2), 3.89 (1H, dd, J = 13.7 and 4.9 Hz, H_{eq}-2), 4.96−5.03 (2H, m, H-4 and H-5), 6.18 (1H, s, H-6), 7.10 (1H, s, Ph_2CH), and 7.26−7.42 (10H, m). Anal. ($C_{28}H_{30}Cl_3N_3O_6$) C, H, N.

16: $[\alpha]^{24}_D$ −32.5° (*c* 0.85, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.44 (9H, s), 1.96 (3H, s), 3.39 (1H, br t, J = 12.0 Hz, H_{ax}-2), 3.52 (1H, dt, J = 12.5 and 4.4 Hz, H-3), 3.69 (1H, dd, J = 12.0 and 4.4 Hz, H_{eq}-2), 5.19 (1H, dd, J = 10.0 and 4.0 Hz, H-4), 5.29 (1H, m, H-5), 5.96 (1H, br s, H-6), 6.94 (1H, s, Ph_2CH), and 7.24−7.43 (10H, m). Anal. ($C_{28}H_{30}Cl_3N_3O_6 \cdot {}^1/_2H_2O$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-6-Acetamido-1-*N*-(*tert*-butoxycarbonyl)-4-(trichloroacetamido)-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (17) and (3*R*,4*S*,5*R*,6*S*)-Isomer 18. To a solution of **15** (1.53 g, 2.5 mmol) in a mixture of pyridine (24 mL) and water (6 mL) was added *p*-toluenesulfonic acid (713 mg, 3.75 mmol), and the mixture was stirred at 80 °C for 2 h. After dilution with chloroform, the solution was washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to a column chromatography on silica gel. Elution with chloroform−methanol (30:1) gave **17** (1.2 g, 77%) as a colorless foam and **18** (135 mg, 9%) as an amorphous solid.

17: $[\alpha]^{24}_D$ +3.8° (*c* 0.95, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.47 (9H, s), 1.99 (3H, s), 3.17 (1H, br t, J = 12.0 Hz, H_{ax}-2), 3.27 (1H, dt, J = 11.5 and 4.2 Hz, H-3), 3.82 (1H, br m, H-5), 4.30 (1H, br d, J = 12.0 Hz, H_{eq}-2), 4.47 (1H, dd, J = 11.5 and 2.0 Hz, H-4), 6.05 (1H, d, J = 2.0 Hz, H-6), 6.84 (1H, s, Ph_2CH), and 7.25−7.38 (10H, m). Anal. ($C_{28}H_{32}Cl_3N_3O_7$) C, H, N.

18: $[\alpha]^{24}_D$ +20.7° (*c* 0.87, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.48 (9H, s), 2.03 (3H, s), 3.02 (1H, dt, J = 11.7 and 3.4 Hz, H-3), 3.08 (1H, br t, J = 12.0 Hz, H_{ax}-2), 3.79 (1H, dd, 10.7 and 4.9 Hz, H-5), 4.15 (1H, br m, H_{eq}-2), 4.40 (1H, t, J = 10.7 Hz, H-4), 6.21 (1H, d, J = 4.9 Hz, H-6), 6.84 (1H, s, Ph_2CH), and 7.24−7.35 (10H, m). Anal. ($C_{28}H_{32}Cl_3N_3O_7$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-6-Acetamido-4-amino-1-*N*-(*tert*-butoxycarbonyl)-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (19). To a solution of **17** (629 mg, 1.0 mmol) in ethanol (13 mL) was added sodium borohydride (114 mg, 3.0 mmol), and the mixture was stirred at room temperature for 50 min. After dilution with chloroform, the solution was washed with saturated aqueous NaCl solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to a column chromatography on silica gel. Elution with chloroform−methanol (9:1) gave **19** (298 mg, 62%) as a colorless foam: $[\alpha]^{24}_D$ +19.7° (*c* 0.78, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.47 (9H, s), 1.95 (3H, s), 2.88 (1H, dt, J = 11.5 and 4.0 Hz, H-3), 3.01 (1H t, J = 12.0 Hz, H_{ax}-2), 3.17 (1H dd, J = 11.5 and 2.4 Hz, H-4) 3.73 (1H, br s, H-5), 4.27 (1H, dd, J = 12.0 and 4.0 Hz, H_{eq}-2), 6.03 (1H, d, J = 2.0 Hz, H-6), 6.92 (1H, s, Ph_2CH), and 7.26−7.41 (10H, m). Anal. ($C_{26}H_{33}N_3O_6 \cdot {}^1/_{10}H_2CO_3$) C, H, N.

(3*R*,4*S*,5*R*,6*S*)-6-Acetamido-4-amino-1-*N*-(*tert*-butoxycarbonyl)-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (20). Compound **20** was obtained by similar procedures used for the preparation of **19**: yield 53%; $[\alpha]^{24}_D$ +19° (*c* 0.85, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.48 (9H, s), 1.98 (3H, s), 2.55 (1H, ddd, J = 12.0, 10.5, and 4.6 Hz, H-3), 2.97 (1H, br t, J = 12.9 Hz, H_{ax}-2), 3.25 (1H, t, J = 10.5 Hz, H-4), 3.44 (1H, dd, J = 10.5 and 5.3 Hz, H-5), 4.19 (1H, br m, H_{eq}-2), 6.18 (1H, d, J = 5.1 Hz, H-6), 6.91 (1H, s, Ph_2CH), and 7.26−7.40 (10H, m). Anal. ($C_{26}H_{33}N_3O_6 \cdot {}^1/_{10}H_2CO_3$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-6-Acetamido-4-amino-5-hydroxypiperidine-3-carboxylic Acid (6). Compound **19** (46 mg, 95 μmol) was dissolved in a solution of 4 M hydrogen chloride in dioxane (1.5 mL), and the solution was stirred at room temperature overnight. The resulting precipitates were collected by centrifugation and washed with ether to give a colorless solid (31 mg). The crude product was chromatographed by a column of Diaion HP 20 (Mitsubishi Kasei Corp., Tokyo). Elution with water gave a colorless solid, which was dissolved in a small amount of methanol. To the solution was added a solution of 1 M hydrogen chloride in dioxane (1 mL) and ether (1 mL), and the mixture was stirred. The resulting precipitates were collected by centrifugation and washed with ether to give a colorless amorphous solid of **6** as its hydrochloride (26 mg, 96%): $[\alpha]^{26}_D$ +31.8° (*c* 0.19, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 2.13 (3H, s), 3.20 (1H, dt, J = 11.5 and 3.9 Hz, H-3), 3.28 (1H, t, J = 12.0 Hz, H_{ax}-2), 3.61 (1H, dd, J = 12.0 and 3.9 Hz, H_{eq}-2), 3.98 (1H, dd, J = 11.5 and 2.7 Hz, H-4), 4.32 (1H, t, J = 2.7 Hz, H-5), and 5.45 (1H, d, J = 2.7 Hz, H-6). Anal. ($C_8H_{15}N_3O_4 \cdot 2HCl \cdot 2H_2O$) C, Cl; H: calcd, 6.49; found, 6.98.

(3*R*,4*S*,5*R*,6*R*)-6-Acetamido-4-amino-5-hydroxypiperidine-3-carboxylic Acid (7). Compound **7** was obtained by the similar procedures used for the preparation of **6**: yield 95%; $[\alpha]^{25}_D$ +32.5° (*c* 0.20, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 2.16 (3H, s), 3.05 (1H, dt, J = 11.2 and 4.4 Hz, H-3), 3.33 (1H, dd, J = 13.7 and 11.2 Hz, H_{ax}-2), 3.65 (1H, dd, J = 13.7 and 4.4 Hz, H_{eq}-2), 3.86 (1H, t, J = 10.8 Hz, H-4), 4.25 (1H, dd, J

= 10.8 and 4.9 Hz, H-5), and 5.66 (1H, d, J = 4.9 Hz, H-6). Anal. ($C_8H_{15}N_3O_4 \cdot 2HCl \cdot 2H_2O$) C, Cl; H: calcd, 6.49; found, 6.95.

(3*R*,4*S*,5*S*,6*S*)-6-Acetamido-4-[*N*,*N*′-bis(*tert*-butoxycarbonyl)guanidino]-1-*N*-(*tert*-butoxycarbonyl)-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (21). To a solution of **19** (242 mg, 0.5 mmol) in DMF (5 mL) were added triethylamine (0.28 mL, 2 mmol), *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (276 mg, 1 mmol), and $HgCl_2$ (272 mg, 1 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. After dilution with a large amount of ethyl acetate, the insoluble matters were removed by centrifugation. The insoluble matters were washed twice with ethyl acetate. The combined solution of the supernatant and the washings was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid, which was subjected to column chromatography on silica gel. Elution with a mixture of toluene−acetone (3:1) gave **21** (320 mg, 88%) as an amorphous solid: $[\alpha]^{23}_D$ −2.3° (*c* 0.72, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.47, 1.48, and 1.49 (9H, each s), 2.01 (3H, s), 2.99 (1H, dt, J = 11.7 and 3.9 Hz, H-3), 3.16 (1H, br m, H_{ax}-2), 3.73 (1H, br m, H-5), 4.17 (1H, br m, H_{eq}-2), 4.68 (1H, dd, J = 11.7 and 2.5 Hz, H-4), 6.02 (1H, d, J = 2.0 Hz, H-6), 6.81 (1H, s, Ph_2CH), and 7.24−7.34 (10H, m, Phx2). Anal. ($C_{37}H_{51}N_5O_{10} \cdot {}^1/_2H_2O$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-6-Acetamido-4-guanidino-5-hydroxypiperidine-3-carboxylic Acid (8). Compound **8** was obtained by the similar procedures used for the preparation of **6**: yield 94%: $[\alpha]^{26}_D$ +16.8° (*c* 0.26, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 2.31 (3H, s), 3.35 (1H, br dt, J = 11.0 and 4.4 Hz, H-3), 3.54 (1H, t, J = 12.0 Hz, H_{ax}-2), 3.69 (1H, dd, J = 12.0 and 4.4 Hz, H_{eq}-2), 4.38 (1H, t, J = 3.2 Hz, H-5), 4.45 (1H, dd, J = 11.0 and 3.2 Hz, H-4), and 5.52 (1H, d, J = 3.2 Hz, H-6). Anal. ($C_9H_{17}N_5O_4 \cdot 2HCl \cdot {}^2/_3H_2O$) C, N, Cl; H: calcd, 6.17; found, 6.59.

(2*S*,3*R*,4*S*,5*R*)-2-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-5-(hydroxymethyl)-3,4-*O*-isopropylidenepiperidine-3,4-diol (23). To a solution of **22**[23] (3.24 g, 10.7 mmol) in CH_3OH (65 mL) were added *N*,*N*-diisopropylethylamine (7.46 mL, 42.8 mmol) and benzyloxycarbonyl chloride (4.58 mL, 32.1 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. Another portion of benzyloxycarbonyl chloride (0.76 mL, 5.35 mmol) was added to the mixture, and the mixture was further stirred at 0 °C for 30 min. Addition of water (1.5 mL) and evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with $NaHCO_3$-saturated aqueous solution and water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with $CHCl_3$−CH_3OH (40:1) gave **23** (4 g, 86%) as a colorless foam: $[\alpha]^{24}_D$ +12.8° (*c* 1.09, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.34 (3H, s), 1.44 (12H, s), 1.94 (1H, t, J = 5.9 Hz, OH), 2.04 (1H, br m, H-5), 3.17 (1H, t, J = 12.2 Hz, H_{ax}-6), 3.45 (1H, dd, J = 12.2 and 4.4 Hz, H_{eq}-6), 3.75 (2H, t, J = 5.9 Hz, CH_2OH), 4.53 (1H, dd, J = 7.8 and 2.4 Hz, H-3), 4.57 (1H, br d, J = 7.8 Hz, H-4), 4.87 (1H, br s, NHCO), 5.08 and 5.13 (2H, AB q, J = 12.0 Hz, $PhCH_2$), 5.65 (1H, dd, J = 6.1 and 2.4 Hz, H-2), and 7.29−7.35 (5H, m). Anal. ($C_{22}H_{32}N_2O_7$) C, H, N.

(3*S*,4*S*,5*R*,6*S*)-6-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-4,5-dihydroxy-4,5-*O*-isopropylidenepiperidine-3-carboxylic Acid (24). To a solution of **23** (2.18 g, 5 mmol) in a mixture of CCl_4 (30 mL) and CH_3CN (30 mL) were added a solution of $NaIO_4$ (3.21 g, 15 mmol) in water (9 mL) and RuO_2 (8.0 mg, 0.06 mmol), and the mixture was vigorously stirred at room temperature for 40 min. The phases were separated. The aqueous phase was extracted twice with ethyl acetate. To the combined organic extracts was added 2-propanol (5 mL), and the mixture was stirred at room temperature for 2 h. After removal of inorganic salts by filtration, the mixture was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid, which was subjected to column chromatography on silica gel. Elution with $CHCl_3$−CH_3OH−concentrated aqueous ammonia (60:10:1) gave **24** (1.79 g, 80%) as an amorphous solid: $[\alpha]^{23}_D$ +5.7° (*c* 0.96, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.32 and 1.37 (3H, each s), 1.44 (9H, s), 2.96 (1H, ddd, J = 12.7, 5.4, and 2.9 Hz, H-3), 3.46 (1H, t, J = 12.7 Hz, H_{ax}-2), 3.55 (1H, dd, J = 12.7 and 5.4 Hz, H_{eq}-2), 4.45 (1H, dd, J = 7.6 and 2.0 Hz, H-5), 4.82 (1H, dd, J = 7.6 and 2.9 Hz, H-4), 5.08 and 5.12 (2H, AB q, J = 12.7 Hz, $PhCH_2$), 5.63 (1H, d, J = 2.0 Hz, H-6), 7.27−7.38 (5H, m). Anal. ($C_{22}H_{30}N_2O_8$) C, H; N; calcd, 6.22; found, 6.62.

(3*S*,4*S*,5*R*,6*S*)-6-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-4,5-dihydroxy-4,5-*O*-isopropylidene-3-[(diphenylmethoxy)carbonyl]piperidine (25). Compound **25** was obtained by the similar procedures used for the preparation of **13**: yield 94% (colorless foam); $[\alpha]^{24}_D$ +3.6° (*c* 0.87, CH_3OH); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.33 and 1.36 (3H, each s), 1.43 (9H, s), 3.49 (1H, t, J = 12.7 Hz, H_{ax}-2), 3.64 (1H, dd, J = 12.2 and 4.9 Hz, H_{eq}-2), 4.52 (1H, dd, J = 7.8 and 2.0 Hz, H-5), 4.93 (1H, dd, J = 7.8 and 2.4 Hz, H-4), 5.07 and 5.13 (2H, AB q, J = 12.2 Hz, $PhCH_2$), 5.66 (1H, d, J = 2.0 Hz, H-6), 6.89 (1H, s, Ph_2CH), 7.25−7.37 (15H, m). Anal. ($C_{35}H_{40}N_2O_8$) C, H, N.

(5*S*,6*S*)-6-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-3,4-didehydro-3-[(diphenylmethoxy)carbonyl]piperidine (26). To a solution of **25** (799 mg, 1.3 mmol) in THF (16 mL) was added potassium *tert*-butoxide (15 mg, 0.13 mmol) at 0 °C under atmosphere of argon gas, and the mixture was stirred for 2 h under the same condition. After dilution with $CHCl_3$, the solution was washed with NH_4Cl-saturated aqueous solution and water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution in the toluene−acetone (7:1) gave **26** (508 mg, 70%) as a colorless foam: $[\alpha]^{24}_D$ +62.9° (*c* 0.89, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.47 (9H, s), 3.81 (1H, d, J = 19.0 Hz, Ha-2), 4.19 (1H, d, J = 5.4 Hz, H-5), 4.53 (1H, d, J = 19.0 Hz, Hb-2), 5.06 and 5.10 (2H, ABq, J = 12.7 Hz, $PhCH_2$), 5.92 (1H, d, J = 1.0 Hz, H-6), 6.95 (1H, s, Ph_2CH), 7.70 (1H, m, H-4), and 7.26−7.40 (15H, m). Anal. ($C_{32}H_{34}N_2O_7$) C, H, N.

(3a*S*,4*R*,7*S*,7a*S*)-7-[(Benzyloxycarbonyl)amino]-6-*N*-(*tert*-butoxycarbonyl)-2-(trichloromethyl)-4-[(diphenylmethoxy)carbonyl]-3a,7a-dihydrooxazolo[5,4-*c*]piperidine (27) and (3a*S*,4*S*,7*S*,7a*S*)-Isomer 28. Compounds **27** and **28** were obtained by the similar procedures used for preparation of **15** and **16** in 70 and 23% yields, respectively.

27: colorless foam; $[\alpha]^{26}_D$ +2.0° (*c* 0.89, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.42 (9H, s), 3.39 (1H, br t, J = 11.7 Hz, H_{ax}-2), 3.54 (1H, ddd, J = 12.7, 4.9 and 4.4 Hz, H-3), 3.64 (1H, dd, J = 11.7 and 4.9 Hz, H_{eq}-2), 5.09 and 5.14 (2H, AB q, J = 11.7 Hz, $PhCH_2$), 5.18 (1H, dd, J = 9.8 and 4.4 Hz, H-4), 5.27 (1H, br d, J = 9.8 Hz, H-5), 5.82 (1H, d, J = 2.0 Hz, H-6), 6.92 (1H, s, Ph_2CH), and 7.23−7.41 (15H, m). Anal. ($C_{34}H_{34}Cl_3N_3O_7$) C, H, N.

28: colorless foam; $[\alpha]^{26}_D$ −18.5° (*c* 0.92, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.44 (9H, s), 2.73 (1H, m, H-3), 3.45 (1H, br dd, J = 12.2 and 8.8 Hz, H_{ax}-2), 3.86 (1H, dd, J = 13.4 and 5.1 Hz, H_{eq}-2), 4.98−5.06 (4H, m, $PhCH_2$, H-4 and H-5), 6.06 (1H, s , H-6), 6.89 (1H, s, Ph_2CH), and 7.24−7.39 (15H, m). Anal. ($C_{34}H_{34}Cl_3N_3O_7$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-6-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-4-(trichloroacetamido)-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (29). Compound **29** was obtained by the similar procedures used for preparation of **17**: yield 95% (colorless foam); $[\alpha]^{26}_D$ −5.7° (*c* 0.92, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.45 (9H, s), 3.14 (1H, br t, J = 12.4 Hz, H_{ax}-2), 3.23 (1H, dt, J = 11.7 and 4.1 Hz, H-3), 3.84 (1H, br s, H-5), 4.24 (1H, br d, J = 12.2 Hz, H_{eq}-2), 4.52 (1H, dd, J = 11.5 and 2.7 Hz, H-4), 5.10 and 5.14 (2H, AB q, J = 12.7 Hz, $PhCH_2$), 5.87 (1H, d, J = 2.0 Hz, H-6), 6.83 (1H, s, Ph_2CH), and 7.24−7.38 (15H, m). Anal. ($C_{34}H_{36}Cl_3N_3O_8$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-4-Amino-6-[(benzyloxycarbonyl)amino-1-*N*-(*tert*-butoxycarbonyl)-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (30). Compound **30** was obtained by the similar procedures used for preparation of **19**: yield 68%; $[\alpha]^{26}_D$ +6.3° (*c* 1.90, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.44 (9H, s), 2.84 (1H, dt, J = 11.4 and 4.1 Hz, H-3), 2.99 (1H, t, J = 12.5 Hz, H_{ax}-2), 3.22 (1H, dd, J = 10.7 and 2.9 Hz, H-4), 3.74 (1H, br s, H-5), 4.22 (1H, dd, J = 13.4 and 4.6 Hz, H_{eq}-2), 5.09 (2H, s, $PhCH_2$), 5.86 (1H, d, J = 1.5

Hz, H-6), 6.90 (1H, s, Ph_2CH), and 7.25−7.40 (15H, m). Anal. ($C_{32}H_{37}N_3O_7$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-6-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-4-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (31). To a solution of **30** (81 mg, 0.14 mmol) in CH_3OH (1.6 mL) were added *N*,*N*′-diisopropylethylamine (73.2 μL, 0.42 mmol) and di-*tert*-butyl dicarbonate (64.3 μL, 0.28 mmol), and the mixture was stirred at room temperature for 1 h. Evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with 10% citric acid aqueous solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid, which was subjected to column chromatography on silica gel. Elution with toluene−acetone (8:1) gave **31** (87 mg, 91%) as a colorless amorphous solid: $[\alpha]^{26}_D$ −3.1° (*c* 0.93, CH_3OH); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.33 and 1.42 (9H, each s), 2.96 (1H, dt, *J* = 11.7 and 4.1 Hz, H-3), 3.11 (1H, t, *J* = 12.7 Hz, H_{ax}-2), 3.77 (1H, br m, H-5), 4.12 (2H, br m, 2-H_{eq} and H-4), 5.08 and 5.11 (2H, AB q, *J* = 12.5 Hz, PhCH_2), 5.83 (1H, d, *J* = ∼2.0 Hz, H-6), 6.81 (1H, s, Ph_2CH), and 7.23−7.37 (15H, m). Anal. ($C_{37}H_{45}N_3O_9$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-6-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-4-[(*tert*-butoxycarbonyl)amino]-5-hydroxy-3-[[(2-methoxyethoxy)methoxy]carbonyl]piperidine (32). To a solution of **31** in CH_3OH (0.4 mL) was added 1 M NaOH aqueous solution (0.15 mL), and the mixture was stirred at room temperature for 1.5 h. Evaporation of the solvent gave a solid, which was dissolved in $CHCl_3$. The solution was washed with 10% citric acid aqueous solution, and the aqueous phase was extracted two times with $CHCl_3$. The organic phases were combined, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was dissolved in CH_2Cl_2 (0.4 mL). To the solution were added *N*,*N*′-diisopropylethylamine (6.3 μL, 36 μmol) and chloromethyl (2-methoxy)ethyl ether (4.1 μL, 36 μmol) at 0 °C, and the mixture was stirred for 1.5 h. After dilution with $CHCl_3$, the solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave an oil, which was subjected to preparative thin-layer chromatography on silica gel developed with toluene−acetone (3:1) to give **32** (15 mg, 83%) as a colorless foam: $[\alpha]^{25}_D$ −2.4° (*c* 0.67, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.42 and 1.44 (9H, s), 2.84 (1H, dt, *J* = 11.7 and 4.4 Hz, H-3), 3.15 (1H, br t, *J* = 13.2 Hz, H_{ax}-2), 3.36 (3H, s, OCH_3), 3.55 and 3.79 (2H, each m, OCH_2CH_2O), 3.77 (1H, br m, H-5), 4.06 (1H, dd, *J* = 11.2 and 2.5 Hz, H-4), 4.14 (1H, dd, *J* = 13.2 and 3.7 Hz, H_{eq}-2), 5.10 and 5.13 (2H, AB q, *J* = 12.5 Hz, PhCH_2), 5.28 and 5.31 (2H, AB q, *J* = 6.1 Hz, CO_2CH_2O), 5.83 (1H, d with a small coupling, H-6), and 7.27−7.39 (5H, m). Anal. ($C_{28}H_{43}N_3O_{11}$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-1-*N*-(*tert*-Butoxycarbonyl)-4-[(*tert*-butoxycarbonyl)amino]-6-(trifluoroacetamido)-5-hydroxy-3-[[(2-methoxyethoxy)methoxy]carbonyl]piperidine (33). The solution of **32** (43 mg, 72 μmol) in CH_3CN (2 mL) was stirred with 10% Pd/C (15 mg) under an atmosphere of H_2 at room temperature for 30 min. After removal of catalysts, evaporation of the solvent gave an oil, which was dissolved in CH_2Cl_2 (0.8 mL). To the solution were added pyridine (13 μL, 0.16 mmol) and trifluoroacetic anhydride (11 μL, 80 μmol), and the mixture was stirred at 0 °C for 20 min. Portions of pyridine (13 μL) and trifluoroacetic anhydride (11 μL) were then added to the mixture, and the mixture was further stirred at 0 °C for 20 min. After dilution with $CHCl_3$, the solution was washed with $NaHCO_3$-saturated aqueous solution and water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative thin-layer chromatography to give **33** (27 mg, 68%) as a colorless foam: $[\alpha]^{27}_D$ +8.8° (*c* 1.08, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.43 and 1.46 (9H, each s), 2.92 (1H, dt, *J* = 11.7 and 4.0 Hz, H-3), 3.27 (1H, br t, *J* = 11.7 Hz, H_{ax}-2), 3.37 (3H, s, OCH_3), 3.55 (2H, m, $OCH_2CH_2OCH_3$), 3.81 (3H, m, OCH_2-CH_2OCH_3 and H-5), 4.04 (1H, br d, *J* = 10.7 Hz, H-4), 4.21 (1H, dd, *J* = 11.7 and 3.9 Hz, H_{eq}-2), 5.30 and 5.33 (2H, AB q, *J* = 6.1 Hz, CO_2CH_2O), and 6.01 (1H, s, H-6). Anal. ($C_{22}H_{36}F_3N_3O_{10}$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-4-Amino-6-(trifluoroacetamido)-5-hydroxypiperidine-3-carboxylic Acid (9). Compound **9** was obtained by the similar procedures used for preparation of **6**: yield 100% (colorless amorphous solid as hydrochloride); $[\alpha]^{29}_D$ +22.1° (*c* 0.36, 1 M HCl); ^{1}H NMR (D_2O, 400 MHz) δ 3.07 (1H, dd, *J* = 11.2 and 4.0 Hz, H-3), 3.15 (1H, t, *J* = 12.7 Hz, H_{ax}-2), 3.50 (1H, dd, *J* = 12.7 and 4.0 Hz, H_{eq}-2), 3.93 (1H, dd, *J* = 11.2 and 2.9 Hz, H-4), 4.25 (1H, t, *J* = 2.9 Hz, H-5), and 5.41 (1H, d, *J* = 2.9 Hz, H-6); FAB-HRMS calcd for $C_8H_{13}F_3N_3O_4$ (M + H) 272.0859, found 272.0861.

(3*R*,4*S*,5*S*,6*S*)-6-[(Benzyloxycarbonyl)amino]-1-*N*-(*tert*-butoxycarbonyl)-4-[*N*,*N*′-bis(*tert*-butoxycarbonyl)guanidino]-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (34). Compound **34** was obtained by the similar procedures used for preparation of **21**: yield 98% (colorless foam); $[\alpha]^{25}_D$ −7.7° (*c* 0.98, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.46, 1.47 and 1.48 (9H, each s), 2.95 (1H, dt, *J* = 11.7 and 3.9 Hz, H-3), 3.13 (1H, br t, *J* = 12.7 Hz, H_{ax}-2), 3.74 (1H, br s, H-5), 4.14 (1H, br d, *J* = 12.7 Hz, H_{eq}-2), 5.11 (2H, br s, PhCH_2), 5.85 (1H, br s, H-6), 6.80 (1H, s, Ph_2CH), and 7.24−7.38 (15H, m). Anal. ($C_{43}H_{55}N_5O_{11}\cdot{}^1/_2H_2O$) C, H, N.

(3*R*,4*S*,5*S*,6*S*)-1-*N*-(*tert*-Butoxycarbonyl)-4-[*N*,*N*′-bis(*tert*-butoxycarbonyl)guanidino]-6-(trifluoroacetamido)-5-hydroxy-3-[(diphenylmethoxy)carbonyl]piperidine (35). Compound **35** was obtained by the similar procedures used for preparation of **33**: 33% yield (conversion yield 52%); $[\alpha]^{24}_D$ −6.7° (*c* 0.78, $CHCl_3$); ^{1}H NMR (CD_3OD, 40 °C, 400 MHz) δ 1.471, 1.477, and 1.480 (9H, each s), 3.01 (1H, dt, *J* = 11.7 and 4.4 Hz, H-3), 3.19 (1H, br t, *J* = 12.2 Hz, H_{ax}-2), 3.84 (1H, br s, H-5), 4.21 (1H, br d, *J* = 12.6 Hz, H_{eq}-2), 6.04 (1H, br d, *J* = 1.5 Hz, H-6), 6.82 (1H, s, Ph_2CH), and 7.26−7.34 (10H, m). Anal. ($C_{37}H_{48}F_3N_5O_{10}\cdot{}^1/_2H_2O$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-6-(Trifluoroacetamido)-4-guanidino-5-hydroxypiperidine-3-carboxylic Acid (10). Compound **10** was obtained by the similar procedures used for preparation of **6**: yield 100% (colorless amorphous solid); $[\alpha]^{29}_D$ +1.3° (*c* 0.47, 1 M HCl); ^{1}H NMR (D_2O, 400 MHz) δ 3.27 (1H, dt, *J* = 11.0 and 4.1 Hz, H-3), 3.38 (1H, t, *J* = 12.0 Hz, H_{ax}-2), 3.55 (1H, dd, *J* = 12.0 and 4.1 Hz, H_{eq}-2), 4.25 (1H, t, *J* = 3.9 Hz, H-5), 4.39 (1H, dd, *J* = 11.0 and 3.9 Hz, H-4), and 5.42 (1H, d, *J* = 3.9 Hz, H-6); FAB-HRMS calcd for $C_9H_{15}F_3N_5O_4$ (M + H) 314.1078, found 314.1056.

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