Articles

Flexible Synthesis and Biological Activity of Uronic Acid-Type *gem*-Diamine 1-*N*-Iminosugars: A New Family of Glycosidase Inhibitors

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An efficient and flexible synthetic route to four *gem*-diamine 1-*N*-iminosugars of uronic acid-type (D-glucuronic, D-mannuronic, L-iduronic, and L-guluronic acid), a new family of glycosidase inhibitor, from l-galactono-1,4-lactone have been developed in an enantiodivergent fashion through a sequence involving as the key steps (a) the formation of *gem*-diamine 1-*N*-iminopyranose ring by the Mitsunobu reaction of an aminal and (b) the introduction of a carboxylic acid group by the Wittig reaction of a ketone, hydroboration and oxidation, and the Sharpless oxidation. D-Glucuronic and D-mannuronic acid-type 1-*N*-iminosugars, (3*S*,4*R*,5*R*,6*R*)- and (3*S*,4*R*,5*R*,6*S*)-4,5-dihydroxy-6-trifluoroacetamido-3-piperidinecarboxylic acid, were proven to be potent inhibitors for *β*-D-glucuronidase (IC_{50} 6.5×10^{-8}M) and to affect human heparanase (*endo*-*β*-glucuronidase).

Introduction

Currently, specific inhibitors of glycosidases aid in developing an understanding of the metabolism of oligosaccharides in glycoconjugates, functional domains for carbohydrate protein interactions involved in a variety of biological functions such as immune response, oncogenesis, metastasis of tumors, viral and bacterial infections, differentiation of neuronal cells, and so forth.[1] The possible applications in antimetastatic,[2] antitumor,[3] antiviral,[4] antibacterial,[5] or immunoregulatory agents[6] stimulate interest into the relationships between structures and specific biological functions. In the course of our study on glycosidase inhibitors, we proposed a new class of glycosidase inhibitors, *gem*-diamine 1-*N*-iminosugars (**1**),[7] in which an anomeric carbon atom is replaced by a nitrogen (Figure 1). We considered that the protonated form of *gem*-diamine 1-*N*-iminosugars (**1**) may mimic glycopyranosyl cation (**3**), the putative transition state of enzymatic glycosidic hydrolysis (Figure 1). We have also demonstrated that *gem*-diamine 1-*N*-iminosugars (**1**), especially 2-trifluoroacetamido-1-*N*-iminosugars (**2**) (Figure 1), are highly potent and specific glycosidase inhibitors[7a−c] and that some of them show the potent suppression of experimental and spontaneous pulmonary metastasis of tumor cells in mice.[7a,b,8]

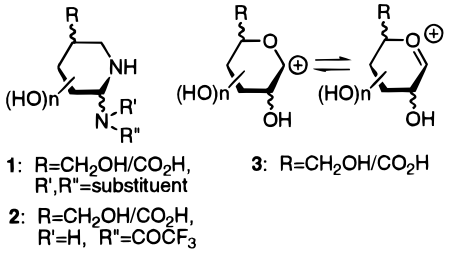

Figure 1. *gem*-Diamine 1-*N*-iminosugars (**1** and **2**) and glycopyranosyl cation (**3**), the putative transition state of enzymatic glycosidic hydrolysis.

On the other hand, uronic acids (D-glucuronic acid (**4**) and L-iduronic acid (**5**)) (Figure 2) are the major constituents of the mammalian glycosaminoglycans (hyalu-

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ronic acid, dermatan sulfate, heparan sulfate, heparin, and chondroitin sulfate) of endotherial basement membranes and extracellular matrix. β-D-Glucuronidase and α-L-iduronidase are known to degrade the mammalian glycosaminoglycans,[9,10] and heparanase (*end*-β-D-glucuronidase) activity in murine B16 melanoma cells correlates with lung colonization ability by degradation of heparan sulfate.[11a] Furthermore, glucuronidase inhibitors inhibit pulmonary colonization of tumor cells in their syngeneic host.[11b,c] Alginate-like polysaccharides, the major constituents of glycocalyx biofilm, secreted by mucoid strains of *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis[12] are also composed of uronic acids (D-mannuronic acid (**6**) and L-guluronic acid (**7**)) (Figure 2).[13] They are known to interfere with the host defense mechanisms,[14] to provide a barrier against penetration of drugs,[15] and to complicate the bronchial obstruction.[16] Inhibitors of alginate biosynthetic enzymes may prove useful in chemotherapy of *P. aeruginosa* infections in cystic fibrosis patients. We now report the extension of our study on glycosidase inhibitor of *gem*-diamine 1-*N*-iminosugar to the divergent synthesis of uronic acid-type 1-*N*-iminosugars, D-glucuronic acid (**8**), L-iduronic acid (**9**), D-mannuronic acid (**10**), and L-guluronic acid (**11**) from L-galactono-1,4-lactone (**12**) (Figure 3).

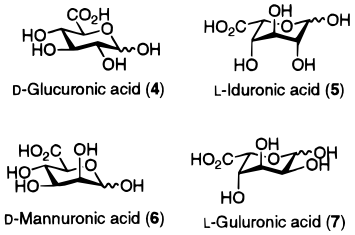

Figure 2. Structures of uronic acids.

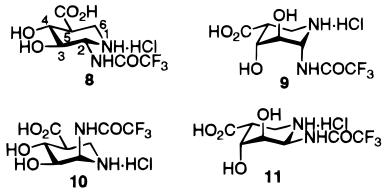

Figure 3. Structures of uronic acid-type *gem*-diamine 1-*N*-iminosugars.

Results and Discussion

Synthesis. Since our interest in this class of inhibitors against metabolic enzymes of glycuronides encompassed four enantiomerically pure stereoisomers, we developed a flexible divergent strategy utilizing L-galactono-1,4-lactone (**12**) as a chiral source (Scheme 1). We have recently reported an efficient approach to the multifunctionalized *gem*-diamine 1-*N*-iminosugars.[7] This methodology, conveniently modified, should allow the introduction of the diastereomeric amino and carboxylic acid substituents at C-2 and C-5, respectively, into the versatile aminal **24** (Scheme 1). The synthesis of the aminal **24** began with the known 5,6-*O*-isopropylidene-L-galactono-1,4-lactone (**13**),[17] which was converted into the diol **15** upon protection and lithium aluminum hydride reduction in good yield. Selective protection of the hydroxymethyl group with the *tert*-butyldiphenylsilyl (TBDPS) group in **15** followed by the Dess−Martin oxidation[18] gave the ketone **17** in 93% yield. One-carbon extension of **17** was carried out by the Wittig reaction with methylenetriphenylphosphorane to afford the methylene **18** in 96% yield. Removal of the isopropylidene group gave the diol **19**, which was successfully transformed into the monoalcohol **20** upon the Luche reduction[19] of the labile aldehyde intermediate obtained by periodate oxidation. Conversion of the hydroxyl group to the amino function proved troublesome until it was discovered that the corresponding sulfonate was converted into the azido **21** by one-pot reaction in situ in 89% yield. Selective reduction of the azide group with metal hydrides such as sodium and lithium borohydride,

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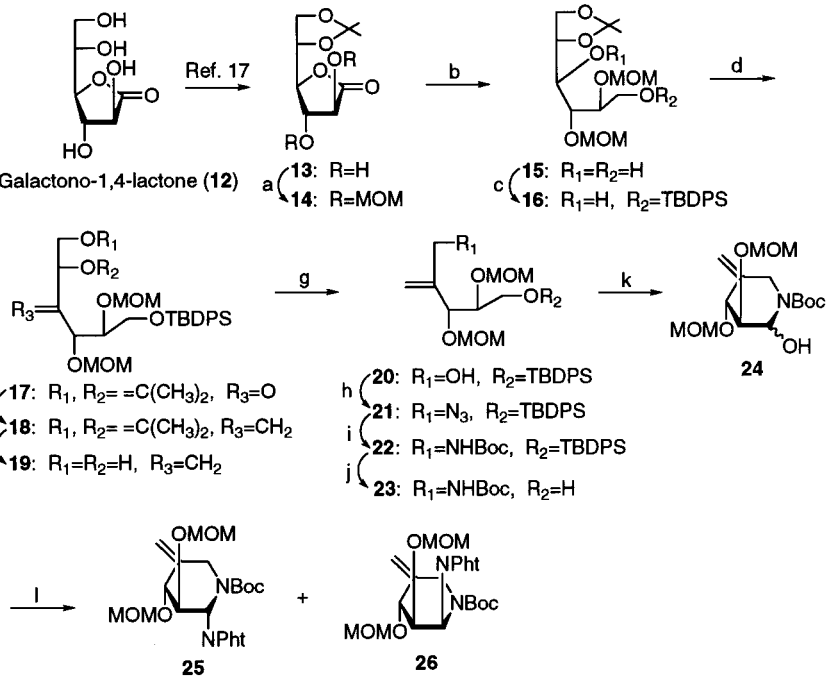
Scheme 1. Synthesis of Uronic Acid-Type *gem*-Diamine 1-*N*-Iminosugars[a]



[a] Key: (a) CH_3OCH_2Cl, *n*-Bu_4NI, *i*-Pr_2NEt, 70 °C, 81%; (b) $LiAlH_4$, THF, 100%; (c) *t*-bu(Ph_2)SiCl, *i*-Pr_2NEt, DMAP, CH_2Cl_2, 99.7%; (d) Dess−Martin periodinane, CH_2Cl_2, 93%; (e) Ph_3PCH_3Br, *n*-BuLi, THF, −78 °C, 96%; (f) 80% AcOH, rt, 99%; (g) $NaIO_4$, CH_3CN/H_2O; $NaBH_4$, $CeCl_3$, MeOH, 88%; (h) MsCl, py; NaN_3, DMF, 88.7%; (i) Te, $NaBH_4$, EtOH; (*t*-$BuCO)_2O$, *i*-Pr_2NEt, DMF, 88%; (j) *n*-Bu_4NF, THF, 100%; (k) $(COCl)_2$, DMSO, CH_2Cl_2, 93%; (l) PPh_3, DEAD, phthalimide, DMF, **25**: 61.4%; **26**: 20%.

lithium aluminum hydride, diisobutylaluminum hydride, and K- and L-Selectride proceeded unfavorably to yield a complicated mixture. This problem was circumvented by catalytic hydrogenation with sodium hydrogentelluride (NaTeH)[20] generated in situ from tellurium and sodium borohydride in ethanol. The desired amide **22** was obtained effectively by the subsequent protection with a *tert*-butyloxycarbonyl (*t*-Boc) group in 88% yield. Removal of a TBDPS group and the Swern oxidation[21] afforded the pivotal intermediate **24** as an epimeric mixture in 93% yield. Replacement of the aminal hydroxyl group to the amino group was achieved by the Mitsunobu reaction[22] (PPh_3, diethyl azodicarboxylate, phthalimide) in *N*,*N*′-dimethylformamide (DMF) to give both the desired epimers of iminophthalimides **25** and **26** in 61 and 20%, respectively. Epimer **25** was crystallized from *n*-hexane to yield a single crystal for X-ray diffraction analysis. The X-ray analysis clearly indicated the desired absolute stereochemistry and a boat conformation (Figure 4). Another epimer, **26**, was consequently assigned its absolute stereochemistry and also its presumed a boat conformation by its ^1H NMR spectrum. Hydroboration of **25** with borane−methyl sulfide complex followed by oxidation with hydrogen peroxide efficiently gave the D-*gluco* isomer **27** and the L-*idulo* isomer **28** in 17 and 77% yield, respectively (Scheme 2). Hydrazinolysis of **27** and conventional trifluoroacetylation furnished the trifluoroacetamide **29** in 90% yield. Conversion of the hydroxymethyl group of **29** 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.[23] in 91% yield. Simultaneous removal of both the methoxymethyl (MOM) and *t*-Boc groups in **30** with 4 M hydrogen chloride in dioxane afforded quanti-

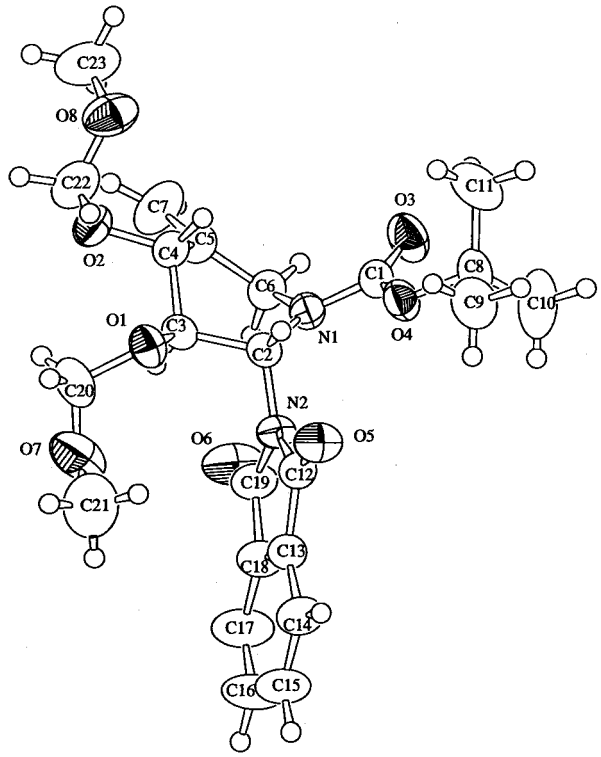


Figure 4. ORTEP drawing of compound **25**.

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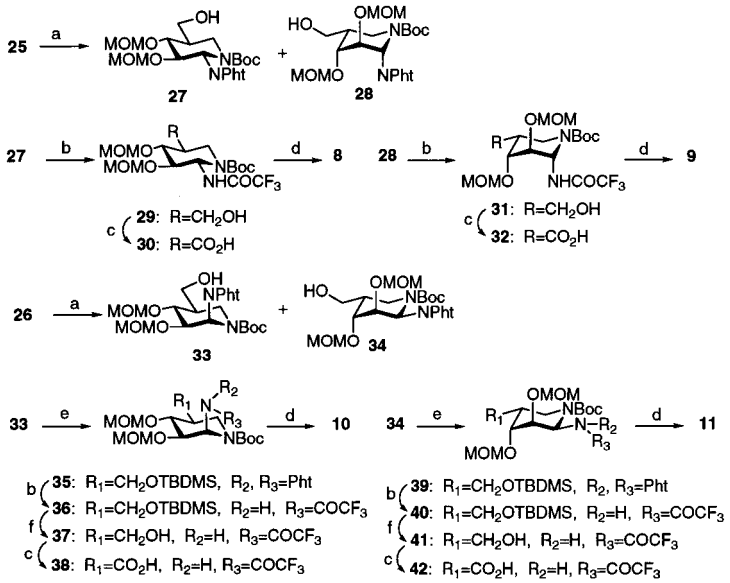
Scheme 2. Synthesis of Uronic Acid-Type *gem*-Diamine 1-*N*-Iminosugars[a]



[a] Key: (a) BH_3·Me_2S, THF; H_2O_2, 2 M NaOH/H_2O, **27**: 16.6% **28**: 77.1%; **33**: 50%, **34**: 38%; (b) $H_2NNH_2.xH_2O$, MeOH; $(CF_3CO)_2O$, py, CH_2Cl_2, **29**: 90%; **31**: 87%; **36**: 88%; **40**: 79%; (c) RuO_2, $NaIO_4$, CCl_4/CH_3CN/H_2O, **30**: 91%; **32**: 90%; **38**: 92%; **42**: 87%; (d) 4 M HCl/dioxane, **8**: 99.7%; **9**: 99%; **10**: 99.7%; **11**: 91%; (e) *t*-Bu(Me_2)SiCl, imidazole, DMF, **35**: 91%; **39**: 100%; (f) *n*-Bu_4NF, THF, **37**: 93%; **41**: 100%.

tatively the final D-glucuronic acid-type 2-trifluoroacetamido-1-*N*-iminosugar **8** as a hydrochloride. The ^{1}H NMR spectrum of D-glucuronic acid-type **8** shows the large coupling constants ($J_{2,3} = J_{3,4} = 9.3$ Hz, $J_{4,5} = 10.5$ Hz and $J_{5,6ax} = 13.0$ Hz) and a small coupling constant ($J_{5,6eq} = 4.4$ Hz), clearly indicative of the $^4C_1$-conformation and the correct stereochemistry. The same sequence of reactions also successfully resulted in L-iduronic acid-type 2-trifluoroacetamido-1-*N*-iminosugar **9** from **28**. The coupling constants ($J_{2,3} = J_{3,4} = 6.4$ Hz, $J_{4,5} = J_{5,6eq} = 3.9$ Hz and $J_{5,6ax} = 7.3$ Hz) of the ^{1}H NMR spectrum of L-iduronic acid-type **9** are indicative of the boat conformation. On the other hand, D-mannuronic acid-type and L-guluronic acid-type 2-trifluoroacetamido-1-*N*-iminosugars (**10** and **11**) were straightforwardly obtained from **26** by a similar sequence of structure changes varying in the protection of the hydroxymethyl groups of **33** and **34** with a *tert*-butyldimethylsilyl (TBDMS) group prior to hydrazinolysis of the phthalimide group for improvement in yield. The protons of H-2 (d, $J_{2,3} = 2$ Hz), H-3 (dd, $J_{3,4} = 4$ Hz), H-4 (t, $J_{4,5} = 4$ Hz), and H-5 (dt, $J_{5,6ax} = 4$ Hz and $J_{5,6eq} = 1.6$ Hz) appear in the ^{1}H NMR spectrum of D-mannuronic acid **10**. This indicated that **10** has the boat conformation as opposed to the $^4C_1$ conformation of D-glucuronic acid **8**. On the other hand, the ^{1}H NMR spectrum of L-guluronic acid **11** shows the protons of H-2 (d, $J_{2,3} = 1.7$ Hz), H-3 (dd, $J_{3,4} = 3.9$ Hz), H-4 (dd, $J_{4,5} = 2.9$ Hz), and H-5 (ddd, $J_{5,6eq} = 5.4$ Hz and $J_{5,6ax} = 13.0$ Hz), clearly indicating the $^1C_4$ conformation with the exact stereochemistry. This is distinct from the boat conformation of L-iduronic acid type **9**.

Biological Activity. The inhibitory activities of uronic acid-type *gem*-diamine 1-*N*-iminosugars (present **8**−**11** and previous **45**) for *β*-glucuronidase (bovine liver), α-glucosidase (bakers' yeast), *β*-glucosidase (almonds), α-mannosidase (jack beans), *β*-mannosidase (snail), *β*-galactosidase (*Aspergillus niger*), α-*N*-acetylgalactosaminidase (chicken liver), and *β*-*N*-acetylglucosaminidase (bovine epididymis) are summarized in Table 1. As expected,

Table 1. Inhibitory Activities (IC_{50} (M)) of 8, 9, 10, 11, and 45 against Glycosidases

enzyme	8	9	10	11	45
β-glucuronidase[a]	6.5×10^{-8}	1.3×10^{-4}	6.5×10^{-8}	NI[i]	6.5×10^{-8}
α-glucosidade[b]	NI	NI	NI	NI	NI
β-glucosidase[c]	9.8×10^{-5}	NI	3.6×10^{-5}	NI	1.3×10^{-5}
α-mannosidase[d]	NI	NI	NI	NI	NI
β-mannosidase[e]	NI	NI	NI	NI	NI
β-galactosidase[f]	NI	NI	NI	NI	1.3×10^{-6}
α-*N*-acetylgalactosaminidase[g]	NI	NI	NI	NI	NI
β-*N*-acetylglucosaminidase[h]	NI	NI	NI	NI	NI

[a] Bovine liver. [b] Bakers' yeast. [c] Almonds. [d] Jack beans. [e] Snail. [f] *Aspergillus niger*. [g] Chicken liver. [h] Bovine epididymis. [i] NI: no inhibition at 3.3×10^{-3} M.

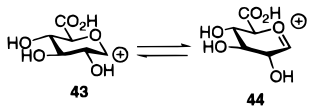


Figure 5. Effect of resonance contributors (**43** and **44**) to the putative glycosyl cation intermediate formed during hydrolysis by *β*-D-glucuronidase.

D-glucuronic acid-type *gem*-diamine 1-*N*-iminosugar **8** affected *β*-D-glucuronidase very potently. This is rationalized that **8** should closely mimic a glycopyranosyl cation **43**, one of the resonance contributors (the chairlike and the flattened conformational cation **43** and **44**, respectively) of the putative glycopyranosyl cation intermediate formed during hydrolysis by *β*-D-glucuronidase (Figure 5).[7d,24] It is interesting that D-mannuronic acid-type *gem*-diamine 1-*N*-iminosugar **10** also inhibited very strongly *β*-D-glucuronidase. On the other hand, the conformation of **10** is indicated to be a boat conformation by its ^{1}H NMR

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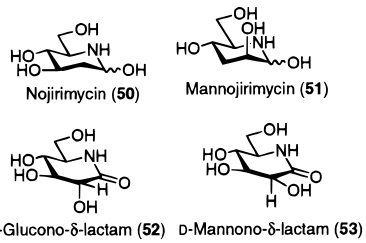

Figure 6. Structures of aza-sugars and δ-lactams.

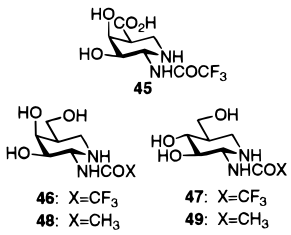

Figure 7. Structures of uronic acid- and glycose-type *gem*-diamine 1-*N*-iminosugars.

spectrum. These facts suggest that **10** is highly likely a mimic of the another glycopyranosyl cation, the flattened conformational cation **44** in the course of the enzymatic reaction (Figure 5). This result is also compatible with the well-known results[25] in which mannojirimycin (**51**) and D-mannono-δ-lactam (**53**) show somewhat stronger inhibitory activities against glucosidase than those of nojirimycin (**50**) and D-glucono-δ-lactam (**52**), respectively (Figure 6). It was also previously reported that D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugar **45** is proved to be a potent glucuronidase inhibitor (Table 1).[26] In the case of *gem*-diamine 1-*N*-iminosugar glycosidase inhibitors, hydrolases recognizing the D-gluco-configuration such as D-glucosidase, *N*-acetyl-D-glucosaminidase, and D-glucuronidase similarly recognize the D-*galacto*-configuration, IC_{50} (M, almond β-glucosidase) 4.7×10^{-7} (**46**)[27] and 4.4×10^{-7} (**47**);[28] IC_{50} (M, bovine epididymis-*N*-acetyl-β-D-glucosaminidase) 1.9×10^{-6} (**48**)[7c] and 1.2×10^{-5} (**49**);[28] IC_{50} (M, bovine liver β-glucuronidase) 6.5×10^{-8} (**8**) and 6.5×10^{-8} (**45**) (Figure 7).[26] These results indicate that the binding group corresponding to the 4-OH in these enzymes is not important for specificity of the inhibitors. Compounds **8**−**11** and **45** were also evaluated the inhibitory activity against recombinant human heparanase (*endo*-β-glucuronidase) from human melanoma A375M cell transfected with pBK-CMV expression vectors containing the heparanase cDNA.[29] Heparanase cleaves the β-1,4-linkage between glucuronic acid (GlcUA) and *N*-acetylglucosamine (GlcNAc) in heparan sulfate.[9c] As shown in Table 2, **8**, **10**, and **45** showed inhibition against the enzyme. The result also proves the relationships between structure and activity similar to those discussed on the inhibition against *exo*-β-glucuronidase from bovine liver. Furthermore, the weak activities against heparanase compared with *exo*-β-glucuronidase indicate that heparanase should recognize simultaneously glucuronic acid and the adjacent glycoses on the both sides. Compounds **8**, **10**, and **45** are also shown to be moderate glucosidase inhibitors, and **45** shows good inhibition against galactosidase, indicating that even a charged carboxyl function assists the binding to these enzymes. On the other hand, as expected, L-uronic acid-type *gem*-diamine 1-*N*-iminosugars **9** and **11** showed no remarkable inhibition against these D-sugar hydrolases. These results indicate that glycohydrolases recognize precisely the absolute configurations of *gem*-diamine 1-*N*-iminosugars corresponding to D- and L-sugars for specificity and potency. Compounds **8**−**11** and **45** were also assayed in vitro for inhibition of biosynthesis of glycocalyx bifilm (alginate-like polysaccharides) composed of D-mannuronic acid (**6**) and L-guluronic acid (**7**) produced by *Pseudomonas aeruginosa* A3. All compounds showed no inhibition at a dose of 3.3×10^{-3}M (see the Experimental Section). However, it is not clear at this stage whether D-mannuronic acid-type and L-guluronic acid-type 1-*N*-iminosugars (**10** and **11**) may inhibit the metabolism of glycocalyx biofilm or penetrate through the cell-membrane of *Pseudomonas aeruginosa*. Further evaluation of biological activities (antimetastatic, antibacterial, etc.) of these analogues are now in progress.

Table 2. Inhibitory Activities (IC_{50}) of 8, 10, and 45 against Human Heparanase[a]

compd	IC_{50} (μM)
8	10.55 ± 1.01
9	NI
10	28.99 ± 11.41
11	NI
45	1.02 ± 0.29

[a] NI: no inhibition at 3.3 mM. Buffer: 50 mM AcNA, pH 4.2, 0.02% CHAPS. Enzyme: heparanase 0.26 μg protein/tube. Substrate: FITC−Heparan sulfate 0.5 μL (5 μg HS). Incubation time: 37 °C, 2 h.

In summary, an efficient and flexible synthetic route to uronic acid-type *gem*-diamine 1-*N*-iminosugars from a readily available L-galactono-1,4-lactone has been developed and has produced the highly potent inhibitors of β-glucuronidase. The present *gem*-diamine 1-*N*-iminosugars may contribute to the study of the involvement of carbohydrates in diseases associated with oligosaccharide biosynthesis and degradation and also to the development of drugs for these diseases. That these *gem*-diamine 1-*N*-iminosugars are potent inhibitors of β-glucuronidase further supports the hypothesis of our design of the new type inhibitor.

Experimental Section

5,6-*O*-Isopropylidene-2,3-di-*O*-(methoxymethyl)-L-*galactono*-1,4-lactone (14). To a solution of **13**[17] (6 g, 27.5 mmol) in *N*,*N*-diisopropylethylamine (57.5 mL, 330 mmol) were added chloromethyl methyl ether (12.5 mL, 110 mmol) and tetra-*N*-butylammonium iodide (10.1 g, 27.5 mmol), and the mixture was stirred at 70 °C for 3 h. Evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (4:1) gave **14** as a colorless solid (7.6 g, 90%). The solid was crystallized from *n*-hexane to give colorless

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crystals of **14**: $[\alpha]^{23}_D$ +13.7° (*c* 0.3, $CHCl_3$); mp 58 °C; NMR ($CDCl_3$, 400 MHz) δ 1.38 and 1.41 (3H, s each), 3.41 and 3.43 (3H, s each), 3.99 (1H, dd, J = 6.8, 7.3 Hz), 4.11 (1H, dd, J = 6.8, 7.3 Hz), 4.20 (1H, dd, J = 2.6, 6.8 Hz), 4.38 (1H, dt, J = 2.6, 6.8 Hz), 4.47 (1H, t, J = 7.0 Hz), 4.53 (1H, d, J = 7.0 Hz), 6.84 and 6.83 (2H, ABq, J = 6.8 Hz) and 4.78 and 5.06 (2H, ABq, J = 6.6 Hz). Anal. Calcd for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.94; H, 7.25.

1,2-*O*-Isopropylidene-4,5-di-*O*-(methoxymethyl)-D-galactitol (15). To a solution of **14** (10.35 g, 33.8 mmol) in THF (200 mL) was added $LiAlH_4$ (2.6 g, 68.5 mmol), and the mixture was stirred at room temperature for 1 h. After the mixture was quenched with a saturated aqueous Na_2SO_4 solution, an organic phase was taken by decantation. Evaporation of the solvent gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (4:1) gave **15** as an oil (10.5 g, 100%): $[\alpha]^{23}_D$ −5.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 and 1.45 (3H, s each), 2.59 (1H, d, J = 8.3 Hz), 3.42 (1H, m), 3.43 and 3.47 (3H, s each), 3.65∼3.80 (3H, m), 3.83 (1H, ddd, J = 6.6, 11.7 Hz), 3.97 (1H, t, J = 7.6 Hz), 4.02 (1H, dt, J = 2.0, 6.6 Hz), 4.06 (1H, t, J = 7.8 Hz), 4.31 (1H, dt, J = 1.5, 7.6 Hz), 4.68 and 4.81 (2H, ABq, J = 6.4 Hz) and 5.73 and 4.77 (2H, ABq, J = 5.8 Hz). Anal. Calcd for $C_{13}H_{26}O_8$: C, 50.31; H, 8.44. Found: C, 50.00; H, 8.52.

6-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-isopropylidene-4,5-di-*O*-(methoxymethyl)-D-galactitol (16). To a solution of **15** (8.8 g, 28.4 mmol) in CH_2Cl_2 (172 mL) were added TBDPSCl (9.6 mL, 36.9 mmol), *N*,*N*-diisopropylethylamine (20.3 mL, 116.5 mmol), and DMAP (4.3 g, 35.2 mmol), and the mixture was stirred at room temperature for 1 h. After the mixture was quenched with H_2O, evaporation of the solvent gave an oil. The oil was dissolved in $CHCl_3$, and the solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (7:1) gave **16** as an oil (12 g, 99.7%): $[\alpha[^{23}_D$ −10.2° (*c* 0.83, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (9H, s), 1.37 and 1.45 (3H, s each), 3.09 (1H, d, J = 7.6 Hz), 3.22 and 3.34 (3H, s each), 3.70 (1H, dt, J = 2.20, 7.6 Hz), 3.77 (1H, dd, J = 2.0, 7.6 Hz), 3.8−3.9 (2H, m), 3.9−4.1 (2H, m), 4.10 (1H, ddd, J = 2.0, 5.6, 6.8 Hz), 4.32 (1H, dt, J = 2.0, 7.6 Hz), 4.62 and 4.72 (2H, ABq, J = 6.6 Hz), 4.71 and 4.83 (2H, ABq, J = 6.6 Hz) and 7.3−7.7 (10H, m). Anal. Calcd for $C_{29}H_{44}O_8Si$: C, 63.47; H, 8.08. Found: C, 63.24; H, 8.06.

D-*threo*-D-*glycero*-6-*O*-(*tert*-Butyldiphenylsilyl)-1,2-*O*-isopropylidene-4,5-di-*O*-(methoxymethyl)-3-hexosulitol (17). To a solution of **16** (59.2 g, 139 mmol) in CH_2Cl_2 (800 mL) was added Dess−Martin periodinane (68.6 g, 161.7 mmol), and the mixture was stirred at room temperature for 2 h. After dilution with a large amount of ether, the solution was washed with a saturated aqueous $NaHCO_3$ solution and H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (7:1) gave **17** as an oil (55 g, 93%): $[\alpha]^{23}_D$ −5.1° (*c* 0.76, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (9H, s), 1.40 and 1.48 (3H, s each), 3.17 and 3.34 (3H, s each), 3.75−3.85 (2H, m), 4.12−4.23 (2H, m), 4.26 (1H, t, J = 8.0 Hz), 4.47 and 4.54 (2H, ABq, J = 6.8 Hz), 4.65 (1H, d, J = 2.4 Hz), 4.68 and 4.71 (2H, ABq, J = 6.6 Hz), 4.91 (1H, dd, J = 5.9, 8.0 Hz) and 7.3−7.7 (10H, m). Anal. Calcd for $C_{29}H_{42}O_8Si$: C, 63.71; H, 7.74. Found: C, 63.94; H, 7.79.

6-*O*-(*t*-Butyldiphenylsilyl)-3-deoxy-1,2-*O*-isopropylidene-4,5-di-*O*-(methoxymethyl)-3-methylene-D-*xylo*-hexitol (18). To a solution of methylenetriphenylphosphorane, prepared from methyltriphenylphosphonium bromide (8.9 g, 24.9 mmol) and *n*-butyllithium (1.6 M/L, 15.6 mL) in tetrahydrofuran (140 mL) from −78 °C to room temperature, was added a solution of **17** (6.8 g, 16.1 mmol), and the resulting mixture was stirred from −78 °C to room temperature overnight. After being quenched with saturated aqueous NH_4Cl solution, the mixture was extracted with ether (3 × 100 mL). The extracts were washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (7:1) gave **18** as an oil (6.5 g, 96%): $[\alpha[^{23}_D$ −34.5° (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (9H, s), 1.40 and 1.44 (3H, s each), 3.24 and 3.32 (3H, s each), 3.61 (1H, t, J = 7.8 Hz), 3.69 (1H, dt, J = 3.9, 5.6 Hz), 3.72−3.85 (2H, m), 4.14 (1H, dd, J = 6.3, 7.8 Hz), 4.30 (1H, d, J = 3.9 Hz), 4.50−4.65 (5H, m), 5.31 and 5.47 (1H, s each) and 7.20−7.80 (10H, m). Anal. Calcd for $C_{30}H_{44}O_7Si$: C, 66.12; H, 8.17. Found: C, 65.73; H, 7.93.

6-*O*-(*tert*-Butyldiphenylsilyl)-3-deoxy-4,5-di-*O*-(methoxymethyl)-3-methylene-D-*xylo*-hexitol (19). Compound **18** (6.5 g, 15.5 mmol) was dissolved in 80% acetic acid (325 mL), and the solution was stirred at room temperature overnight. Evaporation of the solvent gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (5:1) gave **19** as an oil (5.8 g, 99%): $[\alpha]^{23}_D$ −31.5° (*c* 0.82, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (9H, s), 2.50 (1H, br t, J = 5.4 Hz), 3.11 (1H, d, J = 3.9 Hz), 3.33 and 3.35 (3H, s each), 3.71 (1H, br t, J = 5.4 Hz), 3.75−3.90 (3H, m), 4.21 (1H, br q, J = 3.9 Hz), 4.35 (1H, d, J = 2.9 Hz), 4.65 and 4.68 (2H, ABq, J = 6.8 Hz), 4.69 and 4.72 (2H, ABq, J = 6.8 Hz), 5.44 and 5.48 (1H, s each) and 7.35−7.80 (10H, m). Anal. Calcd for $C_{27}H_{40}O_7Si$: C, 64.25; H, 7.99. Found: C, 64.02; H, 8.06.

2-Deoxy-6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(methoxymethyl)-2-methylene-D-*threo*-pentitol (20). To a solution of **19** (4.2 g, 11 mmol) in CH_3CN (110 mL) was added a solution of $NaIO_4$ (2.36 g, 11 mmol) in H_2O (24 mL), and the mixture was stirred at room temperature for 1 h. After dilution with ethyl acetate (200 mL), the resulting inorganic material was filtered off. The filtrate was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil (3.73 g), which was dissolved in CH_3OH (96 mL). To the solution were added $CeCl_3$ (3.9 g, 15.8 mmol) and $NaBH_4$ (592 mg, 15.6 mmol), and the mixture was stirred at room temperature for 1 h. After dilution with ethyl acetate (200 mL), the resulting precipitates were filtered off. The filtrate was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a viscous oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (15:1) gave **20** as an oil (3.4 g, 88%): $[\alpha[^{23}_D$ −41.3° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (9H, s), 2.41 (1H, br t, J = ∼6 Hz), 3.72−3.82 (2H, m), 3.87 (1H, dd, J = 4.4, 4.9 Hz), 4.05−4.420 (2H, m), 4.4 (1H, d, J = 4.4 Hz), 4.58 and 4.63 (2H, ABq, J = 6.6 Hz), 4.66 and 4.71 (2H, ABq, J = 6.6 Hz), 5.23 (1H, s), 5.30 (1H, s) and 7.3−7.8 (10H, m). Anal. Calcd for $C_{26}H_{38}O_6Si$: C, 65.79; H, 8.07. Found: C, 66.20; H, 8.23.

1-Azido-1,2-dideoxy-6-*O*-(*tert*-butyldiphenylsilyl)-3,4-di-*O*-(methoxymethyl)-2-methylene-D-*threo*-pentitol (21). To a solution of **20** (26.3 g, 75 mmol) in a mixture of CH_2Cl_2 (263 mL) and pyridine (53.8 mL, 665 mmol) was added CH_3SO_2Cl (7.72 mL, 99.7 mmol), and the mixture was stirred at room temperature for 1 h. After addition of NaN_3 (36 g, 554 mmol), evaporation of the solvent gave a viscous oil, which was suspended in DMF (263 mL). The resulting mixture was stirred at room temperature for 1 h. Evaporation of the solvent gave a viscous oil, which was dissolved in $CHCl_3$ (500 mL). The solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (10:1) gave **21** as an oil (25 g, 88.7%): $[\alpha[^{23}_D$ −4.8° (*c* 0.66, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (9H, s), 3.32 and 3.38 (3H, s each), 3.7−4.0 (5H, m), 4.48 (1H, d, J = 3.9 Hz), 4.66 and 4.69 (2H, ABq, J = 6.8 Hz), 4.67 and 4.73 (2H, ABq, J = 6.8 Hz), 5.37 (1H, s), 5.41 (1H, s) and 7.4−7.8 (10H, m). Anal. Calcd for $C_{26}H_{37}N_3O_5Si$: C, 62.49; H, 7.46; N, 8.41. Found: C, 62.51; H, 7.52; N, 8.37.

6-*O*-(*tert*-Butyldiphenylsilyl)-1-*tert*-butyloxycarbonylamido-1,2-dideoxy-3,4-di-*O*-(methoxymethyl)-2-methylene-D-*threo*-pentitol (22). To a suspension of tellurium powder (885 mg, 6.9 mmol) in EtOH (39 mL) was added $NaBH_4$ (315 mg, 8.3 mmol), and the mixture was refluxed with stirring under argon for 1 h. To the resulting purple solution was added dropwise a solution of **21** (1.73 g, 4.6 mmol) in EtOH (12 mL) with stirring, and the mixture was stirred at room temperature

for 1 h. After dilution with H_2O (20 mL), the mixture was stirred under air until the purple color disappeared. The resulting insoluble materials were filtered off, and the filtrate was extracted with CH_2Cl_2. The extracts were washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave an oil, which was subjected to flash column chromatography on silica gel. Elution with $CHCl_3$−CH_3OH (10:1) gave an oil (1.47 g). To a solution of the resulting oil in DMF (34 mL) were added *i*-Pr_2NEt (3.2 mL, 18 mmol) and di-*tert*-butyl dicarbonate (2.0 mL, 8.7 mmol), and the mixture was stirred at room temperature overnight. Evaporation of the solvent gave an oil, which was subjected to flash column chromatography on silica gel. Elution with $CHCl_3$−CH_3OH (50:1) gave **22** as an oil (1.82 g, 88%): $[\alpha]^{23}_D$ −34.1° (*c* 0.51, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (9H, s), 1.44 (9H, s), 3.27 and 3.31 (3H, s each), 3.70−3.80 (5H, m), 4.35 (1H, br s), 4.57 and 4.63 (2H, ABq, J = 6.8 Hz), 4.65 and 4.69 (2H, ABq, J = 6.6 Hz), 4.81 (1H, br s), 5.18 (1H, d, J = <1.0 Hz), 5.20 (1H, s) and 7.30−7.70 (10H, m). Anal. Calcd for $C_{31}H_{47}NO_7Si$: C, 64.89; H, 8.26. Found: C, 64.95; H, 8.36.

1-*tert*-Butyloxycarbonylamido-1,2-dideoxy-3,4-di-*O*-(methoxymethyl)-2-methylene-D-*threo*-pentitol (23). A solution of *n*-Bu_4NF (1 M/L, 4.63 mL) was added to a solution of **22** (1.77 g, 3.9 mmol) in THF (35 mL), and the mixture was stirred at room temperature for 1 h. Evaporation of the solvent gave an oil, which was subjected to flash column chromatography on silica gel. Elution with $CHCl_3$−CH_3OH (20:1) gave **23** as an oil (1.32 g, 100%): $[\alpha]^{23}_D$ −13° (*c* 0.53, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s), 2.88 (1H, t, J = 6.1 Hz), 3.39 and 5.48 (3H, s each), 3.55−3.85 (5H, m), 4.25 (1H, d, J = 5.9 Hz), 4.58 and 4.63 (2H, ABq, J = 6.6 Hz), 4.7 and 4.8 (2H, ABq, J = 6.8 Hz), 4.84 (1H, br s) and 5.2 (2H, s). Anal. Calcd for $C_{15}H_{29}NO_7$: C, 53.71; H, 8.72; N, 4.18. Found: C, 53.47; H, 8.75; N, 3.93.

5-*tert*-Butyloxycarbonylamido-4,5-dideoxy-2,3-di-*O*-(methoxymethyl)-4-methylene-D-*threo*-α- and -β-pentopyranose (24). Dimethyl sulfoxide (14.5 mL, 200 mmol) was added to the stirred solution of oxalyl chloride (9.0 mL, 100 mmol) in CH_2Cl_2 (280 mL) at −65 °C, and the mixture was stirred for 5 min. After addition of a solution of **23** (11.2 g, 33.4 mmol) in CH_2Cl_2 (24 mL) at −60 °C within 5 min, the mixture was stirred for 15 min. After addition of triethylamine (70 mL, 500 mmol), the mixture was stirred at the same temperature for 15 min, and then the mixture was allowed to warm to room temperature. After being quenched with water, the mixture was extracted with CH_2Cl_2. The extract was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to flash column chromatography on silica gel. Elution with toluene−acetone (10:1) gave **24** as an oil (10.4 g, 93%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (9H, s, (α and β)), 2.70−2.80 (1/3H, br s, (α)), 2.75−3.00 (2/3H, br s, (β)), 3.39 and 3.41 (3/3H each, s each, (α)), 3.43 and 3.45 (6/3H each, s each, (β)), 3.52 (2/3H, dd, J = 3.7, J = 9.8 Hz, (β)), 3.75 (2/3H, br d, J = 13.7 Hz, (β)), 3.70−3.85 (1/3H, br m, (α)), 3.97 (1/3H, br s, (α)), 4.29 (1/3H, d, J = 3.4 Hz, (α)), 4.20−4.45 (1H, br m, (α and β)), 4.47 (2/3H, d, J = 9.8 Hz, (β)), 4.59 and 4.70 (2/3H, ABq, J = 6.8 Hz, (α)), 4.70 and 4.73 (2/3H, ABq, J = 6.8 Hz, (α)), 4.74 and 4.82 (4/3H, ABq, J = 6.8 Hz, (β)), 4.78 and 4.86 (4/3H, ABq, J = 6.8 Hz, (β)), 5.09 and 5.19 (2/3H each, br s each, (β)), 5.17 and 5.35 (1/3H each, br s each, (α)), 5.55∼5.75 (1/3H, br m, (α)) and 5.80−5.90 (2/3H, br m, (β)).

(+)-(2*S*,3*R*,4*R*)-*N*-(*tert*-Butyloxycarbonyl)-3,4-di-*O*-(methoxymethyl)-5-methylene-2-phthalimido-3,4-piperidinediol (25) and (−)-(2*R*,3*R*,4*R*)-*N*-(*tert*-Butyloxycarbonyl)-3,4-di-*O*-(methoxymethyl)-5-methylene-2-phthalimido-3,4-piperidinediol (26). To the mixture of **24** (10.4 g, 31.2 mmol), triphenylphosphine (24.6 g, 93.8 mmol), and phthalimide (14 g, 93.8 mmol) in DMF (274 mL) was added dropwise diethyl azodicarboxylate (14.8 mL, 94 mmol) under stirring, and the resulting mixture was stirred at room temperature overnight. Addition of water and evaporation of the solvent gave an oil, which was dissolved in ether. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave a viscous solid, which was subjected to the column chromatography on silica gel. Elution with toluene−acetone (15:1) gave a colorless solid of **25** (8.86 g, 61.4%) and **26** as a colorless solid (2.74 g, 20%). **25** was crystallized from *n*-hexane to give colorless crystals of **25**.

25: $[\alpha]^{23}_D$ +98.5° (*c* 0.47, $CHCl_3$); mp 99 °C; NMR ($CDCl_3$, 400 MHz) δ 1.41 (9H, s), 3.02 and 3.43 (3H, s each), 4.28 (1H, dd, J = 1.5, 9.8 Hz), 4.33 (1H, dd, J = 6.8, 9.8 Hz), 4.43 (1H, dq, J = 1.9, 16.3 Hz), 4.61 and 4.85 (2H, ABq, J = 6.8 Hz), 4.68 (1H, br d, J = 16.3 Hz), 4.73 and 4.75 (2H, ABq), 5.18 and 5.28 (1H, br s each), 5.99 (1H, d, J = 6.8 Hz) and 7.70∼7.90 (4H, m). Anal. Calcd for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54; N, 6.06. Found: C, 59.81; H, 6.47; N, 5.94.

26: $[\alpha]^{23}_D$ −33.7° (*c* 0.81, $CHCl_3$); NMR (CD_3OD, 400 MHz, 40 °C) δ 1.38 (9H, br s), 3.30 and 3.43 (3H, s each), 4.02 (1H, dd, J = 6.6, 8.5 Hz), 4.33 and 4.56 (1H, br d each, J = 14.2 Hz), 4.65 and 4.69 (2H, ABq, J = 6.8 Hz), 4.47 and 4.79 (2H, ABq, J = 6.8 Hz), 4.78 (1H, br d, J = 6.6 Hz), 5.23 (1H, br s), 5.29 (1H, dq, J = 1.5 Hz), 6.51 (1H, br d, J = 8.5 Hz) and 7.80−7.95 (4H, m). Anal. Calcd for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54; N, 6.06. Found: C, 59.53; H, 6.36; N, 5.90.

(+)-(2*S*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-phthalimido-3,4-piperidinediol (27) and (+)-(2*S*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-phthalimido-3,4-piperidinediol (28). To a solution of **25** (6.37 g, 13.8 mmol) in THF (170 mL) was added a 2 M solution of BH_3·$(CH_3)_2S$ in THF (1 M/L, 18.4 mL, 18.4 mmol), and the mixture was stirred at room temperature overnight. To the mixture were added 2 M solution of NaOH in water (26.5 mL) and then 30% solution of H_2O_2 in water (17 mL), and the mixture was stirred at room temperature for 2 h. After dilution with CH_2Cl_2, the solution was washed with a saturated solution of NaCl in water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to the column chromatography on silica gel. Elution with toluene-acetone (10:1) gave **27** as a solid (1.1 g, 16.6%) and **28** as a solid (5.1 g, 77.1%).

27: $[\alpha]^{23}_D$ +109.5° (*c* 0.9, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.39 (9H, s), 2.05∼2.15 (1H, m), 2.75−2.85 (1H, br s), 2.87 and 3.41 (3H, s each), 3.48 (1H, dd, J = 5.4, 9.7 Hz), 3.53 (1H, dt, J = 7.3, 11.7 Hz), 3.79 (1H, dt, J = 5.9, 11.7 Hz), 3.95 (1H, dd, J = 5.1, 14.7 Hz), 4.02 (1H, br d, J = 14.7 Hz), 4.53 (1H, t, J = 9.7 Hz), 4.51 and 4.72 (2H, ABq, J = 6.8 Hz), 4.69 and 4.82 (2H, ABq, J = 6.6 Hz), 5.70 (1H, d, J = 9.7 Hz) and 7.70−7.90 (4H, m). Anal. Calcd for $C_{23}H_{32}N_2O_9$: C, 57.49; H, 6.71; N, 5.83. Found: C, 57.90; H, 6.83; N, 5.99.

28: $[\alpha]^{23}_D$ +67° (*c* 0.53, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.37 (9H, s), 2.53 (1H, m), 3.14 and 3.31 (3H, s each), 3.63 (1H, t, J = 13.5 Hz), 3.70−3.80 (2H, m), 3.96 (1H, dd, J = 6.3, 7.3 Hz), 4.04 (1H, dd, J = 6.8, 13.5 Hz), 4.40 (1H, dd, J = 5.7, 7.3 Hz), 4.60 and 4.72 (2H, ABq, J = 6.4 Hz), 4.72 and 4.75 (2H, ABq, J = 6.8 Hz), 5.92 (1H, d, J = 5.7 Hz) and 7.65−7.85 (4H, m). Anal. Calcd for $C_{23}H_{32}N_2O_9$: C, 57.49; H, 6.71; N, 5.83. Found: C, 57.30; H, 6.66; N, 5.67.

(−)-(2*S*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-trifluoroacetamido-3,4-piperidinediol (29). To a solution of **27** (447 mg, 0.93 mmol) in CH_3OH (5.6 mL) was added hydrazine hydrate (0.98 mL, 31.5 mmol), and the mixture was stirred at room temperature overnight. After dilution with $CHCl_3$, the resulting precipitates were filtered off. The filtrate was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to flash column chromatography on silica gel. Elution with $CHCl_3$−CH_3OH (10:1) gave an oil (355 mg). The oil was dissolved in CH_2Cl_2 (4 mL), and to the solution were added pyridine (0.18 mL, 2.2 mmol) and $(CF_3CO)_2O$ (0.16 mL, 1.1 mmol) at −20 °C. The mixture was stirred at room temperature for 30 min. After dilution with CH_2Cl_2, the solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The oil was subjected to preparative thin-layer chromatography on silica gel developed with $CHCl_3$−CH_3OH (15:1) to give **29** as an oil (375 mg, 90%): $[\alpha]^{23}_D$ −26.8° (*c* 0.99, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.48 (9H, s), 2.25−2.35 (1H, m), 2.75 (1H, t, J = 12.7 Hz), 3.42 and 3.44 (3H, s each), 3.59

(1H, dd, J = 8.8, 10.7 Hz), 3.65 (1H, dd, J = 5.9, 10.7 Hz), 3.79 (1H, br s), 3.94 (1H, br d, J = 12.7 Hz), 4.07 (1H, br s), 4.67 and 4.75 (2H, ABq, J = 6.8 Hz), 4.72 and 4.77 (2H, ABq), 6.28 (1H, br d, J = 6.3 Hz) and 8.00 (1H, br s). Anal. Calcd for $C_{17}H_{29}N_2O_8F_3$: C, 45.74; H, 6.55; N, 6.28. Found: C, 45.59; H, 6.40; N, 6.13.

(+)-(3*S*,4*R*,5*R*,6*S*)-*N*-(*tert*-Butyloxycarbonyl)-2,3-didehydro-4,5-di(methoxymethoxy)-6-trifluoroacetamido-3-piperidinecarboxylic Acid (30). To a solution of **29** (285 mg, 0.66 mmol) in a mixture of CCl_4 (3.8 mL) and CH_3CN (3.8 mL) were added a solution of $NaIO_4$ (293 mg, 1.4 mmol) in water (5.8 mL) and RuO_2 (10 mg, 0.075 mmol). The mixture was stirred at room temperature for 2 h. The phases were separated. The aqueous phase was extracted three times with EtOAc. To the combined organic extracts was added 2-propanol (0.2 mL). The mixture was stirred at room temperature for 1 h. The mixture was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid. The solid was subjected to preparative thin-layer chromatography on silica gel developed with $CHCl_3$−CH_3OH (7:1) to give **30** as a solid (275 mg, 91%): $[\alpha]^{22}_D$ +9.2° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.48 (9H, s), 2.81 (1H, br m), 3.22 (1H, br dd, J = 2.7, 15 Hz), 3.38 and 3.44 (3H, s each), 3.84 (1H, br t, J = 2.6 Hz), 4.35−4.55 (2H, br m), 4.58 and 4.64 (2H, ABq), 4.80 (2H, s), 6.84 (1H, br d, J = 6.8 Hz) and 8.02 (1H, br d, J = 6.8 Hz). Anal. Calcd for $C_{17}H_{27}N_2O_9F_3$: C, 44.35; H, 5.91; N, 6.08. Found: C, 44.51; H, 5.57; N, 5.80.

(+)-(3*S*,4*R*,5*R*,6*R*)-4,5-Dihydroxy-6-trifluoroacetamido-3-piperidinecarboxylic Acid (8). Compound **30** (220 mg, 0.48 mmol) was dissolved in HCl in 1,4-dioxane (4 M, 6 mL), and the mixture was kept at room temperature overnight. After addition of Et_2O, the resulting precipitates were taken by centrifugation and washed with Et_2O three times to give a colorless solid of **8** as its hydrochloride (147 mg, 99.7%): $[\alpha]^{22}_D$ +23° (*c* 0.16, H_2O); NMR (D_2O, 400 MHz) δ 2.74 (1H, ddd, J = 4.4, 10.5, 13.0 Hz), 3.24 (1H, t, J = 13.0 Hz), 3.53 (1H, dd, J = 4.4, 13.0 Hz), 3.74 (1H, t, J = 9.3 Hz), 3.85 (1H, dd, J = 9.3, 10.5 Hz) and 5.02 (1H, d, J = 9.3 Hz). Anal. Calcd for $C_8H_{11}N_2O_5F_3 \cdot 1HCl \cdot 1H_2O$: C, 29.41; H, 4.32; N, 8.58; Cl, 10.85. Found: C, 29.58; H, 4.09; N, 8.42; Cl, 11.09.

(+)-(2*S*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-trifluoroacetamido-3,4-piperidinediol (31). Procedures used were similar to those used for the preparation of **29** from **27**; the yield was 87%: $[\alpha]^{23}_D$ +22.8° (*c* 1.3, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.49 (9H, s), 2.05−2.15 (1H, m), 3.22 (1H, dd, J = 3.4, 14.7 Hz), 3.41 (6H, s), 3.62 (1H, dd, J = 6.4, 11.4 Hz), 3.73 (1H, t, J = 2.2 Hz), 3.81 (1H, dd, J = 9.3, 11.4 Hz), 3.89 (1H, br s), 4.02 (1H, br d, J = 14.7 Hz), 4.65 and 4.72 (2H, ABq, J = 6.8 Hz), 4.73 (2H, s), 6.18 (1H, br d, J = 8.3 Hz) and 8.02 (1H, br d, J = 8.3 Hz). Anal. Calcd for $C_{17}H_{29}N_2O_8F_3$: C, 45.74; H, 6.55; N, 6.28. Found: C, 46.04; H, 6.29; N, 6.22.

(+)-(3*R*,4*R*,5*R*,6*S*)-*N*-(*tert*-Butyloxycarbonyl)-2,3-didehydro-4,5-di(methoxymethoxy)-6-trifluoroacetamido-3-piperidinecarboxylic Acid (32). Procedures used were similar to those used for the preparation of **30** from **29**; the yield was 90%: $[\alpha]^{23}_D$ +13.5° (*c* 0.55, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.48 (9H, s), 3.05 (1H, br d, J = 12.0 Hz), 3.19 (1H, br t, J = 12.0 Hz), 3.38 and 3.43 (3H, s each), 3.84 (1H, br s), 4.36 (1H, br s), 4.15−4.40 (1H, br m), 4.65−4.80 (4H, m), 6.26 (1H, br s) and 8.07 (1H, br d, J = 7.8 Hz). Anal. Calcd for $C_{17}H_{27}N_2O_9F_3$: C, 44.35; H, 5.91; N, 6.08. Found: C, 44.30; H, 5.93; N, 5.99.

(+)-(3*R*,4*R*,5*R*,6*R*)-4,5-Dihydroxy-6-trifluoroacetamido-3-piperidinecarboxylic Acid (9). Procedures used were similar to those used for the preparation of **8** from **30**; the yield was 99%: $[\alpha]^{22}_D$ +30° (*c* 0.2, H_2O); NMR (D_2O, 400 MHz) δ 3.20 (1H, dt, J = 3.9, 7.3 Hz), 3.27 (1H, dd, J = 3.9, 13.7 Hz), 3.50 (1H, dd, J = 7.3, 13.7 Hz), 4.07 (1H, t, J = 6.4 Hz), 4.14 (1H, dd, J = 3.9, 6.4 Hz) and 5.14 (1H, d, J = 6.4 Hz). Anal. Calcd for $C_8H_{11}N_2O_5F_3 \cdot 1HCl \cdot 1H_2O$: C, 29.41; H, 4.32; N, 8.58; Cl, 10.85. Found: C, 29.52; H, 4.23; N, 8.44; Cl, 11.08.

(+)-(2*R*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-phthalimido-3,4-piperidinediol (33) and (+)-(2*R*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-phthalimido-3,4-piperidinediol(34). Procedures used were similar to those used for the preparation of **27** and **28** from **25**; the yields of **33** and **34** were 50 and 38%, respectively.

33: $[\alpha]^{23}_D$ +27.4° (*c* 0.72, $CHCl_3$); NMR ($CDCl_3$, 400 MHz); δ 1.45 (9H, s), 2.45−2.60 (1H, br m), 3.60−3.70 (1H, m), 3.70−3.85 (1H, br m), 3.95−4.05 (1H, m), 4.05−4.25 (1H, br m), 4.14 (1H, dd, J = 7.6, 9.8 Hz), 4.61 (2H, s), 4.67 (1H, dd, J = 5.9, 9.8 Hz), 4.74 and 4.78 (2H, ABq, J = 6.3 Hz), 6.72 (1H, br s) and 7.70−7.90 (4H, m). Anal. Calcd for $C_{23}H_{32}N_2O_9$: C, 57.49; H, 6.71; N, 5.83. Found: C, 57.59; H, 6.54; N, 5.65.

34: $[\alpha]^{23}_D$ +37.2° (*c* 0.74, $CHCl_3$); NMR ($CDCl_3$, 400 MHz); δ 1.41 (9H, s), 1.75−1.95 (1H, m), 2.91 (1H, br t, J = 6.8 Hz), 3.55−3.80 (2H, m), 3.90∼4.15 (2H, m), 4.64 (1H, dd, J = 7.0, 9.8 Hz), 4.46 (1H, br t, J = 9.8 Hz), 4.58 and 4.74 (2H, ABq, J = 6.8 Hz), 4.70 and 4.87 (2H, ABq, J = 6.3 Hz), 6.64 (1H, br s) and 7.70−7.90 (4H, m). Anal. Calcd for $C_{23}H_{32}N_2O_9$: C, 57.49; H, 6.71; N, 5.83. Found: C, 57.70; H, 6.61; N, 5.55.

(+)-(2*R*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butyloxycarbonyl)-5-(*tert*-butyldimethylsiloxymethyl)-3,4-di-*O*-(methoxymethyl)-2-phthalimido-3,4-piperidinediol (35). To a solution of **33** (228 mg, 0.475 mmol) in DMF (4.5 mL) were added imidazole (80.7 mg, 1.19 mmol) and TBDMSCl (107.2 mg, 0.71 mmol), and the mixture was stirred at room temperature for 1 h. After the mixture was quenched with water, evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative thin-layer chromatography on silica gel. Elution with toluene−acetone (15:1) gave an oil of **35** (257 mg, 91%): $[\alpha]^{23}_D$ +40.5° (*c* 0.66, $CHCl_3$); NMR ($CHCl_3$, 400 MHz) δ 0.09 (6H, s), 0.93 (9H, s), 1.41 (9H, s), 1.96 (1H, m), 3.33 and 3.42 (3H, s each), 3.44 (1H, dd, J = 12.2, 13.5 Hz), 3.50−3.75 (1H, br m), 3.75−4.25 (2H, br m), 4.00 (1H, dd, J = 7.1, 9.0 Hz), 4.27 (1H, dd, J = 5.4, 13.5 Hz), 4.52 and 4.59 (2H, ABq, J = 6.0 Hz), 4.71 and 4.81 (2H, ABq, J = 6.6 Hz), 6.40−6.65 (1H, br m) and 7.55−7.80 (4H, m). Anal. Calcd for $C_{29}H_{46}N_2O_9Si$: C, 58.56; H, 7.80; N, 4.71. Found: C, 58.25; H, 7.84; N, 4.57.

(+)-(2*R*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butyloxycarbonyl)-5-(*tert*-butyldimethylsiloxymethyl)-3,4-di-*O*-(methoxymethyl)-2-trifluoroacetamido-3,4-piperidinediol (36). Procedures used were similar to those used for the preparation of **29** from **27**; the yield was 88%: $[\alpha]^{23}_D$ +38.6° (*c* 0.59, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 0.05 and 0.06 (3H, s each), 0.89 (9H, s), 1.49 (9H, s), 1.65−1.80 (1H, m), 2.78 (1H, t, J = 14.0 Hz), 3.50−3.70 (3H, m), 3.75 (1H, dd, J = 4.9, 9.3 Hz), 4.02 (1H, dd, J = 4.9, 14.0 Hz), 4.71 and 4.84 (2H, ABq, J = 6.6 Hz), 4.74 and 4.76 (2H, ABq, J = 6.8 Hz), 6.25 (1H, t, J = 4.9 Hz) and 6.58 (1H, br d, J = ∼4.9 Hz). Anal. Calcd for $C_{23}H_{43}N_2O_8SiF_3$: C, 49.27; H, 7.73; N, 5.00. Found: C, 49.15; H, 7.52; N, 4.88.

(+)-(2*R*,3*R*,4*R*,5*R*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-trifluoroacetamido-3,4-piperidinediol (37). Procedures used were similar to those used for the preparation of **23** from **22**; the yield was 93%: $[\alpha]^{23}_D$ +43.3° (*c* 1.5, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.47 (9H, s), 1.76 (1H, m), 2.99 (1H, dd, J = 12.3, 13.6 Hz), 3.17 (1H, br s), 3.39 and 3.47 (2H, s each), 3.57 (1H, br t, J = 11.6 Hz), 3.67 (1H, t, J = 9.6 Hz), 3.82 (1H, dd, J = 5.2, 9.6 Hz), 3.85−4.05 (2H, m), 4.67 and 4.68 (2H, ABq, J = 4.4 Hz), 4.77 and 4.92 (2H, ABq, J = 6.6 Hz) and 6.34 (1H, br t, J = 5.2 Hz). Anal. Calcd for $C_{17}H_{29}N_2O_8F_3$: C, 45.74; H, 6.55; N, 6.28. Found: C, 45.43; H, 6.31; N, 5.83.

(+)-(3*S*,4*R*,5*R*,6*R*)-*N*-(*tert*-Butyloxycarbonyl)-2,3-didehydro-4,5-di-*O*-(methoxymethoxy)-6-trifluoroacetamido-3-piperidinecarboxylic Acid (38). Procedures used were similar to those used for the preparation of **30** from **29**; the yield was 92%: $[\alpha]^{23}_D$ +10.7° (*c* 0.93, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.47 (9H, s), 2.67 (1H, br m), 3.18 (1H, br t, J = 12.0 Hz), 3.10 and 3.39 (3H, s each), 3.83 (1H, dd, J = 5.4, 9.0 Hz), 3.95−4.20 (4H, m), 4.60−4.85 (4H, m), 6.25 (1H, t, J = 5.4 Hz) and 7.58 (1H, br s). Anal. Calcd for $C_{17}H_{27}N_2O_9F_3$: C, 44.35; H, 5.91; N, 6.08. Found: C, 44.51; H, 6.03; N, 6.11.

(−)-(3*S*,4*R*,5*R*,6*S*)-4,5-Dihydroxy-6-trifluoroacetamido-3-piperidinecarboxylic Acid (10). Procedures used were

Table 3.

compd		dose (μg/mL) 100	20	4	0.8	0.16	0.03	0.006	0
erythromycin	G[a]	±	±	+++	+++	+++	+++	+++	+++
	L[b]	0	60	50	>100	>100	>100	>100	
8	G	+++	+++	+++	+++	+++	+++	+++	+++
	L	>100	>100	>100	>100	>100	>100	>100	
9	G	+++	+++	+++	+++	+++	+++	+++	+++
	L	>100	>100	>100	>100	>100	>100	>100	
10	G	+++	+++	+++	+++	+++	+++	+++	+++
	L	>100	>100	>100	>100	>100	>100	>100	
11	G	+++	+++	+++	+++	+++	+++	+++	+++
	L	>100	>100	>100	>100	>100	>100	>100	
45	G	+++	+++	+++	+++	+++	+++	+++	+++
	L	>100	>100	>100	>100	>100	>100	>100	

[a] G: +++ = full growth of *Pseudomonas aeruginosa* A3 detected by MTT. [b] L: length (mm) of alginate slime.

similar to those used for the preparation of **8** from **30**; the yield was 99.7%: $[\alpha]^{22}_D$ −13° (*c* 0.14, H_2O); NMR (D_2O, 400 MHz) δ 2.82 (1H, dt, J = 1.6, 4.0 Hz), 3.40 (1H, dd, J = 4.0, 13.2 Hz), 3.57 (1H, dd, J = 1.6, 13.2 Hz), 3.95 (1H, dd, J = 2, 4.0 Hz), 4.43 (1H, t, J = 4 Hz) and 5.36 (1H, d, J = 2 Hz). Anal. Calcd for $C_8H_{11}N_2O_5F_3$·1HCl·1H_2O: C, 29.41; H, 4.32; N, 8.58; Cl, 10.85. Found: C, 29.67; H, 4.08; N, 8.34; Cl, 11.21.

(−)-(2*R*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-(*tert*-butyldimethylsiloxymethyl)-3,4-di-*O*-(methoxymethyl)-2-phthalimido-3,4-piperidinediol (39). Procedures used were similar to those used for the preparation of **35** from **33**; the yield was 100%: $[\alpha]^{23}_D$ −24.3° (*c* 0.8, $CHCl_3$); δ 0.07 and 0.08 (3H, s each), 0.91 (9H, s), 1.39 (9H, s), 2.25∼2.35 (1H, br m), 3.32 and 3.40 (3H, s each), 3.65 (1H, t, J = 9.6 Hz), 3.74 (1H, br d, J = 13.2 Hz), 3.91 (1H, dd, J = 3.0, 9.8 Hz), 3.90−4.05 (1H, br m), 4.26 (1H, br d, J = 13.2 Hz), 4.50 (1H, dd, J = 5.2, 10.4 Hz), 4.60 and 4.63 (2H, ABq, J = 6.8 Hz), 4.71 and 4.75 (2H, ABq, J = 6.8 Hz), 6.50−6.65 (1H, br m) and 7.60−7.85 (4H, m). Anal. Calcd for $C_{29}H_{46}N_2O_9Si$: C, 58.56; H, 7.80; N, 4.71. Found: C, 58.46; H, 7.80; N, 4.64.

(+)-(2*R*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-(*tert*-butyldimethylsiloxymethyl)-3,4-di-*O*-(methoxymethyl)-2-trifluoroacetamido-3,4-piperidinediol (40). Procedures used were similar to those used for the preparation of **29** from **27**; the yield was 79%: $[\alpha]^{25}_D$ +1.1° (*c* 1.08, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 0.05 and 0.06 (3H, s each), 0.89 (9H, s), 1.48 (3H, s), 2.10−2.30 (1H, m), 2.70 (1H, br t, J = 13.4 Hz), 3.40 and 3.16 (3H, s each), 3.35−3.55 (2H, m), 3.74 (1H, br s), 3.92 (1H, dd, J = 4.6, 13.4 Hz), 4.06 (1H, br s), 4.55−4.80 (4H, m), 6.27 (1H, br d, J = 6.3 Hz) and 8.14 (1H, br s). Anal. Calcd for $C_{23}H_{43}N_2O_8SiF_3$: C, 49.27; H, 7.73; N, 5.00. Found: C, 49.45; H, 7.88; N, 4.90.

(+)-(2*R*,3*R*,4*R*,5*S*)-*N*-(*tert*-Butyloxycarbonyl)-5-hydroxymethyl-3,4-di-*O*-(methoxymethyl)-2-trifluoroacetamido-3,4-piperidinediol (41). Procedures used were similar to those used for the preparation of **23** from **22**; the yield was 100%: $[\alpha]^{23}_D$ +35.8° (*c* 0.54, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.48 (9H, s), 3.04 (1H, dt, J = 4.0, 7.3 Hz), 3.40 (3H, s), 3.42 (3H, s), 4.04 (1H, dd, J = 4.0, 14.3 Hz), 3.78 (1H, dd, J = 7.3, 14.3 Hz), 4.09 (1H, dd, J = 4.0, 6.3 Hz), 4.14 (1H, dd, J = 2.9, 6.3 Hz), 4.55−4.80 (4H, m), 6.03 (1H, brdd, J = 2.9, 5.9 Hz) and 8.40 (1H, br s). Anal. Calcd for $C_{17}H_{29}N_2O_8F_3$: C, 45.74; H, 6.55; N, 6.28. Found: C, 45.45; H, 6.52; N, 6.20.

(+)-(3*R*,4*R*,5*R*,6*R*)-*N*-(*tert*-Butylcarbonyl)-2,3-didehydro-4,5-di-*O*-(methoxymethoxy)-6-trifluoroacetamido-3-piperidinecarboxylic Acid (42). Procedures used were similar to those used for the preparation of **30** from **29**; the yield was 87%: $[\alpha]^{23}_D$ +24.3° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$, 400 MHz) δ 1.48 (9H, s), 3.04 (1H, dt, J = 4.0, 7.3 Hz), 3.40 and 3.42 (3H, s each), 4.04 (1H, dd, J = 4.0, 14.4 Hz), 3.78 (1H, dd, J = 7.3, 14.4 Hz), 4.09 (1H, dd, J = 4.0, 6.3 Hz), 4.14 (1H, dd, J = 2.9 Hz), 4.66 and 4.72 (2H, ABq, J = 6.6 Hz), 4.74 (2H, t, J = 7.3 Hz), 5.86 (1H, dd, J = 2.9, 5.9 Hz) and 8.25−8.50 (1H, br s). Anal. Calcd for $C_{17}H_{27}N_2O_9F_3$: C, 44.35; H, 5.91; N, 6.08. Found: C, 44.47; H, 5.94; N, 6.05.

(−)-(3*R*,4*R*,5*R*,6*S*)-4,5-Dihydroxy-6-trifluoroacetamido-3-piperidinecarboxylic Acid (11). Procedures used were similar to those used for the preparation of **8** from **30**; the yield was 91%: $[\alpha]^{22}_D$ −2° (*c* 0.14, H_2O); NMR (D_2O, 400 MHz) δ 3.19 (1H, ddd, J = 2.9, 5.4, 13.0 Hz), 3.37 (1H, dd, J = 5.4, 13.0 Hz), 3.41 (1H, t, J = 13.0 Hz), 3.99 (1H, dd, J = 1.7, 3.9 Hz), 4.32 (1H, t, J = 2.9, 3.9 Hz) and 5.31 (1H, d, J = 1.7 Hz). Anal. Calcd for $C_8H_{11}N_2O_5F_3$·1HCl·1H_2O: C, 29.41; H, 4.32; N, 8.58; Cl, 10.85. Found: C, 29.74; H, 4.15; N, 8.32; Cl, 10.97.

General Procedures for Enzyme Inhibition Assay. The enzymes, β-glucuronidase (bovine liver), α-glucosidase (bakers' yeast), β-glucosidase (almond), α-mannosidase (jack beans), β-mannosidase (snail), β-galactosidase (*Aspergillus niger*), α-*N*-acetylgalactosaminidase (chicken liver), and β-*N*-acetylglucosaminidase (bovine epididymis) were purchased from Sigma Chemical Co. β-Glucuronidase was assayed using phenolphthalein mono-β-glucuronic acid (3.3×10^{-4}M) as a substrate at pH 5.0 (0.1 M acetate buffer). α- and β-glucosidases were assayed using *p*-nitrophenyl α-D-glucopyranoside (1.5×10^{-3}M) and β-D-glucopyranoside (2×10^{-3}M) as substrates at pH 6.3 (0.025 M citrate-phosphate buffer) and 5.0 (0.025 M acetate buffer), respectively. α- and β-mannosidases were assayed using *p*-nitrophenyl α-D-mannopyranoside (2×10^{-3}M) and β-D-mannopyranoside (2×10^{-3}M) as substrates at pH 4.5 (0.05 M acetate buffer) and 4.0 (0.05 M acetate buffer), respectively. β-Galactosidase was assayed using *p*-nitrophenyl β-D-galactopyranoside (2×10^{-3}M) at pH 4.0 (0.025 M citrate−phosphate buffer). α-*N*-Acetylgalactosaminidase was assayed using *p*-nitrophenyl *N*-acetyl-α-D-galactosaminide (1×10^{-3}M) as a substrate at pH 4.0 (0.025 M citrate−phosphate buffer). β-*N*-Acetylglucosaminidase was assayed using *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide (1×10^{-3}M) at pH 4.0 (0.025 M citrate−phosphate buffer). The reaction mixture contained 0.5 mL of buffer, 0.1 mL of substrate solution, and water or aqueous solution containing the test compound. The mixture was incubated at 37 °C for 3 min, and 0.01 mL of enzyme was added. After 30 min of reaction, 1.0 mL of 0.3 M glycine−sodium hydroxide buffer (pH 10.5) was added, and the absorbance of the liberated nitrophenol or phenolyphthalein measured at 400 or 525 nm, respectively. The percent inhibition was calculated by the formula $(A - B)/A \times 100$, where A is the nitrophenol liberated by the enzyme without an inhibitor and B is that with an inhibitor. The IC_{50} value is the concentration of inhibitor at 50% of enzyme activity.

Heparanase Assay. Recombinant human heparanase activity was determined toward fluorescein isothiocyanate (FITC) labeled heparan sulfate (FITC-HS). The assay was carried out essentially by the method described by Toyoshima and Nakajima.[29]

Inhibition of Biofilm Production by in Vitro Assay with Microplate. Test compounds (e.g., six compounds) with serial dilution (0−100 μg/mL) are put into eight holes of each lane of a 48 hole microplate (8 holes × 6 lanes) containing Miller−Hinton medium. The test organisms producing biofilm (e.g., alginate) are inoculated and incubated at 37 °C for 18 h, and the minimum inhibitory concentration (MIC) for the growth is determined with an aid of formazan color development of MTC (methyl tetrazolim chloride). The production of biofilm is represented by the length of slime produced in each

holes. When 48 plugs of polyethylene (2 mm diameter, 20 mm length) arranged on the cover slip of the microplate are placed in the holes of plate before the incubation, the amount and stickiness of slime production after 18 h incubation can be assayed by measuring the length of slime until the slime is splitted, if the biofilm covered plugs is lifted up gradually from the hole. Thus, as shown in Table 3, erythromycin as the control showed full growth in holes of 4 μg/mL, but scarce growth in 20 and 100 μg/mL. Despite the full growth, the slime production on a plug in the holes of 4 μg/mL was inhibited and splitted at 50 mm length of lifting (the slime production in holes of 0.8 μg/mL or less was not inhibited and splitted at >100 mm length; no slime was produced in 100 μg/mL).

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Supporting Information Available: Copies of ^{1}H NMR spectra of **27**, **28**, **33**, **34**, **8**, **9**, **10**, and **11** and an X-ray crystallographic report for **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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