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REVIEW ARTICLE

Gem-diamine 1-*N*-iminosugars as versatile glycomimetics: synthesis, biological activity and therapeutic potential

Yoshio Nishimura

Iminosugars, which carry a basic nitrogen in the carbohydrate ring, have attracted increasing interest as new glycomimetics. *Gem*-diamine 1-*N*-iminosugars, a new class of iminosugars, have a nitrogen atom in place of the anomeric carbon. Various kinds of 1-*N*-iminosugars have been synthesized from glyconolactones as a chiral source in a totally stereospecific manner and/or by the convergent strategy from siastatin B, a secondary metabolite of *Streptomyces*. The protonated form of 1-*N*-iminosugar mimics the charge at the anomeric position in the transition state of enzymatic glycosidic hydrolysis, resulting in a strong and specific inhibition of glycosidases and glycosyltransferases. They have been recently recognized as a new source of therapeutic drug candidates in a wide range of diseases associated with the carbohydrate metabolism of glycoconjugates, such as tumor metastasis, influenza virus infection, lysosomal storage disorder and so forth.
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Keywords: glycosidase inhibitor; heparanase inhibitor; influenza virus infection; lysosomal storage disease; 1-*N*-iminosugar; siastatin B; tumor metastasis

INTRODUCTION

Iminosugars, which are carbohydrate analogs that most frequently carry the nitrogen atom at the position of the endocyclic oxygen, form the most attractive class of glycomimetics reported so far.[1] Several types of iminosugars have been discovered from natural sources[1,2] since nojirimycin[3] was first isolated as an antibiotic from *Streptomyces* culture in 1966. Many more have also been synthesized on the basis of enzymatic glycoside biosynthesis.[1,4] Of late, they have gained remarkable importance not only as molecular tools to unravel the manner in which glycoconjugates regulate various biological functions, but also as new therapeutic agents in a wide range of diseases associated with the metabolism of carbohydrates.

In 1974, siastatin B (**1**), an unusual iminosugar, was isolated as an inhibitor against neuraminidases (NAs) from *Streptomyces* culture.[5] Siastatin B (**1**) also inhibits β-D-glucuronidase and *N*-acetyl-β-D-glucosaminidase. We recognized from biological activity that siastatin B (**1**) structurally resembles D-glucuronic acid (**2**) and *N*-acetyl-D-glucosamine (**3**), as well as *N*-acetylneuraminic acid (**4**) (Figure 1). It is distinct from the known glycosidase inhibitors, such as nojirimycin, that contain a nitrogen atom in place of the ring oxygen. In the course of our study on siastatin B (**1**), we proposed a new class of glycosidase inhibitors, *gem*-diamine 1-*N*-iminosugars[6–8] (*gem*-diamine 1-aza-carbasugar in IUPAC nomenclature,[9] cyclic methanediamine monosaccharide, **5**) in which the anomeric carbon atom is replaced by nitrogen. We hypothesized that the protonated form of *gem*-diamine 1-*N*-iminosugar **6** may mimic the putative glycopyranosyl cation **7** that was formed during enzymatic glycosidic hydrolysis (Figure 2). This turned out to be the case and led to new findings of highly potent and specific inhibitors of glycosidases and glycosyltransferases, as well as potential therapeutics for tumor metastasis and so forth. On the other hand, the synthetic isofagomine (**8**), another type of 1-iminosugar, was developed by Bols and colleagues in 1994.[10] The isofagomine type 1 iminosugars showed a potent inhibition of their corresponding β-glycosidase.[11] These findings suggest that 1-iminosugars might provide another alternative to the development of therapeutic agents based on the inhibitors of metabolism of glycoconjugates different from the common iminosugars, such as Zavesca (*N*-*n*-butyl-1-deoxynojirimycin)[12] used for the treatment of Gaucher's disease.

This review describes our current progress in the chemistry, biochemistry and pharmacology of *gem*-diamine 1-*N*-iminosugars.

SYNTHESIS

Various types of iminosugar inhibitors, such as polyfunctional piperidines and pyrrolidines, have been designed on the basis of a flattened, half-chair oxocarbenium ion-like transition state in the reaction catalyzed by glycosidases.[13–15] They are all carbohydrate mimics in which the ring oxygen is replaced by nitrogen. On the other hand, 1-*N*-iminosugars have a unique structure with a nitrogen atom in place of the anomeric carbon atom. *Gem*-diamine 1-*N*-iminosugars

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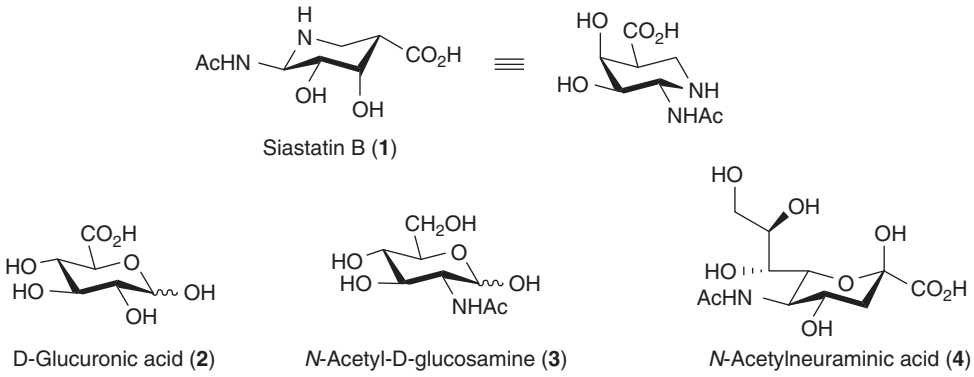


Figure 1 Structural resemblance of siastatin B (**1**) to D-glucuronic acid (**2**), *N*-acetyl-D-glucosamine (**3**) and *N*-acetylneuraminic acid (**4**).

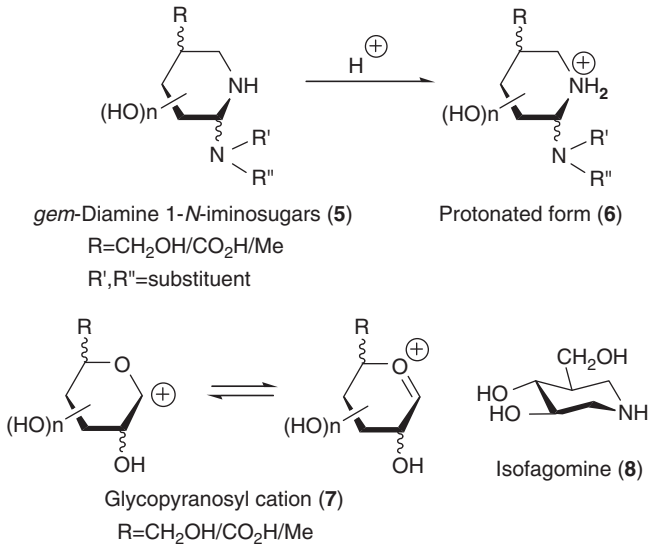


Figure 2 General structures of *gem*-diamine 1-*N*-iminosugars (**5**), its protonated form (**6**), glycopyranosyl cation (**7**), the putative intermediate of enzymatic glycosidic hydrolysis and isofagomine (**8**), another type of 1-iminosugar.

have an especially unusual structure possessing the continuous –CH(OH)–CH(OH)–CH–(NHR)–NH–CH2–CH(CH2OH/COOH)– in a framework. Their multi-functionalized structures with many asymmetric centers in a small molecule and fascinating biological activities have attracted intensive synthetic interest. As the interest in this class of glycomimetics comprised analogs of both D- and L-sugars, we have developed flexible divergent strategies applicable to a wide range of *gem*-diamine 1-*N*-iminosugars using glyconolactones as chiral substrates. Efficient and convenient synthetic methodologies of *gem*-diamine 1-*N*-iminosugars were also developed from natural siastatin B. These convergent strategies using natural siastatin B could be useful and practical for drug development.

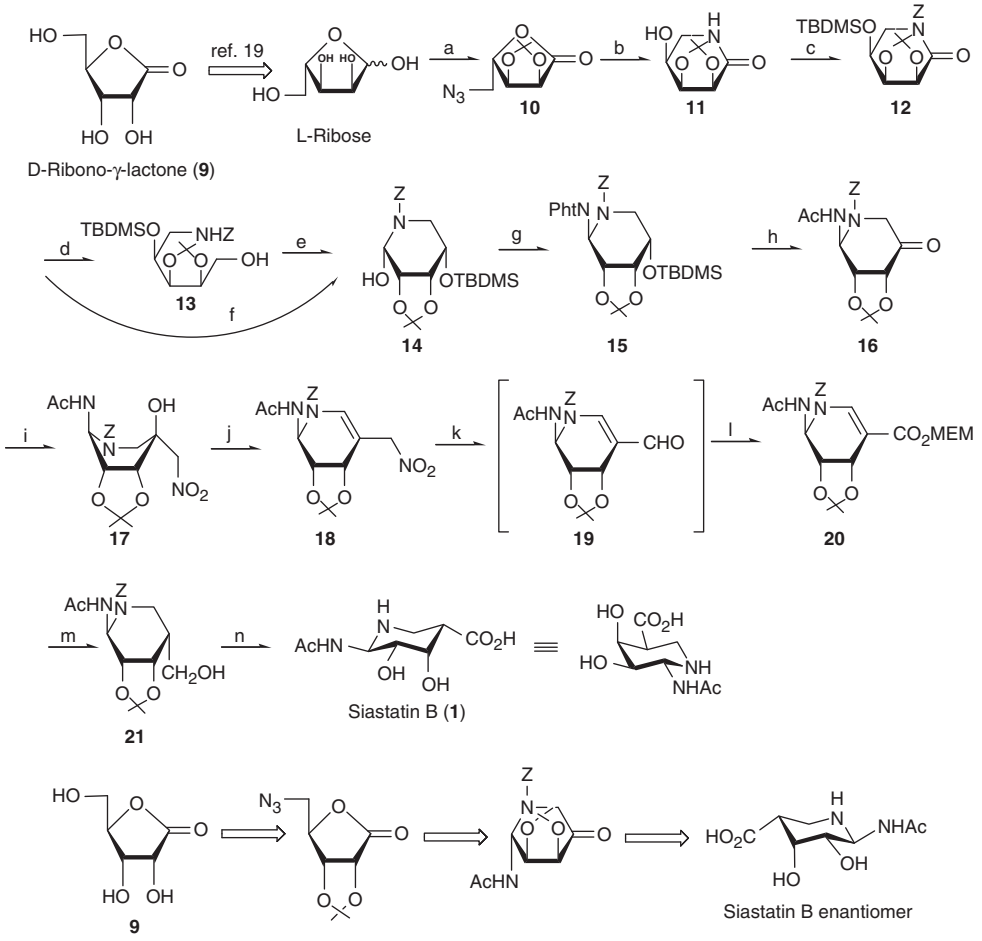
Total synthetic route to *gem*-diamine 1-*N*-iminosugars

The chiron strategy using D-ribono-γ-lactone (**9**) was first adapted for the total synthesis of siastatin B (**1**) and its enantiomer.[16,17] D-galacturonic acid-type 2-acetamido-1-*N*-iminosugar was synthesized in a totally stereospecific and enantiospecific manner as shown in Scheme 1.[18] The strategy involves the formation of the cyclic methanediamine using the Mitsunobu reaction[19] on an aminal (**14**→**15**) and the stereospecific introduction of a carboxylic acid group into a ketone (**16**→**21**) as the key steps. The synthesis of the key intermediate, lactam **11**, commenced with L-ribose, which was transformed into azido-L-ribonolactone **10** by the protection of 2,3-diol, azide formation and oxidation. Hydrogenation of the azide group of **10** resulted in crystalline **11** with ring expansion. Stereospecific introduction of the hydroxyl group at the C-2 position was best achieved by hydride reduction of the protected lactam **12** followed by the Swern oxidation to yield aminal **14**. One-step stereospecific transformation of **12** into **14** was also efficiently achieved by the reduction of L-selectride in tetrahydrofuran. The Mitsunobu reaction with phthalimide in dimethylformamide was proved to quantitatively yield the desired cyclic methanediamine **15**. Replacement of the amino substituent, removal of the *tert*-butyldimethylsilyl (TBDMS) group and oxidation to **16** were carried out straightforwardly. Condensation of **16** with nitromethane was found to proceed smoothly to quantitatively yield **17** as a single stereoisomer. The endocyclic nitro olefin **18** was effectively derived from **17** by acetylation followed by base-catalyzed elimination of the acetoxy group. The crucial intermediate, carboxylate **20**, was obtained through α,β-unsaturated aldehyde **19** generated by simply warming in pyridine. Transformation of **20** into siastatin B (**1**) was best achieved by the following three-step sequences: stereoselective reduction to the α,β-saturated hydroxymethyl compound **21**, oxidation and removal of protecting groups. The antipode of **1** was also synthesized from D-ribono-1,4-lactam using the above-mentioned method. Thus, the total synthesis also elucidated the absolute configuration of siastatin B (**1**) as the (3*S*,4*S*,5*R*,6*R*) isomer.

The strategy of total synthesis of siastatin B (**1**) is applicable to a wide range of D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars (Schemes 2 and 3).[20,21]

Syntheses of D-galacturonic acid-type 2-acetamido- and trifluoroacetamido-1-*N*-iminosugars (**27a** and **27b**) having a hydroxyl group at the C-5 position and their antipodes **27c** and **27d** are achieved in a straightforward manner. The nitromethane condensation of the ketones **23a** and **23b** stereospecifically proceeded to afford adducts **24a** and **24b**. The *S*-configuration at position C-5 was clarified by an X-ray crystallographic analysis of the antipode of **23a**. Successive sequences of catalytic reduction, ninhydrin oxidation of the resultant aminomethyl group, oxidation of the resultant aldehyde with sodium chlorite and removal of the protecting groups afforded the final products. The antipodes **27c** and **27d** were also synthesized starting from D-ribono-1,4-lactone using similar methods that are mentioned above.

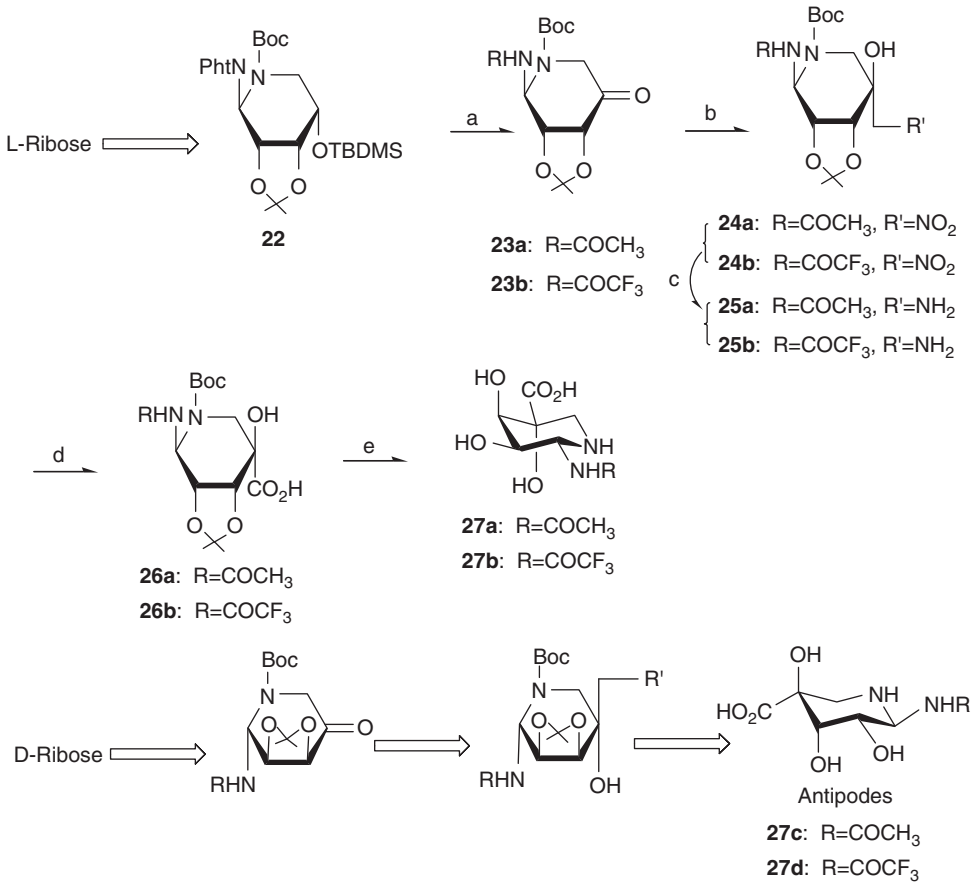
An alternative route from the ketone **23b** to D-galacturonic acid-type 2-trifluoroactamido-1-*N*-iminosugar **32** was also developed



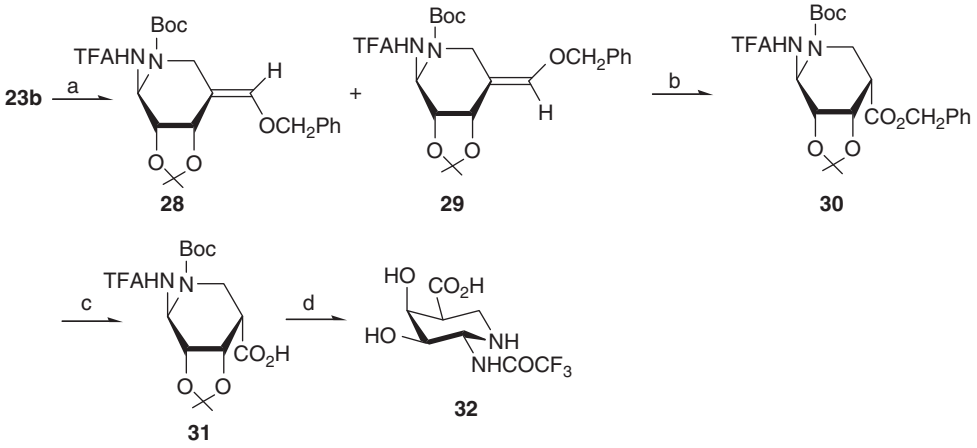
Scheme 1 Reagents and conditions: (a) *p*-TsOH, Me_2CO; MsCl, py; NaN_3, DMSO; CrO_3/py, CH_2Cl_2 (89%); (b) H_2, Raney Ni, MeOH (88%); (c) TBDMSCl, imidazole, DMF; ZCl, NaH, DMF (99%); (d) $NaBH_4$, EtOH (96%); (e) Swern oxidation (88%); (f) L-selectride, THF (88%); (g) phthalimide, Ph_3P, DEAD, DMF (100%); (h) $H_2N{\cdot}NH_2$, MeOH; Ac_2O, py; *n*-Bu_4NF, THF; RuO_4, CH_2Cl_2 (99%); (i) $MeNO_2$, NaH, DME (100%); (j) Ac_2O, *p*-TsOH; K_2CO_3, PhH (100%); (k) py, 38 °C, 1 week (80%); (l) $NaClO_2$–NaH_2PO_4, $CH_3CH{=}CMe_2$, H_2O-*t*-BuOH; MEMCl, *i*-Pr_2NEt, CH_2Cl_2 (55%); (m) $NaBH_4$, THF–CF_3CH_2OH (75%); (n) PDC, DMF; H_2, 10% Pd/C, MeOH; 1 M HCl, then Dowex 50W X_4 (H^+) eluted with 2% NH_4OH (66%).

using the Wacker process oxidation of the enol ethers **28** and **29** as a key step. The Wacker process oxidation stereospecifically proceeded to yield carboxylate **30** as a sole product. The transformation of **30** into **32** was unexceptional.

A flexible synthetic route to four *gem*-diamine 1-*N*-iminosugars of D- and L-uronic acid type (D-glucuronic, D-mannuronic, L-iduronic and L-guluronic acid) from L-galactono-1,4-lactone was also developed in an enantiodivergent manner through a sequence involving as the key steps (1) the formation of *gem*-diamine 1-*N*-iminopyranose ring by the Mitsunobu reaction of an aminal (**44**→**45, 46**) and (2) the flexible introduction of a carboxylic acid group by the Wittig reaction on a ketone, followed by hydroboration and oxidation, as well as the Sharpless oxidation (**45** and **46**→**47**, **48** and **55**, **56**) (Schemes 4 and 5).[22,23] The diastereoselective construction of amino and carboxylic acid substituents at positions C-2 and C-5, respectively, on the versatile aminal **44** led to the formation of four enantiomerically pure stereoisomers (**51**, **54**, **61** and **66**). The Wittig reaction on the ketone **37** derived from L-galactono-1,4-lactone resulted in the methylene derivative **38**, which was converted into the diol **39**. The monoalcohol **40** was successfully obtained by the Luche reduction of the labile aldehyde generated by the periodate oxidation of **39**. Conversion of the hydroxyl group of **40** to the azide group was best achieved from the corresponding sulfonate by one-pot reaction *in situ*. Hydrogenation of the azide group of **41** with sodium hydrogentelluride (NaTeH) was found to proceed preferentially without any effect on the reduction of the methylene group. The pivotal intermediate, aminal **44** was obtained as an epimeric mixture by the removal of a TBDMS group and the Swern oxidation. The Mitsunobu reaction with phthalimide afforded both desired epimers of iminophthalimides **45** and **46** in a 3:1 ratio. The absolute stereochemistry and a boat conformer of **45** were clarified by an X-ray crystallographic analysis. Another epimer **46** was assigned its stereochemistry and boat conformation by the hydrogen-1 nuclear magnetic resonance (^{1}H-NMR) spectrum. Hydroboration of **45** followed by oxidation efficiently yielded the D-*gluco* isomer **47** and the L-*idulo* isomer **48** in a 2:9 ratio. Hydrazinolysis of **47** and conventional trifluoroacetylation furnished the trifluoroacetamide **49**. The ruthenium tetraoxide-catalyzed Sharpless oxidation effectively yielded the carboxylic acid **50**. Removal of the protecting groups of **50** resulted in D-glucuronic acid-type 2-trifluoroacetamido-1-*N*-iminosugar **51**. The same sequences of reactions also successfully resulted in L-iduronic acid-type 2-trifluoroacetamido-1-*N*-iminosugar **54** from **48**. The ^{1}H-NMR spectrum of **51** showed the $^4C_1$-conformation, whereas the ^{1}H-NMR spectrum of **54** indicated the boat conformation. On the other hand, D-mannuronic



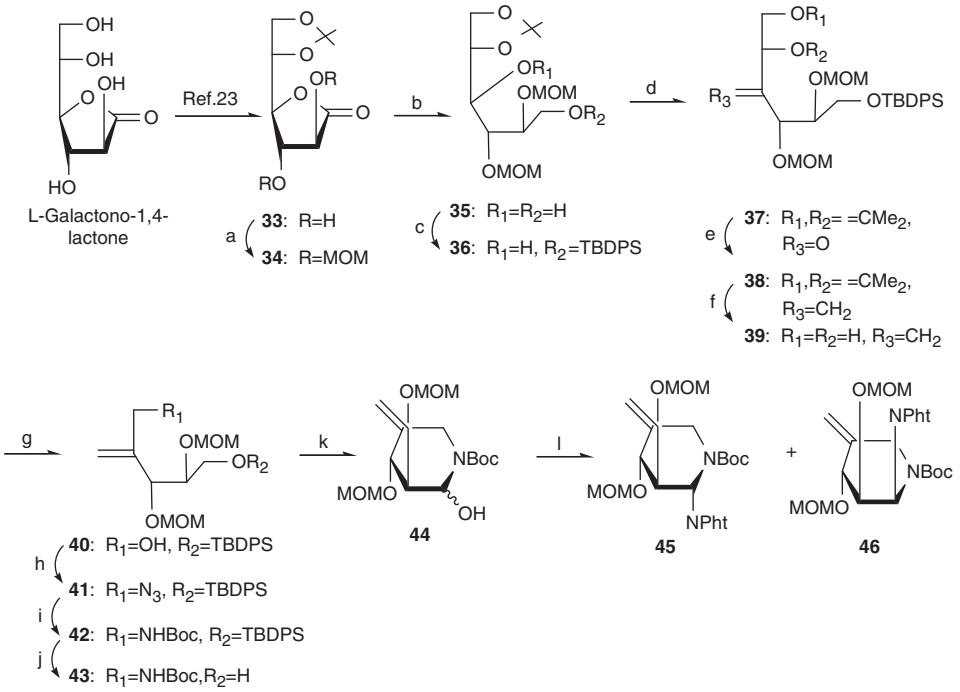
Scheme 2 Reagents and conditions: (a) $H_2N{\cdot}NH_2$, MeOH; Ac_2O, py (or CF_3CO_2Et, *i*-Pr_2NEt, DMF): *n*-Bu_4NF, THF; RuO_4, CH_2Cl_2 (81 and 91%); (b) CH_3NO_2, NaH, DME (69 and 74%); (c) H_2, Raney Ni, MeOH (100 and 98%); (d) ninhydrin, $NaHCO_3$, $MeOH/H_2O$; $NaClO_2$, NaH_2PO_4, $MeCH{=}CMe_2$, *t*-$BuOH/H_2O$ (38 and 43%); (e) 4 M HCl/dioxane (92 and 96%).



Scheme 3 Reagents and conditions: (a) $PhCH_2OCH_2PPh_3Cl$, PhLi, THF (48%); (b) $PdCl_2$, CuCl, O_2, DMF/H_2O (46%); (c) H_2, Pd/C, EtOAc (92%); (d) 4 M HCl/dioxane (96%).

acid-type and L-guluronic acid-type 1-*N*-iminosugars **61** and **66** were prepared in a straightforward manner by a similar sequence of structure transformation, except for the protection of the hydroxyl groups of **55** and **56** before hydrazinolysis of the phthalimide group for improvement in yield. The ^{1}H-NMR spectra of **61** and **66** showed the boat and $^1C_4$-conformations, respectively.

An enantioselective synthesis of L-fucose-type *gem*-diamine 1-*N*-iminosugars from D-ribono-γ-lactone was developed that used the Mitsunobu reaction on an aminal in the *gem*-diamine 1-*N*-iminopyranose ring formation (**74**→**75**) and a stereospecific reduction of an *exo*-methylene group to form the correct configuration of L-fucose (**75**→**76**) (Scheme 6).[24,25] The synthesis of the pivotal intermediate,



Scheme 4 Reagents and conditions: (a) $MeOCH_2Cl$, *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_3PMeBr, *n*-BuLi, THF, −78 °C, 96%; (f) 80% AcOH, rt, 99%; (g) $NaIO_4$, $MeCN/H_2O$; $NaBH_4$, $CeCl_3$, MeOH, 88%; (h) MsCl, py; NaN_3, DMF, 88.7%; (i) Te, $NaBH_4$, EtOH; $(t\text{-}BuCO)_2O$, *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, **45**: 61.4%; **46**: 20%.

aminal **74** began with the known lactam **67**. Transformation of **67** into the diol **70** was unexceptional. The Dess–Martin oxidation of **70** followed by the Wittig reaction with methylenetriphenylphosphorane yielded the *exo*-methylene **72**. Removal of a protecting group of **72** and the Swern oxidation resulted in the desired aminal **74** as a sole product. The Mitsunobu reaction of **74** with phthalimide efficiently afforded the iminophthalimide **75**. The catalytic hydrogenation of **75** yielded the desired product **76**, its epimer **77** and the rearranged derivative **78** in a ratio of 15:1:3. Compound **78** was also successfully converted into the desired **76** on the same hydrogenation. The expected stereochemistry and a boat conformation of **76** were clarified by an X-ray crystallographic analysis. Hydrazinolysis of **76** yielded the amine **79**, which was transformed into the acetamide **80**, the trifluoroacetamide **81** and the trichloroacetamide **82**. Removal of the protecting groups resulted in L-fucose-type 2-acetamido, 2-trifluoroacetamido and 2-trichloroacetamido-1-*N*-iminosugars **83**, **84** and **85**. Other L-fucose-type 1-*N*-iminosugars **86**, **87** and **88** were also obtained from the intermediates **76**, **77** and **75**, respectively, by conventional transformation. The ^{1}H-NMR spectra of **83**, **84**, **85** and **86** showed $^1C_4$-conformations.

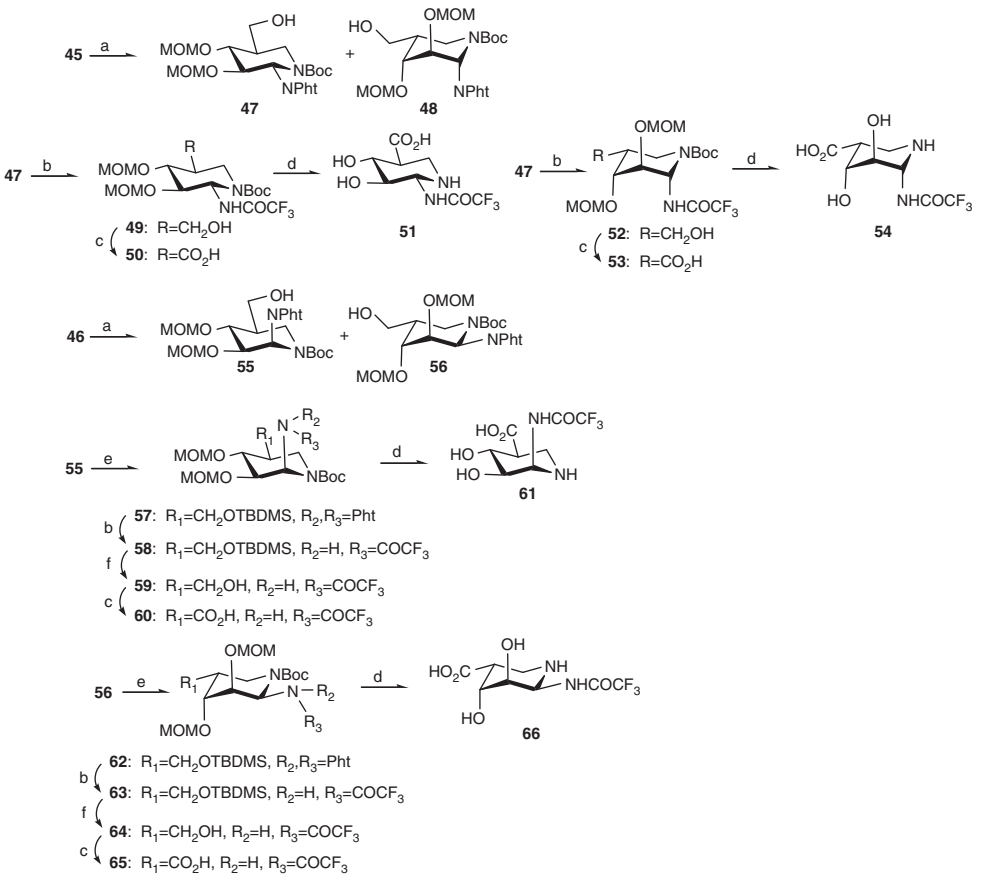
Intermediates prepared during the total synthetic route to uronic acid-type *gem*-diamine 1-*N*-iminosugars are also available for the synthesis of various kinds of glycose and glycosamine-type *gem*-diamine 1-*N*-iminoisugars (Scheme 7).[22]

Semi-synthetic route to *gem*-diamine 1-*N*-iminosugars

Natural siastatin B (**1**) can also serve as a starting material in a simple and easy route to D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars (Scheme 8).[26,27] Transketalization using chlorotrimethylsilane successfully proceeded to yield the ketal **94**. A sequence of esterification, hydride reduction and hydrazinolysis efficiently afforded the amino alcohol **97**, which was converted into the trifluoroacetamide **98**. The ruthenium-catalyzed Sharpless oxidation followed by the removal of the protecting group resulted in the desired product **32**. 2-Trichloroacetamido, guanidino and phthaloyl analogs **106**, **107** and **108** were also prepared using similar methods.

Configurational inversion of the carboxyl group of siastatin B (**1**) leads to *gem*-diamine 1-*N*-iminosugars corresponding to L-sugars.[28,29] The intramolecular Michael addition of *O*-imidate to the α,β-unsaturated ester through *cis* oxiamination[30] (Overman rearrangement, **110**→**111**) as a key step effectively yielded L-uronic acid-type *gem*-diamine 1-*N*-iminosugars (Schemes 9 and 10). The α,β-unsaturated ester **110** readily available from siastatin B (**1**) smoothly underwent *cis* oxiamination through the conjugate addition of the intermediate imidate anion to result in the desired oxazoline **111** in a good yield and a trace amount of its epimer. Hydrolysis of **111** afforded the trichloroacetamides **112** and **113**, which were converted into the amines **114** and **115**, respectively, on reductive cleavage of the trichloroacetamide group. Removal of the protecting groups of **114** and **115** resulted in L-alturonic acid-type and L-mannuronic acid-type 2-acetamido-1-*N*-iminosugars **116** and **117**, respectively. Another type of L-alturonic acid-type 2-acetamido-1-*N*-iminosugar **119** with a guanidine group was also obtained by the conventional method. The ^{1}H-NMR spectra of **116**, **117** and **119** showed $^1C_4$-conformations. 2-Trifluoroactamide analogs **130** and **133** were also prepared by a similar sequence of reactions using the α,β-unsaturated ester **123** readily available from **97**.

Siastatin B (**1**) has the correct configuration corresponding to D-galactose- and D-galactosamine-type gem-diamine 1-*N*-iminosugars. Therefore, the various kinds of D-glycose and D-glycosamine-type *gem*-diamine 1-*N*-iminosugars could be obtainable by a semi-synthetic method starting from **1** (Schemes 11 and 12)[30,31]



Scheme 5 Reagents and conditions: (a) $BH_3{\cdot}Me_2S$, THF; H_2O_2, 2 M NaOH/H_2O, **47**: 16.6%; **48**: 77.1%; **55**: 50%; **56**: 38%; (b) $H_2NNH_2{\cdot}xH_2O$, MeOH; $(CF_3CO)_2O$, py, CH_2Cl_2, **49**: 90%; **52**: 87%; **58**: 88%; **63**: 79%; (c) RuO_2, $NaIO_4$, CCl_4/MeCN/H_2O, **50**: 91%; **53**: 90%, **60**: 92%; **65**: 87%; (d) 4 M HCl/dioxane, **51**: 99.7%; **54**: 99%; **61**: 99.7%; **66**: 91%; (e) *t*-Bu(Me_2)SiCl, imidazole, DMF, **57**: 91%; **62**: 100%; (f) *n*-Bu_4NF, THF, **59**: 93%; **64**: 100%.

Configurational inversions at the C-4 position of **144** and **145** by two-step reactions led to the facile synthesis of D-glucosamine and glucose-type *gem*-diamine 1-*N*-iminosugars **150** and **151**, respectively (Scheme 13).[32]

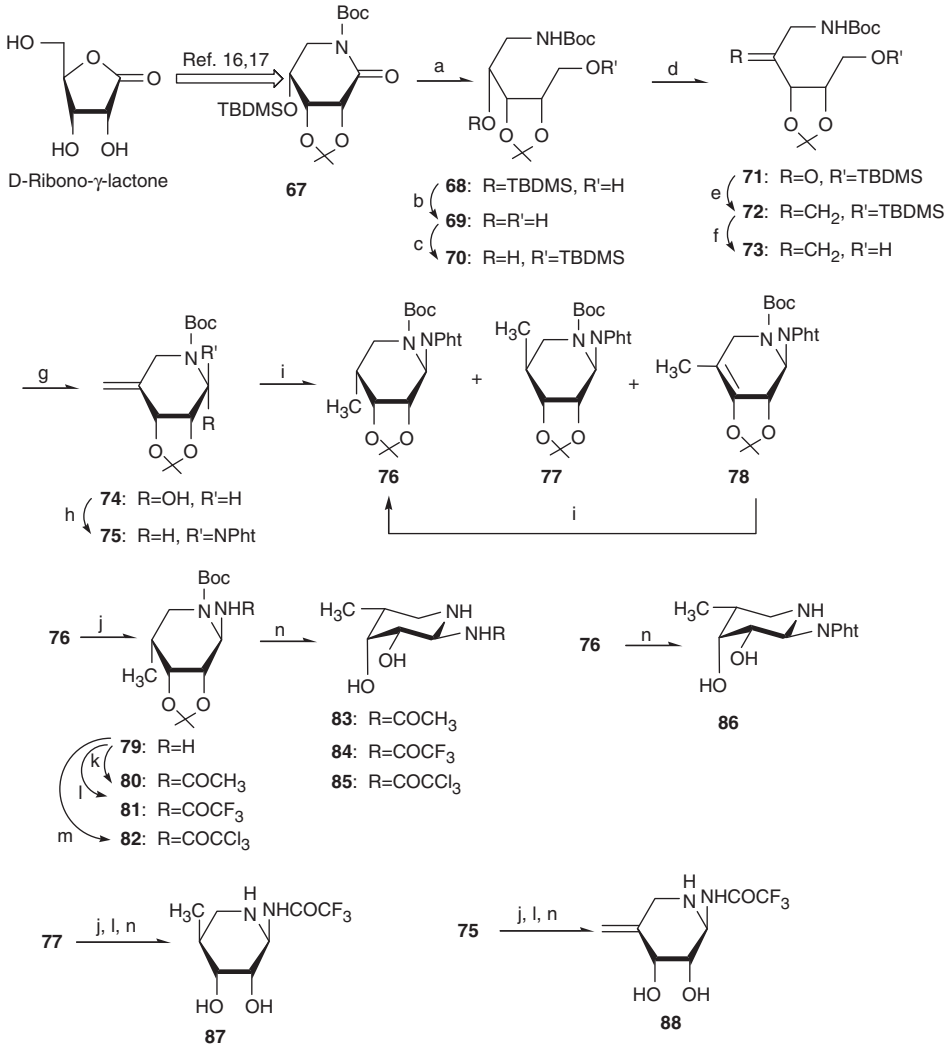
BIOLOGICAL ACTIVITY

Glycoconjugates such as glycoprotein, glycolipid and proteoglycan are ubiquitous in nearly all forms of life and are involved in cell-to-cell communication, cell-to-cell recognition, cell adhesion, cell growth regulation, differentiation and transport. Specific inhibitors of glycosidase and glycosyltransferases are useful for unraveling the manner in which glycoconjugates regulate biological function, and also for developing new drugs for a wide range of diseases associated with both the biosynthesis and degradation of glycoconjugates, namely cancer, tumor metastasis, diabetes, lysosomal storage disorders, viral and bacterial infections and so forth. Iminosugars generally show potent and specific inhibition against glycosidases and glycosyltransferases from various organisms.[1,33–35] Of these, *gem*-diamine 1-*N*-iminosugars have been proven to be highly potent and specific inhibitors of glycosidases, glycosyltransferases and sulfotransferases, and also potential therapeutics for tumor metastasis, lysosomal storage disorders and other diseases.[1,6,36–38]

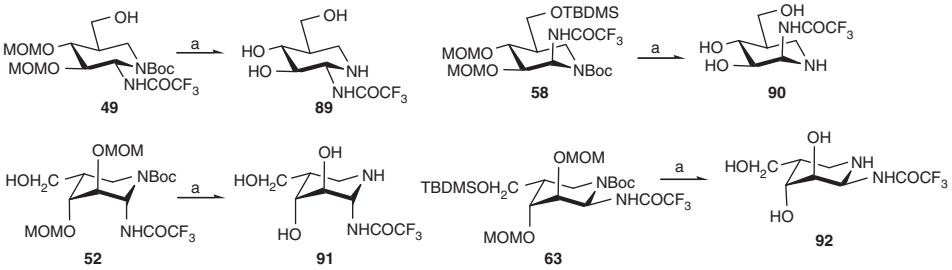
Glycosidase, glycosyltransferase and sulfotransferase inhibitory activity

The inhibitory activities of L-fucose-type *gem*-diamine 1-*N*-iminosugars against glycosidases are summarized in Table 1.[24,25]

The L-fucose-type 2-trifluoroacetamide **84** showed a very strong and specific inhibition against α-L-fucosidase from bovine kidney. Compound **84** was proved to be a competitive inhibitor by the Lineweaver–Burk plot, and the Ki value of **84** was determined as 5×10^{-9} M by the Dixon plot.[25] The 2-trichloroacetamide **85** and 2-phthalimide **86** also strongly affected α-L-fucosidase equivalent to the trifluoroacetamide **84**. Compounds **84**, **85** and **86** have been proven to smoothly undergo the Amadori rearrangement to yield the common intermediates, the hemiaminal **152** and the hydrated ketone **153** at pH 6.3 (Figure 3).[39] The time-course evaluation of inhibitory activities of **84**, **85** and **86** in the medium indicates that the hemiaminal and the hydrated ketone generated in the medium strongly inhibit α-L-fucosidase as the real active form. The ^{1}H-NMR spectra of the hemiaminal **152** and the hydrated ketone **153** also clearly show their $^1C_4$-conformation. Interestingly, the hemiaminal **152** has the same structure as that of synthetic L-fuconoeuromycin.[11,40] L-fuco-noeuromycin shows a potent inhibition against α-L-fucosidase equivalent to compounds **84**, **85** and **86**. On the other hand, the stable acetamide **83** in the medium shows a moderate inhibition against α-L-fucosidase. These results support the hypothesis that the protonated *gem*-diamine 1-*N*-iminosugars may mimic the presumed glycosyl cation **7** in the transition state of the enzymatic reaction as shown in Figure 2. Compounds **87** and **88** show a weak inhibition against α-L-fucosidase. These results also indicate that the 5-methyl group, its stereochemistry and the $^1C_4$-conformation have important roles as major factors for potency and specificity.



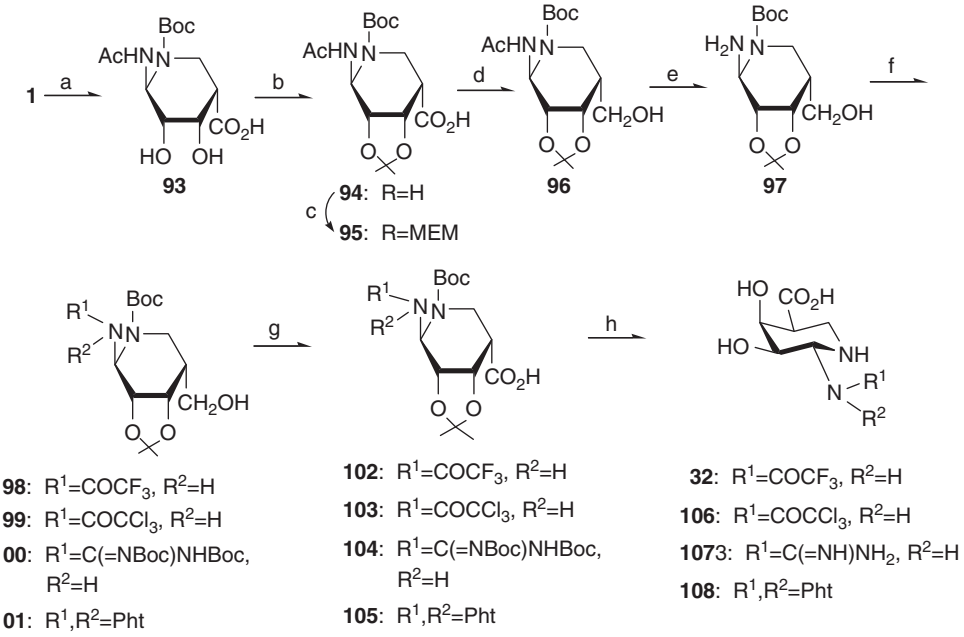
Scheme 6 Reagents and conditions: (a) $NaBH_4$, EtOH, 0 °C to rt; (b) *n*-Bu_4NF, THF, rt; (c) *t*-$BuMe_2SiCl$, imidazole, DMF, rt; (d) Dess–Martin periodinane, CH_2Cl_2; (e) Ph_3PMeBr, $(Me_3Si)_2NLi$, THF, 0 °C to rt; (f) *n*-Bu_4NF, THF, rt; (g) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to rt; (h) phthalimide, Ph_3P, DEAD, DMF, rt; (i) H_2/Pd-C, MeOH, rt; (j) $H_2NNH_2{\cdot}xH_2O$, MeOH, rt; (k) Ac_2O, DMAP, py, rt; (l) $(CF_3CO)_2O$, py, CH_2Cl_2, rt; (m) CCl_3COCl, py, CH_2Cl_2, 0 °C; (n) 4 M HCl/dioxane, 0 °C to rt.



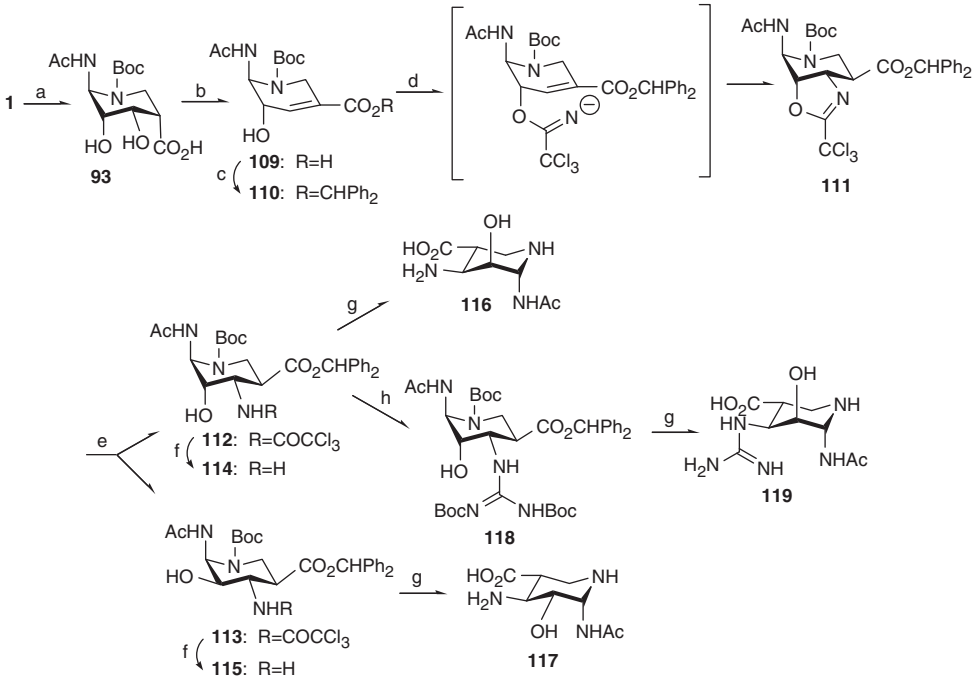
Scheme 7 Reagents and conditions: (a) 4 M HCl/dioxane.

The inhibitory activities of uronic acid-type *gem*-diamine 1-*N*-iminosugars against glycosidases are summarized in Table 2.[7,20–22,26,27]

D-glucuronic acid-type 2-trifluoroacetamido-1-*N*-iminosugar **51** shows a very strong inhibition against β-glucuronidase. D-uronic acid-type *gem*-diamine 1-*N*-iminosugars, similar to the L-fucose-type *gem*-diamine mentioned above, have been proven to smoothly undergo the Amadori rearrangement to yield the hydrated ketone **155** or its derivative **156** through a hemiaminal **154** at pH > 5.0 (Figure 4).[39] The time-course evaluation of the inhibitory activity in the medium also indicates that the hemiaminal and hydrated ketone generated in the medium strongly inhibits glycuronidases. It is reasonable to expect that the hemiaminal and hydrated ketone and

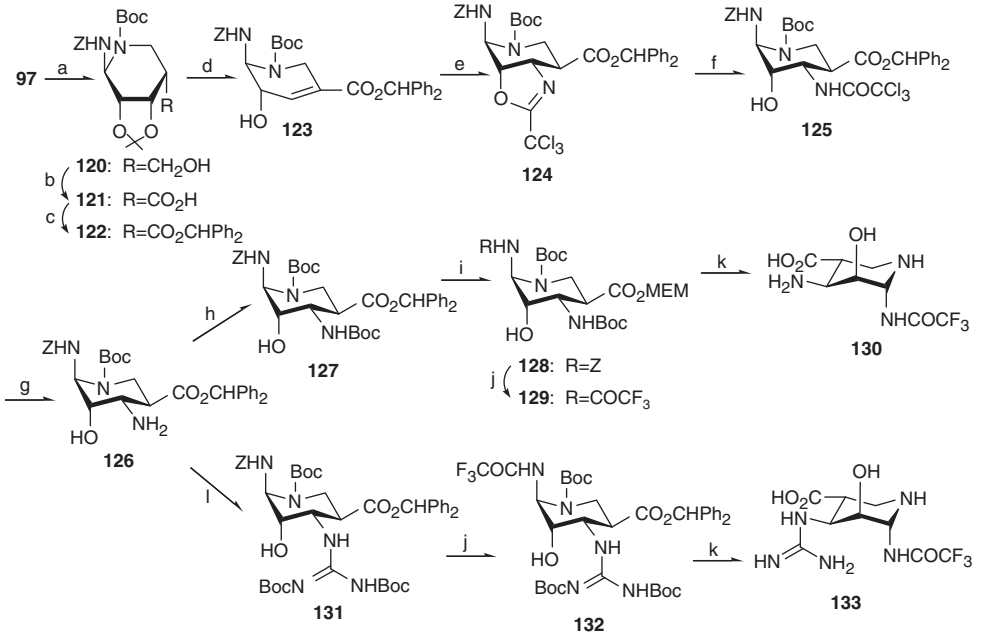


Scheme 8 Reagents and conditions: (a) $(t\text{-BuOCO})_2O$, $i\text{-Pr}_2NEt$, DMF (91%); (b) MeCH(OMe)CH_2OMe, TMSCl, DMF (98%); (c) MEMCl, $i\text{-Pr}_2NEt$, DMF (83%); (d) NaBH4, CF_3CH_2OH/THF (99%); (e) $H_2NNH_2{\cdot}xH_2O$ (54%; recovery, 80%); (f) CF_3CO_2Et, $i\text{-Pr}_2NEt$, DMF; or CCl_3COCl, py, CH_2Cl_2; or $(BocNH)_2CS$, $HgCl_2$, Et_3N, DMF; or Phthalic anhydride, Et3N, DMF, 120 °C (81, 88, 94, 60%); (g) RuO_2, $NaIO_4$, CCl_4/MeCN/H_2O (77, 76, 69, 75%); (h) 4 M HCl/dioxane (97, 100, 94, 95%).

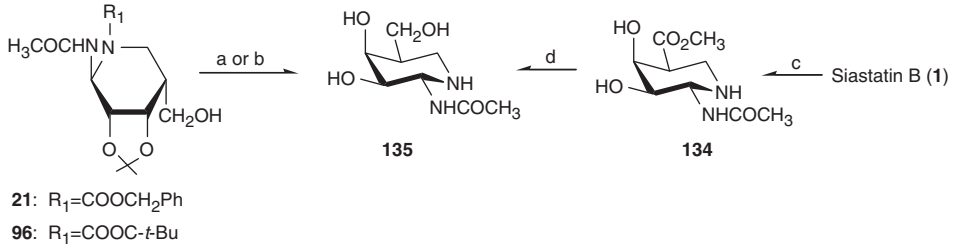


Scheme 9 Reagents and conditions: (a) $(t\text{-BuOCO})_2O$, $i\text{-Pr}_2NEt$, MeOH (93%); (b) Ac_2O, $i\text{-Pr}_2NEt$, DMF; MeONa, MeOH (90%); (c) $PhCN_2$, CH_2Cl_2/MeOH (92%); (d) CCl_3CN, DBU, CH_2Cl_2 (76%); (e) *p*-TsOH, py/H_2O (**112**, 77%; **113**, 9%); (f) $NaBH_4$, EtOH (~62%); (g) 4 M HCl/dioxane (~96%); (h) $(BocNH)_2CS$, $HgCl_2$, Et_3N, DMF (88%).

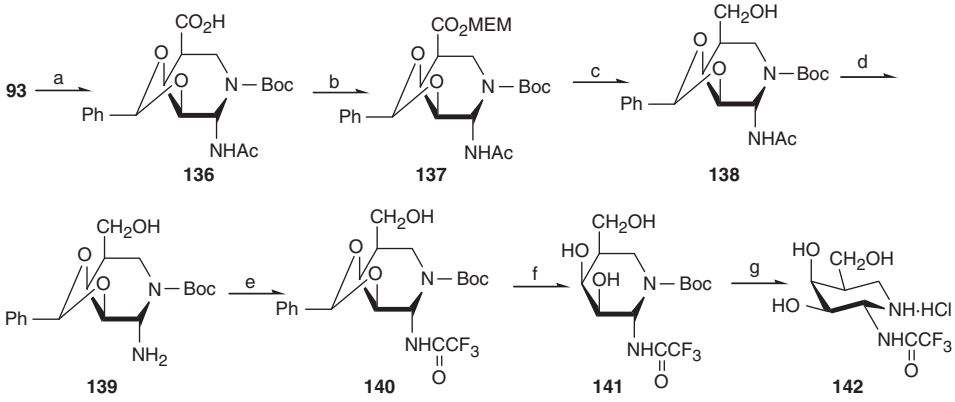
its equivalent generated from **51** in the medium should closely mimic a glycopyranosyl cation that could adapt either a chair-like or a flattened conformational **157** and **158**, respectively, in the putative transition state of β-glucuronidase hydrolysis (Figure 5). D-galacturonic acid-type 2-trifluoroacetamido-, 2-trichloroacetamido-, 2-guanidino- and 2-phthalimido-1-*N*-iminosugars **27b**, **32**, **106**, **107** and **108** also strongly inhibit glucuronidase. These results indicate that β-glucuronidase roughly recognizes the configuration of the 4-OH group of glycopyranose and/or that the binding group of β-glucoronidase to the 4-OH has no important role in the specificity. Interestingly, D-mannuronic acid-type 2-trifluoroacetamido-1-*N*-iminosugar **61** also shows a strong inhibition equivalent to those of



Scheme 10 Reagents and conditions: (a) $Ph_2CH_2CO_2Cl$, *i*-Pr_2NEt, MeOH (86%); (b) RuO_2, $NaIO_4$, MeCN/CCl_4/H_2O (80%); (c) Ph_2CN_2, CH_2Cl_2/MeOH (94%); (d) *t*-BuOK, THF (70%); (e) CCl_3CN, DBU, C6H6 (70%); (f) *p*-TsOH, py/H_2O (95%); (g) $NaBH_4$, EtOH (68%); (h) (*t*-$BuOCO)_2O$, *i*-Pr_2NEt, MeOH (91%); (i) 1 M NaOH/MeOH; MEMCl, *i*-Pr_2NEt, CH_2Cl_2 (83%); (j) H_2, 10% Pd/C, MeCN; $(CF_3CO)_2O$, py (~68%); (k) 4 M HCl/dioxane (~100%); (l) $(BocNH)_2CS$, $HgCl_2$, Et3N, DMF (98%).

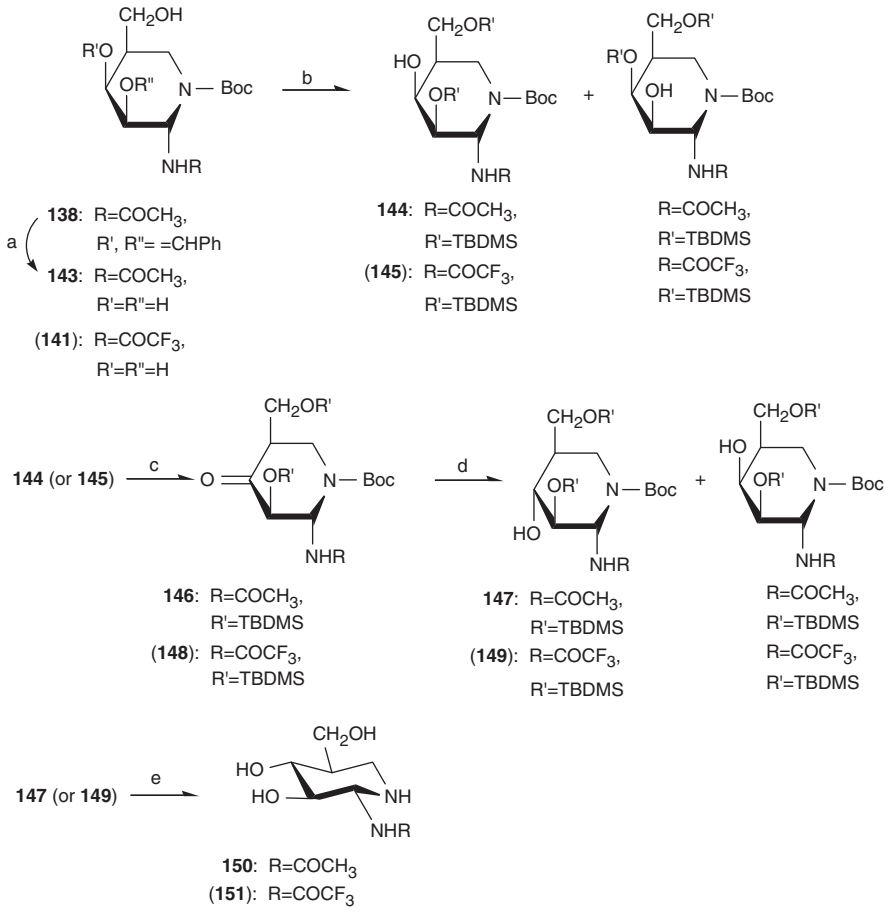


Scheme 11 Reagents and conditions: (a) H_2, 5% Pd/C, MeOH; 4 M HCl/dioxane (78%); (b) 4 M HCl/dioxane(63%); (c) Amberlist 15 (H^+), MeOH (84%); (d) $NaBH_4$, EtOH (90%).



Scheme 12 Reagents and conditions: (a) $(MeO)_2CHPh$, TMSCl, DMF (92%); (b) $MeOCH_2CH_2OCH_2Cl$, *i*-Pr_2NEt, DMF, rt, 98%; (c) $NaBH_4$, CF_3CH_2OH/THF, rt, 94%; (d) $H_2NNH_2{\cdot}xH_2O$, 70 °C, 83%; (e) CF_3CO_2Et, *i*-Pr_2NEt, DMF, 60 °C, 73%; (f) H_2/10% Pd-C, MeOH, rt, 92%; (g) 4 M HCl/dioxane, rt, 80%.

D-glucuronic- and D-galacturonic acid-type 1-*N*-iminosugars **51**, **32**, **106**, **107** and **108**. The ^{1}H-NMR spectrum of **61** shows the adoption of a boat conformation that is different from the chair conformations of D-glucuronic acid- and D-galacturonic acid-type 1-*N*-iminosugars in solution, suggesting that the hemiaminal generated from **61** may mimic the flattened conformation of cation **158** in the transition state



Scheme 13 Reagents and conditions: (a) H_2, 10% Pd/C, MeOH, 94%; (b) TBDMSCl, imidazole, DMF, rt, (**141** → **145**, 58%; **143** → **144**, 50%); (c) Dess–Martin periodinane, CH_2Cl_2, rt, (**145** → **148**,97%; **144** → **146**, 98%); (d) $LiBH_4$, MeCN, −50 °C, (**148** → **149**, 88%; **146** → **147**, 74%); (e) 4 M HCl/dioxane, rt, (**147** → **150**, 91%; **149** → **151**, 80%).

Table 1 Inhibitory activities (IC_{50} (M) and Ki (M)) of L-fucose-type *gem*-diamine 1-*N*-iminosugars against glycosidases

Enzyme	***83***	***84***	***85***	***86***	***87***	***88***
α-L-fucosidase[a]	4.8×10^{-7}	1.1×10^{-8} (5×10^{-9})[j]	9.0×10^{-9}	1.3×10^{-8}	1.8×10^{-6}	7.0×10^{-7}
α-D-glucosidase[b]	1.8×10^{-4}	4.7×10^{-5}	NT	NT	NT	NT
β-D-glucosidase[c]	1.0×10^{-5}	1.2×10^{-4}	NT	NT	NT	NT
α-D-mannosidase[d]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT
β-D-mannosidase[e]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT
α-D-galactosidase[f]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT
β-D-galactosidase[f]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT
β-D-glucuronidase[g]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT
α-D-NAc-galactosaminidase[h]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT
β-D-NAc-glucosaminidase[i]	$>2.2\times10^{-4}$	$>1.8\times10^{-4}$	NT	NT	NT	NT

Abbreviations: IC_{50}, half maximal inhibitory concentration; NT, not tested.
[a]Bovine kidney.
[b]Baker's yeast.
[c]Almonds.
[d]Jack beans.
[e]Snail.
[f]*Escherichia coli.*
[g]Bovine liver.
[h]Chicken liver.
[i]Bovine epididymis.
[j]Ki (M).

(Figure 5). On the other hand, the stable analogs **1** and **27a** in the media expectedly show only a weak inhibitory activity against β-glucuronidase.

The typical analogs (**32**, **51**, **61**) of D-uronic acid-type *gem*-diamine 1-*N*-iminosugars also inhibit recombinant human heparanase from human melanoma A375M cells transfected with

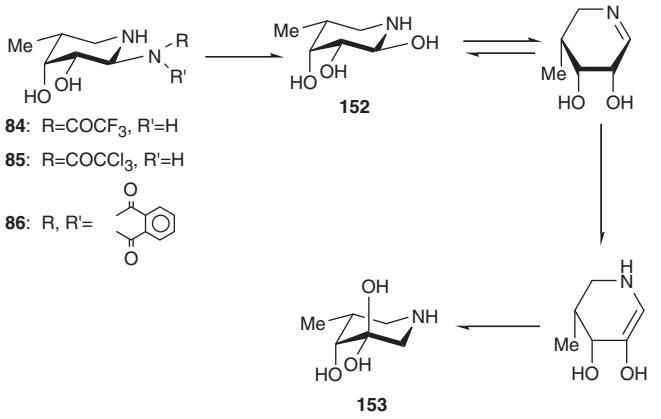


Figure 3 Structural changes of L-fucose-type *gem*-diamine 1-*N*-iminosugars in medium at pH 6.3.

pBK-CMV expression vectors containing the heparanase cDNA (Table 3).[22,41]

Heparanase is an *endo*-β-glucuronidase that specifically cleaves the β-1,4 linkage between D-glucuronic acid and *N*-acetyl-D-glucosamine of heparan sulfate (HS) side chains of HS proteoglycans (HSPGs). The relationships between activity against heparanase and the inhibitor structures are also similar to those discussed regarding the inhibition against *exo*-β-glucuronidase from bovine liver. The weaker activity against heparanase than that against *exo*-β-glucuronidase suggests that heparanase should simultaneously recognize D-glucuronic acid and the adjacent glycoses on both sides of D-glucuronic acid. As expected, all of the L-uronic acid-type *gem*-diamine 1-*N*-iminosugars (**54**, **66**, **116**, **117**, **119**, **130** and **133**) show 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 the D- and L-sugars for specificity and potency.

Table 2 Inhibitory activities (IC_{50} (M)) of D-uronic acid-type *gem*-diamine 1-*N*-iminosugars against D-glycosidase

Enzyme	***1***	***27a***	***27b***	***32***	***106***	***107***	***108***	***51***	***61***
β-D-glucuronidase[a]	7.1×10^{-5}	1.2×10^{-4}	6.2×10^{-8}	6.5×10^{-8}	9.2×10^{-8}	1.3×10^{-7}	6.8×10^{-8}	6.5×10^{-8}	6.5×10^{-8}
α-D-glucosidase[b]	$>3.3\times10^{-3}$ (Ni)	Ni	2.4×10^{-7}	Ni	NT	NT	NT	Ni	Ni
β-D-glucosidase[c]	Ni	Ni	Ni	1.3×10^{-5}	NT	NT	NT	9.8×10^{-5}	3.6×10^{-5}
α-D-mannosidase[d]	Ni	Ni	Ni	Ni	NT	NT	NT	Ni	Ni
β-D-mannosidase[e]	Ni	Ni	Ni	Ni	NT	NT	NT	Ni	Ni
α-D-galactosidase[f]	Ni	Ni	Ni	1.3×10^{-6}	NT	NT	NT	Ni	Ni
β-D-galactosidase[f]	Ni	Ni	Ni	Ni	NT	NT	NT	Ni	Ni
α-D-NAc-galactosaminidase[g]	Ni	Ni	Ni	Ni	NT	NT	NT	Ni	Ni
β-D-NAc-glucosaminidase[h]	Ni	Ni	Ni	Ni	NT	NT	NT	Ni	Ni

Abbreviations: IC_{50}, half maximal inhibitory concentration; Ni, no inhibition at 3.3×10^{-3} M; NT: not tested.
[a]Bovine liver.
[b]Baker's yeast.
[c]Almonds.
[d]Jack beans.
[e]Snail.
[f]*Aspergillus niger.*
[g]Chicken liver.
[h]Bovine epididymis.

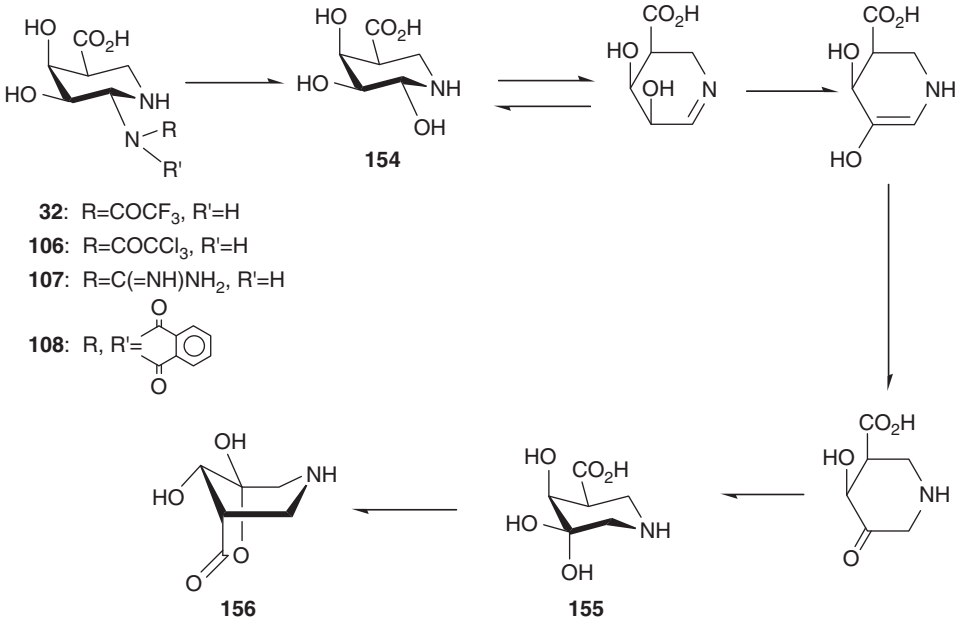
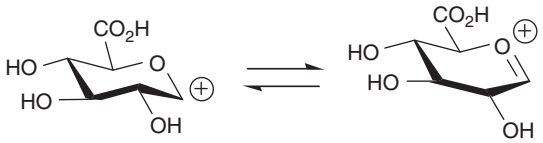


Figure 4 Structural changes of D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars in medium at pH 5.0.



157 **158**

Figure 5 Possible conformations **157** and **158** of the glycosyl cation formed as an intermediate in β-glucuronidase hydrolysis.

Table 3 Inhibitory activities (IC_{50}) of uronic acid-type *gem*-diamine 1-*N*-iminosugars against human heparanase (endo β-D-glucuronidase)

Compounds	*IC_{50} (μM)*
32	1.02 ± 0.29
51	10.5 ± 1.07
54	Ni
61	28.99 ± 11.41
66	Ni

Abbreviations: IC_{50}, half maximal inhibitory concentration; FITC, fluorescein isothiocyanate.
Ni, no inhibition at 3.3 mM; buffer: 50 mM AcNA, pH 4.2, 0.02% CHAPS.
Enzyme: heparanase 0.26 μg protein per tube; substrate: FITC-heparan sulfate 0.5 μl (5 μg HS).
Incubation time: 37 °C, 2 h.

Table 4 Inhibitory activities (IC_{50} (M)) of D-glycose- and D-glycosamine-type *gem*-diamine 1-*N*-iminosugars against D-glycosidases

Enzyme	***135***	***142***	***150***	***151***
α-D-glucosidase[a]	$>3.9\times10^{-4}$	$>3.2\times10^{-4}$	2.9×10^{-6}	1.9×10^{-7}
β-D-glucosidase[b]	7.9×10^{-5}	4.8×10^{-7}	5.4×10^{-6}	4.2×10^{-7}
α-D-galactosidase[c]	2.5×10^{-5}	3.4×10^{-7}	$>3.9\times10^{-4}$	$>3.2\times10^{-4}$
β-D-galactosidase[c]	1.7×10^{-5}	1.7×10^{-7}	$>3.9\times10^{-4}$	1.9×10^{-4}
α-D-mannosidase[d]	$>3.9\times10^{-4}$	$>3.2\times10^{-4}$	2.5×10^{-4}	2.2×10^{-5}
β-D-mannosidase[e]	$>3.9\times10^{-4}$	1.3×10^{-4}	3.8×10^{-5}	3.2×10^{-6}
α-D-NAc-galactosaminidase[f]	3.3×10^{-7}	2.2×10^{-6}	$>3.9\times10^{-4}$	$>3.2\times10^{-4}$
β-D-NAc-glucosaminidase[g]	2.7×10^{-6}	$>3.2\times10^{-4}$	1.2×10^{-6}	$>3.2\times10^{-4}$
β-D-glucuronidase[h]	$>3.9\times10^{-4}$	$>3.2\times10^{-4}$	$>3.9\times10^{-4}$	$>3.2\times10^{-4}$

Abbreviations: IC_{50}, half maximal inhibitory concentration.
[a]Baker's yeast.
[b]Almonds.
[c]*Aspergillus niger.*
[d]Jack beans.
[e]Snail.
[f]Chicken liver.
[g]Bovine epididymis.
[h]Bovine liver.

The inhibitory activities of D-glycose- and D-glycosamine-type *gem*-diamine 1-*N*-iminosugars against glycosidases are summarized in Table 4.[30–32]

As expected, D-glucose-type 2-trifluoroacetamide **151** inhibits α- and β-D-glucosidases very strongly and specifically, and D-glucosamine-type 2-acetamide **150** shows a strong and specific inhibition against β-D-*N*-acetylglucosaminidase. On the other hand, D-galactose-type 2-trifluroacetamide **142** strongly inhibits not only α- and β-D-galactosidases but also β-D-glucosidase. D-galactosamine-type 2-acetamide **135** also strongly inhibits both α-D-*N*-acetylgalactosaminidase and β-D-*N*-acetylglucosaminidase. These results seem to indicate that the hemiaminals of the glycose-type inhibitors generated in the media mimic a transient intermediate **7** (Figure 2) in the hydrolysis of glycosidases, and that the glycosamine-type inhibitors themselves mimic a glycopyranoside in its grand state during the hydrolysis of glycosaminidases. These results also suggest that the axial 4-OH group is the main determinant for specificity and potency of the inhibitors against D-galacto-type hydrolases. However, D-gluco-type hydrolases may roughly recognize the stereochemistry of the 4-OH group and accept both axial and equatorial configurations. *N*-acetylglycosaminidases also recognize the 2-*N*-acetyl group precisely.

Table 5 Effects of 27b and CDP on [^{14}C]NeuAc incorporation into lactosylceramide (LacCer) as an exogenous acceptor

Compound	*Treatment*	*[^{14}C]NeuAc incorporated into GM3 c.p.m. mg^{-1} lipid added*	*%*
—	0	850	100
27b	1.3 mM	961	110
	4.3 mM	659	78
	13 mM	13	1.5
CDP	13 mM	7	0.82

Abbreviations: CDP, cytidine 5′-diphosphate.
The sialyltransferase activity was determined according to the method of Hakomori *et al.*[34] using mouse mammary carcinoma mutant cell line (FUA 169), which shows high activity of CMP-sialic acid:LacCer 2,3-sialosyltransferase.

D-galacturonic acid-type 2-trifluoroacetamido-1-*N*-iminosugar having a hydroxyl group at the C-5 position **27b** inhibits sialyltransferase nearly as well as cytidine 5′-diphosphate, a standard inhibitor, in the mouse mammary carcinoma mutant cell line (FUA169),[42] which has a high transfer activity of sialic acid to lactosylceramide [Galβ1—4Glcβ1—1Cer] to form ganglioside GM3 [NeuAcα2—3Galβ1—4Glcβ1—1Cer] (Table 5). This result suggests that **27b** may resemble a *gem*-diamine 5-*N*-iminosugar and mimic sialic acid (**4**) in the sialyltransferase reaction (Figure 6). This is similar to the method in which siastatin B (**1**) mimics **4** in NA (*N*-acetylsialidase) hydrolysis (Figure 1).

L-altruronic acid-type 2-acetamido-1-*N*-iminosugar **119** and its 1-*N*-2-ethlybutyrylamide (**159**) also inhibit HS 2-*O*-sulfotransferase (HS 2-*O*-ST) over 80% at 25 μM.[43] HS 2-*O*-ST transfers the sulfate group from the sulfate donor, adenosine 3′-phosphate-5′-phosphosulfate, to the 2-OH group of L-iduronic acid of HS, which is composed of a repeating disaccharide unit comprising glucosamine (GlcN) and hexuronic acid (D-glucuronic acid or its C-5 epimer, L-iduronic acid).[44] This result indicates that HS 2-*O*-ST recognizes **119** and **159** as L-iduronic acid. Molecular modeling using PM3 in MOPAC shows the structural similarity between α-L-iduronic acid and **119** (Figure 7).[29] As shown in Figure 7, **119** superimposes well on α-L-iduronic acid and the acetamido and guanidino moieties of **119** are also topographically equivalent to the hydroxyl moieties of α-L-iduronic acid.

Inhibition of esophageal keratinocyte differentiation

Recently, it has been clarified that heparanase is localized in the cell nucleus of the normal esophageal epithelium and esophageal cancer,[45] and that its expression is correlated with cell differentiation.[46] On esophageal cell differentiation, heparanase is translocated from the cytoplasm to the nucleus. On such translocation, heparanase degrades the glycan chain of HS in the nucleus, and changes in the expression of keratinocyte differentiation markers such as p27 and involucrin are observed. D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugar **32** inhibits efficiently this degradation and induction in the nucleus.[47] It has been shown that heparanase regulates the

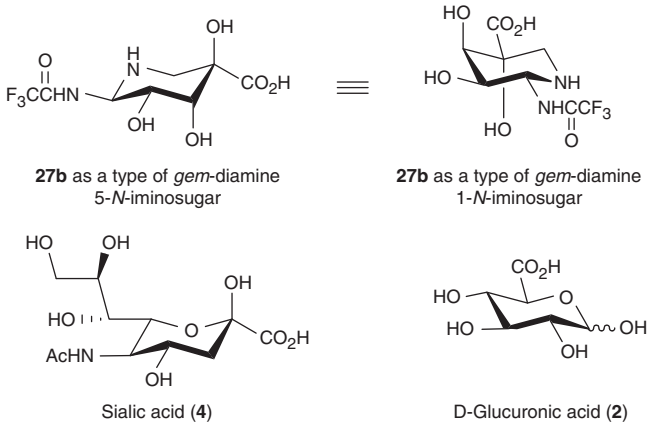


Figure 6 Structural similarity of **27b** to sialic acid (**4**) as *gem*-diamine 5-*N*-iminosugar and to D-glucuronic acid (**2**) as *gem*-diamine 1-*N*-iminosugar.

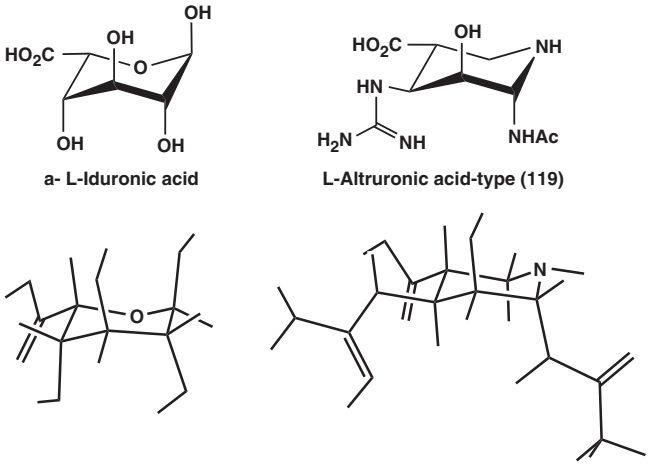


Figure 7 PM3/MOPAC-optimized structures of L-iduronic acid and **119**.

Table 6 Inhibition of experimental pulmonary metastasis of the B16BL6 by *in vitro* treatment with D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars in mice

Compound	*Dose ($\mu g\,ml^{-1}$)*	*Inhibition of metastasis (%)*
Saline (0.9%)	0	0
27b	10	11.9
	30	75.0
	50	80.5**
	100	90.4**
32	10	48.5
	30	61.9
	50	90.8**
106	10	26
	30	29.6
	50	67.3*
107	10	59.1*
	30	74.2*
	50	87.1*

The B16BL6 cells were cultured with or without compounds in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum for 3 days.
The cells were harvested with 0.25% trypsin-1 mM ethylenediaminetetraacetic acid (EDTA) solution from culture dishes and washed twice with phosphate-buffered saline (PBS). The cell suspension (1×10^5) in PBS were implanted intravenously (i.v.) into the tail vein of BDF1 mice. Fourteen days later, the mice were autopsied and the numbers of pulmonary tumor nodules were counted.
*$P<0.01$; **$P<0.001$.

Table 7 Inhibition of experimental metastasis of the B16BL6 by *in vitro* treatment with L-altruronic acid-type *gem*-diamine 1-*N*-iminosugars in mice

Compound	*Dose ($\mu g\,ml^{-1}$)*	*Inhibition of metastasis (%)*
Saline (0.9%)	0	0
116	10	0
	30	12.1
	50	44.3*
119	10	40.1*
	30	91***
	50	97***
130	10	3.8
	30	38.1**
	50	75.5***
133	10	14.1
	30	58.8***
	50	81.0***

The B16BL6 cells were cultured with **116** and **119** for 3 days and with **130** and **133** for 1 day in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum. The cells were harvested with 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) solution. The cells (1×10^5) in 0.1 ml of divalent cation-free Dulbecco's phosphate-buffered saline were collected and injected intravenously (i.v.) into the tail vein of BDF1 mice. Fourteen days later, the mice were autopsied and the pulmonary tumor colonies were counted.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 8 Inhibitory effect of D-galacturonic acid-type 2-trifluoroacetamide 1-*N*-iminosugar 32 on the spontaneous lung metastasis of 3LL cells in mice

Compound	*Administered dose ($mg\,kg^{-1}$)×days*	*Inhibition of metastasis (%)*
Saline (0.9%)	0×5	0
32	10×5	5.1
	50×5	23.5
	100×5	57.1*

Five female C57BL/6 mice per group inoculated with 3LL cells (1×10^6) by intra-footpad injection were administered intravenously (i.v.) with **32** for 5 days starting on the day of the surgical excision of primary tumors on day 9. Mice were killed 10 days after tumor excision.
*$P<0.01$.

differentiation of normal esophageal epithelium through nuclear translocation and nuclear HS cleavage and has an important role in the development of normal esophageal epithelium.

Inhibition of microglial cell migration

Very recently, microglia, the resident macrophages in the brain, have been found to express heparanase mRNA and protein, which can degrade the glycan chain of HSPGs.[48] Heparanase activity is correlated with the *in vitro* transmigration ability of microglia through an artificial basement membrane (BM)/extracellular matrix (ECM) containing HSPGs. D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugar **32** inhibits this process in a dose-dependent manner.[48] The transmigration of microglia through BM/ECM appears to be associated with the degradation of HSPG, and this is also inhibited dose dependently by **32**. The results suggest the involvement of heparanase in the migration or invasion of microglia or brain macrophages across the BM around the brain vasculature.

Table 9 Inhibition of invasive activity of tumor cells by 32

Experimental	*Treatment ($\mu g\,ml^{-1}$)*	*Tumor cell line*	*Inhibition*
1	0	3LL	0
	100	3LL	72.4*
	200	3LL	80.1*
2	0	B16BL6	0
	100	B16BL6	29.1
	300	B16BL6	64.1*

Tumor cells were cultured in the presence of **32** for 72 h (B16BL6) or 15 h (3LL). Numbers of cells that invaded the reconstituted basement membrane Matrigel in 6 h (Experiment 1) or 3 h (Experiment 2) were counted. A laminin coated under the filter surface was used as a cell attractant.
*$P<0.05$.

Table 10 Inhibition of invasive activity of B16BL6 cell by 119 and 133

Experimental	*Compound*	*Treatment ($\mu g\,ml^{-1}$)*	*Inhibition %*
1	**119**	0	0
		200	44.8*
		300	58.9**
2	**133**	0	0
		100	44.4
		200	61.1***
		300	63.9*

The cells were cultured with **119** and **133** for 72 and 24 h, respectively. Numbers of invaded cells on the lower surface of the Matrigel/laminin-coated filters in 3 h (Experiment 1) or 6 h (Experiment 2) were counted.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

THERAPEUTIC POTENTIALS

Tumor metastasis

Recent biochemical studies have shown that cellular function and phenotype are highly influenced by HSPGs of ECM, and that the enzymatic degradation of ECM is involved in fundamental biological phenomena, including angiogenesis and cancer metastasis.[49–53] Proteolytic enzymes (heparanase and matrix metalloproteinases) secreted by tumor cells are capable of degrading ECM and BM components, and their activities are closely related to the metastasis potential of malignant cells.[54–60]

The inhibitory effects of uronic acid-type *gem*-diamine 1-*N*-iminosugars that have inhibitory activities against *exo*-uronidase, heparanase, sulfotransferase and sialyltransferase were also evaluated on tumor metastasis using the experimental and spontaneous pulmonary metastasis in mice.

D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars **27b**, **32**, **106** and **107** that have inhibitory activities against *exo*-uronidase and heparanase significantly suppress in a dose-dependent manner the number of colonies of pulmonary metastasis of B16BL6 cells in the experimental metastasis (Table 6).[6,7,26,27] Of these, 2-trifluoroacetamide **32** inhibits pulmonary metastasis most potently.

L-altruronic acid-type *gem*-diamine 1-*N*-iminosugars **116**, **119**, **130** and **133** that have inhibitory activity against HS 2-*O*-ST also reduce remarkably in a dose-dependent manner the pulmonary colonization of B16BL6 cells in the experimental metastasis (Table 7).[28] Of these, 2-acetamido-4-guanidino-1-*N*- iminosugar **119** inhibits the experimental metastasis very strongly.

As shown in Table 8, the inhibition of spontaneous lung metastasis in mice by the intravenous (i.v.) injection of **32** is more noticeable.[6,61] Compound **32** shows 57% inhibition of metastasis by the administration of 100 mg kg^{-1} per day for 5 days.

Table 11 Inhibitory activity (IC_{50} (M)) of 3-episiastatin B (160) and DDNA (161) against influenza virus *N*-acetylneuraminidase

	Influenza virus neuraminidase		
Compound	*A/FM/1/47 (H1N1)*	*A/Kayano/57 (H2N2)*	*B/Lee/40*
3-Episiastatin B	7.4×10^{-5}	$>1.0\times10^{-5}$ (25.6)	4.2×10^{-5}
DDNA	$<1.0\times10^{-5}$ (93.2)	2.9×10^{-5}	4.9×10^{-5}

Abbreviations: IC_{50}, half maximal inhibitory concentration;
( ): inhibition (%) at 1.0×10^{-5} M.
Neuraminidase inhibition assay was carried out using the method of Aminoff.[71,72]

Table 12 Inhibition (%) of 3-episiastatin B (160) and DDNA (161) against influenza virus A/FM/1/47 (H1N1) infection in MDCK cells

	Plaque forming units (PFU)			*Stained area*		
Compound	*40 μM*	*20 μM*	*10 μM*	*40 μM*	*20 μM*	*10 μM*
3-Episiastatin B	88.9	55.5	35.6	97.1	87.2	64.1
DDNA	100	100	89.6	100	100	98.7

Plaque assay was carried out using the modified method of Schulman and Palese.[71,73]

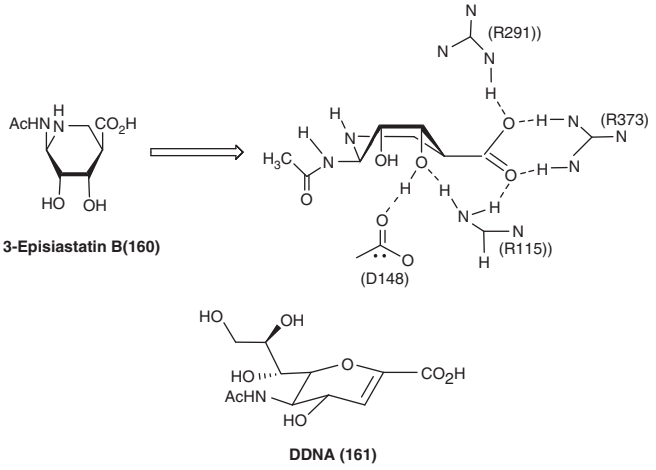


Figure 8 BIOCES[E] /AMBER minimized structure of 3-episiastatin B (**160**) in a pocket of active site residues of the crystal structure of influenza virus B/Beijin/1/87 and structure of DDNA (**161**).

On the other hand, D-galacturonic acid-type **32** and L-altruronic acid-types **119** and **133** inhibit in a dose-dependent manner the transmigration of B16BL6 and 3LL cells through the reconstituted BM (Matrigel) by *in vitro* treatment (Tables 9 and 10).[8,61]

Gem-diamine 1-*N*-iminosugars related to D-glucuronic and L-iduronic acids markedly inhibit the experimentally induced lung metastasis of B16BL6 and/or 3LL cells, and also the spontaneous lung metastasis of 3LL cells after i.v. administration. D-uronic acid-type iminosugars inhibit tumor heparanase activity, an effect that probably results from their resemblance to D-glucuronic acid as a substrate for tumor heparanase. L-uronic acid-type iminosugars inhibit HS 2-*O*-ST activity, an effect that probably results from their resemblance to L-iduronic acid as a substrate for HS 2-*O*-ST. Furthermore, *gem*-diamine 1-*N*-iminosugars prevent the transmigration of B16BL6 and/or 3LL cells through the reconstituted BM with no cytotoxicity. These results suggest that the anti-metastatic effect of the iminosugars may be due to their anti-invasive rather than their

anti-proliferative activities. It is likely that *gem*-diamine 1-*N*-iminosugars related to D-glucuronic and L-iduronic acids act as the mimic of respective uronic acids in the metabolism of ECM and/or BM involved in tumor metastasis. These iminosugars seem to modify the cell surface glycoconjugates of tumor cells simultaneously, thereby altering the cell properties involved in cellular recognition and adhesion.

Influenza virus infection

Some of the uronic acid-type *gem*-diamine 1-*N*-iminosugars could also mimic sialic acid (**4**) in the sialidase (*N*-acetyneuraminidase) reaction as an alternative type of *gem*-diamine 5-*N*-iminosugar such as siastatin B (**1**) (Figure 1).

Two integral membrane glycoproteins, hemagglutinin (HA) and NA, of the influenza virus were proved to have important roles at the beginning of infection and during the spread of the infection,[61–66] respectively, and it has been postulated that the inhibitors of HA and NA should have antiviral properties. NA is a glycosidase that cleaves the α-ketosidic bond linking the terminal sialic acid to the adjacent oligosaccharide residues of glycoproteins and glycolipids.[67,68] In 1992, the binding modes of sialic acid to NAs of the influenza virus B/Beijin/1/87 and A/Tokyo/3/67 were clarified to involve the characteristic α-boat conformation.[69,70]

3-Episiastatin B (**160**) shows specific potent inhibitory activities against influenza virus NAs and the influenza virus infection in the MDK cell *in vitro* (Tables 11 and 12).[71] Its activity is almost comparable with that of DDNA **161**, a standard inhibitor. The lowest energy boat conformer of **160** obtained by molecular modeling using PM3/MOPAC is superimposed onto the α-boat conformer of **4** in a pocket of the active site residue of the crystal structure of influenza virus B/Beijin/1/87 NA complex with **4** by a docking experiment using BIOCES/AMBER[6] (Figure 8). Compound **160** was shown to be a possible lead compound for anti-influenza virus agents.

Lysosomal storage disease

Lysosomal storage disease, in which specific enzymes of glycoconjugate degradation are deficient, is an inherited storage disorder characterized by the accumulation of partially degraded molecules in lysosomes, eventually resulting in cell, tissue and organ dysfunctions.[74] The strategies for overcoming the deficit in enzyme capacity is to provide an endogenous supply of completely functional enzymes by direct infusion or by cellular replacement with cells capable of secreting enzymes (bone marrow replacement) or by gene delivery.[75–77] An alternative to enzyme replacement is to reduce substrate influx to the lysosome by inhibiting the synthesis of glycoconjugates. This strategy has been called substrate reduction therapy.[78–80] By balancing the rate of glycoconjugate synthesis with the impaired rate of glycoconjugate breakdown, the substrate influx–efflux should be regulated to rates that do not lead to storage.

The enzyme deficient in Hunter's syndrome (MPS II) is iduronate 2-*O*-sulfatase, which functions by removing a 2-*O*-sulfate group from the iduronic acid unit of HS.[81–83] As mentioned above in the section 'Total synthetic route to *gem*-diamine 1-*N*-iminosugars,' compounds **119** and **159** inhibit strongly recombinant iduronate 2-*O*-ST over 80% at 25 μM.[43] Therefore, a partial inhibition of iduronate 2-*O*-ST by these iminosugars would reduce the build-up of the sulfated iduronic acid of HS in cells.

On the other hand, an alternative strategy, chemical chaperon therapy has been proposed for lysosomal storage disease, on the basis of a paradoxical phenomenon that states that an exogenous competitive inhibitor of low molecular weight stabilizes the target mutant protein and restores its catalytic activity as a molecular chaperon.[84–87] A competitive inhibitor binds to a misfolded mutant protein as a molecular chaperon in the endoplasmic reticulum/Golgi apparatus of the cell, resulting in the formation of a stable complex at neutral pH and transport of the catalytically active enzyme to lysosomes, in which the complex dissociates under acidic conditions and the mutant enzyme remains stabilized and functional. Some iminosugars have shown remarkable efficacy for chemical chaperon therapy of Fabry and Gaucher's disease in clinical trials.[88] *Gem*-diamine 1-*N*-iminosugars have proven to be highly potent and specific competitive inhibitors against glycosidases, glycosyltransferases and sulfotransferases. These facts suggest that *gem*-diamine 1-*N*-iminosugars would be reasonable candidates for chemical chaperon therapy in lysosomal storage diseases, and that they are in principle applicable to all types of lysosomal storage diseases.

The main advantage of these therapies is the potential ability of the inhibitors as small molecules to cross the blood–brain barrier (BBB) and elicit a favorable response in the central nervous system (CNS). The difficulties in delivering proteins (enzymes) or genes to the CNS are not apparent using a small molecule that can cross the BBB easily. The therapy using iminosugars has the potential to prevent and/or reverse the effects of lysosomal storage disease both in the body and in the brain.

CLOSING REMARKS

This article describes our current progress in the chemical, biochemical and therapeutic potential of *gem*-diamine 1-*N*-iminosugars, a new family of glycomimetics, with a nitrogen atom in place of the anomeric carbon. Mechanistically, the protonated form of new glycomimetics may act as a mimic of a glycopyranosy cation and/or the transition state formed during enzymatic glycosidic hydrolysis. New inhibitors that mimic the charge at the anomeric position of the transition state have proven to be potent and specific inhibitors of various kinds of glycosidases.

New inhibitors that affect some metabolic enzymes of glycoconjugates have been found to participate in tumor metastasis. Uronic acid-type *gem*-diamine 1-*N*-iminosugars certainly contribute to the study regarding the involvement of carbohydrates in malignant cell movement and seem to be a promising new drug candidate for cancer chemotherapy. The *N*-acetylneuraminic acid-type iminosugar has shown potency against influenza virus infection, indicating a possible drug candidate that inhibits NA. It is also likely that *gem*-diamine 1-*N*-iminosugar, a new family of glycomimetics, is a reasonable drug candidate for chemical chaperon therapy and/or substrate reduction therapy in lysosomal storage disorder.

Iminosugars have proven to be a rich source of therapeutic drug candidates in the past several years and have thus become the special focus of research attention. Of these, *gem*-diamine 1-*N*-iminosugars have been recently recognized as a new source of therapeutic drug candidates in a wide range of diseases associated with the carbohydrate metabolism of glycoconjugates.

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