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GEM-DIAMINE 1-*N*-IMINOSUGARS, A NEW FAMILY OF GLYCOSIDASE INHIBITORS: SYNTHESIS AND BIOLOGICAL ACTIVITY+

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Abstract – Specific inhibitors of glycosidases are useful for unraveling how glycoconjugates regulate biological functions, and also for developing the new drugs for the serious diseases associated with both the biosynthesis and the degradation of glycoconjugate such as cancer, tumor metastasis, inflammatory disorders, viral and bacterial infections and so forth. This article describes the synthesis and biological activity of *gem*-diamine 1-*N*-iminosugars, a new class of glycosidase inhibitors, with a nitrogen atom in place of the anomeric carbon. New inhibitors that may mimic the glycopyranosyl cation, the putative intermediate of enzymatic glycosidic hydrolysis, have shown strong and specific inhibition against glycosidases. The inhibitors also illustrate a promising candidate of new drugs for tumor metastasis.

INTRODUCTION

Glycoconjugates such as glycoprotein, glycolipid and proteoglycan are ubiquitous in nearly all forms of life and involved in cell-to-cell communication, cell-cell recognition, cell adhesion, cell growth regulation, differentiation and transport.[1] Specific inhibitors of glycosidases are useful for unraveling how glycoconjugates regulate biological function,[2] and also in developing the new drugs for the serious diseases associated with both the biosynthesis and the degradation of glycoconjugate, namely cancer, tumor metastasis, inflammatory disorders, viral and bacterial infections and so forth.[3] Glycosidase inhibitors have generally structural homology with the natural glycosides, and are the most frequently

+ Dedicated to Professor Barry M. Trost on the occasion of his 65th birthday.

polyhydroxylated six- and five-membered heterocyclic ring in which the ring oxygen often replaced by a nitrogen atom (iminosugars).[4] Various types of iminosugar inhibitors have also been designed on the basis of a flattened, half-chair oxocarbenium ion-like transition state in the reaction catalyzed by glycosidases[5] (Figure 1). In 1974, siastatin B (**1**), an unusual iminosugar was isolated as an inhibitor against neuraminidases from *Streptomyces* culture.[6] Siastatin B (**1**) also inhibits β-D-glucuronidase and *N*-acetyl-β-D-glucosaminidase. We recognized from its glycosidase inhibitory activity that siastatin B (**1**) resembles structurally D-glucuronic acid (**2**) and *N*-acetyl-D-glucosamine (**3**) as well as *N*-acetylneuraminic acid (**4**) (Figure 2). It is distinct from the hitherto known glycosidase inhibitors of heterosugars[4] with 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[7] (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*-iminosugars (**5**) may mimic the glycopyranosyl cation (**6**), the putative intermediates of enzymatic glycosidic hydrolysis (Figure 3). Actually this is the case which leads to the new findings of highly potent and specific inhibition against glycosidases, and also therapeutic potentials for tumor methastasis.

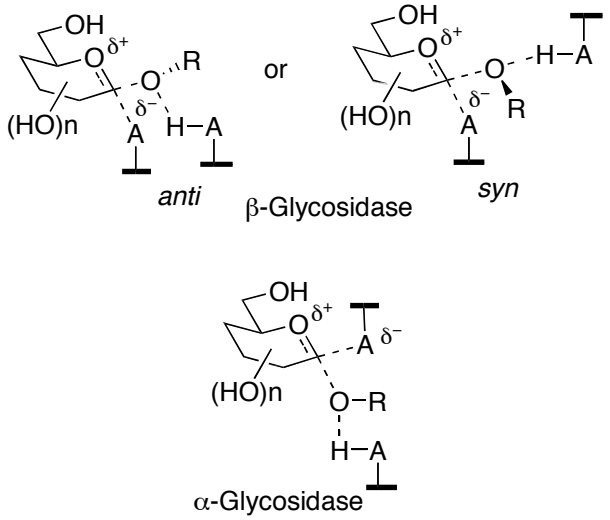


Figure 1. Presumed oxocarbenium ions in a transition state of hydrolysis by α- and β-glycosidases.

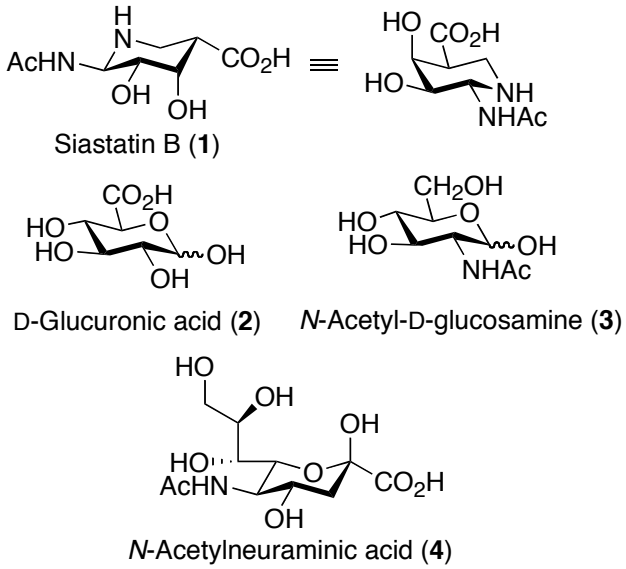


Figure 2. Structural resemblance of siastatin B (**1**) to D-glucuronic acid (**2**), *N*-acetyl-D-glucosamine (**3**) and *N*-acetylneuraminic acid (**4**).

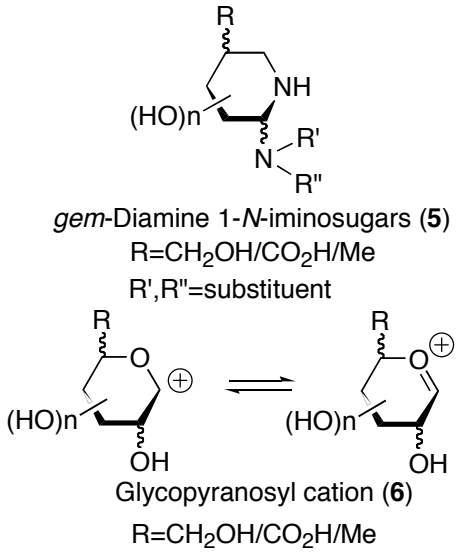
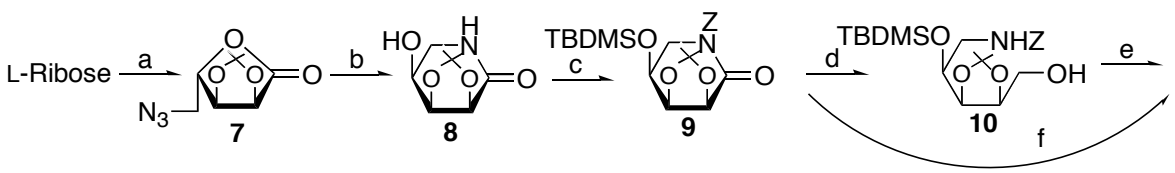


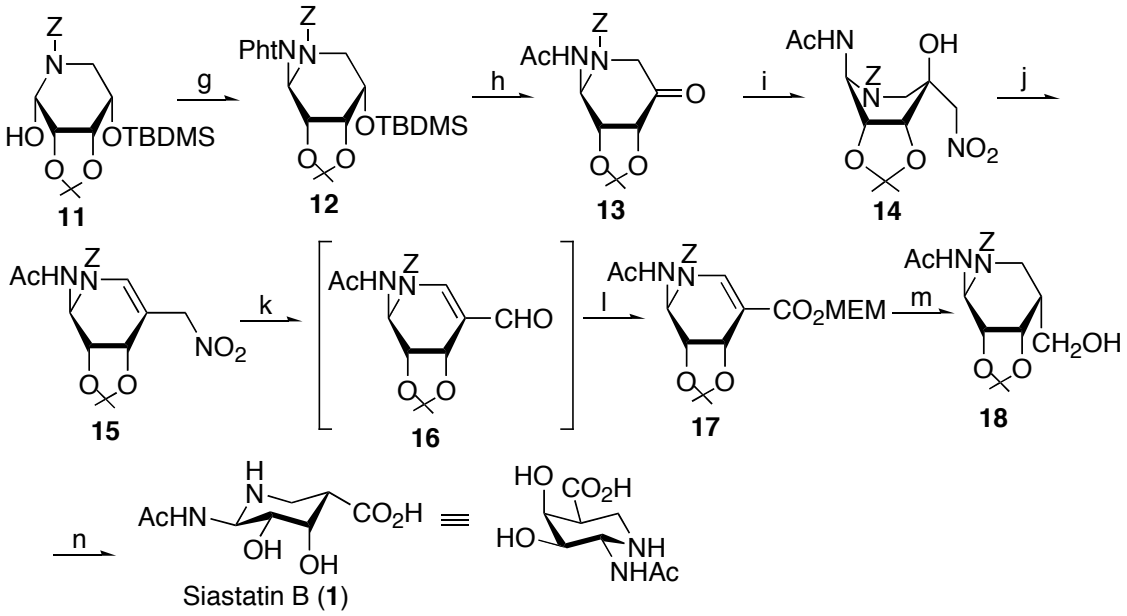
Figure 3. General structures of *gem*-diamine 1-*N*-iminosugars (**5**) and glycopyranosyl cation (**6**), the putative transition state of enzymatic glycosidic hydrolysis.

SYNTHESIS

SYNTHESIS OF URONIC ACID-TYPE *GEM*-DIAMINE 1-*N*-IMINOSUGARS

Total Synthesis Of Siastatin B





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 oxid. (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; 1M HCl, then Dowex 50W X4 (H^+) eluted with 2% NH_4OH (66%)

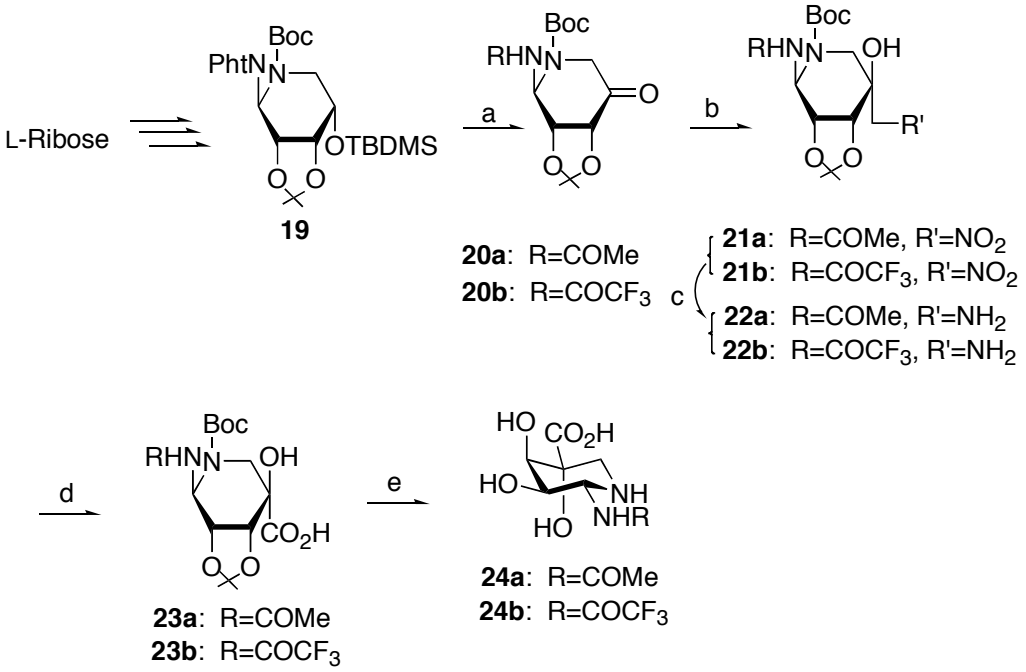
Scheme 1.

The first total synthesis of siastatin B (**1**), D-galacturonic acid-type 2-acetamide-1-*N*-iminosugar was achieved in a totally stereo- and eantiospecific fashion based on a chiron strategy as shown in Scheme1.[8] The synthesis of key intermediate, lactam (**8**) began with L-ribose which was transformed to azido-L-ribonolactone (**7**) by protection of the 2,3-diol, azido formation and oxidation.[9] Hydrogenation of the azido group of **7** afforded crystalline (**8**) with the ring expansion. Stereospecific introduction of an axial hydroxyl group at C-2 position was achieved by hydride reduction of the protected lactam (**9**) to **10**, and Swern oxidation[10] to give animal (**11**). A single isomer controlled by an anomeric effect[11] results from this oxidation. One-step stereospecific transformation from lactam (**9**) into aminal (**11**) was also best achieved by L-selectride reduction in THF. On the other hand, diisobutyl aluminum hydride (DIBAH) reduction in THF gave stereospecifically its epimer. Stereoselectivity in L-selectride reduction was probably caused by hydride attack from a less sterically hindered side (upper side), whereas in DIBAH reduction it was controlled by a metal chelation formed between aluminum and oxygen atoms of isopropylidene group. Displacement of the axial hydroxyl group of **11** to the equatorial amino group proved troublesome until the Mitsunobu reaction[12] using phthalimide in DMF was uncovered to give quantitatively the desired product (**12**). Replacement of the amino substituent, removal the *tert*-butyldimethylsilyl group and oxidation to **13** were unexceptional. Condensation of **13** with nitromethane was found to proceed smoothly to give **14** as a single stereoisomer. The *S*-configuration at

C-5 was best established by analogy with the stereochemistry of synthetic *N*-(*t*-butoxycarbonyl) antipode (**21a**) (*vide infra*) by X-Ray crystallographic analysis.[13] Acetylation of **14** followed by base-catalyzed elimination of the acetoxyl group afforded exclusively the endocyclic nitro olefin (**15**). Transformation of **15** to **17** was achieved *via* the α,β-unsaturated aldehyde (**16**) produced by simply warming in pyridine. Catalytic reduction of **17** accompanied by elimination of the hydroxyl group at C-4, and hydride reduction of the double bond also proceeded unfavorably and without chemoselectivity. Then, **17** was once converted to the α,β-saturated hydroxymethyl compound (**18**) by stereoselective reduction with $NaBH_4$ in trifluoroethanol and THF.[14] The carboxylic acid formed upon oxidation of **18** was converted into crystalline siastatin B (**1**) by removal of protecting groups. The enantiomer of **1** was also synthesized from D-ribono-1,4-lactam by the same method used in the synthesis of **1**. The total synthesis elucidated the absolute configuration of siastatin B as the (3*S*, 4*S*, 5*R*, 6*R*)-isomer (**1**).

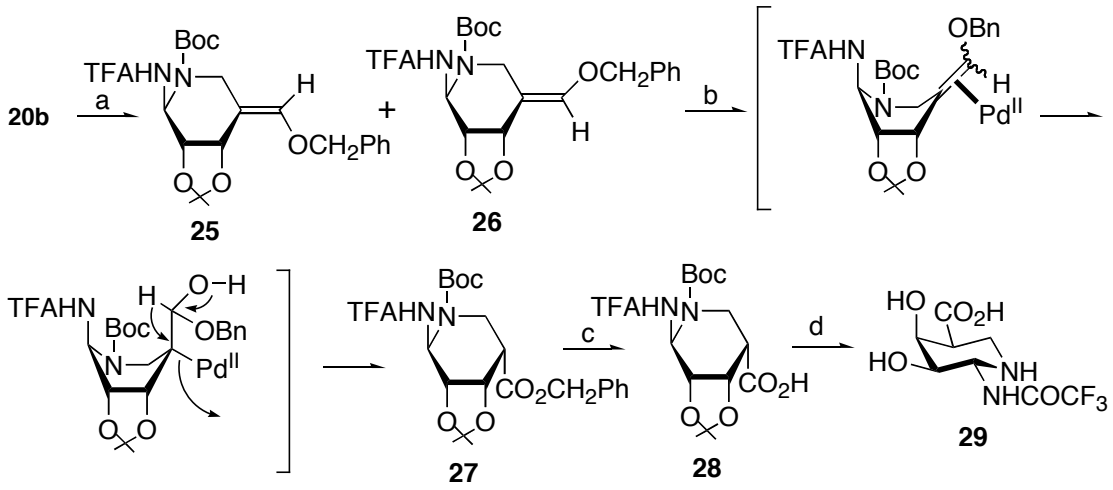
Synthesis Of D-Uronic Acid-Type *Gem*-Diamine 1-*N*-Iminosugars

The strategy of total synthesis of siastatin B (**1**) employing L-ribose as a chiral starting material is applicable to a wide range of D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars (Schemes 2 and 3).



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) 4M HCl/dioxane (92 and 96%)

Scheme 2.



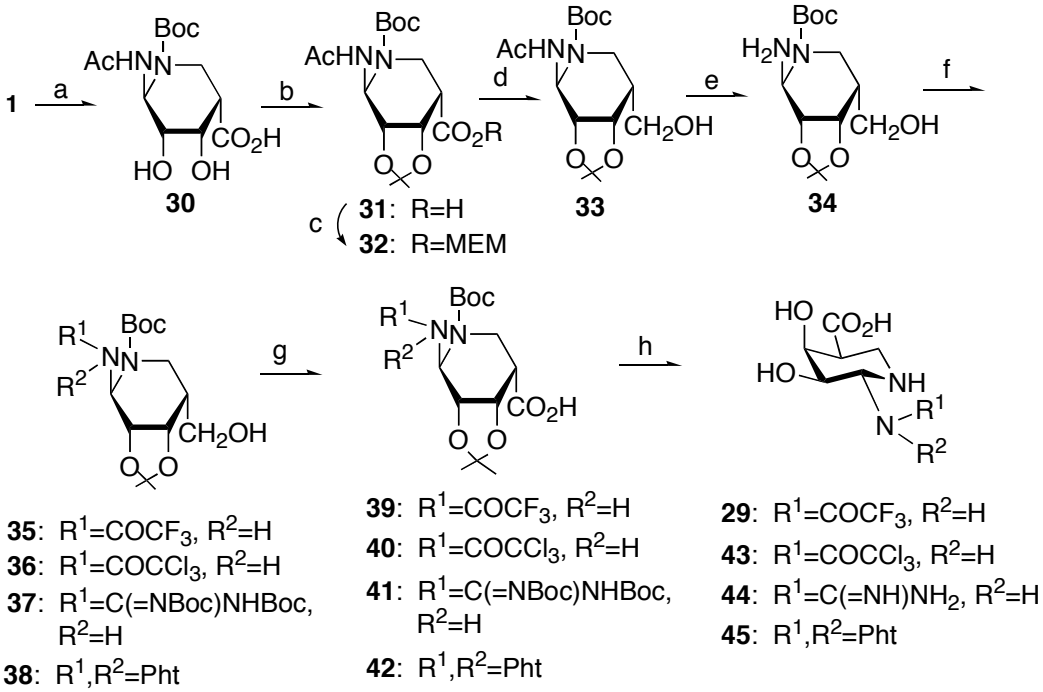
Reagents and conditions: a) $PhCH_2OCH_2PPh_3Cl$, PhLi, THF (48%) b) $PdCl_2$, CuCl, O_2, DMF/H_2O (46%) c) H_2, Pd/C, EtOAc (92%) d) 4M HCl/dioxane (96%)

Scheme 3.

D-Galacturonic acid-type 2-acetamide-1-*N*-iminosugars (**24a** and **24b**) having hydroxyl groups at C-5 position were obtained from L-ribose in a straightforward manner.[13] The acid labile Boc group was employed as the protecting group of imino group different from the Cbz group in the total synthesis.[8] Condensation of the ketones (**20a** and **20b**) with nitromethane proceeded stereospecifically to give the key compounds (**21a**) and (**21b**), respectively. The *S*-configuration at C-5 was proved by X-Ray crystallographic analysis of the antipode of **21a**. Catalytic hydrogenation of each compound with Raney Ni gave the aminomethyl compounds (**22a**) and (**22b**), which were converted into the carboxylic acids (**23a**) and **(23b)** upon ninhydrin oxidation[15] to the aldehydes and subsequent oxidation with sodium chlorite. Removal of the protecting groups of each compound with acid resulted in the desired products. An alternate route using the Wacker process oxidation[16] of the enol ethers (**25**) and (**26**) to D-galacturonic acid-type 2-trifluoroacetamide-1-*N*-iminosugar (**29**) was also developed. Reaction of the ketone (**20b**) with (benzyloxymethylene)triphenylphophorane afforded the (*Z*)-benzyloxy ether **(25**) and (*E*)-isomer (**26**). Oxidation of both **25** and **26** by the Wacker process utilizing palladium chloride and copper(I) chloride in DMF-H_2O gave stereospecifically the ester (**27**). The right stereochemistry may be derived *via* a possible reaction mechanism[17] shown in Scheme 3. The π-complex is formed by attack of the palladium reagent from the less-hindered side of the boat conformers of **25** and **26**, and the subsequent addition of water to the double bond forms the unstable δ-alkyl intermediate which is transformed into the benzyl ester (**27**) by a 1,2-hydride shift and reductive elimination of the palladium. Catalytic hydrogenolysis followed by acid treatment resulted in the desired product.

Siastatin B (**1**), a *Streptomyces* metabolite is applicable for an efficient and practical route[18] to D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars (Scheme 4) as it is. Transketalization using chlorotrimethylsilane[19] proceeded efficiently to give the ketal (**31**). A sequence of esterification, hydride

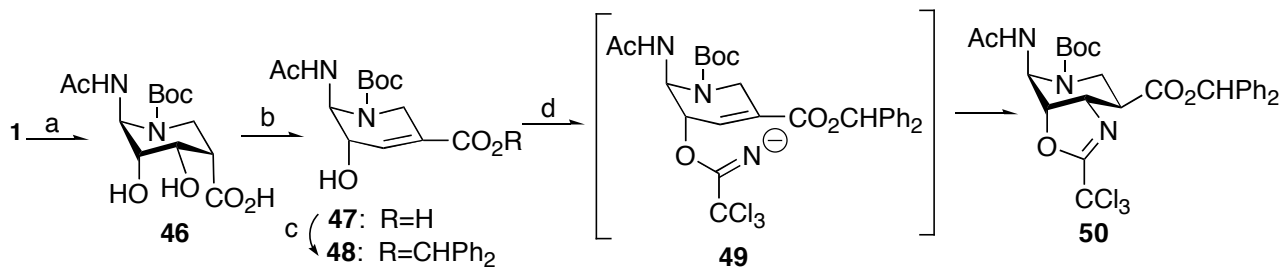
reduction and hydrazinolysis gave the amino alcohol (**34**), which was smoothly converted to the trifluoroacetamide (**35**). Oxidation of **35** was best achieved by ruthenium catalyzed oxidation developed by Sharpless *et al.*[20] to give the carboxylic acid (**39**). Removal of protecting group resulted in the desired product (**29**). 2-Trichloroacetamide, guanidino and phthaloyl analogs (**43**, **44** and **45**) were also obtained by the similar strategy.

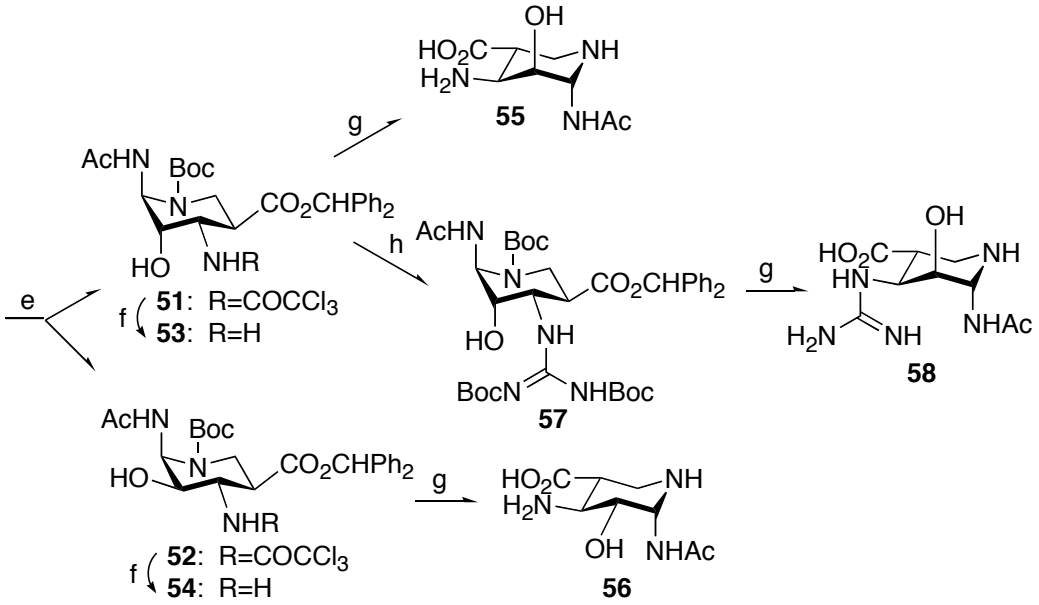


Reagents and conditions: a) (*t*-BuOCO)$_2$O, *i*-Pr$_2$NEt, DMF (91%) b) MeCH(OMe)CH$_2$OMe, TMSCl, DMF (98%) c) MEMCl, *i*-Pr$_2$NEt, DMF (83%) d) NaBH$_4$, CF$_3$CH$_2$OH/THF (99%) e) H$_2$NNH$_2$·xH$_2$O (54%; reconvery y., 80%) f) CF$_3$CO$_2$Et, *i*-Pr$_2$NEt, DMF; or CCl$_3$COCl, py, CH$_2$Cl$_2$; or (BocNH)$_2$CS, HgCl$_2$, Et$_3$N, DMF; or Phthalic anhydride, Et$_3$N, DMF, 120°C (81%, 88%, 94%, 60%) g) RuO$_2$, NaIO$_4$, CCl$_4$/MeCN/H$_2$O (77%, 76%, 69%, 75%) h) 4M HCl/dioxane (97%, 100%, 94%, 95%)

Scheme 4.

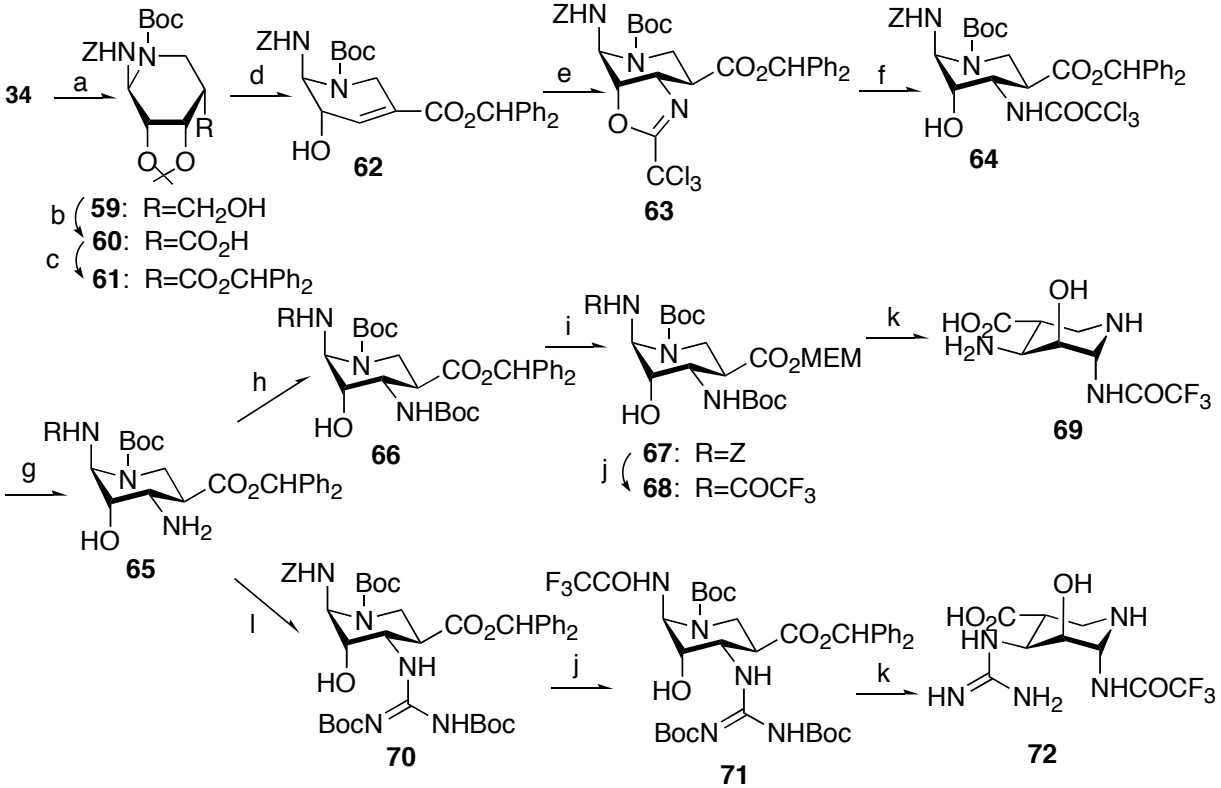
Synthesis Of L-Uronic Acid-Type *Gem*-Diamine 1-*N*-Iminosugars





Reagents and conditions: a) (*t*-BuOCO)$_2$O, *i*-Pr$_2$NEt, MeOH (93%) b) Ac$_2$O, *i*-Pr$_2$NEt, DMF; MeONa, MeOH (90%) c) PhCN$_2$, CH$_2$Cl$_2$/MeOH (92%) d) CCl$_3$CN, DBU, CH$_2$Cl$_2$ (76%) e) *p*-TsOH, py/H$_2$O (**48**, 77%; **49**, 9%) f) NaBH$_4$, EtOH (~62%) g) 4M HCl/dioxane (~96%) h) (BocNH)$_2$CS, HgCl$_2$, Et$_3$N, DMF (88%)

Scheme 5.



Reagents and conditons: a) Ph$_2$CH$_2$CO$_2$Cl, *i*-Pr$_2$NEt, MeOH (86%) b) RuO$_2$, NaIO$_4$, MeCN/CCl$_4$/H$_2$O (80%) c) Ph$_2$CN$_2$, CH$_2$Cl$_2$/MeOH (94%) d) *t*-BuOK, THF (70%) e) CCl$_3$CN, DBU, C$_6$H$_6$ (70%) f) *p*-TsOH, py/H$_2$O (95%) g) NaBH$_4$, EtOH (68%) h) (*t*-BuOCO)$_2$O, *i*-Pr$_2$NEt, MeOH (91%) i) 1M NaOH/MeOH; MEMCl, *i*-Pr$_2$NEt, CH$_2$Cl$_2$ (83%) j) H$_2$, 10% Pd/C, MeCN; (CF$_3$CO)$_2$O, py (~68%) k) 4M HCl/dioxane (~100%) l) (BocNH)$_2$CS, HgCl$_2$, Et$_3$N, DMF (98%)

Scheme 6.

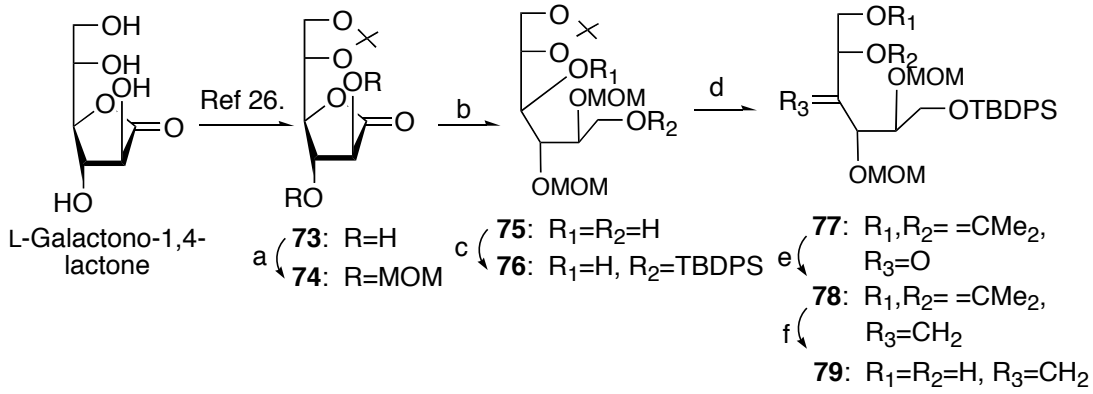
Configurational inversion of the carboxyl group of siastatin B (**1**) leads to *gem*-diamine 1-*N*-iminosugars corresponding to L-sugar.[21] The intramolecular Michael addition of *O*-imidate to the α,β-unsaturated ester (**48**) through *cis* oxiamination[22] (Overman rearrangement) as the key step gave effectively L-uronic acid-type *gem*-diamine 1-*N*-iminosugars (Schemes 5 and 6).

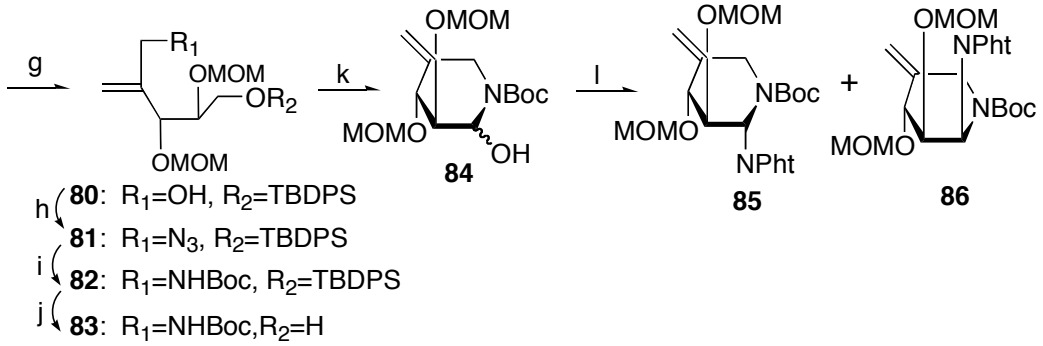
The α,β-unsaturated ester (**48**) was obtained by esterification of the acid (**47**) readily available from **1** with diphenyldiazomethane. Compound (**48**) smoothly underwent *cis* oxiamination to give the desired oxazoline (**50**) in 76% yields and a trace amount of its epimer. The intermediate imidate anion (**49**) generated by reaction with trichloroacetonitrile underwent efficient conjugate addition without the use of an electrophile to trigger oxazoline formation. Hydrolysis of **50** was best achieved by treatment with *p*-toluenesulfonic acid in a mixture of pyridine and water[23] to afford the trichloroacetamides (**51**) and (**52**) in yields of 77 and 9%, respectively. Reductive cleavage of the trichloroacetamide group with $NaBH_4$[24] gave the amines (**53**) and (**54**). Thus obtained compounds (**53**) and (**54**) were smoothly converted into L-altruronic acid- and L-mannuronic acid-type 2-acetamide-1-*N*-iminosugars (**55**) and (**56**) by removal of protecting groups with acid, respectively. The major isomer (**53**) was further transformed into another L-altruronic acid-type 2-acetamido-1-*N*-iminosugar (**58**) with a guanidine group.

2-Trifluoroacetamide analogs of **69** and **72** were similarly prepared using the diphenylmethyl ester **61** readily available from **34**.

Flexible Synthesis Of D- And L-Uronic Acid-Type *Gem*-Diamine 1-*N*-Iminosugars

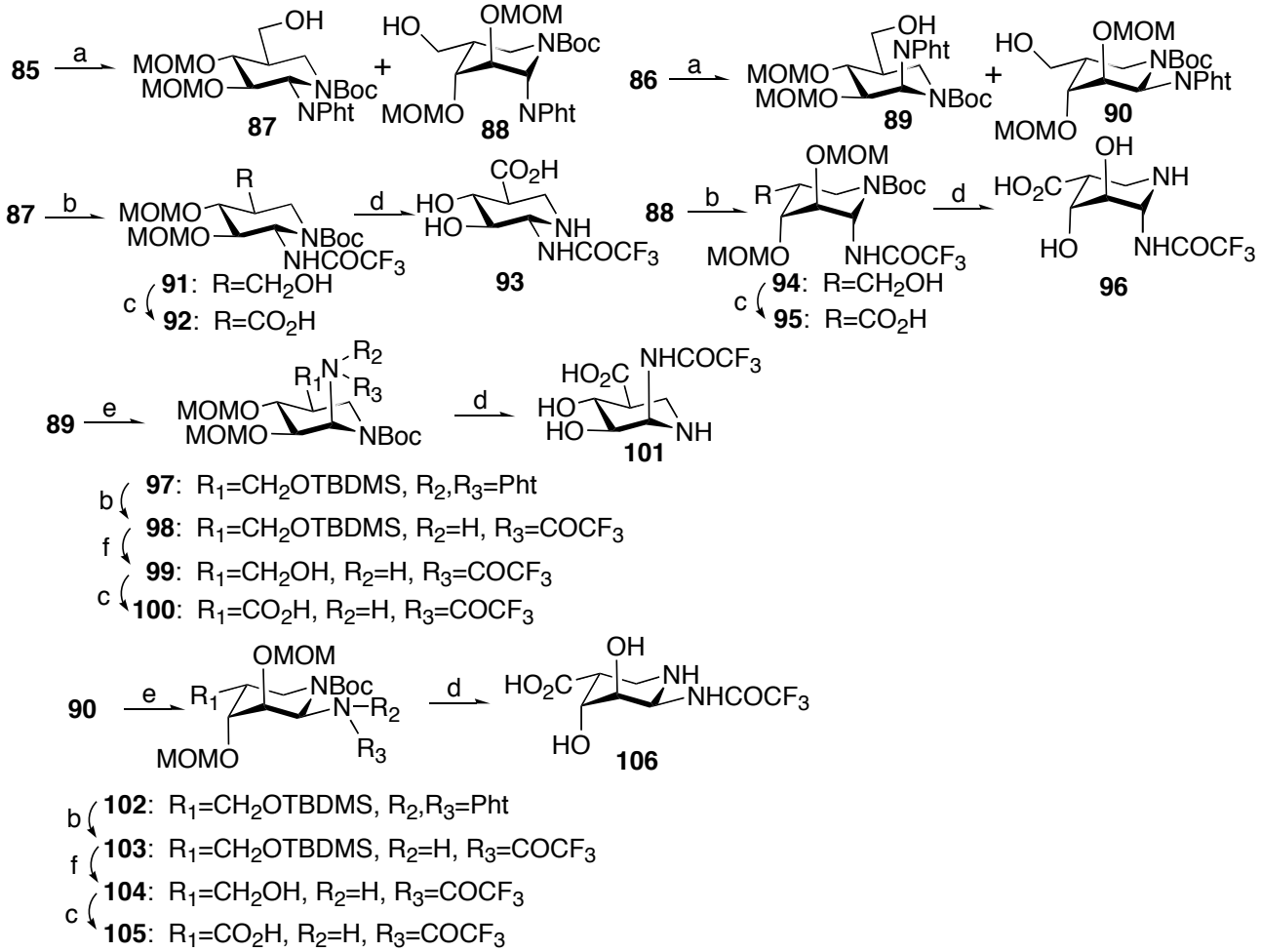
An efficient and flexible synthetic route to four *gem*-diamine 1-*N*-iminosugars of D- and L-uronic acid-type (D-glucuronic, D-mannuronic, L-iduronic, and L-guluronic acid) from L-galactono-1,4-lactone was developed in an enantiodivergent fashion through a sequence involving as the key steps (a) the 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 (Schemes 7 and 8).[25]





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*-$BuCO)_2O$, *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, **85**: 61.4%; **86**: 20%

Scheme 7.



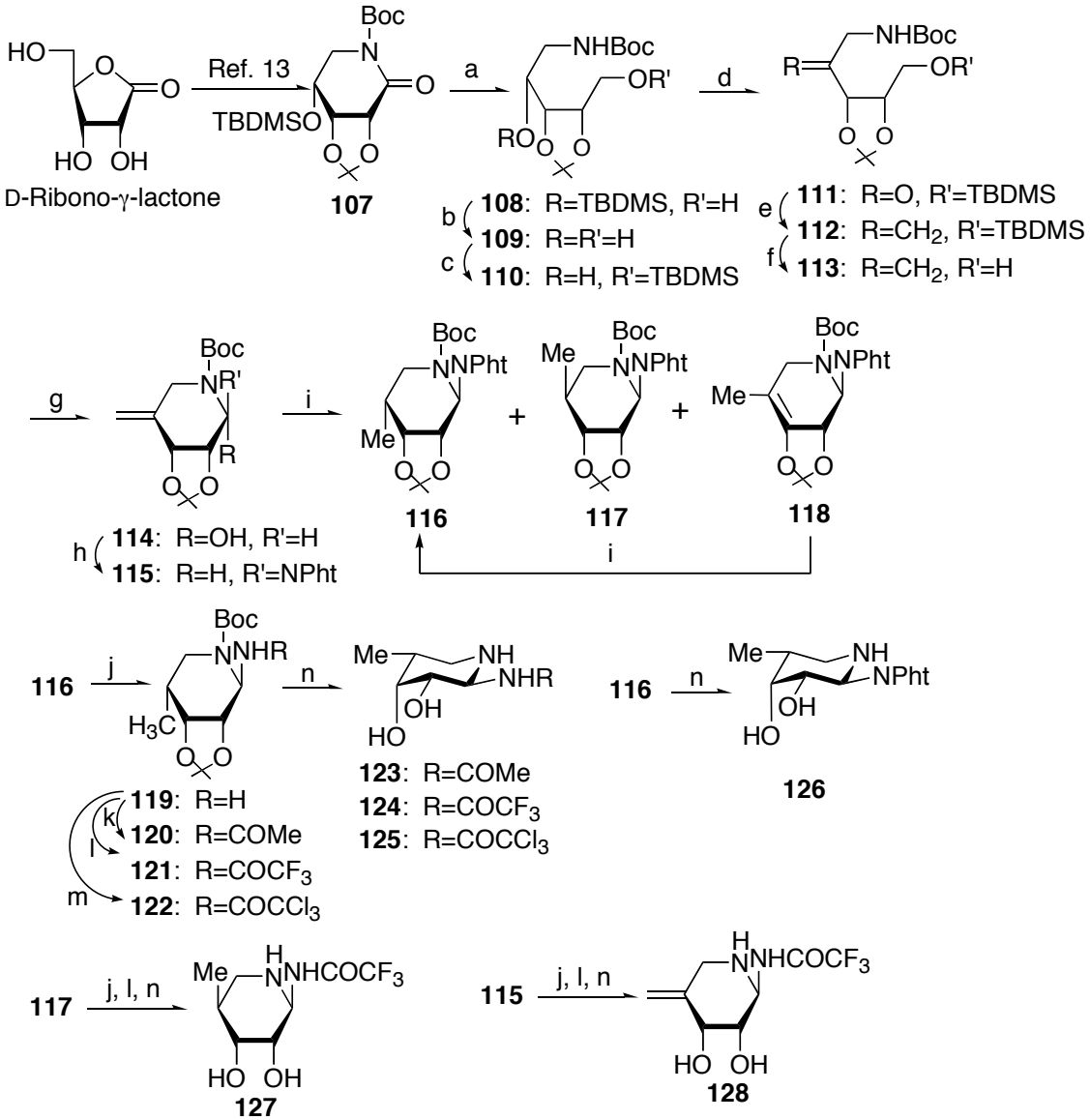
Reagents and conditions: a) $BH_3{\cdot}Me_2S$, THF; H_2O_2, 2M NaOH/H_2O, **87**: 16.6%; **88**: 77.1%; **89**: 50%; **90**: 38% b) $H_2NNH_2{\cdot}xH_2O$, MeOH; $(CF_3CO)_2O$, py, CH_2Cl_2, **91**: 90%; **94**: 87%; **98**: 88%; **103**: 79% c) RuO_2, $NaIO_4$, CCl_4/MeCN/H_2O, **92**: 91%; **95**: 90%, **100**: 92%; **105**: 87% d) 4M HCl/dioxane, **93**: 99.7%; **96**: 99%; **101**: 99.7%; **106**: 91% e) *t*-Bu(Me_2)SiCl, imidazole, DMF, **97**: 91%; **102**: 100% f) *n*-Bu_4NF, THF, **99**: 93%; **104**: 100%

Scheme 8.

The total synthetic approach to the multifuctionalized *gem*-diamine 1-*N*-iminosugars described above allows the introduction of the diastereomeric amino and carboxylic acid substituents at C-2 and C-5, respectively, into the versatile aminal (**84**) to yield four enantiomerically pure stereoisomers. The synthesis of aminal (**84**) began with the known 5,6-*O*-isopropylidene-L-galactono-1,4-lactone (**73**),[26] which was converted into the diol (**75**) upon protection and hydride reduction. Selective protection of the hydroxymethyl group in **75** followed by the Dess-Martin oxidation[27] gave the ketone (**77**). One-carbon extension of **77** by the Wittig reaction afforded the methylene derivative (**78**), which was transformed to the diol (**79**) upon removal of the isopropylidene group. The monoalcohol (**80**) was successfully prepared by the Luche reduction[28] of the labile aldehyde intermediate obtained by periodate oxidation of **79**. Conversion of the hydroxyl group to the amino function was best achieved *via* the corresponding sulfonate to the azide (**81**) by one-pot reaction *in situ*. Selective reduction of the azide group proceeded advantageously by catalytic hydrogenation with sodium hydrogentelluride (NaTeH)[29] generated *in situ* from tellurium and sodium borohydride in ethanol. The desired amide (**82**) was obtained by the subsequent protection with *t*-Boc group. The pivotal intermediate (**84**) was prepared as an epimeric mixture upon removal of protecting group of a TBDMS and the Swern oxidation.[10] Replacement of the aminal hydroxyl group to the amino group was achieved by the Mitsunobu reaction[12] (PPh_3, diethyl azodicarboxylate, phthalimide) to give both the desired epimers of iminophthalimide (**85**) and (**86**) in a ratio of 3 to 1. The absolute stereochemistry and a boat conformer of **85** were clarified by X-Ray crystallographic analysis. Another epimer (**86**) was consequently assigned its absolute stereochemistry and presumed boat conformation by ^{1}H NMR spectrum. Hydroboration of **85** with borane-methyl sulfide complex followed by oxidation with hydrogen peroxide efficiently gave the D-gluco isomer (**87**) and L-idulo isomer (**88**) in a ratio of 2 to 9. Hydrazinolysis of **87** and conventional trifluoroacetylation furnished the trifluoroacetamide (**91**). Conversion of the hydroxymethyl group of **91** to the carboxylic acid was best achieved by the ruthenium tetraoxide-catalyzed Sharpless oxidation.[20] Simultaneous removal of both MOM and *t*-Boc groups in **92** with acid resulted in the D-glucuronic acid-type 2-trifluoroacetamide-1-*N*-iminosugar (**93**). The same sequence of reactions also successfully resulted in L-iduronic acid-type 2-trifluoacetamido-1-*N*-iminosugar (**96**) from **88**. The ^{1}H NMR spectrum of **93** shows the $^4C_1$-conformation, whereas the ^{1}H NMR one of **96** indicates the boat conformation. On the other hand, D-mannuronic acid-type and L-guluronic acid-type 2-trifluoroacetamide-1-*N*-iminosugars (**101** and **106**) were straightforwardly obtained from **86** by a similar sequence of structure transformation varying in the protection of the hydroxymethyl groups of **89** and **90** with TBDMS group prior to hydrazinolysis of the phthalimide group for improvement in yield. While the ^{1}H NMR spectrum of D-mannuronic acid-type (**101**) shows the boat conformation as oppose to the

$^4C_1$-conformation of D-glucuronic acid-type (**93**), ^{1}H NMR one of **106** indicates the $^1C_4$-conformation as oppose to the boat conformation of L-iduronic acid-type **96**.

SYNTHESIS OF L-FUCOSE-TYPE *GEM*-DIAMINE 1-*N*-IMINOSUGARS

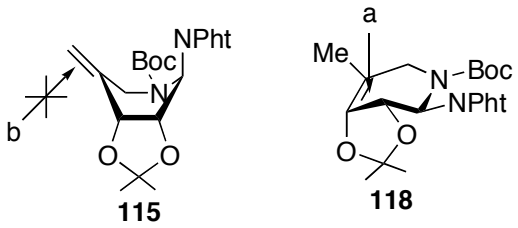


Reagents and coditions: a) $NaBH_4$, EtOH, 0°C to rt b) *n*-Bu_4NF, THF, rt
c) *t*-$BuMe_2SiCl$, imidazole,DMF, rt d) Dess-Martin periodinane, CH_2Cl_2
e) Ph_3PMeBr, $(Me_3Si)_2NLi$, THF, 0°C to rt f) *n*-Bu_4NF, THF, rt g) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78°C to rt h) phthalimide, Ph_3P, DEAD, DMF, 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) 4M HCl/dioxane, 0°C to rt

Scheme 9.

An enantioselective synthesis of L-fucose-type *gem*-diamine 1-*N*-iminosugars from D-ribono-γ-lactone was developed in a formation of a *gem*-diamine 1-*N*-iminopyranose ring by the Mitsunobu reaction of an aminal and a stereospecific reduction of an exo-methylene group to form the right configuration of L-fucose (Scheme 9).[30]

The key intermediate in this synthesis is the aminal (**114**). The synthesis of **114** began with the known lactam (**107**)[13] which was transformed into the diol (**109**) upon hydride reduction and removal of the protecting group. Selective protection of the hydroxymethyl group in **109** followed by the Dess-Martin oxidation[27] gave the ketone (**111**). The Wittig reaction of **111** with methylenetriphenylphophorane afforded the methylene derivative (**112**) which was converted into the mono-alcohol (**113**) by removal of the protecting group. Stereoselective introduction of the hydroxyl group at C-2 was best achieved by the Swern oxidation[10] to give the pivotal intermediate (**114**) as a sole product. Replacement of the axial aminal-hydroxyl group of **114** to the equatorial amino function was successfully carried out by the Mitsunobu reaction[12] to yield the iminophthalimide (**115**). Catalytic hydrogenation of **115** gave the desired product (**116**), its epimer (**117**) and the rearrangement derivative (**118**) in a ratio of 15:1:3. Compound (**118**) was efficiently converted into the desired **116** upon same hydrogenation. The desired absolute stereochemistry and a boat conformation of **116** were clarified by X-Ray crystallographic analysis. The right stereochemistry might be induced through the possible mechanism that the methylene group of **115** easily rearranges under catalytic hydrogenation with palladium on carbon, and that hydrogenation takes place predominantly from the less sterically hindered side (a) of the double bond of **118** with a boat conformation to lead **116** rather than the less sterically hindered side (b) of the methylene group of **115** with an alternative boat conformation to lead **117** (Figure 4). Hydrazinolysis of **116** afforded the amine (**119**) which was converted into the acetamide (**120**), the trifluoroacetamide (**121**) and the trichloroacetamide (**122**) by conventional acetylation, trifluoroacetylation and trichloroacetylation, respectively. Simultaneous removal of both the Ip and *t*-Boc groups in **120**, **121** and **122** with acid resulted in the desired L-fucose-type 2-acetamido-, 2-trifluoroacetamido- and 2-trichloroacetamido-1-*N*-iminosugars (**123**, **124** and **125**), respectively. Another L-fucose-type 2-phthalimido-1-*N*-iminosugar (**126**) was also similarly obtained from **116**. On the other hand, 6-deoxy-D-altrose-type 1-*N*-iminosugar (**127**) (the epimer of **124**) and 5-methylene-D-arabino-hexopyranose-type 1-*N*-iminosugar (**128**) (the 5-methylene isomer of **124**) were prepared by the similar sequences of reaction from **117** and **115**.

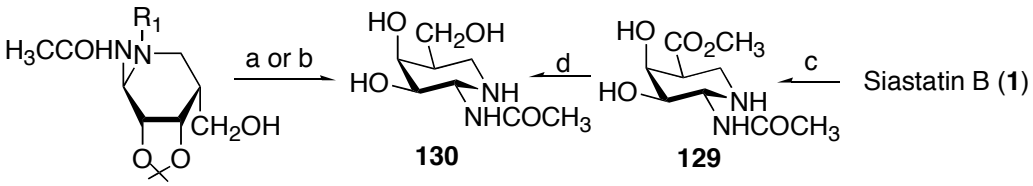


115 **118**

Figure 4. Possible mechanism of the stereochemical outcome in catalytic hydrogenation of **115**.

SYNTHESIS OF GLYCOSE- AND GLYCOSAMINE-TYPE *GEM*-DIAMINE 1-*N*-IMINOSUGARS

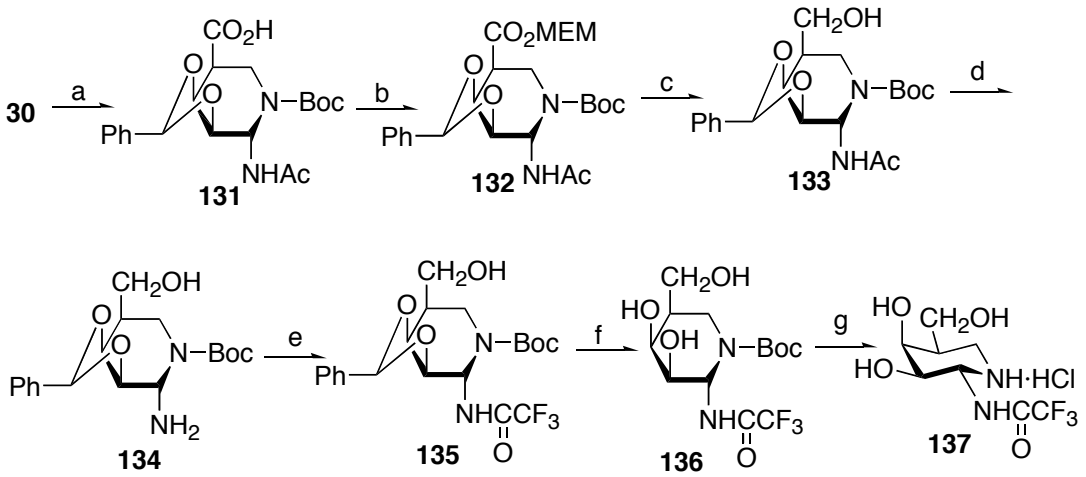
Glycose- and glycosamine-type *gem*-diamine 1-*N*-iminosugars were conveniently obtainable by the semi-synthetic method starting from siastatin B (**1**) (Schemes 10, 11 and 12).[31] They were also easily derived from intermediates in the total synthesis of uronic acid-type *gem*-diamine 1-*N*-iminosugars (Scheme 13).[25] The configurations of siastatin B (**1**) readily available from *Streptomyces* culture correspond to those of D-galactose- and D-galactosamine-type *gem*-diamine 1-*N*-iminosugars, and are also the correct configurations of D-glucose and D-glucosamine except for a configuration at C-4 position. Therefore, **1** is applicable for the facile synthesis of these types of *gem*-diamine 1-*N*-iminosugars.



18: R_1=$COOCH_2Ph$
33: R_1=COOC-*t*-Bu

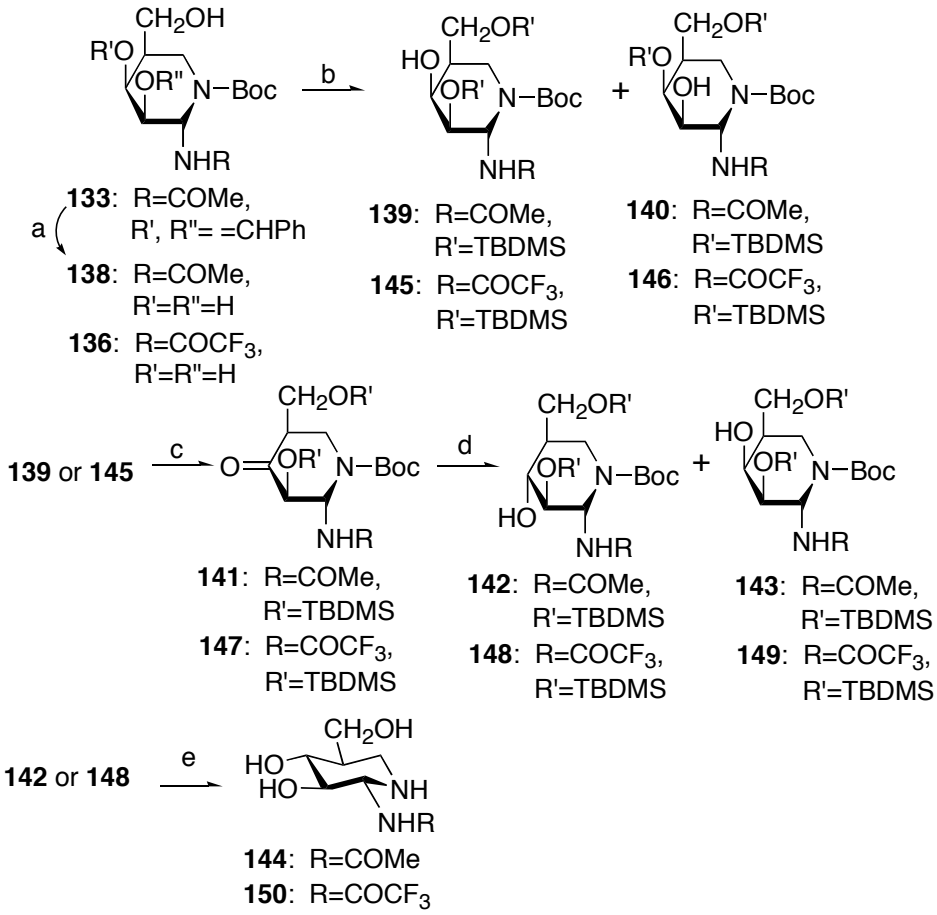
Reagents and conditons: a) H_2, 5% Pd/C, MeOH; 4M HCl/dioxane (78%) b) 4M HCl/dioxane (63%) c) Amberlist 15 (H^+), MeOH (84%) d) $NaBH_4$, EtOH (90%)

Scheme 10.



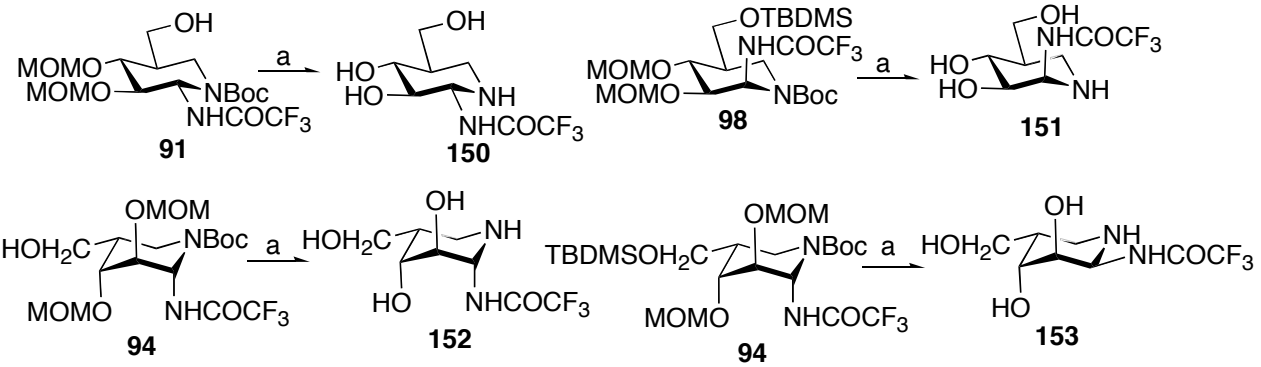
Reagents and conditions: a) $(MeO)_2CHPh$, TMSCl, DMF (92%) b) $MeOCH_2CH_2OCH_2Cl$, *i*-Pr_2NEt, DMF, rt, 98% c) NaBH4, CF_3CH_2OH/THF, rt, 94% d) $H_2NNH_2 \cdot xH_2O$, 70°C, 83% e) CF_3CO_2Et, *i*-Pr_2NEt, DMF, 60°C, 73% f) H_2/10% Pd-C, MeOH, rt, 92% g) 4M HCl/dioxane, rt, 80%

Scheme 11.



Reagents and conditions: a) H_2, 10% Pd/C, MeOH, 94% b) TBDMSCl, imidazole, DMF, rt, (**136**→**145**, 58%; **138**→**139**, 50%) c) Dess-Martin periodinane, CH_2Cl_2, rt, (**145**→**147**, 97%; **139**→**141**, 98%) d) $LiBH_4$, MeCN, -50°C, (**147**→**148**, 88%; **141**→**142**, 74%) e) 4M HCl/dioxane, rt, (**142**→**144**, 91%; **148**→**150**, 80%)

Scheme 12.



Reagents and conditions: a) 4M HCl/dioxane

Scheme 13.

D-Galactosamine-type 2-aceatmido-1-*N*-iminosugar (**130**) was obtained most easily by reduction of siastatin B methyl ester (**129**) with $NaBH_4$. Compound (**130**) was also efficiently prepared from the intermediates (**18**) and (**33**) in the total synthesis[8] of **1** by catalytic hydrogenolysis followed by acid hydrolysis.

The facile synthesis of D-galactose-type 2-trifluoroacetamido-1-*N*-iminosugar (**137**) was easily achieved starting from the known derivative (**131**)[18] obtained from **1**. Protection of the carboxyl group of **131** gave the MEM ester (**132**) which was converted into the alcohol (**133**) by hydride reduction. Hydrazinolysis of **133** followed by conventional trifluoroacetylation furnished the trifluoroacetamide (**135**). Removal of protecting groups by hydrogenolysis and acid hydrolysis resulted in **137**.

The facile synthesis of D-glucosamine-type 2-acetamido-1-*N*-iminosugar (**144**) and D-glucose-type 2-trifluoroacetamido-1-*N*-iminosugar (**150**) was also carried out by epimerization at C-4 position of siastatin B (**1**). The synthesis of **144** and **150** was begun with the known derivatives **133**[7c] and **136**,[31b] respectively, obtained from **1**. Protection of hydroxyl groups in **138** with TBDMS group gave the desired 4-hydroxy derivative (**139**) and the 3-hydroxy derivative (**140**) in a ratio of 2 to 1. An epimerization of the hydroxyl group was best achieved by two-step sequences of oxidation and reduction of the resulting ketone. Dess-Martin oxidation[27] of **139** afforded the ketone (**141**) which was stereoselectively transformed into the desired gluco-isomer (**142**) by reduction with $LiBH_4$ in MeCN. Simultaneous removal of both TBDMS and *t*-Boc groups of **142** by acid hydrolysis resulted in **144**. Compound (**150**) was similarly prepared starting from **136**.

On the other hand, D-glucose-, D-mannose-, L-idose- and L-gulose-type 2- trifluoroacetamido-1-*N*-iminosugars (**150**, **151**, **152** and **153**) were also obtained from the intermediates (**91**, **98**, **94** and **103**), respectively, in the total synthesis[25] of uronic acid-type *gem*-diamine 1-*N*-iminosugars by removal of protecting groups.

BIOLOGICAL ACTIVITY

GLYCOSIDASE INHIBITORY ACTIVITY

Many glycosidase inhibitors have been developed over last three decades, including a number of six- and five-membered heterocyclic rings.[5,32] Of these, *gem*-diamine 1-*N*-iminosugars have proven to be particularly powerful inhibitors of retaining β-D- and α-L-glycosidases.

The inhibitory activities of uronic acid-type *gem*-diamine 1-*N*-iminosugars against glycosidases are summarized in Table 1.[7b,13,18,25]

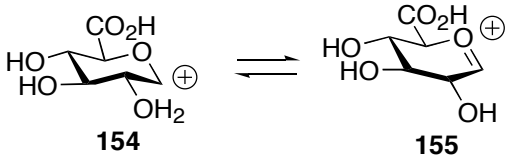
D-Glucuronic 2-trifluoroacetamido-1-*N*-iminosugar (**93**) inhibits very strongly β-glucuronidase. This is rationalized that **93** should closely mimic a glycopyranosyl cation (**154**), one of the resonance contributors (the chair-like and the flattened conformational cations (**154**) and (**155**), respectively) of the putative glycopyranosyl cation intermediate formed during hydrolysis by β-glucuronodase (Figure 5). D-Galacturonic acid-type 2-trifluoroacetamido-, 2-trichloroacetamido-, 2-guanidino-, and 2-phthalimido-1-*N*-iminosugars (**24b**, **29**, **43**, **44** and **45**) also show highly potent inhibition against this enzyme. These results suggest that β-glucuronidase roughly recognizes configuration of 4-OH group of glycopyranose

and/or the binding group corresponding to the 4-OH in β-glucoronidase plays no important role in specificity of the inhibitors. It is interesting that D-mannuronic acid-type 2-trifluoroacetamido-1-*N*-iminosugar (**101**) also shows strong inhibition equivalent to those of D-glucuronic- and D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars (**93**, **29**, **43**, **44** and **45**). D-Mannuronic acid-type *gem*-diamine 1-*N*-iminosugar is shown to have a boat conformer different from chair conformers of D-glucuronic acid- and D-galacturonic acid-type ones in the solution, indicating that **101** is highly likely a mimic of the another glycopyranosyl cation, the flattened conformational cation (**155**) in the course of the enzymatic reaction (Figure 5). Studies on the structure-activity relationship in media of enzyme assays revealed that *gem*-diamine 1-*N*-iminosugars are stable in acidic solution, but they smoothly undergo the Amadari rearrangement to give a hydrate ketone (**157**) or its derivative (**158**) *via* a hemiaminal **156** in the media (pH > 5.0) (Figure 6).[33] The time-course evaluation of inhibitory activity in the media indicates that the hemiaminal and hydrate ketone generated in the media as well as the parent *gem*-diamine 1-*N*-iminosugars inhibit very strongly glycosidases. On the other hand, the stable analogs (**1** and **24a**) in the media show only weak inhibitory activity against β-glucuronidase.

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

Enzyme	**1**	**24a**	**24b**	**29**	**43**	**44**	**45**	**93**	**101**
β-D-Glucuronidase[a)]	$7.1x10^{-5}$	$1.2x10^{-4}$	$6.2x10^{-8}$	$6.5x10^{-8}$	$9.2x10^{-8}$	$1.3x10^{-7}$	$6.8x10^{-8}$	$6.5x10^{-8}$	$6.5x10^{-8}$
α-D-Glucosidase[b)]	$>3.3x10^{-3}$ (Ni)	Ni	$2.4x10^{-7}$	Ni	NT	NT	NT	Ni	Ni
β-D-Glucosidase[c)]	Ni	Ni	Ni	$1.3x10^{-5}$	NT	NT	NT	$9.8x10^{-5}$	$3.6x10^{-5}$
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	$1.3x10^{-6}$	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

a) bovine liver b) baker's yeast c) almonds d) jack beans e) snail f) *Aspergillus niger* g)chicken liver h) bovine epididymis Ni: no inhibition at $3.3x10^{-3}$ M NT: not tested.



154 **155**

Figure 5. Effect of resonance contributors (**154** and **155**) to the putative glycosyl cation intermediate formed during hydrolysis by β-glucuronidase.

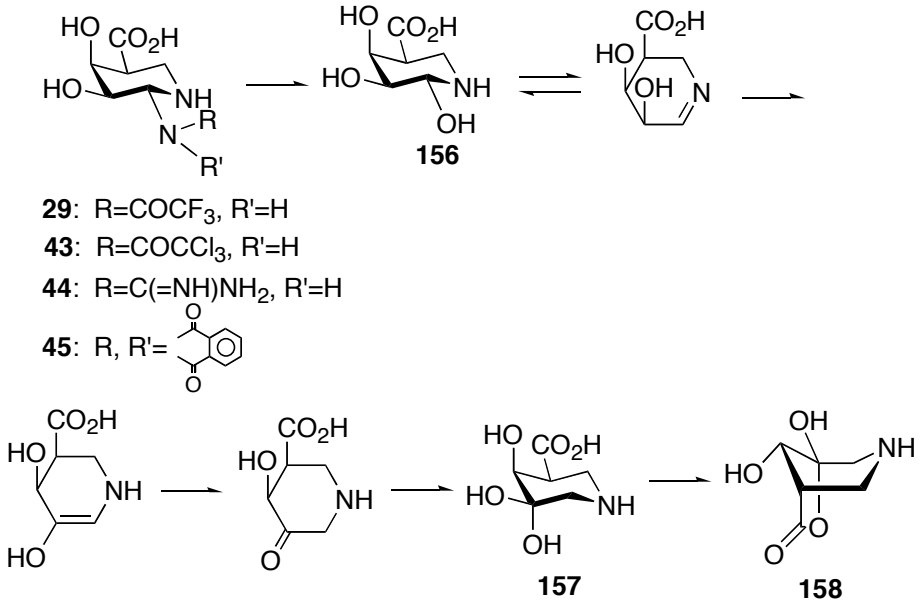


Figure 6. Structural changes of D-galacturonic acid-type *gem*-diamine 1-*N*-iminosugars in media (pH 5.0).

Table 2. Inhibitory activities (IC_{50}) of uronic acid-type *gem*-diamine 1-*N*-iminosugars against human heparanase (endo β-D-glucuronidase).

Compounds	IC_{50} (μM)
29	1.02 ± 0.29
93	10.5 ± 1.07
96	Ni
101	28.99 ± 11.41
106	Ni

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

The typical analogs (**29**, **93**, **101**) of D-uronic acid-type *gem*-diamine 1-*N*-iminosugars also exhibit inhibitory activity against recombinant human heparanase (*endo*-β-glucuronidase) from human melanoma A375M cell transfected with pBK-CMV expression vectors containing the heparanase cDNA (Table 2).[25,34] Heparanase cleaves the β-1,4-linkage between D-glucuronic acid and *N*-acetyl-D-glucosamine in heparan sulfate. The result also proves the structure-activity relationships similar to those discussed on the inhibition against *exo*-β-glucuronidase from bovine liver. The weak activities against heparanase compared with *exo*-β-glucuronidase indicate that heparanase should recognize simultaneously D-glucuronic acid and the adjacent glycoses on the both sides. As expected, all of L-uronic acid-type *gem*-diamine 1-*N*-iminosugars (**55**, **56**, **58**, **69**, **72**, **96** and **106**) 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 D- and L-sugars for specificity and potency.

The inhibitory activities of L-fucose-type *gem*-diamine 1-*N*-iminosugars against glycosidases are summarized in Table 3.[30]

Table 3. Inhibitory activities (IC_{50} M) of L-fucose-type *gem*-diamine 1-*N*-iminosugars against glycosidases.

Enzyme	**123**	**124**	**125**	**126**	**127**	**128**
α-L-Fucosidase[a)]	4.8×10^{-7} (0.11)[j)]	1.1×10^{-8} (0.003)[j)]	9.0×10^{-9} (0.003)[j)]	1.3×10^{-8} (0.004)[j)]	1.8×10^{-6} (0.50)[j)]	7.0×10^{-7} (0.20)[j)]
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

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) IC_{50} (μg/mL) NT: not tested

The L-fucose-type 2-trifluoroacetamide, 2-trichloroacetamide and 2-phthalimide (**124**, **125** and **126**), in analogy with D-glucuronic acd-type ones, show very strong, specific and competitive inhibition against α-L-fucosidase, while the 2-acetamide (**123**) affects moderately the enzyme. Studies on the structure-activity relationship in enzyme assays, in a similar manner as the results shown in D-uronic acid-type *gem*-diamine 1-*N*-iminosugars, revealed that the hemiaminal (**159**) and the hydrate ketone (**160**) generated in the media (pH 6.3) by the Amadari rearrangement as well as the parent *gem*-diamine 1-*N*-iminosugars inhibit very strongly α-L-fucosidase (Figure 7).[33] These results support the hypothesis of which the protonated *gem*-diamine 1-*N*-iminosugars may mimic the presumed glycosyl cation (**6**) in the transition state of enzymatic reaction (Figure 3). On the other hand, **127** and **128** inhibit weakly α-L-fucosidase. These results also indicate that the 5-methyl group, its stereochemistry and the $^1C_4$-conformation play the important roles as the major factors for inhibition against L-fucosidase.

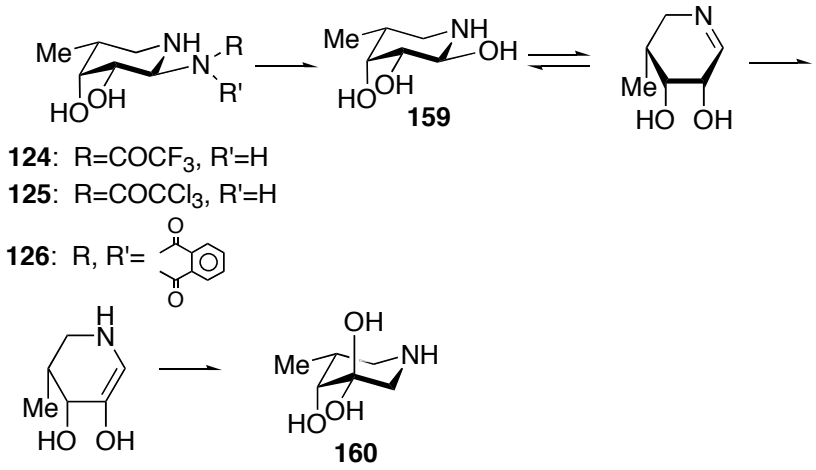


Figure 7. Structural changes of L-fucose-type *gem*-diamine 1-*N*-iminosugars in media (pH 6.3).

The inhibitory activities of D-glycose- and D-glycosamine-type *gem*-diamine 1-*N*-iminosugars against glycosidases are summarized in Table 4.[31]

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

Enzyme	**130**	**137**	**144**	**150**
α-D-Glucosidase[a)]	>3.9x10^{-4}	>3.2x10^{-4}	2.9x10^{-6}	1.9x10^{-7}
β-D-Glucosidase[b)]	7.9x10^{-5}	4.8x10^{-7}	5.4x10^{-6}	4.2x10^{-7}
α-D-Galactosidase[c)]	2.5x10^{-5}	3.4x10^{-7}	>3.9x10^{-4}	>3.2x10^{-4}
β-D-Galactosidase[c)]	1.7x10^{-5}	1.7x10^{-7}	>3.9x10^{-4}	1.9x10^{-4}
α-D-Mannosidase[d)]	>3.9x10^{-4}	>3.2x10^{-4}	2.5x10^{-4}	2.2x10^{-5}
β-D-Mannosidase[e)]	>3.9x10^{-4}	1.3x10^{-4}	3.8x10^{-5}	3.2x10^{-6}
α-D-NAc-Galactosaminidase[f)]	3.3x10^{-7}	2.2x10^{-6}	>3.9x10^{-4}	>3.2x10^{-4}
β-D-NAc-Glucosaminidase[g)]	2.7x10^{-6}	>3.2x10^{-4}	1.2x10^{-6}	>3.2x10^{-4}
β-D-Glucuronidase[h)]	>3.9x10^{-4}	>3.2x10^{-4}	>3.9x10^{-4}	>3.2x10^{-4}

a) baker's yeast b) almonds c) *Aspergillus niger* d) Jack beans e) snail f) chicken liver g) bovine epididymis h) bovine liver

As expected, D-glucose-type 2-trifluoroacetamide (**150**) shows very strong and specific inhibition against α- and β-D-glucosidases, and D-glucosamine-type 2-acetamide (**144**) also affects strongly β-D-*N*-acetylglucosaminidase. On the other hand, D-galactose-type 2-trifluroacetamide (**137**) inhibits very strongly not only α- and β-D-galactosidases but also β-D-glucosidase. D-Galactosamine-type 2-

acetamide (**130**) affects strongly both α-D-*N*-acetylgalactosaminidase and β-D-*N*-acetylglucosaminidase. These results seem to indicate that the inhibitors may mimic a transient intermediate (**6**) (Figure 3) and/or a glycopyranoside of grand-state in hydrolysis of glycosidases and glycosaminidases. These results also suggest that the axial 4-OH group is the main determinant of specificity and potency of the inhibitors against D-galacto-type hydrolases. However, D-gluco-type hydrolases may roughly recognize the configuration of 4-OH group. *N*-Acetylglycosaminidases also recognize precisely the 2-*N*-acetyl group.

GLYCOSYL- AND SULFOTRANSFERASE INHIBITORY ACTIVITY

Table 5. Effects of **24b** and CDP on [^{14}C]NeuAc incorporation into lactosylceramide (LacCer) as an exogenous acceptor.

Compound	Treatment	[^{14}C]NeuAc incorporated into GM3 cpm/mg lipid added	%
—	0	850	100
24b	1.3 mM	961	110
	4.3 mM	659	78
	13 mM	13	1.5
CDP	13 mM	7	0.82

The sialyltransferase activity was determined according to the method of Hakomori *et al.* (*J. Biol. Chem.*, **1993**, *268*, 2211) using mouse mammary carcinoma mutant cell line (FUA 169) which shows high activity of CMP-sialic acid:LacCer 2,3-sialosyltransferase.

As shown in Table 5, in addition to the glucuronidase inhibitory activity mentioned above, D-galacturonic acid-type 2-trifluoroacetamido-1-*N*-iminosugar having a hydroxyl group at C-5 position (**24b**) shows sialyltransferase inhibitory activity nearly equal to that of cytidine 5'-diphosphate (CDP), a standard inhibitor, in the mouse mammary carcinoma mutant cell line (FUA169)[35] which has high activity of transfer of sialic acid to lactosylceramide [Galβ1—4Glcβ1—1Cer] to express ganglioside GM3 [NeuAcα2—3Galβ1—4Glcβ1—1Cer]. This result suggests that **24b** may also mimic sialic acid (**4**) in the sialyltransferase reaction as an alternative type of *gem*-diamine 5-*N*-iminosugar (Figure 8), as well as siastatin B (**1**) mimics **4** in the neuraminidase hydrolysis (Figure 2). The sialic acid content and/or the sialyltransferase activity of tumor cell membranes are also known to closely correlate with the metastatic potential.[36] These facts suggest that **24b** may be capable of inhibiting tumor metastasis as both the sialyltransferase inhibitor and the glucuronidase inhibitor.

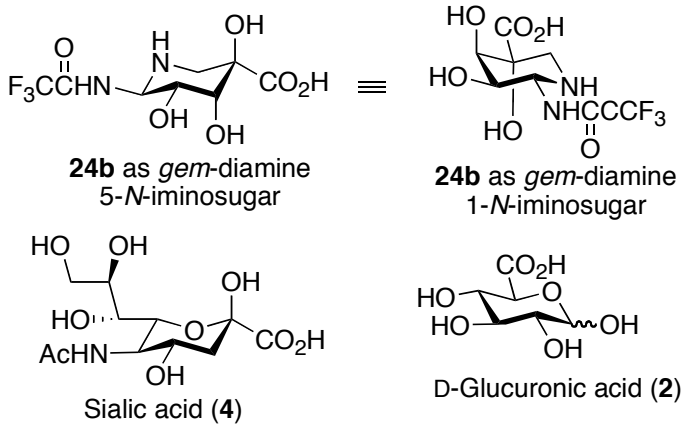


Figure 8. Structural similarity of **24b** to sialic acid (**4**) as *gem*-diamine 5-*N*-iminosugar and to D-glucuronic acid (**2**) as *gem*-diamine 1-*N*-iminosugar.

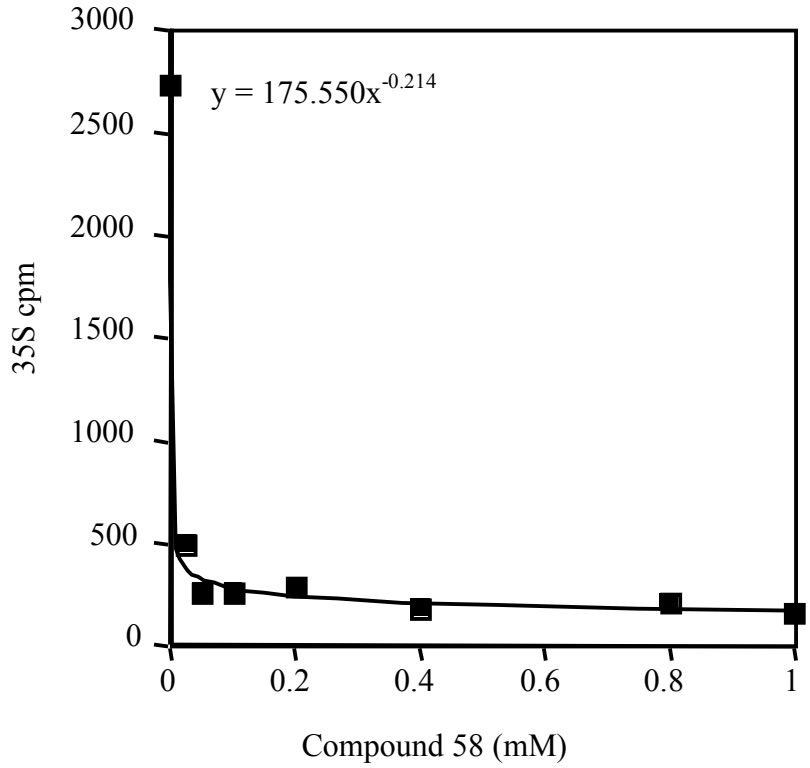


Figure 9. Inhibitory activity of L-altrunonic acid-type 2-acetamido-1-*N*-iminosugar (**58**) against HS 2-*O*-ST: **58** (0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.6 and 1 mM) incubated with 2-*O*-desulfated heparin at 0.1 mM.

As shown in Fig. 9, L-altruronic acid-type 2-acetamido-1-*N*-iminosugar (**58**) shows heparan sulfate 2-*O*-sulfotransferase (HS 2-*O*-ST) inhibitory activity (80% at 25 μM).[37] HS 2-O-ST catalyzes sulfate transfer from the sulfate donor, adenosine 3'-phosphate-5'-phosphosulfate (PAPS) to C-2 hydroxyl group of L-iduronic acid in heparan sulfate (HS) which consists of a disaccharide repeat of glucosamine (GlcN) and hexuronic acid (D-glucuronic acid or its C-5 epimer, L-iduronic acid). This result indicates that HS 2-*O*-ST recognizes **58** as L-iduronic acid.[38]

INHIBITORY EFFECT ON TUMOR METASTASIS

The inhibitory effects of uronic acid-type *gem*-diamine 1-*N*-iminosugars on the tumor metastasis were evaluated using the experimental and spontaneous pulmonary metastasis in mice.

D-Galacturonic acid-type *gem*-diamine 1-*N*-iminosugars (**24b**, **29**, **43** and **44**) significantly suppress in a dose-dependent manner the number of colonies of pulmonary metasatsis of B16BL6 cells in the experimental metastasis (Table 6).[7a,7b,18] Of these, 2-trifluoroacetamide (**29**) inhibits most potently pulmonary metastasis.

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 (μg/mL)	Inhibition of metastasis (%)
Saline (0.9%)	0	0
24b	10	11.9
	30	75.0
	50	80.5**
	100	90.4**
29	10	48.5
	30	61.9
	50	90.8**
43	10	26
	30	29.6
	50	67.3*
44	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 EDTA solution from culture dishes and washed twice with PBS. The cell suspension ($1x10^5$) in PBS were implanted 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. *: $p<0.01$ **: $p<0.001$

L-Altruronic acid-type *gem*-diamine 1-*N*-iminosugars (**55**, **58**, **69** and **72**) also remarkably reduce in a dose-dependent manner the pulmonary colonization of B16BL6 cells in the experimental metastasis (Table 7).[21] Of these, 2-acetamido-4-guanidino-1-*N*- iminosugar (**58**) shows very strong inhibition in the experimental metastasis.

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 (μg/mL)	Inhibition of metastasis (%)
Saline (0.9%)	0	0
55	10	0
	30	12.1
	50	44.3*
58	10	40.1*
	30	91***
	50	97***
69	10	3.8
	30	38.1**
	50	75.5***
72	10	14.1
	30	58.8***
	50	81.0***

The B16BL6 cells were cultured with **55** and **58** for 3 days and with **69** and **72** 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% EDTA solution. The cells ($1x10^5$) in 0.1 mL of divalent cation-free Dulbecco's phosphate-buffered saline were collected and injected 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 (**29**) on the spontaneous lung metastasis of 3LL cells in mice.

Compound	Administered dose (mg/kg)xdays	Inhibition of metastasis (%)
Saline (0.9%)	0 x 5	0
29	10 x 5	5.1
	50 x 5	23.5
	100 x 5	57.1*

Five female C57BL/6 mice per group inoculated with 3LL cells ($1x10^6$) by intrafootpad injection were administered i.v. with **29** 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. *: $p<0.01$

As shown in Table 8, more remarkable is the inhibition of spontaneous lung metastasis in mice by intravenously injection of **29**.[7a,39] Compound (**29**) shows 57% inhibition of metastasis at administration dose of 100 mg/kg.

On the other hand, invasive activities of B16BL6 and 3LL cells through the reconstituted basement membrane (Matrigel) are reduced by *in vitro* treatment with typical compounds of D-galacturonic acid-type (**29**) and L-altruronic acid-types (**58** and **72**) (Table 9 and 10).[7a,21,39]

Table 9. Inhibition of invasive activity of tumor cells by **29**.

Experimental	Treatment (μg/mL)	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 **29** for 72 h (B16BL6) or 15 h (3LL). Numbers of cells which 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 **58** and **72**.

Experimental	Compound	Treatment (μg/mL)	Inhibition %
1	**58**	0	0
		200	44.8*
		300	58.9**
2	**72**	0	0
		100	44.4
		200	61.1***
		300	63.9*

The cells were cultured with **58** and **72** for 72 h and 24 h, respectively. Numbers of invaded cells on the lower surface of the Matrigel/laminin-coated filters in 3 h (Experimental 1) or 6 h (Experimental 2) were counted. *: $p<0.05$ **: $p<0.01$ ***: $p<0.001$

D-Galacturonic acid- and L-alturonic cid-type *gem*-diamine 1-*N*-iminosugars inhibit markedly the experimentally induced lung metastasis of B16BL6 and/or 3LL cells by the *ex vivo* treatment. Spontaneous lung metastasis of s.c. inoculated 3LL cells in mice is also suppressed with i.v. administration of the *gem*-diamine 1-*N*-iminosugars. Furthermore, the *gem*-diamine 1-*N*-iminosugars prevent the invasion of B16BL6 and/or 3LL cells through reconstituted basement membrane. However,

the *gem*-diamine 1-*N*-iminosugars have no effects on cell growth at the concentration used in this study. These results suggest that the anti-metastatic effect of the *gem*-diamine 1-*N*-iminosugars may be due to their anti-invasive rather than anti-proliferative activities. These results suggest that the metabolism of β-D-glucuronide and α-L-iduronide of glycoconjugates participates in tumor metastasis by degrading basement membranes and/or cellular matrix, and the modification of glycoconjugates of tumor cell-surface participates in tumor metastasis by altering cell properties involved in cellular recognition and adhesion.

CLOSING REMARKS

The present article describes our current progress in the chemistry, biochemistry and pharmacology of *gem*-diamine 1-*N*-iminosugars, a new family of glycosidase inhibitors, with a nitrogen atom in place of the anomeric carbon. Mechanistically, new inhibitors may act by forming a protonated charged transition-state analog with the enzyme active site. New inhibitors that mimic charge at the anomeric position of transition-state have been proved to be potent and specific inhibitors of many kinds of glycosidases. New inhibitors that may affect some metabolic enzymes of glycoconjugates have found to participate in tumor metastasis. D- and L- Uronic acid-type *gem*-diamine 1-*N*-iminosugars may contribute to the study of the involvement of carbohydrates in malignant cell movement and show a promising new drug candidate for cancer chemotherapy.

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