Review

Design and synthesis of biologically active carbaglycosylamines: From glycosidase inhibitors to pharmacological chaperones

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(Edited by Kunihiko SUZUKI, M.J.A.)

Abstract: For over 50 years, our group has been involved in synthetic studies on biologically active cyclitols including carbasugars. Among a variety of compounds synthesized, this review focuses on carbaglycosylamine glycosidase inhibitors, highlighting the following: (1) the naturally occurring *N*-linked carbaoligosaccharide α-amylase inhibitor acarbose and related compounds; (2) the novel synthetic β-glycosidase inhibitors, 1′-*epi*-acarviosin and its 6-hydroxy analogue as well as β-valienaminylceramide and its 4′-epimer; (3) the discovery of the β-glycosidase inhibitors with chaperone activity, *N*-octyl-β-valienamine (NOV) and its 4-epimer (NOEV); and (4) the recent development of the potential pharmacological chaperone *N*-alkyl-conduramine F-4 derivatives.

Keywords: synthesis, carbasugars, carbaglycosylamines, *N*-linked carbaoligosaccharides, glycosidase inhibitors, pharmacological chaperones

1. Introduction

In the early 1960s, Prof. Umezawa and co-workers[1] began to work on the total synthesis and chemical modification of the aminocyclitol antibiotic kanamycin, according to their proposed relative structure of the main component kanamycin A (**1**) (Fig. 1a). One of the authors (SO) was conducting joint research with Prof. Umezawa's group at Keio University, synthesizing the model compounds aminocyclohexyl glucosaminides.[2] The work was gradually extended to collaboration with Prof. Suami at the same university and the synthesis of aminocyclitols of biochemical interest,[3),4] including the two main components of aminocyclitol antibiotics, streptamine (**2**) and 2-deoxystreptamine (**3**), leading to a practical synthesis of **3** from *myo*-inositol (**4**).[5] This synthesis was later applied to the preparation of specifically ^{14}C-labeled **3**[6] to elucidate the biosynthetic pathway of the antibiotic neomycin.[7,8] The findings were then utilized to develop the microbial production of novel neomycin analogs (hybrimycins) by replacement of **3** with various aminocyclitols.[9,10] Since then, SO has been involved in the synthesis of new bioactive inositol derivatives, such as anhydro- and dianhydroinositols,[11] and highly oxygenated cyclohexane compounds, such as crotepoxide.[12]

In 1970, the discovery of an antibiotic with potent trehalase inhibitory activity, validamycin A (**5**) (Fig. 1b), was reported by Takeda Chemical Co. Ltd.[13–15] Structural studies demonstrated the presence of the active core *N*-linked carbadisaccharide validoxylamine A (**7**), composed of the aminocarbasugars validamine (**9**)[16] and its unsaturated derivative valienamine (**11**).[17] Carbasugars, originally referred to as pseudo-sugars, are carbocyclic analogues of carbohydrates (Fig. 1b, inset).[18] The α,α-trehalose-like structure of **7** is believed to mimic the transition state of the trehalase reaction. In addition to **5**, validamycin B[14] (**6**) was isolated and found to have a core validoxylamine B (**8**) composed of hydroxyvalidamine[16] (**10**) instead of **9**. Later, the aminocarbasugars **9**, **10**, and **11** were also isolated together with a new aminocarbasugar α-glucosidase inhibitor valiolamine (**12**) from validamycin fermentation broth.[19]

It is noteworthy that 3 years before the discovery of **5**, some carbasugars were chemically synthesized by McCasland and co-workers,[20–22] with

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doi: 10.2183/pjab.98.018
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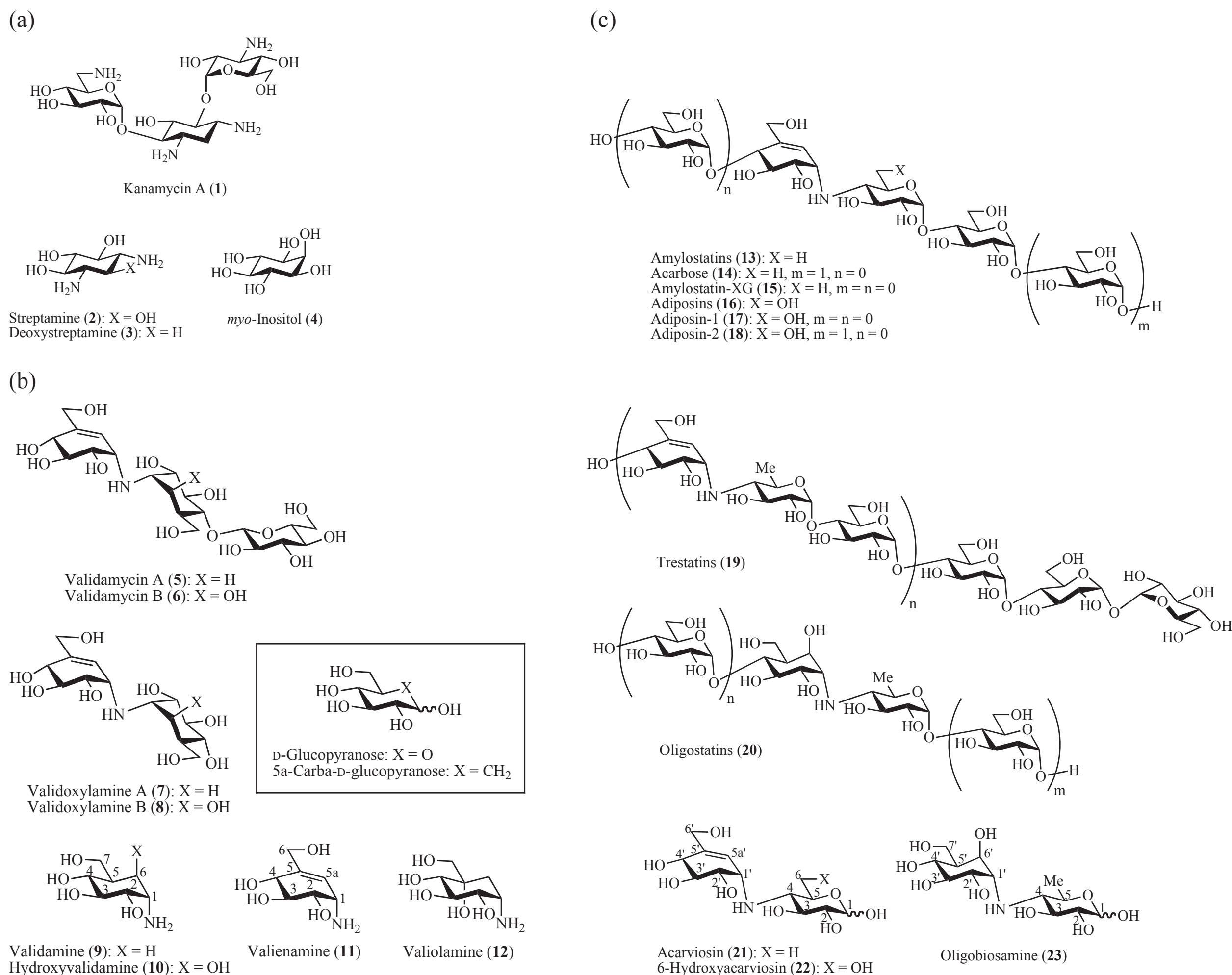


Fig. 1. Naturally occurring aminocyclitol and carbaglycosylamine antibiotics and glycosidase inhibitors. (a) Aminocyclitol antibiotic kanamycin A, two main aminocyclitol components of aminocyclitol antibiotics and naturally abundant cyclitol. (b) Agricultural antibiotics validamycins A and B, their carbadisaccharide active cores and carbaglycosylamine components. Inset: Glucose and carbaglucose with IUPAC nomenclature. (c) Carbaoligosaccharide α-amylase inhibitors and their carbadisaccharide active cores.

the hope that these compounds, due to their structural similarity to natural sugars, might be incorporated into biological systems to exhibit a certain biological activity.

In 1977, a homologous series of α-amylase inhibitors including acarbose (**14**) (Fig. 1c) was discovered by Bayer Co.[23),24)] and characterized as *N*-linked carbaoligosaccharides with the common active core valienamine-containing *N*-linked carbadisaccharide acarviosin[24)] (**21**), which like **7** is considered as a transition-state analogue inhibitor of α-amylase. Among them, **14** was obtainable as a sole product and has been used clinically for the treatment of type II insulin-independent diabetes.[25)] Following the discovery by Bayer Co., other structurally related α-amylase inhibitors, amylostatins (**13**) including amylostatin-XG[26)] (**15**), adiposins[27)] (**16**) including adiposin-1 (**17**) and -2 (**18**), trestatins[28)] (**19**) and oligostatins[29)] (**20**) have been discovered in Japan, all of which contain *N*-linked carbadisaccharide cores: **21** for **13** and **19**, 6-hydroxyacarviosin (**22**) for **16**, and oligobiosamine (**23**) for **20**.

Since the discovery of these carbasugar-containing glycosidase inhibitors, our interest has been extended to include biologically active carbasugars and, as a result, the total synthesis of **5**, **6**, **14**, and related compounds has been achieved.[18),30)] In addition, the concept of the carbaglycosylamine-based transition state analogue inhibitors has been applied to design specific inhibitors of β-glycosidases.

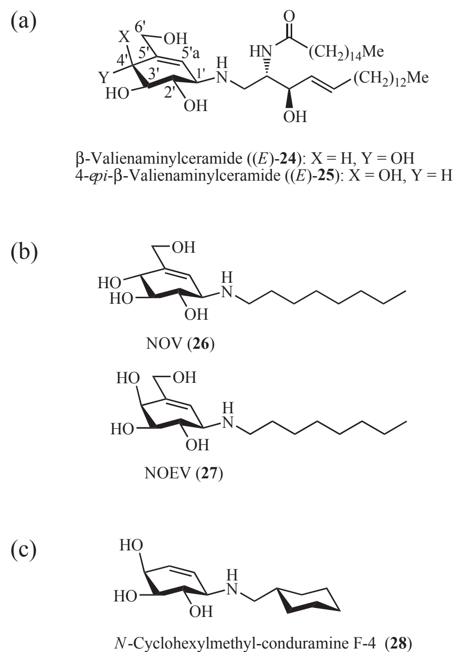


Fig. 2. Synthetic carbaglycosylamine-based transition-state analogue inhibitors of β-glycosidase. (a) Inhibitors of gluco- and galactocerebrosidases. (b) Inhibitors with chaperone activity. (c) Potential pharmacological chaperone for GM1-gangliosidosis.

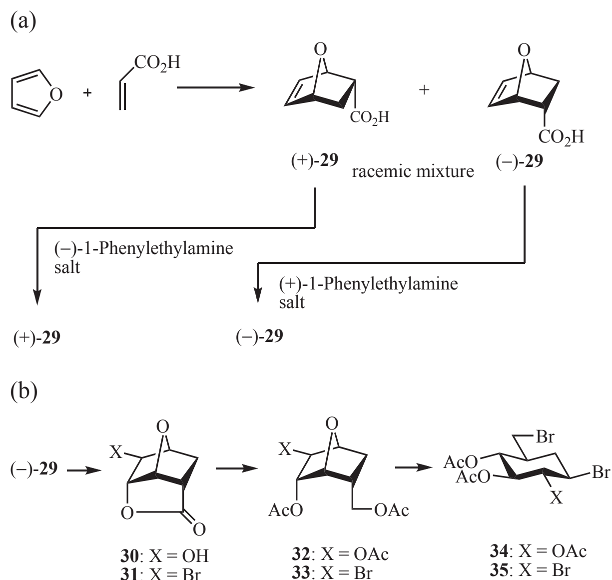


Fig. 3. Synthesis of carbasugars from Diels–Alder *endo*-adduct of furan and acrylic acid. (a) Diels–Alder reaction of furan and acrylic acid, and chiral resolution of the racemic *endo*-adduct. (b) Synthesis of di- and tribromo derivatives of carbaglucose as versatile intermediates of various carbasugars. The structural formulae shown correspond to the enantiomer derived from (−)-**29**.

Particularly, β-valienaminylceramide ((*E*)-**24**) and its 4′-epimer (*E*)-**25** (Fig. 2a) have been shown to be potent and specific inhibitors of gluco- and galactocerebrosidase, respectively.[31]

During the course of the synthetic studies, the structure–activity relationships of **21** and (*E*)-**24** were investigated and have led to the development of the potent glycosidase inhibitor *N*-alkyl-carbaglycosylamines including *N*-octyl-β-valienamine (NOV) (**26**)[32] and its 4-epimer (NOEV) (**27**)[33] (Fig. 2b). To facilitate the accessibility of *N*-alkyl-carbaglycosylamines, an improved and practical synthetic route to chiral carbaglycosylamines from *myo*-inositol (**4**) has been established.[34] Both NOV (**26**) and NOEV (**27**) have later been found to possess chaperone activity for mutant forms of their target glycosidases. Since then, it has become of interest to develop carbaglycosylamine-based pharmacological chaperones for lysosomal glycosidases associated with lysosomal storage disorders (LSDs).[35)–37] Recently, the *N*-alkyl-conduramine F-4 derivatives have been identified as potential pharmacological chaperones for GM1-gangliosidosis, with the *N*-cyclohexylmethyl derivative **28** (Fig. 2c) being the most promising among them.[38),39]

2. Total synthesis of *N*-linked carbaoligosaccharide α-amylase inhibitors

2.1. Preparation of validamine- and valienamine-derived carbaglycosyl acceptors and donors for the construction of *N*-linked carbaoligosaccharides. A practical preparative method for constructing *N*-linked carbaoligosaccharides is the ring opening of carbasugar acceptor epoxides with appropriate donor amines, and vice versa, as demonstrated in the total synthesis of antibiotic validamycins including **5** and **6**.[30] All the carbasugar derivatives used in the study can be prepared from a single starting compound, namely the Diels–Alder *endo*-adduct **29** of furan and acrylic acid (Fig. 3a),[40] which is now obtainable in both chiral forms, (+)-**29** and (−)-**29**, by chiral resolution of racemic **29** with (−)- and (+)-1-phenylethylamines, respectively[41]; thus, all the carbasugar derivatives described are available in both chiral forms. For the sake of simplicity, except noted otherwise, only the D-series of carbasugar derivatives derived from (−) **29** are shown in Figs. 3b and 4. The Diels–Alder adduct **29** was treated with hydrogen peroxide or bromine to give the lactones **30** or **31**, respectively (Fig. 3b),

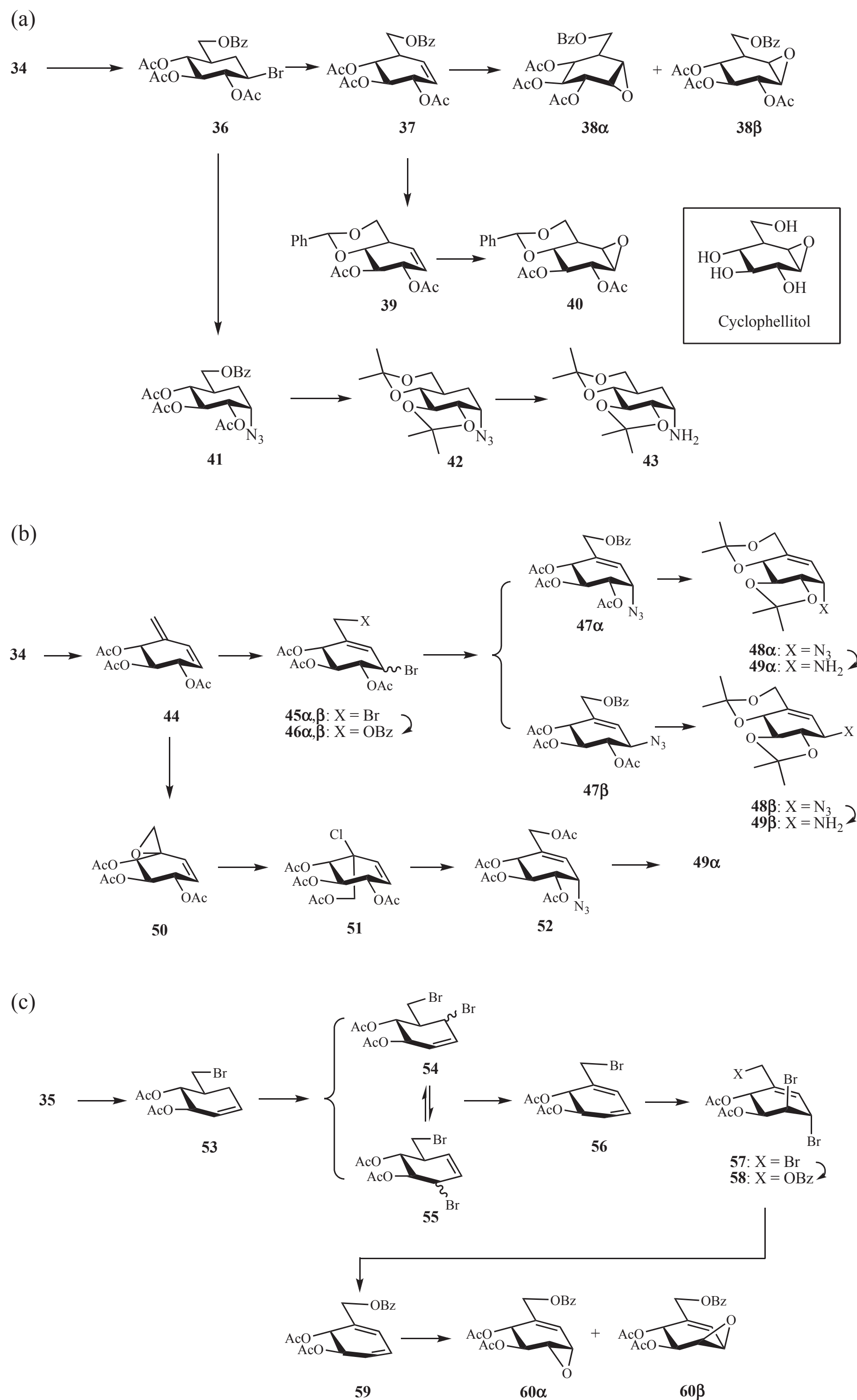


Fig. 4. Synthesis of validamine- and valienamine-derived acceptors and donors for the construction of *N*-linked carbaoligosaccharides. (a) Validamine-derived acceptor and donor from dibromocarbaglucose **34**. Inset: Naturally occurring β-glucosidase inhibitor cyclophellitol. (b) Valienamine-derived donors from dibromocarbaglucose **34**. (c) Valienamine-derived acceptors from tribromocarbaglucose **35**.

both of which were converted into the corresponding 1,4-anhydro-5a-carba-α-glucopyranose derivatives **32** and **33** by reductive opening of the lactone ring with lithium aluminum hydride and subsequent acetylation.[42] The anhydro rings of **32** and **33** were then cleaved with HBr/AcOH giving rise to the dibromocarbaglucose **34** and tribromocarbaglucose **35**, respectively, which are versatile intermediates for the synthesis of various carbasugar derivatives including the validamine- and valienamine-derived acceptors and donors.

The preparation of the validamine-derived acceptor and donor starts from **34** (Fig. 4a).[43)–46] Treatment of **34** with an excess of sodium benzoate resulted in benzoate for the primary bromide substitution (→ **36**) and subsequent elimination of the secondary bromide giving rise to the cyclohexene **37**. Because epoxidation of **37** with *m*-chloroperbenzoic acid (MCPBA) gave a mixture of nearly equal amounts of α- and β-epoxides **38α** and **38β**, the conformationally flexible **37** was first converted to the more rigid **39** by deacylation followed by benzylidenation and acetylation. The MCPBA epoxidation of **39** then proceeded stereoselectively yielding exclusively the acceptor β-epoxide **40**. Notably, after our synthesis of **40**, the strong β-glucosidase inhibitor cyclophellitol[47] (Fig. 4a, inset), which is the deprotected form of **40**, was discovered from the fermentation broth of *Phellinus* sp. When **34** was treated with an equivalent amount of sodium benzoate, the primary benzoate **36** was preferentially formed; subsequent S_N2 substitution of the secondary bromide with an azide anion then yielded **41**. After diacylation and di-*O*-isopropylidenation (→ **42**), the azido group was reduced to give the protected validamine **43** as the donor amine.

The dibromocarbaglucose **34** is also the starting point for the preparation of the valienamine-derived donors (Fig. 4b).[44),45] Dehydrobromination of **34** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the crystalline conjugated alkene **44**,[43] which was reacted with an equivalent amount of bromine in CCl_4 at $-15\,^\circ C$ to provide the 1,4-addition products **45α** and **45β** in a ratio of 1:2; the ratio was reversed to 3:1 when the bromination was carried out at room temperature. Selective replacement of the primary bromide in **45α** with benzoate was accompanied by the epimerization of the allylic secondary bromide yielding an inseparable mixture of **46α** and **46β**, azidation of which led to a separable 1:1.7 mixture of **47α** and **47β**. After deacylation and di-*O*-isopropylidenation, the resulting azides **48α** and **48β** were reduced with H_2S or PPh_3 to afford the protected valienamine **49α** and β-valienamine **49β**, respectively, as the donor amines. The efficient synthesis of **49α** was later achieved from the spiro-epoxide **50** prepared by selective epoxidation of **44** with MCPBA[48]; thus, opening of the epoxide ring in **50** with hydrochloric acid and subsequent acetylation (→ **51**) followed by S_N2' substitution with azide anion gave selectively the α-azide **52**, which was then converted to **49α** in a similar manner as **47α**.

The valienamine-derived acceptors are prepared from the tribromocarbaglucose **35** (Fig. 4c).[45] After vicinal debromination of **35** with zinc powder, the alkene **53** thus obtained was subjected to allylic bromination using a slight excess of *N*-bromosuccinimide (NBS) in CCl_4, resulting in the formation of an equilibrium mixture of the allyl bromides **54** and **55** together with the cyclohexadiene **56** and the tribromocyclohexene **57**. It seemed that both **54** and **55** tend to undergo dehydrobromination (→ **56**) followed by addition of bromine generated *in situ* by the reaction of NBS and HBr (→ **57**). Therefore, the reaction was carried out with an excess of NBS to convert **53** exclusively to **57**. The primary bromide in **57** was then replaced with benzoate, and the resulting monobenzoate **58** was treated with zinc powder yielding the 1,3-cyclohexadiene **59**. Regioselective epoxidation with MCPBA, followed by column chromatography separation, afforded **60α** and **60β** in a ratio of 1:1.5 as the valienamine-derived acceptor epoxides.

2.2. Synthesis of biologically active *N*-linked carbadisaccharide cores. The first effort was made to construct oligobiosamine (**23**) (Fig. 5),[49] which is the biologically active core of oligostatins (**20**) and contains hydroxyvalidamine (**10**). The coupling reaction of the validamine-derived acceptor epoxide **40** (racemic) and the donor amine, methyl 4-amino-4,6-dideoxy-α-D-glucopyranoside[50] (**61**), was carried out in propan-2-ol at 120 °C for 70 h. After de-*O*-benzylidenation followed by acetylation and column chromatography, all four products formed were isolated and their structures and absolute configurations were determined using ^1H NMR analysis and specific rotations. As expected, the epoxide opening proceeded mainly in a *trans*-diaxial fashion to give a pair of diastereoisomers (D, D)-**62** and (L, D)-**62** in 30% yields together with the *trans*-diequatorial isomers (D, D)-**63** and (L, D)-**63** in 14% yield. The use of the chiral D-**40** as the acceptor produced (D, D)-**62** and the isomer (D, D)-**63** in 34% and 16% yields, respectively. Deprotection of (D, D)-**62** af-

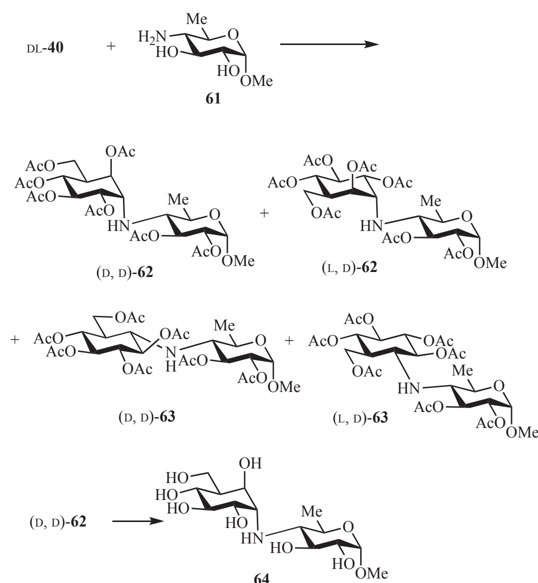


Fig. 5. Synthesis of methyl oligobiosaminide using a validamine-derived acceptor **40**.

forded the α-methyl glycoside of **23**, methyl oligobiosaminide (**64**).

Two other active cores, acarviosin (**21**) and 6-hydroxyacarviosin (**22**), are composed of the valienamine (**11**) moiety. Therefore, a similar coupling reaction was investigated with the valienamine-derived acceptor **60*β*** (Fig. 6a).[45] When an equimolar mixture of **60*β*** (racemic) and **61** was reacted in propan-2-ol at 120 °C in a sealed tube, the ring opening occurred exclusively at the allylic position C-1 giving rise to the *trans*-diaxial products (D, D)-**65** and (L, D)-**65** which, after deprotection, yielded the α-methyl glycoside of the 2′-epimer of **21**, methyl 2′-epiacarviosin (D, D)-**66** (46%) and its diastereomer (L, D)-**66** (31%), respectively.[51] The attempted transformation of (D, D)-**66** to **21** by inversion of the 2′-hydroxy group was unsuccessful due to the neighboring group participation of the nucleophilic imino group.

Next, for the synthesis of **21** and **22**, the protected valienamine **49*α*** was used as the donor amine with the acceptor sugar epoxide, methyl 3,4-anhydro-α-D-galactopyranoside[52] (**67**) (Fig. 6b).[49] The reaction of equivalent amounts of **49*α*** (racemic) and **67** in propan-2-ol at 120 °C for 40 h afforded the desired pair of diastereomers (D, D)-**68** and (L, D)-**68** and their positional isomers (D, D)-**69** and (L, D)-**69** in 37% and 38% yield, respectively. This favorable result was rather unexpected because the formation of the desired pair involved the *trans*-diequatorial opening of the epoxide ring, which is generally less favorable than the *trans*-diaxial opening yielding the pair of the positional isomers. Deprotection of (D, D)-**68** gave the α-methyl glycoside of **22**, methyl 6-hydroxyacarviosin (**70**). A similar approach to prepare **21** using the 6-deoxy derivative of **67**, methyl 3,4-anhydro-6-deoxy-α-D-galactopyranoside[52] (**71**) (Fig. 6c)[54] was, however, unsuccessful because of the exclusive formation of (D, D)-**72** and (L, D)-**72** by *trans*-diaxial opening of the epoxide ring.

Two alternative routes to **21** were, therefore, investigated starting from other *N*-linked carbadisaccharide cores synthesized: (1) deoxygenation of the 6-hydroxy group of **70** (Fig. 7a)[51] and (2) introduction of the C5′–C6′ double bond in **64** by dehydration of the 6′-hydroxy group (Fig. 7b).[53),54] The first route began with the coupling product (D, D)-**68**. After protection of the 2- and 3-hydroxy groups by isopropylidenation (→ **73**), the remaining 6-hydroxy group was converted to *p*-toluenesulfonate (→ **74**) and replaced with iodide (→ **75**). Without the protection of the 3-hydroxy group, the iodide for *p*-toluenesulfonate substitution led to the predominant formation of the 3,6-anhydro ring by intramolecular attack of the 3-hydroxy group. The iodide **75** was treated with lithium triethylborohydride followed by acetylation to give **76**, which, after deacetylation, yielded the α-methyl glycoside of **21**, methyl acarviosin (**77**). For the second route, selective protection of the 6′- and 7′-hydroxy groups in **64** was first carried out using an equivalent amount of 2,2-dimethoxypropane to yield, after acetylation, the desired 6′,7′- and the isomer 4′,7′-*O*-isopropylidene derivatives **78** and **79**, respectively, in a ratio of 1:3. Removal of the isopropylidene group of **78** (→ **80**) was followed by chlorination with sulfuryl chloride affording the dichloride **81**. Because selective substitution of the primary chloride with acetate was accompanied by aziridine formation (→ **82**), the chloro group was reinstalled at C6′ by opening of the aziridine ring with chloride (→ **83**). The C5′–C6′ double bond was then introduced by treatment of **83** with DBU providing **76**, which was deacetylated to give **77**.

2.3. Total synthesis of acarbose and related compounds. The total synthesis of *N*-linked carbatrisaccharide inhibitors, amylostatin-XG (**15**)[55] and adiposin-1 (**17**),[56] was first accomplished (Fig. 8). The coupling of the protected valienamine **49*α*** (racemic) with 1,6-anhydro-4-*O*-(3′,4′-anhydro-6′-deoxy-α-D-galactopyranosyl)-D-glucopyranose (**84**), prepared from 1,6-anhydro-β-D-maltose,[57] was car-

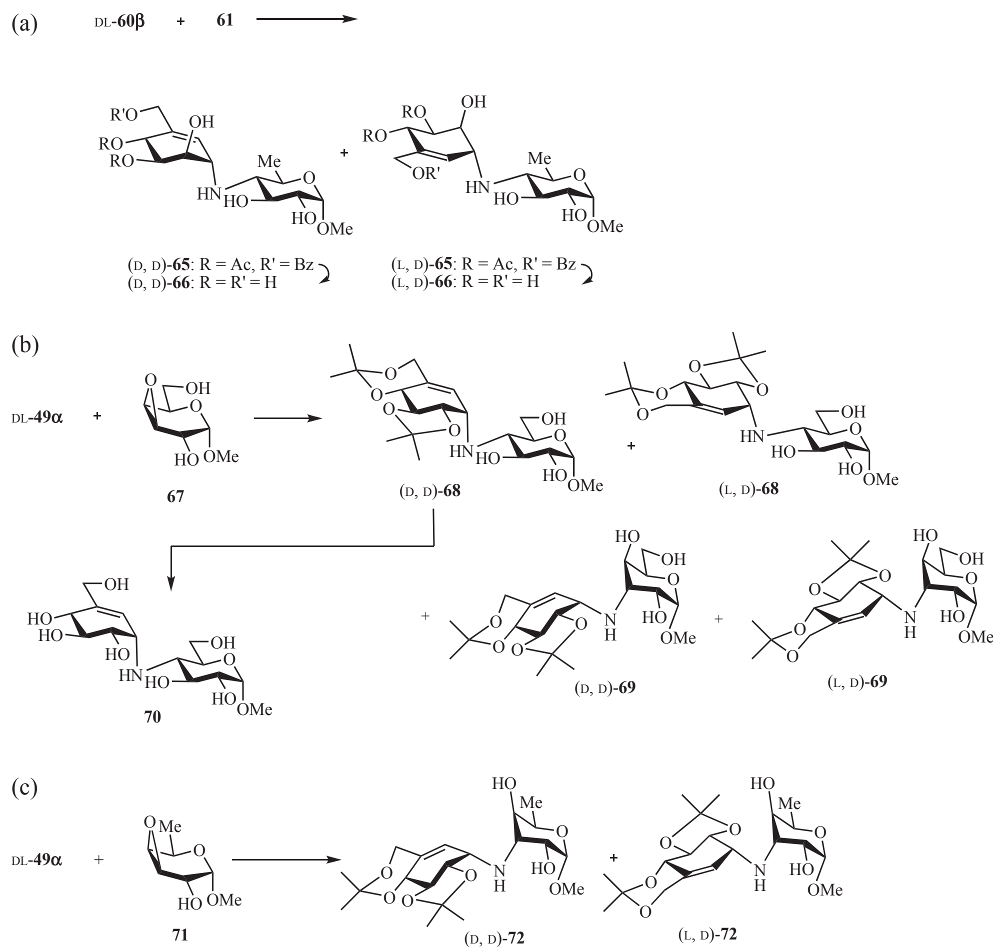


Fig. 6. Synthesis of *N*-linked carbadisaccharide cores composed of valienamine. (a) Attempted synthesis of methyl acarviosin using valienamine-derived acceptor **60*β***. (b) Synthesis of methyl 6-hydroxyacarviosin using valienamine-derived donor **49*α***. (c) Attempted synthesis of methyl acarviosin using valienamine-derived donor **49*α***.

ried out in a 1:8 mixture of *N*,*N*-dimethylformamide and propan-2-ol at 120 °C for 2.5 days. After de-*O*-isopropylidenation followed by acetylation, all diastereoisomers formed were isolated by column chromatography and characterized by the combination of ^{1}H-NMR spectroscopy and specific rotation as a pair of *trans*-diequatorial products, (D, D)-**86** (6.1%) and (L, D)-**86** (5.7%), and a pair of *trans*-diaxial products, (D, D)-**87** (24%) and (L, D)-**87** (32%). Notably, as in the case with the acceptor 6-hydroxy epoxide **67**, a similar coupling reaction of **49*α*** (racemic) with the corresponding 6′-hydroxy derivative **85** facilitated the formation of the desired *trans*-diequatorial products affording (D, D)-**88** (13%) and (L, D)-**88** (14%) together with the *trans*-diaxial products (D, D)-**89** (21%) and (L, D)-**89** (16%). Both (D, D)-**86** and (D, D)-**88** were subjected to acetolysis with AcOH-Ac_2O-H_2SO_4 (30:70:1) at room temperature and subsequent deacetylation with methanolic sodium methoxide to yield **15** and **17**, respectively.

Next, the total synthesis of *N*-linked carbatetrasaccharide inhibitors, acarbose (**14**) and adiposin-2 (**18**), was achieved using the protected chiral valienamine D-**49*α*** (Fig. 9).[58] The coupling reaction of D-**49*α*** with 1,6-anhydro-4′-*O*-(3″,4″-anhydro-6″-deoxy-*α*-D-galactopyranosyl)-*β*-D-maltose (**90**), prepared from 1,6-anhydro-*β*-D-maltotriose,[59] in a 1:1 mixture of *N*,*N*-dimethylformamide and propan-2-ol at 120 °C for 3 days yielded, after de-*O*-isopropylidenation and acetylation, the *trans*-diequatorial and *trans*-diaxial products **92** and **93** in 19% and 30% isolated yields, respectively. Acetolysis followed by deacetylation converted **92** to **14**. Once again, the reaction of D-**49*α*** with the corresponding 6″-hydroxy

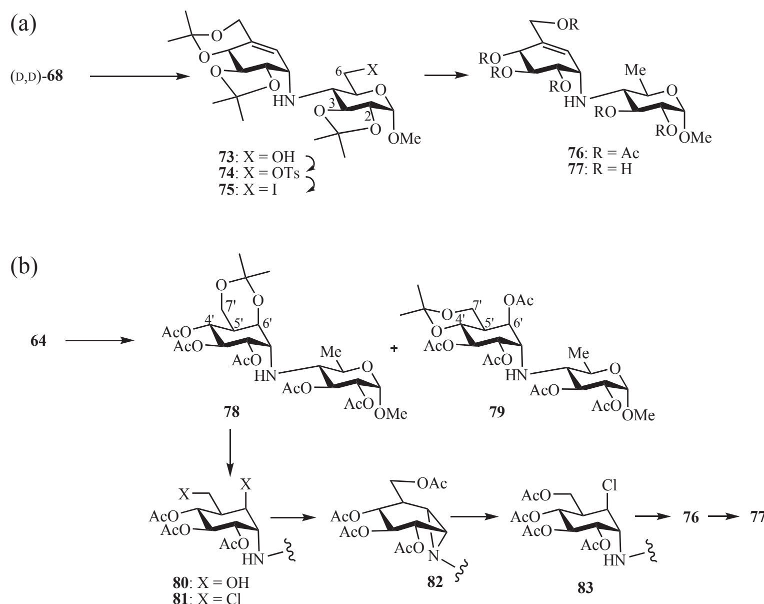


Fig. 7. Synthesis of methyl acarviosin. (a) From methyl 6-hydroxyacarviosin by deoxygenation of the 6-hydroxy group. (b) From methyl oligobiosaminide by dehydration of the 6′-hydroxy group.

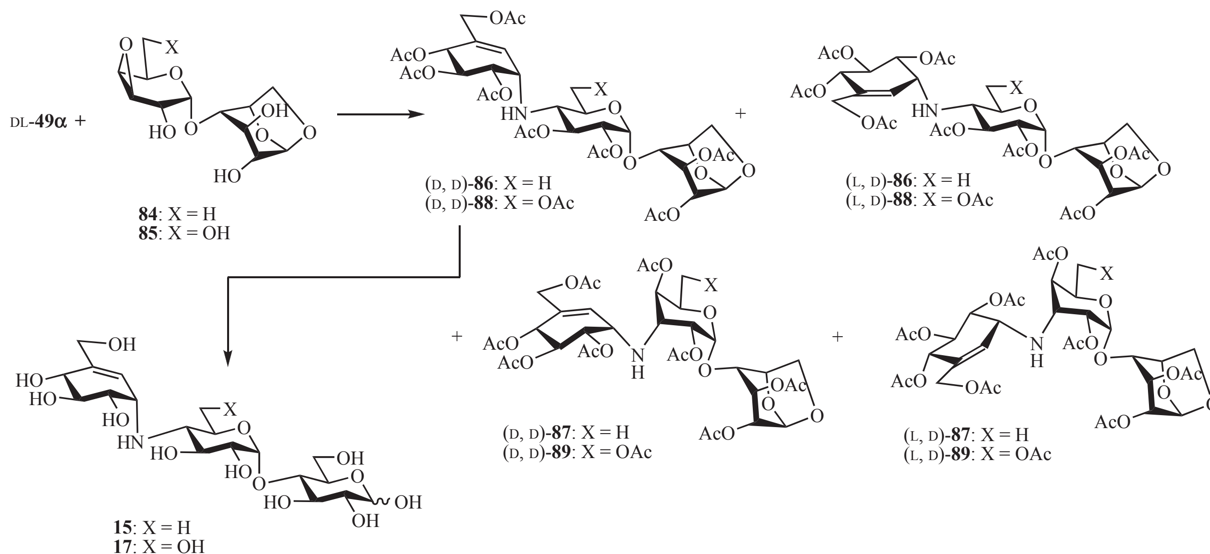


Fig. 8. Total synthesis of amylostatin-XG and adiposin-1.

derivative **91** increased the yield of the *trans*-diequatorial product affording the desired **94** and the *trans*-diaxial product **95** in 33% and 21% isolated yields, respectively, of which **94** was converted to **18** by acetolysis and deacetylation. It is noteworthy that the role of the 6-hydroxy group in favoring the *trans*-diequatorial opening of the 3,4-anhydro ring, possibly through hydrogen bond formation to the epoxide oxygen, needs further investigation.

Although the coupling reaction is not fully optimized, the successful synthesis of *N*-linked carbaoligosaccharides would allow for the development of novel *N*-linked carbaoligosaccharide glycosidase inhibitors.

2.4. Structure–activity relationship of methyl acarviosin. The valienamine moiety of methyl acarviosin (**77**), the active core of acarbose (**14**), is considered to mimic the oxocarbenium ion-like

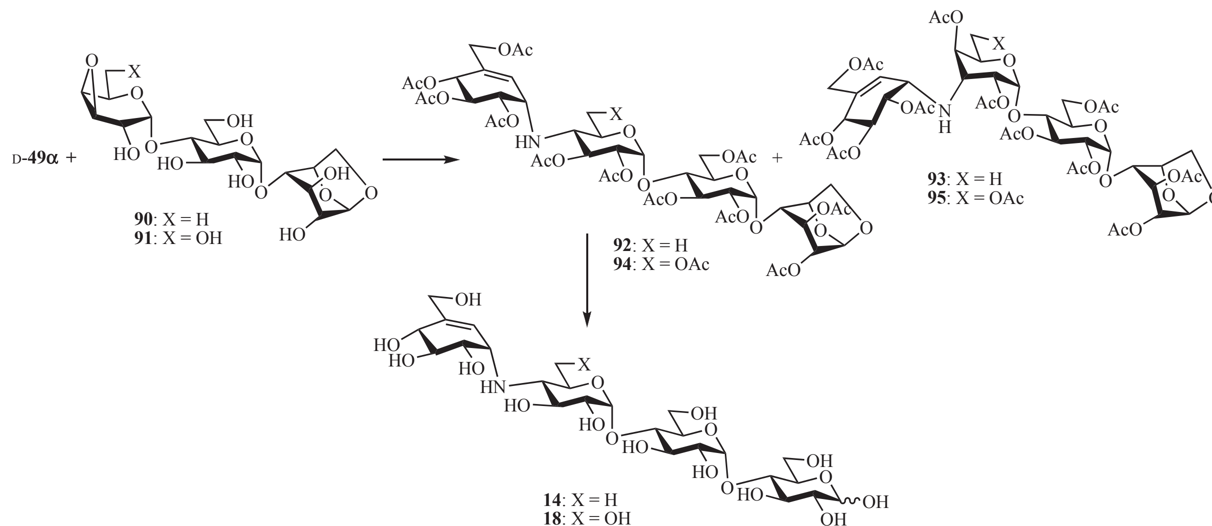


Fig. 9. Total synthesis of acarbose and adiposin-2.

transition state of the α-amylase-catalyzed hydrolysis reaction. However, the role of the sugar moiety in the inhibitory activity was not clear. Therefore, the inhibitory activities of **77** and four related analogues with modified sugar moieties were compared against Baker's yeast α-glucosidase; the analogues included methyl 6-hydroxyacarviosin (**70**) and its 2-*O*-methyl, 3-*O*-methyl, and 3,6-anhydro derivatives (**102a**, **102b**, and **104**, respectively) (Fig. 10 and Table 1a).[60] For the synthesis of the monomethyl derivatives **102a** and **102b**, the epoxide **67** was first converted to either its 2-*O*-methyl or 2-*O*-tetrahydropyranyl derivative (**97** and **98**, respectively) after protection of the 6-hydroxy group as a trityl ether (→ **96**). The coupling of D-**49α** with **97** in propan-2-ol at 120 °C yielded **99**, which, after removal of the trityl and isopropylidene groups, gave **102a**. A similar coupling of D-**49α** with **98** (→ **100**) followed by 3-*O*-methylation (→ **101**) and deprotection provided **102b**. The 3,6-anhydro derivative **104** was prepared from (D, D)-**68** by treatment of its 6-*O*-tosyl derivative **103** with sodium methoxide followed by deprotection.

All four analogues were found to be slightly less active than **77** but were still potent inhibitors. Surprisingly, changing the conformation of the sugar moiety from $^4C_1$ to $^1C_4$ by 3,6-anhydro formation did not have any significant detrimental effect on the activity.

To gain further insight into the structural requirement of the sugar moiety, the 1,6-anhydride of 6-hydroxyacarviosin (**22**), namely **116a**, and its several derivatives **116b–h** were synthesized (Fig. 10b) and their inhibitory activities were compared with that of **77** (Table 1b).[61),62] The 1,6-anhydride **116a** and its 2-epimer **116f** were synthesized by coupling of D-**49α** with 2-*O*-acetyl-1,6:3,4-dianhydro-β-D-galacto-[63] (**105**) and 1,6:3,4-dianhydro-β-D-talopyranoses[63] (**106**), respectively, in propan-2-ol at 120 °C and subsequent deprotection of the corresponding products **111** and **112**. The 2-azido and 2-fluoro derivatives of **116a** (**116b** and **116e**, respectively) were synthesized similarly by coupling of D-**49α** with the 2-azido and 2-fluoro derivatives of **105** (**108** and **109**, respectively), prepared by trifluoromesylaton of **106** (→ **107**) and S_N2 displacement of trifluoromesylate **107** by either azide or fluoride, and subsequent deprotection of the resulting **113** and **114**, respectively. The reduction of **113** and deprotection afforded the 2-amino derivative **116c**, whereas the reduction followed by *N*-acetylation and deprotection yielded the 2-acetamido derivative **116d**. The mono- and dideoxy derivatives (**116g** and **116h**, respectively) were synthesized by coupling of D-**49α** with the 2-thioether **110**, prepared by S_N2 displacement of the 2-trifluoromesylate in **107** by *p*-toluenethiolate, and subsequent desulfurization of the product **115** with Raney-nickel followed by deprotection. The ratio of the two products **116g** and **116h** was 1.4:1; the dideoxy derivative **116h** was presumed to be formed through the intermediate episulfide.

As in the case of the 3,6-anhydro derivative **104**, the $^1C_4$ conformation of the 1,6-anhydro sugar moiety

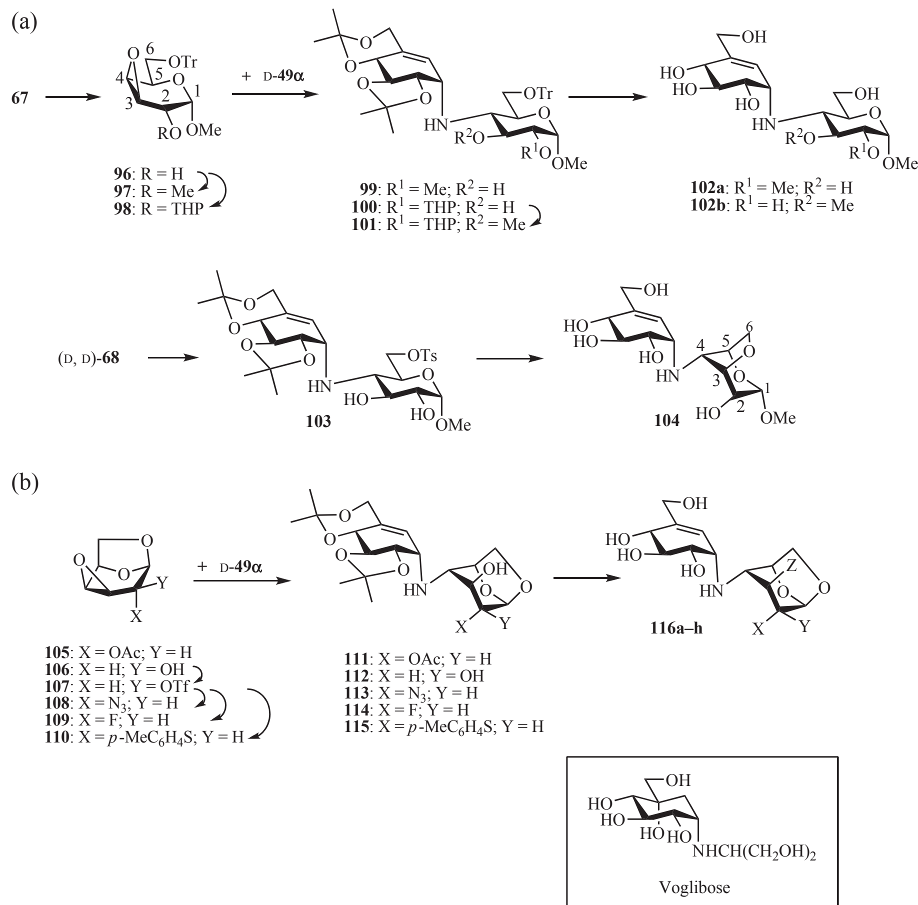


Fig. 10. Chemical modification of 6-hydroxyacarviosin. (a) Synthesis of 2-*O*-methyl, 3-*O*-methyl, and 3,6-anhydo derivatives. (b) Synthesis of 1,6-anhydro and its 2-azido, amino, acetamido, fluoro, and *epi* derivatives. Inset: Potent α-glucosidase inhibitor voglibose.

did not have any negative effect on the inhibitory activity. The 1,6-anhydride **116a**, its 2-epimer **116f**, and its 2-azido and 2-fluoro derivatives (**116b** and **116e**, respectively) showed similar activities to **77**, but the 2-amino derivative **116c** and, particularly, the 2-acetamide derivative **116d** were significantly less active. The most interesting finding was the increased activity of the deoxy derivatives: both mono- and dideoxy-derivatives (**116g** and **116h**, respectively) were nearly 10 times more active than **77**.

The X-ray crystallographic analysis of the acarbose (**14**) and glucoamylase complex was later published by Aleshin *et al.*,[64] revealing: (1) an extensive hydrogen bond network between the valienamine moiety and the surrounding amino acids; (2) a salt bridge between the imino linkage and the putative catalytic Glu; and (3) the hydrophobic interactions between the hydrophobic faces of the sugar moieties and the Tyr/Trp at the binding site. The strong interaction of the valienamine moiety with the enzyme active site probably contributes to the potent activity of the synthetic analogues of **77** except **116c** and **116d**. The poor activity of these two analogues may be attributed to the disruption of the salt bridge interaction by the amino/acetamido group being 1,3-diaxially oriented to the imino linkage. The hydrophobic amino acids present in the sugar binding site may also explain the higher activity of the deoxy analogues **116g** and **116h** than **77**. These results seemed to clearly suggest that the sugar moiety of **77** may be replaced by simple alkyl groups without impairing the inhibitory potency. Kameda *et al.*[65] reported similar findings based on their semi-synthetic studies on valienamine-containing α-glycosidase inhibitors, in which several *N*-alkyl and *N*-aralkyl valienamines were synthesized from valienamine (**11**) prepared by

Table 1. Chemical modification of methyl acarviosin. (a) α-Glucosidase inhibitory activity of several anhydro, deoxy, and *O*-methyl derivatives. (b) α-Glucosidase inhibitory activity of several 1,4-anhydro derivatives **116a–h**.

(a)

Compd	Inhibitory activity (IC_{50}, µM) α-Glucosidase (Baker's yeast)
70	0.98
77	0.38
102a	1.25
b	0.75
104	1.45

(b)

Compd	X	Y	Z	Inhibitory activity (IC_{50}, µM) α-Glucosidase (Baker's yeast)
116a	OH	H	OH	0.10
b	N_3	H	OH	0.29
c	NH_2	H	OH	2.2
d	NHAc	H	OH	33
e	F	H	OH	0.18
f	H	OH	OH	0.50
g	H	H	OH	0.032
h	H	H	H	0.030

microbial degradation of validamycin A (**5**), and the findings were used to guide the development of voglibose[66] (Fig. 10b, inset), which is an *N*-substituted derivative of valiolamine (**12**) and a practical alternative to **14** for the treatment of type 2 diabetes mellitus.

3. Synthesis of novel carbaglycosylamine-based β-glycosidase inhibitors

Several valienamine-type carbaglycosylamine derivatives available in our laboratory were exploited to develop new transition-state analogue inhibitors of β-glycosidases.

3.1. Methyl 1′-*epi*-acarviosin and methyl 1′-*epi*-6-hydroxyacarviosin. By analogy with methyl acarviosin (**77**) and methyl 6-hydroxyacarviosin (**70**), it was assumed that methyl 1′-*epi*-acarviosin (**117**) and 1′-*epi*-6-hydroxyacarvosin (**118**) (Fig. 11) with cellobiose-like structures might be potential transition-state analogue inhibitors of β-glucosidase.[51] Both **117** and **118** were, therefore, synthesized similarly to **77** and **70**, respectively, except that the protected β-valienamine **49β** was used instead of the α-valienamine **49α** (Fig. 11a). Coupling of D-**49β** with **67** yielded the desired **119** (55%). Which, after deprotection, provided **118**. For the synthesis of **117**, the 6-hydroxy group of the coupling product **119** was removed by a sequence of reactions involving selective 6-*O*-tosylation (→ **120**), 2,3-*O*-isopropylidenation (→ **121**), iodide-for-tosylate substitution (→ **122**), and deiodination (→ **123**). The 6-deoxy derivative **123** thus obtained was deprotected to give **117**. The valienamine-derived epoxide **60α** was also found to be effective for the straightforward synthesis of **117** (Fig. 11b).[49] Treatment of **60α** (racemic) with the amine **61** in propan-2-ol at 120 °C in a sealed tube led to the opening of the epoxide exclusively at allylic C-1 yielding *trans*-diequatorial products (D, D)-**124** and (L, D)-**124**, which, after deprotection, provided (D, D)-**117** (25%) and its diastereoisomer (L, D)-**117** (16%), respectively.

The inhibitory activity of **117** and **118** were tested against three glycosidases: yeast α-glucosidase, almond β-glucosidase, and jack bean α-mannosidase (Table 2). Contrary to expectation, both **117** and **118** were found to be poor inhibitors of β-glucosidase but modest inhibitors of α-glucosidase and α-mannosidase. Of note, Stick and co-workers[67),68)] later synthesized the β-methyl glycoside analogue **125** of **117** (Fig. 11a, inset), and reported it to be a potent inhibitor of *A. niger* β-glucosidase but a weak inhibitor of *C. saccharolyticum* β-glucosidase. It seems that the substrate specificity differs widely even within a class of enzymes; hence, caution needs to be taken when evaluating the inhibition results.

3.2. Synthesis of β-valienaminylceramide and 4-*epi*-β-valienaminylceramide. Glucocerebrosidase and galactocerebrosidase are the lysosomal β-glucosidase and β-galactosidase, respectively, and involved in glycolipid metabolism through hydrolysis of glucosyl- and galactosylceramide, respectively. It was reasoned that replacing the carbohydrate moiety of glucosylceramide and galactosylceramide with an appropriate valienamine derivative may provide a transition-state analogue inhibitor of the respective glycosylceramidase.[31] The ring opening of sphingosine-derived aziridines with proper carbaglycosylamines was found to be effective for the synthesis of *N*-carbaglycosylceramides. The protected aziridine (*E*)-**129** was prepared from the known azidosphingosine (*E*)-**126**[69)–71)] in three steps (Fig. 12a): (1)

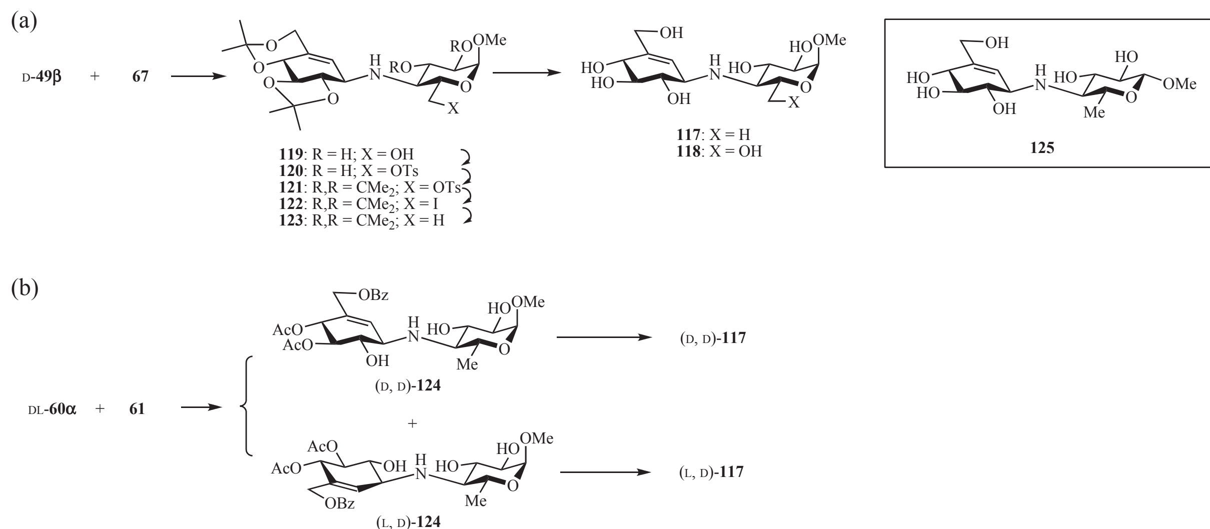


Fig. 11. Synthesis of *N*-linked carbadisaccharides composed of β-valienamine. (a) Synthesis of methyl 1′-*epi*-acarviosin and 1′-*epi*-6-hydroxyacarviosin using valienamine-derived donor **49β**. Inset: β-Methyl glycoside analogue of methyl-1′-*epi*-acarviosin. (b) Straightforward synthesis of 1′-*epi*-acarviosin using valienamine-derived acceptor **60α**.

Table 2. Inhibitory activity of methyl 1′-*epi*-acarviosin **117** and methyl 1′-*epi*-6-hydroxyacarviosin **118**

Compound	Inhibition: I (%) at 1000 μg/mL		
	α-Glucosidase (yeast)	β-Glucosidase (almonds)	α-Mannosidase (Jack beans)
117	72	33	80
118	80	~0	86

selective benzoylation of the primary hydroxy group (→ (*E*)-**127**); (2) protection of the residual hydroxy group as a *tert*-butyldimethylsilyl ether followed by debenzoylation (→ (*E*)-**128**); and (3) aziridine formation by reduction of the azide with triphenylphosphine and subsequent protection of the aziridine with a 2,4-dinitrophenyl group (→ (*E*)-**129**). The aziridine product (*E*)-**129** was formed in 39% yield together with the amino alcohol (*E*)-**130** (45%), which was readily converted to (*E*)-**129** (86%) by iodination (→ **131**) followed by reductive cyclization. The isomer (*Z*)-**129** was similarly prepared from the (*Z*)-**126**.

The 4-*epi*-β-valienamine donor **136** was prepared from the precursor of the β-valienamine donor D-**47β** (Fig. 12b) by epimerization of the 4-hydroxy group, after a series of protecting group manipulations including deisopropylidenation (→ **132**), benzylidenation and subsequent methoxymethylation (→ **133**), and debenzylidenation followed by selective protection of the primary hydroxy group with tetrabutyldimethyl-silyl ether (→ **134** → **135**). Epimerization at the 4 position was then achieved by oxidation of the 4 hydroxy group of **135** with pyridinium chlorochlomate, followed by reduction with diisopropylaluminum hydride, which also reduced the azido group, to give **136**.

The coupling reaction of D-**49β** with (*E*)-**129** was carried out in propan-2-ol at 120 °C for 5 d (Fig. 12c) to afford the protected β-valienaminylsphingosine (*E*)-**137** (60%), which, after selective *N*-deprotection followed by *N*-palmitoylation and deprotection, yielded β-valienaminylceramide (*E*)-**24** (44%). Likewise, the coupling of **136** with (*E*)-**129** (→ (*E*)-**138**; 55%) followed by the same sequence of reactions gave the isomer (*E*)-**25** (49%). The *Z*-isomers (*Z*)-**24** and (*Z*)-**25** were similarly prepared in 27% and 22% overall yields, respectively, using the aziridine (*Z*)-**129**.

Their inhibitory activities were tested against mouse liver glucocerebrosidase and galactocerebrosidase (Table 3). As expected, (*E*)-**24** and (*E*)-**25** were potent and selective inhibitors of glucocerebrosidase and galactocerebrosidase, respectively. Notably, their isomers (*Z*)-**24** and (*Z*)-**25** were also found to be potent and selective inhibitors. Both glucocerebrosidase and galactocerebrosidase appear to have a rather broad specificity for the ceramide moiety.

3.3. *N*-Octyl-β-valienamine and *N*-octyl-4-*epi*-β-valienamine. The above findings suggested

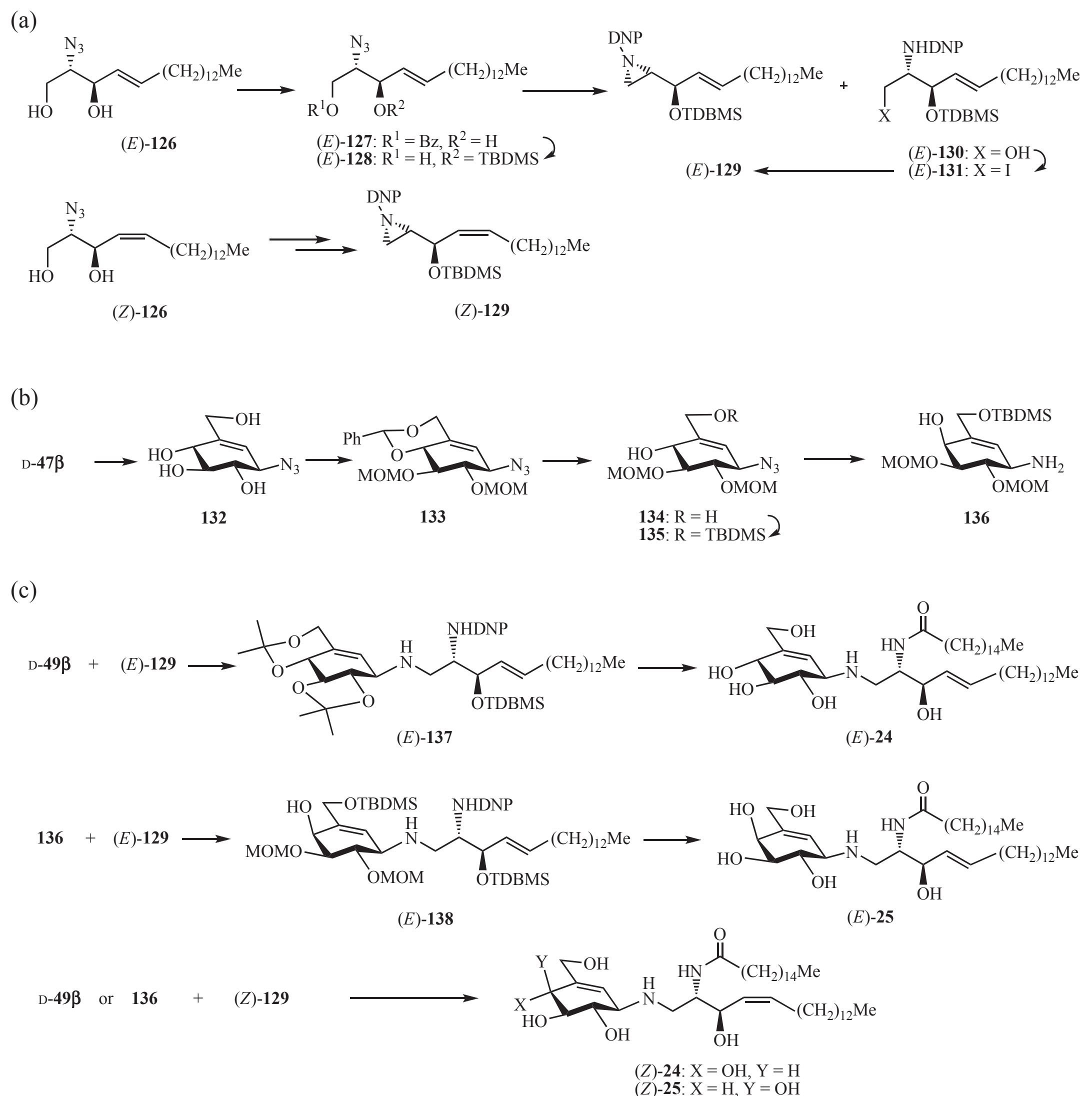


Fig. 12. Synthesis of potent gluco- and galactocerebrosidase inhibitors. (a) Synthesis of sphingosine-derived aziridine and its *Z*-isomer. (b) Synthesis of 4-*epi*-β-valienamine donor. (c) Synthesis of β-valienaminylceramide and 4-*epi*-β-valienaminylceramide.

Table 3. Inhibitory activity of carbaglycosylceramides

Compound	Inhibition at 10 µM (%)	
	β-Glucocerebrosidase (mouse liver)	β-Galactocerebrosidase (mouse liver)
(*E*)-**24**	95.2 (0.3)*	19.4
(*Z*)-**24**	97.7 (0.1)	28.6
(*E*)-**25**	6.7	90.3 (2.7)
(*Z*)-**25**	9.6	78.4 (4.5)

*Number in parentheses denotes IC_{50} (µM) values.

that the ceramide moiety of (*E*)-**24** and (*E*)-**25** can be replaced by simple alkyl chains without compromising their activities. Thus, several *N*-alkyl derivatives of β-valienamine were first prepared by *N*-acylation of the protected β-valienamine D-**49β** with *n*-alkanoyl chlorides (→ **139a**–**e**) followed by lithium aluminum hydride reduction (→ **140a**–**e**) and deprotection (→ **141a**–**e**) (Fig. 13a) and evaluated for their inhibitory activity against mouse liver glucocerebrosidase (Table 4a).[32] All the *N*-alkyl derivatives, except for the shortest alkyl chain derivative **141a**, showed similar or better activity than (*E*)-**24**. The

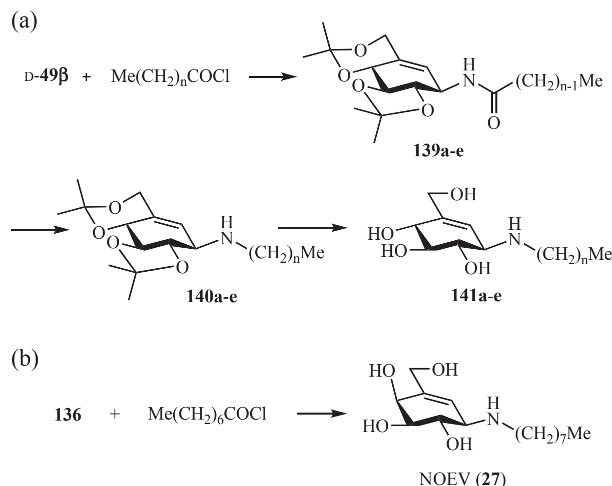


Fig. 13. Synthesis of *N*-alkyl derivatives of β-valienamine and 4-*epi*-β-valienamine.

Table 4. Inhibitory activity of *N*-alkyl derivatives of β-valienamine and 4-*epi*-β-valienamine. (a) Inhibitory activity of *N*-octyl-β-valienamine (NOV) and its *N*-alkyl homologues. (b) Inhibitory activity of *N*-octyl-4-*epi*-β-valienamine (NOEV).

(a)

Compound		Inhibitory activity (IC_{50}, μM) β-Glucocerebrosidase (mouse liver)
26 (NOV)	n = 7	0.03
141a	n = 3	10
b	n = 5	0.3
c	n = 9	0.07
d	n = 13	0.12
e	n = 17	0.3

(b)

Compound	Inhibitory activity (IC_{50}, μM)	
	β-Galactocerebrosidase (mouse liver)	β-Galactosidase (bovine liver)
27 (NOEV)	5.0	0.87

potency seemed to depend on the length of the *N*-alkyl chain: the potency increased rapidly from C_4 (**141a**) to C_6 (**141b**) and to C_8 (**26**) and then decreased gradually for C_{10} (**141c**), C_{14} (**141d**) and C_{18} (**141e**). The most potent C_8 derivative, NOV (**26**), exhibited 10-fold greater activity than (*E*)-**24**. Accordingly, the C_8 derivative of 4-*epi*-β-valienamine, NOEV (**27**), was similarly synthesized from the protected 4-*epi*-β-valienamine **136** and *n*-octanoyl chloride (Fig. 13b) and tested for its inhibitory activity against mouse liver galactocerebrosidase as well as bovine liver β-galactosidase (Table 4b).[33] Of note, NOEV (**27**) was found to be only a moderate inhibitor of galactocerebrosidase but rather a potent inhibitor of β-galactosidase. Moreover, it was later discovered that NOEV is a potent inhibitor of human lysosomal β-galactosidase responsible for the hydrolysis of the terminal β-galactose residue from GM1-ganglioside.[35]

The potent β-galactosidase inhibitory activity of (*E*)-**25** and NOEV (**27**) spawned an increased interest in the *N*-substituted 4-*epi*-β-valienamine and, therefore, a more straightforward synthesis of a suitable 4-*epi*-β-valienamine donor has been developed starting from the tribromocarbaglucose L-**35** derived from the Diels–Alder adduct (+)-**29** (Fig. 14).[37],[72],[73] Treatment of **35** with sodium methoxide gave after acetylation the epoxide **142**, which was subjected to acid hydrolysis followed by acetylation providing the dibromocarbaaltrose **143**. Following the same sequence of reactions described for the preparation of the valienamine-donors **49*α*,*β*** from **45*α*,*β*** (Fig. 4b), the carbaaltrose derivative **143** was converted to the 4-*epi*-β-valienamine donor **149*β*** and its α-isomer **149*α*** via the conjugated alkene **144**, the 1,4-conjugate addition products **145*α*,*β***, the primary benzoates **146*α*,*β***, the allyl azides **147*α*** and **147*β*** (at a ratio of *ca.* 1:1; 50%), and the di-*O*-isopropylidene derivatives **148*α*** and **148*β***. Furthermore, after protecting group manipulation of **144** (deacetylation followed by isopropylidenation and acetylation), the resulting **150** was similarly converted to a mixture of the allyl bromides **151*α*,*β*** and then to a mixture of **152*α*,*β***, which then reacted with alkylamines, providing selectively β-amine **153**.[73] The alkylamination of **152*β*** likely proceeded via a cyclic acetoxonium ion.[71] After deprotection, NOEV (**27**) and **154a**–**c** thus obtained were evaluated for their inhibitory activity (Table 5). Both *N*-decyl and *N*-dodecyl derivatives (**154b** and **154c**, respectively) were found to be more potent β-galactosidase inhibitor than NOEV (**27**). Notably, the most potent inhibitor **154c** was also a potent inhibitor of β-glucosidase.

4. Development of carbaglycosylamine-based pharmacological chaperones for lysosomal storage disorders

4.1. Lysosomal storage disorders and pharmacological chaperones. Lysosomal storage disorders (LSDs) are a group of rare genetic diseases, most of which are caused by mutations in genes

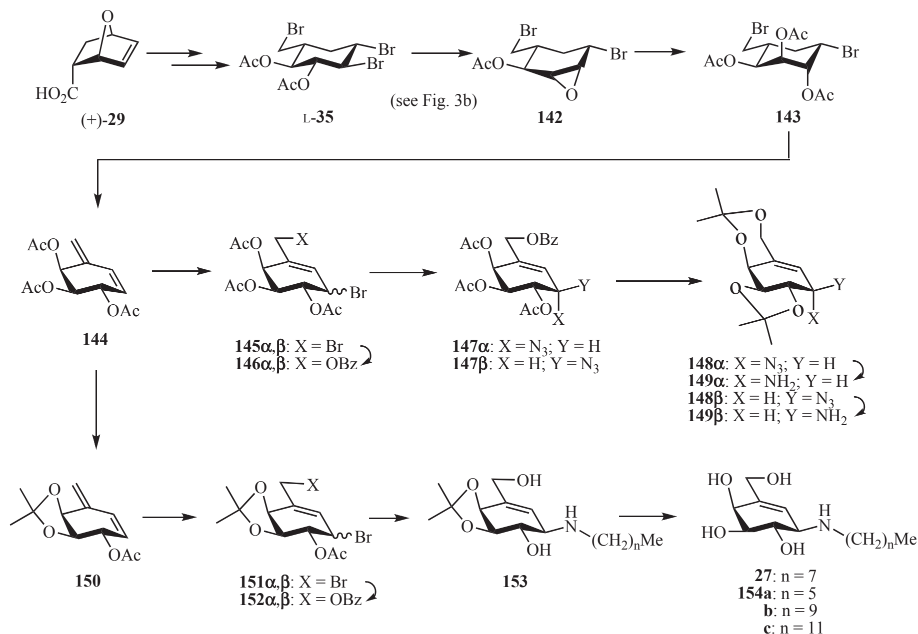


Fig. 14. Straightforward synthesis of 4-*epi*-β-valienamine donor.

Table 5. Inhibitory activity of NOEV (**27**) and its *N*-alkyl homologues against three glycosidases

Compd	Inhibitory activity (IC_{50}, μM)		
	α-Galactosidase (green coffee beans)	β-Galactosidase (bovine liver)	β-Glucosidase (almonds)
27: n = 7	3.1	0.87	3.1
154a: n = 5	2.7	2.3	1.2
b: n = 9	1.9	0.13	2.5
c: n = 11	4.4	0.01	0.87

encoding lysosomal hydrolases.[74),75)] The mutations produce misfolded hydrolases that are retained in the endoplasmic reticulum (ER) and degraded by ER-associated degradation. As a result, lysosomes are deficient in hydrolases, resulting in the progressive accumulation of undegraded substrates and ultimately generalized cell and tissue dysfunction.

Pharmacological chaperone therapy[76)] is an emerging approach to treat LSDs using small-molecule ligands that specifically bind and stabilize mutant enzymes, thereby facilitating proper folding and thus improving lysosomal trafficking and activities.[77),78)] Pharmacological chaperone therapy is particularly advantageous for the treatment of LSDs affecting the central nervous system, because of the potential of small-molecule ligands to cross the blood–brain barrier. As such, reversible competitive inhibitors are obvious candidates for the development of specific pharmacological chaperones. In 1999 the imino sugar 1-deoxygalactonojirimycin (migalastat), an α-galactosidase inhibitor, was shown to function as a pharmacological chaperone for Fabry disease, which is caused by a deficiency in α-galactosidase A activity and subsequent accumulation of the substrate globotriosylceramide.[79)] Its hydrochloride has been approved for the oral treatment of some variants of Fabry disease in several countries including the European Union, U.S.A., and Japan.[80)]

4.2. Discovery of chaperone activities of NOV and NOEV. Gaucher disease and GM1-gangliosidosis are LSDs caused by deficiencies of lysosomal glucocerebrosidase and β-galactosidase, respectively, and characterized by the intracellular accumulation of the corresponding substrates, glucosylceramide and GM1-ganglioside.[81)] The selective inhibitors of these enzymes are potential candidates for the development of specific pharmacological chaperones: the glucocerebrosidase inhibitor NOV (**26**) (see Fig. 2b) for Gaucher disease and the human β-galactosidase inhibitor NOEV (**27**) (see Fig. 2b) for GM1-gangliosidosis.

The increased activity of the mutant enzyme due to the addition of **26**, namely chaperone activity of **26**, was evaluated in fibroblasts expressing F213I mutant glucocerebrosidase, which is a common

Table 6. Inhibitory and chaperone activity of NOV (**26**) and NOEV (**27**). (a) Activity of **26** against wild-type and mutant human β-glucosidase. (b) Activity of **27** against wild-type and mutant human β-galactosidase.

(a)

Compd	IC_{50} (μM) against wild type	Enhanced activity (fold) of mutant with **26** (30 μM) or **26** HCl (3 and 30 μM)	
26	3	F213I	6
26 HCl	0.502	F213I, N188S, G202R, N370S	∼2

(b)

Compd	IC_{50} (μM) against wild type	Enhanced activity (fold) of mutants with **27** (0.2 μM)	
27	0.2	R201C	5.1
		R201H	4.5
		R457Q	2.4
		W273L	2.2
		Y83H	2.0

mutation in patients with Gaucher disease in Japan (Table 6a).[82),83)] After incubation with 30 μM **26**, the maximum enhancement of enzyme activity (∼6-fold compared to without **26**) was observed together with lysosomal localization of the mutant enzyme and intracellular clearance of the substrate glucosylceramide.[82)] This finding was rather surprising, considering that the IC_{50} of **26** for wild-type human glucocerebrosidase was found to be 3 μM. Of note, a similar enhancement of activity was reproduced by 3 μM of the hydrochloride of **26** probably due to its increased water solubility.[83)] In addition to the F213I mutant, the hydrochloride of **26** (3 and 30 μM) was effective against other mutant glucocerebrosidases, including N188S, G202R, and N370S (each ∼2-fold in an ex vivo enzyme assay), but not against the D409H and L444P mutants.

The potent human β-galactosidase inhibitor **27** ($IC_{50} = 0.2$ μM) was evaluated for its chaperone activity in fibroblasts expressing R201C mutant β-galactosidase causing juvenile GM1-gangliosidosis (Table 6b).[35)] Incubation with 0.2 μM **27** resulted in the maximum enzyme activity (5.1-fold compared to without **27**) as well as intracellular reduction of the substrate GM1-ganglioside. The enhancement of activity was also observed in fibroblasts expressing other mutant β-galactosidases including R201H (4.5-fold), R457Q (2.4-fold), W273L (2.2-fold), and Y83H (2.0-fold). An animal study was also performed using GM1-gangliosidosis model mice expressing human R201C mutant β-galactosidase. The oral administration of **27** enhanced the mutant enzyme activity and decreased GM1-ganglioside levels in neuronal cells in the brain.

Computational analysis of the interaction of **26**[84)] and **27**[85)] with their target enzymes indicated that their binding free energies were higher at pH 5 (lysosome) than at pH 7 (ER) because of the reduced number of hydrogen bonds caused by protonation of functional residues in the enzyme active site. Thus, both **26** and **27** bind to the respective mutant enzymes stabilizing them in the ER and dissociate from them thus restoring their activity in the lysosome, which is consistent with the observed chaperone activity of **26** and **27**.

4.3. Practical synthesis of NOV and NOEV from quercitols. The initial synthesis of NOV (**26**) and NOEV (**27**) began with chiral resolution of the racemic Diels–Alder adduct of furan and acrylic acid **29** into (−)-**29** by crystallization with (+)-1-phenylethylamine (Fig. 3a), which is rather cumbersome and time-consuming and, moreover, is not atom-economical because the other enantiomer (+)-**29** is not used. In order to facilitate the advancement of carbaglycosylamine-based glycosidase inhibitors as well as pharmacological chaperones, a more practical synthesis from readily available chiral compounds was investigated.

Quercitol (deoxyinositol) has ten diastereoisomers: four meso and six chiral, of which (−)-*vibo*-(**155**), (+)-*proto*- (**156**) and (−)-*proto*-quercitols are found in nature.[86)] In 1999, Takahashi *et al.*[87)] reported the production of **155**, **156**, and (+)-*epi*-quercitol by fermentation of *myo*-inositol (**4**) with *Salmonella typhimurium*: after fermentation, the three quercitols were isolated and purified by ion-exchange column chromatography and recrystallization providing **155**, **156**, and (+)-*epi*-quercitol in 35%, 5%, and 11% yields, respectively (Fig. 15a). Taking advantage of the close resemblance of the absolute configuration of the hydroxy groups between **155** and β-D-glucopyranose and between **156** and β-D-galactopyranose (Fig. 15a, inset), in addition to their ready availability, **155** and **156** were chosen as the chiral starting materials for the practical synthesis of **26**[88)] and **27**,[89)] respectively.

The first step in the practical synthesis of **26** from **155** involves selective oxidation of the axial hydroxy group in **155**, which was achieved by means of bio-oxidation (Fig. 15b).[88)] Thus, an aqueous solution of **155** was incubated with *Gluconobacter*

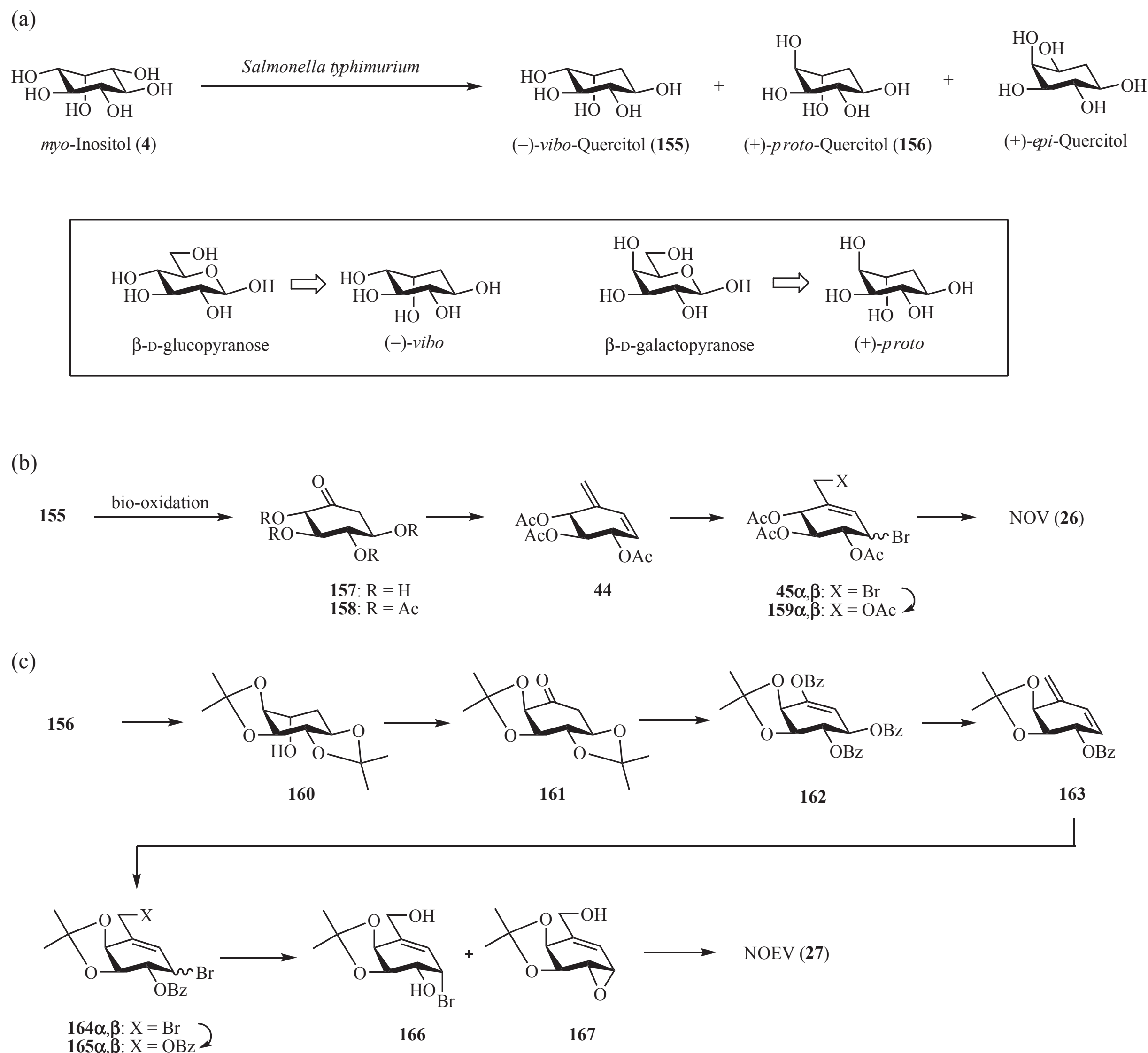


Fig. 15. Practical synthesis of *N*-octyl-β-valienamine (NOV) from (−)-*vibo*-quercitol and 4-*epi*-β-valienamine (NOEV) from (+)-*proto*-quercitol. (a) Bacterial conversion of *myo*-inositol to (−)-*vibo*-quercitol, (+)-*proto*-quercitol and (+)-*epi*-quercitol. Inset: Structural similarity between (−)-*vibo*-quercitol and β-D-glucopyranose and between (+)-*proto*-quercitol and β-D-galactopyranose. (b) Synthesis of NOV from (−)-*vibo*-quercitol. (c) Synthesis of NOEV from (+)-*proto*-quercitol.

sp. at ambient temperature for 1 day to give, after purification by cation- and anion-exchange chromatography, (−)-2-deoxy-*scyllo*-inosose (**157**)[90] in practically quantitative yield. Subsequent acetylation of **157** in acidic conditions was accompanied by β-elimination yielding the α,β-unsaturated ketone **158** in 83% yield. *exo*-Methylenation was then carried out using acidic Nysted reagent to afford the conjugated alkene **44** but in lower yield (25%). Due to the base sensitivity of **158**, conventional Wittig olefination was not applicable. From this point, the synthesis was able to follow the initial synthesis of **26**: **44** → **49** (Fig. 4b) and **49** → **26** (Fig. 13a). Here, a more straightforward approach[73] was used: after 1,4-dibromination of **44** to **45*α*,*β***, the primary bromide was replaced with acetate and the resulting mixture of allyl bromides **159*α*,*β*** was reacted with *n*-octylamine yielding **26** as a sole product in 31% yield. The reaction of **159*β*** likely proceeded via a cyclic acetoxonium ion.

For the practical synthesis of **27** from **156**, isopropylidenation of **156** was first carried out to give

the diisopropylidene derivative **160** (Fig. 15c).[89] The residual hydroxy group was subsequently oxidized with SO_3·Py/DMSO in the presence of Et_3N to provide the ketone **161** in 53% yield from **156**. After selective removal of the *trans*-*O*-isopropylidene group using slightly acidic conditions, benzoylation with benzoyl chloride afforded the enol ester **162** in 60% yield. Using the Wittig reaction conditions, **162** was successfully converted into the conjugated alkene **163** in 66% yield, probably through the following sequence of reactions: (1) debenzoylation of the enol ester by phosphorous ylide; (2) E1cB elimination of the β-benzoyloxy group forming the α,β-unsaturated ketone; and (3) reaction of the α,β-unsaturated ketone with the phosphorous ylide. Bromination of **163** with a slight excess of bromine (→ **164**$\boldsymbol{\alpha},\boldsymbol{\beta}$) followed by selective substitution of the primary bromide with benzoate gave a 1.7:1 mixture of the α- and β-bromides **165**$\boldsymbol{\alpha},\boldsymbol{\beta}$ in 91% yield. Debenzoylation of the mixture in basic conditions led to the formation of the α-bromide **166** and the α-epoxide **167** in 47% and 26% yield, respectively. Because the subsequent coupling reaction of **166** and **167** with *n*-octylamine yielded the same product,[89] the mixture of **166** and **167**, without isolation, was treated with *n*-octylamine followed by deisopropylidenation to give **27** as the sole product in 47% yield.

Recently, Li *et al.*[91] reported an efficient synthesis of **26** and **27** from naturally abundant shikimic acid with overall yields of 31% (14 steps) and 19% (17 steps), respectively.

4.4. *N*-Alkylconduramine F-4 derivatives as potential pharmacological chaperones for GM1-gangliosidosis. NOV (**26**) and NOEV (**27**) have shown promising chaperone activity, yet their potent inhibitory activity might be counterproductive. Therefore, special considerations are necessary to maximize the stabilizing effect and to minimize the inhibitory activity. Mena-Barragán *et al.*[92] have devised sp^2-iminosugar inhibitors bearing pH-sensitive hydrophobic tails by incorporating an orthoester linker; thus, they are strong inhibitors of human glucocerebrosidase or α-galactosidase at pH 7 (in the ER) but become non/less-inhibitory at pH 5 (in the lysosome) due to the loss of the hydrophobic tails, which are necessary for the inhibitory activity, by hydrolysis of the acid-labile orthoester linker.

Suzuki *et al.*[93] reported a comparative crystallographic analysis of human β-galactosidase in complex with **27** and sp^2-iminosugar-type inhibitors, revealing the major interactions of **27** with the enzyme: (1) the hydrogen bond interactions involving the 2-, 3- and 4-hydroxy groups are crucial for the specific binding to the enzyme; (2) the salt bridge between the exocyclic nitrogen and the catalytic Glu is also crucial for the binding and specificity; and (3) the hydrogen bond and hydrophobic interactions involving the 6-hydroxy and *n*-octyl groups, respectively, are important for the stabilization of the complex. Based on these findings, in order to reduce the inhibitory activity of **27** while maintaining a certain affinity to the enzyme for the chaperone activity, modification of the interactions that stabilize the complex appeared to be a rational approach. Therefore, the 6-deoxy and dehydroxymethyl derivatives of **27** (**170** and **175**, respectively) were first prepared to eliminate the hydrogen bond interaction involving the 6-hydroxy group.

The 6-deoxy derivative **170** was synthesized from a mixture of the 1,4-dibromides **164**$\boldsymbol{\alpha},\boldsymbol{\beta}$ (Fig. 16a).[89] Selective reduction of the primary bromide with $NaBH_4$ yielded the mono bromides **168**$\boldsymbol{\alpha}$ and **168**$\boldsymbol{\beta}$ in 68% and 23% yields, respectively. The α-bromide **168**$\boldsymbol{\alpha}$ was then debenzoylated to give **169** (51%), which was subjected to S_N2 replacement of the α-bromide by *n*-octylamine and subsequent de-*O*-isopropylidenation to afford **170** in 90% yield. The dehydroxymethyl derivative **175**, on the other hand, was synthesized from the di-*O*-isopropylidene derivative **160** (Fig. 16b).[38] Mesylation of the hydroxy group (→ **171**) followed by E2 elimination of the mesylate using DBU furnished the cyclohexenetetrol (known as conduritol F) derivative **172** in 76% yield. After selective removal of the *trans*-*O*-isopropylidene group with weak acid, the diol **173** thus obtained was treated with either Martin sulfurane or Mitsunobu reagent to give the α-epoxide **174** as the sole product in 69% and 59% yields, respectively. The epoxide ring opening with *n*-octylamine proceeded regio- and stereo-selectively to yield, after acidic removal of the isoproprylidene group, the hydrochloride of **175** (*N*-octyl-conduramine F-4) quantitatively.

The hydrochlorides of **27**, **170**, and **175** were evaluated for their inhibitory activity against human wild-type β-galactosidase, and their chaperone activity was assessed using fibroblasts expressing R201C mutant β-galactosidase (Table 7).[38] Both **170**, and **175** indeed showed much lower inhibitory activity than **27**; the IC_{50} values of **170** and **175** were 27- and 71-fold higher than that of **27**, respectively. The higher IC_{50} values of **175** than **170** may be attributed to the 5-methyl group in **170**, which might

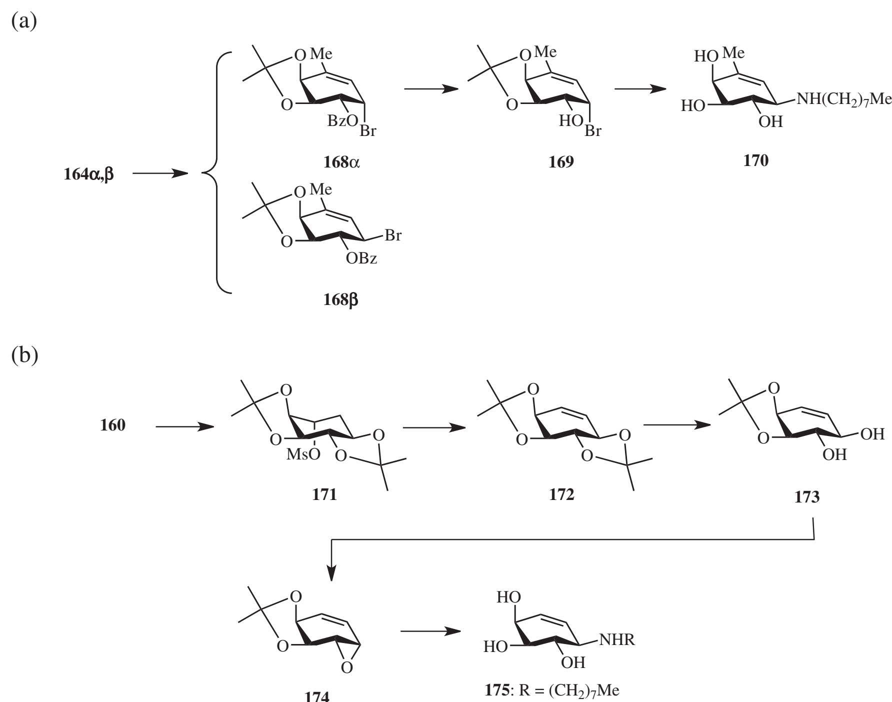


Fig. 16. Synthesis of 6-deoxy and 5-dehydroxymethyl derivatives of *N*-octyl-4-*epi*-β-valienamine (NOEV). (a) Synthesis of *N*-octyl-6-deoxy-4-*epi*-β-valienamine. (b) Synthesis of *N*-octyl-conduramine F-4.

Table 7. Inhibitory and chaperone activity of 6-deoxy and 5-dehydroxymethyl derivatives of NOEV (**170** and **175**, respectively) against wild-type and mutant human β-galactosidase

Compd	IC_{50} (µM) against wild type	Enhanced activity (fold) of the R201C mutant with **27** **170**, and **175**
27 HCl	1.7	3.6
170 HCl	46	5.2
175 HCl	120	5.4

be involved in a hydrophobic interaction with the enzyme. Notably, the chaperone activities of **170** and **175** were slightly higher than that of **27**.

Next, several *N*-alkyl derivatives of conduramine F-4 were prepared similarly to the *N*-octyl derivative **175** using the corresponding alkylamines (Table 8).[38] Because the strength of hydrophobic interactions depends on the size and shape of the hydrophobic molecules, different types of alkylamines were selected including various linear and branched amines.

Linear alkyl groups shorter or longer than C_8 led to slightly more active inhibitors than **175**; meanwhile, the chaperone activity increased gradually with the length of the alkyl chain up to C_8 (Table 8a). It should be noted that the C_{10} derivative showed high cytotoxicity to fibroblasts at 20 µM, probably due to its cationic surfactant character; therefore, linear alkyl groups longer than C_{10} were not investigated.

The binding affinity was significantly impaired when the linear alkyl group was replaced by either hydroxypropyl, 1-ethylpropyl, or cyclohexyl group (Table 8b). The hydrophilic hydroxypropyl group obviously did not form the hydrophobic interaction necessary for binding. On the other hand, both the 1-ethylpropyl and cyclohexyl groups are attached to the nitrogen atom through their secondary carbons; thus, they create more steric hindrance around the nitrogen, which may interfere with the formation of the salt bridge between the NH and the catalytic Glu. Indeed, reduction of the steric hindrance by insertion of a CH_2 group between the nitrogen and the secondary carbon, namely the *N*-2-ethylbutyl and *N*-cyclohexylmethyl derivatives **176** and **28**, respectively, restored the binding affinity (Table 8c). Of note, replacement of the terminal 3-pentyl group in **176** with an isopropyl group and the terminal cyclohexyl group in **28** with a phenyl group resulted in weaker inhibitory and chaperone activities, which

Table 8. Inhibitory and chaperone activity of various *N*-alkyl conduramine F-4 against wild-type and mutant human β-galactosidase

174 + RNH_2 ⟶ HO, HO, NHR, OH

R	IC_{50} (µM) against wild type	Enhanced activity (fold) of the R201C mutant with *N*-alkyl-conduramine F-4 derivatives (20 µM)
(a) *n*-Butyl	50	2.0
n-Pentyl	19	4.0
n-Hexyl	56	4.6
n-Octyl (**175**)	120	5.4
n-Decyl	43	1.6
(b) $(CH_2)_3OH$	>1000	0.9
$CHEt_2$	>1000	1.2
Cy	490	1.4
(c) CH_2CHEt_2 (**176**)	15	7.4
CH_2Cy (**28**)	60	8.5
CH_2CHMe_2	41	4.6
CH_2Ph	180	1.5
$(CH_2)_2CHMe_2$	28	4.6
$(CH_2)_2Ph$	86	4.2

were improved to some degree by insertion of an additional CH_2 group.

Among the *N*-alkyl derivatives synthesized, the *N*-cyclohexylmethyl-conduramine F-4 (**28**) showed the best activity profile for a potential pharmacological chaperone with moderate inhibitory activity ($IC_{50} = 60$ µM) and the highest chaperone activity (8.5-fold activity enhancement). In addition, the *N*-2-ethylbutyl derivative **176** had the second-best activity profile with IC_{50} of 15 µM and 7.4-fold activity enhancement. Further in vivo studies will be needed to confirm the promising chaperone activity of **28** as well as **176**.

5. Prospects

It is now well appreciated that carbohydrates are involved in cellular communication and interaction essential for physiological and pathological events. Despite the important role carbohydrates play, the molecular basis of carbohydrate-mediated recognition processes remains poorly understood. This is in part caused by their structural complexity and diversity, but it is also because their structures are, unlike those of nucleic acids and proteins, generated in a non-template driven manner by various glycosyltransferases and glycosidases. In this context, carbohydrate mimetics that selectively interfere with these carbohydrate-processing enzymes as well as carbohydrate-binding proteins are useful as molecular probes to understand the structure–function relationships of carbohydrates. Among the carbohydrate mimetics, carbasugars and their derivatives are of immense value because of their ability to mimic the oxocarbenium ion-like transition state of glycosidase-catalyzed hydrolysis in addition to the substrates of glycosyltransferases and the ligands of carbohydrate-binding proteins.

In the course of the total synthesis of naturally occurring *N*-linked carbaoligosaccharides, including the amylase inhibitor acarbose and related compounds, a general strategy has been established to link carbasugars to carbohydrates via an imino linkage by epoxide ring opening of either carbasugar epoxides with amino carbohydrates or carbohydrate epoxides with carbaglycosylamines. In addition, the synthesis of chiral carbasugars has been substantially improved with the ready availability of quercitols through the bioconversion of *myo*-inositol in conjunction with the well-established carbasugar synthesis from the Diels–Alder *endo*-adducts of furan and acrylic acid. This practical approach greatly facilitates the availability of carbasugars and their derivatives of interest, including carbaglycosylamines and *N*-linked carbaoligosaccharides, for their applications in glycobiology.

Several carbohydrate-processing enzymes and carbohydrate-binding proteins have been recognized as potential targets for therapeutic interventions. Therefore, carbasugars and their derivatives are also useful for the development of therapeutics against these targets. We were pleased to find the chaperone activity of the carbaglycosylamine-based transition-state analogue inhibitors NOV and NOEV; moreover, chemical modification of NOEV has led to the identification of *N*-cyclohexylmethyl-conduramine F-4 as a potential pharmacological chaperone for GM1-gangliosidosis related human lysosomal β-galactosidase.

Given their ready availability through synthesis, carbasugars and their derivatives now hold great promise for applications in glycobiology and therapeutics.

Acknowledgements

Our synthetic journey towards carbaglycosylamine glycosidase inhibitors described in this review

would not have been possible without the hard work and dedication of students as well as the valuable suggestions of colleagues and collaborators, whose names appear on the cited references. SO is particularly grateful to have had such outstanding students in his laboratory at Keio University.

When we started our synthetic journey, we had no idea it would lead to the development of potential pharmacological chaperones. The outcome of research is indeed unpredictable, but for this reason research is interesting and worth conducting. It is important to focus and execute your research with care and confidence and see what it leads to. Enjoy your journey!

References

1) Umezawa, S. (1974) Structures and syntheses of aminoglycoside antibiotics. Adv. Carbohydr. Chem. Biochem. **30**, 111–182.
2) Suami, T., Ogawa, S. and Umezawa, S. (1963) Studies on antibiotics and related substances. XVII. Syntheses of *trans*-2-aminocyclohexyl-D-glucosaminides. Bull. Chem. Soc. Jpn. **36**, 459–462.
3) Suami, T., Lichtenthaler, F.W. and Ogawa, S. (1966) Aminocyclitols. VIII. A synthesis of inosamines and inosadiamines. Bull. Chem. Soc. Jpn. **39**, 170–178.
4) Ogawa, S., Abe, T., Sano, H., Kotera, K. and Suami, T. (1967) Aminocyclitols. XIV. The synthesis of streptamine and actinamine. Bull. Chem. Soc. Jpn. **40**, 2405–2409.
5) Suami, T., Lichtenthaler, F.W., Ogawa, S., Nakashima, Y. and Sano, H. (1968) Aminocyclitls. XVII. A facile synthesis of 2-deoxystreptamine. Bull. Chem. Soc. Jpn. **41**, 1014–1017.
6) Ogawa, S., Rinehart, K.L., Jr., Kimura, G. and Johnson, R.P. (1974) Chemistry of the neomycins. XIII. Synthesis of aminocyclitols and aminosugars *via* nitromethane condensations. J. Org. Chem. **39**, 812–821.
7) Rinehart, K.L., Jr. and Schimbor, R.F. (1967) Neomycins. *In* Antibiotics. II. Biosynthesis (eds. Gottlieb, D. and Shaw, P.D.). Springer-Verlag, New York, pp. 359–372.
8) Rinehart, K.L., Jr. and Stroshane, R.M. (1976) Biosynthesis of aminocyclitol antibiotics. J. Antibiot. **29**, 319–353.
9) Shier, W.T., Rinehart, K.L., Jr. and Gottlieb, D. (1969) Preparation of four new antibiotics from a mutant of *Streptomyces fradiae*. Proc. Natl. Acad. Sci. U.S.A. **63**, 198–204.
10) Shier, W.T., Ogawa, S., Hichens, M. and Rinehart, K.L., Jr. (1973) Chemistry and biochemistry of the neomycins. XVII. Bioconversion of aminocyclitols to aminocyclitol antibiotics. J. Antibiot. **26**, 551–561.
11) Ogawa, S., Oki, S. and Suami, T. (1979) Inositol derivatives. 11. Synthesis of dianhydroinositols. Bull. Chem. Soc. Jpn. **52**, 1095–1101.
12) Ogawa, S. and Takagaki, T. (1987) Conversion of β-senepoxide to crotepoxide. Total synthesis of (+)-crotepoxide. Bull. Chem. Soc. Jpn. **60**, 800–802.
13) Iwasa, T., Yamamoto, H. and Shibata, M. (1970) Studies on validamycins, new antibiotics. I. *Streptomyces hygroscopicus var. limoneus nov. var.*, validamycin producing organism. J. Antibiot. **23**, 595–602.
14) Iwasa, T., Higashide, E., Yamamoto, H. and Shibata, M. (1971) Studies on validamycins, new antibiotics II. Production and biological properties of validamycins A and B. J. Antibiot. **24**, 107–113.
15) Iwasa, T., Kameda, Y., Asai, M., Horii, S. and Mizuno, K. (1971) Studies on validamycins, new antibiotics. IV. Isolation and characterization of validamycins A and B. J. Antibiot. **24**, 119–123.
16) Horii, S., Iwasa, T., Mizuta, E. and Kameda, Y. (1971) Studies on validamycins, new antibiotics. VI. Validamine, hydroxyvalidamine and validatol, new cyclitols. J. Antibiot. **24**, 59–63.
17) Kameda, Y. and Horii, S. (1972) The unsaturated cyclitol part of the new antibiotic, the validamycins. J. Chem. Soc. Chem. Commun. 746–747.
18) Suami, T. and Ogawa, S. (1990) Chemistry of carba-sugars (pseudo-sugars) and their derivatives. Adv. Carbohydr. Chem. Biochem. **48**, 21–90.
19) Kameda, Y., Asano, N., Yoshikawa, M., Takeuchi, M., Yamaguchi, T., Matsui, K. *et al.* (1984) Valiolamine a new α-glucosidase inhibiting aminocyclitol produced by *Streptomyces hygroscopicus*. J. Antibiot. **37**, 1301–1307.
20) McCasland, G.E., Furuta, S. and Durham, L.J. (1966) Alicyclic carbohydrates. XXIX. The synthesis of a pseudo-hexose (2,3,4,5-tetrahydroxycyclohexanemethanol). J. Org. Chem. **31**, 1516–1521.
21) McCasland, G.E., Furuta, S. and Durham, L.J. (1968) Alicyclic carbohydrates. XXXII. Synthesis of pseudo-β-DL-gulopyranose from a diacetoxybutadiene. Proton magnetic resonance studies. J. Org. Chem. **33**, 2835–2841.
22) McCasland, G.E., Furuta, S. and Durham, L.J. (1968) Alicyclic carbohydrates. XXXIII. Epimerization of pseudo-α-DL-talopyranose to pseudo-α-DL-galactopyranose. Proton magnetic resonance studies. J. Org. Chem. **33**, 2841–2844.
23) Schmidt, D.D., Frommer, W., Junge, B., Müller, L., Wingender, W., Truscheit, E. *et al.* (1977) α-Glucosidase inhibitors. New complex oligosaccharides of microbial origin. Naturwissenschaften **64**, 535–536.
24) Truscheit, E., Frommer, W., Junge, B., Müller, L., Schmidt, D.D. and Wingender, W. (1981) Chemistry and biochemistry of microbial α-glucosidase inhibitors. Angew. Chem. Int. Ed. Engl. **20**, 744–761.
25) Campbell, L.K., White, J.R. and Campbell, R.K. (1996) Acarbose: its role in the treatment of diabetes mellitus. Ann. Pharmacother. **30**, 1255–1262.
26) Fukuhara, K., Murai, H. and Murao, S. (1982)

Amylostatins, other amylase inhibitors produced by *Streptomyces diastaticus subsp. Amylostatics* No. 2476. Agric. Biol. Chem. **46**, 2021–2030.

27) Namiki, S., Kangouri, K., Nagate, T., Hara, H., Sugita, K. and Omura, S. (1982) Studies on the α-glucoside hydrolase inhibitor, adiposin. I. Isolation and physicochemical properties. J. Antibiot. **35**, 1234–1236.

28) Watanabe, K., Furumai, T., Sudoh, M., Yokose, K. and Maruyama, H.B. (1984) New α-amylase inhibitor, trestatins. IV. Taxonomy of the producing strains and fermentation of trestatin A. J. Antibiot. **37**, 479–486.

29) Omoto, S., Itoh, J., Ogino, H., Iwamatsu, K., Nishizawa, N. and Inouye, S. (1981) Oligostatins, new antibiotics with amylase inhibitory activity. II. Structures of oligostatins C, D, and E. J. Antibiot. **34**, 1429–1433.

30) Ogawa, S., Toyokuni, T. and Miyamoto, Y. (2019) Synthesis of *N*-linked carbaoligosaccharides: total synthesis of antibiotic validamycins and related compounds. *In* Studies in Natural Products Chemistry, Vol. 60 (ed. Rhaman, A.). Elsevier, Amsterdam, pp. 123–155.

31) Tsunoda, H., Inokuchi, J., Yamagishi, K. and Ogawa, S. (1995) Pseudosugars, 35. Synthesis of glucosylceramide analogs composed of imino-linked unsaturated 5a-carbaglycosyl residues: potent and specific gluco- and galactocerebrosidase Inhibitors. Liebigs Ann. Chem. **1995**, 279–284.

32) Ogawa, S., Ashiura, M., Uchida, C., Watanabe, S., Yamazaki, C., Yamagishi, K. *et al.* (1996) Synthesis of potent β-D-glucocerebrosidase inhibitors: *N*-alkyl-β-valienamines. Bioorg. Med. Chem. Lett. **6**, 929–932.

33) Ogawa, S., Kobayashi Matsunaga, Y. and Suzuki, Y. (2002) Chemical modification of the β-glucocerebrosidase inhibitor *N*-octyl-β-valienamine: synthesis and biological evaluation of 4-epimeric and 4-*O*-(β-D-galactopyranosyl) derivatives. Bioorg. Med. Chem. **10**, 1967–1972.

34) Ogawa, S. and Kanto, M. (2007) Synthesis of valiolamine and some precursors for bioactive carbaglycosylamines from (−)-*vibo*-quercitol by biogenesis of *myo*-inositol. J. Nat. Prod. **70**, 493–497.

35) Matsuda, J., Suzuki, O., Oshima, A., Yamamoto, Y., Noguchi, A., Takimoto, K. *et al.* (2003) Chemical chaperone therapy for brain pathology in G_{M1}-gangliosidosis. Proc. Natl. Acad. Sci. U.S.A. **100**, 15912–15917.

36) Ogawa, S. (2004) Design and synthesis of carbasugars of biological interest. Trends Glycosci. Glyc. **16**, 33–53.

37) Ogawa, S., Kanto, M. and Suzuki, Y. (2007) Development and medical application of unsaturated carbaglycosylamine glycosidase inhibitors. Mini Rev. Med. Chem. **7**, 679–691.

38) Kuno, S., Higaki, K., Takahashi, A., Nanba, E. and Ogawa, S. (2015) Potent chemical chaperone compounds for G_{M1}-gangliosidosis: *N*-substituted (+)-conduramine F-4 derivatives. MedChemComm **6**, 306–310.

39) Kuno, S. and Ogawa, S. (2016) From quercitols to biologically active valienamine and conduramine derivatives: development of pharmacological chaperones. Trends Glycosci. Glyc. **28**, E13–E22.

40) Suami, T., Ogawa, S., Nakamoto, K. and Kasahara, I. (1977) Synthesis of penta-*N*,*O*-acetyl-DL-validamine. Carbohydr. Res. **58**, 240–244.

41) Ogawa, S., Iwasawa, Y., Nose, T., Suami, T., Ohba, S., Ito, M. *et al.* (1985) Total synthesis of (+)-(1,2,3/4,5)-2,3,4,5-tetrahydroxycyclohexane-1-methanol and (+)-(1,3/2,4,5,)-5-amino-2,3,4-trihydroxycyclohexane-1-methanol [(+)-validamine]. *X*-ray crystal structure of (3*S*)-(+)-2-*exo*-bromo-4,8-dioxatricyclo[4.2.1.0^{3,7}]nonan-5-one. J. Chem. Soc., Perkin Trans. **1**, 903–906.

42) Ogawa, S., Nakamoto, K., Takahara, M., Tanno, Y., Chida, N. and Suami, T. (1979) Pseudo-sugars. 4. A facile synthesis of DL-validamine and its derivative. Bull. Chem. Soc. Jpn. **52**, 1174–1176.

43) Ogawa, S., Toyokuni, T., Omata, M., Chida, N. and Suami, T. (1980) Pseudo-sugars. 5. Synthesis of DL-validatol and DL-deoxyvalidatol, and their epimers. Bull. Chem. Soc. Jpn. **53**, 455–457.

44) Toyokuni, T., Abe, Y., Ogawa, S. and Suami, T. (1983) Synthetic studies on the validamycins. III. Bromination of DL-tri-*O*-acetyl-(1,3/2)-4-methylene-5-cyclohexene-1,2,3-triol. Preparation of several branched-chain unsaturated cyclitols related to valienamine. Bull. Chem. Soc. Jpn. **56**, 505–513.

45) Ogawa, S., Toyokuni, T., Ara, M., Suetsugu, M. and Suami, T. (1983) Synthesis and epoxidation of *trans*-5,6-diacetoxy-1-benzoyloxymethyl-1,3-cyclohexadiene. Bull. Chem. Soc. Jpn. **56**, 1710–1714.

46) Ogawa, S., Chida, N. and Suami, T. (1983) Synthetic studies on the validamycins. 5. Synthesis of DL-hydroxyvalidamine and DL-valienamine. J. Org. Chem. **48**, 1203–1207.

47) Atsumi, S., Umezawa, K., Iinuma, H., Naganawa, H., Nakamura, H., Iitaka, Y. *et al.* (1990) Production, isolation, and structure determination of a novel β-glucosidase inhibitor, cyclophellitol from *Phellinus sp.* J. Antibiot. **43**, 49–53.

48) Toyokuni, T., Ogawa, S. and Suami, T. (1983) Synthetic studies on the validamycins. IV. Synthesis of DL-valienamine and related branched-chain unsaturated amincyclitols. Bull. Chem. Soc. Jpn. **56**, 1161–1170.

49) Ogawa, S., Iwasawa, Y., Toyokuni, T. and Suami, T. (1985) Synthesis of a core structure of the antibiotic oligostatin. Carbohydr. Res. **144**, 155–162.

50) Stevens, C.L., Blumbergs, P., Daniher, F.A., Otterbach, D.H. and Taylor, K.G. (1966) Synthesis and chemistry of 4-amino-4,6-dideoxy sugars. II. Glucose. J. Org. Chem. **31**, 2822–2829.

51) Ogawa, S., Yasuda, K., Takagaki, T., Iwasawa, Y. and Suami, T. (1985) Synthesis of some analogues of methyl dehydro-oligobiosaminide (acarviosin). Carbohydr. Res. **141**, 329–334.

52) Buchanan, J.G. (1958) The behavior of derivatives of 3:4-anhydrogalactose towards acidic reagents. Part II. J. Chem. Soc. 2511–2516.

53) Ogawa, S., Uchida, C. and Shibata, Y. (1992) Alternative synthesis and enzyme inhibitory activity of methyl 1′-epiacarviosin and its 6-hydroxy analog. Carbohydr. Res. **223**, 279–286.
54) Shibata, Y., Ogawa, S. and Suami, T. (1990) Synthesis of methyl hexa-*O*-acethylacarviosin and the 6-acetoxy analogue. Carbohydr. Res. **200**, 486–492.
55) Ogawa, S., Sugizaki, H., Iwasawa, Y. and Suami, T. (1985) Synthesis of amylostatin-XG. Carbohydr. Res. **140**, 325–331.
56) Ogawa, S., Iwasawa, Y., Toyokuni, T. and Suami, T. (1985) Synthesis of adiposin-1 and related compounds. Carbohydr. Res. **141**, 29–40.
57) Mori, M., Haga, M. and Tejima, S. (1975) Synthesis of 4-*O*-α-D-galactopyranosyl-D-glucopyranose and 4-*O*-(6-deoxy-α-D-galactopyranosyl)-D-glucopyranose by chemical modifications of maltose. Chem. Pharm. Bull. **23**, 1480–1487.
58) Ogawa, S. and Shibata, Y. (1988) Total synthesis of acarbose and adiposin-2. J. Chem. Soc. Chem. Commun. 605–606.
59) Takeo, K., Mine, K. and Kuge, T. (1976) Synthesis of methyl α- and β-maltotriosides and aryl β-maltotriosides. Carbohydr. Res. **48**, 197–208.
60) Shibata, Y., Kosuge, Y., Mizukoshi, T. and Ogawa, S. (1992) Chemical modification of the sugar part of methyl acarviosin: synthesis and inhibitory activities of nine analogues. Carbohydr. Res. **228**, 377–398.
61) Ogawa, S., Shibata, Y., Kosuge, Y., Yasuda, K., Mizukoshi, T. and Uchida, C. (1990) Synthesis of potent α-glucosidase inhibitors: methyl acarviosin analogue composed of 1,6-anhydro-β-D-glucopyranose residue. J. Chem. Soc. Chem. Commun. 1387–1388.
62) Ogawa, S. and Aso, D. (1993) Chemical modification of the sugar moiety of methyl acarviosin: synthesis and inhibitory activity of eight analogues containing a 1,6-anhydro bridge. Carbohydr. Res. **250**, 177–184.
63) Pacák, J., Podešva, J., Točík, Z. and Černý, M. (1972) Syntheses with anhydo sugars. XI. Preparation of 2-deoxy-2-fluoro-D-glucose and 2,4-dideoxy-2,4-difluoro-D-glucose. Collect. Czech. Chem. Commun. **37**, 2598–2599.
64) Aleshin, A.E., Firsov, L.M. and Honzatko, R.B. (1994) Refined structure for the complex of acarbose with glucoamylase from *Aspergillus awamori* var. *X100* to 2.4-A resolution. J. Biochem. **269**, 15631–15639.
65) Kameda, Y., Asano, N., Yoshikawa, N., Matsui, K., Horii, S. and Fukase, H. (1982) *N*-Substituted valienamine, α-glucosidase inhibitors. J. Antibiot. **35**, 1624–1626.
66) Horii, S., Fukase, H., Matsuo, T., Kameda, Y., Asano, N. and Matsui, K. (1986) Synthesis and α-D-glucosidase inhibitory activity of *N*-substituted valiolamine derivatives as potential oral antidiabetic agents. J. Med. Chem. **29**, 1038–1046.
67) McAuliffe, J.C., Stick, R.V., Tilbrook, M.G. and Watts, A.G. (1998) The synthesis of a diastereoisomer of methyl acarviosin. Aust. J. Chem. **51**, 91–96.
68) Tilbrook, D.M.G., Stick, R.V. and Williams, S.J. (1999) β-Acarbose. VIII. The synthesis of some *N*-linked carba-oligosaccharides. Aust. J. Chem. **52**, 895–904.
69) Kiso, M., Nakamura, A., Tomita, Y. and Hasegawa, A. (1986) A novel route to D-*erythro*-sphingosine and related compounds from mono-*O*-isopropylidene-D-xylose or D-galactose. Carbohydr. Res. **158**, 101–111.
70) Zimmermann, P. and Schmidt, R.R. (1988) Synthese von *erythro*-sphingosinen über die azidoderivate. Liebigs Ann. Chem. 663–667.
71) Koike, K., Numata, M., Sugimoto, M., Nakahara, Y. and Ogawa, T. (1986) A highly stereoselective synthesis of 2(S), 3(R), 4E- and 2(S), 3(R), 4Z-*N*-tetracosanoylsphingenine from D-glucose. Carbohydr. Res. **158**, 113–123.
72) Ogawa, S., Hattori, T., Toyokuni, T. and Suami, T. (1983) Pseudo-sugars. X. Synthesis of several branched-chain cyclitols and their derivatives. Bull. Chem. Soc. Jpn. **54**, 2077–2081.
73) Ogawa, S., Sakata, Y., Ito, N., Watanabe, M., Kabayama, K., Itoh, M. *et al.* (2004) Convenient synthesis and evaluation of glycosidase inhibitory activity of α- and β-galactose-type valienamines, and some *N*-alkyl derivatives. Bioorg. Med. Chem. **12**, 995–1002.
74) Parkinson-Lawrence, E.J., Shandala, T., Prodoehl, M., Plew, R., Borlace, G.N. and Brooks, D.A. (2010) Lysosomal storage disease: revealing lysosomal function and physiology. Physiology **25**, 102–115.
75) Platt, F.M., d'Azzo, A., Davidson, B.L., Neufeld, E.F. and Tifft, C.J. (2018) Lysosomal storage diseases. Nat. Rev. Dis. Primers **4**, 27.
76) Convertino, M., Das, J. and Dokholyan, N.V. (2016) Pharmacological chaperones: design and development of new therapeutic strategies for the treatment of conformational diseases. ACS Chem. Biol. **11**, 1471–1489.
77) Sánchez-Fernández, E.M., Fernández, J.M.G. and Mellet, C.O. (2016) Glycomimetic-based pharmacological chaperones for lysosomal storage disorders: lessons from Gaucher, G_{M1}-gangliosidosis and Fabry diseases. Chem. Commun. **52**, 5497–5515.
78) Suzuki, Y. (2021) Chaperone therapy for molecular pathology in lysosomal diseases. Brain Dev. **43**, 45–54.
79) Fan, J.-Q., Ishii, S., Asano, N. and Suzuki, Y. (1999) Accelerated transport and maturation of lysosomal α-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. Nat. Med. **5**, 112–115.
80) McCafferty, E.H. and Scott, L.J. (2019) Migalastat: a review in Fabry disease. Drugs **79**, 543–554.
81) Ryckman, A.E., Brockhausen, L. and Walia, J.S. (2020) Metabolism of glycosphingolipids and their role in the pathophysiology of lysosomal storage disorders. Int. J. Mol. Sci. **21**, 6881.
82) Lin, H., Sugimoto, Y., Ohsaki, Y., Ninomiya, H., Oka, A., Taniguchi, M. *et al.* (2004) *N*-Octyl-β-

valienamine up-regulates activity of F213I mutant β-glucosidase in cultured cells: a potential chemical chaperone therapy for Gaucher disease. Biochim. Biophys. Acta **1689**, 219–228.

83) Lei, K., Ninomiya, H., Suzuki, M., Inoue, T., Sawa, M., Iida, M. *et al.* (2007) Enzyme enhancement activity of *N*-octyl-β-valienamine on β-glucosidase mutants associated with Gaucher disease. Biochim. Biophys. Acta **1772**, 587–596.

84) Jo, H., Yugi, K., Ogawa, S., Suzuki, Y. and Sakakibara, Y. (2010) Molecular basis of chemical chaperone effects of *N*-octyl-β-valienamine on human β-glucosidase in low/neutral pH conditions. J. Proteomics Bioinform. **3**, 104–112.

85) Suzuki, Y., Ogawa, S. and Sakakibara, Y. (2009) Chaperone therapy for neuronopathic lysosomal diseases: competitive inhibitors as chemical chaperones for enhancement of mutant enzyme activities. Perspect. Medicin. Chem. **3**, 7–19.

86) McCasland, G.E. (1965) Chemical and physical studies of cyclitols containing four or five hydroxyl groups. Adv. Carbohydr. Chem. **20**, 11–65.

87) Takahashi, A., Kanbe, K., Tamamura, T. and Sato, K. (1999) Bioconversion of *myo*-inositol to rare cyclic sugar alcohols. Anticancer Res. **19**, 3807.

88) Kuno, S., Takahashi, A. and Ogawa, S. (2011) Transformation of quercitols into 4-methylenecyclohex-5-ene-1,2,3-triol derivatives, precursors for the chemical chaperones *N*-octyl-4-*epi*-β-valienamine (NOEV) and *N*-octyl-β-valienamine (NOV). Bioorg. Med. Chem. Lett. **21**, 7189–7192.

89) Kuno, S., Takahashi, A. and Ogawa, S. (2013) Concise syntheses of potent chaperone drug candidates, *N*-octyl-4-*epi*-β-valinenamine (NOEV) and its 6-deoxy derivative, from (+)-*proto*-quercitol. Carbohydr. Res. **368**, 8–15.

90) Yamauchi, N. and Kakinuma, K. (1992) Biochemical studies on 2-deoxy-*scyllo*-inosose, an early intermediate in the biosynthesis of 2-deoxystreptamine I. Chemical synthesis of 2-deoxy-*scyllo*-inosose and [2,2-$^2H_2$]-2-deoxy-*scyllo*-inosose. J. Antibiot. **45**, 756–766.

91) Li, F.-L., Yu, J.-P., Ding, W., Sun, M.-M., He, Y.-G., Zhu, X.-L. *et al.* (2019) Novel stereoselective syntheses of *N*-octyl-β-valienamine (NOV) and *N*-octyl-4-*epi*-β-valienamine (NOEV) from (−)-shikimic acid. RSC Adv. **9**, 42077–42084.

92) Mena-Barragán, T., Narita, A., Matias, D., Tiscornia, G., Nanba, E., Ohno, K. *et al.* (2015) pH-Responsive pharmacological chaperones for rescuing mutant glycosidases. Angew. Chem. Int. Ed. **54**, 11696–11700.

93) Suzuki, H., Ohto, U., Higaki, K., Mena-Barragán, T., Aguilar-Moncayo, M., Mellet, C.O. *et al.* (2014) Structural basis of pharmacological chaperoning for human β-galactosidase. J. Biol. Chem. **289**, 14560–14568.

(Received Mar. 24, 2022; accepted June 2, 2022)

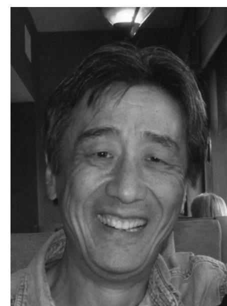
Profile

Seiichiro Ogawa was born in Tokyo in 1937. He graduated from the Department of Applied Chemistry at the Faculty of Engineering of Keio University in 1961 and received a Ph.D. from the same university in 1967. His Ph.D. research concerned the synthesis of the aminocyclitol units of aminocyclitol antibiotics. He then worked as a research fellow with Professor Kenneth L. Rinehart, Jr., at the University of Illinois Urbana-Champaign (1967–1968) and extended his Ph.D. work to develop a practical synthesis of ^{14}C-labeled 2-deoxystreptamine, which helped to elucidate the biosynthesis of neomycins. He was also an Alexander von Humboldt Foundation Fellow with Professor Frieder W. Lichtenthaler at Technische Hochschule Darmstadt (1973–1974), where he studied the synthetic potential of the sugar enolones in the preparation of various sugar derivatives. He was appointed as an Associate Professor in 1973 and promoted to a Full Professor in 1984 at Keio University. Upon his retirement in 2003, he became an Emeritus Professor. Throughout his career, his research has focused on the synthesis of biologically active cyclitol compounds and has contributed to the development of carbasugar derivatives of biochemical and biomedical importance.

Profile

Shinichi Kuno was born in Tokyo, Japan, in 1976. He graduated from International Christian University with a Bachelor of Liberal Arts in 2006 and then from the Graduate School of Science at the University of Tokyo with a Master of Science in 2008. His master's thesis was on mass analysis of sialyl glycosides and development of a chemical probe for identifying natural product-protein interactions. He subsequently joined an agrochemical and industrial chemical company, Hokko Chemical Industry Co., Ltd., and is working on several projects, including the design and synthesis of pharmacological chaperones. In 2012, he entered the Graduate School of Bioscience and Biotechnology at Tokyo Institute of Technology as a working student to study the phosphorescence of organic crystals and received his Ph.D. in Science in 2017. In 2021, he moved to Hokko Chemical America Corporation in the U.S.A. as general manager. His research interest focuses on organic compounds with bioactive or photoactive properties.

Profile



Tatsushi Toyokuni, a retired professor from the University of California, Los Angeles (UCLA), received his Ph.D. in chemistry in 1982 from Keio University under the supervision of Professor Tetsuo Suami and Professor Seiichiro Ogawa, working toward the total synthesis of validamycin A. He subsequently undertook postdoctoral research with Professor Kenneth L. Rinehart, Jr., at the University of Illinois Urbana-Champaign, where he studied the biosynthesis of validamycins identifying that the validamine and valienamine units are derived from the pentose phosphate pathway. In 1987, he was recruited to the Biomembrane Institute, Seattle, WA, as Head of the Organic Chemistry Department and Affiliated Associate Professor of Chemistry at the University of Washington, and led the institute's chemistry projects to help understand the relationship between the cell membrane and the cause of diseases, including the development of fully synthetic vaccines based on tumor-associated carbohydrate antigens, the development of anti-cell adhesion molecules based on cell-surface carbohydrates, and the synthesis of sphingosine derivatives to study the role of sphingolipids in cell signaling. Following the closure of the institute in 1996, he joined the faculty of the Department of Molecular and Medical Pharmacology, UCLA School of Medicine. There, in addition to his continued interest in synthetic glycobiology, his research focused on the design and synthesis of novel radiopharmaceuticals (F-18 labelled and Cu-64 labelled compounds) for positron emission tomography imaging in collaboration with the Nuclear Medicine faculty.