Synthesis and Biological Activity of Natural Aminocyclopentitol Glycosidase Inhibitors: Mannostatins, Trehazolin, Allosamidins, and Their Analogues

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I. Introduction

A. General

The essential role played by oligosaccharides in the proper functioning of living organisms is now well-established.[1] It is thus not surprising that a number of natural and synthetic inhibitors of glycosidases, enzymes implicated in the biosynthesis of these oligosaccharides, have been found to induce far-reaching biological effects.[2] The promise that such effects might be exploited to advantage in medicine and agriculture has encouraged a sustained quest for more potent and specific inhibitors,[3] and screening,

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hand in hand with creative chemical design, has in recent years seen the discovery of an impressive number of mono- and polysaccharide sugar mimics which behave as glycosidase inhibitors.[4]

The exquisite structural diversity manifest in natural glycosidase inhibitors is bewildering,[2,4] especially when one considers that glycosidases themselves often share very similar mechanisms of action. This variety is clearly manifest in the mannostatins **1** and **2**, trehazolin **3**, and the allosamidins **4** (Chart 1), which together constitute yet another class of glycosidase inhibitor and are the subject of this review. Discovered about a decade ago, their structures are conspicuous by the *absence* of the polyhydroxy azasugar motif typical of better known glycosidase inhibitors such as nojirimycin[5] and castanospermine[6] (Chart 1) and are instead characterized by an ami-

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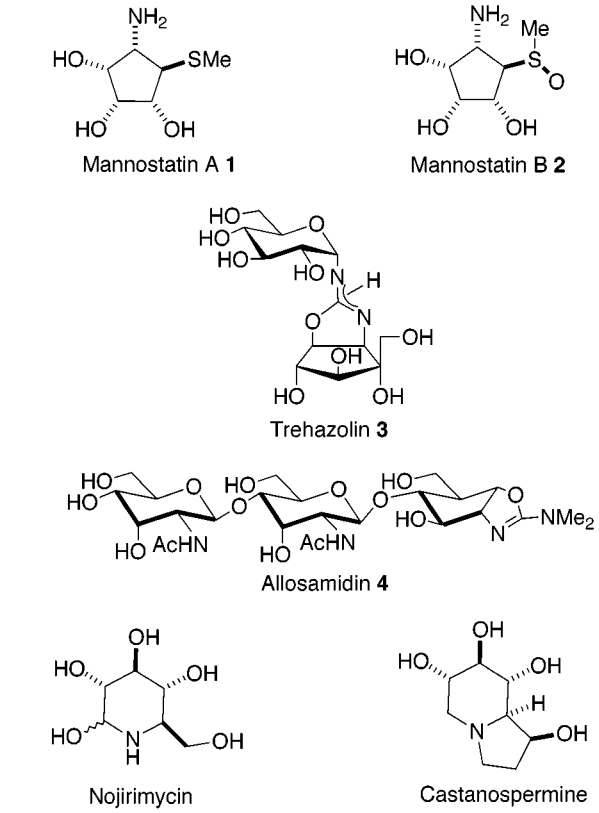
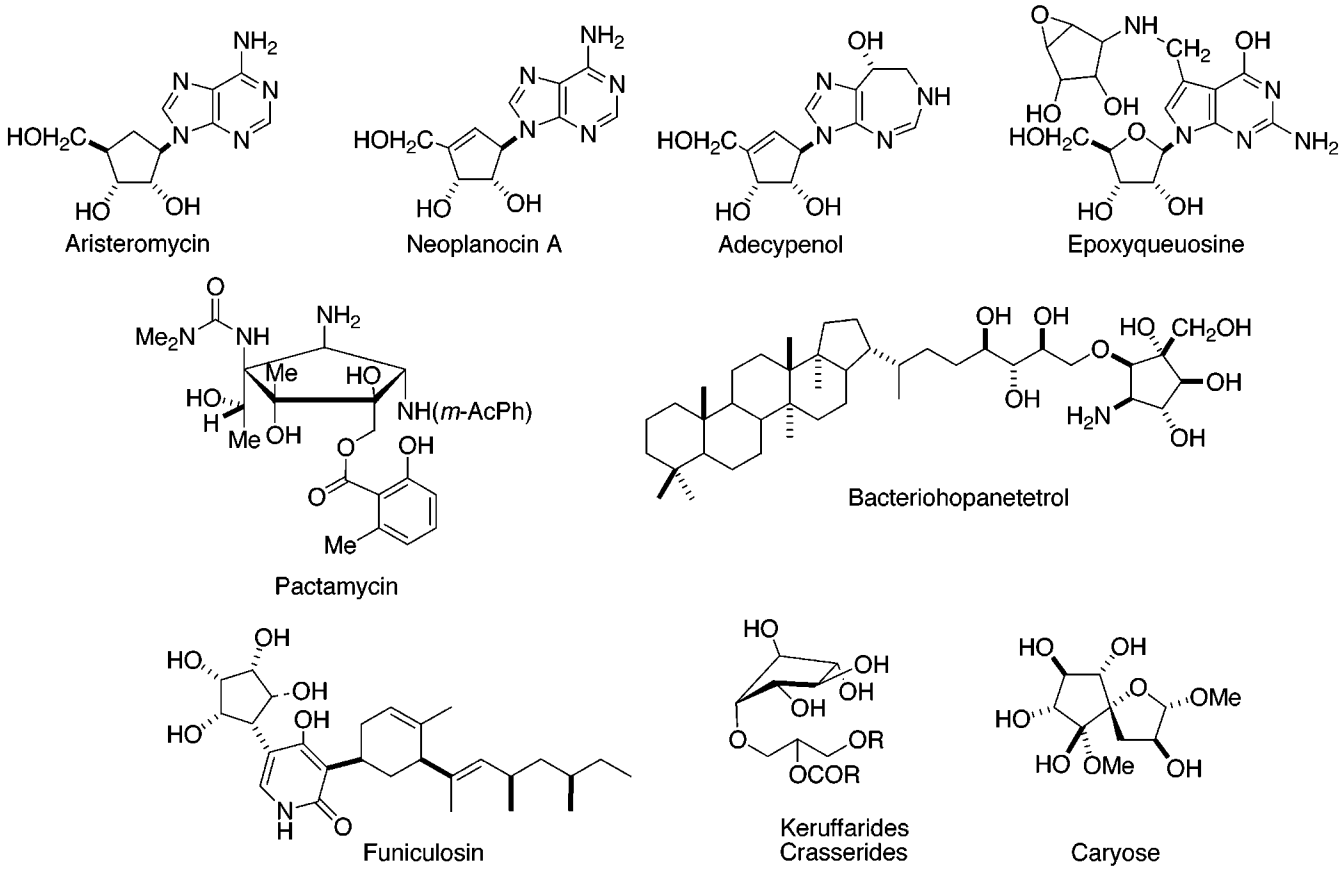
Chart 1



nocyclopentitol moiety. Other polyhydroxycyclopentitol-containing natural products, of which there are relatively few, include pactamycin,[7] bacteriohopanetetrol,[8a] funiculosin,[8b] epoxyqueuosine,[8c] the keruffarides[9a] and crasserides,[9b] caryose,[10] and the carbocyclic

Chart 2



nucleosides aristeromycin,[11] neoplanocin A,[12] and adecypenol[13] (Chart 2).

In addition, the title compounds possess other novel structural features, especially with respect to glycosidase inhibitors, including the methyl thioether and methylsulfenyl functions present in mannostatins and the aminooxazoline moiety which trehazolin and the allosamidins share.

B. Synthesis of Aminocyclopentitol Glycosidase Inhibitors: Overview

The chemical syntheses of densely functionalized aminocyclopentitols, the key structural motif shared by the title compounds, form the nucleus of this review article. Even prior to knowledge of their existence in nature, a number of chemical routes to cyclopentitols had already been studied and developed.[14] In fact, a racemic synthesis of aristeromycin, the first natural cyclopentitol reported, preceded its isolation![15] However, progress in this field had lagged well behind that of their cyclohexitol counterparts until the discovery of natural aminocyclopentitols such as the carbocyclic nucleosides. The synthesis of such nucleosides attracted considerable additional interest following the outbreak of the AIDS epidemic,[16] and the discovery of the aminocyclopentitol glycosidase inhibitors has seen another flourish of activity in the area of aminocyclopentitol synthesis. The endeavors of chemists have been motivated as much by the challenges posed by the syntheses of the title compounds as by an interest in their biological properties. Total synthesis has also helped in confirming the structures of these novel natural products and most notably in establishing the correct structure of trehazolin (see Section III.B.2).

The majority of strategies adopted to obtain aminocyclopentitols can be divided broadly into two groups. The first of these, and the most important historically speaking, is that which comprises carbohydrate-based approaches which can themselves be classified according to the method chosen to form the carbocyclic ring, of which there are three: aldol-like reactions, 1,3-dipolar cycloaddition reactions, and radical reactions.

Aldol-type routes are by far the oldest known chemical methods by which to synthesize polyhydroxy carbocycles.[17] Interestingly, in the biosynthesis of cyclopentitols an aldol condensation is also employed to construct the carbocycle (Section I.C). The dialdehyde−nitromethane cyclization, for example, has been applied to cyclopentitols and studied now for over 30 years.[14] As the dialdehydes in question are derived from *myo*-inositol, the reaction is in effect a ring fragmentation−contraction of a cyclohexitol. Inspite of the fact that racemic mixtures are obtained, this strategy has been exploited in the synthesis of mannostatins, trehazolin, and numerous analogues (e.g., Sections II.B.2 and III.B.2). Another aldol-like reaction involves an intermediate enolate (or enol complex) generated from a ribonolactone-derived 5-*exo*-enol ether to give an enone,[18] which has subsequently served in routes to mannostatin and trehalamine (e.g., Sections II.B.3, II.B.4, and III.B.4).

1,3-Dipolar cycloaddition reactions, which give cyclopentitol−isoxazolidenes via intramolecular trapping of 5-enal-derived nitrones by alkenes, were originally developed with the synthesis of compounds such as natural carbocyclic nucleosides in mind (see Section IV.E.1).[19] The reaction was shown to proceed with good stereoselectivity and in high yield and together with its sister reaction—the [2 + 3] cycloaddition reaction which gives cyclopentitol−isoxazolines from 5-enal-derived nitrile oxides[20]—has been taken advantage of in syntheses of allosamizoline, trehalamine, and their analogues (e.g., Sections III.B.1, IV.C.5, IV.C.7, and IV.C.8).

Early studies on carbohydrate-derived 5-oximinoalkyl radicals were intended *simply* to study their chemistry (Section IV.E.1).[21] These species were found to cyclize to aminocyclopentitols selectively and in high yield and not to suffer the problem of β-scission observed in analogous reactions of 5-oxoalkyl radicals. The stage had been set even before their discovery, and free radical-based strategies have been used in routes to mannostatin, trehalamine, allosamizoline, and their analogues (e.g., Sections II.C.4, III.C.3 and IV.C.6). With the introduction of samarium(II) iodide as a convenient single-electron-transferring agent, ketyl radical cyclizations of carbohydrates have been much studied. The intramolecular reductive cross-coupling of a carbonyl with an oxime ether group, promoted by either tributyltin hydride or samarium(II) iodide, has provided very short routes to trehazolin and its analogues (e.g., Section III.C.3). The related intramolecular reductive coupling of a ketone tethered to an aldehyde (acetal) has also been used to give an allosamizoline-related analogue, but this reaction requires that the necessary nitrogen functionality is in place prior to the cyclization or is introduced later (e.g., Sections IV.E.1).

The second broad category of approaches to natural aminocyclopentitols is that which comprises methods entailing the functionalization of a preformed but 'naked' unsaturated cyclopentane framework. In fact, at least one-third of all reported syntheses of the title compounds fall into this group. This second category can be divided further into methods which involve desymmetrization of substituted cyclopentene-4-*meso*-diols and those involving heterocycloadditions of cyclopentadienes.

The classical method for desymmetrization of *meso*-diols is that using enzymes, and it had already found use[16] in approaches to prostaglandins and carbocyclic nucleosides before being exploited in the synthesis of allosamizoline. However, the enantiotopic sense of the enzymatic hydrolysis of the starting *meso*-acetate originally used for the synthesis of allosamizoline was found at some considerable cost to be opposite to that expected, underscoring the inherent danger of enzymatic desymmetrizations. The error was however discovered and corrected, and thereafter this approach formed the basis of several enantioselective routes to allosamizoline and its analogues (e.g., Sections IV.C.2 and IV.C.4).

Alternatively, enantioselective transition-metal-catalyzed alkylations of *meso*-diols[22] can be used to secure desymmetrization, although they are much less well developed than some related enantioselective transition-metal-catalyzed reactions. The synthesis of racemic allosamizoline and racemic mannostatin, as well as several of their isomers, has been achieved using a desymmetrization of this type, and an enantioselective version has also been suggested to be feasible (e.g., Sections II.B.5 and IV.C.1).

Electrophilic additions of hypohalous acids to *meso*-cyclopentenes have been shown to proceed with good stereoselectivity and have been developed in a route to racemic allosamizoline (Section IV.C.3).

Diels−Alder reactions have been relatively less used than the corresponding [3 + 2] cycloaddition reactions in syntheses of polyhydroxycyclopentitols. However, the asymmetric [4 + 2] heterocycloaddition of a substituted cyclopentadiene and an acylnitroso dienophile has been exploited in an elegant enantioselective synthesis of mannostatin A (Section II.B.1). It should be noted that the heterocycloaddition of singlet oxygen to substituted cyclopentadienes followed by reduction in situ of the transient endoperoxide afford the corresponding *meso*-diols, and these have provided the starting materials for several syntheses, especially of allosamoziline and its analogues (Section IV.C). In addition, the related but relatively little studied [4 + 2] heterocycloaddition of singlet oxygen to fulvene has been applied to a synthesis of trehazolin (Section III.B.5).

Other strategies which have been called upon for syntheses of the title compounds but lie outside the above classification include one which takes advantage of an unprecedented enantioselective spirocyclization in a rather original synthesis of trehazolin from cyclopentadiene (Section III.B.3) and another involving an efficient ring fragmentation−contraction, of a carbohydrate precursor, in a route to allosamizoline (Section IV.C.10).

The approaches used for the coupling of aminocyclopentitol and sugar components, in syntheses of the trisaccharide inhibitor allosamidin and its analogues, serve to highlight the scope and limitations of the available methods of glycosidation[23] (see Section IV.D). Danishefsky's 'sulfonamido glycosylation method' was *specifically* conceived and developed to tackle this problem in the total synthesis of allosamidin: a classic example of the way in which the art of 'total synthesis' can serve as an inspiration for the creation and development of new *general* methods and concepts in organic synthesis (Section IV.D.1).

C. Biosynthesis: Overview

Details of the biosynthesis of the title compounds, except in the case of allosamidin (Section IV.B), have thus far not been unravelled. The biosynthesis of cyclopentitols has in general been rather neglected, and recent work in connection with carbocyclic nucleosides has indeed seen revisions to previously proposed routes.[24] The studies related to allosamizoline have shown it to derive from D-glucose but by a mechanism different from that proposed for the biosynthesis of carbocyclic nucleosides, and it would be interesting to learn by which route the mannostatins and trehazolin are constructed. There have as yet been no details published of how the compo-

nent fragments of trehazolin or the allosamidins are assembled biosynthetically.

D. Biological Activity: Overview

The differences in structure of natural glycosidase inhibitors reflects, among other things, the marked substrate specificity of most glycosidases and also the subtle and intriguing nuances in the mode of action of each member of this large class of enzymes.[25] These structural peculiarities obviously confer (or at one time did confer) important biological advantages on the organism responsible for the biosynthesis of a given inhibitor: The ability of an organism to potently and *discriminately* inhibit a given enzyme could serve to defend it against a pathogenic species, and this advantage would be compounded if the inhibitor was more effective than that of a competitor. Perhaps not surprisingly we have sought to exploit this same defense strategy to our advantage. For example, neither of the natural substrates of trehalases or chitinases (trehalose and chitin, respectively) occur in vertebrates, and this has fuelled the hope that pests and pathogens might be targeted using inhibitors of this type, while at the same time avoiding toxicity to mammals and higher plants (see Sections III.D and IV.F). Furthermore, the fact that mannostatins can disrupt glycoprotein biosynthesis places them in the company of other "trimming enzyme" inhibitors which have attracted attention as potential antiviral agents[2a,5] (Section II.D). Consequently, much energy has been expended in synthesizing analogues of mannostatins, trehazolin, and allosamidins, which might display improved biological activities relative to the lead molecules.

The structures of natural inhibitors give valuable insights into the mode of action of the enzymes they inhibit, and the clues thus gleaned can serve as an important guide in the design of analogues, some of which are very novel.[4,25a] Many inhibitors can be considered as stable mimics of the enigmatic and—*all important*—short-lived intermediates stabilized at the transition states of the enzymic hydrolysis reaction.[26] For example, both pentacyclic polyhydroxy azasugars and sulfonium salts are better inhibitors of certain glycosidases than their six-membered counterparts, and this has been attributed to the closer resemblance of the five-membered ring compounds to the putative carbocationic intermediates implicated in the enzymatic hydrolysis reaction.[4,25a] That the title compounds possess an analogous five-membered cyclic motif suggests that the aminocyclopentitol moiety is, indeed, of particular significance for their inhibitory activity. However, the substrate specificities and modes of action of the enzymes inhibited by the mannostatins, trehazolin, and the allosamidins are quite distinct, and it follows that the precise mechanism by which each inhibits is not identical. This is strikingly exemplified in the allosamidins and trehazolin, in which the aminooxazoline moiety that they have in common interacts in very different ways with the catalytic machinery of the enzymes they inhibit (see Sections IV.F and III.D) and highlights nicely the resourcefulness of nature. Moreover, whereas mannostatin A is monomeric and effectively only resembles part of the polymeric substrate (or intermediate) it mimics, it is nonetheless very selective. It inhibits the Golgi mannosidase II in preference to the Golgi mannosidase I, even though the natural substrates for these enzymes are very similar (see Section II.D). This selectivity is seen to be achieved rather economically in mannostatins when compared to the allosamidins and trehazolin, which bear a much closer structural relationship to the respective polymeric and dimeric substrates (or intermediates) they mimic.

E. Scope of Review

Although the main thrust of this review concerns the chemical syntheses of the mannostatins, trehazolin, and allosamidins, syntheses of aminocyclopentitols related to the natural products will also be covered, even when these were not originally conceived as analogues. An account of syntheses of cyclopentitols is however beyond the scope of this review[27] (e.g., the interesting $[Cp_2Zr(n\text{-}Bu)_2]$-initiated ring contraction introduced by Taguchi and coworkers[28]). The synthesis of glycosidase inhibitors comprising the polyhydroxycyclohexitol motif will not be touched upon either.[14b,29]

The biological activity of the title compounds and their analogues will be treated in depth, only with reference to their inhibition of glycosidases. The possible modes of inhibition of each natural product will also be considered (Sections II.D, III.D, and IV.F). The inhibition data is relatively extensive and is presented, together with other pertinent biological data, in tabulated form.

The article seeks to draw together work published in the area up until the end of 1997 but will exclude altogether the patent literature. A handful of relevant publications have appeared during 1998, and these are discussed in the Addendum (Section V).

II. Mannostatins

A. Isolation and Elucidation of Structure

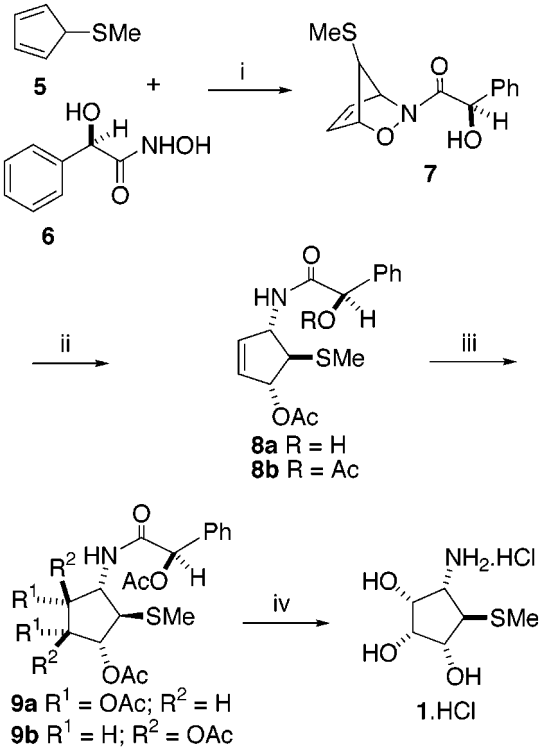
Mannostatins A (**1**) and B (**2**) (Chart 1) were discovered by Aoyagi and collaborators[30] in 1989 and so named because of their marked inhibition of α- and β-mannosidase activity. They were isolated from the soil microorganism *Streptoverticillium verticillus* var. *quintum*, during a screening of culture broths for mannosidase inhibitors. Their novel aminocyclopentitol structures were proposed on the basis of their spectroscopic characteristics and that of mannostatin B tetraacetate, established by X-ray diffraction analysis.[31]

B. Total Synthesis

1. Ganem's Approach

King and Ganem's enantioselective synthesis[32] employed as the key step an asymmetric heterocycloaddition which served to fix three of the five chiral centers in mannostatin A (**1**) (Scheme 1). Thallous cyclopentadienide, when exposed to methylsulfenyl chloride in carbon tetrachloride, gave the known 1-(methylthio)cyclopenta-2,4-diene (**5**). The crude diene was treated with a chiral arylnitroso dienophile generated from (*R*)-mandelohydroxamic acid on oxidation with *tert*-butylammonium periodate. Internal

Scheme 1[a]



[a] Reagents: (i) Bu_4NIO_4, MeOH, 0 °C, 30−35% from MeSCl; (ii) a, Al−Hg, THF−H_2O, b, Ac_2O, pyridine−DMAP, 41%; (iii) a, 1.5 equiv OsO_4, pyridine, 20 h, 74%, b, Ac_2O, pyridine−DMAP; (iv) HCl−MeOH, 60 °C, 65%.

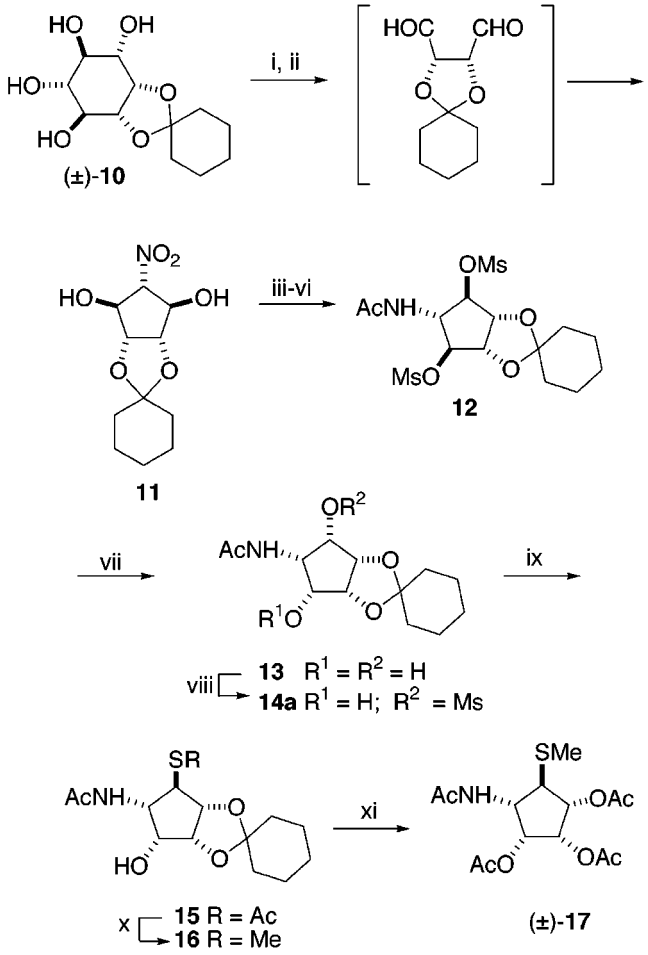
H-bonding in the arylnitroso component was proposed to account for the observed stereocontrol which gave the required diastereomer as the major adduct. Direct *syn*-hydroxylation of the bicyclic Diels−Alder adduct **7** was unsuccessful; thus the N−O bond was reductively cleaved, and the alcohol **8a** thereby obtained was acetylated to give the monocyclic derivative **8b**. Vicinal hydroxylation of this intermediate proved feasible using stoichiometric osmium tetraoxide in pyridine and, moreover, proceeded with the marked facial selectivity expected for bis-allylically substituted cyclopentenes. Acetylation of the mixture produced a 20:1 ratio of tetraacetates **9a** and **9b**, separable by chromatography. Hydrolysis of **9a** gave mannostatin A (**1**), as its hydrochloride. This synthesis required 6 steps and gave an overall yield of 12%.

Ganem and King attempted to obtain mannostatin B (**2**) by direct oxidation of mannostatin A (**1**) with 1 equiv of dimethyldioxirane, but this only gave an inseparable 1:1 mixture of the natural product **2** and its isomeric sulfoxide. Alternatively, the fully protected mannostatin A **9a**, carrying the chiral handle, could be oxidized with periodate, but this again gave a mixture of sulfoxides. This diastereomeric mixture could be separated by chromatography although the conditions employed to hydrolyze these amides led once again to their epimerization and gave an inseparable 1:1 mixture of mannostatin B (**2**) and its isomeric sulfoxide.

2. Ogawa's Approach

Ogawa and Yuming[33] chose to start from *myo*-inositol to obtain racemic mannostatin A (**1**). The key step is a formal ring contraction using a base-catalyzed

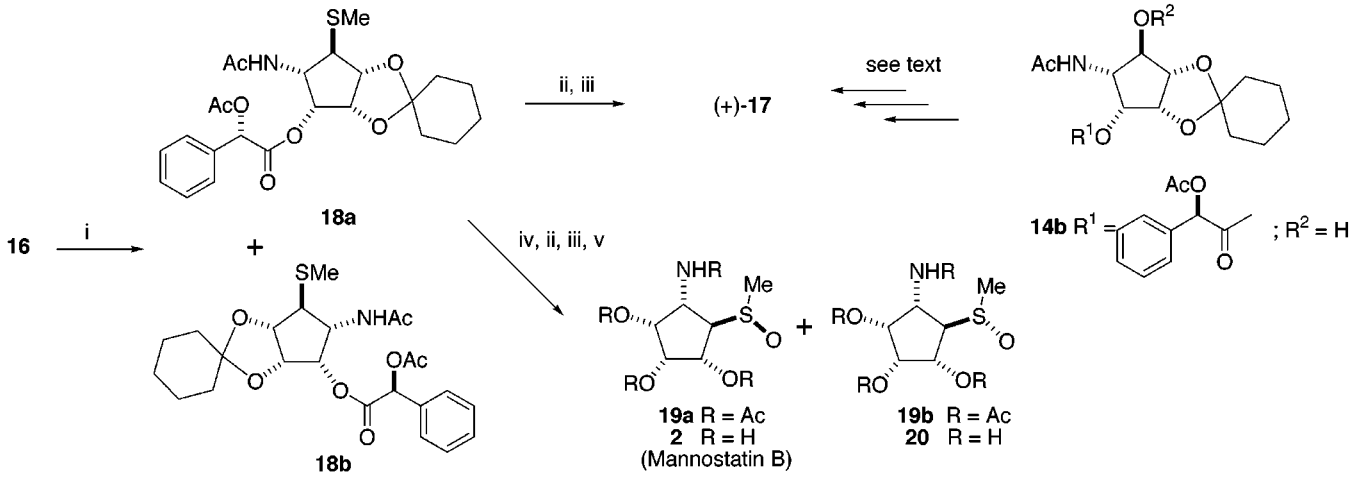
Scheme 2[a]



[a] Reagents: (i) $NaIO_4$, 3.5 h, 0 °C; (ii) $MeNO_2$, MeONa−MeOH, 20 h, 0 °C, then AcOH, pH 4, 60%; (iii) glacial acetic acid, H_2, Adams's catalyst, 24 h; (iv) Ac_2O, pyridine; (v) MeOH, NH_3, 12 h, 61%; (vi) MsCl, pyridine, 12 h, 62%; (vii) 5 equiv AcONa, 80% aq 2-methoxyethanol, 3 h, reflux; (viii) $MeSO_2Cl$, pyridine, 50 min, 0 °C, 31%; (ix) 10 equiv KSAc, DMF, 50 min, 120 °C; (x) MeONa−MeOH, 0 °C, then MeI, MeOH; (xi) 80% aq AcOH, 15 h, 100 °C, then Ac_2O, pyridine, 13.4%.

nitromethane cyclization of the dialdehyde derived from the starting sugar (Scheme 2). Periodate oxidation of (±)-1,2-*O*-cyclohexylidene-*myo*-inositol (**10**) (also used by these same authors in the synthesis of trehazolin (**3**); see Section III.B.2) and reaction of the resulting dialdehyde with nitromethane in the presence of methanolate gave the cyclopentitol derivative **11**. This was transformed in 4 steps as previously published[34] into the dimesylate **12** which on treatment with sodium acetate gave the diol **13**. Displacement of the mesylate groups occurred with inversion and was evidently *not* prone to any neighboring group participation of the 3-acetamido group. Monomesylation gave the desired mesylate **14a** in about 46% yield together with the dimesylate (8%) and unreacted starting material. The monomesylate **14a** underwent decomposition when heated with potassium thioacetate at 120 °C for long periods, but after 1 h the thioacetate **15** could be isolated with unreacted starting material in yields of 20% and 78%, respectively. Base-catalyzed *S*-deacetylation of the crude thioacetate **15** followed by *S*-alkylation gave, quantitatively, the methylthio derivative **16**. The racemic tetra-*N*,*O*-acetyl derivative **17** of mannosta-

Scheme 3[a]



[a] Reagents: (i) 1.2 equiv (*S*)-acetylmandelic acid, DMAP, DCC, −15 °C, CH_2Cl_2; (ii) 1 N HCl, 100 °C; (iii) Ac_2O, DMAP, pyridine; (iv) $NaIO_4$, MeOH, 5 h, 0 °C; (v) NaOH, 100 °C, 20 min.

tin A was finally obtained after acidic cleavage of the cyclohexylidene protecting group followed by acetylation of the intermediate triol. The synthesis was achieved in 10 steps starting from 1,2-cyclohexylidene-*myo*-inositol with an overall yield of 0.9%.

These same authors[35a] disclosed an enantioselective synthesis of mannostatin A (**1**) and mannostatin B (**2**) starting from key intermediate **16** (Scheme 3). Transformation of the racemic mixture of alcohols **16** into its corresponding diastereomeric (*S*)-*O*-acetylmandelyl esters (**18a** and **18b**) allowed their resolution by simple separation by column chromatography. The correct diastereomer **18a** was treated with aqueous hydrogen chloride and the intermediate triol peracetylated to give the tetra-*N*,*O*-acetyl derivative **17** of (+)-mannostatin A.

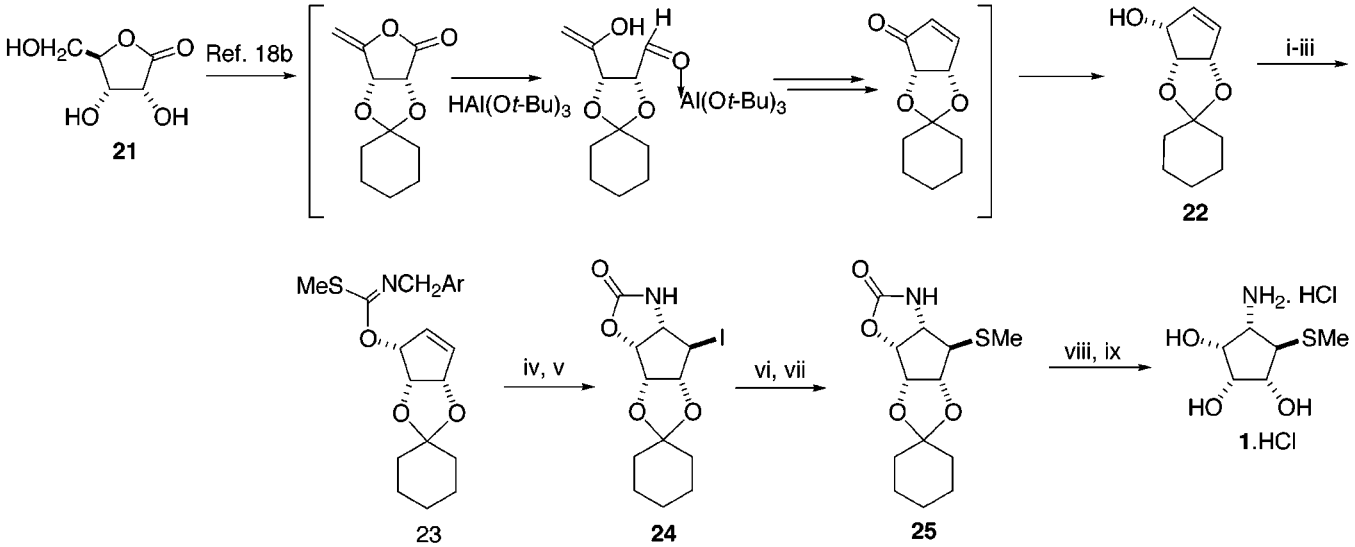
Ogawa and co-workers[35b] have described yet another synthesis of (+)-mannostatin by a modification of their original racemic synthesis. Thus, esterification of diol **13** with (*R*)-(−)-acetylmandelic acid gave predominantly the monoester **14b** (Scheme 3). This could easily be separated, taken through to the (+)-mannostatin tetraacetate derivative **17**, and then through to the natural product, by a route similar to that described (Scheme 2) in their racemic synthesis.

Mannostatin B (**2**) has also been obtained[35a] from mandelate ester **18a** (Scheme 3). Thus, the ester **18a** was oxidized with sodium metaperiodate to give a mixture of sulfoxides which was subjected to acid hydrolysis and per-*O*-acetylation to give the desired tetra-*N*,*O*-acetyl derivative **19a** in 35% yield together with its diastereomer **19b** (39%). Hydrolysis of the mixture with concentrated aqueous sodium hydroxide afforded, after ion-exchange chromatography, pure mannostatin B (**2**) in 90% yield as well as its diastereomer **20**.

3. Knapp's Approach

Knapp and Dhar[36] have disclosed a synthesis of mannostatin A (**1**) using the cyclopentenol **22** (derived from D-ribonolactone (**21**) in 6 steps and 47% overall yield[18b]) as a key intermediate (Scheme 4).

Scheme 4[a]



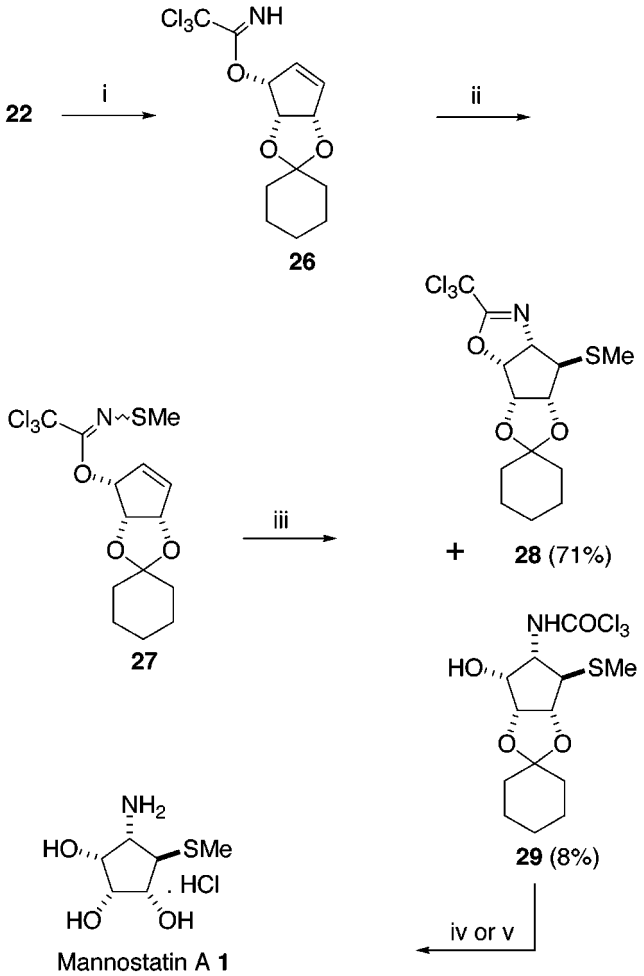
[a] Reagents: (i) NaH, THF; (ii) $ArCH_2NCS$; (iii) CH_3I; (iv) I_2, THF, sieves, 3 h; (v) aq Na_2SO_3, 12 h, 85% from **14**; (vi) CAN, aq MeCN, 1 h, 92%; (vii) NaSMe, DMF, 2 h, 90%; (viii) 2 N KOH, reflux, 2 h; (ix) 6 N HCl, 10 h, 95%.

Reaction of the sodium salt of allylic alcohol **22** with *p*-methoxybenzyl isothiocyanate and alkylation with iodomethane of the intermediate gave the imidothioate **23**. Iodocyclization proceeded smoothly in the presence of iodine to give the bicyclic oxazolidinone **24** expected from a *trans*-alkene addition. Oxidative removal of the *N*-*p*-methoxybenzyl group followed by nucleophilic substitution of the iodide group with sodium methylmercaptide in DMF gave the methylthio derivative **25**. The authors suggested that the retention of configuration observed in this reaction was due to participation by the adjacent nitrogen. Attack of the nucleophile from the less hindered face via an S_N1 substitution is a plausible alternative mechanism. Subsequent removal of the protecting groups gave mannostatin A (**1**) as its hydrochloride salt. This synthesis was accomplished in 15 steps from D-ribonolactone with an overall yield of 32%.

4. Fuchs' Approach

Li and Fuchs[37] sought to improve the synthesis from D-ribose of mannostatin A (**1**) described by Knapp and Dhar (see Section II.B.3) by introducing the nitrogen and sulfur functions simultaneously through use of an *N*-sulfenylimidate (Scheme 5). The cyclopentinol **22** was converted into its trichloroacetimidate **26**. The latter reacted with methylsulfenyl chloride to give the required intermediate *N*-sulfenylimidate **27** but this failed to cyclize to the desired oxazoline **28** even when exposed to a number of relatively forcing conditions. However, methylsulfenyl trifluoromethanesulfonate was found to be sufficiently electrophilic to add to the double bond thereby initiating the regiospecific attack of the *N*-sulfenyl trichloroacetimidate group, to give the required oxazoline **28** together with the trichloroacetamide **29** in a combined yield of 79%. Simultaneous *N*-sulfenylation and cyclization on exposure of the trichloroacetimidate **26** to methylsulfenyl trifluoromethanesulfonate did occur but in poor yield, and the authors favored the two-step method. Treatment of intermediate **28** or **29** with acidic methanol gave mannostatin A (**1**) as its hydrochloride salt in yields of 97% and 90%, respectively. This synthesis was performed in 10 steps with a 39% overall yield.

Scheme 5[a]

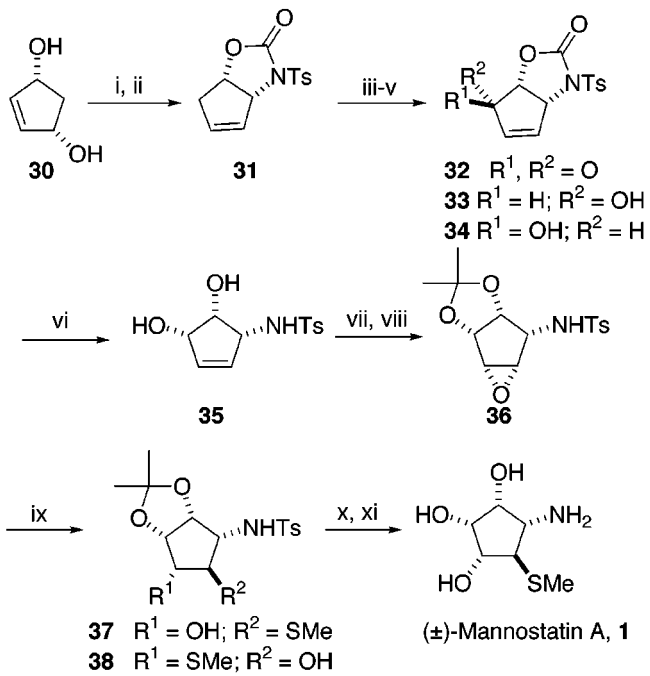


[a] Reagents: (i) NaH, Cl_3CCN, CH_2Cl_2, −10 to −25 °C; (ii) 3.4 equiv $(^iPr)_2NEt$, 3.3 equiv Me_3SOTf, CH_2Cl_2, −72 to 0 °C, 94%; (iii) 1.5 equiv $(^iPr)_2NEt$, 3.5 equiv Me_3SOTf, CH_2Cl_2, −72 to 20 °C; (iv) **28** to **1**·HCl, 7 N HCl−MeOH (1:1), 25 °C, 97%; (v) **29** to **1**·HCl, 7 N HCl−MeOH (1:1), 60−80 °C, 90%.

5. Trost's Approach

Trost and Van Vranken[38] have explored the feasibility of synthesizing racemic mannostatin A (**1**) via vinyloxazolidin-2-ones derived from cyclopentadiene, using Trost's asymmetric organopalladium reaction as the cornerstone of their approach (see also Section IV.C.1). The starting material is cyclopent-2-ene-1,4-diol (Scheme 6). The synthesis began with a tetrakis(triisopropyl phosphite)palladium-catalyzed ionization−cyclization reaction of *meso*-cyclopent-2-ene-1,4-

Scheme 6[a]



[a] Reagents: (i) 2 equiv TsNCO, THF, 60 °C, 2 h; (ii) $[(^iPrO)_3P]_4$-Pd(0), reflux, THF, 35 min, 97%; (iii) SeO_2, diglyme, Na_2HPO_4, reflux 5 h, rt, 3 h; (iv) 4 equiv Dess−Martin periodinane, CH_2Cl_2, 1.6 h, 65%; (v) 1 equiv $CeCl_3$, MeOH, EtOAc, −5 °C, 1 equiv $NaBH_4$, 5 min, 82.9%; (vi) K_2CO_3, MeOH, 18 h, 95%; (vii) 10-CSA, dimethoxypropane, acetone, 25 min, 93%; (viii) Na_2HPO_4, CF_3CO_3H, CH_2Cl_2, 75%; (ix) MeSLi, THF, −78 °C to rt, 9 h, **37** (78%) and **38** (17%); (x) Na/NH_3, −78 °C, 97%; (xi) 60% aq TFA, 60 °C, 6.5 h, 85%.

diol (**30**) with toluenesulfonyl isocyanate. The oxazolidinone **31** thus obtained was oxidized first with selenium dioxide, to give an allylic alcohol (together with some enone **32**), and then with bicarbonate-buffered Dess−Martin periodinane, to achieve complete conversion to the enone **32**. This mild oxidation procedure proved to be more satisfactory than a number of others examined. Reduction of the enone **32** under Luche conditions gave a mixture (7:1) of two alcohols **33** and **34**, the one required in 81% yield. Other methods gave poorer results. The stereochemical assignment of the major epimer, thus formed, was confirmed by its facile rearrangement into a cyclic carbonate upon treatment with base.

Attempted hydroxysulfenylation met with limited success, although a similar strategy was later exploited by Li and Fuchs in their synthesis of mannostatin A (**1**) (see Section II.B.4). Thus, the authors envisaged a diastereoselective *syn*-epoxidation as an alternative strategy. Hydrolysis of the carbamate **33** with methanolic potassium carbonate gave the free diol **35** which on treatment with buffered peroxytrifluoroacetic acid gave, exclusively, the corresponding *syn*-epoxide (not shown). The authors noted that the same selectivity was observed when epoxidation was carried out directly on the bicyclic oxazolidin-2-one **33**, thus confirming that even a single *syn*-directing group is sufficient to direct the diastereofacial selectivity, to the concave face of the bicyclic substrate **33**. The most problematic step in the synthesis proved to be the opening of the epoxide, which proceeded in low yield and gave the undesired regioisomer under a number of conditions. In an attempt to remedy this problem, which was pared down to the conformational flexibility of five-membered substrates relative to their six-membered counterparts, the authors chose to convert the substrate **35** into a rigid, bicyclic analogue, in the hope that this would favorably influence the regioselectivity of epoxide opening. Thus, the thermodynamic ketalization of the *cis*-diol **35** and its subsequent epoxidation provided the expected oxirane **36**. Exposure of this intermediate to lithiomethoxide in THF gave as the major product the mannostatin derivative **37** in 78% yield together with its regioisomer **38**. Deprotection of the major derivative **37** gave free racemic mannostatin A (**1**) which was characterized as its fully acetylated derivative.

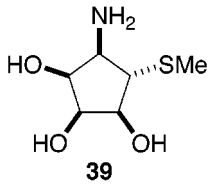
This racemic synthesis of mannostatin A (**1**) was completed in 10 steps in an overall yield of 27%. An enantioselective version was shown, in principle, to be feasible using the same strategy. Thus, performing the initial cyclization/ionization reaction with a chiral palladium catalyst gave the oxazolidinone **31** with 88% ee, but this result was not pursued further.

C. Synthesis: Analogues

1. Enantiomers and Sulfoxide Isomers

Knapp and Dhar[36] obtained the enantiomer **39** of mannostatin A (Chart 3) by applying the synthetic sequence developed for the synthesis of mannostatin A (**1**) to the enantiomer of the allylic alcohol **22** (see Section II.B.3, Scheme 4).

Chart 3

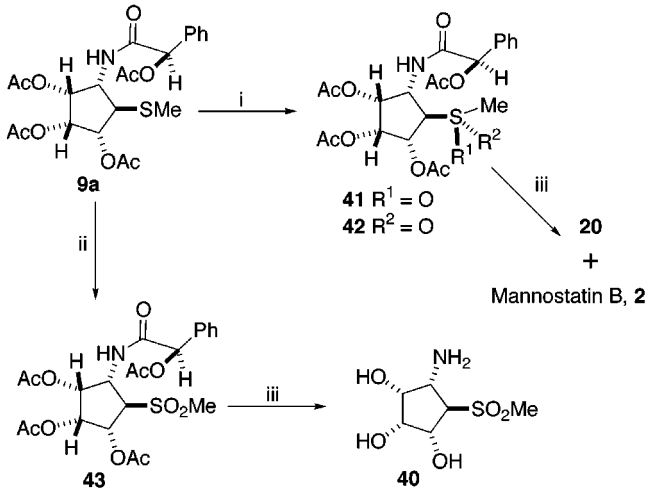


39

Ogawa and Yuming[35] also obtained compound **39** and the isomeric sulfoxide **20** of mannostatin B by resolving racemic **16**, a late intermediate in their total synthesis of mannostatin A (**1**), via the corresponding (*S*)-*O*-acetylmandelates **18a** and **18b** (see Section II.B.2, Scheme 3). Ogawa and co-workers[35b] have described another synthesis of enantiomer **39** by a modification of their original racemic synthesis of the natural product (see Section II.B.2, Schemes 2 and 3). Esterification of diol **13** with (*S*)-(−)-acetylmandelic acid gave predominantly the required monoester which could be easily separated and taken through to the mannostatin enantiomer **39**, by a route similar to that described (Schemes 2 and 3) in their racemic synthesis.

King and Ganem[39] have also obtained the unnatural (*S*)-sulfoxide **20** of mannostatin, which they expected to show better binding to mannosidases than the natural (*R*)-isomer **2**. They argued further that, as some variation in the size of the substituent at C-1 might be tolerated at the reaction center, the sulfone **40** should be targeted as a potential inhibitor (Scheme 7). Thus, exhaustive oxidation of thiomethyl

Scheme 7[a]



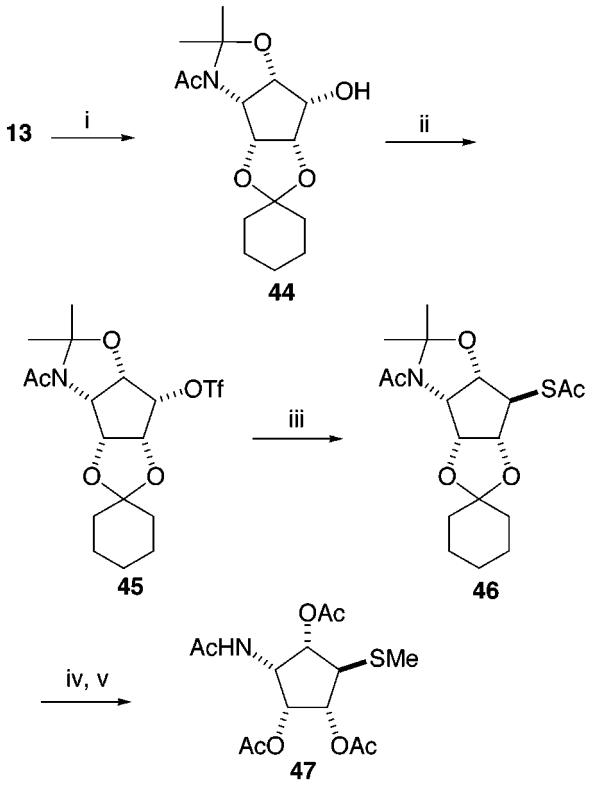
[a] Reagents: (i) $NaIO_4$, **41**/**42** (1:1), 93%; (ii) 2 equiv dimethyldioxirane, acetone, 95%; (iii) 6 N HCl−MeOH, reflux, 2−3 h, 95%.

derivative **9a**, followed by hydrolysis of the resulting protected amide **41**, **42** or **43** with methanolic hydrogen chloride, gave the corresponding sulfoxide **20** and sulfone **40** in a combined yield of 95%.

2. Deoxy, Oxo, Epi, and Positional Analogues

Ogawa and co-workers[33] have reported the synthesis of the 4,5-regiomeric analogue **47** of mannostatin A from the advanced intermediate **13** (see Section II.B.2, Scheme 2) obtained during their total synthesis of mannostatin A (**1**) (Scheme 8). Treatment of intermediate diol **13** with 2,2-dimethoxypropane in

Scheme 8[a]

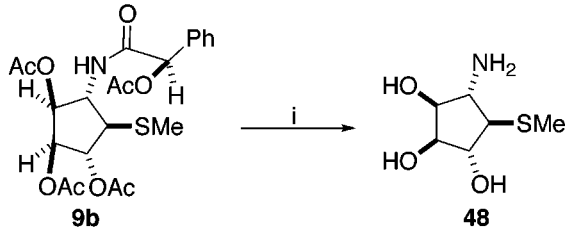


[a] Reagents: (i) $Me_2C(OMe)_2$, *p*-TsOH·H_2O, DMF, 18 h, 60 °C; (ii) $(CF_3SO_2)_2O$, $ClCH_2CH_2Cl$−pyridine 1:5, 15 min, 0 °C; (iii) 5 equiv KSAc, DMF, 5 min, rt; (iv) MeONa−MeOH, 0 °C, then MeI, MeOH; (vi) 80% aq AcOH, 15 h, 100 °C; Ac_2O, pyridine.

DMF and an acidic catalyst saw *N*,*O*-isopropylidene formation with concomitant migration of the cyclohexylidene acetal group, to give only one *N*,*O*-isopropylidene derivative **44**. The latter derivative was transformed into its triflate **45**, which on reaction with potassium thioacetate gave, with inversion, the thio derivative **46**. This was subjected to dethioacetylation, *S*-alkylation, and per-*O*-acetylation, under the usual conditions, to give the target 4,5-regioisomer **47**.

King and Ganem[39] obtained the 3,4-bis-*epi*-analogue **48** (Scheme 9) from the per-*O*-acetylated start-

Scheme 9[a]

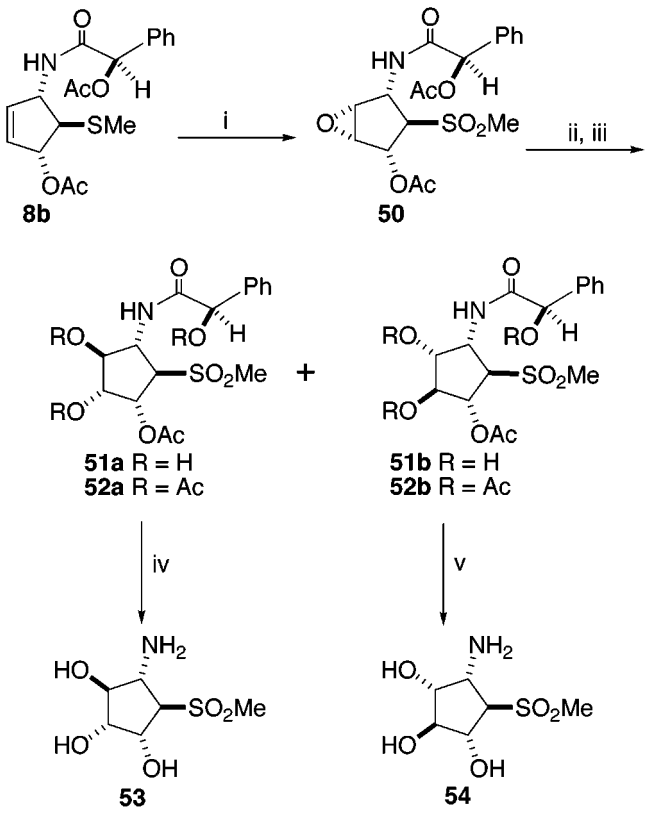


[a] Reagents: (i) 6 N HCl−MeOH, reflux, 2−3 h, 77%.

ing material **9b** (see Section II.B.1), a minor product of an osmylation step in their total synthesis of mannostatin A (**1**). Acid methanolysis of the chiral handle proved difficult for isomer **9b** requiring 5 days as opposed to 1 day needed for the other isomer **9a**, precursor of mannostatin A (**1**). This difference was considered to be due to the impossibility of any anchimeric assistance in the case of the former isomer. Nevertheless, more vigorous conditions saw its smooth conversion into 3,4-bis-*epi*-mannostatin A (**48**) in 77% yield.

The 4-*epi*- and 3-*epi*-mannostatin sulfones (**53** and **54**, respectively) were obtained by Ganem and King[39] via the cyclopentene **8b** (an intermediate in their total synthesis). The authors argued that epimerization at position 4 of mannostatin A (**1**) would give a "*gluco*" analogue, and this 4-*epi*-mannostatin analogue **54** was expected to show glucosidase inhibition on account of its resemblance to glucopyranosylamine (Scheme 10). Thus, exposure of alkene **8b** to 3−4

Scheme 10[a]

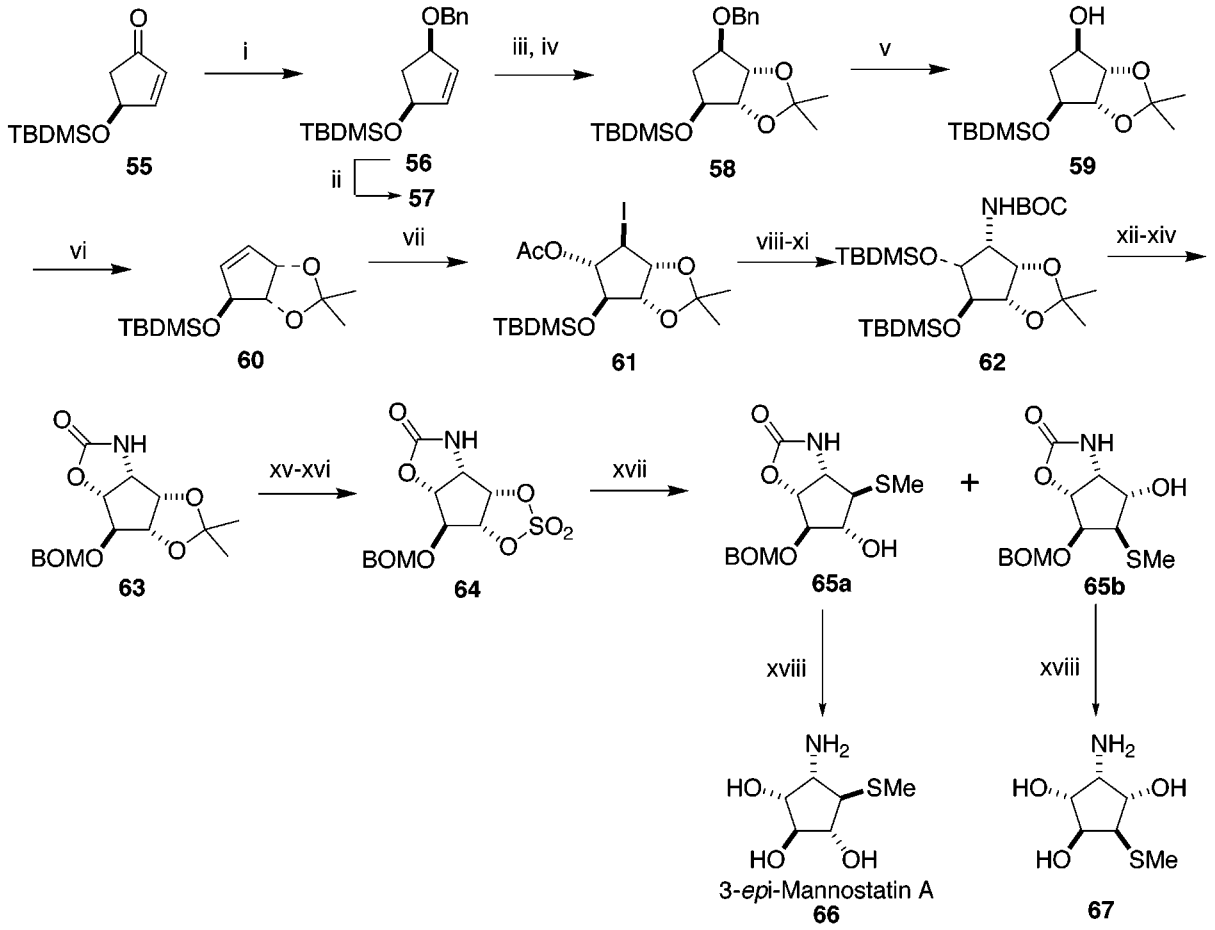


[a] Reagents: (i) 3−4 equiv dimethyldioxirane, acetone, 97%; (ii) 10 equiv TFA, 65 °C, 3 days; (iii) Ac_2O, pyridine; (iv) 6 N HCl, reflux, 3 h, 82%; (v) 0.36 N HCl−MeOH, 14 h, 65 °C, 34%.

equiv of dimethyldioxirane resulted in its oxidation, with complete *syn*-selectivity, to a single epoxy sulfone **50**. As expected for epoxides flanked by electron-withdrawing functions, hydrolysis proved difficult. The use of aqueous trifluoroacetic acid at elevated temperature, although slow and nonregioselective, gave clean *trans*-opening of the epoxide. The resulting triols **51a** and **51b** could be separated with difficulty by HPLC, after acetylation. However, only compound **52b** gave its amine when treated with dilute aqueous acid in methanol. The 3-*epi*-mannostatin isomer **54** was obtained in this way after purification by ion-exchange chromatography. More vigorous conditions (6 N HCl, reflux for 3 h) resulted in hydrolysis of the other isomeric amide **52a** to give the target 4-*epi*-mannostatin sulfone (**53**).

Nishimura and collaborators[40] have synthesized 3-*epi*-mannostatin (**66**), its enantiomer **72**, and their respective positional isomers **67** and **73** (Schemes 11

Scheme 11[a]

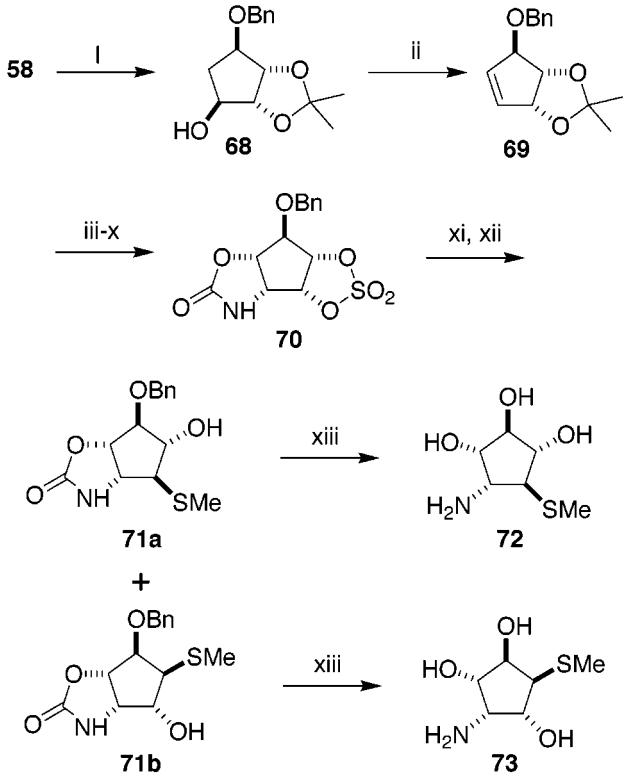


[a] Reagents: (i) DIBALH, benzene, 90%; (ii) BnBr, NaH, Bu_4NI, THF, 98%; (iii) AgOAc, I_2, wet AcOH then MeONa, MeOH, 83%; (iv) $Me_2C(OMe)_2$, CSA, CH_2Cl_2, 95%; (v) H_2, Pd/C, MeOH, 100%; (vi) PPh_3, DEAD, benzene, 72%; (vii) AgOAc, I_2, benzene, 90%; (viii) NH_3–MeOH, 89%; (xi) TBDMSCl, imidazole, DMF, 82%; (x) NaN_3, DMSO; (xi) H_2, Pd/C, AcOEt–MeOH; (^{t}BuOCO)$_2$O, EtN^iPr$_2$, CH_2Cl_2, 79%; (xii) Bu_4NF, THF, 86%; (xiii) NaH, DMF, 89%; (xiv) BOMCl, EtN^iPr$_2$, DMF, 58%; (xv) aq AcOH, 82%; (xvi) $SOCl_2$, pyridine, CH_2Cl_2 then $RuCl_3$, $NaIO_4$, CCl_4, MeCN–H_2O, 73%; (xvii) NaSMe, DMF; (xviii) H_2SO_4, H_2O–THF; $Ba(OH)_2$, H_2O; HCl–dioxane.

and 12), using an enantiodivergent strategy starting from (*S*)-4-[(*tert*-butyldimethylsilyl)oxy]-2-cyclopentenone (**56**). They argued that 1,4-dideoxy-1,4-imino-D-mannitol (see Section II.D, Chart 5), a mannosidase inhibitor, and 1,4-dideoxy-1,4-imino-D-arabinitol, a glucosidase inhibitor, owe their marked difference in selectivity to their difference in configuration at only one stereocenter. Thus, they contended that as mannostatin A (**1**) and 3-*epi*-mannostatin (**66**) share a related difference in configuration, the 3-*epi*-analogue **66** should by analogy show activity as a glucosidase inhibitor. [40b]

Diisobutylaluminum hydride reduction of cyclopentenone **55** (Scheme 11) gave alcohol **56** which was protected as its benzyl ether **57**. Treatment with iodine, silver acetate, and wet acetic acid of the cyclopentene **57**, followed by *O*-deacetylation and reprotection of the newly introduced *cis*-hydroxy function by an *O*-isopropylidene group, provided the pivotal, differentially protected tetrol **58**. Catalytic hydrogenation gave the debenzylated derivative **59**, which smoothly eliminated under Mitsunobu conditions to provide the allylic derivative **60**. Exposure of this allylic alcohol to silver acetate and iodine saw its conversion into the iodide **61**. Protective group manipulation followed by displacement of iodide by azide and hydrogenolysis saw the introduction, with the correct stereochemistry, of the amino function which was protected as its *tert*-butyloxycarbonyl derivative to give carbamate **62**. The authors encountered grave difficulties in chemoselectively removing the isopropylidene group, a necessary prerequisite to introduction of the required thio group. An alternative protecting group strategy was adopted in order to increase the conformational rigidity of the cyclopentane skeleton and thereby to resolve the problem. The *O*-silyl groups when removed induced cyclic carbamate formation, and the remaining hydroxyl group was protected as its benzyloxymethyl ether (BOM) derivative **63**. Chemoselective acid hydrolysis of the isopropylidene group then proved feasible, and the corresponding diol thus obtained was activated as its cyclic sulfate **64** by sequential reaction with thionyl chloride in pyridine and ruthenium tetroxide oxidation. The key intermediate **64** was subjected to nucleophilic opening with sodium mercaptide which proceeded smoothly to give two regiomeric thioethers **65a** and **65b** which were obtained in equal amounts but easily separated. Removal of the protecting groups of the latter thioethers gave the target 3-*epi*-mannostatin (**66**) and its positional isomer **67**, respectively, as their hydrochloride salts. The analogues were synthesized in 18 steps with overall yields of 1.68% and 1.5%, respectively.

Scheme 12[a]



[a] Reagents: (i) Bu_4NF, THF, 100%; (ii) PPh_3, DEAD, benzene, 95%; (iii) AgOAc, I_2, benzene, 82%; (iv) TBDMSCl, imidazole, DMF, 95%; (v) NaN_3, DMSO, 85%; (vi) H_2, Pd/C, AcOEt−MeOH, $(^tBuOCO)_2O$, EtN^iPr_2, CH_2Cl_2, 84%; (vii) Bu_4NF, THF, 98%; (viii) NaH, DMF, 64%; (ix) aq AcOH, 72%; (x) $SOCl_2$, pyridine, CH_2Cl_2 then $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN-H_2O, 89%; (xi) NaSMe, DMF; (xii) H_2SO_4, H_2O−THF; Me_3SiI, CH_2Cl_2, **71a**/**71b** (35:28%); (xiii) $Ba(OH)_2$, H_2O, **72** (57%) and **73** (61%).

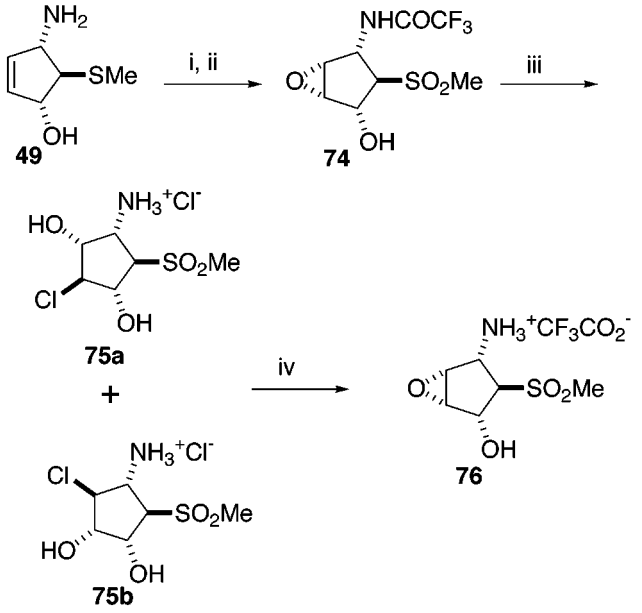
A similar sequence of reactions was applied to the precursor **58** (Scheme 12), the *O*-benzyl group being removed in the penultimate step of the synthesis using iodotrimethylsilane. Thus, the enantiomeric 3-*epi*-mannostatin (**72**) and its positional isomer **73** were each obtained in 18 steps with overall yields of 2.41% and 2.24%, respectively.

3. Other Analogues

King and Ganem[39] synthesized the alkene **49** which they expected to be a mannosidase inhibitor by analogy with conduritols. Alkene **8a**, an intermediate in the synthesis of the natural product (see Section II.B.1), was subjected to base hydrolysis, and the chiral handle was thereby removed, to give the target 3,4-dideoxyalkene analogue **49** in 71% yield (Chart 4).

Because epoxides such as the cyclophellitols have been shown to be powerful inhibitors of glycosidases, King and Ganem[39] targeted the mannostatin-related epoxide **76**. Although epoxidation of alkene **8b** was indeed possible on treatment with 3 equiv of dimethyldioxirane, attempts to hydrolyze the acetoxyphenylacetyl protecting group in a successive step under oxidative or basic conditions, or even enzymatically, failed to give the target compound pure. An alternative strategy, making use of the dideoxy analogue **49** (Chart 4), was therefore explored to obtain the epoxide **76** (Scheme 13). Thus, aminoalkene **49** was

Scheme 13[a]

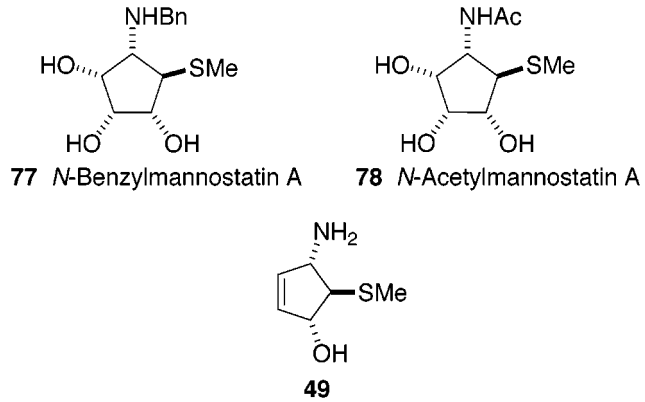


[a] Reagents: (i) TFAA, pyridine, CH_2Cl_2, 0 °C, 1 h, 87%; (ii) 6 equiv dimethyldioxirane, acetone, 91%; (iii) 0.36 N HCl−MeOH, 65 °C, 24 h, 100%; (iv) Amberlite, MeOH, 5 h, TFA−MeOH (1:9), 84%.

protected as its trifluoroacetamide and reacted with dimethyldioxirane to give epoxy sulfone **74**. The *N*-protecting group was cleanly removed in acid with concomitant opening of the epoxide to afford the diastereomeric chlorohydrins **75a** and **75b**, as their hydrochloride salts. These recyclized on being treated with a basic ion-exchange resin to give the target epoxide amine **76**.

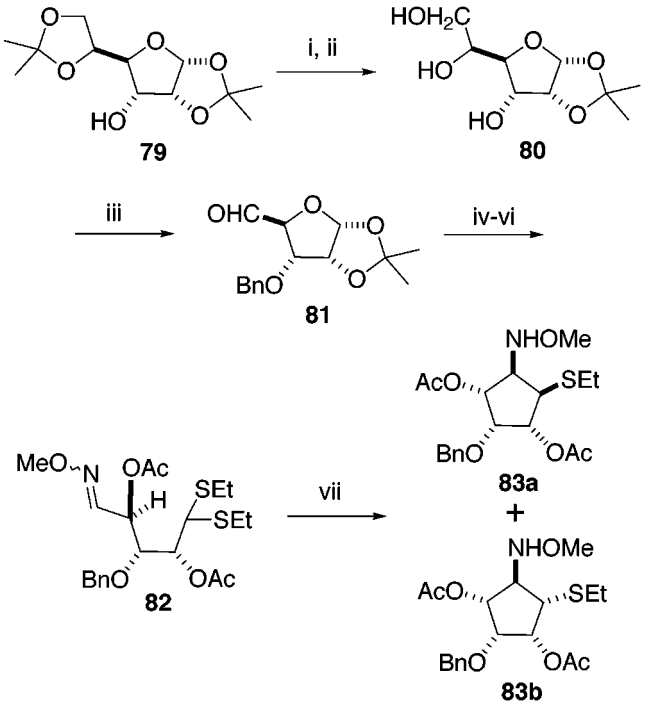
Ganem and King[39] argued that *N*-benzylmannostatin (**77**) would be an interesting mechanistic probe, as a number of glycosidases possess hydrophobic pockets and certain hexopyranosylamines when *N*-protected with aromatic substituents show more effective inhibition than their nonprotected counterparts (Chart 4). *N*-Benzylmannostatin (**77**) was ob-

Chart 4



tained in 89% yield simply by reductive amination of (+)-mannostatin A (**1**) with benzaldehyde using sodium cyanoborohydride. On exposure of natural mannostatin A (**1**) to acetic anhydride in pyridine, Tropea and co-workers[41] obtained *N*-acetylmannostatin A (**78**) (Chart 4).

Scheme 14[a]



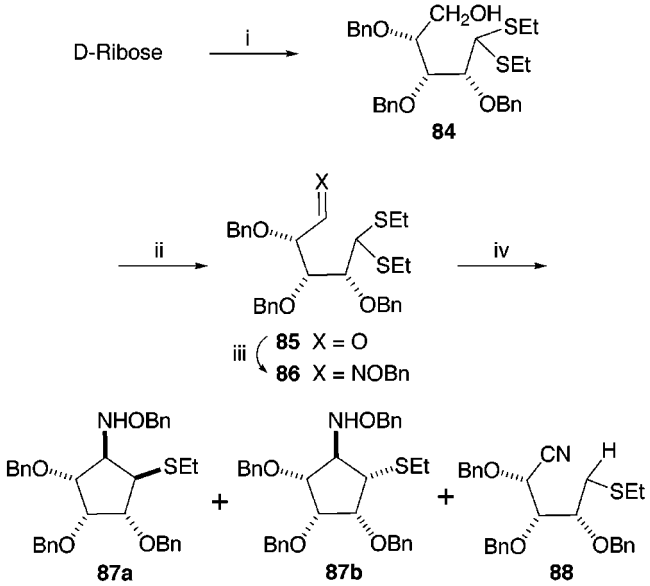
[a] Reagents: (i) BnBr, NaH, THF; (ii) HCl, MeOH, rt, 12 h, 76% from **79**; (iii) $NaIO_4$, H_2O, MeOH; (iv) $MeONH_2$·HCl, pyridine, CH_2Cl_2, rt, 2 h, 72% from **80**; (v) EtSH, $ZnCl_2$, −10 °C, 1 h, 83%; (vi) Ac_2O, DMAP, pyridine, 40 °C, 18 h; (vii) 6 equiv Bu_3SnH, 2.5 equiv AIBN, toluene, 110 °C, 1.5 h, 70%.

4. Miscellaneous

Moore and co-workers[42] adopted a free radical cyclization as the key step in their synthesis of pentasubstituted cyclopentanes related to the mannostatins. Thus, making use of the protected D-allofuranose **79** as starting material, they obtained analogues **83a** and **83b**, as a mixture of diastereomers (Scheme 14). The protected D-allose derivative **79**, obtained from D-glucose in 3 steps, was benzylated and the 5,6-*O*-isopropylidene group selectively removed. The diol **80**, thus obtained, was converted into the aldehyde **81** by oxidative cleavage. Treatment of the latter with *O*-methylhydroxylamine hydrochloride gave an inseparable mixture of (*E*) and (*Z*)-oximes (11:1) in a 75% overall yield. Exposure to ethanethiol and zinc chloride gave the dithioacetal **82** as a mixture of *E*- and *Z*-isomers (3:1) in 85% yield after peracetylation. Radical cyclization of the dithioacetal proved to be effective only under forcing conditions [i.e., with excess tin reagent (6 equiv of tributyltin hydride) and excess initiator (2.5 equiv of AIBN)] giving the pentasubstituted cyclopentanes **83a** and **83b**, albeit as an inseparable mixture.

Using a related strategy, Marco-Contelles and colleagues[43] have reported the syntheses of two new mannostatin A analogues **87a** and **87b**. D-Ribose, which has a configuration corresponding to that of C2−C4 in mannostatin, was used as starting material (Scheme 15). The cyclization precursor **86** was obtained as an inseparable mixture of (*E*)- and (*Z*)-oximes (6:1), on reaction of *O*-benzylhydroxylamine with the aldehyde **85**. The latter was obtained by oxidation of alcohol **84**, itself derived from D-ribose by a known method.[44] Typical tributyltin hydride-

Scheme 15[a]



[a] Reagents: (i) 4 steps (ref 42); (ii) PCC, 4 Å MS, CH_2Cl_2; (iii) NH_2OBn·HCl, pyridine, CH_2Cl_2, 47%; (vii) $SnBu_3H$, AIBN, toluene, 80% **87a**/**87b** (4:1).

mediated radical conditions saw cyclization of oxime **86** to a mixture of carbocycles **87a** and **87b**, together with traces of the nitrile **88**. The authors proposed that the higher ratio of the isomer **87a** was due to the fact that in the transition state of this kinetically controlled 5-*exo*-trig ring closure, the radical species adopts a chairlike conformation where the substituents are in a pseudoequatorial arrangement and able to minimize unfavorable steric interactions. The formation of the reduced product **88** was proposed to be due to a 1,5-hydrogen transfer and elimination of a benzyloxy radical.

D. Biological Activity

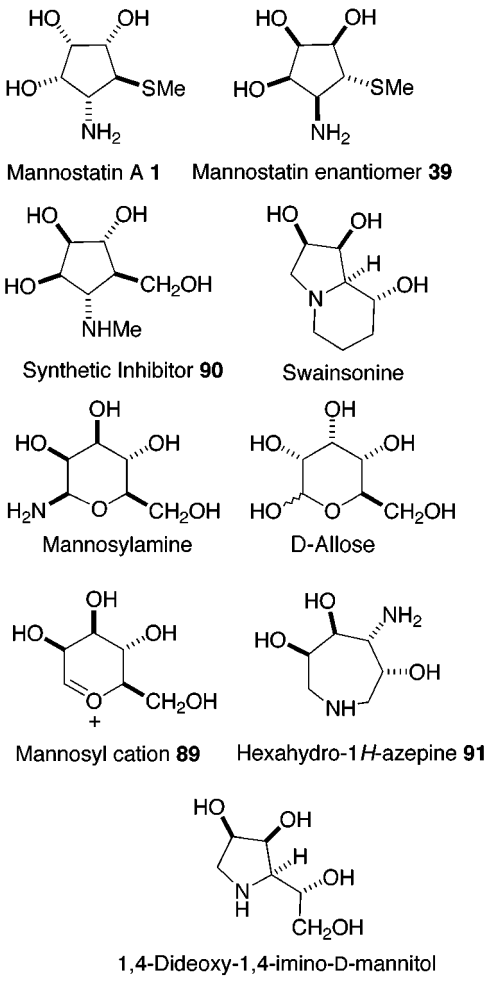
The mannostatins potently and selectively inhibit mannosidases. These enzymes are widespread in nature,[45] but perhaps best known for the key role they play in the biosynthesis of asparagine-linked oligosaccharides.[45a,b] The first committed step in the biosynthesis of these complex biopolymers is the transfer *en bloc* of a triantennary tetradecasaccharide to a nascent polypeptide as it is assembled at the surface of a ribosome. Posttranslational modifications of this common structure ensue, making possible a plethora of unique glycoproteins on the surface of a completed protein. The painstaking care by which the fidelity of these complex structures is ensured reflects the often critical roles played by glycoproteins in the regulation of a variety of physiological processes.[1] The so-called 'trimming' steps which comprise these early posttranslational changes are guaranteed by specific α-glucosidases and α-mannosidases.[45a,b] Of the three mammalian trimming *exo*-α-mannosidases, one resides in the endoplasmic reticulum and two in the Golgi apparatus. Although they share a retaining glycosidase mechanism, their substrate specificities vary.[45a,b] There exists in addition a 'trimming' *endo*-α-mannosidase in the Golgi apparatus. Lysosomal and cytosolic mannosidases are also present in other

compartments of the cell.[45a,b] The deficiency in human lysosomal A and B mannosidases leads to the inherited disease α-mannosidosis, a rare autosomal recessive condition.[2a,45b]

The promise that potent and specific inhibition of 'trimming' enzymes might prove a viable strategy by which to counter viral infection has seen a continued interest in this area.[2a] In addition, specific inhibitors of a given mannosidase have potential as biochemical tools with which to study diseases such as α-mannosidosis[2a] and also to delineate further the details of oligosaccharide biosynthesis.[45a,47] The criteria for selectivity and potency is met by several natural and synthetic mannosidase inhibitors which are, more or less effectively, able to discriminate between a given pair of α-mannosidases.[4] However, the possibility that still more efficient mannosidase inhibitors might be discovered or designed, using, for example, the mannostatins as leads, still holds hope.

Mannostatins A (**1**) and B (**2**) were the first natural α-mannosidase inhibitors to be discovered which possess the cyclopentitol structure. The inhibitors are of the reversible, competitive type[30] and do not show the slow-binding phenomenon[48] exibited by others such as swainsonine and trehazolin (**3**) (see Section III.D). Despite their relative structural simplicity (compared with, for example, trehazolin (**3**) or allosamidin (**4**)), the mannostatins are nevertheless potent and selective. A straightforward correlation of their structures with their inhibitory properties remains nonetheless elusive, at least using classical models. For example, the mode of inhibition of mannosidases by azasugars such as swainsonine,[49a] 1,4-dideoxy-1,4-imino-D-mannitol[49b] (Chart 5), and related analogues has been related to the degree to which they resemble the cation **89**, a putative intermediate in the hydrolysis of mannosides.[4,25a] A molecular graphics study intended to ratify the generality of this hypothesis with respect to mannosidase inhibitors has met with some success, at least in explaining the varying degrees of inhibition shown by a selection of azasugars.[50a] Furthermore, the synthetic aminocyclopentitol **90** (Chart 5, see Section IV.E.1 for synthesis) has been designed as a mannosidase inhibitor using the same model.[51a] Thus, the heteroatoms of the carbocycle **90** surperimpose well onto a 'flap-up' mannosyl carbocation **89**, and as predicted by the transition state theory,[26] this synthetic analogue powerfully inhibits a number of mannosidases.[51a] There has been some argument as to whether the model is adequate in explaining the potent inhibition of the mannostatins A and B. It has been contended that neither of these aminocyclopentitols, in their lowest energy conformations, superimpose very well onto the hypothetical carbocation intermediate.[36,39] [This poor fit at first sight might seem not surprising in that the stereochemistry of the substituents in the mannostatins does not correspond to that of D-mannose but to D-allose (Chart 5)]. These same authors made the further claim[36,39] that the enantiomer of mannostatin apparently shows a better fit with the 'flap-up' cation than does mannostatin itself but is only a weak inhibitor of mannosidases. A subsequent modeling study disputed these latter

Chart 5



assertions and argued that two of the three hydroxyl groups in the mannostatins mapped very well onto the 2- and 3-hydroxyl groups of the mannosyl cation **89**, and it was this correlation that was of paramount importance for determining mannosidase activity in the 'flap-up' model.[50b] Further, although these authors accepted that there exists considerable flexibility in the way active-site amino acids interact with inhibitors,[50a] they nevertheless argued that the enantiomer of mannostatin A maps very poorly onto the 'flap-up' cation and that its poor activity was therefore to be expected.[50b] In this same modeling study it was proposed that the amino group of mannostatin lies in a region of space corresponding to the oxonium ion in mannosyl cation **89**.

In the 'flap-up' model the hydrolysis reaction catalyzed by mannosidases is considered as essentially static, whereas in reality binding at the reaction center is not a passive but a dynamic process.[26,53] It is also assumed that enzymes always interact with inhibitors in their lowest energy conformations.[50] In this connection, it is interesting to note that the hexahydro-1*H*-azepine **91** (Chart 5) is *not* an inhibitor of mannosidases even though in its lowest energy conformation it too maps well onto the 'flap-up' mannosyl carbocation.[51b] This discrepancy was proposed to be due to the fact that the azepine **91** adopts a different conformation in solution to that expected (as observed by H^1 NMR), a conformation that does

Table 1. Survey of Biological Activity of Mannostatins and Their Analogues

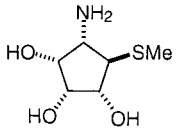
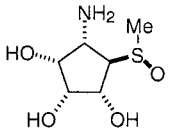
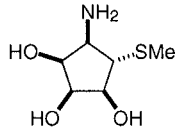
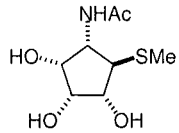
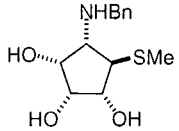
Compounds	Comments	Ref.
 mannostatin A **1**	Highly specific inhibition of jack bean, mung bean, rat liver lysosomal, rat epididymis α-D-mannosidases and Golgi processing mannosidase II (IC_{50} = 70, 450, 160, 112 and 10×10^{-9} mol dm^{-3}, respectively.	30, 35, 39, 41
	Competitive, reversible inhibitor of rat epididymis mannosidase $K_i = 4\times10^{-8}$ M.	
	Potent inhibitor of glycoprotein processing in virally infected cells.	
	For comparison: swainsonine has IC_{50} = 87, 40 and 200×10^{-9} mol dm^{-3} against jack bean, mung bean and Golgi processing mannosidase II, enzymes respectively; kifunensine has IC_{50} = 20-50 nM against mung bean enzyme; deoxymannonojirimycin has IC_{50} = 50 μM against yeast enzyme; 1,4-dideoxy-1,4-imino-D-mannitol has IC_{50} = 0.5 μM against jack bean enzyme (source references for this data are cited in references 45a and 50a)	
	Inhibits rice leaf lectin promoted reception of blast fungus proteoglycomannan by host cells.	
	No activity against bovine liver β-D-galactosidase or *Clostridium perfringens* sialidase.	
	No activity against β-mannosidases, amyloglucosidase, α-galactosidase or β-glucosidase.	
	Low toxicity: no death after 250 mg kg^{-1} intravenous injection to mice.	
	Inhibits *in vitro* tumor cell invasion of mouse melanoma cells	52
	Inhibits *in vitro* invasion of K-ras-NIH3T3 cells and inhibits cellular α-mannosidase activity specifically.	52b
	No antibacterial activity at 100 μg mL^{-1}.	
 mannostatin B **2**	Same activity as mannostatin A particularly against jack bean and rat epididymis α-D-mannosidase [IC_{50} = 0.033 and 0.02 μg mL^{-1} (103×10^{-9} mol dm^{-3})].	30, 35, 41
	Competitive, reversible inhibitor of rat epididymis mannosidase $K_i = 4\times10^{-8}$ M.	
	No activity against bovine liver β-D-galactosidase or *Clostridium perfringens* sialidase.	
	No antibacterial activity at 100 μg mL^{-1}.	
 mannostatin A enantiomer **39**	Weak inhibitor of jack bean α-D-mannosidase (IC_{50} = 61 μg mL^{-1}).	35, 41
 *N*-acetylmannostatin A **78**	Not active against jack bean, mung bean or rat liver lysosomal α-D-mannosidase	41
 *N*-benzylmannostatin A **77**	Competitive inhibitor of jack bean α-D-mannosidase (K_i = 380 ± 81×10^{-9} mol dm^{-3}; for comparison K_i = 150 (and 400) × 10^{-6} mol dm^{-3} for 1-deoxymannojirimycin. Data taken from Ref. 50a).	39

Table 1 (Continued)

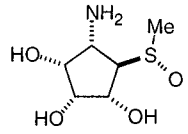
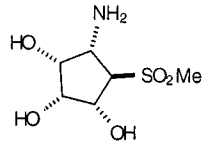
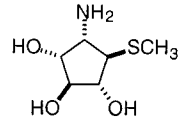
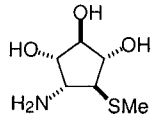
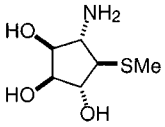
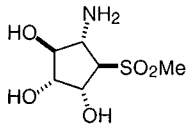
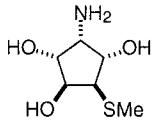
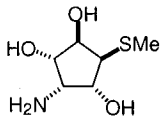
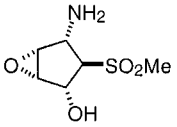
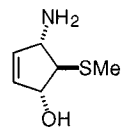
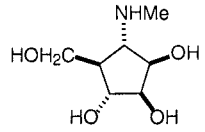
 Stereomeric sulfoxide of mannostatin B **20**	As potent as mannostatin A particularly against jack bean α-D-mannosidase (IC_{50} = 0.11 μg mL^{-1}).	39
 Mannostatin sulfone **40**	Competitive inhibitor of jack bean α-D-mannosidase (K_i = 126 ±16×10^{-9} mol dm^{-3}).	39
 3-*epi*-Mannostatin A **66**	Not active against various α-, β-glucosidases, galactosidases, mannosidases or glucuronidase.	40
 3-*epi*-Mannostatin A enantiomer **72**	Not active against various α- β-glucosidases, galactosidases, mannosidases or glucuronidase.	40
 3,4-di-*epi*-Mannostatin A **48**	Very weak inhibitor of jack bean α-D-mannosidase (K_i = 16 ± 2×10^{-6} mol dm^{-3}).	39
 4-*epi*-Mannostatin A sulfone **53**	Not active against almond β-glucosidase, *Aspergillus niger* amyloglucosidase and jack bean α-D-mannosidase.	39
 analogue **67**	Not active against various α- β-glucosidases, galactosidases, mannosidases or glucuronidase.	40
 analogue **73**	Not active against various α- β-glucosidases, galactosidases, mannosidases or glucuronidase.	40
 analogue **76**	jack bean α-mannosidase irreversible inhibitor (K_i = 153 ± 26×10^{-6} mol dm^{-3}). (Apparent activity K_{inact}/K_i = 160 min^{-1} M^{-1}).	39
 analogue **49**	Not active.	39

Table 1 (Continued)

 **90**	Strong inhibitor of jack bean α-mannosidase (IC_{50} = 62×10^{-9} 53 mol dm^{-3}). Weak inhibitor of mung bean glycoprotein processing α-mannosidase II (IC_{50} = 1×10^{-6} mol dm^{-3}).

not superimpose well onto the mannosyl cation, and is thus not recognized by the enzyme.[50b,51b] It is well-known that the conformations, on the pseudorotational itinerary, of monocyclic five-membered rings often differ very little in energy from one another. It follows that a number of 'high-energy' conformers might be accessible to the mannostatins under physiological conditions. It would thus not be possible to discount *a priori* that it is one of these alternative conformers that resembles a *kinetically significant* intermediate on the reaction coordinate.

An alternative mode of inhibition for mannostatins has been proposed[39] on account of their resemblance to β-mannopyranosylamine (Chart 5); glycopyranosylamines have long been known to be competitive inhibitors of glycosidases.[4a] If mannostatin and β-mannopyranosylamine do sit similarly in a given active site, and the amino group of the inhibitor were to mimic the exocyclic oxygen of the substrate, the mannostatins would then simply owe their activity to being substrate analogues.[39]

However, the substrates of α-mannosidases are α-glycosides, and the enzymatic reaction proceeds with retention of configuration. Preliminary evidence indicates that β-mannosidases adopt a retaining mechanism[25a] and that they are not inhibited by mannostatin A.[41] This raises the possibility that the amino function of the mannostatins mimics the *ring* oxygen of the substrate and that the mannostatins are 'transition-state' analogues. The amino group of the inhibitor would be protonated at physiological pH, expected to interact electrostatically with a catalytic carboxylate group, and thereby inhibit the enzyme.[25a] The analogue **78**, wherein the amino group of mannostatin A is acetylated, shows no inhibitory activity,[41] whereas the synthetic analogue **90**, in which the amino group is monomethylated, is a good mannosidase inhibitor.[51a]

Another question which remains to be addressed is how it is that mannostatin A (**1**) selectively inhibits the Golgi mannosidase II in preference to the Golgi mannosidase I.[41] This selectivity is all the more remarkable given that the natural substrates for these enzymes are very similar and that the mannostatins effectively mimic only a small part (one sugar unit) of their *polymeric* substrates or intermediates (cf. trehazolin (**3**) and allosamidin (**4**), see Sections III.D and IV.F).

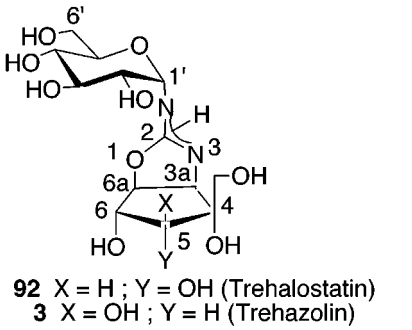
The possible role of the methylthio and methylsulfenyl functions in mannostatins A (**1**) and B (**2**), respectively, is intriguing, all the more so in that these features are unique to these natural glycosidase inhibitors. Thioether groups have been incorporated as structural features in synthetic mannosidase inhibitors,[4d] although the synthetic aminocyclopentitol **90** which lacks this motif is nevertheless a good mannosidase inhibitor.[51a] It has been proposed[50b] that the methyl thioether and methylsulfenyl groups lie near the 4-OH group of the mannosyl cation **89**, but it is also conceivable that these functions are mimics of the hydroxymethyl group in the substrate. However, some disagreement does exist as to the importance of the hydroxymethyl interaction on the overall efficiency of a given inhibitor.[50,51] If the thio functions play a part in the recognition and binding of the mannostatins by mannosidases, the extent to which these structural elements are important remains unclear. It should be noted that mannostatins A (**1**) and B (**2**), the epimer at sulfur **49** of mannostatin B, and the unnatural sulfone analogue **50** all have identical inhibitory activity against mannosidases[39] (Table 1). Although a number of mannostatin analogues have been synthesized, detailed inhibition data is disappointingly sparse. That which is available, together with some related biological data, is presented in tabulated form (Table 1).

III. Trehazolin

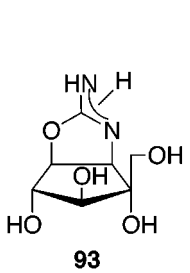
A. Isolation and Elucidation of Structure

In 1991, Nakayama and collaborators[55] reported a compound exhibiting powerful and specific inhibition of various trehalases. Having been isolated from the culture broth of *Amycolaptosis trehalostatica*, the novel inhibitor was named trehalostatin (**92**) (Chart 6). The pseudodisaccharide was proposed to comprise an α-D-glucopyranosyl residue linked to a carbocyclic 5,5-ring-*fused*-aminooxazoline. The aminocyclopentitol moiety was named trehalamine (**93**) (Chart 6). At about the same time, Ando and colleagues[56]

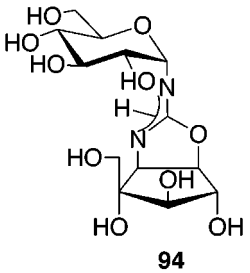
Chart 6



92 X = H ; Y = OH (Trehalostatin)
3 X = OH ; Y = H (Trehazolin)



93



94

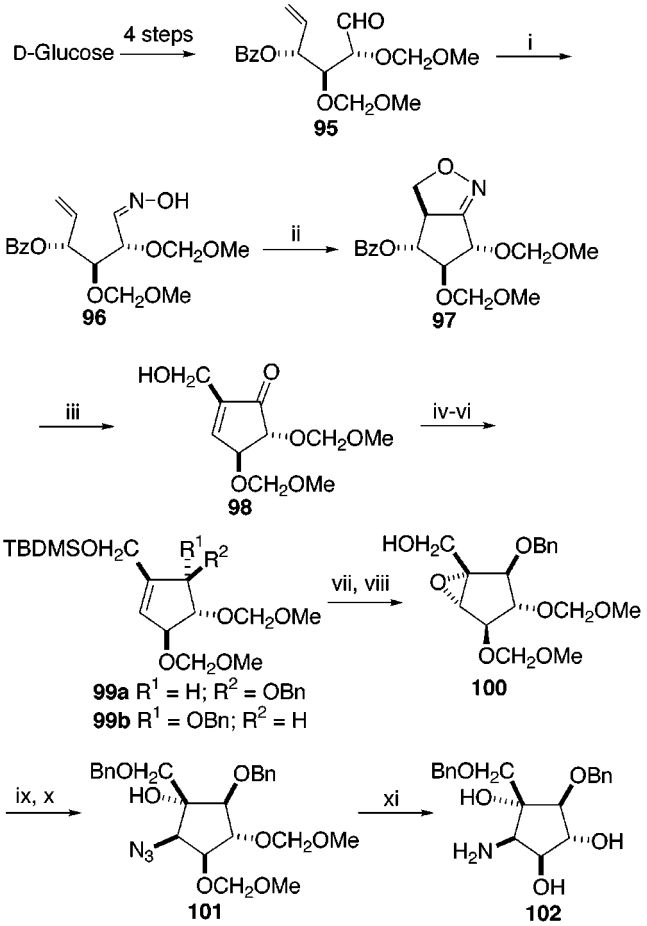
reported the isolation of a substance from *Micronospora* strain SANK 62390, which they called trehazolin (**3**) (Chart 6).

The structures of **92** and **3** differ only in the configuration at position C-5 of the aminocyclopentitol ring. However, their physical properties were postulated to be identical. Total syntheses of these pseudodisaccharides and the trehazolin diastereomer **94** were thus embarked upon in order to unambiguously establish their exact structures and to throw light on this apparent discrepancy (see Sections III.B.2 and III.C.2). These studies established that the molecules in question were in fact identical and that the correct structure of the natural inhibitor corresponds to that originally reported for trehazolin (**3**).

B. Total Synthesis

All three approaches to trehazolin (**3**) thus far reported involve first a synthesis of the polyhydroxylated aminocyclopentitol core, then its coupling with a glucopyranosyl derivative, and finally a ring closure to give the aminooxazoline function via an intermediate carbodiimide. Thus, syntheses of the carbocyclic moiety constitute a formal total synthesis of the natural product and are included in this section (see also Section V for recent syntheses).

Scheme 16[a]



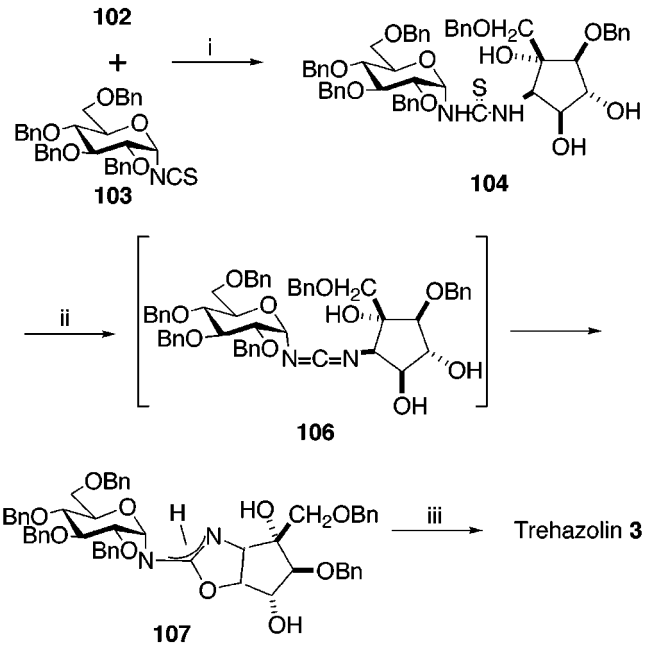
[a] Reagents: (i) 10 equiv $NH_2OH{\cdot}HCl$, 10 equiv Na_2CO_3, Et_2O-H_2O, 8 h, 74%; (ii) aq 5% NaOCl, cat. Et_3N, $CH_2Cl_2-H_2O$, 0 °C, 40 min, 60%; (iii) H_2, Raney Ni, $B(OH)_3$, dioxane−MeOH−H_2O, 5 h, 72%; (iv) 1.5 equiv TBDMSCl, 1.5 equiv imidazole, DMF, 16 h, 88%; (v) 1.5 equiv $NaBH_4$, 1.5 equiv $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 2 h, 53%; (vi) 1.5 equiv BnBr, 1.5 equiv NaH, DMF, 0 °C, 1 h; (vii) 1.5 equiv Bu_4NF, THF, 0 °C, 1 h, 58% from **98**; (viii) 1.4 equiv $Ti(O^iPr)_4$, 1.5 equiv diisopropyl L-tartrate, 2 equiv $^tBuO_2H$, CH_2Cl_2, −20 °C, 5 h, 94%; (ix) 1.5 equiv BnBr, 1.5 equiv NaH, DMF, 2 h, 98%; (x) 12 equiv NaN_3, 12 equiv NH_4Cl, DMF−$HO(CH_2)_2OH$, 125 °C, 48 h, 78%; (xi) 4 equiv LAH, Et_2O, 0 °C, 4 h, then 5% HCl, MeOH, 60 °C, 5 h.

1. Shiozaki's Synthesis

These authors[57] made use of D-glucose as the starting material in their synthesis of trehazolin (**3**) in which an intramolecular [3 + 2] cycloaddition played a key part (Scheme 16). The ethylenic aldehyde **95**,[19a] obtained from D-glucose, was transformed into the corresponding oxime **96** and underwent an intramolecular [3 + 2] cycloaddition to give the cyclopentane skeleton. Reductive opening of the isoxazoline ring, with Raney nickel under atmospheric pressure of hydrogen, was accompanied by hydrolysis of the intermediate imine **97**, and the benzyloxy group was subsequenly eliminated to provide enone **98**. Luche reduction of this intermediate, followed by simple protective group manipulation, provided cyclopentene **99a**. A Sharpless asymmetric epoxidation, another crucial step in this synthesis, gave the alcohol **100**. The primary alcohol funtion was then protected as its benzyl ether and the epoxide regiospecifically opened with sodium azide to give the functionalized carbocycle **101**. Reduction of the azido group and removal of the methoxymethyl groups gave the aminocyclopentitol **102**.

To complete the synthesis, the advanced intermediate **102** was treated with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl isothiocyanate (**103**)[58] to provide the thiourea **104** (Scheme 17). The cyclization was initiated with 2-chloro-3-ethylbenzoxazolin tetrafluoroborate (**105**) (Mukaiyama's reagent) to give, via the carbodiimide **106**, the ring-fused aminooxazoline **107**.

Scheme 17[a]



[a] Reagents: (i) 15 equiv Et_3N, MeOH, 69% (with precedent step); (ii) 17 equiv 2-chloro-3-ethylbenzoxazoline tetrafluoroborate (**105**), MeCN, 0 °C, 1 h, then quenched with 3.2 equiv Et_3N, 0 °C, 1 h, 68%; (iii) H_2, $Pd(OH)_2/C$, MeOH, 60 °C, 30 min, 44%.

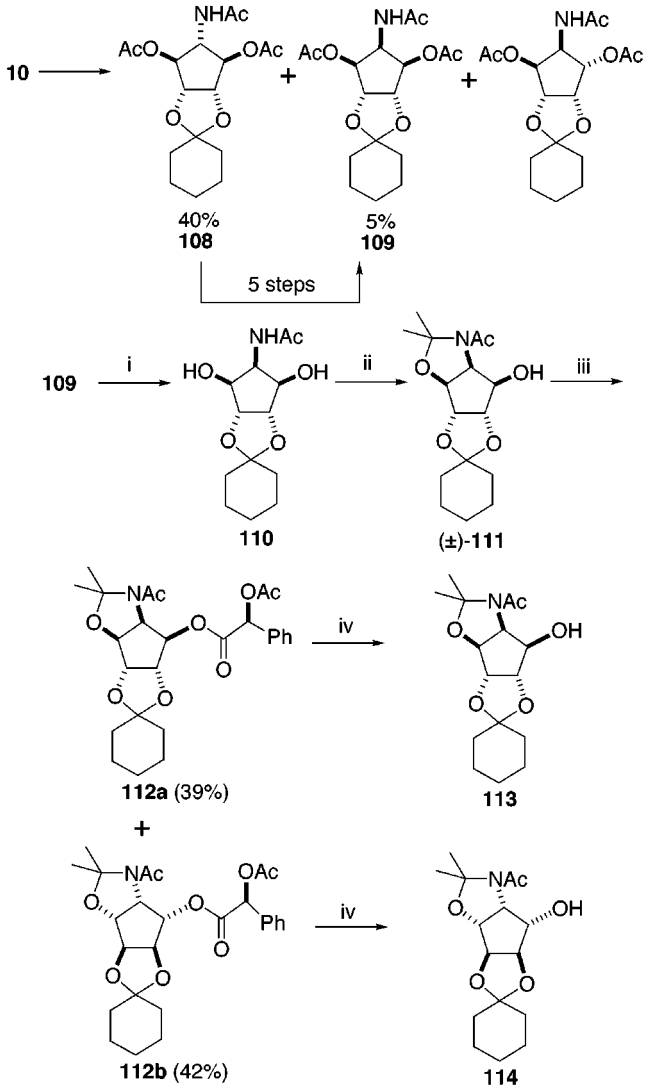
Hydrogenolysis of the latter gave trehazolin (**3**). The synthesis comprises 20 steps and proceeded in 0.3% overall yield.

2. Ogawa's Synthesis

Ogawa and co-workers[59] have, in a series of publications, disclosed the synthesis of the racemic aminocyclopentitol core of trehalamine (**93**) and thereby confirmed its relative stereochemistry.[59a,b] They have also completed the syntheses of trehazolin (**3**) and its diastereomer **94** (Chart 6) using racemic trehalamine (**93**), and thus elucidated the absolute configuration of the natural product.[59c] Having established unambiguously its structure, an enantioselective synthesis of trehazolin (**3**) was undertaken and is commented upon here.[59d]

The synthesis suffers from the difficulty in obtaining the starting cyclopentitol **109** (Scheme 18) and also from the many separation steps needed to purify the various isomeric mixtures obtained. However, these isomers subsequently proved invaluable in the preparation of a large number of trehazolin analogues (see Section III.C).

Scheme 18[a]

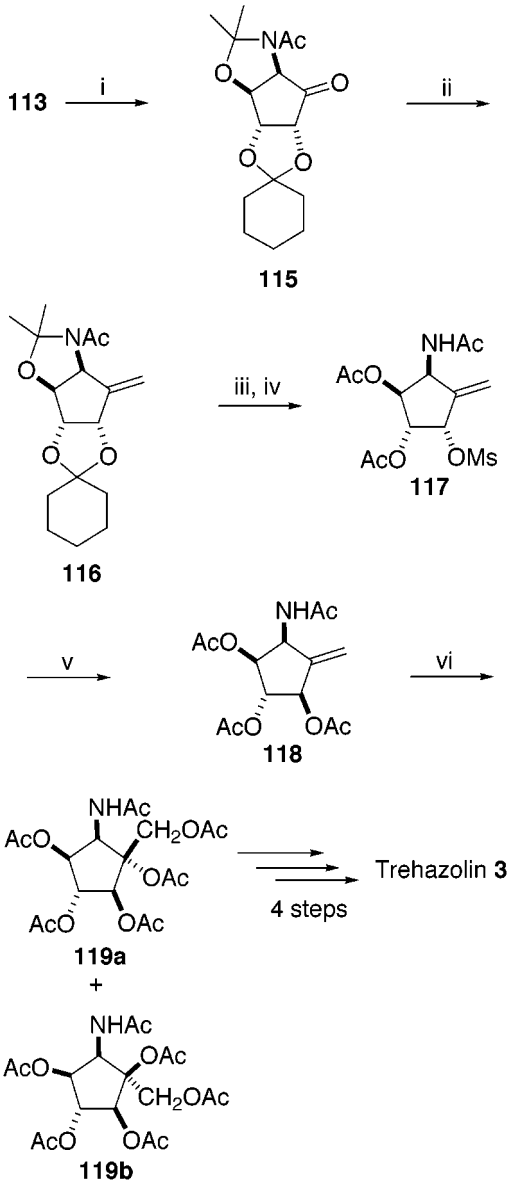


[a] Reagents: (i) MeONa, MeOH; (ii) $Me_2C(OMe)_2$, DMF, *p*-TsOH cat., 14 h, 87%; (iii) (*S*)-acetylmandelic acid, DCC, DMAP, CH_2Cl_2, 0 °C, 15 min, 81%; (iv) MeONa, MeOH, 1 h, 92%.

The required cyclopentitol **109** is obtained as a minor product (5%) from the base-catalyzed nitromethane cyclization of the dialdehyde derived from the oxidative cleavage of 1,2-*O*-cyclohexylidene-*myo*-inositol (**10**) (also used in their synthesis of mannostatin A (**1**), see Section II.B.2). However, conversion of the major isomer **108** in 5 steps into the required epimer **109** allowed its yield to be improved slightly. Zemplén deacetylation followed by acetalation of the *meso*-compound **109** gave the racemic *N*,*O*-isopropylidene acetal **111**. Homochiral alcohol **113** was obtained on derivatization of this racemic mixture as its (*S*)-mandelate esters, separation by column chromatography, and deesterification. The intermediate **113** was obtained in ca 3.9% yield from the *myo*-inositol derivative **10**.

Alcohol **113** (Scheme 19) was oxidized to the corresponding ketone **115** which was converted to the

Scheme 19[a]



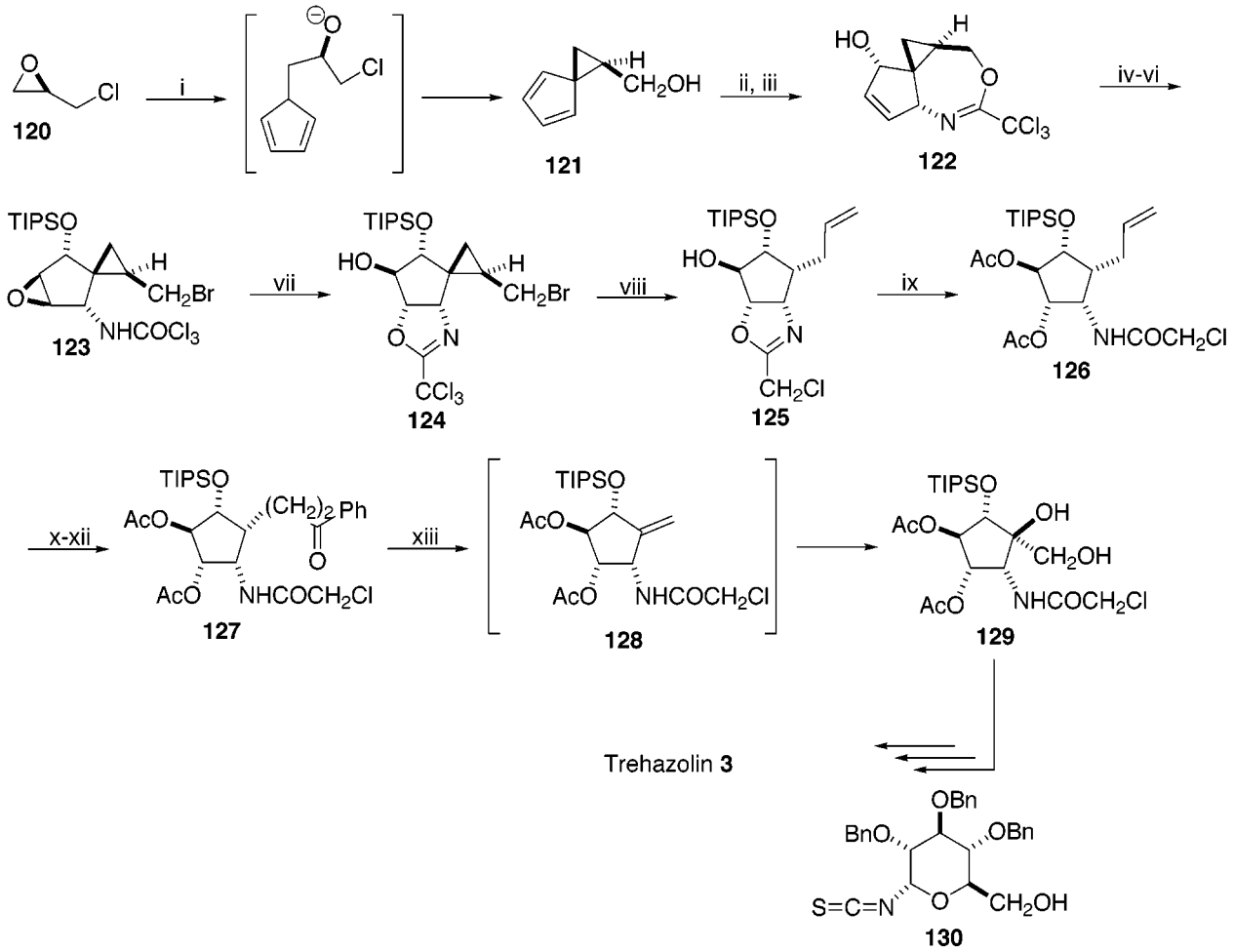
[a] Reagents: (i) PCC, 4 Å MS, CH_2Cl_2, 2 h, 98%; (ii) CH_2N_2, DMSO−Et_2O, then $P(OMe)_3$, 130 °C, 45%; (iii) 80% aq AcOH, 80 °C, 24 h; (iv) MsCl, pyridine, 0 °C, 2 h, then Ac_2O, 1.5 h, 65%; (v) NaOAc, DMF, 80 °C, 19 h, 66%; (vi) OsO_4, NMO, ^{t}BuOH, 20 h, then Ac_2O, pyridine, 85%.

exo-methylenic compound **116** via a spiroepoxide. Exposure to acidic conditions followed by selective mesylation of the allylic alcohol and then *O*-acetylation gave the fully protected mesylate **117**. Nucleophilic substitution with sodium acetate in DMF gave the peracetylated *exo*-methylenic cyclopentitol **118**. Catalytic osmylation allowed the introduction of the required pair of hydroxy groups to give alcohols **119a** and **119b** which were separated by chromatography. The synthesis was finished similarly to that of Shiozaki (see Section III.B.1) except that yellow mercuric oxide was used instead of Mukaiyama's reagent **105** to effect formation of the aminooxazoline ring. Trehazolin (**3**) was thus obtained in 10 steps in 12% overall yield from alcohol **113**.

3. Carreira's Synthesis

Ledford and Carreira[60] have reported an enantioselective total synthesis of trehazolin (**3**) starting with optically active 1-(hydroxymethyl)spiro[2.4]cyclohepta-4,6-diene (**121**), itself derived in a key step from (*R*)-epichlorohydrin (**120**) and cyclopentadiene (Scheme 20). Treatment of epichlorohydrin (**120**) with lithium cyclopentadienide in the presence of sodium hydride led to epoxide opening followed by spirocyclization to give the (*R*)-spirocyclopropane **121** with 91% ee. Other conditions gave racemic mixtures or poorer enantioselectivity. Alcohol **121** was then converted into its trichloroacetamidate, whose subsequent iodocyclization and in situ hydrolysis gave the secondary alcohol **122**, wherein the requisite 1,4-aminocarbinol functionalities with the correct configuration are installed. Following protection of this secondary alcohol, the imidate was submitted to nucleophilic attack with Li_2NiBr_4 and the resulting cyclopentene epoxidized to give the oxirane **123**. The latter underwent an intramolecular attack by the trichloroacetamide promoted by boron trifluoride etherate to give oxazoline **124**. Fragmentation of the cyclopropane ring, accompanied by partial reduction of the trichloromethyl group, was achieved under free radical conditions with tributyltin hydride in the presence of triethylborane to give the 2-(propen-1-yl)cyclopentane **125**. The oxazoline ring was hydrolyzed, and the alcohol functions were esterified. The branched alkenyl chain now had to be transformed into an exomethylene group to allow the remaining pair of hydroxyl groups to be introduced. Thus, the alkene **126** was hydroborated and then oxidized to an aldehyde which was converted into the aryl ketone **127**. Ir-

Scheme 20[a]



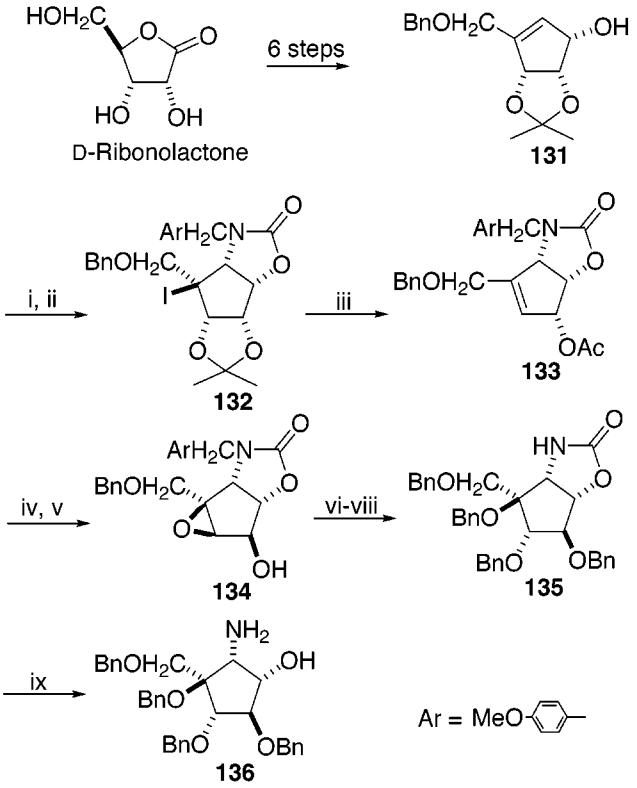
[a] Reagents: (i) CpLi, NaH, THF, 60%; (ii) NaH, CCl_3CN, THF, 95%; (iii) I(*sym*-collidine)$_2ClO_4$, $NaHCO_3$, aq MeCN, 0 °C, 61%; (iv) $^iPr_3SiOTf$, 2,6-lutidine, CH_2Cl_2, 95%; (v) Li_2NiBr_4, THF, 80%; (vi) dimethyldioxirane, acetone, 95%; (vii) BF_3-Et_2O, 87%; (viii) Bu_3SnH, Et_3B, $NaBH_4$, EtOH, 75%; (ix) PPTS, aq MeCN, then Ac_2O, DMAP, 77%; (x) Chx_2BH, H_2O_2, 83%; (xi) $(COCl)_2$, DMSO, Et_3N, 83%; (xii) PhMgBr, LiBr, THF, then $(COCl)_2$, DMSO, Et_3N, 60%; (xiii) *hν*, then OsO_4, NMO, 79%.

radiation gave the desired *exo*-methylenic cyclopentane **128** via a Norrish type II cleavage. This intermediate was directly *cis*-hydroxylated with osmium tetraoxide to provide the diol **129**. The advanced intermediate **129** was thus obtained in 15 steps and 3% overall yield. The synthesis was completed by fully deprotecting intermediate **129** and, following reported procedures (see Section II.B.2), coupling it with isothiocyanate **130**. These last 3 steps proceeded in 31% yield.

4. Knapp's Synthesis: The Aminocyclopentitol

Knapp and collaborators[61] have disclosed the synthesis of the aminocyclopentitol **136**, a precursor of trehalamine (**93**), starting from D-ribonolactone (Scheme 21). The lactone was transformed into the hydroxycyclopentene **131** in 6 steps (see Scheme 4, ref 18b). Condensation of *p*-methoxybenzyl isothiocyanate with alcohol **131** and treating the product with iodine yielded the oxazolidinone **132**. This reaction is described as a rare example of anti-Markovnikov iodocyclization and was said to result from a kinetic preference for the formation of five-membered rather than six-membered rings and for fused rather than bridged ring systems. Reduction of the iodide **132** was accompanied by elimination of the vicinal ether and gave the allylic acetate **133**. Conventional Mitsunobu inversion followed by Sharpless asymmetric epoxidation gave the key intermediate **134**. Regioselective ring opening of this oxirane followed by protecting group manipulations provided the bicyclic carbamate **135**. Base hydrolysis of the oxazolidinone function gave the amino alcohol **136** in quantitative yield. This intermediate was obtained in 11 steps and 42% overall yield from alcohol **131**.

Scheme 21[a]

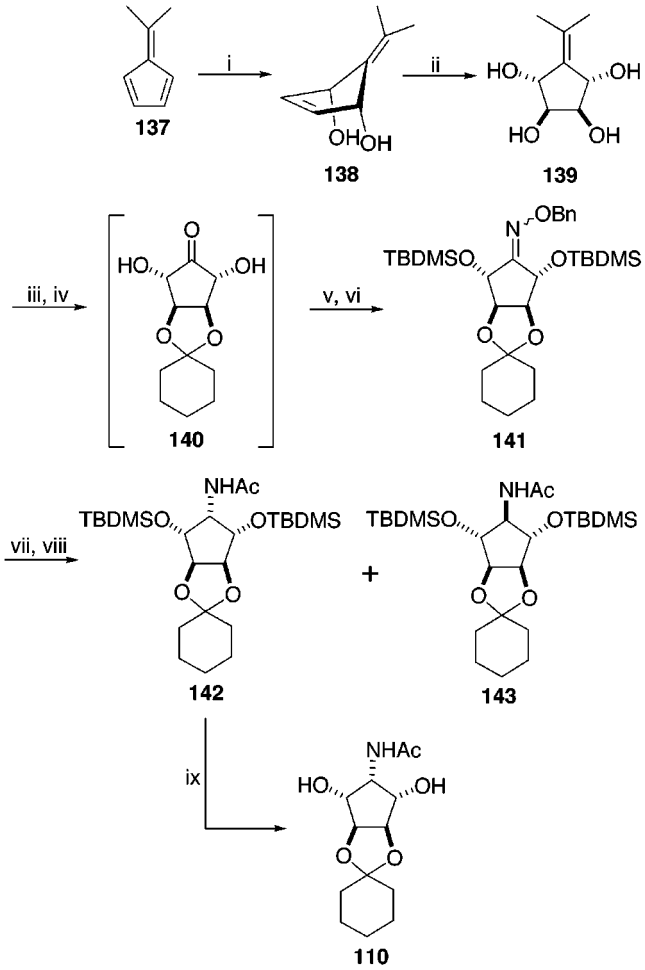


[a] Reagents: (i) NaH, $ArCH_2NCS$, MeI; (ii) I_2, Na_2CO_3, Na_2SO_3, THF, 82%; (iii) Ac_2O, H_2SO_4, Zn, THF, 90%; (iv) K_2CO_3, aq MeOH, $PhCO_2H$, DEAD, PPh_3, toluene, Na_2CO_3, aq MeOH, 83%; (v) CF_3CO_3H, Na_2CO_3, CH_2Cl_2, −20 °C, 90%; (vi) $PhCO_2Na$, aq DMF, 100 °C, 12 h, 89%; (vii) NaH, BnBr, Bu_4NI, THF; (viii) CAN, aq CH_3CN, 86%; (ix) 2 N aq KOH, EtOH, reflux, 12 h.

5. Ganem's Synthesis: The Aminocyclopentitol

Ganem and colleagues[62] have obtained diol **110** (first described by Ogawa, see Section III.B.2), a precursor of the racemic aminocyclopentitol moiety of trehazolin (**3**). A key step in the route is the [4 + 2] heterocycloaddition of singlet oxygen on dimethylfulvene (**137**) (Scheme 22). Rose bengal-sensitized photooxidation of fulvene **137** gave the 1,4-diol **138**. The inclusion of thiourea and an acid trap in the reaction medium suppressed the formation of mixtures of products usually observed in this transformation. Hydroxylation by catalytic osmylation gave tetrol **139**, the observed selectivity being determined by the conformational preference of the diol **138**. Protection of the *cis*-vicinal diol as its cyclohexylidene acetal, followed by ozonolysis of the *exo*-methylenic bond, gave the dihydroxy ketone **140**. This ketone was transformed without purification into the corresponding oxime and then silylated to give the protected oxime **141**. This protection strategy secured

Scheme 22[a]



[a] Reagents: (i) rose bengal, O_2, thiourea, NaOAc, CH_3OH, −30 °C, 2 h, 78%; (ii) OsO_4, NMO, acetone−H_2O (10:1), 22 h, 70%; (iii) 1,1-diethoxycyclohexane, *p*-TsOH, DMF, 95 °C, 62%; (iv) O_3, −78 °C; (v) NH_2OBn; (vi) 3 equiv TBDMSCl, 5 equiv imidazole, DMF, 2 days, 92%; (vii) 15 equiv BH_3−THF, THF, 70 °C, 18 h, 89%; (viii) Ac_2O, pyridine; (ix) 1.8 equiv Bu_4NF, THF, 20 min, 97%.

the stereoselective reduction of the oxime in the sense desired. Thus, oxime **141** on treatment with borane followed by acetylation gave a 6:1 mixture of acetamides **142** and **143**. The target *meso*-diol **110** was subsequently obtained from the major diastereomer **142**. This compound is identical to that employed in Ogawa's total synthesis of trehazolin (**3**) (see Section III.B.2) but was obtained by this present method in a markedly improved yield (21% versus 0.1%).

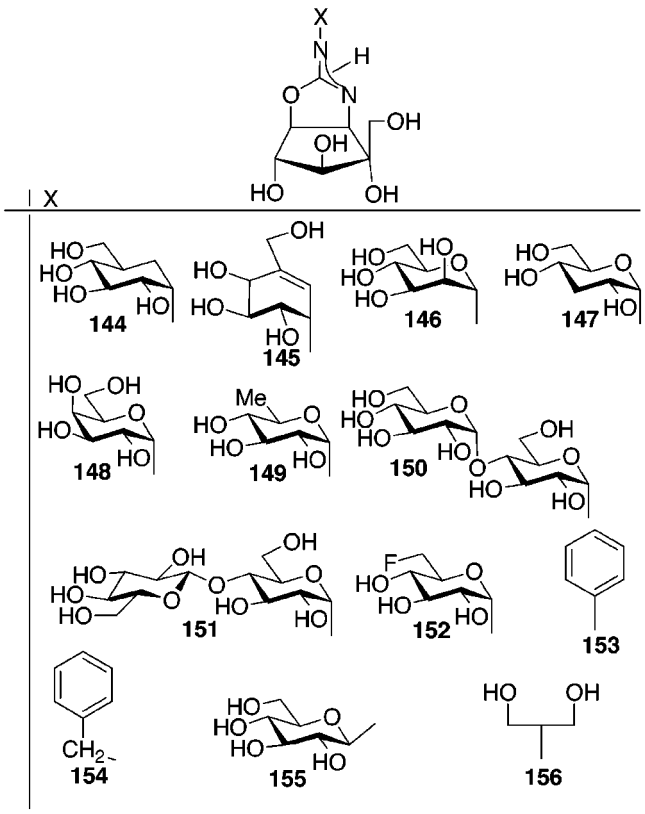
C. Synthesis: Analogues

Many analogues of trehazolin (**3**) and trehalamine (**93**) have been synthesized to date, not only to establish the correct structure of the natural inhibitor but also to obtain inhibitors with improved strength and selectivity toward a given trehalase, compared with those already available. The natural products have also provided leads in the design of glycosidase inhibitors of novel structure and of inhibitors with other specificities.

1. Modification of the Sugar Moiety

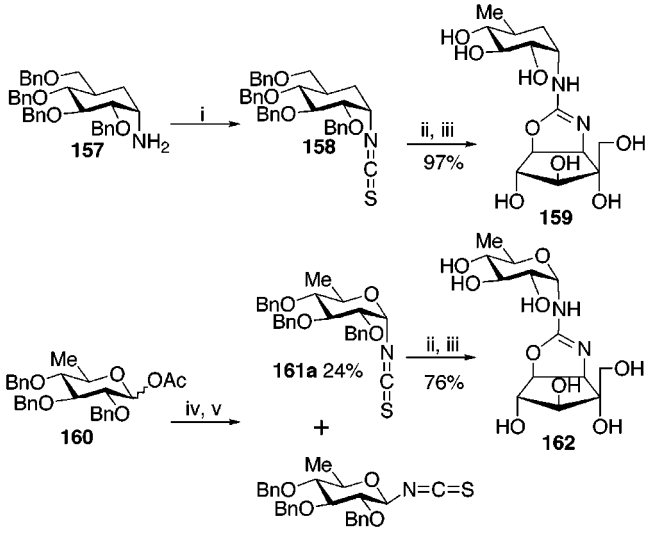
Ogawa's group has undertaken a systematic study in order to assess the contribution of the sugar moiety on the biological activity. Their strategy involves replacing the glucose residue in trehazolin (**3**) by carbasugar,[63] carbohydrate,[64,65] or aryl[64] moieties (Chart 7).

Chart 7



Shiozaki's group has reported the preparation of the trehazolin β-anomer **155** (the C-1′ epimer)[66] and of the aminopropanediol derivative **156**[67] (Chart 7). The classical coupling strategy has been employed for the syntheses of these analogues from trehalamine (**93**) (see Scheme 23 for representative examples).

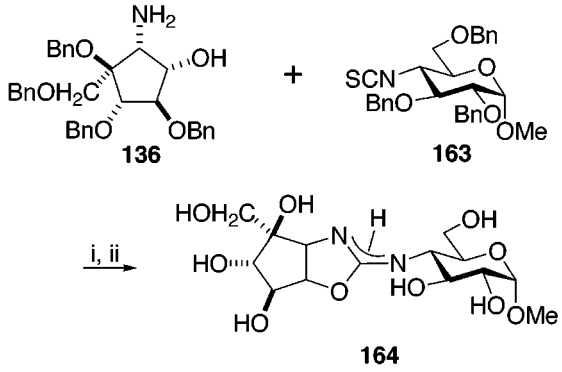
Scheme 23[a]



[a] Reagents: (i) thiocarbonyldiimidazole, 67%; (ii) **102**, aq THF, 5 days, then HgO, Et_2O; (iii) Na, NH_3; (iv) HCl−1,4-dioxane; (v) KSCN.

Knapp and collaborators[61] have reported the (1→4)-linked pseudodisaccharide **164**, the first in a series of novel analogues coined "trehazoloids" (Scheme 24).

Scheme 24[a]

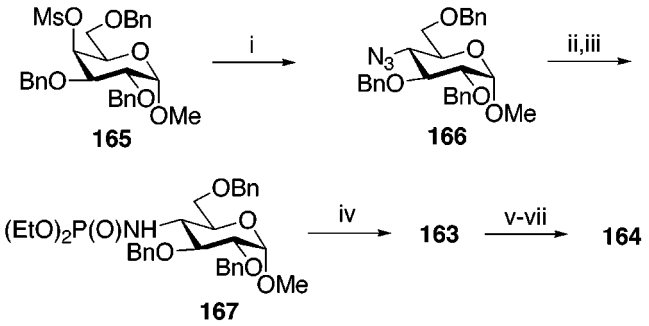


[a] Reagents: (i) Et_3N, MeOH, then HgO yellow; (ii) H_2, $Pd(OH)_2$/C, ~64%.

The latter compound differs from trehazolin (**3**) in that its glucose moiety is linked to the carbocycle via its C-4′ position rather than via its anomeric center and was obtained by coupling the glucose-derived isothiocyanate **163** with the trehalamine derivative **136** by known methods (Scheme 24).

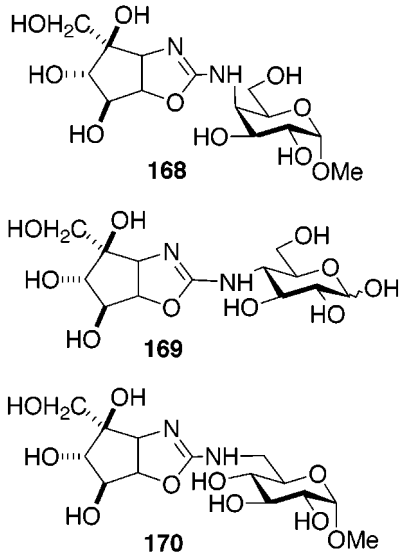
Shiozaki and co-workers[68] have also reported the preparation of the pseudodisaccharide **164**. They, however, used a Wittig−Horner−Emmons-type reaction of precursor **166** to synthesize the isothiocyanate coupling partner **163** (Scheme 25). Thus, the galactose-derived mesylate **165** gave the azide **166** possessing the required *gluco*-configuration, from which the target isothiocyanate **163** was obtained via the imidophosphate **167**. Coupling partners related to analogue **163** have been employed by these same authors[68] in routes to other trehazoloids, including compound **168** and the maltose- and isomaltose-type trehazoloid analogues **169** and **170**, respectively (Chart 8).

Scheme 25[a]



[a] Reagents: (i) NaN_3, 84%; (ii) LAH, 89%; (iii) $(EtO)_2P(O)Cl$, Et_3N, 56%; (iv) NaH, $^nBu_4NBr$, then CS_2, 91%; (v) **93**, 94%; (vi) Mukaiyama's reagent (**105**), Et_3N, 72%; (vii) H_2, $Pd(OH)_2/C$, 57%.

Chart 8



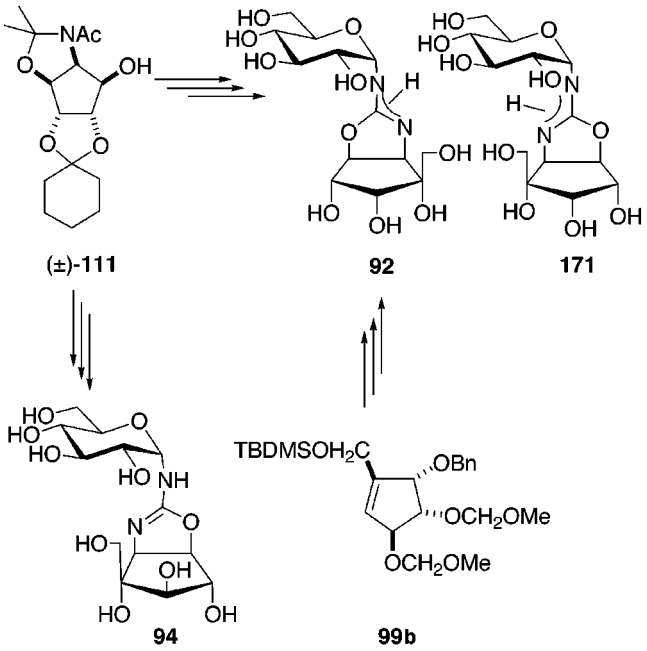
2. Modification of the Aminocyclopentitol

With a view to elucidate the absolute configuration of the natural trehalase inhibitor, Ogawa and co-workers[59c] synthesized the diastereomer **94** of trehalozin (**3**) (Chart 9), starting from the racemic polyhydroxylated aminocyclopentane diacetal **111**. The aminocyclitol **111** in question was obtained by these authors by chromatographic purification of a mixture of oxazolines, described in their synthesis of the natural isomer (see Section 111.B.2, Scheme 18), and coupled by the reported procedures to obtain the target diasteromer **94**.

A similar strategy as that employed for the synthesis of diastereomer **94** was used to obtain trehalostatin (**92**) (5-*epi*-trehazolin) and its diastereomer **171** (Chart 9).[69] These analogues proved simpler to synthesize than trehazolin (**3**) itself (see Section III.B.2), as the route, from intermediate **111**, to their aminocyclopentitol moieties did not require an inversion of configuration (at the C-5 position, trehazolin nomenclature), whereas that of the natural product did.

Trehalostatin (**92**) has also been synthesized by Shiozaki and co-workers[70] (Chart 9) from aminocyclopentitol **99b** (see Section III.B.1, Scheme 16), a minor compound isolated in the course of their total synthesis of trehazolin (**3**). The required aminocyclopentitol component was synthesized by a strategy identical to that reported by these authors for their synthesis of the natural product.

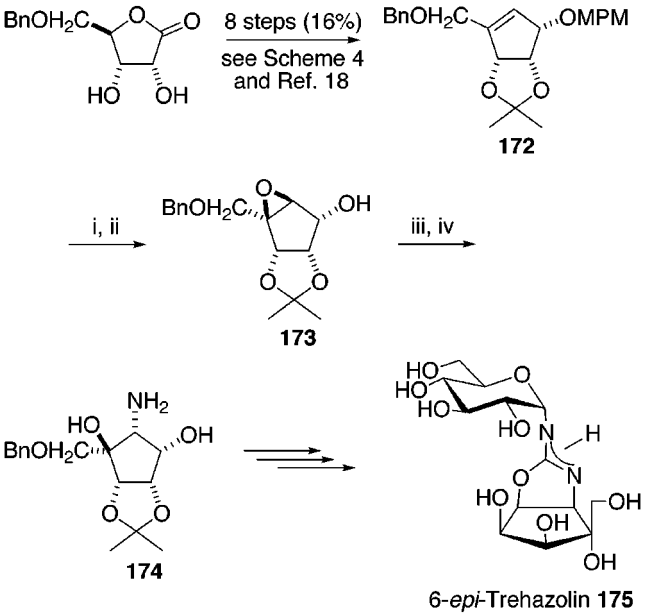
Chart 9



In pursuit of their goal to systematically evaluate the relative importance of the aminocyclitol substitution pattern on the biological activity of trehazolin, Ogawa's and Shiozaki's groups have synthesized a host of epimeric and deoxy analogues of the natural product.[65,71,72]

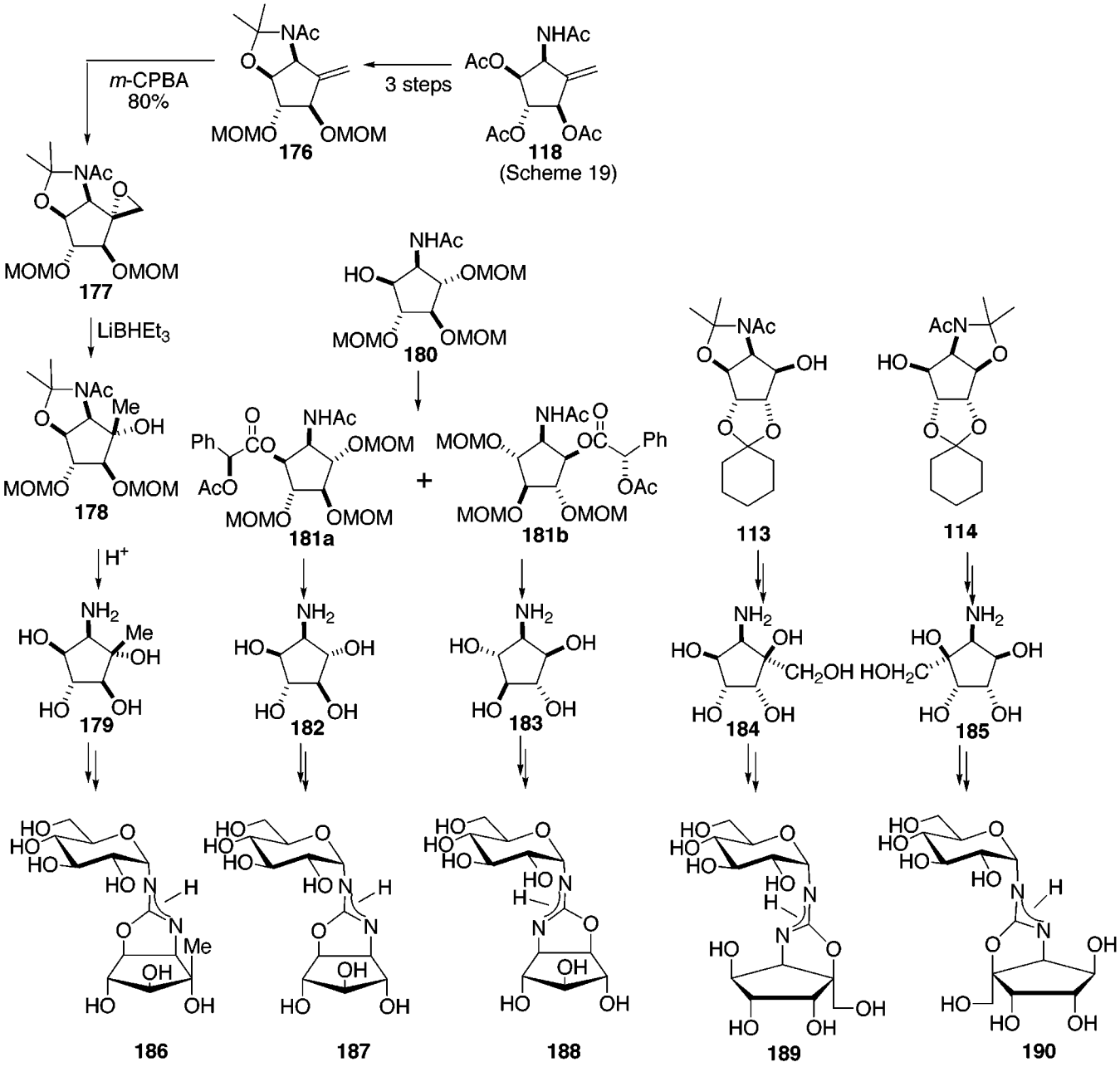
Shiozaki and co-workers[71] have reported the synthesis of 6-*epi*-trehazolin (**175**). A synthesis very similar to that published by Knapp and collaborators[61] (see Section III.B.4) was used to obtain the aminocyclopentitol moiety **174** (Scheme 26). Thus, D-ribonolactone was converted into carbocycle **172** by

Scheme 26[a]



[a] Reagents: (i) *m*-CPBA, 55%; (ii) DDQ, 99%; (iii) NaN_3, 99%; (iv) PPh_3, 97%.

Scheme 27



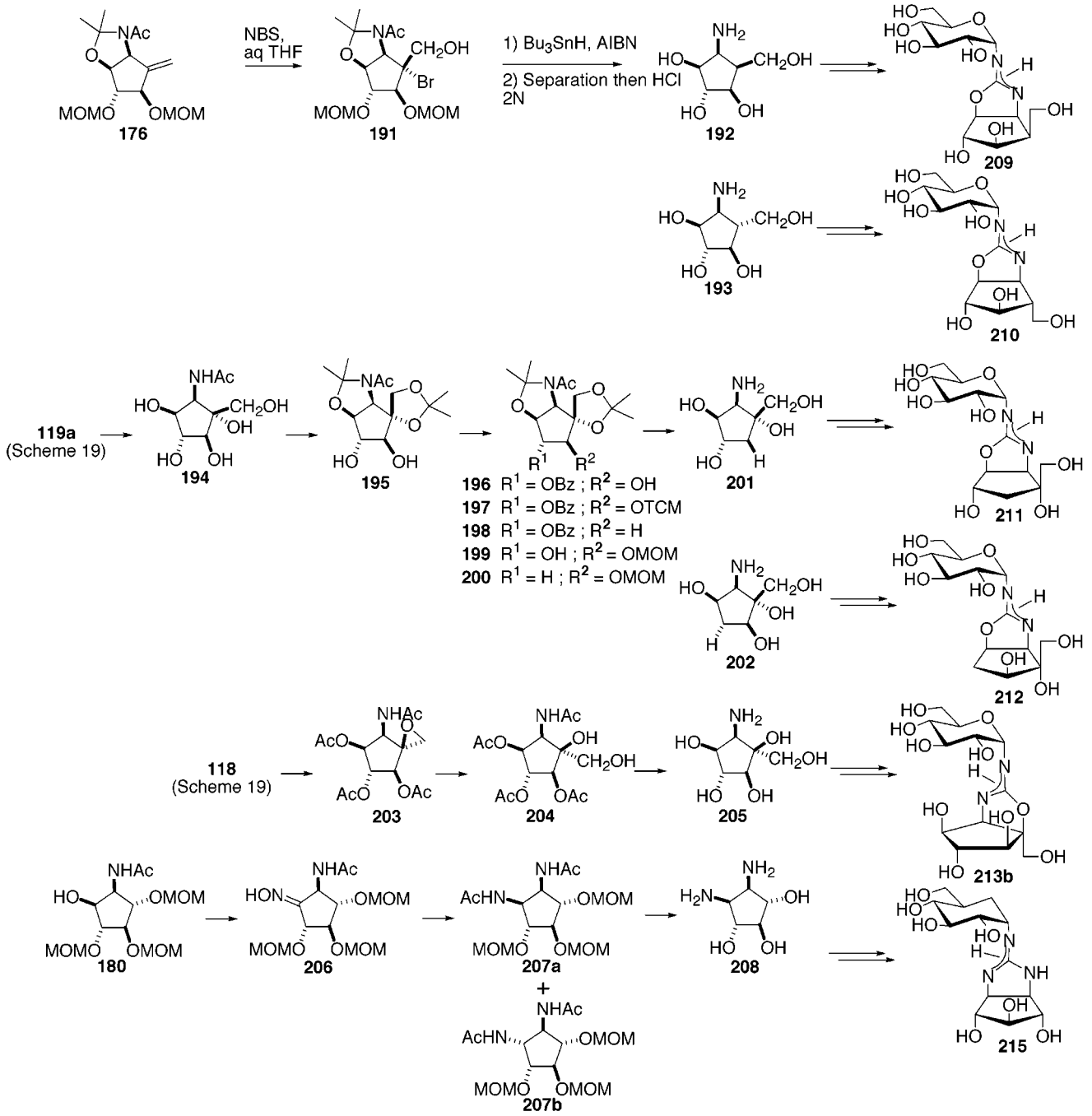
the reported method and then into the corresponding epoxide **173**. The amino group was introduced by nucleophilic ring opening of the latter with sodium azide followed by a Staudinger reduction to give the protected carbocycle **174**. Deprotection followed by conventional coupling gave the target 6-*epi* analogue **175** of trehazolin.

Ogawa's group[59d,65,72] have put the various undesired isomers encountered during their synthesis of trehazolin (**3**) and trehalostatin (**92**) to good use by preparing a number of novel trehazolin analogues from them. These pseudodisaccharides **186**−**190** feature modifications at the 4-hydroxymethyl function of the carbocyclic moiety (Scheme 27). The aminocyclopentitol **179** was obtained from the homochiral *exo*-methylenic cyclopentane **176** (Scheme 27). The latter was epoxidized to give intermediate **177** which was treated with lithium triethylborohydride to provide alcohol **178** as a mixture of rotamers. Acid hydrolysis led to deprotection to give the desired amino tetrol **179**, which was coupled by the usual procedure to give the target dehydroxy derivative **186**.

Carbocycles **182** and **183** were obtained from racemate **180** (Scheme 27). The latter was converted into the diastereomeric (*S*)-acetylmandelate esters **181a** and **181b**. These were purified by chromatography and each deprotected, in turn, to give their corresponding acetamido tetrols **182** and **183**. These aminocyclopentitols were coupled by classical procedures to give the respective de(hydroxymethyl)-trehazolin analogues **187** and **188**.

The aminocyclopentitols **184** and **185** were derived from the homochiral isopropylidene acetals **113** and **114** (Scheme 27). Each of these were converted into the corresponding polyhydroxylated acetamidocyclopentanes **184** and **185**, following the reported procedures (Scheme 19). The latter were transformed, again by the usual coupling method, into the target

Scheme 28



4,5-di-*epi*-trehazolin analogue **189** and its diastereomer **190**, respectively.

The deoxy analogues **209**−**212** and the 4-*epi*-4,6-rearranged product **213b** have also been described. These were obtained on coupling of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl isothiocyanate (**103**) [following the previously disclosed procedure (see Scheme 23)] with the aminocyclopentitols **192**, **193**, **201**, **202**, and **205**, respectively (Scheme 28).

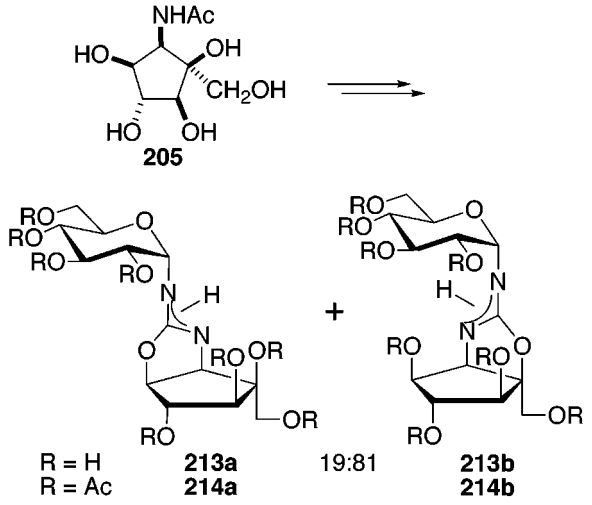
Tetrols **192** and **193** were derived from the *exo*-methylenic cyclopentane **176** (Scheme 28). Bromohydrination of the latter provided intermediate **191** as a mixture of rotamers. This mixture was debrominated and deprotected to give tetrols **192** and **193**, which were coupled, in turn, to give the corresponding target pseudodisaccharides **209** and **210**.

The deoxygenated tetrols **201** and **202** were derived from the intermediate **119a** via the polyhydroxylated acetamidocyclopentane **194** (Scheme 28). The latter was protected as its bis-isopropylidene acetal **195**. Steric factors favored the regioselective monobenzoylation of the latter to give alcohol **196**. This, on treatment with 1,1′-thiocarbonyldiimidazole gave the thionocarbamate **197**, which was reduced under free radical conditions to give the dehydroxy compound **198**. In a parallel route, alcohol **196** was protected as its methoxymethyl ether and then debenzoylated to give alcohol **199**. The latter compound was deoxygenated, as described above, to give the dehydroxy compound **200**. Both the dehydroxy compounds **198** and **200** were deprotected to give the corresponding aminocyclopentanes **201** and **202**, and these were

coupled to give the target pseudodisaccharides **211** and **212**, respectively.

Aminocyclopentitol **205** was derived from the *exo*-methylenic cyclopentane **118** (Scheme 28). Epoxidation of the latter and subsequent ring opening with sodium acetate, known to proceed with participation of the *N*-acetamido group,[59a] led exclusively to diol **204**, which gave alcohol **205** upon Zemplén deacetylation. From this was obtained the target pseudodisaccharide **213b** (also see later, Scheme 29).

Scheme 29



The novel guanidino-carba analogue **215** has also been described (Scheme 28). This was targeted, as coupling of the *meso*-diamine **208** with isothiocyanate **103** gave, on ring formation, an isourea which proved too labile for biological tests. The cyclic guanidine moiety in the corresponding 5a-carba analogue **215** was expected to remain intact. This carba analogue was deemed all the more apt to be targeted, as 5a′-carbatrehazolin (**144**) had been shown to possess a similar range of inhibitory activity to that of trehazolin.[63]

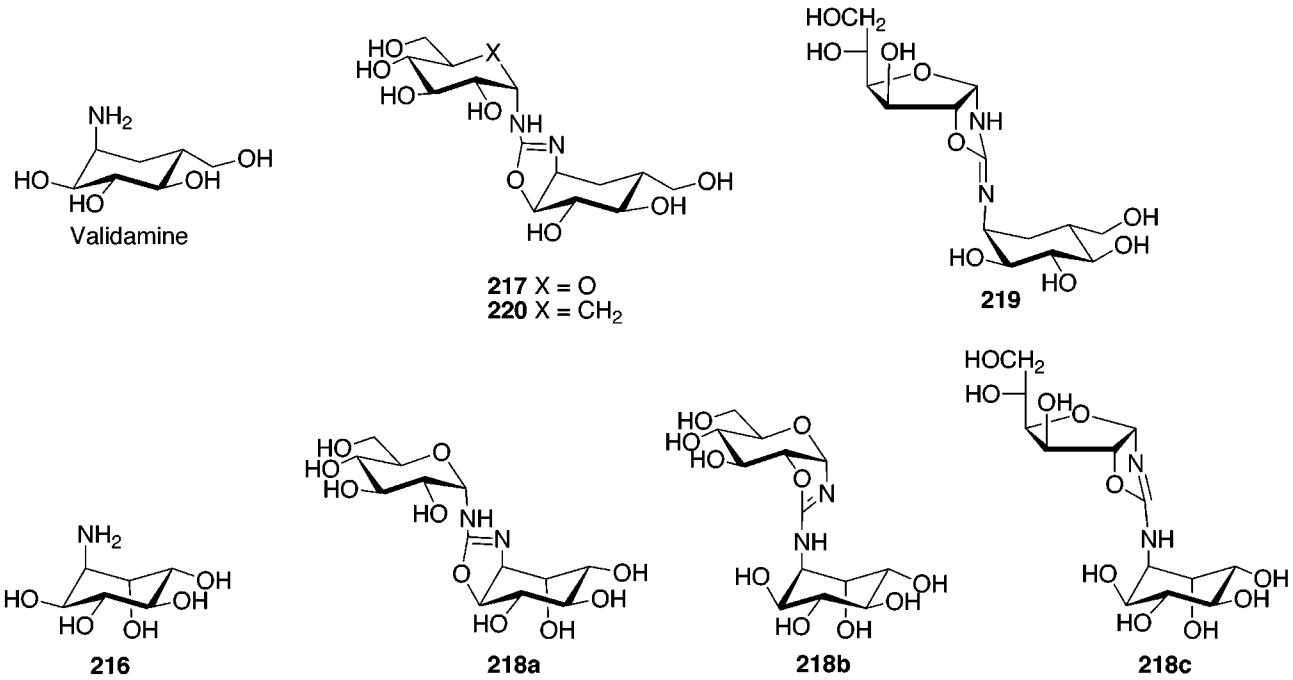
The aminocyclopentitol **208** required was derived from the racemic acetamidocyclopentane **180** (Scheme 28). This racemate was oxidized and the ketone, thus obtained, transformed immediately into its oxime **206**. Reduction followed by acetylation of this oxime **206** gave rise to two *bis*-acetamidocyclopentane isomers (**207a** and **207b**), which were separated. *meso*-Diaminocyclopentitol (**208**) was subsequently obtained from aminocyclopentitol **207a** and coupled by the classical procedure to give the corresponding pseudodisaccharide **215**.

Attempts to form an oxazoline from compound **205** failed, leading instead to a mixture of tautomers **213a** and **213b** (Scheme 29). These compounds were separated by chromatography as their per-*O*-acetates **214a** and **214b**, respectively. Deesterification of acetate **214b** allowed the tautomer **213b** to be obtained pure. Deprotection of acetate **214a** was accompanied by isomerization under the basic reaction conditions and gave the same tautomer **213b** as the major product.

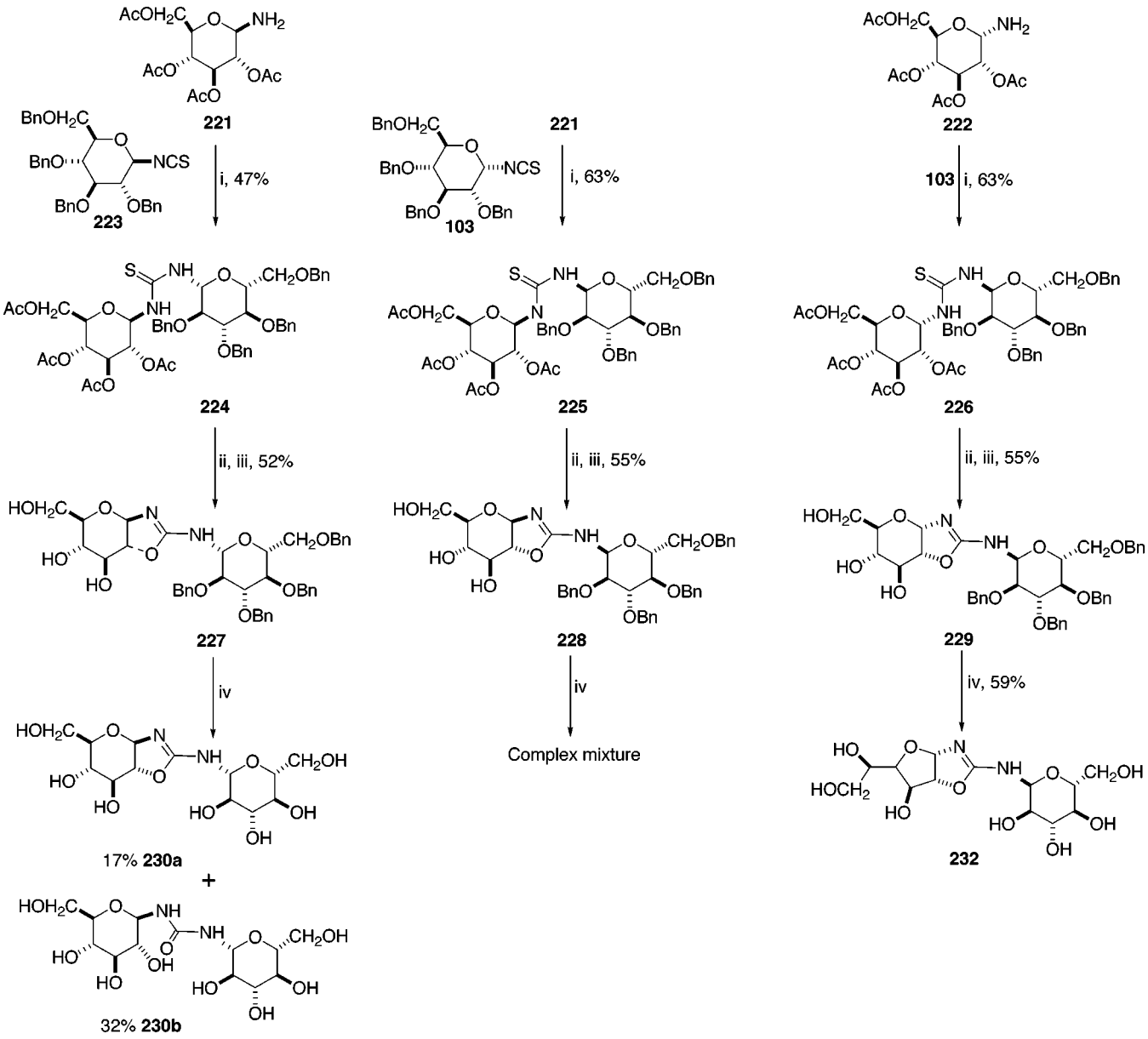
Both Ogawa's[65,72] and Shiozaki's[73] groups have undertaken the synthesis of a series of analogues wherein the aminocyclopentitol ring of trehazolin (**3**) is replaced either by the six-membered carbocycle validamine [constituent of validoxylamine A (see Chart 12), a strong in vitro trehalase inhibitor[74]] or by the aminocyclohexane **216**, with a view to obtaining the pseudodisaccharide analogues **217** and **218a**, respectively (Chart 10).

Reaction of the glucose-derived isothiocyanate **103** with validamine gave, not the target analogue **217**, but unexpectedly, the rearranged tetrahydrofuranic derivative **220** upon attempted deprotection. However, coupling of the carbocyclic glucose analogue **158** (see Section III.B.1, Scheme 23) with validamine, under identical conditions, did give the corresponding

Chart 10



Scheme 30[a]



[a] Reagents: (i) THF, 2 days; (ii) cat. KOH, EtOH, 10 min; (iii) 2-chloro-3-ethylbenzoxaline tetrafluoroborate (**105**), MeCN, 0 °C, 2 h, then Et_3N, 0 °C to rt; (iv) H_2, $Pd(OH)_2$, MeOH, 60 °C, 30 min.

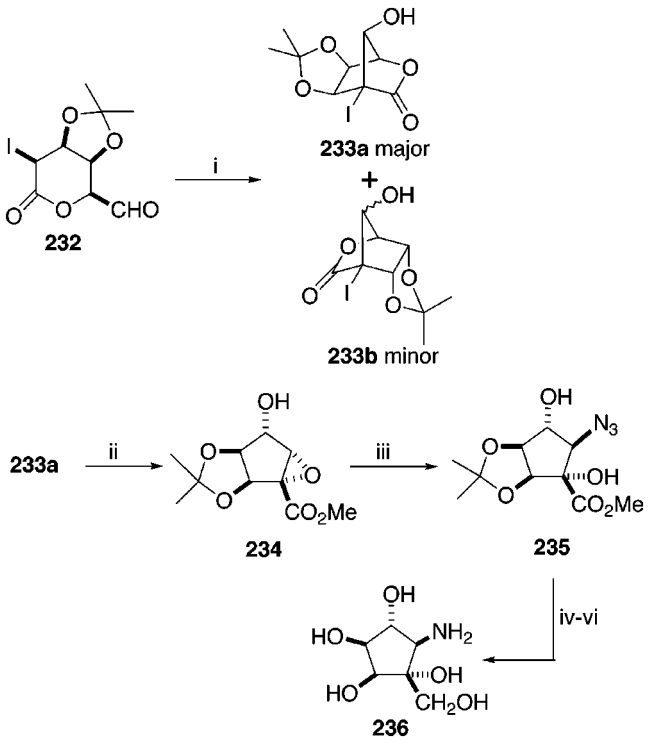
pseudodisaccharide **220**. Difficulties were also encountered in the coupling reaction between the isothiocyanate **103** and the polyhydroxylated aminocyclohexane **216**, which led to an inseparable mixture of the expected aminooxazoline **218a**, together with the rearranged pyranosidic and furanosidic products **218b** and **218c**, repectively, in a 1:1:1−2 ratio (Chart 10).

Shiozaki and co-workers[75] have attempted to prepare 5,6-ring-*trans*-fused-2-aminotetrahydropyrano-[2,3-*d*]oxazole derivatives **227**−**229**, wherein the cyclopentitol moiety in trehazolin (**3**) is replaced by a tetrahydropyran unit (Scheme 30). The α- or *β*-glucopyranosylamine derivative **221** or **222** when coupled with either glucopyranosyl isothiocyanate **103** or **223** gave the corresponding urea derivatives **224**−**226** respectively. These ureas were cyclized under Mukaiyama's coupling conditions to give the target oxazolines **227**−**229**, respectively. Unfortunately, these compounds proved to be rather unstable, derivative **230a** being the only one of the desired molecules to be obtained pure. Thus, deprotection of oxazoline **227** gave derivative **230a**, in an isolated yield of 17% from a mixture containing mainly the corresponding acyclic urea **230b**. The aminooxazoline **229** rearranged, upon attempted deprotection, to its more stable 5,5-ring-fused analogue **231**, and the diastereomer **228** decomposed to a complex mixture upon attempted deprotection.

3. Enantioselective Approaches to the Aminocyclopentitol

The enantioselective syntheses of a fair number of modified aminocyclopentitols related in structure to trehalamine (**93**) have been reported in the past decade. Not all were specifically conceived as analogues of the natural product. An exhaustive discussion of these syntheses is beyond the scope of this review, but a handful deserve to be commented upon here.

Scheme 31[a]

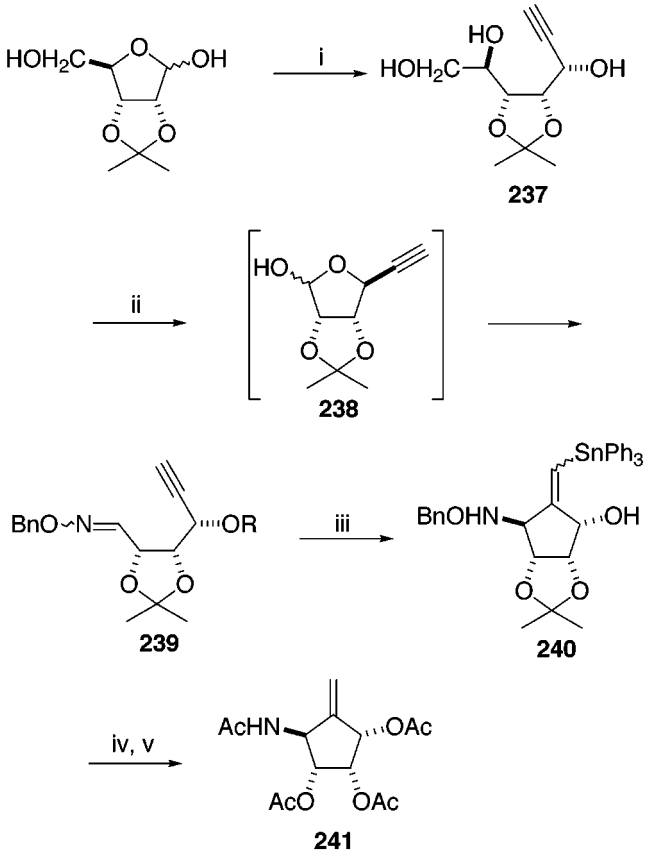


[a] Reagents: (i) KF, 18-crown-6 ether, −6 °C, 95%; (ii) K_2CO_3, MeOH, 100%; (iii) NaN_3, NH_4Cl aq, MeOH, 83%; (iv) $LiBH_4$, THF, 0 °C, 94%; (v) 40% aq CF_3CO_2H, 94%; (vi) H_2, Pd/C, H_2O, 100%.

Fleet and collaborators[76] have disclosed an access to aminocyclopentitol **236**. The carbocyclic skeleton was formed via the iodoformyllactone **232** (Scheme 31). Exposure of **232** to potassium fluoride in the presence of 18-crown-6 ether gave the tricyclic compound **233a** (the minor epimer **233b** was also isolated). The transformation presumably proceeds through a nonreductive aldol condensation. Reaction of isomer **233a** with potassium carbonate led to opening of the lactone. Subsequent displacement of the iodide by the vicinal alcohol gave epoxide **234**. The later was opened with sodium azide to give the polysubstituted cyclopentane derivative **235**. Reduction and deprotection gave the target aminocyclopentitol **236**.

Marco-Contelles and co-workers[77] have described the synthesis aminocyclopentitols, such as amino alcohol **241**, via a free radical cyclization of enantiomerically pure, sugar-derived, alkyne-tethered oxime ethers (Scheme 32). For example, 2,3-*O*-isopropylidene-D-ribose was converted into the hemiacetal **238** via a Grignard reaction followed by oxidative periodate cleavage of the intermediate diol **237**. In situ oximation of hemiacetal **238** gave the pivotal alkyne-tethered oxime **239**. The latter, on treatment with triphenyltin hydride in the presence of triethylborane, cyclized smoothly and with complete diastereoselectivity by a 5-*exo* process to give the carbocycle **240**. Protodestannylation and samarium(II) iodide-promoted reduction of the *O*-benzylhydroxylamine **240** followed by peracetylation gave the *exo*-methylenic cyclopentitol **241**, of potential as an intermediate in the synthesis of various trehazolin analogues (see Scheme 19 for one example).

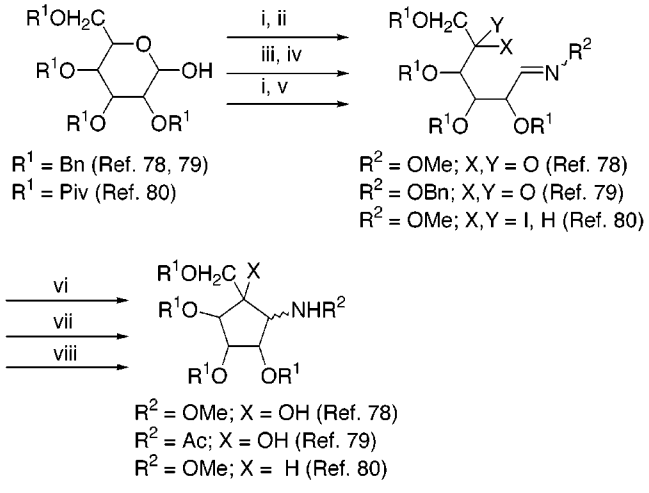
Scheme 32[a]



[a] Reagents: (i) HC≡CMg, 62%; (ii) $NaIO_4$, then $BnONH_2$, HCl, 86%; (iii) Ph_3SnH, Et_3B, 91%; (iv) HCl, EtOH, 92%; (v) SmI_2, then Ac_2O, 79%.

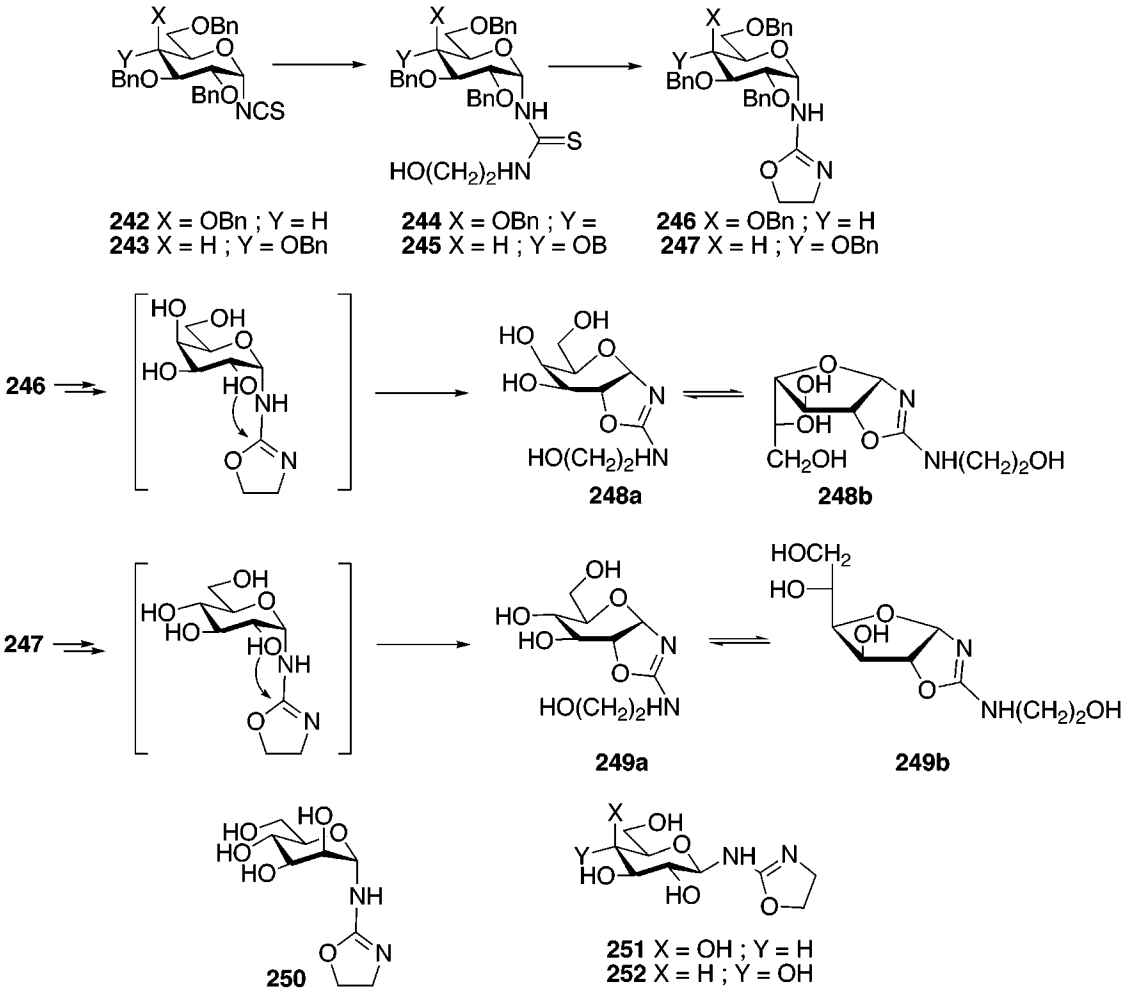
Kiguchi and co-workers[78] have disclosed a very straightforward access to aminocyclopentitols based on a tributyltin hydride-induced radical cyclization of a ketone-tethered oxime ether. Marco-Contelles and colleagues[79] have used samarium(II) iodide as the cyclization promotor in an adaptation of this approach, and Cronjé Grové and Holzapfel[80] have recently reported a similar cyclization of iodo oxime

Scheme 33[a]



[a] Reagents: (i) $MeONH_2$·HCl, pyridine; (ii) CrO_3-pyridine, pyridine; (iii) $BnONH_2$·HCl, pyridine, MeOH, 60 °C; (iv) PCC, 3 Å MS, NaOAc, CH_2Cl_2; (v) PPh_3, imidazole, I_2; (vi) Bu_3SnH, AIBN; (vii) SmI_2, THF, then H_2O, −25 °C to rt, then Ac_2O, pyridine; (viii) SmI_2, HMPA, THF.

Scheme 34



ethers (Scheme 33). The key intermediates in these related approaches were easily obtained by condensation of either *O*-benzylhydroxylamine or *O*-methylhydroxylamine with the appropriate fully protected pyranose and the hydroxyl group thereby released at C-5 (sugar nomenclature), either oxidized or converted into an iodide (Scheme 33).

4. Miscellaneous

Ogawa and collaborators[81] have reported a series of simplified analogues of trehazolin (**3**) in which the aminocyclopentitol ring has been replaced by cyclic isourea moieties. A more appropriate interaction of charge in the catalytic site of glycosidases was expected for these analogues compared with the parent compound (Scheme 34). Thus, the α-manno-, *β*-galacto-, and *β*-gluco-linked derivatives **250**−**252**, respectively, were obtained by coupling of the appropriate *β*-isothiocyanate derivative with 2-aminoethanol (Scheme 34). The same synthetic strategy when applied to the corresponding *β*-isothiocyanate derivatives failed to give the desired α-gluco- and α-galacto-type aminooxazolines **246** and **247**, respectively (Scheme 34). The latter compounds both rearranged spontaneously, as a result of participation of the equatorial C-2 hydroxy function (sugar nomenclature), to give mixtures of the corresponding 6,5-ring- or 5,5-ring-fused aminooxazolines.

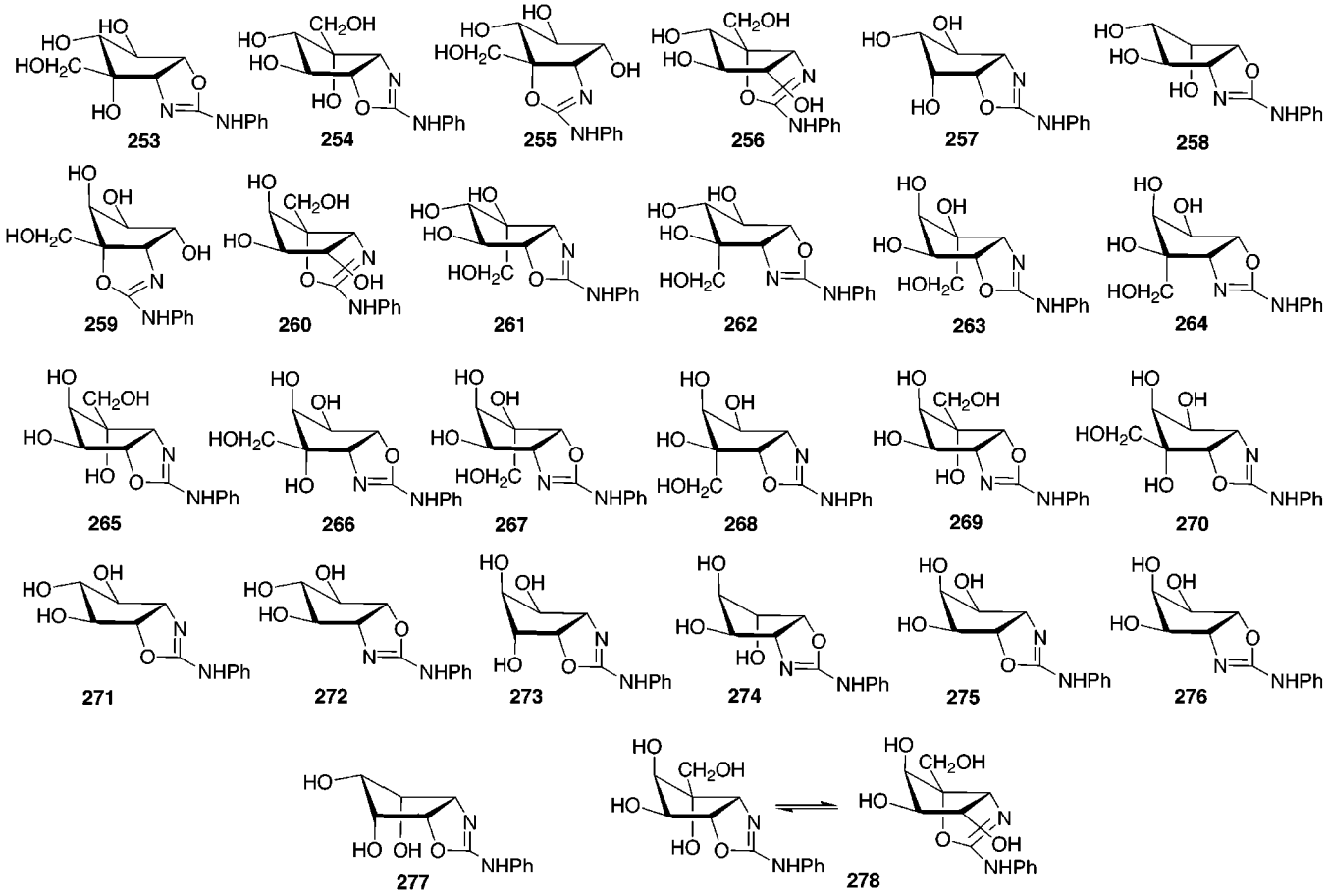
This same research group[82] has also reported a large number of simplified isourea derivatives (Chart 11). These analogues of trehalamine (**93**), in which the carbohydrate moiety of trehazolin (**3**) is absent, were synthesized using a variant of the strategy described in Scheme 34.

D. Biological Activity

Trehazolin (**3**) inhibits trehalases, enzymes which although widespread in nature are only rarely found in vertebrates. Trehalose (Chart 12), the natural substrate of this enzyme, is a unique nonreducing disaccharide comprising two glucose units linked 'head-to-head' and has, thus far, not been identified in vertebrates. As a consequence, inhibitors of trehalases have been recognized as having potential for selectively fighting pests and pathogens. The trehalase inhibitor validamycin A has already been exploited in the control of the pathogenic plant fungus *Rhizoctonia Solani*, the causative agent of blight sheath in rice.[74c] It is also noteworthy that the breakdown of trehalose by trehalase is a very early step in the biosynthesis of chitin (see also discussion in Section IV.F relating to biological activity of allosamidin (**4**)).

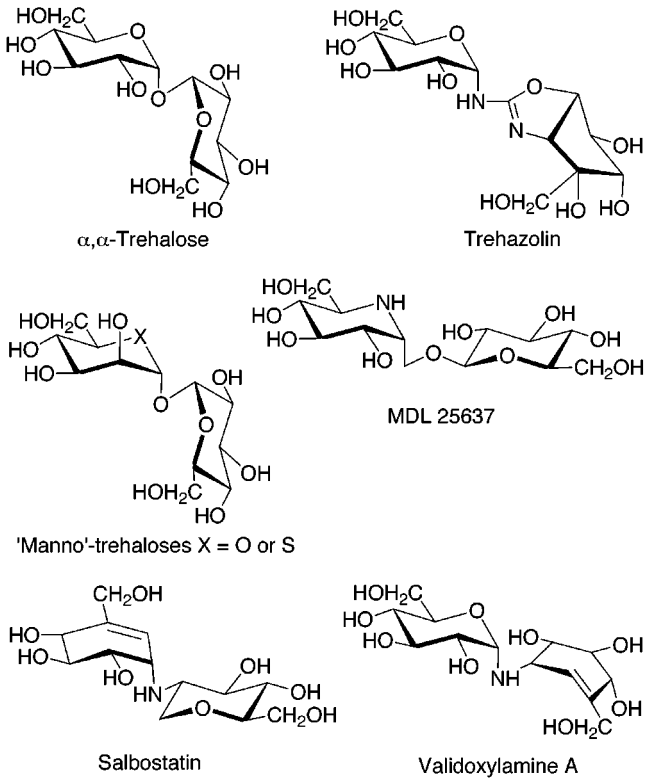
Although a number of natural and synthetic structures have been shown to inhibit trehalases, the majority of these [for example, MDL 25637 (Chart

Chart 11



12), nojirimycin, and castanospermine (Chart 1)] show limited selectivity and inhibit other glucose-recognizing enzymes such as isomaltase, sucrase, glucoamylase, maltase, α-amylase, and lactase (Table 2).[84] Trehazolin (**3**), on the other hand, shows marked specificity for trehalases as well as being potent and is thus of much more interest (Table 2). Other natural inhibitors which show selectivity for trehalase include validoxylamine A[74b,85] and salbostatin[86] (Chart 12, Table 2).

Chart 12



Trehazolin (**3**) is a pseudodisaccharide, and its structural resemblance to the disaccharide trehalose must serve to ensure its selective recognition by trehalases. That one of the two subsites in the enzyme efficiently binds α-D-glucopyranosyl-like structures is supported by studies with other inhibitors, and presumably, it is this subsite that is occupied by the α-glucopyranosylamine unit of trehazolin (**3**). The remaining subsite thus houses the catalytic machinery of the enzyme and has evolved to bind structures which mimic the high-energy intermediates implicated in the hydrolysis reaction.[26] The aminocyclopentitol and aminooxazoline portions of trehazolin (**3**) serve to mimic this latter intermediate. It should be noted that both halves of the inhibitor are essential for its tight binding to trehalases (Table 2).

Trehalases are inverting glycosidases and are an exception in that the vast majority of inverting enzymes are exoglycanases.[25a] Trehalose has an axial leaving group which is cleaved by the enzyme with inversion of configuration.[25a] This implies the presence in the active site of an acidic catalytic group in addition to a nucleophilic water molecule which is

Table 2. Survey of Biological Activity of Trehazolin and Its Analogues

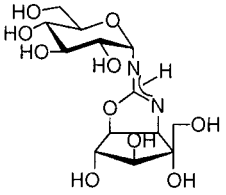
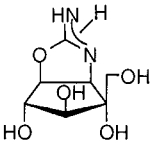
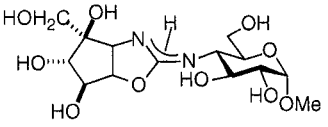
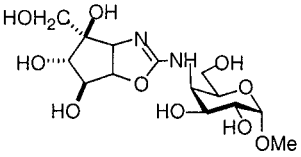
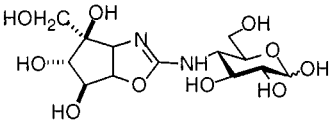
Compounds	Comments	Ref.
 trehazolin **3**	Very strong and specific inhibitor of trehalases: Silkworm trehalase $IC_{50} = 5.5\times10^{-9}$ mol dm^{-3} Porcine trehalase $IC_{50} = 3.7\times10^{-9}$ mol dm^{-3} Blowfly trehalase $IC_{50} = 0.09\times10^{-9}$ mol dm^{-3} (3800 times more potent than deoxynojirimicin) Pig kidney trehalase $IC_{50} = 0.09\times10^{-9}$ mol dm^{-3} [Compare with other inhibitors of pig kidney trehalase (IC_{50}): validoxylamine A = 2.4×10^{-9}; MDL 25637 = 1.9×10^{-7}; castanospermine = 2.5×10^{-6}; deoxynojirimycin = 4.3×10^{-6}; validamycin A = 2.5×10^{-4} mol dm^{-3}]. Only very weak inhibition of yeast α-glucosidase, almond β-glucosidase and rat isomaltase. No activity (at 500 μM) against rat intestinal maltase or sucrase, sweet potato β-amylase, bovine *N*-acetyl-glucosaminidase, *S. griseus* chitinase, yeast invertase, *Penicillium* exo-β-1-3-glucanase or *Rhyzopus niveus* amyloglycosidase. No inhibition observed against β-glucosidase, α-mannosidase, α–galactosidase, β-galactosidase, β-xylosidase, α-fucosidase, β-*N*-acetylglucosaminidase, β-glucuronidase, α-amylase or sialidase.	55a, 56, 88, 89
 trehalamine **93** (Basic structure)	Only a weak inhibitor of silkworm or porcine trehalase (6700 and 27 times less active than trehazolin, respectively). Weak activity against yeast α-glucosidase and *Agrobacterium* β-glucosidase. Very weak activity against rat maltase, rat sucrase and yeast maltase. No activity against β-glucosidase, α-mannosidase, α–galactosidase, β-galactosidase, β-xylosidase, α-fucosidase, β-*N*-acetylglucosaminidase, β-glucuronidase, α-amylase or sialidase. The glucose moiety of trehazolin is essential for the tight binding with trehalases.	61, 67, 88
 (1→4)-linked «trehazoloid» **164**	Designed to inhibit (1→4) glucosidases: only moderate competitive inhibition of yeast α-glucosidase and *Agrobacterium* β-glucosidase was observed. No activity against trehalases.	61, 67, 68
 (1→4)-linked «trehazoloid» **168**	Designed to inhibit α-glucosidases: no inhibitory activity observed against various enzymes.	67, 68
 (1→4)-linked «trehazoloid» **169**	Maltose-like compound: no activity against rat maltase, isomaltase, sucrase or silkworm trehalase except very weak activity against porcine trehalase (IC_{50} = 0.245 μg mL^{-1}) and yeast α-glucosidase.	67, 68

Table 2 (Continued)

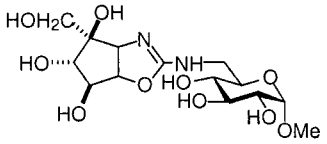
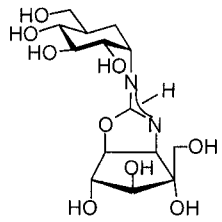
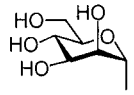
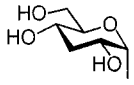
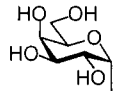
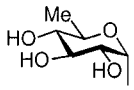
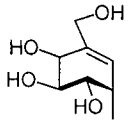
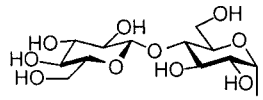
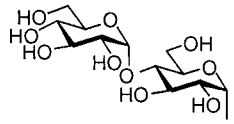
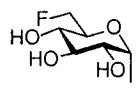
 (1→6)-linked «trehazoloid»**170**	Isomaltose-like compound: weak inhibitor of yeast maltase and rat maltase, isomaltase and sucrase although more potent than trehazolin.	67, 68
Modification of the glucose moiety		
 5a'-carbatrehazolin **144**	Very strong silkworm trehalase inhibitor (IC_{50} = 49×10^{-9} mol dm^{-3}). As potent inhibitor as trehazolin but more stable towards isomerization than the parent compound. No antifungal activity against *Rhizoctonia solani.*	63, 64
 2'-*epi*-trehazolin (mannose type)**146**	Only weak activity against jack bean α-mannosidase. 1000 times less potent than trehazolin against silkworm trehalase.	64, 81
 3'-deoxytrehazolin **147**	100 times less active than trehazolin against silkworm trehalase.	64, 81
 4'-*epi*-trehazolin (galactose type) **148**	No activity observed against various glycosidases except for a weak one against yeast α–galactosidase. 10000 times less active than trehazolin against silkworm trehalase.	64, 81
 6'-deoxytrehazolin **149**	100 times less active than trehazolin against silkworm trehalase.	64
 valienamine type **145**	Validoxylamine A-like conformation; the ring is flattened compared to the 5'a-carba analog **144**. 10 times less active than trehazolin against silkworm trehalase.	64
 4'-*O*-(β-D-glucopyranosyl)trehazolin (cellobiosyl type) **151**	Analogously to validamycin A, this compound was designed to increase antifungal activity by enhancing uptake into cell. It was 10 times less active than trehazolin against silkworm trehalase but was more effective towards *Rhizoctonia solani.*	64
 4'-*O*-(α-D-glucopyranosyl)trehazolin **150**	100 times less active than trehazolin against silkworm trehalase. The 4'-*O*-glucopyranosyl residue was thought to hinder the interaction between the active core and its enzymatic site.	64
 6'-fluoro-6'-deoxytrehazolin **152**	Strong trehalase inhibitor (only 10 times weaker than trehazolin).	65

Table 2 (Continued)

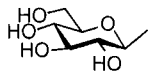
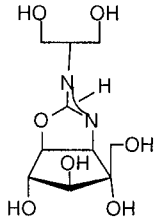
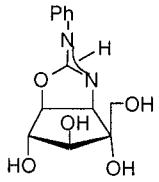
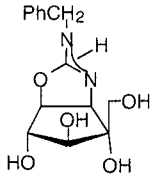
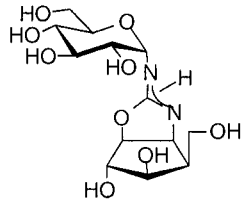
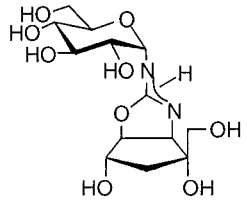
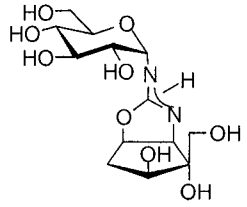
 trehazolin β anomer **155**	Strong inhibitor of porcine trehalase [IC_{50} = 8.0×10^{-9} mol dm^{-3} (only 2 times less potent than trehazolin)]. Weak inhibitor of silkworm (18 times less potent than trehazolin). Also shows some weak activity against yeast maltase.	66, 67
 AO128-like compound **156**	Weak inhibitor of trehalases. Same range of activity against rat maltase or sucrase as trehazolin.	67
 **153**	Expected to be active against α- and β-glucosidases: the oxazole ring was intended to mimic the charge distribution expected for putative hydrolysis intermediate. Inhibitor of yeast α-glucosidase (IC_{50} = 1.3×10^{-6} mol dm^{-3}). Only very weak activity against silkworm trehalase compared with trehazolin. No activity against α-galactosidase, β-glucosidase or α-mannosidase.	64
 **154**	Same observations as for analogue **153**: active against yeast α-glucosidase (IC_{50} = 4.8×10^{-7} mol dm^{-3}).	64
Modification of the pseudosugar moiety		
 4-deoxytrehazolin **209**	1000 times less active than trehazolin against silkworm trehalase.	65
 5-deoxytrehazolin **211**	1000 times less active than trehazolin against silkworm trehalase.	65
 6-deoxytrehazolin **212**	No activity.	65

Table 2 (Continued)

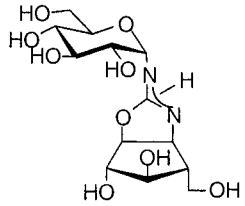
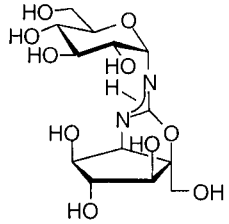
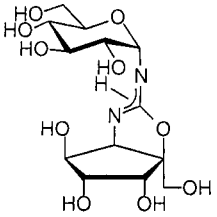
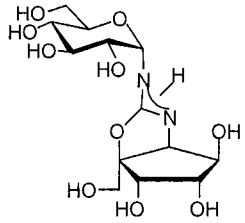
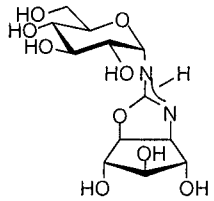
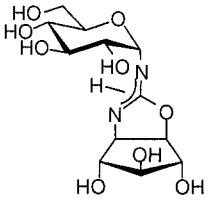
 4-deoxy-4-*epi*-trehazolin **210**	No activity.	65
 «4-*epi*-trehazolin» (rearranged product) **213b**	10 times less active than trehazolin against silkworm trehalase.	65, 67
 «4,5-di-*epi*-trehazolin» (rearranged product) **189**	20 times less active than trehazolin against silkworm trehalase.	59d, 65
 **190** (**189** diastereomer)	600 times less active than trehazolin against silkworm trehalase.	59d, 65
 4-de(hydroxymethyl)trehazolin **187**	100 times less active than trehazolin against silkworm trehalase.	65, 72
 **188** (**187** diastereomer)	10 times less active than trehazolin against silkworm trehalase. This derivative, having an unnatural configuration at its pseudosugar moiety, is more potent than its diastereomer **187**.	65, 72

Table 2 (Continued)

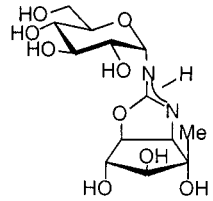
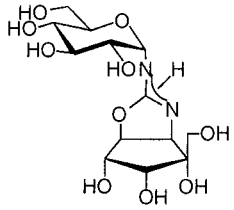
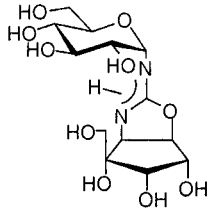
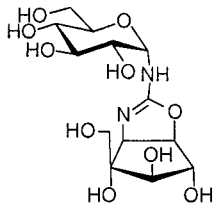
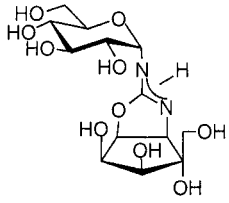
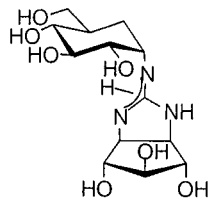
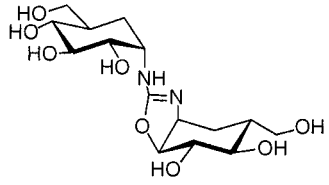
 4-de(hydroxymethyl)-4-methyltrehazolin **186**	Only weak activity against trehalases.	72
 5-*epi*-trehazolin (trehalostatin) **92**	No activity against trehalases. Weak activity against rat isomaltase.	59d, 69, 70, 67
 trehalostatin diastereomer **171**	No activity against trehalases.	59d, 69
 trehazolin diastereomer **94**	Weak inhibitor of silkworm trehalase (IC_{50} = 1.22×10^{-7} mol dm^{-3}) and strong inhibitor of porcine trehalase (IC_{50} = 9.8×10^{-9} mol dm^{-3}), about one third of the activity of trehazolin is preserved.	59c, 59d, 66
 6-*epi*-trehazolin **175**	No activity against silkworm trehalase. Only a poor inhibitor of porcine trehalase although an inhibitor of various α-glycosidases such as rat maltase, isomaltase and sucrase.	67
 guanidino-5'a-carba-analogue **215**	1000 times less potent than trehazolin against silkworm trehalase.	65
 validoxylamine type 5'a-carba-analogue **220**	Not active against silkworm trehalase. Cyclic isourea seems unable to mimic the charge distribution at the transition state when cyclopentane skeleton is replaced by a cyclohexane ring.	65

Table 2 (Continued)

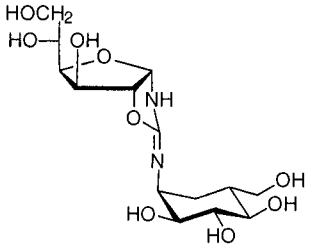
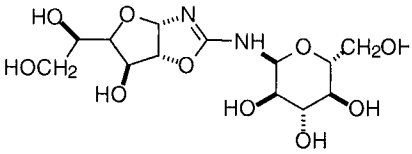
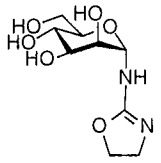
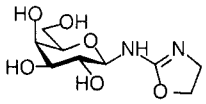
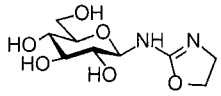
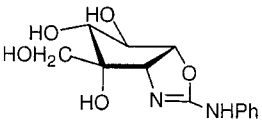
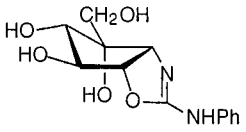
 validoxylamine type analogue (rearranged product) **219**	No activity observed against silkworm or porcine trehalases nor rat isomaltase.	72
cyclohexane type analogue **218a**:**218b**:**218c** (diastereomeric mixture) (see Figure 10 for precise structures)	No activity against trehalase although the structures are closely related to trehazolin.	73
tetrahydropyranic analogue: mixture of **230a** and **230b** (see Scheme 30 for precise structures)	No activity against various glycosidases.	75
 analogue **232**	No activity against various glycosidases.	75
 mannose-like isourea derivative **250**	Not active against various glycosidases. Only weak activity against jack bean α-mannosidases.	81
 galactose-like isourea derivative **251**	Not active against various glycosidases especially *E. coli* and bovine liver β-galactosidases.	81
 glucose-like isourea derivative **252**	Very weakly active against various glycosidases especially almond β-galactosidases.	81
 *N*-phenylcyclic isourea analogue **253**	Weak inhibitor of yeast α-glucosidase and bovine liver β-galactosidase.	82a, 82b
 *N*-phenylcyclic isourea derivative **254** (**253** enantiomer)	Shows very strong specific activity against yeast α-glucosidase (IC_{50} = 2.9×10^{-8} mol dm^{-3}).	82a, 82b

Table 2 (Continued)

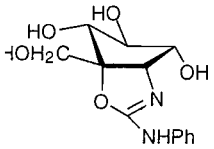
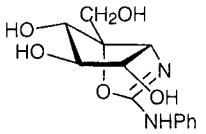
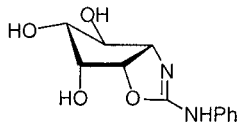
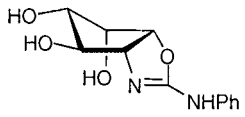
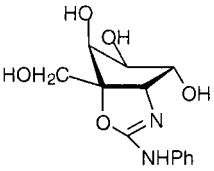
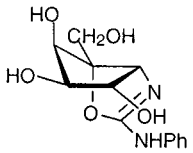
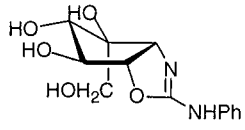
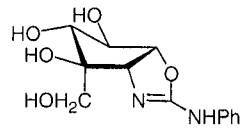
 *N*-phenylcyclic isourea derivative **255**	Strong inhibitor of yeast α-glucosidase and bovine liver β-galactosidase.	82a, 82b
 *N*-Phenylcyclic isourea derivative **256** (**255** enantiomer)	Shows very strong specific activity against yeast α-glucosidase (IC_{50} = 7.6×10^{-9} mol dm^{-3}).	82a, 82b
 *N*-Phenylcyclic isourea derivative **257**	Strong inhibitor of almond β-galactosidase and *E. coli* β-galactosidase (IC_{50} = 2.2×10^{-6} mol dm^{-3} and IC_{50} = 2.0×10^{-7} mol dm^{-3}, respectively).	82a, 82b
 *N*-Phenylcyclic isourea derivative **258** (**257** enantiomer)	Strong inhibitor of almond β-galactosidase and *E. coli* β-galactosidase (IC_{50} = 6.4×10^{-6} mol dm^{-3} and IC_{50} = 9.4×10^{-7} mol dm^{-3}, respectively).	82a, 82b
 *N*-Phenylcyclic isourea derivative **259**	Strong inhibitor of almond β-galactosidase and bovine liver β-galactosidase (IC_{50} = 4.9×10^{-6} mol dm^{-3} and IC_{50} = 5.7×10^{-7} mol dm^{-3}, respectively).	82a, 82b
 *N*-Phenylcyclic isourea derivative **260** (**259** enantiomer)	Strong inhibitor of bovine liver β-galactosidase (IC_{50} = 2.9×10^{-6} mol dm^{-3}).	82a, 82b
 *N*-Phenylcyclic isourea analogue **261**	Inhibitor of baker's yeast α-glucosidase (IC_{50} = 1.3×10^{-6} mol dm^{-3}).	82b
 *N*-Phenylcyclic isourea derivative **262** (**261** enantiomer)	Inhibitor of jack bean α-mannosidase (IC_{50} = 7.9×10^{-5} mol dm^{-3}).	82b

Table 2 (Continued)

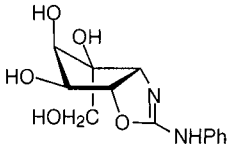
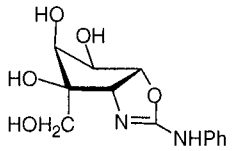
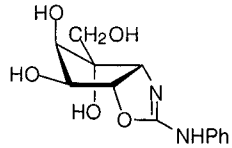
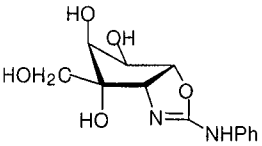
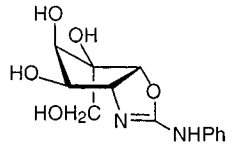
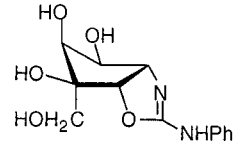
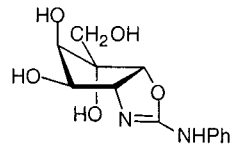
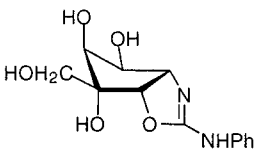
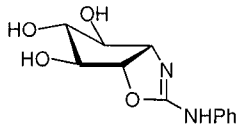
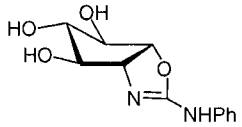
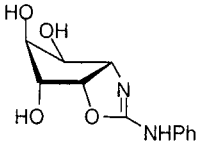
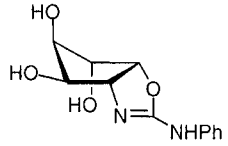
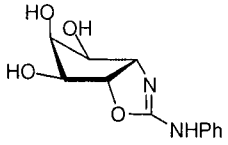
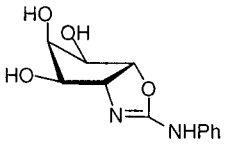
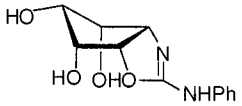
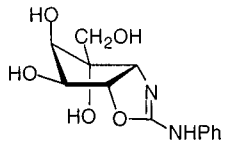
 *N*-Phenylcyclic isourea analogue **263**	Inhibitor of almonds β-glucosidase and of bovine liver β-galactosidase. (IC_{50} = 5×10^{-5} mol dm^{-3} and IC_{50} = 5.8×10^{-5} mol dm^{-3}, respectively).	82b
 *N*-Phenylcyclic isourea derivative **264** (**263** enantiomer)	Same activity as its enantiomer. (IC_{50} = 7.2×10^{-5} mol dm^{-3} and IC_{50} = 8.6×10^{-6} mol dm^{-3}, respectively).	82b
 *N*-Phenylcyclic isourea analogue **265**	Inhibitor of almond β-glucosidase, *E. coli* α-galactosidase and bovine liver β-galactosidase.	82b
 *N*-Phenylcyclic isourea derivative **266** (**265** enantiomer)	Same activity as its enantiomer more pronounced against almond β-glucosidase (IC_{50} = 4.8×10^{-5} mol dm^{-3}).	82b
 *N*-Phenylcyclic isourea analogue **267**	No activity.	82b
 *N*-Phenylcyclic isourea derivative **268** (**267** enantiomer)	No activity.	82b
 *N*-Phenylcyclic isourea analogue **269**	No activity.	82b
 *N*-Phenylcyclic isourea derivative **270** (**269** enantiomer)	Good inhibitor of *E. coli* α-galactosidase (IC_{50} = 4.6×10^{-5} mol dm^{-3}).	82b

Table 2 (Continued)

 *N*-Phenylcyclic isourea analogue **271**	Good inhibitor of almond β-glucosidase.	82b
 *N*-Phenylcyclic isourea derivative **272** (**271** enantiomer)	Inhibitor of almond β-glucosidase.	82b
 *N*-Phenylcyclic isourea analogue **273**	Good inhibitor of baker's yeast α-glucosidase. Some activity against bovine liver β-galactosidase.	82b
 *N*-Phenylcyclic isourea derivative **274** (**273** enantiomer)	Good inhibitor of bovine liver β-galactosidase. Some activity against baker's yeast α-glucosidase.	82b
 *N*-Phenylcyclic isourea analogue **275**	Inhibitor of jack bean α-mannosidase and of bovine liver β-galactosidase.	82b
 *N*-Phenylcyclic isourea derivative **276** (**275** enantiomer)	Slightly less potent than its enantiomer against jack bean α-mannosidase and of bovine liver β-galactosidase. Shows some activity against almond β-glucosidase.	82b
 *N*-Phenylcyclic isourea analogue **277**	Good inhibitor of *E. coli* β-galactosidase ($IC_{50} = 4.2\times10^{-5}$ mol dm^{-3}).	82b
 *N*-Phenylcyclic isourea analogue **278**	Strong inhibitor of bovine liver β-galactosidase and of jack bean α-mannosidase ($IC_{50} = 2.4\times10^{-7}$ mol dm^{-3} and $IC_{50} = 7.8\times10^{-5}$ mol dm^{-3}, respectively). Weak inhibitor of almond β-galactosidase.	82a, 82b

held close to the bond to be hydrolyzed and which interacts with a basic catalytic group.[25a] The inhibition of trehalases by trehazolin (**3**) is of the reversible, competitive type with respect to trehalose.[87,88] Thus, it is plausible that the exocyclic nitrogen of trehazolin (**3**) interacts with the acid catalytic group of the enzyme and mimics the exocyclic oxygen in the substrate (cf. mode of inhibition of glycosylamines).[4a] The endocyclic nitrogen then mimics the endocyclic oxygen (of the substrate), and the oxygen of the aminooxazoline, the nucleophilic water molecule. That it is the endocyclic nitrogen of the aminooxazo-

line ring in trehazolin (**3**) which mimics this water molecule, a variant of this same scenario, is yet another possibility. The importance of a surrogate for the nucleophilic water molecule is highlighted by a number of compounds which seem to owe their inhibition of trehalases solely to the presence of a structural element which mimics this feature [cf. the axial 2-OH group in the potent competitive trehalase inhibitors, α-mannopyranosyl α-D-glucopyranoside, and its thioglycoside analogue (Chart 12)].[89,25a]

The carbocyclic moiety serves to mimic the putative glucopyranosyl high-energy intermediate implicated in the hydrolysis reaction, in common with polyhydroxypyrollidines, several of which inhibit certain glycosidases more potently than their six-membered counterparts (see also discussion in Section II.D).[4,26a] However, trehalamine (**93**) alone is a weaker inhibitor of trehalases than trehazolin and markedly less specific than the pseudodisaccharide (see Table 2). A further interesting feature is the quaternary center present in trehalamine (**93**), which allows an additional hydroxyl group to be integrated into the inhibitor and could thereby further increase its opportunities to bind to the enzyme. Valiolamine, a cyclohexitol sucrase inhibitor, possesses this structural motif which is rare in glycosidase inhibitors.

An additional characteristic of trehazolin (**3**) is that its two 'halves' are separated by three atoms, and not one as in trehalose. This latter feature may well serve to mimic the bond to be cleaved (which would lengthen as hydrolysis of the substrate proceeded) and thereby further liken the inhibitor to a putative transition-state intermediate. The glucosamino fragment in trehazolin (**3**) deserves further comment. This motif, in isolation, is chemically rather reactive and rarely occurs in natural glycosidase inhibitors: nojirimycin, for example, which possesses the chemically related amino alcohol function, decomposes fairly rapidly under physiological conditions as does the unnatural inhibitor glucosylamine.[4a] The aminooxazoline group thus also serves to procure the chemical integrity of trehazolin (**3**) by effectively modifying the chemical reactivity of the glucopyranosyl unit of the inhibitor.

An important feature of the inhibition of trehalases by trehazolin (**3**) is that its onset is slow.[88] Other slow, tight-binding inhibitors include castanospermine (with isomaltase)[48] and the trehalase inhibitor validoxylamine A.[85] Kinetic studies with silkworm trehalase have shown that the association and dissociation of the inhibitor enzyme complex are extremely slow (dissociation constant at 37 °C = 7.3×10^{-2} h^{-1}) even when compared to castanospermine.[88] The kinetic data was proposed not to support the covalent attachment of the inhibitor to the enzyme (even of an intermittent, reversible kind). This would in principle be possible as the aminooxazoline moiety can be considered as an electrophilic locus, and such reactivity has already been reported for allosamidin (**4**) which shares the aminooxazoline motif (see Section IV.F).

Many of the aminocyclopentitol and pseudodisaccharide analogues discussed in Section III.C have been tested as inhibitors of trehalases as well as of several other glycosidases. Trehazolin (**3**) and trehalamine (**93**) have also served as leads in the design of inhibitors of a host of novel synthetic inhibitors. The 1,6-linked 'trehazoloid' analogue **170**, for example, an isomaltose-like compound, shows better inhibition of isomaltase than trehazolin (**3**), albeit weak (see Table 2). More striking still are the simplified *N*-phenyl cyclic isourea analogues **254** and **256** which were designed to inhibit α-glucosidases and do so rather well (IC_{50}/M of ca. 10^{-8}, see Table 2).

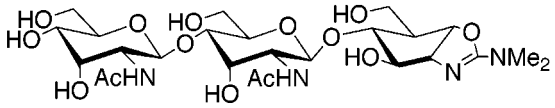
The consequences of the various structural modifications on inhibitory activity of the numerous analogues are too extensive to be commented upon here in detail. The inhibition data is instead presented in tabulated form (see Table 2).

IV. Allosamidins

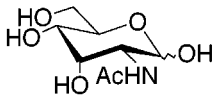
A. Isolation and Elucidation of Structure

In 1986, Suzuki and collaborators,[90] while screening metabolites of actinomycetes, reported the discovery of a potent chitinase inhibitor which they isolated from the mycelial extract of *Streptomyces* sp. No. 1713. Chemical and spectroscopic data as well as degradation studies were used in the elucidation of its novel structure which was shown to be that of a pseudotrisaccharide containing two β-linked *N*-acetyl-2-amino-2-deoxy-D-allopyranoside units. This novel disaccharide is linked to an aminocyclopentitol moiety at its reducing end. The new inhibitor was named allosamidin (**4**) (Chart 13). *N*-Acetyl-D-allosamine

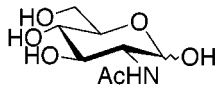
Chart 13



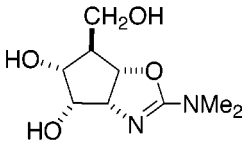
4 Allosamidin



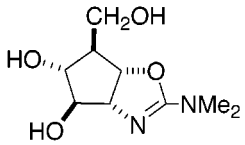
279 *N*-Acetyl-D-allosamine



280 *N*-Acetyl-D-glucosamine



281 Original structure proposed for **282**



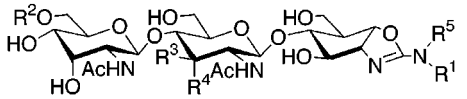
282 allosamizoline

(**279**) is the C-3 epimer of *N*-acetyl-D-glucosamine (**280**) and was previously unknown in nature. The cyclopentitol component features a novel (dimethylamino)oxazoline function, and its relative configuration at C-2, -3, and -4 was initially thought to correspond to that of D-allosamine. Thus, Suzuki and co-workers[90] assigned the structure **281** to the new pseudosugar and named it allosamizoline. More detailed NMR studies revealed however that its relative stereochemistry was actually that of *N*-acetyl-D-glucosamine (**280**), and the original structure was revised to that of aminocyclopentitol **282** but the

name allosamizoline nevertheless retained.[91] The relative stereochemistry was subsequently confirmed by chemical synthesis (see Sections IV.C.1 and IV.E.1). The absolute configuration of the aminocyclopentitol moiety was elucidated to be (−)-allosamizoline (**282**) by the exciton chirality method using its 3,4-bis[*p*-(dimethylamino)benzoyl]-6-trityl derivative[92] and subsequently unequivocally established by chemical synthesis (see Section IV.C.9). In 1987, details were reported of an improved method of screening for chitinase inhibition.[93] Application of the protocol to some 3000 fermentations revealed a potent chitinase inhibitor which they designated A82516 and showed to be identical in structure to allosamidin (**4**).

The discovery of methylallosamidin (**283**) was reported shortly afterward[94] (Chart 14). It differs in

Chart 14



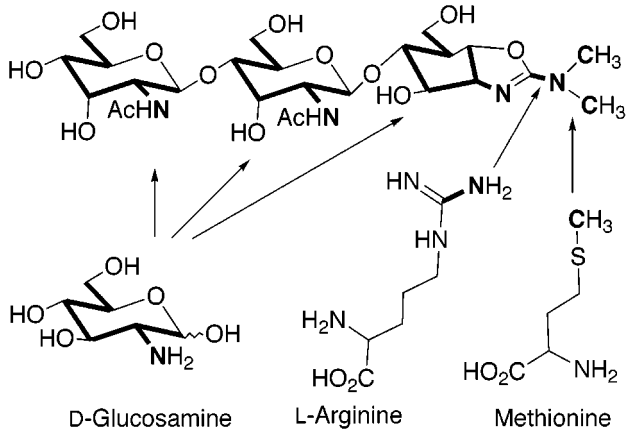
283 Methylallosamidin	R^1, R^2, R^5 = Me; R^3 = H; R^4 = OH
284 *N*-Demethylallosamidin	R^1, R^2, R^3 = H; R^4 = OH; R^5 = Me
285 Methyl-*N*-demethylallosamidin	R^1, R^3 = H; R^2, R^5= Me; R^4 = OH
286a Glucoallosamidin A	R^1, R^2, R^5 = Me; R^3 = OH; R^4 = H
286b Glucoallosamidin B	R^1, R^4 = H; R^2, R^5= Me; R^4 = H
287 Didemethylallosamidin	R^1, R^2,R^3, R^5 = H; R^4 = OH

structure from allosamidin (**4**) only in that its terminal *N*-acetyl-D-allosamine unit is 6-*O*-methylated. Demethylallosamidin (**284**) has also been identified in the mycelial extracts of *Streptomyces*. It differs in structure from the parent molecule **4** in that its aminooxazoline function is only monomethylated. Screening of *Streptomyces* sp. SA-648 brought to light three new chitinase inhibitors related in structure to allosamidin (**4**).[95] Methyl-*N*-demethylallosamidin (**285**) differs from the parent compound in that its terminal *N*-acetyl-D-allosamine unit is 6-*O*-methylated but its aminooxazoline moiety is only monomethylated.[95] The other two compounds, named glucoallosamidin A (**286a**) and glucoallosamidin B (**286b**), are curious in that they both contain *N*-acetyl-D-glucosamine (**280**) as the central sugar (in place of *N*-acetyl-D-allosamine (**279**) in the parent compound).[95] Both are 6-*O*-methylated on the terminal *N*-acetyl-D-allosamine moiety, whereas glucoallosamidin B (**286b**) is only monomethylated on the aminooxazoline moiety. Yet another member of this family of natural products was isolated in 1993 from the mycelia of *Streptomyces* sp. AJ 9463.[96] It was named didemethylallosamidin (**287**) and differs from allosamidin (**4**) in that its aminooxazoline function is not methylated.

B. Biosynthesis

Zhou, Sakuda, and Yamada[97] have studied the biosynthesis of allosamidin (**4**) in *Streptomyces* sp. AJ 9463 and in particular the origin of the carbon and nitrogen atoms, by means of feeding experiments with labeled precursors. Incorporation experiments using D-[1-^{13}C]- and [6-^{13}C]glucose as well as doubly labeled D-[1-^{13}C,2-$^{15}N_2$]glucosamine established that both sugar units as well as the carbocyclic moiety are

Chart 15. Biosynthetic Origin of Allosamidin (4)



derived from D-glucosamine. The origin of the (dimethylamino)oxazoline portion of the carbocycle was explored, using L-[*guanidino*-^{13}C,$^{13}N_2$]arginine and L-[*methyl*-^{13}C]methionine (Chart 15).

Zhou and co-workers[96] have also prepared ^{14}C-labeled allosamidin (**4**), methyl-*N*-demethylallosamidin (**285**), and didemethylallosamidin (**287**) as well as a number of related compounds to investigate the biosynthesis of allosamidin (**4**). The studies suggested that the first *N*-methylation of allosamidin (**4**) occurs before formation of the aminooxazoline ring.

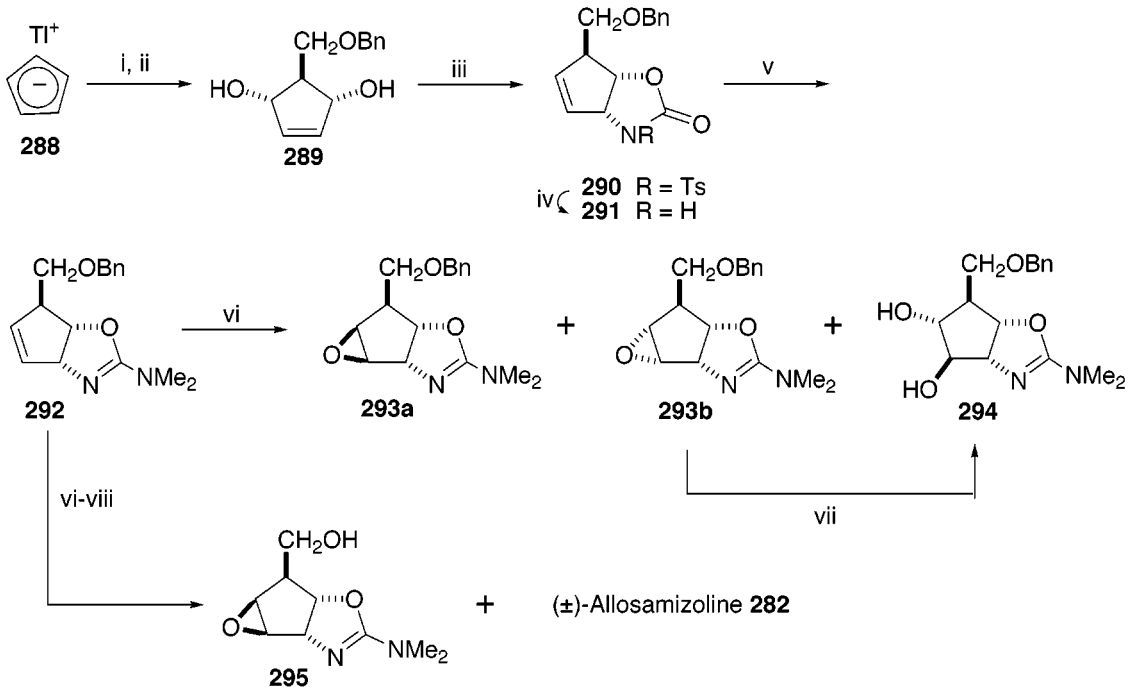
Sakuda and co-workers[98] have studied the biosynthesis of the cyclopentane skeleton in further detail. The results of feeding experiments with D-[3-^{2}H]-, [4-^{2}H]-, [5-^{2}H]-, and [6-^{2}H]glucosamine indicated the loss of deuterium on C-5, and stereospecifically of one of the two deuteriums on C-6. On the basis of these observations, the intermediacy of 6-aldehydo-D-glucosamine was proposed. The authors advanced a mechanism of carbocyclization for allosamizoline (**282**) akin to that observed in the biosynthesis of inositols, which involves an aldol condensation between C-1 and C-5 of D-glucosamine. An alternative Shikimate-like pathway was discounted.[98] The biosynthesis of other aminocyclopentitols such as pactamycin and bacteriohopanetetrol (see Section I, Chart 2) is believed to follow a similar route, whereas in the carbocyclic nucleosides, aristeromycin and neoplanacin A (see Section I, Chart 2), an aldol condensation between C-2 and C-6 of glucose is implicated.[24]

Zhou and co-workers[97] have conjectured that D-allosamine, previously unknown in nature, might be derived from D-glucosamine via the action of an epimerase acting on their respective UDP analogues. However no evidence to support this hypothesis, or as to how the three component units of allosamidin (**4**) are assembled, is yet at hand.

C. Synthesis: Aminocyclopentitol Unit

Successful routes to allosamizoline (**282**) are discussed in this section. Syntheses of protected derivatives of allosamizoline, appropriate for use in total syntheses of allosamidin (**4**), are also presented here. The latter constitute formal total syntheses. However, for the sake of clarity, the total syntheses themselves are discussed in Section IV.D.

Scheme 35[a]



[a] Reagents: (i) $PhCH_2OCH_2Cl$; (ii) O_2, *hν*, methylene blue, thiourea, MeOH, 33%; (iii) 2 equiv TsNCO, THF, $(dba)_3Pd_2{\cdot}CHCl_3$, $(^iPrO)_3P$, 93%; (iv) Na, naphthalene, 91%; (v) MeOTf, Me_2NH, 100%; (vi) 5.4 M CF_3CO_3H−TFA; (vii) 10% TFA, 40 °C, 68%; (viii) H_2, 2 atm, 10% Pd/C, 83%.

1. Trost's Approach

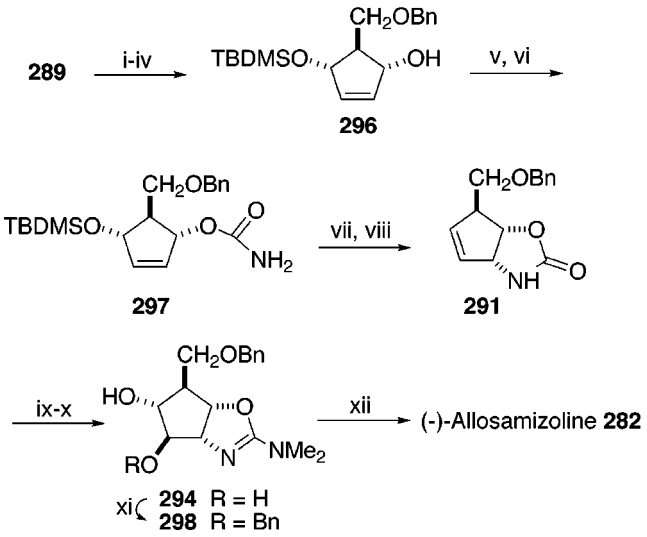
Trost's palladium-catalyzed protocol for vicinal hydroxyamination serves as the means of desymmetrization of a *meso*-diol and is the key step in Trost and Van Vranken's[99,38b,c] synthesis of racemic allosamizoline (**282**) (Scheme 35). The synthesis of all four 3,4-isomers (see Section IV.E.1, Schemes 53 and 54) established the relative configuration of the natural compound. The synthesis starts with cyclopentadienylthallium (**288**) which was alkylated and then treated with thiourea and *cationic* methylene blue to give the *meso*-diol **289** as the major diastereomer (12:1). The stereochemical outcome of the reaction was that anticipated on addition of singlet oxygen to the less hindered face of the monosubstituted cyclopentadiene. Exposure of the *cis*-diol **289** to tolylsulfonyl isocyanate and a palladium(0) catalyst produced the oxazolidin-2-one **290** which was reductively desulfonylated to give cyclopentene **291**. This oxazolidinone gave the key aminooxazoline **292** upon *O*-alkylation and exposure to dimethylamine, and this on treatment with trifluoroperacetic acid gave the diastereomeric epoxides **293a** and **293b**, together with 6-*O*-benzylallosamizoline (**294**). The latter diol arises from partial hydrolysis of epoxide **293b** under the conditions of the epoxidation reaction. Other epoxidation methods proved less fruitful as protonation of the allylic amino function inductively deactivates the olefin **292**. The alkene **292** also proved very unreactive toward typical electrophilic epoxidation reagents. The regioselective opening of epoxide **293b** to give diol **294** was expected on both steric and electronic grounds and occurred completely and rapidly upon exposure of the epoxidation reaction mixture to 10% aqueous trifluoroacetic acid at 40 °C. The unwanted epoxide **293a** was stable to these conditions. Hydrogenolysis of the crude hydrolysis reaction mixture gave racemic allosamizoline (**282**) and the easily separable unreacted epoxide **295** (83% combined yield, 7:2). Racemic allosamizoline **282** was obtained in ca. 19% overall yield.

The possibility of asymmetric induction was demonstrated in the palladium-catalyzed vicinal hydroxyamination step, through the use of palladium(0) in combination with (−)-BINAPO which gave the oxazolidinone **290** with 59−65% ee in 91% yield. This preliminary result was however not explored further.

2. Danishefsky's Approach

Griffith and Danishefsky's[100] approach to (−)-allosamizoline **282** parallels the approach disclosed by Trost (see Section IV.C.1) and took advantage of conditions reported by the latter workers to terminate their synthesis. Desymmetrization was however achieved by an enzymatic resolution step of a *meso*-diol, a key feature of this strategy (Scheme 36). The readily available *meso*-2,3-diol **289** was acetylated and resolved using the acetylcholinesterase from the electric eel (<95% ee). The enantiotopic sense of the enzymic hydrolysis was *opposite* to that expected by the authors and gave the undesired monoester. This enantiotopically pure alcohol was therefore protected as its *tert*-butyldimethylsilyl ether and deacetylated with methanolic ammonia to give the desired enantiomer **296**. The carbamate **297** was formed from the alcohol **296** by the action of benzyl chloroformate followed by treatment with methanolic ammonia. The oxazolidinone **291** was obtained from the intermediate **297** by desilylation, *O*-trifluoroacetylation, and cyclization. The strategy described by Trost (Section IV.C.1) was used to complete the synthesis. Thus oxazolidinone **291**, on *O*-methylation and exposure

Scheme 36[a]



[a] Reagents: (i) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 89%; (ii) electric eel acetylcholinesterase, NaN_3, pH 6.9 phosphate buffer, 95%, >95% ee; (iii) TBDMSCl, imidazole, 100%; (iv) NH_3, MeOH, 100%; (v) $ClCO_2Ph$, pyridine; (vi) NH_3, MeOH, 82%; (vii) aq HF, CH_3CN, 94%; (viii) TFAA, Et_3N, 63%; (ix) MeOTf; Me_2NH, 87%; (x) CF_3CO_3H, TFA, CF_3CO_2H, H_2O, 44%; (xi) Bu_2SnO, MeOH, reflux, BnBr, CsF, DMF, 46%; (xii) H_2, Pd/C.

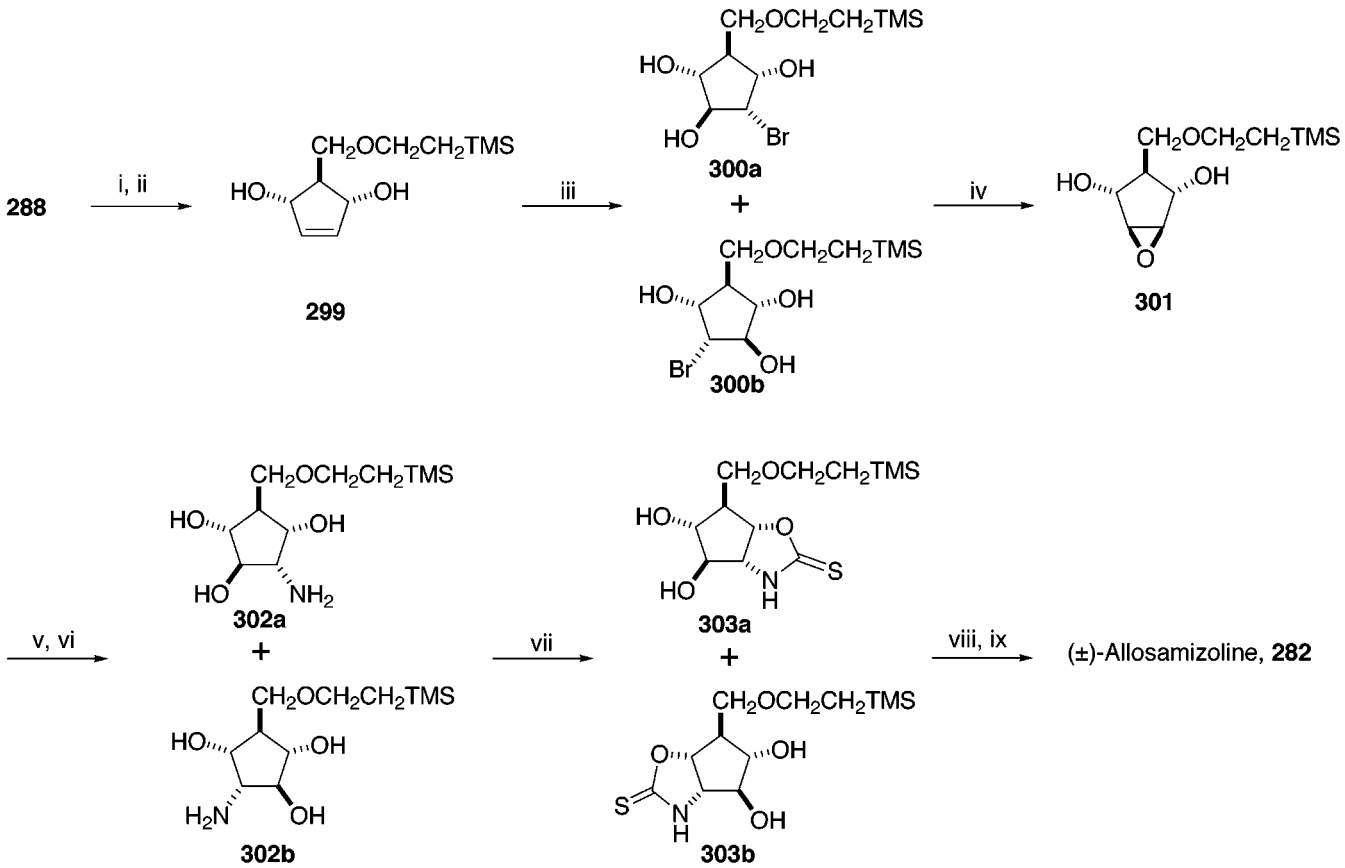
to dimethylamine, followed by hydroxylation with trifluoroperacetic acid and solvolysis with aqueous trifluoracetic acid, gave the diol **294**. Selective benzylation of diol **294** was achieved via its corresponding stannylene intermediate gave the building block **298** employed in the total synthesis reported by these authors (see Section IV.D.1). Catalytic hydrogenation of the diol **294** gave (−)-allosamizoline (**282**).

An alternative strategy to allosamizoline (**282**) was explored using the epoxide derived from the cyclopentene **297** but was discontinued as attempts to open this epoxide by a base-catalyzed intramolecular attack of the carbamate amino group did not give the desired oxazolidinone.

3. Ganem's Approach

Ganem and co-workers' synthesis[101] of racemic allosamizoline (**282**) uses a strategy based on a *meso*-cyclopentenediol as starting material and hypohalous acid additions to achieve desymmetrization (Scheme 37). The *meso*-diol **299** was obtained by the reaction of trimethylsilylethoxymethyl chloride with thallous cyclopentadienide (**288**). As peracid epoxidations of allylic double bonds give exclusively the undesired *syn*-epoxydiols, recourse was made to hypohalous acid additions which have been reported to proceed *via* syn-halonium ions. Thus, reaction of the diol **299** with *N*-bromosuccinimide gave bromohydrins **300a** and **300b**. Addition of hypochlorite was found to give, in a parallel manner, the corresponding chlorohydrins. Either of these derivatives could be transformed smoothly into the required *anti*-epoxydiol **301** by treatment with base. This epoxide **301** was opened smoothly with azide, and the azide was reduced

Scheme 37[a]



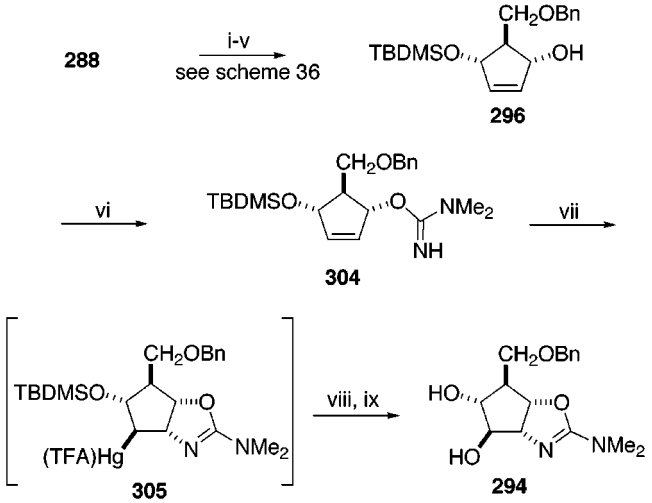
[a] Reagents: (i) SEMCl; (ii) O_2, *hν*, sens, thiourea, 35%; (iii) NBS, aq DMSO; (iv) Na_2CO_3, MeOH, 60%; (v) NaN_3, aq $CH_3O(CH_2)_2OH$, 66%; (vi) H_2, Pd/C, 100%; (vii) $IM_2C{=}S$, 81%; (viii) Me_2NH, MeOH, sealed tube; (ix) $LiBF_4$−MeCN (83% for the 2 steps).

quantitatively to the corresponding aminotriols **302a** and **302b**. Cyclization with thiocarbonyldiimidazole produced the thiooxazolidinones **303a** and **303b** which, on being heated in a sealed tube with dimethylamine, saw the (dimethylamino)oxazoline ring introduced in 1 step. Deprotection gave racemic allosamizoline (**282**).

4. Imperiali's Approach

Shrader and Imperiali's[102] approach to the (−)-allosamizoline (**282**) (6-*O*-benzyl-protected) parallels the approach reported by Danishefsky (Section IV.C.2). Alcohol **296** served as the key intermediate and was derived from a *meso*-diol using an enzymatic resolution step (Scheme 38). Alcohol **296** was treated with

Scheme 38[a]



[a] Reagents: (i) $PhCH_2OCH_2Cl$, O_2, *hν*, methylene blue, thiourea, 60%; (ii) 2 equiv Ac_2O, DMAP, 95%; (iii) electric eel acetylcholinesterase, KH_2PO_4, MeOH, 90%; (iv) TBDMSCl, imidazole, 95%; (v) NH_3, MeOH, 95%; (vi) neat dimethyl cyanamide, NaH, 93%; (vii) $Hg(CF_3CO_2)$; (viii) O_2, $NaBH_4$, NaOH, 69%; (ix) HF, 90%.

sodium hydride and neat dimethyl cyanamide to give the aminoimidate **304**. Mercury(II)-mediated cyclization of the latter gave the alkylmercurium intermediate **305** which underwent a radical oxygenation on reaction with molecular oxygen, which added exclusively to the convex face of the bicyclic ring system, to give after desilylation 6-*O*-benzylallosamizoline (**294**) as a single diastereomer. The protected (−)-allosamizoline derivative **294** was obtained in 58% yield from the optically active alcohol **296**.

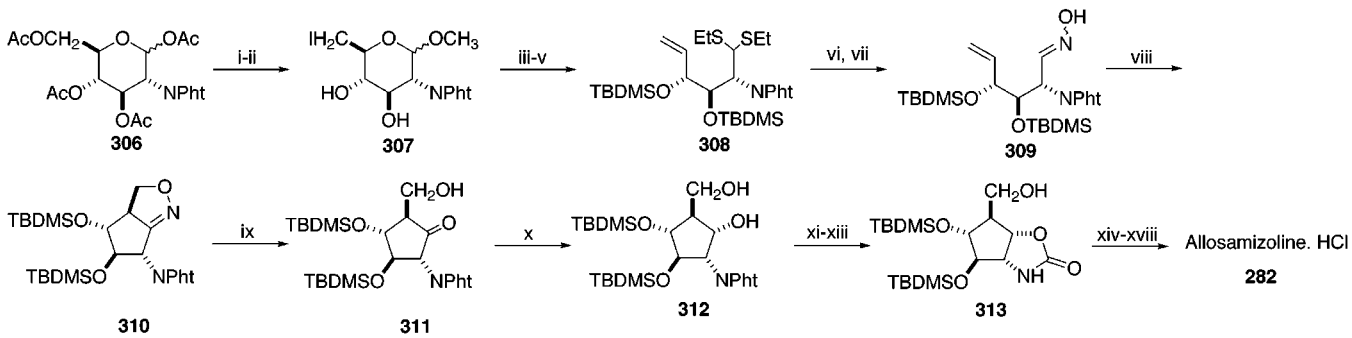
5. Tatsuta's Approach

Tatsuta and collaborators' synthesis[103] of (−)-allosamizoline (**282**) started from D-glucosamine and used an intramolecular nitrile oxide cycloaddition as the key step in their strategy (Scheme 39). The known *N*-phthalamido-per-*O*-acetylated D-glucosamine derivative **306**, prepared from D-glucosamine, was converted into the primary iodide **307**. The latter underwent a zinc-promoted reductive *β*-elimination to give as the major product a 5-enofuranose, which was treated with ethanethiol and then per-*O*-silylated to give the dithioacetal **308**. The thioacetal group was then cleaved and the aldehydo sugar, thus obtained, treated with hydroxylamine hydrochloride to obtain the required oxime **309**. Cycloaddition proceeded best on exposure of oxime **309**, the key intermediate in this approach, to sodium hypochlorite and produced a single isoxazoline **310**. Opening of the latter, however, proved troublesome and was eventually achieved by ozonolytic cleavage to give *β*-hydroxy ketone **311**. Reduction of this ketone with zinc borohydride gave, selectively, the required alcohol **312**. This was converted into the bicyclic derivative **313** by removal of the phthalimido group, benzyloxycarbonylation, and treatment with sodium hydride. The oxazolidinone **313**, upon desilylation and acetylation, followed by treatment with methyltriflate and exposure to dimethylamine, gave (−)-allosamizoline (**282**), which was isolated as its hydrochloride salt.

6. Simpkins' Approach

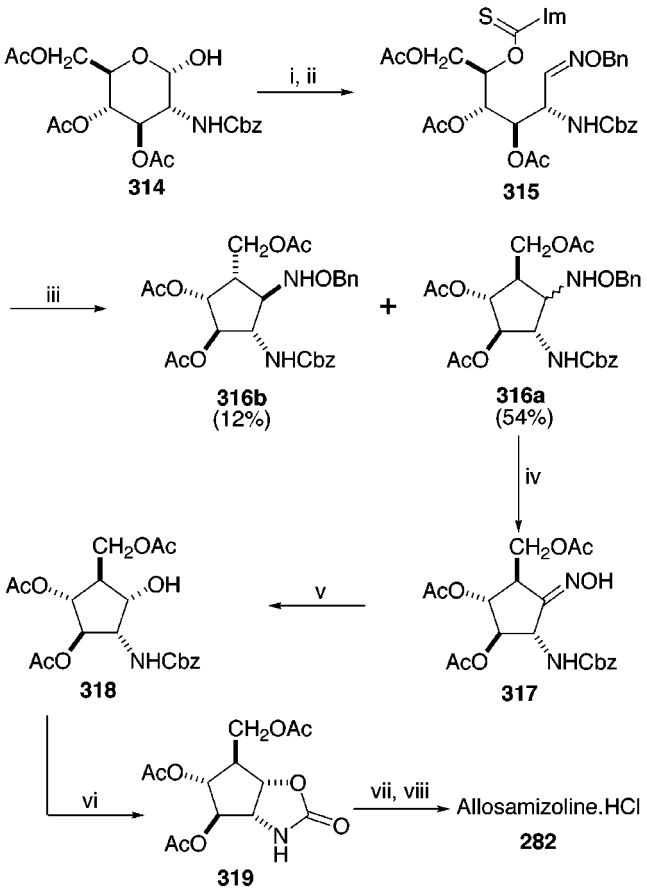
Simpkins' synthesis[104] of (−)-allosamizoline (**282**) again makes use of D-glucosamine as a starting material and has as a key step a radical cyclization onto an oxime ether to form the cyclopentane skeleton (Scheme 40). The known *N*-(benzyloxycarbonyl)-tri-*O*-acetyl-D-glucosamine derivative **314** was synthesized from D-glucosamine in 3 steps. Reaction of the latter with *O*-benzylhydroxylamine hydrochloride gave an acyclic oxime, which was converted into the thiocarbonylimidazolidine derivative **315**. Standard radical cyclization conditions, when applied to this key precursor, gave cleanly a mixture of diastereo-

Scheme 39[a]



[a] Reagents: (i) MeOH, 1% HCl, 60%; (ii) I_2, PPh_3, imidazole, 90%; (iii) Zn, THF; (iv) EtSH, HCl, 61%; (v) TBSOTf, 2,6-lutidine, 90%; (vi) $HgCl_2$, $CaCO_3$, 81%; (vii) $NH_2OH{\cdot}HCl$, pyridine, 81%; (viii) NaOCl, 91%; (ix) O_3/O_2, CH_2Cl_2−MeOH, 60%; (x) $Zn(BH_4)_2$, 100% (xi) $NH_2NH_2{\cdot}H_2O$; (xii) BnOCOCl, Na_2CO_3; (xiii) NaH, 60%; (xiv) 1% HCl-MeOH; (xv) Ac_2O, pyridine, 100%; (xvi) MeOTf; (xvii) $Me_2NH{\cdot}HCl$, Et_3N, CH_2Cl_2; (xviii) 1 M HCl, 50 °C, 80%.

Scheme 40[a]



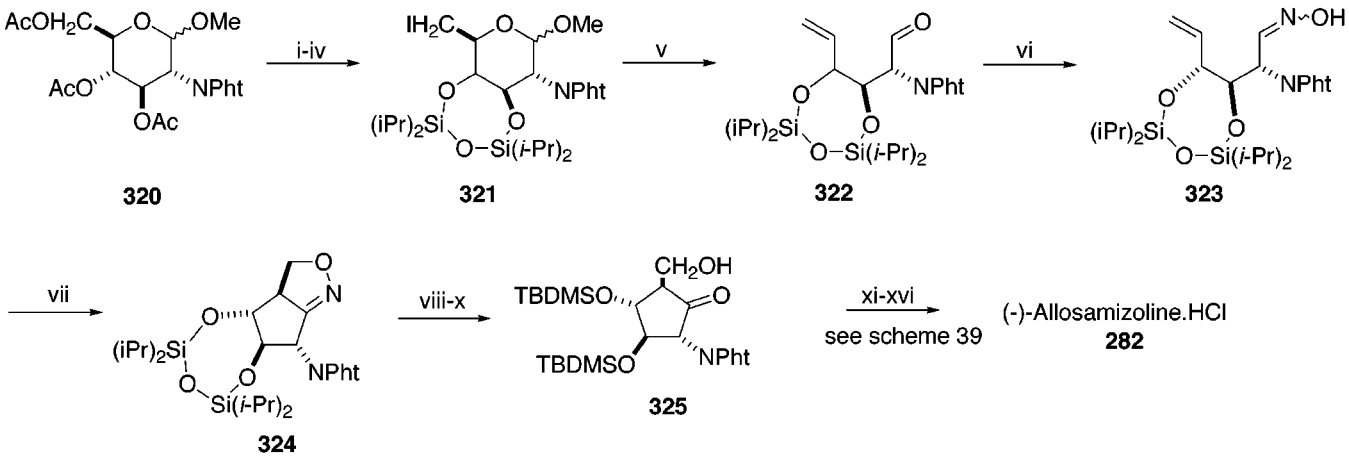
[a] Reagents: (i) $NH_2OBn{\cdot}HCl$, pyridine, 88%; (ii) $Im_2C{=}S$, 82%; (iii) Bu_3SnH, AIBN, C_6H_6; (iv) *m*-CPBA, Na_2CO_3, 79%; (v) O_3, $NaBH_4$, 20−40%; (vi) $SOCl_2$, 82%; (vii) Et_3OBF_4, Me_2NH, 80%; (viii) NaOMe, MeOH, HCl, 98%.

meric carbocycles **316a** and **316b**. The major diastereomer **316a** was that desired and was obtained as a mixture of epimers at C-1 in a yield of 54%. A number of other routes to the aminocyclopentitol core, based on radical cyclizations, were also explored but to no avail. Conversion of the benzyloxyamino group in derivative **316a** into the required alcohol **318** presented considerable problems and was eventually accomplished upon its oxidation to the oxime **317**, followed by reaction of the latter with ozone and direct reductive workup. Despite attempted optimization of this sequence, the alcohol **318** could only be obtained in 20−40% overall yield. Exposure to thionyl chloride of this alcohol **318** gave the oxazolidinone **319** directly, obviating the need for the prior deprotection of the amino function. The oxazolidinone **319** was *O*-alkylated, treated with dimethylamine, deacetylated with sodium methoxide, and exposed to acid to give (−)-allosamizoline (**282**) as its hydrochloride salt.

7. Koseki's Approach

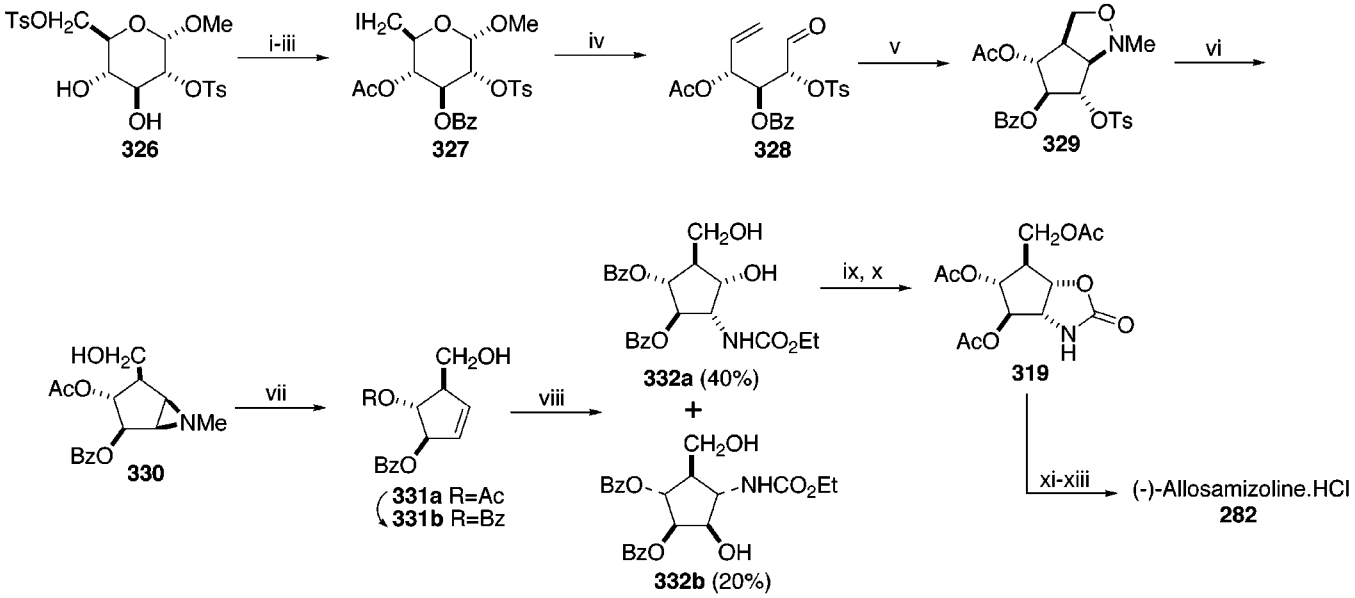
Koseki and collaborators'synthesis[105] of (−)-allosamizoline (**282**) starts from D-glucosamine and parallels somewhat closely Tatsuta's approach (see Section IV.C.5). It employs as a key step an intramolecular nitrile oxide cycloaddition (Scheme 41). The *N*-phthalimido-per-*O*-acetylated-D-glucosamine derivative **320** was deacetylated and regioselectively monotosylated, the two secondary alcohol functions protected as a cyclic tetraisopropyldisiloxane (TIPDS) ether, and then finally reacted with sodium iodide to give the primary iodide **321**. Reductive elimination of the latter gave the expected vinyl aldehyde **322** which was converted into the key precursor **323** on treatment with hydroxylamine hydrochloride. The rigidity of the cyclic TIPDS protecting group was proposed to help reduce any tendency of iodide **321** to degrade via a β-elimination, a problem which was encountered when benzyl protection was used instead. Exposure of the oxime **323** to sodium hypochlorite saw its smooth cyclization into the desired isoxazoline **324**. The latter was converted into its alcohol **325** and then reprotected to give the known bis-TBDMS ether **310** (see Section IV.C.5) apparently to facilitate *N*-deprotection in a subsequent step. The synthesis was completed in essentially the same manner as reported by Tatsuta (see Section IV.C.5, Scheme 39) to give (−)-allosamizoline (**282**) as its hydrochloride salt.

Scheme 41[a]



[a] Reagents: (i) NaOMe, MeOH; (ii) TsCl, pyridine; (iii) $TIPDSiCl_2$, imidazole/DMF, 73%; (iv) NaI, 69%; (v) Zn; (vi) $NH_2OH{\cdot}HCl$, NaOAc/MeOH, 69%; (vii) NaOCl aq, 91%; (viii) 1 M TBAF; (ix) TBDMSCl, imidazole, 60%; (x) O_3, Me_2S, $Zn(BH_4)_2$, 52%; (xi) NH_2NH_2; (xii) BnOCOCl, Na_2CO_3; (xiii) NaH; (xiv) HCl−MeOH: (xv) Ac_2O, pyridine, 46%; (xvi) Et_3OBF_4, Me_2NH, 81%, HCl, 50 °C, 100%.

Scheme 42[a]



[a] Reagents: (i) BzCl, pyridine; (ii) Ac_2O, pyridine, 47%; (iii) NaI, Ac_2O, 83%; (iv) Zn, EtOH; (v) MeNHOH, 57% (vi) H_2/Raney Ni, 68%; (vii) peracid, 81%; (viii) $Hg(CF_3CO_2)$, Na ethyl *N*-chlorocarbamate; (ix) NaOMe, MeOH; (x) Ac_2O, pyridine, 86%; (xi) Me_3OBF_4; (xii) $HNMe_2$, 81%; (xiii) NaOMe, MeOH, HCl, MeOH, 97%.

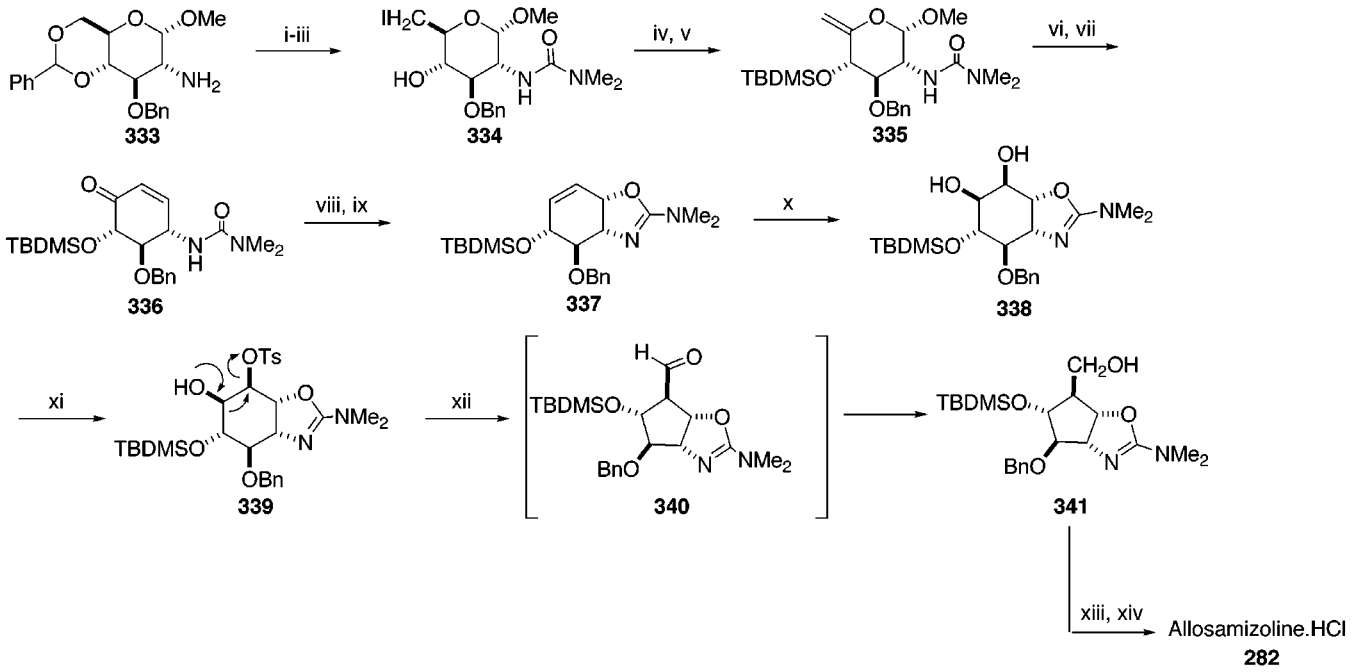
8. Ferrier's Approach

Ferrier and collaborators[106] used an intramolecular nitrone cycloaddition of a precursor derived from D-glucose to construct the pentacyclic skeleton and an oxyamination to introduce the appropriate ring functionalization of (−)-allosamizoline (**282**) (Scheme 42). The known 2,6-bis-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside **326**, prepared from D-glucose, was selectively 3-*O*-benzoylated and then acetylated and treated with sodium iodide to give the primary iodide **327**. Zinc-promoted reductive elimination gave the enal **328** which underwent a smooth cycloaddition on reaction with *N*-methylhydroxylamine. Reduction of the isoxazolidine **329**, thus obtained, gave the corresponding aziridine **330** which upon peracid oxidation gave the cyclopentene **331a**. Oxyamination of the alkene **331a** using Chloramine-T and catalytic osmium tetraoxide did not give favorable results even on varying the manner in which this alkene was protected. Difficulties, including problems in the separation of the products as well as unfavorable ratios of regioisomers, led the authors to consider the Sharpless modification of the oxyamination reaction which employs *N*-chloro-*N*-metallocarbamate instead of Chloramine-T. Thus, reaction of sodio ethyl *N*-chlorocarbamate and silver nitrate with the dibenzoyl-protected alkene **331b** gave the required adducts **332a** and **332b** in 50% yield and a ratio of 1:1, but the most satisfactory results were obtained employing the homogeneous system mercury(II) trifluoroacetate and chlorocarbamate in acetonitrile without added water, which gave these regioisomers in a 60% combined yield and a ratio of 1:2. Exposure of regioisomer **332a** to sodium methoxide saw simultaneous debenzoylation and cyclic carbamate formation, and the triol thus formed was then converted into the acetylated derivative **319** before being transformed into (−)-allosamizoline (**282**) (as its hydrochloride) upon sequential reaction with trimethyloxonium tetrafluoroborate and dimethylamine followed by treatment with sodium methoxide and, finally, exposure to aqueous hydrogen chloride.

9. Kuzuhara's Approach

Kuzuhara and co-workers[107] established the absolute configuration of (−)-allosamizoline (**282**). Their synthesis constitutes as a key step a novel and efficient ring fragmentation−contraction of a cyclohexitol precursor derived from D-glucosamine (Scheme 43). The protected D-glucosamine derivative **333**, obtained in 3 steps from D-glucosamine hydrochloride, was *N*-protected as a urea, its benzylidene group cleaved by acid hydrolysis, and its primary hydroxyl group converted regioselectively to an iodide to give the alcohol **334**. Protection of the remaining hydroxyl function as a *tert*-butyldimethylsilyl ether followed by *tert*-butoxide-promoted dehydroiodination gave the labile enol ether **335** which was submitted to the modified Ferrier reaction conditions. The crude keto enol thus obtained, when exposed to mesyl chloride−pyridine, underwent a *β*-elimination reaction to give the enone **336**. Selective reduction of the latter was possible under Luche conditions, and the allylic alcohol, thus obtained, was reacted with mesyl anhydride to give the desired oxazoline **337**. When this oxazoline was subjected to osmylation, attack of the reagent took place exclusively on its convex face giving the desired diol **338**, which itself underwent tosylation with unexpectedly good regioselectivity to provide the monotosylate **339**. This key intermediate, when treated with L-Selectride, underwent a smooth ring contraction to give the alcohol **341** via the base-sensitive aldehyde intermediate **340**. This aldehyde was reduced in situ by the L-Selectride present, thus circumventing its possible decomposition. Deprotection gave (−)-allosamizoline (**282**) as its hydrochloride salt.

Scheme 43[a]

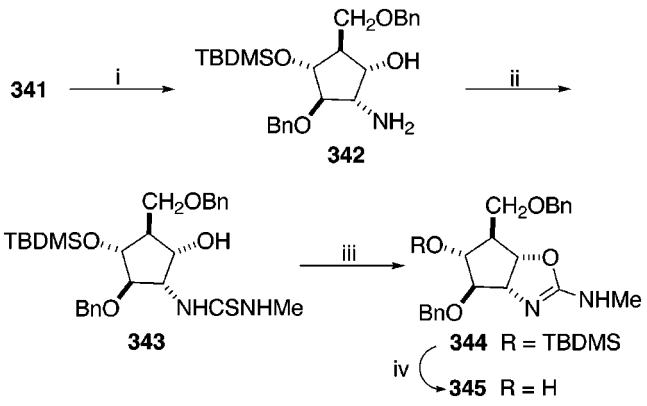


[a] Reagents: (i) Me_2NCOCl, Et_3N; (ii) aq AcOH, 91%; (iii) NIS, PPh_3; (iv) TBDMSOTf, 2,6-lutidine, 71%; (v) ^tBuOK, 96%; (vi) $HgSO_4$, H_2SO_4—acetone; (vii) MsCl, pyridine, 65%; (viii) $NaBH_4$, $CeCl_3 \cdot 7H_2O$; (ix) Ms_2O, Et_3N, 86%; (x) OsO_4, $Me_3N \rightarrow O$, 92%; (xi) TsCl, pyridine, DMAP, 86%; (xii) L-Selectride, 86%; (xiii) 1 M HCl, THF; (xiv) H_2, Pd/C, $HCl-H_2O$, 90%.

10. Takahashi's Approach

Takahashi and co-workers[108] have synthesized the aminocyclopentitol **345**, a protected derivative of demethylallosamizoline, used subsequently in the only reported total synthesis (see Section IV.D.4) of the natural compound demethylallosamidin (**284**). The synthesis of the carbocycle **345** is described here (Scheme 44). Cleavage of the oxazoline ring of protected allosamizoline **341** was achieved using methanolic hydrazine monohydrate—conditions developed by Vasella and Trost in their total synthesis (see Section IV.D.2). The amino alcohol **342**, thus obtained, was treated with methyl isothiocyanate to afford the thiourea **343** in high yield. Formation of oxazoline **344** occurred, with participation of the vicinal hydroxy group, upon treatment of the thiourea **343** with yellow mercury oxide. Desilylation of the oxazoline **344** gave the demethylallosamizoline derivative **345**.

Scheme 44[a]



[a] Reagents: (i) BnBr, NaH, DMF, $NH_2NH_2 \cdot H_2O$, EtOH, 60%; (ii) CH_3NCS, 100%; (iii) HgO, 91%; (iv) 1 M HCl, THF, 89%.

D. Total Synthesis

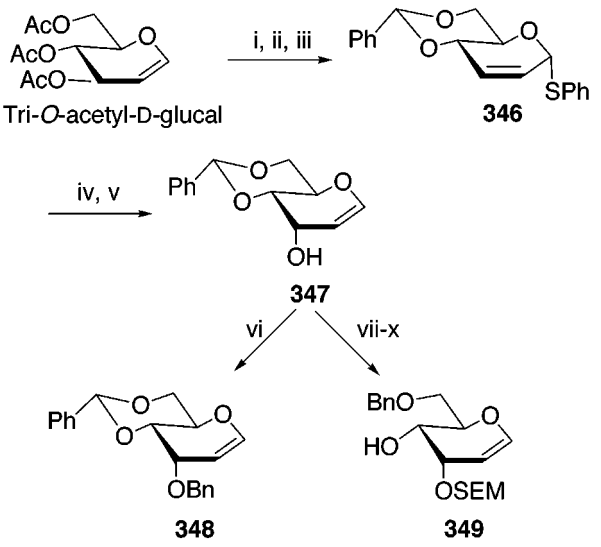
The choice of glycosidation method to assemble the component units of the allosamidins constitutes the essential difference between each of the reported total syntheses. Ensuring exclusive β-selectivity is the primary goal of these coupling methods, and as the outcome is known to be strongly influenced by the protecting group pattern of the glycosyl donors *and* acceptors, these latter choices impose yet further constraints and constitute important differences between the reported strategies. Furthermore, as D-allosamine is not a commonly occurring sugar, strategies by which to obtain it from known carbohydrate precursors have also been an important facet and further complicated the syntheses. Construction of the protected carbocyclic acceptor fragments is considered in the previous Section (Section IV.C).

1. Danishefsky's Synthesis

The first total synthesis of allosamidin (**4**) reported is that described by Griffith and Danishefsky.[100b] Danishefsky's synthesis of the carbocyclic moiety has already been described (see Section IV.C.2), and the authors subsequently developed a novel glycosylation method through which to synthesize the natural product. This method is of particular interest for constructing glycosides comprising β-linked 2-deoxy-D-allosamine units, as the amino function is introduced on the hindered α-face in concert with β-glycoside formation.

Tri-*O*-acetyl-D-glucal was subjected to a Ferrier-type rearrangement with thiophenol, deacetylated, and reprotected as its *O*-benzylidene derivative **346** (Scheme 45). Treatment of this thioglycoside with *m*-chloroperbenzoic acid gave an unexpectedly complex mixture, but exposure to dimethyldioxirane and

Scheme 45[a]



[a] Reagents: (i) PhSH, BF_3Et_2O; (ii) NaOMe, MeOH; (iii) $PhCH(OMe)_2$, *p*-TsOH, 73%; (iv) dimethyldioxirane; (v) Et_2NH, 96%; (vi) BnBr, Bu_4NI, 96%; (vii) SEMCl, $^iPr_2NEt$, 100%; (viii) Na, NH_3; (ix) Bn_2SnO, MeOH, reflux; (x) CsF, BnBr, 69%.

then diethylamine saw its oxidation and smooth [2,3]-sigmatropic rearrangement via an intermediate sulfoxide to give the pivotal 4,6-*O*-benzylidene-D-allal (**347**). The latter could be converted into either of the required starting sugar building blocks. The first, the fully protected allal **348**, was obtained by benzylation. The second, alcohol **349**, was obtained by protection of the benzylideneallal (**347**) as its 2-(trimethylsilyl)-ethoxy (SEM) derivative and then removal of the benzylidene group followed by regioselective benzylation of the primary alcohol function using stannylene chemistry.

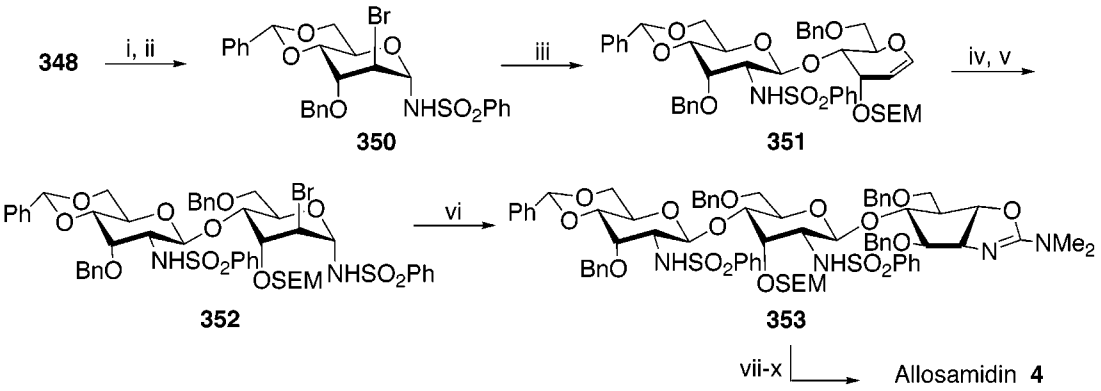
The 3-*O*-benzyl-D-allal derivative **348** (Scheme 46) was treated with *N*,*N*-dibromobenzenesulfonamide to afford the glycosyl donor **350**. Potassium hexamethyldisilazane (KHMDS)-promoted coupling of the latter with the 3-*O*-SEM-D-allal acceptor **349**—conditions typical of Danishefsky's glycosidation method—provided the protected disaccharide **351** in 81% yield. The latter was then converted into the glycosyl donor **352** by treatment with *N*,*N*-dibromobenzenesulfonamide. Although Trost and Vasella found that good regioselectivity is observed when the carbocyclic diol **294** is employed as an acceptor in conjunction with a trichloroacetimidate donor (see Section IV.D.2), in preliminary work the Danishefsky coupling reaction between a model glycosyl donor and diol acceptor gave mixtures of regioisomers. An appropriately protected allosamizoline acceptor, with only its 4-hydroxy group free, was thus deemed necessary in order to ensure regioselectivity. Furthermore, preliminary experimentation demonstrated that a 5-*O*-silyl protecting group was unexpectedly prone to migration under the basic conditions of the coupling reaction and would not be suitable. Thus, the 3,5-di-*O*-benzylated carbocyclic acceptor **298** (see Scheme 36) was subjected to the base-catalyzed coupling reaction with glycosyl donor **352** to give pseudotrisaccharide **353** in 42% yield. Deprotection of the latter by acid hydrolysis of the SEM and benzylidene groups, and by reductive cleavage of the sulfonamide and benzyl groups, followed by per-*O*-acetylation and finally treatment with methanolic ammonia, gave (−)-allosamidin (**4**).

2. Vasella and Trost's Synthesis

The total synthesis reported by Vasella's and Trost's groups[109] employed the trichloroacetimidate glycosidation method to couple the *N*-acetyl-D-allosamine units together and subsequently for coupling to the racemic carbocyclic diol **294**. The synthesis of this latter segment had already been disclosed by Trost (see Section IV.C.1, Scheme 35). The disaccharide donor was obtained in a convergent fashion from D-glucosamine and coupled regioselectively with the aminocyclopentitol diol. But, as the latter component was racemic, the coupling inevitably led late in the synthesis to 50% of an unwanted diastereomer. The latter compound did nevertheless show interesting biological activity (see Section IV.F., Table 3).

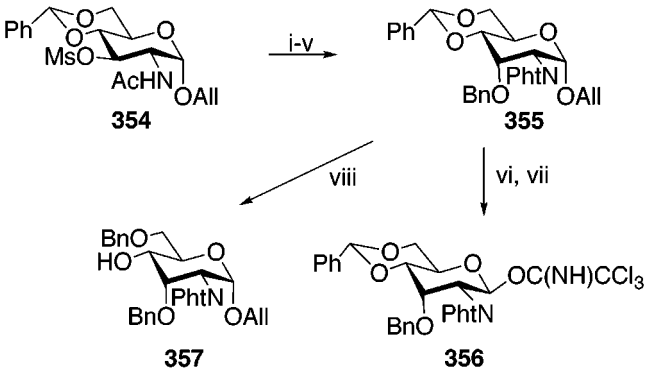
The required D-*allo* configuration of the sugar units was achieved through solvolysis of the 3-*O*-mesylate **354**, derived from the known allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside, itself obtained from *N*-acetyl-D-glucosamine (Scheme 47). This D-allose intermediate was converted, through selective protective group manipulations, into the pivotal *N*-phthaloylated intermediate **355**, which could be transformed into either of the required

Scheme 46[a]



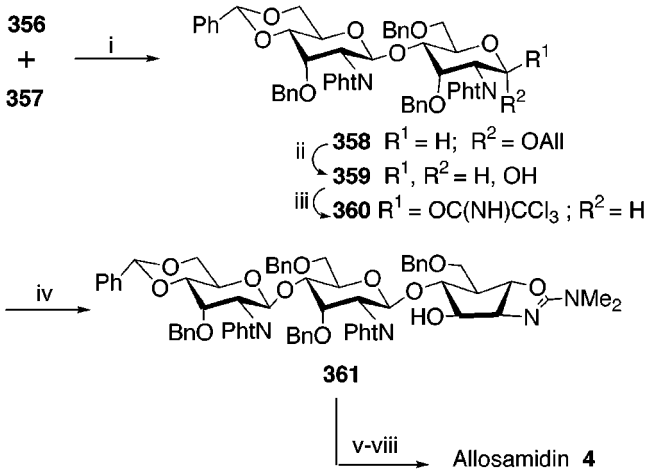
[a] Reagents: (i) Br_2NSO_2Ph; (ii) NH_4I, 63%; (iii) **349**, KHMDS, 81%; (iv) Br_2NSO_2Ph; (v) NH_4I, 57%; (vi) **298**, KHMDS, 42%; (vii) aq HCl—MeOH; (viii) Na, NH_3; (ix) Ac_2O, pyridine, 36%; (x) NH_3, MeOH, 79%.

Scheme 47[a]



[a] Reagents: (i) NaOAc, aq $MeOCH_2OH$, reflux, 81%; (ii) aq NaOH, reflux, 100%; (iii) phthalic anhydride, Et_3N, 95%; (iv) BnBr, NaH, 79%; (v) NaOH, dioxane, Ac_2O, pyridine, 75%; (vi) iridium catalyst, H_2, 75%; (vii) CCl_3CN, K_2CO_3, 81%; (viii) Me_3NBH_3, $AlCl_3$, 84%.

Scheme 48[a]



[a] Reagents: (i) 1.2 equiv TMSOTf, 80%; (ii) iridium catalyst, H_2, 73%; (iii) CCl_3CN, K_2CO_3, 86%; (iv) **294**, TMSOTf, 27% + other diastereomers (24%) and regioisomeric diastereomer (5%); (v) $MeNH_2$, EtOH; (vi) Ac_2O, pyridine, 70%; (vii) MeONa, MeOH, 96%; (viii) H_2, PD/C, 95%.

monosaccharide units. Cleavage of the allyl group and reaction with trichloroacetonitrile converted intermediate **355** into donor **356**. Selective reductive ring opening of the benzylidene group in intermediate **355** gave the acceptor alcohol **357**.

The TMSOTf-promoted coupling of the trichloroacetimidate **356** with the alcohol **357** gave the corresponding β-disaccharide **358** in 81% yield (Scheme 48). This was in turn deallylated and the resulting alcohol **359** converted into the disaccharide imidate donor **360**, which was itself subjected to a TMSOTf-promoted glycosylation with the racemic 6-*O*-benzylallosamizoline diol acceptor **294**. The coupling reaction proceeded in 61% overall yield and with good regioselectivity to give the desired natural pseudotrisaccharide derivative **361** in 27% yield. (Other pseudotrisaccharide products obtained in this glycosidation are discussed in Section IV.E.2, Chart 16). The observed regioselectivity obviated the need to protect more elaborately the carbocyclic acceptor **294** (as was necessary in a number of other approaches, e.g., Danishefsky's approach, Section IV.D.1) and was expected on the grounds that the hydroxyl group *nearest* the electron-withdrawing dihydrooxazole moiety would be less reactive than the other. The pthalimide group in pseudotrisaccharide **361** was cleaved without concomitant opening of the dihydrooxazole ring, using methanolic methylamine, and the amino group was protected as the corresponding acetamide. De-*O*-acetylation and catalytic hydrogenation of the latter gave pure allosamidin (**4**).

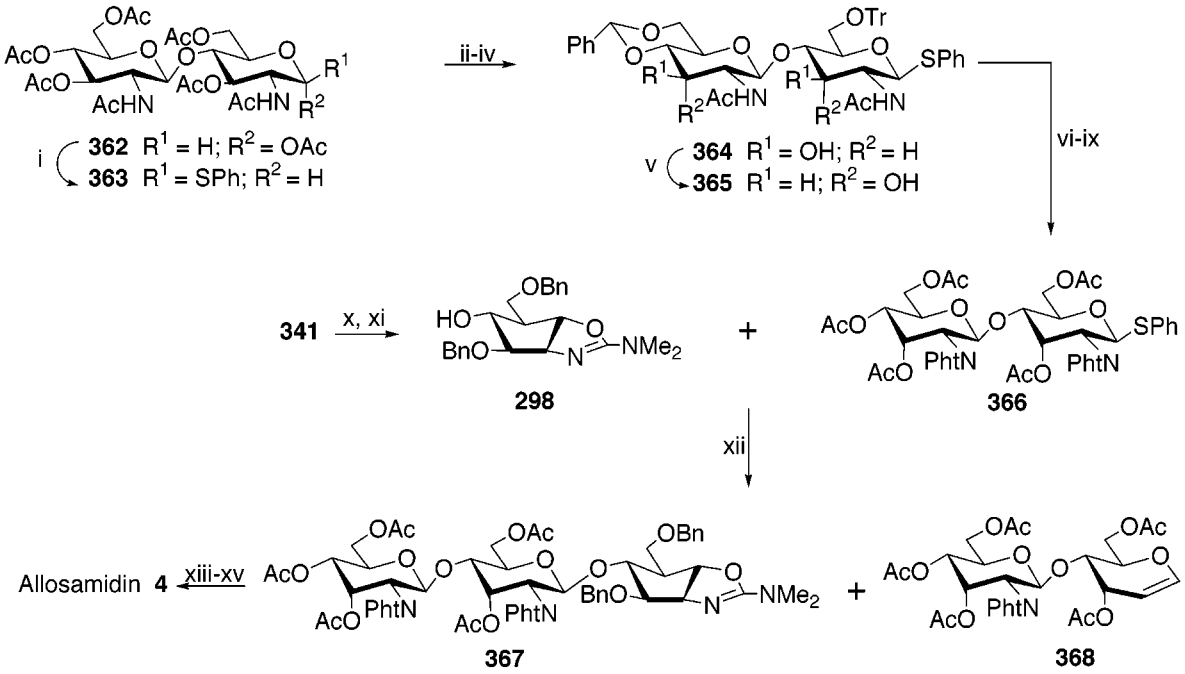
3. Kuzuhara's Synthesis

Kuzuhara's total synthesis[110] involves an *N*-iodosuccinimide (NIS)-promoted coupling of the appropriately protected (−)-allosamizoline derivative [reported earlier (see Section IV.C.5)] with a chitin-derived disaccharide thioglycoside donor (Scheme 49). Chitobiose, obtained by the action of a chitinase (rather obtusely!) on colloidal chitin, was per-*O*-acetylated to give the *N*,*N*-diacetylchitobiose derivative **362** which was treated with thiophenol to give the β-thioglycoside donor **363**, an oxazoline intermediate being invoked to explain the β-selectivity observed. Protecting group manipulation—saponification, benzylidenation, and tritylation—gave the required diol disaccharide derivative **364**, which was di-*O*-mesylated and subjected to solvolysis in the presence of acetate ion, to obtain the disaccharide **365** possessing the desired D-*allo* configuration. Hydrolysis of the benzylidene group, base-catalyzed *N*-deacetylation, and reprotection of the amino groups by *N*-phthaloylation followed by per-*O*-acetylation gave the glycosyl donor **366**. *O*-Benzylation (using benzyl 2,2,2-trichloroacetimidate) and desilylation of the aminocyclopentitol intermediate **341** (described in Section IV.C.5, Scheme 43) gave the protected allosamizoline acceptor **298** which reacted with the disaccharide donor **366** upon catalysis with triflic acid−NIS. The desired β-trisaccharide **367** was thus obtained, but in only 40% yield, as a proportion of the donor underwent elimination to the corresponding disaccharide glycal **368**. *N*-Dephthaloylation with methylamine, *N*-acetylation, and hydrogenolysis of the benzyl groups gave the natural allosamidin (**4**).

4. Takahashi's Synthesis

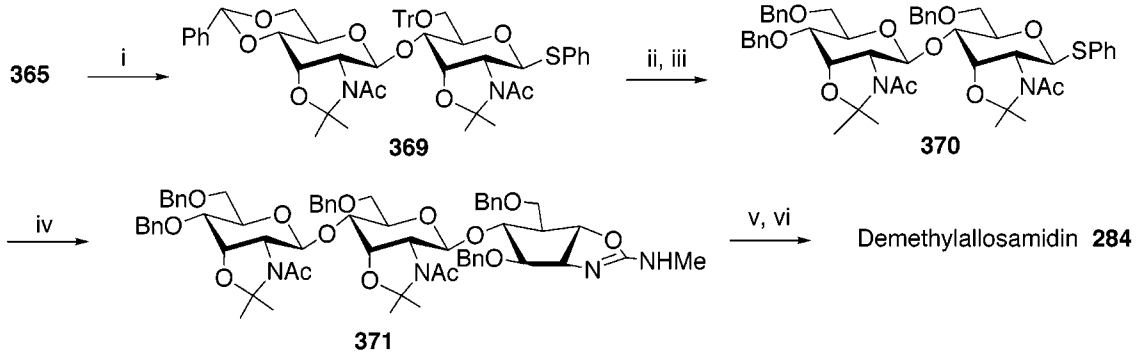
Takahashi and collaborators,[108] using alcohol **345** (see Section IV.C.10, Scheme 44) and a strategy paralleling their approach to allosamidin (**4**) (see Section IV.D.3), reported the first total synthesis of the natural product demethylallosamidin (**284**) (Scheme 50). Isopropylidenation of the known intermediate **365** was achieved with 2-methoxypropene in the presence of camphorsulfonic acid to give acetal **369** in high yield. These conditions did not cleave the acid-labile benzylidene and trityl groups also present. Selective hydrolysis of the latter groups was achieved with 80% acetic acid, and benzylation gave the glycosyl donor **370**. Glycosidation of the latter with the acceptor **345**, promoted by catalytic triflic acid−NIS, gave the desired fully protected demethylallosamidin derivative **371** in 60% yield. The authors ascribed the absence of any α-isomer to the steric hindrance provided by the *N*,*O*-isopropylidene group and noted that the yield of the glycosidation step was much higher than when they had used an *N*-phthal-

Scheme 49[a]



[a] Reagents: (i) PhSTMS, ZnI_2, 87%; (ii) NaOMe, MeOH; (iii) $PhCH(OMe)_2$, *p*-TsOH; (iv) TrCl, pyridine, 71%; (v) MsCl, pyridine, 92%, NaOAc, 82%; (vi) aq MeOH, H^+; (vii) 1 M NaOH; (viii) phthalic anhydride, TEA; (ix) Ac_2O, pyridine, 41%; (x) benzyl 2,2,2-trichloroacetimidate, TfOH; (xi) 1 M HCl, 61%; (xii) NIS, TfOH, 40%; (xiii) aq $MeNH_2$; (xiv) Ac_2O; (xv) H_2, Pd/C, 66%.

Scheme 50[a]



[a] Reagents: (i) ^{i}PrOMe, cat. CSA, 100%; (ii) 80% AcOH; (iii) BnBr, NaH, Bu_4NI, 81% (iv) **345**, NIS, TfOH, 60%; (v) HCl, MeOH, 65%; (vi) H_2, Pd/C, 91%.

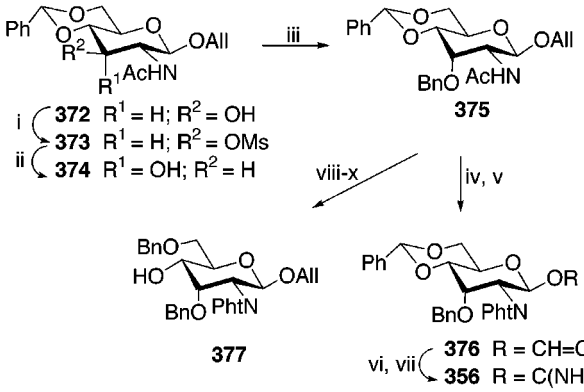
imido protecting group in their synthesis of allosamidin (**4**). The *N*,*O*-protecting group was hydrolyzed with acid, and catalytic hydrogenolysis cleaved the *O*-benzyl group to give demethylallosamidin (**284**).

5. Ferrier's Synthesis

Ferrier and co-workers[106] chose to use the trichloroacetimidate glycosidation method for coupling of their aminocyclopentanol acceptor (described in Section IV.C.8). The latter was obtained by selective protection of carbocycle **332a**, an intermediate in their total synthesis of allosamizoline. The coupling strategy reported by Vasella and Trost (see Section IV.D.2) parallels closely the approach reported by these authors (Scheme 51).

The β-allyl-*O*-glucoside **372** was chosen as starting material as opposed to the corresponding α-anomer employed in Trost and Vasella's approach. Thus, solvolysis of the 3-*O*-mesylated alcohol **373** gave the D-allose derivative **374** which upon benzylation gave

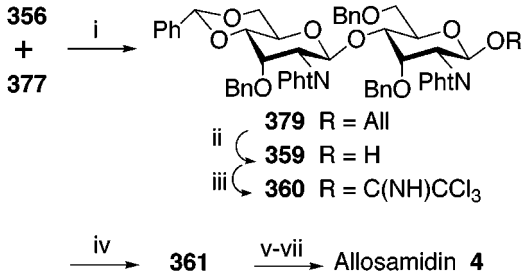
Scheme 51[a]



[a] Reagents: (i) MsCl, pyridine, 89%; (ii) NaOAc, aq CH_3OCH_2-CH_2OH, 91%; (iii) BnBr, $Ba(OH)_2$, BaO, 94%; (iv) KOH, MeOH; (v) phthalic anhydride, 71%; (vi) $HgCl_2$, 91%; (vii) CCl_3CN, DBU, 85%; (viii) KOH, MeOH, H_2O; (ix) phthalic anhydride, 61%; (x) $NaBH_3CN$, HCl, 78%.

the pivotal monosaccharide intermediate **375**. *N*-Deacetylation with methanolic potassium hydroxide

Scheme 52[a]



[a] Reagents: (i) TMSOTf, 85%; (ii) base, rhodium(I) catalyst, 73%; (iii) CCl_3CN, DBU, 84%; (iv) **294**, TMSOTf, 68%; (v) $MeNH_2$, EtOH; (vi) Ac_2O, 79% + 18% regioisomer; (vii) H_2, Pd/C, 93%.

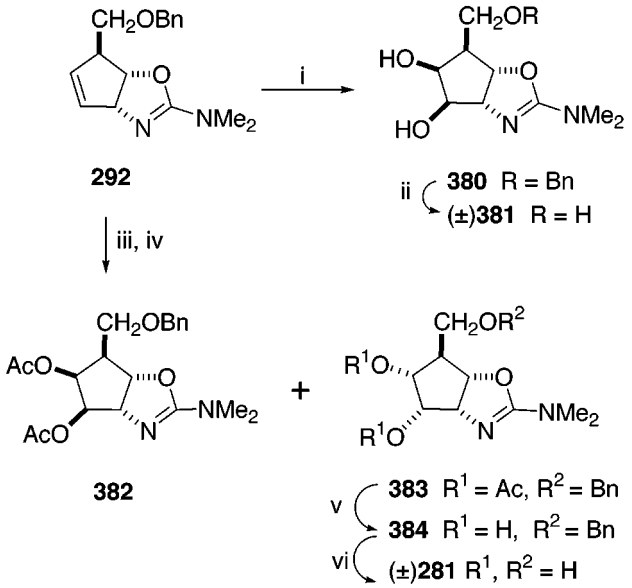
(125 °C, 48 h) followed by *N*-phthaloylation of the latter gave the propenyl glycoside **376**. Isomerization of the allyl function had conveniently occurred under the basic conditions of the *N*-deacetylation reaction. The propenyl group was hydrolyzed and converted into the required trichloroacetimidate donor **356**. Potassium hydroxide-catalyzed *N*-deacetylation (aqueous methanol, 125 °C, 5 days) of the allyl glycoside **375**, conditions which did not cause isomerization of the allyl group, followed by *N*-phthaloylation and selective reduction of the benzylidene group, gave the required D-allose acceptor monosaccharide **377**.

Coupling of the trichloroacetimidate **356** (Scheme 52) with alcohol **377** gave the *O*-allyl disaccharide **379** which was deallylated to give disaccharide hemiacetal **359** and then converted into the trichloroacetimidate **360**, identical to the donor reported by Vasella and Trost (see Section IV.D.2). The synthesis was completed in essentially the same manner as described by Vasella and Trost except that enantiomerically pure 6-*O*-benzylallosamizoline (**294**) was used. The required pseudotrisaccharide **361** was obtained in 60% yield. (Other products obtained in this glycosidation reaction are discussed in Section IV.E, Chart 16.) When smaller amounts of TMSOTf were used, much poorer β-selectivity was observed. Isolation of the desired regioisomer **361** was possible by chromatography only after *N*-dephthaloylation and per-*O*-acetylation of the glycosidation mixture. Catalytic hydrogenolysis gave allosamidin (**4**).

E. Synthesis: Analogues

A number of synthetic carbocyclic analogues have served to verify the structure of allosamizoline (**282**) and the allosamidins. Several others have been synthesized in a search for improved biological activity relative to their natural counterparts, including better species selectivity. A number of analogues with variations to the sugar moieties have been reported, as well as analogues with alterations to the aminooxazoline function. Pseudomono-, pseudodi-, and pseudotrisaccharides are among the analogues described. A variety of analogues have been obtained simply as side products in total syntheses. Novel analogues which inhibit other glycosidase activities have also been designed using the natural compounds as leads. This section also includes methods which give aminocyclopentitols related to allosamizoline (**282**), even though these might not have been explicitly conceived as analogues.

Scheme 53[a]



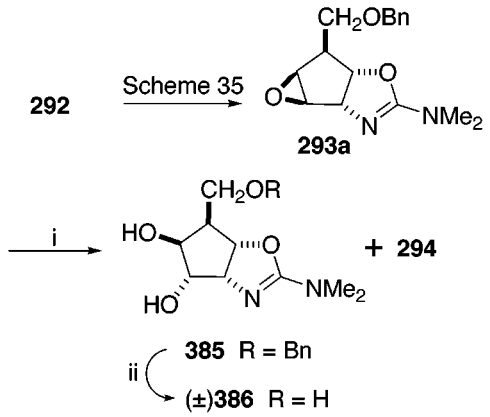
[a] Reagents: (i) NMO, cat. OsO_4, THF−H_2O (2:1), 82%; (ii) H_2, Pd/C; (iii) NMO, cat. OsO_4, THF−H_2O (3:1); (iv) AcCl, pyridine, **382**:**383** = 69%:21%; (v) K_2CO_3, MeOH; (vi) H_2, Pd/C.

1. Enantiomers and Modified Carbocyclic Analogues

Trost and Van Vranken[38,99] have applied a standard catalytic *cis*-hydroxylation to the previously described alkene **292** (see Section IV.C.1, Scheme 35) to obtain analogues **381** and **384**, a pair of 3,4-isomers of allosamizoline (Scheme 53). Catalytic osmylation of racemic alkene **292** gave, as the major isomer, diol **380** isolated in 82% yield. The observed selectivity was that expected upon preferential approach of the reagent on the convex face of the bicyclic skeleton. Hydrogenolysis gave the "*gluco*" triol **381**, an isomer of allosamizoline. Attempts to improve the proportion of the "*allo*" alcohol (hydroxylation from the opposite, concave face) employing other hydroxylation methods were unsuccessful due to the poor nucleophilicity of the double bond under acidic conditions. However, simply diminishing the water content in the osmylation reaction did decrease the proportion of diol **380** (from 5:1 to 3:1). The mixture of alcohols thus obtained was converted into the acetates **382** and **383** and thereby separated. The minor isomer **383** (21% yield) was treated with base to give diol **384** which was debenzylated to give the racemic "*allo*" triol **281** which has a relative stereochemistry corresponding to that originally assigned incorrectly (see Section IV.A) to allosamizoline (**282**). Interestingly, enhancing the steric shielding of the β-face of the cyclopentene **292** by complexation of its benzyl protecting group with β-cyclodextrin in the osmylation reaction reversed the ratio of "*gluco*" diol **380** relative to its "*allo*" isomer (1:1.7, 48% combined yield). This novel result was however not explored further.

The remaining 3,4-isomer of allosamizoline, the "*galacto*" alcohol **386** was obtained by these same authors[38,99] again starting with the alkene **292** (Scheme 54). The epoxide **293a**, obtained as a minor compound during epoxidation of alkene **292** (see Section IV.C.1, Scheme 35), proved to be remarkably

Scheme 54[a]

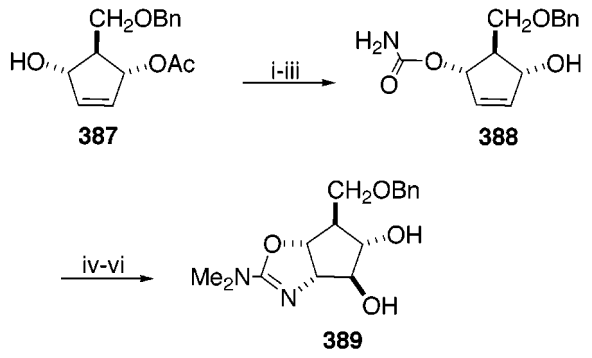


[a] Reagents: (i) 50% aq TFA, 65 °C; (ii) H_2, Pd/C.

robust toward hydrolysis, but did eventually give *trans*-diol **385** in a stereorandom fashion (contaminated with *trans*-diol **294**) upon exposure to rather vigorous solvolysis conditions. Catalytic hydrogenolysis of pure **385** gave racemic triol **386**, the "*galacto*" isomer of allosamizoline.

Griffith and Danishefsky[100b] have reported the synthesis of the 6-*O*-benzylated (+)-allosamizoline (**389**), the enantiomer of the natural aminocyclopentitol (Scheme 55). The optically active monoacetate

Scheme 55[a]

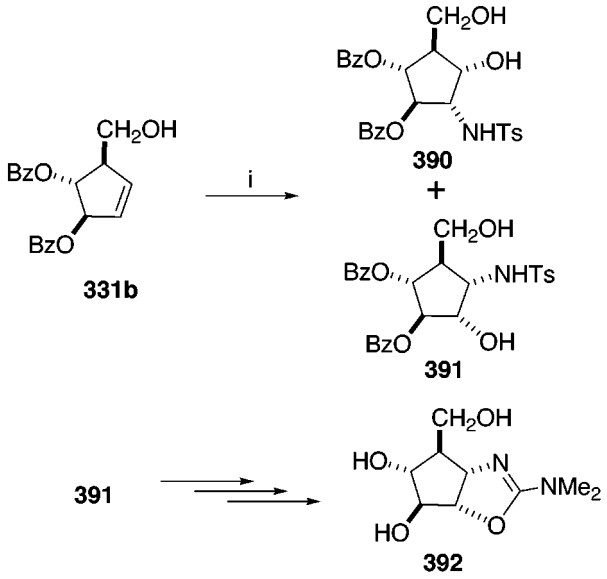


[a] Reagents: (i) PhCOCl, pyridine; (ii) NH_3, MeOH; (iii) K_2CO_3; (iv) TFAA, TFA, 60%; (v) MeOTf, Me_2NH, 77%; (vi) CF_3CO_3H, TFA, TFA, 46%.

387, derived from the *meso*-diol **289** by acetylation and the action of the acetylcholinesterase from the electric eel, was converted into its carbamate **388** and thereafter into the diol **389**, using the strategy described by these authors for the synthesis of the natural aminocyclopentitol (see Section IV.C.1).

Blattner and co-workers[106,111] have reported the synthesis of the allosamizoline regioisomer **392** (Scheme 56). A Sharpless oxyamination of the cyclopentene derivative **331b** (see Section IV.C.9, Scheme 42) gave the isomeric hydroxysulfoamides **390** and **391** in the ratio 1:6.6, from which the regioisomer **391** could be isolated in 25% yield. Oxyaminations of a host of other differently protected alkenes gave other regioisomer ratios, but these mixtures could not be purified. Subjection of the regioisomer **391** to the sequence of reactions reported for the natural equivalent (see Section IV.C.9, Scheme 42) gave the target regioisomer **392**.

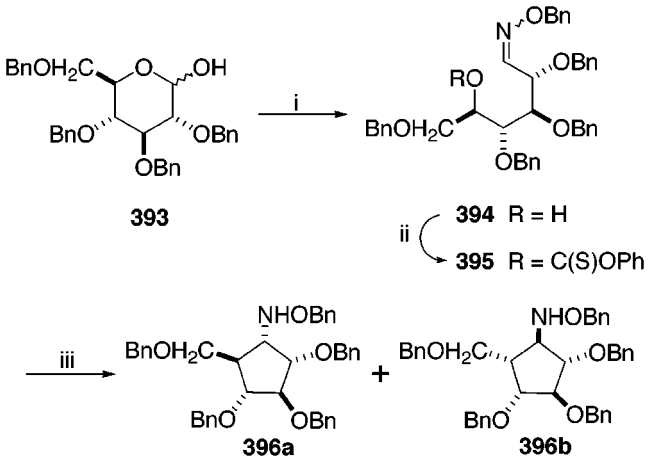
Scheme 56[a]



[a] Reagents: (i) Chloramine T, ^{t}BuOH, OsO_4.

Bartlett and co-workers[21] had established, even before the discovery of allosamizoline (**282**), that radical reactions of oxime ethers give structurally related aminocyclopentitols (Scheme 57). The key

Scheme 57[a]



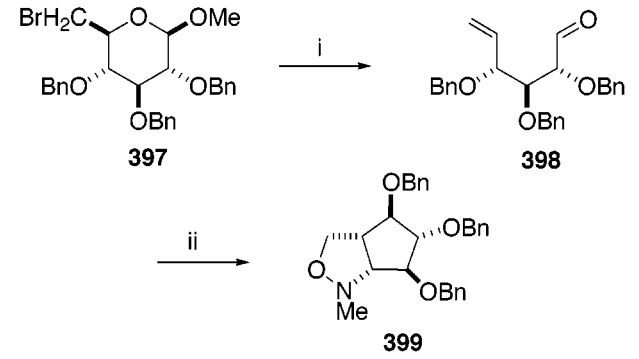
[a] Reagents: (i) $BnONH_3^+Cl^-$, pyridine; (ii) PhOC(=S)Cl, pyridine; (iii) AIBN, $(^n Bu)_3SnH$, reflux, 93%.

phenyl thionocarbonate **395** was obtained from tetra-*O*-benzyl-D-glucopyranose (**393**) via the oxime **394**. This radical precursor cyclized under classical conditions to give a mixture (3:2, 93% combined yield) of carbocycles **396a** and **396b**.

Vasella and Bernet[19a] demonstrated prior to the discovery of allosamizoline (**282**) that related aminocyclopentitols, for example, carbocycle **399**, could be obtained via intramolecular cycloadditions of sugar-derived nitrones (Scheme 58). A reductive elimination provided the required unsaturated derivative **398** from the easily available primary bromo sugar **397**. Exposure of this key aldehyde to *N*-methylhydroxylamine led, via a nitrone intermediate, to the isoxazolidine **399** as the major product.

Farr, Peet, and Kang[51a] made use of a nitrone cycloaddition to obtain aminocyclopentitol **90**, a

Scheme 58[a]

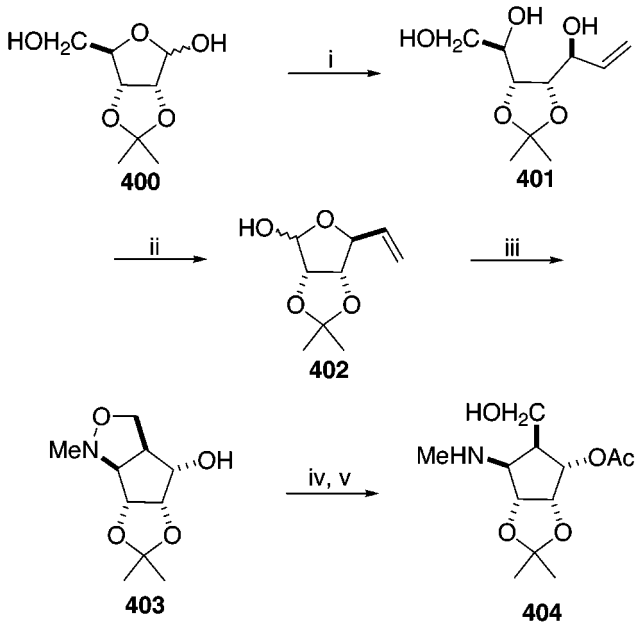


[a] Reagents: (i) Zn, aq EtOH; (ii) MeNHOH, $NaHCO_3$.

potent mannosidase inhibitor (see Section II.D, Chart 5), also starting from precursor **398**.

Shing and collaborators[112] have exploited a nitrone cycloaddition to obtain the allosamizoline-like aminocyclopentitol **404** (Scheme 59). The alkene **401** was

Scheme 59[a]



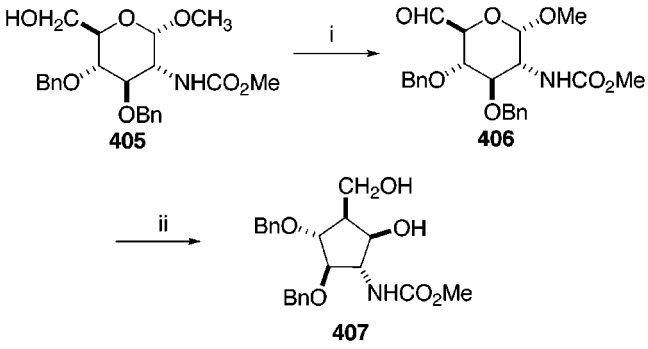
[a] Reagents: (i) $H_2C{=}CHMgBr$, 72%; (ii) $NaIO_4$, aq MeOH, 90%; (iii) MeHNOH·HCl, $NaHCO_3$, 94%; (iv) Ac_2O, pyridine, 85%; (v) $Pd(OH)_2$, H_2, 75%.

obtained through a Grignard reaction on hemiacetal **400**, stereoselectivity arising presumably through chelation control. The latter was exposed to periodate oxidation and the resulting hemiacetal **402** treated with *N*-methylhydroxylamine to give the cycloaddition product **403**. Acetylation and reductive cleavage gave the aminocyclopentitol **404**.

Sinäy and co-workers[113] have reported a samarium(II) iodide-induced ring contraction to obtain analogue **407**, related to allosamizoline **282** (Scheme 60). Swern oxidation of the known amino alcohol derivative **405** gave the corresponding aldehyde **406** which when treated with samarium(II) iodide and HMPA gave the cyclopentitol **407** in 55% yield as a single isomer. The reaction was proposed to follow an aldol-type mechanism.

Imperiali and Shrader[102] have described the synthesis [from an intermediate in their total synthesis

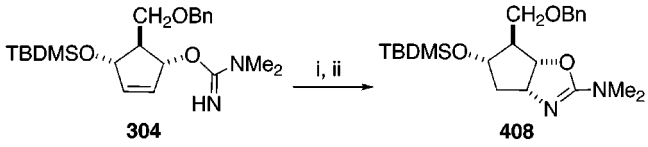
Scheme 60[a]



[a] Reagents: (i) Swern oxidation; (ii) SmI_2–THF, 2 equiv HMPA, 55%.

of allosamizoline (**282**) (Section IV.C.4, Scheme 38)] of the 3-deoxyallosamizoline derivative **408** (Scheme 61). Thus, the aminoimidate **304**, when subjected to

Scheme 61[a]

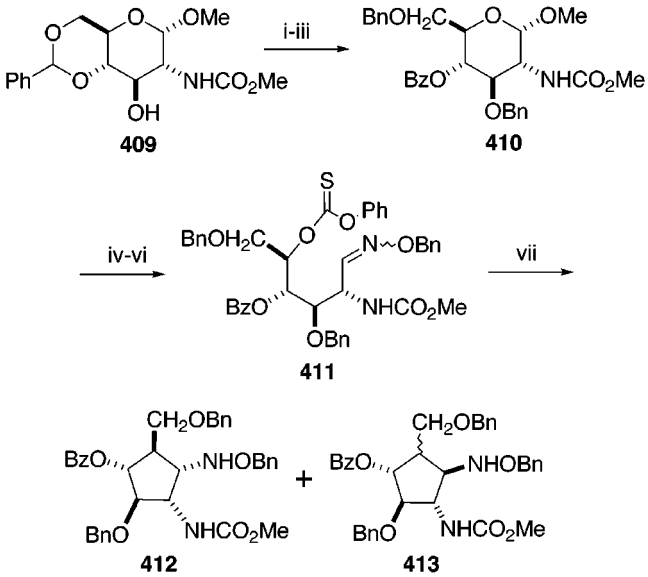


[a] Reagents: (i) $(CF_3CO_2)_2Hg$; (ii) without oxygen, 1 M $NaBH_4$, 2 M NaOH, 75%.

a mercury(II)-mediated cyclization followed by a reductive radical demercuration in a rigorously oxygen-free environment, gave the deoxygenated analogue **408**.

Takahashi and co-workers[114] have obtained the diaminocyclopentitol derivatives **412** and **413** from a sugar precursor via a radical approach (Scheme 62).

Scheme 62[a]



[a] Reagents: (i) BnBr, $Ba(OH)_2$, BaO; (ii) BH_3·Et_3N, $AlCl_3$, 85%; (iii) BzCl, pyridine, 93%; (iv) CSA, AcOH; (v) BnNHOH, pyridine, *anti*:*syn* = 4:1; (vi) PhOCSCl, pyridine; (vii) AIBN, Bu_3SnH, 54%.

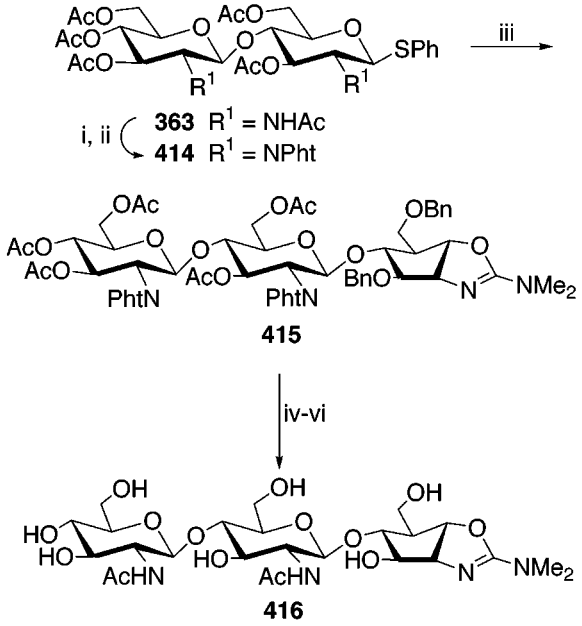
The known alcohol **409**, derived from D-glucosamine, was protected as its benzyl ether, the benzylidene group regioselectively opened, and the thus freed hydroxyl group benzoylated to give the fully protected

derivative **410**. Cleavage of the *O*-methyl group by acetolysis proved unsuccessful. However, acid hydrolysis provided the hemiacetal which was reacted with *O*-benzylhydroxylamine to give the expected oximes as an inseparable mixture of stereoisomers, which were converted into their corresponding thiocarbonate derivatives **411**. The latter mixture was subjected to radical cyclization conditions to give an isomeric mixture of carbocycles **412** and **413**. Chromatography gave compound **412** pure in 15% yield together with stereomer **413** as an inseparable mixture (8:5 by NMR). The *cis*-relationship of the major stereomer **412** was further confirmed by conversion to its acetonide derivative.

2. Isomeric Pseudodi- and Pseudotrisaccharides

Takahashi and co-workers[115] have achieved the synthesis of the pseudotrisaccharide analogue **416** wherein the D-allosamine moieties of allosamidin (**4**) are replaced by *N*-acetyl-D-glucosamine units. This analogue is thus also related to the natural glucoallosamidins **286a** and **286b** (see Chart 14), both of which feature a central glucosamine unit flanked by *N*-acetylallosamine and allosamizoline moieties (Scheme 63). The starting disaccharide **363** was

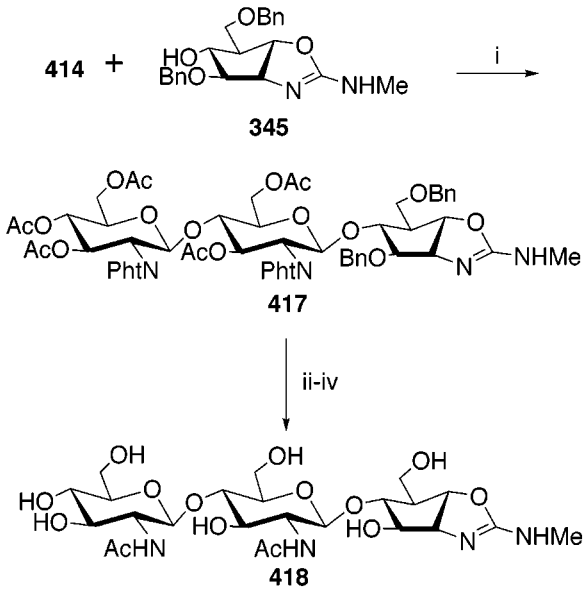
Scheme 63[a]



[a] Reagents: (i) NaOMe, MeOH, NaOH, H_2O, reflux, phthalic anhydride, TEA, 74%; (ii) Ac_2O, pyridine; (iii) **298**, NIS, cat. TfOH, 51% + **368** (17%); (iv) $MeNH_2$, EtOH; (v) Ac_2O; (vi) H_2, Pd/C, 81%.

prepared by enzymatic degradation of chitin followed by appropriate derivatization (see Section IV.D.4). Its exhaustive deacetylation followed by *N*-phthaloylation and per-*O*-acetylation gave the protected glycosyl donor **414**. Coupling of the latter with the partially protected allosamizoline derivative **298**, on activation with catalytic triflic acid−NIS, proceeded to give the desired coupled product **415** in 51% yield together with 17% yield of an unwanted disaccharide glycal **368**. The latter glycal is formed from the donor **414** by elimination and was obtained in 48% yield in the total synthesis of allosamidin (**4**) reported by these

Scheme 64[a]



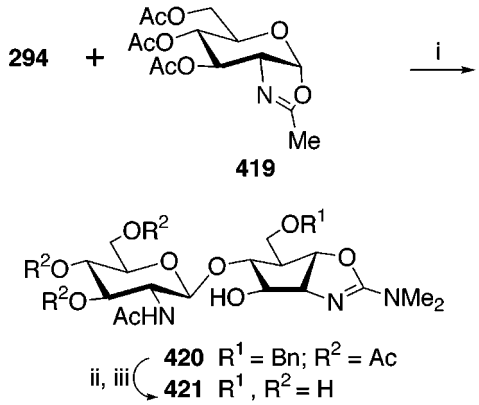
[a] Reagents: (i) NIS, TfOH, 73% + **368** (6%); (ii) EtOH, $MeNH_2$; (iii) Ac_2O, MeOH; (iv) H_2, Pd/C.

same authors (see Section IV.D.4, Scheme 49). Saponification and hydrogenolysis of the coupled product **415** gave the deprotected *all-gluco*-allosamidin analogue **416**.

Takahashi and colleagues[116] have also synthesized the related *N*-demethyl pseudotrisaccharide analogue **418** in a manner similar to that described above (Scheme 64). Thus, the thioglycoside glycosyl donor **414** was coupled with the *N*-demethylallosamizoline analogue **345** (Section IV.C.5, Scheme 44) using catalytic triflic acid−NIS activation to give the pseudotrisaccharide **417** in 73% yield together with 6% yield of an unwanted disaccharide glycal **368**. Deprotection gave the target *all-gluco*-demethylallosamidin analogue **418**.

Imperiali and Shrader[102] have described a synthesis of the disaccharide analogue **421** (Scheme 65).

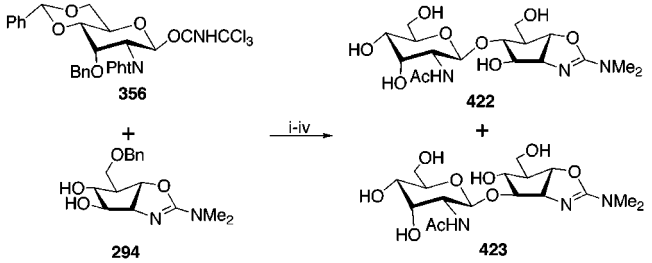
Scheme 65[a]



[a] Reagents: (i) TfOH, (1 equiv), 50%; (ii) H_2, $Pd(OH)_2$, 95%; (iii) aq NH_3, MeOH, 95%.

The known oxazoline glycosyl donor **419** was coupled to 6-*O*-benzylallosamizoline (**294**) (Section IV.C.2, Scheme 36) using catalytic triflic acid to give in 50% yield the desired 4-*O*-regiomeric disaccharide **420** as

Scheme 66[a]



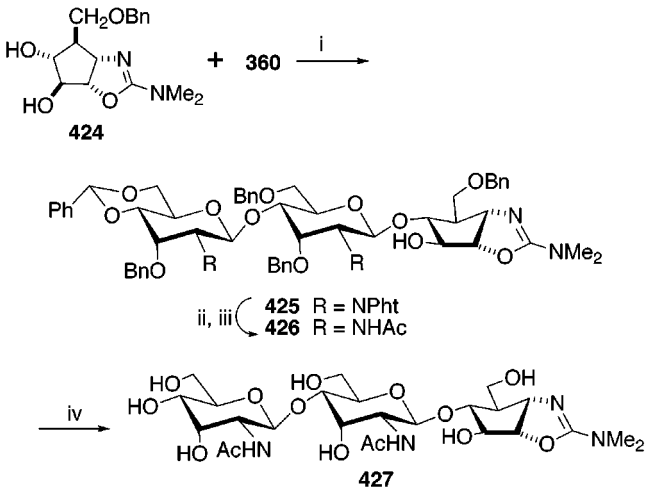
[a] Reagents: (i) TMSOTf (gives regioisomeric mixture); (ii) $MeNH_2$, EtOH (gives regioisomeric mixture); (iii) Ac_2O, MeOH, separation of regioisomers (60% and 11%); (iv) H_2, Pd/C, 84%.

the main component (6:1) and exclusively the β-anomer. Deprotection gave the target analogue **421**.

Blattner and co-workers[111] have reported the synthesis of two pseudodisaccharides, **422** and **423** (Scheme 66). Condensation of trichloroacetimidate **356** (see Section IV.D.5, Scheme 47) with the 6-*O*-benzylallosamizoline (**294**) (Scheme 38) gave a mixture of regiomeric β-pseudodisaccharides which was *N*-dephthaloylated, *N*-acetylated, separated by chromatography, and subjected to hydrogenolysis to obtain the target analogues **422** and **423** in a ratio of ca. 6:1. Sakuda and co-workers[91] found that exposure of either glucoallosamidin A (**286a**) or allosamidin (**4**) to mild acid resulted in hydrolysis of the terminal nonreducing sugar of each to give the corresponding pseudodisaccharides **421** and **422,** respectively.

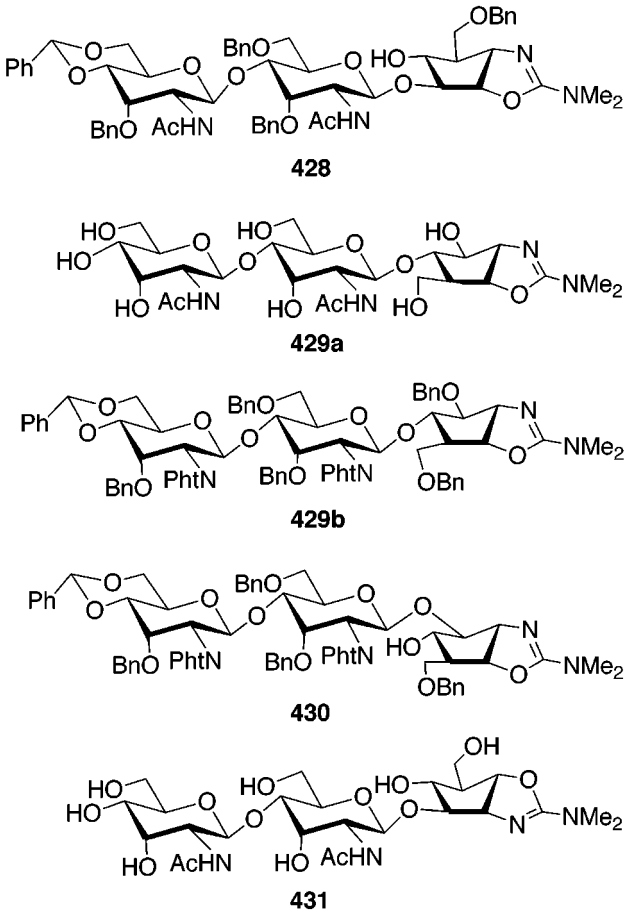
The pseudotrisaccharide analogue **427** carrying the 1,2-regiomeric diol **424** (an intermediate in the synthesis of the corresponding triol **392**, see Scheme 56) has also been obtained by Blattner and co-workers[111] (Scheme 67). The disaccharide trichloroacetimidate **360** (see Section IV.D.5, Scheme 48) was coupled with the diol **424** to form the desired di-*N*-phthaloyl disaccharide **425** in a 2:5 ratio as an inseparable mixture with its regioisomer (see Chart 16 and discussion below for details of secondary products). *N*-Dephthaloylation followed by *N*-acetylation gave a mixture of regiomeric *N*-acetylated

Scheme 67[a]



[a] Reagents: (i) TMSOTf, 59% + 3-*O*-regioisomer; (ii) $MeNH_2$, EtOH; (iii) Ac_2O, MeOH; (iv) H_2, Pd/C.

Chart 16



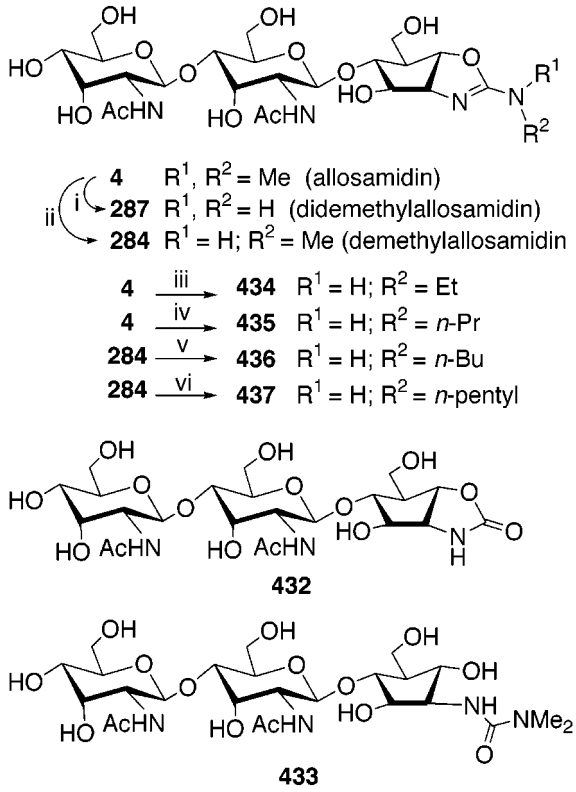
pseudotrisaccharide derivatives from which a sizable proportion of the desired isomer **426** was obtained pure by fractional crystallization (from the chromatographically inseparable mixture). Hydrogenolysis gave the target analogue **427**.

Blattner and co-workers[111] have reported the pseudotrisaccharide derivative **428** (Chart 16), the 3-*O*-regioisomer carrying a 1,2-regiomeric allosamizoline moiety, which was obtained as a contaminant in the coupling reaction to obtain the analogue **427** (see Scheme 67). Danishefsky[100b] has encountered the pseudotrisaccharide **429a** and Trost and Vasella[109a] its protected analogue **429b** (Chart 16) in glycosylation reactions during their total syntheses of allosamidin (**4**) (see Sections IV.D.1 and IV.D.2, respectively). Both analogues comprise, as the aminocyclopentitol component, the enantiomer of allosamizoline. The latter workers also encountered the 3-*O*-regiomeric pseudotrisaccharide **430**, together with its diastereomer (Chart 16), as a mixture in 5% combined yield, in a glycosidation reaction involving racemic 6-*O*-benzylallosamizoline (**294**) in their total synthesis of the natural product (see Section IV.D.2). Ferrier and co-workers also obtained the latter compound in 9% yield in their total synthesis of the natural product (see Section IV.D.5) and deprotected it to give the 3-*O*-regiosomer **431** (Chart 16).

3. Esters and N-Alkylated Derivatives

Yamada and co-workers[117] have described the syntheses of a number of *N*-monoalkylated analogues

Scheme 68[a]

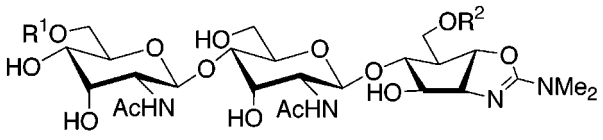


[a] Reagents: (i) 121 °C, aq NH_3, 13% (+ **432** + **433**); (ii) 121 °C, $MeNH_2$, 17% (+ **432** + **433**); (iii) $EtNH_2$, 22%; (iv) $^nPrNH_2$, 5%; (v) $CH_3(CH_2)_3NH_2$, 22%; (vi) $CH_3(CH_2)_4NH_2$, 21%.

starting from allosamidin (**4**) and demethylallosamidin (**284**) (Scheme 68). Didemethylallosamidin (**287**) was obtained in 13% yield by treating allosamidin (**4**) with 28% aqueous ammonia at elevated temperatures. Two byproducts were also observed and identified as the oxazolidinone **432** and the ureido derivative **433**. When allosamidin (**4**) was treated under similar conditions but replacing ammonia with aqueous methylamine, demethylallosamidin (**284**) was formed together with the byproducts. Use of the appropriate amine under identical conditions gave the corresponding *N*-ethyl and *N*-propyl derivatives **434** and **435**, respectively, from allosamidin (**4**), but no reaction occurred when *N*-butylamine was employed as the amine component. However, reaction of demethylallosamidin (**284**) with aqueous *N*-butylamine or *N*-pentylamine did give the desired *N*-butyl and *N*-pentyl derivatives **436** and **437**, respectively.

Yamada and co-workers[117] have also reported the synthesis of a series of 6- and 6″-*O*-acyl derivatives of allosamidin (**4**) (Chart 17). Esterification of the natural product **4** with trichloroethyl butyrate catalyzed by a protease gave selectively the 6″-*O*-butyryl derivative **438**, but only in very low yield. Attempted chemical acylation using 3-octonyl-5-methyl-1,3,4-thiazole-2(3*H*)-thione, in the presence of 4-diazabicyclo-[2.2.2]octane, gave the 6- and 6″-*O*-octylallosamidin derivatives **439** and **440**. Similar conditions allowed the synthesis of a mixture of the 6- and 6″-*O*-alkyl esters **441** and **442**. The 6-*O*-pivaloyl and 6″-*O*-phenylacetal analogues **443** and **444** were also reported. The ratios of products were found to vary with the size and nature of the acyl group.

Chart 17



438 $R^1 = CH_3(CH_2)_2(C{=}O)$-; R^2 = H
439 $R^1 = CH_3(CH_2)_6(C{=}O)$-; R^2 = H
440 R^1 = H; $R^2 = CH_3(CH_2)_6(C{=}O)$-
441 $R^1 = CH_3(OCH_2CH_2)_5(C{=}O)$-; R^2 = H
442 R^1 = H; $R^2 = CH_3(OCH_2CH_2)_5(C{=}O)$-
443 $R^1 = (CH_3)_3C(C{=}O)$-; R^2 = H
444 $R^1 = C_6H_5CH_2(C{=}O)$-; R^2 = H

4. Miscellaneous

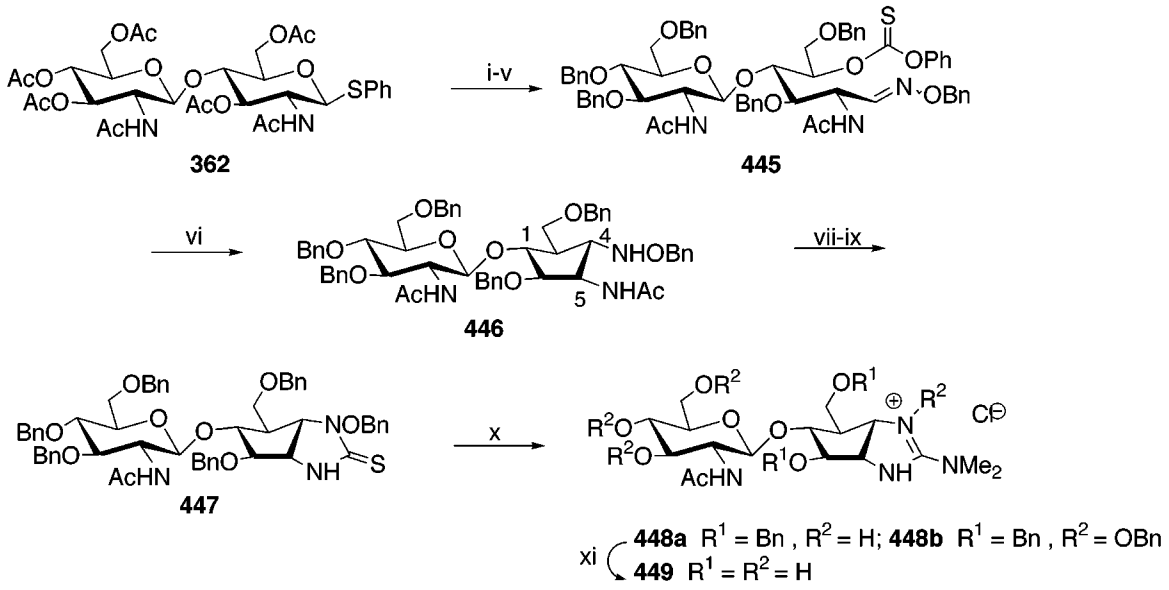
Takahashi and co-workers[118] have described the synthesis of a pseudodisaccharide guanidine derivative **449** starting from *N*-acetylchitobiose. The carbocycle was obtained via a radical reaction, the precursor for which was tethered glycosidically to an *N*-acetylglucosamine unit (Scheme 69). Chitobiose heptaacetate thioglycoside (**362**) was de-*O*-acetylated, per-*O*-benzylated, and treated with NIS in aqueous THF, and the hemiacetal thus obtained was condensed with *O*-benzylhydroxylamine hydrochloride to give an inseparable mixture of (*E*)- and (*Z*)-oximes which was treated with chlorophenyl thionoformate to give the key thiocarbonate intermediates **445**. Radical cyclization of the latter gave the desired hydroxylamine **446** as the major carbocycle, together with three isomers, separable by chromatography. Reaction of the desired isomer **446** with chlorophenyl thiocarbonate followed by treatment of this product with sodium hydride resulted in cyclization with concomitant *N*-deacetylation to give the thiourea **447**. This thiourea **447**, on being refluxed with methyl iodide, treated with dimethylamine, and then exposed to aqueous hydrogen chloride, gave the guanidine derivative **448** together with its *N*-benzyloxy derivative. Each of these derivatives, on hydrogenolysis, gave the novel pseudodisaccharide **449**.

Zhou and co-workers,[96] in investigations related to the biosynthesis of the allosamidins (see Section IV.B), made use of the three ^{14}C-labeled pseudotrisaccharide analogues **450**−**452** in which the aminocyclopentitol moiety was ^{14}C-labeled at C-7 (Chart 18). These analogues were obtained as a mixture upon treating [7-^{14}C]allosamidin with 28% aqueous ammonia at 121 °C in a sealed tube and were separated by HPLC.

Griffith and Danishefsky[100b] have reported the synthesis of a number of pseudodi- and pseudotrisaccharide analogues of allosamidin (**4**), for example, **453** (Chart 19), comprising a homopyrollidine moiety in place of allosamizoline (**282**). A discussion of these is beyond the scope of this review.

Dean and co-workers[119] have described the synthesis of a pair of aminocyclohexitol analogues **454** and **455** (Chart 19) and a series of pseudodisaccharide analogues **456**−**458** (Chart 19) wherein a polyhydroxyaminocyclohexitol moiety replaces the aminocyclopentitol found in allosamidin (**4**). The syntheses will not be described here, but their biological activity is reported in Table 3.

Scheme 69[a]



[a] Reagents: (i) NaOMe, MeOH, 100%; (ii) BnBr, $Bn(OH)_2$, 69%; (iii) NBS, aq THF, 70%; (iv) $BnONH_2$·HCl, pyridine, 81%; (v) PhOCSCl, pyridine, 71%; (vi) Bn_3SnH, AIBN, 31% along with three isomeric compounds at the C-4/C-5 positions; (vii) PhOCSCl, pyridine, 74%; (viii) NaH, DMF, 81%; (ix) CH_3I, reflux; (x) Me_2NH_2OAc, R^2 = H (69%), R^2 = OBn (12%); (xi) H_2, Pd/C, 81% from R = H, 75% from R = OBn.

Chart 18

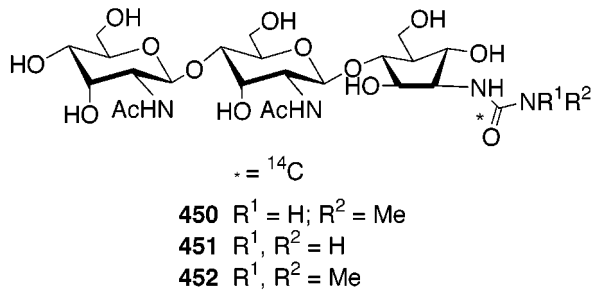
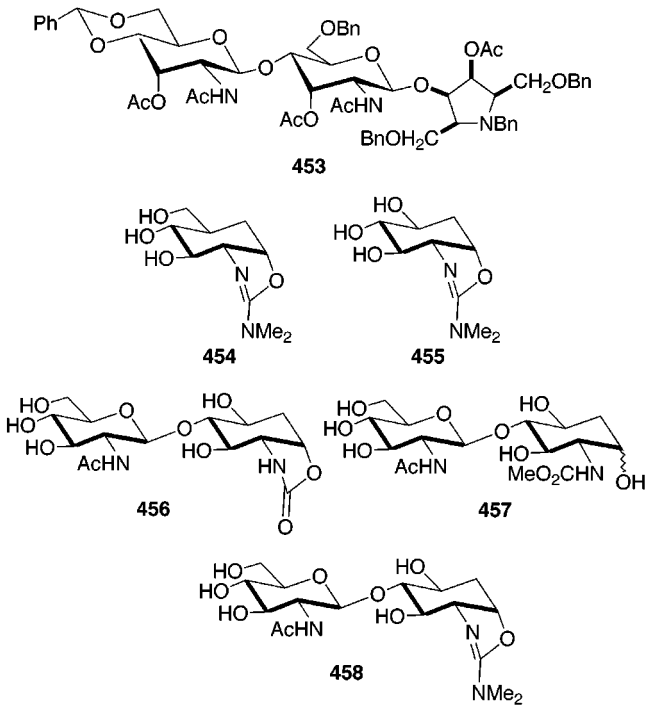


Chart 19



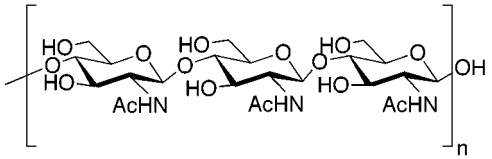
F. Biological Activity

Allosamidin (**4**) was the first compound identified to potently inhibit chitinases,[90,120] enzymes implicated in the hydrolysis of chitin (Chart 20). Chitin, the second most abundant polysaccharide in nature, is a polymer predominantly composed of a β-(1−4)-linked *N*-acetyl-D-glucosamine backbone and is an integral supportive component of most fungal systems and anthropods, but it is not present in vertebrates.[121] Thus disruption of chitin biosynthesis has been recognized as, potentially, a means by which to selectively control pest organisms.[122]

Many inhibitors of chitin synthetase, the enzyme responsible for transferring *N*-acetyl-D-glucosamine units to a growing polysaccharide chain, have already been tested,[121] the most notorious of these being the polyoxins (Chart 20) which have already found use, albeit limited, as pesticides in Japan. However, the discovery of allosamidin (**4**) and its relatives has for the first time opened up the possibility that species-specific chitinase inhibitors might be found.[122,123] It is interesting that the first step in the biosynthesis of chitin[121] is the hydrolysis of trehalose by a trehalase, and the prospect of using a 'cocktail' composed of a trehalase inhibitor such as trehazolin (**3**) (Section III), in combination with a chitinase inhibitor such as allosamidin (**4**), would appear to provide a powerful two-pronged strategy by which to combat pests and possibly prove more effective than using either inhibitor alone (cf. combination therapy).

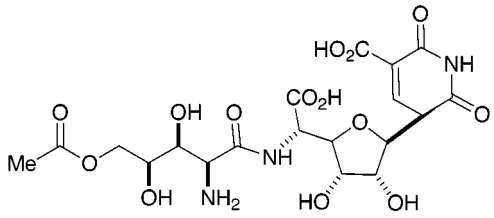
The structure of allosamidin (**4**) resembles somewhat closely that of chitotriose, a trisaccharide fragment of chitin (Chart 20). Glucoallosamidins A (**286a**) and B (**286b**) (see Chart 14) bear an even closer relationship to chitotriose. Because chitinases are endoglycosidases and possess several binding pockets in order to recognize and selectively bind their polymeric substrates,[25a] it would seem that an inhibitor of at

Chart 20

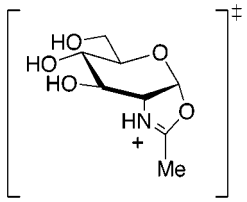


n = 3, chitotriose

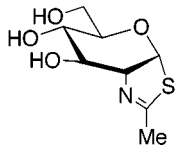
n = large , chitin



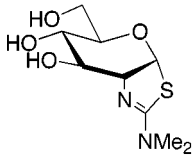
polyoxin D
chitin synthetase inhibitor
$K_i = 9.0 \times 10^{-7}$M



Oxazoline-type
High-energy intermediate



Thiazoline analogue **459**



Isothiourea analogue **460**

least three subunits would be a requirement for good selectivity. However, the pseudodisaccharide analogues **421** and **422**, which lack the terminal *N*-acetyl-D-allosamine unit, nevertheless exibit good inhibition of certain chitinases (see Table 3), although their selectivity profile is much altered to that of the parent pseudotrisaccharides **4** and **286a**, respectively.[95]

An X-ray structure of allosamidin (**4**) bound to hevamine, a plant chitinase/lysozyme (Figure 1), confirms that the *N*-acetyl-D-allosamine units in the inhibitor[124] occupy the same subsites as the *N*-acetyl-D-glucosamine units in the natural substrate.[125] This begs the question as to why nature has employed the rare sugar *N*-acetyl-D-allosamine as a mimic for *N*-acetyl-D-glucosamine as occurs in allosamidin (**4**). Although the C-3 hydroxyl group of the terminal nonreducing unit of allosamidin (**4**) can possibly hydrogen bond to Asn45 helping to enhance binding, the unique configuration of the sugar units in this inhibitor may well serve to modify its selectivity profile (compared to the glucoallosamidins, for example) or, more intriguingly, play a role in lessening the likelihood of the inhibitor being recognized as a substrate by *N*-acetylglucosaminidases and degraded. Pseudotrisaccharide **416**, an "*all-gluco*" analogue of allosamidin (**4**), is apparently susceptible to hydrolysis by chitinases themselves (Table 3).[115] That the C-3 hydroxyl group of the central sugar in allosamidin (**4**) might mimic an incoming nucleophilic water molecule, as has previously been suggested,[25] seems a less plausible explanation: the glucoallosamidins (**286a** and **286b**), both of which contain a central D-glucosamine unit rather than D-allosamine, are better inhibitors of certain chitinases than allosamidin (**4**) itself,[95] and the "*all-gluco*" analogue **418** of demethylallosamidin shows activity[108] comparable to that of the natural compound **284** toward *Candida albicans* chitinase (see Table 3).

The aminocyclopentitol portion of the allosamidins is that which interacts directly with the catalytic machinery of the enzyme. The structure of the hevamine−allosamidin (Figure 1) complex shows that this pseudosugar is held firmly in position by

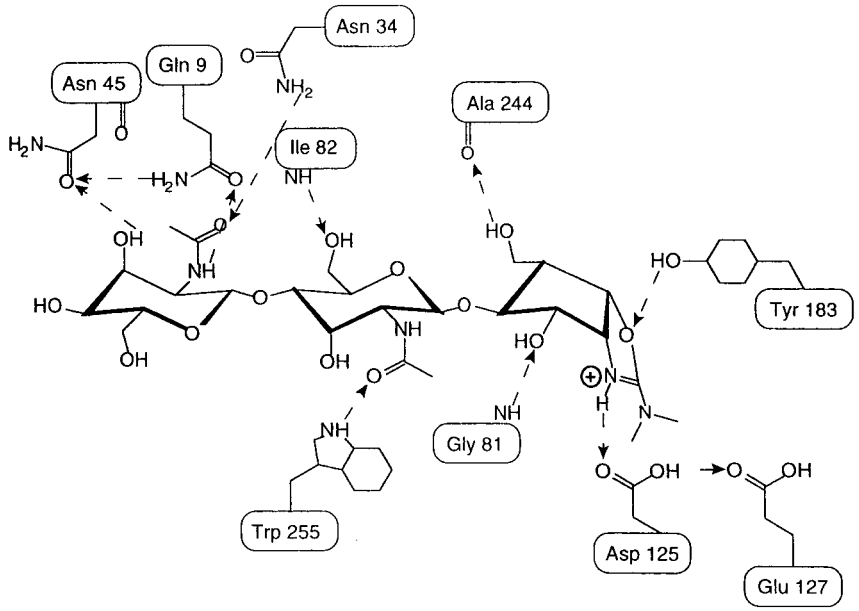


Figure 1. Direct hydrogen bonds between allosamidin and hevamine. (Reprinted from ref 124. Copyright 1995 American Chemical Society.)

Table 3. Survey of Biological Activity of Allosamidins and Their Analogues

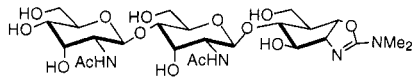
Compounds	Comments	Ref.
Natural products		
 Allosamidin **4**	Inhibits ecdysis in 4th instar larvae of *Bombyx mori* (EI_{50} = 2 μg) (no effect on larvae during feeding to molting stage).	90
	Inhibits ecdysis in 5th and last instar larvae of *Leucania separata* (EI_{50} = 4 and 8 μg).	
	Inhibits ecdysis in 4th instar larvae of *Heliothis virescens* (larvae all dead after 5 days of 0.5 mm^3 of 10% solution of the inhibitor).	106
	Stops malarial parasite transmission in mosquito midgut by blocking oocyst development *in vivo.*	129
	Strongly inhibits chitinolytic activities in the nematode *Heligmosomoides polygyrus* ($IC_{50} = 2.2\times10^{-9}$ mol dm^{-3}), in the nematode *Onchocerca gibsoni* ($IC_{50} = 0.2\times10^{-9}$ mol dm^{-3}) and weakly in the reptilian parasite *Entamoeba invadens* ($IC_{50} = 85\times10^{-9}$ mol dm^{-3}).	130 131
	Inhibition of chitinase activities from *Bombyx mori* is also strong (K_i = 0.07 and 0.11×10^{-6} mol dm^{-3} for the pupal alimentary canal and for the larval integument chitinases) but not observed for the corresponding β-*N*-acetyl-D-glucosaminidases.	132, 94,11
	Inhibition of microbial and plant chitinase activity is more nuanced;	
	e.g. inhibition of chitinase activity from *Streptomyces griseus* and *Serratia marcescens* is weak (500 times less active than for *Bombyx mori* and medium against chitinase from *S. griseus* chitinase from culture strain A82516 ($IC_{50} = 3.7\times10^{-6}$ mol dm^{-3}).	132, 90b 93
	Inhibits competitively most chitinases from opportunistic fungal pathogen *Candida albicans* ($K_i = 0.23\times10^{-6}$ mol dm^{-3}, $IC_{50} = 0.3\times10^{-6}$ mol dm^{-3} against cytosolic chitinase, IC_{50} = 6.2 μg mL^{-1} against chitinases from ATCC10231). Prevents development of house fly larvae to pupae by 100% at 0.27 mg mL^{-1}.	133, 134 95
	Kinetic studies support the formation of reversible complex between inhibitor *at micromolar concentration* and enzyme, followed by irreversible modification of enzyme ($K_{inact} = 5\times10^{-6}$ mol dm^{-3}, $k_2 = 4.6\times10^{-3}$ s^{-1}).	127
	Chitinolytic activity of the dimorphic fungus *Mucor rouxii* is inhibited by allosamidin ($IC_{50} = 6.4\times10^{-6}$ mol dm^{-3}) nevertheless, inhibition failed to stop growth of fungus at 100 times above IC_{50}.	135
	Marked *in vivo* toxicity to larvae of *Tineola bisselliella* (pest of woolen materials) and *Epiphyas postvittina* (pest of fruit crops)	111
	Inhibition of fungal chitinase from *Neurospora crassa* is non-competitive ($IC_{50} = 1.6\times10^{-6}$ mol dm^{-3})	136
	Allosamidin strongly inhibits plant-root chitinase activities of *Pinus sylvestris* L and *Eucalyptus pilularis* Sm and of associated fungi such as *Suillus variegatus* (IC_{50} = 25, 600 and 10×10^{-9} mol dm^{-3}) but not yam chitinase.	137, 90b, 132
	It inhibits yeast *Tricoderma* Sp. (AF6-T8) better than *Saccharomyces cerevisiae* (IC_{50} = 0.8 and 338 μg mL^{-1}, respectively).	95,115

Table 3 (Continued)

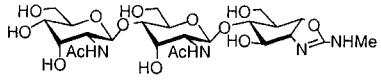
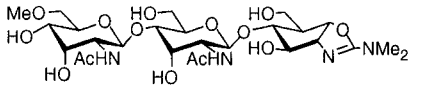
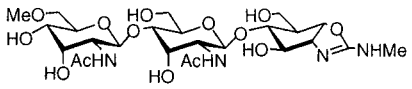
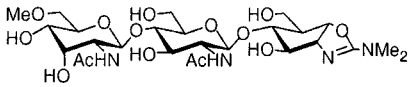
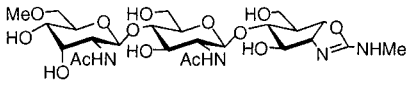
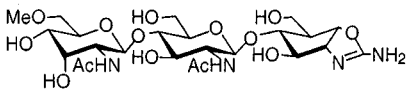
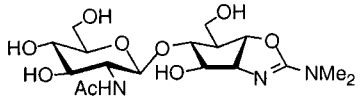
	Exochitinase *Kluyvermomyces lactis* toxin is inhibited by allosamidin.	138
	Allosamidin used as a tool in investigating mechanism of lysis in *Saccharomyces cerevisiae*.	139
	Allosamidin does not inhibit porcine trehalase, silkworm trehalase, rat maltase, rat isomaltase, rat sucrase, yeast maltase, amyloglucosaminidase or a host of other enzyme activities (see Ref. 88 for details).	88
 *N*-Demethylallosamidin **284**	This compound shows much better inhibition of the chitinases from the pathenogenic *Candida albicans* (IC_{50} = 0.7 μg mL^{-1}) and from *Saccharomyces cerevisiae* (IC_{50} = 0.3 μg mL^{-1}) than allosamidin does (about 100 times more active). Nevertheless, that it does not act on yeast growth as a fungicide demonstrated the role of yeast chitinase on cell separation. Similar activity as allosamidin against chitinases from *Bombyx mori* (IC_{50} = 0.25 μg mL^{-1}) and *Trichoderma Sp.* (IC_{50} = 0.8 μg mL^{-1}).	95 120, 115 94
	Used as a tool in investigating mechanism of lysis in *Saccharomyces cerevisiae* (superior to allosamidin)	140
 Methylallosamidin **283**	Similar activity to allosamidin against chitinases from *Candida albicans*, *Saccharomyces cerevisiae* and *Trichoderma* Sp. enzymes.	95
 Methyl-*N*-demethylallosamidin **285**	Similar activity as demethylallosamidin against *Trichoderma* Sp. yeast chitinase but much better against the pathogenic *Candida albicans* enzyme than allosamidin (IC_{50} = 0.6 and 0.4 μg mL^{-1}respectively).	95
 Glucoallosamidin A **286a**	Similar activity to allosamidin against chitinases from *Candida albicans*, *Saccharomyces cerevisiae* and *Trichoderma* Sp.	95
 Glucoallosamidin B **286b**	Better activity than allosamidin against chitinases from *Candida albicans* and *Trichoderma* Sp. but much better against *Saccharomyces cerevisiae* enzyme.	95
 Di-demethylallosamidin **287**	Drop in inhibition by one order of magnitude against insect chitinase. No activity against *Trichoderma* Sp. chitinase. Inhibition of *Saccharomyces cerevisiae* between that of demethylallosamidin and allosamidin.	117
De-glycosylated analogues		
 analogue **421**	Not active against yeast enzymes.	95
	Good activity against chitinase from pathogenic *Candida albicans*.	95
	Inhibits chitinase from *Bombyx mori* (IC_{50} = 0.25 μg mL^{-1}).	94
	Inhibits ability of larvae of *Tineola biselliella* and *Epiphyas postvittana* to shed culicles during moulting resulting in high level of mortality of these pests.	111

Table 3 (Continued)

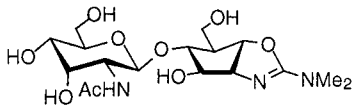
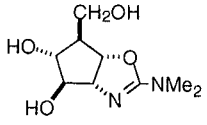
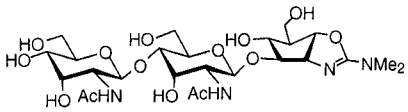
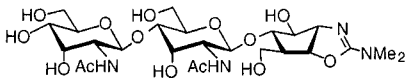
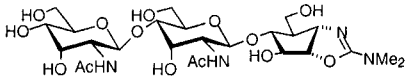
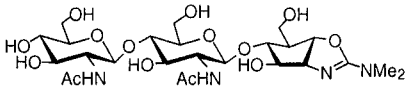
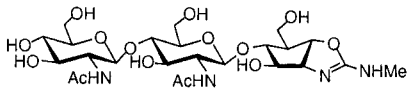
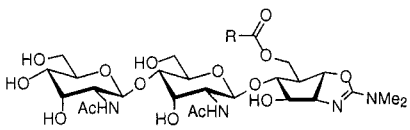
 analogue **422**	Not active against yeast enzymes. Slightly less active against chitinase from *Candida* albicans ATCC10231 than allose analogue **421**.	95 119a
 analogue **282**	Not active against yeast enzyme. No inhibition of chitinase from *Bombyx mori.* Very weak inhibition of *S. griseus* chitinase. Weak inhibition of rat isomaltase, rat maltase, and rat sucrase Inhibits trehalases, *Aspergillus niger* amyloglucosidase and bovine *N*-acetyl-β-glucosaminidase very weakly but more potently than allosamidin. Weak inhibitor of *Candida albicans* chitinase.	95 94 87
Regioisomer, gluco analogs and other epimers		
 3-*O*-regioisomer **431**	Very weak inhibitor of *Streptomyces griseus* chitinase. No death after 5 days injection of 0.5 mm^{-3} of 10% solution of the inhibitor into 4th instar larvae of *Heliothis virescens*, but 33% of larvae smaller than average size.	106
 analogue **429a**	Inhibits *Artemia salina* endochitinase to 62% at 0.25 μM final concentration. No effect on the *Streptomyces* enzyme (compared with value for allosamidin which is 84% at 0.25 μM final concentration).	109b
 1,2-regioisomeric allosamidin **427**	Not active *in vivo* against larvae of *Tineola bisselliella* (pest of woollen materials) nor *Epiphyas postvittana* (fruit crop pest). A number of protected analogues were also tested but did not show promise.	111
 « all-gluco »-allosamidin **416**	Sharp drop in inhibition of yeast chitinase *Trichoderma* Sp. but 10 times less active against insect enzyme *Bombyx mori* (IC_{50} = 0.16 μg mL^{-1}) and only 2 times less active against chitinase from *Saccaromyces cerevisae* compared to allosamidin. Partial decomposition observed of gluco analogues with *Trichoderma* Sp. chitinase, authors suggest enzyme hydrolyses substrate.	115
 « all-gluco »-demethylallosamidin **418**	Inhibition of mold *Trichoderma* Sp. chitinase strongly decreased by gluco change whereas that of *Saccharomyces cerevisiae* is not affected much (IC_{50} = 0.5 μg mL^{-1}) compared to *N*-demethylallosamidin.	116
6 and 6'' esters derivatives of allosamidin		
 R = Heptyl **440** R = $(CH_2CH_2O)_5CH_3$ **442** R = ^{t}Bu **443**		

Table 3 (Continued)

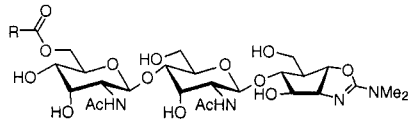
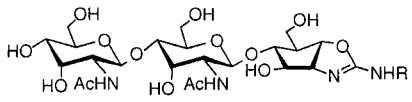
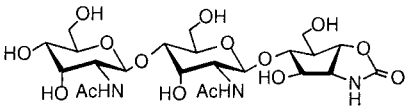
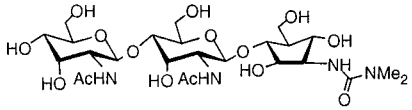
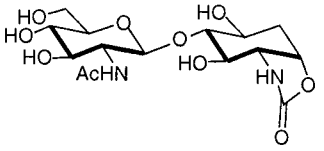
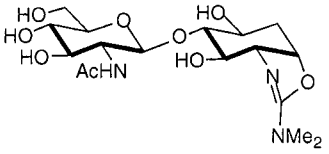
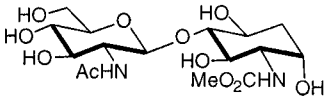
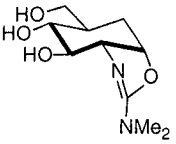
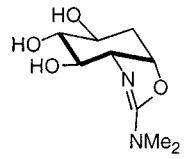
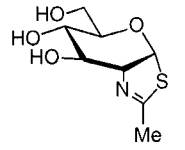
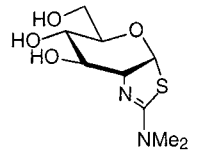
 R = "Pr **438** R = Heptyl **439** R = $(CH_2CH_2O)_5CH_3$ **441** R = Bn **444**	6-*O*-acylation of the terminal sugar or 5-*O*-acylation in the carbocycle has minimal effect on inhibition of *Trichoderma* Sp. although it reduces that of insect enzyme compared to allosamidin by relatively small amounts.	115, 117
N-Alkyl derivatives of allosamidin  R = Et **434** R = "Pr **435** R = "Bu **436** R = Pentyl **437**	*N*-alkylation on the terminal sugar reduces the activity by two orders of magnitude except against yeast *Saccharomyces cerevisiae* chitinase where the activity is much better but decreases with increasing chain length.	117
Modified pseudodisaccharides derivatives  Cyclic carbamate **432**	The analogue is active against chitinases from *Bombyx mori* and *Trichoderma* Sp. (IC_{50} = 5.5 and 26 μg mL^{-1}, respectively) but not against *S. cerevisiae* enzyme. See Ref. for precise values.	117
 Ureido analogue **433**	No inhibition of chitinases, but see Ref. 95 where inhibition by **433** and two related « ring-opened » analogues is observed, albeit weak.	117
 Cyclohexitol **456**	No inhibition of *Candida albicans* chitinase	119
 Cyclohexitol **458**	No inhibition of *Candida albicans* chitinase.	119
 Cyclohexitol **457**	No inhibition of *Candida albicans* chitinase.	119
 analogue **454**	Weak chitinase inhibitor: comparable to allosamizoline.	119

Table 3 (Continued)

 analogue **455**	Not active	119b
 Thiazoline derivative **459**	Competitive inhibition against jack bean *N*-acetylhexosaminidase ($K_i = 280\times10^{-9}$ mol dm^{-3}).	128a
 Aminothiazoline derivative **460**	Inhibits jack bean *N*-acetylhexoaminidase competitively ($K_i = 160\times10^{-6}$ mol dm^{-3}).	128b

hydrogen bonds to amino acids at the active site,[124] several of which are strictly conserved in plant and fungal family 18 chitinase sequences.[126] A number of pyrollidines have been shown to inhibit certain glycosidases more potently than their six-membered counterparts presumably because they resemble more closely the flattened intermediate implicated in the mode of action of retaining enzymes (see also discussion in Section II.D).[4,26a] It is likely that the shape of the cyclopentitol skeleton of the allosamidins affords it similar advantages and for the same arguments.

More intriguing is the question of how the aminooxazoline moiety has a bearing on the potency of this class of inhibitors. Again, this feature can be understood in terms of the probable mode of catalysis of chitinases. In the hevamine−allosamidin complex (Figure 1), Glu127 is very close to the aminooxazoline group, and it is this amino acid which is well-positioned to act as the proton donor in the hydrolysis reaction.[124] The absence in the active site of an appropriately positioned nucleophilic carboxylate, needed to stabilize the expected carbocationic intermediate and to ensure the observed retention of configuration, supports the contention that it is the *N*-acetyl group at C-2 of the substrate which serves this role by assisting anchimerically the enzymatic hydrolysis.[25a] Several lines of argument support this hypothesis,[124] not least the resemblance of the aminooxazoline group in allosamizoline (**4**) to the probable structure of such an oxazoline-type high-energy hydrolysis intermediate (Chart 20). Transformation of the oxazoline function of allosamidin (**4**) into a cyclic carbonate gives analogue **432** which retains some inhibitory activity against chitinases, but the acyclic ureido analogue **433** (Scheme 68) is inactive (see Table 3).

It has also been pointed out that the aminooxazoline function is a potential electrophilic locus and could be viewed as a site by which the chitin-degrading enzyme might be covalently attached by irreversible attack of an active site nucleophile.[100,117] Although the mode of inhibition almost exclusively observed at low inhibitor concentrations is competitive and reversible, at higher concentrations the situation can apparently change. Kinetic studies have shown that at micromolar inhibitor concentrations, a reversible complex forms between the allosamidin (**4**) and a chitinase from *Candida albicans*, and this is followed by irreversible modification of the enzyme.[127] A similar reactivity would in principle be possible for trehazolin (**3**); however, this has not been observed (see discussion in Section III.D).

Already, insights gained by reflecting on the mechanism of inhibition of allosamidin (**4**) and the mode of action of *N*-acetyl-β-hexosaminidases have led to the design of a number of successful synthetic inhibitors of these enzymes. These include the thiazoline derivative **459**[128a] and the isothiourea analogue **460**[128b] (Chart 20). It remains to be seen if pseudodi- or pseudotrisaccharide versions of these synthetic analogues would give an effective inhibitor of chitinases.

The inhibition data reported for natural allosamidins and their analogues is brought together in Table 3. Other selected biological data, related to these molecules, is also included but not intended to be exhaustive.

V. Addendum

Since the completion of this review, a number of reports covering various aspects of the synthesis of the title compounds have appeared. These include an article on the mannostatins (Nishimura, Y. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1997; Vol. 19; p 351). A recent book entitled *Carbohydrate Mimics* (Chapleur, Ed.; Wiley-VCH: New York, 1998) also includes a number of chapters which touch upon various aspects of the syntheses of aminocyclopentitols. In addition, several new syntheses of the title compounds have appeared since the end of 1997, and these will be discussed in brief below.

A novel enantioselective synthesis of mannostatin A has been reported by Ling and Mariano (Ling, R.; Mariano, P. S. *J. Org. Chem.* **1998**, *63*, 6072). The key step was the photolysis of pyridinium perchlorate which yielded an amino diol. This *meso*-diol, after per-*O*-acetylation, could be converted into a nonracemic monoalcohol using an enzymatic desymmetrization. (+)-Mannostatin A was derived from the latter intermediate by a relatively short sequence of reactions, including a stereoselective methylthiolation using Trost's methylthiolation method.

Ogawa and Washida (Ogawa, S.; Washida, K. *Eur. J. Org. Chem.* **1998**, 1929) have reported the synthesis of five aminocyclopentitols to probe further the minimum requirements necessary for α-mannosidase inhibition. None of these carbocycles carry the thiomethyl moiety present in mannostatin A. The aminocyclopentitols were obtained as mixtures, using as a key step a base-catalyzed nitro aldol condensation of nitromethane and the dialdehyde derived by periodate oxidation of DL-1,2-cyclohexylidene-*myo*-inositol. Two of the analogues showed modest inhibition of jack bean α-mannosidase.

Li, Lang, and Ganem Washida (Li, J.; Fengrui, L. *J. Org. Chem.* **1998**, *63*, 3403) have described the formal total synthesis of (+)-trehazolin and a synthesis of the analogue (+)-6-*epi*-trehazolin employing as a key step an asymmetric heterocycloaddition between [(benzyloxy)methyl]cyclopentadiene and the acylnitroso dienophile derived from the periodate oxidation of (*S*)-mandelohydroxamic acid. The quarternary carbon in each target was introduced by a stereoselective osmylation of the respective 2,3,4,5-substituted 1-methylenecyclopentane intermediates. The synthesis of (+)-6-*epi*-trehazolin took 14 steps and proceeded in 11% overall yield (from thallous cyclopentadiene).

Giese and co-workers (Boiron, A.; Zilling, P.; Faber, D.; Giese, B. *J. Org. Chem.* **1998**, *63*, 5877) have reported a total synthesis of (+)-trehazolin starting from D-glucose. Three of the chiral centers of the aminocyclopentitol moeity were derived from the sugar and the remaining two introduced stereoselectively by a reductive cyclization of a keto oxime ether [catalyzed by samarium(II) diiodide] and then the reduction of an oxime ether. The stereoselectivity of the pinacol-type coupling was assured by restricting the transition-state conformations accessible in the 5-*exo*-trig cyclization by proper choice of the protecting groups in the precursor. Trehalamine was obtained in an overall yield of 22% in 8 steps starting from a protected glucose derivative and taken through to the natural product by classical methods.

A pinacol coupling forms the basis of an approach from D-glucose to trehalamine and to one of its diastereomers described by Chiara and co-workers (Storch de Gracia, I.; Dietrich, H.; Bobo, S.; Chiara, J. L. *J. Org. Chem.* **1998**, *63*, 6072). The route to the aminocyclopentitol involved a samarium(II) diiodide-catalyzed coupling of a 1,5-dicarbonyl compound and proceeded in an overall yield of 39%, over 9 steps, from the protected starting sugar. A diastereomeric analogue was also obtained in 57% overall yield over 4 steps under similar conditions, from a ketone oxime ether (derived from D-glucose).

Shiozaki's group (Shiozoki, M.; Obukata, O.; Haruyama, H.; Yoshiiki, R. *Tetrahedron. Lett.* **1998**, *39*, 1925) has described the synthesis of a number of 'hybrid' analogues comprising deoxynojirimycin and trehalamine. The inhibitory activity of these analogues against rat intestinal maltase was reported.

A formal synthesis of (−)-allosamizoline has been described by Iwata and collaborators (Maezaki, N.; Sakamoto, A.; Tanaka, T.; Iwata, C. *J. Org. Chem.* **1998**, *63*, 6072), using as a key step a chemical desymmetrization of a *meso*-cyclopentitol. The latter transformation was achieved by a monodeprotection of a *meso*-diol protected as its *C*2-symmetric bissulfoxide acetal (a chiral auxiliary).

Sakuda and Sakurada (Sakuda, S.; Sakurada, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2987) have described the synthesis of a number of allosamidin derivatives in which the nonreducing sugar moiety is acyclic. Three of these were biotinylated. The analogues showed marked inhibition of chitinases, one of them proving as active as allosamidin against the *Trichoderma* enzyme.

VI. Acknowledgments

This article was initiated while A.S. was on "leave of absence" from the Institut de Chimie des Substances Naturelles, and he gives due thanks to Professors Jean-Yves Lallemand (Ecole Polytechnique) and André Lubineau (Orsay University) for providing laboratory space and the support needed to complete this work during sabatical stays in their respective laboratories. The authors wish to thank Professors A. Vasella (Zurich), M. Sinnott (Manchester), B. Ganem (Ithaca), and S. Ogawa (Yokahoma) for their encouragement and for making helpful comments on the review. Gratitude is expressed to Professor B. Dijkstra and R. de Jong for providing the X-ray image which appears on the cover. The diligent criticisms and corrections to this manuscript offered by the reviewers are also gratefully acknowledged.

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(3) For an example, see: von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418.
(4) For a handful of examples, see: (a) Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319. (b) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182. (c) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340. (d) Siriwardena, A. H.; Chiaroni, A.; Riche, C.; El-Daher, S.; Winchester, B.; Grierson, D. S. *J. Chem. Soc., Chem. Commun.* **1992**, 1532.
(5) For a review, see: Hughes, A. B.; Rudge, A. J. *Nat. Prod. Rep.* **1994**, 135.
(6) For a review, see: Burgess, K.; Henderson, I. *Tetrahedron* **1992**, *48*, 4045.
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