REVIEW

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Natural product family 18 chitinase inhibitors

Ole A. Andersen, Mark J. Dixon, Ian M. Eggleston and Daan M. F. van Aalten*
Division of Biological Chemistry & Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee, Scotland DD1 5EH. E-mail: dava@davapc1.bioch.dundee.ac.uk; Fax: ++ 44 1382 345764

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This review covers the synthesis of natural product chitinase inhibitors, and compares their binding modes with family 18 chitinases from a structural and kinetic viewpoint; 121 references are cited.

Ole Andreas Andersen received his M.Sc and Ph.D from the University of Tromsø, Norway, in 1998 and 2003, respectively. His Ph.D work was supervised by Prof. Edward Hough and comprised crystallographic and biochemical studies on human phenylalanine hydroxylase. Andersen had a postdoctoral fellowship for one year at The Norwegian Structural Biology Centre in Tromsø under the supervision of Prof. Arne Smalås. He is currently working on chitinases using protein crystallography as a tool for inhibitor design in the group of Daan van Aalten at the University of Dundee.

Mark Dixon received his first degree from the University of Leeds in 1997. He was then employed by Nycomed Amersham where he worked on the development of new radiopharmaceuticals for 3 years. He received his Ph.D from the University of Southampton in 2004, where he worked under the supervision of Prof. Mark Bradley on the solid-phase synthesis of novel inhibitors of the parasitic enzyme trypanothione reductase. He is currently working on the synthesis of natural product chitinase inhibitors in the laboratory of Ian Eggleston at the University of Dundee.

Ian Eggleston graduated from the University of Durham in 1987, and obtained his D.Phil degree in 1990 at the University of Oxford, under the supervision of Dr J. H. Jones. Following postdoctoral appointments in the USA, France and Switzerland, he was appointed Lecturer at the University of Dundee in 1997. His main research interests are in the synthesis and design of biologically active peptides, peptidomimetics, and other inhibitors of carbohydrate-processing enzymes, along with the development of novel inhibitors of enzymes associated with organisms responsible for tropical parasitic diseases.

Daan van Aalten graduated with a B.Sc in Chemistry from the University of Nijmegen, Netherlands, followed by a Ph.D in Biochemistry/Biocomputing at the University of Leeds in 1996. After postdoctoral work in protein crystallography at Cold Spring Harbor Laboratory and the University of Oulu, Finland, he is now a Reader at the University of Dundee. His group works on two main topics, using a combination of protein crystallography and inhibitor design. The first area is aimed at understanding, at the molecular level, the function of proteins involved in carbohydrate metabolism. The second area focuses on understanding molecular mechanisms in signal transduction pathways, with a particular focus on the PKB/PDK1 pathways and the LKB1 tumour supressor complex.


Ole Andreas Andersen


Mark Dixon


Ian Eggleston


Daan van Aalten

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1 Introduction

Chitinases catalyze the hydrolysis of β-(1,4)-linked *N*-acetylglucosamine (chitin, Fig. 1). Whereas chitin is not a component of mammalian cells, it occurs widely elsewhere in nature and is abundant in human pathogens. Chitin is used as a key structural component of the fungal cell wall,[1] is found in nematode egg shells[2] and is a structural component of insect exoskeletons.[3] Consequently, chitinase inhibitors have chemotherapeutic potential against fungi,[4,5] insects[6] and malaria transmission.[7,8] Recently, chitinase inhibitors were also suggested to have anti-inflammatory potential against asthma and allergic diseases, including atopic dermatitis and allergic rhinitis.[9]

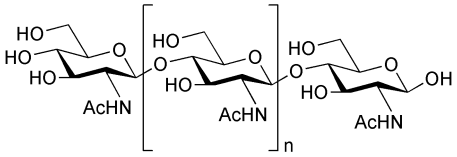


Fig. 1 The structure of chitin.

Although a few synthetic family 18 chitinase inhibitors exist, the majority of effective (*i.e.* nM range) inhibitors are natural products. These vary widely in structure and include pseudotrisaccharides,[6,10,11] cyclic peptides[12–16] and amino acid-derived materials,[17] complex alkaloids[18] and simple purine-derived heterocycles.[19] The need to understand the mode of binding of such inhibitors has led to detailed crystallographic investigations of chitinase–inhibitor complexes along with complementary kinetic, mutational and computational studies. These studies have also elucidated the reaction mechanism of this family of enzymes, and numerous examples of bound inhibitors mimicking reaction intermediates have been observed.[19–27]

2 Family 18 chitinases and their active site architectures

Chitinases belong to the families 18 and 19 of glycosyl hydrolases that differ in amino acid sequence, structure and mechanism.[28,29] Family 18 chitinases contain enzymes from mammals, insects, plants, nematodes, fungi and bacteria and are evolutionarily diverse, whereas family 19 chitinases contain only plant enzymes that are highly conserved. The "plant-type" family 18 chitinases are *endo*-chitinases, cleaving random β-(1,4)-glycosidic bonds within the oligosaccharide chain and leading to oligosaccharide products of varying length.[30–32] The "bacterial-type" enzymes are usually *exo*-chitinases, meaning that specific β-(1,4)-glycosidic bonds are cleaved along the oligosaccharide chain such that acetylchitobiose ($GlcNAc_2$) or acetylchitotriose ($GlcNAc_3$) is progressively released from the non-reducing end of chitin.[30]

Aspergillus fumigatus chitinase B1 (*Af* ChiB1) possesses most of the conserved residues of family 18 chitinases, and is one of several extensively studied enzymes in this family in terms of inhibitor complexes. In the following discussion, therefore, in the interest of simplicity, residue numbering and structural features are discussed consistently using the *Af* ChiB1 structure.

In the "bacterial-type" enzymes, chitin binds to the chitinases by stacking of the *N*-acetylglucosamine (GlcNAc) units on several conserved aromatic residues that line a groove leading to the active site. The core sugar binding subsites are termed −2, −1, +1, +2, with the latter three corresponding to the exposed Trp384, Trp137 and Phe251 residues, respectively, with chitin cleavage occurring between subsites −1 and +1 (panel A, Fig. 2a). The "plant-type" family 18 chitinases, however, are generally smaller and usually contain less clearly identifiable subsites compared to the "bacterial-type" enzymes. Several residues line the active site pocket including a MXYD motif (Met243–Asp246, Fig. 2a) that is conserved in the "bacterial-type" enzymes which defines the bottom of the active site pocket. All family 18 chitinases contain the conserved DXDXE (Asp173–Glu177, Fig. 2a) active site structural motif that is essential for activity.[27,33–35]

3 Catalytic mechanism of family 18 *exo*-chitinases

Structural studies have provided information on the binding modes and specificity of chitinase inhibitors, as well as elucidating the mechanism of the hydrolysis reaction. Effective inhibitors are often observed to mimic reaction intermediates while withstanding catalytic turnover.

The reaction mechanisms of family 18 and 19 chitinases differ significantly, and are both well established. Chitin binds to the active site groove of family 18 chitinases with the GlcNAc units stacking on the solvent exposed tryptophans, with the −1 sugar assuming a boat conformation (step (i), Fig. 3).[25,27,36] Furthermore, chitin binding results in a reorientation of the Asp175 side chain, replacing its hydrogen bond to the carboxyl group of Asp173 with hydrogen bonds to the carboxyl group of the catalytic acid Glu177 and the −1 C2-acetamido group of the substrate. Nucleophilic attack of the carbonyl oxygen of the acetamido group on the anomeric C1 carbon of the same pyranose ring occurs concurrently with protonation of the glycosidic oxygen by the catalytic acid, generating the leaving group from the reducing end of chitin as well as an oxazolinium ion intermediate (step (ii), Fig. 3).[23,25,27,37] This step differs from the classical retaining mechanism of glycosyl hydrolases such as lysozyme, cellulase, xylanase and *endo*-1,3-1,4-β-D-glucan-4-glucanohydrolase that use a carboxylate side chain as the nucleophile.[38]

For the family 19 chitinases, HPLC,[39] NMR[40] and modelling studies[37] have shown that these enzymes use a carboxylate as a base to activate a water molecule that attacks the C1 position resulting in an inversion of configuration at the C1 anomeric carbon as part of a single step mechanism. Formation of the oxazolinium ion intermediate in family 18 chitinases has been questioned on the basis of a combined structural and mutational study on chitinase A from the bacterium *Serratia marcescens* (*Sm*ChiA).[36] However, substrate-assisted catalysis is strongly supported by kinetic data on *Sm*ChiA that show that the polarity of the carbonyl group is essential for enzymatic activity.[41] Formation of the oxazolinium ion intermediate is supported by molecular mechanics calculations,[42] whereas mutation studies have indicated that the formation of the intermediate and its stabilization is assisted by Tyr245 that forms a hydrogen bond to the *N*-acetyl oxygen,[33] thereby generating an interaction with the oxazolinium oxygen.[25,27] The reorientation of Asp175 to the "up conformation", hydrogen bonding with Glu177 and the oxazolinium nitrogen, is believed to be important for stabilization of the positive charge on the intermediate.[27] In the final step, hydrolysis of the oxazolinium ion, regenerating the *N*-acetyl group, results in overall retention of the stereochemistry at the C1 anomeric carbon as well as re-protonation of Glu177, and reorientation of Asp175 to re-generate the hydrogen bond with Asp173 (step (iii), Fig. 3). Retention of configuration at the anomeric carbon is supported by the binary complexes of the pseudotrisaccharide allosamidin **1** with hevamine,[23] *Serratia marcescens* chitinase B (*Sm*ChiB),[27] *Coccidioides immitis* chitinase (*Ci*Chi),[20] and *Sm*ChiA.[21] These complexes have the allosamizoline aglycone moiety in an equivalent position to the oxazolinium ion reaction intermediate.[27] A specific, structurally well-defined water molecule, located opposite the leaving group (O7 oxygen) relative to the C1 anomeric carbon, is a putative C1 attacking group whose position is consistent with retention of the C1 stereochemistry.[27] NMR studies using family 18 chitinases A1 and D from *Bacillus circulans* WL-12 also support a molecular mechanism retaining the anomeric configuration.[43]

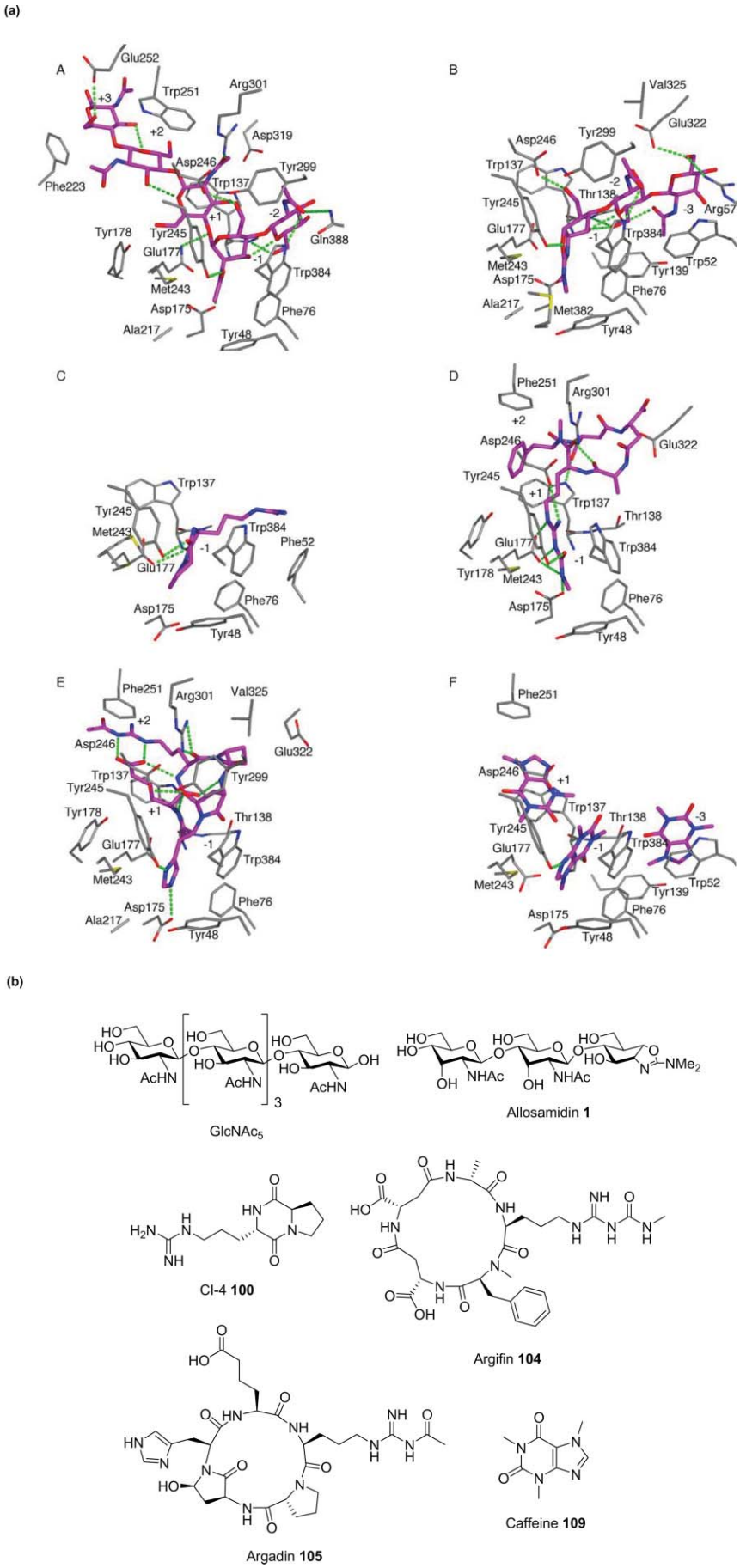


Fig. 2 (a) Ligand binding to chitinase active sites. **A** *Sm*ChiB–$GlcNAc_5$, **B** *Af*ChiB1–allosamidin, **C** *Sm*ChiB–CI-4, **D** *Af*ChiB1–argifin, **E** *Af*ChiB1–argadin and **F** *Af*ChiB1–caffeine. Residues involved in direct ligand interactions are shown (selected residues in **A** omitted for clarity). Residue numbering is according to *Af*ChiB1 for all complexes; hydrogen bonds defined by WHAT IF[120] are shown in green. Ligand carbon, magenta; protein carbon, grey; oxygen, red; nitrogen, blue; sulfur, yellow. Subsites (+2, +1, −1, −2 *etc.*) are also indicated. (b) Chemical structures of ligands depicted in Fig. 2a.

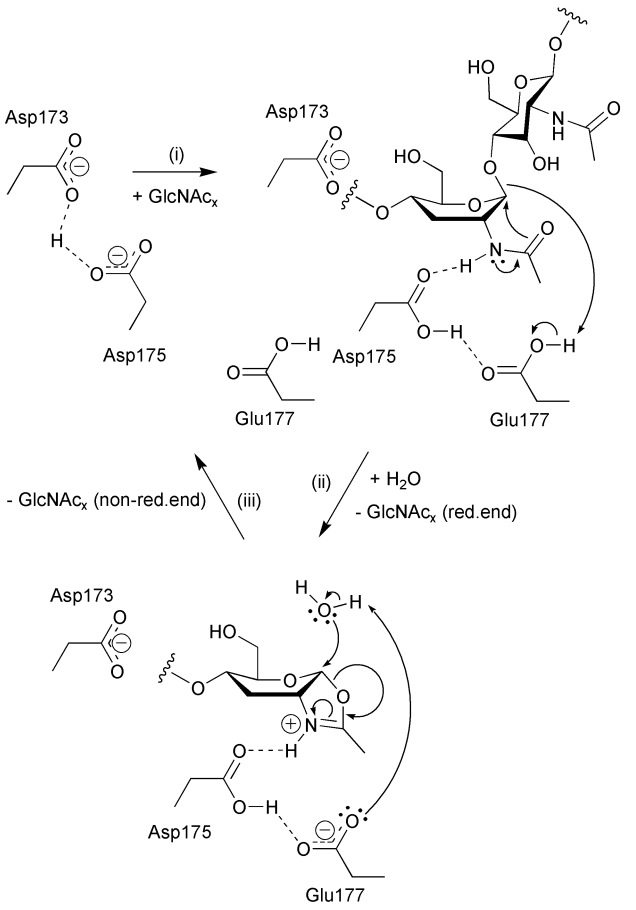


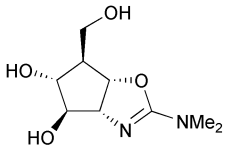
Fig. 3 The catalytic cycle of family 18 *exo*-chitinases. Arrows indicate electron transfers of the subsequent step, whereas dashed lines represent hydrogen bonds. (i) Binding of GlcNAc; (ii) cleavage of GlcNAc releasing product from the reducing end; (iii) release of product from non-reducing end. The figure is based on reaction schemes from Tews *et al.*[25] and van Aalten *et al*.[27] See main text for details.

4 Kinetics, modes of binding and synthesis of natural product family 18 chitinase inhibitors

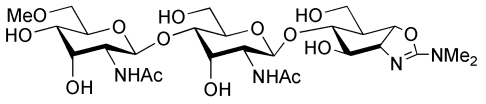
Most known chitinase inhibitors are classical reversible inhibitors that compete with the substrate by blocking its binding site, usually mimicking the stacking to one or more of the subsite tryptophans through π–π or other hydrophobic interactions and generating hydrogen bonds with potential partners in the active site. Oligo-GlcNAcs exhibit this binding capability, but long carbohydrate polymers suffer enzymatic degradation while short oligosaccharides have only low binding affinities.[44] Additionally, the synthesis of carbohydrate oligomers is often challenging, thus precluding the development of drug-like compounds of this type. However, over the past two decades a range of natural product family 18 chitinase inhibitors have been identified and characterized, and in recent years, numerous crystal structures of chitinase–inhibitor complexes have been published.[17,19–22,26,27,45–48] Most of these inhibitor complexes are of the "bacterial-type" chitinases, whereas only one "plant-type" chitinase complex has been published.[23] As detailed below, valuable information has also been obtained as to how the scaffolds provided by the natural products may be chemically modified to optimize inhibitor binding.

4.1 The pseudotrisaccharide allosamidin and its derivatives

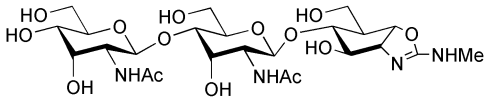
Allosamidin **1** (Fig. 2b), a pseudotrisaccharide first isolated from the mycelium of *Streptomyces* sp.,[6,11] is the most extensively studied chitinase inhibitor. Various biological properties have been reported associated with its activity as a chitinase inhibitor, including inhibition of cell separation in fungi,[49,50] toxicity towards insect larvae[6,11,51] and blocking of malaria parasite penetration into the mosquito midgut.[52] Most recently, compound **1** has been shown to ease lung inflammation in a mouse model of asthma.[9] Compound **1** is in general reported to be a competitive chitinase inhibitor,[53] although non-competitive inhibition has also been reported.[54] The structure of **1** is composed of two *N*-acetylallosamine residues and an aminocyclitol aglycone, allosamizoline **2** (Fig. 4). Investigation of the biosynthesis of



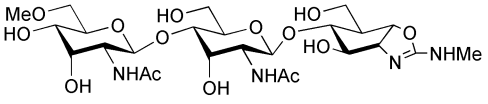
Allosamizoline **2**



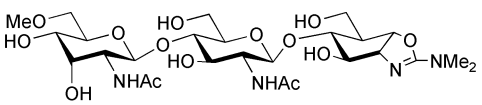
Methylallosamidin **3**



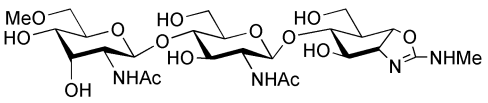
N-Demethylallosamidin **4**



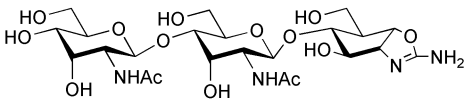
Methyl-*N*-demethylallosamidin **5**



Glucoallosamidin A **6**



Glucoallosamidin B **7**



Didemethylallosamidin **8**

Fig. 4 Allosamizoline **2** and naturally occurring allosamidins **3–8**.

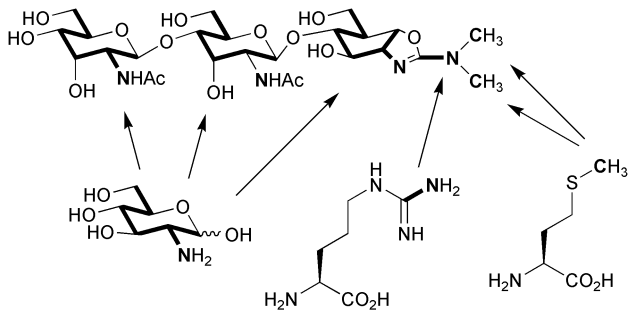


Fig. 5 Biosynthetic origin of allosamidin.

1 in *Streptomyces* sp. has revealed that both *N*-acetylallosamine sugars and the carbocyclic moiety of **2** are derived from D-glucosamine whereas the oxazoline group is derived from L-Arg and L-Met (Fig. 5).[55] Binding of **1** to chitinases has been investigated through crystal structures using hevamine,[23] *Sm*ChiB,[26,27] *Ci*Chi,[20] human chitotriosidase (HCHT),[22] *Sm*ChiA[21] and *Af* ChiB1.[19] It is likely that bound **1** resembles the oxazolinium ion reaction intermediate of the substrate-assisted reaction mechanism, thus generating favourable interactions leading to tight binding. K_i values of 60 nM and 230 nM have been reported for *Candida albicans* chitinase[53] and *Ci*Chi,[20] respectively. IC_{50} values have also been reported for **1** against numerous chitinases (Table 1) ranging from strong inhibition of HCHT[22] and *Bombyx mori* chitinase[56] to relatively weak inhibition of *Saccharomyces cerevisiae* chitinase.[10]

Compound **1** binds to the different chitinases in a similar way, with the two *N*-acetylallosamine sugars occupying the −3 and −2 subsites in an identical fashion to the corresponding *N*-acetylglucosamine substrate units. The allosamizoline unit binds in the −1 subsite as an exact mimic of the oxazolinium ion reaction intermediate, making similar hydrogen bonds from the allosamizoline nitrogen and oxygen to the side chains of Asp175 and Tyr245, respectively (panel B, Fig. 2a).[22,23,26,27] Moreover, Asp175 is in the "up conformation" forming an additional hydrogen bond with Glu177, similar to that observed for the reaction intermediate.[21–23,26,27] In addition to conserved stacking interactions with Trp52 (only present in "bacterial-type" family 18 chitinases) and Trp384, hydrogen bonds are formed between the allosamizoline C6-hydroxyl and the side chain of Asp246, between the allosamizoline C4 hydroxyl and the backbone nitrogen of Trp137, between the acetyl of the −2 sugar and the side chain of Trp384, between the C5 hydroxyl of the −2 sugar and the backbone nitrogen of Thr138, between the acetyl group of the −3 sugar and the side chain of Thr138 and between the C5 hydroxyl of the −3 sugar and the side chains of Arg57 and Glu322. These interactions are almost fully conserved amongst family 18 chitinases.

Several naturally occurring analogues of **1** have been identified and isolated[10] from *Streptomyces* sp., and tested for chitinase inhibition (Fig. 4 and Table 1), namely methylallosamidin **3**,[57] *N*-demethylallosamidin **4**, methyl-*N*-demethylallosamidin **5**, glucoallosamidins A **6** and B **7**,[58] and didemethylallosamidin **8**.[59] Compound **4** has been shown to be a better protector of lysis of *Saccharomyces cerevisiae* daughter cells[1] and is a stronger inhibitor of HCHT than compound **1**.[22] Its complex with HCHT shows that it binds identically to **1**, with no significant backbone conformational change relative to the complex of **1**.[22] The increased affinity of **4** can be explained by the removal of the methyl group of **1** generating less repulsion and increased rotational freedom of the nearby side chains of Asp173 and Asp175.

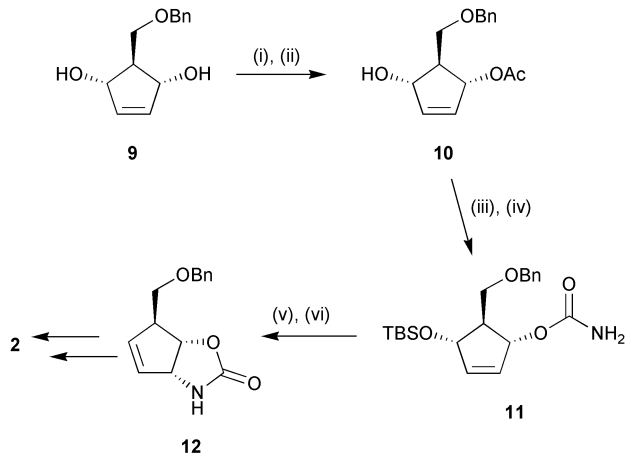
Since the first syntheses of **1** and **2** were reported by the groups of Danishefsky and Griffith[60,61] and Vasella and Trost *et al.*,[62–64] some 12 preparations of the aglycone moiety **2** have been described, along with 4 total syntheses of **1**. Several of these syntheses share a number of common features, hence not all will be described in detail here. Siriwardena *et al.*,[65] in an excellent review in 1999, summarised the syntheses reported at that time of **1** and certain other aminocyclopentitol-based natural products. This review highlighted that synthetic approaches to **2** may be divided into two groups, namely those which employ a carbohydrate starting material to prepare the functionalised cyclopentane unit and those which rely upon the functionalisation and desymmetrisation of a "naked" or preformed cyclopentene. For convenience, this general classification will be adopted here to describe both the preparations of **1** and **2**. For the preparations of **1**, the principle distinguishing features are the choice of glycosylation procedures adopted in order to ensure β-selectivity in the assembly of the pseudotrisaccharide skeleton and also the strategies employed to obtain the rare sugar D-allosamine from readily available carbohydrate precursors. In some cases, as in the approach of Danishefsky (see below), this has inspired the development of highly innovative synthetic solutions to deal with the particular problems that compound **1** poses.

4.1.1 Synthesis of allosamidin: cyclopentene desymmetrisation. The first total synthesis of **1** was described by Griffith and Danishefsky,[60] narrowly preceding that disclosed by Vasella and Trost (see below). As well as featuring an asymmetric synthesis of the aglycone moiety, Danishefsky's approach is particularly notable for the development of a novel "azaglycosylation" methodology[60,61] designed specifically to meet the challenge of creating the β-linked *N*-acetylallosamine-containing pseudotrisaccharide skeleton of **1**.

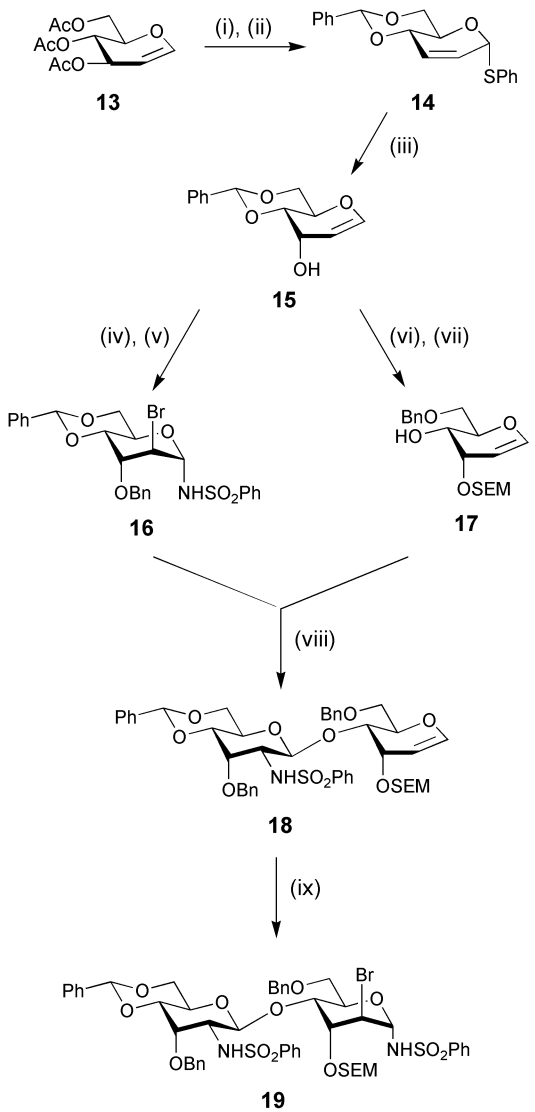
The starting material employed for the asymmetric synthesis of **2** was the same *meso*-diol **9** employed by Trost and van Vranken in their first synthesis of the former in racemic form.[66] The key steps of Griffith and Danishefsky's synthesis are shown in Scheme 1. Resolution and desymmetrisation of **9** was effected by conversion to the diacetate and hydrolysis with electric eel acetylcholinesterase to afford monoacetate **10**. The enzymatic hydrolysis proceeded with the opposite enantiotopic sense to that anticipated, necessitating the protection of **10** as the TBS ether prior to its deacetylation and elaboration into carbamate **11**. Compound **11** was subsequently desilylated and then treated with trifluoroacetic acid anhydride and Et_3N to give oxazolidinone **12**, *via* the intermediate trifluoroacetate.

Table 1 IC_{50} values of allosamidins against various family 18 chitinases (nM)

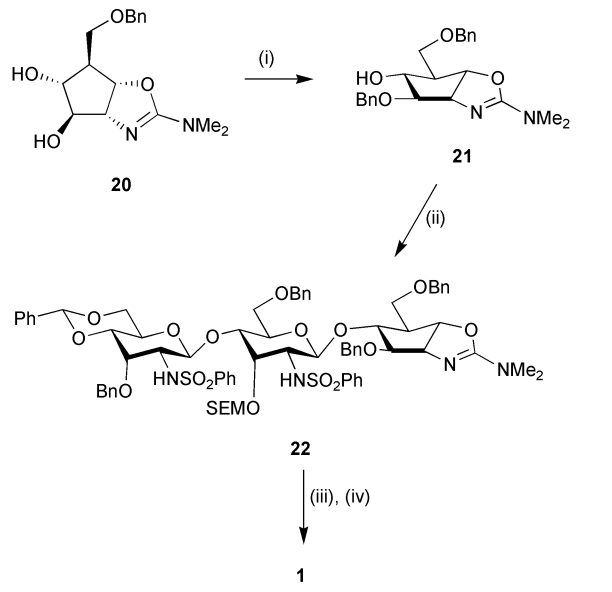
	Allosamidin derivative					
Chitinase	**1**	**3**	**4**	**5**	**6**	**7**
HCHT[22]	40	2.6	1.9	N. d.	N. d.	8.0
Mouse acidic mammalian chitinase[121]	400	N. d.	N. d.	N. d.	N. d.	N. d.
Neurospora crassa[54]	1600	N. d.	N. d.	N. d.	N. d.	N. d.
Trichoderma harzianum[56]	1300	1900	1300	N. d.	N. d.	2600
Bombyx mori[56]	48	65	81	N. d.	N. d.	65
Candida albicans[10,53]	300/10 000	14 000	1200	960	5300	1300
Saccharomyces cerevisiae[10]	54 000	58 000	490	640	49 000	800
Trichoderma sp.[10]	1300	1900	1300	210	1400	2600



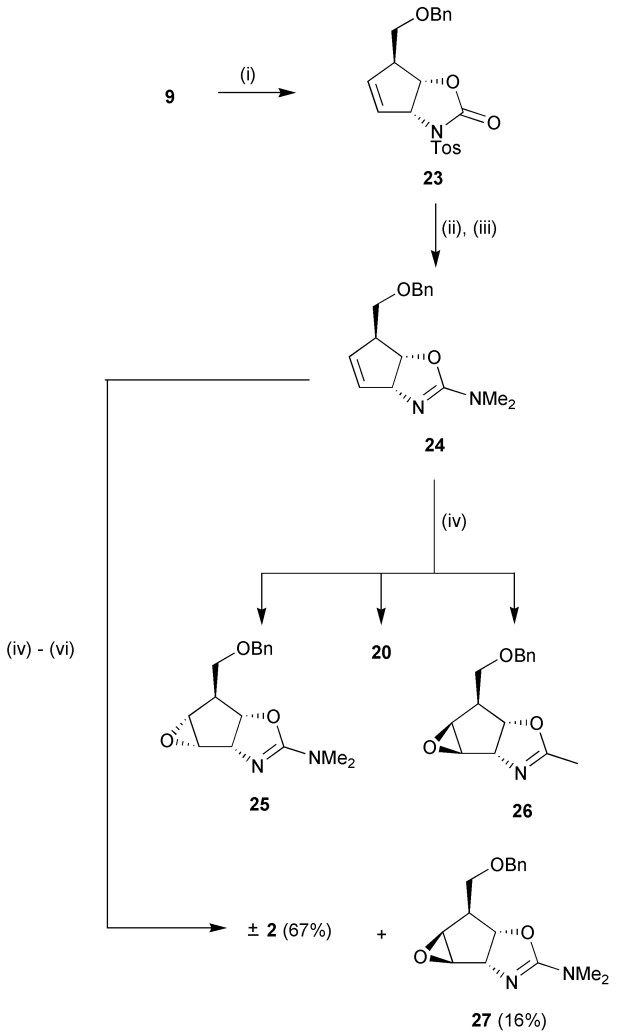
Scheme 1 *Reagents and conditions*: (i) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 89%; (ii) electric eel acetylcholinesterase, NaN_3, pH 6.9 phosphate buffer, 95%, (95% ee); (iii) TBS–Cl, imidazole, CH_2Cl_2; NH_3, MeOH, 100%; (iv) $ClCO_2Ph$, pyridine, CH_2Cl_2, 0 °C; NH_3, MeOH, 82%; (v) aqueous HF, CH_3CN, 94%; (vi) Et_3N, TFAA, −78 °C to r.t., 63%.



Scheme 2 *Reagents and conditions*: (i) PhSH, $BF_3{\cdot}Et_2O$; (ii) NaOMe, MeOH; $PhCH(OMe)_2$, TsOH, DMF, 73%; (iii) 3,3-dimethyldioxirane, CH_2Cl_2, −78 °C; Et_2NH, THF, 96%; (iv) NaH, THF, BnBr, Bu_4NI, 96%; (v) Br_2NSO_2Ph, CH_2Cl_2, 0 °C; NH_4I, EtOH, 63%; (vi) SEMCl, $^iPr_2NEt$, CH_2Cl_2, 100%; (vii) Na, NH_3; Bn_2SnO, MeOH, reflux; CsF, BnBr, DMF, 69%; (viii) KHMDS, DMF, −40 °C to r.t., 81%; (ix) repeat (v), 57%.



Scheme 3 *Reagents and conditions*: (i) Bu_2SnO, MeOH, reflux; BnBr, CsF, DMF, 35%; (ii) **19**, KHMDS, DMF, −40 °C to r.t., 42%; (iii) 5% aq. HCl–MeOH; Na, NH_3, −78 °C; Ac_2O, pyridine, 36%; (iv) NH_3, MeOH, 79%.



Scheme 4 *Reagents and conditions*: (i) 2 eq. TsNCO, THF; $(dba)_3Pd_2{\cdot}CHCl_3$, $(^iPrO)_3P$, THF, 93%; (ii) Na, naphthalene, DME, −78 °C, 91%; (iii) MeOTf, CH_2Cl_2; Me_2NH, 100%; (iv) 5.4 M CF_3CO_3H–TFA; (v) 10% aqueous TFA, 40 °C; (vi) H_2, 10% Pd/C.

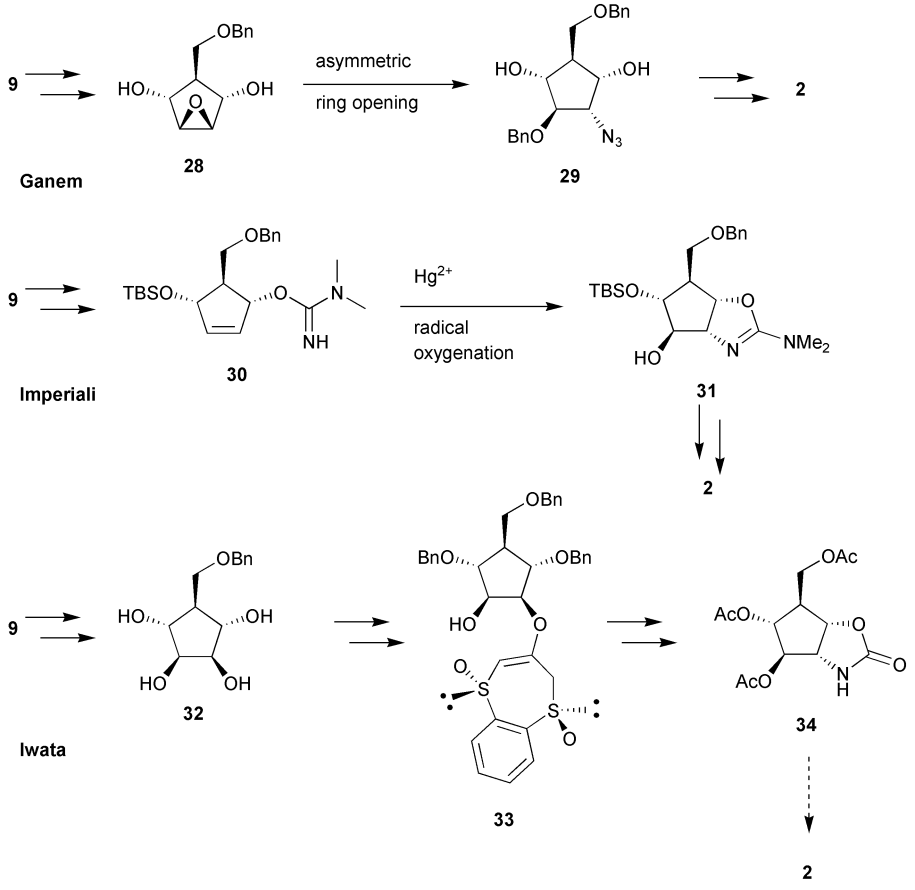
Compound **12** was also an intermediate in the Trost synthesis of **2** described in more detail below, and Griffith and Danishefsky therefore took advantage of the conditions reported to complete their synthesis of **2** in 6.9% overall yield for the 9 step sequence.

For the assembly of the disaccharide portion of **1**, readily available tri-*O*-acetyl-D-glucal **13** provided a convenient starting material from which appropriate glycosyl donor and acceptor units could be elaborated (Scheme 2). Briefly, Ferrier-type rearrangement[67] of **13** with thiophenol followed by deacetylation of the resulting glycal and subsequent reprotection afforded benzylidene derivative **14**. Upon oxidation of **14** to the sulfoxide with 3,3-dimethyldioxirane followed by exposure to Et_2NH, the latter underwent a 2,3-sigmatropic rearrangement[68] to give **15** in 96% yield. This building block could be benzylated and then treated with *N*,*N*-dibromobenzenesulfonamide to provide donor **16** for the subsequent azaglycosylation step. Alternatively, the axial hydroxyl group of **15** could be protected as the SEM ether prior to cleavage of the benzylidene acetal and regioselective benzylation to give **17**, the required acceptor. KHMDS-promoted coupling of **16** and **17** gave the protected disaccharide **18** in 81% yield, which was then converted to the bromosulfonamide donor **19**. This set the stage for the second critical azaglycosylation reaction involving the selectively 4,6-*O*-benzylated allosamizoline **21** which would simultaneously establish the stereochemistry of the remaining allosamine residue (Scheme 3). KHMDS-mediated coupling as previously gave the desired allosamidin derivative **22** in 42% yield. To complete the synthesis, the SEM ether and benzylidene acetal were first cleaved from **22** under acidic conditions, followed by reductive cleavage of the sulfonamides and benzyl ether protection. Peracetylation followed by removal of all *O*-acetates with methanolic NH_3 then gave the natural product.

As already noted, the completion of Danishefsky's synthesis of **2** drew upon transformations that had earlier been reported by Trost and van Vranken[66] in the first synthesis of this molecule in racemic form. Several other groups have also adopted elements of Trost's synthesis in their preparations of **2**, which is shown in Scheme 4.

Trost's approach hinges upon the desymmetrisation of *meso*-diol **9**, *via* a Pd(0)-catalysed hydroxyamination protocol[66,69] to give oxazolidinone **23** in 93% yield. Reductive desulfonylation of **23** with sodium naphthalenide gave the parent oxazolidinone, which was followed by *O*-alkylation with methyl triflate and treatment of the intermediate imidate with Me_2NH to give oxazoline **24**. To complete the synthesis of (±)-**2**, *trans*-dihydroxylation of **24** *via* a sequence of epoxidation–hydrolysis was envisaged, but **24** proved unreactive to typical electrophilic epoxidising agents, ultimately requiring the use of an excess of trifluoroperacetic acid in trifluoroacetic acid. Under these conditions, one of the diastereoisomeric epoxides generated, **25**, was partly solvolysed to the desired *O*-benzylallosamizoline **20**, while the other, **26**, proved surprisingly resistant to exposure to aqueous acid. This outcome was exploited in the following manner [steps (iv) to (vi)]: epoxidation of **24** was followed directly by solvolysis of the crude reaction mixture with 10% trifluoroacetic acid at 40 °C under which conditions the undesired epoxide **26** was stable. Direct hydrogenolysis then gave an easily separable mixture of **27** and (±)-**2** in 16% and 67% respectively from **24**. The overall yield of (±)-**2** for the six stages of the synthesis was 19%.

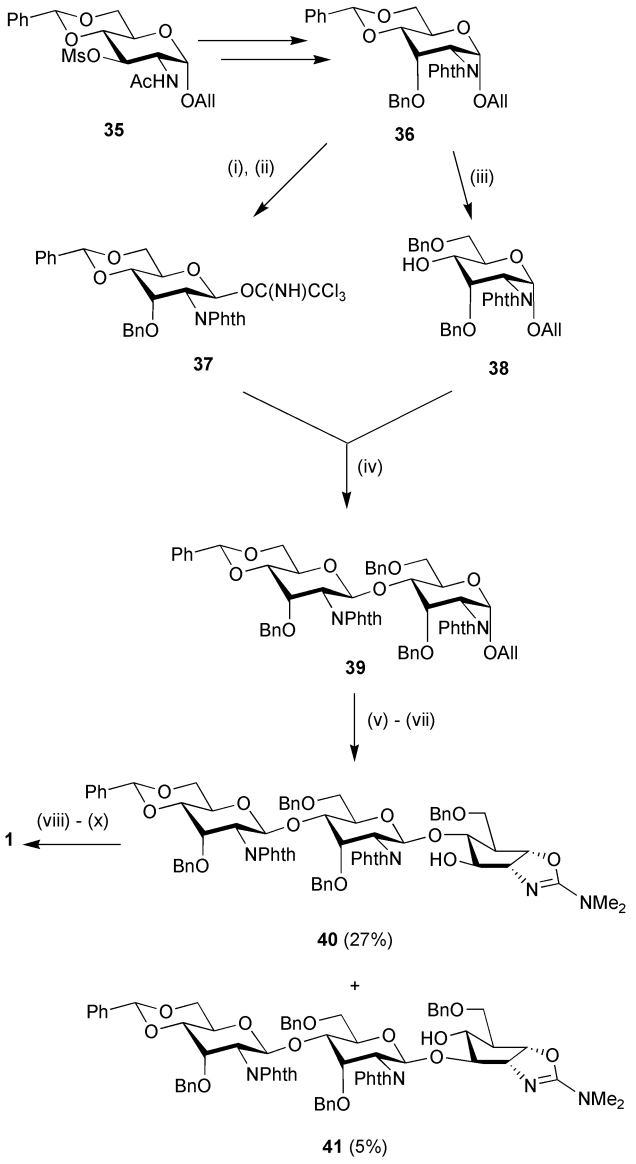
Although Trost's synthesis of **2** yielded the aglycone in racemic form,[70] the general synthetic strategy has been adopted by four other groups for both racemic and asymmetric preparations of **2**. In addition to Griffith and Danishefsky, related approaches have also been reported by Ganem *et al.*,[71,72] Imperiali and Shrader,[73] and Iwata *et al.*[74] (Scheme 5). Ganem has described two approaches based upon the desymmetrisation of a *meso*-epoxide derived from **9** or a closely related derivative, latterly employing an asymmetric Cr(III)–salen-catalysed ring opening of epoxide **28** with $TMSN_3$ to generate the orthogonally protected



Scheme 5 Ganem, Imperiali and Iwata's approaches to aglycone **2**.

cyclopentitol **29**.[72] Compound **29** was subsequently transformed to the acceptor **20** employed by Danishefsky, with the aid of methodology developed in a previous racemic synthesis of **2** by the Ganem group,[71] in an impressive overall yield of 25% (9 steps). Imperiali's approach[73] employs Danishefsky's protocol to desymmetrise *meso*-diol **9**, and then generates the allosamizoline ring system by a highly stereoselective Hg(II)-mediated cyclisation of aminoimidate **30** and subsequent radical oxygenation to give **31**. Iwata's formal synthesis[74] of **2** is particularly elegant, employing a C_2 symmetric bis-sulfoxide as a chiral auxiliary to effect the asymmetric desymmetrisation of a *meso*-cyclopentitol **32** derived from **9**, which was then converted in a straightforward fashion to oxazolidinone **34**, transformable to **2** by Trost's original methodology.

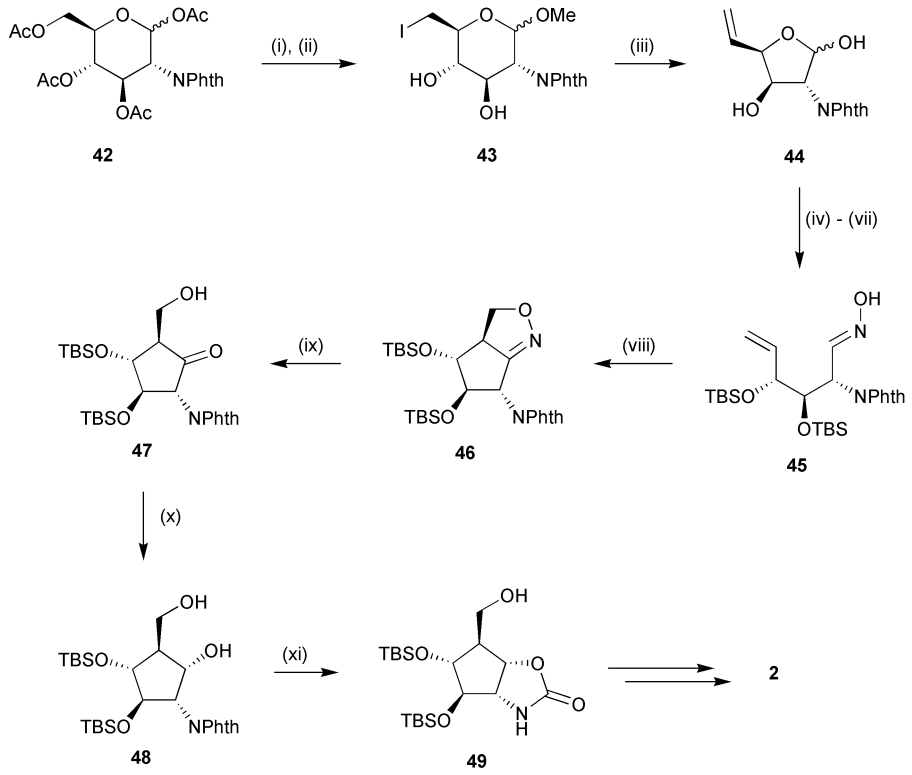
Trost's synthesis of **2** also paved the way for a total synthesis of **1**, achieved in collaboration with the group of Vasella.[62,63] This employs a more conventional, but nonetheless innovative solution to the problem of generating the unusual allosamine stereochemistry, which has been paralleled in subsequent total syntheses described by Kuzuhara *et al.*[75,76] and Ferrier *et al*.[51] The well-known trichloroacetimidate methodology of Schmidt and Kinzy[77] was employed for the key glycosylation steps. Preparation of the disaccharide moiety was achieved in a convergent fashion from D-glucosamine. The effective starting point was mesylate **35**, derived from allyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (Scheme 6). Solvolysis (NaOAc, H_2O, $MeOCH_2OH$, 40 h, 150 °C) of this derivative provided an intermediate with the required D-*allo* configuration, which was subsequently transformed to the key *N*-phthaloylated derivative **36** *via* a 5 stage sequence.[62] Compound **36** was chosen as it could then be transformed into either of the monosaccharide building blocks required. Ir-catalysed deallylation of **36** and treatment of the resulting hemiacetal with trichloroacetonitrile furnished the β-donor **37**, while regioselective reductive opening of the benzylidene grouping of **36** using Garegg's procedure (Me_3NBH_3–$AlCl_3$)[78] provided acceptor **38** in 84% yield. These units were combined in the presence of TMSOTf to give the desired β-disaccharide **39** in 80% yield.[64] Compound **39** was then deallylated and converted to the corresponding trichloroacetimidate donor in an analogous fashion to the conversion of **36** to **37**. TMSOTf-promoted glycosylation of this donor with the racemic 6-*O*-benzylalloxamizoline diol **20** gave a mixture of pseudotrisaccharides in an overall yield of 61%. The regioselectivity of the glycosylation, 5 : 1 in favour of the 4-OH of **20** over 3-OH, was anticipated on the grounds of the relatively lower nucleophilic character of the 3-OH, and led to an isolated yield of 27% for the natural pseudotrisaccharide **40** plus 5% of the regioisomeric diastereoisomer **41**. This feature of Vasella and Trost's synthesis was also subsequently exploited in Ferrier's total synthesis (see below), although Griffith and Danishefsky found it preferable to protect the 4-OH in their key glycosylation step. Dephthaloylation under mild conditions ($MeNH_2$, EtOH), to avoid opening of the dihydrooxazole ring of the aglycone, followed by peracetylation, *Zémplen* de-*O*-acetylation and finally catalytic hydrogenolysis of the *O*-benzyl protection then gave **1**.



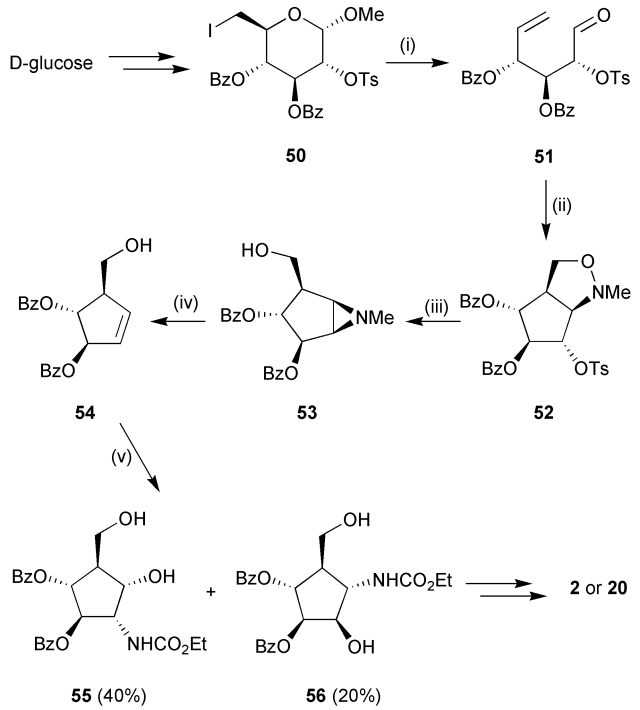
Scheme 6 *Reagents and conditions*: (i) Ir catalyst, H_2, THF; acetone–H_2O, 75%; (ii) Cl_3CCN, K_2CO_3, CH_2Cl_2, 81%; (iii) Me_3NBH_3, $AlCl_3$, THF, 84%; (iv) 1.2 eq. TMSOTf, CH_2Cl_2, 4 Å MS, 80%; (v) repeat (i), 73%; (vi) repeat (ii), 86%; (vii) **20**, 1.2 eq. TMSOTf, 4 Å MS, 27%; (viii) $MeNH_2$, EtOH; Ac_2O, pyridine, 70%; (ix) MeONa, MeOH, 96%; (x) H_2, 10% Pd/C, MeOH, AcOH, 95%.

4.1.2 Synthesis of allosamidin: "chiral pool" approaches. The first carbohydrate-based preparation of **2** was described by Tatsuta *et al.*[79] using D-glucosamine as starting material. The key step that generates the cyclopentane nucleus is an intramolecular cycloaddition of a nitrile oxide to an olefin, a popular general strategy for the synthesis of aminocyclopentitols.[65] The substrate for this transformation in Tatsuta's synthesis was oxime **45**, which was obtained as shown in Scheme 7. The known glucosamine derivative **42** was treated with 1% HCl–MeOH to give a mixture of anomeric methyl glycosides that were then converted to the primary iodide **43**. Zn-promoted reductive β-elimination of **43** gave mainly 5-enofuranose **44**, which was then treated with ethanethiol, silylated and the intermediate thioacetal cleaved to the parent aldehydo sugar, from which **45** was obtained by reaction with hydroxylamine hydrochloride. The pivotal cycloaddition reaction proceeded best using aqueous NaOCl in CH_2Cl_2 to give a single isoxazoline **46**, which was eventually successfully cleaved to the β-hydroxyketone **47** by ozonolysis. Reduction with $Zn(BH_4)_2$ afforded a single diol diastereoisomer **48**, which was converted to the oxazolidinone **49** by removal of the phthaloyl protecting group, formation of the *N*-benzyloxycarbonyl derivative and NaH-induced cyclisation. Completion of the synthesis then closely paralleled the original approach of Trost and van Vranken.[66]

A very similar approach to **2** has also been described by Koseki *et al.*,[80] again starting from D-glucosamine, while a somewhat different cycloaddition strategy has been reported by Ferrier *et al.*[51] as part of a total synthesis of **1**. Ferrier's synthesis of **2** (Scheme 8) involves the preparation of the enantiomerically pure cyclopentene **54**, which was obtained from D-glucose *via* intramolecular cycloaddition of a nitrone derivative generated from aldehyde **51**.[81,82] Compound **51** was obtained from D-glucose *via* an analogous series of reactions to those described by Tatsuta *et al.*[79] and Koseki *et al.*[80] and gave isoxazoline



Scheme 7 *Reagents and conditions*: (i) 1% HCl–MeOH, 60 °C, 60%; (ii) I_2, PPh_3, imidazole, 90%; (iii) Zn, THF; (iv) EtSH, HCl, 67% from **43**; (v) TBSOTf, 2,6-lutidine, CH_2Cl_2, 90%; (vi) $HgCl_2$, $CaCO_3$, aqueous acetone, 81%; (vii) $NH_2OH{\cdot}HCl$, pyridine, 81%; (viii) aqueous NaOCl, CH_2Cl_2, 91%; (ix) O_3/O_2, CH_2Cl_2–MeOH, 60%; (x) $Zn(BH_4)_2$, THF–Et_2O, 100%; (xi) $N_2H_4{\cdot}H_2O$, EtOH; BnOCOCl, Na_2CO_3; NaH, THF, 60%.

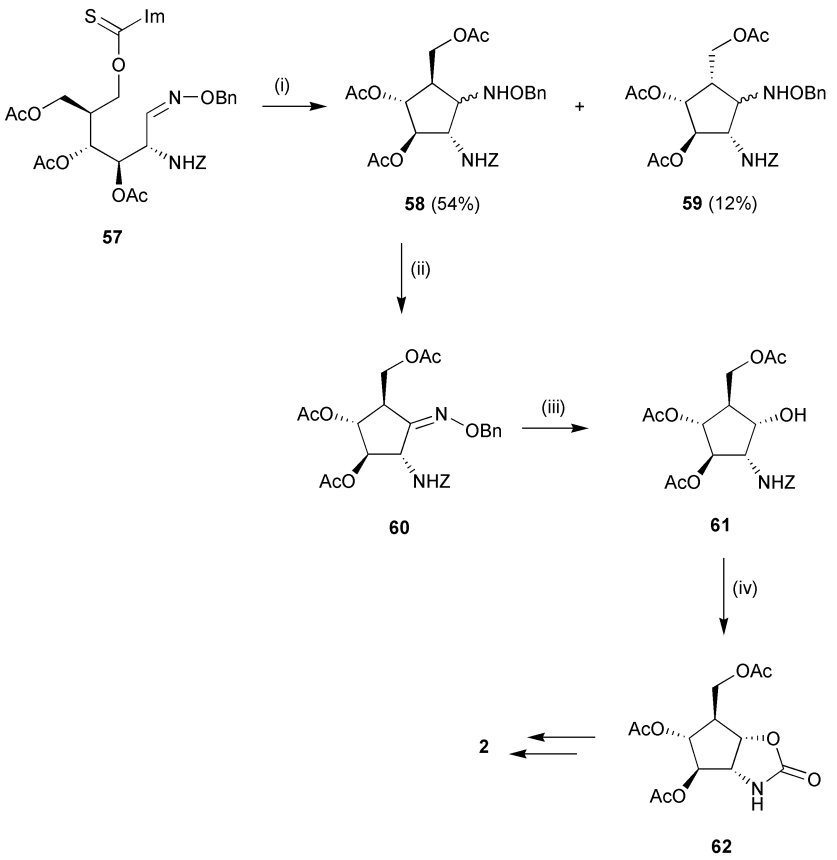


Scheme 8 *Reagents and conditions*: (i) Zn, EtOH; (ii) MeNHOH, 73% (2 steps); (iii) H_2/Raney Ni, 74%; (iv) MCPBA, DCM, 83%; (v) Na ethyl *N*-chlorocarbamate, $Hg(CF_3CO_2)_2$, 60%.

52 upon nitrone formation and intramolecular cycloaddition. Reduction of **52** with hydrogen over Raney nickel converted **52** to the aziridine **53**, which was then oxidised with MCPBA to give **54**. Oxyamination of **54** was effected using the *N*-chloro-*N*-metallocarbamate modification of the Sharpless reaction to give a 2 : 1 ratio of the regioisomers **55** and **56** in a combined yield of 60%. The desired regioisomer was converted both to **2** and also to diol **20**. TMSOTf-promoted coupling of **20** and the disaccharide donor employed in Trost and Vasella's synthesis gave a 68% yield of the two regioisomeric pseudotrisaccharides **40** and **41** in an approximate 5 : 1 ratio (see Scheme 6). The desired major isomer was separated after dephthaloylation and peracetylation of the glycosylation mixture and finally converted to **1** by catalytic hydrogenolysis of the remaining benzyl protection.

Two further approaches to **2** and one synthesis of a closely related aminocyclopentitol have been reported using D-glucosamine as starting material but employing alternative strategies for the formation of the carbocycle. In Simpkins' synthesis (Scheme 9),[83,84] a cyclisation of a carbon-centred radical onto an oxime ether function was employed in the key step. The major product **58**, obtained in 54% yield as a mixture of epimers at C1, was converted to the oxime **60** and then to alcohol **61** *via* reaction with ozone and direct reductive workup, although in disappointingly low yield (20–40%) for the latter step. Treatment of **61** with $SOCl_2$ did however lead directly to oxazolidinone **62** (also an advanced intermediate in Ferrier's synthesis)[51] which was then converted to **2** *via* established procedures.

The preparation of **2** by Kuzuhara *et al.*[85] (Scheme 10) not only established the absolute configuration of the latter, but also formed part of a further total synthesis[75] of **1**, closely following those of Danishefsky and Griffith[61] and Vasella and Trost *et al.*[62–64] The strategy for the synthesis of **2** involved utilising the chiralities of C2, C3 and C4 of D-glucosamine **63**, and accessing the desired ring system *via* ring contraction of an intermediate cyclohexitol. Thus, **64**, obtained in 7 steps from **63**, was converted to enol ether **65** which was then subjected to a modified Ferrier reaction, followed by β-elimination of the crude keto enol obtained to give enone **66**. Stereoselective reduction of **66** under Luche conditions and treatment of the allylic alcohol formed with methanesulfonic anhydride–Et_3N then gave oxazoline **67**, which upon dihydroxylation (OsO_4, Me_3NO), gave exclusively diol **68** in 92% yield. Regioselective tosylation of



Scheme 9 *Reagents and conditions*: (i) Bu_3SnH, AIBN, C_6H_6; (ii) MCPBA, Na_2CO_3, EtOAc, 79%; (iii) O_3, CH_2Cl_2, −40 °C; MeOH, $NaBH_4$, −40 °C to r.t., 20–40%; (iv) $SOCl_2$, 82%.

68 provided the substrate **69** for the projected ring contraction step, which was cleverly carried out with the basic reducing agent L-Selectride® so as to avoid possible decomposition of the aldehyde **70** that was initially generated. Oxazoline **71** was thus obtained in 86% yield and could be transformed in two further steps either to **2** or the glycosyl acceptor **21** employed in Danishefsky's synthesis of **1**.[60]

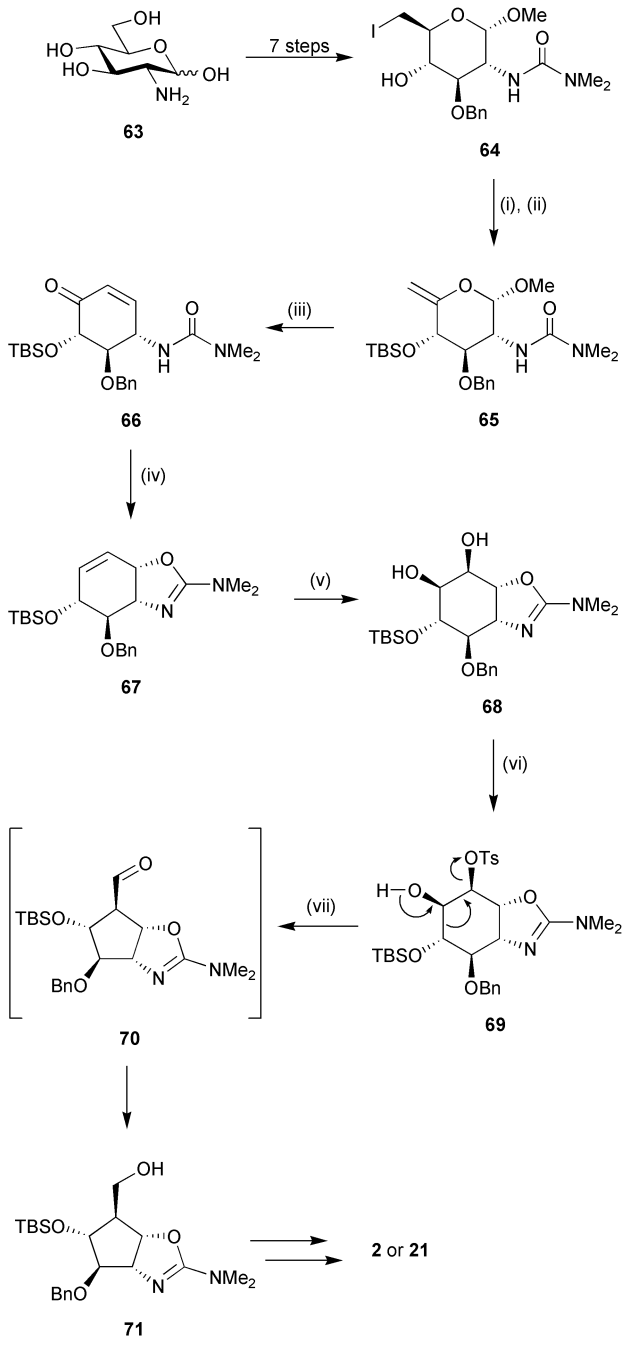
Kuzuhara's subsequent synthesis of **1**[75] (Scheme 11) is noteworthy for the ingenious use of chitin as a starting material for the preparation of the required disaccharide donor unit. Kuzuhara *et al.*[75,86] and other workers[87–91] have devised efficient protocols for the partial enzymatic or chemical degradation of colloidal chitin to *N*,*N*′-diacetylchitobioside **72** or other useful oligosaccharide fragments. Compound **72** obtained in this way was converted to the thioglycoside donor **73**,[75,92] with the essential stereochemical inversions at C3 and C3′ being effected in an analogous manner to the synthesis of Vasella and Trost *et al*.[62] TfOH–NIS-mediated coupling of **73** and **21** proceeded in 40% yield to give the required pseudotrisaccharide **74**, the somewhat low yield being due to significant competing elimination of thiophenol from **73** to give glycal **75**. Subsequent dephthaloylation, *N*-acetylation and hydrogenolysis then furnished **1**.

4.1.3 Synthesis of naturally occurring allosamidin analogues. Takahashi, Kuzuhara and Terayama[76] have also described the synthesis of the aglycone **79** (Scheme 12) of the naturally occurring *N*-demethylated allosamidin analogue **4** and the preparation of the natural product using intermediates from the preceding synthesis of **1**. Vasella and Trost had observed that treatment of pseudotrisaccharide **40** with hydrazine hydrate led not only to dephthaloylation, but also cleavage of the oxazoline ring system,[63] and this was exploited as shown in Scheme 12 by Takahashi and Kuzuhara to convert **76** to amino alcohol **77**. This was then converted to **79**, *via* HgO-induced cyclodesulfurisation of thiourea **78**. Interestingly, the hydrazinolysis step was also later applied by Shiozaki *et al.*[93] in a synthesis of **79** using crude **2** obtained by aqueous HCl hydrolysis of **1** from a *Streptomyces* fermentation broth as the starting material.

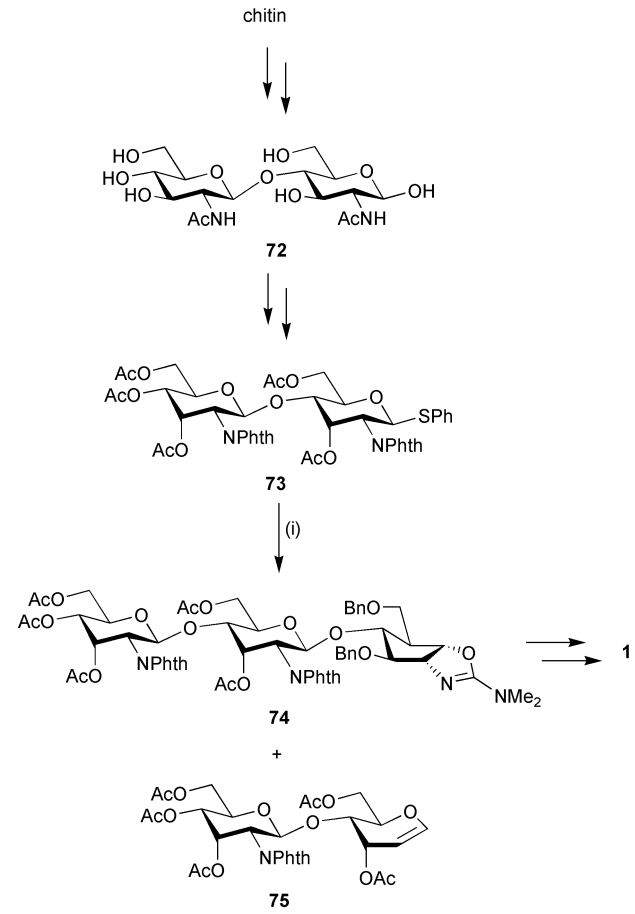
For the preparation of **4**, chitin-derived thioglycoside donor **80** was employed and coupled with acceptor **79** as in the previous synthesis to give the pseudotrisaccharide **81** in 60% yield (Scheme 13). No detectable amounts of the α-isomer were observed in this reaction, which the authors attributed to the steric bulk of the *N*,*O*-isopropylidenyl protection in **80**. This was significant as the change in protecting group strategy (*cf.* Scheme 11) had been specifically devised to prevent participation of the neighbouring acetamido group in the glycosylation step and therefore obviate the need for its temporary replacement with a phthalimido group and subsequent reintroduction (see Schemes 2 and 6).

Yamada *et al.*[94] have described the direct transformation of **1** into **4** by treatment with methylamine, and also the analogous preparation of **8** (Scheme 14). No other preparations have been reported to date for the other naturally occurring members of the allosamidin family for which X-ray structural information has been obtained.

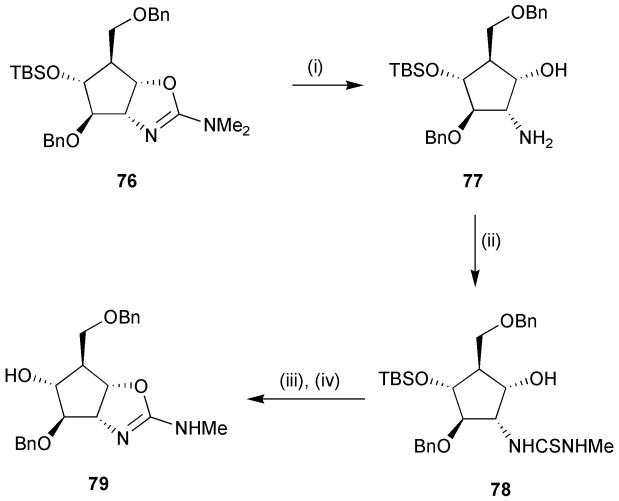
Although it is beyond the scope of this review to describe the preparation and properties of the many unnatural analogues of **1** and **2** that have been reported, it is worth noting that some useful analogues have been obtained as side products in some of the total syntheses discussed above. Allosamidin diastereoisomer **82** (Fig. 6), prepared independently by Danishefsky and Griffith,[60] was originally encountered as a minor product from the glycosylation step in Vasella and Trost's synthesis involving racemic acceptor **20**.[62,63] Ferrier's synthesis,[51] and that of Vasella and Trost, provided access to the regioisomeric derivative **83**, derived from **20** (Fig. 6). Siriwardena *et al.*[65] have provided a comprehensive discussion of the various derivatives synthesised up to 1999, while more recent contributions are to be found in the work of Takahashi *et al.*,[95] Peter *et al.*[96,97] and Lopez *et al*.[98,99]



Scheme 10 *Reagents and conditions*: (i) TBSOTf, 2,6-lutidine, 76%; (ii) ^{t}BuOK, THF, 96%; (iii) $HgSO_4$, H_2SO_4–acetone; MsCl, pyridine, 65%; (iv) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH; Ms_2O, Et_3N, CH_2Cl_2, 86%; (v) OsO_4, Me_3NO, ^{t}BuOH–H_2O, 92%; (vi) TsCl, pyridine, CH_2Cl_2, DMAP, 86%; (vii) L-Selectride®, THF, 65 °C, 86%.



Scheme 11 *Reagents and conditions*: (i) **21**, NIS, TfOH, 4 Å MS, CH_2Cl_2, 0 °C, 40%.

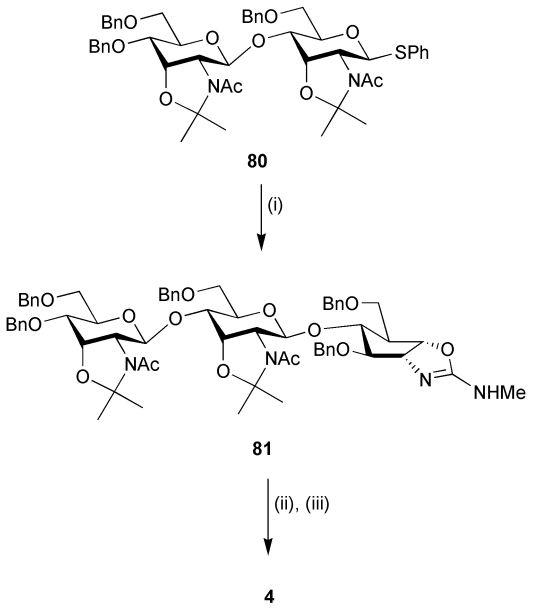


Scheme 12 *Reagents and conditions*: (i) $N_2H_4{\cdot}H_2O$, EtOH, 80 °C, 60%; (ii) CH_3NCS, DMF–EtOH, 100%; (iii) HgO, THF, 91%; (iv) 1 M HCl, THF, 65 °C, 89%.

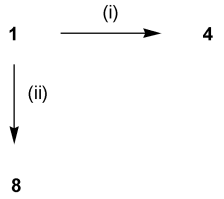
4.2 Psammaplins

Psammaplin A **84** (Fig. 7) is a member of a family of compounds with antibacterial and antitumour properties that are derived biosynthetically from bromotyrosine and cysteine. Compound **84** was originally isolated from the sponge *Psammaplysilla purpurea*, collected in the region of Tonga by Crews *et al.*,[100,101] and subsequently identified independently from other sources by the groups of Scheuer[102] and Schmitz.[103] Tabudravu *et al.*[17] showed that **84** is a modest inhibitor of *Bacillus* sp. chitinase (IC_{50} = 68 μM) giving a mixed non-competitive inhibition at lower concentrations and an uncompetitive inhibition at higher concentrations. Compound **84** was further determined to be a non-competitive inhibitor of *Sm*ChiB with a K_i of 148 μM and an IC_{50} of 100 μM.[17] The closely related derivatives psammaplin K **85** and L **86**, and the dimeric analogue, bisaprasin **87** showed no significant inhibition of *Bacillus* sp. chitinase. Further members of the psammaplin family have since been isolated by Crews *et al.*,[104] but no chitinase inhibitory activity has so far been reported. The crystal structure of *Sm*ChiB in complex with **84** shows undefined electron density in the active site consistent with conformational flexibility and disordered binding of the inhibitor.[17] Asp175 is partially in the "up conformation" pointing towards Glu177 indicating partial binding in the −1 subsite. The precise binding mode of this inhibitor to family 18 chitinases has thus so far not been established.

Hoshino *et al.*[105] have reported the synthesis of **84** from 3-bromotyrosine in 4 steps, and the essential features of this



Scheme 13 *Reagents and conditions*: (i) **79**, NIS, TfOH, 4 Å MS, CH_2Cl_2, −18 °C, 60%; (ii) conc. HCl–MeOH, r.t. to 40 °C, 60%; (iii) H_2, 10% Pd/C, MeOH–AcOH–H_2O, 91%.



Scheme 14 *Reagents and conditions*: (i) aqueous CH_3NH_2, 121 °C, 17%; (ii) aqueous NH_3, 121 °C, 13%.

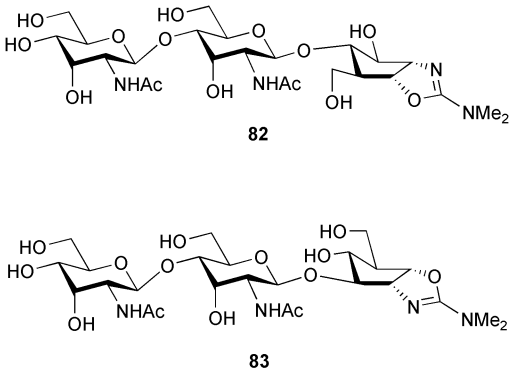
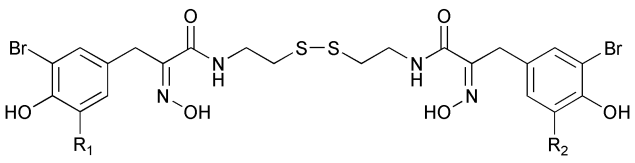
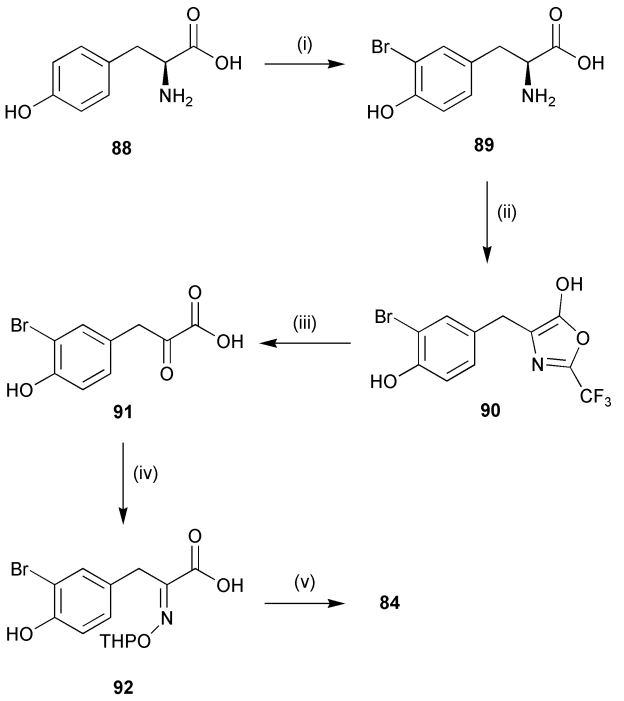


Fig. 6 Regio and diastereomeric allosamidin derivatives.



	R_1	R_2
84 (Psammaplin A)	H	H
85 (Psammaplin K)	H	OH
86 (Psammaplin L)	OH	OH
87 (Bisaprasin)	H	Dimer

Fig. 7 Psammaplins tested for chitinase inhibitory activity.



Scheme 15 *Reagents and conditions*: (i) KBr–$KBrO_3$, H_2O, 81%; (ii) TFAA, 80 °C; (iii) 70% TFA, 60% (2 steps); (iv) THP–ONH_2, EtOH; (v) EDC, NHS, 1,4-dioxane; Et_3N, cystamine·HCl (0.5 eq.), 1,4-dioxane–MeOH; HCl, CH_2Cl_2–MeOH, 60 °C, 36% from **91**.

approach were subsequently incorporated into a general approach to psammaplin A-type derivatives reported by Nicolaou *et al.*,[106] shown in Scheme 15. Monobromination of tyrosine **88** with KBr–$KBrO_3$ gave 3-bromotyrosine **89** in 81% yield, which was treated with trifluoroacetic acid anhydride to form trifluoromethyloxazolone **90** and subsequently hydrolysed to α-keto acid **91** (60% over the two steps). Compound **91** was then converted to the protected oxime **92** by condensation with *O*-(tetrahydro-2*H*-pyran-2-yl)-hydroxylamine and coupled with cystamine *via* activation as the *N*-succinimidyl ester. Cleavage of the THP protection then gave **84** in 36% yield over the 4 steps from **91**. Nicolaou's approach was exploited in the preparation, *via* catalytically-induced disulfide exchange, of a 3828-membered library of psammaplin A analogues which were screened for their antibacterial properties.[107] Again, no reports of the chitinase inhibitory activity of these molecules have appeared so far.

4.3 Styloguanidines

The styloguanidines (Fig. 8) are a small group of natural products that possess a unique hexacyclic bis-guanidine structure. Styloguanidine **93** itself and two brominated analogues **94** and **95** were isolated by Kato *et al.*[18] from the sponge *Stylotella aurantium*, collected in the Yap Sea. Also isolated was the isomeric alkaloid palau'amine **96**, which had previously been obtained by Sheuer *et al.*[108,109] from *Stylotella agminata*, collected in the Western Caroline Islands. Kato reported that **93–95** showed inhibitory activity towards a bacterial chitinase from *Schwanella* sp. and also inhibited the moulting of cyprid larvae of barnacles. Although a range of interesting biological properties has been reported for **96** (cytotoxic, antibiotic, antifungal, immunomodulatory), no inhibition of chitinases has yet been noted.

While the preparation of the highly complex styloguanidine structure has attracted considerable interest, it has not so far yielded to total synthesis. The synthesis of the tetracyclic core of these molecules **97**[110] and several other advanced intermediates (*e.g.* **98** and **99**[111,112]) has however been recently reported by

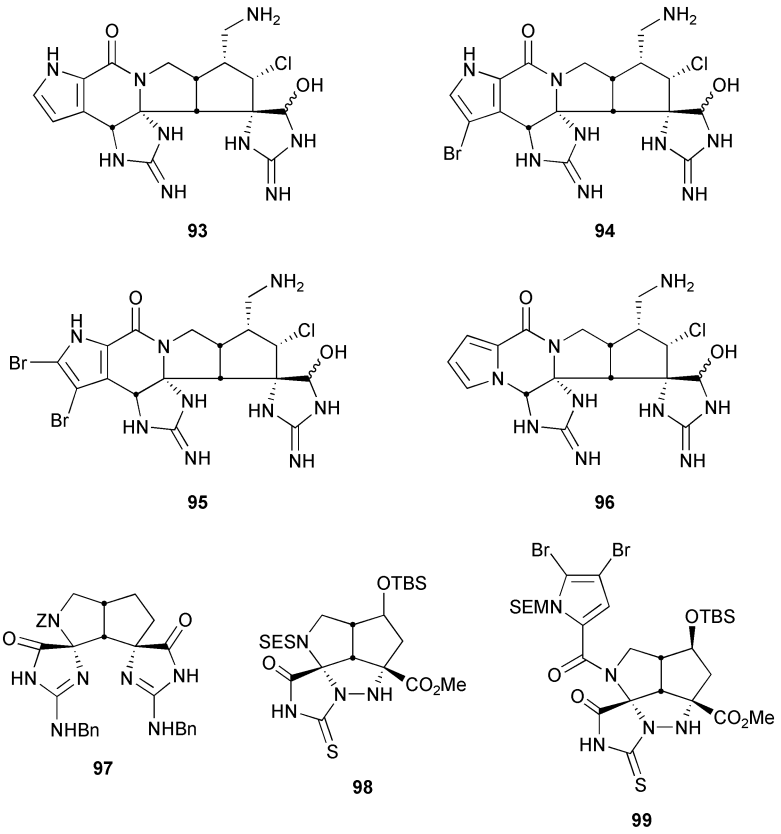
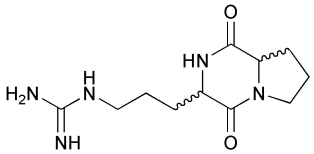


Fig. 8 Styloguanidines, palau'amine and synthetic intermediates.

the group of Overman. Other synthetic efforts towards the styloguanidines and related bis-guanidine natural products have been reviewed by Hoffmann and Lindel.[113]

4.4 Cyclic proline-containing dipeptides

The cyclic dipeptide, cyclo(L-Arg–D-Pro) (CI-4, **100**, Fig. 2b), produced by the marine bacterium *Pseudomonas* sp. IZ208, was isolated and identified as a chitinase inhibitor by Izumida *et al.*[12] Izumida *et al.* prepared **100** and the stereoisomers **101** and **102** (Fig. 9) and evaluated them against chitinase from *Bacillus* sp. Compound **100** gave 17.2% inhibition of this enzyme at a concentration of 1 mM, the L–L diastereoisomer **101** was slightly more potent giving 18.4% inhibition at the same concentration, while **102** only showed 4.9% inhibition.[12] Using the agar plate method, Izumida *et al.*[12] also detected chitinase inhibitory activity for **100** and **101** at a concentration of 50 μg per disk, whereas weaker inhibition was observed for **102**, consistent with the enzyme kinetics data. Compound **100** has since been shown to competitively inhibit *Sm*ChiB with a K_i of 0.65 mM,[47] and an IC_{50} of 1.2 mM,[48] whereas an IC_{50} value of 6.3 mM has been reported for **101**.[48] **100** was also shown to inhibit cell



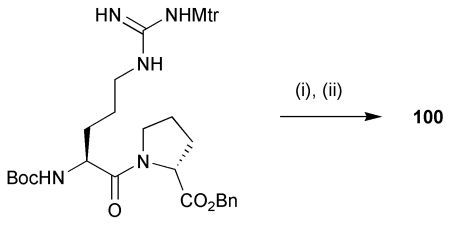
	Arg	Pro
101	L	L
102	D	L

Fig. 9 Stereoisomers of **100** (CI-4) tested for chitinase inhibitory activity.

separation in *Saccharomyces cerevisiae* and blocked morphological changes in *Candida albicans*, presumably through inhibition of chitinases in these organisms.[114] Crystallographic and kinetic studies of other cyclic dipeptides related to **100** have also been reported.[48]

The structure of *Sm*ChiB in complex with **100** shows that the latter mimics the reaction intermediate (panel C, Fig. 2a) with the proline unit and the cyclic dipeptide backbone of the similarly sized two-ring structure coinciding with the oxazoline and pyranose rings of the reaction intermediate, respectively.[47] The hydrogen bond between the D-Pro carbonyl oxygen and the Tyr245 hydroxyl group also imitates the corresponding hydrogen bond involving the oxazoline nitrogen of the reaction intermediate. Likewise, the hydrogen bond between the L-Arg carbonyl oxygen and the main chain nitrogen of Trp137 reproduces the corresponding interaction that involves the hydroxyl group of the pyranose moiety. Additionally, a water-mediated hydrogen bond is observed between the L-Arg backbone nitrogen to the carboxyl group of Asp246, mimicking the direct hydrogen bond from the hydroxymethyl group of the intermediate to the Asp residue. An additional hydrogen bond involves the L-Pro backbone nitrogen and the side chain of Glu175.

The synthesis of cyclic dipeptides such as **100** is very straightforward,[115] and as such they are attractive leads for the development of more potent chitinase inhibitors based upon easily accessible modifications of the core "cyclo(Gly–Pro)" scaffold. Izumida's original preparation of **100** serves as an illustrative example of the general ease of preparation of such compounds (Scheme 16). DCC-mediated coupling of Boc–L-Arg(Mtr)–OH with D-Pro–OBn to give the fully protected dipeptide **103**, was followed by treatment with TFA–anisole to effect cleavage of the Boc and Mtr groups, prior to cyclisation by heating an aqueous solution at pH 6 at 80 °C. **100** was isolated in 43% yield from **103**, with the preparation of two of the other stereoisomers of the natural product being achieved in analogous fashion.



Scheme 16 *Reagents and conditions*: (i) TFA–anisole, 50 °C; (ii) Et_3N, H_2O, pH 6, 80 °C, 43%.

4.5 The cyclopentapeptides argifin and argadin

The cyclopeptide argifin **104** (Fig. 2b), isolated from the fungal strain *Gliocladium* sp. FTD-0668,[13–15] was the first non-sugar molecule to show chitinase inhibition in the submicromolar range.[15] **104** consists of an *N*-methyl carbamoyl-derivatised L-Arg, an *N*-methyl L-Phe, two β-L-Asp and a D-Ala residue. The unusual acylated Arg residue (see below) plays a key role in the binding of **104** to family 18 chitinases. IC_{50} values of 3.7, 1.1 and 4.5 μM were obtained against *Lucilia cuprina* (blowfly) chitinase,[15] *Af*ChiB1 and HCHT,[46] respectively. A K_i of 33 μM was obtained against *Sm*ChiB,[45] whereas a K_d of 0.46 μM has been reported for *Af*ChiB1.[46]

Crystallographic structures of binary enzyme-**104** complexes have been determined for *Sm*ChiB,[45] *Af*ChiB1 and HCHT.[46] The structures reveal that **104** binds to subsites −1, +1 and +2, interacting with residues Trp384, Trp137 and Phe251 that are conserved hydrophobic residues in family 18 chitinases (panel D, Fig. 2a). Compound **104** binds in a similar fashion to the three different chitinases and itself contains one intramolecular hydrogen bond linking the carbonyl group of the D-Ala to the nitrogen of the first β-Asp. The *N*-methyl-carbamoyl moiety packs in the active site pocket so as to interact with the side chains of Tyr48 and Trp384; its nitrogen hydrogen bonds to the carboxyl groups of Asp175 and Glu177, whereas its oxygen hydrogen bonds to the hydroxyl group of Tyr245. The Arg residue also forms a salt-bridge-like interaction with the catalytic acid Glu177. Parts of the binding pattern of the *N*-methyl-carbamoyl group resemble that seen for the natural substrate in which the *N*-acetyl group of the distorted −1 sugar in the *Sm*ChiB–$GlcNAc_5$ (panel A, Fig. 2a) structure overlaps with the *N*-methyl-carbamoyl group thereby retaining the hydrogen bonds to Asp175 and Tyr245. Likewise, one of the hydrogen bonds to the catalytic acid is preserved, while Asp175 is retained in the "up conformation" pointing towards the catalytic acid. An additional hydrogen bond conserved amongst chitinases is observed between the first β-L-Asp side chain and the Trp137 side chain. In addition, stacking interactions are formed between the L-Phe side chain of **104** and the side chains of Phe251 and Trp137. The higher affinity of **104** towards *Af*ChiB1 compared to *Sm*ChiB can be explained by a slight shift in the binding of **104** to *Af*ChiB1 (maximum backbone coordinate shift of 1.9 Å)[46] that generates specific hydrogen bonds between the L-Arg side chain and the Asp246 carboxyl group and between the L-Arg carbonyl and the Arg301 side chain. In addition, a higher number of water-mediated hydrogen bonds linking **104** with the protein are observed in the *Af*ChiB1 complex.

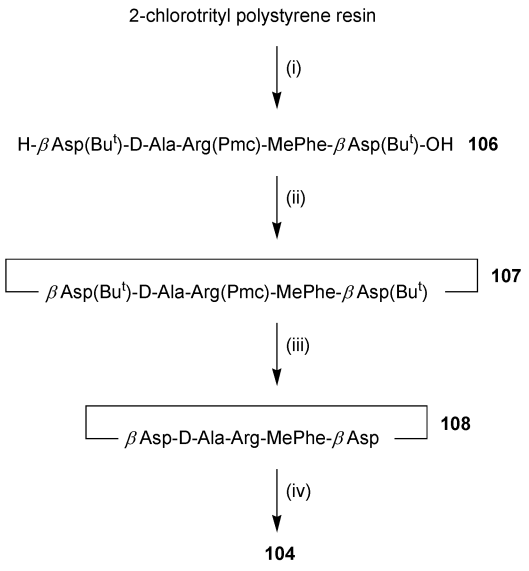
Shortly after the isolation of **104** was reported, a further cyclopentapeptide, argadin **105** (Fig. 2b), was obtained from fungal strain *Clonostachys* sp. FO-7314.[16] **105** consists of an acetylated L-Arg, a D-Pro, a backbone cyclised L-Asp-β-semialdehyde, an L-His and an L-aminoadipic acid residue. The published inhibition and binding constants for **105** against representative chitinases are generally lower compared to **104**, with IC_{50} values of 150 nM against *Lucilia cuprina* chitinase,[16] 0.5 μM against *Af*ChiB1[46] and 13 nM against HCHT.[46] A K_d of 0.81 μM was obtained against *Af*ChiB1 and a K_i of 20 nM was obtained against *Sm*ChiB.[46] Compound **105** has also been shown to arrest the moulting of cockroach larvae upon injection into the ventral abdominal part.[16] Compared to **104**, **105** has a smaller cyclic backbone, generating a more compact structure. The hemiaminal unit and the D-Pro restrict torsion involving the main chain, thus possibly contributing to a more rigid structure for **105**. Moreover, four conserved intramolecular hydrogen bonds (aminoadipic carbonyl to hemiaminal nitrogen, aminoadipic nitrogen to hemiaminal hydroxyl group and two bonds from the aminoadipic carboxyl group to the Arg side chain, panel E, Fig. 2a) are present in the binary *Sm*ChiB,[45] *Af*ChiB1 and HCHT-**105**[46] complexes to further stabilize the inhibitor conformation upon binding to chitinases. An additional intramolecular hydrogen bond between the aminoadipic carboxyl group and the backbone nitrogen of L-Arg is observed in the *Af*ChiB1 and HCHT complexes. While, like **104**, **105** is seen to bind to subsites −1, +1 and +2 in the complexes with *Sm*ChiB, *Af*ChiB1 and HCHT, this binding is surprisingly different compared to **104** with the His side chain instead of the Arg pointing inside the active site pocket. The binding pattern of the His residue of **105** mimics that of the natural substrate in a similar fashion to that observed for binding of **104**, with the L-His side chain hydrogen bonding the side chains of Asp175 and Tyr245; however the Asp175 is in the "down conformation" pointing towards Asp173. The peptide backbone of **105** binds noticeably deeper in the active site than that of **104**, allowing hydrogen bonds between the hemiaminal carbonyl and the side chain of Asp246 whereas two hydrogen bonds are observed between the L-Arg carbonyl and the side chain of Arg301.

Mutation, crystallographic and kinetic studies have determined that the stacking interactions with Trp384, Trp137 and Phe251 (corresponding to the −1, +1 and +2 subsites) are the main contributors for cyclopentapeptide binding and specificity. Consequently, "plant-type" family 18 chitinases are believed to be poor targets for **104** or **105**, as these enzymes do not possess all of these conserved side chains. The published inhibition and binding constants of the cyclopentapeptides show that chitinases (except *Af*ChiB1) have a marked preference towards **105** over **104**. The difference can be explained by the more rigid structure of **105**, and a deeper binding in the active site that displaces more water molecules allowing a larger contact surface with the protein (130–148 $Å^2$) compared to **104** (112–133 $Å^2$).[45,46] The stronger *Af*ChiB1 inhibition of **104** relative to **105** can be explained by Trp384 adopting a different conformation upon binding of **104**, thereby maximizing the stacking with the guanyl-urea moiety. Moreover, a few enzyme-specific differences are observed in the hydrogen-bonding pattern.

Recently, the first synthesis of **104** has been achieved, utilising a combined solid and solution phase approach (Scheme 17).[116] Initially, the partially protected linear peptide **106** was assembled using Fmoc solid-phase chemistry on 2-chlorotrityl polystyrene resin. Following cleavage from the solid support, **106** was cyclised in solution in near quantitative yield to give **107**. Removal of the *tert*-butyl ester and Pmc protecting groups under acidic conditions gave **108**, which was converted to **104** by introduction of the *N*-methyl carbamoyl moiety. This general approach should be amenable to the synthesis of various analogues of **104** as well as the synthesis of **105**.

4.6 Methylxanthines

Methylxanthines have been found in as many as sixty different plant species and include the well known compounds caffeine **109** (Fig. 2b), theophylline **110** and theobromine **111** (Fig. 10). **109** and **110** have recently been identified as chitinase inhibitors, with **110** previously having been shown to act as a bronchodilator, and to have several anti-inflammatory activities related to asthma.[117] **109** and **110** were shown to be competitive *Af*ChiB1 inhibitors and crystallographically determined to bind in an identical fashion in the −1 subsite, mimicking the oxazolinium



Scheme 17 *Reagents and conditions*: (i) Fmoc solid-phase peptide synthesis; (ii) PyBOP, DIPEA, DCM, 16 h; (iii) TFA–thioanisole–DCM–H_2O (16 : 2 : 1 : 1), 2 h; (iv) *N*-succinimidyl *N*-methylcarbamate, DBU, DMF, 40 °C, 2 h.

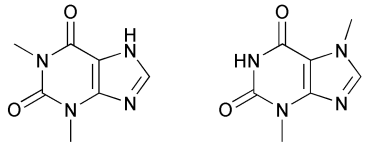


Fig. 10 Methylxanthines theophylline **110** and theobromine **111**.

ion intermediate by hydrogen bonding the side chains of Asp175 (**110** only) and Tyr245 as well as the main chain of Trp137 (panel F, Fig. 2a). Despite the loss of one hydrogen bond and a displacement of Asp175 to the "down conformation" pointing towards Asp173, **109** was shown to be the more potent *Af*ChiB1 inhibitor compared to **110** with IC_{50} values of 469 and 1500 μM, respectively. Inhibition of human acidic mammalian chitinase has also been reported by Rao *et al.*[19] The preparation of xanthine-type molecules related to **109–111** is quite straightforward and there should therefore be considerable scope for the synthesis of a variety of analogues of this kind.

4.7 Miscellaneous inhibitors

A few other reports of chitinase inhibitory activity have appeared for other natural products, but so far no syntheses or crystallographic information have been reported for these molecules. Nitoda *et al.* have reported the identification of a water-soluble polysaccharide from screening of fungal broths that is a potent inhibitor of a chitinase from *Spodoptera litura* (IC_{50} = 28 nM).[118] The isolated molecule, with an estimated molecular weight of 16 kDa, was partially characterised and shown to contain glucose, galactose, *N*-acetylglucosamine and a deoxysugar. Séquin *et al.*[119] isolated four α,β-unsaturated γ-lactones **112–115** (Fig. 11) from *Streptomyces antibioticus* that were weak inhibitors of a chitinase from *S. marcescens*.

5 Summary

The principal purpose of this review has been to assemble the published results on natural product family 18 chitinase inhibitors concerning synthesis, binding mode and inhibitory properties. The studies have revealed that, at present, there are six main classes of inhibitors, although only the allosamidin and cyclopentapeptide (argifin and argadin) classes can be described as highly potent (nM-range). Structural studies of chitinase

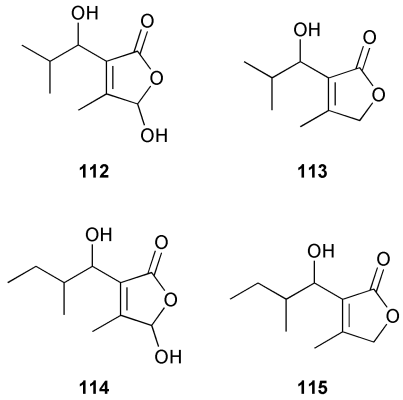


Fig. 11 α,β-Unsaturated γ-lactones isolated from *Streptomyces antibioticus*.

complexes have been reported for most of the inhibitor families, predominantly using the "bacterial-type" subclass of family 18 chitinases.

The synthesis of natural product chitinase inhibitors has attracted considerable interest due to their wide potential as biocontrol agents in medicine and agriculture. The combination of synthetic advances with detailed knowledge of the binding modes of these molecules with their biological targets is now beginning to provide a framework within which inhibitor efficiency and selectivity may be optimised in a rational manner.

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