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Design and Synthesis of Exocyclic Cyclitol Aziridines as Potential Mechanism-Based Glycosidase Inactivators

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Cyclophellitol aziridines have found wide application as mechanism-based, covalent, and irreversible inhibitors of retaining glycosidases. These compounds, like their parent compound, cyclophellitol (a natural product retaining β-glucosidase inactivator), make use of the mechanism of action of retaining glycosidases, which process their substrate through the formation of a transient covalent intermediate. In contrast, inverting glycosidases, the other main family of glycosyl hydrolases, do not employ such a covalent intermediate, and, as a consequence, useful scaffolds for mechanism-based inhibitor design have yet to be discovered. In this work, we explore chemistries that allow for the construction of cyclitol aziridines with the aziridine electrophile attached in an exocyclic fashion, more distal from the anomeric carbon – thus putatively closer to an inverting glycosidase active site nucleophile. The developed chemistries have allowed for the synthesis of a focused library of differently *N*-substituted, α-and β-glucopyranose configured cyclitol aziridines for future evaluation as inhibitors or inactivators of α-and β-glucosidases alike.

Introduction

In 1990, cyclophellitol (Figure 1B, **1**) was isolated from samples of the *Phellinus sp.* mushroom and shown to be a potent, irreversible inactivator of retaining β-glucosidases.[1,2] Structural elucidation revealed cyclophellitol to have a carba-glucose core, functionalized with a β-oriented epoxide spanning the C-1 and C-5a position.[3,4] This epoxide forces the carba-glucose backbone to adopt a half-chair conformation, mimicking the conformation of the oxocarbenium ion transition state of β-D-glucopyranoside during hydrolysis by retaining β-glucosidase enzymes.[5]

Retaining α- and β-glucosidases employ a Koshland double displacement mechanism (Figure 1A).[6–12] The acid/base residues of these enzymes are in close proximity, with a relative distance of roughly 5.5 Å.[9,13] Upon binding of a substrate molecule in the enzyme pocket, in a first nucleophilic substitution reaction the carboxylate residue acts as a nucleophile displacing the substrate aglycon, which is activated through protonation by the acid-base residue. Subsequently, the aglycon leaves the enzyme active site allowing water to enter and upon deprotonation it then engages in a second displacement reaction to deliver the glucopyranose product with net retention of stereochemistry at the anomeric center. The covalently bound adduct formed during hydrolysis has inspired the design of mechanism-based inhibitors that react to form stable, covalent adducts, effectively incapacitating the enzyme.[14,15] This in turn has formed the basis for the design and synthesis of activity based probes (ABPs) as tools to study enzyme activities.[15–18]

In previous studies, we have shown that equipping cyclophellitol **1** and its nitrogen congener cyclophellitol aziridine **2** with a tag (for instance, a fluorophore or biotin) allowed for selective and sensitive profiling of β-glucosidases.[19] Subsequently, inhibitors and probes of the 1,7-epimers (Figure 1B, **3** and **4**) were constructed to selectively inhibit and probe retaining α-glucosidases, revealing **3** and **4** to be irreversible inactivators with micromolar to nanomolar potencies.[20]

In an alternative design, the incorporation of an *N*-2-bromoacetyl warhead on a β-glucose scaffold results in efficient, covalent inactivators of retaining β-glucosidases (Figure 1B, **5**).[21–23] In this inhibitor design the electrophilic site is transpositioned from the anomeric center to the more distal α-bromo amide, which traps the catalytic acid/base residue through a nucleophilic substitution reaction of the bromide to form a stable ester linkage.[23–27]

Inverting glycosidases represent another large group of glycoside hydrolase (GH) and these hydrolases employ a different reaction mechanism than retaining glycosidases.[6,9,11,12] Inverting glycosidases employ a Koshland single displacement mechanism (Figure 1C).[9–11,28,29] The relatively large distance (6–12 Å) between the two catalytic side residues, which usually are two carboxylic acids, enables binding of the substrate and a water molecule.[6,30–32] The active site carboxylate deprotonates the water molecule which concommitantly performs a nucleophilic substitution on the anomeric center expelling the aglycon, which is simultaneously protonated by the enzyme active site carboxylic acid. This results in net inversion of

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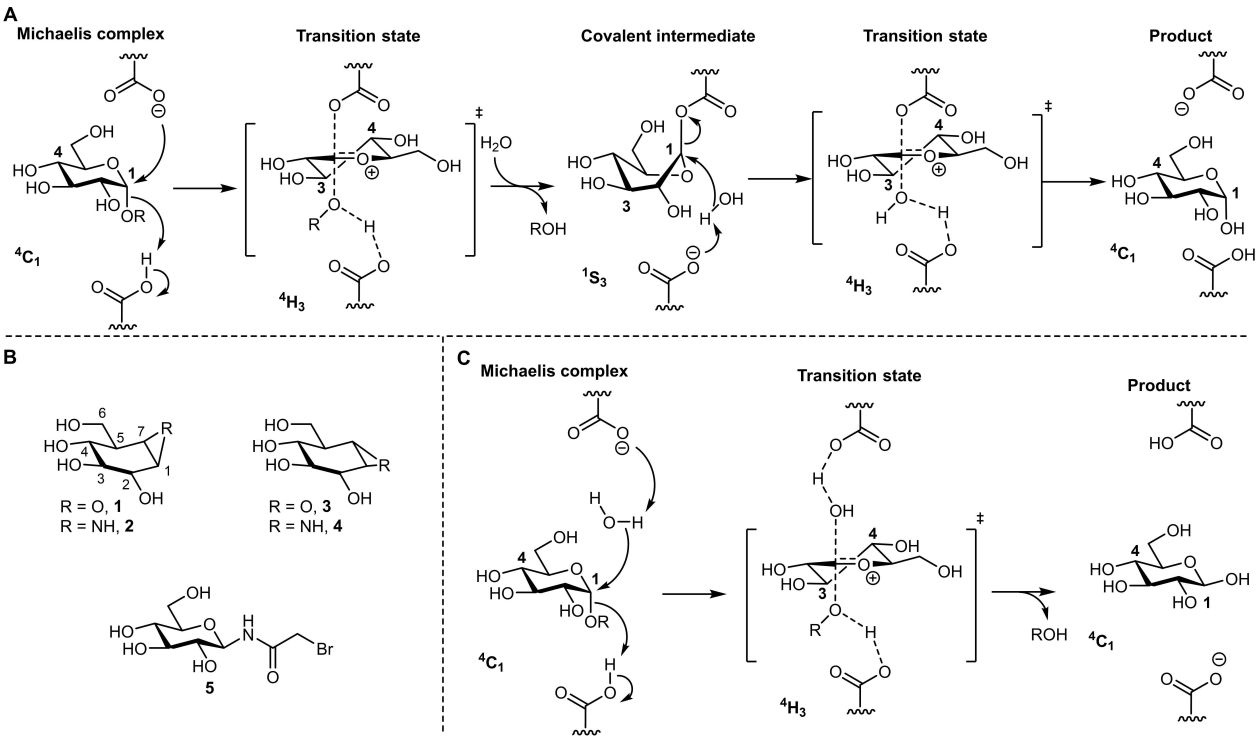

Figure 1. Conformational itinerary of inverting and retaining α-glucosidases via classic Koshland mechanisms, and potent, irreversible inhibitors **1**–**5**.[19,20,28,33] (A) Reaction itinerary of retaining α-glucosidases following a Koshland double displacement mechanism. (B) Potent, irreversible α- and β-glucosidase inhibitors; cyclophellitol **1**, cyclophellitol azirdine **2**, 1,7-*epi*-cyclophellitol **3**, 1,7-*epi*-cyclophellitol azirdine **4** and the structure of glucosyl-1-amine *N*-2-bromoacetyl **5**. (C) Reaction itinerary of inverting α-glucosidases following a Koshland single displacement mechanism.

stereochemistry at the anomeric center of the thus produced glucopyranose.

Due to lack of a covalently bound intermediate during hydrolysis, the design of covalent inhibitors and probes for inverting glycosidases, in analogy to the *modus operandi* of cyclophellitol, is complicated. To date, this has lead to an absence of covalent inhibitors and activity-based probes for selectively targeting inverting glycosidases.

In an attempt to identify such inhibitors, we here propose a series of inhibitors based on 1-*epi*-validamine **6** and validamine **7**,[34–37] which are modified at the amine forming an exocyclic aziridine (Figure 2). This aziridine may act as a distal electrophile, for which it was reasoned there is enough space in the relatively large inverting glycosidase active site. It is hypothesized that the electrophile, further away from the anomeric position can bridge the relatively large distance between the carboxylic acid/carboxylate residues, allowing reaction with one

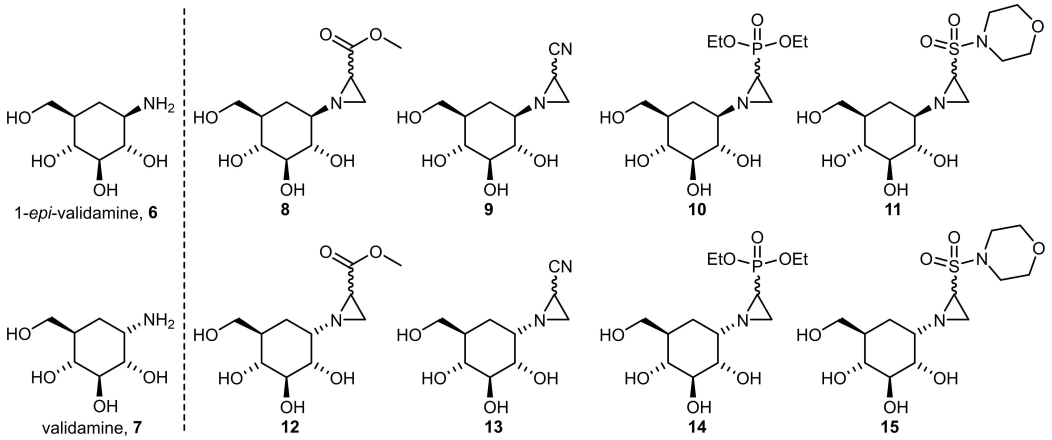

Figure 2. 1-*Epi*-validamine **6** and validamine **7** and eight 1-*N*-aziridine analogues **8**–**15** subject of the here-described studies.

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of these – specifically, the one responsible for deprotonating the water molecule, which is replaced by the inhibitor in the enzyme pocket.

We here describe the synthesis of a panel of inhibitors **8**–**15** using an aza-Michael initiated ring closure reaction (aza-MIRC) as the key step.[38,39] Literature precedent has shown the aza-MIRC aziridine formation on primary amines to be high yielding and taking place under mild conditions.[38,39] To this end, validamine and 1-*epi*-validamine were considered suitable substrates for this transformation. A small series of dibromide coupling partners was composed, equipped with a diverse selection of electron withdrawing groups, all envisioned to be suitable for coupling under aza-MIRC conditions.

In turn, the inhibitor design and synthetic procedures presented here, can fuel future design and synthesis of constructs to act on inverting glycosidases.

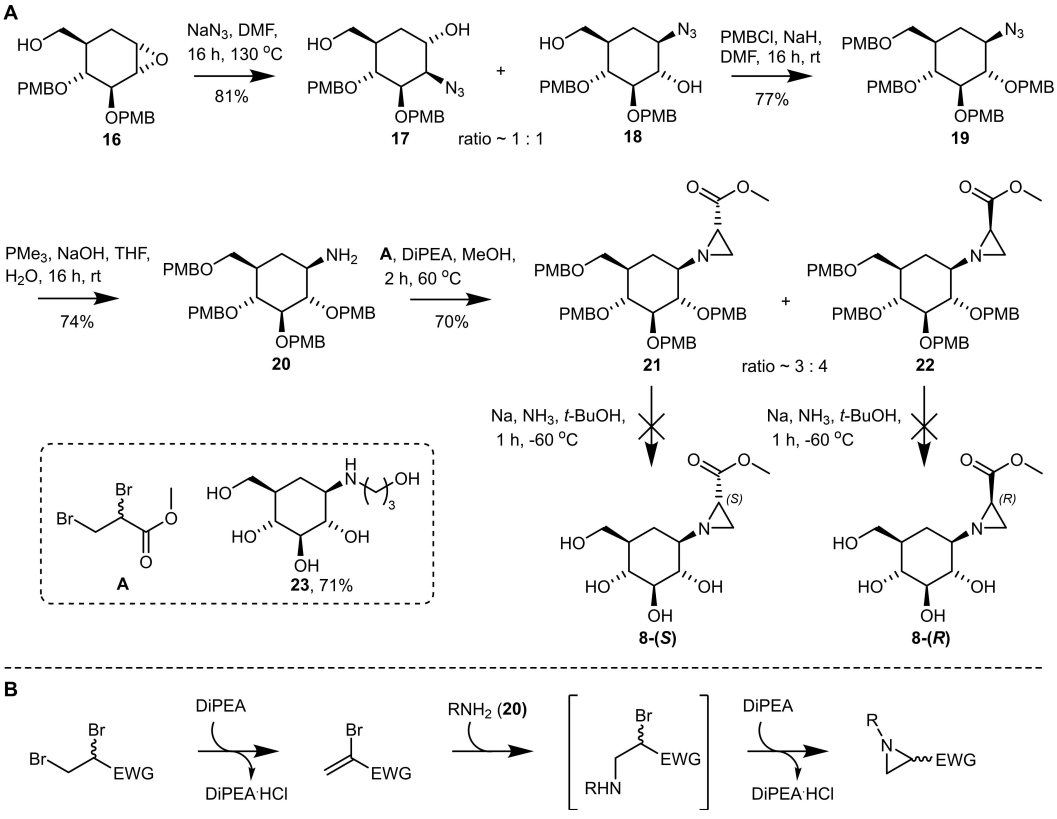
Results and Discussion

The synthesis of the panel of target compounds as depicted in Figure 2 started with the preparation of 4-methoxybenzyl protected 1-*epi*-validamine **20**, which we envisioned would be a suitably protected construct to investigate the aza-MIRC reaction. To this end, epoxide **16**, which could be obtained according to literature procedures,[40] was treated with NaN_3 in DMF at elevated temperatures to yield a separable mixture of regioisomers **17** and **18** in a 1:1 ratio and an overall yield of 81% (Scheme 1A). Subsequent protection of the 2- and 6-OH in **18** under standard Williamson etherification conditions (NaH, PMBCl) yielded fully protected compound **19** in 77% yield. Reduction of the azide under standard Staudinger conditions (PMe_3, aq. NaOH, THF) transformed the azide into the corresponding primary amine **20** (74%).

With protected 1-*epi*-validamine **20** in hand, attention was then turned to the installation of the exocyclic aziridine. The protected 1-*epi*-validamine **20** was reacted with commercially available methyl 2,3-dibromopropanoate (**A**) in a polar, protic solvent (MeOH) using a non-nucleophilic base (DiPEA) to yield a separable mixture of diastereomers **21** and **22** in a 3:4 ratio, and an overall yield of 70%. Observed NOE interactions allowed for identification of both epimers.

The general mechanism of the efficient aziridine formation is shown in scheme 1B.[38,39] First, elimination of the primary bromide results in the in situ formation of the 2-bromovinyl intermediate which bears a Michael acceptor motive ready for a 1,4-addition of the primary amine of the protected 1-*epi*-validamine **20**. Subsequently, in an aza-Darzen reaction, the α-bromide is substituted by the resulting secondary amine to deliver the desired aziridine functionality.

Unfortunately, all attempts to deprotect the exo-cyclic aziridines **21** and **22** resulted in degradation of the starting material, or led to undesired side reactions. Both reductive conditions (Pd/C, H_2) and acidic conditions (TFA, TES) resulted in complete degradation of material, while removal of the PMB ethers under Birch conditions led to the clean formation of



Scheme 1. Attempted synthesis of two exo-aziridine epimers via an aza-MIRC reaction (A) and the mechanism of the aza-MIRC aziridine formation (B).

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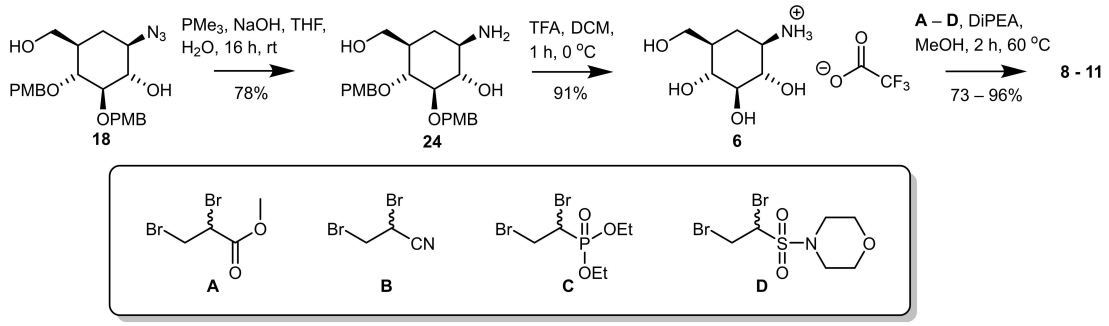


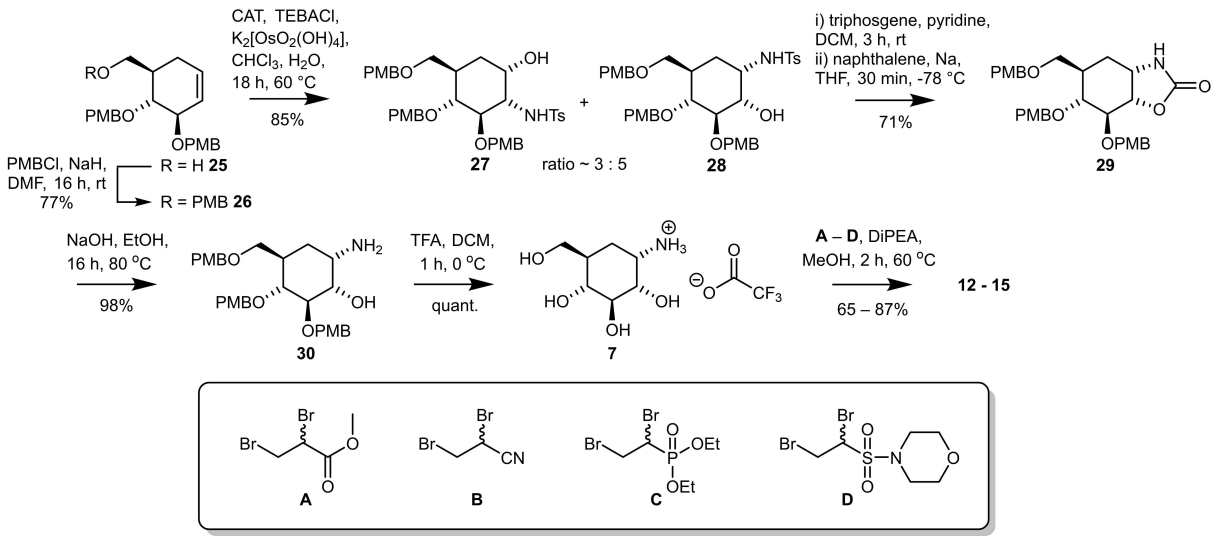
Scheme 2. Construction of target compounds **8**–**11** via an aza-MIRC reaction with 1-*epi*-validamine **6**.

compound **23**,[41] in which the reductive cleavage of the PMB groups was accompanied by reduction of the aziridine and methyl ester to from the *N*-propan-3-ol adduct.

Prompted by the robustness of the aza-MIRC reaction, we hypothesized that the problematic PMB deprotection could be circumvented by the use of unprotected substrates.[39] We therefore explored the use of unprotected 1-*epi*-validamine **6**, which was prepared from azide **18** (Scheme 2). Reduction of the azide under standard Staudinger conditions (PMe_3, NaOH, H_2O, THF) transformed the azide into the corresponding amine **24** (78%), of which the PMB protecting groups were removed under acidic conditions (TFA, TES, DCM) to yield 1-*epi*-validamine **6** as its TFA salt.

Next, 1-*epi*-validamine **6** was reacted, under the agency of DiPEA, with dibromides **A**–**D** (either commercially available (**A**) or easily accessible via known literature procedures (**B**–**D**),[42–44] see Scheme S1, supporting info), in MeOH at elevated temperatures. Gratifyingly, clean conversion towards the desired target structures was observed, yielding target compounds **8**–**11** in moderate to excellent yields after purification (73%–96%).

With effective conditions in hand to generate the exocyclic aziridines we next set out to assemble the diasteroisomeric set of target compounds from validamine **7** (Scheme 3). The primary hydroxyl in compound **25**, obtained according to literature procedures,[40] was protected as a PMB ether under standard Williamson etherification conditions (NaH, PMBCl) to yield fully protected compound **26** in 85% yield. A stereoselective Sharpless aminohydroxylation on alkene **26** ($K_2[OsO_2(OH)_4]$, chloramine-T (CAT), TEBACl) resulted in a separable, regioisomeric mixture of α-*cis*-amino alcohols **27** and **28** in 31% and 54% respectively.[45] The α-*cis*-amino alcohol **28** could be transformed into the corresponding cyclic carbamate by treatment with triphosgene and pyridine. Subsequent removal of the *N*-tosyl functionality under reductive conditions (Na, naphthalene) gave rise to cyclic carbamate **29** in 71%. Cyclic carbamate **29** was then hydrolyzed under alkaline conditions using NaOH in EtOH under elevated temperatures to afford the deprotected amino alcohol **30** (98%). Global deprotection using TFA and TES resulted in validamine **7** which was obtained as its TFA salt in quantitative yield.



Scheme 3. Construction of target compounds **12**–**15** via an aza-MIRC reaction with validamine **7**.

Following the procedures applied to the 1-*epi*-validamine substrate **6**, validamine **7** was transformed into the set of target compounds **12**–**15** using dibromides **A**–**D**. Also these reactions proceeded uneventfully to cleanly provide **12**–**15** which were isolated in 65% to 87% yield.

Conclusion

In conclusion, this report describes the design and synthesis of functionalized validamines **8**–**15**, bearing an exocyclic aziridine motif as putative inhibitors of inverting glucosidases. We designed and synthesized these compounds on the premise that the exo-cyclic aziridine functionality can bridge the distance between the carboxylic acid/carboxylate residues in the enzyme pocket, potentially allowing for the formation of a covalent bond with the enzyme active site nucleophile, effectively incapacitating the enzyme. Key in the synthesis schemes has been an aza-Michael initiated ring closure (aza-MIRC) reaction, which in a single step converts unprotected 1-*epi*-validamine **6** and validamine **7** into target compounds **8**–**15**, proving the sturdiness and robustness of these aziridine forming reactions on complex, unprotected substrates. We are currently developing suitable inhibition assays to probe whether this novel class of carbomimetics is capable of inhibiting inverting glucosidases. If so, our inhibitor design and synthetic procedures presented here, can fuel the future design and synthesis of constructs to effectively act on inverting glycosidases.

Experimental Section

Please find details on synthetic procedures and experimental details in the Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: aziridine · aza-MIRC · cyclophellitol · glucosidase · inhibitor

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