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Activity-based protein profiling of glucosidases, fucosidases and glucuronidases

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The synthesis of cyclophellitol aziridine and its configurational and functional isomers

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2.1 Introduction

The natural product cyclophellitol¹ (**1**, Figure 1A) and its synthetic analogue, cyclophellitol aziridine (**2**)² are potent, mechanism-based and irreversible retaining β -glucosidase inhibitors. Retaining β -glucosidases employ a Koshland double displacement mechanism³ in substrate hydrolysis (Figure 1B), and this process proceeds through a covalent enzyme-glucoside intermediate. Both cyclophellitol (**1**) and cyclophellitol aziridine (**2**) capitalize on this mechanism. They are configurational β -glucopyranose analogues, and outperform the structurally related (and much wider studied) conduritol B epoxide (**3**, CBE – lacking the C8 methylene compared to **1** in potency and selectivity as retaining β -glucosidase inhibitors.⁴ Compounds **1** and **2** adopt a ⁴H₃ half chair conformation, thereby emulating the oxocarbenium ion-like transition state.⁵ This oxocarbenium ion is trapped to yield the glycosyl-enzyme intermediate and the acylal linkage is then hydrolyzed to release β -glucose with double inversion – thus net retention – of the anomeric carbon configuration.⁶ Due to their preferred conformation, cyclitols **1** and **2** fit well

in the retaining β -glucosidase active site and present the epoxide (**1**) or aziridine (**2**) heteroatom for protonation by the acid-base residue in the binding pocket. In an acid-catalyzed nucleophilic attack, the epoxide/aziridine ring opens to form a covalent enzyme-inhibitor adduct. Compared to the acylal linkage featuring during β -glucose hydrolysis, the formed ester is considerably more stable, and the β -glucosidase is irreversibly disabled.

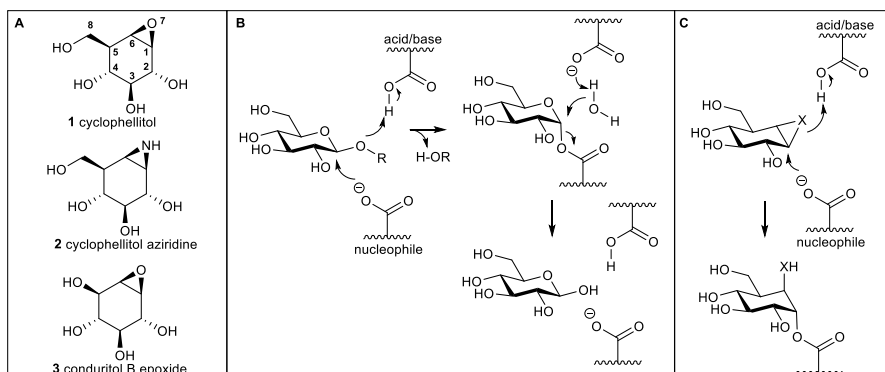


Figure 1. A) Structure of cyclophellitol (**1**), cyclophellitol aziridine (**2**) and conduritol B epoxide (**3**). B) Mechanism employed by retaining β -glucosidases. C) Mechanism-based, irreversible retaining β -glucosidase inhibition by compounds **1** and **2** (X = O or NH).

By virtue of their mechanism of action, cyclophellitol (**1**) and cyclophellitol aziridine (**2**) are selective for retaining β -glucosidases over inverting β -glucosidases. This property distinguishes cyclophellitols from another class of glycosidase inhibitors: the iminosugars (amongst which the archetypal competitive glucosidase inhibitor is deoxynojirimycin).⁷ In recent years, covalent and irreversible inhibitors have received growing attention, as they are ideal starting points for the development of activity-based probes (ABPs).⁸ In the field of activity-based protein profiling (ABPP), covalent and irreversible inhibitors of an enzyme, or enzyme family, are equipped with a reporter entity – either a fluorophore, a biotin or a bioorthogonal tag – and used to profile their target enzymes in complex biological samples. Cyclophellitol (**1**) is on paper suited for this purpose, and has indeed been transformed⁹ into a highly selective ABP highly selective for the human retaining β -glucosidases.

Cyclophellitol aziridine is the more attractive lead for ABP development.¹⁰ It is at least as potent an inhibitor as cyclophellitol, and the aziridine nitrogen can be modified with a range of functional groups, including reporter functionalities, without interfering with recognition by the target enzyme (at least, *exo*-glycosidases to which the target enzymes of **1** and **2** belong are often largely indiscriminate to the nature of the aglycon – the general space also occupied by an aziridine substituent). For this reason, as well as the finding that about half of the

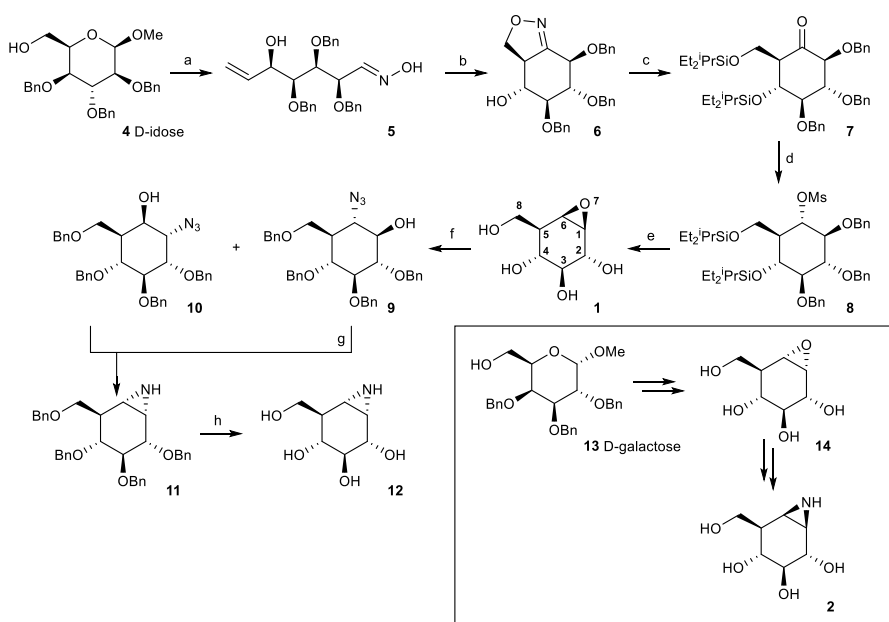
glycosidases known employ a Koshland double replacement mechanism,¹¹ interest in cyclophellitol aziridines has grown considerably in recent years. Their use as inhibitors and ABPs to monitor glycosidases recognizing and processing configurational and functional isomers of glucose requires however effective synthetic routes to cyclophellitol aziridine synthesis. Herein, the synthesis strategies reported to date are reviewed, starting with the known syntheses of cyclophellitol aziridine (**2**), followed by strategies towards configurational and functional analogues and overall with a focus on methodology towards cyclohexitol aziridines mimicking pyranose sugars.

2.2 Synthetic strategies

2.2.1 Tatsuta's synthesis of cyclophellitol, cyclophellitol aziridine and their 1,6-*epi* isomers

The group of Tatsuta was the first to study the synthesis of cyclophellitol derivatives.¹² Closely following the discovery, by Umezawa, Takeuchi and colleagues,¹ of cyclophellitol (**1**) and its annotation as a mechanism-based retaining β -glucosidase inhibitor,¹³ Tatsuta and co-workers disclosed the first synthesis of cyclophellitol (**1**) and cyclophellitol aziridine (**2**), as well as their configurational analogues 1,6-*epi*-cyclophellitol (**14**, Scheme 1) and 1,6-*epi*-cyclophellitol aziridine **12** (atom numbering as indicated in Figure 1, compound **1**). As with most literature syntheses¹⁴ of cyclophellitol, the Tatsuta scheme^{14a} starts from a chiral building block, here partially protected D-idopyranose derivative **4**, which is prepared from L-glucose following established procedures.¹⁵ Swern oxidation and Wittig olefination on the primary alcohol is followed by hydrolysis of the methyl acetal. Subsequent reaction of the liberated hemi-acetal with hydroxylamine provided oxime **5** in a series of standard transformations. *In situ* oxidation of the oxime in **5** to the corresponding nitrile oxide (treatment with sodium hypochlorite in methylene chloride) led to a [2+3] cycloaddition to give the key intermediate, isoxazoline **6** as the single stereoisomer. The isoxazoline N-O bond was reduced by Raney nickel catalyzed hydrogenation, after which the free alcohols were protected as the diethylisopropylsilyl ethers to give cyclohexanone **7**. Reduction of the carbonyl provided the desired alcohol in a 3:1 diastereomeric ratio, which was then transformed into mesylate **8**. Catalytic hydrogenation followed by treatment with base led to removal of all protective groups. Finally, intramolecular S_N2 substitution of the methanesulfonyl group yielded cyclophellitol (**1**). Perbenzylation of **1** followed by opening of the epoxide gave the mixture of *trans*-1,2-azido-alcohols **9** and **10**. Treatment of this mixture with triphenylphosphine in a mixture of THF and water gave, after formation of the phosphazene and expulsion of triphenylphosphine oxide in a Staudinger type reaction¹⁶, tetra-*O*-benzyl-1,6-*epi*-cyclophellitol aziridine **11**. Finally, removal of the benzyl groups under Birch conditions afforded 1,6-*epi*-cyclophellitol aziridine **12**.

Scheme 1. Synthesis of cyclophellitol (**1**), 1,6-*epi*-cyclophellitol (**14**), cyclophellitol aziridine (**2**) and 1,6-*epi*-cyclophellitol aziridine (**12**) by Tatsuta and co-workers.



Reagents and conditions: (a) i) $(\text{COCl})_2$, DMSO, Et_3N , $-78\text{ }^\circ\text{C}$; ii) $\text{Ph}_3\text{P}=\text{CH}_2$, benzene, 75% over two steps; iii) HCl (aq.), dioxane; iv) $\text{HO}-\text{NH}_2$ (HCl salt), pyridine, 80% over two steps; (b) NaOCl , DCM, 70%; (c) i) H_2 (1.0 atm.), Raney Ni, 80%; ii) diethylisopropyl triflate, 2,6-lutidine, DCM; (d) i) $\text{BH}_3\cdot\text{Me}_2\text{S}$, 45% over three steps; e) i) H_2 (1 atm.), $\text{Pd}(\text{OH})_2$, MeOH; ii) NaOH (1.0 M, aq.), 75% over two steps; (f) i) NaH , BnBr , DMF, 90%; ii) NaN_3 , DMF, $110\text{ }^\circ\text{C}$, **9**: 27%, **10**: 41%; (g) i) Ph_3P , $\text{THF}/\text{H}_2\text{O}$; ii) NaOMe , MeOH, 40% over two steps; h) Li , NH_3 (liq.), THF, $-78\text{ }^\circ\text{C}$, 60%.

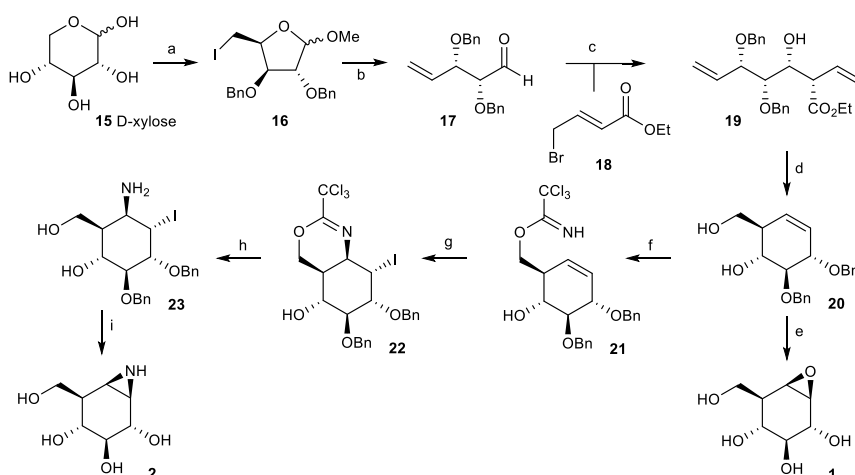
In a similar fashion (though with altered conditions at various stages), Tatsuta and co-workers synthesized 1,6-*epi*-cyclophellitol **14**¹⁷ starting from D-galactopyranose derivative **13** (a configurational isomer of D-idose **3** with the same protective group pattern). The strategy to open the epoxide with sodium azide yielded a mixture of azido-alcohols, which were both transformed into the epimeric (with respect to the epoxide) aziridine using Staudinger conditions, proved to also be effective in the synthesis of cyclophellitol aziridine **2** from **14** in comparable yields to that of the preparation of aziridine **12**. The general strategy – installation of an aziridine in a two-step sequence (epoxide opening with nucleophilic azide followed by Staudinger reduction/cyclisation with inversion of configuration) – features in a number of subsequent syntheses as is described further on in this review.

2.2.2 Synthesis of cyclophellitol aziridines **2** and **12**: Ring-closing metathesis and intramolecular iodo-amination as the key steps

The years following the pioneering synthesis studies of Tatsuta's group witnessed the rising

impact of ring closing metathesis (RCM)¹⁸ – the transformation of two terminal alkenes of an (acyclic) substrate into an internal alkene in a cyclic product – in organic chemistry. Suitable transition metal catalysts became available,¹⁹ which is capable to produce small to medium-sized rings from dienes featuring numerous functional groups. RCM evolved to become a key step in the synthesis of functionalized heterocycles²⁰ and carbocycles²¹, and is compatible with carbohydrate chemistry.²² This holds true as well for the synthesis of cyclophellitol/cyclophellitol aziridine analogues, most of which are prepared nowadays through synthetic routes involving a RCM step.

Scheme 2. Ring-closing metathesis as a key step in the synthesis of compounds **1** and **2**.



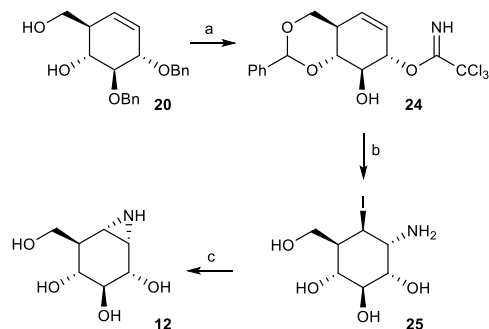
Reagents and conditions: (a) i) HCl, MeOH, 5 °C; ii) I₂, Ph₃P, imidazole, THF, 74%; iii) BnOC(=NH)CCl₃, TfOH, dioxane, 90%; (b) Zn, THF/H₂O, ultrasound, 78%; (c) indium powder, La(OTf)₃, H₂O, ultrasound, 80%; (d) i) Grubbs 2nd generation catalyst, DCM, 40 °C, 89%; ii) DIBAL-H, THF, 0 °C → RT; iii) NaBH₄, H₂O, EtOAc, 94%; (e) i) *m*CPBA, Na₂HPO₄ (aq., 1.0 M), NaH₂PO₄ (aq., 1.0 M), DCE, 50 °C, 55%; ii) H₂, Pd(OH)₂, MeOH, 88%; (f) CCl₃CN, DBU, DCM, 0 °C; (g) I₂, NaHCO₃, H₂O; (h) HCl (37%, aq.), dioxane, 60 °C; (i) i) NaHCO₃, MeOH, 60% over four steps; ii) Li, NH₃ (liq.), THF, -60 °C, 70%.

Madsen and co-workers²³ prepared cyclophellitol (**1**) via cyclohexene intermediate **20**, itself prepared through RCM on the appropriate diene **19** (Scheme 2). In this route (optimized by us recently),²⁴ D-xylose is converted in three steps into iodofuranoside **16**. First, kinetic Fischer glycosylation of D-xylose in methanol provided methyl xylofuranoside. Next the iodine was installed using conditions developed by Garegg and Samuelsson²⁵ after which benzylation under acidic conditions of the two remaining secondary hydroxyls) gave **16**. Vasella fragmentation of **16** with zinc dust under sonication gave aldehyde **17**. Indium-mediated Barbier reaction of **17** with ethyl 4-bromocrotonate **18** yielded diene **19** in good yield and excellent stereochemistry – much better, as stated by the authors²³, than those found when performing the Barbier

allylation with 4-bromobut-2-en-1-ol instead of crotonate **18** (which, if successful, would have obviated the reduction of the methyl ester to the corresponding primary hydroxyl in a later stage). RCM of diene **19** with Grubbs second-generation ruthenium alkylidene catalyst²⁶ and ensuing reduction of the methyl ester to the primary alcohol (treatment with DIBAL-H, followed by sodium borohydride reduction of the intermediate aldehyde) gave cyclohexene **20** in good yield. Madsen and colleagues continued their synthesis to cyclophellitol (**1**) by capitalizing on the homoallylic alcohol in **20** for stereospecific introduction of the epoxide. Therefore, treatment of **20** with *meta*-chloroperbenzoic acid (*m*CPBA) in methylene chloride followed by catalytic hydrogenation yielded cyclophellitol (**1**) in ten steps starting from D-xylose (**15**).

The homoallylic alcohol embedded in partially protected cyclohexene **20** proved also ideal for the stereospecific introduction of an amine functionality, as demonstrated in previous synthesis of cyclophellitol aziridine (**2**).²⁴ Reaction of **20** with trichloroacetonitrile and 1,8-diazabicycloundec-7-ene (DBU) as base gave trichloroacetimidate **21**. Iodocyclisation (with iodine and sodium bicarbonate) followed by acidolysis of the resulting cyclic imidate **22**, yielded stereospecifically *trans*-1-iodo-2-amine **23**. Under mild basic conditions the iodine in **23** is displaced to form the aziridine – again in a stereospecific fashion – after which Birch reduction (lithium in liquid ammonia) gave cyclophellitol aziridine in five steps from the Madsen cyclohexene **20**.

Scheme 3. Transformation of common intermediate **19** into 1,6-*epi*-cyclophellitol aziridine **11**.



Reagents and conditions: (a) i) Li, NH₃ (liq.), THF, -60 °C, 57%; ii) PhCH(OMe)₂, CSA, DMF, 61%; iii) CCl₃CN, DBU, DCM, 0 °C; (b) i) NaHCO₃, I₂, H₂O, 41% over two steps; ii) HCl (37%, aq.), dioxane; (c) NaHCO₃, MeOH, 63% over two steps.

The accessible synthesis of cyclohexene **20**, which can be performed to yield multi-gram quantities, combined with the control in stereochemical outcome exerted by the intramolecular iodo-imidation/iodine displacement sequence, led to the adaptation of the synthetic route of cyclophellitol aziridine (**2**) (Scheme 2) towards 1,6-*epi*-cyclophellitol aziridine (**12**).²⁷ As outlined in Scheme 3, removal of the two benzyl ethers in **20** under Birch conditions was followed by

selective installation of the benzylidene and selective transformation of the allylic alcohol (as opposed to the homo-allylic alcohol) into the trichloroacetimidate, yielding (labile) intermediate **24**. Treatment of **24** with iodine now results in iodo-imidation with delivery of the nitrogen at C1 from the 'alpha' face (in terms of the parent D-glucopyranoside), as compared to C6-beta-delivery of the nitrogen seen in the transformation of **21** into **22** (Scheme 2). Following the same sequence of events, acidolysis of the cyclic imidate yielded *trans*-iodo-amine **25**, which under mild basic conditions gave the desired 1,6-*epi*-cyclophellitol aziridine **12**.

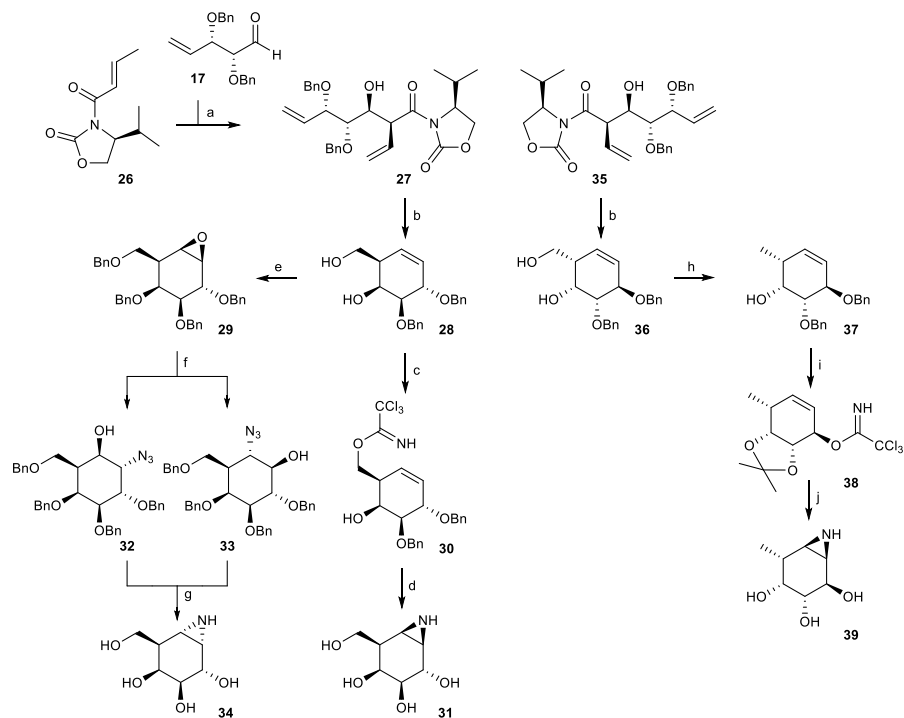
2.2.3 Synthesis of configurational and functional cyclophellitol aziridine isomers: iodo-ination/substitution versus epoxide opening/Staudinger cyclisation

In recent years syntheses of a number of cyclophellitol aziridines differing in configuration and/or substitution pattern from the glucopyranose configured compounds **1** and **14** have appeared in the literature. These syntheses share a number of features with the strategies outlined above. They all use chiral pool building blocks as starting material, RCM may feature as a key step and the aziridine moiety is introduced from either an epoxide precursor (with full stereocontrol and retention of configuration – stereocontrol in epoxide formation is not always complete though) or through intramolecular iodocyclisation from a partially protected (homo)-allylic alcohol precursor.

The synthesis of 4-*epi*-cyclophellitol aziridine **31** (a configurational analogue of β -galactopyranose) and 1,4,6-*epi*-cyclophellitol aziridine **34** (a configurational analogue of α -galactopyranose) starts with a dibutylboryl triflate-catalyzed stereoselective aldol condensation of aldehyde **17** and Evans' oxazolidinone **26** (Scheme 4), in a procedure developed by Llebaria and co-workers.²⁸ Reduction of the amide in **27** to the primary alcohol (with concomitant removal of the chiral Evans' auxiliary) followed by RCM yielded partially protected cyclohexene **28**, which can be regarded as the galactopyranose equivalent of common building block **20** that was used to synthesize both cyclophellitol aziridine **2** (Scheme 2) and 1,6-*epi*-cyclophellitol aziridine (**12**) (Scheme 1). Following the synthetic schemes as outlined for compounds **2** and **12** indeed yielded 5-*epi*-cyclophellitol aziridine (**31**) and 1,2,5-*epi*-cyclophellitol (**34**).²⁹ The synthesis of compound **31** proceeded with an efficiency equal to that observed in the synthesis of cyclophellitol aziridine (**2**) and with absolute stereocontrol as offered by the intramolecular iodo-ination/intramolecular substitution protocol. The synthesis of compound **34** required the preparation of 4-*epi*-cyclophellitol **29**, which was accomplished after perbenzylation of **28** followed by epoxidation with *m*CPBA, which proceeded with remarkable stereoselectivity given that no (homo)-allylic alcohol is present in the precursor to guide the epoxidation. In their original publication²⁸ on the synthesis of **28**, Llebaria and co-workers produced the perbenzylated, *galacto*-configured cyclophellitol **29** through stereoselective dihydroxylation of cyclohexene **28** followed by reaction with excess of the Mattocks-Moffatt reagent,³⁰ 2-acetoxyisobutryl bromide. The resulting crude mixture of *trans*-cyclohexane bromoacetates

was treated with potassium carbonate to give compound **29** in good yield as well.

Scheme 4. Synthesis of *galactose* and *fucose*-configured cyclophellitol aziridines.

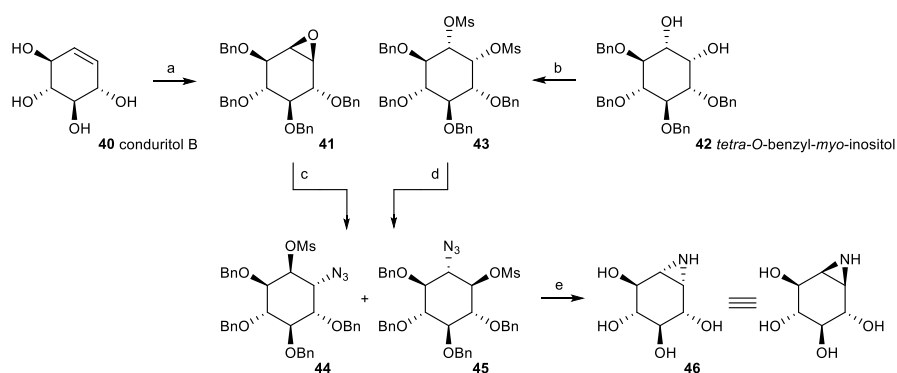


Reagents and conditions: (a) $\text{Bu}_2\text{BSO}_3\text{CF}_3$, Et_3N , DCM, $-78\text{ }^\circ\text{C}$ to $-20\text{ }^\circ\text{C}$, 80%; (b) i) LiBH_4 , THF/ H_2O , $0\text{ }^\circ\text{C}$ →RT, 85%; ii) second-generation Grubbs catalyst, DCM, $40\text{ }^\circ\text{C}$, 78%; (c) CCl_3CN , DBU, DCM, $0\text{ }^\circ\text{C}$; (d) i) I_2 , NaHCO_3 , H_2O ; ii) HCl (37% aq.), MeoH, iii) HCl (37% aq.), dioxane, $60\text{ }^\circ\text{C}$; iv) NaHCO_3 , MeOH, 60% over four steps), (d) Li , NH_3 (liq.), THF, $-60\text{ }^\circ\text{C}$; (e) i) NaH , BnBr , TBAI, DMF, 85%; ii) *m*CPBA, DCM, 63%; (f) NaN_3 , LiClO_4 , MeCN, $80\text{ }^\circ\text{C}$, 73% (**32**:**33** = 1:1.3); (g) i) Ph_3P , MeCN, $80\text{ }^\circ\text{C}$ (26%); ii) Li , NH_3 (liq.), THF, $-60\text{ }^\circ\text{C}$; (h) i) TsCl , Et_3N , DCM, 87%; ii) LiAlH_4 , THF, $0\text{ }^\circ\text{C}$ →RT, 87%; (i) i) Li , NH_3 (liq.), THF, $-60\text{ }^\circ\text{C}$, 73%; ii) 2,2-dimethoxypropane, CSA, 60%; iii) CCl_3CN , DBU, DCM; (j) i) CCl_3CN , DBU, DCM; ii) NaHCO_3 , I_2 , H_2O , 46%; iii) HCl (37% aq.), MeOH, $60\text{ }^\circ\text{C}$; iv) NaHCO_3 , MeOH, 65% over four steps.

One attractive feature of chiral pool material in synthesis is that – providing that the enantiomer is available and affordable – subjecting this enantiomer to the same sequence of events will yield the mirror image products. Both aldehyde **17** and oxazolidinone **26** are readily available in enantiomeric form and their condensation following the Mukayama-aldol procedure developed by Llebaria and co-workers²⁸ gave access to diene **35**, being the mirror image of **27**. Processing **35** similar to **27** would yield a set of L-galactose-configured cyclophellitol aziridines. The 6-deoxy analogue of L-fucose is often encountered – α -linked – in naturally occurring glycoconjugates. With the aim to develop covalent and irreversible α -fucosidase inhibitors, we synthesized 8-

deoxy-2,3,5-*epi*-cyclophellitol aziridine (**39**) following a route related to the one depicted in Scheme 3.²⁹ Following RCM (**35** to **36**), the primary alcohol was reduced to the methyl group (**36** to **37**) after which the debenzoylation, protection of the *cis*-diol and reaction of the remaining allylic alcohol with trichloroacetonitrile yielded imidate **38**. Iodo-imation, acidolysis and base-catalyzed intramolecular iodide substitution provided aziridine (**39**) – a configurational analogue of α -L-fucopyranose. The above schemes represent all configurational and functional cyclophellitol aziridines (namely, **1**, **12**, **31**, **34** and **39**) whose synthesis has been reported to date. Studies on the synthesis of related compounds exists, however, both targeting cyclopentitol aziridines³² (not shown here) and in particular conduritol aziridines.

Scheme 5. Syntheses of conduritol B aziridine.



Reagents and conditions: (a) i) *m*CPBA, DCM; ii) NaH, BnBr, DMF (on racemic conduritol, for enantiopure **40** see³³); (b) MsCl, pyridine; (c) i) NaN₃, MeCN, 2 N LiClO₄, 91%; ii) MsCl, Et₃N, THF, only **45**: 88%; (d) NaN₃, DMF, 42% over two steps; (e) i) LiAlH₄, Et₂O, 0 °C, 60%; ii) Na, NH₃ (liq.), THF, -78 °C, 86%.³⁴

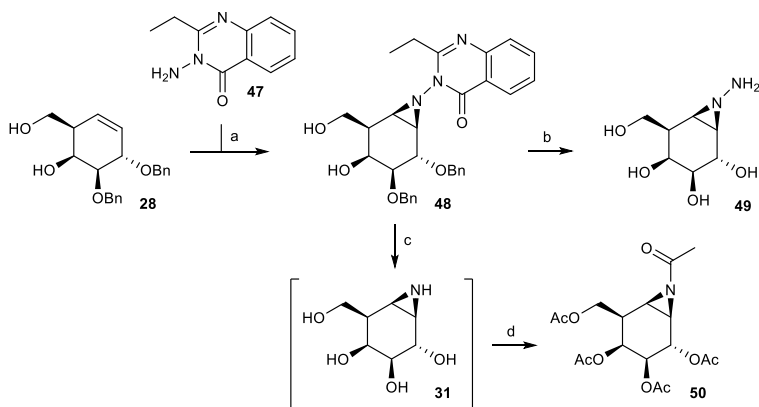
Scheme 5 depicts two recent and representative syntheses of the broad-spectrum retaining glucosidase inhibitor, conduritol B aziridine (**46**) (both α - and β -glucosidase).³⁵ In the first route,³⁶ the natural product, conduritol B (**40**) is perbenzylated followed by epoxidation to afford fully protected conduritol B epoxide **41**. Opening of the epoxide with azide, mesylation of the resulting secondary hydroxyl, Staudinger ring-closure and final debenzoylation provided conduritol B aziridine (**46**). In an alternative route, the mixture of azides **44** and **45** was prepared from another natural product, *myo*-inositol (**42**)³⁷ protected as the *tetra*-*O*-benzyl derivative, via a number of standard protective group and functional group manipulations.

2.3 Discussion

Cyclitol epoxides were widely used in the sixties of the past century as mechanism-based, irreversible glycosidase inhibitors. Conduritol B epoxide (**3**, CBE), discovered and exploited by Legler and co-workers⁴, was the glucosidase inhibitor of choice up until the discovery in 1967 of

the polyhydroxylated alkaloid, deoxynojirimycin.⁷ In the following decades, cyclitol epoxides and cyclitol aziridines received relatively little attention. In 1989, the Withers group³⁵ proposed that a CBE homologue bearing an extra methylene would fit better within a glucosidase active site and suggested that such a compound should be synthesized. Shortly thereafter, cyclophellitol (**1**) was discovered¹ as a natural product and shown to be a potent and highly selective inhibitor of retaining β -glycosidases. These discoveries led to some renewed interest in the cyclitol epoxide/aziridine compound class, and several studies on the synthesis of **1** and evaluation as enzyme inhibitors and its functional and configurational analogues were reported.

In recent years, and in conjunction with a general rise in interest in covalent, irreversible inhibitors as starting point for the development of ABPP methodology,⁸ cyclophellitol (**1**), cyclophellitol aziridine (**2**) and analogous structures are receiving renewed attention. To date, ABPs have been reported that enable selective profiling of retaining glycosidases such as β -glucosidases,³⁷ α -glucosidases,²⁷ α -galactosidases^{29b} and α -fucosidases,³¹ which are designed based on compound **2** bearing *N*-substituents featuring a fluorophore or biotin as a reporter entity. Cyclophellitol aziridine (**2**) has proven to be a superior scaffold compared to 2 (or 5)-deoxy-5-fluoroglycosides³⁷ for *in vitro* and *in situ* ABPP of retaining β -glucosidase activities⁴⁰ and adaptation of the configuration and substitution pattern will likely yield selective ABPs for retaining glycosidase families evolved to recognize and hydrolyze the underlying configurational carbohydrates – next to monosaccharides (*exo*-glycosidases⁴¹) likely also oligosaccharides (*endo*-glycosidases⁴²). To fulfill this promise, though, synthetic methodology needs to be expanded to enable easy access to an array of configurational and functional cyclophellitol/cyclophellitol aziridine analogues. The aziridine moiety in all the syntheses described in this review is installed either by modification of an epoxide precursor or through iodocyclization starting from a (homo) allylic alcohol precursor. Expansion of methodology that enables the introduction of an aziridine⁴³ at various stages of the synthesis is important to access to a wide array of cyclophellitol aziridine analogues. Llebaria and co-workers recently reported⁴⁴ direct aziridination of *galacto*-configured cyclohexene **28** (Scheme 6). Although the objective of this synthetic study was to obtain *N*-amino-aziridine **49**, the authors showed that reduction of the N-N bond yields 5-*epi*-cyclophellitol aziridine (**31**) (the structure of which was established after peracetylation to **50**). Direct aziridination – either by reaction with aminoquinazolines⁴⁴ or by means of other recently published⁴⁵ methodology – will likely evolve to become a complementary method for the synthesis of cyclophellitol aziridine analogues, thus expanding the chemical toolbox of covalent, irreversible glycosidase inhibitors and activity-based glycosidase probes derived thereof.

Scheme 6. Direct aziridination of protected cyclohexene **28**.

Reagents and conditions: (a) PhI(OAc)₂, K₂CO₃, DCM, 54%; (b) i) H₂N-NH₂, 120 °C, 78%; ii) Na, NH₃ (liq.), THF, -78 °C, 91%; (c) i) Na, NH₃ (liq.), THF, -78 °C; ii) Ac₂O, pyridine, 28% over two steps).

2.4 References

- [1] S. Atsumi, K. Umezawa, H. Iinuma, H. Naganawa, H. Nakamura, Y. Iitaka, T. Takeuchi, *J. Antibiotics* **1990**, *43*, 49-53.
- [2] S. Tatsuta, Y. Niwata, K. Umezawa, K. Toshima, M. Nakata, *J. Antibiotics* **1991**, *44*, 912-914.
- [3] D. Koshland, *Biol. Rev.* **1953**, *28*, 416-436.
- [4] a) G. Legler, *Hoppe-Seyler's Z. Physiol. Chem.* **1966**, *345*, 197-214; b) G. Legler, *Hoppe-Seyler's Z. Physiol. Chem.* **1968**, *349*, 767-774.
- [5] G. J. Davies, A. Planas, C. Rovira, *Acc. Chem. Res.* **2012**, *45*, 308-316.
- [6] See for some reviews on the mechanism of glycosyl hydrolases: a) D. J. Vocadlo, G. J. Davies, *Curr. Opin. Chem. Biol.* **2008**, *12*, 539-555; b) G. Davies, B. Henrissat, *Structure* **1995**, *15*, 853-859.
- [7] a) H. Paulsen, *Angew. Chem. Int. Ed.* **1966**, *5*, 495-510; b) M. Yagi, T. Koumo, Y. Aoyagi, H. Murai, *Nippon Nogei Kagaku Kaishi* **1976**, *50*, 571-572; c) Iminosugars as glycosidase inhibitors: nojirimycin and beyond, A. E. Stütz, editor, Wiley VCH, **1999**.
- [8] a) Y. Liu, M. P. Patricelli, B. F. Cravatt, *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14694-14699; b) D. Greenbaum, K. F. Medzihradsky, A. Burlingame, M. Bogoy, *Chem. Biol.* **2000**, *7*, 569-581; c) A. Borodovsky, B. M. Kessler, R. Casagrande, H. S. Overkleeft, K. D. Wilkinson, H. L. Ploegh, *EMBO J.* **2001**, *20*, 5187-5196.
- [9] M. D. Witte, W. W. Kallemeijn, J. Aten, K.-Y. Li, A. Strijland, W. E. Donker-Koopman, B. Blijlevens, G. Kramer, A. M. C. H. van den Nieuwendijk, B. I. Florea, B. Hooibrink, C. E. M. Hollak, R. Ottenhoff, R. G. Boot, G. A. van der Marel, H. S. Overkleeft, J. M. F. G. Aerts, *Nat. Chem. Biol.* **2010**, *6*, 907-913.
- [10] L. I. Willems, J. Jiang, K.-Y. Li, M. D. Witte, W. W. Kallemeijn, T. J. N. Beenakker, S. P. Schröder, J. M. F. G. Aerts, G. A. van der Marel, J. D. C. Codée, H. S. Overkleeft, *Chem. Eur. J.* **2014**, *20*, 10864-10872.
- [11] a) See the CAZypedia website on carbohydrate-active enzymes: http://www.cazypedia.org/index.php/Glycoside_hydrolases; b) See for a description of the CAZypedia website: V. Lombard, H. Golaconda Ramulu, E. Drula, P. M. Coutinho, B. Henrissat, *Nucleic Acids Res.* **2014**, *42*, D490-495.

- [12] K. Tatsuta, Y. Niwata, K. Umezawa, K. Toshima, M. Nakata, *Tetrahedron Lett.* **1990**, *31*, 1171-1172.
- [13] K. Tatsuta, Y. Niwata, K. Umezawa, K. Toshima, M. Nakata, *J. Antibiotics* **1991**, *44*, 456-458.
- [14] a) K. Tatsuta, Y. Niwata, K. Umezawa, K. Toshima, M. Nakata, *Carbohydr. Res.* **1991**, *222*, 189-203; b) See for a review on cyclophellitol synthesis: b) J. Marco-Contelles, *Eur. J. Org. Chem.* **2001**, 1607-1618.
- [15] a) D. Semeria, M. Philippe, J.-M. Delaumeny, A.-M. Sepulchre, S. D. Gero, *Synthesis* **1983**, 710-713; b) A. Lipták, I. Jodal, P. Násáni, *Carbohydr. Res.* **1975**, *44*, 1-11.
- [16] a) P. Pöchlauer, E. P. Müller, *Helv. Chim. Acta* **1984**, *67*, 1238-1247; b) B. Ritzen, M. C. M. van Oers, F. L. van Delft, F. P. J. T. Rutjes, *J. Org. Chem.* **2009**, *74*, 7548-7551.
- [17] a) See for a review of the Tatsuta laboratory on the synthesis of cyclophellitols: K. Tatsuta, *Pure Appl. Chem.* **1996**, *68*, 1341-1446; b) see also references 2 and 13.
- [18] a) R. R. Schrock, J. S. Murdzek, G. C. Bazan, J. Robbins, M. DiMare, M. O'Regan, *J. Am. Chem. Soc.* **1990**, *112*, 3875-3886; b) G. C. Fu, R. H. Grubbs, *J. Am. Chem. Soc.* **1992**, *114*, 7324-7325.
- [19] a) S. Nguyen, R. H. Grubbs, *J. Am. Chem. Soc.* **1993**, *115*, 3800-3801; b) P. Schwab, R. H. Grubbs, J. W. Ziller, *J. Am. Chem. Soc.* **1996**, *118*, 100-110.
- [20] See for example a) M. F. Schneider, H. Junga, S. Blechert, *Tetrahedron* **1995**, *51*, 13003-13014; b) H. S. Overkleeft, U. K. Pandit, *Tetrahedron Lett.* **1996**, *37*, 547-550; c) see for a review: R. H. Grubbs, S. Chang, *Tetrahedron* **1998**, *54*, 4413-4450.
- [21] See for example a) H. Ovaa, J. D. C. Codée, B. Lastdrager, H. S. Overkleeft, G. A. van der Marel, J. H. van Boom, *Tetrahedron Lett.* **1999**, *40*, 5063-5066; b) P. Kapferer, F. Sarabia, A. Vasella, *Helv. Chim. Acta* **1999**, *82*, 645-656.
- [22] M. Jorgensen, P. Hadwiger, R. Madsen, A. E. Stütz, T. M. Wrodnigg, *Curr. Org. Chem.* **2000**, *4*, 565-588.
- [23] F. G. Hansen, E. Bundgaard, R. Madsen, *J. Org. Chem.* **2005**, *70*, 10139-10142.
- [24] K.-Y. Li, J. Jiang, M. D. Witte, W. W. Kallemeijn, H. van den Elst, C.-S. Wong, S. D. Chander, S. Hoogendoorn, T. J. M. Beenakker, J. D. C. Codée, J. M. F. G. Aerts, G. A. van der Marel, H. S. Overkleeft, *Eur. J. Org. Chem.* **2014**, 6030-6043.
- [25] P. J. Garegg, B. Samuelsson, *J. Chem. Soc., Perkin Trans.* **1980**, *1*, 2866-2869.
- [26] M. Scholl, S. Ding, C. W. Lee, R. H. Grubbs, *Org. Lett.* **1999**, *1*, 953-956.
- [27] J. Jiang, C.-L. Kuo, L. Wu, C. Franke, W. W. Kallemeijn, B. I. Florea, E. van Meel, G. A. van der Marel, J. D. C. Codée, R. G. Boot, G. J. Davies, H. S. Overkleeft, J. M. F. G. Aerts, *ACS Central Sci.* **2016**, doi:10.1021/acscentsci.6b00057.
- [28] Y. Harrak, C. M. Barra, A. Delgado, A. R. Castano, A. Llebaria, *J. Am. Chem. Soc.* **2011**, *133*, 12079-12084.
- [29] a) L. I. Willems, T. J. M. Beenakker, B. Murray, B. Gadestein, H. van den Elst, E. R. van Rijssel, J. D. C. Codée, W. W. Kallemeijn, J. M. F. G. Aerts, G. A. van der Marel, H. S. Overkleeft, *Eur. J. Org. Chem.* **2014**, 6044-6056; b) L. I. Willems, T. J. M. Beenakker, B. Murray, S. Scheij, W. W. Kallemeijn, R. G. Boot, M. Verhoek, W. E. Donker-Koopman, M. J. Ferraz, E. R. van Rijssel, B. I. Florea, J. D. C. Codée, G. A. van der Marel, J. M. F. G. Aerts, H. S. Overkleeft, *J. Am. Chem. Soc.* **2014**, *136*, 11622-11625.
- [30] a) A. R. Mattocks, *J. Chem. Soc.* **1964**, 4840-4845; b) A. F. Russell, S. Greenberg, J. G. Moffatt, *J. Am. Chem. Soc.* **1973**, *95*, 4025-4030.
- [31] J. Jiang, W. W. Kallemeijn, D. W. Wright, A. M. C. H. van den Nieuwendijk, V. C. Rohde, E. C. Folch, H. van den Elst, B. I. Florea, S. Scheij, W. E. Donker-Koopman, M. Verhoek, N. Li, M. Schurmann, D. Mink, R. G. Boot, J. D. C. Codée, G. A. van der Marel, G. J. Davies, J. M. F. G. Aerts, H. S. Overkleeft, *Chem. Sci.* **2015**, *6*, 2782-2789.
- [32] O. L. Lopez, J. G. Fernandez-Bolanos, V. H. Lillelund and M. Bols, *Org. Biomol. Chem.* **2003**, *1*, 478-482.

- [33] a) C. Jaramillo, J.-L. Chiara, M. Martín-Lomas, *J. Org. Chem.* **1994**, *59*, 3135-3141; b) P. Serrano, A. Llebaria, A. Delgado, *J. Org. Chem.* **2002**, *67*, 7165-7167.
- [34] Though the final Birch reduction is not described in the referenced paper, this procedure does yield aziridine **46** as we have found (not published) in the stated yield. Alternatively, compound **45** features also as an intermediate in the first synthesis of **46** (see ref 35), where it is stated that **46** can be produced from **45** by catalytic hydrogenation.
- [35] G. Caron, S. G. Withers, *Biochem. Biophys. Res. Commun.* **1989**, *163*, 495-499.
- [36] P. Serrano, A. Llebaria, A. Delgado, *J. Org. Chem.* **2005**, *70*, 7829-7840.
- [37] B. T. Adams, S. Niccoli, M. A. Chowdhury, A. N. K. Esarik, S. J. Lees, B. P. Rempel, C. P. Phenix, *Chem. Commun.* **2015**, *51*, 11390-11393.
- [38] W. W. Kallemeijn, K. Y. Li, M. D. Witte, A. R. Marques, J. Aten, S. Scheij, J. Jiang, L. I. Willems, T. M. Voorn-Brouwer, C. P. van Roomen, R. Ottenhoff, R. G. Boot, H. van den Elst, M. T. Walvoort, B. I. Florea, J. D. Codee, G. A. van der Marel, J. M. Aerts, H. S. Overkleeft, *Angew. Chem. Int. Ed.* **2012**, *51*, 12529-12533.
- [39] a) S. G. Withers, I. P. Street, P. Bird, D. H. Dolphin, *J. Am. Chem. Soc.* **1987**, *109*, 7530-7531; b) S. G. Withers, K. Rupitz, I. P. Street, *J. Biol. Chem.* **1988**, *263*, 7929-7932; c) see for a review on mechanism-based glycosidase inhibitors, including fluoroglycosides: B. P. Rempel, S. G. Withers, *Glycobiology* **2008**, *18*, 570-586.
- [40] a) M. T. C. Walvoort, W. W. Kallemeijn, L. I. Willems, M. D. Witte, J. M. F. G. Aerts, G. A. van der Marel, J. D. C. Codée, H. S. Overkleeft, *Chem. Commun.* **2012**, *48*, 10386-10388; See for studies on the use of fluoroglycosides in activity-based glycosidase profiling studies: b) D. J. Vocadlo, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2004**, *43* 5338-5342; c) O. Hekmat, Y.-W. Kim, S. J. Williams, S. He, S. G. Withers, *J. Biol. Chem.* **2005**, *280*, 35126-35135; d) K. A. Stubbs, A. Scaffidi, A. W. Debowski, B. L. Mark, R. V. Stick, D. J. Vocadlo, *J. Am. Chem. Soc.* **2008**, *130*, 327-335
- [41] See for an example of a 6-phospho-cyclophellitol-sensitive enzyme that would be amenable for 6-phospho-cyclophellitol-aziridine based ABPP experiments: D. H. Kwan, Y. Jin, J. Jiang, H. M. Chen, M. P. Kötzler, H. S. Overkleeft, G. Davies, S. G. Withers, *FEBS Lett.* **2016**, *590*, 461-468.
- [42] See for an example of a glucosyl 1,6-*epi*-cyclophellitol-sensitive endoglucosidase that would be amenable for glucosyl 1,6-*epi*-cyclophellitol aziridine based ABPP experiments: S. Caner, X. Zhang, J. Jiang, H.-M. Chen, N. T. Nguyen, H. Overkleeft, G. D. Brayer, S. G. Withers, *FEBS Lett.* **2016**, *590*, 1143-1151.
- [43] See for recent reviews on aziridine synthesis: a) H. Pellissier, *Tetrahedron* **2010**, *66*, 1509-1555; b) L. Degennaro, P. Trinchera, R. Luisi, *Chem. Rev.* **2014**, *114*, 7881-7929.
- [44] A. Alcaide, A. Trapero, Y. Pérez, A. Llebaria, *Org. Biomol. Chem.* **2015**, *13*, 5690-5697.
- [45] a) J. L. Jat, M. P. Paudyal, H. Gao, Q.-L. Xu, M. Youssufuddin, D. Devarajan, D. H. Ess, L. Kürti, J. R. Falck, *Science* **2014**, *343*, 61-65; b) S. O. Scholz, E. P. Farney, S. Kim, D. M. Bates, T. P. Yoon, *Angew. Chem. Int. Ed.* **2016**, *55*, 2239-2242.

