Back to (non-)Basics: An Update on Neutral & Charge-Balanced Glycosidase Inhibitors

Michela I. Simone^{1,*}, Laura J. Mares¹, Clothilde A. Eveleens¹, Adam McCluskey¹, Brighid B. Pappin², Milton J. Kiefel² and Todd A. Houston^{2,*}

¹Discipline of Chemistry, School of Environmental and Life Sciences, University of Newcastle, University Drive, Callaghan, NSW 2308, Australia

²Institute for Glycomics and School of Natural Sciences, Griffith University, Gold Coast Campus, Southport, QLD 4222, Australia

Abstract: Glycosidases have important anti-cancer, anti-viral and anti-diabetic properties. This review covers the literature in the past 15 years since our initial review in this journal on “neutral” glycosidase inhibitors lacking a basic nitrogen found in iminosugars and azasugars or inhibitors that are neutral by virtue of being “charge-balanced” (zwitterionic). These structurally diverse inhibitors include lactones, lactams, epoxides such as cyclophellitol, and sulfonium ion derivatives of the natural product salacinol. Synthetic efforts toward cyclophillitol, salicinol and derivatives are also highlighted. Importantly, certain metals can inhibit glycosidases and care must be taken to remove residual catalysts from synthetic material to be tested against these enzymes.

1. Introduction

Glycosidases (EC3.2.1-) are a large family of hydrolase enzymes crucial to metabolism and cellular function across the full breadth of living organisms. Glycosidase inhibitors represent an important family of medicinal agents encompassing numerous classes of alkaloids, the largest one of which is the iminosugars. These are considered carbohydrate analogues where the endocyclic oxygen is replaced with an amine and are further sub-classified into five main heterocyclic ring systems: piperidine, pyrrolidine, indolizidine, pyrrolizidine, and nortropane. Nojirimycin (NJ), a piperidine-based iminosugar, was the first natural sugar mimic to be discovered and its 1-deoxy congener (1-DNJ) is recognised a privileged structure and contained in several marketed clinical agents (Figure 1). While 1-DNJ is not particularly basic ($pK_{aH} = 6.7$),¹ it can deprotonate a catalytic carboxylic acid within the glycosidase active site to form a strong complementary electrostatic interaction with the anionic environs that are present in the glycosidase hydrolytic site. Glycosidases have evolved specialised active sites designed to lower the energy barrier for glycosidic bond hydrolysis and consequently increase this reaction rate by a factor of more than 10^{17} through transition state stabilisation and substrate distortion.²⁻¹⁵

Figure 1. Small molecule glycomimetics marketed as clinical agents for management of diabetes (Miglitol, Voglibose and Acarbose), Gaucher disease (Miglustat), and influenza (Oseltamivir, Zanamivir).

Glycosidases (EC3.2.1-) can cleave oligosaccharides at internal glycoside linkages (carried out by endo-glycosidases such as amylase and lysozyme) or more commonly at the non-reducing terminal sugar residue (carried out by exo-glycosidases). Generally, these enzymes are glycoside hydrolases that operate by one of two mechanisms either inverting or retaining the anomeric stereochemistry on replacement of the glycosidic bond with water to produce a reducing sugar (Scheme 1). Retaining enzymes place an acidic group in close proximity to the exo-anomeric oxygen and a nucleophile poised to attack the anomeric carbon to produce a glycosyl-enzyme intermediate. Attack of water then occurs through a virtually identical transition state thus receiving similar stabilization from the active site residues. The vast majority of these active site pairs are carboxylic acids (aspartate/glutamate) spaced roughly 5.5 Å apart. Inverting enzymes stretch the distance between active site carboxylates to roughly 10 Å such that one carboxylate can deprotonate a water molecule to displace the glycosidic bond in a single step as shown.²

Scheme 1. General mechanisms of retaining and inverting glycosidases.

Significant insight to inhibitor design has been provided in the past 15 years from X-ray crystallography of a variety of inhibitors bound to glycosidases. Protonation states of both glycosidases and putative transition-state mimics provide crucial data to inform drug design strategies.¹⁶ An illustrative example was reported by Davies that permitted direct observation of a protonated isofagomine derivative in co-crystallised the active site of an endocellulase (EC 3.2.1.4).¹⁷ This same compound occupies the active site of a cellobiohydrolase (EC 3.2.1.91) in an unusual boat conformation demonstrating the diverse conformational profile of both glycomimetics and natural sugar substrates.¹⁸ Thus, the conformational flexibility of

iminosugars and other glycomimetics demands a more complete understanding of enzyme binding if optimal inhibitors are to be developed.¹⁹ Use of thermodynamic and kinetic analysis in concert with structural studies provides a greater understanding of substrate transition-state mimicry and can allow identification and development of novel classes of inhibitors.²⁰ Natural abundance kinetic isotope effect studies have been used to compare similarities and differences between acid- and enzyme-catalyzed glucoside hydrolysis of both anomeric configurations.²¹ While this evidence suggests the α -glucosidase reaction involves a $D_N^*A_N$ (S_N1 -type) mechanism, the β -glucosidase mechanism appears to undergo a concerted A_ND_N (S_N2) pathway.

While iminosugars remain currently the most abundant class of glycosidase inhibitors in development,²²⁻³⁷ research into other molecular scaffolds has provided new classes of lead compounds. Neutral (non-ionized at physiologic pH) and charge-balanced (zwitterionic at physiologic pH) glycosidase inhibitors were the subject of our initial review in 2003.³⁸ Examples include the neutral D-glucono- δ -lactone (gluconolactone) **1** and (+)-cyclophellitol **2** and the zwitterionic salacinol **3** and recent FDA approved neuraminidase inhibitor peramivir **4** (Figure 2).

Figure 2. Chemical structures of “neutral” (**1** and **2**) and “charge-balanced” (**3** and **4**) glycosidase inhibitors.

An increased understanding of the intricacies of glycosidase-inhibitor interaction over the past decade has provided opportunities for further improving drug design. Antidiabetic drugs such as miglitol and acarbose and the influenza drugs zanamivir (Relenza) and oseltamivir (Tamiflu) demonstrate the biological impact of glycosidase inhibition (Figure 1).³⁹ Lactams derived from glucuronic acid possess potent anticancer activity presumably by inhibition of glycosidases such as heparanase.^{40, 41} Inhibitors such as cyclophellitol **2** have served as foundation for the development of acylaziridines as potent activity-based probes of human galactosidases.⁴² As such, we felt it was appropriate to provide updated review of work on glycosidase inhibitors beyond iminosugars and their congeners, which had already provided several agents to reach the market at the time of our previous review (Figure 2).³⁷

This review examines new lactone, lactam, epoxide, polycyclic aromatic hydrocarbon and sulfonium ion inhibitors of principally *exo*-glycosidases: glucosidase, mannosidase, galactosidase and fucosidase enzymes. At the time of our previous review, many of the most potent glycosidase inhibitors had K_i values in the low micromolar to high nanomolar range. However, in the past decade several nanomolar inhibitors have been identified and one exceptionally potent inhibitor now operates in the picomolar range.⁴³ In terms of a quantitative classification of inhibitory potencies towards glycosidases, we use as a guide the following bands: a weak inhibitor possesses IC_{50} value above 250 μ M, moderate inhibition for 100-249 μ M, good for 10-99 μ M, potent for 0.1-9 μ M and very potent for inhibition under 0.1 μ M. As sugar lactones (e.g. **1**) were the first glycosidase inhibitors identified in the 1940's, these are a fitting starting point for this review.³⁸

2. Lactones, Lactams, and Derivatives

Gluconolactone **1** continues to find application today as it was shown to block the glucanase activity of Gram negative bacteria binding proteins in termites as a means of pest control.⁴⁴ One pharmacological disadvantage of lactones is their susceptibility to hydrolysis, which makes them unlikely to provide suitable leads for potential human therapies. However, lactams are a more hydrolytically robust and useful family of glycosidase inhibitors due to their close relationship to the iminosugar class of natural products. The reduced basicity of the nitrogen means these derivatives will not protonate as readily as iminosugars within the glycosidase active site. Direct comparison of lactams and the iminosugars from which they are derived has provided useful insight into glycosidase binding mechanisms and the extent of transition state mimicry as outlined below.

In 2004, Davies and co-workers reported an X-ray crystallography study of lactam **9** bound in the active site of both β -glucosidases and β -mannosidases which aided the understanding of differences in substrate binding by these related *exo*-glycosidases (Figure 3). The β -glucosidase *TmGH1* and β -mannosidase *CmMan5* distort their substrates into either a half-chair or boat-like conformation, respectively.⁴⁵ Lactam **5** is able to adopt conformations that conform to either transition state structure depending on the predisposition of the active site. The lactam derivatives of iminosugars can serve as more effective transition state analogues than their parent structures has been demonstrated by Withers for the xylanase *Cex*.⁴⁵ While inhibitor **5** and its analogue **6** have similar K_i values (0.33 vs 0.13 μ M), they would be expected to display significantly different pharmacologic profiles due to lack of a basic

secondary amine in **5**. A related study into analogous imidazole-containing inhibitors of glucosidases and mannosidases identified *manno*-imidazoles **7** and **8** as potent inhibitors of α -mannosidase (Jack bean, $K_i = 0.75$ and $1.22 \mu\text{M}$) and excellent selectivity in inhibition of β -mannosidase (snail, $K_i = 0.12$ and $138 \mu\text{M}$). This latter effect was accounted for by the extensive protonation of both **7** and **8** at the low pH of the assay for this enzyme (pH = 4.0-4.5), resulting in the formation of, respectively, the imidazolium salt of **7** and an equilibrium mixture of the corresponding ammonium/imidazolinium salts of **8**. The formation of the ammonium salt is expected to impair the interaction between the inhibitor and the catalytic acid.⁴⁶ Two mannosidase enzymes are of relevance to mammalian glycoprotein processing.^{44, 47-49} The natural product lactam kifunensine **9** inhibits α -mannosidase I and has been used to allow crystallization of a cytotoxic T-lymphocyte antigen by restricting processing of its glycan structures.⁵⁰ Pearson reported a practical synthesis of kifunensine **9** ($\text{IC}_{50} = < 0.03$ [mannosidase I (endoplasmatic reticulum, ER)] and $< 0.03 \text{ mM}$ [mannosidase IA (mouse, Golgi)]) and derivatives, where N-1 substitution correlated with an improved selectivity profile of mannosidase I vs mannosidase IA for this class of analogues, albeit at the cost of loss of potency. For example, **10** inhibits the enzymes with $\text{IC}_{50}\text{s} = 0.08$ and 0.17 mM respectively, **11** exhibiting the greatest selectivity with $\text{IC}_{50}\text{s} = 0.03$ and 0.15 mM , and **12** with $\text{IC}_{50}\text{s} = 0.2$ and 2.1 mM .⁵¹ Introduction of a lactam moiety and a geminal difluoromethylene group on trihydroxy-2-thiaquinolizidines affords difluorotetrahydropyridothiazinone (not shown) with improved selectivity in glycosidase inhibition and an IC_{50} of $2.01 \mu\text{M}$ against β -galactosidase (*E. coli*).⁵² As a caveat, it should be noted that some lactam derivatives of natural iminosugars have dramatically diminished glycosidase inhibition profiles and such substitution is not always warranted.⁵³

Figure 3. Lactam **5** and amine analogue **6**,⁴⁵ *manno*-imidazoles **7** and **8**,⁴⁶ the natural product kifunensine **9** and their analogues (**10-12**) as glycosidase inhibitors.^{51, 54}

Carbamate derivatives **15-18** (Figure 4) of the natural bicyclic alkaloid australine **14** and its monocyclic version (2*R*,3*R*,4*R*,5*R*)-2,5-hydroxy-methyl-3,4-dihydroxypyrrolidine (DMDP) **13** display selective glycosidase inhibition profiles (Table I) over that of DMDP, whilst still retaining similar potency. For example, **15** is selective for β -glucosidase (almonds) and isomaltase (yeast), and the more conformationally restricted australine analogues displayed increased selectivity with **16** for trehalase (pig kidney), **17** and **18** for isomaltase (yeast). The enzyme selectivity in this latter case seems to depend strongly on the degree of sp^2 -hybridisation of the bridgehead-N, with greater basicity of that N being correlated to decreased isomaltase inhibition (see **16** vs **17** and **18**).⁵⁵ Pandey has described an unusual β -lactam iminosugar derivative **19** with selective β -galactosidase inhibition ($K_i = 172 \mu M$) in a panel of glycosidases (β -galactosidase (*Aspergillus oryzae*), α -galactosidase (coffee beans), β -glucosidase/ β -mannosidase (almonds), α -glucosidase (yeast) and α -mannosidase (jack beans)).⁵⁶

Figure 4. A range of natural products (**13**, **14**) and their analogues (**15-18**) as glycosidase inhibitors⁵⁵ and constrained β -lactam iminosugar analogue **19**.⁵⁶

Table I. K_i Values (μM) of derivatives **15-18** and for the parent alkaloids DMDP **13** and australine **14** against various glycosidases.⁵⁵

Compound	α -Glucosidase (yeast)	Isomaltase (yeast)	β -Glucosidase (almond)	β -Glucosidase/ β -Galactosidase (bovine liver)	Trehalase (pig kidney)	Invertase (yeast)	Amyloglucosidase (<i>A. niger</i>)
13	0.73	2.5	1.7	3.3	355	2.9	2.1
14	NI ^a	ND ^b	NI	ND	ND	28	1.5
15	72	28	9.2	39	NI	> 1000	NI
16	NI	251	NI	NI	20	NI	249
17	132	29	340	644	NI	NI	NI
18	408	72	433	347	NI	NI	NI

^a NI = no inhibition detected; ^b ND = not determined.

3. Hydantocidin analogues

Many C-1,⁵⁷⁻⁶¹ C-2,^{62, 63} C-3,⁶⁴⁻⁷² and C-473, 74 hydantoin derivatives (highlighted in red in **20**, Figure 5) have been synthesised and biologically evaluated in the past 10-15 years.

Representative examples of this class of glycosidase inhibitor are highlighted in Figure 5. The D-glucopyranose analogue **21** of hydantocidin **20** is a powerful competitive inhibitor of glycogen phosphorylase (GP) with respect to its natural substrate glucose-1-phosphate with a K_i value of 3.1 μM , whereas the α -epimer inhibits GP with a K_i value of 28.6 μM (Figure 5).⁷⁵ Pal and Vankar synthesised the sugar-derived spiro-aminals **22**, **23** and **24**, **25** which were tested against five glycosidases [α -glucosidase (yeast), β -glucosidase (almonds), α -galactosidase (coffee beans), β -galactosidase (bovine) and α -mannosidase (Jack bean)].⁷⁶ The most potent spiro-aminals were **22** and **24** which showed good inhibition against galactosidases, **24** against α -galactosidase (coffee beans) with an IC_{50} of 77 μM (non-selective), and **22** against β -galactosidase (selective, IC_{50} of 207 μM). Of the epimeric mannose-derived spiro-lactams **23** and **25**, **23** showed no activity against the tested enzymes, and **25** displayed only low levels of activity against β -galactosidase at 400 μM .

Commencing from a D-arabino-configured α -azido ester template, Merino-Montiel et al.,⁷⁷ synthesised the first examples of C-3 spiro-annulated thiohydantoin and selenohydantoin (**26-31**; Figure 6). Thiohydantoin **26** and **27** exhibited weak to moderate, albeit selective, inhibition of GP (type b, rabbit muscle) (**26**; K_i 910 μM) and α -galactosidase (green coffee beans) (**27**; K_i 160 μM).

Figure 5. Natural product hydantocidin **20**, hydantoin functional group highlighted in red. Hydantoin-based (**21**)⁷⁵ and hydantocidin analogues (**22-27**)⁷⁶⁻⁷⁸ evaluated as glycosidase inhibitors.

4. Salacinol and Derivatives

Polyhydroxylated thiolane natural products, e.g. salacinol **3**, are lead candidates for the treatment of Type 2 diabetes via α -glucosidase inhibition (Figure 6).^{59, 60, 64, 73, 77-98} Herein we highlight some more recent developments in the field. Salacinol **3** is a potent competitive α -glucosidase inhibitor with K_i values of 0.93 (rat intestinal maltase), 0.96 (rat intestinal sucrase), and 1.4 (rat intestinal isomaltase) μ M, respectively.^{85, 99-101} The structurally related sulfonium sulfates, kotalanol **37**,¹⁰² ponkoranol **39**¹⁰³ and salaprinol **44**¹⁰⁴ were subsequently isolated. In 2008, neosalacinol **32** and neokotalanol **38** lacking the 3'-sulfate moiety were isolated from *Salacia oblonga* and *Salacia reticulata* respectively. Neokotalanol **38** is the most potent glycosidase inhibitor among the naturally occurring sulfonium salts yet reported (IC_{50} : maltase, 0.23 μ M; sucrase 0.19 μ M).^{94, 105} The permanent positive charge on the sulfate moiety mimics the oxacarbenium ion-like transition state of glycoside hydrolysis, allowing inhibition of a range of glycosidases.¹⁰⁶⁻¹⁰⁹ A number of structure-activity relationship studies, some of which are highlighted below, have shown that the 1,4-anhydro-4-thio-D-arabinitol (D-ATA) **48** core and the side-chain stereochemistries are important for inhibition (Scheme 6).^{101, 110}

Figure 6. A range of polyhydroxylated-thiolanes as glycosidase inhibitors.^{85, 93, 94, 99-105, 111-114}

The isolation of neoponkoranol **40** and neosalaprinol **45** from dried stems of *Salacia chinensis*, and the syntheses of their epimers (5'R-*epi*- **41** and 3'R-*epi*-ponkoranol **42**, and 2'R-*epi*-salaprinol **46**) was reported by Xie et al.¹¹¹ The inhibition of α -glucosidases (rat small intestinal) by this *neo*-series of compounds against was investigated *in vitro* and the outcomes highlighted in Table II.

Table II. IC_{50} Values (μ M) of thiosugar sulfonium salts and related compounds against α -glucosidases.¹¹¹

Compound	α -Glucosidase (rat intestinal)		
	maltase	Sucrase	isomaltase
40	5.1	1.0	1.4
41	4.3	2.9	1.0
42	1.3	0.3	1.0
45	>384	90	6.5
46	>329	>329	>329
47	105	>384	53
Voglibose	1.2 ^b	0.2 ^b	2.1 ^b
Acarbose	1.7 ^a	1.5 ^a	646 ^a

^aFrom ref. 115. ^bFrom ref. 113.

Neoponkoranol **40** was potent against the three enzymes tested, while 2'*R*-*epi*-neosalaprinol **47** inhibited both maltase and isomaltase more effectively than its sulfated analogue 2'*R*-*epi*-salaprinol **46** (Table II). In terms of structure-activity relationships, the de-*O*-sulfonation analogues showed improved inhibition of isomaltase. 2'-Epimerization causes a marked decrease in its inhibitory activity against isomaltase, however the C-3' and/or C-5' configuration showed no significant influence on the inhibitory activity. Thus, in the ponkoranol series, all four compounds were potent inhibitors regardless of the presence of the C-3'-sulfate moiety and also of the C-3' and/or C-5' stereochemistry with 3'*R*-*epi*-ponkoranol **42** being the most potent, and showing almost the same inhibitory capability as voglibose against all enzymes.

The de-*O*-sulfonated analogue neokotalanol **38**,¹¹² which also belongs to the salacinol family, was isolated from *Salacia reticulata* Wight.¹¹⁶ Neokotalanol **38** is a potent α -glucosidase inhibitor of rat intestinal maltase and sucrase (0.23 μ M, 0.19 μ M) and 0.01 μ M isomaltase inhibitor. The combined inhibitory activities of **38**, salacinol **3** (IC₅₀: maltase, 9.6 μ M; sucrase, 2.5 μ M; isomaltase, 1.8 μ M) and kotalanol **37** (IC₅₀: maltase, 6.6 μ M; sucrase, 1.4 μ M; isomaltase, 4.5 μ M), from the same plant genus contribute to reported activity of this plants extracts.^{95, 116} Neokotalanol **38** was also found to lower post-prandial glucose levels *in vivo* via maltose- and sucrose-loading tests on Wistar rats.¹²⁷

Minami et al. investigated the inhibitory activities of five-membered sugar mimics toward glycogen-degrading enzymes and various glucosidases and found evidence that the side-chain sulfate plays an important role in the specificity of enzyme inhibition.⁹³ Salacinol **3**, neosalacinol **32** and core structure D-ATA **48** (Scheme 6) were not found to inhibit glycogen phosphorylase but the former two inhibit amylo-1,6-glucosidase (IC₅₀ 48 and 16 μ M

respectively) (Table III). Neosalacinol **32** is also a potent inhibitor of α -glucosidase (rat intestinal isomaltase, IC₅₀ 0.64 μ M). On the other hand, salacinol **3** showed a much more potent inhibitory activity toward α -glucosidase (rat intestinal maltase, IC₅₀ 0.5 μ M) in Caco-2 cell model system and α -glucosidase (human lysosomal, IC₅₀ 0.34 μ M).

Table III. IC₅₀ Values (μ M) of thiosugar sulfonium salts **3** and **32**, and D-ATA **48** against α -glucosidases.⁹³

Compound	α -Glucosidase				
	rice	rat maltase	rat isomaltase	rat sucrase	human lysosome
3	2.6	2.4	5.7	2.2	0.34
32	8.6	10	0.64	5.2	4.4
48	220	290	1000	NI	NI

The synthesis and biological evaluation of 3'-*O*-alkylated derivatives of salacinol (e.g. **33-36**) and ponkoranol (e.g. **39**) provide SAR data for chemical modifications at the 3'-*O* position. Tanabe et al. produced analogues **33-36**), where replacement of the sulfonate group with an alkyl/aromatic group increases α -glucosidases inhibition (Table IV).¹¹³ The benzylated derivative **36** is more potent than the currently marketed antidiabetics, voglibose and acarbose. This suggests that the introduction of a C-3' hydrophobic moiety may be a viable potency enhancement strategy for this class of inhibitors.

Table IV. IC₅₀ Values (μ M) of 3'-*O*-alkylated salacinol derivatives **33-36** and positive controls voglibose and acarbose against α -glucosidases.¹¹³

Compound	α -Glucosidase (rat intestinal)		
	maltase	isomaltase	sucrase
33	5.3	0.39	0.46
34	1.7	0.27	0.12
35	1.0	0.95	1.3
36	0.44	0.14	0.32
Voglibose	1.2	2.1	0.2
Acarbose	1.7 ^a	646 ^a	1.5 ^a

^a From ref. 115.

OH stereochemistry seems to play an important role in determining selectivity in α -glucosidase inhibition, with **54** and **56** showing enhanced selectivity for this enzyme. *In silico* docking studies of **54** and **56** and of parent compound kotalanol **37** with N-terminal subunit of human maltase-glucoamylase provided evidence for the fundamental requirement for the thiosugar moiety, with additional important determinants identified. Modelling supports that (a) the 2'-S-OH is essential for the activity; (b) a polyhydroxylated side chain longer than four carbons does not significantly enhance the inhibitory activity; (c) the cooperative role of 2'-S-OH and 4'-OH appears critical for optimal inhibition; and the R configuration of OH at C-4' is imperative to inhibitors bearing a side chain of more than four carbons.

Liu and coworkers also coupled of D-ATA **48** with cyclic sulfates to access six carbon chain-extended analogues of salacinol **3**,¹¹⁸ with ring heteroatom variation to also include selenium.^{52, 119} Four compounds were found to inhibit α -glucosidase (recombinant human maltase glucoamylase), one of the key intestinal enzymes involved in the breakdown of glucose oligosaccharides in the small intestine, with low μM K_i values. The salacinol selenosugar analogue, **57**, returned a K_i value of 41 μM , while the corresponding sulfur analogue **58** is not active. Of the non-natural L-arabinitol configuration compounds **59** and **60**, only the sulfur congener **60** is active, with a K_i value of 25 μM (Figure 7).

Figure 7. Polyhydroxylated selenides and thiolanes as glycosidase inhibitors.¹¹⁸

Muroaka et al. synthesised salacinol analogues lacking the polar substituents in the side chain from reaction of D-ATA **48** with cyclic sulfates.¹²⁰ Biological evaluation against α -glucosidase (rat intestinal, maltase and sucrase) showed a marked decrease in inhibitory activity compared to salacinol. These results convey the importance of the cooperative role of the polar substituents in the side chain for the α -glucosidase inhibitory activity. Other systems have been synthesized replacing a monosaccharide's ring oxygen with a sulfonium ion in the search for novel fucosidase inhibitors.¹²¹

Wolf and Weisbrode investigated the toxicological properties and safety of plant extracts from the *Salacia oblonga* supplemented to or processed into a medical food on male Sprague-Dawley rats.¹²² These were assigned among one of three treatments: (1) EN-0178 (control, liquid diet), (2) EN-0178+salacinol (as 1 plus 500 mg of salacinol extract per 253 g diet, which was added to product immediately prior to feeding), (3) EN-0195 (as 1 plus 500 mg of salacinol extract per 253 g diet, which was added during product manufacture). The tested salacinol extract consumed for two weeks in amounts estimated at 10-fold greater than proposed for human intake was found to be safe and did not result in clinical chemistry or histopathologic indications of toxic effects in male Sprague-Dawley rats. The rats had diarrhea (over the first four days) and reduced ($P < 0.05$) weight gain and feed intake as salacinol extracts prevent complete digestion of dietary carbohydrate.¹²³ Reduced feed efficiency ratio was also noted, and effects on the relative weight (% of body weight) of the liver, spleen (lower ($P < 0.05$)) and testes (higher ($P < 0.05$)). Of the serum chemistries analyzed, blood urea nitrogen and alkaline phosphatase were lower ($P < 0.05$) for rats consuming salacinol extract. No differences in blood hematology were found.

5. Electrophilic Species

Affinity-based probes and covalent inhibitors of glycosidases have been the subject of separate reviews recently.^{124, 125} Herein we focus on epoxides, namely (+)-cyclophellitol **2** and its derivatives shown to act as irreversible inhibitors of β -glucosidases, and transition metal complexes which possess α -glucosidase and α -galactosidase inhibitory activity. Irreversible enzyme inhibitors such as epoxides have different pharmacological profiles to reversible enzyme inhibitors and in some cases are more effective at lower doses. Additionally, certain metals alone can inhibit glycosidases (*vide infra*) and may interfere with enzyme assays.

5.1 Epoxides: Cyclophellitol and Related Cyclitols

(+)-Cyclophellitol **2**, a cyclitol analogue bearing an epoxide moiety on the β -face of the molecule, was isolated by Atsumi and coworkers from the mushroom *Phellinus sp.* (Figure 8).^{126, 127} Biological evaluation against a panel of nine glycosidases revealed it to be a specific, competitive and irreversible inhibitor of β -glucosidase (almond, 50% inhibition at a 4.5 μM concentration; with a K_i of 2.8 μM). Furthermore, cyclophellitol inhibited β -glucocerebrosidase (Molt-4 cells) completely at 11 μM with an inhibitory activity 100 times more potent than that of nojirimycin **61**, 1-DNJ **62**, or castanospermine **63**. Mice treated with 1

mg/mouse of cyclophellitol daily for five days exhibited severe nervous system abnormalities similar to those found in the Gaucher's mouse. X-ray crystallography, enzyme kinetics and mass spectrometry study revealed the structural basis for β -glucosidase inhibition by cyclophellitol as irreversible *via* nucleophilic opening of the epoxide moiety by the active site's Glu351 of β -glucosidase (*Thermotoga maritima*) and formation of a covalent bond.¹²⁸

Figure 8. Structures of natural products that modulate glycosidase enzyme activity.

Synthetically, only two preparations of (+)-cyclophellitol from achiral precursors have been reported,^{129, 130} while all other reports rely on carbohydrates as starting materials.¹³¹⁻¹³⁷ A number of groups have used organometallic reactions to develop more efficient synthetic routes from carbohydrates. Notable is the work by Hansen and colleagues,¹³⁸ who reported the shortest synthesis of cyclophellitol to date from D-xylose **66** in nine steps (14% overall yield, Scheme 2). Key to Hansen's concise synthesis is three consecutive organometallic reactions: a zinc-mediated fragmentation of **67** affording **68**, which upon indium-mediated coupling with the bromoester **69**, gives **70** as a single diastereoisomer. In the third step, diene **70** was converted into the corresponding diene **71**, precursor to the final product, by ring-closing metathesis via Grubbs II catalysis. Ishikawa et al.¹³⁷ also used the zinc-mediated fragmentation strategy, but on a D-glucose derived substrate. Under Henry reaction conditions, the aldehyde adduct thus obtained afforded the corresponding epimeric nitro-derivatives. These nitro-derivatives are converted into the corresponding cyclic nitronate intermediates and opened *via* reductive N-O bond cleavage using of $\text{Mo}(\text{CO})_6$.¹³⁹

Scheme 3. Key steps in the synthetic strategy to cyclophellitol **2** devised by Hansen¹³⁸ and, in Kireev's strategy, latent plane of symmetry highlighted on starting material **66**.¹⁴⁰

In a synthesis also starting from D-xylose **66**, Kireev's strategy¹⁴⁰ takes advantage of the latent symmetry principle (latent plane of chirality)¹⁴¹⁻¹⁴³ of the monosaccharide (Scheme 3) to achieve the enantiodivergent synthesis of both (+)- and (-)-cyclophellitol in 12 and 15 steps with 18% and 13% overall yield, respectively. Serrano and coworkers investigated the hydroboration-oxidation sequence from precursor **72** and report the hydroboration step takes place with complete and opposite stereocontrol to that previously described in the literature (Scheme 4).¹⁴⁴ This outcome was rationalized the bulky benzyloxy moiety driving the hydroborating agent approach to the less sterically hindered face, followed by BCl₃ mediated debenzoylation of **72** to afford the hitherto unreported (+)-5-*epi*-cyclophellitol **73**.

Scheme 4. Reaction conditions and reagents for the key hydroboration-oxidation and deprotection steps in Serrano's synthetic strategy.¹⁴⁴

Epoxy-inositol analogues, such as conduritol B epoxide (CBE, 1,2-anhydro-*myo*-inositol) **74**, also irreversibly bind within the active site of glycosidases (Scheme 5, left). Premkumar and coworkers obtained an X-ray co-crystal of CBE **74** irreversibly bound to the acid- β -glucosidase (human) active site, with Glu340 as the catalytic nucleophile.¹⁴⁵ The crystal structure data supports the proposed reaction mechanism, which requires protonation of the epoxide oxygen of CBE **74** by the catalytic acid/base pair, followed by nucleophilic attack at C-1 of the cyclohexitol ring, causing diaxial opening of the epoxide and formation of a nucleophile-cyclohexitol ester bond shown in **75**.^{145, 146} The distance between C-1 of **75** and the Glu340 O ϵ 2 is 1.43 Å, and the binding between **74** and the active site only involves Glu340. The distance between the epoxide oxygen of **74**, oriented and the Glu235 O ϵ , is within H-bonding distance, which is consistent with the role of Glu235 as the acid/base catalyst.

Freeman and Hudlicky synthesized oligomers of (-)-conduritol-F **64** and *muco*-inositol **65** (Figure 8) as non-glycosidically labile oligosaccharide analogues, to exploit their rich combinatorial potential due to the additional chiral center of each monomer relative to their monosaccharide counterparts.¹⁴⁷ These were evaluated against six glycosidases and compounds **76** (IC₅₀: α -galactosidase, 275 μ M; β -galactosidase, 250 μ M) and **77** (IC₅₀: α -glucosidase, 60 μ M; β -galactosidase, 190 μ M) that preserved the conduritol-F motif proved to be more potent inhibitors relative to the monomers or the *muco*-inositol dimer **78** (IC₅₀: α -glucosidase, 800 μ M; β -galactosidase, 600 μ M). The conduritol-F motif resembles more closely the structure of the transition state assumed to operate in glycolysis,¹⁴⁶ and this may explain the higher inhibitory activity in comparison with the fully hydroxylated inositols.

Scheme 5. (Left) Ester **75** is the adduct of the irreversible inhibition of acid- β -glucosidase via nucleophilic addition of active site's Glu340 to epoxy-inositol analogue CBE **74** to give **75**.¹⁴⁵ (Right) Conduritol- and inositol-based oligosaccharide analogues **76-78** as non-glycosidically labile oligosaccharide analogues.¹⁴⁷

Witte and colleagues applied activity-based protein profiling (ABPP), more commonly used in the protease and esterase fields to the glycosidase field, to profiling of β -glucosidases in complex samples. The development of two cyclophellitol-based activity based probes C1-BODIPYred **79** and C2-BODIPYgreen **80** were highly potent and selective for GBA-1 (Figure 9) permitted *in vitro* and *in vivo* visualization of these probes and the monitoring of wild-type and mutant GBA-1 activities. These studies offer the potential for critical insights into acid- β -glucosidase, GBA-1, in Gaucher's disease.¹⁴⁸ Using C-6-azido cyclophellitol analogues followed by either a Staudinger-Bertozzi or Huisgen [3+2] click bio-orthogonal ligation with the C1-BODIPYred **79** and C1-BODIPYgreen **80** alkyne intermediates, to give C-1 and C-2 directly covalently bound to GBA-1. Experiments on isolated enzyme revealed that the ligation step is efficient, but a drop in *in vitro* efficacy was apparent on repeating the same experiments on cell extracts (which may possibly have been a permeability artefact).

Figure 9. Cyclophellitol-based BODIPY ABPP probes.¹⁴⁸

Martínková et al. reported a chemo/enzymatic route to cyclophellitol and *epi*-cyclophellitol in enantiopure forms from racemic starting materials.¹⁴⁹ The synthesis involves a multi-enzymatic resolution/biotransformation steps of the cyano-cyclitol intermediates (1*R*,4*S*,5*R*,6*R*)/(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxycyclohex-2-enecarbonitrile (*rac*)-**81** by a cooperative use of lipase, nitrile hydratase, and amidase (Scheme 6). Preparation of the enantiomeric mixture of intermediate nitrile conduritol (*rac*)-**81** from inexpensive and available *p*-benzoquinone required five synthetic steps. Enantiomerically pure (+)-**81** was obtained after a lipase-catalysed resolution of (*rac*)-**81** in organic solvent to give intermediates (+)-**82** and (-)-**81**, followed by chemical hydrolysis of (+)-**82**. The optimized resolution step employs lipase from *Candida antarctica B* or *Rhizomucor miehei* to afford the primary

monoester product (+)-**82** with yields in the range of 45–48% and an ee >98%, with recovery of unreacted starting material (-)-**81** (>98% ee). Single enantiomers of **81** were incubated with *R. erythropolis* A4 bacterial whole-cell preparations. The two hydrolysis steps occurred sequentially with nitrile hydratase [$-\text{CN} \rightarrow -\text{C}(=\text{O})\text{NH}_2$] and amidase [$-\text{C}(=\text{O})\text{NH}_2 \rightarrow -\text{COOH}$] at the differing rates of 10 hours for (+)-**81** and 24 hours for (-)-**81**, thus avoiding conventional chemical hydrolysis of nitriles in strongly acidic conditions and high temperature that result in degradation of the starting material. Optically pure cyclophellitols were accessed from these materials in three steps: esterification, reduction to the corresponding primary alcohol by LAH and epoxide formation by mCPBA.

Scheme 6. Use of lipase, nitrile hydratase and amidase in the enzyme resolution and biotransformation of cyanocyclitol precursors towards synthesis of cyclophellitol **2** and *epi*-cyclophellitol.¹⁴⁹

5.2 Inorganic Salts and Metal Trace Elements

A limited number of studies have been reported on the action of inorganic salts and metal trace elements on α -glycosidase action.^{150–156} The three main areas of investigation encompass

a) the study of glucosidase and galactosidase inhibition in soils, b) the insulinomimetic activity of transition metal complexes, and c) the glycosidase inhibition by Pd complexes.

Soil borne glucosidase and galactosidase enzymes provide an important energy source for microorganisms. Eivazi et al. reported on the effects of soil treatments using inorganic salts and of trace elements on the activities of these enzymes.¹⁵² In general, the inorganic salts were stronger inhibitors of α -glucosidase and α -galactosidase activities (range 0 to 70%) than the activities of their β -counterparts (range 0 to 44%) [1 g of soil in the incubation medium made 8 mM with respect to the inorganic salt]. The effect of trace elements on the activities of these enzymes varied considerably among the elements, enzymes and soils [1 g of soil in the incubation medium (1 mL) gave a 25 μ M concentration with respect to the trace element under evaluation]. The percentage inhibition of α -glucosidase activity in Webster soil ranged from 11% with Mo^{4+} [as H_2MoO_4] to 66% with Ag^+ [as Ag_2SO_4] and in Luton soil ranged from 9% with V^{4+} [as VOSO_4] to 66% with Cd^{2+} [as CdCl_2].

Yoshikawa and coworkers reported that Zn, Cu and VO sulfate and picolinic acid (pa) complexes exhibit insulinomimetic activity.^{153, 154} The modulation of α -glucosidase inhibitory effect by metal ions and their complexes in the lowering of high blood-glucose levels in diabetic model animals has been reported.¹⁵⁵ *In vitro* analysis revealed that divalent cations such as Zn^{2+} , Cu^{2+} , VO^{2+} , $\text{Zn}(\text{pa})_2$ and $\text{Cu}(\text{pa})_2$ were 4.7, 0.8, 201, 15 and 1.3 μ M inhibitors of α -glucosidase activity, respectively (acarbose was used as a positive control).¹⁵⁵ *In vivo*, the α -glucosidase inhibition was studied in ddy-mice⁴⁷ where it was found that the Cu^{2+} and its complexes produced the same effect as acarbose (~ 80 mg/dL blood glucose level vs ~ 150 mg/dL for the control). These results suggested that Cu^{2+} and $\text{Cu}(\text{pa})_2$ inhibited the α -glucosidase action in the epithelium of the small intestine and reduced disaccharide digestion. Lineweaver–Burk plots show that Cu^{2+} and $\text{Cu}(\text{pa})_2$ act as a non-competitive inhibitors against α -glucosidase (yeast).

Vasella and coworkers¹⁵⁶ found that traces of Pd compounds, generated upon catalytic debenzoylation in their synthesis of glycosidase inhibitors, proved to be themselves strong inhibitors. Palladium chloride $[\text{PdCl}_4]^{2-}$ was determined to be a 40 μ M reversible inhibitor for the β -glucosidases (*Clostridium saccharolyticum* and sweet almonds) and a 19 μ M non-competitive and reversible inhibitor of the α -glucosidase (yeast). In addition, $[\text{PdCl}_4]^{2-}$ inhibits two β -galactosidases (bovine liver and *Escherichia coli*) non-competitively and reversibly with K_i 36 μ M and 1.2 μ M respectively, and is a competitive and reversible inhibitor of both the α -galactosidase (*Aspergillus niger*) with K_i 5.8 μ M, and the α -galactosidase (coffee beans) with

K_i 4.8 μM . Thus particular care must be exercised when synthesising glycosidase inhibitors, *especially in the removal of transition metal catalyst residue*, e.g. from hydrogenation reactions, with flow chemistry approaches with embedded catalysts being potentially highly beneficial in this respect.¹⁵⁷

6. Conclusion

Over 75 years since gluconolactone **1** was first discovered to inhibit glucosidases, research into new classes of glycosidase inhibitors continues to flourish.^{158, 159} This is supported by a broader understanding of the tertiary structures of enzyme targets and the further uncovering of novel mechanisms in this family.^{98, 160, 161} Epoxides such as the natural product cyclophellitol and sulfonium ions like ones found naturally within the salicinol family were both discovered as synthetic classes of glycosidase inhibitor before they were identified in nature.^{84, 146} The irreversible nature of epoxide inhibitors has recently allowed detection of proteins fused to a β -glucosidase in a cellular context.¹⁶² Intriguingly, epoxides have recently been postulated as intermediates in mannoside hydrolysis by glycosidases lacking a nucleophilic group near the anomeric site of cleavage.¹⁶³⁻¹⁶⁵ Research synergies between past and present work continue to drive this field forward. Particularly, the area of polycyclic aromatic natural products covered in our previous review³⁸ has seen significant growth and will be the subject of a separate update.

7. REFERENCES

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TABLES

Table I. K_i Values (μM) of derivatives **15-18** and for the parent alkaloids DMDP **13** and australine **14** against various glycosidases.⁵⁵

Compound	α -Glucosidase (yeast)	Isomaltase (yeast)	β -Glucosidase (almond)	β -Glucosidase/ β -Galactosidase (bovine liver)	Trehalase (pig kidney)	Invertase (yeast)	Amyloglucosidase (<i>A. niger</i>)
13	0.73	2.5	1.7	3.3	355	2.9	2.1
14	NI ^a	ND ^b	NI	ND	ND	28	1.5
15	72	28	9.2	39	NI	> 1000	NI
16	NI	251	NI	NI	20	NI	249
17	132	29	340	644	NI	NI	NI
18	408	72	433	347	NI	NI	NI

^a NI = no inhibition detected; ^b ND = not determined.

Table II. IC_{50} Values (μM) of thiosugar sulfonium salts and related compounds against α -glucosidases.¹¹¹

Compound	α -Glucosidase (rat intestinal)		
	maltase	Sucrase	isomaltase
40	5.1	1.0	1.4
41	4.3	2.9	1.0
42	1.3	0.3	1.0
45	>384	90	6.5
46	>329	>329	>329
47	105	>384	53
Voglibose	1.2 ^b	0.2 ^b	2.1 ^b
Acarbose	1.7 ^a	1.5 ^a	646 ^a

^aFrom ref. 115. ^bFrom ref. 113.

Table III. IC_{50} Values (μM) of thiosugar sulfonium salts **3** and **32**, and D-ATA **48** against α -glucosidases.⁹³

Compound	α -Glucosidase				
	rice	rat maltase	rat isomaltase	rat sucrase	human lysosome
3	2.6	2.4	5.7	2.2	0.34
32	8.6	10	0.64	5.2	4.4
48	220	290	1000	NI	NI

Table IV. IC₅₀ Values (μ M) of 3'-*O*-alkylated salacinol derivatives **33-36** and positive controls voglibose and acarbose against α -glucosidases.¹¹³

Compound	α -Glucosidase (rat intestinal)		
	maltase	isomaltase	sucrase
33	5.3	0.39	0.46
34	1.7	0.27	0.12
35	1.0	0.95	1.3
36	0.44	0.14	0.32
Voglibose	1.2	2.1	0.2
Acarbose	1.7 ^a	646 ^a	1.5 ^a

^a From ref. 115.