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Review

Zwitterionic glycosidase inhibitors: salacinol and related analogues

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Abstract—Natural products with interesting biological properties and structural diversity have often served as valuable lead drug candidates for the treatment of human diseases. Salacinol, a naturally occurring α-glucosidase inhibitor, was shown to be one of the active principles of the aqueous extract of a medicinal plant that has been prescribed traditionally as an Ayurvedic treatment for type II diabetes. Salacinol contains an intriguing zwitterionic sulfonium-sulfate structure that comprises a 1,4-anhydro-4-thio-D-arabinitol core and a polyhydroxylated acyclic chain. Due to the unique structural features and its potential to become a lead drug candidate in the treatment of type II diabetes, a great deal of attention has been focused on salacinol and its analogues. Since the isolation of salacinol, several papers describing various synthetic routes to salacinol and its analogues have appeared in the literature. This review is aimed at highlighting the synthetic aspects of salacinol and related compounds as well as their structure–activity relationship studies.

Keywords: Ammonium ions; Cyclic sulfates; Glycosidase inhibitors; Iminosugars; Salacinol analogues; Selenonium ions; Sulfonium ions

Contents

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1. Introduction

Over the years, glycosidase inhibitors have received considerable attention in the field of chemical and medicinal research.[1] They have many potential therapeutic applications because the glycosidase enzyme-catalyzed hydrolysis of complex carbohydrates is a biologically widespread phenomenon in living systems.[2–4] For example, inhibition of glycosidase enzymes that are involved in the biosynthesis of oligosaccharide chains of the N-linked glycoproteins in the endoplasmic reticulum (ER) and Golgi apparatus has profound effects on maturation, transport, and secretion of these glycoproteins.[5,6] This strategy has potential for many therapeutic applications, such as in the treatment of cancer and viral infections.[5] Inhibition of carbohydrate-hydrolyzing enzymes such as pancreatic α-amylase and α-glucosidases in the digestive tract that leads to a delay in digestion of ingested carbohydrates is one of the therapeutic approaches for the treatment of type II non-insulin-dependent diabetes mellitus (NIDDM).[7,8]

Many naturally occurring monocyclic and bicyclic amines such as 1-deoxynojirimycin (**1**), swainsonine (**2**), and castanospermine (**3**) (Fig. 1) are effective inhibitors of various glycosidase enzymes and have shown potential as therapeutic agents.[9] For example, treatment with the indolizidine alkaloid swainsonine (**2**), a naturally occurring Golgi α-mannosidase II inhibitor, has led to significant reduction of tumor mass in human patients with advanced malignancies, and is a promising drug therapy for patients suffering from breast, liver, lung, and other malignancies.[10,11]

These amine-based glycosidase inhibitors are believed to carry a positive charge at physiological pH and hence are postulated to bind in the active sites of glycosidase enzymes by mimicry of the charge of the oxacarbenium-ion-like transition state formed during hydrolysis of the natural substrate (Fig. 2).[12] The establishment of stabilizing electrostatic interactions between the protonated nitrogen and an active-site carboxylate residue is

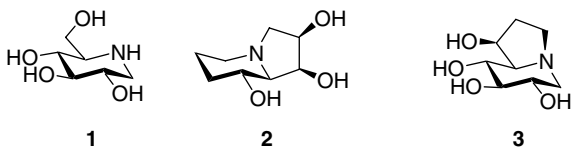

Figure 1. Examples of naturally occurring glycosidase inhibitors: 1-deoxynojirimycin (**1**), swainsonine (**2**), and castanospermine (**3**).

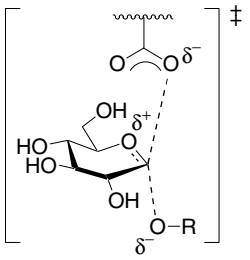

Figure 2. Oxacarbenium-ion-like transition state for the glycosidase-mediated hydrolysis reaction.

considered to be a possible mechanism of action of such inhibitors.[12]

Hence, while designing a synthetic inhibitor, consideration of inclusion of an atom that carries a permanent positive charge at a suitable position was considered as an alternative means of providing the required charge state to establish such electrostatic interactions between the inhibitor and the active-site carboxylate residue. Accordingly, introduction of a permanent positive charge on the nitrogen atoms of azasugars was attempted. For example, Kajimoto et al.[13] synthesized the *N*-oxide analogue of castanospermine (**4**) shown in Figure 3. However, this compound was only a weak inhibitor of β-glucosidase compared to the parent compound, castanospermine (**3**).

For the purpose of establishing such electrostatic pairing, introduction of a positively charged sulfur atom in the structures of potential glycosidase inhibitors was considered next. The approach had precedent in the mimicry by sulfonium ions of tertiary amines with narcotic analgesic properties. For example, Belleau et al.[14–17] reported the syntheses of sulfonium-ion analogues of levorphanol (**5**) and isolevorphanol (**6**), *S*-methyl sulforphanol (**7**), and *S*-methyl isosulforphanol (**8**) (Fig. 3), respectively, and showed that they were agonists or antagonists of morphine for the opiate receptor. In addition, the syntheses of various sulfonium salts such as the bicyclic compounds **9**[18] and **10**,[19] and a sulfimide derivative **11**[20] (Fig. 3) have been reported, and these compounds have been tested for their glycosidase inhibitory activities. We have reported the synthesis of a sulfonium-ion analogue of castanospermine, **12** (Fig. 3), as a model to test the theory that a sulfonium salt carrying a permanent positive charge might be advantageous in providing the necessary electrostatic interactions between the inhibitor and the active-site carboxylate residues.[21] After initiation of this work, a

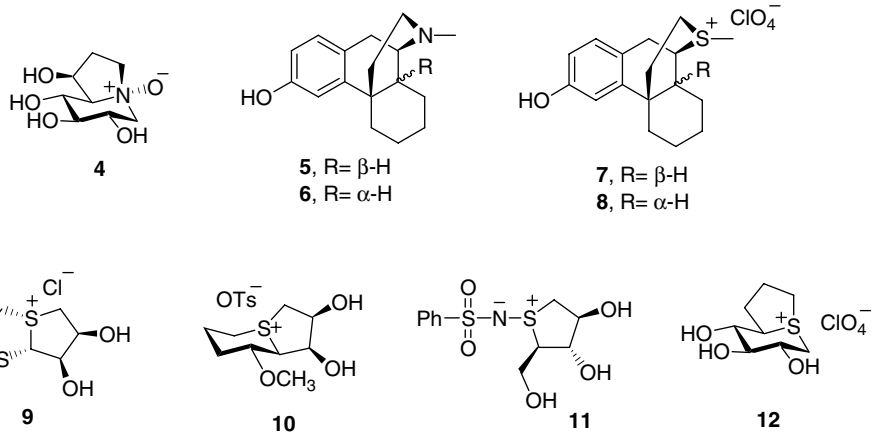

Figure 3. Synthetic glycosidase inhibitors carrying a permanent positive charge.

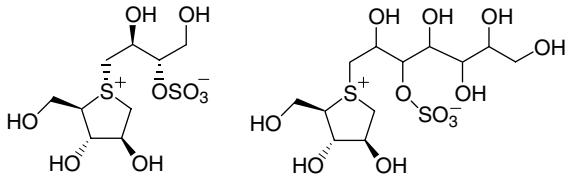

Figure 4. Structures of salacinol (**13**) and kotalanol (**14**).

literature report strongly validated this approach as two naturally occurring glycosidase inhibitors, namely salacinol (**13**)[22] and kotalanol (**14**),[23] both having a sulfonium-ion center (Fig. 4), were isolated from *Salacia reticulata*, known as 'Kotalahimbutu' in Singhalese, a large woody climbing plant widespread in Sri Lanka and South India. Aqueous extracts of this plant have been traditionally used in the Ayurvedic system of Indian medicine for treating type II diabetes.[22–24] Traditionally, Ayurvedic medicine advised that a person suffering from diabetes should drink water stored overnight in a mug carved from Kotalahimbutu wood.

It was demonstrated that these two natural products were active components of the aqueous extracts of this plant. Salacinol (**13**) and kotalanol (**14**) share a common zwitterionic sulfonium-sulfate structure that comprises a 1,4-anhydro-4-thio-D-arabinitol core and a polyhydroxylated acyclic chain, the difference between them being the length of the polyhydroxylated acyclic chain; kotalanol has a seven-carbon chain, with unspecified (to date) stereochemistry at the stereogenic centers, whereas salacinol has a four-carbon chain with defined stereochemistry. Although the exact mechanism of action of salacinol and kotalanol has yet to be elucidated, it is postulated that the positively charged sulfur atom can act in the same way as the protonated ammonium center of the amine-based glycosidase inhibitors when binding in the active site of glucosidases that process carbohydrate polymers and oligomers.[25,26]

2. Salacinol and its analogues

2.1. Isolation and structural elucidation of salacinol

Salacinol (**13**) was originally isolated from *S. reticulata* by Yoshikawa et al.[22] and later also from other plant species of the *Salacia* genus, *Salacia oblonga*[27] and *Salacia chinensis*,[28] by the same group. Through bioassay-guided separation using α-glucosidase inhibitory activities, a potent glycosidase inhibitor that they named salacinol (**13**) was isolated from the water-soluble fractions obtained from the dried roots of *S. reticulata*.[22] The water-soluble fraction inhibited the increase in serum glucose levels after the administration of sucrose or maltose in rats. Column chromatographic purification of the water-soluble fraction followed by repeated HPLC separations gave the pure natural product salacinol (**13**). The purified salacinol showed competitive inhibition for intestinal α-glucosidases in vitro: the glucosidase inhibitory activities of salacinol against maltase and sucrase were nearly equal to those of acarbose, a clinically used α-glucosidase inhibitor of microbial origin (Table 1). However, the inhibitory activity of salacinol against isomaltase was found to be greater than that of acarbose.

The structural elucidation studies of salacinol used various chemical and spectroscopic methods including 1D and 2D NMR experiments and a potassium rhodizonate test (a positive test that confirmed the presence of a sulfate moiety), and suggested the presence of an

Table 1. K_i values of salacinol (**13**) for rat small-intestinal disaccharidases[22]

Enzyme	K_m (M)	K_i (μg/mL)	
		Salacinol (**13**)	Acarbose
Maltase	2.7×10^{-3}	0.31	0.12
Sucrase	2.0×10^{-2}	0.32	0.37
Isomaltase	4.5×10^{-3}	0.47	75

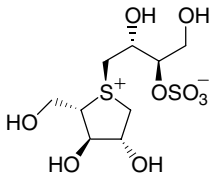

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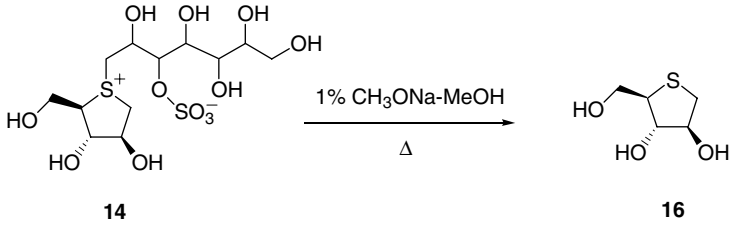
Figure 5. Structure of salacinol as initially proposed by Yoshikawa et al.[22]

unusual zwitterionic sulfonium-sulfate structure. Based on the spectral and X-ray crystallographic analyses of salacinol (**13**), Yoshikawa et al.[22] assigned the initial stereostructure of salacinol as an inner-salt sulfonium structure (**15**) that was composed of a 1,4-anhydro-4-thio-L-arabinitol unit alkylated at sulfur by a 1-deoxy-D-erythritol-3-sulfate unit, as shown in Figure 5.

However, the degradation studies of kotalanol (**14**), also performed by Yoshikawa et al.,[23] led to the revision of the initial stereostructure (**15**) of salacinol. In this study, although the configurations of the stereogenic centers in the longer heptitol side chain of kotalanol (**14**) or at the sulfur atom were not determined, the presence of an anhydro-4-thio-D-arabinitol unit was confirmed based on the degradation product, 1,4-anhydro-4-thio-D-arabinitol (**16**), that was formed upon alkaline treatment of kotalanol (**14**) (Scheme 1). Because salacinol (**13**) and kotalanol (**14**) are of the same plant origin and are likely to arise from similar biosynthetic pathways, the structure of salacinol was then assigned to be the enantiomer (**13**) of the initial stereostructure (**15**).[27,29]

2.2. Synthesis of salacinol

The ambiguity in the structure of salacinol was resolved when Yuasa et al.[30] and we[31] independently reported the total synthesis of salacinol (**13**). In 2000, Yuasa et al.[30] first reported the synthesis of salacinol (**13**) and its diastereomer **17** (Scheme 2). The key step used for the synthesis of salacinol, involved a ring-opening reaction of the cyclic sulfate **18**, derived from L-glucose, with 1,4-anhydro-4-thio-D-arabinitol (**16**),[20] followed by removal of the protecting groups (Scheme 2). This study indicated that the unprotected thioether **16** reacted smoothly only with the cyclic sulfates **18** and **19**, that are protected as an isopropylidene ketal, when compared to other cyclic sulfates protected with benzyl or



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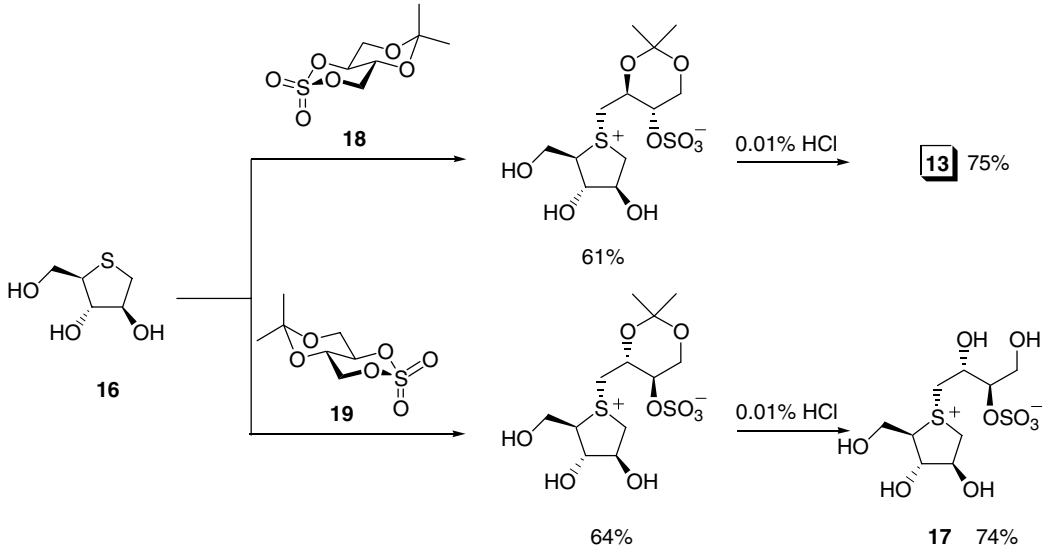
Scheme 1.

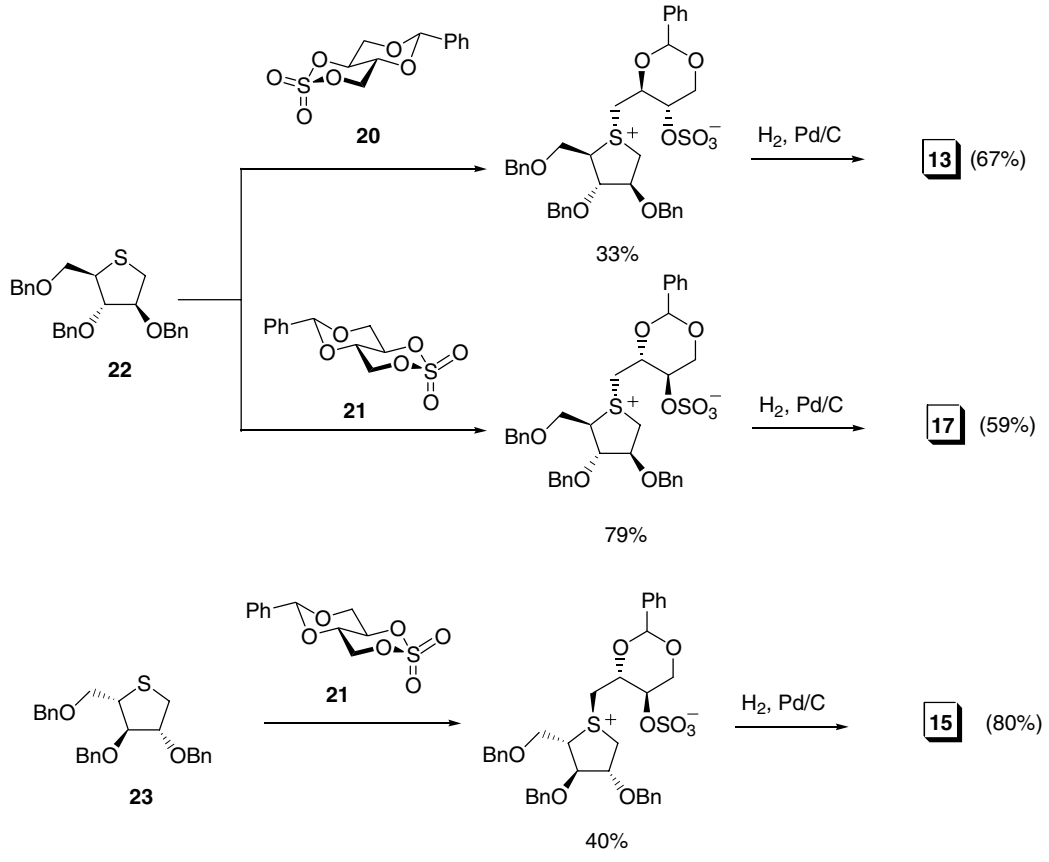


Scheme 2.

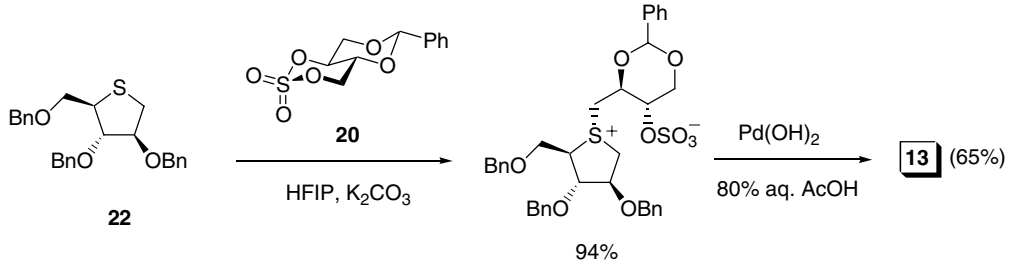
benzylidene groups, or without protecting groups; in the latter cases, decomposition of the cyclic sulfates was observed.

We achieved the synthesis of salacinol (**13**) and its stereoisomers **15** and **17** via nucleophilic ring opening of a benzylidene-protected cyclic sulfate (**20**, derived from L-glucose or **21**, derived from D-glucose), similar to the approach of Yuasa et al.,[30] but using a per-benzylated thioether **22**[32] or **23**[33] (Scheme 3).[31] Finally, the protecting groups were removed by hydrogenolysis. In this synthesis, dry acetone was found to be a more suitable solvent for the coupling reaction and the addition of K_2CO_3 in the reaction mixture was necessary to prevent the unwanted hydrolysis reaction of the cyclic sulfate.

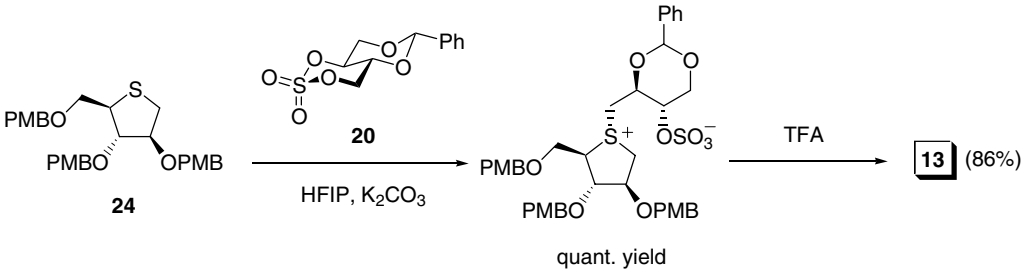
A significant improvement in this synthesis was achieved by exploiting an unusual solvent effect provided by 1,1,1,3,3,3-hexafluoroisopropanol (HFIP).[34] Thus, the reaction of the per-benzylated thioether **22** and the cyclic sulfate **20** in HFIP containing K_2CO_3 gave a significantly increased yield of the alkylated product (94%). The increased yields in HFIP were considered to be the effect of better solvation of the transition states relative to the ground states of the reactants. However, a relatively low yield of the final product (65%) was observed in the hydrogenolysis step (Scheme 4a), likely



Scheme 3.



Scheme 4a.



Scheme 4b.

due to the poisoning of the catalyst by the small amount of the thioether **22** that was formed during the reaction. Ultimately, the efficient synthesis of salacinol (**13**, 75% overall yield) was achieved by reacting the *p*-methoxybenzyl (PMB)-protected thioether **24**, instead of the benzyl-protected thioether **22**, with the cyclic sulfate **20** and the problematic hydrogenolysis step was eliminated as, in this case, all the protecting groups were readily removed using aqueous trifluoroacetic acid (TFA), as shown in Scheme 4b.

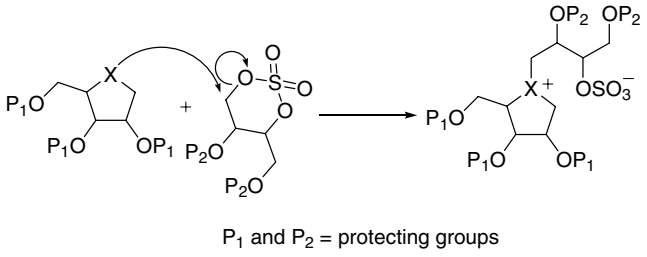
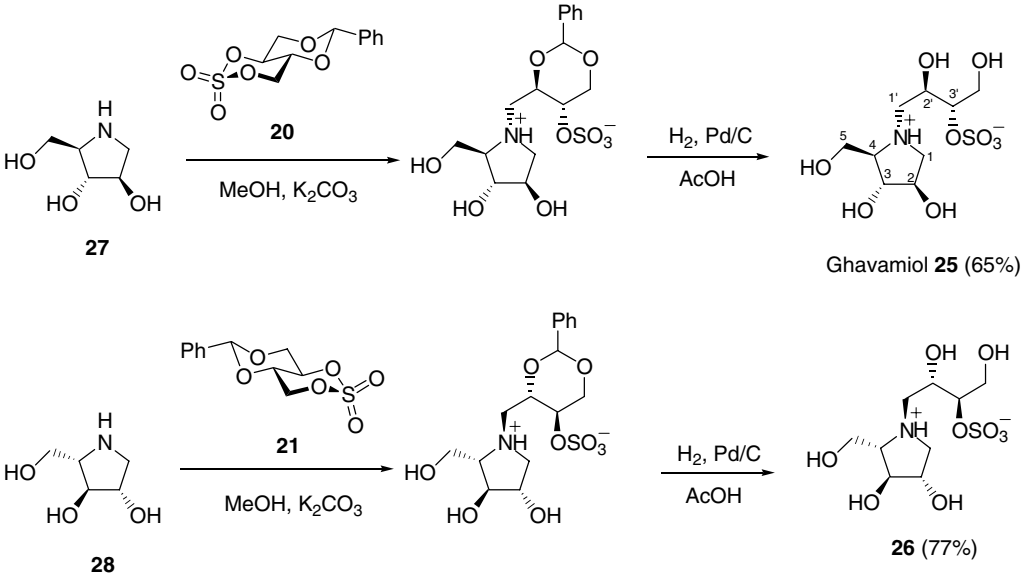


Figure 6. General strategy for the alkylation at the heteroatom of a hetero anhydroalditol.

2.3. Heteroatom analogues of salacinol

The subsequent syntheses of salacinol and its analogues have incorporated many improvements especially with respect to the syntheses of the respective cyclic sulfates derived from a variety of polyhydroxylated heterocyclic ethers that have been employed as coupling partners. However, the key strategy for the formation of the zwitterionic alkylated product (Fig. 6), that is, the nucleophilic ring opening of a protected cyclic sulfate by the heteroatom of a protected or unprotected hetero anhydroalditol, developed by Yuasa et al.[30] and our group,[31,34] remains unchanged.

2.3.1. Nitrogen analogues. In 2001, we reported the syntheses of two nitrogen analogues (**25**, ghavamiol and **26**) of salacinol.[35] Compounds **25** and **26** were synthesized by reactions of the iminoarabinitol **27**[36] with the cyclic sulfate **20** and the iminoarabinitol **28**[37] with the cyclic sulfate **21**, respectively, as shown in Scheme 5. Compounds **25** and **26** were tested against three glucosidase enzymes, namely glucoamylase G2, porcine pancreatic α-amylase (PPA), and barley α-amylase



Scheme 5.

(AMY1), and the effects were compared to those of salacinol (**13**). It was observed that the ammonium compounds **25** and **26** were not active against AMY1 and PPA at concentrations of 5 mM compared to salacinol (**13**) that showed stronger inhibition against AMY1 and PPA ($K_i = 15 \pm 1$ and 10 ± 2 μM, respectively). Salacinol (**13**) showed weak inhibition of glucoamylase G2 ($K_i = 1.7$ mM) whereas the ammonium compounds **25** and **26** inhibited glucoamylase with K_i values in the range ~10-fold higher than salacinol.

Screening of salacinol and the nitrogen analogues against human pancreatic α-amylase (HPA) showed that compound **26** was an inhibitor of this enzyme with a K_i value of 0.4 mM compared to salacinol that showed stronger inhibition with a K_i value of 75 μM. In contrast, ghavamiol (**25**) was not active against this enzyme.[38]

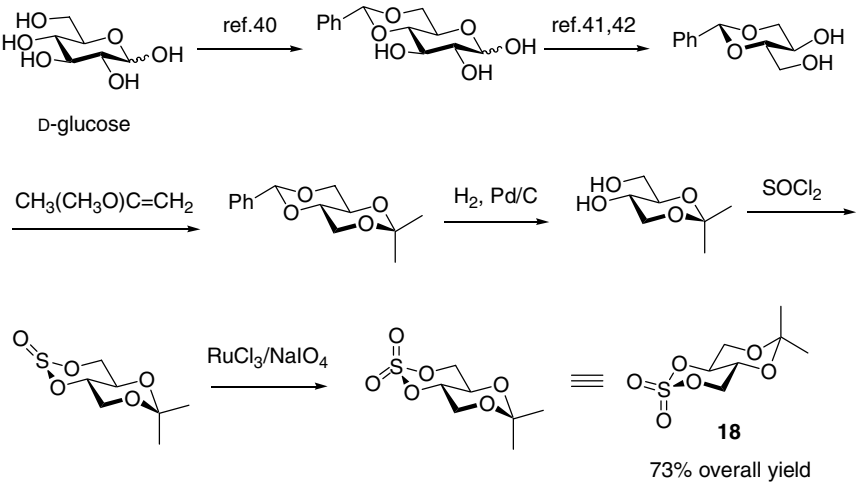
Ghavamiol (**25**) was also independently synthesized by Muraoka et al.[39] and was tested for its α-glucosidase inhibitory activities against rat small-intestinal disaccharidases; maltase, sucrase, and isomaltase. When compared to salacinol (**13**), the inhibitory activities of ghavamiol (**25**) against these enzymes decreased considerably. In approaching the target **25**, the same ring-opening strategy using the iminoarabinitol **27** and the cyclic sulfate **18** was followed; however, unlike the previous syntheses of salacinol[30,31,34] and ghavamiol[35] in which the respective cyclic sulfates **18** and **20** were obtained from L-glucose, an optimized synthesis of the cyclic sulfate **18** starting from a benzylidene-protected diol that was readily obtained from D-glucose[40–42] was presented (Scheme 6).

It is of interest that, at a concentration of 1 mmol/L, ghavamiol (**25**) inhibited ~96% of the recombinant lysosomal acid α-glucosidase (GAA) activity, whereas this enzyme was relatively insensitive to the α-glucosidase inhibitor, acarbose.[43] In addition, ghavamiol showed selective inhibition of the activity of GAA in the detergent extract of human polymorphonuclear leukocytes (PMNs), which also contains a second acid α-glucosidase known as maltase glucoamylase (RAAG). Inhibition studies in which recombinant GAA was added to the PMN extract showed that all of the recombinant enzyme was inhibited by 1 mmol/L ghavamiol in the presence of all the other components from the PMN lysate.[43] This selective inhibition of the enzyme GAA has been used to advantage in the design of a diagnostic test for Pompe's disease.

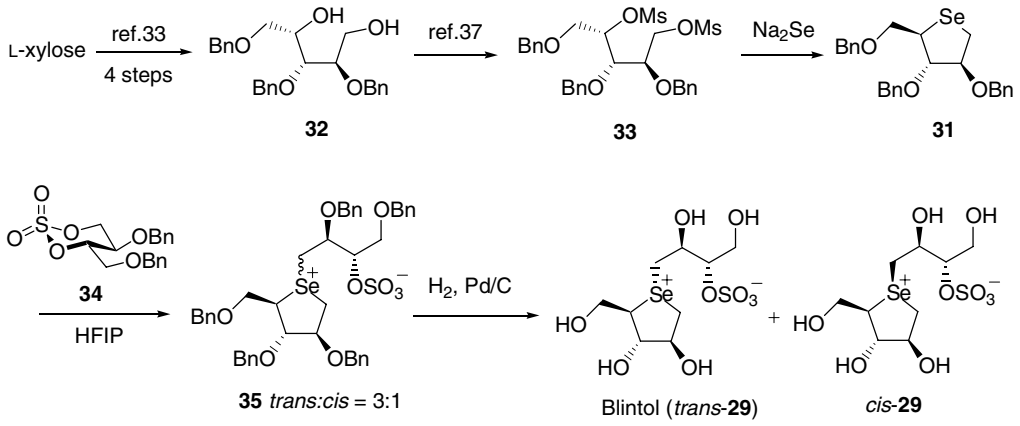
In all of the above syntheses of salacinol and related compounds, the alkylation reactions between the various 1,4-anhydroarabinitol moieties and the cyclic sulfates proceeded stereoselectively irrespective of the reaction conditions. The trans relationship between C-5 and C-1′ was assigned by means of NOE correlations observed between H-4 and H-1′ in NOESY experiments.

2.3.2. Selenium analogues. We have also described the convenient syntheses of two selenium analogues of salacinol, selenosalacinol (*trans*-**29**), and its stereoisomer **30**.[44–46] Compounds *trans*-**29** and **30** were tested against different glycosidases and the results were compared to those of salacinol (**13**). Selenosalacinol (*trans*-**29**, named blintol) was found to be a weak inhibitor of glucoamylase G2 ($K_i = 0.72$ mM) and showed no significant inhibition of AMY1, HPA,[38] or PPA. The stereoisomer **30** of blintol showed no significant inhibition of glucoamylase G2, HPA,[38] PPA, or AMY1. The activity of blintol (*trans*-**29**), ghavamiol (**25**), and salacinol (**13**) against recombinant human intestinal maltase glucoamylase (MGA) will be described later.

The original synthesis of blintol began with the preparation of the per-benzylated selenoether **31** that was derived from the intermediate **32** by its conversion into the corresponding dimesylate **33**[37] and subsequent reaction with freshly prepared Na_2Se (Scheme 7a). The



Scheme 6.

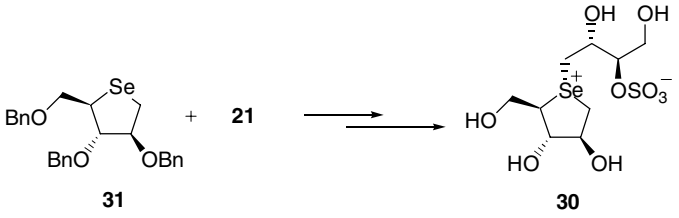


Scheme 7a.

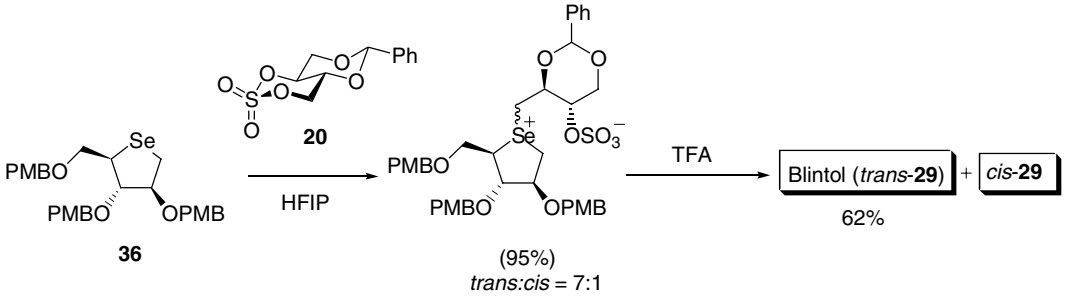
intermediate **32** was prepared from L-xylose in four steps using a literature method.[33] The coupling reaction was again based on similar strategies developed for the synthesis of salacinol and related compounds, except that the selenoether **31** and the cyclic sulfate **34**[30] were used as coupling partners.

Unlike the reported reactions of an anhydrothioarabinitol or iminoarabinitol with various cyclic sulfates (e.g., Schemes 2–6), the reaction of the selenoether**31** with the cyclic sulfate **34** yielded the alkylated selenonium ion **35** as a 3:1 mixture of diastereomers at the stereogenic selenium center (Scheme 7a). The major isomer was identified, by means of 1D-NOESY experiments, as the diastereomer with a trans relationship between C-5 and C-1′. Hydrogenolysis of the mixture (**35**) gave a mixture of diastereomers (*trans*-**29** and *cis*-**29**) that, upon precipitation from MeOH, yielded the pure blintol (*trans*-**29**). The stereoisomer **30** was prepared in a similar fashion from the selenoether **31** and the cyclic sulfate **21** (Scheme 7b).

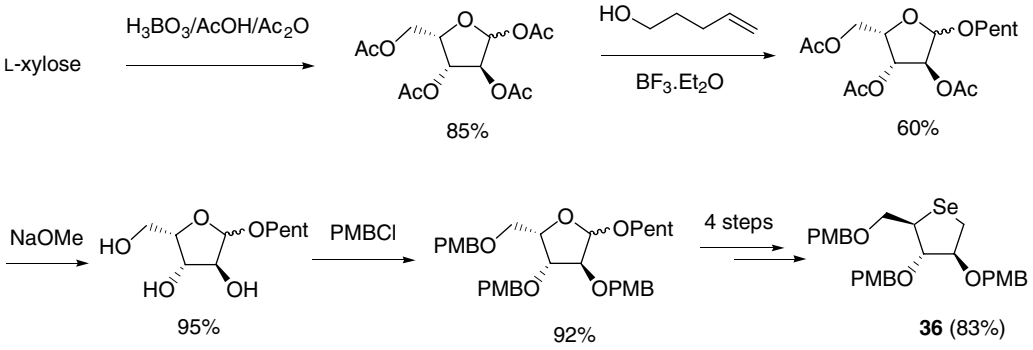
We have also achieved an efficient synthesis of blintol using a PMB-protected selenoether (**36**) and the benzylidene-protected cyclic sulfate (**20**) (Scheme 8).[47] This synthesis required, in turn, an optimized synthesis of the selenoether **36**, which featured the use of boric acid in the acid-catalyzed acetylation of L-xylose to improve the furanoside:pyranoside ratio[48] and the use of an *n*-pentenyl glycoside in an intermediate step[49] (Scheme 9). In addition, the benzylidene-protected cyclic sulfate **20**, the other coupling partner, was successfully prepared from D-glucose instead of expensive L-glucose, as depicted in Scheme 10.



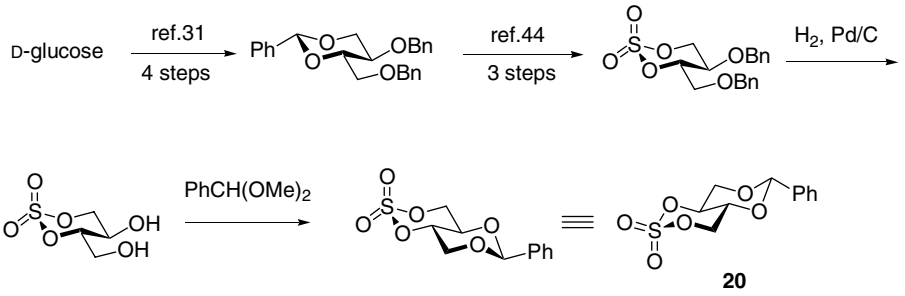
Scheme 7b.



Scheme 8.



Scheme 9.



Scheme 10.

2.4. Five-membered ring analogues with different sugar stereochemistries

To probe the importance of the configurations of the stereogenic centers in the heterocyclic ring of salacinol (**13**), we have described the synthesis of an anhydrothio-D-xylitol analogue (**37**) of salacinol and also the corresponding nitrogen congener (**38**) (Fig. 7).[50] In these compounds, the stereochemistries at C-2 and C-3 were both inverted compared to salacinol. As shown in Scheme 11, the synthesis started with the preparation of the respective coupling partners, anhydrothio- (**39**) and imino- (**40**) xylitols, from a common precursor **41** that was readily prepared in five steps from L-arabinose by use of a literature procedure.[51]

Enzyme inhibition studies indicated that the sulfonium analogue **37** inhibited, albeit weakly when compared to salacinol (**13**), AMY 1 and PPA with K_i values of 109 ± 11 and 55 ± 5 μM, respectively. However, the ammonium-ion analogue **38** showed no significant inhibition against these enzymes.

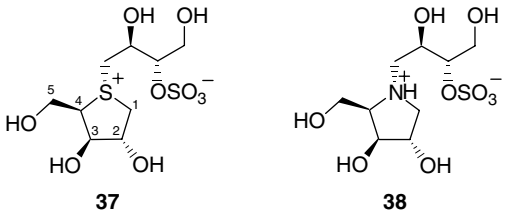
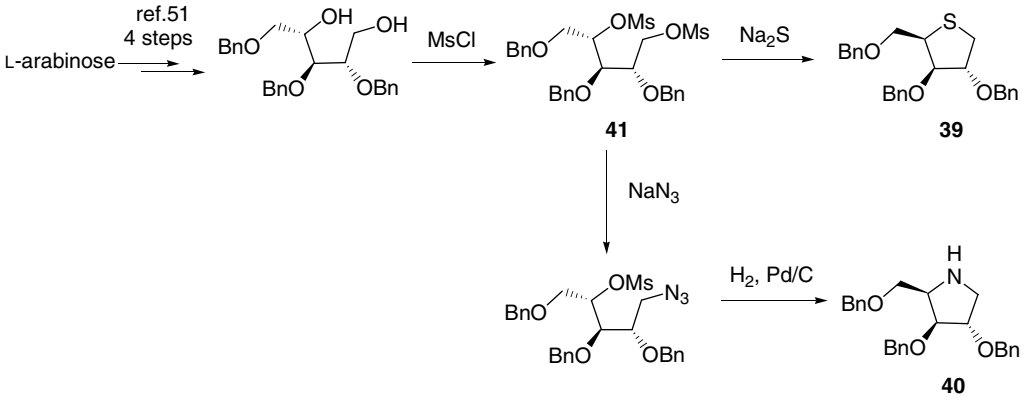


Figure 7. An anhydrothio-D-xylitol analogue (**37**) of salacinol and the corresponding nitrogen congener (**38**).

The syntheses of anhydrothio-D-lyxitol (**42**) and anhydrothio-D-ribitol (**43**) analogues of salacinol (Fig. 8), in which the stereochemistries at C-3 and C-2, respectively, are inverted compared to salacinol, have also been reported.[52] These compounds were synthesized by use of PMB-protected thioethers **44** and **45**, respectively, and the cyclic sulfate **20**. The PMB-protected thioether **44** was conveniently prepared from the intermediate **46**, as shown in Scheme 12. A large-scale synthetic route for the key intermediate **46** was developed starting from cheaper D-lyxose, as opposed to the literature method starting from L-lyxose,[53] by employing a Mitsunobu reaction as a key step to yield the required stereochemistry at C-4 (Scheme 12).

The thioether **45** was obtained from D-ribose by a literature route similar to the above protocol described for the thioether **44**.[54] Enzyme inhibition studies of **42** and **43** against MGA indicated that they were not effective inhibitors against MGA, whereas salacinol inhibited this enzyme with a K_i value of 0.19 μM.[55] Hence, it was concluded that the D-arabinitol configuration in the heterocyclic ring displayed by salacinol is critical for its activity.



Scheme 11.

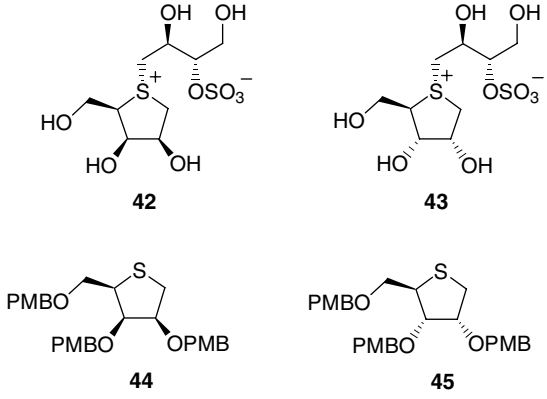
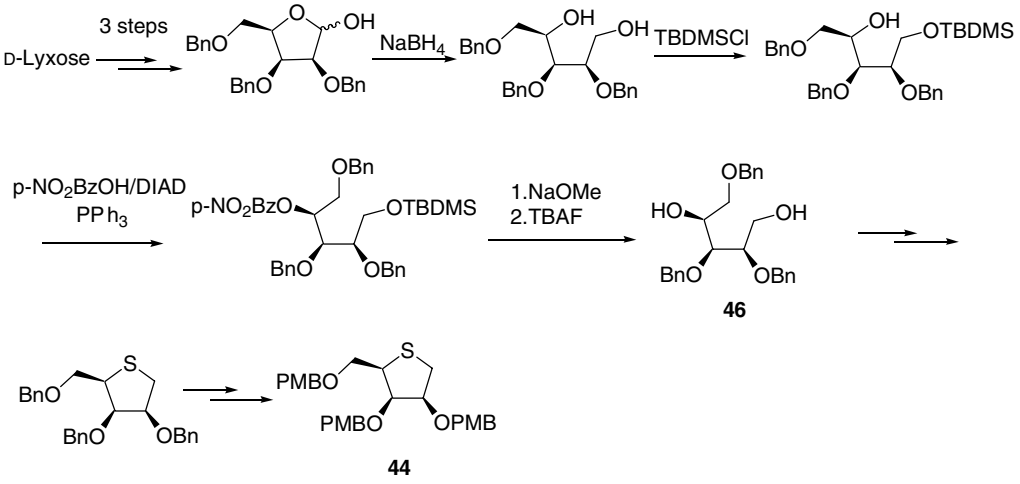


Figure 8. Anhydrothio-D-lyxitol (**42**) and anhydrothio-D-ribitol (**43**) analogues of salacinol and PMB-protected precursors to these structures, **44** and **45**.

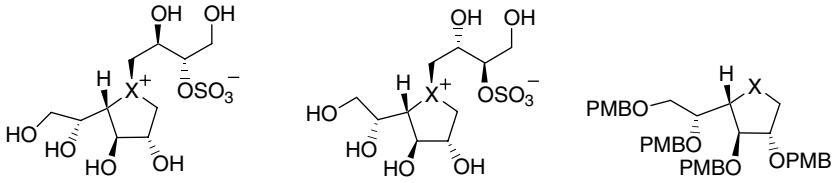
With the aim of deriving potential inhibitors of UDP-galactopyranose mutase, we have also synthesized the related sulfonium salts (**47** and **48**) with 1,4-anhydro-D-galactitol moieties,[56] as well as the corresponding nitrogen (**49** and **50**) and selenium (**51** and **52**) analogues (Fig. 9).[57] The syntheses of the nitrogen and selenium analogues (**49**–**52**) made use of the respective coupling partners **53** and **54**. The synthetic highlight of this report was the synthesis of **54** from D-glucose by a multi-step synthetic route that involved selective deprotection of the anomeric pentenyl glycoside in the presence of acid labile PMB ethers using NBS (Scheme 13); the corresponding imino-D-galactitol (**53**) was prepared by a slightly modified literature method.[58] Enzyme inhibition studies of the final zwitterionic compounds (**47**–**52**) indicated that they did not inhibit UDP-galactopyranose mutase.

2.5. Six-membered ring analogues

To study the effect of the size of the heterocyclic ring on glycosidase inhibitory activity, we have synthesized a series of six-membered ring analogues (**55**–**59**) of salacinol that were modeled after miglitol.[59] Each of these compounds has a six-membered, cyclic alditol structure, with a positively charged heteroatom (sulfur or selenium or nitrogen) and also contains either a D- or L-erythritol-3-sulfate side chain (Fig. 10). The coupling reactions



Scheme 12.

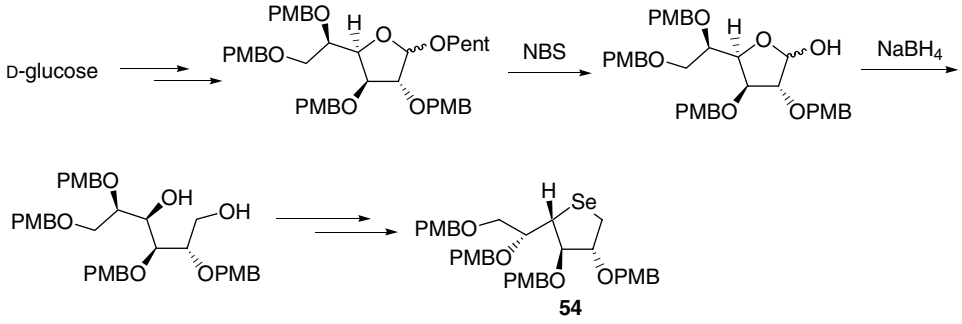


47 X=S
49 X=NH
51 X=Se

48 X=S
50 X=NH
52 X=Se

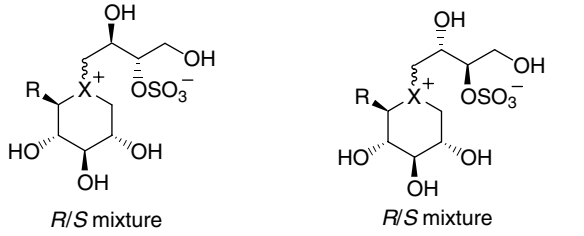
53 X=NH
54 X=Se

Figure 9. 1,4-Anhydro-D-galactitol analogues synthesized as potential inhibitors of UDP-galactopyranose mutase.



54

Scheme 13.

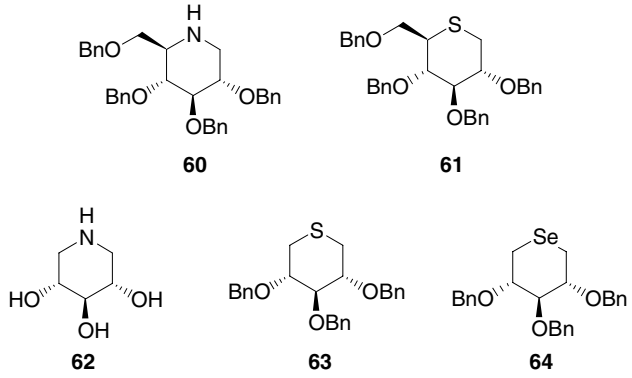


R/*S* mixture

R/*S* mixture

55a	X=NH	R=CH_2OH	**55b**	X=NH	R=CH_2OH
56a	X=NH	R=H	**56b**	X=NH	R=H
57a	X=S	R=H	**57b**	X=S	R=H
58a	X=S	R=CH_2OH	**58b**	X=S	R=CH_2OH
59a	X=Se	R=H	**59b**	X=Se	R=CH_2OH

Figure 10. Six-membered ring analogues of salacinol.



60 **61** **62** **63** **64**

Figure 11. Heterocyclic ethers used in the synthesis of **55**–**59**.

were performed by the standard protocol using the respective heterocyclic ethers **60**–**64** (Fig. 11) and the cyclic sulfates **20** and **21**. Unlike the five-membered ring analogues, the final products were obtained as mixtures of epimers, separable in some cases, differing only at the configurationally stable sulfur or selenium atoms. The required starting materials were prepared using literature methods (**62**)[60] or readily obtained in one (**60**[61] and **61**[21]) or two steps (**63** and **64**)[62] from known intermediates. An important conclusion derived from the enzyme inhibition studies was that the five-membered ring of salacinol (or heteroanalogues) is a necessary and key structural determinant for its activity, as all the six-membered ring analogues were inactive against MGA or HPA.

Recently, Gallienne et al.[63] reported the syntheses of two series of five- and six-membered ring analogues of salacinol involving thioanhydro alditol moieties with *erythro*, D, L-*threo*, *xylo*, *ribo*, D-*arabino*, and D-*manno* configurations (**65**–**74**, Fig. 12), including the previously reported six-membered ring analogue **57b**.[59] Apart from synthesizing these zwitterionic compounds, by following the general strategy for the coupling reaction, they have reported an alternative synthesis of the L- and D-cyclic sulfates (**18** and **21**) from a common intermediate (**75**) that was readily obtained from 4,6-*O*-benzylidene-D-glucose (**76**).[40] This method exploits two one-pot procedures to obtain the required cyclic sulfates from 4,6-*O*-benzylidene-D-glucose (Scheme 14). The synthesis began

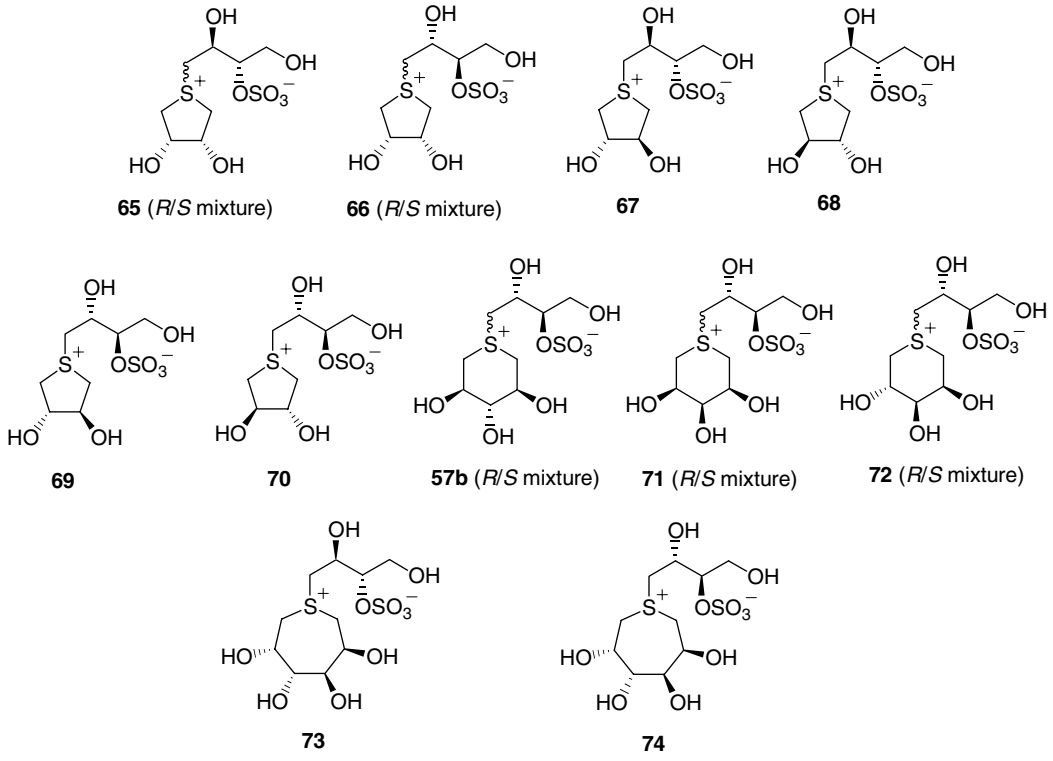
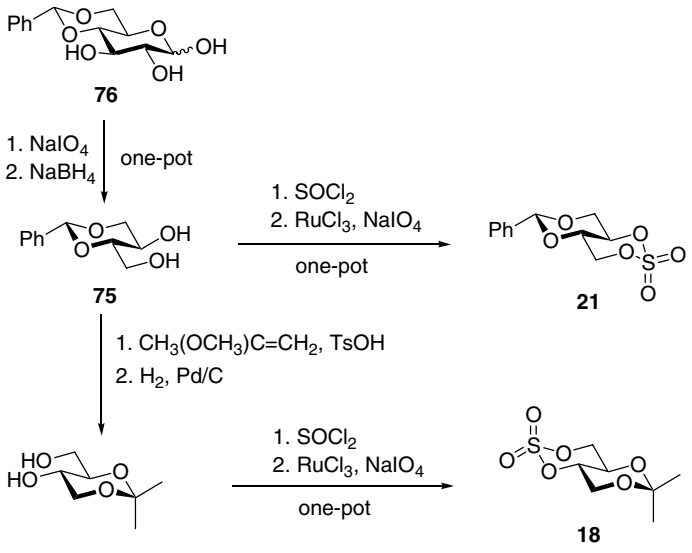


Figure 12. Analogues of salacinol synthesized by Gallienne et al.[63]



Scheme 14.

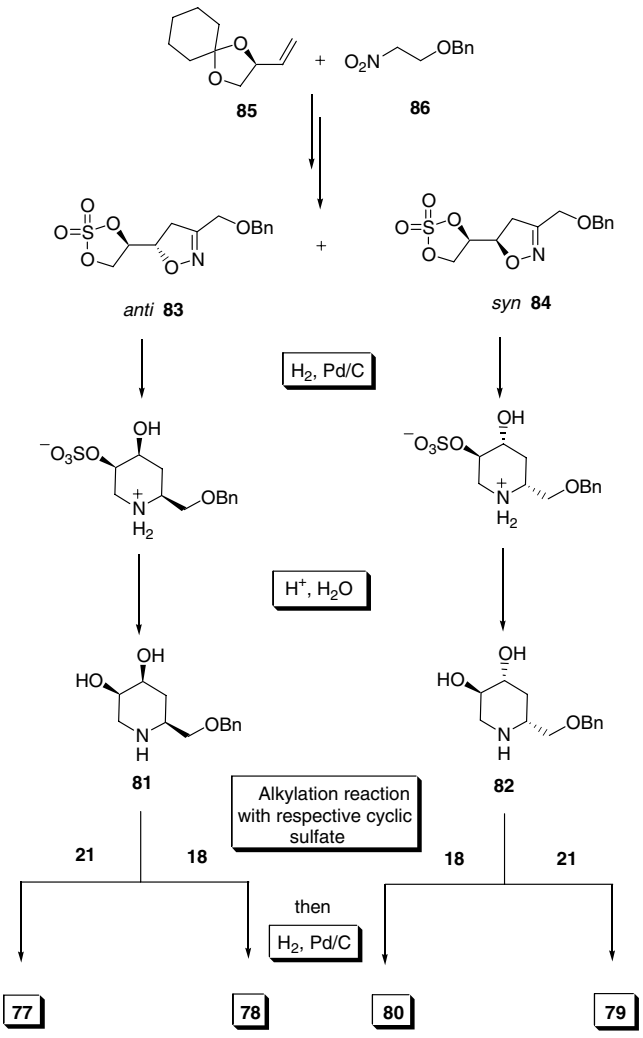
with the periodate oxidation of benzylidene-protected D-glucose **76**, followed by the reduction of the resulting aldehyde with $NaBH_4$ in one pot. The benzylidene-protected diol **75** was transformed into the required D-cyclic sulfate **21** using a one-pot procedure. In the case of the L-cyclic sulfate (**18**), the diol (**75**) was first protected as an isopropylidene ketal and the benzylidene-protecting group was then selectively removed. The resulting isopropylidene-protected diol was subjected to the one-pot procedure, as before, to give the L-cyclic sulfate (**18**).

These compounds (**65**–**74** and **57b**) were screened against six different commercial glycosidases including the rice-derived α-glucosidase. All compounds from the five-membered ring series (**65**–**70**) that are missing the hydroxymethyl group at C-4 when compared to salacinol were inactive against the α-glucosidase from rice,

whereas, salacinol (**13**) had been shown previously to exhibit a strong inhibitory effect toward this enzyme ($IC_{50} = 1.1 \times 10^{-3}$ mM).[64] Hence, it was concluded that the hydroxymethyl group of salacinol is essential for its inhibitory activity toward this particular enzyme. The six- and seven-membered ring analogues exhibited only very weak activities or no activity against the glycosidases tested, except for the xylitol analogue **57b** (*R*/*S* mixture), which showed an inhibitory effect against β-glucosidase from almond. Based on this study and our enzyme inhibition data for the various six-membered ring analogues (**55**–**59**),[59] we conclude that, in general, the ring expansion of the heterocycle displayed in salacinol leads to loss of inhibitory activity.

This conclusion was corroborated, when Gallienne et al.[65] synthesized a new series of salacinol analogues (**77**–**80**, Fig. 13) containing six-membered ring nitrogen heterocycles and tested them against six commercial glycosidases including rice α-glucosidase and baker's yeast α-glucosidase. As observed with the other six-membered ring analogues, none of the synthesized ammonium salts were active against the glycosidases tested.

The synthetic route involved the facile synthesis of two, enantiomerically pure, iminosugars (**81** and **82**) and their further transformation into the ammonium-ion analogues (**77**–**80**) by reacting them with cyclic sulfates **18** and **21** (Scheme 15). The syntheses began with the preparation of the novel isoxazoline cyclic sulfates, **83** and **84**, in three steps starting from the optically pure alkene **85**[66] and the nitro compound **86**.[67] Hydrogenolysis of the isoxazolines **83** and **84**, followed by acid treatment of the resulting zwitterionic intermediates, gave the iminosugars **81** and **82**, respectively. Alkylation reactions of these iminosugars with the cyclic sulfates **18** and **21**, followed by the removal of the protecting group, gave the final zwitterionic ammonium salts.



Scheme 15.

Recently, the synthesis of related six-membered ring sulfonium ions (**87**–**90**), containing 1,5-anhydro-5-thio-L-fucitol as a core structure, as potential α-L-fucosidase inhibitors was described.[68,69] These compounds were obtained from the alkylation reactions of 1,5-anhydro-5-thio-L-fucitol (**91**) with the cyclic sulfates, **20** and **21**, or various alkyl halides (Scheme 16).

The starting material, 1,5-anhydro-5-thio-L-fucitol (**91**), was readily prepared in few steps from a selectively protected mannopyranose derivative (**92**),[70,71] as shown in Scheme 17. The synthesis began with the nucleophilic addition of a methyl group to the intermediate **92** via a Grignard reagent, and then, sodium periodate oxidation and sodium borohydride reduction reactions were performed sequentially to get the diol **93**. This diol was further transformed into the required anhydrothiofucitol derivative (**91**) by forming the corresponding dimesylate followed by its reaction with sodium sulfide.

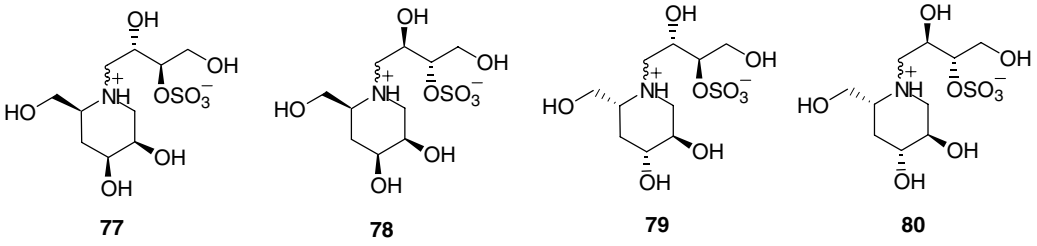
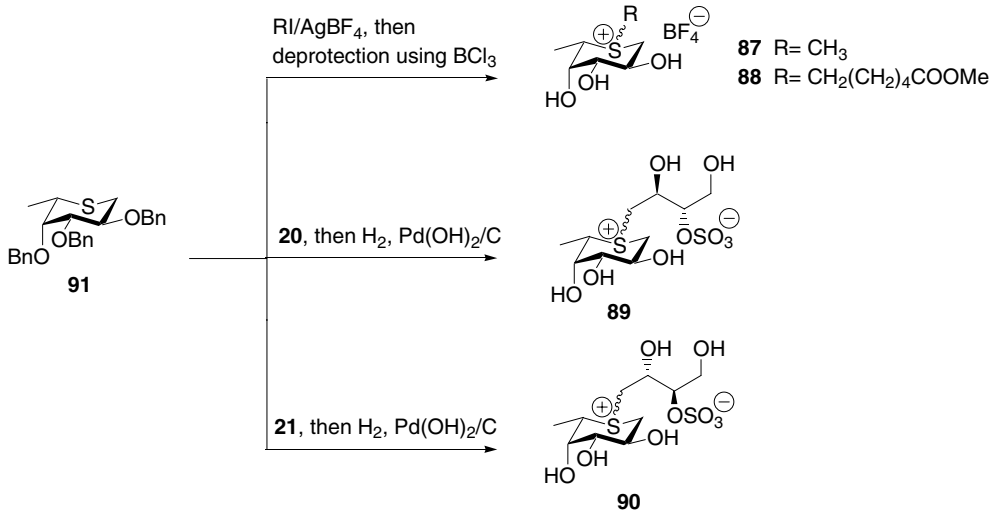
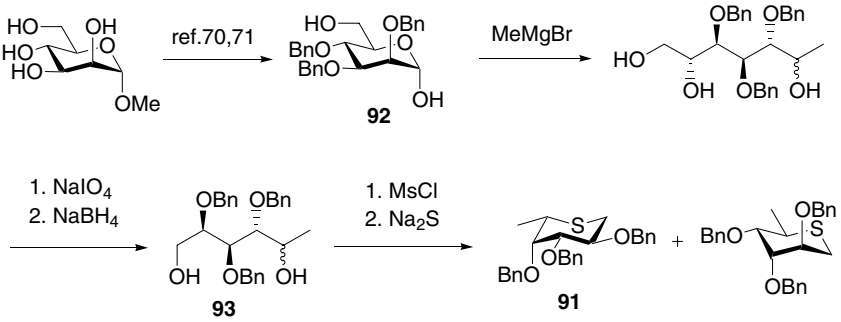


Figure 13. Salacinol analogues synthesized by Gallienne et al.[65] containing a six-membered ring nitrogen heterocycle.



Scheme 16.



Scheme 17.

2.6. Chain-modified analogues

Based on the fact that kotalanol (**14**), with a longer polyhydroxylated side chain, has been reported to possess greater inhibitory activity against certain glucosidase enzymes when compared to salacinol (**13**),[23] much attention has been directed toward the synthesis of acyclic chain-modified analogues of salacinol. Accordingly, several chain-modified analogues have been synthesized and tested for their glucosidase inhibitory properties. These modifications include the extension of the acyclic chain, the change in the position or complete removal of the sulfate group, and the introduction of acyclic chains that are missing one or two hydroxyl groups when compared to salacinol.

2.6.1. Chain-extended analogues. In 2006, we reported the synthesis and enzyme inhibitory activity of four chain-extended homologues (**94**–**97**) of salacinol containing polyhydroxylated, acyclic chains of 5- and 6-carbons, differing in stereochemistry at the stereogenic centers.[72,55] The syntheses involved the reactions of anhydro-4-thio-D-arabinitol (**22**) with cyclic sulfate derivatives of different monosaccharides (**98**–**101**,[73–76] Fig. 14). Deprotection of the benzyl groups followed by reduction of the hemiacetal intermediates (**102**–**105**) gave the final compounds **94**–**97** (Scheme 18). With the exception of compound **94**, all the other compounds **95**–**97** inhibited MGA, with K_i values of 0.25, 0.26, and 0.17 μM, respectively. By comparison of the stereo-

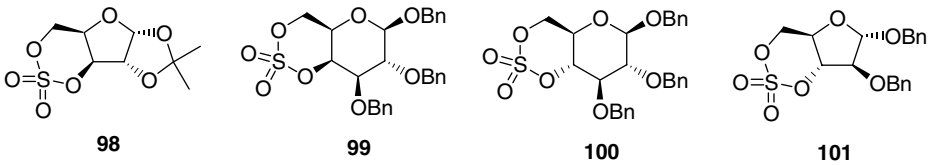
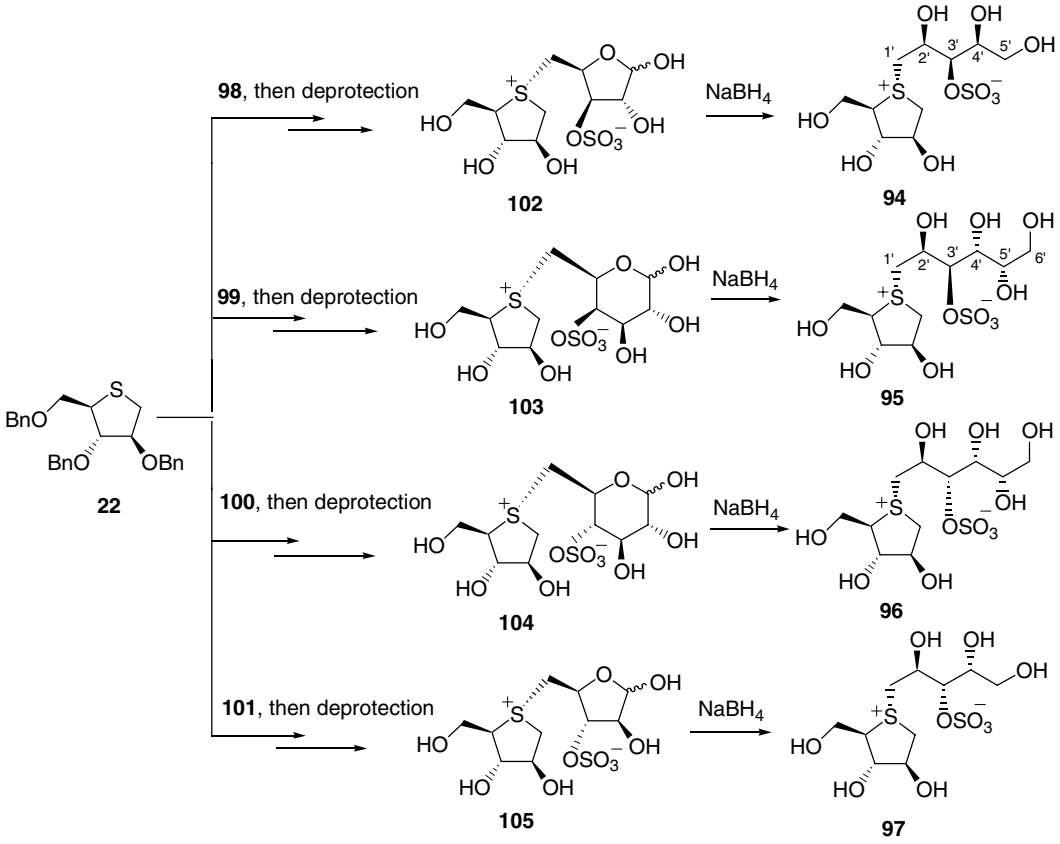


Figure 14. Cyclic sulfates used in the synthesis of chain-extended homologues of salacinol containing polyhydroxylated, acyclic chains of 5- and 6-carbons.



Scheme 18.

chemistry of the stereogenic centers in the extended acyclic chain and the observed inhibitory properties of the four compounds, it was concluded that the *R*-stereochemistry of the C-4′ stereogenic center is critical for activity. The hemiacetal derivatives **102**–**105** were also tested for their glycosidase inhibitory properties; however, these compounds were not active against MGA.

Recently, we also reported a series of six chain-extended analogues (**106**–**111**, Fig. 15) with heteroatom variations, containing polyhydroxylated, extended acyclic chains of 6-carbons.[77] These analogues were prepared by coupling the PMB-protected D- and L-anhydroseleno-, anhydrothio-, and iminoarabinitols (**24**, **36**, and **112**–**115**,[34,47,46] Fig. 15) with the cyclic sulfate **116** that was readily prepared in three steps from D-sorbitol, as shown in Scheme 19.

The enzyme inhibition studies against MGA revealed that the sulfur analogue **107** with the D-arabinitol configuration in the heterocyclic ring did not inhibit MGA, while the corresponding selenium (**106**) and nitrogen (**108**) analogues inhibited MGA with K_i values of 41 and 26 μM, respectively. Interestingly, the sulfur and nitrogen congeners with the L-arabinitol configuration in the heterocyclic ring, **110** and **111**, were also active, with K_i values of 25 and 5 μM, respectively.

Recently, the syntheses of the acyclic, chain-modified, sulfur (**117**) and selenium (**118**) analogues (Fig. 16), with an extended polyhydroxylated acyclic chain of

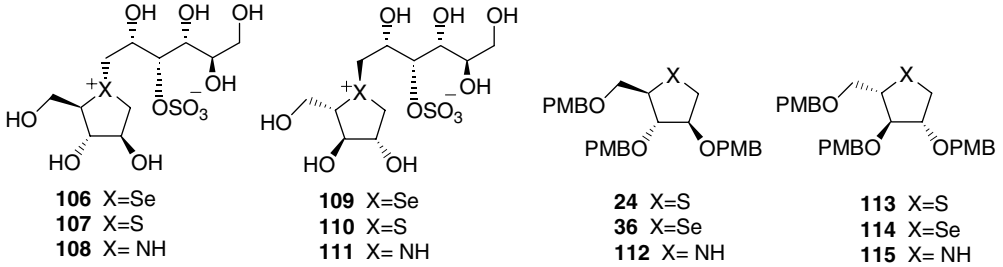
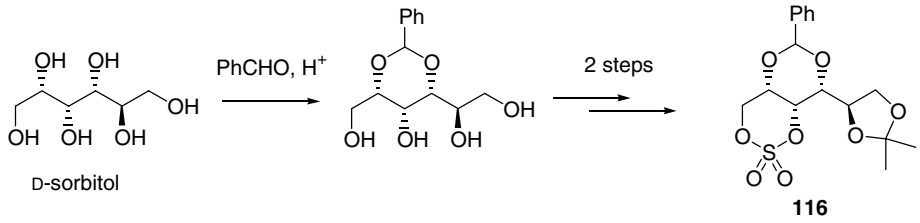


Figure 15. Chain-extended analogues of salacinol with heteroatom variations, containing polyhydroxylated, extended acyclic chains of 6-carbons (**106**–**111**), and precursors used in their synthesis (**24**, **36**, **112**–**115**).



Scheme 19.

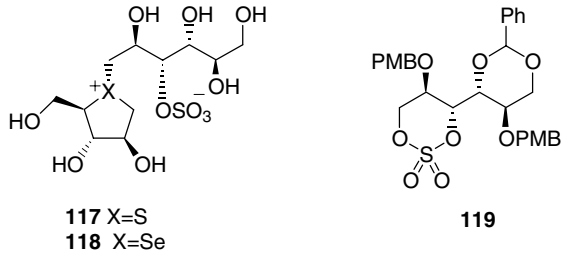


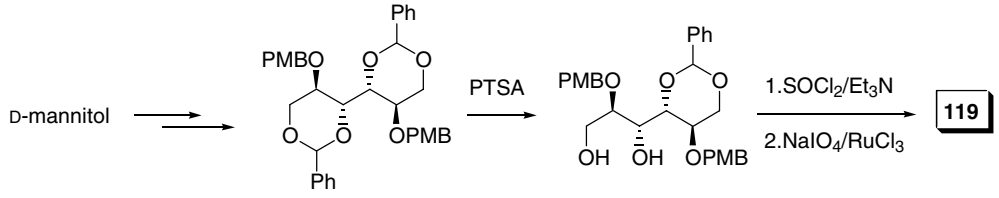
Figure 16. Sulfur (**117**) and selenium (**118**) analogues of salacinol, with an extended polyhydroxylated acyclic chain of 6-carbons and the cyclic sulfate (**119**) used in their synthesis.

6-carbons, have been reported.[78] These analogues were synthesized using the cyclic sulfate **119**. The synthesis of **119** was achieved by the selective acetolysis of a suitably protected D-mannitol derivative followed by 1,3-cyclic sulfate formation, as shown in Scheme 20. The main reason for the use of the cyclic sulfate **119** was to maintain the stereochemistry at C-2′ and C-3′ as in salacinol (**13**), and the stereochemistry at C-4′ as in the other active chain-extended analogues (**95**–**97**) that were reported previously.[72] Enzyme inhibition studies indicated that **117** and **118** were effective inhibitors of MGA, with K_i values of 0.65 and 0.14 μM, respectively.

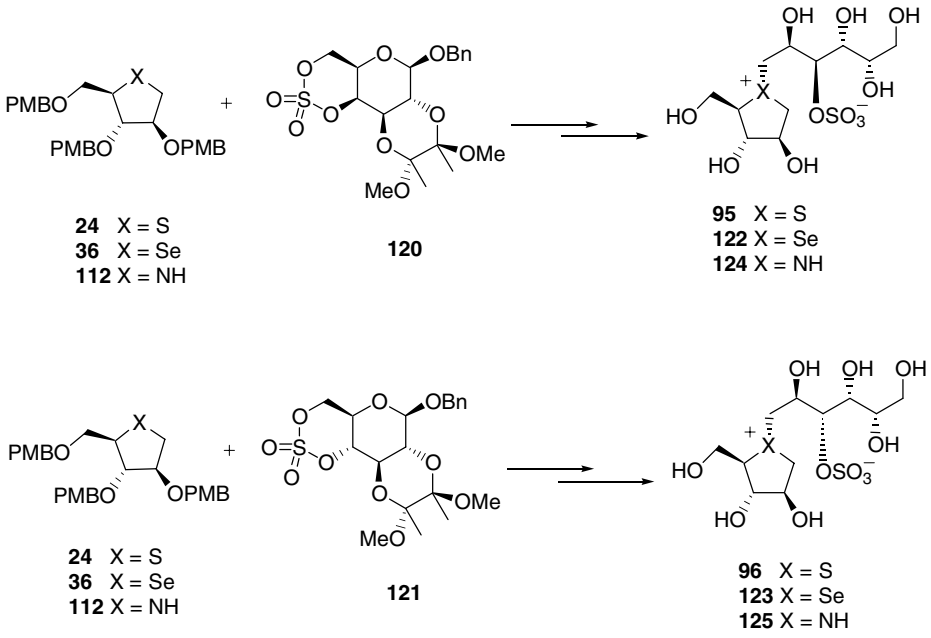
We have developed an alternative synthetic route to the active chain-extended analogues **95** and **96** (Scheme 21) that involves the use of 1,3-cyclic sulfates (**120** and **121**) protected with an acid sensitive, butane diacetal (BDA)-protecting group compared to the previous synthetic route, which used 1,3-cyclic sulfates with benzyl-protecting groups (Scheme 18 and Fig. 14).[72] This new strategy eliminates the difficulties of the previous synthetic route with respect to the removal of the benzyl-protecting groups, which limited our access to the corresponding selenium analogues; hence, by use of BDA-protected cyclic sulfates **120** and **121**, the corresponding selenium analogues, **122** and **123**, were synthesized successfully (Scheme 21).[79] In addition, the corresponding nitrogen analogues, **124** and **125**, were also synthesized. The cyclic sulfates **120** and **121** were readily obtained in two steps from benzyl β-D-galactopyranoside (**126**)[80] and benzyl β-D-glucopyranoside (**127**),[81] respectively, as shown in Scheme 22.

Enzyme inhibition studies indicated that the selenium analogues **122** and **123** inhibited MGA, both with K_i values of 0.10 μM.[79] These compounds constitute the most active to date in this series of zwitterionic glycosidase inhibitors. However, the nitrogen analogues **124** and **125** were less active compared to the corresponding sulfur and selenium analogues, with K_i values of 35 and 8 μM, respectively. These data corroborate our previous conclusion with other derivatives (**95**–**97** and **117**–**118**),[72,78] that is, the configuration at C-3′ does not appear to be critical for inhibitory activity, as each of the pairs with enantiomeric configurations at C-3′, **122** and **123**; **124** and **125** has similar K_i values.

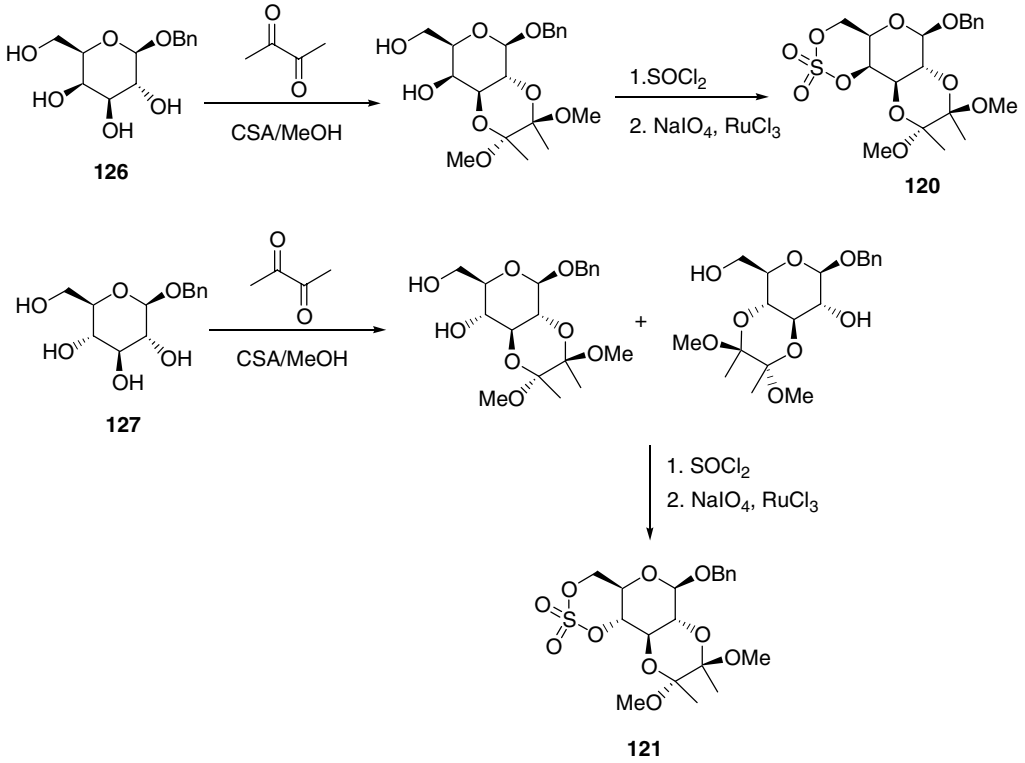
Comparison of the K_i values of the sulfonium and selenonium chain-extended analogues (**94**–**97**, **106**, **107**, **117**, **118**, **122**, and **123**) against MGA indicated that for both the 5- and 6-carbon chain-extended series, the *S*-configuration at C-2′ and the *R*-configuration at C-4′ are critical for activity (the highlighted portions in Table 2).[72,77–79,55] Furthermore, the configuration at C-3′, bearing the sulfate moiety, was found to be unimportant. The configurational requirement at C-5′ was not clear because the trend was found to depend on the heteroatom. However, the overall conclusion was that the effect of the acyclic chain extension did not confer any dramatic change on inhibitory properties for this particular enzyme, because the K_i values of the active chain-extended analogues were in a similar range to those of salacinol (**13**) and blintol (*trans*-**29**, 0.49 μM).



Scheme 20.



Scheme 21.



Scheme 22.

2.6.2. Deoxy-salacinol analogues. Recently, Muraoka et al.[82] reported the syntheses and glycosidase inhibitory properties of three deoxy-salacinols lacking one (**128** and **129**) or two polar (**130**) substituents in the side chain of salacinol (**13**); the syntheses made use of the thioarabinitol **16** and the cyclic sulfates **131**–**133** as coupling partners (Fig. 17). In addition, an alternative synthetic route to **16** was achieved via the selective *tert*-butyldimethylsilyl (TBDMS)-protection of the primary hydroxyl group of the intermediate **134** that was prepared from

Table 2. K_i values against MGA for various chain-extended analogues of salacinol with an identical anhydro-D-arabinitol configuration in the heteroalditol ring and acyclic chains of 5- or 6-carbons with different stereochemistry at the stereogenic centers

Inhibitor	Stereochemistry at the stereogenic centers in the acyclic chain				K_i (μM)	Reference
	C-2′	C-3′	C-4′	C-5′		
94[a]	*S*	*R*	*S*	NA	NI	72
95[a]	*S*	*R*	*R*	*S*	0.25	
96[a]	*S*	*S*	*R*	*S*	0.26	
97[a]	*S*	*S*	*R*	NA	0.17	
106[b]	*R*	*S*	*R*	*R*	41	77
107[a]	*R*	*S*	*R*	*R*	NI	
117[a]	*S*	*S*	*R*	*R*	0.65	78
118[b]	*S*	*S*	*R*	*R*	0.14	
122[b]	*S*	*R*	*R*	*S*	0.10	79
123[b]	*S*	*S*	*R*	S	0.10	

NA = not applicable; NI = no inhibition.

[a] Sulfur analogues.

[b] Selenium analogues.

D-xylose in two steps (Scheme 23). Benzylation of the C-3 hydroxyl of **135**, followed by removal of the other protecting groups and reaction with mesyl chloride, gave the dimesylate **136**. Compound **136** was then converted into the thioarabinitol **16** in three steps, as shown in Scheme 23. The cyclic sulfate **133** was synthesized from 1,3-*O*-benzylidene-L-erythritol (**75**) via deoxygenation of the xanthate intermediate **137**, as shown in Scheme 24, while the other cyclic sulfates (**131** and **132**) were synthesized using modified literature methods.[83–88]

Enzyme inhibition studies indicated that compounds **129** and **130** having acyclic chains that lack the hydroxyl group at C-2′, compared to the acyclic chain of salacinol (**13**), were not effective inhibitors of intestinal α-glucosidases in vitro. However, compound **128**, lacking a hydroxymethyl group at C-3′, retained some inhibitory activity against sucrase ($IC_{50} = 780$ μM). These results suggested that both the hydroxymethyl group at C-3′ and the hydroxyl group at C-2′ with an *S*-configuration are essential for α-glucosidase inhibitory activity.

2.6.3. Frame-shifted analogues. To probe the effect of the positioning of the sulfate group in the acyclic chain, we have synthesized the chain-modified analogues (**138**–**141**, Fig. 18) with an extended chain of 5-carbons in which the position of the sulfate moiety was shifted from C-3′ to C-4′ compared to salacinol (**13**) and other chain-extended analogues (Table 2).[89] The target compounds were synthesized by the reactions of the heteroalditols **24** and **36** with the cyclic sulfates **142** and **143**, followed by removal of the protecting groups. The synthetic highlight was that three of the four required coupling partners, namely, the seven-membered cyclic sulfate (**142**), the PMB-protected thioether (**24**), and selenoether (**36**), were derived from a common diol precursor **144**, as shown in Scheme 25. The other cyclic sulfate **143** was synthesized in a similar manner starting from the

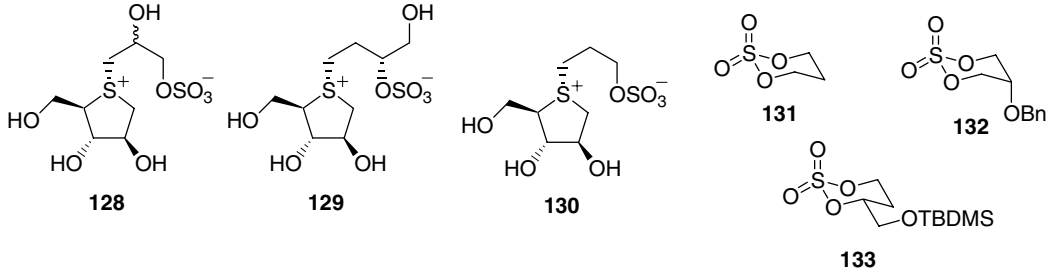
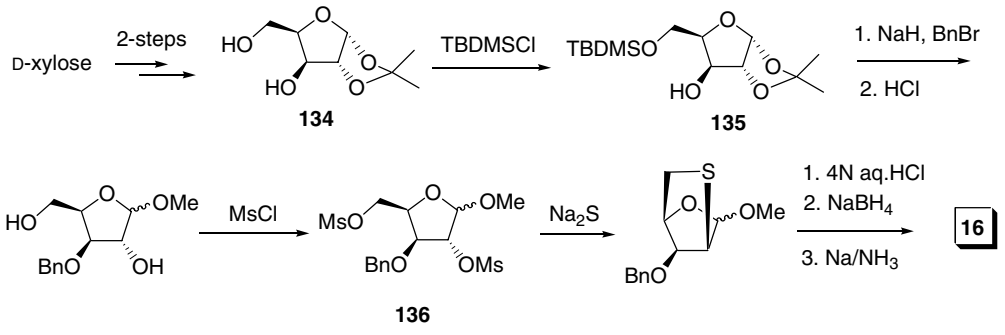
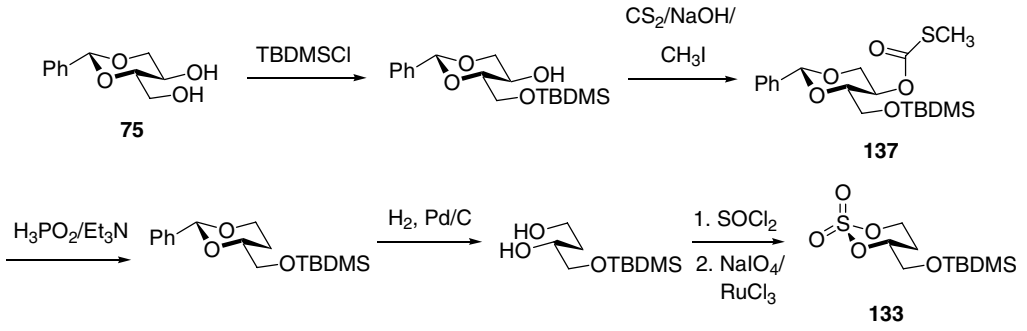


Figure 17. Synthetic deoxy-salacinols lacking one (**128** and **129**) or two polar (**130**) substituents in the side chain and the cyclic sulfates (**131**–**133**) employed as coupling partners.



Scheme 23.



Scheme 24.

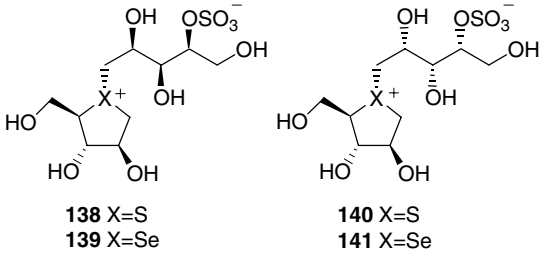
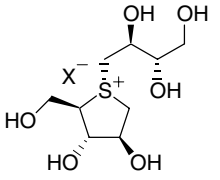


Figure 18. Chain-modified analogues in which the position of the sulfate moiety is shifted from C-3′ to C-4′.

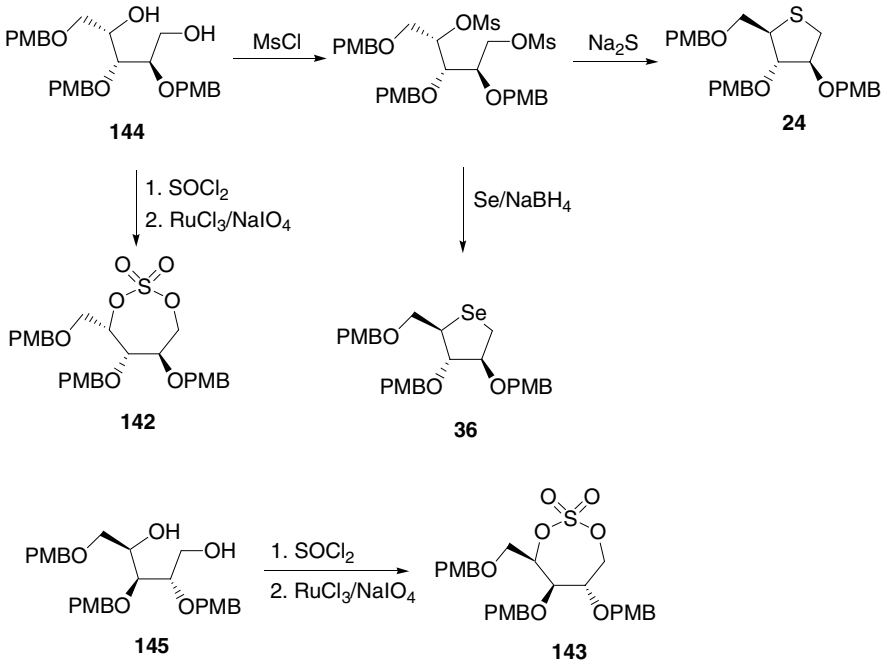
enantiomeric diol **145** (Scheme 25). The diols **144** and **145** were synthesized in turn from D- or L-xylose according to the procedure developed recently in our laboratory.[47] The target compounds **138** and **139** showed inhibition of MGA with K_i values of 20 and 53 μM, respectively. In contrast, compounds **140** and **141** were not active against MGA.[89]

2.6.4. De-O-sulfonated analogues. Very recently, Tanabe et al.[90] reported the de-O-sulfonated analogues of salacinol (**146** and **147**, Fig. 19) and studied the role of



	IC_{50} (μM)	
	Maltase	Sucrase
13	9.6	2.5
146	15.6	3.7
147	14.0	3.5

Figure 19. De-O-sulfonated analogues of salacinol (**146** and **147**) and their inhibitory activities.



Scheme 25.

the sulfate anion moiety in the acyclic chain of salacinol (**13**) on inhibitory activity. The de-O-sulfonated salacinol **146** was synthesized by de-O-sulfonation of salacinol (**13**) using methanolic hydrogen chloride, and compound **147** was obtained by simply exchanging the monomethyl sulfate counterion of **146** with chloride counterion using an ion exchange resin. The enzyme inhibition studies indicated that the internal sulfate counterion is not essential for inhibitory activity as the de-O-sulfonated analogues (**146** and **147**) showed almost equal inhibitory activities against intestinal α-glucosidases (in vitro) when compared to salacinol (**13**), irrespective of the external counterion (Fig. 19). Presumably, Nature biosynthesizes the sulfated analogues for purposes of stability, solubility, or more facile transport into cells.

2.7. Carboxylate analogues

In an attempt to understand the role of the sulfate group present in the acyclic chain of ghavamiol (**25**), we have synthesized an unusual class of amino acids **148** and **149** (Fig. 20).[91] These analogues were considered to be the carboxylate analogues of ghavamiol. Compound **148** was found to be an inhibitor of MGA with a K_i value of 21 μM. In addition, this compound was also active against *Drosophila melanogaster* Golgi mannosidase II (dGMII), with an IC_{50} of 0.3 mM. Compound **149** was not active against either MGA or dGMII. The crystal structure of **148** bound in the active site of dGMII indicated that the hydroxyl groups from the acyclic chain and also the carboxylic acid group form extensive contacts with both side chains and water molecules in the active site.[91] Previously, the role of the sulfate group on glycosidase inhibitory activity was inferred by Yuasa et al.;[92] the docking of salacinol into the binding site of glucoamylase indicated close contacts between the sulfate ion and Arg305.

The key step in the synthesis of compound **148** was the reaction of the iminoarabinitol **150** with the epoxide **151** that was prepared from vitamin C using a simplified literature procedure.[93] Debenzylation of the coupled product **152** followed by stereoselective catalytic reduction of the double bond of the L-ascorbic acid moiety produced the lactone **153**. The desired amino acid **148** was derived from the lactone **153** by hydrolysis followed by ion exchange resin treatment, as depicted in Scheme 26. Compound **149** was prepared in a similar fashion using the iminoarabinitol **154**.

Very recently, using the epoxide **155**[94] and the thioethers **22** and **23**, the sulfonium-ion analogues, **156** and **157** (Fig. 21), with an internal carboxylate counterion were also synthesized by our group.[95] Compound

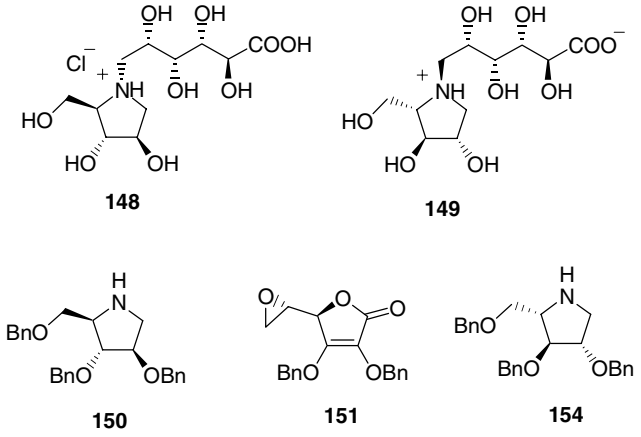


Figure 20. Carboxylate analogues of ghavamiol (**148** and **149**) and compounds used in their preparation (**150**, **151**, and **154**).

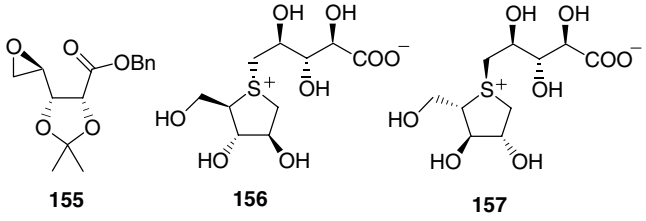
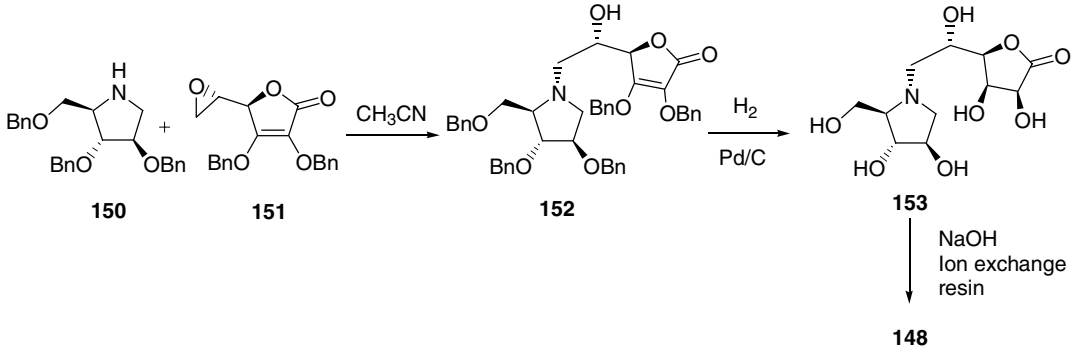


Figure 21. Carboxylate analogues of salacinol (**156** and **157**) and the protected epoxy ester **155** used in their synthesis.



Scheme 26.

156, with the D-arabinitol configuration in the heterocyclic ring, inhibited MGA with a K_i value of 10 μM, whereas compound **157** did not show significant inhibition against MGA.[95]

2.8. Phosphate derivatives

We have synthesized the phosphate derivatives related to salacinol (**158** and **159**, Fig. 22) that are missing the hydroxyl groups in the side chain and screened them against MGA.[26,96] Compounds **158** and **159** were considered to be the prototypes of the phosphate analogue **160** that has the exact structural features of salacinol except for the phosphate group that replaces the sulfate moiety at C-3′ (Fig. 22). However, compounds **158** and **159** were not effective against MGA. We have also attempted the synthesis of the exact phosphate analogue of salacinol **160** using the cyclic phosphate **161** or **162** and the thioether **22**.[26,96] Unfortunately, the coupling reaction did not proceed as planned even after several attempts under various reaction conditions.

2.9. 2-Amido- and 2-amino derivatives

The syntheses of 2-acetamido (**163**) and 2-amino (**164**) derivatives of salacinol were also attempted (Fig. 23).[97] However, it was found that these derivatives were unstable and underwent ring-opening reactions to give the acyclic amido sulfate **165** and ammonium sulfate **166**.

The synthesis started with the key azido intermediate **167**, obtained from 1,4-anhydro-4-thio-D-ribose.[54] As depicted in Scheme 27, sequential reduction and acetylation of the intermediate **167**, followed by a change of protecting groups, gave the required coupling partner **168** for the synthesis of the acetamido derivative **163**. However, the coupling reaction between **168** and the cyclic sulfate **20** did not lead to the desired product, giving instead the ring-opened product, an acyclic amido sulfate **165**.

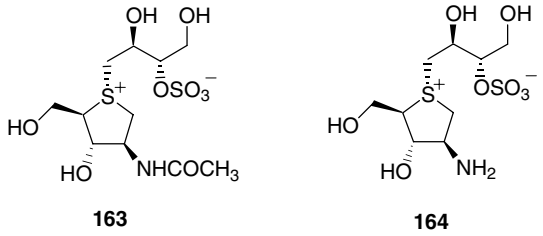


Figure 23. 2-Acetamido (**163**) and 2-amino (**164**) analogues of salacinol.

This ring-opening reaction can be attributed to the nucleophilic attack of the amide oxygen on C-1 of the ring (Scheme 27). In an attempt to avoid this intramolecular reaction, the azido intermediate **169**, derived from the intermediate **167**, was coupled directly with the cyclic sulfate **20** and indeed, the alkylated product **170** was formed in this case. However, during reduction of the azido group by hydrogenation, the ring-opened product **166** was formed, presumably through nucleophilic participation of the free amine (Scheme 27).

It is of interest that the corresponding nitrogen analogues **171**–**176** are stable (Fig. 24).[98]

2.10. Other salacinol-related analogues

Salacinol analogues (**177**–**184**, Fig. 25) based on novel anhydroseleno- and anhydrothio-allitols (**185**–**188**), derived from D-gulono-γ-lactone (**189**) and L-ascorbic acid (**190**), have also been synthesized.[99] Protection of the hydroxyl groups in D-gulono-γ-lactone,[100] followed by sequential reduction and mesylation reactions,[101] produced the dimesylate that, upon treatment with selenium metal and sodium borohydride or Na_2S, produced the anhydroseleno- and anhydrothio-D-allitols, **185** and **186**, respectively, as shown in Scheme 28.

The enantiomeric allitols (**187** and **188**) were also synthesized in a similar manner (Scheme 29), except

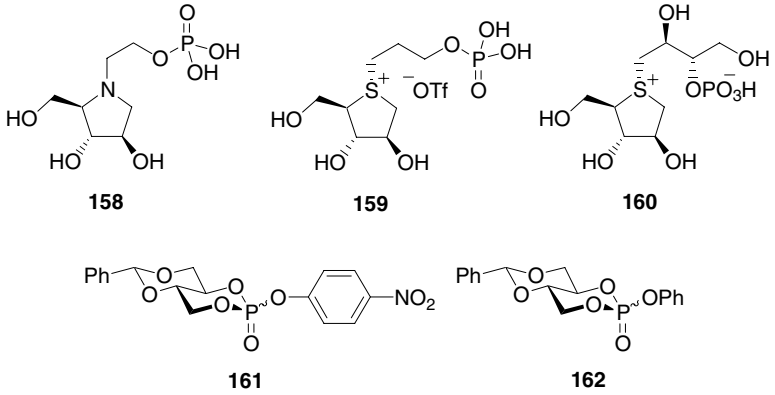
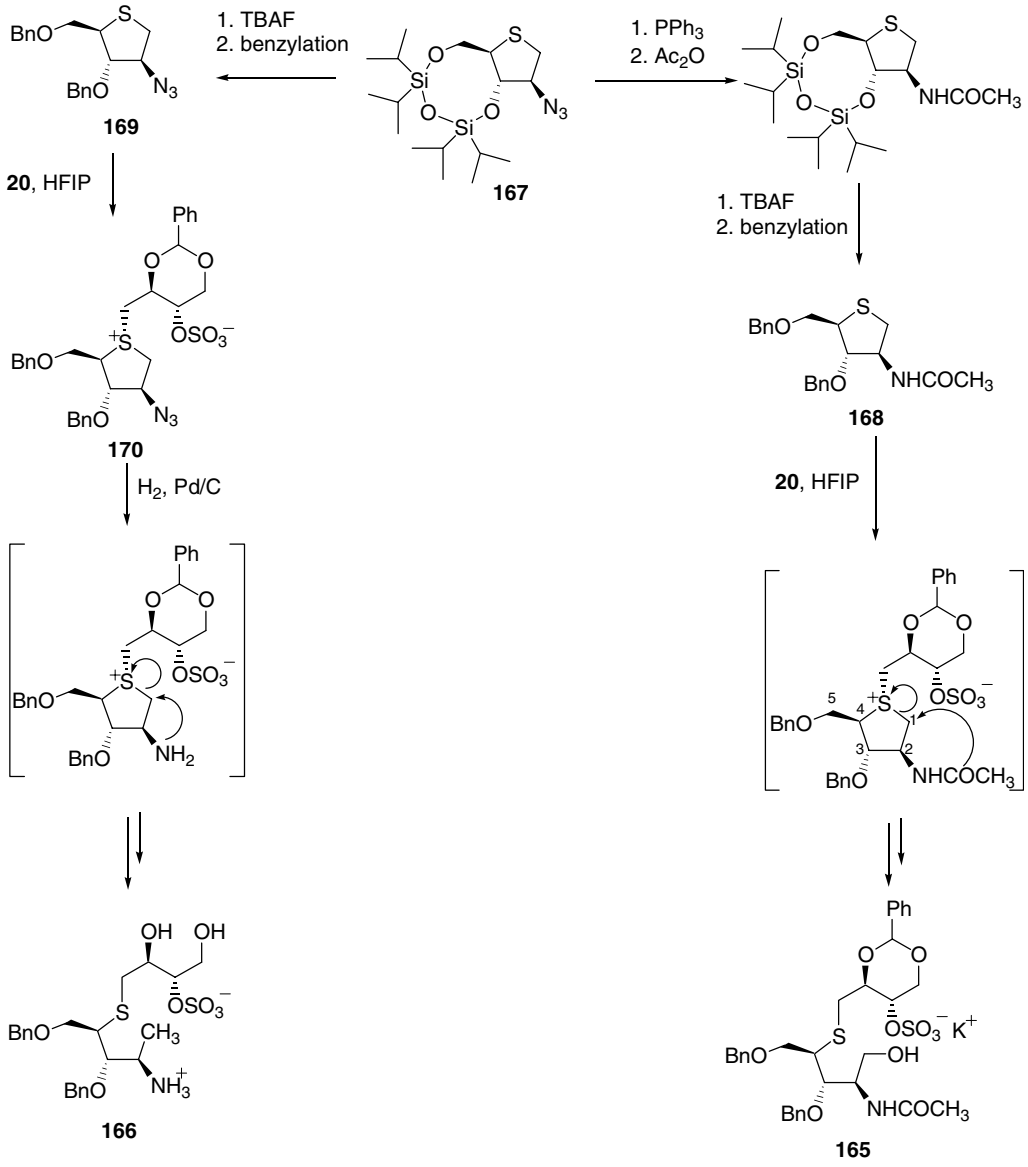


Figure 22. Phosphate derivatives related to salacinol (**158** and **159**) and the cyclic phosphate derivatives (**161** and **162**) used in the attempted synthesis of the exact phosphate analogue of salacinol, **160**.



Scheme 27.

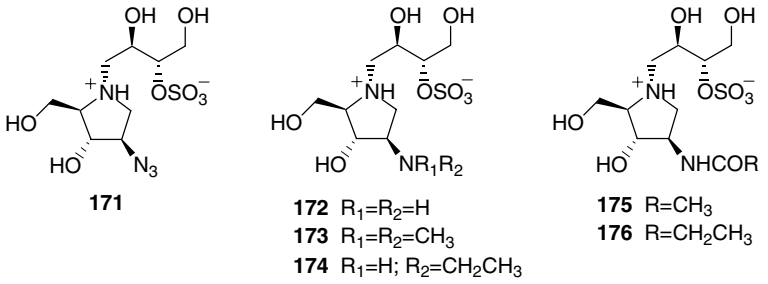


Figure 24. Analogues of ghavamiol with a nitrogen substituent at C-2.

that the syntheses started from L-ascorbic acid (**190**) instead of expensive L-gulono-γ-lactone.[102] These allitols were subjected to coupling reactions with the cyclic sulfates **20** and **116**, and the coupled products were then deprotected to obtain the zwitterionic compounds (**177**–**184**). Enzyme inhibition studies of these compounds indicated that they were not active against MGA.

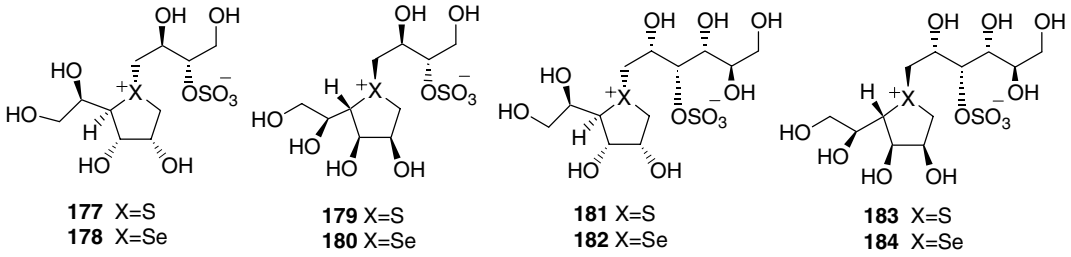
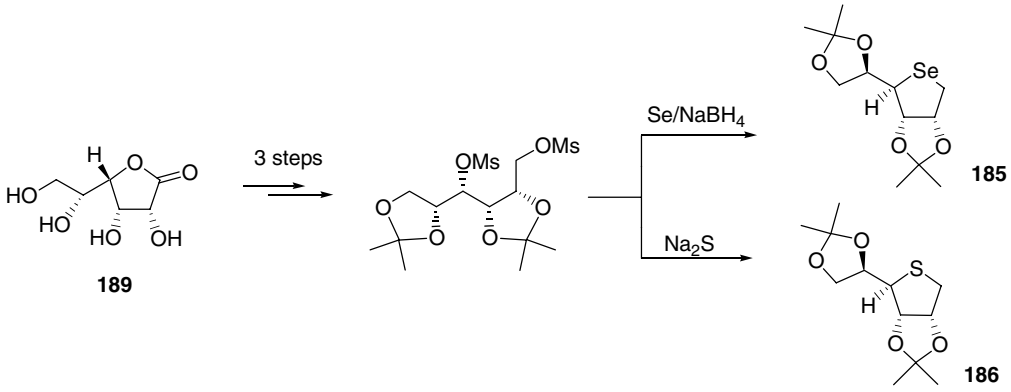
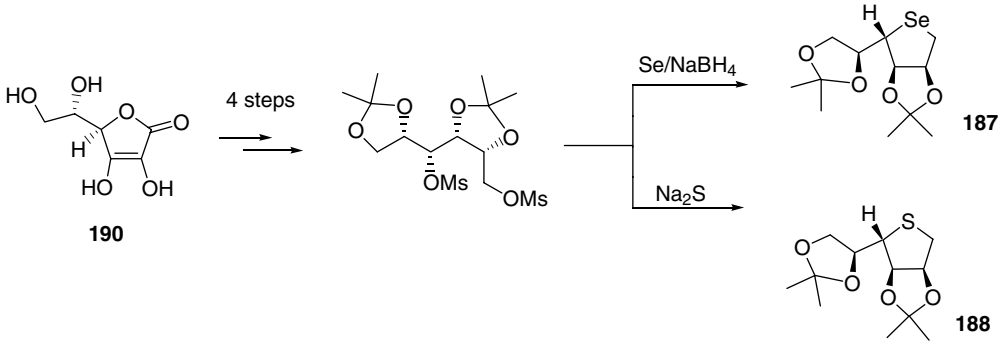


Figure 25. Salacinol analogues (**177**–**184**) derived from D-gulono-γ-lactone and L-ascorbic acid.



Scheme 28.



Scheme 29.

Using the cyclic sulfate **116**, the synthesis of another series of chain-extended analogues (**191**–**194**, Fig. 26), containing a heterocyclic five- and six-membered ring core with polyhydroxylated acyclic chains of six carbons, was reported by our group.[103] The salient feature of this report was that all of the required

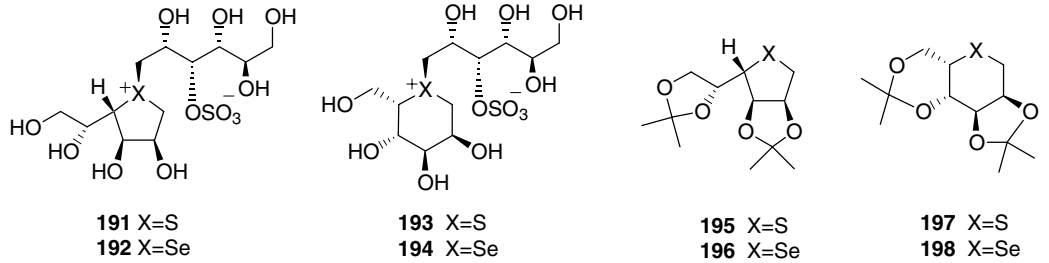
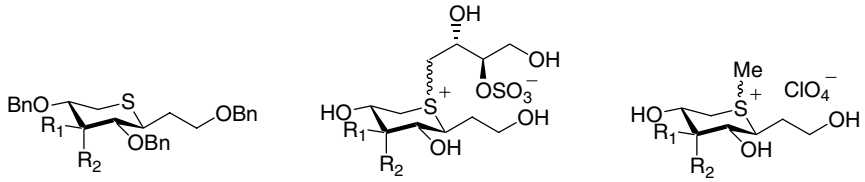


Figure 26. Chain-extended analogues (**191**–**194**) containing a heterocyclic five- and six-membered ring core with polyhydroxylated acyclic chains of six carbons, which were synthesized from **195** to **198**.

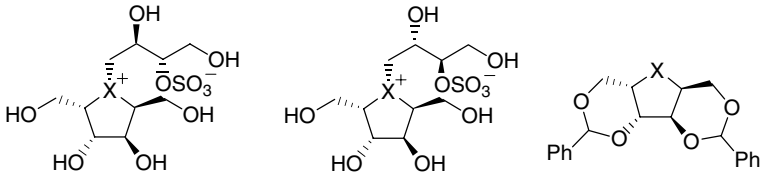


199 R_1=OBn, R_2=H
200 R_1=H, R_2=OBn

201 R_1=OH, R_2=H
202 R_1=H, R_2=OH

203 R_1=OH, R_2=H
204 R_1=H, R_2=OH

Figure 27. 5-Thioglycopyranosides **199** and **200** and the corresponding sulfonium-ion derivatives **201**–**204**.



205 X=S
207 X=NH

206 X=S
208 X=NH

209 X=S
210 X=NH

Figure 28. Sulfonium (**205** and **206**) and ammonium ion (**207** and **208**) analogues of salacinol, containing an additional hydroxymethyl group at C-1, which were obtained from thioether **209** and iminoalditol **210**.

coupling partners (**195**–**198**) were synthesized from D-mannose.

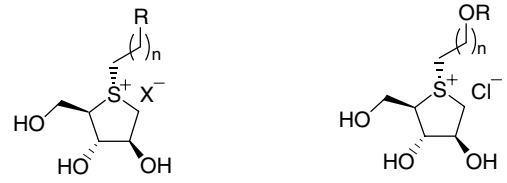
Tian et al.[104] have reported the efficient synthesis of 5-thioglycopyranosides, **199** and **200**, starting from D-xylose and D-ribose, respectively. These compounds were further transformed into the sulfonium derivatives **201**–**204** using the cyclic sulfate **21** and methyl iodide (Fig. 27). In this report, they also proposed a simple ^{13}C NMR-based method to determine the stereochemistry at the stereogenic sulfonium center of the six-membered ring sulfonium salts.

Recently, we reported the synthesis of the sulfonium (**205** and **206**) and ammonium ion (**207** and **208**) analogues of salacinol, containing an additional hydroxymethyl group at C-1, using the thioether (**209**) and iminoalditol (**210**) (Fig. 28).[105]

Our recent additions to the repertoire of salacinol-related compounds include a series of S-alkylated sulfonium ions with varying alkyl chains (**211**–**219**, Fig. 29).[106] These lipophilic compounds were shown to be effective inhibitors of MGA, with K_i values ranging from 6 to 75 μM.

3. Crystallographic and NMR/molecular modeling studies of salacinol and its analogues

We have investigated the crystal structures of salacinol (**13**), blintol (*trans*-**29**), ghavamiol (**25**), and also the stereoisomers of salacinol and blintol, **17** and **30**, respectively, bound in the active site of Golgi α-mannosidase II.[107] Although these compounds show poor



211 $R=CH_3$; X=OTf; n=12
212 $R=CH_3$; X=Cl; n=2
213 $R=CH_3$; X=Cl; n=4
214 $R=CH_3$; X=Cl ;n=6
215 $R=CH_3$; X=Cl; n=12
216 $R=CH_3$; X=Cl; n=16
217 $R=CH(CH_3)_2$; X=Cl; n=1

218 $R=CH_2CH_3$; n=5
219 $R=CH_3$; n=8

Inhibitor	K_i (μM)
211	10
212	32
213	75
214	51
215	6
216	19
217	67
218	52
219	33

Figure 29. S-Alkylated sulfonium ions with varying alkyl chains (**211**–**219**) and their inhibitory activities against MGA.

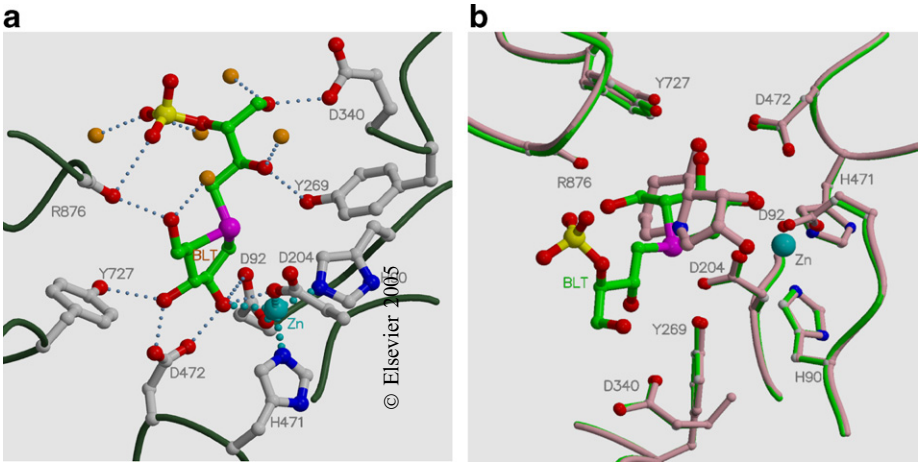


Figure 30. (a) View of the GM II enzyme interactions with compound **30**, (b) overlay of **30** and swainsonine in the active site of GM II. Reprinted with permission from Ref. 107.

inhibitory activity against GM II (in the millimolar range), they form critical interactions with the active-site carboxylate Asp 204 (Fig. 30a), as also observed in the crystal structure of swainsonine (**2**) bound in the active site of GM II. For example, the selenonium center in compound **30** is located in a similar position to the nitrogen atom in swainsonine (**2**). The hydroxyl groups OH-2 and OH-3 overlap very favorably with the OH-1 and OH-8 hydroxyl groups of swainsonine (Fig. 30b).

The transient positive charge of the transition state in the glucosidase-mediated hydrolysis reaction is presumably mimicked by the permanent positive charge provided by the sulfonium, ammonium, or selenonium ions in these compounds. The interactions between these positive centers with the side chain of Asp 204 are indicative of the electrostatic stabilization provided by the enzyme. All these compounds form T_5 coordination with the active-site Zn atom whereas swainsonine forms T_6 coordination with zinc. Hence, the lower inhibitory activities observed for these compounds might be a consequence of the lack of T_6 coordination with the active-site Zn atom.

A combined saturation transfer difference NMR (STD-NMR)/molecular modeling protocol applied to the dGMII-salacinol **13** complex was also published by our group.[108] The experimental STD-NMR results together with theoretical STD values, calculated with the CORCEMA-ST program (complete relaxation and conformational exchange matrix analysis of saturation transfer),[109,110] correlated well with the lowest-energy binding modes shown by molecular modeling. Of the different conformers obtained using Auto Dock 3.0, conformer E_4 exhibited the most favorable docked energy (-11.71 kcal mol^{-1}). This conformation was similar to that of salacinol (**13**) in the complex with dGMII in the crystal structure (Fig. 31).

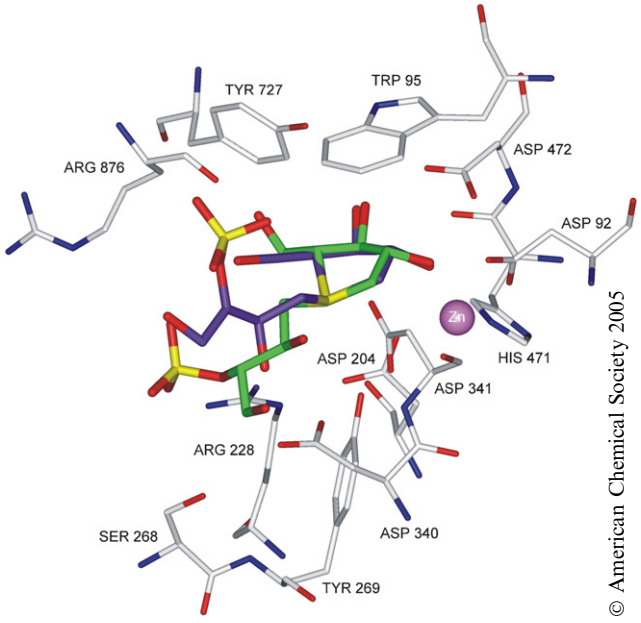


Figure 31. The predicted structure (green carbon atoms) obtained by Auto Dock 3.0, superimposed on the crystal structure of salacinol (**13**) (amethyst carbon atoms) in the active site of dGMII. Reprinted with permission from Ref. 108

4. Concluding remarks

It is of interest that studies with herbal extracts of *S. reticulata* with rats have shown control of blood glucose levels after a carbohydrate meal.[111] Studies have also shown that the oral ingestion of an extract from a *S. reticulata* trunk at a dose of 5000 mg kg^{-1} had no serious acute toxicity or mutagenicity in rats.[112] It is also noteworthy that a double-blind study of the effects of the extract from *S. reticulata* on human patients with type II diabetes mellitus has shown that the extract is an effective treatment of type II diabetes with side effects

Table 3. Glycosidase inhibitory activities of salacinol and related compounds

Inhibitor	α-Glucosidase								β-Glcase[a] IC_{50} (mM)	α-Galase[b] IC_{50} (mM)	β-Galase[c] IC_{50} (mM)	α-Manase[d] IC_{50} (mM)	Ref.
	Rat intestinal disaccharidase K_i (μM)	Gluco-amylase G2 K_i (mM)	PPA K_i (mM)	AMY1 K_i (mM)	MGA K_i (μM)	HPA K_i (mM)	Rice IC_{50} (mM)	Baker's yeast IC_{50} (mM)					
Salacinol (**13**)	0.31 (9.6) Maltase[e] 0.32 (2.5) Sucrase[e] 0.47 (1.8) Isomaltase[e]	1.71	0.01	0.015	0.19	0.075	1.1×10^{-3}	NI	NI	—	—	2.1[f,g] ~7.5[h]	22,35,38,39,46,55,64,107
Kotalanol (**14**)	0.23 Maltase 0.18 Sucrase 1.8 Isomaltase	—	—	—	—	—	—	—	—	—	—	—	23
15	—	2.17	>5	>5	NI	NI	—	—	—	—	—	—	38,46,50
17	—	1.06	>5	>5	NI	NI	0.38	NI	3.4	—	—	3.6[f] ~7.5[h]	38,46,50,64,107
Heteroatom analogues of salacinol													
Ghavamiol (**25**)	306 Maltase[i] 44 Sucrase[i] 136 Isomaltase[i] >315 Trehalase[i]	>2.5	>5	>5	NI	NI	—	—	—	—	—	~7.5[h]	38,39,46,50,107
26	—	>8	>5	>5	NI	0.4	—	—	—	—	—	—	38,46,50
Blintol (**29**)	—	0.72	>5	>5	0.49	>5	—	—	—	—	—	~7.5[h]	38,44,46,55,107
30	—	>9	>5	>5	NI	NI	—	—	—	—	—	~7.5[h]	38,44,46,107
Five-membered ring analogues													
37	—	>5	0.052	0.109	—	—	—	—	—	—	—	—	50
38	—	>30	>5	>5	—	—	—	—	—	—	—	—	50
42	—	—	—	—	NI	—	—	—	—	—	—	—	52
43	—	—	—	—	NI	—	—	—	—	—	—	—	52
65	—	—	—	—	—	—	NI	NI	NI	NI	0.853[j]	NI	63
66	—	—	—	—	—	—	NI	NI	2.47[j,k]	NI	0.716[j]	NI	63
67 + 68	—	—	—	—	—	—	NI	NI	1.87[j,k]	NI	NI	NI	63
69 + 70	—	—	—	—	—	—	NI	NI	NI	NI	1.29[j,k]	NI	63
Six-membered ring analogues													
55	—	NI	—	—	NI	—	—	—	—	—	—	—	55,59
56	—	NI	—	—	—	—	—	—	—	—	—	—	59
57a, **58**, **59a**	—	NI	—	—	NI	—	—	—	—	—	—	—	46,59
57b	—	NI	—	—	NI	>5	1.41[j,k]	NI	0.016[j]	NI	NI	0.467[j]	38,46,55,59,63
59b	—	NI	—	—	NI	>5	—	—	—	—	—	—	38,46,59
71	—	—	—	—	—	—	NI	NI	NI	—	0.85[j,k]	1.34[j,k]	63
72	—	—	—	—	—	—	1.32[j,k]	NI	NI	NI	NI	3.59[j,k]	63
77–80	—	—	—	—	—	—	NI	NI	NI	NI	NI	NI	65
Seven-membered ring analogues													
73	—	—	—	—	—	—	NI	NI	NI	NI	NI	NI	63
74	—	—	—	—	—	—	NI	—	NI	—	NI	1.83[j,k]	63
Chain-extended analogues													
94	—	—	—	—	NI	—	—	—	—	—	—	—	72
95	—	—	—	—	0.25	—	—	—	—	—	—	—	72
96	—	—	—	—	0.26	—	—	—	—	—	—	—	72
97	—	—	—	—	0.17	—	—	—	—	—	—	—	72
106	—	—	—	—	41	—	—	—	—	—	—	—	77

107	—	—	—	—	NI	—	—	—	—	—	—	—	77
108	—	—	—	—	26	—	—	—	—	—	—	—	77
109	—	—	—	—	NI	—	—	—	—	—	—	—	77
110	—	—	—	—	25	—	—	—	—	—	—	—	77
111	—	—	—	—	5	—	—	—	—	—	—	—	77
117	—	—	—	—	0.65	—	—	—	—	—	—	—	78
118	—	—	—	—	0.14	—	—	—	—	—	—	—	78
122	—	—	—	—	0.10	—	—	—	—	—	—	—	79
123	—	—	—	—	0.10	—	—	—	—	—	—	—	79
124	—	—	—	—	35	—	—	—	—	—	—	—	79
125	—	—	—	—	8	—	—	—	—	—	—	—	79
Deoxy-salacinol analogues													
128	>1320 Maltase[i] 780 Sucrase[i]	—	—	—	—	—	—	—	—	—	—	—	82
129	>1260 Maltase[i] >1260 Sucrase[i]	—	—	—	—	—	—	—	—	—	—	—	82
130	>1390 Maltase[i] >1390 Sucrase[i]	—	—	—	—	—	—	—	—	—	—	—	82
Frame-shifted analogues													
138	—	—	—	—	20	—	—	—	—	—	—	—	89
139	—	—	—	—	53	—	—	—	—	—	—	—	89
140	—	—	—	—	NI	—	—	—	—	—	—	—	89
141	—	—	—	—	NI	—	—	—	—	—	—	—	89
De-O-sulfonated analogues													
146	15.6 Maltase[i] 3.7 Sucrase[i]	—	—	—	—	—	—	—	—	—	—	—	90 90
147	14.0 Maltase[i] 3.5 Sucrase[i]	—	—	—	—	—	—	—	—	—	—	—	90
Carboxylate analogues													
148	—	—	—	—	21	—	—	—	—	—	—	0.3[h]	91
149	—	—	—	—	NI	—	—	—	—	—	—	NI[h]	91
156	—	—	—	—	10	—	—	—	—	—	—	—	95
157	—	—	—	—	NI	—	—	—	—	—	—	—	95
Phosphate derivatives													
158	—	—	—	—	NI	—	—	—	—	—	—	—	26,96
159	—	—	—	—	NI	—	—	—	—	—	—	—	26,96
Other salacinol-related analogues													
177–184	—	—	—	—	NI	—	—	—	—	—	—	—	99
211–219	—	—	—	—	6–75	—	—	—	—	—	—	—	106

NI: no inhibition.

[a] β-Glucosidase from almond.

[b] α-Galactosidase from green coffee beans.

[c] β-Galactosidase from *Aspergillus oryzae*.

[d] α-Mannosidase from jack beans.

[e] Values in parentheses indicate IC_{50} values.

[f] α-Mannosidase from almond.

[g] 40% Inhibition.

[h] *Drosophila melanogaster* Golgi mannosidase II.

[i] IC_{50} values in μM.

[j] K_i values in mM.

[k] Preliminary determined with K_m and one K'_m.

comparable to the placebo control group.[113] As it appears that the use of sulfonium salts as glycosidase inhibitors has also been exploited by Nature, the design of sulfonium-ion mimics could be a promising approach in the search for novel inhibitors. Various structure–activity studies of salacinol and related compounds have provided the key structural elements that appear to be necessary in the design of salacinol-based inhibitors. Such knowledge is essential if salacinol or its analogues are to be used in the treatment of type II diabetes as well as to gain further insights into their mode of action. Inhibition data for the compounds described in this review are listed in Table 3.

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