ACCOUNTS
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Naturally Occurring Sulfonium-Ion Glucosidase Inhibitors and Their Derivatives: A Promising Class of Potential Antidiabetic Agents†

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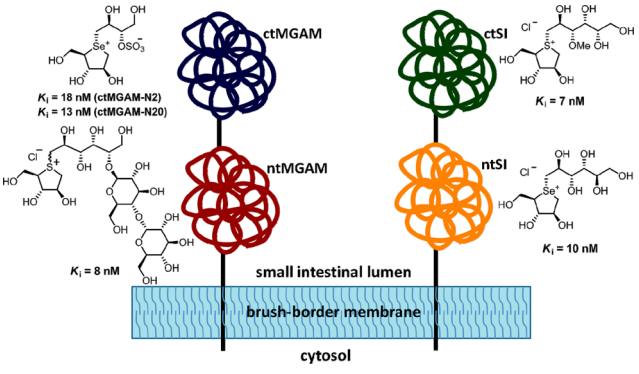
CONSPECTUS



In humans, four different enzymes mediate the digestion of ingested carbohydrates. First salivary and pancreatic α-amylases, the two endoacting retaining glucosidases, break down the complex starch molecules into smaller linear maltose-oligomers (LM) and branched α-limit dextrins (αLDx). Then two retaining exoglucosidases, maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI), convert those molecules into glucose in the small intestine. The small intestinal brush-border epithelial cells anchor MGAM and SI, and each contains a catalytic N- and C-terminal subunit, ntMGAM, ctMGAM, ntSI, and ctSI, respectively. All four catalytic domains have, to varying extents, α-1,4-exohydrolytic glucosidase activity and belong to the glycoside hydrolase family 31 (GH31). ntSI and ctSI show additional activity toward α-1,6 (isomaltose substrates) and α-1,2 (sucrose) glycosidic linkages, respectively. Because they mediate the final steps of starch digestion, both MGAM and SI are important target enzymes for the treatment of type-2 diabetes.

Because of their potent inhibitory activities against the mammalian intestinal α-glucosidases, sulfonium-ion glucosidase inhibitors isolated from the antidiabetic herbal extracts of various *Salacia* species have received considerable attention recently. Thus far, researchers have isolated eight sulfonium-ion glucosidase inhibitors from *Salacia* species: salaprinol, salacinol, ponkoranol, kotalanol, and four of their corresponding de-*O*-sulfonated compounds, the structures of which comprise a 1,4-anhydro-4-thio-D-arabinitol and a polyhydroxylated acyclic side chain. Some of these compounds more strongly inhibit human intestinal α-glucosidases than the currently available antidiabetic drugs, acarbose and miglitol, and could serve as lead candidates in the treatment of type-2 diabetes.

In this Account, we summarize progress in the field since 2010 with this class of inhibitors, with particular focus on their selective inhibitory activities against the intestinal glucosidases. Through structure–activity relationship (SAR) studies, we have modified the natural compounds to derive more potent, nanomolar inhibitors of human MGAM and SI. This structural optimization also yielded the most potent inhibitors known to date for each subunit. Furthermore, we observed that some of our synthetic inhibitors selectively blocked the activity of some mucosal α-glucosidases. Those results led to our current working hypothesis that selective inhibitors can dampen the action of a fast digesting subunit or subunits which places the burden of digestion on slower digesting subunits. That strategy can control the rate of starch digestion and glucose release to the body. Decreasing the initial glucose spike after a carbohydrate-rich meal and extending postprandial blood glucose delivery to the body can be desirable for diabetics and patients with other metabolic syndrome-associated diseases.

1. Introduction

Type-2 diabetes is a metabolic disorder characterized by elevated blood glucose levels with defects in insulin secretion, insulin action, or both. One of the approaches currently in use for the treatment of type-2 diabetes is the reversible inhibition of α-glucosidases involved in starch digestion using glycomimetics.[1−3] In humans, the digestion of ingested carbohydrates is mediated by four different

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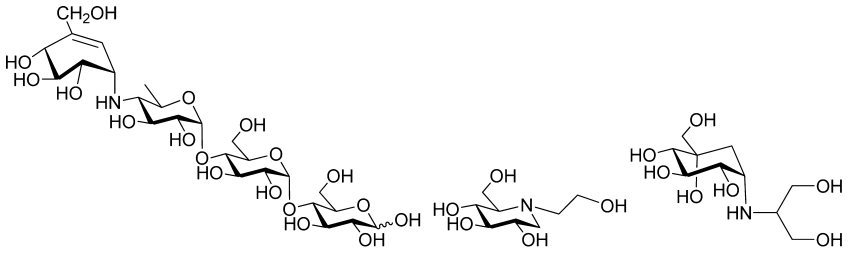
CHART 1. Antidiabetic Drugs Currently in Clinical Use



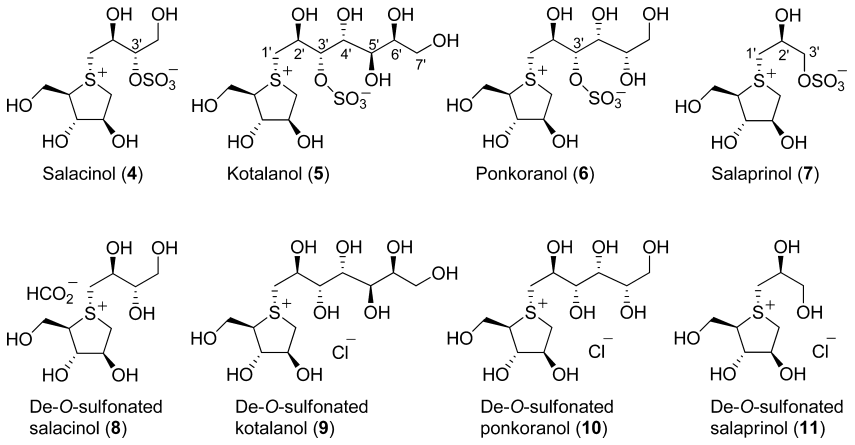
enzymes. First salivary and pancreatic α-amylases, the two endoacting retaining glucosidases, break down the complex starch molecules into smaller linear malto-oligomers (LM) and branched α-limit dextrins (αLDx) which are then broken down into glucose in the small intestine by the action of two retaining exoglucosidases, namely, maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI).[4−6] MGAM and SI are anchored to the small intestinal brush-border epithelial cells, and each contains a catalytic N- and C-terminal subunit, namely ntMGAM, ctMGAM, ntSI, and ctSI, respectively.[7−9] All four catalytic domains have, to varying extents, α-1,4-exohydrolytic glucosidase activity and belong to glycoside hydrolase family 31 (GH31), and ntSI and ctSI show additional activities toward α-1,6 (isomaltose substrates) and α-1,2 (sucrose) glycosidic linkages, respectively.[10] The individual physiological contributions of the four subunits, and the extent to which they act in cooperation under different physical or dietary conditions, are unknown and remain to be elucidated. Regulation of MGAM and SI activities on the hydrolysis of LM-αLDx is considered to be one of the effective approaches for regulating blood glucose levels.[7,8]

Reversible inhibition of these α-glucosidase activities helps to maintain normal blood glucose levels in patients with type-2 diabetes by slowing down the digestion of ingested carbohydrates which in turn leads to controlled release of glucose into the intestinal lumen and its subsequent transport into the bloodstream. There are only three α-glucosidase inhibitors, acarbose **1**,[11] miglitol **2**,[12] and voglibose **3**[13] (only marketed in Japan) that are currently used as drugs for treating type-2 diabetes (Chart 1). Acarbose is known to inhibit mainly α-amylase activity, while the other two compounds inhibit intestinal α-glucosidases. Inhibition of α-amylase can result in gastrointestinal upset because of colonic fermentation of undigested starch.[14]

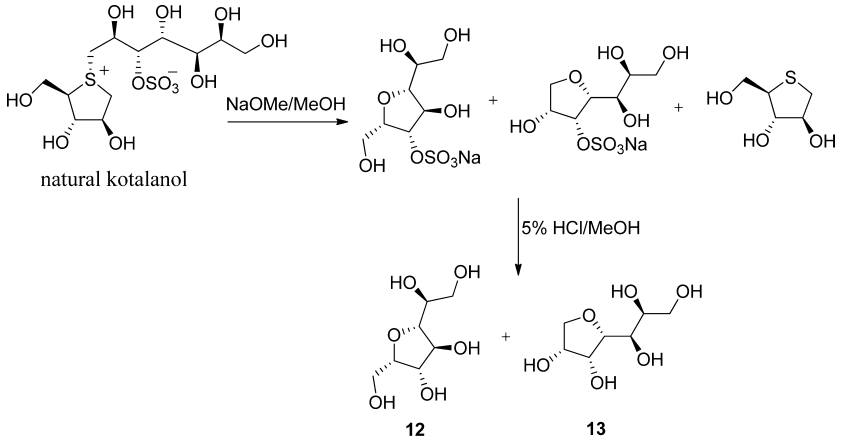
A novel class of sulfonium-ion compounds isolated from plants of the *Salacia* genus (family Celesterae), with unique structural features and potent inhibitory activities against the intestinal α-glucosidases, provides opportunities for the development of new drug candidates for treatment of type-2 diabetes. *Salacia reticulata*, also known as Kothalahimbutu in Singhalese, is the medicinal plant used in traditional medicine in Sri Lanka and South India for the treatment of type-2 diabetes.[15] *S. reticulata* is a large woody, climbing plant widely found in Sri Lanka and southern parts of India. Ayurvedic medicine, the ancient art of treatment with Indian herbal remedies, prescribes the herbal extract, obtained by storing water overnight in a mug made from the root of this plant and other members of the *Salacia* genus, such as *Salacia oblonga*, *Salacia prinoides*, and *Salacia chinensis,* as a remedy for type-2 diabetes.[16−18] Inspired by this medicinal value, in 1997 Yoshikawa et al.[19] isolated the first member of the sulfonium-ion class of glucosidase inhibitors, namely, salacinol **4** (Chart 2), as one of the active components from the aqueous extracts of *S. reticulata* through bioassay guided separation. This was then followed by the isolation of kotalanol **5** (Chart 2), another potent glucosidase inhibitor, from the same plant in 1998.[20] Later on, ponkoranol **6**,[21,22] salaprinol **7**,[22] and de-*O*-sulfonated derivatives of all four parent compounds, de-*O*-sulfonated salacinol **8**,[23,24] de-*O*-sulfonated kotalanol **9**,[25−27] de-*O*-sulfonated ponkoranol **10**,[28,29] and de-*O*-sulfonated salaprinol **11**,[29] were also isolated from *Salacia* genus (Chart 2). For most of these compounds, the absolute stereostructures were not known at the time of their isolation. Synthetic studies by our research group and others have led to the establishment of their absolute stereostructures, shown in Chart 2.[30−32]

Previously, we have reviewed the syntheses of salacinol and its derivatives and also covered the structure elucidation of kotalanol in a separate review article.[30−32] We also note that a mini-review on this class of compounds has appeared very recently.[33] In this Account, we summarize further progress in the field with this class of inhibitors, with particular focus on their selective inhibitory activities against the intestinal glucosidases. Through structure−activity relationship

CHART 2. Sulfonium-Ion Glucosidase Inhibitors **4**–**11** Isolated from *Salacia* Species



SCHEME 1. Alkaline Degradation of Kotalanol



(SAR) studies, we have modified the natural compounds to derive more potent, nanomolar inhibitors of human MGAM and SI. This structural refinement also yielded the most potent inhibitors known to date for each subunit.

2. Structural Features

The structure of this class of sulfonium-ion compounds is unique, comprising a 1,4-anhydro-4-thio-D-arabinitol moiety and a polyhydroxylated side chain. These compounds differ only in the length of the polyhydroxylated side chain. For example, salaprinol (**7**) has a three carbon side chain whereas kotalanol (**5**) has a seven carbon side chain (Chart 2). In the case of the parent compounds, salacinol (**4**), kotalanol (**5**), ponkoranol (**6**), and salaprinol (**7**), C-3′ bears a sulfate group which gives a unique sulfonium-sulfate inner salt structure (Chart 2). It is interesting that the configurations around the heterocyclitol moiety and also the configurations up to the C-3′ position in the side chain are conserved in all of these compounds. We have also shown that changing the configurations at any of these positions, including that at the stereogenic sulfur atom, leads to complete or partial loss of inhibitory activities against the human intestinal α-glucosidases.[30]

3. Structure–Activity Relationship Studies of Kotalanol

Kotalanol (**5**) and de-*O*-sulfonated-kotalanol (**9**) are the most active principles in the aqueous extracts of *S. reticulata*. We first reported the exact stereochemical structures of these two compounds by synthesis and comparison of their physical data to those of the corresponding natural compounds.[25] The candidate structures were based on our previous report on the synthesis of analogues and also the structure–activity relationship studies of lower homologues.[31] Characterization of the degradation products **12** and **13** upon alkaline treatment of kotalanol by Muraoka et al.[34] later provided

SCHEME 2. Synthesis of Kotalanol (**5**) and its C-6′ Diastereomer (**14**)

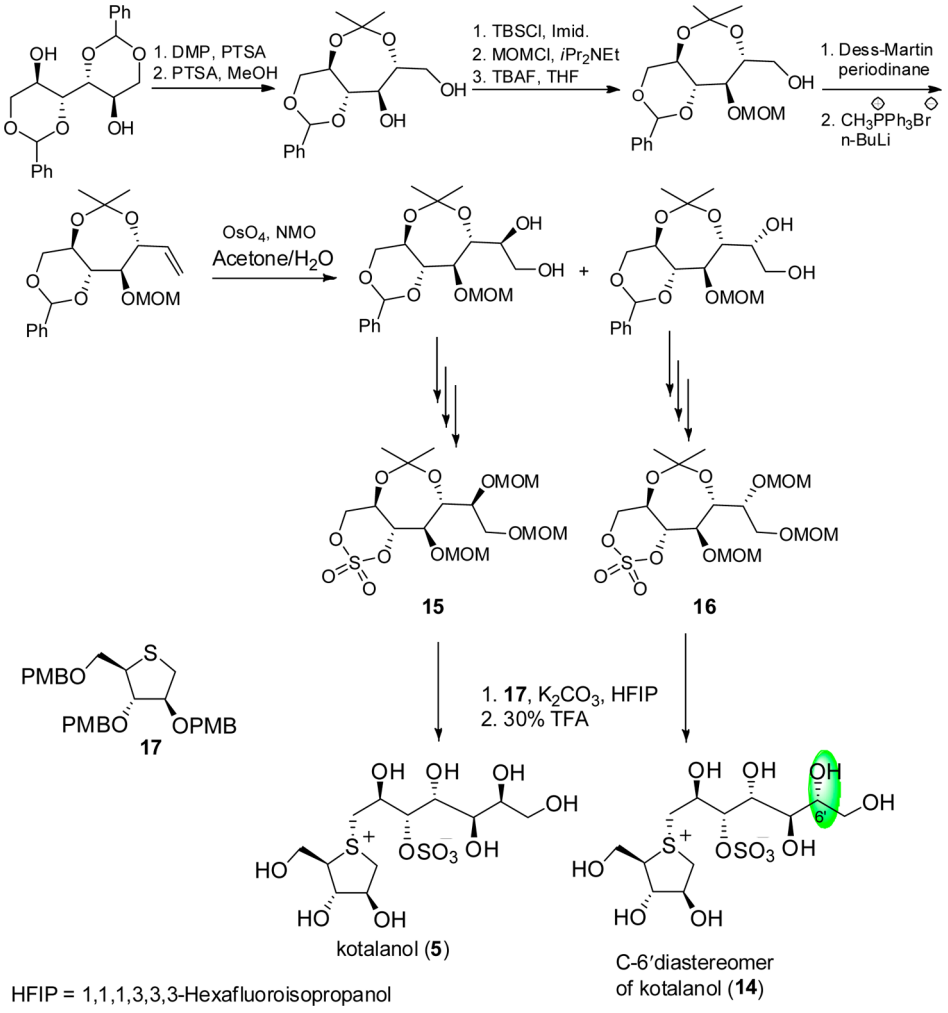


CHART 3. Diastereomers of Kotalanol, **18**, and **19**

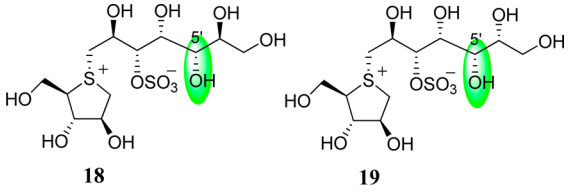
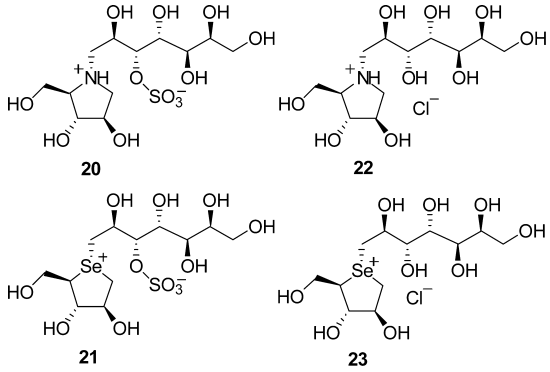


CHART 4. Heteroatom Analogues of Kotalanol and De-*O*-sulfonated Kotalanol

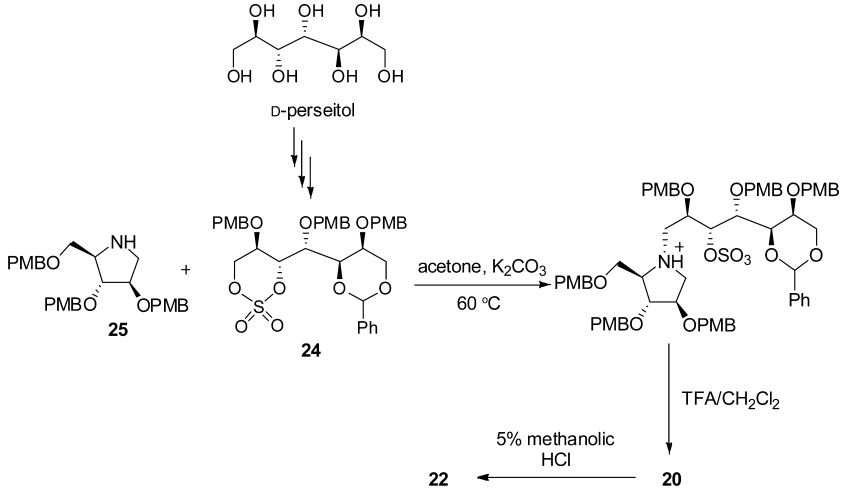


corroborating evidence for the stereochemical structure of kotalanol (Scheme 1).

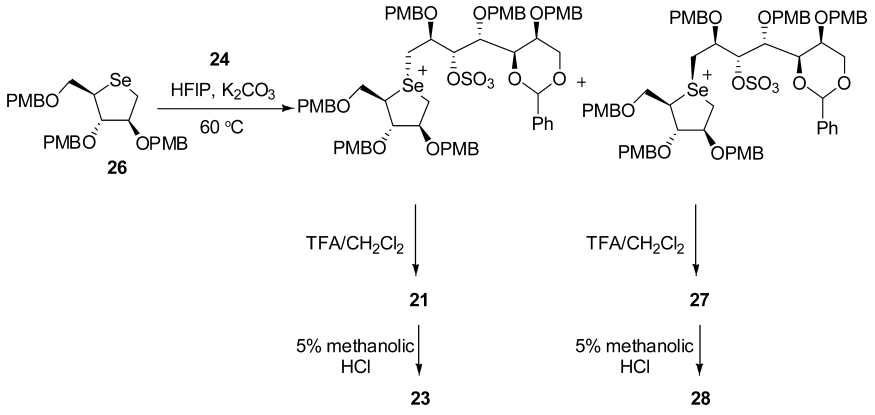
An alternative synthetic route for kotalanol (**5**) and its C-6′ epimer **14** was reported by our group in which cyclic sulfates, **15** and **16**, with an isopropylidene acetal, and PMB (para-methoxybenzyl)-protected 4-thio-D-arabinitol **17** were used as the coupling partners (Scheme 2).[35] The C-6′ epimer **14** showed equal inhibitory activity (K_i = 0.20 μM)[35] against ntMGAM to that of kotalanol (K_i = 0.19 μM).[36] This value is also comparable to the inhibitory activities of other diastereomers of kotalanol **18** and **19** (Chart 3) with opposite configuration at C-5′ (K_i = 0.10 μM and 0.13 μM, respectively).[37]

In order to study the effect of heteroatom substitution on the inhibitory activities of kotalanol and de-*O*-sulfonated kotalanol, we synthesized their nitrogen and selenium

SCHEME 3. Synthesis of Nitrogen Analogues of Kotalanol and De-*O*-sulfonated Kotalanol



SCHEME 4. Synthesis of Selenium Analogues of Kotalanol and De-*O*-sulfonated Kotalanol

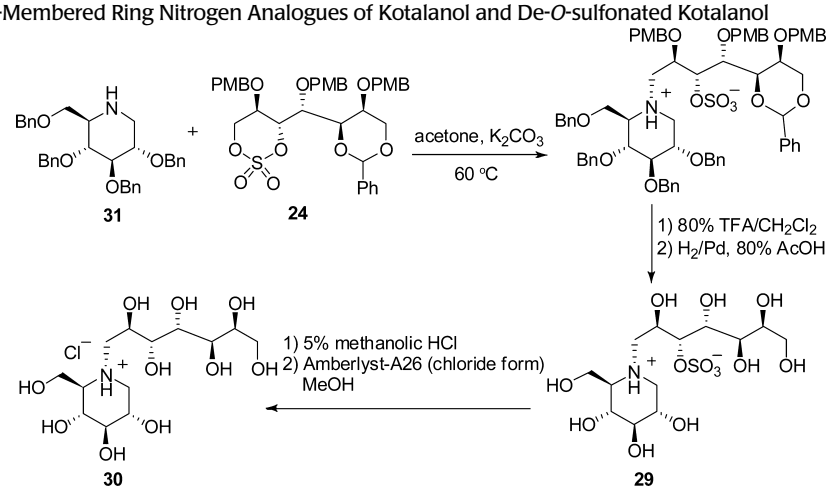


analogues **20**–**23** (Chart 4) using the perseitol-derived cyclic sulfate **24** and respective coupling partners, iminoarabinitol **25** and selenoarabinitol **26**, as shown in Schemes 3 and 4.[38]

Screening against ntMGAM indicated that selenium substitution has minimal effects on the inhibitory activities of both kotalanol (X = Se, K_i = 80 nM; X = S, K_i = 190 nM) and de-*O*-sulfonated kotalanol (X = Se, K_i = 20 nM; X = S, K_i = 30 nM). In contrast, nitrogen substitution drastically decreased the inhibitory activity in the case of kotalanol (K_i = 90 μM) but showed only a minimal effect in the case of de-*O*-sulfonated kotalanol (K_i = 61 nM).[38] The undesired diastereomers **27** and **28**, with respect to the stereogenic selenium center, showed significant decrease in inhibitory activities compared to the natural compounds, indicating the strict requirement of the natural *R* configuration at the positively charged heteroatom center.[38] The de-*O*-sulfonated derivatives derived from biologically active C-5′ diastereomers of kotalanol, **18** and **19** (Chart 3), were found to be more active against ntMGAM than the parent compounds, supporting the general hypothesis that de-*O*-sulfonation leads to an increase in the inhibitory activity against ntMGAM compared to the parent sulfated compounds.[38]

In order to probe the active-site requirements of ntMGAM, we designed two six-membered ring nitrogen analogues **29** and **30** by combining the headgroup, deoxynojirimycin, of miglitol and the 7-carbon side chains of kotalanol (**5**) and de-*O*-sulfonated kotalanol (**9**).[39] The purpose of these hybrid molecules was twofold: (1) to improve the binding interactions of miglitol in the +1 binding site of ntMGAM by replacing the *N*-hydroxyethyl side chain with the polyhydroxylated side chain of kotalanol or de-*O*-sulfonated kotalanol, and (2) to probe the purported transition state mimicry by the kotalanol class of molecules. The target compounds **29** and **30** were synthesized using the perseitol-derived cyclic sulfate **24** and benzyl-protected deoxynojirimycin **31**, as shown in Scheme 5. Screening of these two target

SCHEME 5. Synthesis of Six-Membered Ring Nitrogen Analogues of Kotalanol and De-*O*-sulfonated Kotalanol



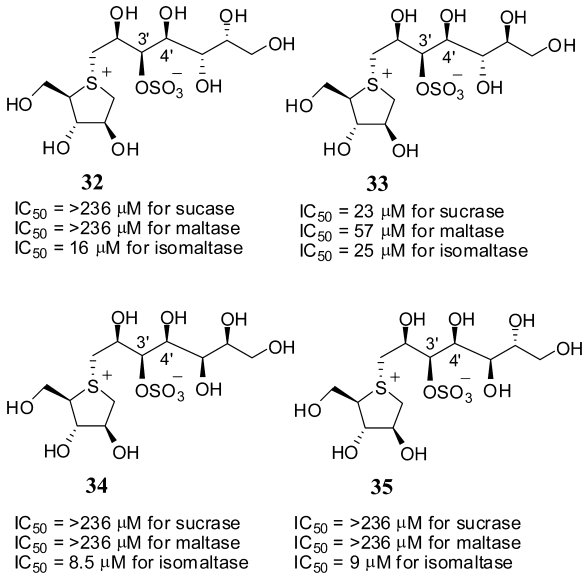
compounds (**29** and **30**) against ntMGAM indicated that they have comparable activities (K_i = 2.3 and 1.4 μM, respectively) to that of miglitol (K_i = 1.0 μM) but less inhibitory activities when compared to kotalanol (K_i = 0.19 μM).[36,39] These results clearly indicate the importance of the five-membered ring thiocyclitol moiety with a permanent positive charge and thus provide further support for transition state mimicry by these sulfonium-ion glucosidase inhibitors.

The role of the side chain stereochemistry on the α-glucosidase inhibitory activity of kotalanol was examined by Xie et al.,[40a] by synthesizing four diastereomers of kotalanol having opposite configurations at C-3 and C-4, **32**–**35** (Chart 5). The required cyclic sulfates **36**–**39** were synthesized from 3,5-di-*O*-benzyl D-xylofuranose **40** via two carbon extension by Wittig reaction followed by osmium-catalyzed dihydroxylation of the resulting *cis*- and *trans*-olefins, as shown in Scheme 6. Compared to kotalanol (IC_{50} values for sucrase = 0.75 μM; maltase = 7.2 μM; and isomaltase = 5.7 μM), all of these diastereomers showed higher IC_{50} values against maltase, sucrase, and isomaltase (see Chart 5 for comparison of IC_{50} values). With the exception of compound **33**, a considerable loss of inhibitory activity (IC_{50} values > 236 μM) was observed against both maltase and sucrase indicating the importance of the *R* configuration at C-4′. Similar results were observed for the other four diastereomers of kotalanol having natural configurations at C-2′ and C-3′ and opposite configuration only at C-4′ reported by Tanabe et al.[40b]

4. Ponkoranol

Unlike salacinol and kotalanol, both ponkoranol (**6**) and salaprinol (**7**) and the corresponding de-*O*-sulfonated derivatives **10** and **11**, were first synthesized, prior to the knowledge of their existence in *Salacia prinoides*.[22] Comparison

CHART 5. Kotalanol Diastereomers (**34**, **35**) Synthesized by Xie et al.[40a]

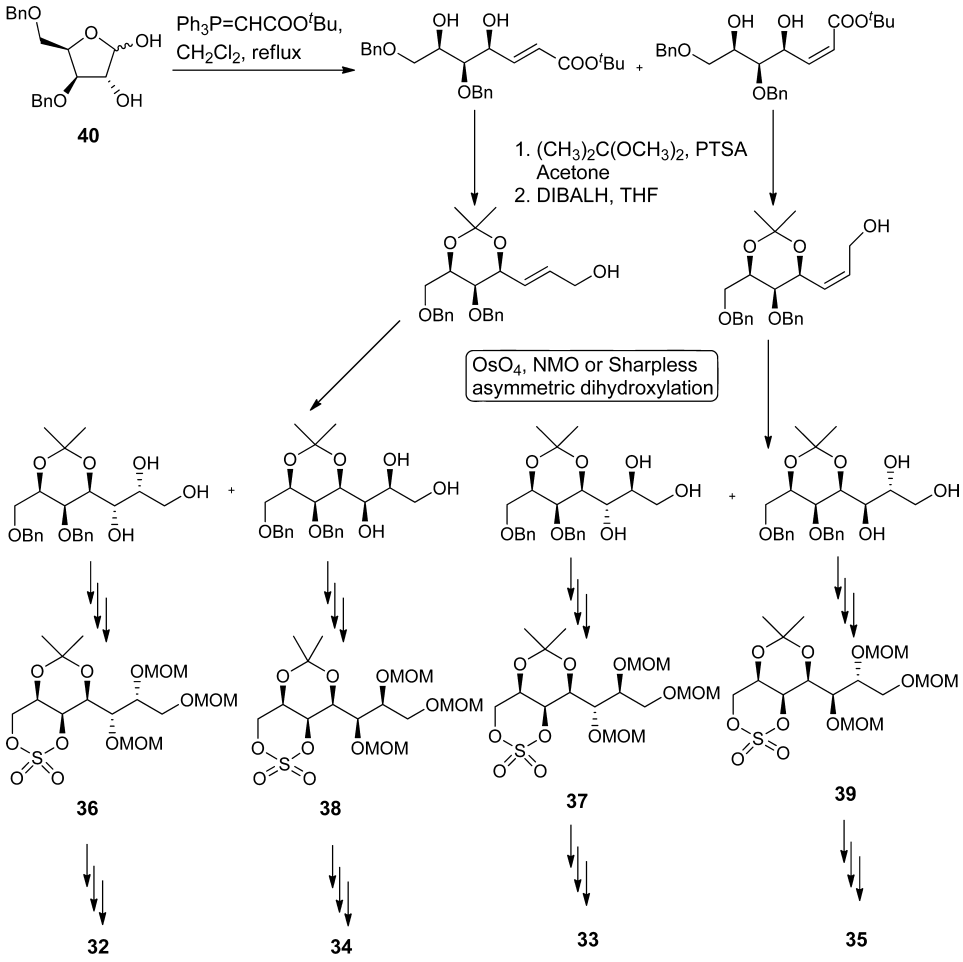


of the spectral data of the six-carbon, chain-extended synthetic analogues synthesized by our group allowed us to establish the absolute stereostructure of ponkoranol (**6**).[21]

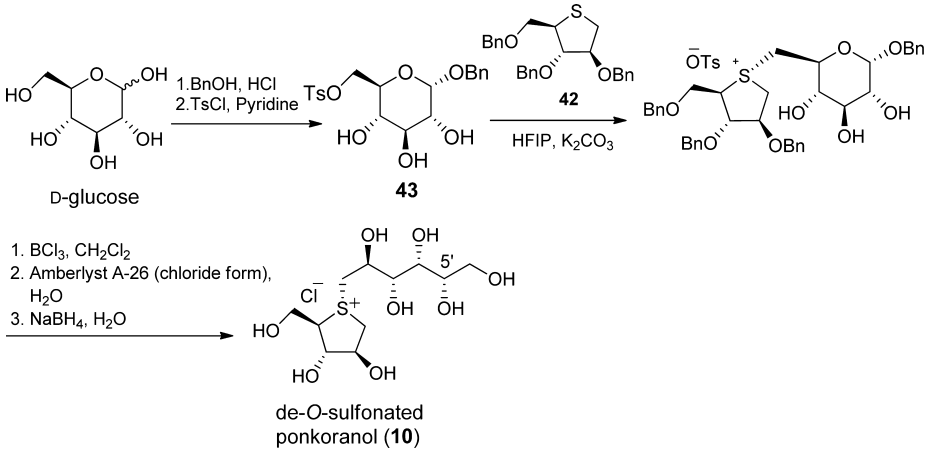
An efficient synthetic strategy afforded de-*O*-sulfonated ponkoranol (**10**) and its C-5′ epimer **41** (Scheme 7 and 8).[28] Thus, coupling of the benzyl-protected 4-thio-D-arabinitol **42** with a suitably protected primary tosylate, **43** or **44**, followed by removal of the protecting groups and reduction of the hemiacetal to the alditol yielded the target compounds, as shown in Schemes 7 and 8.

As seen previously with the de-*O*-sulfonated analogues of kotalanol, the de-*O*-sulfonated ponkoranol (**10**) and its C-5′ epimer (**41**) showed significantly higher inhibitory activities (K_i = 43 and 15 nM, respectively)[28] against ntMGAM compared to the parent sulfated compound, ponkoranol

SCHEME 6. Synthesis of Diastereomers of Kotalanol (**32**–**35**)



SCHEME 7. Synthesis of De-*O*-sulfonated Ponkoranol



(**6**, $K_i = 170$ nM).[21] Comparison of the inhibitory activities of compounds, **10** and **41**, to that of de-*O*-sulfonated kotalanol (**9**) ($K_i = 30$ nM),[36] led to the conclusion that the configuration at C-5′ is not critical for dictating inhibition against ntMGAM, and further extension of the acyclic side chain beyond six carbons is not beneficial.

By following the same protocol, we also synthesized the selenium analogues of de-*O*-sulfonated ponkoranol **45** and

SCHEME 8. Synthesis of C-5′ Epimer of De-*O*-sulfonated Ponkoranol

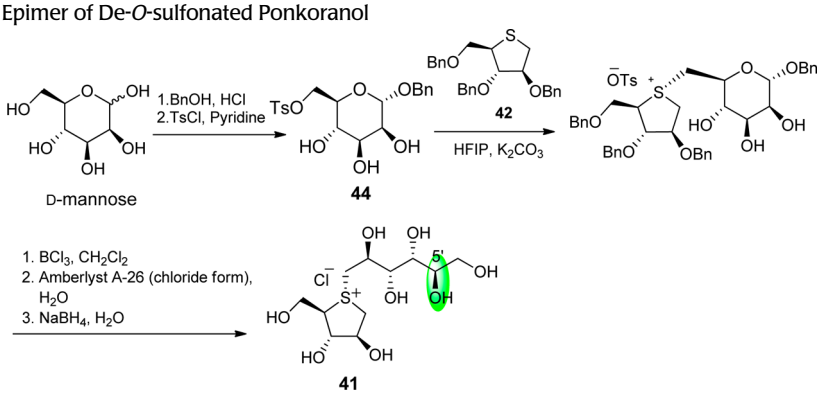
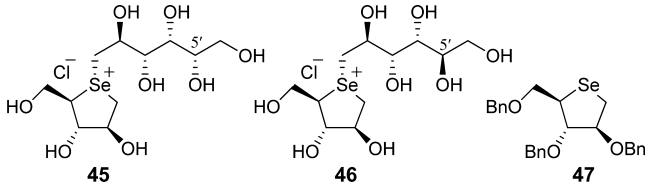


CHART 6. Selenium Analogues of De-*O*-sulfonated Ponkoranol and Selenoether



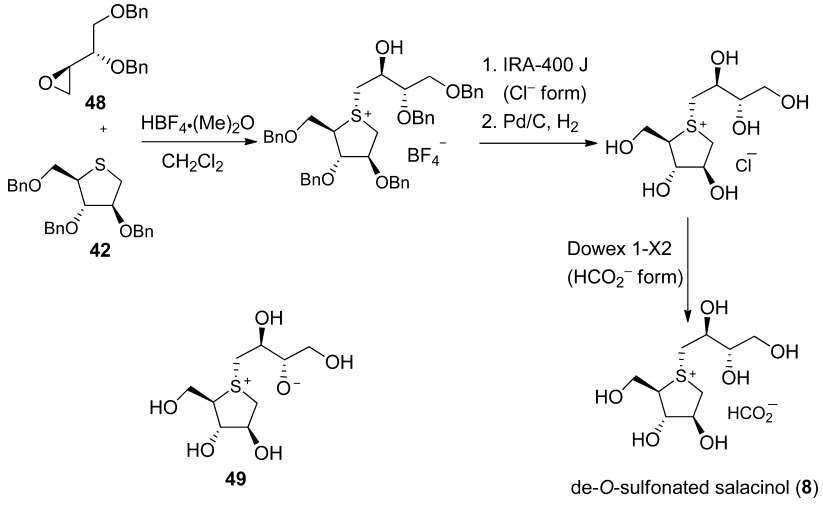
its C-5′ epimer **46** using the benzyl-protected seleno-D-arabinitol **47** and tosylates, **43** and **44** (Chart 6).[41]

These compounds (**45** and **46**) were tested against the N-terminal and C-terminal subunits of maltase glucoamylase and sucrase-isomaltase (ntMAGM,[7] ctMGAM,[42] ntSI,[43] and ctSI[42]). In addition, there are various alternative splicing patterns of ctMGAM in mammals; two spliceforms, namely, ctMGAM-N2 and ctMGAM-N20, derived from mice, which also occur in humans, were screened in the above study.[42,44] The catalytic activities of the two spliceforms are slightly different, as revealed by the kinetic analysis for maltose as a substrate; ctMGAM-N2 ($K_m = 1.59 \pm 0.18$ mM) and ctMGAM-N20 ($K_m = 1.91 \pm 0.23$ mM. Compared to the parent compounds **10** and **41** (ntSI, $K_i = 302$ and 138 nM, respectively; and ctSI, $K_i = 103$ and 132 nM, respectively), substitution of the ring sulfur atom with selenium (**45** and **46**) increased the inhibitory activities against both ntSI ($K_i = 13$ and 10 nM, respectively) and ctSI ($K_i = 18$ and 19 nM, respectively), whereas it did not have much effect in the case of ntMGAM and ctMGAM-N20. Interestingly, all four compounds, both sulfur (**10** and **41**) and selenium (**45** and **46**) analogues, did not inhibit the ctMGAM-N2 spliceform, the first recorded differentiation of the two spliceforms.[41] These results suggested that only subtle changes in the structure of these compounds could selectively inhibit one or a combination of subunits. Tanabe et al.[24] employed a different strategy for the synthesis of de-*O*-sulfonated salacinol **8** in which a primary epoxide precursor **48** was used as the coupling partner, as shown in Scheme 9. This work also clarified the ambiguity in the structure of this natural compound (**8**); a sulfonium-alkoxide inner salt structure **49** (Scheme 9), initially proposed by Minami et al.[23] being incorrect.

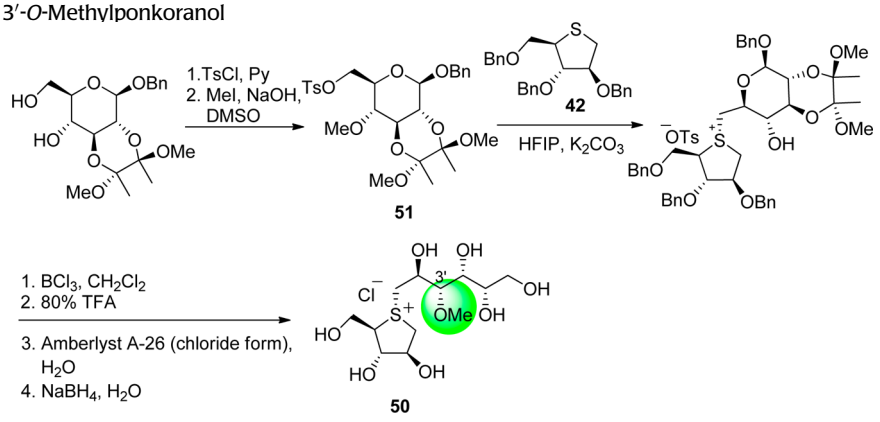
4.1. 3′-*O*-Alkylated Ponkoranol and Salacinol Derivatives. Although the reason for the existence of both sulfated and de-*O*-sulfonated versions of these sulfonium-ions in the plant is not clear, in general, the de-*O*-sulfonated compounds are more active against the intestinal α-glucosidases than the corresponding sulfated counterparts.[28,38] We have explained this difference based on the crystal structures of kotalanol (**5**) and de-*O*-sulfonated kotalanol (**9**) complexed with human ntMGAM.[36] These crystal structures revealed that in the case of kotalanol, the sulfate group at C-3′ occupies a hydrophobic cavity (Y299, W40, and F575) causing a positional constraint, whereas in the case of de-*O*-sulfonated kotalanol, the positional constraint is removed and allows the rest of the polyhydroxylated side chain to make optimal contacts with the ntMGAM active site. These observations also suggested that installation of a hydrophobic methoxy group in place of a sulfate group might increase inhibitory activity against ntMGAM. Accordingly, we synthesized the 3′-*O*-methylponkoranol **50** using the 4-*O*-methylated glucopyranoside derivative **51** and benzyl-protected 4-thio-D-arabinitol (**42**), as shown in Scheme 10.[45] Surprisingly, the 3′-*O*-methylponkoranol (**50**) was less active ($K_i =$ 0.5 μM) against ntMGAM than de-*O*-sulfonated ponkoranol (**10**, $K_i = 43$ nM),[45] indicating that the hydrophobic interactions between the methyl group and the hydrophobic residues in the active site of ntMGAM are not as optimal as the interactions of the latter groups with the rest of the polyhydroxylated chain in the absence of the methyl ether.

However, compound **50** did exhibit some interesting selectivity for the four subunits, ntMGAM, ctMGAM, ntSI,

SCHEME 9. Synthesis of De-*O*-sulfonated Salacinol

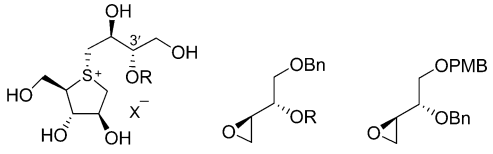


SCHEME 10. Synthesis of 3′-*O*-Methylponkoranol



and ctSI, including two spliceforms of ctMGAM, N2, and N20.[46] Remarkable inhibitory activity against ctSI (K_i = 0.007 μM), which is approximately 70 times higher when compared to ntMGAM (K_i = 0.5 μM), was observed. It also showed a nanomolar range inhibitory activity against ntSI (K_i = 0.035 μM), ctMGAM-N2 (K_i = 0.060 μM), and ctMGAM-N20 (K_i = 0.055 μM) spliceforms. Comparison with the inhibition profile of de-*O*-sulfonated ponkoranol (**10**) against all four subunits clearly suggested that the installation of the methoxy group at C-3′ is beneficial except in the case of ntMGAM where the activity dropped by an order of magnitude. The beneficial effect of a methoxy group at C-3′ is remarkable, giving the most active inhibitor of ctSI to date, with a K_i value of 7 nM (de-*O*-sulfonated ponkoranol (**10**) had a K_i value of 103 nM), and also showing an order of magnitude increased inhibitory activity against ntSI compared to (**10**). Based on the potent inhibitory activity of the 3′-*O*-methyl derivative (**50**) against ctSI, we speculate that

CHART 7. 3′-*O*-Alkylated Salacinol Analogues



52 R = Me; X = Cl
53 R = C_2H_5; X = Cl
54 R = $C_{13}H_{27}$; X= Cl
55 R = Bn; X = BF_4

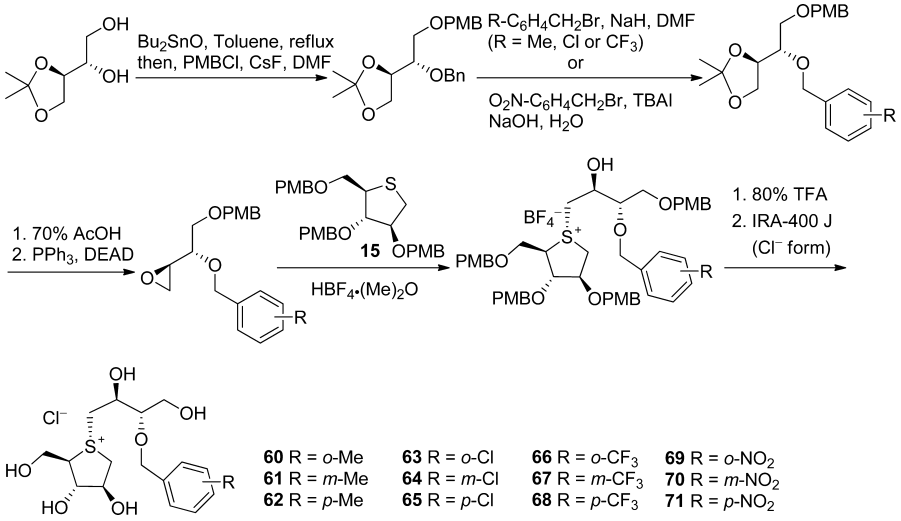
56 R = Me
57 R = C_2H_5
58 R = $C_{13}H_{27}$

59

ctSI will have a hydrophobic pocket in the catalytic site that better accommodates the methyl group and provides favorable hydrophobic interactions relative to the other subunits. Such information is critical in designing inhibitors against ctSI, especially in the absence of its crystal structure.

Tanabe et al.[47] subsequently synthesized a series of salacinol analogues (**52**−**55**, Chart 7) having different hydrophobic groups at C-3′ of the salacinol side chain by applying their strategy (Scheme 9) of regioselective ring-opening of

SCHEME 11. Synthesis of 3′-*O*-Benzylated Analogues of Salacinol



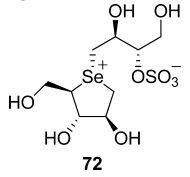
appropriate epoxides (**56**–**59**, Chart 7) with the thioether **42** in the presence of the tetrafluoroboric acid dimethyl ether complex.

All of these compounds showed considerably higher inhibitory activities against rat intestinal α-glucosidases compared to salacinol and its de-*O*-sulfonated derivative. In particular, compound **55** with a benzyloxy group at C-3′ was the most potent inhibitor of maltase (IC_{50} = 0.44 μM) and isomaltase (IC_{50} = 0.14 μM), and compound **53** having an ethoxy group at C-3′ showed highest inhibitory activity against sucrase (IC_{50} = 0.12 μM).[47] Very recently, Tanabe et al.,[48] in an attempt to further optimize the inhibitory activity of the 3′-*O*-benzyl derivative of salacinol (**55**), synthesized twelve analogues (**60**–**71**) having four different substituents (methyl, chloro, trifluoromethyl, and nitro) at different positions (*meta* or *para* or *ortho*) of the phenyl ring (Scheme 11). Inhibition assays against rat intestinal glucosidases indicated that, in general, *ortho* substituted compounds were more active than the corresponding *para* and *meta* substituted compounds. One compound (**69**), with an *ortho*-nitrophenyl group, was found to be the most potent inhibitor of all three rat intestinal glucosidases, maltase (IC_{50} = 0.13 μM), sucrase (IC_{50} = 0.042 μM), and isomaltase (IC_{50} = 0.21 μM).

5. Toggling of Activities of Mucosal α-Glucosidases

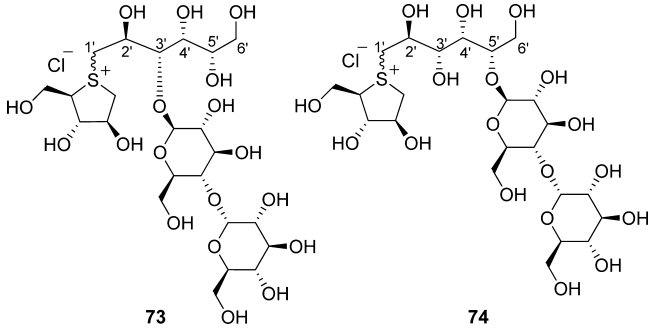
All four subunits, ntMGAM, ctMGAM, ntSI, and ctSI, have α-1,4-exohydrolytic glucosidase activity. ntSI has an additional glucosidase activity on α-1,6 linkages and ctSI has additional glucosidase activity on α-1,2 linkages. This apparent redundancy in the α-1,4-exohydrolytic glucosidase activities in the gut is not well understood. To enhance our understanding of the role these enzymes play in the glucogenesis of starch in vivo, it was of interest to derive compounds that could regulate (toggle) the activities of the four subunits, including two spliceforms of ctMGAM, N2 and N20, individually. As a first step, we studied the effectiveness of a panel of inhibitors including two of the currently available antidiabetic drugs, acarbose (**1**) and miglitol (**2**) and three of the naturally occurring sulfonium-ion glucosidase inhibitors, salacinol (**4**), kotalanol (**5**) and de-*O*-sulfonated kotalanol (**9**) and the selenium analogue of salacinol, namely, blintol (**72**, Chart 8).[42]

CHART 8. Selenium Analogue (Blintol) of Salacinol



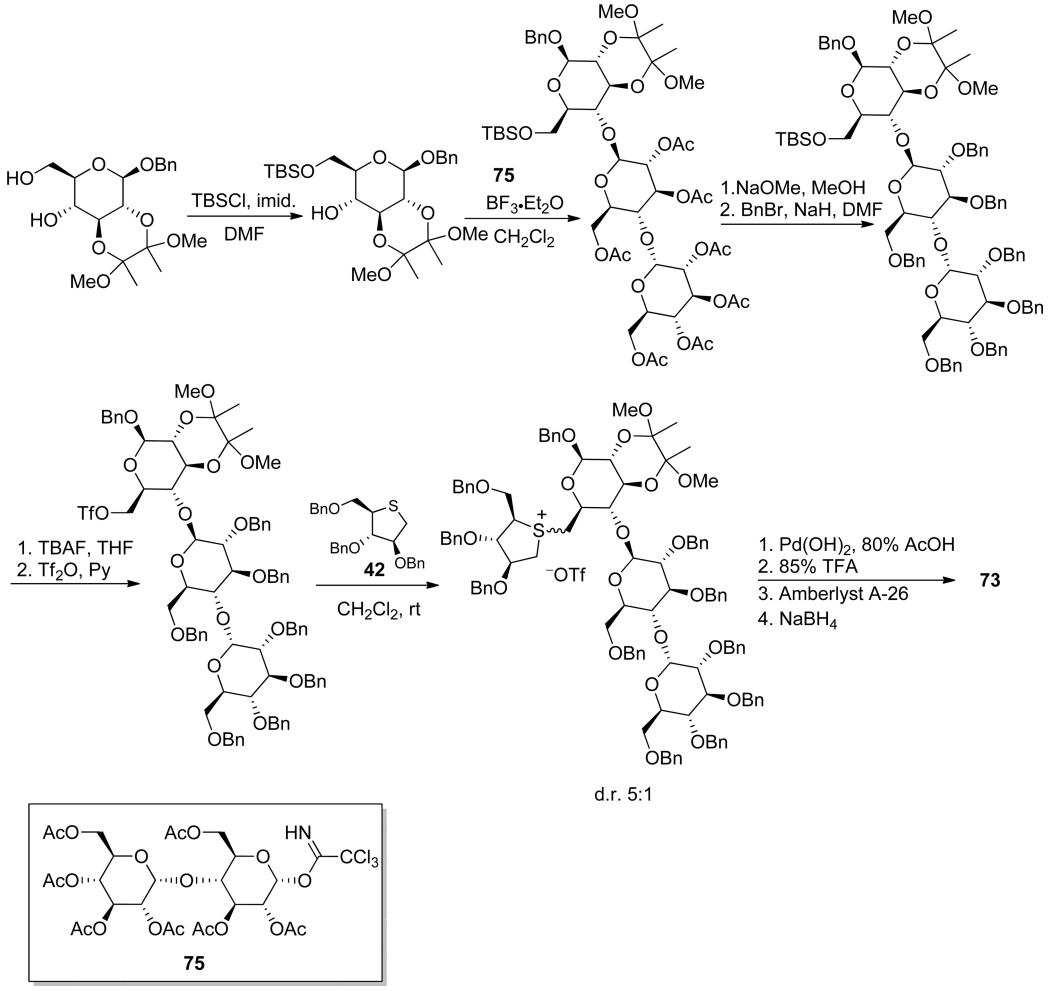
72

CHART 9. 3′- and 5′-Maltose-Extended Analogues of De-*O*-sulfonated Ponkoranol



73 **74**

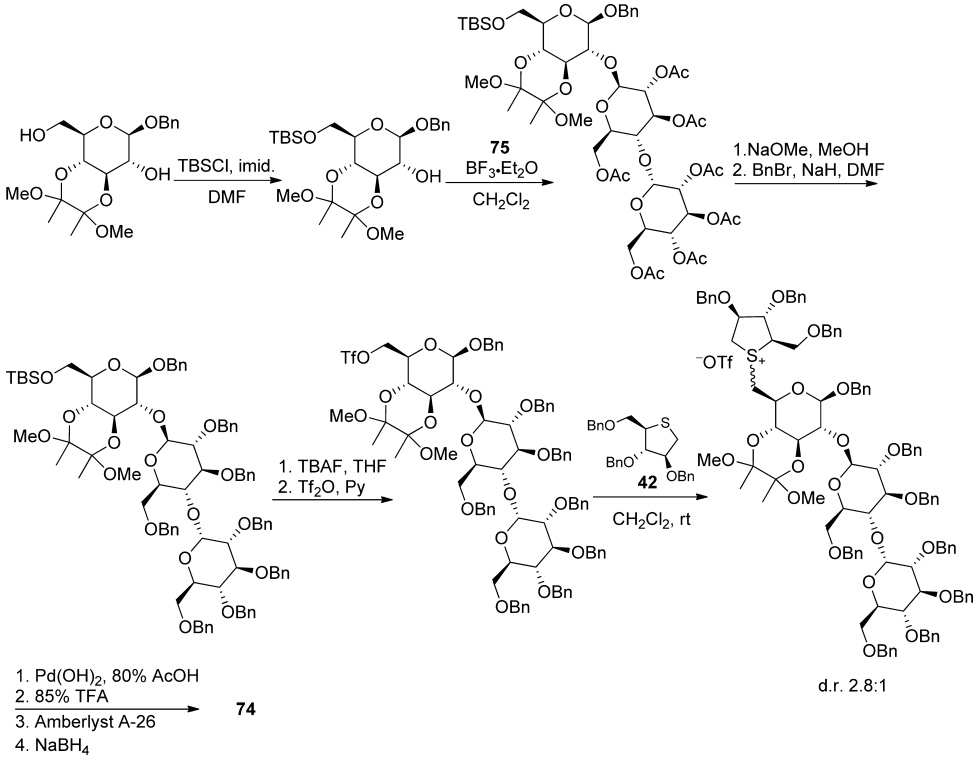
SCHEME 12. Synthesis of 3′-Maltose-Extended Analogues of De-*O*-sulfonated Ponkoranol



This study demonstrated catalytic domain selectivity, helping to differentiate the active site requirements of each catalytic subunit. Acarbose was found to be a poor inhibitor of ntMGAM ($K_i = 62\ \mu M$) and ntSI ($K_i = 14\ \mu M$). In contrast, it was found to be a 1000-fold better inhibitor of ctMGAM-N2 ($K_i = 0.009\ \mu M$) and ctMGAM-N20 ($K_i = 0.028\ \mu M$) splice-forms and 100-fold better inhibitor of ctSI ($K_i = 0.246\ \mu M$). Salacinol and kotalanol showed 4 to 6-fold selectivity for inhibiting C-terminal enzymes, as compared to N-terminal units. Blintol (**72**) showed improved inhibition of ctMGAM-N2 ($K_i = 0.018\ \mu M$) with little changes against other enzymes in comparison to salacinol. Miglitol showed little distinction between the catalytic subunits. This study showed that relatively small structural changes in a compound can result in significant differences in its ability to selectively inhibit one enzyme over the others. The results also reflect different biochemical and structural properties of these enzyme units, despite their overall similarities in amino acid sequence and tertiary structure. Based on the better inhibition profile of acarbose (**1**) against C-terminal enzymes and a 21-amino acid residue extension near the catalytic site in ctMGAM, we hypothesized that C-terminal enzymes might have an extended substrate binding site compared to N-terminal enzymes and thus have stronger affinity for longer oligomers compared to maltose.[7,42] In the case of acarbose, compared to other small inhibitors, the extended sugar-binding site should favor additional interactions with the trisaccharide portion of acarbose, thus leading to a significant increase in its inhibitory activity for C-terminal enzymes over N-terminal enzymes. This prediction was confirmed independently by X-ray crystallography of the ctMGAM-acarbose complex.[49]

To better define the individual roles of the MGAM and SI domains in the process of terminal starch digestion, we proposed further modification to the ponkoranol scaffold to toggle their activities on and off with domain-specific inhibitors. Based on the ~100- to 1000-fold differential inhibition of C-terminal enzymes over N-terminal enzymes observed with acarbose, we decided to probe whether

SCHEME 13. Synthesis of 5′-Maltose-Extended Analogues of De-*O*-sulfonated Ponkoranol

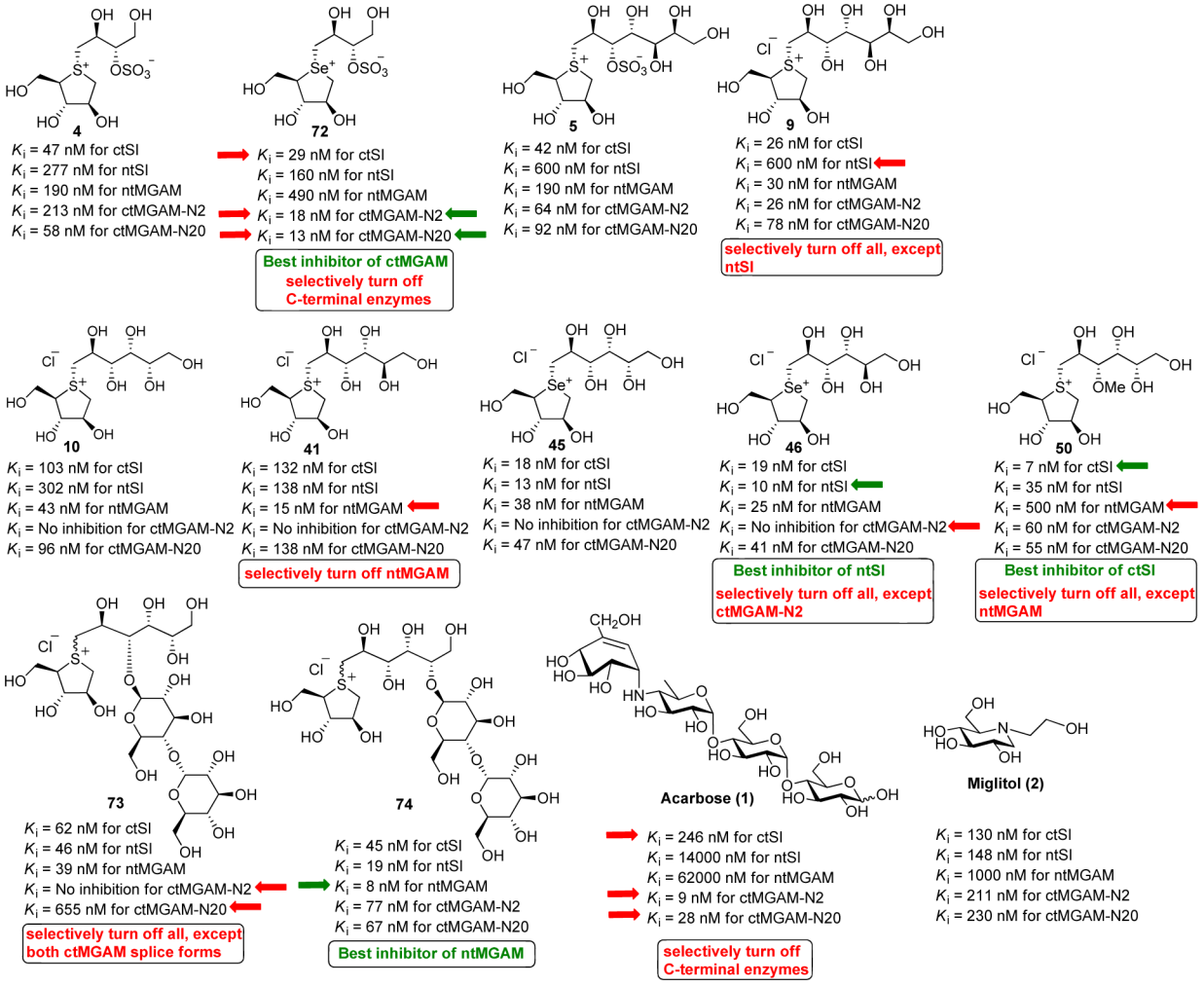


appending glucose residues to the polyhydroxylated side chain of salacinol-based compounds, irrespective of their attachment at the reducing or nonreducing end, would lead to differential inhibition of the four enzyme activities. Accordingly, we synthesized our initial candidates, C-3′- and C-5′-β-maltose-extended analogues of de-*O*-sulfonated ponkoranol, **73** and **74** (Chart 9), respectively, as shown in Scheme 12 and 13 and screened for inhibitory activities against all four subunits.[50]

Although elongation of the scaffold with maltose, in general, does not result in a significant gain in binding energy, it does result in some interesting selectivity in inhibitory activities. Compound **74** inhibits all four subunit activities in the nanomolar range and is the most potent inhibitor of ntMGAM ($K_i = 0.008$ μM) to date. Compound **73**, on the other hand, showed selectivity and differentiated ctMGAM from the others, showing poor inhibition of ctMGAM-N20 ($K_i = 0.655$ μM) and no inhibition against ctMGAM-N2; it inhibited the other subunits similarly (ntMGAM, ntSI, and ctSI: $K_i = 0.039$, 0.046, and 0.062 μM, respectively). Therefore, with compound **73**, it is possible to keep the ctMGAM activity on and dampen the others. In a complementary fashion, acarbose (**1**) can be used to selectively turn off ctMGAM activity and study the effect of three other subunits on starch digestion. The outcome of such studies in conjunction with the recent crystal structure of ctMGAM in complex with acarbose by Ren et al.[49] should enhance our understanding of the exact role of ctMGAM in the terminal starch digestion process, especially the need for and role of several spliceforms. A summary of inhibitory activities of some of the compounds listed in this article and their selectivity profile against the four subunits is shown in Chart 10 (green arrows indicate the best inhibitor known to date for each subunit, and red arrows indicate the potential application of these inhibitors to selectively inhibit one or more subunits over the other).

The inhibition data of all the compounds against recombinant mammalian MGAM (ntMGAM and ctMGAM) and SI (ntSI and ctSI) discussed in the forgoing sections were obtained using maltose as a substrate. However, in the small intestine in vivo, MGAM and SI are exposed to a mixture of linear maltose-oligosaccharides (LM) and branched α-limit dextrins (αLDx), rather than maltose. Hence, we studied the inhibitory profile of acarbose (**1**), de-*O*-sulfonated kotalanol (**9**) and the maltose-extended de-*O*-sulfonated ponkoranol derivatives, **73** and **74**, against all four subunits using LM/αLDx substrates, produced by the α-amylase reaction on waxy corn starch.[51] In this study, acarbose once again showed ~300 to 500 times stronger inhibition of C-terminal enzymes (ctMGAM, $IC_{50} = 0.12$ nM and ctSI, $IC_{50} = 0.4$ nM) compared to N-terminal enzymes (ntMGAM, $IC_{50} = 62$ nM

CHART 10. Potent Inhibitors of ntMGAM, ctMGAM, ntSI, and ctSI and Their Selectivity Profiles



and ntSI, IC_{50} = 135 nM), similar to the trend in inhibition profile obtained using maltose as substrate. De-*O*-sulfonated kotalanol (**9**) showed 20 and 40% inhibition of ctMGAM and ctSI, respectively, at 50 pmol concentration, whereas N-terminal subunits were not inhibited at this concentration. Thus, this compound could be used to toggle off the C-terminal subunits, particularly ctSI with sucrase activity, while having little effect on the N-terminal subunits. The 5′-maltose-extended derivative **74** showed no selectivity (IC_{50} values from 0.12 to 0.54 nM). On the other hand, the 3′-maltose extended derivative **73** showed a similar range of IC_{50} values for ctMGAM, ctSI and ntMGAM (0.53, 0.17, and 0.69 nM, respectively) and ~4−15 times higher IC_{50} value (2.69 nM) for ntSI compared to the other subunits. The more potent inhibition of the enzymes with the α-limit dextrins as substrates compared to maltose is presumably due to the requirements of fitting larger oligosaccharide units in the active sites compared to the small-molecule inhibitors. The selectivity of the enzymes for different inhibitors remains the same.

6. Conclusions

The results summarized here demonstrate that the sulfonium ion-based inhibitors have potential as antidiabetic agents. Through structure−activity relationship studies, we have refined the inhibitory activities of the natural compounds against human MGAM and SI and as a result we have identified several compounds with low nanomolar inhibitory activities. This structural refinement has also yielded the most potent inhibitors to date for each subunit, for example, 3′-*O*-methylponkoranol (**50**), with a K_i value of 7 nM against ctSI, the 5′-maltose-extended derivative **74**, with a K_i value of 8 nM against ntMGAM, and the selenium analogue of the C-5′ epimer of de-*O*-sulfonated ponkoranol (**46**), with a K_i value of 10 nM against ntSI. The selenium analogue of salacinol, blintol (**72**), was found to be the best

inhibitor of both spliceforms of ctMGAM (ctMGAM-N2, K_i = 18 nM and ctMGAM-N20, K_i = 13 nM) which is comparable to acarbose (K_i = 9 nM for ctMGAM-N2 and 28 nM for ctMGAM-N20), and remarkably, the sulfur and selenium analogues of de-*O*-sulfonated ponkoranol differentiated between the spliceforms. Furthermore, we have shown that the sulfonium ion inhibitors inhibit each of the α-glucosidase enzyme units in real starch digestion. The results also support the concept of controlling starch digestion rate through the toggling of activities of the mucosal α-glucosidases by selective enzyme inhibition. The concept of toggling has potential implications in the treatment of type-2 diabetes. We propose, through the approach of differential inhibition by toggling of specific mucosal enzymes, that the starch digestion rate may be modulated to attain similar effects as observed with slowly digestible starch, which has the property of being digested throughout the small intestine.[52] Decreasing the initial peak glucose levels and extending postprandial blood glucose delivery to the body can be desirable for diabetics and possibly to other groups vulnerable to metabolic syndrome-associated diseases. Toggling of mucosal glucosidases activities will also provide a better understanding of the individual role of each subunit in the starch digestion process and in all possible combinations. Interesting and unprecedented enzyme selectivity obtained for our initial candidates sets the stage for improvement of the specificity and affinity of this type of compounds for their development as antidiabetic agents and for controlled release of glucose over a defined time frame, thus avoiding the spike in glucose formation and ensuing stress on insulin producing cells of the pancreas. The crystal structures of all subunits, except for ctSI, have been reported. This structural information will be of added advantage in future design and synthesis of domain-specific inhibitors against MGAM and SI subunits.

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BIOGRAPHICAL INFORMATION

Professor B. Mario Pinto was born in Sri Lanka. He received his Ph.D. in Chemistry from Queen's University, Canada under the direction of Walter A. Szarek, and then pursued postdoctoral studies with Sir Derek Barton at ICSN-CNRS, France and David Bundle at NRC, Ottawa. He joined Simon Fraser University where he became Professor, Chair of Chemistry, and Vice-President, Research. Dr. Pinto is one of the founding members of the Centre for Drug Research and Development in Vancouver. He served as President of the Canadian Society for Chemistry and the International Carbohydrate Organization (ICO), and serves as the Canadian representative to the ICO. Dr. Pinto has received numerous awards and was elected to the Academy of Sciences of the Royal Society of Canada in 2003. His research interests span the study of conformational effects and molecular mimicry, the development of NMR/molecular dynamics protocols for the study of bioactive ligand conformations when bound to proteins, the synthesis of enzyme inhibitors as agents against Type-2 diabetes and related metabolic disorders, viruses, and mycobacteria, the development of bacterial vaccines, and control of intracellular trafficking of glycoproteins and surface expression of carbohydrates.

Sankar Mohan was born in Tiruvannamalai, India in 1978. After receiving his Masters degree (2000) in Chemistry from University of Madras, India, he worked as a research associate in Dr. Reddy's Research Foundation in India (2000−2004). He received his Ph.D. (2010) in Chemistry from Simon Fraser University, Canada under the supervision of Prof. B. Mario Pinto. He then worked as a Senior Research Scientist in Albany Molecular Research Inc, Hyderabad, India and returned to Prof. Pinto's research group in May 2012. He is currently working on synthesis of novel inhibitors of influenza neuraminidase and human intestinal glucosidases.

Razieh Eskandari graduated with a B.Sc. (2003) in chemistry from Yasouj University, Iran, which was followed by a M.Sc. (2006) at Shiraz University. She subsequently obtained her Ph.D. (2012) at Simon Fraser University under the supervision of Prof. B. Mario Pinto. This work involved mapping the enzyme specificities of intestinal maltase-glucoamylase and sucrase-isomaltase. Razieh is currently working with David Vocadlo at Simon Fraser University as a Michael Smith Foundation for Health Research postdoctoral fellow.

FOOTNOTES

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The authors declare no competing financial interest.

†This Account is dedicated, with respect, to the memory of Blair D. Johnston.

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