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Probing the active-site requirements of human intestinal N-terminal maltase glucoamylase: The effect of replacing the sulfate moiety by a methyl ether in ponkoranol, a naturally occurring α-glucosidase inhibitor

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ABSTRACT

Ponkoranol is a naturally occurring glucosidase inhibitor isolated from the plant *Salacia reticulata*. The compound comprises a sulfonium ion with an internal sulfate counter ion. We report here an efficient synthetic route to 3′-*O*-methyl ponkoranol to test the hypothesis that occupation of a hydrophobic pocket by a methyl group instead of the polar sulfate ion within the active site of human N-terminal maltase glucoamylase would be beneficial. The synthetic strategy relies on the nucleophilic attack of 2,3,5-tri-*O*-benzyl-1,4-anhydro-4-thio-D-arabinitol at the C-6 position of benzyl 6-*O*-*p*-toluenesulfonyl β-D-glucopyranoside, followed by deprotection using boron trichloride and reduction with sodium borohydride. The target compound inhibited the N-terminal catalytic domain of intestinal human maltase glucoamylase (ntMGAM) with a K_i value of 0.50 ± 0.04 μM, higher than those of de-*O*-sulfonated ponkoranol (K_i = 43 ± 3 nM), or its 5′-stereoisomer (K_i = 15 ± 1 nM). We conclude that the interaction of the methyl group with hydrophobic residues in the active site is not as beneficial to inhibition of ntMGAM as the other interactions of the polyhydroxylated chain with active-site residues.

Glycosidase inhibitors have many potential therapeutic applications because glycosidase enzyme-catalyzed hydrolysis of complex carbohydrates is biologically widespread, and has been implicated in several disease states.[1,2] For example, inhibition of starch-hydrolyzing enzymes such as pancreatic α-amylase and intestinal α-glucosidases that leads to a delay in digestion of ingested carbohydrates is one of the therapeutic approaches for the treatment of type 2 diabetes.[3,4] Bioactive components isolated from medicinal plants often provide the lead structures for drug development programs.[5,6] For example, a relatively new and interesting class of inhibitors is the sulfonium ion containing inhibitors, which were first isolated from *Salacia reticulata*, a plant, that is, used in traditional Ayurvedic medicine in Sri Lanka and South India for treating type 2 diabetes.[7–9] The active components in *S. reticulata* were found to include salaprinol (**1**),[10] salacinol (**2**),[11] ponkoranol (**3**),[10] kotalanol (**4**),[12] de-*O*-sulfonated kotalanol (**5**),[13] and de-*O*-sulfonated salacinol (**6**)[14] (Fig. 1), whose structures comprise a 1,4-anhydro-4-thio-D-arabinitol core and polyhydroxylated acyclic chain (Fig. 1).

Comparison of the inhibitory activities against the human N-terminal catalytic domain of maltase glucoamylase (ntMGAM) of de-*O*-sulfonated kotalanol (**5**) and some of its stereoisomers versus kotalanol (**4**) and the corresponding sulfated stereoisomers, respectively, revealed that the de-*O*-sulfonated analogs were more potent inhibitors than the parent compounds.[15,16] Furthermore, we have shown recently that de-*O*-sulfonated ponkoranol (**7**) (K_i = 0.043 ± 0.01 μM) and its 5′-stereoisomer (**8**) (K_i = 0.015 ± 0.01 μM) are more potent inhibitors of ntMGAM than ponkoranol (**3**) itself (K_i = 0.17 ± 0.03 μM) (Fig. 2).[17]

Our previous X-ray crystallographic studies of ntMGAM in complex with kotalanol (**4**) and de-*O*-sulfonated kotalanol (**5**) had indicated that removal of the sulfate group affects the conformation of the rest of the polyhydroxylated chain.[18] We concluded that although the stereoconfiguration at C3′ does not affect inhibitory activity, the proximity of the sulfate group to the large hydrophobic groups (Y299, W406, and F575) likely restricts its conformational freedom. Therefore, by removing the sulfate group, the positional constraint imposed by the bulky hydrophobic residues surrounding the C3′ group is relieved, allowing the rest of the polyhydroxylated chain to make optimal contacts with the ntMGAM active site (Fig. 3).[18]

In view of these findings, it was of interest to question whether replacing the sulfate group with a hydrophobic methyl ether in ponkoranol (**9**) (Fig. 4) would increase its inhibitory properties through hydrophobic interactions in the site compared to de-*O*-sulfonated ponkoranol **7**.

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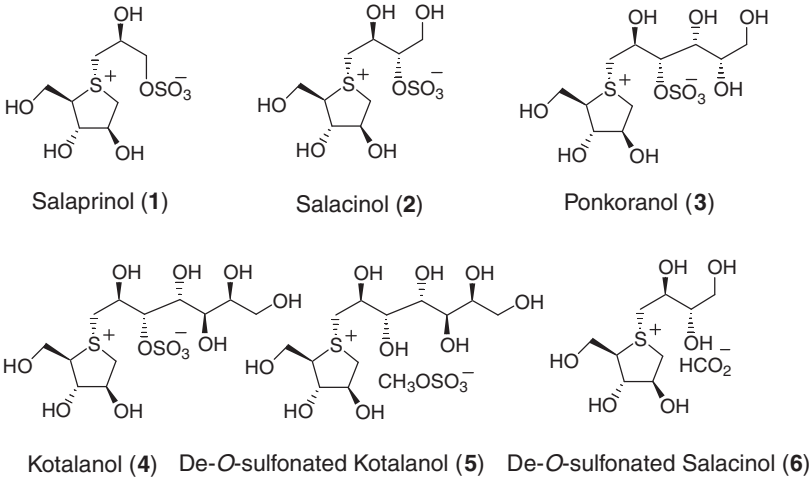


Figure 1. Components isolated from *Salacia* species.

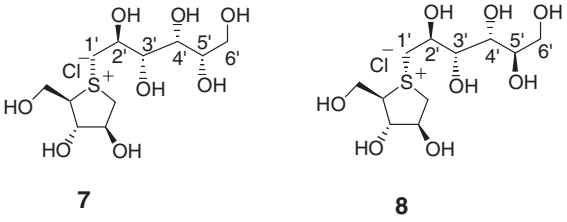


Figure 2. De-*O*-sulfonated ponkoranol and its 5′-stereoisomer.

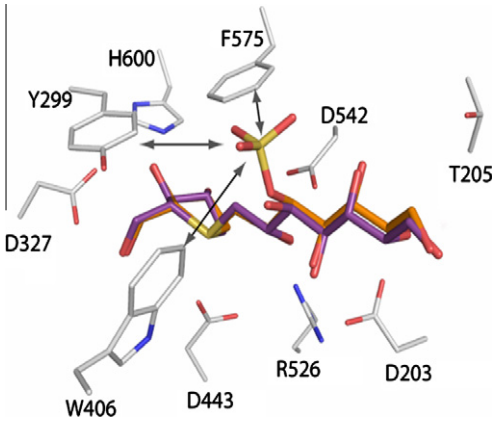


Figure 3. Effect of removing the sulfate group. Superposition of kotalanol (**4**) (orange) and de-*O*-sulfonated kotalanol (**5**) (purple) structures. Double-headed arrows show the proximities of the sulfate group to the surrounding hydrophobic residues Y299, W406, and F575. (Reproduced with permission from *Biochemistry*, **2010**, *49*, 443. Copyright American Chemical Society.)

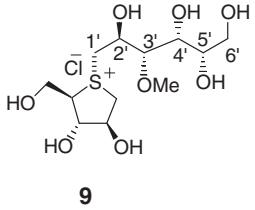
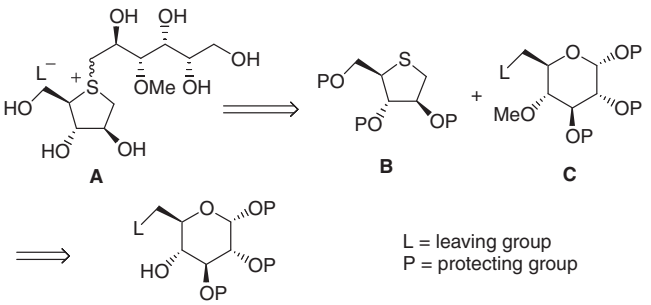


Figure 4. 3′-*O*-Methyl ponkoranol.

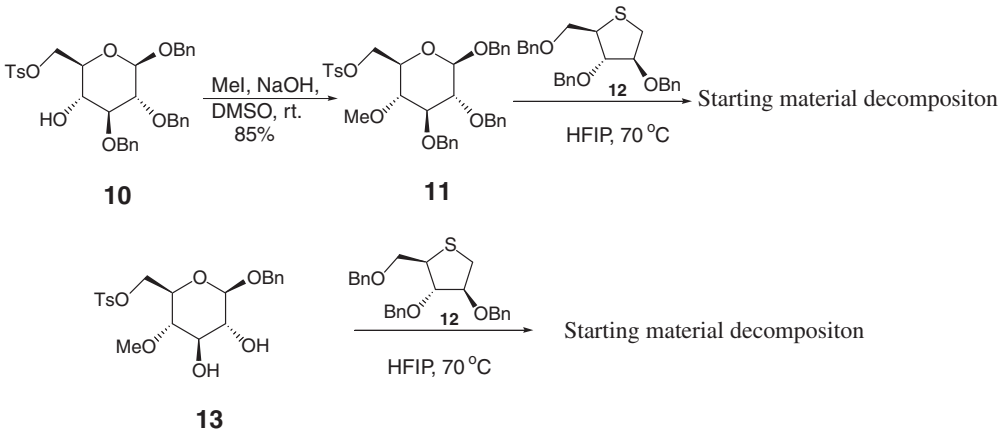
We report here an efficient synthetic route to 3′-*O*-methyl ponkoranol (**9**). Our synthetic strategy involved the alkylation of an appropriate protected anhydrothioarabinitol **B** at the ring sulfur atom with agent **C**. Agent **C** could be obtained by methylation of protected D-glucose at C-4′ (Scheme 1).

Thus, benzyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-tosyl-β-D-glucopyranoside (**11**) was obtained from **10**[19] by treatment with a mixture of methyl iodide in aqueous sodium hydroxide and dimethyl sulfoxide (50% w/w)[20] to afford **11**. Our initial attempt at the coupling reaction employed **11** with the anhydrothioarabinitol (**12**)[21] in 1,1,1,3,3,3-hexafluoroisopropyl alcohol (HFIP) at 70 °C, as in our previous work (Scheme 2).[22] No product formation and decomposition of the starting material were observed by TLC; the coupling reaction of benzyl 4-*O*-methyl-6-*O*-tosyl-β-D-glucopyranoside (**13**) with the anhydrothioarabinitol (**12**) was also unsuccessful, the same result being observed (Scheme 2).

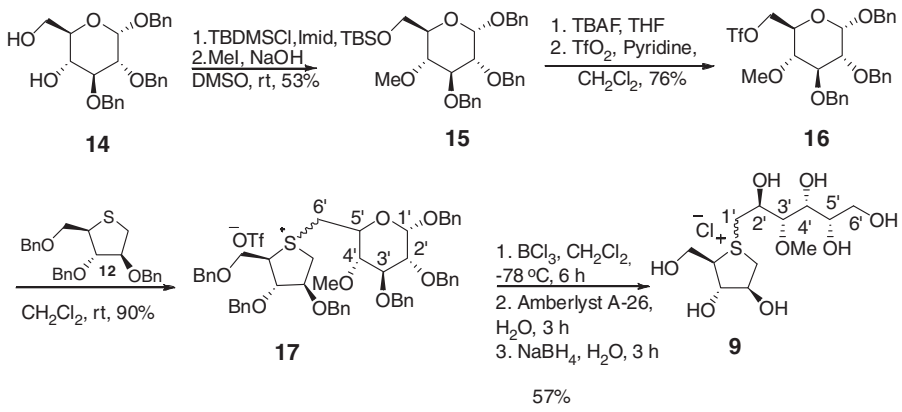
Next, a trifluoromethanesulfonyl (OTf) group was chosen as a leaving group to ensure milder conditions for the coupling reaction. Thus, the primary hydroxyl group in the diol **14**[19] was protected as its TBDMS ether followed by sequential methylation of the secondary hydroxyl group to give **15** in 53% over two steps (Scheme 3). Removal of the TBDMS group using tetrabutylammonium fluoride (TBAF) gave the alcohol which was treated with trifluoromethanesulfonyl anhydride to yield the glycoside **16** in 76% yield over two steps. The coupling reaction of the OBn-protected thioether (**12**) with benzyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-trifluoromethanesulfonyl-α-D-glucopyranoside **16** was carried out in dry CH_2Cl_2 at room temperature to give the corresponding protected sulfonium ion **17** as a 4:1 mixture of diastereomers at the stereogenic sulfur center. However, attempts to separate the mixture of diastereomers, even after deprotection and reduction, were unsuccessful.



Scheme 1. Retrosynthetic analysis.



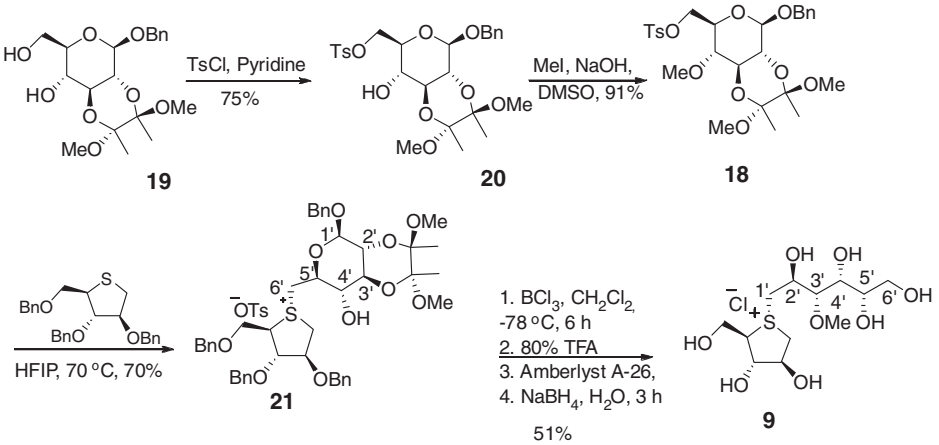
Scheme 2. First attempted syntheses of **9**.



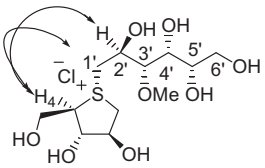
Scheme 3. Second attempted synthesis of **9**.

In another attempt, the tosylate **18**, containing a butane diacetal (BDA) protecting group, was chosen as the coupling partner. As shown in Scheme 4, the tosylate **18** was synthesized in two steps from benzyl 2,3-*O*-[(2*R*,3*R*)-2,3-dimethoxybutane-2,3-diyl]-β-D-glucopyranoside **19** which was, in turn, prepared from D-glucose according to literature procedures.[23] Thus, compound **19** was treated with *p*-toluenesulfonyl chloride to afford the *p*-toluenesulfonyl ester **20** in 75%, which was methylated with methyl iodide to give **18** in 91% yield. The coupling reactions of the OBn-protected 1,4-anhydro-4-thio-D-arabinitol (**12**) with the *p*-toluenesulfonyl ester **18** were carried out in HFIP to give the protected sulfonium ion **21** as a single diastereomer in 70% yield (Scheme 4).

Deprotection of the coupled product **21** was carried out in a two-step procedure. The benzyl group was first removed by treatment with boron trichloride at −78 °C in CH_2Cl_2, followed by sequential BDA deprotection with 80% TFA. During the course of benzyl group deprotection with BCl_3, the *p*-toluenesulfonate counterion was partially exchanged with chloride ion. Similar results were observed in our previous work.[18] Hence, after deprotection, the product was treated with Amberlyst A-26 resin (chloride form) to completely exchange the *p*-toluenesulfonate counterion with



Scheme 4. Synthesis of compound **9**.



9

Figure 5. 1D-NOESY correlations of selected protons in compound **9**.

chloride ion. Finally, the crude product was reduced with $NaBH_4$ to provide the desired 3′-*O*-methyl ponkoranol **9** in 51% yield over three steps (Scheme 4).

The absolute stereochemistry at the stereogenic sulfur center in **9** was established by means of 1D-NOESY experiments (Fig. 5). Correlation between H-1′ and H-4 and also a correlation between H-2′ and H-4 confirmed the anti relationship between the alkyl side chain and the C-4 substituent on the anhydroarabinitol moiety in compound **9**.

Finally, the inhibitory activity of compound **9** was examined against the N-terminal catalytic domain of recombinant human maltase glucoamylase (ntMGAM), a critical intestinal glucosidase for processing starch-derived oligosaccharides into glucose. The 3′-*O*-methyl ponkoranol **9** inhibited ntMGAM with a K_i value of 0.50 ± 0.04 μM. By comparison, de-*O*-sulfonated ponkoranol **7** and its 5′-stereoisomer **8** inhibited ntMGAM with K_i values of 43 ± 3 and 15 ± 1 nM, respectively.[17] We conclude, therefore, that the hydrophobic interactions between the methyl group and the hydrophobic residues Y299, W406, and F575 in the active site are not as optimal as the interactions of the latter groups with the rest of polyhydroxylated chain in the absence of the methyl ether; a similar situation is also observed in the binding of the sulfated compound, ponkoranol (**3**) (K_i = 0.17 ± 0.03 μM).

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Supplementary data

Supplementary data (experimental details and ^1H and ^{13}C NMR spectra of compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.020.

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