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COMMUNICATION

The effect of heteroatom substitution of sulfur for selenium in glucosidase inhibitors on intestinal α-glucosidase activities†‡

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The synthesis of selenium analogues of de-*O*-sulfonated ponkoranol, a naturally occurring sulfonium-ion glucosidase inhibitor isolated from *Salacia reticulata*, and their evaluation as glucosidase inhibitors against two recombinant intestinal enzymes maltase glucoamylase (MGAM) and sucrase isomaltase (SI) are described.

The root and stem extracts of *Salacia reticulata*, a large woody climbing plant found in Sri Lanka and southern India, have traditionally been used for treatment of type-2 diabetes.[1] Human clinical trials on patients with type-2 diabetes, conducted with the extract of *Salacia reticulata*, have shown effective treatment of type-2 diabetes with minimal side effects.[2] Our efforts in recent years have been focused on this novel class of α-glucosidase inhibitors comprising sulfonium ions as putative mimics of the oxacarbenium ion intermediates in glucosidase-mediated hydrolysis reactions.[3] Thus, we have described the synthesis and stereochemical structure elucidation of the naturally occurring glucosidase inhibitors salacinol (**1**),[4] ponkoranol (**2**),[5] kotalanol (**3**),[6] de-*O*-sulfonated kotalanol (**4**)[6] (Fig. 1) from the plant extract of *Salacia reticulata*. Interestingly, one of our synthetic compounds, de-*O*-sulfonated ponkoranol (**5**)[7] (Fig. 1), was isolated recently from the same plant.[8]

The antidiabetic property of these herbal extracts can be attributed, at least in part, to the inhibition of intestinal α-glucosidases by these sulfonium-ion components.[9–11] Crystallographic studies of the *N*-terminal domain of human intestinal maltase glucoamylase (ntMGAM), a family 31 glycoside hydrolase (GH31),[12] with several candidate inhibitors suggested that the permanent positive charge of these entities may mimic the oxacarbenium-ion like transition state of the glucosidase-mediated hydrolysis reactions.[13] We also suggested that the ring conformation of the thiocyclitol in kotalanol (**3**), a $^{3}T_{2}$ conformation, closely resembles the proposed $^{4}H_{3}$ conformation of the oxacarbenium-ion like transition state.[13]

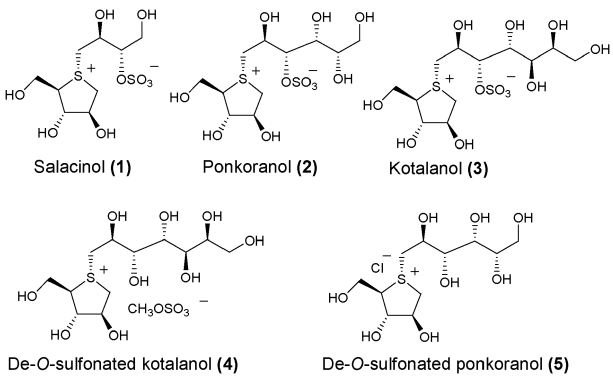

Fig. 1 Components isolated from *Salacia* species.

We have also reported the synthesis of the 5′-stereoisomer of de-*O*-sulfonated ponkoranol **6** (Fig. 2), and have shown that it is a potent (K_i = 15 nM) inhibitor of ntMGAM.[7]

As part of our continuing interest in evaluating the effect of heteroatom substitution in the sugar ring on glucosidase inhibitory activity, we have also synthesized selenium congeners of several candidates as potential glucosidase inhibitors.[14–16]

Comparison of the proposed intermediate in the glucosidase-catalyzed reaction and the selenonium ion, inferred from the X-ray crystal structure of the complex of kotalanol with ntMGAM,[13] shows that the selenonium ion in a $^{3}T_{2}$ conformation superimposes well on the $^{4}H_{3}$ conformation of the oxacarbenium ion intermediate (Fig. 3).

We now report the synthesis and enzyme inhibitory activity of analogues of **5** and **6**, in which the sulfur atom has been replaced by the heavier cognate atom selenium to give **7** and **8**, respectively (Fig. 4).

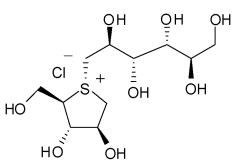

Fig. 2 Structure of the 5′-stereoisomer of de-*O*-sulfonated ponkoranol **6**.

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‡ Electronic supplementary information (ESI) available: Experimental procedures, characterization data and ^{1}H, ^{13}C NMR spectra of compounds **7**, **8**, **11** and **13**, 2D-NOESY spectra of compounds **13** and a representative Lineweaver–Burk plot. See DOI: 10.1039/c1cc13052h

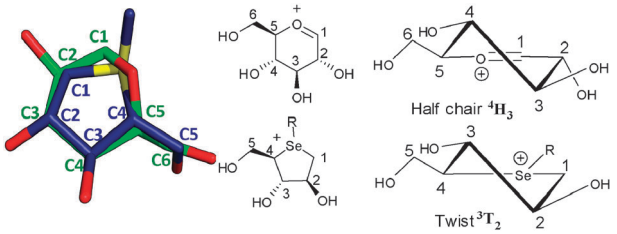


Fig. 3 Superimposition of the ring carbon atoms of the proposed intermediate in glucosidase-catalyzed reactions (in green) and the selenonium ion (in blue).

Retrosynthetic analysis indicated that **7** or its analogue **8** could be obtained by alkylation of an appropriately protected 1,4-anhydro-4-seleno-D-arabinitol **B** at the ring heteroatom with agent **C** (Scheme 1).[7]

The required benzyl (Bn)-protected D-selenoarabinitol (**10**)[14] and benzyl 6-*O*-*p*-toluenesulfonyl-α-D-gluco **9** or manno pyranoside **12**[7] were obtained by literature methods. The selenonium salts **11** and **13** were synthesized by alkylation of **10** with **9** or **12** (1.2 equiv.) in hexafluoroisopropanol (HFIP), containing K_2CO_3, to give **11** and **13** in 55% and 45% yields, respectively (Scheme 2). In the case of compound **11**, just one isomer was obtained at the stereogenic selenium atom, whereas in the case of compound **13**, a mixture of isomers was obtained; ^{1}H NMR spectroscopy showed the presence of two isomers in the ratio of 10 : 1. The benzyl protecting groups were removed with boron trichloride at −78 °C to give the desired sulfonium salts. During the course of deprotection, some of the tosylate counterions were exchanged with the chloride ions. Hence, the deprotected sulfonium salts were treated with Amberlyst A-26 (chloride form) to completely exchange the tosylate counterion with the chloride ion.[7] Finally, the products were reduced with $NaBH_4$ to provide the desired selenium analogues of de-*O*-sulfonated ponkoranol **7** and its 5′ epimer **8** in 52% and 45% yields, respectively, over three steps (Scheme 2).

The stereochemistry at the selenium centre for the major isomer of **13** was confirmed with the aid of a 2D-NOESY

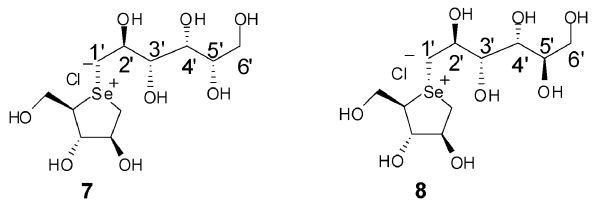
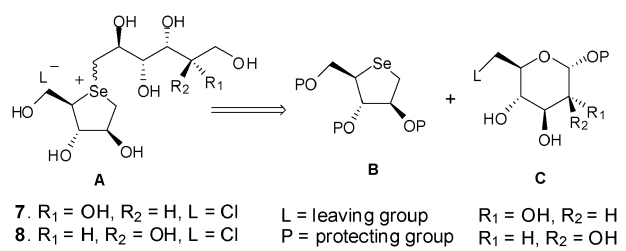
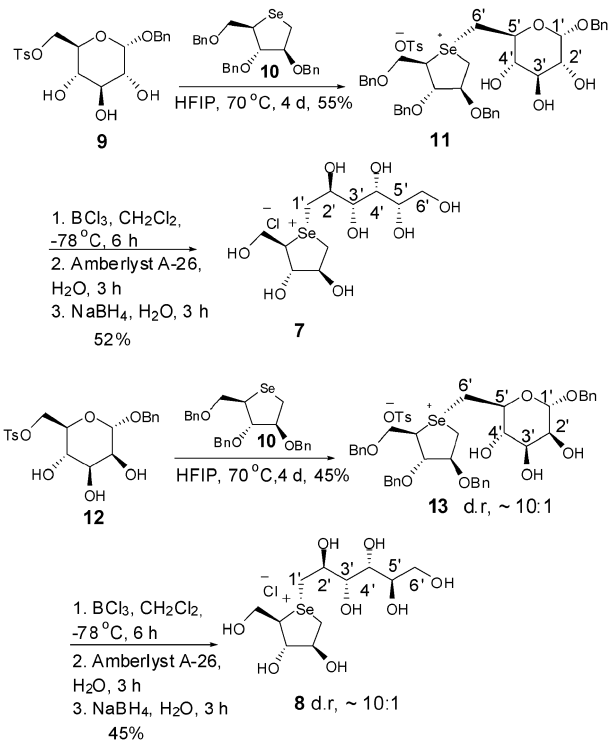


Fig. 4 Selenium analogues of de-*O*-sulfonated ponkoranol and its 5′-stereoisomer.



Scheme 1 Retrosynthetic analysis.



Scheme 2 Synthesis of compounds **7** and **8**.

experiment, which showed a correlation between H-4 and H-6′a, thus indicating that these atoms are *syn*-facial with respect to the sulfonium salt ring (Fig. 5). In the case of **11**, the absolute configuration at the selenium centre was assigned by analogy since a NOESY experiment was not possible owing to overlapping signals for H-4 and H-6′. This assignment is consistent with our previous work.[3]

Finally, we comment on the inhibitory activities of the compounds synthesized in this study and previous study[7] against the two recombinant intestinal enzymes, maltase glucoamylase (MGAM) and sucrase isomaltase (SI), critical for postamylase processing of starch-derived oligosaccharides into glucose. Each of these enzymes exhibits two separate catalytic units (by gene duplication) at their *C*- and *N*-terminal domains, resulting in ntMGAM, ctMGAM, ntSI, and ctSI enzyme activities.[17–22] There are also various alternative splicing patterns of ctMGAM in mammals; two spliceforms from mice are discussed in this communication: ctMGAM-N2 and ctMGAM-N20.[23,24] The experimentally determined inhibition constants (K_is) for the competitive inhibitors de-*O*-sulfonated ponkoranol (**5**) and its synthetic analogues (**6–8**) are listed in Table 1.

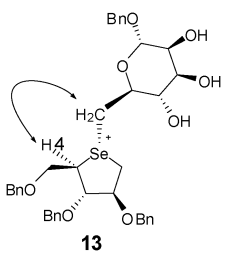


Fig. 5 2D-NOESY correlations of selected protons in compound **13**.

Table 1 Experimentally determined K_i values (μM)[a]

	CtMGAM-N2	ctMGAM-N20	ntMGAM	ctSI	ntSI
5	No inhibition	0.096 ± 0.015	0.043 ± 0.001[7]	0.103 ± 0.037	0.302 ± 0.123
6	No inhibition	0.138 ± 0.068	0.015 ± 0.001[7]	0.132 ± 0.047	0.138 ± 0.012
7	No inhibition	0.047 ± 0.014	0.038 ± 0.008	0.018 ± 0.004	0.013 ± 0.008
8	No inhibition	0.041 ± 0.027	0.025 ± 0.014	0.019 ± 0.010	0.010 ± 0.002

[a] Analysis of inhibition was performed using maltose as the substrate.

Comparison of the data in Table 1 indicates that substitution of the ring sulfur atom in de-*O*-sulfonated ponkoranol (**5**) and its 5′ epimer (**6**) by selenium results in a 13-fold and 23-fold improvement, respectively, in inhibition of ntSI. NtSI demonstrates the broadest substrate specificity of all four subunits and hydrolyses both α(1–4) and α(1–6) linkages.[25] Similarly, substitution of sulfur by selenium leads to an increase in inhibitory activity against ctSI (*cf.* **5** and **6** *vs.* **7** and **8**). In contrast, little discrimination is observed between the congeners for inhibition of ntMGAM or ctMGAM-N20. Interestingly, compounds **5–8** differentiate between the spliceforms of ctMGAM, and none shows inhibition of ctMGAM-N2; the reason for this selectivity must await structural analysis.

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