Potent and Selective Inhibition of Class II α-D-Mannosidase Activity by a Bicyclic Sulfonium Salt

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Recent years have seen a sustained interest in the search for glycosidase inhibitors.[1–3] An important impetus has come from the recognition that the control of oligosaccharide metabolism by using such inhibitors might offer a promising strategy in the treatment of diseases such as cancer, influenza, and diabetes.[4] Further, the use of glycosidase inhibitors as tools with which to delineate the details of oligosaccharide biosynthesis[5] as well as to clarify the catalytic mechanisms of glycosidases[2,3,6] has also provided an important stimulus in this search.

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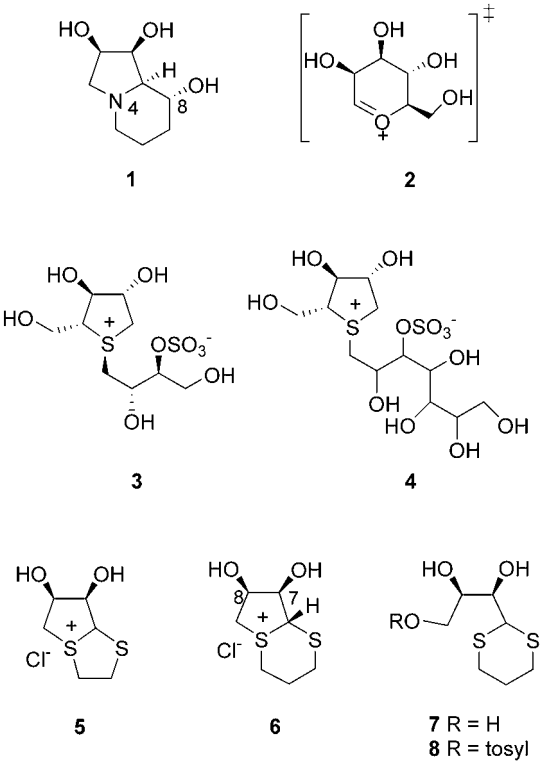
The polyhydroxylated alkaloid swainsonine (**1**),[7] is the archetypal mannosidase inhibitor. This iminosugar has frequently



been instructive in the conception of novel synthetic inhibitors of glycosidases.[8,9] The pronounced activity of swainsonine against α-D-mannosidases is thought to be related to its resemblance, when protonated, to the mannosyl cation **2**.[10–12] Although uncertainties remain as to the exact sequence of events and the precise structure of any intermediates in the mechanism-of-action of α-D-mannosidases, such as the human Golgi mannosidase II (HGMII),[12–14] there is little doubt that a transient species is generated on the reaction coordinate that carries a considerable build up of positive charge in the vicinity of the anomeric center of the substrate. It is thus an interaction of the conjugate acid of the inhibitor ($pK_a \sim 7.4$), with the partially deprotonated enzymic catalytic-carboxyl group (pK_a between 4.1 and 4.6), that is presumed to be the origin of the observed tight binding of **1** by HGMII and related enzymes.[10,15]

Considerations such as these led us to conjecture[16] that properly designed sulfonium salts might be effective mimics of glycosyl cations and, as a consequence, inhibit glycosidases. The recent discovery of the natural polyhydroxy sufonium salts salicinol (**3**) and kotalanol (**4**), and the demonstration that they too are potent inhibitors of glycosidases,[17,18] has convinced us that sugar mimics, such as salt **5**, would provide fresh opportunities for inhibitor design. However, although a number of such analogues have since been proposed, most of these salts have proven to be rather low in activity.[19,20]

This paper describes the synthesis of (1*R*,6*R*,7*R*,8*S*)-7,8-dihydroxy-5-thia-1-thioniabicyclo[4.3.0]nonane chloride (**6**) and demonstrates it to be not only a potent inhibitor of α-D-mannosidase activity, but also markedly more selective than swainsonine.

On treating crude D-erythrose[21] with 1,3-propanethiol and concentrated HCl the cyclic thioacetal, **7**, was obtained after flash chromatography on silica gel. This product cyclized to give the target salt, **6**, simply upon treatment with *p*-toluenesulfonyl chloride in pyridine at 10 °C. The intermediacy of γ-tosylate **8** was shown by ^{13}C NMR spectroscopic examination of aliquots of the reaction mixture, which were removed during a 48 h period (data not reported), but no attempt was made to isolate it. Concentration of the crude reaction mixture after this period revealed that it was composed of salt **6** as the only new sugar-derived component, and unreacted starting materials. The new product, **6**, was isolated as its chloride salt by flash chromatography on silica gel with a yield of ~40%.

The 1*R*,6*R*,7*R*,8*S*-*cis*-fused structure and conformation of **6** were elucidated on the basis of their NMR spectroscopic data. The observed formation of only one sulfonium salt in the cyclization reaction is remarkable in that either sulfur atom might have been expected to participate in tosylate displacement.[22] The proton spectrum of salt **6** shows a large three-bond scalar coupling of 10.6 Hz between H-6 ($\delta = 4.736$) and H-7 ($\delta = 4.606$); this indicates that they have an almost antiperiplanar relationship. The equatorial orientation of H-6 and the $^3C_6$ conformation of its six-membered cycle are consistent with the strong NOEs observed between H-7 and both H-2_{axial} and H-4_{axial}, and also supported by the absence of NOEs between H-6 and either H-2_{axial} or H-4_{axial}. The proposed $^3C_6$ conformation of the six-membered ring in salt **6** is further supported by the large three-bond proton–carbon coupling constants observed between H-6 and C-2 as well as between H-6 and C-4, as would be expected from their *trans* relationship, and are manifested as sizable cross-peaks in the HMBC spectrum of **6** (data not shown). The *cis*-fused structure and conformation of **6** were further affirmed by exploiting a recently developed approach that involves a combination of computational and NMR spectroscopic techniques.[22]

Although the cyclization of γ-activated sugar-derived thioacetals to give five-membered sulfonium salts is known to be facile,[23] the product salts are notoriously susceptible to rearrangements and nucleophilic displacements. In the past their intermediacy was presumed only to account for the various secondary products that were isolated.[24] In contrast, the bicyclic sulfonium salt **5** was found to be relatively robust,[16] as were the more recently described synthetic and natural sulfonium salts.[17–20] This is also true of sulfonium salt **6**, which showed no sign of decomposition even after months at ambient temperature in either methanol or aqueous solution. Salt **6** was not prone to scrambling even when heated overnight at 80 °C in water (data not shown).

Confident of its structure, solution conformation,[22] and stability to assay conditions, we tested salt **6** against both recombinant human-lysosomal α-mannosidase (HLM)[25a] and recombinant human-Golgi α-mannosidase II (HGMII)[25b] using 4-methylumbelliferyl α-D-mannopyranoside as a fluorogenic substrate. Inhibition constants (K_i) were calculated from Lineweav-

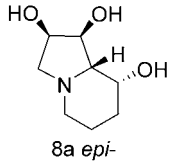
er–Burke plots for various inhibitor concentrations between 160 and 3 mM. Inhibition of recombinant HGMII by compound **6** was found to be competitive with a K_i of 15 μM, but a much better K_i of 800 nM was obtained with the lysosomal enzyme. Swainsonine itself gives a K_i of 35 nM against HLM. Inhibition of a panel of twelve crude human-liver glycosidases by salt **6** was also studied under standard assay conditions (1 mM concentration).[9a] Salt **6** was found to potently inhibit crude α-mannosidase activity in human liver (97%, pH 4). The natural compound **1**, in addition to human-liver α-D-mannosidase activity (100% at pH 4), inhibited crude human-liver α-D-glucosidase, β-D-galactosidase, and α-D-arabinosidase activities by more than 80% at a concentration of 1 mM (Table 1). The synthetic inhibitor **6**, however, did not inhibit other crude human-liver enzymes to any extent, and is therefore seen to be markedly more selective than iminosugar **1** at these concentrations. Most notably, salt **6** shows no inhibition of α-D-fucosidase, an activity that is characteristic of many α-mannosidase inhibitors.

Table 1. *IC_{50}'s (percentage inhibition) obtained for compounds **6**, **1**, and **11** against a panel of crude human-liver lysosomal enzymes. (Inhibitor concentration = 1 mM.)*

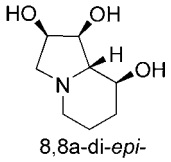
	Compound		
Enzyme	**6**	**1**	**11**
α-D-mannosidase (pH 4)	**97**	**100**	61 (K_i = 0.75 mM)
α-D-mannosidase (pH 6.5)	**100**	**95**	37
β-D-mannosidase	4	4	5
α-D-glucosidase	2	**80**	21
β-D-glucosidase	6	16	3
α-D-galactosidase	1	2	0
β-D-galactosidase	26	**84**	10
α-L-fucosidase	13	6	92 (K_i = 5 μM)
β-D-hexosaminidase	4	1	54
β-D-glucuronidase	7	52	4
β-D-xylosidase	0	61	5
α-D-arabinosidase	15	**83**	5

K_i values are reported for lysosomal enzymes partially purified from human liver. For assay conditions see ref. [9a].

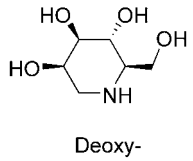
In contrast to **6**, the dihydroxylated iminosugar, 8-deoxyswainsonine is reported not to be an effective glycosidase inhibitor, although they possess the same number of hydroxyl groups.[7c] Furthermore, the activity of the title salt against HLM (K_i of 800 nM) is far higher than that of 8a-*epi*- and 8,8a-di-*epi*-swainsonine (**9** and **10**, respectively), which have the same ring-junction and hydroxyl-group configuration as the sulfonium salt.[9a] Thus, when tested against human lysosomal α-D-mannosidase, salt **6** is seen to be far more potent than 8a-*epi*-swainsonine (**9**; K_i' of 75 μM). Even though salt **6** has one hydroxyl group fewer than **1**, it shows far greater activity to the di-*epi* analogue, **10**. The latter analogue gives a K_i of 20 μM and, although it does possess a hydroxyl group at C-8, its configuration is opposite to that of swainsonine. In this respect, it is interesting to note that 5-fluorogulosyl-β-L-fluoride (5FGulF), the 5-epimer of the pseudosubstrate 5-fluoro-α-D-mannosyl fluoride, was found to bind tightly to the cloned Golgi α-mannosidase from *Drosophila melanogaster*.[12a,13b] Both salt **6** and 5FGulF share an inverted configuration at the site that is equivalent to the C-5 position of D-mannose. Moreover, very little difference between the abilities of deoxymannonojirimycin (DMJ) and 5-*epi*-DMJ to inhibit mannosidases has been reported.[12b] DMJ (**11**) exhibits a K_i of 0.75 mM at pH 5.5, which is much poorer than that observed for sulfonium salt **6**. Furthermore, the K_i's of these two compounds follow the trend that class II α-D-mannosidases are more susceptible to inhibition by D-mannofuranose analogues than by D-mannopyranose analogues.[9a] The selectivity shown by salt **6** towards the panel of lysosomal glycosidases tested might have been anticipated to be less marked than that of the natural product **1**, in that the iminosugar possesses one more hydroxyl function than **6** and a bridgehead configuration opposite to that of the sulfonium salt. Moreover, swainsonine analogues with fewer than three hydroxyl groups have been reported to be ineffective as mannosidase inhibitors. That the sulfonium salt **6** proves to be more selective for HLM than for HGMII when compared with iminosugar **1**, suggests too that the replacement of a hydroxymethyl moiety for a sulfur group, and the ensuing changes expected in bond lengths and angles, could also be the root of the bias shown by analogue **6** for HLM, and might be important to take into consideration when designing selective inhibitors of HGMII.



8a *epi*-swainsonine, **9**



8,8a-di-*epi*-swainsonine, **10**



Deoxymannonojirimycin, **11**

In conclusion, this work reports a convenient synthesis of the synthetic bicyclic polyhydroxylated sulfonium salt **6**. This compound is shown to more selectively inhibit α-mannosidase activity than the natural iminosugar, swainsonine. Moreover, salt **6** is seen to be the most potent *synthetic* sulfonium salt glycosidase inhibitor reported thus far. We believe that the ease with which salt **6** has been synthesized, together with its remarkable potency and selectivity towards class II α-mannosidases, will revive hope that the sulfonium salt motif will prove valuable in the development of effective glycosidase inhibitors.

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