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Synthesis from D-Altrose of (5*R*,6*R*,7*R*,8*S*)-5,7-Dihydroxy-8-hydroxymethylconidine and 2,4-Dideoxy-2,4-imino-D-glucitol, Azetidine Analogues of Swainsonine and 1,4-Dideoxy-1,4-imino-D-mannitol

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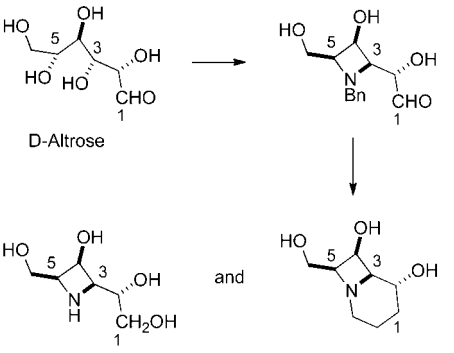
ABSTRACT



Sterically congested azetidine analogues of swainsonine

Ring closure of a 3,5-di-*O*-triflate derived from D-altrose with benzylamine allowed the formation of both monocyclic and bicyclic azetidine analogues of swainsonine.

The biological activity of monocyclic azetidine iminosugars[1] is of current interest.[2] *N*-Alkyl hydroxyazetidines are potent inhibitors of purine nucleoside phosphorylase with subnanomolar K_i,[3] azetidine iminosugar analogues of pentoses have been found to be specific inhibitors of nonmammalian glycosidases,[4,5] and *N*-nonyl trihydroxyazetidines are specific inhibitors of some ceramide-specific glucosyl transferases and glucosidases.[6] The only reported

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bicyclic azetidine iminosugars, designed as glycosidase inhibitors, have no hydroxyl group in the azetidine ring.[7] This paper describes the synthesis of (5*R*,6*R*,7*R*,8*S*)-5,7-dihydroxy-8-hydroxymethylconidine **1**[8] and 2,4-dideoxy-2,4-imino-D-glucitol **2**, which are azetidine analogues of swainsonine **4** and DIM [1,4-dideoxy-1,4-imino-D-mannitol] **5** (Figure 1).[9] The synthesis requires the formation of the azetidine ring by a double displacement of leaving groups at C3 and C5 in a protected derivative of D-altrose **6**, to give the key intermediate aldehyde **3**.

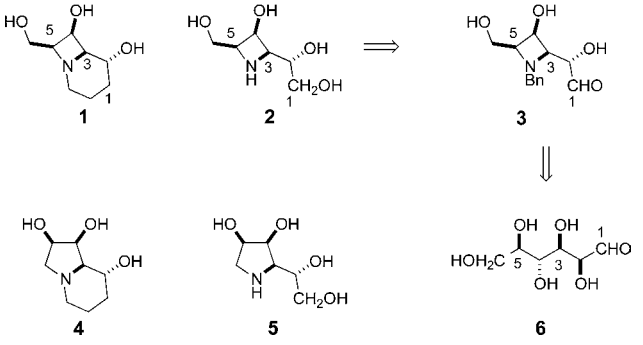
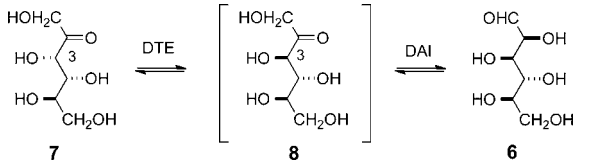


Figure 1. Swainsonine analogues.

Both swainsonine **4**[10] and DIM **5**[11] are specific and potent inhibitors of an α-mannosidase of *N*-linked glycoprotein processing. Swainsonine is currently being studied as a potential chemotherapeutic agent for cancer,[12] for its effect as an immunoregulator,[13] and as a probe in the life cycle of prions.[14] Structure–activity relationships of swainsonine analogues on α-mannosidase inhibition have been studied.[15,16] This paper also reports assays of glycosidase inhibition by the azetidine analogues **1** and **2**.

Scheme 1. Formation of D-Altrose **6** from D-Fructose **7**



Biotechnology, and in particular the isomerization of common to rare hexoses by Izumoring,[17] has greatly increased the number of carbohydrates that may conveniently be used as chirons. D-Altrose **6**, a hitherto inaccessible sugar, was formed in a batch reactor from D-fructose **7** by epimerization of C3 by D-tagatose-3-epimerase (DTE) to D-psicose **8** which was equilibrated *in situ* by D-arabinose isomerase (DAI) to form D-altrose **6** [Scheme 1] without the need for the isolation of intermediates.[18]

The sterically crowded azetidine **14** is a divergent intermediate for the synthesis of **1** and **2** [Scheme 2]. Protection of D-altrose **6** with acetone in the presence of sulfuric acid and anhydrous copper(II) sulfate gave an inseparable mixture of the pyranose **9** and furanose **10** diacetonides in 85% yield in a ratio of 2:3.[19] Partial hydrolysis of the mixture of **9** and **10** with aqueous acetic acid resulted in selective hydrolysis of the side chain acetonide of the furanose isomer **10** which allowed isolation of the required

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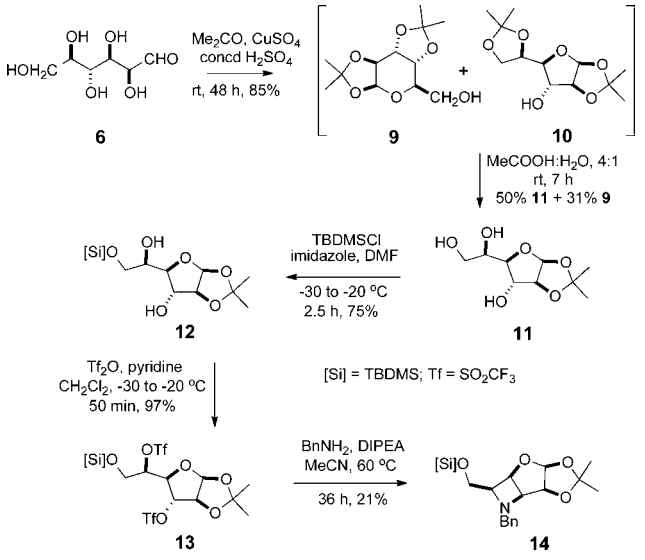
monoacetonide **11** (50%) together with pure pyranose diacetonide **9** (31%).[20]

Scheme 2. Synthesis of Key Intermediate **14**



Treatment of **11** with TBDMS chloride in DMF in the presence of imidazole afforded selective protection of the primary alcohol to give the silyl ether **12** (75%). Esterification of the two remaining hydroxyl groups in **12** with triflic anhydride in dichloromethane in the presence of pyridine formed the ditriflate **13** (97%). Reaction of **13** with benzylamine in acetonitrile in the presence of diisopropylethylamine (DIPEA) gave the key azetidine **14** in low yield (21%).

Cyclizations of 3,5-di-*O*-triflates of protected furanosides with amines usually proceed in excellent yield.[4,21] The extent of steric congestion in azetidine **14** was explored with the gas phase geometries of **15D** and **14** by DFT calculations at the B3LYP/6-31G* level of theory.[22] The overlay of the calculated molecular model of **15D** with its corresponding X-ray structure[23] (shown in green) is shown in Figure 2A;[24] the close fit of the two structures indicates that the molecular modeling provided a realistic representation of the molecule. Therefore azetidine **14** was modeled using the same presets, and its structure is shown in Figure 2B in comparison to the same crystal structure of **15D**. Inclusion of the additional TBDMS protected hydroxymethyl side chain in **14** has displaced both the *N*-benzyl and isopropylidene protecting groups, reinforcing the high degree of spatial crowding in azetidine **14**. The low yield of **14** may be due to steric crowding developing in the transition state of the formation of the azetidine ring.

Hydrolysis of **14** with aqueous trifluoroacetic acid removed both the silyl ether and acetonide protecting groups to give **3** which, on reduction by sodium borohydride in water, gave the *N*-benzylazetidine **16** (80% yield from **14**) [Scheme 3]. Transfer hydrogenation of **16** by ammonium formate in the presence of palladium on carbon gave the azetidine analogue **2** of DIM. Reaction of **3** with the stabilized ylid, $Bu_3P{=}CHCO_2Me$, in 1,4-dioxane afforded the Wittig product **17** (48% from **14**). Transfer hydrogenation of **17** caused debenzylation and reduction of the C=C and gave a mixture of products from which the bicyclic lactam **18** could be isolated in 34% yield. From the IR spectrum of the crude reaction mixture, it was apparent that a lactone was also formed, but it was not possible to obtain a pure sample of any product other than lactam **18**. The low yield of cyclization to **18** may again be due to steric congestion in the highly substituted azetidine ring. In order to avoid competing lactone formation, all the hydroxyl groups in **17** were protected as the corresponding trisilyl ether **19** by treatment with TBDMS triflate in DMF in the presence of 2,6-lutidine (75%). Transfer hydrogenation of **19** in methanol gave a mixture of an ester together with lactam **20**; further heating of the reaction mixture in acetonitrile allowed the isolation of pure fully protected lactam **20** but, again, only in poor yield (30%). Reduction of lactam **20** with borane in THF gave the borane adduct **21** in which four substituents on the azetidine ring are *cis*. Treatment of **21** with acidic ion-exchange resin in methanol destroyed the borane complex and removed the silyl ethers to allow the isolation of the free trihydroxyconidine **1** (92%). It is noteworthy that, though the cyclizations proceeded in low yield, possibly due to steric crowding in the bicyclic azetidine, the azetidine swainsonine analogue **1** was stable to the acid treatment in the final step.

Inhibition by the azetidines **1**, **2**, **16**, and **18** of the following glycosidases was studied:[25] α-glucosidases (rice, yeast, rat intestinal maltase, *A. niger*), β-glucosidases (almond, bovine liver), α-galactosidase (coffee beans), β-galactosidase (bovine liver), α-mannosidase (Jack bean), β-mannosidase (snail), α-L-rhamnosidase (*P. decumbens*),

(20) A crystal structure of compound **11** was obtained. Data were collected at low temp [Cosier, J.; Glazer, A. M. *J. Appl. Crystallogr.* **1986**, *19*, 105107], and the structure was solved using SIR92[Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Carnalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435] and refined using the CRYSTALS software suite[Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K.; Watkin, D. J. *J. Appl. Crystallogr.* **2003**, *36*, 1487]; full refinement details are given in the Supporting Information. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre (CCDC 893177), and copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

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(22) For full details of the performed calculations, please see the Supporting Information.

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(24) Figures were generated using Maestro 9.2 of the Schrödinger Software Suite 2011.

(25) For details of assays, see: (a) Mercer, T. B.; Jenkinson, S. F.; Nash, R. J.; Miyauchi, S.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2009**, *20*, 2368–2373. (b) Best, D.; Wang, C.; Weymouth-Wilson, A. C.; Clarkson, R. A.; Wilson, F. X.; Nash, R. J.; Miyauchi, S.; Kato, A.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2010**, *21*, 311–319. A table of the glycosidase inhibition by the azetidines is shown in the Supporting Information.

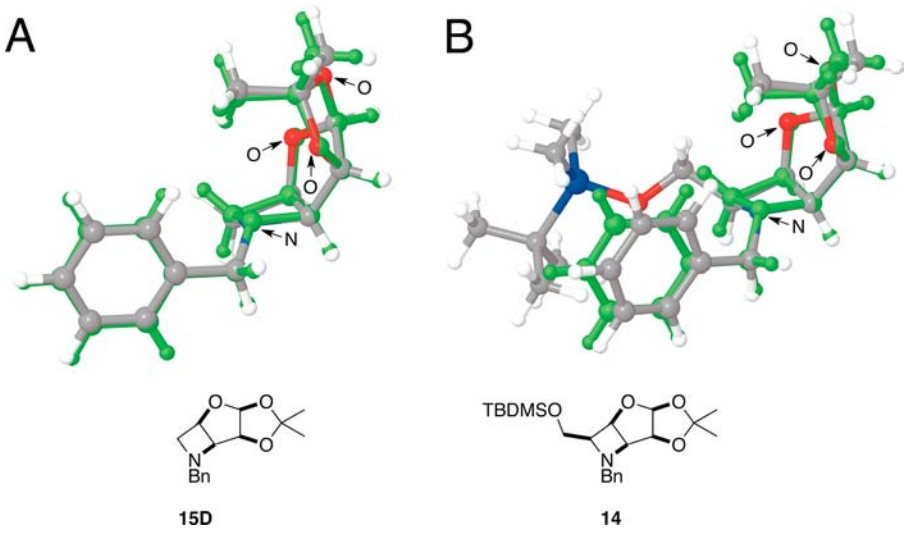
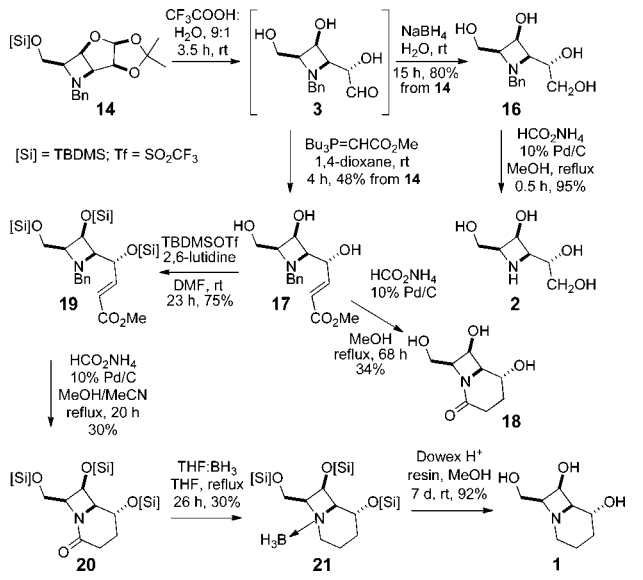


Figure 2. (A) Overlay of calculated and X-ray crystal structure (shown in green) of **15D**, validating the applicability of the DFT calculations to azetidine ring systems. (B) Overlay of calculated structure for **14** with X-ray crystal structure (shown in green) of **15D**, indicating the high degree of spatial crowding in azetidine **14**.

Scheme 3. Formation of Swainsonine Analogues **1**, **2**, and **18**



α-L-fucosidase (bovine kidney), trehalase (porcine kidney), and amyloglucosidase (*A. niger*). The monocyclic azetidine analogue of DIM **2** showed no inhibition of any glycosidase; its *N*-benzyl derivative **16** was a specific weak inhibitor of almond β-glucosidase [IC_{50} 545 μM]. Both the conidine analogue of swainsonine **1** and the corresponding lactam **18** were weak inhibitors of β-galactosidase [IC_{50} 492 and 341 μM, respectively]. Azetidine swainsonine **1** also showed weak inhibition of α-mannosidase [IC_{50} 640 μM].

In summary this paper describes the synthesis of azetidine analogues of the mannosidase inhibitors swainsonine and DIM *via* a highly hindered bicyclic azetidine. The sterically congested and functionalized conidine **1** was remarkably stable to acid, in contrast to the ready acid catalyzed polymerization of conidine itself.[26] None of the azetidines were potent inhibitors of any glycosidase.

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Supporting Information Available. Experimental procedures, full spectroscopic data, full details of the biological assays and computational data. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.