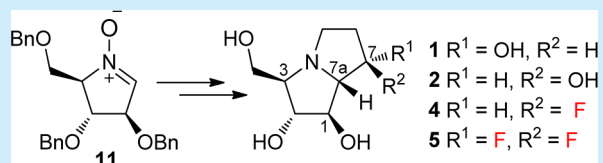


Synthesis and Glycosidase Inhibition of Australine and Its Fluorinated Derivatives

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S Supporting Information

ABSTRACT: Australine (1), 7-*epi*-australine (2), and their C-7-fluorinated derivatives 4 and 5 have been synthesized efficiently from D-arabinose-derived cyclic nitron 11. Fluorination at the C-7 position enhanced the inhibition against *A. niger* α -glucosidase, and this constitutes the first example of fluorination substitution for a hydroxyl increasing the inhibition of any glycosidases. The enantiomers synthesized from nitron *ent*-11 showed no inhibition of the corresponding enzymes.



Australine (1) was isolated from seeds of *Castanospermum australe* in 1988¹ and can be viewed as a conformationally fixed 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, 6) analogue. As one of the most investigated iminosugars, australine (1) is a potent inhibitor of α -glucosidase,² a competitive inhibitor of amyloglucosidase,^{1,3} and the first polyhydroxylated pyrrolizidine inhibiting glycoprotein processing enzyme glucosidase I,^{3b} which also displays antiviral⁴ and anti-HIV activity.⁵ Compared to DMDP (6),⁶ the glycosidase inhibition profile of australine (1) was believed to arise from the locked bicyclic structure which abolished the C_{2v} symmetry of the system.^{6d,7} In the context of our continuing interest in the synthesis and biological activities of DMDP-related iminosugars,⁸ we became interested in the synthesis and structure–activity relationship study of australine and its derivatives, especially C-7-fluorinated derivatives (Figure 1).

Fluorination is a powerful tool in structure–activity relationship studies.^{8d,9} The importance of the C-6 hydroxyl group of DMDP in glycosidase inhibition had been reported by Stütz and co-workers.^{9b} Study of the role of the C-7 hydroxyl of the bicyclic system of australine could prove insightful, since the C-7 hydroxyl of australine corresponds to the C-6 hydroxyl of DMDP. In this regard, fluorination of iminosugar hydroxyls usually decreases glycosidase inhibition except for a few examples.^{9e}

The synthesis and glycosidase inhibition evaluation of 7-deoxy-7-fluoro-australine (3), 7-deoxy-7-fluoro-7-*epi*-australine (4), and 7,7-difluoro-7-deoxyastraline (5) could help us to understand the SAR of australine and that of the DMDP-

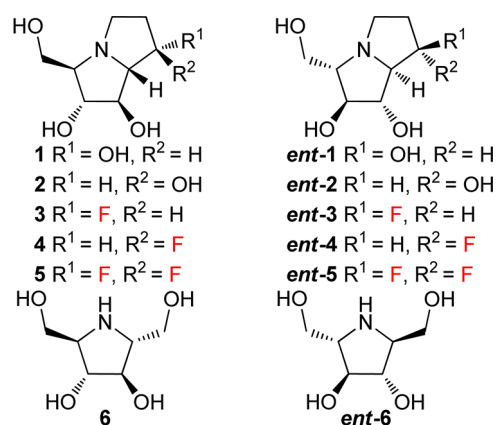


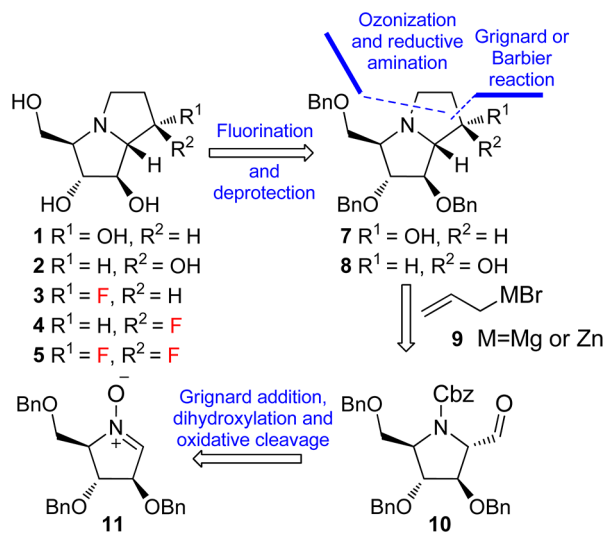
Figure 1. Iminosugars related to australine.

related iminosugars. Moreover, synthetic enantiomers of some naturally occurring iminosugars are more potent glycosidase inhibitors.¹⁰ For example, L-DMDP (*ent*-6) is a more powerful and specific α -glucosidase inhibitor than DMDP (6).^{8d,10a,f} Therefore, the synthesis and glycosidase inhibition evaluation of enantiomers of australine (*ent*-1) and its derivatives (*ent*-2)–(*ent*-5) could help to provide a better understanding of the SAR of australine and DMDP-related iminosugars.

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Synthetic methods for accessing australine or its derivatives have been well documented in the literature via a range of approaches.¹¹ Our strategy was based on the sugar-derived nitron 11¹² which can be easily converted to aldehyde 10, establishing the bicyclic structure in an efficient way (Scheme 1).^{8d,12h} The aldehyde can then be converted to australine

Scheme 1. Retrosynthesis of Australine and Its Derivatives

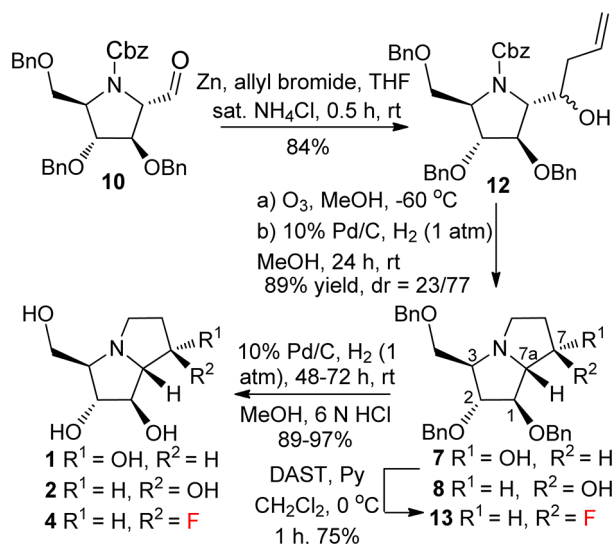


derivatives 7 and 8 by Grignard or Barbier reactions, ozonization, and reductive amination. Direct hydrogenation of 7 and 8 would give australine (1) and its C-7 epimer 2, while fluorination of the suitably protected 7 and 8 and subsequent deprotection would afford fluorinated australine derivatives 3–5.

Thus, the key intermediate, aldehyde 10, which was prepared via a reported method from nitron 11 in 81% overall yield within 4 steps,^{8d,12h} reacted with allylzinc bromide under Barbier conditions¹³ to give 12 as an inseparable mixture of two isomers (dr = 35/65, determined by ¹H NMR). Sequential ozonization and reductive amination of 12 afforded tri-O-benzylated australine derivatives 7 and 8 in 89% yield (dr = 23/77) which were separated by silica gel chromatography. The configuration of the C-7 hydroxyl was determined by comparing the optical rotation and NMR data of 7 and 8 with those reported for 1,2,8-tri-O-benzyl-australine [7: $[\alpha]_D^{20} +9.0$ (c 1.11 in CH₂Cl₂); lit. of 7:^{11d} $[\alpha]_D^{25} +8.4$ (c 1.6 in EtOH); 8: $[\alpha]_D^{20} -9.8$ (c 1.02 in CH₂Cl₂); lit. of 8:¹¹ⁱ $[\alpha]_D^{20} -27$ (c 0.33 in CHCl₃)]. Further hydrogenolysis of 7 and 8 led to australine (1) and 7-*epi*-australine (2), respectively, in high yields. The ¹H and ¹³C NMR spectra of compounds 1 and 2 were identical to those reported for australine and 7-*epi*-australine with optical rotations [1: $[\alpha]_D^{20} +9.0$ (c 1.12, H₂O); 2: $[\alpha]_D^{20} -12.0$ (c 1.84, H₂O)] consistent with literature [australine: $[\alpha]_D^{25} +8.0$ (c 0.35, H₂O);^{11d} 7-*epi*-australine: $[\alpha]_D^{24} -14.1$ (c 0.22, H₂O),^{14a} $[\alpha]_D^{23} -13.2$ (c 1.2, H₂O)^{14b}].

Treatment of the alcohols 7 and 8 (Scheme 2) with DAST (diethylaminosulphur trifluoride) unexpectedly gave the same fluorinated product 13. The structure of 13 was determined by spectroscopic data, and its C-7 configuration was determined as an *R*-configuration through a NOESY experiment (13: H1 and H7; Supporting Information). Fluorination of 8 with DAST may have occurred via an aziridinium ion intermediate,^{11a,15} which opened with a second inversion when attacked by

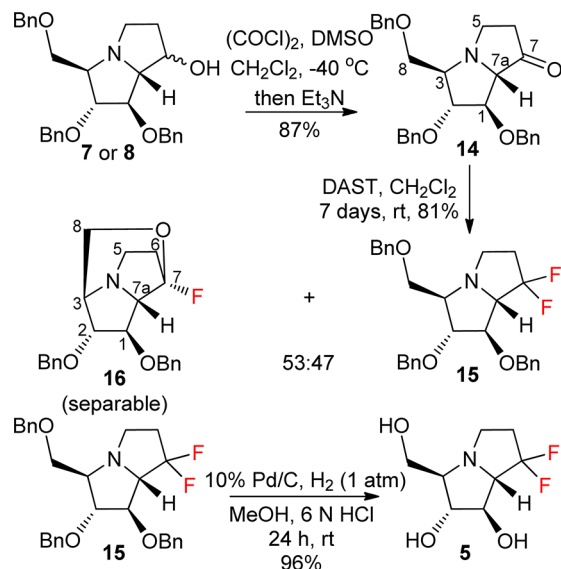
Scheme 2. Synthesis of Australine and Its Derivatives



fluoride, affording the configuration-retained product 13. As for alcohol 7, fluorination proceeded in an ordinary S_N2 pattern, giving the configuration-inverted product, i.e., 13. After hydrogenation of the fluorinated derivative 13, 7-deoxy-7-fluoro-7-*epi*-australine (4) was obtained in high yield.

To prepare the C-7-difluorinated derivative of australine, i.e. 7,7-difluoro-7-deoxyastraline (5), the alcohols 7 and 8 were transformed into ketone 14 via Swern oxidation in 87% yield. Treatment of ketone 14 with DAST afforded the desired C-7-difluorinated derivative 15 together with a tricyclic byproduct 16. Debenzylation of 15 by hydrogenation gave the expected difluorinated product 5 in 96% yield (Scheme 3).

Scheme 3. Synthesis of 7,7-Difluoro-7-deoxyastraline (5)



A plausible reaction pathway for formation of the C-7 difluorinated product 15 and the tricyclic byproduct 16 is shown in Figure 2. Reaction of ketone 14 with DAST formed the intermediates 17a and 17b, where both would give product 15 by an S_N2 attack of fluoride.¹⁶ It is also possible to finish the reaction in another way; that is, an aziridinium system can be formed by losing the leaving group, and the ion is then opened

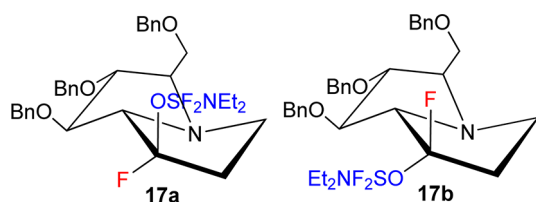


Figure 2. Possible intermediate in formation of 15 and 16.

by a second fluoride to give product 15.^{11a,15} Since the C-8 benzyloxy group can also act as a nucleophile in 17a and 17b, an intramolecular S_N2 reaction in 17b would furnish the cyclic ether in product 16,^{14b,17} while such a reaction is not favorable in 17a.

Through similar procedures, the enantiomers of australine (*ent*-1) and its derivatives *ent*-2, *ent*-4, and *ent*-5 were also synthesized from the D-xylose-derived nitron *ent*-11 smoothly.

These compounds were then assayed as potential inhibitors for a range of glycosidases (see the Supporting Information). Australine (1) and its C-7 diastereomer (2) proved to be potent inhibitors of *A. niger* α -glucosidase (IC_{50} = 6.0 and 1.5 μ M, respectively) and *A. niger* amyloglucosidase (IC_{50} = 37 and 8.0 μ M, respectively). By contrast, *ent*-1 and *ent*-2¹⁸ showed virtually no inhibition of the tested enzymes, which is in sharp contrast to the more potent glycosidase inhibition of L-DMDP (*ent*-6) than DMDP (6).^{8d,10a,f} For fluorinated derivatives, compound 4 turned out to be a potent inhibitor of *A. niger* α -glucosidase (IC_{50} = 0.63 μ M), which is 10 times better than australine (1). Compound 4 also behaved as a moderate inhibitor of porcine kidney trehalase (IC_{50} = 31 μ M), while australine (1) is only a weak inhibitor of the enzyme (IC_{50} = 175 μ M). Difluorinated derivative 5 is not only a potent *A. niger* α -glucosidase inhibitor (IC_{50} = 1.1 μ M) but also a good *A. niger* amyloglucosidase inhibitor (IC_{50} = 5.9 μ M). In comparison, *ent*-4 and *ent*-5 were also completely ineffective toward all tested enzymes.

In summary, we have developed a simple and efficient method for the synthesis of australine (1) as well as its C-7-fluorinated and difluorinated derivatives starting from sugar-derived cyclic nitrones. Australine (1), 7-*epi*-australine (2), and their C-7-fluorinated derivatives 4 and 5 and their enantiomers have been synthesized efficiently by this method. Glycosidase inhibition studies of these compounds showed that C-7-fluorination and difluorination of australine considerably enhanced the potency of its inhibition against α -glucosidase. This enriches the few examples that demonstrate fluorination of a hydroxy group increasing rather than seriously decreasing inhibition. These observations are significant to the understanding of the SAR of australine as well as DMDP-related iminosugars and will be helpful for the design and synthesis of more potent α -glucosidase inhibitors based on this kind of compounds.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization data, ¹H and ¹³C NMR spectra for all new compounds, ¹⁹F NMR spectra and glycosidase inhibition data for australine derivatives. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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