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Synthesis and Glycosidase Inhibition of the Enantiomer of (−)-Steviamine, the First Example of a New Class of Indolizidine Alkaloid

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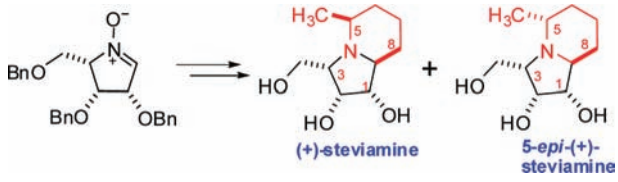
ABSTRACT



(+)-Steviamine, the enantiomer of the natural (−)-steviamine, and its corresponding C5 epimer have been synthesized from the D-ribose-derived cyclic nitrone. (−)-Steviamine was found to be the first naturally occurring iminosugar that causes any inhibition of α-galactosaminidases.

The absolute and relative stereochemistry of the five stereogenic centers in (−)-steviamine (**1**), recently isolated from *Stevia rebaudiana* (Asteraceae) leaves,[1] was established by X-ray crystallographic analysis of the hydrobromide salt.[2] This paper reports the synthesis of the enantiomer of (−)-steviamine (**1**), (+)-steviamine (**2**), and of the corresponding C5 epimer **3** and the glycosidase inhibition profile of **1**−**3** (Figure 1). (−)-Steviamine (**1**) can be viewed as the indolizidine analogue of the pyrrolizidine, hyacinthacine A_5 (**4**), isolated from *Scilla sibirica*;[3] many hyacinthacines have been isolated from a range of plants.[4] (−)-Steviamine (**1**) is

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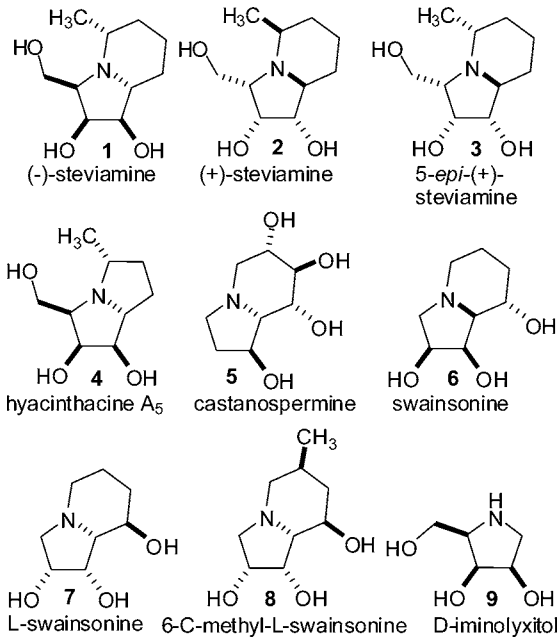


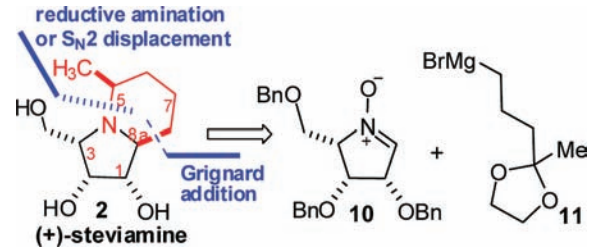
Figure 1. Iminosugars related to (−)-steviamine.

the first example of a new class of indolizidine alkaloid in which an alkyl group is attached to the piperidine ring. Polyhydroxylated indolizidines, such as castanospermine (**5**) from *Castanospermum australe*[5] (an inhibitor of α-glucosidases) and swainsonine (**6**) from *Swainsona canescens*[6] (an inhibitor of α-mannosidases), were among the first sugar mimics recognized. Simple derivatives of castanospermine are in development for the treatments of dengue virus[7] and of HCV infections;[8] swainsonine (**6**) has potential as a chemotherapeutic agent for the treatment of cancer.[9]

Nearly 200 iminosugars in which the ring oxygen of the furanose or pyranose has been replaced by nitrogen have been isolated from plants or bacteria.[10] Many of the enantiomers[11] of the naturally occurring alkaloids are themselves even more potent inhibitors of the same enzymes;[12] alternatively, they may be excellent inhibitors of different glycosidases.[13] For example, L-swainsonine (**7**) inhibits α-rhamnosidase[14] rather than α-mannosidases, and the *C*-methyl analogue **8** is an even more potent inhibitor of naringinase.[15] (−)-Steviamine and its enantiomer may also be viewed as bicyclic analogues of the iminolyxitols **9** and *ent*-**9**; **9** is a very potent, and *ent*-**9** a weak, competitive inhibitor of α-galactosidases.[16]

Retrosynthesis for **2** (Scheme 1) suggested that, starting from the D-ribose-derived cyclic nitrone **10**,[17] (+)-steviamine

Scheme 1. Retrosynthesis of (+)-Steviamine (**2**)



(**2**) could be synthesized efficiently through the diastereoselective addition of Grignard reagent **11**, followed by annulation, via either intramolecular reductive amination or S_N2 displacement.

According to the retrosynthetic analysis, we commenced the synthesis by making the key intermediate, ketone **13**,

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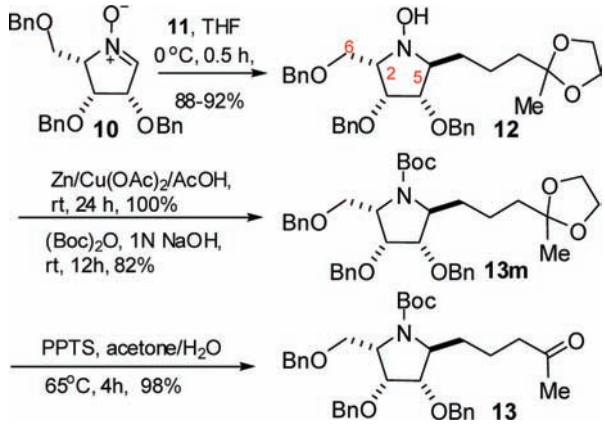
starting from the D-ribose-derived cyclic nitrone **10** (Scheme 2). Thus, Grignard addition of **11**[18] to the *all-cis* cyclic nitrone

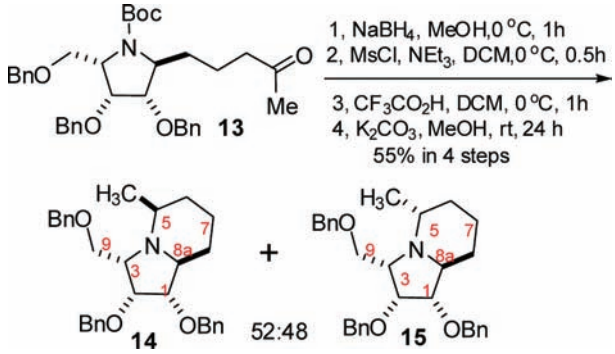
Scheme 2. Synthesis of Ketone **13**



10 at 0 °C furnished the hydroxylamine **12** in excellent yields (88−92%) with high diastereoselectivity (dr >95%). The *trans*-selectivity was determined by NOESY experiment which showed H5 and H6 correlation in **12**. The gratifyingly high *trans*-selectivity can be explained by a Felkin−Anh transition-state model[19] and is in accordance with previous reports.[20] Reduction of the resulting hydroxylamine **12** by Zn−Cu(OAc)$_2$−AcOH system gave the corresponding amine in quantitive yield, which was treated with $(Boc)_2O$ to form the *N*-Boc derivative **13m** in 82% yield. Compound **13m** was then converted to the key intermediate, *N*-Boc ketone **13**, by liberation of the carbonyl group under mild acidic condition.

With ketone **13** in hand, two parallel annulation approaches were under consideration for the construction of the second ring: (1) intramolecular nucleophilic displacement and (2) intramolecular reductive amination. The annulation by intramolecular nucleophilic displacement (Scheme 3) was first

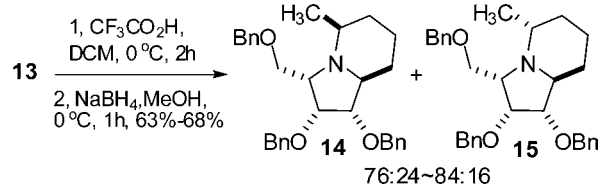
Scheme 3. Construction of (+)-Steviamine (**2**) via S_N2 Displacement



examined. Thus, reduction of *N*-Boc ketone **13** by $NaBH_4$ furnished a mixture of diastereomeric alcohols, and the resulting alcohols were transformed into their corresponding mesylates directly without further purification. Liberation of the amino group by CF_3CO_2H and subsequent treatment of the resulting amine with K_2CO_3 in methanol with catalytic water afforded the two epimeric indolizidines **14** and **15** in nearly 1:1 ratio and moderate yield.

In order to achieve better diastereoselectivity, annulation by intramolecular reductive amination was also examined (Scheme 4). Thus, reduction by $NaBH_4$ of the iminium intermediate after

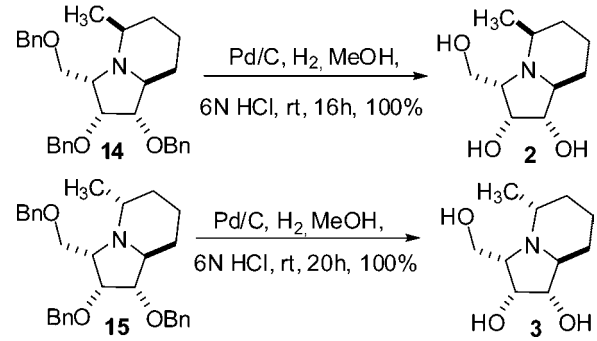
Scheme 4. Synthesis of (+)-Steviamine (**2**) via Reductive Amination



acidic deprotection of *N*-Boc ketone **13** generated a mixture of the two epimers **14** and **15** in good yields with relatively higher diastereoselectivity (**14**/**15** = 76:24−84:16) which favored amine **14**. The stereochemistry of the newly generated stereocenter was determined by the 600 MHz NOESY spectrum of **14** and **15** (**14**: H5 and H9, H5 and H8a; **15**: H3 and H5, H1 and H5; Supporting Information).

Finally, hydrogenolysis of indolizidine **14** and **15** (Scheme 5) gave (+)-steviamine (**2**) and 5-*epi*-(+)-steviamine (**3**) in

Scheme 5. Completion of (+)-Steviamine (**2**) and 5-*epi*-(+)-Steviamine (**3**)



quantitive yields, respectively. The ^1H and ^{13}C NMR spectra of compound **2** were identical (see the Supporting Informa-

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tion) to those reported for the natural (−)-steviamine (**1**), and the optical rotation of **2** [$[\alpha]^{20}_D = +34.0$ (*c* 1.0, MeOH)] was opposite to that of the natural (−)-steviamine [$[\alpha]^{20}_D = -22.0$ (*c* 1.0, MeOH)].

(−)-Steviamine (**1**), (+)-steviamine (**2**), and 5-*epi*-(+)-steviamine (**3**) were assayed as potential glycosidase inhibitors of a range of enzymes (see the Supporting Information). (−)-Steviamine showed weak inhibition of β-glucosidases ($IC_{50} = 454$ μM against β-glucosidases from almond; $IC_{50} = 739$ μM against β-glucosidases from *C. saccharolyticum*) but was a good inhibitor of β-galactosidase (rat intestinal lactase, $IC_{50} = 35$ μM). In spite of its structural similarity to swainsonine (**6**) and the D-iminolyxitol **9**, it showed no significant inhibition of either α-mannosidase or α-galactosidase, respectively. The enantiomer of (−)-steviamine (**2**) and its *C*-5 epimer **3** were weak inhibitors of α-rhamnosidase ($IC_{50} = 484$ and 342 μM, respectively), several orders of magnitude weaker than shown by L-swainsonine (**7**) and the *C*-methyl analogue **8**. The *N*-benzyl derivatives of both of the enantiomers of the iminolyxitol **9** were more potent inhibitors of α-rhamnosidase. However (−)-steviamine shows weak inhibition of an α-galactosaminidase (GalNAcase, $IC_{50} = 814$ μM); there has been no prior report of any natural product inhibiting any GalNAcase. GalNAcase inhibition may allow the design of chaperones for the treatment of Schindler−Kanzaki disease[21] and a strategy for the treatment of cancer by the protection of macrophage activating factor.[22] A synthetic iminosugar analogue of GalNAc has recently been reported as a potent inhibitor of GalNAcases.[23] The specific inhibition GalNAcases by a natural product, and particularly one that does not contain a NAc or any amide group, is remarkable.

In conclusion, the first synthesis of (+)-steviamine (**2**), the enantiomer of the novel natural indolizidine iminosugar (−)-steviamine (**1**), has been accomplished starting from the readily available D-ribose-derived cyclic nitrone **10**. (−)-Steviamine was found to be a weak inhibitor of an α-galactosaminidase (GalNAcase), which is a remarkable finding and might become a starting point for the design and synthesis of more potent inhibitors of α-galactosaminidase.

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Supporting Information Available: Experimental procedures, characterization data, and ^{1}H NMR and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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