

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

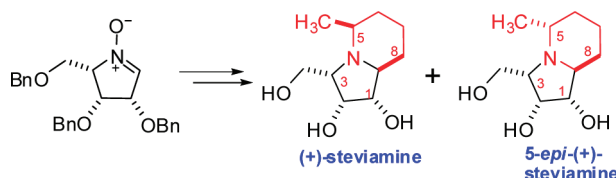
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Received April 2, 2010

ABSTRACT



(+)-Steviamine, the enantiomer of the natural (–)-steviamine, and its corresponding C5 epimer have been synthesized from the d-ribose-derived cyclic nitron. (–)-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 A₅ (**4**), isolated from *Scilla sibirica*;³ many hyacinthacines have been isolated from a range of plants.⁴ (–)-Steviamine (**1**) is

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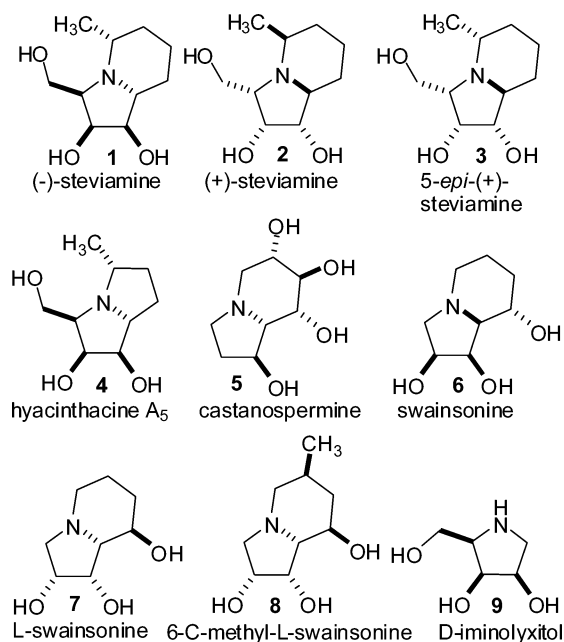


Figure 1. Iminosugars related to (–)-steviamine.

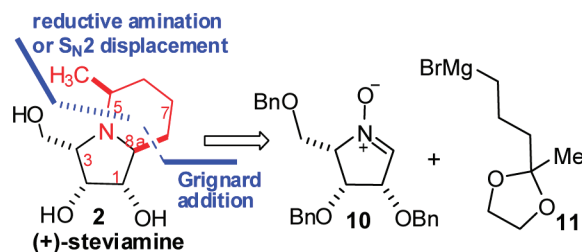
the first example of a new class of indolizidine alkaloid in which an alkyl group is attached to the piperidine ring. Polyhydroxylated indolizidines, such as castanospermine (**5**) from *Castanospermum australe*⁵ (an inhibitor of α -glucosidases) and swainsonine (**6**) from *Swainsona canescens*⁶ (an inhibitor of α -mannosidases), were among the first sugar mimics recognized. Simple derivatives of castanospermine are in development for the treatments of dengue virus⁷ 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Nearly 200 iminosugars in which the ring oxygen of the furanose or pyranose has been replaced by nitrogen have been isolated from plants or bacteria.¹⁰ Many of the enantiomers¹¹ of the naturally occurring alkaloids are themselves even more potent inhibitors of the same en-

zymes;¹² alternatively, they may be excellent inhibitors of different glycosidases.¹³ For example, L-swainsonine (**7**) inhibits α -rhamnosidase¹⁴ 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 nitronone **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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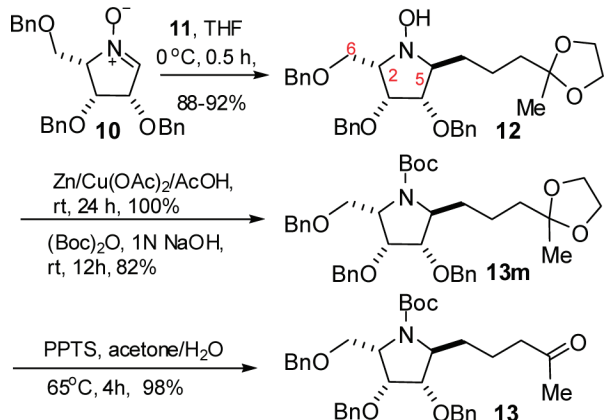
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starting from the D-ribose-derived cyclic nitron **10** (Scheme 2). Thus, Grignard addition of **11**¹⁸ to the *all-cis* cyclic nitron

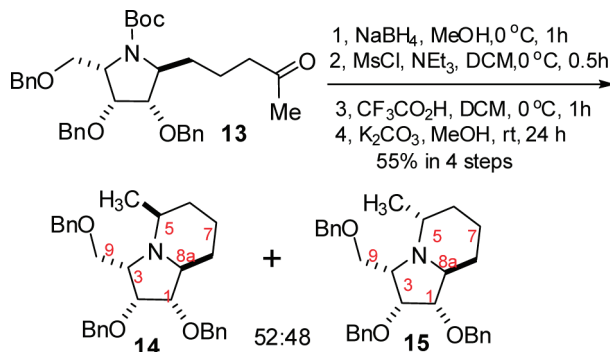
Scheme 2. Synthesis of Ketone **13**



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 model¹⁹ 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 quantitative 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

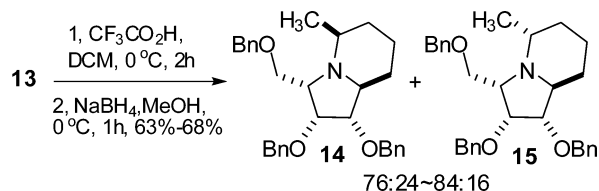


examined. Thus, reduction of *N*-Boc ketone **13** by NaBH₄ furnished a mixture of diastereomeric alcohols, and the

resulting alcohols were transformed into their corresponding mesylates directly without further purification. Liberation of the amino group by CF₃CO₂H and subsequent treatment of the resulting amine with K₂CO₃ 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 NaBH₄ of the iminium intermediate after

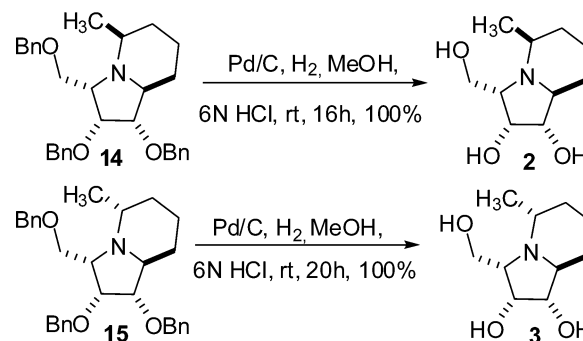
Scheme 4. Synthesis of (+)-Steviamine (**2**) via Reductive Amination



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**)



quantitative yields, respectively. The ¹H and ¹³C 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 ($IC_{50} = 454 \mu M$ against β -glucosidases from almond; $IC_{50} = 739 \mu M$ against β -glucosidases from *C. saccharolyticum*) but was a good inhibitor of β -galactosidase (rat intestinal lactase, $IC_{50} = 35 \mu M$). In spite of its structural similarity to swainsonine (**6**) and the D-iminolixitol **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 $342 \mu M$, 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 iminolixitol **9** were more potent inhibitors of α -rhamnosidase. However (–)-steviamine shows weak inhibition of an α -galactosaminidase (GalNAcase, $IC_{50} = 814 \mu M$); 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 disease²¹ 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 nitron **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.

Acknowledgment. This work is supported by Summit PLC (UK), The National Natural Science Foundation of China (No. 20672117), The National Basic Research Program of China (No. 2009CB526511), The Ministry of Science and Technology and the Ministry of Health of the P.R. China (No. 2009ZX09501-006), and The Chinese Academy of Sciences.

Supporting Information Available: Experimental procedures, characterization data, and ¹H NMR and ¹³C 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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