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

Xiang-Guo Hu,[†,‡] Barbara Bartholomew,[§] Robert J. Nash,[§] Francis X. Wilson,[⊥] George W. J. Fleet,[#] Shinpei Nakagawa,[||] Atsushi Kato,[||] Yue-Mei Jia,[†] Renate van Well,[⊥] and Chu-Yi Yu*[,†]

Beijing National Laboratory for Molecular Science (BNLMS), CAS Key Laboratory of Molecular Recognition and Function, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China, Graduate University of The Chinese Academy of Sciences, Beijing 100049, China, Phytoquest Limited, IBERS, Plas Gogerddan, Aberystwyth SY23 3EB, Ceredigion, Wales, U.K., Department of Hospital Pharmacy, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan, Summit PLC, 91, Milton Park, Abingdon, Oxon OX14 4RY, U.K., and Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford OX1 3TA, U.K.

yucy@iccas.ac.cn

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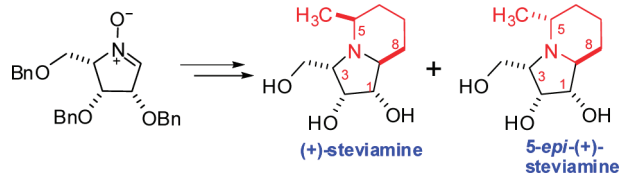
ABSTRACT



(+)-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

[†] Institute of Chemistry, Chinese Academy of Sciences.
[‡] Graduate University of The Chinese Academy of Sciences.
[§] Phytoquest Limited.
[⊥] Summit PLC.
[#] University of Oxford.
[||] University of Toyama.

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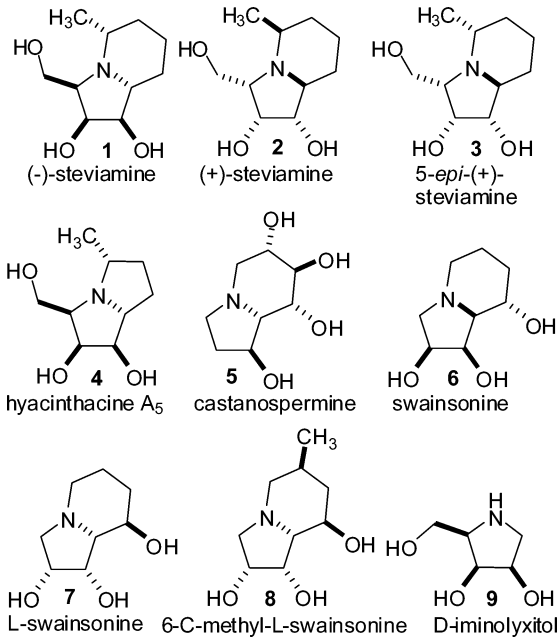


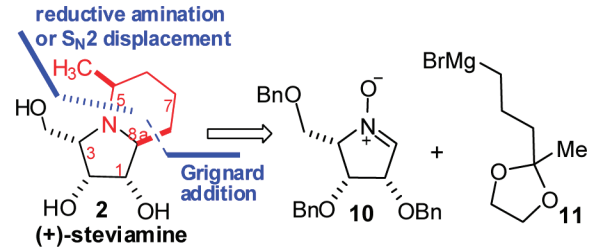
Figure 1. Iminosugars related to (−)-steviamine.

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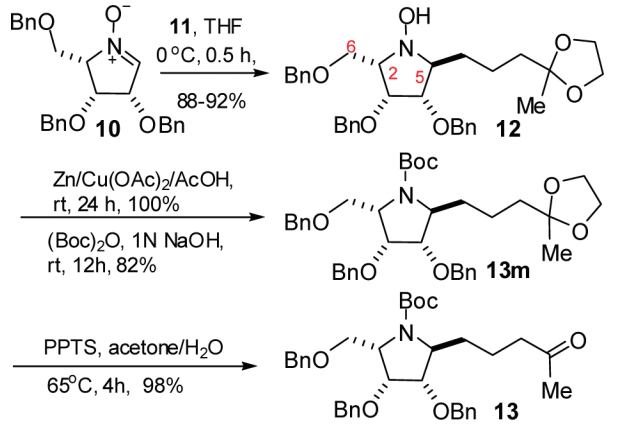
starting from the D-ribose-derived cyclic nitrone **10** (Scheme 2). Thus, Grignard addition of **11**[18] to the *all-cis* cyclic nitrone

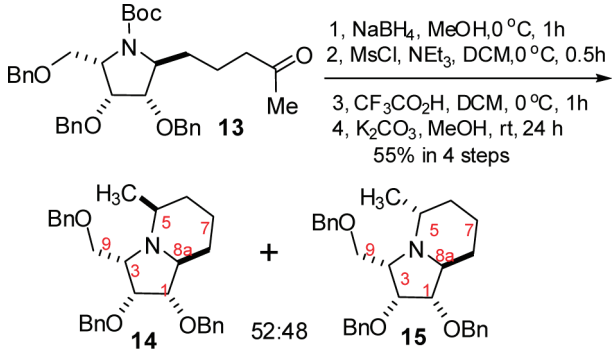
Scheme 2. Synthesis of Ketone **13**



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

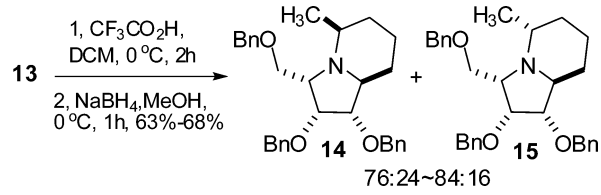
Scheme 3. Construction of (+)-Steviamine (**2**) via S_N2 Displacement



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

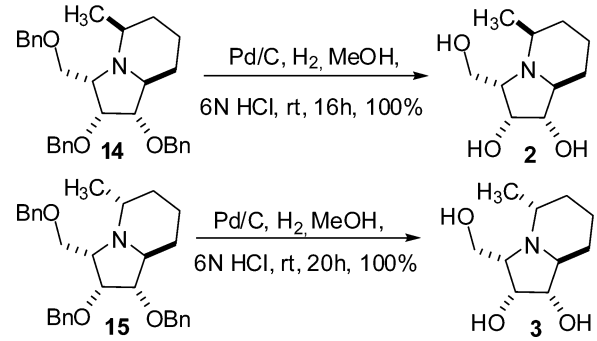
Scheme 4. Synthesis of (+)-Steviamine (**2**) via Reductive Amination



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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