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Total Synthesis and Structural Revision of the Alkaloid Incargranine B**†

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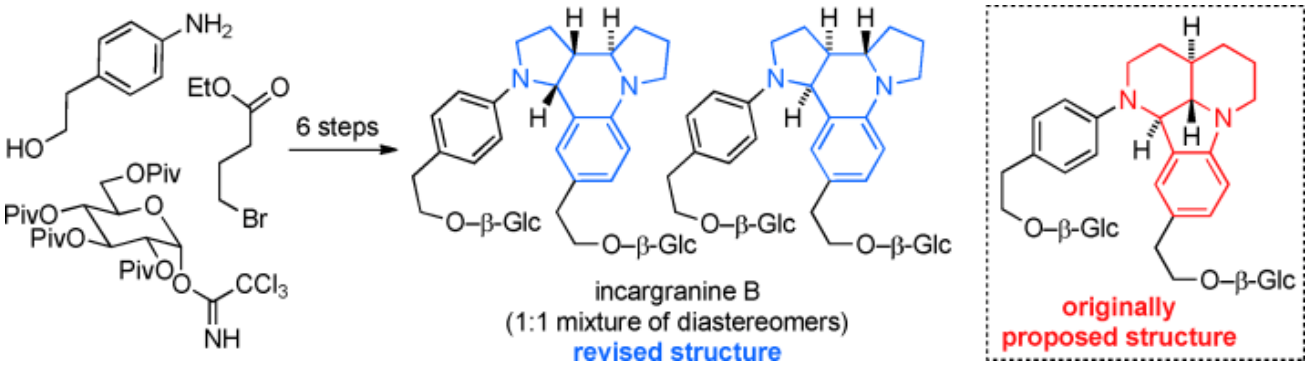
[†]Dedicated to Professor Sir Jack E. Baldwin in celebration of his 75th birthday.

Supporting information:
Supporting information for this article is available on the WWW under http://www.angewandte.org

Graphical abstract



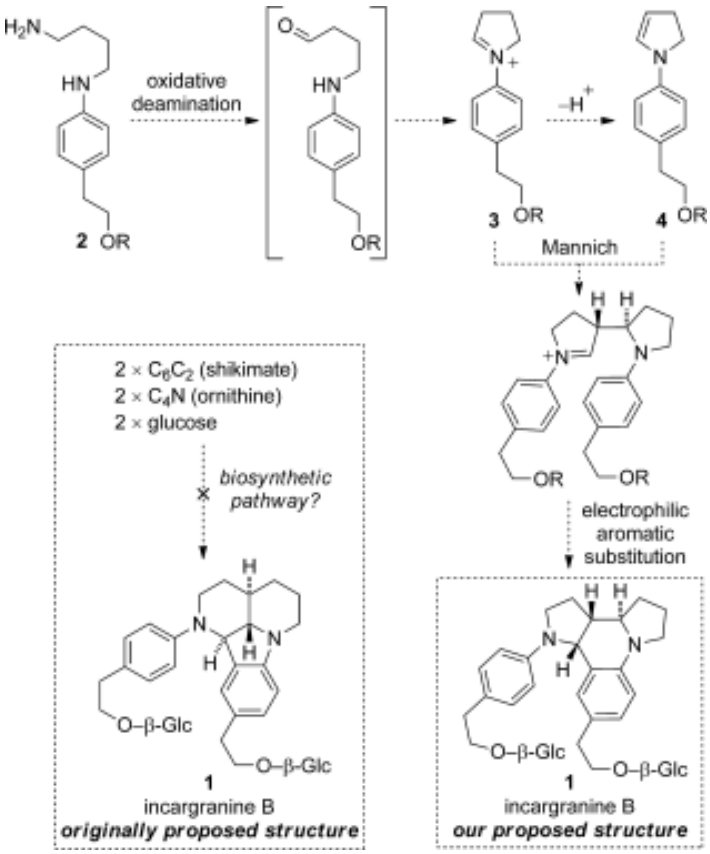
Keywords:
biomimetic synthesis; domino reactions; natural products; alkaloids; structural reassignment

Abstract

Consideration of the likely biosynthetic origins of incargranine B, which was originally assigned an unprecedented indolo[1.7]napthyridine structure, led us to hypothesize that a dipyrrolo-quinoline framework was a more biosynthetically feasible structure. We herein demonstrate that our biosynthetically inspired structural revision is correct through a short biomimetic synthesis of incargranine B.

Main text

Incargranine B (**1**) was isolated from *Incarvillea mairei* var. *grandiflora* in 2010 by Zhang and coworkers.[1] Analysis of the obtained spectroscopic data, particularly 1D and 2D NMR data, led Zhang and co-workers to propose an unprecedented indolo[1.7]napthyridine structure for incargranine B (**1**), as shown in Scheme 1. This proposed structure constituted the first, and still stands as the only, reported alkaloid to contain this framework.



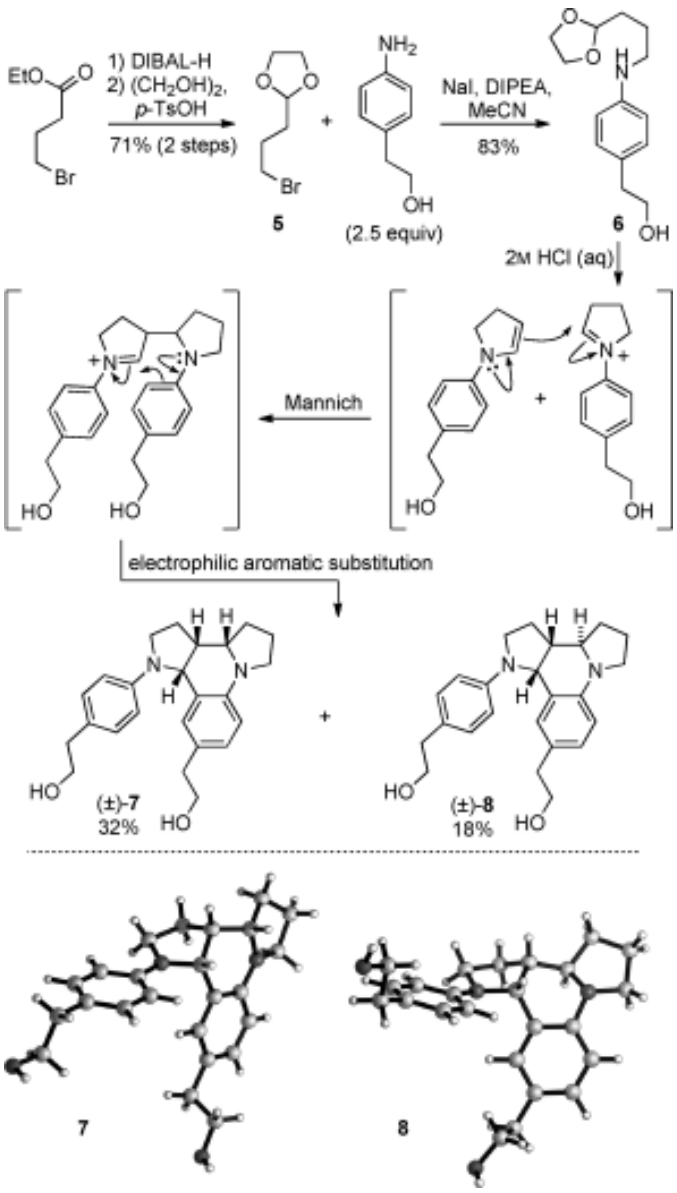
Scheme 1. Our proposed biogenesis and structural revision for incargranine B (**1**).[3]

Consideration of the biosynthetic origins of incargranine B (**1**) led us to hypothesize that the proposed structure was incorrect. Incargranine B (**1**) formulates as a dimer that contains two glucose molecules, two phenylethanoid structures (**C_6C_2**) and two ornithine-derived units (**C_4N**). Our efforts to discern a likely biosynthetic mechanism for a dimerization process were met with great difficulty.[2] The proposed indolo[1.7]napthyridine core would necessitate the cleavage and formation of an unusually high number of bonds within any conventional biosynthesis. Although this insight does not constitute evidence against the proposed structure for incargranine B (**1**), it did lead to the development of the biosynthetic speculation outlined in Scheme 1.

We considered that a phenylethanoid diamine **2** could be a biosynthetic precursor towards incargranine B (**1**) (Scheme 1).[3] Thus, oxidative deamination of compound **2** and subsequent intramolecular condensation would result in iminium ion **3**, which upon deprotonation would afford enamine **4**. Iminium ion **3** and enamine **4** could then combine through a domino Mannich/electrophilic aromatic substitution sequence, to afford a dipyrrolo-quinoline structure. This structure appeared to fit very well with all the spectroscopic data reported by Zhang for incargranine B (**1**).[1] Therefore, we decided to embark upon a biomimetic synthesis of incargranine B (**1**) to investigate the feasibility of our biosynthetic proposal and structural revision.

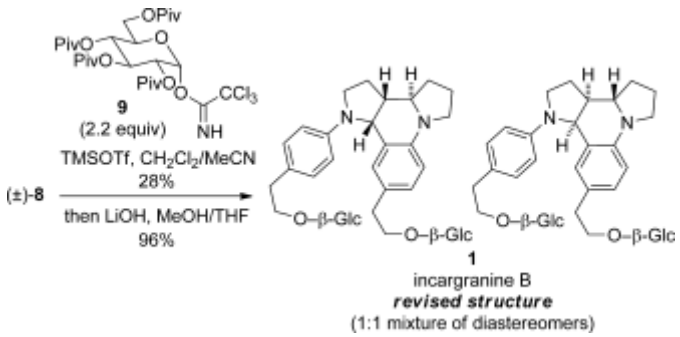
The synthesis began with DIBAL-H reduction of commercially available ethyl 4-bromobutanoate (Scheme 2). The resulting aldehyde was then protected as a cyclic acetal to afford alkyl bromide **5**, isolated in 71% yield over the two steps.[4] Alkyl bromide **5** was then used in a substitution reaction with commercially available 4-aminophenethyl alcohol, using Hünig's base and NaI. It was found that simply using an excess of 4-aminophenethyl alcohol was sufficient to avoid di-alkylation, with mono-alkylated aniline **6** isolated in 83% yield. Aniline **6** was then treated with aqueous hydrochloric acid to deprotect the aldehyde and trigger the proposed biomimetic domino Mannich/electrophilic aromatic substitution sequence. This dimerization process, which forms two new C–C bonds, two new C–N bonds and three new rings in a single operation,[5] afforded two diastereomers **7** and **8** in a combined 50% yield.[6] The relative stereochemistry of each diastereomer was established through single crystal X-ray analyses (Scheme 2).[7]

It should be noted that dimeric structures **7** and **8** were found to be unstable, particularly in solution when exposed to air. This instability was ascribed to autoxidation at the benzylic methine sites. Therefore, handling of these compounds, and derivatives thereof, in air was minimized and BHT (2,6-di-*tert*-butyl-4-methylphenol) was added to stored samples.



Scheme 2. Synthesis of incargranine B aglycone **8**.[7]

The ^{1}H NMR spectrum of diastereomer **8** was strikingly similar to that reported for incargranine B (**1**), thus indicating that dimer **8** was indeed the agylcone of incargranine B (**1**). To definitively prove this, we needed to synthesize an analytical sample of the diglucoside of dimer **8**. A screen of glucosyl donors and activators was undertaken to identify glucosidation conditions with high β-selectivity, in order to avoid the production of unwated diastereomers. It was found that pivaloyl-protected trichloroacetimidate **9** was an effective and highly β-selective glucosyl donor.[8] Dimer **8** was, therefore, treated with glucosyl donor **9**, using TMSOTf as the activator to afford the two expected protected diglucoside diastereomers in 28% yield (Scheme 3).[9] The pivaloyl groups were then removed in excellent yield using LiOH in MeOH/THF.



Scheme 3. Double glucosidation of aglycone **8**.

Professor Zhang very kindly provided pdf files of the processed 1D and 2D NMR spectra for natural incargranine B (**1**). Unfortunately, Professor Zhang was unable to provide us with either the original fid files or an authentic sample of the natural product. Nevertheless, spectroscopic data obtained on our synthetic 1:1 diastereomeric mixture are a close match with the available data from the natural product.[10] Furthermore, the optical rotation for our synthetic diastereomeric mixture, $[\alpha]_D^{20}$ −16.7 (*c* 0.275, MeOH), is in good agreement with that reported for natural incargranine B (**1**), $[\alpha]_D^{20}$ −12 (*c* 0.275, MeOH).[1] We therefore tentatively conclude that the natural product also exists as a diastereomeric mixture, although conclusive proof will require closer scrutiny of a sample isolated from the natural source.[11] It is interesting to note that a biosynthetically related diglucosidic alkaloid, millingtonine,[3b] also exists as a mixture of diastereomers. The production of these *pseudo*-enantiomeric diastereomers in nature is presumably due to either a late-stage biosynthetic glucosidation of racemic (or scalemic) aglycones, or a lack of stereochemical influence exerted by the sugars in non-enzyme mediated biosynthetic reactions.

In summary, consideration of the biosynthetic origins of incargranine B (**1**) led us to suspect that the originally assigned indolo[1.7]napthyridine structure was incorrect. We proposed that a dipyrrolo-quinoline framework was a more biosynthetically feasible structure and have now demonstrated, through total synthesis, that this proposed structural revision was correct.[12] We submit that our rapid synthesis of incargranine B (**1**), which requires a longest linear sequence of just six steps, provides very strong evidence that a similar dimerization process may be occurring in nature. This work not only demonstrates the great utility of biosynthetic considerations in devising synthetic strategies but also in aiding the determination/reassignment of natural product structures. Work is ongoing in our laboratories to extend this biomimetic strategy to other related alkaloids.[3]

Notes and references

[1] Y.-H. Shen, Y.-Q. Su, J.-M. Tian, S. Lin, H.-L. Li, J. Tang, W.-D. Zhang, *Helv. Chim. Acta* **2010**, *93*, 2393–2396.

[2] For our previous biomimetic studies on dimeric natural products, see: a) P. D. Brown, A. C. Willis, M. S. Sherburn, A. L. Lawrence, *Org. Lett.* **2012**, *14*, 4537–4539; b) S. L. Drew, A. L. Lawrence, M. S. Sherburn, *Angew. Chem.* **2013**, *125*, 4315–4318; *Angew. Chem. Int. Ed.* **2013**, *52*, 4221–4224.

[3] The biogenesis of incargranine A and millingtonine can also be traced back to diamine **2**; see the Supporting Information for details. For the isolation of incargranine A, see: a) Y.-Q. Su, Y.-H. Shen, S. Lin, J. Tang, J.-M. Tian, X.-H. Liu, W.-D. Zhang, *Helv. Chim. Acta* **2009**, *92*, 165–170. For the isolation of millingtonine, see: b) T. Hase, K. Ohtani, R. Kasai, K. Yamasaki, C. Picheansoonthon, *Phytochemistry* **1996**, *41*, 317–321.

[4] R. W. Bates, S. Sridhar, *Synlett* **2009**, 1979–1981.

[5] For a review of multi-bond forming processes, see: N. J. Green, M. S. Sherburn, *Aust. J. Chem.* **2013**, *66*, 267–283.

[6] There exists the possibility that this dimerization proceeds via a concerted cycloaddition mechanism, with dimers **7** and **8** the result of *endo* and *exo*-processes respectively.

[7] CCDC 958613 (**7**) and CCDC 958614 (**8**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[8] Glucosyl donor **9** was synthesized in three steps according to the procedure of Schmidt and coworkers: P. Zimmermann, R. Sommer, T. Bär, R. R. Schmidt, *J. Carbohydr. Chem.* **1988**, *7*, 435–452.

[9] [9] Analytical chiral HPLC of the deprotected diglucosides showed a 1:1 ratio of diastereomers, see the Supporting Information for details.

[10] See the Supporting Information for a detailed analysis of the NMR data and a direct comparison of the ^{1}H and ^{13}C NMR spectra of our synthetic material with that of natural incargranine B.

[11] At high field strength the presence of two diastereomers in our synthetic sample is evidenced by the splitting of peaks in the ^{13}C NMR spectra. Unfortunately, the magnitude of this splitting is very small and is not visible on the scale of the processed NMR spectra for natural incargranine B from Professor Zhang.

[12] For an excellent review of the role of chemical synthesis in modern structure elucidation, see: K. C. Nicolaou, S. A. Snyder, *Angew. Chem.* **2005**, *117*, 1036–1069; *Angew. Chem. Int. Ed.* **2005**, *44*, 1012–1044.