Total Synthesis

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

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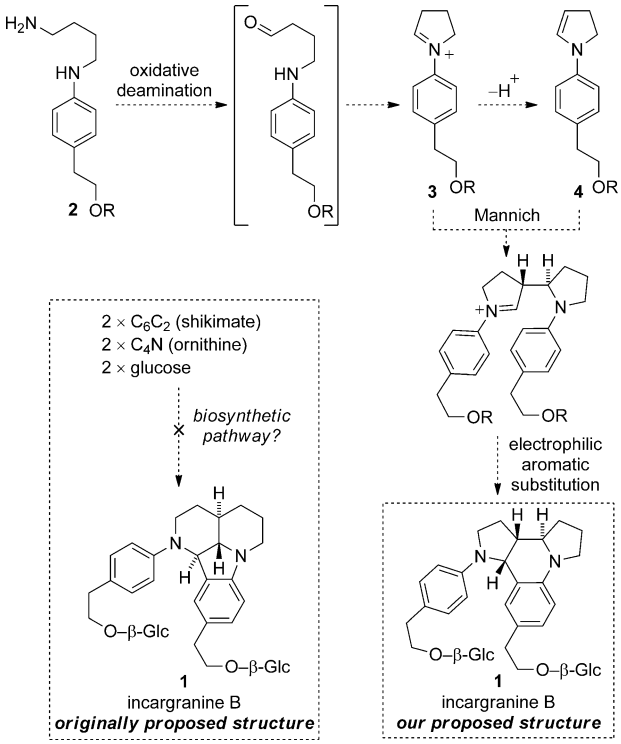
Dedicated to Professor Sir Jack E. Baldwin on the occasion of his 75th birthday

Incargranine B (**1**) was isolated from *Incarvillea mairei* var. *grandiflora* in 2010 by Zhang and co-workers.[1] Analysis of the obtained spectroscopic data, particularly 1D and 2D NMR spectroscopic data, led Zhang and co-workers to propose an unprecedented indolo[1.7]naphthyridine 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.

Consideration of the biosynthetic origins of incargranine B (**1**) led us to hypothesize that the proposed structure was incorrect. Incargranine B (**1**) has the chemical formula of 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 met with great difficulty.[2] The proposed indolo[1.7]naphthyridine 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 to 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 dipyrroloquinoline structure. This structure appeared to fit very well with all the spectroscopic data reported by Zhang and co-workers for incargranine B (**1**).[1] Therefore, we



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

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 the reduction of commercially available ethyl 4-bromobutanoate with DIBAL-H (Scheme 2). The resulting aldehyde was then protected as a cyclic acetal to afford alkyl bromide **5**, which was isolated in 71% yield over the two steps.[4] Alkyl bromide **5** was used in a substitution reaction with commercially available 4-aminophenethyl alcohol in the presence of Hünig's base and NaI. We found that it was sufficient to simply use an excess of 4-aminophenethyl alcohol to avoid dialkylation; thus, monoalkylated aniline **6** was 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 50% combined yield.[6]

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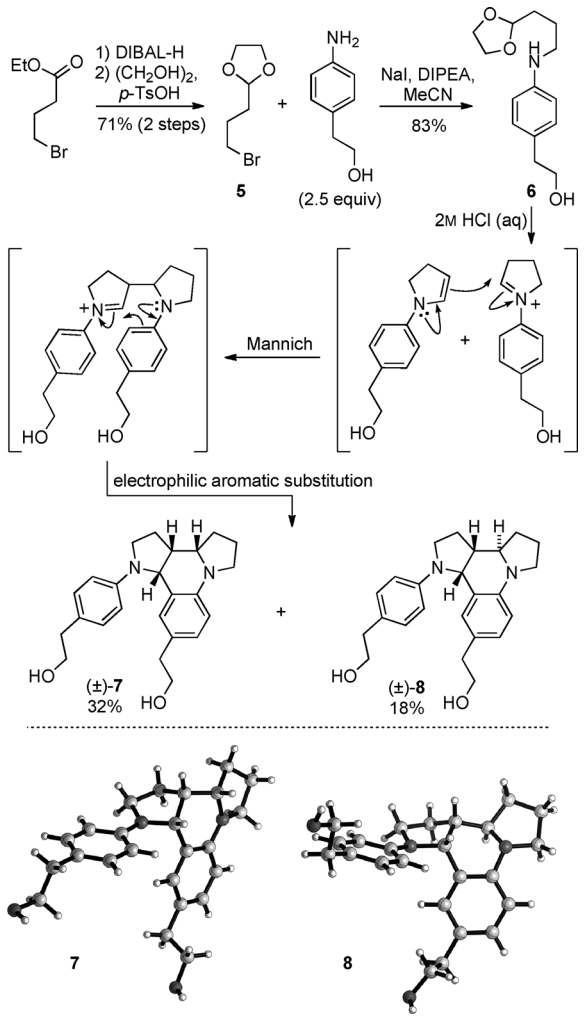
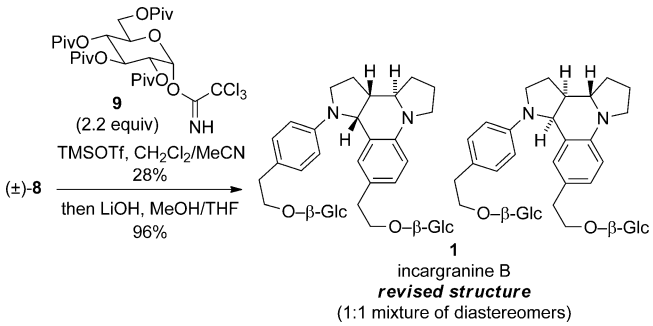


Scheme 2. Synthesis of the incargranine B aglycone **8** and X-ray crystal structures of **7** and **8**.[7] DIBAL-H = diisobutylaluminum hydride, DIPEA = diisopropylethylamine.

The relative configuration of each diastereomer was established through single-crystal X-ray structure analysis (Scheme 2).[7]

The 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 2,6-di-*tert*-butyl-4-methylphenol (BHT) was added to stored samples.

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**). For definitive proof, we needed to synthesize an analytical sample of the diglucoside of dimer **8**. We screened a range of glucosyl donors and activators to identify glucosidation conditions with high β selectivity to avoid the production of unwanted diastereomers. It was found that the pivaloyl-protected trichloroacetimidate **9** was an effective and highly β-selective glucosyl donor.[8] The treatment of dimer **8** with glucosyl donor **9** in the presence of TMSOTf as the activator afforded the two expected protected diglucoside diastereomers in 28% yield (Scheme 3).[9] The pivaloyl groups were then removed in excellent yield with LiOH in MeOH/THF.



Scheme 3. Double glucosidation of aglycone **8**. TMSOTf = trimethylsilyl trifluoromethanesulfonate, Piv = pivaloyl.

Professor Zhang very kindly provided pdf files of the processed 1D and 2D NMR spectra for natural incargranine B (**1**). Unfortunately, he was unable to provide us with either the original fid files or an authentic sample of the natural product. Nevertheless, the spectroscopic data obtained for our synthetic 1:1 diastereomeric mixture were a close match with the available data for the natural product.[10] Furthermore, the optical rotation of 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 pseudoenantiomeric 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]naphthyridine structure was incorrect. We proposed that a dipyrroloquinoline 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 study demonstrates the great utility of biosynthetic considerations not only during the design of synthetic strategies but also in aiding the determination/reassignment of natural product structures. Research is ongoing in our laboratories to extend this biomimetic strategy to related alkaloids.[3]

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[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 through a concerted cycloaddition mechanism, with dimers **7** and **8** the result of *endo* and *exo* processes, respectively.

[7] CCDC 958613 (**7**) and 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] The glucosyl donor **9** was synthesized in three steps according to a reported procedure: P. Zimmermann, R. Sommer, T. Bär, R. R. Schmidt, *J. Carbohydr. Chem.* **1988**, *7*, 435 – 452.

[9] Analytical HPLC of the deprotected diglucosides on a chiral stationary phase 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 spectroscopic data and a direct comparison of the ^{1}H and ^{13}C NMR spectra of our synthetic material with those of natural incargranine B.

[11] At high field strength, the presence of two diastereomers in our synthetic sample was 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.

