



Total synthesis of incargranine A†

Cite this: *Org. Biomol. Chem.*, 2019, **17**, 1698

Patrick D. Brown and Andrew L. Lawrence *

Received 22nd March 2018,

Accepted 4th April 2018

DOI: 10.1039/c8ob00702k

rsc.li/obc

Synthetic studies into the origins of the alkaloid incargranine A have resulted in the development of a four-step (longest linear sequence) total synthesis. This synthesis has been scaled-up to provide gram-scale quantities of material, which would alternatively require extraction of several metric-tons of dried-whole Chinese Trumpet-Creeper plants (*Incarvillea mairei* var. *grandiflora*).

In 2009 Zhang and co-workers isolated the alkaloid incargranine A (**1**) from *Incarvillea mairei* var. *grandiflora*, a Bignonia plant more commonly known as the Chinese Trumpet-Creeper plant (Scheme 1).¹ Incargranine A (**1**) has not yet succumbed to total synthesis and represents a particularly scarce natural product, constituting just 0.0000002% by weight of the dried whole plant. Therefore, a practical – *i.e.*, efficient and scalable – chemical synthesis of incargranine A (**1**) might advance a

better understanding of its biological function. The novel framework of incargranine A (**1**) contains a synthetically daunting bridged-cyclohexane ring, in which all six-carbon atoms are stereogenic. Nevertheless, we were hopeful that if we could gain insight into how nature synthesizes this alkaloid a step-economical biomimetic strategy could be developed.

Our biosynthetic analysis, shown in Scheme 1, reveals incargranine A (**1**) is likely constructed from two shikimate-derived C₆C₂ units linked together by an ornithine-derived C₄N unit. Our previous biomimetic studies on related phenylethanoid alkaloids provide important clues as to the potential origins of incargranine A (**1**).² We recently proposed that a network of pathways, all originating from a simple biosynthetic precursor, diamine **2**, could account for the formation of several structurally distinct phenylethanoid natural products (Scheme 2).^{2d} In our proposal, diamine **2** can participate in a pair of divergent oxidative pathways (Scheme 2; pathways 1 and 2). As shown in Scheme 2, pathway 1 terminates in the formation of incarviditone (**3**)³ and incarvilleatone (**4**),⁴ via the intermediacy of cornoside (**5**)⁵ and rengyolone (**6**),⁶ whereas pathway 2 results in the production of incargranine B (**7**).^{2a-c,7} It was proposed that these two divergent pathways could re-converge to give millingtonine (**8**),⁸ via a crossed-dimerization of cornoside **5**, from pathway 1, and a PLP (pyridoxal phosphate) derived enamine **9**, from pathway 2 (Scheme 2; pathway 3).^{2d} The chemical feasibility of this re-convergent pathway was demonstrated in our seven-step biomimetic total synthesis of millingtonine (**8**).^{2d} Herein, we propose that an additional re-convergent pathway could give rise to incargranine A (**1**) (Scheme 2; pathway 4). Thus, a Michael reaction between PLP-enamine **9** and rengyolone (**6**) would give an intermediate imine **11**, which would ring-close through a condensation/Mannich reaction sequence to give incargranine A (**1**).⁹ To investigate the feasibility of this second re-convergent pathway, and in the hope of establishing a practical solution to the supply problem associated with incargranine A (**1**),¹ we decided to pursue the development of a biomimetic synthetic strategy.



Scheme 1 Structure and biosynthetic analysis of incargranine A.

EaStCHEM School of Chemistry, University of Edinburgh, Joseph Black Building,

David Brewster Road, Edinburgh, EH9 3FJ, UK. E-mail: a.lawrence@ed.ac.uk

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8ob00702k





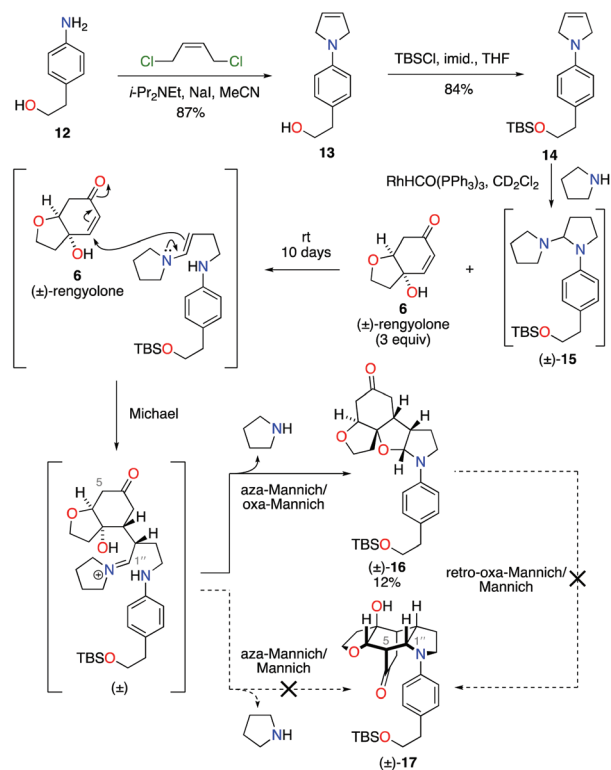
Scheme 2 Proposed network of biosynthetic pathways towards a family of plant-derived phenylethanoid natural products, including incargranine A.

Condensation of 4-aminophenethyl alcohol **12** with (*Z*)-1,4-dichlorobut-2-ene gave *N*-aryl-2,5-dihydropyrrole **13** in 87% yield (Scheme 3).¹⁰ The primary alcohol functional group was then protected under standard conditions as a *tert*-butyldimethylsilyl ether, to give alkene **14** in 84% yield. Exposure of alkene **14** to our previously developed RhHCO(PPh₃)₃ and pyrrolidine reaction conditions gave the expected amination intermediate **15**.^{2d,11} Due to the instability of amination **15**, and in the interests of practicality and efficiency, rengyolone (**6**), which can be readily prepared from tyrosol in 3 steps,^{2a} was added directly to this crude reaction mixture. Monitoring the reaction by ¹H NMR spectroscopy revealed it took 10 days at ambient temperature for amination **15** to be consumed. Purification of the resulting crude reaction mixture by column chromatography resulted in a 12% isolated yield of an unwanted crossed-dimer **16**, with no detectable formation of the desired product **17**. Hemi-aminal **16** is presumably formed *via* a domino Michael/aza-Mannich/oxa-Mannich reaction sequence. In contrast, a final Mannich reaction between C5 and C1' would be required for formation of the incargranine A framework **17** (Scheme 3). Although this result demonstrates the viability of a crossed-dimerization between amination **15** and rengyolone (**6**), several issues presented them-

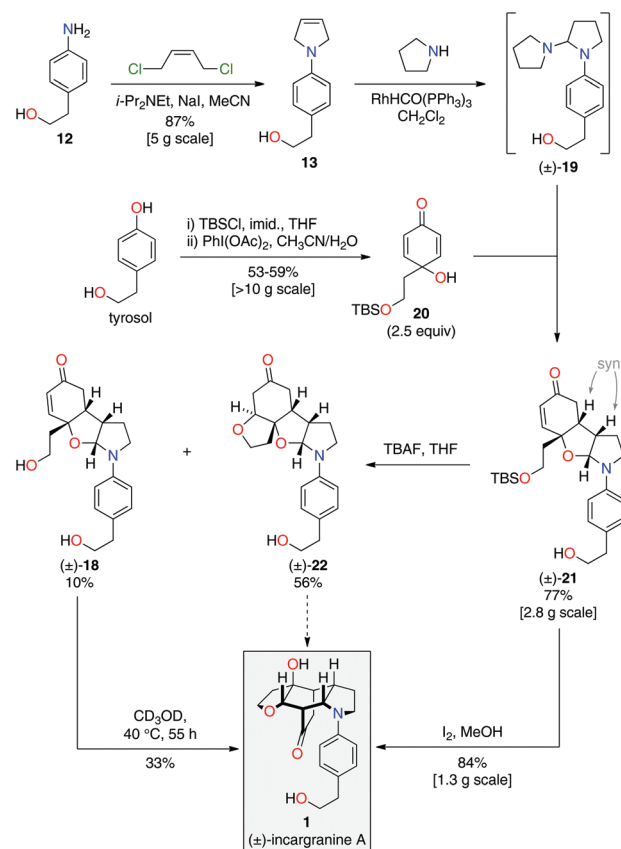
selves with respect to using this strategy to access incargranine A (**1**). Firstly, rengyolone (**6**) proved to be relatively unreactive in the crossed-dimerization, taking over a week to give full consumption of starting material **15**, while comparable reactions with *para*-quinols were generally complete in 24 h.^{2d} Furthermore, the low yield of crossed-dimer **16**, even after these prolonged reaction times, was not a promising start to the development of an efficient synthesis. Finally, and most importantly, our attempts to rearrange hemi-aminal **16** to give the incargranine A framework **17**, *via* a retro-oxa-Mannich/Mannich reaction sequence, were unsuccessful.¹² This prompted us to reconsider our biosynthetic proposal and synthetic strategy.

Upon further evaluation of the incargranine A (**1**) framework it became apparent that it might instead be derived from the *syn*-diastereomer of millingtonine, dia-millingtonine (**10**), which we had previously identified as a potential natural product and direct biosynthetic precursor to millingtonine (**8**) (Scheme 2; pathway 3).^{2d} Specifically, the putative aglycone of dia-millingtonine, diol **18**, could undergo a domino retro-oxa-Mannich/oxa-Michael/Mannich reaction sequence to give incargranine A (**1**) (Scheme 4).¹³ If this pathway could be shown to be chemically feasible it would lend further support

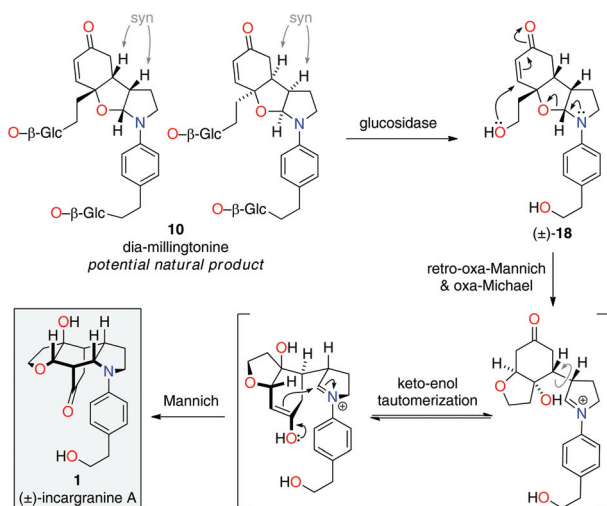




Scheme 3 Failed approach to synthesize incargranine A.



Scheme 5 Total synthesis of incargranine A.



Scheme 4 Revised biosynthetic hypothesis for incargranine A.

to our proposal that dia-millingtonine (**1**) represents an as-yet-undiscovered natural product.^{2d}

During the development of this new strategy, it was discovered that protection of the primary alcohol in *N*-aryl-2,5-dihydropyrrole **13** was not necessary for the subsequent alkene-isomerization/hydroamination reaction. Thus, exposure of free alcohol **13** to RhHCO(PPh₃)₃ and pyrrolidine gave the expected amination intermediate **19** (Scheme 5).^{2d,11} TBS-protected *para*-

quinol **20**, which was prepared in 2 steps from tyrosol,^{2a} was then added directly to this crude reaction mixture resulting in a kinetically-controlled crossed-dimerization to give *syn*-dimer **21** in 77% yield.^{2d}

Attention could now turn to the de-protection of crossed-dimer **21**, a synthetic equivalent of dia-millingtonine (**10**), and its subsequent conversion to incargranine A (**1**). Cleavage of the *tert*-butyldimethylsilyl ether using standard TBAF (tetra-*n*-butylammonium fluoride) conditions gave the expected diol-aglycone **18** in just 10% yield, alongside a cyclized-aglycone **22** in 56% yield (Scheme 5). Remarkably, it was observed that diol-aglycone **18** spontaneously rearranges to give (±)-incargranine A (**1**) when dissolved in methanol at ambient temperature, albeit very slowly. Ultimately, a 33% isolated yield of (±)-incargranine A (**1**) was achieved when a CD₃OD solution of diol-aglycone **18** was warmed to 40 °C for 2 days. The chemical feasibility of our proposed biosynthetic pathway between dia-millingtonine (**10**) and incargranine A (**1**) had thus been established. All efforts, however, to rearrange the cyclized-aglycone **22** to give incargranine A (**1**) were unsuccessful, akin to our failure to rearrange hemi-aminal **16** (Scheme 3).¹²

The low yields and lack of selectivity achieved in the final de-protection and rearrangement steps rendered this synthesis unsuitable for scale-up. Alternative deprotection conditions were therefore screened in the hope of favoring production of



diol **18**, whilst avoiding formation of the seemingly intractable ring-closed aglycone **22**. Vaino and Szarek have reported iodine in methanol as mild reaction conditions for the cleavage of *tert*-butyldimethylsilyl ethers.¹⁴ Unexpectedly, however, exposure of *syn*-dimer **21** to iodine in methanol did not result in the formation of diol **18**, nor ring-closed aglycone **22**, but instead gave (\pm)-incargranine A (**1**) directly. Thus, in a single step, 2 new bonds, 2 new rings and 3 new stereogenic centres are formed in an impressive 84% yield. This synthetic sequence was readily scaled-up to provide gram-scale quantities of (\pm)-incargranine A (**1**), which compares very favorably to the effort required to obtain this material from the natural source; over four metric-tons of dried *Incarvillea mairei* var. *grandiflora* would need to be extracted to isolate one gram of natural incargranine A (**1**).¹

Zhang and co-workers reported an optical rotation for natural incargranine A (**1**), $[\alpha]_D^{22} = +2$ ($c = 0.175$, CHCl_3).¹ However, given our biosynthetic speculation and the small magnitude of the reported optical rotation value, we consider it likely that natural incargranine A (**1**) exists as a racemic mixture. Unfortunately, no authentic sample was available to validate this hypothesis.¹⁵ In all other respects, however, the spectroscopic data for our synthetic material matched that reported for natural incargranine A (**1**).^{1,15} We propose that this successful synthesis provides new evidence in support of the proposal that dia-millingtonine (**10**) is a natural product.^{2d,16} In fact, it is possible that incargranine A (**1**) is only produced from dia-millingtonine (**10**) during the extraction and isolation process. This would not necessarily mean that incargranine A (**1**) is an unimportant artifact of human intervention.¹⁷ It is known, for example, that plants can use glycosidic-metabolites as chemical defense systems, wherein damage to the plant brings glycosidase enzymes into contact with the glycosides to release the active aglycones.¹⁸

Conclusions

In just three-linear steps from 4-aminophenethyl alcohol **12** we have selectively formed 2 new C–N bonds, 2 new C–C bonds, 2 new rings, and 6 new contiguous stereogenic centres, in 56% overall yield.¹⁹ Key to the development of this efficient synthetic strategy has been the probing and refinement of a biosynthetic proposal using chemical synthesis. Ultimately, this has led to new evidence in support of the notion that dia-millingtonine (**10**) is an as-yet-undiscovered natural product.¹⁶ Practical quantities of these metabolites are now available for interested parties to study their biological function.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank Prof. Wei-Dong Zhang (School of Pharmacy, Second Military Medical University, Shanghai) for kindly providing copies of the processed NMR spectra for natural incargranine A. The Royal Society is thanked for the award of a Research Grant. P. D. B. thanks the University of Edinburgh for the provision of a studentship.

Notes and references

- 1 Y.-Q. Su, Y.-H. Shen, S. Lin, J. Tang, J.-M. Tian, X.-H. Liu and W.-D. Zhang, *Helv. Chim. Acta*, 2009, **92**, 165–170.
- 2 For previous biomimetic studies on related dimeric natural products, see: (a) P. D. Brown, A. C. Willis, M. S. Sherburn and A. L. Lawrence, *Org. Lett.*, 2012, **14**, 4537–4539; (b) K. Zhao, G.-J. Cheng, H. Yang, H. Shang, X. Zhang, Y.-D. Wu and Y. Tang, *Org. Lett.*, 2012, **14**, 4878–4881; (c) P. D. Brown, A. C. Willis, M. S. Sherburn and A. L. Lawrence, *Angew. Chem.*, 2013, **125**, 13515–13517, (*Angew. Chem. Int. Ed.*, 2013, **52**, 13273–13275); (d) P. D. Brown and A. L. Lawrence, *Angew. Chem.*, 2016, **128**, 8561–8565, (*Angew. Chem. Int. Ed.*, 2016, **55**, 8421–8425).
- 3 Y. Q. Chen, Y. H. Shen, Y. Q. Su, L. Y. Kong and W. D. Zhang, *Chem. Biodiversity*, 2009, **6**, 779–783.
- 4 Y. P. Gao, Y. H. Shen, S. D. Zhang, J. M. Tian, H. W. Zeng, J. Ye, H. L. Li, L. Shan and W. D. Zhang, *Org. Lett.*, 2012, **14**, 1954–1957.
- 5 J. S. Rosendal, A. Kjaer and N. B. Juhl, *Acta Chem. Scand.*, 1973, **27**, 367–369.
- 6 (a) K. Endo and H. Hikino, *Can. J. Chem.*, 1984, **62**, 2011–2014; (b) I. Messana, M. Sperandei, G. Multari, C. Galeffi and G. B. Marini Bettolo, *Phytochemistry*, 1984, **23**, 2617–2619; (c) J. Tian, Q. S. Zhao, H. J. Zhang, Z. W. Lin and H. D. Sun, *J. Nat. Prod.*, 1997, **60**, 766–769.
- 7 Y.-H. Shen, Y.-Q. Su, J.-M. Tian, S. Lin, H.-L. Li, J. Tang and W. D. Zhang, *Helv. Chim. Acta*, 2010, **93**, 2393–2396.
- 8 T. Hase, K. Ohtani, R. Kasai, K. Yamasaki and C. Picheansoonthon, *Phytochemistry*, 1996, **41**, 317–321.
- 9 For our earliest biosynthetic proposals, see page 36 of the ESI for ref. 2c.
- 10 J. M. Bobbitt, L. H. Amundsen and R. I. Steiner, *J. Org. Chem.*, 1960, **25**, 2230–2231.
- 11 (a) W. A. Herrmann and M. Prinzh, *Applied Homogeneous Catalysis with Organometallic Compounds*, Wiley-VCH, Weinheim, 2nd edn, 2002; (b) T. J. Donohoe, T. J. C. O'Riordan and C. P. Rosa, *Angew. Chem.*, 2009, **121**, 1032–1035, (*Angew. Chem. Int. Ed.*, 2009, **48**, 1014–1017); (c) A. Vasseur, J. Bruffaerts and I. Marek, *Nat. Chem.*, 2016, **8**, 209–219.
- 12 Attempts to rearrange hemi-aminals **16** and **22** failed. Heating solutions in MeOH, EtOH or MeCN returned starting material. Treatment with TFA appeared to give isomerization from the *syn* to the *anti* configuration, with no sign



- of further rearrangement. Treatment with LiOH in refluxing MeOH/H₂O resulted in slow decomposition.
- 13 Treatment of millingtonine with glucosidase enzymes has been shown to result in a retro-oxa-Mannich/oxa-Michael/Mannich reaction sequence to give a diastereomer of incargranine A, see ref. 8.
- 14 A. R. Vaino and W. A. Szarek, *Chem. Commun.*, 1996, **20**, 2351–2352.
- 15 Professor Zhang very kindly provided pdf files of the processed NMR spectra for natural incargranine A, see the ESI† for direct comparisons.
- 16 For recent examples of natural product anticipation from biomimetic studies, see: (a) D. P. O'Malley, K. Li, M. Maue, A. L. Zografos and P. S. Baran, *J. Am. Chem. Soc.*, 2007, **129**, 4762–4775; (b) P. Sharma, B. Lygo, W. Lewis and J. E. Moses, *J. Am. Chem. Soc.*, 2009, **131**, 5966–5972; (c) M. Gavagnin, E. Mollo and G. Cimino, *Rev. Bras. Farmacogn.*, 2015, **25**, 588–591; (d) A. L. Lawrence, R. M. Adlington, J. E. Baldwin, V. Lee, J. A. Kershaw and A. L. Thompson, *Org. Lett.*, 2010, **12**, 1676–1679; (e) V. Sofiyev, J.-P. Lumb, M. Volgraf and D. Trauner, *Chem. – Eur. J.*, 2012, **18**, 4999–5005; (f) S. Strych, G. Journot, R. P. Pemberton, S. C. Wang, D. J. Tantillo and D. Trauner, *Angew. Chem.*, 2015, **127**, 5168–5172, (*Angew. Chem. Int. Ed.*, 2015, **54**, 5079–5083); (g) P. Ellerbrock, N. Armanino, M. K. Ilg, R. Webster and D. Trauner, *Nat. Chem.*, 2015, **7**, 879–882.
- 17 P. Champy, Artifacts and Natural Substances Formed Spontaneously, in *Biomimetic Organic Synthesis*, ed. E. Poupon and B. Nay, Wiley-VCH, Weinheim, 2011, vol. 2, pp. 849–934.
- 18 A. Mithofer and W. Boland, *Annu. Rev. Plant Biol.*, 2012, **63**, 431–450.
- 19 N. J. Green and M. S. Sherburn, *Aust. J. Chem.*, 2013, **66**, 267–283.

