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Total synthesis of incargranine A†

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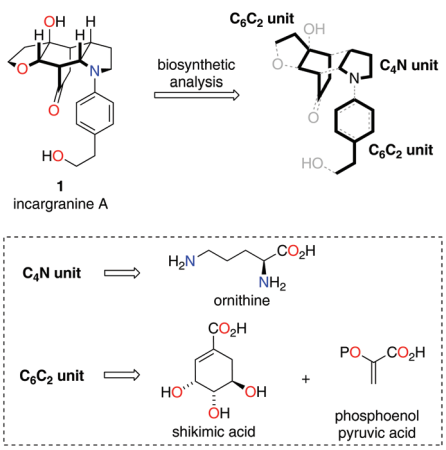
Synthetic studies into the origins of the alkaloid incargranine A have resulted in the development of a four-step (longest linear sequence) total synthesis. This synthesis has been scaled-up to provide gram-scale quantities of material, which would alternatively require extraction of several metric-tons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



Scheme 1 Structure and biosynthetic analysis of incargranine A.

Our biosynthetic analysis, shown in Scheme 1, reveals incargranine A (**1**) is likely constructed from two shikimate-derived C_6C_2 units linked together by an ornithine-derived C_4N 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.

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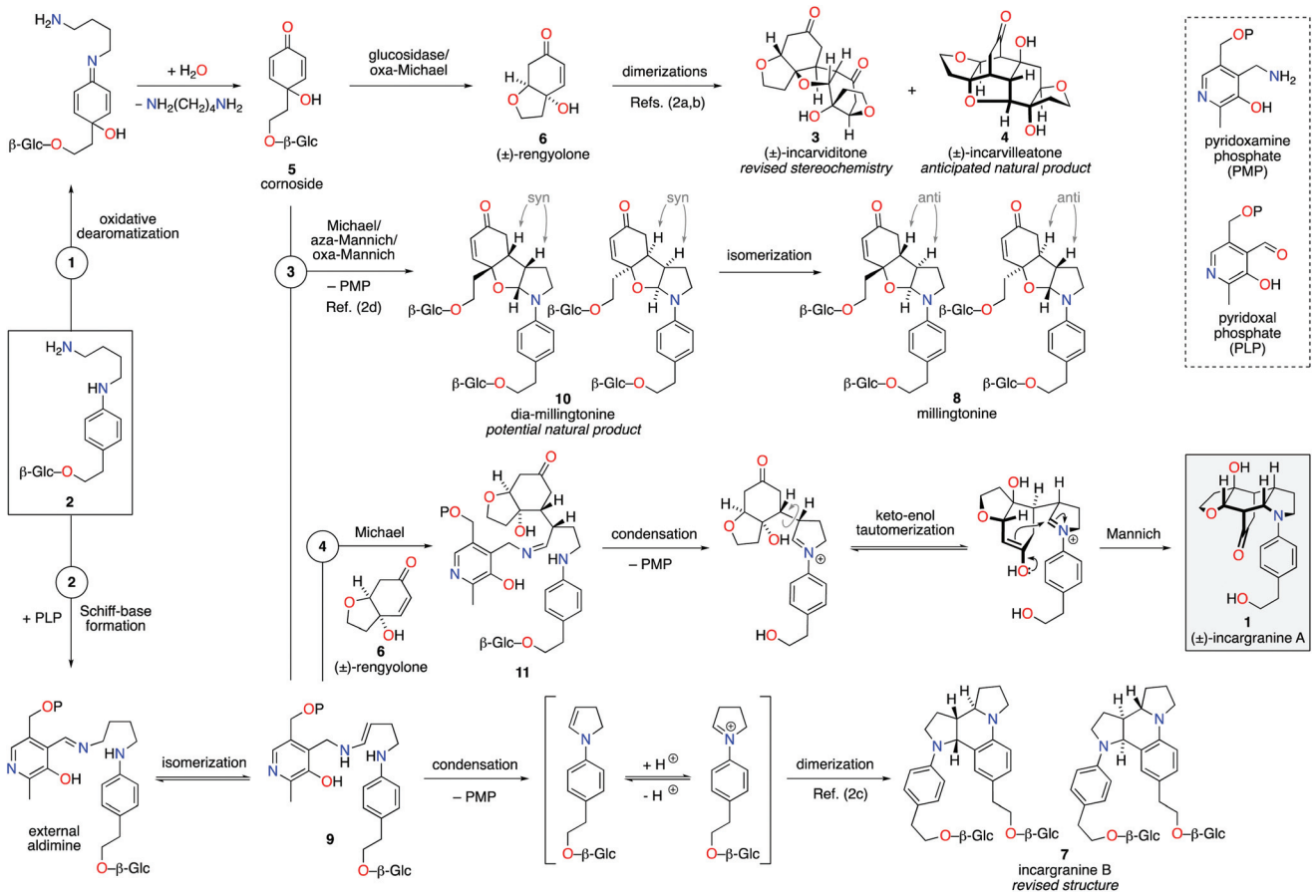
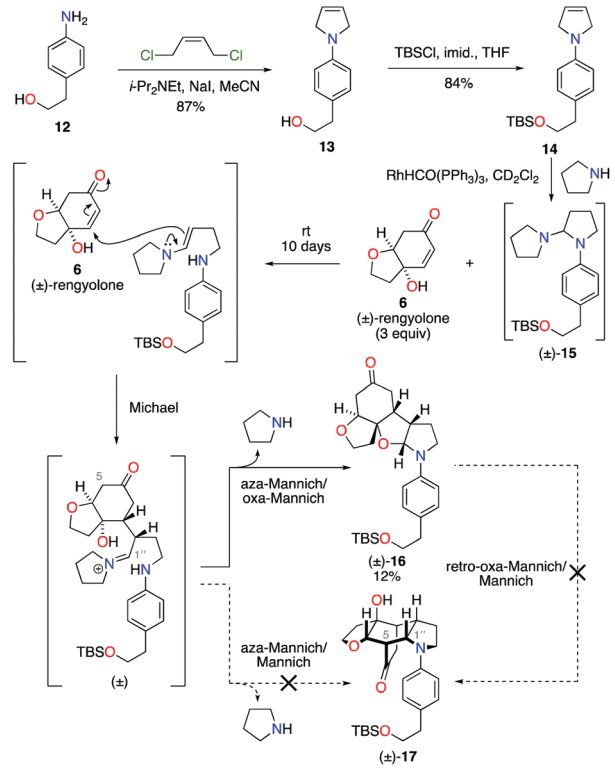


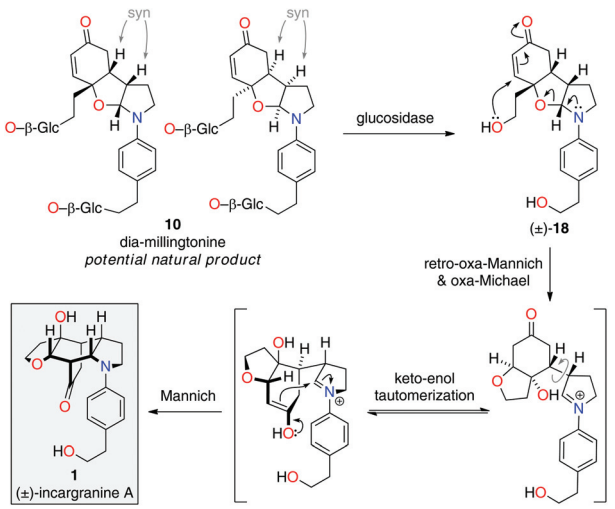
Scheme 2 Proposed network of biosynthetic pathways towards a family of plant-derived phenylethanoid natural products, including incargranine A.

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 $RhHCO(PPh_3)_3$ and pyrrolidine reaction conditions gave the expected aminal intermediate **15**.[2d,11] Due to the instability of aminal **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 ^{1}H NMR spectroscopy revealed it took 10 days at ambient temperature for aminal **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 aminal **15** and rengyolone (**6**), several issues presented themselves 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.

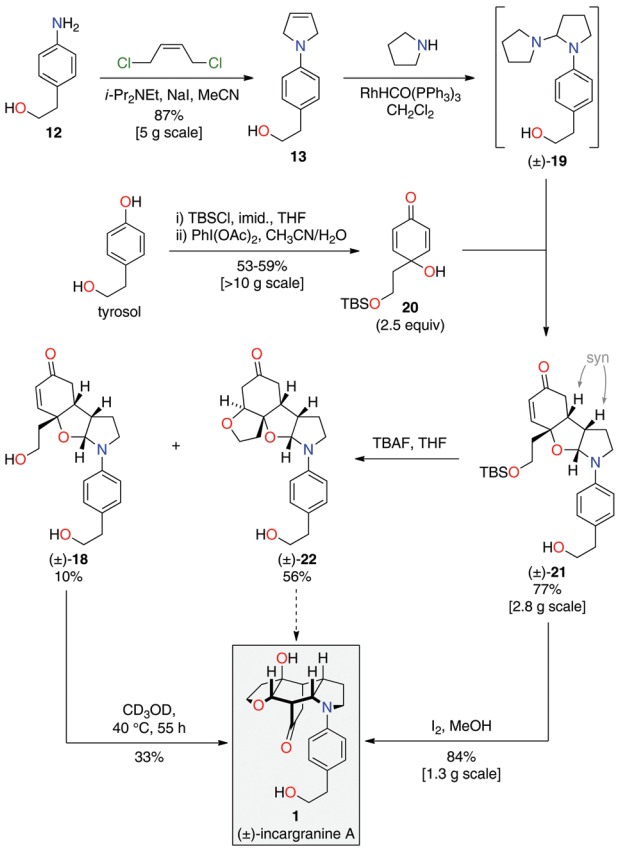
Upon further evaluation of the incargranine A (**1**) framework it became apparent that it might instead be derived from the *syn*-diastereomer of millingtonine, dia-millingtonine (**10**), which we had previously identified as a potential natural product and direct biosynthetic precursor to millingtonine (**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 4 Revised biosynthetic hypothesis for incargranine A.



Scheme 5 Total synthesis of 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 $RhHCO(PPh_3)_3$ and pyrrolidine gave the expected aminal 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 CD_3OD 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 (±)-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 (±)-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 gycosidase 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

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