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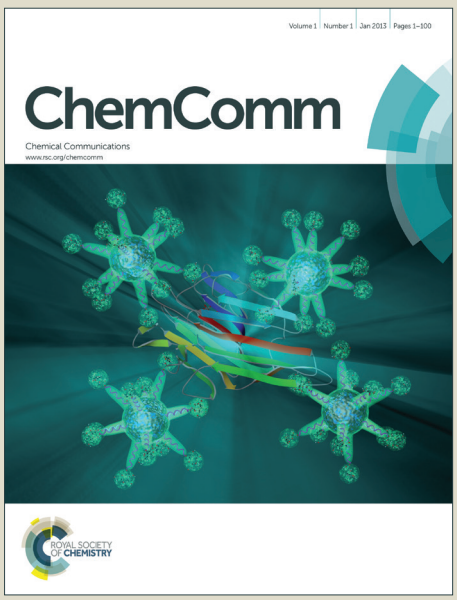

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ARTICLE TYPE

Two-directional synthesis and biological evaluation of alkaloid 5-*epi*-*cis*-275B'

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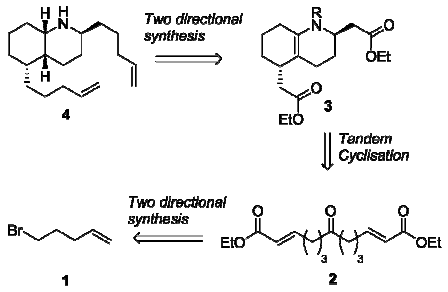
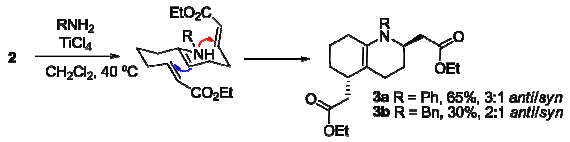
The first total synthesis of myrmicine ant alkaloid 5-*epi*-*cis*-275B' (4, Scheme 1) is presented. A tandem cyclisation established the entire core of the structure in a single transformation as well as the required 2,5-*anti* stereochemistry. Two-directional synthesis was used to furnish the cyclisation precursor 2, as in each of the subsequent steps towards the natural product. The first electrophysiology studies for 4 (against nicotinic acetylcholine receptors) were also conducted, finding modest inhibition of current.



Scheme 1. Retrosynthetic analysis

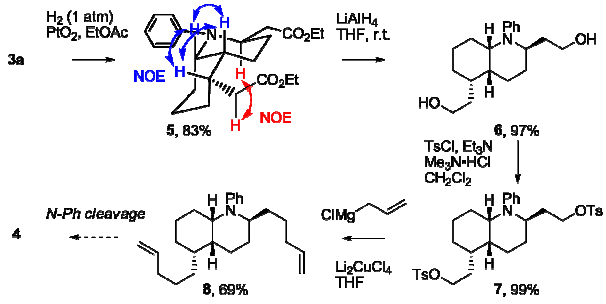
Chemical defences are widespread among living organisms and, although toxins are often biosynthesised locally, in poison frogs they are thought to be trophically derived and sequestered.[1] Unsurprisingly, species that sequester particular substances rely on specific diets, indicative of their complex ecological and evolutionary relationships with other organisms.

2,5-Disubstituted decahydroquinolines (DHQs) are a major class of poison frog alkaloids, some of which have also been identified in myrmicine ants (which is one of the main food sources of such frogs).[2] Toxicity often arises through the disruption of normal neurotransmitter-receptor binding activity, thus leaving muscles in an inactive state of contraction and leading to fibrillation. Poison frogs have classically been used by some indigenous populations of Central America to provide poison for darts but their toxins have garnered modern attention due to their potential development as, for example, analgesics.[3] Considerable synthetic studies have been dedicated towards DHQs, some recent seminal contributions including those from the groups of Blechert,[4] Bosch and Amat[5] and Bonjoch and Bradshaw.[6]



Scheme 2. Tandem cyclisation towards functionalised octahydroquinolines.

Traditionally, targets of synthetic interest are constructed via linear or convergent approaches, which tackle one bond at a time. Two-directional synthesis[7] on the other hand, is capable of elevating the rapidity with which molecular complexity is established, by simultaneously functionalising each molecule *twice* per reaction. Similarly, tandem cyclisations,[8] which constitute several bond formations (each occurring as a direct consequence of the preceding) in a single transformation, are inherently more efficient processes than their stepwise counterparts. Over the past decade, our group have successfully combined these two strategies and applied them in the context of diversity[9] and target oriented synthesis (for example, towards the synthesis of complex heterocyclic natural products).[10]



Scheme 3. Two-directional approach towards **4**.

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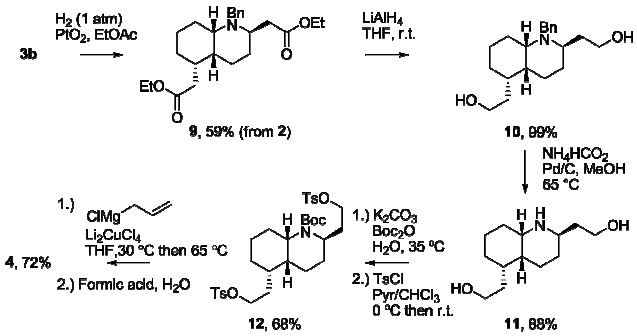
Previously, we reported the efficient synthesis of substituted octahydroquinolines (Scheme 3)[11] and in this report we extend those studies towards the first total synthesis of myrmicine ant DHQ alkaloid 5-*epi*-*cis*-**275B'** (**4**).

Multi-gram quantities of starting material ketodienoate **2** were efficiently accessed through our optimised route, as previously reported.[10a] Given that aniline performed best in trial tandem cyclisations (**3a**),[11] it served as our starting point amine of choice as we proceeded with the synthesis. Hence the subsequent steps (Scheme 4) involved first, hydrogenation of the olefin, then two-directional: reduction of the esters, conversion of the resulting diol to leaving groups and their displacement with allyl Grignard. NOE studies on the major diastereomer isolated from the hydrogenation step (**5**) shed light on its relative stereochemistry. Unsurprisingly, delivery of H_2 led to the required *cis* configuration about the bicycle bridge and approach from the face *opposite* to the contiguous ester, as desired. Reduction to diol **6**, ditosylation to **7** and diallylation with allyl Grignard in a dilithium tetrachlorocuprate (II) solution[12] afforded DHQ **8** in good yield. In order to access the natural product **4**, efficient *N*-phenyl cleavage would need to be enacted. However, after extensive efforts, using primarily various Birch reduction conditions,[13] but also oxidative (and other) approaches, either no reaction at all, or total decomposition was observed. We therefore revised our strategy and looked instead to employ benzylamine as the amine of choice in the tandem cyclisation key step. Although this originally fared worse in terms of yield in the synthesis of octahydroquinoline **3b** (Scheme 2), we envisaged that deprotection (*N*-benzyl cleavage) could be simultaneously brought about by hydrogenolysis[14] under the same conditions used to reduce the bridgehead enamine, thus saving one step. The sequence of transformations had to be adjusted accordingly, to avoid reduction of the terminal olefin groups during hydrogenation (Scheme 4).



Scheme 4. Completion of the synthesis of **4**.

The low yield originally obtained for the tandem cyclisation towards **3b** (Scheme 3) was found to be due to the rapid reversibility of that reaction via *retro* Michael reaction in the presence of moisture. However, when the reaction mixture was quenched anhydrously and followed by hydrogenation (thus removing the potential for retro Michael reaction), a reasonable 59% yield of DHQ **9** (Scheme 4) was obtained over two steps from ketodieonate **2**. To our surprise, no *N*-benzyl cleavage was seen under these hydrogenation conditions. Several, more forcing, conditions were screened[14] and even at 40 atm (and after several days reaction), only very partial deprotection was seen. Quite contrastingly however, under *transfer* hydrogenation conditions,[15] complete deprotection was seen in less than 30 mins. Double ester reduction having proceeded efficiently, the next problem encountered was the pronounced aqueous affinity of the secondary amine/diol **11**. This was overcome by treating the aqueous extract from **11** with potassium carbonate and Boc_2O. Re-protection of **11** as the Boc carbamate was found to be imperative otherwise decomposition was seen in the later stages of the synthesis. Ditosylation then led to **12** in a good 68% yield (from **11**). For the final step, it was found that by first stirring the reaction mixture at 30 °C and then heating to 65 °C, both double-allylation and partial Boc-deprotcection could be instigated and any remaining Boc-protected material was then simply subjected to formic acid, and upon basic workup and chromatography, a clean sample of **4** was obtained in good overall yield. Although the NMR spectra of **4** were not disclosed in the isolation paper, comparison to the closely related *cis*-275B,[2a] which is epimeric at C2, shows a high degree of similarity, as would be expected.

With **4** in hand, we went about exploring its biological properties.[17] The limited research conducted on DHQs so far indicates low vertebrate toxicity, but some have been identified as antagonists of nicotinic acetylcholine receptors (nAChR).[16] We used whole-cell patch-clamp of TE671 human cells expressing embryonic muscle-type nAChR to investigate the inhibition of acetylcholine induced currents by **4**. Application of 10 µM of **4** alone failed to elicit an excitatory response (V_h = -75 mV). Co-application of increasing concentrations of **4** with 10 µM ACh resulted in a minor, concentration dependent inhibition of inward currents (V_h = -75 mV). This low activity meant that an IC_{50} could not be directly determined, but had to be extrapolated from the curve (Fig. 1). The IC_{50} value was found to be 162 µM (95% Cl: 95.8-275 µM). The inhibition of net-charge movement was 25% at 100 µM, whereas the peak current was only reduced by 13%. The modest nAChR activity of racemic **4** warrants further investigation into other, potentially useful bioactivities such as antiparasitic or antimicrobial properties.

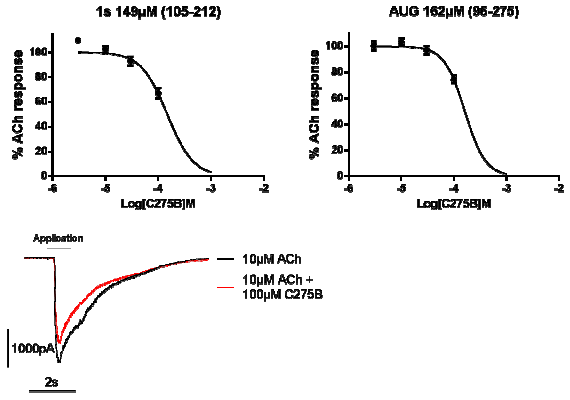


Figure 1. Inhibition of ACh induced currents in TE671 cells expressing human muscle-type nAChR by **4**.

Conclusions

In conclusion, we have demonstrated the first total synthesis of a DHQ alkaloid via a two-directional strategy. Key steps include a tandem cyclisation that established the entire core with the desired stereochemistry. Biological evaluation showed modest nAChR activity.

Acknowledgments

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Notes and references

(1) R. A. Saporito, M. A. Donnelly, P. Jain, H. M. Garraffo, T. F. Spande and J. W. Daly, *Toxicon*, 2007, **50**, 757.

(2) (a) T. F. Spande, P. Jain, H. M. Garraffo, L. K. Pannell, H. J. C. Yeh and J. W. Daly, *J. Nat. Prod.*, 1999, **62**, 5; (b) J. W. Daly, T. F. Spande and H. M. Garraffo, *J. Nat. Prod.*, 2005, **68**, 1556; (c) T. H. Jones, J. S. T. Gorman, R. R. Snelling, J. H. C. Delabie, M. S. Blum, H. M. Garraffo, P. Jain, J. W. Daly and T. F. Spande, *J. Chem. Ecol.*, 1999, **25**, 1179.

(3) J. W. Daly, *J. Med. Chem.*, 2003, **46**, 446.

(4) N. Holub, J. Neihöfer and S. Blechert, *Org. Lett.*, 2005, **7**, 1227.

(5) (a) M. Amat, E. Ghirardi, L. Navío, R. Griera, N. Llor, E. Molins and J. Bosch, *Chem. Eur. J.*, 2013, **19**, 16044; (b) M. Amat, R. Fabregat, R. Griera and J. Bosch, *J. Org. Chem.*, 2009, **74**, 1794; (c) M. Amat, R. Fabregat, R. Griera, P. Florindo, E. Molins and J. Bosch, *J. Org. Chem.* 2010, **75**, 3797; (d) M. Amat, R. Griera, R. Fabregat, E. Molins and J. Bosch, *Angew. Chem. Int. Ed.*, 2008, **47**, 3348.

(6) B. Bradshaw, C. Luque-Corredera and J. Bonjoch, *Org. Lett.*, 2013, **15**, 326.

(7) K. M. G. O'Connell, M. Díaz-Gavilán, W. R. J. D. Galloway and D. R. Spring, *Beilstein J. Org. Chem.*, 2012, **8**, 850.

(8) (a) K. C. Nicolaou, D. J. Edmonds and P. G. Bulger, *Angew. Chem. Int. Ed.*, 2006, **45**, 7134; (b) K. C. Nicolaou, T. Montagnon and S. A. Snyder, *Chem. Commun.*, 2003, 551;

(9) D. Robbins, A. F. Newton, C. Gignoux, J.-C. Legeay, A. Sinclair, M. Rejzek, C. A. Laxon, S. K. Yalamanchili, W. Lewis, M. A. O'Connell and R. A. Stockman, *Chem. Sci.*, 2011, **2**, 2232.

(10) (a) C. Gignoux, A. F. Newton, A. Barthelme, W. Lewis, M.-L. Alcarazb and R. A. Stockman, *Org. Biomol. Chem.*, 2012, **10**, 67; (b) A. Barthelme, D. Richards, I. R. Mellor and R. A. Stockman, *Chem. Commun.*, **2013**, *49*, 10507.

(11) P. Aggarwal, G. Procopiou, D. Robbins, G. Harbottle, W. Lewis and R. A. Stockman, *Synlett*, 2012, **23**, 423.

(12) (a) D. H. Burns, J. D. Miller, H.-K. Chan and M. O. Delaney, *J. Am. Chem. Soc.*, 1997, **119**, 2125; (b) G. Cahiez, C. Chaboche and M. Jézéquel, *Tetrahedron*, 2000, **56**, 2733.

(13) N. Girard, J.-P. Hurvois, C. Moinet and L. Toupet, *Eur. J. Org. Chem.*, 2005, 2269.

(14) T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, Wiley, New York, 3rd edn., 1999, pp. 579-580.

(15) S. Ram and L. D. Spicer, *Tetrahedron Lett.*, 1987, **28**, 515.

(16) J. W. Daly, Y. Nishizawa, W. L. Padgett, T. Tokuyama, P. J. McCloskey, L. Waykole, A. G. Schultz and R. S. Aronstam, *Neurochem. Res.*, 1991, **16**, 1207.

(17) All biological studies were carried out on racemic material. Unfortunately insufficient material was in hand to separate enantiomers and test them seperately.