RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

COMMUNICATION

Access to harmonine, a chemical weapon of the ladybird bioterrorists†

Satish Chandra Philkhana,[a] Prabhu Dhasaiyan,[b] B.L.V. Prasad*[b] and D. Srinivasa Reddy*[a]

Received (in XXX, XXX) XthXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Synthesis of harmonine, a defense alkaloid from the harlequin ladybird is reported by three different routes. The preparation of several new analogs with the same molecular weight and the decoration of gold nanoparticles with harmonines are also part of the present communication.

The harlequin ladybird, also known as a ladybird beetle (*Harmonia axyridis*), has been introduced as a biological control agent against harmful pests of the crops in several countries in Europe and North America.[1] These Asian ladybirds are good eaters of other insects, for example, each beetle consumes roughly 200 aphids per day. Hence, it became popular and is considered as a green way to fight pests and has been commercially available for several decades.[1,2,3] Wiesner's group in Germany recently discovered that a defense alkaloid harmonine **1**, also known as (*17R,9Z*)-1,17-diaminooctadec-9-ene, is the principal compound with a broad spectrum of biological activities from the haemolymph of this species.[4] Compound **1** displayed very interesting biological activities against a variety of pathogens (See ESI for the detailed biological activities). However, in recent times, it became a global problem because of the successful invading potential of the Asian ladybirds, where they outperform native ladybird species.[1,2] Sometimes, they can enter into houses in hundreds, leading to nuisance and allergies. They also effect the fruit industry by altering the quality of products through contamination.[3] These obnoxious Asian ladybirds can be made useful by utilizing harmonine as a lead compound towards discovery of new drugs. We initiated a medicinal chemistry program based on this chemotype and our intention is to make the target compound in good quantity and generate a focused library of compounds around this molecule, to understand SAR and ultimately find a lead compound. Although harmonine was isolated and synthesized[5] two decades ago, very recently, it was in the news[6a] and was highlighted on many internet blogs.[6b] The design and synthesis of harmonine and its analogues using three different approaches are described here.

Retrosynthetically, the target alkaloid harmonine was envisioned in two different strategies as shown in Figure 1. The classical

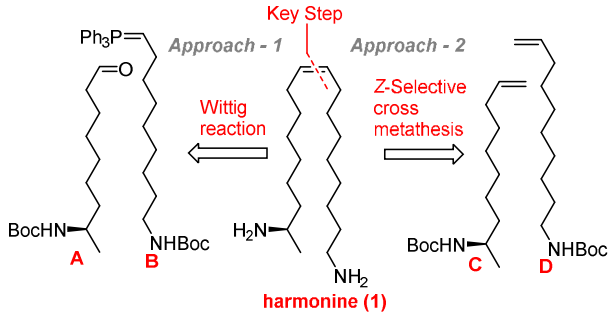
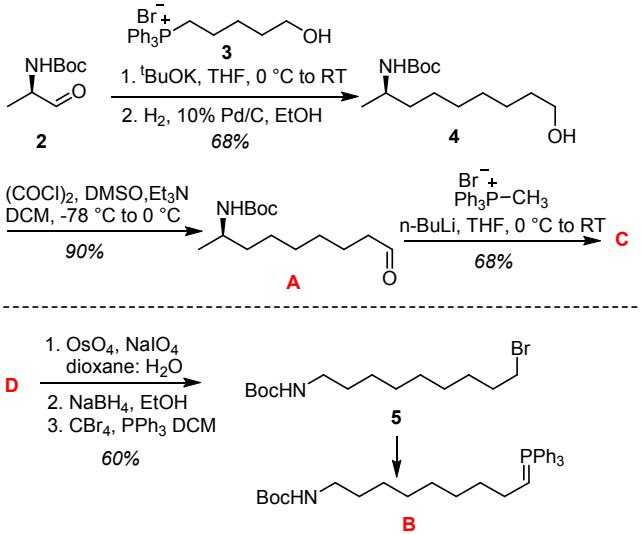


Figure 1. Planned strategies to access harmonine (**1**).

Wittig reaction and the modern Z-selective cross metathesis were chosen as key steps in our approach 1 and 2, respectively. Accordingly, the desired intermediates **A-D** are planned as the key building blocks which could be prepared from readily available starting materials. The only chiral center present in the harmonine can be introduced by use of amino acid D-alanine.



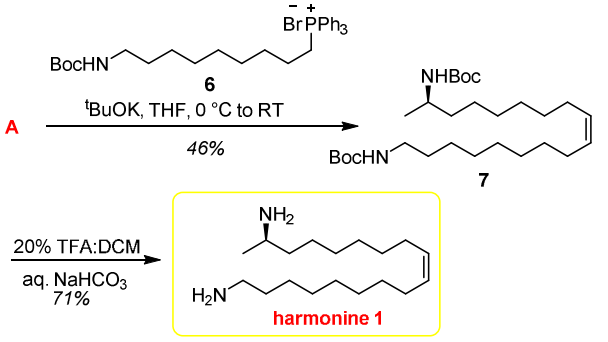
Scheme 1. Synthesis of the key intermediates A-D.

Our efforts to access harmonine began with the preparation of all the key intermediates **A-D** as described in Scheme 1. The reaction of known aldehyde **2**[7] and an ylide **B** generated from triphenylphosphoniumbromide **3**[8] resulted in olefin which on hydrogenation produced the saturated alcohol **4** in 68% yield over two steps. The Swern oxidation of alcohol **3** gave the key intermediate **A**, which on one-carbon Wittig homologation resulted in compound **C**. Compound **D** was prepared as per the published procedures[9] and it was further transformed to compound

(a) Division of Organic Chemistry (b) Division of Physical Chemistry, Address: CSIR-National Chemical Laboratory, Dr.HomiBhabha Road, Pune, 411008, India. Fax: +91 20 25902629; Tel: +91 20 25902445; E-mail: pl.bhagavatula@ncl.res.in; ds.reddy@ncl.res.in

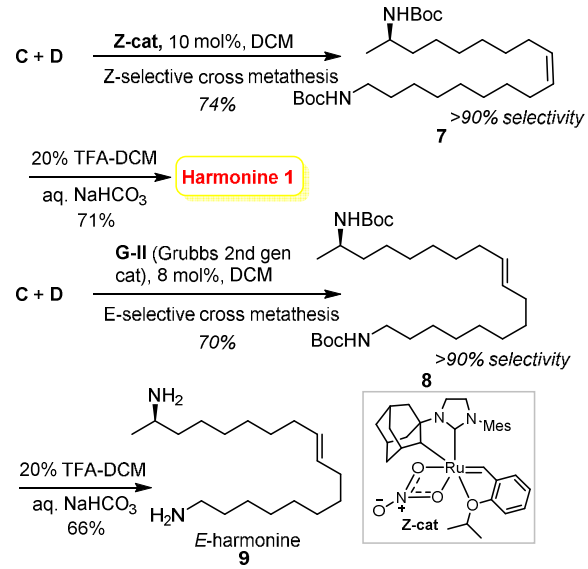
† Electronic Supplementary Information (ESI) available: Characterization data, NMR spectra and detailed experimental procedures. See DOI: 10.1039/b000000x/

B *via* **5** as shown in Scheme 1. Having all the key intermediates **A-D** in hand, we ventured into the synthesis of target compound harmonine **1**. As per the plan in approach-1, the triphenylphosphoniumbromide **6** (prepared from **5**) was reacted with aldheyde **A** in the presence of potassium tertiarybutoxide to furnish the Z-olefin **7** in 46% isolated yield (Scheme 2). It is difficult to distinguish between E- and Z-olefins using ^{1}H NMR pattern, as they were not clear multiplets. However, we have used characteristic allylic ^{13}C signals (absence of signal at ~32 ppm) for the identification of desired Z-olefin **7**.[10] Boc deprotection in **7** using TFA-dichloromethane mixture followed by neutralization with $NaHCO_3$, furnished the target compound harmonine **1** as a gummy material. The spectral data of synthetic harmonine **1** was compared with that of literature and found identical.[4b,5]



Scheme 2. Synthesis of harmonine **1** using Wittig approach.

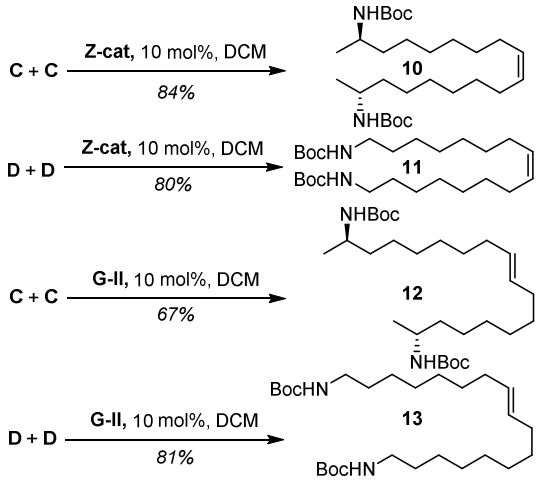
In the second approach, we have explored the cross metathesis reaction using recently developed Z-selective ruthenium catalyst (**Z-cat**).[11] After a few optimizations, slow and simultaneous addition of both olefins **C** and **D** to a solution of Z-cat in DCM resulted in the desired compound **7** with >90% selectivity.[12] The authenticity of **7** prepared using Z-selective cross metathesis was further confirmed by comparing the data with compound **7** prepared through approach-1. This material was also transformed to the harmonine and compared the spectral data from literature (Scheme 3).[4b,5] To access unnatural harmonine with *E*-olefin, the mixture of olefins **C** and **D** was exposed to Grubbs 2nd generation



Scheme 3. Cross metathesis approaches.

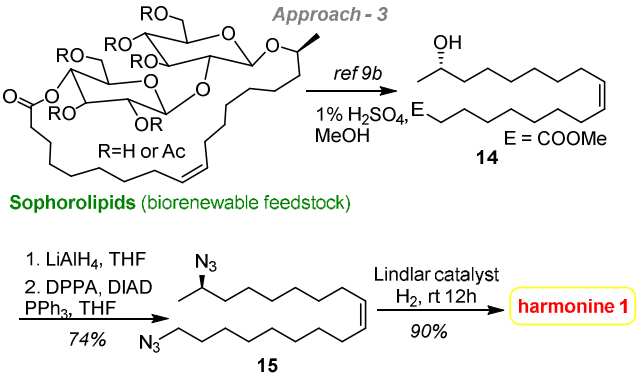
catalyst (**G-II**)[13] in DCM under similar conditions, resulted in *E*-olefin **8** in 70% yield with >90% selectivity. The compound **8** was transformed to *E*-harmonine **9** in a similar manner and spectral data was consistent with the assigned structure.

Towards the generation of closely related library of molecules around harmonine skeleton, we have attempted self metathesis reactions of both olefins separately to deliver homodimers as described in Scheme 4. The homodimers **10** and **11** were synthesized using Z-selective cross metathesis catalyst from olefins **C** and **D**, respectively. Similarly, compounds **12** and **13** were prepared using E-selective Grubbs 2nd generation catalyst from olefins **C** and **D**, respectively. Interestingly, all the hetero dimers (**7** & **8**) and homo dimers (**10**, **11**, **12** & **13**) prepared here have the same molecular weight and are worth exploring the biological activity of the corresponding amines.



Scheme 4. Homodimers towards harmonine analogs.

In another interesting approach, the harmonine **1** was accessed from methyl 17-hydroxy oleate **14**, which in turn could be prepared from bio-renewable feedstock sophorolipids as per the procedures described in literature.[9b] The methyl 17-hydroxy oleate **14** was readily converted to diazide **15** using a two-step protocol (i) $LiAlH_4$ in THF to reduce it to diol and (ii) Mitsunobu reaction conditions (DPPA, DIAD, PPh_3) to convert diol to diazide in 74% yield ~~in~~ two steps. To our delight, the secondary alcohol present in the starting material inverted to give the desired stereocenter present in the target natural product harmonine **1**. Finally, both the primary and secondary azides were reduced



Scheme 5. Access to harmonine from bio-renewable feedstock.

using Lindlar's catalyst[5b] under the blanket of hydrogen atmosphere to furnish the harmonine **1** in 90% yield (Scheme 5). The spectral data including optical rotation were matched with that of the previous ones.

Decorating biologically active molecules on a nanoparticle scaffold is an attractive strategy to improve their bio-availability and enhanced cellular uptake.[14] Considering the structural features present in harmonine, we envisaged that these molecules could be easily decorated on gold nanoparticles, as primary amine groups are known to be noble metal nanoparticle surface capping/passivating agents. The UV-Vis spectrum of the harmonine coated nanoparticle dispersion shows an absorption peak around 578 nm, which is typical for Au nanoparticles[15] (Figure 2). The pure metallic phase of the nanoparticles was also confirmed by powder X - ray diffraction pattern corresponding to the fcc lattice system. The HR - TEM images show that the nanoparticles are spherical in shape. The size distribution plotted from Figure 2 (inset) indicate the mean size of the nanoparticles to be 8.5 nm (S. D = 2.6). The potential applications of the nanoparticles prepared from harmonine or its analogues can be explored further.

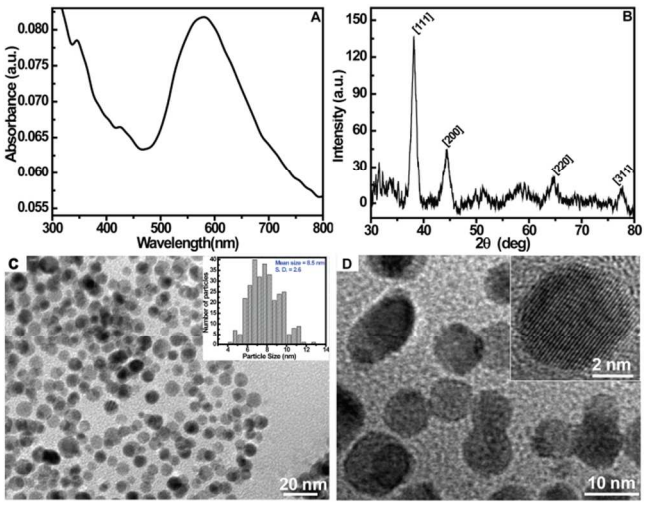


Figure 2. (A) The UV- Visible spectra, (B) XRD pattern, (C) and (D) the TEM images of Harmonine capped AuNPs. The inset in (C) shows the particle size distribution plot and the inset in (D) shows the higher magnification TEM image where the (111) planes of the gold lattice are clearly visible.

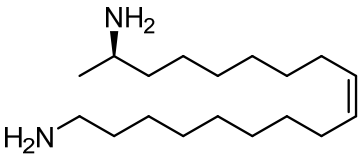
In summary, we have described three novel and different synthetic routes to access the defense alkaloid harmonine of the *harlequin* ladybird. The developed routes are simple and practical to access harmonine and its analogues. All the three are shorter and efficient when compared to the known route. Out of the three routes described here, the approach using sophorolipids is suitable for scale up and the remaining two routes are suitable for analogue synthesis. To expand the scope, synthesized harmonine molecules were decorated on gold nanoparticles and characterized. As we have ready access to harmonine in good quantities, it can be utilized for further understanding of the ladybird biology and possibly have solutions to the existing problems. Also, the related analogues of harmonine are expected to show interesting biological activities and it is worth exploring.

DSR thanks CSIR, New Delhi, for the support through NaPAHA program (XII Five Year Plan, CSC0130). SCP and PD thank UGC, New Delhi, for the award of research fellowship.

Notes and references

1) (a) H. E. Roy and H. Wajnberg, *BioControl*, 2008, **53**, 1-4; (b) H. E. Roy, P. M. Brown, P. Rothery, R. L. Ware and M. E. N. Majerus, *Biocontrol*, 2007, **53**, 265–276; (c) R. L. Koch, *Journal of Insect Science*, 2003, **3**, 16.
2) (a) A. Vilcinskas, K. Stoecker, H. Schmidtberg, C. R. Röhrich and H. Vogel, *Science*, 2013, **340**, 862-863; (b) A. Vilcinskas, *J. Insect Physiol.*, 2013, **59**, 123-129 and refs cited therein.
3) C. Ross, H. Fergusson, M. Keller, D. Walsh, K. Weller and S. Spayd, *Journal of Food Quality*, 2007, **30**, 855-863.
4) (a) M. F. Braconier, J. C. Braekman, D. Daloze and J. M. Pasteels, *Experientia*, 1985, **41**, 519-520; (b) N. Alam, I. S. Choi, K-S. Song, J. Hong, C. O. Lee and J. H. Jung, *Bull. Kor. Chem. Soc.*, 2002**,** **23**, 497–499; (c) L. Sologub, A. Kuehn, S. Kern, J. Przyborski, R. Schillig and G. Pradel, *Cell. Microbiol.*, 2011**,** **13**, 897–912; (d) C. R. Rohrich, C. J. Ngwa, J. Wiesner, H. Schmidtberg, T. Degenkolb, C. Kollewe, R. Fischer, G. Pradel and A. Vilcinskas, *Biol. Lett.*, 2012**,** **8**, 308–311; (e) T. L. Galvan, S. Kells and W. D. Hutchison, *J. Agric. Food Chem.*, 2008, **56**, 1065–1071.
5) (a) D. Enders and D. Bartzen, *Liebigs Ann. Chem.*, 1991, 569-574; (b) M. F. Braconier, J. C. Braekman and D. Daloze, *Bull. Soc. Chim. Belg.*, 1985, **94**, 605-613.
6) Selected links: http://www.the-scientist.com/?articles.view/articleNo/35588/title/Ladybird-Bioterrorists.html. (b) http://www.acs.org/content/acs/en/molecule-of-the-week/archive/molecule-of-the-week-harmonine.html.
7) C. Jahns, T. Hoffmann, S. Müller, K. Gerth, P. Washausen, G. Höfle, H. Reichenbach, M. Kalesse and R. Müller, *Angew. Chem. Int. Ed.*, 2012, **51**, 5239 –5243.
8) J. M. Chong, M. A. Heuft and P. Rabbat, *J. Org. Chem.*, 2000, **65**, 5837–5838.
9) (a) A. D. Jones, D. W. Knight and D. E. Hibbs, *J. Chem. Soc., Perkin Trans.*, 2001**,** **1**, 1182-1203; (b) J. A. Zerkowski and D. K. Y. Solaiman, *J. Am. Oil Chem. Soc.,* 2006, **83**, 621-628.
10) S. Santos and J. Graça, *Phytochem. Anal.*, 2014, **25**, 192–200.
11) (a) M. B. Herbert, V. M. Marx, R. L. Pederson and R. H. Grubbs, *Angew. Chem. Int. Ed.*, 2013, **52**, 310–314; (b) B. L. Quigleya and R. H. Grubbs, *Chem. Sci.*, 2014, **5**, 501-506.
12) It was not very clear that the minor products formed in this reaction are from self dimerization or E-isomer of desired compound. However, the NMR signals correpsond to major compound Z-isomer and are matching with that of compound prepared using Wittig reaction approach.
13) Selected reviews (a) S. J. Connon and S. Blechert, *Angew. Chem. Int. Ed.*, 2003, **42**, 1900 – 1923; (b) K. C. Nicolaou, P. G. Bulger and D. Sarlah, *Angew. Chem., Int. Ed. Engl.*, 2005, **44**, 4490–4527; (c) R. H. Grubbs, *Tetrahedron*, 2004, **60**, 7117–7140.
14) (a) D. A. Giljohann, D. S. Seferos, W. L. Daniel, M. D. Massich, P. C. Patel and C. A. Mirkin, *Angew. Chem. Int. Ed.*, 2010, **49**, 3280 – 3294; (b) E. Boisselier, and D. Astruc, *Chem. Soc. Rev.*, 2009, **38**, 1759–1782.
15) (a) P. Mulvaney, *Langmuir*, 1996, **12**, 788-800; (b) P. Sahu and B. L. V. Prasad, *Chem. Phys. Lett.*, 2012, **525-526**, 101-104.

TOC



Harmonine

Harmonia axyridis

Synthesized by three different routes