

ORIGINAL ARTICLE

Synthesis of deoxyelephantopin analogues

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Deoxyelephantopin is a sesquiterpene lactone that was reported to be as effective in the treatment of mammary tumours and lung metastasis as taxol based on a murine orthotopic cancer model. Its germacrene skeleton harbours three Michael acceptors that can potentially engage a target covalently. Its strained 10-membered ring is densely functionalised and represents an important synthetic challenge. We herein describe our studies towards deoxyelephantopins using a ring-closing metathesis approach and report some unexpected observations.

The Journal of Antibiotics (2018) 71, 248–256; doi:10.1038/ja.2017.132; published online 1 November 2017

INTRODUCTION

Secondary metabolites have been an important source of therapeutics, or leads thereof, and natural product research continues to deliver novel chemical matter for drug discovery.^{1–3} Natural products have also provided a fertile ground for innovations in synthetic chemistry, in addition to a testing ground for the scope and limitations of newly developed synthetic methodologies.⁴ Some biologically interesting natural products have low availability and total synthesis allows the delivery of amounts beyond those isolable from oftentimes-limited natural sources to address their pharmacological profile⁵ and validate or correct their structural assignment.⁶ Secondary metabolite biosynthetic pathways are nature's combinatorial chemistry machines. It is interesting to note that these synthetic pathways often deliver products, which include mildly reactive functionalities that can engage a target protein in a covalent interaction.⁷ The preponderance of such reactive groups amongst secondary metabolites suggests that evolution has selected for this inhibition modality.^{8,9} On this basis, we have pursued the synthesis and discovery of covalent inhibitors^{10–16} and were drawn to sesquiterpene lactones, which often bear an α -*exo*-methylene- γ -butyrolactone moiety and/or Michael acceptor side chain. In a number of cases, these electrophiles act as cysteine traps resulting in a covalent adduct with their target protein.^{17,18} Examples include guaianolides thapsigargin, arglabin, and helenalin or germacranolides parthenolide, eupatolide and cnicin, which selectively act on diverse target classes. The germacranolides are a broad sub-family of sesquiterpene lactones with a germacrene skeleton. The germacrenes have received attention from the synthetic community, but there is to date no general approach to germacrene-based sesquiterpene lactones.¹⁹ Indeed, most methodologies allowing access to the germacrene skeleton either have low functional group tolerance or demand complete re-functionalisation of the carbon framework.^{20–25}

Deoxyelephantopin **1** was found to be the main active ingredient in herbal extracts of *Elephantopus scaber* used in traditional medicine,

along with its C2 epimer isodeoxyelephantopin **2** and parent C4–C5 epoxide elephantopin **3** (Figure 1).²⁶ From a synthetic point of view, deoxyelephantopin is a rather challenging structure owing to its highly strained 10-membered ring flanked with 2 tri-substituted olefins and 4 stereocenters, 3 of which are embedded in two strained 5-membered rings. It is however available from natural sources and previous studies have revealed that it has notable cytotoxicity against a range of cancer cell lines; most interestingly, it was found to have superior efficacy to taxol in orthotopic murine breast cancer models, raising questions about its mode of action.²⁷ Mechanistic studies suggested that deoxyelephantopin suppresses proteasome activity,²⁸ whereas isodeoxyelephantopin inhibits the nuclear factor- κ B pathway.²⁹ Deoxyelephantopin was also found to be a partial peroxisome proliferator-activated receptor- γ (PPAR γ) agonist.³⁰ Based on the fact that covalent ligand of PPAR γ have been reported,^{31,32} we anticipated a covalent engagement paralleling previous covalent ligand of PPAR γ .³³

As no synthesis of deoxyelephantopin had been reported and because modifying its densely functionalised medium-sized ring may negatively impact its target binding and biological activity, we needed structural analogues for a structure–activity relationship study, as well as tagged probes for chemical proteomic studies, to evaluate its covalent interactome. To this end, we envisioned a convergent synthesis taking advantage of a late-stage ring-closing metathesis (RCM) on substrate **4**, which ought to be accessible through a diastereoselective coupling of fragments of aldehyde **5** and bromolactone **6** under Barbier-type allylation conditions (Scheme 1).

Following the proposed synthetic sequence, a range of structural analogues and alkyne-tagged probes were successfully obtained and used for the aforementioned proteomic studies, allowing us to not only identify 11 new covalent cellular targets but also to establish that deoxyelephantopin indeed covalently engages PPAR γ but at cysteine 190 in the zinc-finger domain rather than in the ligand-binding

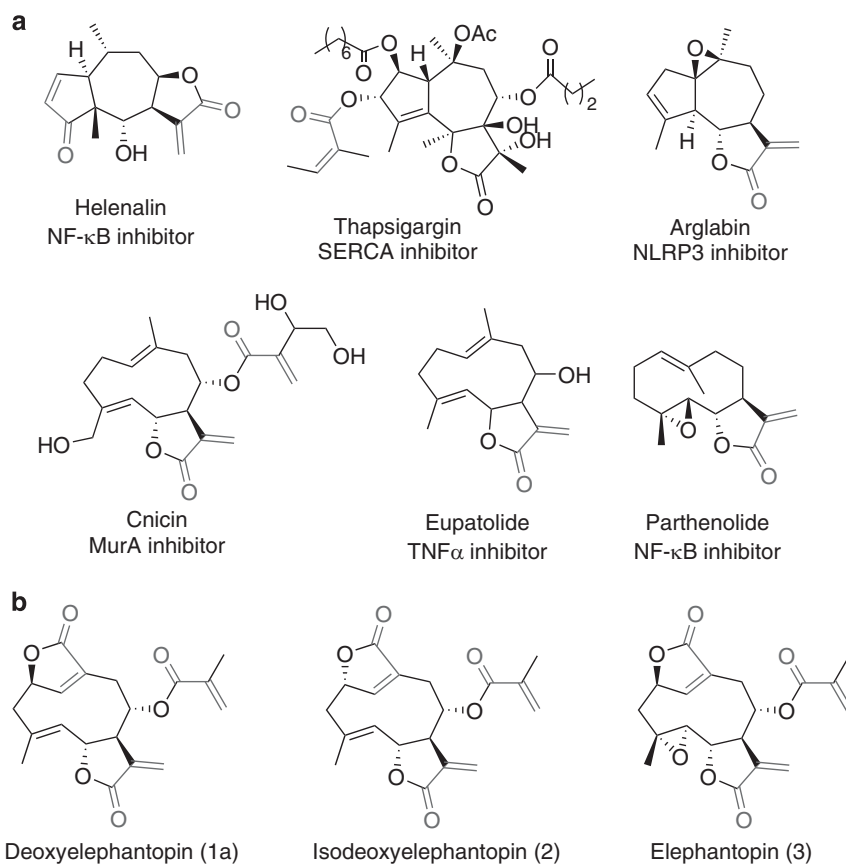
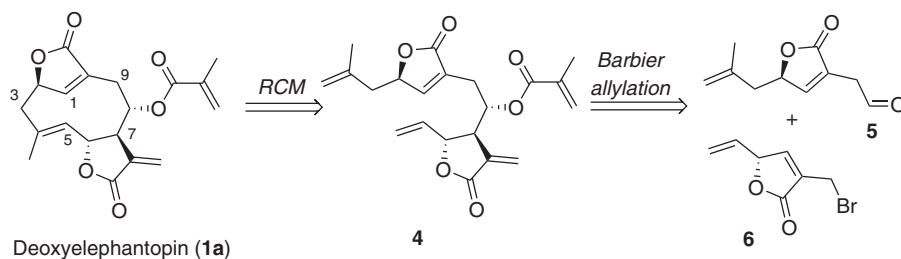


Figure 1 (a) Selected bioactive sesquiterpene lactones known to covalently engage cysteine residues; (b) selected naturally occurring elephantopins. A full colour version of this figure is available at the *Journal of Antibiotics* journal online.



Scheme 1 Retrosynthetic analysis of deoxyelephantopin.

domain as anticipated.³³ Herein we report an account of our synthetic efforts towards this important yet challenging target.

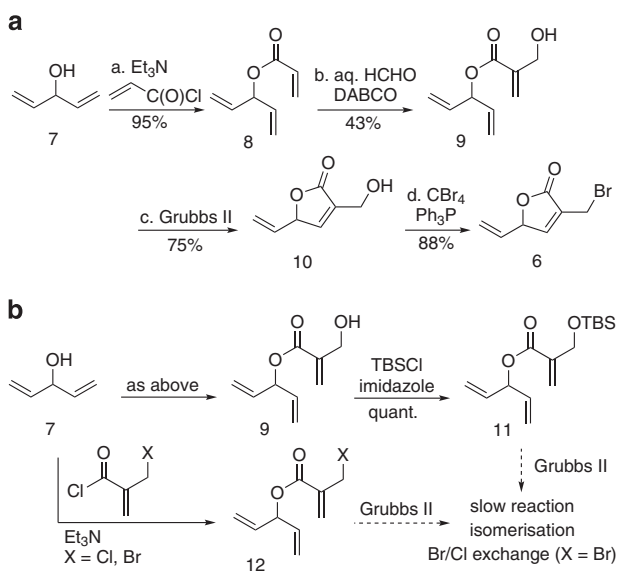
RESULTS AND DISCUSSION

In our synthetic design, the late-stage Barbier-type allylation is a key-step. It represents a point of convergence in a diversity-oriented synthesis by allowing the coupling of a range of aldehydes with bromolactone **6** with high diastereoselectivity. The key intermediate bromolactone **6** is readily available from alcohol **7** in a four-step sequence (Scheme 2a). Commercially available or readily obtained from the addition of vinyl Grignard to acrolein, alcohol **7** is esterified into acrylate **8**, which is in turn converted into allylic alcohol **9** under Morita–Baylis–Hillman conditions using formaldehyde as coupling partner. Triene **9** is then submitted to RCM using Grubbs II³⁴ as

catalyst to afford *endo*-butenolide **10**, which is in turn converted to bromolactone **6** under typical Appel conditions.

It is noteworthy that the allylic alcohol functionality in **9** is important to achieve RCM at an appreciable rate; indeed, the RCM reactions of any other *O*-protected substrates or directly on the allylic chloride or bromide are very sluggish, leading to non-negligible isomerisation issues, in addition to partial Br/Cl exchange for the latter (Scheme 2b).

We then examined the synthesis of the second fragment **5**. As shown in Scheme 3a, aldehyde **15a** was readily obtained by Dess–Martin periodinane (DMP) oxidation of homoallylic alcohol **14a** in Et₂O and directly transferred to a cooled solution of the lithium acetylide of **13**. Isolation of aldehyde **15** was avoided due to its propensity to isomerise to the α,β -conjugated system. *Trans*-hydroalumination of the resulting propargylic alcohol **16a** using LiAlH₄ and



Scheme 2 (a) Synthesis of bromolactone **6**; (b) Unproductive routes. Reagents and conditions: a. acryloyl chloride, Et₃N, CH₂Cl₂, 0 °C; b. aq. HCHO, dioxane, DABCO, rt; c. Grubbs II (10 mol%), CH₂Cl₂ (0.01 M), reflux; d. CBr₄, Ph₃P, CH₂Cl₂, 0 °C.

treatment with elemental iodine afforded vinyl iodide **17a** with perfect regioselectivity. Nickel-mediated carbonylation³⁵ of **17a** afforded the butenolide **18a**. Acetal hydrolysis with iron(III) chloride hexahydrate³⁶ yielded aldehyde **5a**, which was typically used directly after aqueous work-up, i.e. without purification, in the Barbier allylation step (*vide infra*). As for aldehyde **15**, this intermediate carried the risk of olefin isomerisation to the conjugated enal. The same synthetic sequence was used to obtain **5b** from **14b**.

We explored an asymmetric approach towards fragment **5** (Scheme 3b) taking advantage of a Brown allylation³⁷ of aldehyde **20**, readily obtained by MnO₂-mediated oxidation of propargylic alcohol **19**. The allylation proceeded with high enantioselectivity albeit in modest yield. Although the *trans*-hydroalumination/iodination transformation of (*R*)-**16b** was uneventful, partial epimerisation occurred during the subsequent nickel-mediated carbonylation step, presumably due to deprotonation at the γ -position by triethylamine under the reaction conditions to form a prochiral dienolate, which racemises upon re-protonation. The acidity of this proton is accentuated by the aromaticity of the dienolate. With no control over the extent of deprotonation, we devised an alternative asymmetric approach using a palladium-catalysed asymmetric allylic alkylation, which reproducibly provided **18b** with good yields and high enantioselectivity (Scheme 3c).^{38,39} Briefly, *trans*-hydroalumination/iodination of propargylic alcohol **19** followed by Pd-catalysed carbonylation generated *endo*-butenolide **21**, the dienolate of which was treated with allyl chloroformate to provide allyl dienyl carbonate **22**. This central intermediate could be treated with Pd(PPh₃)₄ or Pd₂dba₃ in combination with either (*S,S*)- or (*R,R*)-DACH Phenyl Trost ligands, to access respectively *rac*-**18b**, (*R*)-**18b** or (*S*)-**18b**.⁴⁰ It is noteworthy that the reaction proceeds via α -allylation of the dienolate to form α -butenolide **23**, which is directly submitted to microwave irradiations to undergo [3,3]-Cope rearrangement and provide **18b**.

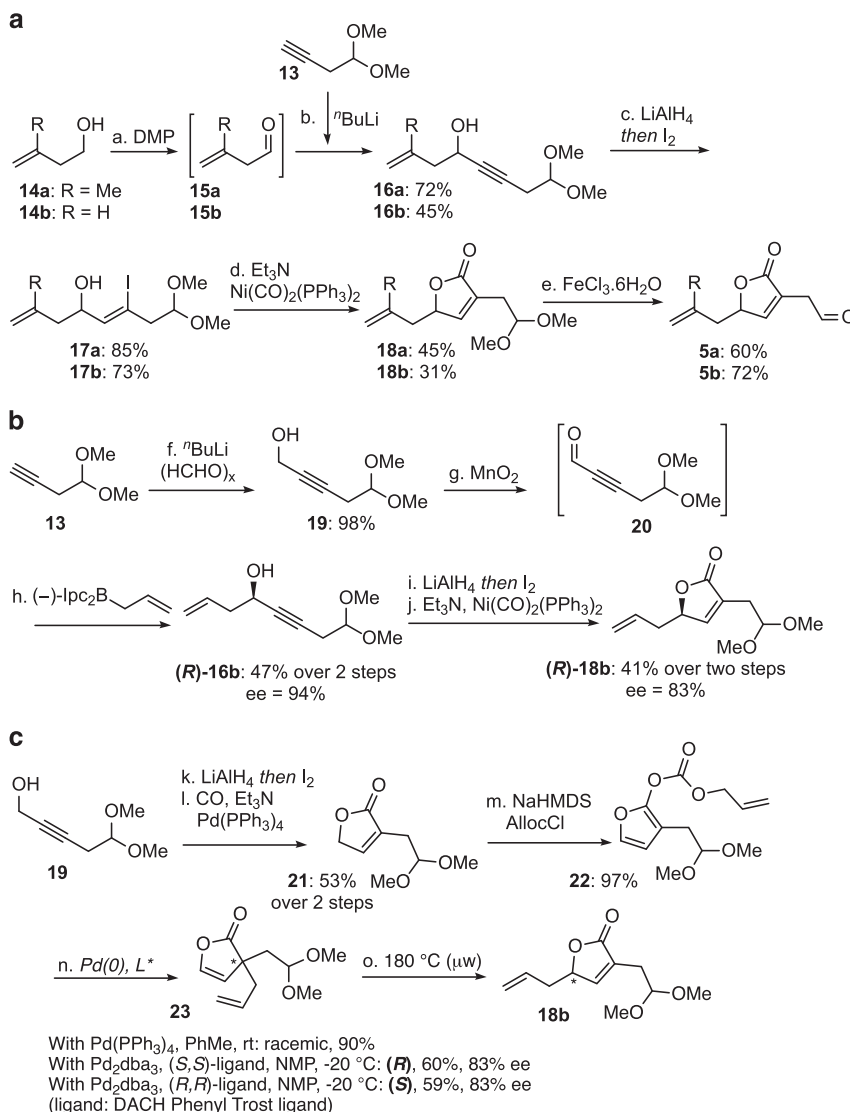
With fragments **5a** and **6** in hand, the zinc-mediated Barbier-type allylation provided secondary alcohol **24a** in good yield and high diastereoselectivity, as anticipated⁴¹ (Scheme 4, the relative

stereochemical assignment is extrapolated from the closely related reaction yielding **24b**, Scheme 5, where cyclisation permitted unequivocal assignment by NMR). RCM attempts on free alcohol **24a** essentially led to partial recovery of **24a** along with some degradation product, and none of the desired RCM product was obtained. This may be due to coordination of the alcohol to the intermediate ruthenium carbene, preventing it from reacting further. We thus esterified the secondary alcohol as the methacrylate **4a**, which in turn was submitted to RCM conditions. Varying catalysts and catalyst loadings (Grubbs I/II, Hoveyda–Grubbs I/II), solvents (dichloromethane, 1,2-dichloroethane, toluene), concentrations (0.1–5 mM), operating temperatures 40–110 °C), order of addition and reaction time, with or without N₂ sparging, led again either to recovery of unreacted starting material or to degradation and oligomerisation products. To ascertain that the methacrylate moiety is not responsible for this failure, secondary alcohol **24a** was silylated and silyl ether **26** submitted to the various RCM conditions to no avail.

Medium-sized rings are notoriously difficult to close under RCM conditions, especially when the RCM leads to a tri-substituted olefin from a terminal olefin and a 1,1-disubstituted olefin.^{42–44} We thus probed whether the failed RCM of **4a** resulted from the presence of the methyl group or the ring tension. To this end we used RCM precursor **4b**, which lacks the methyl group of interest (Scheme 5a). Gratifyingly, when **4b** was treated with Grubbs I in refluxing dichloromethane at 0.5 mM, nordeoxyelephantopin **1b** could be obtained albeit in low yield but as a single diastereomer. A detailed comparison of the relevant coupling constants and NOESY correlations of an authentic sample of **1a** and RCM product **1b** allowed us to confirm that **1b** has the same relative configuration and conformation in solution as **1a**. Most importantly, nordeoxyelephantopin **1b** proved to have comparable cytotoxicity to deoxyelephantopin **1a** against HeLa and MCF-7, suggesting that this methyl group does not contribute to important interactions. Finally, selective epoxidation of the unconjugated olefin in nordeoxyelephantopin **1b** using *meta*-chloroperbenzoic acid (*m*CPBA) afforded the norelephantopin **3b**.

It is noteworthy that only Grubbs I is competent to form the desired cyclisation product; under otherwise identical conditions, Grubbs II solely led to oligomerisation. All attempts to improve the outcome, including solvents, concentration, order of addition or temperature, either resulted in recovery of the starting material or oligomerisation and degradation products. The rationale for this observation is unclear, but the Grubbs I-mediated reaction with a phosphine scavenger (CuCl) as additive did not provide **1b**; it is thus tempting to conclude that free phosphine in the mixture is key to the reaction outcome. Indeed, ruthenium-based carbenes are Schrock carbenes with a double bond character with fixed configuration.⁴⁵ Besides their bulkiness, NHC ligands as in second-generation catalysts cannot be displaced by phosphines; however, for first-generation catalysts, phosphine exchange at ruthenium is possible and would thus lead to inversion of configuration at ruthenium to provide the RCM-competent and -productive carbene. Upon activation, pre-catalysts Hoveyda–Grubbs I⁴⁶ and Piers–Grubbs I⁴⁷ do not generate free phosphine in the reaction mixture, and under otherwise identical conditions as with Grubbs I, none of desired **1b** was formed, which is consistent with the phosphine-exchange hypothesis. Finally, applying the reaction conditions to precursor **4a** did unfortunately not provide **1a**.

As **4b** successfully ring closes into **1b**, while contributing to some extent, ring tension cannot account as the only reason for the failed



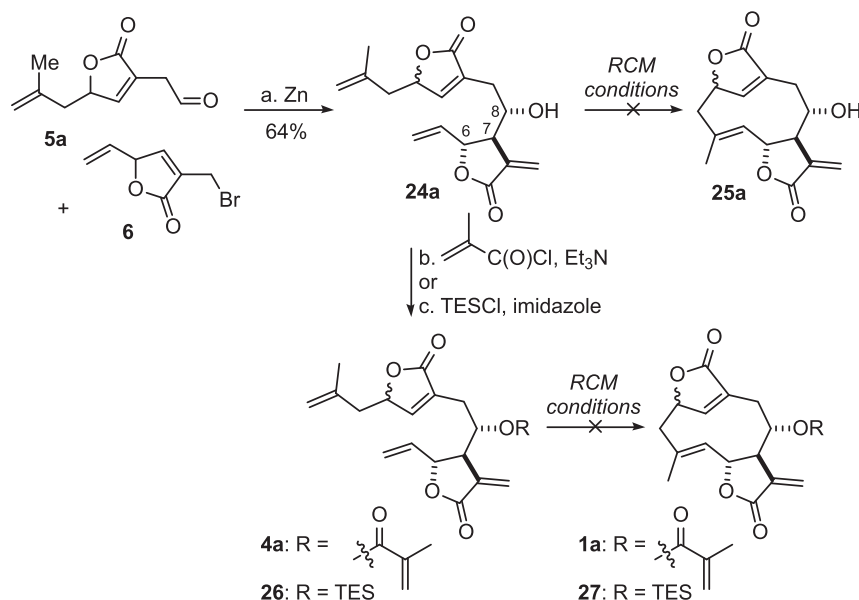
Scheme 3 (a) Synthesis of fragment **5**. (b) Attempted asymmetric synthesis of **18b** by Brown allylation. (c) Asymmetric synthesis of **18b**. Reagents and conditions: a. DMP, Et₂O, 0 °C then decantation of the solids; b. **13**, ⁿBuLi, Et₂O, -78 °C, then solution of **15** added via cannula; c. LiAlH₄, THF, reflux, then EtOAc, 0 °C, then I₂, 0 °C; d. Ni(CO)₂(PPh₃)₂, Et₃N, PhMe, reflux; e. FeCl₃·6H₂O, CH₂Cl₂, reflux; f. ⁿBuLi, THF, -78 °C then (HCHO)_x; g. MnO₂, CH₂Cl₂, rt; h. (-)-Ipc₂Ballyl, pentane, -20 °C to rt; i. LiAlH₄, THF, reflux, then EtOAc, 0 °C, then I₂, 0 °C; j. Ni(CO)₂(PPh₃)₂, Et₃N, PhMe, reflux; k. LiAlH₄, THF, reflux, then EtOAc, 0 °C, then I₂, 0 °C; l. CO, Pd(PPh₃)₄ (1 mol%), Et₃N, MeCN, reflux; m. NaHMDS, THF, -60 °C then Alloc-Cl; n. Pd(PPh₃)₄ (2 mol%), PhMe, rt (racemic) or Pd₂dba₃ (2.5 mol%), chiral ligand (6 mol%), NMP, -20 °C (enantioselective); o. 180 °C (microwave).

RCM of **4a** into **1a**. Directing group-free 1,1-disubstituted olefins are poor substrates for olefin metathesis (type III olefins).⁴⁸ Thus, as linear dimers and the benzylidene cross-metathesis products are detected, metathesis initiation must take place at the terminal mono-substituted olefin (type I olefin) in **4a** to generate ruthenium carbene **4a-Ru-1** (Scheme 6).

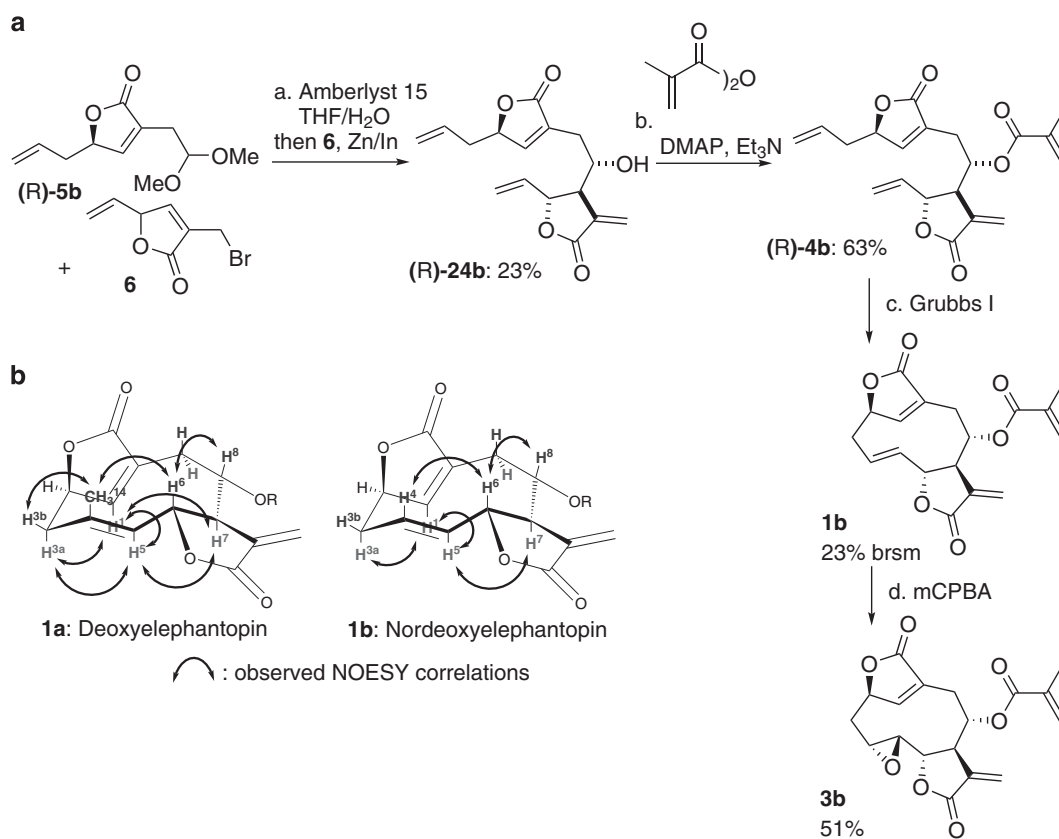
Although it is impossible to rule out that metathesis initiation in **4b** generates intermediate **4b-Ru-1**, it is tempting to consider that the productive carbene intermediate is rather **4b-Ru-2**. Relay RCM (RRCM) has proven valuable to overcome problems with recalcitrant RCM reactions with tri-substituted olefins.^{49–51} Briefly, a terminal allylic ether with enhanced reactivity (type I olefin) is attached to the olefin where one wants to form an otherwise difficult-to-generate ruthenium carbene. Upon initiation at the allyl ether in a substrate such as **28**, rapid RCM generates **29** along with the desired di-substituted ruthenium carbene **4a-Ru-2**, which in turn should achieve RCM.

We thus set out to prepare RRCM precursor **28** (Scheme 7). *In situ* vinylogous Mukaiyama addition of the silyl enolate of **30** with activated trimethyl orthoformate provided enoate **31**. DIBAL reduction of the ester and allylation of the resulting allylic alcohol provided ether **32** with the required RRCM handle in place. TMSOTf/2,6-lutidine-mediated acetal cleavage⁵² to the corresponding aldehyde and treatment with the lithium acetylide of **13** provided propargylic alcohol **33**. RRCM precursor **28** was then obtained as above and submitted to the RCM conditions. Disappointingly, when Grubbs I was used, oligomers were observed along with traces of macrocycle **37** (tentatively assigned based on mass spectrometric data). On the other hand, with Grubbs II as catalyst, **4a** was systematically and quantitatively obtained instead of desired **1a**.

The formation of oligomers suggests that Grubbs I was, in our hands, not competent to undergo RCM for the extrusion of **29** and concomitant generation of intermediate carbene **4a-Ru-2**. On the



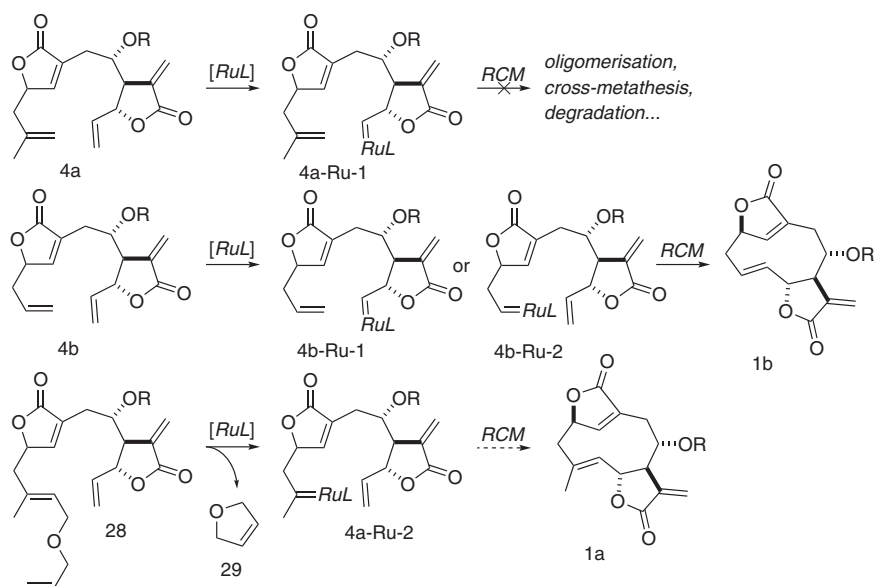
Scheme 4 Coupling of the fragments and RCM attempts. Reagents and conditions: a. Zn, THF, sat. aq. NH_4Cl , rt; b. methacryloyl chloride, DMAP, Et_3N , CH_2Cl_2 , 0°C ; c. TESCl, imidazole, CH_2Cl_2 , rt.



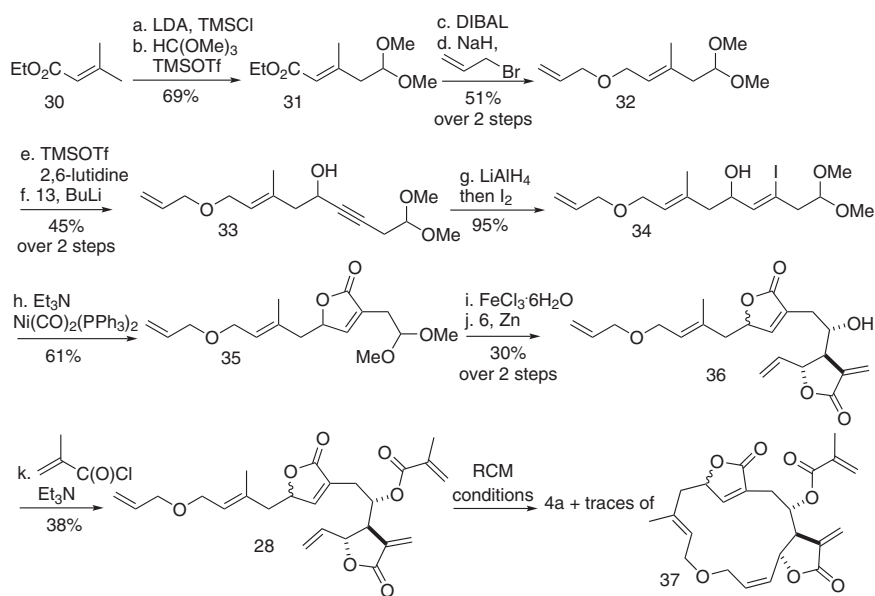
Scheme 5 (a) Successful RCM on nor substrate **4b**. Reagents and conditions: a. Zn, THF, sat. aq. NH_4Cl , rt; b. methacryloyl anhydride, DMAP, Et_3N , CH_2Cl_2 , 0°C ; c. Grubbs I (20 mol%), CH_2Cl_2 (0.5 mm), reflux; d. *m*CPBA, CH_2Cl_2 , rt. (b) Observed NOESY correlations for deoxyelephantopin (**1a**) and nordeoxyelephantopin (**1b**). A full colour version of this scheme is available at the Journal of Antibiotics journal online.

other hand, the formation of **4a** confirms that initiation with Grubbs II took place at the RRCM handle; however, once formed, carbene **4a-Ru-2** does not undergo RCM to close the medium-sized ring, but propagates by cross-metathesis with remaining **28** in a head-to-tail

manner, potentially due to steric hindrance imposed by the di-substitution of the carbene. Furthermore, the trace formation of **37** must be due to initiation at the other olefin and concomitant RCM at the allylic ether.



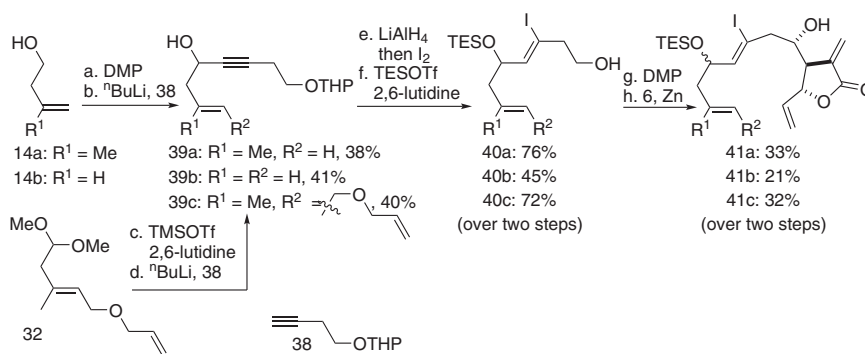
Scheme 6 Proposed ruthenium carbene intermediates for **4a** and **4b**, and rationale for RRCM.



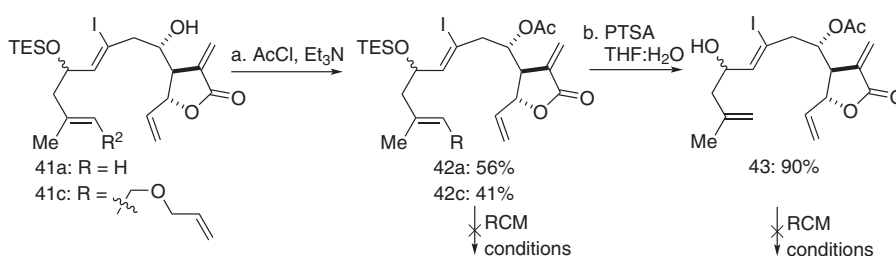
Scheme 7 Synthesis of substrate **28** and RRCM attempts. Reagents and conditions: a. LDA, TMSCl, Et₂O, -78 °C, then filtration; b. HC(OMe)₃, TMSOTf, CH₂Cl₂, -78 °C; c. DIBAL, PhMe, 0 °C; d. allyl bromide, NaH, THF, 0 °C; e. TMSOTf, 2,6-lutidine, CH₂Cl₂, 0 °C; f. **13**, ⁿBuLi, Et₂O, -78 °C; g. LiAlH₄, THF, reflux, then EtOAc, 0 °C, then I₂, 0 °C; h. Ni(CO)₂(PPh₃)₂, Et₃N, PhMe, reflux; i. FeCl₃·6H₂O, CH₂Cl₂, reflux; j. **6**, Zn, THF, sat. aq. NH₄Cl, rt; k. methacryloyl chloride, DMAP, Et₃N, CH₂Cl₂, 0 °C.

In the RCM of **4b** into **1b**, the formation of a single diastereomer suggests that the *endo*-butenolide fragment has a non-negligible impact on the RCM outcome. Its *endo* olefin and γ -stereocentre may indeed impose too much conformational strain and negatively impact the pre-organisation of the intermediate carbene to achieve RCM. We thus envisioned that postponing the carbonylation step to a later stage of the synthesis, that is, after the RCM step, would allow us to carry out the RCM reaction on a substrate with higher conformational freedom for a potentially better outcome. Not surprisingly, this is nature's strategy for the biosynthesis of sesquiterpenes: a flexible carbon skeleton is indeed cyclised before decoration by means of multiple sequential oxidations.

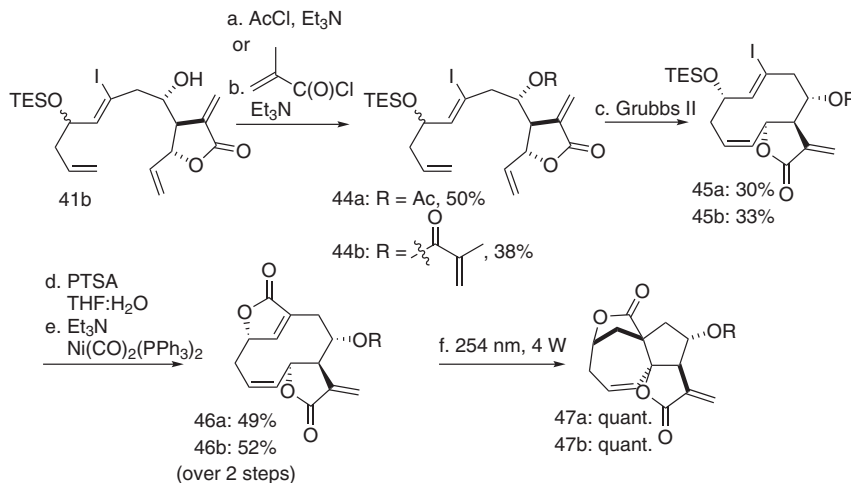
With this in mind, we set out to prepare a more flexible fragment bearing the iodoallylic alcohol for late-stage carbonylation. However, upon acetal cleavage of **17** under a variety of conditions, the product suffered extensive olefin migration to the α,β -unsaturated aldehyde, which forced us to slightly modify our oxidation state/protecting group strategy. As shown in Scheme 8, we thus opted for the tetrahydropyranyl (THP) protecting group and used lithiated acetylene **38** to obtain intermediates **39a–c**. Hydroalumination followed by iodine treatment provided iodoallylic alcohol, which was subsequently masked as triethylsilyl (TES) ether with concomitant cleavage of the THP acetal (**40a–c**). DMP oxidation of the alcohol provided the aldehyde substrate to the Barbier coupling with **6**, leading to secondary alcohol **41a–c**.



Scheme 8 Synthesis of the northern part and Barbier coupling. Reagents and conditions: a. DMP, Et₂O, 0 °C then decantation of the solids; b. **38**, ^tBuLi, Et₂O, -78 °C, then solution of **14** added via cannula; c. TMSOTf, 2,6-lutidine, CH₂Cl₂, 0 °C; d. **38**, ^tBuLi, Et₂O, -78 °C; e. LiAlH₄, THF, reflux, then EtOAc, 0 °C, then I₂, 0 °C; f. TESOTf, 2,6-lutidine, CH₂Cl₂, 0 °C; g. DMP, CH₂Cl₂, 0 °C; h. Zn, THF, sat. aq. NH₄Cl, rt.



Scheme 9 Preparation of the substrates towards deoxyelephantopin and RCM attempts. Reagents and conditions: a. AcCl, Et₃N, CH₂Cl₂, 0 °C; b. PTSA, THF/H₂O, rt.

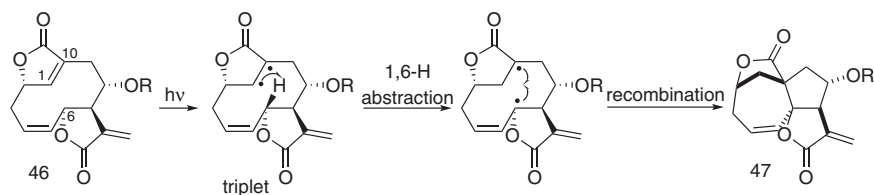


Scheme 10 Preparation of the substrates and RCM attempts. Reagents and conditions: a. AcCl, Et₃N, CH₂Cl₂, 0 °C; b. methacryloyl chloride, DMAP, Et₃N, CH₂Cl₂, 0 °C; c. Grubbs II (30 mol%), CH₂Cl₂ (0.5 mm), reflux; d. PTSA, THF/H₂O, rt; e. Ni(CO)₂(PPh₃)₂, Et₃N, PhMe, reflux; f. h ν (254 nm, 4W), CD₃CN, rt.

In order to probe the RCM step, secondary alcohol **41a** was in the first pass converted into acetate **42a**, which was in turn submitted to various RCM conditions (Scheme 9). It thus appears that releasing ring-strain seemed to have no beneficial effect and none of the desired RCM product was obtained, only leading to oligomerisation. Considering that the TES group in **42a** is somewhat bulky and that a free hydroxyl may have allowed initiation at the more substituted olefin, we deprotected it and submitted **43** to the RCM conditions to no avail. Similarly, RRCM substrate **42c** did not allow formation of the desired RCM product under a variety of conditions, leading as

above either to the quantitative formation of **42a** or degradation and isomerisation.

Disheartened by these results, we examined whether the RCM outcome is improved with respect to the RCM reaction of **4b**. Thus, substrate **41b** was converted to acetate **44a** and submitted to RCM (Scheme 10). Gratifyingly, RCM proceeded more smoothly and took place even in the presence of Grubbs II to yield **45a** obtained as a single diastereomer. Surprisingly, the olefin obtained was found to be *Z* as confirmed by the coupling constant between the olefinic protons (³J = 10.4 Hz for **45a** vs ³J = 16.1 Hz for **1b**). In order to establish



Scheme 11 Proposed mechanism for the light-induced transformation.

the relative configuration at C-2, the TES ether was removed and carbonylation provided the northern butenolide in **46a**. Much to our surprise, NOESY experiments revealed that the relative configuration at C-2 was consistent with that of isodeoxyelephantopin (**2**). The bulkiness of the TES group may explain that in one diastereomer the TES group forces the olefin in close proximity allowing the cyclisation to take place, whereas in the other the olefins are pushed away from each other leading to an unproductive carbene. The recovered starting material enriched in the unreacted diastereomer was submitted to slightly harsher reaction conditions but no RCM product was obtained.

Replacing the acetate for a methacrylate did not affect the RCM outcome and **46b** was obtained as a single diastereomer consistent with isodeoxyelephantopin and with a *Z* olefin. We thus probed the light-induced *cis/trans* isomerisation of the olefin in order to access the *nor* isomer of isodeoxyelephantopin. To this end, compounds **46** were dissolved in CD₃CN and irradiated at 254 nm, and the advancement monitored by ¹H NMR. Unexpectedly, instead of the desired isomerisation, transannular radical cyclisation took place leading very cleanly to unprecedented tetracyclic compounds **47**. From a mechanistic point of view (Scheme 11), UV irradiation activates the C1–C10 double bond as the diradical; the C1-centred radical abstracts a hydrogen atom across the ring in a 1,6-fashion to generate a C6-centred radical, which in turn recombines with the C10-centred radical. This process is highly favoured owing to the proximity of C6 to the C1–C10 double bond, and the weakness of the C6–H bond, stabilised both by the neighbouring olefin and oxygen atom.

CONCLUSION

In summary, the chemistry presented allowed rapid access to nordeoxyelephantopin, norelephantopin and isonordeoxyelephantopin analogues but, despite our efforts, synthetic access to deoxyelephantopin remains elusive. The short and divergent synthetic design, taking advantage of a Barbier allylation and a subsequent RCM, delivered novel analogues in an interesting chemical diversity space. It is noteworthy that analogue **46b** proved to be a better PPAR γ ligand than deoxyelephantopin. The Barbier allylation with intermediate **6** represents an important strategy to access sesquiterpene lactones natural products containing an α -*exo*-methylene- γ -butyrolactone, a moiety present in over 3000 natural products. The chemical orthogonality and ease of practical implementation of the RCM make it a very attractive carbon-carbon bond formation reaction in synthetic route design. Although it has been successfully used for the formation of a number of medium-sized rings, the outcome of the reaction remains however uncertain and is highly substrate-dependent, especially for the formation of tri-substituted olefins. To overcome these reactivity issues, the relay RCM have been developed and successfully implemented in a number of examples but was not applicable to deoxyelephantopin. We hope that the hindsight acquired during our journey towards deoxyelephantopin will help other synthetic chemists in carefully designing their synthetic routes not only towards

germacranes and sesquiterpene lactones but also other challenging natural products, and in being successful in their endeavours.

DEDICATION

This manuscript is dedicated to Professor KC Nicolaou, his tremendous achievements in the area of total synthesis and his dedication to chemical education.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the Swiss National Science Foundation for generous support.

- 1 Pye, C. R., Bertin, M. J., Lokey, R. S., Gerwick, W. H. & Lington, R. G. Retrospective analysis of natural products provides insights for future discovery trends. *Proc. Natl. Acad. Sci. USA* **114**, 5601–5606 (2017).
- 2 Rodrigues, T., Reker, D., Schneider, P. & Schneider, G. Counting on natural products for drug design. *Nat. Chem.* **8**, 531–541 (2016).
- 3 Nicolaou, K. C. & Montagnon, T. *Molecules That Changed the World* (Wiley-VCH, 2008), ISBN 3527309837, 9783527309832.
- 4 Nicolaou, K. C., Vourloumis, D., Winssinger, N. & Baran, P. S. The art and science of total synthesis at the dawn of the twenty-first century. *Angew. Chem. Int. Ed.* **39**, 44–122 (2000).
- 5 Nicolaou, K. C., Hale, C. R. H., Nilewski, C. & Ioannidou, H. A. Constructing molecular complexity and diversity: total synthesis of natural products of biological and medicinal importance. *Chem. Soc. Rev.* **41**, 5185–5238 (2012).
- 6 Nicolaou, K. C. & Snyder, S. A. Chasing molecules that were never there: misassigned natural products and the role of chemical synthesis in modern structure elucidation. *Angew. Chem. Int. Ed.* **44**, 1012–1044 (2005).
- 7 Drahl, C., Cravatt, B. F. & Sorensen, E. J. Protein-reactive natural products. *Angew. Chem. Int. Ed.* **44**, 5788–5809 (2005).
- 8 Potashman, M. H. & Duggan, M. E. Covalent modifiers: an orthogonal approach to drug design. *J. Med. Chem.* **52**, 1231–1246 (2009).
- 9 Baillie, T. A. Targeted covalent inhibitors for drug design. *Angew. Chem. Int. Ed.* **55**, 13408–13421 (2016).
- 10 Dakas, P. Y., Barluenga, S., Totzke, F., Zirgibel, U. & Winssinger, N. Modular synthesis of radicolic A and related resorcylic acid lactones, potent kinase inhibitors. *Angew. Chem. Int. Ed.* **46**, 6899–6902 (2007).
- 11 Dakas, P.-Y., Jogireddy, R., Valot, G., Barluenga, S. & Winssinger, N. Divergent syntheses of resorcylic acid lactones: L-783277, LL-Z1640-2, and hypothemycin. *Chem. Eur. J.* **15**, 11490–11497 (2009).
- 12 Jogireddy, R., Dakas, P.-Y., Valot, G., Barluenga, S. & Winssinger, N. Synthesis of a resorcylic acid lactone (RAL) library using fluorour-mixture synthesis and profile of its selectivity against a panel of kinases. *Chem. Eur. J.* **15**, 11498–11506 (2009).
- 13 Barluenga, S., Jogireddy, R., Koripelly, G. K. & Winssinger, N. *In vivo* efficacy of natural product-inspired irreversible kinase inhibitors. *ChemBioChem* **11**, 1692–1699 (2010).
- 14 Leproult, E., Barluenga, S., Moras, D., Wurtz, J.-M. & Winssinger, N. Cysteine mapping in conformationally distinct kinase nucleotide binding sites: application to the design of selective covalent inhibitors. *J. Med. Chem.* **54**, 1347–1355 (2011).
- 15 Duplan, V. *et al.* Synthesis of sesquiterpene-inspired derivatives designed for covalent binding and their inhibition of the NF-kappa B pathway. *Org. Biomol. Chem.* **12**, 370–375 (2014).
- 16 Daguer, J. P. *et al.* Identification of covalent bromodomain binders through DNA display of small molecules. *Angew. Chem. Int. Ed.* **54**, 6057–6061 (2015).
- 17 Merfort, I. Perspectives on sesquiterpene lactones in inflammation and cancer. *Curr. Drug Targets* **12**, 1560–1573 (2011).
- 18 Ren, Y. L., Yu, J. H. & Kinghorn, A. D. Development of anticancer agents from plant-derived sesquiterpene lactones. *Curr. Med. Chem.* **23**, 2397–2420 (2016).
- 19 Kitson, R. R. A., Millemaggi, A. & Taylor, R. J. K. The renaissance of alpha-methylene-gamma-butyrolactones: new synthetic approaches. *Angew. Chem. Int. Ed.* **48**, 9426–9451 (2009).

- 20 Grieco, P. A. & Nishizawa, M. Total synthesis of (+)-costunolide. *J. Org. Chem.* **42**, 1717–1720 (1977).
- 21 Wender, P. A. & Lechleiter, J. C. Total synthesis of (+/-) isabelin. *J. Am. Chem. Soc.* **102**, 6340–6341 (1980).
- 22 Marshall, J. A., Lebreton, J., Dehoff, B. S. & Jenson, T. M. Stereoselective total synthesis of (+/-)-aristolactone and (+/-)-epiaristolactone via [2,3] Wittig ring contraction. *J. Org. Chem.* **52**, 3883–3889 (1987).
- 23 Takahashi, T., Nemoto, H., Kanda, Y. & Tsuji, J. Macroring contraction methodology .2. total synthesis of haagenolide by [2,3]-wittig rearrangement of 13-membered diallylic ether. *Heterocycles* **25**, 139–144 (1987).
- 24 Foo, K. *et al.* Scalable, enantioselective synthesis of germacrenes and related sesquiterpenes inspired by terpene cyclase phase logic. *Angew. Chem. Int. Ed.* **51**, 11491–11495 (2012).
- 25 Long, J. *et al.* Total syntheses of parthenolide and its analogues with macrocyclic stereocontrol. *J. Med. Chem.* **57**, 7098–7112 (2014).
- 26 Su, M., Wu, X., Chung, H. Y., Li, Y. & Ye, W. Antiproliferative activities of five Chinese medicinal herbs and active compounds in *Elephantopus scaber*. *Nat. Prod. Commun.* **4**, 1025–1030 (2009).
- 27 Huang, C.-C., Lo, C.-P., Chiu, C.-Y. & Shyur, L.-F. Deoxyelephantopin, a novel multifunctional agent, suppresses mammary tumour growth and lung metastasis and doubles survival time in mice. *Br. J. Pharmacol.* **159**, 856–871 (2010).
- 28 Lee, W.-L., Wen, T.-N., Shiau, J.-Y. & Shyur, L.-F. Differential proteomic profiling identifies novel molecular targets of paclitaxel and phytoagent deoxyelephantopin against mammary adenocarcinoma cells. *J. Proteome Res.* **9**, 237–253 (2010).
- 29 Ichikawa, H. *et al.* Isodeoxyelephantopin, a novel sesquiterpene lactone, potentiates apoptosis, inhibits invasion, and abolishes osteoclastogenesis through suppression of nuclear factor-kappa B (NF-kappa B) activation and NF-kappa B-regulated gene expression. *Clin. Cancer Res.* **12**, 5910–5918 (2006).
- 30 Zou, G. *et al.* Deoxyelephantopin inhibits cancer cell proliferation and functions as a selective partial agonist against PPAR gamma. *Biochem. Pharmacol.* **75**, 1381–1392 (2008).
- 31 Bae, H. *et al.* Mechanistic elucidation guided by covalent inhibitors for the development of anti-diabetic PPAR gamma ligands. *Chem. Sci.* **7**, 5523–5529 (2016).
- 32 Itoh, T. *et al.* Structural basis for the activation of PPAR gamma by oxidized fatty acids. *Nat. Struct. Mol. Biol.* **15**, 924–931 (2008).
- 33 Lagoutte, R. *et al.* Divergent synthesis and identification of the cellular targets of deoxyelephantopins. *Nat. Commun.* **7**, 12470 (2016).
- 34 Trnka, T. M. & Grubbs, R. H. The development of L2X2Ru=CHR olefin metathesis catalysts: An organometallic success story. *Acc. Chem. Res.* **34**, 18–29 (2001).
- 35 Semmelhack, M. F. & Brickner, S. J. Intramolecular carbonylation of vinyl halides. *J. Org. Chem.* **46**, 1723–1726 (1981).
- 36 Sen, S. E., Roach, S. L., Boggs, J. K., Ewing, G. J. & Magrath, J. Ferric chloride hexahydrate: a mild hydrolytic agent for the deprotection of acetals. *J. Org. Chem.* **62**, 6684–6686 (1997).
- 37 Brown, H. C., Bhat, K. S. & Randad, R. S. B-allyldiisopinocampheylborane - a remarkable reagent for the diastereoselective allylboration of alpha-substituted chiral aldehydes. *J. Org. Chem.* **52**, 319–320 (1987).
- 38 Fournier, J., Arseniyadis, S. & Cossy, J. A modular and scalable one-pot synthesis of polysubstituted furans. *Angew. Chem. Int. Ed.* **51**, 7562–7566 (2012).
- 39 Fournier, J., Lozano, O., Menozzi, C., Arseniyadis, S. & Cossy, J. Palladium-catalyzed asymmetric allylic alkylation of cyclic dienol carbonates: efficient route to enantioenriched gamma-butenolides bearing an all-carbon alpha-quaternary stereogenic center. *Angew. Chem. Int. Ed.* **52**, 1257–1261 (2013).
- 40 Trost, B. M., Xu, J. Y. & Schmidt, T. Palladium-catalyzed decarboxylative asymmetric allylic alkylation of enol carbonates. *J. Am. Chem. Soc.* **131**, 18343–18357 (2009).
- 41 Gao, Y. Z., Wang, X., Sun, L. D., Xie, L. G. & Xu, X. H. Zinc or indium-mediated Barbier-type allylation of aldehydes with 3-bromomethyl-5H-furan-2-one in aqueous media: an efficient synthesis method for alpha-methylene-gamma-butyrolactone. *Org. Biomol. Chem.* **10**, 3991–3998 (2012).
- 42 Chany, A. C. *et al.* A diverted total synthesis of mycolactone analogues: an insight into buruli ulcer toxins. *Chem. Eur. J.* **17**, 14413–14419 (2011).
- 43 Fürstner, A. & Müller, T. The first synthesis of a 10-membered ring by olefin metathesis: Jasmine ketolactone. *Synlett* **8**, 1010–1012 (1997).
- 44 Nicolaou, K. C., Bulger, P. G. & Sarlah, D. Metathesis reactions in total synthesis. *Angew. Chem. Int. Ed.* **44**, 4490–4527 (2005).
- 45 Occhipinti, G. & Jensen, V. R. Nature of the transition metal-carbene bond in Grubbs olefin metathesis catalysts. *Organometallics* **30**, 3522–3529 (2011).
- 46 Kingsbury, J. S., Harrity, J. P. A., Bonitatebus, P. J. & Hoveyda, A. H. A recyclable Ru-based metathesis catalyst. *J. Am. Chem. Soc.* **121**, 791–799 (1999).
- 47 Romero, P. E., Piers, W. E. & McDonald, R. Rapidly initiating ruthenium olefin-metathesis catalysts. *Angew. Chem. Int. Ed.* **43**, 6161–6165 (2004).
- 48 Chatterjee, A. K., Choi, T. L., Sanders, D. P. & Grubbs, R. H. A general model for selectivity in olefin cross metathesis. *J. Am. Chem. Soc.* **125**, 11360–11370 (2003).
- 49 Hoye, T. R., Jeffrey, C. S., Tennakoon, M. A., Wang, J. Z. & Zhao, H. Y. Relay ring-closing metathesis (RRCM): a strategy for directing metal movement throughout olefin metathesis sequences. *J. Am. Chem. Soc.* **126**, 10210–10211 (2004).
- 50 Hoye, T. R., Danielson, M. E., May, A. E. & Zhao, H. Y. Total synthesis of (-)-callipeltoside A. *J. Org. Chem.* **75**, 7052–7060 (2010).
- 51 Schulte, M. L. *et al.* Total synthesis of stemaphylline N-oxide and related C9a-epimeric analogues. *Chem. Eur. J.* **19**, 11847–11852 (2013).
- 52 Fujioka, H. *et al.* Unexpected highly chemoselective deprotection of the acetals from aldehydes and not ketones: TESOTf-2,6-lutidine combination. *J. Am. Chem. Soc.* **126**, 11800–11801 (2004).

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