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.
Synthesis of Deoxyelephantopin Analogues

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ABSTRACT

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 germacrane skeleton harbours three Michael acceptors that can potentially engage a target covalently. Its strained 10-membered ring is densely functionalized and represents an important synthetic challenge. We herein describe our studies towards deoxyelephantopins using a ring-closing metathesis approach and report some unexpected observations.

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.1,2 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.3 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 profile4 and validate or correct their structural assignment.5 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.6 The preponderance of such reactive groups amongst secondary metabolites suggests that evolution has selected for this inhibition modality.7 On this basis, we have pursued the synthesis and discovery of covalent inhibitors8-10 and were drawn to sesquiterpene lactones which often bear an α:α'-methylene-β:β'-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.11-13 Examples include guaianolides thapsigargin, arglabin, and helenalin or germacranolides parthenolide, eupatolide and cnicin, which selectively act on diverse target classes. The germacrane skeletons are a broad sub-family of sesquiterpene lactones with a germacrane skeleton. The germacrane have received attention from the synthetic community, but there is to date no general approach to germacrane-based sesquiterpene lactones.14 Indeed, most methodologies allowing access to the germacrane skeleton either have low functional group tolerance or demand complete re-functionalisation of the carbon framework.15-20

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).21 From a synthetic point of view, deoxyelephantopin is a rather challenging structure owing to its highly strained 10-membered ring flanked with two tri-substituted olefins and four stereocenters, three 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.22 Mechanistic studies suggested that deoxyelephantopin suppresses proteasome activity23 while isodeoxyelephantopin inhibits the NF-κB pathway.24 Deoxyelephantopin was also found to be a partial PPARγ agonist.25 Based on the fact that covalent ligand of PPARγ have been reported,26,27 we anticipated a covalent engagement paralleling previous covalent ligand of PPARγ.28
Figure 1 (A) Selected bioactive sesquiterpene lactones known to covalently engage cysteine residues; (B) Selected naturally occurring elephantopins.

As no synthesis of deoxyelephantopin had been reported and because modifying its densely functionalized 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 in order 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).

Scheme 1 Retrosynthetic analysis of deoxyelephantopin.

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 eleven new covalent cellular targets but also to establish that deoxyelephantopin indeed covalently engages PPARγ but at cysteine 190 in the zinc-finger domain rather than in the ligand-
binding domain as anticipated. 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 II

\[ \text{Grubbs II} \] as catalyst to afford endobutenolide 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).

![Scheme 2](image)

We then examined the synthesis of the second fragment 5. As shown in scheme 3A, aldehyde 15a was readily obtained by DMP oxidation of homoallylic alcohol 14a in Et₂O and directly transferred to cooled solution of the lithium acetylide of 13. Isolation of aldehyde 15 was avoided due to its propensity to isomerize to the α,β-conjugated system. Trans-hydroalumination of the resulting propargylic alcohol 16a using LiAlH₄ and treatment with elemental iodine afforded vinyl iodide 17a with perfect regioselectivity. Nickel-mediated carbonylation of 17a afforded the butenolide 18a. Acetal hydrolysis with iron(III) chloride hexahydrate 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 isomerization 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 allylation\textsuperscript{37} of aldehyde 20, readily obtained by \textit{MnO}_2-mediated oxidation of propargylic alcohol 19. The alkylation proceeded with high enantioselectivity albeit in modest yield. While, the trans-hydroalumination/iodination transformation of (R)-16b was uneventful, partial epimerisation occurred during the subsequent nickel-mediated carbylation step, presumably due to deprotonation at the y-position by triethylamine under the reaction conditions to form a prochiral dienolate, which racemizes 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).\textsuperscript{38,39} Briefly, trans-hydroalumination/iodination of propargylic alcohol 19 followed by Pd-catalysed carbylation 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(PPh\textsubscript{3})\textsubscript{4} or Pd\textsubscript{2}dba\textsubscript{2} in combination with either (S,S)- or (R,R)-DACH Phenyl Trost ligands, to access respectively rac-18b, (R)-18b or (S)-18b.\textsuperscript{40} It is noteworthy that the reaction proceeds via \(\text{[\text{B]}\text{-allylation of the dienolate to form}\) \(\text{butenolide 23, which is directly submitted to microwave irradianations to undergo [3,3]-Cope rearrangement and provide 18b.}\) With fragments 5a and 6 in hand, the zinc-mediated Barbier-type alkylation provided secondary alcohol 24a in good yield and high diastereoselectivity, as anticipated\textsuperscript{41} (Scheme 4, the relative stereochemical assignment is extrapolated from the closely related reaction yielding 24b, Scheme 5, where cyclization 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 the intermediate ruthenium carbene, preventing it from reacting further. We thus esterified the secondary alcohol as the methacyclate 4a, which in turn was submitted to RCM conditions. Varying catalysts and catalyst loadings (Grubbs I/II, Hoveyda-Grubbs I/III), 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 N\textsubscript{2} sparging, led again either to recovery of unreacted starting material or to degradation and oligomerisation products. To ascertain that the methacyclate 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.\textsuperscript{42-44} We thus prober 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 (schemes 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 the methyl group does not contribute to important interactions. Finally, selective epoxidation of the unconjugated olefin in nordeoxyelephantopin 1b using mCPBA 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 degradtion 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.\textsuperscript{45} Besides their bulkiness, NHC ligands as in 2\textsuperscript{46} generation catalysts cannot be displaced by phosphines; however, for 1\textsuperscript{st} 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\textsuperscript{46} and Piers-Grubbs\textsuperscript{47} 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.
Scheme 3  (A) Synthesis of the fragment 5; (B) Attempted asymmetric synthesis of 18b by Brown allylation; (C) Asymmetric synthesis of by asymmetric allylic alkylation. Reagents and conditions: a. DMP, Et$_2$O, 0 °C then decantation of the solids; b. 13, _n_ BuLi, Et$_2$O, -78 °C, then solution of 15 added via cannula; c. LiAlH$_4$, THF, reflux, then EtOAc, 0 °C, then _I_$_2$, 0 °C; d. Ni(CO)$_2$(PPh$_3$)$_2$, Et$_3$N, PhMe, reflux; e. FeCl$_3$, 6H$_2$O, reflux; f. _n_ BuLi, (HCHO)$_x$, then LiAlH$_4$ then _I_$_2$; g. MnO$_2$, pentane, -20 °C to rt; h. (-)-Ipc$_2$Ballyl, pentane, -20 °C to rt; i. LiAlH$_4$, THF, reflux, then EtOAc, 0 °C, then _I_$_2$, 0 °C; j. Ni(CO)$_2$(PPh$_3$)$_2$, Et$_3$N, PhMe, reflux; k. LiAlH$_4$, THF, reflux, then EtOAc, 0 °C, then _I_$_2$, 0 °C; l. CO, Et$_3$N, Pd(PPh$_3$)$_4$; m. NaHMDS, THF, -60 °C then AllocCl; n. Pd(PPh$_3$)$_4$ (2 mol%), PhMe, rt (racemic) or Pd$_2$dba$_3$ (2.5 mol%), chiral ligand (6 mol%), NMP, -20 °C (enantioselective); o. 180 °C (microwave).
Scheme 4 Coupling of the fragments and RCM attempts. Reagents and conditions: a. Zn, THF, sat. aq. NH₄Cl, rt; b. methacryloyl chloride, DMAP, Et₃N, CH₂Cl₂, 0 °C; c. TESCl, imidazole, CH₂Cl₂, rt.

Scheme 5 (A) Successful RCM on nor substrate 4b. Reagents and conditions: a. Amberlyst 15 then 6, Zn/In; b. DMAP, Et₃N; c. Grubbs I (20 mol%), CH₂Cl₂ [0.5 mM], reflux; d. mCPBA, CH₂Cl₂, rt; (B) Observed NOESY correlations for deoxyelephantopin (1a) and nordeoxyelephantopin (1b).
Since 4b successfully ring-closes into 1b, while contributing to some extent, ring tension cannot account as the only reason for the failed RCM of 4a into 1a. Directing group-free 1,1-disubstituted olefins are poor substrates for olefin metathesis (type III olefins). 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).

While 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 ring-closing metathesis (RRCM) has proven valuable to overcome problems with recalcitrant RCM reactions with tri-substituted olefins. 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 cleavage to the corresponding aldehyde and treatment with the lithium acetylide of 13 provided propargylic alcohol 33. RCM 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 spectroscopy 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 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 fashion, potentially due to steric

Scheme 6 Proposed ruthenium carbene intermediates for 4a and 4b, and rationale for RRCM.
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 7 Synthesis of substrate 28 and RCM attempts. Reagents and conditions: a. LDA, TMSCl, Et₂O, -78 °C, then filtration; b. HC(O)Me₂, 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, BuLi, 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, THF, 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 Z-sterocentre 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, i.e. 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 prior to 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 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 its 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.
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 ($^2J = 10.4$ Hz for 45a vs $^2J = 16.1$ Hz for 1b). In order to establish 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 takes place, while 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 CD$_2$CN and irradiated at 254 nm, and the advancement monitored by $^1$H NMR. Unexpectedly, instead of the desired isomerisation, transannular radical cyclisation took place leading very cleanly to unprecedented tetracyclic and the advancement monitored by $^1$H 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-centered radical abstracts a hydrogen atom across the ring in a 1,6-fashion to generate a C6-centered radical, which in turn recombines with the C10-centered 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.

![Scheme 10](image)

**Scheme 10** Preparation of the substrates and RCM attempts. Reagents and conditions: a. AcCl, Et$_3$N, CH$_2$Cl$_2$, 0 °C; b. methacryloyl chloride, DMAP, Et$_3$N, CH$_2$Cl$_2$, 0 °C; c. Grubbs II (30 mol%), CH$_2$Cl$_2$ [0.5 mM], reflux; d. PTSA, THF/H$_2$O, rt; e. Ni(CO)$_2$(PPh$_3$)$_2$, Et$_3$N, PhMe, reflux; f. h$_0$ (254 nm, 4W), CD$_2$CN, rt.

![Scheme 11](image)

**Scheme 11** Proposed mechanism for the light-induced transformation

**CONCLUSION**

In summary, the chemistry presented allowed rapid access to nordeoxyelephantopin, norelephantopin and isonordeoxyelephantopin analogs 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 ring-closing metathesis, delivered novel analogues in an interesting chemical diversity space. It is noteworthy that analogue 46b proved to be a better PPARγ ligand than deoxyelephantopin. The Barbier allylation with intermediate 6 represents an important strategy to access sesquiterpene lactones natural products containing an $\beta$-exo-methylene-$\beta$-butyrolactone, a moiety present in over 3000 natural products. The chemical orthogonality and ease of practical implementation of the ring-closing metathesis make it a very attractive carbon-carbon...
bond formation reaction in synthetic route design. While 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 ring-closing metathesis 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 germacrane and sesquiterpene lactones but also other challenging natural products, and in being successful in their endeavours.

ACKNOWLEDGEMENTS

We thank the Swiss National Science Foundation for generous support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DEDICATION

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

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