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Total Synthesis and Structure-Activity Relationship Study of the Potent cAMP Signaling Agonist (-)-Alotaketol A

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Abstract

A detailed account of the first total synthesis of alotaketol A, a tricyclic spiroketal sesterterpenoid that potently activates the cAMP signaling pathway, is provided. The synthesis employs both intra- and intermolecular reductive allylation of esters for assembling one of the fragments and their coupling. A Hg(OAc)₂-mediated allylic mercuration is used to introduce the C22-hydroxyl group. The subtle influence of substituents over the course of the spiroketalization process is revealed. The synthesis confirms the relative and absolute stereochemistry of (-)-alotaketol A and allows verification of alotaketol A's effect over cAMP signaling using reporter-based FRET imaging assays with HEK 293T cells. Our studies also revealed alotaketol A's unique activity in selectively targeting nuclear PKA signaling in living cells.

1. Introduction

Nature provides a rich repertoire of small molecules with useful biological properties. Many of these small-molecules target and regulate disease-relevant cellular signaling pathways/processes and find application in drug development for treating human diseases.¹ Indeed, half of clinical anti-cancer drugs are derived from natural products, i.e. they are either analogs of natural products or natural products themselves.² Bioactive natural products that selectively target biological pathways and processes are also used as probes to gain insights of complex biological systems.³ This so-called “small-molecule approach” was instrumental in study of cellular signaling events, including the cellular cyclic adenosine monophosphate (cAMP) signaling pathway.⁴ The activation of this pathway is initiated with hormone binding to cell-surface G protein-coupled receptors (GPCRs), which leads to activation of trimeric guanine-nucleotide binding proteins (G proteins) and subsequent activation of adenylyl cyclases (ACs), the enzyme responsible for converting adenosine triphosphate (ATP) to cAMP. This “second messenger” in turn binds to its downstream effectors, such as cAMP dependent protein kinase (PKA) and exchange proteins activated by cAMP (Epac).⁵ Production of cAMP by ACs is countered by phosphodiesterases (PDEs), which hydrolyze cAMP to give adenosine monophosphate (AMP). Thus, ACs and PDEs collectively determine cellular cAMP levels. In addition to using agonists and antagonists of GPCRs, cAMP signaling also can be pharmacologically regulated using modulators of ACs and PDEs. For example, ACs are activated by the diterpenoid natural product forskolin (1, Scheme 1), which interacts with ACs at the hydrophobic site created by the C1 and C2

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catalytic subunits and activates their enzymatic activity for generating cAMP.⁶ Inhibition of cAMP-specific PDEs by their small-molecule inhibitors also leads to upregulation of cellular cAMP levels. Since cAMP signaling is relevant to a number of disease states, such as heart failure, cancer, and neurodegenerative diseases, development of new modulators of this signaling pathway is therapeutically relevant.⁷

Alotaketals A (**2**) and B (**3**) belong to a new class of terpenoids isolated by Andersen and co-workers from the marine sponge *Hamigera* sp. collected in Papua New Guinea (Figure 1).⁸ These natural products feature an “alotane” sesterterpenoid molecular skeleton that cyclizes into a unique tricyclic spiroketal ring system in which the spiroketal center was simultaneously substituted with a vinyl group and an allyl group. To the best of our knowledge, similarly substituted spiroketals are unprecedented in natural products. Along with their unique molecular structures, these compounds also possess interesting biological activities. For example, using HEK293 cells transformed with pHTS-CRE luciferase reporter genes, alotaketals A and B were found to potently activate the cAMP signaling pathway with EC₅₀ values of 18 nM and 240 nM in the absence of hormone binding. Forskolin also activated cAMP signaling in this reporter gene assay with an EC₅₀ value (3 μM) that is 167-fold less potent than that of alotaketals A. On the other hand, forskolin elicited a stronger response in the reporter gene assay, suggesting that different mode-of-action might be involved. Contemporaneous to the report of Andersen and co-workers, the Rho group reported isolation of phorbaketals A-C (**4-6**) from Korean marine sponge *Phorbas* sp.⁹ These compounds possess a molecular skeleton that is identical to that of alotaketals and are moderately cytotoxic toward human colorectal, hepatoma, and lung cancer cell lines. In addition, a recent study by Hong and co-workers demonstrated that phorbaketal A stimulated murine C3H10T1/2 cells and human mesenchymal stem cells for osteoblast differentiation through TAZ mediated Runx 2 activation, possibly by activation of extracellular signal regulated kinase (ERK).¹⁰ While cross talk between the cAMP and ERK signalings has been well established,¹¹ the effect of phorbaketal A on the cAMP signaling remains unknown and the potential correlation between the bioactivities of alotaketals A and phorbaketal A remains to be elucidated. Our interests in developing small molecule tools for biomedical discoveries prompted us to start a research project to elucidate the mode-of-action and explore potential biomedical applications of this family of natural products. Herein we report in detail our study, which culminated in the first enantioselective total synthesis of (-)-alotaketals A and illuminated the preliminary structure-activity relationship (SAR) of this potent cAMP signaling agonist.^{12,13} Our study also revealed alotaketals A's unique activity in selectively targeting PKA signaling in the nucleus of living cells.

2. Results and Discussions

2.1 Synthetic design

Our convergent synthetic design of alotaketals A relied on a late stage spiroketalization to assemble its tricyclic spiroketal ring system. Two such cyclization processes could be envisioned (Scheme 1), one involved spiroketalization through lactol **7** (process **I**) while the other involved a similar process through lactol **8** (process **II**). The former lactol (**7**) could be assembled by intermolecular reductive allylation of bicyclic lactone **10** with allyl halide **11** while the latter (**8**) could be assembled by intramolecular reductive allylation of ester **9**, which in turn would be synthesized by coupling of **10** and **12**. These building blocks (i.e. **10**, **11** and **12**) would be prepared from 5β-hydroxy carvone (**13**) and ethyl acetoacetate. Unknown at the outset of our study was the stability of the exocyclic Δ^{11,23} alkene under the acidic reaction conditions that are necessary for formation of the spiroketal. It was expected that allylic activation of the C10 methylene by both the Δ^{11,23} alkene and the oxocarbenium intermediates formed during spiroketalization would significantly increase the chance of exo-to-endo migration of the alkene through a process of deprotonation and re-protonation.

Despite such a concern, building upon the assumption that the alkene isomerization could be suppressed by fine-tuning the reaction conditions and the substrates themselves, we initiated our synthetic studies.

2.2 Efforts toward a ring-closing metathesis approach

Our initial efforts focused on developing a ring-closing metathesis approach to bicyclic lactone **10** en route to hydrobenzopyranone **15** (Scheme 2). The metathesis substrates **14a**/**14b** were synthesized by Mitsunobu reaction of acrylic/*trans*-2-pentenoic acid and 5 β -hydroxycarvone **13**,¹⁴ readily prepared from *R*-(-)-carvone by vinylogous oxidation of the corresponding silyl dienol ether under the conditions we recently developed.¹⁵ Unfortunately, only the starting materials were recovered when **14a** or **14b** was subjected to common ring-closing metathesis conditions.¹⁶ We then turned to the “relay metathesis” approach, which was expected to facilitate cyclization by assisted formation of the initiating ruthenium alkylidene species.¹⁷ To this end, ester **14c** was similarly prepared and subjected to reaction with the second-generation Grubbs catalyst.¹⁸ To our surprise, ester **14a** was isolated as the sole product. Formation of **14a** suggested that the β -carbonyl-carbene species [Ru]=CH(CO)R was indeed generated from **14c** and was competent for intermolecular cross metathesis with styrene, but not for the intramolecular ring-closing metathesis.¹⁹ Despite their electronically and sterically deactivated nature, acrylates and 1,1-disubstituted alkenes have been shown to be effective for ring-closing metathesis to give related benzohydropyranones.²⁰ Thus, the difficulty for cyclization of **14** likely was due to unfavourable conformational effects, such as developing 1,2-interactions during formation of the metallocyclobutane intermediate (e.g. **A**). Esters **16a/b**, prepared by diastereoselective Luche reduction of **14b** and TBS-protection of the allyl hydroxyl group thus formed, also remained intact under ring-closing metathesis reaction conditions.

2.3 An intramolecular reductive allylation approach to bicyclic lactone **10**

The difficulty encountered in ring-closing metathesis of **14** and **16** prompted us to develop a second-generation approach to bicyclic lactone **10** based on intramolecular reductive allylation of esters (Scheme 3). It commenced with regioselective allylic chlorination of **13** with hypochlorous acid, generated in situ from calcium hypochlorous and CO₂, to give allyl chloride **18**.²¹ Formate **19** was obtained when **18** was subjected to Mitsunobu reaction with formic acid, DEAD, and PPh₃. The allylic chloride of **18** remained intact in the presence of nucleophilic PPh₃. To prepare for intramolecular reductive allylation, cyclohexenone **19** was subjected to diastereoselective Luche reduction to give **20a**,²² which was converted to allyl iodide **21a** by reaction with NaI under Finkelstein conditions.²³ Despite some initial concerns, the free hydroxyl group of **21a** proved to be compatible with SmI₂-mediated intramolecular reductive allylation to give epimers of bicyclic lactol **22a** under the conditions developed by Keck and co-workers.²⁴ However, despite our efforts, further functionalization of **22a** was found to be problematic. Thus, allyl alcohol **20a** was protected as its *tert*-butyldimethylsilyl ether **20b**, and converted to allyl iodide **21b** by reaction with NaI. Again, SmI₂-mediated reductive allylation of **21b** gave bicyclic lactol **22b**. Subsequent oxidation of **22b** with IBX gave β,γ -unsaturated lactone **23b**. A MOM-protected variant **23c** was similarly prepared from **20c**, obtained by MOM- protection of **20a**. However, it was not further pursued because of the relatively harsh conditions that would be necessary for removal of the MOM- protecting group.

Further functionalization of **23b** required oxidative migration of the $\Delta^{7,22}$ alkene to introduce the C22-allyl hydroxyl group, which proved to be surprisingly difficult. For example, β,γ -unsaturated lactone **23b** was recovered when it was subjected to epoxidation with mCPBA at RT to 40 °C while complex mixtures were obtained when it was treated with DMDO or CF₃CO₃H. Attempts for allylic halogenation of **23** with NIS, I₂, or Br₂ led

to complex reaction mixtures or recovery of **23b**. Efforts to prepare the silyl dienol ether of **23** for vinylogous oxidation were also unsuccessful.¹⁵ Only α,β -unsaturated bicyclic lactone **24** was isolated upon reaction of **23** with TBSOTf-Et₃N or under other silylation conditions. This bicyclic lactone **24** could also be obtained in 95% yield upon treating **23b** with DBU. A solution was eventually identified after extensive experimentation. It started from migratory mercuration of **23** with Hg(OAc)₂ to give allylmercury intermediate **25**, apparently through facile rearrangement of the reversibly formed $\Delta^{7,22}$ mercuronium intermediate under the influence of the ester group. Iodinolysis of **25** with I₂ gave allyl iodide **26**, which was subjected to nucleophilic substitution with sodium formate to give allyl alcohol **27** after basic workup. The hydroxyl group of **27** was protected as its PMB ether to give bicyclic lactone **10**. We also tested the possibility of displacing allyl iodide **26** with *p*-methoxybenzyl alcohol, which was expected to provide access of **10** directly. However, common substitution reaction conditions, such as with NaH, KHMDS, Cs₂CO₃, K₂CO₃, or Ag₂O all failed to deliver **10**. Interestingly, a mixture of **23b** and **24** was obtained under Pd(0)-catalyzed substitution conditions.²⁵ Attempts for direct oxidation of allylmercury chloride **23b** to allyl alcohol **27** gave reductive-demercuration product **24** only.²⁶

2.4 Synthesis of the allyl iodide fragment

Our efforts toward synthesis of fragments **11** and **12** were built upon the ease of access of β -keto ester **28**, through γ -alkylation of the dienolate of ethyl acetoacetate with 4-bromo-2-methylbut-1-ene (Scheme 4).²⁷ We envisioned employing transition metal-catalyzed methylation to convert **28** to *E*-enoate **30** through the intermediacy of *Z*-enol triflate **29**, which could be prepared from **28** under biphasic reaction conditions with aq. LiOH-Tf₂O.²⁸ Whereas Fe-catalyzed methylation of enol triflates often proceeds with excellent yield and stereoselectivity,²⁹ we were surprised that the reaction of **29** under such conditions led to extensive isomerization of the enoate alkene of **30** (*E*:*Z* ~ 3:1 to 1:1). To our relief, such isomerization was suppressed when CuCN-mediated methylation with MeMgBr was used and enoate **30** was obtained in 93% yield as a single isomer.³⁰ Reaction of **30** with *N,O*-dimethylhydroxylamine by the Merck procedure gave Weinreb amide **31**.³¹ It was converted to ketone **33** upon alkylation with allylmagnesium chloride **32**. No isomerization of the β,γ -enone moiety of **33** was observed. Preparation of allyl alcohol **12** required enantioselective reduction of **33**. However, this β,β -disubstituted enone was found to be unreactive under Noyori transfer hydrogenation and LiAlH₄/*R*-(+)-BINOL reduction conditions.^{32,33} While allyl alcohol **12** could be obtained by CBS reduction of **33**,³⁴ no enantioselectivity was observed during this process.

We then turned to an aldol approach to introduce the C13-allyl hydroxyl group. For this purpose, reaction of ester **30** with DIBAL-H followed by oxidation of the resulting allyl alcohol with Dess-Martin periodinane gave aldehyde **34**, which was subjected to Nagao-Fujita aldol reaction with **35** to give **36** in 80% yield and with excellent diastereoselectivity (Scheme 5).³⁵ Protection of **36** as its tert-butyldimethylsilyl ether and methanolysis of the thione chiral auxiliary gave ester **37**, which was subjected to reaction with TMSCH₂Li in the presence of anhydrous CeCl₃.³⁶ The initially formed bis-trimethylsilylmethyl carbinol was stirred with silica gel at room temperature to afford allyl silane **38** in 86% yield. Consistent with previous reports, successful preparation of the allylsilane required that the trimethylsilylmethylcerium reagent be meticulously prepared. Under less stringent conditions, methyl ketone **39** was formed as the product (>95% yield).³⁷ However, it could be converted to **38** via a procedure that consisted of transforming **39** to its enol triflate followed by Pd-catalyzed Kumada coupling with trimethylsilylmethylmagnesium bromide. Various protocols for iodinolysis of allylsilane **38** were tested. The optimal results were obtained when **38** was treated with freshly recrystallized NBS followed by Finkelstein

reaction with NaI to give allyl iodide **11** in 88% yield. It was crucial to maintain the reaction in the dark to suppress radical bromination of the remote terminal 1,1-disubstituted alkene.

2.5 Synthesis of 22-deoxyalotaketal A

To shed light on potential obstacles that we might encounter along the rest of the synthetic route, a pilot study of fragment coupling and subsequent transformations was carried out using **11** and **24** as model substrates. This study not only would illuminate nuances in assembling the unique spiroketal ring system of alotaketal A, it also would provide access to 22-deoxy and other analogues of alotaketal A for structure-activity relationship (SAR) studies. Pathway **I** of our synthetic design (Scheme 1) was employed because of the perceived simplicity of its fragment coupling event. Whereas initial attempts for fragment coupling through the intermediacy of allyllithium or allylmagnesium reagents of **11** were hampered by competing dimerization of the allyl iodide, the two fragments were smoothly coupled under Barbier conditions with SmI_2 through an intermolecular reductive allylation process to give lactol **40** (Scheme 6). No over-reduction was observed even though excess of SmI_2 was used. Our attempts for global desilylation and spiroketalization of **40** under acidic conditions (HF-Py or pTSA) in one-pot were unsuccessful. However, the desired transformations could be effected in a stepwise manner. Thus, global desilylation of crude **40** with TBAF gave lactol **41**, which was subjected to pTSA-mediated spiroketalization without purification. This led to formation of tricyclic spiroketal **42** and its $\Delta^{10,11}$ isomer **43** due to migration of $\Delta^{11,23}$ alkene under the acidic reaction conditions, with the latter isolated as the major (1:3 to 1:6). Interestingly, spiroketalization of **41** by PPTS led to formation of spiroketals **42** and **43** in equal amounts. Since the same oxocarbenium intermediate is involved in the spiroketalization of **41** with either pTSA or PPTS, it is unlikely that the different product distribution reflects the kinetics of the spiroketalization (to give **42**) versus the alkene isomerization (to give **43** after cyclization). Instead, it is more likely that isomerization of the initially formed products contributed to the observed product distribution. Indeed, treatment of **42** with pTSA in CH_2Cl_2 led to formation of **43**. Under otherwise identical conditions, compound **42** remained intact when treated with the less acidic PPTS. Both **42** and **43** were oxidized with IBX to give 22-deoxyalotaketal A (**44**) and its $\Delta^{10,11}$ isomer (**45**), respectively.

2.6 Synthesis of (-)-alotaketal A

Building upon our preliminary studies, the successful synthesis of alotaketal A was carried out as depicted in Scheme 7. The coupling of fragments **10** and **11** went smoothly by SmI_2 -mediated reductive allylation under Barbier conditions to give **46**. This was followed by global desilylation with TBAF and spiroketalization of the reaction crude (**47**) with PPTS. We were pleased to find that only the desired spiroketal **48** was formed and the exo-to-endo isomerization of $\Delta^{7,22}$ alkene was not observed. Since **41** and **47** differ by a *p*-methoxybenzyloxyl substituent only, we speculate that the inductive electron-withdrawing effect of the alkoxyl functionality or the cation- π stabilization of the oxocarbenium by the substituted 4-methoxyphenyl group contributed to the different reactivities of **41** and **47**. Oxidative removal of the PMB protection of **48** by DDQ gave diol **49**, an alotaketal A analogue with the enone moiety reduced to an allyl alcohol. Oxidation of **48** with IBX followed by removal of the PMB protecting group with DDQ gave synthetic alotaketal A (**2**). Its ^1H and ^{13}C NMR spectra were consistent with those reported for the natural product, as was its optical rotation. In addition, synthetic (-)-alotaketal A was found to be identical to an authentic sample of the natural product by TLC and HPLC.

2.7. Investigating the bioactivity of alotaketal A and analogs

The goal of the next series of experiments was to examine the effects of alotaketal A and analogs on the cAMP/PKA signaling pathway. These effects were studied in HEK 293T cells by performing fluorescence imaging using an optimized genetically encoded A-kinase activity reporter (AKAR4).³⁸ As the name suggests, AKAR4 is able to directly report PKA activity by its change in fluorescence. The change in fluorescence is due to the phosphorylation-dependent conformational change of a tandem fusion domain, composed of a substrate peptide for PKA and a phosphorylation recognition domain (Figure 2a). The conformational change alters the distance or orientation between the two fluorescent proteins at the N and C termini of the reporter, generating a change in Förster Resonance Energy transfer (FRET) between them. In the context of AKAR4, the phosphorylation of the substrate domain by activated PKA causes a conformational change that brings the fluorescent proteins in close proximity to produce an increase in FRET which is detected as an increase in ratio of yellow over cyan emission.

First, the HEK293T cells were transfected with AKAR4 for 24 hours and treated with 1 μ M of alotaketal A (**2**) or one of the analogs (**42-45** and **49**). Of the six compounds, **42-45** were unable to induce a significant change in ratio of yellow over cyan emission from the reporter, increasing the emission ratio by $1.07 \pm 0.3\%$ ($n = 11$; n = number of cells), $1.55 \pm 0.8\%$ ($n = 8$), $1.28 \pm 0.6\%$ ($n = 5$), $1.22 \pm 0.4\%$ ($n = 8$), respectively (Figure 2b,c). The relatively small responses from analogs **42-45** were confirmed to be due to the inactivity of the analogs and not from poorly functioning AKAR4 because the probes were able to respond maximally upon addition of a cAMP-elevating cocktail of the AC activator forskolin (Fsk) and general PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX).³⁹ In contrast to analogs **42-45**, **49** and alotaketal A (**2**) elicited responses of $6.7 \pm 2.2\%$ ($n=24$) and $5.3 \pm 2.5\%$ ($n=13$) from AKAR4, respectively (Figure 2b,c). Thus, these results show that **49** and **2** are active and involved in PKA activation. Together with the data showing **49** and **2** induced responses from a FRET-based cAMP indicator,¹² these results suggest that these compounds activate PKA by inducing cAMP production.

The mechanisms by which these compounds induce cAMP production and PKA activation are not clear. But an interesting observation from these imaging experiments suggested that they exhibit subcellular specificity when activating this pathway in living cells. As illustrated in the ratiometric images of the cells treated with **49** (Figure 3a), the nuclear region showed a more drastic color change compared to the cytosol, upon addition of **49** or **2** (Figure 3a). We then quantitated the responses in the two cellular compartments. Compound **49** elicited a response of $7.2 \pm 0.71\%$ ($n = 5$) in the nucleus and a mere $2.5 \pm 0.72\%$ ($n = 5$) in the cytosol, while **2** also had a similar response of $6.3 \pm 2.5\%$ ($n = 6$) in the nucleus and $2.5 \pm 0.21\%$ ($n = 6$) in the cytosol (Figure 3b). These results are quite different from what has been seen when naïve HEK293T cells expressing AKAR4 are treated with forskolin and IBMX. As previously observed,⁴⁰ the magnitude of the AKAR4 responses is smaller and kinetically slower in the nucleus than in the cytosol (Figure 3c), $16.1 \pm 0.11\%$ ($n = 8$) and $70.8 \pm 0.09\%$ ($n = 8$) respectively, due to the activation of cytosolic PKA and subsequent translocation of the catalytic domain of cytosolic PKA to the nucleus. These results show that alotaketals are possibly targeting a different pool of cAMP/PKA signaling components, more specifically those found in the nucleus.

3. Conclusions

We described in detail the first total synthesis of the tricyclic sesterterpenoid (-)-alotaketal A, a potent cAMP signaling agonist that features an unprecedented spiroketal ring system. Two Barbier-type SmI_2 -mediated reductive coupling of allyl iodides and esters were employed in this convergent synthetic route: an intramolecular reductive coupling was

crucial for preparation of the key bicyclic lactone fragment whereas an intermolecular reductive coupling was employed to join the fragments and complete the sesterterpenoid molecular skeleton. Our study revealed subtle interplay of structure and functional group stability/reactivity during spiroketalization to form the unique spiroketal ring system. Also highlighted is Hg(OAc)₂-mediated allylic mercuration of β,γ -unsaturated lactone to introduce the C22-hydroxyl group. Using FRET imaging assays with genetically encoded AKAR4 reporter genes, we verified that the cAMP signaling pathway was activated by alotaketol A. Our preliminary SAR studies revealed that the C22-hydroxyl group to be important to alotaketol A's bioactivity. On the other hand, reduction of the enone moiety to an allyl alcohol was tolerated, suggesting that the enone moiety to be nonessential. Our FRET imaging studies revealed alotaketol A and analog **49** activated the cAMP/PKA pathway in a subcellular specific manner. Recent studies have suggested that cAMP elicits highly specific cellular response to external stimuli via highly compartmentalized cAMP signaling.⁴¹ Evidence of subcellular cAMP signaling domains in specific regions such as the nucleus has been presented through the identification of a functional pool of nuclear PKA holoenzyme, and its regulators, the phosphodiesterase, PDE4, and scaffolding proteins such as A-kinase anchoring proteins (AKAPs).⁴² Therefore, future experiments will focus on the identification of cellular targets of alotaketols and elucidation of their functions within the nucleus. Furthermore, these alotaketols may also serve as molecular tools to provide a better understanding of the function of nuclear PKA as well as the details of the mechanism that underlies compartmentalized cAMP signaling.

4. Experimental Section

(4*S*,5*R*)-4-Hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enone (**13**)

To a solution of nitrosobenzene (10.80 g, 100.7 mmol) in CH₂Cl₂ (210 mL) was added acetic acid (5.76 mL, 100 mmol) and (5-isopropenyl-2-methyl-cyclohexa-1,3-dienyloxy)-trimethylsilane⁴³ (9.33 g, 42.0 mmol) at -78 °C. The solution was stirred at this temperature for 12 h before being warmed to room temperature and further stirred for 4 h. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/2) to provide **13** (4.12 g, 59%) as yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 6.71-6.70 (m, 1H), 4.98-4.97 (m, 1H), 4.95-4.94 (m, 1H), 4.46-4.42 (m, 1H), 2.72-2.67 (m, 1H), 2.50 (dd, *J* = 16.3, 3.9 Hz, 1H), 2.38 (dd, *J* = 16.4, 13.8 Hz, 1H), 1.79-1.78 (m, 3H), 1.76-1.75 (m, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 198.5, 147.4, 143.0, 135.1, 114.8, 68.4, 52.7, 40.8, 19.0, 15.4.

(1*R*,6*R*)-3-Methyl-4-oxo-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl acrylate (**14a**)

To a solution of 5 β -hydroxycarvone **13** (83 mg, 0.5 mmol), acrylic acid (103 μ L, 1.5 mmol) and triphenylphosphine (394 mg, 1.5 mmol) in THF (2.5 mL) was added diethyl azodicarboxylate (0.68 mL, 40% in toluene, 1.5 mmol) dropwise at 0 °C under nitrogen. The resulting mixture was stirred at room temperature for 3 h, diluted with EtOAc (30 mL), washed with H₂O and brine. The organic layer was dried over Na₂SO₄, concentrated and purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/5) to provide **14a** (52 mg, 47%) as light yellow oil. [α]_D²⁰ -98.3 (*c* 1.96, CHCl₃); IR (film, cm⁻¹) 2976, 2919, 1729, 1685, 1407, 1262, 1182, 1042, 900, 809; ¹H NMR (500 MHz, CDCl₃) δ 6.79 (dd, *J* = 5.6, 1.5 Hz, 1H), 6.38 (dd, *J* = 17.3, 1.4 Hz, 1H), 6.07 (dd, *J* = 17.3, 10.4 Hz, 1H), 5.84 (dd, *J* = 10.4, 1.4 Hz, 1H), 5.63-5.61 (m, 1H), 4.94 (s, 1H), 4.75 (s, 1H), 2.86 (d, *J* = 12.6 Hz, 2H), 2.55 (dd, *J* = 12.5, 0.9 Hz, 1H), 1.83 (dd, *J* = 1.4, 0.8 Hz, 3H), 1.78 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) 199.2, 165.4, 142.8, 138.9, 138.6, 131.4, 128.0, 113.0, 66.4, 44.3, 37.9, 21.9, 15.6; HRMS (ESI): calculated for C₁₃H₁₆O₃ [M+Li⁺] 227.1259, found 227.1249.

(E)-(1*R*,6*R*)-3-Methyl-4-oxo-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl pent-2-enoate (14b)

To a solution of 5β-hydroxycarvone **13** (50 mg, 0.3 mmol), 2-pentenoic acid (0.09 mL, 0.9 mmol) and triphenylphosphine (237 mg, 0.9 mmol) in THF (2 mL) was added diethyl azodicarboxylate (0.42 mL, 40% in toluene, 0.9 mmol) dropwise at 0 °C under nitrogen. The resulting mixture was stirred at room temperature for 3 h and then diluted with EtOAc (30 mL), washed with H₂O, brine. The organic layer was dried over Na₂SO₄, concentrated and purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/5) to provide product **14b** (61.8 mg, 83%) as a light yellow oil. $[\alpha]_D^{21}$ -287.77 (*c* 2.14, CHCl₃); IR (film, cm⁻¹) 2970, 2919, 1720, 1679, 1649, 1244, 1176, 1116, 1025, 971; ¹H NMR (300 MHz, CDCl₃) δ 7.05-6.96 (m, 1H), 6.79 (dd, *J* = 5.6, 1.5 Hz, 1H), 5.76 (dt, *J* = 15.7, 1.7 Hz, 1H), 5.61-5.58 (m, 1H), 4.94 (s, 1H), 4.75 (s, 1H), 2.88-2.80 (m, 2H), 2.60-2.47 (m, 1H), 2.27-2.17 (m, 2H), 1.83 (dd, *J* = 1.5, 0.8 Hz, 3H), 1.78 (s, 3H), 1.06 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 199.3, 166.0, 151.8, 142.9, 139.0, 138.7, 119.7, 112.9, 66.1, 44.3, 37.9, 25.4, 21.9, 15.6, 12.0; HRMS (ESI): molecular ion was not observed.

(E)-4,4-Diethyl 1-((1*R*,6*R*)-3-methyl-4-oxo-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl) hepta-1,6-diene-1,4,4-tricarboxylate (14c)

To a solution of triethyl pent-4-ene-1,2,2-tricarboxylate⁴⁴ (5 g, 16 mmol) in EtOH (20 mL) was added 1 N NaOH (19.2 mL, 19.2 mmol) at room temperature. The resulting mixture was stirred for 4 h before it was acidified with 2 N HCl. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over Na₂SO₄, concentrated and purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/2) to provide 3,3-bis(ethoxycarbonyl)hex-5-enoic acid (3.51 g, 77%) as colorless oil. IR (film, cm⁻¹) 2985, 1735, 1700, 1652, 1282, 1208, 1096, 927, 856; ¹H NMR (500 MHz, CDCl₃) δ 6.92 (dt, *J* = 15.5, 7.7 Hz, 1H), 5.87 (dd, *J* = 15.5, 0.8 Hz, 1H), 5.66-5.58 (m, 1H), 5.15 (dd, *J* = 2.8, 0.9 Hz, 1H), 5.12 (d, *J* = 0.9 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 4H), 2.78 (d, *J* = 7.7 Hz, 2H), 2.65 (d, *J* = 7.4 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 171.1, 170.1, 145.8, 131.7, 124.2, 119.8, 61.6, 56.9, 37.4, 35.3, 14.1; HRMS (ESI): calculated for C₁₄H₂₀O₆ [M+Li⁺] 291.1420, found 291.1433.

To a solution of 5β-hydroxycarvone **13** (83 mg, 0.5 mmol), 3,3-bis(ethoxycarbonyl)hex-5-enoic acid (426 mg, 1.5 mmol) and triphenylphosphine (394 mg, 1.5 mmol) in THF (2.5 mL) was added diethyl azodicarboxylate (0.68 mL, 40% in toluene, 1.5 mmol) dropwise at 0 °C under nitrogen. The resulting mixture was stirred at room temperature for 3 h, diluted with EtOAc (30 mL), washed with H₂O and brine. The organic layer was dried over Na₂SO₄, concentrated and purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/5) to provide **14c** (121 mg, 56%) as light yellow oil. $[\alpha]_D^{19}$ -174.17 (*c* 1.81, CHCl₃); IR (film, cm⁻¹) 2985, 1726, 1685, 1649, 1448, 1173, 1045, 915; ¹H NMR (500 MHz, CDCl₃) δ 6.82-6.76 (m, 2H), 5.81 (d, *J* = 15.6 Hz, 1H), 5.65-5.56 (m, 2H), 5.13 (d, *J* = 1.2, 1H), 5.10 (dd, *J* = 9.3, 1.9 Hz, 1H), 4.92 (s, 1H), 4.73 (s, 1H), 4.19-4.16 (m, 4H), 2.88-2.77 (m, 2H), 2.74 (dd, *J* = 7.7, 1.3 Hz, 2H), 2.62 (d, *J* = 7.5 Hz, 2H), 2.53 (dd, *J* = 15.2, 2.6 Hz, 1H), 1.83 (s, 3H), 1.76 (s, 3H), 1.29-1.18 (m, 6H); ¹³C NMR (125 MHz, CDCl₃) δ 199.2, 170.1, 170.1, 165.0, 143.9, 142.9, 138.8, 138.7, 131.7, 124.3, 119.7, 112.9, 66.3, 61.6, 56.9, 44.3, 37.9, 37.3, 35.3, 21.9, 15.6, 14.1; HRMS (ESI): calculated for C₂₄H₃₂O₇ [M+Li⁺] 439.2308, found 439.2288.

(E)-(1*R*,4*R*,6*R*)-4-Hydroxy-3-methyl-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl pent-2-enoate (16a)

To a solution of enone **14b** (19 mg, 0.076 mmol) in MeOH (1.5 mL) was added CeCl₃·7H₂O (43 mg, 0.12 mmol) at room temperature under nitrogen. The resulting suspension was stirred for 10 min, and cooled to -78 °C, then NaBH₄ (3.5 mg, 0.092 mmol) was added in one portion. Once the reaction was complete (typically <10 min), it was immediately quenched with sat. aq. NaHCO₃. The aqueous phase was extracted with EtOAc. The

combined organic layers were dried over Na₂SO₄, concentrated and purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/4) to provide **16a** (17.5 mg, 92 %) as yellow oil. $[\alpha]_D^{19}$ -261.6 (*c* 2.02, CHCl₃); IR (film) 2967, 2875, 2854, 1714, 1694, 1653, 1448, 1285, 1178, 1119, 1093, 1045, 974, 918, 885; ¹H NMR (300 MHz, CDCl₃) δ 6.95 (dt, *J* = 15.6, 6.3 Hz, 1H), 5.80-5.65 (m, 2H), 5.44-5.32 (m, 1H), 4.85 (s, 1H), 4.74 (s, 1H), 4.14 (brs, 1H), 2.31 (d, *J* = 13.1 Hz, 1H), 2.25-2.03 (m, 3H), 1.95-1.84 (m, 1H), 1.83 (s, 3H), 1.75 (s, 3H), 1.04 (t, *J* = 7.4 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 166.3, 150.7, 144.6, 143.5, 121.9, 120.4, 111.6, 70.8, 66.8, 43.3, 32.2, 25.3, 22.0, 18.8, 12.1; HRMS (ESI): calculated for C₁₅H₂₂O₃ [M+Li⁺] 257.1729, found 257.1724.

(E)-(1*R*,4*R*,6*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-3-methyl-6-(prop-1-en-2-yl)cyclohex-2-en-1-yl pent-2-enoate (16b)

To a solution of allyl alcohol **16a** (12 mg, 0.048 mmol) in CH₂Cl₂ (1 mL) was added 2,6-lutidine (14 μ L, 0.12 mmol) and TBSOTf (22 μ L, 0.096 mmol) at 0 °C. After 15 min, the reaction was quenched with a sodium potassium phosphate buffer solution (pH = 7, 1 mL) and extracted with CH₂Cl₂ (3 $\times$ 10 mL). The combined organic layers were washed with sat. aq. NaHCO₃ and dried over Na₂SO₄. After concentration in *vacuo*, the residue was purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/15) to provide **16b** (16.7 mg, 97 %) as yellow oil. $[\alpha]_D^{19}$ -188.8 (*c* 1.58, CHCl₃); IR (film, cm⁻¹) 2958, 2928, 2854, 1717, 1646, 1253, 1179, 1105, 891, 832; ¹H NMR (300 MHz, CDCl₃) δ 6.96 (dt, *J* = 15.7, 6.3 Hz, 1H), 5.74 (dt, *J* = 15.6, 1.6 Hz, 1H), 5.65-5.63 (m, 1H), 5.36-5.33 (m, 1H), 4.85 (s, 1H), 4.73 (s, 1H), 4.14 (t, *J* = 8.0 Hz, 1H), 2.34-2.23 (m, 1H), 2.22-2.14 (m, 2H), 1.94-1.82 (m, 2H), 1.75 (s, 6H), 1.04 (t, *J* = 7.4 Hz, 3H), 0.92 (s, 9H), 0.11 (s, 3H), 0.11 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 166.4, 150.5, 144.9, 144.8, 121.0, 120.5, 111.5, 71.4, 66.8, 43.3, 32.5, 25.9, 25.6, 25.3, 22.1, 19.6, 12.1, -4.1, -4.9; HRMS (ESI): calculated for C₂₁H₃₆O₃Si [M+Li⁺] 371.2594, found 371.2606.

(1*R*,4*R*,6*R*)-6-(3-Chloroprop-1-en-2-yl)-4-(methoxymethoxy)-3-methylcyclohex-2-en-1-yl formate (20c)

To a solution of allyl alcohol **20a** (400 mg, 1.73 mmol) in dichloromethane (11 mL) was added *N,N*-diisopropylethylamine (0.58 mL, 3.46 mmol). The reaction mixture was cooled to 0 °C before methyl chloromethyl ether (2.1 mL, 2.1 M in toluene, 4.35 mmol) was introduced. The solution was stirred at room temperature for 12 h before it was poured into water (20 mL) and extracted with ethyl acetate. The combined organic layers were dried over Na₂SO₄. After concentration in *vacuo*, the residue was purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/5) to provide **20c** (450 mg, 95 %) as yellow oil. $[\alpha]_D^{21}$ -209.4 (*c* 0.71, CHCl₃); IR (film, cm⁻¹) 2949, 2931, 2890, 1720, 1173, 1143, 1101, 1034, 927; ¹H NMR (500 MHz, CDCl₃) δ 7.97 (s, 1H), 5.70 (d, *J* = 5.5 Hz, 1H), 5.43 (brs, 1H), 5.30 (s, 1H), 5.06 (s, 1H), 4.82 (d, *J* = 6.9 Hz, 1H), 4.70 (d, *J* = 6.9 Hz, 1H), 4.16 (d, *J* = 11.9, 1H), 4.14-4.10 (m, 1H), 4.04 (d, *J* = 11.9, 1H), 3.44 (s, 3H), 2.73 (d, *J* = 13.5 Hz, 1H), 2.17-2.12 (m, 1H), 1.96-1.88 (m, 1H), 1.83 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 160.5, 144.2, 143.8, 121.2, 117.1, 95.8, 75.5, 65.9, 55.7, 47.5, 38.4, 28.9, 19.3; HRMS (ESI): molecular ion not observed.

(1*R*,4*R*,6*R*)-6-(3-Iodoprop-1-en-2-yl)-4-(methoxymethoxy)-3-methylcyclohex-2-en-1-yl formate (21c)

To a solution of allyl chloride **20c** (450 mg, 1.64 mmol) in acetone (16 mL) was added anhydrous NaI (2.46 g, 16.4 mmol) at room temperature. The resulting mixture was stirred overnight before it was concentrated. The mixture was suspended in ether (20 mL) and filtered through a short pad of silica gel. After concentration in *vacuo*, the crude allyl iodide **21c** was used without further purification.

(4a*R*,6*R*,8a*R*)-6-(Methoxymethoxy)-7-methyl-4-methylene-3,4,4a,5,6,8a-hexahydro-2*H*-chromen-2-one (23c)

To an ice-cold solution of SmI₂ (93 mL, 0.1 M in THF, 9.28 mmol) was added a solution of allyl iodide **21c** (485 mg, 1.32 mmol) in dry THF (5 mL) via cannula under nitrogen. The deep blue mixture was stirred at 0 °C for 10 min before it was quenched by a mixture of sat. aq. NaHCO₃ (30 mL) and a sodium potassium phosphate buffer solution (pH = 7, 30 mL). After 15 min, the reaction mixture was extracted with EtOAc. The combined organic layers were washed with water, saturated aqueous NaHCO₃, brine, and dried over Na₂SO₄. After concentration in vacuo, the residue was purified by flash column chromatography (silica gel, eluted with ethyl acetate/petroleum ether = 1/2) to provide lactols **22c** (1:1 mixture of epimers, 258 mg, 76%) as colorless oil.

To a solution of lactols **22c** (113 mg, 0.47 mmol) in DMSO (4 mL) was added 2-iodoxybenzoic acid (266 mg, 0.95 mmol) at room temperature. The resulting mixture was stirred at 45 °C. The reaction was quenched with H₂O (3 mL) when it was complete. The mixture was filtered and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine and dried over Na₂SO₄. After concentration in vacuo, the residue was purified by column chromatography (silica gel, eluted with ethyl acetate/petroleum ether = 1/2) to provide **23c** (84 mg, 75%) as light yellow oil. $[\alpha]_D^{21}$ -27.12 (*c* 2.12, CHCl₃); IR (film, cm⁻¹) 2949, 2925, 2889, 1741, 1448, 1241, 1152, 1102, 1028, 968, 906; ¹H NMR (500 MHz, CDCl₃) δ 5.69-5.66 (m, 1H), 5.08-5.06 (m, 2H), 4.79 (d, *J* = 7.0 Hz, 1H), 4.69-4.67 (m, 1H), 4.67 (d, *J* = 7.0 Hz, 1H), 4.12-4.09 (m, 1H), 3.41 (s, 3H), 3.31 (d, *J* = 17.6 Hz, 1H), 3.25 (d, *J* = 17.6 Hz, 1H), 2.72 (d, *J* = 13.2 Hz, 1H), 2.23-2.09 (m, 1H), 1.85 (s, 3H), 1.80-1.73 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 170.2, 144.5, 139.8, 120.2, 113.1, 95.8, 74.6, 73.0, 55.7, 37.9, 36.9, 31.2, 19.4; HRMS (ESI): calculated for C₁₃H₁₈O₄ [M+H⁺] 239.1284, found 239.1288.

(*E*)-*N*-Methoxy-*N*,3,7-trimethylocta-2,7-dienamide (31)

To a solution of *E*-alkenoic ester **30** (904 mg, 4.61 mmol) and *N,O*-dimethylhydroxyamine hydrochloride (899 mg, 9.22 mmol) in THF (12 mL) was added *i*-PrMgCl (10.4 mL, 2 M in THF, 20.75 mmol) dropwise at -5 °C. The mixture was stirred for 30 min and quenched with sat. aq. NH₄Cl (30 mL). The mixture was extracted with ethyl acetate. The combined organic layers were washed with brine and dried over Na₂SO₄. After concentration in vacuo, the residue was purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether = 1/5) to give Weinreb amide **31** (896 mg, 92% yield) as colorless oil. IR (film, cm⁻¹) 2970, 2940, 1658, 1635, 1436, 1362, 1173, 1099, 980, 883; ¹H NMR (300 MHz, CDCl₃) δ 6.10 (s, 1H), 4.71 (s, 1H), 4.67 (s, 1H), 3.65 (s, 3H), 3.20 (s, 3H), 2.15-2.10 (m, 5H), 2.01 (t, *J* = 7.5, 2H), 1.70 (s, 3H), 1.66-1.56 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) 145.3, 114.0, 110.2, 61.3, 40.6, 37.2, 25.4, 22.3, 18.6; HRMS (ESI): calculated for C₁₂H₂₁NO₂ [M+H⁺] 212.1651, found 212.1653.

(*E*)-2-(((4-Methoxybenzyl)oxy)methyl)-6,10-dimethylundeca-1,5,10-trien-4-one (33)

To a well-stirred suspension of magnesium turnings (1.38 g, 57.0 mmol) in anhydrous THF (12 mL) was added 1,2-dibromoethane (0.17 mL, 1.97 mmol) under nitrogen. The reaction mixture was stirred at room temperature for 30 min and a solution of the corresponding allyl chloride (1.77 g, 7.86 mmol) in anhydrous THF (4 mL) was introduced dropwise in 30 min. After an additional 1.5 h, the freshly prepared Grignard reagent was transferred to Weinreb amide **31** (565 mg, 2.68 mmol) in THF (10 mL) at -78 °C via cannula. The reaction mixture was stirred at -78 °C for 30 min and quenched with aq. NH₄Cl. After warming to room temperature, the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with H₂O, brine and dried over Na₂SO₄. After concentration in vacuo, the residue was purified by flash column chromatography (silica gel, ethyl acetate/petroleum

ether, 1/10) to give **33** (910 mg, 98% yield) as colorless oil. IR (film, cm^{-1}) 2937, 2854, 1679, 1611, 1513, 1244, 1173, 1099, 1028, 883, 814; ^1H NMR (300 MHz, CDCl_3) δ 7.28 (d, $J = 8.1$ Hz, 2H), 6.90 (d, $J = 8.7$ Hz, 2H), 6.14 (s, 1H), 5.27 (s, 1H), 5.07 (s, 1H), 4.76 (s, 1H), 4.71 (s, 1H), 4.44 (s, 2H), 4.01 (s, 2H), 3.83 (s, 3H), 3.24 (s, 2H), 2.16–2.11 (m, 5H), 2.03 (t, $J = 7.6$ Hz, 2H), 1.74 (s, 3H), 1.68–1.58 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) 198.2, 159.6, 159.1, 145.2, 140.4, 130.3, 129.3, 122.7, 115.9, 113.7, 110.4, 72.7, 71.8, 55.3, 48.8, 40.7, 37.2, 25.3, 22.3, 18.4; HRMS (ESI): calculated for $\text{C}_{22}\text{H}_{30}\text{O}_3$ [$\text{M}+\text{H}^+$] 343.2273, found 343.2278.

(E)-2-(((4-Methoxybenzyl)oxy)methyl)-6,10-dimethylundeca-1,5,10-trien-4-ol (12)

A solution of (*R*)-(+)-2-methyl-CBS-oxazaborolidine (60 μL , 0.06 mmol) in THF (0.3 mL) was treated with $\text{BH}_3\cdot\text{DMS}$ (30 μL , 2 M in THF, 0.06 mmol) at room temperature. After stirring for 15 min, the mixture was cooled to -78°C and a solution of enone **33** (17 mg, 0.05 mmol) in THF (0.3 mL) was added through cannula. The reaction was stirred at -78°C for 3 h before it was slowly warmed to room temperature. The reaction was quenched by the addition of MeOH followed by H_2O . The aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 . After concentration *in vacuo*, the residue was purified by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/2) to give **12** (14.6 mg, 85% yield) as colorless oil. IR (film, cm^{-1}) 2935, 2854, 1649, 1617, 1513, 1442, 1247, 1170, 1037, 891, 820; ^1H NMR (500 MHz, CDCl_3) δ 7.27 (d, $J = 8.6$ Hz, 2H), 6.88 (d, $J = 8.7$ Hz, 2H), 5.20 (d, $J = 8.4$ Hz, 1H), 5.16 (s, 1H), 5.05 (s, 1H), 4.70 (s, 1H), 4.67 (s, 1H), 4.52–4.48 (m, 1H), 4.47 (d, $J = 11.7$ Hz, 1H), 4.45 (d, $J = 11.5$ Hz, 1H), 3.99 (d, $J = 11.3$ Hz, 1H), 3.95 (d, $J = 11.9$ Hz, 1H), 3.80 (s, 3H), 2.35–2.26 (m, 2H), 1.98 (t, $J = 7.7$ Hz, 4H), 1.71 (s, 3H), 1.66 (d, $J = 1.3$ Hz, 3H), 1.58–1.51 (m, 2H); ^{13}C NMR (125 MHz, CDCl_3) 159.2, 145.8, 142.8, 138.2, 129.9, 129.5, 127.3, 116.3, 113.8, 109.9, 73.2, 72.0, 67.2, 55.3, 42.7, 39.0, 37.3, 25.6, 22.4, 16.6; HRMS (ESI): calculated for $\text{C}_{22}\text{H}_{32}\text{O}_3$ [$\text{M}+\text{Li}^+$] 351.2511, found 351.2503.

(S,E)-4-((tert-Butyldimethylsilyl)oxy)-6,10-dimethylundeca-5,10-dien-2-one (39) was formed as the major product during synthesis of **38** when less stringently prepared $\text{TMSCH}_2\text{Li}-\text{CeCl}_3$ reagent was used.⁴⁵ Isolate by flash column chromatography (silica gel, ethyl acetate/petroleum ether = 1/20) as light yellow oil. $[\alpha]_D^{21}$ -24.28 (c 2.72, CHCl_3); IR (film) 2955, 2931, 2860, 1362, 1256, 1075, 838; ^1H NMR (300 MHz, CDCl_3) δ 5.13 (dd, $J = 8.8, 1.2$ Hz, 1H), 4.87–4.80 (m, 1H), 4.70 (s, 1H), 4.66 (s, 1H), 2.69 (dd, $J = 14.3, 8.4$ Hz, 1H), 2.34 (dd, $J = 14.3, 4.6$ Hz, 1H), 2.15 (s, 3H), 1.96 (dd, $J = 15.1, 7.4$ Hz, 4H), 1.70 (s, 3H), 1.64 (d, $J = 1.3$ Hz, 3H), 1.57–1.47 (m, 2H), 0.84 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 207.7, 145.7, 135.7, 127.9, 109.9, 67.0, 51.8, 38.9, 37.3, 31.9, 25.8, 25.5, 22.4, 18.0, 16.5, -4.3 , -5.0 ; HRMS (ESI): calculated for $\text{C}_{19}\text{H}_{36}\text{O}_2\text{Si}$ [$\text{M}+\text{Li}^+$] 331.2645, found 331.2637.

(S,E)-tert-Butyl((6,10-dimethyl-2-((trimethylsilyl)methyl)undeca-1,5,10-trien-4-yl)oxy)dimethylsilane (38)

A solution of methyl ketone **39** (1.50 g, 4.62 mmol) in THF (10 mL) was cooled to -78°C and treated with a solution of potassium hexamethyldisilazide (5.55 mL, 1.0 M in THF, 5.55 mmol) dropwise. After stirring for 1 h, a solution of *N*-phenyltrifluoromethanesulfonimide (1.98 g, 5.55 mmol) in 4 mL of THF was added dropwise. After 2 h, the solution was poured over 20 mL of saturated aqueous NaHCO_3 and extracted with ethyl acetate (2×50 mL). The combined organic layers were dried with Na_2SO_4 and concentrated *in vacuo*. Purification of the residue by flash column chromatography (silica gel, ethyl acetate/petroleum ether = 1/20) provided **39-enol** triflate (2.06 g, 98 %) as clear oil. $[\alpha]_D^{21}$ -9.56 (c 2.38, CHCl_3); IR (film, cm^{-1}) 2955, 2937, 2863, 1673, 1649, 1418, 1247, 1208, 1146, 1069, 954, 891, 838; ^1H NMR (300 MHz, CDCl_3) δ 5.14–5.11 (m, 2H), 4.97 (d, $J = 3.2$ Hz, 1H), 4.71 (s, 1H), 4.67

(s, 1H), 4.64-4.58 (m, 1H), 2.50 (dd, $J = 14.8, 8.0$ Hz, 1H), 2.39 (dd, $J = 14.9, 4.9$ Hz, 1H), 2.00-1.94 (m, 4H), 1.71 (s, 3H), 1.63 (d, $J = 1.3$ Hz, 3H), 1.61-1.48 (m, 2H), 0.85 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 153.8, 145.7, 136.7, 127.4, 109.9, 106.8, 66.3, 43.3, 38.9, 37.3, 25.7, 25.5, 22.4, 18.1, 16.4, -4.4, -5.1; HRMS (ESI): molecular ion not observed.

A round-bottom flask was charged with LiCl (783 mg, 18.48 mmol), flame-dried under reduced pressure, and purged with argon. The anhydrous LiCl thus prepared was taken into diethyl ether (20 mL) followed by a solution of **39**-enol triflate (2.06 g, 4.52 mmol) in 10 mL of diethyl ether. The suspension was cooled to 0 °C and tetrakis(triphenylphosphine)-palladium(0) (267 mg, 0.23 mmol) was added. This was followed by a solution of (trimethylsilyl)methylmagnesium chloride (1.0 M in Et_2O , 9.24 mL, 9.24 mmol). After 2 h, the yellow suspension was filtered through a pad of celite and eluted with diethyl ether (50 mL). The ether solution was washed with 50 mL of saturated aqueous NaHCO_3 , which was back-extracted with Et_2O (3×50 mL). The combined organic fractions were dried with Na_2SO_4 and concentrated *in vacuo*. Purification of the residue by flash column chromatography (silica gel, ethyl acetate/petroleum ether, 1/20) gave **38** (1.51 g, 85 %) as clear oil.

Supplementary Material

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Acknowledgments

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Fig. 1.
Structures of forskolin, alotaketals and phorbaketals

Fig. 2. Effects of different alotaketral A analogs on cAMP/PKA signaling. (A) Schematic of AKAR4 FRET-based biosensor used in this series of experiments. (B) Bar graph showing AKAR4 responses in HEK293T cells following treatment with **42** (1 μ M; n = 11), **43** (1 μ M; n = 8), **44** (1 μ M; n = 5), **45** (1 μ M; n = 8), **49** (1 μ M; n = 24), and **2** (1 μ M; n = 13). (C) Representative time course graphs depicting AKAR4 responses to the inactive compound **42** (*left*), and active compound **49** (*right*).

Fig. 3. Alotaketol A and **49** preferentially activate nuclear cAMP/PKA signaling. (A) Representative YFP and ratiometric images of AKAR4-expressing HEK293T cells treated with **49**. (B) Time course graph depicting AKAR4 responses in the nucleus and cytoplasm of HEK 293T cells after addition of **49** (top panel) and **2** (bottom panel). (C) YFP and ratiometric images of AKAR4 response after Fsk (50 μ M) and IBMX (100 μ M) treatment (top panel). Corresponding time course graph depicting AKAR4 responses in nucleus and cytoplasm after treatment ($n = 8$) (bottom panel).

Scheme 1.
Synthetic Design

Studies of a ring-closing metathesis approach. a) RCO₂H, DEAD, PPh₃, THF, RT, **14a** 47%, **14b** 83%, **14c** 56%. b) NaBH₄, CeCl₃·7H₂O, MeOH, -78 °C, 92%. c) TBSOTf, 2,6-lutidine, CH₂Cl₂, 97%. DEAD = diethyl azodicarboxylate.

Scheme 3.

Synthesis of bicyclic lactone **10**. a) $\text{Ca}(\text{ClO})_2$, CO_2 , CH_2Cl_2 - H_2O , 64%; b) HCO_2H , DEAD, PPh_3 , THF, 70%; c) NaBH_4 , $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, MeOH, 92%; d) TBSCl, imidazole, CH_2Cl_2 , 73% for 2 steps; e) MOMCl, $i\text{Pr}_2\text{NEt}$, CH_2Cl_2 , 95%; f) NaI, acetone, RT; g) SmI_2 , THF, 73% for **22b** over 2 steps, 76% for **22c** over 2 steps; h) IBX, DMSO, RT, 72% for **23b**, 75% for **23c**; i) DBU, CH_2Cl_2 , RT, 95%; j) $\text{Hg}(\text{OAc})_2$, toluene; aq. KCl; k) I_2 , CH_2Cl_2 , 81% for 2 steps; l) HCO_2H , NaHCO_3 , DMF; MeOH- H_2O , 86%; m) PMBOC(NH) CCl_3 , $p\text{TSA}$, CH_2Cl_2 , 92%

Scheme 4.

Preparation of fragment **12**. a) 2.2 equiv. LDA, THF, -78°C, 82%; b) Tf₂O, aq. LiOH, hexanes, 98%; MeMgBr, CuCN, Et₂O, 95%; d) MeNH(OMe)•HCl, *i*PrMgBr, THF, 92%; e) **32**, THF, 98%; f) (*R*)-(+)-methyl-CBS-oxazaborolidine, BH₃•Me₂S, THF, 85%, e.e. < 5%.

Scheme 5.

Preparation of allyl iodide **11**. a) DIBAL-H, THF; DMP, CH₂Cl₂, 93% for 2 steps; b) 4-isopropyl-*N*-acetyl-1,3-thiazolidine-2-thione, Sn(OTf)₂, *N*-ethylpiperidine, CH₂Cl₂, -50 °C, 4 h, then **35**, CH₂Cl₂, -78 °C, 80%; c) TBSOTf, 2,6-lutidine, CH₂Cl₂; d) K₂CO₃, MeOH, 97% for 2 steps; e) TMSCH₂Li, CeCl₃, THF, -78 °C to RT; silica gel; f) NBS, propylene oxide, THF, RT; NaI, acetone, RT, 12 h, 76% for 3 steps; g) KHMDS, PhNTf₂, THF, 98%; h) TMSCH₂MgBr, LiCl, Pd(PPh₃)₄, Et₂O, 85%.

Scheme 6.

Synthesis of 22-deoxyalotaketals A (**44**). a) SmI_2 , THF, 0 °C; b) TBAF, THF, RT; c) *p*TSA, CH_2Cl_2 , 29% for 3 steps, **42:43** = 1:3 to 6; with PPTS, 31% for 3 steps, **42:43** = 1:1; d) IBX, DMSO, 87% for **44**, 89% for **45**.

Scheme 7.

Synthesis of (-)-alotaketal A (**2**). a) SmI_2 , THF, 0 °C; b) TBAF, THF, RT; c) PPTS, CH_2Cl_2 , RT, 40% for 3 steps; d) ; e) IBX, DMSO, 89%.