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Total Synthesis of the Potent cAMP Signaling Agonist (−)-Alotaketal A

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Supporting Information

ABSTRACT: We have developed a convergent synthetic route to the potent cAMP signaling agonist (−)-alotaketal A that employs two stages of SmI_2-mediated reductive allylation reactions for assembling the polycycle and fragment coupling. Also notable are a $Hg(OAc)_2$-mediated selective alkene oxidation and the subtlety of the formation of the unprecedented spiroketal ring system. The probes AKAR4 and ICUE3 were used to evaluate the cAMP singaling agonistic activity of (−)-alotaketal A and elucidate its structure−activity relationship.

Signaling through cyclic adenosine monophosphate (cAMP), the paradigm for the second messenger concept, is fundamental to a diverse range of cellular processes.[1] Such signaling is typically initiated by the binding of hormones to cell-surface G protein-coupled receptors (GPCRs), which leads to the recruitment of cellular guanine-nucleotide binding proteins (G proteins) and activation of adenylyl cyclases (ACs), the enzymes responsible for converting adenosine triphosphate (ATP) to cAMP. The elevated level of cAMP in turn regulates downstream cellular functions through effectors such as cAMP-dependent protein kinase (PKA) and the cAMP−GTP exchange factor Epac.[2,3] Formation of cAMP by ACs and degradation by cAMP-specific phosphodiesterases (PDEs) collectively determine cellular cAMP levels.

Traditional pharmacological regulation of cAMP signaling has employed GPCR agonists or antagonists and PDE inhibitors. ACs have also been pharmacologically targeted by the diterpenoid forskolin, which binds to ACs and activates their enzymatic activity.[4] Development of new modulators of cAMP signaling has implications for treating heart failure, cancer, and neurodegenerative diseases.[5] Thus, we were intrigued by a recent report from the Andersen lab describing the isolation of alotaketals A (**1**) and B (**2**) from the marine sponge *Hamigera* sp. collected in Papua New Guinea (Figure 1).[6] These compounds were found to cause potent activation of cAMP cell signaling in the absence of hormone binding in a cell-based pHTS-CRE luciferase reporter gene assay with half-maximal effective concentration (EC_{50}) values of 18 and 240 nM, respectively. In contrast, forskolin activates cAMP signaling with an EC_{50} of 3 μM. Alotaketals possess a sesterterpenoid carbon skeleton that cyclizes into a unique tricyclic spiroketal. In particular, simultaneous substitution of the spiroketal center by both allyl and vinyl groups is unprecedented in natural

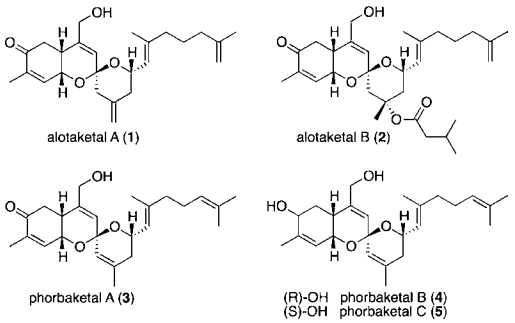

Figure 1. Alotaketals and phorbaketals.

spiroketals. Contemporaneous to the Andersen report, the Rho lab described the isolation of the closely related phorbaketals A−C (**3**−**5**) from the sponge *Phorbas* sp.[7] Their studies suggested that an unknown endosymbiotic microorganism might be the true producer of phorbaketals. We initiated our synthetic study of alotaketals and phorbaketals as part of a research program aimed at functionally characterizing natural products with useful biological properties. Herein we report the results of our efforts, which culminated in the first enantioselective total synthesis of (−)-alotaketal A and elucidation of the structure−activity relationship (SAR) of this potent agonist of cAMP signaling.

Our convergent synthetic design leading to alotaketal A is depicted in Scheme 1. We planned to construct the tricyclic molecular skeleton by spiroketalization of the alcohol derived from silyl deprotection of **6**. Unknown at the outset was the compatibility of the $\Delta^{11,23}$ alkene with the acidic reaction conditions that would be necessary to elaborate this unprecedented spiroketal ring system. Specifically, allylic activation of the C10 methylene by both the $\Delta^{11,23}$ alkene and the C9 oxocarbenium, to be transiently formed during spiroketalization, would cause the $\Delta^{11,23}$ alkene to be susceptible to undesired exo-to-endo isomerization. With the expectation that conditions to suppress such isomerization could be identified, we pursued this route because of the efficiency gained by convergent coupling of bicyclic lactone **7** with allyl iodide **8** to afford the fully functionalized hemiketal **6**. These two fragments would in turn be prepared from 5β-hydroxycarvone (**9**) and ethyl acetoacetate (**10**), respectively.

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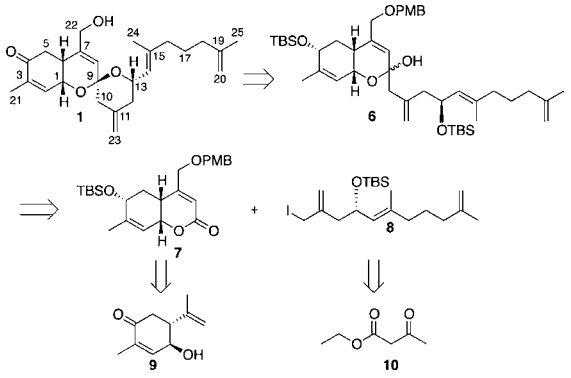
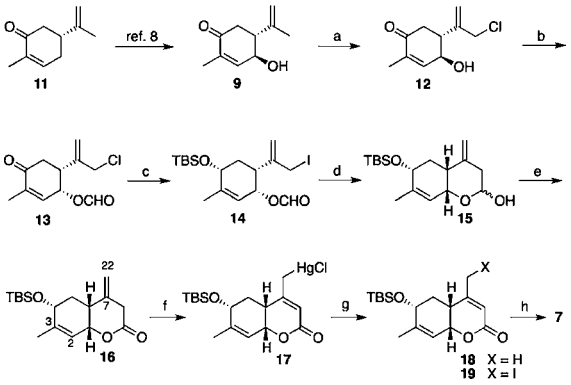
Scheme 1. Synthetic Design



We developed a reductive allylation approach to bicyclic lactone **7** (Scheme 2). Regioselective allylic chlorination of **9**, which was readily prepared from (*R*)-(−)-carvone (**11**) in two steps using the vinylogous *O*-nitroso Mukaiyama aldol approach we recently developed,[8] with HClO gave allylic chloride **12**.[9] Mitsunobu reaction of **12** with formic acid went smoothly to give **13** in 70% yield in the presence of the electrophilic allyl chloride moiety.[10] Diastereoselective Luche reduction of the enone of **13**,[11] protection of the hydroxyl group with TBSCl, and Finkelstein reaction gave iodide **14** as a single diastereomer.[12] As expected, the powerful yet underexplored reductive allylation approach reported by Keck,[13] achieved by treatment of **14** with excess SmI_2, led to smooth cyclization to give lactol **15** as an inconsequential mixture of epimers through intramolecular Barbier-type allylation of the formate. Even though excess SmI_2 was employed, further reduction of **15** was not observed. Oxidation of **15** with 2-iodoxybenzoic acid (IBX) furnished hydrobenzopyranone **16**.

Further functionalization of lactone **16** was complicated by its unexpected low reactivity toward common electrophilic reagents required for selective functionalization of the disubstituted $\Delta^{7,22}$ alkene in the presence of the trisubstituted $\Delta^{2,3}$ alkene. For example, no reaction occurred when **16** was treated with *m*-chloroperoxybenzoic acid (mCPBA) or *N*-iodosuccinimide (NIS) in CH_2Cl_2, while a complex mixture was obtained when dimethyldioxirane or CF_3CO_3H was used. Thus, after extensive experimentation, we were pleased to find that selective functionalization of the $\Delta^{7,22}$ alkene could be achieved through reaction of **16** with $Hg(OAc)_2$ to give allylmercury chloride **17** as the only product. This somewhat surprising chemoselectivity is likely due to facile rearrangement of the reversibly formed $\Delta^{7,22}$ mercurinium intermediate upon enolization of the lactone carbonyl of **16**. Since attempts at direct oxidation ($NaBH_4$, O_2) of the C−Hg bond of **17** led only to proto-demercuration product **18**,[14,15] the C22 hydroxyl group was introduced by iodinolysis of **17** followed by substitution of the resulting allyl iodide **19** with sodium formate. The initially formed formic ester was hydrolyzed upon basic workup. Protection of the C22 hydroxyl group as its *p*-methoxybenzyl (PMB) ether gave **7**.

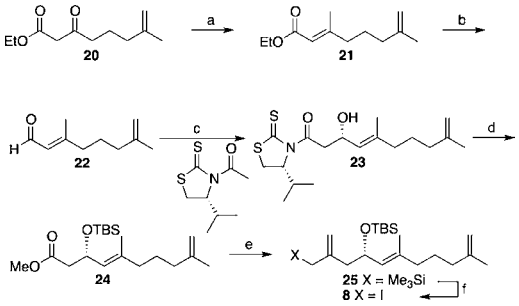
Scheme 2. Synthesis of Bicyclic Lactone 7[a]



[a]Reagents and conditions: (a) HClO, CH_2Cl_2, 64%. (b) HCO_2H, DEAD, PPh_3, THF, 70%. (c) (i) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH; (ii) TBSCl, imidazole, DMF, 88% for two steps; (iii) NaI, acetone. (d) SmI_2, THF, 73% for two steps. (e) IBX, DMSO, 72%. (f) $Hg(OAc)_2$, toluene; aq. KCl. (g) I_2, CH_2Cl_2, 81% for two steps. (h) (i) HCO_2H, $NaHCO_3$, DMF; $MeOH/H_2O$, 86%; (ii) $PMBOC(NH)CCl_3$, pTSA, CH_2Cl_2, 92%.

The synthesis of allyl iodide **8** started from the known *β*-keto ester **20** (Scheme 3).[16] Ester **20** was stereoselectively (>20:1) converted to **21** by the CuCN-mediated methylation of the corresponding (*Z*)-enol triflate,[17] which was prepared by treating **20** with Tf_2O/LiOH(aq) under biphasic conditions.[18] While similar methylation reactions could be catalyzed by $Fe(acac)_3$ in high yield,[19] significant isomerization of the alkene was observed under Fe catalysis. Application of a diisobutylaluminum hydride (DIBAL-H) reduction/Dess−Martin periodinane (DMP) oxidation sequence to ester **21** gave aldehyde **22**, which was subjected to the Nagao−Fujita aldol protocol to give **23** with excellent diastereoselectivity (>20:1 based on ^1H NMR analysis).[20,21] Alcohol **23** was converted to **24** by silylation and methanolysis.[22] Allylsilane **25** was prepared in good yield by reacting **24** with $Me_3SiCH_2Li/CeCl_3$ and then exposing the crude reaction mixture to silica gel for the Peterson elimination of the bis(trimethylsilyl)methylcarbinol intermediate.[23] Treatment of **25** with freshly recrystallized *N*-bromosuccinimide (NBS) at −78 °C in the dark[24] followed by Finkelstein reaction of the allyl bromide intermediate gave **8**.

Scheme 3. Synthesis of 8[a]



[a]Reagents and conditions: (a) (i) Tf_2O, LiOH(aq), hexanes, 98%; (ii) MeMgBr, CuCN, Et_2O, 95%. (b) (i) DIBAL-H, THF; (ii) DMP, CH_2Cl_2, 93% for two steps. (c) 4-Isopropyl-*N*-acyl-1,3-thiazolidine-2-thione, $Sn(OTf)_2$, *N*-ethylpiperidine, CH_2Cl_2, −50 °C, 4 h; then **22**, CH_2Cl_2, −78 °C, 80%. (d) (i) TBSOTf, 2,6-lutidine, CH_2Cl_2; (ii) K_2CO_3, MeOH, 97% for two steps. (e) Me_3SiCH_2Li, $CeCl_3$, THF, −78 °C to RT; silica gel. (f) (i) NBS, propylene oxide, THF, RT; (ii) NaI, acetone, RT, 12 h, 76% for three steps.

To investigate the nuances of this transformation and also to provide the 22-deoxy analogue of alotaketal A for SAR studies, we explored the coupling of **8** (or the corresponding bromide)

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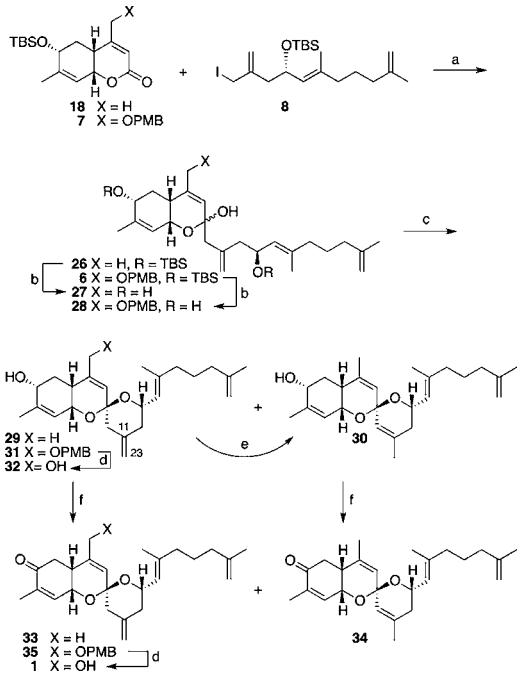
and bicyclic lactone **18** under a variety of conditions (Scheme 4). Whereas all attempts at coupling through the intermediacy

Scheme 4. Synthesis of 22-Deoxyalotaketal A (33) and Alotaketal A (1)[a]

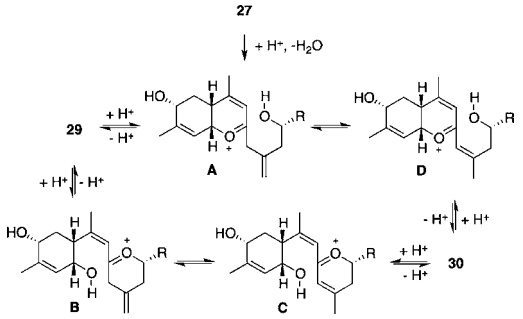


[a]Reagents and conditions: (a) SmI_2, THF. (b) TBAF, THF. (c) pTSA, CH_2Cl_2, 29% for three steps, **29**:**30** = 1:3−6; with PPTS, 31% for three steps, **29**:**30** = 1:1; 40% for **31** over three steps with PPTS. (d) DDQ, CH_2Cl_2/H_2O (10:1), 92% for **32**, 92% for **1**. (e) pTSA, CH_2Cl_2, RT. (f) IBX, DMSO, 87% for **33**, 89% for **34**, 89% for **35**.

of the allyllithium or allyl Grignard reagents prepared in situ were unsuccessful, the desired transformation occurred under Barbier conditions in which the allylsamarium reagent, generated in situ from **8** by treatment with SmI_2, combined with **18** to give **26** smoothly. Again, despite the presence of excess SmI_2, no over-reduction was observed. Since hemiacetal **26** was relatively unstable, it was subjected to desilylation with tetrabutylammonium fluoride (TBAF) to give **27** followed by spiroketalization with *p*-toluenesulfonic acid (pTSA) without purification of any intermediates. The desired spiroketalization to give **29** did occur, but it was accompanied by significant isomerization of the $\Delta^{11,23}$ alkene to afford the $\Delta^{10,11}$ isomer **30** as the major product (1:3−6). **29** and **30** were oxidized with IBX to give 22-deoxyalotaketal A (**33**) and its isomer **34**, respectively. The stereochemistry of the spiroketal centers was assigned by analogy to that of the natural product.

The formation of the $\Delta^{10,11}$ isomer **30** was mechanistically interesting because it could arise either from isomerization of oxocarbenium intermediate **A** by deprotonation/reprotonation to form **D** followed by cyclization to give **30** or from isomerization of **29** through the intermediacy of oxocarbenium **A** and/or **B** under the acidic conditions (Scheme 5). To illuminate the mechanistic subtleties of this process, the spiroketalization of **27** was tested using less acidic pyridinium *p*-toluenesulfonate (PPTS). Isomerization of the $\Delta^{11,23}$ alkene was again observed, but the two isomers **29** and **30** were formed in a ratio of ∼1:1. Further experiments showed that **29** could be readily isomerized to **30** upon treatment with pTSA.

Scheme 5. Isomerization of the $\Delta^{11,23}$ Alkene



However, no isomerization of **29** was observed when it was treated with PPTS. These results suggested that part of the exo-to-endo isomerization of the $\Delta^{11,23}$ alkene occurred through the intermediacy of oxocarbenium **A** prior to spiroketalization. However, the significant isomerization of the alkene in the pTSA-promoted spiroketalization of **27** was to a large extent due to the unchecked equilibration of **29** to its thermodynamically more favorable $\Delta^{10,11}$ isomer **30**.

On the basis of this model study, the completion of the alotaketal A synthesis involved the coupling of **7** and **8** with SmI_2 under Barbier conditions to give hemiketal **6** (Scheme 4). Desilylation of **6** with TBAF to give **28** was again followed by spiroketalization with PPTS. Interestingly, the spiroketalization proceeded smoothly to give **31** as a single diastereomeric product without $\Delta^{11,23}$ alkene isomerization. Since **27** and **28** differ only by the C22 *p*-methoxybenzyloxy group, we speculate that the electron-withdrawing inductive effect of the alkoxy group might be responsible for their differential reactivity profiles for spiroketalization. Alotaketal A was obtained by IBX oxidation of **31** and removal of the PMB protecting group with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). The ^1H and ^{13}C NMR spectra of the synthetic alotaketal A (**1**) were consistent with those of the natural product, as was its specific optical rotation ($[\alpha]_D^{25} = -40.2$ (*c* 0.15, MeOH) for synthetic **1**, $[\alpha]_D^{25} = -38.9$ (*c* 0.01, MeOH) reported for the natural product). Synthetic **1** was also identical to an authentic sample on the basis of TLC and HPLC.[25]

We examined the effects of **1** and its analogues **29**, **30**, and **32**−**34** on cAMP/PKA signaling using a genetically encoded A kinase activity reporter (AKAR4).[26] AKAR4 serves as a surrogate substrate for PKA and reports endogenous PKA activity via a change in Förster resonance energy transfer (FRET). First, we tested each of these compounds in HEK 293T cells transfected with the AKAR4 biosensor. **1** and **32** produced significant increases in the emission ratio of yellow over cyan [6.7 ± 2.2% (n = 24) and 5.3 ± 2.5% (n = 13), respectively; Figure 2], whereas no response was observed with the addition of **29**, **30**, **33**, or **34**. We further evaluated the specificity of the alotaketal-induced AKAR4 responses by utilizing an AKAR4 T/A mutant probe containing a mutated PKA phosphorylation site within the PKA substrate domain. This mutation abolishes PKA phosphorylation and the PKA-activity-induced FRET changes. No response was detected when cells expressing the AKAR4 T/A mutant were treated with **1** and **32**, confirming that these compounds induce PKA activity via the cAMP/PKA signaling pathway (Figure S1 in the Supporting Information).

To examine further the effects of **1** and **32** on cAMP accumulation, we used ICUE3, a FRET-based reporter for

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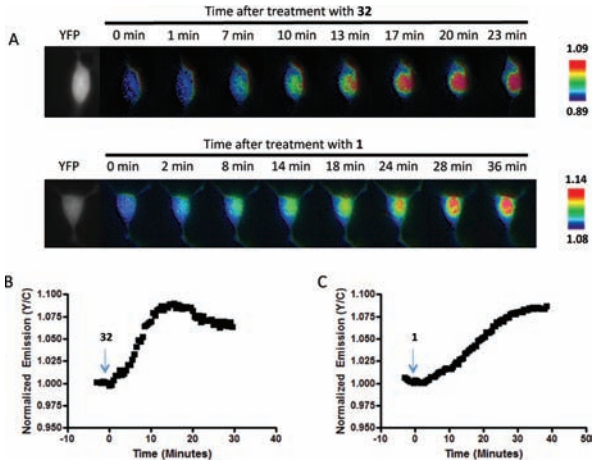

Figure 2. (A) Ratiometric images of HEK 293T cells expressing AKAR4 upon treatment with (top) **32** and (bottom) **1**. (B, C) Representative time-course curves depicting the AKAR4 response to (B) **32** (1 μM, n = 24) and (C) **1** (1 μM, n = 13).

cAMP.[27] The binding of cAMP to ICUE3 induces a conformational change that results in a decrease in FRET, which is detected as an increase in the cyan/yellow emission ratio. When treated with 1 μM **1** and **32**, the cells expressing ICUE3 showed 6.5 ± 0.32% (n = 10) and 4.4 ± 1.1% (n = 6) increases in the cyan/yellow emission ratio, respectively (Figure S2). These data suggest that both **1** and **32** increase PKA activity by increasing cellular levels of cAMP.

In summary, we have completed the first total synthesis of (−)-alotaketal A and confirmed its assigned absolute configuration. The synthesis features two Barbier-type intra- and intermolecular SmI_2-mediated reductive allylations for the efficient formation of two key C−C bonds. These reactions will likely find further applications in complex natural product synthesis. Also notable are the $Hg(OAc)_2$-mediated selective functionalization of the $\Delta^{7,22}$ alkene and the subtlety of the spiroketalization/isomerization of the unprecedented spiroketal ring system. We have also examined the cAMP agonistic activity of alotaketal A using the FRET-based AKAR4 and ICUE3 reporters and revealed the structure−activity relationships of these cAMP signaling pathway modulators. These studies set the stage for further investigations of the mode of action of alotaketal A, which will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(24) Williams, D. R.; Patnaik, S.; Plummer, S. V. *Org. Lett.* **2003**, *5*, 5035. The reaction had to be carried out in the dark with only a slight excess of freshly recrystallized NBS to suppress radical bromination of the remote 1,1-disubstituted alkene.
(25) The retention time of **1** was identical to that of the coinjected authentic alotaketal A using an Agilent ZORBAX Eclipse Plus C18 3.5 μM HPLC column eluted with 90:10 acetonitrile/water.
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