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Total Synthesis of the Potent cAMP Signaling Agonist (–)-Alotaketol A

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Abstract

We developed a convergent synthetic route to the potent cAMP signaling agonist (–)-alotaketol A employing two stages of SmI₂-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 for formation of the unprecedented spiroketal ring system. The AKAR4 and ICUE3 probes were used to evaluate the cAMP signaling agonistic activity of (–)-alotaketol 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 recruitment of cellular guanine-nucleotide binding proteins (G-protein) and activation of adenylyl cyclases (AC), the enzymes responsible for converting 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 adenylyl cyclase and degradation by cAMP-specific phosphodiesterases (PDE) collectively determine cellular cAMP levels.

Traditional pharmacological regulation of the cAMP signaling has been through GPCR agonists or antagonists and PDE inhibitors. The adenylyl cyclases have also been pharmacologically targeted by the diterpenoid forskolin, which binds to adenylyl cyclases and activates their enzymatic activity.⁴ Development of new modulators of the cAMP signaling have implications for treating heart failure, cancer, and neurodegenerative diseases.⁵ Thus, we were intrigued by a recent report from the Andersen lab describing isolation of alotaketol A (**1**) and B (**2**) from the marine sponge *Hamigera* sp. collected in Papua New Guinea (Figure 1).⁶ These compounds were found to potently activate cAMP cell signaling in the absence of hormone binding in a cell-based pHTS-CRE luciferase reporter gene assay with EC₅₀ values of 18 and 240 nM, respectively. In contrast, forskolin activates the cAMP signaling with an EC₅₀ of 3 μM. Alotaketols 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 spiro-ketals. Contemporaneous to the Andersen report, the Rho lab described 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

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

of phorbaketals. We initiated our synthetic study of alotaketals/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 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, 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 could be identified to suppress such isomerization, we pursued this route given 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 5 β -hydroxycarvone **9** and ethyl acetoacetate **10**, respectively.

We developed a reductive allylation approach to the bicyclic lactone **7** as shown in Scheme 2. Regioselective allylic chlorination of 5 β -hydroxycarvone **9**, readily prepared from R-(–)-carvone in 2 steps using the vinylogous *O*-nitroso Mukaiyama aldol approach we recently developed,⁸ with hypochlorous acid 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 under-explored reductive allylation approach reported by Keck,¹³ achieved by treatment of **14** with excess SmI₂ 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 SmI₂ was employed, further reduction of **15** was not observed. Oxidation of **15** with IBX furnished the hydrobenzopyranone **16**.

Further functionalization of lactone **16** was complicated by its unexpected low reactivity toward common electrophilic reagents required to selectively functionalize 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 mCPBA or NIS in CH₂Cl₂ while a complex mixture was obtained when dimethyldioxirane or CF₃CO₃H 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 likely was due to facile rearrangement of the reversibly formed $\Delta^{7,22}$ mercurinium intermediate upon enolization of the lactone carbonyl of **16**. Since attempts for direct oxidation (NaBH₄, O₂) of the C-Hg bond of **17** only led 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 work-up. Protection of the C22-hydroxyl group as its PMB ether gave **7**.

The synthesis of allyl iodide **8** started from the known β -ketoester **20** (Scheme 3).¹⁶ It 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 Tf₂O-aq. LiOH 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. A reduction/oxidation sequence of ester **21** by DIBAL-H/Dess-Martin periodinane gave aldehyde **22** which was subjected to the Nagao-Fujita aldol protocol to give **23** with

excellent diastereoselectivity (>20:1, based on ^1H NMR).^{20,21} Alcohol **23** was converted to **24** by silylation and methanolysis.²² The allylsilane **25** was prepared in good yield by reaction of **24** with $\text{Me}_3\text{SiCH}_2\text{Li}-\text{CeCl}_3$ followed by exposing the crude reaction mixture to silica gel for the Peterson elimination of the bis(trimethylsilyl)methyl carbinol intermediate.²³ The allyl iodide **8** was obtained by treating **25** with freshly recrystallized NBS at -78°C in the dark followed by Finkelstein reaction of the allyl bromide intermediate.²⁴

We anticipated that the fragments of alotaketal A could be joined through allylation of the bicyclic lactone **7** with allyl iodide **8**. To investigate the nuances of this transformation and also provide the 22-deoxy analogue of alotaketal A for SAR studies, we explored the coupling of allyl iodide **8** (or the corresponding bromide) and the bicyclic lactone **18** under a variety of conditions (Scheme 4). Whereas all attempts for coupling through the intermediacy of the allyllithium or the allyl Grignard reagents prepared in situ were unsuccessful, the desired transformation occurred under Barbier conditions in which the allyl samarium 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 TBAF to give alcohol **27** followed by spiroketalization with pTSA without purification of any intermediates. The desired spiroketalization indeed occurred to give **29**, but was accompanied by significant isomerization of the $\Delta^{11,23}$ alkene to afford **30** as the major product (1:3–6). Both **29** and **30** were oxidized with IBX to give 22-deoxyalotaketal A (**33**) and the isomeric **34**. 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 either arise from isomerization of the oxocarbenium intermediate **A** by deprotonation/reprotonation to form **D** followed by cyclization to give **30** or due to isomerization of **29** through the intermediacy of the 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 PPTS. Isomerization of the $\Delta^{11,23}$ alkene was again observed, but the two isomers **29** and **30** were formed in $\sim 1:1$ ratio. Further experiments showed that **29** could be readily isomerized to **30** upon treatment with pTSA. However, no isomerization of **29** was observed when it was treated with PPTS. These results suggested that the partial 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 under the pTSA-promoted spiroketalization of **27** was to a large extent due to unchecked equilibration of **29** to its thermodynamically more favourable $\Delta^{10,11}$ isomer **30**.

Building on this model study, the completion of alotaketal A synthesis involved coupling of **7** and **8** with SmI_2 under Barbier conditions to give hemiketal **6** (Scheme 4). Desilylation with TBAF to give **28** and spiroketalization was again achieved 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** only differ by the C22 p-methoxybenzyloxyl 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 DDQ. The ^1H and ^{13}C NMR spectra of the synthetic alotaketal A were consistent with those of the natural product, as well as its specific optical rotation ($[\alpha]^{25}_{\text{D}} = -40.2$ (c 0.15, MeOH) for synthetic **1**, $[\alpha]^{25}_{\text{D}} = -38.9$ (c 0.01, MeOH) reported

for the natural product). Synthetic alotaketal A (**1**) was also identical to an authentic sample by TLC and HPLC.²⁵

We examined the effects of alotaketal A (**1**) and analogs (i.e. **29**, **30**, **32**, **33**, and **34**) on cAMP/PKA signaling using a genetically encoded A kinase activity reporter (AKAR4).²⁶ AKAR 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. Alotaketal A (**1**) and **32** produced significant increases in the emission ratio of yellow over cyan, $6.7 \pm 2.2\%$ ($n = 24$) and $5.3 \pm 2.5\%$ ($n = 13$) 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 AKAR responses by utilizing an AKAR4 T/A mutant probe that contains 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).

To further examine the effects of **1** and **32** on cAMP accumulation, we used ICUE3, a FRET-based reporter for cAMP.²⁷ The binding of cAMP to ICUE3 induces a conformational change, resulting in a decrease in FRET detected as an increase in emission ratio of cyan over yellow. When treated with $1\mu\text{M}$ of **1** and **32**, the cells expressing ICUE3 showed $6.5 \pm 0.32\%$ ($n = 10$) and $4.4 \pm 1.1\%$ ($n = 6$) increases in cyan-to-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 completed the first total synthesis of (–)-alotaketal A and confirmed its assigned absolute configuration. The synthesis features two Barbier-type intra- and inter-molecular SmI_2 -mediated reductive allylations for efficient formation of two key C-C bonds. These reactions will likely find further applications in complex natural product synthesis. Also notable are the $\text{Hg}(\text{OAc})_2$ -mediated selective functionalization of the $\Delta^{7,22}$ alkene and the subtlety of the spiroketalization/isomerization of the unprecedented spiroketal ring system. We 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Alotaketals and phorbaketals

Figure 2.

(A) Ratiometric images of HEK 293T cells expressing AKAR4 treated with **32** (top panel) and **1** (bottom panel). (B) Representative time course curves depicting AKAR4 response to **32** (1 μ M, $n = 24$) and (C) **1** (1 μ M, $n = 13$).

Scheme 1.
Synthetic design

Scheme 2.**Synthesis of the bicyclic lactone 7**

Reagents and conditions: a) HClO , CH_2Cl_2 , 64%; b) HCO_2H , DEAD, PPh_3 , THF, 70%; c) i. NaBH_4 , $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, MeOH; ii. TBSCl , imidazole, DMF, 88% for 2 steps; iii. NaI , acetone; d) SmI_2 , THF, 73% for 2 steps; e) IBX , DMSO, 72%; f) $\text{Hg}(\text{OAc})_2$, toluene; aq. KCl ; g) I_2 , CH_2Cl_2 , 81% for 2 steps; h) i. HCO_2H , NaHCO_3 , DMF; MeOH- H_2O , 86%; ii. $\text{PMBOC}(\text{NH})\text{CCl}_3$, pTSA , CH_2Cl_2 , 92%.

Scheme 3.**Synthesis of 8**

Reagents and conditions: a) i. TiF_2O , aq. LiOH , hexanes, 98%; ii. MeMgBr , CuCN , Et_2O , 95%; b) i. Dibal-H, THF; ii. DMP, CH_2Cl_2 , 93% for 2 steps; c) 4-isopropyl-*N*-acetyl-1,3-thiazolidine-2-thione, $\text{Sn}(\text{OTf})_2$, *N*-ethylpiperidine, CH_2Cl_2 , $-50\text{ }^\circ\text{C}$, 4h; then **22**, CH_2Cl_2 , $-78\text{ }^\circ\text{C}$, 80%; d) i. TBSOTf, 2,6-lutidine, CH_2Cl_2 ; ii. K_2CO_3 , MeOH, 97% for 2 steps; e) $\text{Me}_3\text{SiCH}_2\text{Li}$, CeCl_3 , THF, $-78\text{ }^\circ\text{C}$ to RT; silica gel; f) i. NBS, propylene oxide, THF, RT; ii. NaI, acetone, RT, 12h, 76% for 3 steps.

Scheme 4.

Synthesis of 22-deoxyalotaketal A (**33**) and alotaketal A

Reagents and conditions: a) SmI_2 , THF; b) TBAF, THF; c) pTSA, CH_2Cl_2 , 29% for 3 steps, **29:30** = 1:3–6 (1:1 with PPTS, 31% for 3 steps); 40% for **31** over 3 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**.

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