Published in final edited form as:

Angew Chem Int Ed Engl. 2013 October 4; 52(41): 10796–10799. doi:10.1002/anie.201304812.

Dehydrogenative Coupling Enables the Enantioselective Total Synthesis of (-)-Simaomicin α

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Keywords

Synthesis; dehydrogenative coupling; simaomicin; xanthone natural products; cytotoxicity

Simaomicin α (**1**) is a polycyclic xanthone natural product with remarkable biological properties. It was isolated by scientists from American Cyanamid Company from the culture broth of an actinomycete, and its structure and relative stereochemistry were established by NMR spectroscopy and X-ray crystallography.^[1] Simaomicin α originally garnered attention for its ability to prevent coccidiosis infection in chickens that were fed meal containing 1 mg drug/kg feed. The isolation group noted that it was ‘the most potent natural-occurring anticoccidial agent reported.’^[2] Fifteen years after its structure and anti-parasitic activity was reported, Tomoda and co-workers revealed that simaomicin α had little effect on Jurkat cells as a single agent at low concentration (0.6 nM), but that it synergized with the DNA-damaging agent bleomycin. Thus, while bleomycin caused G2-arrest, the combination of bleomycin and simaomicin α was cytotoxic against this T lymphocyte cell line. Provocatively, even much higher concentrations of simaomicin α (500 nM) had little effect on a normal HUVEC cell line. The molecular underpinnings for these effects are unknown, but the checkpoint regulators Chk1, Chk2, ATM, ATR and Wee1 are not inhibited.^[3] The natural product was found to suppress phosphorylation of retinoblastoma protein,^[4] but a molecular target has not been identified. The therapeutic implication of these observations is that co-administration of a DNA-damaging agent with a cell-cycle inhibitor could be more efficacious than treatment with a single agent.

Given the potent biological activity and unique structure of simaomicin α , we targeted it for total synthesis.^[5] Our work builds on the discoveries from the Kelly group, which reported the first synthesis of a polycyclic xanthone natural product, cervinomycin A₂ (Scheme 1, top).^[6] Subsequently the Suzuki group disclosed a synthesis of the FD-594 aglycon,^[7] and Porco and coworkers^[8] and our group^[9] independently synthesized kibdelone C. The latter studies were the first to target members of this class that feature a tetrahydroxanthone ring

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system with its attendant stereogenic centers. In all of these syntheses, formation of the B-D biaryl bond represented a critical synthetic step that allowed assembly of the polycyclic ring system from simpler precursors. Early approaches relied on low-yielding photocyclizations of a diaryl olefin while more recent work has focused on transition metal-promoted couplings. For example, Pd-mediated couplings of an aryl iodide with an aryl C-H bond proved successful for both kibdelone C^[9] and FD-594 aglycon,^[7] whereas the Porco synthesis introduced a novel Pt-catalyzed addition of a phenol to a quinone mono-acetal^[8]. Here we disclose an advance in the synthesis of polycyclic xanthone natural products that involves a Pd-mediated direct dehydrogenative coupling of two unfunctionalized aryl rings to forge the key B-D biaryl linkage. This transformation enabled the enantioselective synthesis of simaomicin α and the assignment of its absolute stereochemistry.

Our synthetic strategy (Scheme 1, bottom) envisioned assembling the AB-ring isoquinolinone and the DEF tetrahydroxanthone subunits from simpler precursors. They would then be joined to form an advanced intermediate lacking the central C-ring (**2**). This approach was designed to maximize convergency. The C7 stereocenter (simaomicin numbering) is remote from the C14 and C17 stereocenters within the F-ring, so we planned to exploit asymmetric reduction technology for its establishment. Finally, formation of the B-D biaryl bond would form the C-ring and complete the synthesis of the carbon skeleton of simaomicin α . A variety of options existed for the final biaryl bond formation, but we were attracted to the possibility of a direct dehydrogenative coupling.^[10] A dual C-H functionalization would allow us to avoid introducing reactive groups such as halides or metals at C22 and C23, thereby simplifying the synthesis.

A convergent synthesis of the AB-DEF ring system of simaomicin α is shown in Scheme 2. The absolute stereochemistry of the natural product was established with hydroxyenone **3**, the product of an enzymatic resolution (>95% ee).^[11] Benzylation, iodination and Luche reduction provided the vinyl iodide **4** as an inconsequential mixture of diastereomers. Methyl lithium deprotonated the alcohol in **4**, and *tert*-BuLi induced metal-halogen exchange to generate a vinyl anion, which added cleanly to the o,o'-disubstituted aldehyde **6** to link the D and F-rings. The resulting mixture of diastereomers was oxidized to an enedione, which was stable to aqueous work-up, but not purification. Accordingly, the crude diketone was exposed to perchloric acid at elevated temperatures to effect removal of the MOM protecting group, cyclocondensation with the C13 ketone, and addition of water to C17 to yield the DEF-ring tetrahydroxanthone **8** (d.r. 1:1).^[12] The pure *trans* diastereomer of the tetrahydroxanthone (**10**) could be obtained following an oxidation/Noyori transfer hydrogenation sequence.^[13] Alternatively, the mixture of C17 diastereomers could be separated chromatographically after introduction of a benzyloxymethyl (BOM) group. Completion of the DEF-ring fragment involved allylation and oxidative cleavage to form aldehyde **11**. In the latter transformation, performing the dihydroxylation and periodate cleavage separately proved critical; using conditions that combine OsO₄ and NaIO₄ led to the excision of two carbons and the isolation of the corresponding substituted benzaldehyde.

We planned to join all the carbons of simaomicin α by coupling aldehyde **11** with an appropriate isoquinolinone. To that end, the AB-ring subunit **15** was prepared in good yield through a sequence that involved amidation of *N*-methyl glycinol^[14] with acid **12** and

subsequent oxidation to aldehyde **14**.^[15] Treatment with BCl₃ promoted cyclization and selective removal of one *O*-methyl group. Dehydration with *p*-toluene sulfonic acid and silylation of the phenol then yielded the requisite heterocycle (**15**).^[16]

Nucleophilic addition to aldehyde **11** proved challenging. The C8 methylene is acidified by the adjacent aldehyde and electron deficient aryl ring. For this reason, the lithium reagent derived from aryl bromide **15** simply deprotonated aldehyde **11**. Likewise, transmetalation with MgBr₂, ZnCl₂, Ti(OiPr)₄ and even CeCl₃ proved ineffective as enolization dominated the reaction. Remarkably, inclusion of LiCl along with CeCl₃, or, optimally, LaCl₃, facilitated nucleophilic addition to join the two heterocyclic ring systems. The utility of LaCl₃ 2LiCl, first reported by Knochel and co-workers, is largely due to the enhanced solubility of this reagent compared to LaCl₃ or CeCl₃.^[17]

Completion of the full carbon skeleton of simaomicin α required adjustment of the stereochemistry at C7 and incorporation of the methylene acetal. To this end, a C7 ketone was reduced with Noyori's catalyst,^[13] and the B-ring silyl and methyl groups were removed. The resulting triol was alkylated with CH₂ICl in the presence of Cs₂CO₃, forming the dioxane **2** in good yield. Surprisingly, this transformation required extensive optimization, with other bases and dihalomethanes resulting in substantially lower yields. We speculate that the reaction involves initial alkylation of the C5 phenol followed by cyclization by the C7 alcohol. Weaker bases or poorer electrophiles (e.g. CH₂Br₂) led to low conversion, while reagents with two good leaving groups (e.g. CH₂I₂) appeared to promote polymerization.

We next targeted formation of B-D biaryl bond to forge the C-ring and construct the carbon skeleton of simaomicin α . In the course of our synthetic studies on kibdelone C we had prepared model system **18**. Exposure to Pd(OAc)₂ in DMSO at elevated temperatures effected a direct dehydrogenative coupling in modest yield, although cleanly (eq 1).^[11] Unfortunately, as is common with model systems, this one led us astray: we were unable to achieve the analogous transformation in the context of the entire skeleton of kibdelone C. In particular, the tetrahydroxanthone **20** failed to cyclize under a variety of conditions featuring Pd(II), Cu(II), Fe(III) or I(III) (eq 2). We ascribed the failure of the more advanced substrate (**20**) to cyclize to the presence of an electron-withdrawing carbonyl at C8 (kibdelone C numbering). Presumably the ketone deactivates the D-ring such that it is unable to participate in an electrophilic palladation. Eventually we solved the problem by introducing an iodide at C4 and decorating the C3 phenol with a Boc group. A subsequent Pd-mediated dehydrohalogenative coupling formed the key biaryl bond and enabled a synthesis of kibdelone C.^[9]

Cognizant of the failure of direct dehydrogenative coupling in the synthesis of kibdelone C, we were intrigued by the rapid and high-yielding cyclization of simaomicin model system **22** (eq 3). Specifically, while the kibdelone model **18** cyclized slowly (ca. 50 % conversion after 2 d), the presence of the methylene acetal in substrate **22** led to complete conversion in 12 h.^[18] We attribute the improvement offered by the 1,3-dioxane ring to a conformational bias in which the rigidity imparted by the extra ring brings C22 and C23 (simaomicin numbering) into closer proximity.^[19] With this result as encouragement, we then examined

the reactivity of the more elaborate substrate **2**. Gratifyingly, heating a solution of the free phenol and Pd(OAc)₂ in DMSO resulted in a dehydrogenative coupling of the B and D-rings to form the C-ring and the full skeleton of the natural product. Finally, removal of the benzyl and BOM groups provided optically active simaomicin α . The latter transformation required carefully controlled reaction parameters, with only failure meeting a variety of alternative conditions including hydrogenation, dissolving metal and other Lewis acids. Generally, removal of the D-ring benzyl group occurred rapidly, but the remaining ethers showed significant stubbornness. The optimized conditions featured a large excess of BCl₃ at low temperatures. Ultimately, the natural product was isolated in high yields and purity, with ¹H NMR, ¹³C NMR and mass data consistent with the reported values. Only the optical rotation value differed from the isolation report as it was similar in magnitude but opposite in sign [-759 (synthetic, c = 0.06, DMF) vs. +836 (natural, c = 0.3, DMF)].^[20] We therefore conclude that the natural product is in fact enantiomeric to the structures shown herein. The natural enantiomer of simaomicin α should be easily accessible from *ent*-**3**, which can be obtained from the same enzymatic resolution that is used to generate **3**, and by using (*S,S*)-Ru-**9** for hydrogenation.

Simaomicin α is reported to synergize with the DNA-damaging agent bleomycin at sub-nM concentrations, and arrest cells in the G1 phase at low nM concentrations as a single agent.^[3] Moreover, it reduced cell proliferation by 50% (IC₅₀) at concentrations between 0.3 and 19 nM for a range of breast adenocarcinoma, cervical carcinoma, gastric adenocarcinoma, esophageal carcinoma and colon cancer cell lines. Growth inhibition appears to result from both G1 arrest and apoptosis.^[4] No molecular target has been identified for simaomicin α or other polycyclic xanthone natural products. In this context, we tested the cytotoxicity of synthetic *ent*-simaomicin α against a colon cancer cell line (HCT116) and two non-small cell lung cancer lines (H460, H1819). We found IC₅₀ values of 21, 13 and 87 nM, the first of which is equivalent to the value reported for *nat*-simaomicin α against HCT116 cells (11.0 nM; H2122 and H460 were not reported). At concentrations over 1 μ M, complete cell death was observed. Moreover, *ent*-simaomicin α proved toxic to *Bacillus subtilis* with a minimum inhibitory concentration (MIC) of 280 nM, which is similar to that reported for *nat*-simaomicin α against *B. cereus* (110 M)^[21] Taken together, the toxicity data suggests that the two enantiomers of simaomicin α possess similar biological activity, a surprising result, but one with precedent within the class of type-II polyketides.^[21]

In conclusion, we report the first total synthesis of simaomicin α , and establish its absolute stereochemistry. Noteworthy features of the synthesis include its convergent nature, the use of LaCl₃ 2LiCl in a late-stage fragment union, and a direct dehydrogenative coupling to complete the carbon skeleton of the natural product. Initial biological profiling revealed that (+)- and (-)-simaomicin α display similar biological activity. Ongoing experiments aim to identify a binding partner for this natural product and understand its mode of toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Financial support from the American Cancer Society, the Welch Foundation (I-1612) and the NIH (R01 GM102403). We thank Ms. Maria Goralski and Dr. Deepak Nijhawan for performing cytotoxicity assays and Dr. Michael Nottingham for performing antibiotic assays.

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18. Substrates possessing oxygenation at C7 (OH or OMOM) but not the cyclic acetal did not undergo dehydrogenative coupling, indicated that the rigidity of the dioxane ring rather than the C7 oxygenation is the important difference between substrates **19** and **23**.
19. Molecular modeling indicates that the 1,3-dioxane brings C22 and C23 closer together and orients the B and D rings appropriately for C-C bond formation. See Figure S2.
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Scheme 1.

Top: polycyclic xanthone natural products. Bottom: convergent synthetic approach to simaomicin α .

a) BnBr, Ag₂O, CH₂Cl₂, rt, 95% yield. b) I₂, N,N-dimethylaminopyridine (cat.) CH₂Cl₂/pyridine 2:1, rt. c) NaBH₄, CeCl₃·H₂O, MeOH, -78 °C, 70% yield over 2 steps, 4:1 *anti:syn*. d) Me₂AlCl, (CH₂O)_n, CH₂Cl₂, 0 °C to rt. e) Aliquat 336, NaOH (aq.), CH₃OCH₂Cl, CH₂Cl₂, rt. f) PCC/Al₂O₃, CH₂Cl₂, rt, 77% yield over 3 steps. g) CH₃Li then t-BuLi, THF/toluene (1:5), -78 °C, 78% yield, d.r. ca. 4:4:1:1. h) Dess-Martin periodinane (DMP), CHCl₃, 0 °C to rt. i) 1 M HClO₄, THF, 42 °C, 73% yield, d.r. 1:1. j) DMP, CH₂Cl₂, 0 °C to rt. k) (R,R)-Ru-9, Et₃N, HCO₂H, CH₃CN, rt. l) BOM-Cl, Proton Sponge, NaI, THF, rt. 65% yield over 3 steps, d.r. >10:1. m) BOM-Cl, Proton Sponge, NaI, THF, rt. 30% yield *cis*-10, 30% yield *trans*-10. n) PdCl₂(PhCN)₂ (5 mol%), X-Phos (15 mol%), (Allyl)Sn^{IV}Bu₃, Cs₂CO₃, toluene, 80 °C. o) OsO₄ (15 mol%), N-methylmorpholine-N-oxide, THF/H₂O 1:3, rt. p) NaIO₄/SiO₂, CH₂Cl₂, rt. q) (COCl)₂, DMF (cat), CH₂Cl₂, rt; 13, 2.5 M NaOH/CH₂Cl₂, rt. r) 2,2,6,6-tetramethylpiperidine-1-oxyl (10 mol%), Ph(OAc)₂, CH₂Cl₂, rt. s) BCl₃ (1 then 1.5 equiv), CH₂Cl₂, rt; TsOH, toluene, reflux. 54% over 4 steps. t) TIPSOTf, 2,6-lutidine, rt. 95% yield. u) t-BuLi (1.05 equiv), then LaCl₃(LiCl)₂ (1.1 equiv), THF, -78 °C; then 11. 76% yield, d.r. 1:1. v) DMP, NaHCO₃, CH₂Cl₂, rt. w) (R,R)-Ru-9, Et₃N, HCO₂H, CH₃CN, rt. 92% yield over 2 steps, d.r. 13:1. x) Bu₄NF, THF, 0 °C. y) Ce(NH₄)₂(NO₃)₆, CH₃CN/H₂O 20:3; then Na₂S₂O₄. z) ICH₂Cl, Cs₂CO₃, DMF, 65% over 3 steps. Bn: benzyl; PCC: pyridinium chlorochromate; BOM: benzyloxymethyl; DMF: dimethylformamide.

Scheme 2.

Enantioselective convergent synthesis of the AB-DEF ring system of simaomicin α.

Scheme 3.

Synthesis of simaomicin α via dehydrogenative coupling.