Total Synthesis

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Dehydrogenative Coupling to Enable the Enantioselective Total Synthesis of (−)-Simaomicin α**

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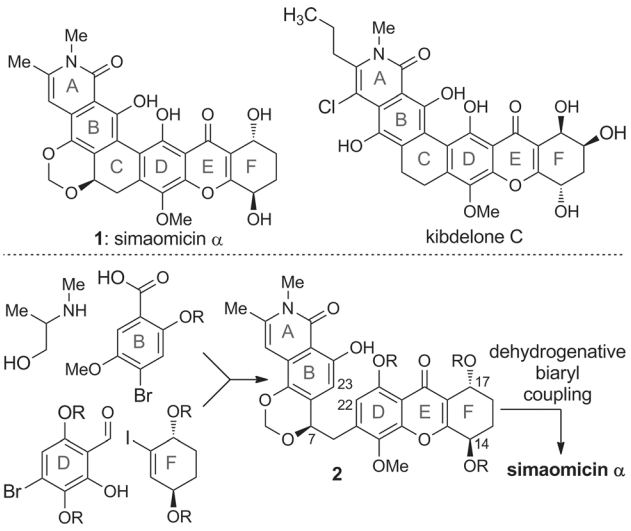
Simaomicin α (**1**) is a polycyclic xanthone natural product with remarkable biological properties. It was isolated by scientists from American Cyanamid Company from the culture broth of an actinomycete, and its structure and relative stereochemistry were established by NMR spectroscopy and X-ray crystallography.[1] 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 A_2.[6] Subsequently the Suzuki group disclosed a synthesis of the FD-594 aglycon,[7] and Porco and co-workers[8] and our group[9] independently synthesized kibdelone C (Scheme 1, top). The latter studies were the first to target members of this class that feature a tetrahydroxanthone ring system with its attendant stereogenic centers. In all of these syntheses, formation of the B–D biaryl bond was 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 was 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 monoacetal.[8] Herein we present 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.



Scheme 1. Top: polycyclic xanthone natural products. Bottom: convergent synthetic approach to simaomicin α.

Our synthetic strategy (Scheme 1, bottom) envisioned assembling the AB-ring isoquinolinone and the DEF tetrahyroxanthone 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

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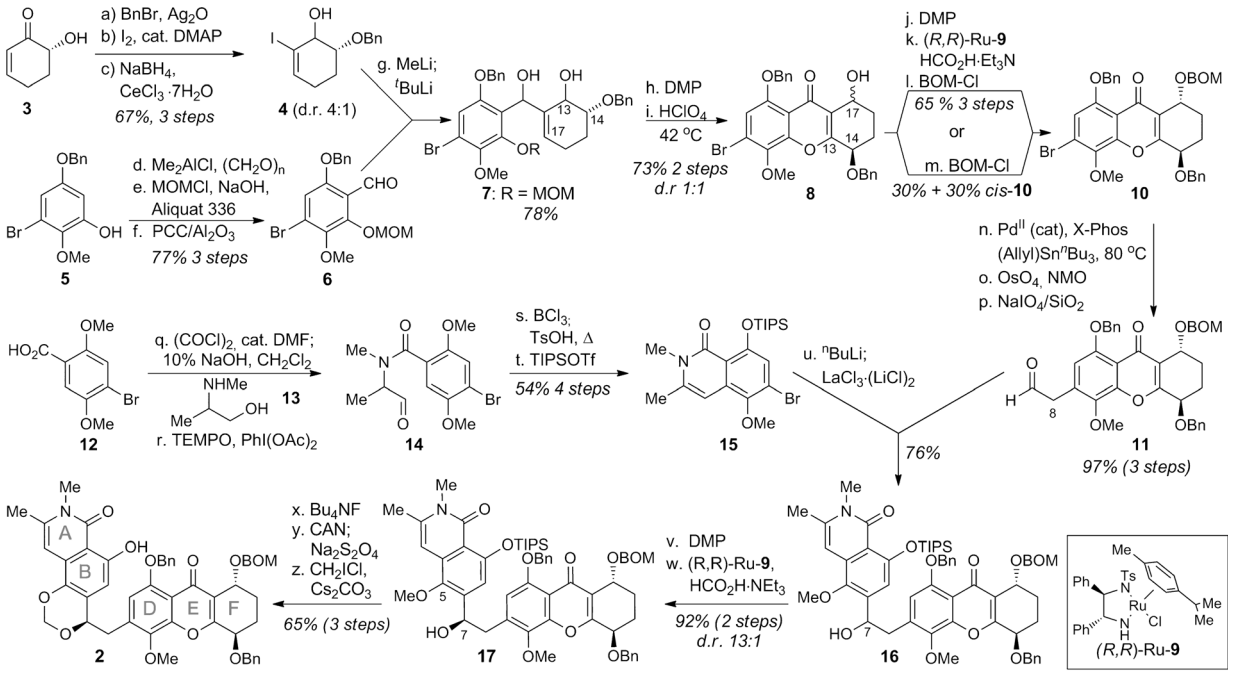


a) BnBr, Ag_2O, CH_2Cl_2, rt, 95% yield. b) I_2, N,N-dimethylaminopyridine (cat.) CH_2Cl_2/pyridine 2:1, rt. c) $NaBH_4$, $CeCl_3{\cdot}H_2O$, MeOH, -78 °C, 70% yield over 2 steps, 4:1 *anti:syn*. d) Me_2AlCl, $(CH_2O)_n$, CH_2Cl_2, 0 °C to rt. e) Aliquat 336, NaOH (aq.), CH_3OCH_2Cl, CH_2Cl_2, rt. f) PCC/Al_2O_3, CH_2Cl_2, rt, 77% yield over 3 steps. g) CH_3Li then ^{t}BuLi, THF/toluene (1:5), -78 °C, 78% yield, d.r. ca. 4:4:1:1. h) Dess-Martin periodinane (DMP), $CHCl_3$, 0 °C to rt. i) 1 M $HClO_4$, THF, 42 °C, 73% yield, d.r. 1:1. j) DMP, CH_2Cl_2, 0 °C to rt. k) (*R,R*)-Ru-**9**, Et_3N, HCO_2H, CH_3CN, 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) $PdCl_2(PhCN)_2$ (5 mol%), X-Phos (15 mol%), (Allyl)Sn^{n}Bu$_3$, Cs_2CO_3, toluene, 80 °C. o) OsO_4 (15 mol%), *N*-methylmorpholine-*N*-oxide, THF/H_2O 1:3, rt. p) $NaIO_4/SiO_2$, CH_2Cl_2, rt. q) $(COCl)_2$, DMF (cat), CH_2Cl_2, rt; **13**, 2.5 M NaOH/CH_2Cl_2, rt. r) 2,2,6,6-tetramethylpiperdine-1-oxyl (10 mol%), $PhI(OAc)_2$, CH_2Cl_2, rt. s) BCl_3 (1 then 1.5 equiv), CH_2Cl_2, rt; TsOH, tol, reflux. 54% over 4 steps. t) TIPSOTf, 2,6-lutidine, rt. 95% yield. u) ^{n}BuLi (1.05 equiv), then $LaCl_3{\cdot}(LiCl)_2$ (1.1 equiv), THF, -78 °C; then **11**. 76% yield, d.r. 1:1. v) DMP, $NaHCO_3$, CH_2Cl_2, rt. w) (*R,R*)-Ru-**9**, Et_3N, HCO_2H, CH_3CN, rt, 92% yield over 2 steps, d.r. 13:1. x) Bu_4NF, THF, 0 °C. y) $Ce(NH_4)_2(NO_3)_6$, CH_3CN/H_2O 20:3; then $Na_2S_2O_4$. z) ICH_2Cl, Cs_2CO_3, 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.

C–H functionalization would allow us to avoid introducing reactive groups such as halides or metals at C22 and C23, thereby simplifying the synthesis.

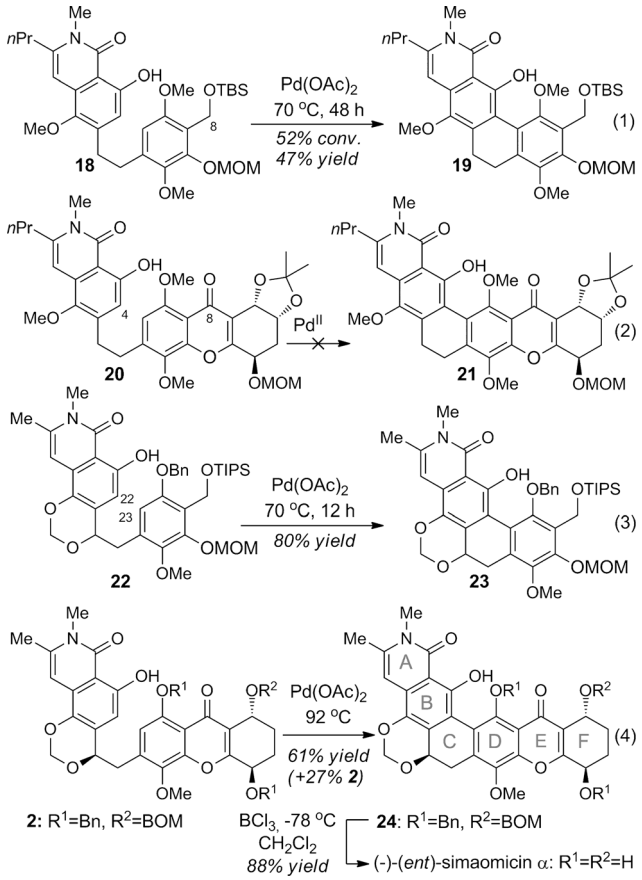
A convergent synthesis of the AB–DEF ring system of simaomicin α is shown in Scheme 2. The absolute stereochemistry of the natural product was established with hydroxyenone **3**, the product of an enzymatic resolution (>95% *ee*).[11] Benzylation, iodination, and Luche reduction provided the vinyl iodide **4** as an inconsequential mixture of diasteromers. Methyllithium 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 to be critical; using conditions that combine OsO_4 and $NaIO_4$ led to the excision of two carbon atoms and the isolation of the corresponding substituted benzaldehyde.

We planned to join all of the carbon atoms 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 carboxylic acid **12** and subsequent oxidation to aldehyde **14**.[15] Treatment with BCl_3 promoted cyclization and selective removal of one *O*-methyl group. Dehydration with *p*-toluenesulfonic 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 $MgBr_2$, $ZnCl_2$, $Ti(OiPr)_4$, and even $CeCl_3$ proved ineffective, as enolization dominated the reaction. Remarkably, inclusion of LiCl along with $CeCl_3$, or, optimally, $LaCl_3$, facilitated nucleophilic addition to join the two heterocyclic ring systems. The utility of $LaCl_3{\cdot}2\,LiCl$, first reported by Knochel and co-workers, is largely due to the enhanced solubility of this reagent compared to $LaCl_3$ or $CeCl_3$.[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 CH_2ICl in the presence of Cs_2CO_3, 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 (for example, CH_2Br_2) led to low conversion, while reagents with two good leaving groups (for example, CH_2I_2) 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** (Scheme 3). Exposure to Pd-



Scheme 3. Synthesis of simaomicin α via dehydrogenative coupling.

$(OAc)_2$ in DMSO at elevated temperatures effected a direct dehydrogenative coupling in modest yield, albeit 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 BCl_3 at low temperatures. Ultimately, the natural product was isolated in high yield and purity, with ^1H and ^{13}C NMR spectroscopy and mass spectrometry 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-nanomolar concentrations, and arrest cells in the G1 phase at low nanomolar concentrations as a single agent.[3] Moreover, it reduced cell proliferation by 50 % (IC_{50}) 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 IC_{50} 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 μM, complete cell death was observed. Moreover, *ent*-simaomicin α proved to be 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 μM).[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 $LaCl_3 \cdot 2\,LiCl$ 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.

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[1] T. M. Lee, G. T. Carter, D. B. Borders, *J. Chem. Soc. Chem. Commun.* **1989**, 1771 – 1772.
[2] W. M. Maiese, J. Korshalla, J. Goodman, M. J. Torrey, S. Kantor, D. P. Labeda, M. Greenstein, *J. Antibiot.* **1990**, *43*, 1059 – 1063.
[3] M. Arai, H. Sato, H. Kobayashi, M. Suganuma, T. Kawabe, H. Tomoda, S. Omura, *Biochem. Biophys. Res. Commun.* **2004**, *317*, 817 – 822.
[4] Y. Koizumi, H. Tomoda, A. Kumagai, X.-p. Zhou, S. Koyota, T. Sugiyama, *Cancer Sci.* **2009**, *100*, 322 – 326.
[5] Review on the chemistry and biology of polycyclic xanthone natural products: D. K. Winter, D. L. Sloman, J. A. Porco, Jr., *Nat. Prod. Rep.* **2013**, *30*, 382 – 391.
[6] T. R. Kelly, C. T. Jagoe, Q. Li, *J. Am. Chem. Soc.* **1989**, *111*, 4522 – 4524.
[7] R. Masuo, K. Ohmori, L. Hintermann, S. Yoshida, K. Suzuki, *Angew. Chem.* **2009**, *121*, 3514 – 3517; *Angew. Chem. Int. Ed.* **2009**, *48*, 3462 – 3465.
[8] a) D. L. Sloman, B. Mitasev, S. S. Scully, J. A. Beutler, J. A. Porco, *Angew. Chem.* **2011**, *123*, 2559 – 2563; *Angew. Chem. Int. Ed.* **2011**, *50*, 2511 – 2515; b) D. L. Sloman, J. W. Bacon, J. A. Porco, *J. Am. Chem. Soc.* **2011**, *133*, 9952 – 9955.
[9] J. R. Butler, C. Wang, J. Bian, J. M. Ready, *J. Am. Chem. Soc.* **2011**, *133*, 9956 – 9959.
[10] Recent reviews: a) C. S. Yeung, V. M. Dong, *Chem. Rev.* **2011**, *111*, 1215 – 1292; b) W. R. Gutekunst, P. S. Baran, *Chem. Soc. Rev.* **2011**, *40*, 1976 – 1991; c) L. Ackermann, R. Vicente, A. R. Kapdi, *Angew. Chem.* **2009**, *121*, 9976 – 10011; *Angew. Chem. Int. Ed.* **2009**, *48*, 9792 – 9826; d) S. H. Cho, J. Y. Kim, J. Kwak, S. Chang, *Chem. Soc. Rev.* **2011**, *40*, 5068 – 5083; e) G. P. McGlacken, L. M. Bateman, *Chem. Soc. Rev.* **2009**, *38*, 2447 – 2464; f) J. A. Ashenhurst, *Chem. Soc. Rev.* **2010**, *39*, 540 – 548; g) D. Alberico, M. E. Scott, M. Lautens, *Chem. Rev.* **2007**, *107*, 174 – 238. The absolute stereochemistry was not assigned in the isolation report.
[11] F. Oğuzkaya, E. Şahin, C. Tanyeli, *Tetrahedron: Asymmetry* **2006**, *17*, 3004 – 3009.
[12] For related cyclizations, see: a) Ref [9]; b) K. C. Nicolaou, A. Li, *Angew. Chem.* **2008**, *120*, 6681 – 6684; *Angew. Chem. Int. Ed.* **2008**, *47*, 6579 – 6582.
[13] R. Noyori, S. Hashiguchi, *Acc. Chem. Res.* **1997**, *30*, 97 – 102.
[14] Y. Minoura, M. Takebayashi, C. C. Price, *J. Am. Chem. Soc.* **1959**, *81*, 4689 – 4692.
[15] A. De Mico, R. Margarita, L. Parlanti, A. Vescovi, G. Piancatelli, *J. Org. Chem.* **1997**, *62*, 6974 – 6977.
[16] Related Pomeranz-Fritsch-type cyclizations: a) Ref [9]; b) E. R. Walker, S. Y. Leung, A. G. M. Barrett, *Tetrahedron Lett.* **2005**, *46*, 6537 – 6540.
[17] A. Krasovskiy, F. Kopp, P. Knochel, *Angew. Chem.* **2006**, *118*, 511 – 515; *Angew. Chem. Int. Ed.* **2006**, *45*, 497 – 500.
[18] Substrates with oxygenation at C7 (OH or OMOM) but not the cyclic acetal did not undergo dehydrogenative coupling, indicating 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 the Supporting Information, Figure S2.
[20] Complete experimental details are provided in the Supporting Information.
[21] D. L. Boger, O. Hueter, K. Mbiya, M. Zhang, *J. Am. Chem. Soc.* **1995**, *117*, 11839 – 11849.

