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# Total Synthesis and Biological Evaluation of Cortistatins A and J and Analogues Thereof

K. C. Nicolaou,* Xiao-Shui Peng, Ya-Ping Sun, Damien Polet, Bin Zou, Chek Shik Lim, and David Y.-K. Chen*

*Chemical Synthesis Laboratory@Biopolis, Institute of Chemical and Engineering Sciences (ICES), Agency for Science, Technology and Research (ASTAR), 11 Biopolis Way, The Helios Block, #03-08, Singapore 138667*

Received April 17, 2009; E-mail: kcn@scripps.edu; david_chen@ices.a-star.edu.sg

***Abstract:*** Total syntheses of the highly selective antiproliferative natural products cortistatins A (**1**) and J (**5**) in their naturally occurring enantiomeric forms are described. The modular and convergent strategy employed relies on an intramolecular oxa-Michael addition/aldol/dehydration cascade reaction to cast the ABCD ring framework of the molecule and both Sonogashira and Suzuki−Miyaura coupling reactions to assemble the necessary building blocks into the required heptacyclic skeleton. A divergent approach from a late-stage epoxy ketone leads to both target molecules in a stereoselective manner. The developed synthetic technologies were applied to the construction of several analogues of the cortistatins which were biologically evaluated and compared to the natural products with regards to their antiproliferative activities against a variety of cancer cells. Analogues **8** and **81**, lacking both the dimethylamino and hydroxyl groups of cortistatin A, were found to exhibit comparable biological activity as the parent compound, leading to the conclusion that such functionalities are not essential for biological activity.

## Introduction

Angiogenesis is a physiological phenomenon that involves the generation of new blood vessels in both healthy and disease tissues of the human body.[1] And while it is an essential and required process for growth and repair, angiogenesis is also responsible for the transition of tumors from a dormant to a malignant state. It is for this reason that the inhibition of angiogenesis in general, and the search for novel molecules possessing such inhibitory properties in particular, became an attractive strategy of intervention for the treatment of cancer patients.[2] In 2006, the Kobayashi group disclosed the cortistatin family of natural products (e.g., cortistatins A−D, **1**−**4**, Figure 1) and their selective antiproliferative properties against human umbilical vein endothelial cells (HUVECs).[3] Most strikingly and as the most potent member of the family ($IC_{50}$ = 1.8 nM), cortistatin A (**1**) demonstrated a selectivity index of more than 3000 against HUVECs in comparison with normal human dermal fibroblast (NHDF) and several other tumor cells (KB3-1, K562, and Neuro2A). More recently, cortistatins J−L (**5**−**7**, Figure 1) were reported by the same group.[4a] Among them, cortistatin J (**5**) exhibited the most potent antiproliferative activity against HUVECs ($IC_{50}$ = 8 nM) with a selectivity index of 300−1100 as compared with NHDF, KB3-1, K562, and Neuro2A cells. Isolated from the sponge *Corticium simplex*, the cortistatins appear to be promising drug candidates or leads, their potential tempered only by their low natural abundance. The impressive biological properties coupled with their unprecedented molecular architectures and scarcity made the cortistatins enticing targets for chemical synthesis.[5] Culminating in the total synthesis of cortistatin A, our studies were reported in a preliminary communication in 2008.[6] In this paper we describe the full account of our work in the cortistatin area that includes, in addition to the total synthesis of several natural (A and J) and designed (e.g., **8** and **81**) members of the class, their biological evaluation as antiproliferative agents.

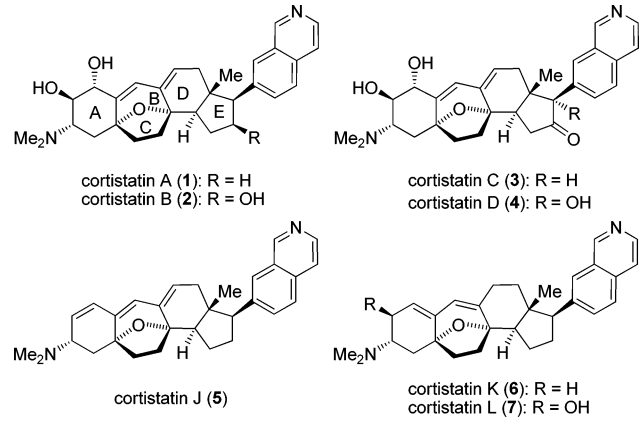



***Figure 1.*** Structures of cortistatins A−D (**1**−**4**) and J−L (**5**−**7**).

## Results and Discussion

The attractiveness of cortistatins A and J and their siblings as targets for chemical synthesis was enhanced by the prospect

(1) Carmeliet, P. *Nature* **2005**, *438*, 932–936.
(2) Ziche, M.; Donnini, S.; Morbidelli, L. *Curr. Drug Targets* **2004**, *5*, 485–493.
(3) Aoki, S.; Watanabe, Y.; Sanagawa, M.; Setiawan, A.; Kotoku, N.; Kobayashi, M. *J. Am. Chem. Soc.* **2006**, *128*, 3148–3149.
(4) For isolation of cortistatins J−L, see: (a) Aoki, S.; Watanabe, Y.; Tanabe, D.; Setiawan, A.; Arai, M.; Kobayashi, M. *Tetrahedron Lett.* **2007**, *48*, 4485–4488. For isolation of cortistatins E−H, see: (b) Watanabe, Y.; Aoki, S.; Tanabe, D.; Setiawan, A.; Kobayashi, M. *Tetrahedron* **2007**, *63*, 4074–4079.

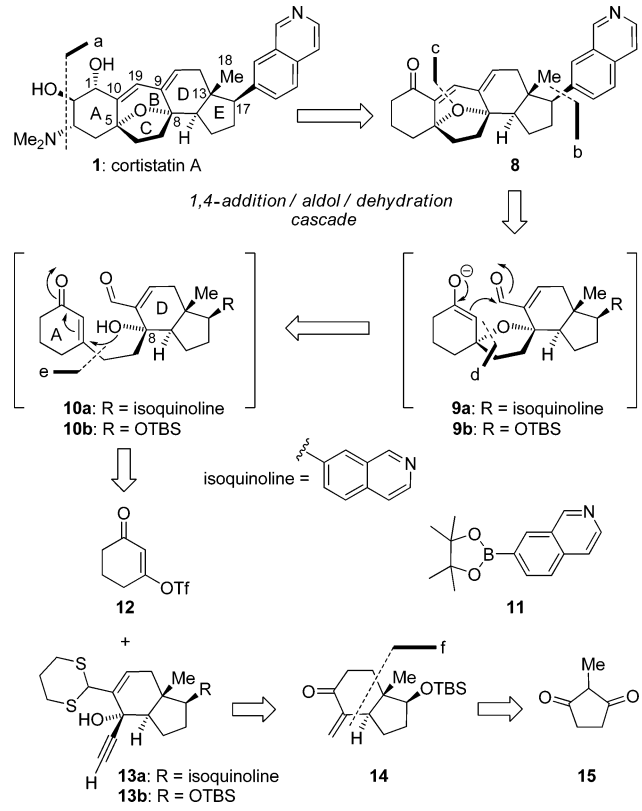
  

of developing new synthetic technologies and strategies and applying them to the construction of analogues for biological evaluation as potential anticancer agents. Of particular interest to us were the development of cascade reactions[7] for total synthesis and the discovery of selective antiproliferative agents for cancer chemotherapy. With these aims in mind we pondered the cortistatin molecule as a synthetic target, starting with its structural motifs and retrosynthetic analysis.

**Retrosynthetic Analysis.** Being the most potent member of the family, cortistatin A (**1**) became our first target for synthesis. Inspection of its structure revealed the unique abeo-9(10−19)-androstane-type steroidal skeleton with substitutions on rings A and E, a structural motif common to all cortistatins. Retrosynthetically removing the dimethylamino and C-2 hydroxyl groups from ring A (a) led to hypothetical precursor ketone **8** as shown in Scheme 1. Disconnecting the isoquinoline moiety from the main framework (b) of the molecule at this stage (or at some stage downstream) simplified the structure further and revealed boronic ester **11** as a potential donor of this group in a Suzuki−Miyaura[8] coupling. At this stage a cascade involving a 1,4-addition/aldol/dehydration sequence (c,d) was envisioned (**10** → **9** → **8**). Imagining an acetylenic unit as the bridge between rings A and D of intermediate **10** allowed a Sonogashira[9] disconnection to reveal vinyl triflate **12** and terminal acetylenes **13a** and **13b** as its potential precursors. These intermediates were then connected to enone **14**,[10] itself being traceable to simple monocyclic diketone **15** via the Hajos−Parrish ketone.[11] The strategy derived from this retrosynthetic analysis had the advantages of high convergency and flexibility for analogue construction, as well as enantioselectivity options. It was with this plan that we embarked on the cortistatin program which included both methodology development and chemical biology studies.

**Model Studies.** The rather daring nature of the designed strategy toward cortistatin A and the uncertainties with regards

***Scheme 1.*** Retrosynthetic Analysis of Cortistatin A (**1**)[a]




[a] Operations: (a) epoxide opening; (b) Suzuki−Miyaura coupling; (c) aldol condensation; (d) 1,4-addition; (e) Sonogashira coupling; (f) Hajos−Parrish ketone construction.

to the timing of the introduction of the isoquinoline and dimethylamino moieties within the growing molecule dictated a number of model studies prior to its finalization. We first set out to test the feasibility of the 1,4-addition/aldol/dehydration cascade to form the central core of cortistatin A containing the oxa bridge and the dienone structural motif by targeting model system **26** as shown in Scheme 2. The key substrate for this cascade, bicyclic hydroxy enone enal **23**, was conveniently prepared in racemic form from cyclohexenone (**16**) through a short sequence. Thus, reaction of **16** with formaldehyde in the presence of 4-DMAP gave hydroxy enone **17** in 72% yield. Silyl protection of the latter compound (TESCl, imid.) furnished TES ether **18** (81% yield), which reacted with lithium TMS acetylide to afford, upon global desilylation (TBAF, 72% yield for the two steps), dihydroxy terminal acetylene **20** via intermediate **19**. Sonogoshira[9] coupling [$Pd(PPh_3)_4$ cat., CuI cat., $Et_3N$] of acetylene **20** with freshly prepared enol triflate **12** (1,3-cyclohexadione, $Tf_2O$, $Et_3N$) followed by DMP oxidation afforded acetylenic aldehyde enone **22** in 58% overall yield for the two steps. Finally, selective hydrogenation of **22** with 10% Pd/C under carefully controlled conditions ($H_2$, MeOH/EtOAc 2:3, 23 °C) led to hydroxy enone enal **23** (61% yield), setting the stage for the much anticipated cascade sequence. While pleased with the remarkable selectivity of this hydrogenation process, we were somewhat surprised by the resistance of the product (**23**) toward spontaneous cyclization. The cascade reaction of hydroxy enone enal **23** was, therefore, investigated under basic and acidic conditions as summarized in Table 1. Interestingly, we found that **23** undergoes the desired transformation to the targeted model system **26**, both under basic (entries 1−3, Table 1) and acidic (entries 4 and 5, Table 1) conditions,

(5) For the first synthesis of cortistatin A, see: (a) Shenvi, R. A.; Guerrero, C. A.; Shi, J.; Li, C.-C.; Baran, P. S. *J. Am. Chem. Soc.* **2008**, *130*, 7241–7243. For a total synthesis of cortistatin A, see: (b) Lee, H. M.; Nieto-Oberhuber, C.; Shair, M. D. *J. Am. Chem. Soc.* **2008**, *130*, 16864–16866. For a formal synthesis of cortistatin A, see: (c) Yamashita, S.; Kitajima, K.; Iso, K.; Hirama, M. *Tet. Lett.* **2009**, *50*, 3277–3279. For studies toward the synthesis of cortistatins, see: (d) Yamashita, S.; Iso, K.; Hirama, M. *Org. Lett.* **2008**, *10*, 3413–3415. (e) Simmons, E. M.; Hardin, A. R.; Guo, X.; Sarpong, R. *Angew. Chem., Int. Ed.* **2008**, *47*, 6650–6653. (f) Dai, M.; Wang, Z.; Danishefsky, S. J. *Tetrahedron Lett.* **2008**, *49*, 6613–6616. (g) Dai, M.; Danishefsky, S. J. *Tetrahedron Lett.* **2008**, *49*, 6610–6612. (h) Kürti, B.; Corey, E. J. *Org. Lett.* **2008**, *10*, 5247–5250. (i) Kotoku, N.; Sumii, Y.; Hayashi, T.; Kobayashi, M. *Tetrahedron Lett.* **2008**, *49*, 7078–7081. (j) Craft, D. T.; Gung, B. W. *Tetrahedron Lett.* **2008**, *49*, 5931–5934. (k) Dai, M.; Danishefsky, S. J. *Heterocycles* **2009**, *77*, 157–161. (l) Liu, J.; Gao, Y.; Che, C.; Wu, N.; Wang, D.; Li, C.; Yang, Z. *Chem. Commun.* **2009**, 662–664.

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(7) For a recent review on cascade reactions in total synthesis, see: (a) Nicolaou, K. C.; Montagnon, T.; Snyder, S. A. *Chem. Commun.* **2003**, *5*, 551–564. (b) Tietze, L. F.; Brasche, G.; Gericke, K. M. *Domino Reactions in Organic Synthesis*; Wiley-VCH: Weinheim, 2006; p 617. (c) Nicolaou, K. C.; Edmonds, D. J.; Bulger, P. G. *Angew. Chem., Int. Ed.* **2006**, *45*, 7134–7186.

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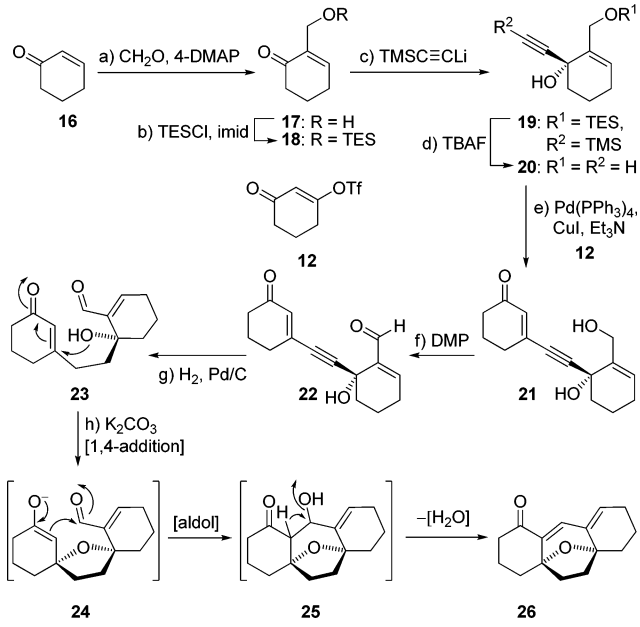
(9) Sonogashira, K. *Handbook of Organopalladium Chemistry for Organic Synthesis*; John Wiley & Sons: New York, 2002; Vol. 1, pp 493−529.

(10) (a) Rychnovsky, S. D.; Mickus, D. E. *J. Org. Chem.* **1992**, *57*, 2732–2736. For the synthesis of *ent*-**14**, see: (b) Isaacs, R. C. A.; Di Grandi, M. J.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 3938–3941.

(11) Olton, Z.; Hajos, G.; Parrish, R. *Org. Synth.* **1990**, *7*, 363–368.

***Scheme 2.*** Synthesis of Model Dienone **26**[a]




[a] Reagents and conditions: (a) $CH_2O$ (37% aq., 5.0 equiv), 4-DMAP (5.0 equiv), THF, 23 °C, 16 h, 72%; (b) TESCl (1.2 equiv), imidazole (2.0 equiv), DMF, 23 °C, 6 h, 81%; (c) TMSC≡CLi (1.5 equiv, prepared from *n*-BuLi and TMSC≡CH), THF, 0 °C, 2 h; (d) TBAF (1.0 M in THF, 1.3 equiv), THF, 23 °C, 0.5 h, 72% for two steps; (e) $Pd(PPh_3)_4$ (0.05 equiv), CuI (0.1 equiv), $Et_3N$ (3.0 equiv), **12** (1.5 equiv, from 1,3-cyclohexadione, $Tf_2O$ and $Et_3N$), DMF, 23 °C, 1 h, 82%; (f) DMP (1.5 equiv), $NaHCO_3$ (6.0 equiv), $CH_2Cl_2$, 23 °C, 0.5 h, 71%; (g) Pd−C (10 wt %/wt, 0.38 equiv), $H_2$ (1 atm), MeOH/EtOAc (2:3), 23 °C, 25 min, 61%; (h) $K_2CO_3$ (1.2 equiv), dioxane, 125 °C, 16 h, 50%.

***Table 1.*** Cascade Cyclization of Hydroxy Enone-Enal **23** to Tetracyclic Dienone **26** (Scheme 2)[a]

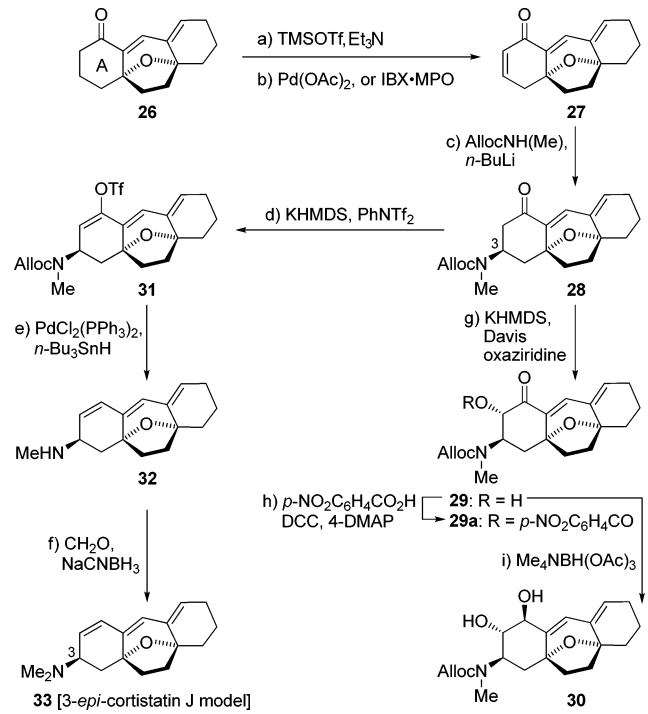
| entry | conditions | product (yield)[b] |
|---|---|---|
| 1 | 10% KOH, MeOH, reflux, 12 h | 31% |
| 2 | piperidine (0.1 equiv), toluene, reflux, 22 h | 21% |
| *3* | *$K_2CO_3$ (1.2 equiv), dioxane, 125 °C, 16 h* | *50%* |
| 4 | PPTS (0.1 equiv), toluene, reflux, 14 h | 22% |
| 5 | *p*-TsOH (0.1 equiv), toluene, reflux, 14 h | 17% |

[a] Reactions were carried out on 0.17−0.26 mmol scale. [b] Yields refer to chromatographically pure materials.

presumably through intermediates **24** and **25** (which, however, were neither isolated nor detected). The optimum conditions for this cyclization cascade involved heating precursor **23** in dioxane at 125 °C in the presence of $K_2CO_3$ for 16 h, yielding product **26** in 50% yield (entry 3, Table 1).

With the tetracyclic dienone structure **26** secured, we then turned our attention to its modification into closer model systems to cortistatins A (i.e., **30**, Scheme 3) and J (i.e., **33**, Scheme 3) through functionalization of ring A as shown in Scheme 3. A primary objective of the envisioned sequence was the stereoselective installment of the dimethylamino group, common to both cortistatins A and J, into the growing molecule. Based on manual molecular models, it was hypothesized that the trajectory of a nucleophilic attack on the corresponding A-ring enone (i.e., **27**) would follow an antiperiplanar path to the bis-methylene bridge of the tetrahydrofuran ring, thereby resulting in the desired α-stereochemistry for the incoming nucleophile. To this end, model system **26** was converted to trienone **27** through

***Scheme 3.*** Synthesis of Cortistatin A and J Model Compounds (**30** and **33**, respectively)[a]




[a] Reagents and conditions: (a) TMSOTf (1.2 equiv), $Et_3N$ (2.0 equiv), −78 → 0 °C, 1.5 h; (b) $Pd(OAc)_2$ (1.5 equiv), $CH_3CN$, 23 °C, 15 h, 43% for two steps; or IBX•MPO (0.4 M in DMSO, 6.0 equiv), DMSO, 23 °C, 6 h, 45% for two steps; (c) LiN(Me)Alloc (5.0 equiv, prepared from *n*-BuLi and AllocNHMe), THF, −78 °C, 15 min, 70%; (d) KHMDS (0.5 M in toluene, 3.0 equiv), $PhNTf_2$ (2.0 equiv), THF, −78 → 0 °C, 0.5 h; (e) $PdCl_2(PPh_3)_2$ (0.05 equiv), *n*-$Bu_3SnH$ (2.0 equiv), AcOH (23 equiv), $CH_2Cl_2$, 23 °C, 1 h, 27% for two steps; (f) $CH_2O$ (37% aq., 6.0 equiv), $NaCNBH_3$ (6.0 equiv), MeOH, 23 °C, 1 h, 56%; (g) KHMDS (0.5 M in toluene, 3.0 equiv), Davis oxaziridine (2.0 equiv), THF, −78 °C, 1 h, 60%; (h) DCC (1.5 equiv), *p*-nitrobenzoic acid (1.5 equiv), 4-DMAP (0.2 equiv), $CH_2Cl_2$, 23 °C, 2 h, 71%; (i) $Me_4NBH(OAc)_3$ (8.0 equiv), AcOH/$CH_3CN$, 1:1, 23 °C, 1 h, 25%.

either a Saegusa[12] protocol [(i) TMSOTf, $Et_3N$; (ii) $Pd(OAc)_2$ (cat.), 43% yield] or oxidation of the intermediate silyl enol ether with IBX•MPO[13] (45% yield). Pleasantly, trienone **27** entered into a facile and chemoselective reaction with the lithio derivative of methylamine allyloxycarbamate [generated from AllocNHMe and *n*-BuLi] to afford keto carbamate **28** in 70% yield and as a single diastereoisomer. The stereochemistry of this product, however, was not easily discernible at this stage and had to await further chemical transformations, which culminated in the synthesis of model system **33** (for cortistatin J) and **30** (for cortistatin A). To this end, ketone **28** was first converted to its vinyl triflate (KHMDS, $PhNTf_2$) and then to its olefinic counterpart **32** [*n*-$Bu_3SnH$, AcOH, $PdCl_2(PPh_3)_2$ cat.][14] in 27% overall yield for the two steps. Finally, reductive amination of the latter compound ($CH_2O$ aq., $NaCNBH_3$) furnished the cortistatin J model system **33** (56% yield), whose stereochemical assignment as a 3-*epi*-cortistatin model system became possible by NMR spectroscopic analysis (see Figure 2).

Thus, in the reported proton NMR data for natural cortistatin J (**5**),[4a] H2 displayed a clear doublet of 9.9 Hz solely due to

(12) Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011–1013.
(13) Nicolaou, K. C.; Gray, D. L. F.; Montagnon, T.; Harrison, S. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 996–1000.
(14) Dangles, O.; Guibe, F.; Balavoine, G.; Lavielle, S.; Marquet, A. *J. Org. Chem.* **1987**, *52*, 4984–4993.

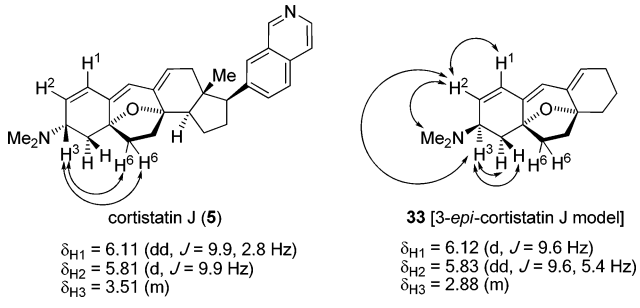



***Figure 2.*** Key $^1$H NMR signals and NOSEY correlations for cortistatin J (**5**) and model compound **33**.

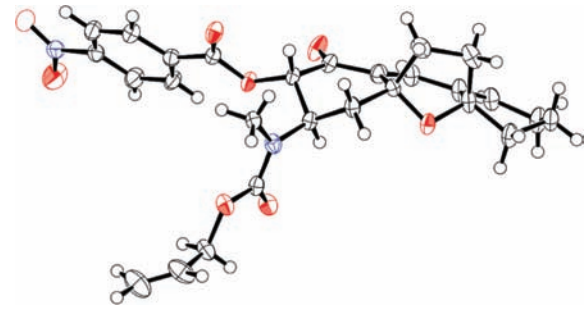


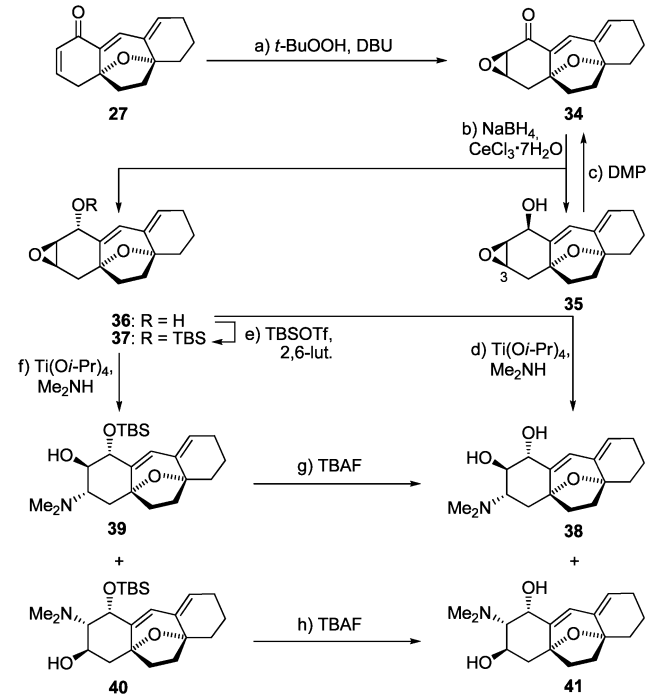
***Figure 3.*** X-ray derived ORTEP drawing of **29a** with thermal ellipsoids shown at the 50% probability level.[17]

$J_{H1-H2}$ coupling, in line with a 90° H2−H3 dihedral angle. In stark contrast, in the synthesized model system **33**, the corresponding H2 exhibited a dd splitting pattern with $J_{H1-H2} = 9.6$ Hz and $J_{H2-H3} = 5.4$ Hz. The H2−H3 dihedral angle predicted, based on manual molecular modeling studies for compound **33**, was considerably smaller than 90° and thus supportive of the observed $J_{H2-H3}$ coupling constant. Furthermore, the notable difference in the chemical shifts observed for H3 in model system **33** ($\delta = 2.88$ ppm, $CDCl_3$) and in natural cortistatin J ($\delta = 3.51$ ppm, $CDCl_3$) also added to our suspicion. Most diagnostically, the signature H3−$H6_{ab}$ NOESY correlation that supported the $C_3$-α stereochemical assignment in the natural product (**5**) was clearly absent in model system **33**.

Despite the realization that the dimethylamino group within intermediate **28** (Scheme 3) possessed the undesired stereochemical configuration, we decided to explore a possible pathway to a cortistatin A model system. We first attempted the introduction of the obligatory hydroxyl group, an objective that was nicely achieved by reaction of the potassium enolate of ketone **28** (KHMDS) with Davis oxaziridine[15] in 60% yield and with complete stereocontrol, as shown in Scheme 3, to afford the desired α-hydroxylated product **29** as a single diastereoisomer. Subsequent 1,2-directed reduction of the carbonyl group [$Me_4NBH(OAc)_3$][16] then produced diol **30** in 25% (unoptimized) yield. The trans relationship of the two hydroxyl groups was supported by the observed coupling constant of the relevant protons ($J_{1,2} = 5.4$ Hz) in the $^1$H NMR spectrum of **30**. Furthermore, and delightfully, the *para*-nitrobenzoate derivative (**29a**, Scheme 3) of cortistatin A model system **29** crystallized beautifully from $CH_2Cl_2$/hexane (**29a**, dec 145 °C), allowing its X-ray crystallographic analysis (see ORTEP drawing, Figure 3), which confirmed all the stereochemical assignments made previously on the basis of NMR spectroscopy.

While the undesired stereochemical outcome ($\beta$) of the conjugate addition of the nitrogen nucleophile to trienone **27** was disappointing, it suggested an alternative tactic for the introduction of the A-ring functionality of cortistatin A. Specifically, it was reasoned that should the *t*-BuOOH-DBU[18] epoxidation of **27** proceed in the same way, then the resulting $\beta$-epoxide would be a potentially useful precursor for our desired objective. Indeed, and as shown in Scheme 4, the *t*-BuOOH-DBU epoxidation of **27** proved to be both chemo- and

***Scheme 4.*** Synthesis of Cortistatin A Model System **38**[a]




[a] Reagents and conditions: (a) *t*-BuOOH (5.5 M in decane, 4.0 equiv), DBU (3.0 equiv), $CH_2Cl_2$, 0 → 23 °C, 5 h, 72%; (b) $NaBH_4$ (1.0 equiv), $CeCl_3 \cdot 7H_2O$ (3.0 equiv), MeOH, 0 °C, 10 min, **35**: 40% and **36**: 40%; (c) DMP (2.0 equiv), $NaHCO_3$ (5.0 equiv), $CH_2Cl_2$, 23 °C, 2 h, 100%; (d) $Me_2NH$ (2.0 M in THF, as solvent), $Ti(Oi\text{-}Pr)_4$ (5.0 equiv), 80 °C, 5 h, **38**: 44% and **41**: 35%; (e) TBSOTf (2.0 equiv), 2,6-lut. (3.0 equiv), $CH_2Cl_2$, 0 °C, 1 h, 80%; (f) $Me_2NH$ (2.0 M in THF, as solvent), $Ti(Oi\text{-}Pr)_4$ (5.0 equiv), 80 °C, 50 h, **39**: 65%, **40**: 16%; (g) TBAF (1.0 M solution in THF, 5.0 equiv), THF, 23 °C, 5 h, 80%; (h) TBAF (1.0 M solution in THF, 5.0 equiv), THF, 65 °C, 3 h, 85%.

stereoselective, delivering epoxide **34** in 72% yield and as a single diastereoisomer. An X-ray crystallographic analysis of **34** (mp 186−187 °C, $CH_2Cl_2$/hexane) confirmed its stereochemical assignment (see ORTEP drawing, Figure 4). Having failed to obtain satisfactory results with $Me_4NBH(OAc)_3$,[16] $Zn(BH_4)_2$, and DIBAL-H, we resorted to the use of Luche[19] conditions ($NaBH_4-CeCl_3$) for the reduction of epoxy ketone **34**, which furnished diastereomeric epoxy alcohols **35** and **36** in 80% yield and *ca.* 1:1 ratio. The two isomers were chromatographically separated, and their stereochemistry was assigned by NMR spectroscopy. Indeed, an X-ray crystallographic analysis of **36** (mp 175−176 °C, $CH_2Cl_2$/hexane)

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(17) CCDC-710300 contains the supplementary crystallographic data for compound **29a**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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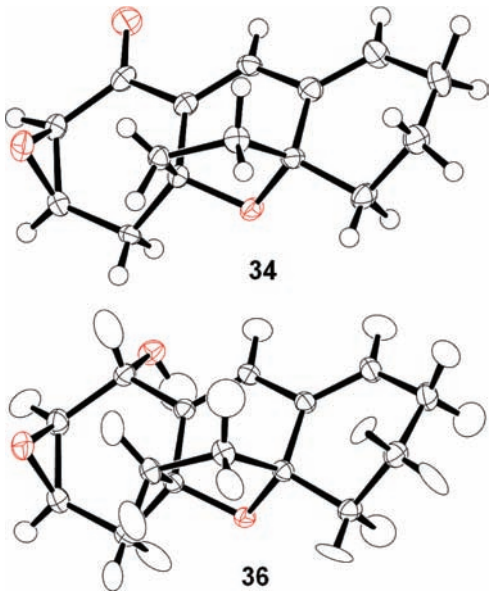
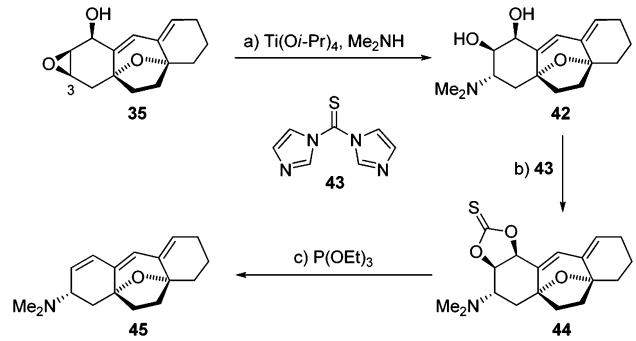



***Figure 4.*** X-ray derived ORTEP drawings of compounds **34** and **36** with thermal ellipsoids shown at the 50% probability level.[21]

confirmed its stereochemistry (see ORTEP drawing, Figure 4). The undesired $\beta$-OH diastereoisomer (**35**) was recycled through oxidation (DMP, 100% yield)−reduction as shown in Scheme 4. Finally, introduction of the dimethylamino group into the molecule (**36**) was accomplished through a titanium-assisted[20] epoxide opening [Ti(O*i*-Pr)$_4$, $Me_2NH$] leading to an isomeric mixture of dihydroxy dimethylamino compounds **38** (44% yield) and **41** (35% yield), which were conveniently separated by preparative thin-layer chromatography (silica). This ratio was improved in favor of the desired isomer **38** by placing a bulky protecting group (i.e., TBS) on the hydroxyl group of substrate **36** (TBSOTf, 2,6-lut., 80% yield) to afford intermediate silyl ether **37**, which upon reaction with Ti(O*i*-Pr)$_4$-$Me_2NH$ furnished the two regioisomers **39** and **40** in *ca.* 4:1 ratio and 81% combined yield. Chromatographic separation of these isomers (preparative TLC, silica) followed by desilylation with TBAF led to cortistatin A model systems **38** (80% yield) and **41** (85% yield), respectively. Pleasingly, the NMR spectra of isomer **38** exhibited encouraging resemblance to those of cortistatin A.

Although hydroxy epoxide **35** possessed the wrong stereochemistry for cortistatin A, it did provide a productive entry into cortistatin J model system **45** as outlined in Scheme 5. Thus, epoxide opening of compound **35** under the previously established conditions [Ti(O*i*-Pr)$_4$-$Me_2NH$] afforded dimethylamine diol **42** in 70% yield as a single regioisomer. The exclusive formation of regioisomer **42** is most likely a result of the Lewis acidic titanium reagent concomitantly coordinating both the epoxide and alcohol oxygens from the same face of epoxy alcohol **35**, thereby leading to steric bias that favors the exclusive nucleophilic attack at C3. With the C3 stereocenter secured, the introduction of the A-ring olefinic bond was subsequently

***Scheme 5.*** Synthesis of Cortistatin J Model System **45**[a]




[a] Reagents and conditions: (a) Ti(O*i*-Pr)$_4$ (5.0 equiv), $Me_2NH$ (2.0 M solution in THF, as solvent), 80 °C, 1 h, 70%; (b) thiocarbonyl diimidazole (**43**) (1.5 equiv), toluene, 110 °C, 12 h, 81%; (c) P(OEt)$_3$ (as solvent), 160 °C, 24 h, 42% (50% recovered starting material).

achieved through the Corey−Winter protocol[22] [(i) thiocarbonyl diimidazole, 81% yield; (ii) P(OEt)$_3$, 42% yield (50% recovered starting material, **44**)] to afford cortistatin J model system **45** via intermediate thiocarbonate **44**. Cortistatin J model system **45** exhibited excellent agreement with the naturally occurring cortistatin J with regards to its $^1$H NMR spectral data spanning rings A−D.[4a]

**Initial Approach to Cortistatin A Involving Early-Stage Installation of Isoquinoline Moiety.** With the initial cortistatin A model studies successfully completed, we then proceeded to apply the developed synthetic technologies to the total synthesis of cortistatin A (**1**). We chose first to test the approach involving early stage installation of the isoquinoline moiety that would allow the key cascade sequence as a subsequent event. Such an approach would have the advantage of minimizing protecting group manipulations and oxidation state adjustments in the late stages of the synthesis. Toward this end, we first investigated the route involving a Hajos−Parrish ketone synthesis[11] and starting from cyclopentadione **15**, as shown in Scheme 6. Thus, **15** was converted to bicyclic enone **14** through modification of published procedures.[10b] The olefinic bond of enone **14** was dihydroxylated with $OsO_4$ (cat.)/NMO and the resulting 1,2-diol (**46**) was protected as its acetonide derivative [$Me_2C(OMe)_2$, *p*-TsOH] to afford compound **47** in 64% yield. Vinyl triflate formation (NaHMDS-$PhNTf_2$) followed by palladium-catalyzed carboxymethylation in DMF/MeOH [Pd(PPh$_3$)$_4$ cat., CO, 72% yield for the two steps] then converted ketone **47** to $\alpha,\beta$-unsaturated methyl ester **48**. In preparation for the attachment of the isoquinoline moiety, compound **48** was desilylated (TBAF, 87% yield), and the resulting alcohol (**49**) was oxidized (DMP, 81% yield) to afford cyclopentanone **50**.

**Installment of the Isoquinoline System.** With the stage now set for the next stage of the synthesis, ketone **50** was converted to its vinyl triflate (NaHMDS, $PhNTf_2$), which entered the intended Suzuki−Miyaura[8] reaction with boronic ester **11**[23] in the presence of Pd(PPh$_3$)$_4$ cat. and $K_2CO_3$ to afford isoquinoline system **51** in 62% overall yield. For the purposes of cortistatin A (and J−K), a stereoselective reduction of the cyclopentene double bond was required. To this end, a number of conditions were investigated, and as shown in Table 2, a satisfactory solution to this problem was found. Thus, catalytic hydrogena-

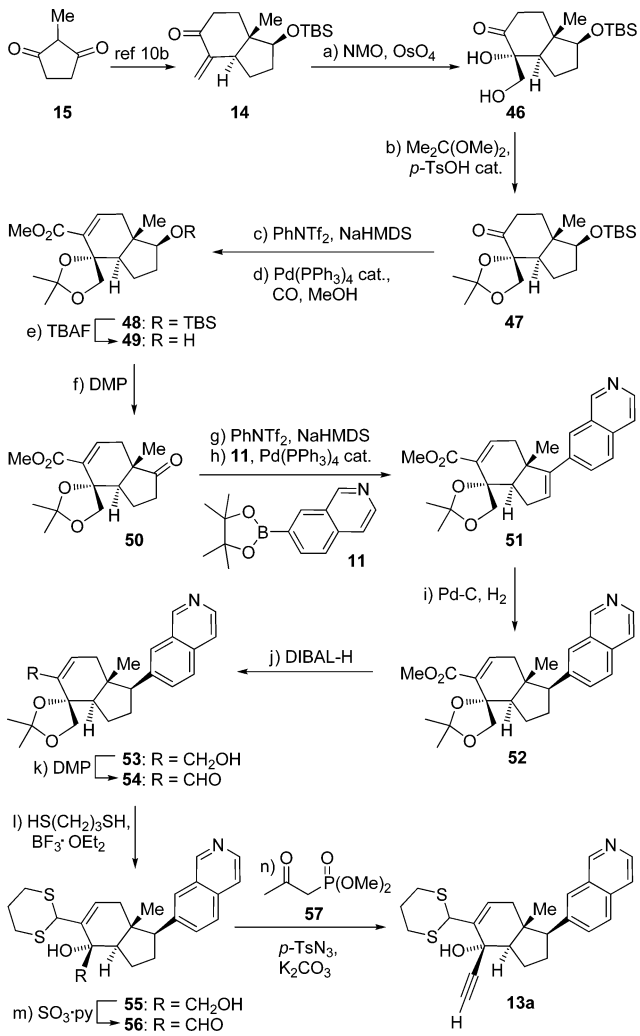
(20) (a) Chong, J. M.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 1560–1563. (b) Alvarez, E.; Nunez, M. T.; Martin, V. S. *J. Org. Chem.* **1990**, *55*, 3429–3431.

(21) CCDC-710299 and CCDC-710298 contain the supplementary crystallographic data for compounds **34** and **36**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(22) Corey, E. J.; Carey, F. A.; Winter, R. A. E. *J. Am. Chem. Soc.* **1965**, *87*, 934–935.

(23) Prepared from 7-bromoisoquinoline via: [Pd(dppf)Cl$_2$], KOAc, bispinacolato diboron, DMSO, 80 °C, 50%. For preparation of 7-bromoisoquinoline, see: Miller, B. R.; Frincke, J. M. *J. Org. Chem.* **1980**, *45*, 5312–5315.

***Scheme 6.*** Synthesis of Terminal Acetylene **13a**[a]




[a] Reagents and conditions: (a) $OsO_4$ (2.5 wt %/wt in *t*-BuOH, 0.02 equiv), NMO (2.5 equiv), acetone/$H_2O$ (1:1), 23 °C, 16 h, 73%; (b) $Me_2C(OMe)_2$ (5.0 equiv), *p*-TsOH (0.04 equiv), acetone, 23 °C, 1 h, 87%; (c) NaHMDS (1.0 M in THF, 1.2 equiv), $PhNTf_2$ (1.1 equiv), THF, 0 °C, 2 h; (d) $Pd(PPh_3)_4$ (0.05 equiv), MeOH (30 equiv), CO, DMF, 70 °C, 4 h, 72% for the two steps; (e) TBAF (1.0 M in THF, 1.2 equiv), THF, 23 °C, 16 h, 87%; (f) DMP (2.0 equiv), $NaHCO_3$ (5.0 equiv), $CH_2Cl_2$, 23 °C, 3 h, 81%; (g) NaHMDS (1.0 M in THF, 1.5 equiv), $PhNTf_2$ (1.2 equiv), THF, −78 → 0 °C, 2 h; (h) $Pd(PPh_3)_4$ (0.1 equiv), **11** (1.3 equiv), $K_2CO_3$ (3.0 equiv), THF, 80 °C, 3 h, 62% for two steps; (i) Pd−C (10 wt %/wt, 0.5 equiv), $H_2$, MeOH, 23 °C, 4 h, 70%; (j) DIBAL-H (1.0 M in toluene, 3.0 equiv), toluene, −78 °C, 3 h, 71%; (k) DMP (1.5 equiv), $NaHCO_3$ (5.0 equiv), $CH_2Cl_2$, 23 °C, 0.5 h, 80%; (l) $HS(CH_2)_3SH$ (6.0 equiv), $BF_3 \cdot OEt_2$ (10.0 equiv), $CH_2Cl_2$, −40 °C, 3 h, 41%; (m) $SO_3 \cdot py$ (4.0 equiv), $CH_2Cl_2$/DMSO (2:1), 23 °C, 1 h, 70%; (n) *p*-$TsN_3$ (1.5 equiv), dimethyl-2-oxopropylphosphonate (**57**) (1.5 equiv), $K_2CO_3$ (4.0 equiv), MeOH/$CH_3CN$/THF (1:2:1), 23 °C, 12 h, **13a**: 28%, **64**: 22%.

tion of compound **51** with 10% Pd/C in MeOH at ambient temperature selectively delivered isoquinoline derivative **52** in 70% yield and as a single diastereoisomer. That this crystalline compound (mp = 204−206 °C, $CH_2Cl_2$/hexanes) possessed the desired $\beta$-stereochemistry was determined through X-ray crystallographic analysis (see ORTEP drawing, Figure 5). Although this stereochemical outcome was expected on the basis of manual molecular modeling, its experimental verification was both gratifying and comforting at this stage. Prior to installing the planned acetylenic moiety, the ester group of the growing molecule was adjusted to the aldehyde oxidation state and protected as a dithiane system. Thus, DIBAL-H reduction of

***Table 2.*** Reduction of Cyclopentenyl Isoquinoline **51** to Cyclopentyl Isoquinoline **52** (Scheme 6)[a]

| entry | conditions | product (yield)[b] |
|---|---|---|
| 1 | Wilkinson's catalyst (0.3 equiv), EtOH, 23 °C, 12 h | no reaction |
| 2 | $Cy_2BH$ (2.5 equiv), AcOH (20 equiv), THF, 12 °C, 10 h | 14% |
| 3 | $BH_3 \cdot SMe_2$ (10 M, 1.5 equiv), THF, 23 °C, 5 h; then AcOH (20 equiv) | 20% |
| *4* | *Pd−C (10 wt %/wt, 0.2 equiv), MeOH, 23 °C, 4 h* | *70%* |
| 5 | KOOCN=NCOOK (3.0 equiv), THF/$H_2O$ (4:1), 23 °C, 6 h; then AcOH (20 equiv) | 25% |

[a] Reactions were carried out on 0.094−0.161 mmol scale under 1 atm of hydrogen. [b] Yields refer to chromatographically pure materials.

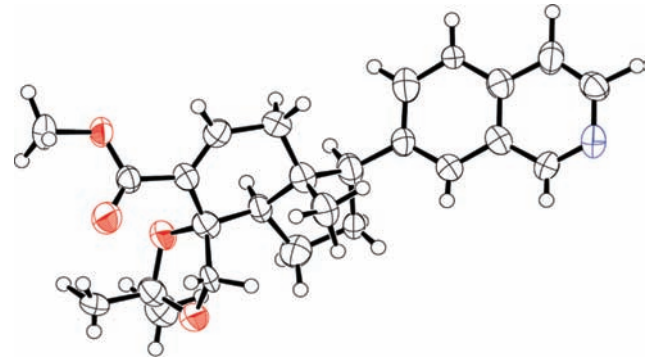


***Figure 5.*** X-ray derived ORTEP drawing of **52** with thermal ellipsoids shown at the 50% probability level.[26]

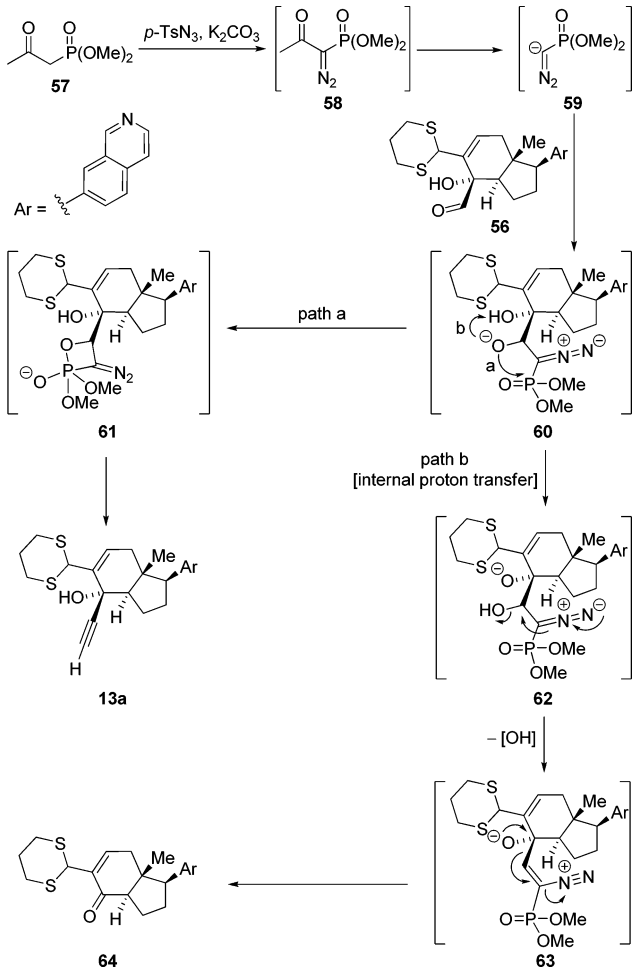
**52** led to alcohol **53** (71% yield), which was oxidized to aldehyde **54** through the action of DMP (80% yield). Treatment of the latter compound with 1,3-propanedithiol and $BF_3 \cdot OEt_2$ resulted in dithiane formation and concomitant acetonide collapse to unveil the molecule's 1,2-diol system, leading to dihydroxy dithiane **55** (41% yield). Finally, oxidation of **55** according to the Parikh−Doering[24] protocol ($SO_3 \cdot$py, 70% yield), followed by Ohira−Bestmann[25] reaction (**57**, *p*-$TsN_3$, $K_2CO_3$) of the resulting hydroxy aldehyde (**56**), furnished the targeted acetylenic compound **13a**, albeit in a modest 28% yield.

The low yielding conversion of hydroxy aldehyde **56** to terminal acetylene **13a** prompted us to investigate the reaction mixture further. Indeed, a second product was discovered (22% yield) whose structure was determined to be that of enone **64** (Scheme 7). This intriguing observation can be explained by the mechanism presented in Scheme 7. Thus, while in the normal mode of the Ohira−Bestmann reaction (**57** → **58** → **59**), the initially formed hydroxy phosphonate (**59** + **56** → **60**) cyclizes rapidly to form the transient intermediate betaine **61**, which undergoes spontaneous collapse to generate the terminal acetylene **13a** (path a), in this instance, intermediate **60** is presumed to rearrange through proton transfer to isomeric alkoxy phosphonate **62** (path b), which could collapse as shown (**62** → **63** → **64**) to afford the observed truncated molecule (**64**).

With the final step of the synthesis of acetylenic compound **13a** pending improvement, and with sufficient quantities of this

(24) Parikh, J. R.; Doering, W. V. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.
(25) (a) Ohira, S. *Synth. Commun.* **1989**, *19*, 561–564. (b) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *Synlett* **1996**, 521–522. (c) Roth, G. J.; Liepold, B.; Müller, S. G.; Bestmann, H. J. *Synthesis* **2004**, 59–62.
(26) CCDC-684134 contains the supplementary crystallographic data for compound **52**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

***Scheme 7.*** Postulated Mechanism for the Formation of Acetylenic Compound **13a** and Byproduct **64**

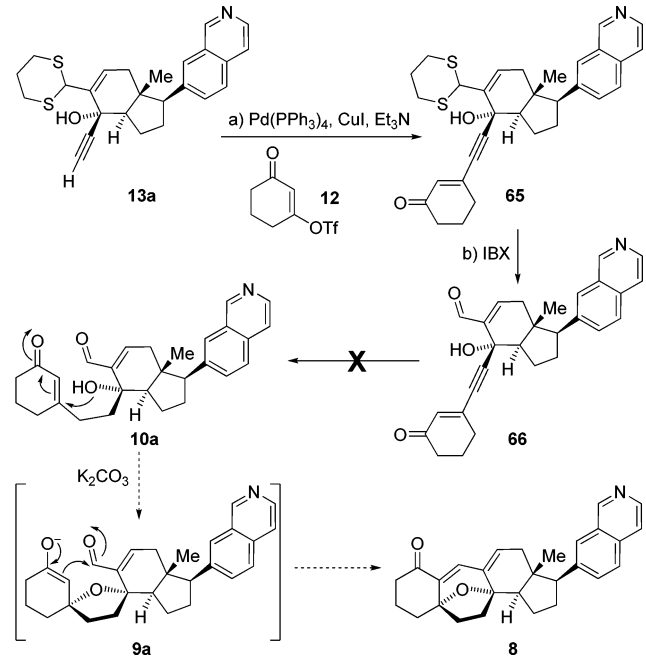



intermediate in hand, we proceeded to explore its possible conversion to the core structure of cortistatin A (**1**) through the proposed cascade depicted in Scheme 1. Our attempted sequence to reach cortistatin structure **8** is shown in Scheme 8. Thus, Sonogoshira[9] coupling of **13a** with freshly prepared vinyl triflate **12** [$Pd(PPh_3)_4$ cat., CuI cat., $Et_3N$], followed by removal of the dithiane protecting group from the resulting coupling product **65** (IBX),[27] led to enal enynone **66** in 43% overall yield for the two steps. Reduction of the acetylenic bond within the latter compound was then investigated as a necessary step prior to the proposed cyclization cascade. Unfortunately, however, only trace amounts of the desired precursor enal enone **10a** were obtained under several tried conditions, notably those that proved successful in the synthesis of model system **26** (**22** → **23**, Scheme 2) previously discussed. From analysis of the reaction mixtures of the various reductions, it was surmised that the difficulties arose from the presence of the isoquinoline moiety within the employed substrate. For these reasons, and due to the previous low-yielding steps already mentioned, this approach to cortistatin A was abandoned in favor of one in which the isoquinoline installment was reserved for a later stage and after the crucial cyclization cascade.

**Revised Strategy and Total Synthesis of Coristatin A.** Recognizing the problematic nature of the isoquinoline moiety in several of the reaction steps of our first attempt toward

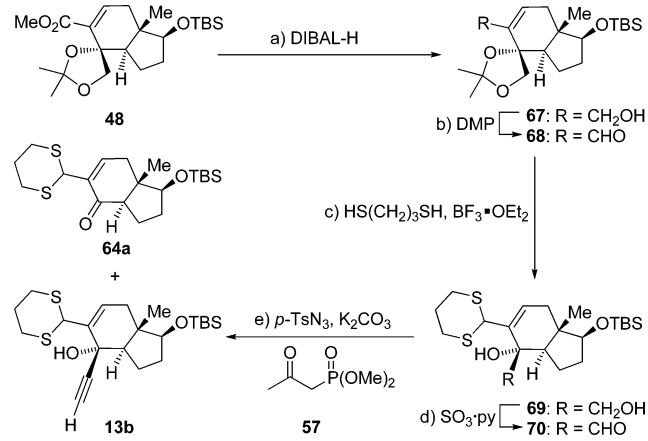
(27) Nicolaou, K. C.; Mathison, C. J. N.; Montagnon, T. *J. Am. Chem. Soc.* **2004**, *126*, 5192–5201.

***Scheme 8.*** Attempted Synthesis of Dienone **8**[a]




[a] Reagents and conditions: (a) $Pd(PPh_3)_4$ (0.1 equiv), CuI (0.1 equiv), $Et_3N$ (3.0 equiv), **12** (1.5 equiv), DMF, 23 °C, 1.5 h, 61%; (b) IBX (6.0 equiv), DMSO, 23 °C, 9 h, 70%.

***Scheme 9.*** Synthesis of Acetylenic Compound **13b**[a]

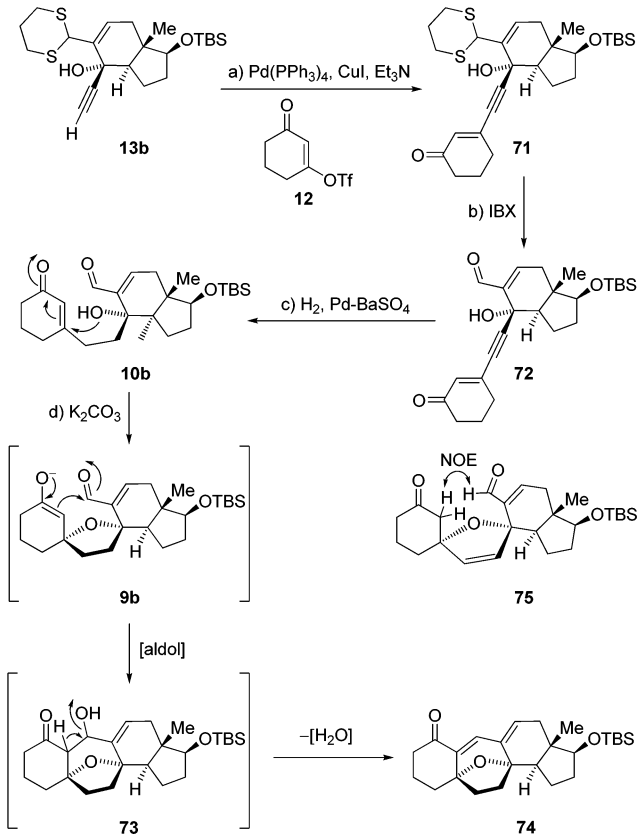



[a] Reagents and conditions: (a) DIBAL-H (1.0 M in toluene, 3.0 equiv), toluene, −78 °C, 3 h, 79%; (b) DMP (1.5 equiv), $NaHCO_3$ (4.6 equiv), $CH_2Cl_2$, 23 °C, 0.5 h, 86%; (c) $HS(CH_2)_3SH$ (3.0 equiv), $BF_3 \cdot OEt_2$ (3.5 equiv), $CH_2Cl_2$, −78 °C, 1.5 h, 70%; (d) $SO_3 \cdot py$ (3.0 equiv), $Et_3N$ (5.0 equiv), $CH_2Cl_2$/DMSO (4:1), 23 °C, 1.5 h, 72%; (e) *p*-$TsN_3$ (1.5 equiv), dimethyl-2-oxopropylphosphonate (**57**) (1.5 equiv), $K_2CO_3$ (3.5 equiv), $CH_3CN$, 23 °C, 2 h; then aldehyde **70**, THF/$CH_3CN$/MeOH (1:2:1); 23 °C, 16 h, **13b**: 45% (10% recovered starting material), **64a**: 20%.

cortistatin A, we retreated back to intermediate **48** (see Scheme 6) with the intention of reaching cortistatin A through intermediate compounds **13b** (Scheme 9) and **74** (Scheme 10). Such an approach would ensure the construction of the mainframe of the molecule prior to the attachment of the isoquinoline group and leave only a few steps in which to deal with its presence.

The preparation of acetylenic compound **13b** from intermediate **48** (Scheme 9) involved an initial reduction (DIBAL-H, 79% yield)−oxidation (DMP, 86% yield) protocol to afford aldehyde **68** via alcohol **67**. Subsequent exposure of **68** to 1,3-propanedithiol in the presence of $BF_3 \cdot OEt_2$ resulted in dithiane formation with concomitant acetonide removal to furnish

***Scheme 10.*** Construction of Dienone **74**[a]




[a] Reagents and conditions: (a) $Pd(PPh_3)_4$ (0.1 equiv), CuI (0.1 equiv), $Et_3N$ (3.0 equiv), **12** (1.4 equiv, freshly prepared from 1,3-cyclohexadione, $Tf_2O$ and $Et_3N$), DMF, 23 °C, 1 h, 85%; (b) IBX (4.0 equiv), DMSO, 0 → 23 °C, 4 h, 81%; (c) Pd−$BaSO_4$ (5 wt %/wt, 0.24 equiv), $H_2$, MeOH/THF (1:1), 23 °C, 0.5 h, 64%; (d) $K_2CO_3$ (1.2 equiv), dioxane, 125 °C, 12 h, 52%.

dihydroxy dithiane **69** in 70% yield. Oxidation of **69** with $SO_3$·py led to hydroxy aldehyde **70** in 72% yield, setting the stage for forging the required acetylenic moiety. Despite attempts to obtain the targeted acetylene through other means, the Ohira−Bestmann[25] method was the most efficient. We were, however, able to improve the yield of this reaction to 45% (10% recovered starting material) after optimization of the reaction conditions [1.5 equiv of **58** generated *in situ*, 3.5 equiv of $K_2CO_3$, THF/$CH_3CN$/MeOH (1:2:1), 23 °C, 16 h], accompanied with enone byproduct **64a** in 20% yield.

Acetylenic substrate **13b** entered the Sonogashira[9] coupling reaction [$Pd(PPh_3)_4$ cat., CuI cat., $Et_3N$] with vinyl triflate **12** with significantly improved efficiency (over that in the first generation study) to afford coupling product **71** in 85% yield. The IBX[27]-induced dithiane removal from the latter compound also proved superior to that involving the isoquinoline containing compound (**65** → **66**, Scheme 8), affording enal enone **72** in 81% yield. The next step involving selective hydrogenation of **72** to the desired cascade precursor **10b** was also expected to proceed more efficiently than the corresponding transformation of the isoquinoline compound (**66** → **10a**, Scheme 8). Indeed, exploration of various protocols (Table 3) led to the identification of optimum conditions for the preparation of **10b** [entry 9, Table 3, 5% Pd−$BaSO_4$, MeOH/THF (1:1), 23 °C, 0.5 h, 64% yield]. Interestingly, partially hydrogenated and cyclized compound **75** (stereochemistry supported by NOE studies) resisted further hydrogenation under certain reaction conditions (entries 1, 5−7, Table 3), and resubjecting the isolated material to further hydrogenation under more forceful conditions led to unattractive mixtures containing over-reduced products.

***Table 3.*** Hydrogenation of Alkyne **72** To Form Enone-Enal **10b** (Scheme 10)[a]

| entry | conditions | product(s) (yield)[b] |
|---|---|---|
| 1 | Wilkinson's catalyst (0.2 equiv), EtOH, 23 °C, 12 h | **75**(30%) + **72**(50%) |
| 2 | Pd−C (10 wt %/wt, 0.04 equiv), EtOAc/MeOH (4:1), 23 °C, 15 min | complex mixture |
| 3 | $Pd(OH)_2$ (10 wt %/wt, 0.03 equiv), EtOAc/MeOH (4:1), 23 °C, 15 min | complex mixture |
| 4 | Pd−$BaSO_4$ (5 wt %/wt, 0.05 equiv), quinoline (1 drop), MeOH, 23 °C, 12 h | no reaction |
| 5 | Pd−$BaSO_4$ (5 wt %/wt, 0.05 equiv), MeOH, 23 °C, 6 h | **75**(50%) |
| 6 | Pd−$BaSO_4$ (5 wt %/wt, 0.05 equiv), MeOH/THF (anhydrous) (5:1), 23 °C, 1 h | **10b**(20%) + **75**(30%) |
| 7 | Pd−$BaSO_4$ (5 wt %/wt, 0.1 equiv), MeOH/THF (3:1), 23 °C, 1 h | **10b**(30%) + **75**(30%) |
| 8 | Pd−$BaSO_4$ (5 wt %/wt, 0.2 equiv), MeOH/THF (3:2), 23 °C, 45 min | **10b**(55%) |
| *9* | *Pd−$BaSO_4$ (5 wt %/wt, 0.24 equiv), MeOH/THF (1:1), 23 °C, 0.5 h* | **10b***(64%)* |

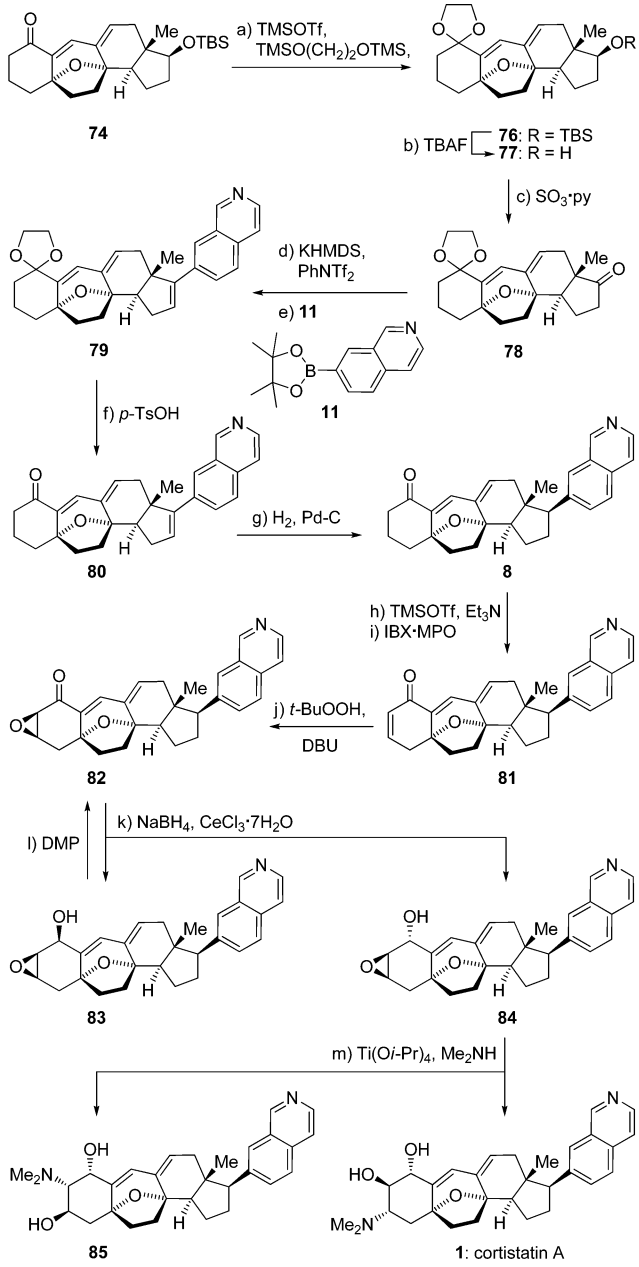
[a] Reactions were carried out on 0.014−0.116 mmol scale under 1 atm of hydrogen. [b] Yields refer to chromatographically pure materials.

Substrate **10b** performed well under the intended conjugate addition/aldol/dehydration cascade as previously developed ($K_2CO_3$, dioxane, reflux) to afford the targeted dienone **74** in 52% yield, presumably through the transient and non detectable intermediates **9b** and **73**, as shown in Scheme 10.

With the construction of the cortistatin dienone **74** completed, we then turned our attention to the final stages of the synthesis of cortistatin A (**1**) that required introduction of the isoquinoline moiety and functionalization of ring A. The successful drive to the target molecule (**1**) is summarized in Scheme 11. Installation of the isoquinoline moiety became our first priority, an objective that required temporary protection of the carbonyl group of dienone **74**. After considerable experimentation it was found that ketal **76** could be generated from **74** through the action of $TMSOCH_2CH_2OTMS$ in the presence of TMSOTf in $CH_2Cl_2$ at −50 → −10 °C together with small amounts (*ca.* 10%) of the next desired intermediate, hydroxy ketal **77**. The latter compound was formed from **76** by treatment of the crude reaction mixture with TBAF in 63% overall yield for the two steps. Oxidation of **77** with $SO_3$·py furnished ketone **78** (80% yield) which was converted to its vinyl triflate (KHMDS, Comins reagent)[28] and thence to vinyl isoquinoline **79** through a Suzuki−Miyaura coupling[8] reaction with boronic ester derivative **11** [$Pd(PPh_3)_4$ cat., $K_2CO_3$] in 60% overall yield. Subsequent removal of the protecting group from **79** with *p*-TsOH gave dienone vinyl isoquinoline **80** (88% yield), whose desired stereo- and chemoselective reduction to dienone isoquinoline **8** was accomplished by catalytic hydrogenation using 10% Pd/C as the catalyst and MeOH as the solvent (50% yield, 30% recovered starting material). The stereochemistry of **8** was tentatively assigned based on the result obtained with the truncated isoquinoline **52** discussed above (Scheme 6) and ultimately confirmed by the successful synthesis of **1** (see below). The next step required introduction of unsaturation in ring A, an operation in which a stark contrast between the

(28) Comins, D. L.; Dehghani, A. *Tetrahedron Lett.* **1992**, *33*, 6299–6302.

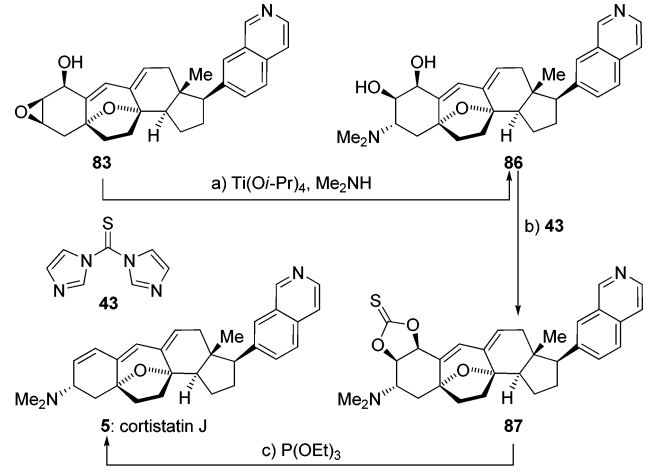
***Scheme 11.*** Completion of the Total Synthesis of Cortistatin A (**1**)[a]




[a] Reagents and conditions: (a) $TMSO(CH_2)_2OTMS$ (5.0 equiv), TMSOTf (1.5 equiv), $CH_2Cl_2$, −50 → −10 °C, 4 h; (b) TBAF (1.0 M in THF, 3.5 equiv), THF, 23 °C, 2 h, 63% for the two steps; (c) $SO_3$·py (6.0 equiv), $Et_3N$ (10.0 equiv), $CH_2Cl_2$/DMSO (3:1), 23 °C, 3 h, 80%; (d) KHMDS (0.5 M in toluene, 2.0 equiv), Comins reagent (2.0 equiv), THF, −78 °C, 1 h; (e) **11** (3.0 equiv), $Pd(PPh_3)_4$ (0.1 equiv), $K_2CO_3$ (3.0 equiv), THF, 80 °C, 3 h, 60% for two steps; (f) *p*-TsOH (1.5 equiv), acetone/$H_2O$ (10:1), 23 °C, 1 h, 88%; (g) Pd−C (10 wt %/wt, 0.3 equiv), $H_2$, MeOH, 23 °C, 1 h, 50% (30% recovered starting material); (h) TMSOTf (14 equiv), $Et_3N$ (30 equiv), THF, −78 → 0 °C, 1.5 h; (i) IBX·MPO (0.4 M in DMSO, 6.0 equiv), DMSO, 23 °C, 6 h, 46% for two steps; (j) *t*-BuOOH (6.0 equiv, 5.5 M in decane), DBU (3.0 equiv), $CH_2Cl_2$, 0 → 23 °C, 5 h, 70%; (k) $NaBH_4$ (1.0 equiv), $CeCl_3$·$7H_2O$ (4.0 equiv), MeOH, 0 °C, 10 min, 80% (*ca.* 1:1 mixture of diastereoisomers); (l) DMP (5.0 equiv), $NaHCO_3$ (10.0 equiv), $CH_2Cl_2$, 23 °C, 2 h, 100%; (m) $Me_2NH$ (2.0 M in THF, as solvent), Ti(O*i*-Pr)$_4$ (5.0 equiv), 80 °C, 5 h, **1**: 45%, **85**: 36%.

Saegusa[12] and the IBX·MPO[13] oxidation methods was observed. Thus, TMS enol ether formation from **8** followed by $Pd(OAc)_2$ addition resulted in significant complexation of palladium to the isoquinoline moiety (as deduced by baseline material on TLC) and no productive reaction, whereas addition of IBX·MPO to this enol ether furnished, upon chromatographic purification, the desired trienone **81** in 46% overall yield from **8**. Similar results were observed with other isoquinoline intermediates. Chemo- and stereocontrolled epoxidation of trienone **81** with *t*-BuOOH-DBU afforded epoxy ketone **82** in 70% yield. Luche[19] reduction ($NaBH_4$−$CeCl_3$) of the latter compound furnished isomeric hydroxy compounds **83** and **84** in 80% combined yield and *ca.* 1:1 ratio. Chromatographic separation of the two isomers allowed recycling of the undesired isomer (**83**) through an oxidation (DMP, 100% yield)−reduction protocol and conversion of the desired isomer **84** to cortistatin A (**1**) and its regioisomer **85**. This final step of the total synthesis was brought about through the action of $Me_2NH$−Ti(O*i*-Pr)$_4$ and delivered **1** in 45% yield and **85** in 36% yield, after chromatographic separation. All physical properties ($^1H$ and $^{13}C$ NMR spectra, MS, and $[\alpha]_D^{25}$) of synthetic cortistatin A (**1**) were in accordance to those reported for the natural substance.[3,5a]

***Scheme 12.*** Completion of the Total Synthesis of Cortistatin J (**5**)[a]




[a] Reagents and conditions: (a) $Me_2NH$ (2.0 M in THF, as solvent), Ti(O*i*-Pr)$_4$ (5.0 equiv), 80 °C, 2 h, 60%; (b) thiocarbonyl diimidazole (**43**) (2.0 equiv), toluene, 110 °C, 12 h, 81%; (c) $P(OEt)_3$ (as solvent), 160 °C, 24 h, 40% (45% recovered starting material).

**Total Synthesis of Cortistatin J.** Having completed the total synthesis of cortistatin A from hydroxy epoxide **84**, we then proceeded to convert the latter intermediate to cortistatin J (**5**) employing our developed technologies as discussed above for the construction of model system **45** from model hydroxy epoxide **35** (see Scheme 5). Thus, exposure of **83** (Scheme 12) to $Me_2NH$−Ti(O*i*-Pr)$_4$ in refluxing THF led to dimethylamino diol **86** in 60% yield. Reaction of the latter with thiocarbonyl diimidazole (toluene, 110 °C) gave thiocarbonate derivative **87** (81% yield), whose treatment with $P(OEt)_3$ (as solvent, 160 °C) led to cortistatin J (**5**) in 40% yield (plus 45% recovered starting material). Synthetic **5** exhibited identical physical properties to those reported for the natural substance.[4a]

**Biological Evaluation of Cortistatins A and J and Analogues.** The inhibitory activities of the synthesized compounds were tested against human umbilical vein endothelial cells (HUVECs), and the active candidates were further tested against a panel of cancer cells, including breast (MCF-7), CNS (SF268), lung (NCI-H460), ovarian (1A9), Taxol-resistant ovarian (PTX22),[29] and epothilone-resistant ovarian (A8)[30] cells using doxorubicin

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***Table 4.*** Antiproliferative Effects of Cortistatin A (**1**), Cortistatin J (**5**), and Selected Synthetic Analogues ($GI_{50}$ Values in $\mu$M)[a]

| entry | cell line/ compound | HUVEC | MCF-7 (SI) | NCI-H460 (SI) | SF268 (SI) | IA9 (SI) | PTX22 (SI) | A8 (SI) |
|---|---|---|---|---|---|---|---|---|
| 1 | synthetic cortistatin A (**1**) | 0.002 ± 0.001 | >10 (>5000.00) | 7.786 ± 1.692 (3893.00) | 4.429 ± 0.664 (2214.50) | 6.518 ± 0.756 (3259.00) | 6.543 ± 0.165 (3271.50) | 5.523 ± 0.045 (2761.50) |
| 2 | synthetic cortistatin J (**5**) | 0.070 ± 0.013 | 27.439 (391.99) | 6.197 ± 0.122 (88.53) | 4.340 ± 0.161 (62.00) | 43.22 (617.43) | 7.055 ± 0.013 (100.79) | 8.538 ± 0.588 (121.97) |
| 3 | **8** | 0.007 ± 0.001 | 7.893 ± 0.991 (1127.57) | 4.693 ± 0.826 (670.43) | 6.326 ± 0.698 (903.71) | 5.236 ± 0.039 (748.00) | 4.905 ± 0.488 (700.71) | 3.714 ± 0.072 (530.57) |
| 4 | **10b** | >10 | - | - | - | - | - | - |
| 5 | **13a** | >10 | - | - | - | - | - | - |
| 6 | **13b** | 5.745 ± 0.173 | 5.085 ± 0.170 (0.89) | 5.26 ± 0.358 (0.92) | 6.681 ± 0.729 (1.17) | 8.400 ± 1.302 (1.46) | >10 (>1.74) | 7.379 ± 0.698 (1.28) |
| 7 | **26** | >10 | - | - | - | - | - | - |
| 8 | **27** | 6.373 ± 0.847 | 6.505 ± 0.211 (1.02) | 5.700 ± 0.173 (0.89) | 5.142 ± 0.337 (0.81) | 6.251 ± 0.071 (0.98) | 6.009 ± 0.401 (0.94) | 5.281 ± 0.305 (0.83) |
| 9 | **28** | 5.753 ± 0.252 | 6.487 ± 0.235 (1.13) | 5.448 ± 0.278 (0.95) | 5.558 ± 0.163 (0.97) | 6.338 ± 0.318 (1.10) | 6.050 ± 0.029 (1.05) | 5.499 ± 0.038 (0.96) |
| 10 | **30** | >10 | - | - | | - | - | - |
| 11 | **32** | >10 | - | - | - | - | - | - |
| 12 | **33** | >10 | - | - | - | - | - | - |
| 13 | **35** | >10 | - | - | - | - | - | - |
| 14 | **36** | >10 | - | - | - | - | - | - |
| 15 | **38** | >10 | - | - | - | - | - | - |
| 16 | **41** | >10 | - | - | - | - | - | - |
| 17 | **44** | >10 | - | - | - | - | - | - |
| 18 | **45** | >10 | - | - | - | - | - | - |
| 19 | **53** | 0.253 ± 0.099 | >10 (>39.53) | >10 (>39.53) | >10 (39.53) | >10 (>39.53) | >10 (>39.53) | 9.861 ± 0.565 (38.99) |
| 20 | **54** | 0.357 ± 0.047 | 6.032 ± 0.324 (16.90) | 4.744 ± 0.453 (13.29) | 5.632 ± 1.152 (15.78) | 5.851 ± 0.349 (16.39) | 5.845 ± 0.057 (16.37) | 6.008 ± 0.281 (16.83) |
| 21 | **55** | >10 | - | - | - | - | - | - |
| 22 | **56** | 8.040 ± 1.756 | >10 (>1.24) | >10 (>1.24) | >10 (>1.24) | >10 (>1.24) | 6.456 ± 0.110 (0.80) | >10 (>1.24) |
| 23 | **64** | >10 | - | - | - | - | - | - |
| 24 | **65** | 5.804 ± 1.059 | 5.652 ± 0.111 (0.97) | 5.218 ± 0.646 (0.90) | 5.322 ± 0.471 (0.92) | 5.971 ± 0.292 (1.03) | 5.660 ± 0.071 (0.98) | 5.609 ± 0.033 (0.97) |
| 25 | **66** | 4.542 ± 0.853 | 5.060 ± 0.081 (1.11) | 5.225 ± 0.132 (1.15) | 5.161 ± 0.199 (1.14) | 3.719 ± 0.905 (0.82) | 4.477 ± 1.054 (0.99) | 4.057 ± 0.643 (0.89) |
| 26 | **71** | 3.995 ± 1.030 | 0.745 ± 0.110 (0.19) | 4.64 ± 0.429 (1.16) | 5.452 ± 0.260 (1.37) | 5.121 ± 0.102 (1.28) | 5.622 ± 0.250 (1.41) | 3.518 ± 0.887 (0.88) |
| 27 | **72** | 0.544 ± 0.052 | 0.532 ± 0.024 (0.98) | 0.729 ± 0.180 (1.34) | 0.524 ± 0.030 (0.96) | 0.519 ± 0.013 (0.95) | 0.512 ± 0.005 (0.94) | 0.578 ± 0.004 (1.06) |
| 28 | **74** | >10 | - | - | - | - | - | - |
| 29 | **77** | >10 | - | - | - | - | - | - |
| 30 | **78** | >10 | - | - | - | - | - | - |
| 31 | **79** | 0.486 ± 0.031 | 6.760 ± 0.117 (13.90) | 4.323 ± 0.265 (8.90) | 5.100 ± 0.392 (10.49) | 5.683 ± 0.036 (11.69) | 5.806 ± 0.168 (11.95) | 5.416 ± 0.004 (11.14) |
| 32 | **80** | 0.039 ± 0.003 | 5.504 ± 0.398 (141.13) | 4.750 ± 0.946 (121.79) | 4.383 ± 0.868 (112.38) | 5.291 ± 0.697 (135.66) | 4.999 ± 0.366 (128.18) | 4.026 ± 1.068 (103.23) |
| 33 | **81** | 0.008 ± 0.001 | 0.676 ± 0.031 (84.5) | 0.511 ± 0.033 (63.88) | 0.384 ± 0.029 (48) | 0.561 ± 0.001 (70.13) | 0.566 ± 0.009 (70.75) | 0.531 ± 0.002 (66.38) |
| 34 | **82** | 0.038 ± 0.007 | 2.18 ± 0.778 (57.37) | 5.363 ± 0.098 (141.13) | 0.627 ± 0.018 (16.5) | 0.638 ± 0.003 (16.79) | 0.656 ± 0.030 (17.26) | 0.906 ± 0.069 (23.84) |
| 35 | **83** | 0.008 ± 0.000 | 7.421 ± 0.286 (927.63) | 7.552 ± 2.068 (944.00) | 7.636 ± 1.989 (954.50) | 6.137 ± 0.327 (767.13) | 7.118 ± 0.237 (889.75) | 7.364 ± 1.163 (920.5) |
| 36 | **84** | 0.076 ± 0.002 | 7.860 ± 0.146 | 5.498 ± 0.060 | 4.375 ± 0.022 | 6.020 ± 0.033 | 6.057 ± 0.553 | 5.356 ± 0.251 |
| 37 | **85** | 0.034 ± 0.004 | 7.803 ± 0.935 (229.50) | 5.589 ± 1.015 (164.38) | 3.799 ± 0.573 (111.74) | 7.303 ± 0.712 (214.79) | 7.638 ± 0.248 (224.65) | 5.963 ± 0.211 (175.38) |
| 38 | **86** | 0.011 ± 0.001 | 46.026 (4184.18) | 55.163 (5014.80) | 6.918 ± 0.550 (628.91) | 7.535 ± 1.320 (685.00) | 7.379 ± 0.221 (670.82) | 6.439 ± 0.373 (585.36) |
| 39 | **87** | 0.060 ± 0.008 | 35.584 (593.07) | 8.539 ± 1.420 (142.32) | 5.856 ± 0.631 (97.60) | 45.571 (759.52) | 36.669 (611.15) | 47.213 (786.88) |
| 40 | Taxol | 0.005 ± 0.001 | 0.007 ± 0.001 (1.4) | 0.006 ± 0.001 (1.20) | 0.006 ± 0.001 (1.2) | 0.005 ± 0.000 (1.00) | 0.058 ± 0.001 (11.6) | 0.052 ± 0.001 (10.4) |
| 41 | Doxorubicin | 0.007 ± 0.001 | 0.040 ± 0.018 (5.71) | 0.052 ± 0.019 (7.43) | 0.045 ± 0.008 (6.429) | 0.018 ± 0.006 (2.57) | 0.053 ± 0.0002 (7.57) | 0.037 ± 0.006 (5.29) |

[a] Antiproliferative effects of cortistatin A (**1**), cortistatin J (**5**), and its synthetic analogues on human umbilical vein endothelial cells (HUVECs), human tumor cell lines, and drug-resistant tumor cell lines in a 96 h growth inhibition assay using WST-8 colorimetric method. The human cancer cell lines included were breast (MCF-7), lung (NCI-H460), CNS (SF268), ovarian (IA9) and its drug-resistant mutants PTX10 (Taxol-resistant) and A8 (epithilone resistant). Growth inhibition of 50% ($GI_{50}$) is calculated as the drug concentration which caused 50% inhibition as compared to untreated control. $GI_{50}$ values for each compound represent mean of 2−5 independent experiments ± SE; Selectivity index (SI = $GI_{50}$ cancer cells/$GI_{50}$ HUVECs cells).

and Taxol as standards. The results are summarized in Table 4. As expected, cortistatin A (**1**) exhibited potent and selective inhibition toward HUVECs, with the selectivity index ranging between 2000 to 5000 against the cancer cell lines examined (entry 1). Synthetic cortistatin J (**5**) was found under the conditions employed in this study to be somewhat less active than originally reported[4a] against the HUVECs and less selective against the cancer cell lines examined (entry 2). Cortistatin model systems **26**−**28** (entry 7−9), **30** (entry 10), **32** (entry 11), **33** (entry 12), **35** (entry 13), **36** (entry 14), **38** (entry 15), **41** (entry 16), **44** and **45** (entries 17 and 18) containing only the ABCD ring systems of the cortistatin core structure showed complete loss of activities against all cell lines tested. Compounds **53** (entry 19) and **54** (entry 20) containing the DE ring systems and the isoquinoline domain, despite their significant loss of activity compared to natural cortistatin A, showed a notable level of selectivity toward the HUVECs. While compounds **74**, **77**, and **78** (entry 28−30) containing solely the ABCDE ring systems of the cortistatins were completely inactive, interestingly, their activities were restored upon attachment of the isoquinoline moiety, as illustrated by compounds **79**−**87** (entry 31−39). Stereochemistry of the isoquinoline appendage seems to play somewhat of a role, although not dramatic, in the observed inhibitory activity toward the HUVECs, as seen by comparing compounds **8** (entry 3) and **80** (entry 32). Compounds **8** (entry 3), **81** (entry 33), and **83** (entry 35) were the most active analogues synthesized. In particular, the activities of advanced intermediates **8** (entry 3) and **81** (entry 33), lacking most of the A-ring functionalities, were of interest. These results lead to the rather surprising conclusion that neither the dimethyl amino nor the hydroxyl functionalities on ring A of the cortistatin molecule are absolutely essential for biological activity. In contrast, the isoquinoline moiety appears to be consistently essential and required for the biological action of these molecules. Considering the easier access through chemical synthesis to such simplified analogues, this finding may prove valuable and pathpointing for future explorations within the field.

## Conclusion

In this article we described the evolution of a modular, convergent synthetic strategy culminating in the total synthesis of the antiangiogenic natural products cortistatins A (**1**) and J (**5**). These syntheses demonstrate the power of cascade reactions and palladium-catalyzed couplings in complex molecule construction and the importance of chemical synthesis in rendering scarce biologically active natural products readily available for chemical biology investigations. Indeed, through application of the developed synthetic routes, a number of analogues of the cortistatins were synthesized and tested, providing important insights into their structure activity relationships (SARs).[31] Of particular interest is the discovery that simplified analogues such as **8** and **81** exhibit comparable antiproliferative activities against HUVECs as the most active naturally occurring cortistatins themselves. These findings are expected to catalyze and facilitate further developments in the area of anticancer research.

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**Supporting Information Available:** Experimental procedures and compound characterization (PDF, CIF). This material is available free of charge via Internet at http://pubs.acs.org.

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