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Synthetic Strategies Directed Towards the Cortistatin Family of Natural Products

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Since their isolation by Kobayashi and co-workers, the cortistatins have captured the attention both of the synthetic chemistry community and of researchers interested in exploiting the potent anti-angiogenic activity of these natural products. The unique rearranged steroidal cortistatin core has become the target of numerous synthetic efforts, which are detailed herein.

1. Introduction

Natural products have long been a source of inspiration for drug development. Many naturally produced molecules are currently on the market as pharmaceuticals, and many small-molecule drugs can trace their structures back to naturally isolated compounds. In fact, nearly half of the drugs on the market today are in some way derived from natural products.[1] As such, the isolation of new classes of natural products from marine and terrestrial sources, coupled with the capability of chemists to synthesize these molecules and their structural analogues, is of central importance to the introduction of new classes of compounds into the pharmaceutical industry.[2]

In 2006, Kobayashi and co-workers reported the structures of four novel steroidal alkaloids, cortistatins A–D (**1**–**4**, Figure 1).[3] Milligram quantities of the cortistatins were isolated through bioassay-guided separation of extracts from the Indonesian marine sponge *Corticium simplex*. Ko-

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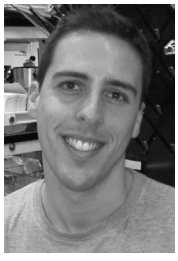

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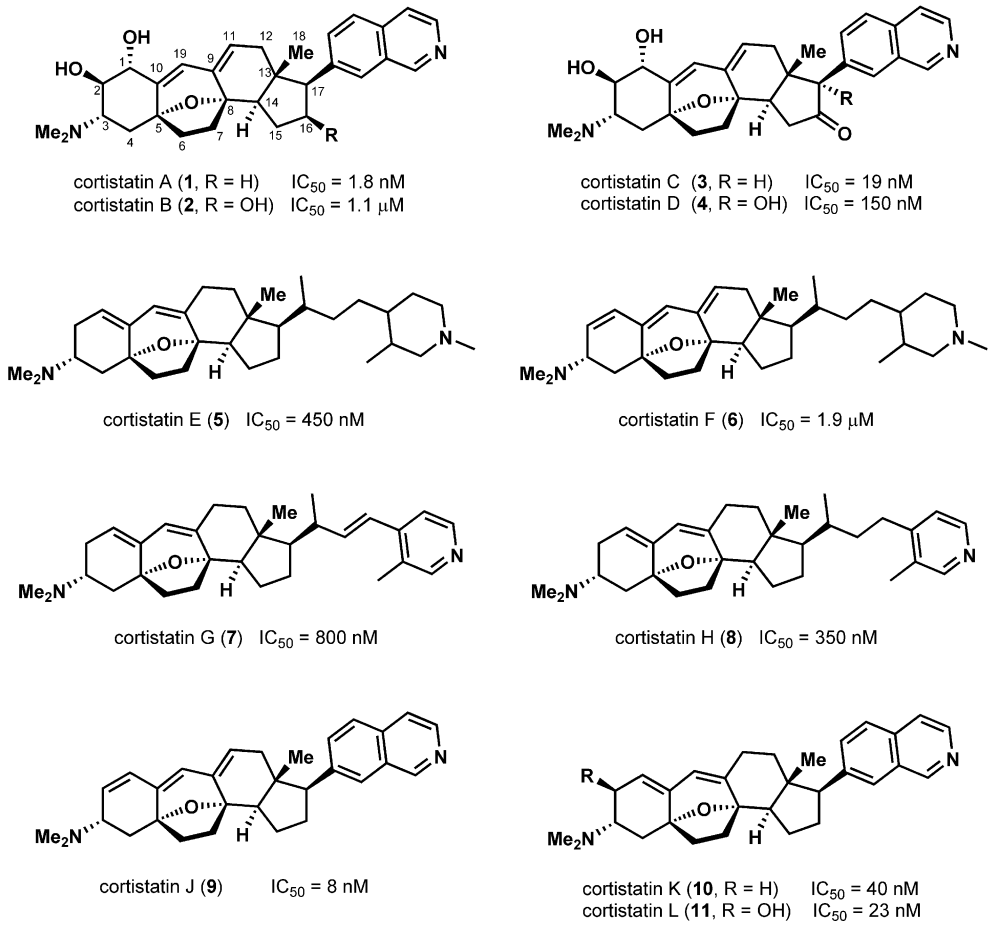
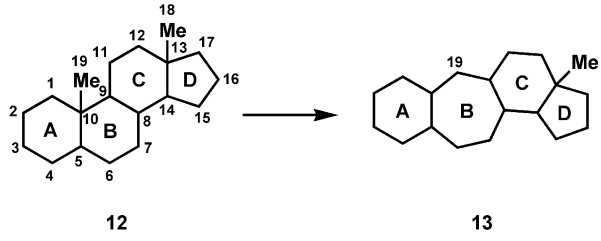


Figure 1. The cortistatin family of natural products.

bayashi et al. elucidated the pentacyclic structure of the cortistatins through detailed 2D NMR studies and further confirmed their connectivity and absolute configuration through X-ray crystallographic and CD spectroscopic analysis of cortistatin A (**1**). Cortistatins A–D display a common rearranged steroidal core (which features the C19 methyl group incorporated into the rare oxabicyclo[3.2.1] octane B ring; Scheme 1) possessing two hydroxy groups and one dimethylamino group on the A ring, identical unsaturation patterns along the northern edge of the B and C rings, and an isoquinoline appendage on the D ring. The sole difference between these four molecules lies in the level of oxidation at C16 and C17: cortistatin A (**1**) is devoid of oxygenation at both positions whereas cortistatins B–D (**2**–**4**) exist at various higher oxidation levels.

In the year after the initial isolation of cortistatins A–D, Kobayashi reported four closely related alkaloids from extracts of the same species.[4] The pentacyclic core is conserved throughout cortistatins A–H, but cortistatins E–H (**5**–**8**) are decorated differently from their previously isolated congeners. One key difference is that cortistatins E–H each possess a side chain bearing either an *N*-methylpiperidine (cortistatins E and F, **5** and **6**, respectively) or a 3-methylpyridine (cortistatins G and H, **7** and **8**) unit on the D ring instead of an isoquinoline group. These *N*-heterocyclic side chains are believed to be precursors to the isoquinoline group in the biosynthetic pathway of cortistatins A–D.[4] In a third communication, Kobayashi and co-workers disclosed an additional three cortistatins: cortistatins J, K, and L (**9**, **10** and **11**).[5] Cortistatins J, K, and L differ from cortistatin A only in the oxidation of the A ring and the pattern of unsaturation along the northern edge of the molecule.



Scheme 1. Rearrangement of the steroidal core to the cortistatin core.

It is undoubtedly the sheer beauty of the cortistatin structure that has, over the past three and a half years, drawn the attention of so many synthetic chemists. However, the relative scarcity of naturally isolated material, coupled with the striking biological activity of these molecules,

truly demands an efficient synthetic approach to the cortistatins and related compounds. At the time of their isolation, members of the cortistatin family demonstrated nanomolar IC_{50} values in an assay evaluating their antiproliferative activity against human umbilical vein endothelial cells (HUVECs), a common model for anti-angiogenic activity.[6] Angiogenesis is a vital cellular process that plays a central role in embryonic development and wound repair, and under normal circumstances is tightly regulated by angiogenic polypeptides.[7,8] However, angiogenesis can also facilitate tumor growth and metastasis. Compounds capable of inhibiting angiogenesis have potential use in cancer treatment because they may reduce the growth of new blood capillaries to tumors. Anti-angiogenic agents have been shown to suppress the recurrence of tumor growth when administered in parallel with traditional chemotherapeutics in some animal models, leading to curative treatment.[9] The excitement surrounding anti-angiogenics as a cancer treatment option stems not only from their low toxicity but also from the lack of observed drug resistance.[8] The recent approval of Bevacizumab, a monoclonal antibody against VEGF, for the treatment of breast and colon cancers constitutes a major step forward for anti-angiogengenics as a promising cancer therapy.[10]

Of the eleven known family members, cortistatins A (**1**) and J (**9**) have proven to be the most potent anti-angiogenic compounds, with IC_{50} values of 1.8 nM and 8 nM, respectively, in an assay measuring the growth inhibition of HUVECs.[6] From the biological evaluation carried out by Kobayashi and co-workers the isoquinoline side-chain appears to be essential for anti-angiogenic activity, and the substituents on the A ring also play a role in modulating the potency of the cortistatins as anti-angiogenic compounds.

2. Synthetic Approaches to the Cortistatins

Over the past three years, more than a dozen research groups have published approaches directed toward the synthesis of the cortistatins.[11] Their collective efforts, commencing from a broad array of starting materials and guided by an assortment of creative strategies, have culminated in one semi-synthesis,[12] three total syntheses,[13–15] and two formal syntheses,[16] as well as a number of syntheses of the pentacyclic cortistatin core and some illuminating model studies.[17] In an attempt to present this synthetic work in a comprehensive yet palatable manner, these approaches are organized herein by the method for B ring formation.

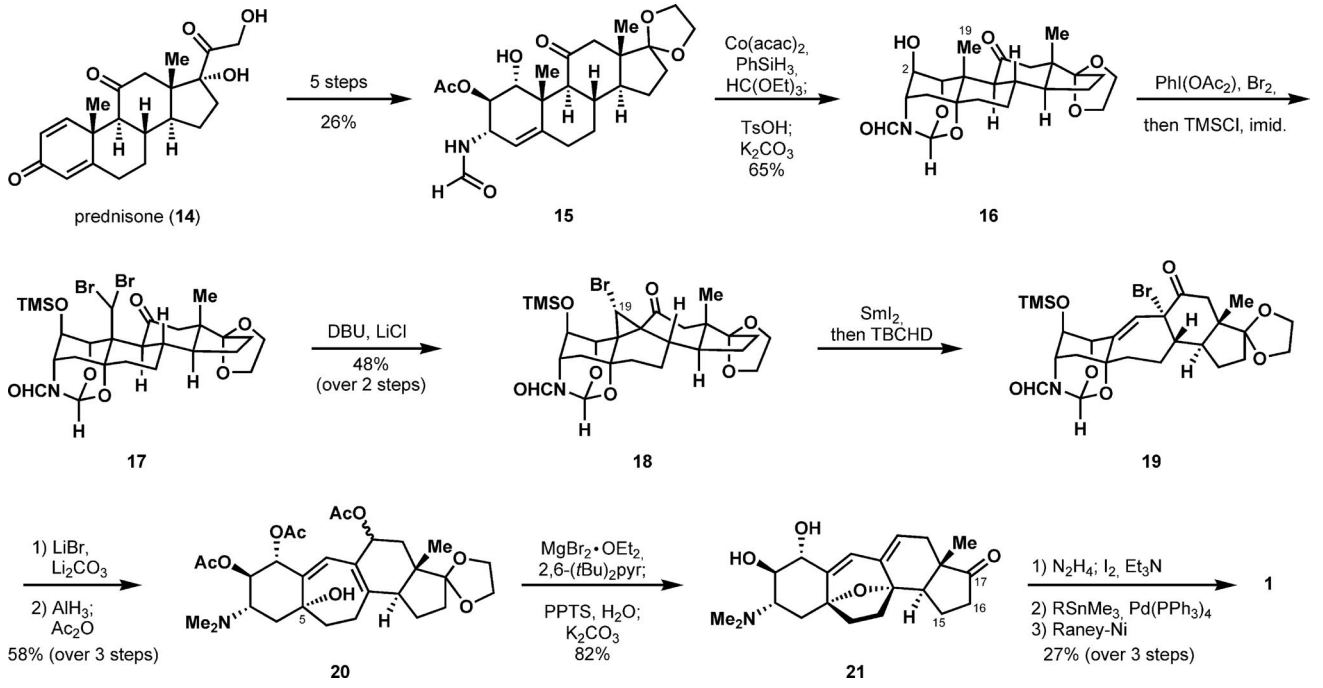
2.1.1. Ring-Expansion Approaches – Syntheses of Cortistatin A

Although organic chemists have been successfully constructing and elaborating the classic steroid core for nearly a century, the unusual rearranged steroidal skeleton (see Scheme 1) possessed by the cortistatins poses a challenge that has inspired multiple solutions. Some have chosen to build the cortistatins from simple starting materials, one ring at a time, whereas others have relied on converting the familiar steroid framework into the cortistatin core by expansion of the B ring through the incorporation of the C19 methyl group.

In the latter vein, Baran and co-workers employed a cyclopropane fragmentation to craft the seven-membered B ring of the cortistatins en route to the first synthesis of (+)-cortistatin A.[12] Their semi-synthetic effort began with the commercially available steroid prednisone (**14**), which was converted into the formamide **15** in five steps and 26% overall yield (Scheme 2). Mukaiyama hydration of alkene **15** with $Co(acac)_2$ and $PhSiH_3$ was then followed by *ortho*-amide formation and subsequent cleavage of the acetate to deliver the secondary alcohol **16** in 65% yield. To set the stage for the key ring-expansion step, an alcohol-directed double C–H functionalization was effected by treatment of **16** with $PhI(OAc)_2$ and Br_2 and irradiation with a sunlamp. Because of the instability of the resulting dibromo alcohol, this intermediate was directly silylated with TMSCl to yield the C19-dibromomethyl species **17**. The carefully selected C–H functionalization conditions lead to the generation of acetoxy hypobromite (AcOBr) in situ, and the ensuing transformation is believed to proceed through a domino process involving formation of a C2 oxygen radical, abstraction of a hydrogen atom from the C19 methyl group, and subsequent bromine atom abstraction by the intermediate alkyl radical. Exposure of **17** to DBU and LiCl then yielded the bromocyclopropane **18** in 48% overall yield.

To complete the ring-expansion sequence and to forge the seven-membered B ring, a regioselective fragmentation of the newly formed cyclopropane was required. This transformation was successfully effected upon treatment of the bromocyclopropane **18** with SmI_2, and the intermediate enolate was trapped by treatment with 2,4,4,6-tetrabromo-2,5-cyclohexadienone (TBCHD) to give the α-bromo ketone **19**. Dehydrobromination of **19** with Li_2CO_3, alane reduction of the ketone and *ortho*-amide moieties, and acylation of the secondary hydroxy groups delivered the triacetate **20** in 58% overall yield from **18**. Intramolecular displacement of the allylic acetate by the C5 hydroxy group installed the ether bridge, and global deprotection provided the ketone **21**, which was dubbed "cortistatinone" by Baran et al. Conversion of **21** into (+)-cortistatin A (**1**) was achieved by conversion of the ketone into an intermediate hydrazone, which was then oxidized to a vinyl iodide that underwent Stille coupling with 7-(trimethylstannyl)isoquinoline to yield Δ^{16}-cortistatin A. Chemoselective reduction of the isolated, trisubstituted double bond with Raney-Ni then delivered (+)-cortistatin A (**1**) in 27% overall yield from cortistatinone (**21**).

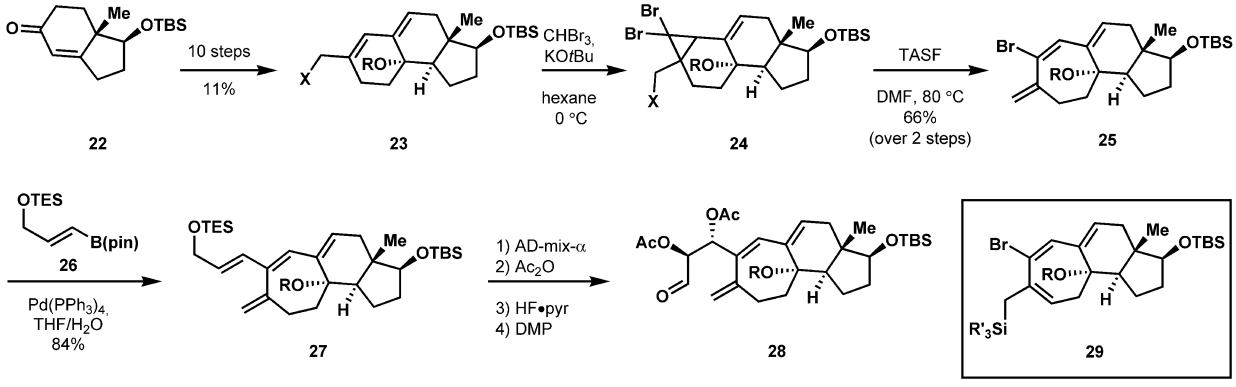
A cyclopropane fragmentation was also exploited by Shair and co-workers in their total synthesis of (+)-cortistatin A (Scheme 3).[14] This sequence began with the known Hajos–Parrish ketone derivative **22**, which was converted in ten steps and 11% overall yield into the tricyclic diene **23** (R = MEM). In preparation for the ring-expanding event, compound **23** was treated with dibromomethylene to effect



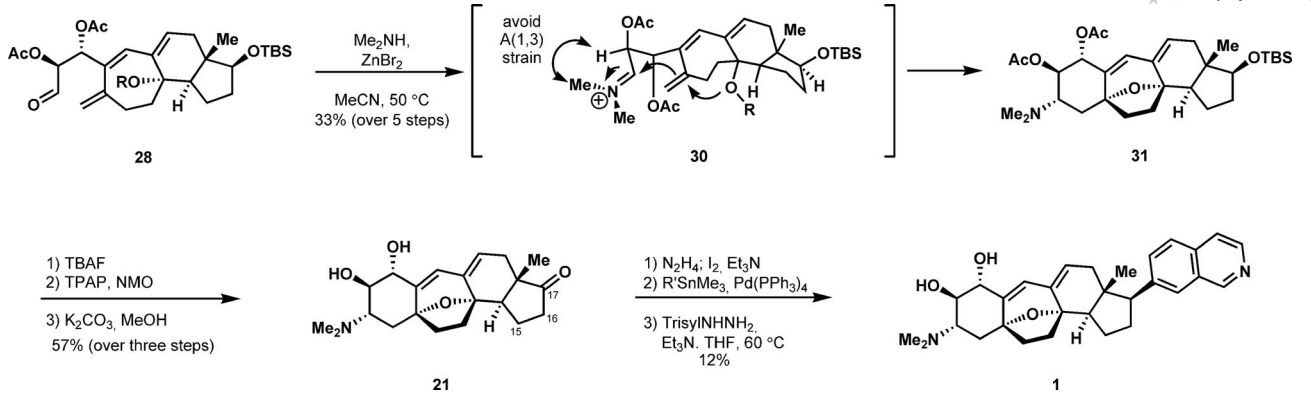
Scheme 2. Baran's semi-synthesis of (+)-cortistatin A. R = 7-isoquinolinyl; acac = acetylacetonyl; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; imid. = imidazole; PPTS = pyridinium *p*-toluenesulfonate; pyr = pyridine; TBCHD = 2,4,4,6-tetrabromo-2,5-cyclohexadienone.

cyclopropanation and to provide the dibromocyclopropane **24**. A fluoride-mediated desilylative ring-expansion of **24** (X = SiR_3) was chosen to provide the vinyl bromide **25**, and the selection of the allylic silyl group was found to play a critical role in the outcome of this transformation. The use of a trimethylsilyl group (X = $SiMe_3$) led to a mixture of the desired ring-expansion product **25** and the endocyclic cycloheptadiene **29**, the latter arising from base-promoted elimination of the presumed pentadienyl cation intermediate. However, through the employment of a methyl(diisopropoxy)silyl group [X = $Si(OiPr)_2Me$], which was expected to have a higher propensity to form a pentacoordinate fluorosilicate species, the vinyl bromide **25** could be isolated as the exclusive product in 66% overall yield from **23**.

With the bromide **25** in hand, a Suzuki–Miyaura coupling of this compound with the boronate **26** gave an 84% yield of the tetraene **27**. This product was elaborated to the aldehyde **28** by a four-step sequence consisting of dihydroxylation, bisacylation, selective silyl ether cleavage, and alcohol oxidation. In the second key step of the synthesis, treatment of **28** with Me_2NH and $ZnBr_2$ triggered an aza-Prins/transannular cyclization to deliver the pentacycle **31** in 33% overall yield from **27** (Scheme 4). The aza-Prins cyclization is presumed to proceed through a boat-like transition state in which A(1,3) strain is minimized (see **30**, Scheme 4), and it is believed that the bridged ether formation occurs prior to loss of the MEM group. Cleavage of the TBS ether moiety of **31** and oxidation of the resulting alcohol, followed by removal of the acetate groups, then



Scheme 3. Shair's elaboration of the Hajos–Parrish ketone to an advanced intermediate for the synthesis of cortistatin A. R = MEM (2-methoxyethoxy)methyl; X = $SiMe_3$ or $Si(OiPr)_2Me$; DMP = Dess–Martin periodinane; pin = pinacolato; pyr = pyridine; TASF = tris(diethylamino)sulfonium difluorotrimethylsilicate.



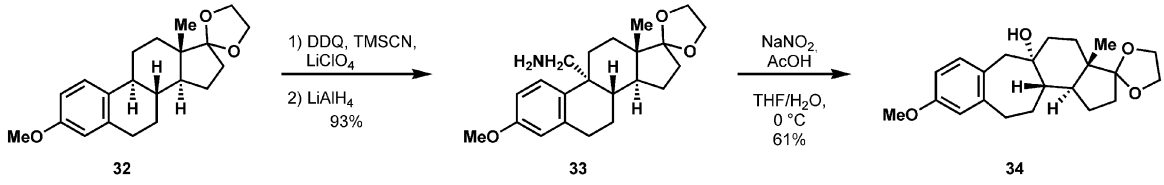
Scheme 4. Shair's completion of (+)-cortistatin A. R = MEM = (2-methoxyethoxy)methyl; R' = 7-isoquinolinyl; NMO = *N*-methylmorpholine *N*-oxide; TBAF = tetra-*n*-butylammonium fluoride; TPAP = tetra-*n*-propylammonium perruthenate; trisyl = 2,4,6-triisopropylbenzenesulfonyl.

gave **21** in 57% yield. Following the precedent of Baran et al., **21** was converted into the corresponding vinyl iodide, which underwent a Stille coupling to provide Δ^{16}-cortistatin A in 61% yield. Selective hydrogenation of this alkene proved to be challenging, and ultimately a 20% yield of (+)-cortistatin A (**1**) was achieved by use of diimide generated in situ from 2,4,6-triisopropylsulfonylhydrazide and Et_3N.

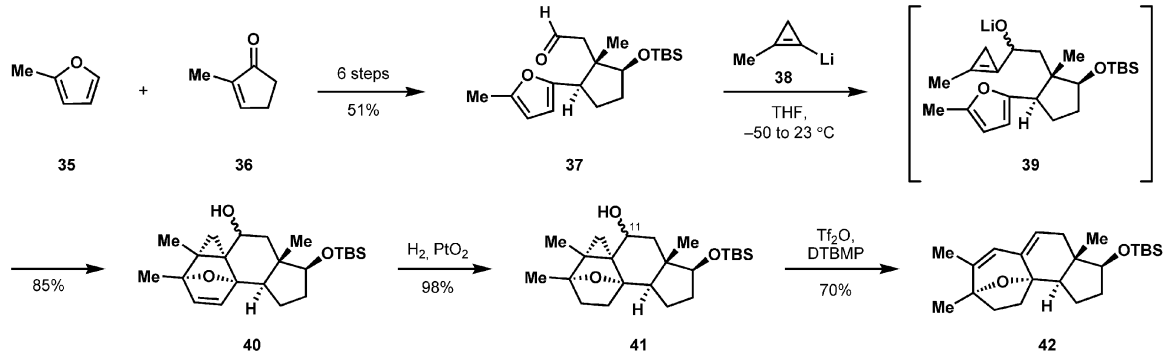
2.1.2. Ring-Expansion – Model Studies

Corey and co-workers employed a Demjanov ring-expansion in a semi-synthetic approach that provides rapid entry to the carbocyclic cortistatin tetracycle.[17h] Compound **32** (Scheme 5) was prepared in two steps from (+)-estrone and subsequently treated with a mixture of DDQ, TMSCN, and $LiClO_4$ to effect a benzylic oxidation and to yield an intermediate nitrile. Reduction of this species with $LiAlH_4$ afforded the amine **33** in 93% overall yield from **32**. Diazotization of **33** with $NaNO_2$/AcOH in THF/H_2O then effected the key Demjanov ring-expansion to provide the tetracycle **34** directly in 61% yield.

Magnus and Littich utilized a cyclopropylcarbinyl rearrangement to access the BCD ring system of cortistatin A.[17k] Their efforts began with 2-methylfuran (**35**, Scheme 6) and 2-methylcyclopent-2-enone (**36**), which were converted in six steps and 51% overall yield into the aldehyde **37**. Addition of the cyclopropenyllithium species **38** to the aldehyde **37** at low temperature led to the initial formation of the lithium alkoxide **39**, which was observed by TLC as the corresponding alcohol. Allowing this species (**39**) to warm to room temperature effected an intramolecular cyclopropene–furan [2+4] cycloaddition to generate the cycloadduct **40** in 85% yield and as a 1:1 mixture of separable diastereomers. Hydrogenation of this mixture with Adams' catalyst yielded **41** as an inseparable diastereomeric mixture. Whereas the C11 axial β-epimer readily underwent cy-



Scheme 5. Corey's ring-expansion model study. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.



Scheme 6. Magnus' construction of the BCD ring system. DTBMP = 2,6-di-*tert*-butyl-4-methylpyridine.

clopropylcarbinyl rearrangement upon treatment with Me_2AlCl, the corresponding C11 equatorial α-epimer proved unreactive under these conditions. However, direct subjection of the 1:1 mixture (**41**) to Tf_2O and DTBMP provided a 70% yield of the diene **42**, which possesses the substitution pattern of the BCD ring system of cortistatin A (**1**).

2.2. Oxidative Dearomatization

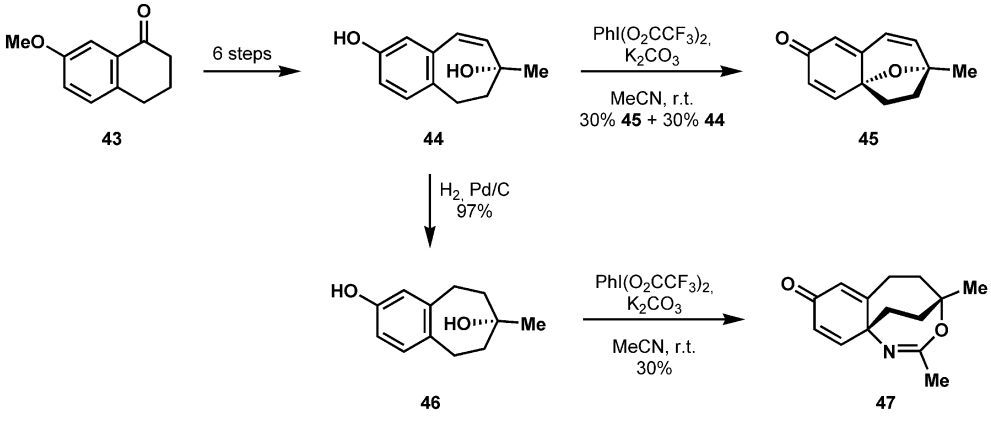
Oxidative dearomatization has proven to be a powerful tool in total synthesis for the formation of bonds to phenolic carbons.[18] In the case of the cortistatins, oxidative dearomatization has been employed for the construction of the bridging ether moiety contained within the B ring. We,[16c,17c] along with the research groups of Sorensen[17l] and Danishefsky,[17a] have utilized different incarnations of this transformation in synthetic studies directed towards the cortistatins.

In the first published synthetic efforts directed towards the cortistatins, a preliminary model study exploring the viability of an oxidative dearomatization strategy to access the cortistatin framework was reported by Dai and Danishefsky.[17a] 7-Methoxy-1-tetralone (**43**) was converted into the phenol **44** in six steps (Scheme 7). Treatment of **44** with $PhI(O_2CCF_3)$ and K_2CO_3 in MeCN induced an oxidative dearomatization to give the desired tricycle **45** in 30% yield, along with recovered **44** (30%), and the structure of **45** was confirmed by X-ray crystallography. Interestingly, attempted cyclization of the saturated analogue **46** under identical conditions did not lead to formation of the desired product, but instead a Ritter reaction took place in which a molecule of MeCN was incorporated into the bridge of the resulting tricyclic product **47**.

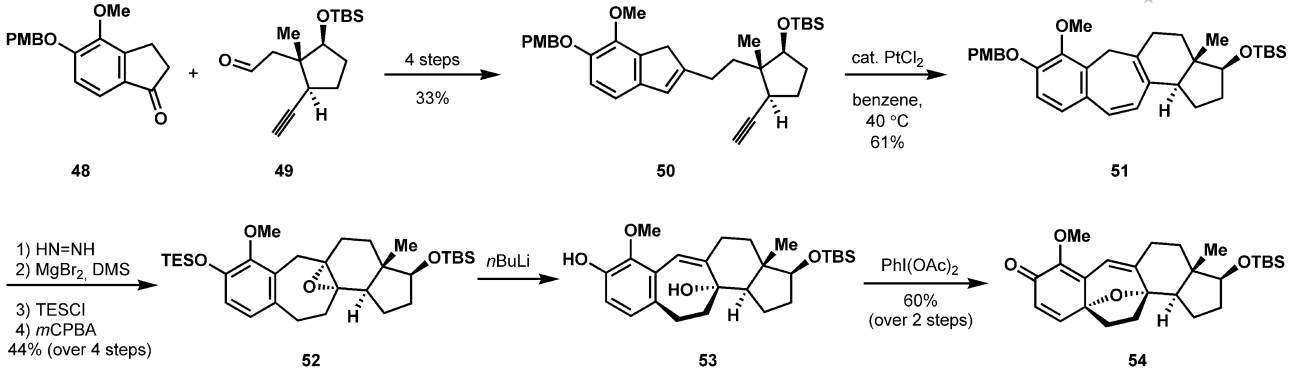
We reported a synthesis of the pentacyclic core of the cortistatins that utilized an enyne cycloisomerization to form the seven-membered B ring followed by an oxidative dearomatization to forge the ether bridge.[17c] The sequence commenced with the indanone **48** (Scheme 8) and the aldehyde **49**, which were converted in four steps and 33% overall yield into the alkynyl indene **50**. Treatment of this compound with catalytic $PtCl_2$ effected an enyne cycloisomerization that provided the benzocycloheptadiene **51** in 61% yield. Chemoselective hydrogenation of the disubstituted double bond of **51** with diimide (generated in situ from tosylhydrazide and Et_3N), followed by protecting group exchange and epoxidation with *m*CPBA, then furnished the epoxide **52** in 44% overall yield. Regioselective opening of the epoxide moiety and concomitant cleavage of the TES ether was achieved by treatment of **52** with *n*BuLi to yield the intermediate allylic alcohol **53**. Oxidative dearomatization of **53** then occurred upon subjection of this species to $PhI(OAc)_2$, which formed the ether bridge and provided the pentacycle **54** in 60% yield over the two-step sequence.

After this preliminary report we devised an improved route to the pentacycle **54** (Scheme 9), which began with the benzyl-protected indanone **55**.[16c] The robustness of this protecting group relative to the previously employed PMB ether in several of the key transformations of the sequence, such as the $PtCl_2$-catalyzed enyne cycloisomerization, led to an overall yield of **54** that was nearly double that of the first effort. With significant quantities of **54** available, initial investigations were directed toward cortistatin A (**1**). Oxidative transposition of the C1,C19 diene moiety of **54** was achieved through a three-step sequence involving epoxidation of the trisubstituted double bond with *m*CPBA, acid-catalyzed opening of the epoxide in the presence of MeOH, and dehydration of the resulting tertiary alcohol with TFAA to provide the C10,C19 diene **56** in 58% overall yield. Luche reduction of **56** and acylation of the intermediate allylic alcohol with Boc_2O afforded a 69% yield of the carbonate **57**, which was subjected to Pd(dppf)-Cl_2 and ammonium formate to yield the tetraene **58**. Selective hydrogenation of the disubstituted double bond of **58** with Wilkinson's catalyst and ensuing enol ether hydrolysis then afforded the dienone **59** in 77% overall yield from **57**. The dienone **59** had previously been converted into cortistatin A (**1**) by Nicolaou and co-workers[13] (see Section 2.4.1), so a formal synthesis of this natural product was achieved.

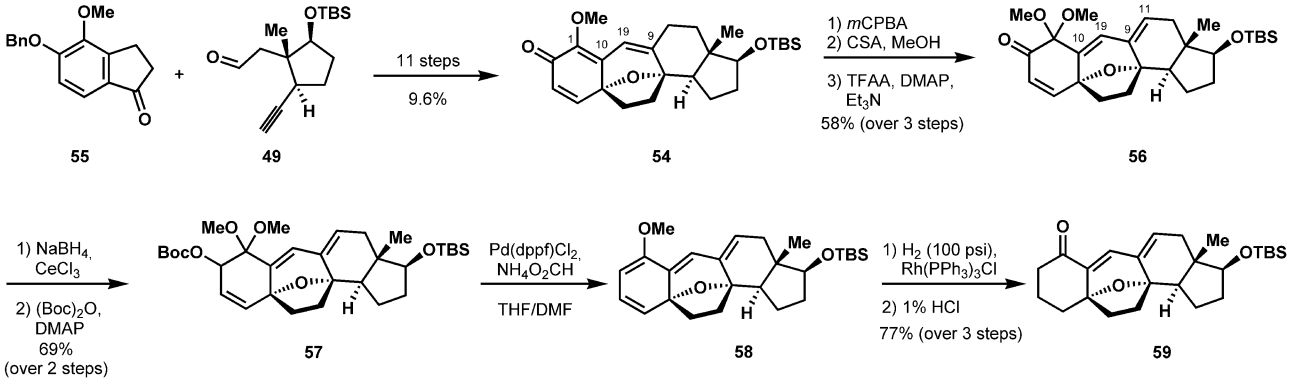
Sorensen and co-workers reported a synthesis of the pentacyclic core of the cortistatins featuring a tandem oxidative dearomatization/intramolecular dipolar cycload-



Scheme 7. Danishefsky's oxidative dearomatization model study.

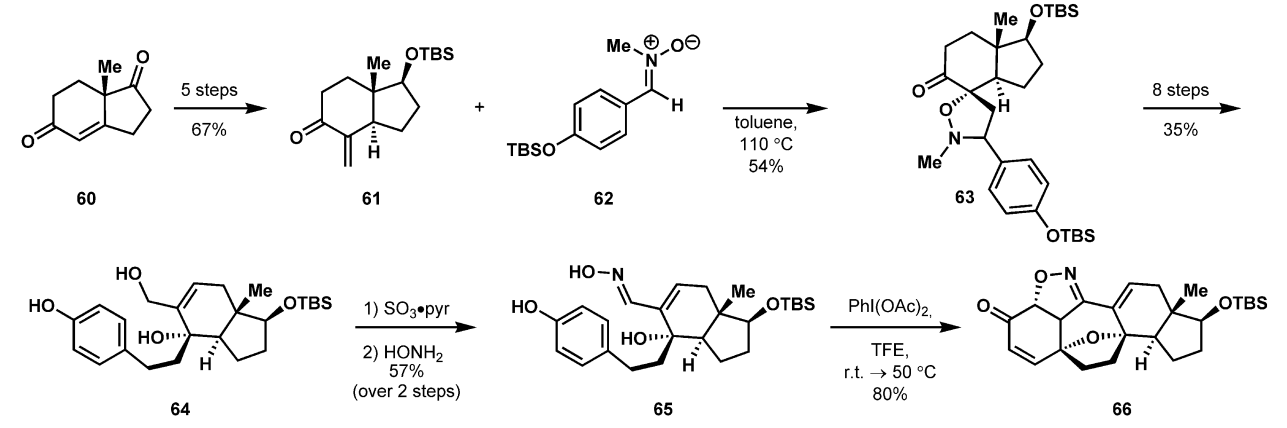


Scheme 8. Sarpong's first-generation synthesis of the pentacyclic core. DMS = dimethylsulfide; *m*CPBA = *m*-chloroperbenzoic acid; PMB = *p*-methoxybenzyl.

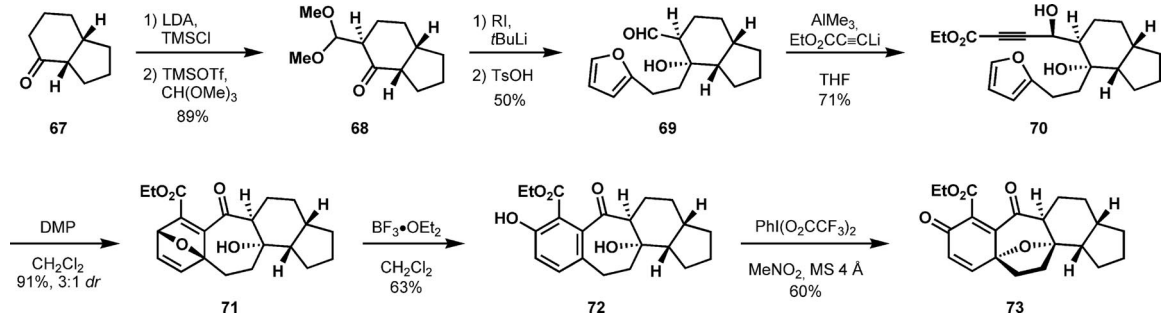


Scheme 9. Sarpong's formal synthesis of (±)-cortistatin A (**1**). Boc = *tert*-butyloxycarbonyl; CSA = camphorsulfonic acid; DMAP = 4-(dimethylamino)pyridine; dppf = 1,1′-bis(diphenylphosphanyl)ferrocene; *m*CPBA = *m*-chloroperbenzoic acid; TFAA = trifluoroacetic anhydride.

dition.[17l] The synthesis employed the Hajos–Parrish ketone (**60**, Scheme 10) as the starting material. This was converted into the known derivative **61**, a compound originally prepared by Danishefsky and co-workers, in 67% yield by an improved five-step sequence. A regioselective, intermolecular dipolar cycloaddition between this species and the nitrone **62** provided the cycloadduct **63** in 54% yield. Compound **63** was further advanced to the phenol **64** in eight steps, which included a vinyl triflate carbonylation and N–O bond cleavage with Zn dust. Preliminary efforts to oxidize the allylic hydroxy group of **64** to the corresponding aldehyde with Dess–Martin periodinane also triggered oxidative dearomatization of the phenol moiety, but the resulting adduct could not be successfully advanced. However, Doering oxidation of **64** provided an aldehyde that was condensed with hydroxylamine to give the oxime **65** in 57% yield. An elaborate, yet efficient, cascade sequence was achieved upon treatment of this species with $PhI(OAc)_2$.



Scheme 10. Sorensen's synthesis of the pentacyclic core. pyr = pyridine; TFE = 2,2,2-trifluoroethanol.



Scheme 11. Li and Yang's [4+2] cycloaddition/oxidative dearomatization model study. R = 2-(2′-furyl)ethyl; DMP = Dess–Martin periodinane.

This reagent was effective in mediating both oxidative dearomatization and oxidation of the oxime to the corresponding nitrile oxide. Ensuing intramolecular 1,3-dipolar cycloaddition directly generated the pentacycle **66** in 80% yield.

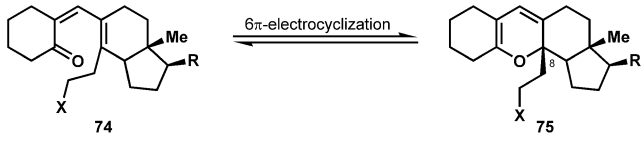
Li and Yang presented an approach to the cortistatins that featured an intramolecular Diels–Alder cycloaddition to form the AB rings, followed by an oxidative dearomatization that installed the ether bridge.[17j] Their efforts commenced with a Mukaiyama-aldol reaction between trimethyl orthoformate and the silyl enol ether derived from **67** to deliver the ketal **68** in 89% yield (Scheme 11). Lithium/halogen exchange of 2-(2′-furyl)-1-iodoethane and subsequent addition to **68** gave the aldehyde **69** in 50% yield after cleavage of the ketal moiety. Complexation of **69** with $AlMe_3$ and addition of the lithium anion of ethyl propiolate provided the alcohol **70** as a single diastereomer in 71% yield. Oxidation of **70** with Dess–Martin periodinane led to spontaneous cycloaddition to generate the tetracycle **71** in 91% yield as a 3:1 mixture of diastereomers. Lewis-acid-promoted aromatization of **71**, followed by oxidative dearomatization of the resulting phenol (**72**) with PhI-$(O_2CCF_3)_2$, gave the pentacycle **73** in 38% overall yield.

2.3. Construction of the B Ring through Pericyclic Transformations

Several groups have turned to pericyclic reactions as an alternative approach to the formation of the [3.2.1]oxabicyclic B ring. This classic set of reactions is commonly utilized in total synthesis to rapidly build complexity. In the context of the synthesis of the cortistatin pentacyclic core, both the electrocyclization and cycloaddition strategies have proven fruitful in efforts reported independently by the groups of Hirama, Danishefsky, and Gung.

2.3.1. Electrocyclization Strategies

The research groups of Hirama[16a] and Danishefsky[17e,17f] have both pursued routes to the core of cortistatin A that employ an oxa-6π-electrocyclization to form a 2*H*-pyran such as **75** (Scheme 12). In each case, the C8 stereocenter is set in this electrocyclization event, placing the two-carbon alkyl chain on the correct face of the molecule for subsequent formation of the seven-membered ring.

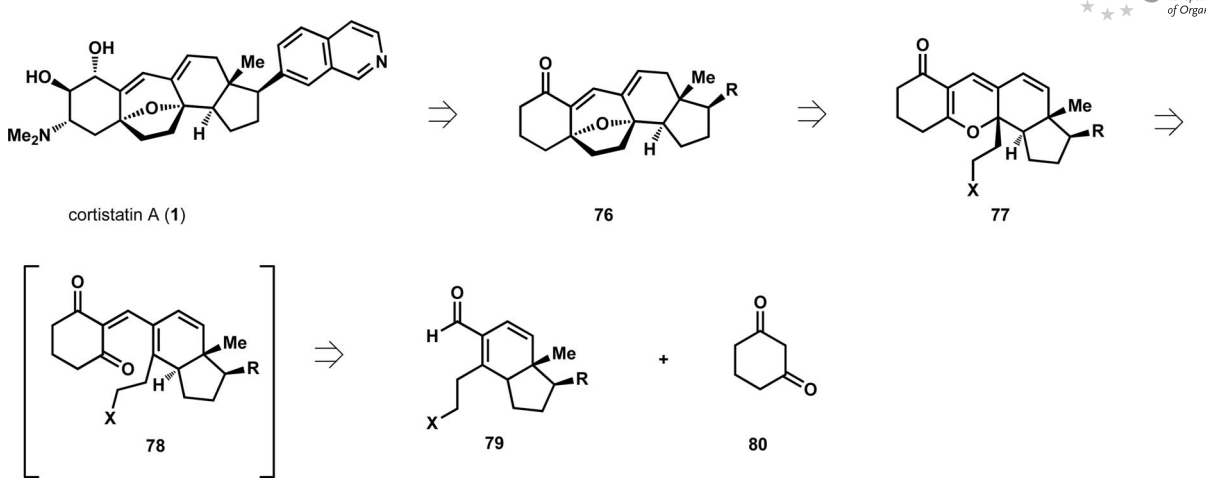


Scheme 12. Oxa-6π-electrocyclization to form a 2*H*-pyran.

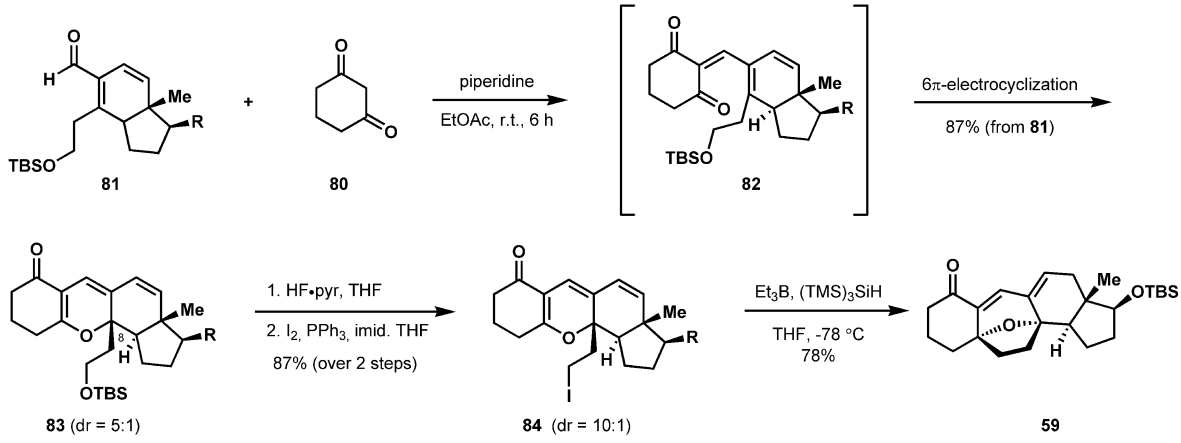
In 2008, Hirama et al. were the first to report an oxa-6π-electrocyclization strategy in pursuit of the cortistatins.[16a] They envisioned accessing cortistatin A (**1**) from the simplified enone **76** (Scheme 13), which accordingly became their initial synthetic target. They planned to arrive at the pentacycle **76** through a radical cyclization of the 2*H*-pyran **77**, which could arise from a 6π-electrocyclization of the intermediate **78**. The oxatriene **78**, in turn, is the direct product of a Knoevenagel condensation of cyclohexane-1,3-dione (**80**) and the bicycle **79**.

Hirama's synthesis began with the construction of the bicycle **81** (Scheme 14) in ten steps from the commercially available enantio-enriched Hajos–Parrish ketone. Their key sequence commenced with a piperidine-catalyzed condensation of the aldehyde **81** (R = OTBS) with cyclohexane-1,3-dione (**80**) to form the intermediate **82**, which under the reaction conditions spontaneously underwent 6π-electrocyclization to form the 2H-pyran **83** in 87% overall yield and as a mixture of diastereomers (5:1 ratio) about C8. The two-step conversion of the TBS ether **83** into the corresponding alkyl iodide provided **84** as a 10:1 mixture of diastereomers. Upon further experimentation, Hirama and co-workers observed that the diastereomeric ratio was dependent on both temperature and solvent, suggesting an equilibration between **83** and the retro-6π-electrocyclization compound **82**. To complete the assembly of the pentacyclic core, a radical cyclization was effected upon treatment of **84** with triethylborane and $(TMS)_3SiH$ to close the seven-membered ring successfully and to provide the dienone **59** in 78% yield.

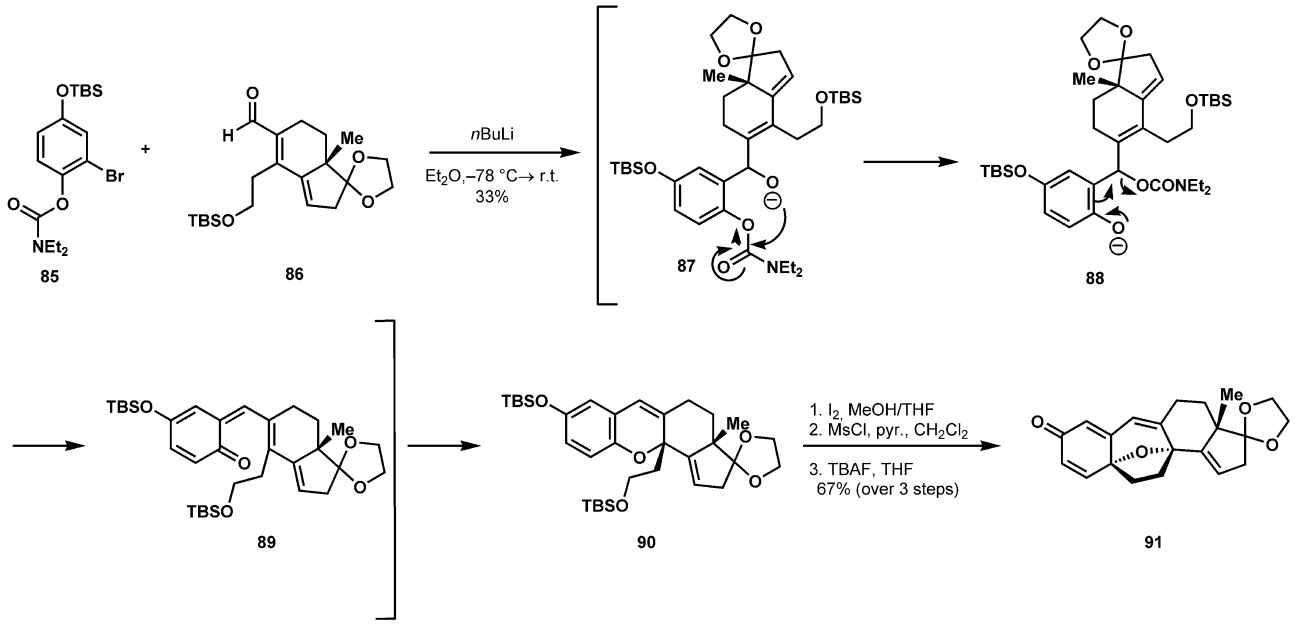
Using a similar electrocylization strategy, Dai and Danishefsky reported a synthesis of the pentacyclic cortistatin core only months after the report by Hirama et al.[17e] Al-



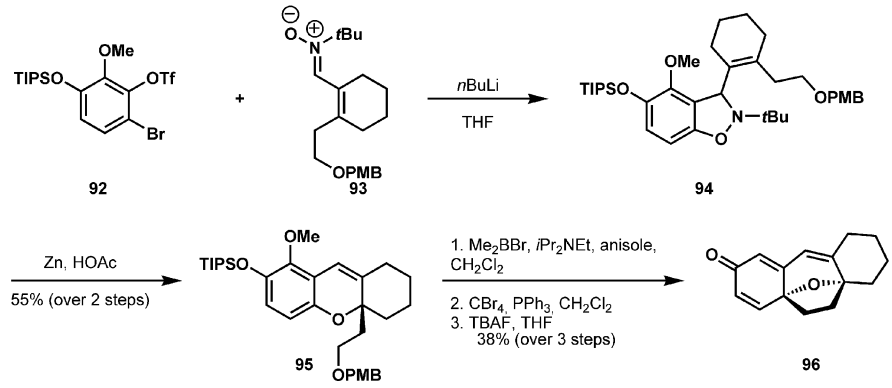
Scheme 13. Hirama's antithetic analysis.



Scheme 14. Hirama's synthesis of the pentacyclic core of cortistatin A. imid. = imidazole; pyr = pyridine.



Scheme 15. Danishefsky's Snieckus/electrocyclization cascade. pyr = pyridine; TBAF = tetrabutylammonium fluoride.



Scheme 16. Danishefsky's 1,3-dipolar cycloadditon/electrocyclization cascade. PMB = *p*-methoxybenzyl; TBAF = tetrabutylammonium fluoride.

though a 6π-electrocyclization is shared as a key step, Danishefsky assembled the electrocyclization substrate through a Snieckus cascade (see **87**→**89**, Scheme 15) to generate intermediate **89**, which spontaneously underwent an oxa-6π-electrocyclization to provide the tetracycle **90**. The final ring was then constructed by conversion of the primary silyl ether into a mesylate and subsequent Masamune alkylation to provide Danishefsky's most advanced intermediate (**91**) toward cortistatin A (**1**).

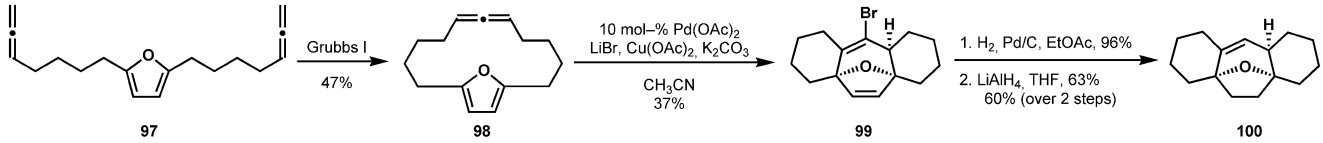
In a separate manuscript published back-to-back with the work described above, Dai and Danishefsky reported an alternative approach to a related 6π-electrocyclization substrate. This strategy involved the 1,3-dipolar cycloaddition of the α,β-unsaturated nitrone **93** and the benzyne derivative obtained from **92** (Scheme 16).[17f] The cycloaddition directly provided the benzoisoxazoline **94**, which upon reductive cleavage of the N–O bond and subsequent thermolysis underwent an oxa-6π-electrocyclization to deliver the tricycle **95**. Dai and Danishefsky again demonstrated the viability of the Masamune alkylation strategy to arrive at the tetracycle **96**, which successfully concluded this model study and paved the way for the potential application of this strategy to the total synthesis of members of the cortistatin family.

2.3.2. Cycloaddition Strategies

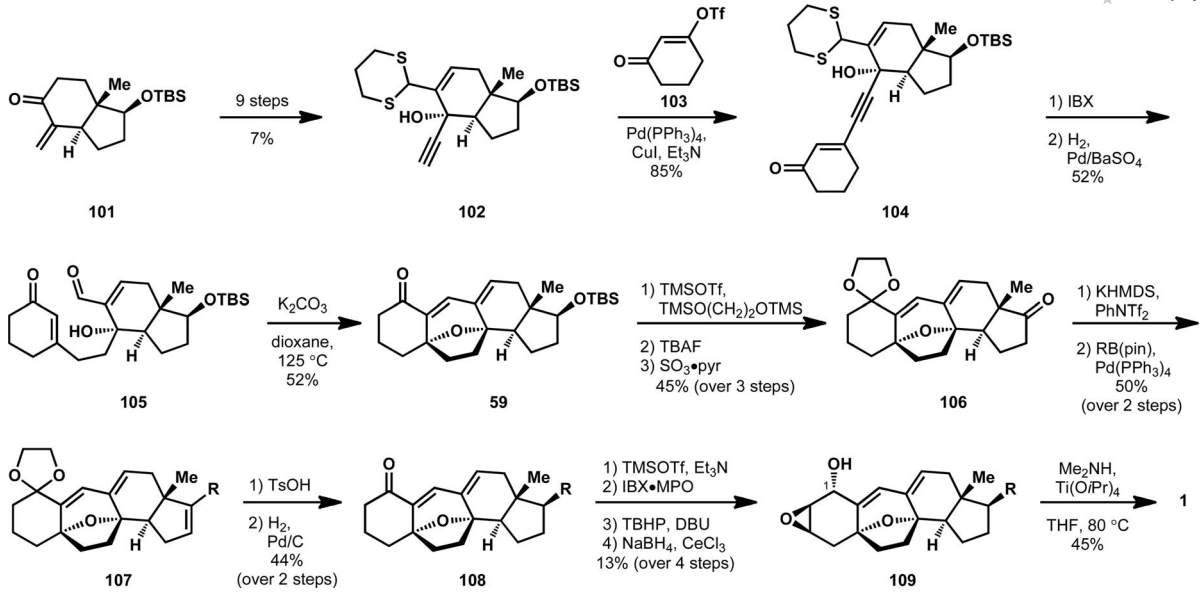
Gung et al. took a unique approach to formation of the cortistatin B ring, proposing a transannular [4+3] cycloaddition to generate the A, B, and C rings simultaneously in a single operation.[17d] They envisioned the three-atom component of this cycloaddition as an allene, whereas the diene comprising the four-atom fragment would be held in an *s-cis* configuration as a part of a furan ring. This strategy has the advantage of concurrently installing the bridging oxygen atom present in the natural product skeleton. In a preliminary model study, the 14-membered macrocycle **98** (Scheme 17) was assembled from the diallene **97** through allene ring-closing metathesis. After significant experimentation, Gung and co-workers were able to effect the desired cycloaddition in the presence of catalytic $Pd(OAc)_2$ and five equivalents of LiBr to obtain the tetracycle **99**, which was further reduced to **100**. This synthetic accomplishment demonstrates the viability of the [4+3] cycloaddition as an approach to the assembly of the ABC ring system of the cortistatin core.

2.4.1. Domino Sequences: Syntheses of Cortistatin A

The total synthesis of (+)-cortistatin A reported by the joint groups of Nicolaou and Chen was the first total synthesis of this natural product to be disclosed. It featured a domino sequence for the simultaneous construction of the seven-membered B ring and the tetrahydrofuran ring.[13] The synthesis began with the Hajos–Parrish ketone derivative **101** (Scheme 18), which was converted in nine steps and 7% overall yield into the alkyne **102**. Sonogashira coupling of **102** with the triflate **103** provided an 85% yield of the enone **104**, which was converted into the keto-aldehyde **105** in 52% yield by IBX-mediated dithiane cleavage and subsequent hydrogenation of the alkyne group. In an elegant cascade sequence, treatment of **105** with K_2CO_3 in dioxane at reflux induced an oxy-Michael addition of the tertiary hydroxy group to the enone moiety, and the intermediate enolate then underwent intramolecular aldol condensation to provide the dienone **59** in 52% yield.



Scheme 17. Gung's model system for transannular [4+3] cycloaddition.



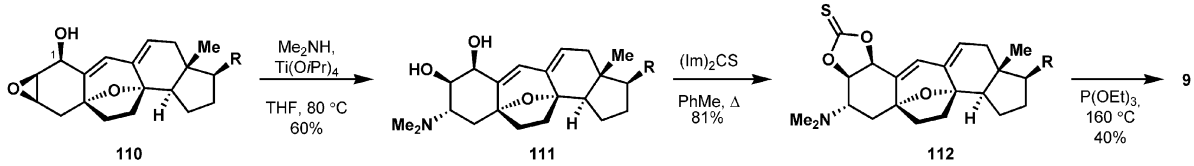
Scheme 18. Nicolaou and Chen's total synthesis of (+)-cortistatin A. R = 7-isoquinolinyl; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; IBX = *o*-iodoxybenzoic acid; KHMDS = potassium bis(trimethylsilyl)amide; MPO = 4-methoxypyridine *N*-oxide; pin = pinacolato; pyr = pyridine; TBAF = tetrabutylammonium fluoride; TBHP = *tert*-butyl hydroperoxide.

The dienone **59** was elaborated to (+)-cortistatin A (**1**), beginning with ketal protection of the carbonyl group followed by TBS ether cleavage and oxidation of the resulting alcohol to provide the cyclopentanone **106** in 45% yield. Triflate formation and cross-coupling with a 7-isoquinolinyl boronic ester derivative gave the alkene **107** (R = 7-isoquinolinyl) in 50% yield. Ketal cleavage and chemoselective hydrogenation then provided the ketone **108** in 44% yield. Oxidation of **108** to the corresponding enone was achieved by sequential treatment with TMSOTf and IBX·MPO. Nucleophilic epoxidation of this enone with TBHP/DBU followed by Luche reduction provided a 13% overall yield of the alcohol **109**, along with an equal amount of the undesired C1 epimer, which could be recycled by an oxidation/reduction sequence. In the final step of the synthesis, epoxide opening of **109** with Me_2NH in the presence of Ti(O*i*Pr)$_4$ gave a 45% yield of (+)-cortistatin A (**1**) along with 36% of the regioisomeric amino alcohol.

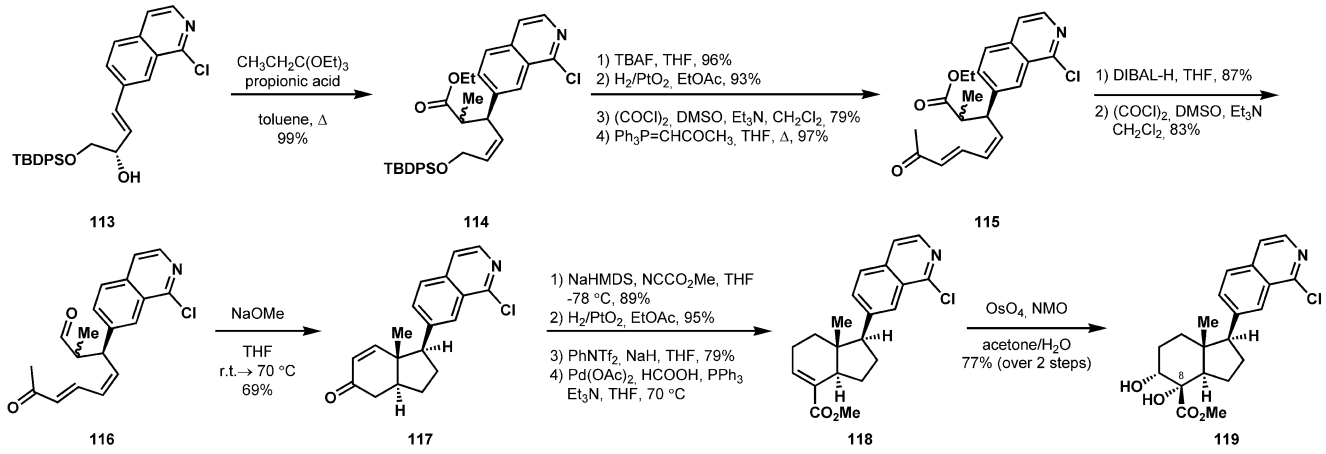
Nicolaou, Chen, and co-workers were able to exploit the 1:1 regioselectivity that was obtained in the Luche reduction step in their synthesis of (+)-cortistatin A to achieve the first total synthesis of (–)-cortistatin J.[15] The epoxide **110** (the C1 epimer of alcohol **109**, Scheme 18) was treated with Me_2NH and Ti(O*i*Pr)$_4$ to provide the aminodiol **111** in 60% yield (Scheme 19). Treatment of the diol **111** with thiocarbonyldiimidazole in toluene at reflux generated the thiocarbonate **112** in 81% yield. A subsequent Corey–Winter olefination was achieved by heating **112** in P(OEt)$_3$ at reflux to give synthetic (–)-cortistatin J (**9**) in 40% yield, along with recovered **112** (45%).

2.5. Other Synthetic Contributions

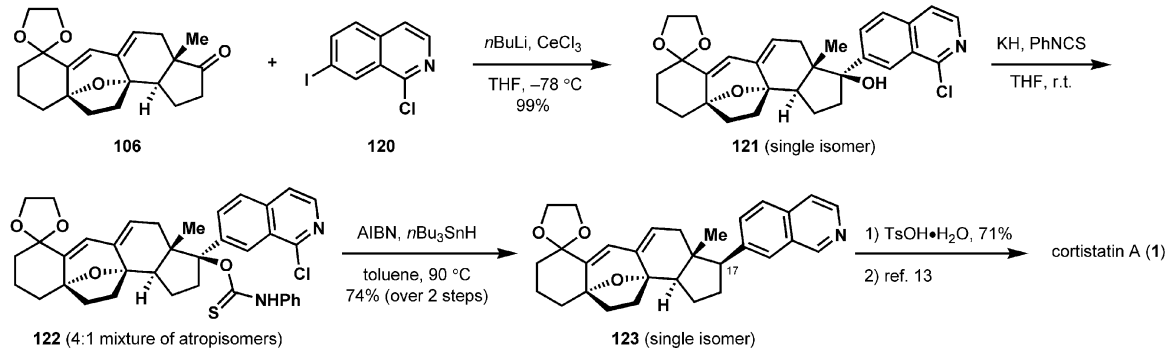
Although the work in this review has so far been framed by the strategy for construction of the unusual cortistatin B ring, important synthetic contributions have also been made on the CD ring fragment of the pentacyclic core. Kobayashi and co-workers disclosed their approach toward cortistatin A, in which they envisioned uniting the A ring fragment with the CD piece derived from the enantioenriched bicycle **119** (Scheme 20).[17g] Their synthesis of the diol **119** commenced with the conversion of D-mannitol into the allylic alcohol **113** in five steps. From the enriched alcohol **113**, the C–O stereochemistry was transformed into C–C stereochemistry by means of a high-yielding Johnson–Claisen rearrangement to arrive at the ester **114**. After a series of functional group manipulations, they arrived at the Michael-aldol substrate **116** and, after some experimentation, found sodium methoxide to be the optimal base to



Scheme 19. Nicolaou and Chen's total synthesis of (–)-cortistatin J. R = 7-isoquinolinyl; Im = 1-imidazolyl.



Scheme 20. Kobayashi's synthesis of the C and D rings. DIBAL-H = diisobutylaluminium hydride; NaHMDS = sodium bis(trimethylsilyl)-amide; NMO = *N*-methylmorpholine *N*-oxide; TBAF = tetrabutylammonium fluoride.



Scheme 21. Hirama's improved installation of the isoquinoline fragment. AIBN = azobis(isobutyronitrile).

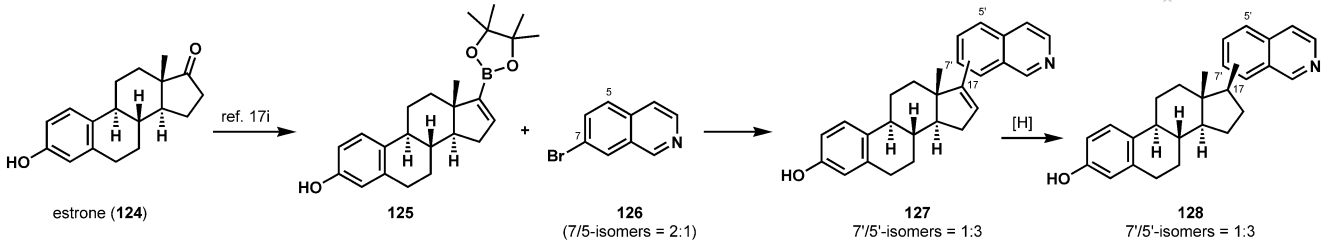
effect the intramolecular Michael-aldol double cyclization, providing the bicycle **117** in 69% yield. The desired *trans* relative stereochemistry of the bicycle **117** was confirmed through nOe studies.

With the *trans*-6,5-fused bicycle **117** to hand, Kobayashi and co-workers focused their attention on introducing a carbon substituent and on oxygenation at C8. Alkylation of the sodium enolate of the enone **117** was followed by hydrogenation of the enone double bond and subsequent vinyl triflate formation. Reduction of the vinyl triflate provided the α,β-unsaturated ester **118**, which was dihydroxylated with catalytic osmium tetroxide to install an oxygen atom on the α-face of the molecule at C8. The newly installed tertiary hydroxy group of **119** is thus positioned to become the bridging oxygen of the B ring of cortistatin A (**1**).

After several reports in which low yields were obtained during the installation of the isoquinoline moiety on the D ring of cortistatin A (**1**), Hirama and co-workers addressed this challenge in the course of a formal synthesis of cortistatin A (**1**).[16b] During initial attempts to generate 7-lithioisoquinoline for subsequent 1,2-addition to the ketone **106**, they observed competitive addition of *n*-butyllithium to the C1-position of 7-iodoisoquinoline. To avoid this problem, 1-chloro-7-iodoisoquinoline (**120**, Scheme 21) was employed. The chlorine atom at the C1-position successfully blocked addition of *n*-butyllithium, instead favoring lithium/halogen exchange at the C7-position, and the desired 1,2-addition proceeded smoothly from the α-face of the molecule to give the tertiary alcohol **121** as a single isomer. With this first hurdle behind them, Hirama et al. next addressed the deoxygenation of **121** with inversion of configuration, which was necessary to set the proper stereochemistry at C17. They accomplished this deoxygenation by converting the tertiary alcohol **121** into the corresponding phenyl thiocarbamate **122** followed by treatment with AIBN and tributyltin hydride in hot toluene. The deoxygenated product **123** was subjected to acid-catalyzed removal of the ketal to provide a ketone intermediate (**108**, Scheme 18), which intersects the Nicolaou–Chen synthesis of cortistatin A (**1**).

3. Cortistatin Analogues/SAR Studies

The synthetic strides made in the pursuit of the cortistatins have enabled studies on the structure/activity relationships of these natural products and strategic analogues pertaining to their anti-angiogenic properties. Efforts by the research groups of Kiyota,[17i] Baran,[19] Corey,[20] and Nicolaou[15] have provided insight into the structural features that play critical roles in the potent anti-angiogenic activity



Scheme 22. Kiyota's synthesis of estrone-derived analogues.

displayed by cortistatins A (**1**) and J (**9**). To date, four facets of the cortistatin structure have been explored: 1) the stereochemistry at C17, 2) the nature of the D ring side chain, 3) the importance of the [3.2.1]oxabicyclic B ring over the standard six-membered ring common to most steroids, and 4) the necessity of the dimethylamine moiety, conserved throughout cortistatins A–L.

Kiyota and co-workers were the first to report studies on the effect of the D ring stereochemistry on the anti-angiogenic activity of cortistatin analogues.[17i] They synthesized estrone analogues by converting estrone (**124**, Scheme 22) into the corresponding vinyl boronic ester **125** and then carried out a Suzuki coupling to introduce the isoquinoline side-chain. The immediate product of the Suzuki coupling, compound **127**, was then hydrogenated to provide the stereochemistry borne by the cortistatin D ring. The two estrone-derived analogues **127** and **128** were subjected to a cell growth inhibition assay for several cell lines. Analogue **128**, with the C17 stereochemistry conserved among the cortistatins, selectively inhibited endothelial cell (HUVEC) proliferation with an IC_{50} value of 9.1 μM, but the Δ-analogue **127** did not display an inhibitory effect on any of the cell lines tested. It is important to note that although analogue **128** does possess activity that is qualitatively analogous to that of cortistatins A (**1**) and J (**9**), its potency is more than three orders of magnitude less than those of the natural products. The main contribution of this study is not in finding a replacement for the naturally occurring compounds, but rather in understanding the importance of the stereochemistry on the D ring with respect to anti-angiogenic activity.

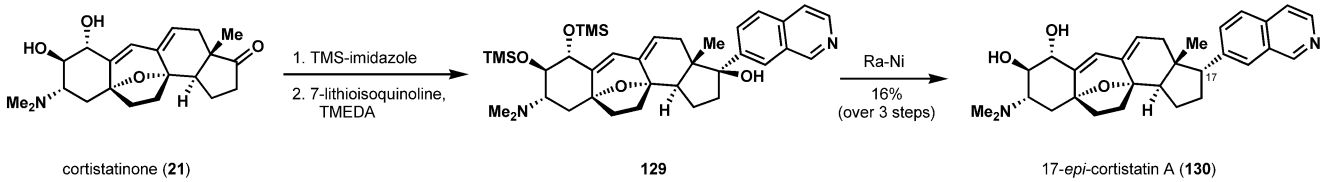
Subsequent to the work of Kiyota and co-workers, Baran et al. examined the impact of the configuration of the D ring on biological activity in further detail by synthesizing estrone analogues as well as the C17 epimer of cortistatin A (**1**).[19] They first developed a strategy for accessing both epimers from a common intermediate. Cortistatinone (**21**), an intermediate in Baran's synthesis of cortistatin A (see Section 2.1.1), was chosen as the divergent point in the synthesis. To arrive at naturally occurring cortistatin A (**1**), cortistatinone (**21**) was converted into the corresponding vinyl iodide and joined with the isoquinoline fragment by a Stille coupling. The natural product was then produced by hydrogenation of the double bond contained within the D ring from the less sterically hindered face of the molecule (see **21**→**1**, Scheme 2). To arrive at 17-*epi*-cortistatin A (**130**), Baran and co-workers devised an alternative strategy to setting the C17 stereochemistry through hydrogenation. First, they added 7-lithioisoquinoline in a 1,2-fashion to cortistatinone (**21**) to access the alcohol **129** (Scheme 23). For deoxygenation with retention of the C17 stereochemistry, they found that treatment of the alcohol **129** with Raney nickel was effective, and they obtained 17-*epi*-cortistatin A (**130**) in 16% yield over three steps.

With synthetic access to both cortistatin A (**1**) and 17-*epi*-cortistatin A (**130**), as well as to Δ^{16}-cortistatin A (**131**), the biological activity of these substrates was examined. Baran and co-workers reported that their synthetic cortistatin A (**1**) gave an IC_{50} value of 2.43 nM in an assay to measure growth inhibition of HUVECs, consistent with natural cortistatin A, which was reported to have a similar IC_{50} value of 1.8 nM (Table 1). Without a stereocenter at C17, Δ^{16}-cortistatin A (**131**) still exhibited growth inhibition of HUVECs at nanomolar concentrations. However, 17-*epi*-cortistatin A (**130**) was not active in the same assay.

Table 1. Selective growth inhibition of HUVECs.

Substrate	IC_{50} [nM]
Natural cortistatin A (**1**)	1.8
Synthetic cortistatin A (**1**)	2.43
Δ^{16}-Cortistatin A (**131**)	3.88
17-*epi*-Cortistatin A (**130**)	>1000

A common finding of the studies conducted to date on the structure/activity relationships of the cortistatins and their anti-angiogenic activity is the importance of the D ring substituent. Corey and co-workers synthesized a plethora of estrone-derived cortistatin analogues that differ only



Scheme 23. Baran's synthesis of 17-*epi*-cortistatin A (**130**). TMEDA = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; Ra-Ni = Raney nickel.

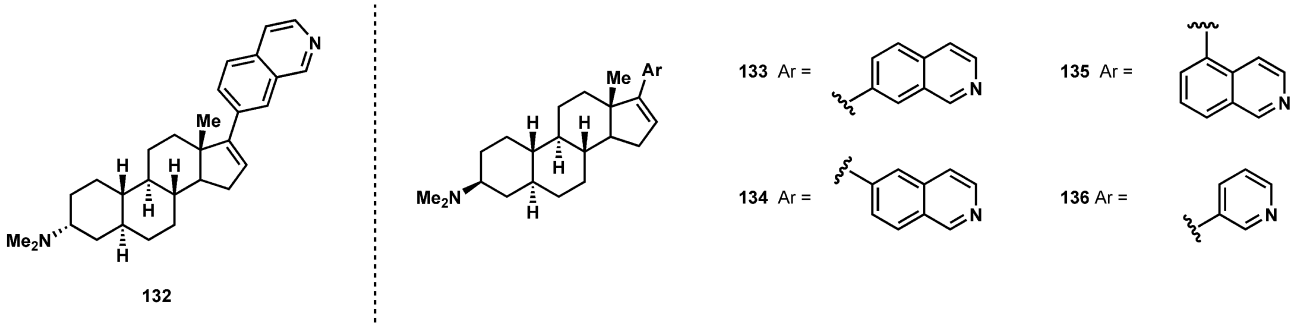


Figure 2. Estrone-derived analogues synthesized by Corey.

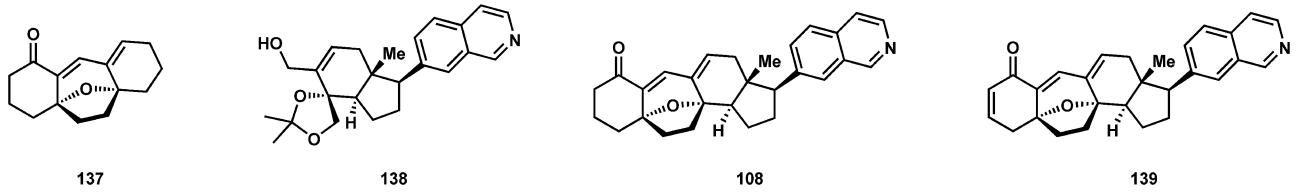


Figure 3. Intermediates and analogues synthesized and tested by Nicolaou and Chen.

in the composition of the D ring substituent (Figure 2).[20] The data from their study suggest that a nitrogen heterocycle at this position is necessary for any inhibition in growth of HUVECs. Although the point of attachment to the steroidal core is not imperative for activity, the presence of an isoquinoline moiety was a common feature of the most active cortistatin-inspired analogues **133**, **134**, and **135** tested by Corey et al. Interestingly, one of the four most active compounds, compound **136**, contained a pyridine moiety attached to the D ring through C3.

Similarly, Nicolaou, Chen, and co-workers suggested that the isoquinoline moiety is required for cortistatins and related compounds to display anti-angiogenic activity against HUVECs.[15] The work by Nicolaou and Chen goes further, suggesting that the substituents present on the A ring (i.e., hydroxy groups as well as the dimethyl amino group conserved in all the cortistatins) do not play a significant role in the biological action of this class of compounds. To that end, they found that molecules devoid of A ring substituents, but which contain an isoquinoline fragment, retained biological activity (**108** and **139**, Figure 3).

A collaborative effort by Cee, Chen, and Nicolaou probed the biological mode of action of cortistatin A.[21] It was found that cortistatin A is a high-affinity ligand for a group of protein kinases including CDK8, CDK11, ROCKI, and ROCKII. Additionally, cortistatin A bound to ROCK and CDK8 (from their crystal structures) was modeled, providing insights into the experimentally observed structure/activity relationships.

4. Summary and Outlook

The flurry of work directed towards the synthesis of the naturally occurring cortistatins and related compounds has enabled preliminary biological studies. Since the disclosure of the structures of cortistatins A–L three and a half years ago by Kobayashi and co-workers, numerous approaches directed towards the cortistatins and four impressive syntheses have been reported. These efforts have supported initial studies of the biological activity of these compounds and have begun to unveil the structure/activity relationships of the cortistatins. The excitement generated by the potent anti-angiogenic properties of cortistatins A and J and related compounds continues to fuel the frenzy to devise more efficient and convergent synthetic paths to this class of compounds.

Acknowledgments

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