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Synthesis of Cortistatins A, J, K, and L

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Abstract

The cortistatins are a recently identified class of marine natural products characterized by an unusual steroidal skeleton and have been found to inhibit differentially the proliferation of various mammalian cells in culture by an unknown mechanism. We describe a comprehensive route for the synthesis of cortistatins from a common precursor, which in turn is assembled from two fragments of similar structural complexity. Cortistatins A and J, and for the first time, K and L, have been synthesized in parallel processes from like intermediates prepared from a single compound. With the identification of facile laboratory transformations linking intermediates in the cortistatin L synthetic series with corresponding intermediates to cortistatins A and J, we have been led to speculate that somewhat related paths might occur in nature, offering potential sequencing and chemical detail for cortistatin biosynthetic pathways.

Since the structure of cortistatin A was elucidated by Kobayashi and coworkers in 2006 more than 10 natural cortistatins have been described comprising a common modified steroidal skeleton with varying substitution of the A- and D-rings,[1–3] many of these exhibiting profound effects upon cultured mammalian cells and human umbilical vein endothelial cells (HUVECs) in particular.[1–4] Baran and coworkers reported the first laboratory synthetic route to cortistatin A,[5] from prednisone, in 2008, and subsequent to this a number of different approaches to the synthesis of cortistatins have been pursued, with focus on the most highly functionalized (and potent) member of the family, cortistatin A (**1**). No fewer than three independent routes to cortistatin A have been reported to date,[5–7] as

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Author contributions

A.N.F., C.S., and A.G.M conceived the synthetic route. A.N.F and C.S. conducted all experimental work and analyzed the results. A.N.F., C.S., and A.G.M. wrote the manuscript.

Additional information

Detailed experimental procedures and tabulated spectroscopic data (^{1}H and ^{13}C NMR, FT-IR, and HRMS) for all new compounds. This material is available free of charge via the Internet at www.nature.com/naturechemistry.

well as a number of synthetic studies[8–18] and formal routes.[19,20] In addition, the Nicolaou–Chen group has described a synthesis of cortistatin J.[21]

Structure-activity relationships among natural cortistatins and synthetic analogs have suggested that the 7-substituted isoquinoline appendage is a key determinant of the phenotypic effects of cortistatins upon cells grown in culture.[4,21–23] The Nicolaou–Chen group recently reported that cortistatin A inhibits the function of a number of different kinases in vitro and proposed that the natural product may occupy the ATP-binding site of at least one of the enzymes they identified.[24] Prior to this, Kobayashi and coworkers had shown that the addition of cortistatin A to cultured HUVECs led to a marked decrease in levels of an unidentified 110 kDa phosphoprotein.[4]

We envisioned that the naturally occurring cortistatins A, J, K, and L (**1**–**4**, respectively) and a diverse array of cortistatin analogs, including compounds suitable for use as biological probes in target identification experiments, could be prepared from the single azido alcohol intermediate **5**, depicted in Figure 2.

We considered it likely that one or more pathways linking the azido alcohol **5** to the cyclohexadienone **6** could be developed. The oxabicyclic core of the latter precursor could then be disconnected by an oxidative cyclization transform, a strategy independently conceived and previously demonstrated by Sarpong and coworkers, as well as others.[8,15,16,18] To allow for a convergent assembly process, we imagined an earlier disconnection of the seven-membered B-ring by an olefin metathesis reaction, giving rise to the triene precursor **7**, which we envisioned could be synthesized by coupling of the *o*-vinyl benzylzinc reagent **8** and the enol triflate intermediate **9**, structures of similar complexity, but less than certain viability as stable chemical entities.

Results

The *o*-vinyl benzylzinc reagent **8**, the A-ring precursor, was derived from the benzyl bromide **14**, which was prepared in amounts up to 47-g by the four-step sequence shown in Figure 3 (via intermediates **10**–**13**). Direct insertion of zinc[25] into the carbon-bromine bond of the benzyl bromide **14** was attempted only once, without success; however, a two-stage process for the preparation of organozinc reagent **8** proved to be both highly reliable and readily scalable. Addition of benzyl bromide **14** to activated magnesium turnings[26] in ether at 23°C led to rapid (<30 min) formation of a deep green solution of the corresponding Grignard reagent, contaminated with <5% of the product of Wurtz coupling. In contrast, formation of the Grignard reagent in tetrahydrofuran as solvent was accompanied by as much as 65% of the Wurtz coupling by-product. Addition of the freshly prepared ethereal Grignard product to a solution of anhydrous zinc chloride in tetrahydrofuran at 23 oC led to the formation of a cloudy white suspension of the presumed *o*-vinyl benzylzinc reagent **8**, which was used directly for coupling with the enol triflate **9** (vide infra).

The enol triflate coupling partner **9**, the CD-ring precursor, was synthesized in a three-step sequence from the known α-methylene ketone **15** (Figure 4). The latter substance was first prepared in its antipodal form by Danishefsky and coworkers[27] (from the Hajos–Parrish enedione)[28] in the context of their synthesis of taxol, and was also selected as a starting material by the Nicolaou-Chen[6] and Sorensen[18] groups in their research directed toward the synthesis of cortistatins. As illustrated in Figure 4, phosphoniosilylation[29],[30] of α-methylene ketone **15** with triphenylphosphine (1.02 equiv) and triethylsilyl trifluoromethanesulfonate (1.02 equiv) at −40°C in chloroform, uniquely effective as a solvent in this particular transformation, afforded the phosphonium salt **16** (containing a tetrasubstituted triethylsilyl enol ether), which was observed to be stable toward aqueous workup and extractive isolation. Heretofore, phosphoniosilylation-Wittig sequences have been conducted without isolation of the intermediate phosphonium salts;[30] this was not an option in the present case, for chloroform was not compatible with formation of the Wittig reagent. The oily phosphonium salt obtained upon extractive isolation and concentration was dissolved in tetrahydrofuran, and after cooling to −78°C, the resulting solution was treated sequentially with *n*-butyllithium (1.1 equiv) and paraformaldehyde (5 equiv), affording the α-vinyl triethylsilyl enol ether **17** in 75% yield over the two steps from the α-methylene ketone **15**. Methodology for triflation developed by Corey and coworkers was used to transform the triethylsilyl enol ether **17** into the corresponding enol triflate derivative **9** (92% yield after purification by flash-column chromatography).[31] This product, a colorless oil, was stored frozen as a solution in benzene for not longer than a few days; decomposition was observed to occur over longer periods of time.

Coupling of the enol triflate **9** (1 equiv) with the benzylzinc reagent **8** (~1.2 equiv, a solution in ether–tetrahydrofuran) in the presence of tris(dibenzylideneacetone)dipalladium (0.045 equiv) and the ligand 2-dicyclohexylphosphino-2′,6′-dimethoxy-biphenyl (S-Phos, 0.18 equiv)[32] in *N*-methylpyrrolidone at 70°C (20 h) afforded the metathesis substrate **7** in 70% yield on a 16-g scale. Warming of a 0.2 M solution of the latter product with the second-generation Grubbs catalyst (0.025 equiv)[33] in dichloromethane at 45°C for 5 h led to highly efficient ring-closing metathesis, forming the tetracyclic diene **18** (>95% yield, 11-g scale). In our initial route design we had planned to hydrogenate selectively the disubstituted alkene within this product and then epoxidize the remaining (tetra-substituted) alkene; however, we were not able to achieve selective 1,2-reduction of the diene. Sarpong and coworkers have reported the successful execution of this exact two-step sequence, albeit using a substrate with different A-ring substituents (1-methoxyl and 2-*p*-methoxybenzyl groups).[8]

We found that we could achieve the desired transformation (**18**→**20**) by reversing the order of the two operations and in so doing we were able to avoid an isolation step. Thus, direct addition of a solution of dimethyldioxirane (1.5 equiv) in acetone[34] to the unpurified metathesis product solution at 0°C led to stereoselective epoxidation of the tetrasubstituted alkene, affording the acid-sensitive tetracyclic diene monoepoxide **19**. Without purification, the epoxide **19** was hydrogenated in benzene under 500 psi hydrogen in the presence of a mixture of Wilkinson’s catalyst (0.15 equiv) and suspended solid sodium bicarbonate (1.5 equiv), the latter added as an acid scavenger. The saturated product, epoxide **20**, then underwent selective eliminative opening with lithium diethylamide (2 equiv) in tetrahydrofuran at −15°C,[35] furnishing the conjugated allylic alcohol **21**, a sufficiently polar intermediate to allow for facile chromatographic isolation (>95% purity, ^{1}H NMR analysis, 50% yield over the four-step sequence). Cleavage of the triisopropylsilyl protective group with tetra-*n*-butylammonium fluoride (1.1 equiv) in tetrahydrofuran and oxidative cyclization of the resulting phenol with [bis(trifluoroacetoxy)iodo]benzene (1.8 equiv) in a mixture of dichloromethane and hexafluoroisopropanol provided the cyclohexadienone **6** in 50% yield.[8],[18],[36]

The synthesis of the key azido alcohol intermediate **5** was achieved in three steps from the cyclohexadienone **6**, a sequence initiated by hydrosilylation with triethylsilane (2 equiv) in the presence of Wilkinson's catalyst (0.05 equiv).[37] The intermediate triethylsilyl enol ether was not isolated; rather, pyridine was added (14% by volume) followed by *N*-bromosuccinimide (2 equiv), forming the (3*R*)-bromo ketone **22** as a single diastereomer in 70% yield. In the absence of pyridine, or with lesser quantities of pyridine, the bromination reaction was much less stereoselective, which evidence suggests was related to the presence of Wilkinson's catalyst during the bromination (bromination of the triethylsilyl enol ether generated in the absence of Wilkinson's catalyst was highly diastereoselective). Bromide displacement with tetramethylguanidinium azide (2 equiv) proceeded with clean inversion of C3-stereochemistry and this was followed by direct reduction of the resulting (3*S*)-α-azido ketone with catecholborane (2 equiv) in the presence of the (*R*)-Corey–Bakshi–Shibata catalyst[38] (0.2 equiv, −40°C) and tetramethylguanidine (1 equiv), producing a 15:1 mixture of the (2*S*)- and (2*R*)-configured alcohols, respectively, in 85% yield after chromatographic isolation. Similar stereocontrol of the C3-center could not be achieved with other reductants examined (lithium tri-*tert*-butoxyaluminum hydride was the next most selective reductant, affording a 5:1 mixture of products favoring the (2*S*)-isomer).

Shown in parallel in Figure 5 are the sequential pathways by which the key intermediate **5** was transformed into the corresponding 17-keto precursors to cortistatins A, J, K, and L. The synthetic route to the 17-keto precursor to cortistatin A (**29**) is depicted first, and was initiated by protection of the 2-hydroxyl substituent of intermediate **5** with excess chloroacetyl chloride in pyridine. Cleavage of the *tert*-butyldimethylsilyl ether within the resulting product occurred upon exposure to aqueous hydrofluoric acid in a 1:1 mixture of acetonitrile and tetrahydrofuran at 0°C, affording the alcohol **23** in 80% yield. Significantly, we observed that if during the latter transformation the reaction mixture was allowed to warm from 0 to 23°C, 1,6-elimination of the chloroacetate ester accompanied deprotection of the *tert*-butyldimethylsilyl ether, an observation later used to advantage in the synthesis of the 17-keto precursor to cortistatin J (**32**). Oxidation of alcohol **23** with the Dess–Martin periodinane (2.4 equiv) in dichloromethane at 0 oC followed by addition of methanol and solid potassium carbonate gave in a single operation the keto alcohol **24**, in 85% yield. Addition of *N*-bromosuccinimide (1.02 equiv) to a solution of keto alcohol **24** in a 4:1 mixture of acetonitrile and methanol, respectively, at 23°C led to stereoselective (*trans*-diaxial) 1,4-bromoetherification of the conjugated diene, forming the bromide **25** which, without purification, was directly subjected to nucleophilic displacement at 0°C with potassium superoxide (2.1 equiv) in a 3:1 mixture of toluene and dimethyl sulfoxide, respectively.[39],[40] After extractive isolation and chromatography, the *trans*-diol methyl ether **26** was obtained in diastereomerically pure form (41% yield, 100-mg scale, two steps). When combined with scandium triflate (0.03 equiv) and trifluoroacetic acid (9.4 equiv) in dioxane at 23°C, this substance was found to undergo efficient 1,2-elimination of methanol, affording the azido diol **27** (94% yield). The methoxyl substituent of substrate **26** is axially oriented and therefore stereoelectronically disposed toward elimination; none of the other allylic C–O bonds of substrate **26** (nor product **27**) is so oriented. Reductive (di)methylation of the azido group and diol protection with chlorotriethylsilane then furnished the protected 17-keto cortistatin A precursor (**29**) in 65% yield for the two steps (75-mg scale).

Syntheses of the 17-keto precursors to cortistatins J, K, and L were substantially more direct (Figure 5). The 17-keto cortistatin J precursor (**32**) was synthesized in just three steps from the azido alcohol **5**. Following reductive (di)methylation to form the dimethylamino alcohol **30** (85% yield), 1,6-elimination of water and deprotection of the *tert*-butyldimethylsilyl ether occurred simultaneously upon stirring the dimethylamino alcohol **30** with a biphasic mixture of chloroform and concentrated hydrochloric acid (20 min, 23°C). Without purification, the product of the latter transformation (**31**) was subjected to oxidation with the Dess–Martin periodinane, providing the 17-keto cortistatin J precursor (**32**) as a white solid (77% yield following chromatographic purification, two steps).

The synthetic route to the 17-keto precursor to cortistatin K (**35**) also proceeded through the dimethylamino alcohol **30** (derived from the azido alcohol **5**, as described in the paragraph above), in a four-step sequence that was initiated by acetylation at 0°C with acetic anhydride (a large excess) and scandium triflate as catalyst (0.05 equiv) in a 1:1 mixture of acetonitrile and dichloromethane (93% yield).[41] Regioselective reductive cleavage of the allylic C–O bond of the resulting acetate occurred in the presence of lithium borohydride (excess) and tetrakis(triphenylphosphine)-palladium (0.2 equiv).[42] This afforded the dimethylamino diene **34** in complex with borane; decomplexation was achieved by treatment of this intermediate with Raney nickel in methanol,[43] providing the free amine as a pale yellow solid (90% yield after chromatographic purification over Davisil® silica gel). Silyl ether cleavage (tetra-*n*-butylammonium fluoride, 87% yield) and oxidation (Dess–Martin periodinane, 90%), then completed the route to the 17-keto cortistatin K precursor (**35**), also obtained as a pale yellow solid.

Lastly, the 17-keto precursor to cortistatin L (**38**) was synthesized from the azido alcohol **5** by a straightforward four-step sequence involving silyl ether deprotection (conducted at 0°C, as in the cortistatin A series, to avoid 1,6-elimination), selective protection,[44] reductive (di)methylation, and oxidation (Figure 5, 60% yield overall).

Depicted in Figure 6 are the parallel paths by which each advanced 17-keto cortistatin precursor was transformed into the corresponding cortistatin. The method we used to introduce stereoselectively the 7-isoquinolyl substituent differs from earlier reported syntheses of cortistatin A, where in each case a variation of the sequence 17-keto→vinyl iodide[5,7] or vinyl triflate[6] followed by cross-coupling with a 7-metalloisoquinoline derivative, then stereoselective hydrogenation was pursued. The hydrogenation step in particular has been established to be challenging to execute with intermediates containing the conjugated diene function of cortistatin A[5,7] and we gauged it as likely that this would be even more problematic with precursors to cortistatins J, K, and L. As a general alternative, we developed the three-step 17-keto addition–reductive deoxygenation sequence shown in Figure 6. While our work was in progress, Hirama and coworkers reported a conceptually similar but differentially executed approach to introduce the isoquinolyl substituent in their formal synthesis of cortistatin A.[19] We observed that lithiation of 7-iodoisoquinoline (5 equiv) with an equimolar amount of *n*-butyllithium in tetrahydrofuran at −78°C followed by sequential addition of *N, N, N′, N′*-tetramethylethylenediamine (TMEDA, 15 equiv) and a 17-keto cortistatin precursor (1 equiv) reproducibly afforded the corresponding (17*S*)-1,2-addition product in 52–62% yield in all cases.[45] In the absence of TMEDA, 1,2-addition did not occur, undoubtedly a consequence of competing enolization of the 17-keto substituent. Enolization likely occurs, but to a much lesser extent, in our optimized conditions as well (in the presence of TMEDA) for we typically recover between 34–40% of each 17-keto precursor. The Hirama group developed a very different protocol for metalation (employing *n*-butyllithium and cerium (III) chloride with 1-chloro-7-iodoisoquinoline as substrate) and with this organometallic reagent observed highly efficient addition to the carbonyl group of the Nicolaou–Chen 17-keto cortistatin A precursor. They then transformed the resulting tertiary alcohol into a thionocarbamate derivative and the latter was deoxygenated using tri-*n*-butyltin hydride.[19]

We briefly explored a similar method for hydroxyl activation in our deoxygenation studies but our attempts to form a thionocarbonyl ester intermediate were unsuccessful. Instead, we adapted methodology by Jang and coworkers for free-radical deoxygenation of alcohols via their trifluoroacetate esters.[46],[47] As shown in Figure 6, each tertiary alcohol intermediate was readily transformed into the corresponding trifluoroacetate ester with trifluoroacetic anhydride and pyridine–*N, N*-dimethylaminopyridine. When heated with tri-*n*-butyltin hydride (excess) and azobisisobutyronitrile in benzene in a sealed tube at 100°C for 1–2 h, each trifluoroacetate ester underwent efficient deoxygenation to provide the (17*S*)-configured reduction product. Attempted deoxygenation under the conditions originally reported by Jang et al. (Ph_2SiH_2, $(t\text{-BuO})_2$, 130°C)[46],[47] led primarily to elimination of the trifluoroacetate ester, a problem that was almost completely circumvented by the use of tri-*n*-butyltin hydride as reductant at 100°C.

In the cortistatin J and K series, deoxygenation led to these substances directly; spectroscopic data for the synthetic materials matched values reported for the natural products. In the cortistatin A and L series, cleavage of the silyl ether protective group(s) with tetra-*n*-butylammonium fluoride at 23°C following deoxygenation provided these targets; spectroscopic data obtained from the synthetic substances were found to be the same as those reported for the natural products. In all four series (A, J, K, and L), the yield for the two- or three-step deoxygenation (deprotection) procedure was 61–72% (1–20-mg amounts of final product).

In addition to spectroscopic characterization, synthetic cortistatins A, J, K, and L were evaluated for their ability to inhibit the growth of HUVECs in culture (96-h incubation). The GI_{50} values we measured are summarized in Figure 1 alongside literature values. For synthetic cortistatin A we observed growth inhibition of HUVECs consistent with reported values, while measurements for synthetic cortistatin J more closely matched reports from the Nicolaou–Chen group than initial reports, and GI_{50} values for synthetic cortistatins K and L fell within the range of those originally reported to slightly higher.[1],[3],[21]

Discussion

In the course of our studies we observed that several compounds closely related to cortistatin L undergo facile 1,6-elimination to form the conjugated triene function of cortistatin J (see **30**→**31**, Figure 5, discussion of intermediate **23** above, and the additional example of the transformation of dienyl alcohol **30** to triene **44**, Figure 7). In an interesting and somewhat related transformation, we observed that reaction of diene **45** (cortistatin L oxidation state) with dimethyldioxirane afforded the highly sensitive epoxide **46** as primary product, which upon standing in benzene solution underwent spontaneous1,4-elimination to form a product (dienyl alcohol **47**) with functionality characteristic of cortistatin A. Consideration of these findings has led us to speculate that cortistatins A and J may derive from cortistatin L or a precursor of the same oxidation state, as opposed to an alternative sequencing such as J→A (although this cannot be ruled out).[2] Cortistatin L might in turn be derived by C2 oxidation of cortistatin K. We note that many sterols bearing 2β-hydroxy and 2β-sulfato groups have been isolated from marine sponges, but detailed biosynthetic routes to these substances have not been determined.[48],[49]

We have shown that the protected azido alcohol intermediate **5**, synthesized in a nine-step sequence beginning with the coupling of the benzylzinc reagent **8** and the enol triflate **9**, is readily transformed into advanced 17-keto precursors to cortistatins A, J, K, and L. Each of these intermediates is in turn converted into the corresponding cortistatin final product by a three- or four-step sequence involving addition of a 7-isoquinolyl organometallic intermediate followed by deoxygenation (deprotection). The latter sequence appears to be a general route to cortistatins with divergent substitutions of the A, B, and C rings and it is anticipated that it will allow for late-stage introduction of diversely substituted isoquinoline groups and other heterocycles at position C17.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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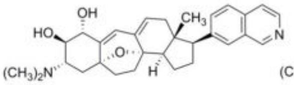
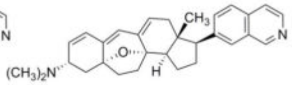
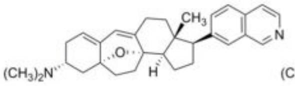
Appendix



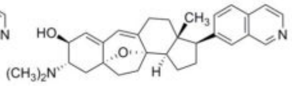
cortistatin A



cortistatin J



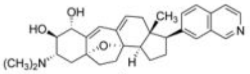
cortistatin K



cortistatin L

1	cortistatin A (**1**)
2	cortistatin J (**2**)
3	cortistatin K (**3**)
4	cortistatin L (**4**)
5	azido alcohol **5**
6	cyclohexatrienone **6**
7	triene **7**
8	*o* –vinyl benzylzinc reagent **8**
9	enol triflate **9**
10	methyl 5-hydroxy-2-iodobenzoate (**10**)
11	methyl 2-iodo-5-(triisopropylsilyloxy)benzoate (**11**)
12	methyl 5-(triisopropylsilyloxy)-2-vinylbenzoate (**12**)
13	(5-(triisopropylsilyloxy)-2-vinylphenyl)methanol (**13**)
14	(3-(bromomethyl)-4-vinylphenoxy)triisopropylsilane (**14**)
15	α-methylene ketone **15**
16	triethylsilyl enol ether phosphonium salt **16**
17	triethylsilyl dienol ether **17**
18	tetracyclic diene **18**
19	tetracyclic diene monoepoxide **19**
20	tetracyclic epoxide **20**
21	allylic alcohol **21**
22	bromo ketone **22**
23	chloroacetate ester **23** (cortistatin A series)
24	dienyl keto alcohol **24** (cortistatin A series)
25	allylic bromide **25** (cortistatin A series)
26	trans diol methyl ether **26** (cortistatin A series)
27	dienyl azido diol **27** (cortistatin A series)
28	dimethylamino keto diol **28** (cortistatin A series)
29	dimethylamino ketone **29** (cortistatin A series)
30	dimethylamino alcohol **30** (cortistatin J and K series)

31	trienyl alcohol **31** (cortistatin J series)
32	dimethylamino ketone **32** (cortistatin J series)
33	acetate **33** (cortistatin K series)
34	*tert*- butyldimethylsilyl ether **34** (cortistatin K series)
35	dimethylamino ketone **35** (cortistatin K series)
36	azido *tert* -butyldimethylsilyl ether **36** (cortistatin L series)
37	dimethylamino alcohol **37** (cortistatin L series)
38	dimethylamino ketone **38** (cortistatin L series)
39	7-lithioisoquinoline (**39**)
40	isoquinolyl alcohol **40** (cortistatin A series)
41	isoquinolyl alcohol **41** (cortistatin L series)
42	isoquinolyl alcohol **42** (cortistatin J series)
43	isoquinolyl alcohol **43** (cortistatin K series)
44	trienyl *tert*- butyldimethylsilyl ether **44**
45	dienyl bromo triethylsilyl ether **45**
46	allylic epoxide **46**
47	dienyl alcohol **47**
48	5-hydroxy-2-iodobenzoic acid (**48**)
49	7-isoquinolyl trifluoromethylsulfonate (**49**)
50	7-trimethylstannylisoquinoline (**50**)
51	7-iodoisoquinoline (**51**)
52	cortistatin A bis(triethylsilyl) ether (**52**)
53	dimethylamino alcohol **53** (cortistatin K series)
54	dienyl diol **54** (cortistatin L series)
55	cortistatin L *tert* -butyldimethylsilyl ether (**55**)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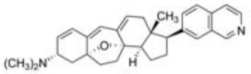
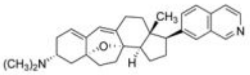
HO
(CH3)2N
O
CH3
H
N

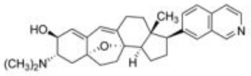
1


(CH3)2N
O
CH3
H
N

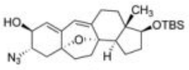
2


(CH3)2N
O
CH3
H
N

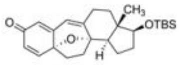
3


HO
(CH3)2N
O
CH3
H
N

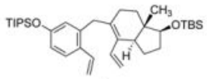
4


HO
N3
O
CH3
OTBS
H

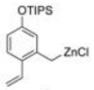
5


O
O
CH3
OTBS
H

6


TIPSO
CH3
OTBS
H

7



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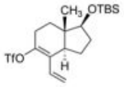
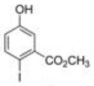

CH3
OTBS
TfO
H

9



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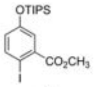
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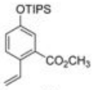
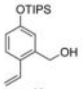
CO2CH3
I

11


OTIPS
CO2CH3

12



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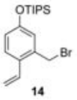

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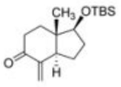

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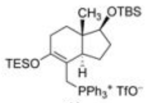
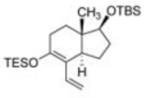
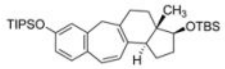

CH3
OTBS
TESO
H
PPh3+ TfO−

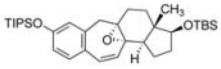
16


CH3
OTBS
TESO
H

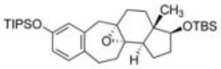
17


TIPSO
CH3
OTBS
H

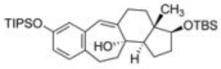
18


TIPSO
CH3
OTBS
O
H

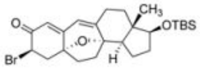
19


TIPSO
CH3
OTBS
O
H

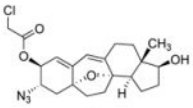
20


TIPSO
CH3
OTBS
HO
H

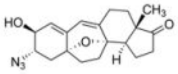
21


O
CH3
OTBS
O
Br
H

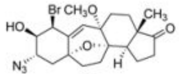
22


Cl
O
O
CH3
OH
O
N3
H

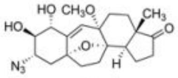
23


HO
CH3
O
O
N3
H

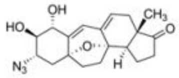
24


Br CH3O
CH3
HO
O
O
N3
H

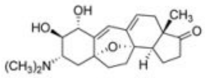
25


HO CH3O
CH3
HO
O
O
N3
H

26


OH
CH3
HO
O
O
N3
H

27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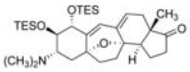
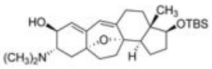
CH3
HO
O
O
(CH3)2N
H

28


OTES
TESO
CH3
O
O
(CH3)2N
H

29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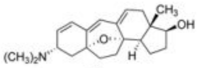
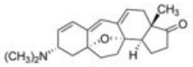
CH3
OTBS
O
(CH3)2N
H

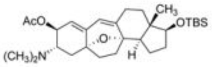
30


CH3
OH
O
(CH3)2N
H

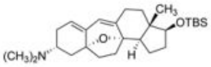
31


CH3
O
O
(CH3)2N
H

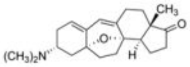
32


AcO
CH3
OTBS
O
(CH3)2N
H

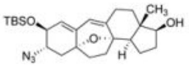
33


CH3
OTBS
O
(CH3)2N
H

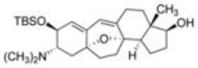
34


CH3
O
O
(CH3)2N
H

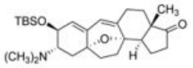
35


TBSO
CH3
OH
O
N3
H

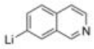
36


TBSO
CH3
OH
O
(CH3)2N
H

37


TBSO
CH3
O
O
(CH3)2N
H

38



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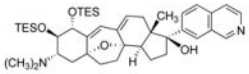
OTES
TESO
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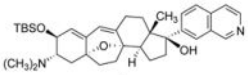
N
O
OH
(CH3)2N
H

40


TBSO
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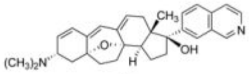
N
O
OH
(CH3)2N
H

41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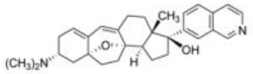
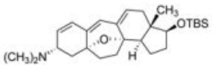
N
O
OH
(CH3)2N
H

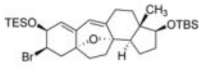
42


CH3
N
OH
O
H
(CH3)2N

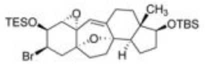
43


CH3
OTBS
O
H
(CH3)2N

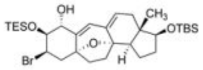
44


TESO
CH3
OTBS
O
Br
H

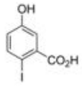
45


TESO
O
CH3
OTBS
O
Br
H

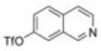
46


OH
TESO
CH3
OTBS
O
Br
H

47



48



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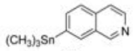
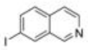
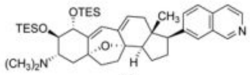


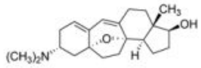
50



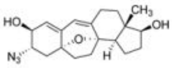
51


OTES
TESO
CH3
N
O
(CH3)2N
H

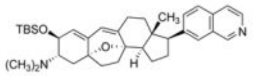
52


CH3
OH
O
(CH3)2N
H

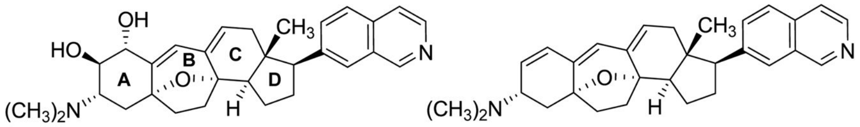
53


HO
CH3
OH
O
N3
H

54


TBSO
CH3
N
O
(CH3)2N
H

55

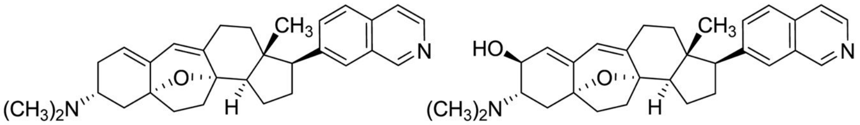


cortistatin A (**1**)

1.8	nM	Ref 1
2 ± 1	nM	Ref 21
2.1 ± 0.4	nM	(this work)

cortistatin J (**2**)

8	nM	Ref 3
70 ± 13	nM	Ref 21
46 ± 36	nM	(this work)



cortistatin K (**3**)

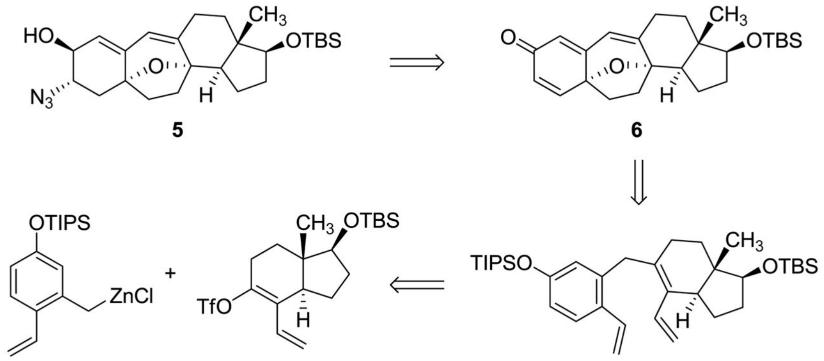
40	nM	Ref 3
112 ± 45	nM	(this work)

cortistatin L (**4**)

23	nM	Ref 3
32 ± 11	nM	(this work)

Figure 1. Cortistatins A, J, K, and L and their IC_{50} values measured in cultured HUVECs

Members of the cortistatin natural product family comprise a common modified steroidal skeleton with varying substitutions, primarily within the A, B, and C rings. These substitutional variations are found to modulate the potencies of natural cortistatins as growth inhibitors of human umbilical vein endothelial cells (HUVECs) in culture.


HO
N3
CH3
OTBS
O
H
O
CH3
OTBS
H
OTIPS
ZnCl
+
CH3
OTBS
TfO
H
TIPSO
CH3
OTBS
H

5 **6** **8** **9** **7**

Figure 2. A key precursor to cortistatins and its retrosynthetic disconnection

The azido alcohol intermediate 5 serves as a common intermediate for the synthesis of cortistatin A, J, K, and L as well as cortistatin analogs. This intermediate arises in turn from the cyclohexadienone 6 and the triene 7. The latter intermediate is formed by coupling of the *o*-vinyl benzylzinc reagent 8 and the enol triflate intermediate 9. TBS = *tert*-butyldimethylsilyl; TIPS = triisopropylsilyl; Tf = trifluoromethanesulfonyl.

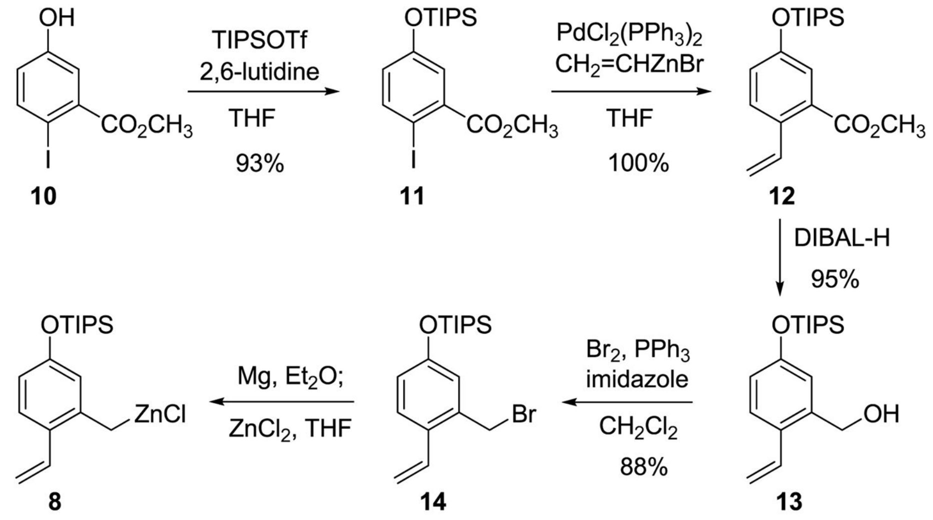

OH
CO_2CH_3
I
10
TIPSOTf
2,6-lutidine
THF
93%
OTIPS
CO_2CH_3
I
11
$PdCl_2(PPh_3)_2$
CH_2=CHZnBr
THF
100%
OTIPS
CO_2CH_3
12
DIBAL-H
95%
OTIPS
OH
13
Br_2, PPh_3
imidazole
CH_2Cl_2
88%
OTIPS
Br
14
Mg, Et_2O;
$ZnCl_2$, THF
OTIPS
ZnCl
8

Figure 3. Preparation of the *o*-vinyl benzylzinc reagent 8

The functionalized benzylzinc reagent 8 is prepared from the corresponding Grignard reagent and anhydrous zinc chloride. The Grignard reagent is readily formed from the benzyl bromide 14, which is prepared in multi-gram amounts by a four-step sequence. THF = tetrahydrofuran; Ph = phenyl; DIBAL-H = diisobutylaluminium hydride.

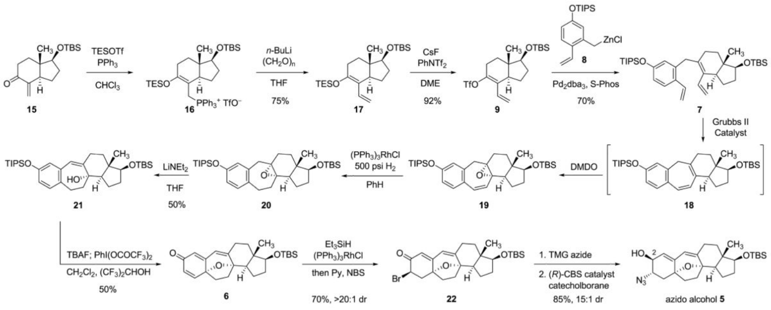


Figure 4. Synthesis of the azido alcohol 5 from the α-methylene ketone 15

The α-methylene ketone 15 is transformed in three steps to the enol triflate intermediate 9. Coupling with the functionalized benzylzinc reagent 8 affords the triene 7, which undergoes efficient ring-closing metathesis and oxidation (*in situ*, with dimethyldioxirane) to form the tetracyclic diene monoepoxide 19. A sequence of hydrogenation, eliminative epoxide opening, silyl ether cleavage, and oxidative cyclization provides the cyclohexadienone 6, which is converted stereoselectively in three steps to the key azido alcohol intermediate 5. TES = triethylsilyl; DME = 1,2-dimethoxyethane; Pd_2dba_3 = tris(dibenzylideneacetone)dipalladium(0); S-Phos = 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl; DMDO = dimethyl dioxirane; TBAF = tetra-*n*-butylammonium fluoride; Py = pyridine; NBS = *N*-bromosuccinimide; TMG azide = tetramethylguanidinium azide; CBS = Corey-Bakshi-Shibata.

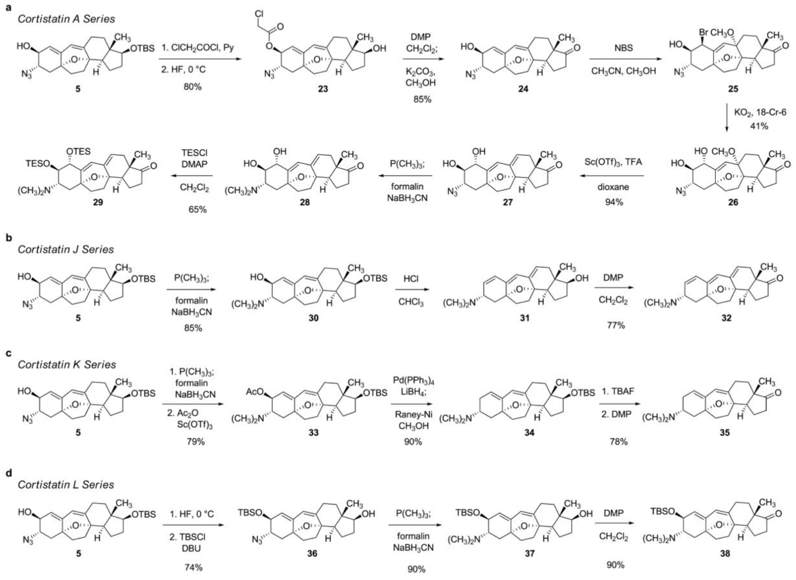

a
Cortistatin A Series
1. ClCH$_2$COCl, Py
2. HF, 0 °C
80%
DMP
CH$_2$Cl$_2$;
K$_2$CO$_3$, CH$_3$OH
85%
NBS
CH$_3$CN, CH$_3$OH
KO$_2$, 18-Cr-6
41%
Sc(OTf)$_3$, TFA
dioxane
94%
P(CH$_3$)$_3$;
formalin
NaBH$_3$CN
TESCl
DMAP
CH$_2$Cl$_2$
65%
5
23
24
25
26
27
28
29
b
Cortistatin J Series
P(CH$_3$)$_3$;
formalin
NaBH$_3$CN
85%
HCl
CHCl$_3$
DMP
CH$_2$Cl$_2$
77%
5
30
31
32
c
Cortistatin K Series
1. P(CH$_3$)$_3$; formalin NaBH$_3$CN
2. Ac$_2$O Sc(OTf)$_3$
79%
Pd(PPh$_3$)$_4$
LiBH$_4$;
Raney-Ni
CH$_3$OH
90%
1. TBAF
2. DMP
78%
5
33
34
35
d
Cortistatin L Series
1. HF, 0 °C
2. TBSCl DBU
74%
P(CH$_3$)$_3$;
formalin
NaBH$_3$CN
90%
DMP
CH$_2$Cl$_2$
90%
5
36
37
38

Figure 5. Synthetic pathways to 17-keto precursors to cortistatins A, J, K, and L from the azido alcohol 5

a, Synthesis of the 17-keto precursor to cortistatin A (29) by an eight-step sequence. b, A three-step sequence to the 17-keto precursor to cortistatin J (32). c, Synthesis of the 17-keto precursor to cortistatin K (35) by a five-step sequence. d, A four-step sequence to the 17-keto precursor to cortistatin L (38). DMP = Dess-Martin periodinane;18-Cr-6 = 18-crown-6; TFA = trifluoroacetic acid; DMAP = 4-dimethylaminopyridine; Ac = acetyl; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

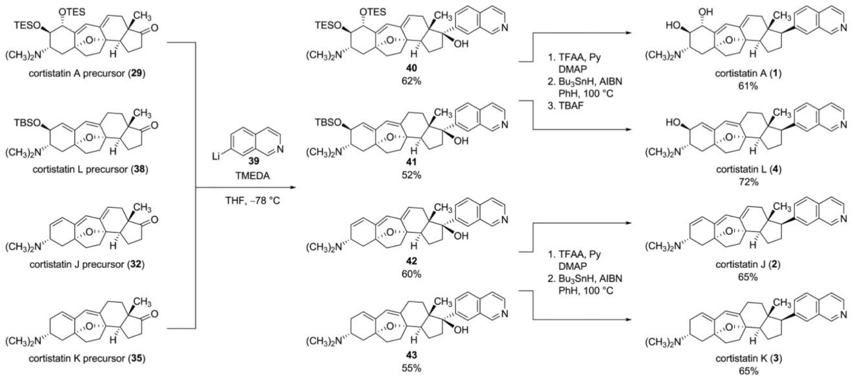

OTES
TESO
(CH3)2N
CH3
O
H
cortistatin A precursor (29)
TBSO
cortistatin L precursor (38)
cortistatin J precursor (32)
cortistatin K precursor (35)
Li
N
39
TMEDA
THF, −78 °C
OH
40
62%
41
52%
42
60%
43
55%
1. TFAA, Py
DMAP
2. Bu3SnH, AIBN
PhH, 100 °C
3. TBAF
HO
cortistatin A (1)
61%
cortistatin L (4)
72%
cortistatin J (2)
65%
cortistatin K (3)
65%

Figure 6. Syntheses of cortistatins A, L, J, and K

Parallel paths of 17-keto addition of 7-lithioisoquinoline followed by reductive deoxygenation transform each 17-keto cortistatin precursor into the corresponding cortistatin. TMEDA = *N*, *N*, *N′*, *N′*-tetramethylethylenediamine; TFAA = trifluoroacetic anhydride; AIBN = 2,2′-azobis(2-methylpropionitrile).

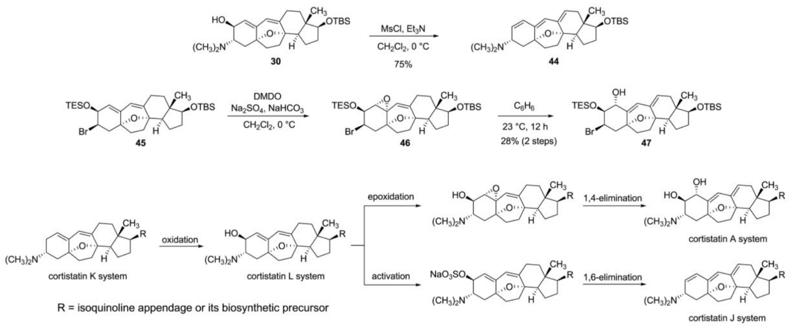

HO
(CH3)2N
CH3
OTBS
H
O
30
MsCl, Et3N
CH2Cl2, 0 °C
75%
44
TESO
Br
45
DMDO
Na2SO4, NaHCO3
CH2Cl2, 0 °C
46
C6H6
23 °C, 12 h
28% (2 steps)
OH
47
cortistatin K system
oxidation
cortistatin L system
epoxidation
activation
R
1,4-elimination
cortistatin A system
NaO3SO
1,6-elimination
cortistatin J system
R = isoquinoline appendage or its biosynthetic precursor

Figure 7. Chemical interconversions among intermediates of cortistatin series A, J, and L and a hypothesized parallel biosynthetic sequence

Intermediates of the cortistatin L series are found to undergo facile laboratory transformations to form corresponding intermediates of the A and J series, suggesting a plausible sequencing for the latter stages of cortistatin biosyntheses, which are unknown. Ms = methanesulfonyl.