VIP Natural Products

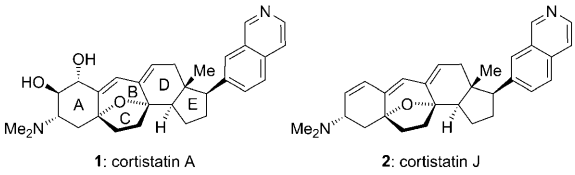
DOI: 10.1002/anie.200803550

Total Synthesis of (+)-Cortistatin A**

K. C. Nicolaou, Ya-Ping Sun, Xiao-Shui Peng, Damien Polet, and David Y.-K. Chen**

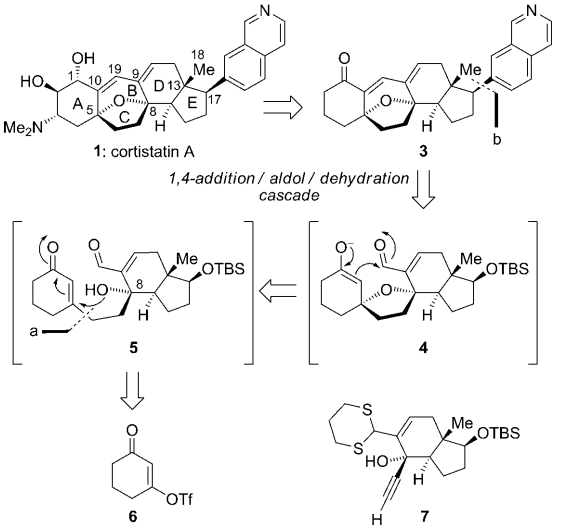
Angiogenesis is an important physiological phenomenon whose imbalance may result in several disease states, including malignant, inflammatory, ischaemic, infectious, and immune disorders.[1] The inhibition of angiogenesis has been considered for some time as an attractive way to improve such conditions. With the recent introduction of the first anti-angiogenic agents to treat cancer and blindness, the search for new inhibitors of angiogenesis assumed a new level of priority and urgency.

In 2006[2] and 2007,[3,4] the Kobayashi research group disclosed a series of novel steroidal alkaloids possessing remarkable anti-angiogenic properties that endow them with potent anti-proliferative activities. Isolated from the sponge *Corticium simplex*, and named cortistatins, these molecules boast a heptacyclic skeleton featuring an oxabicyclo-[3,2,1]octene and, some, an isoquinoline structural motif. From the 11 naturally occurring members of the cortistatin family, cortistatin A (**1**, Scheme 1; $IC_{50} = 1.8$ nM against human umbilical vein endothelial cells (HUVECs)) and cortistatin J (**2**, Scheme 1; $IC_{50} = 8$ nM against HUVECs) are the most potent. Furthermore, these compounds demonstrated a striking selectivity index against HUVECs when their activities against normal human dermal fibroblast (NHDF) and several tumor cells (KB3–1, K562, and Neuro2A) were compared (**1**: selectivity index > 3000; **2**: selectivity index 300–1100). The low natural abundance of these compounds combined with their unprecedented molecular architectures and promising biological properties prompted us to undertake their chemical synthesis. Herein we report a total synthesis of cortistatin A (**1**)[5] through a flexible synthetic strategy which may be applied to the construction of other members of the class, natural or designed.



Scheme 1. Structures of cortistatins A (**1**) and J (**2**).

Upon cursory inspection, cortistatin A (**1**) reveals a unique abeo-9(10-19)-androstane steroidal skeleton onto whose E ring is attached an isoquinoline moiety. Our chosen retrosynthetic analysis (Scheme 2) converted **1** into cyclohexanone derivative **3**, for whose construction we envisaged a cascade reaction[6] involving an intramolecular 1,4-addition/aldol/dehydration sequence to forge the pentacyclic framework of the molecule, followed by a Suzuki–Miyaura[7] coupling to install the isoquinoline structural motif (**5**→**4**→**3**). The required hydroxy dicarbonyl precursor **5** was then dismantled through a retro-Sonogashira[8] reaction into cyclohexenone triflate **6** and terminal acetylene **7**.



Scheme 2. Retrosynthetic analysis of cortistatin A (**1**). a) Sonogashira coupling; b) Suzuki–Miyaura coupling. TBS = *tert*-butyldimethylsilyl; Tf = trifluoromethanesulfonyl.

The construction of the required acetylenic compound **7** is summarized in Scheme 3. Thus, starting with enantiomerically

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[**] We thank Doris Tan (ICES) for high-resolution mass spectrometric assistance and Dr. Tommy Wang Chern Hoe (Institute of Molecular and Cell Biology (IMCB)—A*STAR) for X-ray crystallographic analysis. Financial support for this work was provided by A*STAR, Singapore.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200803550.

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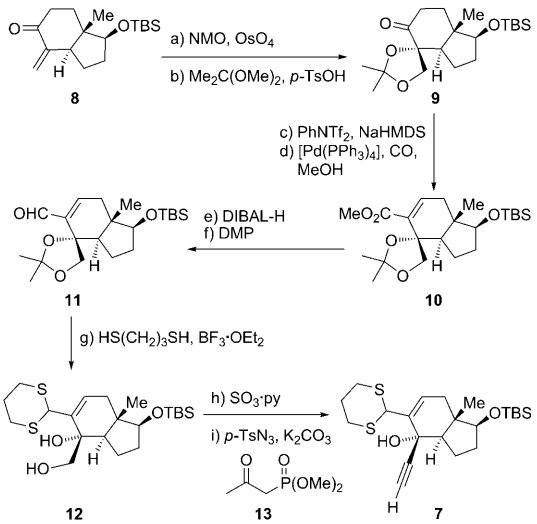
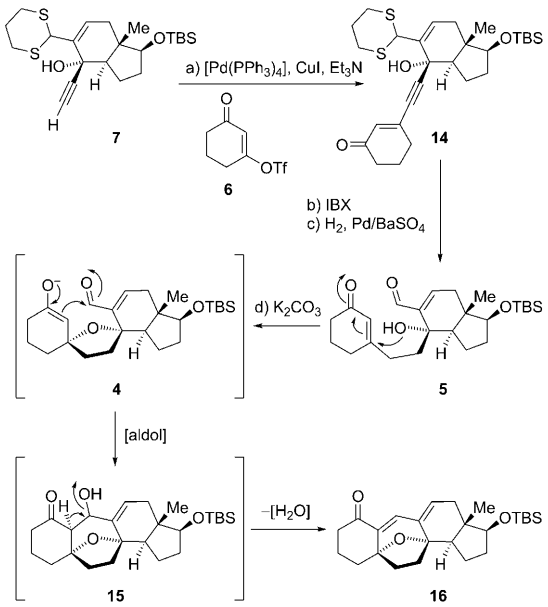


Scheme 3. Construction of acetylene **7**. Reagents and conditions: a) OsO_4 (0.02 equiv), NMO (2.5 equiv), acetone/H_2O (8:1), 23 °C, 16 h, 73 %; b) $Me_2C(OMe)_2$ (5.0 equiv), *p*-TsOH (0.04 equiv), acetone, 23 °C, 1 h, 87 %; c) NaHMDS (1.0 M in THF, 1.2 equiv), $PhNTf_2$ (1.1 equiv), THF, 0 °C, 2 h; d) $[Pd(PPh_3)_4]$ (0.05 equiv), Et_3N (3.0 equiv), CO, DMF/MeOH (5:2), 70 °C, 3 h, 72 % for the two steps; e) DIBAL-H (1.0 M in toluene, 3.0 equiv), toluene, −78 °C, 3 h, 79 %; f) DMP (1.5 equiv), $NaHCO_3$ (4.6 equiv), CH_2Cl_2, 23 °C, 30 min, 86 %; g) $HS(CH_2)_3SH$ (3.0 equiv), $BF_3{\cdot}OEt_2$ (3.5 equiv), CH_2Cl_2, −78 °C, 1.5 h, 70 %; h) $SO_3{\cdot}py$ (3.0 equiv), Et_3N (5.0 equiv), CH_2Cl_2/DMSO (4:1), 23 °C, 1.5 h, 72 %; i) *p*-TsN_3 (1.5 equiv), dimethyl-2-oxopropylphosphonate (**13**) (1.5 equiv), K_2CO_3 (3.5 equiv), CH_3CN, 23 °C, 2 h; then aldehyde from **12**, MeOH/THF/MeCN (1:1:3), 23 °C, 16 h, 45 % after two cycles. NMO = *N*-methylmorpholine *N*-oxide; *p*-TsOH = *para*-toluenesulfonic acid; NaHMDS = sodium hexamethyldisilazide; DMF = *N*,*N*′-dimethylformamide; DMP = Dess–Martin periodinane; DIBAL-H = diisobutylaluminium hydride; DMSO = dimethylsulfoxide; *p*-TsN_3 = *para*-toluenesulfonylazide; py = pyridine.



Scheme 4. Construction of pentacyclic dienone **16** through a hydroxy 1,4-addition/aldol/dehydration cascade. Reagents and conditions: a) $[Pd(PPh_3)_4]$ (0.1 equiv), CuI (0.1 equiv), Et_3N (3.0 equiv), **6** (1.4 equiv, from 1,3-cyclohexadione, Tf_2O, and Et_3N), DMF, 23 °C, 1 h, 85 %; b) IBX (4.0 equiv), DMSO, 0 → 23 °C, 4 h, 81 %; c) $Pd/BaSO_4$ (5 % wt/wt, 0.24 equiv), H_2, MeOH/THF (1:1), 23 °C, 30 min, 64 %; d) K_2CO_3 (1.2 equiv), dioxane, 125 °C, 12 h, 52 %. IBX = *o*-iodoxybenzoic acid.

enriched bicyclic enone **8**,[9] acetonide **9** was prepared in 64 % overall yield through a stereoselective dihydroxylation (NMO, cat. OsO_4) followed by exposure of the resulting diol to $Me_2C(OMe)_2$ in the presence of a catalytic amount of *p*-TsOH. Conversion of ketone **9** into its enol triflate ($PhNTf_2$, NaHMDS) followed by methoxy carbonylation under the standard conditions led to methyl ester **10** in 72 % overall yield. The latter compound was then converted into aldehyde **11** through a reduction/oxidation sequence (1. DIBAL-H, 79 % yield; 2. DMP, 86 % yield). Treatment of aldehyde **11** with $HS(CH_2)_3SH$ in the presence of $BF_3{\cdot}OEt_2$ at −78 °C resulted in protection of the aldehyde moiety and concomitant removal of the acetonide group, thereby affording dihydroxy dithiane **12** in 70 % yield. Finally, oxidation of **12** under the Parikh–Doering[10] conditions ($SO_3{\cdot}py$) furnished the hydroxy aldehyde (72 % yield), which was treated with Ohira–Bestman[11] reagent (ketophosphonate **13**, *p*-TsN_3, K_2CO_3), generated in situ, to afford the desired acetylenic compound **7** in 45 % yield.

Scheme 4 depicts the four-step elaboration of intermediate **7** to pentacyclic framework **16**. Thus, Sonogashira coupling of **7** (cat. $[Pd(PPh_3)_4]$, CuI, Et_3N) with freshly prepared enol triflate **6** (1,3-cyclohexadione, Tf_2O, Et_3N) furnished enynone **14** smoothly in 85 % yield. Unveiling of the aldehyde functionality from **14** with IBX,[12] followed by chemoselective hydrogenation (H_2, $Pd/BaSO_4$), then led to the desired cascade precursor **5** in 52 % overall yield for the two steps. Pleasingly, the much anticipated 1,4-hydroxy enone addition/aldol/dehydration cascade proceeded smoothly upon heating hydroxy enone-enal **5** at reflux in dioxane in the presence of K_2CO_3 to afford pentacyclic dienone **16**[5b] in 52 % yield, presumably through the intermediacy of **4** and **15**, as shown in Scheme 4.

Scheme 5 summarizes the final stages of the synthesis of cortistatin A (**1**) that secured the attachment of the isoquinoline structural motif on ring E and installed the required functional groups on ring A. Our chosen sequence of functionalization necessitated temporary protection of the carbonyl group of **16** as its dioxolane derivative (TMSO-$(CH_2)_2$OTMS, TMSOTf), which was subsequently converted into ketone **17** through desilylation (TBAF, 56 % yield for the two steps) and oxidation ($SO_3{\cdot}py$, 80 % yield). The enol triflate derived from **17**, through the action of $PhNTf_2$ and KHMDS, was then coupled to isoquinoline boronic ester **18**[13] through a Suzuki–Miyaura reaction (cat. $[Pd(PPh_3)_4]$, K_2CO_3) to afford alkenyl isoquinoline **19** in 50 % overall yield for the two steps. Removal of the dioxolane group from **19** (*p*-TsOH, acetone/H_2O, 88 % yield) followed by stereo- and chemoselective hydrogenation (10 % Pd/C, MeOH) led to isoquinoline dienone **3** in 50 % yield (plus 30 % recovered starting material). The desired stereochemical outcome of this reduction was expected on steric grounds, an assumption that was supported by a molecular modeling study,[14] and which was ultimately confirmed by the synthesis of **1** (see

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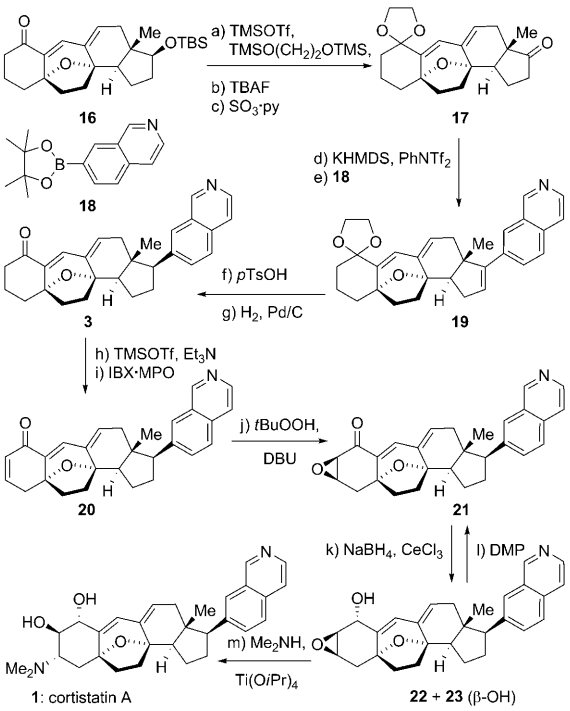

Scheme 5. Completion of the total synthesis of cortistatin A (**1**). Reagents and conditions: a) $TMSO(CH_2)_2OTMS$ (5.0 equiv), TMSOTf (1.5 equiv), CH_2Cl_2, −60→−10 °C, 1.5 h; b) TBAF (1.0 M in THF, 7.0 equiv), THF, 23 °C, 2 h, 56% for the two steps; c) SO_3·py (6.0 equiv), Et_3N (10.0 equiv), CH_2Cl_2/DMSO (3:1), 23 °C, 3 h, 80%; d) KHMDS (0.5 M in toluene, 3.0 equiv), THF, −78 °C, 1 h, then $PhNTf_2$ (5.0 equiv), 0.5 h; e) **18** (3.3 equiv), $[Pd(PPh_3)_4]$ (0.3 equiv), K_2CO_3 (3.0 equiv), THF, 80 °C, 2 h, 50% for the two steps; f) *p*-TsOH (1.5 equiv), acetone/H_2O (10:1), 23 °C, 1 h, 88%; g) Pd/C (10% wt/wt, 0.3 equiv), H_2, MeOH, 23 °C, 1 h, 50% (71% based on recovered starting material); h) TMSOTf (14 equiv), Et_3N (30 equiv), THF, −78→0 °C, 1.5 h; i) IBX·MPO (0.4 M in DMSO, 6.0 equiv), DMSO, 23 °C, 6 h, 46% for the two steps; j) *t*BuOOH (6.0 equiv), DBU (3.0 equiv), CH_2Cl_2, 0→23 °C, 5 h, 70%; k) $NaBH_4$ (1.0 equiv), $CeCl_3$ (4.0 equiv), MeOH, 0 °C, 10 min, 80% (ca. 1:1 mixture of diastereoisomers); l) DMP (5.0 equiv), $NaHCO_3$ (10.0 equiv), CH_2Cl_2, 23 °C, 2 h, 100%; m) Me_2NH (2.0 M in THF, as solvent), $Ti(OiPr)_4$ (5.0 equiv), 80 °C, 5 h, 45%. TBAF = tetra-*n*-butylammonium fluoride; KHMDS = potassium hexamethyldisilazide, TMS = trimethylsilyl; MPO = 4-methoxypyridine *N*-oxide; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

below). All that now remained to reach the target (**1**) was the functionalization of ring A. To this end, dienone **3** was transformed to trienone **20** through exposure of its TMS enolate (TMSOTf, Et_3N) to IBX·MPO[15] (46% overall yield, unoptimized) and the latter compound was subjected to the action of *t*BuOOH in the presence of DBU to afford epoxide **21** (70% yield), whose β stereochemistry was tentatively assigned on the basis of steric considerations and supported by a related model study.[16] This assignment was later confirmed by reaching **1** (see below). Thus, reduction of ketoepoxide **21** with $NaBH_4/CeCl_3$ furnished a mixture of hydroxy epoxide **22** and its β-OH isomer **23** (ca. 1:1 d.r., 80% total yield), which were separated by chromatography. While the β-OH isomer **23** could be recycled by oxidation (DMP, 100% yield)—reduction ($NaBH_4/CeCl_3$), the α-OH isomer **22** was converted into cortistatin A (**1**), together with a chromatographically separable, isomeric epoxide-opened product (36%),[17] through the action of Me_2NH in the presence of $Ti(OiPr)_4$ (45%, unoptimized). The ^1H and ^{13}C NMR spectroscopic and mass spectrometric data of synthetic **1** were consistent with those reported for the natural product.[2,5a] Furthermore, synthetic **1** exhibited $[\alpha]_D^{25} = +30.7\ deg\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.05\ g\,cm^{-3}$, MeOH) [lit. $[\alpha]_D^{20} = +30.1\ deg\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.56\ g\,cm^{-3}$, MeOH).[2,5a]

The described chemistry, in addition to rendering cortistatin A (**1**) readily available for further biological investigations, opens an entry to other members of the cortistatin family, natural and designed, for screening purposes.[18]

Received: July 21, 2008
Published online: August 14, 2008

Keywords: antitumor agents · cascade reactions · natural products · total synthesis

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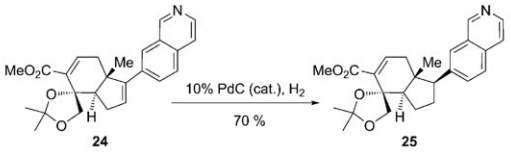
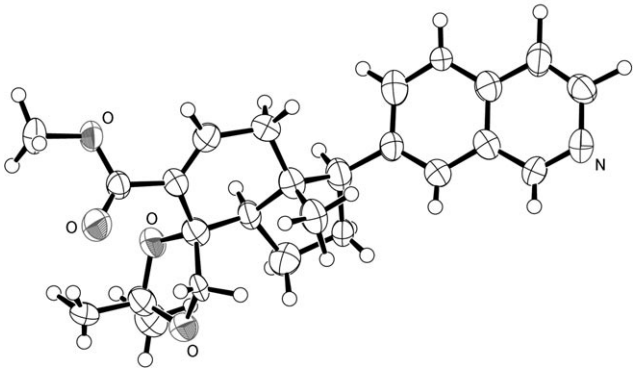
mentary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.





ORTEP drawing of **25** with thermal ellipsoids shown at the 50% probability level.

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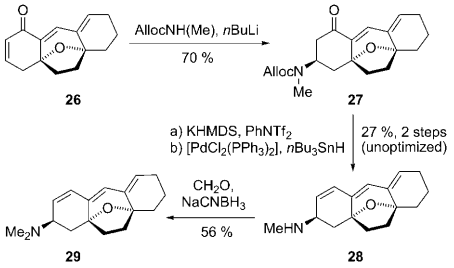
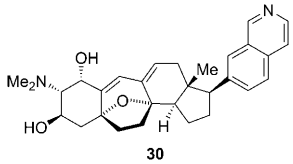
[16] The chemo- and facial selectivity of 1,4-addition to simplified trienone system **26** was first demonstrated in a model study towards cortistatin J, where detailed NMR analysis of trienyl dimethylamine **29** revealed the 1,4-addition took place from the β face.



[17] The isomeric epoxide-opened product is tentatively assigned as the C2,C3 regioisomer **30** of cortistatin A (**1**) on the basis of ^{1}H NMR, ^{13}C NMR, and MS analysis.



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