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Creation of Readily Accessible and Orally Active Analogue of Cortistatin A

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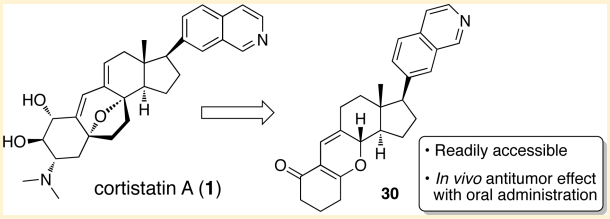
Supporting Information

ABSTRACT: Syntheses of structurally simplified analogues of cortistatin A (**1**), a novel antiangiogenic steroidal alkaloid from Indonesian marine sponge, and their biological activities were investigated. The analogues were designed by considering the 3-D structure of **1**. Compound **30**, in which the isoquinoline moiety was appended to the planar tetracyclic core structure, showed potent antiproliferative activity against human umbilical vein endothelial cells (HUVECs) together with high selectivity and also showed *in vivo* antiangiogenic activity and significant antitumor effect by oral administration.



KEYWORDS: *Cortistatin A, antiangiogenesis, marine sponge, analogue synthesis, structure−activity relationship*

Angiogenesis, the formation of new blood capillaries from preexisting blood vessels, is critical for tumor growth and metastasis. A growing tumor needs an extensive network of capillaries to provide nutrients and oxygen, etc. In addition, the new blood vessels provide a way for tumor cells to enter in the circulation and to metastasize to another organ. Therefore, substances that inhibit angiogenesis have a considerable potential to be novel therapeutic agents for the treatment of cancer.[1]

In the course of our study on bioactive substances from marine organisms, we focused on a search for selective inhibitors of proliferation of human umbilical vein endothelial cells (HUVECs) as antiangiogenic substances and isolated cortistatins,[2−5] a family of novel *abeo*-9(10−19)-androstane-type steroidal alkaloids, from the Indonesian marine sponge of *Corticium simplex* (**1**−**11**, Figure 1). We found that cortistatin A (**1**), a major constituent, showed remarkably selective antiproliferative activity against HUVECs and also inhibited migration and tubular formation of HUVECs induced by VEGF or bFGF.[2] Therefore, cortistatins might have considerable potential as a novel antiangiogenic drug lead.

The unique structure and characteristic biological properties of this compound attracted many synthetic chemists, and a number of synthetic reports, including five total syntheses[6−13] have appeared, even though small quantities of the final compound could be obtained. And there have been no reports about *in vivo* antitumor effects of cortistatins. Then we decided to engage in a synthetic study of structurally simplified and *in vivo* active analogues of cortistatins.[14−16] We report herein about the design, synthesis, and biological evaluation of cortistatin analogues exhibiting an *in vivo* antitumor effect.

Through examination of the growth inhibitory activity of eleven naturally occurring cortistatins,[2−5] we have analyzed the structure−activity relationship as follows: (1) the isoquinoline moiety is crucial for exhibiting potent and selective activity; cortistatins E−H (**5**−**8**) show weak antiproliferative activity against HUVEC with no selectivity, (2) the presence of the hydroxyl group at the D-ring (cortistatins B (**2**) and D (**4**)) diminishes their activity, and (3) structural modifications at the A- or B-ring cause some influence but not a critical one; cortistatins J (**9**), K (**10**), and L (**11**) show comparable potency and selectivity to those of **1**.[5]

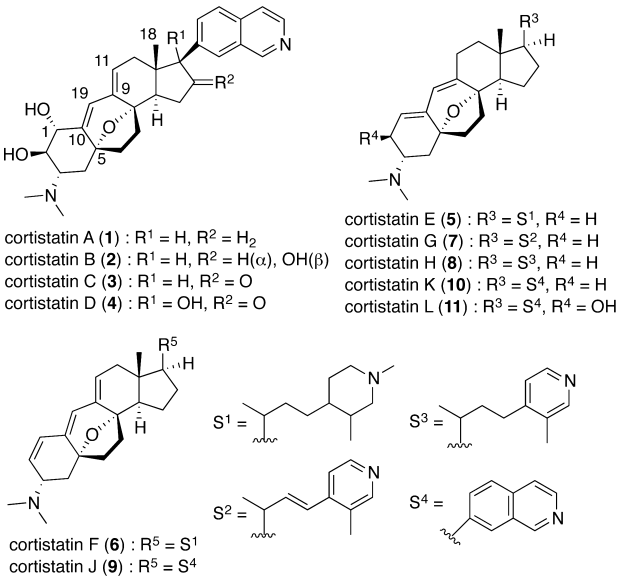


Figure 1. Chemical structure of cortistatins.

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The X-ray crystallographic analysis of cortistatin A (**1**) shows that the core structure of **1**, an *abeo*-9(10−19)-androstane-type rearranged steroidal backbone with an oxa-bridge between the 5- and 8-positions, possesses an anthracene-like planar ABC-ring system (Figure 2). Considering the structural property, we designed two compounds **12** and **13** as novel simplified analogues of cortistatin A.

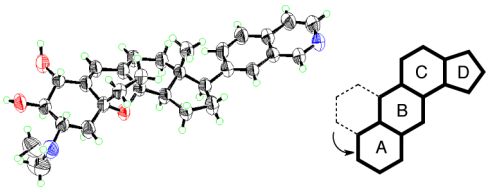


Figure 2. X-ray structure of cortistatin A (**1**).

As shown in Figure 3, the CD-ring part having an isoquinoline moiety and the A-ring part having a dimethylamino group in compound **12** were connected through one sp2 carbon linker, to put the isoquinoline and dimethylamino moieties in the appropriate position. From molecular mechanics (MM) calculation analysis, these two key components were expected to locate in a similar position between **1** and **12**. On the other hand, Nicolaou and Chen reported that an intermediate compound for their total synthesis of **1**, having the ketone group at the C-1 position, exhibited a similar selective antiproliferative activity to that of **1**. This evidence implied that the dimethylamino group commonly existing at the C-3 position in all cortistatins is not an essential moiety.[7] Furthermore, we presumed that the bicyclic B-ring structure might be mimicked by a simple pyran ring. MM calculation revealed that compounds **1** and **13** showed high similarity in their lowest energy conformations (Figure 3).

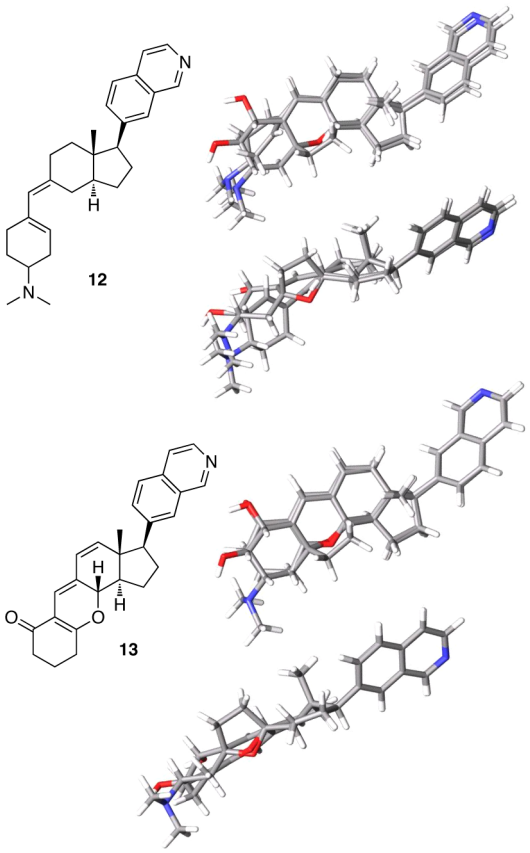
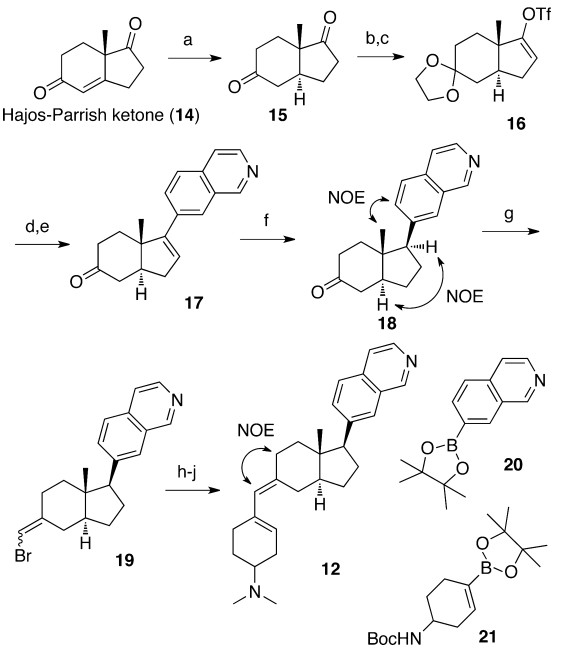


Figure 3. Design of cortistatin analogues **12** and **13**. The top and side views of the imposed 3D structures with cortistatin A (**1**) were also shown.

The synthesis of analogue **12** was executed as shown in Scheme 1. Following the literature, stereoselective conjugate reduction of Hajos−Parrish ketone (**14**) using *tert*-BuMgCl, DIBAL-H, and CuI in the presence of HMPA provided *trans*-hydrindanedione **15** in good yield.[17,18] After the sterically less-hindered ketone group was protected as an ethylene ketal, the other ketone group was further converted to its enol triflate to give compound **16**. Introduction of an isoquinoline moiety with desired stereochemistry was successfully achieved by a Suzuki−Miyaura cross-coupling reaction between compound **16** and isoquinolin-7-yl boronate **20**[7] using standard conditions, removal of the ketal protection, and subsequent hydrogenation of the remaining olefin using Pd−C as a catalyst. An NOE experiment for compound **18** confirmed the desired orientation of the isoquinoline group. Then Wittig olefination using (bromomethyl)triphenylphosphonium bromide provided compound **19** as a *E*/*Z* mixture (1:1). Finally, an A-ring appendage was introduced by Suzuki coupling with boronate **21**, which was prepared from 4-aminocyclohexanol.[19] After deprotection and methylation, the objective analogue **12** and its geometrical isomer **12′** were obtained as a 1:1 mixture. Each isomer was separated by using reversed-phase HPLC.

Scheme 1. Synthesis of Analogue 12[a]



[a]Reagents and conditions: (a) DIBAL-H, CuI, *t*-BuMgCl, HMPA, THF, −78 °C, 77%; (b) ethylene glycol, $(COOH)_2 \cdot 2H_2O$, CH_3CN; (c) $PhNTf_2$, KHMDS, THF, −78 °C, 78% (2 steps); (d) **20**, $Pd(PPh_3)_4$, K_2CO_3, DMF, 50 °C; (e) p-TsOH, acetone/H_2O, 52% (2 steps); (f) H_2, Pd−C, AcOEt, 92%; (g) $BrCH_2PPh_3Br$, NaHMDS, THF, 86%; (h) **21**, $Pd(dppf)Cl_2$, Cs_2CO_3, $AsPh_3$, DMF, 80 °C, 75%; (i) 5 N HCl, THF; (j) $(HCHO)_n$, $NaBH(OAc)_3$, CH_2Cl_2; HPLC separation, 30% (2 steps).

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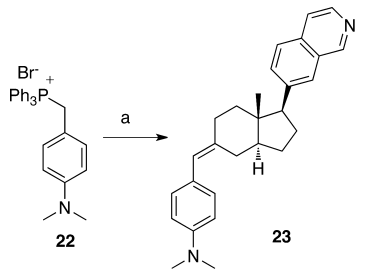
The analogue **23**, having an aromatic A-ring moiety, was also prepared by using a simpler method than that for analogue **12** (see Scheme 2). Thus, Wittig reaction between **18** and

Scheme 2. Synthesis of Analogue 23[a]

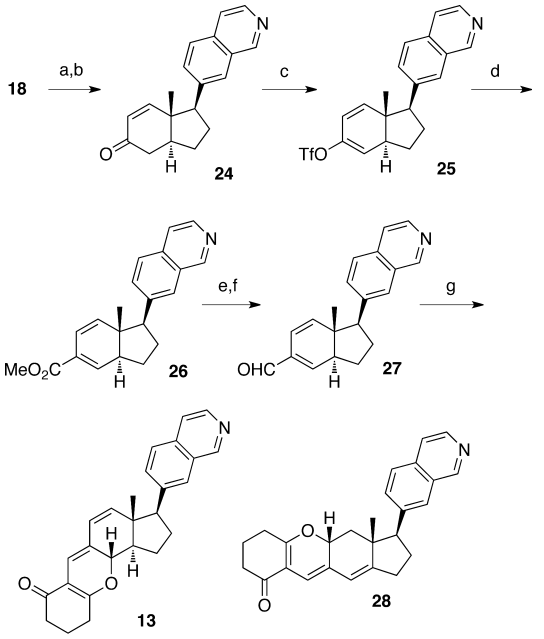


[a]Reagents and conditions: (a) NaH, THF, **18**, 50 °C; HPLC separation, 23%.

phosphonium salt **22**[20,21] in the presence of NaH afforded analogue **23** as an *E*/*Z* mixture, which was also separated by HPLC. The stereochemistry of the product was determined by a downfield shift of the allylic equatorial proton caused by an anisotropic effect, as well as NOE experiment.

Next, preparation of analogue **13** was investigated starting from compound **18** (Scheme 3). IBX oxidation of the

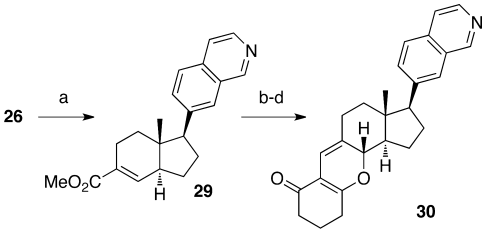
Scheme 3. Synthesis of Analogue 13[a]



[a]Reagents and conditions: (a) TMSCl, NaI, HMDS, CH_3CN; (b) IBX, DMSO, 71% (2 steps); (c) $PhNTf_2$, KHMDS, THF, −78 °C, 94%; (d) CO (gas), $Pd(PPh_3)_4$, MeOH, Et_3N, DMF, 98%; (e) DIBAL-H, CH_2Cl_2, −78 °C; (f) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2, 67% (2 steps); (g) 1,3-cyclohexanedione, ethylenediamine, AcOEt, 97%.

kinetically generated silyl enol ether of **18** gave enone **24**, which was further converted to a dienol triflate **25** in good yield. Then, CO insertion reaction smoothly proceeded to give a dienoate **26**, and subsequent DIBAL-H reduction and Dess−Martin oxidation provided dienal **27**. Finally, the treatment of **27** with 1,3-cyclohexanedione in the presence of ethylenediamine afforded the desired analogue **13**, through Knoevenagel condensation and subsequent electrocyclization,[10] in 14% overall yield. The use of the other amines than ethylenediamine in this reaction, such as piperidine, provided a substantial amount of compound **28** as a byproduct, through isomerization of olefin and the following condensation−cyclization. In the ^1H NMR spectrum of **13**, the oxymethine proton signal was observed as a doublet with J = 11.6 Hz, which indicates that compound **13** has a planar tetracyclic structure similar to that of **1**. The 11,12-dihydro analogue **30** was also prepared using the same method from enoate **29**, which was obtained by regioselective hydrogenation using the diimide of the disubstituted olefin of dienoate **26** (Scheme 4). Hydrogenation using Pd−C as a catalyst resulted in olefin migration.

Scheme 4. Synthesis of Analogue 30[a]



[a]Reagents and conditions: (a) potassium azodicarboxylate, AcOH, 99%; (b) L-Selectride, THF, 80%; (c) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2; (d) 1,3-cyclohexanedione, ethylenediamine, AcOEt, 64% (2 steps).

The antiproliferative activities of the synthetic analogues against endothelial cells (HUVECs) and KB3-1 cells were evaluated (Table 1). This revealed that analogue **12** showed

Table 1. Antiproliferative Activities of Cortistatin Analogues

	IC_{50} (μM)				
cell line	**1**	**12**	**23**	**13**	**30**
HUVEC	0.0018	2.0	15	0.1	0.035
KB3-1	7.0	18	20	10.5	10.5
K562	7.0	n.t.[a]	n.t.	6.0	5.0
Neuro2A	6.0	n.t.	n.t.	10.5	10.5
NHDF	6.0	n.t.	n.t.	10.5	4.0

[a]n.t.: not tested.

moderate antiproliferative activity against HUVEC (IC_{50}: 2.0 μM) with only 9-fold selectivity over KB3-1 cells (IC_{50}: 18 μM) and that compound **23**, having an aromatic A-ring moiety, showed no selectivity (1.5-fold). The geometrical isomers of **12** and **23** showed weaker activity or selectivity (see Supporting Information). On the other hand, analogues **13** and **30** showed good antiproliferative activities against HUVEC (IC_{50}: 0.1 or 0.035 μM) with high selectivity (>100-fold) over KB3-1 cells (IC_{50}: 10.5 μM each). Particularly, analogue **30** exhibited comparable activity against HUVEC, with high selectivity, to that of cortistatin A (**1**). These results imply that the planar structure of the tetracyclic core part would be an essential structural element for HUVEC-selective growth inhibitory activity. The byproduct **28** showed weaker activity than **13** (see Supporting Information), which indicates that the structural resemblance to cortistatin A (**1**) is also an important factor.

Then, we evaluated the *in vivo* activity of analogue **30** on the formation of new blood vessels, by the matrigel plug assay[22] in

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mice. As shown in Figure 4, the oral administration of compound **30** prevented new blood capillary formation in the

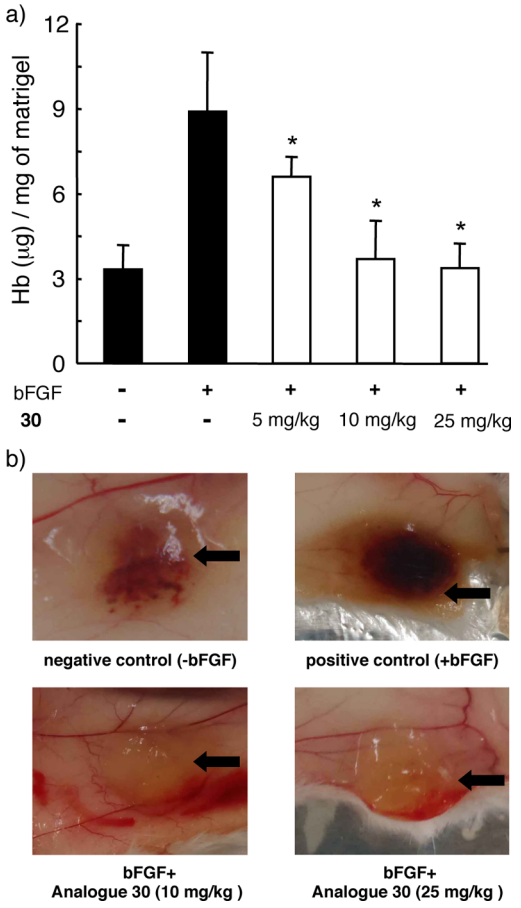

Figure 4. *In vivo* antiangiogenic effect of analogue **30**. (a) Mean ± SD hemoglobin content in the matrigel of each group; *: $P < 0.05$. (b) Images of Matrigel plugs after 10 days.

matrigel plug induced by bFGF. Compound **30** significantly decreased the hemoglobin content in the plug, and the 25 mg/kg dosage of **30** reduced the hemoglobin content to the same level of the negative control. These results indicated that analogue **30** effectively inhibited *in vivo* angiogenesis.

We further examined an *in vivo* antitumor effect of analogue **30**. Compound **30** significantly inhibited growth of the tumor with more than 5 mg/kg of oral administration, and ~90% reduction of tumor weight was observed at the dose of more than 25 mg/kg in comparison with that of control (Figure 5). Moreover, up to 100 mg/kg administration of compound **30** exhibited no significant acute toxicity, such as body weight loss or diarrhea. Considering the result of these *in vivo* experiments, the potent antitumor activity of compound **30** is presumed to come from the intensive inhibition of angiogenesis promoted by the implanted tumor. Compound **13** also showed potent *in vivo* antitumor activity, by oral administration (data not shown).

In summary, we achieved syntheses of readily accessible and orally active analogues of cortistatin A (**1**). Analogue **30** is a first example of the cortistatin-related compound exhibiting a potent *in vivo* antitumor effect through inhibition of angiogenesis. Furthermore, the synthetic scheme of compound **30** was very simple and scalable. Actually, we could prepare >100 mg of the final compound for *in vivo* study without any

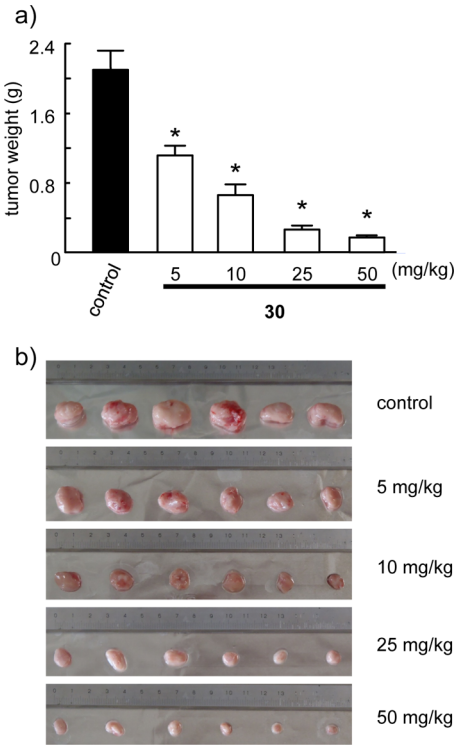

Figure 5. *In vivo* antitumor effect of analogue **30**. (a) Mean ± SD tumor weight of each group; *: $P < 0.05$. (b) Images of isolated tumors after two weeks.

difficulty. We are further investigating to develop more practical and promising anticancer drug candidates based on analogue **30** as a structural template.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures and spectral data of compounds **15**–**30**, and procedures for *in vitro* and *in vivo* evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Carmeliet, P.; Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *407*, 249–257.

(2) Aoki, S.; Watanabe, Y.; Sanagawa, M.; Setiawan, A.; Kotoku, N.; Kobayashi, M. Cortistatins A, B, C, and D, Anti-angiogenic Steroidal Alkaloids, from the Marine Sponge *Corticium simplex*. *J. Am. Chem. Soc.* **2006**, *128*, 3148–3149.

(3) Watanabe, Y.; Aoki, S.; Tanabe, D.; Setiawan, A.; Kobayashi, M. Cortistatins E, F, G, and H, four novel steroidal alkaloids from marine sponge *Corticium simplex*. *Tetrahedron* **2007**, *63*, 4074−4079.
(4) Aoki, S.; Watanabe, Y.; Tanabe, D.; Setiawan, A.; Arai, M.; Kobayashi, M. Cortistatins J, K, L, novel abeo-9(10−19)-androstane-type steroidal alkaloids with isoquinoline unit, from marine sponge *Corticium simplex*. *Tetrahedron Lett.* **2007**, *48*, 4485−4488.
(5) Aoki, S.; Watanabe, Y.; Tanabe, D.; Arai, M.; Suna, H.; Miyamoto, K.; Tsujibo, H.; Tsujikawa, K.; Yamamoto, H.; Kobayashi, M. Structure−activity relationship and biological property of cortistatins, anti-angiogenic spongean steroidal alkaloids. *Bioorg. Med. Chem.* **2007**, *15*, 6758−6762.
(6) Shi, J.; Manolikakes, G.; Yeh, C.-H.; Guerrero, C. A.; Shenvi, R. A.; Shigehisa, H.; Baran, P. S. Scalable Synthesis of Cortistatin A and Related Structures. *J. Am. Chem. Soc.* **2011**, *133*, 8014−8027.
(7) Nicolaou, K. C.; Peng, X.-S.; Sun, Y.-P.; Polet, D.; Zou, B.; Lim, C. S.; Chen, D. Y.-K. Total Synthesis and Biological Evaluation of Cortistatins A and J and Analogues Thereof. *J. Am. Chem. Soc.* **2009**, *131*, 10587−10597.
(8) Lee, H. M.; Nieto-Oberhuber, C.; Shair, M. D. Enantioselective Synthesis of (+)-Cortistatin A, a Potent and Selective Inhibitor of Endothelial Cell Proliferation. *J. Am. Chem. Soc.* **2008**, *130*, 16864−16866.
(9) Flyer, A. N.; Si, C.; Myers, A. G. Synthesis of cortistatins A, J, K and L. *Nat. Chem.* **2010**, *2*, 886−892.
(10) Yamashita, S.; Iso, K.; Kitajima, K.; Himuro, M.; Hirama, M. Total Synthesis of Cortistatins A and J. *J. Org. Chem.* **2011**, *76*, 2408−2425.
(11) Narayan, A. R. H.; Simmons, E. M.; Sarpong, R. Synthetic Strategies Directed Towards the Cortistatin Family of Natural Products. *Eur. J. Org. Chem.* **2010**, *19*, 3553−3567.
(12) Chen, D. Y.-K.; Tseng, C.-C. Chemistry of the cortistatins−a novel class of *anti*-angiogenic agents. *Org. Biomol. Chem.* **2010**, *8*, 2900−2911.
(13) Nising, C. F.; Bräse, S. Highlights in Steroid Chemistry: Total Synthesis versus Semisynthesis. *Angew. Chem., Int. Ed.* **2008**, *47*, 9389−9391.
(14) Shi, J.; Shigehisa, H.; Guerrero, C. A.; Shenvi, R. A.; Li, C.-C.; Baran, P. S. Stereodivergent Synthesis of 17-α and 17-β-Aryl Steroids: Application and Biological Evaluation of D-Ring Cortistatin Analogues. *Angew. Chem., Int. Ed.* **2009**, *48*, 4328−4331.
(15) Czakó, B.; Kürti, L.; Mammoto, A.; Ingber, D. E.; Corey, E. J. Discovery of Potent and Practical Antiangiogenic Agents Inspired by Cortistatin A. *J. Am. Chem. Soc.* **2009**, *131*, 9014−9019.
(16) Sato, Y.; Kamiyama, H.; Usui, T.; Saito, T.; Osada, H.; Kuwahara, S.; Kiyota, H. Synthesis and Anti-Angiogenic Activity of Cortistatin Analogs. *Biosci. Biotechnol. Biochem.* **2008**, *72*, 2992−2997.
(17) Tsuda, T.; Hayashi, T.; Satomi, H.; Kawamoto, T.; Saegusa, T. Methylcopper(I)-Catalyzed Selective Conjugate Reduction of α,β-Unsaturated Carbonyl Compounds by Diisobutylaluminum Hydride in the Presence of Hexamethylphosphoric Triamide. *J. Org. Chem.* **1986**, *51*, 537−540.
(18) Daniewski, A. R.; Kiegiel, J. Stereoselective conjugate reduction of (+)-(7aS)-5,6,7,7a-tetrahydro-7a-methyl-1,5-indandione. *Synth. Commun.* **1988**, *18*, 115−118.
(19) Raju, B.; Anandan, S.; Gu, S.; Herradura, P.; O'Dowd, H.; Kim, B.; Gomez, M.; Hackbarth, C.; Wu, C.; Wang, W.; Yuan, Z.; White, R.; Trias, J.; Patel., D. V. Conformationally restricted analogs of deoxynegamycin. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3103−3107.
(20) Chou, S.-S. P.; Yeh, Y.-H. Synthesis of novel sulfonyl-substituted pyrrole chromophores for second-order nonlinear optics. *Tetrahedron Lett.* **2001**, *42*, 1309−1311.
(21) Zhang, J.-X.; Dubois, P.; Jerome, R. An improved preparation method of benzyl and thenyl triphenylphosphonium salts. *Synth. Commun.* **1996**, *26*, 3091−3095.
(22) Arai, M.; Hayashi, A.; Sobou, M.; Ishida, S.; Kawachi, T.; Kotoku, N.; Kobayashi, M. Anti-angiogenic effect of triterpenoidal saponins from *Polygala senega*. *J. Nat. Med.* **2011**, *65*, 149−156.