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Synthesis of (+)-Cortistatin A

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Steroids have historically elicited attention from the chemical sciences owing to their utility in living systems, as well as their intrinsic and diverse beauty.[1] The cortistatin family (Figure 1, **1**−**7** and others),[2] a collection of unusual, marine 9-(10,19)-*abeo*-androstane steroids, is certainly no exception; aside from challenging stereochemistry and an odd bricolage of functional groups, the salient feature of these sponge metabolites is, inescapably, their biological activity. Cortistatin A, the most potent member of the small family, inhibits the proliferation of human umbilical vein endothelial cells (HUVECs, IC_{50} = 1.8 nM), evidently with no general toxicity toward either healthy or cancerous cell lines ($IC_{50(\text{testing cells})}/IC_{50(\text{HUVECs})} \geq 3300$).[2a] From initial pharmacological studies, binding appears to occur reversibly, but to an unknown target, inhibiting the phosphorylation of an unidentified 110 kDa protein, and implying a pathway that may be unique to known antiangiogenesis compounds.[2d]

Compelled by the pharmacological potential of the cortistatins,[2d,3] together with the unanswered questions surrounding their biological activity,[4] our group embarked on a synthesis of cortistatin A (**1**), aiming for a concise route from inexpensive, commercially available materials and the opportunity to develop new chemistry as the occasion arose. A crucial target structure became the cortistatin A ketonic core, (+)-cortistatinone (**8**, Figure 1), which was anticipated to allow for straightforward elaboration to the natural product, as well as divergence to other family members. A semisynthetic route to these marine steroids was deemed an acceptable strategy largely due to the economy of using prednisone, which at $1.20/g possesses 70% of the carbon atoms and the corresponding, enantiopure chirality of **1**.

Prednisone could be converted to the known steroid core **9** (Scheme 1) in a short two-step sequence (see Supporting Information) and in 92% overall yield after recrystallization. The C1,C2 *trans*-vicinal diol was targeted through the intermediacy of α-disposed epoxide **10**, which was installed using *tert*-butyl hydroperoxide instead of the precedented, but nonscalable, dimethyldioxirane (DMDO) procedure.[5] Reductive amination of the unsaturated ketone proceeded uneventfully, and refluxing the crude reaction mixture with ethyl formate generated formamide **10** in good yield. The epoxide, however, proved intractable to a number of standard procedures for nucleophilic addition. Under acidic aqueous conditions, the nascent diol underwent facile cyclization onto the C11 ketone, followed by dehydration to yield an unproductive dihydrofuran, which itself is a rare motif among steroids.[6] Conversely, basic aqueous conditions led to undesired cleavage of the formyl moiety, a group which would figure prominently as a methyl surrogate of the target molecule. Eventually, it was found that tetra-*n*-butylammonium acetate (TBAA) in refluxing benzene opened the epoxide at C2, producing the *trans*-hydroxy acetate **11** (see X-ray **11′**).

After extensive experimentation, it was found that the key orthoamide **12** (see X-ray **12**) could be synthesized in one pot from

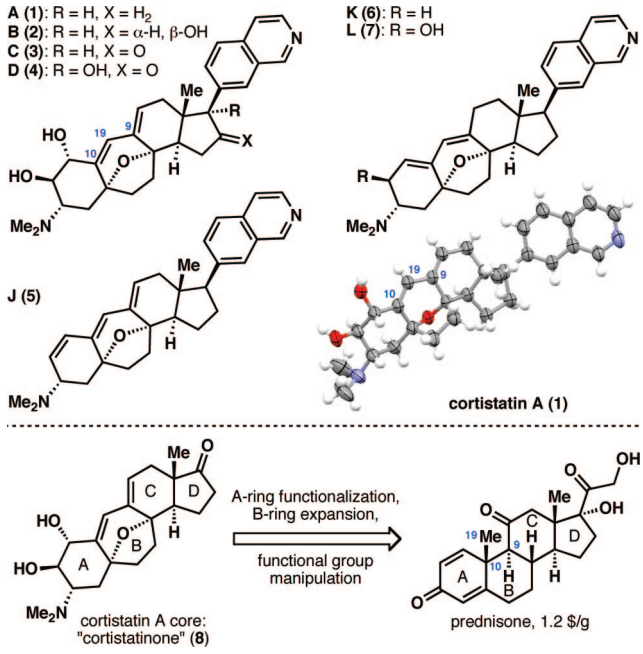
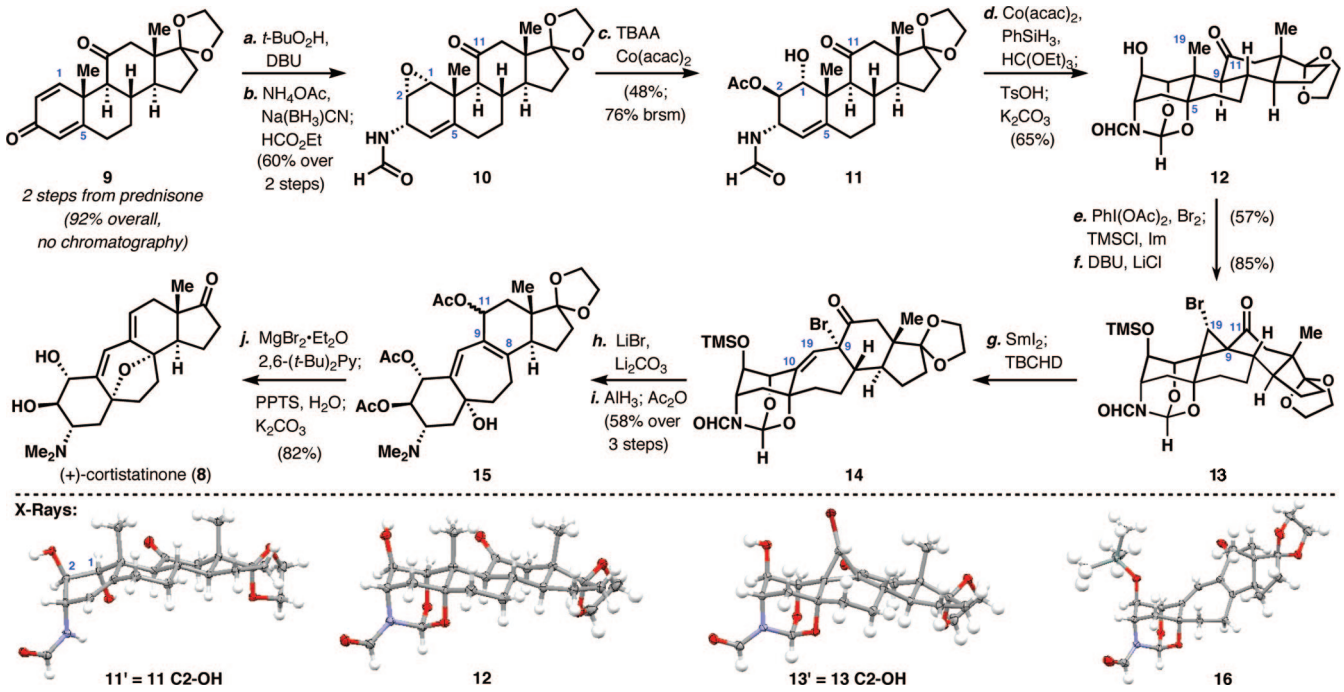


Figure 1. Isoquinoline-bearing cortistatins A−D (**1**−**4**) and J−L (**5**−**7**) (cortistatins E−H bear pyridines or piperidines and exhibit markedly diminished potency), and a general retrosynthetic strategy to target a steroid skeleton, namely, the inexpensive, terrestrial steroid, prednisone.

intermediate **11** by (1) Mukaiyama hydration[7] of the trisubstituted olefin, (2) reaction of the amidodiol with trimethyl orthoformate, and (3) solvolysis of the C2 acetate. Notably, reacting epoxyformamide **10** under identical conditions for Mukaiyama hydration gave a 5:1 stereoisomeric mixture of tertiary alcohols, disfavoring the desired C5 α-stereochemistry.

During preliminary reconnaissance in accessing the cortistatin core, the most difficult functionality to secure turned out to be the C19 methine oxidation state,[8] suggesting the importance of its installation early in the sequence. Unfortunately, existing methods for such a transformation (angular methyl→aldehyde oxidation state) are reported to give generally low yields[9] and, more importantly, proved completely ineffectual in our system. Consequently, a new process was conceived to access a dibrominated 19-carbon, utilizing in situ generated acetoxy hypobromite (AcOBr).[10] Success was realized by significantly lowering the reaction temperature and extending the reaction time, resulting in an iterative, double methyl activation (Figure 2, **17**→**18**→**19**), while suppressing S_N2 attack of the alcohol on the σ^*_{C-Br} orbital of the monobromide (**17**→**20**; possibly S_H2 attack of the transient O-centered radical). To the best of our knowledge, *this is the first example of an alcohol-directed, geminal dihalogenation of an unactivated hydrocarbon*. The selectivity for dibromination (57%) over mono- or tribromination well surpasses what would be expected with only the governance

Scheme 1. Synthesis of the (+)-Cortistatin A Core: Cortistatinone (**8**)[a]



[a] Reagents and conditions: (a) *t*-BuO_2H (2 equiv), DBU (2 equiv), THF, 23 °C, 72 h, 82%; (b) NH_4OAc (15 equiv), $Na(BH_3)CN$ (1.2 equiv), MeOH, THF, 23 °C,18 h; then HCO_2Et (74 equiv), Et_3N (11 equiv), 54 °C, 12 h, 73%; (c) TBAA (5 equiv), $Co(acac)_2$ (0.2 equiv), PhH, 90 °C, 24 h, 48%, 76% brsm; (d) $Co(acac)_2$ (0.2 equiv), $PhSiH_3$ (2.2 equiv), O_2, THF, $HC(OMe)_3$, 23 °C, 12 h; then TsOH•H_2O (3 equiv), rt, 2 h; then K_2CO_3 (5 equiv), MeOH, 6 h, 65%; (e) $PhI(OAc)_2$ (5 equiv), Br_2 (5 equiv), CH_2Cl_2, −30 °C, 10 h; then TMSCl (5 equiv), imidazole (5 equiv), 0 °C, 15 min, 57%; (f) DBU (2 equiv), LiCl (10 equiv), THF, 23 °C, 24 h, 85%; (g) SmI_2 (2.2 equiv), 1:9 DMPU:THF, 23 °C, 5 min; then TBCHD (1.1 equiv), 23 °C, 5 h; (h) LiBr (20 equiv), Li_2CO_3 (20 equiv), DMF, 80 °C, 1 h (65% over 2 steps); (i) AlH_3 (0.5 M in THF, 5 equiv), THF, 23 °C, 1 h; then K_2CO_3 (4 equiv), MeOH, 23 °C, 12 h; then Ac_2O (20 equiv), Et_3N (40 equiv), DMAP (0.1 equiv), CH_2Cl_2, 23 °C, 3 h, 89%; (j) $MgBr_2$•Et_2O (1.1 equiv), 2,6-(*t*-$Bu)_2Py$ (2.1 equiv), PhH, 80 °C, 1 h; PPTS (5 equiv), butanone: H_2O (1:1), 90 °C, 2 h; then K_2CO_3 (10 equiv), 23 °C, 5 h (82%).

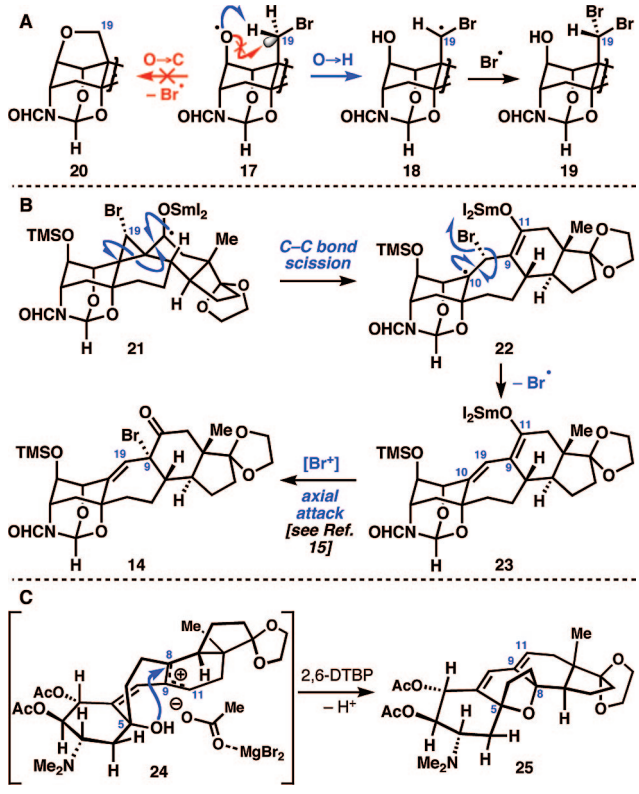


Figure 2. Key transformations en route to (+)-cortistatin A and mechanistic analyses.

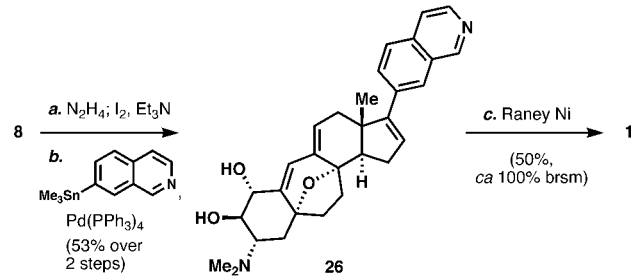
of statistics,[11] which would produce the dihalide in a maximum yield of 27% (see Supporting Information). Use of the well-precedented $PhI(OAc)_2/I_2$ conditions for *mono*iodination[12] resulted in competitive bicyclic ether (**20**) formation, likely due to a much larger coefficient of the σ^*_{C-I} orbital.

The unstable dibromo alcohol was capped with a trimethylsilyl group to prevent intramolecular etherification. Alkylation of the 9,11-enolate with the proximal dibromomethyl proceeded with DBU and lithium chloride to give one bromocyclopropane diastereomer **13** (Scheme 1), whose configuration was confirmed by X-ray diffraction of alcohol **13′**.

After extensive experimentation, a cascade sequence (see Figure 2 for a possible mechanism) was developed to achieve isomerization of bromocyclopropane **13** to cycloheptyl α-bromoketone **14** in high yield, via radical opening of the three-membered ring (**21**→**22**) extrusion of bromine radical (**22**→**23**), and trapping of the extended enolate (**23**→**14**) with 2,4,4,6-tetrabromo-2,5-cyclohexadienone (TBCHD).[13,14] The major diastereomer of the reaction is likely the α-disposed allylic, C9 bromide **14**; although spectroscopy could not assign this stereochemistry unambiguously, ensuing reactions and stereoelectronic considerations suggest this configuration.[15] Thus, the oxidation state deliberately embedded in the 19-methyl dibromide **19** translated smoothly into the olefinic C19 methine of the cortistatin core. Without the carefully placed bromine atom in the cyclopropane ring of **13**, this fragmentation led to a cycloheptyl ketone that could not be brought to the desired oxidation state.

Elimination of the α-bromide **14** with lithium carbonate delivered the cross-conjugated dienone (see X-ray **16**, Scheme 1). Under the action of alane, the heteroadamantane A ring core was reductively unmasked to reveal the entire A ring of cortistatin A; in situ

Scheme 2. Completing the Synthesis of (+)-Cortistatin A[a]



[a] Reagents and conditions: (a) N_2H_4 (10 equiv), Et_3N (10 equiv), EtOH, 50 °C, 6 h, I_2 (2 equiv), Et_3N (3 equiv), THF, 23 °C, 5 min; (b) 7-(trimethylstannyl)-isoquinoline (4 equiv), $Pd(PPh_3)_4$ (0.5 equiv), CuCl (10 equiv), LiCl (10 equiv), DMSO, 23 °C, 10 min, 53% (over 2 steps); (c) Raney Ni (88 wt equiv), *i*-PrOH, H_2O, 50 °C, 1 h, 50% (ca. 100% brsm).

desilylation and triacetylation delivered dimethylaminotriacetate **15** as a diastereomeric mixture at C11. Acetylation of the C11 alcohol served to activate the 8,9-olefin toward conjugate displacement (see **24**, Figure 2), which was achieved upon heating with $MgBr_2 \bullet Et_2O$ in benzene, delivering the bridging bicyclic ether of the cortistatin core **25**. Mild deketalization using pyridinium *p*-toluenesulfonate, followed by solvolytic removal of the A-ring acetates delivered (+)-cortistatinone (**8**), whose proton and carbon NMR spectra bore a satisfying similarity to the reported spectra for cortistatin A.

To complete the synthesis, a challenging task lay ahead: appending the requisite β-disposed C17 isoquinoline in the presence of a tertiary amine, a vicinal diol, and two olefins; these objectives were achieved by the sequence delineated in Scheme 2. First, cortistatinone (**8**) was treated with hydrazine to generate an intermediate C17 hydrazone, which was not isolated, but rather immediately subjected to iodine and triethylamine to form an alkenyl iodide,[16] sparing both the C3 tertiary amine and the internal diene. Second, Stille coupling[17] delivered the isoquinoline **26** without incident; it is projected that this reaction should be amenable to the installation of numerous heterocyclic side chains. Finally, in a consummate example of chemoselective reduction, the benzylic olefin was reduced with Raney nickel in water and isopropanol to yield synthetic (+)-cortistatin **1**, whose spectral characteristics were identical to those reported in the literature.

Thus, (+)-cortistatin A (**1**), the most biologically active member of the marine-derived cortistatin family, was synthesized from the inexpensive terrestrial steroid prednisone (ca. 3% overall yield, unoptimized), which is commercially available in multikilogram quantities. Certain aspects of this synthesis carry important lessons in chemical reactivity, selectivity, and synthesis strategy, including (1) the four-step sequence (**9**→**12**) to install all requisite A ring stereochemistry; (2) a newly invented alcohol-directed, dibromination reaction (**12**→**19**); (3) an isohypsic (oxidation-state conserving) cascade (**13**→**14**) to access the 9-(10,19)-*abeo*-androstane skeleton; (4) an olefin-sparing, heteroadamantane fragmentation to differentiate the tethered aminodiol; (5) a mild S_N' cyclization to close the final ring of the cortistatin skeleton (**15**→**25**); and (6) a selective benzylic hydrogenation to facilitate the transformation of (+)-cortistatinone to (+)-cortistatin A (**8**→**1**). Access to **8** will prove valuable in probing the crucial[2d] heterocyclic domain of the cortistatins; targeting this intermediate should also facilitate future syntheses. Efforts are now underway to establish the antiangiogenic mode of action of cortistatin A and analogues thereof.

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Supporting Information Available: Detailed experimental procedures, copies of all spectral data, and full characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Djerassi, C. Steroids Made it Possible. In *Profiles, Pathways and Dreams*; Seeman, J. I., Ed.; American Chemical Society: Washington, D.C., 1990.
(2) (a) Aoki, S.; Watanabe, Y.; Sanagawa, M.; Setiawan, A.; Kotoku, N.; Kobayashi, M. *J. Am. Chem. Soc.* **2006**, *128*, 3148–3149. (b) Watanabe, Y.; Aoki, S.; Tanabe, D.; Setiawan, A.; Kobayashi, M. *Tetrahedron* **2007**, *63*, 4074–4079. (c) Aoki, S.; Watanabe, Y.; Tanabe, D.; Setiawan, A.; Arai, M.; Kobayashi, M. *Tetrahedron Lett.* **2007**, *48*, 4485–4488. (d) Aoki, S.; Watanabe, Y.; Tanabe, D.; Arai, M.; Suna, H.; Miyamoto, K.; Tsujibo, H.; Tsujikawa, K.; Yamamoto, H.; Kobayashi, M. *Bioorg. Med. Chem.* **2007**, *15*, 6758–6762.
(3) Folkman, J. *Nat. Rev. Drug Discovery* **2007**, *6*, 273–286.
(4) Notably, the natural products themselves are so scarce that no authentic sample of cortistatin A could be spared for comparison to synthetic material, without impeding the biological studies already underway; personal communication with M. Kobayashi.
(5) Bovicelli, P.; Lupattelli, P.; Mincione, E.; Prencipe, T.; Curci, R. *J. Org. Chem.* **1992**, *57*, 2182–2184.
(6) Halsall, T. G.; Troke, J. A. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1758–1764.
(7) Isayama, S.; Mukaiyama, T. *Chem. Lett.* **1989**, 1071–1074.
(8) For the first examples of angular methyl oxidation of a steroid, see: (a) Corey, E. J.; Hertler, W. R. *J. Am. Chem. Soc.* **1958**, *80*, 2903–2904. (b) Buchschacher, P.; Kalvoda, J.; Arigoni, D.; Jeger, O. *J. Am. Chem. Soc.* **1958**, *80*, 2905–2906.
(9) Godula, K.; Sames, D. *Science* **2006**, *312*, 67–72.
(10) González, C. C.; León, E. I.; Riesco-Fagundo, C.; Suárez, E. *Tetrahedron Lett.* **2003**, *44*, 6347–6350.
(11) McQuarrie, D. A.; Simon, J. D. *Physical Chemistry: A Molecular Approach*; University Science Books: Sausolito, CA, 1997.
(12) Cekovic, Z. *Tetrahedron* **2003**, *59*, 8073–8090.
(13) NBS and more orthodox sources of bromonium gave lower diastereoselectivities and/or yields.
(14) For other approaches to the 9-(10−19)-*abeo*-androstane ring system utilizing the scission of a cyclopropane, see: (a) Kupchan, S. M.; Abushanab, E.; Shamasundar, K. T.; By, A. W *J. Am* **1967**, *89*, 6327–6332. (b) Kupchan, S. M.; Findlay, J. W. A.; Hackett, P.; Kennedy, R. M. *J. Org. Chem.* **1972**, *37*, 2523–2532. (c) Barton, D. H. R.; Budhiraja, R. P.; McGhie, J. F. *J. Chem Soc. C* **1969**, 336–338. (d) Sakamaki, H.; Take, M.; Matsumoto, T.; Iwadare, T.; Ichinohe, Y. *J. Org. Chem.* **1988**, *53*, 2622–2624. (e) Neef, G.; Eckle, E.; Muller-Fahrnow, A *Tetrahedron* **1993**, *49*, 833–840.
(15) (a) Corey, E. J. *J. Am. Chem. Soc.* **1954**, *76*, 175–179. (b) Corey, E. J.; Sneen, R. A. *J. Am. Chem. Soc.* **1956**, *78*, 6269–6278.
(16) Barton, D. H. R.; O'Brian, R. E.; Sternhell, S. *J. Chem. Soc.* **1962**, 470–476.
(17) Han, X. J.; Stoltz, B. M.; Corey, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 7600–7605.

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