Natural Products

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Stereodivergent Synthesis of 17-α and 17-β-Aryl Steroids: Application and Biological Evaluation of D-Ring Cortistatin Analogues**

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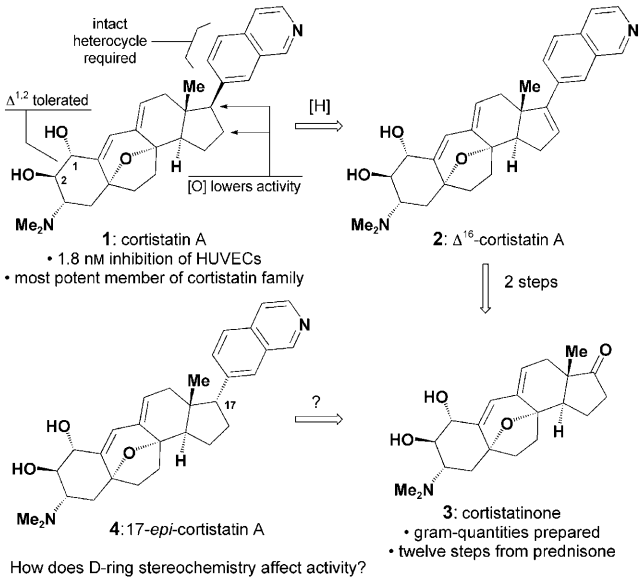
Natural products have always been a successful pool of molecules from which the pharmaceutical industry can find novel medicinal agents.[1] Steroids, in particular, continue to be the subject of medicinal investigations for two reasons: they are "privileged"[2] pharmacophores and their scrutiny for over half a century has resulted in a vast body of knowledge regarding their reactivity.[3] Recently, Kobayashi and co-workers elucidated the structures of cortistatins A–L (see Scheme 1 for the structure of cortistatin A (**1**)) from the sponge *Corticium simplex*.[4] These marine derived steroidal alkaloids exhibit potent anti-angiogenic activity against HUVECs (human umbilical vein endothelial cells) by a possibly unique mechanism.[4] While the Kobayashi research group has already delineated a preliminary structure–activity relationship (SAR) of the family (see Scheme 1), their scarce natural supply renders chemical synthesis as the only means to decipher their medicinal potential. Particularly intriguing is the impact of the isoquinoline moiety on biological activity since its absence significantly lowers activity. Here, we illustrate the critical influence of D-ring configuration on biological activity with the synthesis of 17-*epi*-cortistatin A (**4**). Specifically, we have found that the C-17 stereochemistry may be removed all together as Δ^{16}-cortistatin A (**2**) retains much of the potency of **1**. This line of chemical inquiry has also led to the first useful method for the stereocontrolled preparation of other α-aryl-substituted D-ring steroids.



Scheme 1. Cortistatin A (**1**), its known structure–activity relationship,[4a,d] and relationship to its 17-*epi* relative (**4**).

Several approaches have been reported for the preparation of the core structure of cortistatins[5] and two elegant total syntheses of the most potent member of this natural product class, cortistatin A (**1**), have appeared from the Nicolaou/Chen[6a] and Shair research groups.[6b]

Our own synthetic plan[7] was profoundly affected by the strategic choice to use prednisone—a synthetic corticosteroid produced annually on multi-ton scale by microbial oxidations of naturally occurring steroids—as a starting material. Aside from economical considerations, this choice was made with the knowledge that semi-synthetic approaches have enjoyed decades of success in the pharmaceutical industry. Thus, a twelve-step sequence was utilized from this starting point to arrive at cortistatinone (**3**) in gram quantities.[7] The synthesis concluded with a highly chemo- and stereoselective Raney nickel (Ra-Ni) mediated hydrogenation of Δ^{16}-cortistatin A (**2**). In order to evaluate the importance of a β-oriented isoquinoline moiety, an estrone-derived model (**5**, Scheme 2) was employed as a testbed for a strategy that would deliver both epimers from a common intermediate.

By analogy to the synthesis of **2**, estrone model **5** was converted to the D-ring styrene **6a** as depicted in Scheme 2. Regardless of the reducing conditions, the only observed product was the expected β-aryl substituted product **7a**. Ra-Ni mediated reduction led to a 97% yield of isolated **7a**. This is not surprising given the fact that an overwhelming majority of nucleophilic, electrophilic, and radical substitution reactions at C-17 occur from the α-face.[8] Attention was therefore turned to an alternative approach that began with tertiary alcohol **8a**, derived from addition of PhLi to **5**. Based on preliminary evidence[9] and a report that Ra-Ni reductions of benzylic alcohols occurred with retention of configuration,[10]

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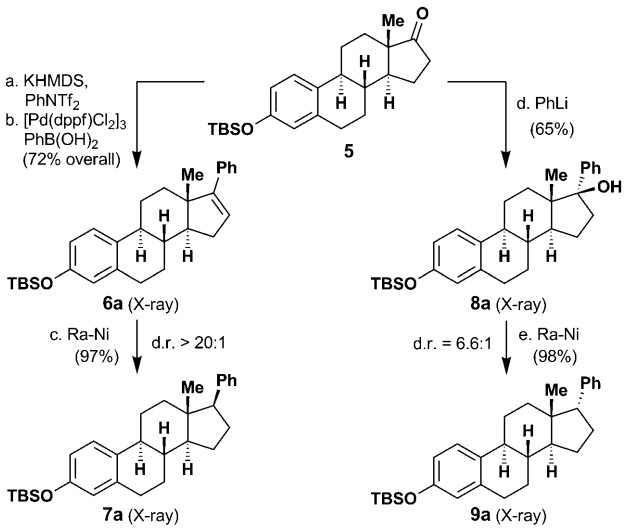


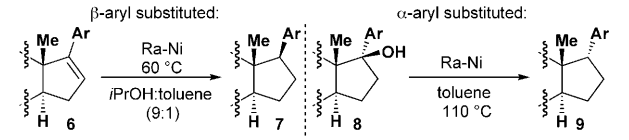
Scheme 2. Divergent access to α- and β- configured C-17 aryl estrone derivatives.

alcohol **8a** was subjected to Ra-Ni in toluene at reflux. A diastereomeric pair of compounds was isolated in a 6.6:1 ratio, the major isomer of which bore the desired α-configuration. The structures of **6a**–**9a** were all verified by X-ray crystallography.[12]

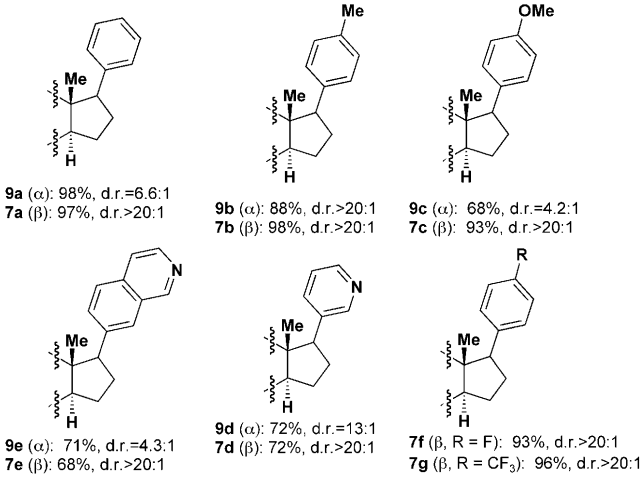
The generality of this reagent system, a synthesis of 17-*epi*-cortistatin A, mechanistic analysis of these reductive processes, and biological evaluation of cortistatin analogues are presented below.

As shown in Table 1, both pathways (**6** to **7** and **8** to **9**) are amenable to the incorporation of electron rich, neutral, and withdrawn arenes, as demonstrated by the successful deoxygenations and hydrogenations of C-17 phenyl, *p*-tolyl, *p*-anisyl, and 3-pyridyl steroids. Hydrogenation of Δ^{16}-17-arylsteroids **6** was carried out in 10% toluene in isopropyl alcohol with Ra-Ni at 60 °C for 2 h, providing **7** with yields varying between 68% and 98%. The diastereoselectivity of this transformation is generally over 20:1 (17-β/17-α). Alternatively, deoxygenation of 17-β-hydroxy-17-α-arylsteroids **8** was carried out in toluene with Ra-Ni at 110 °C for 5 h. Yields varied between 68% and 98% with moderate to good diastereoselectivities.

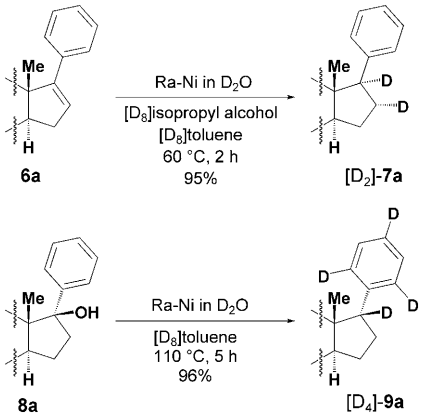
Table 1: Deoxygenation and hydrogenation mediated by Ra-Ni.



Yields and diastereoselectivities



To determine the source of hydrogen in the reductions, deuterium labelling experiments were carried out as shown in Scheme 3. For Ra-Ni mediated transfer hydrogenation, the

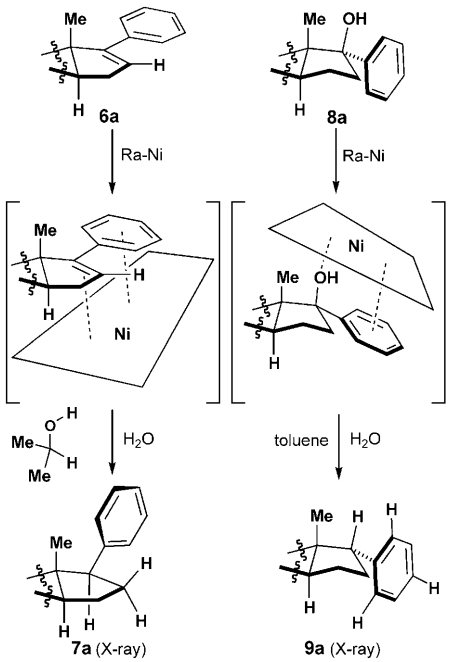


Scheme 3. Deuterium labelling of hydrogenation and deoxygenation.

reaction of **6a** was conducted in deuterated isopropyl alcohol and toluene (toluene or $[D_8]$toluene gave identical results) with D_2O-washed Ra-Ni, affording $[D_2]$-**7a** with deuterium incorporation at C-16 and C-17. For Ra-Ni mediated deoxygenation, the reaction of **8a** employed deuterated toluene with D_2O-washed Ra-Ni. Surprisingly, $[D_4]$-**9a** was obtained as the major product in 96% yield. However, **7a** and **9a** exhibited identical aromatic deuterium substitution when subjected to the same reduction conditions as **8a**, demonstrating that this aromatic deuteration is independent of the deoxygenation process.

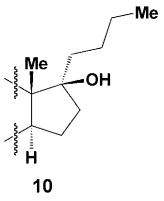
The divergence in observed stereochemical outcome between **7** and **9** seemingly excludes the intermediacy of free-radicals in deoxygenation (**8**→**9**; radical deoxygenation produces β-configuration at C-17). The difference in stereoselectivity between **6** and **8** can be rationalized based on the facial selectivity of chemo-adsorption to the metal surface as depicted in Scheme 4. Previous studies[11] have demonstrated that a high degree of stereoselectivity can be incurred in Ra-Ni mediated reductions based on stereoselective adsorption.

In the case of **6**, adsorption likely occurs most favorably on the relatively flat α-face, away from the angular methyl group (C-18). Hydrogens and/or electrons are then transfered from the metal surface to the preferentially adsorbed face.[11] In the case of **8**, adsorption possibly takes place on the convex face, with interaction occuring between the surface and both the aromatic π-system and the benzylic hydroxy, followed by hydrogen/deuterium delivery from the metal.



Scheme 4. Possible explanation for observed stereochemical divergence.

Finally, the mechanistic requirement of an aryl group at C-17 during deoxygenation is supported by the fact that 17-β-hydroxy-17-α-(*n*-butyl)-estrone (**10**) was inert to deoxygenation using Ra-Ni. In addition to submitting **10** to the reaction condition, a control experiment was carried out with an equimolar mixture of **10** and **8a** premixed in the same reaction vessel and treated with Ra-Ni. While **8a** was completely deoxygenated, **10** was quantitatively recovered.



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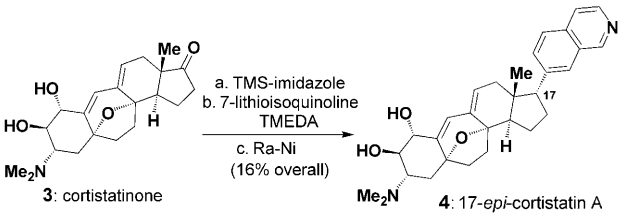
The utility of the present invention is aptly demonstrated by the synthesis of 17-*epi*-cortistatin (**4**), as shown in Scheme 5. Thus, protection of the diol motif in cortistatinone (**3**) with TMS-imidazole, followed by treatment with an excess of 7-lithioisoquinoline in a THF/TMEDA solvent mixture at −78 °C generated an alkanolisoquinoline that was deoxygenated with Ra-Ni to deliver 17-*epi*-cortistatin A (**4**) in 16% yield over three steps.

This substance proved crucial in testing the substrate scope/specificity of cortistatin A's biological target. The importance of this substrate is clear since the greatest modulation of biological activity in the naturally occurring cortistatins stems from structure variations of the C-17 substituent.[4]



Scheme 5. Synthesis of 17-*epi*-cortistatin A.

In an assay to determine activity against HUVECs (carried out by Pfizer Inc.), synthetic cortistatin A exhibited an IC_{50} value of 2.43 nM, which is in good agreement with the reported value (Table 2).[4a] Remarkably, **2** still retains high potency against HUVECs, with an IC_{50} of 3.88 nM. This result is a significant step forward in the simplification of the overall cortistatin structure from a synthesis standpoint. However, **4** does not exhibit useful levels of activity (>1 μM). This profound difference of biological activity clearly indicates that the C-17 stereochemistry is essential for biological behavior. Modeling studies (shown in Figure 1) suggested that **1**, **2**, and **4** exhibit rigid architectures that differ only in the angle in which the isoquinoline moiety would be presented to the active site. Several estrone model compounds were also found to be inactive in the HUVEC screen.

Table 2: Selective growth inhibition of cortistatins against HUVECs.

Substrate	IC_{50} [nM]
cortistatin A (**1**)	2.43[a], 1.8[b]
Δ^{16}-cortistatin A (**2**)	3.88
17-*epi*-cortistatin A (**4**)	>1000
6d–g, **7a–f**, **8e**, **9a**, **9d–e**[c]	>1000

[a] IC_{50} of synthetic cortistatin A tested by Pfizer Inc. [b] IC_{50} of natural cortistatin A tested by Kobayashi group.[4a] [c] The TBS groups were removed prior to testing. The results of **6e** and **7e** are from Ref. [4f].

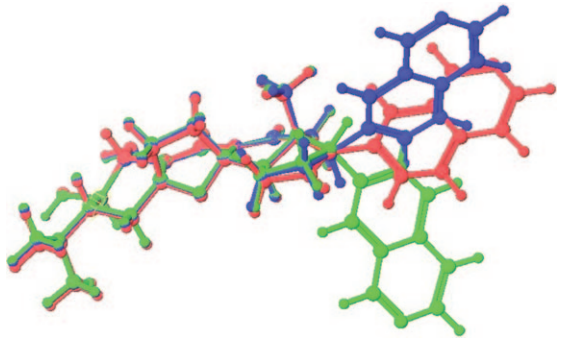


Figure 1. Superimposed structures of the lowest energy conformation of **1** (blue), **2** (red), and **4** (green) by Schrödinger software dihedral drive macromodel.

In summation, two processes were identified for the production of either α- or β-oriented C-17 aryl steroids, conveniently using the C-17 ketone as a common starting material. Because of this divergence, it is now possible to efficiently produce both epimers of C-17 aryl steroids, thus adding the α epimers to the collection of non-natural biomolecules available for biological and other studies. The relevance and utility of such a transformation has been demonstrated by the synthesis of **1**, its epimer **2**, and biological evaluations thereof.[12] Finally, the compelling finding that **2** retains much of the potency of **1** should considerably simplify SAR studies in this family.

Experimental Section

General procedure for the standardization of Raney nickel (Ra-Ni): Raney 2800 Nickel (ca. 1 g of a 1 g mL^{-1} slurry in H_2O, pH 9, Sigma–Aldrich) was placed in a vial. The water was removed by pipette, and the Ra-Ni was washed by 5 s of shaking, followed by removal of the supernatant: first H_2O (2 × 2 mL), then saturated aqueous Rochelle's salt (2 × 2 mL), then H_2O (10 × 2 mL). After all washes, the Ra-Ni aqueous solution (pH 7) was stored under H_2O (1 mL).

General procedure for hydrogenation: To a solution of styrene in *i*PrOH/toluene (9:1, 0.01M) was added the suspension of Ra-Ni prepared above (the Ra-Ni suspension was removed by 5.75″ pipette from the thick bottom layer of the vial; one drop suspension per 0.1 mL solution). The reaction flask was immersed in an oil bath preheated to 60 °C and stirred vigorously for 120 min. After cooling to ambient temperature, the reaction mixture was passed through Celite, the Ra-Ni washed with CH_2Cl_2, and the combined filtrates were concentrated in vacuo. The product was purified by flash column chromatography.

General procedure for deoxygenation: To a solution of alcohol in toluene (0.01M) was added the suspension of Ra-Ni prepared above (the Ra-Ni suspension was removed by 5.75″ pipette from the thick bottom layer of the vial; one drop suspension per 0.1 mL solution). The reaction flask was immersed in an oil bath preheated to 110 °C and stirred vigorously for 5 h. After cooling to ambient temperature, the reaction mixture was passed through Celite, the Ra-Ni washed with CH_2Cl_2, and the combined filtrates were concentrated in vacuo. The product was purified by flash column chromatography.

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[1] D. D. Baker, M. Chu, U. Oza, V. Rajgarhia, *Nat. Prod. Rep.* **2007**, *24*, 1225 – 1244.
[2] *Burger's Medicinal Chemistry and Drug Discovery*, *Vol. 5* (Ed.: M. E. Wolff), Wiley, New York, **1997**, pp. 281 – 376.
[3] J.-F. Biellmann, *Chem. Rev.* **2003**, *103*, 2019 – 2033.
[4] a) S. Aoki, Y. Watanabe, M. Sanagawa, A. Setiawan, N. Kotoku, M. Kobayashi, *J. Am. Chem. Soc.* **2006**, *128*, 3148 – 3149; b) Y. Watanabe, S. Aoki, D. Tanabe, A. Setiawan, M. Kobayashi, *Tetrahedron* **2007**, *63*, 4074 – 4079; c) S. Aoki, Y. Watanabe, D. Tanabe, A. Setiawan, M. Arai, M. Kobayashi, *Tetrahedron Lett.* **2007**, *48*, 4485 – 4488; d) S. Aoki, Y. Watanabe, D. Tanabe, M. Arai, H. Suna, K. Miyamoto, H. Tsujibo, K. Tsujikawa, H. Yamamoto, M. Kobayashi, *Bioorg. Med. Chem.* **2007**, *15*, 6758 – 6762; e) Y. Sato, H. Kamiyama, T. Usui, T. Saito, H. Osada, S. Kuwahara, H. Kiyota, *Biosci. Biotechnol. Biochem.* **2008**, *72*, 2992 – 2997.
[5] a) M. Dai, S. J. Danishefsky, *Heterocycles* **2009**, *77*, 157 – 161; b) E. M. Simmons, A. R. Hardin, X. Guo, R. Sarpong, *Angew. Chem.* **2008**, *120*, 6752 – 6755; *Angew. Chem. Int. Ed.* **2008**, *47*, 6650 – 6653; c) S. Yamashita, K. Iso, M. Hirama, *Org. Lett.* **2008**, *10*, 3413 – 3415; d) D. T. Craft, B. W. Gung, *Tetrahedron Lett.* **2008**, *49*, 5931 – 5934; e) M. Dai, S. J. Danishefsky, *Tetrahedron Lett.* **2008**, *49*, 6610 – 6612; f) M. Dai, Z. Wang, S. J. Danishefsky, *Tetrahedron Lett.* **2008**, *49*, 6613 – 6616; g) N. Kotoku, Y. Sumii, T. Hayashi, M. Kobayashi, *Tetrahedron Lett.* **2008**, *49*, 7078 – 7081; h) L. Kürti, B. Czakó, E. J. Corey, *Org. Lett.* **2008**, *10*, 5247 – 5250; i) S. Yamashita, K. Kitajima, K. Iso, M. Hirama, *Tetrahedron Lett.* DOI: 10.10167j.tetlet.2009.02.038; j) B. W. Gung, D. T. Craft, *Tetrahedron Lett.* **2009**, *50*, 2685 – 2687; k) L. Z. Liu, Y. X. Gao, C. Che, N. Wu, D. Z. G. Wang, C. C. Li, Z. Yang, *Chem. Commun.* **2009**, 662 – 664; l) For a review, see: C. F. Nising, S. Bräse, *Angew. Chem.* **2008**, *120*, 9529 – 9531; *Angew. Chem. Int. Ed.* **2008**, *47*, 9389 – 9391.
[6] a) K. C. Nicolaou, Y. P. Sun, X. S. Peng, D. Polet, D. Y. K. Chen, *Angew. Chem.* **2008**, *120*, 7420 – 7423; *Angew. Chem. Int. Ed.* **2008**, *47*, 7310 – 7313; b) H. M. Lee, C. Nieto-Oberhuber, M. D. Shair, *J. Am. Chem. Soc.* **2008**, *130*, 16864 – 16866.
[7] R. A. Shenvi, C. A. Guerrero, J. Shi, C. C. Li, P. S. Baran, *J. Am. Chem. Soc.* **2008**, *130*, 7241 – 7243.
[8] L. F. Fieser, M. Fieser, *Steroids*, Reinhold, New York, **1959**.
[9] a) C. A. Guerrero, Ph.D. Thesis, The Scripps Research Institute, **2008**; b) R. A. Shenvi, Ph.D. Thesis, The Scripps Research Institute, **2008**.
[10] a) S. Mitsui, S. Imaizumi, *Bull. Chem. Soc. Jpn.* **1961**, *34*, 774 – 778; b) M. E. Krafft, W. J. Crooks III, *J. Org. Chem.* **1988**, *53*, 432 – 434.
[11] S. Mitsui, S. Imaizumi, Y. Esashi, *Bull. Chem. Soc. Jpn.* **1970**, *43*, 2143 – 2152, and references therein.
[12] See Supporting Information for detailed experimental procedures and copies of all spectra. CCDC 721528 (**6a**), 726038 (**7a**), 721529 (**8a**), 721530 (**9a**), and 721527 (**10**) contain the supplementary 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.

