

Scalable and Divergent Total Synthesis of (+)-Colleteoic Acid, a Selective 11 β -Hydroxysteroid Dehydrogenase Type 1 Inhibitor

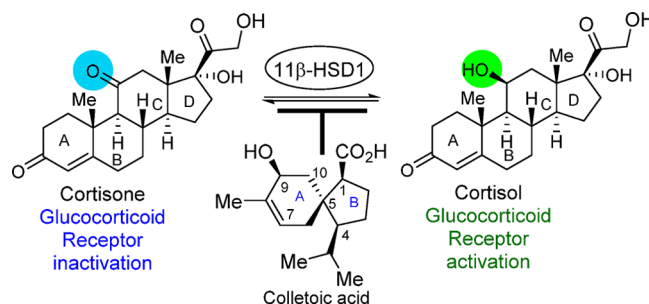
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ABSTRACT



An efficient and divergent total synthesis of (+)-colleteoic acid, a selective 11 β hydroxysteroid dehydrogenase 1 (11 β HSD1) inhibitor, is presented along with its biological activity at the whole-cell level. A scalable, asymmetric synthetic strategy was designed featuring a diversity-oriented synthesis utilizing a diastereoselective intramolecular 5-exo-Heck reaction as the key step to provide the quaternary spirocenter intermediate **9** in multigram scale, thus establishing a platform for further structure–activity relationship studies and providing access to other acorane family members.

Since the validation of the important 11 β -hydroxysteroid type 1 (11 β -HSD1) enzyme as a therapeutic target^{1–3} for metabolic syndrome, interest in the development of small-molecule 11 β -HSD1 inhibitors has grown rapidly. Metabolic syndrome is clinically defined as a cluster of conditions including high blood pressure, high blood sugar level, excess body fat, and abnormal cholesterol level that

occur together to increase the risk of heart disease, stroke, and diabetes.^{1,2} 11 β -HSD1 is a potential target for treatment since it plays a crucial role in regulating intracellular cortisol levels, serving as a molecular switch in cooperation with cofactor NADP(H). 11 β -HSD1 is an ER-localized transmembrane protein that catalyzes conversion of inactive cortisone to cortisol, in turn activating the glucocorticoid receptor and triggering a cascade of cellular events.³ Although primarily a reductase, 11 β -HSD1 can also function as an oxidase in a tissue-dependent manner.^{2,15} Various steroidal natural products inhibit 11 β -HSD1, but with poor discrimination between its two isoforms.³ The natural product colleteoic acid was recently identified as a potent inhibitor of 11 β -HSD1 and selective over its isoform 11 β -HSD2.⁴ Colleteoic acid (**1**) was isolated from the fungus *Colletotrichum gloeosporioides* and is a member of the acorane sesquiterpenoid family, possessing

(1) Ge, R.; Huang, Y.; Liang, G.; Li, X. *Curr. Med. Chem.* **2010**, *17*, 412–422. Wamil, M.; Seckl, J. R. *Drug Discovery Today* **2007**, *12*, 505–520. Zhang, J.; Osslund, T. D.; Plant, M. H.; Clogston, C. L.; Nybo, R. E.; Xiong, F.; Delaney, J. M.; Jordan, S. R. *Biochemistry* **2005**, *44*, 6948–6957.

(2) (a) Scott, J. S.; Bowker, S. S.; deSchoolmeester, J.; Gerhardt, S.; Hargreaves, D.; Kilgour, E.; Lloyd, A.; Mayers, R. M.; McCoull, W.; Newcombe, N. J.; Ogg, D.; Packer, M. J.; Rees, A.; Revell, J.; Schofield, P.; Selmi, N.; Swales, J. G.; Whittamore, P. R. O. *J. Med. Chem.* **2012**, *55*, 5951–5964. (b) Feig, P. U.; Shah, S.; Hermanowski-Vosatka, A.; Plotkin, D.; Springer, M. S.; Donahue, S.; Thach, C.; Klein, E. J.; Lai, E.; Kaufman, K. D. *Diabetes, Obes. Metab.* **2011**, *13*, 498–504. (c) Hughes, K. A.; Manolopoulos, K. N.; Iqbal, J.; Cruden, N. L.; Stimson, R. H.; Reynolds, R. M.; Newby, D. E.; Andrew, R.; Karpe, F.; Walker, B. R. *Diabetes* **2012**, *61*, 1357–1364.

(3) Su, X.; Vicker, N.; Potter, B. V. L. *Prog. Med. Chem.* **2008**, *46*, 29–130.

(4) Aoyagi, A.; Ito-Kobayashi, M.; Ono, Y.; Furukawa, Y.; Takahashi, M.; Muramatsu, Y.; Umetani, M.; Takatsu, T. *J. Antibiot.* **2008**, *61*, 136–141.

a bicyclic framework (Figure 1). It contains an all-carbon spirocenter and three contiguous stereocenters, and the B ring features syn substituent groups.

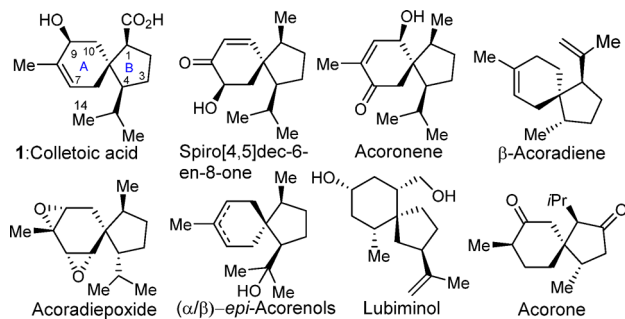


Figure 1. Selected acorane family members.

Not surprisingly, the molecular complexity of colloletoic acid within a deceptively simple carbon framework, and the unique structural territory it offers, have attracted the attention of the synthetic organic chemistry community including a recently reported total synthesis based on a Diels–Alder approach.^{5,6} The synthetic challenge and the possibility of developing therapeutic agents for metabolic syndrome based on this chemotype led us to investigate diversity-oriented routes for its synthesis to evaluate it as a potential 11 β -HSD1 modulator. Colloletoic acid's most distinctive feature is its all-carbon spirocenter, which is difficult to install with stereospecific control due to inherent steric hindrance and electronic factors surrounding the system.^{5b} In many cases, a neighboring functional group (such as a ketone) is required to initiate formation of a spiro-center. Additional challenges, such as enantioselectivity and stereoselectivity must also be considered. Although significant efforts have been devoted to the syntheses of acorane family members, reported methods include alkylation, intramolecular rearrangements and Diels–Alder reaction,^{5,6} no general strategy has been proposed yet. This work describes a diversity-oriented total synthesis of (+)-colloletoic acid, based on a metal-mediated reaction forming a spiro-center and featuring a highly diastereoselective intramolecular cyclic alkene vinylation, providing multigram quantities of the core intermediate **9**, and late-stage functionalization to establish the required chemistry for SAR evaluation. To the best of our knowledge, here we report the first example of the utility of an intramolecular diastereoselective Heck reaction to form the all-carbon spiro-center as the key transformation to the total synthesis of an acorane natural product. Although, Negishi has conducted Heck reactivity studies on a similar system, no diastereoselective control was achieved in respect to the

spirocenter formation.⁷ We have developed a potential unified strategy for the synthesis of the acorane family members.

The retrosynthetic analysis of colloletoic acid features alkylation at C1 to introduce the vinyl iodide moiety, which subsequently forms the spiro-center through a Heck reaction. Our preliminary findings showed that olefin insertion is directed by steric factors from substituents on C1, C4, and C8; thus, palladium-mediated carbon–carbon formation reaction would directly establish the relative stereochemistry between C1 and C5 functional groups (Figure 2). We hypothesized that after the spiro-center was installed, chemoselective epoxidation on the A ring, followed by substrate-directed hydrogenation of the C3–C4 double bond and late-stage functionalization of the A ring, would yield the total synthesis of (+)-colloletoic acid. Our retrosynthetic analysis of colloletoic acid (**1**) took advantage of robust chemical reactions, which allow considerable flexibility in assembling a medicinal chemistry platform.

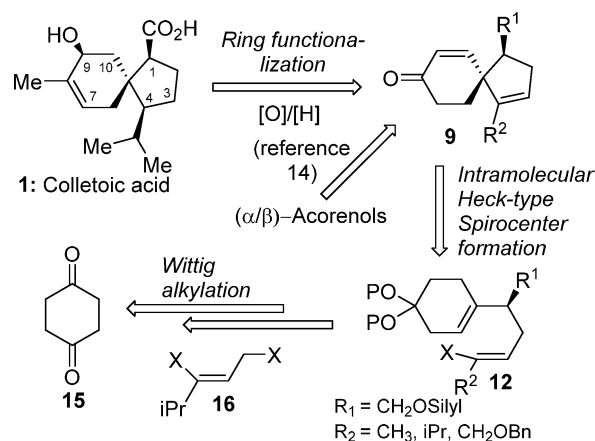


Figure 2. Retrosynthetic analysis of colloletoic acid.

The main building blocks used for this strategy (**12**, **16**) and the key intermediate **9** were produced on a 100 g scale (Figure 2). Our synthesis commenced with Wittig mono-olefination using the stabilized ylide of commercially available cyclohexane-1,4-dione **15**, followed by catalytic ketone protection with pyridinium *p*-toluenesulfonate and 2,2-dimethylpropane 1,3-diol under refluxing conditions and deconjugation by treatment with DBU (see the Supporting Information). Hydrolysis of the ester, followed by coupling with (1*R*,2*R*)-(–)-pseudoephedrine⁸ afforded compound **13** in excellent yield. Treatment of compound **13** with LDA in the presence of stabilizing LiCl salt followed by addition of electrophile **16** afforded the Heck precursor in gram quantities (Scheme 1) and excellent enantiomeric excess (ee \geq 98%). Electrophile **16** was synthesized from ethyl 4-methyl-3-oxopentanoate, which was converted to vinyl iodide after triflate and base

(5) (a) Kotha, S.; Deb, A. C.; Lahiri, K.; Ethirajan, M. *Synthesis* **2009**, 165–193 and references therein. (b) Abelman, M. M.; Overman, L. E.; Tran, V. D. *J. Am. Chem. Soc.* **1990**, *112*, 6959.

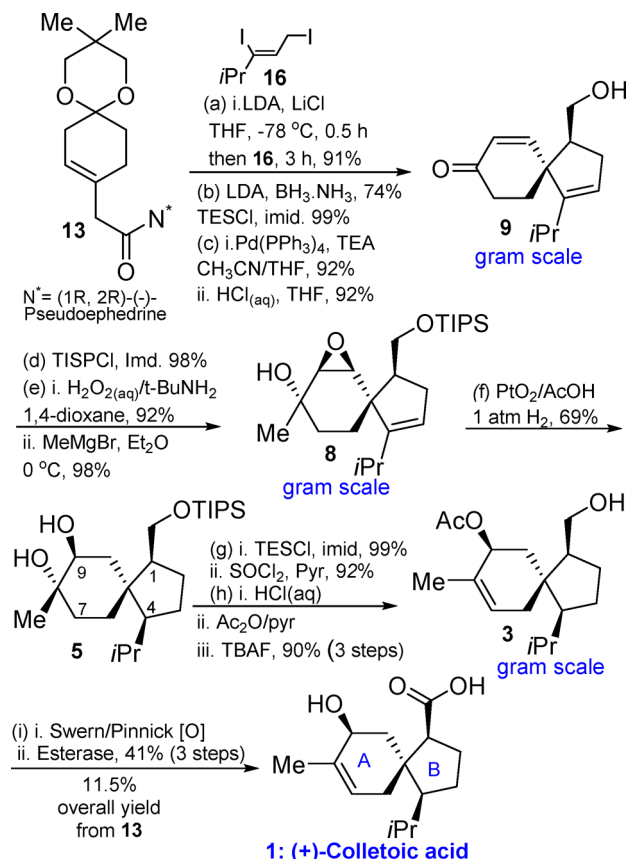
(6) Sawada, T.; Nakada, M. *Org. Lett.* **2013**, *15*, 1004–1007 and references therein.

(7) Negishi, E.; Zhang, Y.; O'Connor, B. *Tetrahedron Lett.* **1988**, 2915–2918.

(8) Myers, A. G.; Yang, B. H.; Chen, H.; McKinsty, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc.* **1997**, *119*, 6496–6511.

treatment, followed by iodine in DMF under refluxing conditions in a one-pot procedure without purification.

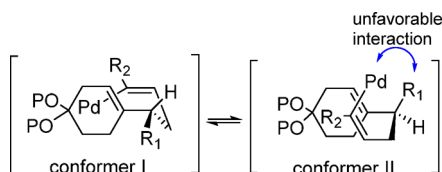
Scheme 1. Total Synthesis of (+)-Colleteic Acid



The corresponding vinyl ester was reduced with DIBAL-H to the primary alcohol, which was displaced by iodide under zirconium chloride catalysis to produce **16** in 20–60g batches (see the Supporting Information) with a 63% overall yield. Several catalysts and solvents were evaluated for the Heck reaction, and our results indicated that the pseudoephedrine chiral auxiliary should be removed before the cyclization reaction to prevent steric hindrance. Once free, the primary hydroxyl group was protected with a silyl group to facilitate the desired spirocenter formation. The Heck reaction was mediated by palladium(0) in acetonitrile/THF at 60 °C, and gratifyingly, a single diastereoisomer compound **9** was isolated after acid treatment to remove all acid-sensitive protecting groups in excellent yields. The palladium 5-exo spirocyclization is presumably going through an envelope conformer I to avoid unfavorable interactions between the

incoming bulky palladium complex with the R¹ group.⁹ The relative stereochemistry of compound **9** was confirmed by 2D NMR and X-ray crystallography of late-stage intermediates. The α -hydroxylation of the ester form of compound **9** was synthesized by Pb(OAc)₄ treatment in refluxing benzene to produce crystalline compound **18** as a single stereoisomer, in 68% yield, which confirmed the stereochemistry through X-ray analysis (Scheme 2). The α,β -unsaturated system **9** was produced almost quantitatively, and the sequence was streamlined to ensure gram quantities of this core material for future analog development. Chemoselective epoxidation under aqueous H₂O₂ and *t*-BuNH₂ provided **8** after treatment with methyl magnesium bromide in excellent yields.¹⁰ The C3–C4 olefin reduction was performed before regioselective opening of the epoxide to avoid mixtures of epimers at C4. After extensive experimentation, we found that one-pot sequential hydrogenation/regioselective reductive opening of epoxide **8**, promoted by Adam's catalyst (PtO₂) in acetic acid,¹¹ produced compound **5** in good yields in multigram scale, therefore establishing the required C4 and C9 stereocenters simultaneously. Alternatively, it was observed that transient protection of C8 tertiary hydroxyl group with TMS followed by DIBAL-H reductive epoxide opening can afford the desired free triol (compound **5B**, see the Supporting Information). Compound **5** was then treated with TESCl followed by elimination reaction¹² promoted by thionyl chloride and protecting group exchange afforded compound **3** in good yields after TBAF treatment in gram scale. This advanced intermediate smoothly underwent two-step oxidation to the corresponding acid and was treated with esterase to remove the acetate group, providing the synthetic natural product in 11.5% overall yield. The hydrolysis of the ester was carried out under enzymatic conditions because the reaction is mild and efficient. The spectroscopic and optic properties of the synthetic material are fully consistent with the reported properties of the natural product. This synthetic strategy also permits to efficiently address the chemical space of rings A and B (Scheme 2). To evaluate the A ring, α -hydroxylation at C7 of the core compound was performed, affording compound **18** as a crystalline substance suitable for X-ray studies. In addition, 1, 3-regioselective epoxide opening with hydrides provided compound **17**, as confirmed by X-ray crystallography.¹³ The stereochemistry of the hydroxyl group at C8 of compound **5** can be reversed by conducting the Grignard addition then allylic epoxidation, followed by our one-pot hydrogenation/regioselective reductive epoxide opening reaction conditions to afford compound **19** in excellent yields. In reference to the B ring, the side chain on C4 can be manipulated prior to

(9) Potential transition states during the Heck reaction



(10) Uyanik, C.; Malay, A.; Ayna, A. S.; Hanson, J. R.; Hitchcock, P. B. *Steroids* **2005**, 71–75.

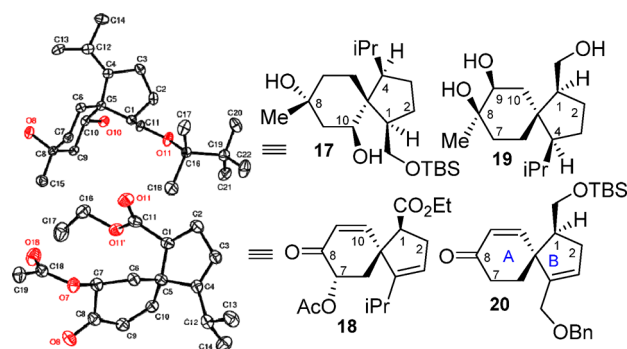
(11) You, Z.; Hoveyda, A. H.; Snapper, M. L. *Angew. Chem. Int. ed.* **2009**, 48, 547–550.

(12) Sha, C.-K.; Huang, S.-J.; Zhan, Z.-P. *J. Org. Chem.* **2002**, 67, 831–836.

(13) X-ray data were deposited at the Cambridge Crystallographic Data Centre (CCDC) and are available under access numbers CCDC 892448 (compound **17**) and CCDC 892449 (compound **18**).

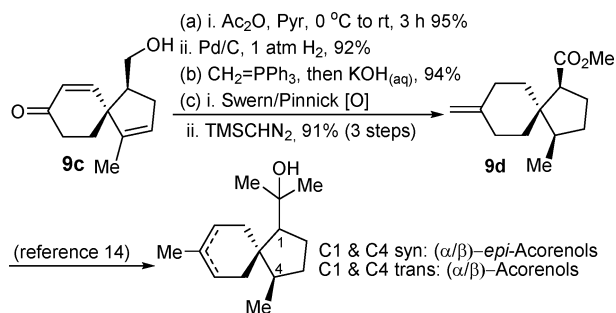
the Heck reaction to afford compound **20** (Scheme 2) and C2 can be hydroxylated prior to ring formation to afford compound **9e** (see the Supporting Information). Therefore, the oxidative stage and substituents on the A ring (C7–C10) and B ring (C1–C4) can be efficiently evaluated as shown (Scheme 2), providing the optimal platform for further SAR studies and a unified synthetic strategy for other acorane members.

Scheme 2. X-rays of Late-Stage Intermediates



To demonstrate the versatility of our synthetic strategy, compound **9c** was converted to its corresponding methyl ester compound **9d**, therefore completing a formal total synthesis of α/β acorenol and α/β *epi*-acorenol natural products (Scheme 3).¹⁴

Scheme 3. Formal Synthesis of Acorenols



The biological activity of colletoic acid was next evaluated utilizing a fluorescence-based assay based on the rate of formation of NADPH (cortisol to cortisone) using a human 11 β -HSD1 mutant model (Figure 3) known to have a net 2-fold decrease in the efficiency ($K_{\text{cat}}/K_{\text{m}}$) of cortisol oxidation.¹⁵ Colletoic acid's inhibition was modest with a higher K_i ($K_i = 0.62 \pm 0.04 \mu\text{M}$, Figure 3B) than the

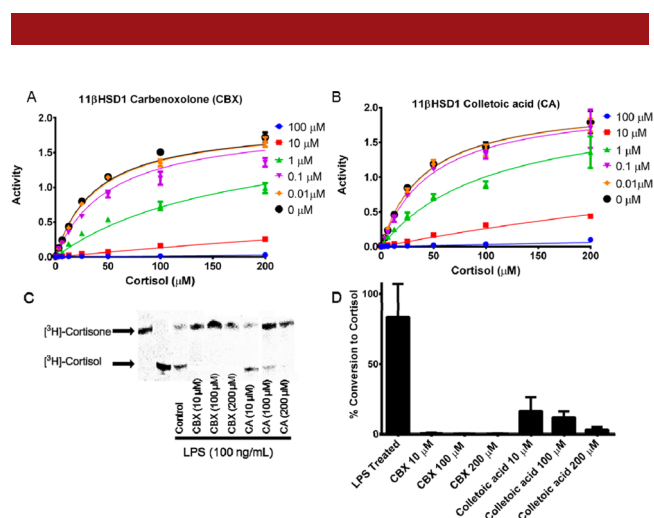


Figure 3. Biochemical and whole-cell evaluation of (+)-colletoic acid (see the Supporting Information for details).

originally reported in a microsomal assay.⁴ However, the positive control, the known unselective inhibitor carbenoxolone (CBX), also showed a similar inhibition pattern ($K_i = 0.29 \pm 0.02 \mu\text{M}$, Figure 3A) to colletoic acid in this assay. After the biochemical activity of colletoic acid was confirmed, its biological activity was assessed at the whole-cell level. Utilizing lipopolysaccharide (LPS)-activated J774.1 macrophage mouse cell line¹⁶ intracellular inhibition of 11 β -HSD1 by colletoic acid was observed at $200 \pm 0.12 \mu\text{M}$ (TLC and quantification shown in Figure 3C,D). Gratifyingly, colletoic acid showed no cytotoxicity at $500 \mu\text{M}$ in J774.1 or other mammalian cells ($> 200 \mu\text{M}$).

In conclusion, natural products remain at the forefront of drug discovery, offering new potential scaffolds such as the one described here. A diversity-oriented total synthesis of (+)-colletoic acid has been described and its biological activity confirmed at the cellular level. Our synthetic strategy establishes the platform for a medicinal chemistry program using the acorane scaffold, which has efficient scalability to support SAR studies and in vivo animal studies, which will be reported in due course.

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Supporting Information Available. Experimental procedures, spectroscopic data, and proof of composition for compounds **1–30**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.