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Enantiomeric Deoxycholic Acid: Total Synthesis, Characterization, and Preliminary Toxicity toward Colon Cancer Cell Lines

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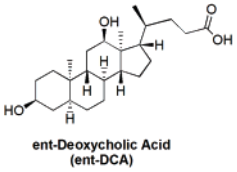

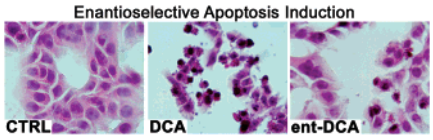

Deoxycholic acid (DCA) is an endogenous secondary bile acid implicated in numerous pathological conditions including colon cancer formation and progression and cholestatic liver disease. DCA involvement in these disease processes results partly from its ability to modulate signaling cascades within the cell, presumably through both direct receptor activation and general detergent mediated membrane changes. To further explore DCA induced changes in cell signaling, we completed a total synthesis of enantiomeric deoxycholic acid (*ent*-DCA) from achiral 2-methyl-1,3-cyclopentanedione. Using a modified method of the synthesis of *ent*-testosterone that proceeds through the (*R*)-(−)-Hajos−Parrish ketone, we have completed the successful synthesis of *ent*-DCA in 25 steps with a yield of 0.3% with all stereochemical assignments of the product confirmed by X-ray crystallography. Our studies toward this synthesis also uncovered the methodology for the development of a novel A,B-cis steroidal skeleton system containing a C3−C9 single bond as well as conditions to selectively ketalize the typically less reactive 12-carbonyl in poly-keto A,B-cis androgens. The critical micelle concentration (cmc) of *ent*-DCA, determined by a dye solubilization method, was identical to the cmc of natural DCA. Toxicity studies toward HT-29 and HCT-116 human colon cancer cell lines demonstrated that *ent*-DCA had similar effects on proliferation, yet showed a markedly decreased ability to induce apoptosis as compared to natural DCA.

Introduction

Bile acids are steroid detergents that are necessary for the proper absorption of fats and fat soluble vitamins within the gastrointestinal tract. Primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized from cholesterol by hepatocytes and can be 7α-dehydroxylated by enteric bacteria to yield the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. Hydrophobic bile acids such as DCA, CDCA, and LCA are thought to contribute to the development and/or pathogenesis of colon cancer, cholestatic liver disease, and bile acid diarrhea.[1−3] Furthermore, these bile acids are known to cause toxicity and apoptosis in many cell types, including colon cancer cells.[4−12] The mechanisms by which the hydrophobic bile acids contribute

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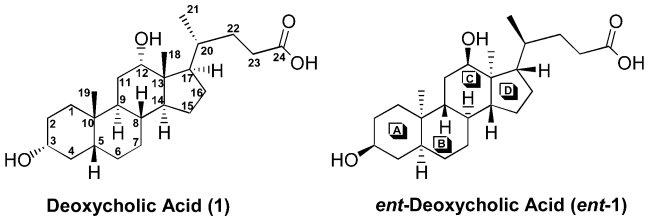
CHART 1. Natural and Enantiomeric Deoxycholic Acid[a]



[a] Numbering of the steroid ring system is indicated on deoxycholic acid, and steroid ring assignment letters are illustrated on *ent*-deoxycholic acid.

to these pathologic conditions and cellular effects are still being investigated; however, they are thought to be partly due to the ability of bile acids to modulate cellular signaling cascades.[1−3] DCA has been shown to be an effective modulator of many signaling pathways including ERK and AKT,[13] COX-2,[14] PKC,[15−17] and EGFR.[18]

Modulation of cell signaling by DCA could result from the specific interaction of this bile acid with a receptor or from general membrane disturbances caused by its detergent properties. One method to probe the mechanism of DCA induced changes in cell signaling is to use the enantiomer of DCA (*ent*-DCA, Chart 1). Two enantiomeric bile acids have already been synthesized by our group, enantiomeric lithocholic acid (*ent*-LCA) and enantiomeric chenodeoxycholic acid (*ent*-CDCA).[19] These *ent*-bile acids were shown to have the same critical micelle concentrations (cmcs) as their natural counterparts, and they were also shown to interact enantioselectively with the FXR, VDR, and PXR nuclear receptors as well as the G-protein coupled receptor TGR5. Thus, enantiomeric bile acids are useful for distinguishing between receptor and nonreceptor mediated effects of natural steroids.

Structurally, DCA differs significantly from LCA and CDCA in that it contains a 12α-hydroxyl group, and to our knowledge, there is no literature precedent in the natural or enantiomeric steroid series addressing the installation of a 12α-hydroxyl group on to the steroid nucleus followed by construction of the bile acid side chain. Therefore, here we report the first total synthesis of enantiomeric deoxycholic acid from achiral 2-methyl-1,3-cyclopentanedione as well as novel steroid methodologies for the creation of a C3−C9 single bond and selective protection of a 12-carbonyl in poly-keto A,B-cis androgen steroid systems. Once synthesized, *ent*-DCA was physically characterized including determination of the cmc, and all properties, except for optical rotation, were identical to the natural steroid. Furthermore, preliminary toxicity studies focusing on proliferation and apoptosis were performed on the colon cancer cell lines HT-29 and HCT-116. These studies provide the first report of cellular toxicity by an enantiomeric bile acid and offer insight into the primary mechanisms responsible for bile acid toxicity.

Results and Discussion

The B, C, and D rings of the *ent*-steroid were constructed using methodology developed for the synthesis of *ent*-testosterone.[20,21] Thus, 2-methyl-1,3-cyclopentanedione was converted into enone **2** in 11 steps with a 22% yield (Scheme 1). Sodium hydride deprotonation of compound **2** led to dienolate formation, which when quenched with methyl iodide gave nonconjugated ketone **3** with migration of the double bond to the Δ^{9-11} position.[22] Cyclization of compound **3** through an intramolecular aldol condensation and removal of the *t*-butyl ether protecting group under acidic conditions led to the formation of *ent*-steroid **4**. Because of its sterically hindered location, the Δ^{9-11} double bond is difficult to hydrogenate.[23] Therefore, hydrogenation of the Δ^{4-5} olefin could be accomplished selectively, leaving the Δ^{9-11} double bond intact. Under basic conditions, which favors the cis fused A,B ring product, the Δ^{4-5} double bond of dienone **4** was selectively hydrogenated to give the mono-olefin **5**.[24] Allylic oxidation of compound **5** to install oxygen functionality at the 12 position proved to be difficult, and of the reagents tested, chromium (VI) oxide proved to be the most successful. This allylic oxidation along with subsequent oxidation of the 17-hydroxyl group gave the diketo enone **6** in a low yield.[25] Higher temperatures and longer reaction times led to increased formation of side products. Therefore, the reaction was stopped before all material had undergone allylic oxidation, which allowed substantial recovery of dione **7**, a compound that could be used again as a starting material for this allylic oxidation.

Initial attempts to saturate the Δ^{9-11} double bond of compound **6** were attempted using standard Li/NH_3(l) reducing conditions to favor the more energetically stable trans ring fusion between the B and the C rings of the *ent*-steroid (Scheme 2). However, after quenching with NH_4Cl and then oxidizing the crude reaction product to remove minor amounts of secondary alcohols resulting from ketone reduction, an inseparable mixture

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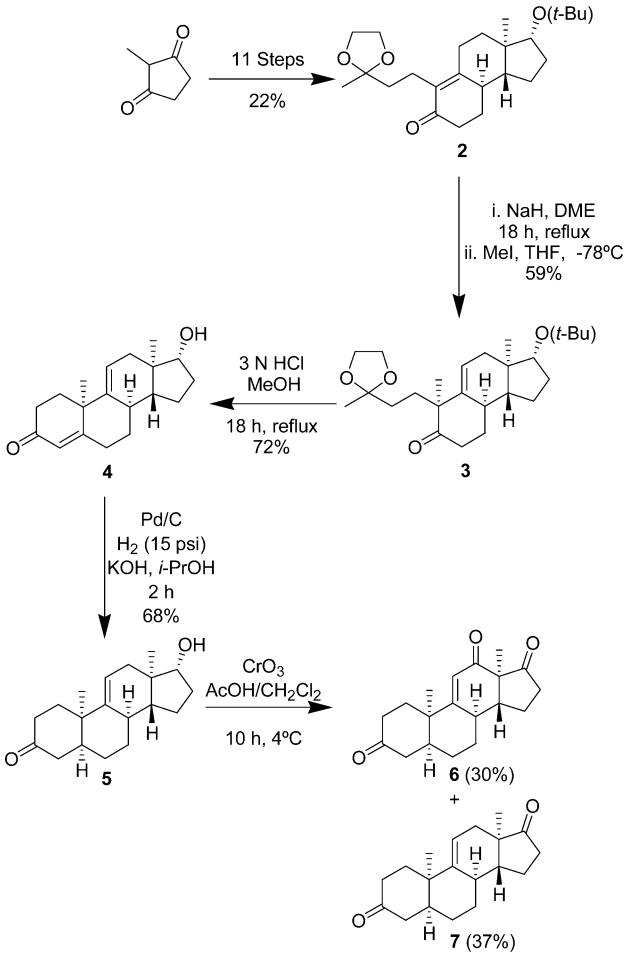
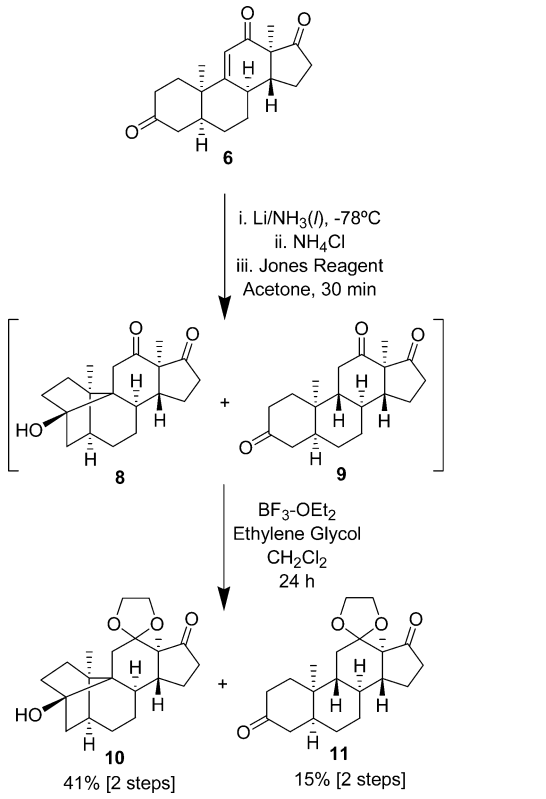
SCHEME 1. Synthesis of 12-Functionalized *ent*-Steroid Ring System



SCHEME 2. Abnormal Δ^{9-11} Reduction and Selective 12-Ketalization



of compounds **8** and **9** remained. The desired product **9**, with only saturation of the Δ^{9-11} double bond, was the minor product of this reduction step, with the major product **8** having a new carbon−carbon bond between C-3 and C-9 as well as a tertiary alcohol on C-3 resistant to Jones oxidation. Following addition of electrons from Li/NH_3(l) to the Δ^{9-11} olefin, formation of this C3−C9 bond resulted from nucleophilic attack of the 3-ketone by electron rich C-9. This created a norbornane system from the A ring of the *ent*-steroid with C-9 acting as the bridging carbon.

The mixture of compounds **8** and **9** could not be separated, but selective ketalization of the 12-carbonyl using boron trifluoride diethyl etherate and ethylene glycol produced the mono-ketals **10** and **11**, which could easily be separated (Scheme 2).[26] This method of selective ketalization showed a preference toward the 12-ketone over the typically more reactive 3- and 17-ketones of the A,B-cis steroid **9** as well as preference over the 17-ketone in compound **8** with the novel steroid backbone system. Furthermore, crystal structures of both compounds **10** (Figure 1) and **11** were obtained to confirm the assigned structures of these compounds. Although some of the desired product **11** was obtained, the yield was too low for a large total synthesis. Furthermore, the 17-carbonyl of diketone **11** was found to be resistant to Wittig reactions (data not reported) due to the steric bulk of the 12-ketal, thus making installation of the bile acid side chain difficult in this system. Although this methodology did not prove to be useful for completion of the synthesis of *ent*-DCA, the formation of the C3−C9 single bond provides a new steroid backbone connectivity that clearly illustrates the flexibility of the steroid A ring in A,B-cis systems. Also, the ability to selectively functionalize the typically less reactive 12-carbonyl in the presence of 3- and 17-carbonyls is yet another new method to exploit selectivity within the steroid ring system.

To avoid the formation of the C3−C9 single bond and to differentiate between the 12- and 17-carbonyls, an attempt was made to selectively ketalize the 3- and 17-ketones of diketo enone **6** under anhydrous acidic conditions (Scheme 3). The reduced reactivity of enones toward ketalization led to the 3,-17-diketal **12** as the slightly major product.[27] However, a significant amount of the 3,12-diketal **13** was formed as well. This side product could easily, and nearly quantitatively, be converted back to starting material **6** under aqueous acidic conditions (Scheme 3). Furthermore, even after prolonged periods of reflux, no tri-ketal formation was noted, presumably due to the steric hindrance around the remaining 12- or 17-ketone resulting from the presence of a ketal on the β carbon.

Reduction of the enone **12** with Li/NH_3(l) led to the formation of the saturated ketone **14** in an excellent yield (Scheme 3). Triethylamine was added to the reaction immediately after quenching with NH_4Cl to ensure that the ketals were not removed by any residual acid. Furthermore, now that the 3-carbonyl was protected as a ketal, there was no nucleophilic attack of the 3-position to form a C3−C9 single bond. Next,

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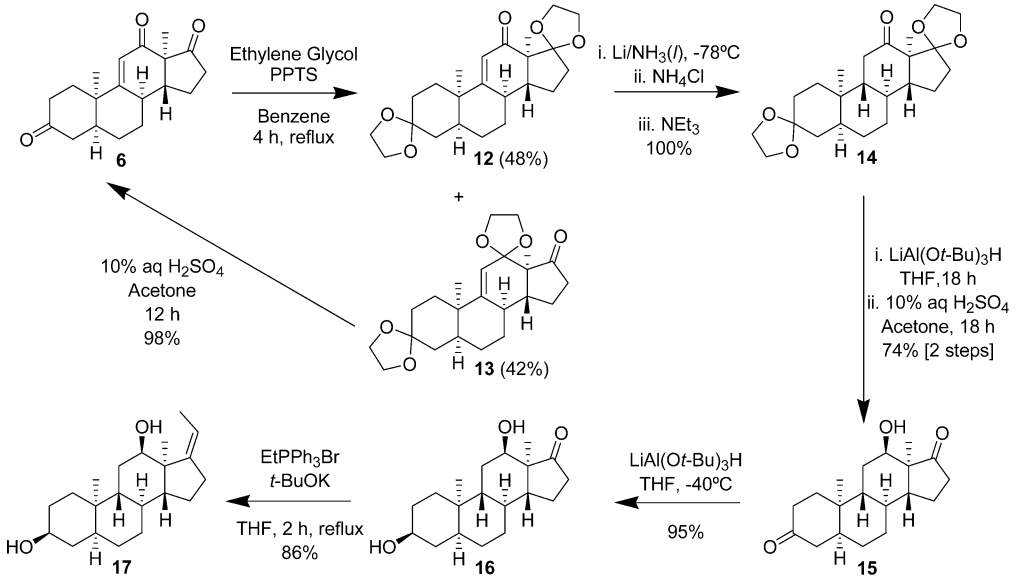
SCHEME 3. Construction of Functionalized *ent*-Deoxycholic Acid Steroid Nucleus



the 12-ketone of compound **14** was reduced with lithium tri-*t*-butoxyaluminohydride to give a 4:1 mixture of the *ent*-12α and *ent*-12β isomers, respectively.[23] These two isomers could not be separated, so the crude mixture was hydrolyzed and purified to give the pure diketone **15**. Selective reduction of the 3-ketone of compound **15** gave nearly quantitatively the diol **16**.[28] Installation of carbons 20 and 21 onto *ent*-steroid **16** through a Wittig reaction gave primarily the *Z*-isomer of the diol **17** in a good yield. Although a ketal in the 12-position was found to prevent the Wittig reaction from taking place (data not reported), the 12β-hydroxyl of the *ent*-steroid **16** did not inhibit this reaction and gave the desired *Z*-isomer as the major product. A small amount of the *E*-isomer was also obtained, and this isomer was not easily separated from the major *Z*-isomer. However, an analytical sample of the pure *Z*-isomer **17** was obtained by recrystallizing the *E*/*Z* mixture twice, and the stereochemistry of the olefin was confirmed by X-ray crystallography. The small amount of the *E*-isomer was not separated from the rest of the material at this stage due to the inefficiency of two recrystallizations and was instead removed at a later stage of the synthesis. Next, the hydroxyl groups of diol **17** were then protected as acetates to give *ent*-steroid **18** in high yield (Scheme 4). This protection was necessary due to the aprotic conditions needed for the subsequent ene reaction.

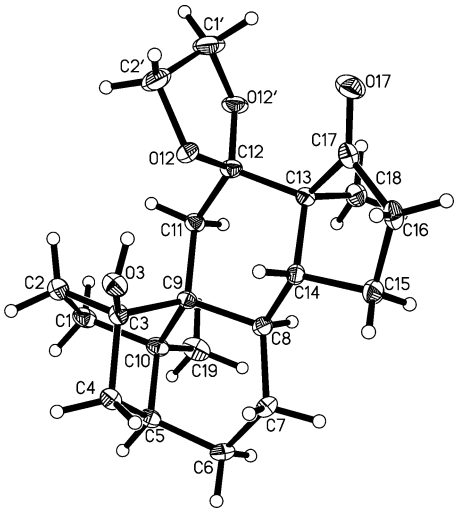


FIGURE 1. X-ray crystal structure of **10**.

The standard ene reaction methodology, used for the synthesis of numerous natural and enantiomeric steroidal side chains, utilizes methyl propiolate, diethyl aluminum chloride, and toluene.[29] However, these conditions resulted in no reaction when used with diacetate **18**. It is possible that the Lewis basicity of the acetates at both C-3 and C-12 and/or the steric bulk of the 12-acetate requires more reactive conditions to allow this ene reaction to proceed. Methyl propiolate was successfully added to diacetate **18** using a stronger Lewis acid, methylaluminum dichloride, and CH_2Cl_2 as the solvent to give compound **19** in a good yield (Scheme 4). Hydrogenation of *ent*-steroid **19** yielded saturated ester **20**, and hydrolysis of the three esters of this compound under basic conditions led to *ent*-DCA (***ent*-1**). To confirm the stereochemistry of C-17 and C-20, attempts were made to obtain a crystal structure of *ent*-DCA; however, crystals with sufficient diffraction could not be obtained. Therefore, a derivative of *ent*-DCA was made through a Fisher esterification of *ent*-DCA followed by Jones oxidation to obtain diketone **21** (Scheme 5). Crystals of this compound were grown from *p*-xylene and indeed confirmed that the correct stereochemistry was present in the side chain at C-17 and C-20. Ultimately, *ent*-DCA was synthesized enantioselectively from achiral 2-methyl-1,3-cyclopentanedione in 25 steps with a 0.3% yield. Furthermore, *ent*-DCA had identical properties when compared to DCA except for optical rotation, which was of opposite direction and equal magnitude.

The cmcs of both DCA and *ent*-DCA were evaluated by a previously validated dye solubilization method.[30] This method utilized an achiral, water insoluble dye, Orange OT, which can be solubilized by bile acid micelles. Furthermore, in these experiments, the sodium salt of each bile acid was used due to the increased aqueous solubility of the salt as compared to the acid.

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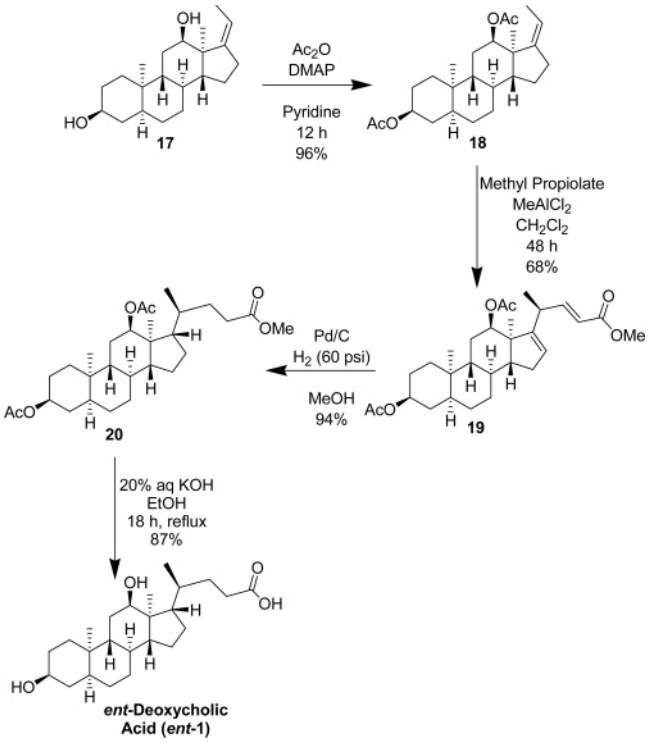
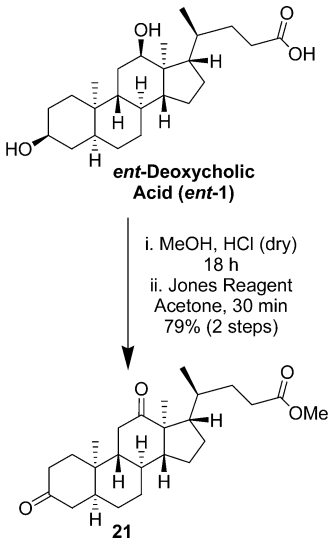
SCHEME 4. *ent*-Deoxycholic Acid Side Chain Addition



SCHEME 5. Synthesis of *ent*-Deoxycholic Acid Derivative for X-ray Structure Analysis



Representative plots of absorbance as a function of concentration looked similar for the salts NaDCA and *ent*-NaDCA (Figure 2A,B). When these absorbance plots were used to calculate the cmcs, it was found that NaDCA had a cmc of 10.73 ± 0.53 mM and *ent*-NaDCA had a cmc of 10.41 ± 0.26 mM (Table 1). These two values are not significantly different ($p > 0.3$) and adequately correspond to previously determined values for the cmc of NaDCA, thus illustrating that *ent*-DCA has the same cmc as DCA.[30]

Preliminary evaluation of DCA and *ent*-DCA effects on cell proliferation were performed on two human colon adenocarcinoma cell lines: HT-29 and HCT-116. At varying physiologically relevant concentrations (10, 50, 100, 250, and 500 μM), DCA and its enantiomer *ent*-DCA showed similar effects on

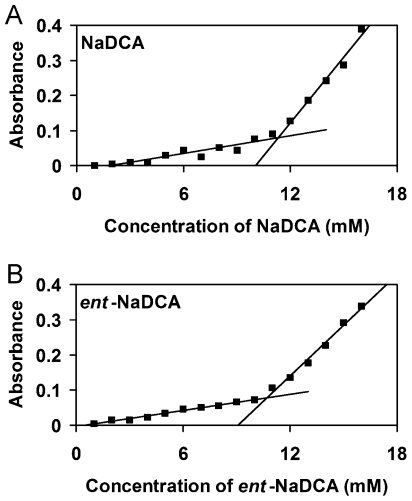


FIGURE 2. The cmc determination of natural and enantiomeric deoxycholic acid. Representative absorbance vs bile salt concentration plots used for the determination of the cmc by the Orange OT dye solubilization method for (A) NaDCA and (B) *ent*-NaDCA. Absorbance readings were taken at 483 nm, and linear best-fit lines are drawn according to the experimental methods.

TABLE 1. cmc Values of NaDCA and *ent*-NaDCA[a]

	cmc (mM)	SD (mM)
NaDCA (**1**)	10.73	0.53
ent-NaDCA (***ent*-1**)	10.41[b]	0.26

[a] cmcs were determined by the Orange OT dye solubilization method. The cmc reported is the average of three independent experiments using a different aqueous bile salt standard for each experiment. SDs calculated with $N = 3$. [b] $p > 0.3$, as compared to the value calculated for NaDCA.

cell proliferation after 48 h (Figure 3A,B). At low concentrations (≤100 μM), both compounds showed little to no decrease in proliferation. In fact, neither compound caused more than a 10% decrease in proliferation as compared to control in both cell lines. However, at high concentrations (250 and 500 μM), both steroids substantially decreased cell proliferation to nearly 50−60% of control at 250 μM and to nearly 20% of control at 500 μM. Proliferation of these cells lines was also examined over various time points (24, 48, and 72 h) at 250 μM (Figure 4A,B). DCA and *ent*-DCA exhibited similar toxicity profiles at each time point, with both compounds decreasing proliferation by over 50% after 72 h. Although some significant differences in toxicity are noted between DCA and *ent*-DCA treated cells, these differences are small in comparison to the large decreases in proliferation seen with both compounds at 250 and 500 μM.

The ability of DCA and *ent*-DCA to induce apoptosis was also examined in HT-29 and HCT-116 cells (Figure 5). Examination of hematoxylin and eosin (H&E) stained HT-29 (Figure 5A) and HCT-116 (Figure 5C) cells showed morphologic signs of apoptosis (condensed nuclei and cytoplasmic blebbing) after treatment with 500 μM DCA or *ent*-DCA for 1 h, whereas control cells showed no morphologic signs of apoptosis. However, in both cell lines, apoptotic cells were much more prevalent following DCA treatment as compared to *ent*-DCA treatment, indicating that the natural bile acid is a more potent inducer of apoptosis. Further confirmation of this difference in apoptosis induction was obtained by examining activation of the executioner caspase, caspase-3 (Figure 5B,D). Cells treated with DCA showed an increased cleavage of inactive procaspase-3 (35 kDa) to the active caspase-3 (19 kDa)

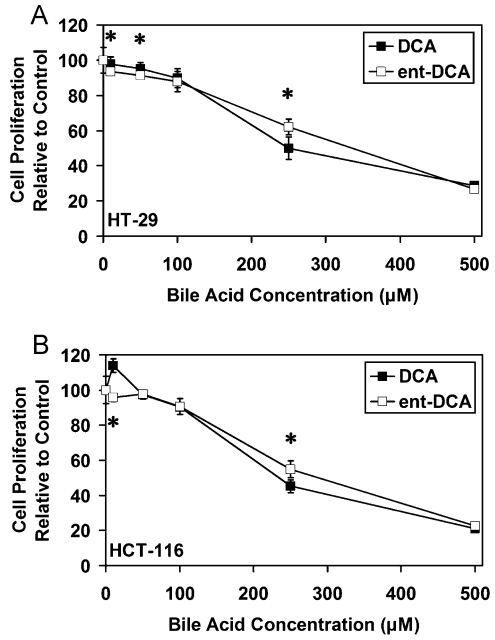


FIGURE 3. Cell proliferation in response to varying concentrations of natural and enantiomeric DCA. Concentrations of 10, 50, 100, 250, and 500 μM DCA and *ent*-DCA were applied for 48 h to (A) HT-29 cells and (B) HCT-116 cells. Proliferation was determined by the hexosaminidase assay[31] and was relative to vehicle treated control (EtOH), $N \geq 6$, values are expressed as mean $\pm$ SD. *$p < 0.05$.

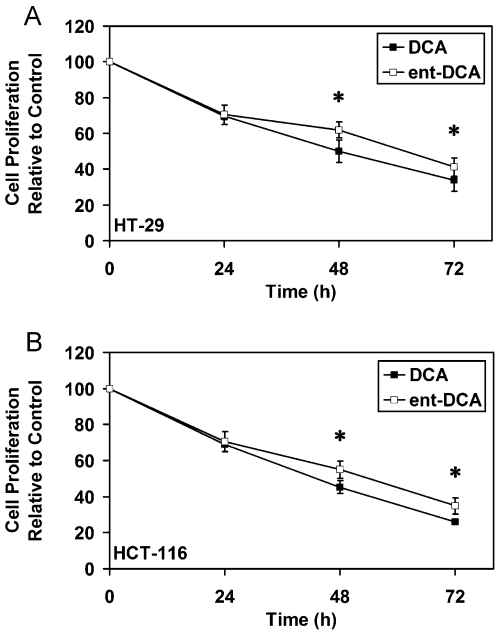


FIGURE 4. Time course analysis of cell proliferation in response to 250 μM natural and enantiomeric DCA. 250 μM DCA or *ent*-DCA was applied for 24, 48, and 72 h to (A) HT-29 cells and (B) HCT-116 cells. Proliferation was determined by the hexosaminidase assay[31] and was relative to vehicle treated control (EtOH), $N \geq 6$, values are expressed as mean $\pm$ SD. *$p < 0.05$.

as compared to *ent*-DCA treated cells, indicating more apoptosis induction in response to the natural bile acid. When comparing the two cell lines, there is more caspase cleavage in DCA treated HT-29 cells as compared to DCA treated HCT-116 cells. This correlates well with the morphologic data, where HT-29 cells treated with DCA show increased apoptosis as compared to

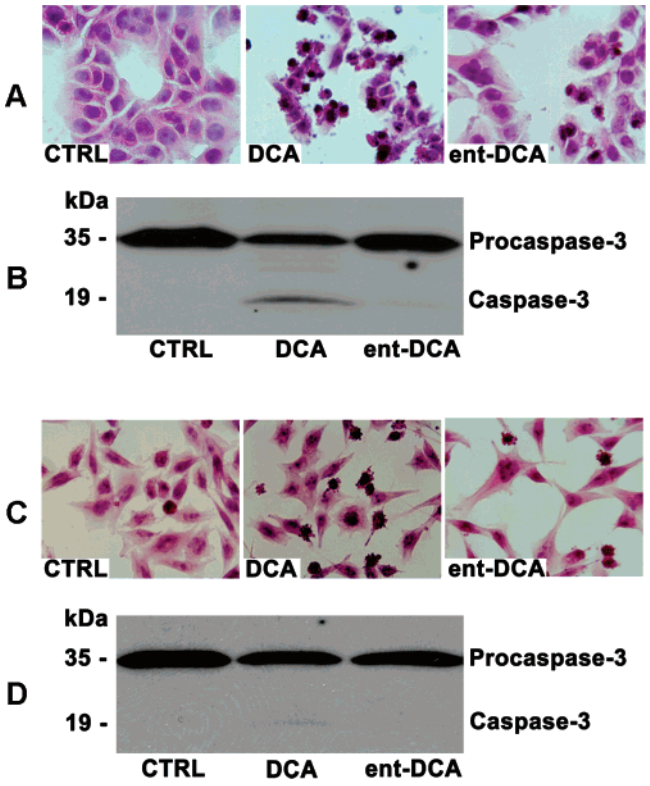


FIGURE 5. Cellular morphology changes and caspase-3 cleavage in HT-29 and HCT-116 cells in response to natural and enantiomeric DCA. (A) H&E stained HT-29 cells. (B) Western blot of procaspase-3 (35 kDa) and cleaved caspase-3 (19 kDa) in HT-29 cells. (C) H&E stained HCT-116 cells. (D) Western blot of procaspase-3 (35 kDa) and cleaved caspase-3 (19 kDa) in HCT-116 cells. In all experiments, cells were treated with 500 μM bile acid or vehicle control (EtOH) for 1 h.

HCT-116 cells exposed to the same bile acid. This illustrates the increased sensitivity of HT-29 cells to DCA and *ent*-DCA mediated apoptosis.

Taken together, the toxicity studies toward the selected human colon adenocarcinoma cell lines demonstrate that natural and enantiomeric DCA have similar effects on cell proliferation, yet the natural bile acid is a much more potent inducer of apoptosis. This indicates that the relative, not absolute, three-dimensional configurations of the bile acid seem to be most important for its effects on proliferation; however, the absolute configuration of the bile acid appears to be vital for the induction of apoptosis. Therefore, it is possible that primarily nonspecific processes (i.e., detergent effects) are responsible for proliferative changes, whereas a more specific process, such as a direct protein interaction, is associated with apoptosis induction. Finally, within the ever expanding field of bile acid biology, application toward bile acid toxicity illustrates only one of a myriad of possible applications for *ent*-DCA as well as other enantiomeric bile acids.

Conclusion

Deoxycholic acid is an important secondary bile acid, and having new probes to explore the biology of this steroid is critically important. The enantiomer of DCA can serve as a novel probe of DCA function, and in this study, we successfully synthesized *ent*-DCA in 25 steps with a 0.3% yield from achiral 2-methyl-1,3-cyclopentanedione. To our knowledge, this synthesis is the first successful attempt at installing a 12α-hydroxyl

(12β in *ent*-steroids) group together with the bile acid side chain while achieving the correct stereochemistry. *ent*-DCA has identical properties as compared to DCA except for optical rotation, which is equal in magnitude and opposite in direction, as expected for the enantiomer. Also, the cmcs were evaluated for both natural and enantiomeric DCA, and the cmcs were found to be equivalent. Finally, the toxicity of *ent*-DCA was evaluated toward several colon adenocarcinoma cell lines and compared to DCA. Both bile acids had similar effects on cell proliferation, whereas natural DCA was found to be a more potent inducer of apoptosis. These results illustrate that the relative configuration of this bile acid is more important for impacting proliferation, whereas the absolute configuration is more important for the specific induction of apoptosis. Finally, with the completion of the total synthesis of *ent*-DCA, this compound becomes accessible for further exploration of the many other roles of DCA in the vast field of bile acid biology.

Experimental Section

ent*-Deoxycholic Acid (*ent*-1).** Compound **20** (101.1 mg, 0.21 mmol) was dissolved in ethanol (20 mL) and refluxed with 20% aqueous KOH (10 mL) for 18 h. After cooling to room temperature, the solution was acidified with 6 N HCl until a precipitate was formed. The resulting suspension was then extracted with CH_2Cl_2 (150 mL × 3). The organic extracts were combined, and the solvent was removed in vacuo to yield a beige solid. Column chromatography (silica gel, 99% EtOAc/1% AcOH) of the product gave a white solid that was recrystallized from MeOH/hexanes to yield *ent*-deoxycholic acid (ent*-1**) (70.3 mg, 87%) as a white solid: mp 171−174 °C (deoxycholic acid lit[32] mp 176−178 °C); $[\alpha]_D^{25}$ = −55.6 (c = 0.2, MeOH) (deoxycholic acid lit[32] $[\alpha]_D^{25}$ = 55 (alcohol)); IR 3306, 1691 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.95−3.94 (m, 1H), 3.53−3.48 (m, 1H), 2.36−2.26 (m, 1H), 2.20−2.10 (m, 1H), 1.00 (d, 3H, J = 6 Hz), 0.92 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (CD_3OD) δ 180.4, 74.2, 72.7, 48.4, 47.8, 43.8, 37.6, 37.4, 37.1, 36.6, 35.5, 35.0, 33.7, 33.1, 31.3, 30.1, 28.8, 28.6, 27.6, 25.0, 23.8, 17.8, 13.4, one additional carbon is buried in the MeOH standard peaks. Analysis for $C_{24}H_{40}O_4$: calcd C, 73.43; H, 10.27. Found C, 73.59; H, 9.99.

(3*R*,3a*R*,9a*R*,9b*R*)]-3-(1,1-Dimethylethoxy)-1,2,3,3a,4,5,8,9,9a,-9b-decahydro-3a-methyl-6-[2-(2-methyl-1,3-dioxolan-2-yl)ethyl]-7*H*-benz[*e*]inden-7-one (2). Enone **2** was synthesized from 2-methyl-1,3-cyclopentanedione in 11 steps with a 22% yield as done previously.[20,21]

(3*R*,3a*R*,6*S*,9a*R*,9b*R*)]-3-(1,1-Dimethylethoxy)-1,2,3,3a,4,6,8,9,-9a,9b-decahydro-3a,6-dimethyl-6-[2-(2-methyl-1,3-dioxolan-2-yl)ethyl]-7*H*-benz[*e*]inden-7-one (3). Enone **2** (6.90 g, 17.7 mmol, 1 equiv) and dry ethylene glycol dimethyl ether (70 mL) distilled from sodium were added to a dry flask under N_2. With stirring, sodium hydride (0.78 g, 19.5 mmol, 1.1 equiv, 60% dispersion in mineral oil) was slowly added, and the solution immediately turned yellow. The mixture was refluxed overnight for 18 h under N_2. After cooling to room temperature, dry THF (40 mL) was added, and the solution was then cooled to −78 °C. Methyl iodide (3.31 mL, 53.1 mmol, 3 equiv) was slowly added to quench the intermediate dieneolate. The reaction was stirred for 5 h at −78 °C, after which it was warmed to 0 °C and stirred for 2 h. Water (50 mL) was added, and the solution was stirred and allowed to warm to room temperature for 1 h. The organic solvent was removed in vacuo, and then water (100 mL) was added. The aqueous solution was extracted with CH_2Cl_2 (3 × 150 mL). The organic extracts were combined and dried over Na_2SO_4, after which the solvent was removed in vacuo to yield a yellow oil. Column chromatography (silica gel, 10% EtOAc/hexanes) of the resulting oil yielded product **3** (4.20 g, 59%) as a yellow oil and starting material **2** (1.56 g, 23%) as a yellow oil. Product **3**: IR 1714 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.54−5.52 (m, 1H), 3.94−3.86 (m, 4H), 3.58 (t, 1H, J = 8.4 Hz), 1.31 (s, 3H), 1.19 (s, 3H), 1.13 (s, 9H), 0.73 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 214.3, 141.8, 120.7, 110.2, 80.6, 72.3, 64.5 (C × 2), 54.0, 47.1, 41.1, 39.0, 38.3, 37.6, 34.0, 31.1, 30.8, 29.3, 28.8 (C × 3), 27.6, 24.5, 23.6, 11.3. Analysis for $C_{25}H_{40}O_4$: calcd C, 74.22; H, 9.97. Found C, 74.36; H, 9.74.

(8α,10α,13α,14β,17α)-17-Hydroxyandrosta-4,9(11)-dien-3-one (4). To a flask was added compound **3** (11.70 g, 29.1 mmol), 3 N HCl (60 mL), and MeOH (150 mL). With stirring, this solution was heated to reflux for 18 h. After cooling to room temperature, the MeOH was removed in vacuo. Water (300 mL) was added to the residue, and the aqueous solution was extracted with CH_2Cl_2 (200 mL × 3). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (300 mL) and brine (300 mL). The organic layer was then dried over Na_2SO_4 and filtered, and then the solvent was removed in vauco. Column chromatography (silica gel, 20% EtOAc/hexanes to 60% EtOAc/hexanes) of the resulting product yielded *ent*-steroid **4** (7.03 g, 72%) as a white solid. An analytical sample of enone **4** was recrystallized from acetone/hexanes (1:1) to give a white solid: mp 157−159 °C; $[\alpha]_D^{25}$ = −91.5 (c = 0.4, $CHCl_3$); IR 3400, 1667, 1613 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.74−5.73 (m, 1 H), 5.53−5.51 (m, 1H), 3.78−3.70 (m, 1H), 1.34 (s, 3H), 0.75 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 199.3, 169.9, 144.8, 123.9, 118.7, 81.7, 47.6, 41.3, 41.0, 38.4, 37.5, 34.2, 33.8, 32.9, 31.7, 30.7, 26.1, 24.2, 10.5. Analysis for $C_{19}H_{26}O_2$: calcd C, 79.68; H, 9.15. Found C, 79.84; H, 9.13.

(5α,8α,10α,13α,14β,17α)-17-Hydroxyandrost-9(11)-en-3-one (5). Potassium hydroxide (1.87 g, 2 equiv) and *i*-PrOH (60 mL) were added to a hydrogenation bottle, and the solution was stirred for 30 min to allow some of the potassium hydroxide to dissolve. Palladium on carbon (0.80 g, 5%) was added to the hydrogenation bottle followed by the addition of dienone **4** (4.79 g, 16.7 mmol, 1 equiv) dissolved in *i*-PrOH (40 mL). Additional *i*-PrOH was added so that the total volume in the hydrogenation flask was ∼120 mL. The solution was hydrogenated for 2 h at 15 psi, and then it was filtered through a pad of Celite, eluting with MeOH. After removal of the solvent in vacuo, a yellow oil remained. To this oil was added brine (200 mL) and Et_2O (300 mL), and the aqueous phase was neutralized with 6 N HCl. After thorough mixing, the aqueous layer was removed, and the organic layer was washed with brine (100 mL × 3) and then dried over Na_2SO_4. This solution was filtered, and then the solvent was removed in vauco to give a white solid. Column chromatography (silica gel, 20% EtOAc/hexanes) of the resulting solid yielded mono-olefin **5** (3.25 g, 68%) as a white solid. An analytical sample of compound **5** was recrystallized from acetone/hexanes (1:1): mp 136−138 °C; $[\alpha]_D^{25}$ = −22.6 (c = 0.3, $CHCl_3$); IR 3418, 1714, 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.57−5.56 (m, 1H), 3.80−3.72 (m, 1H), 1.15 (s, 3H), 0.71 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 213.4, 139.7, 119.2, 81.9, 48.2, 44.6, 43.7, 41.5, 39.0, 38.5, 38.2, 37.5, 36.5, 30.8, 28.9, 26.3, 26.1, 24.2, 10.6. Analysis for $C_{19}H_{28}O_2$: calcd C, 79.12; H, 9.78. Found C, 79.31; H, 9.95.

(5α,8α,10α,13α,14β)-Androst-9(11)-ene-3,12,17-trione (6) and (5α,8α,10α,13α,14β)-Androst-9(11)-ene-3,17-dione (7). *ent*-Steroid **5** (2.41 g, 8.4 mmol, 1 equiv) was dissolved in AcOH (60 mL) and CH_2Cl_2 (60 mL) and stirred at 4 °C for 1 h. Chromium (VI) oxide (5.04 g, 50.4 mmol, 6 equiv) was then added, and the solution was stirred at 4 °C for 10 h. The reaction was quenched by the addition of *i*-PrOH, after which it was allowed to warm to room temperature. The solvent was removed in vacuo, and the resulting residue was dissolved in CH_2Cl_2 (400 mL) and water (400 mL). The organic layer was separated and washed repeatedly with saturated aqueous $NaHCO_3$ (200 mL) until no gas evolution was noted. The organic layer was then dried over Na_2SO_4 and filtered, and then the solvent was removed in vauco to give a solid. Column

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chromatography (silica gel, 50% EtOAc/hexanes to 100% EtOAc) of the resulting solid yielded enone **6** (0.76 g, 30%) as a white solid and compound **7** (0.89 g, 37%) as a white solid that could be used as a starting material for this reaction. Analytical samples of *ent*-steroids **6** and **7** were recrystallized from acetone/hexanes (1:1) to give crystalline white solids.

Product **6**: mp 236−237 °C; $[\alpha]_D^{25} = -198.2$ ($c = 0.5$, $CHCl_3$); IR 1755, 1717, 1677, 1602 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.94−5.93 (m, 1H), 1.26 (s, 3H), 1.09 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 211.2, 210.7, 196.1, 165.9, 123.5, 53.7, 48.3, 44.1, 43.6, 40.6, 37.7, 36. 7 (C × 2), 36.3, 29.4, 25.4, 25.0, 21.0, 14.1. Analysis for $C_{19}H_{24}O_3$: calcd C, 75.97; H, 8.05. Found C, 76.02; H, 8.15. Product **7**: mp 148−151 °C; $[\alpha]_D^{25} = -151.7$ ($c = 0.2$, $CHCl_3$); IR 1736, 1710, 1633 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.58 (d, 1H, $J = 5.4$ Hz), 1.16 (s, 3H), 0.83 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 221.4, 212.9, 140.2, 118.6, 48.6, 46.0, 44.4, 43.6, 39.1, 38.1, 37.4, 36.2, 35.9, 33.4, 28.9, 26.1, 25.6, 22.7, 14.0. Analysis for $C_{19}H_{26}O_2$: calcd C, 79.68; H, 9.15. Found C, 79.80; H, 8.90.

(3*β*,5α,8α,9*R*,10α,13α,14*β*)-3,9-Cyclo-3-hydroxyandrostane-12,17-dione, Cyclic-12-(1,2-ethanediyl acetal) (10) and (5α,8α,9*β*,-10α,13α,14*β*)-Androstane-3,12,17-trione, Cyclic-12-(1,2-ethanediyl acetal) (11). A dry three-necked flask was fitted with a gas condenser, and the entire apparatus was dried under vacuum for 1 h. After back-filling with N_2, the flask was cooled to −78 °C, and NH_3 gas was condensed (50 mL). While stirring, lithium wire (75 mg, 10.8 mmol, 20 equiv) was added, and the liquid ammonia solution turned blue. This solution was stirred for 1 h at −78 °C. Enone **6** (162.1 mg, 0.54 mmol, 1 equiv) was dissolved in dry THF (10 mL) and added dropwise to the reaction over 5 min. The solution was stirred for an addition 2 h at −78 °C during which time the color remained blue. NH_4Cl (1.5 g) was then added to quench the reaction, and then the solution was allowed to warm to room temperature. Water (100 mL) and EtOAc (100 mL) were added to the mixture, and after separating the aqueous layer, it was extracted with EtOAc (100 mL × 2). The combined organic extracts were dried over Na_2SO_4 and filtered, and then the solvent was removed in vacuo to give an oil. This resulting residue was dissolved in acetone (10 mL), and while stirring, Jones reagent was added dropwise to this solution until a yellow color persisted. The solution was stirred at room temperature for 30 min, after which time isopropyl alcohol was added dropwise to quench any remaining Jones reagent. The reaction mixture was poured into brine (50 mL), and the aqueous solution was extracted with EtOAc (50 mL × 3). The organic extracts were combined, dried over Na_2SO_4, and filtered. After solvent removal in vacuo, a brown solid remained. This solid was passed through silica gel, eluting with 50% EtOAc/hexanes to yield an inseparable mixture of saturated *ent*-steroids **8** and **9** (108.1 mg, 67%) as a white solid. This mixture of compounds **8** and **9** (108.1 mg, 0.36 mmol, 1 equiv) was dried thoroughly and dissolved in dry CH_2Cl_2 (8 mL) under N_2. To this solution was added ethylene glycol (60.2 μL, 1.08 mmol, 3 equiv) and boron trifluoride diethyl etherate (182.5 μL, 1.44 mmol, 4 equiv), and the solution was stirred at room temperature under N_2 for 24 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (50 mL), and then this solution was extracted with CH_2Cl_2 (100 mL × 3). The organic extracts were combined, dried over Na_2SO_4, and filtered, and then the solvent was removed in vacuo. Column chromatography (silica gel, 10% EtOAc/hexanes to 30% EtOAc/hexanes) of the resulting product yielded 3,9-cyclo product **10** (77.1 mg, 41% [two steps]) and ketal **11** (27.8 mg, 15% [two steps]), both as white solids. Crystals of both compounds for X-ray structure determination were grown from hexanes/acetone [9:1].

Product **10**: mp 176−177 °C; $[\alpha]_D^{25} = -153.0$ ($c = 0.3$, $CHCl_3$); IR 3420, 1738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.68 (s, 1H), 4.40−4.31 (m, 1H), 4.08−3.93 (m, 3H), 2.83−2.73 (m, 1H), 2.37−2.28 (m, 1H), 1.02 (s, 3H), 0.86 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 216.1, 111.8, 85.3, 66.1, 65.5, 55.5, 50.2, 44.9, 42.8, 42.2, 38.4, 38.3, 36.6, 34.9, 34.8, 33.1, 25.8, 21.7, 21.2, 15.0, 12.0. Analysis for $C_{21}H_{30}O_4$: calcd C, 72.80; H, 8.73. Found C, 73.00; H, 8.92. Product **11**: mp 210−214 °C; $[\alpha]_D^{25} = -155.9$ ($c = 0.2$, $CHCl_3$); IR 1736, 1711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.32−4.25 (m, 1H), 4.02−3.95 (m, 2H), 3.91−3.83 (m, 1H), 2.69 (t, 1H, $J = 14.4$ Hz), 1.02 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 216.6, 212.4, 110.8, 65.7, 65.4, 54.8, 48.6, 43.8, 42.2, 38.3, 36.9 (C × 2), 36.8, 34.7, 34.0, 32.5, 26.3, 24.3, 22.4, 21.1, 12.6. Analysis for $C_{21}H_{30}O_4$: calcd C, 72.80; H, 8.73. Found C, 72.79; H, 8.68.

(5α,8α,10α,13α,14*β*)-Androst-9(11)-ene-3,12,17-trione, Cyclic-3,17-bis(1,2-ethanediyl acetal) (12) and (5α,8α,10α,13α,14*β*)-Androst-9(11)-ene-3,12,17-trione, Cyclic-3,12-bis(1,2-ethanediyl acetal) (13). Enone **6** (1.49 g, 4.96 mmol, 1 equiv) was added to a flask with pyridinium *p*-toluene sulfonate (250 mg, 0.99 mmol, 0.2 equiv), ethylene glycol (1.38 mL, 24.8 mmol, 5 equiv), and dry benzene (60 mL). A Dean−Stark trap was attached, and the reaction was refluxed for 4 h. After cooling to room temperature, the reaction was poured into EtOAc (250 mL). This organic solution was washed with saturated aqueous $NaHCO_3$ (100 mL × 2) and water (100 mL), and then it was dried over Na_2SO_4. After filtering, the solvent was removed in vacuo to give a clear oil. Column chromatography (silica gel, 10% EtOAc/hexanes to 25% EtOAc/hexanes) yielded diketals **12** (0.92 g, 48%) and **13** (0.80 g, 42%), both as clear oils.

Product **12**: $[\alpha]_D^{25} = -76.2$ ($c = 0.3$, $CHCl_3$); IR 1674, 1596 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.75 (d, 1H, $J = 1.5$ Hz), 4.31−4.26 (m, 1H), 4.00−3.78 (m, 7H), 1.14 (s, 3H), 0.92 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 200.8, 166.9, 124.4, 117.0, 109.6, 65.6, 65.3, 64.2, 64.0, 54.7, 47.8, 40.8, 40.2, 37.3, 36.8, 34.7, 34.1, 30.9, 29.8, 25.7, 25.5, 21.0, 13.2. Analysis for $C_{23}H_{32}O_5$: calcd C, 71.11; H, 8.30. Found C, 70.89; H, 8.20. Product **13**: $[\alpha]_D^{25} = -191.7$ ($c = 0.3$, $CHCl_3$); IR 1742, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.14 (s, 1H), 4.28−4.21 (m, 1H), 3.99−3.77 (m, 7H), 2.36−2.27 (m, 1H), 1.07 (s, 3H), 0.85 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 217.7, 145.8, 122.5, 110.0, 108.7, 65.7, 65.3, 64.1, 63.9, 53.2, 46.7, 40.7, 38.7, 37.7, 36.8, 35.6, 34.3, 30.8, 29.3, 25.9, 25.6, 21. 9, 11.5. Analysis for $C_{23}H_{32}O_5$: calcd C, 71.11; H, 8.30. Found C, 70.85; H, 8.57.

(5α,8α,9*β*,10α,13α,14*β*)-Androstane-3,12,17-trione, Cyclic-3,-17-bis(1,2-ethanediyl acetal) (14). A dry three-necked flask was fitted with a gas condenser, and the entire apparatus was dried under vacuum for 1 h. After back-filling with N_2, the flask was cooled to −78 °C, and NH_3 gas was condensed (125 mL). While stirring, lithium wire (248 mg, 35.7 mmol, 15 equiv) was added, and the liquid ammonia solution turned blue. This solution was stirred for 1 h at −78 °C. Diketal **12** (0.92 g, 2.38 mmol, 1 equiv) was dissolved in dry THF (20 mL) and added dropwise to the reaction over 5 min. The solution was stirred for an additional 2 h at −78 °C, during which time the color remained blue. NH_4Cl was then slowly added until the reaction was quenched and the blue color disappeared. This was then followed by the immediate addition of NEt_3 (10 mL). The solution was allowed to warm to room temperature after which it was diluted with saturated aqueous $NaHCO_3$ (200 mL). This solution was then extracted with EtOAc (150 mL × 3). The organic extracts were combined, dried over Na_2SO_4, and filtered, then the solvent was removed in vacuo to yield a clear oil. Column chromatography (silica gel, 20% EtOAc/hexanes to 50% EtOAc/hexanes) of the resulting product yielded compound **14** (0.93 g, 100%) as a clear oil: $[\alpha]_D^{25} = -78.9$ ($c = 0.3$, $CHCl_3$); IR 1709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.20−4.13 (m, 1H), 4.07−4.01 (m, 1H), 3.94−3.78 (m, 6H), 2.27−2.17 (m, 1H), 1.03 (s, 3H), 0.95 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 210.3, 116.7, 109.4, 65.7, 65.3, 64.1, 64.0, 58.1, 49.9, 40.3, 39.9, 38.4, 35.5, 34.8, 34.7, 34.5, 33.8, 29.8, 26.3, 24.9, 22.3, 20.6, 15.1. Analysis for $C_{23}H_{34}O_5$: calcd C, 70.74; H, 8.78. Found C, 70.75; H, 8.80.

(5α,8α,9*β*,10α,12*β*,13α,14*β*)-12-Hydroxyandrostane-3,17-dione (15). To a dry flask was added ketone **14** (0.93 g, 2.38 mmol, 1 equiv) dissolved in dry THF (100 mL). While under N_2, the solution was cooled to 0 °C in an ice bath. Lithium tri-*t*-butoxyaluminohydride (19 mL, 19.04 mmol, 8 equiv, 1 M in THF) was added dropwise, and the solution was allowed to warm to room temperature and then stirred for 18 h under N_2. The reaction was

then cooled to 0 °C and carefully quenched with H_2O, and then it was allowed to warm to room temperature. EtOAc (300 mL) was added, and the organic solution was washed with water (200 mL) and brine (200 mL). The organic layer was then dried over Na_2SO_4 and filtered, and then the solvent was removed in vacuo to give a clear oil. This crude product was then dissolved in acetone (90 mL). To this solution was added 10% aqueous H_2SO_4 (10 mL), and the resulting solution was stirred at room temperature overnight. The organic solvent was removed in vacuo, and then the solution was diluted with water (200 mL). This aqueous solution was extracted with EtOAc (150 mL × 3), and the combined organic extracts were then washed with saturated aqueous $NaHCO_3$ (200 mL), dried over Na_2SO_4, and filtered. Then, the solvent was removed in vacuo to yield a white solid. Column chromatography (silica gel, 30% EtOAc/hexanes to 40% EtOAc/hexanes) of the resulting product yielded compound **15** (0.54 g, 74% over two steps) as a white solid. An analytical sample of *ent*-steroid **15** was recrystallized from a mixture of acetone/hexanes (1:1) to give fine white needles: mp 175−177 °C; $[\alpha]_D^{25} = -166.0$ ($c = 0.5$, $CHCl_3$); IR 3500, 1733, 1711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.11 (d, 1H, J = 3 Hz), 2.70 (t, 1H, J = 14.3 Hz), 1.00 (s, 3H), 0.85 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 220.2, 212.7, 69.2, 53.2, 44.2, 43.4, 42.2, 37.0, 36.8, 36.1, 34.9, 34.9, 34.5, 27.5, 26.2, 24.4, 22.2, 20.9, 13.8. Analysis for $C_{19}H_{28}O_3$: calcd C, 74.96; H, 9.27. Found C, 75.04; H, 9.46.

(3β,5α,8α,9β,10α,12β,13α,14β)-3,12-Dihydroxyandrostan-17-one (16). To a dry flask was added compound **15** (540 mg, 1.77 mmol, 1 equiv) dissolved in dry THF (30 mL). While under N_2, the solution was cooled to −40 °C in a bath of acetonitrile and dry ice. Lithium tri-*t*-butoxyaluminohydride (2.21 mL, 2.21 mmol, 1.25 equiv, 1 M in THF) was added dropwise, and the solution was stirred for 2 h at −40 °C under N_2. After seeing that the reaction was not complete, additional lithium tri-*t*-butoxyaluminohydride was added at −40 °C until no starting material was present. The reaction was carefully quenched with 3 N HCl and then warmed to room temperature. EtOAc (250 mL) was added, and the organic solution was washed with saturated aqueous $NaHCO_3$ (150 mL) and brine (150 mL). The organic layer was dried over Na_2SO_4 and filtered, and then the solvent was removed in vacuo to give a white solid. Column chromatography (silica gel, 50% EtOAc/hexanes to 20% MeOH/EtOAc) of the resulting product yielded diol **16** (515 mg, 95%) as a white solid. An analytical sample of compound **16** was recrystallized from a mixture of acetone/hexane (1:4) to give a fluffly white solid: mp 161−162 °C; $[\alpha]_D^{25} = -157.7$ ($c = 0.3$, $CHCl_3$); IR 3400, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.08 (d, 1H, J = 2.4 Hz), 3.64−3.52 (m, 1H), 2.45−2.36 (m, 1H), 0.90 (s, 3H), 0.82 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 220.6, 71.6, 69.4, 53.2, 43.4, 42.1, 36.4, 36.3, 35.3, 35.3, 34.7, 34.3, 30.4, 27.1, 26.9, 25.2, 23.0, 21.0, 13.9. Analysis for $C_{19}H_{30}O_3$: calcd C, 74.47; H, 9.87. Found C, 74.76; H, 10.00.

(3β,5α,8α,9β,10α,12β,13α,14β,17*Z*)-Pregn-17(20)-ene-3,12-diol (17). Ethyltriphenyl phosphonium bromide (3.12 g, 8.4 mmol, 5 equiv) was added to a flask and dried under high vacuum for 1 h. After filling with N_2, dry THF (50 mL) was added followed by KO(*t*-Bu) (0.94 g, 8.4 mmol, 5 equiv). The entire mixture was heated to reflux under N_2 for 30 min, during which time the mixture turned bright orange as the ylide formed. Diol **16** (515 mg, 1.68 mmol, 1 equiv) was dissolved in dry THF (30 mL) and then added to the refluxing solution, which was then stirred at reflux for 2 h. After cooling to room temperature, the solution was poured into brine (200 mL). The aqueous solution was extracted with ether (150 mL × 3), and then the combined organic extracts were washed with brine (100 mL × 2). The organic layer was then dried over Na_2SO_4 and filtered, and then the solvent was removed in vacuo to yield a beige solid. Column chromatography (silica gel, 10% EtOAc/hexanes to 50% EtOAc/hexanes) of the resulting solid yielded compound **17** (460 mg, 86%) as a white solid. An analytical sample of diol **17** was recrystallized twice from a mixture of acetone/hexanes (1:1) to give fine white needles. Crystals of *ent*-steroid **17** for X-ray structure determination were grown from hexanes/acetone (9:1). Compound **17**: mp 167−169 °C; $[\alpha]_D^{25} = -64.4$ ($c = 0.4$, $CHCl_3$); IR 3292, 1669 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.34−5.26 (qt, 1H, J = 7.2, 2.1 Hz), 4.36−4.34 (m, 1H), 3.62−3.58 (m, 1H), 0.92 (s, 3H), 0.89 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 146.4, 115.0, 72.1, 71.8, 50.0, 48.3, 42.1, 36.4, 35.3, 35.1, 34.5, 34.2, 31.9, 30.5, 27.1, 26.9, 26.1, 23.9, 23.1, 17.3, 12.6. Analysis for $C_{21}H_{34}O_2$: calcd C, 79.19; H, 10.76. Found C, 79.21; H, 10.74.

(3β,5α,8α,9β,10α,12β,13α,14β,17*Z*)-Pregn-17(20)-ene-3,12-diol, Diacetate (18). Diol **17** (460 mg, 1.44 mmol, 1 equiv), 4-dimethylaminopyridine (17.6 mg, 0.144 mmol, 0.1 equiv), acetic anhydride (4.10 mL, 43.2 mmol, 30 equiv), and pyridine (30 mL) were added to a dry flask and stirred under N_2 for 12 h at room temperature. The reaction mixture was poured into water (200 mL), and a white solid formed. The aqueous solution was then extracted with EtOAc (200 mL × 3). The combined organic extracts were then washed with 1 N HCl (200 mL) and brine (200 mL) and then dried over Na_2SO_4. The solution was filtered, and then the solvent was removed in vacuo to yield a yellow oil. Column chromatography (silica gel, 10% EtOAc/hexanes) of the resulting product yielded diacetate **18** (556 mg, 96%) as a clear oil, which still contained a small amount of the *E*-isomer, which was inseparable and removed at a later stage. $[\alpha]_D^{25} = -136.2$ ($c = 0.6$, $CHCl_3$); IR 1737, 1656 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.45−5.44 (m, 1H), 5.20−5.11 (m, 1H), 4.74−4.64 (m, 1H), 2.06 (s, 3H), 2.02 (s, 3H), 0.91 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 170. 7, 170.5, 145.2, 114.4, 75.1, 74.1, 49.5, 47.1, 41.8, 35.1, 34.9, 34.7, 34.2, 32.2, 31.9, 26.9, 26.6, 25.9, 25.6, 23.8, 23.0, 21.4, 21.4, 17.6, 12.9. Analysis for $C_{25}H_{38}O_4$: calcd C, 74.59; H, 9.51. Found C, 74.27; H, 9.78.

(3β,5α,8α,9β,10α,12β,13α,14β,20*S*,22*E*)-3,12-Bis(acetyloxy)-chola-16,22-dien-24-oic Acid, Methyl Ester (19). A flask was dried under high vacuum and then back-filled with argon. To this flask was added dry CH_2Cl_2 (5 mL), methylaluminum dichloride (1.1 mL, 1.1 mmol, 2 equiv, 1 M in hexane), and methyl propiolate (54 μL, 0.61 mmol, 1.1 equiv). This yellow solution was stirred under argon at room temperature for 30 min, after which diacetate **18** (222 mg, 0.55 mmol, 1 equiv) dissolved in dry CH_2Cl_2 (5 mL) was added. After the solution was stirred for 48 h, it was then poured into saturated aqueous $NaHCO_3$ (50 mL). A solid precipitated, and this was removed by filtration through a pad of Celite. The aqueous layer was then extracted with CH_2Cl_2 (150 mL × 3). The organic extracts were combined, washed with brine (100 mL), and then dried over Na_2SO_4. After the solution was filtered, the solvent was removed in vacuo to yield a yellow oil. Column chromatography (silica gel, 10% EtOAc/hexanes to 15% EtOAc/hexanes) of the product gave *ent*-steroid **19** (181.5 mg, 68%) as a clear oil and *ent*-steroid **18** (62.0 mg, 28%) as the recovered starting material. Product **19**: $[\alpha]_D^{25} = -107.9$ ($c = 0.4$, $CHCl_3$); IR 1734, 1653, 1618 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.80 (dd, 1H, J = 15.6, 8.7 Hz), 5.80 (d, 1H, J = 15.6 Hz), 5.51 (m, 1H), 5.10−5.09 (m, 1H), 4.75−4.66 (m, 1H), 3.70 (s, 3H), 2.83 (t, 1H, J = 7.4 Hz), 2.07 (s, 3H), 2.02 (s, 3H), 1.12 (d, 3H, J = 6.9 Hz), 0.92 (s, 3H), 0.78 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 170.6, 170.5, 167.2, 153.7 (C × 2), 125.5, 119.0, 74.1, 73.2, 51.4, 51.1, 49.5, 41.9, 36.1, 35.1, 34.7, 34.3, 34.1, 32.3, 30.4, 26.8, 26.6, 25.9 (C × 2), 23.1, 21.5, 21.4, 19.8, 17.4. Analysis for $C_{29}H_{42}O_6$: calcd C, 71.57; H, 8.70. Found C, 71.76; H, 8.51.

(3β,5α,8α,9β,10α,12β,13α,14β,17α,20*S*)-3,12-Bis(acetyloxy)-cholan-24-oic Acid, Methyl Ester (20). Palladium on carbon (48.9 mg, 5%), unsaturated compound **19** (146.7 mg, 0.30 mmol), and methanol (20 mL) were added to a hydrogenation bottle. The mixture was hydrogenated overnight at 60 psi, after which the solution was passed through Celite to remove the palladium catalyst. The solvent was removed in vacuo to give an oil. Column chromatography (silica gel, 10% EtOAc/hexanes) of the product to remove minor amounts of other hydrogenation products gave compound **20** (138.1 mg, 94%) as a clear oil. $[\alpha]_D^{25} = -62.1$ ($c = 0.3$, $CHCl_3$); IR 1736 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.05−5.04 (m, 1H), 4.70−4.63 (m, 1H), 3.63 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H),

0.87 (s, 3H), 0.77 (d, 3H, J = 6.3 Hz), 0.69 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 174.5, 170.4, 170.3, 75.8, 74.1, 51.4, 49.4, 47.5, 45.0, 41.8, 35.6, 34.6 (C × 2), 34.4, 34.0, 32.2, 30.9, 30.8, 27.3, 26.8, 26.6, 25.8, 25.6, 23.4, 23.0, 21.4, 21.3, 17.4, 12.3. Analysis for $C_{29}H_{46}O_6$: calcd C, 70.99; H, 9.45. Found C, 71.13; H, 9.29.

(5α,8α,9β,10α,13α,14β,17α,20*S*)-3,12-Dioxocholan-24-oic Acid, Methyl Ester (21). Acetyl chloride (1.7 μM, 0.024 mmol, 0.2 equiv) was added to MeOH (5 mL) under nitrogen and stirred for 20 min to generate anhydrous HCl. To this solution was added compound ***ent*-1** (47.3 mg, 0.12 mmol, 1 equiv) in MeOH (5 mL). After stirring overnight, the solvent was removed in vacuo. The resulting residue was dissolved in acetone (10 mL), and while stirring, Jones reagent was added dropwise to this solution until a yellow color persisted. The solution was stirred at room temperature for 30 min after which time *i*-PrOH was added dropwise to quench any remaining Jones reagent. The reaction mixture was poured into brine (100 mL), and the aqueous solution was extracted with EtOAc (100 mL × 3). The organic extracts were combined, dried over Na_2SO_4, and filtered. After solvent removal in vacuo, a yellow solid remained. Column chromatography (silica gel, 10% EtOAc/hexanes) of the product gave compound **21** (38.0 mg, 79%) as a white solid. An analytical sample of product **21** was obtained by recrystallization from 1:9 Et_2O/hexanes to give a white solid. Crystals of *ent*-steroid **21** for X-ray structure determination were grown by slow evaporation of *p*-xylene. mp 131−133 °C; $[\alpha]_D^{25}$ = −94.5 (c = 0.4, $CHCl_3$); IR 1737, 1709 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.64 (s, 3H), 1.09 (s, 3H), 1.03 (s, 3H), 0.83 (d, 3H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$) δ 214.0, 212.0, 174.6, 58.5, 57.5, 51.4, 46.5, 44.2, 43.7, 42.1, 38.3, 36.9, 36.8, 35.6 (C × 2), 35.4, 31.3, 30.5, 27.4, 26.6, 25.4, 24.3, 22.1, 18.6, 11.7. Analysis for $C_{25}H_{38}O_4$: calcd C, 74.59; H, 9.51. Found C, 74.70; H, 9.31.

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Supporting Information Available: General experimental methods (chemistry, cmc determination, and colon cancer cell biology), X-ray crystallographic information for **10**, **11**, **17**, and **21**, as well as ^{1}H NMR spectra for ***ent*-1** as well as **3**−**7** and **10**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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