JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Semisynthesis and Biological Evaluation of Ganodermanontriol and Its Stereoisomeric Triols

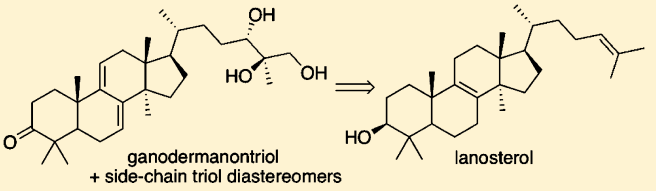
Erin M. Kennedy,[†] Steven J. P'Pool,[†,‡] Jiahua Jiang,[‡] Daniel Sliva,[‡] and Robert E. Minto*[,†]

[†]Department of Chemistry and Chemical Biology, Indiana University–Purdue University Indianapolis, 402 N. Blackford Street, Indianapolis, Indiana 46202, United States

[‡]Methodist Research Institute, Department of Medicine, and Indiana University Simon Cancer Center, School of Medicine, Indiana University, Indianapolis, Indiana 46202, United States

Supporting Information

ABSTRACT: The first synthesis of ganodermanontriol, a bioactive lanostane triterpene from the medicinal mushroom *Ganoderma lucidum*, has been achieved in 15.3% yield over nine steps, along with its three stereoisomeric triols and ganoderol A. The key steps leading to this family of isomers involve the reconstruction of the trisubstituted alkene by stereoselective and chemoselective phosphonate reactions and the formation of the unusual Δ7,9(11)-diene core by the mild acidic opening of a lanosterone-derived epoxide. Ganodermanontriol showed promising activity on the inhibition and proliferation of breast cancer cells. The effect of ganodermanontriol and its isomers on cell proliferation was assayed; IC_{50} values of 5.8 and 9.7 μM on breast cancer cell lines MCF-7 and MDA-MB-231, respectively, were found for ganodermanontriol.



Ganoderma lucidum (Reishi, Lingzhi) has been used in traditional Chinese medicine for thousands of years and is widely available in the form of dietary supplements. Ethnopharmacological studies of this ancient medicinal mushroom have shown that the triterpene extract and its components possess antiandrogenic,[1] anticomplement,[2] antihistamine,[3] anti-inflammatory,[4] antinociceptive,[5] antioxidant,[6] and hypocholestremic[7] properties and inhibit proliferation of cancer cells and tumor growth *in vitro* and *in vivo*.[8] Over 150 highly oxygenated triterpenes have been reported from *G. lucidum*; however, purification of novel steroids from the fruiting bodies of *G. lucidum* is unreliable due to variability of the natural product composition resulting from environmental and geographic factors, age, and handling. The content of the terpenoid acids and alcohols in *G. lucidum* tissue and spores has shown by HPLC with UV detection to fluctuate widely depending on the growth substrate.[9] The limited availability of the *Ganoderma* alcohols, in particular, impedes their detailed evaluation and was the impetus for our study.

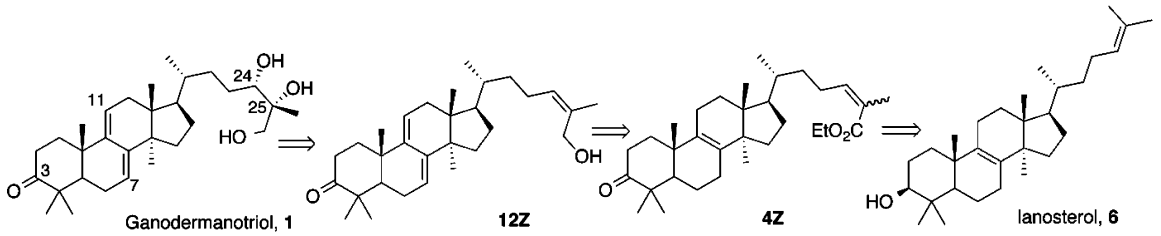
Hattori and co-workers examined the inhibition of human immunodeficiency virus (HIV) by a range of fungal natural products. A methanol extract of the fruiting bodies of *G. lucidum* showed moderate inhibitory effects against HIV-1 and its protease.[10a] In a primary screen for anti-HIV activity, the Δ7,9(11)-lanostadienes ganodermanontriol (**1**) and ganoderiol F inhibited an HIV-1-induced cytopathic effect in MT-4 cells.[10a] A subsequent study showed that ganoderic acid β, lucidumol B, ganodermanodiol, and ganodermanontriol have significant inhibitory activity against recombinant HIV-1 protease and that the hydroxyl groups at C-23 or C-24 and C-25 are essential for strong HIV-1 protease inhibition.[10b] Ganodermanontriol, as well as other triterpene alcohols, had significant anticomplement activity with IC_{50} values of 17.2 μM. The potency of triterpene alcohols in inhibiting classical pathway activity improved when the number of side-chain hydroxymethyl groups increased.

G. lucidum triterpenes also inhibit the invasive behavior and proliferation of breast and prostate cancer cells through the down-regulation of cyclin-D1 expression and suppression of urokinase-plasminogen activator secretion.[11] They inhibit growth, induce apoptosis, and suppress angiogenesis of breast, prostate, hepatoma, and colon cancer cells through a variety of mechanisms including the up-regulation of p21 and Bax expression, suppression of protein kinase C, activation of caspase-3, and inhibition of secretion of vascular endothelial growth factor (VEGF) and transforming growth factor-1 (TGF-1), respectively.[11] Ganodermanontriol suppressed proliferation of human colon cancer cells *in vitro* and inhibited tumor growth in a xenograft model of colon cancer *in vivo*.[10c]

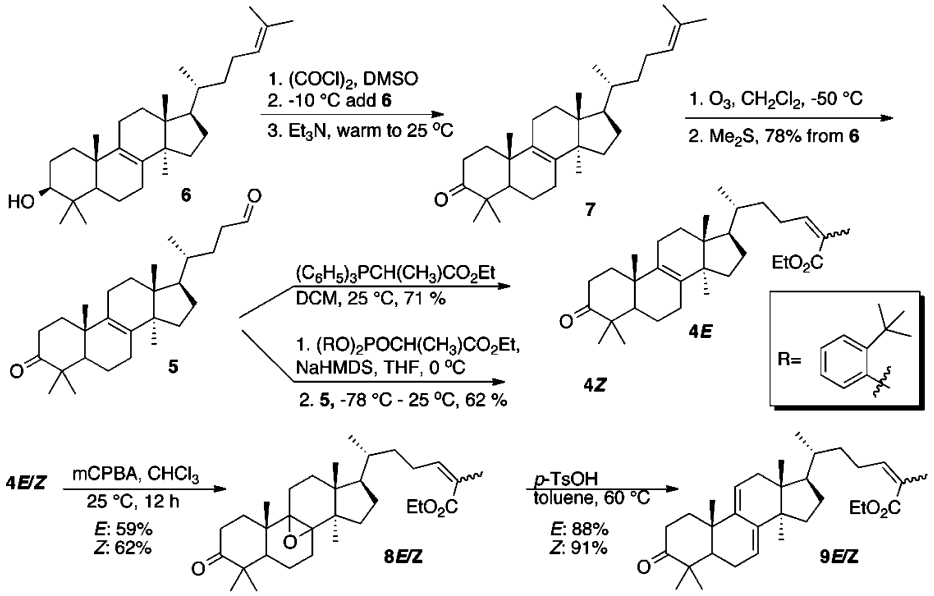
Evaluation of the health effects of *Ganoderma* triterpene products that are widely used for alternative health care is confounded by the lack of standardization and characterization of the extracts. The Sliva lab has recently shown that *G. lucidum* triterpene extract reduces proliferation of estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) breast cancer cells, possibly by the modulation of the estrogen receptor and NF-κB signaling.[8b] The identity of the molecular component(s) that plays the central role in the inhibition and proliferation of these breast cancer cell lines remains to be

Received: March 6, 2011
Published: November 1, 2011

Scheme 1. Retrosynthetic Analysis of Ganodermanontriol



Scheme 2. Construction of Dienone Core



determined, but it is hypothesized that ganodermanontriol was the active component.

Ganodermanontriol (**1**, Scheme 1) is the most abundant 7,9(11)-lanostenol in *Ganoderma* extracts.[9] We report the preparation of ganodermanontriol and three stereoisomeric triols and examine their relative effectiveness in the inhibition and proliferation of breast cancer cells.

RESULTS AND DISCUSSION

Retrosynthetically, we intended to install the diene of the tetracyclic core first (Scheme 1). The starting material, lanosterol (**6**), was the most cost-effective precursor with the correct methyl branch configurations.

However, we ultimately found that side-chain revisions were best interwoven with the diene core preparation through a five-step sequence (Scheme 2). The low solubility of **6** impeded ozonolysis; therefore, **6** (56% pure) was subjected to a Swern oxidation[12] of lanosterol, leading to a mixture of **7** and dihydrolanosterone, which is derived from the main contaminant in commercial lanosterol. Exhaustive ozonolysis of **7** formed aldehyde **5**, which could be easily purified from the 24,25-dihydrosteroid (Scheme 2).

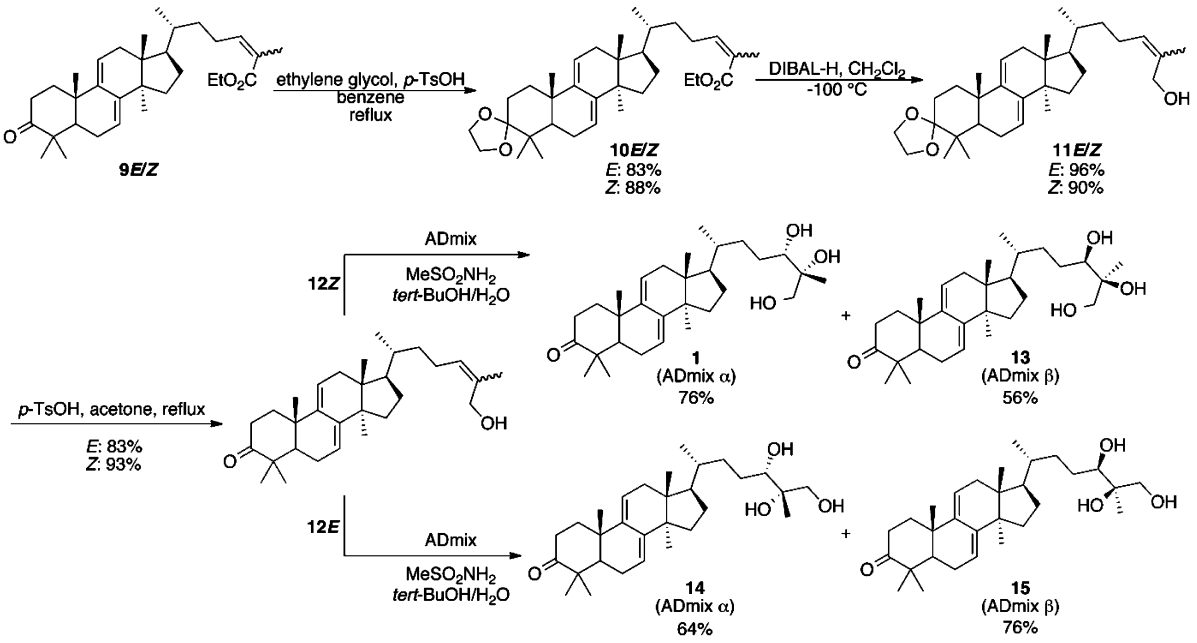
While other more arduous approaches exist,[13] a direct route to the Δ7,9(11)-diene core had been shown to proceed through the epoxide.[14] It has been reported that the 8,9-epoxide can be formed by ozone treatment and converted to the diene in a solvent-dependent fashion; however, our repeated efforts to reproducibly effect this reaction failed.[15] When direct mCPBA epoxidation of **5** was attempted, Baeyer-Villager oxidation occurred at the aldehyde that led to a 5:3:2 mixture of carboxylic acid, epoxide, and **5**. It was expected that the sterically protected, normally unreactive, tetrasubstituted 8,9-olefin would be more suceptible to reaction with peracids than the α,β-unsaturated ester, as it is more electron rich, and this led us to resequence the rebuilding of the side chain. Thus, the stereochemistry of the triol was tied retrosynthetically to the asymmetric dihydroxylation catalyst and trisubstituted alkene precursor (Scheme 1). The *Z*-olefin was generated as a precursor to ganodermanontriol and the (24*R*,25*S*)-diastereomer, while the *E*-olefin was prepared to access the two other isomeric triols.

The reported use of a methyl-branched phosphonate in an Ando-modified Horner-Wadsworth Emmons (HWE) reaction with moderate *Z*-stereoselectivity suggested an approach to **1**.[16] Reaction of the anion generated from ethyl 2-(bis(*o*-*tert*-butylphenoxy)phosphoryl)propanoate occurred at the aldehyde of **5** with excellent stereoselectivity to give *Z*-olefin **4*Z***. The effect of counterions during the deprotonation step was investigated to determine their influence upon *E*- or *Z*-selectivity. It was found that NaHMDS gave only the *Z*-olefin in 62% yield, while KHMDS and LiHMDS both gave an *E*/*Z* ratio of 1:9 in 58% and 43%, respectively. This result is at variance with the higher *Z*/*E*-selectivity previously observed with larger counterions in the formation of α,β-unsaturated esters.[17]

Selective formation of the *E*-olefin through phosphonate chemistry initially presented difficulties. HWE olefination of **5** using triethyl 2-phosphonopropionate in THF with the

dx.doi.org/10.1021/np200205n | *J. Nat. Prod.* 2011, 74, 2332–2337

Scheme 3. Elaboration of the 7,9(11)-Diene Core to Triols



addition of LiBr gave **4** with an *E*/*Z* ratio of 2:1,[18] but complete separation of the *E* and *Z* isomers was not attainable by flash chromatography. Ultimately, we found that the use of (carbethoxyethylidene)triphenylphosphorane gave a much better *E*/*Z* ratio of 22:1 in an isolated yield of 79%.[19] It should also be noted that in neither the phosphonate or phosphorane reactions was there any evidence of a reaction at the ketone. Selectivity toward the aldehyde is presumed to be driven by the greater electrophilicity and lower steric hindrance relative to the ketone. Peracid reactions with the unsaturated esters provided 59−62% yields of **8*E*/*Z***, along with starting material and an overepoxidized side product. Gentle opening of the epoxides with *p*-TsOH in toluene for 1 h provided improved access to the dienes **9*E*/*Z*** without purification in an isolated yield of 88−90%. The rate of epoxidation was greatly accelerated using $CHCl_3$, consistent with acid catalysis.[20]

For the reduction to the allylic alcohols, the ketones were protected with ethylene glycol and *p*-TsOH as **10*E*/*Z*** (Scheme 3). The esters were subsequently reduced with DIBAL under conditions that required careful optimization.[18] Reducing the reaction temperature from 0 °C to −100 °C coupled with a slow, syringe pump addition of DIBAL led to 90−96% yields. Facile acidic deprotection of the ketals gave the isomeric triols from the precursors **12**. Alcohol **12*E*** is ganoderol A, a natural product with significant hypocholestemic activity in mammalian cells potentially through inhibition of lanosterol 14α demethylase.[7b]

The triols were formed by Sharpless asymmetric dihydroxylations (AD) of **12**. Reaction of **12*Z*** and **12*E*** with 2.8 g/mmol of AD-mix-β gave **13** and **15**, respectively. Similarly, and consistent with the AD model that requires placement of the smallest group in the southeast quadrant,[21] oxidation of **12*Z*** and **12*E*** with AD-mix-α gave **1** and **14**, respectively. All spectroscopic data for synthetic **1** were in agreement with literature data.[22] Purification of the AD reactions gave 56−77% yields of the triols. AD distereoselectivities of the *Z*-alkenes were highest; a 17.6:1 diastereomeric ratio was found for the stereomatched reaction with AD-mix-β, and the ratio was 10.5:1 for the production of **1**. Diastereomeric ratios for the *E*-isomers were lower, at 2.7−5.7:1. Our results are consistent with the stereochemical biases for dihydroxylation of lanosterol and desmosterol ester substrates.[21,23] The *E*-isomer of **12** was found to be less reactive toward either AD-mix reagent compared to the *Z*-isomer, allowing the recovery of residual starting material. Inductive deactivation toward dihydroxylation of allylic alcohols and ethers versus alkyl-substituted alkenes has been observed when coordination to osmium is blocked, as is the case for ferricyanide-mediated AD-mix reactions.[24] For **12**, the rate of side-chain oxidation remained substantially greater than for the diene.

Ganodermanontriol showed promising inhibitory activity (IC_{50} = 5.8 μM at 72 h) on the proliferation of MCF-7 cancer cells (Supporting Figure 1), while an IC_{50} value of 9.7 μM was obtained for the MDA-MB-231 cell line (Supporting Figure 2). The other isomers were less active against the proliferation of cell lines MCF-7 and MDA-MB-231, as shown by their respective IC_{50} values (**13**: IC_{50} = 24.1, 33.8 μM; **14**: IC_{50} = 16.3, 36.7 μM; **15**: IC_{50} = 24.1, 11.3 μM).

In summary, the antiproliferative sterol ganodermanontriol was prepared in an overall yield of 15.3% over nine steps, and yields for the stereoisomeric triols ranged between 11.3% and 14.5%.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were performed under a nitrogen atmosphere with dry solvents in oven-baked or flame-dried glassware, unless otherwise noted. Tetrahydrofuran (THF) was dried by refluxing over sodium and benzophenone. Dichloromethane was distilled from calcium hydride. Triethylamine was distilled from calcium hydride. Anhydrous DMSO was obtained from Acros Organics. Ozone was produced using a calibrated corona discharge generator as described in the Supporting Information. Yields are reported for chromatographically homogeneous and spectroscopically pure materials, unless otherwised noted. Unless otherwise indicated, all other reagents were commercially available from Thermo Fisher/ACROS or Sigma-Aldrich and used without further purification. AD-mix-α and -β were furnished by Sigma-Aldrich (cat. 392758 and 392766, respectively). AD-mix-α is a powdered mixture of the ligand $(DHQ)_2PHAL$ (hydroquinine 1,4-phthalazinediyl diether),

dx.doi.org/10.1021/np200205n | J. Nat. Prod. 2011, 74, 2332−2337

K_2CO_3, $K_3Fe(CN)_6$, and $K_2OsO_4 \cdot 2H_2O$ in the molar ratio 0.0016:0.4988:0.4988:0.0007. In AD-mix-β, the ligand is replaced by $(DHQD)_2PHAL$ (hydroquinidine 1,4-phthalazinediyl diether). Lanosterol was purchased from Apin Chemicals Ltd. in Abington, Oxon, UK. Ethyl 2-bis(2-*tert*-butylphenoxy)phosphonopropionate was prepared by the method of Ando.[16b]

Physical Properties and Spectroscopic Measurements. Analytical thin layer chromatography (TLC) was performed on precoated aluminum sheets (200 μm thickness of F_{254} silica gel; Aldrich). The TLC plates were visualized with UV light and/or by staining with *p*-anisaldehyde solution (2.6 mL *p*-anisaldehyde + 1.1 mL acetic acid + 3.6 mL concentrated H_2SO_4 diluted to 100 mL with 95% ethanol) or by $KMnO_4$ (1% w/v + 6.6% w/v K_2CO_3 in 0.082% w/v aqueous NaOH). Silica gel (32−63 μm, Dynamic Adsorbents Inc., Atlanta, GA, USA) was slurry packed for flash column chromatography. Melting points were measured using a Thomas-Hoover capillary melting point apparatus. Optical rotations were determined on a Perkin-Elmer 241 polarimeter at 20 °C and 589 nm (sodium D-line). IR spectra were recorded using a Nicolet Avatar 330 FT-IR spectrophotometer with either KBr pellets or neat films on a NaCl disk prepared by evaporating a CH_2Cl_2 solution of the analyte. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra referenced to residual solvent signals ($CHCl_3$, δ 7.26 ppm and DMSO-d_6, δ 2.50 for ^1H spectra; $CHCl_3$, δ 77.00 ppm for ^{13}C spectroscopic data) were recorded on a Bruker Avance III spectrometer at 500 and 125 MHz, respectively. Filtered (DEPT-135, DEPT-90) and *z*-gradient two-dimensional (2D) NMR experiments (gsHSQC, gsHMBC, and gsCOSY90) were conducted using standard pulse sequences. Atmospheric pressure chemical ionization (APCI) high-resolution mass spectrometry (HRMS) data were recorded on an Agilent 1200 LC-6520 QTOF MS using the manufacturer's internal reference solution.

*(10S,13R,14R,17R)-4,4,10,13,14-Pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-4,5,6,7,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (lanosterone, **7**).* A solution of oxalyl chloride (4.60 mL, 53.4 mmol, 2.2 equiv) in CH_2Cl_2 (61 mL) was placed in a 100 mL three-neck round-bottom flask equipped with a thermometer and a magnetic stir bar. Dimethyl sulfoxide (8.30 mL, 116 mmol, 4.8 equiv) was dissolved in CH_2Cl_2 (12.1 mL) and added slowly by a dropping funnel to the stirred oxalyl chloride solution at a temperature between −50 and −60 °C in a dry ice/acetone bath. This temperature was maintained for 4 min. Next, a solution of lanosterol (**6**; 10.352 g, 24.26 mmol, 1.0 equiv) in DMSO (7 mL) and CH_2Cl_2 (55 mL) was added in one portion by dropping funnel to the stirred reaction mixture. The bath was switched to dry ice/ethylene glycol, and the temperature was increased to ca. −20 °C for 2 min. Triethylamine (16.9 mL, 121 mmol, 5.0 equiv) was added, and the solution stirred for 5 min before removing the cooling bath and allowing the mixture to warm to room temperature. Water (50 mL) was added, the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed twice with 1 N HCl (50 mL) and once each with water (50 mL), a 1% aqueous sodium bicarbonate (50 mL), and saturated NaCl solution (50 mL). The CH_2Cl_2 solution was then dried with anhydrous $MgSO_4$, filtered through a pad of Celite, and concentrated with rotary evaporator to afford crude **7** as a brownish-yellow solid (9.85 g). The product, which contains both lanosterone (**7**) and dihydrolanosterone, was carried to the ozonolysis step without further purification.

*(4R)-4-((10S,13R,14R,17R)-4,4,10,13,14-Pentamethyl-3-oxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanal (**5**).* A solution of crude lanosterone **7** (4.27 g, 9.11 mmol) in CH_2Cl_2 (128 mL) was placed in a three-neck round-bottom flask equipped with a magnetic stir bar, thermometer, a $CaCl_2$ drying tube, and a gas inlet tube. The mixture was stirred and cooled to −50 °C, at which point ozone was passed through the mixture for 17 min (total available ozone 5.53 mmol, 1.08 equiv). The reaction mixture was purged with argon for 20 min and then concentrated *in vacuo* to ca. 5 mL. After diluting the crude material with CH_2Cl_2 (50 mL), the organic phase was washed twice with water (50 mL) and dried with anhydrous $MgSO_4$. The crude product was concentrated with a rotary evaporator and purified by silica gel chromatography (step gradient of 5:1 then 3:1 hexane/EtOAc) to afford **5** as a white solid (1.578 g, 78% based on an initial purity of 56% for the commercial lanosterol **6**).

*Ethyl (6R,Z)-2-Methyl-6-((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-3-oxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate (**4Z**).* To a stirred solution of ethyl 2-bis(2-*tert*-butylphenoxy)phosphonopropionate (0.463 g, 1.04 mmol, 1.0 equiv) in THF (21 mL) cooled in an ice−water bath was added sodium hexamethyldisilazide (NaHMDS; 1.04 mL, 1.0 M in solvent, 1.04 mmol, 1.0 equiv). The deprotonation was allowed to proceed for 1 h. The reaction mixture was cooled to −78 °C; then a solution of aldehyde **5** (0.413 g, 1.04 mmol, 1.0 equiv) in THF (21 mL) was added in one portion. The solution was stirred for 20 min and was then allowed to warm to room temperature, and stirring was continued for 12 h. The resulting solution was diluted with CH_2Cl_2 (50 mL) and washed twice with saturated ammonium chloride solution (50 mL). The organic phase was dried with anhydrous $MgSO_4$ and vacuum filtered, and the filtrate evaporated under reduced pressure. The sample was purified by silica gel chromatography (step gradient of 20:1:1 to 8:1:1 hexane/EtOAc/CH_2Cl_2) to afford **4** as a pure white crystalline solid (0.311 g, 62%).

*Ethyl (6R,E)-2-Methyl-6-((10S,13R,14R,17R)-4,4,10,13,14-pentamethyl-3-oxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)hept-2-enoate (**4E**).* A mixture of aldehyde **5** (0.925 g, 2.32 mmol, 1.0 equiv) and (carbethoxyethylidene)triphenylphosphorane (0.925 g, 2.32 mmol, 1.1 equiv) was stirred overnight in CH_2Cl_2 (23 mL) at 25 °C and then concentrated *in vacuo*. The mixture was then purified by silica gel chromatography (5:1:1 *n*-hexane/EtOAc/CH_2Cl_2) to afford the ester as a clear oil (0.131 g, 71%).

General Epoxidation Procedure. A mixture of ester **4*E*** (0.444 g, 0.920 mmol, 1.0 equiv) and mCPBA (0.212 g, 0.920 mmol, 75%, 1.0 equiv) in $CHCl_3$ (16.0 mL) was stirred for 12 h at 25 °C. The reaction was then quenched by the addition of solid $Ca(OH)_2$ (0.273 g, 3.68 mmol, 4.0 equiv) and Na_2SO_4 (0.523 g, 3.68 mmol, 4.0 equiv) and stirred for 75 min. The solid was then filtered off, and the filtrate was concentrated with a rotary evaporator. The oily residue was then purified by silica gel chromatography (5:1:1 *n*-hexane/EtOAc/CH_2Cl_2) to afford **8*E*** as a clear oil (0.268 g, 59% yield). Ester **8*Z*** was prepared by an analogous procedure to afford **8*Z*** as a white crystal in 62% yield.

General Epoxide Openings. A mixture of epoxide **8*E*** (0.373 g, 0.748 mmol, 1.0 equiv) and *p*-toluenesulfonic acid (0.00142 g, 0.0748 mmol, 0.1 equiv) in toluene was stirred for 1 h at 60 °C. The reaction was then quenched with triethylamine (10.4 μL, 0.0748 mmol, 0.1 equiv). The solid ammonium salt was then filtered through a pad of silica gel and Celite that was rinsed with toluene to afford **9*E*** as a pure white crystalline solid upon concentration *in vacuo* (0.311 g, 88% yield). Diene **9*Z*** was prepared by an analogous procedure in 91% yield.

General Ketalizations. Ketone **9*E*** (0.257 g, 0.535 mmol, 1.0 equiv) was dissolved in benzene (27 mL). *p*-Toluenesulfonic acid (0.0102 g, 0.0535 mmol, 0.1 equiv) and ethylene glycol (0.298 mL, 5.35 mmol, 10 equiv) were added, and the solution was heated to reflux for 20 h using a modified Dean−Stark apparatus. The mixture was allowed to cool to room temperature and was then concentrated using a rotary evaporator. The residue was diluted with CH_2Cl_2 (30 mL), and the solution was washed with saturated sodium bicarbonate solution (3 × 30 mL) and saturated brine (30 mL), dried with $MgSO_4$, and vacuum filtered through a pad of Celite. The filtrate was concentrated with a rotary evaporator to give **10*E*** as a pure white crystalline product (0.246 g, 88% yield). Ketal **10*Z*** was prepared by an analogous procedure in 83% yield.

General Reduction of Esters. In a round-bottom flask containing a magnetic stir bar, ester **10*E*** (0.223 g, 0.425 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (1.7 mL) and cooled to −100 °C. Diisobutylaluminum hydride (1.31 mL, 0.97 M solution in hexanes, 1.28 mmol, 3.0 equiv) was added dropwise with the aid of a syringe

pump over 12 min. After 40 min had elapsed, saturated NH_4Cl solution (4 mL) was added, and the quenched reaction mixture was warmed to room temperature and stirred overnight. A white precipitate ensued, which was removed by filtration with a Celite-packed Büchner funnel. The contents of the funnel were washed with CH_2Cl_2 (15 mL), and the combined organic layers were then separated from the aqueous solution and washed with saturated brine (30 mL). The CH_2Cl_2 solution was dried over $MgSO_4$, vacuum filtered, and concentrated with a rotary evaporator to give **11*E*** as pure white crystals (0.197 g, 96% yield). Alcohol **11*Z*** was made by an analogous procedure in 90% yield.

Deprotection of Ketals. Alcohol **11*E*** (0.165 g, 0.342 mmol, 1.0 equiv) was dissolved in acetone (10 mL). *p*-Toluenesulfonic acid (0.0065 g, 0.034 mmol, 0.01 equiv) was added, and the mixture was stirred for 24 h at reflux. A rotary evaporator was used to remove the acetone, and the residual solid was then diluted with CH_2Cl_2 (10 mL) and washed with saturated sodium bicarbonate solution (2 × 10 mL) followed by saturated brine (10 mL). The organic layer was dried over $MgSO_4$ and vacuum filtered through a pad of Celite, and the resulting filtrate was then concentrated using a rotary evaporator to give **12*E*** as off-white crystals (0.124 g, 83% yield). Ketone **12*Z*** was made by an analogous procedure in 93% yield.

AD-Mix. Alcohol **12*Z*** (0.0138 g, 0.0325 mmol, 1.0 equiv) was dissolved in *t*-BuOH (0.33 mL) and water (0.33 mL). To this solution was added AD-mix-α (0.091 g, 2.8 g/mmol) followed by methanesulfonamide (0.0062 g, 0.065 mmol, 2.0 equiv). The solution was allowed to stir for 6 h at 25 °C. Saturated aqueous sodium sulfite (5 mL) was added, and the quenched reaction mixture was allowed to stir for 1 h. The mixture was then diluted with ethyl acetate (5 mL), the phases were separated, and the aqueous layer was then washed with ethyl acetate (4 × 5 mL). The combined organic layers were washed with saturated brine (20 mL), dried over $MgSO_4$, and vacuum filtered through a pad of Celite, and the resulting filtrate was evaporated to dryness using a rotary evaporator. The solid was then purified using silica gel chromatography (preabsorbed on silica gel, step gradient of 3:1 hexane/EtOAc followed by 100% MeOH) to afford **1** as a pure white crystalline solid (0.0114 g, 76% yield). The other isomeric triols were made by an analogous procedure to afford **13** (0.0100 g, 56% yield from **12*Z*** with AD-mix-β), **14** (0.0137 g, 64% yield **12*Z*** with AD-mix-α), and **15** (0.0169 g, 76% yield from **12*E*** with AD-mix-β).

*(10S,13R,14R,17R)-4,4,10,13,14-Pentamethyl-17-((2R,5S,6R)-5,6,7-trihydroxy-6-methylheptan-2-yl)-4,5,6,10,12,13,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (ganodermanontriol, **1**):* mp 145−147 °C; $[\alpha]_D^{23}$ +33.6; IR (KBr) ν_{max} 3343 (br), 3020, 2958, 2933, 2882, 1706, 1150, 1112, 1048, 812 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.51 (br d, J = 6.5 Hz, 1H), 5.38 (br d, J = 6.2 Hz, 1H), 4.86 (br s, 2H), 3.83 (d, J = 11.2 Hz, 1H), 3.49 (d, J = 11.3 Hz, 1H), 3.45 (br d, 1H), 2.77 (dt, J = 5.7, 14.6 Hz, 1H), 2.34 (m, 1H), 2.30−1.96 (m, 7H), 1.85−1.30 (m, 11H), 1.20 (s, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 1.08 (s, 3H), 0.92 (d, J = 6.4 Hz, 3H), 0.88 (s, 3H), 0.59 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 216.9, 144.5, 142.8, 119.9, 117.2, 79.3, 74.0, 67.6, 51.0, 50.7, 50.3, 47.5, 43.8, 43.4, 37.8, 37.2, 36.6, 36.5, 34.8, 33.5, 31.4, 28.9, 27.9, 25.4, 25.3, 23.7, 22.0, 21.0, 18.6, 15.7; HRMS calcd for $C_{30}H_{49}O_4$ $(M + H)^+$, 473.3625, found 473.3641.

*(10S,13R,14R,17R)-4,4,10,13,14-Pentamethyl-17-((2R,5R,6S)-5,6,7-trihydroxy-6-methylheptan-2-yl)-4,5,6,10,12,13,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (**13**):* mp 142−145 °C; $[\alpha]_D^{23}$ +36.3 (c 0.60, $CHCl_3$); IR (KBr) ν_{max} 3418 (br), 3040, 2964, 2927, 2881, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.51 (d, J = 6.7 Hz, 1H), 5.39 (d, J = 6.3 Hz, 1H), 4.76 (br s, 1H), 3.84 (d, J = 11.0 Hz, 1H), 3.50 (m, 2H), 2.80 (dt, J = 5.8, 14.6 Hz, 1H), 2.50 (br m, 2H), 2.34 (m, 1H), 2.31−1.98 (m, 6H), 1.76 (dt, J = 4.5, 14.0 Hz, 1H), 1.70−1.22 (m, 10H), 1.20 (s, 3H), 1.13 (s, 3H), 1.11 (s, 3H), 1.09 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.88 (s, 3H), 0.60 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 216.9, 144.5, 142.8, 119.9, 117.2, 77.2, 74.1, 69.3, 50.9, 50.7, 50.3, 47.5, 43.7, 37.8, 37.2, 36.6, 36.5, 34.8, 32.8, 31.4, 28.3, 27.9, 25.4, 25.3, 23.7, 22.7, 22.5, 22.0, 19.6, 18.6, 15.7; HRMS calcd for $C_{30}H_{49}O_4$ $(M + H)^+$ 473.3625, found 473.3619.

*(10S,13R,14R,17R)-4,4,10,13,14-Pentamethyl-17-((2R,5S,6S)-5,6,7-trihydroxy-6-methylheptan-2-yl)-4,5,6,10,12,13,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (**14**):* mp 139−141 °C; $[\alpha]_D^{23}$ +34.3 (c 0.87, $CHCl_3$); IR (KBr) ν_{max} 3477 (br), 3024, 2969, 2872, 1699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.51 (d, J = 6.8 Hz, 1H), 5.38 (d, J = 6.1 Hz, 1H), 3.65 (d, J = 11.1 Hz, 1H), 3.55 (m, 1H), 3.49 (d, J = 11.2 Hz, 1H), 2.78 (dt, J = 5.7, 14.5 Hz, 1H), 2.34 (m, 1H), 2.30−1.96 (m, 7H), 1.76 (dt, J = 4.3, 13.2 Hz, 1H), 1.68−1.2 (9H), 1.19 (s, 3H), 1.12 (s, 3H), 1.10 (s, 3H), 1.09 (s, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.87 (s, 3H), 0.59 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 216.8, 144.5, 142.8, 119.9, 117.2, 78.5, 73.9, 67.6, 51.0, 50.7, 50.3, 47.5, 43.7, 37.4, 37.2, 36.6, 34.8, 33.1, 31.4, 28.5, 27.9, 25.4, 25.3, 23.6, 22.0, 21.0, 18.3, 15.7; HRMS calcd for $C_{30}H_{49}O_4$ $(M + H)^+$ 473.3625, found 473.3616.

*(10S,13R,14R,17R)-4,4,10,13,14-Pentamethyl-17-((2R,5R,6R)-5,6,7-trihydroxy-6-methylheptan-2-yl)-4,5,6,10,12,13,14,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3(2H)-one (**15**):* mp 134−135 °C; $[\alpha]_D^{23}$ +25.1 (c 0.42, $CHCl_3$); IR (KBr) ν_{max} 3335, 3018, 2964, 2924, 2883, 1707 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.50 (d, J = 6.9 Hz, 1H), 5.38 (d, J = 6.5 Hz, 1H), 4.86 (br s, 1H), 3.64 (d, J = 11.2 Hz, 1H), 3.61 (m, 1H), 3.52 (d, J = 11.2 Hz, 1H), 2.77 (dt, J = 5.7, 14.6 Hz, 1H), 2.34 (ddd, J = 3.2, 4.4, 14.8 Hz, 1H), 2.21−1.97 (m, 6H), 1.75 (dt, J = 4.5 Hz, 13.7 Hz, 1H), 1.66−1.23 (m, 10H), 1.20 (s, 3H), 1.12 (s, 3H), 1.09 (s, 3H), 1.08 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 0.88 (s, 3H), 0.59 (s, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 216.9, 144.5, 142.8, 119.9, 117.2, 76.2, 74.1, 69.4, 51.0, 50.7, 50.3, 47.5, 43.7, 43.4, 37.8, 37.2, 36.6, 34.8, 32.4, 31.4, 28.0, 27.9, 25.4, 25.3, 23.6, 22.0, 19.6, 18.3, 15.7; HRMS calcd for $C_{30}H_{49}O_4$ $(M + H)^+$ 473.3625, found 473.3615.

Reproducible proton shifts were found in DMSO-d_6 for the hydroxyl proton on C-24 of purified triols (**1**, δ 4.33; **13**, δ 4.25; **14**, δ 4.11; and **15**, δ 4.05; all doublets, J = 6.4 ± 0.4 Hz). These signals were integrated with a relaxation delay of 2 s to determine the de for the asymmetric dihydroxylation reactions.

Cell Culture. The human breast cancer cell lines MCF-7 and MDA-MB-231, obtained from ATCC (Manassas, VA, USA), were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing penicillin (50 U/mL), streptomycin (50 U/mL), and 10% fetal bovine serum. Media and supplements were from Invitrogen (Grand Island, NY, USA).

Cell Proliferation Assay. Ganodermanontriol, **13**, **14**, and **15** (isomers) were dissolved in DMSO (Sigma; St. Louis, MO, USA) at a concentration of 10 mM and stored at 4 °C. Dulbecco's phosphate buffered saline was purchased from Cambrex Bio Science Walkersville, Inc. (Walkersville, MD, USA).

Cell proliferation was determined by the tetrazolium salt method (MTT method), according to the manufacturer's instructions (Promega, Madison, WI, USA). Briefly, MCF-7 and MDA-MB-231 cells (2.5 × 10^3/well) were cultured in a 96-well plate and treated with ganodermanontriol, **13**, **14**, and **15** (0−100 μM) for 24, 48, and 72 h. At the end of the incubation period, the cells were harvested and absorption was determined with an ELISA plate reader at 570 nm. Data points represent mean ± SD in triplicate determinations repeated at least twice. IC_{50} values were determined by using SigmaPlot (Systat Software Inc., San Jose, CA, USA).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Biological assay results for **1**, **13**, **14**, and **15** and analytical data for all of the newly synthesized compounds are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: (317) 274-6869. Fax: (317) 274-4701. E-mail: rminto@iupui.edu.

■ ACKNOWLEDGMENTS

We thank the National Science Foundation for supporting a fellowship to E.M.K. (DUE-0742475) and the NMR and MS instruments (CHE 0619254, DBI 0821661). The authors are grateful for postdoctoral support from Methodist Research Institute (S.J.P.) and research funding from the School of Science, IUPUI (R.E.M.).

■ REFERENCES

(1) (a) Liu, J.; Kurashiki, K.; Shimizu, K.; Kondo, R. *Biol. Pharm. Bull.* **2006**, *29*, 392−395. (b) Liu, J.; Kurashiki, K.; Shimizu, K.; Kondo, R. *Bioorg. Med. Chem.* **2006**, *14*, 8654−8660. (c) Liu, J.; Tamura, S.; Kurashiki, K.; Shimizu, K.; Noda, K.; Konishi, F.; Kumamoto, S.; Kondo, R. *Chem. Biodiversity* **2009**, *6*, 231−243.

(2) Min, B. S.; Gao, J. J.; Hattori, M.; Lee, H. K.; Kim, Y. H. *Planta Med.* **2001**, *67*, 811−814.

(3) Kohda, H.; Tokumoto, W.; Sakamoto, K.; Fujii, M.; Hirai, Y.; Yamasaki, K.; Komoda, Y.; Nakamura, H.; Ishihara, S.; Uchida, M. *Chem. Pharm. Bull* **1985**, *33*, 1367−1374.

(4) (a) Ko, H. H.; Hung, C. F.; Wang, J. P.; Lin, C. N. *Phytochemistry* **2008**, *69*, 234−239. (b) Dudhgaonkar, S.; Thyagarajan, A.; Sliva, D. *Int. Immunopharmacol.* **2009**, *11*, 1272−1280.

(5) Koyama, K.; Imaizumi, T.; Akiba, M.; Kinoshita, K.; Takahashi, K.; Suzuki, A.; Yana, S.; Horie, S.; Wantanabe, K.; Naoi, Y. *Planta Med.* **1997**, *63*, 224−227.

(6) Zhu, M.; Chang, Q.; Wong, L. K.; Chong, F. S.; Li, R. C. *Phytother. Res.* **1999**, *13*, 529−531.

(7) (a) Shiao, M. S. *Chem. Rec.* **2003**, *3*, 172−180. (b) Berger, A.; Rein, D.; Kratky, E.; Monnard, I.; Hajjaj, H.; Meirim, I.; Piguet-Welsch, C.; Hauser, J.; Mace, K.; Niedeberger, P. *Lipids Health Dis.* **2004**, *3*, 2. (c) Hajjaj, H.; Macé, C.; Roberts, M.; Niederberger, P.; Fay, L. B. *Appl. Environ. Microbiol.* **2005**, *71*, 3653−3658.

(8) (a) Gao, J.-J.; Min, B.-S.; Ahn, E.-M.; Nakamura, N.; Lee, H.-K.; Hattori, M. *Chem. Pharm. Bull.* **2002**, *50*, 837−840. (b) Jiang, J.; Silvova, V.; Sliva, D. *Int. J. Oncol.* **2006**, *29*, 695−703. (c) Jiang, J.; Grieb, B.; Thyagarajan, A.; Sliva, D. *Int. J. Mol. Med.* **2008**, *21*, 577−584. (d) Thyagarajan, A.; Jedinak, A.; Nguyen, H.; Terry, C.; Baldridge, L.; Jiang, J.; Sliva, D. *Nutr. Cancer* **2010**, *62*, 630−640.

(9) Gao, J.-J.; Nakamura, N.; Min, B.-S.; Hirakawa, A.; Zuo, F.; Hattori, M. *Chem. Pharm. Bull.* **2004**, *52*, 688−695.

(10) (a) El-Mekkawy, S.; Meselhy, M. R.; Nakamura, N.; Tezuka, Y.; Hattori, M.; Kakiuchi, N.; Shimotohno, K.; Kawahta, T.; Otake, T. *Phytochemistry* **1998**, *49*, 1651−1657. (b) Min, B. S.; Nakamura, N.; Miyashiro, H.; Bae, K. W.; Hattori, M. *Chem. Pharm. Bull* **1998**, *46*, 1607−1612. (c) Jedinak, A.; Thyagarajan-Sahu, A.; Jiang, J.; Sliva, D. *Int. J. Oncol.* **2011**, *38*, 761−767.

(11) Sliva, D. *Leukemia Res.* **2006**, *30*, 767−768.

(12) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480−2482.

(13) (a) Solaja, B.; Dermanovic, M. *J. Serb. Chem. Soc.* **1993**, *58*, 275−279. (b) Solaja, B.; Dermanovic, M.; Lim, D.-M.; Paik, Y.-K.; Tinant, B.; Declerq, J.-P. *Steroids* **1997**, *62*, 709−718.

(14) Brewis, S.; Halsall, T. G.; Sayer, G. C. *J. Chem. Soc.* **1962**, 2763−2766.

(15) Paryzek, Z.; Martynow, J. *Can. J. Chem.* **1988**, *66*, 2130−2136.

(16) (a) Ando, K. *Tetrahedron Lett.* **1995**, *36*, 4105−4108. (b) Ando, K. *J. Org. Chem.* **1998**, *63*, 8411−8416. (c) Franci, X.; Martina, S. L. X.; McGrady, J. E.; Webb, M. R.; Donald, C.; Taylor, R. J. K. *Tetrahedron Lett.* **2003**, *44*, 7735−7740.

(17) Touchard, F. P.; Capelle, N.; Mercier, M. *Adv. Synth. Catal.* **2005**, *347*, 707−711.

(18) Chun, J.; Byun, H. S.; Bittman, R. *J. Org. Chem.* **2003**, *68*, 348−354.

(19) Gemma, S. G.; Gabellieri, E.; Coccone, S. S.; Marti, F.; Taglialatela-Scafati, O.; Novellino, E.; Campiani, G.; Butini, S. *J. Org. Chem.* **2010**, *75*, 2333−2340.

(20) Bach, R. D.; Canepa, C.; Winter, J. E.; Blanchette, P. E. *J. Org. Chem.* **1997**, *62*, 5191−5197.

(21) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.

(22) Arisawa, M.; Fujita, A.; Hayashi, T.; Shimizu, M.; Morita, N.; Kikuchi, T.; Kadota, S.; Tezuka, Y. *J. Nat. Prod.* **1988**, *51*, 54−59.

(23) Okumura, K.; Nakamura, Y.; Takeuchi, S.; Kato, I.; Fujimoto, Y.; Ikekawa, N. *Chem. Pharm. Bull.* **2003**, *51*, 1177−1182.

(24) (a) Xu, D.; Crispino, G. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1992**, *114*, 7570−7571. (b) Xu, D.; Park, C. Y.; Sharpless, K. B. *Tetrahedron Lett.* **1994**, *35*, 2495−2498.