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A Concise Synthesis of *ent*-Cholesterol

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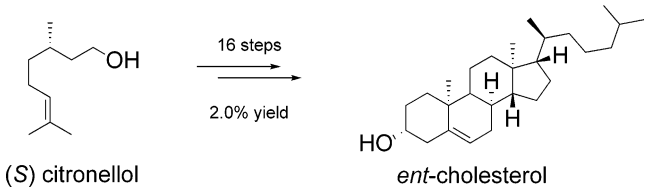

ent-Cholesterol was synthesized in 16 steps from commercially available (*S*)-citronellol. The overall yield for the synthesis was 2.0%. This route is amenable to gram-scale preparation of *ent*-cholesterol. Isotopic incorporation near the end of the synthesis was achieved using labeled methyl iodide. This synthesis is the most practical to date and will make *ent*-cholesterol more readily available to use as a probe of the function and metabolism of cholesterol.

Introduction

Cholesterol is a steroidal biomolecule that plays a vital role in cellular functions. Molecular mechanisms underlying these cellular functions of cholesterol are still largely unclear. The unnatural enantiomer of cholesterol (Figure 1) can serve as a valuable biological probe as its chemical composition, bonding pattern, and relative configuration are the same as the natural cholesterol. Since the enantiomer of cholesterol is not found in nature, it must be prepared by chemical synthesis. Our group reported the first synthesis of *ent*-cholesterol in 1992.[1] It was then used as a probe to study the interactions of cholesterol with amphotericin B and its role in the formation of ion channels.[2] *ent*-Cholesterol has subsequently been synthesized using several different procedures.[3] The most effective route involved the preparation of a D-ring synthon with an intact side chain and subsequent elaboration of the CBA rings. Herein we report a new and more efficient synthesis of *ent*-cholesterol.

Cholesterol plays a central role in cell membranes and is the biogenetic precursor to a number steroid hormones. Its enantiomer, *ent*-cholesterol has been used to probe the role of cholesterol and specifically to differentiate the general role of cholesterol in membranes with stereospecific interactions with proteins and other chiral molecules. Covey and co-workers have led the way in using *ent*-cholesterol to probe the function of

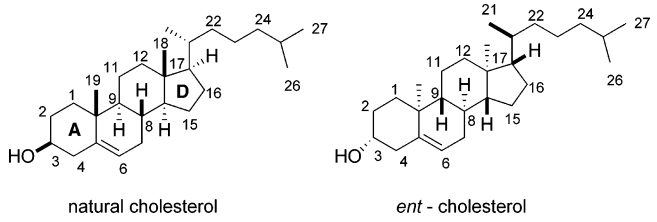

FIGURE 1. Natural cholesterol and *ent*-cholesterol.

cholesterol.[4] Westover and Covey studied the effects of cholesterol on epidermal growth factor (EGF) and found that the effects are not enantioselective.[5] More recently, Cohen and Rychnovsky have conducted experiments to define the structural features of sterols required for mammalian growth. The results indicated that sterols fulfill two roles in mammalian cells: a bulk membrane requirement in which phytosterols can substitute for cholesterol and other processes that specifically require small amounts of cholesterol but are not completely enantioselective.[6] Epand and Rychnovsky have shown that specific peptide chirality is not required for cholesterol containing membranes. However, a specific chirality of membrane lipids is required for peptide-induced formation of cholesterol-rich domains.[7]

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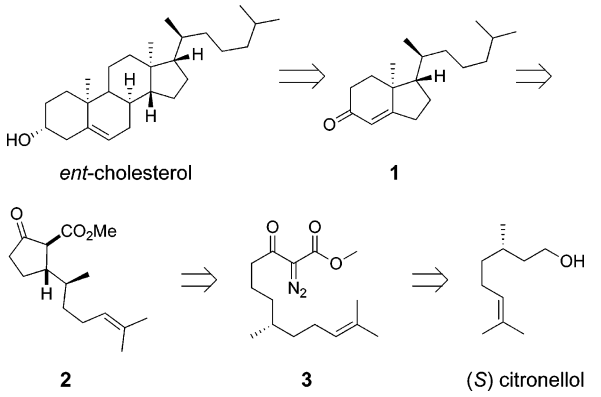


FIGURE 2. Retrosynthetic analysis of *ent*-cholesterol.

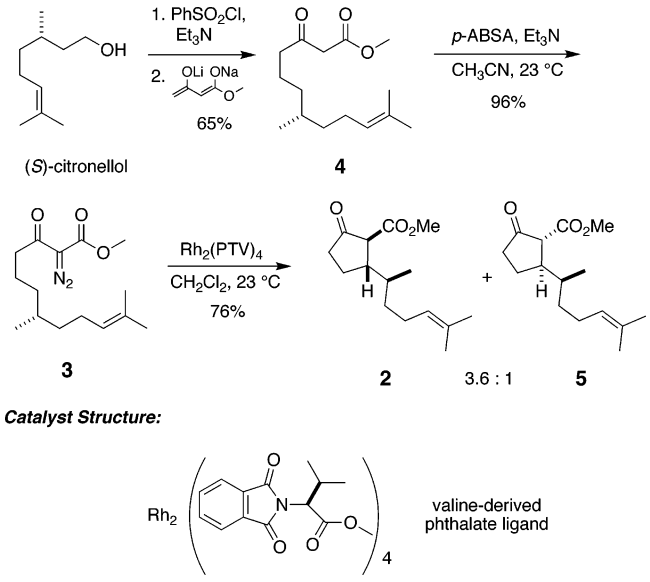
These and other studies have provided new insights into the mechanisms of cellular processes involving cholesterol; however, much remains to be learned.

One approach to probing cholesterol metabolism would be to study *ent*-cholesterol distribution in whole animals. Such studies would require significant quantities of *ent*-cholesterol, and none of the syntheses reported thus far are easily adaptable to preparation of *ent*-cholesterol on a gram scale. Our current efforts were aimed at the development of a practical, scalable route to this useful probe molecule. An ideal synthesis would be short, proceed in high yield, and allow for easy incorporation of heavier isotopes of carbon (^{13}C, ^{14}C) and hydrogen (^{2}H, ^{3}H) to facilitate distribution and metabolism analysis in vivo. We have developed a new route that achieves these goals. The retrosynthetic analysis of our new route is shown in the Figure 2. The synthesis is based on a ring D to C to B to A approach and incorporates the cholesterol side chain early in the synthesis. The synthesis of the D-ring intermediate was inspired by Taber's elegant synthesis of (−)-astrogorgiadiol, in which he prepared the intermediate **2** using CH insertion methodology.[8]

Results

Commercially available (*S*)-citronellol (>97% ee)[9] was converted to the corresponding benzenesulfonate and subsequently alkylated with the dianion of methyl acetoacetate (see Scheme 1).[10] The resultant β-keto ester **4** could be easily distilled on large scale under high vacuum to provide the product in 63% yield over two steps. The β-keto ester **4** was converted into α-diazo-β-keto ester **3** using *p*-acetamidobenzenesulfonic azide (*p*-ABSA) in 96% yield.[11] Taber reported use of Hashimoto's PTPA catalyst[12] for the diastereoselective C−H insertion reaction to provide a ratio of 2.6:1 in favor of the desired diastereomer. This key CH insertion reaction is pivotal to the success of the route, and we hoped to improve this ratio by catalyst optimization. Hence, the precursor for the C−H insertion was subjected to a series of chiral Rh catalysts. Both phthalate and naphthalate imides were investigated, with the smaller phthalates showing consistently higher selectivity (see Supporting Information). The valine-derived phthalate ligand gave the best ratio (3.6:1) and was more selective than ligands with either more or less bulky substituents. The keto ester **2** was crystalline and could be easily recrystallized from ethanol to >99% ee.

SCHEME 1. Synthesis of the C−H Insertion Product



The yield on the C−H insertion step is a bit vague in the literature because the separation of the two diastereomers is difficult. When the reaction was carried out on a small scale and the product was purified by chromatography, we isolated 76% of a 3.6:1 mixture of keto ester **2** and its C13, C14 diastereomer **5**. When conducted on a large scale, the crude product was filtered through a plug of silica gel and crystallized directly to give 29.5% of keto ester **2** with 99% ee and 99% de. The mother liquors contained a great deal more keto ester **2**, accompanied by its diastereomer **5**. Taber had shown that the mother liquor diastereomers (14% de) could be separated using an enantioselective reaction to reduce the ketone **5** selectively.[8] This process resulted in the recovery of 29% of the crude mass with 99% de after recrystallization. Taber's total yield (40% + 16%) is 56%. Our total yield of pure keto ester **2** on large scale is 29.5%, because we have not processed the mother liquors. The overall yield for the synthesis is calculated using 29.5% for this step but could be almost twice that based on precedent from Taber's group.

The presence of the C24−C25 alkene in keto ester **2** could be used to prepare functionalized or substituted sterol side

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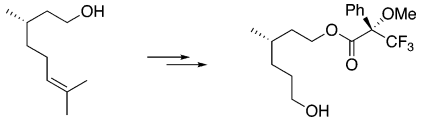
(8) Malcolm, S. C.; Taber, D. F. *J. Org. Chem.* **2001**, *66*, 944−953.

(9) The optical purity of (*S*)-citronellol was found to vary with supplier, and the optical rotation was an unreliable indicator of optical purity. (*S*)-Citronellol was derivatized with (*R*)-Mosher acid chloride to give the (*S*)-Mosher ester. Ozonolysis and $NaBH_4$ reduction gave a primary alcohol. The optical purity of this alcohol could be assayed using HPLC analysis on a Chiracel OD-H column, eluting with 3% *i*-PrOH in hexanes. The optical purity of (S)-citronellol purchased from TCI was found to be ca. 60% ee, whereas the more expensive (*S*)-citronellol from Aldrich was found to be >97% ee.



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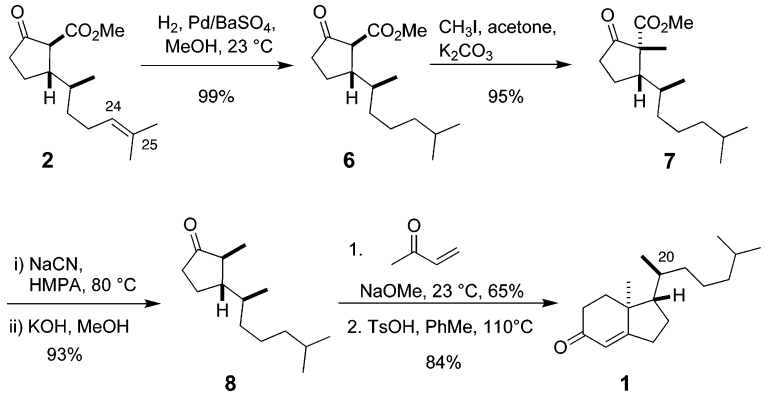
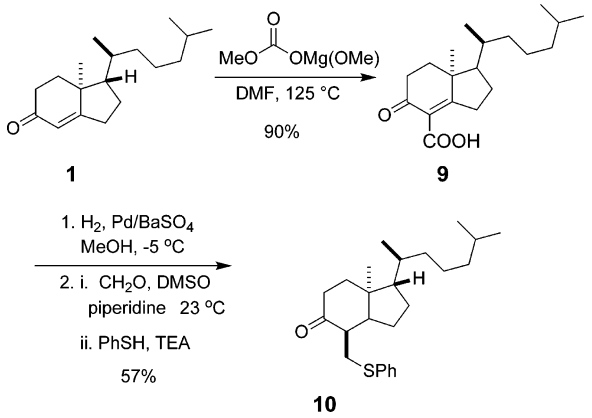
(12) Hashimoto, S.; Watanabe, N.; Sato, T.; Shiro, M.; Ikegami, S. *Tetrahedron Lett.* **1993**, *34*, 5109−5112.

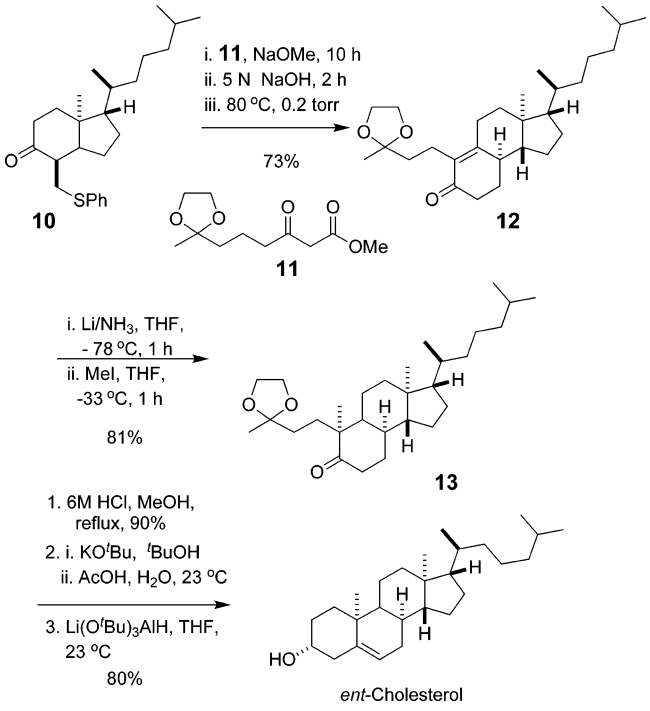
SCHEME 2. Formation of C,D-Ring Enone



SCHEME 3. Synthesis of Thiophenol Adduct



SCHEME 4. Completing the Synthesis of *ent*-Cholesterol



chains; however, for the synthesis of *ent*-cholesterol, the alkene simply needed to be reduced. Hydrogenation of keto ester **2** using Pd/C provided compound **6** with a saturated side chain (Scheme 2). Methylation of β-keto ester **6** using MeI and K_2CO_3, followed by decarbomethoxylation gave the desired α-methyl ketone **8** as a single diastereomer in 86% yield over three steps. Robinson annulation of ketone **8** with methyl vinyl ketone in the presence of NaOMe proceeded from the more substituted thermodynamic enolate. Treatment of the Michael adduct with *p*-toluenesulfonic acid catalyzed the aldol condensation and provided the CD enone **1** in 55% yield from ketone **8**. Taber's C−H insertion strategy drastically shortens the synthesis of the sterol side chain and allows the C20 stereogenic center to be introduced from a chiral pool source.

The initial strategy for the conversion of enone **1** to *ent*-cholesterol used the same BA double annulation strategy developed by Hoffmann La Roche chemists that was used in our first synthesis (Scheme 3).[1,13] Thus, treatment of CD-enone **1** with Stile's reagent[14] provided the desired acid **9** in 90% yield. Purification of the acid was not easy as the hydrophobic side chain made the acid−base extraction difficult. Hydrogenation of the crude acid with $Pd/BaSO_4$ at 10 °C was monitored by TLC and reaction was worked up rapidly. The solvent, methanol, was removed under high vacuum while maintaining the temperature below 0 °C because the saturated β-keto acid was very susceptible to decarboxylation.[15] The crude saturated acid was treated with fresh aqueous formaldehyde in the presence of piperidine, followed by the addition of a solution of thiophenol in CH_2Cl_2 and Et_3N to mask the newly formed *exo*-enone as a thioether. The desired thioether **10** was obtained in 51% overall yield over four steps from CD enone **1**. While this strategy was successful, it required very careful handling to avoid the problematic decarboxylation.[15]

The BA rings were introduced using the β-keto ester **11** as illustrated in Scheme 4.[13] Annulation of the thiophenol adduct **10** with β-keto ester **11**[13c] proceeded as expected to provide the tricyclic enone **12** in 73% yield. Reduction of the enone with Li/NH_3 followed by *in situ* alkylation with MeI installed the C19 methyl group stereoselectively in 80% yield. This step must be run at moderate dilution to avoid over-alkylation and

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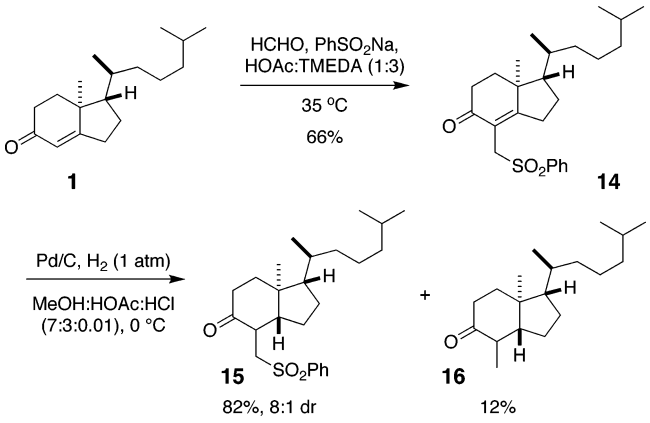
(14) Stile, M. J. *J. Am. Chem. Soc.* **1959**, *81*, 2598−2599.

(15) Covey synthesized CD enone **1** by a different route and reported that its conversion to acid **9** was problematic due to difficulties in the purification.[3] Their attempted conversion of acid **9** to the *exo* enone intermediate by treatment with formaldehyde and piperidine led to a "complex mixture of products." In our hands the sequence was successful with very careful handling but was not easily scalable.

SCHEME 5. Improved Strategy for Preparing the CD-Ring *trans*-Hydrindane



other side reactions. Acid-catalyzed deprotection of the ketal followed by aldol condensation provided the A ring of *ent*-cholestenone in 90% yield. The AB ring functionality was modified by deprotonation using KO^{t}Bu followed by kinetic protonation to provide the deconjugated ketone and diastereoselective reduction of the ketone with Li(O^{t}Bu)$_3$AlH gave *ent*-cholesterol in 80% yield over two steps.[1,16]

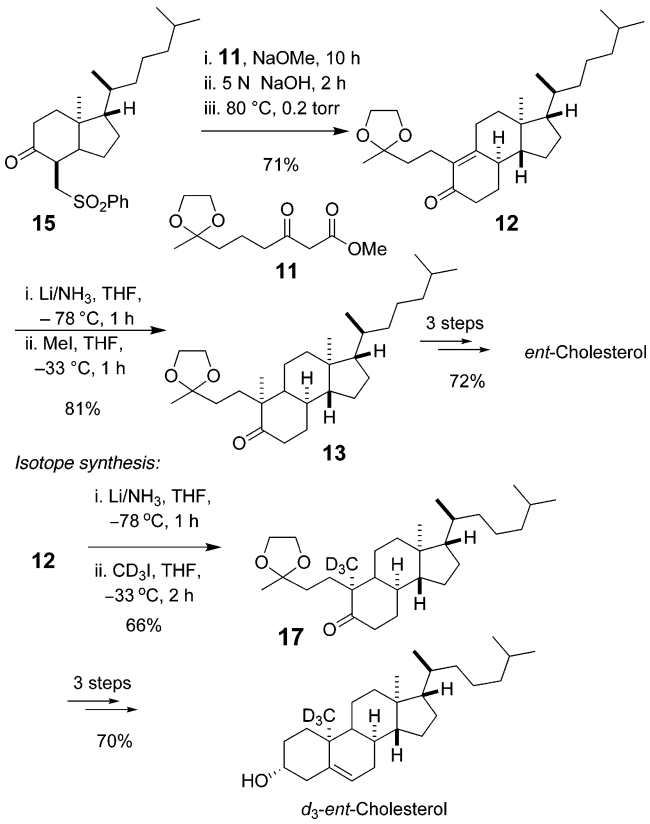
The synthesis described above used a D−C−B−A assembly of the steroid molecule in a 17 steps linear sequence from commercially available (*S*)-citronellol. The synthesis proceeded with a 1.9% overall yield from (*S*)-citronellol. However, conversion of the CD-enone to the thiophenol adduct was a challenging transformation: the temperature control and short handling times were difficult to achieve with larger-scale reactions, and the yields were reduced upon scaling up due to premature decarboxylation. Furthermore, the workup for acid **9** is difficult due to solubility problems. For these reasons, the synthetic approach to *ent*-cholesterol was further refined to avoid the issues inherent in the Stiles' carboxylation route. We explored two separate strategies to replace the Stile's carboxylation route.

The first alternative involved a selective conjugate reduction of enone **1** to provide the *trans*-hydrindane system followed by *in situ* trapping of the enolate with an electrophile (see Supporting Information). The conjugate reduction step was investigated, and although yields were generally high, the best selectivity was only 4:1 in favor of the *trans* ring junction. Hence, we abandoned further consideration of this approach. In the second approach, we turned our attention to an addition−elimination sequence first reported by Sauer for the synthesis of D-norgestrel.[17] The improved route to a CD enone precursor **15** is illustrated in Scheme 5. Treatment of the CD-enone **1** with formaldehyde and benzenesulfinic acid in a mixture of *N*,*N*,*N*′,*N*′-tetramethylenediamine and acetic acid provided sulfone **14** in 66% yield. Hydrogenation using Pd/C in EtOH with 5% aqueous hydrochloric acid provided the saturated sulfone in 2:1 (*trans*:*cis*) facial selectivity. Optimization of hydrogenation conditions is shown in Supporting Information. More polar solvents and the addition of acid improved the *trans*/*cis* ratio of the product, and added acid minimized the formation of the over-reduced product **16**. In the optimized conditions, a combination of methanol, acetic acid and dilute HCl (1 N) in a ratio of 70:30:0.01 gave the *trans*-hydrindane as the major product (8:1) in a combined 82% yield. Recrystallization from isopropanol provided diastereomerically pure sulfone **15** with *trans*-ring junction (62%). Purification of the mother liquor containing about a 1:1 mixture of the two diastereomers using HPLC provided another 9% of the desired *trans*-hydrindane product. A crystal structure of the sulfone **15** confirmed its configuration (see Supporting Information). A major side product (8−12%) in hydrogenation reactions was compound **16**, which results from elimination of the sulfone followed by hydrogenation of the resulting exocyclic olefin. This byproduct was easily removed by column chromatography.

The synthesis was completed by AB ring annulations and enone reduction as outlined in Scheme 6. Annulation of *trans*-sulfone **15** with β-keto ester **11** afforded the desired tricyclic enone in 71% yield. Tricyclic enone **12** was identical by spectroscopy and optical rotation to that obtained by annulation with thiophenyl adduct **10** (Scheme 4). Enone **12** was elaborated to *ent*-cholesterol, as described previously, in four steps and 57% overall yield.

In many cases biochemical studies can be facilitated by isotopically labeling the unnatural compound to facilitate subsequent analysis by MS or NMR spectroscopy. There are several points where one could add an isotopic label in this improved *ent*-cholesterol synthesis. The simplest labeling strategy was to replace the C19 methyl group, which is derived from methyl iodide, with an isotopic variant. This strategy was easily achieved by replacing methyl iodide with CD_3I in the

SCHEME 6. Improved Synthesis of *ent*-Cholesterol and Synthesis of 19*d*$_3$-*ent*-Cholesterol



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(17) Sauer, G.; Eder, U.; Haffer, G.; G.; Neef, G.; Wiechert, R. *Angew. Chem., Int. Ed.* **1975**, *14*, 413−414.

reductive alkylation of enone **12** (Scheme 6). Further elaboration and modification of ring A provided deutero-*ent*-cholesterol in good yields.

Conclusion

The total synthesis of *ent*-cholesterol using C−H insertion as the key step was achieved in 16 steps with an overall yield of 2.0%. The route uses commercially available (*S*)-citronellol and a diastereoselective C−H insertion reaction to provide the D-ring synthon with the *ent*-cholesterol side chain already attached. Two crystalline intermediates (the CH insertion product **2** and the hydrogenated CD-ring sulfone **15**) facilitate purification and separation of minor diastereomers. While the reductive alkylation step must be run at moderate dilution (and thus on a limited scale), this step occurs near the end of the synthesis and does not present a practical limitation for material throughput. Isotopic labels may be introduced late in the synthesis using labeled methyl iodide, a practical and inexpensive source of isotopic atoms. This synthetic pathway has a potential to supply gram quantities of *ent*-cholesterol for biological studies.

Experimental Section

***β*-Ketoester (2).** To a stirred solution of diazo-*β*-ketoester **3** (64 g, 0.22 mol) in dry CH_2Cl_2 (800 mL) cooled in an ice bath was added Rh_2 (*R*)-(PTV)$_4$ (1.5 g, 0.45 mol%) at 10 °C. Immediate evolution of N_2 gas was observed. Temperature was not allowed to rise above room temperature. Reaction mixture was stirred until evolution of the gas ceased (∼2−3 h). Solvent was removed under vacuum, and the crude material was loaded on a silica plug (∼500 mL) and eluted with 20% ether−hexanes mixture (6 × 1000 mL) to afford 56.2 g of crude yellow oil that was seeded with 99% ee crystals to afford 17 g (30%) of the desired diastereomerically pure CH insertion product **2**. Physical and spectroscopic data were consistent with literature data:[8] mp = 54−55 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.07 (1H, m), 3.74 (3H, s), 2.96 (1H, d, J = 11.4 Hz), 2.56 (1H, m), 2.45−2.30 (2H, m), 2.17 (1H, m), 2.05 (1H, m), 1.92 (1H, m), 1.69 (3H, s), 1.61 (3H, s), 1.59−1.40 (3H, m), 1.15 (1H, m), 0.87 (3H, d, J = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.5, 170.9, 131.9, 124.4, 59.8, 52.6, 47.0, 38.8, 37.0, 34.0, 25.9, 25.5, 25.4, 17.8, 17.1; IR (film in $CDCl_3$) 2956, 1755, 1726, 1435 cm^{-1} HRMS (ESI) *m*/*z* calcd for $C_{15}H_{24}O_3Na$ $(M + Na)^+$ 275.1623, found 275.1623.

Enantiopure CD-Enone (1). To a solution of NaOMe (freshly prepared from 30 mL MeOH and 0.6 g of Na) was added a solution of ketone **8** (3.6 g, 17.1 mmol) in MeOH (10 mL) at 0 °C. After stirring for 5 min at 0 °C, methyl vinyl ketone (5 mL, 61.7 mmol) was added, and the yellow mixture was stirred at 23 °C for 6 h. The reaction was quenched with saturated aqueous NH_4Cl (20 mL) and was concentrated. The crude oil was extracted with EtOAc (3 × 25 mL), concentrated, and then passed through a silica plug using a mixture of EtOAc, hexanes, and MeOH (10:89:1) to afford a yellow oil (3.7 g) that was dissolved in toluene (100 mL). *p*-Toluenesulfonic acid (250 mg, 1.4 mmol) was added, and the mixture was heated at reflux temperature for 3 h in a flask attached to Dean−Stark trap and condenser. The reaction mixture was washed with saturated $NaHCO_3$, concentrated, and chromatographed with 10% EtOAc−hexanes to provide 2.45 g (55% over two steps) of the CD enone **1** as a yellow oil. Physical and spectroscopic data were consistent with literature data:[3] $[\alpha]^{24}_D$ −64.3, (c = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.72 (1 H, s), 2.61 (1 H, dd, J = 10.9, 19.7 Hz), 2.51 (1 H, m), 2.40 (1 H, m), 2.34−2.20 (2 H, m), 2.0 (1 H, m), 1.80 (1 H, td, J = 4.9, 13.8, 18.6 Hz), 1.53 (3 H, m), 1.30−1.45 (3 H, m), 1.10−1.25 (4 H, m), 1.08 (3 H, s), 0.97 (3 H, d, J = 6.6 Hz), 0.862 (3 H, d, J = 6.6 Hz), 0.858 (3 H, d, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.5, 180.3, 121.6, 56.0, 45.2, 39.3, 37.2, 35.9, 34.5, 33.7, 29.1, 28.2, 27.0, 23.9, 22.9, 22.7, 18.9, 16.3 IR (film in $CDCl_3$) 2929, 1742, 1463, 1383, 1158 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{18}H_{30}ONa$ $(M + Na)^+$ 285.2194, found 285.2191.

Sulfone (14). Enone **1** (1.6 g, 6.15 mmol, 1.0 equiv) was dissolved in a premixed solution of HOAc (20 mL) and TMEDA (60 mL), and sodium benzenesulfinic acid (recrystallized from EtOH, 1.32 g, 8.39 mmol, 1.36 equiv) and paraformaldehyde (0.36 g, 12 mmol, 1.95 equiv) were added. The reaction mixture was heated to 60 °C for 5 h under inert atmosphere. The reaction was quenched with H_2O (10 mL), and EtOAc (75 mL) was added. The mixture was then washed with H_2O (250 mL), aqueous HCl (10%, 50 mL) and brine (50 mL). The organic layer was dried, concentrated and passed through a 1-in. silica plug to provide 2.7 g of crude oil. Flash chromatography with 10% EtOAc−hexanes provided 1.7 g (66%) of desired sulfone **14** as a colorless oil. The reaction can also be carried out at lower temperature (35 °C); however reaction times are usually longer (∼36 h) and yields are very similar. Sulfone **14**: $[\alpha]^{24}_D$ −67.5, (c = 2.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (2 H, d, J = 7.2 Hz), 7.62 (1 H, t, J = 7.6 Hz), 7.52 (2 H, t, J = 8 Hz), 4.26 (1 H, d, J = 13.6 Hz), 4.02 (1 H, d, J = 13.6 Hz), 2.82 (1 H, dd, J = 10.1, 20.4 Hz), 2.66 (1 H, m), 2.35 (1 H, m), 2.20 (1 H, m), 2.05 (1 H, m), 1.77 (1 H, td, J = 5, 13.9 Hz), 1.40−1.65 (3 H, m), 1.25−1.50 (5 H, m), 1.10−1.22 (4 H, m), 1.09 (3 H, s), 0.96 (3 H, d, J = 6.6 Hz), 0.868 (3 H, d, J = 6.6 Hz), 0.863 (3 H, d, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 182.5, 139.1, 133.8, 129.0, 128.8, 120.3, 56.0, 53.1, 46.5, 39.6, 36.3, 35.9, 34.2, 32.9, 29.0, 28.2, 27.3, 23.9, 22.9, 22.85, 22.74, 19.0, 16.5, 14.3; IR (film in $CDCl_3$) 2929, 1742, 1463, 1383, 1158 cm^{-1} HRMS (ESI) *m*/*z* calcd for $C_{25}H_{36}O_3SNa$ $(M + Na)^+$ 439.2282, found 439.2289.

***trans*-Hydrindane (15).** Sulfone **14** (0.85 g, 2 mmol) was dissolved in a mixture of MeOH (14 mL), acetic acid (4 mL), and aqueous HCl (1 N, 0.2 mL). The 10% Pd/C catalyst (0.80 g) was added, and the mixture was cooled and stirred at 0 °C under an atmosphere of hydrogen for 12 h. The mixture was filtered through Celite to give 0.85 g of crude mixture of two epimers at the ring junction is a ratio of 8:1 in favor of *trans*. The ratio of the two isomers can be easily obtained from GC analysis (column RTX-1701), *cis* isomer (16.72 min) and *trans* isomer (17.44 min). Recrystallization of the crude mixture provided 0.53 g (62%) of the desired *trans* isomer, and this process provided a 1:1 mixture of isomers in the mother liquor along with variable amounts of ketone **16**. The mother liquor was concentrated and purified by chromatography to remove ketone **16**. The partially purified mixture was loaded as a solution in 50% IPA−hexanes on a preparative HPLC system (column 300 × 50 mm, KromaSpher 80, 5 μM silica column; solvent system 0.5% IPA−hexanes; retention times *trans* 37.64 min; *cis* 38.77 min; flow rate 40 mL/min at 41 bar) to provide 77 mg (9%) of desired *trans* isomer and 93 mg of *cis* epimer (10.9%). The combined yield for the *trans* hydrindane system was 71%. *trans* Isomer: ($[\alpha]^{24}_D$ −15.6, c = 1.45, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (2 H, m), 7.62 (1 H, tt, J = 7.2, 2 Hz), 7.55 (2 H, m), 4.0 (1 H, dd, J = 6.5, 14.5 Hz), 3.03 (1 H, ddd, J = 2, 6.5, 11 Hz), 2.87 (1 H, dd, J = 2.5, 14 Hz), 2.50 (1 H, td, J = 6.5, 14.5 Hz), 2.30 (1 H, ddd, J = 14.9, 4.9, 2.0 Hz), 2.17 (1 H, ddd, J = 1, 4.5, 11 Hz), 1.94 (1 H, m), 1.65 (2 H, m), 1.30−1.55 (7 H, m), 1.20−1.10 (4 H, m), 1.04 (3 H, s), 0.95 (1 H, m), 0.90 (3 H, J = 7 Hz), 0.865 (3 H, d, J = 6.6 Hz), 0.860 (3 H, d, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.0, 140.1, 133.8, 129.3 (2C), 128.2 (2C), 55.7, 55.1, 53.0, 45.9, 43.5, 39.6, 38.5, 37.5, 35.9, 35.7, 28.8, 25.0, 23.9, 23.0 (2C), 22.7, 18.7, 11.5; IR (film in $CDCl_3$) 2929, 1742, 1463, 1383, 1158 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{38}O_3SNa$ $(M + Na)^+$ 441.2439, found 441.2430. *cis* Isomer: ($[\alpha]^{24}_D$ +82.6, c = 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (2 H, m), 7.62 (1 H, tt, J = 7.5, 2 Hz), 7.55 (2 H, m), 4.0 (1 H, dd, J = 7.4, 14 Hz), 2.98 (1 H, ddd, J = 1.5, 7.5, 10 Hz), 2.32 (2 H, m), 2.05 (1 H, m), 2.0−1.87 (3 H, m), 1.53−1.26 (9 H, m), 1.17−1.038 (4 H, m), 0.93 (1 H, m), 0.88 (3 H, J = 6.5 Hz), 0.87

(3 H, d, $J = 6.6$ Hz), 0.865 (3 H, d, $J = 6.6$ Hz), 0.841 (3 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.0, 139.9, 133.8, 129.3 (2C), 128.2 (2C), 55.4, 54.3, 53.4, 47.6, 43.2, 39.6, 36.3, 35.7, 34.8, 33.6, 30.8, 29.5, 28.2, 24.1, 23.0, 22.7, 22.6, 19.6; IR (film in $CDCl_3$) 2929, 1742, 1463, 1383, 1158 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{25}H_{38}O_3SNa$ $(M + Na)^+$ 441.2439, found 441.2441.

Tricyclic Enone (12). To a solution of freshly prepared NaOMe (0.133 g, 5.52 mmol, 2.76 equiv) was added β-keto ester **11**[13c] (0.61 g, 2.5 mmol, 1.25 equiv) as a solution in MeOH (5 mL) at 10 °C, and the mixture was stirred for 10 min. A solution of sulfone **15** (0.84 g, 2 mmol, 1 equiv) in dry benzene (10 mL) was added, and the reaction mixture was stirred at room temperature for 12 h. A solution of NaOH (4 g) in H_2O (16 mL) was added to the reaction mixture. Sufficient MeOH (~25 mL) was added to cause formation of one layer. The mixture was stirred for 3−4 h at 23 °C and then concentrated. Toluene (50 mL) and H_2O (50 mL) were added, and the mixture was cooled to 0 °C. An aqueous solution of HOAc (50%) was added dropwise till the pH was ~4. Mixture was extracted with toluene (2 × 25 mL), the organic layers were mixed, dried (Na_2SO_4), and concentrated to provide a yellow oil. The oil was heated at 80 °C for 3 h under vacuum (0.2 Torr). It was necessary to stir the oil for efficient decarboxylation. The crude material was then chromatographed on silica gel with 10% EtOAc−hexanes to provide 0.61 g of desired tricyclic enone **12**. Physical and spectroscopic data were consistent with literature data:[3] $([\alpha]^{24}_D$ +18.7, $c = 3.6$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93 (4H, m), 2.76 (1 H, m), 2.46 (2 H, m), 2.36 (1 H, td, $J = 5.2$, 11.9 Hz), 2.25 (3 H, m), 2.12 (1 H, m), 1.98 (1 H, m), 1.90 (1 H, m), 1.70−1.45 (5 H, m), 1.42 (2 H, m), 1.36 (3 H, s), 1.34−1.27 (3 H, m), 1.20−1.08 (6 H, m), 1.00 (1 H, m), 0.93 (3 H, d, $J = 6.5$ Hz), 0.870 (3 H, d, $J = 6.6$ Hz), 0.865 (3 H, d, $J = 6.6$ Hz), 0.82 (3 H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ198.8, 159.9, 133.6, 110.0, 64.8 (2C), 56.5, 42.1, 39.6, 39.4, 39.1, 38.3, 37.3, 36.2, 35.8, 28.4, 28.1, 27.3, 27.1, 24.6, 23.9, 23.7, 22.9 (2C), 22.7, 20.1, 18.7, 11.4; IR (film in $CDCl_3$) 2952, 1667, 1466, 1373, 1060 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{28}H_{46}O_3Na$ $(M + Na)^+$ 453.3347, found 453.3345.

Tricyclic Ketone (13). To 65 mg of lithium wire (9.3 mmol, 20 equiv) in 100 mL of anhydrous NH_3 (distilled from Na/$FeNO_3$, 100 mg) at −78 °C was added 200 mg of enone **12** (0.46 mmol, 1.0 equiv) in 40 mL of THF. The reaction mixture was stirred for 1 h and MeI (1.62 mL, 27.9 mmol, 60 equiv) was added as a solution in THF (20 mL) over 30 min. The reaction mixture was stirred for 2−3 h at −33 °C and then the reaction was quenched with excess solid NH_4Cl. After allowing the NH_3 to evaporate, water (25 mL) was added. The layers were separated and aqueous layer was extracted with ether (3 × 50). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude oil was purified by flash chromatography (ethyl acetate−hexanes 5:95) to give 165 mg (80%, 0.37 mmol) of colorless oil. Physical and spectroscopic data were consistent with literature data:[3] $([\alpha]^{24}_D$ −32.3, $c = 1.3$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93 (4 H, m), 2.51 (1 H, td, $J = 6.5$, 14.5 Hz), 2.24 (1 H, m), 2.02 (1 H, m), 1.95−1.80 (4 H, m), 1.76−1.38 (8 H, m), 1.35 (3 H, s), 1.32−1.21 (6 H, m), 1.20−1.10 (6 H, m), 1.08 (3 H, s), 1.05−0.95 (1 H, m), 0.91 (3 H, d, $J = 6.5$ Hz), 0.867 (3 H, d, $J = 6.6$ Hz), 0.854 (3 H, d, $J = 6.6$ Hz), 0.71 (3 H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.5, 110.5, 64.7, 64.6, 56.2, 56.0, 50.6, 47.5, 42.7, 39.6 (2C), 38.4, 36.3, 35.9, 35.0, 33.2, 31.4, 29.2, 28.29, 28.20, 24.4, 23.6, 23.0 (2C), 22.75, 21.4, 21.2, 18.8, 12.1; IR (film in $CDCl_3$) 2936, 1707, 1466, 1366 cm^{-1}.

***ent*-Cholest-4-ene-3-one**. A solution of tricyclic ketone **13** (300 mg, 0.67 mmol) in MeOH (20 mL) and 6 N HCl (3 mL) was refluxed for 12 h. Solid $NaHCO_3$ (1 g) was added carefully, and the mixture was concentrated. Water (20 mL) was added, the mixture was extracted with EtOAc (3 × 25 mL), and the organic layers were combined, dried, and concentrated. Chromatography of the crude solid using 5% EtOAc−hexanes gave 234 mg (90%) of desired enone. Physical and spectroscopic data were consistent with literature data:[3] $([\alpha]^{24}_D$ −88.3, $c = 0.5$, $CHCl_3$); δ ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.71 (1 H, s), 2.40−2.25 (4 H, m), 2.05−1.95 (2 H, m), 1.85−1.75 (2 H, m), 1.72 (1 H, td, $J = 4.7$, 13.9 Hz), 1.65−1.20 (10 H, m), 1.16 (3 H, s), 1.05−1.15 (6 H, m), 0.94−1.04 (3 H, m), 0.88 (3 H, d, $J = 6.5$ Hz), 0.867 (3 H, d, $J = 6.6$ Hz), 0.854 (3 H, d, $J = 6.6$ Hz), 0.69 (3 H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ199.7, 171.7, 123.7, 56.1, 55.9, 53.8, 42.4, 39.6, 39.5, 38.6, 36.1, 35.79, 35.72, 35.6, 34.0, 32.9, 32.0, 28.2, 28.0, 24.2, 23.8, 22.8, 22.6, 21.0, 18.6, 17.4, 11.9; IR (film in $CDCl_3$) 2934, 1676, 1618, 1467 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{27}H_{44}ONa$ $(M + Na)^+$ 407.3289, found 407.3274.

***ent*-Cholesterol**. To a solution of *ent*-cholest-4-en-3-one (200 mg, 0.52 mmol) in ^{t}BuOH (10 mL) under inert atmosphere was added KO^{t}Bu (0.35 g, 3.1 mmol, 6 equiv), and the mixture was stirred for 4 h at 23 °C. An aqueous solution of 50% HOAc (5 mL) was added in one portion. The mixture was extracted with EtOAc (3 × 20 mL). The organic layer was dried, (Na_2SO_4), concentrated, and then azeotroped with benzene (2 × 20 mL) to give a crude solid that was taken to the next step. To a solution of the crude solid in THF (10 mL) was added solid Li(O^{t}Bu)$_3$Al-H (0.8 g, 3.1 mmol, 6 equiv). The reaction was stirred for 2 h at 23 °C. TLC analysis showed complete disappearance of the starting material. The reaction was quenched with 5% HCl (20 mL) and extracted with ethyl acetate (3 × 25 mL) to provide 159 mg (80%) yield of the desired *ent*-cholesterol. A small sample was recrystallized from methanol for analysis. Physical and spectroscopic data were consistent with literature data:[1,3] mp = 145−147 °C; $[\alpha]^{23}_D$ +38.8 ($c = 0.5$, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.34 (1 H, m), 3.51 (1 H, t, $J = 6.1$ Hz), 2.27 (2 H, m), 2.01 (2H, M), 1.82 (3 H, m), 1.65−1.45 (8 H, m), 1.35−1.32 (2 H, m), 1.28−1.22 (4 H, m), 1.17−1.05 (8 H, m), 1.03 (3 H, s), 0.96 (3 H, d, $J = 6.5$ Hz), 0.87 (3 H, d, $J = 6.6$ Hz), 0.85 (3 H, d, $J = 6.6$ Hz), 0.67 (3 H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.9, 121.39, 72.0, 56.9, 50.3, 42.5, 40.0, 39.7, 37.4, 36.7, 36.7, 36.4, 36.0, 32.1, 31.8, 29.9, 28.4, 28.2 (2C), 24.51, 24.0, 23.0, 22.7, 21.3, 19.6, 18.9, 12.0; IR (film in $CDCl_3$) 3347, 2969, 2926, 1429, 754 cm^{-1}.

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Supporting Information Available: Experimental procedures for the preparation of compounds **3**, **4**, **6**, **7**, **8**, **10**, **12**, **17**, and 19*d*$_3$-*ent*-cholesterol are included. X-ray data for sulfone **15** in CIF format and NMR spectra of the new compounds are provided, including detailed comparisons between the synthetic and isolated natural products described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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