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Synthesis of side-chain oxysterols and their enantiomers through cross-metathesis reactions of Δ^{22} steroids

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ABSTRACT

A synthetic route that utilizes a cross-metathesis reaction with Δ^{22} steroids has been developed to prepare sterols with varying C-27 side-chains. Natural sterols containing hydroxyl groups at the 25 and (25*R*)-26 positions were prepared. Enantiomers of cholesterol and (3β,25*R*)-26-hydroxycholesterol (27-hydroxycholesterol) trideuterated at C-19 were prepared for future biological studies.

1. Introduction

Side-chain oxysterols have been implicated in multiple important biological processes, including: bile acid synthesis [1], cholesterol regulation and transport [2–6], modulation of estrogen receptor function [7], and apoptosis [8]. Further, these oxygenated cholesterol derivatives are believed to be significant in diseases such as Alzheimer's [9,10], Parkinson's [10,11], multiple sclerosis [10,12], Niemann-Pick type C disease [13], and cataracts [14]. With regards to cholesterol homeostasis, (3β)-cholest-5-ene-3,25-diol (25-hydroxycholesterol, 25-HC, **1**) and (3β,25*R*)-cholest-5-ene-3,26-diol (27-hydroxycholesterol, 27-HC, **3**) are known to be modulators of sterol regulatory element binding proteins (SREBP), liver X receptors (LXR) and hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase (Fig. 1) [4].

We are focused on the importance of the absolute configuration of 25-HC and 27-HC for their modes of action on cholesterol homeostatic-regulating proteins. We previously prepared the enantiomer of 25-HC (*ent*-25-HC) and have compared its biophysical and pharmacological properties with those of 25-HC [15–18]. We are also interested in performing similar comparative activity studies with 27-HC and its enantiomer, *ent*-27-HC. Because *ent*-27-HC has not been prepared previously, we evaluated synthetic strategies for its preparation.

Generally, synthetic methods used for the total synthesis of a natural steroid can be used to make the corresponding *ent*-steroid simply by using a chiral starting material with the opposite absolute configuration of the natural steroid. Thus, for synthesizing *ent*-27-HC, methods used to synthesize 27-HC are relevant. 27-HC has been prepared from the commercially available natural products diosgenin or kryptogenin [19–22]. However, these synthetic methods are not useful for preparing *ent*-27-HC because neither *ent*-diosgenin nor *ent*-kyptogenin occurs naturally and it is impractical to first prepare either of them for subsequent conversion into *ent*-27-HC.

A literature precedent for the synthesis of 27-HC from (3β)-hydroxychol-5-en-24-oic acid has been reported [23]. We have previously reported the total synthesis of *ent*-lithocholic acid [24] and we could have modified this synthesis to prepare the

Abbreviations: 25-HC, (3β)-cholest-5-ene-3,25-diol; 25-HC-d_6, (3β)-cholest-5-ene-26,26,26,27,27,27-,d_6-,3,25-diol; 27-HC, (3β,25*R*)-cholest-5-ene-3,26-diol; *ent*-Chol-d_3, *ent*-[(3β)-Cholest-5-en-19,19,19-,d_3-,3-,ol]; *ent*-27-HC-d_3, *ent*-[(3β,25*R*)-Cholest-5-ene-19,19,19-d_3-3,26-diol].

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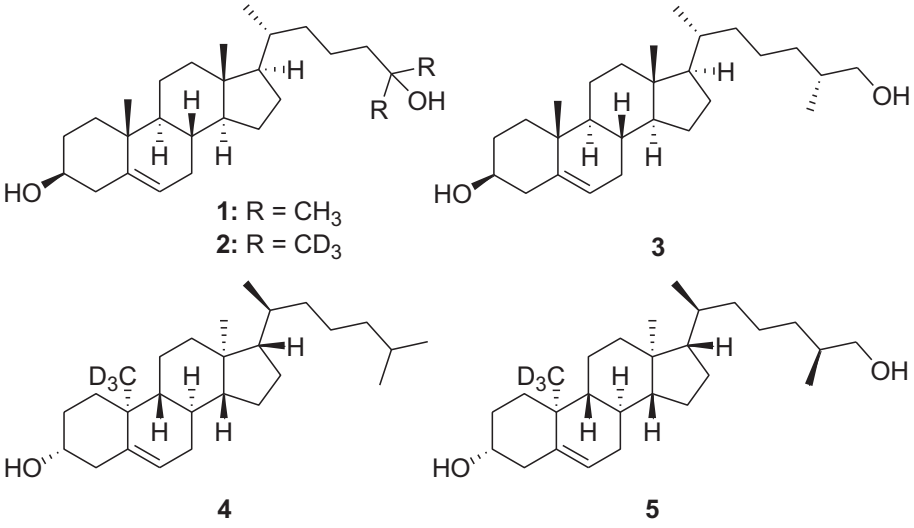


Fig. 1. Structures of 25-HC (**1**), 25-HC-d_6 (**2**), 27-HC (**3**), *ent*-Chol-d_3 (**4**) and *ent*-27-HC-d_3 (**5**).

enantiomer of (3β)-3-hydroxychol-5-en-24-oic acid, then converted it to *ent*-27-HC. While this route could have enabled the total synthesis of *ent*-27-HC, we also sought a method that could prepare a variety of side-chains on the steroid. Therefore, we modified our *ent*-lithocholic acid synthesis to prepare an *ent*-Δ^{22} steroid which allowed us to utilize a cross-metathesis reaction with either 2nd generation Grubbs catalyst or Stewart-Grubbs catalyst to install the remaining portions of the side-chain and complete the synthesis of *ent*-27-HC. We used this synthetic approach to prepare *ent*-27-HC-d_3 (**5**). We also demonstrated the generality of this approach for making side chain modified sterols by preparing 25-HC (**1**), 25-HC-d_6, (**2**), 27-HC (**3**) and *ent*-cholesterol-d_3 (*ent*-Chol-d_3, **4**). The deuterated *ent*-sterols **4** and **5** will be of utility in future biological studies.

2. Experimental

2.1. General methods

Solvents were either used as purchased or dried and purified by standard methods. All air and/or moisture sensitive reactions were carried out under nitrogen environments using oven-dried glassware, which was cooled under nitrogen gas. Flash chromatography was performed using silica gel (32–63 μm) purchased from Scientific Adsorbants (Atlanta, GA). Optical rotations were determined at room temperature on a Perkin-Elmer Model 341 polarimeter. Melting points were determined utilizing a Kofler micro hot stage and are uncorrected. IR spectra were recorded on a NaCl plate with a Perkin-Elmer 1710 FT-IR spectrophotometer. NMR spectra were recorded at ambient temperature in $CDCl_3$ with a 5 mm probe on a Varian Gemini 2000 operating at 300 MHz (^{1}H) or 75 MHz (^{13}C) and were referenced to $CDCl_3$ (7.27 ppm or 77.00 ppm, respectively). Elemental analyses were performed by M-H-W laboratories (Phoenix, AZ). Lithocholic acid was purchased from Steraloids, Newport, RI, USA.

2.1.1. (3β)-Cholest-5-ene-3,25-diol (25-hydroxycholesterol, *1*)

Compound **15** (23 mg, 0.057 mmol) was dissolved in Et_2O (10 mL) and cooled to 0 °C under N_2. CH_3MgBr (0.5 mL, 3 M in Et_2O) was added dropwise. The reaction was allowed to slowly reach room temperature and stirred overnight under N_2. Additional CH_3MgBr (0.2 mL) was added in the morning and the reaction stirred at room temperature for another 4 h. The solution was quenched with satd. aqueous NH_4Cl and the product extracted into Et_2O (3 × 40 mL). The combined extracts were dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 5:1) to obtain compound **1** (21 mg, 60%, 3 steps from compound **14**) as a white solid: mp 172–175 °C; $[\alpha]_D^{25}$ −37.0 (*c* = 0.08, $CHCl_3$); ^{1}H NMR δ 0.66 (3H, s), 0.90 (3H, d, *J* = 6.6 Hz), 3.47 (1H, m), 5.32 (1H, d, *J* = 4.9 Hz); ^{13}C NMR δ 140.9, 121.8, 71.7, 71.2, 56.9, 56.2, 50.3, 44.5, 42.5, 42.3, 39.9, 37.4, 36.6, 36.6, 35.9, 32.0, 31.6, 29.3, 29.2, 28.4, 24.4, 21.2, 20.9, 19.5, 18.8, 12.0; IR υ_{max} 3292, 2933, 2864, 1465, 1377 cm^{-1}.

2.1.2. (3β)-Cholest-5-ene-26,26,26,27,27,27-d_6-3,25-diol (25-hydroxycholesterol-d_6, *2*)

Compound **15** (27 mg, 0.067 mmol) was dissolved in Et_2O (10 mL) and CD_3Li (1.5 mL, 0.5 M in Et_2O, stabilized with LiI) was added slowly while stirring under N_2 for 4 h. Additional CD_3Li solution (1.5 mL) was added and the reaction stirred at room temperature overnight under N_2. A third addition of CD_3Li solution (1.5 mL) was then added and stirring continued for another 4 h. The reaction was quenched with water, then 0.5 M HCl, and the product extracted into Et_2O (3 × 40 mL). The combined extracts were dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with CH_2Cl_2:EtOAc, 8:1) to obtain compound **2** (24 mg, 59%, 3 steps from compound **13**) as a white solid: mp 172–174 °C; $[\alpha]_D^{25}$ −33.3 (*c* = 0.08, $CHCl_3$); ^{1}H NMR δ 0.69 (3H, s), 0.94 (3H, d, *J* = 6.3 Hz), 1.01 (3H, s), 2.01 (2H, m), 2.28 (2H, m), 3.53 (1H, m), 5.36 (1H, d, *J* = 5.1 Hz); ^{13}C NMR δ 12.0, 18.8, 19.5, 20.9, 21.2, 24.4, 28.4, 31.8, 32.0, 35.9, 36.6, 36.6, 37.4, 39.9, 42.5, 42.5, 44.5, 50.3, 56.2, 56.9, 71.0, 72.0, 121.8, 140.9; IR υ_{max} 3307, 2934, 2902, 2865, 1465, 1376 cm^{-1}. HRMS (ESI) calcd. for $C_{27}H_{40}D_6O_2$ (M-H_2O+H^+) 391.3847; Found 391.3852.

2.1.3. (3β,25R)-Cholest-5-ene-3,26-diol (27-hydroxycholesterol, *3*)

Compound **22** (85 mg, 0.191 mmol) was dissolved in acetic anhydride (20 mL). NaI (530 mg) was added, N_2 was bubbled through the solution for 30 min, and then the reaction was cooled to 0 °C. $SiMe_3Cl$ (0.4 mL) was added dropwise and the reaction was stirred at 0 °C under N_2 for 30 min, then at room temperature for 3 h. The acetic anhydride was removed *in vacuo* and satd. aqueous $NaHCO_3$ was added to the resulting residue. The product was extracted into EtOAc (3 × 40 mL) and the combined extracts were

washed with 5% sodium thiosulfate solution, dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The dienol acetate intermediate was partially purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 9:1). EtOH (50 mL) was added to the partially purified intermediate, followed by $NaBH_4$ (197 mg, 5.20 mmol) and the reaction was stirred overnight. The EtOH was removed *in vacuo* and the product dissolved in EtOAc and washed with 1 M HCl, satd. aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain compound **23** which was further purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 6:1 to 3:1). Compound **23** was dissolved in MeOH (50 mL), K_2CO_3 (250 mg, 1.81 mmol) was added and the reaction was refluxed overnight under N_2. The MeOH was removed *in vacuo* and 1 M HCl (50 mL) was poured over the residue. The product was extracted into EtOAc (3 × 40 mL) and the combined extracts were dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with CH_2Cl_2:EtOAc, 3:1) to obtain compound **3** (44 mg, 57%, 3 steps from compound **22**) as a white solid: ^{1}H NMR δ 0.68 (3H, s), 2.27 (2H, m), 3.50 (3H, m), 5.37 (1H, m); ^{13}C NMR δ 140.9, 121.8, 72.0, 68.9, 56.9, 56.3, 50.3, 42.5, 39.9, 37.4, 36.7, 36.3, 36.0, 35.9, 33.7, 32.1, 31.8, 28.4, 24.4, 23.6, 21.2, 19.5, 18.8, 16.5, 12.0.

*2.1.4. ent-[(3β)-Cholest-5-en-19,19,19-d_3-3-ol] (ent-cholesterol-d_3, **4**)*

Compound **4** (80 mg, 65%) was prepared from compound **35** (117 mg, 0.302 mmol) using the two step deconjugation procedure described as part of three step procedure for the preparation of compound **3**. Compound **4** was a white solid: ^{1}H NMR δ 0.68 (3H, s), 0.86 (6H, d, J = 6.9 Hz), 0.99 (3H, d, J = 9.9 Hz), 2.28 (2H, m), 3.51 (1H, m), 5.35 (1H, s); ^{13}C NMR δ 12.0, 18.9, 21.2, 22.7, 23.0, 24.0, 24.4, 28.2, 28.4, 31.8, 32.1, 36.0, 36.3, 36.4, 37.3, 39.7, 39.9, 42.5, 50.2, 56.3, 56.9, 72.0, 121.9, 140.9; IR υ_{max} 3369, 2931, 2867, 2901, 1459, 1378 cm^{-1}. HRMS (ESI) calcd. for $C_{27}H_{43}D_3O$ (M+H$^+$) 390.3810; Found. 390.3813.

*2.1.5. ent-[(3β,25R)-Cholest-5-ene-19,19,19-d_3-3,26-diol] (ent-27-hydroxycholesterol-d_3, **5**)*

Compound **5** (65 mg, 60%) was prepared from compound **36** (120 mg, 0.261 mmol) using the three step procedure described for the preparation of compound **3**. Compound **5** was a white solid: mp 174–175 °C; $[\alpha]_D^{25}$ +33.5 (c = 0.25, $CHCl_3$); ^{1}H NMR δ 0.69 (3H, s), 0.92 (6H, d, J = 6.3 Hz), 2.28 (2H, m), 3.46 (3H, m), 5.35 (1H, m); ^{13}C NMR δ 12.0, 16.7, 18.8, 21.2, 23.6, 24.4, 28.4, 31.8 (2 × C), 32.1, 33.7, 35.9, 36.0, 36.3, 36.5, 37.3, 39.9, 42.6, 50.2, 56.3, 56.9, 68.7, 72.0, 121.9, 140.9; HRMS (ESI) calcd. for $C_{27}H_{43}D_3O_2$ (2M+H$^+$) 811.7445; Found. 811.7446.

*2.1.6. (3α,5β)-24-norchol-22-en-3-ol (**10**)*

(3α,5β)-24-Norchol-22-en-3-ol, 3-acetate (**6**, 6.17 g, 16.56 mmol, 1 eq) prepared according to the literature [25], was dissolved in hot MeOH (300 mL) and benzene (50 mL). K_2CO_3 (10.3 g, 74.5 mmol, 4.5 eq) was added and the mixture was heated to reflux and stirred overnight. The solvent was removed *in vacuo* and 1 M HCl was added. The product was extracted into CH_2Cl_2 (3 × 200 mL), dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with hexanes: EtOAc, 8:1 to 6:1) to give compound **10** (4.75 g, 87%) as a white solid: mp 137–140 °C; $[\alpha]_D^{25}$ +123.7 (c = 0.40, $CHCl_3$); ^{1}H NMR δ 0.67 (3H, s), 0.93 (3H, s), 1.02 (3H, d, J = 6.6 Hz), 3.61 (1H, m), 4.92 (2H, m), 5.69 (1H, s); ^{13}C NMR δ 145.5, 111.6, 72.0, 56.7, 55.8, 42.3, 41.4, 40.7, 40.3, 36.6, 36.0, 35.5, 34.8, 30.7, 28.6, 27.3, 26.6, 24.4, 23.5, 21.0, 20.2, 12.4; IR υ_{max} 3307, 2931, 2865, 1637, 1449, 1261 cm^{-1}. Anal. Calcd for $C_{23}H_{38}O$: C, 83.57; H, 11.59; Found: C, 83.74; H, 11.43.

*2.1.7. (3α,5β)-3-Hydroxychol-22-en-24-oic acid, methyl ester (**11**)*

Compound **10** (2.52 g, 7.62 mmol) was dissolved in CH_2Cl_2 (200 mL), *trans*-3-hexenedioic acid dimethyl ester (1.2 mL, 7.67 mmol) and Grubbs catalyst, 2nd generation (660 mg, 0.78 mmol) were added. The reaction was heated to reflux and allowed to stir for 40 h. CH_2Cl_2 was removed *in vacuo* and the product was partially purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 6:1 to 4:1) to recover a mixture of *cis*/*trans* isomers to give compound **11** (2.17 g) as a light brown solid which was converted to compound **12** without further purification or characterization.

*2.1.8. (3α,5β)-3-Hydroxycholane-24-carboxylic acid, methyl ester (**12**)*

Compound **11** (2.01 g, 4.99 mmol) was dissolved in EtOAc (150 mL) and Pd/C (1.0 g, 10% on charcoal) was added to a round-bottomed flask equipped with a balloon and evacuated and purged with H_2 (3x). The reaction was stirred while bubbling H_2 through the solvent for 3 h. The solution was filtered through celite and the solvent removed *in vacuo* to yield compound **11** (2.01 g, 70%, 2 steps from compound **6**): mp 105–108 °C; $[\alpha]_D^{25}$ +265.8 (c = 0.32, $CHCl_3$); ^{1}H NMR δ 0.63 (3H, s), 0.91 (6H, 2s), 2.28 (2H, m), 3.64 (1H, m), 3.66 (3H, s); ^{13}C NMR δ 12.2, 18.7, 21.0, 21.7, 23.5, 24.4, 26.6, 27.4, 28.4, 30.7, 34.7, 34.7, 35.5, 35.6, 35.7, 36.0, 36.6, 40.3, 40.6, 42.3, 42.8, 51.6, 56.2, 56.7, 72.0, 174.5; IR υ_{max} 3368, 2934, 2864, 1742, 1448 cm^{-1}. Anal. Calcd for $C_{26}H_{44}O_3$: C, 77.18; H, 10.96; Found: C, 77.20; H, 11.05.

*2.1.9. (5β)-3-Oxocholane-24-carboxylic acid, methyl ester (**13**)*

Compound **12** (2.01 g) was dissolved in acetone (250 mL) and N_2 was bubbled through the solution for 30 min. Jones reagent (30% H_2SO_4, 30% chromic acid) was added dropwise until a persistent yellow color remained. The reaction was allowed to stir at room temperature under N_2 for 2 h. Water was added and the acetone removed *in vacuo*. Brine (100 mL) was added and the product was extracted into EtOAc (3 × 250 mL). The combined extracts were washed with brine (200 mL), dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 9:1) to obtain compound **13** (1.93 g, 96%) as a white solid: mp 92–93 °C; $[\alpha]_D^{25}$ +28.4 (c = 0.50, $CHCl_3$); ^{1}H NMR δ 0.67 (3H, s), 0.91 (3H, d, J = 6.3 Hz), 1.01 (3H, s), 2.69 (1H, t, J = 14.3 Hz), 3.66 (3 H, s); ^{13}C NMR δ 213.5, 174.5, 56.6, 56.2, 51.6, 44.5, 42.9, 42.5, 40.9, 40.2, 37.4, 37.2, 35.7, 35.6, 35.5, 35.0, 34.6, 28.3, 26.8, 25.9, 24.3, 22.8, 21.7, 21.4, 18.7, 12.2; IR υ_{max} 2932, 2865, 1736, 1716, 1445, 1377 cm^{-1}. Anal. Calcd for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51; Found: C, 77.69; H, 10.35.

*2.1.10. 3-Oxochol-4-ene-24-carboxylic acid, methyl ester (**14**)*

Compound **13** (110 mg, 0.273 mmol) was dissolved in AcOH (50 mL) and pyridinium tribromide (102 mg, 0.319 mmol) was added. The mixture was stirred, under N_2 at room temperature for 1 h. The AcOH was removed *in vacuo* and satd. aqueous $NaHCO_3$ was added to the residue. The product was extracted into EtOAc (3 × 40 mL), dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The brominated intermediate was partially purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 6:1). The partially purified intermediate was dissolved in DMF (40 mL) and LiCl (450 mg, 10.62 mmol) was added. The solution was heated to 100 °C and stirred for 2 h under N_2. DMF was removed *in vacuo* and 1 M HCl (50 mL) was added. The product was extracted into EtOAc (3 × 40 mL) and the combined extracts dried over anhydrous Na_2SO_4, and the solvents removed under reduced pressure. The product was purified by

flash column chromatography (silica gel eluted with hexanes: EtOAc, 8:1) to obtain compound **14** (50 mg, 46%) as a white solid: mp 118–122 °C; $[\alpha]_D^{25}$ +47.6 (*c* = 0.24, $CHCl_3$); ^{1}H NMR δ 0.71 (3H, s), 0.90 (3H, d, *J* = 1.9 Hz), 3.67 (3H, s), 5.73 (1H, s); ^{13}C NMR δ 199.7, 174.4, 171.7, 123.9, 56.0, 55.9, 53.9, 51.6, 42.5, 39.7, 38.7, 35.8, 35.7, 35.6, 35.5, 34.6, 34.1, 33.1, 32.2, 28.2, 24.3, 21.7, 21.2, 18.7, 17.5, 12.1; IR υ_{max} 2937, 1736, 1671, 1616 cm^{-1}. Anal. Calcd for $C_{26}H_{40}O_3$: C, 77.95; H, 10.06; Found: C, 78.23; H, 10.21.

2.1.11. (3β)-3-Hydroxychol-5-ene-24-carboxylic acid, ethyl and methyl ester (15)

Compound **15** (27 mg, a 3:1 mixture of ethyl and methyl esters as determined by ^{1}H NMR) was prepared from compound **14** (41 mg, 0.102 mmol) using the procedure described for the preparation of compound **3.** Compound **15** was characterized only by its ^{1}H NMR spectrum and was subsequently converted to either compound **1** or compound **2**.

*2.1.12. (2R,7R)-2,7-bis(methyl)-1,8-bis[(4S)-2-oxo-4-(phenylmethyl)-3-oxazolidinyl]-4-octene-1,8-dione (**17**)*

Compound **16** [26] (5.6 g, 20.5 mmol) was dissolved in CH_2Cl_2 (250 mL) and Grubbs catalyst, 1st generation (1.5 g, 1.8 mmol) was added. The reaction was heated to reflux and stirred under N_2 for 40 h. The CH_2Cl_2 was removed *in vacuo* and the product purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 4:1 to 2:1) to obtain partially purified compound **17** (4.6 g, 86%) as a grey solid: ^{1}H NMR δ 1.18 (6H, m), 2.23 (2H, m), 2.33 (2H, m), 2.48 (2H, m), 2.71 (2H, m), 3.31 (2H, d, *J* = 13.2 Hz), 4.21 (4H, m), 4.69 (2H, m), 5.55 (2H, m), 7.31 (10H, m).

*2.1.13. (2R,7R)-2,7-dimethyl-oct-4-ene-1,8-diol (**18**)*

Partially purified compound **17** (4.45 g, 8.58 mmol) was dissolved in dry THF and cooled to 0 °C while stirring under N_2. $LiAlH_4$ (55 mL, 2 M in THF) was added and the reaction was allowed to reach room temperature and stirred overnight. The $LiAlH_4$ was quenched by sequentially adding water (4.2 mL), 8.4 mL 10% NaOH (8.4 mL) and 12.6 mL water (12.6 mL). Precipitates were removed by filtration and the product extracted into EtOAc (3 x 200 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was partially purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 2:1) to obtain compound **18** (1.4 g, 65%) as a mixture of *E* and *Z* isomers which was used directly in the Grubbs cross-metathesis reaction with compound **19**.

*2.1.14. (5β)-24-Norchol-22-en-3-one (**19**)*

Compound **19** (1.37 g, 93%) was prepared from compound **6** (1.49 g, 4.51 mmol) using the procedure described for the preparation of compound **13**. Compound **19** was a white solid: mp 126–127 °C; $[\alpha]_D^{25}$ +154.6 (*c* = 0.49, $CHCl_3$); ^{1}H NMR δ 0.70 (3H, s), 1.01 (3H, s), 1.03 (3H, s), 2.69 (1H, t, *J* = 14.3 Hz), 4.86 (2H, m), 5.65 (1H, m); ^{13}C NMR δ 213.3, 145.2, 111.8, 56.6, 55.8, 44.5, 42.9, 42.5, 41.3, 41.0, 40.1, 37.3, 37.2, 35.7, 35.1, 28.6, 26.8, 25.9, 24.3, 22.8, 21.3, 20.2, 12.4; IR υ_{max} 2939, 2857, 1716, 1445 cm^{-1}. Anal. Calcd for $C_{23}H_{36}O$: C, 84.09; H, 11.04; Found: C, 84.01; H, 10.91.

*2.1.15. (5β,25R)-26-Hydroxycholestan-3-one (**20**)*

Compound **19** (1.16 g, 3.53 mmol), compound **17** (610 mg, 3.56 mmol) and Grubbs catalyst, 2nd generation (300 mg, 0.25 mmol) were dissolved CH_2Cl_2 (300 mL), heated to reflux, and stirred for 72 h. Additional Grubbs catalyst, 2nd generation (300 mg, 0.25 mmol) was added, heated to reflux, and stirred for 24 h. The solvent was removed *in vacuo*, and partially purified by flash column chromatography (silica gel eluted with hexanes: EtOAc, 6:1). A portion of this product (740 mg, 1.84 mmol) was dissolved in EtOAc (150 mL) and hydrogenated (70 psi) using 10% Pd/C as catalyst. The hydrogenation was run overnight. After filtration through celite and solvent removal under reduced pressure, the product was purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 4:1) to yield compound **20** (660 mg, 54%, 2 steps from compound **19**) as a white solid: mp 87–90 °C; $[\alpha]_D^{25}$ +36.6 (*c* = 0.47, $CHCl_3$); ^{1}H NMR δ 0.68 (3H, s), 2.30 (1H, m), 2.70 (1H, t, *J* = 14.0 Hz), 3.49 (2H, m); ^{13}C NMR δ 213.6, 68.6, 56.6, 56.5, 44.5, 42.9, 42.5, 40.9, 40.2, 37.3, 37.2, 36.3, 35.9, 35.8, 35.7, 35.0, 33.7, 28.4, 26.8, 25.9, 24.3, 23.6, 22.8, 21.4, 18.8, 16.7, 12.2; IR υ_{max} 3418, 2932, 2865, 1715, 1455, 1378 cm^{-1}. Anal. Calcd for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52; Found: C, 80.59; H, 11.28.

*2.1.16. (5β,25R)-26-(Acetyloxy)-cholestan-3-one (**21**)*

Compound **20** (170 mg, 0.420 mmol) was dissolved in pyridine (30 mL). Acetic anhydride (0.6 mL, 6.35 mmol) and 4-(dimethylamino)pyridine (20 mg, 0.16 mmol) were added and the mixture stirred under N_2 at room temperature for 3.5 h. The pyridine was removed *in vacuo*. Water was poured over the residue and the product extracted into EtOAc (3 × 40 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with hexanes: EtOAc, 12:1) to obtain compound **21** (164 mg, 87%) as a white solid: mp 62–65 °C; $[\alpha]_D^{25}$ +21.1 (*c* = 0.19, $CHCl_3$); ^{1}H NMR δ 0.65 (3H, s), 2.31 (1H, m), 2.71 (1H, t, *J* = 14.1 Hz), 3.90 (2H, m); ^{13}C NMR δ 213.3, 171.3, 69.7, 56.6, 56.4, 44.5, 42.9, 42.5, 40.9, 40.2, 37.3, 37.2, 36.1, 35.8, 35.7, 35.0, 33.9, 32.6, 28.4, 26.8, 25.9, 24.3, 23.4, 22.8, 21.3, 21.1, 18.8, 16.9, 12.2; IR υ_{max} 2936, 2865, 1740, 1716, 1467, 1377, 1238 cm^{-1}. Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88; Found: C, 78.57; H, 10.69.

*2.1.17. (25R)-26-(Acetyloxy)-cholest-4-en-3-one (**22**)*

Compound **22** (74 mg, 51%) was prepared from compound **21** (146 mg, 0.323 mmol) using the procedure described for the preparation of compound **14**. Compound **22** was an oil: $[\alpha]_D^{25}$ +53.5 (*c* = 0.12, $CHCl_3$); ^{1}H NMR δ 0.68 (3H, s), 0.89 (3H, d, *J* = 4.9 Hz), 3.91 (2H, m), 5.69 (1H, s); IR υ_{max} 2935, 2868, 1740, 1676, 1465, 1375, 1236 cm^{-1}. Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47; Found: C, 78.58; H, 10.69.

*2.1.18. 2,7-dimethyloct-4-ene (**24**)*

4-Methyl-1-pentene (20 mL, 157.8 mmol) was dissolved in CH_2Cl_2. Grubbs catalyst, 2nd generation (189 mg, 0.23 mmol) was added and the reaction was stirred under N_2 at room temperature for 12 h. The product was purified by flash column chromatography (silica gel eluted with hexanes) to obtain compound **24** as an oil (5.7 g, 51%): ^{1}H NMR δ 0.88 (12H, m), 1.56 (2H, septet), 1.90 (2H, m), 5.38 (2H, m); IR υ_{max}. 2899, 2927, 2870, 1466, 1384, 1367 cm^{-1}.

*2.1.19. (2S,7S)-2,7-bis(methyl)-1,8-bis[(4R)-2-oxo-4-(phenylmethyl)-3-oxazolidinyl]-4-octene-1,8-dione (**26**)*

Compound **25** (5.02 g, 21.5 mmol) was dissolved in CH_2Cl_2 (250 mL) and Grubbs catalyst, 1st generation (1.5 g, 1.8 mmol) was added. The reaction was heated to reflux and stirred under N_2 for 40 h. The CH_2Cl_2 was removed *in vacuo* and the product partially purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 4:1 to 2:1) to obtain compound **26** (3.94 g, 83%) as a gray solid, which was not characterized before being converted to compound **27**.

*2.1.20. (2S,7S)-2,7-dimethyl-oct-4-ene-1,8-diol (**27**)*

Compound **27** (1.13 g, 82%) was prepared from compound **26** (3.94 g, 8.03 mmol) using the procedure described for the prepara-

tion of compound **18**. Compound **27** was not characterized before being converted to compound **28**.

*2.1.21. (2S,7S)-2,7-dimethyl-4-octene-1,8-diol, diacetate (**28**)*

Compound **28** (1.11 g, 66%) was prepared from compound **27** (1.13 g, 6.56 mmol) using the procedure described for the preparation of compound **21**. Compound **28** had: ^{1}H NMR δ 0.89 (6H, m), 2.03 (6H, s), 3.88 (4H, m), 5.37 (2H, m); ^{13}C NMR δ 16.5, 16.6, 20.8, 30.8, 32.7, 32.9, 36.3, 68.7, 128.6, 129.6, 171.1; IR υ_{max} 2962, 1739, 1463, 1367, 1239 cm^{-1}; HRMS (ESI) calcd. for $C_{14}H_{24}O_4$ (M+H$^+$) 257.1747; Found: 257.1748.

*2.1.22. ent-[(3α,5β)-chola-16,22-diene-3,24-diol] (**30**)*

Compound **29** (1.09 g, 2.32 mmol), was prepared by the same procedure reported previously for the natural abundance form [24], then dissolved in dry THF and cooled to −78 °C. DIBAL-H (1.5 M in THF, 25 mL) was added and the reaction was stirred under N_2 at −78 °C for 1 h. Sodium potassium tartrate was added slowly until bubbling ceased. The product was extracted into EtOAc (2 × 250 mL), then washed with brine (100 mL), dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 2:1) to yield compound **30** (840 mg, 97%) as a white solid: $[\alpha]_D^{25}$ −28.0 (c = 0.49, $CHCl_3$); ^{1}H NMR δ 0.75 (3H, s), 1.12 (3H, d, J = 6.9 Hz), 2.85 (1H, m), 3.61 (1H, m), 4.10 (2H, d, J = 4.5 Hz), 5.34 (1H, t, J = 1.65 Hz), 5.64 (2H, m); ^{13}C NMR δ 16.6, 20.7, 26.6, 27.3, 30.6, 31.1, 34.6, 34.7, 35.3 (2 × C), 35.6, 36.6, 41.2, 42.3, 47.4, 57.6, 63.9, 72.0, 122.5, 126.8, 138.5, 159.1; HRMS (ESI) calcd. for $C_{29}H_{45}D_3O_3$ (M-H_2O+H$^+$) 344.3033; Found: 344.3025.

*2.1.23. ent-[(3α,5β)-Cholesta-16,22-diene-19,19,19-d_3-3-ol](**31**)*

Compound **30** (780 mg, 2.08 mmol) was dissolved in CH_2Cl_2 (300 mL) and compound **24** (945 mg, 6.74 mmol) was added, followed by the addition of 1,3-bis(2-methylphenyl)-2-imidazolidinylidene]dichloro(2-isopropoxyphenylmethylene)ruthenium (II) (478 mg, 0.84 mmol). The reaction was heated to reflux and stirred under N_2 for 72 h. The solvent was removed *in vacuo* and the product partially purified by flash column chromatography (silica gel eluted with hexanes:EtOAc, 9:1 to 2:1) to obtain semi-purified compound **31** (630 mg, 78%) which was not characterized before being converted to compound **33**.

*2.1.24. ent-[(3α,5β,25R)-Cholesta-16,22-diene-19,19,19-d_3-3,26-diol, 26-acetate] (**32**)*

Partially purified compound **32** (630 mg, 63%) was prepared from compound **30** (840 mg, 2.24 mmol) and compound **28** (650 mg, 2.53 mmol) using the procedure described for the preparation of compound **31**. The product was not characterized before being converted to compound **34**.

*2.1.25. ent-[(5β)-cholestan-3-one-19,19,19-d_3] (**33**)*

Compound **31** (630 mg, 1.63 mmol) was dissolved in EtOAc (150 mL) and hydrogenated (70 psi) using 10% Pd/C (414 mg) as catalyst. The hydrogenation was run overnight. The product was filtered through celite, and the solvent removed *in vacuo*. This product was dissolved in acetone, and Jones reagent added until a persistent yellow color was achieved. This reaction was stirred under N_2 for 30 min, brine was added and the product extracted into EtOAc (3 × 100 mL). The combined extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure. The product was purified by flash column chromatography (silica gel eluted with hexanes: EtOAc, 18:1) to obtain compound **33** (440 mg, 54%, 3 steps from compound **30**) which had: $[\alpha]_D^{25}$ −32.5 (c = 0.82, $CHCl_3$); ^{1}H NMR δ 0.69 (3H, s), 0.91 (3H, d, J = 6.3 Hz), 0.91 (3H, d, J = 6.9 Hz), 2.29 (1H, t of d, J = 5.4 Hz, J = 9.0 Hz), 2.70 (1H, t, J = 14.1 Hz); ^{13}C NMR δ 12.2, 18.8, 21.4, 22.7, 23.0, 24.0, 24.3, 25.9, 26.8, 28.2, 28.4, 34.8, 35.7, 35.9, 36.3, 37.1, 37.4, 39.7, 40.2, 40.9, 42.5, 42.9, 44.5, 56.5, 56.6, 213.7; IR υ_{max} 2950, 2867, 1717, 1455, cm^{-1}; HRMS (ESI) calcd. for $C_{27}H_{43}D_3O$ (M+H$^+$) 390.3810; Found: 390.3814.

*2.1.26. ent-[(5β,25R)-26-(Acetyloxy)-cholestan-3-one-19,19,19-d_3] (**34**)*

Compound **34** (350 mg, 35%) was prepared from compound **32** (630 mg, 1.41 mmol) using the procedure described for the preparation of compound **33**. Compound **34** had: $[\alpha]_D^{25}$ −64.9 (c = 0.68, $CHCl_3$); ^{1}H NMR δ 0.68 (3H, s), 0.92 (3H, d, J = 6.6 Hz), 0.92 (3H, d, J = 6.9 Hz), 2.06 (3H, s), 2.33 (1H, m), 2.65 (1H, t, J = 14.4 Hz), 3.96 (2H, m); ^{13}C NMR δ 12.2, 16.9, 18.8, 21.1, 31.3, 23.4, 24.3, 25.9, 26.8, 28.4, 32.6, 33.9, 34.8, 35.7, 35.8, 36.1, 37.1, 37.3, 40.2, 40.8, 42.5, 42.9, 44.4, 56.4, 56.6, 69.7, 171.4, 213.6; IR υ_{max} 3369, 2933, 2865, 1740, 1716, 1455, 1377, 1238 cm^{-1}; HRMS (ESI) calcd. for $C_{29}H_{45}D_3O_3$ (M+H$^+$) 448.3865; Found: 448.3866.

*2.1.27. ent-[Cholest-4-ene-3-one-19,19,19-d_3] (**35**)*

Compound **35** (177 mg, 45%) was prepared from compound **33** (400 mg, 1.03 mmol) using the procedure described for the preparation of compound **14**. Compound **35** was a white solid: mp 58–60 °C; $[\alpha]_D^{25}$ −89.6 (c = 0.34, $CHCl_3$); ^{1}H NMR δ 0.71 (3H, s), 0.86 (6H, d, J = 6.6 Hz), 0.91 (3H, d, J = 6.6 Hz), 2.03 (2H, m), 2.40 (4H, m), 5.73 (1H, s); ^{13}C NMR δ 12.1, 18.8, 21.2, 22.7, 23.0, 24.0, 24.3, 28.2, 28.3, 32.2, 33.1, 34.1, 35.8, 35.9, 36.3, 38.6, 39.6, 39.8, 42.5, 53.9, 56.0, 56.2, 123.9, 171.9, 199.9; IR υ_{max} 2950, 2867, 1675, 1467, 1448, 1379, 1264, 1226 cm^{-1}; HRMS (ESI) calcd. for $C_{27}H_{41}D_3O$ (M+H$^+$) 388.3653; Found: 388.3654.

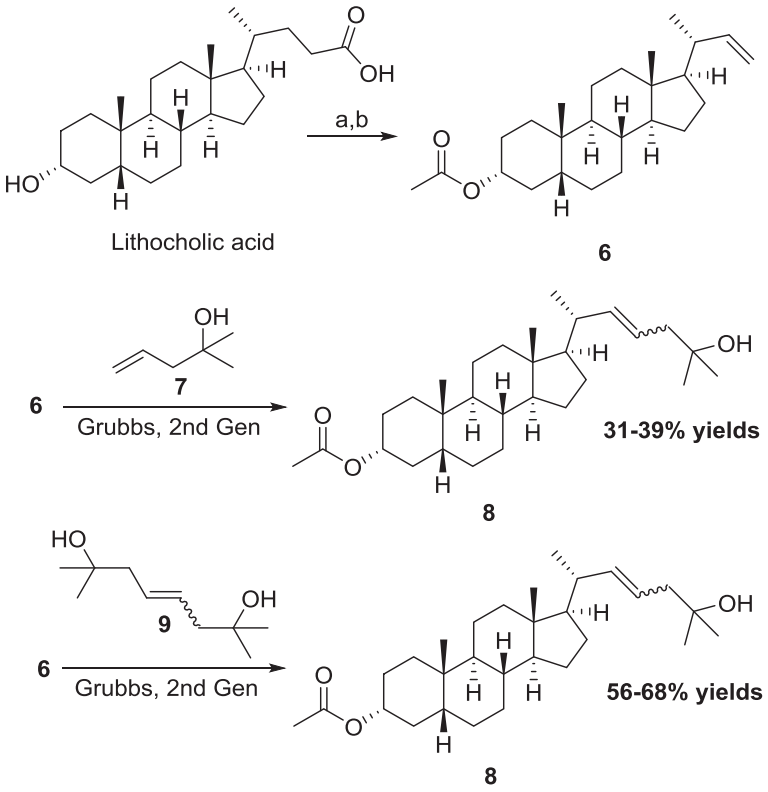
*2.1.28. ent-[(25R)-26-(Acetyloxy)-cholest-4-ene-3-one-19,19,19-d_3] (**36**)*

Compound **36** (125 mg, 42%,) was prepared from compound **34** (324 mg, 0.73 mmol) using the procedure described for the preparation of compound **14**. Compound **36** was a white solid: $[\alpha]_D^{25}$ −64.9 (c = 0.43, $CHCl_3$); ^{1}H NMR δ 0.70 (3H, s), 0.91 (3H, d, J = 6.9 Hz), 0.91 (3H, d, J = 6.3), 2.05 (3H, s), 3.86 (2H, m), 5.72 (1H, s); ^{13}C NMR δ 12.1, 16.9, 18.7, 21.2, 23.4, 24.3, 28.3, 32.2, 32.6, 33.1, 33.9, 34.1, 35.8, 36.1, 38.5, 39.8, 42.5, 53.9, 56.0, 56.2, 69.7, 123.9, 171.5, 171.9, 199.8; IR υ_{max} 3459, 2935, 2870, 1739, 1674, 1617, 1449, 1376, 1240 cm^{-1}; HRMS (ESI) calcd. for $C_{29}H_{43}D_3O_3$ (M+H$^+$) 446.3708; Found: 446.3699.

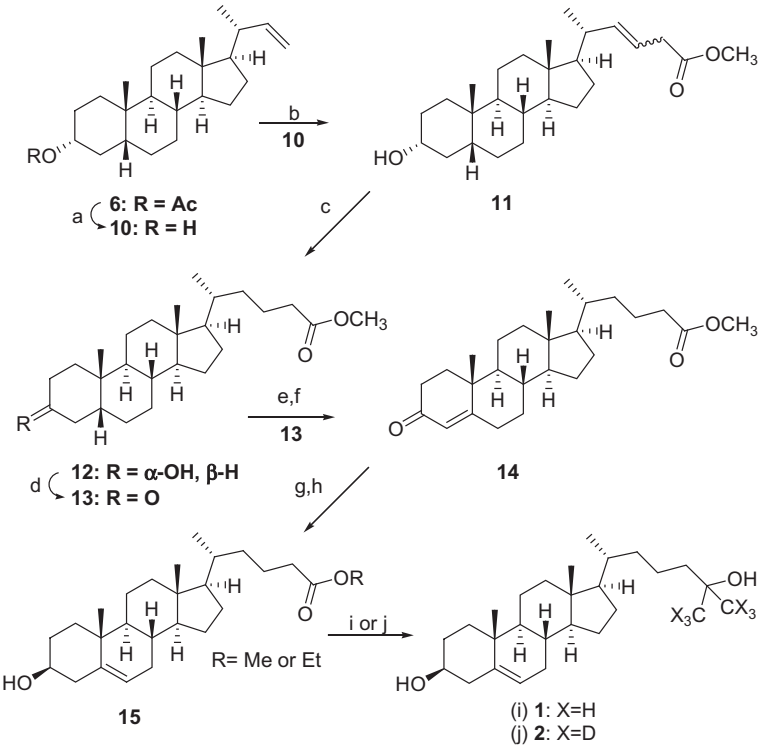
3. Results and discussion

Our goal was to develop a synthetic route that could be utilized to synthesize a variety of side-chain oxysterols in both the natural and unnatural (*ent*) sterol series. We chose to start with either lithocholic acid or *ent*-lithocholic acid as starting materials; the former is commercially available and the latter was previously synthesized [24]. The synthetic strategy was to convert these steroid enantiomers to their corresponding Δ^{22} steroids and subsequently utilize a cross-metathesis reaction to convert them to our target steroids (Fig. 1). Methods were first developed in the natural steroid series because of its ready availability.

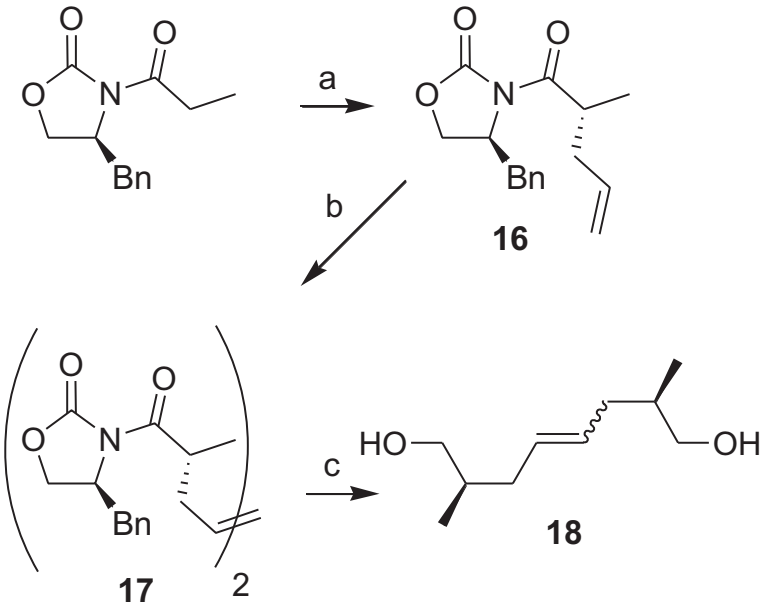
In order to generate the Δ^{22}-steroid for the Grubbs cross-metathesis, lithocholic acid was acetylated at the 3-position and then subjected to oxidative decarboxylation using $Pb(OAc)_4$, $Cu(OAc)_2$ and catalytic pyridine to obtain steroid **6** as described previously [25]. A series of reactions were attempted to cross-metathesize steroid **6** with 2-methylpent-4-en-2-ol (**7**, synthesized as previously described [27]), using Grubbs catalyst, 2nd Generation (Scheme 1). Unfortunately, the yields for steroid **8** (characterized only by ^{1}H NMR) were poor (31–39%), despite attempts to



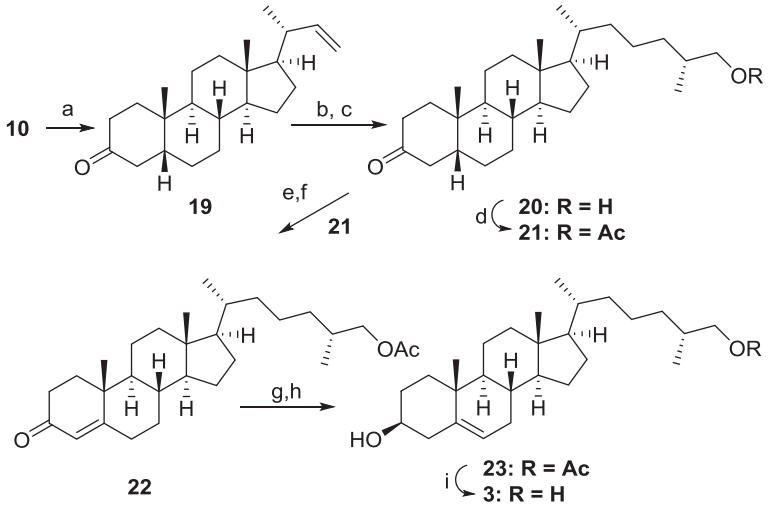
Scheme 1. Attempts to cross-metathesize the Δ^{22} steroid **6** to generate compound **8**. Reagents and Conditions: (a) acetic anhydride, DMAP, pyridine, 12 h, ca. 100%; (b) $Pb(OAc)_4$, $Cu(OAc)_2$, benzene, cat. pyridine, reflux, 6 h, 69%. Steroid **6** was then treated with Grubbs catalyst, 2nd generation, CH_2Cl_2, reflux, 40 h with either compound **7** or compound **9**.



Scheme 2. Reagents and Conditions: (a) K_2CO_3, Methanol/benzene (6/1), reflux, 12 h, 87%; (b) *trans*-3-hexenedioic acid dimethyl ester, Grubbs catalyst, 2nd generation, CH_2Cl_2, reflux, 40 h; (c) H_2, Pd/C (10%), EtOAc, 3 h, 70%, (2 steps); (d) Jones reagent, acetone, 25 °C, 2 h, 96%; (e) pyridinium tribromide, AcOH, 1 h; (f) LiCl, DMF, 100 °C, 2 h, 46% (2 steps); (g) acetic anhydride, TMSCl, NaI, 0 °C to 25 °C, 3 h; (h) $NaBH_4$, EtOH, 25 °C, 12 h; (i) MeMgBr, diethyl ether, 0 °C to 25 °C, 20 h, 60% (3 steps); (j) CD_3Li, ether, 20 h, 59% (3 steps).



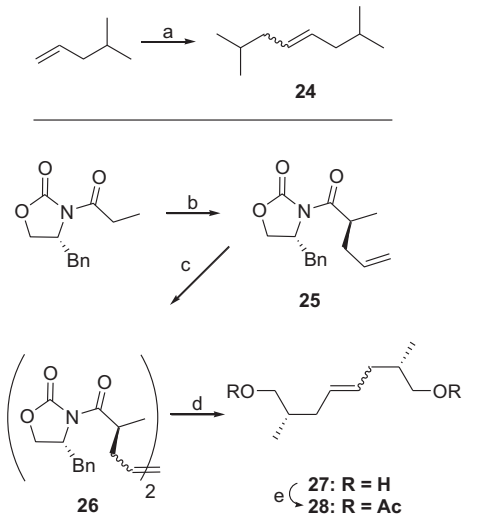
Scheme 3. Reagents and Conditions: (a) allyl magnesium iodide, NaHMDS, THF, −78 °C, 4 h, 82%; (b) Grubbs catalyst, 1st generation, CH_2Cl_2, reflux, 24 h, 77%; (c) $LiAlH_4$, THF, 0 °C, 12 h, 65%.



Scheme 4. Reagents and Conditions: (a) Jones reagent, acetone, 25 °C, 2 h, 93%; (b) **18**, Grubbs catalyst, 2nd generation, CH_2Cl_2, reflux, 72 h; (c) H_2, Pd/C (10%), EtOAc, 12 h, 54%, 2 steps; (d) acetic anhydride, DMAP, pyridine, 3.5 h, 87%; (e) pyridinium tribromide, AcOH, 1.5 h; (f) LiCl, DMF, 100 °C, 2 h, 51% (2 steps); (g) acetic anhydride, TMSCl, NaI, 0 °C to 25 °C, 3 h; (h) $NaBH_4$, EtOH, 12 h; (i) K_2CO_3, MeOH, reflux, 12 h, 57% (3 steps).

optimize this reaction with varying solvent conditions, temperature, reaction time and equivalents of either olefin **7** or the Grubbs catalyst. In all attempts, the main product was homo-metathesized olefin **9** (previously prepared by other methods [28]) and unreacted steroid **6**. This is consistent with a recent report attempting similar cross-metathesis reactions on Δ^{22}-steroids [29]. However, we found that if olefin **9** was used in place of olefin **7**, the cross-metathesis with the Δ^{22}-steroid proceeded smoothly. The high reactivity of terminal olefin **7** with the catalyst apparently resulted in the exhaustion of the catalytic cycle prior to involvement of **6**. We conclude that the less reactive internal olefins perform better than terminal olefins in cross-metathesis reactions with Δ^{22}-steroids.

In order to generate 25-HC (**1**) and 25-HC-d_6 (**2**), steroid **6** was deacetylated (higher yields were obtained with the deacetylated steroid) with K_2CO_3 to obtain steroid **10** in 87% yield (Scheme 2). Steroid **10** was then treated with *trans*-3-hexenedioic acid dimethyl ester and Grubbs catalyst, 2nd generation to form the cross-metathesis product **11** as a mixture of *E* and *Z* isomers (*E*:*Z* ratio >95:5), which upon hydrogenation generated steroid **12** in good yield (70%, 2 steps). Steroid **12** was then oxidized to the 3-ketosteroid **13** using Jones reagent (96%). The 4-position of steroid **13** was selectively brominated using pyridinium tribromide in HOAc and subsequently converted with LiCl in DMF to the known Δ^4-3-ketosteroid **14** [30] in 46% yield. The Δ^4-3-ketosteroid **14** was converted to the Δ^5-3β-hydroxysteroid **15** through a dienol acetate intermediate (formed by treatment with acetic anhydride, NaI and trimethyl silyl chloride) which was then stereoselectively reduced with $NaBH_4$ [31]. Steroid **15** was purified as a combination of methyl and ethyl esters (the latter formed during the $NaBH_4$ reduction in

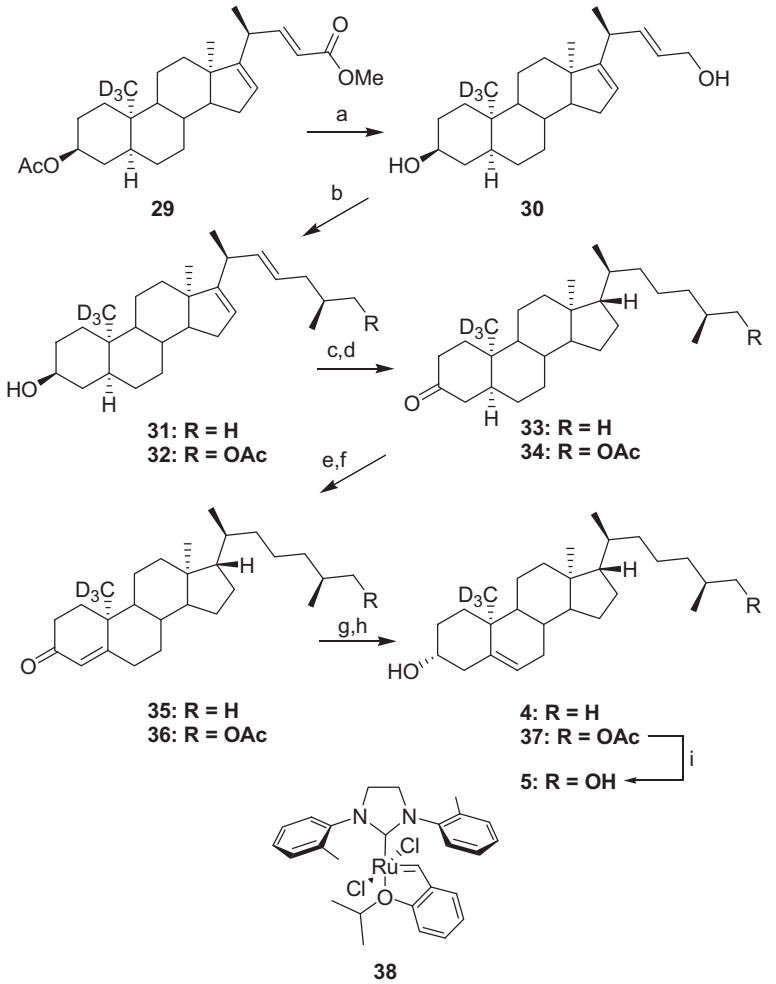


Scheme 5. Reagents and Conditions: (a) Grubbs catalyst, 1st generation, CH_2Cl_2, 12 h, 51%; (b) allyl magnesium iodide, NaHMDS, THF, −78 °C, 4 h, 93%; (c) Grubbs catalyst, 1st generation, CH_2Cl_2, reflux, 24 h, 83%; (d) $LiAlH_4$, THF, 0 °C, 12 h, 82%; (e) acetic anhydride, DMAP, pyridine, 2 h, 66%.

EtOH). Lastly, steroid **15** was either treated with methyl magnesium bromide to generate 25-HC (**1**, 60% from **14**) or trideuterated methyllithium to generate 25-HC-d_6 (**2**, 59% from **14**).

Olefinic diol **18** (Scheme 3), used to generate the side-chain for 27-HC, was synthesized by treating commercially available (4*S*)-3-(1-oxopropyl)-4-(phenylmethyl)-2-oxazolidinone with allyl iodide and NaHMDS to form the diastereoselectively pure product **16** (82%) [26]. Compound **16** was then homo-metathesized with Grubbs catalyst, 1st generation to generate compound **17** in 77% yield as a mixture of *E* and *Z* isomers. The oxazolidinone chiral auxiliary was removed from compound **17** using $LiAlH_4$ to form the olefinic diol **18** as a mixture of *E* and *Z* isomers (65%).

Steroid **10** was oxidized with Jones reagent to form the 3-ketosteroid **19** in 93% yield (Scheme 4). Steroid **19** was cross-metathesized with Grubbs catalyst, 2nd generation and the olefinic diol to form the Δ^{22}-steroid intermediate which after hydrogenation yielded steroid **20** (54%, 2 steps). Steroid **20** was acetylated to obtain steroid **21** (87%) and then converted into steroid **22** (51%) by the earlier described bromination/elimination sequence. The earlier described sequence for the conversion of Δ^4-3-ketosteroids to Δ^5-3β-hydroxysteroids yielded steroid **23** which was not characterized, but was immediately deacetylated to yield 27-HC (**3**, 57% 3 steps).



Scheme 6. Reagents and Conditions: (a) DIBAL-H (1 M in THF), THF, −78 °C, 1 h, 97%; (b) **38** with **24** for synthesis of **31** or **38** with **28** for synthesis of **32**, CH_2Cl_2, reflux, 72 h; (c) H_2/Pd, EtOAc, 4 h; (d) Jones reagent, acetone, 30 min, 35–53% for 3 steps; (e) pyridinium tribromide, AcOH, 1.5 h; (f) LiCl, DMF, 100 °C, 2 h, 42–45% (2 steps); (g) acetic anhydride, TMSCl, NaI, 0 °C to 25 °C, 3 h; (h) $NaBH_4$, EtOH, 12 h, 65% for **4** (2 steps); (i) K_2CO_3, MeOH, reflux, 12 h, 60% for **5** (3 steps).

The synthetic route utilized for the *ent*-steroids was very similar to that described for the natural steroids. The reagent used to generate the side-chain for *ent*-Chol-d_3 (**4**) in the cross-metathesis reaction was synthesized by treating 4-methylpent-1-ene with Grubbs catalyst, 1st generation to obtain 2,7-dimethyloct-4-ene **24** as a mixture of *E* and *Z* isomers in 51% yield (Scheme 5). The side-chain used to construct *ent*-27-HC-d_3 (**5**) was synthesized similarly to that for 27-HC. (4*R*)-3-(1-oxopropyl)-4-(phenylmethyl)-2-oxazolidinone was allylated, treated with 1st generation Grubbs catalyst to generate the disubstituted olefin, and hydrolyzed to synthesize the diol **27**. This compound was then acetylated with acetic anhydride, DMAP, and pyridine to generate the *bis*-acetylated product **28** in 50% overall yield for the four steps.

ent-Testosterone-19,19,19-d_3 was synthesized as previously reported [32]. The deuterium labeled *ent*-testosterone was then converted to *ent*-steroid **29** (Scheme 6) in the same manner that unlabeled *ent*-testosterone was converted to the analogous unlabeled steroid [24]. In order to simplify the synthesis, we attempted to perform the cross-metathesis reaction directly on the natural enantiomer of compound **29** using both 2nd generation Grubbs catalyst and the more active ruthenium catalyst, **38**. This simplification was unsuccessful; however, when the natural enantiomer of compound **29** was reduced to the natural enantiomer of compound **30** using DIBAL-H, we found that the ruthenium-based catalyst **38** successfully catalyzed its cross-metathesis reaction with olefin **24**. Therefore, *ent*-steroid **29** was reduced to *ent*-steroid **30** with DIBAL-H (97%) and was then treated with the ruthenium-based catalyst **38** and either olefin **24** or **28** to form *ent*-steroids **31** or **32**, respectively. Both *ent*-steroids were then hydrogenated and oxidized with Jones reagent to generate *ent*-steroids **33** and **34** in 54% and 35% yield, respectively over 3 steps. Treatment of *ent*-steroids **33** and **34** with pyridinium tribromide in HOAc followed by LiCl in DMF yielded *ent*-steroids **35** and **36** in 45% and 42% yield, respectively. These Δ^4-3-ketones were then converted either to *ent*-Chol-d_3 (65%) or to the acetylated intermediate **37** as described for the preparation of steroid **15**. Treatment of *ent*-steroid **37** with K_2CO_3 yielded *ent*-27-HC-d_3 in 60% yield from *ent*-steroid **35**. *ent*-Chol-d_3 has previously been prepared by a different total synthesis method [33].

In summary, a cross-metathesis reaction has been successfully utilized to synthesize 25-HC (**1**), 25-HC-d_6 (**2**), 27-HC (**3**), *ent*-Chol-d_3 (**4**) and *ent*-27-HC-d_3 (**5**). We believe the methodology developed will be of general use for synthesizing other analogues with side-chains that are different from those described here. Further studies utilizing the newly synthesized *ent*-27-HC-d_6 to probe the mechanism for its regulation of cholesterol homeostasis are anticipated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2017.03.002.

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