Synthesis of Potential Mechanism-Based Inactivators of Lanosterol 14α-Methyl Demethylase

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Lanosterol 14α-methyl demethylase is a key enzyme in the degradation of lanosterol to cholesterol. This cytochrome P-450 monooxygenase catalyzes the oxidative removal of the methyl group at C-14 of lanosterol. Inhibitors of this enzyme may prove useful as cholesterol-lowering agents and as antimycotics. This paper describes the design and synthesis of seven potential mechanism-based inactivators (compounds **9**–**15**) of lanosterol 14α-methyl demethylase. The appropriately protected aldehydes **20a** and **20b** were the key intermediates in the synthesis of compounds **9**, **11**, **12**, **14**, **15**. A method was developed for the homologation of these aldehydes to give **25a** and **25b**, which were converted to lanosterol analogues **10** and **13**.

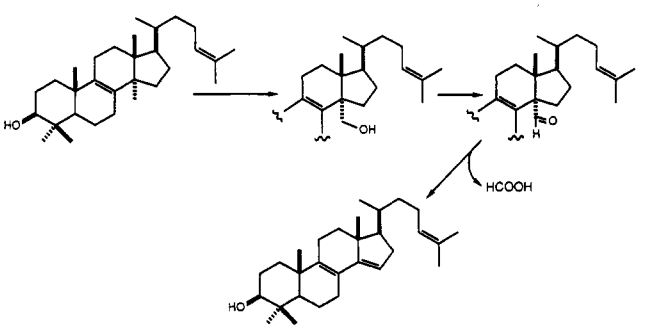
A key step in the biosynthesis of cholesterol and ergosterol is the removal of the C-14 methyl group of lanosterol by the enzyme lanosterol 14α-methyl demethylase.[1] Mammalian lanosterol 14α-methyl demethylase is a cytochrome P-450 monooxygenase which oxidatively removes C-32 of lanosterol in three O_2-NADPH dependent steps. The 14α-methyl group is first hydroxylated and then oxidized to the aldehyde; the nature of the third oxidation, which results in the loss of C-32 as formic acid and the formation of the 8,14-diene, is still unclear (Scheme I).[2] Both lanosterol and its Δ^7 isomer are efficiently converted to cholesterol,[3] and the double bond in the side chain is not mandatory for turnover.[4]

Inhibitors of lanosterol 14α-methyl demethylase are not only of potential use as cholesterol-lowering agents, but also as antimycotics. We have designed and synthesized a series of potential mechanism-based inactivators[5] of lanosterol 14α-methyl demethylase based on the first two steps in the removal of C-32 (Figure 1). The first approach involves a lanosterol derivative having two leaving groups (X and Y) attached to C-32. Enzymatic oxidation of **1** would give an unstable geminal halohydrin which, upon spontaneous loss of HY, should yield the reactive acylating species **2**. Reaction of this electrophile with an active-site nucleophile should inactivate the enzyme as a result of covalent bond formation.

The second approach is based on the enzymatic formation of the α,β-unsaturated ketone **5a**, which could be formed from **3** by two enzymatic oxidations, or from **4** (X = leaving group) by one oxidation followed by loss of HX. Michael addition of an enzymatic nucleophile to the α,β-unsaturated ketone **5a** would lead to covalent linkage of steroid to the enzyme. Another possibility is enzymatic oxidation of the multiple bonds in **3** and **4** to give reactive oxirenes and oxiranes respectively (**5b**, **5c**).

A third approach to the mechanism-based inactivation of lanosterol 14α-methyl demethylase is the enzymatic generation of an α-halo ketone **7**. In this case, two oxidations of the 32-halomethyl analogue **6** would result in the formation of reactive species **7** which might covalently modify the enzyme.

Scheme I



The above approaches suggested the preparation of 32-functionalized lanost-7-en-3β-ols **9**–**15** (Figure 2). The Δ^7-sterols were chosen in preference to the Δ^8-isomers because they are easily accessible from the steroidal tetrahydrofuran **8**, and, as mentioned earlier, lanost-7-en-3β-ol is efficiently converted to cholesterol. The vinyl and acetylenic compounds **14** and **15** were also prepared on the basis of Ortiz de Montellano's demonstration that terminal vinyl and acetylenic compounds cause oxygen-dependent inactivation of cytochromes P-450.[6]

Results and Discussion

The aforementioned 32-functionalized lanost-7-en-3β-ols were synthesized from lanost-7-ene-3β,32-diol **16**.[7a] We have developed an improved method for the preparation of diol **16** from tetrahydrofuran **8**. As noted by Schroepfer[7b] and by Sato,[7c] the reported method for the conversion of **8** to **16** yields a mixture of double bond isomers which are difficult to separate on a preparative scale. We have found that treatment of compound **8** with acetyl chloride, acetic anhydride, and pyridine (1 equiv)[8] furnishes isom-

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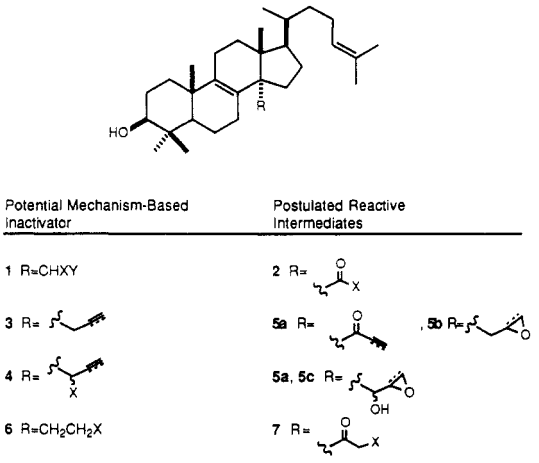


Figure 1.

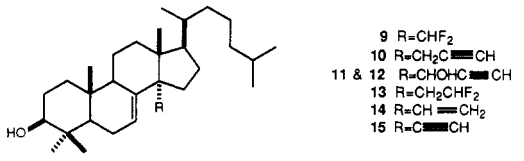
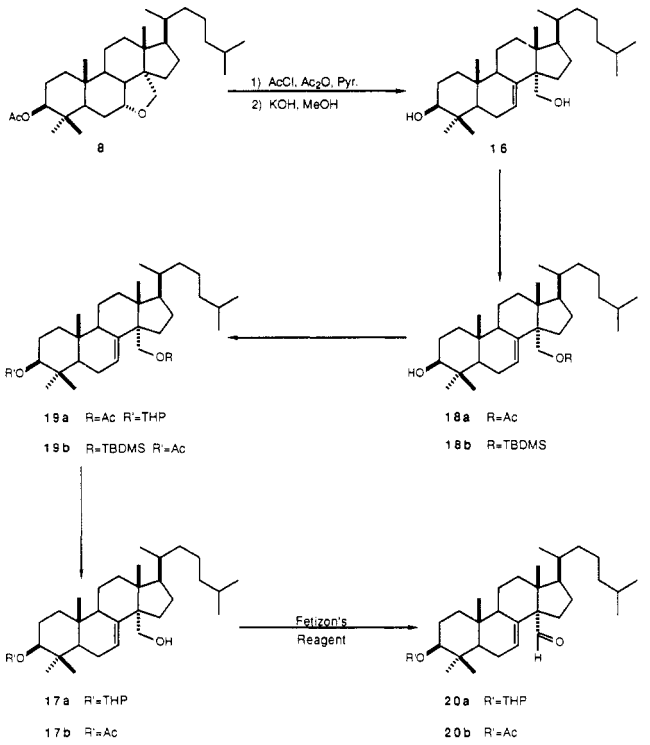


Figure 2.

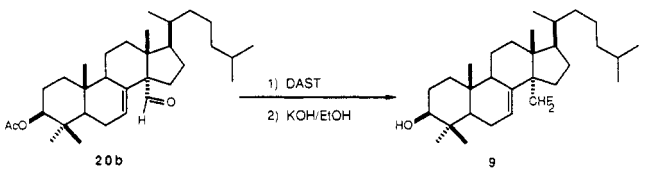
Scheme II



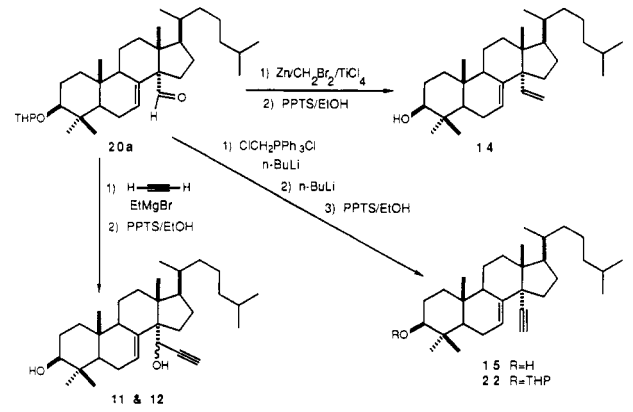
erically pure **16** after deprotection, in an overall yield of 52% (Scheme II).

The desired 3-protected 3,32-diols **17a** and **17b** were obtained from diol **16** by blocking the primary 32-hydroxyl followed by protection of the secondary 3-alcohol, and regeneration of free 32-hydroxyl (Scheme II). Thus, the diol **16** was treated with acetic anhydride and pyridine[9] to give a mixture of the 32-acetate **18a** and the 3,32-diacetate (**19c**: R = R′ = Ac), which were easily separated by flash chromatography. Diacetate **19c** could be quantitatively cleaved back to the diol **16** with potassium hydroxide in methanol. The monoprotected diol **18a** was then reacted with dihydropyran and pyridinium *p*-toluenesulfonate (PPTS) in methylene chloride to give bisprotected **19a**.[10] The acetate at C-32 was then removed by treatment with lithium aluminum hydride to give **17a**.[11] The position of protection was easily discerned by ^{1}H NMR spectroscopy, and the integrity of the steroid nucleus was verified by conversion of **17a** to the starting diol **16**. In a similar manner, the 3β-acetoxylanost-7-en-32-ol **17b** was prepared by protection of the 32-hydroxyl as the *tert*-butyldimethylsilyl (TBDMS) ether, acetylation,[12] and removal of the silyl protecting group.[13] Oxidation with Fetizon's reagent (silver carbonate on celite) was the optimal method for conversion of alcohols **17a** and **17b** to aldehydes **20a** and **20b** (Scheme II).[14,15]

Scheme III



Scheme IV



The difluoromethyl compound **9** was prepared by treating the acetoxy aldehyde **20b** with (diethylamino)-sulfur trifluoride (DAST)[16] at 80 °C followed by hydrolysis of the acetate (Scheme III, overall yield 25%). The vinyl compound **14** was synthesized from compound **20a** (Scheme IV) by using Oshima's methylenation reagent ($Zn/CH_2Br_2/TiCl_4$)[17] followed by deprotection with PPTS in abs. ethanol.[10] The acetylenic analogue **15** was obtained from aldehyde **20a** (Scheme IV) by reaction with the ylide of chloromethyltriphenylphosphonium chloride followed by treatment with *n*-butyllithium.[18] The desired product **15** was then obtained by removal of the THP-protecting group of compound **22**.[10] The diastereomeric propargylic alcohols **11** and **12** were prepared by treating the THP-

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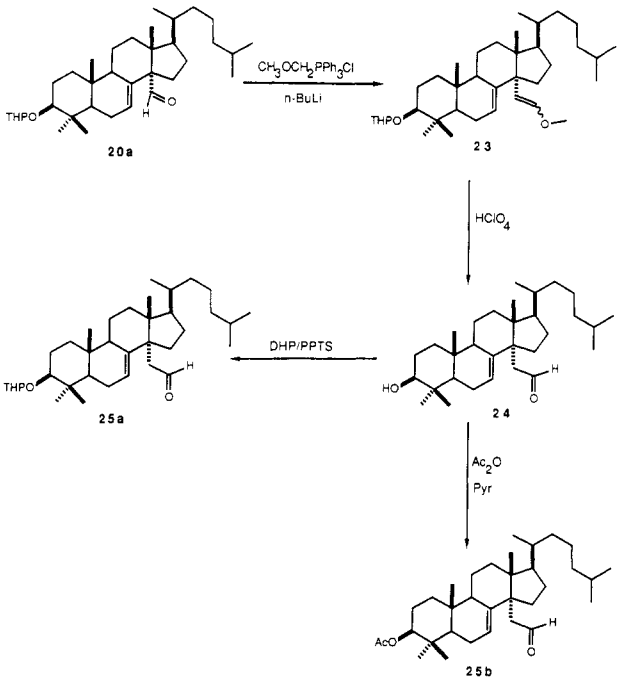
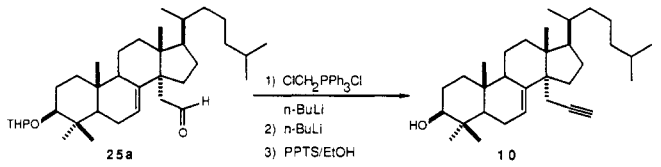
Scheme V



Scheme VI

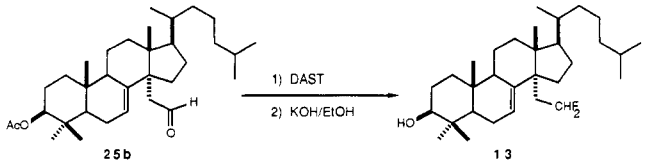


protected aldehyde **20a** with the Grignard reagent of acetylene[19] followed by deprotection (Scheme IV).[10] The diastereomers could be separated easily by either careful flash chromatography or preparative HPLC. The absolute configuration at C-32 of these alcohols has not yet been determined.

The synthesis of the propargyl compound **10** and the difluoroethyl compound **13** necessitated the preparation of the previously undescribed homologated aldehydes **25a** and **25b** (Scheme V). Thus, reaction of aldehyde **20a** with the ylide of (methoxymethyl)triphenylphosphonium chloride, followed by hydrolysis of the resultant enol ether **23** with perchloric acid gave homologated aldehyde **24**.[20] This unprotected homologated aldehyde could be reprotected as either the 3-THP ether or the 3-acetate to give the aldehydes **25a** (94% yield from **23**) and **25b** (68% yield from **23**), respectively. With the homologated aldehydes in hand, propargyl compound **10** and difluoroethyl compound **13** were prepared in a manner analogous to the preparation of the acetylenic compound **15** and the difluoromethyl compound **9**, respectively (Schemes VI and VII).[18,16]

In summary, we have synthesized a series of novel 32-functionalized lanost-7-en-3β-ols (**9–15**) which are potential mechanism-based inactivators of lanosterol 14α-methyl demethylase. All of these compounds proved to be effective competitive inhibitors of the enzyme, and the data have been presented in a preliminary account[21] of this work. Enzyme inactivation studies are in progress.

Scheme VII



Experimental Section

Melting points were determined on a hot-stage apparatus and are uncorrected. ^{19}F NMR chemical shifts are reported as ppm downfield from Freon-11 ($CFCl_3$). Analytical thin-layer chromatography (TLC) was performed on Machery-Nagel Polygram Silica G/UV_{254} precoated plastic sheets. Preparative TLC was performed on Analtech preparative silica gel G Uniplates 20 × 20 cm square. Flash chromatography was carried out on silica gel as described by Still.[22] Reactions requiring anhydrous conditions and an inert atmosphere were carried out in oven-dried flasks equipped with stir bar, serum cap, and gas-needle inlet, under argon, as described for compound **10**.

The following reagents were purchased from Aldrich Chemical Co. and were used as received: (diethylamino)sulfur trifluoride (DAST), dibromomethane, titanium(IV) chloride, (chloromethyl)triphenylphosphonium chloride, lithium aluminum hydride (LAH), *tert*-butyldimethylsilyl chloride (TBDMSCl), 4-(dimethylamino)pyridine (DMAP), (methoxymethyl)triphenylphosphonium chloride, ethylmagnesium bromide (2 M in THF), *n*-butyllithium (1.96 M in hexanes), and tetra-*n*-butylammonium fluoride (1.0 M in THF). Dihydropyran (DHP) was purchased from Aldrich Chemical Co. and was distilled prior to use. Acetylene was obtained from Matheson, Inc., and prior to use was passed through a tower of alumina and then through concentrated H_2SO_4. Acetic anhydride was distilled prior to use. Benzene and methylene chloride were distilled from calcium hydride prior to use. Tetrahydrofuran (THF) and ether were distilled from sodium benzophenone ketyl. Pyridine was distilled from potassium hydroxide. Pyridinium *p*-toluenesulfonate (PPTS) and Fetizon's Reagent were prepared as described by Grieco[10] and Fetizon,[14] respectively. All other reagents were of reagent grade and were used as received.

32,32-Difluorolanost-7-en-3β-ol (9). Aldehyde **20b** (78.4 mg, 0.162 mmol) and neat DAST (1.5 mL, 12.3 mmol) in a 10-mL flask were stoppered tightly with a Teflon stopper and were heated at 80 °C for 3 h. Care must be taken in the use of DAST; explosions have been reported[23] during the distillation of DAST and while solvent was being removed from the product. The reaction was then cooled to 0 °C, a stir bar was added, and water was introduced cautiously, with stirring, until the reaction was quenched. The resultant mixture was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was submitted to flash chromatography (5:1 hexanes–ethyl acetate), giving 20.8 mg (25%) of the desired acetate protected difluoro compound: ^1H NMR ($CDCl_3$, 80 MHz) δ 4.95 (t, J = 56.4 Hz, 1 H), 5.41–5.33 (m, 1 H), 4.51 (dd, J = 5.5 and 9.4 Hz, 1 H), 2.05 (s, 3 H), 2.1–0.5 (m, 47 H); mass spectrum, m/e 506 (M^+), 455 (M - CHF_2).

A solution of the above acetate-protected difluoro compound (20.8 mg) in methanolic KOH (50 mL, 5%) and absolute ethanol (10 mL) was stirred at room temperature overnight and then concentrated in vacuo. Water and CH_2Cl_2 were added to the residue, and the pH was adjusted to 6 with dilute acetic acid. The mixture was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was submitted to flash chromatography (9:1 hexanes–ethyl acetate), giving 19.0 mg (quant.) of 32,32-difluorolanost-7-en-3β-ol (**9**): mp 106.5–107.0 °C; IR ($CHCl_3$) 3620, 3450, 2900, 1720, 1682, 1460, 1382, 1365, 1160, 1095, 1030, 890, 860 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.95 (t, J = 56.5 Hz, 1 H), 5.37 (m, 1 H), 3.25 (dd, J = 4.50 and 11.0 Hz, 1 H), 2.1–0.70 (m, 46 H); ^{19}F NMR ($CDCl_3$, 75

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MHz) δ −117.85 (dd, J = 56.8 and 277.0 Hz), −123.61 (dd, J = 57.1 and 277 Hz); mass spectrum, m/e 464 (M^+), 449 (M − CH_3), 446 (M − H_2O), 431 (M − CH_3 − H_2O), 414 (M − CF_2). Anal. Calcd for $C_{30}H_{50}OF_2$: C, 77.54; H, 10.85; F, 8.18. Found: C, 77.58; H, 10.96; F, 8.17.

32-Ethynyllanost-7-en-3β-ol (10). An oven-dried 10-mL one-neck round-bottom flask with stirring bar was charged with (chloromethyl)triphenylphosphonium chloride (71.8 mg, 0.21 mmol), equipped with a serum cap and a gas-needle inlet, and flushed with argon. Dry THF (1.4 mL) was introduced followed by the dropwise addition of *n*-butyllithium (84 μL, 1.96 M in hexanes, 0.164 mmol). The resultant orange-red solution was stirred at room temperature for 2 h. Homologated aldehyde **25a** (9.2 mg, 0.017 mmol) in dry THF (1.8 mL) was added dropwise to the above mixture. The flask was equipped with a condenser, and the solution was heated under reflux, under argon, for 2 h. The reaction was allowed to cool to room temperature, and aqueous saturated NH_4Cl was added. The THF was removed in vacuo, and the residue was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$). Filtration, concentration, and purification by preparative TLC (20 × 20 cm, 250 μm, 50:1 hexanes–ethyl acetate) gave a mixture of the isomeric vinyl chlorides (8.7 mg, 89%): ^1H NMR ($CDCl_3$, 80 MHz) δ 6.01 (d, J = 7.1 Hz), 5.71 (d, J = 7.1 Hz), 5.72–5.38 (m), 5.24–5.07 (m, 1 H), 4.82–4.50 (m, 1 H), 4.2–2.9 (m, 3 H), 2.5–0.6 (m, 54 H).

A solution of the above vinyl chlorides (8.7 mg, 0.016 mmol) in dry THF (1.0 mL) was stirred under argon, *n*-butyllithium (77 μL, 1.96 M in hexanes, 0.15 mmol) was added dropwise, and the mixture was stirred at room temperature for 2.3 h. Aqueous saturated NH_4Cl was added dropwise, and the THF was removed in vacuo. The residue was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4). Filtration, concentration, and purification by preparative TLC (20 × 20 cm, 250 μm, 9:1 hexanes–ethyl acetate) gave the THP-protected propargyl compound (5.7 mg, 70%): ^1H NMR ($CDCl_3$, 80 MHz) δ 5.4–5.2 (m, 1 H), 4.8–4.7 and 4.6–4.5 (m, 1 H), 4.3–2.9 (m, 3 H), 2.5–0.6 (m, 55 H).

A solution of the THP-protected propargyl compound (5.7 mg, 0.011 mmol) in absolute ethanol (10 mL) containing PPTS (5.2 mg, 0.106 mmol) was stirred at room temperature overnight. The ethanol was removed in vacuo, and the residue was partitioned between CH_2Cl_2 and water. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$). Filtration, concentration, and purification by preparative TLC (20 × 20 cm, 250 μm, 9:1 hexanes–ethyl acetate) gave compound **10** (4.8 mg, quant.) as a white crystalline solid: mp 142.0–142.5 °C; IR ($CHCl_3$) 3610, 3440, 3305, 2890, 2110, 1715, 1665, 1465, 1382, 1365, 1165, 1090, 1050, 1020, 995, 908, 858 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.28 (t, J = 2.4 Hz, 1 H), 3.26 (dd, J = 4.9 and 11.2 Hz, 1 H), 2.5 (dt, J = 2.40 and 16.4 Hz, 1 H), 2.16 (dd, J = 2.4 and 4.2 Hz, 1 H), 2.1–0.7 (m, 47 H); mass spectrum, m/e 452 (M^+), 437 (M − CH_3), 413 (M − $CH_2C{\equiv}CH$), 395 (M − $CH_2C{\equiv}CH$ − H_2O). Anal. Calcd for $C_{32}H_{52}O$: C, 84.89; H, 11.58. Found: C, 84.73; H, 11.74.

32-Ethynyllanost-7-ene-3β,32-diols (11 and 12). Acetylene was bubbled through stirred THF (10 mL) for 5 min. Ethylmagnesium bromide (1.90 mL, 2.0 M in THF, 3.8 mmol) was added dropwise. After the addition was complete, the solution was stirred at room temperature for 1.5 h with acetylene continuously bubbling through the mixture. The solution was cooled to 0 °C, and the aldehyde **20a** was introduced dropwise in a total of 2.0 mL of dry THF. As soon as the addition was complete, the reaction was allowed to warm to room temperature and stirred for 1 h with continuous introduction of acetylene. The resultant solution was poured into aqueous saturated NH_4Cl, the THF was removed in vacuo, and the residue was extracted well with ethyl acetate. The combined organic layers were dried ($MgSO_4$). Filtration, concentration, and purification by flash chromatography (9:1 hexanes–ethyl acetate) gave a mixture of the diastereomeric THP-protected propargyl alcohols as a clear oil (42.1 mg, 92%): ^1H NMR ($CDCl_3$, 80 MHz) δ 5.5–5.3 (m, 1 H), 4.8–4.3 (m, 2 H), 4.3–2.9 (m, 3 H), 2.58–2.5 and 2.46–2.37 (m, 1 H), 2.3–0.6 (m, 52 H).

A solution of the above THP-protected propargyl alcohols (25.0 mg, 0.045 mmol) in absolute ethanol (5.0 mL) containing PPTS (18.4 mg) was stirred at room temperature. The reaction was followed by TLC, and PPTS was added over a 3-day period until the reaction was complete (total amount of PPTS added was 43.2 mg, 1.72 mmol). The ethanol was removed in vacuo, and the residue was partitioned between ethyl acetate and 10% HCl. The ethyl acetate layer was washed with 10% HCl, the combined aqueous layers were back extracted with ethyl acetate, and the combined organic layers were dried ($MgSO_4$). Filtration, concentration, and purification by flash chromatography (3:1 hexanes–ethyl acetate) gave the diastereomeric propargyl alcohols **11** and **12** (13.8 and 8.3 mg, respectively; quant.). **11**: mp 161–162 °C; IR ($CHCl_3$) 3600, 3450, 3300, 2925, 2875, 1720, 1465, 1382, 1370, 1160, 1090, 1010, 980, 910 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.45–5.25 (m, 1 H), 4.41 (br d, J = 7.44 Hz, 1 H), 3.25 (dd, J = 8.9 and 5.3 Hz, 1 H), 2.55 (d, J = 2.3 Hz, 1 H), 2.5–0.6 (m, 46 H); mass spectrum, m/e 468 (M^+), 453 (M − CH_3), 413 (M − CHOHC≡CH), 395 (M − CHOHC≡CH − H_2O); high-resolution mass spectrum calcd 468.3970, found 468.3966. **12**: mp 135–136 °C; IR ($CHCl_3$) 3470, 3305, 2940, 2860, 1720, 1465, 1382, 1090, 1015 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.56–5.37 (m, 1 H), 4.61–4.48 (m, 1 H), 3.4–3.1 (m, 1 H), 2.41 (d, J = 2.1 Hz, 1 H), 2.36–0.60 (m, 46 H); mass spectrum, m/e 468 (M^+), 453 (M − CH_3), 413 (M − CHOHC≡CH), 395 (M − CHOHC≡CH − H_2O); high-resolution mass spectrum calcd 468.3970, found 468.3997.

32-(Difluoromethyl)lanost-7-en-3β-ol (13). The homologated aldehyde–acetate **25b** (41.3 mg, 0.083 mmol) and neat DAST (0.76 mL, 6.2 mmol) were heated at 80 °C for 2 h in a 10-mL flask stoppered tightly with a Teflon stopper. The mixture was allowed to cool to room temperature and slowly poured into stirred ice water. The resultant mixture was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was submitted to flash chromatography (35:1 hexanes–ethyl acetate), giving 19.5 mg of a clear glass which was a mixture of the desired product and an unknown compound.

The above mixture (19.5 mg) in methanolic KOH (100 mL, 5%) was stirred at room temperature overnight and worked up exactly as for the hydrolysis of **9**. The residue was submitted to flash chromatography (9:1 hexanes–ethyl acetate), giving a mixture of the desired 32-(difluoromethyl)lanost-7-en-3β-ol (**13**) and an unknown compound. These two compounds were separated by HPLC (Whatman M9/50 Partisil 10, 5:1 hexanes–ethyl acetate, 4 mL/min, t_R 19.4 min), giving 6.3 mg (16% from **25b**) of the desired product **13**: mp 111.5–112.5 °C; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.52 (tdd, J = 57, 4.8, 2.8 Hz, 1 H), 5.43–5.23 (m, 1 H), 3.25 (dd, J = 5.5 and 9.0 Hz, 1 H), 2.3–0.6 (m, 48 H); ^{19}F NMR ($CDCl_3$, 75 MHz) δ −109.95 (ddd, J = 57, 32, and 25 Hz); mass spectrum, m/e 478 (M^+), 458 (M − HF), 445 (M − CH_3 − H_2O), 414 (M − $CHCHF_2$), 413 (M − CH_2CHF_2), 396 (M − $CHCHF_2$ − H_2O), 395 (M − CH_2CHF_2 − H_2O); high-resolution mass spectrum calcd 478.3989, found 478.3961.

4,4-Dimethyl-14α-ethenylcholest-7-en-3β-ol (14). To a stirred solution of **20a** (40.5 mg, 0.077 mmol) and dry CH_2Cl_2 (7 mL) at room temperature was added ca. 1 mL of the CH_2Br_2/Zn/$TiCl_4$ complex.[17] After the mixture was stirred for 5 min, the reaction was complete as judged by TLC. A 2:1 solution of saturated aqueous $NaHCO_3$–water was added, and the resultant solution was extracted four times with ether. The combined organic layers were washed with 2:1 saturated $NaHCO_3$–water and dried ($MgSO_4$). Filtration and rotary evaporation gave a crude product, which was submitted to flash chromatography (25:1 hexanes–ethyl acetate) to give the THP-protected vinyl compound as a clear glass (23.0 mg, 57%): ^1H NMR ($CDCl_3$, 80 MHz) δ 6.47–6.05 (m, 1 H), 5.37–5.21 (m, 1 H), 4.80–4.71 (m, 1 H), 4.77–4.46 (m, 1 H), 4.18–2.86 (m, 3 H), 2.3–0.6 (m, 52 H).

A solution of the above THP-protected vinyl compound (23.0 mg, 43.8 mmol) in absolute ethanol (5 mL) containing PPTS (29.7 mg, 0.12 mmol) was stirred for 24 h. The ethanol was removed in vacuo, and the residue was partitioned between ethyl acetate and aqueous saturated NH_4Cl. The aqueous layer was washed with ethyl acetate, and the combined organic layers were dried ($MgSO_4$). Filtration, concentration, and purification by flash chromatography (9:1 hexanes–ethyl acetate) gave 19.2 mg (quant.) of **14** as a white crystalline solid: mp 106.5–107.0 °C; IR ($CHCl_3$) 3610, 3350, 2920, 1722, 1665, 1611, 1465, 1382, 1365, 995, 912 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 6.28 (dd, J = 10.7 and 17.3 Hz, 1 H), 5.36–5.30 (m, 1 H), 5.01 (dd, J = 17.3 and 1.8 Hz, 1 H), 4.953 (dd, J = 10.7 and 1.8 Hz, 1 H), 2.07–0.66 (m, 46 H); mass spectrum,

m/e 440 (M^+), 425 (M - CH_3), 422 (M - H_2O); high-resolution mass spectrum calcd 440.4021, found 440.4027.

4,4-Dimethyl-14α-ethynylcholest-7-en-3β-ol (15). To a solution of (chloromethyl)triphenylphosphonium chloride (171.8 mg, 0.495 mmol) in dry THF (4 mL) under argon was added dropwise *n*-butyllithium (253 μL, 1.55 M, 0.392 mmol). The resultant red slurry was stirred at room temperature for 2 h, under argon. The THP-protected aldehyde **20a** (49.1 mg, 0.093 mmol) in 1.5 mL of dry THF was added dropwise. The flask was equipped with a condenser, and the mixture was heated under argon at reflux for 1 h. The solution was allowed to cool to room temperature, dilute NH_4Cl was added, and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4). Filtration, concentration, and purification by flash chromatography (9:1 hexanes–ethyl acetate) gave 59.7 mg of the isomeric vinyl chlorides, contaminated with a small amount of triphenylphosphine oxide: ^1H NMR ($CDCl_3$, 80 MHz) δ 6.02 (d, *J* = 7.1 Hz), 5.71 (d, *J* = 7.1 Hz), 5.63 (d, *J* = 6.5 Hz), 5.46 (d, *J* = 6.5 Hz, 6.02–5.46 integrates to 2 H), 5.23–5.05 (m, 1 H), 4.82–4.51 (m, 1 H), 4.15–2.91 (m, 3 H), 2.4–0.7 (m, 52 H).

To a stirred solution of the above THP-protected vinyl chlorides (59.7 mg) under nitrogen in dry THF (2.5 mL) was added *n*-butyllithium (0.60 mL, 1.55 M, 0.93 mmol) dropwise, and the mixture stirred at room temperature for 1.5 h. Aqueous saturated NH_4Cl was added, and the THF was removed in vacuo. The residue was extracted with CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4). Filtration, concentration, and purification by flash chromatography (35:1 hexanes–ethyl acetate) gave the THP-protected acetylenic compound (31.1 mg, 64% from **20a**): ^1H NMR ($CDCl_3$, 80 MHz) δ 5.50–5.33 (m, 1 H), 4.84–4.53 (m, 1 H), 4.13–3.92 (m, 3 H), 2.4–0.5 (m, 53 H).

A solution of the above THP-protected acetylenic compound (31.1 mg, 0.075 mmol) in absolute ethanol (10 mL) containing PPTS (6.8 mg, 0.027 mmol) was stirred overnight at room temperature. PPTS was added over the next 2 days (total amount PPTS added was 28.3 mg, 0.11 mmol), and the reaction was followed by TLC. When complete, 10% aqueous $NaHCO_3$ solution was added, the ethanol was removed in vacuo, CH_2Cl_2 was added to the residue, and the mixture was brought to pH 6 with 10% aqueous HCl. The CH_2Cl_2 was removed, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$). Filtration, concentration, and purification by flash chromatography (9:1 hexanes–ethyl acetate) gave acetylenic compound **15** (25.4 mg, 94%): mp 168.0–168.5 °C; IR ($CHCl_3$) 3620, 3302, 2925, 2870, 2095, 1730, 1665, 1465, 1382, 995 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.47–5.40 (m, 1 H), 3.29 (dd, *J* = 11.3 and 4.8 Hz, 1 H), 2.17 (s, 1 H), 2.25–0.8 (m, 46 H); mass spectrum, m/e 438 (M^+), 423 (M - CH_3), 420 (M - H_2O), 405 (M - CH_3 - H_2O). Anal. Calcd for $C_{31}H_{50}O$: C, 84.87; H, 11.49. Found: C, 84.97; H, 11.54.

Lanost-7-ene-3β,32-diol (16). A stirred solution of compound **8** (2.29 g, 4.71 mmol) in acetic anhydride (46.0 mL), acetyl chloride (2.25 mL, 31.6 mmol), and dry pyridine (0.39 mL, 4.8 mmol) was heated to reflux under argon for 24 h. The resultant solution was poured into ice water, allowed to stand for 1.5 h, and extracted with ether. The combined ether layers were washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and water and dried ($MgSO_4$). Filtration, concentration, and flash chromatography (12:1 hexanes–ethyl acetate) gave 1.97 g of the desired diacetate contaminated with a compound of unknown structure.

A solution of the above mixture and KOH (13.5 g) in absolute methanol (540 mL) was heated under reflux for 3 h. After cooling to room temperature, the solution was concentrated in vacuo and poured into aqueous saturated NH_4Cl. The resultant slurry was acidified (pH 5) with 1 N aqueous HCl and extracted with 10% CH_2Cl_2 in ether. The combined organic layers were dried ($MgSO_4$). Filtration, concentration, and flash chromatography (15% ethyl acetate in toluene) gave lanost-7-ene-3β,32-diol (**16**) (1.09 g, 52% from **8**) as a white crystalline solid with physical and spectral properties identical with those reported in the literature.[7]

Lanost-7-ene-3β,32-diol 32-Acetate (18a). An oven-dried 5-mL reactivial with stirring vane was charged with lanost-7-ene-3β,32-diol (**16**) (48.3 mg, 0.11 mmol), acetic anhydride (12.8 μL, 0.14 mmol), and 1.0 mL of dry pyridine. The vial was sealed and stirred at room temperature for 24 h. The crude product was streaked directly onto preparative TLC plates (2 × 2000 μm), which were dried, developed (3:1 hexanes–ethyl acetate), scraped, and eluted with ethyl acetate. Filtration and rotary evaporation gave **18a** as a white crystalline solid (31.8 mg, 60%): mp 129–130 °C; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.35–5.20 (m, 1 H), 4.59 (d, *J* = 10.9 Hz, 1 H), 4.00 (s, 1 H), 3.69 (d, *J* = 10.9 Hz, 1 H), 3.24 (dd, *J* = 5.0 and 8.6 Hz, 1 H), 2.2–0.7 (m, 46 H). Anal. Calcd for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 79.16; H, 11.27.

Lanost-7-ene-3β,32-diol 3β-(Tetrahydropyranyl (THP) ether) 32-Acetate (19a). A solution of **18a** (519.9 mg, 1.07 mmol) in dry CH_2Cl_2 (17 mL) and DHP (1.11 mL, 12.2 mmol) containing PPTS (43.8 mg, 174 mmol) under argon was stirred at room temperature overnight. The resultant solution was introduced directly onto a flash chromatography column; elution with 5:1 hexanes–ethyl acetate gave **19a** (680.1 mg, quant.) as a clear glass: IR ($CHCl_3$) 3000, 1730, 1465, 1385, 1230, 1138, 1080, 1024, 980, 920, 900, 875 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.3–5.1 (m, 1 H), 5.0–4.5 (m, 2 H), 4.1–3.0 (m, 4 H), 2.2–0.6 (m, 52 H).

Lanost-7-ene-3β,32-diol 3β-(THP ether) (17a). An oven-dried one-neck round-bottom flask with stirring bar was charged with LAH (22.7 mg, 0.598 mmol), equipped with a serum cap and a gas-needle inlet, and flushed with argon. Dry THF (1.6 mL) was introduced. To the resultant slurry, **19a** (69.3 mg, 0.124 mmol) was added in a total of 2.4 mL of dry THF. The mixture was stirred for 1 h at room temperature and cooled to 0 °C, and ethyl acetate was added slowly until the excess LAH was quenched. Saturated aqueous Na_2SO_4 was introduced until only white solid remained. Anhydrous Na_2SO_4 was added, the slurry was filtered, and the cake was washed well with ethyl acetate. The filtrate was evaporated in vacuo and the residue was submitted to flash chromatography (5:1 hexanes–ethyl acetate), giving **17a** as a colorless solid (63.8 mg, quant.): IR ($CHCl_3$) 2945, 2855, 1720, 1660, 1460, 1380, 1360, 1075, 1015 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.50–5.35 (m, 1 H), 5.05–4.50 (m, 2 H), 4.15–2.9 (m, 5 H), 2.3–0.5 (m, 52 H); mass spectrum, m/e 528 (M^+), 498 (M - CH_2O), 497 (M - CH_2OH), 414 (M - CH_2O - THP + H), 413 (M - CH_2OH - THP + H).

Lanost-7-ene-3β,32-diol 32-(*tert*-Butyldimethylsilyl ether) (18b). An oven-dried 25-mL one-neck round-bottom flask with stirring bar, addition funnel, a serum cap, and a gas-needle inlet was charged with **16** (157.3 mg, 0.35 mmol) and flushed with argon. The diol was dissolved in dry CH_2Cl_2 (3 mL), and solution A [TBDMSCl (80.7 mg, 2.7 mmol), DMAP (64.8 mg, 2.4 mmol), dry pyridine (2.0 mL)] was introduced. Solution A was introduced every 24 h until the reaction was complete by TLC (a total of four aliquots of solution A were used). After a total of 5 days, the reaction was diluted with CH_2Cl_2 and washed with water. The aqueous layer was back extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and filtered, and the solvent was evaporated in vacuo. The residue was submitted to flash chromatography (9:1 hexanes–ethyl acetate) to give **18b** as a clear solid (136.1 mg, 69%): ^1H NMR ($CDCl_3$, 80 MHz) δ 5.17–5.15 (m, 1 H), 3.65 (d, *J* = 8.6 Hz, 1 H), 3.5–3.1 (m, 2 H), 2.2–0.6 (m, 55 H), 0.01 (s, 6 H).

Lanost-7-ene-3β,32-diol 3β-Acetate 32-(TBDMS ether) (19b). To a stirred solution of compound **18b** (136.1 mg, 0.243 mmol), under argon, in dry CH_2Cl_2 (1 mL) and dry pyridine (1 mL), was then added quickly DMAP (10 mg). The flask was reflushed with argon, and acetic anhydride (138 μL, 1.46 mmol) was added. The solution was stirred at room temperature for 7 h. The resultant mixture was placed directly onto a flash chromatography column and eluted (5:1 hexanes–ethyl acetate), giving **19b** as a white solid (139.0 mg, 95%): ^1H NMR ($CDCl_3$, 80 MHz) δ 5.25–5.05 (m, 1 H), 4.53 (d, *J* = 9.9 Hz, 1 H), 3.64 (d, *J* = 9.9 Hz, 1 H), 3.46–3.32 (m, 1 H), 2.2–0.6 (m, 58 H), 0.02 (s, 6 H).

Lanost-7-ene-3β,32-diol 3β-Acetate (17b). Compound **19b** (40.2 mg, 0.067 mmol) was dissolved in dry THF (1.0 mL), and tetra-*n*-butylammonium fluoride (201 μL, 1.0 M in THF, 0.201 mmol) was introduced. The reaction was stirred under argon at room temperature for 3 days. Tetra-*n*-butylammonium fluoride (67 μL, 1.0 M, 0.067 mmol) was once again introduced, and the mixture was stirred overnight at room temperature under argon. The resultant solution was submitted to flash chromatography (5:1 hexanes–ethyl acetate) to give **17b** as a white crystalline solid (29.8 mg, 92%): mp 155.0–155.5 °C; IR ($CHCl_3$) 3510, 2940, 1722, 1682, 1465, 1375, 1225, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 5.45–5.25 (m, 1 H), 4.48 (dd, *J* = 9.1 and 4.8 Hz, 1 H), 3.63 (d,

J = 10.0 Hz, 1 H), 3.22 (d, J = 10.0 Hz, 1 H), 2.03 (s, 1 H), 2.3–0.5 (m, 50 H); mass spectrum, m/e 486 (M^+), 471 (M – CH_3), 456 (M – CH_2O), 455 (M – CH_2OH), 397 (M – CH_2O – OAc). Anal. Calcd for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 78.96; H, 11.39.

14α-Formyl-4,4-dimethylcholest-7-en-3β-ol 3β-(THP ether) (20a). An oven-dried one-neck 25-mL round-bottom flask with stirring bar was charged with **17a** (43.9 mg, 0.083 mmol) and Fetizon's reagent[14] (1.92 g, 1.75 mmol/g, 3.36 mmol). The flask was equipped with a one piece distilling head and flushed with argon. Dry C_6H_6 (18 mL) was introduced, the slurry was brought to reflux, and 9 mL of C_6H_6 was distilled off. The remaining mixture was heated under reflux for 20 h, allowed to cool to room temperature, and filtered. The cake was washed well with ethyl acetate, and the solvents were removed from the combined filtrates to give **20a** as a colorless solid (43.7 mg, quant.): ^1H NMR ($CDCl_3$, 80 MHz) δ 9.61 (s, 1 H), 5.50–5.30 (m, 1 H), 4.15–1.90 (m, 3 H), 1.3–0.5 (m, 53 H); mass spectrum, m/e 526 (M^+), 498 (M – CO), 497 (M – CHO), 414 (M – CO – THP + H), 413 (M – CHO – THP + H).

14α-Formyl-4,4-dimethylcholest-7-en-3β-ol 3β-Acetate (20b). A mixture of **17b** (53.2 mg, 0.110 mmol) and Fetizon's reagent[14] (1.08 g, 1.66 mmol) in dry C_6H_6 (13 mL) was brought to reflux as above. Part of the C_6H_6 (4.5 mL) was distilled off, and the remaining mixture was kept at reflux for 3.5 h, cooled to room temperature, and filtered. The cake was washed with ethyl acetate, and the solvents were removed from the combined filtrates to give crude **20b**[15] as a white solid (51.3 mg, 97%): mp 126.0–128.5 °C; IR ($CHCl_3$) 2940, 2860, 1722, 1460, 1370, 1220, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 9.56 (s, 1 H), 5.4–5.2 (m, 1 H), 4.5–4.3 (m, 1 H), 2.3–0.6 (m, 50 H); mass spectrum, m/e 484 (M^+), 456 (M – CO), 455 (M – CHO), 397 (M – CO – OAc), 395 (M – CHO – HOAc); high-resolution mass spectrum calcd 484.3919, found 484.3922.

32-Formyllanost-7-en-3β-ol (24). To a solution of (methoxymethyl)triphenylphosphonium chloride (1.04 g, 3.02 mmol) under nitrogen in dry THF (4 mL) was added dropwise, with stirring, n-butyllithium (1.3 mL, 1.96 M, 2.55 mmol). The resultant dark red mixture was stirred at room temperature for 30 min. The THP-protected aldehyde **20a** (100.4 mg, 0.191 mmol) in 1.5 mL of dry THF was added dropwise. The flask was equipped with a condenser, and the mixture refluxed under nitrogen for 2 h. The reaction was allowed to cool to room temperature, and aqueous saturated NH_4Cl solution was added. The THF was removed in vacuo, and the residue was extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The resultant oil was triturated with hexanes to remove a large portion of the triphenylphosphine oxide. The hexanes solution was removed and concentrated, and the residue was submitted to flash chromatography (50:1 hexanes–ethyl acetate) to give a mixture of isomeric THP-protected methyl enol ethers **23** (73.9 mg, 70%): ^1H NMR ($CDCl_3$, 80 MHz) δ 5.61 (d, J = 7.2 Hz, 1 H), 5.43–5.23 (m, 1 H), 4.51 (d, J = 7.2 Hz, 1 H), 4.8–4.6 (m, 1 H), 4.2–2.8 (m, 3 H), 3.44 (s, 3 H), 2.2–0.6 (m, 52 H).

An oven-dried 5-mL reactivial was charged with the above mixture of enol ethers (73.9 mg, 0.133 mmol) and dry ether (2.35 mL). The vial was equipped with a serum cap and a gas-needle inlet and flushed with nitrogen. The solution was cooled to 0 °C, and $HClO_4$ (290 μL, 70%, 3.34 mmol) was added dropwise. The mixture was stirred at 0 °C for 15 min, and 5% $NaHCO_3$ was added carefully until the reaction was quenched. The resultant mixture was extracted with ether. The combined organic layers were dried ($MgSO_4$). Filtration and concentration gave **24** (82.8 mg, wax), which was used without further purification. An analytical sample was purified by flash chromatography (5:1 hexanes–ethyl acetate): IR ($CHCl_3$) 2952, 2932, 2868, 2720, 1710, 1463, 1380, 1364, 1090, 1023, 996 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 9.53 (t, J = 3.7 Hz, 1 H), 5.47–5.28 (m, 1 H), 3.37–3.13 (m, 1 H), 2.7–0.6 (m, 48 H); mass spectrum (CI, isooctane), m/e 457 (M + 1), 439 (M + 1 – H_2O), 421 (M + 1 – $2H_2O$), 413 (M + 1 – CH_2CHOH), 395 (M + 1 – H_2O – CH_2CHOH). Recrystallization from ether and methanol gave **24** as a hydrate: mp 145–146 °C. Anal. Calcd for $C_{31}H_{52}O_2 \cdot H_2O$: C, 78.43; H, 11.47. Found: C, 78.12; H, 11.68.

32-Formyllanost-7-en-3β-ol 3β-(THP ether) (25a). A solution of crude **24** (82.8 mg) in CH_2Cl_2 (25 mL) and DHP (244 μL, 2.66 mmol) containing PPTS (9.8 mg, 0.039 mmol) under nitrogen was stirred at room temperature for 2.5 days and then was placed on a short column of florisil. The column was eluted with CH_2Cl_2 until all of the desired product had come off. The solution was concentrated, and the residue was submitted to flash chromatography (12:1 hexanes–ethyl acetate), giving THP-protected homologated aldehyde **25a** (67.3 mg, 94%) as a clear glass: IR ($CHCl_3$) 2920, 1725, 1460, 1375, 1020 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 9.52 (t, J = 4 Hz, 1 H), 5.44–5.26 (m, 1 H), 4.82–4.48 (m, 1 H), 4.1–2.9 (m, 3 H), 2.7–0.6 (m, 54 H); mass spectrum (CI, isooctane), m/e 541 (M + 1), 497 (M + 1 – CH_2CHOH), 457 (M + 1 – C_5H_8O), 439 (M + 1 – HOTHP), 421 (M + 1 – HOTHP – H_2O), 413 (M + 1 – CH_2CHOH – C_5H_8O), 395 (M + 1 – HOTHP – CH_2CHOH).

32-Formyllanost-7-en-3β-ol 3β-Acetate (25b). A 25-mL one-neck round-bottom flask with stirring bar was charged with the crude 32-formyllanost-7-en-3β-ol (**24**) (80.8 mg, from 80.4 mg of methyl enol ether), then equipped with a serum cap and gas-needle inlet, and flushed with nitrogen. The steroid was dissolved in dry CH_2Cl_2 (10 mL), and then acetic anhydride (0.137, 1.45 mmol), dry pyridine (2.0 mL), and DMAP (cat.) were added. The mixture was stirred at room temperature for 1.5 days, CH_2Cl_2 was added, and the solution was washed with 5% HCl and water. The organic layer was dried ($MgSO_4$). Filtration, concentration, and purification by flash chromatography (12:1 hexanes–ethyl acetate) gave **25b** (49.0 mg, 68%) as a clear glass: IR ($CHCl_3$) 2920, 1720, 1460, 1375, 1220, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 9.52 (t, J = 3.1 Hz, 1 H), 5.43–5.23 (m, 1 H), 5.50 (dd, J = 9.8 and 5.6 Hz, 1 H), 2.8–0.6 (m, 51 H); mass spectrum (CI, isooctane), m/e 499 (M + 1), 481 (M + 1 – H_2O), 455 (M + 1 – CH_2CHOH), 439 (M + 1 – HOAc), 421 (M + 1 – HOAc – H_2O), 395 (M + 1 – HOAc – CH_2CHOH).

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Registry No. **8**, 4489-03-6; **9**, 116194-52-6; **9** (acetate), 124126-54-1; **10**, 116194-51-5; **10** (R = (*E*)-CH=CHCl, THP ether), 124126-55-2; **10** (R = (*Z*)-CH=CHCl, THP ether), 124126-56-3; **10** (THP ether), 116194-50-4; (32*R*)-**11**, 116194-42-4; (32*R*)-**11** (3-THP ether), 116194-39-9; (32*S*)-**11**, 116194-43-5; (32*S*)-**11** (3-THP ether), 116194-44-6; **13**, 116194-49-1; **13** (acetate), 124126-60-9; **14**, 116209-62-2; **14** (THP ether), 124126-61-0; **15**, 116194-45-7; **15** (R = (*E*)-CH=CHCl), 124126-62-1; **15** (R = (*Z*)-CH=CHCl), 124126-63-2; **16**, 5713-35-9; **16** (diacetate), 4489-04-7; **17a**, 116194-37-7; **17b**, 52648-02-9; **18a**, 124126-57-4; **18b**, 124126-64-3; **19a**, 124126-58-5; **19b**, 124126-65-4; **20a**, 116194-38-8; **20b**, 4573-26-6; **22**, 116194-40-2; (*E*)-**23**, 124153-31-7; (*Z*)-**23**, 124126-59-6; **24**, 116194-46-8; **25a**, 116194-47-9; **25b**, 116194-48-0; HC≡CH, 74-86-2; EtMgBr, 925-90-6; $ClCH_2PPh_3^+Cl^-$, 5293-84-5; $CH_3OCH_2PPh_3^+Cl^-$, 4009-98-7; lanosterol 14α-methyl demethylase, 60063-87-8.