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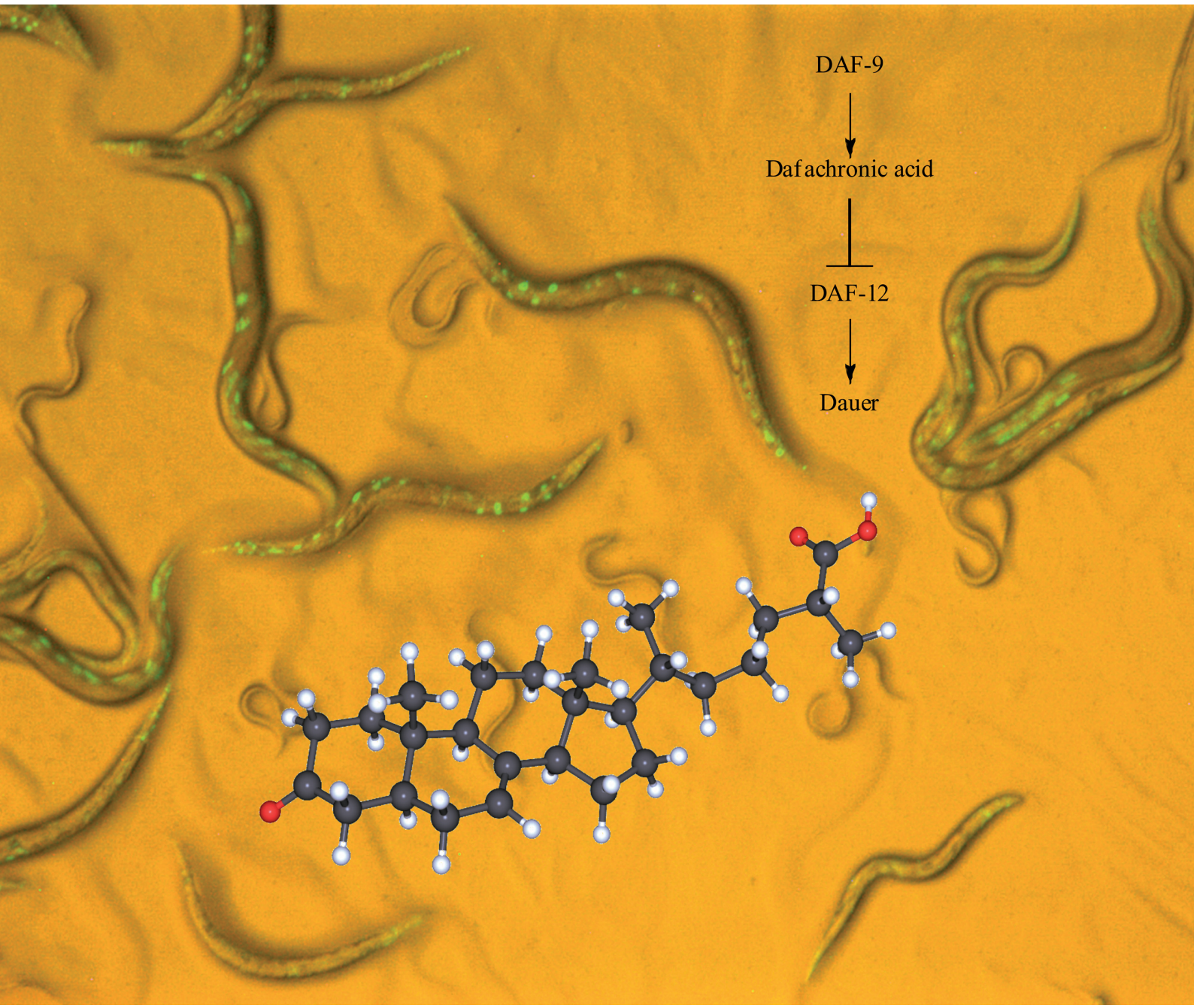

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Synthesis and biological activity of the (25*R*)-cholesten-26-oic acids—ligands for the hormonal receptor DAF-12 in *Caenorhabditis elegans*†

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We describe the stereoselective transformation of diosgenin (**4a**) to (25*R*)-Δ^4-dafachronic acid (**1a**), (25*R*)-Δ^7-dafachronic acid (**2a**), and (25*R*)-cholestenoic acid (**3a**), which represent potential ligands for the hormonal receptor DAF-12 in *Caenorhabditis elegans.* Key-steps of our synthetic approach are a modified Clemmensen reduction of diosgenin (**4a**) and a double bond shift from the 5,6- to the 7,8-position. In the 25*R*-series, the Δ^7-dafachronic acid **2a** exhibits the highest hormonal activity.

Introduction

The nematode *Caenorhabditis elegans* has been established as an important model organism for studying developmental and metabolic processes and moreover, for the functional characterization of novel drug targets.[1,2] Upon starvation or overcrowding it enters diapause and forms specialized larvae called dauer (enduring). The activity of a single nuclear hormone receptor, DAF-12, plays a major role in the dauer formation process.[3,4] In the absence of ligand, DAF-12 is activated and initiates the dauer formation program. However, the products of the cytochrome P450 DAF-9 are ligands for the DAF-12 receptor. In the presence of these ligand(s), DAF-12 is inactivated and a program for reproductive development is initiated.[3] Recently, Mangelsdorf *et al.* identified novel steroidal metabolites which they called dafachronic acids (**1a**, **1b**, **2a**, and **2b**; Fig. 1).[3d–f] These hormones function as ligands for the hormonal receptor DAF-12. The dafachronic acids are the Δ^4- and Δ^7- isomers of (25*R*)- and (25*S*)-3-ketocholesten-26-oic acid. However, the 25*R*-diastereoisomer **1a** showed significantly lower activity in comparison to the 25*S*-diastereoisomer **1b**.[3d–f] In the same year, Gill *et al.* reported that (25*S*)-cholestenoic acid (**3b**) also effectively binds to DAF-12.[5] In contrast, (25*R*)-cholestenoic acid (**3a**) was not active (Fig. 1).

Corey and Giroux described the first synthesis of (25*S*)-Δ^7-dafachronic acid (**2b**), which they called dafachronic acid A, from β-stigmasterol using a stereoselective ruthenium-catalyzed hydrogenation as the key-step.[6] In 2008, the same group completed the synthesis of (25*R*)-Δ^7-dafachronic acid (**2a**) in 10 steps and 13% overall yield starting from β-ergosterol.[7] Previously, Khripach *et al.* published the synthesis of both diastereoisomers (**1a** and **1b**) of Δ^4-dafachronic acid as well as both diastereoisomers (**3a** and **3b**) of cholestenoic acid *via* a stereoselective assembly of the steroid side chain.[8]

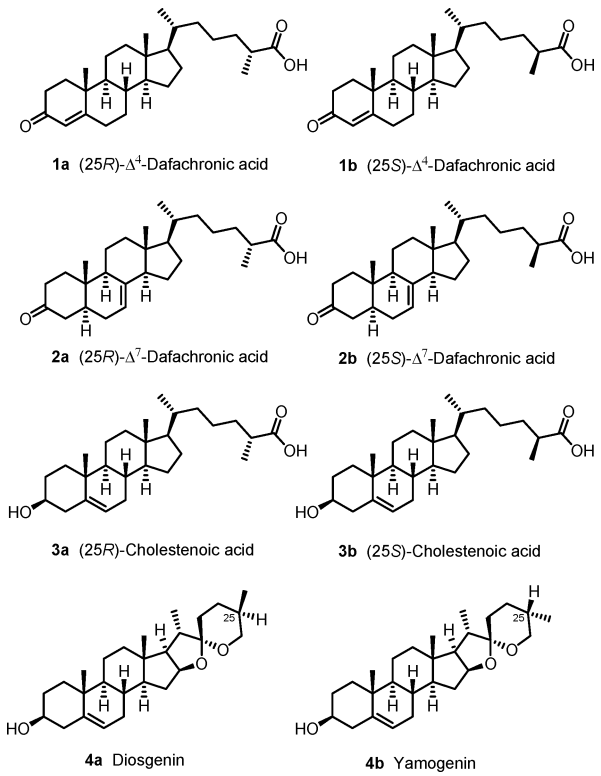

Fig. 1 (25*R*)- and (25*S*)-cholesten-26-oic acids **1a–3b**, diosgenin (**4a**), and yamogenin (**4b**).

Results and discussion

In the course of our studies directed towards the synthesis of hormonally active steroids,[4,9] we became interested in the synthesis of the three (25*R*)-cholesten-26-oic acids **1a–3a**. The commercially available sapogenin diosgenin (**4a**) was used as a perfect starting material as it provides the 25*R*-configuration present in the target compounds.[10]

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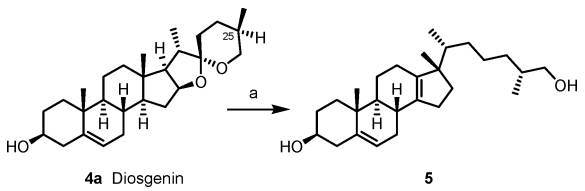
Clemmensen reduction of diosgenin (**4a**) with zinc dust and concentrated hydrochloric acid, according to the procedure described by Williams,[11–13] provided the rearrangement product **5** (9% yield) as the only compound that could be isolated (Scheme 1).[14] The structural assignment of **5** was unequivocally confirmed by an X-ray crystal structure determination (Fig. 2).†[15]



Scheme 1 Clemmensen reduction of diosgenin (**4a**). *Reagents and conditions*: (a) 147 equiv. Zn dust, conc. HCl, EtOH, reflux, 4.5 h, 9%.

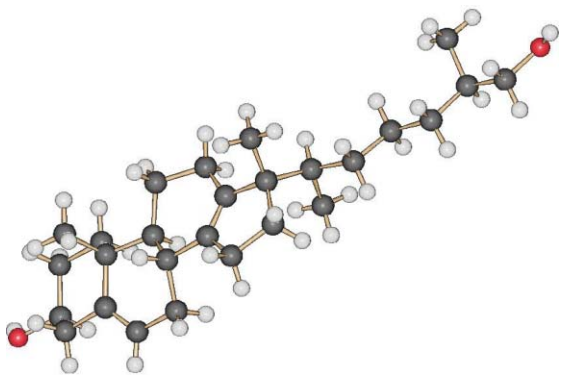
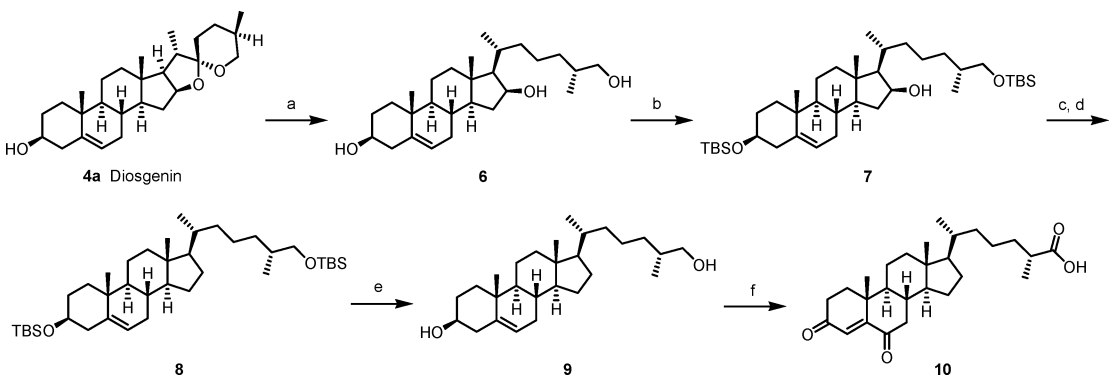


Fig. 2 Molecular structure of the rearrangement product **5** in the crystal form.

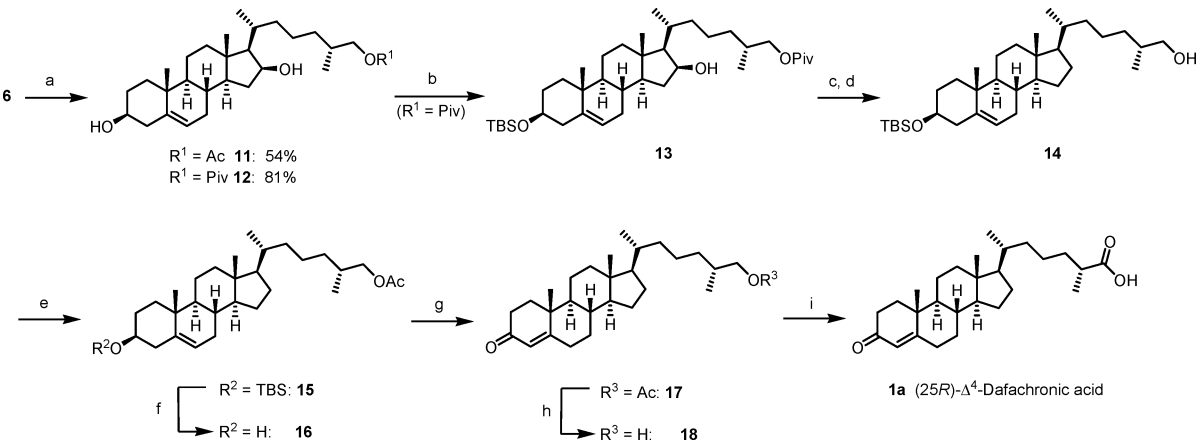
In an optimization of the Clemmensen reduction of **4a**, we found out that using half-concentrated hydrochloric acid instead of concentrated hydrochloric acid led to the desired triol **6** in 85% yield on large scale (Scheme 2). Treatment of the triol **6** with *tert*-butylchlorodimethylsilane (TBSCl) and DBU provided the disilyl ether **7** in 85% yield.[11,14] Mesylation at the 16β-hydroxy group of **7** and subsequent reduction with lithium aluminium hydride afforded **8** in 89% yield.[13] Removal of the silyl groups with TBAF in THF at reflux provided (25*R*)-26-hydroxycholesterol (**9**) in 89% yield.[11] However, treatment of **9** with an excess of Jones reagent did not provide the desired (25*R*)-Δ^4-dafachronic acid (**1a**) but the (25*R*)-3,6-diketocholest-4-en-26-oic acid (**10**) in 56% yield. Using PDC in *N*,*N*-dimethylformamide[16] instead of Jones reagent, the diketo acid **10** was obtained in 74% yield. Earlier reports described that oxidation of 3β-hydroxycholest-5-enes with Jones reagent,[17] PCC,[18] and TPAP/NMO[19] provided the corresponding cholest-4-ene-3,6-diones. More recently, a similar transformation has been reported for diosgenin (**4a**).[20] In order to avoid allylic oxidation, it was necessary to achieve a sequential oxidation of the hydroxy groups at C-3 and C-26. Therefore, the introduction of orthogonal protecting groups at these positions was required.

Since a selective monodesilylation of compound **8** proved to be impossible, a differentiation between all three hydroxy groups of **6** had to be achieved. Selective acetylation of **6** with acetic anhydride provided the C-26 acetate **11** in remarkable 54% yield (Scheme 3). The sterically more demanding pivaloyl chloride represents an excellent reagent for selective acylation of primary alcohols in the presence of secondary alcohols.[21] Williams and Gong reported a double pivaloylation at C-3β and C-26 of the triol **6**.[13] Using 1.1 equivalents of pivaloyl chloride we achieved a differentiation between the primary and the secondary hydroxy groups of the triol **6**, providing the C-26 pivalate **12** in 81% yield. Treatment of **12** with 1.1 equivalents of *tert*-butylchlorodimethylsilane and DBU led to selective silylation at C-3β. Compound **13** represents an orthogonally diprotected derivative of the triol **6** in which all three hydroxy groups have been differentiated. Mesylation of the 16β-hydroxy group followed by treatment with lithium aluminium hydride afforded the alcohol **14** in 89% yield by removal of the hydroxy group at C-16 with concomitant cleavage of the pivalate at C-26. Acetylation of **14** and subsequent desilylation of the acetate **15** provided the 3β-alcohol **16** in 88% yield. In order to avoid allylic oxidation at C-6 (*cf.* Scheme 2), we used a classical Oppenauer oxidation to obtain the cholest-4-en-3-one **17** (70% yield). Saponification of the acetate **17** to the 26-alcohol **18** (93% yield) with catalytic amounts of potassium carbonate in methanol and subsequent Jones oxidation provided (25*R*)-Δ^4-dafachronic acid (**1a**) in 79% yield.

For the synthesis of (25*R*)-Δ^7-dafachronic acid (**2a**) we envisaged a shift of the 5,6-double bond into the 7,8-position. Treatment of compound **8** with PDC and *tert*-butyl hydroperoxide in benzene according to Chandrasekaran and Chidambaram's procedure[22]



Scheme 2 Synthesis of (25*R*)-3,6-diketocholest-4-en-26-oic acid (**10**). *Reagents and conditions*: (a) 125 equiv. Zn dust, 19% HCl, EtOH, reflux, 4 h, 85%; (b) 5.0 equiv. TBSCl, 3.8 equiv. DBU, THF, 25 °C, 16 h, 85%; (c) 3.8 equiv. MsCl, pyridine, 0 °C to 25 °C, 16 h; (d) 10.0 equiv. $LiAlH_4$, Et_2O, reflux, 4 h, 89% for 2 steps; (e) 3.0 equiv. TBAF, THF, reflux, 20 h, 89%; (f) 10.0 equiv. PDC, DMF, 25 °C, 18 h, 74%.



Scheme 3 Synthesis of (25*R*)-Δ^4-dafachronic acid (**1a**). *Reagents and conditions*: (a) 10 mol% DMAP, 1.1 equiv. Ac_2O, 2.2 equiv. Et_3N, THF, 25 °C, 16 h, 54% of **11**; 1.1 equiv. PivCl, 2.2 equiv. Et_3N, THF, 25 °C, 20 h, 81% of **12**; (b) 1.1 equiv. TBSCl, 2.2 equiv. DBU, THF, 25 °C, 17 h, 79%; (c) 3.8 equiv. MsCl, pyridine, 0 °C to 25 °C, 16 h; (d) 5.0 equiv. $LiAlH_4$, Et_2O, reflux, 4 h, 89% for 2 steps; (e) 10 mol% DMAP, 2.0 equiv. Ac_2O, 4.0 equiv. Et_3N, THF, 16 h, 100%; (f) 1.2 equiv. TBAF, THF, reflux, 17 h, 88%; (g) 1.7 equiv. $Al(O\textit{i}\text{-}Pr)_3$, toluene/acetone (9:1), reflux, 15 h, 70%; (h) 10 mol% K_2CO_3, MeOH, 25 °C, 40 h, 93%; (i) 5.0 equiv. Jones reagent, acetone, 0 °C, 1 h, 79%.

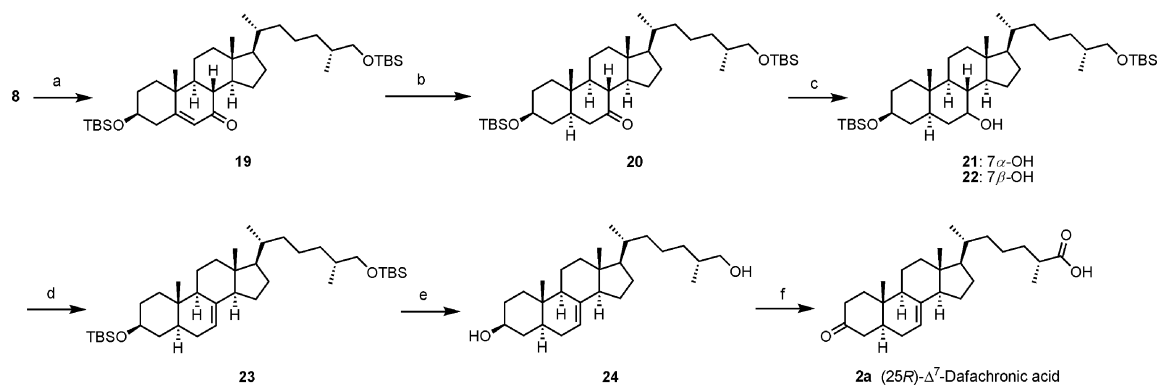
afforded the cholest-5-en-7-one **19** in 57% yield (Scheme 4). Allylic oxidation of **8** with chromium trioxide and 3,5-dimethyl-1*H*-pyrazole provided a similar result (56% yield of **19**).[23] Using catalytic amounts of manganese(III) acetate in the presence of *tert*-butyl hydroperoxide led to **19** in 47% yield.[24] Transfer hydrogenation of **19** with ammonium formate and Pd/C afforded the ketone **20** in 90% yield.

For stereoelectronic reasons we required the 7α-alcohol **21** to introduce the Δ^7-double bond *via* elimination of the hydroxy group. Reduction of the ketone **20** using lithium aluminium hydride at low temperature provided the 7α-alcohol **21** in 59% yield along with 31% of the undesired 7β-alcohol **22** (Table 1). The epimeric alcohols **21** and **22** were easily separable using flash chromatography on silica gel. Grignard reduction with isopropylmagnesium chloride led diastereoselectively to the 7α-alcohol **21** in 70% yield along with 10% of recovered starting material **20**. Treatment of the 7α-alcohol **21** with thionyl chloride in pyridine afforded quantitatively the cholest-7-ene **23**.[25] Removal of the silyl protecting groups with TBAF to the diol **24** (93% yield) followed by Jones oxidation provided (25*R*)-Δ^7-dafachronic acid (**2a**) in 74% yield.

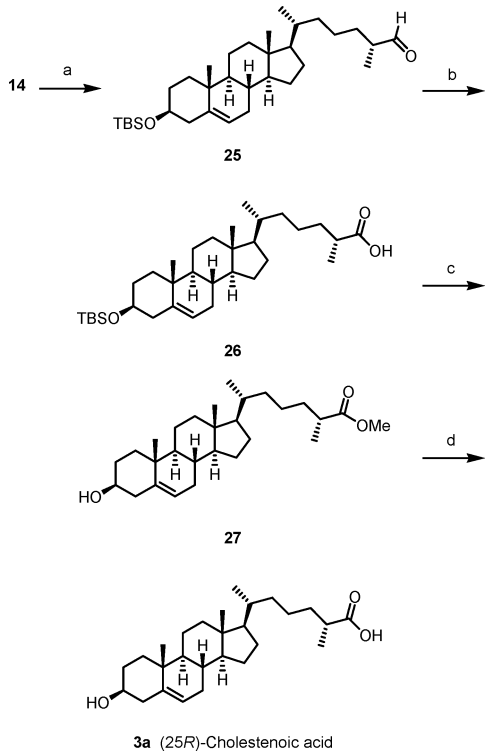
Table 1 Reduction of the ketone **20**

Reaction conditions	Yield
1.0 equiv. $LiAlH_4$, THF, –78 °C to 25 °C, 24 h	59% of **21**, 31% of **22**
1.2 equiv. *i*-PrMgCl, Et_2O, 25 °C, 1 h	70% of **21**, 10% of **20**

The alcohol **14** has been readily converted to (25*R*)-cholestenoic acid (**3a**) in four steps (Scheme 5). Oxidation of **14** using tetrapropylammonium perruthenate in the presence of *N*-methylmorpholine *N*-oxide (TPAP/NMO)[26] afforded the aldehyde **25** in only moderate yield (54%). However, oxidation of **14** with pyridinium dichromate (PDC)[16] led to the aldehyde **25** in 89% yield. Oxidation of the aldehyde **25** to the silyl-protected (25*R*)-cholestenoic acid **26** has been achieved with sodium chlorite. Following the standard protocol (aqueous *tert*-butanol as the solvent) provided low yields of **26** due to the poor solubility of our starting material.[27] In contrast, using aqueous tetrahydrofuran as solvent the acid **26** could be obtained in 92% yield. Cleavage of the silyl ether with TBAF afforded only impure **3a** in moderate yield. Chromatographic purification of (25*R*)-cholestenoic acid



Scheme 4 Synthesis of (25*R*)-Δ^7-dafachronic acid (**2a**). *Reagents and conditions*: (a) 4.0 equiv. PDC, 4.0 equiv. *t*-BuOOH, Celite®, benzene, 0 °C to 25 °C, 28 h, 57%; (b) 10% Pd/C, 4.0 equiv. ammonium formate, EtOAc/MeOH (3:4), reflux, 8 h, 90%; (c) 1.4 equiv. *i*-PrMgCl, Et_2O, 25 °C, 1 h, 70%; (d) 3.0 equiv. $SOCl_2$, pyridine, 0 °C, 1 h, 100%; (e) 2.2 equiv. TBAF, THF, reflux, 20 h, 93%; (f) 5.0 equiv. Jones reagent, acetone, 0 °C, 1 h, 74%.



Scheme 5 Synthesis of (25*R*)-cholestenoic acid (**3a**). *Reagents and conditions*: (a) 2.0 equiv. PDC, MS 4 Å, CH_2Cl_2, 25 °C, 3.5 h, 89%; (b) 10.0 equiv. 2-methyl-2-butene, 1.3 equiv. KH_2PO_4, 1.5 equiv. $NaClO_2$, THF/H_2O (3:1), 25 °C, 16 h, 92%; (c) cat. H_2SO_4, MeOH, reflux, 16 h, 84%; (d) 3.0 equiv. LiOH, THF/MeOH/H_2O (2:1:1), 25 °C, 16 h, 97%.

(**3a**) proved to be difficult. Therefore, the silyl-protected (25*R*)-cholestenoic acid **26** was converted to the methyl ester **27** with concomitant cleavage of the silyl ether by treatment with catalytic amounts of concentrated sulfuric acid in methanol at reflux. The (25*R*)-cholestenoic acid methyl ester (**27**) could be easily purified by column chromatography on silica gel. Finally, saponification of the methyl ester **27** with lithium hydroxide provided pure (25*R*)-cholestenoic acid (**3a**) in 97% yield.

Conclusion

We have developed a highly efficient synthesis of the three (25*R*)-cholesten-26-oic acids **1a–3a** from diosgenin (**4a**) as a cheap commercially available starting material. The (25*R*)-Δ^4-dafachronic acid (**1a**) was synthesized in 10 steps and 22% overall yield based on **4a**. The perfect differentiation of the three hydroxy groups present in the triol **6** led to the orthogonally diprotected alcohol **13** and was the key to solve all regioselectivity problems. This intermediate has been also exploited for the synthesis of (25*R*)-cholestenoic acid (**3a**) (9 steps and 32% overall yield based on **4a**). Chandrasekaran oxidation followed by reduction and elimination resulted in a shift of the 5,6-double bond to the 7,8-position, which represents the key-step of the transformation into (25*R*)-Δ^7-dafachronic acid (**2a**) (10 steps and 16% overall yield based on **4a**). The spectroscopic data of all three natural products **1a–3a** were in full agreement with those reported previously.[3d,7,8] Another sapogenin natural product is yamogenin (**4b**), which represents the C-25 diastereoisomer of diosgenin (**4a**). Thus, application of the present methodology to **4b** should provide a stereoselective access to the (25*S*)-cholesten-26-oic acids **1b–3b**. Synthetic studies in this direction are currently underway in our laboratories and will be reported in due course.

In order to study the activity of the (25*R*)-compounds on dauer formation *in vivo*, they have been used for feeding mutant worms *daf-9(dh6)* that lack the activity of DAF-9, the cytochrome P450 that is involved in the production of dafachronic acids. Mutant worms *daf-9(dh6)* do not produce the ligand of DAF-12 and arrest as dauer-like larvae (Fig. 3A, B). In addition, *daf-9(dh6)* can be distinguished by the absence of green fluorescence which is carried by the rescuing *dhEx24* extrachromosomal array in the parental strain and siblings *daf-9(dh6)*; *dhEx24*.[3c,d] Therefore, appearance of non-fluorescent worms going over dauer arrest can be used as an assay for the presence of DAF-9 product activity. We found both compounds **1a** and **2a** to show activity towards rescuing dauer arrest in *daf-9(dh6)* (Fig. 3C–H). More active was **2a**, which at 0.5 μM was sufficient for *daf-9(dh6)* to develop at the same speed as control fluorescent worms (Fig. 3G). A similar activity was observed for compound **1a** at 5 μM (Fig. 3E). Compound **1a** at 0.5 μM (Fig. 3D) and compound **2a** at 0.05 μM (Fig. 3F) were sufficient for *daf-9(dh6)* to pass through the arrest but the worms developed slower than the control and often migration of the gonad phenotypes could be seen. Thus, (25*R*)-Δ^7-dafachronic acid (**2a**) is one order of magnitude more active than (25*R*)-Δ^4-dafachronic acid (**1a**). Similar to a previous study,[5] little or no activity was detected with (25*R*)-cholestenoic acid (**3a**) at all concentrations applied (Fig. 3I–L). Taken together, we can conclude that although the (25*R*)-diastereoisomers are less active as compared with the (25*S*)-diastereoisomers,[3d–f,5] they can be used for the clarification of basic principles of dauer larvae formation (Fig. 3).

Experimental

General

All reactions were carried out in oven-dried glassware under an argon atmosphere. Tetrahydrofuran, ethyl acetate, dichloromethane, and diethyl ether were dried using a solvent purification system (MBraun-SPS). Acetone was distilled from phosphorus(V) oxide and stored over 3 Å molecular sieves. Benzene was dried over sodium. Toluene was purchased from ACROS Organics (water content less than 50 ppm). Pyridine was obtained from FLUKA (water content less than 50 ppm). Dry methanol was purchased from VWR Prolabo (water content less than 20 ppm). All other chemicals were used as received from commercial sources. Flash chromatography was performed using silica gel from ACROS Organics (0.063–0.200 mm). Thin layer chromatography was performed with TLC plates from Merck (60 F_{254}) using anisaldehyde solution for visualization. Melting points were measured on an Electrothermal IA9100 and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Avatar 360 FT-IR using the ATR technique (Attenuated Total Reflectance). Mass spectra were recorded on a Finnigan MAT-95 (electron impact, 70 eV) or by GC/MS-coupling using an Agilent Technologies 6890 N GC System equipped with a 5973 Mass Selective Detector (electron impact EI, 70 eV). ESI-MS spectra were recorded on an Esquire LC with an ion trap detector from Bruker. Positive and negative

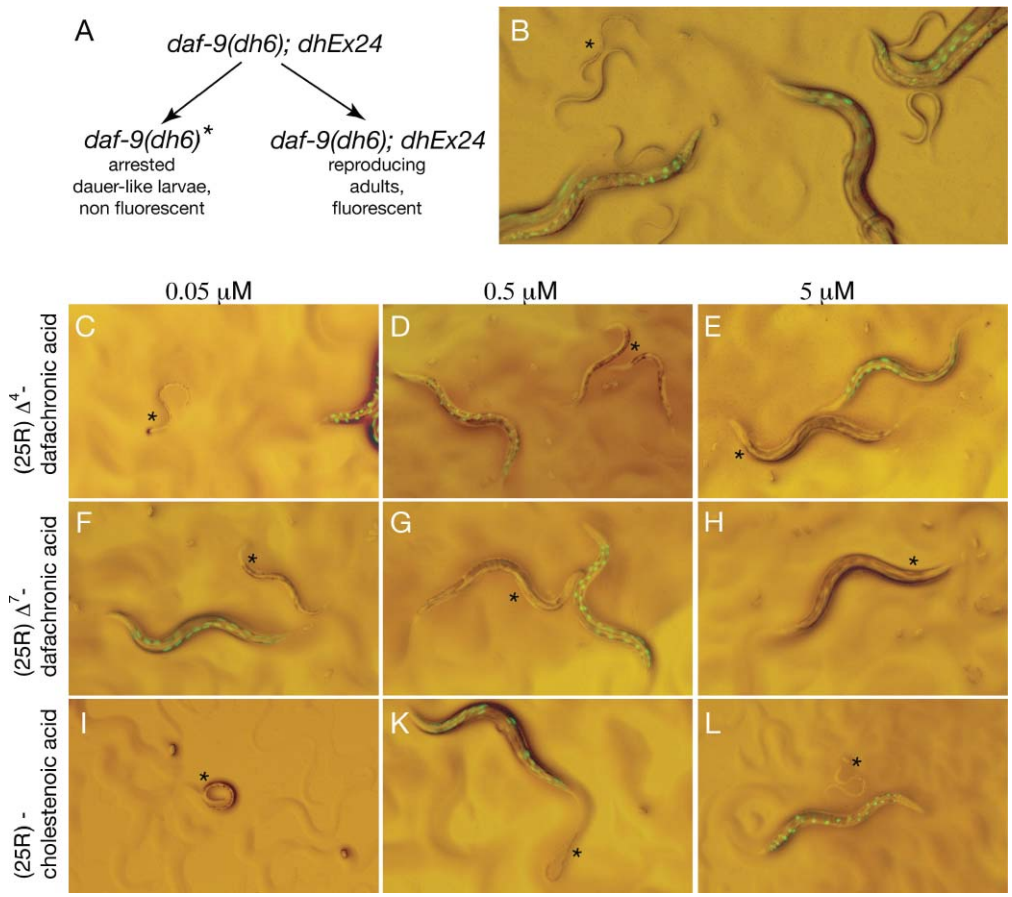


Fig. 3 Hormonal activity of **1a**, **2a** and **3a** at 0.05 μM, 0.5 μM and 5 μM; asterisks—non fluorescent *daf-9(dh6)*; the sibling *daf-9(dh6); dhEx24* which are wild type for *daf-9* gene can be easily distinguished by the green fluorescence.

ions were detected. Elemental analyses were measured on an EuroVector EuroEA3000. NMR spectra were recorded on a Bruker DRX 500. Complete assignment of ^{1}H and ^{13}C signals was done using HSQC experiments. Chemical shifts are reported in ppm with the deuterated solvent as internal standard. The following abbreviations have been used: s: singlet, d: doublet, dd: doublet of doublets, dt: doublet of triplets, t: triplet, q: quartet, m: multiplet, br: broad. X-ray analyses: Bruker-Nonius Kappa CCD with Oxford Cryosystems and STOE IPDS 2 image plate. Software: Collect (Nonius BV, 1999), Dirax/lsq (Duisenberg, 1992), SHELXS-97 (G. M. Sheldrick, 1990), EvalCCD (Duisenberg *et al.*, 2003), SADABS version 2.10 (G. M. Sheldrick, Bruker AXS Inc., 2002), SHELXL-97 (G. M. Sheldrick, 1997), Schakal-99 (E. Keller, 1999).

(3*S*,8*R*,9*S*,10*R*,17*S*)-2,3,4,7,8,9,10,11,12,15,16,17-Dodecahydro-17-((2*R*,6*R*)-7-hydroxy-6-methylhept-2-yl)-10,17-dimethyl-1*H*-cyclopenta[*a*]phenanthren-3-ol (5). Zinc dust (80.0 g, 1.23 mol) and diosgenin (**4a**) (3.47 g, 8.37 mmol) were mixed in ethanol (250 mL) and concentrated aqueous hydrochloric acid (800 mL) was added under vigorous stirring over a period of 4 h. The resulting mixture was then heated under reflux for further 30 min. Unreacted zinc was removed by filtration of the hot reaction mixture. After cooling to room temperature, water (500 mL) and then diethyl ether (500 mL) were added until the layers separated. The aqueous layer was extracted with diethyl ether (2 × 300 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate (100 mL) and brine (100 mL) and then dried over sodium sulfate. Removal of the solvent and recrystallization of the crude product from ethanol/water (1:1) provided pure **5**, yield: 300 mg (9%). Colorless solid; mp: 143–145 °C; IR (ATR): ν = 3289, 2929, 2857, 1463, 1434, 1373, 1331, 1249, 1203, 1134, 1039, 1004, 943, 906, 848, 808 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69 (d, J = 6.7 Hz, 3 H), 0.90 (d, J = 6.7 Hz, 3 H), 0.97 (s, 3 H), 0.98 (s, 3 H) 1.07–1.62 (m, 14 H), 1.70–1.87 (m, 4 H), 1.91–1.98 (m, 2 H), 2.03–2.24 (m, 5 H), 2.31 (ddd, J = 12.8, 4.8, 2.1 Hz, 1 H), 3.41 (dd, J = 10.4, 6.5 Hz, 1 H), 3.49 (dd, J = 10.4, 6.0 Hz, 1 H), 3.52 (m, 1 H), 5.40 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 14.68 (CH_3), 16.48 (CH_3), 18.71 (CH_3), 22.93 (CH_2), 23.22 (CH_2), 24.97 (CH_3), 25.64 (CH_2), 30.94 (CH_2), 31.40 (CH_2), 31.46 (CH_2), 31.62 (2 CH_2), 33.05 (CH), 33.44 (CH_2), 35.79 (CH), 36.91 (C), 37.02 (CH_2), 39.13 (CH), 42.14 (CH_2), 49.20 (CH), 52.78 (C), 68.51 (CH_2), 72.00 (CH), 121.94 (CH), 136.26 (C), 140.32 (C), 141.34 (C); GC-MS (70 eV): m/z (%) = 271 (100) [(M − $C_8H_{17}O$)$^+$], 253 (17), 175 (6), 157 (9), 147 (7), 133 (11).

Crystallographic data for 5

$C_{27}H_{44}O_2$, crystal size: 0.87 × 0.24 × 0.17 mm^3, M = 400.62 g mol^{-1}, monoclinic, space group $P2_1$, a = 6.6550(10), b = 21.3180(11), c = 17.247(2) Å, β = 91.568(11)°, V = 2445.9(5) Å^3, Z = 4,

$\rho_{calcd} = 1.088$ g cm^{-3}, $\mu = 0.066$ mm^{-1}, $\lambda = 0.71073$ Å, $T = 198(2)$ K, θ range = 3.04–25.39°, reflections collected: 27537, independent: 4577 ($R_{int} = 0.0349$), 535 parameters. The structure was solved by direct methods and refined by full-matrix least-squares on F^2; final R indices [$I > 2\sigma(I)$]: $R_1 = 0.0490$; $wR_2 = 0.1236$; maximal residual electron density: 0.538 e Å^{-3}. CCDC 697766.

(25*R*)-Cholest-5-ene-3*β*,16*β*,26-triol (6). Zinc dust (90.0 g, 1.376 mol) and diosgenin (**4a**) (4.4 g, 11.0 mmol) were mixed in ethanol (1 L) and the mixture was heated at reflux. Half-concentrated aqueous hydrochloric acid (800 mL) was added to the reaction mixture over a period of 1 h and heating was continued for a further 30 min. Unreacted zinc was removed by filtration of the hot reaction mixture. After cooling to room temperature, water (600 mL) and then diethyl ether (approximately 500 to 1000 mL) were added until the layers separated. The two layers were separated and the aqueous layer was extracted with diethyl ether (2 × 300 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate (100 mL) and brine (100 mL) and then dried over magnesium sulfate. Removal of the solvent and recrystallization of the crude product from ethanol/water (1:1) provided pure **6**, yield: 3.9 g (85%). Colorless solid; mp: 170–174 °C; ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.84–0.91 (m, 1 H), 0.97–1.19 (m, 4 H), 1.05 (d, $J = 6.7$ Hz, 3 H), 1.06 (s, 3 H), 1.09 (d, $J = 6.7$ Hz, 3 H), 1.13 (s, 3 H), 1.21–1.33 (m, 2 H), 1.44–1.70 (m, 8 H), 1.76–1.86 (m, 3 H), 1.97 (m, 2 H), 2.08 (m, 2 H), 2.27–2.36 (m, 2 H), 2.62 (m, 2 H), 3.65 (dd, $J = 10.3$, 6.5 Hz, 1 H), 3.74 (dd, $J = 10.3$, 5.8 Hz, 1 H), 3.85 (m, 1 H), 4.55 (m, 1 H), 5.41 (d, $J = 4.7$ Hz, 1 H), 5.68 (br s, 1 H), 5.93 (br s, 1 H), 6.20 (br s, 1 H); ^{13}C NMR and DEPT (125 MHz, pyridine-d_5): δ = 13.43 (CH_3), 17.32 (CH_3), 18.58 (CH_3), 19.63 (CH_3), 21.15 (CH_2), 24.59 (CH_2), 30.39 (CH), 31.95 (CH), 32.27 (CH_2), 32.64 (CH_2), 34.35 (CH_2), 36.71 (CH_2), 36.75 (CH), 36.96 (C), 37.80 (CH_2), 38.02 (CH_2), 40.37 (CH_2), 42.59 (C), 43.51 (CH_2), 50.60 (CH), 54.92 (CH), 62.12 (CH), 67.75 (CH_2), 71.07 (CH), 71.30 (CH), 121.22 (CH), 142.03 (C).

(25*R*)-3*β*,26-Bis(*tert*-butyldimethylsilyloxy)cholest-5-en-16*β*-ol (7). DBU (700 μL, 4.6 mmol) was added dropwise to a stirred solution of TBSCl (900 mg, 6.0 mmol) and **6** (500 mg, 1.2 mmol) in THF (20 mL). After stirring for 16 h at room temperature, the mixture was quenched with water (50 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried with magnesium sulfate and the solvent was removed *in vacuo.* Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 10:1) furnished **7**, yield: 660 mg (85%). Colorless solid; mp: 122–124 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 6 H), 0.04 (s, 6 H), 0.85 (d, $J = 6.7$ Hz, 3 H), 0.87 (s, 3 H), 0.875 (s, 9 H), 0.879 (s, 9 H), 0.90–1.59 (m, 17 H), 0.97 (d, $J = 6.7$ Hz, 3 H), 0.99 (s, 3 H), 1.17 (dd, $J = 13.3$, 4.5 Hz, 1 H), 1.70 (m, 1 H), 1.79 (dt, $J = 13.3$, 3.3 Hz, 1 H), 1.82–1.84 (m, 1 H), 1.95–2.01 (m, 2 H), 2.13–2.26 (m, 3 H), 3.35 (dd, $J =$ 9.7, 6.5 Hz, 1 H), 3.42 (dd, $J = 9.7$, 5.9 Hz, 1 H), 3.46 (m, 1 H), 4.33 (m, 1 H), 5.30 (d, $J = 5.2$ Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = −5.34 (2 CH_3), −4.60 (2 CH_3), 13.01 (CH_3), 16.68 (CH_3), 18.18 (CH_3), 18.25 (C), 18.37 (C), 19.41 (CH_3), 20.67 (CH_2), 23.75 (CH_2), 25.93 (3 CH_3), 25.96 (3 CH_3), 29.75 (CH), 31.48 (CH), 31.83 (CH_2), 32.04 (CH_2), 33.60 (CH_2), 35.79 (CH), 36.29 (CH_2), 36.49 (CH_2), 36.58 (C), 37.31 (CH_2), 39.87 (CH_2), 42.19 (C), 42.78 (CH_2), 50.17 (CH), 54.53 (CH), 61.38 (CH), 68.49 (CH_2), 72.47 (CH), 72.59 (CH), 120.91 (CH), 141.66 (C).

(25*R*)-3*β*,26-Bis(*tert*-butyldimethylsilyloxy)cholest-5-ene (8). Methanesulfonyl chloride (1.1 mL, 1.56 g, 13.58 mmol) was added dropwise at 0 °C to a solution of **7** (2.38 g, 3.59 mmol) in pyridine (20 mL). Stirring was continued for 16 h and the reaction mixture was allowed to warm to room temperature. The resulting orange solution was carefully quenched with ice-water (50 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with ice-cold 1 N HCl (50 mL), water (50 mL), a saturated solution of sodium hydrogen carbonate (100 mL), and brine (100 mL). After drying with magnesium sulfate and removal of the solvent the crude mesylate was obtained in quantitative yield (2.60 g). The mesylate (500 mg, 680 μmol) was dissolved in diethyl ether (25 mL) and carefully treated with lithium aluminium hydride (260 mg, 6.8 mmol) at 0 °C. The resulting suspension was refluxed for 4 h. After cooling to room temperature, the reaction mixture was carefully quenched by addition of water (10 mL) and ethyl acetate (10 mL). An ice-cold 10% aqueous solution of sulfuric acid (20 mL) was slowly added until the precipitate disappeared. The aqueous layer was extracted with diethyl ether (3 × 100 mL) and the combined organic layers were washed with a saturated solution of sodium hydrogen carbonate (100 mL), water (50 mL), and brine (50 mL). Drying with magnesium sulfate and evaporation of the solvent gave the crude product which was purified by flash chromatography on silica gel (petroleum ether/diethyl ether, 40:1) to obtain **8**, yield: 382 mg (89%). Colorless solid; mp: 92–93 °C; IR (ATR): ν = 2930, 2897, 2856, 1471, 1383, 1368, 1249, 1079, 1023, 1006, 957, 938, 885, 869, 833, 773 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 6 H), 0.04 (s, 6 H), 0.66 (s, 3 H), 0.84 (d, $J =$ 6.7 Hz, 3 H), 0.876 (s, 9 H), 0.881 (s, 9 H), 0.90 (d, $J = 6.5$ Hz, 3 H), 0.92 (m, 1 H), 0.97–1.37 (m, 13 H), 0.98 (s, 3 H), 1.42–1.55 (m, 7 H), 1.70 (m, 1 H), 1.77–1.85 (m, 2 H), 1.94–2.00 (m, 2 H), 2.13–2.17 (m, 1 H), 2.26 (m, 1 H), 3.34 (dd, $J = 9.7$, 6.6 Hz, 1 H), 3.42 (dd, $J = 9.7$, 5.9 Hz, 1 H), 3.47 (m, 1 H), 5.30 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = −5.34 (2 CH_3), −4.60 (2 CH_3), 11.83 (CH_3), 16.65 (CH_3), 18.27 (C), 18.36 (C), 18.67 (CH_3), 19.42 (CH_3), 21.04 (CH_2), 23.33 (CH_2), 24.27 (CH_2), 25.93 (3 CH_3), 25.95 (3 CH_3), 28.23 (CH_2), 31.88 (CH), 31.92 (CH_2), 32.07 (CH_2), 33.56 (CH_2), 35.69 (CH), 35.73 (CH), 36.16 (CH_2), 36.57 (C), 37.36 (CH_2), 39.78 (CH_2), 42.30 (C), 42.80 (CH_2), 50.18 (CH), 56.11 (CH), 56.79 (CH), 68.56 (CH_2), 72.64 (CH), 121.16 (CH), 141.56 (C); MS (70 eV): m/z (%) = 616 (3) [(M − Me)$^+$], 574 (100) [(M − *tert*-Bu)$^+$], 572 (10), 442 (10); HRMS: m/z calc. for $C_{35}H_{65}O_2Si_2$ [(M − *tert*-Bu)$^+$]: 573.4518, found: 573.4532; Anal. calc. for $C_{39}H_{74}O_2Si_2$: C 74.21, H 11.82, found: C 74.25, H 11.92%.

(25*R*)-26-Hydroxycholesterol (9). A 1.0 M solution of TBAF in THF (2.3 mL, 2.3 mmol) was added to a solution of the disilyl ether **8** (484 mg, 0.77 mmol) in THF (40 mL). The resulting mixture was heated at reflux for 20 h. After cooling to room temperature, water (100 mL) was added and the reaction mixture was extracted with diethyl ether (3 × 100 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. Purification of the residue by flash chromatography on silica gel (petroleum ether/ethyl acetate, 2:1) furnished the known (25*R*)-26-hydroxycholesterol (**9**), yield: 275 mg (89%). Colorless

solid; mp: 172–173 °C; IR (ATR): ν = 3292, 2959, 2932, 2897, 2850, 1463, 1435, 1374, 1354, 1254, 1228, 1194, 1126, 1057, 1042, 1023, 990, 955, 841, 798, 737 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.90 (d, J = 6.7 Hz, 6 H), 0.99 (s, 3 H), 1.03–1.60 (m, 21 H), 1.80–1.85 (m, 3 H), 1.94–2.02 (m, 2 H), 2.20–2.30 (m, 2 H), 3.41 (dd, J = 10.5, 6.4 Hz, 1 H), 3.49 (dd, J = 10.5, 5.8 Hz, 1 H), 3.51 (m, 1 H), 5.34 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.85 (CH_3), 16.49 (CH_3), 18.65 (CH_3), 19.39 (CH_3), 21.06 (CH_2), 23.38 (CH_2), 24.27 (CH_2), 28.24 (CH_2), 31.63 (CH_2), 31.87 (CH), 31.88 (CH_2), 33.50 (CH_2), 35.70 (CH), 35.78 (CH), 36.13 (CH_2), 36.48 (C), 37.22 (CH_2), 39.75 (CH_2), 42.27 (CH_2), 42.30 (C), 50.08 (CH), 56.09 (CH), 56.73 (CH), 68.53 (CH_2), 71.78 (CH), 121.70 (CH), 140.74 (C); MS (70 eV): m/z (%) = 402 (93) [M^+], 400 (16), 387 (34), 385 (100), 369 (33), 317 (36), 300 (23), 299 (35), 291 (62), 273 (22), 271 (90); HRMS: m/z calc. for $C_{27}H_{46}O_2$ [M^+]: 402.3498, found: 402.3505; Anal. calc. for $C_{27}H_{46}O_2$: C 80.54, H 11.51, found: C 80.61, H 11.65%.

(25*R*)-3,6-Diketocholest-4-en-26-oic acid (10). PDC (700 mg, 1.86 mmol) was added to a solution of (25*R*)-26-hydroxycholesterol (**9**) (75 mg, 0.186 mmol) in DMF (20 mL) and the resulting mixture was stirred for 18 h at room temperature. Water (100 mL) was added and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried with magnesium sulfate. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 2:1) to provide compound **10**, yield: 59 mg (74%). Colorless solid; mp: 105–110 °C; IR (ATR): ν = 2940, 2868, 1699, 1462, 1415, 1379, 1221, 1024, 922, 868 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.70 (s, 3 H), 0.91 (d, J = 6.5 Hz, 3 H), 1.15 (s, 3 H), 1.17 (d, J = 7.0 Hz, 3 H), 1.19–1.51 (m, 12 H), 1.61 (m, 3 H), 1.80–1.92 (m, 4 H), 2.02 (dd, J = 16.0, 12.4 Hz, 1 H), 2.08 (dt, J = 13.0, 3.3 Hz, 1 H), 2.13 (ddd, J = 13.4, 5.1, 2.6 Hz, 1 H), 2.43–2.53 (m, 3 H), 2.66 (dd, J = 16.0, 4.1 Hz, 1 H), 6.16 (s, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.87 (CH_3), 16.76 (CH_3), 17.49 (CH_3), 18.55 (CH_3), 20.84 (CH_2), 23.59 (CH_2), 23.93 (CH_2), 27.97 (CH_2), 33.83 (CH_2), 33.94 (CH_2), 34.16 (CH), 35.50 (CH, CH_2), 35.60 (CH_2), 39.08 (CH_2), 39.21 (CH), 39.78 (C), 42.52 (C), 46.77 (CH_2), 50.91 (CH), 55.83 (CH), 56.48 (CH), 125.45 (CH), 161.04 (C), 182.27 (C=O), 199.55 (C=O), 202.34 (C=O); MS (70 eV): m/z (%) = 428 (100) [M^+], 410 (11), 400 (19), 386 (7), 277 (10), 243 (18); HRMS: m/z calc. for $C_{27}H_{40}O_4$ [M^+]: 428.2927, found: 428.2914.

(25*R*)-26-Acetoxycholest-5-ene-3β,16β-diol (11). Triethylamine (230 µL, 1.65 mmol), acetic anhydride (78 µL, 825 µmol), and DMAP (9 mg, 76 µmol) were added to a solution of **6** (316 mg, 760 µmol) in THF (30 mL). The resulting mixture was stirred at room temperature for 16 h, subsequently quenched by addition of water (50 mL), and the aqueous layer was extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with brine (50 mL) and dried with magnesium sulfate. Removal of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 1:2) provided the acetate **11**, yield: 188 mg (54%). Colorless solid; mp: 89–90 °C; IR (ATR): ν = 3381, 2932, 2859, 1739, 1628, 1465, 1437, 1375, 1363, 1336, 1231, 1152, 1043, 985, 954 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.81–0.99 (m, 3 H), 0.87 (s, 3 H), 0.90 (d, J = 6.7 Hz, 3 H), 0.96 (d, J = 6.7 Hz, 3 H), 1.00 (s, 3 H), 1.02–1.54 (m, 14 H), 1.75–1.84 (m, 4 H), 1.95–2.01 (m, 2 H), 2.04 (s, 3 H), 2.19–2.29 (m, 3 H), 3.50 (m, 1 H), 3.79 (dd, J = 10.7, 7.1 Hz, 1 H), 3.97 (dd, J = 10.7, 5.9 Hz, 1 H), 4.33 (dt, J = 4.5, 7.6 Hz, 1 H), 5.33 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 13.01 (CH_3), 16.86 (CH_3), 18.14 (CH_3), 19.36 (CH_3), 20.66 (CH_2), 20.99 (CH_3), 23.55 (CH_2), 29.75 (CH), 31.43 (CH), 31.57 (CH_2), 31.76 (CH_2), 32.34 (CH), 33.59 (CH_2), 35.91 (CH_2), 36.47 (C), 36.65 (CH_2), 37.14 (CH_2), 39.80 (CH_2), 42.16 (C), 42.22 (CH_2), 50.03 (CH), 54.44 (CH), 61.35 (CH), 69.53 (CH_2), 71.70 (CH), 72.35 (CH), 121.41 (CH), 140.84 (C), 171.40 (C=O); MS (70 eV): m/z (%) = 460 (7) [M^+], 442 (97), 427 (24), 424 (79), 409 (50), 383 (23), 331 (17), 271 (100), 253 (54). HRMS: m/z calc. for $C_{29}H_{46}O_3$ [$(M - H_2O)^+$]: 442.3447, found: 442.3454; Anal. calc. for $C_{29}H_{48}O_4$: C 75.61, H 10.50, found: C 75.15, H 10.34%.

(25*R*)-26-Pivaloyloxycholest-5-ene-3β,16β-diol (12). Triethylamine (80 µL, 572 µmol), pivaloyl chloride (35 µL, 286 µmol), and DMAP (3 mg, 26 µmol) were added to a solution of **6** (107 mg, 260 µmol) in THF (10 mL). The resulting solution was stirred at room temperature for 20 h and then water was added. The mixture was extracted with diethyl ether three times and dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 1:2) provided the pivalate **12**, yield: 106 mg (81%). Colorless solid; mp: 119–121 °C; IR (ATR): ν = 3395, 2928, 2850, 1722, 1632, 1479, 1462, 1397, 1375, 1364, 1336, 1282, 1164, 1138, 1045, 1020, 983, 954, 936, 815, 770 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.82–0.86 (m, 1 H), 0.87 (s, 3 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.94–0.99 (m, 2 H), 0.96 (d, J = 6.7 Hz, 3 H), 1.00 (s, 3 H), 1.02–1.17 (m, 5 H), 1.19 (s, 9 H), 1.25–1.32 (m, 3 H), 1.45–1.55 (m, 6 H), 1.77–1.85 (m, 4 H), 1.96 (m, 1 H), 1.99 (dt, J = 12.6, 3.5 Hz, 1 H), 2.20–2.30 (m, 3 H), 3.51 (m, 1 H), 3.79 (dd, J = 10.7, 7.0 Hz, 1 H), 3.97 (dd, J = 10.7, 5.6 Hz, 1 H), 4.33 (dt, J = 4.2, 7.6 Hz, 1 H), 5.34 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 13.02 (CH_3), 16.94 (CH_3), 18.17 (CH_3), 19.37 (CH_3), 20.67 (CH_2), 23.55 (CH_2), 27.21 (3 CH_3), 29.78 (CH), 31.45 (CH), 31.58 (CH_2), 31.78 (CH_2), 32.52 (CH), 33.68 (CH_2), 36.03 (CH_2), 36.49 (C), 36.66 (CH_2), 37.15 (CH_2), 38.84 (C), 39.81 (CH_2), 42.17 (C), 42.22 (CH_2), 50.04 (CH), 54.46 (CH), 61.32 (CH), 69.20 (CH_2), 71.74 (CH), 72.38 (CH), 121.45 (CH), 140.84 (C), 178.71 (C=O); MS (70 eV): m/z (%) = 502 (2) [M^+], 484 (33), 469 (10), 466 (68), 451 (17), 271 (100); HRMS: m/z calc. for $C_{32}H_{52}O_3$ [$(M - H_2O)^+$]: 484.3916, found: 484.3907.

(25*R*)-3β-(*tert*-Butyldimethylsilyloxy)-26-pivaloyloxy-cholest-5-en-16β-ol (13). *tert*-Butylchlorodimethylsilane (885 mg, 5.874 mmol) and DBU (1.76 mL, 11.748 mmol) were added to a solution of **12** (2.687 g, 5.34 mmol) in THF (50 mL). The mixture was stirred at room temperature for 17 h and then water (100 mL) was added. The resulting solution was extracted with diethyl ether (3 × 100 mL) and the combined organic layers were washed with brine (50 mL) and dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 20:1) provided the silyl ether **13**, yield: 2.618 g (79%). Colorless solid; mp: 108 °C; IR (ATR): ν = 3547, 2931, 2852, 1722, 1464, 1397, 1382, 1367, 1281, 1251, 1167, 1077, 1022, 985, 959, 938, 886, 870, 838, 777 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.81–0.86 (m, 1 H), 0.869 (s, 3 H), 0.874 (s, 9 H), 0.89–0.98 (m, 2 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.96 (d, J= 6.7 Hz, 3 H), 0.99 (s, 3 H), 1.01–1.16 (m, 5 H), 1.19 (s, 9 H), 1.21–1.34 (m,

2 H), 1.38–1.57 (m, 7 H), 1.68–1.72 (m, 1 H), 1.77–1.87 (m, 3 H), 1.95–2.00 (m, 2 H), 2.15 (ddd, J = 13.4, 4.9, 2.1 Hz, 1 H), 2.20–2.28 (m, 2 H), 3.46 (m, 1 H), 3.79 (dd, J = 10.7, 6.9 Hz, 1 H), 3.97 (dd, J = 10.7, 5.6 Hz, 1 H), 4.33 (m, 1 H), 5.30 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 13.02 (CH_3), 16.94 (CH_3), 18.17 (CH_3), 18.26 (C), 19.41 (CH_3), 20.66 (CH_2), 23.55 (CH_2), 25.92 (3 CH_3), 27.22 (3 CH_3), 29.77 (CH), 31.46 (CH), 31.82 (CH_2), 32.03 (CH_2), 32.53 (CH), 33.69 (CH_2), 36.04 (CH_2), 36.57 (C), 36.69 (CH_2), 37.29 (CH_2), 38.84 (C), 39.85 (CH_2), 42.18 (C), 42.76 (CH_2), 50.14 (CH), 54.51 (CH), 61.32 (CH), 69.19 (CH_2), 72.39 (CH), 72.58 (CH), 120.90 (CH), 141.66 (C), 178.68 (C=O); MS (70 eV): m/z (%) = 598 (2) [(M – H_2O)$^+$], 559 (86) [(M – *tert*-Bu)$^+$], 541 (12), 483 (14), 467 (9), 457 (13), 383 (29), 365 (100), 271 (9); HRMS: m/z calc. for $C_{34}H_{59}O_4Si$ [(M – *tert*-Bu)$^+$]: 559.4183, found: 559.4182; Anal. calc. for $C_{38}H_{68}O_4Si$: C 73.97, H 11.11, found: C 74.01, H 11.33%.

(25*R*)-3*β*-(*tert*-Butyldimethylsilyloxy)cholest-5-en-26-ol (14). Methanesulfonyl chloride (1.02 mL, 13.22 mmol) was added dropwise to a solution of **13** (2.15 g, 3.48 mmol) in pyridine (30 mL) at 0 °C. Stirring was continued for 16 h and the reaction mixture was allowed to warm to room temperature. The resulting orange solution was carefully quenched with ice-water (50 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with ice-cold 1 N HCl (50 mL), water (50 mL), a saturated solution of sodium hydrogen carbonate (50 mL), and brine (50 mL). After drying with magnesium sulfate and removal of the solvent the crude mesylate was obtained in quantitative yield (2.42 g). The mesylate (2.42 g, 3.48 mmol) was dissolved in diethyl ether (40 mL) and carefully treated with lithium aluminium hydride (660 mg, 17.4 mmol) at 0 °C. The resulting suspension was heated at reflux for 4 h. After cooling to room temperature, the reaction mixture was carefully quenched by addition of water (10 mL) and ethyl acetate (10 mL). An ice-cold 10% aqueous solution of sulfuric acid (20 mL) was slowly added until the precipitate disappeared. The aqueous layer was extracted with diethyl ether (3 × 100 mL) and the combined organic layers were washed with a saturated solution of sodium hydrogen carbonate (50 mL), water (50 mL), and brine (50 mL). Drying with magnesium sulfate and evaporation of the solvent gave the crude product, which was purified by flash chromatography on silica gel (petroleum ether/diethyl ether, 5:1) to afford the alcohol **14**, yield: 1.601 g (89%). Colorless solid; mp: 163–164 °C; IR (ATR): ν = 3306, 2929, 2901, 2857, 1462, 1381, 1252, 1196, 1086, 1039, 1006, 989, 959, 938, 887, 869, 835, 802, 773 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.88 (s, 9 H), 0.90 (d, J = 6.7 Hz, 6 H), 0.91–1.63 (m, 20 H), 0.98 (s, 3 H), 1.70 (m, 2 H), 1.77–1.83 (m, 2 H), 1.93–2.01 (m, 2 H), 2.16 (ddd, J = 13.3, 4.9, 2.2 Hz, 1 H), 2.25 (m, 1 H), 3.41 (dd, J = 10.5, 6.5 Hz. 1 H), 3.44–3.51 (m, 1 H), 3.48 (dd, J = 10.5, 4.6 Hz, 1 H), 5.31 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.84 (CH_3), 16.49 (CH_3), 18.27 (C), 18.66 (CH_3), 19.42 (CH_3), 21.04 (CH_2), 23.37 (CH_2), 24.27 (CH_2), 25.93 (3 CH_3), 28.25 (CH_2), 31.88 (CH), 31.92 (CH_2), 32.06 (CH_2), 33.51 (CH_2), 35.69 (CH), 35.79 (CH), 36.13 (CH_2), 36.56 (C), 37.36 (CH_2), 39.78 (CH_2), 42.31 (C), 42.80 (CH_2), 50.17 (CH), 56.09 (CH), 56.78 (CH), 68.54 (CH_2), 72.63 (CH), 121.15 (CH), 141.56 (C); MS (70 eV): m/z (%) = 516 (0.4) [M$^+$], 501 (2), 459 (100), 457 (38); HRMS: m/z calc. for $C_{29}H_{51}O_2Si$ [(M – *tert*-Bu)$^+$]: 459.3658, found: 459.3650; Anal. calc. for $C_{33}H_{60}O_2Si$: C 76.68, H 11.70, found: C 76.85, H 11.79%.

(25*R*)-26-Acetoxy-3*β*-(*tert*-butyldimethylsilyloxy)cholest-5-ene (15). Triethylamine (863 μL, 6.2 mmol), acetic anhydride (292 μL, 3.1 mmol), and DMAP (19 mg, 0.155 mmol) were added to a solution of the alcohol **14** (800 mg, 1.55 mmol) in THF (30 mL). The resulting mixture was stirred at room temperature for 16 h and then water (100 mL) was added. The solution was extracted with diethyl ether (3 × 100 mL), the combined organic layers were washed with brine (50 mL) and dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 20:1) provided the acetate **15**, yield: 866 mg (100%). Colorless solid; mp: 105–106 °C; IR (ATR): ν = 2933, 2893, 2857, 1742, 1471, 1362, 1240, 1195, 1092, 1031, 988, 963, 927, 890, 870, 836, 804, 774 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.87 (s, 9 H), 0.900 (d, J = 6.4 Hz, 3 H), 0.903 (d, J = 6.7 Hz, 3 H), 0.98 (s, 3 H), 1.00–1.55 (m, 20 H), 1.69–1.82 (m, 4 H), 1.93–2.01 (m, 2 H), 2.04 (s, 3 H), 2.15 (ddd, J = 13.3, 4.9, 2.2 Hz, 1 H), 2.25 (m, 1 H), 3.47 (m, 1 H), 3.83 (dd, J = 10.7, 7.0 Hz, 1 H), 3.93 (dd, J = 10.7, 6.0 Hz, 1 H), 5.30 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.84 (CH_3), 16.78 (CH_3), 18.27 (C), 18.65 (CH_3), 19.42 (CH_3), 21.00 (CH_3), 21.04 (CH_2), 23.20 (CH_2), 24.27 (CH_2), 25.93 (3 CH_3), 28.24 (CH_2), 31.88 (CH), 31.92 (CH_2), 32.07 (CH_2), 32.48 (CH), 33.73 (CH_2), 35.66 (CH), 36.02 (CH_2), 36.56 (C), 37.36 (CH_2), 39.77 (CH_2), 42.31 (C), 42.80 (CH_2), 50.17 (CH), 56.05 (CH), 56.77 (CH), 69.61 (CH_2), 72.63 (CH), 121.14 (CH), 141.56 (C), 171.33 (C=O); GC-MS (70 eV): m/z (%) = 501 (64) [(M – *tert*-Bu)$^+$], 441 (14), 367 (100).

(25*R*)-26-Acetoxycholest-5-en-3*β*-ol (16). A 1 M solution of TBAF in THF (1.74 mL, 1.74 mmol) was added to a solution of the silyl ether **15** (811 mg, 1.45 mmol) in THF (25 mL) and the mixture was heated at reflux for 17 h. After cooling to room temperature, water (100 mL) was added and the mixture was extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine (50 mL) and dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 3:2) provided the alcohol **16**, yield: 563 mg (88%). Light yellow oil, which slowly solidified on cooling to give a colorless solid; mp: 74 °C; IR (ATR): ν = 3264, 2931, 2901, 2864, 1739, 1463, 1365, 1233, 1195, 1108, 1058, 1036, 986, 954, 926, 841, 800, 740 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.90 (d, J = 6.7 Hz, 6 H), 0.92–1.62 (m, 20 H), 0.99 (s, 3 H), 1.73–1.85 (m, 4 H), 1.93–2.06 (m, 2 H), 2.04 (s, 3 H), 2.22–2.30 (m, 2 H), 3.51 (m, 1 H), 3.82 (dd, J = 10.7, 7.0 Hz, 1 H), 3.93 (dd, J = 10.7, 6.0 Hz, 1 H), 5.34 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.84 (CH_3), 16.78 (CH_3), 18.64 (CH_3), 19.38 (CH_3), 21.00 (CH_3), 21.05 (CH_2), 23.21 (CH_2), 24.26 (CH_2), 28.23 (CH_2), 31.63 (CH_2), 31.87 (CH, CH_2), 32.47 (CH), 33.72 (CH_2), 35.67 (CH), 36.01 (CH_2), 36.48 (C), 37.22 (CH_2), 39.74 (CH_2), 42.27 (CH_2), 42.30 (C), 50.08 (CH), 56.05 (CH), 56.72 (CH), 69.61 (CH_2), 71.77 (CH), 121.68 (CH), 140.74 (C), 171.35 (C=O); GC-MS (70 eV): m/z (%) = 444 (15) [M$^+$], 429 (16), 426 (100), 411 (27), 384 (16), 359 (27), 333 (47), 271 (12).

(25*R*)-26-Acetoxycholest-4-en-3-one (17). Aluminium isopropoxide (232 mg, 1.14 mmol) was added to a solution of the alcohol **16** (303 mg, 680 μmol) in a mixture of acetone (1 mL) and toluene (9 mL). The reaction mixture was heated at reflux for 15 h and after cooling to room temperature, treated with dilute HCl (20 mL). The two layers were separated and the aqueous layer was extracted with diethyl ether (2 × 100 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 4:1) furnished the ketone **17**, yield: 210 mg (70%). Yellow oil; IR (ATR): ν = 2934, 2868, 1737, 1674, 1616, 1465, 1375, 1330, 1230, 1187, 1034, 986, 933, 865, 778 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3 H), 0.88–0.93 (m, 1 H), 0.897 (d, J = 6.6 Hz, 3 H), 0.904 (d, J = 6.7 Hz, 3 H), 0.96–1.15 (m, 7 H), 1.17 (s, 3 H), 1.18–1.84 (m, 14 H), 1.98–2.03 (m, 2 H), 2.04 (s, 3 H), 2.23–2.44 (m, 4 H), 3.82 (dd, J = 10.7, 7.0 Hz, 1 H), 3.93 (dd, J = 10.7, 6.0 Hz, 1 H), 5.71 (s, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.93 (CH_3), 16.77 (CH_3), 17.36 (CH_3), 18.57 (CH_3), 20.99 (CH_3, CH_2), 23.20 (CH_2), 24.14 (CH_2), 28.17 (CH_2), 32.00 (CH_2), 32.47 (CH), 32.93 (CH_2), 33.70 (CH_2), 33.97 (CH_2), 35.58 (CH), 35.63 (CH), 35.66 (CH_2), 35.94 (CH_2), 38.58 (C), 39.59 (CH_2), 42.37 (C), 53.76 (CH), 55.83 (CH), 56.01 (CH), 69.59 (CH_2), 123.72 (CH), 171.34 (C=O), 171.73 (C), 199.71 (C=O); MS (70 eV): m/z (%) = 442 (100) [M$^+$], 440 (8), 427 (7), 425 (15), 400 (23), 319 (15), 271 (11), 269 (19); HRMS: m/z calc. for $C_{29}H_{46}O_3$ [M$^+$]: 442.3447, found: 442.3433.

(25*R*)-26-Hydroxycholest-4-en-3-one (18). Potassium carbonate (6 mg, 45 μmol) was added to a solution of the acetate **17** (200 mg, 450 μmol) in methanol (20 mL) and the reaction mixture was stirred at room temperature for 20 h. A second portion of potassium carbonate (6 mg, 45 μmol) was added and stirring was continued for further 20 h. Water (50 mL) was added and the solution was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 1:8) provided the alcohol **18**, yield: 167 mg (93%). Colorless crystals; mp: 125 °C; IR (ATR): ν = 3415, 2928, 2860, 1660, 1615, 1447, 1434, 1376, 1332, 1276, 1231, 1188, 1112, 1038, 958, 932, 861, 779, 740 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3 H), 0.88–1.15 (m, 8 H), 0.90 (d, J = 6.7 Hz, 6 H), 1.17 (s, 3 H), 1.21–1.63 (m, 11 H), 1.68 (dt, J = 4.6, 14.0 Hz, 1 H), 1.79–1.88 (m, 2 H), 1.98–2.06 (m, 2 H), 2.23–2.44 (m, 4 H), 3.41 (dd, J = 10.5, 6.4 Hz, 1 H), 3.49 (dd, J = 10.5, 5.8 Hz, 1 H), 5.71 (s, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.94 (CH_3), 16.48 (CH_3), 17.36 (CH_3), 18.57 (CH_3), 20.99 (CH_2), 23.37 (CH_2), 24.15 (CH_2), 28.18 (CH_2), 32.01 (CH_2), 32.93 (CH_2), 33.48 (CH_2), 33.97 (CH_2), 35.58 (CH), 35.65 (CH, CH_2), 35.77 (CH), 36.05 (CH_2), 38.58 (C), 39.60 (CH_2), 42.37 (C), 53.77 (CH), 55.84 (CH), 56.05 (CH), 68.51 (CH_2), 123.72 (CH), 171.72 (C), 199.69 (C=O). For further spectroscopic data see ref. 8.

(25*R*)-3-Ketocholest-4-en-26-oic acid – (25*R*)-Δ^4-dafachronic acid (1a). Freshly prepared Jones reagent, consisting of CrO_3 (238 mg, 2.4 mmol in 400 μL water) and concentrated sulfuric acid (209 μL, 3.77 mmol in 500 μL water), was added to a solution of the diol **18** (193 mg, 480 μmol) in acetone (30 mL) at 0 °C. After stirring for 1 h at 0 °C, 2-propanol (10 mL) and brine (10 mL) were added, and the resulting mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried with magnesium sulfate and the solvent was evaporated. Purification of the residue by flash chromatography on silica gel (petroleum ether/ethyl acetate, 60:40 +1% AcOH) provided pure (25*R*)-Δ^4-dafachronic acid (**1a**), yield: 158 mg (79%). Colorless solid; mp: 148–150 °C; $[\alpha]_D^{20}$ = +66.0 (c = 1.0, $CHCl_3$); IR (ATR): ν = 2934, 2865, 2849, 1734, 1703, 1673, 1638, 1614, 1465, 1445, 1435, 1374, 1331, 1269, 1233, 1194, 1161, 1141, 1115, 1029, 933, 866, 781, 734 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3 H), 0.88–0.93 (m, 1 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.96–1.63 (m, 17 H), 1.163 (d, J = 6.9 Hz, 3 H), 1.164 (s, 3 H), 1.67 (dt, J = 4.9, 14.0 Hz, 1 H), 1.78–1.86 (m, 2 H), 1.98–2.02 (m, 2 H), 2.25 (ddd, J = 14.5, 4.0, 2.4 Hz, 1 H), 2.30–2.47 (m, 4 H), 5.72 (s, 1 H), 11.15 (br s, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.93 (CH_3), 16.74 (CH_3), 17.35 (CH_3), 18.55 (CH_3), 20.99 (CH_2), 23.60 (CH_2), 24.14 (CH_2), 28.15 (CH_2), 32.00 (CH_2), 32.93 (CH_2), 33.86 (CH_2), 33.94 (CH_2), 35.57 (2 CH), 35.64 (2 CH_2), 38.58 (C), 39.26 (CH), 39.57 (CH_2), 42.37 (C), 53.75 (CH), 55.81 (CH), 55.99 (CH), 123.71 (CH), 171.85 (C), 182.48 (C=O), 199.83 (C=O); ESI-MS: m/z = 415 [(M + H)$^+$]; Anal. calc. for $C_{27}H_{42}O_3$: C 78.21, H 10.21, found: C 78.03, H 10.46%.

(25*R*)-3β,26-Bis(*tert*-butyldimethylsilyloxy)cholest-5-en-7-one (19). A 5.5 M solution of *tert*-butyl hydroperoxide in decane (500 μL, 2.54 mmol) was added to a mixture of the disilyl ether **8** (400 mg. 630 μmol), Celite® (760 mg) and PDC (950 mg, 2.54 mmol) in benzene (20 mL) at 0 °C. The ice-bath was removed and stirring was continued at room temperature for further 24 h. A second portion of *tert*-butylhydroperoxide (500 μL, 2.54 mmol) was added and stirring was continued for 4 h. The crude mixture was filtered through a short pad of silica gel (elution with diethyl ether) and the solvent was removed. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 20:1) provided the enone **19**, yield: 231 mg (57%). Colorless solid; mp: 147–148 °C; IR (ATR): ν = 2930, 2896, 2857, 1669, 1633, 1472, 1376, 1360, 1317, 1283, 1248, 1189, 1081, 1059, 1006, 952, 940, 889, 876, 864, 836, 803, 772 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 6 H), 0.05 (s, 6 H), 0.66 (s, 3 H), 0.84 (d, J = 6.7 Hz, 3 H), 0.875 (s, 9 H), 0.879 (s, 9 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.96–1.38 (m, 13 H), 1.17 (s, 3 H), 1.44–1.66 (m, 5 H), 1.78–1.91 (m, 3 H), 2.01 (dt, J = 12.6, 3.2 Hz, 1 H), 2.22 (dd, J = 12.1, 11.1 Hz, 1 H), 2.36–2.40 (m, 3 H), 3.33 (dd, J = 9.7, 6.7 Hz, 1 H), 3.42 (dd, J = 9.7, 5.9 Hz, 1 H), 3.58 (m, 1 H), 5.65 (d, J = 1.1 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = −5.34 (2 CH_3), −4.70 (CH_3), −4.67 (CH_3), 11.94 (CH_3), 16.65 (CH_3), 17.28 (CH_3), 18.14 (C), 18.35 (C), 18.83 (CH_3), 21.17 (CH_2), 23.35 (CH_2), 25.82 (3 CH_3), 25.95 (3 CH_3), 26.29 (CH_2), 28.55 (CH_2), 31.71 (CH_2), 33.52 (CH_2), 35.62 (CH), 35.71 (CH), 36.16 (CH_2), 36.39 (CH_2), 38.33 (C), 38.69 (CH_2), 42.51 (CH_2), 43.06 (C), 45.38 (CH), 49.92 (CH), 49.97 (CH), 54.76 (CH), 68.54 (CH_2), 71.31 (CH), 125.79 (CH), 165.88 (C), 202.49 (C=O); MS (70 eV): m/z (%) = 644 (0.4) [M$^+$], 629 (3), 587 (100), 585 (8), 455 (6), 441 (3), 265 (5); HRMS: m/z calc. for [(M − *tert*-Bu)$^+$]: 587.4310, found: 587.4313; Anal. calc. for $C_{39}H_{72}O_3Si_2$: C 72.61, H 11.25, found: C 72.83, H 11.52%.

(25*R*)-3β,26-Bis(*tert*-butyldimethylsilyloxy)-5α-cholestan-7-one (20). Ammonium formate (40 mg, 620 μmol) was added to a mixture of the enone **19** (100 mg, 155 μmol) and Pd/C (10%, 16 mg, 15 μmol Pd) in methanol (3 mL) and ethyl acetate (4 mL). The mixture was heated at reflux for 8 h and then cooled to

room temperature. Filtration over a short pad of Celite® (elution with diethyl ether) and removal of the solvent gave the crude product, which was purified by flash chromatography on silica gel (petroleum ether/diethyl ether, 40:1 to 20:1) to provide **20**, yield: 90 mg (90%). Colorless crystalline solid; mp: 102–103 °C; IR (ATR): ν = 2928, 2856, 1706, 1671, 1471, 1376, 1361, 1252, 1174, 1098, 1053, 1006, 941, 909, 872, 835, 774, 735, 667 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.021 (s, 6 H), 0.025 (s, 6 H), 0.63 (s, 3 H), 0.84 (d, J = 6.7 Hz, 3 H), 0.84–1.10 (m, 5 H), 0.86 (s, 9 H), 0.88 (s, 9 H), 0.89 (d, J = 7.7 Hz, 3 H), 1.06 (s, 3 H), 1.16–1.58 (m, 16 H), 1.71 (m, 2 H), 1.87 (m, 1 H), 1.95–2.00 (m, 2 H), 2.16 (m, 1 H), 2.33 (m, 2 H), 3.33 (dd, J = 9.7, 6.7 Hz, 1 H), 3.42 (dd, J = 9.7, 5.9 Hz, 1 H), 3.53 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –5.34 (2 CH_3), –4.66 (2 CH_3), 11.86 (CH_3), 12.03 (CH_3), 16.64 (CH_3), 18.20 (C), 18.35 (C), 18.74 (CH_3), 21.84 (CH_2), 23.29 (CH_2), 24.93 (CH_2) 25.87 (3 CH_3), 25.95 (3 CH_3), 28.40 (CH_2), 31.52 (CH_2), 33.50 (CH_2), 35.55 (CH), 35.70 (CH), 36.01 (C), 36.11 (CH_2), 36.21 (CH_2), 38.44 (CH_2), 38.75 (CH_2), 42.47 (C), 46.21 (CH_2), 47.13 (CH), 48.83 (CH), 50.01 (CH), 55.01 (CH), 55.45 (CH), 68.53 (CH_2), 71.51 (CH), 212.44 (C=O); MS (70 eV): m/z (%) = 646 (0.2) [M$^+$], 631 (2), 589 (100), 587 (9), 459 (16), 445 (12); HRMS: m/z calc. for $C_{35}H_{63}O_3Si_2$ [(M – *tert*-Bu)$^+$]: 589.4467, found: 589.4478; Anal. calc. for $C_{39}H_{74}O_3Si_2$: C 72.38, H 11.53, found: C 72.56, H 11.57%.

(25*R*)-3β,26-Bis(*tert*-butyldimethylsilyloxy)-5α-cholestan-7α-ol (21). A 2.0 M solution of isopropylmagnesium chloride in diethyl ether (110 µL, 220 µmol) was further diluted with diethyl ether (2 mL), and added dropwise over a period of 30 min to a solution of the ketone **20** (100 mg, 155 µmol) in diethyl ether (8 mL) at room temperature. After stirring for 1 h at room temperature, the reaction mixture was quenched by addition of a saturated solution of ammonium chloride (25 mL) and the layers were separated. The aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), and dried with magnesium sulfate. Removal of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 10:1) provided diastereoselectively the 7α-alcohol **21**, yield: 70 mg (70%). Colorless solid; mp: 95–98 °C; IR (ATR): ν = 3455, 2928, 2856, 1471, 1376, 1361, 1250, 1098, 1078, 1031, 1006, 948, 834, 772, 667 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 6 H), 0.03 (s, 6 H), 0.64 (s, 3 H), 0.78 (s, 3 H), 0.84 (d, J = 6.7 Hz, 3 H), 0.86 (s, 9 H), 0.88 (s, 9 H), 0.89 (d, J = 8.2 Hz, 3 H), 0.96–1.68 (m, 27 H), 1.83–1.87 (m, 1 H), 1.93 (dt, J = 12.6, 3.2 Hz, 1 H), 3.33 (dd, J = 9.7, 6.7 Hz, 1 H), 3.42 (dd, J = 9.7, 6.0 Hz, 1 H), 3.57 (m, 1 H), 3.81 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –5.33 (2 CH_3), –4.58 (2 CH_3), 11.27 (CH_3), 11.81 (CH_3), 16.64 (CH_3), 18.25 (C), 18.36 (C), 18.61 (CH_3), 20.96 (CH_2), 23.26 (CH_2), 23.64 (CH_2), 25.95 (6 CH_3), 28.20 (CH_2), 31.85 (CH_2), 33.52 (CH_2), 35.57 (C), 35.68 (CH), 35.71 (CH), 36.10 (CH_2), 36.28 (CH_2), 36.90 (CH_2), 37.21 (CH), 38.18 (CH_2), 39.51 (CH_2), 39.54 (CH), 42.66 (C), 45.92 (CH), 50.58 (CH), 56.09 (CH), 68.12 (CH), 68.55 (CH_2), 71.97 (CH); GC-MS (70 eV): m/z (%) = 591 (84) [(M – *tert*-Bu)$^+$], 573 (25), 459 (17), 75 (100); Anal. calc. for $C_{39}H_{76}O_3Si_2$: C 72.15, H 11.80, found: C 72.16, H 11.91%.

(25*R*)-3β,26-Bis(*tert*-butyldimethylsilyloxy)-5α-cholestan-7β-ol (22). Lithium aluminium hydride (5.5 mg, 144 µmol) was added to a solution of **20** (93 mg, 144 µmol) in THF (10 mL) at –78 °C. The reaction mixture was allowed to warm to room temperature over a period of 24 h. Diethyl ether (50 mL), water (10 mL), and 10% sulfuric acid (5 mL) were added. The layers were separated and the aqueous layer was extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate (50 mL) and brine (50 mL), and then dried over magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 10:1) provided as the less polar fraction the 7α-alcohol **21** (59 mg, 85 µmol, 59%) (spectral data, see above) and the more polar 7β-alcohol **22**, yield: 29 mg (31%). Colorless solid; mp: 88–94 °C; IR (ATR): ν = 3488, 2928, 2856, 1471, 1374, 1249, 1187, 1100, 1074, 1026, 1005, 943, 870, 833, 771, 667 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 6 H), 0.03 (s, 6 H), 0.66 (s, 3 H), 0.81 (s, 3 H), 0.84 (d, J = 6.6 Hz, 3 H), 0.87 (s, 9 H), 0.88 (s, 9 H), 0.89 (d, J = 6.7 Hz, 3 H), 0.97–1.89 (m, 28 H), 1.97 (m, 1 H), 3.31–3.36 (m, 1 H), 3.34 (dd, J = 9.7, 6.6 Hz, 1 H), 3.42 (dd, J = 9.7, 6.0 Hz, 1 H), 3.53 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –5.34 (2 CH_3), –4.58 (2 CH_3), 12.13 (CH_3), 12.47 (CH_3), 16.65 (CH_3), 18.24 (C), 18.36 (C), 18.75 (CH_3), 21.40 (CH_2), 23.35 (CH_2), 25.92 (3 CH_3), 25.95 (3 CH_3), 26.90 (CH_2), 28.73 (CH_2), 31.86 (CH_2), 33.54 (CH_2), 34.94 (C), 35.59 (CH), 35.72 (CH), 36.17 (CH_2), 37.05 (CH_2), 38.09 (CH_2), 38.17 (CH_2), 39.99 (CH_2), 42.16 (CH), 43.43 (CH), 43.60 (C), 52.55 (CH), 55.20 (CH), 55.72 (CH), 68.54 (CH_2), 71.91 (CH), 75.24 (CH); GC-MS (70 eV): m/z (%) = 591 (90) [(M – *tert*-Bu)$^+$], 589 (10), 573 (12), 459 (22), 75 (100); Anal. calc. for $C_{39}H_{76}O_3Si_2$: C 72.15, H 11.80, found: C 72.36, H 11.93%.

(25*R*)-3β,26-Bis(*tert*-butyldimethylsilyloxy)-5α-cholest-7-ene (23). Thionyl chloride (33 µL, 450 µmol) was added to a solution of the 7α-alcohol **21** (100 mg, 150 µmol) in pyridine (10 mL) at 0 °C. After stirring for 1 h at 0 °C, water (50 mL) was added and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 20:1) provided **23**, yield: 95 mg (100%). Colorless crystals; mp: 68–78 °C; IR (ATR): ν = 2929, 2890, 2855, 1738, 1471, 1375, 1360, 1248, 1160, 1099, 1007, 939, 869, 836, 773, 668 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.02 (s, 6 H), 0.04 (s, 6 H), 0.51 (s, 3 H), 0.77 (s, 3 H), 0.84 (d, J = 6.7 Hz, 3 H), 0.87 (s, 9 H), 0.88 (s, 9 H), 0.90 (d, J = 6.6 Hz, 3 H), 0.97–1.06 (m, 5 H), 1.17–1.88 (m, 22 H), 2.00 (dt, J = 12.5, 3.4 Hz, 1 H), 3.34 (dd, J = 9.7, 6.7 Hz, 1 H), 3.42 (dd, J = 9.7, 5.9 Hz, 1 H), 3.53 (m, 1 H), 5.14 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –5.34 (2 CH_3), –4.58 (2 CH_3), 11.81 (CH_3), 13.08 (CH_3), 16.65 (CH_3), 18.28 (C), 18.36 (C), 18.80 (CH_3), 21.50 (CH_2), 22.94 (CH_2), 23.41 (CH_2), 25.95 (6 CH_3), 27.95 (CH_2), 29.69 (CH_2), 31.86 (CH_2), 33.54 (CH_2), 34.21 (C), 35.73 (CH), 36.10 (CH, CH_2), 37.31 (CH_2), 38.43 (CH_2), 39.57 (CH_2), 40.38 (CH), 43.37 (C), 49.51 (CH), 55.03 (CH), 56.12 (CH), 68.55 (CH_2), 71.89 (CH), 117.49 (CH), 139.59 (C); GC-MS (70 eV): m/z (%) = 615 (2) [(M – Me)$^+$], 573 (100), 497 (8), 441 (7), 258 (14); Anal. calc. for $C_{39}H_{74}O_2Si_2$: C 74.21, H 11.82, found: C 73.75, H 12.33%.

(25*R*)-3β-Hydroxy-5α-cholest-7-en-26-ol (24). TBAF (1.0 M in THF, 330 µL, 330 µmol) and water (18 µL, 990 µmol) were added to a solution of **23** (94 mg, 150 µmol) in THF (10 mL) and the resulting mixture was heated at reflux for 20 h. After cooling to

room temperature, water (50 mL) was added and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine (50 mL) and dried with magnesium sulfate. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 1:10) provided the diol **24**, yield: 56 mg (93%). Colorless solid; mp: 148–150 °C; IR (ATR): ν = 3289, 2925, 2851, 1734, 1464, 1450, 1373, 1344, 1246, 1160, 1134, 1097, 1040, 984, 940, 847, 829, 797, 729 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.52 (s, 3 H), 0.78 (s, 3 H), 0.90 (d, J = 6.7 Hz, 3 H), 0.91 (d, J = 6.6 Hz, 3 H), 0.99–1.88 (m, 27 H), 2.01 (dt, J = 12.5, 3.3 Hz, 1 H), 3.41 (dd, J = 10.5, 6.5 Hz, 1 H), 3.49 (dd, J = 10.5, 5.9 Hz, 1 H), 3.58 (m, 1 H), 5.15 (dd, J = 4.6, 2.2 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.84 (CH_3), 13.04 (CH_3), 16.49 (CH_3), 18.78 (CH_3), 21.52 (CH_2), 22.93 (CH_2), 23.47 (CH_2), 27.96 (CH_2), 29.62 (CH_2), 31.45 (CH_2), 33.49 (CH_2), 34.18 (C), 35.79 (CH), 36.07 (CH_2), 36.11 (CH), 37.11 (CH_2), 37.96 (CH_2), 39.53 (CH_2), 40.21 (CH), 43.37 (C), 49.40 (CH), 55.01 (CH), 56.09 (CH), 68.53 (CH_2), 71.05 (CH), 117.44 (CH), 139.56 (C); ESI-MS: m/z = 385 [(M − H_2O + H)$^+$]; Anal. calc. for $C_{27}H_{46}O_2$: C 80.54, H 11.51, found: C 80.42, H 11.63%.

(25*R*)-3-Keto-5*α*-cholest-7-en-26-oic acid – (25*R*)-Δ^7-Dafachronic acid (2a). Freshly prepared Jones reagent, consisting of CrO_3 (103 mg, 1.03 mmol in 100 μL water) and concentrated sulfuric acid (90 μL, 1.62 mmol in 400 μL water), was added to a solution of the diol **24** (83 mg, 206 μmol) in acetone (50 mL) at 0 °C. After stirring for 1 h at 0 °C, 2-propanol (10 mL) was added and the resulting mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried with magnesium sulfate and the solvent was removed *in vacuo*. Purification of the residue by flash chromatography on silica gel (petroleum ether/ethyl acetate, 3:1) provided pure (25*R*)-Δ^7-dafachronic acid (**2a**), yield: 63 mg (74%). Colorless solid; mp: 174–175 °C; $[\alpha]_D^{20}$ = +13.3 (c = 0.5, $CHCl_3$); IR (ATR): ν = 3093, 2955, 2932, 2862, 1731, 1690, 1446, 1414, 1378, 1328, 1301, 1263, 1233, 1210, 1210, 1196, 1170, 1117, 1101, 948, 874, 847, 835, 807, 764, 736, 637 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.54 (s, 3 H), 0.91 (d, J = 6.5 Hz, 3 H), 1.00 (s, 3 H), 1.01–1.05 (m, 1 H), 1.17 (d, J = 7.0 Hz, 3 H), 1.18–1.29 (m, 5 H), 1.35–1.65 (m, 10 H), 1.71–1.91 (m, 5 H), 2.03 (dt, J = 12.6, 3.3 Hz, 1 H), 2.11 (ddd, J = 13.3, 5.9, 2.3 Hz, 1 H), 2.20–2.28 (m, 3 H), 2.40 (dd, J = 14.6, 6.0 Hz, 1 H), 2.45 (dd, J = 13.5, 6.8 Hz, 1 H), 5.17 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.87 (CH_3), 12.44 (CH_3), 16.72 (CH_3), 18.74 (CH_3), 21.66 (CH_2), 22.91 (CH_2), 23.68 (CH_2), 27.90 (CH_2), 30.02 (CH_2), 33.85 (CH_2), 34.35 (C), 35.62 (CH_2), 36.01 (CH), 38.09 (CH_2), 38.73 (CH_2), 39.24 (CH), 39.38 (CH_2), 42.82 (CH), 43.33 (C), 44.20 (CH_2), 48.78 (CH), 54.87 (CH), 56.01 (CH), 116.99 (CH), 139.46 (C), 182.51 (C=O), 212.19 (C=O); MS (70 eV): m/z (%) = 414 (100) [M$^+$], 399 (27), 397 (7), 381 (8), 368 (7), 277 (5), 271 (54); HRMS: m/z calc. for $C_{27}H_{42}O_3$ [M$^+$]: 414.3134, found: 414.3142; Anal. calc. for $C_{27}H_{42}O_3$: C 78.21, H 10.21, found: C 78.42, H 10.41%.

(25*R*)-3*β*-(*tert*-Butyldimethylsilyloxy)cholest-5-en-26-al (25). PDC (143 mg, 0.38 mmol) was added to a mixture of the alcohol **14** (100 mg, 0.19 mmol) and 4 Å molecular sieves (95 mg) in dichloromethane (5 mL). The reaction mixture was stirred at room temperature for 3.5 h and then the solvent was evaporated. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 10:1) afforded the aldehyde **25**, yield: 87 mg (89%). Colorless solid; mp: 138–141 °C; IR (ATR): ν = 2930, 2896, 2856, 1727, 1462, 1369, 1251, 1197, 1087, 1006, 960, 925, 888, 869, 835, 804, 772, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.88 (s, 9 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.98 (s, 3 H), 1.08 (d, J = 7.0 Hz, 3 H), 1.10–1.72 (m, 21 H), 1.76–1.83 (m, 2 H), 1.93–2.00 (m, 2 H), 2.15 (ddd, J = 13.3, 5.0, 2.2 Hz, 1 H), 2.23–2.34 (m, 2 H), 3.47 (m, 1 H), 5.30 (m, 1 H), 9.60 (d, J = 2.0 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = −4.60 (2 CH_3), 11.83 (CH_3), 13.27 (CH_3), 18.27 (C), 18.63 (CH_3), 19.41 (CH_3), 21.03 (CH_2), 23.36 (CH_2), 24.25 (CH_2), 25.93 (3 CH_3), 28.23 (CH_2), 30.91 (CH_2), 31.87 (CH), 31.91 (CH_2), 32.06 (CH_2), 35.59 (CH), 35.87 (CH_2), 36.56 (C), 37.36 (CH_2), 39.76 (CH_2), 42.31 (C), 42.79 (CH_2), 46.38 (CH), 50.15 (CH), 55.95 (CH), 56.76 (CH), 72.62 (CH), 121.12 (CH), 141.56 (C), 205.48 (CHO); GC-MS (70 eV): m/z (%) = 514 (0.5) [M$^+$], 499 (3), 457 (100); Anal. calc. for $C_{33}H_{58}O_2Si$: C 76.98, H 11.35, found: C 76.92, H 11.44%.

(25*R*)-3*β*-(*tert*-Butyldimethylsilyloxy)cholest-5-en-26-oic acid (26). A solution of sodium chlorite (38 mg, 0.42 mmol) in water (1 mL) was added to a solution of the aldehyde **25** (144 mg, 0.280 mmol), 2-methyl-2-butene (304 μL, 2.8 mmol) and potassium dihydrogen phosphate (50 mg, 0.37 mmol) in a mixture of THF (6 mL) and water (1 mL). The resulting mixture was stirred at room temperature for 16 h. The solution was acidified with dilute HCl (20 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with a saturated solution of sodium hydrogen carbonate (50 mL) and brine (50 mL), and then dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 4:1 to 2:1) provided the acid **26**, yield: 137 mg (92%). Colorless crystals; mp: 175 °C; IR (ATR): ν = 2929, 2895, 2854, 1706, 1463, 1427, 1371, 1249, 1198, 1082, 1007, 963, 940, 926, 890, 870, 835, 804, 772 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.88 (s, 9 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.91–0.97 (m, 2 H), 0.98 (s, 3 H), 0.99–1.15 (m, 5 H), 1.17 (d, J = 7.0 Hz, 3 H), 1.18–1.84 (m, 16 H), 1.93–2.01 (m, 2 H), 2.15 (ddd, J = 13.3, 4.9, 2.1 Hz, 1 H), 2.23–2.28 (m, 1 H), 2.45 (dd, J = 13.7, 6.9 Hz, 1 H), 3.47 (m, 1 H), 5.30 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = −4.60 (2 CH_3), 11.84 (CH_3), 16.73 (CH_3), 18.27 (C), 18.63 (CH_3), 19.41 (CH_3), 21.04 (CH_2), 23.61 (CH_2), 24.27 (CH_2), 25.93 (3 CH_3), 28.22 (CH_2), 31.88 (CH), 31.91 (CH_2), 32.06 (CH_2), 33.90 (CH_2), 35.62 (CH), 35.72 (CH_2), 36.56 (C), 37.36 (CH_2), 39.19 (CH), 39.76 (CH_2), 42.31 (C), 42.79 (CH_2), 50.16 (CH), 56.05 (CH), 56.75 (CH), 72.64 (CH), 121.14 (CH), 141.55 (C), 182.19 (C=O); MS (70 eV): m/z (%) = 515 (2) [(M − Me)$^+$], 473 (100), 471 (16), 455 (18); HRMS: m/z calc. for $C_{29}H_{49}O_3Si$ [(M − *tert*-Bu)$^+$]: 473.3451, found: 473.3418; Anal. calc. for $C_{33}H_{58}O_3Si$: C 74.66, H 11.01, found: C 74.82, H 11.10%.

(25*R*)-3*β*-Hydroxycholest-5-en-26-oic acid methyl ester (27). One drop of concentrated sulfuric acid was added to a solution of the acid **26** (134 mg, 0.252 mmol) in methanol (10 mL). The reaction mixture was heated at reflux for 16 h, then neutralized with a saturated solution of sodium hydrogen carbonate (30 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with brine (50 mL) and dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum

ether/ethyl acetate, 6:1) provided the ester **27**, yield: 91 mg (84%). Colorless solid; mp: 65 °C; IR (ATR): ν = 3416, 2929, 2864, 2846, 1736, 1461, 1435, 1375, 1363, 1254, 1198, 1163, 1108, 1055, 1022, 985, 955, 926, 882, 840, 799, 740 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.91–1.20 (m, 7 H), 0.99 (s, 3 H), 1.13 (d, J = 7.0 Hz, 3 H), 1.23–1.63 (m, 13 H), 1.76–1.85 (m, 3 H), 1.94 (m, 1 H), 1.98 (dt, J = 12.6, 3.5 Hz, 1 H), 2.23 (m, 1 H), 2.28 (ddd, J = 13.1, 5.0, 2.0 Hz, 1 H), 2.42 (dd, J = 13.9, 6.9 Hz, 1 H), 3.51 (m, 1 H), 3.66 (s, 3 H), 5.34 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.84 (CH_3), 16.95 (CH_3), 18.61 (CH_3), 19.38 (CH_3), 21.05 (CH_2), 23.67 (CH_2), 24.26 (CH_2), 28.20 (CH_2), 31.62 (CH_2), 31.87 (CH), 31.91 (CH_2), 34.17 (CH_2), 35.61 (CH), 35.72 (CH_2), 36.47 (C), 37.22 (CH_2), 39.44 (CH), 39.73 (CH_2), 42.27 (CH_2), 42.30 (C), 50.07 (CH), 51.46 (CH_3), 56.03 (CH), 56.71 (CH), 71.77 (CH), 121.67 (CH), 140.74 (C), 177.45 (C=O); MS (70 eV): m/z (%) = 430 (54) [M$^+$], 415 (26), 412 (42), 397 (24), 345 (22), 319 (51), 271 (100); HRMS: m/z calc. for $C_{28}H_{46}O_3$ [M$^+$]: 430.3447, found: 430.3444.

(25*R*)-3β-Hydroxycholest-5-en-26-oic acid – (25*R*)-Cholestenoic acid (3a). Lithium hydroxide (16 mg, 654 µmol) was added to a solution of the methyl ester **27** (91 mg, 211 µmol) in a mixture of tetrahydrofuran/methanol/water (2:1:1) (4 mL). The reaction mixture was stirred at room temperature for 16 h. Further water (10 mL) was added and the reaction mixture was extracted with dichloromethane (3 × 20 mL) to remove traces of unreacted starting material. The aqueous layer was acidified with dilute HCl (20 mL) and then extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated to afford pure (25*R*)-cholestenoic acid (**3a**), yield: 85 mg (97%). An analytically pure sample was prepared by recrystallization from acetonitrile. Colorless solid; mp: 168–170 °C (MeCN); $[\alpha]_D^{20}$ = –30.0 (c = 0.1, $CHCl_3$); IR (ATR): ν = 3399, 2934, 2899, 2862, 1702, 1460, 1377, 1333, 1178, 1106, 1052, 1022, 987, 955, 911, 881, 840, 804, 735 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.92–1.14 (m, 7 H), 0.99 (s, 3 H), 1.16 (d, J = 6.9 Hz, 3 H), 1.20–1.27 (m, 2 H), 1.36–1.63 (m, 11 H), 1.77–1.84 (m, 3 H), 1.93–2.01 (m, 2 H), 2.22–2.30 (m, 2 H), 2.45 (dd, J = 13.7, 6.9 Hz, 1 H), 3.52 (m, 1 H), 5.34 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.85 (CH_3), 16.74 (CH_3), 18.63 (CH_3), 19.38 (CH_3), 21.05 (CH_2), 23.62 (CH_2), 24.27 (CH_2), 28.21 (CH_2), 31.58 (CH_2), 31.87 (CH, CH_2), 33.91 (CH_2), 35.62 (CH), 35.72 (CH_2), 36.47 (C), 37.22 (CH_2), 39.27 (CH), 39.73 (CH_2), 42.21 (CH_2), 42.30 (C), 50.07 (CH), 56.05 (CH), 56.70 (CH), 71.82 (CH), 121.71 (CH), 140.69 (C), 182.46 (C=O); MS (70 eV): m/z (%) = 416 (100) [M$^+$], 401 (36), 399 (73), 398 (76), 383 (52), 331 (44), 305 (99), 299 (24), 277 (22), 271 (43), 255 (33), 231 (18), 213 (43); HRMS: m/z calc. for $C_{27}H_{44}O_3$ [M$^+$]: 416.3290, found: 416.3297; Anal. calc. for $C_{27}H_{44}O_3$: C 77.83, H 10.64, found: C 77.26, H 10.33%.

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