DOI: 10.1002/ejoc.200900443

Synthesis and Hormonal Activity of the (25*S*)-Cholesten-26-oic Acids – Potent Ligands for the DAF-12 Receptor in *Caenorhabditis elegans*

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Keywords: Diastereoselectivity / Hormones / Oxidation / Protecting groups / Steroids

Using a highly stereoselective Evans aldol reaction for the introduction of the stereogenic center at C-25, we describe an efficient synthesis of the orthogonally diprotected (25*S*)-26-hydroxycholesterol **11**. In a few synthetic steps, this crucial intermediate **11** has been converted into the four (25*S*)-cholesten-26-oic acids **1**–**4**, which have been obtained in 12–15 steps and 19–53 % overall yield based on commercially available 3β-hydroxychol-5-en-24-oic acid (**5**). Our biological studies of the compounds **1**–**4** reveal that (25*S*)-Δ^7-dafachronic acid (**1**) represents the most active steroidal ligand for the hormonal receptor DAF-12 in *Caenorhabditis elegans*. Moreover, the saturated (25*S*)-dafachronic acid (**3**) represents a new ligand for this receptor and the (25*S*)-steroidal acids are more active as compared to their corresponding (25*R*)-counterparts.

Introduction

Reproductive development of nematodes such as *Caenorhabditis elegans* and *Pristionchus pacificus* is controlled by steroidal ligands, called dafachronic acids (Figure 1).[1,2] In *C. elegans*, the biosynthesis of these steroids requires activity of the cytochrome P450 DAF-9.[3] Dafachronic acids are ligands which inactivate the nuclear hormone receptor DAF-12 and thus, lead to reproductive development of worms. In *daf-9* mutant worms, incapable of dafachronic acid biosynthesis, DAF-12 is active and worms enter the diapause state generating dauer larvae. Another ligand known to bind at DAF-12 is (25*S*)-cholestenoic acid (**4**).[4]

Mangelsdorf and colleagues prepared (25*S*)-Δ^4-dafachronic acid (**2**) and its C-25 epimer from the corresponding 26-hydroxycholesterols.[1a] In 2007, the structure of the other ligand, (25*S*)-Δ^7-dafachronic acid (**1**), has been confirmed by a synthesis from Corey and Giroux.[5] Moreover, it has been shown that (25*S*)-Δ^7-dafachronic acid (**1**) represents the most active ligand known so far.[1a,5] Interestingly, the synthesis of both C-25 epimers of **2** and **4** was described previously by Khripach et al.[6] Our investigations on the synthesis and biological activity of cholesterol derivatives,[7] led us to an elegant and concise synthesis of the 25*R*-diastereoisomers of **1**, **2** and **4** starting from commercially available diosgenin.[8,9] Also in the 25*R*-series, the Δ^7-dafachronic acid exhibited the highest hormonal activity.[9] Since yamogenin, the C-25 epimer of diosgenin, was not available from commercial sources, we devised a novel stereoselective construction of the side chain for the synthesis of all three (25*S*)-cholesten-26-oic acids **1**, **2** and **4**, as well as the saturated (25*S*)-dafachronic acid (**3**).[10] The synthesis of (25*S*)-dafachronic acid (**3**) was also reported by Corey and co-workers.[11]

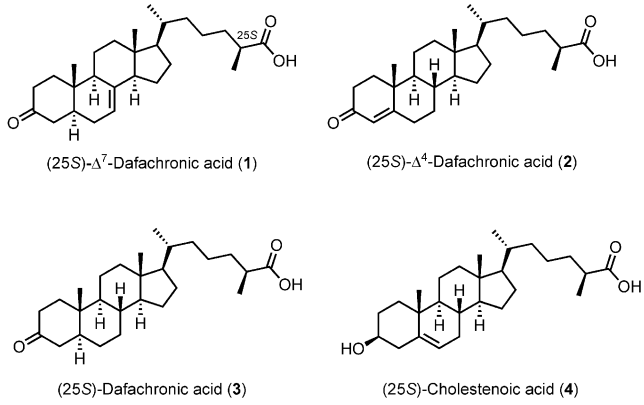

Figure 1. Hormonally active steroidal acids **1**–**4**.

Results and Discussion

The Evans aldol reaction represents a powerful synthetic method for the enantioselective construction of stereogenic carbon centers.[12,13] However, applications to stereoselective synthesis of steroid side chains are rare.[14] For our pur-

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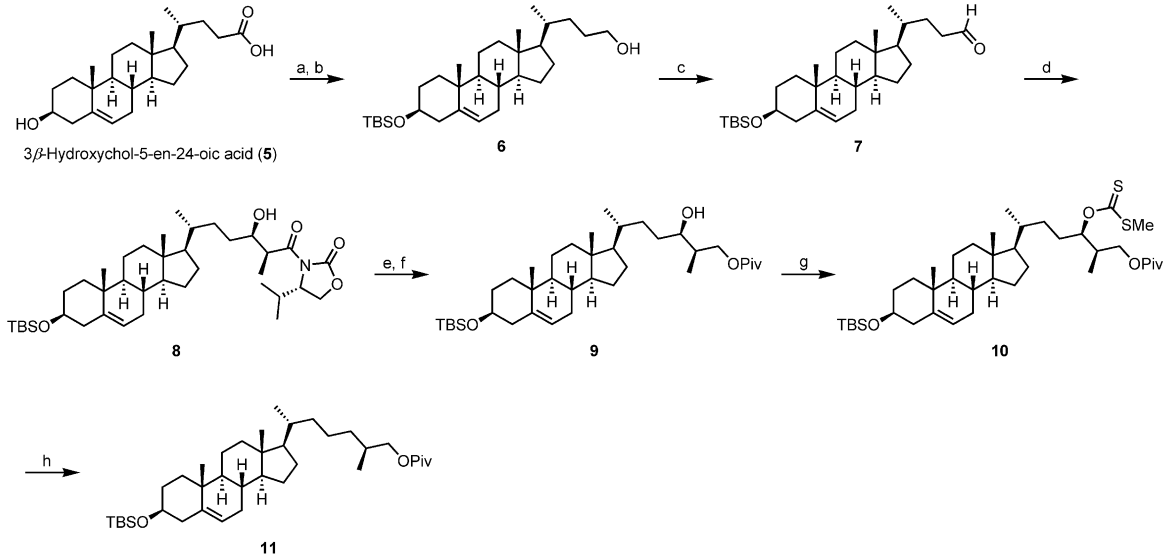
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900443.



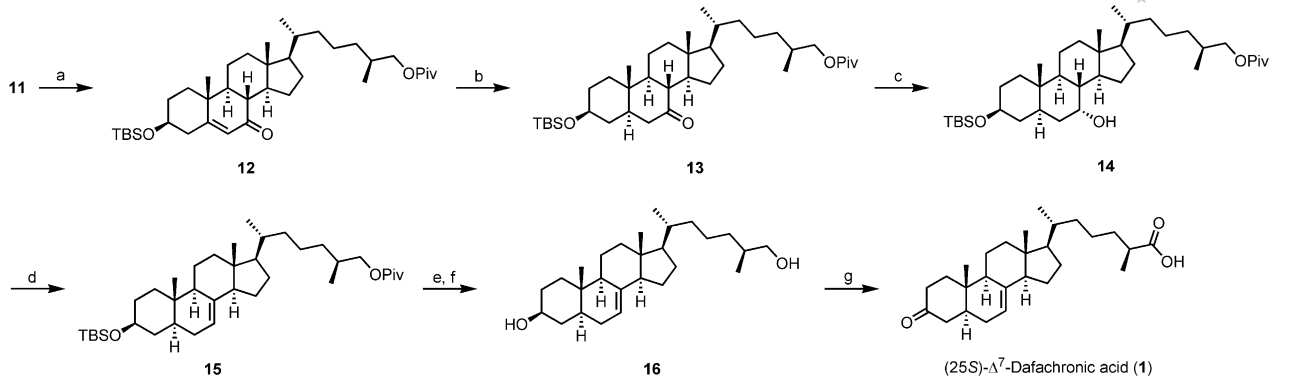
Scheme 1. Stereoselective synthesis of the crucial intermediate **11**. *Reagents and conditions:* (a) 3.2 equiv. TBSCl, 8.1 equiv. imidazole, 2.2 equiv. DMAP, DMF, 25 °C, 18 h; (b) 4.0 equiv. $LiAlH_4$, THF, 25 °C, 17 h, 96% for 2 steps; (c) 4.0 equiv. DMSO, 2.0 equiv. $(COCl)_2$, CH_2Cl_2, –78 °C, then **6**, 20 min, 5.0 equiv. Et_3N, to 25 °C, 98%; (d) 1.3 equiv. (*S*)-(+)-4-isopropyl-3-propionyl-2-oxazolidinone, 1.43 equiv. Bu_2BOTf, 1.69 equiv. Et_3N, CH_2Cl_2, 0 °C, 1 h, then –78 °C, **7**, 30 min, then 0 °C, 80 min, 95%; (e) 1.1 equiv. $LiBH_4$, 1.0 equiv. H_2O, Et_2O, 0 °C, 2 h; (f) 1.3 equiv. PivCl, pyridine/CH_2Cl_2 (1:1), 0 °C, 2 h, 81% for 2 steps; (g) 1.0 equiv. NaHMDS, 20.0 equiv. CS_2, THF, –78 °C to 0 °C, then 2.0 equiv. MeI, 30 min, 98%; (h) 10 mol-% AIBN, 15.0 equiv. Bu_3SnH, reflux, 5 min, 93%.

poses, commercially available 3β-hydroxychol-5-en-24-oic acid (**5**) appeared to be the ideal starting material (Scheme 1).[15] Treatment of **5** with *tert*-butylchlorodimethylsilane (TBSCl) in the presence of imidazole and DMAP led to the intermediate silyl ester which on subsequent reduction using lithium aluminium hydride provided almost quantitatively the 24-hydroxy derivative **6**. Oxidation of the alcohol **6** with PDC afforded the aldehyde **7** in 96% yield.

Moreover, Swern oxidation of **6** provided the aldehyde **7** in 98% yield even on large scale.[16] By using the standard conditions reported by Evans,[12] (1.3 equiv. of commercial (*S*)-(+)-4-isopropyl-3-propionyl-2-oxazolidinone, triethylamine and dibutylboron triflate), the aldol product **8** was available on large scale as a single stereoisomer in 95% yield. Reduction of **8** with lithium borohydride followed by selective pivaloylation of the primary hydroxy group afforded compound **9** in 81% yield over both steps. In the next step, the hydroxy group at C-24 had to be removed. Mesylation of the hydroxy group followed by treatment with lithium aluminium hydride afforded the corresponding C-26 hydroxy compound along with the C-24/C-25 olefin. Both compounds were not separable by flash chromatography on silica gel. The reaction of xanthates with tributylstannane in the presence of AIBN provides deoxygenated products in high yields and thus, represents a promising alternative.[17] For the synthesis of the corresponding xanthate, alcohol **9** was treated with NaHMDS and carbon disulfide at low temperature. Addition of iodomethane provided the xanthate **10** almost quantitatively. After a reaction time of only 5 min, Barton deoxygenation of **10** afforded the deoxygenated compound **11** in 93% yield. In order to achieve good results in this transformation, recrystallisation of commercial AIBN from methanol is recommended. Using the strategy described above, the orthogonally diprotected (25*S*)-26-hydroxycholesterol **11** is readily available in 8 steps and 66% overall yield. Compound **11** represents the central intermediate of our synthesis and has been converted into the four (25*S*)-steroidal acids **1**–**4**.

For an access to (25*S*)-Δ^7-dafachronic acid (**1**), a double bond shift was required. Allylic oxidation following Chandrasekaran's procedure afforded the enone **12** in 75% yield (Scheme 2).[18] Palladium-catalyzed hydrogenation using ethyl acetate as solvent led to the ketone **13**.

The reduction of cyclohexanones using sterically hindered reducing agents under kinetic conditions provides the axial alcohols.[19] Thus, treatment of ketone **13** with L-Selectride® afforded stereoselectively the 7α-alcohol **14** in 90% yield. Using the commercially available Burgess reagent in benzene under reflux, the cholest-7-ene **15** could be isolated in 78% yield (Table 1).[20] In the 25*R*-series, elimination of a structurally related alcohol with thionyl chloride in pyridine provided quantitatively the corresponding Δ^7-olefin.[8,9] Treatment of **14** with 3.0 equiv. of thionyl chloride in pyridine provided the olefin **15** in only 72% yield. However, increasing the amount of thionyl chloride to 5.0 equiv. afforded **15** in 87% yield. Removal of the silyl and pivaloyl protecting groups was achieved by treatment with first TBAF and then lithium aluminium hydride to afford the diol **16** in 82% yield. Changing the sequence of the removal of the protecting groups resulted in only 69% yield of **16**. At this stage of our synthesis, the 25*S*-configuration of the 26-hydroxycholest-7-en-3β-ol (**16**) has been unambiguously confirmed by an X-ray crystal structure determination (Figure 2).[10] Finally, Jones oxidation of the diol **16** provided (25*S*)-Δ^7-dafachronic acid (**1**) in 89% yield.



Scheme 2. Synthesis of (25*S*)-Δ^7-dafachronic acid (**1**) from the intermediate **11**. *Reagents and conditions:* (a) 4.0 equiv. PDC, 8.0 equiv. *t*BuOOH, Celite®, benzene, 0 °C to 25 °C, 41 h, 75%; (b) 10% Pd/C, H_2, EtOAc, 25 °C, 16 h, 95%; (c) 1.3 equiv. L-Selectride®, THF, –78 °C, 1.5 h, 90%; (d) 5.0 equiv. $SOCl_2$, pyridine, 0 °C, 40 min, 87%; (e) 1.5 equiv. TBAF, THF, reflux, 16 h; (f) 4.0 equiv. $LiAlH_4$, THF, 0 °C to 25 °C, 17 h, 82% for 2 steps; (g) 5.0 equiv. Jones reagent, acetone, 0 °C, 90 min, 89%.

Table 1. Elimination of the 7α-hydroxy group from **14**.

Reaction conditions	% Yield of **15**
2.0 equiv. Burgess reagent, benzene, reflux, 2 h	78
3.0 equiv. $SOCl_2$, pyridine, 0 °C, 30 min	72
5.0 equiv. $SOCl_2$, pyridine, 0 °C, 40 min	87

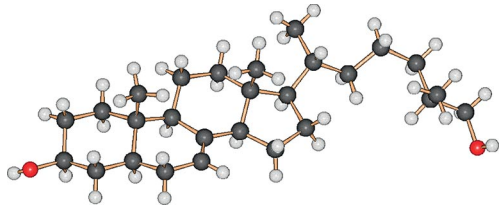


Figure 2. X-ray crystal structure of the diol **16** (orthorhombic, $P2_12_12_1$).

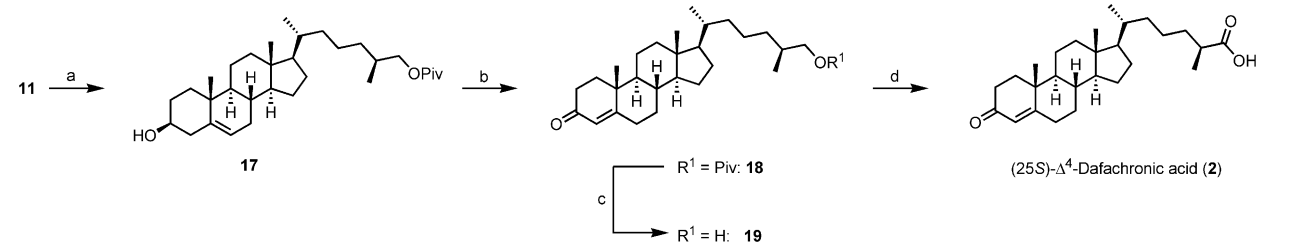
Transformation of intermediate **11** to (25*S*)-Δ^4-dafachronic acid (**2**) required only a few steps (Scheme 3). As we have described previously for the 25*R* series,[8,9] treatment of 26-hydroxycholesterol with Jones reagent leads by concomitant allylic oxidation to the undesired 3,6-diketocholest-4-en-26-oic acid. Therefore, we decided to achieve a sequential oxidation of the two hydroxy groups at C-3 and C-26. Selective removal of the silyl ether with TBAF in THF under reflux afforded the 3β-alcohol **17**. Oppenauer oxidation of the hydroxy group at C-3 occurred with concomitant isomerisation of the double bond and afforded the cholest-4-en-3-one **18** in 86% yield. Cleavage of the pivalate proved to be difficult (Table 2). Reaction of the pivalate **18** with bis[tributyltin(IV)] oxide in benzene under reflux resulted in 100% recovery of starting material **18**.[21] Saponification of **18** with lithium hydroxide afforded only 29% of the desired 26-hydroxy derivative **19**. Using one equivalent of potassium carbonate in methanol at room temperature provided after 6 d the alcohol **19** in 39% yield along with 20% of starting material **18**. The best result was obtained by treatment of **18** with sodium methoxide in methanol at room temperature and afforded the alcohol **19** in 56% yield along with 15% of starting material. Completion of the synthesis was achieved by Jones oxidation of the alcohol **19** to provide (25*S*)-Δ^4-dafachronic acid (**2**) in 65% yield.

Table 2. Removal of the pivaloyl protecting group from **18**.

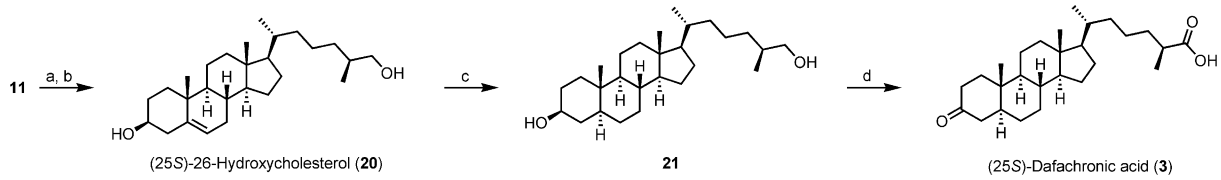
Reaction conditions	% Yield of **19**
3.0 equiv. LiOH, MeOH/H_2O (10:1), 50 °C, 18 h	29
1.0 equiv. K_2CO_3, MeOH, 25 °C, 6 d	39[a]
2.0 equiv. NaOMe, MeOH, 25 °C, 5 d	56[b]

[a] 20% of starting material recovered. [b] 15% of starting material recovered.

For the synthesis of (25*S*)-dafachronic acid (**3**), both protecting groups had to be removed from our crucial intermediate **11** (Scheme 4). Removal of the pivalate using lithium aluminium hydride and subsequent desilylation by treatment with TBAF afforded the known (25*S*)-26-hydroxycho-



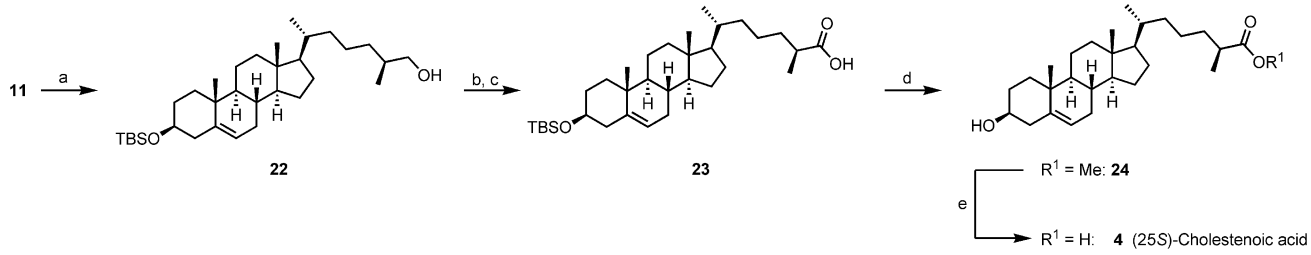
Scheme 3. Synthesis of (25*S*)-Δ^4-dafachronic acid (**2**). *Reagents and conditions:* (a) 1.5 equiv. TBAF, THF, reflux, 17 h, 92%; (b) 1.5 equiv. $Al(OiPr)_3$, acetone/toluene (1:9), 100 °C, 5 h, 86%; (c) 2.0 equiv. NaOMe, MeOH, 25 °C, 5 d, 56% of **19**, 15% of **18**; (d) 5.0 equiv. Jones reagent, 0 °C, 90 min, 65%.



Scheme 4. Synthesis of (25*S*)-dafachronic acid (**3**). *Reagents and conditions:* (a) 1.5 equiv. TBAF, THF, reflux, 17 h; (b) 4.0 equiv. $LiAlH_4$, THF, 25 °C, 16 h, 93% for 2 steps; (c) 10% Pd/C, H_2, MeOH/CH_2Cl_2 (1:1), 25 °C, 24 h, 99%; (d) 5.0 equiv. Jones reagent, acetone, 0 °C, 60 min, 88%.

lesterol (**20**) in only 69% yield.[22] However, as described above for the synthesis of the diol **16**, a reversal of the sequence by first removing the silyl and then the pivaloyl protecting group gave a much better result and provided (25*S*)-26-hydroxycholesterol (**20**) in 93% yield over both steps. Palladium-catalyzed hydrogenation of **20** led to (25*S*)-5α-cholestan-3β,26-diol (**21**). Finally, Jones oxidation of **21** afforded (25*S*)-dafachronic acid (**3**) in 88% yield.[10,11]

For the conversion of compound **11** to (25*S*)-cholestenoic acid (**4**), the pivaloyl protecting group had to be removed selectively. Treatment of **11** with lithium aluminium hydride afforded the alcohol **22** in 91% yield (Scheme 5). Swern oxidation of **22** followed by oxidation of the crude aldehyde with sodium chlorite provided the silyl-protected (25*S*)-cholestenoic acid **23** in 89% yield over two steps. In our studies of the 25*R*-series, we found that chromato-



Scheme 5. Transformation of **11** into (25*S*)-cholestenoic acid (**4**). *Reagents and conditions:* (a) 4.0 equiv. $LiAlH_4$, THF, 25 °C, 17 h, 91%; (b) 4.0 equiv. DMSO, 2.0 equiv. $(COCl)_2$, CH_2Cl_2, –78 °C, then **22**, 20 min, 5.0 equiv. Et_3N, to 25 °C; (c) 2.0 equiv. $NaClO_2$, 10.0 equiv. 2-methyl-2-butene, KH_2PO_4, THF/H_2O (3:1), 25 °C, 24 h, 89% for 2 steps; (d) cat. H_2SO_4, MeOH, reflux, 16 h, 87%; (e) 3.0 equiv. LiOH, THF/MeOH/H_2O (1:1:1), 25 °C, 24 h, 99%.

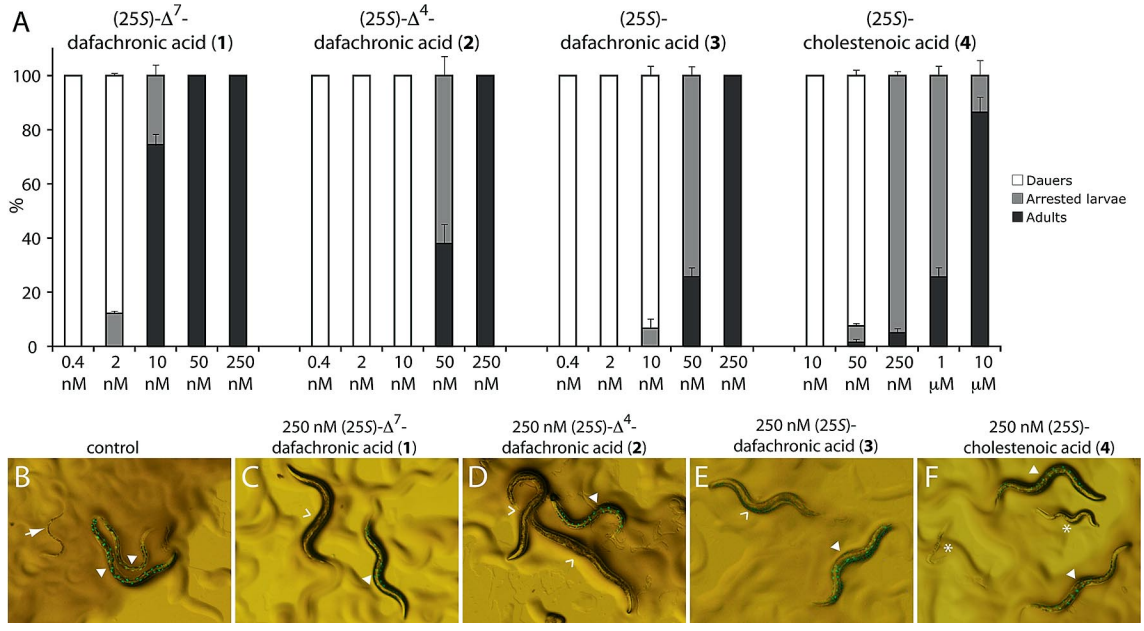


Figure 3. Bioactivity of (25*S*)-dafachronic acids **1**–**3** and (25*S*)-cholestenoic acid (**4**). **A**: rescue of diapause in *daf-9(dh6)* mutant worms by feeding with the indicated (25*S*)-cholesten-26-oic acids. **B**: without addition of (25*S*)-cholesten-26-oic acids, *daf-9(dh6)* mutant worms (no fluorescence) arrest as dauer like larvae (white arrow). **C**–**E**: 250 nM (25*S*)-dafachronic acids **1**–**3** rescue *daf-9(dh6)* mutant worms to adults (Λ). **F**: 250 nM (25*S*)-cholestenoic acid (**4**) rescues only partially *daf-9(dh6)* mutant worms forming arrested larvae, often with molting defects (*). White triangles in all images indicate fluorescent *daf-9(dh6);dhEx24* mutant worms which develop to adults without requirement of exogenous (25*S*)-dafachronic or (25*S*)-cholestenoic acid.

graphic purification of cholestenoic acid is difficult.[8,9] Thus, esterification was carried out with concomitant cleavage of the silyl ether using catalytic amounts of concentrated sulfuric acid in methanol under reflux. The methyl ester **24** was purified by flash chromatography on silica gel and isolated in 87% yield. A subsequent saponification of the ester with lithium hydroxide provided almost quantitatively pure (25*S*)-cholestenoic acid (**4**).

The bioactivity of 25*S*-steroidal acids **1**–**4** was investigated by rescue of *daf-9* mutant worms from dauer arrest (Figure 3). The *daf-9(dh6)* mutant worms lacking DAF-9 protein activity cannot generate dafachronic acids.[1a,3b] In consequence, these mutant worms arrest as dauer-like larvae (Figure 3, A, B). In the experimental setup, some *daf-9(dh6)* mutant worms are obtained as progeny from the strain *daf-9(dh6);dhEx24*.[3b] The *daf-9(dh6)* mutant worms are identified by the absence of green fluorescence, which is carried by the extrachromosomal array *dhEx24*. In the parental strain *daf-9(dh6);dhEx24*, the extrachromosomal array *dhEx24* rescues the *daf-9(dh6)* mutation (Figure 3, B).[3b] Normal reproductive development of *daf-9(dh6)* mutant worms is only possible by exogenous supply of dafachronic acid (Figure 3, A–E).

If the supplied amount of dafachronic acid is not sufficient for complete rescue, arrested larvae, often with molting defects, are observed (Figure 3 A, F). Our experiments show that the rescue of *daf-9(dh6)* mutant worms from dauer arrest is dependent on the concentration of steroidal acids. The rescue of *daf-9(dh6)* mutant worms from dauer arrest by feeding with the 25*S*-steroidal acids **1**–**4** demonstrates the difference in activity for these ligands (Figure 3, A). Our results emphasize that (25*S*)-Δ^7-dafachronic acid (**1**) is the most active ligand. Moreover, it became obvious that (25*S*)-Δ^4-dafachronic acid (**2**) and (25*S*)-dafachronic acid (**3**) have a moderate activity, which is about one order of magnitude lower than the activity of **1**. The least active compound in this series is (25*S*)-cholestenoic acid (**4**) with an activity about one order of magnitude lower than found for the ligands **2** and **3**. We have compared the activities of the (25*S*)-steroidal acids **1**–**4** with those of the (25*R*)-dafachronic acids, which have been reported in our previous publication.[9] As a result, the activity of (25*R*)-Δ^7-dafachronic acid is in the same range as observed for (25*S*)-Δ^4-dafachronic acid (**2**) and (25*S*)-dafachronic acid (**3**). While (25*R*)-Δ^4-dafachronic acid has an activity comparable to that of (25*S*)-cholestenoic acid (**4**).

Conclusions

We have developed a highly efficient synthetic route to all four of the (25*S*)-cholesten-26-oic acids **1**–**4** by using a completely stereoselective Evans aldol reaction as key-step. Thus, compound **8** was obtained as a single stereoisomer even on a multigram scale and our crucial intermediate **11** became available in 8 steps and 66% overall yield. Intermediate **11** has been exploited to prepare all four (25*S*)-steroidal acids: (25*S*)-Δ^7-dafachronic acid (**1**) (15 steps, 27% overall yield), (25*S*)-Δ^4-dafachronic acid (**2**) (12 steps, 19% overall yield), (25*S*)-dafachronic acid (**3**) (12 steps, 53% overall yield) and (25*S*)-cholestenoic acid (**4**) (13 steps, 46% overall yield). Our present synthesis of the (25*S*)-cholesten-26-oic acids **1**–**4** is clearly superior with respect to overall yields and efficiency as compared to the previous approaches to these compounds.[5,6,11] The present methodology for the assembly of the side chain can be applied to the synthesis of other (25*S*)-steroids.

Our efficient access to the (25*S*)-cholesten-26-oic acids **1**–**4** also set the stage for detailed biological studies towards their hormonal activity in controlling the life cycle of *C. elegans*. Our study of the biological activity of (25*S*)-dafachronic acids emphasizes the importance of the 3-hydroxy group and suggests that worms can not efficiently oxidise the 3-hydroxy group of (25*S*)-cholestenoic acid (**4**). The fact that (25*S*)-Δ^4-dafachronic acid (**2**) and (25*S*)-dafachronic acid (**3**) show a similar activity indicates that either the double bond at C-4 is not important for the activity or, that the worms very efficiently convert **3** into **2**. The latter reaction might be carried out by a steroid dehydrogenase.[23] The comparison of the biological activities of the (25*S*)-steroidal acids described in the present publication with those of the (25*R*)-dafachronic acids reported in our previous paper[9] emphasizes that the (25*S*)-steroidal acids are more active than their corresponding (25*R*)-diastereoisomers. Moreover, it is shown that (25*S*)-Δ^7-dafachronic acid (**1**) has the highest biological activity among all these steroidal acids. The bioactivity of (25*R*)-Δ^7-dafachronic acid (**1**) is in the range of the bioactivity observed for (25*S*)-Δ^4-dafachronic acid (**2**) and (25*S*)-dafachronic acid (**3**). Whereas (25*R*)-Δ^4-dafachronic acid is almost as active as (25*S*)-cholestenoic acid (**4**). Thus, by our biological studies the functional groups of the steroids and their stereochemistry required for efficacious signaling can be concluded.

Experimental Section

General: All reactions were carried out in dry solvents and oven-dried glassware under argon atmosphere. Tetrahydrofuran, ethyl acetate, dichloromethane, and diethyl ether were dried in a solvent purification system (MBraun-SPS). Acetone was distilled from phosphorus pentoxide and stored over molecular sieves (3 Å). Benzene was dried with sodium. Toluene was purchased from Acros Organics (water content less than 50 ppm). Dry methanol was purchased from VWR Prolabo (water content less than 20 ppm). Pyridine was obtained from Fluka (water content less than 50 ppm). Triethylamine was heated under reflux with calcium hydride for 48 h and stored over 3 Å molecular sieves. Commercial AIBN was recrystallised from methanol. Dibutylboron triflate was obtained from Acros Organics as a 1 M solution in dichloromethane. 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 melting point apparatus. Specific rotation values were obtained from a Perkin–Elmer 341 polarimeter. Infra-

red spectra were recorded on a Thermo Nicolet Avatar 360 FT-IR spectrometer using the ATR method (Attenuated Total Reflectance). NMR spectra were recorded on a Bruker DRX 500 NMR spectrometer. Complete assignment of the ^1H and ^{13}C signals was achieved by HSQC experiments. Chemical shifts δ 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, sext: sextet, sept: septet, m: multiplet, br: broad. Mass spectra were recorded on a Finnigan MAT-95 spectrometer (electron impact, 70 eV) or by GC/MS-coupling using an Agilent Technologies 6890N 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 ions were detected. Elemental analyses were measured on an EuroVector EuroEA3000 elemental analyser. X-ray analyses: Bruker–Nonius Kappa CCD equipped with a 700 series Cryostream low temperature device from 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).

3β-(*tert*-Butyldimethylsilyloxy)chol-5-en-24-ol (6): *tert*-Butylchlorodimethylsilane (3.78 g, 25.09 mmol), imidazole (4.32 g, 63.50 mmol) and DMAP (2.2 g, 17.25 mmol) were added to a solution of 3β-hydroxychol-5-en-24-oic acid (**5**) (2.936 g, 7.84 mmol) in DMF (50 mL). Additional DMF (50 mL) was added and the solution was stirred at room temperature for 18 h. After addition of water (250 mL), the resulting mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (2 × 100 mL), brine (100 mL) and then dried with magnesium sulfate. Evaporation of the solvent gave the crude product which was dissolved in THF (50 mL). Lithium aluminium hydride (1.19 g, 31.36 mmol) was added in portions to this solution at 0 °C and the resulting mixture was stirred at room temperature for 17 h. Water (50 mL) was slowly 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 removed. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 4:1) provided the alcohol **6**, yield 3.561 g (96%). Colourless solid; m.p. 171–173 °C (ref.[15] 146.5–152 °C). ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.67 (s, 3 H), 0.88 (s, 9 H), 0.93 (d, J = 6.6 Hz, 3 H), 0.95–1.17 (m, 6 H), 0.98 (s, 3 H), 1.21–1.27 (m, 2 H), 1.39–1.72 (m, 11 H), 1.79 (dt, J = 13.3, 3.5 Hz, 1 H), 1.82–1.84 (m, 1 H), 1.93–2.01 (m, 2 H), 2.15 (ddd, J = 13.3, 4.9, 2.2 Hz, 1 H), 2.25 (m, 1 H), 3.47 (m, 1 H), 3.60 (m, 2 H), 5.30 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.84 (CH_3), 18.27 (C), 18.66 (CH_3), 19.42 (CH_3), 21.03 (CH_2), 24.25 (CH_2), 25.93 (3 CH_3), 28.22 (CH_2), 29.37 (CH_2), 31.82 (CH_2), 31.87 (CH), 31.90 (CH_2), 32.06 (CH_2), 35.55 (CH), 36.56 (C), 37.36 (CH_2), 39.76 (CH_2), 42.31 (C), 42.79 (CH_2), 50.15 (CH), 55.95 (CH), 56.76 (CH), 63.60 (CH_2), 72.63 (CH), 121.13 (CH), 141.54 (C) ppm. $C_{30}H_{54}O_2Si$ (474.83): C 75.88, H 11.46, found: C 75.94, H 11.34%. For further spectroscopic data see ref.[15]

3β-(*tert*-Butyldimethylsilyloxy)chol-5-en-24-al (7): Oxalyl chloride (396 μL, 4.68 mmol) was added slowly to a solution of DMSO (665 μL, 9.36 mmol) in dichloromethane (10 mL) at –78 °C. After 5 min, a solution of the alcohol **6** (1.109 g, 2.34 mmol) in dichloromethane (20 mL) was added and stirring was continued at –78 °C for 20 min. Then, triethylamine (1.63 mL, 11.70 mmol) was added, the solution was warmed to room temperature and stirring was continued for additional 10 min. The reaction mixture was quenched by addition of a saturated aqueous solution of ammonium chloride (20 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (2 × 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, 10:1) afforded the aldehyde **7**, yield 1.081 g (98%). Colourless solid; m.p. 137–139 °C (ref.[15] 132–137 °C). ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.87 (s, 9 H), 0.91 (d, J = 6.5 Hz, 3 H), 0.95–1.18 (m, 6 H), 0.98 (s, 3 H), 1.24–1.34 (m, 3 H), 1.40–1.60 (m, 6 H), 1.68–1.86 (m, 4 H), 1.93–2.00 (m, 2 H), 2.15 (ddd, J = 13.3, 4.9, 2.2 Hz, 1 H), 2.25 (m, 1 H), 2.35 (m, 1 H), 2.44 (m, 1 H), 3.47 (m, 1 H), 5.30 (m, 1 H), 9.76 (t, J = 1.9 Hz, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.85 (CH_3), 18.26 (C), 18.40 (CH_3), 19.41 (CH_3), 21.02 (CH_2), 24.23 (CH_2), 25.93 (3 CH_3), 27.94 (CH_2), 28.17 (CH_2), 31.87 (CH, CH_2), 32.05 (CH_2), 35.32 (CH), 36.55 (C), 37.35 (CH_2), 39.72 (CH_2), 40.92 (CH_2), 42.37 (C), 42.78 (CH_2), 50.11 (CH), 55.76 (CH), 56.73 (CH), 72.60 (CH), 121.09 (CH), 141.54 (C), 203.25 (CHO) ppm. $C_{30}H_{52}O_2Si$ (472.82): C 76.21, H 11.09, found: C 76.27, H 11.14%. For further spectroscopic data see ref.[15].

(4*S*,24′*R*,25′*S*)-3-[3′β-(*tert*-Butyldimethylsilyloxy)-24′-hydroxycholest-5′-en-26′-oyl]-4-isopropyloxazolidin-2-one (8): A 1.0 M solution of dibutylboron triflate (3.28 mL, 3.28 mmol) was added to a solution of (*S*)-(+)-4-isopropyl-3-propionyl-2-oxazolidinone (505 μL, 2.98 mmol) in dichloromethane (10 mL) at 0 °C. After 5 min, triethylamine (539 μL, 3.87 mmol) was added dropwise and the resulting mixture was stirred at 0 °C for 1 h. The reaction mixture was cooled to –78 °C and a solution of the aldehyde **7** (1.081 g, 2.29 mmol) in dichloromethane (10 mL) was added dropwise. Stirring was continued at –78 °C for 30 min. Then, the mixture was warmed to 0 °C and stirring was continued for additional 80 min. Methanol (10 mL) and 30% aqueous H_2O_2 (10 mL) were added and the resulting mixture was stirred at 0 °C for 30 min. Water (100 mL) was added and the layers were separated. The aqueous layer was extracted with dichloromethane (2 × 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, 4:1 to 2:1) afforded the aldol product **8** as a single stereoisomer, yield 1.428 g (95%). Colourless solid; m.p. 172–174 °C. IR (ATR): $\tilde{\nu}$ = 3533, 2928, 2858, 1773, 1701, 1680, 1458, 1386, 1367, 1302, 1236, 1207, 1143, 1078, 1057, 1017, 959, 886, 870, 835, 807, 774, 719 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.87 (d, J = 6.5 Hz, 3 H), 0.87 (s, 9 H), 0.89–1.28 (m, 9 H), 0.91 (d, J = 7.1 Hz, 6 H), 0.98 (s, 3 H), 1.24 (d, J = 7.1 Hz, 3 H), 1.38–1.60 (m, 9 H), 1.70 (m, 1 H), 1.79 (dt, J = 13.3, 3.3 Hz, 1 H), 1.82–1.85 (m, 1 H), 1.93–2.00 (m, 2 H), 2.15 (ddd, J = 13.3, 4.8, 2.1 Hz, 1 H), 2.25 (m, 1 H), 2.33 (dsept, J = 4.0, 7.0 Hz, 1 H), 2.91 (br. d, J = 2.1 Hz, 1 H), 3.46 (m, 1 H), 3.76 (dq, J = 2.6, 7.1 Hz, 1 H), 3.87 (m, 1 H), 4.21 (dd, J = 8.6, 3.3 Hz, 1 H), 4.27 (t, J = 8.6 Hz, 1 H), 4.46 (ddd, J = 8.6, 4.0, 3.3 Hz, 1 H), 5.30 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.61 (2 CH_3), 10.78 (CH_3), 11.84 (CH_3), 14.66 (CH_3), 17.90 (CH_3), 18.25 (C), 18.66 (CH_3), 19.41 (CH_3), 21.02 (CH_2), 24.25 (CH_2), 25.92 (3 CH_3), 28.18 (CH_2), 28.30 (CH), 30.07 (CH_2), 31.87 (CH), 31.89 (CH_2), 32.03 (CH_2), 32.06 (CH_2), 35.45 (CH), 36.55 (C), 37.34 (CH_2), 39.73 (CH_2), 42.08 (CH), 42.30 (C), 42.79 (CH_2), 50.13 (CH), 55.79 (CH), 56.73 (CH), 58.18 (CH), 63.29 (CH_2), 71.54 (CH), 72.61 (CH), 121.14 (CH), 141.52 (C), 153.50 (C=O), 177.93 (C=O) ppm. ESI-MS: m/z = 658.5 [$(M + H)^+$], 640.4, 508.4. $C_{39}H_{67}NO_5Si$ (658.04): C 71.18, H 10.26, N 2.13, found: C 71.43, H 10.34, N 2.25%.

(24*R*,25*R*)-3β-(*tert*-Butyldimethylsilyloxy)-26-(pivaloyloxy)cholest-5-en-24-ol (9): A 2.0 M solution of lithium borohydride in THF (908 μL, 1.815 mmol) and water (30 μL, 1.65 mmol) were added to a solution of the aldol product **8** (1.085 g, 1.65 mmol) in diethyl ether (25 mL) at 0 °C. After stirring for 2 h at 0 °C, water (20 mL) and 2 N NaOH (20 mL) were slowly added to the reaction mixture, which was subsequently extracted with dichloromethane (2 × 50 mL), diethyl ether (2 × 50 mL) and ethyl acetate (2 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. The crude diol was dissolved in pyridine (15 mL) and dichloromethane (15 mL). After addition of pivaloyl chloride (264 μL, 2.145 mmol) at 0 °C, the resulting mixture was stirred at 0 °C for 2 h and then water (50 mL) was added. The mixture was extracted with dichloromethane (3 × 50 mL) and the combined organic layers were dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 6:1) provided the pivalate **9**, yield 818 mg (81%). Colourless solid; m.p. 133–136 °C. IR (ATR): $\tilde{\nu}$ = 3525, 2958, 2926, 2898, 2851, 1710, 1471, 1459, 1382, 1287, 1247, 1174, 1131, 1076, 1029, 1010, 975, 940, 892, 873, 834, 805, 777 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.88 (s, 9 H), 0.90–1.33 (m, 8 H), 0.911 (d, J = 7.0 Hz, 3 H), 0.915 (d, J = 6.4 Hz, 3 H), 0.98 (s, 3 H), 1.20 (s, 9 H), 1.37–1.58 (m, 10 H), 1.69–1.74 (m, 1 H), 1.79 (dt, J = 13.3, 3.3 Hz, 1 H), 1.82–1.87 (m, 2 H), 1.94 (m, 1 H), 1.99 (dt, J = 12.4, 3.3 Hz, 1 H), 2.15 (ddd, J = 13.3, 4.9, 2.2 Hz, 1 H), 2.25 (m, 1 H), 3.47 (m, 1 H), 3.53 (m, 1 H), 3.92 (dd, J = 11.0, 5.8 Hz, 1 H), 4.19 (dd, J = 11.0, 7.6 Hz, 1 H), 5.30 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 10.27 (CH_3), 11.84 (CH_3), 18.26 (C), 18.67 (CH_3), 19.41 (CH_3), 21.03 (CH_2), 24.25 (CH_2), 25.93 (3 CH_3), 27.21 (3 CH_3), 28.26 (CH_2), 30.60 (CH_2), 31.87 (CH), 31.90 (CH_2), 32.06 (CH_2), 32.28 (CH_2), 35.50 (CH), 36.56 (C), 37.36 (CH_2), 37.95 (CH), 38.83 (C), 39.75 (CH_2), 42.31 (C), 42.79 (CH_2), 50.14 (CH), 55.83 (CH), 56.75 (CH), 66.83 (CH_2), 71.88 (CH), 72.62 (CH), 121.13 (CH), 141.54 (C), 178.89 (C=O) ppm. GC-MS (70 eV): m/z (%) = 559 (29) [(M – tBu)$^+$], 383 (18), 365 (16), 341 (27), 331 (11), 255 (15), 211 (20), 175 (11), 171 (11), 161 (33), 159 (100). $C_{38}H_{68}O_4Si$ (617.03): C 73.97, H 11.11, found: C 74.04, H 11.19%.

***O*-(24*R*,25*R*)-3β-(*tert*-Butyldimethylsilyloxy)-26-(pivaloyloxy)cholest-5-en-24-yl *S*-Methyl Xanthate (10):** A 1.0 M solution of NaHMDS in THF (136 μL, 136 μmol) and carbon disulfide (164 μL, 2.72 mmol) were added to a solution of the alcohol **9** (84 mg, 136 μmol) in THF at –78 °C. After stirring for 30 min at –78 °C, the solution was warmed to 0 °C and iodomethane (17 μL, 272 μmol) was added. Stirring was continued for additional 30 min, water (50 mL) was added and the solution was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was removed to provide the xanthate **10**, yield 94 mg (98%). Yellow oil (which crystallised on standing). ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.65 (s, 3 H), 0.85–0.93 (m, 1 H), 0.88 (s, 9 H), 0.91 (d, J = 6.5 Hz, 3 H), 0.95–1.26 (m, 7 H), 0.98 (s, 3 H), 1.02 (d, J = 7.0 Hz, 3 H), 1.20 (s, 9 H), 1.36–1.57 (m, 8 H), 1.70 (m, 1 H), 1.77–1.83 (m, 3 H), 1.93–1.98 (m, 2 H), 2.12–2.27 (m, 4 H), 2.54 (s, 3 H), 3.47 (m, 1 H), 3.97 (d, J = 6.5 Hz, 2 H), 5.30 (m, 1 H), 5.78 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.85 (2 CH_3), 18.27 (C), 18.57 (CH_3), 18.87 (CH_3), 19.41 (CH_3), 21.03 (CH_2), 24.23 (CH_2), 25.93 (3 CH_3), 27.05 (CH_2), 27.20 (3 CH_3), 28.02 (CH_2), 31.31 (CH_2), 31.88 (CH, CH_2), 32.06 (CH_2), 35.17 (CH), 36.08 (CH), 36.56 (C), 37.36 (CH_2), 38.78 (C), 39.74 (CH_2), 42.31 (C), 42.79 (CH_2), 50.14 (CH), 55.49 (CH), 56.73 (CH), 65.56 (CH_2), 72.61 (CH), 84.56 (CH), 121.12 (CH), 141.55 (C), 178.41 (C=O), 215.94 (C=S) ppm.

(25*S*)-3β-(*tert*-Butyldimethylsilyloxy)-26-(pivaloyloxy)cholest-5-ene (11): AIBN (3.5 mg, 21.1 μmol) was added to a solution of the xanthate **10** (149 mg, 211 μmol) in toluene (5 mL). The flask containing this mixture was put in an oil bath, which was already heated at 110 °C. A solution of tributylstannane (851 μL, 3.165 mmol) in toluene (2 mL) was slowly added to the hot reaction mixture. After 5 min, the yellow solution became colourless, indicating complete conversion. After cooling to room temperature, a saturated aqueous solution of sodium hydrogen carbonate (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, 100:1 to 70:1) afforded compound **11**, yield 118 mg (93%). Colourless solid; m.p. 116–118 °C. IR (ATR): $\tilde{\nu}$ = 2929, 2895, 2856, 1728, 1471, 1397, 1368, 1282, 1252, 1152, 1090, 1033, 989, 959, 939, 890, 870, 835, 804, 771, 666 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.86–1.28 (m, 10 H), 0.88 (s, 9 H), 0.90 (d, J = 6.6 Hz, 3 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.98 (s, 3 H), 1.19 (s, 9 H), 1.30–1.59 (m, 10 H), 1.69–1.83 (m, 4 H), 1.94 (m, 1 H), 1.99 (dt, J = 13.0, 3.3 Hz, 1 H), 2.15 (ddd, J = 13.3, 4.9, 2.1 Hz, 1 H), 2.26 (m, 1 H), 3.47 (m, 1 H), 3.82 (dd, J = 10.7, 6.7 Hz, 1 H), 3.93 (dd, J = 10.7, 5.6 Hz, 1 H), 5.30 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.82 (CH_3), 17.07 (CH_3), 18.27 (C), 18.70 (CH_3), 19.42 (CH_3), 21.04 (CH_2), 23.20 (CH_2), 24.27 (CH_2), 25.93 (3 CH_3), 27.22 (3 CH_3), 28.22 (CH_2), 31.88 (CH), 31.92 (CH_2), 32.07 (CH_2), 32.70 (CH), 33.86 (CH_2), 35.67 (CH), 36.14 (CH_2), 36.57 (C), 37.36 (CH_2), 38.84 (C), 39.77 (CH_2), 42.30 (C), 42.80 (CH_2), 50.17 (CH), 56.01 (CH), 56.77 (CH), 69.11 (CH_2), 72.63 (CH), 121.14 (CH), 141.56 (C), 178.64 (C=O) ppm. MS (70 eV): m/z (%) = 600 (1) [M$^+$], 585 (3), 543 (73) [(M – tBu)$^+$], 441 (12), 367 (100), 295 (11), 255 (14), 161 (20), 159 (79). HRMS: m/z calcd. for $C_{34}H_{59}O_3Si$ [(M – tBu)$^+$]: 543.4233, found: 543.4246.

(25*S*)-3β-(*tert*-Butyldimethylsilyloxy)-26-(pivaloyloxy)cholest-5-en-7-one (12): A 5.5 M solution of *tert*-butyl hydroperoxide in decane (629 μL, 3.46 mmol), PDC (1.30 g, 3.46 mmol) and Celite® (1.03 g) were added to a solution of compound **11** (520 mg, 0.865 mmol) in benzene (30 mL) at 0 °C. The resulting mixture was stirred at room temperature for 24 h. A second portion of *tert*-butyl hydroperoxide (629 μL, 3.46 mmol) was added and stirring was continued for 17 h. The reaction mixture was filtered through a short pad of silica gel with diethyl ether (500 mL) and the solvent was removed. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 20:1) provided the enone **12**, yield 398 mg (75%). Colourless crystals; m.p. 153–155 °C. IR (ATR): $\tilde{\nu}$ = 2934, 2858, 1730, 1666, 1626, 1461, 1375, 1282, 1253, 1153, 1091, 1033, 955, 937, 892, 877, 835, 804, 771 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.05 (s, 6 H), 0.66 (s, 3 H), 0.88 (s, 9 H), 0.90 (d, J = 6.4 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H), 1.00–1.66 (m, 17 H), 1.17 (s, 3 H), 1.19 (s, 9 H), 1.74–1.82 (m, 2 H), 1.85–1.91 (m, 2 H), 2.01 (dt, J = 12.7, 3.3 Hz, 1 H), 2.22 (dd, J = 12.3, 10.9 Hz, 1 H), 2.33–2.43 (m, 3 H), 3.59 (m, 1 H), 3.83 (dd, J = 10.7, 6.7 Hz, 1 H), 3.93 (dd, J = 10.7, 5.6 Hz, 1 H), 5.65 (d, J = 1.4 Hz, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.70 (CH_3), –4.67 (CH_3), 11.93 (CH_3), 17.07 (CH_3), 17.28 (CH_3), 18.15 (C), 18.85 (CH_3), 21.16 (CH_2), 23.26 (CH_2), 25.82 (3 CH_3), 26.28 (CH_2), 27.22 (3 CH_3), 28.54 (CH_2), 31.71 (CH_2), 32.70 (CH), 33.83 (CH_2), 35.61 (CH), 36.15 (CH_2), 36.39 (CH_2), 38.34 (C), 38.68 (CH_2), 38.85 (C), 42.51 (CH_2), 43.05 (C), 45.38 (CH), 49.90 (CH), 49.95 (CH), 54.66 (CH), 69.10 (CH_2), 71.31 (CH), 125.78 (CH), 165.89 (C), 178.65

(C=O), 202.47 (C=O) ppm. GC-MS (70 eV): *m/z* (%) = 614 (2) [M$^+$], 557 (41), 381 (100), 269 (25). $C_{38}H_{66}O_4Si$ (615.01): C 74.21, H 10.82, found: C 74.24, H 10.92%.

(25*S*)-3β-(*tert*-Butyldimethylsilyloxy)-26-(pivaloyloxy)cholest-5α-an-7-one (13): A solution of the enone **12** (100 mg, 163 μmol) in ethyl acetate (10 mL) was added to a Schlenk flask, loaded with Pd/C (10%, 17.3 mg, 16.3 μmol Pd). The resulting mixture was stirred at room temperature for 16 h under an hydrogen atmosphere. Filtration of the mixture over a short pad of Celite® with ethyl acetate, evaporation of the solvent and purification of the crude product by flash chromatography on silica gel (petroleum ether/diethyl ether, 10:1) afforded the ketone **13**, yield 95 mg (95%). Colourless solid; m.p. 116–117 °C. IR (ATR): $\tilde{\nu}$ = 2929, 2857, 1726, 1704, 1471, 1375, 1284, 1250, 1162, 1099, 1054, 1007, 986, 943, 873, 835, 797, 772 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.025 (s, 3 H), 0.026 (s, 3 H), 0.63 (s, 3 H), 0.86 (s, 9 H), 0.89 (d, J = 6.7 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H), 0.93–1.56 (m, 20 H), 1.06 (s, 3 H), 1.19 (s, 9 H), 1.69–1.78 (m, 3 H), 1.85–1.89 (m, 1 H), 1.94–2.00 (m, 2 H), 2.16 (m, 1 H), 2.33 (m, 2 H), 3.53 (m, 1 H), 3.82 (dd, J = 10.7, 6.7 Hz, 1 H), 3.92 (dd, J = 10.7, 5.6 Hz, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.66 (2 CH_3), 11.85 (CH_3), 12.02 (CH_3), 17.05 (CH_3), 18.20 (C), 18.75 (CH_3), 21.83 (CH_2), 23.23 (CH_2), 24.92 (CH_2), 25.87 (3 CH_3), 27.21 (3 CH_3), 28.40 (CH_2), 31.52 (CH_2), 32.71 (CH), 33.83 (CH_2), 35.55 (CH), 36.01 (C), 36.12 (CH_2), 36.21 (CH_2), 38.44 (CH_2), 38.74 (CH_2), 38.84 (C), 42.47 (C), 46.20 (CH_2), 47.13 (CH), 48.83 (CH), 49.99 (CH), 54.92 (CH), 55.44 (CH), 69.10 (CH_2), 71.50 (CH), 178.64 (C=O), 212.44 (C=O) ppm. GC-MS (70 eV): *m/z* (%) = 601 (3) [(M – Me)$^+$], 561 (8), 559 (59), 457 (28), 383 (29), 365 (25), 271 (31), 253 (11), 177 (11), 161 (35), 159 (89), 75 (100), 57 (73). $C_{38}H_{68}O_4Si$ (617.03): C 73.97, H 11.11, found: C 74.06, H 11.22%.

(25*S*)-3β-(*tert*-Butyldimethylsilyloxy)-26-(pivaloyloxy)cholest-5α-an-7α-ol (14): A 1.0 M solution of L-Selectride® in THF (754 μL, 754 μmol) was added to a solution of the ketone **13** (358 mg, 580 μmol) in THF (20 mL) at –78 °C. Stirring was continued at the same temperature for 1.5 h. The reaction mixture was quenched by addition of a saturated aqueous solution of sodium hydrogen carbonate (10 mL), methanol (5 mL) and 30% aqueous H_2O_2 (5 mL), and was warmed to 0 °C. After stirring for 30 min, water (50 mL) and diethyl ether (50 mL) were added and the layers were separated. The aqueous layer was extracted with diethyl ether (2 × 50 mL) and the combined organic layers were dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 10:1) provided the 7α-alcohol **14** as a single stereoisomer, yield 324 mg (90%). Colourless solid; m.p. 90–92 °C. IR (ATR): $\tilde{\nu}$ = 3501, 2929, 2855, 1728, 1708, 1471, 1398, 1380, 1285, 1250, 1162, 1101, 1075, 1032, 1006, 975, 947, 871, 835, 814, 772 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.03 (s, 6 H), 0.63 (s, 3 H), 0.78 (s, 3 H), 0.86 (s, 9 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.91 (d, J = 6.8 Hz, 3 H), 0.98–1.05 (m, 2 H), 1.06–1.16 (m, 6 H), 1.19 (s, 9 H), 1.21–1.68 (m, 17 H), 1.56 (dt, J = 12.6, 3.1 Hz, 1 H), 1.74–1.86 (m, 2 H), 1.92 (dt, J = 12.6, 3.2 Hz, 1 H), 3.57 (m, 1 H), 3.81 (m, 1 H), 3.82 (dd, J = 10.7, 6.7 Hz, 1 H), 3.93 (dd, J = 10.7, 5.6 Hz, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (CH_3), –4.58 (CH_3), 11.26 (CH_3), 11.80 (CH_3), 17.05 (CH_3), 18.25 (C), 18.63 (CH_3), 20.95 (CH_2), 23.17 (CH_2), 23.63 (CH_2), 25.94 (3 CH_3), 27.21 (3 CH_3), 28.19 (CH_2), 31.85 (CH_2), 32.71 (CH), 33.85 (CH_2), 35.57 (C), 35.66 (CH), 36.10 (CH_2), 36.29 (CH_2), 36.90 (CH_2), 37.20 (CH), 38.17 (CH_2), 38.84 (C), 39.50 (CH_2), 39.53 (CH), 42.65 (C), 45.91 (CH), 50.57 (CH), 55.99 (CH), 68.11 (CH), 69.11 (CH_2), 71.96 (CH), 178.64 (C=O) ppm. GC-MS (70 eV): *m/z* (%) = 603 (2) [(M – Me)$^+$], 561 (13), 485 (18), 451 (12), 383 (13), 378 (15), 367 (17), 345 (14), 343 (10), 331 (76), 281 (24), 273 (16), 269 (32), 259 (14), 257 (22), 255 (13), 213 (14), 211 (23), 75 (100). $C_{38}H_{70}O_4Si$ (619.05): C 73.73, H 11.40, found: C 73.84, H 11.48%.

(25*S*)-3β-(*tert*-Butyldimethylsilyloxy)-26-(pivaloyloxy)-5α-cholest-7-ene (15): Thionyl chloride (41 μL, 565 μmol) was added to a solution of the 7α-alcohol **14** (70 mg, 113 μmol) in pyridine (5 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 40 min and the solvent was evaporated. Purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 50:1) afforded the cholest-7-ene **15**, yield 59 mg (87%). Colourless solid; m.p. 79–80 °C. IR (ATR): $\tilde{\nu}$ = 2930, 2854, 1728, 1471, 1398, 1377, 1282, 1252, 1161, 1102, 1084, 1006, 982, 940, 871, 835, 815, 795, 772 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.51 (s, 3 H), 0.77 (s, 3 H), 0.87 (s, 9 H), 0.90 (d, J = 6.4 Hz, 3 H), 0.92 (d, J = 6.7 Hz, 3 H), 0.99–1.80 (m, 26 H), 1.19 (s, 9 H), 1.83–1.87 (m, 1 H), 2.00 (dt, J = 12.3, 3.2 Hz, 1 H), 3.53 (m, 1 H), 3.83 (dd, J = 10.7, 6.8 Hz, 1 H), 3.93 (dd, J = 10.7, 5.6 Hz, 1 H), 5.14 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.58 (2 CH_3), 11.80 (CH_3), 13.07 (CH_3), 17.06 (CH_3), 18.28 (C), 18.83 (CH_3), 21.49 (CH_2), 22.93 (CH_2), 23.28 (CH_2), 25.94 (3 CH_3), 27.21 (3 CH_3), 27.94 (CH_2), 29.69 (CH_2), 31.86 (CH_2), 32.69 (CH), 33.84 (CH_2), 34.21 (C), 36.08 (CH, CH_2), 37.31 (CH_2), 38.43 (CH_2), 38.84 (C), 39.56 (CH_2), 40.37 (CH), 43.37 (C), 49.50 (CH), 55.02 (CH), 56.02 (CH), 69.11 (CH_2), 71.89 (CH), 117.53 (CH), 139.53 (C), 178.65 (C=O) ppm. GC-MS (70 eV): *m/z* (%) = 600 (6) [M$^+$], 585 (7), 545 (9), 543 (74), 468 (21), 467 (20), 441 (22), 367 (15), 255 (10), 161 (19), 159 (69), 75 (100). $C_{38}H_{68}O_3Si$ (601.03): C 75.94, H 11.40, found: C 75.82, H 11.40%.

(25*S*)-26-Hydroxy-5α-cholest-7-en-3β-ol (16): A 1.0 M solution of tetrabutylammonium fluoride in THF (0.53 mL, 530 μmol) was added to a solution of the cholest-7-ene **15** (214 mg, 356 μmol) in THF (15 mL). The mixture was heated under reflux for 16 h. After cooling to room temperature, water was added (50 mL) and the resulting mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. The crude product was dissolved in THF (10 mL) and lithium aluminium hydride (54 mg, 1.424 mmol) was added at 0 °C. The resulting mixture was stirred at room temperature for 17 h. Then, 10% sulfuric acid (10 mL) and water (50 mL) were added. The mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/ethyl acetate, 3:1) provided the diol **16**, which has been recrystallised from ethyl acetate, yield 118 mg (82%). Colourless crystals; m.p. 165–167 °C. IR (ATR): $\tilde{\nu}$ = 3303, 2914, 2865, 1464, 1447, 1381, 1366, 1347, 1100, 1052, 1032, 1019, 976, 941, 848, 832, 729 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.52 (s, 3 H), 0.78 (s, 3 H), 0.91 (d, J = 6.7 Hz, 6 H), 0.98–1.30 (m, 9 H), 1.33–1.64 (m, 12 H), 1.69–1.90 (m, 6 H), 2.01 (dt, J = 12.7, 3.4 Hz, 1 H), 3.41 (dd, J = 10.5, 6.5 Hz, 1 H), 3.50 (dd, J = 10.5, 5.7 Hz, 1 H), 3.58 (m, 1 H), 5.14 (dd, J = 4.7, 2.2 Hz, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.84 (CH_3), 13.04 (CH_3), 16.72 (CH_3), 18.85 (CH_3), 21.52 (CH_2), 22.93 (CH_2), 23.51 (CH_2), 27.95 (CH_2), 29.62 (CH_2), 31.45 (CH_2), 33.63 (CH_2), 34.18 (C), 35.81 (CH), 36.17 (CH, CH_2), 37.11 (CH_2), 37.95 (CH_2), 39.53 (CH_2), 40.21 (CH), 43.36 (C), 49.40 (CH), 55.01 (CH), 56.07 (CH), 68.34 (CH_2), 71.04 (CH), 117.44 (CH), 139.56 (C) ppm. MS (70 eV): *m/z* (%) = 402 (100) [M$^+$], 387 (30), 273 (15), 255 (46), 231 (16), 229 (11), 213 (15), 161 (11). HRMS: *m/z* calcd. for $C_{27}H_{46}O_2$ [M$^+$]: 402.3498, found: 402.3490.

Crystallographic Data for Compound 16: $C_{27}H_{46}O_2$, M = 402.64 g mol^{-1}, crystal size: 0.27 × 0.12 × 0.10 mm^3, orthorhombic, space

group $P2_12_12_1$, a = 34.899(7), b = 9.3865(19), c = 7.5000(15) Å, V = 2456.8(9) Å^3, Z = 4, $\rho_{calcd.}$ = 1.089 g cm^{-3}, μ = 0.066 mm^{-1}, λ = 0.71073 Å, T = 223(2) K, θ range = 3.19–25.40°, reflections collected: 35264, independent: 2611 (R_{int} = 0.0748), data/restraints/parameters: 1947/0/266. 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 = 0.0468; wR_2 = 0.1065; maximal residual electron density: 0.242 e Å^{-3}. CCDC-697683 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(25*S*)-Δ^7-Dafachronic Acid [(25*S*)-3-Keto-5α-cholest-7-en-26-oic Acid] (1): A freshly prepared solution of Jones reagent (CrO_3: 156 mg, 1.565 mmol; concd. H_2SO_4: 137 μL, 2.46 mmol) in water (1 mL) was added to a solution of the diol **16** (126 mg, 313 μmol) in acetone (12 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 90 min, then 2-propanol (5 mL) and water (50 mL) were added. The resulting dark green mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 3:1 + 1% acetic acid) to provide (25*S*)-Δ^7-dafachronic acid (**1**), yield 116 mg (89%). Colourless solid; m.p. 139–143 °C (ref.[5] 143 °C). $[\alpha]_D^{20}$ = +33.9 (c = 0.49, $CHCl_3$). IR (ATR): $\tilde{\nu}$ = 2939, 2872, 2804, 1724, 1705, 1439, 1420, 1383, 1254, 1233, 1208, 1184, 1159, 1143, 1122, 1013, 974, 938, 844, 832, 793, 749, 729 cm^{-1}. ^{1}H 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), 1.02–1.06 (m, 1 H), 1.17 (d, J = 7.0 Hz, 3 H), 1.19–1.89 (m, 20 H), 2.03 (dt, J = 12.6, 3.4 Hz, 1 H), 2.12 (ddd, J = 13.4, 6.0, 2.5 Hz, 1 H), 2.20–2.28 (m, 3 H), 2.40 (dd, J = 14.6, 6.0 Hz, 1 H), 2.45 (m, 1 H), 5.17 (d, J = 2.3 Hz, 1 H), 11.12 (br. s, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.89 (CH_3), 12.45 (CH_3), 17.01 (CH_3), 18.74 (CH_3), 21.68 (CH_2), 22.92 (CH_2), 23.79 (CH_2), 27.92 (CH_2), 30.04 (CH_2), 34.01 (CH_2), 34.38 (C), 35.68 (CH_2), 36.04 (CH), 38.11 (CH_2), 38.75 (CH_2), 39.36 (CH), 39.40 (CH_2), 42.83 (CH), 43.35 (C), 44.22 (CH_2), 48.81 (CH), 54.90 (CH), 56.02 (CH), 117.00 (CH), 139.48 (C), 182.55 (C=O), 212.17 (C=O) ppm. ESI-MS: m/z = 415.3 [(M + H)$^+$]. $C_{27}H_{42}O_3$ (414.62): C 78.21, H 10.21, found: C 78.21, H 10.31%.

(25*S*)-26-(Pivaloyloxy)cholest-5-en-3β-ol (17): A 1.0 M solution of tetrabutylammonium fluoride in THF (1.25 mL, 1.25 mmol) was added to a solution of the silyl ether **11** (500 mg, 0.832 mmol) in THF (20 mL). The mixture was heated under reflux for 17 h. After cooling to room temperature, 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/ethyl acetate, 6:1) provided the alcohol **17**, yield 373 mg (92%). Colourless solid; m.p. 101–103 °C. IR (ATR): $\tilde{\nu}$ = 3416, 2962, 2934, 2903, 2865, 1729, 1479, 1461, 1397, 1376, 1365, 1283, 1160, 1057, 1023, 984, 957, 926, 841, 799, 770, 742 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.90 (d, J = 7.6 Hz, 3 H), 0.91 (d, J = 6.9 Hz, 3 H), 0.93–1.30 (m, 10 H), 0.99 (s, 3 H), 1.19 (s, 9 H), 1.31–1.59 (m, 10 H), 1.75–1.85 (m, 4 H), 1.94–2.00 (m, 2 H), 2.20–2.30 (m, 2 H), 3.51 (m, 1 H), 3.82 (dd, J = 10.7, 6.7 Hz, 1 H), 3.93 (dd, J = 10.7, 5.6 Hz, 1 H), 5.34 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.83 (CH_3) 17.07 (CH_3), 18.69 (CH_3), 19.38 (CH_3), 21.05 (CH_2), 23.21 (CH_2), 24.26 (CH_2), 27.21 (3 CH_3), 28.22 (CH_2), 31.63 (CH_2), 31.87 (CH, CH_2), 32.69 (CH), 33.85 (CH_2), 35.67 (CH), 36.14 (CH_2), 36.48 (C), 37.22 (CH_2), 38.84 (C), 39.73 (CH_2), 42.27 (CH_2), 42.29 (C), 50.08 (CH), 56.01 (CH), 56.72 (CH), 69.11 (CH_2), 71.77 (CH), 121.67 (CH), 140.75 (C), 178.66 (C=O) ppm. ESI-MS: m/z = 509.4 [(M + Na)$^+$]. $C_{32}H_{54}O_3$ (486.77): C 78.96, H 11.18, found: C 79.02, H 10.95%.

(25*S*)-26-(Pivaloyloxy)cholest-4-en-3-one (18): Aluminium isopropoxide (152 mg, 746 μmol) was added to a solution of the alcohol **17** (242 mg, 497 μmol) in acetone (1 mL) and toluene (9 mL). The resulting mixture was stirred at 100 °C for 5 h. After cooling to room temperature, water (50 mL) and diethyl ether (50 mL) were added, and the layers were separated. The aqueous layer was extracted with diethyl ether (2 × 50 mL) and the combined organic layers were 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) provided the enone **18**, yield 207 mg (86%). Pale yellow solid; m.p. 63–64 °C. IR (ATR): $\tilde{\nu}$ = 2933, 2868, 2851, 1727, 1671, 1616, 1478, 1466, 1433, 1397, 1377, 1332, 1282, 1227, 1157, 1031, 977, 959, 933, 860, 770, 684 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3 H), 0.85–0.92 (m, 1 H), 0.89 (d, J = 6.6 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.93–1.62 (m, 17 H), 1.16 (s, 3 H), 1.19 (s, 9 H), 1.68 (dt, J = 4.8, 14.0 Hz, 1 H), 1.74–1.84 (m, 3 H), 1.98–2.03 (m, 2 H), 2.25 (ddd, J = 14.5, 4.0, 2.3 Hz, 1 H), 2.32 (dt, J = 16.7, 3.8 Hz, 1 H), 2.36–2.44 (m, 2 H), 3.82 (dd, J = 10.7, 6.8 Hz, 1 H), 3.93 (dd, J = 10.7, 5.6 Hz, 1 H), 5.71 (s, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.92 (CH_3), 17.06 (CH_3), 17.35 (CH_3), 18.61 (CH_3), 20.98 (CH_2), 23.21 (CH_2), 24.14 (CH_2), 27.21 (3 CH_3), 28.15 (CH_2), 32.00 (CH_2), 32.69 (CH), 32.92 (CH_2), 33.83 (CH_2), 33.96 (CH_2), 35.57 (CH), 35.64 (CH, CH_2), 36.07 (CH_2), 38.57 (C), 38.83 (C), 39.57 (CH_2), 42.36 (C), 53.76 (CH), 55.82 (CH), 55.96 (CH), 69.08 (CH_2), 123.72 (CH), 171.72 (C), 178.64 (C=O), 199.69 (C=O) ppm. GC-MS (70 eV): m/z (%) = 484 (88) [M$^+$], 469 (10), 442 (17), 382 (10), 361 (23), 271 (25), 245 (12), 244 (13), 229 (45), 124 (100). $C_{32}H_{52}O_3$ (484.75): C 79.29, H 10.81, found: C 79.23, H 10.79%.

(25*S*)-26-Hydroxycholest-4-en-3-one (19): Sodium methoxide (43 mg, 792 μmol) was added to a solution of compound **18** (192 mg, 396 μmol) in methanol (3 mL). The mixture was stirred at room temperature for 5 d, then 2 N HCl (20 mL) and ethyl acetate (50 mL) were added. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 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/ethyl acetate, 5:1 to 2:1) afforded recovered pivalate **18**, yield 29 mg (15%) and the more polar alcohol **19**, yield 89 mg (56%). Colourless solid; m.p. 126–127 °C. IR (ATR): $\tilde{\nu}$ = 3414, 2932, 2866, 2850, 1659, 1612, 1462, 1446, 1376, 1332, 1273, 1231, 1190, 1043, 956, 933, 864, 686 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3 H), 0.86–0.96 (m, 1 H), 0.896 (d, J = 6.5 Hz, 3 H), 0.903 (d, J = 6.7 Hz, 3 H), 0.97–1.14 (m, 8 H), 1.16 (s, 3 H), 1.22–1.29 (m, 1 H), 1.32–1.63 (m, 9 H), 1.67 (dt, J = 4.8, 14.0 Hz, 1 H), 1.78–1.86 (m, 2 H), 1.98–2.06 (m, 2 H), 2.25 (ddd, J = 14.6, 4.1, 2.4 Hz, 1 H), 2.32 (dt, J = 17.0, 3.8 Hz, 1 H), 2.34–2.44 (m, 2 H), 3.40 (dd, J = 10.5, 6.5 Hz, 1 H), 3.49 (dd, J = 10.5, 5.7 Hz, 1 H), 5.71 (s, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.92 (CH_3), 16.70 (CH_3), 17.34 (CH_3), 18.63 (CH_3), 20.98 (CH_2), 23.41 (CH_2), 24.13 (CH_2), 28.16 (CH_2), 32.00 (CH_2), 32.92 (CH_2), 33.61 (CH_2), 33.95 (CH_2), 35.56 (CH), 35.64 (CH_2), 35.71 (CH), 35.79 (CH), 36.15 (CH_2), 38.57 (C), 39.58 (CH_2), 42.35 (C), 53.76 (CH), 55.82 (CH), 56.01 (CH), 68.29 (CH_2), 123.70 (CH), 171.78 (C), 199.74 (C=O) ppm. MS (70 eV): m/z (%) = 400 (100) [M$^+$], 385 (12), 366 (12), 358 (31), 277 (25), 276 (11), 271 (13), 229 (59), 124 (91). HRMS: m/z calcd. for $C_{27}H_{44}O_2$ [M$^+$]: 400.3341, found: 400.3329.

(25*S*)-Δ^4-Dafachronic Acid [(25*S*)-3-Ketocholest-4-en-26-oic Acid] (2): A freshly prepared solution of Jones reagent (CrO_3: 111 mg,

1.11 mmol; concd. H_2SO_4: 97 μL, 1.743 mmol) in water (0.5 mL) was added to a solution of the alcohol **19** (89 mg, 222 μmol) in acetone (12 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 90 min, then 2-propanol (5 mL) and water (50 mL) were added. The resulting dark green mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 2:1) to provide (25*S*)-Δ^4-dafachronic acid (**2**), yield 60 mg (65%). Colourless solid; m.p. 173–174 °C (ref.[6] 172–175 °C). $[\alpha]_D^{20}$ = +61.9 (*c* = 0.47, $CHCl_3$). IR (ATR): $\tilde{\nu}$ = 2929, 2848, 1725, 1649, 1610, 1467, 1450, 1413, 1375, 1362, 1333, 1306, 1282, 1237, 1199, 1168, 1117, 1030, 947, 932, 901, 871, 843, 781 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3 H), 0.82–0.93 (m, 1 H), 0.89 (d, *J* = 6.5 Hz, 3 H), 0.96–1.29 (m, 8 H), 1.16 (s, 3 H), 1.17 (d, *J* = 6.9 Hz, 3 H), 1.31–1.69 (m, 9 H), 1.68 (dt, *J* = 4.7, 13.9 Hz, 1 H), 1.78–1.86 (m, 2 H), 1.98–2.03 (m, 2 H), 2.25 (ddd, *J* = 14.6, 4.0, 2.4 Hz, 1 H), 2.32 (dt, *J* = 17.3, 4.0 Hz, 1 H), 2.36–2.44 (m, 2 H), 2.45 (sext, *J* = 6.9 Hz, 1 H), 5.71 (s, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.93 (CH_3), 17.01 (CH_3), 17.35 (CH_3), 18.54 (CH_3), 20.99 (CH_2), 23.69 (CH_2), 24.14 (CH_2), 28.15 (CH_2), 32.00 (CH_2), 32.93 (CH_2), 33.94 (CH_2), 34.00 (CH_2), 35.58 (2 CH), 35.64 (CH_2), 35.68 (CH_2), 38.58 (C), 39.34 (CH), 39.58 (CH_2), 42.37 (C), 53.75 (CH), 55.82 (CH), 55.98 (CH), 123.71 (CH), 171.86 (C), 182.35 (C=O), 199.84 (C=O) ppm. ESI-MS *m*/*z* = 415.3 [(M + H)$^+$]. $C_{27}H_{42}O_3$ (414.62): C 78.21, H 10.21, found: C 78.21, H 10.12%.

(25*S*)-26-Hydroxycholesterol [(25*S*)-Cholest-5-en-3β,26-diol] (20): A 1.0 M solution of tetrabutylammonium fluoride in THF (249 μL, 249 μmol) was added to a solution of compound **11** (100 mg, 166 μmol) in THF (10 mL). The mixture was heated under reflux for 17 h. After cooling to room temperature, water (50 mL) was added and the resulting mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. The crude product was dissolved in THF (15 mL) and lithium aluminium hydride (25 mg, 664 μmol) was added. The mixture was stirred at room temperature for 16 h, then water (10 mL) and 10% HCl were added (10 mL). The mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried with magnesium sulfate. Evaporation of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/ethyl acetate, 3:1) provided the diol **20**, yield 62 mg (93%). Colourless solid; m.p. 173–175 °C (ref.[22] 171–174 °C). IR (ATR): $\tilde{\nu}$ = 3318, 2931, 2864, 1464, 1376, 1231, 1193, 1132, 1107, 1053, 1039, 1022, 987, 954, 926, 839, 799, 736 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.88–1.17 (m, 9 H), 0.90 (d, *J* = 6.8 Hz, 6 H), 0.99 (s, 3 H), 1.20–1.27 (m, 1 H), 1.33–1.63 (m, 11 H), 1.77–1.85 (m, 3 H), 1.93–2.01 (m, 2 H), 2.23 (m, 1 H), 2.28 (ddd, *J* = 13.0, 5.0, 1.9 Hz, 1 H), 3.40 (dd, *J* = 10.5, 6.5 Hz, 1 H), 3.48–3.54 (m, 1 H), 3.50 (dd, *J* = 10.5, 5.7 Hz, 1 H), 5.33 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.84 (CH_3), 16.70 (CH_3), 18.71 (CH_3), 19.38 (CH_3), 21.04 (CH_2), 23.42 (CH_2), 24.26 (CH_2), 28.22 (CH_2), 31.61 (CH_2), 31.86 (CH), 31.87 (CH_2), 33.63 (CH_2), 35.75 (CH), 35.80 (CH), 36.22 (CH_2), 36.47 (C), 37.21 (CH_2), 39.73 (CH_2), 42.26 (CH_2), 42.28 (C), 50.07 (CH), 56.06 (CH), 56.72 (CH), 68.32 (CH_2), 71.76 (CH), 121.68 (CH), 140.73 (C) ppm. MS (70 eV): *m*/*z* (%) = 402 (100) [M$^+$], 387 (38), 384 (70), 369 (43), 317 (47), 291 (76), 273 (24), 255 (32), 231 (20), 213 (42). HRMS: *m*/*z* calcd. for $C_{27}H_{46}O_2$ [M$^+$]: 402.3498, found: 402.3494. $C_{27}H_{46}O_2$ (402.65): C 80.54, H 11.51, found: C 80.71, H 11.59%.

(25*S*)-5α-Cholestan-3β,26-diol (21): A solution of the diol **20** (127 mg, 315 μmol) in dichloromethane (5 mL) was added to a mixture of Pd/C (10%, 33.5 mg, 31.5 μmol Pd) in methanol (5 mL). The reaction mixture was stirred under an hydrogen atmosphere at room temperature for 24 h and then filtered with ethyl acetate over a short pad of Celite®. The solvent was evaporated to provide the pure saturated diol **21**, yield 126 mg (99%). Colourless solid; m.p. 169–171 °C. IR (ATR): $\tilde{\nu}$ = 3240, 2969, 2932, 2848, 1463, 1449, 1385, 1369, 1358, 1332, 1320, 1256, 1236, 1171, 1127, 1080, 1049, 1030, 1005, 987, 956, 917, 797, 736, 719 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.58–0.61 (m, 1 H), 0.63 (s, 3 H), 0.79 (s, 3 H), 0.80–0.93 (m, 1 H), 0.89 (d, *J* = 6.6 Hz, 3 H), 0.90 (d, *J* = 6.7 Hz, 3 H), 0.94–1.15 (m, 9 H), 1.17–1.49 (m, 12 H), 1.51–1.66 (m, 4 H), 1.69 (dt, *J* = 13.2, 3.6 Hz, 1 H), 1.75–1.81 (m, 2 H), 1.94 (dt, *J* = 12.6, 3.4 Hz, 1 H), 3.40 (dd, *J* = 10.5, 6.5 Hz, 1 H), 3.50 (dd, *J* = 10.5, 5.7 Hz, 1 H), 3.57 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.06 (CH_3), 12.31 (CH_3), 16.71 (CH_3), 18.67 (CH_3), 21.23 (CH_2), 23.43 (CH_2), 24.19 (CH_2), 28.25 (CH_2), 28.71 (CH_2), 31.50 (CH_2), 32.07 (CH_2), 33.64 (CH_2), 35.44 (C), 35.48 (CH), 35.77 (CH), 35.81 (CH), 36.22 (CH_2), 36.98 (CH_2), 38.19 (CH_2), 40.01 (CH_2), 42.57 (C), 44.83 (CH), 54.32 (CH), 56.21 (CH), 56.46 (CH), 68.35 (CH_2), 71.37 (CH) ppm. MS (70 eV): *m*/*z* (%) = 404 (100) [M$^+$], 389 (22), 388 (14), 386 (16), 371 (25), 278 (11), 248 (22), 234 (40), 233 (90), 217 (52), 215 (78). HRMS: *m*/*z* calcd. for $C_{27}H_{48}O_2$ [M$^+$]: 404.3654, found: 404.3652. $C_{27}H_{48}O_2$ (404.67): C 80.14, H 11.96, found: C 79.53, H 11.66%.

(25*S*)-Dafachronic Acid [(25*S*)-3-Keto-5α-cholestan-26-oic Acid] (3): A freshly prepared solution of Jones reagent (CrO_3: 124 mg, 1.24 mmol; concd. H_2SO_4: 111 μL, 1.99 mmol) in water (0.7 mL) was added to a solution of the diol **21** (100 mg, 247 μmol) in acetone (12 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 60 min, then 2-propanol (5 mL) and water (50 mL) were added. The resulting dark green mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 3:1 + 1% acetic acid) to provide (25*S*)-dafachronic acid (**3**), yield 91 mg (88%). Light yellow solid; m.p. 123–126 °C. $[\alpha]_D^{20}$ = +44.9 (*c* = 1.0, $CHCl_3$). IR (ATR): $\tilde{\nu}$ = 2931, 2863, 1702, 1443, 1416, 1379, 1292, 1232, 1173, 1030, 955, 804, 732 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.68–0.73 (m, 1 H), 0.84–0.92 (m, 1 H), 0.88 (d, *J* = 6.5 Hz, 3 H), 0.97–1.41 (m, 16 H), 0.99 (s, 3 H), 1.17 (d, *J* = 7.0 Hz, 3 H), 1.47–1.58 (m, 3 H), 1.63–1.70 (m, 2 H), 1.76–1.83 (m, 1 H), 1.95–2.02 (m, 2 H), 2.07 (ddd, *J* = 14.7, 3.8, 2.2 Hz, 1 H), 2.24 (d, *J* = 14.7 Hz, 1 H), 2.26–2.30 (m, 1 H), 2.35 (dd, *J* = 13.9, 6.5 Hz, 1 H), 2.45 (sext, *J* = 7.0 Hz, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.45 (CH_3), 12.05 (CH_3), 16.99 (CH_3), 18.56 (CH_3), 21.42 (CH_2), 23.71 (CH_2), 24.19 (CH_2), 28.21 (CH_2), 28.94 (CH_2), 31.68 (CH_2), 34.00 (CH_2), 35.36 (CH), 35.61 (C, CH), 35.71 (CH_2), 38.18 (CH_2), 38.53 (CH_2), 39.37 (CH), 39.86 (CH_2), 42.57 (C), 44.71 (CH_2), 46.67 (CH), 53.74 (CH), 56.15 (CH), 56.23 (CH), 182.70 (C=O), 212.41 (C=O) ppm. MS (70 eV): *m*/*z* (%) = 416 (33) [M$^+$], 402 (19), 398 (14), 387 (13), 370 (18), 246 (23), 233 (13), 232 (42), 231 (100), 218 (19), 217 (53). HRMS: *m*/*z* calcd. for $C_{27}H_{44}O_3$ [M$^+$]: 416.3290, found: 416.3291.

(25*S*)-3β-(*tert*-Butyldimethylsilyloxy)cholest-5-en-26-ol (22): Lithium aluminium hydride (63 mg, 1.664 mmol) was slowly added to a solution of the pivalate **11** (250 mg, 0.416 mmol) in THF (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 17 h, then water (10 mL) and 10% sulfuric acid were added. The mixture was extracted with diethyl ether (3 × 100 mL) and the combined organic layers were dried with magnesium sulfate. Removal of the solvent and purification of the residue by flash chromatog-

raphy on silica gel (petroleum ether/diethyl ether, 5:1) provided the alcohol **22**, yield 195 mg (91%). Colourless solid; m.p. 165–166 °C. IR (ATR): $\tilde{\nu}$ = 3302, 2929, 2857, 1462, 1381, 1367, 1250, 1196, 1093, 1024, 1006, 989, 958, 886, 869, 835, 803, 775, 732, 669 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.04 (s, 6 H), 0.66 (s, 3 H), 0.87–0.94 (m, 1 H), 0.88 (s, 9 H), 0.90 (d, J = 6.6 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H), 0.95–1.17 (m, 8 H), 0.98 (s, 3 H), 1.24 (m, 1 H), 1.33–1.62 (m, 11 H), 1.68–1.72 (m, 1 H), 1.77–1.85 (m, 1 H), 1.79 (dt, J = 13.4, 3.6 Hz, 1 H), 1.92–2.01 (m, 2 H), 2.15 (ddd, J = 13.3, 4.9, 2.2 Hz, 1 H), 2.26 (m, 1 H), 3.41 (dd, J = 10.5, 6.6 Hz, 1 H), 3.47 (m, 1 H), 3.50 (dd, J = 10.5, 5.7 Hz, 1 H), 5.30 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.84 (CH_3), 16.72 (CH_3), 18.27 (C), 18.72 (CH_3), 19.42 (CH_3), 21.04 (CH_2), 23.41 (CH_2), 24.27 (CH_2), 25.93 (3 CH_3), 28.24 (CH_2), 31.88 (CH), 31.92 (CH_2), 32.06 (CH_2), 33.65 (CH_2), 35.76 (CH), 35.82 (CH), 36.24 (CH_2), 36.56 (C), 37.36 (CH_2), 39.78 (CH_2), 42.30 (C), 42.79 (CH_2), 50.17 (CH), 56.06 (CH), 56.77 (CH), 68.35 (CH_2), 72.63 (CH), 121.15 (CH), 141.55 (C) ppm. GC-MS (70 eV): m/z (%) = 459 (100) [(M – tBu)$^+$], 389 (17), 377 (54), 343 (10), 331 (19), 281 (21), 273 (12), 269 (14). $C_{33}H_{60}O_2Si$ (516.91): C 76.68, H 11.70, found: C 76.82, H 11.88%.

(25*S*)-3β-(*tert*-Butyldimethylsilyloxy)cholest-5-en-26-oic Acid (23): Oxalyl chloride (84 μL, 0.994 mmol) was added to a solution of DMSO (141 μL, 1.988 mmol) in dichloromethane (5 mL) at –78 °C. After 5 min, a solution of the alcohol **22** (257 mg, 0.497 mmol) in dichloromethane (10 mL) was added and the reaction mixture was stirred at –78 °C for 20 min. Then, triethylamine (346 μL, 2.485 mmol) was added dropwise, the solution was warmed to room temperature and stirring was continued for 10 min. A saturated aqueous solution of ammonium chloride (50 mL) was added and the layers were separated. The aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated to afford the aldehyde. 2-Methyl-2-butene (527 μL, 4.97 mmol) and KH_2PO_4 (200 mg) were added to the solution of the aldehyde in THF (6 mL) and water (1 mL). After addition of a solution of sodium chlorite (90 mg, 0.994 mmol) in water (1 mL), the mixture was stirred at room temperature for 24 h. Then, 10% HCl (25 mL) and diethyl ether (50 mL) were added, and the layers were separated. The aqueous layer was extracted with diethyl ether (2 × 50 mL) and the combined organic layers were dried with magnesium sulfate. Removal of the solvent and purification of the residue by flash chromatography on silica gel (petroleum ether/diethyl ether, 3:1) provided the acid **23**, yield 235 mg (89%). Colourless solid; m.p. 183–185 °C. IR (ATR): $\tilde{\nu}$ = 2931, 2880, 2854, 1704, 1464, 1418, 1380, 1294, 1248, 1226, 1198, 1083, 988, 957, 888, 870, 835, 803, 774, 733, 669 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.05 (s, 6 H) 0.65 (s, 3 H), 0.86–0.94 (m, 1 H), 0.88 (s, 9 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.95–1.28 (m, 8 H), 0.98 (s, 3 H), 1.17 (d, J = 7.0 Hz, 3 H), 1.31–1.59 (m, 10 H), 1.63–1.72 (m, 2 H), 1.76–1.83 (m, 2 H), 1.92–1.98 (m, 1 H), 1.98 (dt, J = 12.6, 3.2 Hz, 1 H), 2.15 (ddd, J = 13.3, 4.9, 2.1 Hz, 1 H), 2.26 (m, 1 H), 2.45 (sext, J = 7.0 Hz, 1 H), 3.47 (m, 1 H), 5.30 (m, 1 H), 11.08 (br. s, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.84 (CH_3), 16.99 (CH_3), 18.27 (C), 18.62 (CH_3), 19.41 (CH_3), 21.04 (CH_2), 23.71 (CH_2), 24.27 (CH_2), 25.93 (3 CH_3), 28.22 (CH_2), 31.88 (CH), 31.91 (CH_2), 32.05 (CH_2), 34.03 (CH_2), 35.63 (CH), 35.76 (CH_2), 36.56 (C), 37.36 (CH_2), 39.35 (CH), 39.76 (CH_2), 42.31 (C), 42.79 (CH_2), 50.16 (CH), 56.04 (CH), 56.76 (CH), 72.64 (CH), 121.14 (CH), 141.55 (C), 182.60 (C=O) ppm. ESI-MS: m/z = 553.4 [(M + Na)$^+$]. $C_{33}H_{58}O_3Si$ (530.90): C 74.66, H 11.01, found: C 74.75, H 10.94%.

Methyl (25*S*)-3β-Hydroxycholesten-5-en-26-oate (24): A catalytic amount of concentrated sulfuric acid was added to a solution of the acid **23** (227 mg, 0.428 mmol) in methanol (10 mL) and the mixture was heated under reflux for 16 h. After cooling to room temperature, a saturated aqueous solution of sodium hydrogen carbonate (25 mL) and ethyl acetate (50 mL) were added. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate, 6:1) to provide the methyl ester **24**, yield 161 mg (87%). Colourless crystals; m.p. 118–119 °C. IR (ATR): $\tilde{\nu}$ = 3443, 2931, 2901, 2886, 2866, 1732, 1460, 1378, 1363, 1312, 1222, 1192, 1164, 1140, 1107, 1054, 1041, 1025, 1011, 987, 959, 841, 804, 765, 741 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.87–1.16 (m, 8 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.99 (s, 3 H), 1.13 (d, J = 7.0 Hz, 3 H), 1.19–1.66 (m, 12 H), 1.76–1.85 (m, 3 H), 1.93–1.99 (m, 1 H), 1.99 (dt, J = 12.6, 3.5 Hz, 1 H), 2.22–2.24 (m, 1 H), 2.28 (ddd, J = 13.0, 5.1, 2.0 Hz, 1 H), 2.42 (sext, J = 7.0 Hz, 1 H), 3.51 (m, 1 H), 3.66 (s, 3 H), 5.34 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.84 (CH_3), 17.23 (CH_3), 18.60 (CH_3), 19.38 (CH_3), 21.05 (CH_2), 23.76 (CH_2), 24.26 (CH_2), 28.20 (CH_2), 31.62 (CH_2), 31.86 (CH), 31.87 (CH_2), 34.31 (CH_2), 35.61 (CH), 35.75 (CH_2), 36.47 (C), 37.22 (CH_2), 39.51 (CH), 39.73 (CH_2), 42.27 (CH_2), 42.29 (C), 50.07 (CH), 51.45 (CH_3), 56.02 (CH), 56.72 (CH), 71.77 (CH), 121.68 (CH), 140.74 (C), 177.45 (C=O) ppm. $C_{28}H_{46}O_3$ (430.66): C 78.09, H 10.77, found: C 78.06, H 10.88%.

(25*S*)-Cholestenoic Acid [(25*S*)-3β-Hydroxycholest-5-en-26-oic Acid] (4): Lithium hydroxide (11.5 mg, 480 μmol) was added to a solution of the methyl ester **24** (69 mg, 160 μmol) in THF/methanol/water (1:1:1, 6 mL) and the mixture was stirred at room temperature for 24 h. Methanol and THF were removed in vacuo and the residue was thoroughly extracted with dichloromethane (3 × 30 mL) to remove traces of unreacted starting material. The combined dichloromethane layers were discarded. Then, 10% hydrochloric acid was added to the aqueous residue (pH < 4) and the mixture was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried with magnesium sulfate and the solvent was evaporated to provide pure (25*S*)-cholestenoic acid (**4**), yield 67 mg (99%). An analytically pure sample was obtained by recrystallization from acetonitrile. Colourless solid; m.p. 172–175 °C (MeCN) (ref.[6] 157–160 °C). $[\alpha]_D^{20}$ = –22.9 (c = 0.14, $CHCl_3$). IR (ATR): $\tilde{\nu}$ = 3262, 2934, 2922, 2890, 2862, 1674, 1457, 1434, 1421, 1374, 1277, 1225, 1137, 1113, 1088, 1052, 1020, 987, 953, 927, 890, 841, 819, 797, 737, 658 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.87–1.27 (m, 9 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.99 (s, 3 H), 1.17 (d, J = 7.0 Hz, 3 H), 1.31–1.59 (m, 10 H), 1.63–1.69 (m, 1 H), 1.77–1.85 (m, 3 H), 1.93–2.01 (m, 2 H), 2.22–2.30 (m, 2 H), 2.46 (sext, J = 7.0 Hz, 1 H), 3.52 (m, 1 H), 5.34 (m, 1 H) ppm. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.84 (CH_3), 17.02 (CH_3), 18.62 (CH_3), 19.38 (CH_3), 21.05 (CH_2), 23.70 (CH_2), 24.26 (CH_2), 28.21 (CH_2), 31.61 (CH_2), 31.87 (CH, CH_2), 34.05 (CH_2), 35.63 (CH), 35.75 (CH_2), 36.48 (C), 37.22 (CH_2), 39.21 (CH), 39.73 (CH_2), 42.24 (CH_2), 42.30 (C), 50.07 (CH), 56.03 (CH), 56.71 (CH), 71.81 (CH), 121.71 (CH), 140.71 (C), 181.58 (C=O) ppm. ESI-MS: m/z = 399.4 [(M + H – H_2O)$^+$]. $C_{27}H_{44}O_3$ (416.64): C 77.83, H 10.64, found: C 77.99, H 10.77%.

Supporting Information (see also the footnote on the first page of this article): Copies of the ^1H and ^{13}C NMR spectra for compound **10** and copies of the HSQC spectra for the (25*S*)-steroidal acids **1**–**4**.

Acknowledgments

We thank Dr. Margit Gruner for 2D NMR experiments and Prof. Adam Antebi for the *daf-9(dh6);dhEx24* strain. We are grateful to JADO Technologies GmbH, Dresden, for support.

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Received: April 21, 2009
Published Online: June 10, 2009

