4α-Bromo-5α-cholestan-3β-ol and nor-5α-cholestan-3β-ol derivatives—stereoselective synthesis and hormonal activity in *Caenorhabditis elegans*†

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We describe the stereoselective synthesis of 4α-bromo-5α-cholestan-3β-ol, 21-nor-5α-cholestan-3β-ol, 27-nor-5α-cholestan-3β-ol and 21,27-bisnor-5α-cholestan-3β-ol. In order to clarify the *in vivo* metabolism of cholesterol, these compounds have been used for feeding experiments in *Caenorhabditis elegans*. Our preliminary results provide important insights into the metabolism of cholesterol in worms.

Introduction

In recent years, sterols have been shown to play a pivotal role for the development and ageing of the nematode *Caenorhabditis elegans*.[1–4] Supply with exogenous sterols is absolutely required for the worm life cycle,[5] which allows the testing of different sterols for biological activity by mixing them with the food.[1,6] Thus, we could demonstrate that, when feeding *C. elegans*, cholesterol can be substituted by dihydrocholesterol (5α-cholestan-3β-ol) (**1a**) (Fig. 1).[2,7] Moreover, we found that lophanol (4α-methyl-5α-cholestan-3β-ol) (**1b**) can support the life cycle of *C. elegans*, but is not sufficient to favour reproductive development over diapause.[2,7]

We are engaged in a project directed towards the synthesis of hormonally active cholesterol derivatives for the study of metabolic processes of *C. elegans*.[2,7–9] Recently, we described the stereoselective synthesis of the (25*R*)-dafachronic acids (*e.g.* **2a**)[8] as well as their 25*S*-epimers (*e.g.* **2b**).[9] The (25*S*)-dafachronic acids are the more active ligands for the hormonal receptor DAF-12 in *C. elegans*.[3,4,9] Feeding *C. elegans* with lophanol (**1b**), the 4α-methyl derivative of **1a**, induces dauer larvae formation. The same result has been obtained with 4α-fluoro-5α-cholestan-3β-ol (**3**) and further 5α-cholestan-3β-ol derivatives.[2,7] The sterols **1b** and **3** cannot be metabolised *in vivo* to promote reproduction. In consequence, wild-type worms grown on **1b** or **3** enter diapause and generate dauer larvae. However, all other sterol-derived functions are maintained when feeding wild-type worms with compounds **1b** or **3**. This has been confirmed by the following experiment. Mutant worms lacking the DAF-12 receptor, which do not require dafachronic acid, show normal growth when fed with sterols **1b** or **3**.

Following sterol metabolism in *C. elegans* is very difficult, because only very small amounts of sterols are formed *in vivo*.

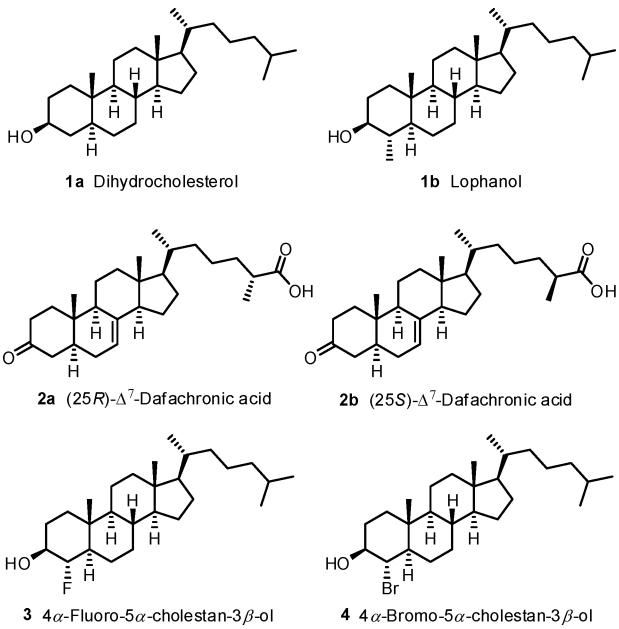

Fig. 1 Hormonally active cholestanols **1**, **3** and **4** and dafachronic acids **2**.

However, bromo derivatives are easily detected using mass spectroscopy by the characteristic signal pattern resulting from their two isotopes ($m/z = 81$ and 79, ratio: 49:51). Due to the similar van der Waals radii of methyl (2.00 Å) and bromine (1.85 Å), we expected that lophanol (**1b**) can be replaced by 4α-bromo-5α-cholestan-3β-ol (**4**) when feeding *C. elegans*. Therefore, the bromo analogue of 4α-fluoro-5α-cholestan-3β-ol (**3**) should be suitable for studying the metabolic degradation of sterols in *C. elegans* by mass spectroscopy.

Moreover, we envisaged the nor-5α-cholestan-3β-ols **5–7** as interesting compounds to study the metabolism of 5α-cholestan-3β-ol (**1a**) (Fig. 2). Synthesis of the norcholestanols **5** and **7** should be feasible by introduction of a steroid side chain lacking the corresponding methyl groups. Commercially available 3-*epi*-androsterone (**8**) appeared to be a perfect starting material. Wittig olefination at the C-17 ketone to the corresponding olefins

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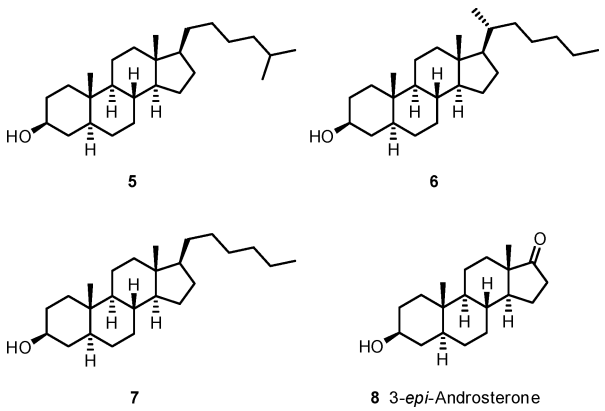
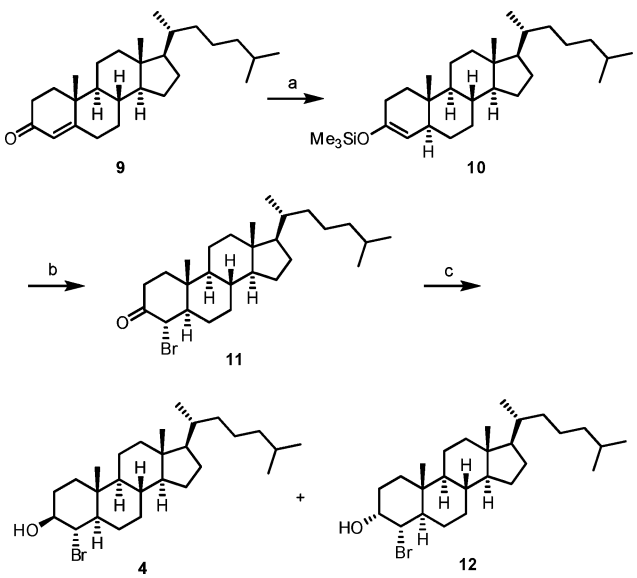


Fig. 2 Target molecules **5–7** and 3-*epi*-androsterone (**8**).

followed by hydrogenation should lead diastereoselectively to the desired compounds **5** and **7**. Synthesis of the 27-nor-5α-cholestan-3β-ol (**6**) required a precursor with an *R*-configuration at C-20 of the side chain.

Results and discussion

For the synthesis of the 4α-bromocholestanol **4**, commercially available cholest-4-en-3-one (**9**) was converted to the known trimethylsilyl enol ether **10**.[2,7] Reduction of **9** with lithium in liquid ammonia using a modified work-up provided compound **10** on large scale in an increased yield of 85% (previously:[7] 55%) (Scheme 1). The easily accessible silyl enol ether **10** represents an excellent intermediate for the synthesis of 4α-substituted 5α-cholestan-3β-ol derivatives.[7] Treatment of **10** with *N*-bromosuccinimide (NBS) and TBAF provided diastereo- and regioselectively the 4α-bromoketone **11** in 65% yield. Reduction



Scheme 1 Synthesis of the epimeric 4α-bromocholestanols **4** and **12**. *Reagents and conditions*: (a) –78 °C, THF/NH_3, then –35 °C, 2.2 equiv. Li, to 25 °C, then –10 °C, 4.0 equiv. Et_3N, 3.9 equiv. Me_3SiCl, 2 h, 85% (b) 1.5 equiv. NBS, 1.0 equiv. TBAF, THF, 25 °C, 2 h, 65%; (c) 2.0 equiv. $NaBH_4$, MeOH, 25 °C, 15 h, 66% of **4**, 33% of **12**.

of the ketone **11** using sodium borohydride afforded quantitatively the 3β-alcohol **4** and the 3α-alcohol **12** in a 2:1 ratio. Both diastereoisomers can be easily separated by flash chromatography on silica gel. Due to concomitant debromination, application of lithium aluminium hydride or DIBAL-H as reducing agents resulted in lower yields of the desired compound **4**. The assignment of the proton signals in the ^1H NMR spectra of **4** and **12** is based on COSY and HSQC spectra (see ESI). The relative configurations at C-3 and C-4 have been determined based on NOE experiments with the diastereoisomeric alcohols **4** and **12** (Fig. 3, see also ESI). The structure of 4α-bromo-5α-cholestan-3β-ol (**4**) has been

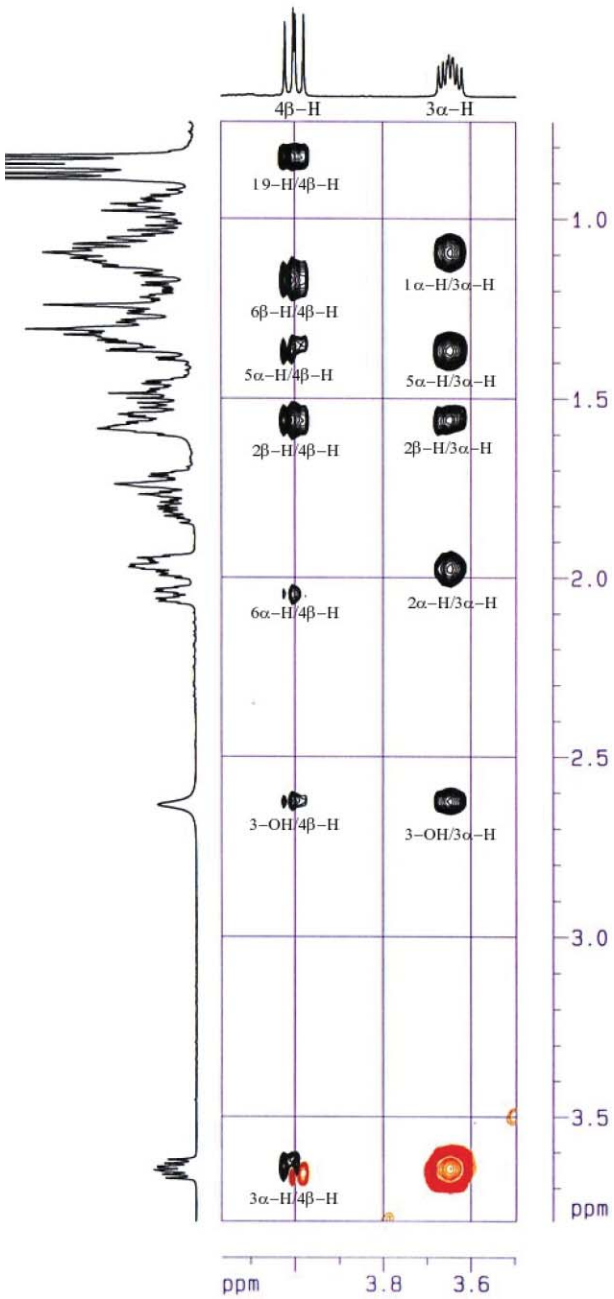


Fig. 3 Expansion of the NOESY Spectrum (500 MHz, $CDCl_3$) of 4α-bromo-5α-cholestan-3β-ol (**4**) showing the characteristic signals.

unambiguously confirmed by an X-ray analysis of single crystals, obtained by recrystallisation from methanol (Fig. 4).

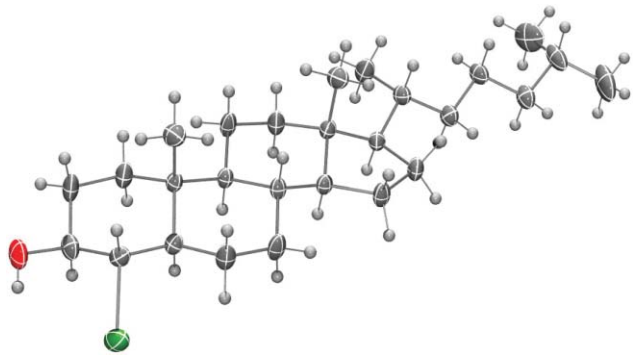
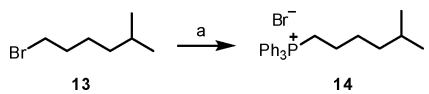
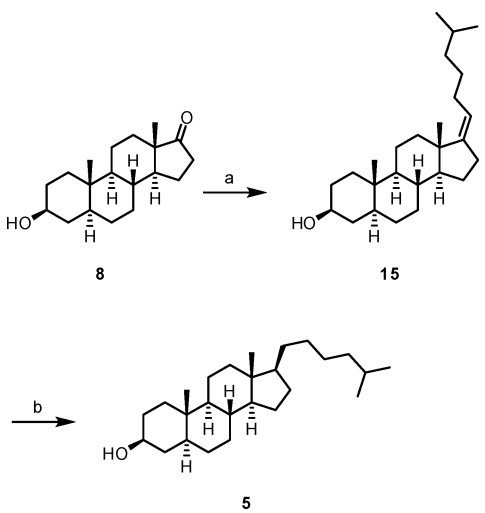


Fig. 4 Molecular structure of the 4α-bromo-5α-cholestan-3β-ol (**4**) in the crystal (ORTEP plot; ellipsoids at 50% probability level).

The non-commercial alkylphosphonium bromide **14** was prepared by reaction of triphenylphosphane with 1-bromo-5-methylhexane (**13**) in toluene under reflux (Scheme 2). Initial experiments for the Wittig reaction of 3-*epi*-androsterone (**8**) and the phosphonium salt **14** using standard conditions (*tert*-BuOK, THF, reflux) led only to the recovery of starting material. However, using an alternative procedure (sodium hydride in dimethyl sulfoxide at elevated temperature)[10] afforded the olefin **15** in 56% yield as a non-separable mixture of the 17*Z*- and 17*E*-isomers (ratio: 11:1) (Scheme 3). The moderate yield is explained by steric hindrance resulting from the C-19 methyl group. Catalytic hydrogenation of the *E*/*Z*-mixture of **15** led diastereoselectively to 21-nor-5α-cholestan-3β-ol (**5**).

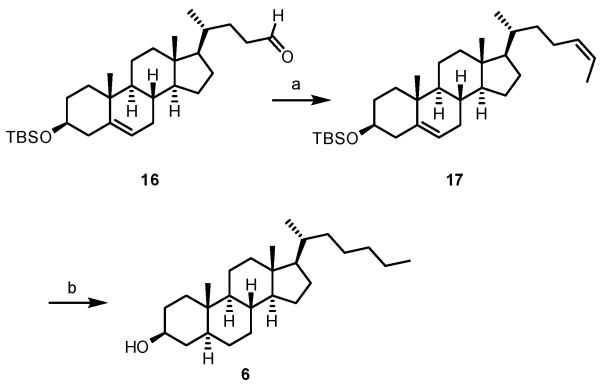


Scheme 2 Synthesis of the alkylphosphonium bromide **14**. *Reagents and conditions*: (a) 1.0 equiv. of **13**, 1.0 equiv. PPh_3, toluene, reflux, 6 d, 85%.



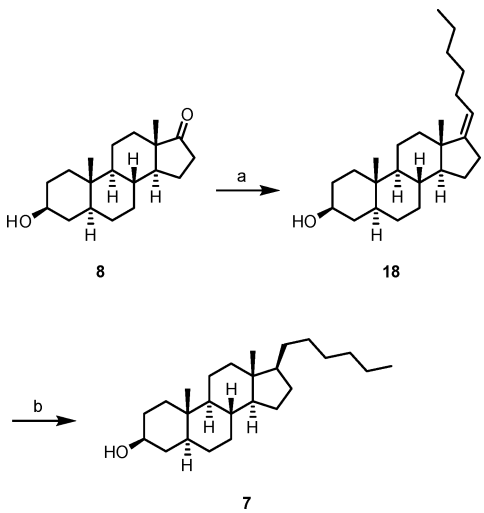
Scheme 3 Stereoselective synthesis of 21-nor-5α-cholestan-3β-ol (**5**). *Reagents and conditions*: (a) 4.5 equiv. NaH, DMSO, 75 °C, 1 h, then 4.5 equiv. **14**, 15 min, then **8**, 75 °C, 3 d, 56% (*Z*/*E* = 11:1); (b) 10% Pd/C, H_2, $MeOH/CH_2Cl_2$ (1:1), 25 °C, 24 h, 99%.

For the synthesis of **6**, we envisaged aldehyde **16** as starting material as it provides the correct stereochemistry at C-20. Aldehyde **16** is readily prepared in three steps from the commercially available 3β-hydroxychol-5-en-24-oic acid (94% overall yield).[9,11] Wittig reaction of **16** and ethyltriphenylphosphonium iodide afforded quantitatively the diene **17** as a non-separable mixture of the *Z* and *E* double bond stereoisomers (ratio: 83:17, as determined by GC-MS) (Scheme 4). Catalytic hydrogenation of the *E*-**17**/*Z*-**17** mixture in methanol/dichloromethane (1:1) led by concomitant desilylation[12] in 84% yield directly to 27-nor-5α-cholestan-3β-ol (**6**).



Scheme 4 Synthesis of 27-nor-5α-cholestan-3β-ol (**6**). *Reagents and conditions*: (a) 4.0 equiv. NaH, DMSO, 75 °C, 1 h, then 4.0 equiv. $[EtPPh_3]^+ I^-$, 50 °C, 30 min, then **16**, 50 °C, 20 min, 100% (*Z*/*E* = 83:17); (b) 10% Pd/C, H_2, $MeOH/CH_2Cl_2$ (1:1), 25 °C, 24 h, 84%.

Wittig reaction of **8** and hexyltriphenylphosphonium bromide afforded in 55% yield the non-separable *Z*/*E*-mixture of the olefin **18** (ratio: 11:1) (Scheme 5). Subsequent catalytic hydrogenation provided diastereoselectively 21,27-bisnor-5α-cholestan-3β-ol (**7**).



Scheme 5 Synthesis of 21,27-bisnor-5α-cholestan-3β-ol (**7**). *Reagents and conditions*: (a) 4.5 equiv. NaH, DMSO, 70 °C, 1 h, then 4.5 equiv. $[hexylPPh_3]^+ Br^-$, 70 °C, 15 min, then **8**, 70 °C, 40 h, 55% (*Z*/*E* = 11:1); (b) 10% Pd/C, H_2, $MeOH/CH_2Cl_2$ (1:1), 25 °C, 22 h, 97%.

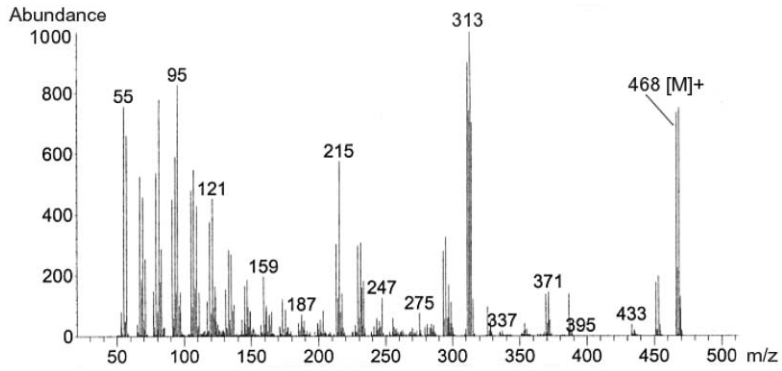


Fig. 5 GC-MS (70 eV) spectrum of 4α-bromo-5α-cholestan-3β-ol (**4**). $R_f = 66.63$ min. GC conditions: injector temperature 250 °C; 100 min at 250 °C, then 4 °C/min to 280 °C, hold 15 min at 280 °C, then 4 °C/min to 340 °C, hold for 10 min.

Biological studies

On feeding experiments with the nematode *Caenorhabditis elegans*, the 5α-cholestan-3β-ol derivatives **4–7** displayed interesting activities. 4α-Bromo-5α-cholestan-3β-ol (**4**) can be easily detected using mass spectroscopy by the characteristic signal pattern of the bromine isotopes (Fig. 5). In order to investigate the bioactivity of **4**, we fed wild-type and *daf-12* mutant worms in the absence of any other sterol.[2] It could be demonstrated that the 4α-bromocholestanol **4** represents a food substitute for lophanol (**1b**). Feeding wild-type *C. elegans* with compound **4** resulted in dauer larvae formation, as previously observed with lophanol (**1b**) and 4α-fluoro-5α-cholestan-3β-ol (**3**).[2,7] Feeding *daf-12* mutant worms with **4** led to normal reproductive development, which is also in agreement with our previous feeding experiments using compounds **1b** and **3**. The present findings indicate that the 4α-bromocholestanol **4** can be metabolised *in vivo* to all required sterols except the DAF-12 ligands.

The requirement of the methyl groups at the cholestanol side chain was investigated by feeding wild-type and *daf-12* mutant worms with the norcholestanols **5–7**. Wild-type worms grown on 27-norcholestanol **6** entered diapause. However, *daf-12* mutant worms exhibited normal growth and thus, demonstrated that the C-27 methyl group is required for the production of dafachronic acids. Surprisingly, the presence of the C-21 methyl group is very important for development, since wild-type worms grown on 21-norcholestanol **5** could not form complete dauer larvae, but arrested as dauer-like larvae. Interestingly, *daf-12* mutant worms grown on 21-norcholestanol **5** developed only into sick adults. Feeding wild-type and *daf-12* mutant worms with 21,27-bisnorcholestanol **7** provided similar results as obtained with 21-norcholestanol **5**. The present results indicate that the C-21 methyl group is crucial for production of sterols other than dafachronic acids. Obviously, these steroids are required for normal development.

Conclusion

The present synthetic methodology provides a simple and stereoselective route to 5α-cholestan-3β-ols, useful for the study of cholestanol metabolites in biological systems. Our previous studies had shown that feeding with 4α-methyl-5α-cholestan-3β-ol (lophanol) (**1b**) is sufficient for all biological functions in *C. elegans*, except DAF-12 dependent reproductive development. The present study demonstrates that 4α-bromo-5α-cholestan-3β-ol (**4**) can be used to substitute lophanol (**1b**) in the food of worms. The bromo substituent might be detectable by MS in downstream metabolic products. Therefore, 4α-bromo-5α-cholestan-3β-ol (**4**) could be used as a probe for the clarification of biological modifications of cholesterol.

Using nor-5α-cholestan-3β-ols lacking different methyl groups at the sterol side chain, we could demonstrate the importance of these methyl groups for the generation of reproduction-promoting sterols (like the dafachronic acids) and sterols required for other functions in *C. elegans*. Thus, we found that the C-27 methyl group is required for the production of the hormonally active dafacronic acids. The C-21 methyl group is of fundamental importance, as feeding with the norcholestanols **5** and **7** did neither lead to dauer formation nor to normal growth of dauer-deficient *daf-12* mutant worms. Future studies along these lines using labelled sterols could lead to the detection and identification of further sterol-derived metabolites involved in the reproductive development of *C. elegans*.

Experimental section

General

All reactions were carried out in oven-dried glassware under an argon atmosphere. Tetrahydrofuran was dried using a solvent purification system (MBraun-SPS). Toluene was purchased from Acros Organics (water content less than 50 ppm). Dry DMSO was obtained from Fluka (water content less than 50 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 F254) using anisaldehyde solution for visualization. Melting points were measured on an Electrothermal IA9100. Infrared spectra were recorded using 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, 70 eV). Elemental analyses were measured on an EuroVector EuroEA3000. NMR spectra were recorded on a Bruker DRX 500.

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, non: nonet, 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), EvalCCD (Duisenberg *et al.*, 2003), SADABS version 2.10 (G. M. Sheldrick, Bruker AXS Inc., 2002), SHELXL-97 (G. M. Sheldrick, 1997), ORTEP-3 for Windows, version 2.02 (L. J. Farrugia, 1997–2008).

3-Trimethylsilyloxy-5α-cholest-3-ene (10)

Cholest-4-en-3-one (**9**) (3.00 g, 7.80 mmol) was placed in a three-necked flask equipped with a condenser. Tetrahydrofuran (60 mL) was added to the flask, the reaction mixture was cooled to −78 °C and ammonia (50 mL) was condensed. After addition of lithium (120 mg, 17.3 mmol), the solution was warmed to −35 °C and stirred at that temperature for 2 h. Ammonia was evaporated by warming to room temperature overnight. After addition of THF (20 mL), the mixture was cooled to −10 °C, and triethylamine (4.38 mL, 31.5 mmol) and chlorotrimethylsilane (3.9 mL, 30.7 mmol) were added dropwise. The mixture was stirred for 2 h at −10 °C, then transferred to a 500 mL flask and the solvent was removed. The residue was purified by flash chromatography on activated neutral aluminium oxide (7.2% water added) (petroleum ether with 1% triethylamine) to afford the trimethylsilyl enol ether **10** as colourless crystals, yield: 3.05 g (85%). For full characterisation, see ref. 7.

4α-Bromo-5α-cholestan-3-one (11)

N-Bromosuccinimide (1.16 g, 6.52 mmol) was added to a solution of the silyl enol ether **10** (2 g, 4.36 mmol) in THF (20 mL). The resulting mixture was stirred at room temperature for 10 min. A 1 M solution of TBAF in tetrahydrofuran (4.36 mL, 4.36 mmol) was added and stirring was continued for additional 110 min. After addition of water (25 mL), 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 crude product by flash chromatography on silica gel (petroleum ether–diethyl ether, 20:1) provided the α-bromoketone **11**, yield: 1.32 g (65%). Colourless solid; mp: 137–139 °C; IR (ATR): ν = 2929, 2866, 1722, 1652, 1467, 1417, 1378, 1333, 1309, 1290, 1232, 1171, 1108, 1078, 1034, 1000, 957, 937, 829, 717 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (s, 3 H), 0.75 (m, 1 H), 0.849 (d, J = 6.6 Hz, 3 H), 0.854 (d, J = 6.6 Hz, 3 H), 0.89 (d, J = 6.6 Hz, 3 H), 0.92–1.16 (m, 8 H), 1.06 (s, 3 H), 1.21–1.60 (m, 12 H), 1.66 (dt, J = 3.0, 12.4 Hz, 1 H), 1.74–1.84 (m, 2 H), 1.98 (dt, J = 12.8, 3.4 Hz, 1 H), 2.04 (ddd, J = 13.4, 6.3, 2.6 Hz, 1 H), 2.12 (m, 1 H), 2.53 (dt, J = 6.2, 14.5 Hz, 1 H), 2.64 (ddd, J = 15.0, 5.1, 2.6 Hz, 1 H), 4.58 (d, J = 12.8 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.04 (CH_3), 12.53 (CH_3), 18.62 (CH_3), 21.44 (CH_2), 22.54 (CH_3), 22.81 (CH_3), 23.79 (CH_2), 24.11 (CH_2), 28.00 (CH), 28.07 (CH_2), 28.22 (CH_2), 31.46 (CH_2), 34.99 (CH), 35.74 (CH), 36.09 (CH_2), 37.43 (CH_2), 38.22 (CH_2), 38.98 (C), 39.47 (CH_2), 39.76 (CH_2), 42.52 (C), 53.96 (CH), 55.21 (CH), 56.08 (CH), 56.15 (CH), 62.74 (CH), 201.96 (C=O); MS (EI): m/z (%) = 466 (89), 464 (87) [M$^+$], 451 (11), 449 (11), 386 (51), 312 (67), 311 (96), 310 (71), 309 (85), 297 (27), 295 (28), 232 (46), 231 (100); HRMS: m/z calc. for $C_{27}H_{45}BrO$ [M$^+$]: 464.2654, found: 464.2649.

4α-Bromo-5α-cholestan-3α-ol (12) and 4α-bromo-5α-cholestan-3β-ol (4)

Sodium borohydride (16.3 mg, 431 μmol) was added to a solution of the ketone **11** (100 mg, 215 μmol) in dry methanol (5 mL) and the resulting mixture was stirred at room temperature for 15 h. The reaction mixture was quenched by addition of dilute hydrochloric acid (25 mL) and extracted with dichloromethane (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, 10:1 to 8:1) provided the 3α-alcohol **12**, yield: 33 mg (33%) and the more polar 3β-alcohol **4**, yield: 66 mg (66%).

12. Colourless oil; IR (ATR): ν = 3557, 3478, 2933, 2865, 1727, 1444, 1419, 1380, 1290, 1270, 1205, 1168, 1118, 1057, 1014, 950, 933, 912, 853, 823, 723, 688 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.63 (s, 3 H), 0.76–1.37 (m, 16 H), 0.83 (s, 3 H), 0.847 (d, J = 6.6 Hz, 3 H), 0.852 (d, J = 6.6 Hz, 3 H), 0.88 (d, J = 6.6 Hz, 3 H), 1.43–1.58 (m, 6 H), 1.70 (m, 1 H), 1.77–1.84 (m, 3 H), 1.86–1.97 (m, 3 H), 2.35 (br s, 1 H), 4.07 (m, 1 H), 4.36 (dd, J = 12.1, 2.5 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.03 (CH_3), 12.20 (CH_3), 18.62 (CH_3), 20.81 (CH_2), 22.55 (CH_3), 22.81 (CH_3), 23.80 (CH_2), 24.07 (CH_2), 26.80 (CH_2), 27.92 (CH_2), 28.00 (CH), 28.22 (CH_2), 31.46 (CH_2), 31.60 (CH_2), 35.21 (CH), 35.76 (CH), 36.12 (CH_2), 39.49 (CH_2), 39.86 (CH_2), 40.25 (C), 42.41 (C), 46.45 (CH), 54.26 (CH), 56.12 (CH), 56.35 (CH), 68.07 (CH), 70.55 (CH); MS (EI): m/z (%) = 468 (78), 466 (77) [M$^+$], 453 (14), 451 (15), 371 (21), 369 (14), 328 (14), 326 (15), 314 (66), 313 (100), 312 (68), 311 (92), 231 (45); HRMS: m/z calc. for $C_{27}H_{47}BrO$ [M$^+$]: 466.2810, found: 466.2803.

4. Colourless crystals; mp: 122–124 °C; IR (ATR): ν = 3579, 2947, 2930, 2904, 2849, 1726, 1486, 1467, 1419, 1371, 1337, 1270, 1255, 1235, 1199, 1137, 1119, 1097, 1073, 1050, 1004, 956, 933, 908, 860, 830, 739, 705, 652, 629 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.64 (s, 3 H), 0.65–0.71 (m, 1 H), 0.83 (s, 3 H), 0.848 (d, J = 6.6 Hz, 3 H), 0.852 (d, J = 6.6 Hz, 3 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.92–1.39 (m, 17 H), 1.45–1.60 (m, 5 H), 1.71–1.84 (m, 3 H), 1.97 (m, 2 H), 2.03–2.07 (m, 1 H), 2.62 (br s, 1 H), 3.65 (ddd, J = 14.9, 9.6, 5.3 Hz, 1 H), 4.00 (dd, J = 11.6, 9.7 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.02 (CH_3), 12.92 (CH_3), 18.61 (CH_3), 21.13 (CH_2), 22.53 (CH_3), 22.79 (CH_3), 23.78 (CH_2), 24.08 (CH_2), 26.79 (CH_2), 27.98 (CH), 28.21 (CH_2), 29.09 (CH_2), 31.60 (CH_2), 34.94 (CH), 35.67 (CH_2), 35.73 (CH), 36.10 (CH_2), 39.40 (CH_2), 39.46 (C), 39.84 (CH_2), 42.49 (C), 51.88 (CH), 54.28 (CH), 56.16 (CH), 56.26 (CH), 70.17 (CH), 76.41 (CH); MS (EI, 220 °C): m/z (%) = 468 (100), 466 (99) [M$^+$], 453 (15), 451 (16), 386 (12), 385 (14), 369 (14), 328 (14), 326 (15), 314 (67), 313 (90), 312 (69), 311 (83), 297 (19), 295 (23), 293 (17), 233 (17), 231 (16), 229 (23), 215 (45), 213 (18); GC-MS (EI, 230 °C): m/z (%) = 468 (76), 466 (74) [M$^+$], 453 (16), 451 (18), 386 (14), 371 (19), 369 (14), 314 (70), 313 (100), 312 (74), 311 (90), 295 (33), 293 (28), 231 (30), 229 (26), 215 (57), 213 (30); Anal. calc. for $C_{27}H_{47}BrO$: C 69.36, H 10.13, found: C 69.55, H 10.28%.

Crystallographic data for compound 4

$C_{27}H_{47}BrO$, crystal size: 0.48 × 0.36 × 0.21 mm^3, M = 467.56 g mol^{-1}, monoclinic, space group $P2_1$, a = 11.802(1), b = 7.528(1), c = 15.271(2) Å, β = 110.66(1)°, V = 1269.5(3) Å^3, Z = 2, ρ_{calcd} = 1.223 g cm^{-3}, μ = 1.634 mm^{-1}, λ = 0.71073 Å, T = 198(2) K, θ range = 3.06–27.99°, reflections collected: 55589, independent: 5973 (R_{int} = 0.0465), 265 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 = 0.0268; wR_2 = 0.574; maximal residual electron density: 0.173 e Å^{-3}. CCDC 721761.

(5-Methylhexyl)triphenylphosphonium bromide (14)

Triphenylphosphane (2.70 g, 10.3 mmol) was added to a solution of 1-bromo-5-methylhexane (**13**) (1.675 mL, 10.3 mmol) in toluene (20 mL) and the reaction mixture was heated at reflux for 6 d. After cooling to room temperature, toluene was removed and the residue was purified by flash chromatography on silica gel (dichloromethane–methanol, 20:1) to provide the phosphonium salt **14**, yield: 3.84 g (85%). Colourless solid; mp: 227–229 °C; IR (ATR): ν = 3074, 3049, 3008, 2944, 2890, 2865, 2787, 1586, 1483, 1466, 1433, 1408, 1385, 1366, 1313, 1217, 1153, 1112, 1018, 995, 867, 849, 823, 787, 760, 745, 724, 690 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.77 (d, J = 6.6 Hz, 6 H), 1.09 (m, 2 H), 1.42 (non, J = 6.6 Hz, 1 H), 1.58 (m, 4 H), 3.74 (m, 2 H), 7.66–7.70 (m, 6 H), 7.75–7.83 (m, 9 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 22.40 (2 CH_3), 22.70 (d, J = 49.8 Hz, CH_2), 22.77 (d, J = 4.7 Hz, CH_2), 27.56 (CH), 28.20 (d, J = 15.7 Hz, CH_2), 38.26 (CH_2), 118.19 (d, J = 86.0 Hz, 3 C), 130.41 (d, J = 12.9 Hz, 6 CH), 133.53 (d, J = 10.3 Hz, 6 CH), 134.94 (d, J = 2.5 Hz, 3 CH); Anal. calc. for $C_{25}H_{30}BrP$: C 68.03, H 6.85, found: C 68.07, H 6.93%.

(17*Z*)-21-Nor-5α-cholest-17-en-3β-ol (15)

Sodium hydride (48 mg, 1.20 mmol, 60% dispersion in mineral oil) was added to DMSO (5 mL) and the mixture was heated at 75 °C for 1 h. Then, a solution of the phosphonium salt **14** (531 mg, 1.20 mmol) in DMSO (5 mL) was added and the resulting red mixture was stirred at 75 °C for 15 min. Finally, a solution of 3-*epi*-androsterone (**8**) (100 mg, 344 μmol) in DMSO (4 mL) was added and the reaction mixture was stirred at 75 °C for 3 d. After cooling to room temperature, water was added (50 mL) and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (2 × 50 mL) and dried with magnesium sulfate. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (petroleum ether–ethyl acetate, 6:1) to afford the olefin **15**, yield: 72 mg (56%, Z/E = 11:1). Colourless solid; mp: 116–118 °C; IR (ATR): ν = 3236, 2925, 2854, 1467, 1448, 1371, 1348, 1321, 1304, 1251, 1172, 1138, 1082, 1044, 953, 937, 904, 864, 799, 736, 664 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.66 (ddd, J = 12.3, 10.5, 4.2 Hz, 1 H), 0.80 (s, 3 H), 0.84 (s, 3 H), 0.85 (d, J = 6.7 Hz, 6 H), 0.87–1.00 (m, 2 H), 1.08–1.17 (m, 5 H), 1.23–1.63 (m, 13 H), 1.67–1.72 (m, 2 H), 1.77–1.80 (m, 1 H), 1.98–2.20 (m, 4 H), 2.33–2.38 (m, 1 H), 3.58 (m, 1 H), 5.00 (tt, J = 7.4, 1.9 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.28 (CH_3), 17.38 (CH_3), 21.43 (CH_2), 22.62 (CH_3), 22.66 (CH_3), 24.34 (CH_2), 27.77 (CH_2), 27.91 (CH), 28.58 (CH_2), 28.66 (CH_2), 31.50 (2 CH_2), 31.90 (CH_2), 35.05 (CH), 35.51 (C), 36.93 (CH_2), 37.35 (CH_2), 38.17 (CH_2), 38.81 (CH_2), 44.29 (C), 44.78 (CH), 54.33 (CH), 56.23 (CH), 71.32 (CH), 120.13 (CH), 149.33 (C); Anal. calc. for $C_{26}H_{44}O$: C 83.80, H 11.90, found: C 83.23, H 12.24%.

21-Nor-5α-cholestan-3β-ol (5)

A solution of the olefin **15** (51 mg, 138 μmol) in dichloromethane (5 mL) was added to a Schlenk flask, preloaded with 10% palladium on charcoal (17 mg). After addition of methanol (5 mL), the mixture was stirred at room temperature under an hydrogen atmosphere for 24 h. The reaction mixture was filtered with ethyl acetate (250 mL) over a short pad of Celite. Removal of the solvent and purification of the residue on silica gel (petroleum ether–ethyl acetate, 6:1) provided compound **5**, yield: 51 mg (99%). Colourless solid; mp: 91–93 °C; IR (ATR): ν = 3492, 3282, 2918, 2852, 1659, 1448, 1371, 1336, 1297, 1171, 1135, 1075, 1036, 952, 939, 889, 858, 727 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.54 (s, 3 H), 0.63 (ddd, J = 12.4, 10.6, 4.1 Hz, 1 H), 0.80 (s, 3 H), 0.85 (d, J = 6.6 Hz, 6 H), 0.87–1.43 (m, 21 H), 1.47–1.72 (m, 8 H), 1.76–1.82 (m, 2 H), 3.58 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.35 (CH_3), 12.59 (CH_3), 21.02 (CH_2), 22.64 (CH_3), 22.69 (CH_3), 24.64 (CH_2), 27.92 (CH_2), 27.98 (CH), 28.51 (CH_2), 28.74 (CH_2), 29.13 (CH_2), 30.36 (CH_2), 31.53 (CH_2), 32.21 (CH_2), 35.53 (C), 35.56 (CH), 37.04 (CH_2), 38.02 (CH_2), 38.22 (CH_2), 39.08 (CH_2), 42.17 (C), 44.93 (CH), 51.04 (CH), 54.80 (CH), 55.89 (CH), 71.37 (CH); MS (EI): m/z (%) = 374 (100) [M$^+$], 359 (45), 341 (30), 273 (10), 248 (38), 234 (95), 233 (99.7), 215 (87); HRMS: m/z calc. for $C_{26}H_{46}O$ [M$^+$]: 374.3549, found: 374.3546.

(24*Z*)-3β-(*tert*-Butyldimethylsilyloxy)-27-norcholesta-5,24-diene (17)

Sodium hydride (82 mg, 2.04 mmol, 60% dispersion in mineral oil) was dissolved in DMSO (5 mL). The resulting mixture was heated at 75 °C for 1 h and subsequently, a solution of ethyltriphenylphosphonium iodide (853 mg, 2.04 mmol) in DMSO (5 mL) was added at 50 °C. After 30 min, a solution of the aldehyde **16** (241 mg, 0.51 mmol) in DMSO (5 mL) was added and stirring was continued at 50 °C for further 20 min. 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 washed with water (2 × 100 mL), brine (50 mL) and then dried with magnesium sulfate. Evaporation of the solvent and purification of the crude product by flash chromatography on silica gel (petroleum ether–diethyl ether, 50:1) provided the olefin **17**, yield: 247 mg (100%, Z/E = 83:17). Colourless solid; mp: 110–116 °C; IR (ATR): ν = 2928, 2894, 2880, 2853, 1471, 1461, 1370, 1251, 1196, 1084, 1024, 1005, 963, 926, 890, 870, 834, 803, 773, 667 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.05 (s, 6 H), 0.67 (s, 3 H), 0.83–1.29 (m, 8 H), 0.88 (s, 9 H), 0.93 (d, J = 6.6 Hz, 3 H), 0.99 (s, 3 H), 1.37–1.63 (m, 8 H), 1.60 (d, J = 6.1 Hz, 3 H), 1.68–1.72 (m, 1 H), 1.80 (dt, J = 13.5, 3.6 Hz, 1 H), 1.80–1.85 (m, 1 H), 1.93–2.06 (m, 4 H), 2.15 (ddd, J = 13.3, 4.9, 2.2 Hz, 1 H), 2.26 (m, 1 H), 3.47 (m, 1 H), 5.31 (m, 1 H), 5.35–5.42 (m, 2 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = –4.60 (2 CH_3), 11.84 (CH_3), 12.73 (CH_3), 18.27 (C), 18.57 (CH_3), 19.42 (CH_3), 21.04 (CH_2), 23.52 (CH_2), 24.28 (CH_2), 25.93 (3 CH_3), 28.22 (CH_2), 31.88 (CH), 31.92 (CH_2), 32.07 (CH_2), 35.52 (CH), 35.74 (CH_2),

36.57 (C), 37.36 (CH_2), 39.78 (CH_2), 42.35 (C), 42.80 (CH_2), 50.17 (CH), 56.03 (CH), 56.78 (CH), 72.63 (CH), 121.15 (CH), 123.39 (CH), 131.23 (CH), 141.55 (C); GC-MS (EI): m/z (%) = 427 (100) [$(M - t\text{Bu})^+$], 351 (7), 75 (37); Anal. calc. for $C_{32}H_{56}OSi$: C 79.27, H 11.64, found: C 79.59, H 11.79%.

27-Nor-5α-cholestan-3β-ol (6)

A solution of the olefin **17** (190 mg, 392 μmol) in dichloromethane (4 mL) was added to a Schlenk flask, preloaded with 10% palladium on charcoal (42 mg). After addition of methanol (4 mL), the mixture was stirred at room temperature under an hydrogen atmosphere for 24 h. The reaction mixture was filtered with dichloromethane (250 mL) over a short pad of Celite. Removal of the solvent and purification of the crude product by flash chromatography on silica gel (petroleum ether–ethyl acetate, 6:1) provided **6**, yield: 124 mg (84%). Colourless solid; mp: 83–86 °C; IR (ATR): ν = 3327, 2927, 2850, 1466, 1375, 1233, 1171, 1135, 1076, 1040, 957, 930, 903, 724 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.58–0.63 (m, 1 H), 0.63 (s, 3 H), 0.79 (s, 3 H), 0.87 (t, J = 7.1 Hz, 3 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.93–1.42 (m, 23 H), 1.45–1.55 (m, 3 H), 1.62–1.71 (m, 2 H), 1.76–1.83 (m, 2 H), 1.95 (dt, J = 12.5, 3.2 Hz, 1 H), 3.58 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.05 (CH_3), 12.30 (CH_3), 14.13 (CH_3), 18.64 (CH_3), 21.23 (CH_2), 22.74 (CH_2), 24.19 (CH_2), 25.73 (CH_2), 28.22 (CH_2), 28.72 (CH_2), 31.52 (CH_2), 32.07 (CH_2), 32.37 (CH_2), 35.43 (C), 35.48 (CH), 35.74 (CH), 35.87 (CH_2), 36.97 (CH_2), 38.20 (CH_2), 40.01 (CH_2), 42.56 (C), 44.82 (CH), 54.32 (CH), 56.22 (CH), 56.47 (CH), 71.37 (CH); MS (EI): m/z (%) = 374 (85) [M^+], 359 (45), 358 (32), 343 (28), 341 (14), 248 (28), 234 (55), 233 (76), 232 (12), 219 (20), 218 (41), 217 (100), 216 (25), 215 (70); HRMS: m/z calc. for $C_{26}H_{46}O$ [M^+]: 374.3549, found: 374.3537.

(17*Z*)-21,27-Bisnor-5α-cholest-17-en-3β-ol (18)

Sodium hydride (62 mg, 1.55 mmol, 60% dispersion in mineral oil) was added to DMSO (3 mL). The resulting mixture was heated at 70 °C for 1 h to afford a dark green solution and a solution of hexyltriphenylphosphonium bromide (645 mg, 1.51 mmol) in DMSO (10 mL) was added at 70 °C. After 15 min of stirring, a solution of 3-*epi*-androsterone (**8**) (100 mg, 344 μmol) in DMSO (4 mL) was added and stirring was continued for 40 h at 70 °C. 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 washed with water (2 × 50 mL), 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 olefin **18**, yield: 68 mg (55%, Z/E = 11:1). Colourless solid, mp: 86–88 °C; IR (ATR): ν = 3228, 2920, 2851, 1466, 1448, 1371, 1339, 1321, 1304, 1172, 1138, 1082, 1043, 953, 935, 904, 798, 768, 730 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.63–0.68 (m, 1 H), 0.80 (s, 3 H), 0.81–0.92 (m, 1 H), 0.84 (s, 3 H), 0.87 (t, J = 7.0 Hz, 3 H), 0.96 (dt, J = 4.1, 13.5 Hz, 1 H), 1.06–1.49 (m, 16 H), 1.53–1.63 (m, 3 H), 1.67–1.72 (m, 2 H), 1.76–1.82 (m, 1 H), 2.00–2.20 (m, 4 H), 2.33–2.38 (m, 1 H), 3.58 (m, 1 H), 5.00 (tt, J = 7.4, 2.0 Hz, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.27 (CH_3), 14.10 (CH_3), 17.38 (CH_3), 21.43 (CH_2), 22.62 (CH_2), 24.33 (CH_2), 27.50 (CH_2), 28.66 (CH_2), 30.49 (CH_2), 31.49 (2 CH_2), 31.67 (CH_2), 31.89 (CH_2), 35.05 (CH), 35.51 (C), 36.92 (CH_2), 37.35 (CH_2), 38.17 (CH_2), 44.29 (C), 44.78 (CH), 54.33 (CH), 56.23 (CH), 71.33 (CH), 120.12 (CH), 149.31 (C); MS (EI): m/z (%) = 358 (9) [M^+], 343 (5), 287 (100), 269 (16); HRMS: m/z calc. for $C_{25}H_{42}O$ [M^+]: 358.3236, found: 358.3228.

21,27-Bisnor-5α-cholestan-3β-ol (7)

A solution of the olefin **18** (58.5 mg, 163 μmol) in dichloromethane (2 mL) was added to a Schlenk flask, preloaded with 10% palladium on charcoal (17.3 mg). Methanol (2 mL) was added and the resulting mixture was stirred at room temperature under an hydrogen atmosphere for 22 h. The reaction mixture was filtered with dichloromethane (250 mL) over a short pad of Celite. Removal of the solvent and purification of the crude product by flash chromatography on silica gel (petroleum ether–ethyl acetate, 6:1) afforded **7**, yield: 57 mg (97%). Colourless solid; mp: 95–98 °C; IR (ATR): ν = 3284, 2920, 2851, 1448, 1374, 1337, 1234, 1171, 1136, 1082, 1035, 994, 952, 936, 799, 725 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 0.54 (s, 3 H), 0.63 (ddd, J = 12.4, 10.7, 4.1 Hz, 1 H), 0.80 (s, 3 H), 0.85–1.63 (m, 27 H), 0.87 (t, J = 7.0 Hz, 3 H), 1.64–1.72 (m, 3 H), 1.76–1.82 (m, 2 H), 3.57 (m, 1 H); ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 12.35 (CH_3), 12.59 (CH_3), 14.13 (CH_3), 21.02 (CH_2), 22.70 (CH_2), 24.63 (CH_2), 28.51 (CH_2), 28.74 (CH_2), 28.82 (CH_2), 29.85 (CH_2), 30.33 (CH_2), 31.54 (CH_2), 31.93 (CH_2), 32.21 (CH_2), 35.53 (C), 35.56 (CH), 37.05 (CH_2), 38.02 (CH_2), 38.23 (CH_2), 42.17 (C), 44.93 (CH), 51.04 (CH), 54.80 (CH), 55.89 (CH), 71.36 (CH); MS (EI): m/z (%) = 360 (80) [M^+], 345 (35), 327 (21), 248 (23), 234 (100), 233 (77), 219 (14), 217 (32), 216 (29), 215 (69), 165 (49); HRMS: m/z calc. for $C_{25}H_{44}O$ [M^+]: 360.3392, found: 360.3394.

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