Stereoselective synthesis of the hormonally active (25*S*)-Δ^7-dafachronic acid, (25*S*)-Δ^4-dafachronic acid, (25*S*)-dafachronic acid, and (25*S*)-cholestenoic acid†

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We report a stereoselective synthesis of the (25*S*)-cholestenoic-26-acids which are highly efficient ligands for the hormonal receptor DAF-12 in *Caenorhabditis elegans*.

The life cycle and longevity of the nematode *Caenorhabditis elegans* are regulated by several genes, among them *daf*-9.[1,2] DAF-9 protein, a cytochrome P450, is involved in the biosynthesis of the dafachronic acids. Mangelsdorf *et al.* identified these new steroidal hormones, products of DAF-9 activity, as ligands for the nuclear hormone receptor DAF-12. In the presence of dafachronic acids, DAF-12 is inactive and reproductive development occurs, whereas in the absence of the hormone the dauer larva is formed. The dauer-pathway genes and concomitant hormones also influence the life span of worms.[2] The steroids were assigned as (25*S*)-Δ^7- and (25*S*)-Δ^4-dafachronic acid (**1**) and (**2**) (Fig. 1).[2]

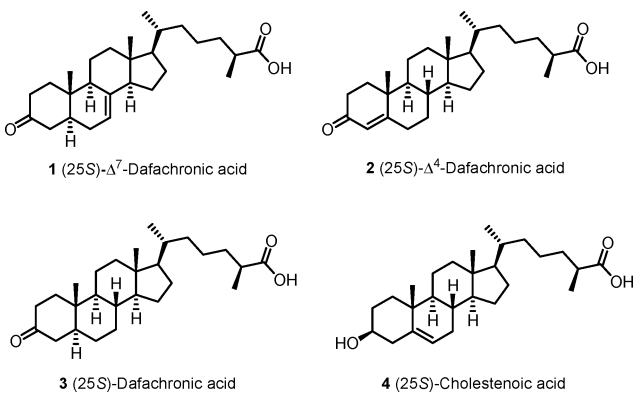

Fig. 1 (25*S*)-Cholestenoic-26-acids **1**–**4**.

The (25*R*)-dafachronic acids are much weaker ligands for DAF-12 compared with the 25*S*-isomers **1** and **2**.[2] Independently, Gill *et al.* identified (25*S*)-cholestenoic acid (**4**) as a ligand for DAF-12.[3] Recently, we described the total syntheses of the (25*R*)-dafachronic acids and (25*R*)-cholestenoic acid starting from diosgenin.[4] As we found that cholesterol can be replaced by cholestanol,[1] the saturated (25*S*)-dafachronic acid **3** appeared to be also an attractive target molecule. Herein, we report a flexible synthesis of the (25*S*)-configurated steroidal acids **1**–**4**.

Recently, Corey and Giroux described the synthesis of both diastereoisomers of **1**, which they called dafachronic acid A.[5,6] For the (25*S*)-diastereoisomer they started from β-stigmasterol using a diastereoselective ruthenium-catalyzed hydrogenation.[5] The (25*R*)-diastereoisomer was synthesized from β-ergosterol.[6] In an independent synthetic study, Khripach and co-workers reported the synthesis of both diastereoisomers of **2** and **4** using a diastereoselective elaboration of the steroid side chain.[7]

Starting from commercially available 3β-hydroxychol-5-en-24-oic acid (**5**) we have developed a highly stereoselective synthesis of one crucial intermediate providing access to all three (25*S*)-dafachronic acids **1**–**3** and (25*S*)-cholestenoic acid (**4**) as ligands for DAF-12. Transformation of **5** to the known aldehyde **6** was achieved by disilylation of **5** with *tert*-butyldimethylsilyl chloride followed by reduction of the silyl ester using lithium aluminium hydride (Scheme 1).[8] Swern oxidation of the alcohol provided the aldehyde **6** in 3 steps and 94% overall yield.[9] For introduction of the stereogenic center at C-25 we applied a stereoselective Evans aldol reaction using commercially available (*S*)-(+)-4-isopropyl-3-propionyl-2-oxazolidinone (**7**).[10] The resulting aldol product **8** was obtained in 95% yield as a single stereoisomer. Reduction of **8** using lithium borohydride followed by esterification of the C-26 hydroxy group with pivaloyl chloride provided compound **9** in high yield. For transformation into the natural products, removal of the hydroxy group at C-24 was required. Mesylation of **9** and subsequent reduction with lithium aluminium hydride afforded the desired alcohol along with the C24/C25-olefin as an inseparable mixture. However, Barton deoxygenation of **9** provided **10** in excellent yield.[11] It should be emphasized that radical removal of the C-24 hydroxy group occurred without detectable epimerization at C-25. The orthogonally diprotected (25*S*)-26-hydroxycholesterol **10**, which is available in 8 steps and 66% overall yield, represents our key intermediate and has been converted to all four (25*S*)-steroidal acids. Allylic oxidation of **10** provided the cholest-5-en-7-one **11** in 75% yield.[12] Palladium-catalyzed hydrogenation of **11** and subsequent reduction with L-Selectride® at −78 °C diastereoselectively provided the 7α-alcohol **12** in excellent yield. Elimination of **12** using thionyl chloride in pyridine[13] followed by removal of both protecting groups afforded the diol **13** in 71% overall yield. As the (25*S*)-26-hydroxy-Δ^7-cholesterol **13** is unprecedented in this series, we confirmed the configuration at C-25 by an X-ray crystal structure determination (Fig. 2).‡ Finally, Jones oxidation of **13** led to (25*S*)-Δ^7-dafachronic acid (**1**) in 89% yield.§

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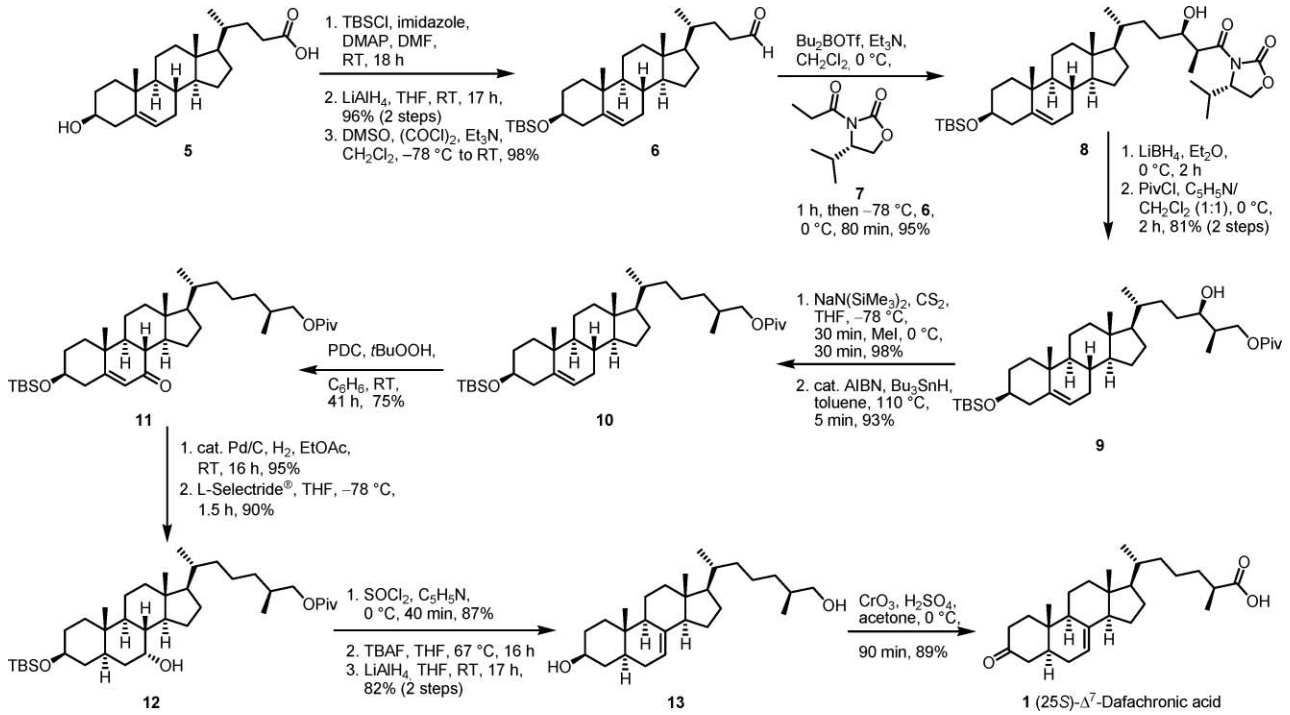
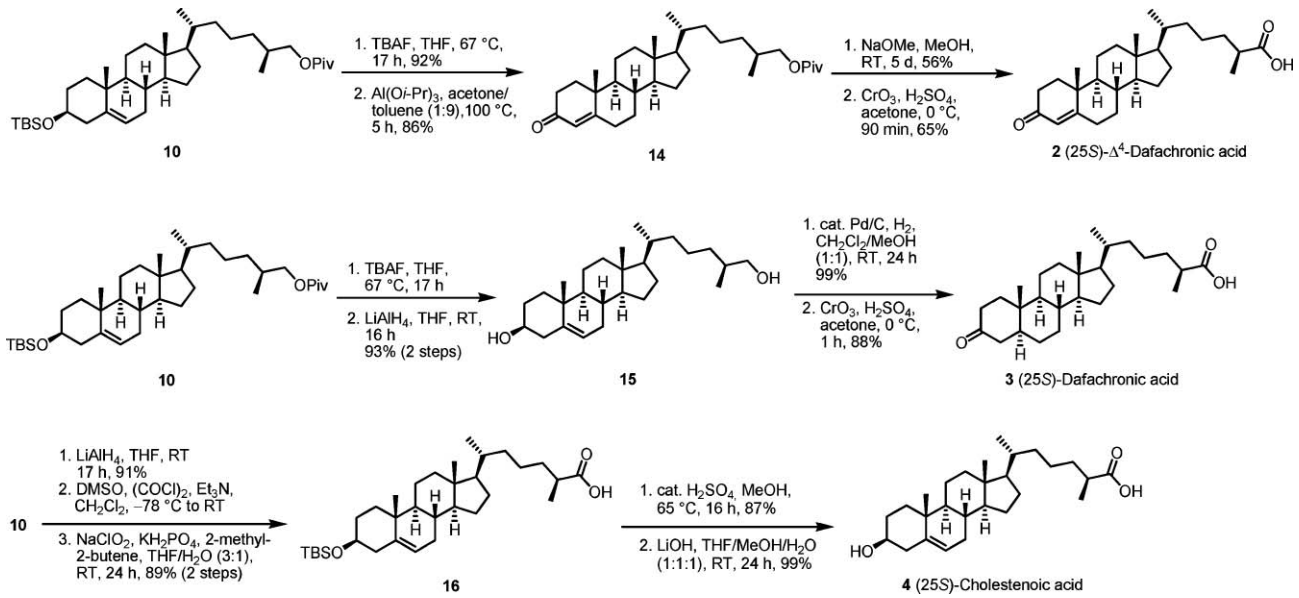


Scheme 1 Synthesis of the orthogonally diprotected diol **10** as the crucial intermediate and transformation into (25*S*)-Δ^7-dafachronic acid (**1**).



Scheme 2 Transformation of the crucial intermediate **10** into (25*S*)-Δ^4-dafachronic acid (**2**), (25*S*)-dafachronic acid (**3**), and (25*S*)-cholestenoic acid (**4**).

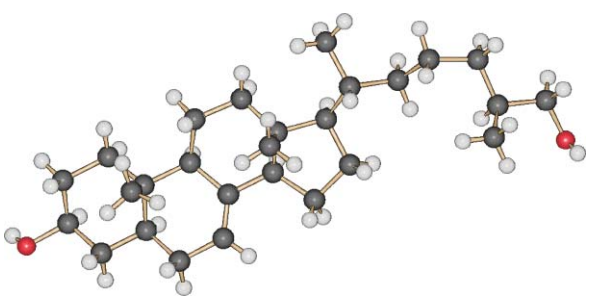


Fig. 2 Molecular structure of the diol **13** in the crystal form.

For the transformation of compound **10** into **2**, removal of the silyl protecting group and Oppenauer oxidation of the C-3 hydroxy group afforded the cholest-4-en-3-one **14**. Subsequent cleavage of the pivaloyl protecting group and Jones oxidation of the C-26 hydroxy group provided (25*S*)-Δ^4-dafachronic acid (**2**).¶

Sequential removal of both protecting groups by desilylation of **10** with TBAF followed by treatment with lithium aluminium hydride led to the known (25*S*)-26-hydroxycholesterol (**15**) (Scheme 2).[14] Catalytic hydrogenation of the diol **15** and subsequent Jones oxidation provided (25*S*)-dafachronic acid (**3**) in 81% yield over 4 steps.‖

For the synthesis of **4**, the pivaloyl protecting group of **10** was removed using lithium aluminium hydride. Subsequent Swern oxidation[9] of the C-26 hydroxy group and further oxidation with sodium chlorite led to the C-3 silyl-protected acid **16** in 81% yield over 3 steps. In the 25*R*-series, we found that chromatographic separation of cholestenoic acid proved to be very difficult.[4] Therefore, compound **16** was converted to the 3-hydroxy 26-methyl ester by treatment with catalytic amounts of concentrated sulfuric acid in methanol at reflux. After chromatographic purification, cleavage of the methyl ester with lithium hydroxide provided pure (25*S*)-cholestenoic acid (**4**) in 99% yield.**

In conclusion, we have developed a highly efficient and stereoselective synthesis of the crucial intermediate **10** which serves as precursor for the hormonally active steroids **1**–**4** in excellent yields (**1**: 15 steps, 27% overall yield; **2**: 12 steps, 19% overall yield; **3**: 12 steps, 53% overall yield; **4**: 13 steps, 46% overall yield). The Evans aldol reaction of aldehyde **6** as the key-step of our synthesis proceeded with complete stereoselectivity and provided compound **8** as a single stereoisomer, also on a large scale. Our approach is superior with respect to efficiency and overall yields as compared with the previously reported syntheses. Moreover, the present route is highly flexible as it provides all 4 steroidal acids in amounts of up to 1 g *via* a common intermediate and paves the way for detailed biological studies. Thus, it opened up a simple route to the non-natural (25*S*)-dafachronic acid (**3**). In a preliminary biological assay, the hormonal activity of **3** was comparable to that of the other (25*S*)-diastereoisomers **1**, **2**, and **4**. Further investigations in this direction are currently underway.

Acknowledgements

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Notes and references

‡ Crystallographic data for the diol **13**: $C_{27}H_{46}O_2$, $M = 402.64$ g mol^{-1}, crystal size: $0.27 \times 0.12 \times 0.10$ 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}, $\mu = 0.066$ mm^{-1}, $\lambda = 0.71073$ Å, $T = 223(2)$ K, θ range = 3.19–25.40°, reflections collected: 35264, independent: 2611 ($R_{int} = 0.0748$), 266 parameters. The structure was solved by direct methods and refined by full-matrix least-squares on F^2; final R indices [$I > 2\sigma(I)$]: $R_1 = 0.0468$; $wR_2 = 0.1065$; 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.

§ Characteristic spectroscopic data for (25*S*)-Δ^7-dafachronic acid (**1**): colorless solid, mp 139–143 °C; ^{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); anal. calc. for $C_{27}H_{42}O_3$: C 78.21, H 10.21; found: C 78.21, H 10.31%.

¶ Characteristic spectroscopic data for (25*S*)-Δ^4-dafachronic acid (**2**): colorless solid, mp 173–174 °C; ^{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); anal. calc. for $C_{27}H_{42}O_3$: C 78.21, H 10.21; found: C 78.21, H 10.12%.

‖ Characteristic spectroscopic data for (25*S*)-dafachronic acid (**3**): light yellow solid, mp 123–126 °C; ^{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_3), 46.67 (CH), 53.74 (CH), 56.15 (CH), 56.23 (CH), 182.70 (C=O), 212.41 (C=O); HRMS: m/z calc. for $C_{27}H_{44}O_3$ [M$^+$]: 416.3290; found: 416.3291.

** Characteristic spectroscopic data for (25*S*)-cholestenoic acid (**4**): colorless solid, mp 172–175 °C; ^{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); anal. calc. for $C_{27}H_{44}O_3$: C 77.83, H 10.64; found: C 77.99, H 10.77%.

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