Steroid hormones controlling the life cycle of the nematode *Caenorhabditis elegans*: stereoselective synthesis and biology

René Martin,[a] Eugeni V. Entchev,[b] Teymuras V. Kurzchalia[b] and Hans-Joachim Knölker*[a]

Received 8th September 2009, Accepted 26th October 2009
First published as an Advance Article on the web 3rd December 2009
DOI: 10.1039/b918488k

Cholesterol-derived hormones, the dafachronic acids, play a major role in controlling the life cycle and initiating dauer larva formation of the nematode *Caenorhabditis elegans*. This Perspective describes recent progress in the synthesis of these steroid hormones and their biological function.

Introduction

The nematode *Caenorhabditis elegans* is a widely used model organism in genetics, cell biology and biochemistry. *C. elegans* lives in soil. Simple cultivation, short life cycle and the fact that it contains relatively few but differentiated cells brought *C. elegans* quickly into the focus of biological research.[1,2] In 1998, the complete genome sequence of *C. elegans* was reported and more than 19000 genes have been revealed.[3] The life cycle of *C. elegans* has six recognized stages: embryo, first (L1) to fourth (L4) stage of larva and adult (Fig. 1). In addition, at specific points there are options for alternative developmental programmes or reversible arrest (diapause). Physiological and environmental factors, including food, temperature and population density, can pause or divert postembryonic development at several points.[4,5,6] For normal worm development it is essential that sterols are supplied exogenously, since nematodes are not able to synthesize sterols *de novo*.[7] Genes for squalene cyclase and squalene synthase are missing in nematodes. In fact, only the final products of the plant and mammalian biosynthetic sterol pathways (*e.g.* ergosterol, sitosterol, cholesterol or their close precursors) can fully support worm growth. Several processes in nematodes such as growth, locomotion, moulting and formation of enduring (dauer) larvae require sterols.[7] Under favourable conditions, the worms develop successively from embryos to reproductive adults in three days. In contrast, under non-favourable conditions, they remain as sexually immature larvae and enter an alternative third stage, the dauer diapause.[8] Dauer larvae are characterised by reorganization of body morphology and changes in metabolism which ensure increased stress resistance and longevity. Thus, by its morphology and behaviour, the dauer larva is specialised for extended diapause and dispersal, allowing survival until conditions improve. The formation of dauer larvae is regulated *via* the *da*uer-*f* ormation (*daf*) genes. Moreover, several ascarylose-derived pheromones have been identified which induce dauer larva formation.[9,10] Genetic studies have revealed three pathways (TGF-β, cyclic GMP and insulin-like IGF-1) controlling the formation of dauer larvae.[11] The genes *daf-9* and *daf-12* have been found to play a major role in this process. It has been shown that *daf-9* encodes the DAF-9 protein, a cytochrome P450 oxidase, which produces steroidal acid ligands for the hormonal receptor

[a]*Department Chemie, Technische Universität Dresden, Bergstrasse 66, 01069 Dresden, Germany. E-mail: hans-joachim.knoelker@tu-dresden.de; Fax: +49 351-463-37030*
[b]*Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauer-strasse 108, 01307 Dresden, Germany*

René Martin

René Martin studied chemistry at the Technical University of Dresden (2001–2006). He received his Ph.D. under the supervision of Professor H.-J. Knölker at the Technical University of Dresden, working on the synthesis of polyhalogenated pyrrole alkaloids as novel myosin AT-Pase inhibitors and on the synthesis of hormonally active steroids (2006–2008). Since 2009, he is working for ChiroBlock GmbH in Wolfen, Germany.

Eugeni V. Entchev

Eugeni V. Entchev received an M.Sc. in molecular biology from the University of Sofia, Bulgaria, in 1997. In 2002, he completed his Ph.D. at the Max Planck Institute for Molecular Cell Biology and Genetics in Dresden. Subsequently, he was as a postdoctoral fellow in the research group of Dr. T. V. Kurzchalia at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (2003–2009), where he investigated different aspects of lipid metabolism and lipid signaling using the nematode Caenorhabditis elegans. *Currently, he is a research associate at MRC Centre for Developmental Neurobiology, King's College London, UK.*

DAF-12.[12] In the presence of these ligands, DAF-12 is inactivated and the nematode undergoes normal reproductive development. However, in the absence of ligands, DAF-12 is activated and initiates the dauer larva formation programme. Thus, mutant worms lacking *daf-12* can never form dauer larvae and do not require DAF-12 ligands for reproductive development. The concentration of the steroidal acid ligands in wild-type worms is controlled by an irreversible methylation at C-4 of a sterol precursor compound. This remarkable methylation of the sterol nucleus regulates dauer larva formation and growth of the nematodes. Therefore, the present findings suggest potential ways to control the growth of pathogenic nematodes, which represent a serious problem for crops (*e.g.* potatoes) as well as for animal and human health.

Feeding *C. elegans* with cholestanol derivatives: synthesis and bioactivity

Substitution of dietary cholesterol (cholest-5-en-3β-ol) in the nematode growth medium by other naturally occurring or synthetic sterols shows whether these compounds can replace cholesterol in all or at least in some of the functions described above. Thus, feeding experiments with *C. elegans* revealed that cholesterol is completely substituted by cholestanol (5α-cholestan-3β-ol, dihydrocholesterol) (Fig. 2).[13,14] However, substitution of cholesterol by its natural metabolite lophenol (4α-methyl-5α-cholest-7-en-3β-ol) led to formation of regular dauer larvae. Addition of the non-methylated sterols cholesterol and lathosterol (5α-cholest-7-en-3β-ol) in amounts as low as 20 nM, prevented worms fed with lophenol from forming dauer larvae. The observation that small amounts of non-methylated sterols prevent dauer larva formation of worms fed with lophenol suggests that the dauer larva formation is caused by the lack of a cholesterol derivative which is required for reproductive development and which cannot be produced from lophenol. In order to investigate the possibility that 4α-methylation of sterols is necessary for dauer larva formation and lophenol itself induces this process, we required several derivatives of lophenol. We decided to use saturated sterols for our studies, since they are much more readily available than the Δ^7-sterols (Scheme 1).

Reduction of commercially available cholest-4-en-3-one (**1**) with lithium in liquid ammonia led to the lithium enolate **2**. Trapping of **2** with alkyl halides and subsequent reduction with lithium aluminium hydride provided stereoselectively lophanol (4α-methyl-5α-cholestan-3β-ol) (**3**) and 4α-ethyl-5α-cholestan-3β-ol (**4**). The structure of lophanol (**3**) has been confirmed by an X-ray crystal structure determination of its corresponding *para*-bromobenzoate (Fig. 3). Reaction of the enolate **2** with chlorotrimethylsilane afforded in high yield the trimethylsilyl enol ether **5**. The silyl enol ether **5** represents an excellent intermediate for the stereoselective synthesis of a variety of 4α-substituted cholestanols. Treatment of **5** with Selectfluor® and subsequent reduction with lithium aluminium hydride afforded 4α-fluoro-5α-cholestan-3β-ol (**6**). Reaction of **5** with *N*-bromosuccinimide (NBS) followed by reduction with sodium borohydride led to 4α-bromo-5α-cholestan-3β-ol (**7**).[15] The structure of the bromo derivative **7** has been unequivocally confirmed by an X-ray analysis of a single crystal (Fig. 4). 4α-Hydroxy-5α-cholestan-3β-ol (**8**) has been prepared by oxidation of the silyl enol ether **5** with *meta*-chloroperbenzoic acid (*m*-CPBA) and subsequent reduction using sodium borohydride. Feeding experiments with *C. elegans* confirmed that compounds **3**, **4**, **6**, **7** and **8** induce dauer larva formation (Table 1). This is a very interesting result, since fluoro- and also bromo-substituted compounds are not susceptible to biological modifications by living organisms. Therefore, 4α-fluoro-5α-cholestan-3β-ol (**6**) and 4α-bromo-5α-cholestan-3β-ol (**7**) cannot be methylated. The present results suggest that 4α-methylation *per se* is not required for dauer larva

Teymuras V. Kurzchalia

Teymuras V. Kurzchalia studied biochemistry at Tbilisi State University in Georgia (1971–1976) and he received his Ph.D. at the Institute of Protein Research in Pushtchino-na-Oke in Russia (1976–1981). Subsequently, he worked as a research scientist at the Institute of Plant Biochemistry in Tbilisi (1981–1983), the Department of Cell Biology of the Academy of Sciences, GDR (1983–1990), at the European Molecular Biology Laboratory in Heidelberg (1990–1992) and as a group leader at the Max Delbrück Centre for Molecular Medicine in Berlin (1992–1998). Since 1998, he is a group leader at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden. His research interests include the role of different classes of lipids in the functioning of the cell membrane and organism.

Hans-Joachim Knölker

Hans-Joachim Knölker studied chemistry at the Universities of Göttingen and Hannover (1978–1983) and received his Ph.D. under the direction of Prof. E. Winterfeldt at the University of Hannover (1983–1985). After postdoctoral studies with Prof. K. P. C. Vollhardt at the Department of Chemistry, University of California in Berkeley (1986), he returned to the University of Hannover for his habilitation (1987–1990). He was a Full Professor of Organic Chemistry at the University of Karlsruhe (1991–2001) and Chairman of the Chemistry Department at the University of Karlsruhe (1995–1997). Since 2001, he is Professor for Organic Chemistry at the Technical University of Dresden. He is a cofounder of the biotech company JADO Technologies in Dresden and an Ordinary Member of the Saxon Academy of Sciences. His research interests are organometallic chemistry, biomolecular chemistry and natural product synthesis.

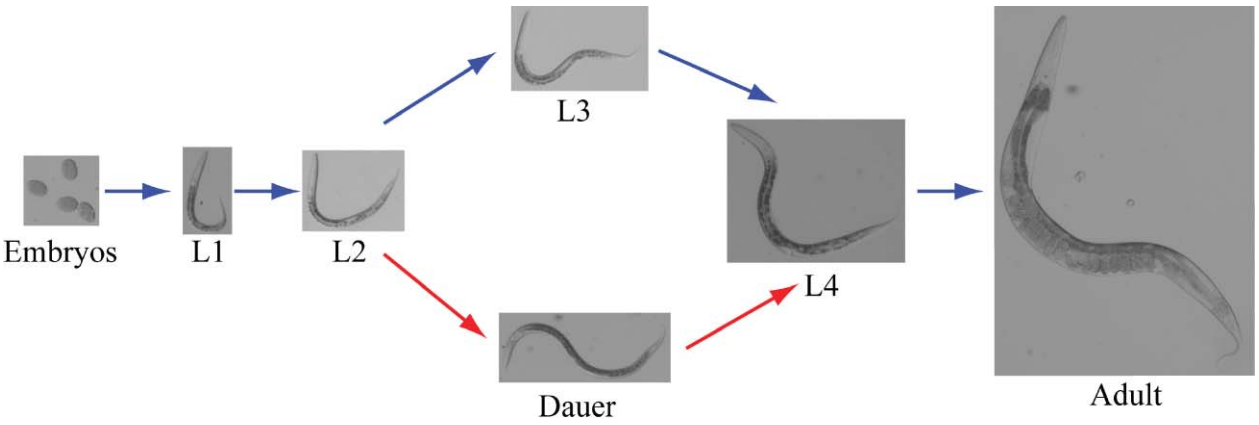
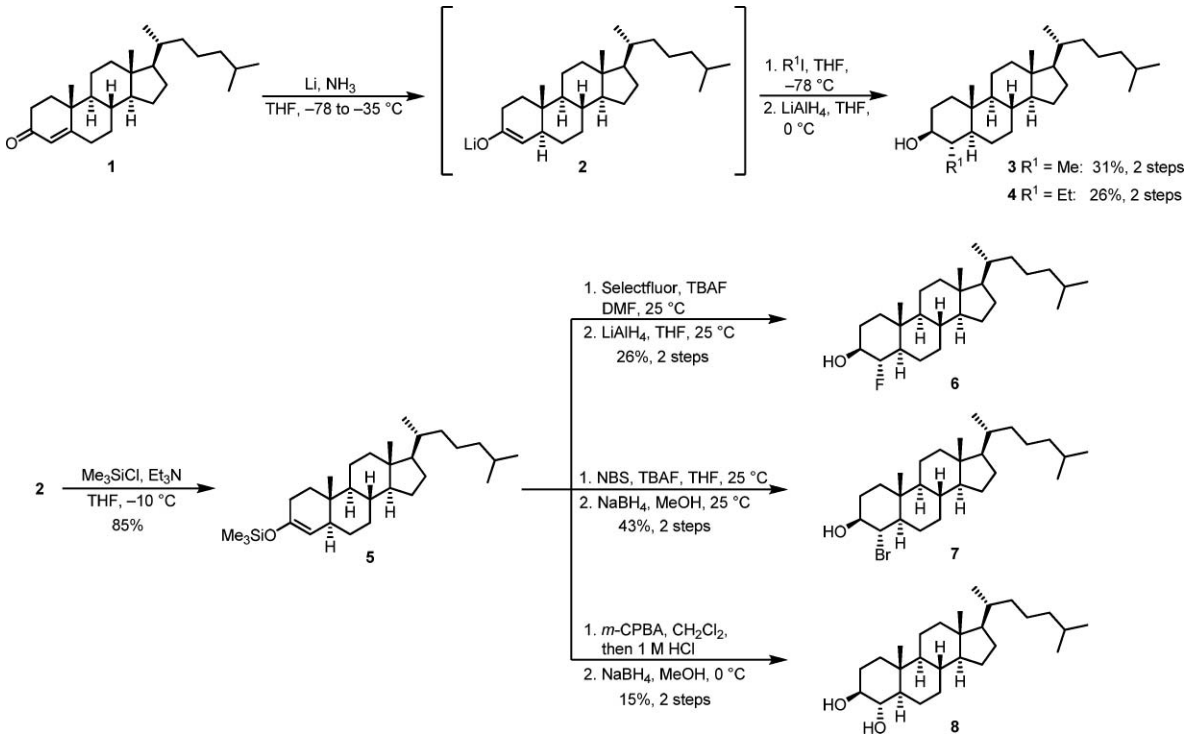


Fig. 1 Life cycle of *Caenorhabditis elegans*: embryos can develop *via* the larval stages L1 to L4 or the dauer larvae to reproductive adults.



Scheme 1 Stereoselective synthesis of the dauer-promoting 4α-substituted 5α-cholestan-3β-ols **3**, **4** and **6–8**.

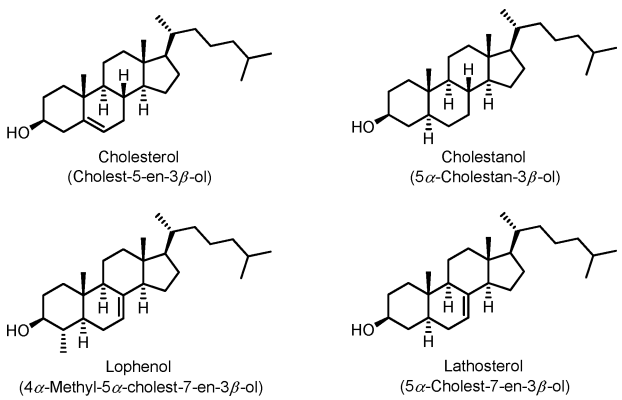


Fig. 2 Cholesterol, cholestanol, lophenol and lathosterol.

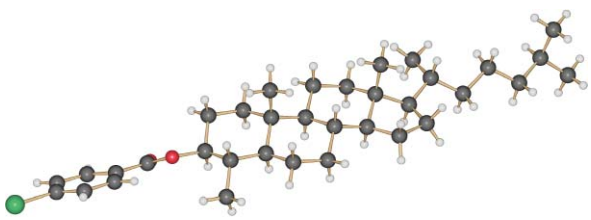


Fig. 3 Molecular structure of lophanyl *p*-bromobenzoate in the crystal.

formation. However, the availability of an unsubstituted 4-position at the sterol framework is obviously essential for biosynthesis of a hormone which promotes normal reproductive growth of nematodes. It is interesting to note, that we have identified several 4-substituted cholestanol derivatives which show a membrane domain-disrupting effect in model membranes.[16]

Table 1 Results for feeding experiments of *C. elegans* with cholestanols

Sterol	wild type	*daf-12* mutant
cholestanol	reproductive growth	reproductive growth
3, **4**, **6**, **7**, **8**, **11**, **12**, **13**, **14**, **16**	dauer larvae	reproductive growth
15, **17**	arrested larvae	sick adults

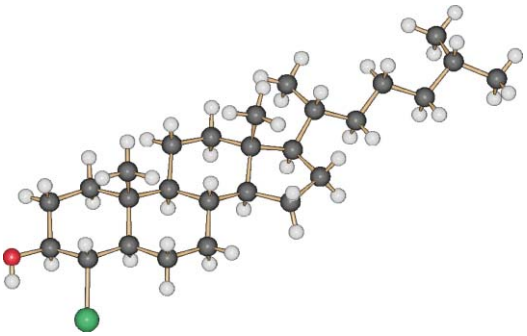


Fig. 4 Molecular structure of 4α-bromo-5α-cholestan-3β-ol (**7**) in the crystal.

Starting from 7-ketocholesterol (**9**) and 6-keto-5α-cholestan-3β-ol (**10**), we prepared the cholesterol derivatives **11–14** (Scheme 2). Remarkably, the sterols **11–14** have no substituents at C-4 and still promote the formation of dauer larvae when fed to *C. elegans* (Table 1). This result emphasizes that methylation at C-4 is not required for dauer larvae formation. Obviously, using the sterols **11–14** with substituents at C-6 and C-7, the production of the reproductive hormone is blocked in a different way. Therefore, we suggest that the natural metabolism of cholesterol in nematodes occurs in two distinct pathways. One pathway generates lophenol and the second leads to a steroid hormone which represents the ligand for the DAF-12 receptor and thus is required for reproductive growth. This steroid hormone cannot be produced from 4α-methylated sterols.[13,14] In agreement with these conclusions, *daf-12* mutant worms, which cannot form dauer larvae and do not need the reproductive growth hormone, show normal growth when fed with lophenol.

In order to gather information on the function of the methyl groups at the side chain of cholesterol for the development of nematodes, we decided to synthesize 21-nor-5α-cholestan-3β-ol (**15**), 27-nor-5α-cholestan-3β-ol (**16**) and 21,27-bisnor-5α-cholestan-3β-ol (**17**) (Fig. 5).[15] These compounds had not been described in the literature so far. Wittig reaction of 3-*epi*-androsterone with the appropriate alkylphosphonium halide followed by catalytic hydrogenation provided stereoselectively the cholestanols **15** and **17**. For the synthesis of 27-nor-5α-cholestan-3β-ol (**16**) 3β-(*tert*-butyldimethylsilyloxy)chol-5-en-24-al was used as starting material.[15] Feeding *C. elegans* with the norcholestanols **15–17** provided interesting information on the biological role of the methyl groups at the sterol side chain (Table 1). Wild-type worms grown on the norcholestanols **15** and **17** arrested as dauer-like larvae. Interestingly, feeding *daf-12* mutants of *C. elegans*, which lack the DAF-12 activity, with **15** and **17** resulted in the formation of sick adults. However, feeding wild-type *C. elegans* with 27-nor-5α-cholestan-3β-ol (**16**) resulted in formation of dauer larvae. Development of normal reproductive adults was observed when feeding *daf-12* mutant worms with compound **16**. These observations gave important insights into cholesterol metabolism of *C. elegans*. It became evident that the C-27 methyl group of cholesterol is required for the production of a steroidal hormone which binds to the DAF-12 receptor and thus, is promoting normal reproductive growth in wild-type worms of *C. elegans*. In contrast, the C-21 methyl group is of more general importance for worm development. Thus, feeding wild-type worms with the cholestanols **15** or **17** led to the formation of arrested dauer-like larvae and *daf-12* mutant worms fed with **15** or **17** developed only into sick adults.[15] These findings indicate that the C-21 methyl group is required for production of further, yet unidentified sterols, which are crucial for normal development.

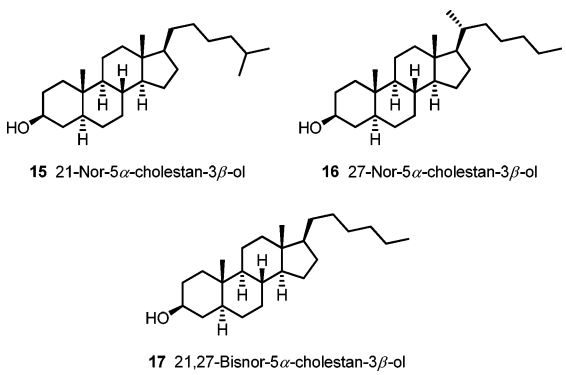
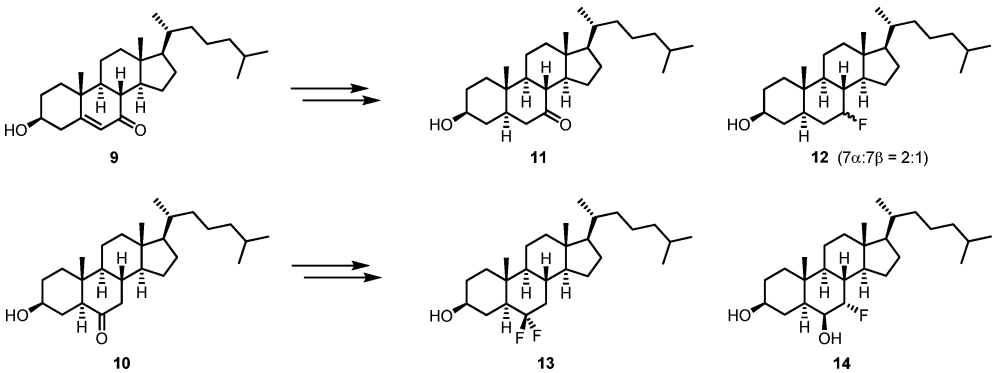


Fig. 5 Synthetic normethyl-5α-cholestan-3β-ols **15–17**.



Scheme 2 Stereoselective synthesis of the dauer-promoting B-ring substituted 5α-cholestan-3β-ols **11–14**.

Synthetic approaches to dafachronic acids—ligands for the hormonal receptor DAF-12

In 2006, the identification of sterol-derived ligands **18–23** for the hormonal receptor DAF-12 was reported by the groups of Mangelsdorf and Gill (Fig. 6).[17,18] These ligands were assigned to be (25*R*)- and (25*S*)-cholesten-26-oic acids differing in the position of the double bond (Δ^4, Δ^5 or Δ^7) and the oxidation state at C-3 (carbinol or ketone). The 3-ketosteroid ligands were designated as dafachronic acids. Regulation of reproductive growth by dafachronic acids controls the development to L3 larvae and represents an evolutionary conserved mechanism for formation of dauer and infective larvae, since it has been identified recently also in parasitic nematodes.[19] The development of infective larvae in several parasitic nematodes is blocked by (25*S*)-Δ^7-dafachronic acid (**19**) due to the inhibition of the dauer pathway. Therefore, dafachronic acids and related compounds may pave the way to novel strategies for controlling populations of parasitic nematodes and even to novel therapies for nematode infections.

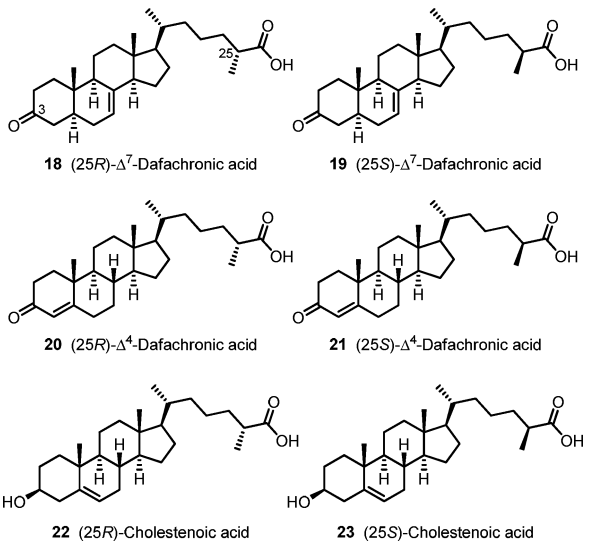
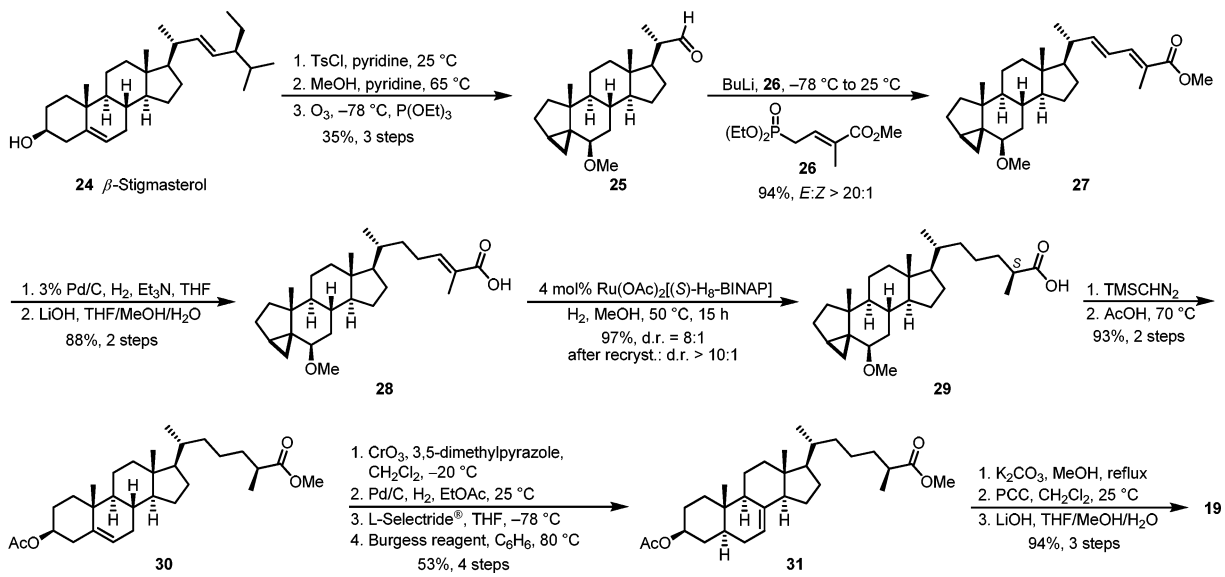


Fig. 6 Naturally occurring ligands for the hormonal receptor DAF-12: (25*R*)- and (25*S*)-cholesten-26-oic acids **18–23**.

Syntheses of sterols functionalised at C-26 have a long tradition and early work dates back to 1956, when Scheer *et al.* obtained (25*R*)-26-hydroxycholesterol by reduction of kryptogenin.[20] In 1975, Caspi and co-workers reported the stereoselective hydroboration of a cholest-5,25-diene and subsequent transformation into (25*S*)-26-hydroxycholesterol with about 83% optical purity (based on CD spectra).[21] They also described for the first time a synthesis of (25*R*)-cholestenoic acid (**22**) and (25*S*)-cholestenoic acid (**23**). Six years later, Gut *et al.* achieved a stereoselective synthesis of (25*S*)-26-hydroxycholesterol by using (2*S*)-3-*tert*-butoxy-2-methylpropan-1-ol as chiral synthon for the sterol side chain.[22] Santaniello *et al.* prepared (25*S*)-26-hydroxycholesterol starting from stigmasterol *via* a related approach.[23] Using a similar strategy, Khripach and co-workers described in 2005 the stereoselective synthesis of the steroidal acids **20–23**.[24] Mangelsdorf prepared (25*R*)-Δ^4-dafachronic acid (**20**) and (25*S*)-Δ^4-dafachronic acid (**21**) by Jones oxidation of the corresponding 26-hydroxycholest-4-en-3-ones.[17a]

The structure of (25*S*)-Δ^7-dafachronic acid (**19**) was unequivocally proven by the stereoselective synthesis reported by Corey and Giroux in 2007 (Scheme 3).[25] The key-step of Corey's approach to **19** is the stereoselective ruthenium-catalyzed hydrogenation of an α,β-unsaturated acid to generate the 25*S*-configuration. β-Stigmasterol (**24**) was converted to aldehyde **25** following a known three-step procedure. Horner–Wadsworth–Emmons reaction of **25** with the phosphonate **26** provided the (22*E*,24*E*)-diene ester **27** in 94% yield ($E:Z > 20:1$). Regioselective catalytic hydrogenation of the C-22/C-23 double bond and subsequent ester cleavage led to the α,β-unsaturated acid **28**. Homogenous hydrogenation of **28** using 4 mol% of Noyori's chiral catalyst $Ru(OAc)_2[(S)\text{-}H_8\text{-}BINAP]$[26] under an hydrogen atmosphere in methanol at 50 °C provided the (25*S*)-steroidal acid **29** in a diastereoisomeric ratio of 8 : 1 (97% yield). Recrystallisation of this mixture from diisopropyl ether increased the diastereoisomeric purity of **29** (d.r. > 10 : 1).



Scheme 3 Corey's synthesis of (25*S*)-Δ^7-dafachronic acid (**19**) starting from β-stigmasterol (**24**) (16 steps, 13% overall yield).[25]

Esterification of **29** with trimethylsilyldiazomethane followed by treatment with acetic acid afforded the 3β-acetoxy-Δ^5-steroidal ester **30**. Allylic oxidation of **30** with chromium trioxide in the presence of 3,5-dimethylpyrazole and subsequent hydrogenation of the resulting enone led to the saturated 7-ketosteroid. Reduction of the ketone with L-Selectride® to the 7α-hydroxysteroid followed by elimination with Burgess reagent afforded the Δ^7-steroid **31**. Finally, cleavage of the acetate, oxidation to the 3-ketosteroid with PCC and saponification of the methyl ester led to (25*S*)-Δ^7-dafachronic acid (**19**). The Corey synthesis provides **19** in 16 steps and 13% overall yield based on β-stigmasterol (**24**).[25]

Subsequently, Corey and Giroux described a synthesis of (25*R*)-Δ^7-dafachronic acid (**18**) starting from naturally occurring β-ergosterol.[27] Key-steps of this approach are the regioselective reduction of the C-5/C-6 double bond using lithium and *tert*-amyl alcohol in liquid ammonia and a stereoselective Claisen rearrangement for installation of the 25*R*-configuration. Thus, (25*R*)-Δ^7-dafachronic acid (**18**) was prepared in 10 steps and 13% overall yield starting from β-ergosterol.[27]

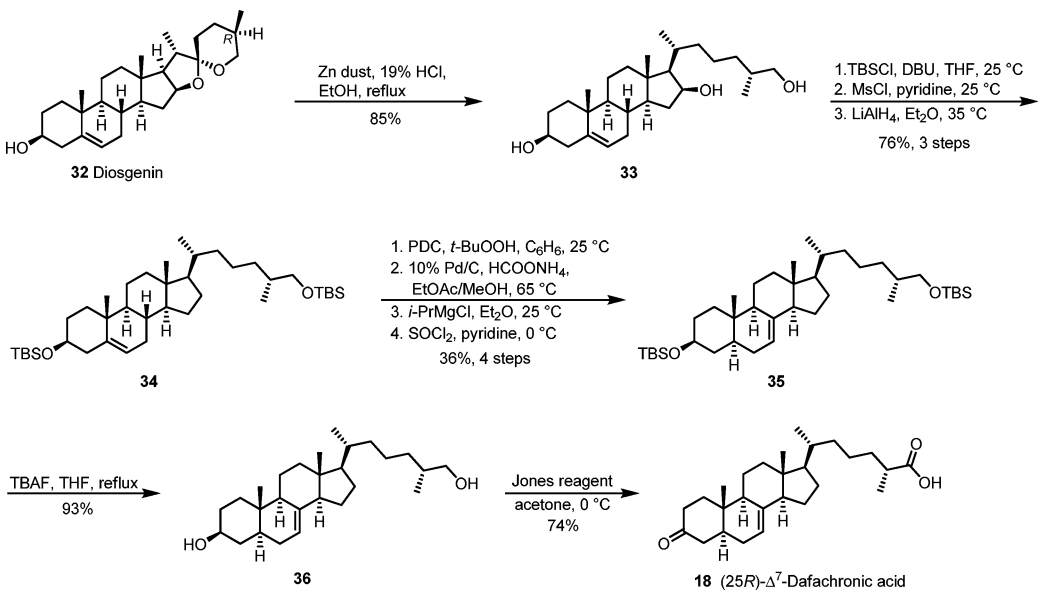
We thought that the most simple approaches to dafachronic acids would start from appropriate precursors with the desired configuration present in the starting material. Thus, during the course of our studies directed towards the synthesis of the dafachronic acids, we found an elegant approach to the (25*R*)-steroidal acids **18**, **20** and **22** starting from diosgenin (**32**) (Scheme 4).[28,29] Using a modified literature procedure,[30] Clemmensen reduction of diosgenin (**32**) provided the triol **33** in a high yield. Selective silylation of the two sterically less hindered hydroxy groups at C-3 and C-26 followed by reductive removal of the hydroxy group at C-16 *via* its mesylate led to the diprotected diol **34**. A virtual shift of the double bond in compound **34** from the 5,6-position to the 7,8-position has been achieved by the following sequence: Allylic oxidation of **34** to the corresponding cholest-5-en-7-one, transfer hydrogenation of the double bond, diastereoselective Grignard reduction using isopropylmagnesium chloride to the 7α-hydroxysteroid and elimination with thionyl chloride to afford the cholest-7-ene **35**. Removal of the silyl protecting groups with TBAF to **36** followed by Jones oxidation provided (25*R*)-Δ^7-dafachronic acid (**18**) in 10 steps and 16% overall yield.[28,29]

For the synthesis of the steroidal acids **20** and **22**, the triol **33** was converted to the regioselectively monoprotected diol **37** (Scheme 5). A differentiation between the three hydroxy groups of compound **33** was achieved by selective pivaloylation of the primary hydroxy group at C-26 in the presence of the secondary alcohols, subsequent selective silylation of the hydroxy group at C-3 and mesylation of the hydroxy group at C-16. Reduction of the mesylate with concomitant removal of the pivaloyl group afforded compound **37**. The monoprotected diol **37** represents a crucial intermediate in our total synthesis of (25*R*)-Δ^4-dafachronic acid (**20**) and (25*R*)-cholestenoic acid (**22**). Acetylation of **37** followed by removal of the silyl protecting group with TBAF led to (25*R*)-26-acetoxycholesterol which on Oppenauer oxidation afforded the enone **38**. Saponification of the ester and subsequent Jones oxidation of the intermediate 26-hydroxy compound provided (25*R*)-Δ^4-dafachronic acid (**20**) in 10 steps and 22% overall yield.[28,29]

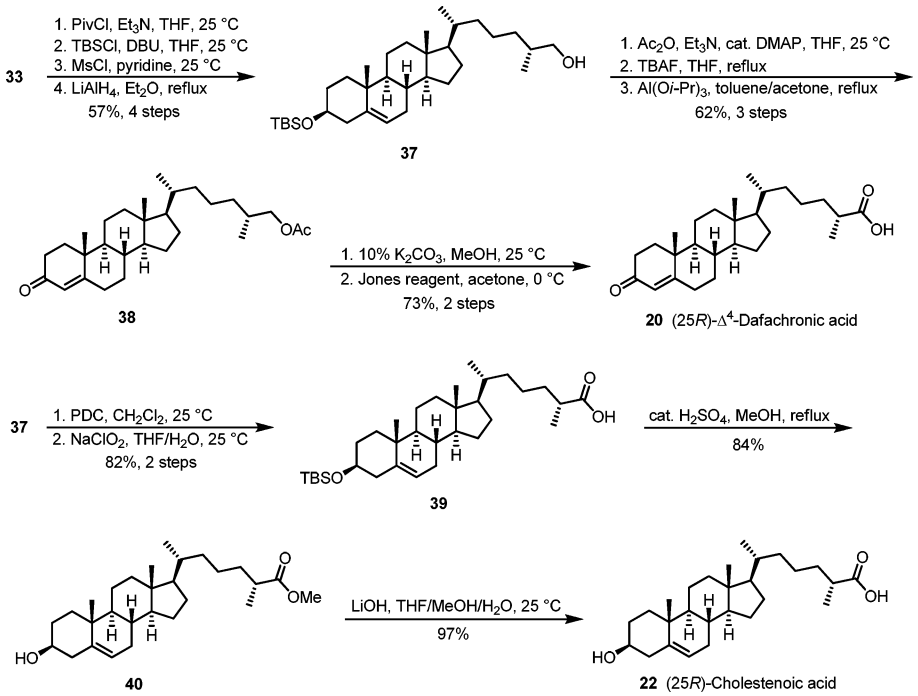
Oxidation of **37** using PDC and then treatment with sodium chlorite afforded the silyl-protected (25*R*)-cholestenoic acid **39** (Scheme 5). Reaction of compound **39** with catalytic amounts of concentrated sulfuric acid in methanol under reflux provided by concomitant cleavage of the silyl ether methyl (25*R*)-cholestenoate (**40**). Saponification of the methyl ester afforded (25*R*)-cholestenoic acid (**22**) in a total number of only 9 steps and 32% overall yield.[28,29]

In summary, starting from diosgenin (**32**), a cheap commercial steroidal sapogenin, we have developed a highly efficient synthesis of the (25*R*)-cholesten-26-oic acids **18**, **20** and **22**.[28,29] Our approach provides the hormonally active (25*R*)-steroidal acids in excellent overall yields even on large scale and thus, has been subsequently applied by others as well.[31]

Starting from yamogenin, the (25*S*)-diastereoisomer of diosgenin (**32**),[32] our methodology described above would lead to the corresponding (25*S*)-steroidal acids. However, compared to diosgenin, the occurrence of yamogenin in nature is rare. Therefore,

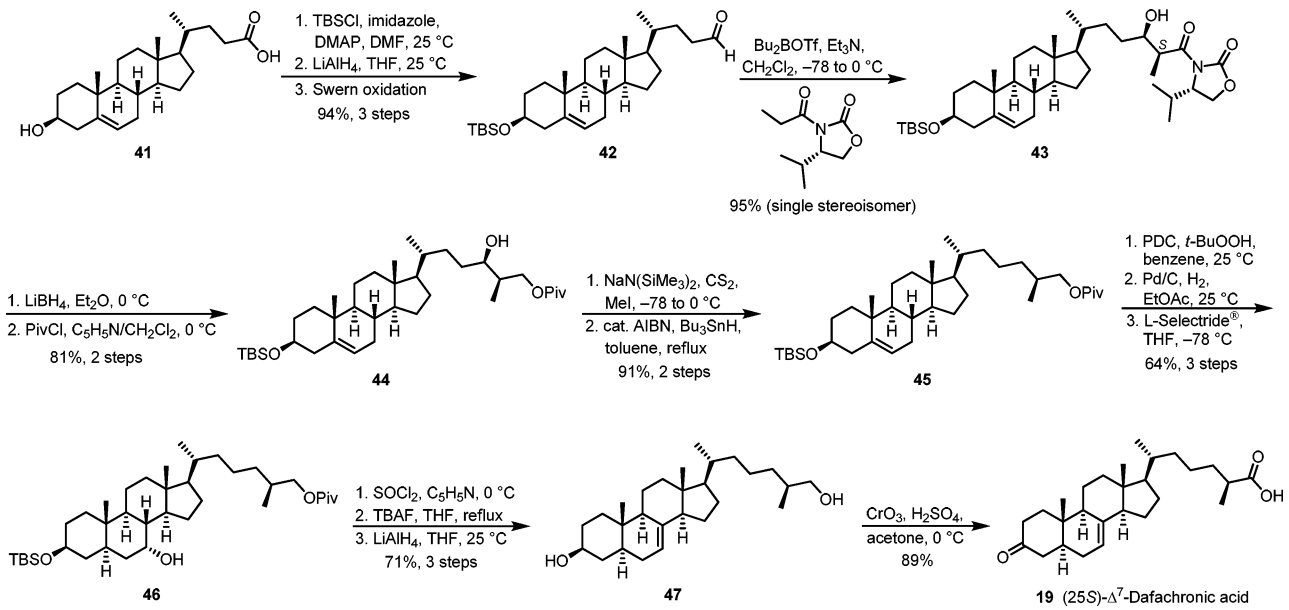


Scheme 4 Knölker's synthesis of (25*R*)-Δ^7-dafachronic acid (**18**) starting from diosgenin (**32**) (10 steps, 16% overall yield).[28,29]



Scheme 5 Synthesis of (25*R*)-Δ^4-dafachronic acid (**20**) (10 steps, 22% overall yield) and (25*R*)-cholestenoic acid (**22**) (9 steps, 32% overall yield).[28,29]

we envisaged to establish the 25*S*-configuration in the cholesteryl side chain using a stereoselective Evans aldol reaction.[33] 3*β*-Hydroxychol-5-en-24-oic acid (**41**) was found to be the perfect starting material (Scheme 6).[34,35] Using a three-step procedure of *O*-silylation, reduction and Swern oxidation, the acid **41** was converted to the aldehyde **42**. An Evans aldol reaction of **42** under standard conditions afforded compound **43** in 95% yield as a single stereoisomer even on large scale (up to 6 g). Removal of the chiral auxiliary followed by selective pivaloylation of the primary hydroxy group at C-26 provided compound **44**. Deoxygenation at C-24 using the classical Barton–McCombie procedure afforded compound **45**. It is important to note that the removal of the hydroxy group at C-24 *via* its xanthogenate under radical reaction conditions proceeded without detectable epimerisation at the adjacent stereogenic centre (C-25). The orthogonally protected diol **45** is available in 8 steps and 66% overall yield starting from a commercially available precursor. Compound **45** has been successfully transformed into all four (25*S*)-cholesten-26-oic acids described below and thus, is the key intermediate of our synthesis. Moreover, we regard the orthogonally protected diol **45** as an excellent intermediate for the synthesis of further cholesterols with an 25*S*-stereogenic centre.



Scheme 6 Knölker's synthesis of (25*S*)-Δ^7-dafachronic acid (**19**) (15 steps, 27% overall yield) *via* the orthogonally protected diol **45**.[34,35]

For the synthesis of (25*S*)-Δ^7-dafachronic acid (**19**) a shift of the double bond from the 5,6-position to the 7,8-position was required (Scheme 6). Allylic oxidation of compound **45** to the corresponding enone followed by catalytic hydrogenation and stereoselective reduction using L-Selectride® provided the 7α-hydroxy compound **46**. Dehydration of the alcohol **46** with thionyl chloride in pyridine to the cholest-7-ene was followed by removing first the silyl and second the pivaloyl protecting group to afford (25*S*)-26-hydroxy-5α-cholest-7-en-3β-ol (**47**). It is noteworthy, that a reversal of the sequence for removal of the protecting groups resulted in a lower yield for this two-step transformation (69% instead of 82% yield). An X-ray crystal structure determination of the diol **47** unequivocally confirmed the structural assignment for this sterol including the *S*-configuration at C-25 (Fig. 7). Jones oxidation of **47** led directly to (25*S*)-Δ^7-dafachronic acid (**19**).[34,35] Our synthesis provides **19** in 15 steps and 27% overall yield based on commercial 3β-hydroxychol-5-en-24-oic acid (**41**) and thus, is superior to alternative procedures.[24,25]

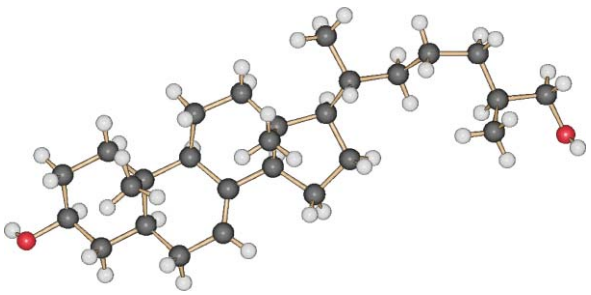
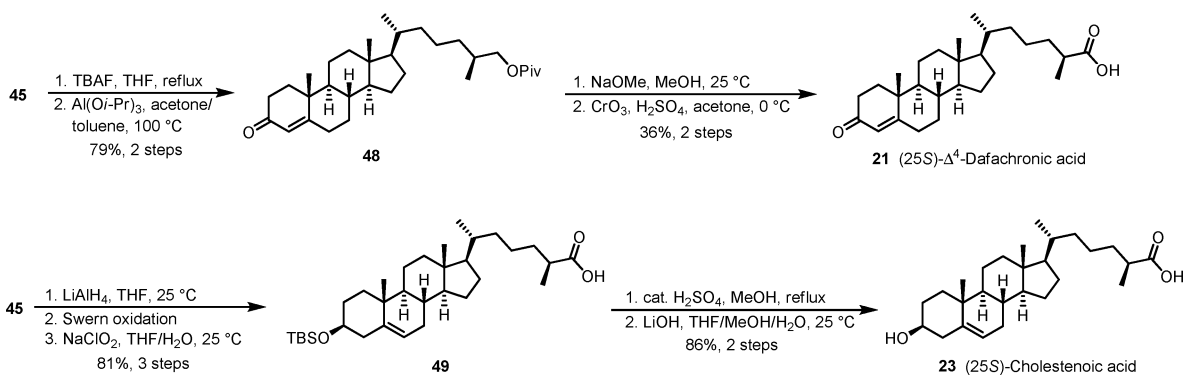


Fig. 7 Molecular structure of the diol **47** in the crystal.

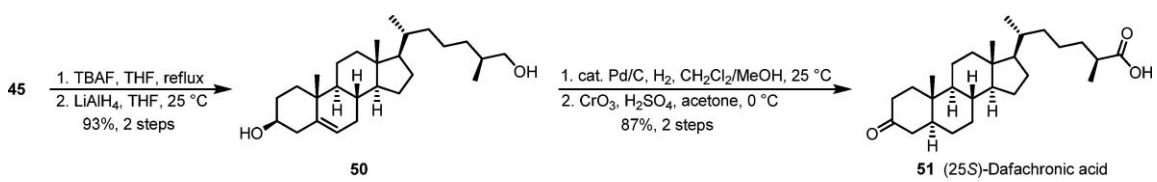
The conversion of our intermediate **45** to (25*S*)-Δ^4-dafachronic acid (**21**) requires only four synthetic steps. Removal of the silyl protecting group and subsequent Oppenauer oxidation afforded by concomitant isomerisation of the double bond the cholest-4-en-3-one **48**. Cleavage of the pivalate followed by Jones oxidation provided (25*S*)-Δ^4-dafachronic acid (**21**) in 12 steps and 19% overall yield based on compound **41**.[34,35]

Starting from intermediate **45**, (25*S*)-cholestenoic acid (**23**) was available *via* the following five-step sequence (Scheme 7). Removal of the pivaloyl protecting group followed by sequential oxidation of the resulting 26-hydroxy compound first to the aldehyde and then to the acid provided the silyl-protected (25*S*)-cholestenoic acid **49**. Esterification of **49** with concomitant cleavage of the silyl ether by treatment with methanol in the presence of catalytic amounts of sulfuric acid provided methyl (25*S*)-cholestenoate, which on saponification afforded (25*S*)-cholestenoic acid (**23**). Our route provides **23** in 13 steps and excellent 46% overall yield starting from the commercial precursor **41**.[34,35]

In our previous studies, we have found that cholesterol can be completely substituted by its saturated analogue dihydrocholesterol (cholestanol).[13,14] On feeding experiments with *C. elegans*, wild-type and *daf-12* mutant worms both showed normal reproductive growth when fed with either cholesterol or dihydrocholesterol. Thus, the presence of the double bond had no effect on the development of the nematodes in their life cycle. Therefore, we considered the saturated (25*S*)-dafachronic acid as a potential new ligand for the DAF-12 receptor. Our central synthetic intermediate, the orthogonally protected diol **45**, represents also a precursor for this non-natural ligand (Scheme 8). Sequential deprotection by removal of first the silyl group with TBAF and then the pivaloyl group with lithium aluminium hydride afforded (25*S*)-26-hydroxycholesterol (**50**) in 93% yield. As described above for the deprotection of compound **46**, a reversal of this deprotection sequence by first removing the pivaloyl and then the silyl group led to a considerably lower yield of the product (69%). Catalytic hydrogenation of the diol **50** and subsequent Jones oxidation provided (25*S*)-dafachronic acid (**51**) in only 12 steps and 53% overall yield based on compound **41**.[34,35] An alternative synthesis, recently reported by Corey and co-workers, afforded the saturated steroidal acid **51** in 13 steps and 8% overall yield based on β-stigmasterol (**24**).[36]



Scheme 7 Conversion of **45** to (25*S*)-Δ^4-dafachronic acid (**21**) (12 steps, 19% overall yield) and (25*S*)-cholestenoic acid (**23**) (13 steps, 46% overall yield).



Scheme 8 Transformation of the orthogonally protected diol **45** into (25*S*)-dafachronic acid (**51**) (12 steps and 53% overall yield based on **41**).[34,35]

Table 2 Comparison of characteristic ^{1}H NMR and ^{13}C NMR data and specific rotation values for 25*R*- and 25*S*-cholesten-26-oic acids **18–23**, and **51**[29,35]

Compound	δ ([C-27]H_3)[a]	δ (C-26)[b]	δ (C-27)[b]	$[\alpha]_D^{20}$[c]
(25*R*)-Δ^7-dafachronic acid (**18**)	1.17 (d, J = 7.0 Hz)	182.51	16.72	+13.3 (*c* 0.50, $CHCl_3$)
(25*R*)-Δ^4-dafachronic acid (**20**)	1.16 (d, J = 6.9 Hz)	182.48	16.74	+66.0 (*c* 1.0, $CHCl_3$)
(25*R*)-cholestenoic acid (**22**)	1.16 (d, J = 6.9 Hz)	182.46	16.74	–30.0 (*c* 0.10, $CHCl_3$)
(25*S*)-Δ^7-dafachronic acid (**19**)	1.17 (d, J = 7.0 Hz)	182.55	17.01	+33.9 (*c* 0.49, $CHCl_3$)
(25*S*)-Δ^4-dafachronic acid (**21**)	1.17 (d, J = 6.9 Hz)	182.35	17.01	+61.9 (*c* 0.47, $CHCl_3$)
(25*S*)-cholestenoic acid (**23**)	1.17 (d, J = 7.0 Hz)	181.58	17.02	–22.9 (*c* 0.14, $CHCl_3$)
(25*S*)-dafachronic acid (**51**)	1.17 (d, J = 7.0 Hz)	182.70	16.99	+44.9 (*c* 1.0, $CHCl_3$)

[a] ^{1}H NMR signal (500 MHz, $CDCl_3$) for the (C-27)H_3 group [ppm]. [b] ^{13}C NMR signals (125 MHz, $CDCl_3$) for C-26 and C-27 [ppm]. [c] Specific rotation.

By the two elegant routes described above, we have obtained the complete series of the naturally occurring (25*R*)- and (25*S*)-cholesten-26-oic acids **18–23** and also the synthetic (25*S*)-dafachronic acid (**51**). Thus, we have compared the characteristic spectroscopic and physical data for both diastereoisomeric series (Table 2). There are no clear differences between the (25*R*)- and the (25*S*)-steroidal acids for the ^{1}H NMR signal of the methyl group (C-27) at the stereogenic centre and for the ^{13}C NMR signal of the carboxyl group (C-26). However, it is remarkable that the ^{13}C NMR signal of the methyl group (C-27) shows a significant downfield shift for the (25*S*)-cholesten-26-oic acids by an average value of 0.28 ppm as compared to their corresponding (25*R*)-diastereoisomers. The comparison of the specific rotation values for the steroidal acids **18–23** and **51** revealed another interesting feature. The dafachronic acids **18–21** from both diastereoisomeric series (25*R* and 25*S*) and **51**, all with a 3-keto group, have a positive value for the specific rotation. In contrast, for (25*R*)-cholestenoic acid (**22**) and (25*S*)-cholestenoic acid (**23**), both with a 3β-hydroxy group, we found a specific rotation with a negative value (Table 2).

We have investigated and compared the hormonal activities of the different cholesten-26-oic acids by the rescue of *daf-9* mutant worms from dauer arrest (Fig. 8 and 9).[29,35] The *daf-9*(*dh6*) mutant worms are lacking the activity of the protein DAF-9, the cytochrome P450 which is involved in the production of dafachronic acids. Therefore, these mutant worms arrest as dauer-like larvae. In our experiment, some *daf-9*(*dh6*) mutant worms were obtained as progeny from the strain *daf-9*(*dh6*);*dhEx24*.[12] The *daf-9*(*dh6*) mutant worms are identified by the absence of

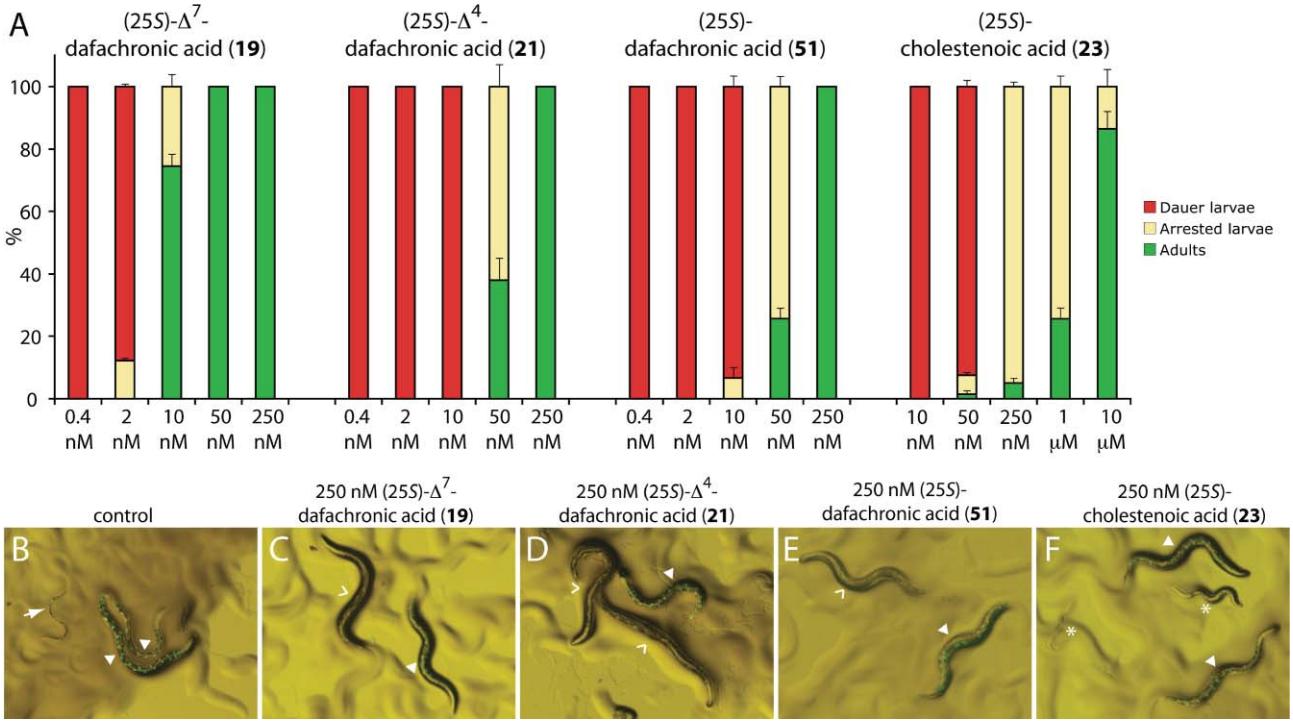


Fig. 8 Hormonal activity of the (25*S*)-dafachronic acids **19**, **21** and **51** and of (25*S*)-cholestenoic acid (**23**). White triangles (▲) indicate the fluorescent *daf-9*(*dh6*);*dhEx24* mutant worms which develop into adults without the requirement of exogenous (25*S*)-dafachronic acids or (25*S*)-cholestenoic acid. **A**: rescue of the *daf-9*(*dh6*) mutant worms from diapause by feeding with the indicated (25*S*)-cholesten-26-oic acids. **B**: without addition of (25*S*)-cholesten-26-oic acids the *daf-9*(*dh6*) mutant worms (no fluorescence) arrest as dauer-like larvae (white arrow). **C**–**E**: 50 nM of (25*S*)-Δ^7-dafachronic acid (**19**) and 250 nM of the (25*S*)-dafachronic acids **21** and **51** rescue the *daf-9*(*dh6*) mutant worms to adults (∧). **F**: 250 nM of (25*S*)-cholestenoic acid (**23**) rescue the *daf-9*(*dh6*) mutant worms only partially, forming arrested larvae often with moulting defects (*).

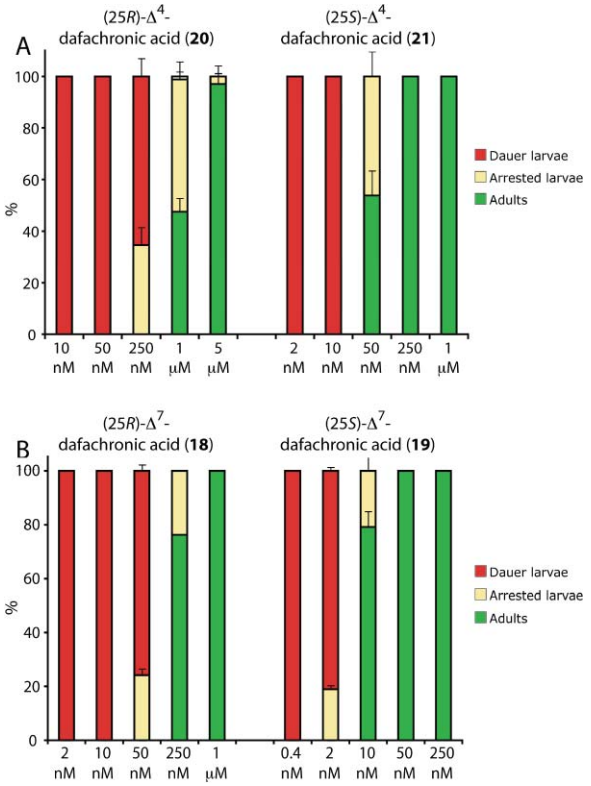


Fig. 9 Comparison of the hormonal activities of the 25*R*-series with the 25*S*-series for Δ^4-dafachronic acid (**A**) and Δ^7-dafachronic acid (**B**).

the green fluorescence, which is carried by the extrachromosomal array *dhEx24*. For the parental strain *daf-9*(*dh6*);*dhEx24*, the extrachromosomal array *dhEx24* rescues the *daf-9*(*dh6*) mutant worms (Fig. 8B). A normal reproductive development of *daf-9*(*dh6*) mutant worms is possible only by exogenous supply of dafachronic acid (Fig. 8A–E). If the supplied amount of dafachronic acid is not sufficient for complete rescue, arrested larvae, often with moulting defects, are generated (Fig. 8A and F). The results emphasize that the rescue of *daf-9*(*dh6*) mutant worms from dauer arrest depends on the concentration of the steroidal acids which are supplied. The rescue of *daf-9*(*dh6*) mutant worms from dauer arrest by feeding with (25*S*)-steroidal acids shows the differences in activity for these ligands (Fig. 8A). (25*S*)-Δ^7-Dafachronic acid (**19**) is the most potent inhibitor for the DAF-12 receptor. The non-natural (25*S*)-dafachronic acid (**51**) is also an efficient inhibitor for DAF-12 with an activity comparable to that of (25*S*)-Δ^4-dafachronic acid (**21**). In the series of the (25*S*)-steroidal acids, (25*S*)-cholestenoic acid (**23**) shows the lowest activity as ligand for the DAF-12 receptor.

Moreover, we have compared the biological activities of the (25*S*)-dafachronic acids with those of their corresponding (25*R*)-diastereoisomers (Fig. 9). As known for other nuclear receptor ligands, the stereochemistry of the DAF-12 ligand is an important factor for binding. Thus, we found that the (25*S*)-dafachronic acids are significantly more potent DAF-12 ligands as compared to the (25*R*)-dafachronic acids. Only very little hormonal activity was found for (25*R*)-cholestenoic acid (**22**) and thus, this ligand is not considered in this comparison. (25*S*)-Δ^4-Dafachronic acid (**21**) is about 20 times more potent than (25*R*)-Δ^4-dafachronic acid (**20**) (Fig. 9A). (25*S*)-Δ^7-Dafachronic acid (**19**) is about 25 times more potent than (25*R*)-Δ^7-dafachronic acid (**18**) (Fig. 9B). In summary, the potency of (25*S*)-Δ^4-dafachronic acid (**21**) and (25*S*)-dafachronic acid (**51**) as DAF-12 ligands is about the same and is approximately 20 times higher than observed for (25*S*)-cholestenoic acid (**23**) and (25*R*)-Δ^4-dafachronic acid (**18**) (Fig. 8 and 9).

STRM-1—a novel methyltransferase regulates dauer larva formation in *C. elegans* by methylation of the sterol nucleus

More than 25 years ago, it has been shown that nematodes produce 4α-methylated sterols from cholesterol.[37] However, the mechanism and biological significance of this sterol methylation remained unknown. The fact that feeding *C. elegans* with 4α-methylated sterols leads to dauer larva formation suggested that sterol methylation and dauer larva formation might be physiologically linked.[7b,13] Recently, we have identified the methylating enzyme, a novel type of sterol methyltransferase: STRM-1 (*St*erol A-*R*ing *M*ethyltransferase), which is responsible for the production of 4α-methylated sterols in *C. elegans*.[38] Mutant worms of the type *strm-1*(*tm1781*) are lacking the sterol methyltransferase STRM-1 and thus, cannot produce 4α-methylated sterols. Studying these mutant worms allowed us to unravel the biological importance of 4α-methylation. We could show that mutant worms of the type *strm-1*(*tm1781*) have reduced capability for dauer larva formation. STRM-1 and the cytochrome P450 protein DAF-9 use the same substrates to produce 4α-methylated sterols and dafachronic acids, respectively.[38] Consistently, *strm-1*(*tm1781*) mutant worms have increased concentrations of dafachronic acids which explains the inefficiency of dauer larva formation for these mutant worms. Therefore, by using the same substrates as required for the biosynthesis of dafachronic acids, STRM-1 can reduce the concentration of the hormone which is promoting reproduction. Thus, methylation of sterols at the 4α-position represents a novel mechanism for regulation of dauer larva formation in nematodes. The process of sterol methylation is an evolutionary conserved process in nematodes which has been confirmed by the presence of 4α-methylated sterols on metabolic labelling in the insect parasitic nematode *Steinernema feltiae*. Moreover, sequences homologous to *strm-1* have been also found in *Brugia malayi*, a parasitic nematode causing lymphatic filariasis. With more than one billion people infected, parasitic nematodes are causing a tremendous worldwide health problem. The uniqueness of 4α-sterol methylation and its importance for regulating nematode life cycle suggests STRM-1 as potential target for novel therapeutic strategies to treat nematode infections.[38]

Synthesis of an ascaroside pheromone

Recently, several pheromones which induce dauer larva formation of *C. elegans* have been isolated and synthesized.[9,10] As a common structural feature, these pheromones represent glycosides of the 3,6-dideoxysugar ascarylose (**52**) (Fig. 10). In 2005, Paik *et al.* described the isolation and synthesis of the first compound in this series and called it daumone (**53**).[9] An alternative synthesis of daumone (**53**) has been subsequently reported by O'Doherty and co-workers.[39] This pheromone initiates diapause entry of

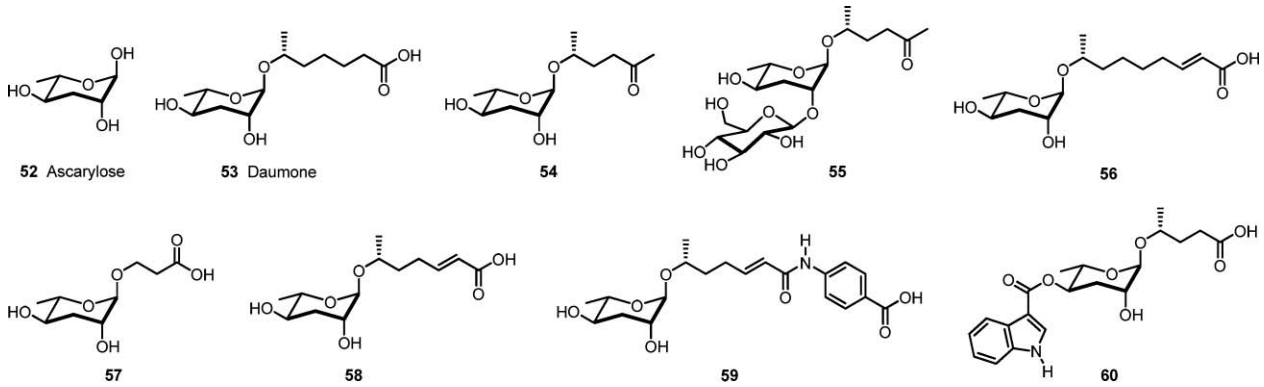
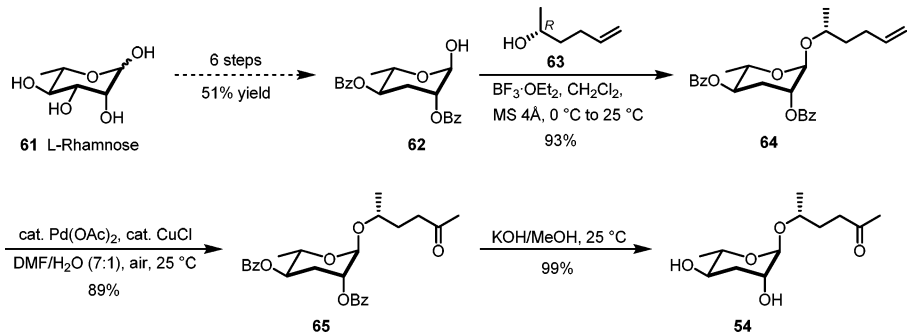


Fig. 10 Pheromones deriving from the 3,6-dideoxysugar ascarylose (**52**): daumone (**53**) and the ascarosides **54–60**.



Scheme 9 Synthesis of the ascaroside **54** *via* Wacker oxidation of **64** as key-step (3 steps and 82% yield based on **62**).[40]

C. elegans. More recently, the identification and synthesis of a series of different ascarosides **54–60** has been reported (Fig. 10).[10] It has been demonstrated, that the ascarosides **54–56** exhibit a significantly stronger dauer-promoting activity than daumone (**53**) itself.[10] Moreover, the blend of ascarosides **54–56** regulates dauer larva formation and male attraction. At concentrations (pM) more than 10 000 times lower than required for dauer induction (nM–μM), ascarosides **54–56** function as male attractants. At higher concentrations (nM–μM), the male attractant effect is lost and hermaphrodites are deterred.[10c]

The methyltransferase STRM-1 is regulating the dauer larva formation by controlling the *in vivo* concentration of dafachronic acids.[38] To confirm this mechanism, we required sufficient amounts of an ascaroside pheromone which is involved in the process leading to formation of dafachronic acids. Thus, we have developed an optimised synthetic route to the ascaroside **54**.[40] Starting from L-rhamnose (**61**), 2,4-di-*O*-benzoylascarylose (**62**) is readily available in 6 steps and 51% overall yield (Scheme 9).[9,40,41] Compound **62** represents an important intermediate for the synthesis of daumone (**53**)[9] as well as the ascarosides **54–60**.[10] Clardy *et al.* have obtained the ascaroside **54** in 4 steps starting from compound **62**.[10a] In contrast, our synthetic approach requires only 3 steps from **62** and proceeds in a considerably higher overall yield (Scheme 9). Glycosidation of **62** with (2*R*)-hex-5-en-2-ol (**63**) in the presence of boron trifluoride etherate afforded the hexenyl ascaroside **64** in 93% yield. The olefin **64** was subjected to Wacker oxidation using catalytic amounts of palladium(II) acetate and cuprous chloride in the presence of air providing compound **65** in high yield. Final saponification of **65** afforded quantitatively the ascaroside **54**. A comparison with Clardy's route to **54** (4 steps and 36% yield based on **62**),[10a] emphasizes the efficiency of our approach which affords the ascaroside **54** in only 3 steps and 82% overall yield starting from 2,4-di-*O*-benzoylascarylose (**62**).[40]

Conclusions

The developmental switch between reproductive life cycle and the entry into diapause of the nematode *C. elegans* is regulated by the nuclear hormonal receptor DAF-12. The dafachronic acids, cholesterol-derived hormonal ligands for DAF-12, inactivate DAF-12 and the nematodes show a normal reproductive development. By the absence of these hormonal ligands, DAF-12 is activated and the nematodes generate dauer larvae. The sterol ring methylating enzyme STRM-1 controls the concentration of dafachronic acids *in vivo* and thus, regulates dauer larva formation and growth of nematodes. Our findings suggest STRM-1 as a potential target for the development of novel therapies for infections with pathogenic nematodes. The design and execution of elegant stereoselective syntheses for the dafachronic acids paved the way to study the biological function of these hormonal ligands. Thus, the work summarised in this review has led to highly interesting and fundamental results in developmental biology and emphasizes the importance of modern organic synthesis at the interface between chemistry and biology.

References

1 S. Brenner, *Genetics*, 1974, **77**, 71–94.
2 R. A. Ankeny, *Nat. Rev. Genet.*, 2001, **2**, 474–479.
3 The *C. elegans* Sequencing Consortium, *Science*, 1998, **282**, 2012–2018.
4 J. W. Golden and D. L. Riddle, *Dev. Biol.*, 1984, **102**, 368–378.

5 T. E. Johnson, D. H. Mitchell, S. Kline, R. Kemal and J. Foy, *C. elegans. Mech. Ageing Dev.*, 1984, **28**, 23–40.
6 P. Arasu, B. Wightman and G. Ruvkun, *Genes Dev.*, 1991, **5**, 1825–1833.
7 (*a*) T. V. Kurzchalia and S. Ward, *Nat. Cell Biol.*, 2003, **5**, 684–688; (*b*) E. V. Entchev and T. V. Kurzchalia, *Semin. Cell Dev. Biol.*, 2005, **16**, 175–182.
8 A. Antebi, J. G. Culotti and E. M. Hedgecock, *Development*, 1998, **125**, 1191–1205.
9 P.-Y. Jeong, M. Jung, Y.-H. Yim, H. Kim, M. Park, E. Hong, W. Lee, Y. H. Kim, K. Kim and Y.-K. Paik, *Nature*, 2005, **433**, 541–545.
10 (*a*) R. A. Butcher, M. Fujita, F. C. Schroeder and J. Clardy, *Nat. Chem. Biol.*, 2007, **3**, 420–422; (*b*) T. J. Baiga, H. Guo, Y. Xing, G. A. O'Doherty, A. Dillin, M. B. Austin, J. P. Noel and J. J. La Clair, *ACS Chem. Biol.*, 2008, **3**, 294–304; (*c*) J. Srinivasan, F. Kaplan, R. Ajredini, C. Zachariah, H. T. Alborn, P. E. A. Teal, R. U. Malik, A. S. Edison, P. W. Sternberg and F. C. Schroeder, *Nature*, 2008, **454**, 1115–1119; (*d*) R. A. Butcher, J. R. Ragains, E. Kim and J. Clardy, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 14288–14282; (*e*) C. Pungaliya, J. Srinivasan, B. W. Fox, R. U. Malik, A. H. Ludewig, P. W. Sternberg and F. C. Schroeder, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 7708–7713; (*f*) R. A. Butcher, J. R. Ragains and J. Clardy, *Org. Lett.*, 2009, **11**, 3100–3103.
11 D. L. Riddle and P. S. Albert, in *C. elegans II*, eds. D. L. Riddle, T. Blumenthal, J. B. Meyer and J. R. Priess, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1997, p. 739–768.
12 B. Gerisch, C. Weitzel, C. Kober-Eisermann, V. Rottiers and A. Antebi, *Dev. Cell*, 2001, **1**, 841–851.
13 V. Matyash, E. V. Entchev, F. Mende, M. Wilsch-Bräuninger, C. Thiele, A. W. Schmidt, H.-J. Knölker, S. Ward and T. V. Kurzchalia, *PLoS Biol.*, 2004, **2**, e280.
14 A. W. Schmidt, T. Doert, S. Goutal, M. Gruner, F. Mende, T. V. Kurzchalia and H.-J. Knölker, *Eur. J. Org. Chem.*, 2006, 3687–3706.
15 R. Martin, R. Saini, I. Bauer, M. Gruner, O. Kataeva, V. Zagoriy, E. V. Entchev, T. V. Kurzchalia and H.-J. Knölker, *Org. Biomol. Chem.*, 2009, **7**, 2303–2309.
16 D. C. Carrer, A. W. Schmidt, H.-J. Knölker and P. Schwille, *Langmuir*, 2008, **24**, 8807–8812.
17 (*a*) D. L. Motola, C. L. Cummins, V. Rottiers, K. V. Sharma, T. Li, Y. Li, K. Suino-Powell, H. E. Xu, R. J. Auchus, A. Antebi and D. J. Mangelsdorf, *Cell*, 2006, **124**, 1209–1223; (*b*) V. Rottiers, D. L. Motola, B. Gerisch, C. L. Cummins, K. Nishiwaki, D. J. Mangelsdorf and A. Antebi, *Dev. Cell*, 2006, **10**, 473–482; (*c*) B. Gerisch, V. Rottiers, D. Li, D. L. Motola, C. L. Cummins, H. Lehrach, D. J. Mangelsdorf and A. Antebi, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 5014–5019.
18 J. M. Held, M. P. White, A. L. Fisher, B. W. Gibson, G. J. Lithgow and M. S. Gill, *Aging Cell*, 2006, **5**, 283–291.
19 (*a*) A. Ogawa, A. Streit, A. Antebi and R. J. Sommer, *Curr. Biol.*, 2009, **19**, 67–71; (*b*) Z. Wang, X. E. Zhou, D. L. Motola, X. Gao, K. Suino-Powell, A. Conneely, C. Ogata, K. K. Sharma, R. J. Auchus, J. B. Lok, J. M. Hawdon, S. A. Kliewer, H. E. Xu and D. J. Mangelsdorf, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 9138–9143.
20 (*a*) I. Scheer, M. J. Thompson and E. Mosettig, *J. Am. Chem. Soc.*, 1956, **78**, 4733–4736; (*b*) A. F. Kluge, M. L. Maddox and L. G. Partridge, *J. Org. Chem.*, 1985, **50**, 2359–2365; (*c*) H.-S. Kim, W. K. Wilson, D. H. Needleman, F. D. Pinkerton, D. K. Wilson, F. A. Quiocho and G. J. Schroepfer, *J. Lipid Res.*, 1989, **30**, 247–261.
21 R. K. Varma, M. Koreeda, B. Yagen, K. Nakanishi and E. Caspi, *J. Org. Chem.*, 1975, **40**, 3680–3686.
22 C.-Y. Byon, M. Gut and V. Toome, *J. Org. Chem.*, 1981, **46**, 3901–3903.
23 P. Ferraboschi, A. Fiecchi, P. Grisenti and E. Santaniello, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1749–1752.
24 V. A. Khripach, V. N. Zhabinskii, O. V. Konstantinova, N. B. Khripach, A. V. Antonchick, A. P. Antonchick and B. Schneider, *Steroids*, 2005, **70**, 551–562.
25 S. Giroux and E. J. Corey, *J. Am. Chem. Soc.*, 2007, **129**, 9866–9867.
26 T. Ohta, H. Takaya, M. Kitamura, K. Nagai and R. Noyori, *J. Org. Chem.*, 1987, **52**, 3174–3176.
27 S. Giroux and E. J. Corey, *Org. Lett.*, 2008, **10**, 801–802.
28 R. Martin, A. W. Schmidt, G. Theumer, T. V. Kurzchalia and H.-J. Knölker, *Synlett*, 2008, 1965–1968.
29 R. Martin, A. W. Schmidt, G. Theumer, T. Krause, E. V. Entchev, T. V. Kurzchalia and H.-J. Knölker, *Org. Biomol. Chem.*, 2009, **7**, 909–920.
30 (*a*) Y. Ni, H.-S. Kim, W. K. Wilson, A. Kisic and G. J. Schroepfer, *Tetrahedron Lett.*, 1993, **34**, 3687–3690; (*b*) J. R. Williams, D. Chai and D. Wright, *Steroids*, 2002, **67**, 1041–1044; (*c*) J. R. Williams, D. Chai, J. D. Bloxton, H. Gong and W. R. Solvibile, *Tetrahedron*, 2003, **59**, 3183–3188; (*d*) H. Gong and J. R. Williams, *Org. Lett.*, 2006, **8**, 2253–2255.
31 K. K. Sharma, Z. Wang, D. L. Motola, C. L. Cummins, D. J. Mangelsdorf and R. J. Auchus, *Mol. Endocrinol.*, 2009, **23**, 640–648.
32 (*a*) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith and C. H. Ruof, *J. Am. Chem. Soc.*, 1947, **69**, 2167–2230; (*b*) A. Uomori, S. Seo, T. Sato, Y. Yoshimura and K. Takeda, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1713–1718.
33 (*a*) D. A. Evans, J. Bartroli and T. L. Shih, *J. Am. Chem. Soc.*, 1981, **103**, 2127–2129; (*b*) D. A. Evans, J. V. Nelson, E. Vogel and T. R. Taber, *J. Am. Chem. Soc.*, 1981, **103**, 3099–3111; (*c*) D. A. Evans, J. M. Takacs, L. R. McGee, M. D. Ennis, D. J. Mathre and J. Bartroli, *Pure Appl. Chem.*, 1981, **53**, 1109–1127; (*d*) D. A. Evans, *Aldrichimica Acta*, 1982, **15**, 23–32; (*e*) J. R. Gage and D. A. Evans, *Org. Synth.*, 1990, **68**, 83–87.
34 R. Martin, F. Däbritz, E. V. Entchev, T. V. Kurzchalia and H.-J. Knölker, *Org. Biomol. Chem.*, 2008, **6**, 4293–4295.
35 R. Martin, E. V. Entchev, F. Däbritz, T. V. Kurzchalia and H.-J. Knölker, *Eur. J. Org. Chem.*, 2009, 3703–3714.
36 S. Giroux, A. Bethke, N. Fielenbach, A. Antebi and E. J. Corey, *Org. Lett.*, 2008, **10**, 3643–3645.
37 D. J. Chitwood, W. R. Lusby, R. Lozano, M. J. Thompson and J. A. Svoboda, *Steroids*, 1983, **42**, 311–319.
38 J. T. Hannich, E. V. Entchev, F. Mende, H. Boytchev, R. Martin, V. Zagoriy, G. Theumer, I. Riezman, H. Riezman, H.-J. Knölker and T. V. Kurzchalia, *Dev. Cell*, 2009, **16**, 833–843.
39 H. Guo and G. A. O'Doherty, *Org. Lett.*, 2005, **7**, 3921–3924.
40 R. Martin, T. Schäfer, G. Theumer, E. V. Entchev, T. V. Kurzchalia and H.-J. Knölker, *Synthesis*, 2009, 3488–3492.
41 (*a*) O. J. Varela, A. F. Cirelli and R. M. De Lederkremer, *Carbohydr. Res.*, 1979, **70**, 27–35; (*b*) O. Han and H.-w. Liu, *Tetrahedron Lett.*, 1987, **28**, 1073–1076; (*c*) R. N. Russell, T. M. Weigel, O. Han and H.-w. Liu, *Carbohydr. Res.*, 1990, **201**, 95–114.

