Stereoselective Synthesis of (25*R*)-Dafachronic Acids and (25*R*)-Cholestenoic Acid as Potential Ligands for the DAF-12 Receptor in *Caenorhabditis elegans*

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Abstract: Commercially available diosgenin has been used as starting material for a highly efficient synthesis of (25*R*)-dafachronic acids and (25*R*)-cholestenoic acid, potential ligands for the receptor DAF-12 in the nematode *Caenorhabditis elegans*.

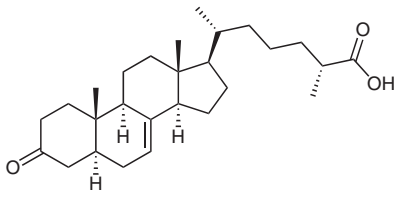
Key words: carboxylic acids, oxidations, protecting groups, stereoselective synthesis, steroids

Under unfavorable conditions, such as scarcity of food or overcrowding, the nematode *Caenorhabditis elegans* enters diapause and forms so-called dauer larvae. The hormonal receptor DAF-12 appears to play a key role in this process.[1] Recently, Mangelsdorf et al. reported novel steroidal metabolites from *C. elegans* identified as 3-ketocholesten-26-oic acids. These steroids function as ligands for the orphan receptor DAF-12 and have been described as (25*R*)-Δ^7-dafachronic acid (**1**) and (25*R*)-Δ^4-dafachronic acid (**2**) as well as their 25*S*-diastereomers (Figure 1).[2]

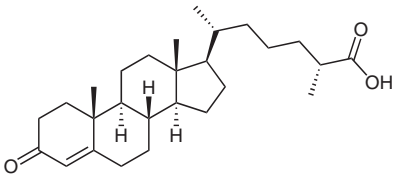
The synthesis of both diastereomers of Δ^4-dafachronic acid starting from the noncommercial (25*R*)- and (25*S*)-26-hydroxycholesterol was described by Mangelsdorf et al.[2] It was found that (25*R*)-Δ^4-dafachronic acid (**2**) was significantly less active than its 25*S*-isomer. Gill et al. have shown that (25*S*)-cholestenoic acid represents another ligand for DAF-12.[3] However, the 25*R*-isomer **3** exhibited no activity at all. In an independent synthetic study, Khripach et al. reported the syntheses of (25*R*)- and (25*S*)-Δ^4-dafachronic acid as well as (25*R*)- and (25*S*)-cholestenoic acid via a multistep elaboration of the steroid side chain.[4] In 2007, Corey et al. described the synthesis of (25*S*)-Δ^7-dafachronic acid via a diastereoselective ruthenium-catalyzed hydrogenation.[5] More recently, they reported a synthesis of (25*R*)-Δ^7-dafachronic acid (**1**) from β-ergosterol (10 steps and 13% overall yield).[6]

In the course of our project directed towards the synthesis of hormonally active cholesterol derivatives,[1,7] we became interested in a diastereoselective route to the (25*R*)-cholesten-26-oic acids **1**–**3**. We recognized commercially available diosgenin (**4**) as perfect starting material since it provides the 25*R*-configuration present in the target compounds **1**–**3**.

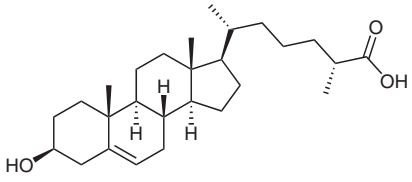
Using a modification of the procedure reported by Williams, the Clemmensen reduction of diosgenin afforded the triol **5** in 85% yield on large scale (Scheme 1).[8] Selective protection of the C-3β and C-26 hydroxy groups using TBSCl and DBU to **6** followed by removal of the C-16 hydroxy group led to the disilyl ether **7** in 76% overall yield.[8] Several methods are known for allylic oxidations at C-7 of steroids (Table 1).[9–11] We found that Chan-



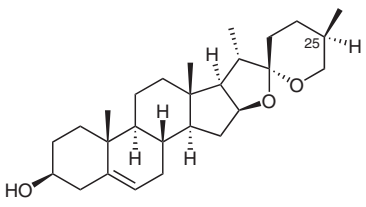
(25*R*)-Δ^7-dafachronic acid (**1**)



(25*R*)-Δ^4-dafachronic acid (**2**)



(25*R*)-cholestenoic acid (**3**)



diosgenin (**4**)

Figure 1 (25*R*)-Cholesten-26-oic acids (**1**–**3**) and diosgenin (**4**)

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Table 1 Allylic Oxidation of **7** to the Cholest-5-en-7-one **8**

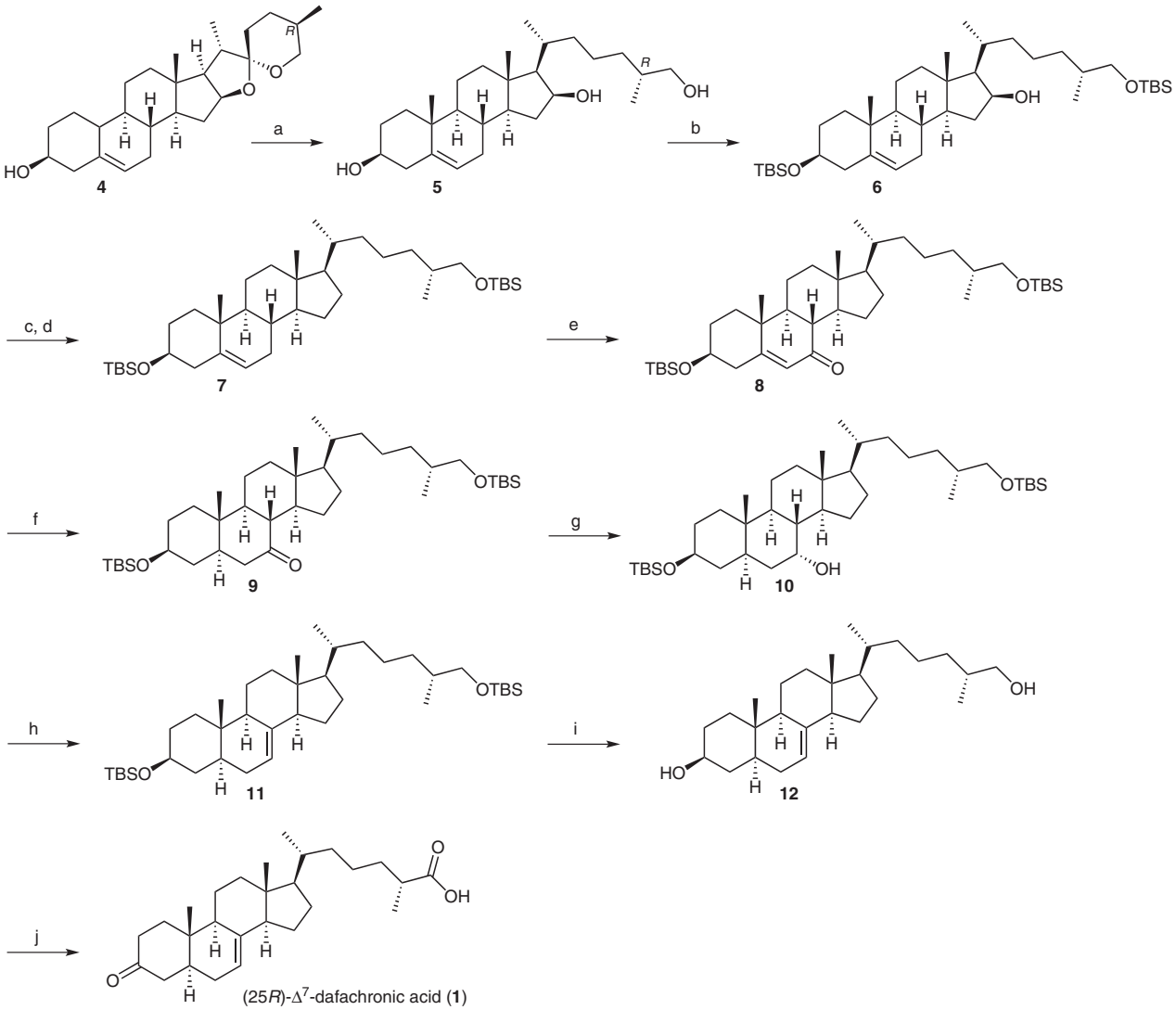
Reaction conditions	Yield (%)
PDC (4.0 equiv), *t*-BuOOH (4.0 equiv), C_6H_6, 0 °C to r.t., 28 h[9]	57
$Mn(OAc)_3$ (10 mol%), *t*-BuOOH (5.1 equiv), EtOAc, r.t., 2 d[10]	47
CrO_3 (18 equiv), DMP (18 equiv),[a] CH_2Cl_2, –20 °C to –10 °C, 1 h[11]	56

[a] DMP = 3,5-dimethyl-1*H*-pyrazole

drasekaran's procedure[9] provides the best results for allylic oxidation of **7** to the cholest-5-en-7-one **8**.

Transfer hydrogenation of **8** provided the ketone **9** in high yield. In order to establish the Δ^7-double bond, we required only the 7α-alcohol **10** for stereoelectronic reasons. However, reduction with lithium aluminum hydride at low temperature provided the 7α-alcohol **10** in 59% yield along with the corresponding 7β-alcohol in 31% yield. Grignard reduction using isopropylmagnesium chloride in diethyl ether afforded diastereoselectively the 7α-alcohol **10** in 70% yield. Elimination of the 7α-alcohol **10** with thionyl chloride in pyridine led quantitatively to the cholest-7-ene **11**.[12] Finally, desilylation with TBAF to the diol **12** and subsequent Jones oxidation provided (25*R*)-Δ^7-dafachronic acid (**1**).[13]

A stereoselective route to (25*R*)-Δ^4-dafachronic acid (**2**) proved to be more difficult. Desilylation of **7** provided (25*R*)-26-hydroxycholesterol in 89% yield (Scheme 2). However, Jones oxidation of (25*R*)-26-hydroxycholesterol did not provide the desired (25*R*)-Δ^4-dafachronic acid (**2**) but the (25*R*)-3,6-diketocholest-4-en-26-oic acid (**13**) in 56% yield. Using PDC in *N*,*N*-dimethylformamide as oxidizing agent[14] provided the undesired compound **13** in 74% yield.[13] Oxidation of cholesterols was known to afford cholest-4-en-3,6-diones using Jones reagent,[15] PCC,[16] or TPAP/NMO.[17] Therefore, we decided to oxidize the C-3 and C-26 hydroxy groups in two different



Scheme 1 Synthesis of (25*R*)-Δ^7-dafachronic acid (**1**). *Reagents and conditions*: a) Zn, 19% HCl, EtOH, reflux, 2 h, 85%; b) TBSCl, DBU, THF, r.t., 16 h, 85%; c) MsCl, pyridine, 0 °C to r.t., 16 h; d) $LiAlH_4$, Et_2O, 0 °C to reflux, 4 h, 89% over 2 steps; e) PDC, *t*-BuOOH, benzene, 0 °C to r.t., 28 h, 57%; f) Pd/C, ammonium formate, MeOH–EtOAc (3:4), reflux, 8 h, 90%; g) *i*-PrMgCl, Et_2O, r.t., 1 h, 70%; h) $SOCl_2$, pyridine, 0 °C, 1 h, 100%; i) TBAF, THF, reflux, 24 h, 93%; j) CrO_3, H_2SO_4, acetone, 0 °C, 1 h, 74%.

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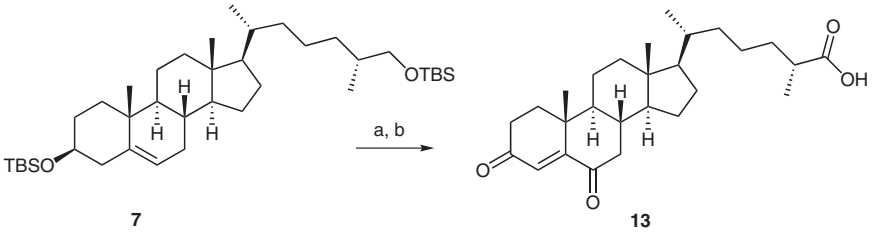


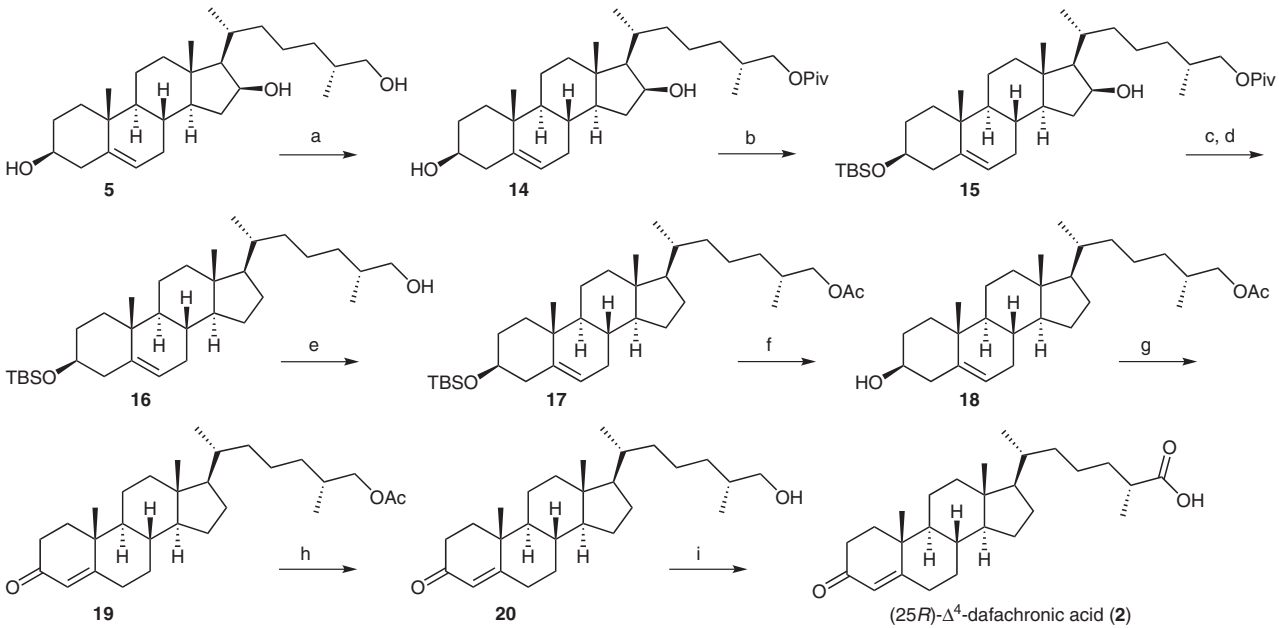
Scheme 2 Synthesis of (25*R*)-3,6-diketocholest-4-en-26-oic acid (**13**). *Reagents and conditions*: a) TBAF, THF, reflux, 20 h, 89%; b) PDC, DMF, r.t., 18 h, 74%.

steps. This strategy required a differentiation between all three hydroxy groups at the stage of the triol **5**.

Pivaloyl chloride represents an excellent reagent for selective acylations of primary in the presence of secondary hydroxy groups.[18] However, Williams reported a double pivaloylation of the triol **5** at C-3β and C-26.[8c] Treatment of **5** with only 1.1 equivalents of pivaloyl chloride led to a selective esterification at C-26 to provide **14** in 81% yield (Scheme 3). Silylation of the C-3β hydroxy group of **14** using TBSCl provided **15** in 79% yield. Thus, sequential introduction of the pivaloyl and *tert*-butyldimethylsilyl protecting groups resulted in a perfect differentiation between all three hydroxy groups. Mesylation of the hydroxy group at C-16 and subsequent reduction removed also the pivaloyl group at C-26 to afford the 26-hydroxy derivative **16** in 89% yield. Acylation of **16** to **17** followed by cleavage of the silyl ether using TBAF provided **18** in 88% yield. Using the classical Oppenauer oxidation was found to avoid allylic oxidation at C-6 (see Scheme 2) and afforded the ketone **19** in 70% yield. Saponification of the acetate to **20** followed by Jones oxidation provided (25*R*)-Δ^4-dafachronic acid (**2**).[13]

Compound **16** represents a crucial intermediate, which has been exploited also for the synthesis of (25*R*)-cholest-5-en-26-oic acid (**3**) (Scheme 4). Oxidation of **16** using PDC in dichloromethane[14] to the aldehyde followed by further oxidation with sodium chlorite afforded the acid **21** in 82% yield over both steps. Direct oxidation of **16** to **21** by using PDC in DMF as solvent gave only 45% yield. Desilylation of the acid **21** by the usual method (TBAF in THF at reflux) led only to impure (25*R*)-cholestenoic acid (**3**) (67% yield). Chromatographic purification of **3** proved to be difficult. Therefore, desilylation of **21** was carried out with catalytic amounts of concentrated sulfuric acid in methanol at reflux to provide the methyl ester **22** in 84% yield. Purification of **22** by flash chromatography and subsequent saponification using lithium hydroxide afforded pure (25*R*)-cholestenoic acid (**3**) in 97% yield.[13]

In conclusion, we have developed highly efficient and stereoselective syntheses of (25*R*)-Δ^7-dafachronic acid (**1**) (10 steps, 16% overall yield), (25*R*)-Δ^4-dafachronic acid (**2**) (10 steps, 22% overall yield), and (25*R*)-cholestenoic acid (**3**) (9 steps, 32% overall yield) starting from commercially available diosgenin (**4**). These compounds are much less active than described for the corresponding



Scheme 3 Synthesis of (25*R*)-Δ^4-dafachronic acid (**2**). *Reagents and conditions*: a) PivCl, Et_3N, cat. DMAP, THF, r.t., 20 h, 81%; b) TBSCl, DBU, THF, r.t., 17 h, 79%; c) MsCl, pyridine, 0 °C to r.t., 18 h; d) $LiAlH_4$, Et_2O, 0 °C to reflux, 4 h, 89% over 2 steps; e) Ac_2O, Et_3N, cat. DMAP, THF, r.t., 16 h, 100%; f) TBAF, THF, reflux, 17 h, 88%; g) Al(O*i*-Pr)$_3$, acetone–toluene (1:9), reflux, 15 h, 70%; h) cat. K_2CO_3, MeOH, r.t., 40 h, 93%; i) CrO_3, H_2SO_4, acetone, 0 °C, 1 h, 79%.

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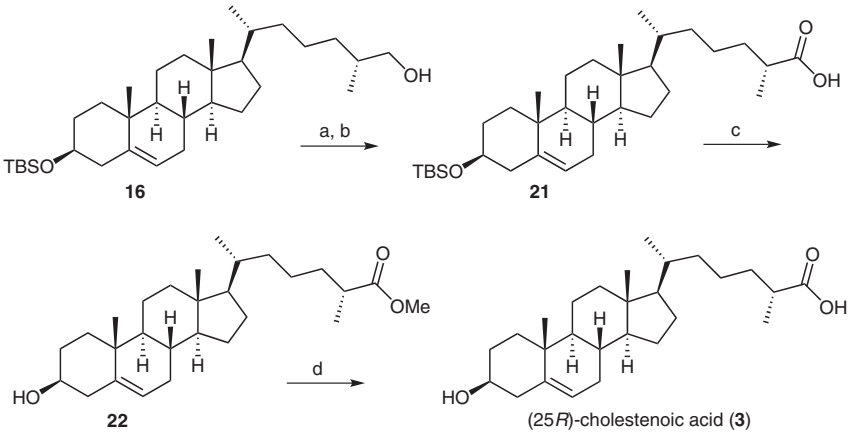

Scheme 4 Synthesis of (25*R*)-cholestenoic acid (**3**). *Reagents and conditions*: a) PDC, CH_2Cl_2, r.t., 3.5 h, 89%; b) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, THF–H_2O (3:1), r.t., 16 h, 92%; c) cat. H_2SO_4, MeOH, reflux, 16 h, 84%; d) LiOH, THF–MeOH–H_2O (2:1:1), r.t., 15 h, 97%.

25*S*-diastereoisomers.[2] However, they could be used for the clarification of basic principles of dauer larva formation and biological studies in this direction are underway. By use of the present methodology, yamogenin, the 25*S*-diastereoisomer of diosgenin (**4**), provides a direct and stereoselective access to the (25*S*)-cholesten-26-oic acids.

References and Notes

(1) Matyash, V.; Entchev, E. V.; Mende, F.; Wilsch-Bräuninger, M.; Thiele, C.; Schmidt, A. W.; Knölker, H.-J.; Ward, S.; Kurzchalia, T. V. *PLoS Biol.* **2004**, *2*, 1561.

(2) Motola, D. L.; Cummins, C. L.; Rottiers, V.; Sharma, K. K.; Li, T.; Li, Y.; Suino-Powell, K.; Xu, H. E.; Auchus, R. J.; Antebi, A.; Mangelsdorf, D. J. *Cell* **2006**, *124*, 1209.

(3) Held, J. M.; White, M. P.; Fisher, A. L.; Gibson, B. W.; Lithgow, G. J.; Gill, M. S. *Aging Cell* **2006**, *5*, 283.

(4) Khripach, V. A.; Zhabinskii, V. N.; Konstantinova, O. V.; Khripach, N. B.; Antonchick, A. V.; Antonchick, A. P.; Schneider, B. *Steroids* **2005**, *70*, 551.

(5) Giroux, S.; Corey, E. J. *J. Am. Chem. Soc.* **2007**, *129*, 9866.

(6) Giroux, S.; Corey, E. J. *Org. Lett.* **2008**, *10*, 801.

(7) Schmidt, A. W.; Doert, T.; Goutal, S.; Gruner, M.; Mende, F.; Kurzchalia, T. V.; Knölker, H.-J. *Eur. J. Org. Chem.* **2006**, 3687.

(8) (a) Williams, J. R.; Chai, D.; Wright, D. *Steroids* **2002**, *67*, 1041. (b) Williams, J. R.; Chai, D.; Bloxton, J. D.; Gong, H.; Solvibile, W. R. *Tetrahedron* **2003**, *59*, 3183. (c) Gong, H.; Williams, J. R. *Org. Lett.* **2006**, *8*, 2253.

(9) Chidambaram, N.; Chandrasekaran, S. *J. Org. Chem.* **1987**, *52*, 5048.

(10) Shing, T. K. M.; Yeung, Y.-Y.; Su, P. L. *Org. Lett.* **2006**, *8*, 3149.

(11) Salmond, W. G.; Barta, M. A.; Havens, J. L. *J. Org. Chem.* **1978**, *43*, 2057.

(12) Knölker, H.-J.; Ecker, A.; Struwe, P.; Steinmeyer, A.; Müller, G.; Neef, G. *Tetrahedron* **1997**, *53*, 91.

(13) **Characteristic Spectroscopic Data for the (25*R*)-Cholesten-26-oic Acids 1, 13, 2, and 3**

(25*R*)-Δ^7-Dafachronic acid (**1**): colorless solid; mp 174–175 °C. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.87 (CH_3), 12.44 (CH_3), 16.72 (CH_3), 18.74 (CH_3), 21.66 (CH_2), 22.91 (CH_2), 23.68 (CH_2), 27.90 (CH_2), 30.02 (CH_2), 33.85 (CH_2), 34.35 (C), 35.62 (CH_2), 36.01 (CH), 38.09 (CH_2), 38.73 (CH_2), 39.24 (CH), 39.38 (CH_2), 42.82 (CH), 43.33 (C), 44.20 (CH_2), 48.78 (CH), 54.87 (CH), 56.01 (CH), 116.99 (CH), 139.46 (C), 182.51 (C=O), 212.19 (C=O). Anal. Calcd (%) for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.42; H, 10.41.

(25*R*)-3,6-Diketocholest-4-en-26-oic acid (**13**): colorless solid; mp 105–110 °C. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.87 (CH_3), 16.76 (CH_3), 17.49 (CH_3), 18.55 (CH_3), 20.84 (CH_2), 23.59 (CH_2), 23.93 (CH_2), 27.97 (CH_2), 33.83 (CH_2), 33.94 (CH_2), 34.16 (CH), 35.50 (CH, CH_2), 35.60 (CH_2), 39.08 (CH_2), 39.21 (CH), 39.78 (C), 42.52 (C), 46.77 (CH_2), 50.91 (CH), 55.83 (CH), 56.48 (CH), 125.45 (CH), 161.04 (C), 182.27 (C=O), 199.55 (C=O), 202.34 (C=O). HRMS: *m/z* calcd for $C_{27}H_{40}O_4$ [M^+]: 428.2927; found: 428.2914.

(25*R*)-Δ^4-Dafachronic acid (**2**): colorless solid; mp 148–150 °C. ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.93 (CH_3), 16.74 (CH_3), 17.35 (CH_3), 18.55 (CH_3), 20.99 (CH_2), 23.60 (CH_2), 24.14 (CH_2), 28.15 (CH_2), 32.00 (CH_2), 32.93 (CH_2), 33.86 (CH_2), 33.94 (CH_2), 35.57 (2 CH), 35.64 (2 CH_2), 38.58 (C), 39.26 (CH), 39.57 (CH_2), 42.37 (C), 53.75 (CH), 55.81 (CH), 55.99 (CH), 123.71 (CH), 171.85 (C), 182.48 (C=O), 199.83 (C=O). Anal. Calcd (%) for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.03; H, 10.46.

(25*R*)-Cholestenoic acid (**3**): colorless solid; mp 168–170 °C (MeCN). ^{13}C NMR and DEPT (125 MHz, $CDCl_3$): δ = 11.85 (CH_3), 16.74 (CH_3), 18.63 (CH_3), 19.38 (CH_3), 21.05 (CH_2), 23.62 (CH_2), 24.27 (CH_2), 28.21 (CH_2), 31.58 (CH_2), 31.87 (CH, CH_2), 33.91 (CH_2), 35.62 (CH), 35.72 (CH_2), 36.47 (C), 37.22 (CH_2), 39.27 (CH), 39.73 (CH_2), 42.21 (CH_2), 42.30 (C), 50.07 (CH), 56.05 (CH), 56.70 (CH), 71.82 (CH), 121.71 (CH), 140.69 (C), 182.46 (C=O). Anal. Calcd (%) for $C_{27}H_{44}O_3$: C, 77.83; H, 10.64. Found: C, 77.26; H, 10.33.

(14) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.

(15) (a) Šolaja, B. A.; Milić, D. R.; Došen-Mićović, L. I. *Steroids* **1994**, *59*, 330. (b) Chodounská, H.; Pouzar, V.; Buděšínský, M.; Slavíková, B.; Kohout, L. *Steroids* **2004**, *69*, 605.

(16) Brunel, J. M.; Loncle, C.; Vidal, N.; Dherbomez, M.; Letourneux, Y. *Steroids* **2005**, *70*, 907.

(17) (a) Miranda Moreno, M. J. S.; Sá e Melo, M. L.; Campos Neves, A. S. *Tetrahedron Lett.* **1991**, *32*, 3201. (b) Acosta, C. K.; Rao, P. N.; Kim, H. K. *Steroids* **1993**, *58*, 205.

(18) (a) Nagaoka, H.; Rutsch, W.; Schmid, G.; Iio, H.; Johnson, M. R.; Kishi, Y. *J. Am. Chem. Soc.* **1980**, *102*, 7962. (b) Boschelli, D.; Takemasa, T.; Nishitani, Y.; Masamune, S. *Tetrahedron Lett.* **1985**, *26*, 5239. (c) Nicolaou, K. C.; Webber, S. E. *Synthesis* **1986**, 453.

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