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Divergent and stereoselective synthesis of dafachronic acids

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ABSTRACT

A new divergent synthesis of DAF-12 ligands, namely Δ^4- and Δ^7-dafachronic acid, is described starting from the natural bile acid hyodeoxycholic acid. Homologation of the side chain followed by stereoselective reduction of the Δ^{24} olefinic linkage, 6α-dehydroxylation and C_3-oxidation affords dafachronic acids in good yields and high diastereoselectivity making this approach easily accessible and scalable.

1. Introduction

Upon unfavorable conditions and difficult environmental plights, many organisms from worms to mammals respond by arresting their development and initiating programs of reversible states of dormancy.[1] Several strategies of diapause have evolved even within the same species, allowing them to survive until conditions improve and they can return to normal reproductive life. Recent studies on the nematode *Caenorhabditis elegans* are beginning to provide crucial insights into the diversity and complexity of these alternate life strategies, as well as into the molecular mechanisms governing these responses.[2] It has been reported, in particular, that upon environmental cues both G protein-coupled receptors (GPCRs) and nuclear hormone receptors (NHRs) are involved in modulating diapause programs in the adult reproductive and larval stage, respectively.[1a,2a,3]

The most investigated among these arrests are the larval stage one (L1) and the third dauer diapause (L3). In both these cases, a key hormonal role is played by dafachronic acids (DAs, **1**–**4**) (Fig. 1),[4] 3-keto-steroids derived from cholesterol.[5] Upon the binding to the NHR DAF-12 ('*DA*uer larva *F*ormation-12'),[6a] DAs activate *daf-12* genes and prevent the entry of *C. elegans* into the dauer recovery, while the absence of the ligands induces the quiescent L3 stage, in which the nematode exhibits more stress resistance, an extended larval survival, and, interestingly, an increased life span.[2,3,6] At this regard, it has been reported that among the four DA isomers (Fig. 1), *S*-Δ^4-DA (**1**) and *S*-Δ^7-DA (**2**) are more potent toward DAF-12 than the corresponding 25-*R*-epimers **3** and **4**, with **2** being the most potent ligand so far reported.[4a,6b]

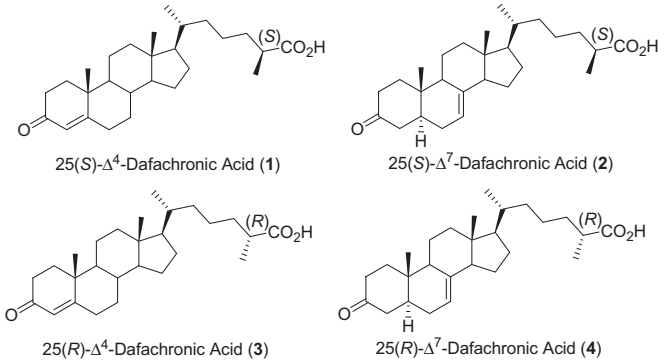

Fig. 1. Structure of dafachronic acids.

In addition, while the dauer diapause represents a survival strategy, it was reported to help dispersal of the *C. elegans*, a phenomenon, which has suggested an important evolutionary link with parasitism.[7] Indeed, it has been recently demonstrated that the DA/DAF-12 signal is crucial during infection caused by parasitic nematodes. As in free-living *C. elegans*, DAs activate parasitic DAF-12 orthologs and promote the recovery of the third stage of infective larvae (iL3) in *Strongyloides stercoralis* and hookworm.[7a,b] In particular, administration of *S*-Δ^7-DA (**2**) causes a concomitant and significant reduction in the infection progression and in the pathogenic dauer-like iL3 population of the *S. stercoralis*. These important results, which indicate a conserved NHR signaling pathway governing the stage 3 larvae, suggest a potential therapeutic utility

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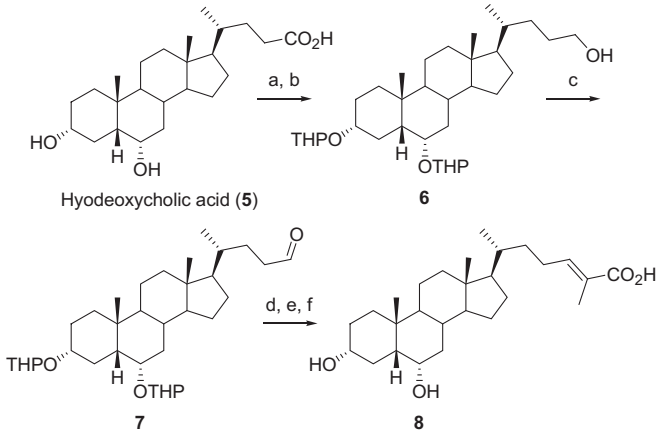
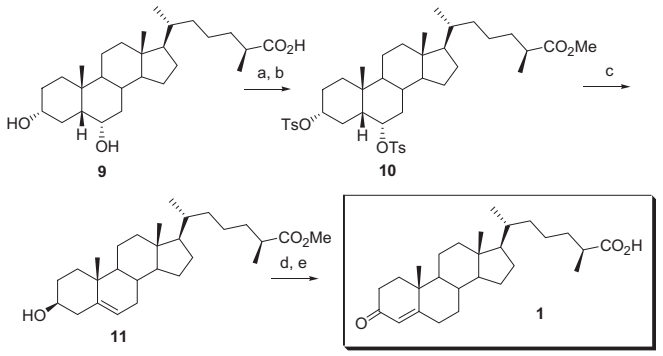
of DAs and DA derivatives in the treatment of disseminated strongyloidiasis.[7a,b,8]

On the basis of these findings, the readily availability of DAs became of primary importance not only to fully define the biological relevance of DAF-12 but also to grasp the endocrine circuitry and molecular mechanism that govern its actions. To this end, in the last few years a significant effort has been directed toward the development of facile and scalable procedures for the synthesis of the DAs **1**–**4**.[9–13] The first synthesis of Δ^4-DA (**1**) was reported in 2005 as a multistep elaboration of 20(*S*)-3β-acetoxy-pregn-5-en-20-carboxylic acid side chain to give **1** in 11 steps with an overall yield of 4%.[9] The first diastereoselective synthesis of the biologically active 25(*S*)-Δ^7-DA (**2**) was published by Corey in 2007, starting from the plant sterol β-stigmasterol (16 steps, 13%).[10] A divergent synthesis of both 25(*S*)-Δ^4- and 25(*S*)-Δ^7-DAs (**1**, **2**) has been reported from 3β-hydroxychol-5-en-24-oic acid.[11] In this case, the stereogenic center at C_{25} was achieved by stereoselective Evans aldol reaction and gave the desired derivatives **1** and **2** in 12 (19%) and 15 steps (18%), respectively. Moreover, while diosgenin has been largely employed as starting material for the preparation of the *R*-epimers **3** and **4**, as it provides the 25(*R*)-configuration,[12] a stereocontrolled synthesis of the 25(*R*)-Δ^7-DA (**4**) was successfully achieved from β-ergosterol in 10 steps with an overall yield of 13%.[13]

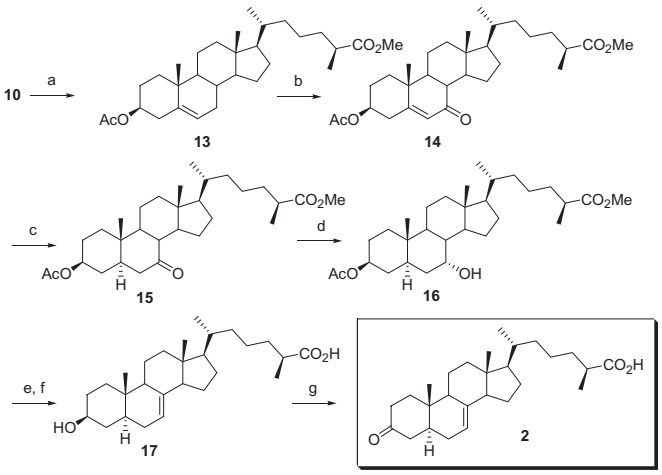
With the aim to provide a new and efficient synthetic route for the preparation of DA isomers **1**–**4**, we report here an alternate and a divergent synthesis of DAs using the naturally occurring bile acid hyodeoxycholic acid (**5**) as starting material (Schemes 1–3).



Scheme 1. Synthesis of 3α,6α-dihydroxy-25-methyl-24-bishomo-5β-chol-24-en-26-oic acid (**8**). Reagents and conditions: (a) 3,4-DHP, *p*-TSA, dioxane, quantitative. (b) 1. $ClCO_2Et$, Et_3N, THF; 2. $NaBH_4$, H_2O, 95%. (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 92%. (d) $(Ph_3P)CH_3CHCO_2EtBr$, *t*-BuOK, THF. (e) HCl, MeOH. (f) NaOH, MeOH, 89% from **7**.



Scheme 2. Synthesis of 25(*S*)-Δ^4-dafachronic acid (**1**). Reagents and conditions: (a) *p*-TSA, MeOH. (b) TsCl, pyridine, 96%. (c) AcOK, DMF, H_2O, 62%. (d) $Al(OiPr)_3$, cyclohexanone, toluene. (e) NaOH, MeOH, 83% from **11**.



Scheme 3. Synthesis of 25(*S*)-Δ^7-dafachronic acid (**2**). Reagent and conditions: (a) AcOK, AcOH, reflux, 60%. (b) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2, 83%. (c) H_2, Pd/C, EtOAc, 93%. (d) L-Selectride, THF, 88%. (e) $SOCl_2$, pyridine. (f) NaOH, MeOH, 86% from **16**. (g) Jones oxidation, 87%.

2. Results and discussion

The strategy employed to access DAs was based on the preparation of a common intermediate **8** obtained from the homologation of hyodeoxycholic acid (**5**) side chain by Wittig reaction (Scheme 1).

Thus, **5** was protected at the $C_{3\alpha}$- and $C_{6\alpha}$-hydroxy groups by using 3,4-dihydro-2*H*-pyran in the presence of catalytic amount of *p*-TSA at room temperature to furnish the corresponding dihydropyranyl derivative in quantitative yield (Scheme 1). The terminal carboxylic group was then reduced to alcohol by treatment with $ClCO_2Et$ and Et_3N in THF at 0 °C, followed by the addition of a suspension of $NaBH_4$ in H_2O at room temperature. The alcoholic intermediate **6** was converted into the corresponding aldehyde **7** by Swern oxidation (92% yield). The following Wittig reaction was performed with [(1-carboethoxy)ethyl]triphenylphosphonium bromide[14] in THF using *t*-BuOK as a base. The resulting adduct was submitted to acidic (HCl/MeOH) and basic hydrolysis (NaOH/MeOH) to give (*E*)-3α,6α-dihydroxy-25-methyl-24-bishomo-5β-chol-24-en-26-oic acid (**8**) (*E*/*Z*>20/1), in excellent yield (89%) (Scheme 1). The stereochemistry of **8** was assigned based on NMR COSY and NOE analysis (Fig. 2).

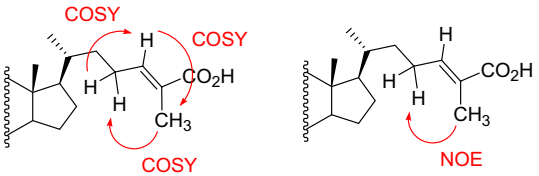
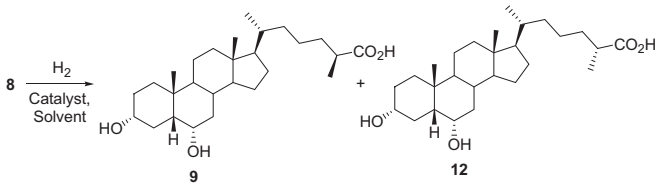


Fig. 2. Diagnostic ^1H NMR COSY and NOE for **8**.

Stereoselective reduction of the C_{24}–C_{25} double bond was attempted according to diverse reaction conditions (Table 1). For the preparation of 3α,6α-dihydroxy-25(*S*)-methyl-24-bishomo-5β-cholan-26-oic acid (**9**), the best result was obtained by using (*S*)-Ru(OAc)$_2$(BINAP) as a catalyst (90% yield, *de*: 100%, Table 1, entry 3), which although retains the selectivity and efficiency of the parent (*S*)-Ru(OAc)$_2$(H_8-BINAP), is consistently cheaper. Next, in order to provide a procedure to synthesize the corresponding 25(*R*)-epimer **12**, the key intermediate for the synthesis of the 25(*R*)-DA **3** and **4**, we sought to use (*R*)-Ru(OAc)$_2$(BINAP). While in this case the

Table 1
Stereoselective reduction of **8**



Entry	Catalyst	Solv.	T, Press.	Yield[a] (*S*/*R* Ratio)[b]
1	PtO_2	AcOH	rt, 3 atm	98% (55/45)
2	(*S*)-Ru(OAc)$_2$(H_8-BINAP)	MeOH	50 °C, 2 atm	90% (100/0)
3	(*S*)-Ru(OAc)$_2$(BINAP)	MeOH	50 °C, 2 atm	90% (100/0)
4	(*R*)-Ru(OAc)$_2$(BINAP)	MeOH	50 °C, 2 atm	85% (40/60)
5	(*R*)-RuCl[(*p*-cymene)(BINAP)]Cl	MeOH/Et_3N (1 equiv)	50 °C, 5 atm	97% (3/97)

[a] Calculated after purification by flash chromatography on silica gel.
[b] S/R ratio was calculated by HPLC analysis of the crude reaction mixture.

reaction proceeded with low levels of selectivity (*S*/*R* ratio: 40/60), hydrogenation conducted with (*R*)-RuCl[(*p*-cymene)(BINAP)]Cl in the presence of Et_3N in MeOH at 50 °C afforded **12** with high diastereoisomeric ratio (Table 1, entry 5). The absolute configuration assignment to epimers **9** and **12** was based upon ^{13}C NMR comparison.[15]

Reaction of the 3α,6α-dihydroxy-25(*S*)-methyl-24-bishomo-5β-cholan-26-oic acid (**9**) with tosyl chloride in pyridine at room temperature afforded the corresponding 3α,6α-ditosylate **10** (quantitative yield), which was then transformed into 3β-hydroxy-Δ^5-methyl ester **11** by 6α-tosylate elimination and Walden inversion achieved by refluxing **10** in a H_2O/DMF solution of AcOK (Scheme 2).[16] Finally, Oppenauer oxidation and subsequent ester hydrolysis provided 25(*S*)-Δ^4-DA (**1**), in 35% overall yield (12 steps).

For the preparation of 25(*S*)-Δ^7-DA (**2**) we envisaged to change the reaction condition for detosylation (Scheme 3). Thus, when **10** was treated with AcOK in boiling AcOH, methyl 3β-acetoxy-25(*S*)-methyl-24-bishomochol-5-en-26-oate (**13**) was isolated in 60% yield along with the corresponding $\Delta^{3,5}$-diene derivative obtained as by-product of the reaction.[17] Allylic oxidation of **13** with CrO_3 and 3,5-dimethylpyrazole in CH_2Cl_2 at room temperature furnished the enone **14**, in 83% yield (Scheme 3).[18]

Palladium-catalyzed hydrogenation of the C_5–C_6 double bond allowed to obtain the 5α-steroid **15**, in high yields. Stereoselective reduction of the 7-keto group with L-Selectride in THF at −78 °C followed by the treatment of the 7α-hydroxy intermediate **16** with thionyl chloride in pyridine at room temperature and alkali hydrolysis (NaOH/MeOH) furnished 3β-hydroxy-25-methyl-24-bishomo-5α-chol-7-en-26-oic acid (**16**), in 71% yield.[12c] Finally, Jones oxidation gave the desired 25(*S*)-Δ^7-DA (**2**) in 22% overall yield (16 steps).

3. Conclusions

In summary, we have developed a new efficient diastereoselective synthesis of DAs starting from the readily available hyodeoxycholic acid (**5**). The method, which includes a Wittig reaction and a stereoselective hydrogenation, furnishes 25(*S*)-DAs (**1**–**2**) in good yield and high isomeric purity. Our approach results to be flexible and a valuable alternative to the previous reported methodology as it can easily provide DA isomers **1**–**4** via a common intermediate, obtained in high overall yield also on a large scale synthesis.

4. Experimental section

4.1. General methods

All reagents were commercially available unless otherwise noted. All reactions were carried out in dried glassware under a dry nitrogen atmosphere. The final products were purified by chromatography on silica gel (70–230 mesh). TLC was performed on aluminium backed silica plates (silica gel 60 F_{254}). Spots on TLC were visualized by using UV and by staining and warming with phosphomolybdate reagent (5% solution in EtOH). All the reactions were performed using distilled solvent. ^1H NMR spectra were recorded at 200 and 400 MHz, ^{13}C NMR spectra were recorded at 50.3 and 100.6 MHz, respectively, using the solvents indicated below. Chemical shifts are reported in parts per million. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; br s, broad signal. Melting points were determined with an electrothermal apparatus and are uncorrected. For HRMS a Micromass spectrometer was used. Purity of the DAs was >95% according to NMR and HPLC analysis. The HPLC analyses were carried out on apparatus with a chromatography data software, a pump, a system controller, a low pressure gradient formation unit, an on-line degasser and an injector with a 20 mL stainless steel loop. An evaporative light scattering detector (ELSD) was used as detector. An interface allowed the analog-to-digital conversion of the output signal from the ELSD. The column temperature was controlled through a heater/chiller thermostat. Column: RP-18 (250×4.6 mm, 5 μm). Eluent: H_2O/MeCN/MeOH, 50/45/5 (v/v/v)+ NH_4HCO_2=40 mM pH 3.5. Tcol: 25 °C. Flow-rate: 0.700 mL/min. Detector: ELSD (Tvap=50 °C, Tneb=30 °C, Gas Flow-rate=1.50 L/min, Gain=2.00).

4.2. Synthesis of 3α,6α-dihydroxy-25-methyl-24-bishomo-5β-chol-24-en-26-oic acid (8)

*4.2.1. 3α,6α-Ditetrahydropyranyloxy-24-hydroxy-5β-cholane (**6**)*[19]. To a solution of hyodeoxycholic acid (**5**) (15.00 g, 38.20 mmol) and *p*-toluensulfonic acid (0.7 g, 3.64 mmol) in 1,4-dioxane (100 mL), 3,4-dihydro-2*H*-pyran (48.20 g, 570 mmol) was added dropwise in 5 h. At the end of the addition the reaction mixture was diluted with water (120 mL) and extracted with EtOAc (3×100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The resulting yellow oil was dissolved in a solution

of MeOH (100 mL) and 5% aqueous NaOH, and stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure, the resulted residue was dissolved in H_2O (150 mL) and washed with Et_2O (2×100 mL). The aqueous phase was neutralized by addition of 3N HCl and then extracted with EtOAc (3×100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to furnish 3α,6α-ditetrahydropyraloxy-5β-cholan-24-oic acid (21.40 g, 38.10 mmol) as white solid that was used for the next step without further purifications. The 3,6-diprotected acid thus obtained was dissolved in dry THF (200 mL) and Et_3N (13.25 g, 131.18 mmol) at 0 °C, and a solution of $EtCO_2Cl$ (13.40 g, 123.50 mmol) in dry THF (10 mL) was added dropwise. The resulting mixture was stirred at room temperature for 3 h. A suspension of $NaBH_4$ (15.70 g, 415.34 mmol) in H_2O (50 mL) was added dropwise. The resulting mixture was stirred at room temperature overnight and then diluted with H_2O (500 mL) and extracted with EtOAc (3×100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to furnish a yellow oil that was purified by flash chromatography eluting with $CHCl_3$ to obtain **6** as mixture of diastereoisomers (19.84 g, 36.10 mmol, 95%). Mp: 102–103 °C; R_f (30% EtOAc/PET) 0.38; δ_H (200 MHz, $CDCl_3$) 0.61 (3H, s, 18-CH_3), 0.88–0.91 (6H, m, 19-CH_3+21-CH_3), 0.99–2.03 (41H, m), 3.45–3.48 (2H, m, OCH_2-THP), 3.57–3.60 (2H, m, 24-CH_2), 3.69–3.72 (1H, m, 3-CH), 3.83–3.93 (2, m, OCH_2-THP), 3.95–34.00 (1H, m, 6-C*H*), 4.59–4.66 (1H, m, OC*H*O-THP), 4.72–4.76 (1H, m, OC*H*O-THP); δ_C (100.6 MHz, $CDCl_3$) 11.9, 18.6, 19.8, 20.0, 20.1, 20.2, 20.7, 23.5, 24.2, 25.5, 25.8, 26.3, 26.4, 27.6, 27.9, 28.2, 28.4, 28.5, 29.4, 31.1, 31.2, 31.3, 31.8, 33.2, 34.7, 34.8, 35.4, 35.5, 35.6, 35.7, 35.9, 36.0, 39.8, 39.9, 42.8, 44.9, 45.1, 47.7, 47.9, 56.1, 56.2, 62.5, 62.8, 63.0, 63.1, 63.5, 72.0, 72.1, 72.3, 72.4, 75.1, 75.2, 75.5, 75.8, 95.9, 96.0, 96.2, 96.4, 96.5, 96.9, 97.1.

*4.2.2. 3α,6α-Ditetrahydropyranyloxy-5β-cholan-24-al (**7**)[20].* To a solution of $(COCl)_2$ (4.49 g, 35.60 mmol) in dry CH_2Cl_2 (30 mL) at −50 °C a solution of DMSO (5.35 g, 68.55 mmol) in CH_2Cl_2 (10 mL) was added dropwise in 30 min and then reacted at −50 °C for 15 min. A solution of the alcohol **6** (15.00 g, 27.42 mmol) in CH_2Cl_2 (30 mL) was then added dropwise. After 2 h at −50 °C Et_3N (13.90 g, 137.36 mmol) was added and the reaction was allowed to warm at room temperature overnight. The reaction mixture was poured into 2N KOH (200 mL) and extracted with CH_2Cl_2 (3×60 mL). The combined organic layers were washed with H_2O (100 mL), brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography eluting with from 5 to 10% EtOAc in petroleum ether to obtain the desired aldehyde **7** (13.70 g, 25.14 mmol, 92%) as mixture of diastereoisomers. Mp: 92–94 °C; R_f (20% EtOAc/PET) 0.62; δ_H (200 MHz, $CDCl_3$) 0.62 (3H, s, 18-CH_3), 0.89–0.91 (6H, m, 19-CH_3+21-CH_3), 1.08–1.95 (36H, m), 2.36–2.38 (1H, m, 23-CH_aH_b), 2.41–2.45 (1H, m, 23-CH_aH_b), 3.46–3.49 (2H, m, OCH_2-THP), 3.58–3.63 (1H, m, 3-C*H*), 3.87–3.95 (2H, m, OCH_2-THP), 3.96–4.02 (1H, m, 6-C*H*), 4.61–4.68 (1H, m, OC*H*O-THP), 4.72–4.76 (1H, m, OC*H*O-THP), 9.76 (1H, s, C*H*O); δ_C (100.6 MHz, $CDCl_3$) 12.0, 18.3, 19.8, 20.0, 20.2, 20.7, 23.5, 24.1, 25.5, 25.8, 26.4, 27.6, 27.9, 28.2, 28.4, 28.5, 31.1, 31.2, 33.2, 34.7, 34.8, 35.3, 35.4, 35.6, 35.9, 36.0, 39.8, 39.9, 40.9, 42.8, 44.9, 45.1, 47.6, 47.9, 55.9, 56.2, 62.5, 62.9, 63.0, 63.1, 72.0, 72.1, 72.3, 75.1, 75.2, 75.4, 75.8, 95.9, 96.0, 96.2, 96.4, 96.5, 96.9, 97.1, 203.2.

*4.2.3. 3α,6α-Dihydroxy-25-methyl-24-bishomo-5β-chol-24-en-26-oic acid (**8**)[21].* A suspension of (1-(ethoxycarbonyl)ethyl) triphenylphosphonium bromide[14] (21.40 g, 48.27 mmol) in dry THF (100 mL) was reacted with potassium *t*-butoxide (44.0 ml, 1 M in THF) at room temperature overnight. A solution of the aldehyde **7** (8.00 g, 14.68 mmol) in dry THF (100 mL) was then added and refluxed for 4 h. The reaction mixture was allowed to cool at room temperature, was diluted with hexane (100 mL) and H_2O/MeOH (150 mL, 1:1, v/v), and the resulting solution was extracted with EtOAc (2×100 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residual brown oil was dissolved in 5% HCl in MeOH (150 mL) and stirred at room temperature for 2 h. The solvent was removed under reduced pressure, the residue was dissolved in H_2O (120 mL) and extracted with $CHCl_3$ (3×60 mL). The combined organic layers were washed with H_2O (80 mL), brine (80 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography to obtain ethyl 3α,6α-dihydroxy-25-methyl-24-bishomo-5β-chol-24-en-26-oate as white solid. The ester thus obtained was dissolved in 60 mL of 5% NaOH in MeOH and the resulting mixture was stirred at 60 °C overnight. The solvent was evaporated under reduced pressure, the residue was dissolved into H_2O (100 mL), acidified with 3N HCl and extracted with EtOAc (3×60 mL). The combined organic layers were washed with H_2O (100 mL), brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure to afford the desired acid **8** (5.70 g, 13.17 mmol, 89%, E/*Z*>20/1) as pure white solid. Mp: 203–205 °C; R_f (10% MeOH/$CHCl_3$+0.1% AcOH) 0.29; ν_{max} (KBr) 3255, 2937, 2867, 1685, 1458, 1378, 1261, 1030, 751 cm^{-1}; δ_H (400 MHz, $CDCl_3$+CD_3OD) 0.55 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 0.86 (3H, d, *J* 6.4 Hz, 21-CH_3), 0.94–1.67 (22H, m), 1.73 (3H, s, 27-CH_3), 1.88–2.11 (4H, m), 3.47 (1H, m, 3-C*H*), 3.68 (3H, br s, O*H*), 3.92 (1H, dt, *J* 4.7, 11.9 Hz, 6-C*H*), 6.70 (1H, t, *J* 7.4 Hz, 24-CH); δ_C (100.6 MHz, $CDCl_3$) 11.7, 11.9, 18.2, 20.5, 23.2, 24.0, 25.3, 28.0, 28.6, 29.6, 34.3, 34.6, 35.3, 35.4, 35.7, 39.6, 39.8, 42.6, 48.2, 55.8, 55.9, 67.6, 71.0, 126.9, 143.7, 170.6.

4.3. General procedure for stereoselective reduction of 8

To a solution of 0.50 mmol of **8** in 10 mL of freshly distilled solvent, 5 mol % of the catalyst was added. The resulting mixture was reacted for 48 h according to the reaction conditions reported in Table 1. The solution was then filtered through a Celite pad, evaporated to dryness and purified by flash chromatography using a solution of $CHCl_3$/MeOH 95:5 (v/v).

*4.3.1. 3α,6α-Dihydroxy-25(S)-methyl-24-bishomo-5β-cholan-26-oic acid (**9**)[22].* Mp: 207–208 °C; R_f (10% MeOH/$CHCl_3$+0.1% AcOH) 0.29; ν_{max} (KBr) 3469, 3237, 2931, 1735, 1466, 1345, 1029, 731 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.58 (3H, s, 18-CH_3), 0.83–0.85 (6H, m, 19-CH_3+27-CH_3), 0.96–1.00 (5H, m), 0.99 (3H, d, *J* 6.4 Hz, 21-CH_3), 1.10–1.95 (26H, m), 2.34–2.37 (1H, m, 25-C*H*), 3.52 (1H, m, 3-C*H*), 3.97 (1H, dt, J_1 4.7 Hz, J_2 11.9 Hz, 6-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.8, 16.9, 18.3, 20.6, 23.3, 23.6, 24.0, 28.0, 28.73, 29.72, 34.0, 34.4, 34.7, 35.4, 35.5, 35.6, 35.7, 39.2, 39.7, 39.8, 42.7, 48.2, 56.0, 56.1, 67.7, 71.1, 179.6.

*4.3.2. 3α,6α-Dihydroxy-25(R)-methyl-24-bishomo-5β-cholan-26-oic acid (**12**)[22].* Mp: 192–195 °C; R_f (10% MeOH/$CHCl_3$+0.1% AcOH) 0.29; ν_{max} (KBr) 3457, 3248, 2931, 1734, 1463, 1346, 1185, 1019, 720 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.57 (3H, s, 18-CH_3), 0.82–0.83 (6H, m, 19-CH_3+27-CH_3), 0.94–1.04 (5H, m), 1.09 (3H, d, *J* 7.0 Hz, 21-CH_3), 1.14–1.92 (26H, m), 2.34–2.35 (1H, m, 25-C*H*), 3.50 (1H, m, 3-C*H*), 3.95 (1H, dt, J_1 4.6 Hz, J_2 11.9 Hz, 6-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.8, 16.7, 18.4, 20.6, 23.3, 23.6, 24.0, 28.1, 28.73, 29.70, 33.9, 34.4, 34.7, 35.4, 35.5, 35.6, 35.8, 39.2, 39.7, 39.8, 42.6, 48.2, 2× 56.0, 67.7, 71.1, 179.8.

4.4. Synthesis of Δ^4-(*S*)-dafachronic acid (1)

*4.4.1. Methyl 3α,6α-ditosyloxy-25(S)-methyl-24-bishomo-5β-cholan-26-oate (**10**).* To a solution of **9** (0.70 g, 1.61 mmol) in MeOH (30 mL), *p*-toluensulfonic acid (30 mg, 0.16 mmol) was added and the mixture was refluxed for 2 h. The solvent was evaporated under reduce pressure, the residue was dissolved in $CHCl_3$ (50 mL),

washed with aqueous $NaHCO_3$ saturated solution (2×50 mL), H_2O (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to afford the methyl ester derivative as white solid. The residue was dissolved in dry pyridine (4 mL) at 0 °C and a solution of TsCl (0.67 g, 3.54 mmol) in dry pyridine (4 mL) was added dropwise. The resulting solution was stirred at room temperature for 48 h. The reaction mixture was then poured into crushed ice and extracted with EtOAc (3×50 mL). The combined organic layers were sequentially washed with 1N HCl (3×100 mL), H_2O (100 mL), brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure to furnish the ditosylated derivative **10** (1.20 g, 1.54 mmol, 96%) as off white solid. Mp: 146–148 °C; R_f (20% EtOAc/PET) 0.77; ν_{max} (KBr) 2946, 2873, 1732, 1456, 1358, 1175, 923, 849, 669 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.58 (3H, s, 18-C*H*$_3$), 0.80 (3H, s, 19-C*H*$_3$), 0.85 (3H, d, *J*=6.4 Hz, 21-C*H*$_3$), 0.90–1.07 (7H, m), 1.14 (3H, d, *J* 6.9 Hz, 27-C*H*$_3$), 1.19–1.94 (21H, m), 2.41–2.49 (7H, m, 25-C*H*+2× Ar-C*H*$_3$), 3.67 (3H, s, CO_2CH_3), 4.30 (1H, m, 3-C*H*), 4.78 (1H, dt, *J* 4.8, 12.0 Hz, 6-C*H*), 7.35 (4H, t, *J* 8.0 Hz, Ar*H*), 7.72 (2H, d, *J* 8.3 Hz, Ar*H*), 7.79 (2H, d, *J* 8.3 Hz, Ar*H*); δ_C (100.6 MHz, $CDCl_3$) 11.9, 17.2, 18.4, 20.4, 2× 21.6, 22.8, 23.7, 23.8, 26.4, 27.3, 28.0, 32.0, 34.2, 2× 34.7, 35.4, 35.5, 36.1, 39.4, 39.4, 39.5, 42.7, 46.2, 51.4, 55.7, 56.0, 79.6, 81.7, 4× 127.5, 4× 129.7, 2× 134.4, 2× 144.6, 177.3. HRMS (ESI^+) *m*/*z* 757.3810 [$(M+H)^+$; calcd for $C_{42}H_{61}O_8S_2$ 757.3808].

*4.4.2. Methyl 3β-hydroxy-25(S)-methyl-24-bishomochol-5-en-26-oate (**11**).* To a solution of **10** (0.80 g, 1.04 mmol) in DMF (12 mL) potassium acetate (82 mg, 0.84 mmol) dissolved in H_2O (1.8 mL) was added and the resulting mixture was refluxed overnight. The reaction mixture was then cooled to room temperature and poured into crushed ice. The aqueous phase was extracted with EtOAc (3×60 mL) and the combined organic layers were stirred with a 2 M aqueous solution of K_2CO_3 for 30 min. The phases were separated, the organic phase was washed with H_2O (100 mL), brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The resulting solid was purified by flash chromatography eluting with from 5 to 40% EtOAc in petroleum ether to obtain the desired compound **11** (0.28 g, 0.64 mmol, 62%) as off white solid. Mp: 102–104 °C; R_f (40% EtOAc/PET) 0.28; ν_{max} (KBr) 3450, 2935, 2867, 2356, 1735, 1461, 1378, 1364, 1193, 1165 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.62 (3H, s, 18-C*H*$_3$), 0.85 (3H, d, *J* 6.4 Hz, 21-C*H*$_3$), 0.95 (3H, s, 19-C*H*$_3$), 0.99–1.04 (5H, m), 1.08 (3H, d, *J* 6.6 Hz, 27-C*H*$_3$), 1.20–1.99 (23H, m), 2.37 (1H, q, *J* 6.9 Hz, 25-C*H*), 3.40–3.48 (1H, m, 3-C*H*), 3.61 (3H, s, CO_2CH_3), 5.28 (1H, bd, *J* 4.7 Hz, 6-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.7, 16.82, 18.4, 19.2, 23.6, 24.1, 28.0, 31.4, 2× 31.7, 34.0, 34.18, 35.5, 35.6, 36.36, 37.1, 39.3, 39.6, 2× 42.1, 49.9, 51.3, 55.9, 56.6, 71.4, 121.4, 140.7, 177.3. HRMS (ESI^+) *m*/*z* 431.3526 [$(M+H)^+$; calcd for $C_{28}H_{47}O_3$ 431.3525].

*4.4.3. 25(S)-Δ^4-Dafachronic acid (**1**).* To a solution of **11** (0.27 g, 0.63 mmol) in dry toluene (15 mL), cyclohexanone (0.26 g, 2.63 mmol) and aluminium isopropoxide (0.20 g, 0.96 mmol) were added and the resulting mixture was refluxed overnight. The reaction mixture was then diluted with EtOAc (50 mL) and 1% aqueous H_2SO_4 (50 mL), and stirred at room temperature for 30 min. The phases were separated and the aqueous phase was extracted with EtOAc (3×50 mL). The combined organic layers were washed with H_2O (100 mL), brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude was dissolved in 5% NaOH in MeOH (12 mL) and was treated with MW (*T*=70 °C, *P*=200 psi, power 100 W) for 10 min. The brown reaction mixture was evaporated to dryness, dissolved into water (100 mL) and extracted with diisopropyl ether (2×50 mL). The organic phase was separated and acidified to pH=4 with 3N HCl and extracted with EtOAc (3×50 mL). The combined organic layers were washed with H_2O (100 mL), brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography eluting with a solution of $CHCl_3$/MeOH (92/8, v/v) to obtain the desired compound **1** (0.22 g, 0.51 mmol, 83%) as off white solid. Mp: 173–175 °C; R_f (5% MeOH/$CHCl_3$+0.1% AcOH) 0.31; α_D^{16} 60.9 (*c* 1.9, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.70 (3H, s, 18-C*H*$_3$), 0.91 (3H, d, *J* 6.6 Hz, 21-C*H*$_3$), 0.95–1.16 (6H, m), 1.18–1.19 (6H, m, 19-C*H*$_3$+27-C*H*$_3$), 1.25–2.04 (18H, m), 2.20–2.38 (1H, m, 25-C*H*), 2.35–2.39 (2H, m, 6-C*H*$_2$), 2.42–2.47 (2H, m, 2-C*H*$_2$), 5.73 (1H, s, 4-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.9, 17.0, 17.3, 18.5, 21.0, 23.7, 24.1, 28.1, 32.0, 32.9, 33.9, 34.0, 2× 35.5, 2× 35.6, 38.5, 39.3, 39.6, 42.3, 53.7, 55.8, 56.0, 123.7, 171.8, 182.4, 199.8. HRMS (ESI^+) *m*/*z* 415.3226 [$(M+H)^+$; calcd for $C_{27}H_{43}O_3$ 415.3212].

4.5. Synthesis of Δ^7-(*S*)-dafachronic acid (2)

*4.5.1. Methyl 3β-acetoxy-25(S)-methyl-24-bishomochol-5-en-26-oate (**13**)[10].* To a solution of **10** (1.74 g, 2.30 mmol) in AcOH (80 mL), anhydrous potassium acetate (6.81 g, 69.5 mmol) was added and the resulting mixture was refluxed for 5 h. The reaction mixture was then allowed to cool at room temperature and the solvent was removed under vacuum. The crude was dissolved into H_2O (150 mL) and extracted with EtOAc (3×80 mL). The combined organic layers were washed with 5% aqueous Na_2CO_3 solution (5×80 mL), brine (150 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography eluting with from 5 to 30% EtOAc in petroleum ether to furnish the desired compound **13** (0.65 g, 1.38 mmol, 60%) as whitish solid. Mp: 112–114 °C; R_f (10% EtOAc/PET) 0.54; δ_H (400 MHz, $CDCl_3$) 0.68 (3H, s, 18-C*H*$_3$), 0.89 (3H, d, *J* 6.4 Hz, 21-C*H*$_3$), 1.03 (3H, s, 19-C*H*$_3$), 1.03–1.10 (5H, m), 1.14 (3H, d, *J* 6.9 Hz, 27-C*H*$_3$), 1.18–2.01 (21H, m), 2.02 (3H, s, $COCH_3$), 2.31 (1H, d, *J* 7.0 Hz, 4-C*H*), 2.40–2.45 (1H, m, 25-C*H*), 3.68 (3H, s, CO_2CH_3), 4.56–4.64 (1H, m, 3-C*H*), 5.36 (1H, bd, *J* 4.4 Hz, 6-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.8, 17.1, 18.5, 19.2, 20.9, 21.3, 23.7, 24.2, 27.7, 28.1, 2× 31.8, 34.2, 35.5, 35.7, 36.5, 36.9, 38.0, 39.4, 39.6, 42.2, 49.9, 51.4, 56.0, 56.6, 74.0, 122.5, 139.6, 170.6, 177.5.

*4.5.2. Methyl 3β-acetoxy-7-keto-25(S)-methyl-24-bishomochol-5-en-26-oate (**14**)[10].* To a suspension of CrO_3 (1.50 g, 15.20 mmol) in CH_2Cl_2 (20 mL) at −20 °C, 3,5-dimethylpyrazole (1.46 g, 15.20 mmol) was added and the resulting mixture was stirred at −20 °C for 20 min. A solution of **13** (0.40 g, 0.88 mmol) in CH_2Cl_2 (10 mL) was then added and the mixture was allowed to warm at room temperature and reacted for 2 h. The reaction mixture was then filtered through a Celite pad, washing the filter with CH_2Cl_2, and evaporated to dryness. The crude was purified by flash chromatography eluting with from 5 to 10% EtOAc in petroleum ether to furnish the desired compound **14** (0.34 g, 0.70 mmol, 83%) as off white solid. Mp: 110–112 °C; R_f (10% EtOAc/PET) 0.37; δ_H (400 MHz, $CDCl_3$) 0.67 (3H, s, 18-C*H*$_3$), 0.91 (3H, d, *J* 6.4 Hz, 21-C*H*$_3$), 1.02–1.10 (3H, m), 1.15 (3H, d, *J* 6.9 Hz, 27-C*H*$_3$), 1.21 (3H, s, 19-C*H*$_3$), 1.24–2.01 (19H, m), 2.05 (3H, s, $COCH_3$), 2.20–2.25 (1H, m, 25-C*H*), 2.39–2.54 (3H, m, 4-C*H*$_2$+8-C*H*), 3.67 (3H, s, CO_2CH_3), 4.70 (1H, m, 3-C*H*), 5.70 (1H, br s, 6-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.9, 2× 17.2, 18.7, 21.1, 21.2, 23.7, 26.2, 27.3, 28.4, 34.2, 35.5, 35.7, 35.9, 37.7, 38.2, 38.6, 39.4, 43.0, 45.3, 49.7, 49.9, 51.4, 54.6, 72.1, 126.6, 163.8, 170.2, 177.3, 201.8.

*4.5.3. Methyl 7-keto-3β-acetoxy-25(S)-methyl-24-bishomo-5α-cholan-26-oate (**15**)[10].* To a solution of **14** (0.30 g, 0.62 mmol) in EtOAc (30 mL) Pd/C 10% w/w (20 mg, 0.04 mmol) was added and the resulting mixture was hydrogenated at 1 atm for 5 h. The reaction mixture was then filtered through a Celite pad, washing the filter with EtOAc. The filtrate was evaporated under reduced pressure to give the desired 5α-derivative **15** (0.28 g, 0.56 mmol, 93%) as white solid that was used for the next step without further purification. Mp: 116–118 °C; R_f (10% EtOAc/PET) 0.41; δ_H (400 MHz, $CDCl_3$) 0.64 (3H, s, 18-C*H*$_3$), 0.88 (3H, d, *J* 6.4 Hz, 21-C*H*$_3$), 0.93–1.07 (4H, m), 1.09 (3H, s, 19-C*H*$_3$), 1.13 (3H, d, *J* 6.9 Hz, 27-C*H*$_3$), 1.18–1.99

(19H, m), 2.02 (3H, s, $COCH_3$), 2.15–2.25 (1H, m, 25-C*H*), 2.30 (2H, bt, *J* 12.0 Hz, 6-CH_2), 2.42 (1H, q, *J* 6.8 Hz, 8-C*H*), 3.67 (3H, s, CO_2CH_3), 4.67 (1H, m, 3-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.6, 12.0, 17.2, 18.6, 21.3, 21.7, 23.7, 24.9, 27.0, 28.3, 33.8, 34.2, 35.4, 35.6, 35.7, 35.9, 38.6, 39.4, 42.4, 45.8, 46.4, 48.8, 49.9, 51.4, 54.9, 54.9, 72.7, 170.4, 177.3, 211.5.

*4.5.4. Methyl 7α-hydroxy-3β-acetoxy-25(S)-methyl-24-bishomo-5α-cholan-26-oate (**16**).* To a solution of the 7-keto derivative **15** (0.26 g, 0.52 mmol) in dry THF (10 mL) at −78 °C L-selectride® (0.8 mL) was added dropwise. The resulting mixture was stirred at −78 °C for 2 h, and at −30 °C for 12 h. A mixture of H_2O/EtOH (1:4, v/v, 20 mL) was added and the reaction mixture was allowed to warm at 0 °C. At this temperature the reaction was poured into an aqueous saturated solution of NH_4Cl (60 mL), warmed at room temperature and extracted with EtOAc (3×40 mL). The combined organic layers were washed with H_2O (50 mL), brine (2×50 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude was purified by flash chromatography eluting with from 5 to 20% EtOAc in petroleum ether to furnish the corresponding 7α-OH derivative **16** (0.22 g, 0.46 mmol, 88%) as off white solid. Mp: 107–109 °C; R_f (15% EtOAc/PET) 0.26; ν_{max} (KBr) 3523, 2944, 2865, 2357, 2138, 1734, 1457, 1375, 1261, 751 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 0.65 (3H, s, 18-CH_3), 0.82 (3H, s, 19-CH_3), 0.89 (3H, d, *J* 6.5 Hz, 21-CH_3), 1.01–1.13 (4H, m), 1.16 (3H, d, *J* 6.5 Hz, 27-CH_3), 1.25–1.97 (22H, m), 2.02 (3H, s, $COCH_3$), 2.41–2.46 (1H, m, 25-C*H*), 3.67 (3H, s, CO_2CH_3), 3.82 (1H, bd, *J* 2.6 Hz, 7-C*H*), 4.71 (1H, m, 3-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.1, 11.7, 17.1, 18.5, 20.9, 21.4, 23.6, 23.7, 27.3, 28.1, 33.5, 34.2, 35.5, 35.6, 35.7, 36.2, 36.4, 36.9, 39.4, 39.4, 42.6, 45.7, 50.5, 51.4, 56.0, 67.8, 73.5, 170.5, 177.3. HRMS (ESI^+) *m*/*z* 491.3734 [$(M+H)^+$; calcd for $C_{30}H_{51}O_5$ 491.3737].

*4.5.5. 3β-Hydroxy-25(S)-methyl-24-bishomo-5α-chol-7-en-26-oic acid (**17**)[10].* Methyl 7α-hydroxy-3β-acetoxy-25(*S*)-methyl-24-bishomo-5α-cholan-26-oate (**16**) (0.16 g, 0.32 mmol) was dissolved in dry pyridine (6 mL) and $SOCl_2$ (95 mg, 0.80 mmol) was added at 0 °C. The resulting solution was stirred at room temperature for 48 h, and then cautiously poured into crushed ice (40 mL) and extracted with Et_2O (3×50 mL). The combined organic phases were washed with $NaHCO_3$ saturated solution (40 mL), 1 N HCl (2×40 mL), H_2O (40 mL), brine (40 mL), dried over Na_2SO_4 and evaporated under reduced pressure to furnish the corresponding Δ^7-derivative (0.14 g, 0.28 mmol, 90%) that was submitted to hydrolysis without further purification. The thus obtained crude was dissolved into 5% NaOH in MeOH (4 mL) and reacted at room temperature overnight. The solvent was evaporated under reduced pressure, the residue was dissolved into H_2O (40 mL), washed with iPr_2O (2×20 mL), acidified with 2 N HCl and extracted with EtOAc (3×40 mL). The combined organic layers were washed with H_2O (50 mL), brine (50 mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield the desired product **17** (0.11 mg, 0.27 mmol, 96%) as white solid. Mp: 94–96 °C; R_f (5% MeOH/$CHCl_3$+0.1% AcOH) 0.28; δ_H (400 MHz, $CDCl_3$) 0.52 (3H, s, 18-CH_3), 0.78 (3H, s, 19-CH_3), 0.90 (3H, d, *J* 6.4 Hz, 21-CH_3), 0.92–1.06 (4H, m), 1.15 (3H, d, *J* 6.9 Hz, 27-CH_3), 1.21–1.97 (23H, m), 2.42–2.51 (1H, m, 25-C*H*), 2.50–2.75 (2H, br s, OH+CO_2H), 3.64 (1H, m, 3-C*H*), 5.17 (1H, br s, 7-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.7, 12.9, 17.0, 18.7, 21.4, 22.9, 23.7, 27.8, 29.6, 31.2, 34.10, 34.15, 35.6, 36.0, 37.0, 37.74, 39.2, 39.5, 40.1, 43.3, 49.3, 54.9, 56.0, 70.9, 117.4, 139.5, 180.0.

*4.5.6. 25(S)-Δ^7-dafachronic acid (**2**).* A fresh made Jones reagent (1.0 mL) was added dropwise to a stirred solution of **17** (80 mg, 0.19 mmol) in acetone (15 mL) at 0 °C and the mixture was stirred at room temperature for 2 h. Methanol (15 mL) was then added and the oxidized product was extracted with EtOAc (2×30 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure The residue was purified by flash chromatography using 5–40% EtOAc in petroleum ether to afford the desired compound **2** (68 mg, 0.17 mmol, 87%) as off white solid. Mp: 139–141 °C; R_f (5% MeOH/$CHCl_3$+0.1% AcOH) 0.42; α_D^{16} 42.6 (*c* 0.8, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 0.56 (3H, s, 18-CH_3), 0.95 (3H, d, *J* 6.4 Hz, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.21 (3H, d, *J* 6.9 Hz, 27-CH_3), 1.25–2.18 (1H, m, CH_{24}), 2.15–2.25 (2H, m, 2-CH_2), 2.30–2.35 (1H, m, 25-C*H*), 2.42–2.47 (2H, m, 4-CH_2), 5.19 (1H, br s, 7-C*H*); δ_C (100.6 MHz, $CDCl_3$) 11.8, 12.4, 17.0, 18.7, 21.6, 22.9, 23.7, 27.9, 30.0, 34.0, 34.3, 35.6, 36.0, 38.1, 38.7, 39.3, 39.4, 42.8, 43.3, 44.2, 48.8, 54.9, 56.0, 117.0, 139.4, 182.1, 212.0. HRMS (ESI^+) *m*/*z* 415.3215 [$(M+H)^+$; calcd for $C_{27}H_{43}O_3$ 415.3212].

Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.01.022. These data include MOL file and InChIKey of the most important compounds described in this article.

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