Stereocontrolled Synthesis of Dafachronic Acid A, the Ligand for the DAF-12 Nuclear Receptor of *Caenorhabditis elegans*

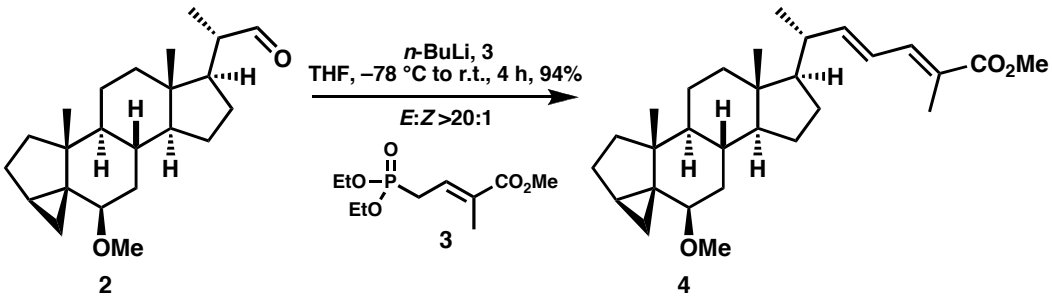
Simon Giroux and E. J. Corey*
Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street Cambridge, Massachusetts 02138

Supporting Information

1. Materials and Methods. Unless stated otherwise, reactions were performed in flame-dried glassware under a positive pressure of nitrogen using freshly distilled dry solvents. Commercial grade reagents and solvents were used without further purification except as indicated below. MeOH was distilled over $CaSO_4$. Dichloromethane was distilled from calcium hydride. Toluene, Et_2O and THF were purified by Seco Solvent Systems. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F_{254} precoated plates (0.25 mm). Flash chromatography was performed using Baker silica gel (40 μm particle size). ^{1}H NMR spectra were recorded on Varian Mercury 400 (400 MHz) or Unity/INOVA 500 (500 MHz) spectrometers and chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as internal (δ 7.26 ppm for $CDCl_3$). ^{13}C NMR spectra were recorded on Varian Mercury 400 (100 MHz) or Unity/INOVA 500 (125 MHz) spectrometers with proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent as internal (δ 77.16 ppm for $CDCl_3$). IR spectra were recorded on Avatar 360 FT-IR spectrometer. Low-resolution and high-resolution mass spectral analyses were performed at the Harvard University Mass Spectrometry Center. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at the indicated temperature with a sodium lamp (D line, 589 nm). Melting points (m.p.) are uncorrected and were recorded on a Thomas-Hoover Unimelt capillary melting point apparatus.

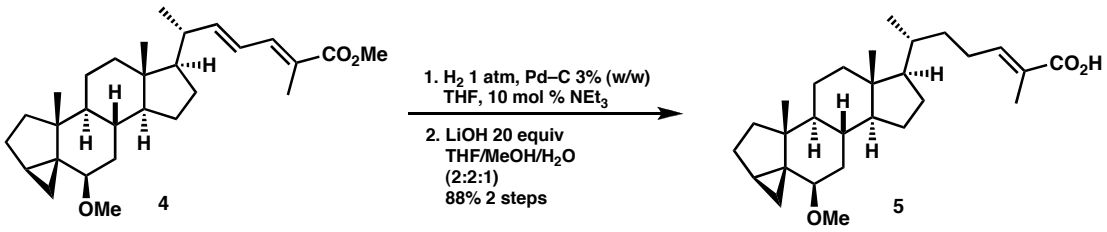
2. Experimental procedures.

E,E-dienoate (4)



To a stirred solution of phosphonate **3**[1] (3.11g, 12.5 mmol) in THF (40 mL) was added *n*-BuLi (4.64 mL, 2.5 *M* in hexanes, 11.6 mmol) at -78 °C. The resulting bright yellow solution was stirred for 30 min at -78 °C and the aldehyde **2**[2] (2.86 g, 8.3 mmol) was slowly added *via* canula (20 mL, THF and 2 x 3 mL rinse). The pale yellow solution was stirred at -78 °C for 1h and warmed gradually to r.t. over 3 h and quenched with a saturated solution of aq. NH_4Cl (50 mL). The aqueous phase was extracted with Et_2O (3 x 50 mL) and the organic phase was dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography (95:5 → 9:1; hexanes/EtOAc) to give the dienoate **4** (3.43 g, 94%, *E*/*Z* > 20:1) as a white solid. $[\alpha]_D$ + 42.7 (*c* 0.11, $CHCl_3$); m.p. 96-98 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, 1H, *J* = 11.2 Hz), 6.24 (dd, 1H, *J* = 11.2 and 15.1 Hz), 5.92 (dd, 1H, *J* = 8.8 and 15.1 Hz), 3.73 (s, 3H), 3.31 (s, 3H), 2.76 (s, 1H), 2.24 (m, 1H), 1.96 (dt, 1H, *J* = 3.4 and 12.6 Hz), 1.91 (s, 3H), 1.86 (dt, 1H, *J* = 2.9 and 13.6 Hz), 1.74 (m, 2H), 1.71-1.44 (m, 5H), 1.41 (m, 2H), 1.21 (m, 3H), 1.07 (d, 3H, *J* = 6.8 Hz + 1H), 1.02 (s, 3H + 1H), 0.85 (m, 3H), 0.74 (s, 3H), 0.64 (t, 1H, *J* = 4.4 Hz), 0.42 (dd, 1H, *J* = 5.4 and 8.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3, 149.7, 139.5, 124.8, 123.6, 82.5, 56.8, 56.6, 55.8, 51.9, 48.2, 43.6, 43.2, 40.9, 40.3, 35.4, 35.2, 33.5, 30.7, 28.6, 25.1, 24.4, 22.9, 21.6, 20.1, 19.5, 13.3, 12.8, 12.7; IR (film, cm^{-1}) 2930, 1706, 1635, 1434, 1244, 1096; MS (ES +) *m*/*z* (%): 441.7 (100).

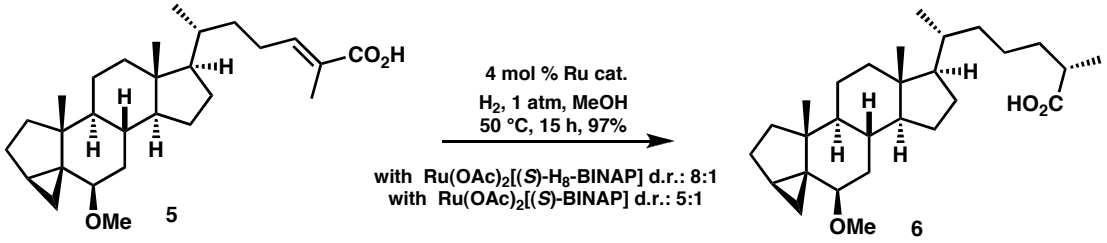
α,β-unsaturated acid (5)



To a stirred solution of dienoate **4** (1.85 g, 4.19 mmol) in THF (40 mL) and NEt_3 (0.058 mL, 0.419 mmol) was added Pd/C (55 mg, 3% w/w). A hydrogen-filled balloon (1 atm) was placed over the solution and the mixture was stirred for 20 min, by which time TLC analysis indicated that the reaction was completed. The mixture was filtered through a pad of Celite® and washed with EtOAc (25 mL). Evaporation gave the methyl ester of **5** that was directly submitted to hydrolysis without any further purification.

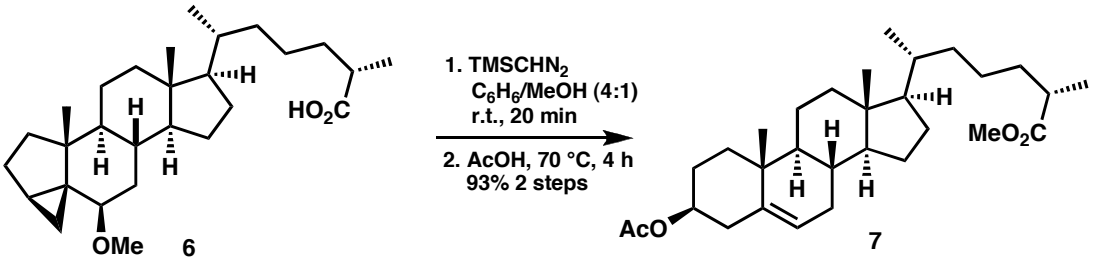
To a stirred solution of the methyl ester of **5** in THF/MeOH/H_2O (2:2:1) (85 mL) was added LiOH (3.51 g, 83.8 mmol) at room temperature. The resulting mixture was stirred at r.t. for 15 h. The reaction mixture was acidified to ca. pH 2 by addition of 1*N* HCl (20 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase were dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (8:2 → 7:3; hexanes/EtOAc) to give the title acid **5** (1.59 g, 88% 2 steps) as a white solid. $[\alpha]_D$ + 41.5 (*c* 0.06, $CHCl_3$); m.p. 87-88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (t, 1H, *J* = 7.3 Hz), 3.32 (s, 3H), 2.77 (s, 1H), 2.22 (m, 1H), 2.12 (m, 1H), 1.97 (m, 1H), 1.87 (m, 1H), 1.83 (s, 3H), 1.74 (m, 3H), 1.60 (m, 1H), 1.51 (m, 3H), 1.41 (m, 3H), 1.25 (m, 2H), 1-15-1.03 (m, 5H), 1.01 (s, 3H), 0.95 (d, 3H, *J* = 6.2 Hz), 0.89-0.83 (m, 4H), 0.71 (s, 3H), 0.64 (t, 1H, *J* = 4.4 Hz), 0.43 (dd, 1H, *J* = 5.1 and 8.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 146.0, 126.8, 82.6, 56.8, 56.7, 56.3, 48.2, 43.6, 43.0, 40.4, 35.9, 35.4, 35.2, 34.7, 33.5, 30.6, 285, 25.9, 25.1, 24.3, 22.9, 21.7, 19.5, 187, 13.3, 12.4, 12.1; IR (film, cm^{-1}) 2931, 1684, 1558, 1456, 1096; MS (ES +) *m/z* (%): 429.5 (100).

Acid (6)



The acid **5** (833 mg, 1.94 mmol) is dissolved in MeOH (18 mL, degassed with 3 freeze-pump-thaw cycle). To this solution was added a solution of $Ru(OAc)_2$[(*S*)-BINAP] (62 mg, 0.04 mmol) in MeOH (1 mL). The resulting yellow solution was stirred at 50 °C under H_2 for 15 h. The mixture was evaporated and the residue was purified by flash chromatography (dichloromethane/MeOH; 95:5) to give the acid **4** (814 mg, 97%; dr.: 5:1) as a beige solid. Recrystallization with $(i\text{Pr})_2O$ affords colorless needles with d.r. > 10:1. The same reaction using $Ru(OAc)_2$[(*S*)-H_8- BINAP] as catalyst affords the desired acid with a d.r. of 8:1 in 97% yield. $[\alpha]_D$ + 58.0 (*c* 0.06, $CHCl_3$); m.p. 161-162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.31 (s, 3H), 2.77 (s, 1H), 2.44 (qt, 1H, *J* = 6.9 Hz), 1.96 (dt, 1H, *J* = 3.3 and 12.4 Hz), 1.88 (dt, 1H, *J* = 2.9 and 13.5 Hz), 1.85-1.56 (m, 5H), 1.50 (m, 2H), 1.38 (m, 6H), 1.24 (m, 2H), 1.17 (d, 3H, *J* = 6.9 Hz), 1.16-0.98 (m, 9H), 0.89 (d, 3H, *J* = 6.6 Hz), 0.87-0.73 (m, 4H), 0.70 (s, 3H), 0.63 (t, 1H, *J* = 4.4 Hz), 0.42 (dd, 1H, *J* = 5.1 and 7.7 Hz);^{13}C NMR (125 MHz, $CDCl_3$) δ 183.1, 82.7, 56.7, 56.4, 48.2, 43.6, 43.0, 40.5, 39.6, 39.5(min), 35.9, 35.8, 35.4, 35.2, 34.2, 34.1(min), 33.5, 30.6, 28.5, 25.1, 24.3, 23.9, 23.8(min), 22.9, 21.7, 19.5, 18.8, 17.2, 16.9(min), 13.3, 12.4.; IR (film, cm^{-1}) 2933, 2866, 1733, 1700, 1558, 1456, 1096; HRMS calcd for $C_{28}H_{48}O_4$ (M + H_2O) 448. 3791 found: 448. 3770.

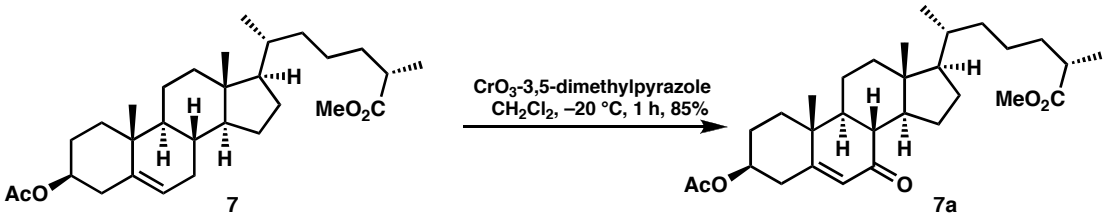
Δ^5 (7)



To a solution of the acid **6** (1.46 g, 3.39 mmol) in C_6H_6/MeOH (4:1) (34 mL) was added $TMSCHN_2$ (2 *M* in Et_2O, 2.03 mL, 4.07 mmol). The resulting solution was stirred for 20 min at r.t. and evaporated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc; 9:1) to give the ester (1.49 mg, 99%) which was submitted to the next reaction.

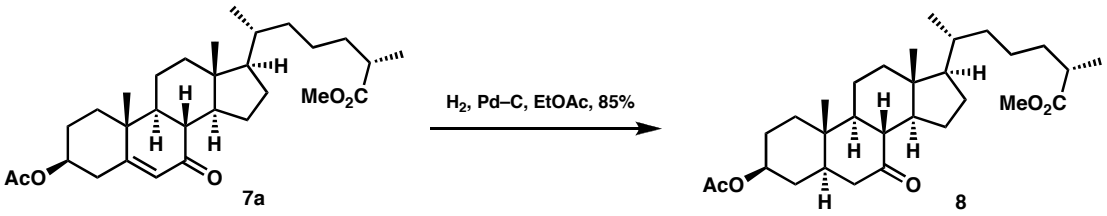
A solution of the above ester (1.50 g, 3.37 mmol) in glacial AcOH (33 mL)was heated for 4 h at 90 °C. The solution was cooled to r.t., diluted with H_2O (20 mL), and slowly neutralized with saturated aq. $NaHCO_3$. The aqueous phase was extracted with EtOAc and the organic phase was dried with Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc; 9:1) to give the **7** (1.5 g, 94%) as a white solid. $[\alpha]_D$ -30.0 (*c* 0.06, $CDCl_3$); m.p. 110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.33 (s, 1H), 4.57 (m, 1H), 3.63 (s, 3H), 2.40 (m, 1H), 2.27 (m, 2H), 1.99 (s, 3H), 1.96 (m, 2H), 1.85-1.75 (m, 3H), 1.64-1.40 (m, 7H), 1.31 (m, 4H), 1.22 (m, 1H), 1.15-1.03 (m, 6H containing a doublet of 3H, *J* = 7.3 Hz), 1.03-0.87 (m, 8H containing a singlet of 3H), 0.86 (d, 3H, *J* = 6.4 Hz), 0.63 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.5, 170.6, 139.8, 122.8, 74.1, 56.8, 56.2, 51.6, 50.2, 42.5, 39.9, 39.7, 38.3, 37.1, 36.7, 35.9, 35.8, 34.5, 32.1, 32.0, 28.4, 27.9, 24.4, 24.0, 21.6, 21.2, 19.5, 18.8, 17.4, 12.0; IR (film, cm^{-1}) 2937, 1728, 1456, 1373, 1246; MS (ES +) *m/z* (%): 473.7 (M + H^+, 20).

Enone (7a)



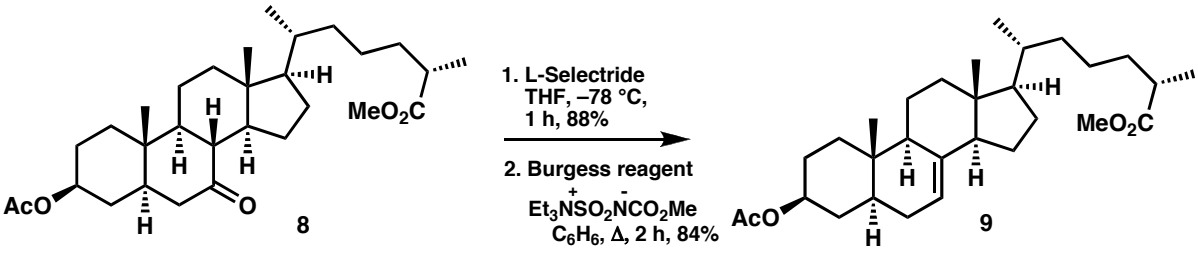
To a stirred solution of CrO_3 (2.16 g, 21.7 mmol) in CH_2Cl_2 (22 mL) was rapidly added 3,5-dimethylpyrazole (2.08 g, 21.7 mmol) at -20 °C. The resulting reddish solution was stirred at -20 °C for 20 min and **7** (570 mg, 1.2 mmol) was directly added in one portion. The solution was stirred for 1 h while allowing to warm to -15 °C. The solution was filtered through a pad of Celite® and evaporated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc; 8:2) to give the Δ^5-7-enone **7a** (498 mg, 85%) as a white solid. $[\alpha]_D$ -90.0 (*c* 0.1, $CHCl_3$); m.p. 108-110 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.67 (s, 1H); 4.68 (m, 1H), 3.64 (s, 3H), 2.53 (m, 1H), 2.50-2.34 (m, 3H), 2.20 (t, 1H, *J* = 11.2 Hz), 2.02 (s, 3H), 1.95 (m, 3H), 1.86 (m, 1H), 1.65 (m, 2H), 1.54 (m, 4H), 1.40-1.18 (m, 12H containing a singlet of 3H), 1.12 (d, 3H, *J* = 6.8 Hz), 1.04 (m, 2H), 0.88 (d, 3H, *J* = 6.4 Hz), 0.65 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.9, 177.4, 170.3, 164.0, 126.2, 72.3, 54.8, 51.6, 50.1, 49.9, 45.5, 43.2, 39.6, 38.8, 38.4, 37.9, 36.1, 35.9, 35.7, 34.4, 28.7, 27.5, 26.4, 23.9, 21.4, 21.3, 18.9, 17.4, 12.1; IR (film, cm^{-1}) 2949, 1728, 1669, 1457, 1243, 1181, 1034; HRMS calcd for $C_{30}H_{47}O_5$ ($M + H^+$) 487. 3423 found: 487. 3413.

Ketone (8)



To a stirred solution of **7a** (1.16 g, 2.38 mmol) in THF (24 mL) was added Pd/C (58 mg, 5% w/w). A hydrogen-filled balloon (1 atm) was placed over the solution and the mixture was stirred for 3h by which time TLC analysis indicated that the reaction was completed. The mixture was filtered through a pad of Celite® and washed with EtOAc (10 mL). After evaporation, the residue was purified by flash chromatography (hexanes/EtOAc; 8:2) to give **8** (990 mg, 85%) as a white solid. $[\alpha]_D$ -4.5 (*c* 0.22, $CHCl_3$); m.p. 122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.60 (m, 1H), 3.59 (s, 3H), 2.36 (q, 1H, *J* = 6.8 Hz), 2.27 (t, 2H, *J* = 12.2 Hz), 2.13 (m, 1H), 1.95 (s, 3H), 1.90 (m, 2H), 1.81 (m, 2H), 1.73 (m, 1H), 1.60 (m, 2H), 1.52-1.38 (m, 5H), 1.38-1.21 (m, 6H), 1.18 (m, 2H), 1.06 (d, 3H, *J* = 7.3 Hz), 1.03 (s, 3H), 1.00 (m, 4H), 0.85 (m, 1H), 0.82 (d, 3H, *J* = 6.4 Hz), 0.57 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.7, 177.4, 170.5, 72.9, 55.1(2), 51.5, 50.0, 49.0, 46.6, 46.0, 42.6, 39.6, 38.8, 36.1, 36.0, 35.9, 35.6, 34.4, 34.0, 28.5, 27.2, 25.1, 23.9, 21.9, 21.5, 18.8, 17.4, 12.2, 11.8; IR (film, cm^{-1}) 2942, 1733, 1705, 1558, 1456, 1239, 1024; MS (ES +) *m/z* (%): 489.7 (M + H$^+$, 40).

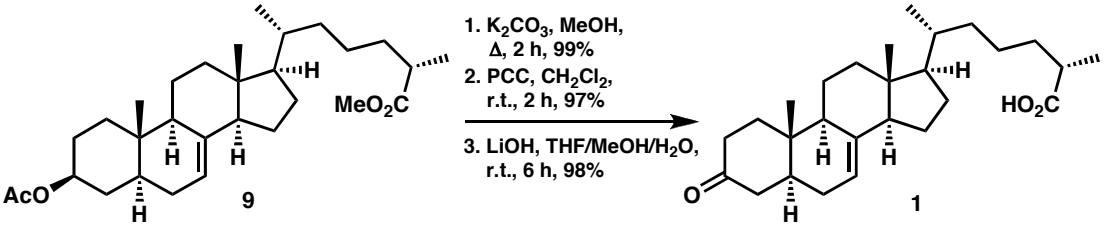
Δ^7-Unsaturated Ester (9)



To a stirred solution of **8** (990 mg, 0.116 mmol) in THF (20 mL) was slowly added L-Selectride® (2.62 mL, 2.62 mmol, 1*M* in THF) at -78 °C. The resulting solution was stirred at that temperature for 1 h and quenched with sat. $NaHCO_3$(aq) (10 mL), H_2O_2 (50% aq.) (10 mL) and diluted with EtOAc (20 mL). The solution was stirred at r.t. for 1 h and the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic phases were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography

(hexanes/EtOAc; 7:3) to give the 7-α-OH compound as a single diastereomer (878 mg, 88%) that was directly submitted to the next step. To a stirred solution of the above alcohol (52 mg, 0.105 mmol) in C_6H_6 (1 mL) was added Burgess reagent ($Et_3N^+SO_2N^-CO_2Me$)(50 mg, 0.211 mmol) at room temperature. The solution was then heated at reflux temperature for 2 h by which time TLC analysis showed complete conversion. The solution was directly submitted to flash chromatography (hexanes/EtOAc; 9:1) to give **9** (41 mg, 84%) as a white solid. $[\alpha]_D$ +16.0 (*c* 0.05, $CHCl_3$); m.p. 95 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.12 (s, 1H), 4.67 (m, 1H), 3.65 (s, 3H), 2.41 (q, 1H, *J* = 6.3 Hz), 2.00 (s, 3H), 1.98 (m, 1H), 1.86-1.83 (m, 4H), 1.81 (m, 2H), 1.80-1.60 (m, 4H), 1.60-1.40 (m, 4H), 1.34 (m, 6H), 1.11 (m, 2H), 1.12 (d, 3H, *J* = 6.8 Hz) + (m, 2H) , 1.01 (m, 1H), 0.89 (d, 3H, *J* = 6.8 Hz), 0.79 (s, 3H), 0.50 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.6, 170.9, 139.7, 117.5, 73.6, 56.2, 55.1, 51.6, 49.4, 43.5, 40.2, 39.7(2), 37.0, 36.2, 35.9, 34.5, 34.4, 34.0, 29.7, 28.9, 28.1, 27.7, 24.0, 23.1, 21.6, 18.9, 17.4, 13.1, 12.0; IR (film, cm^{-1}) 2949, 1718, 1558, 1249, 1215, 1030; HRMS calcd for $C_{30}H_{49}O_4$ (M + H^+) 473.3631 found: 473.3635.

Dafachronic acid A (1)



To a stirred solution of 3-β-acetate **9** (574 mg, 0.198 mmol) in MeOH (12 mL) was added K_2CO_3 (84 mg, 0.607 mmol) and the solution was refluxed for 2 h. The solution was cooled to r.t. and acidified with 1*N* HCl (4 mL). The aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic phase were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc; 7:3) to give the deacetylated

3-β-alcohol (514 mg, 99%), which was submitted directly to the oxidation step. To a stirred solution of the 3-β-alcohol (514 mg, 1.19 mmol) in CH_2Cl_2 (15 mL) was added pyridinium chlorochromate (PCC) (334 mg, 1.55 mmol) at r.t. The solution was stirred at that temperature for 2h and filtered through a short pad of Celite® which was washed with CH_2Cl_2. After evaporation of the filtrate, the residue was purified by flash chromatography (hexanes/EtOAc; 8:2) to give the corresponding 3-ketoester (490 mg, 97%) which was submitted directly to the hydrolysis step. To a stirred solution of the above ester (490 mg, 1.14 mmol) in THF/MeOH/H_2O (2:1:1) (11 mL) was added $LiOH{\cdot}H_2O$ (956 mg, 3.48 mmol) at r.t. and the reaction was stirred for 6h by which time TLC analysis showed complete conversion. The reaction mixture was acidified by 3*M* HCl and the aqueous phase was extracted with EtOAc (3 x 25 mL). The combined organic phase were dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography (hexanes/EtOAc; 7:3) to give **1** (466 mg, 98%) as a white solid. $[\alpha]_D$ +23.4 (*c* 2.0, $CDCl_3$); m.p. 143 °C ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.16 (s, 1H), 2.45 (m, 1H), 2.40 (dd, 1H, *J* = 5.8 and 14.6 Hz), 2.25 (m, 3H), 2.11 (m, 1H), 2.05 (m, 1H), 1.81 (m, 5H), 1.78-1.58 (m, 4H), 1.52 (m, 3H), 1.48-1.32 (m, 7H), 1.30-1.18 (m, 5H), 1.16 (d, 3H, *J* = 6.8 Hz), 1.01 (m, 1H), 0.90 (d, 3H, *J* = 6.8 Hz), 0.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 212.3, 183.3, 139.7, 117.2, 56.2, 55.1, 49.0, 44.4, 43.5, 43.0, 39.6(2), 38.9, 38.3, 36.2, 35.9, 34.6, 34.2, 30.2, 28.1, 24.0, 23.1, 21.9, 18.9, 17.2, 12.6. 12.1; IR (film, cm^{-1}) 2939, 2869, 1733, 1701, 1684, 1653, 1558, 1457, 1215; HRMS calcd for $C_{27}H_{43}O_3$ (M + H^+) 415.3206 found: 415.3211.

3. References.

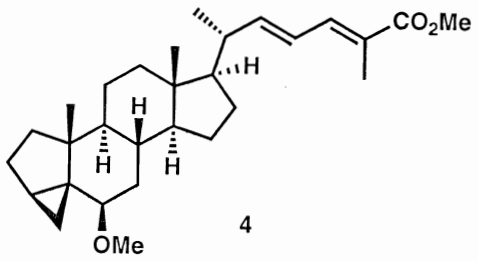
(1) The phosphonate **3** was prepared according to a literature procedure: Kitahara, T.; Horiguchi, A.; Mori, K. *Tetrahedron* **1988**, *44*, 4713.

(2) Aldehyde **2** was prepared in large amounts according to literature procedures in: (a) Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571-572. (b) Salmond, W. G.; Sobala, M. C. *Tetrahedron Lett.* **1977**, *18*, 1695-1698.

STANDARD PROTON PARAMETERS

exp1 s2pul

SAMPLE		DEC. & VT	
date	Feb 19 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	32
ACQUISITION		dof	0
sfrq	500.176	dm	nnn
tn	H1	dmm	c
at	2.048	dmf	8770
np	32768	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	8	temp	22.0
ss	2	PROCESSING	
tpwr	58	lb	0.10
pw	5.0	wtfile	
d1	0	proc	ft
tof	0	fn	not used
nt	10000	math	f
ct	64		
alock	n	werr	
gain	not used	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	48.3		
wp	4897.9		
vs	127		
sc	0		
wc	250		
hzmm	19.59		
is	536.08		
rfl	1499.0		
rfp	0		
th	7		
ins	1.000		
nm cdc ph			

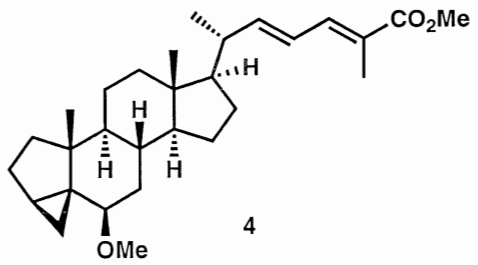


4

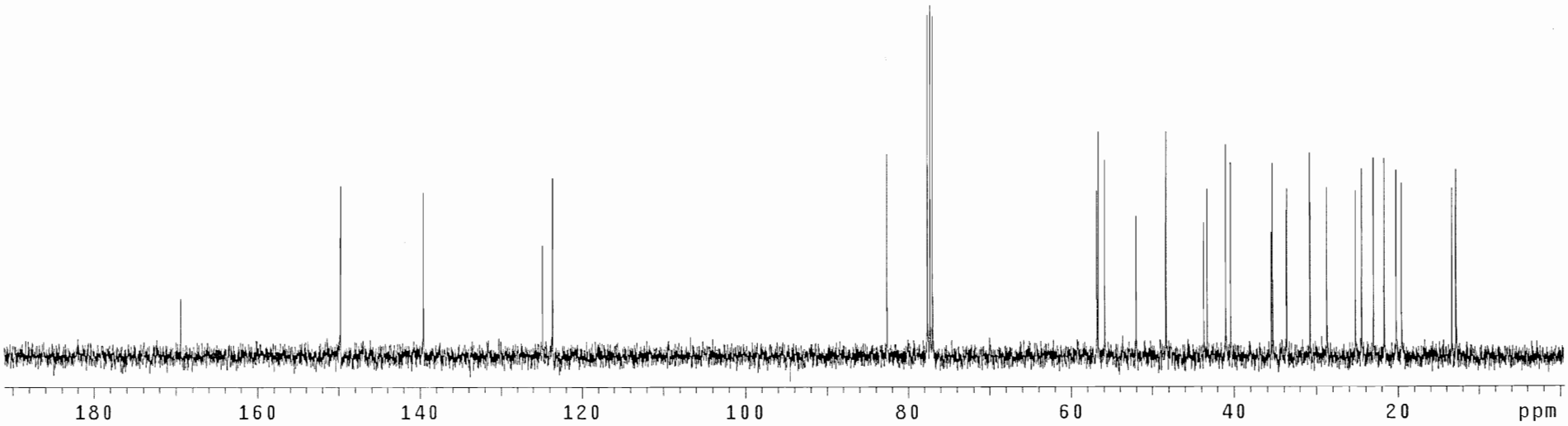
13C OBSERVE

exp2 std13c

SAMPLE		DEC. & VT	
date	Jan 13 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	0
sfrq	100.447	dm	yyy
tn	C13	dmm	w
at	1.311	dmf	9000
np	62862	temp	23.0
sw	23980.8	PROCESSING	
fb	13200	lb	1.00
bs	16	wtfile	
ss	2	proc	ft
tpwr	60	fn	not used
pw	4.0		
d1	0	werr	
tof	0	wexp	
nt	1024	wbs	
ct	160	wnt	
alock	n		
gain	30		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-52.3		
wp	19242.0		
vs	56		
sc	0		
wc	250		
hzmm	76.97		
is	500.00		
rfl	2493.7		
rfp	0		
th	20		
ins	100.000		
nm no ph			



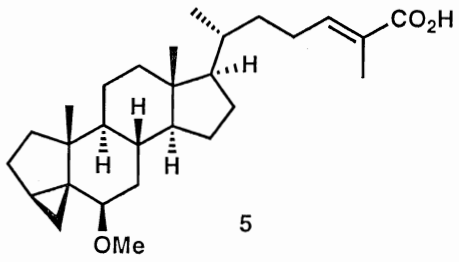
4



STANDARD 1H OBSERVE

exp1 std1h

SAMPLE		DEC. & VT	
date	Jan 25 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	43
ACQUISITION		dof	0
sfrq	399.433	dm	nnn
tn	H1	dmm	c
at	2.731	dmf	12200
np	32768	temp	23.0
sw	5998.8	PROCESSING	
fb	3400	lb	0.10
bs	8	wtfile	
ss	2	proc	ft
tpwr	59	fn	not used
pw	12.0		
d1	0	werr	
tof	0	wexp	
nt	1000	wbs	
ct	88	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	54.6		
wp	3110.9		
vs	100		
sc	0		
wc	250		
hzmm	12.44		
is	820.86		
rfl	1031.8		
rfp	0		
th	20		
ins	1.000		
nm	ph		

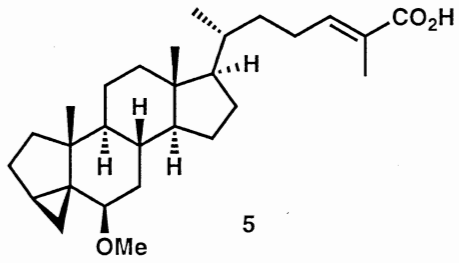


5

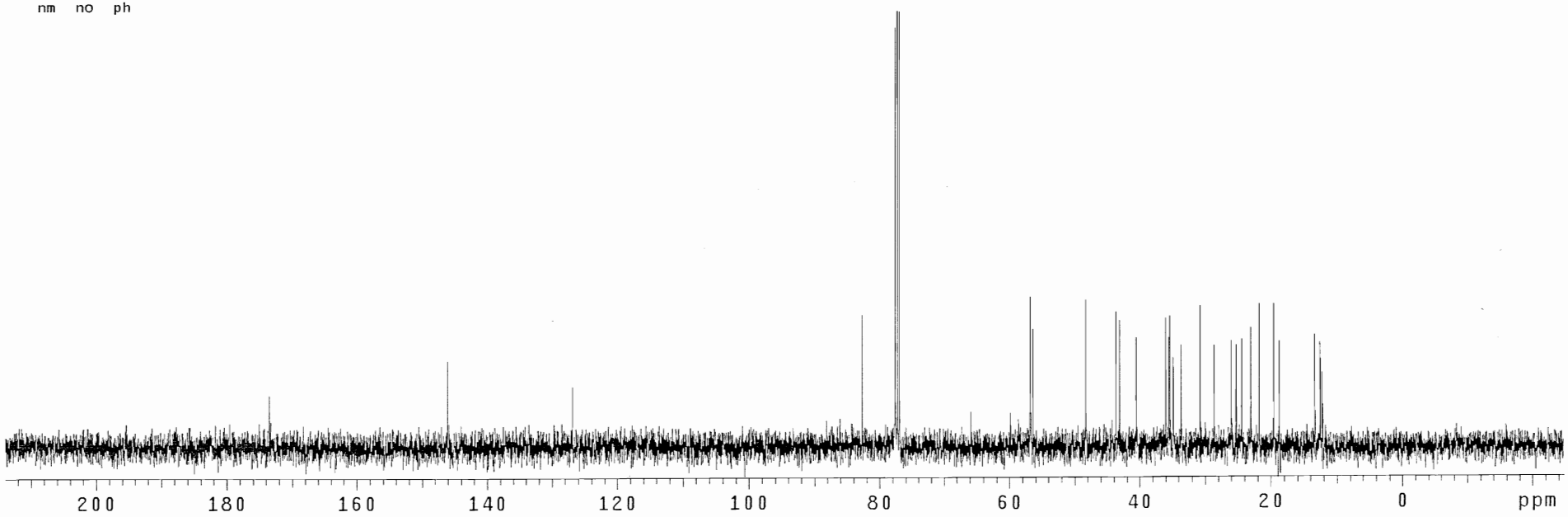
13C OBSERVE

exp1 std13c

SAMPLE		DEC. & VT	
date	Jan 25 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	0
sfrq	100.447	dm	yyy
tn	C13	dmm	w
at	1.311	dmf	8000
np	62862	temp	23.0
sw	23980.8	PROCESSING	
fb	13200	lb	1.00
bs	16	wtfile	
ss	2	proc	ft
tpwr	60	fn	not used
pw	4.0		
d1	0	werr	
tof	0	wexp	
nt	1024	wbs	
ct	128	wnt	
alock	n		
gain	30		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-2493.7		
wp	23980.8		
vs	70		
sc	0		
wc	250		
hzmm	95.92		
is	500.00		
rfl	2493.7		
rfp	0		
th	6		
ins	100.000		
nm no ph			



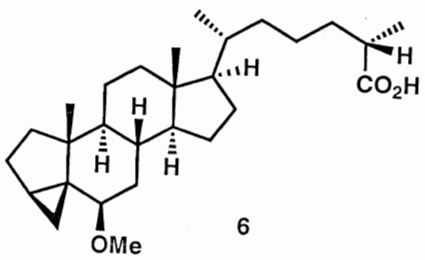
5



STANDARD PROTON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Mar 16 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	32
ACQUISITION		dof	0
sfrq	500.176	dm	nnn
tn	H1	dmm	c
at	2.048	dmf	8770
np	32768	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	8	temp	22.0
ss	2	PROCESSING	
tpwr	58	lb	0.10
pw	5.0	wtfile	
d1	0	proc	ft
tof	0	fn	not used
nt	10000	math	f
ct	64		
alock	n	werr	
gain	not used	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-98.1		
wp	3810.5		
vs	127		
sc	0		
wc	250		
hzmm	15.24		
is	339.33		
rfl	1499.0		
rfp	0		
th	7		
ins	1.000		
nm cdc ph			

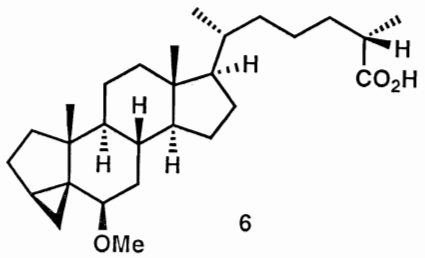


6

STANDARD CARBON PARAMETERS

exp1 s2pul

SAMPLE		DEC. & VT	
date	Mar 29 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	38
ACQUISITION		dof	0
sfrq	125.781	dm	yyy
tn	C13	dmm	w
at	1.170	dmf	15970
np	65536	dseq	
sw	28001.4	dres	1.0
fb	15000	homo	n
bs	16	temp	22.0
tpwr	57	PROCESSING	
pw	8.0	lb	1.00
d1	0.100	wtfile	
tof	0	proc	ft
nt	1000	fn	not used
ct	352	math	f
alock	n		
gain	56	werr	
FLAGS		wexp	
il	n	wbs	
in	n	wnt	
dp	y		
hs	nn		
DISPLAY			
sp	1153.4		
wp	22544.3		
vs	149		
sc	0		
wc	250		
hzmm	90.18		
is	500.00		
rfl	2051.1		
rfp	0		
th	68		
ins	100.000		
nm cdc ph			

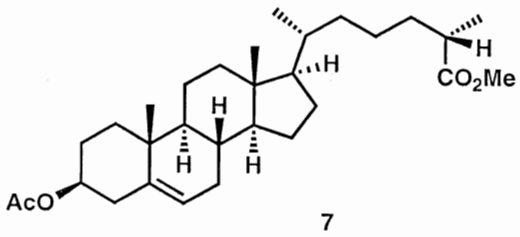


6

STANDARD PROTON PARAMETERS

exp1 s2pul

SAMPLE		DEC. & VT	
date	Feb 18 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	32
ACQUISITION		dof	0
sfrq	500.176	dm	nnn
tn	H1	dmm	c
at	2.048	dmf	8770
np	32768	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	8	temp	22.0
ss	2	PROCESSING	
tpwr	58	lb	0.10
pw	5.0	wtfile	
d1	0	proc	ft
tof	0	fn	not used
nt	1000	math	f
ct	64		
alock	n	werr	
gain	not used	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	247.1		
wp	3397.0		
vs	152		
sc	0		
wc	250		
hzmm	13.59		
is	1456.31		
rfl	1499.0		
rfp	0		
th	7		
ins	1.000		
nm cdc ph			



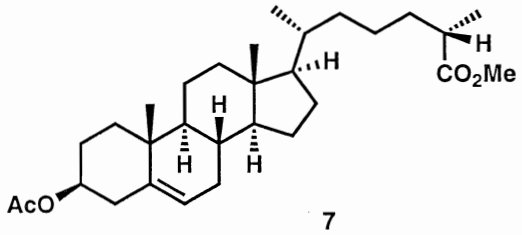
7

STANDARD CARBON PARAMETERS

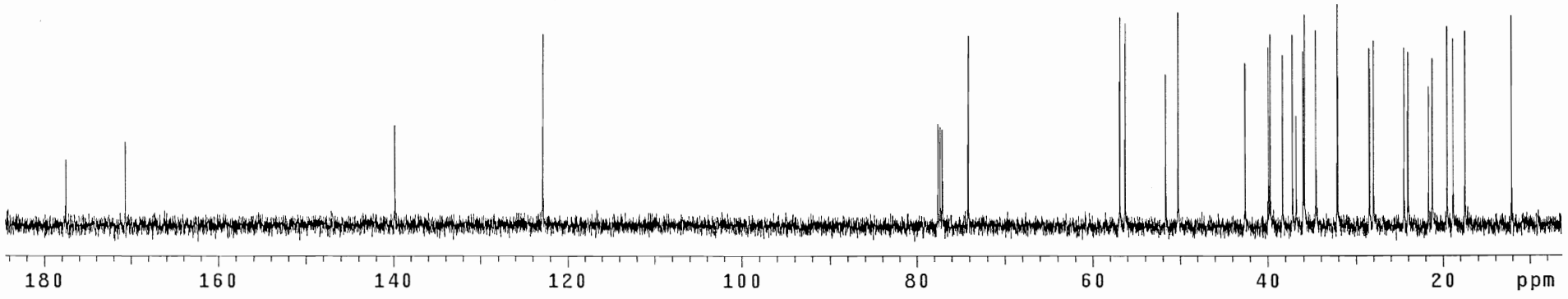
exp3 s2pul

SAMPLE		DEC. & VT	
date	Apr 13 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	38
ACQUISITION		dof	0
sfrq	125.781	dm	yyy
tn	C13	dmm	w
at	1.170	dmf	15970
np	65536	dseq	
sw	28001.4	dres	1.0
fb	15000	homo	n
bs	16	temp	22.0
tpwr	57	PROCESSING	
pw	8.0	lb	1.00
d1	0.100	wtfile	
tof	0	proc	ft
nt	1000	fn	not used
ct	96	math	f
alock	n		
gain	56	werr	
FLAGS		wexp	
il	n	wbs	
in	n	wnt	
dp	y		
hs	nn		
DISPLAY			
sp	793.6		
wp	22405.1		
vs	36		
sc	0		
wc	250		
hzmm	89.62		
is	500.00		
rfl	2051.1		
rfp	0		
th	3		
ins	100.000		

nm cdc ph



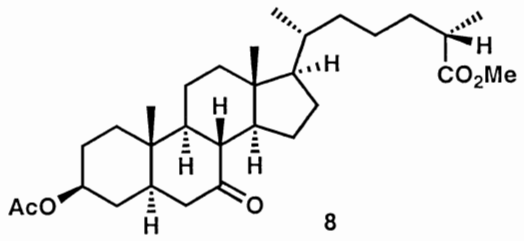
7



STANDARD PROTON PARAMETERS

exp1 s2pul

SAMPLE		DEC. & VT	
date	Feb 23 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	32
ACQUISITION		dof	0
sfrq	500.176	dm	nnn
tn	H1	dmm	c
at	2.048	dmf	8770
np	32768	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	8	temp	22.0
ss	2	PROCESSING	
tpwr	58	lb	0.10
pw	5.0	wtfile	
d1	0	proc	ft
tof	0	fn	not used
nt	10000	math	f
ct	64		
alock	n	werr	
gain	not used	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-27.3		
wp	3684.1		
vs	127		
sc	0		
wc	250		
hzmm	14.74		
is	355.32		
rfl	1499.0		
rfp	0		
th	7		
ins	1.000		
nm cdc ph			

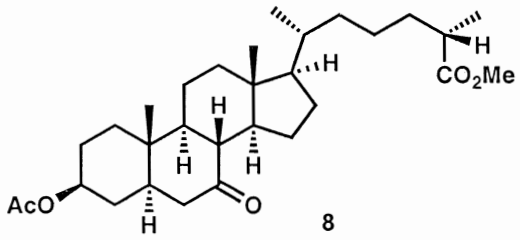


8

13C OBSERVE

exp1 std13c

SAMPLE		DEC. & VT	
date	Apr 23 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	0
sfrq	100.447	dm	yyy
tn	C13	dmm	w
at	1.311	dmf	8000
np	62862	temp	23.0
sw	23980.8	PROCESSING	
fb	13200	lb	1.00
bs	16	wtfile	
ss	2	proc	ft
tpwr	60	fn	not used
pw	4.0		
d1	0	werr	
tof	0	wexp	
nt	1024	wbs	
ct	192	wnt	
alock	n		
gain	30		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	401.5		
wp	21044.6		
vs	151		
sc	0		
wc	250		
hzmm	84.18		
is	500.00		
rfl	2493.7		
rfp	0		
th	11		
ins	100.000		
nm no ph			



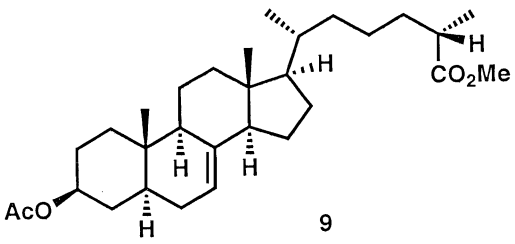
8

STANDARD PROTON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Apr 27 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	32
ACQUISITION		dof	0
sfrq	500.176	dm	nnn
tn	H1	dmm	c
at	2.048	dmf	8770
np	32768	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	8	temp	22.0
ss	2	PROCESSING	
tpwr	58	lb	0.10
pw	5.0	wtfile	
d1	0	proc	ft
tof	0	fn	not used
nt	1000	math	f
ct	104		
alock	n	werr	
gain	not used	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	165.5		
wp	3489.3		
vs	127		
sc	0		
wc	250		
hzmm	13.96		
is	526.00		
rfl	1499.0		
rfp	0		
th	3		
ins	1.000		

nm cdc ph

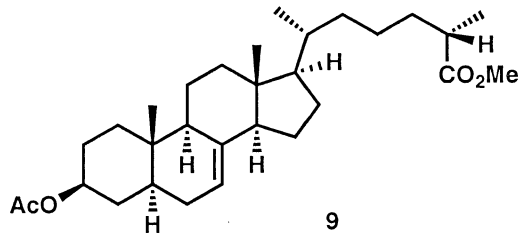


9

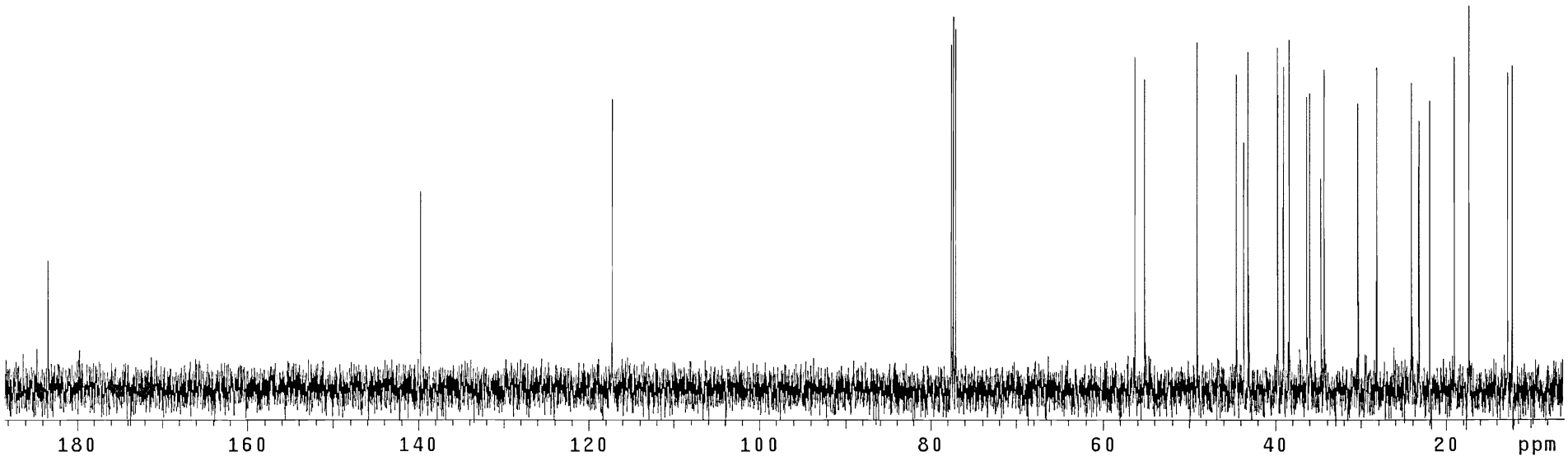
STANDARD CARBON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Apr 27 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	38
ACQUISITION		dof	0
sfrq	125.781	dm	yyy
tn	C13	dmm	w
at	1.170	dmf	15970
np	65536	dseq	
sw	28001.4	dres	1.0
fb	15000	homo	n
bs	16	temp	22.0
tpwr	57	PROCESSING	
pw	8.0	lb	1.00
d1	0.100	wtfile	
tof	0	proc	ft
nt	1000	fn	not used
ct	96	math	f
alock	n		
gain	56	werr	
FLAGS		wexp	
il	n	wbs	
in	n	wnt	
dp	y		
hs	nn		
DISPLAY			
sp	768.0		
wp	22926.3		
vs	62		
sc	0		
wc	250		
hzmm	91.71		
is	500.00		
rfl	2051.1		
rfp	0		
th	11		
ins	100.000		
nm cdc ph			



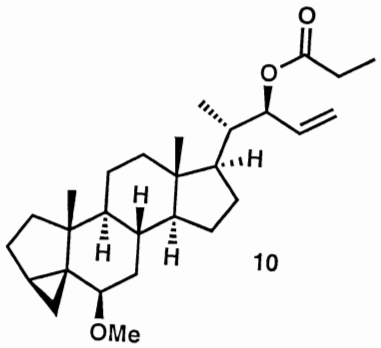
9



STANDARD 1H OBSERVE

exp1 std1h

SAMPLE		DEC. & VT	
date	Jun 5 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	43
ACQUISITION		dof	0
sfrq	399.433	dm	nnn
tn	H1	dmm	c
at	2.731	dmf	12200
np	32768	temp	23.0
sw	5998.8	PROCESSING	
fb	3400	lb	0.10
bs	8	wtfile	
ss	2	proc	ft
tpwr	59	fn	not used
pw	12.0		
d1	0	werr	
tof	0	wexp	
nt	10000	wbs	
ct	80	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	0.0		
wp	3259.6		
vs	162		
sc	0		
wc	250		
hzmm	13.04		
is	240.88		
rfl	1031.8		
rfp	0		
th	20		
ins	1.000		
nm	ph		



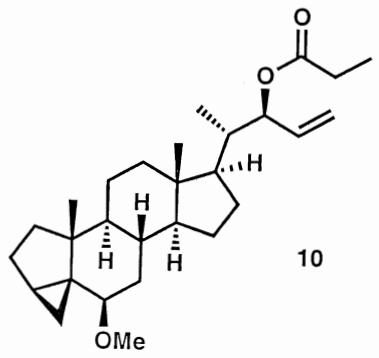
10

13C OBSERVE

exp1 std13c

SAMPLE		DEC. & VT	
date	Jun 5 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	0
sfrq	100.447	dm	yyy
tn	C13	dmm	w
at	1.311	dmf	8000
np	62862	temp	23.0
sw	23980.8	PROCESSING	
fb	13200	lb	1.00
bs	16	wtfile	
ss	2	proc	ft
tpwr	60	fn	not used
pw	4.0		
d1	0	werr	
tof	0	wexp	
nt	1024	wbs	
ct	176	wnt	
alock	n		
gain	30		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	124.1		
wp	18664.6		
vs	151		
sc	0		
wc	250		
hzmm	74.66		
is	500.00		
rfl	2493.7		
rfp	0		
th	12		
ins	100.000		

nm no ph

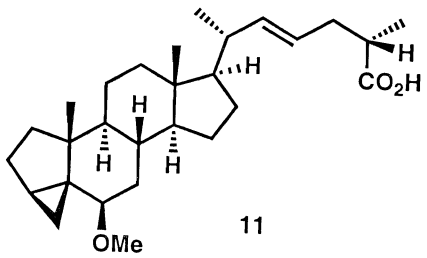


10

STANDARD PROTON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Jun 22 2007	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	32
ACQUISITION		dof	0
sfrq	500.176	dm	nnn
tn	H1	dmm	c
at	2.048	dmf	8770
np	32768	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	8	temp	22.0
ss	2	PROCESSING	
tpwr	58	lb	0.10
pw	5.0	wtfile	
d1	0	proc	ft
tof	0	fn	not used
nt	10000	math	f
ct	48		
alock	n	werr	
gain	not used	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	89.4		
wp	3731.4		
vs	127		
sc	0		
wc	250		
hzmm	14.93		
is	403.90		
rfl	1499.0		
rfp	0		
th	7		
ins	1.000		
nm cdc ph			



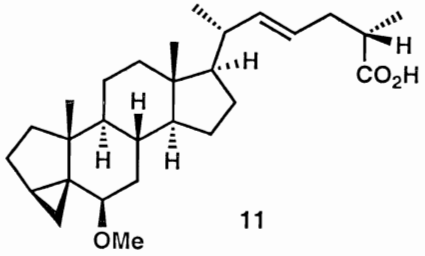
11

13C OBSERVE

exp3 std13c

SAMPLE		DEC. & VT	
date	Jun 6 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	0
sfrq	100.447	dm	yyy
tn	C13	dmm	w
at	1.311	dmf	8000
np	62862	temp	23.0
sw	23980.8	PROCESSING	
fb	13200	lb	1.00
bs	16	wtfile	
ss	2	proc	ft
tpwr	60	fn	not used
pw	4.0		
d1	0	werr	
tof	0	wexp	
nt	1024	wbs	
ct	208	wnt	
alock	n		
gain	30		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	757.2		
wp	17886.6		
vs	151		
sc	0		
wc	250		
hzmm	71.55		
is	500.00		
rfl	2493.7		
rfp	0		
th	13		
ins	100.000		

nm no ph

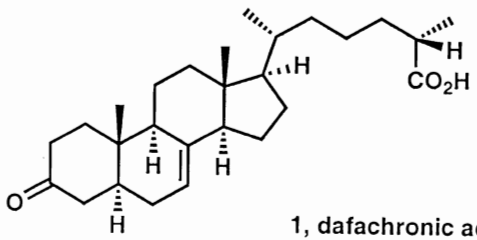


11

STANDARD 1H OBSERVE

exp1 std1h

SAMPLE		DEC. & VT	
date	Jun 13 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	43
ACQUISITION		dof	0
sfrq	399.433	dm	nnn
tn	H1	dmm	c
at	2.731	dmf	12200
np	32768	temp	23.0
sw	5998.8	PROCESSING	
fb	3400	lb	0.10
bs	8	wtfile	
ss	2	proc	ft
tpwr	59	fn	not used
pw	12.0		
d1	0	werr	
tof	0	wexp	
nt	10000	wbs	
ct	48	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	147.6		
wp	2945.8		
vs	108		
sc	0		
wc	250		
hzmm	11.78		
is	500.00		
rfl	1031.8		
rfp	0		
th	20		
ins	1.000		
nm	ph		



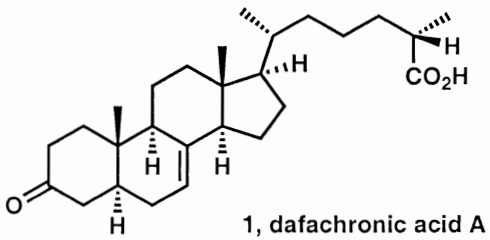
1, dafachronic acid A

13C OBSERVE

exp1 std13c

SAMPLE		DEC. & VT	
date	Jun 13 2007	dfrq	399.433
solvent	CDCl3	dn	H1
file	exp	dpwr	41
ACQUISITION		dof	0
sfrq	100.447	dm	yyy
tn	C13	dmm	w
at	1.311	dmf	8000
np	62862	temp	23.0
sw	23980.8	PROCESSING	
fb	13200	lb	1.00
bs	16	wtfile	
ss	2	proc	ft
tpwr	60	fn	not used
pw	4.0		
d1	0	werr	
tof	0	wexp	
nt	1024	wbs	
ct	320	wnt	
alock	n		
gain	30		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	596.9		
wp	20867.5		
vs	63		
sc	0		
wc	250		
hzmm	83.47		
is	500.00		
rfl	2493.7		
rfp	0		
th	20		
ins	100.000		

nm no ph



1, dafachronic acid A