Pergamon

Tetrahedron, Vol. 53, No. 2, pp. 617-624, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)01023-X

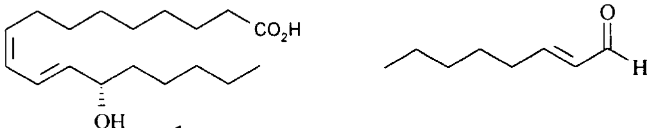
The Chemoenzymatic Synthesis of (*S*)-13-Hydroxyoctadeca-(9*Z*, *11E*)-dienoic Acid using the Hydroxynitrile Lyase from *Hevea brasiliensis*

Dean V. Johnson and Herfried Griengl*

Spezialforschungsbereich Biokatalyse, Institut für Organische Chemie der Technischen Universität Graz,
Stremayrgasse 16, A-8010 Graz, Austria

Abstract: (*S*)-13-Hydroxyoctadeca-(9*Z*, 11*E*)-dienoic acid (**1**) was synthesized in nine steps starting from (*E*)-2-octenal (**2**). The (*S*)-hydroxynitrile lyase cloned from *Hevea brasiliensis*, that has been overexpressed in *Pichia pastoris*, was used to interconvert **2** into (*S*)-cyanohydrin **3**. The subsequent butyrate **6** was rearranged using the palladium-*bis*-(acetonitrile)-dichloride to give the unsaturated nitrile **7** which was fully elaborated to the hydroxy acid **1** in an overall 11% yield and with 99% e.e.

(*S*)-13-Hydroxy-(9*Z*, 11*E*)-octadecadienoic acid (**1**) (13-*S*-HODE) (Figure 1), also known as coriolic acid, is an oxygenated metabolite of linoleic acid which possesses a number of important biological activities. These properties include acting as a self defense agent against rice blast disease,[1] displaying unique calcium ionophoric acitivity[2] and more notably by acting as a prostacyclin mimic.[3] These properties have prompted numerous of synthetic[4] and chemo-enzymatic[5] approaches to this valuable compound. We envisaged that this compound was accessible by employing an alternative chemo-enzymatic method in which hydrogen cyanide (HCN) could be added to a nonchiral aldehyde, such as **2** (Figure 1). This enantioselective processes would generate the chiral center required for the target compound **1**.

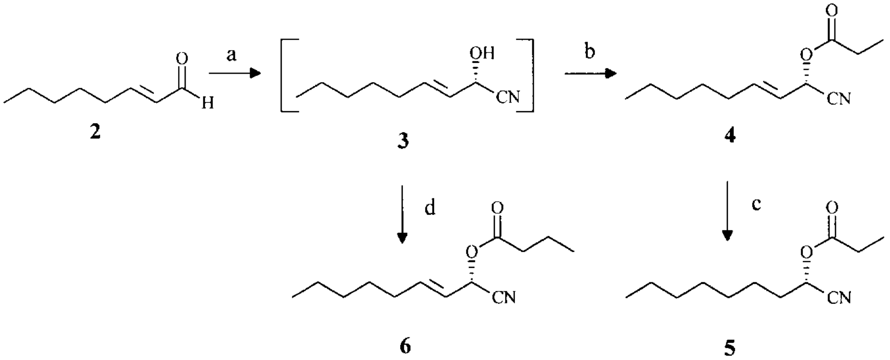


1 **2**

Figure 1

Results and Discussion

The stereoselective addition of HCN to unsaturated aldehydes, to yield unsaturated (*S*)-cyanohydrins, using the hydroxynitrile lyase (Hnl) from *Hevea brasiliensis* has been previously reported by our group.[6] Latterly, the enzyme has been successfully overexpressed in *Pichia pastoris*[7] providing large quantities of the enzyme for synthesis. Thus, the key synthon **6** (Scheme 1) was generated by treatment of commercially available (*E*)-2-octenal **2** with HCN and a partially purified extract[7] of overexpressed Hnl (1000 IU/mmol of substrate) in diisopropyl ether (DIPE). Esterification of the crude cyanohydrin **3** (Scheme 1) with butyric anhydride, pyridine, catalytic dimethylaminopyridine (DMAP) in DIPE provided the butyrate **6** in good yield (80%) and excellent enantiomeric excess (99% e.e.), as determined by chiral gas chromatography (GC) (Chrompack, C-P-Chirasil-Dex-CB). It is noteworthy to that the butyrate **6** can be prepared on a gram scale (20 mmole) and thus this represents a useful synthetic method. It was the butyrate **6** that was required for further synthetic elaboration, however it possessed no easily accessible derivative of known configuration. Hence, a portion of the crude cyanohydrin **3** was also derivatized to the propionate ester **4**. Hydrogenation of **4** with H_2/Pd-C in ethyl acetate (68% yield) gave the saturated cyanohydrin **5** ($[\alpha]_D^{20}$ -50.8, c 1.50, benzene) whose optical rotation value was consistent with the literature value ($[\alpha]_D^{20}$ -51.0, c 3.00, benzene)[8] for the (*S*)-isomer.



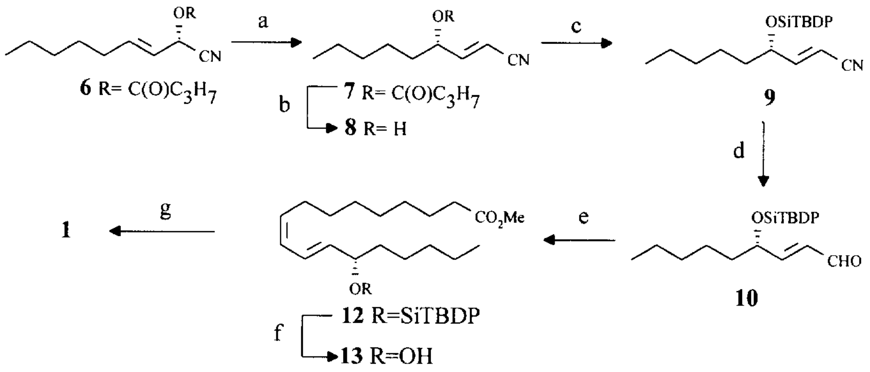
(a) HCN (4 equiv.), Hnl (1000 IU/mmol of substrate), DIPE, 0-4°C, 5h. (b) propionic anhydride, TEA, catalytic. DMAP, CH_2Cl_2. (c) H_2/Pd-C, ethyl acetate. (d) butyric anhydride, pyridine, catalytic DMAP, DIPE.

Scheme 1

The palladium catalyzed [3,3]-sigmatropic rearrangement of **6** (Scheme 2), with $Pd(CH_3CN)_2Cl_2$,[9] proceeded smoothly (80%) to yield the unsaturated nitrile **7**. Enantiomeric separation of **7** by chiral GC (Chrompack, C-P-Chirasil-Dex-CB) confirmed that **7** possessed an enantiomeric excess of 99%. Treatment of **7** with K_2CO_3 in methanol gave the alcohol **8**, ($[\alpha]_D^{20}$ +37.4, c 1.12, $CHCl_3$), {literature value ($[\alpha]_D^{20}$ +37.5, c 0.99, $CHCl_3$, as the *S*-form)}[10] with the (*S*)-configuration. Thus it was shown that the process of chirality transfer from C-2 in the butyrate **6** to C-4 in the unsaturated nitrile **7** (Scheme 1) had occurred with complete retention of configuration and enantiomeric excess.

The observed Pd^{II}-mediated transformation in which the chirality transfer proceeds with complete retention of stereochemistry, is in accordance with previous transformations of allylic acetates[9] and unsaturated cyanohydrin derivatives.[11]

Protection of the alcohol **8** with *tert*-butyldiphenylchlorosilane (TBDPSi), imidazole, DMF, yielded the silyl derivative **9** which was enantiomerically separated by chiral high performance liquid chromotography (HPLC) (Chiracel OD-H, *n*-heptane/2-propanol 99.75:0.25, 0.6 ml/min, 254nm). This revealed that **9** possessed an enantiomeric excess of 99%, and thus the hydrolysis of **7** (to yield the alcohol **8**) had also occurred without racemisation. Thus, with the optical purity of **9** now established the synthesis of **1** was efficiently completed. Diisobutylaluminuimhydride (DIBAL-H) reduction of **9** followed by acidic hydrolysis (SiO_2/H_2O) of the *in situ* imine gave the unsaturated aldehyde **10** (70%), which was coupled *via* a Wittig reaction[12]with the triphenylphosphonium salt **11**[13] (1.4 equiv.) to yield the diene **12** in moderate yield (67%) but with excellent isomeric purity (*E*:*Z* >98:2 by NMR). Desilylation of **12** with *n*-tetrabutylammonium fluoride (TBAF) (3.3 equiv., rt, 5.5h, THF) (78%) afforded (*S*)-methyl coriolate (**13**) which showed good correlation to a previously prepared sample[12] ($[\alpha]_D^{20}$ + 6.26 (c 0.87, $CHCl_3$), lit.[12] +6.50 (c 0.70, $CHCl_3$). Saponification of the methyl ester (LiOH, IPA-H_2O, 65%) and careful acidification (1M HCl) gave **1**, which was identical in all respects to (*S*)-coriolic acid {$[\alpha]_D^{20}$ +6.75 (c 0.80, $CHCl_3$), lit.[4e] +6.20 (c 0.30, $CHCl_3$)}, in an overall 11% yield starting from (*E*)-2-octenal in 9 steps.



(a) 12 mol % $Pd(CH_3CN)_2Cl_2$, 80°C, 5h, THF. (b) K_2CO_3, MeOH, rt, 5h. (c) TBDPSiCl, imidazole, rt, 1.5h, DMF. (d) DIBAL-H, -78°C, 1.5 h, toluene, then SiO_2/H_2O, 1.5 h. (e) $Ph_3^+Br^-(CH_2)_8CO_2Me$ (**11**), Li(HMDS), THF/HMPA (8:1), -78°C. (f) TBAF, 3.3 equiv., 23h, THF. (g) LiOH/H_2O/IPA, followed by acidification to pH 7.0 with HCl.

Scheme 2

Experimental

General: ^1H NMR and ^{13}C NMR were recorded on Varian Gemini 200 MHz and Bruker MSL 300 MHz instruments in $CDCl_3$ using tetramethylsilane as an internal standard. IR Spectra were recorded on a Bomen Michelson 100 instrument and mass spectra on a Hewlett Packard HP6890 mass selective detector. Optical rotations were measured using a Perkin Elmer 341 instrument. Enantiomeric separations of **6** and **7** by chiral gas chromatography used a 25 m x 0.32 mm Chirasil-DEX-CB fused silica capillary column (0.25μm; carrier gas hydrogen) and a temperature gradient from 100 °C to 160 °C (3°/min). HPLC Enantiomeric separation of **9** was performed using a Jasco 880-PU intelligent pump and a Jasco 875-UV intelligent UV/VIS detector connected to a CHIRALCEL OD-H chiral HPLC column (25 cm x 0.46 cm) as chiral selector. The mobile phase was a mixture of *n*-heptane/2-propanol (99.75:0.25), 0.6 ml/min, with detection at 254nm.

Tetrahydrofuran was distilled from sodium benzophenone ketyl, hexamethylphosphorotriamide (HMPA) from calcium hydride, toluene from sodium, DMF from calcium sulphate and methanol from magnesium. All organic layers were dried using anhydrous sodium sulphate. All products were purified by silica gel column chromatography using Merck Kieselgel 60 (230-400 mesh). All reagents were obtained from Sigma-Aldrich and were used as purchased. Petroleum ether refers to the fraction of boiling point 60-80 °C. *Trans*-2-octenal (**2**) was distilled under reduced pressure prior to use. A recombinant (*S*)-hydroxynitrile lyase, which is homologous to the natural enzyme from *Hevea brasiliensis*, was prepared by overexpression in *Pichia pastoris*. A crude cytosolic extract[7] was used for the biotransformation of **2**.

(+)-(2*S*,3*E*)-2-Butanoylnon-3-enenitrile (6): A solution of Hnl (25000 IU) in water (25ml) was added in one portion to a cooled solution (5°C) of freshly distilled *trans*-2-octenal (3.16 g, 25 mmol) in DIPE (50 ml) and the mixture was stirred for 10 min on an ice bath. HCN (3.8 ml, 99 mmol) was added quickly in one portion and the flask was tightly sealed. After 5 h stirring at 0-5°C, the reaction mixture was extracted with DIPE (4 x 50 ml). The ether extracts were combined and centrifuged (10 min at 5000 rpm) for good phase separation, collected and dried using anhydrous sodium sulphate. After removal of the sodium sulphate by filtration, the solution of crude cyanohydrin **3** was derivatized directly by reaction with butyric anhydride (20 ml, 122 mmol), pyridine (10 ml, 124 mmol) and a catalytic amount of DMAP with stirring overnight at room temperature. The organic layer was washed sequentially with 5% H_2SO_4, distilled water and saturated sodium bicarbonate (50 ml each), dried and concentrated *in vacuo*. The crude residue was purified by column chromatography using cyclohexane/ethyl acetate (30:1-20:1), to yield pure **6** (4.48 g, 20.1 mmol, 80% yield) as a colourless oil; $[\alpha]_D^{20}$ +22.3 (c 1.25, $CHCl_3$). IR (neat)/cm^{-1} 2941, 2864 (C-H); 1752 (C=O); 1156 (C-O). ^{1}H NMR: δ(ppm) 0.83-0.97 (2 x t, 6H, J = 6.73, 5.94 Hz, 9-CH_3, 4'-CH_3); 1.22-1.43 (m, 6H, 8-CH_2, 7-CH_2, 6-CH_2); 1.73-1.58 (m, 2H, 3'-CH_2); 2.04-2.13 (q, 2H, J = 6.77 Hz, 5-CH_2); 2.30-2.35 (t, 2H, J = 6.98 Hz, 2'-CH_2); 5.45-5.48 (qt, 1H, J = 15.34, 6.63, 1.53 Hz, 3-CH); 5.76-5.83 (dd, 1H, J = 6.63, 1.04 Hz, 2-CH); 6.04-6.20 (dtd, 1H, J = 15.57, 6.77, 1.02 Hz, 4-CH).^{13}C NMR δ 171.63 (C=O); 140.62 (3-CH); 120.09 (4-CH); 115.91 (CN); 61.28 (2-CH); 35.57 (2'-CH_2); 31.99 (CH_2); 31.26 (CH_2); 31.26 (CH_2); 28.02 (CH_2); 22.43 (CH_2); 18.20 (CH_2); 13.97 (CH_3); 13.51 (CH_3). MS: m/z (relative intensity %): 223 (M^+, 1); 153 (4); 96 (23); 86 (14); 71 ($O=C^+$-C_3H_7, 100); 43 (64).

(+)-(2*S*,3*E*)-2-Propanoylnon-3-enenitrile (4): Propionic anhydride (72 mg, 0.553 mmol), triethylamine (93 mg, 0.62 mmol) and a catalytic quantity of DMAP was added to a cooled solution (5 °C) of the crude cyanohydrin **3** (77 mg, 0.50 mmol) in CH_2Cl_2 (7 ml). The mixture was allowed to slowly warm to room temperature, at which it was stirred overnight. The mixture was diluted with CH_2Cl_2 (30 ml) and washed sequentially with 1M HCl (10 ml), water (10 ml), saturated $NaHCO_3$ solution (10 ml) and saturated sodium chloride solution (10 ml), dried and concentrated *in vacuo*. The crude residue was purified by column chromatography with petroleum ether/diethyl ether (12:1), to yield **4** (57 mg, 0.27 mmol, 30% yield) as a colourless oil; $[\alpha]_D^{20}$ +23.1 (c 1.15, $CHCl_3$); ^{1}NMR δ (ppm) 0.87 (t, 3H, J = 7.45 Hz, 9-CH_3); 1.15-1.20 (t, 3H, J = 7.45 Hz, 3'-CH_3); 1.22-1.48 (bm, 6H, 6-CH_2, 7-CH_2, 8-CH_2); 2.08-2.15 (q, 2H, J = 7.06, 5-CH_2); 2.37-2.45 (q, 2H, J = 7.38 Hz, 2'-CH_2); 5.50-5.58 (ddt, 1H, J = 15.39 and 6.66 and 1.11 Hz, 3-CH); 5.80-5.83 d, 1H, *J* = 6.52 Hz, 2-CH); 6.10-6.19 (dt, 1H, *J* = 15.24 and 7.07 Hz, 4-CH); ^{13}C-NMR δ 172.52 (C=O), 140.72 (3-CH); 120.18 (4-CH); 115.99 (CN); 61.47 (2-CH); 32.06, 31.34, 28.10, 27.24, 22.50 (CH$_2$); 14.04 (CH$_3$), 8.84 (CH$_3$).

(+)-(2*S*)-2-Propanoylnonanenitrile (5)[8]: A mixture of **4** (54 mg, 0.258 mmol) and 10 % palladium on activated carbon (3.4 mg) in ethyl acetate (2 ml) under a hydrogen atmosphere (1 bar) was stirred at room temperature for 2.5 h. The catalyst was then removed by filtration and mixture concentrated *in vacuo*. The crude residue was purified by column chromatography with petroleum ether/diethyl ether (12:1), to yield **5** (37 mg, 10.84 mmol, 68% yield) as a colourless oil; $[\alpha]_D^{20}$ -50.8 (c 1.5, benzene), Lit.[8] -51.0 (c 3.00, benzene). ^{1}NMR δ (ppm) 0.86-0.91 (t, 3H, *J* = 7.45 Hz, 9-CH$_3$); 1.15-120 (t. 3H, *J* = 7.45 Hz, 1.23-1.57, 3'-CH$_3$) (bm, 10H, 4-CH$_2$, 5-CH$_2$, 6-CH$_2$, 7-CH$_2$, 8-CH$_2$); 1-86-1.93 (m, 2H, 3-CH$_2$); 2.33-2.45 (q, 2H, *J* = 7.48 Hz, 2'-CH$_2$); 5.30-5.25 (t, 1H, *J* = 6.74 Hz, 2-CH); ^{13}C-NMR d 172.84 (C=O; 117.24 (CN); 61.22 (2-CH); 32.55, 31.80 29.11, 28.97, 27.30, 24.74, 22.76 (CH$_2$); 14.21 (3'-CH$_3$); 8.97 (9-CH$_3$).

(-)-(4*S*,3*E*)-4-Butanoyl-non-3-enenitrile (7): Palladium-*bis*-(acetonitrile)-dichloride (493 mg, 1.57 mmol) was added to a solution of **6** (3.56 g, 15.9 mmol) in dry THF (80 ml) and the mixture heated at 80 °C for 5h. The mixture was cooled, diluted with petroleum ether (100 ml) and filtered through a pad of silica. The silica was washed with a mixture of petroleum ether/diethyl ether (1:1, 80 ml) and the organic extracts combined and concentrated under reduced pressure. The crude residue was purified by column chromatography with petroleum ether/diethyl ether (12:1-9:1), to yield **7** as a colourless oil (2.89 g, 13.0 mmol, 81% yield); $[\alpha]_D^{20}$ -13.8 (c 2.14, CHCl$_3$). IR (neat)/cm^{-1} 2945 (C-H); 2226 (CN); 1739 (C=O); 1174 (C-O). ^{1}H NMR δ (ppm) 0.84-0.98 (dt, 6H, *J* = 7.36 Hz and 6.69 Hz, 4'-CH$_3$, 9-CH$_3$); 1.22-1.38 (m, 6H, 6-CH$_2$, 7-CH$_2$, 8-CH$_2$); 1.61-1.70 (m, 4H, 5-CH$_2$, 3'-CH$_2$); 2.30-2.35 (t, 2H, *J* = 7.46 Hz, 2'-CH$_2$); 5.34-5.40 (dq, 1H, *J* = 5.18 and 6.57 Hz, 4-CH); 5.47-5.53 (dd, 1H, *J* = 16.39 and 1.26 Hz, 2-CH), 6.60-6.67 (dd, 1H, *J* = 16.42, 5.12, 3-CH). ^{13}C NMR δ 172.64 (C=O); 152.36 (3-CH); 116.84 (CN); 100.71 (2-CH); 33.72 (CH$_2$); 31.52 (CH$_2$); 24.64 (CH$_2$); 22.52 (CH$_2$); 18.63 (CH$_2$), 14.08 (CH$_3$); 13.81 (CH$_3$). MS: m/z (relative intensity %): 223 (M$^+$, 1); 153 (5); 96 (13); 80 (8); 71 (O=C$^+$-C$_3$H$_7$, 100); 43 (46).

(+)-(4*S*,3*E*)-4-Hydroxy-non-3-enenitrile (8)[10]: Solid potassium carbonate was added (88 mg, 0.629 mmol) to a solution of **7** (2.88g, 12.77 mmol) in dry methanol and stirring at room temperature continued for 5h. Solid dry silica (700 mg) was added and the stirring continued for 5 min afterwhich the silica was removed by filtration and the methanol removed under reduced pressure. The crude residue was purified by column chromatography with (3:1-1:1) petroleum ether/diethyl ether, to yield **8** (1.66 g, 10.84 mmol, 85 % yield) as a colourless oil; $[\alpha]_D^{20}$ +37.4 (c 1.12, CHCl$_3$), Lit.[14] +37.5 (c 2.10, CHCl$_3$). IR (neat)/cm^{-1} 3432 (OH); 2937 (CH); 2229 (CN) 1634 (conjugated C=C); 1459 (C-O). ^{1}H NMR δ (ppm) 0.87-0.91 (t, 3H, *J* = 6.59 Hz, 9-CH$_3$); 1.23-1.61 (m, 8H, 5-CH$_2$, 6-CH$_2$, 7-CH$_2$, 8-CH$_2$); 2.05 (s, 1H, OH); 4.27-4.33 (m, 1H, 4-CH); 5.64-5.70 (dd, 1H, *J* = 16.41 and 1.41 Hz, 2-CH), 6.72-6.79 (dd, 1H, *J* = 16.2 and 4.09 Hz, 3-CH). ^{13}C NMR δ 157.47 (3-CH); 117.55 (CN); 98.34 (2-CH); 70.87 (4-CH); 36.28 (CH$_2$); 31.58 (CH$_2$); 24.84 (CH$_2$); 22.54 (CH$_2$); 14.02 (CH$_3$).

(-)-(4*S*,3*E*)-4-*tert*-Butyldiphenylsilyloxy-non-3-enenitrile (9): *Tert*-butyldiphenylchlorosilane (3.77 g, 3.44 ml, 13.47 mmol) followed by imidazole (1.76 g, 25.87 mmol), portionwise, was added to a solution of **8** (1.65 g, 10.78 mmol) in dry DMF (4 ml) and the mixture stirred at room temperature for 1.5 h. Dichloromethane (150 ml) was then added and the organic layer washed with water (3 x 40 ml) followed by saturated sodium chloride solution (40 ml). The organic layer was dried, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with (95:5) petroleum ether/diethyl ether, to yield **9** (3.55 g, 9.09 mmol, 84 % yield) as a colourless oil; $[\alpha]_D^{20}$ -30.0 (c 1.05, $CHCl_3$). IR (neat)/cm^{-1} 3070, 2942, 2868 (CH); 2224 (CN); 1638 (conjugated C=C). ^{1}NMR δ (ppm) 0.83-0-87 (t, 3H, *J* = 6.79 Hz, 9-CH_3); (m and overlapping s, 6H and 9H, 6-CH_2, 7-CH_2, 8-CH_2, *t*-Bu), 1.44-1.48 (m, 2H, 5-CH_2); 4.35-4.39 (m, 1H, 4-CH_2); 5.53-5.59 (dd, 1H, *J* = 16.25 and 1.76 Hz, 2-CH); 6.65-6.71 (dd, 1H, *J* = 16.06 and 4.45 Hz, 3-CH); 7.39-7.50 (m, 6H, H aromatic, *meta* and *para*); 7.61-7.70 (2 x dd, 4H, *J* = 6.49 and 1.33 Hz, H aromatic *ortho*). ^{13}C NMR δ 156.76 (3-CH); 135.8, 135.75 (Ph_{ortho}-CH); 133.43, 133.08 (Ph-C); 130.09 (Ph_{para}-CH); 127.87, 127.81 (Ph_{meta}-CH); 117.54 (CN); 98.84 (2-CH), 72.54 (4-CH); 36.41 (5-CH_2); 31.64 (6-CH_2); 27.06 (*t*-Bu); 23.55 (7-CH_2); 22.44 (8-CH_2); 19.41 (1'-C); 14.01 (9-CH_3).

(-)-(4*S*,3*E*)-4-*tert*-Butyldiphenylsilyloxy-non-3-enealdehyde (10)[12]: To a solution of **9** (1.5 g, 3.84 mmol) in absolute toluene (125 ml) at -78 °C under argon, was added DIBAL-H (1.0 M in CH_2Cl_2, 4.62 ml, 4.62 mmol) and the mixture stirred for 1.5 h. Ethyl acetate (20 ml) was then added and the mixture stirred for 5 min to quench the excess DIBAL-H. A silica/water suspension (20g in 100ml) was added and the mixture allowed to warm to room temperature at which it was stirred for 1.5 h. The mixture was clarified by suction filtration and the aqueous layer extracted with toluene (120 ml). The organic extracts were combined, washed with saturated sodium chloride solution (30 ml), dried, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with petroleum ether/diethyl ether (95:5-90:10), to yield **10** (1.04 g, 2.64 mmol, , 70 % yield) as a colourless oil; $[\alpha]_D^{20}$ -20.1 (c 1.90, $CHCl_3$), Lit.[12] -19.0 (c 1.67, $CHCl_3$). IR (neat)/cm^{-1} 3070, 2942, 2852 (C-H); 1692 (C=O). ^{1}NMR δ (ppm) 0.79-0.87 (t, 3H, *J* = 7.14 Hz, 9-CH_3); 1.05-1.55 (m and overlapping s, 17H, 5-CH_2, 6-CH_2, 7-CH_2, 8-CH_2, *tert*-Bu); 4.45-4.51 (1H, *J* = 5.23 and 6.27 Hz, 4-CH); 6.10-6.23 (ddd, 1H, *J* = 15.5, 7.98 and 1.39 Hz, 2-CH); 6.65-6.75 (dd, 1H, *J* = 15.5 and 5.13 Hz, 3-CH); 7.31-7.44 (m, 6H, H aromatic, *meta* and *para*); 7.60-7.71 (2 x dd, 4H, *J* = 6.49 and 1.33 Hz, H aromatic *ortho*); 9.44-9.49 (d, 0.8H, *J* = 7.95, C<u>H</u>O). ^{13}C NMR δ 193.65 (C=O), 159.41 (3-CH); 135.88, 135.86 (Ph_{ortho}-CH); 133.70, 133.42 (Ph-C); 130.98 (2-CH), 129.96 (Ph-CH); 127.72 (Ph-CH); 72.58 (4-CH); 36.76 (5-CH_2); 31.68 (6-CH_2); 27.06 (*t*-Bu); 23.81 (7-CH_2); 22.46 (8-CH_2); 19.39 (1'-C); 14.01 (9-CH_3).

(-)-(13*S*,9*Z*,11*E*)-13-*tert*-Butyldiphenylsilyoxy-9,11-ocatadecadienoic acid methyl ester (12)[12]: To a solution of phosphonium salt **11** (356 mg, 0.697 mmol) in dry THF (15.1 ml) at -78 °C under argon, was added LiHMDS (1.0m in THF, 647μl, 0.647 mmol) and the mixture stirred for 25 min during which time the solution became dark orange. To this solution was added HMPA (2.21 ml) and the mixture stirred at -78°C for a further 2 min. A solution of the aldehyde **10** (196 mg, 0.497 mmol) in dry THF (2.60 ml) was added and stirring continued for a further 45 min. The mixture was quenched by the addition of warmed (45°C) water

(12 ml), the cooling bath removed and the mixture allowed to rapidly warm to room temperature, at which it was stirred for 30 min. The mixture was extracted with pentane (3 x 40 ml), the organic extracts combined, washed with saturated sodium chloride solution (20 ml), dried and concentrated *in vacuo*. The crude residue was resuspended in pertroleum ether and the precipitant triphenylphosphine oxide removed by filtration. The solvent was removed by evaporation under reduced pressure and the crude residue purified by column chromatography with petroleum ether/diethyl ether (98:2-96:4), to yield **12** (180mg, 0.328 mmol, 67 % yield) as a colourless oil; $[\alpha]_D^{20}$ -16.5 (c 1.23, $CHCl_3$), Lit.[14] -20.5 (c 141, $CHCl_3$). IR (neat)/cm^{-1} 2909 (C-H str); 1734 (C=O); 1446 (C-H def, alkane); 110 (C-O str). ^{1}NMR δ (ppm) 0.79 - 0.86 (t, 3H, *J* = 6.27, 18-CH_3); 1.07 (s, 9H, *tert*-Bu), 1.15-1.62 (m, 18H, 3-CH_2, 4-CH_2; 5-CH_2, 6-CH_2, 7-CH_2, 14-CH_2, 15-CH_2, 16-CH_2, 17-CH_2); 1.97-2.06 (m, 2H, 8-CH_2); 2.26-2.33 (t, 2H, *J* = 7.29 Hz, 2-CH_2), 3.67 (2, 3H, OMe); 4.15-4.23 (q, 1H, *J* = 6.37 and 5.58, 13-CH); 5.27-5.40 (dt, 1H, *J* = 10.62 and 7.65, 9-CH); 5.53-5.63 (dd, 1H, *J* = 15.02 Hz and 6.74 Hz, 12-CH); 5.82.5.93 (t, *J* = 10.94 Hz, 10-CH); 6.11-6.24 (dd, 1H, *J* = 15.02 and 11.03 Hz, 11-CH); 7.29-7.47 (m, 6H, aromatic$_{meta\text{ and }para}$-C-H); 7.63-7.77 (m, 4H, aromatic$_{ortho}$-CH). ^{13}C NMR δ 174.35 (C=O), 136.06 (135.98 (Ph$_{ortho}$-CH), 135.87 (11-CH); 134.65, 134.40 (Ph-C), 131.76 (10-CH); 129.51, 129.42 (Ph-CH); 128.11 (12-CH); 127.45, 127.35 (Ph-CH); 125.34 (9-CH); 74.25 (13-CH), 51.49 (OMe), 37.96 (2-CH_2); 34.15 (8-CH_2); 31.81, 29.66, 29.20, 29.16, 29.12, 27.70 (CH_2); 27.12 (*tert*-Bu); 25.00, 24.34, 22.57 (CH_2); 19.42 (ipso C); 14.07 (18-CH_3).

(-)-(13*S*,9*Z*,11*E*)-13-Hydroxy-9,11-ocatadecadienoic acid methyl ester (13)[12]: To a cooled solution (5°C) of **12** (0.078 mmol) in dry THF (0.3 ml) under an argon atmosphere, was added TBAF (0.77M in THF, 333 μl, 0.249 mmol). The mixture was allowed to warm to room temperature at which it was stirred for 23 h. Ethyl acetate (1 ml) was added followed by saturated sodium bicarbonate solution (2 ml) and the mixture extracted with ethyl acetate (2 x 15 ml). The organic extracts were combined, washed with saturated sodium chloride solution (10 ml), dried, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with petroleum ether/diethyl ether (3:1-2:1), to yield **13** (19 mg, 0.061mmole , 78 % yield) as a colourless oil; $[\alpha]_D^{20}$ + 6.26 (c 0.87, $CHCl_3$), Lit.[14] +6.50 (c 0.70, $CHCl_3$). ^{1}NMR δ (ppm) 0.82 - 0.89 (t, 3H, *J* = 6.24, 18-CH_3); 1.17-1.62 (m, 18H, 3-CH_2, 4-CH_2; 5-CH_2, 6-CH_2, 7-CH_2, 14-CH_2, 15-CH_2, 16-CH_2, 17-CH_2); 1.95 (bs, 1H, OH); 2.09-2.18 (m, 2H, 8-CH_2); 2.16-2.30 (t, 2H, *J* = 7.51 Hz, 2-CH_2); 3.63 (2, 3H, OMe); 4.07-4.16 (q, 1H, *J* = 6.41, 13-CH); 5.32-5.45 (dt, 1H, *J* = 10.82 and 7.54, 9-CH); 5.57-5.68 (dd, 1H, *J* = 15.16 Hz and 6.86 Hz, 12-CH); 5.88-5.99 (t, *J* = 11.22 Hz, 10-CH); 6.37-6.52 (ddt, 1H, *J* = 15.14, 11.04 and 1.04 Hz, 11-CH). ^{13}C NMR δ 174.34 (C=O), 136.07 (11-CH); 132.62 (10-CH); 127.92 (12-CH); 125.64 (9-CH); 72.81 (13-CH); 51.46 (OMe), 37.35 (2-CH_2); 34.06 (8-CH_2); 31.81, 29.50, 29.06, 29.06, 28.96, 27.68, 25.15, 24.90, 22.62 (CH_2); 14.05 (18-CH_3).

(-)-(13*S*,9*Z*,11*E*)-13-Hydroxy-9,11-ocatadecadienoic acid (1)[4e]: Solid lithium hydroxide (34 mg, 0.80 mmol) was added to a solution of methyl ester **13** (50 mg, 0.16 mmol) in isopropanol/water (14.5 ml of a 2:1 mixture) and stirring continued at room temperature for 1.5 h, afterwhich the mixture was very carefully neutralized with 1M HCl (approximately 0.8 ml). The mixture was then extracted with diethyl ether (2 x 25 ml) and the organic extracts combined, dried, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with dichloromethane/methanol (95:5-97:3), to yield **1** (31 mg, 0.104 mmol, 65% yield) as a colourless oil, with spectral characteristics identical to those previously reported.

Acknowledgment: The authors wish to thank Carina Illaszewicz and Hansjörg Weber for measuring the nmr spectra and Michael Schmidt for his assistance in preparing the compound **6**.

References

1. Kato, T.; Yamaguchi, Y.; Hirano, T.; Yokoyama, T.; Uyehara, T.; Namai, T.; Yamanaka, T.; Uyehara, T.; Namai, T.; Yamanaka, S.; Harada, N.; *Chem. Lett.*, **1984**, 409-412.
2. Blondin, G.A.; *Ann. NY. Acad. Sci.*, **1975**, 264, 98.
3. Buchanan, M.R.; Haas, T.; Laganda, M.; Guichardant, M.; *J. Biol. Chem.*, **1985**, 16056-16059.
4. For recent references see: a) Gunn, B.P.; *Heterocycles*, **1985**, 23 (12), 3061-3067; b) Kobayashi, Y.; Okamoto, S.; Shimazaki, T.; Ochiai, Y.; Sato, F.; *Tetrahedron Lett.*, **1987**, 28 (34), 3959-3962; c) Tranchepain, I.; Le Berre, F.; Dureault, A.; Le Merrer, Depezay, J.C.; *Tetrahedron*, **1989**, 45 (7), 2057-2065; d) Bloch, R.; Perfetti, M-T.; *Tetrahedron Lett.*; **1990**, 31 (18), 2577-2580; e) Yadav, J.S.; Deshpande, P.K.; Sharma, G.V.M.; *Tetrahedron*, **1992**, 48 (21), 4465-4474; f) Bennani, Y.L.; Sharpless, B.K.; *Tetrahedron Lett.*; **1993**, 34 (12), 2083-2086; g) Chemin, D.; Linstrumelle, G.; *Synthesis*, **1993**, 377.
5. a) Suememe, H.; Hayashi, N.; Funakoshi, K.; Akita, H.; Oishi, T.; Sakai, K.; *Chem. Pharm. Bull.* **1985**, 33, 2168-2170; b) Chan, C.; Cox, P.B.; Roberts, S.M.; *Biocatalysis*, **1990**, 3, 111-118; c) Bhalerao, U.T; Dasarachi, L.; Muralikishan, C.; Fadnavis, N.W; *Tetrahedron Lett.*, **1993**, 34 (14), 2359-2360; d) Maguire, N.M.; Read, G.; Richardson, P.F.; Roberts, S.M., *J. Chem. Research (S)*, **1994**, 376-377, *J. Chem. Research (M)*, **1994**, 2065-2079.
6. Klempier, N.; Pichler, U.; Griengl, H., *Tetrahedron: Asymmetry*, **1995**, 6 (4), 845-848.
7. Hasslacher, M.; Schall, M., Hayn, M.; Kohlwein, S.D.; Griengl, H.; *Manuscript in preparation.*
8. Kanerva, L.T.; Kiljunen, E.; Huuhtanen, T.; *Tetrahedron: Asymmetry*, **1993**, 4 (11), 2355-2361.
9. a) Greico, P.A.; Takigawa, T.; Bongers, S.L.; Tanaka, H.; *J. Am. Chem. Soc.*, **1980**, 7587-7588; b) Oehlschlarger, A.C.; Mishra, P.; Dhami, S.; *Can. J. Chem.*, **1984**, 62, 791-797.
10. Kang, S. K.; Lee, D. H.; Kim, Y. S.; Kang, S.C.; *Synth. Commun.*, **1992**, 22(8), 1109-1113.
11. Abe, H.; Nitta, H.; Mori, A.; Inoue, S.; *Chem. Lett.*, **1992**, 2443-2446.
12. De Montarby, L.; Tourbah, H.; Gree, R.; *Bull. Soc. Chim. Fr.*, **1989**, 126, 419-432.
13. The phosphonium salt (**11**) was prepared in a three step sequence according to: Genard, S; Patin, H.; *Bull. Chim. Soc. Fr.*, **1991**, 128, 397-406.

(*Received in Germany* 1 *October* 1996; *accepted* 31 *October* 1996)