Chemoenzymatic synthesis of (+)-docosa-4,15-dien-1-yn-3-ol, a component of the marine sponge *Cribrochalina vasculum*, and confirmation of the structure and absolute configuration of the acetylenic alcohol, by lipase-catalysed biotransformations

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The (4*E*,15*Z*)- and (4*E*,15*E*)-isomers of (+)-docosa-4,15-dien-1-yn-3-ol **1**, isolated from the marine sponge *Cribrochalina vasculum*, were synthesized in highly enantiomerically pure form by lipase-mediated biotransformation with Novozym 435, and the structure of **1** from the natural product was proved to be (4*E*,15*Z*)-docosa-4,15-dien-1-yn-3-ol **1*Z***. The absolute configuration of C-3 in compound (+)-**1*Z*** was assigned as *S* on the basis of the conversion of (+)-**1*Z*** into (*R*)-(−)-docosan-3-ol **17**, which was also prepared from oct-7-en-3-ol **12** *via* biotransformation with lipase Novozym.

We previously reported in a preliminary form the first enantioselective synthesis of (+)-(4*E*,15*E*)-docosa-4,15-dien-1-yn-3-ol **1*E*** by lipase-mediated acylation.[1] Compound **1** is one of the chiral acetylenic alcohols possessing a characteristic 4-en-1-yn-3-ol skeleton isolated from the Caribbean sponge *Cribrochalina vasculum* in Belize by Gunasekera and Faircloth[2] and in the Bahamas Islands by Aiello and co-workers,[3] independently, and shows *in vitro* immunosuppressive and antitumour activities.

Recently Hallock and co-workers reported the isolation of several structurally related acetylenic alcohols including (+)-**1** from an organic extract of the sponge *C. vasculum* collected in the Bahamas Islands and assigned the configuration of the C-15 double bond in natural **1** as *Z*, using the ^{13}C NMR spectral data;[4] Gunasekera and Faircloth had assigned *E* geometry to the same double bond in the natural **1** isolated from the sponge from Belize on the basis of the absence of any IR absorptions between 730 and 675 cm^{-1}.[2] Hallock *et al*. also demonstrated that the natural (+)-**1** they obtained had the *S* configuration at C-3 *via* the modified Mosher's NMR method and that on the basis of direct comparison of their natural (+)-**1** and the natural **1**† isolated from the Bahamas by Aiello *et al*.,[3,5] these two natural products provided opposite absolute configurations at C-3.

The present investigation reflects a continuous effort towards the chemoenzymatic synthesis of optically active natural products utilizing lipases as asymmetric catalysts[1,6] and was carried out to confirm synthetically the structure and absolute configuration of natural (+)-**1**.

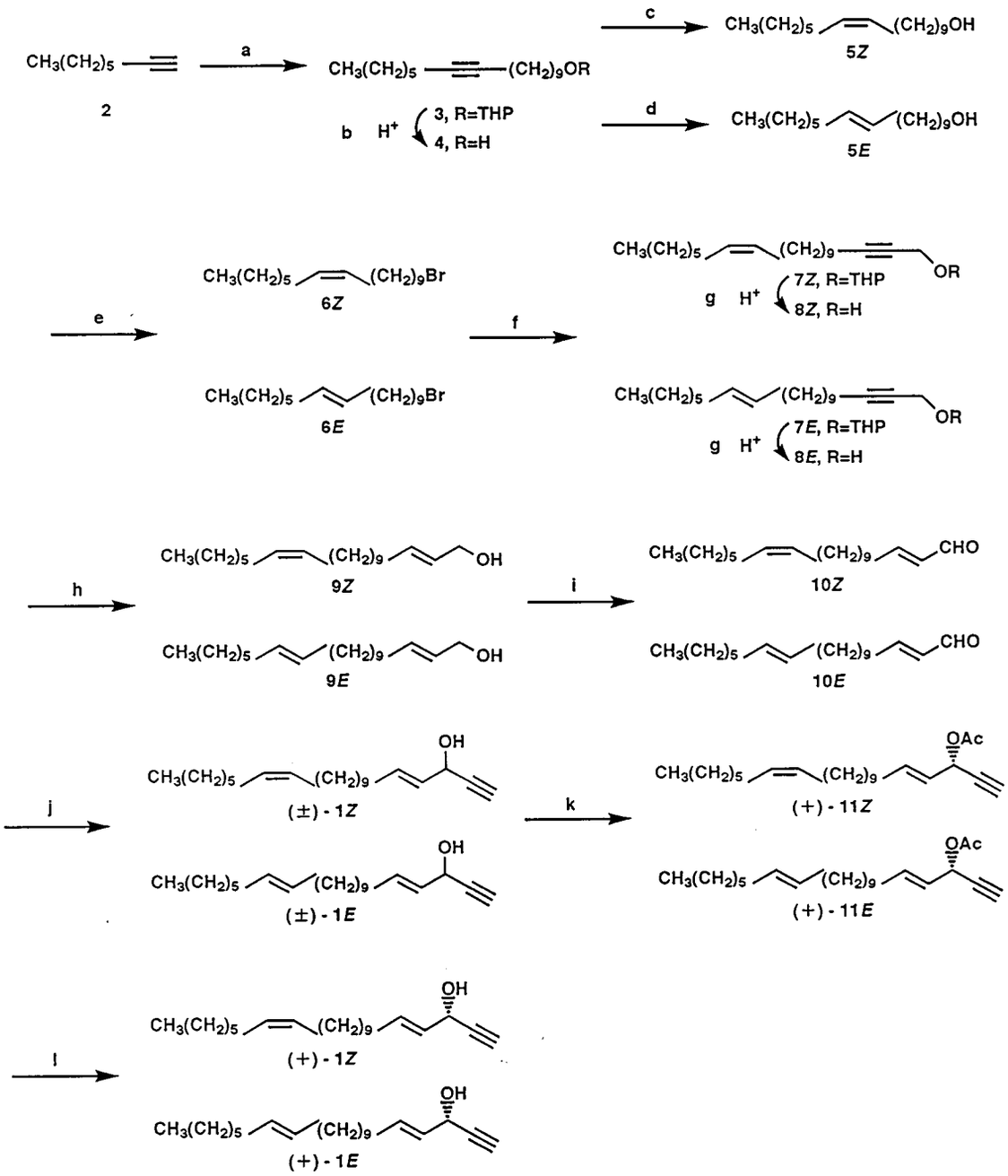
Results and discussion

As shown in Scheme 1, oct-1-yne **2** was alkylated with BuLi and the tetrahydropyran-2-yl (THP) ether derived from 9-bromononan-1-ol, followed by treatment with toluene-*p*-sulfonic acid (*p*-TsOH) to give acetylenic alcohol **4** in 68% yield based on **2**. Alcohol **4** was converted by hydrogenation with P-2 nickel into (*Z*)-olefinic alcohol **5*Z*** in 98% yield, while the alcohol **4** was transformed by $LiAlH_4$ (LAH) reduction into (*E*)-olefinic alcohol **5*E*** in 92% yield; both the olefinic alcohols showed a geometric purity of *ca*. 99% as estimated by ^{1}H NMR analysis. (*Z*)-Alcohol **5*Z*** was treated with triphenylphosphine dibromide to afford (*Z*)-bromide **6*Z*** in 94% yield. Coupling of **6*Z*** with the THP ether of prop-2-yn-1-ol and subsequent treatment with *p*-TsOH gave (*Z*)-enyne alcohol **8*Z*** in 75% yield from **6*Z***, which was converted by LAH reduction into the diene alcohol **9*Z*** in 86% yield. A Swern oxidation of alcohol **9*Z*** produced the aldehyde **10*Z*** in 93% yield, and the latter was treated with ethynylmagnesium chloride to yield (±)-(4*E*,15*Z*)-alcohol **1*Z*** in 85% yield. The (±)-(4*E*,15*E*)-alcohol **1*E*** was prepared from (*E*)-alcohol **5*E*** in the same manner as described for the preparation of (±)-**1*Z***. To complete the chemoenzymatic synthesis of (+)-**1**, the lipase-catalysed biotransformation of racemic **1*Z*** was carried out with Novozym 435 (*Candida antarctica*) and vinyl acetate in *tert*-butyl methyl ether, the acylated product (4*E*,15*Z*)-acetate **11*Z*** and the unreacted alcohol **1*Z*** being formed in 95% ee and 65% ee, respectively. The former chiral product **11*Z*** was submitted to a second biotransformation with Novozym 435 in aqueous solution to give the hydrolysed product (+)-(4*E*,15*Z*)-alcohol **1*Z*** with an enantiomeric purity of >98% ee. Similarly, racemic **1*E*** was acylated with Novozym 435, and the resulting chiral acetate **11*E*** was subsequently subjected to lipase hydrolysis with Novozym 435 to yield (+)-(4*E*,15*E*)-alcohol **1*E*** of >98% ee. In the acylation of (±)-**1*E*** the unreacted alcohol **1*E*** was also obtained with 81% ee.

The ^{13}C NMR spectrum of alcohol **5*Z*** showed the signals of the double bond carbons at δ 129.78 and 129.84 and that of carbons allylic to the *Z*-double bond at δ 27.15,‡ and the

† The absolute configuration of Aiello's acetylenic alcohol **1** was suggested to be *R* on the basis of CD spectral data[3] and the Mosher NMR analysis of the Aiello sample made by Guo and co-workers[5] agreed with the stereochemistry assigned by Aiello *et al*. The value of specific rotation for Aiello's **1** was not given.

‡ A lower chemical shift value would be expected for carbons allylic to a double bond with *E*-geometry.[3,8] In the ^{13}C NMR spectra of **5*E*** and **1*E***, there were no absorptions near δ 27.



Scheme 1 *Reagents*: (a) $Br(CH_2)_9OTHP$, BuLi, THF–DMPU; (b) *p*-TsOH, MeOH; (c) H_2/P-2Ni, EtOH; (d) $LiAlH_4$, diglyme; (e) $Ph_3P{\cdot}Br_2$, CH_2Cl_2; (f) $HC{\equiv}CCH_2OTHP$, BuLi, THF–DMPU; (g) *p*-TsOH, MeOH; (h) $LiAlH_4$, diethyl ether; (i) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2; (j) $HC{\equiv}CMgCl$, THF; (k) lipase Novozym 435, vinyl acetate, *tert*-butyl methyl ether; (l) lipase Novozym 435, acetone–phosphate buffer.

spectrum of the *E* isomer **5*E*** revealed the signals of the double bond carbons at δ 130.27 and 130.35. From the NMR spectra of **5*Z*** and **5*E***, we could establish the assignment of the NMR signals of the isolated double bond carbons and/or that of carbons allylic to the double bond in our synthetic (+)-**1*Z*** and -**1*E***. These signals were observed at δ 129.84 and 129.89 and δ 27.17 and 27.19 in **1*Z*** and at δ 130.30 and 130.35 in **1*E***. The ^{13}C NMR spectral data of our synthetic **1*Z*** were identical with those of the natural **1** isolated independently by Gunasekera[2] and Hallock,[4] indicating that both Gunasekera's **1** and Halloch's **1** have a *Z*-geometry at the C-15 double bond.

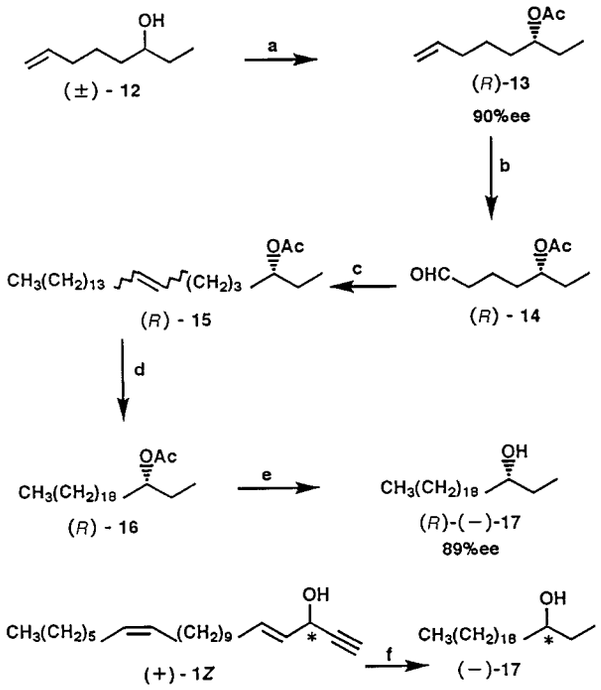
For the determination of the absolute configuration of (+)-**1*Z***, oct-7-en-3-ol **12** was acylated with lipase Novozym 435 and vinyl acetate to give the known chiral acetate (*R*)-(+)-**13**,[7] which was converted into (*R*)-(−)-docosan-3-ol **17** *via* a four-step sequence, including osmium tetroxide oxidation, Wittig reaction, hydrogenation, and alkaline hydrolysis (Scheme 2). Because our synthetic (+)-**1*Z*** provided (−)-**17** by reduction with a combination of LAH–$CoCl_2$, the chirality at C-3 of (+)-**1*Z*** was determined to be *S*. Our results were consistent with the stereochemical view showed by Hallock *et al.*[4]

In conclusion, we have proved, based on the chemoenzymatic synthesis of (+)-**1*Z*** by lipase-catalysed biotransformations, that the structure of the natural **1** is (4*E*,15*Z*)-docosa-4,15-dien-1-yn-3-ol and that the absolute configuration of (+)-**1*Z*** is *S*.

Experimental

General

IR spectra were determined on a Fourier transform Perkin-Elmer 1720 IR spectrometer. ^1H and ^{13}C NMR spectra were obtained on a Fourier transform Bruker AMX-R400 spectrometer in $CDCl_3$ solutions, using Me_4Si as an internal stand-



Scheme 2 *Reagents*: (a) lipase Novozym 435, vinyl acetate, *tert*-butyl methyl ether; (b) OsO_4, $NaIO_4$, THF–H_2O; (c) $Ph_3P{=}CH(CH_2)_{13}CH_3$, DME; (d) H_2–Pd/C, MeOH; (e) KOH, MeOH; (f) $LiAlH_4$, $CoCl_2$, THF.

ard. *J* Values are given in Hz.§ Gas chromatography was carried out on a Hitachi G-5000 gas chromatograph equipped with a TC-1 30 m × 0.25 mm capillary column (GL Sciences) or a Hitachi G-3000 gas chromatograph equipped with a DB-WAX 30 m × 0.25 mm capillary column (J & W Scientific), using He as the carrier gas. Column chromatography was performed with 70–230 mesh silica gel (Merck Kieselgel 60 Art. No. 7734) and 230–400 mesh silica gel (Merck, Kieselgel 60 Art. No. 9385). Optical rotations were measured on a Horiba SEPA-300 high-sensitivity polarimeter; $[a]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. HPLC analyses were performed on a Waters 510 liquid chromatograph equipped with a UV detector (254 nm). Analytical samples were prepared by a combination of column chromatography and micro-vacuum distillation with a Kugelrohr distillation apparatus. The NMR spectra of compounds **6*Z***, **9*E***, **9*Z***, and (*R*)-**14** were recorded on samples contaminated with up to 15% diethyl ether. Lipase from *Candida antarctica* was used (Novozym 435, Novo Nordisk Bioindustrial A/S, Denmark).

Determination of enantiomeric purity

The enantiomeric purity of the chiral alcohols, (*R*)- and (*S*)-**1*Z***, (*R*)- and (*S*)-**1*E***, and (*R*)-**17**, was determined by HPLC analysis of their corresponding 3,5-dinitrophenylurethane derivatives prepared by treatment with 3,5-dinitrophenyl isocyanate.[9] A Sumichiral OA 2100I 4.0 × 250 mm column (Sumica Chemical Analysis Service, Osaka) was used at a flow rate of 1.0 cm^3 min^{-1} [hexane–1,2-dichloroethane–EtOH (80 : 10 : 0.4)]. The urethane derivatives derived from (±)-**1*Z***, -**1*E***, and -**17** were separated into two equal peaks. For (±)-**1*Z***: t_R 37.2 min [(*R*)-(−)-**1*Z***] and 44.0 min [(*S*)-(+)-**1*Z***]. For (±)-**1*E***: t_R 38.1 min [(*R*)-(−)-**1*E***] and 45.5 min [(*S*)-(+)-**1*E***]. For (±)-**17**: t_R 16.1 min [(*S*)-(+)-**17**] and 18.2 min [(*R*)-(−)-**17**]. The enantiomeric purity of the chiral acetates (*S*)-**11*Z*** and -**11*E*** was based on that of their corresponding alcohols; these two acetates were converted into the respective alcohols by alkaline hydrolysis, the enantiomeric excesses of the alcohol being determined as described above. The ee of the chiral acetate (*R*)-**13** was estimated by GLC analysis after converting the acetate into the corresponding alcohol (*R*)-**12** as reported previously.[7]

Heptadec-10-yn-1-ol 4

To a stirred and cooled (−70 °C) solution of oct-1-yne **2** (16 g, 0.145 mol) and dry *N*,*N*′-dimethylpropyleneurea (3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1*H*)-one, DMPU) (200 cm^3) in dry THF (300 cm^3) under argon was added 140 cm^3 of 1.6 mol dm^{-3} BuLi in hexane. After the solution had been stirred for 1.5 h at −30 °C, the THP ether of 9-bromononan-1-ol (44 g, 0.14 mol) in dry DMPU (100 cm^3) was added. The reaction mixture was allowed to warm gradually to room temperature while being stirred and stirring was continued for 2 h. The mixture was subsequently poured into ice-cooled water and extracted with diethyl ether. The extract was washed successively with water and brine, dried over Na_2SO_4 and concentrated. The crude yellow liquid (45 g) thus obtained was dissolved in dry MeOH (400 cm^3) containing *p*-TsOH (10 g), and the mixture was heated under reflux for 4 h with stirring. After cooling, the mixture was concentrated and diluted with diethyl ether. Usual work-up of the ethereal solution gave a crude product, which was purified by column chromatography on silica gel (800 g) with hexane–ethyl acetate (25 : 1) to give compound **4** as a colorless liquid (24 g, 68% based on alkyne **2**); ν_{max}(neat)/cm^{-1} 3341, 2930, 2857, 1464, 1378, 1334, 1058, 890 and 725; δ_H 0.89 (3H, t, *J* 7.1), 1.26–1.38 (16H, m), 1.47 (4H, m), 1.56 (2H, m), 1.89 (1H, s), 2.13 (4H, m) and 3.62 (2H, t, *J* 6.7); δ_C 80.18, 80.11, 62.93, 32.68, 31.31, 29.44, 29.33, 29.05, 28.75, 28.46, 25.67, 22.51, 18.67 and 13.97.

(10*Z*)-Heptadec-10-en-1-ol 5*Z*

Hydrogenation of **4** (9 g, 35 mmol) in EtOH (100 cm^3) was carried out in the presence of ethylenediamine (0.6 g) and a P-2 nickel catalyst, which was prepared as described previously.[10] After hydrogenation was complete (3 h), the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and diluted with diethyl ether. Usual work-up of the ethereal solution gave a crude product, which was purified by column chromatography on silica gel (200 g) with hexane–ethyl acetate (25 : 1) to give compound **5*Z*** as a colorless liquid (8.8 g, 98%); ν_{max}(neat)/cm^{-1} 3328, 2926, 2855, 1466, 1058 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.27–1.33 (20H, m), 1.55 (2H, m), 1.75 (1H, br s), 2.01 (4H, m), 3.62 (2H, t, *J* 6.7) and 5.35 (2H, m); δ_C 129.84, 129.78, 62.82, 32.73, 32.54, 31.73, 29.70, 29.44, 29.39, 28.93, 27.15, 25.71, 22.60 and 14.01.

(10*E*)-Heptadec-10-en-1-ol 5*E*

A solution of **4** (14 g, 55 mmol) in dry diglyme [bis(2-methoxyethyl) ether] (50 cm^3) was added to a stirred suspension of LAH (8.5 g, 0.224 mol) in dry diglyme (150 cm^3) under nitrogen at room temperature. The mixture was heated at 160–170 °C for 24 h while being stirred. After cooling, the suspension was treated with a minimum of water to decompose the excess of the hydride under nitrogen and diluted with diethyl ether. The resulting mixture was filtered through Celite and the filtrate was washed successively with water and brine, dried and concentrated. The crude product obtained was purified by column chromatography on silica gel (300 g) as described for **5*Z*** to give compound **5*E*** as a colorless liquid (12.9 g, 92%); ν_{max}(neat)/cm^{-1} 3333, 2926, 2855, 1464, 1058, 968 and 724; δ_H 0.88 (3H, t, *J* 7.0), 1.22–1.31 (20H, m), 1.56 (2H, m), 1.63 (1H, s), 1.96 (4H, m), 3.63 (2H, t, *J* 6.7) and 5.38 (2H, m); δ_C 130.35, 130.27, 62.97, 32.74, 32.58, 31.73, 29.60, 29.55, 29.42, 29.40, 28.81, 25.71, 22.62 and 14.07.

§ ^1H and ^{13}C NMR spectra of compounds **4**, **5*E***, **5*Z***, **8*E***, **8*Z***, **9*E***, **9*Z***, **10*E***, **10*Z***, (*R*)-**14** and (*R*)-**15** as supplementary data (SUPPL. NO. 57477, pp. 24) from the British Library. For details of the Supplementary Publications Scheme, see 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 1*, available *via* the RSC web page (http://www.rsc.org/authors).

(7*Z*)-17-Bromoheptadec-7-ene 6*Z*

Bromide **6*Z*** was synthesized by treating **5*Z*** (18.5 g, 73 mmol) with triphenylphosphine dibromide (dibromotriphenylphosphorane), which was prepared from triphenylphosphine (19.2 g, 73 mmol) and Br_2 (11.7 g, 73 mmol), in the presence of dry pyridine (6 g) in dry CH_2Cl_2 (250 cm^3). Purification of the product by column chromatography on silica gel (400 g) with hexane–ethyl acetate (60 : 1) gave **6*Z*** as a colorless liquid (21.8 g, 94%); ν_{max}(neat)/cm^{-1} 2926, 2855, 1465 and 723; δ_H 0.88, (3H, t, *J* 7.1), 1.26–1.48 (20H, m), 1.85 (2H, m), 2.06 (4H, m), 3.40 (2H, t, *J* 6.8) and 5.35 (2H, m); δ_C 129.92, 129.78, 34.00, 32.81, 31.77, 29.71, 29.39, 29.21, 28.97, 28.74, 28.15, 27.19, 27.15, 22.65 and 14.09.

(7*E*)-17-Bromoheptadec-7-ene 6*E*

This compound was prepared by treating **5*E*** (12.9 g, 51 mmol) with triphenylphosphine dibromide in dry CH_2Cl_2 (150 cm^3) as described for compound **6*Z***. Purification by column chromatography as described above gave **6*E*** as a colorless liquid (15 g, 93%); ν_{max}(neat)/cm^{-1} 2957, 2926, 1466, 967 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.28–1.45 (20H, m), 1.85 (2H, m), 1.98 (4H, m), 3.40 (2H, t, *J* 6.8) and 5.39 (2H, m); δ_C 130.38, 130.23, 33.99, 32.81, 32.59, 32.56, 31.74, 29.59, 29.37, 29.06, 28.82, 28.73, 28.15, 22.63 and 14.09.

(13*Z*)-Icos-13-en-2-yn-1-ol 8*Z*

To a stirred and cooled (−70 °C) solution of the THP ether of propargyl (prop-2-ynyl) alcohol (9.3 g, 66 mmol) and dry DMPU (180 cm^3) in dry THF (130 cm^3) under argon was added 62 cm^3 of 1.6 mol dm^{-3} BuLi in hexane. After the solution had been stirred for 1.5 h at −30 °C, a solution of bromide **6*Z*** (21 g, 66 mmol) in dry DMPU (50 cm^3) was added. The mixture was allowed to warm gradually to room temperature and stirred for 2 h. It was subsequently poured into ice-cooled water and extracted with diethyl ether. The ethereal solution was worked up in the usual manner to give a yellow liquid (30 g), which, without purification, was dissolved in dry MeOH (350 cm^3) containing *p*-TsOH (10 g). The mixture was then heated under reflux for 4 h with stirring. Usual work-up of the reaction mixture gave a crude product, which was purified by column chromatography on silica gel (400 g) with hexane–ethyl acetate (25 : 1) to give compound **8*Z*** as a colorless liquid (14.5 g, 75% based on **6*Z***); ν_{max}(neat)/cm^{-1} 3328, 2927, 2855, 2227, 1466, 1138, 1015 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.28–1.35 (20H, m), 1.50 (2H, m), 1.84 (1H, s), 2.01 (4H, m), 2.20 (2H, m), 4.25 (2H, br s) and 5.35 (2H, m); δ_C 129.88, 129.80, 86.53, 78.22, 51.31, 31.74, 29.71, 29.69, 29.46, 29.33, 29.24, 29.10, 28.95, 28.84, 28.57, 27.17, 27.15, 22.62, 18.69 and 14.07.

(13*E*)-Icos-13-en-2-yn-1-ol 8*E*

This compound was prepared by coupling **6*E*** (15 g, 47 mmol) with the THP ether of propargyl alcohol (6.6 g, 47 mmol) and subsequently treating with *p*-TsOH according to the procedure described for **8*Z***. Purification of the product by column chromatography on silica gel (300 g) as described above gave **8*E*** as a white solid (10.7 g, 77% based on **6*E***), which was recrystallised from hexane; mp 43–45 °C; ν_{max}(KBr)/cm^{-1} 3310, 2996, 2918, 2850, 2219, 1471, 1134, 1028, 964 and 719; δ_H 0.88 (3H, t, *J* 7.0), 1.27–1.34 (20H, m), 1.50 (2H, m), 1.59 (1H, s), 1.97 (4H, m), 2.21 (2H, m), 4.25 (2H, m) and 5.39 (2H, m); δ_C 130.39, 130.30, 86.69, 78.21, 51.45, 32.59, 31.74, 29.61, 29.46, 29.11, 28.86, 28.83, 28.58, 22.64, 18.71 and 14.10.

(2*E*,13*Z*)-Icosa-2,13-dien-1-ol 9*Z*

A solution of **8*Z*** (7 g, 24 mmol) in dry diethyl ether (50 cm^3) was added to a stirred suspension of LAH (2.7 g, 72 mmol) in dry diethyl ether (100 cm^3) at room temperature. The mixture was heated under reflux for 24 h while being stirred. After cooling, the reaction mixture was treated with small amounts of water and filtered through Celite. Usual work-up of the filtrate gave a crude product, which was purified by column chromatography on silica gel (150 g) with hexane–ethyl acetate (25 : 1) to give **9*Z*** as a colorless liquid (6.02 g, 86%); ν_{max}(neat)/cm^{-1} 3328, 2925, 2854, 1466, 1090, 1005, 970 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.27–1.39 (22H, m), 1.55 (1H, s), 2.02 (6H, m), 4.08 (2H, d, *J* 5.1), 5.35 (2H, m), 5.62 (1H, dt, *J* 15.5, 5.1) and 5.69 (1H, dt, *J* 15.5, 5.7); δ_C 133.51, 129.87, 129.83, 128.75, 63.76, 32.19, 31.75, 29.72, 29.70, 29.54, 29.51, 29.48, 29.26, 29.10, 28.95, 27.17, 22.62 and 14.07.

(2*E*,13*E*)-Icosa-2,13-dien-1-ol 9*E*

This compound was prepared by reducing **8*E*** (4.97 g, 17 mmol) with LAH (2 g, 51 mmol) in dry diethyl ether (150 cm^3) as already described for **9*Z***. Purification of the product by column chromatography as described above gave **9*E*** as a white solid (4.04 g, 80%), which was recrystallised from hexane; mp 39–41 °C; ν_{max}(KBr)/cm^{-1} 3293, 2956, 2921, 2850, 1464, 1001, 966 and 730; δ_H 0.88 (3H, t, *J* 7.0), 1.26–1.46 (23H, m), 1.94–2.06 (6H, m), 4.08 (2H, d, *J* 5.2), 5.39 (2H, m), 5.63 (1H, dt, *J* 15.5, 5.2) and 5.70 (1H, dt, *J* 15.5, 5.7); δ_C 133.57, 130.35, 130.31, 128.74, 63.80, 32.59, 32.20, 31.73, 29.62, 29.60, 29.54, 29.48, 29.46, 29.16, 29.12, 28.82, 22.63 and 14.08.

(2*E*,13*Z*)-Icosa-2,13-dienal 10*Z*

To a stirred and cooled (−70 °C) solution of oxalyl chloride (1.68 g, 13 mmol) in dry CH_2Cl_2 (60 cm^3) was added a solution of DMSO (1.03 g, 13 mmol) in dry CH_2Cl_2 (10 cm^3). The mixture was stirred for 15 min, and a solution of **9*Z*** (2.62 g, 8.9 mmol) in dry CH_2Cl_2 (20 cm^3) was added over 5 min. After being stirred for 15 min, the reaction mixture was treated with triethylamine (4.5 g, 45 mmol) in dry CH_2Cl_2 (60 cm^3); stirring was continued for 2 h. The mixture was subsequently poured into ice-cooled water and extracted with CH_2Cl_2. The extract was successively washed with water and brine, dried and concentrated. The residue was purified by column chromatography on silica gel (60 g) with hexane–diethyl ether (60 : 1) to give **10*Z*** as a colorless liquid (2.45 g, 93%); ν_{max}(neat)/cm^{-1} 2926, 2855, 2729, 1697, 1654, 1466, 975 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.23–1.55 (22H, m), 2.01 (4H, m), 2.33 (2H, m), 5.35 (2H, m), 6.12 (1H, dd, *J* 15.6, 7.9), 6.86 (1H, dt, *J* 15.6, 6.8) and 9.50 (1H, d, *J* 7.9); δ_C 194.09, 158.99, 132.92, 129.89, 129.77, 32.70, 31.74, 29.69, 29.44, 29.43, 29.30, 29.21, 29.10, 28.95, 27.80, 27.17, 27.14, 22.62 and 14.07.

(2*E*,13*E*)-Icosa-2,13-dienal 10*E*

This compound was prepared by a Swern oxidation of **9*E*** (4 g, 13 mmol) according to the procedure described for **10*Z***. Purification of the product by column chromatography as described for **10*Z*** gave **10*E*** as a colorless liquid (3.37 g, 85%); ν_{max}(neat)/cm^{-1} 2926, 2855, 2730, 1696, 1640, 1464, 971 and 725; δ_H 0.88 (3H, t, *J* 7.0), 1.21–1.35 (20H, m), 1.54 (2H, m), 1.99 (4H, m), 2.35 (2H, m), 5.39 (2H, m), 6.12 (1H, dd, *J* 15.5, 8.0), 6.85 (1H, dt, *J* 15.5, 6.8) and 9.51 (1H, d, *J* 8.0); δ_C 194.16, 159.08, 132.92, 130.37, 32.72, 32.58, 32.56, 31.73, 29.59, 29.42, 29.31, 29.11, 29.08, 28.81, 27.80, 22.62 and 14.08.

(3*RS*,4*E*,15*Z*)-Docosa-4,15-dien-1-yn-3-ol [(±)-1*Z*]

A 0.5 mol dm^{-3} THF solution of ethynylmagnesium chloride (30 cm^3) was cooled to 0 °C. To this solution was added dropwise a solution of **10*Z*** (1.5 g, 5.1 mmol) in dry THF (20 cm^3), and the mixture was stirred for 30 min. It was then allowed to warm to room temperature during 30 min while being stirred and stirring was continued for one hour. The mixture was subsequently poured into saturated aq. NH_4Cl and extracted with diethyl ether. The ethereal solution was washed with water and

brine, dried and concentrated. The crude product obtained was purified by column chromatography on silica gel (80 g) with hexane–ethyl acetate (60:1) to give (±)-**1*Z*** as a colorless liquid (1.38 g, 85%); ν_{max}(neat)/cm^{-1} 3400, 3311, 2926, 2854, 2119, 1466, 1091, 1014, 970 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.27–1.45 (23H, m), 1.92–2.13 (6H, m), 2.56 (1H, d, *J* 2.1), 4.84 (1H, br s), 5.35 (2H, m), 5.61 (1H, dd, *J* 15.3, 6.2) and 5.92 (1H, dt, *J* 15.3, 6.2); δ_C 134.51, 129.88, 129.83, 128.33, 83.34, 73.89, 62.73, 31.91, 31.76, 29.73, 29.71, 29.52, 29.50, 29.43, 29.27, 29.16, 28.96, 28.80, 27.17, 22.63 and 14.08 (Found: C, 82.91; H, 11.95. $C_{22}H_{38}O$ requires C, 82.95; H, 12.03%).

(3*RS*,4*E*,15*E*)-Docosa-4,15-dien-1-yn-3-ol [(±)-1*E*]

This compound was prepared by treating **10*E*** (1.5 g, 5.1 mmol) with ethynylmagnesium chloride according to the procedure described for (±)-**1*Z***. Purification by column chromatography as already described for (±)-**1*Z*** gave **1*E*** as a colorless liquid (1.38 g, 85%); ν_{max}(neat)/cm^{-1} 3400, 3311, 2924, 2854, 2117, 1466, 1092, 1016, 967 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.23–1.43 (23H, m), 1.95–2.10 (6H, m), 2.56 (1H, d, *J* 2.1), 4.84 (1H, br s), 5.39 (2H, m), 5.61 (1H, dd, *J* 15.3, 6.1) and 5.91 (1H, dt, *J* 15.3, 6.9); δ_C 134.55, 130.34, 130.29, 128.28, 83.30, 73.91, 62.73, 32.57, 31.91, 31.72, 29.60, 29.58, 29.51, 29.46, 29.42, 29.15, 29.11, 28.80, 22.62 and 14.08 (Found: C, 82.86; H, 12.08. $C_{22}H_{38}O$ requires C, 82.95; H, 12.03%).

(3*S*,4*E*,15*Z*)-Docosa-4,15-dien-1-yn-3-ol [(+)-1*Z*]

A mixture of (±)-**1*Z*** (1.0 g, 3.1 mmol), Novozym 435 (0.4 g), viny acetate (0.8 g, 9 mmol), and *tert*-butyl methyl ether (30 cm^3) was stirred for 2 h at 30 °C (47% conversion). The reaction mixture was filtered through Celite, and the filtrate was washed with brine, dried and concentrated. Purification of the product by column chromatography on silica gel (10 g) with hexane–ethyl acetate (30:1) gave acetate (*S*)-(+)-**11*Z*** with 95% ee as a colorless liquid (0.5 g, 45%); $[\alpha]_D^{25}$ +17.96 (*c* 2.45, MeOH) and alcohol (*R*)-(−)-**1*Z*** with 65% ee as a colorless liquid (0.49 g, 49%); $[\alpha]_D^{25}$ −16.53 (*c* 2.85, MeOH). The enantiomeric ratio for this biocatalytic acylation (*E* value), $E = 85$, was calculated according to the method of Chen *et al.*[11] Acetate (+)-**11*Z***: ν_{max}(neat)/cm^{-1} 3312, 2926, 2855, 1746, 1466, 1371, 1229, 1015, 968 and 735; δ_H 0.88 (3H, t, *J* 7.0), 1.21–1.42 (22H, m), 2.01 (6H, m), 2.09 (3H, s), 2.56 (1H, d, *J* 2.1), 5.35 (2H, m), 5.54 (1H, dd, *J* 15.2, 6.2), 5.83 (1H, d, *J* 6.2) and 6.02 (1H, dt, *J* 15.2, 6.7); δ_C 169.64, 137.18, 129.87, 129.81, 124.20, 79.83, 74.64, 64.04, 31.96, 31.75, 29.72, 29.70, 29.49, 29.39, 29.26, 29.12, 28.95, 28.59, 27.18, 27.16, 22.63, 21.03 and 14.08 (Found: C, 79.86; H, 11.29. $C_{24}H_{40}O_2$ requires C, 79.94; H, 11.18%). The IR and NMR spectra of (−)-**1*Z*** were identical with those of (±)-**1*Z***.

Acetate (+)-**11*Z*** (0.3 g, 0.84 mmol) was added to a mixture of Novozym 435 (0.12 g), acetone (3.6 cm^3), and 0.1 mol dm^{-3} phosphate buffer (pH 7) (5.4 cm^3). The mixture was stirred for 2.5 h at 30 °C, GLC analysis showed a conversion of 83%. Column chromatography as already described for (±)-**1*Z*** gave (+)-**1*Z*** with >98% ee as a colorless liquid (0.2 g, 78%); $[\alpha]_D^{25}$ +24.43 (*c* 3.15, MeOH) {lit.,[2] $[\alpha]_D^{25}$ +4.9 (*c* 4.5, MeOH); lit.,[4] $[\alpha]_D^{25}$ +21.5 (*c* 1.1, MeOH)}; δ_C 134.59, 129.89, 129.84, 128.29, 27.19 and 27.17. The IR and ^{1}H NMR spectra were identical with those of (±)-**1*Z***.

(3*S*,4*E*,15*E*)-Docosa-4,15-dien-1-yn-3-ol [(+)-1*E*]

As described for (±)-**1*Z***, (±)-**1*E*** (1.0 g, 3.1 mmol) was treated with Novozym 435 (0.4 g) in the presence of vinyl acetate (0.8 g, 9 mmol); GLC analysis showed 45% conversion. Column chromatography gave acetate (*S*)-(+)-**11*E*** with 94% ee as a colorless liquid (0.46 g, 41%); $[\alpha]_D^{25}$ +16.17 (*c* 3.5, MeOH) and alcohol (*R*)-(−)-**1*E*** with 81% ee as a colorless liquid (0.51 g, 51%); $E = 76$. Acetate (+)-**11*E***: ν_{max}(neat)/cm^{-1} 3312, 2926, 2854, 1746, 1466, 1371, 1228, 1015, 968 and 723; δ_H 0.88 (3H, t, *J* 7.0), 1.25–1.43 (22H, m), 1.99–2.05 (6H, m), 2.10 (3H, s), 2.56 (1H, d, *J* 2.1), 5.39 (2H, m), 5.53 (1H, dd, *J* 15.3, 6.1), 5.82 (1H, d, *J* 6.1) and 6.02 (1H, dt, *J* 15.3, 6.8); δ_C 169.69, 137.23, 130.36, 130.31, 124.21, 79.86, 74.65, 64.07, 32.59, 31.98, 31.74, 29.63, 29.60, 29.51, 29.47, 29.13, 28.82, 28.60, 22.63, 21.06 and 14.09 (Found: C, 79.81; H, 11.28. $C_{24}H_{40}O_2$ requires C, 79.94; H, 11.18%). The IR and NMR spectra of (−)-**1*E*** were identical with those of (±)-**1*E***.

Acetate (+)-**11*E*** (0.3 g, 0.84 mmol) was hydrolysed with Novozym 435 (0.12 g) in a mixture of acetone and 0.1 mol dm^{-3} phosphate buffer as described for (+)-**11*Z*** (80% conversion). Column chromatography gave (+)-**1*E*** with >98 ee as a colorless liquid (0.19 g, 72%); $[\alpha]_D^{25}$ +22.80 (*c* 1.21, MeOH); δ_C 134.58, 130.35, 130.30 and 128.30. The IR and ^{1}H NMR spectra were identical with those of (±)-**1*E***.

(*R*)-1-Ethylhex-5-enyl acetate [(*R*)-13]

A mixture of (±)-**12** (5.0 g, 39 mmol), Novozym 435 (2.7 g), vinyl acetate (10 g, 0.116 mol), and *tert*-butyl methyl ether (50 cm^3) was stirred for 1.75 h at 30 °C (47% conversion). Purification of the product by column chromatography on silica gel (160 g) with hexane–diethyl ether (30:1) gave (*R*)-**13** with 90% ee as a colorless liquid (2.2 g, 44%); $[\alpha]_D^{20}$ +8.06 (*c* 2.70, pentane) {lit.,[7] $[\alpha]_D^{20}$ −7.24 (*c* 3.01, pentane) for (*S*)-**13**}. The IR and ^{1}H NMR spectra were identical with those of (±)-**13** prepared previously.[7]

(*R*)-1-Ethyl-4-formylbutyl acetate [(*R*)-14]

To a stirred solution of (*R*)-**13** (1.7 g, 10 mmol) in dry THF (40 cm^3) under nitrogen was added dropwise a solution of osmium tetroxide (25 mg) in water (10 cm^3) at room temperature. After stirring for 2 h, finely powdered sodium periodate (6.4 g) was added to the solution over one hour and the mixture was stirred for 3 h. The mixture was extracted with diethyl ether, and the ethereal solution was successively washed with saturated aq. Na_2SO_3, saturated aq. $NaHCO_3$ and brine, dried and concentrated. Purification of the residue by column chromatography on silica gel (80 g) with hexane–diethyl ether (30:1) gave (*R*)-**14** as a colorless liquid (1.0 g, 64%); ν_{max}(neat)/cm^{-1} 2969, 2881, 2725, 1733, 1460, 1375, 1296, 1021 and 957; δ_H 0.89 (3H, t, *J* 7.5), 1.54–1.66 (6H, m), 2.06 (3H, s), 2.48 (2H, m), 4.82 (1H, quintet, *J* 6.1) and 9.77 (1H, t, *J* 1.4); δ_C 202.03, 170.90, 74.74, 43.47, 32.82, 26.83, 21.12, 17.77 and 9.49.

(*R*,5*EZ*)-1-Ethylicos-5-enyl acetate [(*R*)-15]

Pentadecyltriphenylphosphonium bromide, which was freshly prepared from triphenylphosphine (0.8 g, 3 mmol) and 1-bromopentadecane (0.85 g, 3 mmol), was dissolved in 1,2-dimethoxyethane (DME) (20 cm^3). To the solution at −30 °C was added 2.0 cm^3 of 1.6 mol dm^{-3} BuLi in hexane and the mixture was then allowed to warm gradually to room temperature while being stirred, and was stirred for one hour. After the resulting ylide solution had been cooled to −30 °C, a solution of (*R*)-**14** (0.44 g, 2.56 mmol) in dry DME (10 cm^3) was added. The reaction mixture was allowed to warm gradually to room temperature with stirring; stirring was continued for 3 h. The mixture was successively poured into saturated aq. NH_4Cl and extracted with diethyl ether. Usual work-up of the extract gave a yellow liquid, which was purified by column chromatography on silica gel (40 g) with hexane–ethyl acetate (40:1) to give (*R*)-**15** as a colorless liquid (0.39 g, 41%); ν_{max}(neat)/cm^{-1} 3006, 2925, 1741, 1461, 1373, 1243, 1090, 1019, 956 and 722; δ_H 0.88 (6H, t, *J* 7.2), 1.26–1.36 (26H, m), 1.55 (4H, m), 1.98–2.05 (7H, including s at 2.04), 4.82 (1H, quintet, *J* 6.2) and 5.28–5.40 (2H, m); δ_C 170.89, 130.46, 129.09, 75.32, 33.13, 31.90, 29.71, 29.67, 29.64, 29.55, 29.34, 29.30, 27.21, 26.95, 25.34, 22.66, 21.18, 14.08 and 9.53.

(*R*)-1-Ethylicosyl acetate [(*R*)-16]

Catalytic hydrogenation of (*R*)-**15** (0.25 g, 0.68 mmol) in MeOH (15 cm^3) was carried out in the presence of 5% Pd/C (0.1 g). Purification of the product by column chromatography on silica gel (15 g) with hexane–ethyl acetate (25:1) gave (*R*)-**16** as a white solid (0.23 g, 93%), which was recrystallised from hexane; mp 39–41 °C; ν_{max}(neat)/cm^{-1} 2955, 2916, 2849, 1729, 1474, 1463, 1379, 1248, 1030, 1020, 959, 890, 790 and 730; δ_H 0.88 (6H, t, *J* 7.2), 1.20–1.31 (34H, m), 1.48–1.64 (4H, m), 2.05 (3H, s) and 4.80 (1H, quintet, *J* 6.2); δ_C 170.99, 75.55, 33.58, 31.92, 29.69, 29.66, 29.57, 29.54, 29.36, 26.92, 25.32, 22.69, 21.26, 14.11 and 9.57 (Found: C, 78.16; H, 13.08. $C_{24}H_{48}O_2$ requires C, 78.19; H, 13.13%).

(*R*)-Docosan-3-ol [(*R*)-(−)-17]

This compound was prepared by treating (*R*)-**16** (0.1 g, 0.27 mmol) with 6.0 mol dm^{-3} KOH in MeOH (10 cm^3). Purification of the product by column chromatography on silica gel (10 g) with hexane–ethyl acetate (30:1) gave (*R*)-(−)-**17** with 89% ee as a white solid (0.085 g, 96%), which was recrystallised from hexane; mp 68–69 °C; $[\alpha]_D^{20}$ −6.92 (*c* 1.07, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3310, 2959, 2916, 2849, 1472, 1380, 1346, 1139, 971, 949 and 719; δ_H 0.88 (3H, t, *J* 7.0), 0.94 (3H, t, *J* 7.0), 1.20–1.52 (39H, m) and 3.53 (1H, m); δ_C 73.33, 36.96, 31.96, 30.13, 29.70, 25.67, 22.69, 14.12 and 9.87 (Found: C, 81.02; H, 14.26. $C_{22}H_{46}O$ requires C, 80.90; H, 14.20%).

Preparation of (−)-17 from (+)-1*Z*

To a stirred and cooled (−70 °C) suspension of $CoCl_2$ (1.33 g, 10 mmol) and LAH (0.53 g, 14 mmol) in dry THF (30 cm^3) under argon was added dropwise a solution of (+)-**1*Z*** (0.5 g, 1.6 mmol) in dry THF (10 cm^3). The mixture was then allowed to warm gradually to room temperature while being stirred. After being stirred for an additional 24 h, the mixture was cooled to 0 °C, treated with 10% HCl and extracted with diethyl ether. Usual work-up of the product and subsequent purification by column chromatography as described above gave (−)-**17** with 95% ee as a white solid (0.25 g, 49%), which was recrystallised from hexane; mp 66–68 °C; $[\alpha]_D^{20}$ −7.06 (*c* 1.02, $CHCl_3$) (Found: C, 80.76; H, 14.28. $C_{22}H_{46}O$ requires C, 80.90; H, 14.20%). The IR and NMR spectra were identical with (*R*)-(−)-**17** synthesized from (*R*)-**13** as already described.

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