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Total synthesis of acetylenic carotenoids.

3.* First total synthesis of optically active 9-*Z*-(3*R*,3′*R*)-diatoxanthin and 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin.

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Abstract: Optically active 9-*Z*-(3*R*,3′*R*)-diatoxanthin and 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin were synthesised in an overall yield of 9% and 11% respectively, with a seven step $C_9+C_6+C_{25}=C_{40}$ strategy. A key intermediate was the previously undescribed C_{15}-acetylenic aldehyde 2-*Z*-5-((4′*R*)-4′-hydroxy-2′,6′,6′-trimethylcyclohex-1′-enyl)-3-methyl-2-penten-4-yn-1-al.

Observed isomerisation shifts for the title compounds relative to the all-*E* isomers in ^{1}H NMR and ^{13}C NMR were as expected. CD properties are discussed.

Introduction

Biological functions of *Z*-carotenoids have received increasing attention in recent years, and *Z*-carotenoids have consequently become interesting target compounds in total synthesis.[1] The total synthesis of optically active 9-*Z* carotenoids including (3*R*,3′*S*,5′*R*)-mytiloxanthin,[2] (3*S*,3′*S*)- and (3*R*,3′*R*)-tetradehydroasta-xanthin[3,4] and (3*S*,3′*S*)-didehydroastaxanthin,[3] as well as optically neutral 9-*Z* isomers of crocoxanthin and the diacetylenic alloxanthin[5] have previously been reported.

The first total synthesis of the monoacetylenic all-*E*-(3*R*,3′*R*)-diatoxanthin (**1a**) and all-*E*-(3*R*)-7,8-didehydrocryptoxanthin (**2a**), see Scheme 1, was recently achieved.[6,7] Diatoxanthin (**1a**) is a common microalgal carotenoid.[8,9] 7,8-Didehydrocryptoxanthin (**2a**) on the other hand has so far not been encountered in Nature, and is not included in the list of more than 600 carotenoids of known structure.[10]

It is well known that the 9-*Z* isomer of 7,8-didehydrocarotenoids and 9-*Z* as well as 9,9′-di-*Z* isomers of 7,8,7′,8′-tetradehydrocarotenoids usually are more stable than the corresponding all-*E* compounds.[11-14] Such acetylenic *Z*-carotenoids are therefore accessible by geometrical isomerisation of the all-*E* compound. In the iodine catalysed stereomutation mixture of natural diatoxanthin (**1**) was observed a 48 : 52 ratio of **1a** and a neo U isomer, tentatively assigned the 9-*Z* configuration (**1b**) from VIS and CD data.[14] With relevant synthons available from the total synthesis of **1a** and **2a**,[7] it was considered of interest to prepare the optically active 9-*Z*-(3*R*,3′*R*)-diatoxanthin (**1b**) and 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) by specific synthesis.

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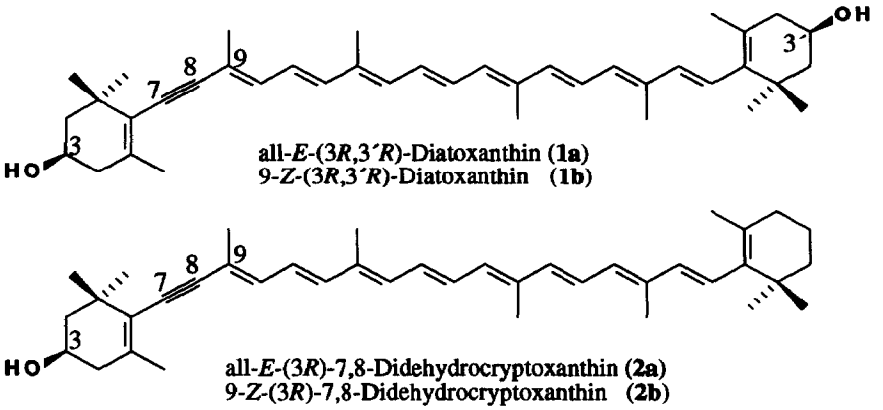


Scheme 1.

Results and discussion

Weedon and co-workers[2,5] and the Roche group[3,4] employed *Z* and/or *E* acetylenic phosphonium salts in their syntheses of acetylenic 9-*Z* carotenoids. It was of interest to employ a different strategy in the present work. With C_{25}-phosphonium salts available from the total synthesis of all-*E*-(3*R*,3'*R*)-diatoxanthin (**1a**) and all-*E*-(3*R*)-7,8-didehydrocryptoxanthin (**2a**), a $C_9+C_6+C_{25}=C_{40}$ strategy with a $C_9+C_6=C_{15}$ Grignard acetylide coupling reaction and a final $C_{15}+C_{25}=C_{40}$ Wittig condensation, was chosen for the construction of the carbon skeleton. According to the theory[15-17] should the final Wittig reaction with a stabilised phosphonium ylide favour the *E* configuration for the generated carbon-carbon double bond, as observed in the total synthesis of the corresponding all-*E* compounds **1a** and **2a** by a similar strategy.[7]

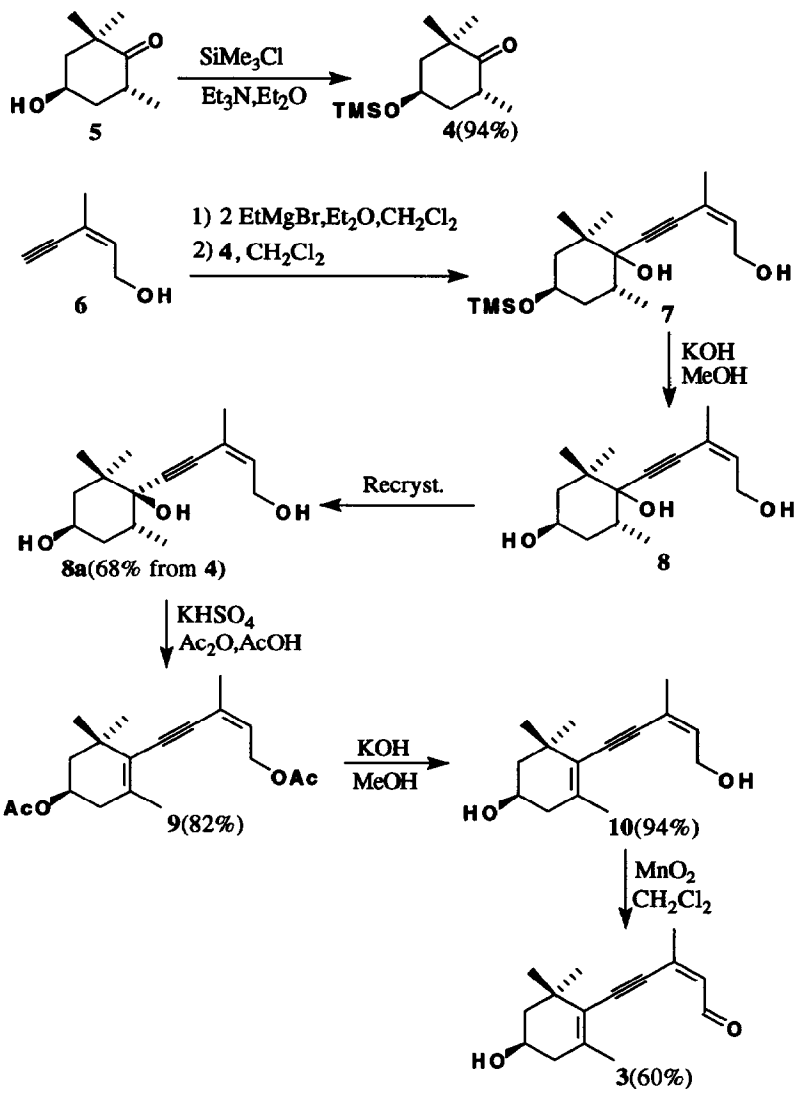
The optically active C_{15}-acetylenic aldehyde 2-*Z*-5-((4'*R*)-4'-hydroxy-2',6',6'-trimethyl-cyclohex-1'-enyl)-3-methyl-2-penten-4-yn-1-al was a key intermediate in the present total synthesis of acetylenic 9-*Z* carotenoids.

Synthesis of optically active 2-Z-5-((4'R)-4'-hydroxy-2',6',6'-trimethylcyclohex-1'-enyl)-3-methyl-2-penten-4-yn-1-al

The synthesis of the 2-*Z*-(4'*R*) hydroxy aldehyde **3** was achieved by a strategy similar to that recently employed in the synthesis of the corresponding 2-*E* compound,[6] *cf.* Scheme 2. The optically active TMS-ether **4** was obtained by standard silylation of optically active (4*R*,6*R*)-actinol (**5**), as a colourless oil in 94% yield. Treatment of the unprotected acetylenic *Z* alcohol **6** with ethylmagnesium bromide, generated from magnesium and ethyl bromide, yielded the proper acetylide Grignard reagent, which was reacted with the TMS-ether **4** in refluxing diethyl ether - dichloromethane. The resulting diol **7** was without purification treated with alkaline methanol and yielded the triol **8** as a yellow-white powder in 95% overall yield from **4**.

The triol **8** was, as judged by ^{1}H NMR analysis, obtained as a 4 : 1 mixture of the two C-1' epimers 2-*Z*-(1'*S*,4'*R*,6'*R*)-**8** and 2-*Z*-(1'*R*,4'*R*,6'*R*)-**8**, resulting from preferred axial attack by the acetylenic Grignard reagent. The corresponding C-1' epimeric 2-*E* acetylenic triols have been synthesised by several research groups.[5,6,18-20] Weedon and co-workers[5] assigned the (1'*S*,4'*R*,6'*R*) relative configuration to the major diastereomer in the *E* series by a mechanistic evaluation of the Grignard reaction. This assignment was

subsequently confirmed by NOE-NMR experiments[20] and X-ray crystallographic analysis.[6] It is reasonable to assume that the corresponding (1′*S*,4′*R*,6′*R*)-**8** diastereomer was the dominant isomer also in the *Z* series. Fractional crystallisation from diethyl ether furnished the pure 2-*Z*-(1′*S*,4′*R*,6′*R*) acetylenic triol (**8a**) as a white semi-crystalline powder, in 68% overall yield from **4**.



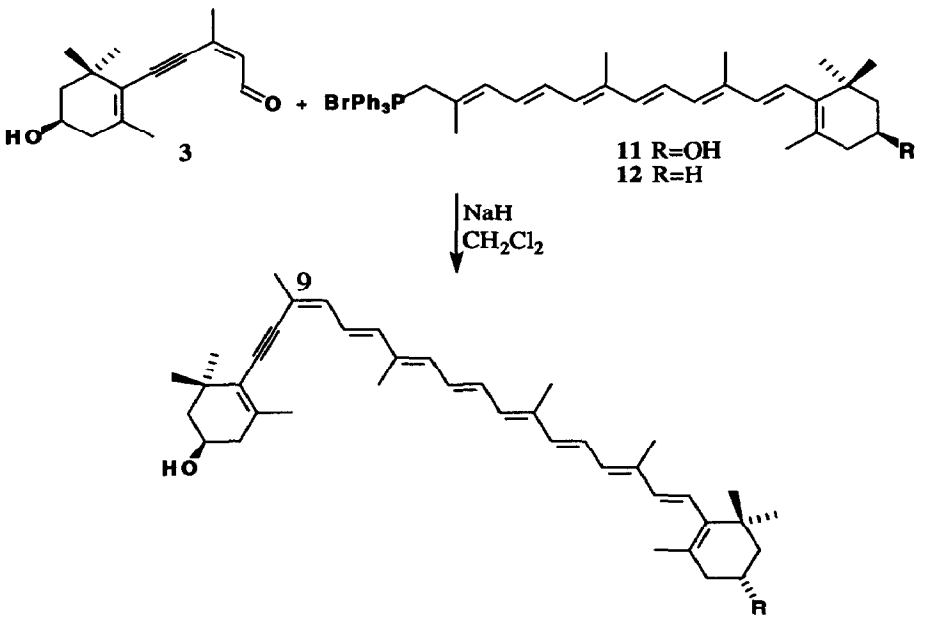
Scheme 2.

The *syn* elimination of the tertiary hydroxy group in **8a** was effected by treatment with acetic anhydride and acetic acid in the presence of potassium hydrogen sulphate, according to a procedure reported by Abrams and Milborrow.[21] Subsequent purification by column chromatography afforded the acetylenic 2-*Z*-(4′*R*)

diacetate **9** as a light yellow oil in 82% yield. Hydrolysis of **9** with methanolic potassium hydroxide furnished the pure acetylenic diol **10**, which upon allylic oxidation with manganese dioxide and subsequent purification by column chromatography yielded the pure optically active 2-*Z*-(4′*R*) hydroxy aldehyde **3** as a light yellow oil, in 30% overall yield from optically active (4*R*,6*R*)-actinol (**5**). This work represents the first synthesis of 2-*Z*-5-((4′*R*)-4′-hydroxy-2′,6′,6′-trimethylcyclohex-1′-enyl)-3-methyl-2-penten-4-yn-1-al (**3**).

Synthesis of optically active 9-Z-(3R,3′R)-diatoxanthin and 9-Z-(3R)-7,8-didehydrocryptoxanthin

A Wittig reaction between the available C_{25}-(3*R*)-phosphonium salt **11**[7] and the C_{15}-2-*Z*-(4′*R*) hydroxy aldehyde **3**, see Scheme 3, followed by CC furnished 9-*Z*-(3*R*,3′*R*)-diatoxanthin (**1b**) in a mixture with several geometrical isomers of (3*R*,3′*R*)-diatoxanthin (**1**), as a red oil in 78% yield. The 9-*Z*-(3*R*,3′*R*) isomer (**18b**) was the major product. The all-*E* compound was not encountered in the CC purified mixture, as evident from HPLC analysis including co-injection with authentic all-*E*-(3*R*,3′*R*)-diatoxanthin (**1a**). Preparative TLC of the CC purified product mixture provided 95% pure (HPLC) 9-*Z*-(3*R*,3′*R*)-diatoxanthin (**18b**) in an overall yield of 9% based on actionol (**5**). Attempted crystallisation of **1b** from diethyl ether - methanol was not successful.



9-*Z*-(3*R*,3′*R*)-Diatoxanthin (**1b**) R=OH (30%)
9-*Z*-(3*R*)-7,8-Didehydrocryptoxanthin (**2b**) R=H (34%)

Scheme 3.

In a similar way as described for 9-*Z*-(3*R*,3′*R*)-diatoxanthin (**1b**) above, a Wittig reaction between the available C_{25}-phosphonium salt **12**[7] and the optically active C_{15} 2-*Z*-(4′*R*) hydroxy aldehyde **3**, see Scheme 3, followed by CC furnished 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) as a red oil in 53% yield. HPLC analysis and VIS spectra recorded on-line during chromatographic analysis of the CC purified product, demonstrated the presence of two geometrical isomers in addition to the target compound. The all-*E* isomer (**2a**) was not detected

in the product mixture. Preparative HPLC of the CC purified compound afforded 96% pure (HPLC) 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) in an overall yield of 11% based on actionol (**5**). Attempted crystallisation of **2b** from diethyl ether - methanol was not successful.

Spectroscopic characterisation of 9-Z-(3R,3'R)-diatoxanthin and 9-Z-(3R)-7,8-didehydrocryptoxanthin

The IR and mass spectral data of 9-*Z*-(3*R*,3'*R*)-diatoxanthin (**1b**) and 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) were as expected similar to the corresponding data recorded for the all-*E* isomers, **1a** and **2a**[7] respectively.

The 9-*Z* isomers (**1b**,**2b**) exhibited 7-9 nm hypsochromic shifts of the main absorption maximum relative to the corresponding all-*E* isomers (**1a**,**2a**), depending on the solvent. Increased spectral fine structure, measured as %III/II,[22] was observed for the *Z* isomers (**1b**,**2b**) compared to the all-*E* compounds (**1a**,**2a**) in the HPLC eluent, in accordance with published data for other 9-*Z*-7,8-didehydro carotenoids.[13] Reduced spectral fine structure in acetone was ascribed to isomerisation of the 9-*Z* isomers (**1b**,**2b**) during the manipulations required for change of solvent. The VIS absorption data, including *cis*-peak intensities, were compatible with the 9-*Z* configuration of the products **1b** and **2b**. The data reported for natural all-*E*-(3*R*,3'*R*)-diatoxanthin (**1a**) and a neo U isomer tentatively identified as 9-*Z* (**1b**) in acetone solution,[14] deviate slightly from the present data.

The chemical shift assignments of the ^{1}H and ^{13}C NMR spectra of 9-*Z*-(3*R*,3'*R*)-diatoxanthin (**1b**) and the ^{1}H NMR spectrum of 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) were made on the basis of ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra, and from a comparison with the spectra of the corresponding all-*E* isomers **1a** and **2a**[7] and relevant available NMR data.[23] Isomerisation shifts $\Delta=\delta_Z - \delta_E$[23] in ^{1}H NMR (>0.03 ppm) and ^{13}C NMR (>1.0 ppm) for 9-*Z*-(3*R*,3'*R*)-diatoxanthin (**1b**) and in ^{1}H NMR (>0.03 ppm) for 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) are given in Table 1. The ^{1}H NMR isomerisation shifts for **1b** and **2b** were as expected identical, and the data were in accordance with expected values[23] for 9-*Z* acetylenic carotenoids.

Table 1. Isomerisation shifts Δ in ^{1}H NMR (>0.03 ppm) and ^{13}C NMR (>1.0 ppm) for 9-*Z*-(3*R*,3'*R*)-diatoxanthin (**1b**) and in ^{1}H NMR (>0.03 ppm) for 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**).

Nucleus		$\Delta=\delta_Z - \delta_E$ (ppm)		
		9-*Z*-(3*R*,3'*R*)-diatoxanthin (**1b**)		9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**)
^{1}H	^{13}C	^{1}H NMR	^{13}C NMR	^{1}H NMR
H-10	C-7	-0.16	*ca.* 5	-0.16
H-11	C-8	0.31	*ca.* 5	0.31
H-16	C-9	0.04	1.3	0.04
H-17	C-11	0.05	3.4	0.05
	C-19		5.7	

The CD spectra of 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) and 9-*Z*-(3*R*,3'*R*)-diatoxanthin (**1b**) are compared with the spectra of the corresponding all-*E* isomers **2a** and **2a**[7] in Figures 1 and 2 respectively. (3*R*)-7,8-Didehydrocryptoxanthin (**2**) contains one single chiral center located in the acetylenic end group. It is well known that asymmetric acetylenic end groups in carotenoids provide no intrinsically chiral chromophore since the acetylenic moiety does not generate a preferred conformation for the junction between the polyene chain and

the chiral end group. Thus, the resulting CD spectrum is typically non-conservative,[24-26] as evident from the spectra of all-*E*-(3*R*)- (**2a**)[7] and 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) in Figure 1.

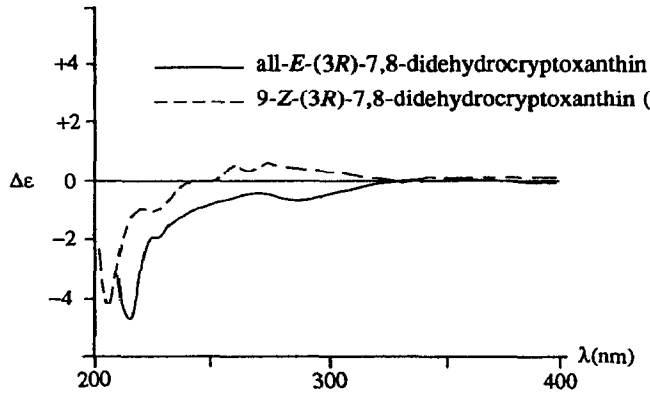


Figure 1. Comparison of the CD spectra of all-*E*-(3*R*)- (**2a**)[7] and 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) recorded in EPA solution at room temperature.

(3*R*,3′*R*)-Diatoxanthin (**1**) contains a nonacetylenic 3-hydroxy-β-end group which, due to steric interaction between the methyl group at C-5′ and the olefinc proton at C-8′, generates an intrinsically chiral chromophore.[24-26] The spectra of all-*E*-(3*R*,3′*R*)- (**1a**)[7] and 9-*Z*-(3*R*,3′*R*)-diatoxanthin (**1b**) were consequently conservative, and the expected sign inversion of the Cotton effects for a mono-*Z* relative to the corresponding all-*E* isomer was observed, *cf.* Figure 2. The CD data are consistent with previous data for natural diatoxanthin (**1a**) and a neo U isomer, assigned the 9-*Z* structure (**1b**), obtained by iodine catalysed stereomutation.[14,24,26]

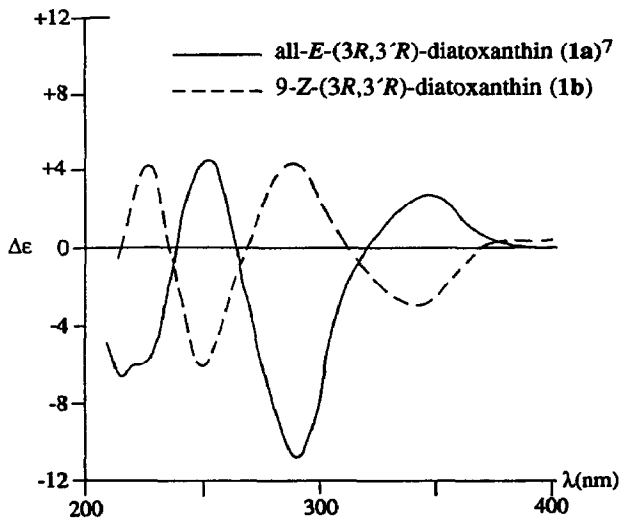


Figure 2. Comparison of the CD spectra of all-*E*-(3*R*,3′*R*)- (**1a**)[7] and 9-*Z*-(3*R*,3′*R*)-diatoxanthin (**1b**) recorded in EPA solution at room temperature.

Experimental

General methods. All solvents were of *p.a.* quality. Diethyl ether used for extraction was filtered through alumina (neutral). Diethyl ether used as solvent in reactions was distilled over solid sodium. Pyridine was distilled over solid potassium hydroxide. Dichloromethane was dried over freshly activated 3 Å molecular sieves. Sodium hydride was washed with hexane and dry dichloromethane before use. Solvents were evaporated under reduced pressure at 20-35 °C.

Thin layer chromatography was performed on silica gel 60 F_{254} (Merck Art. 5554) with ethyl acetate - heptane 2 : 3 (System 1) or 1 : 1 (System 2) as eluents. Column chromatography was performed on silica gel 60 (Merck Art. 7734) with mixtures of hexane - ethyl acetate as eluents. Analytical high performance liquid chromatography was carried out on a Hewlett Packard series 1050 instrument and a Spherisorb S 5-W silica column with hexane - dichloromethane - isopropyl alcohol -*N*-ethyldiisopropylamine 89.9 : 7 : 3 : 0.1 as the eluent. The flow is specified in each case. Simultaneous detection at 250, 280, 320, 350, 380 and 450 nm was employed. Preparative HPLC was performed on a Perkin Elmer Series 2 LC instrument equipped with an ISCO absorbance monitor model 1840 and a Crompack Chromspher 5 Si semi-preparative column. The detection wavelength was 490 nm. Gas liquid chromatography was carried out on a Varian 3700 instrument with an unpolar BP-1 capillary column (25 m x 0.25 mm) and a flame ionisation detector, temperature program: 40 °C 4 min; 10 °C min^{-1} to 280 °C; 10 min.

UV-VIS spectra were recorded on a Perkin Elmer 552 spectrophotometer, solvents are specified in each case. Spectral fine structure and *cis*-peak intensities are expressed as %III/II and $\%D_B/D_{II}$ respectively.[22] IR spectra were recorded in KBr discs of solids or of liquids as a film between NaCl discs, on a Nicolet 20 SXC FT-IR spectrophotometer. CD spectra were recorded on a Jobin Yvon Auto Dichrograph Mark IV in EPA (diethyl ether - isopentane - ethanol 5 : 5 : 2) solution at room temperature. Mass spectra were recorded on an AEI 902 spectrometer with direct inlet to the ion source. ^1H NMR, ^{13}C NMR, ^1H-^1H COSY and ^1H-^{13}C COSY spectra were recorded in $CDCl_3$ solutions on a 400 MHz (100 MHz for ^{13}C) Jeol EX-400 instrument. Optical rotation was measured in methanol solution on a Perkin Elmer 241 polarimeter. Melting points are uncorrected.

Synthesis of optically active 2-Z-5-((4'R)-4'-hydroxy-2',6',6'-trimethylcyclohex-1'-enyl)-3-methyl-2-penten-4-yn-1-al

(4R,6R)-2,2,6-Trimethyl-4-trimethylsiloxycyclohexan-1-one (4). (4*R*,6*R*)-4-Hydroxy-2,2,6-trimethylcyclohexan-1-one (actinol) was silylated under standard conditions[27] with diethyl ether as the solvent. A solution of (4*R*,6*R*)-actinol (**5**, $[\alpha]^{20}_D$= -113.7, c= 0.0214 (MeOH) in accordance with reported data,[28] 30.0 g, 0.19 mol) in dry diethyl ether (190 ml) was added triethylamine (26.0 g) and the mixture was cooled to 0 °C. Trimethylsilyl chloride (25.0 ml, 0.20 mol) was added dropwise, and the reaction mixture kept at 0 - 20 °C for 15h. Ice-water was added and the product was extracted with diethyl ether. The organic phase was washed with brine and water and dried over anhydrous sodium sulphate. Solvents were evaporated and the residue distilled under reduced pressure, *ca.* 20 mmHg. The protected actinol **4**, bp. 105-107 °C, was obtained as a colourless oil in 94% yield (41.0 g, 0.18 mol) 99% pure as demonstrated by GLC.

MS [IP 70 eV, 150 °C; *m/z* (% rel. int.)]: 228 (44, [M]), 170 (29, [M-58]), 146 (63, [M-82]), 130 (25, [M-98]), 82 (17), 75 (64), 73 (100); ^1H NMR ($CDCl_3$) δ 0.137 [*s*, 9H, Me in TMSO-], 1.005 [*d*, 3H, *J* 6.4 Hz, Me-6], 1.009 [*s*, 3H, Me-2], 1.321 [*s*, 3H, Me-2], 1.60 [*td*, 1H, *J* 2.9 Hz, *J* 13.2 Hz, H-5ax], 1.67 [*dd*,

1H, *J* 3.9 Hz, *J* 14.7 Hz, H-3ax], 1.89 [*dt*, 1H, *J* 2.9 Hz, *J* 14.7 Hz, H-3eq], 2.01 [*m*, 1H, H-5eq], 3.17 [*m*, 1H, H-6], 4.10 [*m*, 1H, H-4].

2-Z-5-((1S,4'R,6'R)-1',4'-Dihydroxy-2',2',6'-trimethylcyclohexyl)-3-methyl-2-penten-4-yn-1-ol (8a). Ethyl bromide (26.16 g, 0.24 mol) was added dropwise to magnesium (6.1 g, 0.25 mol) in dry diethyl ether (500 ml) over 1h to maintain a gentle reflux. The mixture was refluxed for another 1h and cooled to 20 °C during 30min. A solution of the C_5 acetylenic 2-Z alcohol **6** (11.1 g, 0.12 mol) in dry dichloromethane (60 ml) was added over 1h to maintain a gentle reflux. The reaction mixture was refluxed for another 10min and cooled to 20 °C during 30min. The protected actinol **4** (18.3 g, 0.08 mol) in dry dichloromethane (60 ml) was added over 30min at 20 °C. The resulting reaction mixture was refluxed for 14h and subsequently cooled to 0 °C. Cold saturated aqueous ammonium chloride (*ca.* 300 ml) was added and the mixture was stirred for 1h. The product was extracted with diethyl ether, the organic phase was washed with brine and water, dried over anhydrous sodium sulphate and the solvents were evaporated. The yellow oily diol **7** (26.6 g) was dissolved in 5% potassium hydroxide in methanol (110 ml) and stirred at 20 °C, in the dark, under an N_2 atm. for 2h. The volume was reduced to *ca.* 50 ml and the product extracted with diethyl ether. The organic phase was washed with brine and water until neutral, dried over anhydrous sodium sulphate and the solvents were evaporated, yielding **8** as a yellow-white powder (19.1 g, 75.8 mmol). ^{1}H NMR indicated a 4 : 1 mixture of the two possible C-1' epimers as judged from the signals for the olefinic proton H-2 (5.87 ppm for the (1'*S*) and 5.96 ppm for the (1'*R*) epimer). The remaining ^{1}H NMR chemical shifts for the two C-1' epimers were identical or almost so. Fractional crystallisation from diethyl ether by slow cooling of a saturated solution from 35 °C to -20 °C afforded the (1'*S*) epimer **8a** as a white crystal powder in 68% yield (13.7 g, 54.4 mmol), 100% pure as judged by ^{1}H NMR. The product decomposed during GLC analysis.

Mp. 139-140 °C; UV λ_{max} (ethanol) 226 nm; IR (KBr) cm^{-1} 3356 s (OH), 2986-2876 s (CH), 2197 w (C≡C), 1452 w, 1392 m, 1234 m, 1062 m, 1034 s, 1016 s, 930 m; MS [IP 20 eV, 170 °C; *m/z* (% rel. int.)]: 252 (4, [M]), 234 (14, [M-18]), 203 (5), 178 (15), 165 (24), 148 (100), 134 (17), 122 (45), 107 (33), 99 (12), 83 (13), 43 (9); ^{1}H NMR ($CDCl_3$) δ 1.084 [*d*, 3H, *J* 6.4 Hz, Me-6'], 1.112 [*s*, 3H, Me-2'], 1.251 [*s*, 3H, Me-2'], 1.57-1.78 [*m*, 4H, H-3'ax, H-3'eq, H-5'ax and H-5'eq], 1.903 [*s*, 3H, Me-3], 2.37 [*m*, 1H, H-6'], 4.05 [*m*, 1H, H-4'], 4.32 [*d*, 2H, *J* 6.4 Hz, H-1], 5.87 [*tq*, 1H, *J* 1.5 Hz, *J* 5.9 Hz, H-2]; $[\alpha]^{25}_D$= -23.9, c= 0.0134 (MeOH).

2-Z-5-((4'R)-4'-Acetoxy-2',6',6'-trimethylcyclohex-1'-enyl)-3-methyl-2-penten-4-yn-1-ol 1-acetate (9). The acetylenic triol **8a** (8.8 g, 35.0 mmol) was dissolved in concentrated acetic acid (108 ml). Acetic anhydride (72 ml) and potassium hydrogen sulphate, *cf.* ref. 21, (5.0 g, 36.8 mmol) were added. The reaction mixture was kept at 70 °C, under an N_2 atm. for 1 3/4h. The mixture was subsequently cooled to 20 °C, water was added and the product extracted with diethyl ether. Sodium bicarbonate (s) was added until gas evolution ceased. Water was added and the product extracted with diethyl ether. The organic phase was washed with brine and water, dried over anhydrous sodium sulphate and solvents were evaporated. The resulting brownish residue was subjected to column chromatography with gradient elution, hexane - ethyl acetate 4 : 1 to 7 : 3, giving the acetylenic diacetate **9** as a light yellow oil in 82% yield (9.06 g, 28.5 mmol), 100% pure as judged by ^{1}H NMR and TLC (System 1). The product decomposed during GLC analysis.

UV λ_{max} (ethanol) (260), 269, (282) nm; IR (KBr) cm^{-1} 2963-2868 s (CH), 2188 m (C≡C), 1740 s (acetate), 1436 m, 1367 m, 1238 s (acetate), 1026 s, 979 m; MS [IP 20 eV, 170 °C; *m/z* (% rel. int.)]: 258 (100, [M-60]), 216 (5, [M-42-60]), 198 (16, [M-60-60]), 183 (64), 43 (27); ^1H NMR ($CDCl_3$) δ 1.165 [*s*, 3H, Me-6′], 1.187 [*s*, 3H, Me-6′], 1.57 [*m*, 1H, H-5′ax], 1.83 [*m*, 1H, H-5′eq], 1.896 [*s*, 3H, Me-2′], 1.950 [*d*, 3H, *J* 1.0 Hz, Me-3], 2.039 [*s*, 3H, Me in AcO], 2.053 [*s*, 3H, Me in AcO], 2.13 [*dd*, 1H, *J* 7.8 Hz, *J* 16.6 Hz, H-3′ax], 2.49 [*dd*, 1H, *J* 5.9 Hz, *J* 17.1 Hz, H-3′eq], 4.78 [*d*, 2H, *J* 7.3 Hz, H-1], 5.02 [*m*, 1H, H-4′], 5.75 [*tq*, 1H, *J* 1.5 Hz, *J* 6.8 Hz, H-2]; $[\alpha]^{23}_D$= -46.1, c= 0.0240 (MeOH).

2-Z-5-((4′R)-4′-Hydroxy-2′,6′,6′-trimethylcyclohex-1′-enyl)-3-methyl-2-penten-4-yn-1-ol (10). The diacetate **9** (0.97 g, 3.05 mmol) was treated with 5% potassium hydroxide in methanol (50 ml), in the dark at 20 °C and under an N_2 atm. for 1h. The volume was subsequently reduced to *ca.* 5 ml, water was added and the product was extracted with diethyl ether. The organic phase was washed with brine and water until neutral, dried over anhydrous sodium sulphate and the solvents were evaporated, giving **10** as a light yellow oil in 94% yield (0.67 g, 2.86 mmol). No further purification was carried out. ^1H NMR, TLC (System 1) and HPLC (flow=1.0 ml/min) indicated a mixture of the 2-*Z* (**10**) and 2-*E* (*cf.* ref. 6) isomers in a 5 : 1 ratio. The spectroscopic characterisation was performed with this 5 : 1 mixture. The ^1H NMR chemical shift assignments for the 2-*E* isomer are given elsewhere.[6]

UV λ_{max} (ethanol) (258), 268, 272 nm, %III/II=2; IR (KBr) cm^{-1} 3338 s (OH), 2962-2866 s (CH), 2186 m (C≡C), 1706 w, 1432 m, 1361 m, 1053 s, 1007 s; MS [IP 50 eV, 160 °C; *m/z* (% rel. int.)]: 234 (100, [M]), 216 (80, [M-18]), 198 (50, [M-18-18]), 183 (58), 173 (20), 168 (14), 159 (24), 147 (25), 143 (21), 131 (22), 128 (21), 119 (24), 115 (25), 105 (30), 95 (23), 91 (38), 77 (28), 43 (27); ^1H NMR ($CDCl_3$) δ 1.134 [*s*, 3H, Me-6′], 1.186 [*s*, 3H, Me-6′], 1.43 [*m*, 1H, H-5′ax], 1.83 [*m*, 1H, H-5′eq], 1.907 [*s*, 3H, Me-2′], 1.937 [*d*, 3H, *J* 1.0 Hz, Me-3], 2.06 [*dd*, 1H, *J* 8.3 Hz, *J* 16.1 Hz, H-3′ax], 2.43 [*dd*, 1H, *J* 5.4 Hz, *J* 17.6 Hz, H-3′eq], 3.99 [*m*, 1H, H-4′], 4.34 [*d*, 2H, *J* 6.4 Hz, H-1], 5.83 [*tq*, 1H, *J* 1.5 Hz, *J* 6.8 Hz, H-2]; $[\alpha]^{27}_D$= -90.9, c= 0.0243 (MeOH).

2-Z-5-((4′R)-4′-Hydroxy-2′,6′,6′-trimethylcyclohex-1′-enyl)-3-methyl-2-penten-4-yn-1-al (3). The acetylenic diol **10** (0.61 g, 2.61 mmol) was dissolved in dry dichloromethane (60 ml). Manganese dioxide (8.0 g) was added and the reaction mixture was stirred vigorously at 20 °C, in the dark, under an N_2 atm. for 20h. The mixture was filtered, the solvents evaporated and the resulting orange oily residue submitted to column chromatography with gradient elution, hexane - ethyl acetate 7 : 3 to 3 : 2. The optically active acetylenic hydroxy aldehyde **3** was obtained as a light yellow oil in 60% yield (0.36 g, 1.56 mmol), 100% pure as judged by ^1H NMR and HPLC (flow=1.0 ml/min).

UV λ_{max} (ethanol) 322 nm; IR (KBr) cm^{-1} 3414 s (OH), 2962-2867 s (CH), 2173 s (C≡C), 1674 s, 1585 m, 1148 m, 1053 m; MS [IP 50 eV, 160 °C; *m/z* (% rel. int.)]: 232 (85, [M]), 214 (33, [M-18]), 199 (100), 173 (56), 147 (58), 133 (34), 115 (31), 105 (41), 91 (43), 77 (49), 69 (80), 43 (36); ^1H NMR ($CDCl_3$) δ 1.142 [*s*, 3H, Me-6′], 1.195 [*s*, 3H, Me-6′], 1.50 [*m*, 1H, H-5′ax], 1.90 [*m*, 1H, H-5′eq], 1.941 [*s*, 3H, Me-2′], 2.09 [*dd*, 1H, *J* 9.8 Hz, *J* 18.1 Hz, H-3′ax], 2.173 [*d*, 3H, *J* 1.5 Hz, Me-3], 2.47 [*dd*, 1H, *J* 4.4 Hz, *J* 18.1 Hz, H-3′eq], 4.01 [*m*, 1H, H-4′], 6.13 [*dd*, 2H, *J* 1.0 Hz, *J* 8.3 Hz, H-2], 10.08 [*d*, 1H, *J* 8.3 Hz, H-1]; $[\alpha]^{29}_D$= -88.4, c= 0.0172 (MeOH).

Synthesis of optically active 9-cis-(3R,3'R)-diatoxanthin and 9-cis-(3 R)-7,8-didehydrocryptoxanthin

The Wittig reactions described below were adapted from a procedure described by Kjøsen and Liaaen-Jensen.[29]

9-Z-(3 R,3'R)-Diatoxanthin (1b). The hydroxy phosphonium salt **11** (100 mg, 0.14 mmol) and the acetylenic hydroxy aldehyde **3** (35 mg, 0.15 mmol) dissolved in dry dichloromethane (40 ml) were added dropwise to a suspension of sodium hydride (150 mg, unwashed) in dry dichloromethane (40 ml), at 20 °C in the dark and under an N_2 atm.. The reaction was monitored by TLC (System 2). After 48h the reaction mixture was cooled to 0° C and ice (s) was added carefully to decompose excess sodium hydride. Water was subsequently added and the product extracted with dichloromethane. The organic phase was washed with water, dried over anhydrous sodium sulphate and the solvents were evaporated. The resulting red oily residue was dissolved in a minimal volume of benzene (*ca.* 2 ml) and subjected to column chromatography with gradient elution, hexane - ethyl acetate 4 : 1 to 3 : 2. (3*R*, 3'*R*)-Diatoxanthin (**1**) was obtained as a red oil in 71% yield (56.5 mg, 0.10 mmol) as a mixture of six geometrical isomers. The main product was isolated in 30% yield (23.9 mg, 0.042mmol) by preparative TLC (System 2), 95% pure (HPLC, flow=2.0 ml/min), and identified as the target compound 9-*Z*-(3*R*,3'*R*)-diatoxanthin (**1b**). Crystallisation from diethyl ether - methanol was not successful.

R_T(HPLC, flow 1.0 ml/min)= 18.7 min. VIS λ_{max} (acetone) 340, 420, 444, 469 nm, %III/II=3, $\%D_B/D_{II}$=15, λ_{max} (hexane) 337, 418, 441, 468 nm, %III/II=25, $\%D_B/D_{II}$=9, λ_{max} (HPLC eluent) 341, 421, 445, 474 nm, %III/II=44, $\%D_B/D_{II}$=11; IR (KBr) cm^{-1} 3426 s (OH), 2959-2854 s (CH), 2171 w (C≡C), 1049 m, 965 m; MS [IP 70 eV, 210 °C; *m/z* (% rel. int.)]: 566 (100, [M]), 548 (15, [M-18]), 474 (6, [M-92]), 460 (2, [M-106]), 283 (12, [M/2]); ^{1}H NMR ($CDCl_3$) δ 1.071 [*s*, 6H, Me-16' and Me-17'], 1.189 [*s*, 3H, Me-16], 1.253 [*s*, 3H, Me-17], 1.48 [*m*, 2H, H-2ax and H-2'ax], 1.735 [*s*, 3H, Me-18'], 1.77 [*m*, 1H, H-2'eq], 1.86 [*m*, 1H, H-2eq], 1.926 [*s*, 3H, Me-18 or Me-20], 1.968 [*s*, 9H, Me-19', Me-20' and Me-18 or Me-20], 1.997 [*s*, 3H, Me-19], 2.04 [*m*, 1H, H-4'ax], 2.09 [*dd*, 1H, *J* 5.8 Hz, *J* 19.5 Hz, H-4ax], 2.39 [*dd*, 1H, *J* 5.4 Hz, *J* 16.6 Hz, H-4'eq], 2.46 [*dd*, 1H, *J* 5.4 Hz, *J* 17.0 Hz, H-4eq], 4.01 [*m*, 2H, H-3 and H-3'], 6.10 [*s*, 2H, H-7' and H-8'], 6.15 [*d*, 1H, $J_{10',11'}$ 11.7 Hz, H-10'], 6.25 [*d*, 2H, $J_{14,15}$ and $J_{14',15'}$ ~11 Hz, H-14 and H-14'], 6.30 [*d*, 1H, $J_{10,11}$ 10.8 Hz, H-10], 6.35 [*d*, 1H, $J_{11,12}$ 15.1 Hz, H-12], 6.36 [*d*, 1H, $J_{11',12'}$ 14.7 Hz, H-12'], 6.62-6.67 [*m*, 3H, H-15, H-11' and H-15'], 6.83 [*dd*, 1H, $J_{10,11}$ 11.2 Hz, $J_{11,12}$ 15.1 Hz, H-11]; ^{13}C NMR ($CDCl_3$) δ 12.9-13.1 [C-20, C-19' and C-20'], 21.9 [C-18'], 22.9 [C-18], 23.8 [C-19], 29.1 and 29.2 [C-16 and C-16'], 30.6 [C-17'], 30.9 [C-17], 36.9 [C-1], 37.4 [C-1'], 41.8 [C-4], 42.9 [C-4'], 47.0 [C-2], 48.8 [C-2'], 65.2 and 65.4 [C-3 and C-3'], 94.1 and 95.0 [H-7 and H-8], 120.2 [C-9], 124.6 [C-6], 125.3 [C-11'], 125.9 [C-7'], 126.5 [C-5'], 127.5 [C-11], 129.7 [C-15'], 130.3 [C-15], 131.6 [C-10'], 132.8 [C-14'], 133.4 [C-14], 135.7 [C-10], 136.1 [C-9'], 136.6 [C-13'], 136.9 [C-13], 137.4 [C-5], 137.8, 137.9 and 138.8 [C-12, C-6' and C-12'], 138.8 [C-8']; CD nm (Δε, based on the same molar extinction coefficient as for all-*E*-(3*R*,3'*R*)-diatoxanthin[7]) 216 (0), 226 (+4.1), 236 (0), 252 (-6.3), 268 (0), 290 (+4.1), 313 (0), 345 (-2.8), 367 (0), > 367 (+).

9-Z-(3R)-7,8-Didehydrocryptoxanthin (2b). The hydroxy phosphonium salt **12** (41 mg, 0.061 mmol) and the acetylenic hydroxy aldehyde **3** (15 mg, 0.065 mmol) dissolved in dry dichloromethane (25 ml) were added dropwise to a suspension of sodium hydride (100 mg, unwashed) in dry dichloromethane (40 ml) at 20 °C in the dark and under an N_2 atm. The reaction was monitored by TLC (System 2). After 44h the reaction

mixture was cooled to 0 °C and ice (s) was added carefully to decompose excess sodium hydride. Water was subsequently added and the product extracted with dichloromethane. The organic phase was washed with water, dried over anhydrous sodium sulphate and the solvents were evaporated. The resulting red oily residue was dissolved in a minimal volume of benzene (*ca.* 1 ml) and subjected to column chromatography with gradient elution, hexane - ethyl acetate 4 : 1 to 7 : 3. The target compound 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) was obtained as a red oil in 53% yield (17.3 mg, 0.032 mmol), as a 7 : 4 mixture of **2b** and two other, unidentified geometrical isomers. Attempted crystallisation of **2b** from diethyl ether - methanol was not successful. Preparative HPLC (flow= 2.0 ml/min) of the CC purified reaction mixture afforded 9-*Z*-(3*R*)-7,8-didehydrocryptoxanthin (**2b**) in 34% yield (11.0 mg, 0,02 mmol), 96% pure (analytical HPLC, flow= 1.0 ml/min) .

R_T(HPLC, flow 0.25 ml/min)= 10.7 min. VIS λ_{max} (acetone) 337, 418, 443, 468 nm, %III/II=8, %D_B/D_{II}=22, λ_{max} (hexane) 337, 418, 438, 466 nm, %III/II=20, %D_B/D_{II}=16, λ_{max} (HPLC eluent) 341, 420, 445, 475 nm, %III/II=37, %D_B/D_{II}=18; IR (KBr) cm^{-1} 3433 s (OH), 2958-2860 s (CH), 2171 w (C≡C), 1383 m, 1031 w, 965 m; MS [IP 70 eV, 210 °C; *m/z* (% rel. int.)]: 550 (100, [M]), 532 (36, [M-18]), 458 (6, [M-92]), 275 (21, [M/2]); ^{1}H NMR ($CDCl_3$) δ 1.026 [*s*, 6H, Me-16´ and Me-17´], 1.190 [*s*, 3H, Me-16], 1.250 [*s*, 3H, Me-17], 1.46 [*m*, 2H, H-2´], 1.48 [*m*, 1H, H-2ax], 1.61 [*m*, 2H, H-3´], 1.715 [*s*, 3H, Me-18´], 1.84 [*m*, 1H, H-2eq], 1.924 [*s*, 3H, Me-18 or Me-20], 1.970 [*s*, 9H, Me-19´, Me-20´ and Me-18 or Me-20], 1.996 [*s*, 3H, Me-19], 2.04 [*m*, 2H, H-4´], 2.09 [*dd*, 1H, *J* 5.6 Hz, *J* 18.7 Hz, H-4ax], 2.44 [*dd*, 1H, *J* 5.4 Hz, *J* 17.1 Hz, H-4eq], 3.99 [*m*, 2H, H-3 and H-3´], 6.10-6.17 [*m*, 3H, H-7´, H-8´ and H-10´], 6.25 [*d*, 2H, $J_{14,15}$ and $J_{14',15'}$ ~11 Hz, H-14 and H-14´], 6.30 [*d*, 1H, $J_{10,11}$ 11.2 Hz, H-10], 6.35 [*d*, 2H, $J_{11,12}$ and $J_{11',12'}$ 15.1 Hz, H-12 and H-12´], 6.60-6.70 [*m*, 3H, H-15, H-11´ and H-15´], 6.82 [*dd*, 1H, $J_{10,11}$ 11.2 Hz, $J_{11,12}$ 14.7 Hz, H-11]; CD nm (Δε, based on the same molar extinction coefficient as for all-*E*-(3*R*)-7,8-didehydrocryptoxanthin[7]) 206 (-4.3), 220 (-0.8), 225 (-0.9), 240 (0), 260 (+0.5), 267 (+0.3), 275 (+0.6), 311 (+0.1), 367 (0), > 311 (+).

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