Synthesis and Characterization of *all-E*-(4,4′-$^{13}C_2$)-Astaxanthin Strategies for Labelling the C_{15}-End Groups of Carotenoids

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The *all-E* isomer of (4,4′-$^{13}C_2$)astaxanthin (**1a**) has been prepared by total synthesis starting from commercially available 99% ^{13}C enriched acetonitrile. The labelled astaxanthin was obtained in high purity and with high isotope incorporation. For this synthesis, the $C_{15} + C_{10} + C_{15}$ strategy was used. The central C_{10}-synthon, 2,7-dimethylocta-2,4,6-triene-1,8-dial (**3**), was coupled with ^{13}C-enriched C_{15}-phosphonium salt **2a**. The new synthetic scheme for the preparation of the C_{15}-phosphonium salt is discussed in this paper; the same scheme can be used to label all positions and combinations of positions of the C_{15}-phosphonium salt.

Introduction

α-Crustacyanin is the carotenoprotein complex of the lobster, which is responsible for the deep blue colour of the lobster's carapace (λ_{max} = 632 nm).[1] The 320 kDa protein complex is an octamer of dimers with each of the dimers containing two noncovalently bound astaxanthin molecules as chromophores.[1,2]

Upon boiling the lobster, the protein complex denatures and the astaxanthin chromophore is released, giving the lobster the red colour of free astaxanthin (λ_{max} = 469 nm). The binding of the astaxanthin in α-crustacyanin results in a large bathochromic shift[1] in the absorption spectrum of 5500 cm^{-1}. For explaining this large shift, detailed information on the structure of the chromophore in the active site and information on the noncovalent interactions between the chromophore and the protein is necessary. The method of choice to obtain structural information at the atomic level of the chromophore in the active site in chromoproteins is to prepare specific isotopically enriched chromophores, introduce them in the active site of the protein[3] and study these isotopically (^{13}C, ^{2}H) labelled systems with non-invasive physical techniques such as solid state ^{13}C NMR and laser resonance Raman spectroscopy. Astaxanthins specifically labelled with ^{13}C in the central C_{10}-part have previously been synthesised by us.[4] These astaxanthins have been reconstituted into α-crustacyanin and have been studied with solid state ^{13}C NMR,[5–7] resonance Raman and Fourier Transform Raman spectroscopy.[8]

It is known that chemical modification of the end groups of astaxanthin prevents reconstitution with the apoprotein to form the coloured protein complex: both oxo-groups at the positions 4 and 4′ need to be present.[1] It is thought that the oxo-groups undergo an interaction with the protein complex: the working hypothesis is that in the protein both oxo-groups are protonated.[5,6] Although a charge at C-4 is not directly reflected in the chemical shift, modern NMR techniques allow for the determination of the charge density at this location.[5,6] We have therefore set out to label these positions with ^{13}C isotopes in order to be able to study these important positions in the molecule.

In this paper we wish to report the synthesis and characterisation of [4,4′-$^{13}C_2$]astaxanthin. The presented scheme allows for the labelling of all positions and combinations in the six-membered ring and, with minor modifications, of all carbons of the C_{15}-end groups. Moreover, this synthetic scheme can be used to label a great number of other carotenoids. The results from the resonance Raman and solid state NMR spectra of the α-crustacyanin reconstituted with the (4,4′) labelled astaxanthin, will be the subject of separate articles.[5,6,9]

Synthesis

For the preparation of the enriched astaxanthin, we use the convergent $C_{15} + C_{10} + C_{15}$ scheme to construct the C_{40}-carotenoid. The central C_{10}-unit of this Scheme is 2,7-dimethylocta-2,4,6-triene-1,8-dial. By the means of a double Wittig reaction with two identical C_{15}-phosphonium salts the C_{10}-synthon is converted in one step into the labelled carotenoid (Scheme 2).

We have developed a scheme for specifically labelling the end groups of astaxanthin (**1**). For this, the C_{15}-phosphonium salt **2** is prepared in an isotopically enriched form (see Scheme 1). The procedure is based on the schemes that have been used to prepare vitamin A and vitamin A derivatives isotopically labelled in the six-membered rings. The C_{10}-compound 3,7-dimethyl-2,6-octadienenitrile (**7**) is prepared by a $C_5 + C_2 + C_1 + C_2$ scheme, starting from 3,3-dimethylallyl bromide (**4**), acetonitrile and methyllithium, by the method of Courtin et al.[10,11] The open-chain compound **7** is subsequently cyclized to the desired six-membered ring, 2,6,6-trimethyl-2-cyclohexenecarbonitrile **(8)**, by the method described by Oh et al.,[12] with some modifica-

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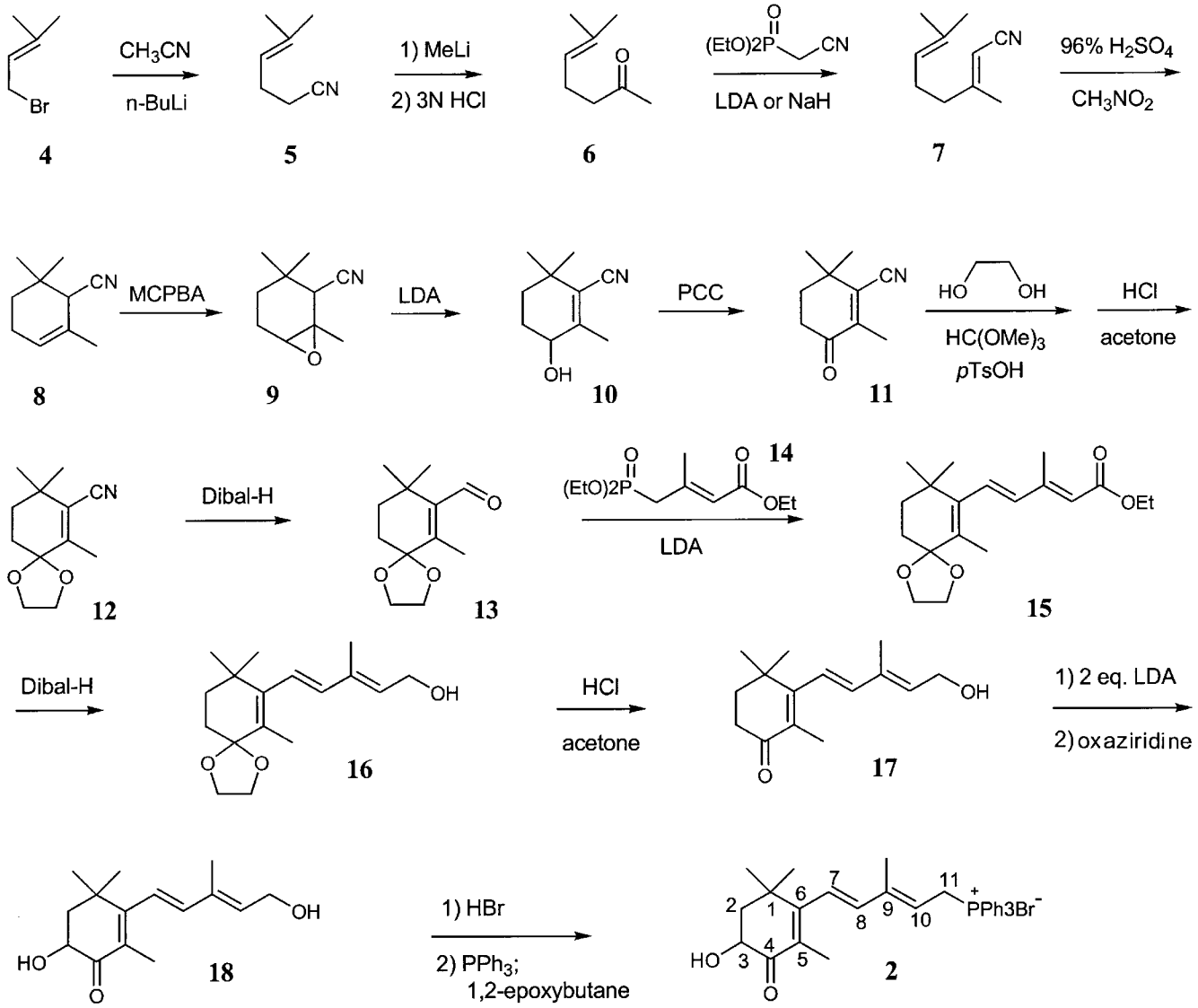


Scheme 1. Synthesis of the C_{15}-phosphonium salt **2**; when (2-^{13}C)acetonitrile is used as starting material, (4-^{13}C)phosphonium salt **2a** is obtained

tions in the workup procedure; this cyclization reaction has also been used by Groesbeek et al.[13] to prepare vitamin A isotopically labelled in the end groups. After these steps, the scheme diverges from the scheme for the synthesis of vitamin A, with the introduction of the carbonyl and hydroxyl groups and the chain elongation step to give the C_{15}-compound.

In the first step (see Scheme 1), acetonitrile is coupled to the commercially available 3,3-dimethylallyl bromide (1-bromo-3-methylbut-2-ene; **4**) giving 5-methyl-4-hexenenitrile (**5**) in 71% yield. The nitrile **5** is converted into 6-methyl-5-hepten-2-one (**6**) by treatment with methyllithium to give ketone **6** in 70% yield. A chain-elongation reaction with the anion of diethyl cyanomethylphosphonate gives the C_{10}-nitrile **7** in 89% yield. The open-chain nitrile **7** is then cyclised to the cyclic C_{10}-nitrile **8**. The ring closure mainly gives the nitrile with the double bond in the α-position. In the workup procedure, it proved to be important to keep the conditions acidic to avoid a double bond shift to the β-position. After purification, the cyclonitrile is obtained as a mixture of α-cyclonitrile (90–95%) and β-cyclonitrile (5–10%) in an overall yield of 86%. The α/β mixture cannot be separated by column chromatography and so the mixture is used in the following steps. The double bond of nitrile **8** is epoxidised with *m*-chloroperbenzoic acid (MCPBA) to give the epoxide **9**. The epoxide **9** is easily opened with LDA: the relatively acidic proton adjacent to the nitrile is abstracted, followed by the opening of the epoxide, giving a double bond in the β-position and a deprotonated hydroxyl group at the 4-position. The alcohol **10** is obtained by protonation during workup and is oxidised with PCC in dichloromethane, without purification, to give 2,6,6-trimethyl-3-oxo-1-cyclohexenecarbonitrile (**11**) in 61% yield based on the α-cyclonitrile **8** (3 steps). At this stage the side-product of the

cyclization reaction, the β-cyclonitrile, which has remained unchanged during the MCPBA oxidation, epoxide opening and PCC oxidation, is separated from the product **11**.

The ketone **11** is protected as the acetal of ethylene glycol **12**. The mixed ortho formate ester of ethylene glycol and methanol formed in this reaction is removed by stirring the product mixture for five minutes in acidified acetone. Under these conditions the acetal **12** is stable, while the orthoester is quickly hydrolyzed. C_{10}-Acetalnitrile **12** is obtained in 84% yield. The nitrile **12** is reduced with Dibal-H, giving the aldehyde **13** in 72% yield. The C_{10}-aldehyde **13** is subsequently elongated by reacting it in an HWE reaction with the anion of the C_5-phosphonate **14** (phosphonate **14** is prepared by coupling the anion of commercially available triethyl phosphonoacetate with chloroacetone, which is also commercially available; the resulting C_5-chloroester is converted into **14** by refluxing it with triethyl phosphite in an Arbuzov reaction, yielding the C_5-phosphonate).[14] The C_{15}-ester **15** is obtained in 80% yield. The ester **15** is reduced to the alcohol by Dibal-H giving the C_{15}-alcohol **16** in 52% yield: this reaction is carried out at low temperature since the acetal group is highly sensitive to reduction to the corresponding ether.[15,16] The 4-acetal group is easily removed by stirring for a few minutes in acidified acetone (yield: 97%). The 3-hydroxyl group is introduced by oxidation of the enolate of **17**. Two equivalents of LDA are used to deprotonate the primary alcohol and to form the enolate of **17**. The enolate is reacted with the commercially available (+)-(dichlorocamphorylsulforyl)oxaziridine; no chiral reduction is observed due to the highly basic conditions and the relatively high temperature needed to convert anion **17**.[17] The racemic α-hydroxy ketone **18** is obtained in 69% yield.

The final conversion into the phosphonium salt is accomplished in two steps: first, the primary alcohol of **18** is selectively converted into the allylic bromide by HBr in dichloromethane. After workup with brine and extraction with ethyl acetate, the excess of HBr is removed with 1,2-epoxybutane. The reaction of the bromide with triphenylphosphane in ethyl acetate is carried out in the presence of 1,2-epoxybutane to prevent the degradation of the unchanged bromide.[18] After an overnight reaction, the C_{15}-phosphonium salt **2** is formed as a white solid in 62% yield based on **18**.

The reaction scheme was first optimised using unlabelled material. The scheme was applied in the synthesis of the (4-^{13}C)C_{15}-phosphonium salt **2a**: starting from (2-^{13}C)acetonitrile (2 g) and 3,3-dimethylallyl bromide (**4**), (4-^{13}C)C_{15}-phosphonium salt **2a** (0.32 g) was obtained. The overall yield of phosphonium salt relative to acetonitrile **2a** is 1.2% (16 steps).

The 4-^{13}C labelled phosphonium salt **2a** was used in the synthesis of (4,4′-$^{13}C_2$)astaxanthin (**1a**) (see Scheme 2). Two equivalents are coupled with the C_{10}-dialdehyde **3** in a double Wittig reaction.[18] The (4,4′-$^{13}C_2$)astaxanthin (**1a**) was purified by a combination of column chromatography and repeated crystallisation from dichloromethane/methanol and dichloromethane/*n*-hexane. In this way, a sample of pure *all-E* (4,4′-$^{13}C_2$)astaxanthin (**1a**) (46 mg) was obtained (yield: 28%, based on the phosphonium salt **2a**).2

Characterization

Chromatography and UV/Vis Spectroscopy

The purity of the astaxanthins was checked by thin layer chromatography. The synthetic astaxanthins showed one spot (detection with visible and UV light) with the same R_f value as astaxanthin from natural sources. Both unlabelled **1** and the labelled astaxanthin **1a** have a single, solvent-dependent absorption maximum in the UV/Vis spectra: λ_{max} (*n*-hexane) = 469 nm and λ_{max} (chloroform) = 490 nm. These values are in excellent agreement with literature values.[19] They indicate that the synthetic astaxanthin is in the *all-E* form: the presence of *Z*-isomers would lower the absorption maximum.[19]

Mass Spectrometry

The electron impact mass spectrum of naturally occurring astaxanthin has its molecular ion peak at 596 mass units. The molecular ion peak of the $^{13}C_2$ enriched astaxanthin (**1a**) is shifted two mass units to 598 mass units because of the presence of the two isotopes. The double-focus mass spectra were recorded for the $^{13}C_2$ enriched astaxanthin (**1a**). The mass found by peak matching of the molecular ion peak is in excellent agreement with the calculated value of 598.3927 for $^{13}C_2{}^{12}C_{38}H_{52}O_4$; the value found for [4,4′-$^{13}C_2$]astaxanthin (**1a**) is 598.3942.

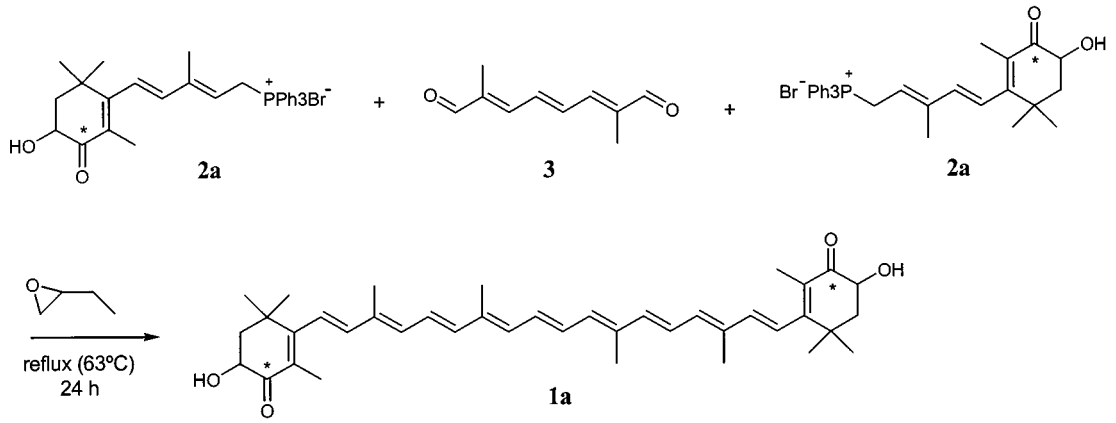
The determination of the isotope enrichment of the labelled astaxanthin (**1a**) was only possible with low accuracy (isotope enrichment >95%) from the [$M^+ - 2$] peak (15%) of the fragment from which hydrogen is eliminated. The high isotopic enrichment of >95% was confirmed by NMR spectroscopy (see below).

^{1}H NMR Spectroscopy

The ^{1}H NMR spectrum (at 300 MHz) was used to determine the purity of the astaxanthins and the positions of the label in the $^{13}C_2$ astaxanthin (**1a**). The ^{1}H NMR spectrum of unlabelled astaxanthin (**1**) confirms the high purity and the *all-E* structure. Within experimental error, no *Z*-isomers could be detected. The spectral values determined are identical with the published data of Englert et al.[19] The signal of H-14/H-14′ overlaps with the doublet H-10/H-10′ and the signal of H-15/H-15′ overlaps with the double doublet of H-11/H-11′: due to this overlap the coupling constants with these protons could not be determined from spectral simulation of the AA′BB′ spectra. The synthetic astaxanthins consist of a mixture of 25% (3*R*,3′*R*), 50% (3*R*,3′*S*) and 25% (3*S*,3*S*′); as expected, no differences between the different stereoisomers were observed.

The olefinic part of the spectrum is identical to unlabelled astaxanthin (**1**) since no coupling between the 4- and 4′-carbonyl carbons with the olefinic protons are observed.

Additional couplings between the ^{13}C labels and the protons in the ring confirm the position and high incorpora-



Scheme 2. Synthesis of (4,4′-$^{13}C_2$)astaxanthin (**1a**) from (4-^{13}C)phosphonium salt (**2a**); labelled positions are marked with an asterisk

tion of the labels. A three-bond coupling (^3J = 2.9 Hz) of the ^{13}C label at the 4- and 4′-position splits the triplet of the axial H-2 and H-2′. The triplet of the equatorial H-2 and H-2′ is split by a three-bond coupling (^3J = 8.2 Hz); the coupling of the equatorial proton, which lies in a plane with C-4 and C-3, is larger than the coupling with the axial proton, which is rotated out of the plane with a smaller dihedral angle between C-3, C-4 and H-2. The signal of the 5- and 5′-methyl groups (18 and 18′) is split by a three-bond ^{13}C-^{1}H coupling of 3.4 Hz. A three-bond ^{13}C-^{1}H coupling of 3.8 Hz is observed between the proton of the 3- and 3′-hydroxyl group resulting in a double doublet. The proton at the positions 3 and 3′ shows four couplings: three ^{1}H-^{1}H couplings (with H-2$_{eq}$, H-2$_{ax}$ and 3-OH) and one ^{13}C-^{1}H coupling of 2.1 Hz. Table 1 summarises these J_{CH} couplings.

Table 1. J_{CH} coupling constants from the ^{13}C labelled astaxanthin (**1a**) as determined by ^{1}H NMR spectroscopy

	Coupled protons	J (Hz)
(4,4′-$^{13}C_2$)astaxanthin (**1a**)	H-3/H-3′	^2J = 2.1
	H-2$_{eq}$/H-2′$_{eq}$	^3J = 8.2
	H-2$_{ax}$/H-2′$_{ax}$	^3J = 2.9
	3-OH/ 3′-OH	^3J = 3.8
	H-18/H-18′	^3J = 3.4

^{13}C NMR Spectroscopy

The ^{13}C{^{1}H} NMR spectra (at 50 MHz) of unlabelled astaxanthin (**1**) and the $^{13}C_2$ enriched astaxanthin (**1a**) have been recorded. In the spectrum of naturally occurring astaxanthin 20 peaks are observed. The chemical shift values found are in exact agreement with the values found by Englert et al. for *all-E* astaxanthin.[19] ^{13}C NMR spectroscopy is the method of choice to determine the position of the label and to check that no scrambling of the label has taken place. Furthermore, the isomeric purity of the sample can be easily determined, since the signals of the *Z*-isomers resonate at higher field. No differences in the spectra of the diastereoisomers are observed. In the spectrum of the labelled astaxanthin (**1a**) a single peak with high intensity is observed at δ = 200.4.

Discussion

In this article the synthesis of racemic astaxanthin has been described. In contrast to both the oxo groups, the hydroxyl groups at the positions 3 and 3′, which induce chirality in the molecule, are not essential for binding and colour shift, nor does the stereoconfiguration: all three isomers (*RR*, *SS* and *RS*) bind in the protein complex.[1] Because of the great distance between the two stereocentres, no differences in the spectra of these stereoisomers are observed.[20–22] For these reasons, in first instance a racemic mixture of astaxanthin has been prepared. If chirality will prove to be important from the spectroscopy experiments, the isomers can be separated according to a literature method.[10]

The synthetic scheme, described here has been used to prepare *all-E* (4,4′-$^{13}C_2$)astaxanthin (**1a**) starting from commercially available 99% ^{13}C enriched (2-^{13}C)acetonitrile. The same synthetic scheme, with some modifications, can also be used to label the C_{15}-phosphonium salt (**2**) at any position or combination of positions. The positions 4, 5, 6 and 7 can be ^{13}C enriched using labelled acetonitrile. The starting material 3,3-dimethylallyl bromide (**4**) can be prepared in labelled form starting from acetone and ethyl bromoacetate.[23] When labelled **4** is used in this scheme (Scheme 1) the positions 1, 2, 3, 16, 17 and any combination of positions in the ring can be labelled with ^{13}C. In Scheme 1, the 5-methyl carbon is introduced using methyllithium. Methyllithium is not available in labelled form; to introduce a ^{13}C label at the 5-Me position, methylmagnesium iodide, prepared in situ from commercially available 99% (^{13}C)methyl iodide and magnesium, can be used. For labelling the positions 8, 9, 10, 11 and 19 (9-CH_3), the reactions that have been developed for the preparation of isotopically enriched vitamin A and vitamin A derivatives can be used.[23]

The same scheme can be used to prepare β-carotene and canthaxanthin labelled in the C_{15}-end groups. For this, the corresponding C_{15}-phosphonium salts are prepared in their labelled form. The synthesis of the phosphonium salt of canthaxanthin, the C_{15}-hydroxy ketone **17** is not oxidized with oxaziridine but directly converted into the desired 4-

oxo-C_{15}-phosphonium salt. For the synthesis of the C_{15}-phosphonium salt for the synthesis of β-carotene labelled in the C_{15}-end groups, all three oxidation steps (epoxidation, PCC and oxaziridine oxidation) are omitted from the synthetic scheme. In this way, all the positions and all combinations of positions of the C_{15}-phosphonium salt can be labelled. In combination with the synthetic schemes for labelling the central C_{10}-dialdehyde **3**,[4,23] astaxanthin (**1**), β-carotene and canthaxanthin can now be labelled at any position or combination of positions.

Experimental Section

All experiments were carried out in a dry nitrogen atmosphere. Reaction vessels were flame-dried under nitrogen prior to use. The astaxanthins were handled in dim red light. The following solvents were distilled prior to use: THF was freshly distilled from $LiAlH_4$; *n*-pentane was distilled from P_2O_5 and stored over sodium wire. Petroleum ether refers to low-boiling petroleum ether 40–60 °C. – For column chromatography, Merck silica gel 60, (230–400 Mesh) was used. – TLC analyses were performed on Schleicher Schuell TLC-plates F1500/LS 254. Evaporation of solvents was carried out in vacuo (1.4×10^3 Pa). – ^{1}H NMR spectra were recorded on a Jeol FX-200, a Bruker WM-300 spectrometer or a Bruker AM-600 using tetramethylsilane (TMS; 0 ppm) as internal standard. – ^{1}H-noise decoupled ^{13}C NMR spectra were recorded on a Jeol FX-200 at 50.1 MHz. NMR data are reported for purified *all-E* isomers. – UV/Vis spectra were recorded with a Varian DMS 200 spectrophotometer. – Mass spectra were recorded on a V.G. Micromass ZAB-2HF mass spectrometer. The experimental conditions and spectral assignments are given for the unlabelled compounds. For labelled compounds, only the changes relative to the corresponding unlabelled compounds are given. [2-^{13}C]Acetonitrile was purchased from Cambridge Isotope Laboratories U.S.A. All other reagents were purchased from Aldrich or Acros.

5-Methyl-4-hexenenitrile (5): To a solution of acetonitrile (3.0 g, 73.2 mmol) in 75 mL THF at –70 °C was added *n*BuLi (46 mL, 1.6 N, 75.2 mmol) dropwise with a syringe. After 15 min 3,3-dimethylallyl bromide (1-bromo-3-methyl-but-2-ene; **4**) (12.6 g, 80 mmol, 95%) in 50 mL THF was added dropwise at the same temperature. After addition, the reaction mixture was allowed to warm to room temperature. Workup was accomplished by adding saturated NH_4Cl and extraction with diethyl ether. The combined organic layers were washed with saturated NaCl and dried with $MgSO_4$. After concentration in vacuo, the crude product was purified by column chromatography (silica gel; 20% diethyl ether/petroleum ether; TLC-detection $KMnO_4$) yielding **5** (4.31 g, 39.2 mmol, 82%) as a colourless oil. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.66 (s, 3 H, 5*E*-CH_3), 1.73 (s, 3 H, 5*Z*-CH_3), 2.34 (m, 4 H, H-2/H-3), 5.13 (m, 1 H, H-4).[10,11]

(2-^{13}C)5 (5a): Similarly (2-^{13}C)acetonitrile (2 g, 48.8 mmol) yielded **5a** (4.31 g, 39.2 mmol, 82%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.66 (s, 3 H, 5*E*-CH_3), 1.73 (s, 3 H, 5*Z*-CH_3), 2.34 (quintet, J = 7 Hz, 2 H, H-3), 2.34 (dt, $^1J_{CH}$ = 136.3 Hz, J_{HH} = 7.0 Hz, 2 H, H-2), 5.13 (m, 1 H, H-4). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 17.7 (C-2).

6-Methyl-5-hepten-2-one (6): At 0 °C, a solution of **5** (5.66 g, 52.0 mmol) in 80 mL diethyl ether was added dropwise to 80 mL MeLi (1.6 M solution in diethyl ether, 130 mmol). The reaction mixture was stirred for 1 h at 0 °C. TLC analysis (50% CH_2Cl_2/petroleum ether; detection $KMnO_4$) showed complete conversion of the starting material. At 0 °C, a cooled solution of conc. HCl (60 mL) in 140 mL water was carefully added and the mixture was stirred at 0 °C for 2 h. Solid $NaHCO_3$ was carefully added until the mixture was neutral. The mixture was extracted with diethyl ether. The combined organic layers were washed with saturated NaCl and dried with $MgSO_4$. After concentration in vacuo, flash chromatography (silica gel, 25% diethyl ether/petroleum ether) yielded **6** (4.58 g, 36.4 mmol, 70%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.62 (s, 3 H, 6*Z*-CH_3), 1.68 (s, 3 H, 6*E*-CH_3), 2.14 (s, 3 H, H-1), 2.25 (q, J = 7 Hz, 2 H, H-4), 2.45 (t, J = 7 Hz, 2 H, H-3), 5.06 (t, J = 7 Hz, 1 H, H-5).[10,11]

(3-^{13}C)6 (6a): Similarly **5a** (4.31 g, 39.2 mmol) yielded **6a** (3.18 g, 25.1 mmol, 82%). – ^{1}H NMR (200 MHz, $CDCl_3$), as for **6**; in addition: δ = 2.45 (dt, $^1J_{CH}$ = 126 Hz, J_{HH} = 7 Hz, 2 H, H-3). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 43.7 (C-3).

3,7-Dimethyl-2,6-octadienenitrile (7): The mineral oil of NaH (1.9 g, 60% in mineral oil, 47 mmol) was removed by washing three times with petroleum ether: in the reaction flask, 20 mL dry petroleum ether was added and the suspension was stirred for a few seconds. After the NaH had settled, the petroleum ether was carefully removed with a pipette. After removing the remaining petroleum ether under a stream of nitrogen, 150 mL THF was added. The suspension was cooled to 0 °C and diethyl cyanomethylphosphonate (9.0 g, 51 mmol) in 35 mL THF was added dropwise. The reaction mixture was stirred at room temperature until all the NaH had disappeared. At 0 °C **6** (4.58 g, 36.4 mmol) in 20 mL THF was slowly added. The mixture was stirred for an additional 2 h at room temperature. TLC analysis (25% diethyl ether/petroleum ether; detection $KMnO_4$) showed complete conversion of the starting material. Workup was accomplished by adding saturated NH_4Cl and extracting the mixture twice with diethyl ether. The combined organic layers were washed with saturated NaCl and dried with $MgSO_4$. Flash chromatography (silica gel, 25% diethyl ether/petroleum ether) yielded **7** (4.84 g, 32.5 mmol, 89.4%) as a mixture of 2-*E*/2-*Z* isomers. – ^{1}H NMR (200 MHz, $CDCl_3$) of *E*-isomer: δ = 1.60 (s, 3 H, 7*Z*-CH_3), 1.69 (s, 3 H, 7*E*-CH_3), 2.05 (s, 3 H, 3-CH_3), 2.19 (br s, 4 H, H-4/H-5), 5.03 (m, 1 H, H-6), 5.10 (s, 1 H, H-2).[10,11]

(4-^{13}C)7 (7a): Similarly, **6a** (3.18 g, 25.1 mmol) yielded **7a** (1.90 g, 12.7 mmol, 51%). – ^{1}H NMR (200 MHz, $CDCl_3$) of *E*-isomer, as for **7**; in addition: δ = 2.05 (d, $^3J_{CH}$ = 3.8 Hz, 3 H, 3-CH_3), 2.18 (q, J_{HH} = 7 Hz, 2 H, H-5), 2.20 (dt, $^1J_{CH}$ = 129 Hz, J_{HH} = 7 Hz, 2 H, H-4), 5.10 (d, $^3J_{CH}$ = 6.2 Hz, 1 H, H-2). – ^{13}C NMR (50.1 MHz, $CDCl_3$); δ = 36.3 (C-4; *Z*-isomer), 38.6 (C-4; *E*-isomer).

2,6,6-Trimethyl-2-cyclohexenecarbonitrile (8): A solution of **7** (4.84 g, 32.5 mmol) in 20 mL nitromethane was slowly added to a solution of 5 mL concentrated sulfuric acid in 50 mL nitromethane at 0 °C. After stirring for 30 min at 0 °C, TLC analysis (10% diethyl ether/petroleum ether; detection $KMnO_4$) showed complete conversion of the starting material. Workup was accomplished by adding 100 mL water and extracting the reaction mixture three times with diethyl ether. The combined organic layers were washed twice with saturated NaCl and once with a saturated solution of sodium acetate in water. After drying the organic layers with $MgSO_4$, the solution was concentrated in vacuo. Flash chromatography (silica gel, 10% diethyl ether/petroleum ether) yielded 4.17 g (28.0 mmol) of a mixture of α-cyclonitrile **8** and β-cyclonitrile (86%). – ^{1}H NMR (200 MHz, $CDCl_3$) of **8** (α-cyclonitrile): δ = 1.04 (s, 3 H, 1-CH_3),

1.14 (s, 3 H, 1-CH_3), 1.5–1.6 (m, 2 H, H-2), 1.83 (s, 3 H, 5-CH_3), 2.05 (m, 2 H, H-3), 2.77 (br s, 1 H, H-6), 5.59 (br s, 1 H, H-4).[13]

(3-^{13}C)8 (8a): Similarly, **7a** (1.90 g, 12.7 mmol) yielded **8a** (1.73 g, 11.5 mol, 91%). – ^{1}H NMR (200 MHz, $CDCl_3$) of **8a** (α-cyclonitrile), as for **8**; in addition: δ = 1.83 (d, $^3J_{CH}$ = 5.5 Hz, 3 H, 5-CH_3), 5.59 (d br s, $^1J_{CH}$ = 155.5 Hz, 1 H, H-4). – ^{13}C NMR (50.1 MHz, $CDCl_3$); δ = 124.5 (C-4).

2,3-Epoxy-2,6,6-trimethylcyclohexanecarbonitrile (9): A solution of **8** (4.17 g, 28.0 mmol) in 40 mL ethyl acetate, was added to a solution of *m*-chloroperbenzoic acid (12.4 g, 50%, 36 mmol) in 40 mL ethyl acetate at 0 °C. The reaction mixture was stirred overnight at room temperature. The solution was concentrated in vacuo and 200 mL petroleum ether was added. The reaction mixture was stirred at 0 °C and triethylamine (2.7 g, 45 mmol) was slowly added. After stirring for 10 min, the reaction mixture was filtered over silica gel. The silica gel was washed with 25% diethyl ether/3% triethylamine/petroleum ether and the filtrate was concentrated in vacuo. In this way, 4.39 g crude product (a mixture of **9** and β-cyclonitrile) was obtained (95%). – **Compound 9:** ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.05 (s, 3 H, 1-CH_3), 1.09 (s, 3 H, 1-CH_3), 1.2–1.3 (m, 2 H, H-2), 1.55 (s, 3 H, 5-CH_3), 2.0 (m, 2 H, H-3), 2.75 (s, 1 H, H-6), 3.05 (s, 1 H, H-4).

(3-^{13}C)9 (9a): Similarly, **8a** (1.73 g, 11.5 mol) yielded 1.53 g product (mixture of **9a** and β-cyclonitrile) (80%). – ^{1}H NMR (200 MHz, $CDCl_3$) of **9a**, as for **9**; in addition: δ = 1.55 (d, $^3J_{CH}$ = 4.1 Hz, 3 H, 5-CH_3), 3.05 (d, $^1J_{CH}$ = 174 Hz, 1 H, H-4). – ^{13}C NMR (50.1 MHz, $CDCl_3$); δ = 58.6 (C-4).

3-Hydroxy-2,6,6-trimethyl-1-cyclohexenecarbonitrile (10): At –40 °C, a solution of **9** (4.39 g, 26.6 mmol) in 10 mL THF was added dropwise to a solution of 30 mmol LDA in 50 mL THF [prepared in situ from *n*BuLi (18.8 mL, 1.6 M solution, 30 mmol) and diisopropylamine (3.2 g, 32 mmol)]. The reaction mixture was allowed to warm to room temperature. Workup was accomplished by adding saturated NH_4Cl and extraction of the mixture with diethyl ether. The combined organic layers were washed with saturated NaCl and dried with $MgSO_4$. After concentration in vacuo, 4.24 g crude product was obtained (96.5%). – ^{1}H NMR (200 MHz, $CDCl_3$) of **10**: δ = 1.15 (s, 3 H, 1-CH_3), 1.20 (s, 3 H, 1-CH_3), 1.3–2.0 (m, 4 H, H-2/H-3), 2.10 (s, 3 H, 5-CH_3), 4.06 (t, *J* 6 Hz, 1 H, H-4).

(3-^{13}C)10 (10a): Similarly, **9a** (1.53 g, 0.92 mmol) yielded 1.92 g crude product. – ^{1}H NMR (200 MHz, $CDCl_3$) of **10a**, as for **10**; in addition: δ = 2.10 (d, $^3J_{CH}$ = 3.4 Hz, 3 H, 5-CH_3), 4.06 (dt, $^1J_{CH}$ = 172 Hz, J_{HH} = 6 Hz, 1 H, H-4). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 68.1 (C-4).

2,6,6-Trimethyl-3-oxo-1-cyclohexenecarbonitrile (11): A solution of **10** (4.24 g, 25.7 mmol) in 20 mL dry CH_2Cl_2 was added dropwise to a suspension of pyridinium chlorochromate (PCC) (7.2 g, 33.4 mmol) in 80 mL dry CH_2Cl_2 at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred until TLC analysis (80% diethyl ether/petroleum ether; detection $KMnO_4$/UV) showed complete conversion of the starting material (after approximately 1 h). Workup was accomplished by cooling the mixture to 0 °C and adding ice-cooled diethyl ether. After stirring for 15 min, the organic layers were decanted. This procedure was repeated until the tar-like reduced PCC was a finely dispersed black solid. The combined organic layers were filtered twice over silica; the silica was rinsed with diethyl ether. Column chromatography (silica gel, gradient 20–40% diethyl ether/petroleum ether) yielded **11** (2.77 g, 17.0 mmol, 66%) and β-cyclonitrile (0.46 g, 3.0 mmol, 12%). – ^{1}H NMR (200 MHz, $CDCl_3$) of **11**: δ = 1.34 (s, 6 H, 1-CH_3), 1.95 (t, *J* 6.8 Hz, 2 H, H-2), 2.06 (s, 3 H, 5-CH_3), 2.56 (t, *J* 6.8 Hz, 2 H, H-3).

(3-^{13}C)11 (11a): Similarly, crude **10a** (1.92 g) yielded **11a** (0.91 g, 5.5 mmol, 60%) and β-cyclonitrile (0.18 g, 1.2 mmol, 13%). – ^{1}H NMR (200 MHz, $CDCl_3$) of **11a**, as for **11**; in addition: δ = 1.95 (q, J_{CH} = 6.8 Hz, J_{HH} = 6.8 Hz, 2 H, H-2), 2.06 (d, J_{CH} = 3.4 Hz, 3 H, 5-CH_3), 2.56 (dt, J_{CH} = 5.8 Hz, J_{HH} = 6.8 Hz, 2 H, H-3). – ^{13}C NMR (50.1 MHz, $CDCl_3$); δ = 196.4 (C-4).

2,10,10-Trimethyl-4,7-dioxaspiro[4,5]dec-1-enecarbonitrile (12): To a mixture of **11** (2.77 g, 17.0 mmol) were added trimethyl orthoformate (5.0 g, 34 mmol), ethylene glycol (3.2 g, 51 mmol) and a catalytic amount of *p*-toluenesulfonic acid. After fifteen minutes, TLC analysis (25% diethyl ether/petroleum ether) showed complete conversion of the starting material. The reaction mixture was poured into a saturated $NaHCO_3$ solution. The mixture was extracted three times with diethyl ether. The combined organic layers were washed with a saturated NaCl solution, dried with $MgSO_4$ and concentrated in vacuo. The crude product was dissolved in 75 mL acetone and one drop 1 N HCl was added. After 5 min at room temperature, solid K_2CO_3 and $MgSO_4$ were added. The solids were filtered off and washed with diethyl ether. After removal of the solvents in vacuo and purification by column chromatography (2% triethylamine/25% diethyl ether/petroleum ether) **12** (3.13 g, 15.1 mmol, 89%) was obtained. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.19 (s, 6 H, 1-CH_3), 1.62–1.83 (m, 4 H, H-2/H-3), 1.97 (s, 3 H, 5-CH_3), 4.03 (s, 4 H, OCH_2CH_2O).

(3-^{13}C)12 (12a): Similarly, **11a** (0.91 g, 5.5 mmol) yielded **12a** (1.06 g, 5.1 mmol, 92%). – ^{1}H NMR (200 MHz, $CDCl_3$), as for **12**, in addition: δ = 1.97 (d, J_{CH} = 3.4 Hz, 3 H, 5-CH_3), 4.03 (d, J_{CH} = 3.4 Hz, 4 H, OCH_2CH_2O). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 105.5 (C-4).

2,10,10-Trimethyl-4,7-dioxaspiro[4,5]dec-1-enecarbaldehyde (13): A solution of **12** (3.13 g, 15.1 mmol) in 60 mL dry petroleum ether was cooled to –60 °C and 19.6 mL 1 N (19.6 mmol) Dibal-H was added with a syringe. The reaction mixture was allowed to warm to room temperature during one hour. After half an hour at room temperature, the reaction mixture was cooled to –20 °C and a homogeneous mixture of 26 g silica gel and 10 mL H_2O was added. The mixture was stirred for an additional hour at 0 °C. Solid K_2CO_3 and $MgSO_4$ were then added. Subsequently, the solids were filtered off and thoroughly washed with diethyl ether. After concentration in vacuo, the product was purified by flash chromatography (50 % diethyl ether in petroleum ether) to yield **13** (2.67 g, 10.8 mmol, 72%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.21 (s, 6 H, 1-CH_3), 1.54 –1.64 (m, 2 H, H-2), 1.75–1.82 (m, 4 H, H-3), 2.01 (s, 3 H, 5-CH_3), 4.07 (s, 4 H, OCH_2CH_2O), 10.16 (s, 1 H, CHO).

(3-13C)13 (13a): Similarly, **12a** (1.06 g, 5.1 mmol) yielded **13a** (0.55 g, 2.61 mmol, 51%). – ^{1}H NMR (200 MHz, $CDCl_3$), identical as for **13**, in addition: δ = 2.01 (d, J_{CH} = 3.8 Hz, 3 H, 5-CH_3), 4.07 (d, J_{CH} = 3.5 Hz, 4 H, OCH_2CH_2O). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 107.2 (C-4).

Ethyl (2*E*,4*E*)-3-Methyl-5-(2,10,10-trimethyl-4,7-dioxaspiro[4,5]dec-1-en-1-yl)-2,4-pentadienoate (15): A 50-mL three-necked round-bottomed flask equipped with a dropping funnel, septum inlet and a low-temperature thermometer, was charged with diisopropylamine (1.5 g, 15 mmol) and 75 mL THF. The mixture was cooled to –20 °C in an alcohol/liquid nitrogen bath, and *n*BuLi (8.8 mL, 1.6 N, 14 mmol) was added. At –20 °C, a solution of triethyl 3-methyl-4-phosphonocrotonate (**14**) (3.7 g, 14 mmol) in 10 mL THF was added dropwise. At 0 °C, **13** (2.67 g, 10.8 mmol) in 10 mL THF

was slowly added. The reaction mixture was allowed to warm to room temperature. After one hour, TLC analysis (25% diethyl ether/petroleum ether) showed complete conversion of the starting material. The reaction mixture was poured into a saturated NaCl solution. The mixture was extracted three times with diethyl ether and the combined organic layers were washed with saturated NaCl and dried with $MgSO_4$. Concentration in vacuo yielded a yellow oil. Column chromatography (silica gel, 10% diethyl ether/petroleum ether/2% triethylamine) gave **15** (2.75 g, 8.59 mmol, 79%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.03 (s, 6 H, 1-CH_3), 1.29 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 1.57–1.83 (m, 4 H, H-2/H-3), 1.66 (s, 3 H, 5-CH_3), 2.33 (s, 3 H, 9-CH_3), 4.02 (s, 4 H, OCH_2CH_2O), 4.23 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 5.76 (s, 1 H, H-10), 6.12 (d, J = 6.1 Hz, 1 H, H-8), 6.47 (d, J = 6.1 Hz, 1 H, H-7).

(3′-^{13}C) 15 (15a): Similarly, **13a** (0.55 g, 2.61 mmol) yielded **15a** (0.73 g, 2.3 mmol, 88%). – ^{1}H NMR (200 MHz, $CDCl_3$), as for **15**, in addition: δ = 1.66 (d, J_{CH} = 3.1 Hz, 3 H, 5-CH_3), 4.02 (d, J_{CH} = 3.1 Hz, 4 H, OCH_2CH_2O). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 107.6 (C-4).

(2*E*,4*E*)-3-Methyl-5-(2,10,10-trimethyl-4,7-dioxaspiro-[4,5]dec-1-en-1-yl)-2,4-pentadienol (16): A solution of **15** (2.75 g, 8.59 mmol) in 150 mL dry petroleum ether was cooled to –80 °C in an alcohol/liquid nitrogen bath and Dibal-H (1.9 mL, 1 M, 1.9 mmol) was slowly added with a syringe. The mixture was allowed to warm to –20 °C. TLC analysis (50% diethyl ether/petroleum ether) showed complete conversion of the starting material. A homogeneous mixture of 2.6 g silica gel and 1 mL H_2O was slowly added and the mixture was stirred for one hour at 0 °C. Solid K_2CO_3 and $MgSO_4$ were added and the solids were filtered off and thoroughly washed with diethyl ether. After concentration in vacuo, the product was purified by column chromatography (silica gel, gradient 50–98% diethyl ether/petroleum ether/2% triethylamine), giving 1.23 g (4.4 mmol; 52%) **16**. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.04 (s, 6 H, 1-CH_3), 1.55–1.90 (m, 4 H, H-2/H-3),1.67 (s, 3 H, 5-CH_3), 1.84 (s, 3 H, 9-CH_3), 4.02 (s, 4 H, OCH_2CH_2O), 4.29 (d, J = 6.5 Hz, 2 H, H-11), 5.64 (t, J = 6.5 Hz, 1 H, H-10), 6.08 (s, 2 H, H-7/H-8).

(3′-^{13}C)16 (16a): Similarly, **15a** (0.73 g, 2.3 mmol) yielded **16a** (0.37 g, 1.3 mmol, 88%). – ^{1}H NMR (200 MHz, $CDCl_3$), as for **16**, in addition: δ = 1.67 (d, J_{CH} = 3.4 Hz, 3 H, 5-CH_3), 4.02 (d, J_{CH} = 3.7 Hz, 4 H, OCH_2CH_2O). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 108.1 (C-4).

(2*E*,4*E*)-3-Methyl-5-(2,6,6-trimethyl-3-oxo-1-cyclohexen-1-yl)-2,4-pentadienol (17): To a solution of **16** (1.23 g, 4.4 mmol) in 125 mL acetone was added one drop of 1 N HCl. TLC analysis (50% diethyl ether/petroleum ether) showed complete conversion of the starting material after a few minutes. Workup was accomplished by adding solid K_2CO_3 and $MgSO_4$. The solids were filtered off and washed with diethyl ether. Concentration in vacuo gave the crude product, which was purified by column chromatography (silica gel, 100% diethyl ether) to give **17** (1.00 g, 4.3 mmol, yield 97%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.17 (s, 6 H, 1-CH_3), 1.83 (s, 3 H, 5-CH_3), 1.86 (t, J = 6.9 Hz, 2 H, H-2), 1.88 (s, 3 H, 9-CH_3), 2.51 (t, J = 6.9 Hz, 2 H, H-3), 4.35 (d, J = 6.9 Hz, 2 H, H-11), 5.76 (t, J = 6.9 Hz, 1 H, H-10), 6.21 (s, 2 H, H-7/H-8).

(3′-^{13}C)17 (17a): Similarly, **16a** (0.37 g, 1.33 mmol) yielded **17a** (0.31 g, 1.31 mmol, 98%). – ^{1}H NMR (200 MHz, $CDCl_3$), as for **17**, in addition: δ = 1.86 (m, J_{CH} not determined due to overlap), 1.83 (d, J_{CH} = 3.4 Hz, 3 H, 5-CH_3), 2.51 (q, J_{CH} = 6.9 Hz, J_{HH} = 6.9 Hz, 2 H, H-3). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 199.4 (C-4).

(2*E*,4*E*)-3-Methyl-5-(4-hydroxy-2,6,6-trimethyl-3-oxo-1-cyclohexen-1-yl)-2,4-pentadienol (18): In a 50-mL three-necked round-bottomed flask equipped with a dropping funnel, septum inlet and a low-temperature thermometer, a solution of LDA (12.9 mmol) was prepared at –20 °C from diisopropylamine (1.4 g, 14 mmol) dissolved in 150 mL freshly distilled THF and *n*BuLi (8.0 mL, 1.6 M, 12.9 mmol). After ten minutes at –20 °C, the solution was cooled to –80 °C and a solution of **17** (1.00 g, 4.29 mmol) in 25 mL THF was added dropwise. After 30 minutes, (+)-(8,8-dichlorocamphorylsulfonyl)oxaziridine (3.8 g, 12.9 mmol) in 25 mL THF was added. The reaction mixture was allowed to warm to room temperature in approximately one hour. As soon as TLC analysis (100% diethyl ether) showed complete conversion (after approximately fifteen minutes at room temperature), the reaction mixture was worked up. Workup was accomplished by adding saturated NH_4Cl and sodium thiosulfate. The mixture was extracted three times with diethyl ether and the combined diethyl ether layers were washed with saturated NaCl and dried with $MgSO_4$. Concentration in vacuo gave a mixture of (+)-(8,8-dichlorocamphorylsulfonyl)imine and **18**. Column chromatography (gradient: 50–100% diethyl ether/petroleum ether), gave **18** (0.74 g, 2.96 mmol, 69%). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.18 (s, 3 H, 1-CH_3), 1.30 (s, 3 H, 1-CH_3), 1.81 (t, J = 13.4 Hz, 1 H, H-2_{ax}), 1.87 (s, 3 H, 9-CH_3), 1.90 (s, 3 H, 5-CH_3), 2.16 (dd, J = 12.7, 5.5 Hz, H-2_{eq}), 3.72 (s, 1 H, OH), 4.32 (q, J = 13.8, 5.5 Hz, H-3), 4.36 (d, J = 6.5 Hz, 2 H, H-11), 5.78 (t, J = 6.5 Hz, 1 H, H-10), 6.16 (d, J = 16.1 Hz, 1 H, H-7), 6.29 (d, J = 16.1 Hz, 1 H, H-8).

(3′-^{13}C)18 (18a): Similarly, **17a** (0.31 g, 1.31 mmol) yielded **18a** (0.22 g, 0.88 mmol) (68%). – ^{1}H NMR (200 MHz, $CDCl_3$), as for **18**, in addition: δ = 1.81 (dt, $^3J_{CH}$ = 2.7 Hz, J_{HH} = 13.4 Hz, 1 H, H-2_{ax}), 1.90 (d, $^3J_{CH}$ = 3.8 Hz, 3 H, 5-CH_3), 2.16 (ddd, $^3J_{CH}$ = 8.0 Hz, J_{HH} = 12.7, 5.5 Hz, H-2_{eq}), 4.32 (m, coupling constants not determined due to overlap with signal of H-11, H-3). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 200.4 (C-4).

[(2*E*,4*E*)-3-Methyl-5-(4-hydroxy-2,6,6-trimethyl-3-oxo-1-cyclohexen-1-yl)-2,4-pentadien-1-yl]phosphonium Bromide (2): A solution of **18** (0.74 g, 2.96 mmol) in 50 mL dichloromethane was cooled with an ice-bath to 0 °C and HBr (0.7 mL, 47%, 5.9 mmol) was added in small portions. The reaction mixture was stirred until TLC analysis (100% diethyl ether) showed complete conversion of the starting material. If the reaction was not complete after half an hour, an additional equivalent of HBr was added. The reaction mixture was poured into a saturated NaCl solution and extracted three times with ethyl acetate. The combined organic layers were dried with $MgSO_4$ and 1,2-epoxybutane was added until the solution was neutral. The solution was concentrated in vacuo to a volume of 20 mL and ethyl acetate (30 mL) and 1,2-epoxybutane (0.5 mL) were added. Subsequently, the solution was again concentrated in vacuo to a volume of 20 mL. This solution, containing the product (2*E*,4*E*)-1-bromo-3-methyl-5-(4-hydroxy-2,6,6-trimethyl-3-oxo-1-cyclohexen-1-yl)-2,4-pentadiene, was slowly added to a solution of triphenylphosphane (0.78 g, 2.98 mmol) in 20 mL ethyl acetate/0.2 mL 1,2-epoxybutane and the mixture was stirred at room temperature for 24 h. The clear solution formed a white suspension. TLC analysis (100% diethyl ether) showed complete disappearance of the starting material. The solids were filtered off and washed with diethyl ether. After drying in vacuo at 50 °C, **2** (1.06 g, 1.83 mmol, 62%) was obtained. – ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.11 and 1.25 (2s, 6 H, 1-CH_3), 1.49 (d, J_{PH} = 3.5 Hz, 3 H, 9-CH_3), 1.77 (t, J = 13.2 Hz, 1 H, H-2_{ax}), 1.81 (s, 3 H, 5-CH_3), 2.14

(dd, J = 13.2, 5.6 Hz, 1 H, H-2$_{eq}$), 3.62 (d, J = 1.9 Hz, 1 H, 3-OH), 4.29 (ddd, J = 13.2, 5.6, 1.9 Hz, 1 H, H-3), 5.53 (q, J_{HH} = 7.7 Hz, J_{PH} = 7.7 Hz, 1 H, H-10), 4.99 (dt, J_{HH} = 15.7, 8.3 Hz, J_{PH} = 15.7 Hz, 1 H, H-11), 5.11 (dt, J_{HH} = 15.7, 7.7 Hz, J_{PH} = 15.7 Hz, 1 H, H-11), 6.06 (d, J = 16.5 Hz, 1 H, H-7), 6.14 (d, J = 16.5 Hz, 1 H, H-8), 7.64–7.92 (m, 15 H, Phenyl).

(3′-^{13}C)2 (2a): Similarly, **18a** (0.22 g, 0.88 mmol) yielded **2a** (0.32 g, 0.56 mmol, 63%). – ^{1}H NMR (200 MHz, $CDCl_3$), as for **2**, in addition: δ = 1.77 (dt, J_{CH} = 3.0 Hz, J_{HH} = 13.2 Hz, 1 H, H-2$_{ax}$), 1.81 (d, J_{CH} = 3.3 Hz, 3 H, 5-CH$_3$), 2.14 (ddd, J_{CH} = 8.2 Hz, J_{HH} = 13.2, 5.6 Hz, 1 H, H-2$_{eq}$), 3.62 (dd, J_{CH} = 3.7 Hz, J_{HH} = 1.9 Hz, 1 H, 3-OH), 4.29 (dddd, J_{CH} = 1.9 Hz, J_{HH} = 13.2, 5.6, 1.9 Hz, 1 H, H-3). – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 200.3 (C-4).

(*all-E*)-3,3′-Dihydroxy--carotene-4,4′-dione (Astaxanthin, 1): With exclusion of light, dialdehyde **3** (200 mg, 1.22 mmol) and C_{15} Wittig salt **2** (1.66 g, 2.89 mmol) in 1,2-epoxybutane (15 mL) were heated under reflux (63 °C) for 20 h under a nitrogen atmosphere. TLC analysis (50% diethyl ether/petroleum ether) showed complete conversion of the dialdehyde **3**. All subsequent handling of the astaxanthins was performed in dim red light and with exclusion of oxygen. The 1,2-epoxybutane was removed by distillation and abs. ethanol added. The crystallised astaxanthin was filtered off and washed with cold ethanol. The astaxanthin was washed off the filter with dichloromethane. The dichloromethane was removed by distillation and *n*-heptane added. The suspension of astaxanthin crystals was then heated under reflux (98 °C) for 20 h under a nitrogen atmosphere. After cooling to 0 °C, the astaxanthin was filtered off and washed with *n*-pentane. Repeated recrystallization from CH_2Cl_2/MeOH and CH_2Cl_2/*n*-hexane afforded 398 mg (0.67 mmol) pure *all-E* astaxanthin (55%). – M.p. 216–218 °C (ref.[3] 217–219 °C). – UV/Vis: λ = 469 nm (*n*-hexane), 490 nm ($CHCl_3$). – MS (EI; 70 eV); *m/z* (%): 488 (1.9), 489 (0.9), 490 (10.7), 491 (2.2), 492 (1.0), 502 (2.0), 503 (0.9), 504 (10.6), 505 (2.2), 506 (1.0), 594 (28.2), 595 (12.2), 596 (100; $M^{+\cdot}$), 597 (43.0), 598 (16.1). – ^{1}H NMR (600 MHz, $CDCl_3$; $\delta_H = \delta_{H'}$): δ = 1.21 + 1.32 (s, 12 H, H-16/H-17), 1.82 (t, J = 13.3 Hz, 2 H, H-2$_{ax}$), 1.95 (s, 6 H, H-18), 1.99 (s, 6 H, H-20), 2.00 (s, 6 H, H-19), 2.16 (dd, J = 5.7, 12.6 Hz, 2 H, H-2$_{eq}$), 3.70 (d, J = 1.8 Hz, 2 H, 3-OH), 4.33 (ddd, J = 1.8, 5.7, 13.9 Hz, 2 H, H-3), 6.21 (d, J = 16.0 Hz, 2 H, H-7), 6.30 (d, J = 11.4 Hz, 2 H, H-10), 6.30–6.32 (m, 2 H, H-14), 6.43 (d, J = 16.0 Hz, 2 H, H-8), 6.48 (d, J = 14.8 Hz, 2 H, H-12), 6.66 (dd, J = 11.4, 14.8 Hz, 2 H, H-11), 6.64–6.68 (m, 2 H, H-15). – ^{13}C NMR (50.1 MHz, $CDCl_3$; $\delta_C = \delta_{C'}$): δ = 12.6 (C-19), 12.8 (C-20), 14.0 (C-18), 26.2 and 30.7 (C-16/C-17), 36.8 (C-1), 45.4 (C-2), 69.2 (C-3), 123.3 (C-7), 124.6 (C-11), 126.8 (C-5), 130.6 (C-15), 133.8 (C-14), 134.6 (C-9), 135.2 (C-10), 136.8 (C-13), 139.7 (C-12), 142.3 (C-8), 162.2 (C-6), 200.4 (C-4).[19]

(4,4′-$^{13}C_2$)Astaxanthin (1a): Similarly, **3** (33 mg, 0.2 mmol) and **2a** (0.32 g, 0.56 mmol) yielded 46 mg (0.08 mmol) of *all-E* **1a** (38% based on **3**; 28% based on **2a**). – UV/Vis: λ = 469 nm (*n*-hexane), 490 nm ($CHCl_3$). – Exact mass: 598.3942 (calculated for $^{13}C_2{}^{12}C_{38}H_{52}O_4$: 598.3927). – MS (EI; 70 eV); *m/z* (%): 490 (1.7), 491 (1.1), 492 (4.1), 493 (1.7), 494 (1.8), 504 (1.6), 505 (1.0), 506 (5.0), 507 (1.8), 508 (1.9), 596 (15.7), 597 (8.9), 598 (100; $M^{+\cdot}$), 599 (44.8), 601 (25.6). – ^{1}H NMR: see characterisation. – ^{13}C NMR (50.1 MHz, $CDCl_3$): δ = 200.4 (C-4/C-4′).

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