Synthesis of 8-, 9-, 12-, and 13-mono-13C-retinal

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This paper is dedicated to Professor Camille Sandorfy on the occasion of his 65th birthday

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The 8-, 9-, 12-, and 13-mono-^{13}C-retinals were synthesized with >98% chemical purity and 93% ^{13}C incorporation from ^{13}C-labelled acetonitrile. Their ^{13}C−^{13}C and ^{13}C−^{1}H nmr coupling constants were determined.

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Les 8-, 9-, 12- et 13-mono-^{13}C rétinènes ont été synthétisés avec plus de 98% de pureté chimique et 93% d'incorporation de ^{13}C à partir d'acetonitrile marqué au ^{13}C. Leurs constants ^{13}C−^{13}C et ^{13}C−^{1}H de couplage de rmn ont été déterminées.
[Traduit par le journal]

Introduction

The retinylidene group occurs as the chromophoric part of the membrane proteins bacteriorhodopsin and rhodopsin, and is formed by reaction of all-*trans* or 11-*cis* retinal with a lysine ϵ-amino group of the peptide chain under formation of a protonated Schiff base bond as shown in Fig. 1 (1, 2). We have synthesized a number of ^{13}C-labelled retinals (3, 4) and prepared ^{13}C-labelled bacteriorhodopsins from these materials. Recently we obtained solid state cross polarization magic angle spinning ^{13}C nmr (CP−MAS ^{13}C nmr) spectra of bacteriorhodopsins with the ^{13}C-enriched chromophore (5, 6). These spectra gave the first *in situ* ^{13}C nmr information of the chromophore within a protein, establishing that in dark-adapted bacteriorhodopsin the chromophore occurs in a 40% all-*trans* protonated Schiff base and 60% 13-*cis*, 15-*cis* protonated Schiff base structure which are not interconverting on the ^{13}C nmr time scale. These results were strongly supported by resonance Raman (RR) (7, 8) and Fourier transform infrared difference (FTIR diff.) (9) vibrational studies of the ^{13}C-labelled bacteriorhodopsins and further detailed information concerning the chromophore structure was obtained. In order to extend these studies we needed bacteriorhodopsins with a ^{13}C-label at positions 9 and 13. These are quaternary positions with a substantial positive charge in a protonated Schiff base structure (10). ^{13}C nuclear magnetic resonance spectroscopy may give insight into how these positions of the chromophore interact with the protein part. Position 8 can give us information on how the 7,8 double bond interacts with the peptide, since this interaction is essential for the colour of light- and dark-adapted bacteriorhodopsin (11). In the rhodopsin bleaching sequence, isomerization takes place around the 11,12 double bond, and to investigate this isomerization process by CP−MAS ^{13}C nmr, RR and FTIR diff. studies we needed retinal with a ^{13}C-label at position 12.

In this paper we describe the synthetic schemes by which we prepared retinal ^{13}C-labelled at positions 8 and 9 and at positions 12 and 13 with a level of ^{13}C-enrichment of more than 90%.

Synthesis and spectroscopic characterization

For the preparation of 8-^{13}C and 9-^{13}C retinal, we used the reaction sequence as presented in Scheme 1. The starting aldehyde β-cyclocitral **3** necessary for this synthesis is obtained by cyclization of the *N*-phenylimine of citral **2** with 95% sulfuric acid at −20°C by the method of Gedye *et al.* (12). Addition at −60°C of ^{13}C lithioacetonitrile, obtained by the reaction of 1 equiv. of n-butyllithium with ^{13}C acetonitrile, to β-cyclocitral **3** gives the alcohol-nitrile **4***a*,*b* in 95% yield. Starting with 2-^{13}C-acetonitrile introduces the label at position 8 (**4***a*) and with 1-^{13}C-acetonitrile at position 9 (**4***b*). For the dehydration we used a two-step method under basic conditions. Quantitative conversion of the alcohol **4***a*,*b* into its acetate with acetic anhydride (Ac_2O) and 4-dimethylaminopyridine (DMAP) is followed by deacetylation using diazabicyclononene (DBN) in toluene. Performing the deacetylation at room temperature − which is completed in 3 days − the nitrile **5***a*,*b* is obtained in 80% yield as a 7-*E*/*Z* (3:1) mixture (determination by ^{1}H nmr spectroscopy), which is converted to ^{13}C-labelled β-ionone **6***a*,*b* in three steps. The nitrile is reduced with diisobutylaluminium hydride (Dibal) to the aldehyde, after which conversion the complete double bond isomerization has taken place, leading to the 7-*E* aldehyde only. Reaction with excess methyl magnesium iodide (CH_3MgI) and oxidation of the α,β-unsaturated alcohol with active manganese dioxide (MnO_2) yields the ^{13}C-labelled β-ionone **6***a*,*b* in 60% overall yield from **5***a*,*b*. The β-ionone ^{13}C-labelled at position 8 or 9 is transformed to the C15-aldehyde **7***a*,*b* with high 9-*E* content, in 80% yield, in two steps by reaction with the anion of diisopropylphosphonoacetonitrile (13) followed by Dibal reduction of the nitrile. The C15-aldehyde **7***a*,*b* is converted into retinal **1***a*,*b* by Horner−Emmons coupling with the C5-synthon **8** (14), leading to retinonitrile, followed by Dibal reduction, providing 8-^{13}C-retinal **1***a* and 9-^{13}C-retinal **1***b* in 80% yield from the C15-aldehyde **7***a*,*b*.

For the synthesis of 12-^{13}C (**1***c*) and 13-^{13}C-retinal (**1***d*) an analogous reaction scheme is used, with the C15-aldehyde **7** as the starting aldehyde (Scheme 2). Addition of 9-*E* C15-

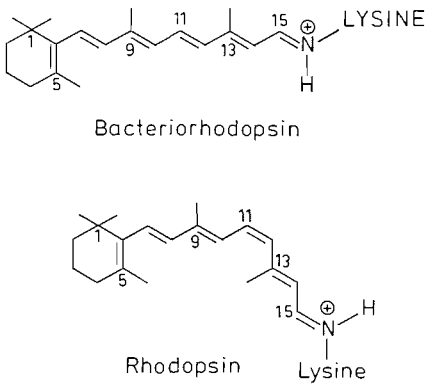
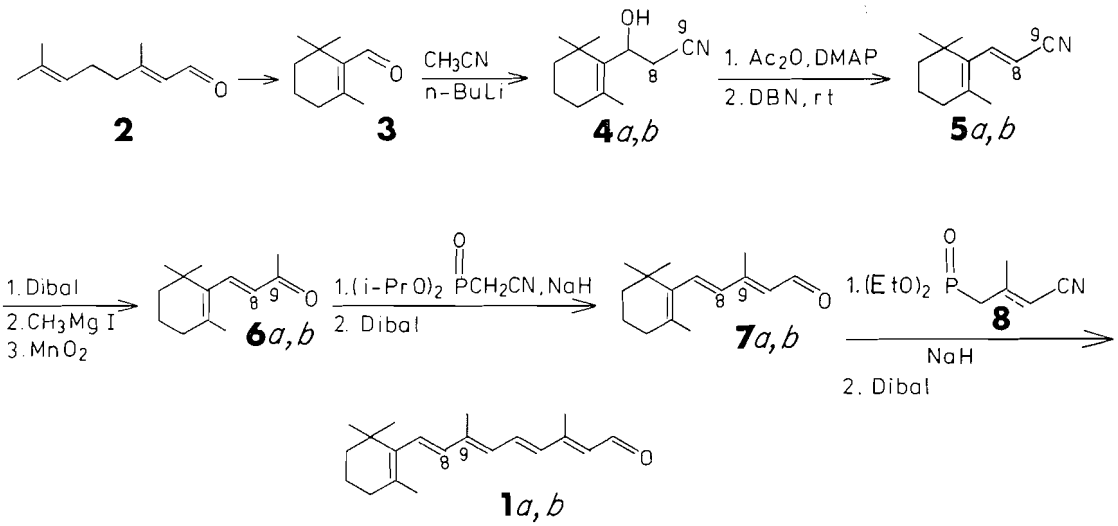
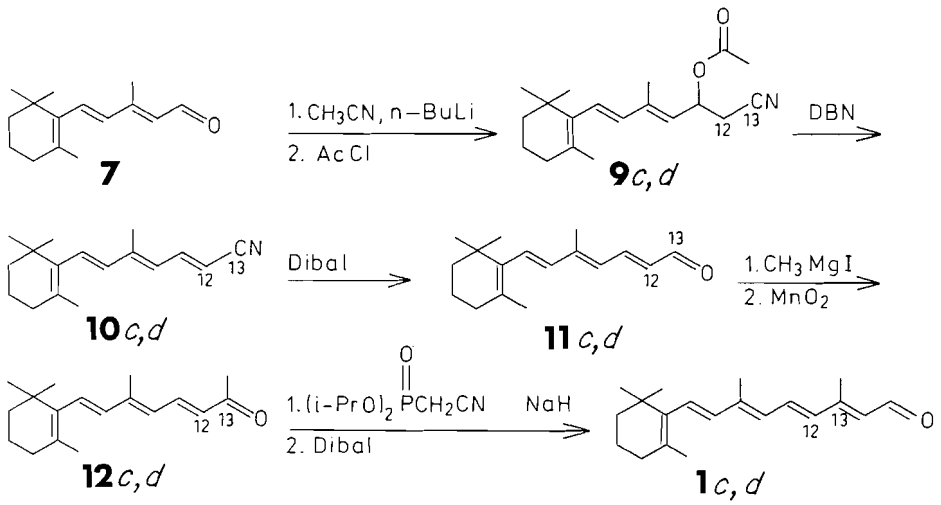


FIG. 1. Chromophoric groups of bacteriorhodopsin and rhodopsin.



SCHEME. 1. Synthesis of (8-^{13}C) **1***a* and (9-^{13}C) retinal **1***b*; *a*, $^{13}CH_3CN$; *b*, $CH_3{}^{13}CN$.



SCHEME 2. Synthesis of (12-^{13}C) **1***c* and (13-^{13}C) retinal **1***d*; *c*, $^{13}CH_3CN$; *d*, $CH_3{}^{13}CN$.

aldehyde **7** to lithioacetonitrile is followed by quenching the reaction mixture with acetyl chloride, leading to the secondary acetate **9***c*,*d* in a one-pot reaction. The addition of the C15-aldehyde **7** to lithioacetonitrile has to be performed at −90°C to prevent base-catalyzed side reactions of the aldehyde (15). The quenching is then performed at −60°C to give **9***c*,*d* in 85% yield after column chromatography. Base-induced acetic acid elimination with DBN in refluxing toluene gives the tetraenenitrile **10***c*,*d* in 88% yield. Dibal reduction leads to the C17-aldehyde **11***c*,*d* (95%) and reaction with excess methyl magnesium iodide (CH_3MgI) and subsequent MnO_2 oxidation gives the desired C18-ketone **12***c*,*d* in 60% yield. Horner–Emmons coupling of **12***c*,*d* with diisopropylphosphonoacetonitrile followed by Dibal reduction of the resulting retinonitrile affords the ^{13}C-labelled retinal in 90% yield, with 85% 13-*E* configuration. Starting with 2-^{13}C-acetonitrile leads to 12-^{13}C-retinal **1***c* and with 1-^{13}C-acetonitrile leads to 13-^{13}C-retinal **1***d* in 38% overall yield from the ^{13}C-labelled acetonitriles. From 0.25 g labelled acetonitrile, 0.64 g ^{13}C-labelled retinal is obtained in eight steps.

The all-*trans* isomers of the labelled retinals **1***a*–*d* can be separated by column chromatography. Irradiation of the all-*trans* isomer with visible light in acetonitrile gives an isomeric mixture which is separated by preparative hplc to yield pure 9-*cis*, 11-*cis*, 13-*cis*, and all-*trans* isomers. The uv–vis spectra of these retinals are identical with those of the unmodified retinal isomers. The mass spectra of the ^{13}C-labelled retinals **1***a*–*d* show the expected 92–93% ^{13}C-incorporation, based on the incorporation of the starting materials.

From the multiplet splittings in the 300-MHz ^{1}H nmr spectra of **1***a*–*d* the location of the ^{13}C is immediately evident. For 8-^{13}C (**1***a*) and 12-^{13}C-retinal (**1***c*) the signals of the H-atoms directly bonded to the labelled position show large splittings with the ^1J(C–H) values of 155.7 Hz for position 8 and 154.6 Hz for position 12. The intensity ratio is 93% ^{13}C-enriched to 7% non-enriched retinal, as has been found by mass spectrometery. For 9-^{13}C (**1***b*) and 13-^{13}C-retinal (**1***d*), containing a quaternary ^{13}C-atom, no one-bond C—H couplings occur. For all four ^{13}C-retinals two- and three-bond C—H couplings are determined from the spectra and these values are presented in Table 1.

The ^{1}H noise-decoupled ^{13}C nmr spectra of the ^{13}C-labelled retinals clearly show the position of ^{13}C-enrichment. They display one strong single line due to the labelled carbon atom at the chemical shift value known from the natural abundance ^{13}C nmr spectrum of unmodified retinal (16). The enriched

TABLE 1. ^{13}C-^{1}H nuclear magnetic resonance coupling constants in all-*trans* retinal as obtained from **1***a*–*d*. Signs not determined

$^{1}J(^{13}C-^{1}H)$		$^{2}J(^{13}C-^{1}H)$		$^{3}J(^{13}C-^{1}H)$	
C(8)–H(8)	155.7	C(8)–H(7)	N.d.*	C(8)–H(10)	8.5
C(12)–H(12)	154.6	C(9)–H(8)	3.2	C(8)–H(19)	3.8
		C(9)–H(10)	0	C(9)–H(7)	6
		C(9)–H(19)	6.2	C(9)–H(11)	3.3
		C(12)–H(11)	0	C(12)–H(10)	5.0
		C(13)–H(12)	2.7	C(12)–H(14)	8.0
		C(13)–H(14)	0	C(12)–H(20)	4.0
		C(13)–H(20)	6.1	C(13)–H(11)	5.7
				C(13)–H(15)	0

*Not determined due to complex signal because of long-range proton–proton couplings.

TABLE 2. $^{13}C-^{13}C$ nuclear magnetic resonance coupling constants in all-*trans* retinal as obtained from **1***a*–*d*. Signs not determined.

$^{1}J(^{13}C-^{13}C)$		$^{2}J(^{13}C-^{13}C)$		$^{3}J(^{13}C-^{13}C)$	
C(7)–C(8)	71.1	C(6)–C(8)	Obsc.*	C(1)–C(8)	0
C(8)–C(9)	56.0	C(7)–C(9)	0	C(5)–C(8)	0
C(9)–C(10)	70.4	C(8)–C(10)	2.4	C(6)–C(9)	5.4
C(9)–C(19)	43.3	C(8)–C(19)	1.9	C(8)–C(11)	7.1
C(11)–C(12)	69.8	C(9)–C(11)	0	C(9)–C(12)	9.4
C(12)–C(13)	54.2	C(10)–C(12)	0	C(10)–C(13)	7.5
C(13)–C(14)	66.7	C(11)–C(13)	0	C(12)–C(15)	7.2
C(13)–C(20)	40.4	C(12)–C(14)	0		
		C(12)–C(20)	1.9		
		C(13)–C(15)	3.0		

*Obscured by the enriched signal.

materials also contain the natural abundance (1.1%) of ^{13}C at each of the non-enriched positions. The singly labelled materials therefore contain two ^{13}C isotopes at the 1.0% level (0.93 × 1.1%), in which one position is enriched and the other is at the natural abundance level. From the ^{1}H noise-decoupled ^{13}C nmr spectra the $^{13}C-^{13}C$ coupling constants are obtained, as shown in Table 2.

Discussion

The four ^{13}C-labelled retinals with the required purity were prepared in high yield by the methods shown in Schemes 1 and 2. In both reaction schemes, by choosing the appropriately ^{13}C-labelled acetonitrile, we can label two different polyene carbon positions. This, together with the fact that in our syntheses we get a high percentage of the all-*trans* isomer, is an advantage over the published synthesis of 9-^{13}C-retinal (17). A further advantage of our methods is that in the first step a simple synthon is added to a higher molecular weight compound in high yield. As after coupling the product has in general only to undergo a number of high yield conversions, the ^{13}C-labelled material is put to an efficient use.

For the introduction of a sp^2-^{13}C label at all retinal chain positions 8 to 15, we used ^{13}C-labelled acetonitrile, whose anion, obtained with n-butyllithium, is coupled in an aldol-type reaction with ketones (3, 4) as well as aldehydes in good yield. To obtain good yields low temperatures have to be used for this addition reaction, especially in the case of the C15-aldehyde **7** (−90°C, Scheme 2). After introduction of the labelled synthon by the aldol-type reaction, dehydration is the first step to be performed. To prevent dehydration taking place in the direction of the trimethylcyclohexyl ring, with formation of retro compounds (18), we needed to use the basic reaction conditions described.

From these labelled retinals, nmr parameters such as $J(^{13}C-^{1}H)$ and $J(^{13}C-^{13}C)$ are easily obtained from the high-field nmr spectra. The $J(^{13}C-^{1}H)$ values found are in agreement with values known for sp^2 bonded hydrogens in a hydrocarbon chain (19) and those we found before for the other retinal chain positions (3, 4). The $^{1}J(^{13}C-^{13}C)$ values in particular show a good relation to the double or single bond character of the bond between two carbon atoms. The values found for the various types of bonds in this large polyene chain are around 70 Hz for carbons bonded via a double bond, around 55 Hz for a single bond in the chain, and 40 Hz for the sp^2-sp^3 single bonds to the 9- and 13-methyl group (Table 2). These values are in good agreement with values known for small systems like butadiene and 1-methyl-cyclohexene (20) and are a measure of the hybridization and bond lengths of the C—C bond in question.

From the ^{13}C-labelled retinals, specifically ^{13}C-labelled visual pigments and bacteriorhodopsin can be obtained. The isotopic substitution does not introduce ambiguities due to changes in the electronic structure of the chromophore or protein–chromophore interaction, nor are changes in quantum yields and photostationary state compositions to be expected. These highly ^{13}C-labelled retinals serve as the basic materials both for the solid state CP–MAS ^{13}C nmr studies of the chromophore of the retinylidene pigments as well as for the unambiguous assignments of the resonance Raman and FTIR difference vibrational spectra. Some of the spectroscopic results obtained from the isotopically labelled rhodopsins and bacteriorhodopsins have been published (8, 21, 22).

Experimental

All experiments were carried out in a dry nitrogen atmosphere, and the purified polyenes were handled in dim red light. Distilled dry solvents were used; pet. ether refers to low boiling petroleum ether 40–60°C. Unless otherwise stated, purification was performed by flash chromatography (23) (Merck silica gel 60, 230–400 mesh) using ether/pet. ether mixtures. The tlc analyses were performed on Schleicher & Schüll F 1500/LS 254 silica gel plates using ether/pet. ether mixtures. Evaporation of the solvents was carried out *in vacuo* (10 Torr; 1 Torr = 133.3 Pa).

The ^{1}H nmr spectra were recorded on a Brucker WM-300 or on a JEOL PS-100 spectrometer using tetramethylsilane (TMS; δ 0 ppm) as internal standard. The ^{13}C nmr spectra were recorded on a Brucker WM-300 spectrometer at 75.5 MHz or on a JEOL PFT system at 25 MHz using tetramethylsilane (TMS; δ 0 ppm) as internal standard. The mass spectra were recorded using an AEI MS 902 instrument. The ir spectra were obtained using a Pye–Unicam SP3-200 and the uv–vis spectra using a Cary 219 spectrophotometer. The hplc separations were performed using a Dupont 830 equipped with a Dupont spectrophotometer (360 nm) and a 25 cm × 22.5 mm Zorbax Sil column. Elution was effected using 10% ether in pentane at a flow rate of 20 mL/min. The glc analyses were performed using a Hewlett–Packard 5700 with a 50-m OV101 capillary column at 160°C. The experimental conditions and spectral assignments are given for the unlabelled compounds. For the labelled compounds only the changes relative to the unlabelled compounds are given. Spectra signal designations were based on the retinoid numbering system (24). (1-^{13}C) Acetonitrile and (2-^{13}C) acetonitrile (90% ^{13}C enrichment) were purchased from Merck Sharp and Dohme Isotopes.

C12-Alcohol-nitrile **4***: 3-hydroxy-3(2,6,6-trimethyl-1-cyclohexenyl)-propanenitrile*

To a stirred solution of 6 mmol of acetonitrile (0.25 g) in 20 mL dry THF, 6 mmol of n-butyllithium (4.3 mL of a 1.4 *M* solution in hexane)

were added dropwise at −60°C and warmed to −20°C. After 15 min, 6 mmol of β-cyclocitral **3** (12) (0.9 g) in 5 mL dry THF were added dropwise at −60°C. The mixture was warmed to 0°C in 1 h, poured into half-saturated NH_4Cl, and extracted with ether. The organic layer was washed with water followed by brine, and then dried ($MgSO_4$). Evaporation of the solvent afforded 1.1 g (95%) of pure C12-alcohol-nitrile **4**; ir (KBr): 3470 cm^{-1} (OH stretch), 2250 cm^{-1} (C≡N stretch); ^1H nmr (100 MHz, $CDCl_3/CCl_4$ 1:1), δ: 0.98−1.09 (2s, $(CH_3)_2C$), 1.79 (s, 5-CH_3), 2.44 (dd, *J* 17 Hz, *J* 4.5 Hz, H8), 2.87 (dd, *J* 17 Hz, *J* 10 Hz, H8), 3.04 (s, OH), 4.60 (dd, *J* 10 Hz, *J* 4.5 Hz, H7).

C12-Acetate-nitrile: 3-acetoxy-3-(2,6,6-trimethyl-1-cyclohexenyl)-propanenitrile

To a solution of 5.7 mmol of the C12-alcohol-nitrile (1.1 g) in 30 mL dry toluene and 10 mL pyridine were added 11.4 mmol of acetic anhydride (2 equiv., 1.2 g) and a catalytic amount of 4-(dimethylamino)pyridine and the mixture stirred at room temperature. After 20 h, when the reaction was complete according to glc, the solids were filtered and washed with ether. The organic filtrates were washed with water, followed by brine, and then dried ($MgSO_4$). Evaporation of the solvents afforded the acetate in quantitative yield; ir (KBr): 2250 cm^{-1} (C≡N stretch), 1745 cm^{-1} (C=O stretch); ^1H nmr (100 MHz, $CDCl_3/CCl_4$ 1:1), δ: 1.03−1.08 (2s, $(CH_3)_2C$), 1.79 (s, 5-CH_3), 2.04 (s, CO—CH_3), 2.61 (dd, *J* 17.3 Hz, *J* 5.2 Hz, H8), 2.94 (dd, *J* 17.3 Hz, *J* 10.0 Hz, H8), 5.75 (dd, *J* 10.0 Hz, *J* 5.2 Hz, H7).

β-Cyclocitrylideneacetonitrile 5: 3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenenitrile

Two equivalents of diazabicyclononene (DBN) (11 mmol, 1.4 g) were added to a solution of 5.5 mmol C12-acetate-nitrile (1.3 g) in toluene and the mixture stirred at room temperature for 72 h, when the reaction according to glc was complete, and then poured into water. The aqueous layer was washed with ether and the organic layers were washed with water until neutral, followed by brine, and then dried ($MgSO_4$) and concentrated. The residue was purified by chromatography using 5% ether/pet. ether to yield 0.8 g (80%) of a 3:1 *E*/*Z* mixture of **5**; ir (KBr): 2210 cm^{-1} (C≡N stretch); ^1H nmr (100 MHz, $CDCl_3/CCl_4$ 1:1), 7-*E*, δ: 1.08 (s, $(CH_3)_2C$), 1.75 (s, 5-CH_3), 5.27 (d, *J* 17.5 Hz, H8), 7.08 (d, *J* 17.5 Hz, H7); 7-*Z*, δ: 5.48 (d, *J* 12.0 Hz, H8), 6.72 (d, *J* 12.0 Hz, H7).

5*a*: ^1H nmr, 7-*E*, δ: 5.27 (dd, ^1J(C8−H8) 172 Hz, *J* 17.5 Hz, H8), 7.08 (dd, *J* 17.5 Hz, ^2J(C8−H7) 1.9 Hz, H7); 7-*Z*, δ: 5.48 (dd, ^1J(C8−H8) 172 Hz, *J* 12.0 Hz, H8), 6.72 (dd, *J* 12.0 Hz, ^2J(C8−H7) 1.6 Hz, H7).

5*b*: ^1H nmr, 7-*E*, δ: 5.27 (dd, *J* 17.5 Hz, ^2J(C9-H8) 3.0 Hz, H8), 7.08 (dd, *J* 17.5 Hz, ^3J(C9−H7) 8.0 Hz); 7-*Z*, δ: 5.48 (dd, *J* 12.0 Hz, ^2J(C9−H8) 3.0 Hz, H8), 6.72 (dd, *J* 12.0 Hz, ^3J(C9−H7) 12.0 Hz, H7).

β-Ionone 6

A 1 *M* solution (1.5 equiv.) of diisobutylaluminium hydride (Dibal) in hexane (6.9 mL) was added dropwise by syringe to a solution of 4.6 mmol **5** (0.8 g) in dry pet. ether at −60°C and the mixture warmed to −20°C in 1 h. A suspension of 1:5 water/silicagel in ether/pet. ether 1:1 was added and the mixture stirred at room temperature for 1 h. After drying with $MgSO_4$, the solids were filtered off and washed with dry ether. Evaporation of the solvents yielded the C12-aldehyde in 95% yield; ^1H nmr (100 MHz, $CDCl_3/CCl_4$ 1:1), δ: 6.14 (dd, *J* 16.0 Hz, *J* 8.0 Hz, H8), 7.27 (d, *J* 16.0 Hz, H7), 9.52 (d, *J* 8.0 Hz, H9). The C12-aldehyde (4.2 mmol, 0.75 g) in dry ether was added dropwise to a solution of 2 equiv. of methyl magnesium iodide in dry ether, freshly prepared from methyl iodide (1.2 g) and magnesium (0.2 g). The solution was refluxed for 1 h, poured into half-saturated NH_4Cl, and extracted with ether. The organic layer was washed with water followed by brine, and then dried ($MgSO_4$). Evaporation of the solvent afforded the β-ionol in 90% yield. The β-ionol dissolved in hexane was added to an 8-fold excess of active manganese dioxide in hexane and stirred at room temperature until no further β-ionol could be detected by tlc. The solids were filtered off through Celite, washed with ether, and the filtrates concentrated to afford an oil which was purified by preparative hplc to give the β-ionone in 70% yield; ^1H nmr (100 MHz, $CDCl_3/CCl_4$ 1:1), δ: 1.09 (s, $(CH_3)_2C$), 1.76 (s, 5-CH_3), 2.26 (s, 9-CH_3), 6.09 (d, *J* 17.0 Hz, H8), 7.28 (d, *J* 17.0 Hz, H7).

6*a*: ^1H nmr, δ: 6.09 (dd, ^1J(C8−H8) 156 Hz, *J* 17.0 Hz, H8).

6*b*: ^1H nmr, δ: 2.26 (d, ^2J(C9−(9-CH_3)) 6 Hz, 9-CH_3), 6.09 (dd, *J* 17.0 Hz, ^2J(C9−H8) 3.3 Hz, H8), 7.28 (dd, *J* 17.0 Hz, ^3J(C9−H7) 6.5 Hz, H7).

β-Ionylidene acetaldehyde 7: (4E)-3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-2,4-pentadienal

Sodium hydride (NaH, 120 mg, 2.7 mmol, 55% in mineral oil) was washed three times with dry pet. ether to remove the mineral oil, and suspended in 10 mL dry THF. A solution of 3.1 mmol of diisopropylphosphonoacetonitrile (0.65 g) in dry THF was added dropwise and stirred for 30 min at room temperature. After cooling to 0°C a solution of 2.1 mmol β-ionone (0.4 g) in dry THF was added dropwise and the mixture stirred and warmed to room temperature. After 2 h the solution was poured into half-saturated NH_4Cl and extracted with ether. The organic layers were washed with water followed by brine, and dried ($MgSO_4$). After filtration through a layer of silica gel the solvents were evaporated to afford β-ionylidene acetonitrile which was reduced with Dibal as described for the reduction of **5** to yield 0.36 g (80%) of β-ionylidene acetonitrile **7** as a 9-*E*/*Z* 85:15 mixture; ^1H nmr (100 MHz, $CDCl_3/CCl_4$ 1:1), 9-*E*, δ: 1.01 (s, $(CH_3)_2C$), 1.70 (s, 5-CH_3), 2.28 (s, 9-CH_3), 5.87 (d, *J* 8.0 Hz, H10), 6.15 (d, *J* 16.0 Hz, H8), 6.68 (d, *J* 6.0 Hz, H7), 10.19 (d, *J* 8.0 Hz, H11); 9-*Z*, δ: 1.03 (s, $(CH_3)_2$), 1.75 (s, 5-CH_3), 2.09 (s, 9-CH_3), 5.80 (d, *J* 8.0 Hz, H10), 6.56 (d, *J* 16.0 Hz, H7), 7.11 (d, *J* 16.0 Hz, H8), 10.23 (d, *J* 8.0 Hz, H11).

7*a*: ^1H nmr, 9-*E*, δ: 5.87 (dd, *J* 8.0 Hz, ^3J(C8−H10) 6.0 Hz, H10), 6.15 (dd, ^1J(C8−H8) 162 Hz, *J* 16.0 Hz, H8).

7*b*: ^1H nmr, 9-*E*, δ: 2.28 (d, ^2J(C9−(9-CH_3)) 6.0 Hz, 9-CH_3), 5.87 (dd, *J* 8.0 Hz, ^2J(C9−H10) 1.0 Hz, H10), 6.15 (dd, *J* 16.0 Hz, ^2J(C9−H8) 3.2 Hz, H8), 6.68 (dd, *J* 16.0 Hz, ^3J(C9−H7) 6.4 Hz, H7).

*Retinal 1*a,b

Sodium hydride (NaH, 80 mg, 1.8 mmol, 55% in mineral oil) was washed three times with dry pet. ether to remove the mineral oil, and suspended in 10 mL dry THF. A solution of 2.1 mmol diethyl-3-cyano-2-methylprop-2-enylphosphonate **8** (14) (0.45 g) in dry THF was added dropwise and stirred for 30 min at room temperature. After cooling to 0°C a solution of 1.4 mmol β-ionylidene acetaldehyde **7** (0.3 g) in dry THF was added dropwise and the mixture stirred and warmed to room temperature. After 2 h the solution was poured into half-saturated NH_4Cl and extracted with ether. The organic layers were washed with water followed by brine, dried ($MgSO_4/K_2CO_3$), and then concentrated. The residue was purified by chromatography using 5% ether/pet. ether to yield the pure retinonitrile, which was reduced with Dibal as described for the reduction of **5** to provide the crude retinal in 80% yield (0.3 g). The all-*trans* isomer **1** was separated by column chromatography (silica gel >230 mesh, 4% ether/pet. ether) and further purified by prep. hplc. The 300-MHz ^1H nmr spectra of **1***a*,*b* are identical to those described by Patel (25) except for the additional ^{13}C-^1H coupling constants as shown in Table 1. The 75.5-MHz ^{13}C nmr spectra of **1***a*,*b* display the enriched signals and are further identical to those described by Englert (16) except for the additional $^{13}C-^{13}C$ coupling constants as shown in Table 2; ms: **1***a* and **1***b* *m*/*z* 285 (M^+); level of ^{13}C enrichment calculated from the mass spectra: 93%.

C17-Acetate-nitrile 9: (4E,6E)-3-acetoxy-5-methyl-7-(2,6,6-trimethyl-1-cyclohexenyl)-4,6-heptadienenitrile

To a stirred solution of 6 mmol of acetonitrile (0.25 g) in 20 mL dry THF, 6 mmol of n-butyllithium (4.3 mL of a 1.4 *M* solution in hexane) were added dropwise at −60°C and stirred at −20°C for 15 min. The reaction mixture was cooled to −90°C and 6 mmol of 7*E*,9*E*-β-ionylidene acetaldehyde (1.3 g) in 10 mL dry THF were slowly added dropwise. The mixture was warmed to −60°C in 30 min and 12 mmol acetyl chloride (0.9 g) was added dropwise. After warming

to 10°C, 20 mL of half-saturated $NaHCO_3$ solution was added and the mixture extracted with ether. The organic layer was washed with water followed by brine, and then dried ($MgSO_4$). Evaporation of the solvent afforded an oil which was purified by chromatography using 15% ether/pet. ether to yield 1.5 g (85%) of C17-acetate-nitrile **9**; ir (KBr): 2250 cm^{-1} (C≡N stretch), 1745 cm^{-1} (C=O stretch); ^1H nmr (100 MHz, $CDCl_3/CCl_4$ 1:1), δ: 0.99 (s, $(CH_3)_2C$), 1.65 (s, 5-CH_3), 1.90 (s, 9-CH_3), 2.06 (s, CO—CH_3), 2.64 (d, *J* 6.0 Hz, H12), 5.35 (d, *J* 10.0 Hz, H10), 5.80 (dt, *J* 10.0 Hz, *J* 6.0 Hz, H11), 5.94 (d, *J* 16.5 Hz, H8), 6.22 (d, *J* 16.5 Hz, H7); ^{13}C nmr (25 MHz, $CDCl_3$), δ: 13.1, 19.2, 21.0, 21.6, 24.0, 28.8, 32.9, 34.2, 39.5, 66.0, 116.2, 123.7, 129.5, 129.7, 135.6, 137.1, 140.1, 169.8.

9*c*: ^1H nmr, δ: 2.64 (dd, ^1J(C12−H12) 137.0, *J* 6.0 Hz, H12), 5.80 (dtd, *J* 10.0 Hz, *J* 6.0 Hz, ^2J(C12−H11) 1.9 Hz, H11); ^{13}C nmr, 92% enriched signal, δ: 24.0.

9*d*: ^1H nmr, δ: 2.64 (dd, ^2J(C13−H12) 9.0 Hz, *J* 6.0 Hz, H12); ^{13}C nmr, 92% enriched signal, δ: 116.2.

*C17-Nitrile **10**: 5-methyl-7-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6-heptatrienenitrile*

Two equivalents of diazabicyclononene (DBN) were added to a solution of the C17-acetate-nitrile **9** in toluene and the mixture was brought to a gentle reflux. After 90 min (no acetate left by tlc) the mixture was cooled, poured into water, and extracted with ether. The organic layers were washed with water until neutral, followed by brine, and then dried ($MgSO_4$) and concentrated. The residue was purified by chromatography using 5% ether/pet. ether. Yield: 88% of a 11-*E*/*Z* mixture which was used in the next step.

*β-C18-ketone **12**: 6-methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatriene2-one*

The β-C18-ketone **12** was prepared in two steps from the C17-nitrile **10** in 60% yield as described for the preparation of **6** from **5**; ^1H nmr (300 MHz, $CDCl_3$), δ: 1.04 (s, $(CH_3)_2C$), 1.72 (s, 5-CH_3), 2.07 (s, 3H, 9-CH_3), 2.30 (s, 13-CH_3), 6.16 (d, *J* 11.7 Hz, H10), 6.17 (d, *J* 15.4 Hz, H12), 6.17 (d, *J* 16.1 Hz, H8), 6.42 (d, *J* 16.1 Hz, H7), 7.55 (dd, *J* 15.4 Hz, *J* 11.7 Hz, H11).

*Retinal **1**c,d*

The retinal **1***c*,*d* was prepared in two steps from the β-C18-ketone **12***c*,*d* in 85% yield as described for the synthesis of **7** from **6**. The all-*trans* isomer **1** was separated by column chromatography (silicagel >230 mesh, 4% ether/pet. ether) and further purified by preparative hplc; ms: **1***c* and **1***b* *m*/*z* 285 (M^+); level of ^{13}C enrichment calculated from the mass spectra: 93%. For ^1H nmr, compare **1***a*,*b*.

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