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Synthesis of (12,13-$^{13}C_2$)retinal and (13,14-$^{13}C_2$)retinal: a strategy to prepare multiple-^{13}C-labeled conjugated systems

M. Groesbeek, G. A. Rood and J. Lugtenburg

Department of Organic Chemistry, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

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Abstract. (12,13-$^{13}C_2$)Retinal, (13,14-$^{13}C_2$)retinal, (19-^{13}C)retinal and (20-^{13}C)retinal (**1**) were prepared in a simple fashion in high yield via a consecutive strategy. The key step is the reaction of a *N*-methoxy-*N*-methylamide with an alkyllithium or a Grignard reagent. The preparation of the required *N*-methoxy-*N*-methylamide is discussed. In this scheme, only three commercially available ^{13}C-labeled starting materials (ethyl bromoacetate, acetonitrile and methyl iodide) are sufficient to construct retinals with any possible combination of ^{13}C labeling in the conjugated tail end. This strategy is applicable to the preparation of many other conjugated systems, such as retinoids, carotenoids and polyenes.

Introduction*

Rhodopsin (Rh) and bacteriorhodopsin (bR) belong to the important class of retinal membrane proteins. Rhodopsin plays a central role in vision[1,2]. Bacteriorhodopsin acts as a light-driven proton pump that allows the conversion of the energy of light into that of a proton gradient which is used by the bacterium *Halobacterium halobium* to drive its life processes[3,4] under anaerobic conditions.

Solid-state NMR spectroscopy of rhodopsin and bacteriorhodopsin with mono-^{13}C-labeled chromophores is a powerful technique for obtaining structural and functional information at the atomic level without pertubation of the system[5]. Recently, rotational-resonance solid-state NMR spectroscopy has been developed[6]. Using doubly labeled chromophores, internuclear distances can be measured in membrane proteins and other systems, from which solution NMR and diffraction techniques yield limited information. Using this technique for (8,18-$^{13}C_2$)bacteriorhodopsin, we found a distance of 4.2 ± 0.3 Å between carbon atoms 8 and 18 in the chromophore[6]. This is in agreement with a planar 6,7-*s-trans* conformation of the chromophore in the active site of the protein[7].

It is clear that very important questions about the structure of Rh and bR and their photoproducts can be addressed by studying molecules with various doubly ^{13}C-labeled retinals. In order to study the structure of the tail end of the retinal chromophore with rotational-resonance ^{13}C NMR we required (12,13-$^{13}C_2$)retinal and (13,14-$^{13}C_2$)retinal. The chemical-shift value of C13 is sensitive to the protonation state of the Schiff-base linkage, whereas the chemical-shift values of C12 and C14 give information about the configuration around the C13=C14, C15=N double bonds, respectively. Using rotational-resonance NMR on photocycle intermediates, containing the chromophore labeled on C12 and C13 or on C13 and C14, will allow us to obtain this structural information.

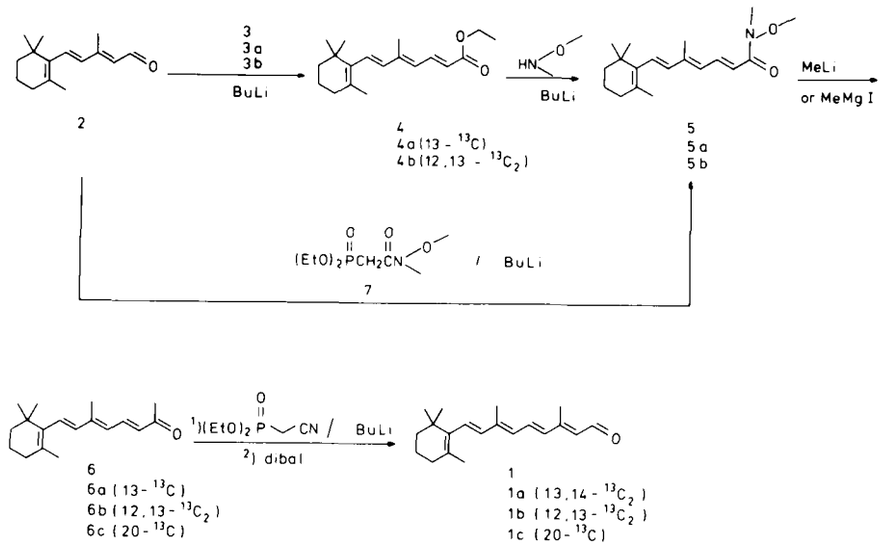
In this paper, we describe the preparation of the specifically labeled (13,14-$^{13}C_2$)retinal (**1a**) and (12,13-$^{13}C_2$)retinal (**1b**) via a simple straight-forward strategy. This method also allows improved preparation of (20-^{13}C)retinal (**1c**) and (19-^{13}C)retinal (**1d**). It can be extended to all combinations of ^{13}C labels in the conjugated tail end of retinal.

* Abbreviations and IUPAC names:

citral = 3,7-dimethyl-2,6-octadienal
sym-collidine = 2,4,6-trimethylpyridine
β-cyclocitral = 2,6,6-trimethyl-1-cyclohexenecarboxaldehyde
dibal = diisobutylaluminum hydride
EI-MS = electron-impact mass spectrometry
β-ionone = (*E*)-4-(2,6,6-trimethyl-1-cyclohexenyl)-3-buten-2-one
β-ionylideneacetaldehyde = (*E*,*E*)-3-methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-2,4-pentadienal
LDA = lithium diisopropylamide
THF = tetrahydrofuran
TMSCl = trimethylsilyl chloride

Synthesis

For the preparation of the required $^{13}C_2$-labeled retinals, we first optimized the reactions depicted in Scheme 1. β-Ionylideneacetaldehyde (**2**), easily prepared from the commercially available β-ionone[8], was reacted in a Horner–Emmons reaction with the anion of ethyl (diethoxyphosphinyl)acetate (**3**). The Horner–Emmons reaction proceeded in 85% yield to give the ester **4**. To convert the ester into the methyl ketone **6** in the most efficient way, we used a novel procedure. 1.1 equivalents of *N*,*O*-dimethylhydroxylamine were dissolved in THF and 1.1 eq. of *n*-butyllithium were added



Scheme 1. Synthesis of retinal (**1**) *via intermediate* **5**.

at −20°C. A solution of 1.0 equivalent of ester **4** was then added slowly. The ester was instantaneous and quantitatively converted into the corresponding amide **5**.

To obtain the free *N,O*-dimethylhydroxylamine we heated *N,O*-dimethylhydroxylamine hydrochloride[9] in 1.5 eq. of *sym*-collidine at the melting point of the salt, and then increased the temperature gradually to the boiling point of collidine. The amine distilled gently and was collected in a cooled receiver flask. In this way, the salt was converted into the free amine in more than 90% yield.

The amide **5** could also be obtained from **2** in one step by coupling **2** in a Horner–Emmons reaction to (diethoxyphosphinyl)-*N*-methoxy-*N*-methylacetamide[10] (**7**). The required phosphonate **7** was prepared from bromoacetyl bromide. *N,O*-Dimethylhydroxylamine (2.0 eq.) in CH_2Cl_2 was added to bromoacetyl bromide (**11**) in CH_2Cl_2 at 0°C. The rate of addition was kept low to prevent ketene formation and subsequent polymerization. The reaction proceeded quantitatively to give bromo-*N*-methoxy-*N*-methylacetamide (**12**). *N,O*-Dimethylhydroxylamine hydrobromide (1 eq.) was filtered off and recycled. **12** was added to 1.0 eq. of triethyl phosphite and heated to give the phosphonate quantitatively.

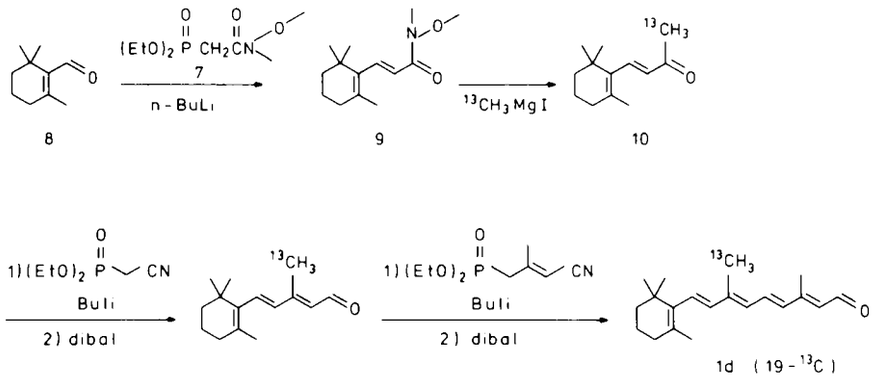
The amide **5** was treated with 1.1 eq. of methyllithium to give the required methyl ketone **6** in 87% yield, based on the ester **4**. The methyl ketone was then added to 1.1 eq. of the anion of (diethoxyphosphinyl)acetonitrile to give the retinonitrile as a mixture of isomers. The nitriles were converted to the retinal by dibal reduction in 53% yield based on **2**. SiO_2 column chromatography gave the isomerically pure all-*E* retinal (**1**).

To prepare (13,14-$^{13}C_2$)retinal (**1a**) according to Scheme 1, we reacted **2** in a Horner–Emmons reaction with 0.20 g of specifically labeled ethyl (1-^{13}C)(diethoxyphosphinyl)acetate (**3a**). This phosphonate is easily obtained by Arbuzov reaction of the commercially available triethyl phosphite and ethyl (1-^{13}C)bromoacetate. In this way, 1-^{13}C ester **4a** was obtained. The ester was treated with lithium *N,O*-dimethylhydroxylamide to give amide **5a**, which was then treated with methyllithium to give methyl (2-^{13}C)-ketone **6a**. **6a** Was treated with the *in-situ* prepared (2-^{13}C)(diethoxyphosphinyl)acetonitrile anion. (2-^{13}C)Acetonitrile was added at −60°C to 2 eq. of LDA, the first eq. of LDA reacting to give the anion of the acetonitrile. At −40°C, 1.0 eq. of diethyl chlorophosphate was then added to react with the acetonitrile anion to give the required phosphonate, which is immediately deprotonated by the second equivalent of LDA. At 0°C, methyl ketone **6a** was then added to yield the (13,14-$^{13}C_2$)retinonitrile as an isomeric mixture. The nitriles were converted to (13,14-$^{13}C_2$)retinal by dibal reduction in 56% yield based on **2**. The pure all-*E* (13,14-$^{13}C_2$)retinal was obtained by SiO_2 column chromatography.

For the synthesis of (12,13-$^{13}C_2$)retinal **1b**, **2** was treated with ethyl (1,2-$^{13}C_2$)(diethoxyphosphinyl)acetate (**3b**; prepared from triethyl phosphite and ethyl (1,2-$^{13}C_2$)bromoacetate) to give 1,2-$^{13}C_2$ ester **4b**. The ester was added to a solution of lithium *N,O*-dimethylhydroxylamide to give 1,2-$^{13}C_2$ amide **5b**. The amide was treated with MeLi to give the 2,3-$^{13}C_2$ methyl ketone **6b** in 89% from the 1,2-$^{13}C_2$ ester **4b**. **6b** Was elongated in a Horner–Emmons reaction with (diethoxyphosphinyl)acetonitrile; subsequent dibal reduction of the resulting nitriles gave 0.293 g of (12,13-$^{13}C_2$)retinal as a mixture of isomers in 63% yield from **2**. The isomerically pure all-E **1b** was obtained after SiO_2 column chromatography.

The high-yield conversion of the ester **4** to methyl ketone **6** prompted us to investigate the introduction of a ^{13}C-labeled methyl group at postion C13. To prepare (20-^{13}C)retinal[11] (**1c**), we converted β-ionylideneacetaldehyde (**2**) to amide **5** in a Horner–Emmons reaction with (diethoxyphosphinyl)-*N*-methyl-*N*-methoxyacetamide (**7**) to give **5** in 72% yield. To introduce the ^{13}C-labeled methyl group, we used (^{13}C)methylmagnesium iodide, as (^{13}C)methyllithium is not available. (^{13}C)Methylmagnesium iodide was prepared from (^{13}C)methyl iodide and magnesium in diethyl ether. To this solution, we added amide **5** to give the 1-^{13}C methyl ketone **6c** in 74% yield based on (^{13}C)methyl iodide. The methyl ketone was elongated to the (20-^{13}C)retinal by Horner–Emmons coupling with (diethoxyphosphinyl)acetonitrile and subsequent dibal reduction of the resulting nitriles. (20-^{13}C)Retinal was obtained in 58% yield, based on (^{13}C)methyl iodide. The isomerically pure all-*E* **1c** was obtained by SiO_2 column chromatography and found to be spectroscopically identical to the material in our earlier publication[11].

For the synthesis of (19-^{13}C)retinal[11] (**1d**), we used β-cyclocitral (**8**, prepared by cyclization of citral[12]) as starting material (Scheme 2). **8** was reacted in 71% yield in a Horner–Emmons reaction with (diethoxyphosphinyl)-



Scheme 2. Synthesis of (19-^{13}*C)*retinal (**1d**).

-*N*-methoxy-*N*-methylacetamide (**7**) to give **9**. Amide **9** (1 eq.) was reacted with 1 eq. of (^{13}C)methylmagnesium iodide to give (1-^{13}C)β-ionone (**10**) in 70% yield based on (^{13}C)methyl iodide. **10** was elongated in a Horner–Emmons reaction with (diethoxyphosphinyl)acetonitrile and subsequent dibal reduction of the resulting nitriles to yield (19-^{13}C)**2**. (19-^{13}C)β-Ionylideneacetaldehyde was converted into (19-^{13}C)retinal by Horner–Emmons coupling with 4-(diethoxyphosphinyl)-3-methyl-2-butenenitrile and dibal reduction of the resulting (19-^{13}C)retinonitriles. (19-^{13}C)-Retinal (**1d**) was obtained as a mixture of isomers in 39% yield, based on (^{13}C)MeI. The isomerically pure all-*E* **1d** was obtained after SiO_2 column chromatography and found to be spectroscopically identical to the material in our earlier publication[11].

Discussion

The doubly labeled retinals, (12,13-$^{13}C_2$)retinal and (13,14-$^{13}C_2$)retinal, were prepared via a consecutive reaction scheme starting from β-ionylideneacetaldehyde and two commercially available isotopically labeled compounds; ethyl bromoacetate and acetonitrile.

The key step of the novel scheme is the conversion of *N*-methoxy-*N*-methylamides **5** into the corresponding methyl ketones. Therefore, esters **4** were first converted into their *N*-methoxy-*N*-methylamide by treatment of the esters with lithium *N*,*O*-dimethylhydroxylamide. Addition of methyllithium to the amides gave the methyl ketone in about 90% yield. Even when using a large excess (10 eq.) of methyllithium, no overaddition to the tertiary alcohol was observed, as with other *N*-methoxy-*N*-methylamides[13]. For the conversion of an ester into its methyl ketone, we used a recently published method[14], in which an ester is treated with methyllithium at −100 °C in the presence of 5 eq. of TMSCl. Using that method, we were able to convert esters into their methyl ketone in about 70% yield. The present method is clearly to be preferred, as it does not require stringent low-temperature control and gives high yields. With alkyllithium reagents, the *N*-methoxy-*N*-methylamides also react with Grignard reagents to form ketones in a high yield.

Commercially available (^{13}C)methyl iodide can easily be converted to the corresponding (^{13}C)methylmagnesium idodide. [(^{13}C)Methyllithium is not easily available.] Reaction of the ^{13}C Grignard reagent with the appropriate *N*-methoxy-*N*-methylamide (**5** or **9**) leads to the introduction of ^{13}C labels in about 70% yield based on (^{13}C)methyl iodide, at positions 19 or 20 in retinal. In this way, (20-^{13}C)retinal and (19-^{13}C)retinal were prepared in 58% and 39% yields, respectively, based on (^{13}C)methyl iodide. The sole alternative for the introduction of 9- or 13-$^{13}CH_3$ in the synthesis of retinals is the addition of (^{13}C)MeMgI to an aldehyde and oxidation of the resulting tertiary alcohol. That method gives a substantially lower yield.

Horner–Emmons reaction with (diethoxyphosphinyl)--*N*-methoxy-*N*-methylacetamide (**7**) is an efficient method to optimize the chain extension not involving isotopes (as yet isotopically labeled forms of **7** are not available in high yield). This reagent reacts smoothly with aldehydes **2** and **8**. However, with unsaturated ketones (such as β-ionone or **6**), it does not react. For the introduction of the ^{13}C labels, we used the ^{13}C-labeled phosphonates **3a** or **3b**, which were obtained quantitatively from the commercially available ^{13}C-labeled ethyl bromoacetate.

Using the procedures described in this paper, any combination of ^{13}C labels in the conjugated tail end of the retinal can now be synthesised in high yield. The present study also shows that the *N*-methoxy-*N*-methylamides are potent intermediates with a broad scope. They present a good starting material for many retinal analogues via various other Grignard reagents. This strategy may find general application in the synthesis of many other conjugated polyene systems, both in natural abundance and in (multiple-) ^{13}C--labeled forms, *e.g.*, carotenoids, vitamin K, fecapentaenes, etc.

Experimental

General

The following solvents were distilled prior to use: CH_2Cl_2, from P_2O_5; THF, from $LiAlH_4$; ethanol, from Mg turnings; petroleum ether (b.p. 40–60 °C), from P_2O_5. Ethyl (^{13}C)bromoacetates and ($^{13}C_2$)acetonitrile (>99% enrichment) were purchased from Cambridge Isotope Lab. in the U.S.A. and used as such. Other chemicals were bought from Janssen Chimica or Aldrich (reagent grade) and used without further purification. Dibal was used as an 1.0M solution in hexanes, n-BuLi as an 1.6M solution in hexanes, MeLi as an 1.6M solution in diethyl ether, and MeMgCl as a 2.9M solution in diethyl ether.

All reactions were carried out in flame-dried apparatus in a N_2 atmosphere. Unless stated otherwise, purification is performed by SiO_2 flash column chromatography (Merck silica gel 60, 230–400 Mesh) using ether/petroleum ether (b.p. 40–60 °C) mixtures as eluent. Evaporation of the solvents was carried out *in vacuo* (20 mmHg). The retinals were handled in dim red light.

NMR spectra were run in $CDCl_3$ with tetramethylsilane (0 ppm) as internal standard on either a Bruker MSL-400 or a Jeol FX-200 (operating at resp. 400.1 and 199.5 MHz for ^{1}H NMR and at 100.1 and 50.1 MHz for ^{13}C NMR). ^{1}H NMR[15] and ^{13}C NMR[16] signals were assigned by comparison with those of the corresponding unlabeled compounds. The retinoid numbering system[17] is used for spectral-signal designations. For labeled compounds only the

^{1}H NMR spectral changes relative to the unlabeled compounds are given and for ^{13}C NMR only the resonances arising from the ^{13}C label. At intermediate stages, an analytical amount of material was separated into its isomerically pure form for spectroscopic characterization.

EI-MS spectra were recorded at 70 eV on a V.G. Micromass ZAB-2HF mass spectrometer. UV/Vis spectra were run on a Varian DMS-200 spectrophotometer, using ethanol (spectroscopic grade) as solvent.

N,O-*Dimethylhydroxylamine*

N,O-Dimethylhydroxylamine hydrochloride (40.0 g, 0.41 mol) was dissolved in 80 ml (0.6 mol) of *sym*-collidine. The mixture was heated, first at an oil-bath temperature of 115°C, later at 178°C. The free *N,O*-dimethylhydroxylamine was distilled through a small vigreux and was collected in an ice-cooled receiver flask. B.p. 44°C; density 0.832 g/ml; yield 22.7 g (91%). ^{1}H NMR (200 MHz): δ 6.50 ppm, br. s, (N–H); 3.51, s (–O–CH_3); 2.70, s (–N–CH_3).

Ethyl (1-^{13}C)(diethoxyphosphinyl)acetate (**3a**)

3a was prepared by heating 1.0 g (6.0 mmol) of ethyl (1-^{13}C)-bromoacetate with 1.0 g (6.0 mmol) of triethyl phosphite for 30 min at 180°C, yielding 1.3 g of **3a** (100%). ^{1}H NMR (200 MHz): δ 4.25–4.10, m (O–CH_2); 2.97, dd, J_{HP} 21.6 Hz, J_{CH} 130.0 Hz (P–CH_2); 1.39–1.31, m (O–C–CH_3).

Ethyl (1,2-$^{13}C_2$)(diethoxyphosphinyl)acetate (**3b**)

3b was prepared by heating 1.0 g (6.0 mmol) of ethyl (1,2-$^{13}C_2$)-bromoacetate with 1.0 g (6.0 mmol) of triethyl phosphite for 30 min at 180°C, yielding 1.3 g of **3b** (100%). ^{1}H NMR (200 MHz): δ 4.25–4.10, m (O–CH_2); 2.97, ddd, J_{HP} 21.6 Hz, J_{CH} 130.0 Hz, J_{CH} 7.7 Hz (P–CH_2); 1.39–1.31, m (O–C–CH_3).

Bromo-N-*methoxy*-N-*methylacetamide* (**12**)

To a solution of 1.0 eq. bromoacetyl bromide (**11**) in CH_2Cl_2 at 0°C were added 2.0 eq. of *N,O*-dimethylhydroxylamine dropwise. After stirring for 30 min at 0°C, the mixture was filtered, yielding 1.0 eq. of the amine·HBr. The solvent in the filtrate was evaporated, yielding 95–100% of **12**; b.p. 103°C at 20 mmHg. ^{1}H NMR (200 MHz): δ 4.11 ppm, s (Br–CH_2); 3.78, s (N–CH_3); 3.25, s (O–CH_3). ^{13}C NMR (50 MHz): δ 166.9 ppm (C=O), 61.3 (C–O–N), 40.7 (C–Br), 32.2 (C–N).

(Diethoxyphosphinyl)-N-*Methoxy*-N-*methylacetamide* (**7**)

Bromo-*N*-methoxy-*N*-methylacetamide (**12**) (11.6 g, 64 mmol) and 10.6 g (64 mmol) of triethyl phosphite were heated to 180°C (decomposition may result at temperatures above 210°C) for 30 min. Yield 15.0 g (98%). ^{1}H NMR (300 MHz): δ 4.24–4.14 ppm, m (H_2C–O); 3.79, s (H_3C–O); 3.22, s (H_3C–N); 3.19, d, J_{HP} 31.6 Hz (H_2C–P); 1.35, t, *J* 7.0 Hz (H_3C–C–O). ^{13}C NMR (50 MHz): δ 165.6 ppm (C=O); 62.1 (C–C–O); 61.6 (C–O–N); 32.1, d, *J* 32.2 Hz (P–C); 29.7 (C–N); 16.0 (C–C–O).

Ethyl 5-methyl-7-(2,6,6-trimethyl-1-cyclohexenyl)heptatrienoate (**4**)

3 (0.20 g, 1.1 mmol) was dissolved in THF at 0°C. BuLi (1.1 mmol) was added dropwise. After stirring the mixture for 30 min, 0.25 g (1.1 mmol) of **2** in THF was added dropwise. After reacting 2 h at room temperature, the mixture was poured into a saturated NH_4Cl solution. The organic layer was separated, and the water layer extracted three times with ether. The combined organic layers were dried (brine, $MgSO_4$). The solids were filtered off and the solvents were evaporated. After purification ester **4** was obtained as a mixture of *E*/*Z* isomers. Yield 0.27 g (85%). ^{1}H NMR (200 MHz): all-*E* **4**: δ 1.03, s (1-CH_3); 1.31, t, *J* 14.4 Hz (O–C–CH_3); 1.50, m (2-CH_2); 1.63, m (3-CH_2); 1.71, s (5-CH_3); 2.02, m (4-CH_2); 2.05, s (9-CH_3); 4.21, m (O–CH_2); 5.87, d, *J* 13.4 Hz (12-CH); 6.15, d, *J* 14.4 Hz (8-CH + 10-CH); 6.38, d, *J* 15.2 Hz (7-CH); 7.72, m (11-CH).

*(1-^{13}C)***4** (**4a**). Using the same method as for **4**, 0.2 g (1.1 mmol) of **3a** and 0.25 g (1.1 mmol) of **2** were converted to 0.27 g of a mixture of isomers of **4a** (85%). ^{1}H NMR (200 MHz): as for **4**, and at δ 7.72, m (11-CH); 4.21, m (O–CH_2). ^{13}C NMR (50 MHz): strong peak at δ 167.4 ppm (COOEt).

*(1,2-$^{13}C_2$)***4** (**4b**). Using the same method as for **4**, 0.3 g (1.7 mmol) of **3b** and 0.37 g (1.7 mmol) of **2** were converted to 0.44 g of a mixture of isomers of **4b** (90%). ^{1}H NMR (200 MHz): as for **4**, and at δ 5.87, dd, $^1J_{HC}$ 161.4 Hz, J_{HH} 13.9 Hz (12-CH); 7.72, m (11-CH). ^{13}C NMR (200 MHz): strong peaks at δ 120.0 ppm, d, *J* 76.2 Hz (12-C); 167.5, d, *J* 76.2 Hz (COOEt).

N-*Methoxy*-N,*5-dimethyl-7-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6-heptanetrienamide* (**5**)

7 (3.1 g, 13 mmol) was dissolved in 50 ml of THF. At 0°C, 13 mmol of BuLi were added via a syringe. After stirring for 15 min, 2.67 g (13 mmol) of a mixture of **4** in 20 ml of THF were added dropwise. The mixture was stirred for 2 h at room temperature. Then water was added. The water layer was extracted three times with ether. The combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off over SiO_2. The solvents were evaporated and the crude yield was purified. Yield 3.71 g (72%). ^{1}H NMR (200 MHz): 9-*Z*-**5**: δ 7.85 ppm, dd, *J* 12.1 Hz, *J* 14.9 Hz (11-CH); 6.79, d, *J* 15.9 Hz (8-CH); 6.41, d, *J* 15.2 Hz (7-CH); 6.33, d, *J* 14.8 Hz (12-CH); 6.13, d, *J* 12.1 Hz (10-CH); 3.71, s (O–CH_3); 3.26, s (N–CH_3); 2.02, s (9-CH_3); 2.01, m (4-CH_2); 1.71, s (5-CH_3); 1.63, m (3-CH_2); 1.48, m (2-CH_2); 1.02, s (1-CH_3). All-*E*-**5**: δ 7.77 ppm, dd, *J* 12.1 Hz, *J* 14.9 Hz (11-CH); 6.48, d, *J* 14.9 Hz (7-CH); 6.37, d, *J* 15.0 Hz (12-CH); 6.22, d, *J* 13.4 Hz (10-CH); 6.15, d, *J* 16.2 Hz (8-CH), 3.71, s (O–CH_3); 3.27, s (N–CH_3); 2.05, s (9-CH_3); 2.01, m (4-CH_2); 1.71, s (5-CH_3); 1.64, m (3-CH_2); 1.50, m (2-CH_2); 1.03, s (1-CH_3). ^{13}C NMR (50 MHz): 9-*Z*-**5**: δ 167.5 ppm; 142.4; 138.3; 137.6; 131.3; 130.3; 129.5; 126.3; 117.1; 61.7; 39.4; 34.1; 32.9; 32.4; 28.9; 21.8; 20.8; 19.1. All-*E*-**5**: δ 167.5 ppm; 143.6; 139.4; 137.4; 136.8; 130.3; 130.1; 127.8; 117.8; 61.6; 39.5; 34.1; 33.0; 32.4; 28.9; 21.6; 19.1; 12.9 ppm. UV/Vis: 9-*Z*-**5**: λ_{max} 327.8 nm; all-*E*-**5**: λ_{max} 332.8 nm.

6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one (**6**)

5 (0.91 g, 3 mmol) was dissolved in 50 ml of THF. At –20°C, 2.5 ml (4 mmol) of MeLi were added via a syringe. The mixture was stirred for 1 h at 0°C. Then a slurry of 5 g of SiO_2 and 2 g of water was added and the mixture was stirred for 2 h at 0°C. $MgSO_4$ was added and the solids were filtered off. After evaporation of the solvents and purification 0.69 g (89%) of **6** was obtained. ^{1}H NMR (200 MHz): all-*E*-**6**: δ 1.04, s (1-CH_3); 1.50, m (2-CH_2); 1.61, m (3-CH_2); 1.72, s (5-CH_3); 2.04, m (4-CH_2); 2.06, s (9-CH_3); 2.29, s (13-CH_3); 6.15, d, *J* 15.4 Hz (12-CH); 6.15, d, *J* 10.3 Hz (10-CH); 6.17, d, *J* 16.1 Hz (8-CH); 6.43, d, *J* 15.9 Hz (7-CH); 7.58, dd, *J* 10.4 Hz, *J* 15.4 Hz (11-CH).

*(2-^{13}C)***6** (**6a**). *N,O*-Dimethylhydroxylamine (0.17 g, 2.8 mmol) was dissolved in THF. 2.7 mmol of BuLi was added via a syringe and the mixture was cooled to –20°C. 0.27 g (0.93 mmol) of **4a** in THF was added dropwise. The mixture was stirred for 30 min. Water was added and the layers were separated. The water layer was extracted three times with ether and the combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off and the solvents were evaporated, yielding **5a**. ^{1}H NMR (200 MHz): as for **5**, and at: δ 6.37 ppm, dd, *J* 15.0 Hz, J_{CH} 4.1 Hz (12-CH); 7.77, m (11-CH). ^{13}C NMR (50 MHz): δ 167.5 ppm. The amide was redissolved in THF and at –60°C 1.8 mmol of MeLi were added via a syringe. The mixture was stirred for 30 min and the temperature rose to –20°C. Then a satd. NH_4Cl solution was added and the mixture was stirred vigorously for 5 min. The layers were separated and the water layer was extracted three times with ether. The combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off and the solvents were evaporated. After purification 0.21 g (87%, based on **4a**) of **6a** was obtained. ^{1}H NMR (200 MHz): all-*E*-**6a**, as for **6**, and at: δ 2.29, d, J_{CH} 5.2 Hz (13-CH_3); 6.15, dd, J_{CH} 2.2 Hz, J_{HH} 15.4 Hz (12-CH); 7.58, m (11-CH). ^{13}C NMR (50 MHz): all-*E*-**6a**: strong peak at δ 198.3 ppm.

*(2,3-^{13}C)***6** (**6b**). **4b** (0.44 g, 1.5 mmol) was treated with 4.4 mmol of lithium *N,O*-dimethylhydroxylamide to give the amide **5b** in the same way as **4a** was converted to **5a**. ^{1}H NMR (200 MHz): as for **5**, and at: δ 7.77, m (11-CH); 6.37, m (12-CH). ^{13}C NMR (50 MHz): δ 167.3 ppm, d, *J* 68.9 Hz (C=O); 117.7, d, *J* 68.9 Hz (12-C). The amide was redissolved in THF and treated with 3 mmol of MeLi to give 0.35 g (89%) of **6b** as a mixture of isomers.

^{1}H NMR (200 MHz): all-*E*-**6b**, as for all-*E*-**6**, and at: δ 2.29 ppm, d, J_{CH} 5.1 Hz, 3H (13-CH_3); 6.15, ddd, $^1J_{HC}$ 175.3 Hz, $^3J_{CH}$ 2.6 Hz, J_{HH} 15.4 Hz, 1H (12-CH); 7.58, m (11-CH). ^{13}C NMR (50 MHz): all-*E*-**6b**: strong peak at: δ 129.2 ppm, d, *J* 55.7 Hz (12-C); 198.3, d, *J* 55.7 Hz (13-C).

*(1-^{13}C)***6 (6c)**. In a three-necked roundbottom flask equipped with a reflux condensor 0.5 g (3.5 mmol) of (^{13}C)methyl iodide was added via a syringe in small portions to 85 mg (3.5 mmol) of Mg turnings in 2 ml of diethyl ether in N_2 atmosphere. As soon as the reaction started, the mixture was cooled on an ice-bath. After stirring for 30 min no turnings were left and 1.06 g (3.5 mmol) of **4** in diethyl ether was added dropwise. The mixture was stirred for 2 h. Satd. NH_4Cl solution (10 ml) was added and the organic layer was separated. The water layer was extracted twice with ether and the combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off. After evaporation of the solvents and purification, 0.67 g of **6c** was obtained (74% yield). ^{1}H NMR (200 MHz): all-*E*-**6c**, as for all-*E*-**6**, and at: δ 2.29 ppm, d, J_{CH} 127.0 Hz, 3H (13-CH_3). ^{13}C NMR (50 MHz): all-*E*-**6c**: strong peak at: δ 27.7 ppm.

*All-*E*-(13,14-$^{13}C_2$)retinal (all-*E* **1a**)*

To a solution of 1.54 mmol of LDA (prepared from 0.2 g of diisopropylamine and 1.54 mmol BuLi) in THF at −60°C were added dropwise 0.32 ml (0.77 mmol) of a solution containing 0.10 g of (2-^{13}C)acetonitrile per 1.0 ml in THF. After stirring for 20 min, 0.132 g (0.77 mmol) of diethyl chlorophosphate in 5 ml of THF were added dropwise at −40°C and the mixture was stirred for 30 min. At 0°C, 0.2 g (0.77 mmol) of **6a** was then added dropwise. The mixture was stirred at room temperature for 2 h. Then a satd. NH_4Cl solution was added and the layers were separated. The water layer was extracted three times with ether and the combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off over SiO_2 and the solvents were evaporated, yielding 0.18 g of the nitriles (83%). ^{13}C NMR (50 MHz): δ 156.7 ppm, d, *J* 73.3 Hz (C-13); 96.3, d, *J* 73.3 Hz (C-14). The nitriles (0.64 mmol) were redissolved in petroleum ether and cooled to −60°C. Dibal (1.0 mmol) was added via a syringe. After stirring for 15 min, a slurry of SiO_2 and water was added and the mixture was stirred for 2 h at 0°C. Then $MgSO_4$ was added and the solids were filtered off. After evaporation of the solvents and purification, 166 mg of (13,14-$^{13}C_2$)retinal was obtained (91%) as a mixture of isomers. The pure all-*E* isomer was obtained from SiO_2 column chromatography. ^{1}H NMR (400 MHz): δ 10.10 ppm, dd, *J* 8.1 Hz, J_{CH} 24.5 Hz 15-CH); 7.14, ddd, J_{HH} 15.2, 11.5 Hz, J_{CH} 5.7 Hz 11-CH); 6.37, ddd, J_{HH} 15.2 Hz, J_{CH} 5.3, 2.6 Hz (12-CH); 6.35, d, *J* 16.0 Hz (7-CH); 6.19, d, *J* 11.5 Hz (10-CH); 6.17, d, *J* 16.0 Hz (8-CH); 5.97, dd, J_{HH} 8.1 Hz, J_{CH} 157.6 Hz (14-CH); 2.33, m (13-CH_3); 2.03, m (4-CH_2); 2.03, s (9-CH_3); 1.72, s (5-CH_3); 1.62, m (3-CH_2); 1.47, m (2-CH_2); 1.04, s, (1-CH_3). ^{13}C NMR (100 MHz): a strong AB pattern at: δ 154.7 ppm, d, *J* 66.6 Hz (C-13); 128.9, d, *J* 66.6 Hz (C-14). At the natural abundance level additional C−C couplings were observed: $J_{C14\ C15}$ 56.4 Hz, $J_{C15\ C20}$ 40.4 Hz, $J_{C12\ C13}$ 54.3 Hz, $J_{C11\ C14}$ 7.6 Hz. Mass spectrometry: measured M$^+$ 286.2215 (calcd. 286.2207); ^{13}C incorporation >99%.

*All-*E*-(12,13-$^{13}C_2$)retinal (all-*E* **1b**)*

0.35 g (2.0 mmol) of (diethoxyphosphinyl)acetonitrile were dissolved in THF and 1.9 mmol of BuLi were added via a syringe at 0°C. After stirring for 15 min 0.33 g (1.3 mmol) of **6b** in THF were added dropwise. The mixture was stirred for 2 h at room temperature. Water was then added and the layers were separated. The water layer was extracted twice with diethyl ether and the combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off over SiO_2. The solvents were evaporated. Crude yield 0.33 g (89%) of nitriles. ^{13}C NMR (50 MHz): all-*E*: δ 156.6 ppm, d, *J* 55.7 Hz (C-13); 130.9, d, *J* 55.7 Hz (C-12). The nitriles were redissolved in petroleum ether and cooled to −60°C. 1.5 mmol of dibal were added via a syringe. After stirring for 15 min, a slurry of SiO_2 and water was added and the mixture was stirred for 2 h at 0°C. Then $MgSO_4$ was added and the solids were filtered off. After evaporation of the solvents and purification 293 mg (89%) of (12,13-$^{13}C_2$)retinal was obtained as a mixture of isomers. The isomerically pure all-*E* **1b** was obtained by SiO_2 column chromatography. ^{1}H NMR (400 MHz): δ 10.10 ppm, d, *J* 8.2 Hz (15-CH); 7.14, ddd, J_{HH} 15.2, 11.5 Hz, J_{CH} 5.7 Hz (11-CH); 6.37, ddd, J_{HH} 15.2 Hz, J_{CH} 154.1, 2.7 Hz (12-CH); 6.35, d, *J* 16.0 Hz (7-CH); 6.19, d, *J* 11.5 Hz (10-CH); 6.17, d, *J* 16.0 Hz (8-CH); 5.97, dd, J_{HH} 8.2 Hz, J_{CH} 8.2 Hz (14-CH); 2.33, m (13-CH_3); 2.03, m (4-CH_2); 2.03, s (9-CH_3); 1.72, s (5-CH_3); 1.62, m (3-CH_2); 1.47, m (2-CH_2); 1.04, s (1-CH_3). ^{13}C NMR (100 MHz): a strong AB pattern at: δ 154.7 ppm, d, *J* 54.7 Hz (C-13); 134.5, d, *J* 54.7 Hz (C-14). At the natural abundance level additional C−C couplings were observed: $J_{C12\ C15}$ 6.9 Hz, $J_{C13\ C15}$ 3.8 Hz, $J_{C13\ C20}$ 40.6 Hz, $J_{C12\ C20} < 1$ Hz, $J_{C12\ C14}$ 1.4 Hz, $J_{C13\ C14}$ 66.5 Hz, $J_{C11\ C12}$ 69.5 Hz. Mass spectrometry: measured M$^+$ 286.2210 (calcd. 286.2207); ^{13}C incorporation >99%.

*All-*E*-(20-^{13}C)retinal (all-*E* **1c**)*

6c (0.67 g, 2.6 mmol) was converted to 0.58 g of **1c** (79%) as a mixture of *E*/*Z* isomers, analogously as **6b** was converted into **1b**. The isomerically pure all-*E* **1c** was obtained via SiO_2 column chromatography. ^{1}H NMR (400 MHz): δ 10.10 ppm, d, *J* 8.2 Hz (15-CH); 7.14, dd, *J* 15.2, 11.5 Hz (11-CH); 6.37, dd, J_{HH} 15.2 Hz, J_{CH} 5.0 Hz (12-CH); 6.35, d, *J* 16.0 Hz (7-CH); 6.19, d, *J* 11.5 Hz (10-CH); 6.17, d, *J* 16.0 Hz (8-CH); 5.97, dd, J_{HH} 8.2 Hz, J_{CH} 7.2 Hz (14-CH); 2.33, d, J_{CH} 127.8 Hz (13-CH_3); 2.03, m (4-CH_2); 2.03, s (9-CH_3); 1.72, s (5-CH_3); 1.62, m (3-CH_2); 1.47, m (2-CH_2); 1.04, s, (1-CH_3). ^{13}C NMR (100 MHz): strong signal at δ 13.1 ppm. At the natural abundance level additional C−C couplings were observed: $J_{C20\ C15}$ 4.6 Hz, $J_{C20\ C11}$ 3.3 Hz, $J_{C20\ C13}$ 40.6 Hz, $J_{C20\ C12}$ 2.0 Hz.

(E)-N-Methoxy-N-methyl-3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenamide (**9**)

BuLi (46 mmol) was added via a syringe to a solution of 11.0 g (46 mmol) of **7** in THF at 0°C. After 10 min, a solution of 7.0 g (46 mmol) of β-cyclocitral (**8**) in 10 ml of THF were added dropwise. The mixture was stirred at room temperature for 24 h, then poured into a satd. NH_4Cl solution. The water layer was extracted three times with ether and the combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off, the solvents were evaporated and the product purified. Yield 7.8 g (71%). ^{1}H NMR (200 MHz): δ 7.45 ppm, d, *J* 16.1 Hz (7-CH); 6.41, d, *J* 16.1 Hz (8-CH); 3.68, s (N−CH_3); 3.27, s (O−CH_3); 2.06, m (4-CH_2); 1.76, s (5-CH_3); 1.62, m (3-CH_2); 1.47, m (2-CH_2); 1.04, s (1-CH_3).

β-Ionone

3.3 mmol of MeLi was added via a syringe to 0.71 g (3.0 mmol) **9** in 20 ml of THF at 0°C. The mixture was stirred for 1 h. A slurry of 5 g of SiO_2 and 2 g of H_2O in ether were then added and the mixture was stirred for 1 h. $MgSO_4$ was then added and the solids were filtered off. After evaporation of the solvents and purification, 0.51 g (89%) of β-ionone was obtained.

*(19-^{13}C)β-Ionone** (**10**)

In a three-necked roundbottom flask equipped with a reflux condensor 0.5 g (3.5 mmol) of (^{13}C)methyl iodide was added via a syringe in small portions to 85 mg (3.5 mmol) of Mg turnings in 2 ml of diethyl ether in N_2 atmosphere. After stirring for 30 min, no turnings were left and 0.83 g (3.5 mmol) of **9** in diethyl ether was added dropwise. The mixture was stirred for 2 h. A satd. NH_4Cl solution (10 ml) was then added and the organic layer was separated. The water layer was extracted twice with ether and the combined layers were washed and dried (brine, $MgSO_4$). The solids were filtered off. After evaporation of the solvents and purification, 0.47 g of product was obtained in 70% yield. ^{1}H NMR (200 MHz); δ 7.28, d, *J* 16.0 Hz (7-CH); 6.08, dd, J_{HH} 16.0 Hz, J_{CH} 4.2 Hz (8-CH); 2.23, d, J_{CH} 126.9 Hz (9-CH_3); 2.03, m (4-CH_2); 1.72, s (5-CH_3); 1.62, m (3-CH_2); 1.47, m (2-CH_2); 1.04, s (1-CH_3). ^{13}C NMR (50 MHz): strong signal at δ 27.1 ppm.

*(19-^{13}C)β-Ionylideneacetaldehyde (19-^{13}C-**2**)*

The conversion of 0.47 g of (19-^{13}C)β-ionone (**10**) into 0.39 g (73%) of (19-^{13}C)**6** is performed analogously to the conversion of β-ionone

* Retinoid numbering.

into β-ionylideneacetaldehyde (**2**). The product was found to be identical to the material in our earlier publication[11]. ^{1}H NMR (200 MHz): δ 10.23 ppm, d, *J* 8.2 Hz (11-CH); 7.11, d, *J* 16.0 Hz (8-CH); 6.56, dd, J_{HH} 15.2, J_{CH} 5.1 Hz (8-CH); 5.80, dd, *J* 8.2, 8.1 Hz (10-CH); 2.09, d, J_{CH} 126.9 Hz (9-CH_3); 2.03, m (4-CH_2); 1.72, s (5-CH_3); 1.62, m (3-CH_2); 1.47, m (2-CH_2); 1.04, s, (1-CH_3). ^{13}C NMR (50 MHz): strong signal at δ 13.0 ppm.

All-E-*(19-*^{13}*C)retinal (all*-E **1d***)*

BuLi (2.0 ml) was added to 0.45 g (2.1 mmol) of 4-(diethoxyphosphinyl)-3-methylbutenenitrile in THF at 0°C via a syringe. After stirring for 10 min, 0.39 g (1.8 mmol) of **16** in THF were dropwise. Then the mixture was stirred for 2 h. Water was added and the layers were separated. The water layer was extracted twice with ether and the combined organic layers were washed and dried (brine, $MgSO_4$). The solids were filtered off and the solvents were evaporated. 0.45 g of the nitriles were obtained after purification in 89% yield. ^{13}C NMR (50 MHz): all-*E*: δ 12.9 ppm. To the nitriles (1.6 mmol) in petroleum ether 2.0 mmol of dibal were added via a syringe at −60°C. After 15 min, a slurry of 4 g of SiO_2 and 1 g of water was added and the mixture was stirred for 2 h. Then $MgSO_4$ was added, the solvents evaporated and the crude product purified, yielding 0.39 g of the (19-^{13}C)retinal (86%). The isomerically pure **1d** was obtained via SiO_2 column chromatography. ^{1}H NMR (400 MHz): δ 10.10 ppm, d, *J* 8.2 Hz (15-CH); 7.14, dd, *J* 15.2, 11.5 Hz (11-CH); 6.37, d, *J* 15.2 Hz (12-CH); 6.35,d, *J* 16.0 Hz (7-CH); 6.19, dd, J_{HH} 11.5 Hz, J_{CH} 7.1 Hz (10-CH); 6.17, J_{HH} 16.0 Hz, J_{CH} 4.7 Hz (8-CH); 5.97, d, *J* 8.2 Hz (14-CH); 2.33, (13-CH_3); 2.03, m (4-CH_2); 2.03, d, J_{CH} 126.8 Hz (9-CH_3); 1.72, s (5-CH_3); 1.62, m (3-CH_2); 1.47, m (2-CH_2); 1.04, s (1-CH_3).^{13}C NMR (100 MHz): strong signal at δ 13.0 ppm. At the natural abundance level additional C–C couplings were observed: J_{C19-C8} 1.6 Hz, J_{C19-C7} 3.3 Hz, J_{19-C9} 43.3 Hz, $J_{C19-C11}$ 4.3 Hz.

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