Synthetic Scheme for the Preparation of ^{13}C-Labeled 3,4-Didehydro-Retinal, 3-Hydroxyretinal, and 4-Hydroxyretinal up to Uniform ^{13}C-Enrichment

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Keywords: Retinoids / Isotopic labeling / Synthesis design / Natural products

A modular synthetic scheme has been developed for the synthesis of ^{13}C-labeled naturally occurring visual pigment chromophores; 3,4-didehydroretinal, 3-hydroxyretinal, and 4-hydroxyretinal. These compounds can now be made with $> 99\%$ ^{13}C enrichment at any position or combination of positions. We used the common $C_{10}+C_5+C_5$ scheme for the synthesis of retinals, and by making variations in the C_{10} part we can now prepare the desired retinal derivatives with selective or uniform ^{13}C enrichment.

Introduction

The chromophores of the visual pigments of important groups of animals are derived from systems closely related to retinal. These chromophores include, for example, 3,4-didehydroretinal (also known as vitamin A_2-aldehyde), in amphibians and fresh-water fish,[1,2] (3*R*)-3-hydroxyretinal in flies and butterflies,[3−5] and (4*R*)-4-hydroxyretinal in squid.[6−8] These retinal derivatives are shown in Figure 1.

In order to obtain the ^1H and ^{13}C NMR solid-state spectroscopic data, distance measurements and vibrational analysis from 3,4-didehydro-, (*R*)-3-hydroxy-, and (*R*)-4-hydroxyrhodopsin and -isorhodopsin with ^{13}C-enriched chromophores, the corresponding site-directed ^{13}C isotopomers of the chemically modified retinoids have to be prepared.

The basis for these syntheses is the chemistry we explored for the preparation of uniformly ^{13}C-labeled *all-E* retinal via a modular total organic synthetic strategy that allows the preparation of any ^{13}C isotopomer up to the uniformly labeled form.[9] The central modules in this synthesis are [$^{13}C_{10}$]-α-cyclocitronitrile (**5**, Scheme 1) and [$^{13}C_5$]-4-(diethylphosphono)-3-methyl-2-butenenitrile (**12**, Scheme 2). In this paper we discuss the efficient conversion of **5** into racemic 4-hydroxycyclocitral (**9**), safranal (**10**), and racemic 3-hydroxycyclocitral (**11**). The cyclocitral derivatives serve as C_{10}-building blocks for the synthesis of the corresponding retinal derivatives 3,4-didehydroretinal (**2**), (*R*)-3-hydroxyretinal (**3**), and (*R*)-4-hydroxyretinal (**4**) (see Figure 1). The resolution of **3** and **4** into the optically pure forms has been reported.[3,10−13]

These C_{10}-cyclocitral derivatives fit perfectly in the synthetic scheme for the synthesis of ^{13}C-labeled retinals, and by making variations in the C_{10}-part, we can easily obtain a complete cassette of retinal derivatives with ^{13}C-enrichment at the desired positions.

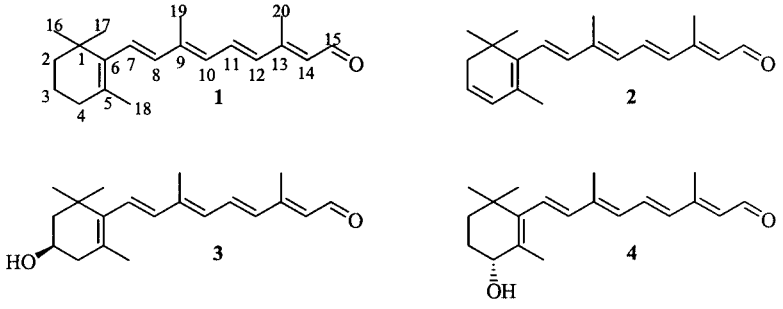

Figure 1. (*all-E*)-retinal (**1**) with IUPAC numbering, (*all-E*)-3,4-didehydroretinal (**2**), (3*R*,*all-E*)-3-hydroxyretinal (**3**), and (4*R*,*all-E*)-4-hydroxyretinal (**4**)

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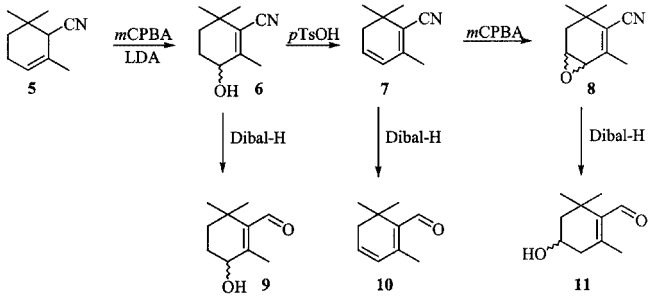
Results and Discussion

In this paper the synthetic scheme for the synthesis of ^{13}C-enriched 3,4-didehydroretinal (**2**), (*RS*)-3-hydroxyretinal (**3**), and (*RS*)--4-hydroxyretinal (**4**) (see Figure 1) is

worked out at the natural abundance level. The scheme (see Scheme 1) starts with the easily accessible α-cyclocitronitrile (**5**),[9] which is converted into racemic 4-hydroxy-β-cyclocitral (**6**) by treatment with *meta*-chloroperbenzoic acid (*m*CPBA) and subsequent opening of the formed epoxide with base, as previously described by our group.[14] Acid-catalyzed dehydration of **6** gives safronitrile (**7**) in high yield.[15] We now find that subsequent epoxidation with *m*CPBA gives 3,4-epoxy-β-cyclocitronitrile (**8**) in good yield. It is interesting that the C5=C6 double bond, conjugated with the nitrile group, is sufficiently deactivated that only the C3=C4 double bond of safronitrile (**7**) reacts. The β-cyclocitronitriles **6**, **7**, and **8** are converted in high yield into the corresponding aldehydes by reduction with Dibal-H. Safronitrile (**7**) is treated with 1.3 equivalents of Dibal-H to give safranal (**10**). We found that for the reduction of 4-hydroxy-β-cyclonitrile (**6**), an additional equivalent Dibal-H is needed to give 4-hydroxy-β-cyclocitral (**9**). Similar reduction of 3,4-epoxy-β-cyclocitronitrile (**8**) with 2.3 equivalents of Dibal-H converts the nitrile to an aldehyde in one step, and we observed that the epoxide opens in the same step to give the hydroxyl group selectively at the 3-position, yielding a racemic mixture of 3-hydroxy-β-cyclocitral (**11**).



Scheme 1. Synthesis of racemic 4-hydroxy-β-cyclocitral (**9**), safranal (**10**), and racemic 3-hydroxy-β-cyclocitral (**11**)

The β-cyclocitral derivatives **9**, **10**, and **11** can easily be converted into the corresponding retinals **2**, **3**, and **4** by a Horner–Wadsworth–Emmons (HWE) coupling with phosphonate **12** and subsequent Dibal-H reduction to obtain the intermediate β-ionylidene acetaldehydes. Repetition of the phosphonate coupling reaction and reduction gives the retinal derivatives **2**, **3**, and **4** as depicted in Scheme 2.

This sequence worked without problems for safranal (**10**). However, the presence of the hydroxyl groups in **9** and **11** interferes with the HWE-coupling reaction. For this reason, the hydroxyl derivatives were treated with NaH first, to convert the hydroxyl groups to alkoxides. Using another equivalent of NaH to deprotonate the phosphonate (**12**), these alkoxides reacted in the HWE-coupling reactions without difficulties.

The retinals were obtained as a mixture of 13*Z*- and *all-E* isomers, with the *all-E* isomers as the predominant products (60:40).[16] The *all-E* retinals were isolated by purification of the mixture of isomers via column chromatography or HPLC, according to literature procedures.[3,11,17,18] The final products were characterized using ^{1}H and ^{13}C NMR spectroscopy; the analytical data proved to be identical to the data reported for the authentic compounds.[3,11,19,20]

Conclusion

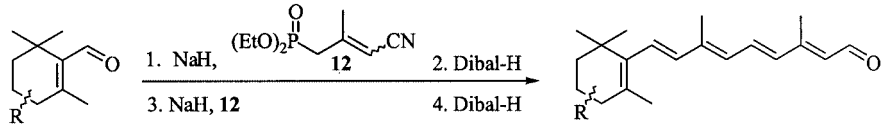
Based on our modular strategy to prepare the full cassette of ^{13}C isotopomers of (*all-E*)-retinal, we have described the chemical modification of the α-cyclocitral module to prepare 3,4-didehydro-, 3-hydroxy-, and 4-hydroxyretinal.

Experimental Section

General Remarks: Unless stated otherwise, dry reactions were carried out under a dry nitrogen atmosphere; reaction vessels were flame-dried prior to use. Solvents were dried by distillation (low-boiling petroleum ether 40–60 °C from P_2O_5, toluene from CaH_2) and kept dry by storage over sodium wire. Dichloromethane (DCM) was dried with CaH_2, and stored on 4 Å molecular sieves. Solutions of NaCl and NH_4Cl refer to saturated solutions of the salts in water. SiO_2/H_2O is a homogeneous mixture of SiO_2 (400 g) with water (120 mL). Ether refers to diethyl ether. PE refers to distilled, low-boiling petroleum ether, b.p. 40–60 °C. Dibal-H refers to a 1.0 M solution of diisobutylaluminium hydride in hexanes.

Reactions were monitored using thin-layer chromatography (TLC), on Merck F_{254} silica gel 60 aluminum sheets, 0.2 mm; spots were visualized with UV-light (254 nm) or treated with an oxidizing spray [$KMnO_4$ (2 g)and K_2CO_3 (4 g) in water (100 mL)]. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 nm, 230–400 mesh).

^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM-600, Bruker AV-400, or a Bruker WM-300 apparatus with tetramethylsilane (TMS; δ = 0.00 ppm) or $CDCl_3$ (δ = 77.00 ppm) as internal standard. For the NMR assignment, the IUPAC retinal numbering (as shown in Figure 1) is used for the retinals and their synthetic intermediates. The intermediates of retinal were obtained as mixtures of *E*/*Z*-isomers, with the *all-E* as



9 = 4-hydroxy-β-cyclocitral
10 = safranal
11 = 3-hydroxy-β-cyclocitral

2 = 3,4-didehydroretinal
3 = 3-hydroxyretinal
4 = 4-hydroxyretinal

Scheme 2. Synthesis of C_{20}-retinal derivatives

the predominant compound. The NMR assignments of the retinals and the intermediates refer to the *all-E* isomers.

All chemicals were purchased from Aldrich, Fluka, or Acros Chimica.

3-Hydroxy-2,6,6-trimethylcyclohex-1-enecarbonitrile (6): A solution of α-cyclocitronitrile **5** (5.76 g, 38.6 mmol) in ethyl acetate (30 mL) was slowly added to a solution of 70% *m*CPBA (11.4 g, 46.3 mmol) in ethyl acetate (30 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature (r.t.). The solvent was removed in vacuo and PE (100 mL) was added. The solution was cooled to 0 °C and triethylamine (TEA, 10 mL) was slowly added. After stirring for 10 min a white precipitate was formed, which was removed by filtration through silica gel. The silica was washed with 25% ether/3% TEA/PE and the filtrate was concentrated in vacuo, to obtain 2,3-epoxy-2,6,6-trimethyl-2-cyclohexanecarbonitrile (5.46 g, 33.0 mmol, 88%) as a mixture of isomers. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.05 (s, 3 H, 1-CH_3), 1.09 (s, 3 H, 1-CH_3), 1.2−1.3 (m, 2 H, 2-H), 1.55 (s, 3 H, 5-CH_3), 2.00 (m, 2 H, 3-H), 2.75 (s, 1 H, 6-H), 3.05 (br. s, 1 H, 4-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 20.7/21.5 (5-CH_3), 23.3 (C-3), 28.4 (C-1), 29.3/30.3 (1-CH_3), 31.3 (C-2), 44.7 (C-6), 55.3 (C-5), 58.6 (C-4), 118.5 (CN).

Lithium diisopropylamide (LDA) was prepared at −20 °C by adding *n*BuLi (1.6 M solution in hexane, 24.8 mL, 39.6 mmol) to a solution of diisopropylamine (DIPA, 6.0 mL, 43.0 mmol) in dry THF. A solution of 2,3-epoxy-2,6,6-trimethyl-2-cyclohexanecarbonitrile (5.46 g, 33.0 mmol) in dry THF (10 mL) was slowly added at −40 °C, and the reaction mixture was allowed to warm to r.t. overnight. The mixture was neutralized by adding NH_4Cl (sat), and the mixture was extracted three times with ether. The combined organic layers were washed with NaCl (sat) and dried with $MgSO_4$. After filtration and concentration in vacuo, the crude product 3-hydroxy-2,6,6-trimethylcyclohex-1-enecarbonitrile **6** (5.05 g, 30.6 mmol, 93%) was obtained. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.15 (s, 3 H, 1-CH_3), 1.20 (s, 3 H, 1-CH_3), 1.3−2.0 (m, 4 H, 2-H/3-H), 2.10 (s, 3 H, 5-CH_3), 2.45 (br. s, 1 H, OH), 4.06 (t, J = 6.0 Hz, 3-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 19.8 (5-CH_3), 27.9 (1-CH_3), 28.2 (C-3), 33.0 (C-2), 33.7 (C-1), 68.2 (C-4), 117.1 (CN), 119.7 (C-6), 152.5 (C-5).

2,6,6-Trimethylcyclohexa-1,3-dienecarbonitrile (7): 4-Hydroxy-β-cyclocitronitrile (**6**) (5.0 g, 30.5 mmol) was dissolved in toluene and *p*-toluenesulfonic acid (*p*TsOH, 2.0 g) was added. The mixture was refluxed overnight and the water formed during the reaction was trapped with a Dean−Stark apparatus. Workup was carried out by adding ether (100 mL) and $NaHCO_3$ (75 mL sat). The organic layer was collected and washed again with $NaHCO_3$ (sat) and NaCl (sat), dried with $MgSO_4$ and concentrated in vacuo to give 2,6,6-trimethylcyclohexa-1,3-dienecarbonitrile **7** (3.46 g, 23.5 mmol, 77%). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.15 (s, 6 H, 1-CH_3), 2.05 (s, 3 H, 5-CH_3), 2.19 (dd, 2 H, J = 4.4/1.9 Hz, 2-H), 5.92 (dt, J = 9.6/1.9 Hz, 1 H, 4-H), 6.06 (dt, J = 9.6/4.4 Hz, 1 H, 3-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 21.2 (5-CH_3), 26.6 (1-CH_3), 31.2 (C-1), 37.3 (C-2), 113.6 (CN), 117.5 (C-6), 126.1 (C-4), 131.3 (C-3), 145.5 (C-5).

3,4-Epoxy-2,6,6-trimethylcyclohex-1-enecarbonitrile (8): 2,6,6-Trimethylcyclohexa-1,3-dienecarbonitrile **7** (2.09 g, 14.2 mmol) was dissolved in ethyl acetate (30 mL), *m*CPBA (4.2 g, 17 mmol) in ethyl acetate (30 mL) was added at r.t. and the mixture was refluxed overnight. The solvent was removed by evaporation in vacuo, and PE (150 mL) was added. The suspension was cooled to 0 °C and TEA (9 mL) was added. After stirring for 15 min the white precipitate of *m*-chlorobenzoic acid was removed by filtration through silica gel. The silica was washed with 25% ether/3% TEA/PE and the filtrate concentrated in vacuo, to yield 3,4-epoxy-2,6,6-trimethylcyclohex-1-enecarbonitrile **8** (2.04 g, 12.1 mmol, 85%) as a mixture of isomers. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.19/1.21 (2s, 6 H, 1-CH_3), 1.63/1.71 (m, 1 H, 2-H), 2.12/2.21 (d, J = 2.4 Hz, 1 H, 2-H), 2.25 (s, 3 H, 5-CH_3), 3.23/3.25 (m, 1 H, 3-H), 3.59 (m, 1 H, 4-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 22.0 (5-CH_3), 29.6/31.4 (1-CH_3), 32.9 (C-6), 35.2 (C-2), 49.6 (C-3), 55.2 (C-4), 116.3 (CN), 119.6 (C-1), 147.5 (C-5).

3-Hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde (9): A solution of 3-hydroxy-2,6,6-trimethylcyclohex-1-enecarbonitrile **6** (1.0 g, 6.1 mmol) in dry toluene was cooled to −60 °C and Dibal-H (14 mL, 14.0 mmol) was added. The solution was allowed to warm to r.t. and the reaction was followed on TLC. After 30 min at r.t. the mixture was cooled to −20 °C and SiO_2/H_2O (24.4 g) was added. The suspension was stirred for another hour at 0 °C, K_2CO_3 and $MgSO_4$ were added, and after stirring for 15 min the solids were removed by filtration, washed with ether and the filtrate was concentrated in vacuo to yield, after flash column chromatography (60% ether/PE), 3-hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde **9** (0.74 g, 4.4 mmol, 73%). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.16/1.21 (2s, 6 H, 1-CH_3), 1.3−2.0 (m, 4 H, 2-H/3-H), 2.20 (s, 3 H, 5-CH_3), 3.72 (br. s, 1 H, -OH), 4.07 (t, J = 6.2 Hz, 1 H, 4-H), 10.11 (s, 1 H, CHO). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 15.2 (5-CH_3), 27.0 (1-CH_3), 27.6 (C-3), 33.1 (C-1), 35.7 (C-2), 70.2 (C-4), 140.8 (C-6), 154.4 (C-5), 193.5 (CHO).

2,6,6-Trimethylcyclohexa-1,3-dienecarbaldehyde (10): 2,6,6-Trimethylcyclohexa-1,3-dienecarbonitrile **7** (2.0 g, 13.6 mmol) was dissolved in dry PE (75 mL) and Dibal-H (17.7 mL, 17.7 mmol) was added at −60 °C. The solution was allowed to warm to r.t. and stirred for 30 min. The mixture was cooled to −20 °C, SiO_2/H_2O (30.9 g) was added and the suspension was stirred for 1 h at 0 °C. K_2CO_3 and $MgSO_4$ were added, the solids were removed by filtration and the filtrate was concentrated in vacuo. Flash-column purification (10% ether/PE) yielded aldehyde **10** (2.04 g, 12.1 mmol, 92%). ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.19 (s, 6 H, 1-CH_3), 2.15 (dd, J = 4.5/1.9 Hz, 2 H, 2-H), 2.17 (s, 3 H, 5-CH_3), 5.91 (dt, J = 9.5/1.9 Hz, 2 H, 4-H), 6.15 (dt, J = 9.5/4.5 Hz, 1 H, 3-H), 10.14 (s, 1 H, CHO). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 17.6 (5-CH_3), 26.2 (1-CH_3), 32.6 (C-1), 40.9 (C-2), 129.8 (C-4), 134.4 (C-3), 137.4 (C-6), 146.8 (C-5), 191.7 (CHO).

4-Hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde (11): Dibal-H (36.3 mL, 36.3 mmol) was added to a solution of 3,4-epoxy-2,6,6-trimethyl-cyclohex-1-enecarbonitrile **8** (2.04 g, 12.1 mmol) in dry PE at −60 °C. The solution was allowed to warm to r.t. in 1 h and stirred for another 2 h at r.t. It was cooled to −20 °C, SiO_2/H_2O (63.6 g) was added and the suspension was stirred at 0 °C for 1 h, after which $MgSO_4$ and K_2CO_3 were added. The solids were removed by filtration and the solvent removed by evaporation in vacuo. After flash-chromatography (60% ether/PE) 3-hydroxy-β-cyclocitral **11** (1.60 g, 9.53 mmol, 79%) was obtained. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.23/1.25 (2s, 6 H, 1-CH_3), 1.47 (dt, J = 12.0/8.9/2.6 Hz, 1 H, 2-H_{ax}), 1.71 (d, J = 2.7 Hz, 1 H, 2-H_{eq}), 2.13 (s, 3 H, 5-CH_3), 2.25 (dd, J = 18.4/9.2 Hz, 4-H_{eq}), 2.53 (dd, J = 2.9/2.2 Hz, 4-H_{ax}), 3.3 (br. s, 1 H, -OH), 3.98 (m, 1 H, 3-H), 10.10 (s, 1 H, CHO). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 19.0 (5-CH_3), 27.5/28.6 (1-CH_3), 35.5 (C-1), 44.2 (C-4), 49.0 (C-2), 63.4 (C-3), 139.6 (C-6), 153.4 (C-5), 191.6 (CHO).

3,4-Didehydroretinal (2)

3-Methyl-5-(2,6,6-trimethylcyclohexa-1,3-dienyl)penta-2,4-dienal: A suspension of NaH (60% in mineral oil, 0.43 g, 10.7 mmol) was

washed three times with dry PE and transferred into a flame-dried flask. The suspension was once more washed with dry PE, PE was removed with a N_2 flow and dry THF (75 mL) was added. At 0 °C, 4-(diethylphosphono)-3-methyl-2-butenenitrile **12** (2.14 g, 9.9 mmol) in THF was added and during 30 min of stirring at r.t. the phosphonate anion was formed. The solution was cooled to 0 °C and 2,6,6-trimethyl-cyclohexa-1,3-dienecarbaldehyde **10** (1.23 g, 8.21 mmol) in THF was added. After stirring overnight at r.t., the reaction was quenched by adding NH_4Cl (sat) and the mixture was extracted three times with ether. The combined organic layers were washed with NaCl (sat), dried with $MgSO_4$ and concentrated in vacuo. After purification on a silica-gel column (20% ether/PE), pure 3-methyl-5-(2,6,6-trimethylcyclohexa-1,3-dienyl)penta-2,4-dienenitrile (1.06 g, 5.0 mmol, 61%) was obtained as a mixture of isomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.04 (s, 6 H, 1-CH_3), 1.86 (s, 3 H, 5-CH_3), 2.09 (m, 2 H, 2-H), 2.21 (s, 3 H, 9-CH_3), 5.20 (s, 1 H, 10-H), 5.81 (dt, *J* = 9.7/4.2 Hz, 1 H, 3-H), 5.86 (d, *J* = 9.7 Hz, 1 H, 4-H), 6.27 (d, *J* = 16.1 Hz, 1 H, 8-H), 6.56 (d, *J* = 16.1 Hz, 1 H, 7-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 16.3 (9-CH_3), 20.1 (5-CH_3), 26.5 (1-CH_3), 33.8 (C-1), 39.7 (C-2), 96.3 (C-10), 117.9 (C-11), 122.0 (C-3), 126.7 (C-5), 129.4 (C-4), 131.7 (C-7), 134.2 (C-8), 140.3 (C-6), 156.8 (C-9).

A solution of 3-methyl-5-(2,6,6-trimethylcyclohexa-1,3-dienyl)-penta-2,4-dienenitrile (1.06 g, 5.0 mmol) in dry PE was cooled to −60 °C and Dibal-H (6.5 mL, 6.5 mmol) was added. The mixture was warmed to r.t. in 1 h, and stirred for another 30 min at r.t. After cooling to −20 °C, SiO_2/H_2O (11.3 g) was added, and the mixture was stirred for 1 h at 0 °C. K_2CO_3 and $MgSO_4$ were added, the solids were removed by filtration and washed with ether. The solvents were removed in vacuo, and the product was purified on a silica-gel column (20% ether/PE) to give 3-methyl-5-(2,6,6-trimethylcyclohexa-1,3-dienyl)penta-2,4-dienal (1.03 g, 4.8 mmol, 96%) as a mixture of isomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.06 (s, 6 H, 1-CH_3), 1.88 (s, 3 H, 5-CH_3), 2.12 (d, *J* = 4.2 Hz, 2 H, 2-H), 2.33 (s, 3 H, 9-CH_3), 5.82 (dt, *J* = 9.6 Hz/4.2 Hz, 1 H, 3-H), 5.88 (d, *J* = 9.6 Hz, 4-H), 5.94 (d, *J* = 8.2 Hz, 1 H, 10-H), 6.33 (d, *J* = 16.5 Hz, 1 H, 8-H), 6.73 (d, *J* = 16.5 Hz, 1 H, 7-H), 10.13 (d, *J* = 8.2 Hz, 1 H, 11-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 12.4 (9-CH_3), 20.0 (5-CH_3), 26.4 (1-CH_3), 33.7 (C-1), 39.5 (C-2), 126.4 (C-3), 127.4 (C-5), 128.5 (C-10), 129.5 (C-4), 133.7 (C-7), 134.5 (C-8), 136.9 (C-6), 154.1 (C-9), 190.5 (C-11).

C_5-Phosphonate **12** (1.24 g, 5.7 mmol) in dry THF (10 mL) was added to a suspension of washed NaH (0.25 g, 6.2 mmol) in dry THF at 0 °C. After stirring at r.t. for 30 min the NaH had reacted with the phosphonate and 3-methyl-5-(2,6,6-trimethylcyclohex-1,3-dienyl)penta-2,4-dienal (1.03 g, 4.8 mmol) in THF was added to the formed phosphonate anion at 0 °C. The solution was stirred overnight, and NH_4Cl (sat) was added. The mixture was extracted three times with diethyl ether, the combined organic layers washed with NaCl (sat) and dried with $MgSO_4$. The solid was removed by filtration and the filtrate concentrated in vacuo to give, after column purification (10% diethyl ether/PE), 3,7-dimethyl-9-(2,6,6-trimethylcyclohexa-1,3-dienyl)nona-2,4,6,8-tetraenenitrile (1.3 g, 4.6 mmol, 96%) as a mixture of isomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.04 (s, 6 H, 1-CH_3), 1.86 (s, 3 H, 5-CH_3), 2.02 (s, 3 H, 9-CH_3), 2.09 (d, *J* = 4.4 Hz, 2 H, 2-H), 2.20 (s, 3 H, 13-CH_3), 5.17 (s, 1 H, 14-H), 5.75 (dt, *J* = 9.4/4.4 Hz, 1 H, 3-H), 5.85 (d, *J* = 9.4 Hz, 1 H, 4-H), 6.15 (d, *J* = 11.4 Hz, 1 H, 10-H), 6.29 (d, *J* = 15.0 Hz, 1 H, 12-H), 6.31 (2s, 2 H, 7-H/8-H), 6.95 (dd, *J* = 15.0/11.4 Hz, 1 H, 11-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 12.6 (9-CH_3), 16.3 (13-CH_3), 20.1 (5-CH_3), 26.5 (1-CH_3), 33.7 (C-1), 39.7 (C-2), 96.3 (C-14), 117.8 (C-15), 125.3 (C-3), 127.4 (C-5), 128.1 (C-7), 129.2 (C-10), 129.6 (C-4), 132.2 (C-11), 133.8 (C-12), 136.8 (C-8), 137.9 (C-6), 140.8 (C-9), 156.6 (C-13).

The 3,4-didehydro-C_{20}-nitrile (1.27 g, 4.6 mmol) was dissolved in dry toluene and Dibal-H (6.0 mL, 6.0 mmol) was added at −80 °C. The mixture was allowed to warm to −50 °C in 20 min and SiO_2/H_2O (10.5 g) was added. The suspension was stirred for 1 h at 0 °C and K_2CO_3 and $MgSO_4$ were added. The solids were removed by filtration, washed with ether and the solvents removed in vacuo. After column chromatography (20% ether/PE) 3,4-didehydroretinal **2** (1.2 g, 4.3 mmol, 94%) was obtained as a mixture of isomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.05 (s, 6 H, 1-CH_3), 1.88 (s, 3 H, 5-CH_3), 2.04 (s, 3 H, 9-CH_3), 2.09 (dd, *J* = 4.3 Hz, 2 H, 2-H), 2.33 (s, 3 H, 13-CH_3), 5.76 (dt, *J* = 9.5/4.3 Hz, 1 H, 3-H), 5.86 (d, *J* = 9.5 Hz, 1 H, 4-H), 5.97 (d, *J* = 8.1 Hz, 14-H), 6.23 (d, *J* = 11.5 Hz, 1 H, 10-H), 6.33 (2s, 2 H, 7-H/8-H), 6.38 (d, *J* = 15.1 Hz, 1 H, 12-H), 7.15 (dd, *J* = 15.1/11.5 Hz, 1 H, 11-H), 10.11 (d, *J* = 8.1 Hz, 1 H, 15-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 12.8 (9-CH_3), 13.0 (13-CH_3), 20.2 (5-CH_3), 26.7 (1-CH_3), 33.9 (C-1), 39.8 (C-2), 125.5 (C-3), 127.7 (C-5), 128.4 (C-7), 128.9 (C-14), 129.8 (C-4), 129.8 (C-10), 132.4 (C-11), 134.6 (C-12), 136.4 (C-8), 138.1 (C-6), 141.0 (C-9), 154.6 (C-13), 190.9 (C-15).

(3*RS*)-Hydroxyretinal (3)

3-Methyl-5-(4-hydroxy-2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal: In a procedure similar to the one described above, C_5-phosphonate **12** (2.59 g, 11.9 mmol) in THF was added to a suspension of washed NaH (0.91 g, 22.8 mmol) in dry THF at 0 °C. After 30 min at r.t., the suspension was cooled back to 0 °C and 4-hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde **11** (1.67 g, 9.9 mmol) in THF (10 mL) was added. After stirring overnight at room temp., NH_4Cl (sat) was added and the mixture was extracted three times with ether. The organic layer was washed with NaCl (sat), dried with $MgSO_4$, filtered, and concentrated. Column purification (60% ether/PE) yielded 3-methyl-5-(4-hydroxy-2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienenitrile (0.96 g, 4.2 mmol, 42%) as a mixture of isomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.06/1.07 (2s, 6 H, 1-CH_3), 1.4 (m, 1 H, 2-H_{ax}), 1.8 (m, 1 H, 2-H_{eq}), 1.72 (s, 3 H, 5-CH_3), 2.0 (m, 1 H, 4-H_{ax}), 2.4 (m, 1 H, 4-H_{eq}), 2.20 (s, 3 H, 9-CH_3), 2.72 (br. s, 1 H, -OH), 3.96 (m, 1 H, 3-H), 5.19 (s, 1 H, 10-H), 6.15 (d, *J* = 16.1 Hz, 1 H, 8-H), 6.69 (d, *J* = 16.1 Hz, 1 H, 7-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 16.2 (9-CH_3), 22.7 (5-CH_3), 28.1/29.5 (1-CH_3), 35.2 (C-1), 40.6 (C-4), 45.1 (C-2), 64.0 (C-3), 96.4 (C-10), 117.1 (C-11), 126.3 (C-5), 134.7 (C-7), 136.1 (C-8), 139.2 (C-6), 156.9 (C-9).

In a similar Dibal-H reduction to the one described earlier, 3-methyl-5-(4-hydroxy-2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienenitrile (0.82 g, 3.6 mmol) was reduced with Dibal-H (8.2 mL, 8.2 mmol) in dry toluene. After column purification (60% ether/PE), the corresponding 3-methyl-5-(4-hydroxy-2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal (0.75 g, 3.2 mmol, 90%) was obtained as a mixture of isomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.09/1.10 (2s, 6 H, 1-CH_3), 1.5 (m, 1 H, 2-H_{ax}), 1.8 (m, 1 H, 2-H_{eq}), 1.75 (s, 3 H, 5-CH_3), 2.33 (s, 3 H, 9-CH_3), 2.0 (m, 1 H, 4-H_{ax}), 2.4 (m, 1 H, 4-H_{eq}), 3.15 (br. s, 1 H, -OH), 3.98 (m, 1 H, 3-H), 5.95 (d, *J* = 8.4 Hz, 1 H, 10-H), 6.22 (d, *J* = 16.1 Hz, 1 H, 8-H), 6.73 (d, *J* = 16.1 Hz, 1 H, 7-H), 10.10 (d, *J* = 8.4 Hz, 1 H, 11-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 12.4 (9-CH_3), 21.1 (5-CH_3), 28.2/30.1 (1-CH_3), 36.5 (C-1), 42.1 (C-4), 47.7 (C-2), 63.7 (C-3), 128.6 (C-10), 129.4 (C-5), 134.8 (C-7), 136.7 (C-8), 139.6 (C-6), 154.8 (C-9), 191.2 (C-11).

By using a similar method to that described above for (**2**), the anion of C_5-phosphonate **12**, formed by adding **12** (0.83 g, 3.8 mmol) to

a suspension of washed NaH (0.29 g, 7.3 mmol) in dry THF at 0 °C, was reacted with 3-methyl-5-(4-hydroxy-2,6,6-trimethylcyclohex-1-enyl)-penta-2,4-dienal (0.75 g, 3.2 mmol). After workup and purification (silica gel, 60% ether/PE), 3,7-dimethyl-9-(4-hydroxy-2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenenitrile (0.65 g, 2.2 mmol, 69%) was obtained as a mixture of isomers. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.03/1.08 (2s, 6 H, 1-CH_3), 1.4 (m, 1 H, 2-H_{ax}), 1.73 (s, 3 H, 5-CH_3) 1.8 (m, 1 H, 2-H_{eq}), 2.01 (s, 3 H, 9-CH_3), 2.1 (m, 1 H, 4-H_{ax}), 2.20 (s, 3 H, 13-CH_3), 2.4 (m, 1 H, 4-H_{eq}), 2.99 (br. s, 1 H, -OH), 3.96 (m, 1 H, 3-H), 5.20 (s, 1 H, 14-H), 6.13 (d, J = 11.6 Hz, 1 H, 10-H), 6.14 (d, J = 16.0 Hz, 1 H, 8-H), 6.27 (d, J = 16.0 Hz, 1 H, 7-H), 6.31 (d, J = 15.0 Hz, 1 H, 12-H), 6.96 (dd, J = 15.0/11.6 Hz, 1 H, 11-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 12.8 (9-CH_3), 16.4 (13-CH_3), 21.4 (5-CH_3), 28.5/30.0 (1-CH_3), 36.8 (C-1), 42.2 (C-4), 48.0 (C-2), 64.2 (C-3), 96.3 (C-14), 117.2 (C-15), 127.1 (C-5), 128.4 (C-7), 131.3 (C-10), 132.3 (C-11), 133.2 (C-12), 137.1 (C-6), 137.5 (C-8), 140.8 (C-9), 156.9 (C-13).

The C_{20}-nitrile (0.54 g, 1.8 mmol) was reduced to the corresponding aldehyde **3** with Dibal-H (4.5 mL, 4.5 mmol) in dry toluene, using the same procedure as described before. After purification on a silica-gel column (60% ether/PE), 3-hydroxy-retinal (**3**) (0.48 g, 1.6 mmol, 89%) was obtained as a mixture of isomers. ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.03/1.08 (2s, 6 H, 1-CH_3), 1.50 (t, J = 12.0 Hz, 1 H, 2-H_{ax}), 1.74 (s, 3 H, 5-CH_3), 1.79 (t, J = 4.1 Hz, 1 H, 2-H_{eq}), 2.03 (s, 3 H, 9-CH_3), 2.08 (t, J = 10.3 Hz, 1 H, 4-H_{ax}), 2.33 (s, 3 H, 13-CH_3), 2.40 (d, J = 5.2 Hz, 1 H, 4-H_{eq}), 2.90 (br. s, 1 H, -OH), 4.00 (m, 1 H, 3-H), 5.97 (d, J = 8.2 Hz, 1 H, 14-H), 6.16 (d, J = 16.1 Hz, 1 H, 8-H), 6.20 (d, J = 11.5 Hz, 1 H, 10-H), 6.29 (d, J = 16.1 Hz, 1 H, 7-H), 6.38 (d, J = 15.0 Hz, 1 H, 12-H), 7.15 (dd, J = 15.0/11.5 Hz, 1 H, 11-H), 10.08 (d, J = 8.2 Hz, 1 H, 15-H). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 12.7 (9-CH_3), 12.9 (13-CH_3), 21.4 (C-18), 28.5/30.0 (1-CH_3), 36.8 (C-1), 42.3 (C-4), 48.0 (C-2), 64.3 (C-3), 127.3 (C-5), 128.5/128.7 (C-7/C-14), 129.6 (C-10), 132.3 (C-11), 134.5 (C-12), 137.1 (C-6), 137.4 (C-8), 140.8 (C-9), 154.8 (C-13), 191.0 (C-15)

(4*RS*)-Hydroxyretinal (4)

3-Methyl-5-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal: In a similar way to that described above, C_5-phosphonate **12** (1.14 g, 5.3 mmol) was deprotonated with NaH (0.4 g, 10.1 mmol) and coupled to 3-hydroxy-2,6,6-trimethylcyclohex-1-enecarbaldehyde **9** (0.74 g, 4.4 mmol). After purification (50% ether/PE), 3-methyl-5-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)-penta-2,4-dienenitrile (0.82 g, 3.6 mmol, 81%) was obtained as a mixture of isomers. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.01/1.04 (2s, 6 H, 1-CH_3), 1.4 (m, 1 H, 2-H_{eq}), 1.7 (m, 2 H, 2-H_{ax}/3-H_{ax}), 1.81 (s, 3 H, 5-CH_3), 1.9 (m, 1 H, 3-H_{eq}), 2.20 (s, 3 H, 9-CH_3), 2.64 (br. s, 1 H, -OH), 3.99 (br. s, 1 H, 4-H), 5.21 (s, 1 H, 10-H), 6.17 (d, J = 16.0 Hz, 1 H, 8-H), 6.52 (d, J = 16.0 Hz, 1 H, 7-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 15.9 (9-CH_3), 18.0 (5-CH_3), 27.0/27.8 (1-CH_3), 28.3 (C-3), 34.0 (C-2), 34.0 (C-1), 69.1 (C-4), 96.6 (C-10), 117.1 (C-11), 131.8 (C-5), 133.1 (C-7), 134.5 (C-8), 139.4 (C-6), 156.3 (C-9).

By using the procedure described before, 3-methyl-5-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienenitrile (0.60 g, 2.6 mmol) in dry toluene was reduced with Dibal-H (6.0 mL, 6.0 mmol). After purification on a silica-gel column (60% ether/PE), 3-methyl-5-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)penta-2,4-dienal (0.57 g, 2.4 mmol, 94%) was obtained as a mixture of isomers. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.03/1.06 (2s, 6 H, 1-CH_3), 1.4 (m, 1 H, 2-H_{eq}), 1.7 (m, 2 H, 2-H_{ax}/3-H_{ax}), 1.84 (s, 3 H, 5-CH_3), 1.9 (m, 1 H, 3-H_{eq}), 2.32 (s, 3 H, 9-CH_3), 2.70 (br. s, 1 H, -OH), 3.99 (br. s, 1 H, 4-H), 5.92 (d, J = 7.9 Hz, 10-H), 6.23 (d, J = 16.0 Hz, 1 H, 8-H), 6.70 (d, J = 16.0 Hz, 1 H, 7-H), 10.11 (d, J = 7.9 Hz, 1 H, 11-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 12.8 (9-CH_3), 18.4 (5-CH_3), 27.4/28.2 (1-CH_3), 28.7 (C-3), 34.4 (C-2), 34.6 (C-1), 69.6 (C-4), 129.0 (C-10), 132.3 (C-5), 134.8 (C-7), 136.6 (C-8), 140.3 (C-6), 154.5 (C-9), 191.3 (C-11).

C_5-Phosphonate **12** (0.63 g, 2.9 mmol) in dry THF (10 mL) was added to a suspension of NaH (0.22 g, 5.6 mmol) in dry THF at 0 °C. After 30 min at r.t., 3-methyl-5-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)-2,4-pentadienal (0.57 g, 2.4 mmol) in THF was added and the mixture was stirred overnight at r.t. After workup and purification by column chromatography (60% ether/PE), 3,7-dimethyl-9-(3-hydroxy-2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenenitrile (0.60 g, 2.0 mmol, 83%) was obtained as a mixture of isomers. ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.02/1.06 (2s, 6 H, 1-CH_3), 1.43 (m, 1 H, 2-H_{eq}), 1.69 (m, 1 H, 2-H_{ax}), 1.72 (m, 1 H, 3-H_{ax}), 1.84 (s, 3 H, 5-CH_3), 1.90 (m, 1 H, 3-H_{eq}), 2.01 (s, 3 H, 9-CH_3), 2.20 (s, 3 H, 13-CH_3), 3.12 (br. s, 1 H, -OH), 4.00 (m, 1 H, 4-H), 5.20 (s, 1 H, 14-H), 6.09 (d, J = 11.5 Hz, 1 H, 10-H), 6.18 (d, J = 16.3 Hz, 1 H, 8-H), 6.28 (d, J = 16.3 Hz, 1 H, 7-H), 6.31 (d, J = 15.1 Hz, 1 H, 12-H), 6.95 (dd, J = 15.1/11.5 Hz, 1 H, 11-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 12.7 (9-CH_3), 16.3 (13-CH_3), 20.7 (5-CH_3), 27.3/28.7 (1-CH_3), 28.2 (C-3), 34.4 (C-2), 34.5 (C-1), 69.7 (C-4), 96.4 (C-14), 117.8 (C-15), 128.5 (C-7), 129.2 (C-10), 129.2 (C-12), 130.5 (C-5), 131.4 (C-11), 137.7 (C-8), 140.6 (C-9), 140.9 (C-6), 156.7 (C-13).

By using the procedure described above for (**2**), reduction of the 4-hydroxy-C_{20}-nitrile (0.60 g, 2.0 mmol) in dry toluene with Dibal-H (5.0 mL, 5.0 mmol) gave, after purification (60% ether/PE), the corresponding 4-hydroxyretinal **4** (0.53 g, 1.8 mmol, 87%) as a mixture of isomers. ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.03/1.06 (2s, 6 H, 1-CH_3), 1.43 (m, 1 H, 2-H_{eq}), 1.68 (m, 1 H, 2-H_{ax}), 1.73 (m, 1 H, 3-H_{ax}), 1.85 (s, 3 H, 5-CH_3), 1.89 (m, 1 H, 3-H_{eq}), 2.03 (s, 3 H, 9-CH_3), 2.33 (s, 3 H, 13-CH_3), 2.90 (br. s, 1 H, -OH), 4.02 (m, 1 H, 4-H), 5.97 (d, J = 8.1 Hz, 1 H, 14-H), 6.19 (d, J = 16.0 Hz, 1 H, 8-H), 6.21 (d, J = 11.3 Hz, 1 H, 10-H), 6.31 (d, J = 16.0 Hz, 1 H, 7-H), 6.38 (d, J = 15.1 Hz, 1 H, 12-H), 7.14 (dd, J = 15.1/11.3 Hz, 1 H, 11-H), 10.07 (d, J = 8.1 Hz, 1 H, 15-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 12.7 (9-CH_3), 12.8 (13-CH_3), 18.4 (5-CH_3), 27.4/28.7 (1-CH_3), 28.2 (C-3), 34.4 (C-2), 34.4 (C-1), 69.6 (C-4), 128.6/128.7 (C-14/C-7), 129.5 (C-10), 130.7 (C-5), 132.2 (C-11), 134.6 (C-12), 137.7 (C-8), 140.5/140.7 (C-6/C-9), 154.7 (C-13), 190.9 (C-15).

Acknowledgments

The authors wish to thank Kees Erkelens and Fons Lefeber for their help in recording the NMR spectra.

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Received September 25, 2002
[O02523]