Simple and Efficient Preparation of [10,20-$^{13}C_2$]- and [10-CH_3,13-$^{13}C_2$]-10-Methylretinal: Introduction of Substituents at the 2-Position of 2,3-Unsaturated Nitriles

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In this paper, we present the synthesis of [10,20-$^{13}C_2$]-10-methylretinal and [10-CH_3,13-$^{13}C_2$]-10-methylretinal, two doubly ^{13}C-labeled chemically modified retinals that have been recently used to study the structural and functional details behind the photocascade of bovine rhodopsin (Verdegem et al. *Biochemistry* **1999**, *38*, 11316; de Lange et al. *Biochemistry* **1998**, *37*, 1411). To obtain both doubly ^{13}C-labeled compounds, we developed a novel synthetic method to directly and regiospecifically introduce a methyl substituent on the 2-position of 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile. Encouraged by these results, we investigated the scope of this novel reaction by developing a general method for the introduction of a variety of substituents to the 2-position of 3-methyl-2,3-unsaturated nitriles, paving the way for simple and efficient synthesis of a wide variety of 10-, 14-, and 10,14-substituted chemically modified retinals, and other biologically important compounds.

Introduction

Retinoids form an important class of biologically active compounds. Retinal (**1**), a C_{20} conjugated aldehyde, the structure of which is depicted in Figure 1, is the most prominent species of this class and has several distinct functions in nature. For vertebrates, mollusks, and anthropods, the 11-*Z* isomer serves as the chromophore of rhodopsin, the visual signal transduction membrane protein.[1] The absorption of a photon by this colored cofactor, results in the highly efficient triggering of a biochemical photocascade, eventually leading to excitation of the optic nerve and visual awareness.[2,3] For other organisms, like bacteria, the light-absorbing characteristics of this molecule are put to use for entirely different processes. Bacteriorhodopsin, the light-driven proton pump in *Halobacterium salinarium*, uses the *all-E* form of retinal as its chromophore. Activation of this protein by light results in the generation of a net proton gradient across the cell membrane, which can be exploited to generate energy.[4,5] Other intrinsic membrane proteins with retinylidene chromophores include halorhodopsin,[6] a chloride ion pump, and sensory rhodopsin,[7] a phototaxis system for bacteria. Retinoids are also known to play an important role in embryo development[8] and are used for specific forms of cancer treatment.[9,10]

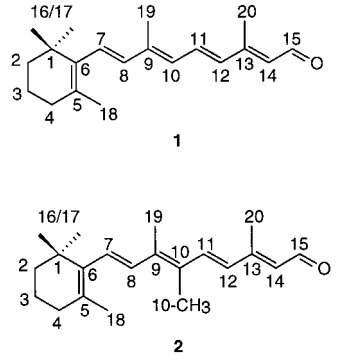

Figure 1. Structure of *all-E*-retinal (**1**) and *all-E*-10-methylretinal (**2**) including the IUPAC-approved numbering.[50]

The details behind the functioning of retinoids as chromophores of intrinsic membrane proteins have been a topic of research since its discovery. A variety of biophysical techniques has been applied to tackle this question.

A well-established approach in retinal proteins function research is the use of chemically modified retinoids.[11] It is widely accepted that the intrinsic structural and electronic characteristics of retinal are essential for the highly efficient processes it drives in nature. Hence,

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replacement of particular atoms or groups in the retinal skeleton by means of organic synthesis, followed by incorporation studies and functional studies in the protein, can deliver a wealth of information on the intriguing structure−activity relationships.

One member of the class of chemically modified retinals, 10-methylretinal (**2**) (See Figure 1), has recently been studied by de Lange et al.[12] In this study, it was anticipated that the presence of an extra methyl moiety on the 10-position of retinal would lead to an increased out-of-plane distortion of the chromophore of bovine rhodopsin. It is widely accepted that this out-of-plane distortion is essential for the fast and efficient kinetics of the rhodopsin to bathorhodopsin transformation in the visual phototransduction cascade. To set out a profound basis for such studies, a detailed study of the geometric structure of the 10-methylretinilydene chromophore is a prerequisite. Recently, some of us developed a novel MAS NMR method that enables the measurement of intramolecular distances in ligands, such as the retinylidene chromophore of rhodopsin, between pairs of ^{13}C atoms.[13] For the determination of the out-of-plane distortion of 10-methylrhodopsin, we focused on the measurement of the C10···C20 and 10-CH_3···C13 distances. For this study, the availability of [10,20-$^{13}C_2$]-10-methylretinal (**2a**) and [10-CH_3,13]-$^{13}C_2$-10-methylretinal (**2b**) was a prerequisite.

Two different syntheses of 10-methylretinal (**2**) have been reported in the literature. These include a procedure by Tanis et al.[14] and a procedure developed by Liu et al.,[15] involving Peterson olefination reactions. In these approaches the modification of the retinal structure is effected by linear synthesis using C_1 to C_3 building blocks. The published synthetic sequences by Tanis and Liu, however, do not allow the incorporation of ^{13}C labels at predetermined positions in 10-methylretinal, because the starting synthons are not available in ^{13}C-labeled form. We decided to investigate the possibility of introducing the 10-methyl directly at the conjugated chain of retinal, with ^{13}C-iodomethane, that is a relatively cheap ^{13}C-labeled synthon.

Our research was boosted by the discovery of a base-induced self-condensation of retinal, that was recently described by us in the literature.[16] In this reaction, the C20 methyl moiety of retinal (see Figure 1) is deprotonated using a strong nonnucleophilic base and subsequently the anion reacts with a neutral retinal molecule at C13, forming a C_{40} condensation compound. On the basis of this observation, we started to investigate the synthetic possibilities of the anion of the corresponding nitrile compound, 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal, for the synthesis of the two ^{13}C-labeled 10-methylretinals. The anion itself only yielded a self-condensation product, when treated with methyl iodide under various experimental conditions. Therefore, we focused on the corresponding nitrile compound 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**). It is known that the anions of such nitriles are less prone to self-condensation. Moreover, the nitrile moiety can be readily converted to the aldehyde functionality after the substitution reaction. The aldehyde moiety can subsequently be extended to the complete retinal skeleton via known synthetic methods.[11]

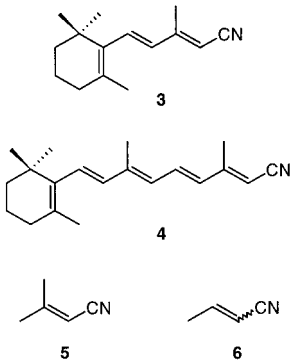

Figure 2. The four 3-methyl-2-butenenitriles that where tested in the 2-functionalization reaction. 3-Methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**); 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**); 3-methyl-2-butenenitrile (**5**); 2-butenenitrile (**6**).

We realized that the development of such a synthetic procedure for 10-methylretinals does not limit itself to these compounds, but could serve to develop novel ways of introducing substituents at the 10 position of retinal. Moreover, novel 10-substituted retinals could become available. The 2,3-unsaturated nitrile moiety, that is present in **3**, is also present in 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**), another well-known intermediate for retinal syntheses. Hence, novel 14-substituted retinals could also become available. Moreover, by combining the two approaches, 10,14-disubstituted retinals could be synthesized. A complete investigation to all possibilities described above is beyond the scope of our research, but we focused on the two nitriles described above: 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**) and 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**). In addition, we studied 3-methyl-2-butenenitrile (**5**) and 2-butenenitrile (**6**) (see Figure 2). Compounds **5** and **6** represent the most simple members of the 3-methyl-2-butenenitrile family. With the successful functionalization of these smaller compounds, it may be expected that a large number of compounds with the same functionality will react in a similar way.

To explore the scope of the synthetic applications of these anions, we applied three distinct electrophiles. From the wide variety of electrophiles available, iodomethane, methyl thiocyanate, and elemental iodine were chosen. This restrictive choice is an effort to cover the characteristics of the electrophile variety. Iodomethane represents a member of the alkyl halogenide family and serves as a paradigm for the introduction of alkyl substituents at the 2-position. Methyl thiocyanate is a sulfur electrophile and stands for the family of O, S,and Se electrophiles, that can introduce heteroatoms in the molecular structure. Iodine is a paradigm for the elemental halogenides, such as, for example, bromine.

All synthesized end products were purified using HPLC

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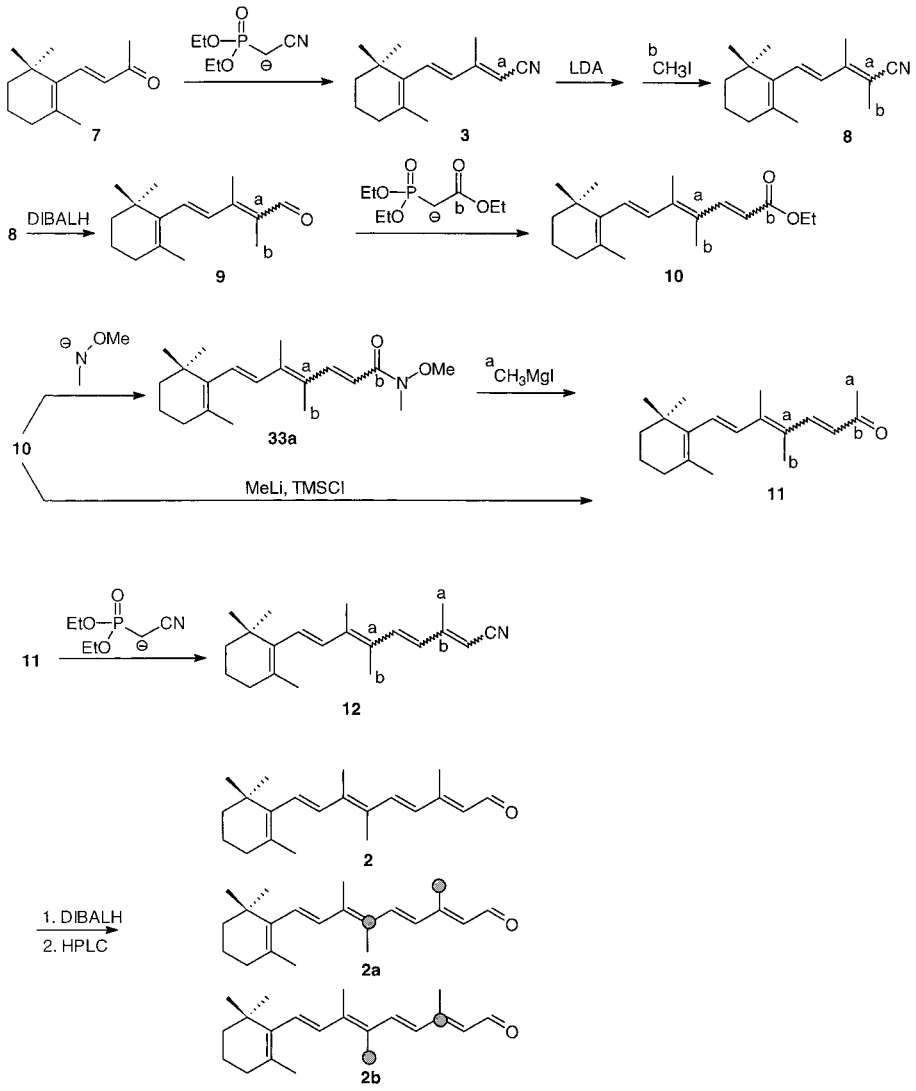
Scheme 1. Synthetic Scheme of 10-Methylretinal (2) (a, b = ^{12}C), [10,20-$^{13}C_2$]-10-Methylretinal (2a) (a = ^{13}C), and [10-CH_3,13-$^{13}C_2$]-10-Methylretinal (2b) (b). The Solid Balls Indicate the Position of the ^{13}C Labels



and subsequently characterized using mass spectrometry and high-resolution ^{1}H and ^{13}C NMR.

Results

Synthesis

10-Methylretinal and Its Isotopomers. The synthetic sequence for the isotopomers of 10-methylretinal was optimized using nonlabeled compounds. β-Ionone (**7**) is the synthon of choice for the synthesis of 10-methylretinal (see Scheme 1). In a Horner−Emmonds reaction sequence, **7** is reacted with the anion of diethyl cyanomethylphosphonate in dry THF at −80 °C. In this way, 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**) is formed in 79% yield as a mixture of *E* and *Z* isomers. To introduce the methyl substituent at the 2-position, this nitrile synthon is deprotonated using a strong nonnucleophilic base in an anhydrous environment at low temperature. We applied lithium diisopropylamide (LDA) in dry tetrahydrofuran at low temperature (−70 °C to −90 °C). At these conditions the nitrile was smoothly deprotonated. At the same low temperature, the resulting anion was reacted with a solution of iodomethane in THF. After about 2 h of stirring at low temperature, TLC analysis showed no presence of the starting nitrile compound. The reaction mixture was quenched with water at −80 °C and subsequently extracted with diethyl ether. After silica gel column chromatography, the 2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**8**) was isolated as an *E*/*Z* mixture in 96% yield. This nitrile was subsequently reduced using DIBALH following known procedures.[17] In this way, 2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (**9**) was obtained in 92% yield as an *E*/*Z*-mixture. The C_{16} aldehyde **9** was subsequently reacted with the anion of triethylphosphonoacetate in a Horner−Emmonds reaction to form 4,5-dimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienoate (**10**) in 72% yield, again as a mixture of *E*/*Z*-isomers. The ester **10** can be reacted with methyllithium to form the corresponding methyl ketone (**11**). In this reaction procedure overalkylation is a particular problem. Gebhard et al.[18,19] have developed a procedure that prevents this problem by performing the reaction at strictly −100 °C in dry THF containing 5 equiv of freshly distilled chlorotrimethylsilane. After the addition

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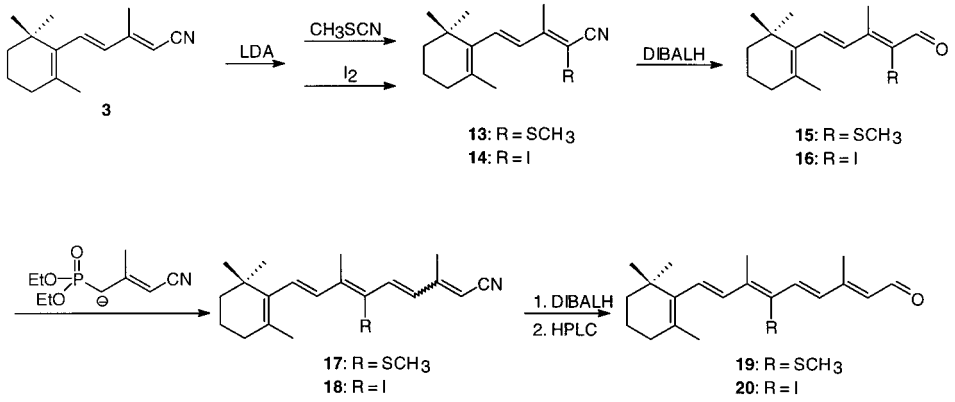
(18) Gebhard, R. Thesis, Leiden University, 1991.
(19) Cooke, M. P., Jr. *J. Org. Chem.* **1986**, *51*, 951.

Scheme 2. Synthetic Scheme for the Preparation of 10-Thiomethylretinal (19) and 10-Iodoretinal (20)



of the methyllithium, the formed alcoholate is trapped by the chlorotrimethylsilane, to form the respective trimethylsilyl alcohol, which is stable under these conditions. Subsequent hydrolysis yields the desired 5,6-dimethyl-8-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-3,5,7-octatrien-2-one (**11**) in 80% yield. To finish the retinal skeleton, **11** is reacted with commercially available cyanomethyl diethylphosphonate to form the C_{21} nitrile 2,3,7-trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**12**) (95% yield), that is subsequently reduced using DIBALH to form 10-methylretinal (**2**) in 83% yield. In this way, 10-methylretinal can be synthesized starting from *β*-ionone (**7**) in 31% overall yield. The product is isolated from the reaction mixture by column chromatography as an *E*/*Z*-mixture. The *all-E*-isomer was isolated from this mixture with straight-phase HPLC and subsequently characterized.

For the synthesis of both doubly ^{13}C-labeled 10-methylretinals, some of the reagents in Scheme 1 were substituted for their ^{13}C-labeled isotopomer. For [10-CH_3,13-$^{13}C_2$]-10-methylretinal (**2b**), the labels are introduced by ^{13}C-iodomethane, that is commercially available, and [1-^{13}C]-triethylphosphonoacetate. This labeled synthon is synthesized starting from [1-^{13}C]-acetic acid according to literature procedures.[19,20] In short, [1-^{13}C]-acetic acid is reacted with bromine and phosphorus tribromide in a Hell−Vollhardt−Zelinsky reaction to form [1-^{13}C]-bromoacetyl bromide, which is subsequently quenched with ethanol to form [1-^{13}C]-ethyl bromoacetate in 86% overall yield, after distillation of the product under reduced pressure. [1-^{13}C]-triethylphosphonoacetate is formed by an Arbusov reaction of [1-^{13}C]-ethyl bromoacetate and triethyl phosphite in 90% yield.

For [10,20-$^{13}C_2$]-10-methylretinal (**2a**), the labels are introduced by ([2-^{13}C]-cyanomethyl)-diethylphosphonate and by ^{13}C-iodomethane in a Grignard reaction. ([2-^{13}C]-Cyanomethyl)diethylphosphonate is prepared starting from commercially available [2-^{13}C]-acetonitrile. In a one-pot reaction, [2-^{13}C]-acetonitrile is reacted with 2 equiv of LDA in THF at −80 °C. The first equivalent of LDA is used to deprotonate [2-^{13}C]-acetonitrile. Subsequently 1 equiv of diethyl chlorophosphate is added to the reaction mixture to form ([2-^{13}C]-cyanomethyl)diethylphosphonate, which is deprotonated by the remaining second equivalent of LDA. Then *β*-ionone (**7**) is added, so that the Horner−Emmonds reaction commences. The introduction of the C13 methyl in 10-methylretinal is effected by methyllithium, but since methyllithium is not available in ^{13}C-labeled form, the introduction of the 20 label cannot be effected by the methylation of ester **10a** as for the synthesis of natural abundance 10-methylretinal. A good alternative has been found in the Grignard reagent methylmagnesium iodide, which can be obtained in ^{13}C-labeled form starting from ^{13}C-iodomethane. When the Grignard reagent is reacted with the ester **10a** directly, the addition of chlorotrimethylsilane cannot prevent overalkylation of the ester moiety. A successful approach has been found in the transformation of the ester **10a** to the *N*-methyl-*N*-methoxy amide **33a** by reaction of the ester with the anion of *N*-methyl-*N*-methoxyamine (yield 74%).[21,22] During the subsequent reaction of this amide with ^{13}C-methylmagnesium iodide, the intermediately formed alcoholate is stabilized by the ligating effect of the magnesium between the alcohol anion and the amide nitrogen. After hydrolysis of this intermediate during workup, the methyl ketone (**11a**) is isolated in 17% yield. Subsequently, the synthetic sequence is followed as for the natural abundance 10-methylretinal to complete the retinal skeleton of **11a**.

Other Chemically Modified Retinoids. Encouraged by the success obtained with the synthesis of 10-methylretinal, we continued to investigate the synthetic scope of the 2-functionalization reaction for 2,3-unsaturated-3-methylnitriles. We decided to react the anion of **3** with methyl thiocyanate and iodine, leading to 10-thiomethylretinal (**19**) and 10-iodoretinal (**20**) as depicted in Scheme 2. The anion of **3** was reacted with 1.5 equiv of methyl thiocyanate or iodine under identical experimental conditions as for the synthesis of 10-methylretinal. Typically, 3 h of stirring at the low temperature was needed to completely react the electrophile according to thin-layer chromatography. Concomitantly, 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2-thiomethyl-2,4-pentadienenitrile (**13**) and 2-iodo-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**14**) were prepared in 97% and 90% yield, respectively. The nitrile moieties of **13** and **14** are subsequently reduced using DIBALH to yield the respective aldehydes **15** and **16**. In this case the completion of the retinal C_{20} skeleton can be effected by a shorter synthetic route, by reacting the aldehydes with the anion of 4-(diethylphosphono)-3-methyl-2-butenenitrile. The resulting nitriles are again reduced using DIBALH to yield 10-thiomethylretinal (**19**) and 10-iodoretinal (**20**) as a mixture of isomers in the

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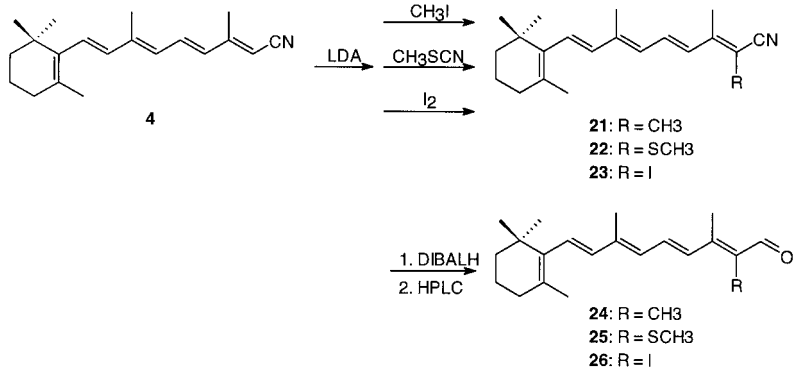
(21) Groesbeek, M.; Lugtenburg, J. *Photochem. Photobiol.* **1992**, *56*, 903.

(22) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.

Scheme 3. Synthetic Scheme for the Preparation of 14-Methylretinal (24), 14-Thiomethylretinal (25), and 14-Iodoretinal (26)



respective overall isolated yields of 11% and 23%. The low overall yield for the synthesis of 10-iodoretinal is mainly due to the low reaction yield for the DIBALH reduction of **18**. After the reaction, a considerable amount of **3** was found, indicating that dehalogenation by DIBALH is an important side reaction, which is in agreement with literature reports.[23]

For the synthesis of 14-substituted retinals, depicted in Scheme 3, 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**) (obtained by literature procedure.[21] The synthesis and characterization of this compound has been previously described in the literature[24]) was deprotonated using LDA at −85 °C. Subsequently one of the three electrophiles, methyl iodide, methyl thiocyanate, or iodine, was added (1.5 equiv). Again, about 3 h of stirring at the low temperature was needed to completely react the electrophile according to thin-layer chromatography. In this way 2,3,7-trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**21**), 3,7-dimethyl-2-thiomethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**22**), and 3,7-dimethyl-2-iodo-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**23**) were prepared in the respective yields of 80%, 96%, and 70%. Subsequent DIBALH reduction of the nitrile moieties of **21**, **22**, and **23** results in the formation of 14-methylretinal (**24**), 14-thiomethylretinal (**25**), and 14-iodoretinal (**26**) in the respective overall yields of 61%, 66%, and 11%. Again, the reductive removal of the substituent by DIBALH is responsible for the lower overall yield of **26**.[23]

Smaller Functionalized Nitriles. For the synthesis of the 2-substituted 3-methyl-2-butenenitriles and butenenitriles depicted in Scheme 4, compounds **5** and **6** were deprotonated using the same reaction conditions and were reacted with the same electrophiles, again adding 1.5 equiv. 2,3-Dimethylbutenenitrile (**27**), 3-methyl-2-thiomethylbutenenitrile (**28**), and 2-iodo-3-methylbutenenitrile (**29**) were formed in 53%, 80%, and 89% yield. The volatility of **27** presumably explains the lower isolated yield of this compound. This effect is even stronger for the 2-substituted butenenitrile **30**. This compound, of which its presence in the reaction mixture was inferred from the proton NMR spectrum, could not be isolated after standard reaction workup. Compounds **31** and **32**, that have a higher molecular weight than **30**, were isolated in 28% and 14%, respectively.

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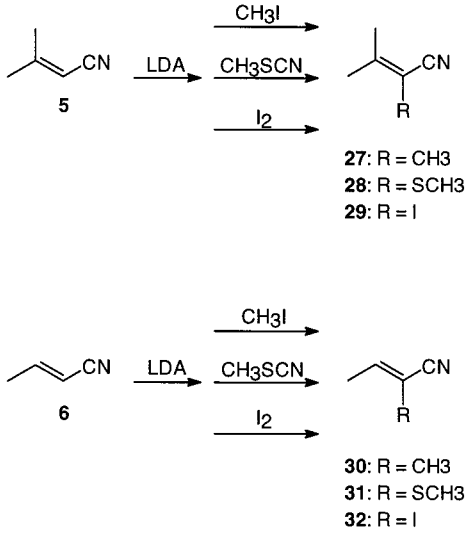
(24) Pardoen, J. A.; Winkel, C.; Mulder, P. P. J.; Lugtenburg, J. *Rec. Trav. Chim. Pays-Bas* **1984**, *103*, 135.

Scheme 4. Synthetic Scheme for the Preparation of 2,3-Dimethyl-2-butenenitrile (27), 3-Methyl-2-thiomethyl-2-butenenitrile (28), 3-Methyl-2-iodo-2-butenenitrile (29), 2-Methyl-2-butenenitrile (30), 2-Thiomethyl-2-butenenitrile (31), and 2-Iodo-2-butenenitrile (32)



It can be concluded that for all nitriles, the 2-functionalization reaction gives exclusively 2-reaction in very good yields. Moreover, no remaining starting material can be observed in the reaction mixtures.

Characterization of the Novel Retinal Compounds. We limit the discussion of the characterization of the compounds to the novel retinal compounds. All spectroscopic data of the other compounds can be found in the Experimental Section. All retinal compounds were isolated as mixtures of geometric isomers. Preparative HPLC (see Experimental Section for procedures) allowed us to purify the *all-E* isomers. After separation of the isomers, the fraction containing the *all-E*-isomer was run again, resulting in a single-peak HPLC trace, proving that the isolated *all-E*-isomers of all compounds were obtained with purity close to unity. The *all-E*-isomers were subsequently characterized using mass spectroscopy and high-resolution NMR.

Mass Spectrometry. The single focus electron impact mass spectra of 10-methylretinal (**2**) appears as a typical retinoid mass spectrum with the base peak at $m/z = 298$, the molecular ion peak. The spectra of the isotopomers of **2**, [10,20-$^{13}C_2$]-10-methylretinal (**2a**) and [(10-CH_3),13-$^{13}C_2$]-10-methylretinal (**2b**) reveal their base

Table 1. Experimentally Determined and Calculated Molecular Masses of 10-Methylretinal (2), [10,20-$^{13}C_2$]-10-Methylretinal (2a), [10-CH_3,13-$^{13}C_2$]-10-Methylretinal (2b), 10-Thiomethylretinal (19), 10-Iodoretinal (20), 14-Methylretinal (24), 14-Thiomethylretinal (25), 14-Iodoretinal (26), 2,3-Dimethyl-2-butenenitrile (27), 3-Methyl-2-thiomethyl-2-butenenitrile (28), 2-Iodo-3-methyl-2-butenenitrile (29), 2-Methyl-2-butenenitrile (30), 2-Thiomethyl-2-butenenitrile (31), 2-Iodo-2-butenenitrile (32). The Data Are Obtained Using Electron Impact (EI, 70 eV) Double Focus Mass Spectrometry

compd	molecular formula	calcd mass (a.m.u.)	experimental mass (a.m.u.)
2	$^{12}C_{21}{}^{1}H_{30}{}^{16}O_1$	298.22967	298.23106
2a	$^{12}C_{20}{}^{13}C_1{}^{1}H_{30}{}^{16}O_1$	300.23638	300.23682
2b	$^{12}C_{20}{}^{13}C_1{}^{1}H_{30}{}^{16}O_1$	300.23638	300.23855
19	$^{12}C_{21}{}^{1}H_{30}{}^{16}O_1{}^{32}S_1$	330.20174	330.20029
20	$^{12}C_{20}{}^{1}H_{27}{}^{127}I_1{}^{16}O_1$	410.11054	410.11400
24[b]	$^{12}C_{21}{}^{1}H_{30}{}^{16}O_1$	299.23749[c]	299.23618[c]
25	$^{12}C_{21}{}^{1}H_{30}{}^{16}O_1{}^{32}S_1$	330.20174	330.20015
26	$^{12}C_{20}{}^{1}H_{27}{}^{127}I_1{}^{16}O_1$	410.11054	410.11180

[a] No data available. [b] Measurement performed using chemical ionization. [c] Mass of $[M + H]^{+\bullet}$.

peak at $m/z = 300$, providing evidence that the two ^{13}C labels have been incorporated into the molecular skeleton of the compounds. The single focus electron impact mass spectra of the other analogues of retinal, 10-thiomethylretinal (**19**), 10-iodoretinal (**20**), 14-methylretinal (**24**) (CI method used), 14-thiomethylretinal (**25**), and 14-iodoretinal (**26**) appear also as typical retinoid mass spectra, with their respective base peaks at $m/z = 330$, 410, 330, 299, and 410, the molecular ion peaks. All spectra of the retinal analogues showed a peak due to the expulsion of a CH_3 unit and a strong peak at $m/z = 149$, assigned to the β-cyclocitral ethene cation, as for retinal.[9] The spectrum of **20** reveals the presence of an iodine substituent in the molecular structure by a peak at $m/z = 283$, assigned to the fragment that is formed by expulsion of the iodine atom. The molecular ion peak in the spectrum of **24** is assigned to the $[M + H]^{+\bullet}$, caused by the use of the chemical ionization technique. The use of the electron impact as ionization technique resulted in the absence of the $M^{+\bullet}$ peak in the mass spectrum, probably due to complete fragmentation of the molecular ion before it is detected.

Double focus mass spectrometry is used to determine the exact mass of the synthesized compounds. Table 1 lists the experimentally determined molecular masses of all synthesized retinal compounds. The experimentally found exact masses of the synthesized compounds are all according to expectations. The difference between the calculated and experimentally determined exact masses is never greater than 0.0008%. The degree of incorporation of the ^{13}C labels in the isotopomers of 10-methylretinal are inferred from the double-focus mass spectra. This is performed by comparing the exact mass of the $M^{+\bullet}$ peak with the $[M - 1]^{+\bullet}$ and $[M - 2]^{+\bullet}$ peak, after correction for the natural abundance contributions to the intensity of the peaks. Both doubly ^{13}C-labeled isotopomers have a label incorporation of ≥98%. This implies that the label incorporation at the individual positions in the isotopomers amounts to ≥99%. This is also shown by the ^1H NMR spectra of the labeled compounds (see below). The commercially available ^{13}C-labeled starting compounds we used for the synthesis of the retinals have a label incorporation of ≥99%, indicating that no loss of label has occurred during the synthetic steps.

NMR. The NMR spectra which are discussed in this section are enclosed in the Supporting Information.

The proton spectra of the *all-E* isomers of 10-methylretinal (**2**), [10,20-$^{13}C_2$]-10-methylretinal (**2a**) and [(10-CH_3),13-$^{13}C_2$]-10-methylretinal (**2b**), were recorded in chloroform at a proton resonance frequency of 300 MHz. The spectra can be assigned by comparison to the ^1H spectrum of retinal, that has been known in the literature for a long time.[9]

The substitution at the C10 position of retinal by a methyl moiety as for 10-methylretinal can be inferred from the proton spectrum. The H10 response, with $\delta =$ 6.18 ppm for retinal, is not present in the spectrum of **2**. Moreover, the AMX pattern including the H11 response in the retinal spectrum has changed to an AX pattern comprising the resonance of H11 at $\delta = 7.42$ ppm and H12 at $\delta = 6.44$ ppm. In the downfield region of the spectrum of natural abundance *all-E*-10-methylretinal, an aldehyde and five other resonances can be observed, corresponding to the five inequivalent vinylic protons, present in the molecular structure.

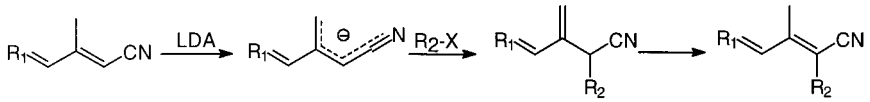
The incorporation of the ^{13}C labels can be inferred from the proton spectra by comparing them to the spectrum of the natural abundance 10-methylretinal. The incorporation of the ^{13}C label at the C10 position can be clearly observed in the resonance of H8 at $\delta = 6.68$ ppm: an extra splitting of the doublet due to the J coupling is observed. The resonance of H12 at $\delta = 6.44$ ppm has changed from a doublet in natural abundance 10-methylretinal to a double doublet, due to the extra three-bond couplings with both $^{13}C10$ and $^{13}C20$. The presence of $^{13}C20$ is also inferred from the coupling of $^{13}C20$ with the H14 resonance. In the aliphatic region of the spectrum of *all-E*-[10,20-$^{13}C_2$]-10-methylretinal, the H20 singlet is now split into a doublet, confirming the position of the ^{13}C methyl label. Within experimental error, no signal in the middle of the doublet can be observed, proving that the label incorporation is very high. In a similar fashion, the spectrum of *all-E*-[10-CH_3,13-$^{13}C_2$]-10-methylretinal can be explained. The presence of both ^{13}C labels is reflected in the H11 resonance, that is split into a double double doublet, due the combined three-bond couplings with the ^{13}C at the C10 methyl moiety and the $^{13}C13$. The remaining vinylic resonances are unaffected by the ^{13}C incorporation.

The proton spectra of *all-E*-10-methylretinal clearly prove that the 10 position is substituted with a methyl group. Moreover, the chemical shifts and J coupling constants are in agreement with an *all-E* configuration. The spectra of *all-E*-[10,20-$^{13}C_2$]-10-methylretinal and *all-E*-[10-CH_3,13-$^{13}C_2$]-10-methylretinal indicate that the ^{13}C labels are at their predetermined positions and, within experimental error, incorporated for 99%.

The 300.1 MHz ^1H NMR spectra of the *all-E* isomers of the other retinal analogues 10-thiomethylretinal (**19**), 10-iodoretinal (**20**), 14-methylretinal (**24**), 14-thiomethylretinal (**25**), and 14-iodoretinal (**26**) have been recorded in deuterated chloroform.

The substitution at the C10 position of retinal by a thiomethyl moiety and an iodine atom, as for **19** and **20**, respectively, can be inferred from the proton spectrum. For 10-thiomethylretinal no H10 resonance is present. Instead, the resonance of H11 appears in an AB pattern, due to the J coupling with H12 at $\delta = 7.26$. The presence

Scheme 5. Proposed Reaction Mechanism for the 2-Functionalization of 3-Methyl-2-butenenitriles



of the 10-thiomethyl moiety is reflected in the spectrum by the strong additional singlet at $\delta = 2.38$ ppm. The strong magnetic anisotropy effect of the iodine atom is reflected in the upfield shift for the H11 resonance[25] compared to the chemical shifts for the H11 proton in *all-E*-retinal at $\delta = 7.14$ ppm.

Substitution at the C14 position of the retinal chain, as for 14-methylretinal (**21**), 14-thiomethylretinal (**22**), and 14-iodoretinal (**23**), results in the disappearance of the H14 signal. For all three compounds, the spectra lack the H14 signal, and contain the 15 aldehyde resonance appearing as a singlet, since the three-bond *J* coupling with H14 is no longer present. The introduction of the iodine atom at C14 in **23** is reflected in the upfield shift of the aldehyde resonance to $\delta = 9.26$ ppm, as compared to the H15 shift for *all-E*-retinal. The overall structure of all three 14-substituted retinals is supported by the ^{1}H NMR data.

The ^{1}H-noise-decoupled 75.4 MHz ^{13}C NMR spectra of *all-E*-10-methylretinal (**2**), *all-E*-[10,20-$^{13}C_2$]-10-methylretinal (**2a**), and *all-E*-[10-CH_3,13-$^{13}C_2$]-10-methylretinal (**2b**) were also recorded in chloroform. The spectrum of natural abundance *all-E*-10-methylretinal shows 20 carbon resonances, in agreement with the structure of *all-E*-10-methylretinal, that comprises 21 carbon atoms, of which two (C16 and C17) are equivalent. The resonance at $\delta = 190.99$ originates from the C15 aldehyde carbon. The vinylic region of the spectrum contains five resonances with a large amplitude, representing the vinylic carbons possessing a proton and five smaller amplitude resonances, from the quaternary carbons. The aliphatic region of the spectrum shows the remaining nine carbon resonances, representing the ionone ring and the methyl substituent carbons, confirming the structure of *all-E*-10-methylretinal.

^{13}C NMR is used to prove that the incorporation of the ^{13}C isotopes in *all-E*-10-methylretinal at their predetermined positions has been successful. Both spectra of the labeled compounds show two strong resonances, flanked by weak signals from the natural abundance background ^{13}C. Comparison of the position of the label signals in the spectrum with the spectrum of natural abundance *all-E*-10-methylretinal, indicates the position of the label.

From the NMR experiments we can conclude that the all *all-E*-isomers of the synthesized compounds were obtained without presence of other isomers or compounds. Concomitantly, the purity of the samples is close to 100%.

Discussion

The mechanism for the 2-functionalization reaction for 3-methyl-2-butenenitriles presented in this paper is depicted in Scheme 5. One of the γ hydrogens at the 3-methyl moiety, that are relatively acidic because of the conjugation effect of the corresponding anion with the nitrile group, is abstracted by the LDA as a strong base. Because of the conjugation effect, negative charge density will be spread out in the region between the nitrogen atom and the γ position. Hence, for a deprotonated 3-methyl-2-butenenitrile, a nucleophilic attack can either occur from the 4- or the 2-position. The nitriles applied in this research, however, *exclusively* give 2-substitution products, the expected product of a kinetic reaction between the nitrile anion and electrophiles. The conditions are such that no unreacted material is present nor further reaction to disubstitution takes place. This substantially increases the synthetic versatility of this reaction. After nucleophilic coupling, the structure of the product must be a β,γ-unsaturated nitrile. With our reaction conditions, however, the 2-substituted products all give immediate isomerization to the "reconjugated" compound, presumably promoted by the basic conditions in the reaction mixture.

It is particularly important to maintain the reaction temperature at values close to −80 °C throughout the whole reaction. If the reaction temperature is allowed to rise to room temperature after the addition of the electrophile, a complex mixture of products is observed that might be explained by competition by a Thorpe type reaction,[26] resulting in nitrile adduct products. If the temperature is raised to room temperature before the addition of the electrophiles, no substitution products can be observed at all, but only unidentified products.

This novel reaction allowed us to introduce the ^{13}C methyl moiety in a one-step procedure. This reduces the costs for the preparation of the labeled compound substantially. In the recent past, our group has developed efficient routes for the introduction of ^{13}C labels at any position or combination of positions in the skeleton of retinal.[21] These methods can easily be adapted to the synthesis of labeled 10-methylretinals. Thus, ^{13}C-labeled 10-methylretinals can be synthesized with labels at any position or combination of positions.

It is anticipated that the 2-functionalization reaction for 3-methyl-2-butenenitriles as presented in this paper will have a wide applicability in the synthesis of chemically modified retinals. Retinal analogues have played an important role in retinal protein structure and function research during the past decades.[11] A large variety of 10- and 14-substituted retinals has been synthesized. Among them, 10-chlororetinal,[27] 10-fluororetinal,[28,29] 10-methylretinal,[28,30] 10-ethylretinal,[28] 14-methylretinal, and 14-fluororetinal.[31,32] A second set of retinal analogues contains a ring system, attached to the C10 position in retinal, among them, 10,20-methanoreti-

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nal,[33,34] 10,20-ethanoretinal,[35,36] 10,20-propanoretinal,[37,38] 10,12-ethanoretinal,[39] and 10,12-propanoretinal.[44] A large variety of synthetic steps has been applied to introduce the substituent at the C10 or C14 position. The reaction presented in this paper appears to be a versatile method to introduce a variety of substituents on the 10 and 14 positions of retinal in excellent yields. Moreover, the scheme can be adapted to introduce substituents at both the 10- and the 14-position of retinal. To demonstrate the scope of the reaction we reacted 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**) and 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**) with a selection of electrophiles. From the wide variety of electrophiles available, we chose iodomethane, methyl thiocyanate, and elemental iodine. This choice is not trivial. We tried to cover the characteristics of different electrophiles. Iodomethane is a soft electrophile that represents the family of alkyl halogenides. Since the introduction of the methyl moiety in 10-methylretinal is so effective, one may anticipate that other alkyl halogenides, such as iodoethane, will react in a similar fashion. In this way the sequence will allow for the easy synthesis of, for example, 10-ethylretinal or 14-ethylretinal, two retinal analogues that are known in the literature.[45]

Another class of well-studied chemically modified retinoids include ring structures.[11] The newly developed 2-functionalization reaction for 3-methyl-2-butenenitriles will facilitate the synthesis of these kind of ring-containing retinal analogues via coupling with bifunctional electrophiles, that allow ring closure of the system.

Methyl thiocyanate is an electrophile with a cyanide moiety as leaving group. With this electrophile, 10-thiomethylretinal (**19**) and 14-thiomethylretinal (**25**) have been synthesized. In a similar fashion, different heteroatom-containing retinal analogues can be synthesized by applying different electrophiles.

10-Iodoretinal is synthesized by using iodine as electrophile. This novel analogue adds to the list of 10-halogenated retinal analogues. 10-Fluororetinal[29,30] and 10-chlororetinal[27] are already described in the literature. Applying the same reaction procedure with bromine as electrophile will allow for the synthesis of 10-bromoretinal. The presence of a iodo substituent in retinal may be very important, if good three-dimensional crystals of rhodopsin will be available in the future. These crystals will be investigated using X-ray crystallography. This technique, probing the overall structure of the protein, is only successful if the so-called phase problem can be solved. This requires the presence of a heavy atom at a known position in the crystal. The iodine atom, with a nuclear mass of 127, can serve to solve the phase problem when the 10-iodoretinal analogue is bound in the binding pocket of the rhodopsin crystals.

The novel 2-functionalization reaction for 3-methyl-2-butenenitriles does not limit itself to the synthesis of retinals. Other biomolecules of which the molecular skeleton comprises isoprene units, such as carotenoids, possess the same structural units as for retinals. The function of proteins containing these carotenoids as chromophores are also investigated using chemically modified skeletons.[46−48] The new procedure can be exploited for efficient synthesis of known and novel chemically modified carotenoids.

As shown, the reaction is also applicable to the smaller nitriles **5** and **6**, in the same high yields. Since the nitrile moiety of these substituted unsaturated compounds can be functionalized to different groups, e.g., carboxylic acids, ketones, or aldehydes, this synthetic approach opens the way to efficient introduction of substituents on vinylic carbons.

Conclusion

In this paper we presented an efficient method for the introduction of substituents at the 2-position of 3-methyl-2-butenenitriles. The procedure is simple, can be applied with very different electrophiles, and yields exclusively the desired 2-functionalization in high yields. We demonstrated the versatility of the reaction with the preparation of two doubly ^{13}C-labeled 10-methylretinals and five 10- and 14-substituted retinals, four of which are novel compounds which have a wide application in retinal proteins function research. In this way, starting from 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile and 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile, 10-methylretinal (31% overall yield), [10,20-$^{13}C_2$]-10-methylretinal (3% overall yield), [10-CH_3,13-$^{13}C_2$]-10-methylretinal (5% overall yield), 14-methylretinal (61% overall yield), 14-thiomethylretinal (66% overall yield), 14-iodoretinal (11% overall yield), 10-thiomethylretinal (11% overall yield), and 10-iodoretinal (23% overall yield) were synthesized. [10,20-$^{13}C_2$]-10-Methylretinal, [10-CH_3,13-$^{13}C_2$]-10-methylretinal, 10-thiomethyl-, 14-thiomethyl, 10-iodo-, and 14-iodoretinal are novel retinal compounds. 10-Methylretinal and 14-methylretinal are known modified retinals that have been presented in the literature, albeit synthesized via different approaches.

Starting from 3-methyl-2-butenenitrile and 2-butenenitrile, we synthesized 2,3-dimethyl-2-butenenitrile (53% yield), 3-methyl-2-thiomethyl-2-butenenitrile (80% yield),

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2-iodo-3-methyl-2-butenenitrile (89% yield), 2-methyl-2-butenenitrile no pure compound was isolated), 2-thiomethyl-2-butenenitrile (28% yield), and 2-iodo-2-butenenitrile (14% yield). To our knowledge, 3-methyl-2-thiomethyl-2-butenenitrile, 2-iodo-3-methyl-2-butenenitrile, 2-thiomethyl-2-butenenitrile, and 2-iodo-2-butenenitrile are novel compounds.

Experimental Section

General Experimental Procedures. All commercially available chemicals were purchased at either Acros, Aldrich, Fluka, or Merck and were used without further purification. The ^{13}C-labeled compounds were purchased at Cambridge Isotope Laboratories (MA). In all cases, the *chemically pure* or higher quality of the chemicals was used. Petroleum ether refers to the distillate with boiling range 40−60 °C. Dry solvents and reagents were stored under a dry argon atmosphere. Dry argon and nitrogen was prepared by leading the gas through a column of sodium hydroxide pellets. Dry tetrahydrofuran (THF) was prepared by storing THF one week on 4 Å molecular sieves. Dry petroleum ether was prepared by distilling from phosphorus pentoxide and was stored on sodium wire. Dry diisopropylamine, dichloromethane, and chlorotrimethylsilane were prepared by distilling from freshly grinded calcium hydride and stored on 4 Å molecular sieves. Reactions were followed with thin-layer chromatography using Merck silica gel (60 F_{254}). Spots were detected with UV-light (254 nm) or by spraying the chromatogram with a coloring spray (2 g of potassium permanganate and 4 g of sodium hydrogen carbonate in 100 mL of water). Silica gel column chromatography was performed using Merck silica gel 60 (230−400 Mesh). HPLC purifications were performed using a preparative Zorbax silica gel column 21.2 mm × 25 cm (Du Pont, Delaware). ^1H NMR spectra were recorded at 199.50 and 300.13 MHz, respectively, and were internally referenced to the protons of tetramethylsilane that resonate at $\delta = 0$ ppm. ^{13}C NMR spectra were recorded using ^1H decoupling at 50.10 and 75.48 MHz, respectively, and were internally referenced to the carbon of deuteriochloroform which resonates at $\delta =$ 77.00 ppm. Mass spectra were recorded on a Finnigan MAT 900, equipped with a programmable direct insertion probe (DIP). Temperature was raised from 30 to 300 °C in 5 min. Exact mass determination was performed using the peak matching procedure with perfluorokerosine as internal calibration compound. UV−vis spectra were recorded on a Varian DMS 200 spectrophotometer. All manipulations with isomerically pure retinoids were performed in dim red light ($\lambda > 620$ nm) or in the dark. All compounds are named according to the IUPAC convention on organic nomenclature. Compounds comprising the retinoid structure are named according to the IUPAC convention on carotenoid nomenclature.

General Procedure for Conversion of 2-Butenenitriles into 2-Substituted Derivatives via Anion Formation and Treatment with Electrophiles (from now on referred to as procedure 1). A three-necked round-bottomed flask of suitable size, was equipped with a Teflon magnetic stirrer, a pentane thermometer, and a dropping funnel. The complete setup was flame-dried using a Bunsen burner, while continuously flushed with dry nitrogen, using two needles through septa as inlet and outlet for the gas stream. The nitrogen flow was continued during the complete reaction until aqueous workup. The flask was loaded with a mixture of 1.55 equiv of dry diisopropylamine and dry THF. The solution was cooled to −85 °C, and via a syringe, 1.5 equiv of 1.6 M butyllithium in hexanes were added and the mixture was stirred for 15 min. At this temperature, 1.0 equiv of the 3-methyl-2-butenenitrile in 20 mL of dry THF was added via the dropping funnel. The mixture was stirred for 20 min and while still at −85 °C 1.5 equiv of electrophile was added. The temperature was maintained at −85 °C until the starting compound had disappeared according to TLC. Subsequently, a saturated solution of ammonium chloride was added. The mixture was allowed to warm to room temperature, and the aqueous and organic layers were separated in a separation funnel. The aqueous layer was extracted three times with diethyl ether and subsequently the organic layers were combined and washed with a saturated sodium chloride solution. The organic layer was dried over magnesium sulfate and after filtration of the solids, the crude product was concentrated in vacuo. The product was purified using silica gel column chromatography, using diethyl ether/petroleum ether as eluent.

General Procedure for the Phosphonate Coupling of an Aldehyde (from now on referred to as procedure 2). A three-necked round-bottomed flask of suitable size was equipped with a Teflon magnetic stirrer, a pentane thermometer, and a dropping funnel. The complete setup was flame-dried using a Bunsen burner, while continuously flushed with dry nitrogen, using two needles through septa as inlet and outlet for the gas stream. The nitrogen flow was continued during the complete reaction until aqueous workup. The flask was loaded with a mixture of 1.5 equiv of the phosphono compound and approximately 100 mL of dry THF. The solution was cooled to −80 °C, and via a syringe, 1.55 equiv of 1.6 M butyllithium in hexanes was slowly added. The mixture was stirred for 20 min at −80 °C, and via the dropping funnel, 1.0 equivalent of aldehyde in 15 mL of dry THF was added. The mixture was allowed to warm to room temperature and stirred for 2 h. To quench anions, a saturated ammonium chloride solution was added, and the aqueous and organic layers were separated in a separation funnel. The aqueous layer was extracted three times with diethyl ether, and subsequently the organic layers were combined and washed with a saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, and after filtration of the solids, the crude product was concentrated in vacuo. Removal of excess phosphono compound was effected with a silica gel flash column using diethyl ether/petroleum ether (1:1 v/v) as eluent. The eluate was subsequently concentrated in vacuo.

General Procedure for the DIBALH Reduction of a Nitrile (from now on referred to as procedure 3). A three-necked round-bottomed flask of suitable size was equipped with a Teflon magnetic stirrer and a pentane thermometer. The complete setup was flame-dried using a Bunsen burner, while continuously flushing with dry nitrogen, using two needles through septa as inlet and outlet for the gas stream. The nitrogen flow was continued during the complete reaction until aqueous workup. The flask was loaded with a mixture of 1.0 equiv of nitrile and approximately 100 mL of dry petroleum ether. The solution was cooled to −50 °C. Via a syringe, 2.5 equiv of 1.0 M DIBALH in hexanes was slowly added. The mixture was stirred for 20 min at −50 °C. Subsequently 1.75 g of silica gel slurry (100 g of SiO_2 and 37.5 mL of distilled water), for each milliliter of DIBALH used, was added. The thick slurry was stirred at room temperature for 2 h. To absorb the water content, magnesium sulfate was added. The solids were filtered off and thoroughly rinsed with diethyl ether. The filtrate was concentrated in vacuo, and the product was purified using silica gel column chromatography, using diethyl ether/petroleum ether (1:4 v/v) as eluent.

General Procedure for the Separation of a Isomer Mixture of Retinoids Using HPLC. Typically, 1−2 mL of the stock solution of the mixture of isomers in *n*-pentane (∼10 mg/mL) was injected on the silica gel column and separated using diethyl ether/petroleum ether (1:4 v/v) as mobile phase. The working pressure was approximately 60 bar and the flow rate 15 mL/min. The eluted isomers were detected using a UV−vis detector operating at 360 nm. The *all-E* isomer is always the fraction with the longest retention time. The pure isomers were collected in a round-bottomed flask, that was packed in aluminum foil to prevent isomerization. Subsequently, the isomers were spectroscopically characterized vide infra. The isomers were concentrated in vacuo in the dark and stored in *n*-pentane at −80 °C.

10-Methylretinal (2). 1.41 g (4.77 mmol) of 3,6,7-trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**12**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield

1.12 g (79%) as a yellow oil. *all-E*-**2**:^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.13 (d, 1H, J = 8.2 Hz); 7.42 (d, 1H, J = 15.5 Hz); 6.68 (d, 1H, J = 16.0 Hz); 6.44 (d, 1H, J = 15.5 Hz); 6.39 (d, 1H, J = 16.0 Hz); 6.03 (d, 1H, J = 8.2 Hz); 2.36 (s, 3H); 2.09 (s, 3H); 2.04 (t, 2H, J = 6.0 Hz); 1.99 (s, 3H); 1.75 (s, 3H); 1.63 (m, 2H); 1.49 (m, 2H); 1.05 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 190.99; 155.37; 138.24; 137.10; 135.43; 132.64; 130.55; 130.44; 130.12; 129.48; 129.02; 39.49; 34.16; 33.07; 28.94; 21.81; 19.12; 14.16; 13.83; 13.17 ppm. UV−vis (*n*-hexane) λ_{max} 371 nm.

[10,20-$^{13}C_2$]-10-Methylretinal (2a). 130 mg (0.44 mmol) of [(3-CH_3),6-$^{13}C_2$]-3,6,7-trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**12a**) was reacted with DIBALH following procedure 2. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 115 mg (88%) as a yellow oil. *all-E*-**2a**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.13 (d, 1H, J = 8.2 Hz); 7.42 (d, 1H, J = 15.5 Hz); 6.68 (dd, 1H, J = 16.0 Hz, J = 3.6 Hz); 6.44 (ddd, 1H, J = 15.5 Hz, J = 5.1 Hz, J = 5.1 Hz); 6.39 (d, 1H, J = 16.0 Hz); 6.03 (dd, 1H, J = 8.2 Hz, J = 7.8 Hz); 2.36 (d, 3H, J = 127.7 Hz); 2.09 (d, 3H, J = 6.1 Hz); 2.04 (t, 2H, J = 6.0 Hz); 1.99 (d, 3H, J = 4.9 Hz, 10-CH_3); 1.75 (s, 3H); 1.63 (m, 2H); 1.49 (m, 2H); 1.05 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 129.53; 13.23 ppm. MS (EI, 70 eV): $^{12}C_{19}{}^{13}C_2{}^1H_{30}{}^{16}O_1$; experimental mass 300.23682; theoretical mass 300.23638; m/z 300 ($M^{+\bullet}$).

[(10-CH_3),13-$^{13}C_2$]-10-Methylretinal (2b). 260 mg (0.88 mmol) of [3,(6-CH_3)-$^{13}C_2$]-3,6,7-trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**12b**) was reacted with DIBALH following procedure 2. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 230 mg (88%) as a yellow oil. *all-E*-**2b**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.13 (d, 1H, J = 8.2 Hz); 7.42 (ddd, 1H, J = 15.5 Hz, J = 5.6 Hz, J = 5.6 Hz); 6.68 (d, 1H, J = 16.0 Hz); 6.44 (dd, 1H, J = 15.5 Hz, J = 2.3 Hz); 6.39 (d, 1H, J = 16.0 Hz); 6.03 (d, 1H, J = 8.2 Hz); 2.36 (d, 3H, J = 6.0 Hz); 2.09 (s, 3H); 2.04 (t, 2H, J = 6.0 Hz); 1.99 (d, 3H, J = 126.0 Hz, 10-CH_3); 1.75 (s, 3H); 1.63 (m, 2H); 1.49 (m, 2H); 1.05 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 155.47; 13.90 (10-CH_3) ppm. MS (EI, 70 eV): $^{12}C_{19}{}^{13}C_2{}^1H_{30}{}^{16}O_1$; experimental mass 300.23835; theoretical mass 300.23638; m/z 300 ($M^{+\bullet}$).

3-Methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (3). 17.0 g (89 mmol) of β-ionone (**7**) was reacted with 2-(diethylphosphono)acetonitrile as phosphonate following procedure 2. Yield 15.0 g (79%) as a light yellow oil. *all-E*-**3**: ^{1}H NMR (199.5 MHz, $CDCl_3$): δ 6.57 (d, 1H, J = 16.1 Hz); 6.15 (d, 1H, J = 16.1 Hz); 5.16 (s, 1H); 2.20 (s, 3H); 2.05 (t, 2H, J = 6.1 Hz); 1.76 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.03 (s, 6H) ppm. The synthesis and characterization of this compound has been previously described in the literature.[49]

[2-^{13}C]-3-Methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (3a). A dry reaction setup was loaded with a mixture of 3.50 mL (25.0 mmol) of dry lithium diisopropylamide and 100 mL of dry THF. The solution was cooled to −80 °C using a cooling bath with a mixture of ethanol and liquid nitrogen. Via a syringe, 15.2 mL (24.3 mmol) of 1.6 M butyllithium in hexanes was slowly added. The mixture was stirred for 20 min at −80 °C, and via the dropping funnel, 0.50 g of (11.9 mmol) [2-^{13}C]-acetonitrile in 15 mL dry THF was added. The mixture was stirred for 20 min at −80 °C, and subsequently 1.80 mL (12.5 mmol) diethyl chlorophosphate in 15 mL of dry THF was added via the dropping funnel. The mixture was allowed to warm to room temperature and stirred for 1 h. Via the dropping funnel, 2.51 g (13.7 mmol) of β-ionone (**7**) in 25 mL of dry THF was added. After 2 h stirring at room temperature, a saturated ammonium chloride solution was added, and the aqueous and organic layers were separated in a separation funnel. The aqueous layer was extracted three times with diethyl ether, and subsequently the organic layers were combined and washed with a saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, and after filtration of the solids, the crude product was concentrated in vacuo. Removal of excess phosphono compound was effected with a silica gel flash column using diethyl ether/petroleum ether (1:1 v/v) as eluent. The eluate was subsequently concentrated in vacuo. Yield 2.48 g (96%) as a light yellow oil. *all-E*-**3a**: ^{1}H NMR (199.5 MHz, $CDCl_3$): δ 6.57 (d, 1H, J = 16.1 Hz); 6.15 (dd, 1H, J = 16.1 Hz, J = 5.5 Hz); 5.16 (d, 1H, J = 172.0 Hz); 2.20 (d, 3H, J = 5.8 Hz); 2.05 (t, 2H, J = 6.1 Hz); 1.76 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.03 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 95.94 ppm.

2,3-Dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (8). 0.70 g (3.2 mmol) of *all-E*-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**) was reacted with iodomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. Yield 0.72 g (96%) as a light yellow oil. *all-E*-**8**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.52 (d, 1H, J = 16.0 Hz); 6.42 (d, 1H, J = 16.0 Hz); 2.20 (s, 3H); 2.04 (t, 2H, J = 6.0 Hz); 1.98 (s, 3H); 1.72 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.03 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 148.21; 137.17; 134.51; 131.64; 128.27; 120.65; 103.92; 39.30; 33.94; 32.93; 28.71; 21.55; 18.88; 18.07; 15.46 ppm.

[2-^{13}C]-2,3-Dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (8a). 2.95 g (13.6 mmol) of [2-^{13}C]-2*E*,4*E*-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3a**) was reacted with iodomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 3.08 g (98%) as a light yellow oil. *all-E*-**8a**: ^{1}H NMR (199.5 MHz, $CDCl_3$): δ 6.52 (d, 1H, J = 16.0 Hz); 6.42 (dd, 1H, J = 16.0 Hz, J = 3.1 Hz); 2.20 (d, 3H, J = 6.5 Hz); 2.04 (t, 2H, J = 6.0 Hz); 1.98 (d, 3H, J = 2.5 Hz); 1.72 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.03 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 103.42 ppm.

[(2-CH_3)-^{13}C]-2,3-Dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (8b). 3.54 g (16.4 mmol) of 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**) was reacted with [^{13}C]-iodomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 3.60 g (95%) as a light yellow oil. *all-E*-**8b**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.52 (d, 1H, J = 16.0 Hz); 6.42 (d, 1H, J = 16.0 Hz); 2.20 (s, 3H); 2.04 (t, 2H, J = 6.0 Hz); 1.98 (d, 3H, J = 133.4 Hz); 1.72 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.03 (s, 6H) ppm. ^{13}C NMR (75.4 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 15.47 ppm.

2,3-Dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (9). 6.0 g (26 mmol) of *all-E*-2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**8**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. Yield 5.6 g (92%) as a light yellow oil. *all-E*-**9**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.27 (s, 1H); 6.76 (d, 1H, J = 16.1 Hz); 6.68 (d, 1H, J = 16.1 Hz); 2.33 (s, 3H); 2.06 (t, 2H, J = 6.0 Hz); 1.88 (s, 3H); 1.77 (s, 3H); 1.63 (m, 2H); 1.49 (m, 2H); 1.07 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 191.57;0.149.43; 137.49; 134.55; 132.11; 132.00; 131.97; 39.34; 33.96; 33.00; 28.73; 21.58; 18.85; 12.38; 10.39 ppm.

[2-^{13}C]-2,3-Dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (9a). 3.60 g (15.6 mmol) of 2*E*/*Z*,4*E*-[2-^{13}C]-2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**8a**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. Yield 2.00 g (55%) as a light yellow oil. *all-E*-**9a**: ^{1}H NMR (199.5 MHz, $CDCl_3$): δ 10.27 (d, 1H, J = 23.0 Hz); 6.76 (d, 1H, J = 16.1 Hz); 6.68 (dd, 1H, J = 16.1 Hz, J = 2.4

(49) Pardoen, J. A.; Neijenhesch, H. N.; Mulder, P. P. J.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 341.

(50) IUPAC Commission on Nomenclature of Organic Chemistry and IUPAC IUB Commission on Biochemical Nomenclature. *Pure Appl. Chem.* **1975**, *41*, 407.

Hz); 2.33 (d, 3H, J = 4.1 Hz); 2.06 (t, 2H, J = 6.0 Hz); 1.88 (d, 3H, J = 6.2 Hz); 1.77 (s, 3H); 1.63 (m, 2H); 1.49 (m, 2H); 1.07 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^1H-noise-decoupled, $CDCl_3$): δ 132.14 ppm.

[(2-CH_3)-^{13}C]-2,3-Dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (9b). 3.08 g (13.4 mmol) of [(2-CH_3)-^{13}C]-2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**8a**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. Yield 2.10 g (67%) as a light yellow oil. *all-E*-**9b**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 10.27 (d, 1H, J = 2.7 Hz); 6.76 (d, 1H, J = 16.1 Hz); 6.68 (d, 1H, J = 16.1 Hz); 2.33 (s, 3H); 2.06 (t, 2H, J = 6.0 Hz); 1.88 (d, 3H, J = 127.7 Hz); 1.77 (s, 3H); 1.63 (m, 2H); 1.49 (m, 2H); 1.07 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^1H-noise-decoupled, $CDCl_3$): δ 10.48 ppm.

Ethyl 4,5-Dimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienoate (10). 5.60 g (24.8 mmol) of 2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (**9**) was reacted with ethyl 2-(diethylphosphono)acetate as phosphonate following procedure 2. Yield 4.99 g (70%) as a yellow oil. *all-E*-**10**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 8.02 (d, 1H, J = 15.5 Hz); 6.66 (d, 1H, J = 15.8 Hz); 6.42 (d, 1H, J = 15.8 Hz); 5.93 (d, 1H, J = 15.8 Hz); 4.23 (q, 2H, J = 7.2 Hz); 2.11 (s, 3H); 2.04 (t, 2H, J = 6.1 Hz); 1.94 (s, 3H); 1.74 (s, 3H); 1.61 (m, 2H); 1.49 (m, 2H); 1.31 (t, 3H, J = 7.2 Hz); 1.04 (s, 6H) ppm.

Ethyl [4-^{13}C]-4,5-Dimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienoate (10a). 2.00 g (8.60 mmol) of [2-^{13}C]-2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (**9a**) was reacted with ethyl 2-(diethylphosphono)acetate as phosphonate following procedure 2. Yield 1.95 g (74%) as a yellow oil. *all-E*-**10a**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 8.02 (dd, 1H, J = 15.5 Hz, J = 16.1 Hz); 6.66 (dd, 1H, J = 15.8 Hz, J = 3.4 Hz); 6.42 (d, 1H, J = 15.8 Hz); 5.93 (dd, 1H, J = 15.8 Hz, J = 5.1 Hz); 4.23 (q, 2H, J = 7.2 Hz); 2.11 (d, 3H, J = 5.8 Hz); 2.04 (t, 2H, J = 6.1 Hz); 1.94 (d, 3H, J = 6.2 Hz); 1.74 (s, 3H); 1.61 (m, 2H); 1.49 (m, 2H); 1.31 (t, 3H, J = 7.2 Hz); 1.04 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^1H-noise-decoupled, $CDCl_3$): δ 128.17 ppm.

Ethyl [1,4-CH_3-$^{13}C_2$]-4,5-Dimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienoate (10b). 2.10 g (9.0 mmol) of [(2-CH_3)-^{13}C]-2,3-dimethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (**9b**) was reacted with ethyl [1-^{13}C]-2-(diethylphosphono)acetate as phosphonate following procedure 2. To prevent loss of ^{13}C label, only 1.1 equiv of phosphonate and 1.15 equiv of butyllithium were used. Yield 1.27 g (46%) as a yellow oil. *all-E*-**10b**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 8.02 (ddd, 1H, J = 15.5 Hz, J = 5.7 Hz, J = 5.7 Hz); 6.66 (d, 1H, J = 15.8 Hz); 6.42 (d, 1H, J = 15.8 Hz); 5.93 (dd, 1H, J = 15.5 Hz, J = 2.4 Hz); 4.23 (q, 2H, J = 7.2 Hz); 2.11 (s, 3H); 2.04 (t, 2H, J = 6.1 Hz); 1.94 (d, 3H, J = 126.7 Hz); 1.74 (s, 3H); 1.61 (m, 2H); 1.49 (m, 2H); 1.31 (t, 3H, J = 7.2 Hz); 1.04 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^1H-noise-decoupled, $CDCl_3$): δ 167.63; 13.72 ppm.

[4-^{13}C]-N-Methoxy-N,4,5-trimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienamide (33a). A dry reaction setup was loaded with a mixture of 0.51 g (8.4 mmol) of *N*-methoxymethylamine and 100 mL of dry THF. The solution was cooled to −15 °C using an ice/salt cooling bath. Via a syringe, 5.22 mL (8.4 mmol) of 1.6 M butyllithium in hexanes was slowly added. The mixture was stirred for 20 min at −15 °C, and via the dropping funnel, 1.95 g (6.4 mmol) of ethyl [4-^{13}C]-4,5-dimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienoate (**10a**) in 15 mL of dry THF was added. The mixture was allowed to warm to room temperature and stirred for 2 h. To quench anions, a saturated ammonium chloride solution was added, and the aqueous and organic layers were separated in a separation funnel. The aqueous layer was extracted three times with diethyl ether, and subsequently the organic layers were combined and washed with a saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, and after filtration of the solids, the crude product was concentrated in vacuo. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. The eluate was subsequently concentrated in vacuo. Yield 1.95 g (74%) as a yellow oil. *all-E*-**33a**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 7.80 (dd, 1H, J = 14.6 Hz, J = 2.8 Hz); 6.37 (d, 1H, J = 16.1 Hz); 6.15 (d, 1H, J = 16.1 Hz); 6.07 (d, 1H, J = 14.6 Hz); 3.70 (s, 3H); 3.26 (s, 3H); 2.05 (s, 3H); 2.05 (s, 3H); 2.03 (t, 2H, J = 6.1 Hz); 1.71 (s, 3H); 1.61 (m, 2H); 1.62 (m, 2H); 1.03 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^1H-noise-decoupled, $CDCl_3$): δ 117.69 ppm.

5,6-Dimethyl-8-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-3,5,7-octatrien-2-one (11). A dry reaction setup was loaded with a mixture of 4.99 g (17.9 mmol) of ethyl 4,5-dimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienoate (**10**) and 30 mL of dry THF. The reaction mixture was cooled to −100 °C, and via a syringe, 10.3 mL (82.1 mmol) freshly distilled chlorotrimethylsilane was slowly added. 10.31 mL of 1.0 M methyllithium was added in aliquots of 0.25 equiv. The mixture was stirred for 2 h. To quench anions, a saturated ammonium chloride solution was added, and the aqueous and organic layers were separated in a separation funnel. The aqueous layer was extracted three times with diethyl ether, and subsequently the organic layers were combined and washed with a saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, and after filtration of the solids, the crude product was concentrated in vacuo. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. The eluate was subsequently concentrated in vacuo. Yield 3.69 g (80%) as a yellow oil. *all-E*-**11**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 7.80 (d, 1H, J = 15.6 Hz); 6.68 (d, 1H, J = 16.1 Hz); 6.48 (d, 1H, J = 16.1 Hz); 6.26 (d, 1H, J = 15.6 Hz); 2.32 (s, 3H); 2.13 (s, 3H); 2.05 (s, 3H); 2.03 (t, 2H, J = 6.1 Hz); 1.95 (s, 3H); 1.75 (s, 3H); 1.61 (m, 2H); 1.52 (m, 2H); 1.05 (s, 6H) ppm.

[1,5-$^{13}C_2$]-5,6-Dimethyl-8-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-3,5,7-octatrien-2-one (11a). A dry reaction setup was loaded with a mixture of 73 mg (3.0 mmol) of magnesium pallets 30 mL of dry diethyl ether. Via a syringe, 0.51 mL (3.5 mmol) of [^{13}C]-iodomethane was slowly added. During the addition of the iodomethane, the nitrogen flow was stopped to prevent evaporation of reactant. The mixture was slowly stirred while the diethyl ether started boiling, indicating the formation of the Grignard reagent. After 20 min, when all solid magnesium had reacted, 0.90 g (2.8 mmol) of [4-^{13}C]-*N*-methoxy-*N*,4,5-trimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienamide (**33a**) in 15 mL of dry THF was added via the dropping funnel. The mixture was stirred for 2 h. To quench anions, a saturated ammonium chloride solution was added, and the aqueous and organic layers were separated in a separation funnel. The aqueous layer was extracted three times with diethyl ether, and subsequently the organic layers were combined and washed with a saturated sodium chloride solution. The organic layer was dried over magnesium sulfate, and after filtration of the solids, the crude product was concentrated in vacuo. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. The eluate was subsequently concentrated in vacuo. Yield 130 mg (17%) as a yellow oil. *all-E*-**11a**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 7.80 (dd, 1H, J = 15.5 Hz, J = 1.8 Hz); 6.68 (dd, 1H, J = 16.1 Hz, J = 3.5 Hz); 6.48 (d, 1H, J = 16.1 Hz); 6.26 (dd, 1H, J = 15.5 Hz, J = 4.9 Hz); 2.32 (d, 3H, J = 127.0 Hz); 2.13 (d, 3H, J = 4.8 Hz); 2.05 (s, 3H); 2.03 (t, 2H, J = 6.1 Hz); 1.95 (d, 3H, J = 5.9 Hz); 1.75 (s, 3H); 1.61 (m, 2H); 1.52 (m, 2H); 1.05 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^1H-noise-decoupled, $CDCl_3$): δ 128.47; 27.82 ppm.

[2,(5-CH_3)-$^{13}C_2$]-5,6-Dimethyl-8-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-3,5,7-octatrien-2-one (11b). 1.27 g (4.2 mmol) ethyl of [1,(4-CH_3)-$^{13}C_2$]-4,5-dimethyl-7-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6-heptatrienoate (**10b**), 2.63 mL (20.9 mmol) of freshly distilled chlorotrimethylsilane, and 2.61 mL of 1.0 M methyllithium were reacted to yield **11b**. Yield 260 mg (23%) as a yellow oil. *all-E*-**11b**: ^1H NMR (199.5 MHz, $CDCl_3$): δ 7.80 (ddd, 1H, J = 15.6 Hz, J = 6.2 Hz, J = 6.2 Hz); 6.68 (d, 1H, J = 16.1 Hz); 6.48 (d, 1H, J = 16.1 Hz); 6.26 (dd, 1H, J = 15.6 Hz, J = 2.8 Hz); 2.32 (d, 3H, J = 5.8 Hz); 2.13 (s, 3H); 2.05 (s, 3H); 2.03 (t, 2H, J = 6.1 Hz); 1.95 (d, 3H, J =

126.7 Hz); 1.75 (s, 3H); 1.61 (m, 2H); 1.52 (m, 2H); 1.05 (s, 6H) ppm. ^{13}C NMR (50.0 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 198.57; 13.83 ppm.

3,6,7-Trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (12). 1.0 g (4.3 mmol) of 5,6-dimethyl-8-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-3,5,7-octatrien-2-one (**11**) was reacted with diethyl cyanomethylphosphonate as phosphonate following procedure 2. Yield 1.2 g (95%) as a light yellow oil. *all-E*-**12**: ^{1}H NMR (199.5 MHz, $CDCl_3$): δ 7.22 (d, 1H, J = 15.8 Hz); 6.67 (d, 1H, J = 16.1 Hz); 6.37 (d, 1H, J = 15.8 Hz); 6.30 (d, 1H, J = 16.1 Hz); 5.22 (s, 1H); 2.23 (s, 3H); 2.06 (s, 3H); 1.95 (s, 3H); 2.02 (t, 2H, J = 6.0 Hz); 1.75 (s, 3H); 1.62 (m, 2H); 1.48 (m, 2H, H′5); 1.06 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 157.04; 138.33; 137.10; 135.20; 132.29; 130.09; 130.01; 129.07; 127.21; 118.21; 96.48; 39.65; 34.27; 33.19; 29.05; 21.92; 19.29; 16.72; 14.24; 13.89 ppm.

[(3-CH_3),6-$^{13}C_2$]-3,6,7-Trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (12a). 130 mg (0.47 mmol) of [1,5-$^{13}C_2$]-5,6-dimethyl-8-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-3,5,7-octatrien-2-one (**11a**) was reacted with 2-(diethylphosphono)acetonitrile as phosphonate following procedure 1. Yield 100 mg (72%) as a yellow oil.

[(3,(6-CH_3)-$^{13}C_2$]-3,6,7-Trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (12b). 260 mg (0.95 mmol) of [2,(5-CH_3)-$^{13}C_2$]-5,6-dimethyl-8-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-3,5,7-octatrien-2-one (**11b**) was reacted with 2-(diethylphosphono)acetonitrile as phosphonate following procedure 1. Yield 250 mg (89%) as a yellow oil.

3-Methyl-2-thiomethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (13). 0.70 g (3.3 mmol) of 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**) was reacted with thiocyanomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 0.83 g (97%) as a light yellow oil. *all-E*-**13**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.90 (d, 1H, J = 16.1 Hz); 6.63 (d, 1H, J = 16.1 Hz); 2.41 (s, 3H); 2.27 (s, 3H); 2.04 (t, 2H, J = 6.0 Hz); 1.76 (s, 3H); 1.63 (m, 2H); 1.48 (m, 2H); 1.06 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 151.32; 136.93; 136.48; 133.35; 128.68; 116.17; 104.88; 39.54; 33.97; 33.27; 28.76; 21.64; 18.85; 18.85; 16.96 ppm.

2-Iodo-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (14). 2.50 g (11.6 mmol) of 3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**3**) was reacted with a solution of iodine in THF as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:19 v/v) as eluent. Yield 3.58 g (90%) as a light yellow solid. *all-E*-**14**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.80 (d, 1H, J = 15.8 Hz); 6.70 (d, 1H, J = 15.8 Hz); 2.19 (s, 3H); 2.03 (t, 2H, J = 6.2 Hz); 1.74 (s, 3H); 1.62 (m, 2H); 1.48 (m, 2H); 1.04 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 159.34; 136.60; 136.60; 133.57; 129.26; 117.87; 56.61; 39.39; 34.00; 33.25; 28.85; 22.65; 21.84; 18.88 ppm.

3-Methyl-2-thiomethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (15). 0.50 g (1.9 mmol) of 3-methyl-2-thiomethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**13**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:4 v/v) as eluent. Yield 0.17 g (34%) as a light yellow oil. *all-E*-**15**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.15 (s, 1H); 7.40 (d, 1H, J = 16.0 Hz); 6.89 (d, 1H, J = 16.0 Hz); 2.41 (s, 3H); 2.21 (s, 3H); 2.09 (t, 2H, J = 6.2 Hz); 1.80 (s, 3H); 1.65 (m, 2H); 1.50 (m, 2H); 1.10 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 188.56;0.156.11; 137.37; 136.63; 133.98; 133.08; 133.08; 39.64; 34.13; 33.43; 28.87; 21.80; 18.89; 17.65; 14.62 ppm.

2-Iodo-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (16). 0.52 g (1.5 mmol) of *all-E*-2-iodo-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienenitrile (**14**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 0.36 g (70%) as a light yellow solid. *all-E*-**16**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 9.27 (s, 1H); 6.79 (s, higher order AB, 1H); 6.79 (s, higher order AB, 1H); 2.54 (s, 3H); 2.11 (t, 2H, J = 6.2 Hz); 1.83 (s, 3H); 1.63 (m, 2H); 1.50 (m, 2H); 1.12 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 184.97;0.156.84; 139.27; 138.53; 136.98; 135.64; 108.61; 39.73; 34.02; 33.62; 28.84; 21.95; 18.77; 16.74 ppm.

3,7-Dimethyl-6-thiomethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (17). 0.19 g (0.72 mmol) of 3-methyl-2-thiomethyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (**15**) was reacted with 4-(diethylphosphono)-3-methyl-2-butenenitrile as phosphonate following procedure 2. Yield 0.12 g (51%) as a yellow oil. *all-E*-**17**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 7.39 (d, 1H, J = 16.2 Hz); 7.08 (d, 1H, J = 15.0 Hz); 7.00 (d, 1H, J = 15.0 Hz); 6.53 (d, 1H, J = 16.2 Hz); 5.32 (s, 1H); 2.26 (s, 3H); 2.13 (s, 3H); 2.10 (s, 3H); 2.06 (t, 2H, J = 6.0 Hz); 1.77 (s, 3H); 1.64 (m, 2H); 1.49 (m, 2H); 1.07 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 156.91; 144.73; 137.80; 133.80; 132.96; 132.21; 131.49; 131.36; 130.80; 118.08; 97.46; 39.65; 34.18; 33.27; 29.83; 21.81; 19.06; 18.48; 16.93; 15.38 ppm.

3,7-Dimethyl-6-iodo-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (18). 0.35 g (1.0 mmol) of 2-iodo-3-methyl-5-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4-pentadienal (**16**) was reacted with 4-(diethylphosphono)-3-methyl-2-butenenitrile as phosphonate following procedure 2. Yield 0.29 g (70%) as a yellow oil. *all-E*-**18**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.86 (d, 1H, J = 16.0 Hz); 6.72 (s, higher order AB, 1H); 6.72 (s, higher order AB, 1H); 6.55 (d, 1H, J = 16.0 Hz); 5.33 (s, 1H); 2.32 (s, 3H); 2.26 (s, 3H); 2.07 (t, 2H, J = 5.8 Hz); 1.80 (s, 3H); 1.63 (m, 2H); 1.49 (m, 2H); 1.09 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 155.93; 143.68; 140.15; 137.27; 137.18; 133.91; 133.81; 132.83; 117.69; 102.24; 98.38; 39.67; 34.04; 33.39; 28.90; 21.96; 18.94; 17.34; 16.55 ppm.

10-Thiomethylretinal (19). 100 mg (0.31 mmol) of 3,7-dimethyl-6-thiomethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**17**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 66 mg (65%) as a yellow oil. The individual regioisomers were separated using HPLC (see procedure 4). *all-E*-**19**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.14 (d, 1H, J = 8.1 Hz); 7.41 (d, 1H, J = 16.3 Hz); 7.26 (d, 1H, J = 15.1 Hz); 7.06 (d, 1H, J = 15.1 Hz); 6.54 (d, 1H, J = 16.3 Hz); 6.10 (d, 1H, J = 8.1 Hz); 2.38 (2, 3H); 2.15 (s, 2H); 2.12 (s, 3H); 2.07 (t, 2H, J = 6.2 Hz); 1.78 (s, 3H); 1.64 (m, 2H); 1.49 (m, 2H); 1.08 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 191.15; 154.72; 144.62; 137.90; 134.59; 133.95; 132.81; 132.26; 131.59; 131.44; 129.79; 39.71; 34.25; 33.34; 29.00; 21.88; 19.12; 18.53; 15.49; 13.52 ppm.

10-Iodoretinal (20). 233 mg (0.57 mmol) of 3,7-dimethyl-6-iodo-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**18**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 124 mg (53%) as a yellow oil. The individual regioisomers were separated using HPLC (see procedure 4). *all-E*-**20**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.14 (d, 1H, J = 8.0 Hz); 6.88 (d, 1H, J = 16.0 Hz); 6.79 (d, 1H, J = 14.5 Hz); 6.56 (d, 1H, J = 16.0 Hz); 6.19 (d, 1H, J = 14.5 Hz); 6.10 (d, 1H, J = 8.0 Hz); 2.38 (s, 3H); 2.25 (s, 3H); 2.08 (t, 2H, J = 6.0 Hz); 1.81 (s, 3H); 1.64 (m, 2H); 1.49 (m, 2H); 1.09 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 191.00; 153.63; 143.59; 140.47; 140.41; 137.43; 134.01; 133.62; 132.78; 130.32; 102.98; 39.78; 34.17; 33.50; 29.01; 22.07; 19.04; 16.70; 14.02 ppm.

2,3,7-Trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (21). 0.70 g (2.5 mmol) of 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**) was reacted with iodomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum

ether (1:19 v/v) as eluent. Yield 0.58 g (80%) as a yellow oil. *all-E*-**21**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.93 (dd, 1H, J = 12.0 Hz, J = 15.0 Hz); 6.55 (d, 1H, J = 15.0 Hz); 6.31 (d, 1H, J = 15.8 Hz); 6.18 (d, 1H, J = 12.0 Hz); 6.15 (d, 1H, J = 15.8 Hz); 2.21 (s, 3H); 2.02 (t, 2H, J = 6.0 Hz); 2.00 (s, 3H); 1.99 (s, 3H); 1.71 (s, 3H); 1.62 (m, 2H); 1.48 (m, 2H); 1.03 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 147.99; 140.27; 137.33; 136.83; 131.57; 129.73; 129.35; 128.92; 126.74; 104.04; 39.30; 33.96; 32.85; 28.70; 21.50; 18.94; 18.07; 15.46; 12.68 ppm.

3,7-Dimethyl-2-thiomethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (22). 0.65 g (2.3 mmol) of 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**) was reacted with thiocyanomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:19 v/v) as eluent. Yield 0.73 g (96%) as a yellow oil. *all-E*-**22**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 7.09 (dd, 1H, J = 12.1 Hz, J = 16.9 Hz); 6.90 (d, 1H, J = 16.9 Hz); 6.34 (d, 1H, J = 16.0 Hz); 6.20 (d, 1H, J = 12.1 Hz); 6.16 (d, 1H, J = 16.0 Hz); 2.41 (s, 3H); 2.28 (s, 3H); 2.02 (t, 2H, J = 6.0 Hz); 2.00 (s, 3H); 1.72 (s, 3H); 1.62 (m, 2H); 1.48 (m, 2H); 1.03 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 150.68; 141.17; 137.36; 136.85; 132.64; 130.03; 129.46; 129.41; 127.46; 116.33; 105.09; 39.37; 34.02; 32.94; 28.77; 21.58; 18.98; 18.74; 16.95; 12.79 ppm.

3,7-Dimethyl-2-iodo-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (23). 1.17 g (4.2 mmol) of 3,7-dimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**4**) was reacted with a solution of iodine in THF as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:19 v/v) as eluent. Yield 1.19 g (70%) as a yellow solid. *all-E*-**23**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 7.11 (dd, 1H, J = 12.9 Hz, J = 14.8 Hz); 6.55 (d, 1H, J = 14.8 Hz); 6.41 (d, 1H, J = 16.0 Hz); 6.23 (d, 1H, J = 12.9 Hz); 6.18 (d, 1H, J = 16.0 Hz); 2.30 (s, 3H); 2.02 (t, 2H, J = 5.9 Hz); 2.02 (s, 3H); 1.73 (s, 3H); 1.63 (m, 2H); 1.48 (m, 2H); 1.04 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 155.62; 143.02; 137.31; 136.64; 135.97; 132.55; 130.72; 130.44; 128.92; 118.84; 54.89; 39.38; 34.00; 33.01; 28.80; 21.65; 18.96; 18.85; 12.97 ppm. MS: $^{12}C_{20}{}^1H_{26}{}^{14}N_1{}^{127}I_1 + {}^1H_1$; experimental mass 407.99659; theoretical mass 408.11870; m/z 282 ([[M − I] + H]$^+$); 408 ([M + H]$^+$).

14-Methylretinal (24). 310 mg (1.05 mmol) of 2,3,7-trimethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**21**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 239 mg (76%) as a yellow oil. The individual regioisomers were separated using HPLC (see procedure 4). *all-E*-**24**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.26 (s, 1H); 7.17 (dd, 1H, J = 11.6 Hz, J = 15.0 Hz); 6.81 (d, 1H, J = 15.0 Hz); 6.34 (d, 1H, J = 16.2 Hz); 6.26 (d, 1H, J = 11.6 Hz); 6.18 (d, 1H, J = 16.2 Hz); 2.34 (s, 3H); 2.04 (s, 3H); 2.01 (t, 2H, J = 6.0 Hz); 1.91 (s, 3H); 1.73 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.04 (s, 6H) ppm.

14-Thiomethylretinal (25). 400 mg (1.22 mmol) of 3,7-dimethyl-2-thiomethyl-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**22**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 278 mg (69%) as a yellow oil. The individual regioisomers were separated using HPLC (see procedure 4). *all-E*-**25**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 10.13 (s, 1H); 7.50 (d, 1H, J = 15.2 Hz); 7.26 (dd, 1H, J = 12.0 Hz, J = 15.2 Hz); 6.38 (d, 1H, J = 16.0 Hz); 6.33 (d, 1H, J = 12.0 Hz); 6.20 (d, 1H, J = 16.0 Hz); 2.42 (s, 3H); 2.23 (s, 3H); 2.05 (s, 3H); 2.04 (t, 2H, J = 6.6 Hz); 1.73 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.04 (s, 6H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 188.39; 155.72; 141.95; 137.59; 137.08; 133.98; 133.56; 132.31; 130.76; 130.24; 130.00; 39.56; 33.17; 28.96; 21.79; 19.14; 17.71; 14.86; 13.07 ppm.

14-Iodoretinal (26). 400 mg (0.98 mmol) of 3,7-dimethyl-2-iodo-9-(2′,6′,6′-trimethyl-1′-cyclohexen-1′-yl)-2,4,6,8-nonatetraenenitrile (**23**) was reacted with DIBALH following procedure 3. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 63 mg (16%) as a yellow oil. The reaction mixture contained substantional amounts of retinal, that originated from dehalogenation reaction (see text for discussion). Major isomer **26**: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 9.26 (s, 1H); 7.00 (d, 1H, J = 15.0 Hz); 7.35 (dd, 1H, J = 11.5 Hz, J = 15.0 Hz); 6.43 (d, 1H, J = 16.1 Hz); 6.34 (d, 1H, J = 11.5 Hz); 6.22 (d, 1H, J = 16.1 Hz); 2.56 (s, 3H); 2.06 (s, 3H); 2.04 (t, 2H, J = 6.6 Hz); 1.74 (s, 3H); 1.63 (m, 2H); 1.47 (m, 2H); 1.05 (s, 6H) ppm.

2,3-Dimethyl-2-butenenitrile (27). 0.50 g (6.2 mmol) of 3-methyl-2-butenenitrile (**5**) was reacted with iodomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 0.31 g (53%) as a yellow oil. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 2.06 (q, 3H, ^4J = 1.4 Hz); 1.87 (q, 3H, J = 1.4 Hz); 1.84 (s, 3H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 152.02; 119.72; 103.18; 24.19; 19.83; 15.81 ppm. MS (EI, 70 eV): m/z 95 ($M^{+\bullet}$).

3-Methyl-2-thiomethyl-2-butenenitrile (28). 0.45 g (5.6 mmol) of 3-methyl-2-butenenitrile (**5**) was reacted with methylthiocyanate as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 0.56 g (80%) as a yellow oil. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 2.38 (s, 3H); 2.16 (q, 3H, ^4J = 0.5 Hz); 2.04 (q, 3H, J = 0.5 Hz) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 156.41; 115.25; 104.18; 24.67; 21.53; 19.57 ppm. MS (EI, 70 eV): m/z 127 ($M^{+\bullet}$).

2-Iodo-3-methyl-2-butenenitrile (29). 0.50 g (6.2 mmol) of 3-methyl-2-butenenitrile (**5**) was reacted with a solution of iodine in THF as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 1.14 g (89%) as a yellow solid. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 2.24 (s, 3H); 2.07 (s, 3H) ppm. ^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 163.25; 117.22; 53.25; 28.60; 24.28 ppm. MS (EI, 70 eV): m/z 207 ($M^{+\bullet}$).

2-Methyl-2-butenenitrile (30). 0.50 g (7.2 mmol) of 2-butenenitrile (**6**) was reacted with iodomethane as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Due to the low-boiling point no pure **30** could be isolated.

2-Thiomethyl-2-butenenitrile (31). 0.50 g (7.2 mmol) of 2-butenenitrile (**6**) was reacted with methyl thiocyanate as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 0.23 g (28%) as a yellow oil. Major isomer: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.63 (q, 1H, J = 7.1 Hz); 2.44 (s, 3H); 1.93 (d, 3H, J = 7.1 Hz) ppm.^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 143.88; 115.49; 111.49; 16.45; 15.86 ppm. Minor isomer: ^{1}H NMR (300.1 MHz, $CDCl_3$): δ 6.58 (q, 1H, J = 7.1 Hz); 2.38 (s, 3H); 2.06 (d, 3H, J = 7.1 Hz) ppm.^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, $CDCl_3$): δ 145.32; 112.22; 110.03; 17.89; 15.63 ppm. MS (EI, 70 eV): m/z 113 ($M^{+\bullet}$).

2-Iodo-2-butenenitrile (32). 0.50 g (7.2 mmol) of 2-butenenitrile (**6**) was reacted with a solution of iodine in THF as electrophile following procedure 1. The product was purified using silica gel column chromatography with diethyl ether/petroleum ether (1:9 v/v) as eluent. Yield 0.19 g (14%) as a yellow solid. Major isomer: ^{1}H NMR (300.1 MHz, CD_3COCD_3): δ 7.14 (q, 1H, J = 7.1 Hz); 1.93 (d, 3H, J = 7.1 Hz) ppm.^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, CD_3COCD_3): δ 155.57; 118.84; 62.07; 22.79 ppm. Minor isomer: ^{1}H NMR (300.1 MHz, CD_3COCD_3): δ 7.31 (q, 1H, J = 7.1 Hz); 1.99 (d, 3H, J = 7.1 Hz) ppm.^{13}C NMR (75.5 MHz, ^{1}H-noise-decoupled, CD_3COCD_3): δ 159.18; 116.84; 52.25; 21.08 ppm. MS (EI, 70 eV): m/z 193 ($M^{+\bullet}$).

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Supporting Information Available: The Supporting Information contains ^{1}H and ^{13}C NMR assignments and *J* couplings of the synthesized retinal compounds. NMR spectra, as well as the mass spectra of all synthesized 10- and 14-substituted retinals are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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