(9*Z*)- and (11*Z*)-8-Methylretinals for Artificial Visual Pigment Studies: Stereoselective Synthesis, Structure, and Binding Models

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Abstract: Artificial visual pigment formation was studied by using 8-methyl-substituted retinals in an effort to understand the effect that alkyl substitution of the chromophore side chain has on the visual cycle. The stereoselective synthesis of the 9-*cis* and 11-*cis* isomers of 8-methylretinal, as well as the 5-demethylated analogues is also described. The key bond formations consist of a thallium-accelerated Suzuki cross-coupling reaction between cyclohexenylboronic acids and dienyliodides (C6–C7), and a highly stereocontrolled Horner–Wadsworth–Emmons or Wittig condensation (C11–C12). The cyclohexenylboronic acid was prepared by trapping the precursor cyclohexenyllithium species with $B(OiPr)_3$ or $B(OMe)_3$. The cyclohexenyllithium species is itself obtained by *n*BuLi-induced elimination of a trisylhydrazone (Shapiro reaction), or depending upon the steric hindrance of the ring, by iodine–metal exchange. In binding experiments with the apoprotein opsin, only 9-*cis*-5-demethyl-8-methylretinal yielded an artificial pigment; 9-*cis*-8-methylretinal simply provided residual binding, while evidence of artificial pigment formation was not found for the 11-*cis* analogues. Molecular-mechanics-based docking simulations with the crystal structure of rhodopsin have allowed us to rationalize the lack of binding displayed by the 11-*cis* analogues. Our results indicate that these isomers are highly strained, especially when bound, due to steric clashes with the receptor, and that these interactions are undoubtedly alleviated when 9-*cis*-5-demethyl-8-methylretinal binds opsin.

Keywords: cross-coupling · docking models · membrane proteins · receptors

Introduction

The light-sensitive visual rhodopsin (Rhs) pigments are members of the G-protein coupled receptor (GPCR) superfamily, which also consists of proteins sensitive to cell stimuli as diverse as calcium ions, neurotransmitters, hormones, and even other proteins.[1] Common to this large family of receptors is a protein backbone architecture of seven α-helices, which span the lipid bilayer of the receptor cell membrane. In contrast to other GPCR subfamilies, the ligand in Rhs is covalently attached to the protein as an inverse agonist. In the binding pocket of bovine Rhs (a 40 kDa protein with 348 aminoacids) the light-sensitive chromophore 11-*cis*-retinal **1** (Figure 1) is attached to Lys296 in helix VII through a protonated Schiff base, while Glu113 in helix III acts as a counterion.[2] Upon light absorption, the 11-*cis* bond of the protonated Schiff base photoisomerizes to the *trans* geometry; this is one of the fastest (less than 200 fs)[3] and most efficient (quantum yield of 0.67) chemical reactions known. As a result, the cyclohexenyl ring and several of the polyene sidechain substituents are displaced to other regions within the binding pocket. These perturbations are linked to the protein structure alterations (such as displacement of helices and reorganization of citoplasmic loops), the Schiff base deprotonation (with concomitant proton uptake by Glu134), and detachment of *trans*-retinal **2** (Figure 1) from the apoprotein.[4,5] The isolation of intermediates, which were spectrometrically detected at low temperature, and their carefully monitored interconversion provides evidence for these changes.[2] The Meta II intermediate, which contains a deprotonated *trans*-retinal Schiff base, is the

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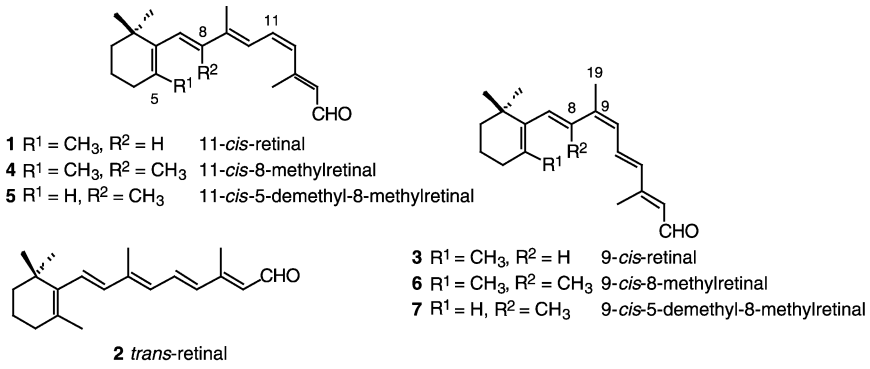


Figure 1. 9-*cis*- and 11-*cis*-Retinal analogues with a methyl substituent at C_8 of the parent retinal isomer.

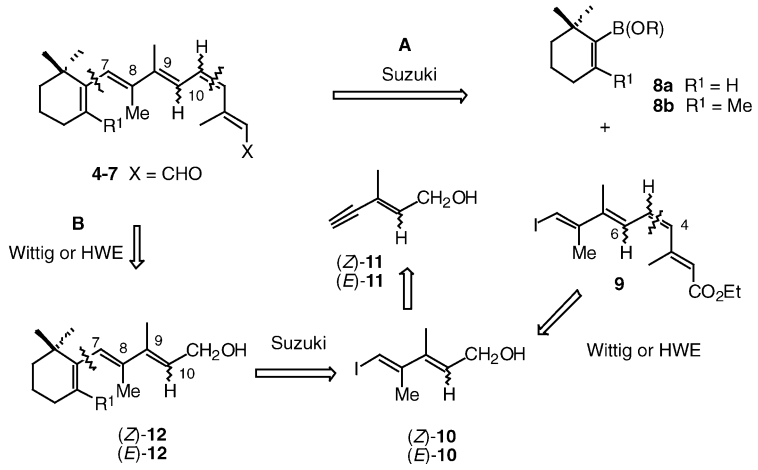
active state of Rhs that interacts with a heterotrimeric G-protein, transducin, through the extracellular loops that connect the seven transmembrane helices. The protein–protein interaction then triggers a biochemical cascade which leads to neural signals that provide the sensation of vision.[2,4,5]

Advances in protein extraction protocols have facilitated the growth of Rhs crystals suitable for X-ray analysis. Although the ground-state (inactive) structure of Rhs[6a] reveals detailed information about the chromophore–opsin interaction, the resolution (2.8 Å) is still insufficient for the 11-*cis*-retinal conformation to be unambiguously assigned.[6] In particular, some discrepancies of the C6–C7 bond[7] have been noted with respect to the values reported for the 6-s-*cis* (by X-ray diffraction)[6a] or 6-s-*trans* conformer (by solid-state deuterium NMR spectroscopy using a labeled chromophore).[8] Therefore, the use of synthetic retinals[9] that have structural modifications in the cyclohexenyl ring or in the adjacent side chain might help clarify the role that conformational changes of the chromophore play in the visual cycle.[10] Interestingly, recent results suggest that the 6-s-*cis* to 6-s-*trans* transition, which occurs by a ring flip following the 11-*cis* to *trans* photoisomerization, is involved in the activation step of the visual process.[11–13] Indeed, the X-ray structure of Rhs shows C5–CH_3 and C8–H to be in close proximity.[6] We reasoned that by substituting the native hydrogen atom at C8 with a methyl group (8-methylretinal), steric interactions of greater severity would occur between C8–CH_3 and C5–CH_3, and that this might lead to changes in the C6–C7 conformation.[14] Furthermore, it was considered that positional exchange of C5–CH_3 and C8–H (5-demethyl-8-methylretinal) might result in a small shift of steric bulk within the chromophore,[6] and would afford a derivative with an opsin-bound conformation close to that of the native chromophore.

Since both 11-*cis*-retinal **1** and 9-*cis*-retinal **3** are known to form visual pigments (rhodopsin and isorhodopsin, respectively),[15] we herein describe the stereoselective synthesis of each of these isomers of 8-methylretinal and 5-demethyl-8-methylretinal (compounds **4**–**7**, Figure 1). Opsin-binding studies have revealed the presence of polyene distortions, especially in the 11-*cis* derivatives **4** and **5**, which fail to bind the apoprotein. These are a result of the structural perturbation introduced by the methyl group at C_8. Structural and molecular mechanic docking simulation studies of the apoprotein–ligand interaction based on the crystal structure of rhodopsin are also presented. The binding rankings of compounds **4** and **5** were calculated relative to **1**, and thus allowed us to put forth a rationale for the binding experiment results observed for the 11-*cis*- and 9-*cis*-retinal analogues. Our results indicate that the 11-*cis* derivatives do not bind because of the strain induced in these molecules from steric clashes between the putative ligands and the Trp265 residue, which is located in the binding pocket of the receptor. Therefore, the superior binding behavior of one of the 9-*cis*-retinal analogues (compound **7**) arises because of the smaller strain energy it encounters when bound.

Results and Discussion

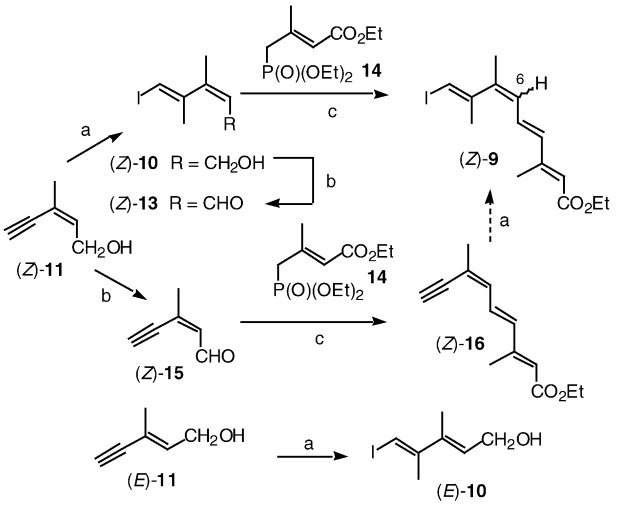
Synthesis: Our previously described protocol for the preparation of 9-*cis*-retinoic acid was considered to be the most convenient approach to the desired analogues.[16] This straightforward stereocontrolled synthesis involves the construction of the C6–C7 bond by convergent Suzuki coupling[17] of a cyclohexenylboronic acid **8** and a stereodefined ω-iodotetraenyl ester **9** (path A, Scheme 1).



Scheme 1. Retrosynthesis of compounds **4**–**7**.

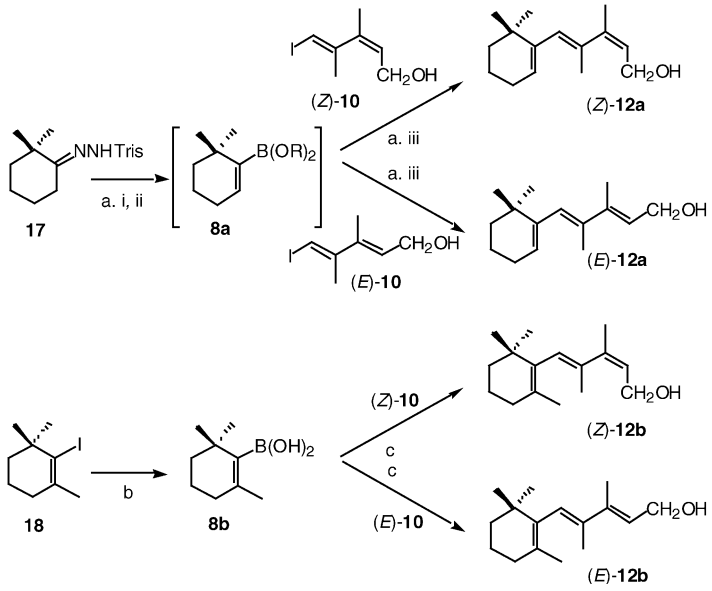
The appropriate choice of isomer of the commercially available 3-methylpent-4-yn-1-ol [(*E*)-**11** or (*Z*)-**11**] starting material should control the C_9–C_{10} geometry of the retinal. Functionalization of these enynols by methylalumination–iodination,[18] which proceeds by *syn*-addition, should afford (*E*)-**10** or (*Z*)-**10**, and would provide the C7–C8 *trans* geometry required in the desired analogues **4**–**7**. Moreover, stereoselective Wittig or related condensations can be utilized to attach the terminal five-carbon fragment of the polyenic side chain, and thus, depending upon the order of steps in the sequence, set the C11–C12 configuration in either **4**–**7** (path B) or **9** (path A).

The attempted stereocontrolled route to ω-iodotetraenylester (*Z*)-**9** is shown in Scheme 2. Zirconium-promoted methylalumination[18] of *cis*-enynol (*Z*)-**11** and subsequent treatment of the resultant dimethylalkenylallane intermediate with iodine in THF at −40 °C afforded iododienol (*Z*)-**10** (52 % yield). Owing to its degradation after rapid valence isomerization to the α-pyran,[19] aldehyde (*Z*)-**13**, which was obtained by MnO_2 oxidation of (*Z*)-**10** in the presence of K_2CO_3,[20] was used immediately without purification. Horner–Wadsworth–Emmons (HWE) condensation of (*Z*)-**13** with the anion derived from phosphonate **14** in the presence of DMPU[21] provided tetraenyliodide **9**. The C4–C5 bond formation was highly *trans* stereoselective, but the ^{1}H NMR spectrum of **9** displayed signals for both geometric isomers at the C8–C9 bond (1:2 ratio) (Scheme 2); this resulted from the reversible valence isomerization indicated above. Although reports describing the successful carboalumination of polyene–ynes[18,22] exist, iodide (*Z*)-**9** could not be obtained from (*Z*)-**16** (itself prepared as indicated in Scheme 2 by HWE condensation of enynal (*Z*)-**15**,[23] and the phosphonate anion obtained from **14**) by this procedure. These and related observations highlight the instability of ω-iodotetraenyl esters, which should be able to be prepared from the corresponding tetraenylstannanes by tin–iodine exchange prior to their subsequent cross-coupling with organometallic reagents.[16]



Scheme 2. a) i) [$ZrCp_2Cl_2$], Me_3Al, CH_2Cl_2, 25 °C; ii) I_2, THF, −50 °C [(*Z*)-**10**, 52 %; (*E*)-**10**, 60 %]; b) MnO_2, K_2CO_3, CH_2Cl_2, 25 °C, 1.5 h [(*Z*)-**15**, 84 %]; c) phosphonate **14**, *n*BuLi, DMPU, THF, −78 → −35 °C (**9**, 75 %; (*Z*)-**16**, 61 %).

With the shorter iododienols (*Z*)-**10** and (*E*)-**10** in hand, attention was directed to the sequence indicated in path B of Scheme 1. Here, the Suzuki reaction to afford (*Z*)-**12** and (*E*)-**12** precedes the condensation step (C11–C12 bond in retinoid numbering) that completes the polyenic side chain.[16] Cyclohexenylboronic acids such as **8** are routinely acquired from the corresponding organolithium species by a lithium–boron exchange process. The desired alkenyllithium compound can be obtained in two ways from commercially available ketones; the choice depends on the steric hindrance of the precursor carbonyl group. Since the Shapiro reaction of unhindered cyclohexanone hydrazones is known,[24] trienols (*Z*)-**12a** and (*E*)-**12a** were prepared in this manner, as shown in Scheme 3. 2,2-Dimethylcyclohexanone trisylhydrazone **17**[25] was treated with *n*BuLi, and the resultant alkenyllithium species was trapped with $B(OiPr)_3$ to afford boronate **8a**. Sequential addition of [$Pd(PPh_3)_4$], iodide (*Z*)-**10** or (*E*)-**10**, and a 10 % aqueous TlOH solution[26] provided, after stirring for 4 h at 25 °C, alcohols (*Z*)-**12a** or (*E*)-**12a** in 60 and 98 % combined yields, respectively (Scheme 3).

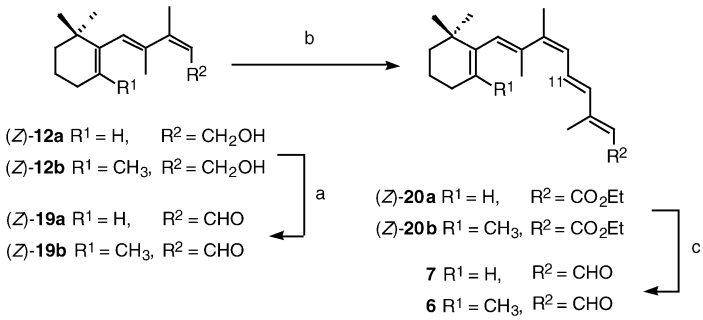


Scheme 3. a) i) *n*BuLi, THF, −78 °C; ii) $B(OiPr)_3$, 0 °C; iii) [$Pd(PPh_3)_4$], iodide (*Z*)-**10** or (*E*)-**10**, 10 % aq. TlOH [(*Z*)-**12a**, 60 %; (*E*)-**12a**, 98 %]; b) i) *t*BuLi, THF, −78 °C; ii) $B(OMe)_3$, 0 °C; iii) H_2O (77 %); c) [$Pd(PPh_3)_4$], iodide (*Z*)-**10** or (*E*)-**10**, 10 % aq. TlOH [(*Z*)-**12b**, 60 %; (*E*)-**12b**, 65 %].

Synthesis of the 8-methylretinal analogues illustrates the alternative method available for the generation of the alkenyllithium precursor.[25] The halogen–lithium exchange reaction is suitable for hydrazones that are derived from hindered ketones in which deprotonation of the C_α tertiary carbon with *n*BuLi, as in the Shapiro reaction, is inefficient. Oxidation of the hydrazone derived from 2,2,6-trimethylcyclohexanone with iodine using Barton's procedure[27] (Scheme 3) afforded the cycloalkenylboronic acid **8b**[25] pre-

cursor, cycloalkenyliodide **18** (77% yield). Compound **8b** was immediately coupled to iodide (*Z*)-**10** or its geometric isomer in the same manner as that described for the demethylated analogues (Scheme 3) to afford, after stirring for 8 h, trienols (*Z*)-**12b** and (*E*)-**12b** in 60 and 65% yield, respectively.

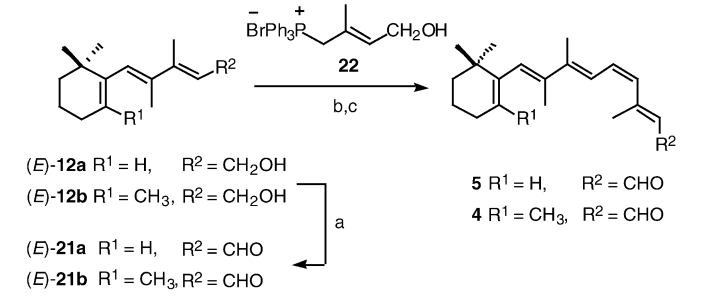
It was envisaged that the geometry of the C11–C12 double bond, which is required to complete the polyene side chain, could be controlled by the use of stereoselective variations of the Wittig and HWE reactions. Trienols (*Z*)-**12a** and (*Z*)-**12b** were oxidized with catalytic quantities of tetra-*n*-propylammonium perruthenate (TPAP) in CH_2Cl_2 in the presence of *N*-methylmorpholine *N*-oxide (NMO) as co-oxidant (Scheme 4).[28] The resultant aldehydes (*Z*)-**19a** and (*Z*)-**19b** were then treated with the anion of phosphonate **14**



Scheme 4. a) TPAP, NMO, 4 Å MS, CH_2Cl_2, 25°C [(*Z*)-**19a**, 77%; (*Z*)-**19b**, 91%]; b) i) hosphonate **14**, *n*BuLi, DMPU, THF, 0°C; ii) aldehyde (*Z*)-**19a** or (*Z*)-**19b**, −78→−40°C [(*Z*)-**20a**, 95%; (*Z*)-**20b**, 94%]; c) i) DIBAL-H, THF, −78°C; ii) MnO_2, Na_2CO_3, CH_2Cl_2, 25°C (**7**, 79%; **6**, 90%).

as indicated[21] to afford the (11*E*)-pentaenyl esters (*Z*)-**20a** and (*Z*)-**20b** in high yields. Subsequent functional-group manipulation, which included a DIBAL-H reduction and MnO_2 oxidation, afforded the desired retinals **6** and **7**.

For the stereoselective preparation of the (11*Z*)-retinoids, we turned our attention to the procedure described by Kobayashi;[29] this uses the (*E*)-oxidoallylic phosphorane reagent derived from phosphonium salt **22** (Scheme 5). Although this (*Z*)-selective Wittig condensation is rarely used, we found it to be a potentially useful and reliable method when a freshly opened bottle of KHMDS was used. Treatment of aldehydes (*E*)-**21a** and (*E*)-**21b** at −78°C with the phosphorane derived from **22** provided the unstable 11-*cis*-retinols. These were immediately oxidized under basic MnO_2 conditions to afford retinals **4** and **5** in 60 and 51% combined yields, respectively, after purification by HPLC (Scheme 5). Examination of the peak intensities in the HPLC trace allowed the *Z*/*E* stereoselectivity for the Wittig reaction to be estimated at a remarkable 20–22:1 ratio.



Scheme 5. a) TPAP, NMO, 4 Å MS, CH_2Cl_2, 25°C [(*E*)-**21a**, 92%; (*E*)-**21b**, 67%]; b) i) phosphonium salt **22**, KHMDS, THF, −78→25°C; ii) aldehyde (*E*)-**21a** or (*E*)-**21b**, THF, −78→25°C; c) MnO_2, Na_2CO_3, CH_2Cl_2, 25°C (**5**, 51%; **4**, 60%).

Structural studies: It was anticipated that the increase in steric bulk at C8 relative to the native retinal would alter the conformation of the bonds that are proximal to the hydrophobic ring, particularly for the 8-methyl retinal isomers **4** and **6**. On the other hand, we considered that exchange of the C5 and C8 substituents in the 5-demethyl-8-methylretinal isomers **5** and **7** would mimic the steric interactions present in the same region of the parent retinal (Figure 1). Distortions as a result of the C8 methyl substituent in the retinal polyene conformation are supported by spectroscopic and molecular mechanic studies. For example, broad signals for the C1–$(CH_3)_2$ groups appeared in the room temperature ^{1}H NMR spectra of **6**, as well as in the simpler systems (*E*)-**12b** and (*E*)-**21b**; this indicates a low-exchange regime. When a solution of **6** in [D_8]toluene was cooled to −35°C, individual methyl resonance signals were observed, and a dynamic NMR study revealed that these signals coalesced at −15°C. From these experiments, the free energy of activation ($\Delta G^{\neq}$) at the coalescence temperature was estimated to be about 14.2 ± 0.5 kcal mol^{-1}.[30] For the shorter trienal model (*E*)-**21b**, coalescence occurs at room temperature, while at −20°C, the two C1 methyl groups appear as sharp singlets ($\Delta G^{\neq}$ is about 14.9 ± 0.5 kcal mol^{-1}). In principle, the barrier to interconversion could be ascribed either to cyclohexene ring inversion or to C6–C7 bond rotation. The retinal cyclohexene has a half-chair conformation,[31] but there are indications that a dynamic process occurs even in the solid state, in which ring inversion through a cyclohexene boat-form transition state with an energy barrier of about 6.3 kcal mol^{-1} interconverts these half-chair conformations. The conformation of the C6–C7 bond has been found to be close to s-*cis* (i.e., in the crystal structure of 11-*cis*-retinal).[32–34] Ab initio studies have also addressed the conformational equilibria of retinal models derived from β-ionone.[35] In the latter, diastereomeric minima were found in which the C6–C7 s-*cis* conformation exhibited dihedral angles of 60.7 and −64.9°, and barrier heights of about 5 kcal mol^{-1} were estimated for bond rotation about the C6–C7 bond.

The retinal derivatives (**4** and **6**) synthesized in this study contain an additional methyl group at C_8. It is reasonable to assume that this sterically-demanding methyl group has less influence on the cyclohexene ring inversion (i.e., sequential movement of C2 and C3 through the plane of the double bond) than on the rotation of the ring sidechain bond, as in the latter there is a severe steric interaction with the C5–CH_3 group. Inspection of Dreiding molecular models clearly shows that upon rotation about the C6–C7 bond, steric clashes occur between C8–CH_3 and C5–CH_3 on one side, and C8–CH_3 and C1–CH_3 on the other side.

Therefore, we consider that the dynamic process observed by ^{1}H NMR spectroscopy corresponds to interconversion of the most stable conformers. Since the energy barrier is considerably higher than that calculated for the model system,[36] we computed the energy profile that corresponds to the C6–C7 bond rotation of compound **6** by using molecular mechanic calculations on a CHARMM force field (see Experimental Section).[37] Two energy minima, which showed a high departure from planarity, were found at −40 and 120°. The barrier for interconversion was calculated to be 13.8 kcal mol^{-1}; this value is similar to that found for the experimental free energy of activation at the coalescence temperature discussed above.

To further document the existence of steric clashes in the C8–CH_3–C11–H region, nOe difference spectra were recorded. The nuclear Overhauser effect study for 9-*cis*-retinals **6** and **7** in CD_3OD solution at ambient temperature reveals a highly distorted polyene, in particular, around the C6–C7 and C8–C9 bonds. The most relevant data are depicted in Figure 2. The through-space interactions of C8–

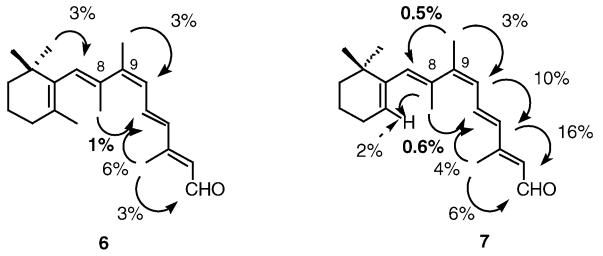


Figure 2. Relevant through-space interactions (nOe difference values) for compounds **6** and **7**.

CH_3 with C11–H, and C9–CH_3 with C7–H are negligible (less than 1% nOe); this indicates that the C8–C9 bond of the unbound chromophore has a twisted conformation. Through-space interaction is not apparent between C8–CH_3 and the ring methyl substituents, as is expected in a predominantly twisted conformation.

The UV spectra of analogues **6** and **7** (listed in Table 1) also suggest that the C8 methyl group causes a loss of planarity since the absorption maxima displays a 30 nm blue-shift with respect to 9-*cis*-retinal **3**. The 11-*cis*-analogues, **4** and **5**, show a moderate shift of around 10 nm relative to the parent system **1**. Therefore, we surmised that the hypsochromic shift in **6** and **7** originates from the C8–CH_3···C11–H steric interaction, and that this is lessened in the C8–CH_3···C10–H region of the 11-*cis*-retinal analogues. Taken together, the spectroscopic data for the 8-methylretinal series **4**–**7** indicates that the ring-chain steric crowding is somehow alleviated by both a twist of the adjacent C8–C9 single bond and by the simultaneous retention of a partial conjugative interaction of all the polyene double bonds.

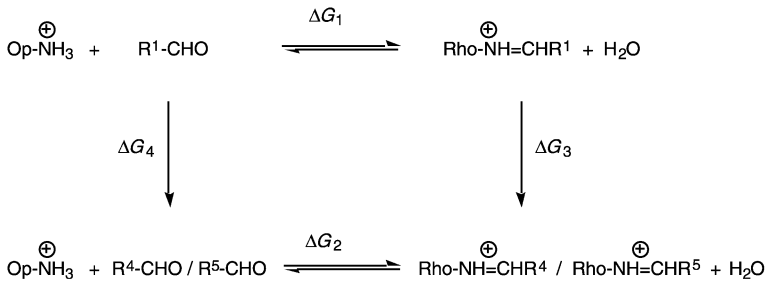
Table 1. Absorption maxima of the parent retinal isomers and their analogues.

Retinal	Absorption maxima [nm]	Retinal	Absorption maxima [nm]
1[a]	365[b], 380[d]	**5**	371[c]
3[a]	363[b], 373[d]	**6**	342[c], 344[d]
2[a]	368[c], 383[d]	**7**	345[c], 343[d]
4	368[c]		

[a] Taken from ref. [38]. [b] In hexane. [c] In methanol. [d] In ethanol.

Protein binding—molecular modelling studies: Preliminary opsin-binding experiments revealed that the added steric bulk at C8 in compounds **4** and **5** with respect to native 11-*cis*-retinal **1** was detrimental to apoprotein binding, since pigment formation was not detected for **4**, while the yield for **5** was very low (~5%); this discouraged further studies.[39] At first it was speculated that the added steric bulk at C8 had induced a conformational change around the C6–C7 bond, particularly in the more substituted derivative **4**, and that this had prevented its entry into the binding pocket. However, a conformational search of **4** and **5** using molecular mechanic calculations (see Figure 3 below) revealed that the polyene side chain is folded in a manner similar to that adopted in the crystal structure of free and opsin-bound 11-*cis*-retinal.[6,32] As expected, the absorption spectra of analogues **4** and **5** (Table 1) do not significantly differ from that of **1**; a small blue-shift (around 12 nm) is observed for the more substituted **4**. Therefore, on the assumption that the conjugated polyene structure is close to that of **1**, the retinals **4** and **5** fail to form pigments because of enhanced ligand–protein steric interactions. Given the recent availability of a rhodopsin crystal structure,[6] we performed molecular modelling calculations on the free and bound ligand (see Experimental Section), and attempted to find a rationale for the results based on energy criteria.

The energy difference for Schiff base formation with opsin between the cognate retinal and its analogues can be calculated and analyzed by using the thermodynamic cycle shown in Scheme 6,[40] in which Op–$^+NH_3$ is the opsin molecule, R^1–CHO and R^4–CHO/R^5–CHO are the cognate 11-*cis*-retinal **1** and analogues **4** and **5**, respectively, and



Scheme 6. Thermodynamic cycle for analysis of the energy difference for Schiff base formation with opsin between the cognate retinal and its analogues.

Rho–$^{+}$NH=CHR are the native and artificial rhodopsins, that is, the putative Schiff base adducts. ΔG_1 and ΔG_2 are the reaction energies for Schiff base formation, ΔG_3 is the energy when the Schiff base adduct of 11-*cis*-retinal **1** transforms into the Schiff base adducts of analogues **4** and **5**, and ΔG_4 gives the energy difference between native retinal and an analogue in the unbound state.

The closure property of this cycle yields the differential binding energy between any two ligands [Eq. (1)]:

$$\Delta\Delta G_{bin} = \Delta G_2 - \Delta G_1 = \Delta G_3 - \Delta G_4 \quad (1)$$

In other words, as Karplus has pointed out,[40a] the binding free-energy change can be calculated either from the "alchemical" vertical legs or from the "chemical" horizontal legs of the thermodynamic cycle.

The ranking binding free-energy function is made up of an internal energy [$\Delta\Delta G_{bin}$(int)] term, which can be calculated by molecular mechanics, and a solvation term [$\Delta\Delta G_{bin}$(solv)]. Entropy contributions have been neglected, because we expect that the analogue modification would have negligible effects. Hence, the binding ranking can be written as a sum of two terms [Eq. (2)]:

$$\Delta\Delta G_{bin} = \Delta\Delta G_{bin}(int) + \Delta\Delta G_{bin}(solv) \quad (2)$$

The solvation term contains a polar component, which penalizes the desolvation of polar groups, as well as a term for hydrophobic desolvation, which favors binding.[41] The ligands studied here are highly apolar. Hence, we have approximated the desolvation energy in a hydrophobic term that is directly proportional (the proportionality constant has been suggested by Sharp et al.[42]) to the solvent accessibility.

Table 2 lists the differential binding energy values for analogues **4**, **5**, and native 11-*cis*-retinal **1**, as given by Equations (1) and (2). As seen from Table 2, the internal energy differences for the rhodopsin adducts (ΔG_3) in the thermodynamic cycle are positive for both 11-*cis*-retinal analogues; this indicates that the Schiff base formation will be adversely effected when the cognate 11-*cis*-retinal **1** is replaced with analogues **4** or **5**. The general trend in ΔG_3(int) did not change when interactions were restricted only to those residues that were used in the energy minimization (10 Å), or when the whole molecule was taken into consideration for our calculations (see values in parenthesis, Table 2). The values for ΔG_4 are rather small relative to ΔG_3. As a result, they do not affect the outcome, that is, the condensation reaction of analogues **4** and **5** is highly unfavorable in comparison to Schiff base formation with 11-*cis*-retinal.

Table 2. Differential binding free energies for the schiff base formation of ligands **4** and **5** with opsin relative to **1**.

Energy difference[a]	**4**	**5**
ΔG_3(int)[b]	110.2 (53.0)[d]	118.3 (51.3)
ΔG_4(int)	3.0	−3.3
$\Delta\Delta G_{bind}$(int)	107.2 (50.0)	121.6 (54.6)
$\Delta\Delta G_{bind}$(solv)[c]	−1.3	−2.0
$\Delta\Delta G_{bind}$	105.9 (48.7)	119.6 (52.6)

[a] Energies in Kcal mol^{-1}. [b] The first two rows list the difference in the internal energy component for the terms indicated in Equation (1). [c] Differential in solvation energy. [d] The numbers in parenthesis were obtained taking the full molecule into account.

The rationale behind the preferential binding can be found by analyzing the internal energy of the bound ligands. Table 3 lists the internal energy differences for the opsin-bound analogues (**4** and **5**) relative to the native ligand. This quantity contributes to $\Delta\Delta G_{bin}$(int) [see Eq. (2)], and corresponds to the change in strain energy between the native ligand and its analogues when bound. As can be seen, when the Schiff bases are formed the internal energy of the analogues is much greater than that of 11-*cis*-retinal; this indicates that the binding of these analogues is burdened by a higher amount of strain than the original ligand. To estimate the direct effect the additional methyl group has on the strain energy of the molecule, we evaluated the strain energies of the unoptimized bound retinal analogue structure without the C8 methyl group. The difference in strain energy with respect to the native ligand was still found to be large. This indicates that the strain resides not only around the modified region (at the C8 position), but is also transmitted to the rest of the molecule, such as the ring moiety and the polyenic system. To visualize the distortions induced by binding we have superimposed the resultant models for the free and bound structures for every analogue. The results are displayed in Figure 3A–C and allow the extent of the distortion that the ligands undergo upon binding to be compared. In agreement with the energy results, the smallest changes in ligand conformation upon binding arise in the native ligand 11-*cis*-retinal **1**. The largest changes brought about by binding can be found in the ring conformation and in the orientation of the methyl groups attached to the polyenic system of analogues **4** and **5**, both of which are critical structural determinants for efficient binding.[6,8]

Table 3. Differences in internal energy between 11-*cis*-retinal and its analogues [Kcal mol^{-1}]

	4	**5**
including Me group (bound)	52.5	55.6
excluding Me group (bound)	29.6	27.4
unbound	3.0	−3.3

The strain energy difference is considerably smaller for the unbound analogues (see Tables 2 and 3). Hence, our results indicate that **4** and **5** fail to form artificial visual pigments because of the strain they experience upon being bound to the apoprotein opsin. The source of this strain can be explained upon inspection of the unoptimized artificial rhodopsin models. Figure 3D shows a close up of the binding region for the structure on which the rhodopsin adducts were modeled, and includes the bound analogue **4** and residue Trp265, which is the closest fragment to the additional methyl group. As can be seen, the additional methyl group is located well below van der Waals contact distances from the methyl group located off the ligand ring, as well as from the indole ring of residue Trp265. Structure optimization of

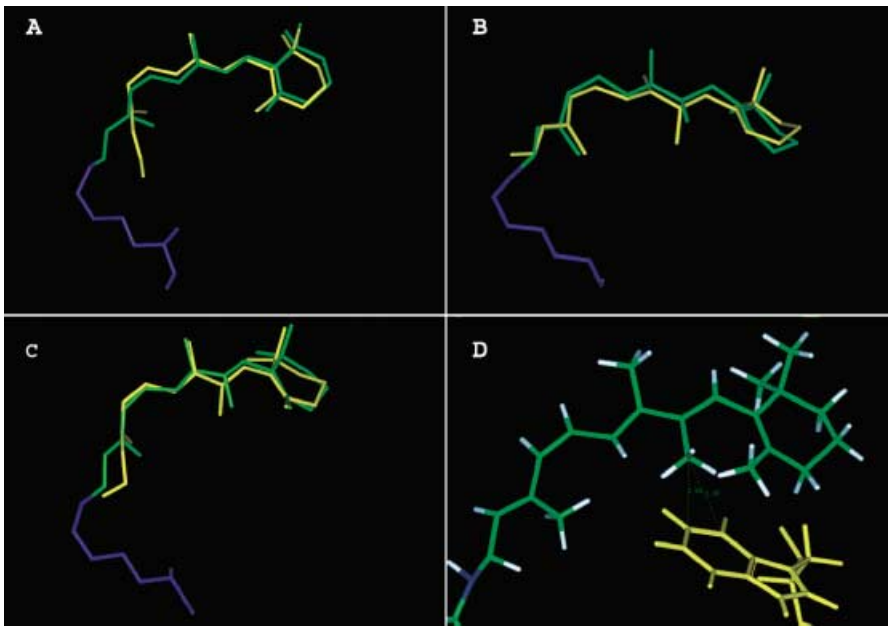


Figure 3. A–C) Modeled structure of the 11-*cis*-retinals (**1**, **4**, and **5**) as they form a Schiff base with Lys296 (magenta) in rhodopsin; this has been superimposed over the modelled free ligands (coloured by their atom types). For clarity, hydrogens have been left out of the displayed structures. The upper left (A), upper right (B), and lower left (C) panels correspond to native 11-*cis*-retinal **1** and its analogues **4** and **5**, respectively. D) The lower right panel depicts a close up of the unoptimized rhodopsin complex binding site in the presence of analogue **4**. The retinal adduct and the closest residue (Trp265) to the additional methyl substituent are included.

4 and **5** may relieve the steric clashes that preclude them from binding by substantially increasing the internal strain energy of these analogues.

Interestingly, when analogue **7**, in which the C8–H and C5–CH_3 groups from the parent 9-*cis*-retinal **3** have been exchanged, was incubated with apoprotein, the artificial pigment 5-demethyl-8-methylisorhodopsin was formed, albeit only in 20% relative yield with respect to the native 9-*cis*-retinal. The pigment showed roughly similar UV maxima (525 nm) to that displayed by native isorhodopsin; this indicates that the structural changes were adequately compensated by the protein binding-pocket flexibility. The properties of the pigment so generated have been described elsewhere.[39]

Since a crystal structure is not yet available for isorhodopsin, we can only hypothesize that the bound conformation of compound **7** has reduced steric clashes with the receptor, and is therefore, not highly strained.

Since only residual pigment formation was detected upon incubating **6** with opsin, the additional methyl group probably causes a greater alteration of the chromophore structure, which in turn makes it unable to fit in the binding pocket.

In summary, an additional methyl substituent at the C8 sidechain position abrogates the ability of the retinal to bind to the apoprotein opsin. Molecular modeling studies indicate that the strain penalty energy that analogues **4** and **5** must incur upon binding precludes them from forming a stable artificial pigment. If the structural perturbation is compensated by removal of the C5 methyl group, the 9-*cis* isomer **7**, but not the 11-*cis* isomer **6**, forms the corresponding artificial visual pigment, albeit in low yield.

Experimental Section

General: All reactions were carried out under an atmosphere of argon, and those that did not involve aqueous reagents were carried out in oven-dried glassware. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl, and dichloromethane was distilled over calcium hydride. Flash column chromatography was carried out under pressure using Merck Kieselgel 60 (230–400 mesh). High-performance liquid chromatography was performed on a Waters machine with a dual-wave detector (254 and 390 nm). Analytical thin-layer chromatography (TLC) was performed on aluminum plates with Merck Kieselgel 60 F_{254}, and were visualised by UV irradiation (254 nm) or by staining with a solution of phosphomolybdic acid. UV/Vis spectra were recorded on a HP5989A spectrophotometer. Infrared spectra of thin films deposited onto NaCl glass were obtained on a MIDAC Prospect FTIR spectrophotometer. Electron ionization mass spectra were obtained on a Hewlett-Packard HP59970 instrument operating at 70 eV. High-resolution mass spectra (HRMS) were taken on a VG Autospec instrument. ^{1}H NMR spectra were recorded in $CDCl_3$ or C_6D_6 at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz using residual protic solvent as the internal reference ($CHCl_3$, $\delta_H = 7.23$; C_6D_6, $\delta_H = 7.26$); chemical shifts (δ) are given in ppm, and coupling constants (J) are given in Hz. The ^{1}H NMR spectra are reported as follows: δ (multiplicity, coupling constant J, number of protons; assignment). ^{13}C NMR spectra were recorded in $CDCl_3$ at ambient temperature on the same spectrometer at 100 MHz using the central peak of $CHCl_3$ ($\delta = 77.0$) or C_6H_6 ($\delta = 128.0$) as the internal reference. DEPT135 spectra aided the assignment of signals in the ^{13}C NMR spectra; difference nOe experiments were also performed in a number of cases.

General procedure for methylalumination–iodination

(2*Z*,4*E*)-3,4-Dimethyl-5-iodopenta-2,4-dien-1-ol ((*Z*)-10): Trimethylaluminum (3.1 mL, 31.26 mmol) and (*Z*)-**11** (1.0 g, 10.42 mmol) were added to a cooled (0 °C) suspension of $[ZrCp_2Cl_2]$ (3.03 g, 10.42 mmol) in CH_2Cl_2 (20 mL). After stirring for 12 h, the mixture was cooled (−50 °C), and a solution of iodine (7.93 g, 31.26 mmol) in THF (27 mL) was added. The reaction was carefully poured over THF/H_2O (50:50, v/v) and was then extracted with Et_2O (3×). The combined organic layers were washed with saturated $Na_2S_2O_3$ solution (3×) and H_2O (3×), dried over Na_2SO_4, and the solvent was removed. The residue was purified by column chromatography (silica gel, 80:20 hexane/EtOAc) to afford a yellow oil (1.29 g, 52%). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.54 (s, 3H; CH_3), 1.74 (d, J(H,H) = 1.2 Hz, 3H; CH_3), 3.90 (d, J(H,H) = 6.8 Hz, 2H; CH_2), 5.30 (t, J(H,H) = 6.8 Hz, 1H; CH), 5.91 ppm (q, J(H,H) = 1.2 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 22.1 (q), 23.3 (q), 59.7 (t), 78.9 (d), 127.4 (d), 140.0 (s), 147.3 ppm (s); IR (NaCl): $\tilde{\nu}$ = 3600–3100, 2966, 2917, 2872, 1595, 1437, 1252, 998 cm^{-1}; MS: *m/z* (%): 221 (18) [M^+−17], 189 (3), 173 (6), 149 (12), 127 (7), 111 (20), 94 (100), 79 (64), 77 (28); HMRS *m/z*: calcd for $C_7H_{11}O$: 111.0810; found: 111.0809 [M^+−I].

(2*E*,4*E*)-3,4-Dimethyl-5-iodopenta-2,4-dien-1-ol ((*E*)-10): (*E*)-**11** (0.5 g, 5.21 mmol) was treated with $[ZrCp_2Cl_2]$ (1.51 g, 5.21 mmol), Me_3Al

(1.6 mL, 15.6 mmol), and iodine (3.96 g, 15.6 mmol) following the general procedure for methylalumination–iodination to give, after purification by column chromatography (silica gel, 80:20 hexane/EtOAc), a yellow oil (0.74 g, 60%). ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta = 1.83$ (s, 3H; CH_3), 2.05 (d, J(H,H) = 0.8 Hz, 3H; CH_3), 4.27 (d, J(H,H) = 6.4 Hz, 2H; CH_2), 5.81 (dd, J(H,H) = 6.4, 0.8 Hz, 1H; CH), 6.42 ppm (s, 1H; CH); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): $\delta = 14.6$ (q), 22.8 (q), 59.8 (t), 80.1 (d), 130.6 (d), 135.7 (s), 148.8 ppm (s); IR (NaCl): $\tilde{\nu} = 3500$–3200, 2923, 2864, 1570, 1442, 1376, 1003 cm^{-1}; MS: m/z (%): 238 (36) [M^+], 236 (7), 219 (13), 149 (22), 148 (17), 131 (42), 119 (25), 111 (63), 94 (31), 93 (33), 78 (28), 69 (100); HMRS m/z: calcd for $C_7H_{11}IO$: 237.9855; found: 237.9849.

General procedure for Shapiro–Suzuki cross coupling

(2*Z*,4*E*)-5-(6,6-Dimethylcyclohex-1-en-1-yl)-3,4-dimethylpenta-2,4-dien-1-ol ((*Z*)-12a): A cooled (−78°C) solution of **17**[25] (0.87 g, 2.14 mmol) in THF (6 mL) was treated with *n*BuLi (2.6 mL, 2.47 M in hexane, 6.43 mmol). After stirring for 30 min, $B(OiPr)_3$ (0.98 mL, 4.250 mmol) was added, and the temperature was raised to 0°C. The mixture was stirred for 1 h at 0°C and for 10 min at 25°C. The solution was then added, by cannula, to a separate flask that contained a solution of $[Pd(PPh_3)_4]$ (0.2 g, 0.17 mmol) and (*Z*)-**10** (0.41 g, 1.70 mmol) in THF (4 mL). After addition of a 10% aqueous TlOH solution (14.7 mL, 6.57 mmol), the mixture was stirred for 4 h at 25°C. The reaction mixture was diluted with Et_2O (8 mL) and was then washed with aqueous $NaHCO_3$ (3×). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 80:15:2 hexane/EtOAc/Et_3N) to afford a yellow oil (0.22 g, 60%). ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 0.99$ (s, 3H; CH_3), 1.00 (s, 3H; CH_3), 1.40–1.70 (m, 4H; $2CH_2$), 1.76 (d, J(H,H) = 1.4 Hz, 3H; CH_3), 1.83 (d, J(H,H) = 1.1 Hz, 3H; CH_3), 2.00–2.20 (m, 2H; CH_2), 4.15 (d, J(H,H) = 6.4 Hz, 2H; CH_2), 5.20–5.50 (m, 2H; CH_2), 5.67 ppm (s, 1H; CH); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 16.9$ (q), 19.1 (t), 22.9 (q), 25.8 (t), 28.2 (q, 2×), 33.8 (s), 38.9 (t), 60.4 (t), 123.9 (d), 125.2 (d), 127.6 (d), 136.5 (s), 142.2 (s), 144.6 ppm (s); IR (NaCl): $\tilde{\nu} = 3600$–3100, 2957, 2926, 1434, 1374, 999 cm^{-1}; UV (MeOH): $\lambda_{max} = 234$ nm; MS: m/z (%): 203 (6) [$M^+ - 17$], 189 (11), 167 (18), 149 (88), 119 (100), 97 (82), 71 (86); HMRS m/z: calcd for $C_{15}H_{24}O$: 220.1827; found: 220.1824.

(2*E*,4*E*)-5-(6,6-Dimethylcyclohex-1-en-1-yl)-3,4-dimethylpenta-2,4-dien-1-ol ((*E*)-12a): Following the general procedure for the Shapiro–Suzuki cross coupling reaction, (*E*)-**12a** was obtained in 98% yield after purification by column chromatography (silica gel, 80:15:2 hexane/EtOAc/Et_3N). ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta = 0.92$ (s, 6H; $2CH_3$), 1.40–1.50 (m, 2H; CH_2), 1.50–1.60 (m, 2H; CH_2), 1.83 (s, 3H; CH_3), 1.84 (s, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 4.30 (d, J(H,H) = 6.7 Hz, 2H; CH_2), 5.28 (td, J(H,H) = 3.8, 1.4 Hz, 1H; CH), 5.73 (t, J(H,H) = 6.7 Hz, 1H; CH), 6.13 ppm (s, 1H; CH); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 14.6$ (q), 15.8 (q), 19.6 (t), 26.3 (t), 28.7 (2q), 34.6 (s), 39.4 (t), 60.5 (t), 125.4 (d), 125.7 (d), 127.7 (d), 137.2 (s), 139.9 (s), 143.5 ppm (s); IR (NaCl): $\tilde{\nu} = 3600$–3200, 2930, 2865, 1455, 1377, 1019 cm^{-1}; UV (MeOH): $\lambda_{max} = 247$ nm. MS: m/z (%): 221 (28) [M^+], 1203 (7), 193 (13), 181 (31), 177 (21), 175 (26), 165 (31), 137 (27), 133 (21), 123 (18), 119 (10bb0), 109 (26), 108 (28), 105 (20), 95 (24); HMRS m/z: calcd for $C_{15}H_{24}O$: 220.1827; found: 220.1822.

2,6,6-Trimethylcyclohex-1-en-1-ylboronic acid (8b): *t*BuLi (2.34 mL, 1.7 M in pentane, 3.99 mmol) was added to a cooled (−78°C) solution of alkenyliodide **18**[25] (0.48 g, 1.90 mmol) in THF (22 mL) at −78°C, and the resultant mixture was stirred for 30 min. Trimethylborate (2.16 mL, 18.9 mmol) was then added, and the reaction was stirred for 2 h at 25°C. Water (5 mL) was subsequently added, and the mixture was stirred for 2 h. The solution was diluted with Et_2O, the layers were separated, and the aqueous layers were extracted with *tert*-butyl methyl ether (TBDME; 3×). The combined organic layers were washed with 1 N HCl (2×), dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 80:20 hexane/EtOAc) to afford a white solid (0.24 g, 77%) (m.p. 108–109°C, hexane/EtOAc). ^{1}H NMR (400.13 MHz, CD_3OD): $\delta = 1.25$ (s, 6H; $2CH_3$), 1.50–1.60 (m, 2H; CH_2), 1.80–1.90 (m, 2H; CH_2), 1.87 (s, 3H; CH_3), 2.08 (t, J(H,H) = 6.1 Hz, 2H; CH_2), 5.05 ppm (s, 2H; CH_2); ^{13}C NMR (100.62 MHz, CD_3OD): $\delta = 21.0$ (d), 25.1 (q), 31.1 (q, 2×), 34.5 (d), 35.7 (s), 39.7 (d), 135.1 ppm (s, 2×); IR (NaCl): $\tilde{\nu} = 3500$–3300, 2925, 1321 cm^{-1}; MS: m/z (%): 169 (5)[$M^+ + 1$], 168 (33) [M^+], 154 (15), 153 (99), 152 (23), 123 (13), 110 (12), 109 (100), 81 (15); HMRS m/z: calcd for $C_9H_{17}BO_2$: 168.1322; found: 168.1322.

General procedure for Suzuki cross coupling

(2*Z*,4*E*)-3,4-Dimethyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dien-1-ol ((*Z*)-12b): A solution of alkenylboronic acid **8b** (0.23 g, 1.36 mmol), $[Pd(PPh_3)_4]$ (0.125 g, 0.108 mmol), and 10% aqueous TlOH (9.2 mL, 4.16 mmol) in THF (7 mL) was added to a solution of (*Z*)-**10** (0.26 g, 1.08 mmol) in THF (7.0 mL). After stirring for 8 h at 25°C, the mixture was diluted with Et_2O (6 mL) and washed with aqueous $NaHCO_3$ (3×). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 80:15:2 hexane/EtOAc/Et_3N) to afford a yellow oil (0.15 g, 60%). ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta = 0.95$ (s, 6H; $2CH_3$), 1.40–1.70 (m, 4H; $2CH_2$), 1.53 (s, 3H; CH_3), 1.56 (d, J(H,H) = 1.3 Hz, 3H; CH_3), 1.85 (s, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 4.20 (d, J(H,H) = 6.8 Hz, 2H; CH_2), 5.40 (tq, J(H,H) = 6.8, 1.3 Hz, 1H; CH), 5.61 ppm (s, 1H; CH); ^{13}C NMR (100.61 MHz, $CDCl_3$): $\delta = 16.9$ (q), 19.3 (t), 21.2 (q), 23.4 (q), 28.4 (2q), 31.9 (t), 34.7 (s), 39.1 (t), 60.6 (t), 123.8 (d), 126.2 (d), 128.3 (s), 135.3 (s), 137.6 (s), 144.3 ppm (s); IR (NaCl): $\tilde{\nu} = 3600$–3100, 2927, 2864, 1436, 1371, 1001 cm^{-1}; UV (MeOH): $\lambda_{max} = 226$ nm; MS: m/z (%): 234 (2) [$M^+ - 17$], 217 (64), 203 (41), 161 (18), 147 (31), 133 (100), 119 (26), 109 (15), 105 (16), 95 (16), 83 (20), 69 (42); HMRS m/z: calcd for $C_{16}H_{26}O$: 234.1984; found: 234.1979.

(2*E*,4*E*)-3,4-Dimethyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dien-1-ol ((*E*)-12b): Following the general procedure for the Suzuki cross coupling, (*E*)-**12b** was obtained in 65% yield after purification by column chromatography (silica gel, 80:15:2 hexane/EtOAc/Et_3N). ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta = 0.70$–1.20 (m, 6H; $2CH_3$), 1.38 (s, 3H; CH_3), 1.40–1.60 (m, 4H; $2CH_2$), 1.60 (s, 3H; CH_3), 1.82 (s, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 4.26 (d, J(H,H) = 6.6 Hz, 2H; CH_2), 5.69 (t, J(H,H) = 6.6 Hz, 1H; CH), 6.04 ppm (s, 1H; CH); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 14.2$ (q), 15.3 (q), 19.4 (t), 21.1 (q), 28.3 (q, 2×), 31.9 (t), 34.9 (s), 39.2 (t), 60.1 (t), 124.4 (d), 126.2 (d), 128.1 (s), 136.3 (s), 137.7 (s), 139.1 ppm (s); IR (NaCl): $\tilde{\nu} = 3600$–3200, 2926, 2863, 1448, 1373, 1011 cm^{-1}; MS: m/z (%): 234 (11) [M^+], 203 (17), 147 (17), 134 (12), 133 (100), 119 (21), 84 (15), 83 (11), 73 (60); HMRS m/z: calcd for $C_{16}H_{26}O$: 234.1984; found: 234.1976.

General procedure for alcohol oxidation using TPAP/NMO

(2*Z*,4*E*)-5-(6,6-Dimethylcyclohex-1-en-1-yl)-3,4-dimethylpenta-2,4-dienal ((*Z*)-19a): A solution of (*Z*)-**12a** (0.22 g, 1.01 mmol) in CH_2Cl_2 (3 mL) was added to a cooled (0°C) and stirred suspension of *N*-methylmorpholine *N*-oxide (0.18 g, 1.51 mmol) and 4 Å molecular sieves in CH_2Cl_2 (6 mL). After stirring for 10 min, TPAP (0.017 g, 0.05 mmol) was added and the mixture was stirred at 25°C for 4 h. The mixture was diluted with CH_2Cl_2 (10 mL) and was washed with aqueous Na_2SO_3 (3×). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 93:5:2 hexane/EtOAc/Et_3N) to afford a yellow oil (0.17 g, 77%). ^{1}H NMR (400.13 MHz, C_6D_6): $\delta = 0.93$ (s, 6H; $2CH_3$), 1.40–1.60 (m, 4H; CH_2), 1.60 (d, J(H,H) = 1.3 Hz, 3H; CH_3), 1.62 (d, J(H,H) = 1.3 Hz, 3H; CH_3), 1.80–2.00 (m, 2H; CH_2), 5.30 (dt, J(H,H) = 3.8, 1.3 Hz, 1H; CH), 5.92 (dq, J(H,H) = 7.8, 1.3 Hz, 1H; CH), 5.95 (s, 1H; CH_3), 10.04 ppm (d, J = 7.8 Hz, 1H; CH); ^{13}C NMR (100.61 MHz, C_6D_6): $\delta = 16.6$ (q), 19.4 (t), 23.1 (q), 26.1 (t), 28.2 (q, 2×), 33.9 (s), 39.0 (t), 126.6 (d), 129.4 (d), 132.3 (d), 135.2 (s), 141.9 (s), 164.6 (s), 191.1 ppm (d); IR (NaCl): $\tilde{\nu} = 2958$, 2929, 2865, 2833, 1678, 1611, 1384, 1140 cm^{-1}; UV (MeOH): $\lambda_{max} = 234$, 282, 338 nm; MS: m/z (%): 218 (18) [M^+], 189 (34), 133 (19), 119 (100), 109 (28), 105 (18), 91 (17), 79 (15), 77 (16); HMRS m/z: calcd for $C_{15}H_{22}O$: 218.1671; found: 218.1676.

(2*Z*,4*E*)-3,4-Dimethyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dienal ((*Z*)-19b): Following the general procedure for TPAP/NMO oxidation, reaction of (*Z*)-**12b** (0.17 g, 0.71 mmol) with TPAP (0.011 g, 0.034 mmol) and NMO (0.13 g, 1.06 mmol) in CH_2Cl_2 (18 mL) afforded, after purification by column chromatography (silica gel, 93:5:2 hexane/EtOAc/Et_3N), a yellow oil (0.15 g, 91%). ^{1}H NMR (400.13 MHz, C_6D_6): $\delta = 0.89$ (s, 6H; $2CH_3$), 1.37 (s, 3H; CH_3), 1.39 (d, J = 1.2 Hz, 3H; CH_3), 1.40–1.60 (m, 4H; $2CH_2$), 1.58 (s, 3H; CH_3), 1.70–1.90 (m, 2H; CH_2), 5.85 (t, J(H,H) = 1.0 Hz, 1H; CH), 5.90 (dq, J(H,H) = 7.9, 1.2 Hz, 1H; CH), 10.12 ppm (d, J(H,H) = 7.9 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, C_6D_6): $\delta = 16.6$ (q), 19.5 (t), 21.3 (q), 23.5 (q), 28.4 (q, 2×), 32.1 (t), 34.8

(s), 39.3 (t), 129.4 (s), 129.5 (d), 131.2 (d), 135.2 (s), 136.5 (s), 164.1 (s), 190.9 ppm (d); IR (NaCl): $\tilde{\nu}$ = 2927, 2864, 2830, 1679, 1612, 1438, 1383, 1139 cm^{-1}; UV (MeOH): λ_{max} = 226, 272, 334 nm; MS: *m/z* (%): 232 (4) [M^+], 203 (40), 161 (17), 147 (17), 134 (15), 133 (100), 119 (22), 109 (29), 105 (12), 91 (16), 83 (19), 77 (12); HMRS *m/z*: calcd for $C_{16}H_{24}O$: 232.1827; found: 232.1833.

General procedure for Horner–Wadsworth–Emmons olefination

Ethyl (2*E*,4*E*,6*Z*,8*E*)-9-(6,6-dimethylcyclohex-1-en-1-yl)-3,7,8-trimethylnona-2,4,6,8-tetraenoate ((*Z*)-20a): A cooled (0 °C) solution of **14** (0.22 g, 0.82 mmol) and DMPU (0.19 g, 1.61 mmol) in THF (2 mL) was treated with *n*BuLi (2.03 mL, 2.35 M in hexane, 0.87 mmol) and stirred for 20 min. The mixture was cooled to −78 °C and a solution of (*Z*)-**19a** (0.13 g, 0.60 mmol) in THF (2 mL) was added. The resultant mixture was allowed to warm to −40 °C, at which temperature H_2O (4 mL) was added. The reaction was extracted with Et_2O (3×) and the organic layers were washed with brine (3×), dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 95:5 hexane/EtOAc) to afford a yellow oil (0.19 g, 95%). ^1H NMR (400.13 MHz, $CDCl_3$): δ = 1.02 (s, 6H; $2CH_3$), 1.27 (t, *J*(H,H) = 7.1 Hz, 3H; CH_3), 1.40–1.60 (m, 4H; $2CH_2$), 1.81 (d, *J*(H,H) = 1.4 Hz, 3H; CH_3), 1.93 (s, 3H; CH_3), 2.00–2.20 (m, 2H; CH_2), 2.27 (d, *J*(H,H) = 0.8 Hz, 3H; CH_3), 4.16 (q, *J*(H,H) = 7.1 Hz, 2H; CH_2), 5.30–5.50 (td, *J*(H,H) = 3.8, 1.3 Hz, 1H; CH), 5.73 (s, 1H; CH), 5.82 (d, *J*(H,H) = 1.3 Hz, 1H; CH), 5.95 (d, *J*(H,H) = 11.0 Hz, 1H; CH), 6.19 (d, *J*(H,H) = 15.3 Hz, 1H; CH), 6.92 ppm (dd, *J*(H,H) = 15.3, 11.0 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 13.8 (q), 14.3 (q), 16.9 (q), 19.2 (t), 23.6 (q), 25.9 (t), 28.3 (q, 2×), 34.0 (s), 38.9 (t), 59.5 (t), 117.9 (d), 125.2 (d), 125.3 (d), 129.1 (d), 132.9 (d), 133.2 (d), 136.7 (s), 142.2 (s), 148.4 (s), 153.2 (s), 167.2 ppm (s); IR (NaCl): $\tilde{\nu}$ = 2959, 2931, 2866, 1711, 1603, 1444, 1350, 1239, 1150, 967 cm^{-1}; UV (MeOH): λ_{max} = 320 nm; MS: *m/z* (%): 328 (100) [M^+], 313 (47), 267 (19), 255 (25), 201 (65), 185 (33), 175 (24), 173 (51), 171 (38), 161 (36), 139 (57); HMRS *m/z*: calcd for $C_{22}H_{32}O_2$: 328.2402; found: 328.2387.

Ethyl (2*E*,4*E*,6*Z*,8*E*)-3,7,8-trimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenoate ((*Z*)-20b): Following the general procedure for HWE olefination, (*Z*)-**20b** was obtained in 94% yield after purification by column chromatography (silica gel, 95:5 hexane/EtOAc). ^1H NMR (400.13 MHz, $CDCl_3$): δ = 1.01 (s, 6H; $2CH_3$), 1.28 (t, *J*(H,H) = 7.1 Hz, 3H; CH_3), 1.40–1.70 (m, 4H; $2CH_2$), 1.60 (s, 3H; CH_3), 1.62 (d, *J*(H,H) = 1.2 Hz, 3H; CH_3), 1.96 (s, 3H; CH_3), 2.00–2.20 (m, 2H; CH_2), 2.27 (d, *J*(H,H) = 0.8 Hz, 3H; CH_3), 4.16 (t, *J*(H,H) = 7.1 Hz, 2H; CH_2), 5.74 (s, 1H; CH), 5.76 (s, 1H; CH), 5.98 (d, *J*(H,H) = 10.9 Hz, 1H; CH), 6.20 (d, *J*(H,H) = 15.4 Hz, 1H; CH), 7.00 ppm (dd, *J*(H,H) = 15.4, 10.9 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 13.8 (q), 14.3 (q), 16.8 (q), 19.3 (t), 21.1 (q), 23.8 (q), 28.4 (q, 2×), 32.0 (t), 34.8 (s), 39.1 (t), 59.5 (t), 117.9 (d), 125.2 (d), 127.7 (d), 128.4 (s), 133.0 (d), 133.1 (d), 135.3 (s), 138.0 (s), 148.0 (s), 153.1 (s), 167.2 ppm (s); IR (NaCl): $\tilde{\nu}$ = 2960, 2928, 2864, 1710, 1603, 1442, 1238, 1151, 969 cm^{-1}; UV (MeOH): λ_{max} = 322 nm; MS: *m/z* (%): 342 (100) [M^+], 327 (20), 281 (21), 269 (23), 215 (22), 199 (39), 187 (27), 185 (33), 159 (26), 157 (19), 139 (61), 133 (25), 119 (19), 105 (14), 91 (16); HMRS *m/z*: calcd for $C_{23}H_{34}O_2$: 342.2559; found: 342.2559.

General procedure for reduction of esters–oxidation of alcohols

(2*E*,4*E*,6*Z*,8*E*)-9-(6,6-Dimethylcyclohex-1-en-1-yl)-3,7,8-trimethylnona-2,4,6,8-tetraenal (7) [(9*Z*)-5-demethyl-8-methylretinal]: DIBAL-H (0.7 mL, 1 M in hexane, 0.7 mmol) was added to a solution of (*Z*)-**20a** (0.06 g, 0.18 mmol) in THF (2 mL) at −78 °C, and the resultant suspension was stirred for 2 h. After careful addition of H_2O, the mixture was extracted with Et_2O (3×), and the organic layers were dried over Na_2SO_4 and concentrated. The residue was oxidized without further purification.

Manganese dioxide (0.27 g, 3.08 mmol) and Na_2CO_3 (0.33 g, 3.08 mmol) were added to a solution of the above compound in CH_2Cl_2 (4 mL), and the suspension was stirred for 4 h. The mixture was filtered throught Celite and the solvent was removed. The residue was purified by column chromatography (silica gel, 95:5 hexane/EtOAc) to afford a yellow oil (0.04 g, 79%). ^1H NMR (400.13 MHz, C_6D_6): δ = 1.01 (s, 6H; $2CH_3$), 1.40–1.50 (m, 2H; CH_2), 1.50–1.60 (m, 2H; CH_2), 1.75 (d, *J*(H,H) = 1.3 Hz, 3H; CH_3), 1.76 (s, 3H; CH_3), 1.80 (d, *J*(H,H) = 1.0 Hz, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 5.46 (td, *J*(H,H) = 3.9, 1.4 Hz, 1H; CH), 5.80 (d, *J*(H,H) = 10.9 Hz, 1H; CH), 5.94 (s, 1H; CH), 5.98 (d, *J*(H,H) = 7.9 Hz, 1H; CH), 6.02 (d, *J*(H,H) = 15.4 Hz, 1H; CH), 7.03 (dd, *J*(H,H) = 15.4, 10.9 Hz, 1H; CH), 9.98 ppm (d, *J*(H,H) = 7.9 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 14.1 (q), 18.2 (q), 20.9 (t), 24.7 (q), 27.5 (t), 29.7 (q, 2×), 35.7 (s), 40.6 (t), 127.2 (d), 127.3 (d), 130.6 (d), 130.9 (d), 134.8 (d), 135.8 (d), 138.9 (s), 143.9 (s), 151.4 (s), 156.2 (s), 192.2 ppm (d); IR (NaCl): $\tilde{\nu}$ = 2927, 2850, 1663, 1594, 1449, 1196 cm^{-1}; UV (MeOH): λ_{max} = 345 nm; MS: *m/z* (%): 285 (24) [M^++1], 284 (84) [M^+], 269 (37), 202 (25), 199 (21), 187 (54), 185 (28), 176 (44), 173 (68), 171 (35), 161 (69), 159 (57), 157 (37), 145 (47), 133 (39), 119 (100), 105 (38), 95 (64); HMRS *m/z*: calcd for $C_{20}H_{28}O$: 284.2140; found: 284.2135.

(2*E*,4*E*,6*Z*,8*E*)-3,7,8-Trimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenal (6) [(9*Z*)-8-methylretinal]: Following the general combined procedure for DIBAL-H reduction/MnO_2 oxidation, compound **6** was obtained in 75% yield after purification by column chromatography (silica gel, 95:5 hexane/EtOAc). ^1H NMR (400.13 MHz, C_6D_6): δ = 1.01 (s, 6H; CH_3), 1.40–1.50 (m, 2H; CH_2), 1.54 (s, 3H; CH_3), 1.58 (s, 3H; CH_3), 1.60–1.70 (m, 2H; CH_2), 1.78 (s, 3H; CH_3), 1.80 (s, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 5.81 (d, *J*(H,H) = 11.0 Hz, 1H; CH), 5.85 (s, 1H; CH), 6.00 (d, *J*(H,H) = 7.9 Hz, 1H; CH), 6.02 (d, *J*(H,H) = 15.4 Hz, 1H; CH), 7.08 (dd, *J*(H,H) = 15.4, 11.0 Hz, 1H; CH), 9.99 ppm (d, *J*(H,H) = 7.9 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 14.2 (q), 18.2 (q), 21.0 (t), 22.5 (q), 25.0 (q), 29.8 (q, 2×), 33.6 (t), 36.5 (s), 40.9 (t), 127.3 (d), 129.4 (d), 130.4 (s), 130.6 (d), 134.9 (d), 135.9 (d), 137.0 (s), 140.3 (s), 151.1 (s), 156.2 (s), 192.2 ppm (d); UV (MeOH): λ_{max} = 344 nm; MS: m/z (%): 299 (23) [M^++1], 298 (100) [M^+], 216 (20), 213 (20), 201 (37), 187 (24), 173 (20), 171 (23), 159 (37), 147 (27), 145 (28), 91 (28); HMRS *m/z*: calcd for $C_{21}H_{30}O$: 298.2297; found: 298.2299.

(2*E*,4*E*)-5-(6,6-Dimethylcyclohex-1-en-1-yl)-3,4-dimethylpenta-2,4-dienal ((*E*)-21a): Following the general procedure for oxidation of alcohols with TPAP/NMO, reaction of (*E*)-**12a** (0.15 g, 0.68 mmol) with TPAP (0.012 g, 0.034 mmol) and NMO (0.12 g, 1.02 mmol) in CH_2Cl_2 (7 mL) afforded, after purification by column chromatography (silica gel, 95:5 hexane/EtOAc), (*E*)-**21a** as a yellow oil (0.14 g, 92%). ^1H NMR (400.13 MHz, CD_2Cl_2): δ = 1.04 (s, 6H; $2CH_3$), 1.50–1.60 (m, 2H; CH_2), 1.60–1.70 (m, 2H; CH_2), 1.95 (d, *J*(H,H) = 1.1 Hz, 3H; CH_3), 2.12 (m, 2H; CH_2), 2.35 (d, *J*(H,H) = 0.9 Hz, 3H; CH_3), 5.45 (td, *J*(H,H) = 3.9, 1.5 Hz, 1H; CH), 6.08 (d, *J*(H,H) = 7.9 Hz, 1H; CH), 6.67 (s, 1H; CH), 10.16 ppm (d, *J*(H,H) = 7.9 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, CD_2Cl_2): δ = 14.5 (q), 15.5 (q), 19.4 (t), 26.3 (t), 28.4 (q, 2×), 34.5 (s), 39.2 (t), 126.5 (d), 127.4 (d), 134.1 (d), 137.0 (s), 143.1 (s), 158.7 (s), 192.2 ppm (d); UV (MeOH): λ_{max} = 305 nm; MS: *m/z* (%): 218 (9) [M^+], 203 (11), 189 (23), 179 (15), 148 (12), 133 (16), 119 (100), 105 (11), 91 (12); HMRS *m/z*: calcd for $C_{15}H_{22}O$: 218.1671; found: 218.1674.

(2*E*,4*E*)-3,4-Dimethyl-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dienal ((*E*)-21b): Following the general procedure for TPAP/NMO oxidation, reaction of (*E*)-**12b** (0.061 g, 0.26 mmol) with TPAP (0.004 g, 0.011 mmol) and NMO (0.05 g, 0.39 mmol) in CH_2Cl_2 (3 mL) afforded, after purification by column chromatography (silica gel, 95:5 hexane/EtOAc), (*E*)-**21b** as a yellow oil (0.04 g, 67%). ^1H NMR (400.13 MHz, $CDCl_3$): δ = 0.80–1.10 (br m, 6H; $2CH_3$), 1.45 (s, 3H; CH_3), 1.40–1.50 (br t, *J*(H,H) = 5.7 Hz, 2H; CH_2), 1.60–1.70 (m, 2H; CH_2), 1.72 (s, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 2.36 (s, 3H; CH_3), 6.13 (d, *J*(H,H) = 7.8 Hz, 1H; CH), 6.62 (s, 1H; CH), 10.17 ppm (d, *J*(H,H) = 7.8 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 14.3 (q), 15.2 (q), 19.2 (t), 21.1 (q), 28.4 (q, 2×), 31.9 (t), 35.0 (s), 39.0 (t), 125.6 (d), 129.5 (s), 133.6 (d), 135.7 (s), 137.7 (s), 157.6 (s), 192.1 ppm (d); UV (MeOH): λ_{max} = 289 nm; MS: *m/z* (%): 232 (3) [M^+], 205 (12), 203 (20), 191 (16), 177 (15), 162 (17), 161 (15), 147 (21), 134 (100), 119 (32), 69 (84); HMRS *m/z*: calcd for $C_{16}H_{24}O$: 232.1827; found: 232.1824.

General procedure for Wittig olefination and oxidation

(2*E*,4*Z*,6*E*,8*E*)-9-(6,6-Dimethylcyclohex-1-en-1-yl)-3,7,8-trimethylnona-2,4,6,8-tetraenal (5) [(11*Z*)-5-demethyl-8-methylretinal]: KHMDS (2.52 mL, 0.5 M in toluene, 1.23 mmol) was added to a cooled (−78 °C) suspension of phosphonium salt **22** (0.25 g, 0.56 mmol) in THF (4 mL). After stirring for 10 min at −78 °C and 1 h at 25 °C, the mixture was cooled to −78 °C and a solution of (*E*)-**21a** (0.14 g, 0.62 mmol) in THF (4 mL) was added. The resultant mixture was stirred for 30 min at −78 °C and for 30 min at 25 °C. The mixture was poured over H_2O and extracted with Et_2O (4×). The combined organic layers were dried over Na_2SO_4

and the solvent was evaporated. The residue was purified by column chromatography (80:18:2 hexane/EtOAc/Et_3N) to afford a yellow oil, which was used immediately in the next reaction.

Manganese dioxide (0.49 g, 5.66 mmol) and Na_2CO_3 (0.60 g, 5.66 mmol) were added to a solution of the above compound in CH_2Cl_2 (15 mL), and the suspension was stirred at room temperature for 3 h. The mixture was filtered through Celite and the solvent was removed. The residue was purified by column chromatography (silica gel, 94:3:3 hexane/EtOAc/Et_3N) to afford a yellow oil (0.09 g, 51%). ^{1}H NMR (400.13 MHz, C_6D_6): δ = 1.03 (s, 6H; $2CH_3$), 1.40–1.70 (m, 4H; $2CH_2$), 1.81 (d, J(H,H) = 0.8 Hz, 3H; CH_3), 1.82 (s, 3H; CH_3), 1.93 (d, J(H,H) = 0.8 Hz, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 5.46 (td, J(H,H) = 3.8, 1.5 Hz, 1H; CH), 5.62 (d, J(H,H) = 11.7 Hz, 1H; CH), 6.13 (d, J(H,H) = 7.7 Hz, 1H; CH), 6.40–6.50 (m, 1H; CH), 6.45 (t, J(H,H) = 11.8 Hz, 1H; CH), 6.82 (d, J(H,H) = 11.8 Hz, 1H; CH), 9.98 ppm (d, J(H,H) = 7.7 Hz, 1H; CH); ^{13}C NMR (100.62 MHz, C_6D_6): δ = 14.0 (q), 15.6 (q), 17.5 (q), 19.5 (t), 26.2 (t), 28.5 (q, 2×), 34.5 (s), 39.2 (t), 122.9 (d), 126.2 (d), 129.7 (d), 130.6 (d), 131.2 (d), 131.6 (d), 137.7 (s), 143.2 (s), 143.3 (s), 154.1 (s), 189.8 ppm (d); IR (NaCl): $\tilde{\nu}$ = 2928, 1662, 1594, 1445, 1116 cm^{-1}; UV (MeOH): λ_{max} = 255, 371 nm; MS: m/z (%): 284 (54) [M^+], 269 (23), 202 (21), 187 (47), 176 (39), 173 (58), 171 (28), 161 (60), 159 (51), 145 (42), 133 (37), 119 (100), 105 (34), 95 (67), 91 (43); HMRS m/z: calcd for $C_{20}H_{28}O$: 284.2140; found: 284.2150.

(2*E*,4*Z*,6*E*,8*E*)-3,7,8-Trimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenal (4) [(11*Z*)-8-methylretinal]: Following the general procedure for Wittig olefination and oxidation, compound **4** was obtained in 60% yield after purification by column chromatography (silica gel, 94:3:3 hexane/EtOAc/Et_3N). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 0.80–1.10 (br m, 6H; $2CH_3$), 1.49 (s, 6H; $2CH_3$), 1.50–1.60 (m, 2H; CH_2), 1.70–1.80 (m, 2H; CH_2), 1.83 (s, 3H; CH_3), 1.86 (s, 3H; CH_3), 1.90–2.00 (m, 2H; CH_2), 5.66 (d, J(H,H) = 11.6 Hz, 1H; CH), 6.18 (d, J(H,H) = 7.5 Hz, 1H; CH), 6.35 (s, 1H; CH), 6.48 (t, J(H,H) = 11.6 Hz, 1H; CH), 6.87 (d, J(H,H) = 11.6 Hz, 1H; CH), 9.97 ppm (d, J(H,H) = 7.5 Hz, 1H; CH); ^{13}C NMR (100.63 MHz, $(CD_3)_2CO$): δ = 14.2 (q), 15.7 (q), 18.1 (q), 20.1 (d), 21,5 (q), 28.9 (q, 2×), 32.5 (d), 35.7 (s), 39.9 (d), 123.0 (d), 129.2 (d), 130.9 (d), 131.9 (d), 132.4 (d), 137.2 (s), 139.4 (s, 2×), 143.5 (s), 156.2 (s), 191.4 ppm (d); IR (NaCl): $\tilde{\nu}$ = 2923, 2852, 1661, 1594, 1463 cm^{-1}; UV (MeOH): λ_{max} = 253, 348, 368 nm; MS: m/z (%): 298 (28) [M^+], 284 (14), 201 (25), 175 (28), 173 (35), 171 (29), 159 (58), 147 (34), 145 (42), 133 (99), 119 (63), 109 (65), 97 (60), 95 (74), 83 (51), 73 (61), 71 (61), 69 (100), 67 (60); HMRS m/z: calcd for $C_{21}H_{30}O$: 298.2297; found: 298.2292.

Calculation methods: The structure of bovine rhodopsin (opsin bound to 11-*cis*-retinal), pdb file 1HXZ, was used as the starting point for our calculations. Hydrogen atoms were added and a CHARMM potential was used throughout our calculations.[37] The unbound structures were modeled by excising the retinal fragment from the complex and converting the polar end of the polyene side chain to an aldehyde using the Builder module found in the InsightII suite of programs (from Accelrys, Inc.).[43] The retinal analogues, in their free and bound forms, were modeled by adding (4) and then deleting (5) methyl groups from the original structure. To avoid having to deal with the residue gaps observed in the protein surface of the original crystallographic structure, the rhodopsin complexes with retinals **1**, **4**, and **5** were optimized only 10 Å around the reaction center; this left the surface gaps out of the calculation. All the rhodopsin structures were energy minimized by using the Adopted Basis Newton Raphson (ABNR) protocol.[44]

The final structure of the unbound retinals was obtained from a 120 ps NVT molecular dynamic (MD) simulation. The MD production stage lasted 100 ps and generated a set of 100 frames that were ordered by their internal energy. The lowest energy conformer obtained in the production stage was taken as an energy minimum that yielded the model used in our thermodynamic-cycle calculations.

Rotation barrier calculation: The rotation barrier around the C6–C7 bond in compound **6** was evaluated using the CHARMM molecular modeling suite.[37] A set of rotamers in 10° increments around this bond was set up for the unbound analogue, and an energy minimization, in which the torsion angle around this bond was frozen, was carried out for all conformers. In all cases, an energy tolerance of 10^{-4} to 10^{-5} was reached.

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