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The Role of the 11-*cis*-Retinal Ring Methyl Substituents in Visual Pigment Formation

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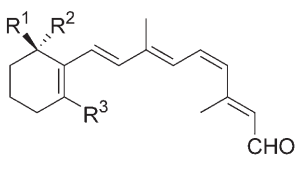
*Artificial visual pigment formation from ring-demethylated retinals was studied in an effort to understand the effect that methyl groups on the chromophore cyclohexenyl ring have on the visual cycle. The stereoselective synthesis of the 11-*cis*-ring-demethylated analogues involves thallium-accelerated Suzuki cross-coupling reactions and highly stereocontrolled Wittig reactions to form key bonds. Only 11-*cis*-1,1,5-trisdemethylretinal (**2**) failed to form an artificial pigment, whilst variable pigment-formation yields were determined for the remaining analogues, increasing with the number (and location) of the chromophore hydrophobic ring methyl groups. Our results with the monodemethylated analogues 11-*cis*-5-demethylretinal (**4**) and 11-*cis*-1-demethylretinal (**5**) show that the C1–2-CH_3 groups are more important for pigment formation than the C5-CH_3 substituent. This is reflected in the absorption maxima of the artificial pigments, with values closer to that of native rhodopsin for **4**. Docking studies based on a rhodopsin crystal structure, however, predict a lower pigment stability for **4** than for **5**. Gas-phase DFT (B3LYP/6-31G*) computations of the free-ligand geometries, conformational searches about the C6–C7 bond, and docking studies revealed that, although the conformation of bound **5** is close to that of the native chromophore, the ligand needs to overcome the energy cost of shifting the unbound favored 6-*s*-trans conformation to the bound 6-*s*-cis form. In addition, the presence of an extra methyl group at C18 (11-*cis*-18-methylretinal, **7**) is tolerated well and adds further stability to the complex, most probably due to increased hydrophobic interactions.*

Introduction

Rhodopsin (Rh) is the protein in the vertebrate retina that initiates the visual transduction cascade under dim light conditions. It is a member of the G protein-coupled receptor (GPCR) superfamily, which also includes proteins that are sensitive and responsive to cell stimuli such as calcium ions, neurotransmitters, hormones, and neuropeptides.[1] The backbone architecture of GPCRs consist of seven transmembrane α-helices (helix I to helix VII).[2] Unlike in other GPCR subfamilies, the chromophore (11-*cis*-retinal, **1**, Scheme 1) in Rh is covalently bound to the protein through a protonated Schiff base to Lys296 in helix VII, with Glu113 in helix III acting as counterion.

Visual transduction is the process by which visual retina cells convert light into a neural signal, which is in turn transmitted to the brain along the optic nerve.[3] It is initiated by photoisomerization of 11-*cis*-retinal (**1**) to all-*trans*-retinal upon light absorption. This event gives rise to a series of conformational changes in the chromophore, linked to changes in the protein structure (such as displacement of helices and reorganization of cytoplasmic loops), deprotonation of the Schiff base (with concomitant proton uptake by Glu134), and hydrolysis of *trans*-retinal from the apoprotein.[4,5] After bleaching, different spectrophotometrically detectable Rh intermediates are formed and their interconversion kinetics can be successfully studied at low temperature. In vertebrate vision, the blue-shifted metarhodopsin II (Meta II) intermediate ($\lambda_{max} = 380$ nm) can be regarded as the agonist-activated form, interacting with the heterotrimeric G protein transducin. The biochemical cascade generates a neural signal that provides the sensation of vision.[4–6]

The X-ray structure of bovine Rh (a 40 kDa protein with 348 amino acids and the bound ligand 11-*cis*-retinal **1**) in its inactive state has been solved;[7] it revealed a highly compact binding pocket in which the chromophore acts as an inverse



1 R^1, R^2, R^3 =Me (11-*cis*-retinal)
2 R^1, R^2, R^3 = H
3 R^1 = Me, R^2, R^3 = H
4 R^1, R^2 = Me, R^3 = H
5 R^1 = H, R^2, R^3 = Me
6 R^1, R^2 = H, R^3 = Me
7 R^1, R^2 =Me, R^3 = Et

Scheme 1. 11-*cis*-Retinal (**1**), its ring-demethylated analogues **2**–**6**, and its C18-methyl derivative **7**.

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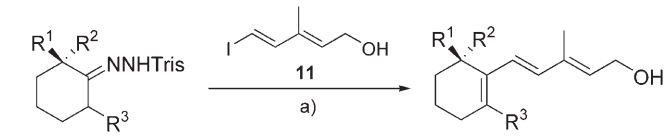
agonist of opsin.[7,8] Additional structural details relating to the geometric changes during the temporal sequencing of the primary isomerization event that converts rhodopsin into the bathorhodopsin intermediate have been gleaned from recent femtosecond-stimulated Raman spectroscopy.[9] Further structural information is lacking, still leaving some uncertainties as to the changes in protein and retinal conformation before the latter leaves the binding site upon hydrolysis of the Schiff base.

The use of synthetic retinals[10] with structural modifications on the cyclohexenyl ring or on the adjacent side chain might help in clarifying the role played by conformational changes of the chromophore in the visual cycle. The regeneration of a pigment incorporating an acyclic retinal[11] has established that the cyclohexenyl moiety is not required for reconstitution, though its reduced photoactivity in relation to the native pigment suggests a key structural role for the methyl groups in the visual process.

We have recently reported the consequences of steric perturbation of the C6–C7 conformational equilibrium of the visual pigment chromophore through the incorporation of methyl groups at the C8-position, with the finding that the perturbation extends to the C9–C10 bond, thus impairing reconstitution. In order to understand the effect of structural modifications on the cyclohexenyl ring region on the visual cycle, we now report the stereoselective synthesis of several ring-demethylated analogues of the parent chromophore (**2**–**6**) and of its C18-methyl derivative **7**, as well as preliminary opsin-binding experiments. The results reveal the role that cyclohexene ring methyl substituents of native 11-*cis*-retinal (**1**) have in artificial pigment regeneration.

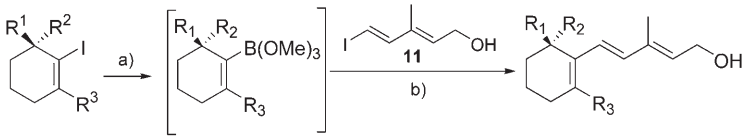


8 R^1, R^2, R^3 = H
9 R^1 = Me; R^2, R^3 = H
10 R^1, R^2 = Me; R^3 = H

12 R^1, R^2, R^3 = H
13 R^1 = Me R^2, R^3 = H
14 R^1, R^2 = Me, R^3 = H

Scheme 2. a) i) *n*BuLi, $B(OiPr)_3$, THF, −78→0 °C. ii) $Pd(PPh_3)_4$, iodide **11**, 10% aq. TlOH, THF, 25 °C (**12**, 74%; **13**, 98%, **14**; 77%).

For the generation of more highly substituted cycloalkenyl lithium species from hindered hydrazones—which showed inefficient and unselective deprotonation of the C_α tertiary carbon with *n*BuLi—the alternative halogen–lithium exchange reaction was used instead (Scheme 3). Oxidation of the hydrazone derived from treatment of the corresponding cyclohexanone with iodine by Barton's procedure[16] afforded iodides **15**[17] and **16**, which were treated with *t*BuLi at −78 °C with subsequent addition of $B(OMe)_3$ to generate the corresponding organoboron species. Sequential addition of iodide **11**, $Pd(PPh_3)_4$, and 10% TlOH to the above boronates provided trienols **17** and **18** uneventfully in 86 and 78% overall yields, respectively.



15 R_1 = H R_2, R_3 = Me
16 R_1, R_2 = Me, R_3 = Et

17 R_1 = H R_2, R_3 = Me
18 R_1, R_2 = Me, R_3 = Et

Scheme 3. a) i) *t*BuLi, $B(OMe)_3$, THF, −78→0 °C. ii) $Pd(PPh_3)_4$, iodide **11**, 10% aq. TlOH, THF, 25 °C (**17**, 86%; **18**, 71%).

Results and Discussion

Synthesis

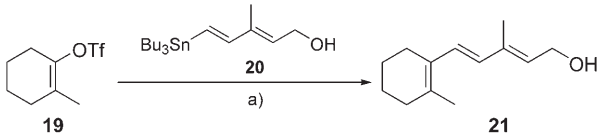
In our previously described protocol for the preparation of 9-*cis*- and 11-*cis*-retinal derivatives with structural perturbations induced through the presence of an extra methyl group at C8,[12] two key disconnections were selected: C11–C12, calling for a *cis*-selective Wittig reaction, and C6–C7, a thallium-accelerated Suzuki cross-coupling reaction between cyclohexenylboronic acids and dienyliodides, to connect the ring and the polyene chain.

One-pot Shapiro–Suzuki reactions starting from unhindered cyclohexanone hydrazones[13] were selected for the construction of the C6–C7 bonds in alcohols **12**–**14**, as shown in Scheme 2. Cyclohexenylboronates were prepared by $B(OiPr)_3$ or $B(OMe)_3$ trapping of the corresponding organolithium species, themselves obtained through *n*BuLi-induced elimination of trisylhydrazones **8**–**10** (Scheme 2).

Sequential addition of $Pd(PPh_3)_4$, dienyl iodide **11**,[14] and a 10% aqueous TlOH solution[15] to the above boronates, with stirring for 4 h at 25 °C, provided the desired alcohols **12**–**14** in 74–98% combined yields.

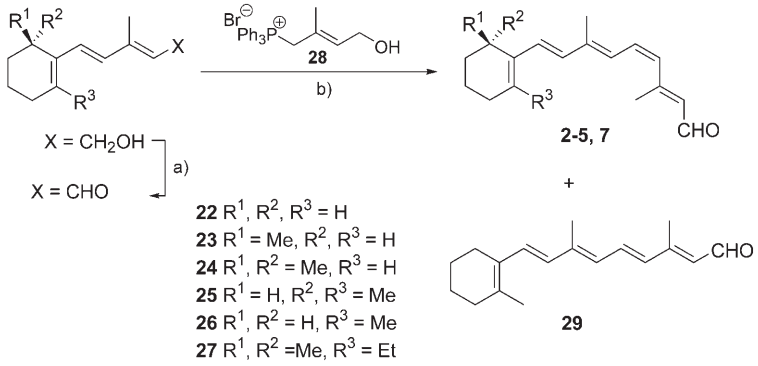
The preparation of the 1,1-bis-demethyl derivative by the above sequence encountered insurmountable regioselectivity problems. Despite our attempts, we could not induce the elimination of the hydrogen atom at the most substituted position in the Shapiro reaction. As an alternative, a Stille coupling between cyclohexenyl triflate **19**[18] and tributylstannyldienol **20**[19] in the presence of $Pd_2dba_3/AsPh_3$ as catalyst in NMP delivered alcohol **21** in 93% yield (Scheme 4).

Trienols **12**–**14**, **17**–**18**, and **21** were oxidized with substoichiometric (0.05 equiv) quantities of tetra-*n*-propylammonium perruthenate (TPAP) in CH_2Cl_2 and *N*-methylmorpholine *N*-



19 **21**

Scheme 4. a) Pd_2dba_3, $AsPh_3$, NMP, 25 °C, 93%.



Scheme 5. a) TPAP, NMO, CH_2Cl_2, 25 °C (**22**, 62%; **23**, 75%; **24**, 62%; **25**, 71%; **26**, 80%; **27**, 60%). b) i) KHMDS, **28**, THF, −78→25 °C. ii) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C. iii) HPLC purification (**2**, 26%; **3**, 36%; **4**, 33%; **5**, 39%; **7**, 37%; **29**, 33%).

oxide (NMO) as co-oxidant (1.5 equiv),[20] to furnish the aldehydes **22**–**27** in good yields (Scheme 5).

For the stereoselective preparation of retinoids with the (11*Z*) geometry, we adhered to the procedure described by Kobayashi:[21,12] a Wittig condensation with the (*E*)-oxido-allylic phosphorane reagent derived from phosphonium salt **28** (Scheme 5). Treatment of aldehydes **22**–**27** with **28** and KHMDS at −78 °C provided the sensitive 11-*cis*-retinol analogues, which without isolation were immediately oxidized with MnO_2 under basic conditions at 25 °C to afford the corresponding retinals **2–5** and **7** and further purified by HPLC. Despite the precautions, the isomerization of the C11—C12 double bond in **6** could not be avoided (all-*trans*-1,1-bis-demethylretinal **29** was isolated instead), so this analogue was unavailable for opsin-binding studies.

Protein binding—molecular modeling studies

Opsin-binding[22] experiments (Figure 1) revealed that the removal of the cyclohexenyl methyl groups was detrimental to apoprotein binding. No pigment formation was detected in the case of 11-*cis*-1,1,5-tris-demethylretinal (**2**), confirming that at least one of the ring methyl substituents is required for pigment stability, since 11-*cis*-1,5-bis-demethylretinal (**3**) does form an artificial rhodopsin, albeit with an efficacy four times lower (24% yield). The differential contributions of the methyl substituents to pigment formation, as examined with the monodemethylated analogues **4**[23] and **5**, with 70% and 33% yields of pigment formation, respectively, point to a more significant role for the geminal C1–2-CH_3 groups. This might be due to enforcement of the C6—C7 s-*cis* conformation—required for the chromophore in the opsin-binding pocket—by 1,2-allylic strain by these substituents, which would be consistent with the absorption values for the pigments (Figure 2). The pigment formed with 11-*cis*-5-demethylretinal (**4**) shows an absorption maximum slightly blue-shifted (492 nm) relative to Rh (500 nm), whereas **5**, in which the C5-methyl group is present, exhibits a ca. 20 nm red-shifted maximum, indicative of greater planarity (more extended conjugation) of the chromophore in the retinal binding pocket (Table 1).

To investigate the energetics of binding and the effects of modifications of the chromophore on pigment stability further, we performed a comprehensive study of the ring-demethylated analogues, initially involving solution conformational studies by NMR and nOe, computations of the ligand conformations both in gas phase and in solution, as well as conformational searches about the C6—C7 bond, and finally docking of the minimized structures into the opsin binding pocket.

Conformational analysis

nOe difference spectra were recorded for all synthetic chromophores in order to determine their predominant conformations in solution (Table 2). The nOe experiments indicate favored 6-s-*trans* conformations for all the ring-demethylated analogues, in contrast to the predominant 6-s-*cis* conformations of both 11-*cis*-retinal (**1**) and 11-*cis*-C18-methylretinal (**7**).

The geometries of the ligands were then optimized by density functional theory at the B3LYP/6-31G* level in the gas phase with the Gaussian03 suite of programs,[24] in an attempt to make an estimate of how and to what extent the substituents determine the conformation of the ligand. Table 2 lists the relative Gibbs free energies for the minima found by conformational searching around the C6—C7 bond. According to these computations, the s-*trans* conformation is preferred for the C6—C7 bonds in all the 11-*cis*-ring-demethylated retinals with the exception of **4** (note also the same preference for **7**). In this case, the s-*cis* conformer is slightly more stable (0.56 kcal mol^{-1}),

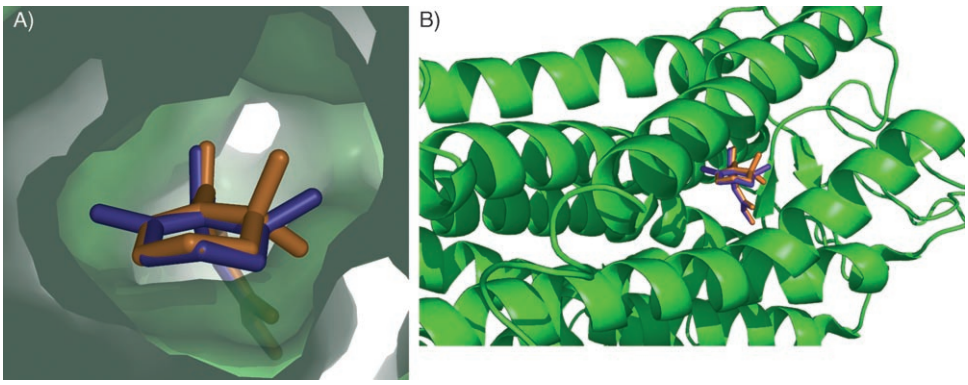


Figure 1. Left: Close-up view of the opsin retinal binding site (green) showing the ligand hydrophobic region for the most favored conformations (s-*cis*) of analogues **4** (orange) and **5** (blue) obtained by docking studies. The restricted volume around C18 (left) disfavors the s-*trans* conformation of **4**. Right: The same view, showing the ligand disposition between the transmembrane helices of the protein.

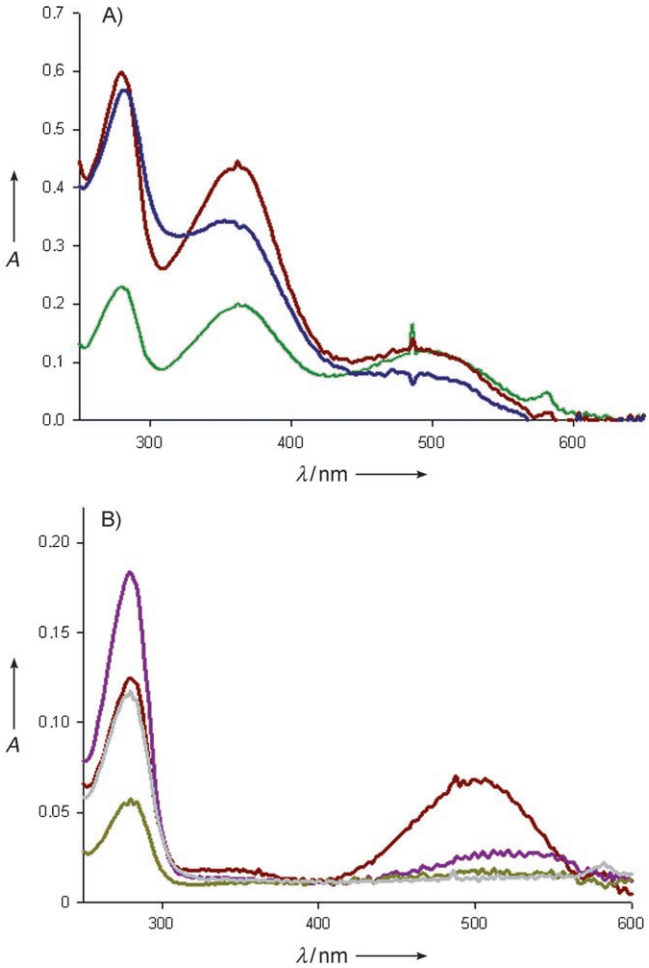


Figure 2. UV spectra of formed pigments. Red: **1**, green: **7**, blue: **4**, pink: **5**, yellow: **3**, gray: **2**.

Table 1. Yields of pigment formation and spectroscopic data for 11-*cis*-ring(*de*)methylated retinal analogues and artificial rhodopsins formed from them.

	UV retinal λ_{max} [MeOH, nm], (ε)	Incubation with opsin Yield [%]	UV rhodopsin λ_{max} [nm]
7	377 (16000)	105	500
1	**379 (25600)**	**100**	**500**
4	385 (20200)	70	492
5	394 (18900)	33	522
3	389 (21500)	24	516
2	387 (21500)	–[a]	–

[a] No pigment formation was detected.

with a barrier to bond rotation of ca. 4.78 kcal mol^{-1}. It can be concluded that the C1–2-CH_3 groups in retinal enforce the C6–C7 s-*cis* conformation, and that the lack of even one of these substituents changes the conformational preference to s-*trans*.

In order to find a rationale for the results based on energy criteria and pigment stability of the artificial rhodopsins derived from **2**–**5**, we performed docking calculations using Autodock3[25] (see Experimental Section). The X-ray diffraction structure of rhodopsin (at 2.2 Å resolution)[8c] was used as starting point to compute ΔG after addition of the charges, solvation, and hydrogen atoms. The Gibbs free energy for the ring-modified analogues evaluated by docking calculations and their variation relative to native 11-*cis*-retinal are shown in Table 3.

Pigment stability is clearly very sensitive to the occupancy of the opsin binding pocket. Whereas the C16- and C17-methyl groups appear to be more critical than the C18-Me substituent for pigment formation, stability might be more related to differential hydrophobic interactions (Table 1). There is an inver-

Table 2. Gas-phase conformational analysis of ring-modified retinal analogues **1**–**7** (B3LYP/6-31G*), energies of the C6–C7 conformers relative to the minimum and their interconversion energies, and predominant C6–C7 conformations determined in solution by NOE experiments.

Analogue	Energy[a]	C5–C6–C7–C8 Dihedral[b] [°]	Conformation A[c]	ΔE[d] s-*cis*–s-*trans*	ΔE[d] s-*trans*–s-*cis*	Conformation B[e]
retinal (**1**)	0.29	−48	6-s-*cis*			–
	0	**48**	**6-s-*cis***	2.68	1.14	
	1.55	180	6-s-*trans*			
11-*cis*-1,1,5-tris-demethylretinal (**2**)	3.22	−24	6-s-cis			6-s-*trans*
	2.94	30	6-s-*cis*	5.11	8.05	
	0	**180**	**6-s-*trans***			
11-*cis*-1,5-bis-demethylretinal (**3**)	1.09	−36	6-s-*cis*			6-s-*trans*
	1.52	30	6-s-*cis*	4.44	5.53	
	0	**162**	**6-s-*trans***			
11-*cis*-5-demethylretinal (**4**)	**0**	**−30**	**6-s-*cis***			6-s-*trans*
	0.06	36	6-s-*cis*	4.79	4.23	
	0.56	168	6-s-*trans*			
11-*cis*-1-demethylretinal (**5**)	1.54	−42	6-s-*cis*			6-s-*trans*
	1.73	42	6-s-*cis*	2.03	3.57	
	0	**162**	**6-s-*trans***			
11-*cis*-1,1-bis-demethylretinal (**6**)	3.81	−30	6-s-*cis*			–[f]
	2.89	36	6-s-*cis*	3.00	5.90	
	0	**174**	**6-s-*trans***			
11-*cis*-5-ethylretinal (**7**)	**0**	**−42**	**6-s-*cis***			6-s-*cis*
	0.42	54	6-s-*cis*	3.13	1.93	
	1.18	162	6-s-*trans*			

[a] In kcal mol^{-1}. [b] C5–C6–C7–C8 dihedral angles (in degrees) for the most stable conformers. [c] Most stable conformer (bold type) obtained by DFT calculations (B3LYP/6-31G*). [d] Conformational barriers (in kcal mol^{-1}) to C6–C7 bond rotation obtained by DFT calculations (B3LYP/6-31G*). [e] Most populated conformer in solution, as determined by nOe experiments. [f] Not available.

Table 3. Docking energy results of free (fd) versus locked (ld) C5–C6–C7–C8 dihedral, and energy penalty due to fixing of the angle of the ligand in solution to its minimum.

Analogue	Free dihedral angle	E_{fd}	ΔE_{fd}	Locked dihedral angle	E_{ld}	ΔE_{ld}	Energy penalty $\Delta\Delta E_{ld-fd}$
1	−51.93	−11.52	0.00	48	−10.49	0.00	1.03
2	−122.07	−8.36	3.16	180	−9.42	1.07	−1.06
3	−11.30	−9.75	1.77	162	0.51	11.00	10.26
4	−66.05	−10.83	0.69	−30	−10.83	−0.34	0.00
5	−60.49	−11.04	0.48	162	–	10.49	–
6	71.42	−10.63	0.89	180	−8.01	2.48	2.62
7	−35.87	−11.69	−0.17	42	−6.52	3.97	5.17

[a] Computed with Autodock3. [b] No comparison can be made with the remaining analogues.

sion in the yield of pigment formation for the 11-*cis*-1-demethylated analogue **5** relative to 11-*cis*-5-demethylretinal (**4**), on comparison with their computed ligand–receptor interaction energies. The rate of pigment formation (incubation times are the same for all experiments) might be related to selection of the 6-s-*cis* conformation of the ligand by the apoprotein. Therefore, although the ligand–receptor interaction free energy of **5** upon incubation with opsin is predicted to be higher (more negative) than that of **4**, 11-*cis*-1-demethylretinal (**5**) would have to pay a penalty (the s-*trans* conformation is favored by 1.74 kcal mol^{-1}) for adopting the s-*cis* conformation required by the opsin binding pocket (Figure 3) and to overcome a barrier of 3.38 kcal mol^{-1} (see Figure 4).

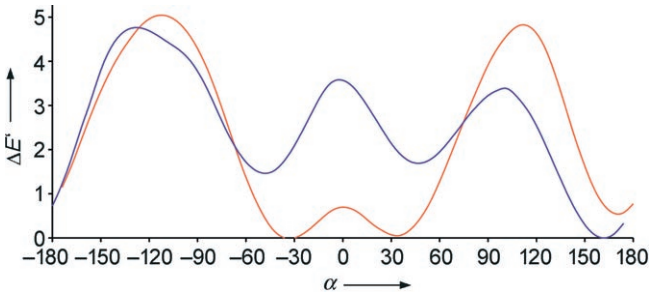


Figure 3. Torsional energy differences (relative to the most stable conformation in each case) for the C5–C6–C7–C8 dihedral of the monodemethylated 11-*cis*-retinal analogues **4** (orange) and **5** (blue). The optimal docking conformation falls around the −45° (s-*cis*) minima in both cases, but the solution conformation for **4** is s-*cis* whilst for **5** it is s-*trans*.

The torsion energy penalty due to the binding conformation, relative to that of the free ligand, was estimated by docking the conformation with the C5–C6–C7–C8 dihedral fixed to the optimal value with the apoprotein in solution; results are shown in Table 3. Whereas **4** gave the same value (−10.83 kcal mol^{-1}) as for the unconstrained conformation, **5** gave a value of −8.76 kcal mol^{-1} only when the clustering level was lowered to 25%, due to the difficulties found on docking the constrained conformation. In addition, the energy value remained virtually unchanged when the population level of the starting freely rotating sample was lowered, supporting the above hypothesis.

Minima were also calculated in methanol as solvent, with the aid of two continuum models. Results obtained by the

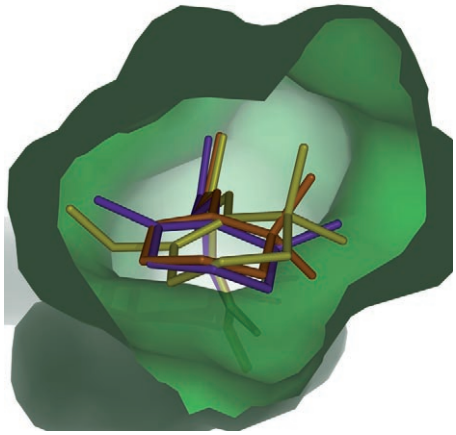


Figure 4. Opsin binding site (green surface) with analogues **4** (orange), **5** (blue), and **7** (yellow).

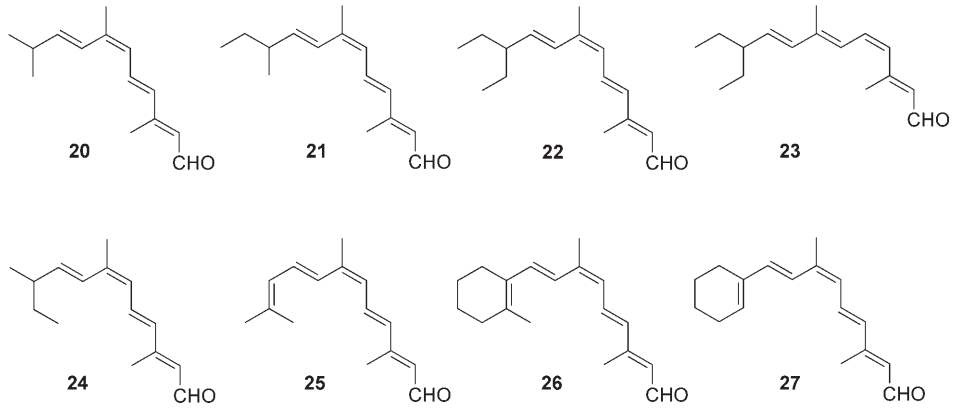
SCIPCM method were consistently about 6 kcal mol^{-1} lower than those in the gas phase. Greater stabilization (about 23 kcal mol^{-1}) was computed with IPCM for all the conformations in each analogue. Nevertheless, the relative variations between different analogues were small (~3 kcal mol^{-1} for SCIPCM, ~2 kcal mol^{-1} for ICPMC), reflecting the observation that the skeleton remained unaffected when solvent was included. The dihedral angles varied slightly from gas to solution phase (less than 6.8 degrees for **7**, less than 5 degrees for most cases), so the solvent contribution to the conformational preferences can be regarded as negligible.

As additional confirmation of the importance of the ring methyl substituents in pigment formation, we determined that incubation of opsin with 11-*cis*-5-ethylretinal (**7**) is more efficient than with native **1**. The increase in hydrophobic interaction energy associated with the added methyl group in **7**, exhibiting the same conformational preferences in a region known to accept structural modifications without alteration of the overall structure, results in a more stable pigment, according to the energy values (Table 3). The computed conformation of **7** bound to opsin is depicted in Figure 4 and compared with those of **4** and **5**. Dispersion interactions inside the protein binding pocket increase in order to overcome the torsion

energy penalty due to the extra space necessary to accommodate the C18 substituent.

Conclusion

Some retinal analogues (**20**–**25**) lacking portions of the hydrophobic ring have been described (Scheme 6).[26,11,27] Despite the absence of the C2 and C3 ring atoms, derivatives **21**–**23** provided partially active pigments upon incubation with opsin



Scheme 6. Acyclic and cyclic retinal analogues incubated with opsin.

(artificial isorhodopsins for the 9-*cis*-retinal analogues **21** and **22**, with rates three times slower than 9-*cis*-retinal, and artificial rhodopsin for **23**).[26] It was concluded that at least the C5-CH_3 and/or one of the C1–2-CH_3 substituents were required for pigment formation, since **20** did not form pigment. In a recent report, Vogel, Sheves, and co-workers described opsin binding by several ring-modified analogues (**21**, **22**, **24**–**27**) and analysis of the conformational equilibria of the Meta I/Meta II photoproducts obtained from the artificial pigments that confirm the above results.[27,28] Interestingly, the artificial isorhodopsins from 9-*cis*-1,1-bisdemethylretinal (**26**) and 9-*cis*-1,1,5-trisdemethylretinal (**27**) could be formed, although with very low regeneration efficacies (10% and 5%, respectively). The considerable reduction in regeneration and efficiency with these ring-demethylated retinal analogues, a likely indication of slow equilibrium between binding and unbinding, together with the results for the acyclic analogues (**21**, **22**, **24**), demonstrated the importance of the ring methyl groups in anchoring the chromophore in the binding site as an inverse agonist.

Together, these reports highlight the importance of substituents mimicking the ring-methyl groups in analogues with truncation in the hydrophobic ring. Our data in Table 2 appear to confirm these findings even in the case of intact cyclohexenyl skeletons. The rate of visual pigment formation is critically dependent upon the number and positions of the ring methyl groups in its 11-*cis*-retinal (**1**) chromophore. A combination of suboptimal occupancy of the binding pocket and the energy penalty entailed in shifting the preferred 6-s-*trans* conformation of the free ligand to the restricted environment of the binding site, enforcing the 6-s-*cis* conformation, explain the ordering of pigment formation of analogues **2**–**5**. As additional evidence of the relevance of the ring methyl substituents in pigment formation, incubation of opsin with 11-*cis*-C18-methylretinal (**7**) yields a more stable pigment than **1**, with greater efficiency. The similarity of the absorption maxima of **1** and **7** points to an increase in stabilizing hydrophobic interactions imparted by the additional methyl group in a region that seems to accept structural modifications without distortion of the overall structure.

Retinal analogues **2**–**7** incorporate modifications in the vicinity of the ring-chain connection, a region previously shown to be important through its implication in the control of the decay rates of early photointermediates. These structural modifications could in turn induce conformational changes around the C6–C7 bond, resulting in sub-optimal protein–ligand interaction. Work to determine the features of the artificial pigment photocycle and the effect that each of the ring methyl substituents has on the rate of formation and the stability of the different photointermediates is in progress. Since solid-state NMR studies have shown that the C1–2-CH_3 groups are more strongly fixed by the protein moiety in Meta II, it may be expected that the artificial pigments described here should significantly affect that critical signaling step, becoming useful tools for further expansion of structural and functional studies to provide better understanding of the vertebrate visual cycle.

Experimental Section

General. Solvents were dried by published methods and distilled before use. HPLC-grade solvents were used for the HPLC purification. All other reagents were commercial compounds of the highest purity available. All reactions were carried out under argon, and those not involving aqueous reagents were carried out in oven-dried glassware. Analytical thin layer chromatography (TLC) was performed on aluminium plates with Merck Kieselgel 60F254 with viewing under UV irradiation (254 nm) or by staining with solution of phosphomolybdic acid. Flash column chromatography was carried out on Merck Kieselgel 60 (230–400 mesh) under pressure. High-performance liquid chromatography was performed on a Waters instrument with use of a dualwave detector (254 and 300 nm), a Preparative Nova Pak HR (silica, 60 Å, 19×300 mm), and hexane/ethyl acetate 95:5 as eluent. UV/Vis spectra were recorded on a Cary 100 Bio spectrophotometer in MeOH as solvent. Infrared spectra were obtained on JASCO FTIR 4200 spectrophotometer, from a thin film deposited onto a NaCl glass. Specific rotations were measured on a JASCO P-1020 machine. Mass spectra were obtained on a Hewlett–Packard HP59970 instrument operating at 70 eV by electron ionization. High-resolution mass spectra were taken on a VG Autospec instrument. ^{1}H NMR spectra were recorded in $CDCl_3$, C_6D_6, and $(CD_3)_2CO$ at ambient temperature on a Bruker

AMX 400 spectrometer at 400 MHz with residual protic solvent as the internal reference [$CDCl_3$, $\delta_H = 7.26$ ppm; C_6D_6, $\delta_H = 7.16$ ppm; $(CD_3)_2CO$, $\delta_H = 2.05$ ppm]; chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz). The proton spectra are reported as follows: δ (multiplicity, coupling constant J, number of protons, assignment); ^{13}C NMR spectra were recorded in $CDCl_3$ and C_6D_6 at ambient temperature on the same spectrometer at 100 MHz, with the central peaks of $CDCl_3$ ($\delta_C = 77.0$ ppm) or C_6D_6 ($\delta_C = 128.0$ ppm) as the internal references. DEPT135 was used to aid in the assignment of signals in the ^{13}C NMR spectra.

(2*E*,4*E*)-5-(Cyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (12)—General Procedure for the Shapiro–Suzuki reaction: A cooled (−78 °C) solution of cyclohexanone trisylhydrazone (**8**, 0.44 g, 1.16 mmol) in THF (3 mL) was treated with *n*BuLi (3.12 mL, 1.09 M in hexane, 3.39 mmol). After the mixture had been stirred for 30 min, $B(OiPr)_3$ (0.53 mL, 2.23 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. This solution was then transferred to a separate flask containing a solution of $Pd(PPh_3)_4$ (0.10 g, 0.089 mmol) and (2*E*,4*E*)-5-iodo-3-methylpenta-2,4-dien-1-ol (**11**, 0.17 g, 0.77 mmol) in THF (3 mL). After addition of an aqueous TlOH solution (10%, 7.7 mL, 3.45 mmol), the mixture was stirred for 4 h at 25 °C and was then diluted with Et_2O (5 mL) and washed with aqueous $NaHCO_3$ (3×). The organic layer was dried (Na_2SO_4), the solvent was evaporated, and the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 90:10) to afford a yellow oil (0.10 g, 74%) identified as (2*E*,4*E*)-5-(cyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (**12**). ^1H NMR (400.16 MHz, $CDCl_3$): $\delta = 6.22$ (d, $J = 16.1$ Hz, 1H; H_5), 6.13 (d, $J = 16.1$ Hz, 1H; H_4), 5.78 (s, 1H; $H_{2'}$), 5.62 (t, $J = 6.8$ Hz, 1H; H_2), 4.24 (d, $J = 6.8$ Hz, 2H; $2H_1$), 2.2–2.1 (m, 4H; 2H3 + 2H6′), 1.79 (s, 3H; C3–CH_3), 1.7–1.6 (m, 2H; 2H4′), 1.6–1.5 ppm (m, 2H; 2H5′); ^{13}C NMR (100.63 MHz, CDCl3): $\delta = 136.3$ (s), 135.8 (s), 131.8 (d), 130.1 (d), 129.2 (d), 128.9 (d), 59.1 (t), 26.0 (t), 24.6 (t), 22.6 (t), 22.5 (t), 12.6 ppm (q) ; FTIR (NaCl): $\nu = 3600$–3100 (br, OH), 2932 (s, C–H), 2861 (s, C–H), 1669, 1380 cm^{-1}; UV (MeOH): $\lambda_{max} = 280$, 269, 259 nm; MS (EI^+): *m/z* (%): 179 $[M+1]^+$ (21), 178 $[M]^+$ (53), 160 (11), 151 (28), 147 (40), 135 (25), 121 (26), 105 (100), 95 (39), 93 (34), 91 (47), 83 (20), 81 (49), 79 (51), 77 (33); HRMS (EI^+): calcd for $C_{12}H_{18}O$: 178.1358; found: 178.1360.

(2*E*,4*E*)-5-(Cyclohex-1-enyl)-3-methylpenta-2,4-dienal (22)—General Procedure for the TPAP/NMO oxidation: A solution of (2*E*,4*E*)-5-(cyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (**12**, 0.1 g, 0.56 mmol) in CH_2Cl_2 (2 mL) was added to a cooled (0 °C), stirred solution of *N*-methylmorpholine *N*-oxide (0.1 g, 0.84 mmol) in CH_2Cl_2 (3 mL) containing 4 Å molecular sieves. After the system had been stirred for 10 min, TPAP (0.01 g, 0.03 mmol) was added and the mixture was stirred at 25 °C for 3.5 h. The mixture was diluted with CH_2Cl_2 (4 mL) and washed with aqueous Na_2SO_3 (3×). The organic layer was dried (Na_2SO_4), the solvent was removed, and the residue was purified by column chromatography (silica gel, hexane/ethyl acetate/Et_3N 93:5:2) to afford a yellow solid (0.06 g, 62%) identified as (2*E*,4*E*)-5-(cyclohex-1-enyl)-3-methylpenta-2,4-dienal (**22**). ^1H NMR (400.16 MHz, $CDCl_3$): $\delta = 10.03$ (d, $J = 8.2$ Hz, 1H; H1), 6.65 (d, $J = 15.9$ Hz, 1H; H5), 6.17 (d, $J = 15.9$ Hz, 1H; H4), 5.99 (s, 1H; H2′), 5.90 (d, $J = 8.2$ Hz, 1H; H2), 2.21 (s, 3H; C3–CH_3), 2.2–2.1 (m, 4H; 2H3′ + 2H6′), 1.7–1.6 (m, 2H; 2H4′), 1.6–1.5 ppm (m, 2H; 2H5′); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 191.1$ (d), 155.3 (s), 139.6 (d), 136.0 (d), 135.8 (s), 128.9 (d), 127.7 (d), 26.6 (t), 24.3 (t), 22.2 (t, 2×), 13.0 ppm (q) ; FTIR (NaCl): $\nu = 2926$ (s, C–H), 2857 (m, C–H), 1661 (s, C=O), 1599 cm^{-1}; UV (MeOH): $\lambda_{max} = 327$ nm; MS (EI^+): *m/z* (%): 176 $[M]^+$ (53), 161 (24), 147 (41), 121 (23), 105 (100), 96 (28), 95 (70), 91 (49), 85 (54), 83 (83), 79 (32), 77 (28); HRMS (EI^+): calcd for $C_{12}H_{16}O$: 176.1201; found: 176.1201.

(11*Z*)-1,1,5-Trisdemethylretinal (2)—General procedure for the Wittig oxidation reaction sequence: KHMDS (4.9 mL, 0.5 M in toluene, 2.45 mmol,) was added to a cooled (−78 °C) suspension of (4-hydroxy-2-methylbut-2-enyl)triphenylphosphonium bromide (**28**, 0.79 g, 1.15 mmol) in THF (28 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 (2*E*,4*E*)-5-(cyclohex-1-enyl)-3-methylpenta-2,4-dienal (**22**, 0.13 g, 0.77 mmol) in THF (14 mL) was added. The resulting mixture was stirred for 30 min at −78 °C and for 45 min at 25 °C, poured over H_2O, and extracted with Et_2O (4×). The combined organic layers were dried (Na_2SO_4), the solvent was evaporated, and the residue was purified by column chromatography (hexane/ethyl acetate/Et_3N 80:18:2) to afford a yellow oil which was used immediately.

MnO_2 (0.66 g, 7.66 mmol) and Na_2CO_3 (0.81 g, 7.66 mmol) was added to a solution of this compound in CH_2Cl_2 (14 mL) and the suspension was stirred at room temperature for 3 h. The mixture was filtered through Celite, the solvent was removed, and the residue was purified by HPLC to afford a yellow oil (47 mg, 26%) identified as (11*Z*)-1,1,5-trisdemethylretinal (**2**). ^1H NMR (400.16 MHz, C_6D_6): $\delta = 9.94$ (d, $J = 7.7$ Hz, 1H; H15), 6.66 (d, $J = 12.4$ Hz, 1H; H10), 6.5–6.4 (m, 1H; H11), 6.41 (d, $J = 15.5$ Hz, 1H; H7), 6.29 (d, $J = 15.5$ Hz, 1H; H8), 6.15 (d, $J = 7.7$ Hz, 1H; H14), 5.75 (s, 1H; H5), 5.59 (d, $J = 11.8$ Hz, 1H; H12), 2.0–1.9 (m, 4H; $2CH_2$), 1.81 (d, $J = 1.0$ Hz, 3H; C13–CH_3), 1.74 (s, 3H; C9–CH_3), 1.5–1.4 ppm (m, 4H; $2CH_2$); UV (MeOH): $\lambda_{max} = 387$ ($\varepsilon = 21\,500$), 275 nm; MS (EI^+): *m/z* (%): 243 $[M+1]^+$ (19), 242 $[M]^+$ (100), 227 (24), 213 (27), 160 (21), 157 (27), 147 (25), 146 (21), 145 (25), 143 (28), 133 (17), 131 (50), 129 (25), 128 (22), 119 (32), 118 (20), 117 (28), 115 (25), 105 (81), 96 (42), 95 (42), 93 (21), 92 (14), 91 (70); HRMS (EI^+): calcd for $C_{17}H_{22}O$: 242.1671; found: 242.1701.

(2*E*,4*E*)-3-Methyl-5-(6-methylcyclohex-1-enyl)penta-2,4-dien-1-ol (13): Treatment of 2-methylcyclohexanone trisylhydrazone (**9**, 0.46 g, 1.16 mmol) in THF (6 mL) with *n*BuLi (3.12 mL, 1.09 M in hexane, 3.39 mmol,), $B(OiPr)_3$ (0.53 mL, 2.23 mmol), (2*E*,4*E*)-5-iodo-3-methylpenta-2,4-dien-1-ol (**11**, 0.17 g, 0.78 mmol), $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol), and an aqueous TlOH solution (10%, 7.7 mL, 3.45 mmol) by the general procedure for Shapiro–Suzuki cross coupling afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 90:10), a colorless oil (0.14 g, 98%) identified as (2*E*,4*E*)-3-methyl-5-(6-methylcyclohex-1-enyl)penta-2,4-dien-1-ol (**13**). ^1H NMR (400.16 MHz, $CDCl_3$): $\delta = 6.21$ (d, $J = 16.2$ Hz, 1H; H5), 6.13 (d, $J = 16.2$ Hz, 1H; H4), 5.73 (t, $J = 4.0$ Hz, 1H; H2′), 5.66 (t, $J = 6.8$ Hz, 1H; H2), 4.29 (d, $J = 6.8$ Hz, 2H; 2H1), 2.6–2.5 (m, 1H; H6′), 2.2–2.1 (m, 2H; 2H3′), 1.83 (s, 3H; C3–CH_3), 1.7–1.6 (m, 2H; 2H4′), 1.6–1.5 (m, 2H; 2H5′), 1.10 ppm (d, $J = 5.0$ Hz, 3H; C6′–CH_3); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 140.7$ (s), 136.6 (s), 131.0 (d), 129.3 (d), 129.1 (d), 128.9 (d), 59.2 (t), 29.7 (t), 27.6 (d), 26.0 (t), 19.9 (q), 17.5 (t), 12.3 ppm (q); FTIR (NaCl): $\nu = 3650$–3100 (br, OH), 2933 (s, C–H), 2869 (s, C–H), 1671, 1454 cm^{-1}; UV (MeOH): $\lambda_{max} = 283$, 270, 260 nm; MS (EI^+): *m/z* (%): 193 $[M+1]^+$ (44), 163 (21), 153 (20), 137(29), 135 (21), 123 (46), 121 (36), 109 (75), 107 (14), 97 (33), 95 (58), 93 (40), 91 (39), 86 (65), 84 (100), 83 (34), 81 (61), 80 (10), 79 (44), 77 (33); HRMS (EI^+): calcd for $C_{13}H_{20}O$: 192.1514; found: 192.1521.

(2*E*,4*E*)-5-(6-Methylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (23): Treatment of (2*E*,4*E*)-3-methyl-5-(6-methylcyclohex-1-enyl)penta-2,4-dien-1-ol (**13**, 0.1 g, 0.51 mmol) with TPAP (9.3 mg, 0.026 mmol) and NMO (0.09 g, 0.77 mmol) in CH_2Cl_2 (4.5 mL) by

the General Procedure for TPAP/NMO oxidation afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 93:7), a yellow solid (73 mg, 75%) identified as (2*E*,4*E*)-3-methyl-5-(6-methylcyclohex-1-enyl)penta-2,4-dienal (**23**). ^{1}H NMR (400.16 MHz, $CDCl_3$): $\delta = 10.10$ (d, $J = 8.2$ Hz, 1H; H1), 6.61 (d, $J = 16.1$ Hz, 1H; H5), 6.27 (d, $J = 16.1$ Hz, 1H; H4), 5.97 (s, 1H; H2′), 5.96 (t, $J = 8.1$ Hz, 1H; H2), 2.6–2.5 (m, 1H; H6′), 2.27 (d, $J = 0.8$ Hz, 3H; C3–CH_3), 2.2–2.1 (m, 2H; 2H3′), 1.7–1.6 (m, 2H; 2H4′), 1.6–1.5 (m, 2H; 2H5′), 1.10 ppm (d, $J = 7.0$ Hz, 3H; C6′–CH_3); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 191.5$ (d), 155.8 (s), 141.3 (s), 139.4 (d), 135.8 (d), 129.3 (d), 128.3 (d), 29.9 (t), 27.9 (d), 26.9 (t), 20.3 (q), 17.7 (t), 13.4 ppm (q); FTIR (NaCl): $\nu = 2932$ (s, C–H), 2869 (s, C–H), 1663 (s, C=O), 1598, 1123 cm^{-1}; UV (MeOH): $\lambda_{max} = 329$ nm; MS (EI$^+$): *m/z* (%): 190 $[M]^+$ (27), 175 (24), 161 (27), 105 (100), 95 (36), 93 (14), 91 (32), 79 (19); HRMS (EI$^+$): calcd for $C_{13}H_{18}O$: 190.1358; found: 190.1352.

(11*Z*)-1,5-Bisdemethylretinal (3): Treatment of (2*E*,4*E*)-3-methyl-5-(6-methylcyclohex-1-enyl)penta-2,4-dienal (**23**, 18.8 mg, 0.1 mmol) with KHMDS (0.64 mL, 0.5 M in toluene, 0.32 mmol), (4-hydroxy-2-methylbut-2-enyl)triphenylphosphonium bromide (**28**, 65 mg, 0.15 mmol) in THF (3.5 mL) by the general procedure for the Wittig oxidation sequence afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate/Et_3N 80:18:2), a yellow oil, which was used immediately. Treatment of this compound in CH_2Cl_2 (5 mL) with MnO_2 (89 mg, 1.02 mmol) and Na_2CO_3 (0.1 g, 1.02 mmol) for 2 h afforded, after purification by HPLC, a yellow oil (9 mg, 36%) identified as (11*Z*)-1,5-bisdemethylretinal (**3**). ^{1}H NMR (400.16 MHz, C_6D_6): $\delta = 9.93$ (d, $J = 7.7$ Hz, 1H; H15), 6.67 (d, $J = 11.9$ Hz, 1H; H10), 6.4–6.3 (m, 1H; H11), 6.38 (d, $J = 16.3$ Hz, 1H; H7), 6.30 (d, $J = 16.3$ Hz, 1H; H8), 6.15 (d, $J = 7.7$ Hz, 1H; H14), 5.7–5.6 (m, 1H; H5), 5.58 (d, $J = 11.7$ Hz, 1H; H12), 2.5–2.4 (m, 1H; H1), 2.0–1.9 (m, 2H; 2H4), 1.79 (s, 3H; CH_3), 1.74 (s, 3H; CH_3), 1.7–1.6 (m, 2H; 2H3), 1.5–1.4 (m, 2H; 2H2), 1.06 ppm (d, $J = 7.0$ Hz, 3H; C1–CH_3); UV (MeOH): $\lambda_{max} = 389$ ($\varepsilon = 21\,500$), 276 nm; MS (EI$^+$): *m/z* (%): 257 $[M+1]^+$ (20), 256 $[M]^+$ (100), 241 (17), 185 (22), 183 (23), 161 (23), 159 (22), 145 (52), 133 (25), 131 (25), 119 (34), 105 (62), 96 (26), 95 (41), 93 (27), 91 (55), 83 (22), 81 (39), 79 (31), 77 (38); HRMS (EI$^+$): calcd for $C_{18}H_{24}O$: 256.1827; found: 256.1821.

(2*E*,4*E*)-5-(6,6-Dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (14): Treatment of 2,2-dimethylcyclohexanone trisylhydrazone (**10**, 0.47 g, 1.16 mmol) in THF (6 mL) with *n*BuLi (3.12 mL, 1.09 M in hexane, 3.39 mmol), B(O*i*Pr)$_3$ (0.53 mL, 2.23 mmol), (2*E*,4*E*)-5-iodo-3-methylpenta-2,4-dien-1-ol (**11**, 0.17 g, 0.78 mmol), $Pd(PPh_3)_4$ (0.10 g, 0.09 mmol), and an aqueous TlOH solution (10%, 7.7 mL, 3.45 mmol) by the general procedure for Shapiro–Suzuki cross coupling afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 90:10), a colorless oil (0.123 g, 77%) identified as (2*E*,4*E*)-3-methyl-5-(6-methylcyclohex-1-enyl)penta-2,4-dien-1-ol (**14**). ^{1}H NMR (400.16 MHz, $CDCl_3$): $\delta = 6.37$ (d, $J = 15.7$ Hz, 1H; H5), 6.19 (d, $J = 15.7$ Hz, 1H; H4), 5.8–5.7 (m, 1H; H2′), 5.66 (t, $J = 6.9$ Hz, 1H; H2), 4.30 (d, $J = 6.9$ Hz, 2H; 2H1), 2.1–2.0 (m, 2H; 2H3′), 1.82 (d, $J = 0.4$ Hz, 3H; C3–CH_3), 1.6–1.5 (m, 2H; 2H4′), 1.5–1.4 (m, 2H; 2H5′), 1.07 ppm (s, 6H; C6′–$(CH_3)_2$); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 144.0$ (s), 137.0 (s), 132.0 (d), 128.8 (d), 128.4 (d), 123.1 (d), 59.5 (t), 39.4 (t), 33.5 (s), 28.6 (q, 2×), 26.4 (t), 19.1 (t), 12.6 ppm (q); FTIR (NaCl): $\nu = 3500$–3100 (br, OH), 2956 (s, C–H), 2927 (s, C–H), 2865 (s, C–H), 1453, 1360 cm^{-1}; UV (MeOH): $\lambda_{max} = 270$ nm; MS (EI$^+$): *m/z* (%): 207 $[M+1]^+$ (10), 177 (19), 151 (36), 135 (21), 123 (100), 109 (23), 95 (42), 81 (26), 77 (19); HRMS (EI$^+$): calcd for $C_{14}H_{22}O$: 206.1626; found: 206.1679.

(2*E*,4*E*)-5-(6,6-Dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (24): Treatment of (2*E*,4*E*)-5-(6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (**14**, 0.12 g, 0.60 mmol) with TPAP (11 mg, 0.03 mmol) and NMO (0.1 g, 0.9 mmol) in CH_2Cl_2 (8 mL) by the general procedure for TPAP/NMO oxidation afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 93:7), a yellow solid (76 mg, 62%) identified as (2*E*,4*E*)-5-(6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (**24**). ^{1}H NMR (400.16 MHz, $CDCl_3$): $\delta = 10.11$ (d, $J = 8.0$ Hz, 1H; H1), 6.72 (d, $J = 15.7$ Hz, 1H; H5), 6.48 (d, $J = 15.8$ Hz, 1H; H4), 6.02 (t, $J = 4.1$ Hz, 1H; H2′), 5.96 (d, $J = 8.0$ Hz, 1H; H2), 2.28 (d, $J = 1.0$ Hz, 3H; C3–CH_3), 2.2–2.1 (m, 2H; 2H3′), 1.7–1.6 (m, 2H; 2H4′), 1.6–1.4 (m, 2H; 2H5′), 1.10 ppm (s, 6H; C6′–$(CH_3)_2$); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 191.3$ (s), 155.1 (s), 143.5 (s), 136.4 (d), 130.7 (d), 128.9 (d), 127.7 (d), 39.3 (t), 33.5 (s), 28.5 (q, 2×), 26.6 (t), 18.8 (t), 13.1 (q), 13.5 ppm (q); FTIR (NaCl): $\nu = 2933$ (s, C–H), 2868 (s, C–H), 1718 (s, C=O), 1665 cm^{-1} (s); UV (MeOH): $\lambda_{max} = 326$ nm; MS (EI$^+$): *m/z* (%): 204 $[M]^+$ (19), 189 (27), 123 (45), 105 (100), 95 (67), 93 (25), 91 (32), 79 (21), 77 (23); HRMS (EI$^+$): calcd for $C_{14}H_{20}O$: 204.1514; found: 204.1514.

(11*Z*)-5-Demethylretinal (4): Treatment of (2*E*,4*E*)-5-(6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (**24**, 0.07 g, 0.33 mmol) with KHMDS (1.28 mL, 0.5 M in toluene, 0.64 mmol) and (4-hydroxy-2-methyl-but-2-enyl)triphenylphosphonium bromide (**28**, 0.13 g, 0.30 mmol) in THF (18 mL) by the general procedure for the Wittig oxidation sequence afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate/Et_3N 80:18:2), a yellow oil that was used immediately. Treatment of this compound in CH_2Cl_2 (5 mL) with MnO_2 (0.40 g, 5.45 mmol) and Na_2CO_3 (0.58 g, 5.45 mmol) for 2 h afforded, after purification by HPLC, a yellow oil (30 mg, 33%) identified as (11*Z*)-5-demethylretinal (**4**). ^{1}H NMR (400.16 MHz, C_6D_6): $\delta = 9.93$ (d, $J = 7.7$ Hz, 1H; H15), 6.62 (d, $J = 12.3$ Hz, 1H; H10), 6.53 (d, $J = 15.6$ Hz, 1H; H7), 6.4–6.3 (m, 2H; H8 + H11), 6.12 (d, $J = 7.7$ Hz, 1H; H14), 5.80 (t, $J = 4.5$ Hz, 1H; H5), 5.59 (d, $J = 11.7$ Hz, 1H; H12), 2.0–1.9 (m, 2H; 2H4), 1.80 (s, 3H; CH_3), 1.72 (s, 3H; CH_3), 1.6–1.5 (m, 2H; 2H3), 1.5–1.4 (m, 2H; 2H2), 1.08 (s, 3H; C1–CH_3), 1.07 ppm (s, 3H; C1–CH_3); UV (MeOH): $\lambda_{max} = 385$ ($\varepsilon = 20\,100$), 277 nm; MS (EI$^+$): *m/z* (%): 271 $[M+1]^+$ (15), 270 $[M]^+$ (71), 255 (31), 159 (55), 149 (62), 147 (34), 145 (32), 131 (33), 121 (21), 119 (46), 105 (100), 96 (40), 95 (79), 93 (37), 91 (71), 81 (35), 79 (38), 77 (44); HRMS (EI$^+$): calcd for $C_{19}H_{26}O$: 270.1984; found: 270.1980.

(2*E*,4*E*)-5-(2,6-Dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (17)—General Procedure for Suzuki reaction: A cooled (−78 °C) solution of 2-iodo-1,3-dimethylcyclohexene (**15**, 0.34 g, 1.45 mmol) in THF (8 mL) was treated with *t*BuLi (1.8 mL, 1.7 M in pentane, 3.01 mmol). After the system had been stirred for 30 min, B(OMe)$_3$ (0.33 mL, 2.90 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, and this solution was transferred to a separate flask containing a solution of 5-iodo-3-methylpenta-2,4-dien-1-ol (**11**, 0.25 g, 1.12 mmol) and $Pd(PPh_3)_4$ (0.13 g, 0.11 mmol) in THF (12 mL). After addition of an aqueous TlOH solution (10%, 9.8 mL, 4.24 mmol), the mixture was stirred for 4 h at 25 °C, diluted with Et_2O (20 mL), and washed with aqueous $NaHCO_3$ (3×). The organic layer was dried (Na_2SO_4), the solvent was removed, and the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 90:10) to afford a yellow oil (0.2 g, 86%) identified as (2*E*,4*E*)-5-(2,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (**17**). ^{1}H NMR (400.16 MHz, $CDCl_3$): $\delta = 6.59$ (d, $J = 16.2$ Hz, 1H; H5), 6.21 (d, $J = 16.2$ Hz, 1H; H4), 5.67 (t, $J = 7.0$ Hz, 1H; H2), 4.29 (d, $J = 7.0$ Hz, 2H; 2H1), 2.7–2.6 (m, 1H; H6′), 2.1–2.0 (m, 2H; 2H3′), 1.86 (s, 3H; C3–CH_3), 1.78 (s, 3H; C2′–CH_3), 1.7–1.6 (m, 2H; 2H4′), 1.6–1.4 (m, 2H; 2H5′), 1.06 ppm (d, $J = 6.9$ Hz, 3H; C6′–CH_3); ^{13}C NMR

(100.63 MHz, $CDCl_3$): $\delta = 136.2$ (s), 134.4 (s), 133.2 (s), 132.5 (d), 131.7 (d), 126.3 (d), 59.7 (t), 34.1 (t), 31.2 (t), 29.1 (d), 21.0 (q), 20.1 (q), 19.7 (t), 17.7 ppm (q); FTIR (NaCl): $\nu = 3500$–3100 (br, OH), 2929 (s, C–H), 2871 (s, C–H), 1653, 1454, 1375 cm^{-1}; UV (MeOH): $\lambda_{max} =$ 283, 242 nm; MS (EI^+): *m/z* (%): 207 $[M+1]^+$ (10), 109 (100), 95 (14), 93 (16), 91 (14), 86 (26), 84 (39), 81 (15), 79 (12), 77 (12); HRMS (EI^+): calcd for $C_{14}H_{22}O$: 206.1671; found: 206.1664.

(2*E*,4*E*)-5-(2,6-Dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (25): Treatment of (2*E*,4*E*)-5-(2,6-dimethylcyclohex-1-yl)-3-methylpenta-2,4-dien-1-ol (**17**, 0.09 g, 0.44 mmol) with TPAP (7.7 mg, 0.02 mmol) and NMO (77 mg, 0.66 mmol) in CH_2Cl_2 (4 mL) by the general procedure for TPAP/NMO oxidation afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 95:5), a yellow solid (63.8 mg, 71%) identified as (2*E*,4*E*)-5-(2,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (**25**). ^1H NMR (400.16 MHz, $CDCl_3$): $\delta = 10.11$ (d, $J = 8.2$ Hz, 1H; H1), 7.15 (d, $J = 16.0$ Hz, 1H; H5), 6.31 (d, $J = 16.0$ Hz, 1H; H4), 5.99 (d, $J =$ 8.2 Hz, 1H; H2), 2.8–2.7 (m, 1H; H6′), 2.33 (s, 3H; C3–CH_3), 2.3–2.1 (m, 2H; 2H3′), 1.86 (s, 3H; C2′–CH_3), 1.8–1.7 (m, 2H; 2H4′), 1.7–1.6 (m, 2H; 2H5′), 1.07 ppm (d, $J = 7.0$ Hz, 3H; C6′–CH_3); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 191.0$ (d), 155.9 (s), 139.7 (s), 133.7 (d), 133.4 (s), 128.6 (d), 128.3 (d), 33.6 (t), 29.7 (t), 27.8 (d), 20.0 (q), 19.9 (q), 17.4 (t), 13.0 ppm (q); FTIR (NaCl): $\nu = 2930$ (s, C–H), 2866 (s, C–H), 1661 (s, C=O), 1594 cm^{-1} (s, C=O); UV (MeOH): $\lambda_{max} = 343$ nm; MS (EI^+): *m/z* (%): 204 $[M]^+$ (56), 189 (25), 161 (20), 149 (24), 147 (44), 135 (88), 133 (32), 119 (92), 109 (75), 107 (44), 105 (62), 97 (26), 96 (45), 95 (100), 93 (53), 91 (69), 82 (42), 81 (36), 79 (43), 77 (42); HRMS (EI^+): calcd for $C_{14}H_{20}O$: 204.1514; found: 204.1515.

(11*Z*)-1-Demethylretinal (5): Treatment of (2*E*,4*E*)-5-(2,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (**25**, 0.04 mg, 0.19 mmol) with KHMDS (0.365 mL, 0.5 M in toluene, 0.73 mmol), (4-hydroxy-2-methylbut-2-enyl)triphenylphosphonium bromide (**28**, 74 mg, 0.17 mmol) in THF (9 mL) by the general procedure for the Wittig oxidation sequence afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate/Et_3N 80:18:2), a yellow oil that was used immediately. Treatment of this compound in CH_2Cl_2 (5 mL) with MnO_2 (0.27 g, 3.13 mmol) and Na_2CO_3 (0.33 g, 3.13 mmol) for 3 h afforded, after purification by HPLC, a yellow oil (20 mg, 39%) identified as (11*Z*)-1-demethylretinal (**5**). ^1H NMR (400.16 MHz, C_6D_6): $\delta = 9.94$ (d, $J = 7.7$ Hz, 1H; H15), 6.82 (d, $J =$ 16.0 Hz, 1H; H7), 6.73 (d, $J = 12.2$ Hz, 1H; H10), 6.48 (t, $J = 12.2$ Hz, 1H; H11), 6.43 (d, $J = 11.5$ Hz, 1H; H8), 6.17 (d, $J = 7.7$ Hz, 1H; H14), 5.62 (d, $J = 11.8$ Hz, 1H; H12), 2.6–2.5 (m, 1H; H1), 1.9–1.8 (m, 2H; 2H4), 1.82 (s, 6H; C13–CH_3 + C9–CH_3), 1.68 (s, 3H; C5–CH_3), 1.7–1.6 (m, 2H; 2H3), 1.5–1.4 (m, 2H; 2H2), 1.08 ppm (d, $J = 7.0$ Hz, 3H; C1–CH_3); UV (MeOH): $\lambda_{max} = 394$ ($\varepsilon = 18\,900$), 283 nm; MS (EI^+): *m/z* (%): 286 $[M]^+$ (42), 270 (12), 149 (100), 135 (22), 109 (45), 105 (24), 95 (24), 91 (31), 81 (22), 77 (19); HRMS (EI^+): calcd for $C_{19}H_{26}O$: 270.1984; found: 270.1988.

(2*E*,4*E*)-5-(2-Ethyl-6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (18): Treatment of 1-ethyl-2-iodo-3,3-dimethylcyclohexene **16** (0.22 g, 0.82 mmol) in THF (11.4 mL) with *t*BuLi (1 mL, 1.7 M in pentane, 1.70 mmol), $B(OMe)_3$ (0.18 mL, 1.65 mmol), 5-iodo-3-methylpenta-2,4-dien-1-ol (**11**, 0.14 g, 0.64 mmol), $Pd(PPh_3)_4$ (73 mg, 0.064 mmol), and an aqueous TlOH solution (10%, 5.33 mL, 2.41 mmol) by the general procedure for Suzuki cross-coupling afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 90:10), a colorless oil (0.11 g, 71%) identified as (2*E*,4*E*)-5-(2-ethyl-6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (**18**). ^1H NMR (400.16 MHz, $CDCl_3$): $\delta = 6.13$ (d, $J = 16.2$ Hz, 1H; H5), 6.02 (d, $J = 16.2$ Hz, 1H; H4), 5.61 (t, $J = 6.9$ Hz, 1H; H2), 4.30 (d, $J = 6.9$ Hz, 2H; 2H1), 2.05 (q, $J = 7.5$ Hz, 2H; C2′–CH_2CH_3), 2.0–1.9 (m, 2H; 2H3′), 1.84 (s, 3H; C3–CH_3), 1.7–1.6 (m, 2H; 2H4′), 1.5–1.4 (m, 2H; 2H5′), 0.99 (s, 6H; C6′–$(CH_3)_2$), 0.96 ppm (t, $J = 7.5$ Hz, 3H; C2′–CH_2CH_3); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta =$ 137.2 (s), 136.7 (s), 135.9 (d), 134.5 (s), 138.3 (d), 127.1 (d), 59.2 (t), 39.5 (t), 34.1 (s), 29.5 (t), 28.8 (q, 2×), 27.8 (t), 19.2 (t), 13.5 (t), 12.4 ppm (q); FTIR (NaCl): ν 3550–3050 (br, OH), 2960 (s, C–H), 2928 (s, C–H), 2865 cm^{-1} (m, C–H); UV (MeOH): $\lambda_{max} = 235$, 260 nm; MS (EI^+): *m/z* (%): 234 $[M]^+$ (13), 207 (23), 177 (41), 161 (26), 137 (28), 133 (24), 123 (49), 109 (24), 107 (28), 105 (37), 95 (34), 93 (23), 91 (25), 86 (28), 84 (38), 81 (44), 77 (19), 69 (100); HRMS (EI^+): calcd for $C_{16}H_{26}O$: 234.1988; found: 234.1984.

(2*E*,4*E*)-5-(2-Ethyl-6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (26): Treatment of (2*E*,4*E*)-5-(2-ethyl-6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dien-1-ol (**18**, 74 mg, 0.32 mmol) with TPAP (6 mg, 0.016 mmol) and NMO (55 mg, 0.47 mmol) in CH_2Cl_2 (2.8 mL) by the general procedure for TPAP/NMO oxidation afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 95:5), a yellow solid (58.6 mg, 80%) identified as (2*E*,4*E*)-5-(2-ethyl-6,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (**26**). ^1H NMR (400.16 MHz, C_6D_6): $\delta = 10.00$ (d, $J = 7.8$ Hz, 1H; H1), 6.49 (d, $J = 16.1$ Hz, 1H; H5), 6.07 (d, $J = 16.1$ Hz, 1H; H4), 5.93 (d, $J = 7.7$ Hz, 1H; H2), 1.99 (q, $J = 7.5$ Hz, 2H; C2′–CH_2CH_3), 2.0–1.9 (m, 2H; 2H3′), 1.73 (s, 3H; C3–CH_3), 1.6–1.5 (m, 2H; 2H4′), 1.5–1.4 (m, 2H; 2H5′), 0.99 (s, 6H; C6′–$(CH_3)_2$), 0.88 ppm (t, $J =$ 7.5 Hz, 3H; C2′–CH_2CH_3); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 191.2$ (d), 154.9 (s), 138.2 (s), 136.6 (s), 135.6 (d), 134.7 (d), 128.8 (d), 39.6 (t), 34.2 (s), 29.9 (t), 28.9 (q), 27.9 (t), 19.0 (t), 13.5 (t), 12.9 ppm (q); FTIR (NaCl): $\nu = 2960$ (s, C–H), 2930 (s, C–H), 2866 (m, C–H), 1665 cm^{-1} (s, C=O); UV (MeOH): $\lambda_{max} = 323$, 277 nm; MS (EI^+): *m/z* (%): 233 $[M+1]^+$ (12), 232 $[M]^+$ (50), 217 (30), 203 (21), 161 (31), 149 (53), 147 (49), 137 (22), 135 (32), 133 (58), 121 (46), 119 (42), 109 (21), 107 (35), 105 (100), 97 (29), 96 (16), 95 (47), 93 (36), 91 (58), 85 (25), 83 (48), 82 (21), 79 (33), 77 (32); HRMS (EI^+): calcd for $C_{16}H_{24}O$: 232.1827; found: 232.1830.

(11*Z*)-18-Methylretinal (7): Treatment of (2*E*,4*E*)-5-(2,6-dimethylcyclohex-1-enyl)-3-methylpenta-2,4-dienal (**26**, 74 mg, 0.30 mmol) with KHMDS (1.92 mL, 0.5 M in toluene, 0.96 mmol) and (4-hydroxy-2-methyl-but-2-enyl)triphenylphosphonium bromide (**28**, 0.19 g, 0.45 mmol) in THF (12 mL) by the general procedure for the Wittig oxidation sequence afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate/Et_3N 80:18:2), a yellow oil that was used immediately. Treatment of this compound in CH_2Cl_2 (5 mL) with MnO_2 (0.262 g, 3.01 mmol) and Na_2CO_3 (0.32 g, 3.01 mmol) for 2 h afforded, after purification by HPLC, a yellow oil (15 mg, 37%) identified as (11*Z*)-18-methylretinal (**7**). ^1H NMR (400.16 MHz, C_6D_6): $\delta = 9.90$ (d, $J = 7.7$ Hz, 1H; H15), 6.59 (d, $J = 12.2$ Hz, 1H; H10), 6.4–6.3 (m, 2H; H7 + H11), 6.28 (d, $J =$ 16.1 Hz, 1H; H8), 6.09 (d, $J = 7.6$ Hz, 1H; H14), 5.58 (d, $J = 11.7$ Hz, 1H; H12), 2.13 (q, $J = 7.5$ Hz, 2H; C5–CH_2CH_3), 2.0–1.9 (m, 2H; 2H4), 1.76 (d, $J = 1.0$ Hz, 3H; CH_3), 1.74 (s, 3H; CH_3), 1.6–1.5 (m, 2H; 2H3), 1.5–1.4 (m, 2H; 2H2), 1.07 (s, 6H; C1–$(CH_3)_2$), 0.96 ppm (t, $J = 7.5$ Hz, 3H; C5–CH_2CH_3); UV (MeOH): $\lambda_{max} = 377$ ($\varepsilon = 16\,000$), 253 nm; MS (EI^+): *m/z* (%): 299 $[M+1]^+$ (24), 298 $[M]^+$ (100), 187 (43), 173 (24), 161 (25), 159 (20), 148 (25), 147 (43), 145 (22), 133 (30), 131 (22), 121 (20), 119 (36), 109 (21), 107 (29), 105 (38), 95 (38), 93 (20), 91 (35); HRMS (EI^+): calcd for $C_{21}H_{30}O$: 298.2297; found: 298.2291.

(2*E*,4*E*)-3-Methyl-5-(2-methylcyclohex-1-enyl)penta-2,4-dien-1-ol (21): A solution of 1-methyl-2-(trifluoromethanesulfonyloxy)cyclohex-1-ene (**19**, 0.24 g, 0.97 mmol) in NMP (3.6 mL) was added to a solution of $Pd_2(dba)_3$ (0.027 g, 0.03 mmol) and $AsPh_3$ (0.07 g, 0.23 mmol) in NMP (0.7 mL). After the system had been stirred for

10 min, (2*E*,4*E*)-5-(tri-*n*-butylstannyl)-3-methylpenta-2,4-dien-1-ol (**20**, 0.49 g, 1.26 mmol) in NMP (0.7 mL) was added and the mixture was stirred for 4 h at 25 °C. An aqueous solution of KF (2 mL) was added and the mixture was stirred for 10 min and then extracted with *t*BuOMe (3×). The combined organic layers were washed with H_2O (3×) and dried (Na_2SO_4), the solvent was removed, and the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 90:10) to afford a yellow oil (0.17 g, 93%) identified as (2*E*,4*E*)-3-methyl-5-(2-methylcyclohex-1-enyl)penta-2,4-dien-1-ol (**21**). ^{1}H NMR (400.16 MHz, $CDCl_3$): $\delta = 6.75$ (d, $J = 15.9$ Hz, 1H; H5), 6.21 (d, $J = 15.9$ Hz, 1H; H4), 5.68 (t, $J = 7.0$ Hz, 1H; H2), 4.30 (d, $J = 7.0$ Hz, 2H; 2H1), 2.2–2.1 (m, 2H; 2H3′), 2.1–2.0 (m, 2H; 2H6′), 1.86 (s, 3H; CH_3), 1.82 (s, 3H; CH_3), 1.7–1.5 ppm (m, 4H; 2H4′ + 2H5′); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 137.4$ (s), 134.4 (s), 129.3 (d), 128.7 (d), 127.8 (s), 127.1 (d), 59.5 (t), 47.9 (t), 33.2 (t), 25.5 (t), 22.9 (t), 19.4 (q), 12.6 ppm (q); MS (EI$^+$): *m/z* (%): 193 $[M+1]^+$ (11), 192 $[M]^+$ (32), 177 (12), 165 (21), 119 (19), 109 (43), 107 (22), 105 (100), 97 (22), 95 (28), 91 (31), 79 (33), 77 (25); HRMS (EI$^+$): calcd for $C_{13}H_{20}O$: 192.1514; found: 192.1517; UV (MeOH): $\lambda_{max} = 325$, 284, 236 nm.

(2*E*,4*E*)-3-Methyl-5-(2-methylcyclohex-1-enyl)-penta-2,4-dienal (27): Treatment of (2*E*,4*E*)-3-methyl-5-(2-methylcyclohex-1-enyl)penta-2,4-dien-1-ol (**21**, 0.32 g, 1.66 mmol) with TPAP (29 mg, 0.08 mmol) and NMO (0.3 g, 2.49 mmol) in CH_2Cl_2 (24 mL) by the general procedure for TPAP/NMO oxidation afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate 93:7), a yellow solid (0.19 g, 60%) identified as (2*E*,4*E*)-3-methyl-5-(2-methylcyclohex-1-enyl)penta-2,4-dienal (**27**). ^{1}H NMR (400.16 MHz, $CDCl_3$): $\delta = 10.11$ (d, $J = 8.2$ Hz, 1H; H1), 7.26 (d, $J = 15.8$ Hz, 1H; H5), 6.27 (d, $J = 15.8$ Hz, 1H; H4), 5.98 (d, $J = 8.2$ Hz, 1H; H2), 2.32 (s, 3H; C3–CH_3), 2.3–2.2 (m, 4H; 2H3′ + 2H6′), 1.89 (s, 3H; C2′–CH_3), 1.7–1.6 ppm (m, 4H; 2H4′ + 2H5′); ^{13}C NMR (100.63 MHz, $CDCl_3$): $\delta = 191.5$ (s), 156.3 (s), 141.0 (s), 134.7 (d), 129.1 (d), 128.6 (d), 128.3 (s), 34.0 (t), 25.8 (t), 23.0 (t), 22.9 (t), 20.1 (q), 13.5 ppm (q); FTIR (NaCl): $\nu = 2932$ (s, C–H), 2865 (s, C–H), 1662 (s, C=O), 1597 cm^{-1}; UV (MeOH): $\lambda_{max} = 337$ nm; MS (EI$^+$): *m/z* (%): 191 $[M+1]^+$ (8), 152 (18), 139 (27), 135 (19), 127 (29), 123 (49), 111 (27), 109 (21), 99 (20), 98 (20), 97 (31), 96 (24), 95 (67), 93 (25), 91 (24), 83 (28), 82 (21), 81 (100), 79 (27), 77 (20); HRMS (EI$^+$): calcd for $C_{13}H_{18}O$: 190.1358; found: 190.1358.

all-*trans*-1,1-Bisdemethylretinal (29): Treatment of (2*E*,4*E*)-3-methyl-5-(2-methylcyclohex-1-enyl)penta-2,4-dienal (**27**, 16.3 mg, 0.86 mmol) with KHMDS (0.55 mL, 0.5 M in toluene, 0.27 mmol) and (4-hydroxy-2-methylbut-2-enyl)triphenylphosphonium bromide **28** (55 mg, 0.13 mmol) in THF (4.3 mL) by the general procedure for the Wittig oxidation sequence afforded, after purification by column chromatography (silica gel, hexane/ethyl acetate/Et_3N 80:18:2), a yellow oil, which was used immediately. Treatment of this compound in CH_2Cl_2 (2 mL) with MnO_2 (74.5 mg, 0.86 mmol) and Na_2CO_3 (91 mg, 0.86 mmol) for 2 h afforded, after purification by HPLC, a yellow oil (7.3 mg, 33%) identified as (2*E*,4*E*,6*E*,8*E*)-9-(2-methylcyclohex-1-enyl)-3,7-dimethylnona-2,4,6,8-tetraenal (**29**). ^{1}H NMR (400.16 MHz, $(CD_3)_2CO$): $\delta = 9.99$ (d, $J = 8.0$ Hz, 1H; H15), 7.17 (dd, $J = 15.1$, 11.6 Hz, 1H; H11), 6.81 (d, $J = 15.9$ Hz, 1H; H7), 6.34 (d, $J = 15.1$ Hz, 1H; H12), 6.22 (d, $J = 16.0$ Hz, 1H; H8), 6.21 (d, $J = 11.3$ Hz, 1H; H10), 5.78 (d, $J = 7.9$ Hz, 1H; H14), 2.2–2.1 (m, 2H; CH_2), 2.1–2.0 (m, 2H; CH_2), 1.93 (s, 3H; CH_3), 1.92 (t, $J = 2.2$ Hz, 3H; CH_3), 1.71 (s, 3H; CH_3), 1.6–1.5 (m, 4H; 2CH_2) ppm; MS (EI$^+$): *m/z* (%): 257 $[M+1]^+$ (21), 256 $[M]^+$ (100), 241 (28), 230 (24), 227 (23), 215 (36), 187 (48), 171 (26), 161 (21), 159 (43), 157 (47), 148 (23), 147 (25), 145 (91), 143 (37), 141 (22), 133 (40), 131 (56), 129 (37), 128 (32), 119 (48), 117 (35), 115 (35), 105 (96), 96 (26), 95 (42), 93 (37), 91 (80), 85 (23), 83 (32), 81 (43), 79 (43), 77 (46), 69 (43), 68 (51), 67 (38); HRMS (EI$^+$): calcd for $C_{18}H_{24}O$: 256.1827; found: 256.1837.

Preparation of artificial rhodopsins: Opsin was prepared from rod outer segments (ROSs) by a published method.[22] Regeneration of artificial visual pigments began with the addition of an aliquot of a concentrated chromophore stock solution in DMF to a ROS suspension of opsin. The amount added was sufficient to give a two- to threefold excess of chromophore over opsin and small enough that DMF remained less than 5% of the resulting solution. Regenerating mixtures were incubated in the dark at room temperature for 20 h. An aliquot was withdrawn to measure the extent of regeneration spectrophotometrically after dilution in 1% DM buffer (final concentration DM 1%).

Calculation methods: The structure of bovine rhodopsin (opsin bound to 11-*cis*-retinal)—PDB code 1U19 (obtained experimentally by X-ray diffraction with 2.2 Å resolution)[8c]—was used as the starting point for our calculations. Differences of binding interaction energy between rhodopsin and opsin bound to retinal analogues **2**–**7** were calculated with the aid of Autodock3.[25] Polar hydrogen atoms and solvation parameters were added to the protein structure. The retinal analogues **2**–**7** were modeled by addition and/or deletion of the methyl groups to the retinal original structure. The preferred binding site location was verified to be the same as for 11-*cis*-retinal for each ligand. Calculations were refined further by using the Local Search method implemented in Autodock3, in 20 series of 100 runs for each ligand, starting from the 11-*cis*-retinal X-ray structure. Clustering tolerance was set to 0.5 Å, and only clusters with 50% or greater percentage of optimizations for each run were considered. In order to take account of the partial flexibility of the chain, we set the center for rigid rotation of the entire ligand at C6 and only the C6–C7 and C8–C9 bonds of the side chain were allowed to rotate freely.

Rotational barrier calculation: The conformational search of the C5–C6–C7–C8 dihedral for the retinal analogues was evaluated in the gas phase by use of the Gaussian03 package (with DFT methods at the B3LYP/6-31G* level).[24] Torsion was incremented in steps of 6° from −180° to 180°. The minima and maxima obtained were optimized and then refined in methanol solution ($\varepsilon = 32.63$) by use of the Isodensity Polarized Continuum Model (IPCM) and the Self Consistent IPCM.

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