51. A solution of CrO_3 (65 mg, 0.65 mmol) and pyridine (10 μL, 0.13 mmol) in 5 mL of CH_2Cl_2 was stirred at room temperature for 15 min (persistent red color) after which a solution of ^{14}C- and ^{13}C-labeled alcohol (27 mg, 0.64 mmol) in 5 mL of CH_2Cl_2 was added dropwise. After 15 min, Et_2O (25 mL) was added and the solution was decanted. The residue was washed with Et_2O (2 × 20 mL). Drying and concentration under vacuo gave 40 mg of residue which after column purification yielded 18 mg (67%) of methyl ketone **51** (total radioactivity = 70 μCi).

Acknowledgment. We thank Professors W. C. Still and D. Collum for kindly providing experimental details and spectra of synthetic intermediates and the Eli Lilly Co. for a generous gift of monensin A. This investigation was supported in part by Grant GM33149 of the National Institutes of Health.

Role of Retinal Isomerizations and Rotations in the Photocycle of Bacteriorhodopsin

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Abstract: Artificial bacteriorhodopsin (bR) pigments based on synthetic retinal analogues with selectively blocked single and double bonds were prepared. It was shown that rotations around single bonds C_{12}–C_{13} and C_{10}–C_{11} and isomerizations of C_{11}=C_{12} and C_9=C_{10} are not required either for initiating the photocycle of *all-trans*-bR or for forming its M_{412} intermediate. The results are discussed in light of mechanisms for the primary event (based on the C_{13}=C_{14} isomerization) involving a concerted double-bond and single-bond rotation around adjacent C,C bonds. Similarly, the photoreaction of the 13-cis isomer of bacteriorhodopsin does not require isomerization about the C_{11}=C_{12} double bond or rotation around C_{12}–C_{13}. It is also shown that 13-cis ⇔ all-trans (light–dark adaptation) reaction of bacteriorhodopsin does not involve additional rotations or isomerizations involving the C_9–C_{13} section of the molecule.

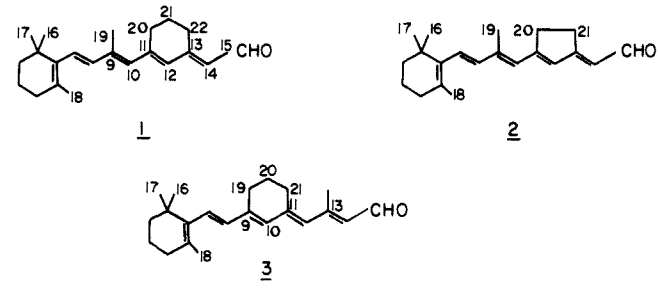
The light-adapted modification of bacteriorhodopsin (bR—the protein pigment in the purple membrane of *Halobacterium halobium*) contains an *all-trans*-retinyl chromophore bound to the protein via a protonated Schiff base linkage with a lysine residue.[1] The photosynthetic activity of bR_t is associated with a light-driven proton pump induced by a photoprocess centered in the polyene chromophore.[1] In analogy to visual pigments [characterized by a similar (11-cis) retinal–protein complex], light absorption is followed by a sequence of structural transformations involving both the polyene and the protein.[2] A detailed description of all these events is required for formulating a molecular model for the function of bacteriorhodopsin.

Of major importance is the primary event, associated with the red-shifted K_{610} intermediate, analogous to bathorhodopsin in the visual photocycle.[2] By use of artificial bacteriorhodopsins based on synthetic retinal analogues, it was recently concluded that only the terminal C_{12}–N part of the polyene is essential for initiating the bR photocycle, directly implying that the freedom to isomerize about the C_{13}=C_{14} double bond is the major prerequisite for generating K_{610}.[3,4]

Several studies have previously led to the suggestion that both visual and bateriorhodopsin photocycles are initiated by isomerization around at least two bonds. Such studies include arguments based on the observation of two independent photocycles for bR_t and for its 13-cis isomer, $bR_{13\text{-cis}}$,[5] Warshel's bicycle-pedal model for isomerization in a constrained medium,[6] and the approaches of Schulten[7] and Liu,[8] requiring simultaneous twisting of two adjacent bonds. The suggested combinations are C_{11}=C_{12} and C_{10}–C_{11} in the case of visual pigments[8a] and C_{13}=C_{14} and C_{14}–C_{15} for bR_t.[7,8b]

In addition to establishing the critical role of the C_{13}=C_{14} isomerization in generating the photocycle, our previous work with bR_t[4,9] has excluded the need of isomerizations and rotations about all other polyene bonds, except for C_{12}–C_{13}, C_{14}–C_{15}, and C_{15}=N, for formation of the primary (K) intermediate. In the present work, based on synthetic retinals **1** and **2** (Chart I), we directly analyze the role played by the C_{12}–C_{13} single bond in initiating the photocycle. These chromophores, which maintain the basic

Chart I



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[‡] Hebrew University.
[§] Incumbent of the Morris and Ida Wolf Career Development Chair.

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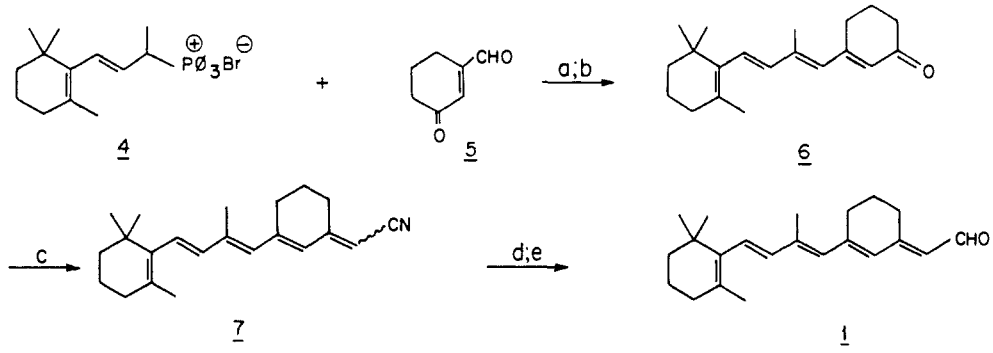
Scheme I[a]



[a] Key: (a) *n*-BuLi/THF, 1 h, 0 °C; (b) separation of trans and 9-cis isomers; (c) $(EtO)_2POCH_2CN$/NaH, THF, 25 °C, 3 h; (d) DIBAH/hexane, −78 °C, 1 h/silica, H_2O; (e) separation of all-trans and 13-cis isomers.

Table I. ^1H NMR Data of Retinals **1–3**: Chemical Shifts and Coupling Constants (Hz) Measured in $CDCl_3$

compd	1,1-$(CH_3)_2$	H-C_2	H-C_3	H-C_4	5-CH_3	9-CH_3	13-CH_3	7-H	8-H	10-H	12-H	14-H	15-H	addtnl chem shifts
all-trans-**1**	1.03	1.46	1.59	2.02	1.71	2.03		6.28	6.10	5.96	6.25	5.81	10.06	1.82 (21-H)
								$J_{7,8}$ =	16.1			$J_{14,15}$ =	8.2	2.49 (20-H)
														2.87 (22-H)
13-*cis*-**1**	1.03	1.46	1.60	2.02	1.72	2.09		6.28	6.12	5.98	7.18	5.71	10.17	1.82 (21-H)
								$J_{7,8}$ =	16.1			$J_{14,15}$ =	7.8	2.47 (20-H, 22-H)
all-trans-**2**	1.04	1.47	1.62	2.03	1.72	2.12		6.37	6.16	6.36	6.09	5.96	9.82	2.97 (20-H)
								$J_{7,8}$ =	16.0			$J_{14,15}$ =	8.0	3.11 (21-H)
13-*cis*-**2**	1.04	1.46	1.61	2.03	1.72	2.13		6.36	6.16	6.25	7.07	5.82	9.94	2.86 (20-H)
								$J_{7,8}$ =	16.1			$J_{14,15}$ =	8.2	2.49 (21-H)
all-trans-**3**	1.03	1.47	1.62	2.02	1.72		2.31	6.31	6.12	5.84	6.09	5.98	10.03	1.80 (20-H)
								$J_{7,8}$ =	16.1			$J_{14,15}$ =	8.1	2.37 (19-H)
														2.63 (21-H)
11-*cis*-**3**[a]	1.02	1.47	1.59	2.02	1.71		2.30	6.32	6.13	6.62	5.76	5.92	10.03	2.40 (19-H, 21-H)
								$J_{7,8}$ =	16.0			$J_{14,15}$ =	8.1	1.84 (20-H)
13-*cis*-**3**	1.02	1.47	1.62	2.00	1.72		2.05	6.27	6.03	6.13	6.24	5.89	9.63	1.80 (20-H)
								$J_{7,8}$ =	16.4			$J_{14,15}$ =	8.0	2.37 (19-H, 21-H)
11,13-di-*cis*-**3**								6.24	6.05	6.13	5.81	5.95	9.63	1.80 (20-H)
	1.00	1.47	1.61	2.00	1.68		2.03	$J_{7,8}$ =	16.4			$J_{14,15}$ =	7.8	2.37 (19-H, 21-H)

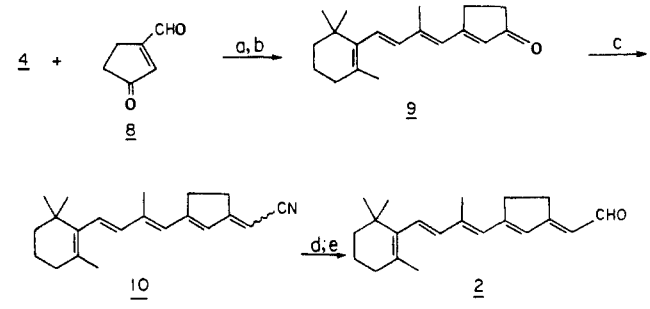
[a] Measured in CD_2Cl_2.

polyene structure of retinal, are free to isomerize around C_{13}=C_{14} but cannot rotate around C_{12}–C_{13}. Another aspect involves the requirements for forming the M_{412} photointermediate.[2] This species, which appears to be directly involved in the proton-pump mechanism, was not observed in the photocycle of an artificial bR_t based on a furanoid retinal analogue with a blocked C_9–C_{12} region.[4] This observation raises questions related to the role of the C_9=C_{10} and C_{11}=C_{12} double bonds and of the C_{10}–C_{11} single bond, at the stage of M_{412}. For the purpose of clarifying this point, we prepared retinal **3** in which all bond rotations between C_9 and C_{11} are blocked by a six-membered ring.

Results and Discussion

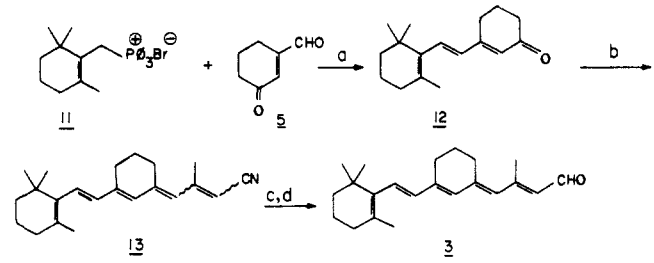
Chromophores **1–3** were synthesized by applying similar basic steps, with 3-formylcyclohexenone (**5**)[10] or 3-formylcyclopentenone (**8**) as the key intermediates. The aldehyde group was condensed with the corresponding phosphonium salt, while ketone protection was avoidable due to the higher reactivity of the aldehyde, as compared to the ketone. Thus, compound **1** was prepared by applying the Wittig reaction between the phosphonium salt of β-ionone **4** and **5**. Extension of the polyene chain afforded (after separation of isomers) chromophore **1** (Scheme I). The cyclopentane derivative **2** was prepared in a similar way (Scheme II). Chromophore **3** was prepared[11] (Scheme III) by condensation of the phosphonium salt of β-cyclocitral **11** with 3-formylcyclohexenone (**5**), followed by Emmons–Horner reaction with diethyl 3-methyl-4-phosphonocrotononitrile, reduction with diisobutylaluminum hydride, and separation of four isomers (all-trans, 13-cis, 11-cis, 11,13-di-cis). The various isomers were characterized by their ^1H NMR chemical shifts (Table I) and NOE experiments, as described in the Experimental Section. Chromophores **1–3**

Scheme II[a]



[a] Key: (a) *n*-BuLi/THF, 1 h, 0 °C; (b) separation of trans and 9-cis isomers; (c) $(EtO)_2POCH_2CN$/NaH, THF, 25 °C, 3 h; (d) DIBAH/hexane, −78 °C, 1 h/silica, H_2O; (e) separation of all-trans and 13-cis isomers.

Scheme III[a]



[a] Key: (a) *n*-BuLi/THF, 20 min, 0 °C; (b) $(EtO)_2POCH_2C(CH_3)$=CHCN/NaH, THF, 25 °C, 20 h; (c) DIBAH/hexane, −78 °C, 90 min/silica, H_2O; (d) separation of isomers.

(10) Quesada, M. L.; Schlessinger, R. H. *Synth. Commun.* **1976**, *6*, 555–557.

(11) The compound has been synthesized independently by R. S. H. Liu by a different method. Private communication of R. S. H. Liu to M. Sheves.

Table II. Absorption Maxima[a] of Retinal Analogues and Their Artificial Pigments

chromophore	aldehyde[b]	SBH^+[c]	pigment	OS,[d] cm^{-1}
retinal	380	438	568	5200
1	378	436	556	5000
2[e]	384	438	525	3780
3	384	448	570	4800

[a] In nanometers. [b] In methanol. [c] *n*-Butylamine protonated Schiff base with Cl^- as counteranion in methanol. [d] Opsin shift between SBH^+ and pigment as defined in the text. [e] 13-*cis* isomer.

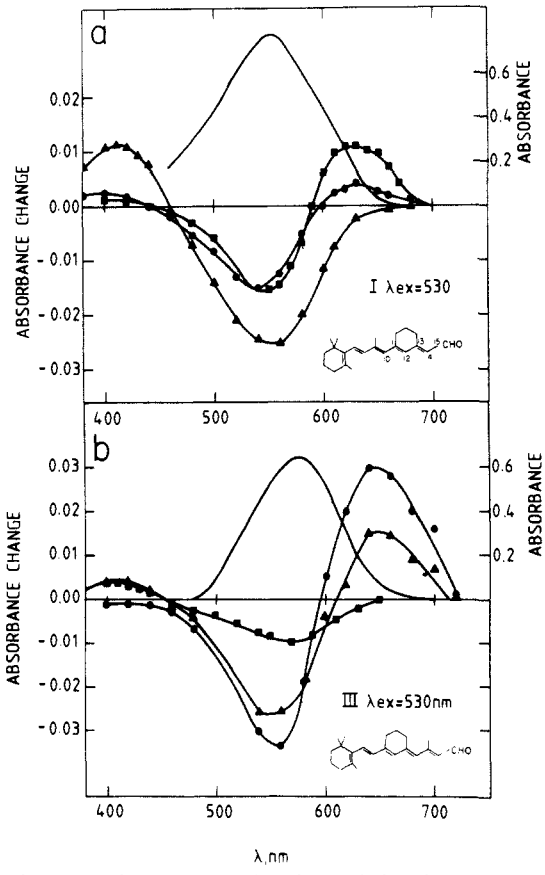


Figure 1. Absorption spectra (—) and laser-induced transient spectra. (a) Pigment I: (■) 1 μs, (▲) 250 μs, (●) 2 ms. (b) Pigment III: (●) 1.5 μs, (▲) 10 μs, (■) 1 ms.

exhibit unusual low extinction coefficients, and the 13-cis isomer of **3** absorbs at a low wavelength [λ_{max} (hexane) 320 nm (ϵ 16000)]. Similar unusual absorption in the UV region has already been observed by Chandraratna et al. for 12-s-cis locked retinals.[12]

Retinal analogues **1** and **3** (all-trans isomers) were incubated with bacterioopsin at 25 °C (Hepes buffer, pH 6.5) for 30 min, resulting in the formation of pigments (denoted as I and III) absorbing close to natural bR (Figure 1; Table II). This implies that no serious steric restrictions due to the added rings are present in I and III, so as to affect the basic opsin shift[13] of the pigments. (The opsin shift is defined as the energy shift between the absorption of a protonated Schiff base of the chromophore in methanol solution and that of the corresponding pigments. It reflects electrostatic and steric interactions of the retinyl moiety with its protein environment.) In variance with bR_t, we did not observe light–dark adaptation changes in the corresponding absorption spectra of the artifical pigments. However, chromophore extraction from pigments I and III with methylene chloride, followed by HPLC analysis, revealed a mixture of ca. 1:1 all-trans to 13-cis in the dark-adapted form and 9:1 in the light-adapted

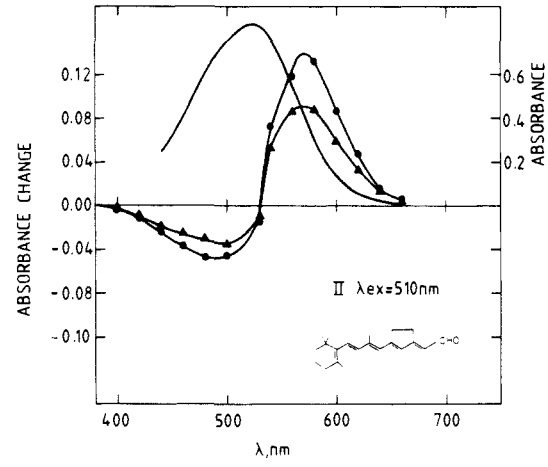


Figure 2. Absorption spectrum (—) and laser-induced transient spectra of pigment II: (●) 100 μs, (▲) 2.5 ms.

modification. These observations, revealing an isomer composition similar to that of natural bacteriorhodopsin,[14] were found to be independent of whether the all-trans or the 13-cis isomer was used in the initial incubation with bacterioopsin.

Pulsed-laser photolysis experiments with light-adapted pigments I and III, performed as previously described,[15] reveal general patterns similar to those of bR_t. As shown in Figure 1a,b, an initial rise in absorption in the red is indicative of the short-lived K intermediate whereas a long-lived M intermediate appears in the blue region, around 410 nm. With previously described procedures,[16] normal proton translocation was observed upon irradiation of pigments I and III incorporated in vesicles.

A generally different behavior was observed in the case of pigment II derived from chromophore **2**. Incubation of the all-trans isomer of **2** with bacterioopsin results in a prepigment species absorbing at 440 nm, which, upon further incubation in the dark (for ~48 h), converts to a pigment (II) absorbing at 525 nm (Figure 2). Alternatively, the prepigment may be converted to the 525-nm pigment by irradiation with sunlight for ~2 min. Incubation of the 13-cis isomer of **2** yields the 525-nm pigment (II) as the major component in about 20 min. Only a minor contribution of the 440-nm band (which converts to the pigment upon irradiation) is now present. In the case of pigment II, denaturation of the protein followed by extraction of the chromophore with methylene chloride and HPLC analysis afforded only the 13-cis isomer of **2**. As shown in Figure 2, exposure of pigment II to laser flash photolysis reveals a photocycle that lacks the blue-shifted M intermediate characteristic of bR_t. It, however, exhibits a long-lived red-shifted species, analogous to the L_{610} transient in the photocycle of $bR_{13\text{-}cis}$.[17] The lack of proton translocation, following irradiation of pigment II incorporated into vesicles, is reminiscent of the behavior of $bR_{13\text{-}cis}$[5,18] and is in keeping with the 13-cis nature of pigment II. We note that no photocycling was observed (within the 0.5-μs resolution of our apparatus) upon exposing the 440-nm prepigment derived from **2** to pulsed-laser photolysis.

The normal behavior described for pigment I, in both photocycle patterns and proton-pumping activity, proves that isomerization of the $C_{11}=C_{12}$ double bond is not required, either for initiating the photocycle (confirming our previous conclusion in this respect[4]) or for forming the M_{412} intermediate. Moreover, the observations

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directly exclude the need of significant rotation around the single C_{12}–C_{13} bond for the formation of K_{610} or other species in the photocycle of bR_t. In a similar way, the normal behavior of pigment III excludes isomerization of C_9=C_{10} and bond rotation about C_{10}–C_{11} in all phases of the photocycle. This extends our previous conclusion[4] that such conformational changes are not essentially involved in the generation of K_{610}.

The fact that pigment II predominantly consists of a 13-cis chromophore that exhibits a photocycle practically identical with that of $bR_{13\text{-cis}}$ indicates that the latter photoreaction does not require isomerization about the C_{11}=C_{12} double bond, or rotation around C_{12}–C_{13}. We note that the restrictions imposed on both motions by the cyclopentane ring are even more severe than those associated with the six-membered ring in I. An additional conclusion relates to the thermal light–dark adaptation reaction of bR, associated with the all-trans ⇔ 13-cis interconversion.[2] As reported above, attainment of this equilibrium is not affected by blocking of bond rotations in the C_9–C_{13} region. As shown recently by a combined NMR[19] and resonance Raman study,[20] it appears to be associated with a C_{15}=N syn–anti interconversion. The steric effects that influence the 13-cis/all-trans equilibrium, favoring the 13-cis isomer in II,[21] are still unclear.

Conclusions

Our present and previous studies of artificial bacteriorhodopsins with blocked double and single bonds limit the possibility of substantial bond rotations in the photocycle of bR_t to the C_{13}–N region of the molecule. Since NMR[19] and resonance Raman experiments[20] exclude isomerization about C_{15}=N in the primary event, only the C_{14}–C_{15} single-bond rotation is left as the possible additional polyene conformational transformation accompanying the C_{13}=C_{14} trans → cis photoprocess. The latter mechanism originally suggested by Schulten[7] has been recently elaborated by Liu et al.,[8b] who introduced the terminology H.T.-*n* for describing a concerted double- and single-bond rotation around two adjacent C,C bonds, centered at C_n. Thus, the results of the present work, although excluding the possibility of an H.T.-13 mechanism, are not inconsistent with the H.T.-14 model.[8b] On the basis of the present synthetic retinals, the road is also open for directly investigating the photoreactions of visual pigments in which the primary event involves a cis → trans isomerization about C_{11}=C_{12} (or C_9=C_{10} in the case of isorhodopsin). Specific models such as Warshel's "bicycle-pedal"[6] or Liu's concerted-twist motion[8a] may be directly tested by applying chromophores such as **1**, **2**, and **3** (9- or 11-cis isomers). Work along this line is under progress in our laboratory.

Experimental Section

Spectroscopic measurements were carried out with the following instruments: UV, Kontron 810; NMR, Varian FT 80A and Bruker 270 MHz with chemical shifts reported in ppm on the δ scale relative to a Me_4Si internal standard in $CDCl_3$; MS, Varian Mat 731 and Finnigan 4500. Chromatographies were performed by using the flash column technique with Merck silica gel 60 (230–400 mesh ASTM) with the solvents mentioned. NaH used was 80% in white oil. The carbons of the synthetic intermediates were numbered as in the retinal skeleton.

2-Cyclohexenone-3-carbaldehyde (5) was prepared from 1,3-cyclohexanedione in 66% overall yield according to Quesada and Schlessinger.[10] UV (MeOH): λ_{max} 231 nm (ϵ 14 000). ^{1}H NMR: δ 2.10 (m, 1, 5-H), 2.48 (m, 4, 4-H, 6-H), 6.55 (t, J = 1.5 Hz, 1, 2-H), 9.78 (s, 1, CHO). Mass Spectrum ($C_7H_8O_2$): *m/e* 124 (M^+), 96 (M − CO), 68 (M − CH_2CH_2CO).

2-Cyclopentenone-3-carbaldehyde (8) was prepared from 1,3-cyclopentanedione in 36% overall yield according to Quesada and Schlessinger.[10] ^{1}H NMR: δ 2.49–2.62 (m, 2, 4-H), 2.72–2.87 (m, 2, 5-H), 6.80 (t, J = 1.9 Hz, 1, 2-H), 10.23 (s, 1, CHO).

Phosphonium salts 4 and 11 were prepared from β-ionone and β-cyclocitral,[22] respectively, according to Olive et al.[23]

Tetraenone 6. Phosphonium salt **4** (680 mg, 1.31 mmol) was dissolved in 5 mL of dry THF. The mixture was stirred at 0 °C under argon atmosphere, and 0.75 mL of a 1.5 M hexane solution of *n*-butylithium was added dropwise. The solution turned red. After 5 min, aldehyde **5** (80 mg, 0.65 mmol) in 1 mL of dry THF was added. The color faded into yellow. After 1 h, water was added, and the mixture was extracted twice with ether. Usual workup and chromatography with ether–hexane (10:90) gave tetraenone **6** (100 mg, 55% yield) in two fractions in a 57:43 trans:cis ratio. UV ($CHCl_3$): λ_{max} 342 nm (ϵ 20 000) (all-trans), 341 (ϵ 16 000) (9-cis). ^{1}H NMR: (trans) δ 1.03 (s, 6, 1-CH_3), 1.46 (t, J = 6 Hz, 2, 2-H), 1.60 (m, 2, 3-H), 1.71 (s, 3, 5-CH_3), 2.03 (m, 4, 4-H, ring protons), 2.06 (s, 3, 9-CH_3), 2.41 (t, J = 7.2 Hz, 2, 11-CH_2), 2.48 (t, J = 7.2 Hz, 2, 13-CH_2), 5.93 (s, 1, 12-H), 6.02 (s, 1, 10-H), 6.12 (d, J = 16.0 Hz, 1, 8-H), 6.34 (d, J = 16.0 Hz, 1, 7-H); (9-cis) δ 1.02 (s, 6, 1-CH_3), 1.45 (t, J = 6.3 Hz, 2, 2-H), 1.59 (m, 2, 3-H), 1.71 (s, 3, 5-CH_3), 2.03 (m, 4, 4-H, ring protons), 2.03 (s, 3, 9-CH_3), 2.40 (t, J = 6.3 Hz, 2 11-CH_2), 2.43 (t, J = 6.3 Hz, 2, 13-CH_3), 5.87 (s, 1, 10-H), 5.96 (s, 1, 12-H), 6.38 (d, J = 16.1 Hz, 1, 7-H), 6.52 (d, J = 16.1 Hz, 1, 8-H). NOE: Irradiation of 9-CH_3 increased integration of 10-H by 20% in the cis isomer. No such effect was observed in the trans isomer. High-resolution mass spectrum ($C_{20}H_{28}O$): found, 284.2133; calcd, 284.2126.

Pentaenenitrile 7. *all-trans*-Tetraenone **6** (30 mg, 0.11 mmol) was reacted with the sodium salt of diethyl (cyanomethyl)phosphonate (21 mg, 0.12 mmol) in 3 mL of dry THF at 25 °C under argon atmosphere. After 3 h, water was added, and the mixture extracted twice with ether. Usual workup and chromatography with ether–hexane (15:85) gave pentaenenitrile **7** (25 mg, 77% yield) as a mixture of the two isomers. The nitrile was reduced directly, without separation of isomers. ^{1}H NMR: δ 1.02 (s, 6, 1-CH_3), 1.71 (s, 3, 5-CH_3), 2.00 (s, 3, 9-CH_3), 4.90 (s, 14-H cis), 4.95 (s, 14-H trans), 5.80–6.70 (m, 4, 7-H, 8-H, 10-H, 12-H). High-resolution mass spectrum ($C_{22}H_{29}N$): found, 307.2290; calcd, 307.2285.

6-Membered Ring Retinal 1. Pentaenenitrile **7** (13 mg, 0.042 mmol) was dissolved in 5 mL of dry hexane under argon atmosphere. The solution was cooled to −78 °C, and 0.07 mL of a 1 M hexane solution of diisobutylaluminum hydride was added. After 1 h, ether (10 mL) and silica (1.5 g) were added, and the mixture was kept stirring at 4 °C for 15 h. The reaction mixture was filtered through Celite, and the solvent was evaporated. Chromatography with ether–hexane (15:85) gave retinal **1** (13 mg, 99% yield) in two fractions in 8:5 trans:cis ratio. UV (hexane): λ_{max} 356 nm (ϵ 11 000) (all-trans), 350 nm (ϵ 19 000) (13-cis). High-resolution mass spectrum ($C_{22}H_{30}O$): found, 310.2285; calcd, 310.2296.

Tetraenone 9. Phosphonium salt **4** (316 mg, 0.6 mmol) was dissolved in 2 mL of dry THF. The mixture was stirred at 0 °C under argon atmosphere, and 0.4 mL of a 1.5 M hexane solution of *n*-butyllithium was added dropwise. The solution turned red. After 5 min, aldehyde **8** (27 mg, 0.25 mmol) in 2 mL of dry THF was added. The color faded into yellow. After 1 h, water was added, and the mixture was extracted twice with methylene chloride. Usual workup and chromatography with ethyl acetate–hexane (10:90) gave tetraenone **9** (41 mg, 62% yield) in two fractions in 1:1 trans:cis ratio. UV (CH_2Cl_2): λ_{max} 339 nm (ϵ 17 000) (all-trans), 338 (ϵ 12 000) (9-cis). ^{1}H NMR: (all-trans) δ 1.04 (s, 6, 1-CH_3), 1.48 (t, J = 5.5 Hz, 2, 2-H), 1.63 (m, 2, 3-H), 1.72 (s, 3, 5-CH_3), 2.04 (t, J = 5.8 Hz, 2, 4-H), 2.13 (s, 3, 9-CH_3), 2.45 (t, J = 4.9 Hz, 2, 11-CH_2), 2.86 (t, J = 4.7 Hz, 2, 13-CH_2), 6.14 (s, 1, 12-H), 6.18 (s, 1, 10-H), 6.18 (d, J = 16.5 Hz, 1, 8-H), 6.45 (d, J = 16.0 Hz, 1, 7-H); (9-cis) δ 1.04 (s, 6, 1-CH_3), 1.48 (t, J = 5.9 Hz, 2, 2-H), 1.63 (m, 2, 3-H), 1.73 (s, 3, 5-CH_3), 2.04 (t, J = 6.0 Hz, 2, 4-H), 2.10 (s, 3, 9-CH_3), 2.45 (t, J = 5.0 Hz, 2, 11-CH_2), 2.82 (t, J = 5.0 Hz, 2, 13-CH_3), 6.07 (s, 1, 12-H), 6.11 (s, 1, 10-H), 6.48 (d, J = 16.4 Hz, 1, 7-H), 6.68 (d, J = 16.0 Hz, 1, 8-H). NOE: Irradiation of 9-CH_3 increased integration of 7-H by 18% in all-trans and 20% in 9-cis. It also increased integration of 10-H by 24% in 9-cis, while no effect was observed in the all-trans isomer. High-resolution mass spectrum ($C_{19}H_{26}O$): found, 270.1596; calcd, 270.1984.

Pentaenenitrile 10. *all-trans*-Tetraenone **9** (13 mg, 0.048 mmol) was reacted with the sodium salt of diethyl (cyanomethyl)phosphonate (36 mg, 0.2 mmol) in 3 mL of dry THF at 25 °C under argon atmosphere. After 3 h, water was added, and the mixture was extracted twice with methylene chloride. Usual workup and chromatography with ethyl acetate–hexane (10:90) gave pentaenenitrile **10** (7 mg, 50% yield) as a mixture of two isomers. The nitrile was reduced directly, without separation of isomers. ^{1}H NMR: δ 1.03 (s, 6, 1-CH_3), 1.45–1.65 (m, 4, 2-H,

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3-H), 1.71 (s, 3, 5-CH_3), 2.04 (t, J = 5.0 Hz, 2, 4-CH_2), 2.09 (s, 3, 9-CH_2), 2.90 (m, 4, 11-CH_2, 13-CH_2), 4.87 (s, 14-H cis), 5.05 (s, 14-H trans), 5.95–6.70 (m, 4, 7-H, 8-H, 10-H, 12-H). High-resolution mass spectrum ($C_{20}H_{27}N$): found, 293.2101; calcd, 293.2144.

5-Membered Ring Retinal 2. Pentaenenitrile **10** (7 mg, 0.024 mmol) was dissolved in 3 mL of dry hexane under argon atmosphere. The solution was cooled to −78 °C, and 0.04 mL of a 1 M hexane solution of diisobutylaluminum hydride was added. After 1 h, ether (10 mL) and silica (1.5 g) were added, and the mixture was kept stirring at 4 °C for 15 h. Water was added, and the mixture was extracted twice with ether. Usual workup and chromatography with ether–hexane (15:85) gave retinal **2** (6 mg, 86% yield) in two fractions in 60:40 cis:trans ratio. UV (hexane): λ_{max} 362 nm (ϵ 22 000) (all-trans), 361 (ϵ 8000) (13-cis). ^{1}H NMR–NOE Experiment: Irradiation of 14-H increased integration of 12-H by 30% and did not exhibit any effect on 13-CH_2 in the trans isomer, while it gave 17% increase in 11-CH_2 + 13-CH_2 and no effect on 12-H in the cis isomer. Irradiation of 13-CH_2 increased integration of 15-H by 18% and had no effect on 14-H in the trans isomer, while it increased 14-H by 18% and had no effect on 15-H in 13-cis isomer. High-resolution mass spectrum ($C_{21}H_{28}O$): found, 296.2185; calcd, 296.2140.

Trienone 12. Phosphonium salt **11** (269 mg, 0.61 mmol) was dissolved in 4 mL of dry THF. The mixture was stirred at 0 °C under argon atmosphere, and 0.42 mL of a 1.5 M hexane solution of *n*-butyllithium was added dropwise. The solution turned red. After 5 min, aldehyde **5** (73 mg, 0.59 mmol) in 2 mL of dry THF was added. The color faded into yellow. After 20 min, water was added, and the mixture was extracted twice with ether. Usual workup and chromatography with ether–hexane (10:90) gave trienone **12** (95 mg, 66% yield) as one isomer (trans) only. UV ($CHCl_3$): λ_{max} 325 nm (ϵ 17 000). ^{1}H NMR δ 1.04 (s, 6, 1-CH_3), 1.48 (m, 2, 2-H), 1.62 (m, 2, 3-H), 1.73 (s, 3, 5-CH_3), 2.04 (m, 4, 4-H, ring protons), 2.43 (t, J = 6.4 Hz, 2, 11-CH_2), 2.53 (t, J = 6.0 Hz, 2, 13-CH_2), 5.92 (s, 1, 10-H), 6.19 (d, J = 15.4 Hz, 1, 8-H), 6.66 (d, J = 16.4 Hz, 1, 7-H). High-resolution mass spectrum ($C_{17}H_{24}O$): found, 244.1824; calcd, 244.1820.

Pentaenenitrile 13. Trienone **12** (197 mg, 0.81 mmol) was reacted with the sodium salt of diethyl 3-methyl-4-phosphonocrotononitrile (500 mg, 2.33 mmol) in 30 mL of dry THF at 25 °C under argon atmosphere. After 20 h, water was added, and the mixture was extracted twice with ether. Usual workup and chromatography with ether–hexane (10:90) gave pentaenenitrile **13** (180 mg, 73% yield) as a mixture of isomers. ^{1}H NMR: δ 1.04 (s, 6, 1-CH_3), 1.46 (m, 2, 2-H), 1.58 (m, 2, 3-H), 1.72 (s, 3, 5-CH_3), 1.83 (m, 2, ring protons), 2.03 (m, 2, 4-H), 2.23 (s, 3, 13-CH_3), 2.36 (m, 4, 9-CH_2, 11-CH_2), 5.14 (s, 14-H cis), 5.19 (s, 14-H trans), 5.67–6.56 (m, 4, 7-H, 8-H, 10-H, 12-H). High-resolution mass spectrum ($C_{22}H_{29}N$): found, 307.2293; calcd, 307.2285.

6-Membered Ring Retinal 3. Pentaenenitrile **13** (176 mg, 0.57 mmol) was dissolved in 30 mL of dry hexane under argon atmosphere. The solution was cooled to −78 °C, and 1 mL of a 1 M hexane solution of diisobutylaluminum hydride was added. After 90 min, ether (10 mL) and silica (3 g) were added. The mixture was stirred for 4 h at 25 °C followed by filtration through Celite and solvent evaporation. Chromatography with ether–hexane (1:99) gave retinal **3** (139 mg, 78% yield) in four fractions in ~6:4:3:2 all-trans: 13-cis:11-cis:11,13-di-cis ratio. UV (hexane): λ_{max} 365 nm (ϵ 28 500) (all-trans), 354 (ϵ 9500) (11-cis), 320 (ϵ 16 000) (13-cis). UV (CH_2Cl_2): λ_{max} 369 nm (ϵ 13 500), 306 (ϵ 13 000) (13-cis), 384 (ϵ 5000) (11,13-di-cis). ^{1}H NMR–NOE Experiment: Irradiation of 13-CH_3 increased integration of 15-H by 18% and had no effect on 14-H in the all-trans and 11-cis isomers. It increased integration of 14-H by 16% and had no effect on 15-H in the 13-cis and 11,13-di-cis isomers. Irradiation of 8-H increased integration of 10-H by 20% in the 11-cis isomer. High-resolution mass spectrum ($C_{22}H_{30}O$): found, 310.2336; calcd, 310.2296.

Preparation and Spectroscopy of Artificial Pigments. Artificial bacteriorhodopsins were prepared by reconstituting the apomembrane with the synthetic retinals. Preparations of apomembrane and pigment reconstitution were carried out by using previously described methods.[24]

Pulsed-laser photolysis experiments of membrane suspensions were carried out on a UV-14DL-200 Molectron dye laser system (8 ns, 0.5 mJ) previously described.[13] Data digitized with a Biomation 8100 transient recorder were averaged in a Nicolet 1170 computing system. The samples were studied in a 10-mm cell and contained a pigment concentration of 10^{-5} M.

Isomer composition of the pigments was determined by methylene chloride extraction,[25] followed by HPLC analysis (μPorosil column with 1:1 methylene chloride–hexane and 1% acetonitrile).

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Registry No. *all-trans*-**1**, 102586-39-0; 13-*cis*-**1**, 102574-05-0; *all-trans*-**2**, 102586-40-3; 13-*cis*-**2**, 102586-41-4; *all-trans*-**3**, 102574-06-1; 11-*cis*-**3**, 102574-07-2; 13-*cis*-**3**, 102574-08-3; 11,13-dicis-**3**, 102574-09-4; **4**, 66556-69-2; **5**, 62952-40-3; *all-trans*-**6**, 102574-10-7; 9-*cis*-**6**, 102574-11-8; 13-*trans*-**7**, 102574-12-9; 13-*cis*-**7**, 102574-13-0; **8**, 102574-14-1; *all-trans*-**9**, 102574-15-2; 9-*cis*-**9**, 102574-16-3; 13-*trans*-**10**, 102574-17-4; 13-*cis*-**10**, 102574-18-5; **11**, 56013-01-5; **12**, 102574-19-6; *all-trans*-**13**, 102574-20-9; 11-*trans*-13-*cis*-**13**, 102574-21-0; 11-*cis*-13-*trans*-**13**, 102574-22-1; 11,13-*dicis*-**13**, 102574-23-2; $(EtO)_2POCH_2CN$(Na salt), 73639-51-7; $(EtO)_2POCH_2C(CH_3)$=CHCN(Na salt), 86948-70-1.

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