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Full Papers

Three bacteriorhodopsins with ring-didemethylated 6-*s*-locked chromophores and their properties

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Abstract. Three novel, 6-*s*-locked rigidified retinals, racemic all-*E* 1,5-didemethyl-8,16-methanoretinal, all-*E* 1,1-didemethyl-8,18-methanoretinal and all-*E* 8a,18-didehydro-1,1-didemethyl-8,18-methanoretinal were prepared in good yield in high purity on a 100-mg scale. For the preparation of the key intermediate in the synthesis of 1,5-didemethyl-8,16-methanoretinal, reductive cyanation of an unsaturated cyanohydrin to the corresponding conjugated nitrile was accomplished using triethylsilane and trifluoroacetic acid. The three locked retinals interact with bacterioopsin to form bacteriorhodopsin with about the same rate as the native chromophore. This work proves that steric interaction of the 1,1-dimethyl group in the chromophore is an important factor in binding to bacterioopsin.

Introduction

The membrane protein bacteriorhodopsin (bR) belongs to the important class of retinal proteins. It is present in the purple membrane of *Halobacterium halobium*[1,2]. bR is folded into seven transmembrane helices and functions as a light-driven proton pump that converts the energy of light into that of a proton gradient over the bacterial membrane. The bacterium uses this energy to generate ATP to drive its life processes. The chromophore is all-*trans* retinal bound to the ϵ-amino group of lysine-216 via a protonated-Schiff-base (PSB) linkage[3], see Figure 1. The λ_{max} value of light-adapted bR (568 nm) is much larger than that of the model PSB compound from all-*trans* retinal and butylamine (440 nm in methanol). The red shift in absorption maximum of bR, relative to its model PSB, is due to interaction of the chromophore with the protein chain. The difference in wave numbers (5100 cm^{-1}) has been called the opsin shift[4].

A few factors contribute to the opsin shift, amongst them delocalization of the positive charge and interaction with the counter-ion[5]. Solid-state ^{13}C-NMR spectroscopy of light-adapted bRs containing a specifically carbon-13-enriched retinylidene group established that the chromophore of bR occurs in a planar 6-*s*-*trans* conformation[6]. In solution, protonated all-*trans* retinylidenebutylamine occurs in the thermodynamically more stable twisted *s*-*cis* conformation[7].

In a bio-organic approach, bR analogues are regenerated by reaction of retinal analogue with the free protein, bacterioopsin (bO), obtained by bleaching of bR in the presence of hydroxylamine[8]. We have studied the pair of isomeric bR analogues with the 8,16- and 8,18-methanoretinylidene chromophore[9]. The 8,16- and 8,18-methano bridges lock the chromophore in almost planar 6-*s*-*trans* and 6-*s*-*cis* conformations, respectively, without substantially perturbing the electronic factors. (The mere presence of an extra alkyl substituent has only a small effect on the λ_{max} of the retinal[10].) 8,16-Methanoretinal (**I**; see Figure 2) forms a bR analogue that closely resembles natural bR (rate of binding, λ_{max} 570 nm, light–dark adaptation and proton-pump activity 90%). The binding of 8,18-methanoretinal (**II**), however, is slow and complex. 8,18-Methano-bR deviates considerably from the natural system (low rate of binding and a proton-pump activity of 20%). From the opsin shift of 8,18-methano and 8,16-methano-bR, it can be derived that a planar 6-*s*-*trans* conformation contributes approximately 1200 cm^{-1} to the opsin shift[11].

A molecular model of bR has only recently become available with the aid of high-resolution cryo-electron microscopy[12]. The resolution of this model is still limited and the chromophore could not be located. (The location of the chromophore in the protein has now been established by neutron-diffraction techniques[13].) The model suggests that the steric environment around the ring can accommodate the two 1-methyl groups only in the 6-*s*-*trans* conformation. The two 1-methyl groups in the 6-*s*-*cis* conformation would collide with helices D and F near residues methionine 118 and proline 186[14]. Steric hindrance of the two 1-CH_3 groups in 8,18-methanoretinal is then likely to account for the slow binding of 8,18-methanoretinal to bO.

Further insight into the steric requirements of the binding pocket can be attained by studying the interaction of bO

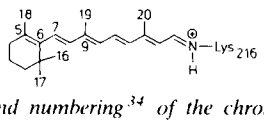

Fig. 1. Structure and numbering[34] *of the chromophore in bacteriorhodopsin.*

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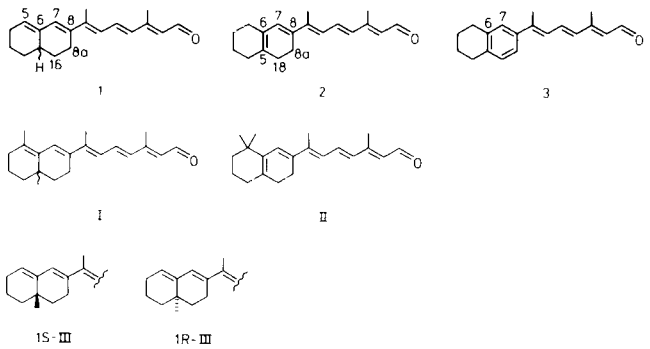


Fig. 2. Structure and numbering of all-E 1,5-didemethyl-8,16-methanoretinal (**1**), *all-E 1,1-didemethyl-8,18-methanoretinal* (**2**), *all-E 8a,18-didehydro-1,1-didemethyl-8,18-methanoretinal* (**3**), *all-E 8,16-methanoretinal* (**I**), *all-E 8,18-methanoretinal* (**II**), *and all-E 5-demethyl-8,16-methanoretinal* (**III**).

with retinal analogues that have a locked 6-*s* conformation and that do not have methyl groups attached to the bicyclic ring part. The novel racemic all-*E* 1,5-didemethyl-8,16-methanoretinal (**1**, see Figure 2), all-*E* 1,1-didemethyl-8,18-methanoretinal (**2**) and all-*E* 8a,18-didehydro-1,1-didemethyl-8,18-methanoretinal (**3**) are the retinal analogues we have chosen for this study.

All-*E* 1,5-didemethyl-8,16-methanoretinal and all-*E* 1,1-didemethyl-8,18-methanoretinal form a pair of retinal analogues that contain the 6-*s-trans*- and 6-*s-cis*-locked conformations, respectively. **1** and **2** differ from all-*E* 8,16-methanoretinal (**I**) and all-*E* 8,18-methanoretinal (**II**) in that they lack methyl groups in the bicyclic part and unequal steric interaction arising from these methyl groups is ruled out. Comparison of their interactions with bacterioopsin will increase our understanding of the size of the binding pocket and the effects of the 6-*s* conformation. The aromatic analogue of **2**, all-*E* 8a,18-didehydro-1,1-didemethyl-8,18-methanoretinal (3) occupies similar space, compared to **1** and **2**, but differs in electronic factors, due to the presence of the aromatic ring.

In this paper, we describe the synthesis of **1**, **2** and **3**. **1** has a chiral carbon atom (C1); it is obtained as a racemate in the total synthesis. The novel compounds are characterized and their interaction with bO is discussed.

Synthesis

All-*E* 1,5-didemethyl-8,16-methanoretinal (**1**), all-*E* 1,1-didemethyl-8,18-methanoretinal (**2**) and all-*E* 8a,18-didehydro-1,5-didemethyl-8,18-methanoretinal (**3**) each have a modified β-ionylidene part with respect to the parent all-*E* retinal. For the synthesis of **1**, **2** and **3**, we first had to prepare the novel β-ionone analogues 1-(3,4,4a,5,6,7-hexahydro-2-naphthalenyl)ethanone **8**, 1-(3,4,5,6,7,8-hexahydro-2-naphthalenyl)ethanone **13** and 1-(5,6,7,8-tetrahydro-2-naphthalenyl)ethanone **14**, see Figure 3. We anticipated that each of the β-ionone analogues could be extended to the corresponding retinal analogue via a well documented four-step chain-elongation sequence that has been used in the synthesis of retinal and many chemically modified retinals[9,15,16].

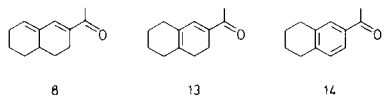
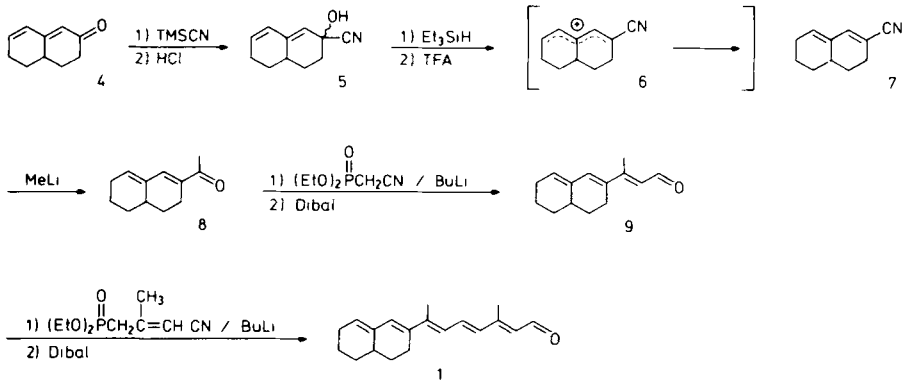


Fig. 3. Structure of novel β-ionone analogues **8**, **13** *and* **14**.

All-E 1,5-didemethyl-8,16-methanoretinal (**1**)

The high-yield two-step preparation of racemic 4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone (**4**) from 2-methoxynaphthalene has recently been described[17]. It contains the hexahydronaphthalenyl structure that serves as an ideal starting material for all-*E* 1,5-didemethyl-8,16-methanoretinal (**1**), see Scheme 1. Conversion of tetrahydronaphthalenone **4** into cyanohydrin **5** was effected by treating **4** with trimethylsilyl cyanide[18] and $AlCl_3$ as a catalyst to give the corresponding trimethylsilyloxy nitrile, which was hydrolysed with dilute HCl. Reductive elimination of the alcohol group of the cyanohydrin with concomitant double-bond shift into conjugation converts the unsaturated cyanohydrin into the conjugated nitrile **7** that contains the required naphthalenyl moiety. It is known that alcohols that can form a stabilized carbonium ion undergo reduction upon treatment with a protic acid and a hydride ion donor[19]. We found that, if cyanohydrin **5** is first suspended in triethylsilane and then treated with trifluoroacetic acid, the alcohol group is eliminated and regioselective hydride addition at C-7 takes place, leading to the required conjugated nitrile **7**. The nitrile group of **7** was converted into a methyl ketone function by treatment with methyllithium, giving the β-ionone analogue **8** in 46% yield based on **4**.

8 was coupled in a Horner–Emmons reaction to the anion of (diethylphosphono)acetonitrile to give the β-ionylideneacetonitrile analogue, which was submitted to reduction by diisobutylaluminum hydride (dibal). The resulting aldehyde **9** was reacted with the anion of 4-(diethylphosphono)-3-methyl-2-butenenitrile in a second Horner–Emmons reaction to give the retinonitrile. A final reaction of the retinonitrile with dibal gave an isomeric mixture, with **1** as the main constituent. The all-*E* isomer is substantially less soluble in diethyl ether than the Z isomers. Pure **1**, which is racemic, was obtained by washing the solid product with diethyl ether.



Scheme 1. Synthesis of all-E 1,5-didemethyl-8,16-methanoretinal (**1**).

All-E 1,1-didemethyl-8,18-methanoretinal (**2**)

β-Ionone analogue 1-(3,4,5,6,7,8-hexahydro-2-naphthalenyl)ethanone (**13**), indispensable for the synthesis of all-*E* 1,1-didemethyl-8,18-methanoretinal (**2**), was prepared in two steps, as depicted in Scheme 2. Diels–Alder reaction of 3-bromo-3-buten-2-one[20] (**11**) and 1,2-bis-(methylene)cyclohexane[21] (**10**) gives unsaturated α-bromo ketone **12** that contains the carbon skeleton of **13** with one carbon–carbon double bond in the right place. The second double bond is introduced by elimination of hydrogen bromide. Treatment of **12** with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) under strict exclusion of oxygen gave the required β-ionone analogue **13**.
β-Ionone analogue **13** contains the required sensitive dihydrobenzene structure. In subsequent reactions leading to **2** excess base and oxygen must be avoided to prevent aromatization. Coupling **13** in a Horner–Emmons reaction to the (diethylphosphono)acetonitrile anion gave the β-ionylideneacetonitrile analogue, which was submitted to reduction by dibal. The resulting aldehyde **15** was treated with the anion of 4-(diethylphosphono)-3-methyl-2-butenenitrile to give the retinonitrile. A final reaction with dibal gave an isomeric mixture with **2** as the main constituent. Although cyclohexadiene systems are prone to aromatization, after the four-step procedure no aromatized product was observed. Pure **2** was obtained after SiO_2 column chromatography.

All-E 8a,18-didehydro-1,5-didemethyl-8,18-methanoretinal (**3**)

3 was obtained from the aromatic β-ionone analogue **14**. This β-ionone analogue is accessible by oxidation of the cyclohexadiene β-ionone analogue **13** with 2,3-dichloro-5,6-dicyano-1,4-quinone (DDQ), as indicated in Scheme 2. Horner–Emmons coupling of **14** to the anion of (diethylphosphono)acetonitrile and subsequent dibal reduction yielded aldehyde **16**. This aldehyde was submitted to coupling to the anion of 4-(diethylphosphono)-3-methyl-2-butenenitrile and subsequent dibal reduction, which gave a mixture of geometric isomers containing mainly **3**. Pure **3** was obtained after SiO_2 column chromatography.

Spectroscopic characterization

Mass spectrometry

The fast-atom-bombardment mass spectra of **1**, **2** and **3** show the parent peaks $[M + H]^+$ at, respectively, *m/z* 269.1885, 269.1943 and 267.1750. They are, within experimental error, fully in agreement with the calculated values for the corresponding molecular formulae (calculated for $C_{19}H_{25}O$ 269.1905 and for $C_{19}H_{23}O$ 267.1747). The low-resolution FAB mass spectra at 70 eV all show the parent peak of M^+, and characteristic peaks at M^+-15 and M^+-29 due to the loss of CH_3 and CHO, respectively. A similar fragmentation pattern is found in retinal and many retinal analogues[9,16,22].

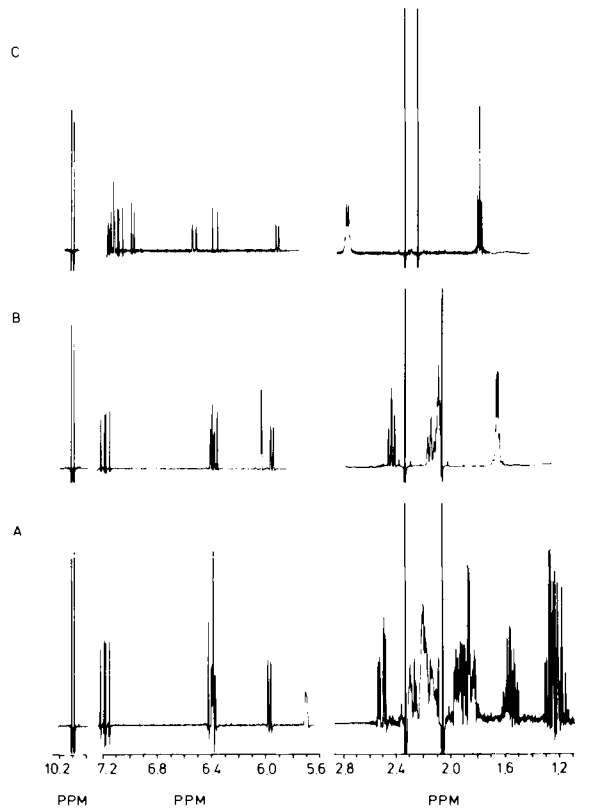
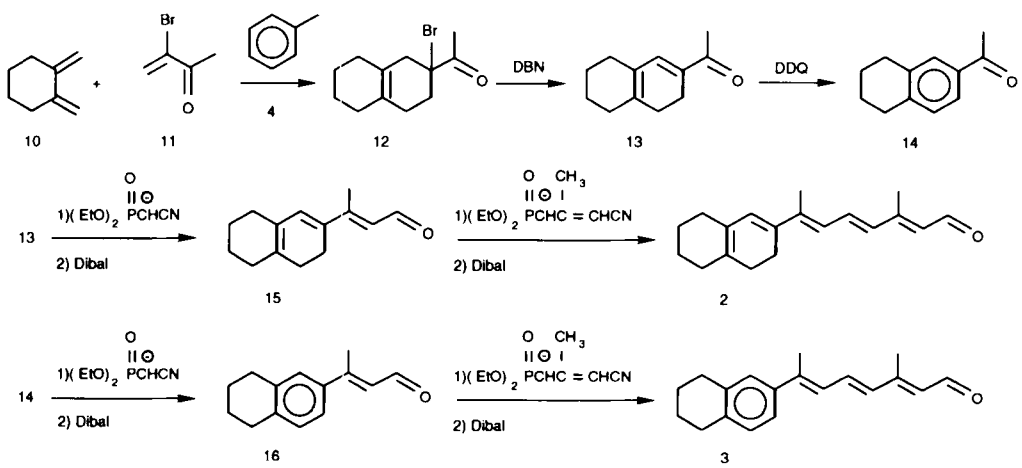


Fig. 4. 400-MHz ^{1}H-NMR spectrum of **1** *(A),* **2** *(B) and* **3** *(C) in $CDCl_3$.*

^{1}H-NMR spectroscopy

The 400-MHz ^{1}H-NMR spectrum of **1** (in $CDCl_3$) is shown in Figure 4a. The chemical-shift values and coupling constants of the signals in the vinylic region confirm the all-*trans* structure[23] of **1**. The AX patterns of 15-H and 14-H appear at δ_{H15} 10.11 and δ_{H14} 5.97 ppm ($J_{H15-H14}$ 8.2 Hz). The AMX pattern of 10-H, 11-H and 12-H is present at δ_{H10} 6.38, δ_{H11} 7.18 and δ_{H12} 6.40 ppm ($J_{H10-H11}$



Scheme 2. Synthesis of all-E 1,1-didemethyl-8,18-methanoretinal (**2**) *and all-E 8l,18-didehydro-1,1-didemethyl-8,18-methanoretinal* (**3**).

11.3 Hz, $J_{H11-H12}$ 15.0 Hz). The signal of 7-H is present at δ 6.38 ppm, and 5-H appears as a broad singlet at δ 5.71 ppm.

The signals of the protons attached to the bicyclic moiety form a complex pattern in the aliphatic region. The signals were assigned using the ^{1}H–^{1}H COSY and the ^{1}H-^{13}C COSY spectra. The spectrum was simulated to confirm the assignment. The presence of the chiral carbon atom (C-1) is reflected by large differences in chemical shift between protons on the same carbon atom *cis* or *trans* to 1-H. This difference amounts to 0.69 ppm for both methylene groups next to the chiral carbon atom, 2-CH_2 and 16-CH_2; 0.28 ppm and 0.21 ppm for the methylene groups two carbon bonds away from the chiral carbon atom, 3-CH_2 and 8a-CH_2, respectively; and only 0.02 ppm for 4-CH_2, which is the methylene group that is farthest away from the chiral center. (The protons *trans* to 1-H are tagged with a prime in Table II.) The 9-CH_3 and 13-CH_3 groups are easily recognisable and appear at δ_{H19} 2.06 ppm and δ_{H20} 2.33 ppm ($J_{H14-H20}$ 0.7 Hz). In Tables I and II, the ^{1}H-NMR chemical-shift values of **1**, together with those of **2** and **3**, are collated. In the ^{1}H-NMR spectrum of **1**, compared to that of **III**[16], removal of the 1-CH_3 group results in large chemical-shift differences for the β and γ hydrogens. The equatorial protons have shifted downfield, the axial protons upfield, in accordance with the substituent effect of the methyl group.

The 400-MHz ^{1}H-NMR spectrum of **2** (in $CDCl_3$) is shown in Figure 4b. The all-*trans* structure of **2** is confirmed by the chemical shift and coupling constants of the vinylic signals. The AX pattern of 15-H and 14-H is present at δ_{H15} 10.10 and δ_{H14} 5.97 ppm, J 8.2 Hz. 10-H, 11-H and 12-H give rise to an AMX pattern at δ_{H10} 6.41, δ_{H11} 7.19 and δ_{H12} 6.39 ppm ($J_{H10-H11}$ 11.3 Hz, $J_{H11-H12}$ 15.0 Hz). 7-H appears at δ 6.05 ppm as a singlet.

The high-field region reveals that the bicyclic part of the molecule contains the correct structure. 18-CH_2 and 8a-CH_2 appear as an AA'BB' sub-spectrum which is found at δ 2.14 and 2.43 ppm, respectively, based on comparison of these values with those in the spectrum of all-*E* 8,18-methanoretinal and the NOE effect of 10-H on 8a-H. 2-CH_2 and 3-CH_2 give rise to a four-proton multiplet centered at δ 1.65 ppm, and the allylic 1-CH_2 and 4-CH_2 form a four-proton multiplet at δ 2.09 ppm. Two sharp singlets of 9-CH, and 13-CH_3 are easily recognised at δ 2.06 and 2.33 ppm, respectively.

The 400-MHz ^{1}H-NMR spectrum of **3** (in $CDCl_3$) is shown in Figure 4c. In the low-field region, the vinylic and aromatic signals appear. The vinylic signals confirm the all-*trans* structure of **3**. The AX pattern of 14-H (δ 6.00 ppm; $J_{H14-H15}$ 8.2 Hz) and 15-H (δ 10.13 ppm) is present. 12-H, 11-H and 10-H form an AMX pattern, appearing at δ 6.46, 7.17 and 6.61, respectively ($J_{11H-12H}$ 15.0 Hz, $J_{10H-11H}$ 11.2 Hz). The 10-H signal is split by additional couplings with 9-CH_3 and the aromatic protons.

The aromatic signals indicate a 1,2,4-trisubstituted benzene ring. The signal of 18-H appears at δ 7.06 ppm and displays *ortho* coupling with 8a-H (J 8.0 Hz), the signal of 8a-H appears at δ 7.23 ppm and displays *ortho* coupling with 18-H (J 8.0 Hz) and *meta* coupling with 7-H (J 2.2 Hz) and the signal of 7-H appears as a broad singlet at δ 7.20 ppm. The ^{1}H–^{1}H COSY spectrum confirms the coupling between 7-H and 8a-H.

In the high-field region, the signals of the cyclohexene

Table I *400-MHz ^{1}H-NMR chemical-shift values (in $CDCl_3$ vs. TMS) of* **1**, **2** *and* **3**.

H [a]	1	2	3
1	[b]	2.08	2.78
2	[b]	1.66	1.81
3	[b]	1.66	1.81
4	[b]	2.08	2.78
5	5.71	–	–
7	6.38	6.05	7.20
8a	[b]	2.14	7.23
10	6.38	6.41	6.61
11	7.18	7.19	7.17
12	6.40	6.39	6.46
14	5.97	5.97	6.00
15	10.11	10.10	10.13
16	[b]	–	–
18	–	2.43	7.06
19	2.06	2.06	2.26
20	2.33	2.33	2.36

[a] Retinoid numbering used; see **1**, **2**, **3** and Ref. 34. [b] See Table II.

Table II *Chemical-shifts (ppm) and coupling constants (Hz) values of all-trans 1,5-didemethyl-8,16-methanoretinal (1) in C_6D_6 (vinylic protons and methyl groups) and $CDCl_3$ (aliphatic ring protons)*

H [a]	δ(ppm)	$^2J_{HH}$ (Hz)		$^3J_{HH}$ (Hz)		$^4J_{HH}$ (Hz)	
		H–H	J	H–H	J	H–H	J [b]
2 [c]	1.89	2–2′	–12.9	2–3	3.2	2–4	–1.2
2′	1.20	3–3′	–13.6	2–3′	3.4	3′–5	–1.1
3	1.57	4–4′	–19.6 [c]	2′–3	14.0	1–7	+
3′	1.85	16–16′	–12.9	2′–3′	3.4	5–7	+
4	2.18	8a–8a′	–16.7	3–4	6.3 [c]	7–8a [d]	–1.3
4′	2.20			3–4′	11.1 [c]	10–19	+
5 [d]	5.64			3′–4	1.2	14–20	+
7 [d]	6.42			3′–4′	7.1 [c]	12–14	+
1	2.14			4–5	4.9 [c]		
16	1.95			4′–5	3.3 [c]		
16′	1.26			16–8a	5.2		
8a	2.30			16–8a′	2.0		
8a′	2.51			16′–8a	12.4		
10 [d]	6.28			16′–8a′	4.9		
11 [d]	6.91			10–11 [d]	11.3		
12 [d]	6.09			11–12 [d]	15.0		
14 [d]	6.00			14–15 [d]	7.8		
15 [d]	10.04			1–2	4.5		
19 [d]	1.84			1–2′	11.2		
20 [d]	1.75			1–16	4.0		
				1–16′	12.5		

[a] Retinoid numbering used; see **1**, **2**, **3** and Ref. 34. [b] + present, but not determined accurately. [c] $5J_{8a-19}$ +. [d] Values obtained from spectrum in benzene-d_6. [e] Constrained value from (1*S*)-5-demethyl-8,16-methanoretinal.

ring confirm the presence of this structural element. The 1-CH_2 and 4-CH_2 next to the aromatic ring are almost equivalent and give rise to a multiplet at δ 2.78 ppm. The 2-CH_2 and 3-CH_2 are also very similar and give a multiplet at δ 1.81 ppm. The signals of 9-CH_3 (δ 2.26 pmm) and 13-CH_3 (δ 2.36 ppm) both appear as a doublet; 9-CH_3 couples with 10-H (J 1.3 Hz) and 13-CH_3 couples with 14-H (J 1.2 Hz). The presence of the aromatic ring induces large chemical shifts in the allylic protons (1-H and 4-H). Comparing the ^{1}H-NMR chemical shift value of the allylic protons in the bicyclic part of **2** and **3**, a difference of 0.70 ppm is found for 1-H and 4-H and a difference of 0.15 ppm is found for 2-H and 3-H.

^{13}C-NMR spectroscopy

^{13}C-NMR spectroscopy is a powerful tool for establishing the structure of the carbon backbone. The 100-MHz ^{1}H-noise-decoupled ^{13}C-NMR spectrum of **1** is composed of 8 signals in the sp^3 region and 11 signals in the sp^2 region, in accordance with the 19 different carbon atoms. The APT spectrum reveals three negative peaks in the sp^3 region, the two methyl groups at δ 14.2 and 13.1 ppm and the signal of 1-C at 35.2 ppm. The five methylene carbon atoms of the bicyclic ring part appear at 30.1 (2-C), 30.3 (16-C), 22.3 (3-C), 26.3 (4-C) and 26.1 ppm (8a-C). The signals of the proton-bearing carbon atoms were unambiguously identified from the ^{1}H-^{13}C COSY spectrum.
In the sp^2 region, 15-C is easily recognized based on its chemical shift (δ 191.0 ppm). 5-C, 7-C, 10-C, 11-C, 12-C and 14-C are identified from the ^{1}H-^{13}C COSY spectrum. The assignment of the peaks of the quaternary carbons was obtained from the long-range ^{1}H-^{13}C COSY spectrum. The ^{13}C-NMR chemical-shift values of **1**, **2** and **3** and their assignments are given in Table III. In the ^{13}C-NMR spectrum of **1**, as compared to that of **III**, removal of the 1-methyl group induced an expected[24] α effect of 3.5 ppm downfield, β effect of 6.9 (for C-2) and 6.5 ppm (for C-16) upfield and a γ effect of 4.1 (for C-3) and 3.1 ppm (for C-8a) downfield.
The 100-MHz ^{1}H-noise-decoupled ^{13}C-NMR spectrum of **2** shows 19 signals, in agreement with the 19 different carbon atoms. In the sp^3 region the expected 8 signals are present, which were assigned form the ^{1}H-^{13}C COSY spectrum and the long-range ^{1}H-^{13}C COSY spectrum.

Table III *100-MHz ^{13}C-NMR chemical-shift values (in $CDCl_3$ vs. TMS) of* **1**, **2** *and* **3**

H [a]	1	2	3
1	35.2	30.1	29.2 [c]
2	30.1	22.8 [b]	23.2
3	22.3	23.1 [b]	23.2
4	26.3	28.6	29.6 [c]
5	127.7	134.6	137.5 [d]
6	138.3	127.6	137.2 [d]
7	129.1	127.8	126.5
8	136.8	133.5	139.4 [d]
8a	26.1	24.1	123.0
9	142.0	141.6	142.1
10	124.6	124.1	125.8
11	133.1	133.3	132.8
12	134.5	134.3	134.9
13	155.0	155.0	154.8
14	128.8	128.8	129.1
15	191.0	191.1	191.1
16	30.3	–	–
18	–	29.2	129.3
19	14.2	14.2	16.4
20	13.1	13.1	13.2

[a] Retinoid numbering used; see **1**, **2**, **3** and Ref. 34. [b,c,d] Assignment may be interchanged.

Table IV *λ_{max} values for* **1**, **2** *and* **3**, *for their (protonated) butylimines and their bR analogues and the calculated opsin shift (OS).*

	Retinal (methanol)	λ_{max}			OS
		SB	PSB	Pigment	
1	395	369	467	552	3300
2	416	391	490	582	3230
3	372	355	428	501	3400

8a-C and 18-C appear at δ 24.1 and 29.2 ppm, respectively. The signals of the allylic carbon atoms in the cyclohexene moiety appear close together at δ 30.1 (1-C) and 28.6 ppm (4-C). The signals of 2-C and 3-C in the cyclohexene part are also close together at δ 22.8 and 23.1 ppm.
In the sp^2 region, the expected 11 vinylic carbon signals are present. 7-C, 10-C, 11-C, 12-C, 14-C and 15-C were assigned from the ^{1}H-^{13}C correlated spectrum. The quaternary 5-C and 6-C are at δ 134.6 and 127.6 ppm, respectively; δ 133.5 ppm was assigned to 8-C. Compared to **II**, the removal of the two 1-methyl groups has a pronounced influence on the chemical shifts of the bicyclic ring part of the molecule.
The 100-MHz ^{1}H-noise-decoupled ^{13}C-NMR spectrum of **3** shows 18 signals. In the sp^3 region, five signals are present. The signals of 19-C and 20-C, negative peaks in the APT spectrum, are identified at δ 16.4 and 13.2 ppm, respectively. The remaining three signals, positive peaks in the APT spectrum, are assigned to the carbons in the cyclohexene ring. At δ 23.2 ppm, the signals of 2-C and 3-C coincide, according to the ^{1}H-^{13}C COSY spectrum. The signals at δ 29.2 and 29.6 ppm belong to 1-C and 4-C.
In the sp^2 region, the expected 13 signals are present. The signals of the proton-bearing carbon atoms were easily identified from the ^{1}H-^{13}C COSY spectrum. The signals of the quaternary aromatic 5-C, 6-C and 8-C (δ 137.2, 137.5 and 139.4 ppm) could not be assigned unambiguously. Comparing the ^{13}C-NMR spectrum of **3** with that of **2**, the chemical-shift differences for the allylic carbon atoms and the β-carbon atoms in the bicyclic part are only very small (less than 1 ppm).

UV/Vis spectroscopy

The λ_{max} values of **1**, **2** and **3** are 395 nm, 416 nm and 372 nm, respectively. The value for **2** is within experimental error the same as that of **II** (415 nm) and that of **1** is 5 nm smaller than that of **I**. This shows that the removal of a methyl group from an sp^3 carbon atom, as expected, does not change the λ_{max} value. The removal of a methyl group from an sp^2 carbon atom gives an expected 5 nm hypsochromic shift. The λ_{max} values of the protonated Schiff base of **1** (467 nm) and **2** (490 nm) are also very close to that of **I** (465 nm) and **II** (485 nm).
The λ_{max} value of **3** (372 nm) is 42 nm smaller than that of **2**, showing that the tetrahydronaphthalene part in **3** contributes less to the conjugation of the system than the C5 to C8 diene moiety in **2**. The λ_{max} value of **3** agrees with the λ_{max} values of the retinal with the naphthalene system (3,7-dimethyl-7-naphthalenyl-2,4,6-heptatrienal[25]; 368 nm). The λ_{max} values of **1**, **2** and **3** are collated in Table IV.

Analogue pigment studies

Bacterioopsin reacts with a slight excess of **1** at room temperature to form, within seconds, the bR analogue bR(**1**) with a λ_{max} at 552 nm (ϵ_{max} 54.10^3). After 15 minutes, the conversion is complete.

Similarly, a slight excess of retinal analogue **2** reacts with bO within seconds at room temperature. First, a broad absorption is observed with a λ_{max} at 582 nm. Before binding is complete, bR(**2**) undergoes further reaction to a pigment with λ_{max} value of 509 nm. After 30 minutes, binding is complete. In the next $1\frac{1}{2}$ hours, the intensity of the absorption decreases slightly and the λ_{max} shifts further down to a final value of 504 nm. No further changes are then observed.

A slight excess of retinal analogue **3** reacts, within seconds at room temperature, with bO to form bR(**3**) with a λ_{max} at 501 nm (ϵ_{max} 47.10^3). After 30 minutes, the conversion is complete and no more increase of the absorption at 501 nm is observed.

If bR(**1**), bR(**2**) and bR(**3**) are treated with excess of all-*E* retinal, no increase in absorption at 568 nm is detected, indicating that, in each case, regeneration is complete and stable towards displacement by retinal. This means that, in each of the three bR analogues, the retinal analogue occupies the binding pocket.

BR(**1**) does show light–dark adaptation. Leaving it to stand in the dark for a few hours shifts the λ_{max} value to 548 nm. It completely reverts to the light-adapted form (λ_{max} 552 nm) upon exposure to visible light. Due to quick further reaction of bR(**2**), light adaptation of bR(**2**) cannot be studied. BR(**3**) does not show light–dark adaptation.

Binding of the retinal analogue pair **1** and **2** with bO to form bR(**1**) and bR(**2**) shows about the same kinetics as the binding of retinal to form bR. This is in strong contrast to the retinal analogue pair **I** and **II**, where the binding of **I** is as rapid as retinal, but the binding of **II** is very slow. These facts show that steric hindrance between the 1,1-dimethylgroups in the 6-*s-cis* retinal **II** and the peptide chain of bR is an important factor in the binding. When this factor is absent, such as in the pair **1** and **2**, the binding of the retinal analogues is as efficient as retinal itself.

From the λ_{max} values of light-adapted bR(**1**) and the butylamine protonated Schiff base of **1**, the opsin shift of bR(**1**) is calculated to amount to 3300 cm^{-1}. The opsin shift of bR(**2**) amounts to 3230 cm^{-1} and the opsin shift of bR(**3**) amounts to 3400 cm^{-1}. The λ_{max} values of **1**, **2**, **3**, their Schiff bases and their analogue bRs are collated in Table IV.

Proton pump action

We reconstituted bacteriorhodopsin in soybean phospholipid vesicles. Illumination of vesicles with visible light rapidly increased the pH of the external medium. The resulting proton gradient decreases the velocity of proton uptake asymptotically until a steady state is reached, in which light-driven proton uptake equals the passive back leakage. The extent of proton uptake is measured as the difference between the pH before illumination and the pH reached at the steady state, expressed as (nmol H^+/mg bR). When the light is then turned off, the protons re-equilibrate until the proton gradient has disappeared.

bR(**1**), bR(**2**) and bR(**3**), as well as native bR and bO were reconstituted in liposomes. For natural bR, a proton-pump action of 211 nmol H^+/mg was recorded. For bR(**1**), bR(**2**) and bR(**3**), values of 107, 42 and 48 nmol H^+/mg were recorded, respectively. The value recorded for the proton-pump action of bR(**1**) amounts to approximately 51% of the value recorded for bR. The values recorded for bR(**2**) and bR(**3**) are equal within experimental error and amount to approximately 20% of the value recorded for bR.

Discussion

We have prepared all-*E* 1,5-didemethyl-8,16-methanoretinal (**1**), all-*E* 1,1-didemethyl-8,18-methanoretinal (**2**) and all-*E* 8a,18-didehydro-1,1-didemethyl-8,18-methanoretinal (**3**). **1** has been prepared in a fair overall yield of 16%, based on 4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone (**4**), and **2** and **3** have been prepared in 40% and 26% yield, respectively, based on 1,2-bis(methylene)cyclohexane (**10**).

The novel hexahydronaphthalenenitrile **7** is a crucial intermediate in the synthesis of **1**. In a first approach to the synthesis of **7**, we started from easily available 4,4a,5,6,7,8-hexahydro-2(3*H*)-naphthalenone[26], which could efficiently be converted into its silyloxy nitrile. Elimination of the elements of water should give **7**. This reaction worked well for the silyloxy nitrile prepared from 3,4,4a,5,6,7-hexahydro-4a-methyl-2-napthalenenitrile[16] (4a-methyl-**7**). However, treating our silyloxy nitrile with $POCl_3$ in pyridine gave a complex mixture containing some **7**, from which **7** could not be separated on preparative scale.

We then used **4** as a starting material for synthesis of **7**. We first tried reductive introduction of the nitrile function by treating **4** with tosylmethyl isocyanide (tosMIC). It is known that, in some cases, tosMIC can be used for the reductive conversion of ketones into a nitrile[27]. We obtained a complex mixture of products in which the desired product could not be identified.

In a later approach, we first prepared cyanohydrin **5** from **4**. Treating **5** with trifluoroacetic acid and subsequent addition of triethylsilane effected reductive elimination of water to give a mixture of **7** and some of its isomers, which could not be separated. First suspending **5** in an excess of triethylsilane and subsequent treatment of this suspension with trifluoroacetic acid quantitatively gave nitrile **7**. Presumably, the carbonium ion **6** is formed, which immediately reacts with triethylsilane selectively on position C-7 to form the conjugated nitrile **7**. Using acetic acid instead of trifluoroacetic acid did not give this conversion; the acidity of acetic acid is probably too low to generate the carbonium ion **6**.

The next step is the conversion of the nitrile function of **7** into the methyl ketone function of β-ionone derivative **8**. This conversion was effected by treatment of **7** with methyl-lithium, as in the case of the 4a-methyl derivative of **7**. Generally, this reaction is very efficient only for aromatic and saturated aliphatic nitriles and does not work well for unsaturated nitriles. However, in the case of the unsaturated, aliphatic **7** and 4a-methyl-**7** this reaction works very well.

Further reactions starting from β-ionone analogue **8** must be carried out in dim red light. The presence of the allylic bridgehead hydrogen atom at position C-1 causes light instability in these systems. As far as we know, this is the first case where this type of light sensitivity has been observed during a retinal synthesis. Retinal **1** also shows this light-induced destruction, which was found to occur more rapidly in acetonitrile than in ethanol. For the preparation of retinal **2**, care must be taken to prevent aromatization of the dihydrobenzene structure. The solvents should be degassed and no excess base should be present. Under these conditions, pure retinal **2** can be prepared with no trace of the corresponding aromatic system **3** being present. **3** can be prepared by treating **2** with 2,3,5,6-tetrachloro-1,4-quinone. However, it is more convenient to prepare **3** starting from β-ionone derivative **14**, which can easily be prepared starting from the dihydrobenzene β-ionone derivative **13** by DDQ oxidation.

The binding rates of **1** and **2** to form bR(**1**) and bR(**2**) are both quick and in the same range as that of retinal and **I**, whereas the binding of **II** to bO is much slower. It is clear that the presence of the two 1-methyl groups in **II** and

their absence in **2** is responsible for this difference in behavior between the retinal pairs **1** and **2** on the one hand and **I** and **II** on the other hand. This shows that there is a steric interference between the two 1-methyl groups and groups in the peptide chain of bR. This steric factor is probably the same factor that forces the native chromophore in bR in the characteristic 6-*s-trans* conformation. Both **1** and **I** have been prepared in racemic form. The fact that we had to use racemic mixtures does not change the conclusions. We studied the corresponding optically pure (1*R*)-**III** and (1*S*)-**III**, and they show only a small difference (factor of two) in binding rate, the racemic form of **III** binds with an intermediate rate. Furthermore, chirality is only reflected in the ϵ_{max} value but not in the λ_{max} value of the bR analogue.

Unlike bR, bR(**1**) and bR(**I**), bR(**2**) is not stable but converts into a product with a smaller λ_{max}. This change is due to an as yet unknown factor in the interaction of the chromophore and the protein. In this case, no covalent interaction is involved because, from this product, bO can be obtained by bleaching the pigment in the presence of hydroxylamine.

The opsin shift of bR(**1**) is 3300 cm^{-1}. Exactly the same value has been found for bR(**III**). This is 650 cm^{-1} less than the opsin shift of bR(**I**) (3950 cm^{-1}), a contribution of the 5-methyl group to the opsin shift, related to the fact that, in the chromophore in bR (and analogues), more positive charge is present on C-5 than in the corresponding model systems[28]. The electron-donating ability of the 5-methyl group stabilizes the charge in 8,16-methano-bR [bR(**I**)] more than in 1,5-didemethyl-8,16-methano-bR [bR(**1**)].

The proton-pump activity of bR(**1**) is 51% of that of bR, the same value as for the proton-pump activity of bR(**III**) and 5-demethyl-bR. The proton-pump activity of bR(**I**) is 90%. This result agrees with the fact that the 5-methyl group in bR contributes about 50% to the proton-pump activity.

Retinal **2** and retinal **3** have virtually the same stereochemistry, but differ in electronic structure (dihydrobenzene *vs.* benzene part). Both show an expected rapid reaction with bO to form bR(**2**) and bR(**3**). bR(**2**) and bR(**3**) have similar opsin shifts (3230 and 3400 cm^{-1}, respectively). The difference in electronic structure between **2** and **3** appears to have no influence on the proton-pump activity and the opsin shift. The low λ_{max} value of the free retinal and the chromophore in bR(**3**) is in agreement with the fact that an aromatic system does not interact as well with the rest of the conjugated chain as a diene in a dihydrobenzene structure.

Experimental

General

Chemicals were purchased as reagent-grade from Janssen Chimica (Belgium) or Aldrich (MO, USA). Trimethylsilyl cyanide was prepared according to *Reetz* et al.[29]. The following solvents were distilled prior to use: tetrahydrofuran (THF; from $LiAlH_4$), diethyl ether (from P_2O_5), petroleum ether (bp. 40–60°C; from P_2O_5), toluene (from Na), CH_2Cl_2 (from CaH_2). Reactions were generally carried out in a nitrogen atmosphere. Diisobutylaluminum hydride (dibal) was used as 1.0M solution in hexanes; butyllithium (BuLi) as 1.6M solution in hexanes.

NMR spectra were run in $CDCl_3$ (with tetramethylsilane as internal standard, δ 0 ppm) at a Jeol FX-200 or a Bruker MSL-400 (operating at 199.5 MHz and 400.1 MHz, respectively, for ^1H; 50.1 MHz and 100.6 MHz for ^{13}C). UV/Vis spectra were run on a Varian DMS-200, using ethanol or *n*-hexane (spectroscopic grade) as solvent. Bacterioopsin was prepared according to published procedures[30].

The FAB mass spectra were recorded on a V.G. Micromass ZAB-2HF mass spectrometer, an instrument with reverse geometry, fitted with a high-field magnet and coupled to a V.G. 11/250 data system. The samples were introduced via a direct insertion probe into the ion source. The ion-source temperature was generally 150°C. During high-resolution FAB-MS measurements, a resolving power of 20000 (10% valley definition) was used.

Evaporation of solvents was performed *in vacuo* (20 mmHg). Purification was performed by flash column SiO_2 chromatography, using ether/petroleum-ether as eluent, unless stated otherwise.

4,4a,5,6-Tetrahydro-2(3H)-naphthalenone (**4**)

18.5 g (117 mmol) of 2-methoxynaphthalene and 75 ml of ethanol were added to 600 ml of liquid NH_3. 18.5 g (0.80 mol) of Na was added in small portions. The NH_3 was then allowed to evaporate. Water was added to the residue and the aqueous layer was extracted three times with ether. The combined organic layers were washed with brine and dried over $MgSO_4$. The solvents were then evaporated. The residue was dissolved in a mixture of 150 ml of THF and 300 ml of 35% $HClO_4$. The mixture was stirred for 5 h and then washed with satd. NaCl. Water was added and the organic layer was neutralized with solid $NaHCO_3$. The water layer was extracted with ether and the combined organic layers were washed with brine and dried over $MgSO_4$. The solvents were evaporated and the product was purified, yielding 16.0 g (92%). ^{13}C NMR (50 MHz): δ 199.5 (C=O); 157.9 (8a-C); 138.9; 129.9; 123.4; 37.5; 35.0; 29.8; 28.8; 25.8.

2,3,4,4a,5,6-Hexahydro-2-hydroxy-2-naphthalenecarbonitrile (**5**)

1.0 g (6.7 mmol) of **4** was dissolved in 4 ml of toluene. 1.0 g (10 mmol) of TMSCN and 50 mg of $AlCl_3$ were added. The mixture was heated to 66°C for 24 h. The solvent was then evaporated and the product purified (giving two pairs of diastereomers). ^1H NMR (200 MHz): δ 6.02–5.92, m (7-H+8-H); 5.37, s (1-H); 2.34–2.20, m; 2.20–2.08, m; 2.93–1.67, m; 1.58–1.18, m; 0.16, s ($Si-CH_3$). ^{13}C NMR (50 MHz): δ 141.5 ppm (8a-C); 132.8; 127.6; 122.5; 121.5 (CN); 69.1 (2-C); 37.5; 35.0 (4a-C); 29.4; 27.9; 25.8; 1.5 (Si-C). The siloxy nitrile was dissolved in 20 ml of THF. 10 ml of 3N HCl was added and the mixture was stirred for 30 min. The layers were then separated and the water layer was extracted with ether. The combined organic layers were neutralized with satd. $NaHCO_3$, washed with brine and dried over $MgSO_4$. The solvents were evaporated, yielding 1.01 g (86%) of a white solid. ^1H NMR (200 MHz): δ 6.05–5.93, m, (7-H+8-H); 5.40, s (1-H); 4.1–3.9, br. s (OH); 2.52–2.35, m; 2.24–2.11, m; 2.00–1.75, m; 1.65–1.28, m. ^{13}C NMR (50 MHz): δ 142.5 (8a-C); 133.2; 127.4; 121.5 (CN); 120.7; 67.7 (C-OH); 36.1; 35.0; 29.3; 27.7; 25.8.

3,4,4a5,6,7-Hexahydro-2-naphthalenecarbonitrile (**7**)

1.0 g (5.7 mmol) of **5** was suspended in 6 ml of triethylsilane at room temperature. 10 ml of trifluoro acetic acid was added slowly. The mixture was stirred for 1 h. Water was added and then solid $NaHCO_3$ to neutralize the mixture. The layers were separated. The water layer was extracted with ether and the combined ethereal layers were washed with brine and dried over $MgSO_4$. The solvent was evaporated and the product was purified. Yield 0.86 g (95%). ^1H NMR (200 MHz): δ 6.67, s (1-H); 5.83, m (8-H); 2.35–2.08, m; 1.92–1.77, m; 1.72–1.46, m; 1.35–1.12, m. ^{13}C NMR (50 MHz): δ 143.3 (C-H); 135.6; 133.3 (C-H); 120.2; 108.2; 34.1; 29.6; 28.9; 27.2; 26.1; 21.8.

1-(3,4,4a,5,6,7-Hexahydro-2-naphthalenyl)ethanone (**8**)

1.0 g (6.3 mmol) of **7** was dissolved in 25 ml of THF. 12 mmol of methyllithium were added via a syringe at −50°C. The mixture was transferred to room temperature and stirred for 30 min. 18 ml of 1 N HCl was added and the mixture was stirred vigorously for 30 min. $NaHCO_3$ was added and the layers were separated. The aqueous layer was extracted twice with ether. The combined organic layers were washed with brine and SiO_2 was added. The mixture was stirred for 1 h, then $MgSO_4$ was added. The solids were filtered off and the solvents were evaporated. The product was purified, yield 0.69 g (62%). ^1H NMR (200 MHz) [a]: δ 6.98, s (H-7); 5.95, br. s (H-5); 2.69–2.56, m (4-CH_2); 2.32, s (19-CH_3); 2.28–2.04, m; 1.98–1.79, m; 1.69–1.42, m; 1.28–1.11, m. ^{13}C NMR (50 MHz): δ 199.2 (C-9); 139.8; 137.4 (C-H); 136.6 (C-H); 133.6; 35.0; 29.8; 29.4; 26.4; 24.9; 23.4; 22.0.

[a] Retinoid numbering used, see **1** and Ref. 34.

3-(3,4,4a,5,6,7-Hexahydro-2-naphthtalenyl)-2-butenal (**9**)

0.53 g (3.0 mmol) of (diethylphosphono)acetonitrile was dissolved in 20 ml of THF and 2.5 mmol of BuLi were added via a syringe at 0°C. After stirring for 10 min, a solution of 0.40 g (2.3 mmol) of **8** in 10 ml of THF was added dropwise. The mixture was stirred for 2 h, then water was added. The layers were separated and the aqueous layer was extracted three times with ether. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered over SiO_2. The solvents were evaporated and the residue was purified, yielding 0.36 g (80%) of nitrile (as a 8:1 mixture of 9-*E* and 9-*Z* isomers). ^{1}H NMR (200 MHz)[34]: 9-*E*: δ 6.47, s (7-H); 5.81, br. s (5-H); 5.24, s (10-H); 2.21, s (9-CH_3); 9-*Z*: δ 6.40, s (7-H); 5.72, br. s (5-H); 5.10, s (10-H); 2.01, s (9-CH_3). ^{13}C NMR (50 MHz): 9-*E*: δ 157.7; 137.0; 133.1; 132.7; 130.8; 118.0; 92.3; 34.3; 29.5; 29.3; 25.6; 25.1; 21.7; 17.4.
0.36 g (1.8 mmol) of the nitrile was dissolved in dry petroleum ether and cooled to −60°C. 2.5 mmol of dibal were added via a syringe. The mixture was stirred for 15 min, then a slurry of 10 g of SiO_2 and 5 g of water was added and the mixture was allowed to warm to room temperature. After 1 h, $MgSO_4$ was added and the solids were filtered off. After evaporation of the solvent and purification, 0.30 g (82%) of aldehyde (as a mixture of 9-*E* and 9-*Z* isomers) was obtained. ^{1}H NMR (200 MHz)[34]: 9-*E*: δ 10.14 ppm, d *J* 8.2 Hz (11-H); 6.64, s (7-H); 6.07, d, *J* 8.2 Hz (10-H); 5.83, br. s (5-H); 2.33, s (9-CH_3); 2.39–1.97, m; 1.95–1.84, m; 1.65–1.45, m; 1.33–1.12, m; 9-*Z*: δ 9.65, d, *J* 8.2 Hz (11-H); 2.05, s (9-CH_3). ^{13}C NMR (50 MHz): 9-*E*: δ 191.2; 155.8; 137.6; 135.5; 132.9; 130.8; 124.4; 34.7; 29.7; 29.7; 26.1; 25.7; 21.9; 13.5.

All-E 1,1-didemethyl-8,16-methanoretinal (**1**)

0.54 g (2.5 mmol) of 4-(diethylphosphono)-3-methyl-2-butenenitrile was dissolved in 20 ml of THF and 2.0 mmol of BuLi were added via a syringe at 0°C. After stirring for 10 min, a solution of 0.30 g (1.5 mmol) of **9** in 10 ml of THF was added dropwise. The mixture was stirred overnight, then water was added. The layers were separated and the aqueous layers was extracted three times with ether. The combined organic layers were washed with brine, dried over $MgSO_4$ and then filtered over SiO_2. The solvents were evaporated and the residue was purified, yielding 0.23 g (58%) of an isomeric mixture of nitriles. ^{1}H NMR (200 MHz)[34]: 9-*E*: δ 5.69 ppm, br. s (7-H); 5.16, s (14-H); 2.21, s (13-CH_3); 2.03, s (9-CH_3).
At −60°C, 2 mmol of dibal was added via a syringe to a solution of 0.23 g (0.87 mmol) of the nitriles in CH_2Cl_2. After stirring for 30 min at −40°C, a slurry of 2 g of SiO_2 and 1 g of water was added. The mixture was stirred for 2 h at 0°C, then $MgSO_4$ was added. The solids were filtered off and the solvents were evaporated. 0.20 g (85%) of an *E*/*Z* mixture was obtained. In order to purify the all-*E* isomer, it was stirred in diethyl ether for 30 min. Pure racemic **1** (0.1 g) did not dissolve. The ^{1}H- and ^{13}C-NMR characteristics of **1** are presented in Tables I, II and III. The UV/Vis and mass spectra are described in the section spectroscopic characterization.

1,2-Bis(methylene)cyclohexane (**10**)

154 g (1.00 mol) of 1,2-cyclohexanedicarboxylic anhydride were dissolved in 4 l of THF. 38 g (1.0 mol) of $LiAlH_4$ was added at 0°C in small portions. Stirring was then continued for 1 h. 100 g of water was then added dropwise and the mixture was stirred overnight at room temperature. The products were filtered over a glass-fritted funnel. The filtrate was dried over $MgSO_4$ and neutralised with NH_4Cl and the solvents were evaporated. The remaining yellow viscous oil was distilled, yielding 79 g (55%) of 1,2-bis(hydroxymethylene)-cyclohexane, a colorless viscous sirup. B.p. 125°C at 0.3 mmHg. ^{1}H NMR (300 MHz): δ 4.9–4.6, br. s (2 C-OH); 3.78–3.47, m, 4H; 1.93–1.84, m, 2H; 1.56–1.35, m, 8H.
1.7 g (55 mmol) of red phosphorus was dissolved in 150 ml of toluene under N_2. 20.6 g (164 mol) of iodine was added and the mixture was refluxed for $\frac{1}{2}$ h 10 g (69 mmol) of 1,2-bis(hydroxymethyl)cyclohexane in 20 ml of toluene was then added. The mixture was refluxed for 8 h. After cooling the mixture was washed with satd. $NaHS_2O_3$ soln., satd. $NaHCO_3$ soln., and with brine. After drying over $MgSO_4$ the solids were filtered off and the solvent was evaporated. The product was purified by distillation, b.p. 141°C at 0.5 mmHg. Yield 22.5 g (90%) of 1,2-bis(iodomethyl)cyclohexane. ^{1}H-NMR (200 MHz): δ 3.14, dd, 4H; 2.15–2.0, m, 2H; 1.7–1.2, m, 8H.
10 g (28 mmol) of product was added to a solution of 4.6 g of KOH (82 mmol) in 10 g of dry methanol. The solution was stirred vigorously and refluxed for 4 h. After cooling, the lower layer was discarded and the upper layer dried with $MgSO_4$. The solids were filtered off, yielding 2.0 g (67%) of **10**. ^{1}H NMR (200 MHz): δ 4.92, s, 2H (2 C=CH); 4.64, s, 2H (2 C=CH); 2.25, br. s, 4H (2 C=C-CH_2); 1.63, br. s, 4H (2 CH_2).

3-bromobutenone (**11**)

21.0 g (0.3 mol) of freshly distilled 3-buten-2-one were added to 150 ml of water. 48.0 g (0.6 mol) of bromine were added dropwise at 10-15°C. The mixture was then heated and distilled, yielding a yellow oil. The aqueous layer was discarded and the organic layer was dried over $MgSO_4$. This layer contained a mixture of 3,4-dibromobutanone and 3-bromobutenone (5-10%). Yield 26 g (58%). An analytical amount was purified, the bulk being used in the next step without further purification. ^{1}H NMR (300 MHz): δ 6.85, d, *J* 2.5 Hz; 6.47, d, *J* 2.5 Hz; 2.49, s. ^{13}C-NMR (50 MHz): δ 191.5 (2-C); 131.7 (3-C); 129.5 (4-C); 28.4 (1-C).

1-(2-bromo-1,2,3,4,5,6,7,8-octahydro-2-naphthalenyl)ethanone (**12**)

2.0 g (19 mmol) of **10** were dissolved in 15 ml of toluene. At room temperature, 2.76 g (19 mmol) of **11** were added. The mixture was heated to 60°C for 12 h. After evaporation of the solvent, the crude product was purified. Yield 3.6 g (76%). ^{1}H NMR (200 MHz): δ 2.76–2.44, m, 2H; 2.40, s, 2H; 2.28–1.96, m, 2H; 1.86, br. s 4H; 1.68–1.52, m, 4H. ^{13}C NMR (50 MHz): δ 201.4 (C=O); 127.1; 124.4; 67.3 (C-Br); 41.1; 32.7; 29.9; 29.3; 28.9; 23.6; 22.6.

1-(3,4,5,6,7,8-Hexahydro-2-naphthalenyl)ethanone (**13**)

3.0 g (12 mmol) of **12** were dissolved in 25 ml of deoxygenated toluene, 1.3 g (12 mmol) of 1,5-diazabicyclo[4.3.0]non-5-ene were added at 0°C and the mixture was stirred at room temperature for 24 h. The solids were then filtered off and the solvent was evaporated. After purification, 1.9 g (93%) of product was obtained. ^{1}H NMR (200 MHz): δ 6.71, s, 1H; 2.46–2.35, t, 2H; 2.31, s (CH_3); 2.16–2.08, m, 6H; 1.71–1.61, m, 4H. ^{13}C NMR (50 MHz): δ 197.8; 140.0 (q); 138.6 (q); 133.4 (q); 126.4; 30.3; 28.6; 28.0; 24.7; 22.6; 22.2; 20.1.

3-(3,4,5,6,7,8-Hexahydro-2-naphthalenyl)-2-butenal (**15**)

2.1 g (12 mmol) of (diethylphosphono)acetonitrile was dissolved in 50 ml of THF and 11 mmol of BuLi were added via a syringe at 0°C. After stirring for 10 min, a solution of 1.9 g (11 mmol) of **13** in 10 ml of THF was added dropwise. The mixture was stirred for 2 h, then water was added. After extraction of the product with ether, the organic layer was washed with brine, dried over $MgSO_4$ and filtered over SiO_2. The solvents were evaporated and the residue was purified, yielding 1.9 g (80%) of nitrile. ^{1}H NMR (200 MHz): δ 6.16 ppm, s, 1H; 5.25, s, 1H (HC-CN); 2.34–2.20, t, 2H; 2.23, s (CH_3); 2.16–2.08, m, 6H; 1.71–1.61, m, 4H.
1.9 g (10 mmol) of the nitrile was dissolved in dry petroleum ether and cooled to −60°C. 13 mmol of dibal was added via a syringe. The mixture was stirred for 15 min, then a slurry of 10 g of SiO_2 and 5 g of water was added and the mixture was allowed to warm to room temperature. After 1 h, $MgSO_4$ was added and the solids were filtered off. After evaporation of solvent and purification, 1.8 g (94%) of aldehyde was obtained. ^{1}H NMR (200 MHz): all-*E*: δ 10.15, d, *J* 8.2 Hz, 1H (CHO); 6.35, s, 1H; 6.09, d, *J* 8.2 Hz, 1H; 2.35–2.25, m, 2H; 2.33, s (CH_3); 2.04, br.s, 6H; 1.65, br.s, 4H.

All-E 1,1-didemethyl-8,18-methanoretinal (**2**)

11 mmol of BuLi was added to a solution of 2.6 g (12 mmol) of 4-(diethylphosphono)-3-methyl-2-butenenitrile in 50 ml of THF at 0°C. After stirring for 10 min. 1.8 g (9 mmol) of **15** in 5 ml of THF was added dropwise. After stirring for 3 h, the mixture was poured into satd. NH_4Cl solution; the organic layer was separated and the water layer extracted three times with ether. The combined ethereal layers were washed with brine and dried over $MgSO_4$. The solids were filtered off, the solvents evaporated and the residue was purified, yielding 2.1 g (89%) of an E/Z mixture of nitriles. At −60°C, 12 mmol of dibal was added via a syringe to a solution of 2.1 g (8 mmol) of the nitriles in CH_2Cl_2. After stirring for 30 min. at −60°C, a slurry of 10 g of SiO_2 and 5 g of water in diethyl ether was added. The mixture was stirred for 1 h at 0°C, then $MgSO_4$ was added. The solids were filtered off and the solvents were evaporated. After purification, 1.8 g (85%) of an E/Z mixture was obtained. The isomers were separated SiO_2 column chromatography. The ^{1}H- and ^{13}C-NMR characteristics of **2** are presented in Tables I and III. The UV/Vis and mass spectra are described in the section spectroscopic characterization.

2-(5,6,7,8-Tetrahydro-2-naphthalenyl)ethanone (**14**)

0.7 g (4 mmol) of **13** was dissolved in 20 ml of CH_2Cl_2 and 0.8 g (4 mmol) of DDQ was added. The mixture was stirred for 4 h at 0°C. The solids were filtered off and the solvent was evaporated. The residue was purified and yielded 0.46 g (66%) of **14**. ^{1}H NMR (200 MHz)[34]: δ 7.66–7.53, m (8a-CH + 18-CH); 7.07, m (7-CH); 2.82–2.65, m (1-CH_2 + 4-CH_2); 2.50, s (CH_3); 1.81–1.72, m (2-CH_2 + 3-CH_2).

3-(5,6,7,8-Tetrahydro-2-naphthalenyl)-2-butenal (**16**)

0.46 g (2.6 mmol) of (diethylphosphono)acetonitrile was dissolved in 20 ml of THF and 2.5 mmol of BuLi were added via a syringe at 0°C. After stirring for 10 min, a solution of 0.44 g (2.5 mmol) of **14** in 10 ml of THF was added dropwise. The mixture was stirred for 2 h, then water was added. The layers were separated and the aqueous layer was extracted three times with ether. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered over SiO_2. The solvents were evaporated and the residue was purified, yielding 0.36 g (73%) of nitrile (as a 7:1 mixture of 9-*E* and 9-*Z* isomers). ^{1}H-NMR (200 MHz)[34]: 9-*E*: δ 7.16–7.04, m (7-H + 8a-H + 18-H); 5.53, s (10-H); 2.75, m (1-CH_2 + 4-CH_2); 2.40, s (9-CH_3); 1.78, m (2-CH_2 + 3-CH_2).
0.36 g (1.8 mmol) of the nitrile was dissolved in dry petroleum ether and cooled to −60°C. 2.5 mmol of dibal were added via a syringe. The mixture was stirred for 15 min, then a slurry of 10 g of SiO_2 and 5 g of water was added and the mixture was allowed to warm to room temperature. After 1 h, $MgSO_4$ was added and the solids were filtered off. After evaporation of solvent and purification, 0.30 g (82%) of aldehyde (as a mixture of 8-*E*/*X* isomers) was obtained. ^{1}H NMR (200 MHz)[34]: 9-*E*: δ 10.15, d, *J* 8.2 Hz (11-H); 7.25, s (7-H); 7.29, m (18-H); 7.15, m (8a-H); 6.39, d, *J* 8.2 Hz (10-H); 2.77, m (1-H + 4-H); 2.53, s (9-CH_3); 1.80, m (2-H + 3-H).

All-E 8a,18-didehydro-1,1-didemethyl-8,18-methanoretinal (**3**)

Method A. 0.54 g (2.5 mmol) of 4-(diethylphosphono)-3-methyl-2-butenenitrile was dissolved in 20 ml of THF and 2.0 mmol of BuLi were added via a syringe at 0°C. After stirring for 10 min, a solution of 0.30 g (1.5 mmol) of **16** in 10 ml of THF was added dropwise. The mixture was stirred overnight, then water was added. The layers were separated and the aqueous layer was extracted three times with ether. The combined organic layers were washed with brine, dried over $MgSO_4$ and filtered over SiO_2. The solvents were evaporated and the residue was purified, yielding 0.23 g (58%) of an isomeric mixture of nitriles. At −60°C, 2 mmol of dibal was added via a syringe to a solution of 0.23 g (0.87 mmol) of the nitriles in CH_2Cl_2. After stirring for 30 min at −40°C, a slurry of 2 g of SiO_2 and 1 g of water was added. The mixture was stirred for 2 h at 0°C, and $MgSO_4$ was then added. The solids were filtered off and the solvents were evaporated. 0.20 g (86%) of an E/Z mixture was obtained.

Method B. 0.50 g (1.9 mmol) of **2** was dissolved in CH_2Cl_2 and 0.50 g (2.0 mmol) of 2,3,5,6-tetrachloro-1,4-benzoquinone in 5 ml of CH_2Cl_2 was added. The mixture was refluxed for $1\frac{1}{2}$ h. Water was then added and the organic phase was separated and dried over $MgSO_4$. After evaporation of the solvents and purification, 0.36 g (73%) of **3** was obtained. The ^{1}H- and ^{13}C-NMR characteristics are presented in Tables I and III. The UV/Vis and mass spectra are described in the section spectroscopic characterization.

Schiff base and protonated Schiff base of **1**, **2** *and* **3**

The Schiff bases were prepared in a cuvette in the UV/Vis photospectrometer by addition of excess of butylamine to a dilute solution of **1**, **2** or **3** in methanol. Addition of a drop of concentrated hydrochloric acid to this methanolic solution completely converted the Schiff base into the protonated form.

Binding experiments

Binding experiments were performed as described earlier at room temperature[29]. Regeneration was followed in cuvettes with a path length of 2 mm. Light–dark addaptation was performed as described earlier[31].

Incorporation of bR analogues in phospholipid vesicles and light-driven proton-pump action

2 mg of bO was regenerated with all-E-retinal, **1**, **2** and **3**. bR, bR(**1**), bR(**2**), bR(**3**) and bO were then precipitated, the pellet was taken up in millipore water and precipitated. This was repeated twice. The precipitate was then taken up in 3 ml of a solution of 0.15M KCl and 2mM EDTA (pH 7). The concentration was determined photospectroscopically 2 ml of the bR solution was added to 50 mg of purified soybean phospholipids (asolectin)[32]. The suspension was then sonicated using a MSE probe-type ultrasonifier (probe diameter 2 mm, freq. 21 kHz, ampl. 5 μm) for 15 s, followed by 45 s of cooling during 1 h[33]. The mixture was kept under nitrogen and cooled in ice during sonication. According to this procedure, liposomes containing bO, bR, bR(**1**), bR(**2**) and bR(**3**) were prepared. The light-dependent pH changes were measured in a 2.5-ml temperature-controlled multipurpose cuvette (25°C) equipped with a stirring device and containing 600 μl freshly prepared (modified) bR liposomes and 1,4 ml 0.15M KCl, 2mM EDTA pH 7 and 4 μg valinomycin. The pH of the medium was measured continuously using an Ingold glass electrode connected to an amplifier (Radiometer PHM 63) and recorded on a Pantos U-228 unicorder. The cuvette was illuminated with a cold light source. The pH changes upon illumination were calibrated by the addition of 50 mmol oxalic acid.

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