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# Synthesis of six novel retinals and their interaction with bacterioopsin

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**Abstract.** The synthesis and spectroscopic characterization of six novel chemically modified retinals, is described, prepared by a single strategy: all-*E*-10,12-ethanoretinal (**1**), all-*E*-10,12-propanoretinal (**2**), all-*E*-10,12-ethano-20-norretinal (**3**), all-*E*-20-nor-10,12-propanoretinal (**4**), all-*E*-12,14-ethano-20-norretinal (**5**), and all-*E*-20-nor-12,14-propanoretinal (**6**). For this strategy we developed two novel synthons, 2-(diethoxyphosphinyl) hexanedinitrile and 2-(diethoxyphosphinyl) heptanedinitrile which were prepared in their deprotonated form from hexanedinitrile and heptanedinitrile. Bacterioopsin was incubated with each of **1** to **6**. Only **1** and **3** form bacteriorhodopsin analogues (bR-**1** and bR-**3**). bR-**1** shows light–dark adaptation and an approx. 50% light-driven proton-pump action. bR-**3** does not show light–dark adaptation or proton-pump action. It is also shown that the protein can efficiently accommodate retinal with the 10,12-ethano group whereas the corresponding retinal with the 10,12-propano group does not fit in the binding site.

## 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 in 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[3]. The bacterium uses this energy to generate ATP which drives its life processes. The chromophore is all-*E*-retinal (**7**) bound to the 6-amino group of lysine 216 via a protonated-Schiff-base (PSB) linkage[4] (see Figure 1).

The $\lambda_{max}$ value of light-adapted bR (568 nm) is much larger than that of the model PSB compound from all-*E*-retinal and *n*-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[5].

The only way to obtain information on the steric and electronic factors that are involved in the spectral tuning and functioning of the pigment is a bioorganic approach in which chemically modified retinals are incorporated in the active site of bacteriorhodopsin. We are currently carrying out a programme to collect this information. In the first study in our group, 8,16-methano- and 8,18-methanoretinal were incorporated and we established that in native bR 1200 $cm^{-1}$ of the opsin shift is due to a change in conformation around the C6–C7 single bond upon binding of the chromophore in the active site[6]. This conformation is planar 6-s-*trans* in bR[7] and predominantly 6-s-*cis* in the free protonated Schiff base in solution[8]. Our study of 17,18-dinor-8,16-methano- and 16,17-dinor-8,18-methano-bR established that steric hindrance of the 1,1-dimethyl group in bR forces the C6–C7 bond in

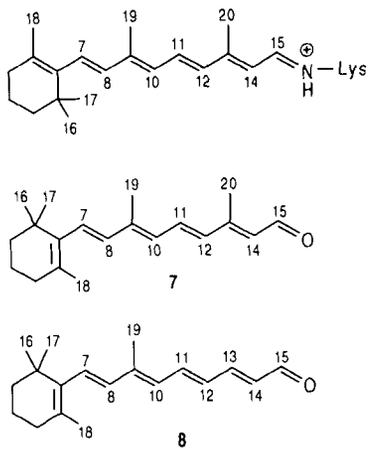


*Fig. 1. Structure and numbering of the chromophore in bR and structure and numbering of all-E-retinal (7) and all-E-20-norretinal (8).*

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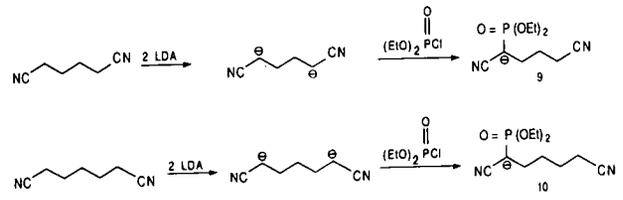


*Scheme 1. Synthesis of phosphonates* **9** *and* **10**.

the 6-s-*trans* conformation[9]. Our study of 5-bromo-18-nor-bR established that the contribution of the 5-methyl group to the light-driven proton-pump action and the opsin shift is due to electronic effects[10].

In this study we have investigated steric and electronic interactions in the central part of the retinal chromophore. The novel all-*E*-10,12-ethanoretinal (**1**; see Figure 2), all-*E*-10,12-propanoretinal (**2**), all-*E*-10,12-ethano-20-norretinal (**3**), all-*E*-20-nor-10,12-propanoretinal (**4**), all-*E*-12,14-ethano-20-norretinal (**5**), and all-*E*-20-nor-12,14-propanoretinal (**6**) are the systems of choice to study the effects of a locked conformation on the opsin shift and light-driven proton-pump activity in the presence or absence of the 13-methyl group.

The introduction of an ethano bridge forces the conjugated chain in a locked conformation with minimal steric changes. The propano bridge leads to a locked conformation with a slightly bigger steric requirement. For the introduction of the ethano and propano bridges in retinal we developed two novel, easily prepared synthons, *viz*, the anion of 2-(diethoxyphosphinyl)1,6-hexanedinitrile (**9**) and the anion of 2-(diethoxyphosphinyl)-1,7-heptanedinitrile (**10**).

In this paper we describe the synthesis and characterization of the new retinal analogues **1**, **2**, **3**, **4**, **5** and **6** and their interaction with bacterioopsin (bO), the apoprotein, as well as the opsin shift and light-driven proton-pump action of bR-1 and bR-3.

## Synthesis

The anion of 2-(diethoxyphosphinyl)-hexanedinitrile (**9**) is the synthon of choice for the introduction of ethano groups in the conjugated chain of retinal. It is prepared starting from hexanedinitrile via the reactions depicted in Scheme 1. Hexanedinitrile is first reacted with two equivalents of lithium diisopropylamide (LDA) in THF which leads to efficient formation of the dianion of hexanedinitrile. Addition of one equivalent of diethyl chlorophosphate gives an $S_N2$ reaction to form, after proton translocation, 2-(diethoxyphosphinyl)-hexanedinitrile. The phosphonate is immediately deprotonated by the nitrile anion to form the phosphonate anion **9**. In a similar way, the anion of 2-(diethoxyphosphinyl)-heptanedinitrile (**10**) is prepared starting from heptanedinitrile.

β-Ionone (**11**) is added to the solution of the phosphonate anion **9**. Horner-Emmons reaction takes place, resulting

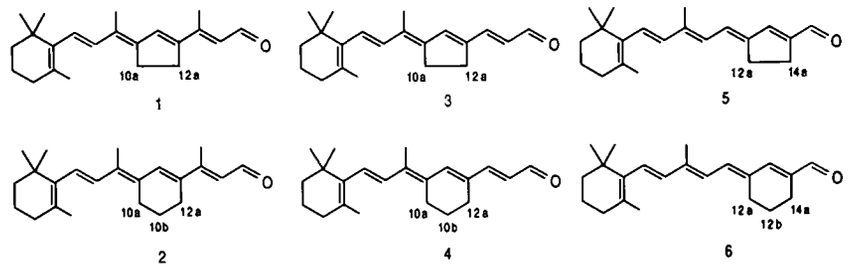
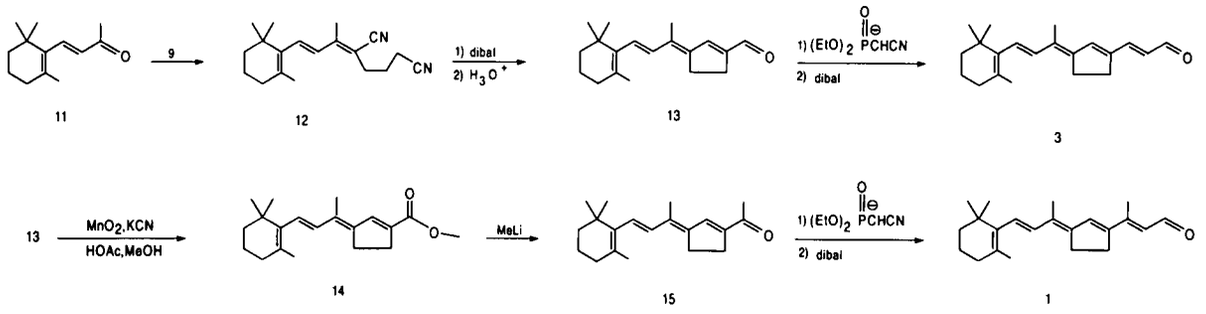
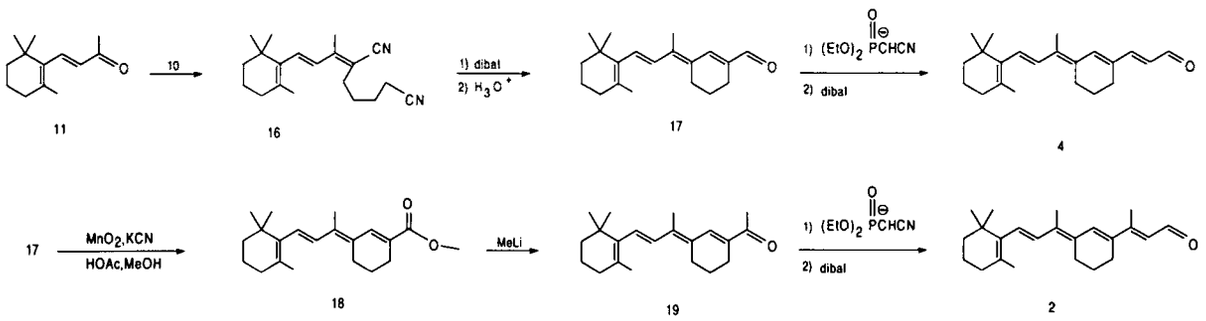


*Fig. 2. Structure and numbering of novel retinals* **1**, **2**, **3**, **4**, **5** *and* **6**.



*Scheme 2. Synthesis of* **1** *and* **3**.



*Scheme 3. Synthesis of* **2** *and* **4**.

in 2-(β-ionylidene)-hexanedinitrile (**12**) and its *Z* isomer (see Scheme 2). The pure all-*E*-isomer was obtained after $SiO_2$ column chromatography. After reduction of the nitrile groups with diisobutylaluminium hybride (dibal) to the aldehyde functions, this system has the required carbon skeleton and functionalities to form the 10,12-ethano bridge. Ring closure was effected by an acid-catalysed internal aldol condensation to give the ethano-bridged conjugated aldehyde **13**. Horner-Emmons condensation of **13** with (diethoxyphosphinyl)acetonitrile and subsequent dibal reduction of the resulting nitrile gave all-*E*-10,12-ethano-20-norretinal (**3**) and its 13-*Z* isomer. The isomerically pure **3** was obtained after $SiO_2$ column chromatography in 22% overall yield based on β-ionone.

For the preparation of all-E-10,12-ethanoretinal (**1**) the aldehyde function of **13** is first converted into the methyl ketone function. This was effected in a two-pot procedure[11,12]. First, the aldehyde is treated with KCN, $MnO_2$ and acetic acid in methanol to form the corresponding methyl ester **14**. This ester is subsequently treated with methyllithium in the presence of excess trimethylsilyl chloride to give methyl ketone **15**. Horner-Emmons reaction of the methyl ketone with (diethoxyphosphinyl) acetonitrile and subsequent dibal reduction of the resulting nitriles gives **1** and its 13-*Z* isomer. The isomerically pure **1** was obtained after $SiO_2$ column chromatography in 9% overall yield based on β-ionone.

A similar reaction scheme starting with the anion of 2-(diethoxyphosphinyl)heptane-dinitrile (**10**) and β-ionone leads to all-*E*-10,12-propanoretinal (**2**) and all-*E*-20-nor-10,12-propanoretinal (**4**) in 6% and 11% overall yield, respectively, based on β-ionone. The reactions are depicted in Scheme 3.

For the preparation of all-*E*-12,14-ethano-20-norretinal (**5**) and all-*E*-20-nor-12,14-propanoretinal (**6**) all-*E*-(β-ionylidene)acetaldehyde (**20**; easily available from β-ionone[13]) serves as the starting material, using synthons **9** and **10** (see Scheme 4). **5** and **6** were prepared in 11% and 7% overall yield, respectively, based on β-ionone.

## Spectroscopic characterization

### *Mass spectrometry*

The high-resolution fast-atom-bombardment mass spectra of **1–6** all show the parent peak at $m/z$ $[M + H]^+$. The experimental values are, within experimental error, fully in agreement with the calculated values for the corresponding molecular formulae, see Table I.

### *$^1$H-NMR spectroscopy*

The 400-MHz $^1$H chemical-shift values of **1**, **2**, **3**, **4**, **5**, **6**, **7** and all-*E*-20-norretinal (**8**) are collated in Table II. The assignments are based on the NOE spectra.

Comparison of the spectrum of all-*E*-10,12-ethanoretinal (**1**) with that of all-*E*-retinal (**7**) clearly shows the presence of the 10,12-ethano bridge in **1**. In the low-field part the signals of the 10-H and 12-H are absent and 11-H appears as a singlet, indicating substitution at 10-C and 12-C. In the high-field part the signals from the methylene groups of the ethano bridge arise as AA'BB' multiplets at $\delta_{10aH}$ 2.69 and $\delta_{12aH}$ 2.78 ppm.

The introduction of the 10a-$CH_2$ group leads to a 0.23-ppm downfield shift of 8-H (γ-effect[14]). The introduction of the 12a-$CH_2$ group does not lead to an observable shift of 14-H. The signals of 7-H and 11-H have shifted 0.10 ppm and 0.05 ppm upfield, respectively, compared to their values in **7**.

Similarly, the spectrum of all-*E*-10,12-propanoretinal (**2**) clearly shows the presence of the 10,12-propano bridge. The signals of the propano bridge are present at $\delta_{10aH}$ 2.34, $\delta_{10bH}$ 1.79 and $\delta_{12aH}$ 2.51 ppm and show that the protons form an AA'BB'CC' spin system.

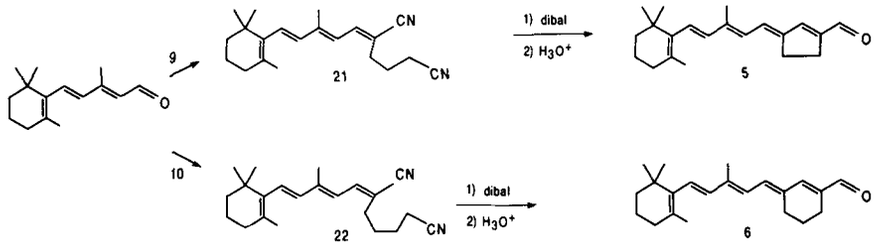
The introduction of the 10,12-propano bridge leads to downfield shifts of 7-H (0.01 ppm), 8-H (0.48 ppm), 14-H (0.17 ppm), 11-H (0.16 ppm) and 15-H (0.06 ppm). The larger downfield shifts of 8-H and 14-H in **2** compared to those in **1** are in agreement with the presence of a propano bridge in **2** compared to an ethano bridge in **1**, as

*Table 1*
*Experimental and calculated mass values for* **1**, **2**, **3**, **4**, **5** *and* **6**.

| | Elementary composition | *m/z* | |
|---|---|---|---|
| | | Calculated | Experimental |
| **1** | $C_{22}H_{30}O$ | 311.2375 | 311.2382 |
| **2** | $C_{23}H_{32}O$ | 325.2531 | 325.2519 |
| **3** | $C_{21}H_{28}O$ | 297.2218 | 297.2231 |
| **4** | $C_{22}H_{30}O$ | 311.2375 | 311.2375 |
| **5** | $C_{21}H_{28}O$ | 297.2218 | 297.2204 |
| **6** | $C_{22}H_{30}O$ | 311.2375 | 311.2368 |

*Table 2*
*400-MHz $^1$H-NMR chemical-shift values ($CDCl_3$, TMS as reference) of* **1**, **2**, **3**, **4**, **5**, **6**, **7** *and* **8**.

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
|---|---|---|---|---|---|---|---|---|
| 2 | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 | 1.49 |
| 3 | 1.63 | 1.63 | 1.63 | 1.63 | 1.63 | 1.63 | 1.63 | 1.63 |
| 4 | 2.04 | 2.04 | 2.04 | 2.04 | 2.04 | 2.04 | 2.04 | 2.04 |
| 7 | 6.24 | 6.35 | 6.26 | 6.39 | 6.30 | 6.34 | 6.34 | 6.38 |
| 8 | 6.39 | 6.64 | 6.39 | 6.65 | 6.20 | 6.21 | 6.16 | 6.17 |
| 10 | – | – | – | – | 6.13 | 6.36 | 6.19 | 6.18 |
| 10a | 2.69 | 2.34 | 2.69 | 2.31 | – | – | – | – |
| 10b | – | 1.79 | – | 1.80 | – | – | – | – |
| 11 | 7.09 | 7.30 | 6.96 | 7.07 | 6.76 | 6.71 | 7.14 | 7.07 |
| 12 | – | – | – | – | – | – | 6.37 | 6.46 |
| 12a | 2.78 | 2.51 | 2.80 | 2.53 | 2.74 | 2.35 | – | – |
| 12b | – | – | – | – | – | 1.76 | – | – |
| 13 | – | – | 7.40 | 7.22 | 7.07 | 6.98 | – | 7.21 |
| 14 | 5.97 | 6.14 | 6.09 | 6.16 | – | – | 5.97 | 6.15 |
| 14a | – | – | – | – | 2.74 | 2.55 | – | – |
| 15 | 10.13 | 10.17 | 9.61 | 9.59 | 9.81 | 9.47 | 10.11 | 9.57 |
| 16,17 | 1.05 | 1.05 | 1.05 | 1.05 | 1.04 | 1.04 | 1.04 | 1.04 |
| 18 | 1.76 | 1.75 | 1.76 | 1.75 | 1.73 | 1.73 | 1.72 | 1.72 |
| 19 | 2.02 | 2.05 | 2.00 | 2.05 | 1.99 | 2.03 | 2.03 | 2.03 |
| 20 | 2.40 | 2.40 | – | – | – | – | 2.33 | – |




*Scheme 4. Synthesis of* **5** *and* **6**.

the propano group has a larger steric requirement than the ethano bridge. Also the 0.21-ppm downfield shift of 11-H in **2** compared to that in **1** is related to the steric effect of the propano group, because the 9- and 13-methyl group are pushed closer to 11-H in **2** than in **1**, leading to a larger $\gamma$-effect.

Comparison of the spectrum of all-*E*-10,12-ethano-20-norretinal (**3**) with that of all-*E*-20-norretinal[15] (**8**) clearly shows that **3** is the all-*E*-20-norretinal containing the 10,12-ethano bridge. In the low-field part the signals of the 10-H and 12-H are absent and 11-H appears as a singlet ($\delta$ 6.96 ppm), indicating substitution at 10-C and 12-C. In the high-field part the signals of the methylene groups of the ethano bridge arise as AA'BB' multiplets at $\delta_{10aH}$ 2.69 and $\delta_{12aH}$ 2.80 ppm. These values are almost identical to those of the ethano bridge in 1.

The introduction of the 10a-$CH_2$ group leads to a 0.22-ppm downfield shift of 8-H and the introduction of the 12a-$CH_2$ group leads to a 0.06 ppm downfield shift of 14-H ($\gamma$-effect). The signals of 7-H and 11-H have shifted upfield (0.12 and 0.11 ppm, respectively) and 13-H and 15-H have shifted downfield (0.19 and 0.05 ppm, respectively). These values are similar to those found for the introduction of the 10,12-ethano bridge in **1**.

Comparison of the spectrum of all-*E*-20-nor-10,12-propanoretinal (**4**) with that of **8** shows the presence of the 10,12-propano bridge in **4**. The signals of the propano bridge are present at $\delta_{10aH}$ 2.31, $\delta_{10bH}$ 1.80 and $\delta_{12aH}$ 2.53 ppm (AA'BBj7CC' spin system). These values are almost identical to those of the 10,12-propano bridge in **2**.

The introduction of the 10,12-propano bridge leads to downfield shifts of 7-H (0.01 ppm), 8-H (0.48 ppm), 14-H (0.01 ppm) and 15-H (0.02 ppm). For 11-H no shift is observed. The downfield shifts of 7-H and 8-H are identical to those in **2** (compared to 7). The difference in downfield shifts observed for 11-H, 14-H and 15-H between **4** (as compared to **8**) and **2** (as compared to **7**) is related to the steric effect of the 13-methyl group, because the presence of the 13-H in **4** gives less steric repulsion at that side of the molecule than the presence of the 13-methyl group in **2**.

Comparison of the spectrum of all-*E*-12,14-ethano-20-norretinal (**5**) with that of **8** reveals the presence of the 12,14-ethano bridge in **5**. The signals of 12-H and 14-H are absent, 11-H appears as a doublet and 13-H and 15-H both appear as a singlet, indicating substitution at 12-C and 14-C. The signals of the methylene groups of the ethano bridge arise as an AA'BB' multiplet at $\delta$ 2.74. This value is very close to those of the ethano bridge in **1** and **3**.

The introduction of the 12,14-ethano bridge leads to an upfield shift of 10-H (0.05 ppm), 11-H (0.31 ppm) and 13-H (0.14 ppm) and 15-H has shifted 0.25 ppm downfield.

Comparison of the spectrum of all-*E*-20-nor-12,14-propanoretinal (**6**) with that of **8** shows the presence of the 12,14-propano bridge. The signals of the propano bridge appear at $\delta_{12aH}$ 2.35, $\delta_{12bH}$ 1.76 and $\delta_{14aH}$ 2.55 ppm (AA'BB'CC' spin system). These values are very close to those of the propano bridge in **2** and **4**.

The introduction of the 12,14-propano bridge leads to an upfield shift of 10-H (0.18 ppm), 11-H (0.36 ppm), 13-H (0.23 ppm) and 15-H (0.09 ppm).

*UV / Vis spectroscopy*

Each of the electronic absorption spectra of the novel retinals **1–6** in ethanol shows a broad bell-shaped absorbance curve without vibrational fine-structure. The $\lambda_{max}$ in the absorbance of all-*E*-10,12-ethanoretinal (**1**) is at 411 nm. This is a 28-nm bathochromic shift compared to the parent all-*E*-retinal (**7**; 383 nm). The $\lambda_{max}$ of all-*E*-10,12-propanoretinal (**2**) is at 395 nm, 12 nm bathochromically shifted compared to **7**. The factors that contribute to these shifts are the presence of the electron-donating alkyl substituents at 10-C and 12-C as well as differences in conjugation as a result of the changes in conformation. The fact that the introduction of the ethano bridge (in **1**) causes a 16 nm larger shift than the introduction of the propano bridge (in **2**) reflects the fact that the five-membered ring is more planar than the six-membered ring, which apparently leads to a better overall conjugation in the polyene with the five-membered ring.

The $\lambda_{max}$ values of all-*E*-10,12-ethano-20-norretinal (**3**;411 nm) and all-*E*-20-nor-10,12-propanoretinal (**4**; 395 nm) are identical to those of their 13-methyl analogues **1** and **2**. This shows that the substitution of the 13-hydrogen for the 13-methyl group does not influence the $\lambda_{max}$. The substituent effect associated with this replacement is counterbalanced by a better conjugation, indicating a more planar conformation of the tail end of the molecule.

The $\lambda_{max}$ values of all-*E*-12,14-ethano-20-norretinal (**5**; 381 nm) and all-*E*-20-nor-12,14-propanoretinal (**6**; 374 nm) are close to that of all-*E*-20-norretinal (380 nm).

The $\lambda_{max}$ values are collated in Table III.

*Table 3*
*$\lambda_{max}$ values of* **1**, **2**, **3**, **4**, **5**, **6**, **7** *and* **8**, *their PSB, their corresponding bacteriorhodopsin and the opsin shift.*

| | $\lambda_{max}$ | PSB | bR | Opsin shift |
|---|---|---|---|---|
| **1** | 411 | 480 | 624 | 4800 |
| **2** | 395 | 465 | – | – |
| **3** | 411 | 475 | 608 | 4600 |
| **4** | 395 | 466 | – | – |
| **5** | 381 | 464 | – | – |
| **6** | 374 | 460 | – | – |
| **7** | 383 | 440 | 568 | 5100 |
| **8** | 380 | 440 | 565 | 5030 |

**Bacteriorhodopsin analogues**

Bacterioopsin (bO) reacts with a slight excess of **1** at room temperature to form, within seconds, the bR analogue bR-**1** with a $\lambda_{max}$ at 624 nm ($\epsilon_{max}$ $63 \cdot 10^3$). After 30 minutes the conversion is complete. bR-**1** shows light-dark adaptation. Upon standing in the dark for a few days the $\lambda_{max}$ value shifts to 608 nm. It completely reverts to the light-adapted form ($\lambda_{max}$ 624 nm) upon exposure to visible light.

A slight excess of retinal analogue **3** reacts at room temperature with bO to form bR-**3** with a $\lambda_{max}$ at 608 nm. The conversion is complete after two weeks. Light-dark adaptation of bR(**3**) was not observed.

When bR-**1** and bR-**3** are treated with an excess of all-*E*-retinal (**7**), no increase in absorbance at 568 nm is detected, indicating that in both cases the regeneration is complete and that the pigment analogue is stable towards displacement by retinal. This means that in both bR analogues, the retinal analogue occupies the binding pocket.

From the $\lambda_{max}$ values of light-adapted bR-**1** and the *n*-butylamine protonated Schiff base of **1**, the opsin shift of bR-**1** is calculated to amount to 4800 $cm^{-1}$ (see Table III). The opsin shift of bR – **3** similarly amounts to 4600 $cm^{-1}$. The values obtained for **7** and **8** in this study are in agreement with those previously published[16].

Bacterioopsin was also incubated with a slight excess of each of the retinal analogues **2**, **4**, **5** and **6**. After standing at room temperature for two weeks, no change in the

UV/Vis absorption spectrum had occurred. After two weeks of incubation with the retinal analogue, to each of the solutions of bO with **2**, **4**, **5** and **6**, a two-fold excess of all-*E*-retinal was added. A pigment with a $\lambda_{max}$ at 568 nm was formed immediately, indicating that the bO was active but did not form a pigment with **2**, **4**, **5** or **6**.

### Light-driven proton-pump activity

We reconstituted bacteriorhodopsin in soybean phospholipid vesicles. Illumination of the 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** and bR – **3**, as well as native bR and bO were reconstituted in liposomes. For natural bR a light-driven proton-pump activity of 211 nmol $H^+$/mg was recorded, with an initial velocity of 530 nmol $H^+$ (mg min)$^{-1}$. For bR-**1** a value of 95 nmol $H^+$/mg was recorded. This amounts to approximately 45% of the value recorded for the proton-pump activity of bR. The initial proton uptake of bR-**1** amounts to 277 nmol $H^+$ (mg–min)$^{-1}$, 52% of the value recorded for natural bR. The liposomes containing bR – **3** and those containing bacterioopsin do not show light-driven proton-pump activity.

### Discussion

We have developed a novel and convenient two-pot four-step annulation sequence. First, a phosphonate anion was prepared from either 1,6-hexanedinitrile (giving **9**) or 1,7-heptanedinitrile (giving **10**) and this was reacted in a subsequent Horner-Emmons reaction with β-ionone or (β-ionylideneacetaldehyde to form a tetrasubstituted alkene. The generation of **9** and subsequent Horner-Emmons coupling proceeded in higher overall yields (80-90%) than the generation and coupling of phosphonate anion **10** (40–50%). The lower yields of **10** are probably due to intramolecular condensation of 10 to form a five-membered ring system.
The coupling with β-ionone, a methyl ketone, gave a product mixture with an about 30% *Z* and 70% *E* configuration around the newly formed double bond. The coupling with (β-ionylidene) acetaldehyde gave a product mixture with a slight excess of the *Z* configuration (about 55%). Neither **9** nor **10** reacted with β-cyclocitral.
Dibal reduction of the resulting dinitriles and subsequent internal aldol condensation gave the annulated aldehyde in a yield of 54–71%. The intermediate dialdehyde could not be isolated due to its instability. The overall yield of the annulation sequence ranges between 23% to 55% in the cases reported here. We assume that many other polyenes with a five- or six-membered ring can be prepared via this method.
All-*E*-10,12-ethanoretinal (**1**) reacts with bO to form bR-**1** with the same order of rate of binding as the native chromophore. This means that the introduction of the 10,12-ethano bridge does not lead to substantial steric hindrance with the protein. BR-**1** has an opsin shift of 4800 cm$^{-1}$, which is only 300 cm$^{-1}$ smaller than that of bR. The light-driven proton-pump activity is 45% of that of native bR and the initial velocity of proton translocation was found to be 52% of the value recorded for native bR. The reduction in light-driven proton-pump action may be due to differences in the electronic structure of the polyene chain caused by the introduction of the five-membered ring or to steric hindrance during the photocycle.
In contrast to the quick binding of **1**, incubation of bacterioopsin with all-*E*-10,12-propanoretinal (**2**) does not yield a pigment. This shows that bO is capable of making a sharp discrimination between chromophores with only a small difference in size.
The novel all-*E*-12,14-ethano-20-norretinal (**3**) reacts with bO to form bR – **3** very sluggishly. Compared to the rapid binding of **1**, the interaction of **3** shows that bO is capable of making a sharp discrimination between chromophores that differ in the presence or absence of the 13-methyl group and that the 13-methyl group fulfills an important steric requirement for binding of the chromophore. This is in agreement with an earlier report showing that a similar difference in binding rate is found for all-*E*-retinal (**7**) *versus* the very sluggishly binding 13-demethyl analogue all-*E*-20-norretinal[16] (**8**).
bR – **3** has an opsin shift of 4600 cm$^{-1}$, which is 430 cm$^{-1}$ smaller than that of 20-nor-bR. bR – **3** does not show any light-driven proton-pump activity. This must be due to the absence of the 13-methyl group and shows that the 13-methyl group also fulfills an important requirement (steric and/or electronic) for proton translocation. This is in agreement with the fact that 20-nor-bR also does not show light-driven proton-pump action[16].
The retinal analogue that combines the presence of the 10,12-propano group and the absence of the 13-methyl group, all-*E*-20-nor-10,12-propanoretinal (**4**), also does not yield a bR analogue.
Neither of the retinal analogues all-*E*-12,14-ethano-20-norretinal (**5**) and all-*E*-20-nor-12,14-propanoretinal (**6**) form a bR analogue. Interestingly, all-*E*-12,14-ethanoretinal has been reported to yield a bR analogue after two weeks of incubation[17].
This study clearly shows that the presence of the 13-methyl group is of utmost importance and that bO is capable of recognizing the absence of this important methyl group.

### Experimental details

*General*

The following solvents and reagents were distilled prior to use: trimethylsilyl chloride (from $CaH_2$), tetrahydrofuran (THF; from $LiAlH_4$), diethyl ether (from $P_2O_5$), petroleum ether (bp. 40–60°C; from $P_2O_5$), diisopropylamine (from $CaH_2$). Reactions were carried out in a nitrogen atmosphere. Diisobutylaluminium hydride (dibal) was used as an 1.0M solution in hexanes, n-butyllithium (BuLi) as an 1.6M solution in hexanes, methyllithium (MeLi) as an 1.6M solution in diethyl ether. These solutions were added via a syringe into the reaction mixture. Chemicals were bought from Janssen Chimica (Belgium) or Aldrich (MA, USA). (β-Ionylidene) acetaldehyde (20) was prepared according to Dugger and Heathcock[13].
NMR spectra were run in $CDCl_3$ [with tetramethylsilane (δ 0 ppm) as internal standard] at a Jeol FX-200 or a Bruker MSL-400 (operating at 199.5 MHz and 400.1 MHz, respectively, for $^1H$ and 50.1 MHz and 100.4 MHz, respectively, for $^{13}C$). For signal designations the IUPAC retinoid numbering system is used[18]. UV/Vis spectra were run on a Varian DMS-200, using ethanol as a solvent. Fast-Atom-Bombardment mass spectrometry (FABMS) was carried out using a V.G. Micromass ZAB-HFQ mass spectrometer, coupled to a V.G. 11/250 data system. The samples were loaded in a glycerol/2-mercepto-1,3-propanediol solution onto a stainless steel probe and bombarded with xenon atoms with an energy of 8 keV. During the high-resolution FABMS measurements a resolving power of 10000 (10% valley definition) was used. Cesium iodide and/or glycerol was used to calibrate the mass spectrometer.
Evaporation of solvents is performed *in vacuo* (at 20 mmHg). Purification is performed by flash column $SiO_2$ chromatography, using ether/petroleum ether as eluent. Reactions were followed by TLC analysis.

*2-[1-Methyl-3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenylidene]-hexanedinitrile (**12**)*

To a stirred solution of 1.58 g (15.6 mmol) of diisopropylamine in 50 ml of THF at −20°C, 15.6 mmol of BuLi were added and the mixture was stirred for 20 min. After cooling to −80°C, 0.84 g (7.8 mmol) of 1,6-hexanedinitrile in 10 ml of THF was added dropwise. After stirring for 30 min, 1.35 g (7.8 mmol) of diethyl chlorophosphate in 15 ml of THF were added at −80°C at such a rate that the addition took 1 h. The mixture was stirred for 1 1/2 during which the temperature rose to −45°C. HMPA [c] (1 ml) was added and after 10 min 1.0 g (5.2 mmol) of 11 in 10 ml of THF was added dropwise. Then the mixture was allowed to warm to room temperature and was stirred for 3 h. Saturated $NH_4Cl$ solution (40 ml) was added and the aqueous layer was extracted with ether three times. The combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the product was purified, yielding 0.83 g of **12** and 0.30 g of *E,E*-**12**. $^1H$ NMR [d] (200 MHz): $\delta$ 6.62 ppm, $\delta$, $J$ 15.9 Hz (8-H); 6.41, $\delta$, $J$ 15.9 Hz (7-H); 2.52, t, $J$ 7.2 Hz ($CH_2$); 2.41, t, $J$ 7.2 Hz ($CH_2$); 2.25, s (9-$CH_3$); 2.05–1.88, m (2 $CH_2$); 1.73, s (5-$CH_3$); 1.63–1.50, m (3-$CH_2$); 1.49–1.46, m (2-$CH_2$); 1.03, s (2 1-$CH_3$).

*E,E-3-[1-Methyl-3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenylidene]-1-cyclopentenecarboxaldehyde (**13**)*

12 (0.90 g, 3.2 mmol) was dissolved in 400 ml of petroleum ether and cooled to −70°C Dibal (10 mmol) was added. The mixture was stirred for 1 h and warmed to −15°C. After complete disappearance of starting material (according TLC), the mixture was cooled to −70°C and a slurry of 20 g of $SiO_2$, 5 ml of water and 1 ml of glacial acetic acid in 200 ml of ether was added. This mixture was stirred for 7 days. The solids were filtered off over celite, and rinsed with ether. After evaporation of the solvents, the residue was purified. Yield 0.61 g (71%) of **13**. $^1H$ NMR (200 MHz): $\delta$ 9.85 ppm, s (13-H); 7.40, s (11-H); 6.37, s (7-H+8-H); 2.78, m ($CH_2$); 2.72, m ($CH_2$); 2.05, s (9-$CH_3$); 2.01, m (4-$CH_2$); 1.75, s (5-$CH_3$); 1.63–1.54, m (3-$CH_2$); 1.49–1.46, m (2-$CH_2$); 1.05, s (2 1-$CH_3$).

*All-E-10,12-ethano-20-norretinal (**3**)*

0.19 g (1.1 mmol) of (diethoxy phosphinyl) acetonitrile was dissolved in 35 ml of THF and 1.0 mmol of BuLi was added dropwise at 0°C. The mixture was stirred for 30 min. Then 0.20 g (0.74) mmol of **13** in 10 ml of THF was added dropwise and stirring was continued for 2 h at room temperature. Saturated $NH_4Cl$ solution was added and the layers were separated. The aqueous layer was extracted three times with ether and the combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the residue was purified yielding 0.14 g (65%) of all-E nitrile. $^1H$ NMR (200 MHz): $\delta$ 7.10 ppm, d, $J$ 16.4 Hz (7-H); 6.90, s (11-H); 6.63, d, $J$ 15.9 Hz (13-H); 6.36, d, $J$ 15.9 Hz (8-H); 5.23, d, $J$ 15.9 Hz (14-H); 2.50, m ($CH_2$); 2.20, m ($CH_2$); 2.01, s (9-$CH_3$); 2.04, m (4-$CH_2$); 1.74, s (5-$CH_3$); 1.63–1.54, m (3-$CH_2$); 1.49–1.46, m (2-$CH_2$); 1.04, s (2×1-$CH_3$). $^{13}C$ NMR (50 MHz): 152.6 ppm; 138.1; 135.8; 135.5; 134.6; 131.5; 131.0; 130.0; 119.2; 92.1; 39.5; 34.1; 33.0; 28.9; 25.7; 23.7; 21.8; 21.8; 19.1; 13.4. To 0.14 g (0.48 mmol) of the thus procured nitrile in 20 ml of petroleum ether 0.7 mmol of dibal was added at −70°C. After stirring for 1 h, a slurry of 1.1 g of $SiO_2$ and 0.3 ml of water was added and the mixture was stirred for 1 h at 0°C. Then $MgSO_4$ was added and the solids were filtered off. The solvent was evaporated and the residue was purified, yielding 0.12 g (85%) of **3**. The electronic absorption spectrum, the high-resolution mass spectrum and the 400-MHz $^1H$-NMR spectrum are described in the section spectroscopic characterization. $^{13}C$-NMR 100-MHz, (in $CDCl_3$): $\delta$ 193.5 ppm, 147.5, 145.7, 144.4, 141.1, 138.2, 132.8, 130.2, 129.6, 128.7, 128.5, 39.6, 34.2, 33.2, 29.5, 29.0, 27.7, 21.8, 19.2, 14.9.

*E,E-1-[3-[1-Methyl-3-(2.6.6-trimethyl-1-cyclohexenyl)-2-propenylidene]-1-cyclopentenyl]-ethanone (**15**)*

**13** (0.40 g, 1.5 mmol) was added to a mixture of 0.83 g (12.7 mmol) of KCN, 4.23 g (48.6 mmol) of $MnO_2$ and 0.25 g (4.23 mmol) of acetic acid in 50 ml of methanol and the mixture was stirred at room temperature for 12 h. The solids were filtered off over $SiO_2$ and Celite and the solvent was evaporated. The residue was partitioned between ether and water. The organic layer was washed (brine) and dried ($MgSO_4$) and the solvent was evaporated to give 0.38 g (87%) of all-*E* ester **14** which was pure enough to be used in the next step. $^1H$ NMR (200 MHz): $\delta$ 7.39 ppm, s (11-H); 6.35, d, $J$ 16.1 Hz (7-H); 6.23, d, $J$ 16.1 Hz (8-H); 3.76, s ($OCH_3$); 2.75, s (2-$CH_2$); 2.03, m (4-$CH_2$); 1.98, s (9-$CH_3$); 1.74, s (5-$CH_3$); 1.64, m (3-$CH_2$); 1.45, m (2-$CH_2$); 1.0, s (2 1-$CH_3$). $^{13}C$ NMR (50 MHz): $\delta$ 166.0 ppm, 143.2, 141.0, 138.3, 137.9, 132.6, 130.0, 129.7, 128.5, 51.2, 39.5, 34.0; 33.0, 30.2, 28.9, 27.7, 21.7, 19.1, 14.7. 0.38 g (1.3 mmol) of ester was dissolved in 50 ml of THF. The solution was stirred and cooled to −100°C and 0.8 ml of $Me_3SiCl$ was added. Then 3.3 mmol of MeLi were added, keeping the temperature at −100°C. After stirring for 10 min a slurry of 7 g of $SiO_2$ and 2 g of water was added at −90°C. The suspension was allowed to warm to 0°C and stirred for 1 h. Then the solids were filtered off and the organic layer was added to a saturated $NH_4Cl$ solution. The layers were separated and the water layer was extracted twice with ether. The combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the residue was purified, yielding 0.19 g (52%) of ketone **15**. $^1H$ NMR (200 MHz): $\delta$ 7.32 ppm, s (11-H); 6.39, d, J 15.9 Hz (7-H); 6.28, d, J 15.9 Hz (8-H); 2.72, s (2 $CH_2$); 2.37, s (19-$CH_3$); 2.03, m (4-$CH_2$); 2.01, s (19-$CH_3$); 1.75, s (5-$CH_3$); 1.64, m (3-$CH_2$); 1.45, m (2-$CH_2$); 1.05, s (2 1-$CH_3$). $^{13}C$ NMR (50 MHz): $\delta$ 196.0 ppm, 147.3, 144.0, 140.8, 137.7, 132.5, 131.2, 129.9, 128.9, 39.4, 33.9, 32.9, 29.2, 29.0, 28.8, 27.3, 26.3, 21.6, 19.0, 14.7.

*All-E-10,12-ethanoretinal (**1**)*

(Diethoxyphosphinylacetonitrile (0.17 g, 0.96 mmol) in 40 ml of THF and 0.9 mmol BuLi was added at 0°C. The mixture was stirred for 30 min. 0.18 g (0.63) mmol of **15** in 10 ml of THF was added dropwise and stirring was continued for 2 h at room temperature. Saturated $NH_4Cl$ solution was added and the layers were separated. The aqueous layer was extracted three times with ether and the combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the residue was purified yielding 0.12 g (62%) of all-E nitrile. $^1H$ NMR (200 MHz): $\delta$ 6.40 ppm, s (11-H); 6.40, d, $J$ 15.9 Hz (7-H); 6.22, d, $J$ 15.9 Hz (8-H); 5.14, s (14-H); 2.80, m ($CH_2$); 2.64, m ($CH_2$); 2.41, s (20-CH3); 2.04, m (4-$CH_2$); 2.02, s (9-$CH_3$); 1.75, s (5-$CH_3$); 1.64, m (3-$CH_2$); 1.45, m (2-$CH_2$); 1.05, s (2 1-$CH_3$). To 0.12 g (0.39 mmol) of nitrile in 20 ml of petroleum ether 0.7 mmol of dibal was added at −70°C. After stirring for 1 h, a slurry of 1.2 g of $SiO_2$ and 0.3 ml of water was added and the mixture was stirred for 1 h at 0°C. Then $MgSO_4$ was added and the solids were filtered off. The solvent was evaporated and the residue was purified, yielding 0.10 g (82%) of 1. The electronic absorption spectrum, the high-resolution mass spectrum and the 400-MHz $^1H$-NMR spectrum are descibed in the section spectroscopic characterization. $^{13}C$-NMR 100 MHz, (in $CDCl_3$): $\delta$ 191.4 ppm, 151.6, 148.8, 144.6, 138.1, 135.7, 132.9, 130.0, 128.6, 128.1, 125.8, 39.6, 34.1, 33.2, 30.5, 29.0, 27.6, 21.8, 19.1, 14.8, 14.4.

*2-[1-Methyl-3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenylidene] heptanedinitrile (**16**)*

To a stirred solution of 3.16 g (31.2 mmol) of diisopropylamine in 100 ml of THF at −20°C, 31.2 mmol of BuLi were added and the mixture was stirred for 20 min. After cooling to −80°C, 1.89 g (15.5 mmol) of heptanedinitrile in 15 ml of THF were added dropwise. After stirring for 25 min, 2.69 g (15.5 mmol) of diethyl chlorophosphate in 15 ml of THF were added at −80°C at such a rate that the addition took 1 h. Then the mixture was stirred for 1½ h, during which the temperature rose to −45°C. Two ml of HMPA were added and after 10 min 2.0 g (10.4 mmol) of 11 in 10 ml of THF were added dropwise. Then the mixture was allowed to warm to room temperature and was stirred for 15 h. 100 ml of a saturated $NH_4Cl$ solution were added and the aqueous layer was extracted with ether three times. The combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the product was purified, yielding 0.71 g of *E,E*-**16** and 0.30 g of 0′*Z*, 2′*E*-**16**. $^1H$ NMR (200 MHz): $\delta$ 6.58 ppm, d, J 15.9 Hz (8-H); 6.36, d, J 15.9 Hz (7-H); 2.40–2.35, m (2 $CH_2$); 2.23, s (9-$CH_3$); 2.05-1.99, m (4-$CH_2$); 1.72, s (5-$CH_3$); 1.74–1.50, m (3-$CH_2$ + 2 $CH_2$); 1.49–1.46, m (2-$CH_2$); 1.03, s (2 1-$CH_3$).

*E,E-3-[1-Methyl-3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenylidene]-1-cyclohexenecarboxaldehyde (**17**)*

0.50 g (1.7 mmol) of **16** was dissolved in 200 ml of petroleum ether and cooled to −70°C. Then 5.4 mmol of dibal were added. The mixture was stirred for 1 h and warmed to −15°C. After complete

[c] Hexamelthyl phosphonic triamide
[d] IUPAC retinoid numbering [18].

disappearance of starting material (according to TLC), the mixture was cooled to −70°C and a slurry of 12 g of $SiO_2$, 3 ml of water and 0.6 ml of glacial acetic acid in 100 ml of ether was added. The mixture was stirred for 7 days. The solids were filtered off over celite, and rinsed with ether. After evaporation of the solvents, the residue was purified. Yield 0.35 g (71%) of 17. $^1$H NMR (200 MHz): δ 9.52 ppm, s (13-H); 7.47, s (11-H); 6.66, d, *J* 16.0 Hz (8-H); 6.48, d, *J* 16.0 Hz (7-H); 2.57, m ($CH_2$); 2.34, m ($CH_2$); 2.11, s ( )-$CH_3$); 2.05, m (4-$CH_2$); 1.76, s (5-$CH_3$); 1.74–1.51, m (2 $CH_2$); 1.49–1.46, m (2-$CH_2$); 1.04, s (2 1-$CH_3$). $^{13}$C NMR (50 MHz): δ 193.5 ppm; 144.9; 139.3; 138.1; 137.4; 131.8; 131.5; 130.9; 130.7; 39.5; 34.2; 33.1; 29.0; 26.5; 21.8; 21.7; 19.1; 13.8.

*All-E-20-nor-10.12-propanoretinal* (**4**)

To 0.32 g (1.8 mmol) of (diethoxyphosphinyl)acetonitrile in 40 ml of THF 1.7 mmol of BuLi were added at 0°C. The mixture was stirred for 10 min. Then 0.35 g (1.2) mmol of **17** in 10 ml of THF was added dropwise and stirring was continued for 2 h at room temperature. Saturated $NH_4Cl$ solution was added and the layers were separated. The aqueous layer was extracted three times with ether and the combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the residue was purified yielding 0.27 g (73%) of all-*E* nitrile. $^1$H NMR (200 MHz): δ 7.27 ppm, d, *J* 16.4 Hz (13-H); 6.81, s (11-H); 6.37, d, *J* 15.9 Hz (7-H); 6.24, d, *J* 15.9 Hz (8-H); 5.20, d, *J* 16.4 Hz (14-H); 2.76, m ($CH_2$); 2.60, m ($CH_2$); 2.02, s (4-$CH_2$); 1.98, m (9-$CH_3$); 1.91, m ($CH_2$); 1.75, s (5-$CH_3$); 1.63, m (3-$CH_2$); 1.46, m (2-$CH_2$); 1.04, s (2 1-$CH_3$). $^{13}$C NMR (50 MHz): 145.7 ppm; 144.3; 143.7; 139.5; 138.0; 132.7; 130.0; 129.4; 128.5; 118.8; 94.8; 39.6; 34.1; 33.1; 28.9; 28.9; 27.5; 21.8; 19.1; 14.7. To 0.27 g (0.88 mmol) of the nitrile in 50 ml of petroleum ether 1.5 mmol of Dibal were added at −70°C. After stirring for 1 h, a slurry of 3.0 g of $SiO_2$ and 0.75 ml of water was added and the mixture was stirred for 1 h at 0°C. Then $MgSO_4$ was added and the solids were filtered off. The solvent was evaporated and the residue was purified, yielding 0.25 g (92%) of **4**. The electronic absorption spectrum, the high-resolution mass spectrum and the 400-MHz $^1$H-NMR spectrum are descibed in the section Spectroscopic characterization. $^{13}$C-NMR 100 MHz, (in $CDCl_3$): δ 193.4 ppm, 155.2, 138.2, 137.2, 135.7, 134.6, 131.6, 130.1, 126.0, 39.4, 34.0, 33.0, 28.8, 25.7, 24.5, 21.9, 21.7, 19.0, 13.4.

*E,E-1-[3-[1-Methyl-3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenylidene]-1-cyclohexenyl]ethanone* (**19**)

**17** (0.50 g, 1.76 mmol) was added to a mixture of 0.98 g (15 mmol) of KCN, 5.02 g (57.7 mmol) of $MnO_2$ and 0.30 g (5 mmol) of glacial acetic acid in 120 ml of methanol and the mixture was stirred at room temperature for 12 h. The solids were filtered off over $SiO_2$ and Celite and the solvent was evaporated. The residue was partitioned between ether and water. The organic layer was washed (brine) and dried ($MgSO_4$) and the solvent was evaporated to give 0.50 g (90%) of ester **18** which was pure enough to be used in the next step. $^1$H NMR (200 MHz): δ 7.78 ppm, s (11-H); 6.62, d, *J* 15.9 Hz (7-H); 6.38, d, *J* 15.9 Hz (8-H); 3.78, s ($OCH_3$); 2.48, m ($CH_2$); 2.40, m ($CH_2$); 2.04, m (4-$CH_2$); 2.00, s (9-$CH_3$); 1.75, m ($CH_2$); 1.74, s (5-$CH_3$); 1.62, m (3-$CH_2$); 1.46, m (2-$CH_2$); 1.04, s (2 1-$CH_3$). 0.48 (1.5 mmol) of ester 18 were dissolved in 100 ml of THF. The solution was stirred and cooled to −100°C and 1.0 ml of TMSCl was added. Then 3.3 mmol of MeLi was added, keeping the temperature at −100°C. After stirring for 10 min a slurry of 7 g of $SiO_2$ and 2 g of water was added at −90°C. The suspension was allowed to warm to 0°C and stirred for 1 h. Then the solids were filtered off and the organic layer was added to a saturated $NH_4Cl$ solution. The layers were separated and the water layer was extracted twice with ether. The combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the residue was purified, yielding 0.27 g (60%) of **19**. $^1$H NMR (200 MHz); δ 7.66 ppm, s (11-H); 6.62, d, *J* 15.9 Hz (7-H); 6.42, d, *J* 15.9 Hz (8-H); 2.50, s (2x $CH_2$); 2.38, s (20-$CH_3$); 2.36, m ($CH_2$); 2.04, m (4-$CH_2$); 2.01, s (19-$CH_3$); 1.75, s (5-$CH_3$); 1.64, m (3-$CH_2$); 1.45, m (2-$CH_2$); 1.05, s (2x 1-$CH_3$).

*All-E-10,12-propanoretinal* (**2**)

To 0.15 g (0.88 mmol) of (diethoxyphosphono)acetonitrile in 40 ml of THF 0.8 mmol of BuLi was added at 0°C. The mixture was stirred for 10 min. Then 0.18 g (0.59) mmol of **19** in 10 ml of THF was added dropwise and stirring was continued for 2 h at room temperature. Saturated $NH_4Cl$ solution was added and the layers were separated. The aqueous layer was extracted three times with ether and the combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the residue was purified yielding 0.14 g (75%) of all-E nitrile. $^1$H NMR (200 MHz): δ 7.11 ppm, s (11-H); 6.64, d, *J* 15.9 Hz (7-H); 6.37, d, *J* 15.9 Hz (8-H); 5.28, s (14-H); 2.48, m ($CH_2$); 2.30, s (20-CH3); 2.04, m (4-$CH_2$); 2.02, s (9-$CH_3$); 1.77, m ($CH_2$); 1.74, s (5-$CH_3$); 1.64, m (3-$CH_2$); 1.45, m (2-$CH_2$); 1.05, s (2x 1-$CH_3$). To 0.14 g (0.44 mmol) of nitrile in 30 ml of petroleum ether 0.7 mmol of dibal were added at −70°C. After stirring for 1 h, a slurry of 1.4 g of $SiO_2$ and 0.35 ml of water was added and the mixture was stirred for 1 h at 0°C. Then $MgSO_4$ was added and the solids were filtered off. The solvent was evaporated and the residue was purified, yielding 0.125 g (89%) of retinal **2**. The electronic absorption spectrum, the high-resolution mass spectrum and the 400-MHz $^1$H-NMR spectrum are descibed in the section spectroscopic characterization. $^{13}$C-NMR 100 MHz, (in $CDCl_3$): δ 191.6 ppm, 156.3, 138.2, 137.8, 133.5, 131.9, 130.0, 130.0, 129.9, 129.4, 124.7, 39.5, 34.1, 33.0, 28.9, 25.9, 25.7, 22.5, 21.8, 19.1, 13.7, 13.4.

*2-[3-Methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-2,4-pentadienylidene]hexanedinitrile* (**21**)

To a stirred solution of 0.764 g (7.6 mmol) of diisopropylamine in 40 ml of THF at −20°C, 7.6 mmol of BuLi were added and the mixture was stirred for 20 min. After cooling to −80°C, 0.37 g (3.4 mmol) of hexanedinitrile in 10 ml of THF was added dropwise. After stirring for 30 min, 0.59 g (3.4 mmol) of diethyl chlorophosphate in 15 ml of THF were added at −80°C at such a rate that the addition took 3/4 h. Then the mixture was stirred for 1/2 h, during which the temperature rose to −50°C HMPA (0.5 ml) was added and after 10 min 0.50 g (2.3 mmol) of 20 in 10 ml of THF was added dropwise. The mixture was allowed to warm to room temperature and was stirred for 12 h. Saturated $NH_4Cl$ solution (40 ml) was added and the aequous layer was extracted three times with ether. The combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the product was purified, yielding 0.30 g of **21** and 0.36 g of all-*E* 21 (51%). $^1$H NMR (200 MHz): 0′*E*,2′*E*,14′*E*: δ 7.23 ppm, d, *J* 12.3 Hz (10-H or 11-H); 6.48, d, *J* 15.9 Hz (7-H); 6.26, d, *J* 12.3 Hz (10-H or 11-H); 6.20, d, *J* 15.9 Hz (8-H); 2.56–2.38, m (2x $CH_2$); 2.02, s (9-$CH_3$); 2.02-1.92, m (2 $CH_2$); 1.72, s (5-$CH_3$); 1.69–1.52, m (3-$CH_2$); 1.49–1.46, m (2-$CH_2$); 1.04, s (2 1-$CH_3$).

*All-E-12,14-ethano-20-norretinal* (**5**)

**21** (0.25 g, (0.81 mmol) was dissolved in 100 ml of petroleum ether and cooled to −70°C. Then 3.0 mmol of dibal as added. The mixture was stirred for 1 h and warmed to −20°C. After complete disappearance of the starting material (according to TLC), the mixture was cooled to −70°C and a slurry of 6 g of $SiO_2$, 1.5 ml of water and 0.3 ml of glacial acetic acid in 50 ml of ether was added. The mixture was stirred for 3 days. The solids were filtered off over celite, and rinsed with ether. After evaporation of the solvents, the residue was purified. Yield 0.13 g (54%) of **5**. The electronic absorption spectrum, the high-resolution mass spectrum and the 400-MHz $^1$H-NMR spectrum are descibed in the section Spectroscopic characterization. $^{13}$C-NMR (100 MHz, in $CDCl_3$): δ 188.8 ppm, 151.0, 148.9, 147.6, 139.2, 137.6, 137.4, 130.0, 128.5, 127.1, 125.4, 39.5, 34.1, 33.0, 28.8, 27.6, 26.6, 21.7, 19.1, 12.5.

*2-[3-Methyl-5-(2,6,6-trimethyl-1-cyclohexenyl)-2,4-pentadienylidene]heptanedinitrile* (**22**)

To a stirred solution of 1.11 g (11.0 mmol) of diisopropylamine in 40 ml of THF at −20°C, 11.0 mmol of BuLi as added and the mixture was stirred for 20 min. After cooling to −80°C, 0.67 g (5.5 mmol) of heptanedinitrile in 15 ml of THF were added dropwise. After stirring for 30 min, 0.95 g (5.5 mmol) of diethyl chlorophosphate in 15 ml of THF were added at −80°C at such a rate that the addition took $\frac{3}{4}$ h. Then the mixture was stirred for 1 h, during which the temperature rose to −50°C. HMPA (1 ml) was added and after 10 min 0.80 g (3.7 mmol) of 20 in 10 ml of THF was added dropwise. Then the mixture was allowed to warm to room temperature and it was stirred for 12 h. 40 ml of a saturated $NH_4Cl$ solution were added and the aqueous layer was extracted with ether three times. The combined organic layers were washed (brine) and dried ($MgSO_4$). The solvents were evaporated and the product was purified, yielding 0.370 g of 0′Z, 2′E, 16′E 22 and 0.217 g of **22**. $^1$H NMR (200 MHz): all-*E*: δ 7.17 ppm, d, *J* 12.3 Hz (10-H or 11-H); 6.47, d, *J* 15.9 Hz (7-H); 6.18, d, *J* 12.3 Hz (10-H or 11-H); 6.16, d, *J* 15.9 Hz (8-H); 2.50–2.35, m (2 $CH_2$); 2.08–2.00, m ($CH_2$); 2.01, s (9-$CH_3$); 1.72, s (5-$CH_3$); 1.72–1.50, m (3-$CH_2$ + $CH_2$); 1.49–1.46, m (2-$CH_2$); 1.04, s (2 1-$CH_3$).

*All-E-20-nor-12,14-propanoretinal (**6**)*

0.22 g (0.68 mmol) of **22** were dissolved in 100 ml of petroleum ether and cooled to −70°C. Then 2.1 mmol of dibal was added. The mixture was stirred for 1 h and warmed to −20°C. After complete disappearance of the starting material (according to TLC), the mixture was cooled to −70°C and a slurry of 6 g of $SiO_2$, 1.5 ml of water and 0.2 ml of glacial acetic acid in 50 ml of ether was added. The mixture was stirred for 3 days. The solids were filtered off over celite, and rinsed with ether. After evaporation of the solvents, the residue was purified. Yield 0.13 g (62%) of **6**. The electronic absorption spectrum, the high-resolution mass spectrum and the 400-MHz $^1$H-NMR spectrum are descibed in the section Spectroscopic characterization. $^{13}$C-NMR (100 MHz, in $CDCl_3$) $\delta$ 193.1 ppm, 150.0, 140.1, 139.0, 137.6, 137.5, 135.6, 131.9, 130.3, 129.2, 125.3, 39.5, 34.2, 33.0, 28.9, 25.4, 21.8, 21.7, 21.3, 19.1, 12.7.

*Preparation of the Schiff bases and protonated Schiff bases*

The Schiff base was prepared in a cuvette in the UV/Vis photospectrometer by addition of excess of *n*-butylamine to a dilute solution of **1**, **2**, **3**, **4**, **5**, **6**, **7** or **8** in methanol. Addition of a drop of concentrated hydrochloric acid to this methanol solution completely converted the Schiff base into the protonated form.

*Bacterioopsin*

Bacteriorhodopsin was obtained from *Halobacterium Halobium* cultures (strain R1S9) as published before[19,20,21]. Bacteriorhodopsin was bleached to give bacterioopsin as previously published.

*Binding experiments*

Binding experiments were performed as described earlier at room temperature[22]. Regeneration was followed in 2-mm-path-length cuvettes. Light–dark adaptation was performed as described earlier[22].

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

bR (8 mg) was bleached to bO. The solution was pelleted and taken up in 8 ml of millipore water. All-*E*-retinal, **1** and **3** (in 10 $\mu$l ethanol) were each added to 2 ml of the bO solution. After overnight incubation, equal amounts of bR, bR-**1**, bR−**3** and bO were obtained and subsequently precipitated. The pellet was taken up in 3 ml of a solution of 0.15M KCl and 2mM EDTA (pH 7). Two ml of the bR solution were added to 50 mg of soybean phospholipids (from Sigma, MO, USA). The suspension was then sonified using a MSE probe-type ultrasonifier (probe diameter 2 mm, freq. 21 kHz, ampl. 5 $\mu$m) for 15-s periods followed by 45 s of cooling for 1 h[23]. The mixture was kept under nitrogen and cooled in ice during sonication. Using 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 multi-purpose cuvette (25°C) equipped with a stirring device and containing 200 $\mu$l freshly prepared (modified) bR liposomes and 1.8 ml of a solution of 0.15M KCl and 2mM EDTA at pH 7. The pH of the medium was measured continuously using an Ingold glass calomel 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 (20 V). The pH changes upon illumination were calibrated by the addition of 50 nmol oxalic acid.

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