$Mo(CO)_2(MeCN)_2]BF_4$.[8] Attempts to grow single crystals of the complex were unsuccessful because it decomposed in solution in ~8 h. This result suggests that CO is a less stable side-on bridging ligand than CS. Also the fact that complex **1** contains a side-on bonded CS rather than CO indicates that CS has a greater preference for a side-on bridging site than CO. Thus, in all four types of bridging situations (A, B, C, and D), the CS is favored over CO as the bridging ligand.

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**Supplementary Material Available:** Tables of crystal data, positional and thermal parameters, complete bond angles and distances, coordinates (calculated) for hydrogen atoms, least-squares planes, and root-mean-square amplitudes of thermal vibration (16 pages); table of observed and calculated structure factors (26 pages). Ordering information is given on any current masthead page.

## Probes Which Reflect the Distance between the Retinal Chromophore and Membrane Surface in Bacteriorhodopsin (bR). Direction of Retinal 9-Methyl in bR

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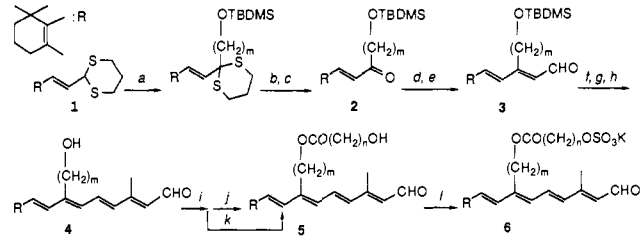
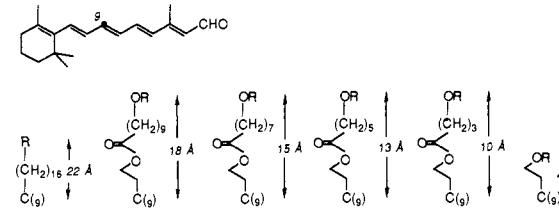
Bacteriorhodopsin (bR),[1] the purple membrane contained in *Halobacterium halobium*, consists of 248 amino acids comprising seven $\alpha$-helices and functions as a light-driven proton pump.[2] Its *all-trans*-retinal chromophore is linked through a protonated Schiff base to the $\epsilon$-amino group of Lys-216.[3] Despite the importance of the bR tertiary structure and location of the chromophore within the binding site in clarifying its mode of action, these are still unsettled problems although several three-dimensional models have been proposed on the basis of the amino acid sequence,[4] diffraction data,[5] susceptibilities of certain regions to proteolysis,[6] spectroscopy,[7] fluorescence energy transfer,[8] neutron diffraction,[9] photoaffinity labeling,[10] and point-mutation techniques.[11]

**Scheme I**



[a] (a) *n*-BuLi/$I(CH_2)_mOTBDMS$/THF, -78 °C, 44%; (b) $HgCl_2$-HgO/95% MeOH reflux, 78%; (c) TBDMSCl/$Et_3N/CH_2Cl_2$, quantitative; (d) $(EtO)_2POCH_2CN$/NaH/THF, 92%; (e) Dibal/$Et_2O$, -78 °C, 64%, flash chromatography; (f) $(EtO)_2POCH_2C(CH_3)$=CHCN/NaH/THF, 73%; (g) Dibal/$Et_2O$, -78 °C, 30%, HPLC (Li-Chrosorb, 30% EtOAc in hexane); (h) *n*-$Bu_4NF$/THF, 97%; (i) TBDMS-*O*-$(CH_2)_nCOOH$/DCC/DMAP/$CH_2Cl_2$, quantitative; (j) *n*-$Bu_4NF$/THF when $n = 9$; (k) $C_5H_5N$ + *n*-$Bu_4NF$/THF, when $n = 3$ or 5 or 7, HPLC as in *h*; (l) $SO_3{\cdot}C_5H_5N/C_5H_5N$, aqueous KCl, combined yields for steps *i*, *j*, *k* and *l* are ca. 35%.

**Chart I.** Absorption Maxima of Pigment Analogues Formed from Retinal Analogues Containing Indicated Chains at C-9[a]



| | | | | | |
|---|---|---|---|---|---|
| R = -H | 7 450 | 8 452 | 9 452 | 10 452 | 11 452 | 12 560 |
| R = -$SO_3K$ | 13 475 | 14 475 | 15 475 | 16 NO | 17 NO | 18 NO |

[a] Approximate C-9 side-chain distances are shown in italics. Pigments were reconstituted from bleached bR-opsin and retinal analogues in 10 mM HEPES buffer, pH 7.0, 25 °C, dark.

In the following we show that 9-substituted retinal analogues with terminal sulfate groups behave as probes reflecting the distance between the chromophore and the membrane surface. Namely, retinal sulfates with sufficiently long spacers bind to give functional pigments, while those with short chains do not bind, presumably because the electrostatic affinity between the sulfate anions and positive charges on the membrane surface prevent the retinal to reach the binding site. Hydroxyalkyl ketone **2**, prepared from dithiane **1**[12] was converted into 9-hydroxyalkylretinal **4** through Emmons reaction, dibal reduction, deprotection, and chromatographic separation of isomers. Retinal **4** was converted into **6** by esterification, deprotection, and sulfonation[13] (Scheme I[14]).

Incubation in HEPES buffer, pH 7.0, in the dark, of bleached bR-opsin[15] with retinals **7**-**12** having terminal hydroxyl groups, resulted in smooth formation of pigments (Chart I). Retinal sulfates **13** and **14** also immediately formed pigments,[16] while

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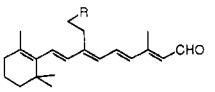
Table I. Absorbance Maxima of Pigments and $H^+$ Pumping Ability



| | R | pigment[a] ($\lambda_{max}$ nm) | $H^+$ pump[b] (%) |
|---|---|---|---|
| **12** | -OH | 560 | 40 |
| **19** | -OCOH | 452 | *c* |
| **20** | $-OCOCH_3$ | 452 | |
| **21** | $-OCOCHN_2$ | 452 | 12 |
| **22** | -OCO-Pr | 452 | 12 |
| **23** | $-OCO(CH_2)_8CH{=}CH_2$ | 452 | 10 |
| **24** | adamantanecarbonyl-O- | 452 | 7 |
| **25** | retinoyl-O- | 450 | |
| **26** | $-(CH_2)_{13}CH_3$ | 452 | |
| **8** | $-OCO(CH_2)_9OH$ | 452 | 7 |
| **14** | $-OCO(CH_2)_9OSO_3K$ | 475 | 12 |

[a] Pigment with bleached bR-opsin, 10 mM HEPES buffer pH 7.0, room temperature, dark. [b] $H^+$ pumping ability of pigments, relative to *all-trans*-retinal (100%), generated with JW2N cell vesicles, irradiation >435 nm; >530 nm for pigment **12**. [c] Not tested.

sulfate **15** yielded a pigment only after 6 h; in contrast, retinal sulfates **16–18** did not reconstitute pigments after 16–24 h, thus suggesting that the chain length in retinal sulfate **15** is the shortest necessary for the chromophore to reach the binding site.[17] This result, together with the fact that the direction of proton translocation in these vesicles is normal (pH decreases upon irradiation, Table I, below) shows that the *substituents at C-9, including 9-methyl in native bR, face the extracellular side of the membrane*. Since the Stokes or hydrodynamic radius of the sulfate group is around 2.30 Å[18] and the C–C bond length in an alkyl chain (zigzag) is 1.25 Å,[19] the distance between C-9 of *all-trans*-retinal and the sulfate oxygen, or *the depth of C-9 from the extracellular side of the membrane surface, can then be estimated to be ca. 15 Å*.

The pigment from hydroxyethyl analogue **12** absorbs at 560 nm and is similar to native bR, while other pigments reconstituted from **7–11**/**19–26** (Table I), *all* absorb around 450 nm, including $C_{16}$–OH analogue **7**, hydroxyethyl formate **19** and -O-CO-adamantyl analogue **24**. This trend indicates that interaction between the C-9 hydroxyethyl (**12**) and surrounding bR $\alpha$-helices is similar to that of native bR, while substituents extending beyond ca. 4 Å, independent of the nature of the neutral group beyond the ester bond, lead to similar perturbations in the interactions between the chromophore and binding site.[20] Retinal sulfates **13–15** gave 23-nm red-shifted pigments compared to corresponding alcohols **7–9**, presumably because electrostatic interaction of the sulfate group with the membrane surface gives rise to chromophoric dislocation.

Proton pumping abilities of pigments reconstituted from retinal analogues **8**, **12**, **14**, **21**, **22**, **23**, and **24** with white membrane cell vesicles were measured (Table I). All 9-alkylretinal pigments tested showed pH decreases resulting from proton extrusion, which were completely blocked by the uncoupler nigericin (data not shown); however, the efficiency of proton translocation was lower than the *all-trans*-retinal pigment. The fact that functional bR analogues can be reconstituted efficiently from retinal analogues with long C-9 alkyl chains indicate that such analogues can be used for affinity labeling studies of bR and related photoreceptors; the latter aspect is under investigation.[21,22]

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(16) When pigments **7–11**, **13**, and **14** were incubated with *all-trans*-retinal in the dark, 25 °C, pH 7.0, the 9-alkyl chromophore was replaced; after >24 h the $\lambda_{max}$ was shifted to 562 nm. All pigments were not stable when treated with 0.1 M $NH_2OH$ and 450-nm irradiation; $t_{1/2}$ 5–10 min.

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(22) Experimental and other details of this communication will be submitted shortly for publication.

## DNA Cleavage by a Metal Chelating Tricationic Porphyrin

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The demonstrated chemotherapeutic effects of porphyrins[1] have stimulated recent investigations into the nature of porphyrin-cell interactions. That *meso*-tetra(4-*N*-methylpyridyl)porphine (TMPyP) intercalates the base pairs of DNA selectively at G-C rich regions has been well-established.[2] $^1H$ and $^{31}P$ NMR experiments with oligonucleotides have indicated that the preferred sequence for intercalation of TMPyP is CpG.[2h] The axially ligated metalloderivatives of TMPyP {Fe(III), Mn(III), and Co(III)} have also been determined to bind to DNA; however, these metalloporphyrins were observed to bind A-T rich regions in the minor groove.[2d,e] In the presence of high intensity visible light and oxygen, various water-soluble porphyrins, including TMPyP, were found to induce single-strand scissions in DNA.[3] DNA cleavage was also observed with the metalloderivatives of TMPyP {Fe(III), Mn(III), and Co(III)}, but these porphyrins generally required reduced forms of oxygen for activity.[4,5] The broad spectrum reactivity of related iron hemes has been utilized in the design of functional bleomycin models[6] and heme–oligonucleotide adducts which are capable of cleaving complementary single-stranded DNA.[7]

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