Experimental and Theoretical Analysis of the Steric Tolerance of the Binding Site of Bacterioopsin with the Use of Side-Chain Methyl-Shifted Retinal Analogs[⊥]

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Abstract: Four positional isomers of *trans*-retinal (**1**) differing in the location of the side-chain methyl groups have been prepared by a combination of Wittig and highly stereocontrolled Suzuki coupling reactions. The incubation of 9-demethyl-10-methylretinal (**5**) with bacterioopsin yielded an artificial pigment with an *opsin shift* of 4630 cm^{-1}. The other three analogs, namely 13-demethyl-14-methylretinal (**3**), 13-demethyl-12-methylretinal (**4**), and 9-demethyl-8-methylretinal (**6**) did not bind to the apoprotein. In order to rationally address the intrinsic structural differences among analogs which could be relevant to the discrimination exhibited by the protein binding site, *ab initio* calculations with complete optimization at the 3-21G level were performed on model *N*-methylretinal iminium salts derived from aldehydes **1** and **3**−**6**. The validity of the approach was inferred from the remarkable coincidence between the minimized structure of *N*-methylretinal Schiff base (**PSB-1**) and the structural parameters displayed by *N*-methyl-*N*-phenylretinal iminium perchlorate (**38b**). Computations clearly show that the location of the methyl groups on the polyene side chain is of the utmost importance in determining the overall shape of the retinal ligands. Those structural effects, added to the dominant steric and electronic restrictions of the binding pocket, would explain the observed discrimination among the analogs **3**−**6**, with minor structural changes, and perhaps among other retinals reported in the literature. Additionally, the theoretical and experimental results obtained with 9-demethyl-8-methylretinal (**6**) provide further indirect evidence of the importance of the 6-*s*-*trans* conformation for the native chromophore in bacteriorhodopsin.

Introduction

The use of retinal derivatives to study the binding site of retinal proteins has been a valuable tool in the provision of extensive data about the retinal−protein interactions.[1] This bioorganic approach represents a complementary method to functional data emanating from site-directed mutagenesis studies[2] as well as structural data obtained mainly through electron cryomicroscopy.[3] The ultimate goal of this multidisciplinary approach is to understand the variety of photobiological functions of the retinal proteins, such as visual transduction (rhodopsin), photosynthesis (bacteriorhodopsin BR and halorhodopsin), phototaxis (sensory rhodopsin), and photoisomerization (retinochrome).

The membrane protein bacteriorhodopsin (BR_{568}), present in the outer purple membrane of *Halobacterium salinarium* (formerly *H. halobium*), functions as a light-driven proton pump, converting light energy into a proton gradient which is used by the cell as an energy source to activate ATP synthase.[4] Structurally, it folds into seven transmembrane helices, one of them containing the residue Lys_{216} at which retinal **1** (Figure 1) binds via a protonated Schiff base. Light absorption leads to the intermediate K_{630}, in which the configuration of the retinal chromophore is 13-*cis* **2**. The photochemical event initiates a

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(1) For general monographs, see: (a) *Chemistry and Biology of Synthetic Retinoids*; Dawson, M. I., Okamura, W. H., Eds.; CRC Press: Boca Raton, FL, 1990. (b) *Methods in Enzymology*; Packer, L., Ed.; Academic Press: New York, 1990; Vol. 189, Retinoids Part A. (c) *Methods in Enzymology*; Packer, L., Ed.; Academic Press: New York, 1991; Vol. 190, Retinoids Part B. (d) *The Retinoids*; Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds.; Academic Press: New York, 1984; Vol. 1 and 2. (e) *The Retinoids: Biology, Chemistry and Medicine*, 2nd ed.; Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds.; Raven Press: New York, 1993.

(2) For some leading references pertaining to BR, see: (a) Khorana, H. G.; Braiman, M. S.; Chao, B. H.; Doi, T.; Flitsch, S. L.; Gilles-Gonzales, M. A.; Hackett, N. R.; Karnik, S. S.; Mogi, T.; Nassal, M. M.; Stern, L. J. *Chem. Scr.* **1987**, *27B*, 137. (b) Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1166. (c) Friedman, N.; Druckmann, S.; Lanyi, J.; Needleman, R.; Lewis, A.; Ottolenghi, M.; Sheves, M. *Biochemistry* **1994**, *33*, 1971. (d) Feng, Y.; Menick, D. R.; Katz, B. M.; Beischel, C. J.; Hazard, E. S.; Misra, S.; Ebrey, T. G.; Crouch, R. K. *Biochemistry* **1994**, *33*, 11624. (e) Sonar, S.; Lee, C.-P.; Coleman, M.; Patel, N.; Liu, X.; Marti, T.; Khorana, H. G.; RajBhandary, U. L.; Rothschild, K. J. *Nature Struct. Biol.* **1994**, *1*, 512.

(3) (a) For BR, see: Henderson, R.; Baldwin, J. M.; Ceska, T. A.; Zemlin, F.; Beckmann, E.; Downing, K. H. *J. Mol. Biol.* **1990**, *213*, 899. (b) For rhodopsin, see: Schertler, G. F. X.; Villa, C.; Henderson, R. *Nature* **1993**, *362*, 770.

(4) For reviews, see: (a) Birge, R. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 315. (b) Stoeckenius, W.; Bogomolni, R. A. *Annu. Rev. Biochem.* **1982**, *52*, 587. (c) Ebrey, T. In *Thermodynamics of Membranes, Receptors and Channels*; Jackson, M., Ed.; CRC Press: Boca Raton, FL, 1993; pp 353−387. (d) Rothschild, K. J. *J. Bioenerg. Biomembr.* **1992**, *24*, 147. (e) Oesterhelt, D., Tittor, J.; Bamberg, E. *J. Bioenerg. Biomembr.* **1992**, *24*, 181. (f) Lanyi, J. K. *Biochim. Biophys. Acta* **1993**, *1183*, 241.

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cycle involving *cis/trans* isomerization of the retinal chromophore about the C(13)−C(14) double bond and deprotonation−reprotonation of the Schiff base. The photocycle drives proton translocation from the inside to the outside of the bacterial cell and produces a pH gradient. The stoichiometry of the translocation process (one proton per photocycle) strongly suggests the involvement of the Schiff base proton in the photocycle.[2,4] The central role of the retinal chromophore in the proton translocation process has generated additional interest to discern its native conformation and orientation into the protein binding site. Although information on BR at the molecular level is available through electron cryomicroscopy studies,[3a] only the tertiary structure in the direction parallel to the membrane plane (3.5 Å resolution) has been elucidated. The electron density map reveals that the chromophore is attached to Lys_{216} of helix G. Unfortunately, the low 10 Å resolution perpendicular to the membrane precluded the exact determination of the conformation and orientation adopted by the retinal. As a consequence, the need exists to use other approaches mentioned above, in order to gather additional information, both on the conformation of the retinal and on the steric and electronic requirements of the binding site as well as on the role of the critical amino acids involved in the functioning of the pigment.[1,2]

With regard to the bioorganic approach, a relatively high number of retinal analogs have been synthesized and incorporated at the binding site of bacterioopsin, the apoprotein of BR.[1,2] The wild and the artificial bacteriorhodopsins display absorption maxima with a strong bathochromic shift as compared to model protonated *n*-butylamine Schiff bases. This shift is considered a reflection of the ligand−protein interactions at the binding site. The role of the protein in modulating the absorption properties of the retinal is expressed using the *opsin shift*,[5] quantified as the difference (in cm^{-1}) between the absorption maxima of the model protonated Schiff base and the pigment.

Suitably tailored retinal analogs, containing minor structural modifications, provide the opportunity to extract meaningful information from the incubation results. In this regard, the use of isotopomers of retinal,[6] the series of dihydroretinals,[5] double- and/or single-bond-locked retinals,[1a,7] fluororetinals,[8] and demethylretinals[9] have provided substantial information on the steric and electronic properties of the binding site, including earlier explanations to the *opsin shift*.[5,10] From the model studies with synthetic retinals,[1a] it is currently accepted that the ring portion of the chromophore, which interacts with the "hydrophobic pocket", does not affect the extension of binding. In contrast, the region near the Schiff base displays severe steric limitations.

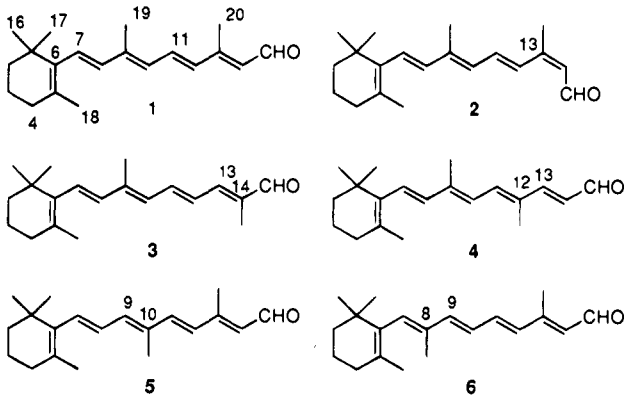


Figure 1. Methyl-shifted retinal analogs **3**−**6**.

It has been suggested that the ring conformation positions the chromophore among the protein α-helices, enabling the functional parts of the retinal to be at the proper distance from the protein residues involved in the proton transfer. Accordingly, the length of the polyene chain and its orientation relative to the plane of the membrane are important factors in the appropriate functioning of the pigment.

In a number of cases reported to date, explanations of the failure to reconstitute an artificial pigment have been based on reasonable assumptions of steric and/or electronic interactions of the retinal analog with protein residues at the binding site. Given the empirical nature of the chromophore modifications for bioorganic studies, we undertook a comprehensive study of retinal side-chain positional isomers **3**−**6**. It was expected that the systematic modification of the retinal side chain, placing the methyl groups at neighboring positions (even-numbered carbons) relative to retinal, would provide a tool to explore the binding pocket dimensions and its steric tolerance in BR. In addition, the study of these series of analogs could help to understand the role of the ligand side-chain methyl groups in the functioning of the pigment. Despite early reports on its failure to bind bacterioopsin,[11] 13-demethyl-14-methylretinal (**3**) was also prepared and incubated with bacterioopsin under the same experimental conditions used for the new analogs **4**−**6**. In order to more precisely locate the effect of the structural changes on the chain conformation, particularly the ring orientation relative to the protonated nitrogen, we also performed *ab initio* calculations of these selected retinal analogs and their model protonated *N*-methylamine retinal Schiff bases.

Results and Discussion

1. Synthesis of Retinal Analogs 3−6. The synthesis of the retinal side chain follows a combination of classical double-bond forming reactions[12] and highly stereoselective transition metal-catalyzed alkenyl-alkenyl couplings, specifically the Suzuki reaction.[13,14]

Synthesis of 13-Demethyl-14-methylretinal (3). Previous syntheses of 13-demethyl-14-methylretinal (**3**) have used Hor-

(5) (a) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945. (b) Derguini, F.; Dunn, D.; Eisenstein, L.; Nakanishi, K.; Odashima, K.; Rao, V. J.; Sastry, L.; Termini, J. *Pure Appl. Chem.* **1986**, *58*, 719. (c) Lugtenburg, J.; Muradin-Szweykowska, M.; Heeremans, C.; Pardoen, J. A.; Harbison, G. S.; Herzfeld, J.; Griffin, R. G.; Smith, S. O.; Mathies, R. A. *J. Am. Chem. Soc.* **1986**, *108*, 3104.

(6) For a review, see: (a) Lugtenburg, J.; Mathies, R. A.; Griffin, R. G.; Herzfeld, J. *Trends Biochem. Sci.* **1988**, *13*, 388. (b) For a recent application of double labeling (protein and retinal), see: Lakshmi, K. V.; Auger, M.; Raap, J.; Lugtenburg, J.; Griffin, R. G.; Herzfeld, J. *J. Am. Chem. Soc.* **1993**, *115*, 8515.

(7) (a) Fang, J.-M.; Carriker, J. D.; Valogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 5162. (b) Kölling, E.; Gärtner, W.; Oesterhelt, D.; Ernst, L. *Angew. Chem., Int. Ed. Engl.* **1985**, *47*, 509. (c) Sheves, M.; Friedman, N.; Albeck, A.; Ottolenghi, M. *Biochemistry* **1985**, *24*, 1260. (d) Albeck, A.; Friedman, N.; Sheves, M.; Ottolenghi, M. *J. Am. Chem. Soc.* **1986**, *108*, 4614. (e) van der Steen, R.; Biesheuvel, P. L.; Mathies, R. A.; Lugtenburg, J. *J. Am. Chem. Soc.* **1986**, *108*, 6410.

(8) (a) Tierno, M. E.; Mead, D.; Asato, A. E.; Liu, R. S. H.; Sekiya, N.; Yoshihara, K.; Chang, C.-W.; Nakanishi, K.; Govindjee, R.; Ebrey, T. G. *Biochemistry* **1990**, *29*, 5948. (b) Steinberg, G.; Sheves, M.; Bressler, S.; Ottolenghi, M. *Biochemistry* **1994**, *33*, 12439.

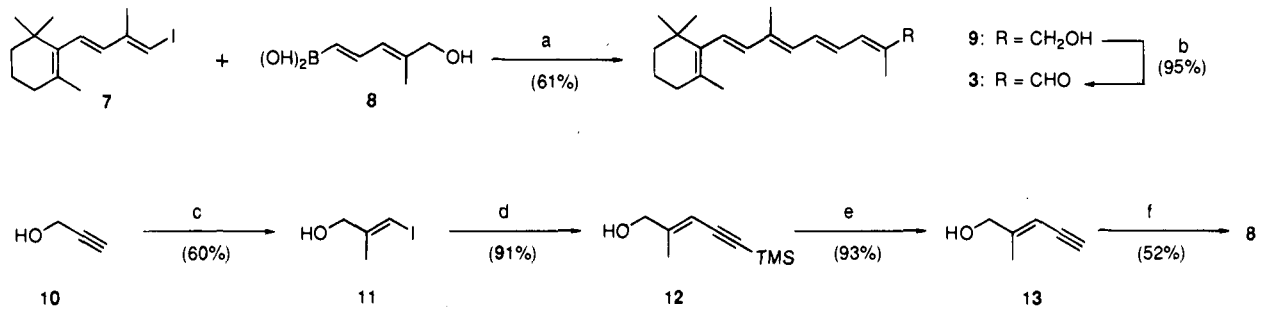
(9) Gärtner, W.; Towner, P.; Hopf, H.; Oesterhelt, D. *Biochemistry* **1983**, *22*, 2637.

(10) A complete account of the *opsin shift* in BR by combination of ring-chain coplanarization and weakening of the Schiff base counterion has recently been published, and it demostrates that the effects are synergistic rather than additive; see: Hu, J.; Griffin, R. G.; Herzfeld, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8880.

(11) (a) Oesterhelt, D.; Christoffel, V. *Biochem. Soc. Trans.* **1976**, *4*, 556. The failure of 13-demethyl-14-methylretinal **3** to give a stable pigment was mentioned here, although no data was reported. (b) Stoeckenius, W.; Lozier, R. H.; Bogomolni, R. A. *Biochim. Biophys. Acta* **1979**, *505*, 215.

(12) (a) Liu, R. S. H.; Asato, A. E. *Tetrahedron* **1984**, *40*, 1931. (b) Dawson, M. I.; Hobbs, P. D. In *The Retinoids: Biology, Chemistry and Medicine*, 2nd ed.; Sporn, M. B., Roberts, A. B., Goodman, D. S., Eds.; Raven Press: New York, 1993; pp 5−178.

Scheme 1[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, 10% aqueous TlOH, THF, room temperature, 15 h; (b) MnO_2, CH_2Cl_2, room temperature, 2 h; (c) (1) Cl_2ZrCp_2, $AlMe_3$, CH_2Cl_2, 0 °C, 12 h, (2) ICN, THF, 0 °C, 1 h; (d) TMS-acetylene, pyrrolidine, CuI, $Pd(PPh_3)_4$, room temperature, 30 min; (e) $(n\text{-}Bu)_4NF$, THF, room temperature, 2 h; (f) (1) catecholborane, (2) H_2O, room temperature, 1 h.

ner–Emmons and Wittig reactions, generally leading to mixtures of isomers.[15] As a quick entry into this analog, we considered a modification of the previously described convergent stereocontrolled approach to vitamin A,[14b] based on the formation of the C(10)–C(11) bond by palladium-catalyzed coupling of alkenyl fragments, namely alkenyl iodide **7**[14b,16] and alkenyl boronic acid **8** (Scheme 1). The previously unknown **8** was derived from propargyl alcohol **10** as depicted on Scheme 1. Thus, as described for the preparation of **7**,[14b] zirconium-catalyzed methylalumination of **10** afforded alkenyl iodide **11** in 60% yield. Sonogashira-type coupling of **11** with trimethylsilylacetylene under the modified conditions described by Linstrumelle[17] (CuI/pyrrolidine, 91% yield), followed by deprotection of the so formed **12** (93% yield), led to enynol **13**. Finally, transformation of **13** into boronic acid **8** was achieved uneventfully using the mild conditions reported for related enynols,[18] that is treatment with doubly distilled catecholborane at room temperature followed by hydrolysis without isolation of the boronate intermediate.

The coupling between **7** and **8** took place at room temperature in the presence of 10% aqueous TlOH solution (Kishi's modification[19] of the Suzuki reaction[13]) affording 13-demethyl-14-methylretinol **9** as a single stereoisomer in 61% yield. Examination of the coupling constants for the vinylic hydrogens allowed the stereochemical assignment of **9** as the anticipated all-(*E*)-isomer. Essentially complete retention of configuration of the coupling partners was observed in this and related Suzuki-type couplings described below, thus showing the potential of this procedure for stereocontrolled polyene synthesis.[14]

The required aldehyde **3** was obtained from **9** in 95% yield by allylic oxidation with MnO_2 in CH_2Cl_2.

Synthesis of 13-Demethyl-12-methylretinal (4). The synthesis of this analog was envisaged as the result of a Wittig condensation of β-ionyltriphenylphosphonium bromide (**19**)[20] with the aldehyde **18**. Functional group manipulations in **14** by the standard sequence of reactions shown on Scheme 2 provided, in 70% overall yield, component **18** of the Wittig condensation. The coupling of phosphonium salt **19** with aldehydes is known[21] to provide mixtures of stereoisomers at the newly-formed trisubstituted double bond. In this case, generation of the phosphorane by treatment of phosphonium salt **19** with *n*-BuLi at −20 °C followed by addition of aldehyde **18** at −78 °C and additional stirring at −40 °C for 7.5 h, provided a 1:1.4 mixture of the (9*Z*)- and (9*E*)-isomers of TBDMS-protected 13-demethyl-12-methylretinol (**20** and **21**, respectively) in 68% yield. After deprotection of the mixture with $(n\text{-}Bu)_4NF$, alcohols **22** and **23** were separated by HPLC, and the latter was treated with MnO_2 to provide the desired 13-demethyl-12-methylretinal (**4**) (Scheme 2).

Synthesis of 9-Demethyl-10-methylretinal (5). A Wittig condensation between phosphonium salt **28** (derived from β-cyclogeraniol)[22] and trienal **27** (Scheme 3) is the key step on the synthetic sequence leading to analog **5**. Component **27** was obtained by MnO_2 oxidation of precursor alcohol **26**, which in turn was prepared stereoselectively by palladium-catalyzed cross-coupling between the known alkenylboronic acid **24**[18] and protected vinyl bromide **25**[23] in 82% yield. Generation of the phosphorane by treatment of phosphonium salt **28** with *n*-BuLi at −30 °C followed by addition of aldehyde **27** provided, in 65% yield, protected 9-demethyl-10-methylretinol (**29**) as a single stereoisomer. Deprotection to **30** and oxidation as described for its positional isomer **3** afforded 9-demethyl-10-methylretinal (**5**) (Scheme 3).

Synthesis of 9-Demethyl-8-methylretinal (6). Disconnection of the retinoid side-chain again at the C(10)–C(11) bond[14] led to alkenylboronic acid **24**[18] and alkenyl iodide **35** (C_{14} + C_6 route[12a]) as starting materials for the preparation of 9-demethyl-8-methylretinol (**36**) (Scheme 4). Anticipated precur-

(13) (a) Suzuki, A. *Acc. Chem. Res.* **1982**, *15*, 178. (b) Suzuki, A. *Pure Appl. Chem.* **1985**, *57*, 1749. (c) Suzuki, A. *Pure Appl. Chem.* **1986**, *58*, 629. (d) Suzuki, A. *Top. Curr. Chem.* **1986**, *130*, 23. (e) Suzuki, A. *Pure Appl. Chem.* **1991**, *63*, 419. (f) Martin, A. R.; Yang, Y. *Acta Chem. Scand.* **1993**, *47*, 221. (g) Suzuki, A. *Pure Appl. Chem.* **1994**, *66*, 213.

(14) (a) de Lera, A. R.; Torrado, A.; Iglesias, B.; López, S. *Tetrahedron Lett.* **1992**, *33*, 6205. (b) Torrado, A.; Iglesias, B.; López, S.; de Lera, A. R. *Tetrahedron* **1995**, *51*, 2435.

(15) (a) Chan, W. K.; Nakanishi, K.; Ebrey, T. G.; Honig, B. *J. Am. Chem. Soc.* **1974**, *96*, 3642. (b) Ebrey, T.; Govindjee, R.; Honig, B.; Pollock, E.; Chan, W.; Crouch, R.; Yudd, A.; Nakanishi, K. *Biochemistry* **1975**, *14*, 3933. (c) Bestmann, H. J.; Ermann, P.; Rüppel, H.; Sperling, W. *Liebigs Ann. Chem.* **1986**, 479. (d) Ito, M.; Katsuta, Y.; Yamano, Y.; Tsukida, K. *J. Chem. Soc. Perkin Trans. 1* **1993**, 987.

(16) Negishi, E.; Owczarczyk, Z. *Tetrahedron Lett.* **1991**, *32*, 6683.

(17) (a) Alami, M.; Ferri, F.; Linstrumelle, G. *Tetrahedron Lett.* **1993**, *34*, 6403. (b) Alami, M.; Crousse, B.; Linstrumelle, G. *Tetrahedron Lett.* **1994**, *35*, 3543.

(18) Roush, W. R.; Brown, B. B. *J. Am. Chem. Soc.* **1993**, *115*, 2268.

(19) (a) Uenishi, J. I.; Beau, J.-M.; Armstrong, R. W.; Kishi, Y. *J. Am. Chem. Soc.* **1987**, *109*, 4756. (b) Armstrong, R. W.; Beau, J.-M.; Cheon, S. H.; Christ, W. J.; Fujioka, H.; Ham, W.-H.; Wawkins, L. D.; Jin, H.; Kang, S. H.; Kishi, Y.; Martinelli, M. J.; McWhorter, W. W., Jr.; Mizuno, M.; Nakata, M.; Stutz, A. E.; Talamas, F. X.; Taniguchi, M.; Tino, J. A.; Ueda, K.; Uenishi, J.; White, J. B.; Yonaga, M. *J. Am. Chem. Soc.* **1989**, *111*, 7525.

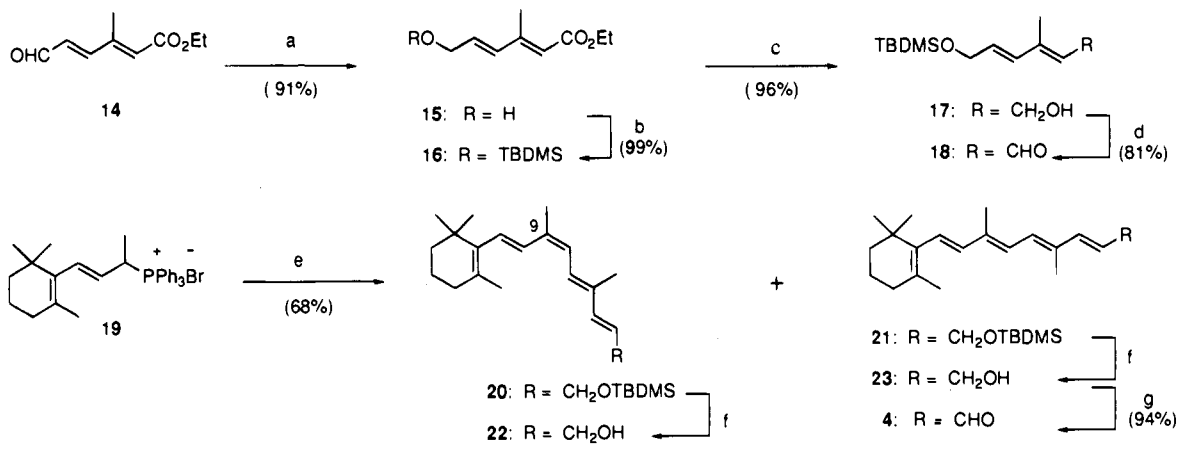
(20) Olivé, J.-L.; Mousseron-Canet, M.; Dornand, J. *Bull. Soc. Chim. Fr.* **1969**, 3247.

(21) See, for example: Sueiras, J.; Okamura, W. H. *J. Am. Chem. Soc.* **1980**, *102*, 6255.

(22) (a) Isler, O.; Gutmann, H.; Lindlar, H.; Montaron, M.; Rüegg, R.; Ryser, G.; Zeller, P. *Helv. Chim. Acta* **1956**, *39*, 463. (b) Broek, A. D.; Muradin-Szweykowska, M.; Courtin, J. M. L.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 46.

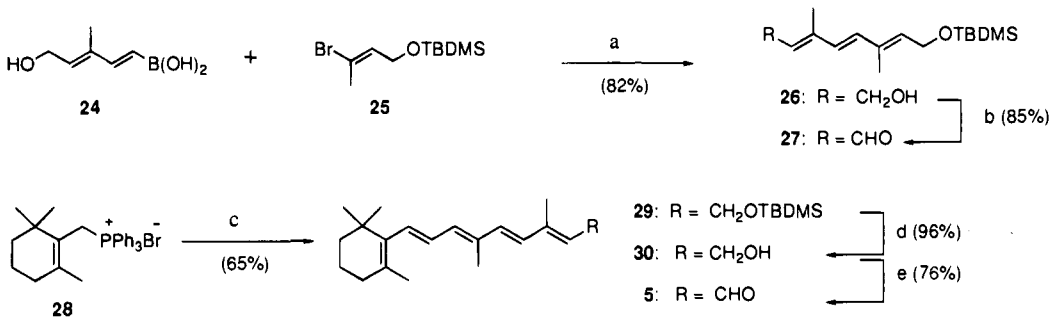
(23) (a) Schlosser, M.; Hammer, E. *Helv. Chim. Acta* **1974**, *57*, 2549. (b) Corey, E. J.; Bock, M. G.; Kozikowski, A. P.; Rama Rao, A. V.; Floyd, D.; Lipshutz, B. *Tetrahedron Lett.* **1978**, 1051.

Scheme 2[a]



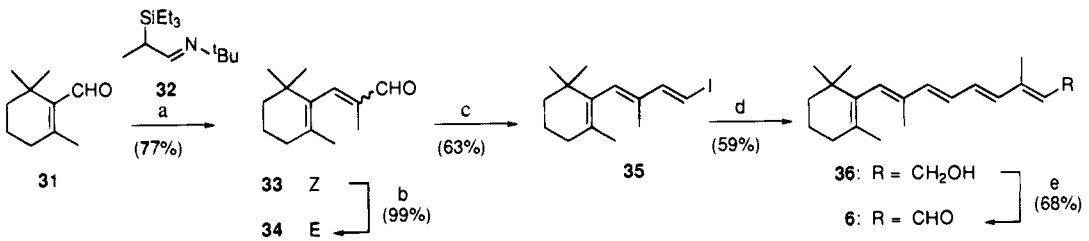
[a] Reagents and conditions: (a) $NaBH_4$, 1:1 EtOH/H_2O, room temperature, 6.5 h; (b) TBDMSCl, Imidazole, DMF, room temperature, 5 h; (c) DIBALH, THF, 0 °C, 1 h; (d) MnO_2, Et_2O, room temperature, 2 h; (e) *n*-BuLi, THF, −20 °C; then −78 °C, addition of **18**, −40 °C, 7.5 h; (f) $(n\text{-Bu})_4NF$, THF, room temperature, 3 h; then HPLC separation of **22** and **23**, 93%; (g) MnO_2, CH_2Cl_2, room temperature, 5 h.

Scheme 3[a]



[a] Reagents and conditions: (a) $Pd(PPh_3)_4$, 10% aqueous TlOH, THF, room temperature, 2 h; (b) MnO_2, Et_2O, room temperature, 6 h; (c) *n*-BuLi, THF, −30 °C → 0 °C, then aldehyde **27**, room temperature, 4 h; (d) $(n\text{-Bu})_4NF$, THF, room temperature, 1.5 h; (e) MnO_2, CH_2Cl_2, room temperature, 3.5 h.

Scheme 4[a]



[a] Reagents and conditions: (a) (1) *s*-BuLi, THF, −78 °C to −20 °C, 5 h, (2) CF_3COOH, THF, −20 °C, 1 h, then H_2O, 0 °C, 8 h; (b) I_2 (cat), hexane, room temperature, 30 min; (c) $CrCl_2$, CHI_3, THF, room temperature, 2 h; (d) $Pd(PPh_3)_4$, boronic acid **24**, 10% aqueous TlOH, THF, room temperature, 1 h; (e) MnO_2, CH_2Cl_2, room temperature, 7 h.

sors of alkenyl iodide **35** were alternatively β-cyclocitral **31** (C_{10} component), a derivative of the trimethylcyclohexene ring (C_9 component) or an open-chain precursor (C_{13} component). The palladium-catalyzed vinylation[24] of the triflate derived from 2,2,6-trimethylcyclohexanone with methacrolein was unsuccessful. The synthesis of the so-called isomethyl- and methylionones from the acid catalyzed cyclization of the condensation product of citral and butanone[25] was, in our hands, of little synthetic utility. We also faced the lack of reactivity of β-cyclocitral **31** with the common Wittig and Horner−Emmons reagents. At last, Peterson olefination of β-cyclocitral **31** with the anion derived from silylimine **32**[26] (*sec*-BuLi, −78 °C) followed by hydrolysis with anhydrous trifluoroacetic acid[27] provided the desired aldehyde, in 77% yield, as a *ca.* 2:1 mixture of the (*Z*)- and (*E*)-isomers **33** and **34**, respectively. The pure (*E*)-aldehyde **34** was obtained by iodine-catalyzed isomerization of the above mixture and stereoselectively converted to (*E*)-iodide **35** by Takai's chromium-mediated iodoolefination[28] ($CrCl_2$, CHI_3, 63% yield). Coupling of boronic acid **24**[18] and alkenyl iodide **35**, following the general procedure described above, provided 9-demethyl-8-methylretinol (**36**) in 59% yield.

(24) (a) Scott, W. J.; Peña, M. R.; Swärd, K.; Stoessel, S. J.; Stille, J. K. *J. Org. Chem.* **1985**, *50*, 2302. (b) Breining, T.; Schmidt, C.; Polos, K. *Synth. Commun.* **1987**, *17*, 85.

(25) (a) Núñez, A.; Rosado, A.; Magraner, J.; Rodríguez, C.; Montejo, L. *Rev. Cienc. Quím.* **1984**, *15*, 197. (b) Núñez, A.; Magraner, J.; Rosado, A.; Vélez, H.; Montejo, L.; Pérez, B. *Rev. Cienc. Quím.* **1984**, *15*, 207. (c) Guo, M.; Xiao, Y.; Xu, G.; Yi, D.; Zeng, Y. *Huaxue Xuebao* **1981**, *39*, 855. (*Chem. Abstr.* **1982**, *97*, 144433).

(26) Campbell, K. N.; Sommers, A. H.; Campbell, B. K. *J. Am. Chem. Soc.* **1944**, *66*, 82.

(27) Schlessinger, R. H.; Poss, M. A.; Richardson, S.; Lin, P. *Tetrahedron Lett.* **1985**, *26*, 2391.

(28) Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *118*, 7408.

Table 1. ^{1}H NMR Chemical Shifts (in $CDCl_3$), Coupling Constants (in parenthesis), and Signal Assignment (Based on NOE Data) for Retinals **1** and **3**–**6**

	C_{1-2CH_3} (C_{16}, C_{17})	C_{5-CH_3} (C_{18})	CH_3[a]	CH_3[b]	H_7	H_8	H_9	H_{10}	H_{11}	H_{12}	H_{13}	H_{14}	H_{15}
1[c]	1.04	1.72	2.03	2.33	6.36 (16.1)	6.18 (16.1)		6.19 (12.1)	7.14 (15.2, 12.1)	6.37 (15.2)		5.98 (8.0)	9.45 (8.0)
3[d]	1.03	1.72	2.01	1.87	6.36 (16.2)	6.17 (16.2)		6.22 (11.9)	7.07 (14.2, 11.9)	6.65 (14.2, 11.7)	6.96 (11.7)		9.45
4	1.04	1.74	2.04	1.96	6.40 (16.3)	6.23 (16.3)		6.38 (12.5)	6.89 (12.5)		7.24 (15.2)	6.19 (15.2, 7.8)	9.59 (7.8)
5	1.06	1.77	1.95	2.32	6.45 (15.0)	6.50 (15.0, 10.3)	6.43 (10.3)		6.84 (15.6)	6.35 (15.6)		6.00 (8.1)	10.12 (8.1)
6	0.96	1.47	1.67	2.30	6.16		6.61 (15.3)	6.34 (15.3, 10.8)	6.85 (15.3, 10.8)	6.38 (15.3)		5.96 (8.2)	10.10 (8.2)

[a] Methyl group at position C(8) for **6** or C(10) for **5**. [b] Methyl group at position C(12) for **4** or C(14) for **3**. [c] Taken from ref 12a. [d] Data coincidental with those reported on ref 19.

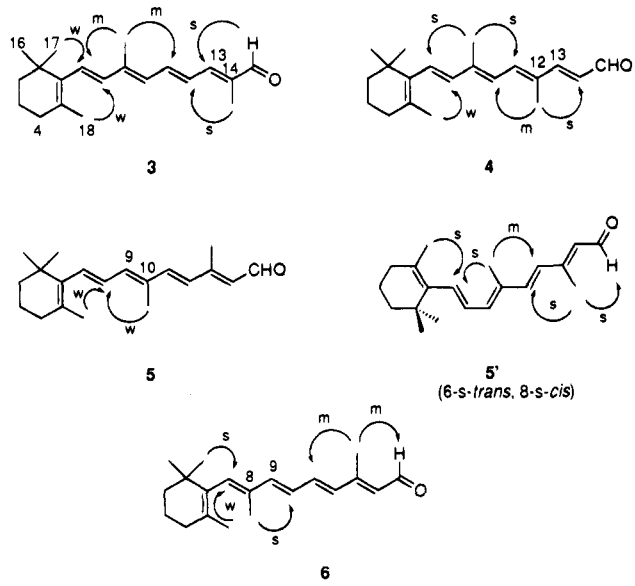


Figure 2. Nuclear Overhauser effects (NOE) observed for the retinal analogs **3**–**6** used in this study. The relative enhancements, represented by s (strong, >7%), m (medium, 3–7%), and w (weak, 1–3%) refer to each individual compound. Conformers shown are those deduced from interpretation of the NOE data. As indicated, compound **5** displayed a predominant 6-*s*-*trans*, 8-*s*-*cis* conformation (**5'**).

Finally, allylic oxidation of precursor **36** provided 9-demethyl-8-methylretinal (**6**) in 68% yield (Scheme 4).

2. Structure Determination. Although ^{1}H NMR chemical shifts and coupling constants for the hydrogens of the retinal polyene chain are well established,[12a] the positional change of the side-chain methyl groups in **3**–**6** required a reexamination of the assignments of the ^{1}H NMR signals and their coupling patterns. This reexamination was also prompted by the lack of stereocontrol in some of the double bond-forming reactions used in our syntheses, which afforded mixtures of products whose relative stereochemistry needed to be confirmed. Accordingly, the signal assignment in the ^{1}H NMR spectra of retinal analogs **3**–**6** (Table 1), particularly that of the methyl groups, relied mainly on NOE experiments.

A summary of the NOE data is collected in Figure 2. For the double bonds of the polyene side chain, the anticipated all-(*E*) configuration was fully supported. In solution the s-*trans* is the preferred conformation of the polyene side-chain for most of the analogs. Earlier work had suggested a conformation closer to 14-*s*-*cis* (mainly based on the shielding of H_{15} in the ^{1}H NMR spectrum, δ = 9.45 ppm) for 13-demethyl-14-methylretinal (**3**). Our NOE experiments support a different rationale; the lack of correlation between C(14)–CH_3 and H_{15} together with the enhancement of H_{13} upon saturation of H_{15} support the 14-*s*-*trans* conformation for analog **3**. With regard to the crucial C(6)–C(7) bond, the *s*-*cis* conformation was generally favored in solution. Evidence of a predominant 6-*s*-*trans*, 8-*s*-*cis* conformation for 9-demethyl-10-methylretinal (depicted as **5'**) in solution was gathered from the enhancement of H_7 upon independent saturation of C(5)–CH_3 and C(10)–CH_3.

In agreement with these solution results, *ab initio* calculations (*vide infra*) in the gas phase favor the extended *s*-*trans* conformation for the bonds of the polyene chain and the skewed *s*-*cis* for the C(6)–C(7) bond.

3. Preparation of the Schiff Bases and Protonated Schiff Bases. Using standard methodology in the field,[29] *n*-butylamine-Schiff bases were prepared by reacting the retinal analog with the amine in ether in the presence of molecular sieves 4Å at room temperature for 30 min. Filtration and solvent evaporation under vacuum provided the desired Schiff base. Their protonation was effected in the UV cell by addition of a drop of 3% HCl in anhydrous MeOH[30] to the solution of the Schiff base in alcoholic solvent. The values of the absorption maxima for the retinals, their Schiff bases, and protonated Schiff bases are shown on Table 2. Compared to the parent retinal **1**, only the 9-demethyl-8-methylretinal (**6**) is blue shifted (360 nm), likely due to the severe steric interaction of C(8)–CH_3 with the cyclohexenyl moiety.

4. Incubation of the Synthetic Retinals (3–6) with Bacterioopsin. Incubation of the synthetic retinals (**3**–**6**) in ethanol with bacterioopsin was done in 0.02 M phosphate buffer, pH 7.0. UV–vis absorption spectra were recorded using bleached purple membrane as the reference.[31]

Only 9-demethyl-10-methylretinal (**5**) afforded a stable pigment absorbing at 540 nm. The *opsin shift* (O.S.) of the artificial pigment is close (4630 cm^{-1}) to that of the native purple membrane (5225 cm^{-1}, Table 2). Consistent with the earlier results,[11] 13-demethyl-14-methylretinal (**3**) failed to yield an artificial pigment. Instead, a major band peaking at 420/440 nm was observed. By comparison with results obtained with the close analog 14-methylretinal (**37**) (structure shown on Figure 6), the proximity of the methyl group to the aldehyde was considered the determining factor preventing the formation of the Schiff base with Lys_{216}.[15a] Our calculations, however (*vide infra*), support an alternative rationale. The other two isomers, namely 13-demethyl-12-methylretinal (**4**) and 9-demethyl-8-methylretinal (**6**), gave rise, after six days, to absorption maxima at 420 and 440 nm,[32] respectively, reminiscent of the behavior described for 13-demethyl-14-methylretinal (**3**).[11]

(29) de Lera, A. R.; Reischl, W.; Okamura, W. H. *J. Am. Chem. Soc.* **1989**, *111*, 4051.
(30) Mori, K.; Takigawa, T.; Matsuo, T. *Tetrahedron* **1979**, *35*, 933.
(31) Oesterhelt, D.; Stoeckenius, W. *Methods Enzymol.* **1974**, *31*, 667.

Table 2. Absorption Maxima (in nm) of Retinals **1** and **3**–**6**, Their *N*-Butyl Schiff Bases (SB), and Protonated Iminium Chlorides (PSB) in Methanol[c]

	λ_{max} (retinal)	λ_{max} (SB)	λ_{max} (PSB)	incubation with bacterioopsin	O.S. (cm^{-1})
1[a]	380	360	438	568	5225
3	378[b]	366	438	420/440	
4	382	368	444	420	
5	380	354	432	540	4630
6	360	346	424	440	

[a] Taken from ref 7d. [b] The value previously reported was 377 nm.[15a,c] [c] The absorption data obtained upon incubation with bacterioopsin and the value of the *opsin shift* (O.S.) are also collected. For reference, the values for the native chromophore and pigment are also shown.

In the currently accepted three-step model for pigment reconstitution,[33] a noncovalent interaction of the protein with retinal takes place first. As a result, an absorption at ~400 nm is immediately observed, which has been attributed to the planarization of the cyclohexene and the polyene side chain. In the second step the pigment shows absorption at 430/460 nm of still undefined nature, the rate-limiting step being the formation of the protonated Schiff base, with a pK_a > 13.[34] The analogy in absorption patterns obtained for retinals **3**, **4**, and **6** points to either incomplete Schiff base formation with Lys_{216} or, alternatively, random Schiff base formation with lysine residues of the protein other than Lys_{216} (*vide infra*).

5. *Ab Initio* Studies. In order to get a better understanding of the factors responsible for the selectivity of binding, we performed *ab initio*[35] calculations[36,37] of retinals **1**, **3**–**6**, and their protonated Schiff bases derived from methylamine (**PSBs**, Figure 3). One of our main objectives was to estimate the intrinsic structural differences among the retinal analogs, which could affect their binding to the apoprotein. Calculations were performed using the Gaussian 92 series of programs,[35] with complete optimization at the 3-21G level, which for truncated models of retinal iminium salts has been reported to provide results comparable to those using larger basis sets.[37e]

At the outset, we knew of the ability of retinoids with all-(*E*) configurations to adopt both the 6-*s*-*cis* and the 6-*s*-*trans* conformations.[38] The extended π-electron conjugation of the s-*trans* conformation counterbalances the steric interaction between H_8 and the C(1)-dimethyl group, which forces the ring out of the polyene plane. For example, *all-trans*-retinoic acid

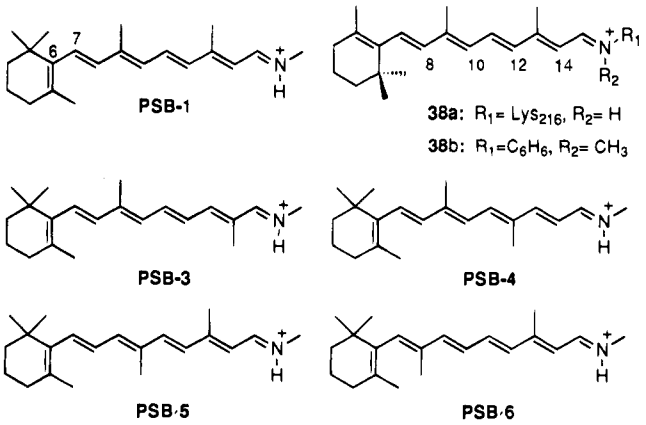


Figure 3. Model *N*-methyl protonated Schiff bases derived from the methyl-shifted retinal analogs used in the *ab initio* study. They are depicted in their most stable 6-*s*-*cis* conformation, compared to the 6-*s*-*trans* conformation exhibited by model *N*-methyl-*N*-phenylretinal iminium perchlorate **38b** and the native system **38a**.

adopts the 6-*s*-*cis* (−35°) in the triclinic modification[38a] and the s-*trans* in the monoclinic form.[38d] However, both in the crystal lattice (−59°)[38b] and in solution[39] *all-trans*-retinal (**1**) shows a preferred 6-*s*-*cis* conformation. With regard to the conformation of the ligands bound to their retinal receptors, there is increasing evidence that *all-trans*-retinal (**1**) in BR is 6-*s*-*trans* (as tentatively assigned from electron cryomicroscopy studies[3]) whereas 11-*cis*-retinal in the visual pigment rhodopsin is 6-*s*-*cis*.[40]

Our *ab initio* calculations indicate that the most stable conformation of *all-trans*-retinal (**1**) and analogs **3**–**6** in the gas phase is invariably the extended s-*trans* for the bonds of the polyene chain and the skewed s-*cis* for the C(6)–C(7) bond joining the cyclohexenyl ring to the side-chain (data not shown). In order to address the conformation of the retinal chromophore in BR, *ab initio* studies at the same (3-21G) computational level were performed on the protonated Schiff bases derived from methylamine.[36,37] Calculations show that the preferred conformation of protonated Schiff bases **PSBs** (Figure 3) is unambiguously the 6-*s*-*cis* (with the ring-chain dihedral angle ranging from −60.7° for **PSB-5** to −70.2° for **PSB-6**) for all the analogs studied. Selected bond angles for the 6-*s*-*cis* conformations of protonated Schiff bases **PSBs** are collected on Table 3. Forcing the systems to adopt the 6-*s*-*trans* conformation (dihedral angle C_5–C_6–C_7–C_8 set to 180°, as depicted on **38a** for the native pigment, Figure 3) a new series of minimized structures were obtained (Table 3). For comparison (*vide infra*), values of selected bond angles measured in the crystal structure of *N*-methyl-*N*-phenylretinal iminium perchlorate (**38b**)[38g] are also included in Table 3.

Table 4 displays the absolute energies corresponding to each conformation, with indication of the torsional angle C_5–C_6–C_7–C_8 for the skewed 6-*s*-*cis* conformers. Of special interest is the computed energies of the 6-*s*-*trans* relative to the most stable 6-*s*-*cis* conformation.

(32) Preliminary experiments showed that addition of *all-trans*-retinal (**1**) to these complexes immediately eliminated the peaks at 420–450 nm and yielded a band at 560 nm, indicating the formation of native BR.

(33) Schweiger, U.; Tittor, J.; Oesterhelt, D. *Biochemistry* **1994**, *33*, 535.

(34) Druckmann, S.; Ottolenghi, M.; Pande, A.; Pande, J.; Callender, R. H. *Biochemistry* **1982**, *21*, 4953.

(35) Gaussian 92. Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1992.

(36) For representative semiempirical calculations on truncated models of retinal, retinal Schiff bases, and retinal iminium salts, see: (a) Orlandi, G.; Schulten, K. *Chem. Phys. Lett.* **1979**, *64*, 370. (b) Tavan, P.; Schulten, K.; Oesterhelt, D. *Biophys. J.* **1985**, *47*, 415. (c) Birge, R. R.; Murray, L. P.; Zidovetzki, R.; Knapp, H. M. *J. Am. Chem. Soc.* **1987**, *109*, 2090. (d) Dormans, G. J. M.; Groenenboom, G. C.; van Dorst, W. C. A.; Buck, H. M. *J. Am. Chem. Soc.* **1988**, *110*, 1406. (e) Gilson, H. S. R.; Honig, B. H. *J. Am. Chem. Soc.* **1988**, *110*, 1943.

(37) For representative *ab initio* calculations on truncated models of retinal, retinal Schiff bases, and retinal iminium salts, see: (a) Hodoscek, M.; Hadzi, D. *Can. J. Chem.* **1985**, *63*, 1528. (b) Sánchez-Marín, J.; Malrieu, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 1985. (c) Poirer, R. A.; Yadav, A. *Chem. Phys. Lett.* **1989**, *156*, 122. (d) Bond, D. *J. Am. Chem. Soc.* **1991**, *113*, 385. (e) Toto, J. L.; Toto, T. T.; de Melo, C. P.; Robins, K. A. *J. Chem. Phys.* **1994**, *101*, 3945.

(38) (a) Stam, C. H.; MacGillavry, C. H. *Acta Crystallogr.* **1963**, *16*, 62. (b) Gilardi, R.; Karle, I. L.; Karle, J.; Sperling, W. *Nature* **1971**, *232*, 187. (c) Hamanaka, T.; Mitsui, T.; Ashida, T.; Kakudo, M. *Acta Crystallogr.* **1972**, *B28*, 214. (d) Stam, C. H. *Acta Crystallogr.* **1972**, *B28*, 2936. (e) Simmons, C. I.; Liu, R. S. H.; Denny, M.; Seff, K. *Acta Crystallogr.* **1981**, *B37*, 2197. (f) Simmons, C. I.; Asato, A. E.; Liu, R. S. H. *Acta Crystallogr.* **1986**, *C342*, 711. (g) Santarsiero, B. D.; James, M. N. G.; Mahendran, M.; Childs, R. F. *J. Am. Chem. Soc.* **1990**, *112*, 9416.

(39) Honig, B.; Hudson, B.; Sykes, B. D.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1289.

(40) For a recent photoaffinity study to determine the orientation of the chromophore in bovine rhodopsin, see: Zhang, H.; Lerro, K. A.; Yamamoto, T.; Lien, T. H.; Sastry, L.; Gawinowicz, M. A.; Nakanishi, K. *J. Am. Chem. Soc.* **1994**, *116*, 10165.

Table 3. Selected Bond Angles (deg) Computed at the 3-21G Level for the Most Stable Skewed 6-*s-cis* and for the 6-*s-trans* Conformers of *N*-Methylretinal Iminium Salt and Analogs (**PSBs**)[a]

	6-*s-cis*					6-*s-trans*				
	PSB-1	**PSB-3**	**PSB-4**	**PSB-5**	**PSB-6**	**38b (exp)**	**PSB-1**	**PSB-3**	**PSB-4**	**PSB-5**
C(5)−C(6)−C(7)	121.91	121.93	121.92	121.89	121.80	118.0	116.78	116.73	116.72	116.86
C(6)−C(7)−C(8)	123.89	123.93	123.90	124.70	127.13	129.7	130.79	130.77	130.75	131.27
C(7)−C(8)−C(9)	125.34	125.28	125.35	121.66	117.67	125.6	124.04	123.99	124.06	120.24
C(8)−C(9)−C(10)	117.99	117.97	117.79	127.97	126.29	117.8	117.87	117.85	117.67	128.08
C(9)−C(10)−C(11)	126.38	126.36	125.73	117.05	121.63	127.7	126.54	126.52	125.91	117.05
C(10)−C(11)−C(12)	123.10	123.80	127.19	125.81	124.15	121.1	123.20	123.90	127.26	125.89
C(11)−C(12)−C(13)	124.91	121.10	116.61	124.49	124.91	127.3	124.93	121.12	116.68	124.53
C(12)−C(13)−C(14)	118.01	127.94	126.49	117.94	118.00	116.2	118.12	128.03	126.54	118.06
C(13)−C(14)−C(15)	125.14	116.12	119.91	125.13	125.10	125.0	125.25	116.24	120.00	125.24
C(14)−C(15)−N	124.66	125.98	125.86	124.64	124.64	124.2	124.78	126.07	125.99	124.76
C(15)−N−C	125.08	124.92	125.09	125.09	125.10		125.02	124.85	125.03	125.03

[a] For comparison, selected experimental data[38g] on *N*-methyl-*N*-phenylretinal iminium perchlorate (**38b**) are also included.

Table 4. Absolute Energies (hartrees) of the Optimized Structures in the *6-s-cis* and *6-s-trans* Conformations[a]

	skewed 6-*s-cis* $C_5-C_6-C_7-C_8$ (deg)			**forced 6-*s-trans*** ($C_5-C_6-C_7-C_8 = 180°$)		
		E (au)	*r* (Å)	*E* (au)	Δ*E* (*trans*−*cis*) (kcal·mol^{-1})	*r* (Å)
PSB-1	−63.2	−863.250 66	0.00	−863.244 72	3.73	0.00
PSB-3	−62.5	−863.251 80	4.40	−863.246 07	3.60	4.46
PSB-4	−62.5	−863.250 24	4.55	−863.244 54	3.58	4.60
PSB-5	−60.7	−863.250 33	2.59	−863.245 18	3.23	2.67
PSB-6	−70.2	−863.251 95	2.10	−863.228 55	14.68	

[a] The energy of the *6-s-trans* relative to their most stable *6-s-cis* conformers are expressed in kcal·mol^{-1}. The torsion angles (deg) defining the skewed most stable C(6)−C(7) *s-cis* conformation, and the distances (Å) between the centroids of the cyclohexenyl rings of **PSBs** and **PSB-1** are also included.

Clearly the structural parameters of the optimized protonated Schiff base **PSB-1** are in excellent agreement with those of *N*-methyl-*N*-phenylretinal iminium perchlorate **38b**.[38g] Bond angles in the optimized structure **PSB-1** (6-*s-trans*) are close to the experimental values in **38b**, the difference not exceeding 2.4°. The calculated bond lengths for **PSB-1** agree well with the experimental values measured in the crystallographic structure of **38b**. The shortening of the C−C single bonds and the lenghtening of the C=C double bonds relative to neutral species (an effect due to charge delocalization from N to the unsaturated chain, which falls off with distance) is also reproduced.[38g] The main deviations from the crystal structure correspond to bond lengths in the proximity of the protonated nitrogen. Calculated distances (Figure 4) are remarkably similar to those observed, with the exception of C(13)−C(14) and C(14)−C(15). The computed distance is *ca.* 0.02 Å larger for C(13)−C(14) and *ca.* 0.02 Å shorter for C(14)−C(15) than the corresponding bond distances in the crystal structure. Although it has been argued[38g] that the phenyl ring in **38b** provides little delocalization to the positive charge (the dihedral angle between the phenyl group and the chain is 46.2°), some differences in bond lengths are expected between model **PSB-1** and structure **38b**. Additionally, packing interactions in the crystal cannot be excluded, which could contribute to the greater curvature (in-plane bending) of the side chain in **38b**. Thus, the calculated geometries of the model conformer **PSB-1** (6-*s-trans*) agree very well with crystallographic structure **38b** (except for the region where both structures differ in chemical composition) in spite of the fact that the calculations simulate gas-phase structures of a related compound. Evidently, many geometric characteristics of the conjugated polyene are intrinsic features, which are moderately affected by the molecular or the physical environment.

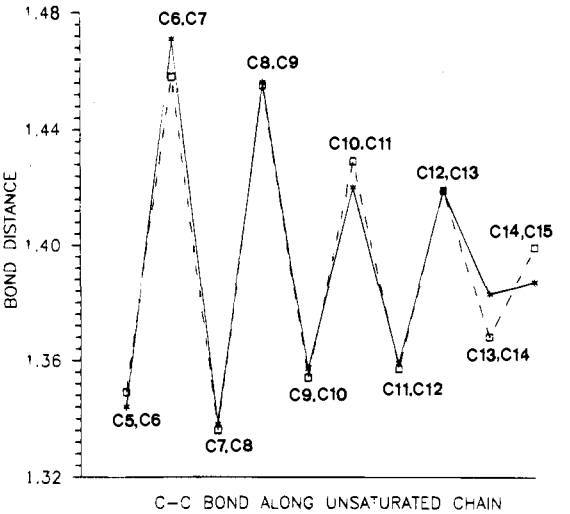


Figure 4. Comparison of C−C bond distances (Å) along the unsaturated chain for the *N*-methyl-*N*-phenylretinal iminium perchlorate (**38b**, dashed line, squares) and model *N*-methylretinal iminium salt (**PSB-1** 6-*s-trans*, solid line, stars). Values are collected in the supporting information.

Applications of molecular graphics analysis allowed the data of Table 3 (the complete data can be found in the supporting information) to be interpreted more completely. When the iminium bonds of the optimized structures were superimposed (Figure 5, top, 6-*s-cis*; bottom, 6-*s-trans*), an interesting trend was revealed: the closer the structural modification to the polar group, the greater the deviation at the cyclohexenyl ring region relative to model **PSB-1**. Side-by-side comparison of the structures superimposed on Figure 5 qualitatively indicates the positional changes of the hydrophobic rings relative to the fixed iminium bond. This effect could be conveniently expressed (Table 4) as the distance (in Å) between the centroids of the cyclohexenyl ring of analog **PSBs** and **PSB-1**. Therefore, retinal analogs with structural modifications near the hydrophobic terminus (**5** and **6**, in our case) bear more resemblance to native *all-trans*-retinal (**1**) than analogs **3** and **4**.

Ab initio calculations show that the positioning of the hydrophobic ring relative to the protonated imine, in any of the C(6)−C(7) conformations available to **PSB-5**, is relatively close to the native chromophore, which should be beneficial to its binding. In line with this reasoning is the observation that analog **5** gives rise to an artificial pigment upon incubation with the apoprotein. However, the experimental finding that 9-demethyl-8-methylretinal (**6**) failed to provide an artificial pigment, despite its similarity to retinal (**1**) in their 6-*s-cis* conformation (Figure 5, top), further reinforces the need of a 6-*s-trans* conformation for the chromophore in BR. Our calculations

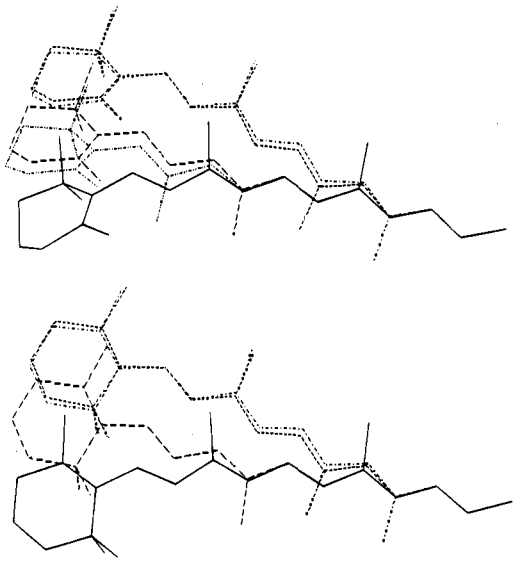


Figure 5. Superimposition of the 6-*s-cis* (top) and 6-*s-trans* (bottom) conformations exhibited by the *N*-methylretinal iminium salts **PSBs** computed at the 3-21G level. For comparative purposes, the protonated Schiff bases (C(15)−N^+−CH_3 fragment) were arbitrarily anchored together. Identification lines are as follows: **PSB-1**, (−); **PSB-3**, (- − - −); **PSB-4**, (- -); **PSB-5**, (− −); **PSB-6**, (- - − - -).

show that the energy difference between the skewed 6-*s-cis* and the 6-*s-trans* conformation is relatively small in all cases (less than 4 kcal·mol^{-1}, Table 4), except in the case of *N*-methyl-9-demethyl-8-methylretinal iminium salt (**PSB-6**), in which it is considerably higher (14.68 kcal·mol^{-1}). The steric interactions of C(8)−CH_3 and the ring-methyl groups in **6**, especially severe with the C(1)-dimethyl, precludes the planarization of the chromophore, either to 6-*s-trans* or 6-*s-cis* conformation. Similar conclusions have been reached using other bioorganic approaches.[7e,41] The importance of the 6-*s-trans* conformation in the functioning of the native protein is now firmly established, mainly through spectroscopic studies using solid-state NMR.[42,43]

For the protonated Schiff bases derived from 13-demethyl-14-methylretinal (**PSB-3**) and 13-demethyl-12-methylretinal (**PSB-4**), examination of the computed location of the cyclohexenyl ring relative to the protonated nitrogen in Figure 5 (6-*s-trans*) shows considerable deviations (see Table 4) from its native position in **PSB-1**. The effect is mainly due to the absence of the C(13)-methyl group which plays a determinant role on the orientation of the chromophore relative to the anchoring point of the protein. Table 3 show that the bond angles opposite the side-chain methyl groups (regardless of their location along the chain) are compressed by an average of 10° relative to other bond angles at hydrogen-substituted carbons. Moreover, placing the native C(9)- and C(13)-methyl groups at the neighboring even-numbered carbons leads to a net displacement of the side-chain toward the direction of the odd-numbered carbons. The effect falls off with distance of the

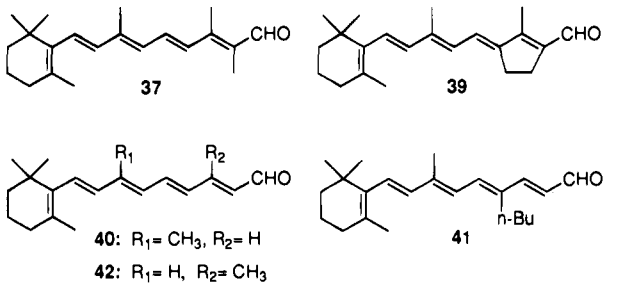


Figure 6. Retinal analogs with additional side-chain modifications pertinent to this study.

structural modification relative to the protonated imine, as observed with analogs **PSB-4** and **PSB-5**. In that regard, the role of the C(13)-methyl group on positioning the chromophore in the binding site of BR must be emphasized. Analogs with additional methyl substituent at C(14), such as 14-methyl retinal (**37**)[15a] and the C(12)−C(13) s-*trans*-locked analog **39**[44] both bind to bacterioopsin, the latter sluggishly (Figure 6). Besides determining the orientation of the hydrophobic terminus relative to the Schiff base, the C(13)-methyl group of the retinal ligand has previously been suggested to play a critical steric and/or electronic role in the binding ability of the analogs.[44b] On the other hand, 13-demethylretinal (**40**) is known to isomerize to a 15% all-*trans*/85% 13-*cis* mixture upon interaction with the protein, the latter isomer being considered largerly responsible for the slow formation of the pigment.[45] Another example of a retinal analog lacking the C(13)-methyl group is the more sterically-demanding 13-demethyl-12-butylretinal (**41**) which, congruently with our findings, has also been reported to be inactive.[46]

The theoretical model, however, must be interpreted with caution. It explains differences in gas-phase conformations[47] but ignores the role of the binding pocket on the selection of the bound conformation of the ligand. Present knowledge points to in-plane curvature and possibly an out-of-the-plane twist[43c] of the polyene in BR. Additionally, the presence of s-*cis* conformers near C(9) has been pointed out in order to explain data emerging from photoaffinity labeling studies.[48]

Finally, the parallel behavior of analogs **3**, **4**, and **6**, leading to species with absorption maxima in the range 420−460 nm, deserves additional comments. The planarization of the chromophore, considered the first step in the interaction with the binding pocket, is unlikely for analog **6**, suggesting instead the possibility of nonselective Schiff base formation of the retinal analogs with other ϵ-amino groups of a lysine other than Lys_{216}. Alternatively, the noncovalent binding of retinal to regions of the apoprotein other than the usual binding site has been advanced by others.[2c]

Conclusions

To fully understand the mechanistic details of the function of a retinal protein, it is necessary to establish its detailed three-

(41) (a) van der Steen, R.; Biesheuvel, P. L.; Erkelens, C.; Mathies, R. A.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 83. (b) Iwasa, T.; Ito, M.; Tokunaga, F. *Photochem. Photobiol.* **1992**, *56*, 921.

(42) (a) Harbison, G. S.; Smith, S. O.; Pardoen, J. A.; Courtin, J. M. L.; Lugtenburg, J.; Herzfeld, J.; Mathies, R. A.; Griffin, R. G. *J. Am. Chem. Soc.* **1985**, *107*, 4809. (b) Creuzet, F.; McDermott, A. E.; Gebhard, R.; van der Hoef, K.; Spijker-Assink, M.; Herzfeld, J.; Lugtenburg, J.; Levitt, M. H.; Griffin, R. G. *Science* **1991**, *251*, 783. (c) McDermott, A. E.; Creuzet, F.; Gebhard, R.; van der Hoef, K.; Levitt, M. H.; Herzfeld, J.; Lugtenburg, J.; Griffin, R. G. *Biochemistry* **1994**, *33*, 6129.

(43) (a) Ulrich, A. S.; Heyn, M. P.; Watts, A. *Biochemistry* **1992**, *31*, 10390. (b) Copié, V.; McDermott, A. E.; Beshah, K.; Williams, J. C.; Spijker-Assink, M.; Gebhard, R.; Lugtenburg, J.; Herzfeld, J.; Griffin, R. G. *Biochemistry* **1994**, *33*, 3280. (c) Ulrich, A. S.; Watts, A.; Wallat, I.; Heyn, M. P. *Biochemistry* **1994**, *33*, 5370.

(44) (a) Fang, J.-M.; Carriker, J. D.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 5162. (b) Groesbeek, M.; Kirillova, Y. G.; Boeff, R.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 45.

(45) (a) Gärtner, W.; Hopf, H.; Hull, W. E.; Oesterhelt, D.; Scheutzow, D.; Towner, P. *Tetrahedron Lett.* **1980**, *21*, 347. (b) Trissl, H.-W.; Gärtner, W. *Biochemistry* **1987**, *26*, 751.

(46) Crouch, R. K.; Scott, R.; Ghent, S.; Govindjee, R.; Chang, C.-H.; Ebrey, T. *Photochem. Photobiol.* **1986**, *43*, 297.

(47) Similar *ab initio* calculations on protonated Schiff base derived from analog **39**[44a] and methylamine reveals a remarkable coincidence in both C(6)−C(7) conformations with those computed for model system **PSB-1** (see supporting information).

(48) Zhang, H.; Lerro, K. A.; Takekuma, S.; Baek, D.-J.; Moquin-Pattey, C.; Boehm, M.; Nakanishi, K. *J. Am. Chem. Soc.* **1994**, *116*, 6823.

dimensional structure, specially the binding site and any of the associated prosthetic groups. Knowledge of the detailed geometry and orientation of the retinal chromophore and its interaction with the surrounding amino acids is essential for the elucidation of the protein functions. This work was set out to investigate the effect of minor chemical modifications of the retinal chromophore in the binding to bacterioopsin. To this end, side-chain positional isomers of retinal **3**−**6** have been synthesized in highly stereocontrolled fashion and have been fully characterized. Our incubation results, with exclusive binding of 9-demethyl-10-methylretinal (**5**), show that the apoprotein is capable of discriminating between retinals **3**−**6** with minor structural changes. The location of the methyl group in the conjugated chain is the discriminating factor for binding to bacterioopsin. The experimental findings were rationally addressed by performing *ab initio* calculations on model protonated *N*-methylretinal Schiff bases (**PSBs**). The calculations further highlight that the side-chain methyl groups are of the utmost importance on the adequate orientation of the retinal in the binding pocket of the apoprotein. The compression of the bond angles opposite the methyl groups is largely responsible for the in-plane bending observed in experimental (**38b**) and theoretical (**PSB-1**) protonated retinal Schiff bases models. In the absence of the methyl groups at the native position, the hydrophobic terminus "drifts" toward the direction of the odd-numbered carbons, effect that falls off with distance to the protonated nitrogen. Although the "relay" effect could be qualitatively recognized, its quantification was somehow surprising. The absence of the C_{20}-methyl group is thus considered responsible for the failure of analogs **3** and **4** to bind the apoprotein. The role of the C_{20}-methyl group in **1** on the formation of pigments has also been emphasized by others.[44b] The C_{19}-methyl group in **1** has a less determinant role and can be either deleted (9-demethylretinal **42**, Figure 6, provides an artificial pigment[9]) or changed to the C(10) position as in **5**. The failure of 9-demethyl-8-methylretinal (**6**) to afford an artificial pigment, despite its similarity to **1** in the 6-*s*-*cis* conformation, must in turn be adscribed to the skewed conformation of the cromophore at the cyclohexenyl ring, preventing the C(6)−C(7) bond to attain the *s*-*trans* conformation present in the native system. Additionally, it provides indirect evidence of the 6-*s*-*trans* conformation for native retinal (**1**) in BR. It is concluded that the retinylidene chromophore has a limited space available within the binding pocket of the surrounding protein. In that regard, the efficient and regioselective *trans*−*cis* isomerization directed by the protein is a remarkable process.

An additional advantage of our approach is that the steric mapping of the retinal chromophore helps define the positions of the side-chain which, upon structural modifications, are more likely to yield artificial pigments for further studies. Gratifyingly, retinal analogs with extended side chains at position C(10) ending on a photoactivable group have been successfully used to study the orientation of the methyl groups relative to the membrane in BR.[48] The theoretical results can also be of interest for the study of the binding of retinoids to other retinal proteins[49] and to the increasingly important retinoid receptors (RARs, RXRs).[50]

(49) Effects on polyene conformation, photoisomerization, and formation of visual pigments analogs due to altered methyl substitution pattern have been observed with 11-*cis*-9-demethyl-11-methylretinal and 11-*cis*-9,13-didemethyl-11-methylretinal; see: Colmenares, L. U.; Liu, R. S. H. *Tetrahedron* **1991**, *47*, 3711.

(50) For a recent review, see: Pfahl, M. In *From Molecular Biology to Therapeutics*; Bernard, B. A., Shroot, B., Eds; Karger: Basel, 1993; pp 83−93.

Experimental Section

General Experimental Procedures.[51] Proton magnetic resonance spectra (^{1}H NMR) were recorded on Bruker WM-250 (250 MHz) or Bruker AMX300 (300 MHz) instruments. Carbon magnetic resonance spectra (^{13}C NMR) were recorded on Bruker WM-250 (63 MHz MHz) or Bruker AMX300 (75 MHz) instruments. Infrared spectra (IR) were recorded in 0.1-mm path length sodium chloride cavity cells on Perkin Elmer 1420 or MIDAC Prospect FTIR spectrometers. Absorbance frequencies are reported in reciprocal centimeters (cm^{-1}). UV spectra were recorded on a Hewlett-Packard HP8452A spectrophotometer. High-resolution mass spectra (HRMS) data were recorded on a Kratos MS-50 instrument at an ionizing current of 98 mA and an ionizing voltage of 70 eV. Standard deviation was determined to be ±3.1 ppm (σ = 1.6 ppm).

Analytical thin-layer chromatography was performed on Merck silica gel plates with F-254 indicator. Visualization was accomplished by UV light, iodine, or a 15% ethanolic phosphomolibdic acid solution. Flash chromatography was performed using E. Merck silica gel 60 (230−400 mesh). High-performance liquid chromatography (HPLC) was performed with a Waters 510 liquid chromatograph equipped with a μPorasil column.

Bulb-to-bulb distillations were carried out on a Büchi GKR-50 Kugelrohr; boiling points refer to air bath temperatures and are uncorrected.

All reactions were performed under a dry argon atmosphere in oven and/or flame-dried glassware. Transfer of anhydrous solvents or mixtures was accomplished with oven-dried syringes or cannula. Solvents and reagents were distilled before use: dichloromethane and dimethylformamide from calcium hydride and ether, tetrahydrofuran from sodium benzophenone ketyl. "Brine" refers to saturated aqueous solution of NaCl.

All reactions involving retinoids as final products or starting materials were done under subdued red light.

Computations. The geometry of the protonated Schiff base was completely optimized at the *ab initio* 3-21G level, without constraints in its most stable conformation (all-*trans*, except the $C_5-C_6-C_7-C_8$ which is skewed *cis*). The geometry of the forced 6-*s*-*trans* conformation was computed with the $C_5-C_6-C_7-C_8$ angle fixed to 180°, leaving the remainig part of the molecule with no restrictions. The Gaussian 92 default criteria for convergence was adopted in all cases.

Synthesis. (*E*)-3-Iodo-2-methylprop-2-en-1-ol 11. A suspension of Cl_2ZrCp_2 (4.16 g, 14.2 mmol) in CH_2Cl_2 (32 mL) was treated at 0 °C with trimethylaluminum (4.1 mL, 42.6 mmol) followed by addition of a solution of prop-2-yn-1-ol **10** (0.80 g, 14.2 mmol) in CH_2Cl_2 (16 mL). The reaction mixture was stirred at room temperature for 12 h and then cooled down to 0 °C. A solution of ICN (6.51 g, 42.6 mmol) in THF (48 mL) was added over 1 h. Addition of 100 mL of a 1:1 THF/H_2O mixture was followed by extraction with Et_2O. The organic layer was washed with aqueous $Na_2S_2O_3$ and water, dried over $MgSO_4$, and evaporated. Distillation of the residue (83 °C/0.6 mmHg) afforded 1.7 g (60% yield) of compound **11** as a yellow oil: ^{1}H NMR (250 MHz, C_6D_6) δ 1.58 (3H, s), 3.52 (2H, d, J = 5.6 Hz), 6.01 (1H, s); ^{13}C NMR (63 MHz, C_6D_6) δ 21.5, 67.1, 77.5, 147.9; IR (neat) ν 3600−3200 cm^{-1}.

(*E*)-2-Methyl-5-(trimethylsilyl)pent-2-en-4-yn-1-ol 12. Trimethylsilylacetylene (1.68 g, 17.08 mmol) was slowly added to a degassed solution of iodide **11** (1.69 g, 8.54 mmol), $Pd(PPh_3)_4$ (0.49 g, 0.43 mmol), and CuI (0.16 g, 0.84 mmol) in pyrrolidine (32 mL). After stirring at room temperature for 30 min, the reaction mixture was treated with a saturated aqueous NH_4Cl solution (50 mL) and extracted with Et_2O (4 × 20 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. Distillation of the residue (85 °C/0.7 mmHg) afforded 1.31 g (91%) of compound **12** as a brown oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.19 (9H, s), 1.90 (3H, s), 4.09 (2H, s), 5.6 (1H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ −0.04, 16.5, 66.7, 98.5, 102.4, 104.8, 151.6; IR (neat) ν 3600−3280, 2140, 1253, 850 cm^{-1}.

(51) The purity of all new compounds were judged by a combination of HPLC and ^{1}H NMR and ^{13}C NMR analysis before mass spectral determinations. The level of purity is indicated by the inclusion of copies of NMR spectra presented in the supporting information.

(*E*)-2-Methylpent-2-en-4-yn-1-ol 13. Tetrabutylammonium fluoride (9.48 mL, 1 M in THF, 9.48 mmol) was added to a solution of silyl ether **12** (0.8 g, 4.74 mmol) in THF (25 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into H_2O (30 mL) and extracted with Et_2O (3 × 20 mL). The organic extracts were dried over Na_2SO_4 and evaporated. Distillation of the residue (68 °C/0.7 mmHg) afforded 0.42 g (93%) of compound **13**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.80 (3H, s), 2.93 (1H, s), 3.65 (2H, s), 5.65 (1H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 16.3, 66.5, 80.9, 81.0, 103.5, 152.2; IR (neat) ν 3900−3500, 2350 cm^{-1}; HRMS calcd for C_6H_8O 96.0575, found 96.0577.

[(*E*,*E*)-(5-Hydroxy-4-methylpenta-1,3-dien-1-yl)]boronic Acid 8. Doubly distilled catecholborane (0.2 mL, 1.87 mmol) was slowly added to alkynol **13** (0.09 g, 0.94 mmol) placed in a Schlenk flask at 0 °C under argon, allowing for slow release of hydrogen. The closed reaction flask was stirred at room temperature for 2.5 h. The reaction mixture was kept at −20 °C for 17 h, then cold water (15 mL) was added, and the resulting white suspension was stirred at room temperature for 1 h. The mixture was saturated with NaCl and extracted with ethyl acetate (5 × 15 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated. Purification by chromatography on silica gel (elution gradient: from 1:1 hexane/ethyl acetate—to eliminate the catechol—to 95:5 CH_2Cl_2/MeOH) afforded 0.07 g (52%) of compound **8**, which was used in the next step without further purification: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.92 (3H, s), 4.11 (2H, s), 5.78 (1H, d, J = 17.2 Hz), 6.27 (1H, d, J = 11.2 Hz), 7.36 (1H, dd, J = 17.2, 11.2 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 14.3, 68.1, 127.6, 142.7, 144.1, 145.4; IR (neat) ν 3600−3400, 1332 cm^{-1}.

13-Demethyl-14-methylretinol 9. All solutions for the palladium-coupling experiments were thoroughly degassed before use. A solution of iodide **7** (90 mg, 0.28 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) in THF (2 mL) was stirred at room temperature for 10 min. A solution of boronic acid **8** (50 mg, 0.35 mmol) in THF (2.5 mL) was then added, followed by 10% aqueous TlOH (2.4 mL). After stirring the mixture at room temperature for 15 h, it was diluted with ether and filtered through Celite. The solution was washed with aqueous $NaHCO_3$ (10 mL) and extracted with ether (5 × 15 mL). The combined organic extracts were dried over $MgSO_4$, and the solvent was removed *in vacuo*. Chromatography (SiO_2, 80:20 hexane/ethyl acetate) of the residue afforded 49 mg (61%) of compound **9**. An analytical sample was obtained after purification by HPLC (μPorasil, 83:17 hexane/ethyl acetate, 2.25 mL/min): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.02 (6H, s), 1.2−1.6 (4H, m), 1.71 (3H, s), 1.82 (3H, s), 1.93 (3H, s), 2.01 (2H, d, J = 6.0 Hz), 4.10 (2H, s), 6.0−6.2 (4H, m), 6.46 (1H, dd, J = 14.4, 10.7 Hz), 6.60 (1H, dd, J = 14.4, 10.7 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.6, 14.3, 19.2, 21.7, 28.9, 33.0, 34.2, 39.7, 68.6, 125.9, 126.9, 128.2, 129.4, 129.6, 130.2, 136.1, 137.2, 137.7, 138.0; UV (MeOH) λ_{max} (ϵ) 278 (15 000), 330 (12 000) nm; IR (neat) ν 3600−3300 cm^{-1}; HRMS calcd for $C_{20}H_{30}O$ 286.2296, found 286.2292.

13-Demethyl-14-methylretinal 3. Manganese dioxide (0.11 g, 1.26 mmol) was added in one portion to a solution of alcohol **9** (0.02 g, 0.07 mmol) in CH_2Cl_2 (4 mL). After stirring at room temperature for 2 h, the reaction mixture was filtered through Celite, and the solvent was removed *in vacuo* to afford 0.019 g (95% yield) of compound **3**. An analytical sample was obtained after purification by HPLC (μPorasil, 98:2 hexane/ethyl acetate, 2.25 mL/min): ^{1}H NMR (250 MHz, $CDCl_3$) Table 1; ^{13}C NMR (63 MHz, $CDCl_3$) δ 9.5, 12.9, 19.2, 21.7, 28.9, 33.1, 34.3, 39.6, 126.9, 129.5, 130.1, 130.7, 136.7, 137.1, 137.7, 138.0, 141.8, 149.1, 194.6; UV (MeOH) Table 2; IR (neat) ν 1600 cm^{-1}; HRMS calcd for $C_{20}H_{28}O$ 284.2140, found 284.2134.

Ethyl (*E*,*E*)-6-Hydroxy-3-methylhexa-2,4-dienoate 15. Aldehyde **14** (1.0 g, 5.95 mmol) was added in one portion to a solution of sodium borohydride (0.11 g, 2.98 mmol) in 1:1 EtOH/H_2O (3 mL) at 0 °C. The mixture was stirred at room temperature for 6.5 h, saturated with NaCl, and extracted with ether (5 × 5 mL). The combined organic extracts were dried over $MgSO_4$, and the solvents were concentrated *in vacuo*. The residue was purified by chromatography (SiO_2, 60:40 hexane/ethyl acetate) to afford 0.92 g (91%) of alcohol **15** (colorless oil, bp 115 °C/0.2−0.3 mmHg): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 2.27 (3H, s), 4.16 (2H, q, J = 7.1 Hz), 4.29 (2H, d, J= 4.6 Hz), 5.77 (1H, s), 6.21 (1H, dt, J = 15.7, 4.6 Hz), 6.32 (1H, d, J = 15.7 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 13.7, 14.1, 59.8, 62.9, 119.5, 133.6, 134.6, 151.4, 167.1; IR ($CHCl_3$) ν 3600−3300, 1710 cm^{-1}; HRMS calcd for $C_9H_{14}O_3$ 170.0943, found 170.0945.

Ethyl (*E*,*E*)-6-[(*tert*-Butyldimethylsilyl)oxy]-3-methylhexa-2,4-dienoate 16. To a cooled (0 °C) solution of alcohol **15** (0.81 g, 4.77 mmol) in DMF (15 mL) was added *tert*-butyldimethylsilyl chloride (0.8 g, 5.31 mmol) and imidazole (0.36 g, 5.29 mmol). The reaction mixture was stirred at room temperature for 5 h and then extracted with ether (3 × 50 mL). The combined organic extracts were washed with H_2O (3 × 100 mL), dried over $MgSO_4$, and evaporated. The residue was purified by distillation (bp 115−120 °C/0.3−0.5 mmHg) to afford **16** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (6H, s), 0.92 (9H, s), 1.28 (3H, t, J = 7.1 Hz), 2.28 (3H, d, J = 1.0 Hz), 4.16 (2H, q, J = 7.1 Hz), 4.31 (2H, d, J = 4.2 Hz), 5.76 (1H, s), 6.16 (1H, dt, J = 15.6, 4.2 Hz), 6.33 (1H, d, J = 15.6 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ −5.4, 13.8, 14.2, 18.3, 25.8, 59.6, 63.2, 119.0, 132.5, 135.2, 151.8, 167.2; IR ($CHCl_3$) ν 1710, 1610, 1160, 840 cm^{-1}; HRMS calcd for $C_{15}H_{28}O_3Si$ 284.1808, found 284.1804.

(*E*,*E*)-6-[(*tert*-Butyldimethylsilyl)oxy]-3-methylhexa-2,4-dien-1-ol 17. To a solution of ester **16** (1.1 g, 3.87 mmol) in THF (20 mL) at 0 °C was added dropwise DIBALH (11.2 mL, 1.0 M in hexane, 11.2 mmol), and the reaction mixture was stirred at 0 °C for 1 h. After adding water carefully, the solution was extracted with ether (5 × 25 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (100 mL), H_2O (100 mL), and brine (100 mL), dried over $MgSO_4$, and evaporated. The residue was purified by distillation (85 °C/0.2 mmHg) to afford 0.93 g (96%) of **17** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (6H, s), 0.92 (9H, s), 1.80 (3H, s), 4.2−4.3 (4H, m), 5.64 (1H, t, J = 6.8 Hz), 5.77 (1H, dt, J = 15.6, 5.2 Hz), 6.26 (1H, d, J = 15.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, 12.8, 18.7, 26.2, 59.4, 64.1, 128.5, 130.1, 134.0, 135.7; IR ($CHCl_3$) ν 3600−3300, 1260, 980, 840 cm^{-1}; HRMS calcd for $C_{13}H_{26}O_2Si$ 242.1703, found 242.1705.

(*E*,*E*)-6-[(*tert*-Butyldimethylsilyl)oxy]-3-methylhexa-2,4-dienal 18. A solution of alcohol **17** (0.8 g, 3.31 mmol) in ether (100 mL) was treated with MnO_2 (11.0 g, 0.12 mol) as indicated for **3**. The residue was purified by distillation (bp 100 °C/0.2 mmHg) to afford 0.64 g (81%) of aldehyde **18** as a yellow oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.08 (6H, s), 0.92 (9H, s), 2.26 (3H, s), 4.34 (2H, d, J = 3.8 Hz), 5.95 (1H, d, J = 8.1 Hz), 6.32 (1H, dt, J = 15.7, 3.8 Hz), 6.44 (1H, d, J = 15.7 Hz), 10.11 (1H, d, J = 8.1 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ −5.4, 13.1, 18.3, 25.8, 63.2, 129.4, 131.9, 137.3, 153.9, 191.5; IR ($CHCl_3$) ν 1670, 1110, 840 cm^{-1}; HRMS calcd for $C_{13}H_{24}O_2Si$ 240.1546, found 240.1545.

(9*Z*)- and (9*E*)-13-Demethyl-12-methylretinyl *tert*-butyldimethylsilyl Ether 20 and 21. To a cooled (−20 °C) stirred suspension of β-ionyltriphenylphosphonium bromide **19** (0.48 g, 0.92 mmol) in THF (6 mL) was added dropwise *n*-butyllithium (0.6 mL, 1.6 M in hexanes, 0.96 mmol). The red solution was kept at that temperature for 30 min and at −78 °C for an additional 30 min, before adding a solution of the aldehyde **18** (0.20 g, 0.83 mmol) in THF (0.5 mL), via cannula at −78 °C. The reaction mixture was slowly warmed up to −40 °C for 7.5 h, water (5 mL) was added, and the resulting mixture was extracted with hexane (3 × 10 mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (98:2 hexane/ethyl acetate) to afford 0.23 g (68%) of retinoids **20** and **21** as a 1:1.4 mixture, as shown by ^{1}H NMR.

(9*Z*)- and (9*E*)-13-Demethyl-12-methylretinol 22 and 23. To the residue obtained above (0.2 g, 0.51 mmol) in THF (12 mL) was added tetrabutylammonium fluoride (0.6 mL, 1.0 M in THF, 0.60 mmol), and the mixture was stirred at room temperature for 3 h. After adding ethyl acetate (10 mL) the mixture was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated. The product was purified by chromatography on silica gel (80:20 hexane/ethyl acetate) to afford 0.14 g (93%) of (9*Z*)- and (9*E*)-13-demethyl-12-methylretinol (**22** and **23**, respectively) in a 1:1.4 ratio, which were separated by HPLC (μPorasil, 97:3 hexane/isopropyl alcohol, 2 mL/min).

Isomer (9*Z*) 22: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.02 (6H, s), 1.4−1.7 (4H, m), 1.73 (3H, s), 1.88 (3H, s), 1.99 (3H, s), 1.9−2.0 (2H, m), 4.23 (2H, t, J = 6.0 Hz), 5.84 (1H, dt, J = 15.5, 6.0 Hz), 6.20 (1H, d, J = 16.0 Hz), 6.24 (1H, d, J = 12.2 Hz), 6.36 (1H, d, J = 15.5 Hz),

6.53 (1H, d, J = 12.2 Hz), 6.65 (1H, d, J = 16.0 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.3, 19.2, 21.0, 21.7, 28.9, 33.0, 34.1, 39.5, 63.9, 124.3, 126.6, 126.7, 129.1, 129.6, 129.8, 133.2, 135.3, 137.0, 138.2; IR ($CHCl_3$) ν 3600−3300, 1250 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 328 (26 000) nm; HRMS calcd for $C_{20}H_{30}O$ 286.2296, found 286.2298.

Isomer (9*E*) 23: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.02 (6H, s), 1.4−1.6 (4H, m), 1.71 (3H, s), 1.90 (3H, s), 1.94 (3H, s), 2.01 (2H, t, J = 5.9 Hz), 4.24 (2H, t, J = 6.0 Hz), 5.84 (1H, dt, J = 16.0, 6.0 Hz), 6.17 (2H, app. s), 6.32 (1H, d, J = 12.0 Hz), 6.34 (1H, d, J = 16.0 Hz), 6.43 (1H, d, J = 12.0 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.4, 12.6, 19.2, 21.6, 28.9, 33.0, 34.2, 39.6, 63.9, 125.9, 126.7, 127.0, 128.0, 129.3, 134.1, 136.8, 136.9, 137.9, 138.0; IR ($CHCl_3$) ν 3600−3300, 1220 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 332 (15 500) nm; HRMS calcd for $C_{20}H_{30}O$ 286.2296, found 286.2288.

13-Demethyl-12-methylretinal 4. Following the general procedure, a solution of alcohol **23** (14 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) was oxidized with MnO_2 (106 mg, 1.22 mmol) at room temperature for 5 h. Purification by chromatography (SiO_2, 95:5 hexane/ethyl acetate) afforded 13 mg (94%) of compound **4**. An analytical sample was obtained after purification by HPLC (μPorasil, 95:5 hexane/ethyl acetate, 2 mL/min): ^{1}H NMR (300 MHz, $CDCl_3$) Table 1; ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.5, 12.8, 19.1, 21.7, 28.9, 33.1, 34.3, 39.6, 125.5, 126.9, 130.2, 130.7, 133.3, 136.9, 137.1, 137.5, 142.5, 157.3, 193.8; IR ($CHCl_3$) ν 1665 cm^{-1}; UV (MeOH) Table 2; HRMS calcd for $C_{20}H_{28}O$ 284.2140, found 284.2146.

(*E,E,E*)-8-[(*tert*-Butyldimethylsilyl)oxy]-3,6-dimethylocta-2,4,6-trien-1-ol 26. A degassed suspension of boronic acid **24**[18] (1.30 g, 9.16 mmol) in 10% aqueous TlOH (24 mL, 11.0 mmol)−THF (28 mL) solution was added, via cannula, to a solution of bromide **25**[23] (1.5 g, 5.5 mmol) and $Pd(PPh_3)_4$ (1.35 g, 1.17 mmol) in degassed THF (18 mL). The heterogeneous mixture was stirred at room temperature for 2 h. Standard workup as described for **9** afforded a residue which was purified by chromatography (SiO_2, 80:20 hexane/ethyl acetate) to afford 1.33 g (82%) of alcohol **26** as a yellow oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.08 (6H, s), 0.91 (9H, s), 1.78 (3H, s), 1.83 (3H, s), 4.30 (2H, d, J = 7.1 Hz), 4.34 (2H, d, J = 6.4 Hz), 5.64 (1H, t, J = 6.4 Hz), 5.70 (1H, t, J = 7.1 Hz), 6.23 (1H, d, J = 16.0 Hz), 6.29 (1H, d, J = 16.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, 12.8, 12.9, 18.6, 26.2, 59.6, 60.6, 130.3, 131.8, 132.3, 132.9, 134.4, 136.6; IR ($CHCl_3$) ν 3600−3300, 1280, 1130, 1080, 860 cm^{-1}; HRMS calcd for $C_{16}H_{30}O_2Si$ 282.2016, found 282.2011.

(*E,E,E*)-8-[(*tert*-Butyldimethylsilyl)oxy]-3,6-dimethylocta-2,4,6-trienal 27. A solution of alcohol **26** (0.30 g, 1.06 mmol) in ether (30 mL) was stirred at room temperature for 6 h with MnO_2 (3.5 g, 40.43 mmol) as described for **3**. The residue after workup was purified by chromatography (SiO_2, 95:5 hexane/ethyl acetate) to afford 0.25 g (85%) of compound **27**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.89 (9H, s), 1.79 (3H, s), 2.27 (3H, s), 4.35 (2H, d, J = 6.1 Hz), 5.83 (1H, t, J = 6.1 Hz), 5.96 (1H, d, J = 8.1 Hz), 6.28 (1H, d, J = 15.8 Hz), 6.72 (1H, d, J = 15.8 Hz), 10.09 (1H, d, J = 8.1 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ −5.3, 12.5, 13.0, 18.2, 25.8, 60.3, 129.5, 130.1, 133.7, 137.5, 140.1, 154.6, 191.1; IR ($CHCl_3$) ν 1700 cm^{-1}; HRMS calcd for $C_{16}H_{28}O_2Si$ 280.1858, found 280.1858.

9-Demethyl-10-methylretinyl *tert*-Butyldimethylsilyl Ether 29. To a cooled (−30 °C) suspension of phosphonium salt **28** (125 mg, 0.26 mmol) in THF (3 mL) was added *n*-butyllithium (0.18 mL, 1.6 M in hexanes, 0.29 mmol). The resulting deep red solution was stirred at 0 °C for 1 h. A solution of aldehyde **27** (80 mg, 0.29 mmol) in THF (1 mL) was then added via cannula, and the reaction mixture was stirred at room temperature for 4 h. Water (5 mL) was added, and the solution was extracted with hexane (3 × 10 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography (SiO_2, 97:3 hexane/ethyl acetate) to afford 68 mg (65%) of compound **29**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (6H, s), 0.91 (9H, s), 1.04 (6H, s), 1.4−1.7 (4H, m), 1.75 (3H, s), 1.80 (3H, s), 1.90 (3H, s), 2.03 (2H, t, J = 6.0 Hz), 4.35 (2H, d, J = 6.4 Hz), 5.63 (1H, t, J = 6.4 Hz), 6.18 (1H, d, J = 15.3 Hz), 6.19 (1H, d, J = 11.1 Hz), 6.28 (1H, d, J = 16.0 Hz), 6.31 (1H, d, J = 16.0 Hz), 6.44 (1H, dd, J = 15.3, 11.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.8, 12.9, 13.0, 18.7, 19.5, 22.1, 26.2, 29.2, 33.5, 34.4, 40.0, 60.7, 130.0, 130.4, 131.6, 131.8, 132.1, 132.7, 132.8, 133.9, 134.9, 138.1; IR ($CHCl_3$) ν 1100, 840 cm^{-1}; HRMS calcd for $C_{26}H_{44}OSi$ 400.3161, found 400.3152.

9-Demethyl-10-methylretinol 30. According to the general procedure described for **23**, silyl ether **29** (136 mg, 0.34 mmol) in THF (10 mL) was treated with tetrabutylammonium fluoride (0.4 mL, 1M in THF, 0.4 mmol) at room temperature for 90 min. Workup and purification by chromatography (SiO_2, 80:20 hexane/ethyl acetate) afforded 93 mg (96%) of compound **30**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.04 (6H, s), 1.4−1.7 (4H, m), 1.75 (3H, s), 1.85 (3H, s), 1.91 (3H, s), 2.05 (2H, t, J = 5.8 Hz), 4.31 (2H, d, J = 7.0 Hz), 5.72 (1H, t, J = 7.0 Hz), 6.20 (1H, d, J = 15.4 Hz), 6.21 (1H, d, J = 10.8 Hz), 6.39 (1H, dd, J = 15.4, 10.8 Hz), 6.43 (1H, d, J = 15.8 Hz), 6.46 (1H, d, J = 15.8 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.5, 12.6, 19.1, 21.7, 28.9, 33.2, 34.1, 39.7, 59.5, 126.9, 129.6, 129.7, 130.3, 131.2, 132.3, 133.0, 133.5, 137.0, 137.9; IR ($CHCl_3$) ν 3600−3300, 1220, 970 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 308 (14 400) nm; HRMS calcd for $C_{20}H_{30}O$ 286.2296, found 286.2285.

9-Demethyl-10-methylretinal 5. Alcohol **30** (40 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) was treated with MnO_2 (0.19 g, 2.24 mmol) at room temperature for 3.5 h. Workup as described for **3**, and purification by chromatography (SiO_2, 95:5 hexane/ethyl acetate) afforded 30 mg (76%) of compound **5**: ^{1}H NMR (250 MHz, $CDCl_3$) Table 1; ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.6, 13.0, 19.1, 21.8, 28.9, 33.4, 34.1, 39.7, 128.4, 129.1, 129.3, 129.6, 131.8, 133.1, 135.4, 137.8, 141.0, 155.1, 191.2; IR ($CHCl_3$) ν 1730 cm^{-1}; UV (EtOH) Table 2; HRMS calcd for $C_{20}H_{28}O$ 284.2140, found 284.2132.

(2*Z*)- and (2*E*)-2-Methyl-3-(2,6,6-trimethylcyclohex-1-en-1-yl)-prop-2-enal 33 and 34. To a solution of silylimine **32** (8.17 g, 35.88 mmol) in THF (32 mL) at −78 °C was added dropwise *sec*-BuLi (34.5 mL, 1.0 M in cyclohexane, 34.5 mmol). The mixture was stirred at −78 °C for 30 min before addition of a solution of β-cyclocitral **31** (2.1 g, 13.8 mmol) in THF (17 mL). The resulting mixture was stirred at −20 °C for 5 h before adding trifluoroacetic acid (55.3 mL, 71.76 mmol). After stirring at −20 °C for 1 h, water (83 mL) was added, and the mixture was stirred at 0 °C for 8 h. The mixture was then poured into saturated aqueous $NaHCO_3$ solution (30 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (100 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by chromatography (SiO_2, 98:2 hexane/ethyl acetate) followed by distillation (50−55 °C/0.1−0.2 mmHg) to afford 2.03 g (77%) of the *Z/E* aldehyde mixture (**33**, **34**) in a 2:1 ratio.

Iodine-Induced Isomerization. A solution of iodine (0.06 equiv) in hexane was added to the residue obtained above, and the mixture was stirred at room temperature for 30 min. After washing the solution with a saturated aqueous sodium thiosulfate solution, the organic layer was dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. The residue was purified by chromatography on silica gel as above to afford pure **34**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.99 (6H, s), 1.45 (3H, s), 1.5−1.7 (4H, m), 1.62 (3H, d, J = 1.2 Hz), 2.02 (2H, t, J = 5.4 Hz), 6.97 (1H, m), 9.51 (1H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 10.8, 19.0, 21.2, 28.3, 31.8, 34.6, 38.8, 130.6, 134.3, 141.3, 151.6, 195.5; IR ($CHCl_3$) ν 1680 cm^{-1}; HRMS calcd for $C_{13}H_{20}O$ 192.1514, found 192.1510.

(*E,E*)-2-(4-Iodo-2-methylbuta-1,3-dien-1-yl)-1,3,3-trimethylcyclohex-1-ene 35. A solution of aldehyde **34** (26 mg, 0.13 mmol) and iodoform (165 mg, 0.42 mmol) in anhydrous THF (2 mL) was added dropwise to a cooled (0 °C) suspension of anhydrous $CrCl_2$ (100 mg, 0.81 mmol) in THF (2 mL). After stirring at room temperature for 2 h, the reaction mixture was poured into water (5 mL) and extracted with hexane (3 × 5 mL). The combined organic extracts were washed with saturated aqueous sodium thiosulfate solution (3 mL) and brine (5 mL), dried over $MgSO_4$, and concentrated *in vacuo*. Purification by chromatography (SiO_2, 98:2 hexane/ethyl acetate) afforded 0.027 g (63%) of iodide **35** as a colorless oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.93 (6H, s), 1.44 (3H, d, J = 0.9 Hz), 1.4−1.6 (4H, m), 1.58 (3H, d, J = 1.1 Hz), 1.9−2.0 (2H, m), 5.97 (1H, br s), 6.19 (1H, d, J = 14.7 Hz), 7.14 (1H, dd, J = 14.7, 0.9 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 13.2, 19.2, 21.2, 28.3, 31.9, 34.6, 39.0, 73.6, 129.2, 132.5, 135.2, 136.6, 149.7; HRMS calcd for $C_{14}H_{21}I$ 316.0689, found 316.0683.

9-Demethyl-8-methylretinol 36. Following the general procedure, boronic acid **24**[18] (56 mg, 0.39 mmol) in THF (2 mL), 10% aqueous

TlOH solution (1.7 mL), and a solution of iodide **35** (83 mg, 0.26 mmol) containing $Pd(PPh_3)_4$ (30 mg, 0.026 mmol) in THF (1.5 mL) were stirred at room temperature for 1 h. Standard workup and purification by chromatography (SiO_2, 75:25 hexane/ethyl acetate) afforded 44 mg (59%) of compound **36**: ^1H NMR (250 MHz, C_6D_6) δ 1.08 (6H, s), 1.4−1.7 (4H, m), 1.57 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 1.95 (2H, t, J = 5.5 Hz), 4.08 (2H, d, J = 6.5 Hz), 5.67 (1H, t, J = 6.5 Hz), 6.17 (1H, s), 6.3−6.5 (4H, m); ^{13}C NMR (63 MHz, C_6D_6) δ 12.4, 14.0, 19.6, 21.4, 28.5, 32.1, 35.0, 39.4, 59.3, 128.0, 128.2, 129.0, 129.5, 131.5, 135.9, 136.3, 136.5, 137.0, 137.9; IR ($CHCl_3$) ν 3600−3300, 1200 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 306 (27 300) nm; HRMS calcd for $C_{20}H_{30}O$ 286.2296, found 286.2291.

9-Demethyl-8-methylretinal 6. Following the general procedure, alcohol **36** (25 mg, 0.09 mmol) in CH_2Cl_2 (3.5 mL) was oxidized with MnO_2 (190 mg, 2.18 mmol) at room temperature for 7 h. Purification of the residue (SiO_2, 95:5 hexane/ethyl acetate) afforded 17 mg (68%) of compound **6**. An analytical sample was obtained after purification by HPLC (μPorasil, 95:5 hexane/ethyl acetate, 2 mL/min): ^1H NMR (250 MHz, $CDCl_3$) Table 1; ^{13}C NMR (63 MHz, $CDCl_3$) δ 12.9, 13.7, 19.2, 21.2, 28.3, 31.9, 34.8, 39.1, 126.4, 129.0, 129.4, 134.0, 135.1, 135.7, 136.4, 137.2, 142.7, 154.7, 191.1; IR ($CHCl_3$) ν 1650 cm^{-1}; UV (MeOH) Table 2; HRMS calcd for $C_{20}H_{28}O$ 284.2140, found 284.2143.

Preparation of Bacterioopsin. Purple membrane was obtained from *H. salinarium* strain S9 as described.[31] Purple membrane in 4 M NaCl, 1 M $NH_2OH{\cdot}HCl$, pH 8.0 (BR concentration, 2 mg/mL), was illuminated at room temperature with filtered light (520 nm cut-off) under continuous stirring, until the suspension became colorless. It was then washed three times with water and resuspended in the appropriate buffer.

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Supporting Information Available: Complete spectroscopic characterization, reproduction of ^1H NMR or ^{13}C NMR spectra for the compounds described in the text, and tables with bond lengths and bond angles computed for the protonated Schiff bases **PSBs** (44 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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