Synthesis of Retinals Fluorinated at Odd-Numbered Side-Chain Positions and of the Corresponding Fluorobacteriorhodopsins

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Conventional Horner−Wadsworth−Emmons and Wittig condensations were used to fluorinate the odd-numbered positions of the retinal side chain past C_7. The stereochemically labile *cis*-fluororetinals were easily converted into the most stable *trans*-fluororetinals, which were incubated with bacterio-opsin. Contrary to expectations, the fluorinated retinals provided artificial pigments with near normal absorption properties, showing that any electrostatic interactions between the fluorine atoms and protein groups were insufficient to prevent normal binding. The new artificial pigments had smaller opsin shifts than did native bacteriorhodopsin, which is interpreted as due either to greater electrostatic interaction between the protonated imine and its counterion, or to local interactions between the fluorine substituents and nearby polar protein groups.

Introduction

The light-transducing protein bacteriorhodopsin (BR) is found in nature in the purple outer membrane of *Halobacterium salinarium*[1] and has been used in opto-electronic devices.[2] Its chromophore is the protonated Schiff base (PSB) formed by *trans*-retinal (**1**) and the Lys_{216} residue of the apoprotein, bacterio-opsin. In the bacterial cell, absorption of a quantum of light induces *trans*/*cis* isomerization of the retinal to the 13-*cis*-retinal configuration (**2**),[1] followed by loss of the iminium proton to an aspartate residue located to the extracelular side of the chromophore. The apoprotein then undergoes a conformational change that exposes the chromophore to a protonated aspartate residue on its cytoplasmic side, and reprotonation of the chromophore is followed by back-isomerization; the photocycle closes with the reversion of the apoprotein to its initial conformation. The net result is to pump a proton across the membrane.

While the gross geometrical changes of the BR retinylidene chromophore in the primary photochemical step are well stablished,[1] little is known about retinal−apoprotein interactions other than the imino bond, or about how such interactions are related to the changes in apoprotein structure that enable proton transport. Efforts to understand these interactions have often attempted to interpret the effects of modifying retinal on the "opsin shift",[3] the difference between the wavenumbers of the absorption maximum of the model PSB formed with *n*-butylamine and that of the pigment formed with bacterio-opsin. The factors determining the absorption wavelength of BR are thought certainly to include both ring-chain coplanarization (*i.e.* adoption of the 6-*s*-*trans* conformation) and the existence of a weak interaction between the Schiff base and its counterion.[4a] In addition, the large bathochromic opsin shift of the BR analogue containing 13,14-dehydroretinal strongly suggests that for BR and BR analogues in general other factors may also be involved, such as a lowering of the energy of the excited state due to interactions between the polyene chain and polar or polarizable side chains on the protein.[4b]

We recently reported the preparation of retinal analogues with relocated side-chain methyl groups.[5] Their incubation with bacterio-opsin failed to produce a BR analogue if the retinal modification affected the region near the terminal CHO, and *ab initio* calculations on corresponding model PSBs suggested that the importance of the C-13 methyl group lies in its narrowing the C12−C13−C14 bond angle, which ensures proper placement of the cyclohexene relative to the imino group.

We have now addressed the issue of possible interactions between retinal and polar residues in the BR molecule by using fluorine as a retinal-bound reporter group with high electronegativity and no steric demands. (For other recent applications of vinylic fluorine and fluorinated olefins, see refs 6−8). The polyolefinic nature of retinal allows incorporation of fluorine at virtually any position of the side chain.[9] In fact, *trans*-retinal analogues with fluorine substituents at even numbered

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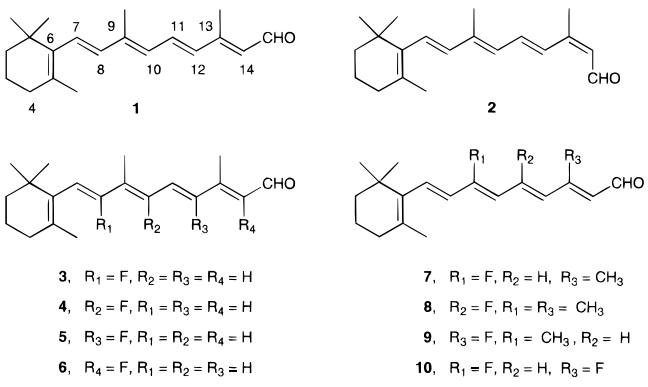

Figure 1. Natural chromophores in BR and side-chain fluorinated retinal analogues.

carbons (**3**−**6**) have already been synthesized[10] and successfully reacted with bacterio-opsin to form BR analogues.[10c−h] In this work we synthesized, and formed models PSBs with, retinals with fluorines at odd-numbered side-chain carbons, namely 9-demethyl-9-fluororetinal **7**, 11-fluororetinal **8**, 13-demethyl-13-fluororetinal **9**, and 9,13-didemethyl-9,13-difluororetinal **10**. In these fluororetinals, unlike those previously studied (**3**−**6**)[10c−h] the fluorine atoms are located at positions which in the native pigment are conjugated with the electron-withdrawing iminium ion of the BR PSB. It has been predicted, on the basis of studies of BR analogues and their model PSBs,[11] that these positions must share the positive charge of the PSB base proton through delocalization along the polyene side chain. We hoped that alterations, by the fluorine atoms, of any interactions between the polyene side-chain and polar bacterio-opsin side chains might show up as alterations of the absorption properties of the pigment and/or of its opsin shift with respect to model PSBs.

Results and Discussion

1. Synthesis of Retinal Analogs 7−10. The use of fluorinated biomolecules in biological research has stimulated the development, mostly in recent years,[12] of a number of stereoselective approaches to functionalized fluoroolefins. When we began this work, the fluorinated versions of the classical Peterson,[12e] Refortmatsky,[12f] and Horner−Wadsworth−Emmons (HWE)[12h] reactions were well known. For this work we chose the HWE reaction because of its mild conditions and the existence of precedents of its use for other fluororetinoids.[10] However, since the conditions used in these latter syntheses have usually led to an approximately 3:1 mixture of *E* and *Z* isomers with respect to the newly formed double bond,[10] we instead used conditions that had been reported to allow highly stereoselective preparation of (*E*)-α-fluoro-α,*β*-unsaturated esters by condensation of fluorinated HWE reagents with either presynthesized aldehydes[12h] or aldehydes obtained *in situ* by DIBALH reduction of the corresponding esters.[12d] We envisaged that the stereochemically pure *cis* compounds so obtained would easily be isomerizable to their more stable *trans* isomers[13] and would additionally provide *cis*-fluororetinoids of interest for research on the biological activities of this type of compounds (13-*cis*-retinal in relation to BR,[1,14] 11-*cis*-retinal for rhodopsin,[14] and 9-*cis*-retinoic acid for research on retinoid receptors[14,15]).

The synthesis of the desired retinals was based on Wittig reaction[16] between a cyclohexene moiety bearing a functionalized side chain and a fluorinated fragment that was itself prepared by reaction of a fluorinated HWE reagent with the appropriate aldehyde representing an even-numbered carbon of the final side chain (Scheme 1). Condensation of diethyl (fluorocarbethoxymethyl)-phosphonate[12h] with aldehydes **12**[17a] and **16**[17b] (*n*-BuLi, THF, −78 °C, and then room temperature)[12d,h] provided the anticipated (*E*)-α-fluoro-α,*β*-unsaturated esters **13** and **17**, respectively, in excellent yield. For their conversion to **15** and **19**, the reagents used in the subsequent Wittig reactions, *in situ* reduction, and olefination[12d] worked poorly, but conversion via the alcohols **14** and **18**, respectively, was satisfactory.

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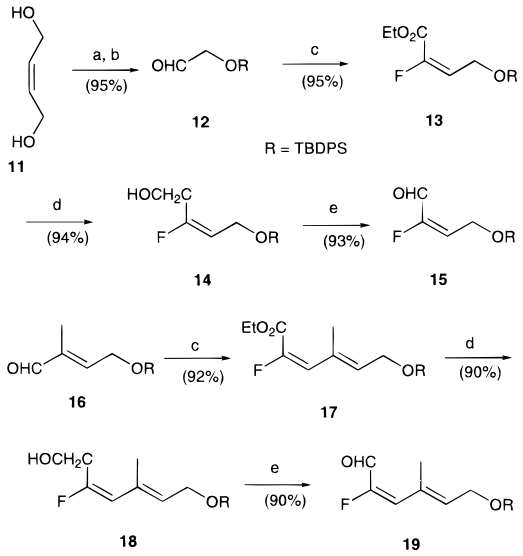
Scheme 1[a]



[a] Reagents and conditions: (a) ClTBDPS, imidazole, DMF, 25 °C, 12 h; (b) O_3, MeOH/CH_2Cl_2 (50:50), −78 °C, then Ph_3P; (c) diethyl (fluorocarbethoxymethyl)phosphonate, *n*-BuLi, THF, −78 °C, then 25 °C, 3 h; (d) DIBALH, THF, −78 °C, then 0 °C, 1 h; (e) $BaMnO_4$, CH_2Cl_2, 25 °C, 12 h.

Synthesis of 9-Demethyl-9-fluororetinal (7). Treatment of aldehyde **15** with the phosphonium salt **20** (derived from β-cyclocitral[18]) under the conditions described above[12d] provided the (2*E*,4*E*)-fluorinated silyl ether **21** as a precursor of C9-fluorinated retinoids (Scheme 2). Aldehyde **23** was prepared by standard *n*-Bu_4NF deprotection[19] of **21** and MnO_2 oxidation of the resulting alcohol **22** under basic conditions (MnO_2/Na_2CO_3 in CH_2Cl_2[20a]). Aldehyde **23** was coupled (*n*-BuLi, DMPU, −78 °C, and then 0 °C, 10 h)[21a] to phosphonate **24**[21b] to afford a 74% yield of ethyl 9-demethyl-9-fluororetinoate (**25**) as the anticipated *all*-(*E*)-isomer (as shown by the coupling constants of the vinylic hydrogens; see below). DIBALH reduction of **25**, followed by allylic oxidation with MnO_2 in the presence of Na_2CO_3 in CH_2Cl_2,[20a] gave the aldehyde **26** in 54% overall yield. Despite careful manipulation in the dark, compound **26** was slowly converted into its isomer **7**, which is *trans* (9*Z*) with respect to the C-9−C-10 double bond.

An alternative route to **7** was based on the finding that stereomutation of the terminal double bond of **22** was easily induced by treatment with HBr·Ph_3P. Coupling of aldehyde **16**[17b] with the β-fluoroallyl phosphorane anion derived from phosphonium salt **27** afforded a 68:32 mixture of the polyene isomers **28** and **29**. After deprotection[19] of the mixture and separation of the retinols **30** and **31** by HPLC, the desired (9*Z*,11*E*)-9-demethyl-9-fluororetinal (**7**) was obtained in 88% yield by oxidation of **30** with $BaMnO_4$.[20b,c]

Synthesis of 11-Fluororetinal (8). The synthesis of this analogue was envisaged as the result of Wittig condensation between aldehyde **19** and the anion derived from phosphonium salt **32**.[22] Generation of the phosphorane with *n*-BuLi at −78 °C, followed by addition of fluoroaldehyde **19** at −78 °C and additional stirring at room temperature for 5 h, provided a 57% yield of the (9*E*)-TBDPS-protected 11-fluororetinol **33** as the only isomer, which was deprotected with *n*-Bu_4NF,[19] to afford the alcohol **34** (Scheme 3); the stereoselectivity of the coupling with fluoroaldehyde **19** is strikingly different[23] from that achieved when the phosphonium salt **32** is coupled with aldehydes,[24] which provides ∼3:2 mixtures of *E* and *Z* stereoisomers at the newly formed trisubstituted double bond. Treatment of **34** with MnO_2 and Na_2CO_3[20a] provided the (9*E*)-11-fluororetinal **35** in 80% yield, which slowly converted into **8** on manipulation. Alternatively, $BaMnO_4$ treatment of alcohol **34** provided in 94% yield the desired *trans*-11-fluororetinal **8**.

Synthesis of 13-Demethyl-13-fluororetinal (9). Wittig condensation between the phosphonium salt **36** (derived from β-ionone)[25] and the enal **15** (Scheme 1) is the key step leading to retinal analogue **9**. Treatment of **36** with *n*-BuLi at −78 °C, followed by addition of aldehyde **15** and stirring at room temperature for 5 h, provided protected 13-demethyl-13-fluororetinol in 69% yield as a 60:40 mixture of the (11*E*) and (11*Z*) stereoisomers **37** and **38**, respectively (Scheme 4). Deprotection[19] and HPLC separation afforded fluororetinols **39** and **40**. Whereas oxidation of **39** with MnO_2/Na_2CO_3[20a] afforded a mixture of **41** and **9** in a ca. 4:1 ratio, the desired 13-*trans* isomer **9** was obtained under barium manganate oxidation conditions.[20b,c]

Synthesis of 9,13-Didemethyl-9,13-difluororetinal (10). Coupling between aldehyde **15** and the fluorophosphonium salt **27** (obtained as in Scheme 2), carried out by the general procedure described above, provided a 89% yield of a 75:25 mixture of **42** and **43**, the (11*E*)- and (11*Z*)-isomers of protected 9,13-didemethyl-9,13-difluororetinol (Scheme 5). Treatment of the unseparated mixture with *n*-Bu_4NF,[19] followed by HPLC separation, provided alcohols **44** and **45** in 76% yield. Allylic oxidation of fluororetinol **44** afforded, under basic MnO_2 conditions,[20a] the 9,13-didemethyl-9,13-difluororetinal isomers **46** and **10** in *ca.* 4:1 ratio, whereas treatment with $BaMnO_4$ took place with isomerization of the terminal double bond to the desired (13*Z*)-isomer **10** in 92% yield. Despite our precautions, isomerization of the (13*E*)-13-fluororetinals of both series (**41** and **46**) to the most stable (13*Z*)-isomers (**9** and **10**) always occurred upon manipulation (*e.g.* after NMR spectroscopy following HPLC separation, evaporation of the solvent gave rise to extensive isomerization).

2. Structure Determination. The ^1H NMR data of the fluororetinal isomers are listed in Table 1. Assignment rests on the ^1H NMR chemical shifts and coupling constants for the hydrogens of the retinal polyene chain[26a] and, for the configuration of the fluoroolefin isomers, on the magnitude of the three bond H−F coupling constants

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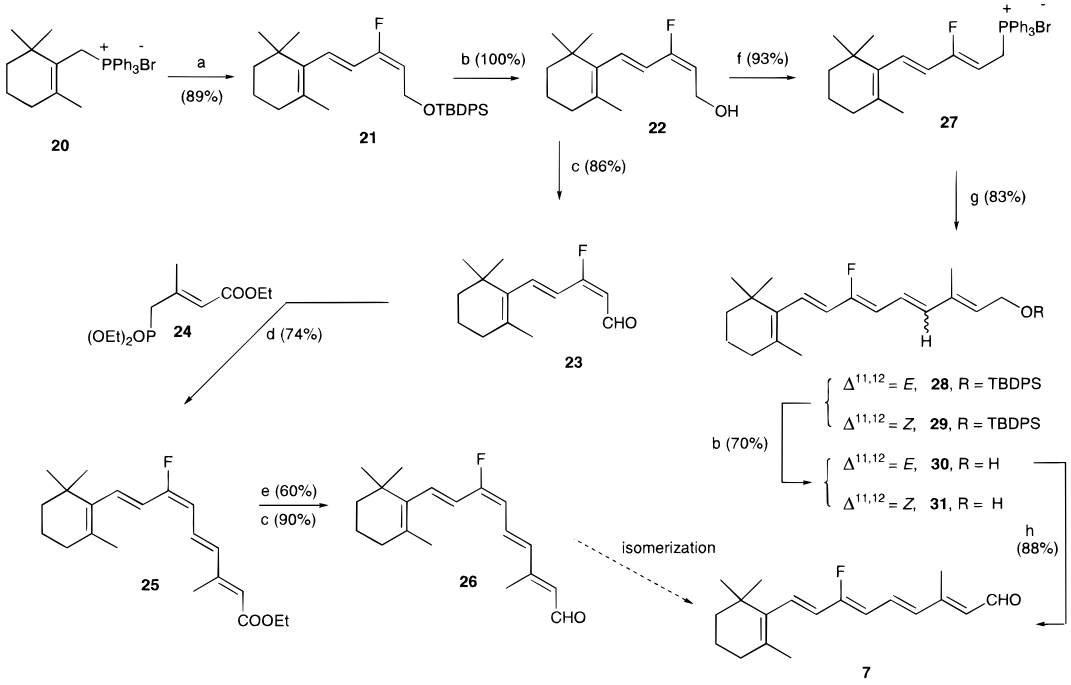
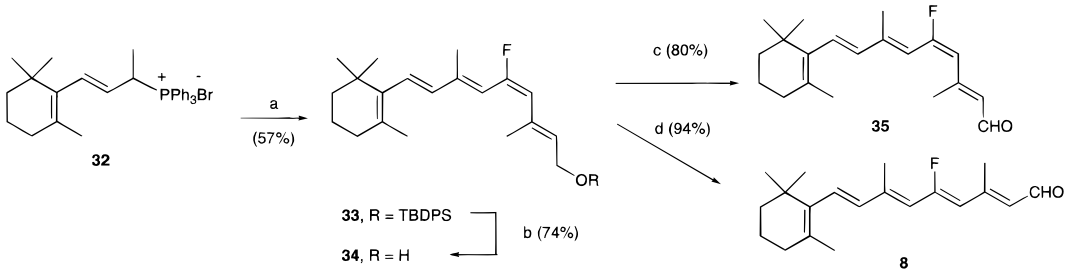
Scheme 2[a]



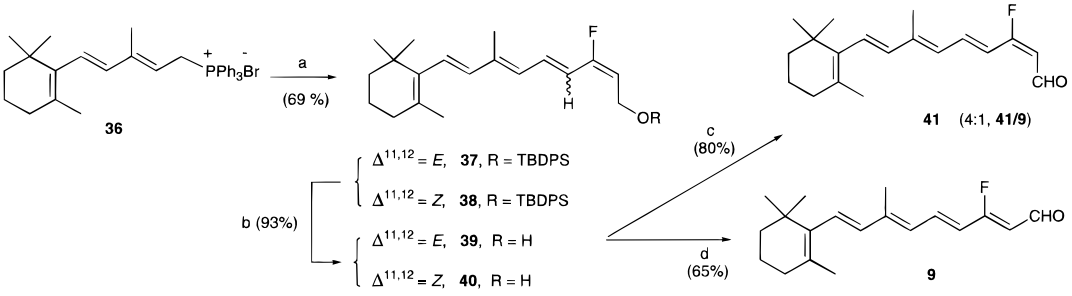
[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then aldehyde **15**, 25 °C, 7 h; (b) *n*-Bu_4NF, Et_2O, 25 °C, 2 h; (c) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C, 10 h; (d) *n*-BuLi, DMPU, THF, −78 °C; then aldehyde **23**, 0 °C, 5 h; (e) DIBALH, THF, −78 °C, then 0 °C, 1 h; (f) HBr·Ph_3P, MeOH, 25 °C, 48 h; (g) *n*-BuLi, **16**, THF, −78 °C, then 25 °C, 6 h; (h) $BaMnO_4$, CH_2Cl_2, 25 °C, 10 h.

Scheme 3[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then aldehyde **19**, 25 °C, 5 h; (b) *n*-Bu_4NF, Et_2O, 25 °C, 1 h; (c) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C, 8 h; (d) $BaMnO_4$, CH_2Cl_2, 25 °C, 10 h.

Scheme 4[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then aldehyde **15**, 25 °C, 5 h; (b) *n*-Bu_4NF, Et_2O, 25 °C, 1 h; (c) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C, 6 h; (d) $BaMnO_4$, CH_2Cl_2, 25 °C, 7 h.

($^3J_{HF}$ (*cis*) ∼ 20−25 Hz, $^3J_{HF}$ (*trans*) ∼ 30−40 Hz).[26b−d] Additionally, for the retinals with fluorine at C-13, the aldehydes with (13*E*) geometry showed a significant $^4J_{HF}$ ∼ 4 Hz, which was absent (or very small) in the corresponding (13*Z*) isomers. The stereochemistry of the trisubstituted double bonds was further confirmed by NOESY experiments.

3. Preparation of Model Schiff Bases (SBs), Protonated Schiff Bases (PSBs), and Pigments. Model Schiff bases were prepared by reacting each retinal analogue (∼0.5 mg) with *n*-butylamine (40 μL) in ether (1 mL) in the presence of 4 Å molecular sieves at room temperature for 30 min. Filtration and evaporation of solvent and excess amine under vacuum provided the desired Schiff base,[27] which was dissolved in methanol

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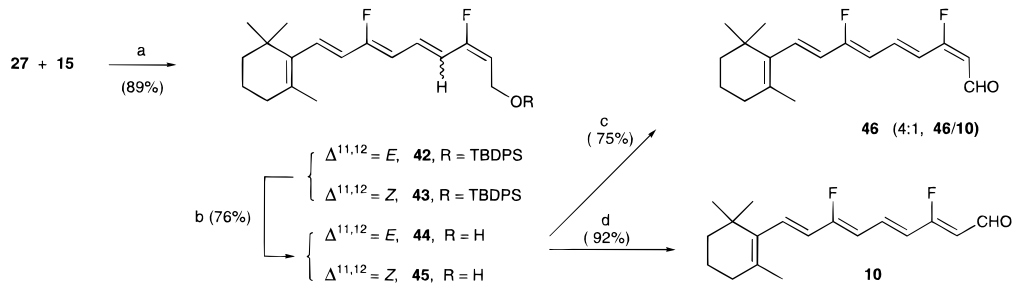
Scheme 5[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then aldehyde **15**, 25 °C, 5 h; (b) *n*-Bu_4NF, Et_2O, 25 °C, 1 h; (c) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C, 6 h; (d) $BaMnO_4$, CH_2Cl_2, 25 °C, 10 h.

Table 1. Selected ^1H NMR Chemical Shifts (in $CDCl_3$) and $^1H-^1H$ Coupling Constants ($^{19}F-^1H$ Coupling Constants in Parentheses) of Fluororetinals[a]

fluororetinal	C-5 CH_3	C-9 CH_3	C-13 CH_3	H_7	H_8	H_{10}	H_{11}	H_{12}	H_{14}	H_{15}	$J_{7,8}$	$J_{10,11}$	$J_{11,12}$	$J_{14,15}$
trans-9-demethyl-9-fluororetinal (**7**)	1.75	–	2.32	6.67	5.89 (26.2)	5.57 (32.9)	7.10	6.33	5.95	10.11	15.9	11.4	15.6	8.1
9-*cis*-9-demethyl-9-fluororetinal (**26**)	1.79	–	2.31	6.70	6.30 (27.1)	5.92 (18.8)	6.86	6.24	5.96	10.10	15.8	11.7	15.2	8.1
trans-11-fluororetinal (**8**)	1.76	2.11	2.43 (3.0)	6.41	6.08	5.74 (30.0)	–	5.44 (36.3)	5.99	10.06	16.0	–	–	8.1
11-*cis*-11-fluororetinal (**35**)[b]	1.62	1.69	2.08	6.42	6.17	6.08 (26.8)	–	5.53 (22.2)	5.97	9.84	15.8	–	–	7.7
trans-13-demethyl-13-fluororetinal (**9**)	1.75	2.09	–	6.47	6.21	6.19	7.42	6.09 (26.6)	5.47 (32.8)	10.08	16.0	12.0	14.9	8.0
13-*cis*-13-demethyl-13-fluororetinal (**41**)	1.76	2.09	–	6.47	6.25	6.20	7.43	6.83 (29.0)	5.76 (18.3)	9.97 (3.7)	16.0	12.0	14.9	7.0
trans-9,13-didemethyl-9,13-difluororetinal (**10**)	1.76	–	–	6.79	5.92 (27.2)	5.58 (32.8)	7.36	6.03 (26.8)	5.51 (26.0)	10.06	16.2	11.4	15.4	8.0
13-*cis*-9,13-didemethyl-9,13-difluororetinal (**46**)	1.77	–	–	6.80	5.94 (26.0)	5.64 (32.3)	7.37	6.79 (28.7)	5.77 (18.0)	9.92 (4.0)	16.1	11.6	15.1	6.8

[a] Chemical shifts in ppm, coupling constants in hertz. For signal assignments, see text. [b] In C_6D_6.

Table 2. Absorption Maxima (in nm) of Fluororetinals, Their Unprotonated (SB) and Protonated (PSB) *N*-Butyl Schiff Bases, and the Pigments Formed upon Incubating Them with Bacterio-Opsin. Also Shown Are the Corresponding *Opsin Shifts* (OS), the Difference between the Absorption Wavenumbers of Model PSB and Pigment. For Comparison, Values Corresponding to Unfluorinated Retinal Are Also Shown[10h]

retinal	λ_{max} (retinal)	λ_{max} (SB)	λ_{max} (PSB)	λ_{max} pigment	OS (cm^{-1})
trans-retinal (**1**)[a]	380	360	443	567	4940
14-F-retinal (**6**)[a]			455	587	4940
				680	7270
13-demethyl-13-F-retinal (**9**)[b]	382	362	446	548	4170
12-F-retinal (**5**)[a]			447	591	5450
11-F-retinal (**8**)[b]	370	354	434	540	4520
10-F-retinal (**4**)[a]			442	562	4830
9-demethyl-9-F-retinal (**7**)[b]	368	355	428	518	4060
8-F-retinal (**3**)[a]			427	530	4550
9,13-didemethyl-9,13-diF-retinal (**10**)[b]	372	355	429	520	4080
13-demethyl-13,14-diF-retinal[a]			466	600	4790

[a] Taken from ref 10h. [b] This work.

for UV spectroscopy. Protonation of the SB was effected in the UV cell by addition of trichloroacetic acid[10h] to this solution. The wavelengths of the absorption maxima of the *trans*-retinals and their SBs and PSBs are listed in Table 2, together with values reported for PSBs derived from retinals fluorinated at the even-numbered carbons.[10h]

It is generally accepted that strongly electronegative groups on the retinal polyene side chain increase the energy of the excited state relative to that of native retinal. However, from the values of the absorption maxima of retinals **7**−**10** and their SBs listed on Table 2 it can be concluded that the overall effect of the substitution of methyl (or hydrogen) by fluorine is negligible for **9** and very small for **7**, **8**, and **10**. It seems likely that fluorine compensates the destabilization of a neighboring positive charge due to its inductive effect with its ability to stabilize the charge through n-π back donation.[28] The small hypsochromic shifts exhibited by the retinal analogues and their SBs with respect to that of native retinal, with values increasing with the distance of the fluorine atom from the polar group, should be interpreted as due to reduction of delocalization along the polyene side chain by the presence of the electron-withdrawing group.

Table 2 shows that this trend is indeed followed both among PSBs fluorinated at even-numbered carbons and among PSBs fluorinated at odd-numbered carbons, but the latter invariably absorb at shorter wavelengths than PSBs fluorinated at neighboring even-numbered carbons. Furthermore, placing a fluorine atom at C-13 (in retinal **9** and its derivatives) does not seem to alter the absorption properties of the native system.

The above difference between the series of PSBs fluorinated at odd- and even-numbered carbons may be attributed to a difference with regard to the distribution

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Table 3. INDO Total Charge Distribution Per Atom (in |*e*|) in the Ground State and in the First Excited State for Selected Carbons of *trans*-Retinal Protonated *N*-Butyl Schiff Base Side Chain[11]

atom number	charge ground state	charge excited state
C-15	0.264	0.144
C-14	−0.078	0.001
C-13	0.150	−0.074
C-12	−0.057	0.096
C-11	0.129	−0.069
C-10	−0.056	0.108
C-9	0.113	0.008
C-8	−0.043	0.057

of the positive charge of the PSB proton over the polyene side-chain positions in both the ground and the excited state. Table 3 lists the values of charge distribution along the polyene side chain in the ground and the first excited state computed (INDO) for the protonated *N*-butylamine retinal Schiff base. Although in the ground state the positive charge is mostly localized on the region close to the protonated imino group (C-11- - -NH^+) in the first excited state the charge is delocalized more along the molecule. The excited state therefore shows a weaker charge separation than the ground state. Furthermore, odd- and even-numbered carbons invert their charge upon excitation (i.e. C-13 changes from +0.150 in the ground state to −0.074 in the excited state, whereas C-12 changes from −0.057 to +0.096). Accordingly, electronegative fluorine substituents at an even-numbered carbon of the retinal PSB side chain should stabilize the excited state, thus contributing to charge delocalization and as a consequence the absorption maxima should shift to longer wavelengths. The opposite effect must prevail for PSBs of retinals **7**−**10** with fluorine atoms positioned at odd-numbered carbons, although the effect of shortening the absorption wavelength is somehow balanced by the greater stabilization of the ground state.

When retinals **7**−**10**, previously dissolved in ethanol, were incubated with bacterio-opsin in 0.02 M phosphate buffer of pH 7.0, all formed the expected artificial pigments. Their absorption peak wavelengths (UV-vis absorption spectra were recorded using bleached purple membrane as the reference[29]) show the same pattern as those of the PSBs: shortening of absorption wavelength (a) as the fluorine atom moves toward the cyclohexene ring, and (b) with respect to BRs in which the retinal is fluorinated at neighboring even-numbered positions. The fact that within the series **7**−**10** trend a shows the same regular dependence on fluorine positions as among the corresponding PSBs suggests that there can be no strong electrostatic interaction between the fluorine atom and polar or charged groups on protein side chains, since any such interaction ought to depend on the position of the fluorine atom and hence disrupt the regularity of the shift.

The opsin shift of native BR is thought to be due largely to the PSB−counterion interaction being weaker in BR than for its model PSB in solution. This weakening, which is attributed to the orientation and distance of the counterion (the carboxylate group of an aspartate residue) being such that water molecules can bridge between counterion and PSB,[30−32] allows delocalization of the PSB proton charge along the retinal polyene chain. In the same way as for model compounds, charge delocalization in the pigments derived from analogues **7**−**10** should be reduced by the presence of a fluorine atom at odd-numbered positions of the retinal side chain, as indicated by the opsin shift values in Table 2. Therefore, the smaller opsin shifts of the new BR analogues compared to the native BR may be due to the PSB−counterion interaction being greater in the analogues than in native BR. Other interpretations cannot yet be ruled out, such as conformational changes induced by the protein or additional interactions of the fluorine atoms with charged or polar protein side-chain groups. In particular, it has been suggested[30] that the presence of a positive charge rather more than 3 Å from C-7 and C-9 would explain the ^{13}C NMR data of BR.

Finally we note that whereas the BR analogues based on compounds **7**−**10** all absorb at shorter wavelengths than native BR, the analogues with fluorines at C-12 and C-14 absorb at longer wavelengths. In the case of the 14-fluorinated analogue, a minor absorption peak at 680 nm has been attributed to an *all-trans*,15-*cis* conformation which allows electrostatic interaction with a nearby carboxylate; this conformer decays into the major form absorbing at 587 nm.[10h]

Conclusions

In this work we synthesized retinals with fluorine atoms at the odd-numbered side-chain positions past C-7. The retinal structure was assembled by means of Wittig reactions between building blocks, one or more of which had been selectively functionalized by HWE condensation. The resulting (*E*)-*cis*-fluororetinals were converted into the more stable corresponding (*Z*)-*trans*-fluororetinals, which were (a) reacted with *n*-butylamine to form model PSBs, and (b) incubated with bacterio-opsin to form BR analogues. The successful formation of the latter, which is concluded from the similarity between their absorption properties and those of native BR, show that any electrostatic interaction between protein groups and the fluororetinal fluorine atoms is not sufficient to prevent the fluororetinal from docking in the bacterio-opsin binding site.

Hypsochromic shifts with respect to native BR and its model PSB are exhibited by the BR analogues and model PSBs of all the new retinals except 13-demethyl-13-fluororetinal (**9**); the BR analogue of **9** exhibits this shift, but not its model PSB. The fact that the new BR analogues have smaller opsin shifts than native BR may be due to the PSB−counterion interaction being greater in the analogues than in native BR, or to local interactions between the fluorine substituents and nearby polar protein groups.

It is hoped that more information on the conformation of compounds **7**−**10** in their BR analogues will be provided by ^{19}F NMR spectroscopy, which has recently afforded evidence of restricted rotation of opsin-bound 11-

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cis-fluororetinal aromatic analogue around the C-6−C-7 bond in the visual pigment rhodopsin.[33]

Experimental Section

General Experimental Procedures.[5,34] **[(*tert*-Butyldiphenylsilyl)oxy]ethanal (12).** *tert*-Butylchlorodiphenylsilane (10.6 g, 38.5 mmol) was added dropwise at 0 °C to a solution of (*Z*)-but-2-ene-1,4-diol (**11**; 1.54 g, 17.5 mmol) and imidazole (2.62 g, 38.5 mmol) in DMF (40 mL). The solution was stirred at 25 °C for 12 h, diluted with H_2O (50 mL), and extracted with hexane (4 × 50 mL). The combined organic layers were washed with H_2O (2 × 50 mL), dried ($MgSO_4$), and concentrated. The resulting residue was purified by distillation (180 °C/1 mmHg) to afford 9.69 g (98%) of the crude silyl ether as a colorless oil which was used in the next step without further purification.

A fraction of the crude silyl ether (6.51 g, 11.5 mmol) was dissolved in MeOH/CH_2Cl_2 (50:50, 200 mL) and treated with O_3 at −78 °C for 20 min. The reaction was quenched with Ph_3P (4.53 g, 17.7 mmol) at −78 °C, and the resulting mixture was allowed to warm to 25 °C. After stirring at 25 °C for 0.5 h, the solvent was removed and the residue was purified by chromatography (silica, 85:15 hexane/ethyl acetate) to afford 6.54 g (95%) of aldehyde **12** as a colorless oil. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.16 (9H, s), 4.26 (2H, s), 7.4−7.5 (6H, m), 7.7−7.8 (4H, m), 9.75 (1H, s). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 19.1, 26.6 (3×), 69.9, 127.9 (4×), 130.0 (2×), 132.5 (2×), 135.5 (4×), 201.5. IR (NaCl): υ 1740 (s, C=O) cm^{-1}. HMRS: calcd for $C_{18}H_{22}O_2Si$, 298.1389; found, 298.1388.

Ethyl (2*E*)-4-[(*tert*-Butyldiphenylsilyl)oxy]-2-fluorobut-2-enoate (13). The following olefination procedure is representative. *n*-BuLi (10.6 mL, 1.6 M in hexane, 16.9 mmol) was added with a syringe to a cooled (−78 °C) solution of diethyl (fluorocarbethoxymethyl)phosphonate (4.11 g, 16.9 mmol) in THF (30 mL). After stirring for 30 min at −78 °C, a solution of aldehyde **12** (4.6 g, 15.4 mmol) in THF (15 mL) was added dropwise with a cannula. After stirring the resulting solution for 1 h at −78 °C and 3 h at 25 °C, a solution of 10% HCl was added until pH was neutral, and the resulting mixture was extracted with Et_2O (3 × 25 mL). The combined organic layers were washed with H_2O and brine, dried ($MgSO_4$), and concentrated. Purification by chromatography (silica, 90:10 hexane/ethyl acetate) afforded ester **13** (6.20 g, 95%) as a yellowish oil. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.07 (9H, s), 1.21 (3H, t, J = 7.2 Hz), 4.16 (2H, q, J = 7.2 Hz), 4.68 (2H, dd, J_{H-H} = 5.7 Hz, $^4J_{H-F}$ = 3.4 Hz), 6.11 (1H, dt, $^3J_{H-F}$ = 19.8 Hz, J_{H-H} = 5.7 Hz), 7.4−7.5 (6H, m), 7.6−7.7 (4H, m). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 13.8, 19.1, 26.7 (3×), 59.1 ($^3J_{C-F}$ = 6.8 Hz), 61.5, 124.0 ($^2J_{C-F}$ = 18.0 Hz), 127.8 (4×), 129.8 (2×), 133.3 (2×), 135.6 (4×), 145.6 ($^1J_{C-F}$ = 255.8 Hz), 160.5 ($^2J_{C-F}$ = 35.7 Hz). IR (NaCl): υ 1735 (s, C=O) cm^{-1}. HMRS: calcd for ([$C_{22}H_{27}FO_3Si$] − [C_4H_9(*t*-Bu)]), 329.1009; found, 329.1006.

(2*E*)-4-[(*tert*-Butyldiphenylsilyl)oxy]-2-fluorobut-2-en-1-ol (14). The following reduction procedure is representative. To a solution of ester **13** (5.2 g, 13.4 mmol) in THF (10 mL) at −78 °C was added DIBALH (28.2 mL, 1 M in hexane, 28.2 mmol), and the resulting suspension was stirred for 1 h at 0 °C. After careful addition of H_2O, the mixture was extracted with ether (3 × 40 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (silica, 85:15 hexane/ethyl acetate) to afford alcohol **14** (4.36 g, 94%) as a colorless oil. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.04 (9H, s), 4.03 (2H, dd, $^3J_{H-F}$ = 19.7 Hz, J_{H-H} = 5.1 Hz), 4.21 (2H, dd, J_{H-H} = 7.3 Hz, $^4J_{H-F}$ = 2.0 Hz), 5.41 (1H, dt, $^3J_{H-F}$ = 20.1 Hz, J_{H-H} = 7.3 Hz), 7.4−7.5 (6H, m), 7.6−7.7 (4H, m). ^{13}C-NMR (75.47 MHz, $CDCl_3$): δ 19.6, 26.6 (3×), 57.5 ($^2J_{C-F}$ = 30.5 Hz), 58.4 ($^3J_{C-F}$ = 13.4 Hz), 107.9 ($^2J_{C-F}$ = 20.1 Hz), 127.7 (4×), 129.8 (2×), 133.0 (2×), 135.5 (4×), 159.7 ($^1J_{C-F}$ = 254.5 Hz). IR (NaCl): υ 3600−3070 (br, OH) cm^{-1}. HMRS: calcd for ([$C_{20}H_{25}FO_2Si$] − [C_4H_9 (*t*-Bu)]): 287.0904; found, 287.0909.

(2*E*)-4-[(*tert*-Butyldiphenylsilyl)oxy]-2-fluorobut-2-enal (15). The following oxidation procedure is representative. $BaMnO_4$ (10.4 g, 90%, 0.041 mol) was added in one portion to a solution of alcohol **14** (1.4 g, 4.1 mmol) in CH_2Cl_2 (40 mL). After stirring at 25 °C for 12 h, the reaction mixture was filtered through Celite, and the solvents were removed. The residue was purified by chromatography (silica, 90:10 hexane/ethyl acetate) to provide aldehyde **15** (1.3 g, 93%) as a colorless oil. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.09 (9H, s), 4.60 (2H, dd, J_{H-H} = 6.4 Hz, $^4J_{H-F}$ = 3.3 Hz), 6.25 (1H, dt, $^3J_{H-F}$ = 18.2 Hz, J_{H-H} = 6.4 Hz), 7.4−7.5 (6H, m), 7.6−7.7 (4H, m), 9.66 (1H, d, $^3J_{H-F}$ = 15.5 Hz). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 18.9, 26.6 (3×), 57.7 ($^3J_{C-F}$ = 10.1 Hz), 124.7 ($^2J_{C-F}$ = 17.0 Hz), 127.9 (2×), 130.1 (4×), 132.4 (2×), 135.4 (4×), 153.4 ($^1J_{C-F}$ = 257.9 Hz), 183.1 ($^2J_{C-F}$ = 29.0 Hz). IR (NaCl): υ 1712 (s, C=O) cm^{-1}. HRMS: calcd for ([$C_{20}H_{23}FO_2Si$] − [C_4H_9(*t*-Bu)]), 285.0747; found, 285.0751.

Ethyl (2*E*,4*E*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-2-fluoro-4-methylhexa-2,4-dienoate (17). Ester **17** was obtained as a colorless oil in 92% yield by the procedure described for **13**. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.08 (9H, s), 1.31 (3H, t, J = 7.2 Hz), 1.67 (3H, s), 4.27 (2H, q, J = 7.2 Hz), 4.33 (2H, d, J = 6.1 Hz), 5.49 (1H, tt, $^3J_{H-H}$ = 6.1 Hz, $^5J_{H-F}$ = $^4J_{H-H}$ = 1.2 Hz), 6.37 (1H, d, $^3J_{H-F}$ = 22.5 Hz), 7.4−7.5 (6H, m), 7.7−7.8 (4H, m). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 14.0, 16.3, 19.1, 26.7 (3×), 61.1, 61.4, 123.7 ($^2J_{C-F}$ = 23.7 Hz), 127.6 ($^3J_{C-F}$ = 9.3 Hz), 127.7 (4×), 129.7 (2×), 133.5 (2×), 134.1 ($^4J_{C-F}$ = 5.0 Hz), 135.5 (4×), 146.7 ($^1J_{C-F}$ = 253.1 Hz), 160.6 ($^2J_{C-F}$ = 36.4 Hz). IR (NaCl): υ 1736 (s, C=O) cm^{-1}. HRMS: calcd for ([$C_{25}H_{31}FO_3Si$] − [(C_4H_9 (*t*-Bu)]), 369.1322; found, 369.1328.

(2*E*,4*E*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-2-fluoro-4-methylhexa-2,4-dien-1-ol (18). Alcohol **18** was obtained as a colorless oil in 90% yield, by the general procedure. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.07 (9H, s), 1.58 (3H, s), 4.29 (2H, d, J = 6.3 Hz), 4.27 (2H, d, $^3J_{H-F}$ = 25.6 Hz), 5.55 (1H, tt, $^3J_{H-H}$ = 6.3 Hz, $^5J_{H-F}$ = $^4J_{H-H}$ = 1.2 Hz), 5.76 (1H, d, $^3J_{H-F}$ = 21.3 Hz), 7.4−7.5 (6H, m), 7.7−7.8 (4H, m). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 16.7, 19.1, 26.7 (3×), 57.9 ($^2J_{C-F}$ = 30.0 Hz), 60.8, 114.3 ($^2J_{C-F}$ = 25.3 Hz), 127.7 (4×), 129.3 ($^3J_{C-F}$ = 12.0 Hz), 129.7 (2×), 130.4 ($^4J_{C-F}$ = 3.2 Hz), 133.7 (2×), 135.6 (4×), 158.5 ($^1J_{C-F}$ = 251.3 Hz). IR (NaCl): υ 3500−3100 (br, OH) cm^{-1}. HRMS: calcd for $C_{23}H_{29}FO_2Si$, 384.1922; found, 384.1924.

(2*E*,4*E*)-6-[(*tert*-Butyldiphenylsilyl)oxy]-2-fluoro-4-methylhexa-2,4-dienal (19). Aldehyde **19** obtained as a colorless oil in 90% yield, by the general procedure, was used in the next step without further purification. ^{1}H NMR (250.13 MHz, C_6D_6): δ 1.17 (9H, s), 1.21 (3H, s), 4.12 (2H, d, J = 6.0 Hz), 5.59 (1H, tt, $^3J_{H-H}$ = 6.0 Hz, $^5J_{H-F}$ = $^4J_{H-H}$ = 1.3 Hz), 6.11 (1H, d, $^3J_{H-F}$ = 17.8 Hz), 7.2−7.3 (6H, m), 7.7−7.8 (4H, m), 9.40 (1H, d, $^3J_{H-F}$ = 20.0 Hz). IR (NaCl): υ 1692 (s, C=O) cm^{-1}.

(2*E*,4*E*)-*tert*-Butyldiphenylsilyl 3-Fluoro-5-(2,6,6-trimethylcyclohex-1-en-1-yl)penta-2,4-dien-1-yl Ether (21). Following the general procedure for olefination, treating phosphonium salt **20** (0.7 g, 1.46 mmol) in THF (8 mL) with *n*-BuLi (0.91 mL, 1.6 M in hexane, 1.46 mmol) and adding aldehyde **15** (0.45 g, 1.32 mmol) in THF (5 mL) provided, after 7 h, a residue which was purified by chromatography (silica, first with 50:50 hexane/Et_2O, and then on a second column with 99:1 hexane/Et_2O) to give silyl ether **21** (0.54 g, 89%) as a yellow oil. ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 0.98 (6H, s), 1.04 (9H, s), 1.4−1.6 (4H, m), 1.61 (3H, s), 1.99 (2H, t, J = 6.0 Hz), 4.25 (2H, dd, J_{H-H} = 7.5 Hz, $^4J_{H-F}$ = 1.3 Hz), 5.31 (1H, dt, $^3J_{H-F}$ = 20.4 Hz, J_{H-H} = 7.5 Hz), 5.89 (1H, dd, $^3J_{H-F}$ = 17.2 Hz, J_{H-H} = 16.0 Hz), 6.48 (1H, d, J = 16.0 Hz), 7.3−7.5 (6H, m), 7.6−7.7 (4H, m). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 19.0, 19.1, 21.5, 26.7 (3×), 28.8 (2×), 33.0, 34.0, 39.5, 58.5 ($^3J_{C-F}$ = 14.8 Hz), 106.5 ($^2J_{C-F}$ = 22.3 Hz), 120.3 ($^2J_{H-F}$ = 22.0 Hz), 127.8 (4×), 129.7 (2×), 130.1 ($^3J_{C-F}$ = 5.1 Hz), 130.9 (2×), 133.5, 135.6 (4×), 136.8, 157.0 ($^1J_{C-F}$ = 242.6 Hz). HRMS: calcd for $C_{30}H_{39}FOSi$, 462.2754; found, 462.2749.

(2*E*,4*E*)-3-Fluoro-5-(2,6,6-trimethylcyclohex-1-en-1-yl)-penta-2,4-dien-1-ol (22). The following procedure for deprotection is general. Tetrabutylammonium fluoride (0.97 mL,

(34) The purity of all new compounds was judged by a combination of HPLC and ^{1}H and ^{13}C NMR analysis before mass spectral were recorded and is indicated in the supporting information by copies of the ^{1}H NMR /^{13}C NMR spectra of selected compounds.

1.0 M in THF, 0.97 mmol) was added to a solution of silyl ether **21** (0.39 g, 0.85 mmol) in Et_2O (3 mL). After stirring at 25 °C for 2 h, ether (15 mL) was added, the resulting solution was washed with saturated $NaHCO_3$ and brine and dried ($MgSO_4$), and the solvent was removed. The residue was purified by chromatography (silica, 70:30 hexane/ether) to afford alcohol **22** (0.91 g, 100%) as an orange oil. ^{1}H NMR (250.13 MHz, C_6D_6): δ 1.05 (6H, s), 1.4−1.6 (4H, m), 1.70 (3H, s), 1.91 (2H, t, J = 6.0 Hz), 2.66 (1H, broad), 4.02 (2H, d, J = 7.9 Hz), 5.37 (1H, dt, $^3J_{H-F}$ = 20.0 Hz, J_{H-H} = 7.9 Hz), 6.24 (1H, dd, $^3J_{H-F}$ = 17.8 Hz, J_{H-H} = 16.0 Hz), 6.81 (1H, d, J = 16.0 Hz). ^{13}C NMR (62.89 MHz, C_6D_6): δ 19.4, 21.5, 28.8 (2×), 33.2, 34.2, 39.7, 56.6 ($^3J_{C-F}$ = 13.3 Hz), 107.1 ($^2J_{C-F}$ = 21.9 Hz), 120.4 ($^2J_{C-F}$ = 22.0 Hz), 130.7 ($^3J_{C-F}$ = 5.1 Hz), 131.4, 137.1, 158.6 ($^1J_{C-F}$ = 247.5 Hz). IR (NaCl): υ 3510−3040 (br, OH) cm^{-1}. HRMS: calcd for $C_{14}H_{21}FO$, 224.1576; found, 224.1578.

(2*E*,4*E*)-3-Fluoro-5-(2,6,6-trimethylcyclohex-1-en-1-yl)-penta-2,4-dienal (23). To a solution of alcohol **22** (0.4 g, 1.78 mmol) in CH_2Cl_2 (30 mL) were added, in one portion, 2.79 g (32.12 mmol) of MnO_2 and 3.41 g (32.12 mmol) of Na_2CO_3. After stirring at 25 °C for 10 h, the reaction mixture was filtered through Celite and the solvents were removed. The residue was purified by chromatography (silica, 95:5 hexane/ethyl acetate) to provide aldehyde **23** (0.34 g, 86%) as a yellow oil, accompanied by the 2*Z* isomer (0.04 g, 9%). ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.08 (6H, s), 1.4−1.6 (4H, m), 1.80 (3H, s), 2.08 (2H, t, J = 6.0 Hz), 5.72 (1H, dd, $^3J_{H-F}$ = 18.4 Hz, J_{H-H} = 7.1 Hz), 6.72 (1H, dd, $^3J_{H-F}$ = 27.3 Hz, J_{H-H} = 16.0 Hz), 7.08 (1H, d, J = 16.0 Hz), 9.92 (1H, dd, J_{H-H} = 7.1 Hz, $^4J_{H-F}$ = 3.4 Hz). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 18.7, 21.6, 28.7 (2×), 33.6, 34.1, 39.6, 108.9 ($^2J_{C-F}$ = 20.2 Hz), 118.1 ($^2J_{C-F}$ = 18.5 Hz), 136.4, 136.5, 138.8 ($^3J_{C-F}$ = 6.6 Hz), 172.1 ($^1J_{C-F}$ = 274.8 Hz), 188.8 ($^3J_{C-F}$ = 20.1 Hz). IR (NaCl): υ 1684 (s, C=O) cm^{-1}. HRMS: calcd for $C_{14}H_{19}FO$, 222.1420; found, 222.1420.

Ethyl (7*E*,9*E*,11*E*,13*E*)-9-Demethyl-9-fluororetinoate (ethyl 9-*cis*-9-demethyl-9-fluororetinoate) (25). To a cooled (−78 °C) solution of phosphonate **24**[21b] (0.16 g, 0.535 mmol) in THF (4 mL) were added *n*-BuLi (0.22 mL, 2.7 M in hexane, 0.594 mmol, via a syringe) and DMPU (0.14 mL, 1.1 mmol). After stirring at −78 °C for 30 min, a solution of aldehyde **23** (0.066 g, 0.298 mmol) in THF (5 mL) was added through a cannula. After stirring the resulting solution at −78 °C for 1 h and at 0 °C for 5 h, a solution of saturated NH_4Cl was added, and the resulting mixture was extracted with ether (3 × 25 mL). The combined organic layers were washed with H_2O and brine, dried ($MgSO_4$), and concentrated. Purification by chromatography (silica, 95:5 hexane/ethyl acetate) afforded ester **25** (0.073 g, 74%) as a yellow oil. ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.06 (6H, s), 1.29 (3H, t, J = 7.0 Hz), 1.4−1.6 (4H, m), 1.78 (3H, s), 2.06 (2H, t, J = 6.0 Hz), 2.33 (3H, s), 4.17 (2H, q, J = 7.0 Hz), 5.77 (1H, s), 5.89 (1H, dd, $^3J_{H-F}$ = 18.8 Hz, J_{H-H} = 11.8 Hz), 6.24 (1H, d, J = 15.2 Hz), 6.28 (1H, dd, $^3J_{H-F}$ = 27.4 Hz, J_{H-H} = 15.9 Hz), 6.64 (1H, d, J = 15.9 Hz), 6.71 (1H, dd, J = 15.2 Hz, 11.8 Hz). ^{13}C NMR (62.89 MHz, $CDCl_3$): δ 13.7, 14.3, 19.0, 21.7, 28.9 (2×), 33.2, 34.2, 39.5, 59.7, 109.5 ($^2J_{C-F}$ = 30.2 Hz), 119.1, 119.3 ($^2J_{C-F}$ = 20.9 Hz), 127.1 ($^3J_{C-F}$ = 11.4 Hz), 131.6 ($^4J_{C-F}$ = 4.5 Hz), 132.4, 135.1 (dd, $^3J_{C-F}$ = 9.6 Hz), 137.1, 151.9, 160.0 ($^1J_{C-F}$ = 272.8 Hz), 167.2. HRMS: calcd for $C_{21}H_{29}FO_2$, 332.2151; found, 332.2150.

(7*E*,9*E*,11*E*,13*E*)-9-Demethyl-9-fluororetinal (9-*cis*-9-demethyl-9-fluororetinal) (26). DIBALH (0.45 mL, 1 M in hexane, 0.45 mmol) was added to a solution of ester **25** (0.070 g, 0.211 mmol) in THF (5 mL) at −78 °C, and the resulting suspension was stirred for 1 h at 0 °C. After careful addition of H_2O, the mixture was extracted with ether (10 × 3 mL). The combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (silica, 85:15 hexane/ethyl acetate) to afford the corresponding alcohol (0.037 g, 60%) as a yellow oil. This compound (0.037 g, 0.127 mmol) in CH_2Cl_2 (5 mL) was oxidized with MnO_2 (0.198 g, 2.28 mmol) and Na_2CO_3 (0.246 g, 2.28 mmol) at 25 °C for 6 h. Workup and purification as described above (silica 90:10 hexane/ether) provided aldehyde **26** (0.033 g, 90%). A sample for analysis was obtained by HPLC purification (98:2 hexane/ethyl acetate, t_R = 33.1 min). ^{1}H NMR (250.13 MHz): Table 1. IR (NaCl): υ 1654 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} 288, 306, 320 nm. HRMS: calcd for $C_{19}H_{25}FO$, 288.1889; found, 288.1890.

(2*Z*,4*E*)-[3-Fluoro-5-(2,6,6-trimethylcyclohex-1-en-1-yl)-penta-2,4-dienyl]triphenylphosphonium Bromide (27). A solution of alcohol **22** (1.1 g, 4.9 mmol) in MeOH (20 mL) was added through a cannula to a suspension of triphenylphosphine hydrobromide (1.69 g, 4.9 mmol) in MeOH (17 mL). After stirring at 25 °C for 48 h, the solvent was removed under vacuum and the residue was crystallized to provide phosphonium salt **27** (2.46 g, 93%) as yellow crystals (mp 76−78 °C). ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 0.93 (6H, s), 1.4−1.6 (4H, m), 1.61 (3H, s), 1.96 (2H, t, J = 6.0 Hz), 4.7−5.0 (3H, m), 5.70 (1H, dd, $^3J_{H-F}$ = 26.3 Hz, J_{H-H} = 15.9 Hz), 6.29 (1H, d, J = 15.9 Hz), 7.7−7.9 (15H, m). HRMS: calcd for ([$C_{32}H_{35}BrFP$] − [Br]), 469.2461; found, 469.2468.

(7*E*,9*Z*,11*E*,13*E*)- and (7*E*,9*Z*,11*Z*,13*E*)-*tert*-Butyldiphenylsilyl 9-Demethyl-9-fluororetinyl Ethers (*trans*- and 11-*cis*-*tert*-butyldiphenylsilyl 9-demethyl-9-fluororetinyl ethers) (28 and 29). Following the general procedure described above for **13**, a mixture of **28** and **29** was prepared from phosphonium salt **27** (0.42 g, 0.76 mmol) in THF (10 mL), *n*-BuLi (0.48 mL, 1.6 M in hexane, 0.76 mmol), and aldehyde **16**[17b] (0.24 g, 0.69 mmol) in THF (5 mL). The residue obtained after chromatography (0.30 g, 83%, 68:32 11*E*/11*Z* isomer ratio) was used in the next step without further purification.

(7*E*,9*Z*,11*E*,13*E*)- and (7*E*,9*Z*,11*Z*,13*E*)-9-Demethyl-9-fluororetinols (*trans*- and 11-*cis*-9-demethyl-9-fluororetinols) (30 and 31). Application of the general procedure to a mixture of the silyl ethers **28** and **29** (0.30 g, 0.56 mmol) afforded, after chromatography (silica, 70:28:2 hexane/ether/triethylamine), the corresponding mixture of alcohols (0.12 g, 70%). The 11*E* and 11*Z* fluororetinol isomers **30** and **31** were separated by HPLC (83:17 hexane/ethyl acetate, t_R (11*Z*) = 26 min, t_R (11*E*) = 34 min). **(7*E*,9*Z*,11*E*,13*E*)-9-Demethyl-9-fluororetinol (*trans*-9-demethyl-9-fluororetinol) (30).** ^{1}H NMR (250.13 MHz, C_6D_6): δ 1.10 (6H, s), 1.4−1.6 (4H, m), 1.58 (3H, s), 1.73 (3H, s), 1.93 (2H, t, J = 6.0 Hz), 4.03 (2H, d, J = 6.4 Hz), 5.41 (1H, dd, $^3J_{H-F}$ = 34.4 Hz, J_{H-H} = 11.2 Hz), 5.65 (1H, t, J = 6.4 Hz), 5.92 (1H, dd, $^3J_{H-F}$ = 26.5 Hz, J_{H-H} = 16.0 Hz), 6.25 (1H, d, J = 15.5 Hz), 6.82 (1H, d, J = 16.0 Hz), 6.85 (1H, dd, J = 15.5, 11.2 Hz). IR (NaCl): υ 3570−3220 (br, OH) cm^{-1}. HRMS: calcd for $C_{19}H_{27}FO$, 290.2046; found, 290.2052. **(7*E*,9*Z*,11*Z*,13*E*)-9-Demethyl-9-fluororetinol (11-*cis*-9-demethyl-9-fluororetinol) (31).** ^{1}H NMR (250.13 MHz, C_6D_6): δ 1.07 (6H, s), 1.4−1.6 (4H, m), 1.64 (3H, s), 1.66 (3H, s), 1.90 (2H, t, J = 5.7 Hz), 3.98 (2H, d, J = 6.6 Hz), 5.67 (1H, t, J = 6.6 Hz), 5.83 (1H, d, J = 11.2 Hz), 5.90 (1H, dd, $^3J_{H-F}$ = 26.5 Hz, J_{H-H} = 16.0 Hz), 6.12 (1H, dd, $^3J_{H-F}$ = 33.4 Hz, J_{H-H} = 12.2 Hz), 6.64 (1H, t, J = 11.9 Hz), 6.83 (1H, d, J = 16.0 Hz). ^{13}C NMR (62.89 MHz, C_6D_6): δ 16.6, 19.4, 21.6, 28.8 (2×), 33.3, 34.3, 39.8, 59.2, 107.1 ($^3J_{C-F}$ = 11.7 Hz), 120.4 ($^4J_{C-F}$ = 6.8 Hz), 124.7 ($^2J_{C-F}$ = 22.2 Hz), 129.6 ($^3J_{C-F}$ = 4.8 Hz), 131.6 ($^2J_{C-F}$ = 29.9 Hz), 133.6, 133.7, 135.4, 137.3, 158.3 ($^1J_{C-F}$ = 257.7 Hz). UV (MeOH): λ_{max} (ϵ) 322 (6 800) nm.

(7*E*,9*Z*,11*E*,13*E*)-9-Demethyl-9-fluororetinal (*trans*-9-demethyl-9-fluororetinal) (7). Following the general procedure described for oxidation of alcohol **14**, retinol **30** (0.11 g, 0.38 mmol) was reacted with $BaMnO_4$ (1.12 g, 90%, 3.9 mmol) in CH_2Cl_2 (5 mL), affording retinal **7** (0.10 g) in 88% yield as an orange solid (mp 60−62 °C; hexane/ethyl acetate). HPLC (96:4, hexane/ethyl acetate; t_R= 34.5 min). ^{1}H NMR (300.13 MHz): Table 1. IR (NaCl): υ 1660 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} (ϵ) 368 (8 200) nm. HRMS: calcd for $C_{19}H_{25}FO$, 288.1889; found, 288.1880.

(7*E*,9*E*,11*E*,13*E*)-*tert*-Butyldiphenylsilyl 11-Fluororetinyl Ether (11-*cis*-*tert*-butyldiphenylsilyl 11-fluororetinyl ether) (33). Following the general procedure for Wittig olefination, the title compound was obtained in 57% yield, after HPLC purification (99.9:0.1 hexane/ethyl acetate; t_R = 38 min). ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.02 (6H, s), 1.04 (9H, s), 1.4−1.6 (4H, m), 1.59 (3H, s), 1.68 (3H, s), 2.00 (2H, t, J = 6.4 Hz), 2.08 (3H, s), 4.29 (2H, d, J = 6.2 Hz), 5.64 (1H, d, $^3J_{H-F}$ = 22.2 Hz), 5.65 (1H, d, J = 6.2 Hz), 6.07 (1H, d, J = 15.8 Hz), 6.20 (1H, d, $^3J_{H-F}$ = 20.8 Hz), 6.27 (1H, d, J = 15.8 Hz), 7.3−

7.4 (6H, m), 7.6−7.7 (4H, m). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 14.1 ($^4J_{C-F}$ = 10.1 Hz), 17.0, 19.1, 19.2, 21.6, 26.7 (3×), 28.8 (2×), 32.9, 34.2, 39.5, 61.1, 114.9 ($^2J_{C-F}$ = 30.6 Hz), 118.4 ($^2J_{C-F}$ = 20.4 Hz), 127.6, 127.7 (4×), 127.9 ($^3J_{C-F}$ = 12.1 Hz), 128.9, 129.6 (2×), 130.7 ($^4J_{C-F}$ = 5.1 Hz), 133.8 (2×), 134.8, 135.6 (4×), 137.7, 139.0 ($^3J_{C-F}$ = 3.3 Hz), 157.5 ($^1J_{C-F}$ = 245.6 Hz). HRMS: calcd for $C_{36}H_{47}FOSi$, 542.3380; found, 542.3383.

(7*E*,9*E*,11*E*,13*E*)-11-Fluororetinol (11-*cis*-11-fluororetinol) (34). Application of the general procedure to silyl ether **33** afforded a 74% yield of alcohol **34** after HPLC purification (87:13, hexane/ethyl acetate; t_R = 28.9 min). ^{1}H NMR (250.13 MHz, C_6D_6): δ 1.09 (6H, s), 1.4−1.6 (4H, m), 1.53 (3H, s), 1.73 (3H, s), 1.95 (2H, t, J = 6.0 Hz), 2.20 (3H, s), 3.90 (2H, d, J = 6.6 Hz), 5.58 (1H, dt, J_{H-H} = 6.6 Hz, $^5J_{H-F}$ = 1.2 Hz), 5.79 (1H, d, $^3J_{H-F}$ = 22.9 Hz), 6.27 (1H, d, J = 16.0 Hz), 6.34 (1H, d, $^3J_{H-F}$ = 29.3 Hz), 6.39 (1H, d, J = 16.0 Hz). ^{13}C NMR (62.89 MHz, C_6D_6): δ 14.3 ($^4J_{C-F}$ = 10.1 Hz), 16.7, 19.5, 21.7, 28.9 (2×), 33.1, 34.4, 39.7, 59.2, 115.5 ($^2J_{C-F}$ = 30.4 Hz), 119.1 ($^2J_{C-F}$ = 20.3 Hz), 127.3, 129.4, 131.4 ($^4J_{C-F}$ = 5.1 Hz), 131.7 ($^3J_{C-F}$ = 12.1 Hz), 137.9, 138.1, 139.6 ($^3J_{C-F}$ = 3.4 Hz), 158.0 ($^1J_{C-F}$ = 245.6 Hz). IR (NaCl): υ 3500−3100 (br, OH) cm^{-1}. HRMS: calcd for $C_{20}H_{29}FO$, 304.2202; found, 304.2208.

(7*E*,9*E*,11*E*,13*E*)-11-Fluororetinal (11-*cis*-11-fluororetinal) (35). Following the general procedure, a solution of 11-fluororetinol **34** (0.04 g, 0.14 mmol) in CH_2Cl_2 (5 mL) was treated for 8 h with MnO_2 and Na_2CO_3 at 25 °C to afford, after chromatography, retinal **35** (32 mg, 80%). Purification by HPLC (97:3 hexane/ethyl acetate, t_R = 31.9 min) provided a sample for analysis. ^{1}H NMR (250.13 MHz, C_6D_6): Table 1. HRMS: calcd for $C_{20}H_{27}FO$, 302.2046; found, 302.2052.

(7*E*,9*E*,11*Z*,13*E*)-11-Fluororetinal (*trans*-11-fluororetinal) (8). In accordance with the general procedure, a solution of 11-fluororetinol **34** (0.072 g, 0.23 mmol) in CH_2Cl_2 (5 mL) was treated for 10 h with $BaMnO_4$ (0.61 g, 90%, 2.14 mmol) at 25 °C to afford, after chromatography, retinal **8** (0.067 g, 94%). Purification by HPLC (97:3, hexane/ethyl acetate; t_R = 34.0 min) provided a sample for analysis. ^{1}H NMR (250.13 MHz): Table 1. IR (NaCl): 1660 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} (ϵ) 370 (11 800) nm. HRMS: calcd for $C_{20}H_{27}FO$, 302.2046; found, 302.2044.

(7*E*,9*E*,11*E*,13*E*)- and (7*E*,9*E*,11*Z*,13*E*)-*tert*-Butyldiphenylsilyl 13-Demethyl-13-fluororetinyl Ethers (13-*cis*- and 11,13-di-*cis*-*tert*-butyldiphenylsilyl 13-demethyl-13-fluororetinyl ethers) (37 and 38). The yellow oil afforded by the general procedure was a 69% combined yield of a 60:40 mixture of the 11*E* and 11*Z* isomers **37** and **38**, which were used in the next step without separation.

(7*E*,9*E*,11*E*,13*E*)- and (7*E*,9*E*,11*Z*,13*E*)-13-Demethyl-13-fluororetinols (13-*cis*- and 11,13-di-*cis*-13-demethyl-13-fluororetinols) (39 and 40). Application of the general procedure described above for **22** to a 60:40 mixture of the silyl ethers **37** and **38** (0.34 g, 0.56 mmol, *E*/*Z* mixture) afforded, after chromatography (silica, 80:18:2 hexane/ether/triethylamine), the corresponding mixture of alcohols **39** and **40** (0.17 g, 93%) which were separated by HPLC (87:13 hexane/ethyl acetate, t_R (11*Z*) = 23.5 min, t_R (11*E*) = 37 min). **(7*E*,9*E*,11*Z*,13*E*)-13-Demethyl-13-fluororetinol (11,13-di-*cis*-13-demethyl-13-fluororetinol) (40).** ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.02 (6H, s), 1.4−1.6 (4H, m), 1.71 (3H, s), 1.96 (3H, s), 2.02 (2H, t, J = 6.2 Hz), 4.22 (2H, d, J = 7.0 Hz), 5.40 (1H, dt, $^3J_{H-F}$ = 20.6 Hz, J_{H-H} = 7.0 Hz), 5.90 (1H, dd, $^3J_{H-F}$ = 32.5 Hz, J_{H-H} = 11.4 Hz), 6.15 (1H, d, J = 16.1 Hz), 6.29 (1H, d, J = 16.1 Hz), 6.55 (1H, t, J = 11.6 Hz), 6.70 (1H, d, J = 12.0 Hz). IR (NaCl): υ 3600−3100 (br, OH) cm^{-1}. HRMS: calcd for $C_{19}H_{27}FO$, 290.2046; found, 290.2049. **(7*E*,9*E*,11*E*,13*E*)-13-Demethyl-13-fluororetinol (13-*cis*-13-demethyl-13-fluororetinol) (39).** ^{1}H NMR (250.13 MHz, $CDCl_3$): δ 1.02 (6H, s), 1.4−1.6 (4H, m), 1.71 (3H, s), 1.98 (5H, m), 4.23 (2H, d, J = 7.5 Hz), 5.40 (1H, dt, $^3J_{H-F}$ = 20.5 Hz, J_{H-H} = 7.5 Hz), 6.10 (1H, d, J = 11.6 Hz), 6.11 (1H, d, J = 15.9 Hz), 6.25 (1H, dd, $^3J_{H-F}$ = 27.8 Hz, J_{H-H} = 15.0 Hz), 6.28 (1H, d, J = 15.9 Hz), 7.00 (1H, dd, J = 15.0, 11.6 Hz). HRMS: calcd for $C_{19}H_{27}FO$, 290.2046; found, 290.2050.

(7*E*,9*E*,11*E*,13*E*)- and (7*E*,9*E*,11*E*,13*Z*)-13-Demethyl-13-fluororetinals (13-*cis*- and 13-*trans*-13-demethyl-13-fluororetinals) (41 and 9). A solution of retinol **39** (0.032 g, 0.011 mmol) in CH_2Cl_2 (10 mL) was treated with activated MnO_2 (0.17 g, 1.98 mmol) and Na_2CO_3 (0.21 g, 1.98 mmol). After stirring at 25 °C for 3 h, the mixture was filtered through Celite, and the solvents were removed in vacuo, to afford a mixture of retinals **41** and **9** (0.025 g, 80%) in a 4:1 ratio, which were separated by HPLC (98:2 hexane/ethyl acetate, t_R (13*E*) = 23.6 min, t_R (13*Z*) = 32.4 min). **(7*E*,9*E*,11*E*,13*E*)-13-Demethyl-13-fluororetinal (13-*cis*-13-demethyl-13-fluororetinal) (41).** ^{1}H NMR (500.13 MHz): Table 1. HRMS: calcd for $C_{19}H_{25}FO$, 288.1889; found, 288.1885. **(7*E*,9*E*,11*E*,-13*Z*)-13-Demethyl-13-fluororetinal (*trans*-13-demethyl-13-fluororetinal) (9).** ^{1}H NMR (500.13 MHz): Table 1. IR (NaCl): υ 1669 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} (ϵ) 382 (10 600) nm. HRMS: calcd for $C_{19}H_{25}FO$: 288.1889; found, 288.1890.

(7*E*,9*Z*,11*E*,13*E*)- and (7*E*,9*Z*,11*Z*,13*E*)-*tert*-Butyldiphenylsilyl 9,13-Didemethyl-9,13-difluororetinyl Ethers (13-*cis*- and 11,13-di-*cis*-*tert*-butyldiphenylsilyl 9,13-didemethyl-9,13-difluororetinyl ethers) (42 and 43). The general procedure effected coupling between phosphonium salt **27** (0.67 g, 1.23 mmol), treated at −78 °C with *n*-BuLi (0.77 mL, 1.6 M en hexane, 1.23 mmol), and aldehyde **15** (0.38 g, 1.12 mmol). Chromatography of the resulting crude on silica, first with 50:50 hexane/ether, and a second column with 99:1 hexane/ether, afforded an 89% yield (0.53 g) of a 75:25 mixture of the 11*E* and 11*Z* isomers **42** and **43**, which was used in the next step without separation.

(7*E*,9*Z*,11*E*,13*E*)- and (7*E*,9*Z*,11*Z*,13*E*)-9,13-Didemethyl-9,13-difluororetinols (13-*cis*- and 11,13-di-*cis*-9,13-didemethyl-9,13-difluororetinols) (44 and 45). The general procedure afforded a residue which was purified (silica, 75:25 hexane/ether) to afford 0.091 g (76%) of a mixture of the 11*E* and 11*Z* isomers **44** and **45**, which were separated by HPLC (85:15, hexane/ethyl acetate; t_R (11*Z*) = 34 min, t_R (11*E*) = 42 min). **(7*E*,9*Z*,11*E*,13*E*)-9,13-Didemethyl-9,13-difluororetinol (13-*cis*-9,13-didemethyl-9,13-difluororetinol) (44).** ^{1}H NMR (250.13 MHz, C_6D_6): δ 1.07 (6H, s), 1.4−1.6 (4H, m), 1.70 (3H, s), 1.91 (2H, t, J = 6.0 Hz), 3.81 (2H, d, J = 7.7 Hz), 5.1−5.3 (2H, m), 5.88 (1H, dd, $^3J_{H-F}$ = 26.0 Hz, J_{H-H} = 16.1 Hz), 6.10 (1H, dd, $^3J_{H-F}$ = 27.4 Hz, J_{H-H} = 15.5 Hz), 6.83 (1H, d, J = 16.1 Hz), 7.35 (1H, dd, J =15.5, 11.6 Hz). ^{13}C NMR (62.89 MHz, C_6D_6): δ 19.3, 21.5, 28.8 (2×), 33.4, 34.3, 39.9, 56.8 ($^3J_{C-F}$ = 13.5 Hz), 108.7 ($^2J_{C-F}$ = 21.0 Hz), 109.3 ($^2J_{C-F}$ = 13.6 Hz), 118.6 ($^2J_{C-F}$ = 23.7 Hz, $^4J_{C-F}$ = 3.4 Hz), 123.8 ($^3J_{C-F}$ = 11.7 Hz), 123.9 ($^2J_{C-F}$ = 13.7 Hz), 130.8 ($^3J_{C-F}$ = 4.3 Hz), 132.2, 137.2, 147.7 ($^1J_{C-F}$ = 252.2 Hz), 158.7 ($^1J_{C-F}$ = 247.5 Hz). IR (NaCl): υ 3520−3150 (br, OH) cm^{-1}. HRMS: calcd for $C_{18}H_{24}F_2O$, 294.1795; found, 294.1793. **(7*E*,9*Z*,11*Z*,13*E*)-9,13-Didemethyl-9,13-difluororetinol (11,13-di-*cis*-9,13-didemethyl-9,13-difluororetinol) (45).** ^{1}H NMR (250.13 MHz, C_6D_6): δ 1.03 (6H, s), 1.4−1.6 (4H, m), 1.60 (3H, s), 1.87 (2H, t, J = 6.1 Hz), 3.72 (2H, d, J = 7.7 Hz), 5.24 (1H, dt, $^3J_{H-F}$ = 21.3 Hz, J_{H-H} = 7.7 Hz), 5.66 (1H, dd, $^3J_{H-F}$ = 34.2 Hz, J_{H-H} = 10.9 Hz), 5.80 (1H, dd, $^3J_{H-F}$ = 26.7 Hz, J_{H-H} = 16.3 Hz), 6.48 (1H, dd, $^3J_{H-F}$ = 32.0 Hz, J_{H-H} = 12.3 Hz), 6.64 (1H, t, J =11.6 Hz), 6.86 (1H, d, J = 16.3 Hz).

(7*E*,9*Z*,11*E*,13*Z*)-9,13-Didemethyl-9,13-difluororetinal (*trans*-9,13-didemethyl-9,13-difluororetinal) (10). Retinol **44** (26 mg, 88.3 mmol) was oxidized with $BaMnO_4$ (0.25 g, 90%, 0.88 mmol) and Na_2CO_3 (93 mg, 0.88 mmol) for 7 h at 25 °C. Purification of the residue (silica, 90:10 hexane/ether) provided 23 mg (92%) of retinal **10** as a solid (mp 58−60 °C, hexane/ethyl acetate) which was further purified by HPLC (96.5:3.5, hexane/ethyl acetate; t_R = 33.5 min). ^{1}H NMR (250.13 MHz): Table 1. IR (NaCl): υ 1675 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} (ϵ) 372 (6 500) nm. HRMS: calcd for $C_{18}H_{22}F_2O$, 292.1639; found, 292.1636.

(7*E*,9*Z*,11*E*,13*E*)-9,13-Didemethyl-9,13-difluororetinal (13-*cis*-9,13-didemethyl-9,13-difluororetinal) (46). This isomer was obtained as the major component in the MnO_2/Na_2CO_3 oxidation (75% yield) of alcohol **44**. ^{1}H NMR (250.13 MHz): Table 1. UV (MeOH): λ_{max} (ϵ) 370 (16 800) nm.

General Procedure for Schiff Base and Protonated Schiff Base Formation. *n*-Butylamine (3 mL, 30.9 μmol) was added at 25 °C to a solution of fluororetinal (8.1 mg, 28.1 μmol) in Et_2O (2 mL) containing 4 Å molecular sieves. After

stirring at 25 °C for 30 min, the solids were filtered out, and the solvent and excess amine were removed under vacuum. The resulting Schiff base was dissolved in MeOH (3 mL) for UV spectroscopy. The Schiff base was protonated in the same solvents by addition of trichloroacetic acid (4.6 mg, 28.1 μmol).

Preparation of Bacterio-opsin. Purple membrane was obtained from *H. salinarium* strain S9 as described.[29] Purple membrane in 4M NaCl, 1 M $NH_2OH \cdot HCl$ of 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, including reproduction of the ^1H or ^{13}C NMR spectra of selected compounds described in the text (40 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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