Synthesis of Retinals with Eight- and Nine-Membered Rings in the Side Chain. Models for Rhodopsin Photobleaching Intermediates

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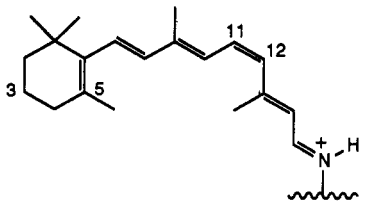
The triggering process of rhodopsin activation leading to visual transduction has remained a key problem in the chemistry of vision. In order to provide probes to explore this process, retinal analogs ret8 **3** and ret9 **4**, with the 11-ene *cis*-locked by 8- and 9-membered rings, have been synthesized. These could lead to pigment analogs which might accomodate a *transoid* chromophore upon irradiation, thus providing unique models for rhodopsin activation studies. Enzyme assays and flash photolysis of rhodopsin analogs incorporating ret8 and other retinal analogs showed that the triggering process requires complete 11-*cis* to *trans* isomerization involving the entire polyene moiety. The syntheses of ret8 **3** and ret9 **4** double bond isomers are described.

In bovine rhodopsin (Rh), 11-*cis*-retinal is linked to Lys-296 of a 40-kDa protein opsin via a protonated Schiff base (PSB) **1**.[1] Irradiation of Rh leads to 11-ene *cis* → *trans*



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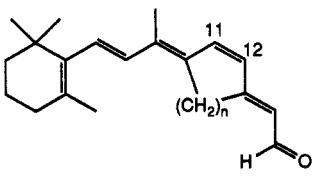
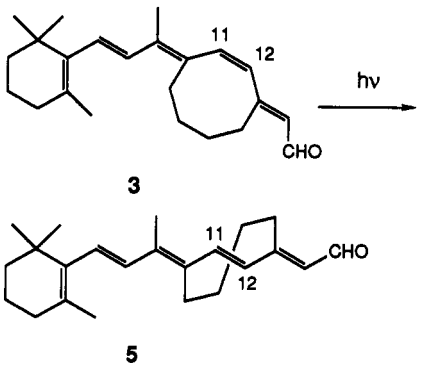
isomerization[2] and a series of intermediates culminating in enzymatic activation and visual transduction. However, the triggering process remains experimentally ambiguous: is it the isomerization or is it the electronic redistribution in the excited state that is responsible for triggering rhodopsin activation?[3] Ret7 (n = 3 in **2**), the



2 n = 1 Ret 5
n = 2 Ret 6
n = 3 Ret 7
3 n = 4 Ret 8
4 n = 5 Ret 9

first 11,12-*cis*-locked analog, gave the pigment Rh7, which was nonbleachable and nonfunctional;[4] flash photolysis detected a transient primary photoproduct (but in only 25% quantum yield of native rhodopsin), which reverted to starting Rh7.[5] It was conceived that the more flexible ret8 **3** and Ret9 **4** may accommodate a *trans* double bond upon irradiation to produce a stable intermediate such as



5 which might induce transduction. Ret8 indeed formed an unbleachable Rh8, which when irradiated gave a photoproduct and a further advanced intermediate that reverted to ground state Rh8 in 50 ns.[6] Since irradiation of Rh8 led to a photoproduct with high quantum yields and spectral properties similar to that of native Rh, charge separation occurs in the photoexcited state of Rh8. However, Rh8 was enzymatically inactive.[7] These and other results demonstrate that both mechanisms are operating, but full isomerization of the polyene system is required for initiating efficient enzymatic reactions.[7] It has so far not been possible to prepare Rh9. The syntheses of Ret8 and Ret9 are reported.[8-10]

(1) (a) Khorana, H. G. *J. Biol. Chem.* **1992**, *267*, 1. (b) Hargrave, P. A.; McDowell, J. H. *FASEB J.* **1992**, *6*, 2323.

(2) (a) Yoshizawa, T.; Wald, G. *Nature* **1963**, *197*, 1279. (b) Wald, G. *Nature* **1968**, *219*, 800. (c) Wald, G. *Science* **1968**, *162*, 230.

(3) (a) Salem, L.; Bruckmann, P. *Nature* **1975**, *258*, 526. (b) Salem, L. *Acc. Chem. Res.* **1979**, *12*, 87. (c) Albert, I. D. L.; Ramasesha, S. *J. Phys. Chem.* **1990**, *94*, 6540.

(4) (a) Akita, H.; Tanis, S. P.; Adams, M.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 6370. (b) Mao, B.; Tsuda, M.; Ebrey, T. G.; Akita, V.; Balogh-Nair, V.; Nakanishi, K. *Biophys. J.* **1981**, *35*, 543. (c) Birge, R. R.; Murray, L. P.; Pierce, B. M.; Akita, H.; Balogh-Nair, V.; Findsen, L. A.; Nakanishi, K. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4117. (d) Crouch, R.; Nodes, B. R.; Perlman, J. I.; Pepperberg, D. R.; Akita, H.; Nakanishi, K. *Inv. Ophthalm. Vis. Sci.* **1984**, *25*, 419. (e) Zankel, T.; Ok, H.; Johnson, R.; Chang, C. W.; Sekiya, N.; Naoki, H. Yoshihara, K.; Nakanishi, K. *J. Am. Chem. Soc.* **1990**, *112*, 5387.

(5) Kandori, H.; Matuoka, S.; Shichida, Y.; Yoshizawa, T.; Ito, M.; Tsukida, K.; Balogh-Nair, V.; Nakanishi, K. *Biochemistry* **1989**, *28*, 6460.

(6) Mizukami, T.; Kandori, H.; Shichida, Y.; Chen, A.-H.; Derguini, F.; Caldwell, C.; Bigge, C.; Nakanishi, K.; Yoshizawa, T. *Proc. Natl. Acad. Sci. USA* **1992**, in press.

(7) Hu, S.; Franklin, P. J.; Wang, J.; Ruiz Silva, B.; Derguini, F.; Nakanishi, K. *Science*, submitted.

(8) The syntheses had already been achieved in the early 1980's.[9] However, except for a short synthetic outline of ret9 and some notes on preliminary binding studies, no data has been published because of the erratic results obtained with recontituted pigments, both in detergent and more so in membranes (the latter being essential for bioassays). Synthetic details and other results are published now because the obstacles in binding and bioassay studies have been overcome.[6,7,10]

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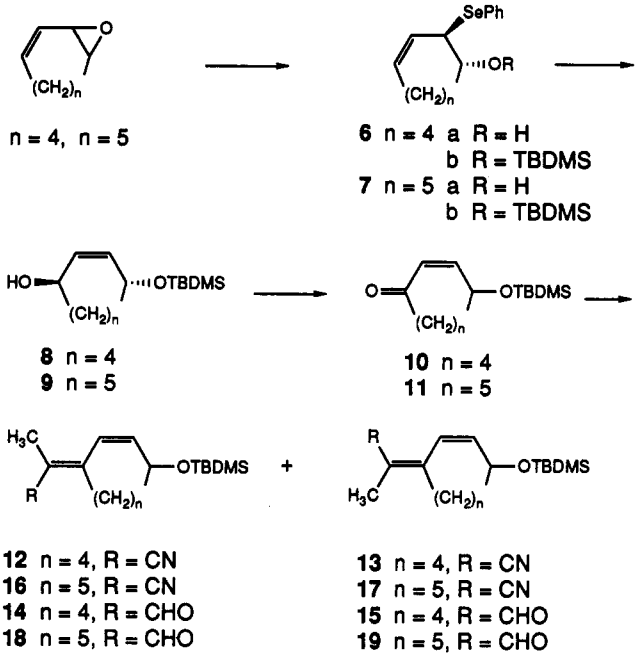
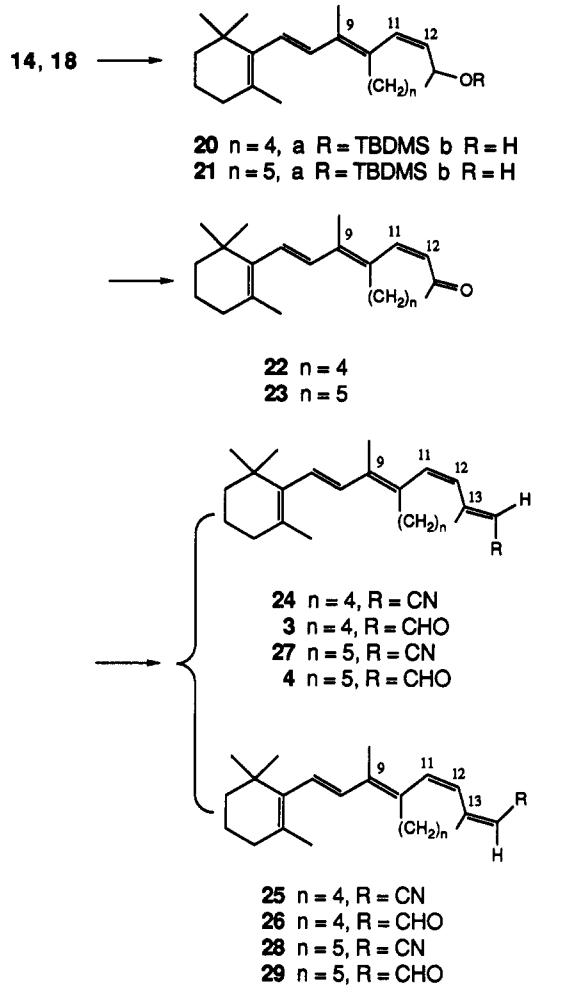
Scheme I



The syntheses started from 1,3-cyclooctadiene and 1,3-cyclononadiene.[11] Cyclooctadiene monoepoxide[12] (Scheme I) reacted with phenylselenide anion[13] to give alcohol **6a** (84%). Protection of the hydroxyl group as the *tert*-butyldimethylsilyl ether[14] **6b** and oxidation with hydrogen peroxide[13] furnished allylic alcohol **8** (74%), which was oxidized to ketone **10** with pyridinium dichromate[15] in DMF (84%). Condensation of **10** with the anion of 2-(diethylphosphono)propionitrile[16] (NaH, DME) produced a 7:3 mixture of nitriles **12** and **13** (90%), separable by flash chromatography. Treatment of the respective nitriles with DIBAL gave aldehydes **14** (97%) and **15** (50%).

A Wittig reaction of aldehyde **14** (Scheme II) with β-cyclogeranyltriphenylphosphonium bromide[17] (t-BuOK, THF) gave the *E*-olefination product **20a** (85%); deprotection[14] (Bu_4NF, THF) and oxidation[18] ($BaMnO_4$, CH_2Cl_2) of the resulting alcohol **20b** gave enone **22** (74%). Elongation of **22** using silylated acetaldehyde *tert*-butylimine anion[19] gave very low yields of retinal analogs **3** and **26**. Alternatively, reaction of **22** with diethyl cyanomethylphosphonate (NaH, DME) gave nitriles **24** and its 13-*cis* isomer **25** (1:1, 80%) which were reduced by DIBAL (87%) to yield 11-*cis*-ret8 **3** and 11,13-di-*cis*-ret8 **26** separable by HPLC. Significant isomerization of **3** to **26** occurs upon storage, even at −70 °C under argon. Similarly elongation of aldehyde **15** yielded 9,11-di-*cis*-ret8 and 9,-11,13-tri-*cis*-ret8.

Scheme II



11-*Cis*-locked nine-membered ring retinal (ret9) **4** was synthesized following a similar procedure. 11-*cis*-Ret9 **4** exhibits an absorption maximum at 280 nm; this blue-shifted maximum, compared to the λ_{max} of the 11-*cis* eight-membered ring homolog (346 nm), reflects the increased nonplanarity of the chromophore.

Experimental Section

General. All reactions were conducted under dried argon atmosphere. Unless otherwise stated, reagents were obtained from commercial suppliers and used without further purification. CH_2Cl_2 was distilled from CaH_2, and THF and benzene were distilled from sodium/benzophenone prior to use. Boiling points and melting points are uncorrected. Flash chromatography was carried out with E. Merck silica gel 60. Samples for spectroscopic analyses were further purified by HPLC (YMC-Pack sil 5 mm, 4.6 × 250 mm, 4% ethyl acetate in hexane; retention times (t_R in min) are reported for a flow rate of 3 mL/min. ^1H NMR spectra were recorded on Varian VXR-400 spectrometer ($CDCl_3$ solution), the chemical shifts are given in ppm (δ reference peak $CHCl_3$ 7.24 ppm) and coupling constants (J) in hertz. CI and HRFAB mass spectra were measured on NERMAG R1010 and JEOL DX-303 HF spectrometers, respectively. Absorption spectra were recorded on Perkin-Elmer Lambda 4B UV/vis spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer.

Synthesis of 11-*Cis*-Locked Eight-Membered Ring Retinals (11-*cis*-Ret8). Hydroxy Selenide 6a. $NaBH_4$ (1.34 g, 35.4 mmol) was added, over a period of 10 min, to a suspension of diphenyl diselenide (5.54 g, 17.7 mmol) in absolute ethanol (90 mL). Cyclooctadiene monoepoxide[12] (4.40 g, 35.4 mmol) was added to the resulting almost colorless solution, which was then

(9) (a) Nakanishi, N. *Pure Appl. Chem.* **1985**, *57*, 769. (b) Balogh-Nair, V.; Nakanishi, K. In *Chemistry and Biology of Synthetic Retinoids*; Dawson, M. I., Okamura, W. H., Eds.; CRC Press: Boca Raton, 1990; pp 147–176.
(10) Hu, S.; Wang, J.; Chen, A.-H.; Franklin, P. J.; Derguini, F.; Nakanishi, K. submitted to *Biochemistry*.
(11) Agarwal, S. K.; Moorthy, S. N.; Mehrotra, I.; Devaprabhakara, D. *Indian J. Chem.* **1977**, *15B*, 563.
(12) Crandall, J. K.; Banks, D. B.; Colyer, R. A.; Watkins, R. J.; Arrington, J. P. *J. Org. Chem.* **1968**, *33*, 423.
(13) Sharpless, K. B.; Lauer, R. F. *J. Am. Chem. Soc.* **1973**, *95*, 2697.
(14) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(15) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399.
(16) Raggio, M. L.; Watt, D. S. *J. Org. Chem.* **1976**, *41*, 1873.
(17) Isler, O.; Gutmann, H.; Lindlar, H.; Montavon, M.; Rüegg, R. Ryser, G. Zeller, P. *Helv. Chim. Acta.* **1956**, *39*, 463.
(18) Firouzabadi, H.; Ghaderi, E. *Tetrahedron Lett.* **1978**, 839.
(19) Corey, E. J.; Enders, D.; Bock, M. G. *Tetrahedron Lett.* **1976**, 7.

stirred 2 h at 25 °C and 4 h at 60 °C. The reaction mixture was poured into an ether/water mixture (50 mL Et_2O/200 mL H_2O), and the phases were separated. The water layer was extracted with ether (3 × 50 mL); the combined organic layers were washed with H_2O (1 × 50 mL) and brine (2 × 50 mL), dried over anhydrous Na_2SO_4, and evaporated. Distillation of the residue through a 1 × 10 cm Vigreux (0.1 mmHg) gave 8.40 g (84%) of a pale yellow oil, which solidified upon standing: mp 51–53 °C; ^{1}H NMR δ 7.56 (m, 2H), 7.28 (m, 3H), 5.61 (m, 2H), 4.00 (m, 1H), 3.62 (dt, J = 10.7, 4.0, 1H), 2.98 (broad d, 1H), 2.18 (m, 1H), 2.04 (m, 1H), 1.65–1.88 (m, 5H), 1.24 (m, 1H).

***tert*-Butyldimethylsilyl Ether 6b.** *tert*-Butyldimethylsilyl chloride (5.14 g, 34.1 mmol) was added, over a period of 15 min, to a solution of hydroxy selenide **6a** (8.00 g, 28.4 mmol) and imidazole (4.84 g, 71.1 mmol) in DMF (20 mL). The reaction mixture was stirred overnight at 25 °C. After filtration of the resulting precipitate, the reaction mixture was poured into hexane (75 mL). The organic layer was washed with water (3 × 50 mL), saturated $NaHCO_3$ (1 × 25 mL), and brine (1 × 25 mL), and then dried over anhydrous Na_2SO_4. Filtration, evaporation, and recrystallization from acetonitrile gave 8.55 g of white crystals (80%). HPLC, t_R = 6.7; mp 56–58 °C; UV (CH_3CN) 202, 248, 270 nm; ^{1}H NMR δ 7.46 (m, 2H), 7.18 (m, 3H), 5.54 (m, 2H), 4.40 (t, J = 9.5, 1H), 3.91 (dt, J = 10.6, 3.2, 1H), 2.21 (m, 1H), 2.04 (m, 1H), 1.76 (m, 4H), 1.56 (m, 1H), 1.25 (m, 1H), 0.91 (s, 1H), 0.13 (s, 3H), 0.06 (s, 3H); HRFAB (matrix PEG 400) 396.1378 (M^+), calcd 396.1388.

Allylic Alcohol 8. Powdered K_2CO_3 (1.0 g, 7.5 mmol) was added to a solution of protected selenide **6b** (1.28 g, 3.25 mmol) in THF (10 mL)/EtOH (5 mL). The mixture was cooled to 0 °C and 30% H_2O_2 (1.13 mL, 13.2 mmol) was added. Stirring for 2 h at 25 °C was followed by filtration into Et_2O (100 mL)/H_2O (20 mL). The solid phase was washed with ether; the combined organic phases were washed with H_2O (2 × 20 mL), brine (2 × 20 mL), and dried with anhydrous Na_2SO_4. After evaporation, 0.94 g of a red brown oil was obtained. Flash chromatography (hexane/acetone, 80/15) led to 0.69 g of a colorless oil (82%): ^{1}H NMR δ 5.54 (m, 2H), 4.87 (m, 1H), 4.71 (dd, J = 9.8, 6.0, 1H), 1.81 (m, 1H), 1.42–1.70 (m, 8H), 0.87 (m, 9H), 0.04 (s, 6H); HRMS 256.1875 (M^+), calcd 256.1859.

***tert*-Butyldimethylsiloxy Ketone 10.** Pyridinium dichromate (1.39 g, 4.0 mmol) was added at 0 °C to a solution of allylic alcohol 8 (0.684 g, 2.67 mmol) in DMF (15 mL). The reaction mixture was stirred at 25 °C for 19 h and poured into ether (25 mL)/hexane (25 mL). After washing with H_2O (3 × 25 mL), saturated aqueous $NaHCO_3$ (1 × 25 mL), and brine (1 × 25 mL), the organic phase was dried over anhydrous Na_2SO_4 and evaporated to give a yellow residue. Flash chromatography (acetone/hexane, 20/80) gave 0.64 g of a colorless oil (94%): HPLC, t_R = 16.7; UV (CH_3CN) 224 nm; ^{1}H NMR δ 6.25 (dd, J = 12.8, 5.5, 1H), 5.98 (dm, J = 12.8, 1H), 2.63 (sextet, 1H), 2.52 (m, 1H), 1.80 (m, 2H), 1.40–1.61 (m, 4H), 0.87 (s, 9H), 0.06 (s, 6H); HRMS 254.1685 (M^+), calcd 254.1702.

***tert*-Butyldimethylsilyloxy Nitriles 12 and 13.** To a suspension of NaH (50% in mineral oil, 270 mg, 5.6 mmol) in freshly distilled DME (15 mL) was added dropwise a solution of 2-(diethylphosphono)propionitrile (1.15 g, 6.0 mmol) in DMF (15 mL). The mixture was refluxed for 5 min and cooled to room temperature; a solution of ketone **10** (1.04 g, 4.12 mmol) in DME was then added and the reaction mixture was stirred for 45 min. After dilution with pentane (100 mL), the organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Flash chromatography (ether/pentane, 5/95) gave 751 mg of (*E*)-nitrile **12** and 315 mg of (*Z*)-nitrile **13** (90%). **(*E*)-Nitrile 12:** ^{1}H NMR δ 6.23 (d, J = 12.6, 1H), 5.72 (dd, J = 12.6, 5.6, 1H), 5.03 (m, 1H), 2.88 (m, 1H), 2.50 (m, 1H), 1.95 (s, 3H), 1.84–1.68 (m, 2H), 1.66–1.49 (m, 3H), 1.45–1.32 (m, 1H), 0.87 (s 9H), 0.045 (s, 3H), 0.042 (s, 3H); CI (NH_4^+) 292 (M + 1), 309 (M + 18). **(*Z*)-Nitrile 13:** ^{1}H NMR δ 6.62 (d, J = 14.6, 1H), 5.67 (dd, J = 14.6, 7.0, 1H), 5.17 (m, 1H), 2.65 (m, 1H), 2.33 (m, 1H), 1.93 (s, 3H), 1.79 (m, 1H), 1.66–1.50 (m, 4H), 1.40 (m, 1 H), 0.88 (s, 9H), 0.054 (s, 3H), 0.047 (s, 3H); HRMS 291.1997 (M^+), calcd 291.2018.

(*E*)-*tert*-Butyldimethylsilyloxy Aldehyde 14. To a solution of silyloxy nitrile **12** (0.73 g, 2.5 mmol) in anhydrous ether (20 mL), cooled to ice bath temperature, was added dropwise a 0.9 M solution of DIBAL (3.3 mmol). After stirring for 30 min, the reaction was quenched with several drops of H_2O and filtered through a pad of wet silica gel twice. After washing the silica gel with ether and evaporating the solvent, the residue was purified by flash chromatography (pentane/ether, 90/10), leading to 713 mg of a colorless oil (97%): HPLC, t_R = 9.4; UV (CH_3CN) 289 nm; ^{1}H NMR δ 10.13 (m, 1H), 6.48 (d, J = 13.4, 1H), 5.82 (dd, J = 13.4, 5.6, 1H), 5.12 (m, 1H), 3.25 (m, 1H), 2.46 (m, 1H), 1.84 (s, 3H), 1.80 (m, 1H), 1.67 (m, 2H), 1.58 (m, 2H), 1.45 (m, 1H), 0.89 (m, 9H), 0.074 (m, 3H), 0.066 (m, 3 H); HRFAB (matrix PEG 400) 394.1987 (M^+), calcd 394.2015.

(*Z*)-*tert*-Butyldimethylsilyloxy Aldehyde 15. Silyloxy nitrile **13** (273 mg, 0.94 mmol), treated as described above for **12**, led to 135 mg of a colorless oil (49%): ^{1}H NMR (Brucker 250-MHz spectrometer) δ 10.15 (s, 1H), 6.46 (d, J = 13.4, 1H), 5.81 (dd, J = 13.4, 5.6, 1H), 4.56 (m, 1H), 3.22 (m, 1H), 2.50 (m, 1H), 1.80 (s, 3H), 1.90–1.40 (m, 6H), 0.85 (s, 9H), –0.04 (s, 3H), –0.05 (s, 3H).

(*E*)-*tert*-Butyldimethylsilyloxy tetraene 20a. β-Cycloger-anyltriphenylphosphonium bromide (610 mg, 1.27 mmol) and powdered potassium *tert*-butoxide (118 mg, 1.05 mmol) were weighed in a glove bag filled with argon and transferred into a 25-mL round-bottom flask. The flask was sealed with a septum and THF (4 mL) was added; the solution turned deep red immediately, but stirring was persued for an additional 15 min. (*E*)-*tert*-butyldimethylsilyloxy aldehyde **14** (144 mg, 0.5 mmol in 4 mL THF) was then added. After stirring for 8 h at 25 °C, the mixture was poured into 50 mL of hexane, washed with H_2O (2 × 25 mL) and brine (1 × 25 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated to give a yellow residue. Purification by flash chromatography on silica gel (ether/hexane, 2/98) produced 180 mg of a colorless oil (89%): HPLC, t_R = 15; UV (CH_3CN) 302 nm; ^{1}H NMR (Brucker 250 MHz) δ 6.45 (d, J = 15.2, 1H), 6.45 (d, J = 12.5, 1H), 6.18 (d, J = 15.2, 1H), 5.41 (dd, J = 12.5, 5.0, 1H), 5.16 (m, 1H), 2.71 (m, 1H), 2.34 (m, 1H), 2.01 (t, J = 5.7, 2H), 1.94 (s, 3H), 1.68 (s, 3H), 1.90–1.43 (m, 10H), 1.01 (s, 3H), 1.00 (s, 3H), 0.88 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H); HRMS 414.3325 (M^+), calcd 414.3318.

(*E*)-Tetraenol 20b. Tetra-*n*-butylammonium fluoride (7.0 mL, 1.0 M in THF) was added to a solution of (*E*)-TBDMS-tetraene **20** (446 mg, 1.08 mmol) in THF (7 mL) and stirred for 2 h at 25 °C. The reaction mixture was then poured into ether (50 mL); the organic layer was separated, washed with H_2O (2 × 25 mL) and brine (1 × 15 mL), and dried over anhydrous Na_2SO_4. After filtration and evaporation, purification by flash chromatography (ether/hexane, 4/6) gave 294 mg of a white solid (90%): HPLC, t_R = 15.5; UV (CH_3CN) 302 nm; ^{1}H NMR δ 6.50 (d, J = 12.8, 1H), 6.45 (d, J = 16.0, 1H), 6.19 (d, J = 16.0, 1H), 5.42 (dd, J = 12.8, 5.6, 1H), 5.18 (m, 1H), 2.70 (dt, J = 14.4, 4.6, 1H), 2.33 (m, 1H), 2.00 (t, J = 6.1, 2H), 1.93 (s, 3H), 1.69 (s, 3H), 1.63–1.49 (m, 9H), 1.45 (m, 2H), 1.01 (s, 3H), 0.99 (s, 3H); HRFAB (matrix PEG 400) 300.2453 (M^+), calcd 300.2453.

(*E*)-Tetraenone 22. Barium manganate (1.06 g, 4.14 mmol) was added to a solution of (*E*)-tetraenol (35.4 mg, 0.118 mmol) in CH_2Cl_2 (6 mL). After 6 h stirring at room temperature, the suspension was filtered through a Celite pad. The Celite was rinsed with CH_2Cl_2 and the filtrate was evaporated. Flash chromatography (ether/hexane, 2/8) gave 31.5 mg of a yellow oil (90%): HPLC, t_R = 11.8; UV (CH_3CN) 334 nm; ^{1}H NMR δ 7.14 (d, J = 12.9, 1H), 6.56 (d, J = 16.5, 1H), 6.23 (d, J = 16.5, 1H), 5.72 (d, J = 12.9, 1H), 2.51 (m, 2H), 2.39 (t, J = 6.2, 2H), 2.00 (t, J = 6.0, 2H), 1.95 (s, 3H), 1.80 (m, 2H), 1.70 (s, 3H), 1.67 (m, 2H), 1.44 (m, 2H), 1.01 (s, 6H); HRFAB (matrix PEG 400) 299.2378 (M + 1), calcd 299.2375.

(13*E*/13*Z*)-Retinonitriles 24/25. To a suspension of sodium hydride (60% in mineral oil, 58.1 mg, 1.45 mmol) in THF (3 mL) was added, at ice bath temperature, diethyl cyanomethylphosphonate (0.28 g, 1.59 mmol). After stirring for 15 min at 25 °C, a solution of (*E*)-tetraenone **22** (37.8 mg, 0.127 mmol) in THF (2 mL) was added and stirring continued for 2 h. The solution was concentrated, taken up with pentane, and applied onto a flash silica gel column. Elution with ether/hexane (1/9) gave 34.9 mg of a 1/1 mixture 11-*cis* and 11,13-di-*cis*-retinonitrile (86%): HPLC, t_R = 7.3 (**24** and **25**); UV (CH_3CN) 263, 334 nm; ^{1}H NMR δ 6.68 (d, J = 12.4, 1H), 6.52 (m, 4H), 6.26 (d, J = 5.2, 1H), 6.23 (d, J = 5.2, 1H), 6.16 (d, J = 12.4, 1H), 5.16 (s, 1H, 14-H [13*E*-

isomer]), 5.09 (s, 1H, 14-H [13-*Z* isomer]), 2.68 (m, 2H), 2.45 (m, 6H), 2.00 (t, J = 5.6, 4H), 1.88 (s, 3H), 1.85 (s, 3H), 1.71 (s, 3H), 1.70 (s, 3H), 1.70–1.56 (m, 10 H), 1.46 (m, 6), 1.02 (m, 6H), 1.01 (m, 6H); HRFAB (matrix PEG 400) 321.2464 (M^+), calcd 321.2457.

11-*cis* and 11,13-di-*cis*-Ret8 3 and 26. DIBAL (0.9 N in hexane, 0.32 mL, 0.30 mmol) was added, at ice bath temperature, to a solution of retinonitriles **24/25** (34 mg, 0.11 mmol) in ether (3 mL). After 2 h at 25 °C the reaction was quenched with several drops of ethyl acetate and H_2O. The mixture was filtered through a pad of wet silica gel; after washing the silica gel with ether, the organic phase was dried over anhydrous Na_2SO_4 and evaporated. Purification by flash chromatography (ether/hexane, 1/9) gave 28 mg of a 1/1 mixture of 11-*cis*-ret8 **3** and 11,13-di-*cis*-ret8 **26** (87%), which were separable by HPLC (ether/hexane, 5/95). **11-*cis*-Ret8 3**: HPLC, t_R = 26.6; UV (hexane) 276 nm (ϵ 17700), 346 (13700), Schiff base (SB) with *n*-BuNH$_2$ (MeOH) 285, 335 nm, protonated SB (SBH^+, in MeOH) 315, 435 nm; ^{1}H NMR (Brucker 250 MHz) δ 10.03 (d, J = 8.8, 1H, 15-H), 6.57 (d, J = 12.2, 1H), 6.55 (d, J = 15.1, 1H), 6.27 (d, J = 12.2, 1H), 6.24 (d, J = 15.1, 1H), 5.89 (d, J = 8.8, 1H), 2.86 (m, 2H), 2.46 (m, 2H), 2.01 (t, J = 5.7, 2H), 1.87 (s, 3H), 1.72 (s, 3H), 1.75–1.55 (m, 6H), 1.47 (m, 2H), 1.02 (s, 6H); HRFAB (matrix 3-nitrobenzyl alcohol) 324.2451 (M^+), calcd 324.2453. **11,13-Di-*cis*-ret8 26**: HPLC, t_R = 34.8; UV (hexane) 286 nm (ϵ 11200), 337 (14900), 336 (18100); (SB) with *n*-BuNH$_2$ (MeOH) 318 nm, SBH^+ (MeOH) 430–440 nm; ^{1}H NMR (Brucker 250 MHz) δ 10.04 (d, J = 7.5, 1H), 6.93 (d, J = 12.5 1H), 6.55 (d, J = 12.5, 1H), 6.50 (d, J = 15.0, 1H), 6.31 (d, J = 15.0, 1H), 5.99 (d, J = 7.5, 1H), 2.53 (m, 4H), 2.01 (m, 2H), 1.99 (s, 3H), 1.71 (s, 3H), 1.53–1.70 (m, 6H), 1.46 (m, 2H), 1.01 (s, 6H); HRFAB (matrix 3-nitrobenzyl alcohol) 324.2446 (M^+), calcd 324.2453.

Elongation of (*Z*)-*tert*-butyldimethylsilyloxy aldehyde **15** led accordingly to 9,11-di-*cis*-ret8 and 9,11,13-tri-*cis*-ret8.

9,11-Di-*cis*-ret8: UV (hexane) 275, 350 nm; ^{1}H NMR (Brucker 250) δ 10.01 (d, J = 8.3, 1H, 15-H), 6.55 (d, J = 13, 1H, 11-H), 6.38 (d, J = 16, 1H, 7-H), 6.29 (d, J = 13, 1H, 12-H), 6.09 (d, J = 16, 1H, 8-H), 5.88 (d, J = 8.3, 1H, 14-H), 1.90 (s, 3H, 9-CH_3), 1.63 (s, 3H, 5-CH_3), 1.02 (s, 6H, 1-$(CH_3)_2$); CI (NH_4^+) 325 (M + 1).

9,11,13-Tri-*cis*-ret8: UV (hexane) 278, 335 nm; ^{1}H NMR (Brucker 250) δ 10.07 (d, J = 8.3, 1H, 15-H), 6.89 (d, J = 16, 1H, 11-H), 6.68 (d, J = 15.5, 1H, 7-H), 6.56 (d, J = 16, 1H, 12-H), 6.16 (d, J = 15.5, 1H, 8-H), 5.82 (d, J = 8.3, 1H, 14-H), 1.91 (s, 3H, 9-CH_3), 1.70 (s, 3H, 5-CH_3), 1.02 (s, 6H, 1-$(CH_3)_2$); Cl ($NH4^+$) 325 (M + 1).

Synthesis of 11-*Cis*-Locked Nine-Membered Ring Retinal (11-*cis*-Ret9). Unless otherwise stated, the synthetic steps leading to 11-*cis*-ret9 **4** were identical to those described for 11-*cis*-ret8.

Cyclononadiene Monoepoxide. 1,3-Cyclononadiene[11] (94 g, 0.77 mol) treated according to literature procedure[12] led to 51.2 g (48%) of monoepoxide: bp 70–75 °C (15 mm); IR (CH_2Cl_2) 3054, 2986, 1648, 1270, 896 cm^{-1}; ^{1}H-NMR δ 5.10 (m, 2H), 3.56 (d, J = 3.3, 1H), 3.05 (m, 1H), 2.50–1.90 (3m, 4H), 1.80–1.10 (3m, 6H); HRMS 138.1039 (M^+), calcd 138.1045.

Hydroxy Selenide 7a. Cyclononadiene monoepoxide (12.2 g, 88 mmol) treated as described for cyclooctadiene monoepoxide gave after flash chromatography (ether/hexane, 1/1) 15.4 g of **7a** (59%). IR (neat) 3448, 1645, 1578, 1264, 739, 692 cm^{-1}; ^{1}H NMR (Brucker 250) δ 7.55 (m, 2H), 7.20 (m, 3H), 5.45 (m, 2H), 4.13 (m, 1H), 3.69 (m, 1H), 2.15–1.20 (m, 10H); HRMS 296.0680 (M^+), calcd 296.0679.

***tert*-Butyldimethylsilyl Ether 7b.** Imidazole (1.22 g, 18 mmol) and *tert*-butyldimethylsilyl chloride (1.84 g, 12 mmol) were added to a solution of hydroxy selenide **7a** (1.75 g, 5.9 mmol) in DMF (5 mL). After stirring overnight at room temperature, water (20 mL) was added, and the mixture was extracted with ether (3 × 20 mL). The extracts were dried over anhydrous $MgSO_4$ and concentrated. Flash chromatography (ether/hexane, 1/1) led to 2.16 g of **7b** (89%): IR (CH_2Cl_2) 1643, 1156, 1077, 837 cm^{-1}; ^{1}H NMR δ 7.50 (m, 2H), 7.20 (m, 3H), 5.41 (m, 2H), 4.60 (t, J = 14.9, 1H), 3.95 (m, J = 14.9, 1H), 2.15 (m, 1H), 1.95 (m, 1H), 1.90–1.20 (m, 8H), 0.95 (s, 9H), 0.20 (s, 3H), 0.10 (s, 3H); HRMS 410.1551 (M^+), calcd 410.1544.

Allylic Alcohol 9. *tert*-Butyldimethylsilyl ether **7b** (5 g, 12 mmol) treated as described for *tert*-butyldimethylsilyl ether **6b** gave 3.14 g of **9** (95%): IR (CH_2Cl_2) 3449, 1631, 1090, 1059, 838 cm^{-1}; ^{1}H NMR δ 6.63 (dd, J = 11.4, 9.0, 1H), 5.48 (dd, J = 11.4, 9.0, 1H), 4.91 (m, 1H), 4.60 (m, 1H), 1.60 (m, 10H), 0.90 (s, 9H), 0.04 (s, 6H); HRMS 270.2019 (M^+), calcd 270.2015.

***tert*-Butyldimethylsilyl Ketone 11.** To a solution of allylic alcohol **9** (393 mg, 1.45 mmol) in acetone (20 mL) was added activated manganese(IV) oxide (6 g, 69 mmol). The suspension was stirred at room temperature for 4 h and then filtered through a pad of Celite, which was subsequently washed with acetone. Evaporation of the solvent followed by flash chromatography (ethyl acetate/hexane, 4/96) gave 319 mg of a colorless oil (82%): IR (neat) 1694, 1660, 1084, 1053 cm^{-1}; ^{1}H NMR (Varian 300) δ 6.17 (dd, J = 12.1, 8.7, 1H), 5.92 (m, J = 12.1, 1H), 5.05 (m, 1H), 2.70 (m, 1H), 2.50 (m, 1H)), 2.00–1.15 (m, 8H), 0.90 (s, 9H), 0.10 (s, 6H); HRMS 268.1850 (M^+), calcd 268.1859.

***tert*-Butyldimethylsilyl Nitriles 16 and 17.** To a suspension of NaH (84 mg 50% suspension in oil, 1.75 mmol) in DME (6 mL) was added at 0 °C a solution of 2-(diethylphosphono)propionitrile (536 mg, 2.8 mmol) in DME (3 mL). The mixture was warmed to reflux for 10 min and allowed to cool slightly. A solution of ketone **11** (272 mg, 1.01 mmol) in DME (3 mL) was added, and the mixture was heated to reflux for 10 min. After cooling to 25 °C, the reaction was poured into ether (25 mL), washed with saturated aqueous sodium bicarbonate (2 × 25 mL) and brine (25 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent followed by flash chromatography (ethyl acetate/hexane, 1/9) gave 195 mg of (*E*)-*tert*-butyldimethylsilyl nitrile **16** and 94 mg of (*Z*)-*tert*-butyldimethylsilyl nitrile **17** (93%). **16**: IR (CH_2Cl_2) 2209, 1621, 1070 cm; ^{1}H NMR δ 5.95 (d, J = 12, 1H), 5.66 (dd, J = 12, 8.8, 1H), 4.55 (m, 1H), 2.73–2.45 (m, 2H), 1.90 (s, 3H), 1.89–1.30 (m, 8H), 0.90 (s, 9H), 0.05 (s, 6H); HRMS 305.2164 (M^+), calcd 305.2175. **17**: ^{1}H NMR δ 6.41 (d, J = 13.2, 1H), 5.65 (dd, J = 13.2, 8.8, 1H), 4.90 (m, 1H), 2.45 (m, 2H), 1.95 (s, 1H), 1.85–1.20 (m, 8H), 0.90 (s, 9H), 0.05 (s, 6H).

(*E*)-*tert*-Butyldimethylsilyloxy Aldehyde 18. (*E*)-*tert*-Butyldimethylsilyl nitrile **16** (151 mg, 0.50 mmol) treated as described for (*E*)-*tert*-butyldimethylsilyl nitrile **12** led to 147 mg of aldehyde **18** (96%): IR (Et_2O) 1720, 1676, 1615, 1128 cm^{-1}; ^{1}H NMR (Brucker 250 MHz) δ 10.18 (s, 1H), 6.10 (d, J = 11.9, 1H), 5.62 (dd, J = 11.9, 5.95, 1H), 4.52 (m, 1H), 2.95 (m, 1H), 2.45 (m, 1H), 1.76 (s, 3H), 1.90–1.30 (m, 8H), 0.90 (s, 9H), 0.05 (s, 6H); HRMS 308.2178 (M^+), calcd 308.2172.

(*E*)-*tert*-Butyldimethylsilyloxy Tetraene 21a. (*E*)-*tert*-butyldimethylsilyloxy aldehyde **18** (1.73 g, 5.62 mmol) treated as described for (*E*)-*tert*-butyldimethylsilyloxy aldehyde **14** gave 1.71 g of **21a** (71%): IR (CH_2Cl_2) 1604, 1065 cm^{-1}; ^{1}H NMR δ 6.42 (d, J = 15.5, 1H), 6.15 (d, J = 15.5, 1H), 6.10 (d, J = 12, 1H), 5.40 (dd, J = 12, 7.1, 1H), 4.60 (m, 1H), 2.56 (m, 1H), 2.05 (m, 3H), 1.90 (s, 3H), 1.71 (s, 3H), 1.70–1.40 (m, 12H), 1.04 (s, 3H), 1.02 (s, 3H), 0.90 (s, 9H), 0.09 (2s, 6H); HRMS 428.3497 (M^+), calcd 428.3474.

(*E*)-Tetraenol 21b. (*E*)-*tert*-Butyldimethylsilyloxy tetraene (98 mg, 0.23 mmol) **21a** treated as described for **20a** gave 63 mg of (*E*)-tetraenol **21b** (88%): IR (CH_2Cl_2) 3414, 1604, 1017 cm^{-1}; ^{1}H NMR (Varian 300 MHz) δ 6.38 (d, J = 15.1, 1H), 6.14 (d, J = 12.1, 2H), 5.39 (dd, J = 12.1, 6, 1H), 4.63 (m, 1H), 2.58 (m, 2H), 2.41 (t, J = 7.3, 2H), 2.10–1.30 (3m, 12H), 1.89 (s, 3H), 1.71 (s, 3H), 1.05 (2s, 6H); HRMS 314.2609 (M^+) calcd 314.2610.

(*E*)-Tetraenone 23. **(*E*)-Tetraenol 21b** (3.3 mg, 10.5 μmol) treated as described for **20b** gave 1.6 mg of ketone **23** (49%): IR (CH_2Cl_2) 1734, 1700, 1684, 1654, cm^{-1}; ^{1}H NMR δ 6.95 (d, J = 12.9, 1H), 6.48 (d, J = 15.5, 1H), 6.26 (d, J = 15.5, 1H), 6.00 (d, J = 12.9, 1H), 2.68 (t, J = 6.5, 2H), 2.38 (t, J = 6.5, 2H), 2.04 (t, J = 6.5, 2H), 1.89 (s, 3H), 1.74 (s, 3H), 1.73–1.50 (m, 10H), 1.05 (s, 6H); HRMS 312.2454 (M^+), calcd 312.2453.

11-*cis*- and 11,13-Di-*cis*-retinonitriles 27 and 28. (*E*)-Tetraenone **23** (44.6 mg, 143 μmol) treated as described for (*E*)-tetraenone **22**, using DME as solvent, yielded 55 mg of a 3/1 mixture of 11-*cis*- and 11,13-di-*cis*-retinonitriles **27** and **28**, respectively (100%): IR (CH_2Cl_2) 2211, 1733, 1684, 1670, 1659 cm^{-1}; ^{1}H NMR (Varian 300) δ 6.63 (d, J = 11.7, 1H), 6.41 (d, J = 16.4, 1H), 6.29 (d, J = 16.4, 1H), 6.19 (d, J = 11.7, 1H), 5.19 (s, 1H, H-14 [11-*cis* **27**]), 5.10 (s, 1H, H-14 [11,13-di-*cis* **28**]), 2.78 (t, J = 7.0, 2H), 2.32 (t, J = 7.0, 2H), 2.02 (t, J = 6.6, 2H), 1.78 (s, 3H), 1.72 (s, 3H), 1.70–1.40 (m, 10H), 1.02 (s, 6H); HRMS 335.2631 (M^+), calcd 335.2613.

11-*cis*- and 11,13-Di-*cis*-ret9 4/29. Retinonitriles **27/28** (55 mg, 164 μmol) treated in dim light, as described for retinonitriles **24/25**, gave 28.7 mg of 11-*cis*- and 11,13-di-*cis*-ret9 (52%) which were separated by HPLC (ethyl acetate/hexane, 4/96). 11-*cis*-Ret9 **4**: UV (hexane) 245 (shoulder), 281 (ε 27500), 330 nm (shoulder); SB with *n*-$BuNH_2$, (MeOH), 285 nm; SBH^+ (MeOH) 324 nm; IR (CH_2Cl_2) 1718, 1662, 1616 cm^{-1}; ^1H NMR (Brucker 250 MHz) δ 10.10 (d, J = 6.9, 1H), 6.45 (d, J = 12.5, 1H), 6.38 (d, J = 15.6, 1H), 6.14 (d, J = 15.6, 1H), 6.10 (d, J = 12.5, 1H), 5.82 (d, J = 6.9, 1H), 2.95 (t, J = 6.3, 2H), 2.31 (t, J = 6.3, 2H), 2.00 (t, J = 5.6, 2H), 1.81 (s, 3H), 1.71 (s, 3H), 1.70–1.40 (m, 10H), 1.02 (s, 6H); HRMS 338.2626 (M^+), calcd 338.2610.

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Supplementary Material Available: ^1HNMR spectra of compounds **3, 4, 6a, 6b, 7a, 7b, 8-14, 16-18, 20a, 20b, 21a, 21b, 22, 23, 24/25, 26,** and **27/28** (25 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.