Synthesis of (+)-(4S)- and (−)-(4R)-(11Z)-4-Hydroxyretinals and Determination of the Absolute Stereochemistry of a Visual Pigment Chromophore in the Firefly Squid, *Watasenia scintillans*†

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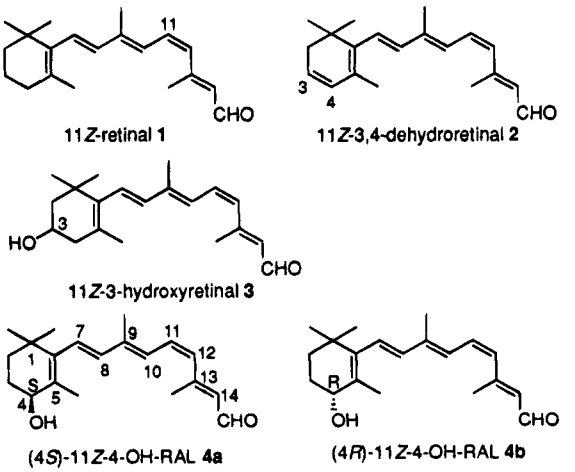
First syntheses of (+)-(4S)- and (−)-(4R)-(11Z)-4-hydroxyretinals (4-OH-RALs) (**4a** and **4b**) were accomplished from (4S)- and (4R)-4-hydroxy-β-ionones, respectively, to determine the absolute configuration of a visual pigment chromophore in *Watasenia scintillans*, which has been isolated as a new chromophore in the animal kingdom. The CD spectra of the native chromophore and its *anti*-oxime agreed with those of synthetic (4R)-compounds. Application of the CD exciton chirality method to the *p*-(dimethylamino)cinnamate of (4R)-4-OH-RAL also established the absolute configuration of the native chromophore as **4b**.

It has long been believed that only two retinal (RAL) analogs, (11Z)-retinal (**1**) and (11Z)-3,4-dehydroretinal (**2**), are used as chromophores of visual pigments in the animal kingdom. However, a new chromophore was extracted from the compound eyes of a member of the class of Insecta and was determined to be (11Z)-3-hydroxyretinal (**3**) by our group.[1] Further, in each class of Insecta, we confirmed the absolute stereochemistry at C-3 of **3**.[2] Recently, another new chromophore, (11Z)-4-OH-RAL (**4**), was isolated from the firefly squid by Kito et al.[3] The firefly squid, *Watasenia scintillans*, has three visual pigments.[4] The major pigment, based on retinal (λ_{max} = 484 nm), is distributed over the whole retina, the second pigment is found in the proximal outer segment layer of the ventral retina and is based on 3,4-dehydroretinal ($\lambda_{max} \approx$ 500 nm), and the third pigment ($\lambda_{max} \approx$ 470 nm) is localized in the distal outer segment layer of the ventral retina. The third pigment layer can act as a color filter for the photoreceptor cells containing the second pigment. This filtering suggests that bioluminescent squid may discriminate between different wavelengths of light in the environment and that the light generation may be a form of communication.[4,5] The chromophore ((11Z)-OH-RAL (**4**)[6]) of the third pigment and its *anti*-oxime **5b**[3] both showed negative Cotton effects in their CD spectra; however, the absolute configuration at C-4 remained to be determined.

In a previous communication,[7] we reported the synthesis of (4S)- and (4R)-(11Z)-4-OH-RALs **4a** and **4b**, and the chirality of the *anti*-oxime extracted from *W. scintillans* by the oxime method[8] was determined by the use of the synthetic (4R)-(11Z)-4-OH-RAL *anti*-oxime. Here we describe a full account of the experimental details and provide some additional data.



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(1) Ito, M.; Matsuoka, N.; Tsukida, K.; Seki, T. *Chem. Pharm. Bull.* **1988**, *36*, 78.

(2) Seki, T.; Isono, K.; Ito, M.; Katsuta, Y. *Eur. J. Biochem., submitted.*

(3) Matsui, S.; Seidou, M.; Uchiyama, I.; Sekiya, N.; Hiraki, K.; Yoshihara, K.; Kito, Y. *Biochim. Biophys. Acta* **1988**, *966*, 370.

(4) Seidou, M.; Sugahara, M.; Uchiyama, H.; Hiraki, K.; Hamanaka, T.; Michinomae, M.; Yoshihara, K.; Kito, Y. *J. Comp. Phys.* **1990**, *A 166*, 769.

(5) Denton, E. J.; Locket, N. A. *J. Mar. Biol. Ass. U.K.* **1989**, *69*, 409.

(6) Kito, Y.; Partridge, J. C.; Seidou, M.; Narita, K.; Hamanaka, T.; Michinomae, M.; Sekiya, N.; Yoshihara, K. *Vision Res.* **1992**, *32*, 3.

(7) Katsuta, Y.; Yoshihara, K.; Nakanishi, K.; Ito, M. *Tetrahedron Lett.* **1994**, *35*, 905.

(8) Groenendijk, G. W. T.; Jansen, P. A. A.; Bonting, S. L.; Daemen, F. J. M. *Methods in Enzymology*; McCormick, D. B., Wright, L. D., Eds.; Academic Press: New York, 1980; Vol. 67, p 203.

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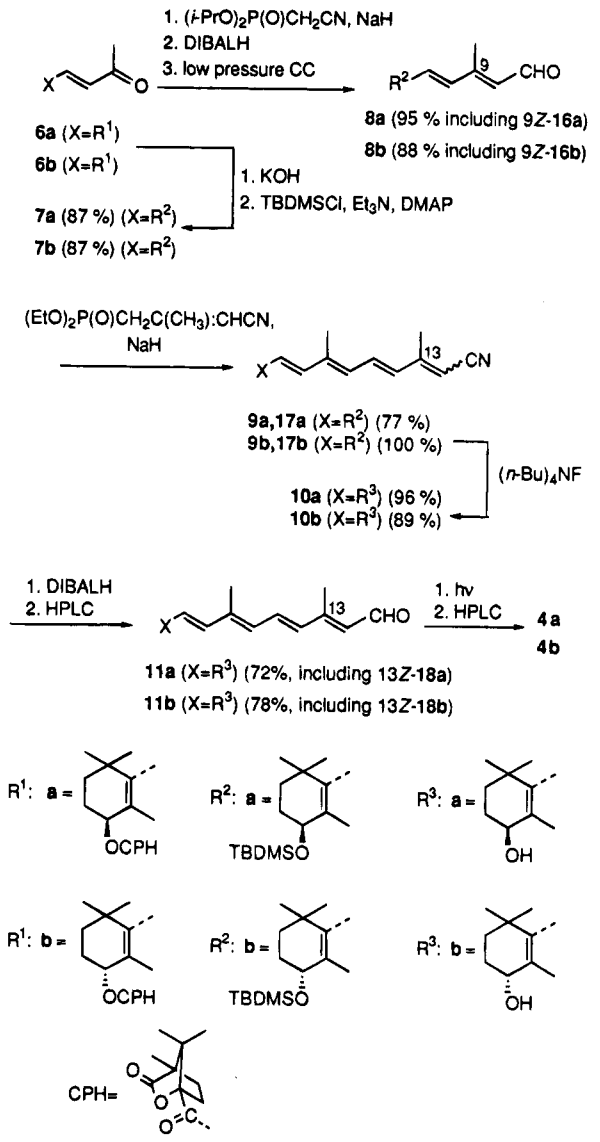
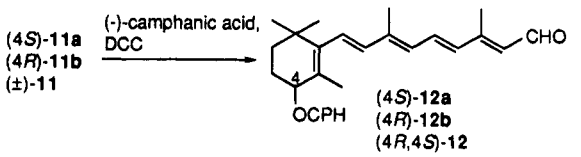
Scheme 1

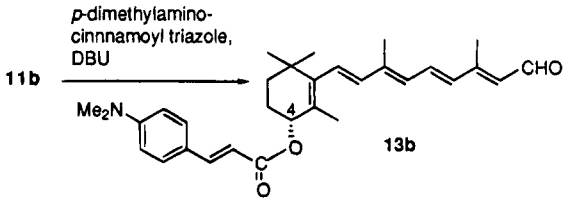


Optically active 4-OH-RALs were prepared as shown in the Scheme 1. (4*S*)-Camphanate **6a** and (4*R*)-camphanate **6b** were derived from racemic 4-hydroxy-β-ionone by means to Haag's procedure.[9] All spectral data for **6a** and **6b**, including their chiroptical properties, were in fair agreement with those in the literature,[9] and the data led to the identification of the chirality at C-4 of **6a** and **6b**. In addition, the stereostructure of **6b** (4*R*) was confirmed independently by X-ray crystallography.[10] The structure was determined by the direct method (SIR88). The positional parameters of non-H atoms were refined by a block-diagonal least-squares method first with isotropic thermal parameters and then with anisotropic ones. The geometrically ideal positions of the H atoms were calculated and included in the calculation of final structure factors with isotropic thermal parameters. Final least-squares refinement using 1888 unique reflections with $[F_o > 3\sigma(F_o)]$ gave $R = 0.059$.

Scheme 2



Scheme 3



After hydrolysis of the camphanate group in **6a**, the resulting hydroxyl group was protected with a TBDMS group. Condensation of **7a** with diisopropyl cyanomethylphosphonate (NaH) and subsequent reduction (DIBALH) of the resulting nitrile group gave aldehyde **8a** and its 9*Z*-isomer, which were cleanly separated by low pressure column chromatography (CC). The Emmons–Horner reaction of **8a** with C_5-phosphonate (NaH) provided nitrile **9a** as a mixture of 13*Z*- and all-*E*-isomers in a 1:5 ratio. After deprotection (n-Bu_4NF) of the silyl group in **9a** without separation, 4-hydroxyretinonitrile **10a** was reduced (DIBALH) to give 4-OH-RAL as a mixture of the all-*E*-isomer **11a** and its 13*Z*-isomer in a 2:1 ratio, which were isolated in pure form by preparative HPLC in the dark.

Isomer **11b** was also prepared from **6b** by means of the route used for **11a**. The optical purities of **11a** and **11b** were determined by HPLC analysis of camphanates (4*S*)-**12a** (88% ee) and (4*R*)-**12b** (84% ee) (Scheme 2), whose peaks were identified by cochromatography with (4*R*,4*S*)-**12** prepared from (±)-(*all-E*)-4-OH-RAL.[11] Owing to instability of 4-OH-RAL, employment of camphanic acid chloride instead of (−)-camphanic acid and DCC resulted in the decomposition of 4-OH-RAL, which was also not recovered after hydrolysis (KOH) of camphanates **12**. Hence it follows that optically active 4-OH-RALs cannot be derived from racemic 4-OH-RAL directly.

p-(Dimethylamino)cinnamate **13b** derived from **11b** (Scheme 3) by means of the established method[12] showed typical exciton split CD Cotton effects (Figure 1). Negative first and positive second Cotton effects were expected, since the α-*p*-(dimethylamino)cinnamoyl group and the pentaenal chromophore constitute a counterclockwise screw, and the results confirmed the absolute configuration of (4*R*)-4-OH-RAL **4b**. This is the first application of the CD exciton chirality method to the determination of retinoid structure, and the CD data were augmented by crystallographical data of starting material **6b**.

(4*R*)-11*Z*-Isomer **4b** was obtained from the photoisomerization[13] mixture (Figure 2) of **11b**, which contained 11*Z*,13*Z*-, 13*Z*-, all-*E*-, 11*Z*-, and 9*Z*-isomers (**19b**, **18b**, **11b**, **4b**, and **20b**), (4*S*)-(11*Z*)-4-OH-RAL (**4a**) was prepared similarly. The geometrical structures of these isomers were confirmed by comparison of their UV-visible

(9) Haag, A.; Eschenmoser, W.; Eugster, C. H. *Helv. Chim. Acta* **1980**, *63*, 10.

(10) The authors have deposited atomic coordinates for this compound with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

(11) Renk, G.; Grover, T.; Crouch, R.; Mao, B.; Ebrey, T. G. *Photochem. Photobiol.* **1981**, *33*, 489.

(12) Cai, G.; Bozhkova, N.; Odingo, J.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1993**, *115*, 7192.

(13) Tsukida, K.; Kodama, A.; Ito, M. *J. Chromatogr.* **1977**, *134*, 331.

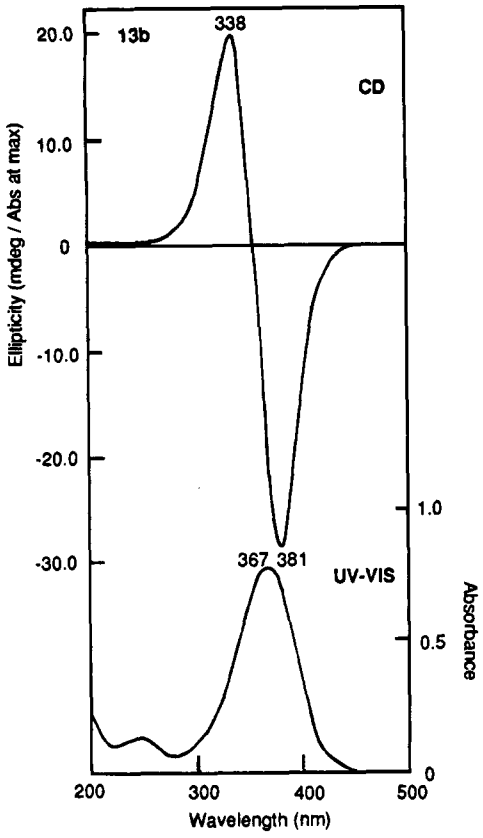


Figure 1. UV-vis and CD spectra of *p*-(dimethylamino)-cinnamate **13b** in CH_3CN.

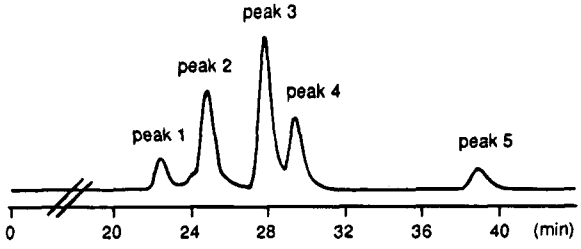


Figure 2. Analytical HPLC (LiChrosorb Si-60 (5 μm) 4 × 300 mm; 0.5% EtOH 15% EtOAc in *n*-hexane, 1 mL/min) for photoisomerization products of **11b**: peak 1, (11*Z*,13*Z*)-**19b**; peak 2, (13*Z*)-**18b**; peak 3, (all-*E*)-**11b**, peak 4, (11*Z*)-**4b**, peak 5, (9*Z*)-**20b**.

(vis) and ^{1}H NMR data to those of the respective retinal isomers.[14] These are the first syntheses of optically active 4-OH-RALs.

That the UV-vis/CD data (Figure 3) of **4b** agreed with those of native chromophore[5] extracted from *W. scintillans* establishes the C-4 chirality of the third visual pigment chromophore as being 4*R*. In addition, the negative CD spectrum (Figure 4) of the *anti*-isomer of (4*R*)-(11*Z*)-4-OH-RAL oxime **5b**, prepared from **4b** (Scheme 4), agreed with that of *anti*-isomer of native (11*Z*)-4-OH-RAL oxime[3] isolated from *W. scintillans*. The data presented here established the absolute stereochemistry of native (11*Z*)-4-OH-RAL as 4*R*.

Experimental Section

General. CH_2Cl_2 and *n*-hexane were distilled in the presence of $CaCl_2$, and THF and Et_2O were distilled from sodium. CC was carried out on Merck silica gel 60 (70–230 mesh ASTM). Preparative TLC was performed with Merck silica gel 60 TLC plates (0.5 mm thickness). Low pressure CC was carried out with a Merck Lobar LiChroprep Si-60 column (Grösse B). 4-OH-RAL isomers were separated by

(14) Liu, R. S. H.; Asato, A. E. *Tetrahedron* **1984**, *40*, 1931.

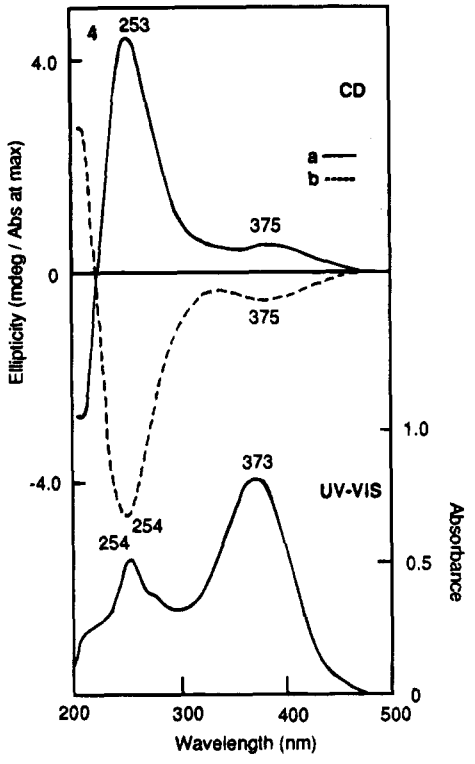


Figure 3. UV-vis and CD spectra in EtOH of (4*S*)-(11*Z*)-4-OH-RAL (**4a**) (solid line) and (4*R*)-(11*Z*)-4-OH-RAL (**4b**) (dashed line).

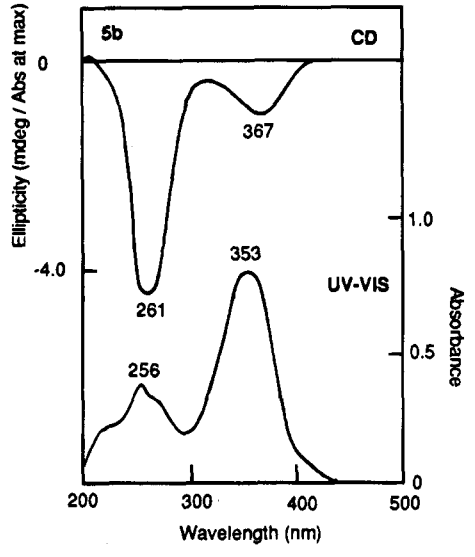
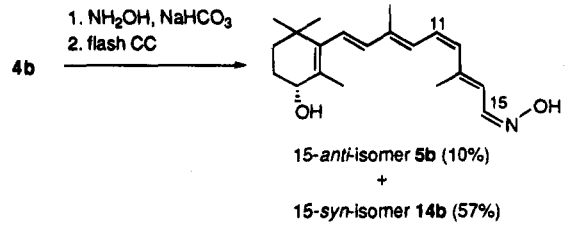


Figure 4. UV-vis and CD spectra in EtOH of the *anti*-oxime **5b**.

Scheme 4



preparative HPLC (Merck LiChrosorb Si-60 (7 μm) 1.0 × 25 cm, EtOH–EtOAc–hexane, 0.5:15:84.5); retention times (t_R in min) are reported for a flow rate of 3 mL/min. Unless specified otherwise, UV–vis spectra were taken in EtOH and ^{1}H NMR spectra in $CDCl_3$ at 200 MHz. J values are given in hertz. Solvent removal was accomplished below 30 °C under reduced pressure using a rotary evaporator.

(4*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]-β-ionylideneacetaldehyde (8b). To a suspension of NaH (60% oil dispersion; 293 mg, 7.33 mmol) in dry THF (10 mL) at 0 °C was added a solution of diisopropyl cyanomethylphosphonate (1.50 g, 7.33 mmol) in dry THF (15 mL). After the mixture stirred at rt for 30 min, a solution of (4*R*)-4-[(*tert*-butyldimethylsilyl)oxy]-β-ionone **7b**[14] (1.18 g, 3.66 mmol) in dry THF (12 mL) was added at 0 °C, and the mixture was stirred at rt for 2 h. The reaction mixture was concentrated to give a residue, which was directly purified by CC (EtOAc–hexane, 1:19) to provide (4*R*)-4-[(*tert*-butyldimethylsilyl)oxy]-β-ionylideneacetonitrile (**15b**) (1.26 g, quant.) as a mixture of 9*E*- and 9*Z*-isomers in a ratio of 5:1 by ^{1}H NMR analysis of the 9-Me peaks (9*Z*-isomer: δ 2.04; 9*E*-isomer: δ 2.18). IR ($CHCl_3$): 2210 cm^{-1}. UV–vis: 293, 254 nm. ^{1}H NMR δ: 0.08 (6 H, s), 0.89 (9 H, s), 0.97 and 1.02 (each 3 H, each s), 1.70 (5/6 × 3 H, s), 1.75 (1/6 × 3 H, s), 2.04 (1/6 × 3 H, s), 2.18 (5/6 × 3 H, s), 4.00 (1 H, m), 5.12 (1/6 × 1 H, s), 5.17 (5/6 × 1 H, s), 6.14 (5/6 × 1 H, d, J = 16), 6.50 (5/6 × 1 H, d, J = 16), 6.54 (1/6 × 1 H, d, J = 16), 6.71 (1/6 × 1 H, d, J = 16). HRMS 345.2492 (M$^+$), calcd 345.2487.

To a solution of nitrile **15b** (1.25 g, 3.62 mmol) in dry hexane (15 mL) at −60 °C was added DIBALH (1.0 M; 5.43 mL, 5.43 mmol) and the mixture was stirred at −60 to −40 °C for 15 min. The reaction was quenched with SiO_2:H_2O (1:4, 15 g), and the mixture was stirred at −60 to 0 °C for 1 h. The suspension was filtered through Celite, and the filtrate was concentrated to give a residue, which was purified by CC (EtOAc–hexane, 1:9) to provide a 5:1 mixture of 9*E*- and 9*Z*-isomers as a pale yellow oil. The isomers were separated by low pressure CC (EtOAc–hexane, 1:19) to afford 919 mg of 9*E*-isomer **8b** (73%), $[\alpha]^{23}_D$ = −46.8° (c = 1.38, $CHCl_3$) [lit.[15] $[\alpha]^{22}_D$ = −45.2° (c = 1.27, $CHCl_3$)] and 187 mg of 9*Z*-isomer **16b** (15%), $[\alpha]^{24}_D$ = −37.9° (c = 0.60, $CHCl_3$), as a pale yellow oil, respectively. **9*E*-Isomer 8b:**[15] IR ($CHCl_3$): 1665, 1609 cm^{-1}, UV-vis: 309 nm. ^{1}H NMR δ: 0.08 (6 H, s), 0.89 (9 H, s), 0.99, 1.04 (each 3 H, each s), 1.72 (3 H, s), 2.29 (3 H, s), 4.01 (1 H, m), 5.93 (1 H, d, J = 8), 6.21 (1 H, d, J = 16), 6.68 (1 H, d, J = 16), 10.13 (1 H, d, J = 8). HRMS 348.2495 (M$^+$), calcd 348.2483. **9*Z*-Isomer 16b:** IR ($CHCl_3$): 1665, 1616 cm^{-1}. UV-vis: 303 nm. ^{1}H NMR δ: 0.09 (6 H, s), 0.90 (9 H, s), 1.00, 1.06 (each 3 H, each s), 1.75 (3 H, s), 2.11 (3 H, s), 4.02 (1 H, m), 5.86 (1 H, d, J = 8), 6.57 (1 H, d, J = 16), 7.08 (1 H, d, J = 16), 10.16 (1 H, d, J = 8). HRMS 348.2491 (M$^+$), calcd 348.2483.

(4*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]retinonitrile (9b). To a suspension of NaH (60% oil dispersion; 253 mg, 6.33 mmol) in dry THF (10 mL) at 0 °C was added a solution of C_5-phosphonate (1.37 g, 6.33 mmol) in dry THF (15 mL). After the mixture stirred at rt for 30 min, a solution of aldehyde **8b** (734 mg, 2.11 mmol) in dry THF (10 mL) was added at 0 °C, and the mixture was stirred ar rt for 20 min. The reaction mixture was concentrated to give a residue, which was directly purified by CC (EtOAc–hexane, 1:19) to provide a colorless oil (866 mg, quant.) as a 5:1 mixture of all-*E*- and 13*Z*-isomers, some of which were separated by CC to give all-*E*-isomer **9b** and its 13*Z*-isomer **17b**. **All-*E*-isomer 9b**: IR ($CHCl_3$): 2210, 1578 cm^{-1}. UV-vis: 354 nm. ^{1}H NMR δ: 0.08 (6 H, s), 0.89 (9 H, s), 0.98, 1.02 (each 3 H, each s), 1.72 (3 H, s), 1.99 (3 H, s), 2.20 (3 H, s), 4.01 (1 H, t-like, J = 5.5), 5.17 (1 H, s), 6.11 (1 H, d, J = 11.5), 6.14 (1 H, d, J = 16.5), 6.28 (1 H, d, J = 15.5), 6.28 (1 H, d, J = 16.5), 6.93 (1 H, dd, J = 15.5, 11.5). HRMS 411.2967 (M$^+$), calcd 411.2956. **13*Z*-Isomer 17b:** ($CHCl_3$): 2210, 1578 cm^{-1}. UV-vis: 354 nm. ^{1}H NMR δ: 0.08 (6 H, s), 0.90 (9 H, s), 0.98, 1.04 (each 3 H, each s), 1.73 (3 H, s), 1.99 (3 H, s), 2.05 (3 H, s), 4.02 (1 H, t-like, J = 5), 5.07 (1 H, s), 6.17 (1 H, d, J = 16), 6.23 (1 H, d, J = 11), 6.29 (1 H, d, J = 16), 6.80 (1 H, d, J = 15), 6.98 (1 H, dd, J = 15, 11). HRMS 411.2947 (M$^+$), calcd 411.2955.

(4*R*)-4-Hydroxyretinonitrile (10b). To a solution of nitrile (a mixture of **9b** and **17b**) (795 mg, 1.93 mmol) in dry THF (10 mL) at 0 °C was added (n-Bu)$_4$NF (1.0 M THF solution; 5.80 mL, 5.80 mmol). After stirring at rt for 3 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give a residue, which was purified by CC (EtOAc–hexane, 3:7) to provide **10b** (510 mg, 89%) as a mixture of all-*E*- and 13*Z*-isomers in a ratio of 5:2 by ^{1}H NMR analysis of the 13-Me peaks (all-*E*-isomer: δ 2.20; 13*Z*-isomer: δ 2.06). IR ($CHCl_3$): 3620, 3480, 2210, 1580 cm^{-1}. UV-vis: 354, 227 nm. ^{1}H NMR δ: 1.00, 1.03 (each 3 H, each s), 1.82 (3 H, s), 2.00 (5/7 × 3 H, s), 2.03 (2/7 × 3 H, s), 2.06 (2/7 × 3 H, s), 2.20 (5/7 × 3 H, s), 4.01 (1 H, m), 5.08 (2/7 × 1 H, s), 5.18 (5/7 × 1 H, s), 6.12 (5/7 × 1 H, d, J = 11.5), 6.14 (5/7 × 1 H, d, J = 16.5), 6.16 (2/7 × 1 H, d, J = 16), 6.27 (1 H, d, J = 16), 6.30 (5/7 × 1 H, d, J = 15), 6.81 (2/7 × 1 H, d, J = 15), 6.92 (5/7 × 1 H, dd, J = 15, 11.5), 6.97 (2/7 × 1 H, dd, J = 15, 11.5). HRMS 297.2081 (M$^+$), calcd 297.2091.

(4*R*)-4-OH-RAL (11b). To a solution of nitrile **10b** (504 mg, 1.70 mmol) in dry Et_2O (10 mL) at −60 °C was added DIBALH (1.0 M hexane solution; 2.55 mL, 2.55 mmol) and the mixture was stirred at −60–0 °C for 4.5 h. The reaction was quenched with SiO_2:H_2O (1:4, 5 g), and the mixture was stirred at −60–0 °C for 1 h. The suspension was filtered through Celite, and the filtrate was concentrated to give the residue, which was purified by CC (EtOAc–hexane, 7:13) to provide a 2:1 mixture of the all-*E*-isomer and the 13*Z*-isomer (399 mg, 78%) as a yellow oil. The isomers were separated by preparative HPLC to afford all-*E*-isomer **11b** (263 mg, 52%), $[\alpha]^{26}_D$ = −78.1° (c = 0.63, EtOH) and its 13*Z*-isomer (130 mg, 26%) as a yellow oil. **All-*E*-isomer 11b:** HPLC, t_R = 54.3 FT-IR (KBr): 3400, 1659, 1574 cm^{-1}. UV-vis: 377 nm. ^{1}H NMR δ: 1.01, 1.04 (each 3 H, each s), 1.83 (3 H, s), 2.01 (3 H, s), 2.32 (3 H, s), 4.00 (1 H, m), 5.97 (1 H, d, J = 8), 6.16 (1 H, d, J = 16), 6.19 (1 H, d, J = 11.5), 6.29 (1 H, d, J = 16), 6.38 (1 H, d, J = 15), 7.12 (1 H, dd, J = 15, 11.5), 10.11 (1 H, d, J = 8). HRMS 300.2085 (M$^+$), calcd 300.2087. **13*Z*-Isomer 18b:** HPLC, t_R = 49.8. FT-IR (KBr): 3400, 1659, 1582 cm^{-1}. UV-vis: 371, 258 nm. ^{1}H NMR δ: 1.01, 1.04 (each 3 H, each s), 1.83 (3 H, s), 2.01 (3 H, s), 2.13 (3 H, s), 4.02 (1 H, m), 5.85 (1 H, d, J = 8), 6.17 (1 H, d, J = 16), 6.23 (1 H, d, J = 11.5), 6.29 (1 H, d, J = 16), 7.01 (1 H, dd J = 15, 11.5), 7.29 (1 H, d, J = 15), 10.20 (1 H, d, J = 8). HRMS 300.2094 (M$^+$), calcd 300.2087.

Preparation of Camphanates (4*S*)-12a, (4*R*)-12b, and (4*R*,4*S*)-12. To a solution of (4*R*)-(*all-E*)-4-OH-RAL (**11b**) (10 mg, 0.033 mmol) in dry CH_2Cl_2 (1 mL) at 0 °C were added DMAP (3.57 mg, 0.033 mmol), camphanic acid (6.60 mg, 0.033 mmol), and DCC (6.87 mg, 0.033 mmol), and the mixture was stirred at rt overnight. CH_2Cl_2 was evaporated and the residue was purified by preparative TLC (EtOAc–hexane, 1:2) to give camphanate (4*R*)-**12b** (5.6 mg, 35%) and recovered (4*R*)-**11b** (6.0 mg, 60%) without geometrical isomerization. (4*R*,4*S*)-**12** and (4*S*)-**12a** were obtained with the same procedure from (±)-**11** and (4*S*)-**11a**, respectively. **(4*R*)-Isomer 12b:** HPLC (LiChrosorb Si-60 (5 μm) 0.4 × 30 cm, MeOH–$CHCl_3$–hexane, 0.2:20:80, 1 mL/min, t_R = 20.5). FT-IR (KBr): 1796, 1746, 1725, 1659, 1580 cm^{-1}. UV-vis: 380 nm. ^{1}H NMR δ: 0.98, 1.03, 1.06, 1.07, 1.11 (each 3 H, each s), 1.68 (3 H, s), 2.02 (3 H, s), 2.32 (3 H, s), 5.39 (1 H, t-like, J = 4), 5.98 (1 H, d, J = 8), 6.16 (1 H, d, J = 16), 6.21 (1 H, d, J = 11.5), 6.28 (1 H, d, J = 16), 6.39 (1 H, d, J = 15), 7.12 (1 H, dd, J = 15, 11.5), 10.11 (1 H, d, J = 8). HRMS 480.2875 (M$^+$), calcd 480.2874. **(4*S*)-Isomer 12a:** FT-IR (KBr): 1796, 1746, 1725, 1659, 1580 cm^{-1}. UV-vis: 380 nm. ^{1}H NMR δ: 0.98, 1.02, 1.06, 1.11 (total 15 H, each s), 1.70 (3 H, s), 2.02 (3 H, s), 2.32 (3 H, s), 5.39 (1 H, t-like, J = 4), 5.98 (1 H, d, J = 8), 6.17 (1 H, d, J = 16), 6.21 (1 H, d, J = 11.5), 6.28 (1 H, d, J = 16), 6.39 (1 H, d, J = 15), 7.12 (1 H, dd, J = 15, 11.5), 10.11 (1 H, d, J = 8). HRMS 480.2876 (M$^+$), calcd 480.2874. **(4*R*,4*S*)-12:** HPLC (LiChrosorb Si-60 (5 μm) 0.4 × 30 cm, MeOH–$CHCl_3$–hexane, 0.2:20:80, 1 mL/min, t_R = 19.2 and 20.5). FT-IR (KBr): 1796, 1746, 1725,

(15) Haag, A.; Eugster, C. H. *Helv. Chim. Acta* **1982**, *65*, 1795.

1659, 1580 cm^{-1}. UV-vis: 380 nm. ^1H NMR δ: 0.98, 1.02, 1.06, 1.07, 1.11 (total 15 H, each s), 1.68 (1/2 × 3 H, s), 1.70 (1/2 × 3 H, s), 2.02 (3 H, s), 2.32 (3 H, s), 5.39 (1 H, t-like, J = 3.5), 5.97 (1 H, d, J = 8), 6.16 (1 H, d, J = 16), 6.21 (1 H, d, J = 11.5), 6.27 (1 H, d, J = 16), 6.39 (1 H, d, J = 15.5), 7.12 (1 H, dd, J = 15.5, 11.5), 10.11 (1 H, d, J = 8). HRMS 480.2874 (M^+), calcd 480.2874.

(4*R*)-*p*-(Dimethylamino)cinnamate (13b). To a solution of (4*R*)-(all-*E*)-4-OH-RAL (**11b**) (11 mg, 0.037 mmol) and *p*-(dimethylamino)cinnamoyl triazole (27 mg, 0.11 mmol) in dry CH_2Cl_2 (1 mL) was added a 0.01 M solution of DBU in CH_2Cl_2 (0.73 mL, 0.073 mmol). After stirring overnight at rt, the reaction mixture was concentrated and purified by CC (EtOAc–hexane, 3:7) to provide **13b** (1 mg) as a yellow oil. FT-IR (KBr): 1700, 1660, 1600 cm^{-1}. UV-vis (CH_3CN): 367 nm. ^1H NMR (500 MHz) δ: 1.04, 1.09 (each 3 H, each s), 1.73 (3 H, s), 2.03 (3 H, s), 2.32 (3 H, s, J = 1.0), 3.01 (6 H, s), 5.37 (1 H, t, J = 4.5), 5.97 (1 H, br d, J = 8.0), 6.21 (1 H, d, J = 11.5), 6.22 (1 H, d, J = 16.5), 6.25 (1 H, d, J = 16.0), 6.31 (1 H, d, J = 16.5), 6.38 (1 H, d, J = 15.0), 6.66 (2 H, d-like, J = 9.0), 7.12 (1 H, dd, J = 15.0, 11.5), 7.42 (2 H, d-like, J = 9.0), 7.63 (1 H, d, J = 16.0), 10.10 (1 H, d, J = 8.0). HRMS 473.2946 (M^+), calcd 473.2928.

Photoisomerization of (4*R*)-(*all-E*)-4-OH-RAL (11b). (4*R*)-(*all-E*)-4-OH-RAL (**11b**) (10 mg, 0.033 mmol) in EtOH (10 mL) was irradiated for 30 min with a slide projector lamp behind a Pyrex glass at 0 °C to give a mixture of geometrical isomers. Evaporation of EtOH and subsequent preparative HPLC of the residue in the dark provided 11*Z*,13*Z*-, 13*Z*-, all-*E*-, 11*Z*-, ($[\alpha]^{25}_D$ = −76.0° (*c* = 0.66, EtOH)), and 9*Z*-isomers in a ratio of *ca.* 13:26:35:16:10. **11Z,13Z-Isomer 19b:** HPLC, t_R = 45.1. FT-IR (KBr): 3430, 1660, 1580 cm^{-1}. UV-vis: 359 (sh), 295, 227 nm. ^1H NMR δ: 0.98, 1.01 (each 3 H, each s), 1.79 (3 H, s), 1.95 (3 H, s), 2.06 (3 H, s), 3.98 (1 H, m), 5.96 (1 H, d, J = 8), 6.06 (1 H, d, J = 16), 6.11 (1 H, d, J = 11.5), 6.18 (1 H, d, J = 11.5), 6.21 (1 H, d, J = 16), 6.75 (1 H, t, J = 11.5), 9.67 (1 H, d, J = 8). HRMS 300.2096 (M^+), calcd 300.2088. **11Z-Isomer 4b:** HPLC, t_R = 59.9. FT-IR (KBr): 3385, 1659, 1574 cm^{-1}. UV-vis: 373, 254 nm. ^1H NMR δ: 1.00, 1.03 (each 3 H, each s), 1.82 (3 H, s), 1.97 (3 H, s), 2.34 (3 H, s), 4.00 (1 H, m), 5.95 (1 H, d, J = 11), 6.07 (1 H, d, J = 8), 6.14 (1 H, d, J = 16), 6.28 (1 H, d, J = 16), 6.53 (1 H, d, J = 12), 6.67 (1 H, t-like, J = 12), 10.08 (1 H, d, J = 8). HRMS 300.2084 (M^+), calcd 300.2087. **9Z-Isomer 20b:** HPLC, t_R = 80.4. FT-IR (KBr): 3420, 1659, 1584 cm^{-1}. UV-vis: 370 nm. ^1H NMR δ: 1.03, 1.05 (each 3 H, each s), 1.86 (3 H, s), 2.01 (3 H, s), 2.31 (3 H, s), 4.05 (1 H, m), 5.97 (1 H, d, J = 8.5), 6.12 (1 H, d, J = 11.5), 6.27 (1 H, d, J = 16), 6.31 (1 H, d, J = 15), 6.68 (1 H, d, J = 16), 7.18 (1 H, dd, J = 15, 11.5), 10.10 (1 H, d, J = 8.5). HRMS 300.2079 (M^+), calcd 300.2087.

(4*R*)-(11*Z*)-4-OH-RAL Oxime (5b). A MeOH solution (3.5 mL) of (4*R*)-(11*Z*)-4-OH-RAL (**4b**) (3.0 mg, 0.01 mmol) was mixed with 100-fold molar excess of aqueous hydroxylamine hydrogen carbonate (1.5 mL) (prepared by neutralization of the aqueous hydrochloride with saturated aqueous $NaHCO_3$). The solution was stirred in the dark at rt for 5 min and then extracted twice with CH_2Cl_2–MeOH–H_2O (1:1:1). The lower layer was washed with brine, dried over Na_2SO_4, and evaporated to give a residue, which was purified by SiO_2 CC (EtOAc–hexane, 3:7) to provide *syn*-oxime **14b** (1.8 mg, 57%) and *anti*-oxime **5b** (0.3 mg, 10%). ***syn*-Isomer 14b:** FT-IR (KBr): 3270 cm^{-1}. UV-vis: 348, 256 nm. ^1H NMR δ: 1.00, 1.03 (each 3 H, each s), 1.82 (3 H, s), 1.94 (3 H, s), 2.05 (3 H, s), 4.00 (1 H, t-like, J = 4.5), 5.94 (1 H, d, J = 11), 6.14 (1 H, d, J = 16), 6.18 (1 H, d, J = 10.5), 6.20 (1 H, d, J = 16), 6.44 (1 H, t, J = 11), 6.57 (1 H, d, J = 11), 8.14 (1 H, d, J = 10.5). HRMS 315.2199 (M^+), calcd 315.2197. ***anti*-Isomer 5b:** FT-IR (KBr): 3270 cm^{-1}. UV-vis: 353, 256 nm. ^1H NMR δ: 1.00, 1.03 (each 3 H, each s), 1.82 (3 H, s), 1.94 (3 H, s), 2.09 (3 H, s), 4.00 (1 H, t-like, J = 4.5), 5.99 (1 H, d, J = 11), 6.13 (1 H, d, J = 16), 6.21 (1 H, d, J = 16), 6.50 (1 H, t, J = 11), 6.62 (1 H, d, J = 11), 6.74 (1 H, d, J = 9.5), 7.47 (1 H, d, J = 9.5). HRMS 315.2209 (M^+), calcd 315.2197.

(4*S*)-4-[(*tert*-Butyldimethylsilyl)oxy]-β-ionone (7a). Camphanate **6a** (573 mg, 1.48 mmol) treated as described by Haag et al.[9] gave 412 mg of (4*S*)-4-[(*tert*-butyldimethylsilyl)-oxy]-β-ionone (**7a**) (87%), $[\alpha]^{20}_D$ = −8.0° (*c* = 1.00, EtOH), $[\alpha]^{20}_D$ = −4.7° (*c* = 1.07, $CHCl_3$). IR ($CHCl_3$): 1661, 1604 cm^{-1}. UV-vis: 287, 216 (sh) nm. ^1H NMR δ: 0.08 (6 H, s), 0.89 (9 H, s), 1.00, 1.06 (each 3 H, each s), 1.74 (3 H, s), 2.28 (3 H, s), 4.01 (1 H, t-like, J = 4.5), 6.11 (1 H, d, J = 16.5), 7.19 (1 H, d, J = 16.5). HRMS 322.2331 (M^+), calcd 322.2327.

(4S)-4-[(*tert*-Butyldimethylsilyl)oxy]-β-ionylideneacetaldehyde (8a). (4*S*)-4-[(*tert*-Butyldimethylsilyl)-oxy]-β-ionone (**7a**) (82 mg, 0.25 mmol) treated as described for **8b** gave 88 mg of (4*S*)-4-[(*tert*-butyldimethylsilyl)oxy]-deoxo-β-ionylideneacetonitrile (quant.). ^1H NMR spectrum of the nitrile was identical with that of the 4*R*-isomer. The nitrile (88 mg, 0.25 mmol), treated as described for **8b**, gave 84 mg of (4*S*)-4-[(*tert*-butyldimethylsilyl)oxy]-β-ionylideneacetaldehyde (**8a**) (95%) as a mixture of 9*Z*- and 9*E*-isomers (1:5), which was separated by low pressure CC as described for **8b**. Spectral properties of **8a** were identical with those of the 4*R*-isomer of **8b**. **9E-Isomer 8a:** $[\alpha]^{20}_D$ = +45.8° (*c* = 1.38, $CHCl_3$). **9Z-Isomer 16a:** $[\alpha]^{20}_D$ = +38.8° (*c* = 1.61, $CHCl_3$).

(4S)-4-[(*tert*-Butyldimethylsilyl)oxy]retinonitrile (9a and 17a). (4*S*)-4-[(*tert*-Butyldimethylsilyl)oxy]-β-ionylideneacetaldehyde (**8a**) (210 mg, 0.60 mmol) treated as described for **9b** gave 190 mg of (4*S*)-4-[(*tert*-butyldimethylsilyl)oxy]-retinonitrile as a mixture of all-*E* isomer **9a** and its 13*Z*-isomer **17a** (5:1) (77%). Spectral properties of **9a** were identical with those of the (4*R*)-isomers **9b** and **17b**.

(4S)-4-Hydroxyretinonitrile (10a). (4*S*)-4-[(*tert*-Butyldimethylsilyl)oxy]retinonitrile (**9a**) (130 mg, 0.32 mmol) treated as described for **10b** gave 90 mg of (4*S*)-4-hydroxyretinonitrile (**10a**) as a mixture of all-*E*- and 13*Z*-isomers (2:1) (96%). Spectral properties of **10a** were identical with those of the (4*R*)-isomer **10b**.

(4S)-(*All-E*)-4-OH-RAL (11a). (4*S*)-4-Hydroxyretinonitrile (**10a**) (85 mg, 0.29 mmol) treated as described for **11b** gave 62 mg of (4*S*)-4-OH-RAL (**11a**) (all-*E*) and **18a** (13*Z*) (72%). Spectral properties of **11a** and **18a** were identical with those of (4*R*)-isomers **11b** and **18b**. **11a**: $[\alpha]^{27}_D$ = +73.1° (*c* = 0.59, EtOH).

Photoisomerization of (4S)-(*All-E*)-4-OH-RAL (11a). By means of the procedure used for photoisomerization of **11b**, (4*S*)-(*all-E*)-4-OH-RAL (**11a**) (20 mg, 0.066 mmol) was isomerized to give 11*Z*,13*Z*-, 13*Z*-, all-*E*-, 11*Z*-, and 9*Z*-isomers (**19a**, **18a**, **11a**, **4a**, and **20a**). **11Z-Isomer 4a:** $[\alpha]^{25}_D$ = +74.2° (*c* = 0.30, EtOH).

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Supplementary Material Available: ^1H NMR spectra with peak assignments of compounds **4a**, **7a**–**12a**, **15a**, **16a**, **18a**–**20a**, **4b**, **5b**, **8b**–**20b**, and **12** (29 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.