On the Bioactive Conformation of the Rhodopsin Chromophore: Absolute Sense of Twist around the 6-*s*-*cis* Bond

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Abstract: Incubation of opsin with synthetic 6-s-locked retinoids **2a** and **2b** only led to pigment formation from the alpha-locked **2a**, the CD spectrum of which was similar to that of native rhodopsin (Rh). This establishes that the 6-*s*-bond of the chromophore in rhodopsin is *cis*, and that its helicity is negative. Earlier cross-linking studies showed that the 11-*cis* to all-*trans* photoisomerization occurring in the batho-Rh to lumi-Rh conversion induces a flip over of the side carrying the ring moiety. The GTP-binding assay of pigment Rh-(**2a**), incorporating retinal analogue **2a**, has shown that its activity is 80% that of the native pigment. That is, the overall conformation around the 6-*s* bond is retained in the steps leading to G-protein activation.

Keywords: chirality · circular dichroism · conformation analysis · receptors · retinal · rhodopsin

Introduction

The visual pigment rhodopsin (Rh), a membrane protein belonging to the family of G-protein (guanyl-nucleotide-binding protein) coupled receptors (GPCR), consists of seven transmembrane α-helices, helix A to helix G (or helices 1–7).[1–5] The X-ray structure of the 40 kDa bovine Rh, **1a**, comprising the 348 amino acid receptor opsin and its ligand 11-*cis*-retinal, **1b**, has recently been solved (Figure 1). This is a first for a GPCR,[6, 7] although the 2.8 Å resolution may not fully define the conformation of the chromophore.

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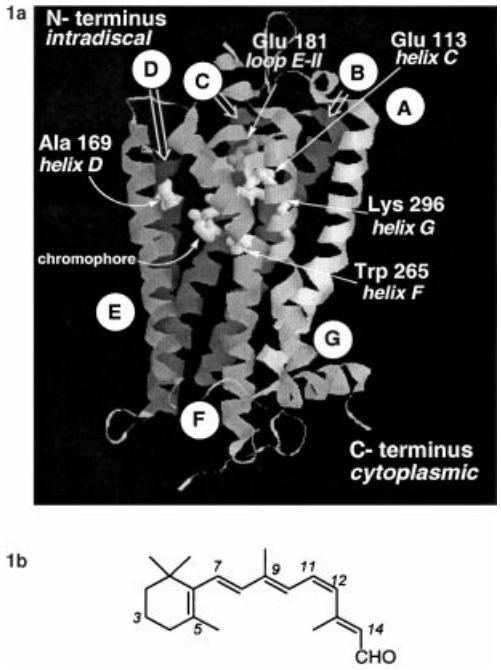

Figure 1. a) Rh crystal structure adopted from ref. [6], chromophore is shown behind helix F; b) 11-*cis* retinal

The chromophore **1b** is present as the positively charged retinylidene chromophore **1c** bound to Lys 296 in helix G via a protonated Schiff base (PSB),[8] the counterion of which is Glu-113 in helix C.(Figure 1a)[9, 10] Due to the steric interactions between 5-Me/8-H and 13-Me/10-H, the chromophore

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is twisted around the C6/C7 and C12/C13 single bonds; that is, planes A/B and B/C in **1c** cannot be coplanar (see Scheme 1, below).[11, 12] This noncoplanarity together with other factors has a direct bearing on the absorption maxima of visual pigments; for example, 500 nm for bovine Rh. Namely, the opsin-binding-site environment dictates: i) the protonated Schiff base–counterion distance, ii) the interaction between an "external point charge" and the polyene chain;[13] and iii) the extent of noncoplanarity of the chromophore. In the X-ray structure shown in Figure 2, this "external point charge" is represented by Glu-181 that resides in the extramembrane E-II loop that links helices D and E (see Figure 1) on the intradiscal side; the salt bridge between the PSB and its counter ion Glu-113 can be seen in the X-ray structure.

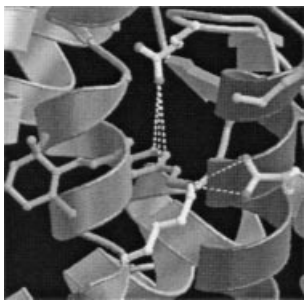
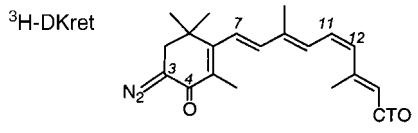


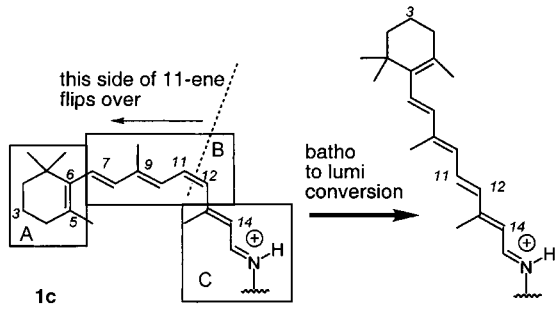
Figure 2. "External point charge" model showing proximity of Glu-181 to the 11,12-ene and counterion Glu-113 interacting with the protonated Schiff base.

Visual transduction is the process by which visual cells convert light into a neural signal, which in turn is transmitted to the brain along the optic nerve. Absorption of light by Rh leads to *cis* → *trans* isomerization of the chromophore; this results in sequential thermal relaxation of the pigment through the intermediates: photo-Rh (λ_{max} 555 nm) → batho- (−140 °C, 535nm) → lumi- (−40 °C, 497 nm) → meta-I- (−15 °C, 480 nm) → meta-II-Rh (0 °C, 380 nm). Except for photo-Rh, the intermediates can be sequestered at the temperatures indicated and submitted to various spectroscopic and other studies. In vertebrate vision, the shape of the protein at the meta-II-Rh stage initiates a cascade of enzymatic reactions, starting with activation of >1000 molecules of G protein (called transducin in vision), that, through phosphodiesterase, result in hydrolysis of 100000 molecules of cyclic guanidine monophosphate (cGMP). This drop in cGMP closes the cation-specific channels in the rod outer segment of visual cells and leads to a buildup of electric potential, which results in generation of the neural signal.[14–20]

Photo cross-linking and sequencing studies were performed with 11-*cis*-3-diazo-4-ketoretinal (DKret) on each of the



transduction intermediates derived from the pigment incorporating cross-linked DKret: namely, at −196 °C for batho, −80 °C for lumi, −40 °C for Meta-I and 0 °C for Meta-II. These studies showed that:[21, 22] i) in ground state Rh[23] and batho-Rh, the C-3 of the ionone ring is close to Trp265 in helix F (see Figure 1a), but that ii) in the batho-Rh to lumi-Rh conversion step, in which the 11-*cis* to all-*trans* double bond isomerization takes place, an unexpected flip-over on the side of the ring moiety occurs (Scheme 1) so that the chromophore C-3 now becomes cross-linked solely to the remote Ala169 in helix D (Figure 1a); the cross-linking site remains the same in the subsequent meta-I- and meta-II-Rh intermediates.



Scheme 1. Conversion of chromophore configuration from ground state Rh and batho-Rh to lumi-Rh.

These changes necessarily induce large helical movements, including twists of helices and changes in conformations of extramembrane interhelical loops, especially in the batho → lumi conversion (Scheme 1). The conformation of the cytoplasmic extramembrane loop at the meta-II-Rh stage activates the heterotrimeric G-protein transducin (G_t); this leads to vision.

Determination of the absolute sense of twist around the 12-*s*-bond adjacent to the *cis*–*trans* isomerization site of the chromophore is critical, since the helicity direction should determine the chromophore movement after the photoisomerization. However, the nature of the twist around this 12-*s*-*cis* bond is controversial. Namely, a negative helicity of the retinal chromophore around the 12-*s*-bond, that is, with 13-Me in the rear of plane B, was proposed by theoretical calculations,[24] solid-state NMR studies,[25, 26] the CD of rhodopsin with 11,12-dihydro chromophores,[27] and studies with 11,12-cyclopropyl-Rh.[28] On the other hand, more recent theoretical studies have led to a positive helicity around the C12–C13 bond,[29, 30] that is, 13-Me in front of plane B. An analysis of chiroptical data by Buss using the best available theoretical analysis as well as more approximate methods has led to a correlation between a positive Rh CD α-band and a positive twist of the 12-s-bond (see Figure 7b, below).[31] Based on the 2.8 Å resolution X-ray structure[32] and ab initio data,[31] the 12-*s*-bond helicity is most likely positive as depicted in Figure 3.

With respect to the 6-*s* bond, in the following we describe results that establish the absolute sense of twist between planes A/B of the Rh chromophore as negative.

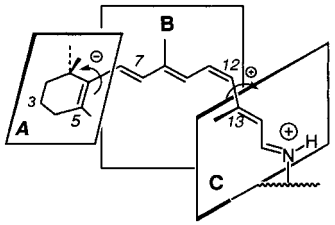
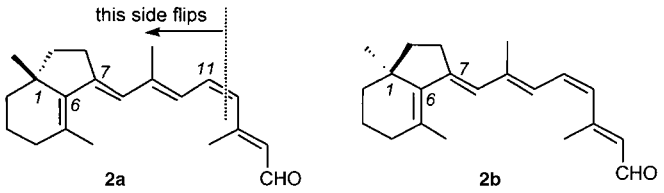


Figure 3. Pigment conformation of rhodopsin.

Results and Discussion

Incubation studies with 6-*s*-*cis*-locked retinoids **2a** and **2b** showed that only the former (1*R*) enantiomer gave a pigment, the CD of which was similar to that of native Rh. This is in agreement with the earlier NMR magic-angle spinning results that showed the C6-C7 bond to be 6-*s*-*cis*[33, 34] but is against the recently reported 6-*s*-*trans* conformation.[35] More importantly, the present study determines the absolute sense of helicity between the cyclohexene ring and the polyene side chain as negative, as seen in Figure 3.

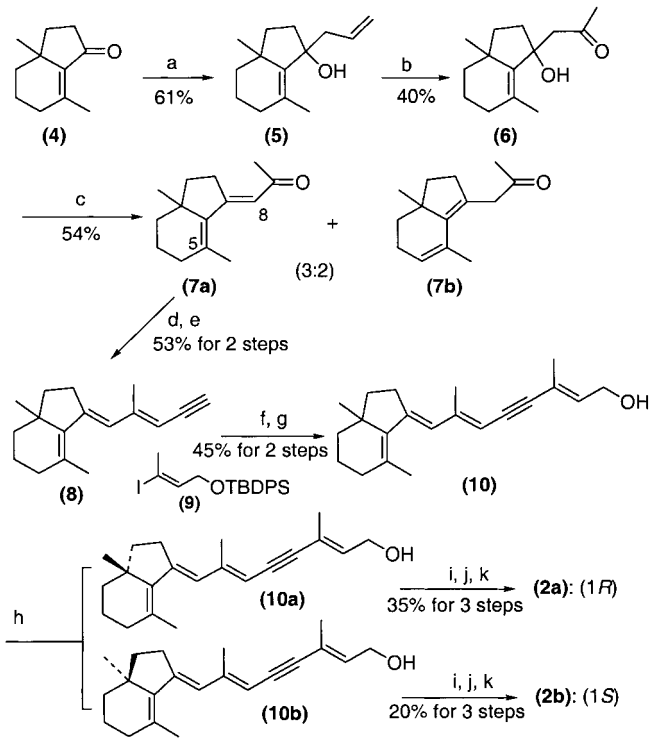
In order to determine the absolute sense of twist around the 6-s-bond in Rh, the enantiomeric α- and β-locked retinal analogues **2a** and **2b**, with established absolute configura-



tions, were prepared and submitted to incorporation studies with opsin. Only the α-locked retinal **2a** formed a pigment Rh-(**2a**), the CD of which was very similar to that of native Rh (Figure 7, below), thus establishing the conformation around the 6-*s*-*cis* bond as negative (Figure 3).

The synthesis and determination of configurations of target retinals α-locked **2a** and β-locked **2b** were performed by resolving racemic allylic alcohol **10** into enantiomeric alcohols **10a**/**10b** (Scheme 2, below), preparing 5-desmethyl analogue **16** from methyl (*R*)-1-methyl-2-oxocyclohexane proprionate **11** of established configuration (Scheme 3, below, and comparing the CD of **16**/**10a** and **16**/**10b** (Figure 4, below).

Syntheses of **2a** and **2b** are outlined in Scheme 2. The readily accessible enone **4**, prepared from 2,6-dimethylcyclohexanone,[36, 37] was treated with allylmagnesium chloride to give allylic alcohol **5**. Wacker oxidation[38] afforded ketol **6**, but only in moderate yield, 24% for two steps. Ketol **6** was exposed to methanesulfonyl chloride and DMAP (for abbreviations, see legends in Schemes) to generate a 3:2 mixture of two isomers, **7a** and **7b** in 54% yield. The steric interaction between 5-Me and 8-H in **7a** led to the formation of isomerized product **7b**; the 7-*cis* alkene is not observed due to severe steric repulsion. Horner–Wadsworth–Emmons (HWE) reaction of **7a** with dimethyl-(3-trimethylsilyl-2-propynyl)phosphonate,[39] followed by deprotection, gave



Scheme 2. Synthesis of retinals **2a** and **2b**. a) allyl MgCl, THF, 0°C–RT, 61%; b) $PdCl_2$, CuCl, O_2, 80% aq. DMF, 6 h, 40%; c) MsCl, DMAP, CH_2Cl_2, pyridine, 54%; d) $(MeO)_2POCH_2C{\equiv}CTMS$, KHMDS, THF; e) Bu_4NF, THF, RT, 53% (two steps); f) vinyl iodide **9**, $(Ph_3P)_4Pd$, CuI, $iPrNH_2$; g) Bu_4NF, THF, 45% (two steps); h) Enantioselective HPLC separation (CHIRALPAK AD); i) Zn(Cu/Ag), MeOH, H_2O, RT, 21 h; j) TPAP, NMO, CH_2Cl_2, RT, 2 h; k) silica gel, HPLC, 35% for **2a**, 20% for **2b** (two steps). MsCl = methanesulfonyl chloride, DMAP = 4,4-dimethylaminopyridine, KHMDS = potassium salt of 1,1,1,3,3,3-hexamethyldisilazane, NMO = 4-methyl-morpholine-*N*-oxide, TPAP = tetrapropylammoniumperruthenate, TBDPS = *tert*-butyldiphenylsilyl.

alkyne **8** in 53% yield. Sonogashira coupling[40] of **8** with vinyl iodide **9**,[41] followed by deprotection, afforded **10** in 45% yield. Racemic **10** was resolved into enantiomers **10a** and **10b** by CHIRALPAK AD (Chiral Technologies Inc., subsidiary of Daicel, semi-preparative, 250 × 10 mm I.D. 10 μm), which were then reduced with Cu/Ag-activated zinc dust in methanol/water at RT to form the corresponding 11-*cis*-retinoids.[42] Subsequent oxidation with NMO/TPAP (tetrapropylammonium perruthenate) gave retinal analogues **2a** and **2b** in yields of 35% and 20%, respectively, after HPLC purification.

The synthesis of the desmethyl alcohol **16** (Scheme 3) started from commercially available (*R*)-1-Methyl-2-oxocyclohexane proprionate, **11**, which was cyclized to ketone **12** with samarium(II) iodide[43] in 64% yield. This was followed by enol acetate formation and OsO_4 catalyzed dihydroxylation to α-hydroxyketone **13** with 50% yield for two steps. Although direct transformation of **11** to **13** with SmI_2 appears to be plausible, it was necessary to use excess (>5 equiv) SmI_2 to push the reaction to completion, this resulted in the formation of over-reduced ketone **12**. Treatment of hydroxy ketone **13** with CSA under reflux gave enone **14** in 86% yield. Grignard addition to **14**, followed by Wacker oxidation and treatment with methanesulfonyl chloride and DMAP afforded dienone

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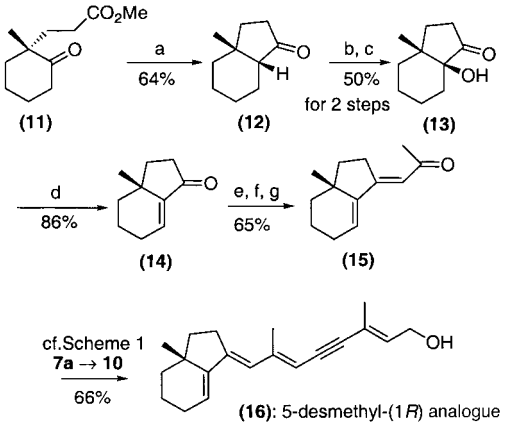

Scheme 3. Synthesis of alcohol **16**. a) SmI_2, HMPA, THF, RT, 64%; b) CSA (catalyst), isopropenyl acetate, reflux, overnight, 52%; c) OsO_4, NMO, acetone/H_2O, RT, 96%; d) CSA (catalyst), toluene, reflux, 8 h, 86%; e) allyl MgCl, THF, 0 °C – RT, 98%; f) $PdCl_2$, CuCl, O_2, 80% aq. DMF, 6 h (quant.); g) MsCl, DMAP, CH_2Cl_2, pyridine (66%). HMPA = hexamethylphosphoramide, CSA = camphorsulfonic acid, DMF = *N*,*N*-dimethylformamide.

15 in 65% overall yield. Double-bond rearrangement accompanying the elimination step, as seen with **7b**, was not seen here. Transformations of **15** to **16** occurred similarly to those described in Scheme 2 (from **7a** to **10**); the yield of **16** for the four steps being 66%. The absolute configurations of 6-*s*-*cis* locked enantiomers **10a** and **10b** were then assigned by comparisons of their CD (Figure 4, right) with that of 5-desmethyl analogue **16**, which has established configuration at C-1 (Figure 4, left). The CD of enantiomeric retinals **2a** and **2b** are shown in Figure 4, bottom.

The twist around the 6-*s*-bond: Upon incubation of the two analogues **2a** and **2b** with bovine opsin, the α-locked **2a** yielded in 1.5 hours a pigment that absorbed at 539 nm; however, in sharp contrast, no pigment was formed from β-locked analogue **2b**, even after 4 hours (Figure 5). Molecular modeling by Macro Model (MM3 force field) yielded the lowest energy conformation of the unbound chromophore **2a** with a -35° twist around the C6–C7 bond (Figure 6). The CD of pigment from **2a**, Rh-**2a**, with two positive CEs (Cotton effects) at 536 nm ($\Delta\varepsilon = +4$) and 329 nm ($\Delta\varepsilon = +6.5$) (Figure 7a) closely resembles that of native rhodopsin with CEs at 500 nm ($\Delta\varepsilon = +9.5$) and 336 nm ($\Delta\varepsilon = +15$) (Figure 7b).

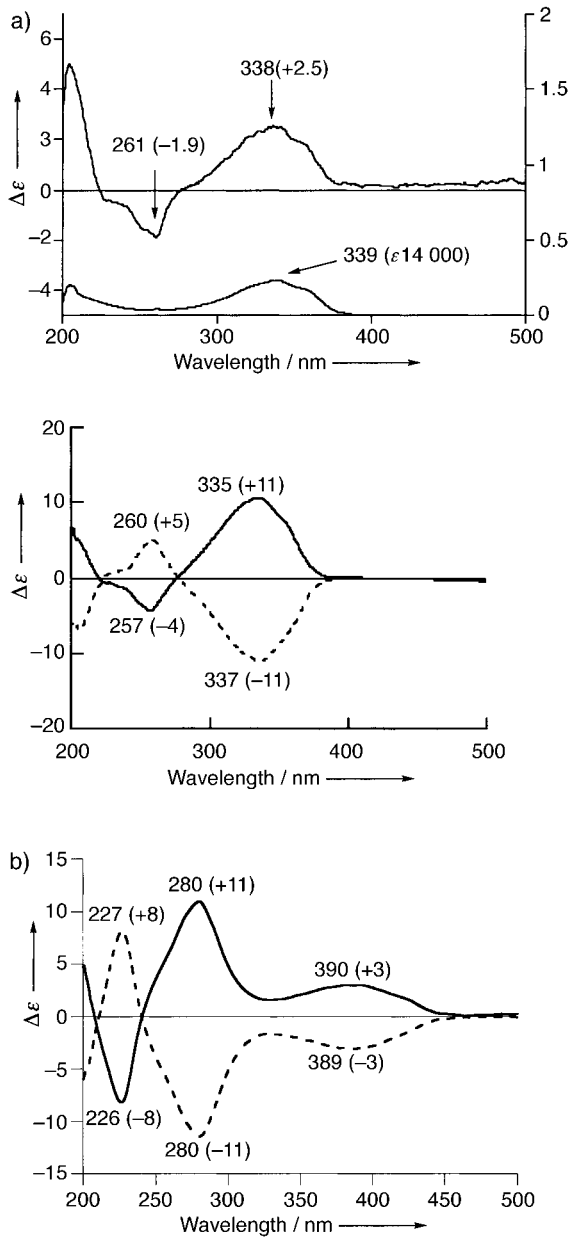

Figure 4. a) CD and UV spectra of **16** (top) and CD spectra of resolved **10a** (——) and **10b** (----) (middle); b) CD spectra of α-locked retinal analogue **2a** (——)and β-locked retinal analogue **2b** (----). All spectra were measured in methylcyclohexane. Numbers in brackets indicate the intensity of the CD signals.

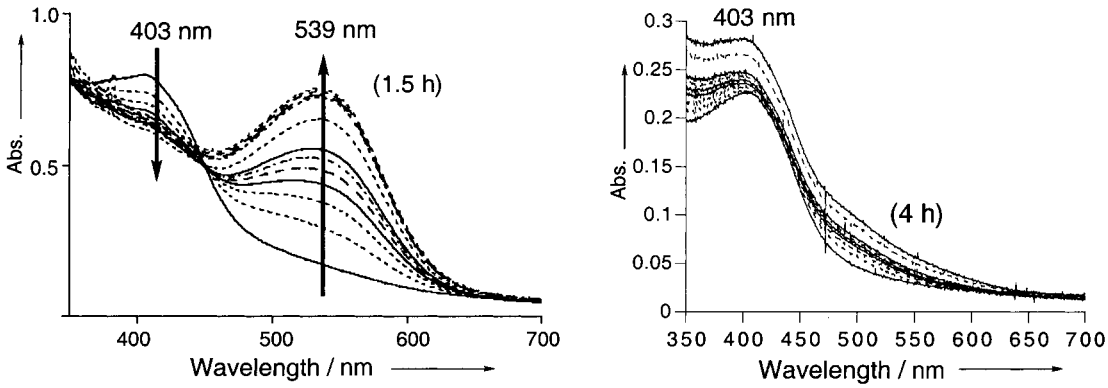

Figure 5. UV spectra of the binding progress upon independent incubation of the α-locked retinal analogue **2a** (left) and the β-locked **2b** (right) with opsin.

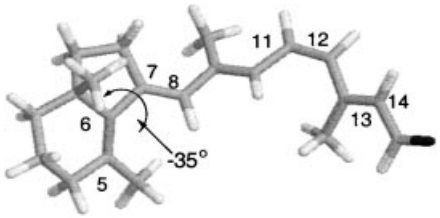

Figure 6. The lowest energy conformation of the **2a** calculated by Macro Model 6.0; (the 12-*s* twist depicted here is irrelevant since it will be determined by the protein environment in the pigment)

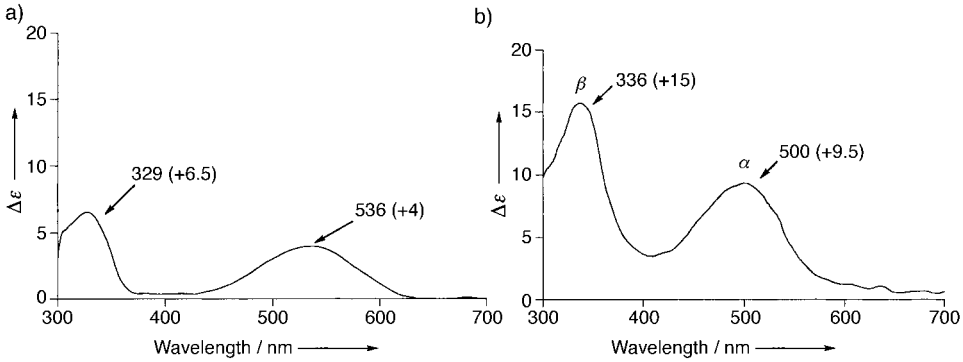

Figure 7. a) CD spectrum of **2a**; b) CD spectrum of native Rh. All spectra were measured in 10 mM CHAPSO/HEPES buffer (pH 6.6). Numbers in brackets indicate the intensity of the CD signals.

It should be noted that a GTP-binding assay showed Rh-**2a** to be 80% as active as the native pigment. The present results, that is, incorporation of **2a** but not **2b**, the close similarities in the CD of **2a** pigment and native pigment, and the bioactivity of the Rh-**2a** pigment with a fixed 6-*s*-linkage, establish the absolute sense of helicity around the 6-*s*-*cis* bond as being negative. As described above[21, 22] the 11-*cis* to all-*trans* isomerization results in the flip-over of the ring-containing moiety. Moreover, the 80% efficiency of rigid 6-*s*-*cis* locked Rh-**2a** in activating transducin shows that, after *cis* to *trans* isomerization, the conformation of the left half of the chromophore remains more or less rigid. Note that the apoprotein differentiates retinals **2a** and **2b** that differ only slightly in geometry around the 6-*s*-bond, that is, $\pm 35^\circ$.

Conclusion

In summary, with the 6-*s*-*cis* locked retinoids only the α-analogue and not its enantiomer gives rise to a pigment, the CD of which closely resembles that of native Rh. This establishes that the 6-*s* bond in Rh is *cisoid* and that the C6–C7 bond has a negative helicity in the protein. In the refined X-ray structure of Rh, which clarified the overall pigment structure, this 6-*s*-twist is -56.6°.[32]

Experimental Section

Materials and general procedures: Centrifugation was performed on Beckman L-70 and L8-M ultracentrifuges with appropriate rotors. HPLC analysis and purification of retinal analogues were performed in the dark on a Rainin HP solvent-delivery system with absorbance detector model UV-D. NMR spectra were recorded on Bruker DMX 500, DRX 400 or DPX 300 instruments and performed in $CDCl_3$ or in [D_6]benzene. Low-resolution and high-resolution FAB mass spectra were measured on a JEOL JMS-DX303 HF mass spectrometer by using a glycerol matrix and Xe ionizing gas. CI mass spectra were measured on a NERMAG R10–10 spectrometer with NH_3 as ionizing gas. ESI mass spectra were measured on JEOL JMS-LC mate LCMS system. IR spectra were recorded on PERKIN ELMER FT-IR spectrometer PARAGON 1000. CD spectra were measured by JASCO J-720 and JASCO J-810 spectropolarimeters with 1 cm light-path cell. UV spectra were measured on a Perkin-Elmer Lambda 40 spectrophotometer. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter at room temperature.

Synthesis of retinal analogues

*Allyl-3a,7-dimethyl-2,3,3a,4,5,6-hexahydro-1H-inden-1-ol (**5**)*: Allylmagnesium chloride (2.0 M solution in THF, 6.1 mL, 12.2 mmol) was added to a solution of enone **4** (1.00 g, 6.09 mmol) in THF (20 mL) at 0 °C under an Ar atmosphere. The reaction mixture was stirred for 2 h at 24 °C, then a saturated NH_4Cl solution (50 mL) was added to it. The mixture was extracted with Et_2O (50 mL × 3), and the combined extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes/EtOAc 10:1) to give compound **5** (762 mg, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.09$ (s, 3H), 1.14–1.26 (m, 2H), 1.56 (dd, $J = 6.7$, 12.2 Hz, 1H), 1.63–1.73 (m, 3H), 1.79–1.95 (m, 2H), 2.01–2.05 (m, 2H), 2.41 (dd, $J = 6.8$, 13.0 Hz, 1H), 2.62 (dd, $J = 7.8$, 13.0 Hz, 1H), 5.11–5.19 (m, 2H), 5.79–5.89 (m, 1H). APCI-MS: 207.2 $[M+H]^+$.

*1-(1-Hydroxy-3a,7-dimethyl-2,3,3a,4,5,6-hexahydro-1H-inden-1-yl)propan-2-one (**6**)*: CuCl (500 mg, 5.05 mmol) and $PdCl_2$ (121 mg, 0.68 mmol) were added to a solution of alcohol **5** (694 mg, 3.36 mmol) in 80% aqueous DMF (15 mL). The reaction mixture was stirred at RT for 8 h under an O_2 atmosphere. The suspension was filtered through Celite, and the filtrate was concentrated in vacuo. The solution was diluted with brine (15 mL), and the mixture was extracted with Et_2O (15 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes/EtOAc 5:1) to give compound **6** (296 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.08$ (s, 3H), 1.62–1.74 (m, 4H), 1.82–2.10 (m, 6H), 1.83 (s, 3H), 2.20 (s, 3H), 2.62 (d, $J = 17.8$ Hz, 1H), 3.08 (d, $J = 17.8$ Hz, 1H), 4.09 (s, 1H); HRMS(FAB): 245.1526 $[M+Na]^+$.

*1-(3a,7-Dimethyl-2,3,3a,4,5,6-hexahydroinden-1-ylidene)propan-2-one(**7**)*: 4-DMAP (17 mg, 0.14 mmol) and MsCl (0.31 mL, 4.0 mmol) were added to a solution of compound **6** (296 mmol, 1.33mmol) in CH_2Cl_2 (5 mL) and pyridine (5 mL). The reaction mixture was stirred at RT for 19 h and was then concentrated in vacuo. The residue was diluted with brine (20 mL), and the aqueous phase was extracted with Et_2O (20 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes/EtOAc 50:1) to give a mixture of compound **7a** (147 mg, 0.72 mmol, 54%) and its isomer **7b** (**7a**/**7b** 3:2).

7a: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.93$ (s, 3H), 1.21–1.41 (m, 4H), 1.67–1.80 (m, 3H), 1.84 (dt, $J = 12.6$, 3.3 Hz, 1H), 1.93 (s, 3H), 2.02–2.22 (m, 2H), 2.27 (s, 3H), 2.88 (dddd, $J = 20.3$, 10.8, 7.9, 2.5 Hz, 1H), 2.99 (dd, $J = 20.3$, 1.4 Hz, 1H), 6.34 (s, 1H); ^{13}C NMR (75 Hz, $CDCl_3$): $\delta = 19.1$, 21.1, 24.6, 30.9, 31.8, 33.9, 36.1, 39.2, 41.0, 121.3, 134.9, 141.4, 160.6, 198.3. HRMS (EI): calcd for $C_{14}H_{20}O$ $[M]^+$: 204.1514, found: 204.1512.

7b: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.93$ (s, 3H), 1.43 (dt, $J = 6.0$, 12.3 Hz, 1H), 1.48–1.56 (m, 1H), 1.64–1.73 (m, 2H), 1.96 (s, 3H), 2.03–2.14 (m, 2H), 2.13 (s, 3H), 2.19–2.29 (m, 1H), 2.55 (dt, $J = 16.7$, 9.3 Hz, 1H), 3.48 (s, 2H), 5.49 (br s, 1H) APCI-MS: 205 $[M+H]^+$.

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*3a,7-Dimethyl-1-(2-methylpent-2-en-4-ynylidene)-2,3,3a,4,5,6-hexahydro-1H-indene (**8**)*: KHMDS (0.5 M solution in toluene, 2.3 mL, 1.15 mmol) was added to a solution of $(MeO)_2POCH_2C{\equiv}CTMS$ (256 mg, 1.16 mmol) in THF (9.0 mL) at 0 °C under an Ar atmosphere. The mixture was stirred at RT for 30 min to give a red solution, to which was added a solution of **7a** (118 mg, 0.575 mmol) in THF (2.0 mL) at 0 °C. The reaction mixture was stirred at RT for 2 h, then a saturated NH_4Cl solution (10 mL) was added to it. The aqueous solution was extracted with Et_2O (10 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes/EtOAc 50:1) to give the crude coupling product. Bu_4NF (1.0 M solution in THF, 0.70 mL, 0.70 mmol) was added to a solution of this crude compound (200 mg) in THF (6.0 mL) at 0 °C under an Ar atmosphere. The mixture was stirred at room temperature for 2 h, then saturated NH_4Cl solution (5 mL) was added to it. The aqueous solution was extracted with Et_2O (5 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes/EtOAc 50:1) to give compound **8** (69.0 mg, 53% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.94$ (s, 3H), 1.20–1.41 (m, 2H), 1.65–1.83 (m, 4H), 1.86 (s, 3H), 2.22 (s, 3H), 2.67–2.72 (m, 2H), 3.32 (d, $J = 2.2$ Hz, 1H), 5.48 (s, 1H), 6.16 (s, 1H); HRMS (EI): calcd for $C_{17}H_{22}$ $[M]^+$ 226.1721, found: 226.1727.

*8-(3a,7-Dimethyl-2,3,3a,4,5,6-hexahydroinden-1-ylidene)-3,7-dimethylocta-2,6-dien-4-yn-1-ol (**10**)*: $(Ph_3P)_4Pd$ (18.2 mg, 0.016 mmol) and CuI (8.4 mg, 0.044 mmol) were added to a solution of **8** (68.6 mg, 0.303 mmol) and vinyl iodide **9** (163 mg, 0.387 mmol) in $iPrNH_2$ (4 mL). The mixture was stirred at room temperature for 13 h under an Ar atmosphere in the dark and was then concentrated in vacuo. The residue was diluted with brine (10 mL), and the aqueous layer was extracted with Et_2O (10 mL × 3). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes/Et_2O 50:1) to give the crude coupling product (202 mg). Bu_4NF (1.0 M solution in THF, 0.45 mL, 0.45 mmol) was added to a solution of crude compound (202 mg) in THF (4.5 mL) at 0 °C. The mixture was stirred in the dark at room temperature for 4 h under an Ar atmosphere and was then concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes/EtOAc 5:1 → 3:1) and HPLC (hexanes/EtOAc 85:15) to give compound **10** (40.0 mg, 45% for 2 steps). ^{1}H NMR (400 MHz, C_6D_6) : $\delta = 0.90$ (s, 3H), 1.24–1.40 (m, 2H), 1.35–1.37 (m, 1H), 1.37 (s, 3H), 1.51–1.70 (m, 4H), 1.74 (s, 3H), 1.84 (ddd, $J = 18.7$, 10.9, 7.4 Hz, 1H), 1.99 (dd, $J = 5.7$, 18.7 Hz, 1H), 2.27 (s, 3H), 2.40–2.50 (m, 2H), 3.84 (t, $J = 6.1$ Hz, 2H), 5.81 (s, 1H), 6.04 (dt, $J = 1.3$, 6.7 Hz, 1H), 6.27 (s, 1H); HRMS (FAB): calcd for $C_{21}H_{28}O$ $[M]^+$: 226.1721, found 226.1727.

The enantiomers were separable with enantioselective HPLC from the racemic mixture of **10a** and **10b**. It was done with CHIRALCEL AD (Chiral Technologies, Inc., the subsidiary of Daicel, 250 × 4.6 mm I.D. (10 μm)), with the mobile phase hexanes/propan-2-ol (98.5:1.5) and the flow rate (3 mL min^{-1}) to give **10a** and **10b** from the racemic **10a/10b**. **10a**: UV: λ_{max} (ε_{max}) = 336 nm (24000 mol^{-1} dm^3 cm^{-1}); CD: 335 nm (+11), 257 nm (−4) (in methylcyclohexane). **10b**; UV: λ_{max} (ε_{max}) = 336 nm (24000 mol^{-1} dm^3 cm^{-1}); CD: 337 nm (−11), 260 nm (+5) (in methylcyclohexane).

*(3aS,7aS)-3a-Methyloctahydroinden-1-one (**12**)*: HMPA (7 mL, 40 mmol) and SmI_2 (0.1 M in THF, 100 mL, 10 mmol) were added to a solution of methyl (*R*)-1-methyl-2-oxocyclohexanepropionate, **11**, (250 mg, 1.25 mmol) in THF (8 mL) at RT under an Ar atmosphere. The reaction mixture was stirred for 8 h at room temperature, then Et_2O (ca. 200 mL) was added to it. The mixture was washed with HCl (0.5 M, 200 mL), saturated $Na_2S_2O_3$ solution (200 mL), and brine (200 mL). The solution was dried over Na_2SO_4 and concentrated in vacuo. Column chromatography on silica gel (hexanes/EtOAc 10:1) gave compound **12** (122 mg, 64%); IR (neat): $\tilde{\nu} = 1736$ cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.06–1.14$ (m, 1H), 1.20 (s, 3H), 1.22–1.29 (m, 1H), 1.34–1.52 (m, 5H), 1.63 (dt, $J = 12.9$, 9.8 Hz, 1H), 1.80 (ddd, $J = 12.0$, 7.0, 5.2 Hz, 1H), 1.86 (br t, $J = 1.8$ Hz, 1H), 1.96–2.25 (m, 1H), 2.28–2.32 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 21.30, 21.96, 23.14, 26.22, 34.02, 34.65, 35.39, 38.67, 56.18, 220.70; ESI-MS: 153 $[M+H]^+$.

*(3aS,7aS)-7a-Hydroxy-3a-methyloctahydroinden-1-one (**13**)*: CSA (25.8 mg, 0.11 mmol) was added to a solution of compound **12** (171 mg, 1.12 mmol) in isopropenyl acetate (11 mL), and the mixture was refluxed for 14 h. The mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (hexanes/EtOAc 20:1) to afford the enol acetate (114 mg, 52%). OsO_4 (0.1 M solution in *t*BuOH, 0.16 mL, 0.016 mmol) and NMO (38.1 mg, 0.33 mmol) were added to a solution of this enol acetate (31 mg, 0.163 mmol) in 80% aqueous acetone (2 mL). The reaction mixture was stirred at RT for 20 h. Saturated $NaHSO_3$ solution (5 mL) was added to the reaction mixture, and the aqueous layer was extracted with Et_2O (5 mL × 3). The extracts were dried over Na_2SO_4 and concentrated in vacuo. Column chromatography on silica gel (hexanes/EtOAc 5:1 to 3:1) gave compound **13** (26 mg, 0.156 mmol, 96%): IR (neat): $\tilde{\nu} = 3464$, 1741 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.92$ (s, 3H), 1.33–1.36 (m, 1H), 1.42–1.64 (m, 8H), 2.05 (ddd, $J = 15.5$, 9.8, 2.4 Hz, 1H), 2.24 (br s, 1H), 2.29–2.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 21.15$, 21.65, 22.81, 28.15, 31.03, 31.51, 33.29, 40.85, 80.71, 220.35; ESI-MS: 169 $[M+H]^+$.

*(3aS)-3a-Methyl-2,3,3a,4,5,6-hexahydroinden-1-one (**14**)*: CSA (0.12 mmol) was added to a solution of compound **13** (197 mg, 1.21 mmol) in toluene (4 mL), and the mixture was heated under reflux for 4 h. The solution was concentrated in vacuo, and the residue was purified by column chromatography on silica gel (hexanes/EtOAc 10:1) to afford compound **14** (157 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) : $\delta = 1.13$ (s, 3H), 1.27–1.45 (m, 2H), 1.58 (dt, $J = 12.2$, 8.6 Hz, 1H), 1.71–1.80 (m, 2H), 1.90 (dt, $J = 12.2$, 3.4 Hz, 1H), 2.00 (dd, $J = 12.2$, 8.7 Hz, 1H), 2.14–2.36 (m, 3H), 2.41 (ddd, $J = 18.8$, 12.2, 8.7 Hz, 1H), 6.61 (t, $J = 3.7$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 18.65, 25.18, 25.69, 35.70, 36.44, 36.58, 38.84, 132.06, 145.89, 207.64; ESI-MS: 151 $[M+H]^+$.

*(3aS)-1-(3a-Methyl-2,3,3a,4,5,6-hexahydroinden-1-ylidene)propan-2-one (**15**)*: The synthesis of **15** is similar to that of **7a**, starting from **11** (Fluka). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.99$ (s, 3H), 1.23–1.30 (m, 1H), 1.38 (ddd, $J = 20.7$, 11.8, 9.0 Hz, 1H), 1.68–1.90 (m, 4H), 2.18–2.24 (m, 2H), 2.25 (br s, 3H), 2.81 (dddd, $J = 20.1$, 17.0, 8.1, 2.8 Hz, 1H), 3.07 (ddd, $J =$ 20.1, 8.7, 1.0 Hz, 1H), 6.22 (t, $J = 4.0$ Hz, 1H), 6.48 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 18.93$, 24.64, 26.04, 30.94, 31.90, 36.15, 39.96, 40.88, 115.76, 123.47, 148.36, 160.61, 198.71; ESI-MS: *m*/*z*: 191 $[M+H]^+$.

The synthesis of **16** is similar to that of **10**. ^{1}H NMR (400 MHz, C_6D_6): $\delta =$ 0.88 (s, 3H), 1.13–1.68 (m, 6H), 1.46 (s, 3H), 1.84–1.99 (m, 2H), 2.25 (s, 3H), 2.44–2.47 (m, 2H), 3.84 (br t, $J = 5.7$ Hz, 2H), 5.68 (t, $J = 3.9$ Hz, 1H), 5.82 (s, 1H), 6.04 (dt, $J = 1.3$, 5.7 Hz, 1H), 6.43 (s, 1H); ^{13}C NMR (75 MHz, C_6D_6): $\delta = 17.9$, 19.2, 19.6, 24.5, 25.8, 29.3, 30.6, 36.5, 40.4, 59.3, 88.1, 100.0, 109.9, 117.6, 122.0, 129.0, 135.9, 143.4, 148.1, 149.9. CI-MS: *m*/*z*: 283 $[M+H]^+$.

*(3aR)-(2E,4Z,6E)-8-(3a,7-Dimethyl-2,3,3a,4,5,6-hexahydroinden-1-ylidene)-3,7-dimethylocta-2,4,6-trienal (**2a**)*: Activated Zn dust was prepared as described by Boland et al.[44] Argon was bubbled through a suspension of Zn dust (500 mg) in distilled water (2 mL) for 15 min. $Cu(OAc)_2$ (50 mg) was added, and the flask was sealed immediately. The mixture was stirred vigorously for 15 min. $AgNO_3$ (50 mg) was then added, and the mixture was stirred for 30 min. The activated Zn was then filtered and washed successively with water, MeOH, and Et_2O and was transferred to a flask of the reaction solvents (H_2O, 2 mL; MeOH, 2 mL). Compound **10a** (4.0 mg) was added to this mixture, which was then stirred at RT in the dark for 21 h. The zinc dust was filtered through Celite with Et_2O and H_2O. The organic phases were separated, washed with saturated NaCl, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give crude alcohol. This compound was dissolved in CH_2Cl_2 (2 mL) and NMO (3.3 mg, 0.028 mmol) and TPAP (catalytic amount) were added to the solution. The mixture was stirred at room temperature in the dark for 2 h, then filtered through Celite with diethyl ether and was concentrated. Column chromatography on silica gel (hexanes/ethyl acetate 4:1) gave (3a*R*)-(2*E*,4*Z*,6*E*)-8-(3a,7-dimethyl-2,3,3a,4,5,6-hexahydroinden-1-ylidene)-3,7-dimethylocta-2,4,6-trienal, **2a**, in a mixture with other isomers. Compound **2a** was further purified with HPLC: YMC Semipreparative HPLC column (10 mm × 25 cm); solvent, hexanes/ethyl acetate (95:5); flow rate, 2 mL min^{-1}; detection wavelength, 380 and 330 nm; retention time for **2a**, 22.1 min. ^{1}H NMR (400 MHz, C_6D_6): $\delta = 0.88$ (s, 3H), 1.13–1.68 (m, 3H), 1.76 (s, 3H), 1.78 (s, 3H), 1.84 (s, 3H), 1.90–2.10 (m, 4H), 2.25 (s, 3H), 5.62 (d, $J = 11.7$ Hz, 1H), 6.16 (d, $J = 7.8$ Hz, 1H), 6.28 (s, 1H), 6.44 (t, $J =$ 12.0 Hz, 1H), 6.73 (d, $J = 12.4$ Hz, 1H), 9.95 (d, $J = 7.8$ Hz, 1H). HRMS(FAB): calcd for $C_{21}H_{28}O$ $[M+H]^+$: 297.2218, found 297.2207.

Reconstitution of the pigment with the retinals and opsin: Opsin was prepared from rod outer segments (ROS)[45, 46] by a published method.[47]

Binding experiment with retinal analogue 2a and 2b: (Figure 5). A stock solution of opsin in CHAPSO/HEPES buffer (1 mL, 10 mM, pH 6.6) was added to a UV cuvette—CHAPSO/HEPES buffer (1 mL, 10 mM, pH 6.6) was used as a reference—and the background of the UV spectrometer was scanned. 0.7 equiv of retinal analogue **2a** or **2b** in EtOH (5 μL) was added to the sample cuvette and to the control cuvette at 25 °C. The UV spectra were monitored over 1.5 and 4 h for analogue **2a** and **2b**, respectively.

GTPγ35S binding assay: Rh-(**2a**) was reconstituted in 0.1% dodecyl maltoside/10 mM HEPES buffer in the same manner as described above. Transducin assay solution containing Gt (600 nM), Rho analogue (5 nM) and GTPγ^{35}S (1 μM) was irradiated with yellow light (> 500nm) for 30 s at 0 °C. Aliquots (40 μL) were collected and filtered through nitrocellulose membranes at 1 and 2 min after photo-irradiation. GTPγS uptake was monitored at RT. The membranes were then washed with washing buffer (5 mL × 3) and dissolved in of scintillation fluid (10 mL; Filtron-X). Their radioactivities were measured by a liquid scintillation counter (TRI-CARB 2200CA, Packard). The amount of GTP bound for 1 min was calculated and compared to the data that was obtained with native rhodopsin.

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