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Synthesis of 11-Cis-Locked Bicyclo[5.1.0]octanyl Retinal and an Enantioselective Binding to Bovine Opsin

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ABSTRACT Both enantiomers of 13-(*E*) and 13-(*Z*) isomers of 11-cis-locked bicyclo[5.1.0]octanyl retinal were prepared by an improved synthesis and incubated with bovine opsin. The synthesis also establishes the absolute configuration of the enantiomers. Only one of the enantiomers binds to opsin, thus showing the steric restrictions regarding the middle polyene moiety of the retinoid molecule; this is in sharp contrast to the known leniency of the ring moiety binding site of retinoids. However, although one enantiomer is incorporated into the pigment, the circular dichroic spectrum of the pigment incorporating the bound enantiomer yields only a very weak Cotton effect, showing that, once incorporated, the bicyclo[5.1.0]octanyl chromophore is flattened by the opsin binding site. The titled retinoid was synthesized for study of the absolute conformation of the retinal pigment in rhodopsin. *Chirality 14:340–346, 2002.*

KEY WORDS: cyclopropyl retinoid; rhodopsin; circular dichroic spectroscopy (CD); chiral separation; chiral discrimination; exciton coupled CD

G-protein (guanyl-nucleotide binding protein)-coupled receptors (GPCRs) are membrane proteins consisting of seven transmembrane α-helices helix A to helix G (or 1 to 7), which are responsible for transmitting numerous external signals, e.g., olfaction, neurotransmitters, and peptide hormones to the cell. The rhodopsins (Rh), the photoreceptor pigments in the rod outer segment of visual cells in the retina of vertebrate eyes, are the GPCRs studied most intensely. The 40 kDa bovine Rh consists of opsin, a 348 amino acid apoprotein, and the positively charged 11-*cis*-retinylidene chromophore (Fig. 1) bound to the terminal amino group of Lys296 in helix G via a protonated Schiff base (PSB). Upon irradiation, the 11-cis bond isomerizes to trans, which initiates conformational changes in the seven helices and this in turn activates the G-protein around the cytoplasmic extramembrane leading to vision.[1–6]

The fact that absorption maxima of the visual pigments cover the range of 350–680 nm is quite extraordinary considering the fact that this wide range simply arises from the interaction between the retinal chromophore and their receptors. Many unique aspects of this chromophore make it ideally suited for controlling the subtle wavelength regulation of visual pigments so as to adjust vision to suit the environment.[7] Namely, wavelength regulation of the pigment is dependent on delocalization of the positive charge, which in turn is governed by factors such as the distance between the positively charged Lys296 nitrogen and its counterion, the local electrostatic field within the binding site,[8] and the geometric restrictions imposed on the pigment by the opsin. This geometric constraint of the binding site defines the conformation of the chromophore, which cannot be planar around the 6-s- and 12-s-bonds due to steric hindrances between 5-Me/8-H and between 13-Me/10H (Fig. 1). The 1,1-gem-dimethyl and 9-methyl groups are all essential for the hydrophobic binding to opsin and for the conformational changes of the pigment triggered by the cis/trans isomerization.

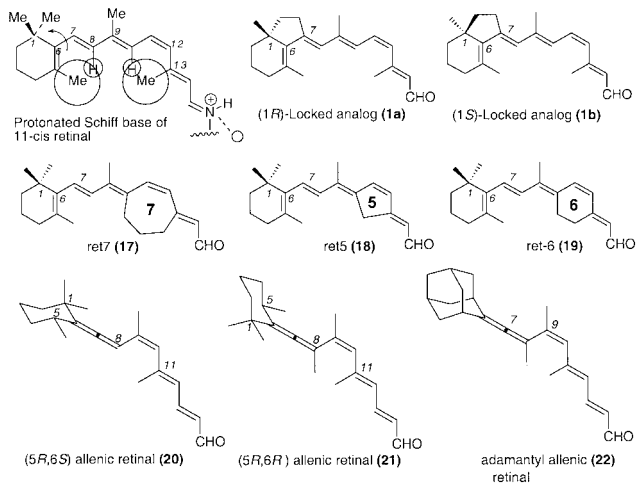

Fig. 1. Retinoids.

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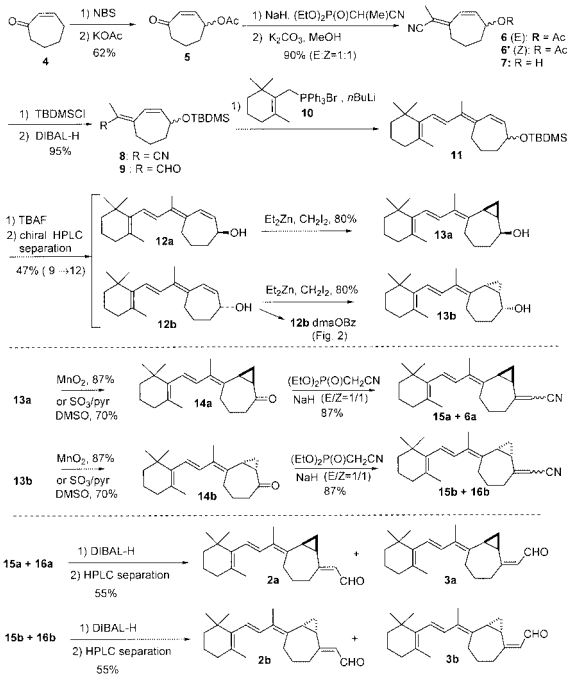
The success in crystallizing Rh in a form suited for X-ray diffraction analysis[9] has led to its 2.8 Å resolution crystal structure, the first ever for a GPCR.[5] Independent of this important determination of the overall structure of Rh, we have been studying the absolute conformation of the chromophores as it resides in Rh. In this connection, the absolute sense of twist around the 6-s-bond has recently been determined as negative.[10] Namely, incubation of the two synthetic enantiomers, 6-s-cis-α-locked retinoid **1a** and 6-*s*-*cis*-β-locked retinoid **1b** showed that of the two enantiomers only the α-locked retinoid **1a** (1*R*) binds to yield Rh-**1a**; the β-locked retinoid **1b** (1*S*) does not bind. The CD of the pigment derived from **1a** (Rh-**1a**) is similar to that of native Rh. Thus, in 10 mM CHAPSO/HEPES pH 6.6 buffer the CD of native Rh shows Cotton effects (CE) at 500 nm, Δε +9.5 (α-band) and 336 nm, Δε +15 (β-band) as seen in Figure 3b (below), while that of Rh-**1a** exhibits CEs at 536 nm, Δε +4, and 329 nm, Δε +6.5. Moreover, the GTP binding assay of Rh-**1a** was 80% that of the native pigment, thus implying that the overall conformation around the 6-*s*-bond does not change throughout the steps leading to G-protein activation.[10] The nature of the twist around the 12-*s*-bond, which may not be that large, is still controversial (see also discussion in Ref. 10). In the refined X-ray structure,[11] this is +156.2s°, namely, 13-Me is at the "front" of the paper plane, or when depicted as in the first structure in Figure 1, 13-Me is in front of the 10-H.

In a previous attempt to solve the twist around the 12-s bond, the bicyclo[5.1.0]octane retinoids **2a** and **2b** (Scheme 1) were incubated with opsin to investigate the 12-*s*-bond twist.[12] Reconstitution studies indicated that only the β-cyclopropyl analog **2a**, but not its enantiomer, gave a pigment. However, although **2a** yielded the corresponding pigment with a clear isosbestic point (Fig. 3, below), the retinoid appeared to be unstable under various incorporation conditions so that the amount of pigment finally secured was insufficient for further studies, particularly for proper measurement of the pigment CD. The cyclopropyl retinoids **2a/2b** and **3a/3b** have therefore been resynthesized via an improved route and incubated with opsin. The separated enantiomeric allylic alcohols **12a/12b**, the absolute configurations of which were determined from exciton-coupled CD (Fig. 2), yielded the β- and α-cyclopropyl alcohols **13a/13b**, which in turn were converted into the desired retinoids **2/3**. The CD of the unique retinoids **2a**, **2b**, **3a**, and **3b** containing a cyclopropyl ring flanked by two chromophoric groups indicated that the two chromophores are exciton-coupled through the cyclopropyl moiety. This aspect together with the conformation of the bicyclo[5.1.0]-ring in the retinal analog will be discussed in a forthcoming article. The four retinoids **2a**, **2b**, **3a**, and **3b** were then incubated with bovine opsin.

In the earlier synthesis, asymmetric induction was performed enzymatically at a very early step in the synthesis and, moreover, 22 steps were necessary to prepare enantiomers **2a** and **2b** in a yield of 0.6% for each compound. These steps have been reduced to 12 and the yield has been improved to 2.3% after enantiomeric HPLC separation. The synthesis is outlined in Scheme 1. Bromination followed by acetylation of cycloheptenone **4** yielded acetate **5**, which was reacted with sodium hydride/diethyl (1-cyanoethyl) phosphonate; deprotection of the acetyl group gave allylic alcohol **6**. Reprotection of alcohol **7** with TBDMSCl followed by DIBAL-H reduction yielded aldehyde **9**, which was submitted to Wittig reaction with phoshonium bromide **10**/*t*-BuOK to afford the protected alcohol, which upon deprotection gave alcohol **12**. At this stage, enantioselective HPLC, i.e., CHIRALPAK AD (Chiral Technologies, Eaton, PA), 250 × 10 mm I.D. 10 μm, Hexanes/2-propanol = 99.85/0.15, 3 mL/min, was used for the separation of enantiomers **12a** and **12b**.

Enantiomer **12b** with its allylic hydroxyl group was used to determine the absolute configuration by conversion to an appropriate acylate for interpreting the resulting



Scheme 1. Synthesis of retinal analogs **2a, 2b, 3a**, and **3b**.

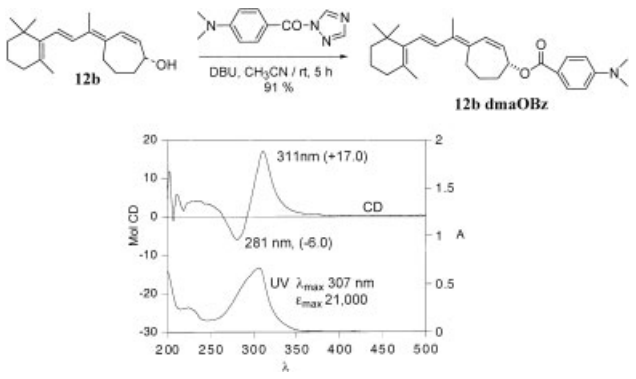


Fig. 2. CD of dimethylamino benzoate of **12b** (**12b** dmaOBz) in methylcyclohexane.

exciton-coupled CD[13]. The dimethylaminobenzoate group (dmaOBz) with an absorption at 311 nm (ε 30,400) (in EtOH)[14] close to that of the substrate **12b** at 295 nm (ε 13,000) (in methylcyclohexane) was selected as the chromophore. The observed positive exciton CD couplet of benzoate **12b** dmaOBz (Fig. 2), 311 nm (Δε +17.0/281 nm Δε −6.0) showed that the dimethylaminobenzoate and tetraene moieties constitute a positive twist, and hence the hydroxyl configuration in **12b** is α. After enantioselective HPLC separation, intermediates **12a** and **12b** were separately converted into the final compounds, as shown in Scheme 1. Simmons-Smith reaction of **12a** afforded a single diastereomer of cyclopropyl alcohol **13a** with syn-selectivity; similarly, **12b** gave **13b**. The syn-geometry was confirmed by NMR experiments (NOESY and COSY). Oxidation of cyclopropyl alcohols **13a** and **13b** yielded ketones **14a** and **14b**. Horner-Emmons reaction of these ketones to **15a/15b** followed by DIBAL reduction give an *E/Z* = 1/1 mixture which was separated affording pure **2a/3a** and **2b/3b**. The four retinoids could also be secured by separating racemic retinals **2** and **3** from the mixture of **2/3** by normal phase HPLC, and then separating the enantiomers of **2** and **3**, respectively, by chiral HPLC with CHIRALPAK AD, 250 × 4.6 mm I.D. 10 μm (Daicel, Tokyo, Japan), Hexanes/2-propanol = 99.85/0.15, 1 mL/min, to give **2a/2b** and **3a/3b**.

The enantiomeric 13-(*E*) β-cyclopropyl retinoids **2a** and **2b**, and 13-*Z* α-cyclopropyl retinoids **3a** and **3b**, were then incubated with bovine opsin and the spectra were recorded (Fig. 3). As shown in the difference UV (Fig. 3a) for the 13-(*E*) β-cyclopropyl analog **2a**, the original aldehyde peak at 260 nm decreases and is slowly replaced by the pigment peak at 315 nm for 6 h. In contrast, the 13-*E* α-cyclopropyl analog **2b** does not yield a pigment (Fig. 3a, inset). From this it is seen that the shape of the cytoplasmic cleft of the apoprotein will accommodate the β-cyclopropyl but not the α-enantiomer. Similarly, of the 13-(*Z*) isomers **3a** and **3b**, only the β-cyclopropyl isomer **3a** yields a pigment with a maximum at 316 nm but the α isomer is not incorporated. Thus, there is clear enantioselective discrimination between the opsin cleft and the conformation of the bicyclic moiety of the retinal analog. In contrast to the clear selectivity in favor of the β- over the α-cyclopropyl retinoids, 13-*E* **2a** vs. **2b**, the steric tolerance around C-13 is lenient and, hence, as in the case of 13-E **2a**, opsin readily incorporates the 13-*Z* β-isomer **3a**, but not the 13-*Z* α-isomer **3b**. Note that the 13-cis isomers were also incorporated in the case of the native 11-cis retinal and ret7 **17**.[15]

The UV of pigments Rh-**2a** and Rh-**3a** appear as a weak shoulder at 315 nm on the stronger 280 nm protein band, a pattern that is seen in the CD as well (Fig. 3b). In the case of Rh, the α- and β-bands (see Fig. 3b) have been assigned, respectively, to twists around the 12-s- and 6-s-bonds.[16,17] In the case of pigment Rh-**2a**, the large difference in shapes of the UV/VIS and CD spectra preclude any similar analysis, except that the weak CE suggests that after entering the opsin binding site, an opsin compression effect forces the chromophore to be flat, i.e., the polyene systems flanking the cyclopropyl are close to being in the same plane when in the pigment. Rh incorporating planar chromophores exhibit no conspicuous CE as exemplified by Rh-ret5 **18**[16] and Rh-ret6 **19**,[18] having planar 5- and 6-membered rings bridging C-10 and C-13. The present results show that conformations of chromophores **2a** and **2b** in solution and in the rhodopsin are different.

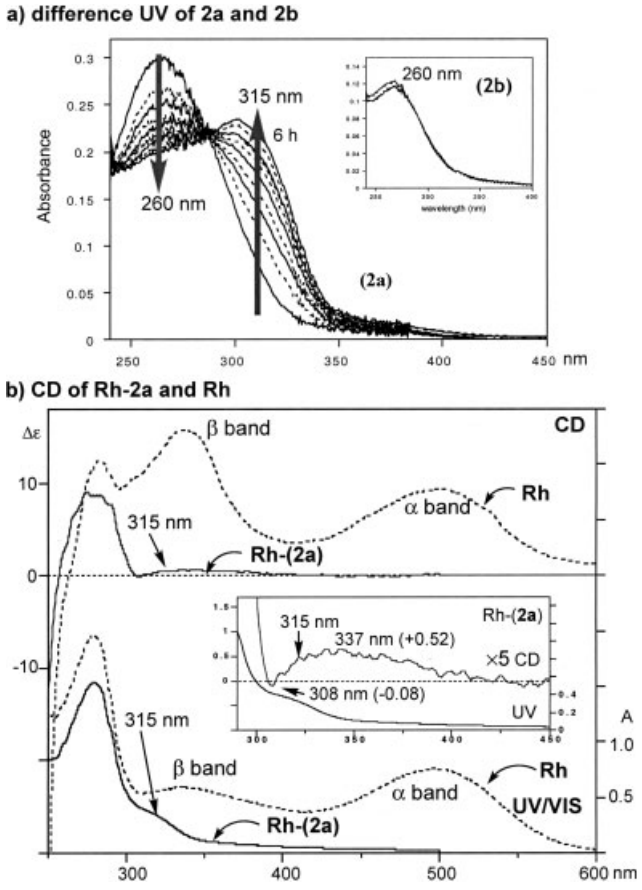


Fig. 3. Reconstitution of retinal analog **2a** and **2b** with apoprotein opsin. **a**: Changes in UV accompanying incubation of opsin with **2a** or **2b**. The retinal analog was bound by incubating 0.7 mol. equiv. of analog with a suspension of bovine opsin in 10 mM CHAPSO/HEPES buffer (pH 7.0) at 25°C for 6 h. The change was plotted every 30 min with opsin as control. The 260 absorbance is that of unbound chromophore **2a** while the band at 315 nm is due to protonated Schiff base (PSB) formation. The UV of analog **2b** at 260 nm was left unchanged. **b**: UV/VIS and CD of native Rh and pigment Rh-**2a**. The spectra were measured in CHAPSO/HEPES buffer at pH 7.0.

The discrimination between retinoids **2a** vs. **2b** in pigment reconstitution shows that the *steric allowance around the 11-ene of the entering molecule* is critical for recognition. Earlier studies[19] which were performed to clarify the mechanism of transient dark activity of 13-desmethylretinal implied that incorporation of retinal into opsin involves the "β-ionone binding site I,"[20,21] "Schiff base site III," and that this is followed by the "polyene site II."[19] It appears that the conformation of the flexible polyene section plays a dominant role as it enters site II. In the present case, this site II does not allow entry of the α-cyclopropylretinoid. This

restriction is in sharp contrast to the studies with the allenic retinoids that showed considerable leniency in the binding capabilities of the ionone binding site I. Thus, opsin could incorporate diastereomers **20**, **21**, and their respective enantiomers,[22] as well as the adamantyl retinoid **22**.[23]

EXPERIMENTAL

All reactions were carried out under argon unless otherwise stated. For light-sensitive compounds, reactions were performed under dim red light. Reagent-grade chemicals were purchased from Aldrich (Milwaukee, WI) unless otherwise specified. HPLC-grade solvents were obtained through Fisher Scientific (Fair Lawn, NJ). Tetrahydrofuran (THF) and ethyl ether (Et_2O) were distilled from sodium/benzophenone; methylene chloride (CH_2Cl_2) was distilled from calcium hydride. Other solvents and reagents were used directly without further purification unless otherwise specified. Dark-adapted retinae were obtained from W. Lawson Co. (Lincoln, NE). Centrifugation was performed on Beckman L-70 and L8-M ultracentrifuge using appropriate rotors. The progress of reactions was checked by thin-layer chromatography (TLC) using 0.25 mm precoated E. Merck (Darmstadt, Germany) silica gel plates 60 F-254, which was visualized by UV light. Flash column chromatography was performed with 32–63 mesh silica gel from Selectro Scientific (Sunnyvale, CA). HPLC analysis and purification of retinal analogs were performed in the dark on a Rainin HP solvent delivery system with absorbance detector model UV-D. The normal phase HPLC columns were YMC-Pack SIL (YMC, Wilmington, NC; 230 × 10 mm I.D., S-5 μm, 120A) and HYPERSIL SILICA 3U (Alltech, Deerfield, IL; 150 × 4.6 mm I.D.). The chiral HPLC columns were CHIRALCEL AD (Chiral Technologies) 250 × 4.6 mm I.D. analytical column and 250 × 10 mm I.D. semiprep column (10 μm). NMR spectra were recorded on Bruker (Fremont, CA) DMX 500, DRX 400, or DPX 300 instruments and performed in $CDCl_3$ or in benzene-d_6. Chemical shifts (δ) are reported in ppm downfield from internal TMS, or calibrated using residual undeuterated solvent peak, $CHCl_3$ (7.27 ppm) or benzene (7.16 ppm), as an internal reference. The coupling constants (*J*) are reported in Hz. Low-resolution and high-resolution FAB mass spectra (MS) were measured on a JEOL JMS-DX303 HF MS using a glycerol matrix and Xe ionizing gas. CI MS were measured on NERMAG R10-10 spectrometer with NH_3 as ionizing gas. ESI MS were measured on a JEOL JMS-LC mate LCMS system. IR spectra were recorded on a Perkin Elmer (Oak Brook, IL) 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.

4-Acetoxy-2-cyclohepten-1-one (5)

To a solution of 2-cyclohepten-1-one **(4)** (80%, 5 g, 36 mmol) in carbon tetrachloride (59 mL) was added N-bromosuccinimide (9 g, 50 mmol) and benzoyl peroxide (15 mg). The mixture was heated on an oil bath and refluxed under argon for 1 h. The solution turned dark brown and cooled to room temperature. Petroleum ether (100 mL) was added to precipitate the succinimide which was removed by filtration and washed with additional petroleum ether (50 mL). The filtrate was concentrated by rotary evaporator to remove most of the ether and some CCl_4 to give a solution of crude 4-bromo-2-cycloheptenone in CCl_4 with a final volume of ca. 20 mL. An aqueous solution of potassium acetate (15 g, 150 mmol) and the phase transfer catalyst Aliquat 366 (tri-(n-octyl)methylammonium chloride, 1 g) in 25 mL water was mixed with the CCl_4 solution and stirred overnight at room temperature. The mixture was diluted with 200 mL Et_2O and washed with water (2 × 30 mL) and brine (30 mL). After drying the organic layer over anhydrous Na_2SO_4 the mixture was filtered and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (silica gel, Hexanes/AcOEt = 20/1–10/1) to give 4-acetoxy-2-cycloheptenone **5** (3.8g, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.44 (dd, *J* = 12.6, 3.3 Hz, 1 H), 6.03 (dd, *J* = 12.6, 2.2 Hz, 1 H), 5.59 (m, 1 H), 2.63 (m, 2 H), 2.19 (m, 1 H), 2.11 (s, 3 H), 1.98 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.3, 169.9, 144.3, 131.2, 71.8, 42.7, 31.5, 20.9, 17.9 ppm. CI-MS (NH_3): m/z 186 $[M+18]^+$. HRMS (FAB) calcd for $C_9H_{13}O_3$ ($[M+H]^+$): 169.0865, found: 169.0858.

2-(4-Acetoxy-cyclohept-2-en-(E)-ylidene Propionitrile (6)

To a suspension of sodium hydride (400 mg, 10 mmol) in anhydrous THF (300 mL) was added a solution of diethyl (1-cyanoethyl)phosphonate (1.95 g, 10 mmol) in THF (100 mL) at 0°C for 15 min. The mixture was stirred at room temperature for 30 min and a solution of 4-acetoxy-2-cycloheptenone (**5**) (1.5 g, 8.9 mmol) in THF (50 mL) was introduced. The reaction proceeded at room temperature overnight and the mixture was then poured into iced water (100 mL) and extracted with Et_2O (3 × 50 mL). The combined organic layer was washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash column chromatography (silica gel, Hexanes/AcOEt = 20/1–10/1) to give 2-(4-acetoxy-cyclohept-2-enylidene)-propionitrile (**6** and **6′**) (1.65 g, 90%). The nitriles are a 1:1 mixture of *Z* (**6′**) / *E* (**6**) isomers which were separated by flash column chromatography (silica gel, Hexane/AcOEt = 20/1–10/1). The configurations of the two enantiomers was determined from the CD of the two dmethylamino benzoates of **(12)** (see Fig. 2).

6: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (dd, *J* = 12.1, 2.1 Hz, 1 H), 5.95 (dd, *J* = 12.1, 2.1 Hz, 1 H), 5.50 (dd, *J* = 10.0, 2.7 Hz, 1 H), 2.85 (m, 1 H), 2.57 (m, 1 H), 2.08 (s, 3 H), 1.98 (m, 3 H), 1.94 (s, 3 H), 1.83 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 151.9, 138.3, 127.2, 119.7, 106.4, 72.1, 34.2, 32.1, 22.0, 21.2, 16.4 ppm. CI-MS (NH_3): m/z 223 $[M+18]^+$. HRMS (FAB) calcd for $C_{12}H_{15}O_2N_1$ ($[M+H]^+$): 206.1181, found: 206.1180. **6′**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (dd,

J = 12.1, 0.6 Hz, 1 H), 5.89 (dd, *J* = 12.1, 2.9 Hz, 1 H), 5.53 (m, 1 H), 2.62 (m, 1 H), 2.38 (m, 1 H), 2.08 (s, 3 H), 2.02 (m, 1 H), 1.97 (s, 3 H), 1.78 (m, 3 H). CI-MS (NH_3): m/z 223 $[M+18]^+$.

2-(4-Hydroxycyclohept-2-en-(E)-ylidene)propionitrile (7)

To a solution of (2*E*)-2-(4-acetoxy-cyclohept-2-enylidene)-propionitrile (**6**) (700 mg, 3.4 mmol) in anhydrous methanol (25 mL) was added potassium carbonate (250 mg, 1.8 mmol) at 0°C. The mixture was stirred at room temperature overnight and poured into iced water (50 mL) and extracted with EtOAc (3 × 40 mL). The combined organic layer was washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash column chromatography (silica gel, Hexanes/AcOEt = 3/2) to give (2*E*)-2-(4-Hydroxy-cyclohept-2-enylidene)-propionitrile (**7**) (547 mg, 99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.28 (dd, *J* = 12.1, 2.4 Hz, 1 H), 6.08 (dd, *J* = 12.1, 2.5 Ha, 1 H), 4.50 (m, 1 H), 2.82 (m, 1 H), 2.51 (m, 1 H), 2.03 (m, 1 H), 1.94 (s, 3 H), 1.82 (m, 2 H), 1.73 (m, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.5, 142.7, 125.7, 119.9, 105.8, 70.3, 35.9, 34.3, 22.2, 16.3 ppm. CI-MS (NH_3): m/z 181 $[M+18]^+$. HRMS (FAB) calcd for $C_{10}H_{14}NO$ ($[M+H]^+$): 164.1075, found: 164.1072.

2-[4-(tert-Butyldimethylsilyloxy)cyclohept-2-en-(E)-ylidene]propionitrile (8)

To a solution of cycloheptenylidene propionitrile (**7**) (540 mg, 3.3 mmol), imidazole (1g, 14.7 mmol), and 4-dimethylaminopyridine (10 mg in anhydrous CH_2Cl_2 (20 mL) was added *tert*-butyldimethylsilyl chloride (600 mg, 3.9 mmol) at 0°C. The mixture was stirred at room temperature for 2 h. Water (50 mL) was added to the reaction mixture which was extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash column chromatography (silica gel, Hexane/AcOEt = 10/1) to give 2-[4-(*tert*-butyldimethylsilyloxy)cyclohept-2-en-(*E*)-ylidene] propionitrile (**8**) (915 mg, quant.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (dd, *J* = 12.1, 2.0 Hz, 1 H), 6.02 (dd, *J* = 12.1, 1.8 Hz, 1 H), 4.44 (m, 1 H), 2.80 (m, 1 H), 2.51 (m, 1 H), 1.92 (s, 3 H), 1.90 (m, 1 H), 1.76 (m, 3 H), 0.90 (s, 9 H), 0.08 (s, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.0, 144.3, 125.0, 120.0, 105.1, 70.6, 35.8, 34.3, 31.5, 25.8, 22.6, 22.2, 18.1, 16.2, 14.1, -3.6, -4.9 ppm. CI-MS (NH_3): m/z 295 $[M+18]^+$. HRMS (FAB) calcd for $C_{16}H_{27}NOSi$ ($[M+H]^+$): 278.1940, found: 278.1927.

2-[4-(tert-Butyldimethylsilyloxy)cyclohept-2-en-(E)-ylidene]propionaldehyde (9)

To a solution of 2-[4-(*tert*-butyldimethylsilyloxy)-cyclohept-2-en-(*E*)-ylidene]propionitrile (**8**) (530 mg, 1.9 mmol) in anhydrous Et_2O (15 mL) was added diisobutylaluminum hydride (1.0 M solution in hexanes, 2.8 mL, 2.8 mmol) for 5 min at −78°C. The mixture was warmed gradually to 0°C for 2 h. The reaction was quenched by addition of EtOAc (5 mL) at 0°C and the mixture was poured into a slurry of wet silica gel suspension in Et_2O. The suspension was stirred at room temperature for 1 h. The silica gel was then removed by filtration and washed with EtOAc (100 mL). The filtrate was concentrated under reduced pressure and purified by flash column chromatography (silica gel, Hexane/AcOEt = 5/1) to give the title compound (**9**) (514 mg, 96%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1 H), 6.48 (d, *J* = 12.0 Hz, 1 H), 6.07 (dd, *J* = 12.0, 1.8 Hz,1 H), 4.47 (m, 1 H), 3.22 (m, 1 H), 2.44 (m, 1 H), 1.89 (m, 1 H), 1.80 (m, 3 H), 1.79 (s, 3 H), 0.91 (s, 9 H), 0.10 (s, 6 H). CI-MS (NH_3): m/z 298 $[M+18]^+$. HRMS (FAB) calcd for $C_{16}H_{28}O_2Si$ ($[M+H]^+$): 280.1859, found: 280.1864.

tert-Butyldimethyl-{4-[(1E,2E)-1-methyl-3-(2,6,6-trimethylcyclohex-1-enyl)prop-2-en-(E)-yliden]cyclohept-2-enyloxy}-silane (11)

To a solution of β-cyclocitral triphenylphosphonium bromide **10** (362 mg, 0.76 mmol) in anhydrous CH_2Cl_2 (5 mL) were added potassium *tert*-butoxide (85 mg, 0.76 mmol) and 18-crown-6 (5 mg) at room temperature under argon. The deep red solution was stirred for an additional 15 min. A solution of 2-[4-(*tert*-butyldimethylsilyloxy)cyclohept-2-en-(*E*)-ylidene]propionaldehyde (**9**) (106 mg, 0.38 mmol) in CH_2Cl_2 (5 mL) was introduced, also at room temperature. The mixture was stirred in the dark for 5 h. The reaction mixture was poured into iced water (20 mL) and extracted with Et_2O (3 × 20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash column chromatography (silica gel, Hexane/AcOEt = 50/1) to give crude *tert*-butyl-dimethyl-{4-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene-cyclohept-2-enyloxy}-silane (**11**) (110 mg).

^{1}H NMR (400 MHz, C_6D_6) δ 6.72 (d, *J* = 6.0 Hz, 1 H), 6.46 (d, *J* = 11.8 Hz, 1 H), 6.33 (d, *J* = 16.0 Hz, 1 H), 5.75 (d, *J* = 11.8 Hz, 1 H), 4.21 (m, 1 H), 2.67 (m, 1 H), 2.16 (m, 1 H), 1.96 (t, *J* = 6.0 Hz, 2 H), 1.88 (s, 3 H), 1.79 (s, 3 H), 1.45-1.68 (m, 8H), 1.10 (s, 6 H), 0.90 (s, 9 H), 0.08 (s, 6 H). ^{13}C NMR (75 MHz, C_6D_6) δ 138.9, 137.3, 134.6, 132.5, 131.1, 130.6, 71.3, 39.8, 36.0, 34.5, 33.2, 30.3, 29.1, 26.1, 24.2, 21.9, 19.7, 18.3, 14.6, -4.5 ppm. CI-MS (NH_3): m/z 418 $[M+18]^+$. HRMS (FAB) calcd for $C_{26}H_{44}OSi$ ($[M]^+$): 400.3161, found: 400.3151.

4-[(1E,2E)-1-Methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-cyclohept-2-enol (12)

Tetrabutylammonium fluoride (1.0 M solution in THF, 0.5 mL, 0.5 mmol) was added to a solution of *tert*-butyldimethyl-{4-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethylcyclohex-1-enyl)-allylidene-cyclohept-2-enyloxy}-silane (**11**) (ca. 100 mg, 0.25 mmol) in THF (5 mL) at 0°C in the dark. After being

stirred at room temperature for 2 h, the mixture was poured into iced water (20 mL) extracted with Et_2O (3 × 15 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash column chromatography (silica gel, Hexane/AcOEt = 4/1−2/1) to give 4-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-cyclohept-2-enol (**12**) (47 mg, 47% (two steps from **9**)). UV (methylcyclohexane): (max 295 nm, (max 13,000, ^{1}H NMR (400 MHz, C6D6) δ 6.70 (d, *J* = 16.0 Hz, 1 H), 6.45 (dd, *J* = 11.8, 1.4 Hz, 1 H), 6.32 (d, *J* = 15.9 Hz, 1 H), 5.75 (dd, *J* = 11.8, 1.9 Hz, 1 H), 4.23 (m, 1 H), 2.66 (dt, 14.0, 5.1 Hz, 1 H), 2.17 (m, 1 H), 1.96 (t, *J* = 6.1 Hz, 2 H), 1.87 (s, 3 H), 1.78 (s, 3 H), 1.67 (m, 2 H), 1.57 (m, 3 H), 1.48 (m, 3 H), 1.11 (s, 6 H). CI-MS (NH_3): m/z 304 $[M+18]^+$, FABMS: m/z 287 $[M+1]^+$, HRMS (FAB) calcd for $C_{20}H_{31}O$ ($[M+H]^+$): 287.2475, found: 287.2361.

Enantioselective HPLC separation was carried out with CHIRALCEL AD (Chiral Technologies; 250 × 10 mm I.D. semiprep column, 10 μm), with the mobile phase (hexanes/2-propanol = 99.85/0.15) and the flow rate (3 mL/min) to give **12a** and **12b**. **12a**; $[\alpha]_D^{20.6}$ +11 (C 0.167, CH_2Cl_2), **12b**; $[\alpha]_D^{17.7}$ -10 (C 0.162, CH_2Cl_2), CD (methylcyclohexane): 249 nm, (Δε −2.8).

6-[(1E,2E)-1-Methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]octan-2-ol (13)

Diethylzinc (1.0 M solution in hexane, 34 μl, 34 μmol) was added slowly to a solution of diiodomethane (18 mg, 68 μmol) in anhydrous CH_2Cl_2 (220 μl) at 0°C. After additional stirring of the mixture at this temperature for 10 min, it was cooled to −78°C and the solution of 4-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-cyclohept-2-enol (**12**) (7.4 mg, 26 μmol) in CH_2Cl_2 (150 μl) was then slowly introduced. The reaction mixture was stirred at −78°C for 30 min and warmed to 0°C over 30 min and then stirred for 3 h. The reaction was quenched by addition of saturated aqueous ammonium chloride solution (200 μl) at 0°C and stirred for 10 min. The mixture was extracted with Et_2O (2 × 5 mL) and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash column chromatography (silica gel, Hexane/AcOEt = 5/1) to give 6-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]octan-2-ol (**13**) (6.2 mg, 80%). ^{1}H NMR (400 MHz, C_6D_6) δ 6.62 (d, *J* = 16.0 Hz, 1 H), 6.25 (d, *J* = 16 Hz, 1 H), 4.01 (m, 1 H), 2.37 (m, 1 H), 2.03 (m, 1 H), 2.01 (s, 3 H), 1.95 (t, *J* = 6.3 Hz, 2 H), 1.80 (s, 3 H), 1.26-1.62 (m, 8H), 1.13 (s, 6 H), 1.10 (m, 1H), 0.88 (m, 1H), 0.77 (m, 1H), 0.58 (m, 1H). ESI-MS m/z 301 $[M+1]^+$, 283. HRMS (FAB) calcd for $C_{21}H_{32}O$ ($[M]^+$): 300.2453, found: 300.2459.**13b**; UV (methylcyclohexane): λ_{max} 267nm, ε 15,000. CD (methylcyclohexane): 270 nm (Δε +3.5).

6-[(1E,2E)-1-Methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]octan-2-one (14)

MnO_2 (Aldrich, <5 μ, activated, ~85%) was preheated in a 250°C oven overnight and cooled to room temperature in a desiccator before use. To the solution of 6-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo [5.1.0]octan-2-ol (**13**) (100 mg, 33 μmol) in anhydrous CH_2Cl_2 (5 mL) at −20°C was added MnO_2 (0.58 g, 6.6 mmol.) The cooling bath was removed and the reaction mixture was warmed up while stirring to room temperature for 5 min (TLC monitored.) At completion, it was filtered through a pad of Celite. The Celite cake was washed with CH_2Cl_2 three times. The combined solution was concentrated under reduced pressure and purified by flash column chromatography (silica gel, Hexane/AcOEt = 10/1) to give 6-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]octan-2-one (**14**) (86 mg, 87%). ^{1}H NMR (400 MHz, C_6D_6) δ 6.47 (d, *J* = 16 Hz, 1 H), 6.27 (d, *J* = 16 Hz, 1 H), 2.49 (m, 1 H), 1.96 (t, *J* = 6 Hz, 2 H), 1.91 (m, 1 H), 1.89 (s, 3 H), 1.87 δ 1.78 (m, 2 H), 1.76 (s, 3 H), 1.65-1.57 (m, 3 H), 1.2-1.4 (m, 4 H), 1.124 (s, 3 H), 1.117 (s, 3 H), 0.92-0.82 (m, 2 H). ESI-MS m/z 299 $[M+1]^+$. HRMS (FAB) calcd. for $C_{21}H_{30}O$ ($[M]^+$):298.2297, found: 298.2296.

(E/Z)-{6-[(1E,2E)-1-Methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]oct-2-ylidene}-acetonitrile (15, 16)

To a suspension of sodium hydride (60% in mineral oil, 40 mg, 1 mmol) in anhydrous THF (5 ml) was added diethyl cyanomethylphosphonate (180 mg, 1 mmol) in THF (5 mL) at 0°C under argon. The mixture was stirred for 20 min at room temperature. A solution of 6-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo [5.1.0]octan-2-one (**14**) (30 mg, 0.1 mmol) in THF (3 mL) was introduced. The reaction was allowed to proceed at room temperature for 2 h and was quenched by addition of iced water (20 mL). The mixture was extracted with Et_2O (3 × 20 mL) and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash column chromatography (silica gel, Hexane/AcOEt = 5/1) to yield (*E/Z*)-{6-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]oct-2-ylidene}-acetonitrile (**15**)/ (**16**) (28 mg, 87%). ^{1}H NMR (400 MHz, C_6D_6) δ 6.47 (d, *J* = 16.0 Hz, 1 H), 6.26 (d, *J* = 16 Hz, 1 H), 4.66 (s, 1 H), 2.45 (m, 1 H), 2.35 (m, 1 H), 1.95 (m, 2 H), 1.88 (s, 3 H), 1.76 (s, 3 H), 1.58 (m, 2 H), 1.48 (m, 2 H), 1.36 (m, 4 H), 1.12 (s, 3 H), 1.11 (s, 3 H), 0.88 (m, 2 H), 0.69 (m, 1 H), 0.54 (m, 1 H). CI-MS (NH_3) m/z 322 $[M+1]^+$, 339 $[M+18]^+$.

(E/Z)-{6-[(1E,2E)-1-Methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]oct-2-ylidene}-acetaldehyde (2, 3)

To a solution of (*E/Z*)-{6-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]oct-2-ylidene}-acetonitrile (**15**)/(**16**) (10 mg, 0.031 mmol) in anhydrous Et_2O (5 mL) was added diisobutylaluminum hydride (1.0 M solution in Hexane, 0.2 mL, 0.2 mmol) at −78°C under argon. The reaction mixture was warmed up

to room temperature and poured into slurry of wet silica gel suspension in Et_2O. The suspension was stirred at room temperature for 30 min. The silica gel was removed by filtration and washed with additional Et_2O (100 mL). The filtrate was concentrated and purified by HPLC (YMC-Pack SIL, 230 × 10 mm I.D., S-5 mm, 120A), Hexane/Et_2O = 95/5) to *(E)*- and *(Z)*-{6-[(1*E*,2*E*)-1-methyl-3-(2,6,6-trimethyl-cyclohex-1-enyl)-allylidene]-bicyclo[5.1.0]oct-2-ylidene}-acetaldehyde (**2** and **3**) (9 mg, 90%).

2: ^{1}H NMR (500 MHz, C_6D_6) δ 9.90 (d, *J* = 8.0 Hz, 1 H), 6.52 (d, *J* = 16 Hz, 1 H), 6.29 (d, *J* = 16 Hz, 1 H), 6.08 (d, *J* = 8 Hz, 1H), 2.51(m, 1 H), 2.45 (m, 1H), 1.97 (m, 2 H), 1.87(s, 3H), 1.80 (m, 1 H), 1.79 (s, 3 H), 1.60 (m, 3 H), 1.51 (m, 2 H), 1.31 (m, 1 H), 1.30 (m, 1 H), 1.24 (m, 1 H), 1.21 (m, 1H[1]), 1.13 (s, 3 H), 1.12 (s, 3 H), 079 (m, 1 H), 0.66 (m, 1 H); (500 MHz, CD_3OD) δ 9.90 (d, *J* = 8.3 Hz, 1 H), 6.40 (d, 16 Hz, 1 H), 6.15 (d, 16 Hz, 1 H), 6.10 (d, 8.3 Hz, 1H), 3.03 (m, 1H), 2.7 (m, 1H), 2.23 (m, 1 H), 2.1-2.0 (m, 3 H), 2.01 (s, 3 H), 1.82 (m, 1 H), 1.69 (s, 3 H), 1.64 (m, 3 H), 1.6 (m, 1 H), 1.52-1.40 (m, 3 H), 1.19 (m, 1H). APCI-MS m/z 325 $[M+1]^+$,. HRMS (FAB) calcd. for $C_{23}H_{33}O$ ($[M+H]^+$): 325.2531, found: 325.2520.

3: ^{1}H NMR (500 MHz, C_6D_6) δ 10.10 (d, *J* = 7.6 Hz, 1 H), 6.52 (d, *J* = 16 Hz, 1 H), 6.28 (d, *J* = 16 Hz, 1 H), 5.88 (d, *J* = 7.6 Hz, 1H), 2.40-2.23 (m, 2 H), 1.97 (m, 2 H), 1.92 (s, 3 H), 1.79 (s, 3 H), 1.71-1.64 (m, 1H), 1.62-1.56 (m, 2 H), 1.52-1.42 (m, 3 H), 1.36-1.20 (m, 4 H), 1.14 (s, 3 H), 1.13 (s, 3 H), 082 (m, 1 H), 0.73 (m, 1 H). APCI-MS m/z 325 $[M+1]^+$,. HRMS (FAB) calcd for $C_{23}H_{33}O$ ($[M+H]^+$): 325.2531, found: 325.2516.

Enantioselective HPLC separation was performed with CHIRALCEL AD (Chiral Technologies; 250 × 4.6 mm I.D. (10 μm)), with the mobile phase (Hexane/2-propanol = 99.85/0.15) and flow rate (1 mL/min) to give **2a** and **2b** from the racemic **2**, and **3a** and **3b** from the racemic **3**.

2a: (in methylcyclohexane) UV: λ_{max} 254 nm, ε 24,000. CD: 239 nm (+9.3), 272 nm (-6.4), (in MeOH) UV: λ_{max} 268 nm, ε 25,000. CD: 245 nm (+8.9), 281 nm (-7.4).

2b: (in methylcyclohexane) UV: λ_{max} 254 nm, ε 24,000. CD: 241 nm (-10.1), 274 nm (+6.1), (in MeOH) UV: λ_{max} 268 nm, ε 25,000. CD: 245 nm (-10.1), 274 nm (+8.5).

3a: (in methylcyclohexane) UV: λ_{max} 260 nm, ε 22,000. CD: 246 nm (+11.5), 283 nm (-4.1), (in MeOH) UV: λ_{max} 268 nm, ε 23,000. CD: 250 nm (+11.7), 287 nm (5.6).

3b: (in methylcyclohexane) UV: λ_{max} 260 nm, ε 22,000. CD: 246 nm (-11.8), 279 nm (+4.6), (in MeOH) UV: λ_{max} 268 nm, ε 23,000. CD: 250 nm (-11.7), 284 nm (+5.9).

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