Synthesis of enantiopure C_3- and C_4-hydroxyretinals and their enzymatic reduction by ADH8 from *Xenopus laevis*

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(*R*)-all-*trans*-3-hydroxyretinal **1**, (*S*)-all-*trans*-4-hydroxyretinal **3** and (*R*)-all-*trans*-4-hydroxyretinal **5** have been synthesized stereoselectively by Horner–Wadsworth–Emmons and Stille cross-coupling as bond-forming reactions. The CBS method of ketone reduction was used in the enantioface-differentiation step to provide the precursors for the synthesis of the 4-hydroxyretinal enantiomers. The kinetic constants of *Xenopus laevis* ADH8 with these retinoids have been determined.

Introduction

Vitamin A (retinol) and its metabolites (retinoids) are essential to the proper function of a number of biological processes. The visual cycle[1] is perhaps the best characterized[2] with 11-*cis*-retinal acting as the inverse agonist of the apoprotein opsin in vertebrates. Reproduction,[3] cell growth and differentiation, embryonic development (*e.g.* limbs, nervous system, heart, kidney), immune response[4] and intermediary metabolism are regulated by all-*trans*-retinoic acid and 9-*cis*-retinoic acid. These gene transcription regulators are the ligands for two classes of nuclear receptors, acting as ligand-dependent transcription factors: the retinoic acid receptors (RARα, β and γ) and the retinoid X receptors (RXRα, β and γ).[5]

An increasing number of studies attribute a physiological role as signalling molecules to ring-oxidized derivatives at position C_4, which are major metabolites of retinoids.[6] In fact, C_4-oxidized retinoids are natural ligands of several RAR receptors.[6a, 6b, 7] Studies in *Xenopus* embryos revealed that 4-hydroxyretinol and 4-hydroxyretinoic acid can transactivate RAR receptors.[7] Retinol metabolism to 4-hydroxy and 4-oxoretinol is detected in murine stem cells[8] and a wide variety of cancer cell lines.[7a, 9] In addition, 4-hydroxyretinol endogenously occurs in serum and liver of normal neonatal rats.[10] A specific role in the onset of neuronal differentiation is postulated for 4-hydroxyretinoic acid,[11] and both 4-hydroxyretinoic and 4-oxoretinoic acid exhibit biological activity in skin retinoid responsive systems *in vivo*.[12] In *Xenopus* eggs and early embryos, the major bioactive retinoids are 4-oxoretinol and, particularly, 4-oxoretinal, which are critical for proper cell differentiation,[6b] whereas 4-oxoretinoic acid is a potent modulator of positional specification.[6a]

Retinoic acid is synthesized from retinol by a metabolic sequence involving two consecutive oxidation reactions, in which the reversible oxidation of retinol to retinal is considered to be the rate-limiting step. Further oxidation of retinal to retinoic acid appears to be irreversible.[13] Three different enzyme types, grouped as superfamilies, have been suggested to be responsible for the reversible conversion of retinol to retinal: alcohol dehydrogenases (ADH) of the medium-chain dehydrogenase/reductase (MDR), retinol dehydrogenases of the short-chain dehydrogenase/reductase (SDR)[13] and several members of the aldo-keto reductases (AKR).[14]

In vertebrates, the ADH family comprises eight different classes, involved in ethanol metabolism and also in the transformation of a variety of alcohols and aldehydes of physiological relevance, such as retinoids, steroids and cytotoxic aldehydes.[15] ADH1 and ADH4 have been the best studied in mammals because of their wide expression in tissues and their activity towards ethanol and retinol. While ADH1 is present in all vertebrate groups, ADH4 has only been reported in mammals. In amphibians, an enzyme showing kinetic properties similar to those of ADH4 has been described, but it exhibits specificity towards NADP(H) instead of NAD(H), the common cofactor for these enzymes. ADH8, isolated from the stomach of *Rana perezi*, displayed high activity towards retinal.[16] This property and the coenzyme requirement suggested that this enzyme might have a significant role in retinal metabolism, probably as a retinal reductase.

We are interested in deciphering the substrate specificity of ADHs towards retinoids in order to understand more deeply the biochemical pathways for the formation of vitamin A metabolites through oxidation/reduction reactions. Previous reports have dealt with the kinetic constants of ADH1 and ADH4 from humans and mice towards some ring-oxidized derivatives (*rac*-4-hydroxyretinol, 4-oxoretinal, 3,4-didehydroretinol and 3,4-didehydroretinal). All these compounds are good substrates for ADHs, judging from the low and similar K_m values measured in all cases. The 4-hydroxyretinol derivative appeared to be the most active, showing a catalytic efficiency *ca.* 30 times higher than parent compound retinol. The data supported the involvement of the enzymes in the red-ox metabolism of the C_4-oxidized retinoids.[17]

The availability of recombinant ADH8 from *Xenopus laevis*, the interest of this species to developmental studies, the high activity of ADH8 with retinal, and the physiological presence of several ring-oxidized retinoids in *Xenopus* prompted us to study the specificity and catalytic efficiency of this enzyme with chemically-modified retinals of biological significance.

Whereas in previous reports we determined the kinetic characteristics of *racemic* 4-hydroxyretinoids[17], we consider the preparation of the corresponding enantiopure substrates to be

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of interest in order to determine whether enantiomeric discrimination by the enzyme can occur. In addition to the C_4-hydroxyl derivatives, the study was extended to C_3-hydroxyretinal, easily obtained from enantiopure starting material. The C_4 and C_3-ring-oxidized retinoids of the *R* configuration are components of the visual cycle in the animal kingdom, namely in the bioluminescent squid *Watasenia scintillans*[18] and in most insects,[19] respectively.

We report herein the stereoselective synthesis of (*R*)-all-*trans*-3-hydroxyretinal **1**, (*S*)-all-*trans*-4-hydroxyretinal **3**, (*R*)-all-*trans*-4-hydroxyretinal **5**, and the kinetic constants measured upon incubation of these retinoids with *Xenopus* ADH8. Due to the pharmacological interest of 13-*cis*-retinoids, we also isolated the 13-*cis* isomers (**2**, **4** and **6**, respectively) of the above compounds, which allowed a comparison of the catalytic efficiencies of C_{13}–C_{14}-double-bond stereoisomers (Fig. 1).

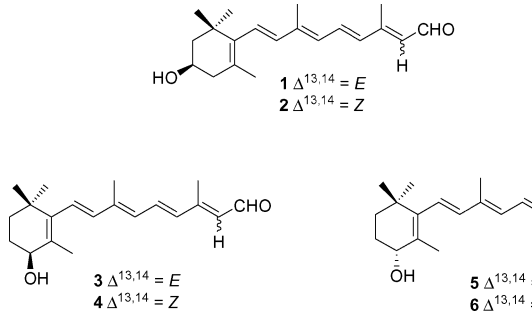


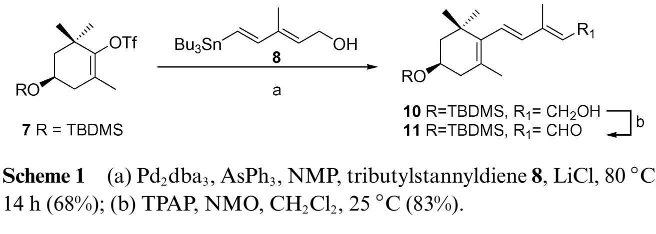
Fig. 1 All-*trans* and 13-*cis*-isomers of enantiopure C_3- and C_4-hydroxyretinals.

For the construction of the polyene side chain of these analogs, we took advantage of palladium-catalyzed cross-coupling reactions, a group of processes that have been found in general to be chemo- and stereoselective and to take place with retention of configuration of the coupling partners. We selected the Stille cross-coupling reaction for the construction of the C_6–C_7 bond [(*R*)-3-hydroxyretinal] and C_8–C_9 bond [(*S*)- and (*R*)-4-hydroxyretinal] guided by our comprehensive study on the scope and limitations of this process as applied to the synthesis of retinoids.[20]

Results and discussion

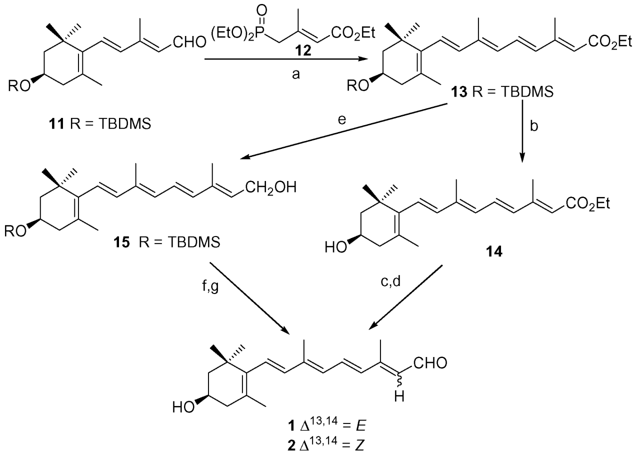
Synthesis of (*R*)-3-hydroxyretinal

The Stille cross-coupling reaction of the already described triflate **7**[21] and tributylstannyldienol **8**,[22] using $Pd_2dba_3/AsPh_3$ as a catalyst and a LiCl additive, provided, after stirring for 14 h at 80 °C, alcohol **10** in 68% yield. Oxidation of **10** with catalytic quantities of tetra-*n*-propylammonium perruthenate (TPAP) and N-methylmorpholine *N*-oxide (NMO) as co-oxidant,[23] afforded aldehyde **11** in good yield (Scheme 1).



Scheme 1 (a) Pd_2dba_3, $AsPh_3$, NMP, tributylstannyldiene **8**, LiCl, 80 °C, 14 h (68%); (b) TPAP, NMO, CH_2Cl_2, 25 °C (83%).

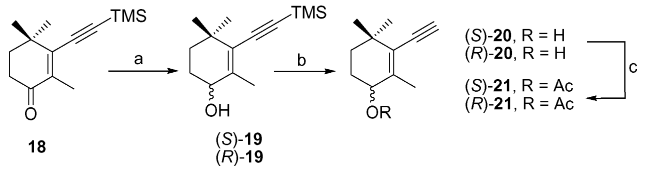
The pentaene ester **13** was obtained by the Horner–Wadsworth–Emmons reaction between aldehyde **11** and phosphonate **12**, using *n*-BuLi as a base in THF and DMPU as the cosolvent, in excellent yield after purification (99%).[24] Deprotection of **13** with TBAF in THF at 0 °C afforded **14** in 58% yield (Scheme 2), which was uneventfully reduced to the corresponding alcohol with Dibal-H in THF at −78 °C and the latter oxidized using MnO_2 (52% combined yield) to afford a 3 : 1 mixture of **1**[25] and **2**, which was separated by HPLC. No improvement could be achieved when the order of reactions (reduction–deprotection and oxidation) in the last functional group interconversion steps was reversed. Reduction of ester **13** with Dibal-H in THF at −78 °C afforded alcohol **15** in 89% yield, deprotection of which (68%), followed by oxidation with MnO_2 under basic conditions at 25 °C afforded a mixture of **1** and **2** in lower yield (42%) and stereoselectivity (2 : 1 ratio) (Scheme 2).



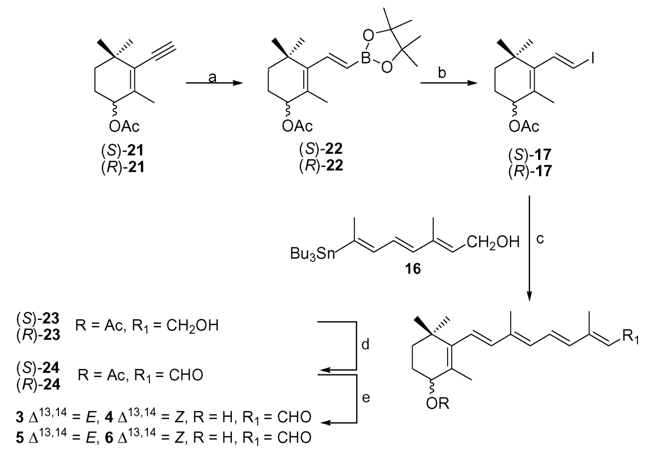
Scheme 2 (a) THF, *n*-BuLi, DMPU, phosphonate **12**, −78 − > 25 °C, 14 h (99%); (b) n-Bu_4NF, THF, 25 °C (58%); (c) Dibal-H, THF, −78 °C (66%); (d) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C (52%); (e) Dibal-H, THF, −78 °C (89%); (f) n-Bu_4NF, THF, 25 °C (68%); (g) MnO_2, Na_2CO_3, CH_2Cl_2, 25 °C (42%).

Synthesis of (*S*) and (*R*)-4-hydroxyretinal

The C_8–C_9 Stille disconnection for the preparation of (*S*)-4-hydroxyretinal **3** and (*R*)-4-hydroxyretinal **5** required the condensation of known stannylated trienol **16**[20] (Scheme 4) and dienyliodides (*S*)-**17** or (*R*)-**17**, both having *trans* geometries. The ketone **18**[17b] already described was envisaged as the precursor of the electrophilic component of the Stille cross-coupling. For the enantioselective reduction of enynone **18**, we selected the CBS chiral oxazaborolidine catalysts.[26] Treatment of enynone **18** with (*R*)- or (*S*)-2-methyl-CBS-oxazaborolidine and $BH_3{\cdot}SMe_2$ in THF at −30 °C afforded alcohols (*S*)-**19** or (*R*)-**19** in excellent yields (97% and 94%, respectively). Enantiomeric ratios [95% *ee* for (*S*)-**19** and 91% *ee* for (*R*)-**19**] were determined by chiral HPLC (Chiralcel OD-H; 0,46 cm ϕ × 15 cm, cellulose on 5 μm silica gel and 95 : 5 hexane/MeOH as eluent) (Scheme 3). The CBS model for enantioface differentiation[27] was adopted to assign the absolute configuration of the major enantiomer in each case.



Scheme 3 (a) (*R*) or (*S*)-2-methyl-CBS-oxazaborolidine, $BH_3·SMe_2$, THF, 21 h, −30 °C [97% for (*S*)-**19** and 94% for (*R*)-**19**]; (b) K_2CO_3, MeOH, 25 °C, 3.5 h [91% for (*S*)-**20** and 98% for (*R*)-**20**]; (c) Ac_2O, DMAP, Et_3N, CH_2Cl_2 [96% for (*S*)-**21** and 94% for (*R*)-**21**].



Scheme 4 (a) *i.* pinacol, $BH_3·SMe_2$, CH_2Cl_2, 0–>25 °C, *ii.* Enyne (*S*)-20 or (*R*)-20, CH_2Cl_2, 0–>50 °C [54% for (*S*)-**22** and 57% for (*R*)-**22**]; (b) *i.* MeONa, MeOH, THF, −78 °C, *ii.* ICl, CH_2Cl_2, −78 °C [81% for (*S*)-**17** and 86% for (*R*)-**17**]; (c) trienylstannane **16**, $Pd_2(dba)_3$, $AsPh_3$, NMP, 40 °C, 7 h [70% for (*S*)-**23** and 65% for (*R*)-**23**]; (d) Dess–Martin Periodinane, CH_2Cl_2, pyridine, 25 °C, 6 h, [71% for (*S*) and 63% for (*R*)]; e) K_2CO_3, MeOH, 25 °C, 5 h [73% for **3** and 67% for **5**].

Deprotection of (*S*)-**19** and (*R*)-**19** with K_2CO_3 in MeOH provided efficiently unstable alkynes (91% and 98%, respectively), which were protected as acetates using Ac_2O, DMAP and Et_3N in CH_2Cl_2 in excellent yield (Scheme 3).

We attempted first to prepare the alkenyliodides by tin-iodide exchange of the corresponding alkenylstannanes, themselves obtained from the terminal alkynes by radical addition of tributyltin hydride,[28] a methodology already used in the synthesis of the racemic material,[17b] but we encountered a number of difficulties, obtaining mixtures of alkenylstannanes with *E* and *Z* geometry as a result of the quality of the tributyltinhydride used being the most significant. In order to circumvent this problem, the alkenyl boronate was chosen instead of the alkenylstannane. Hydroboration of alkynes (*S*)-**21** and (*R*)-**21** using pinacol borane regio- and stereoselective provided the boronic esters (*S*)-**22** and (*R*)-**22** (54% and 57%, respectively, based on recovered starting material). In large-scale reactions, variable amounts of the minor regioisomer are obtained. Boron–halogen exchange with retention of configuration was effected by treatment of **22** with MeONa at −78 °C followed by addition of ICl. Alkenyl iodides (*S*)-**17** and (*R*)-**17** were isolated in very high yields, and their *E* geometry was confirmed by the value of the coupling constant ($J = 14.8$ Hz) for the alkenyl protons in their ^{1}H-NMR spectrum (Scheme 4).

Coupling of iodides **17** with stannanyltrienol **16** under Farina's conditions [$Pd_2(dba)_3$, $AsPh_3$, NMP] required 7 h at 40 °C and afforded (*S*)-**23** and (*R*)-**23** in 70 and 65% yield, respectively. Oxidation of diols (*S*)-**23** and (*R*)-**23** was carried out with the Dess–Martin reagent in CH_2Cl_2-pyridine to minimize isomerization at the terminal double bond. Despite these precautions, 8.5 : 1 and 6.5 : 1 mixtures of the *trans*:13-*cis*-isomers for the (*S*) and (*R*) enantiomers were obtained in 71 and 63% yield, respectively. Although they could be separated at this stage by column chromatography if desired, additional isomerization of the C_{13}–C_{14} bond took place upon deprotection of the acetates with K_2CO_3 in MeOH, affording 3 : 1 mixtures of all-*trans*:13-*cis*-isomers of both (*S*)-4-hydroxyretinal and (*R*)-4-hydroxyretinal. These isomers were separated by HPLC (Preparative Nova Pak® HR silica, 60 Å, 19 × 300 mm and 95:5 hexane/ethyl acetate as eluent) (Scheme 4). The *ee* measured for the final compounds (89% *ee* for **3** and 93% *ee* for **4**)[29] indicates that enantiopurity has been mostly preserved along the sequence.

Kinetics of ADH8 with retinoids

The kinetics of *Xenopus* ADH8 with these C_3- and C_4-oxidized derivatives of all-*trans*-retinal and 13-*cis*-retinal are shown in Table 1. All the hydroxyretinals assayed were very active, suggesting that they can be physiological substrates for ADH8. When compared with the corresponding parent compounds, the k_{cat} values for the ring-oxidized retinals showed a 10-fold increase, while K_m values remained essentially constant. This effect was independent of the position of the hydroxyl group since no substantial differences were found between the C_3- and the C_4-hydroxyl derivatives of either all-*trans*-retinal or 13-*cis*-retinal. Furthermore, no differences were observed between *R* and *S*

Table 1 Kinetic constants of *Xenopus laevis* ADH8 with retinoids **1–6**[a]

Substrate	K_m/μM	k_{cat}/min^{-1}	k_{cat}/K_m/mM^{-1}·min^{-1}
All-*trans*-retinal	21.5 ± 3.5	270 ± 13	12560 ± 2100
(*R*)-all-*trans*-4-hydroxy-retinal	13.2 ± 1.8	2080 ± 90	157600 ± 23500
(*S*)-all-*trans*-4-hydroxyretinal	12.6 ± 1.3	2360 ± 70	187300 ± 20000
(*R*)-all-*trans*-3-hydroxyretinal	10.1 ± 0.9	2460 ± 70	243600 ± 23800
13-*cis*-retinal[b]	15	63	4200
(*R*)-13-*cis*-4-hydroxyretinal	23.7 ± 2.3	860 ± 30	36300 ± 3700
(*S*)-13-*cis*-4-hydroxyretinal	20.3 ± 2.3	860 ± 30	42400 ± 5000
(*R*)-13-*cis*-3-hydroxyretinal	21.9 ± 2.5	760 ± 30	34700 ± 4200
Hexanal	79.4 ± 11.8	18600 ± 900	234000 ± 37000

[a] Activities were determined in 0.1 M sodium phosphate, pH 7.5, 0.02% Tween 80, using 0.6 mM NADPH, at 25 °C. [b] Constants measured for the ADH8 enzyme of *Rana perezi.*[16]

enantiomers of the C_4-hydroxyl derivatives of both all-*trans*-retinal and 13-*cis*-retinal, thus concluding that ADH8 does not exhibit chiral discrimination, at least at position C_4. On the other hand, the *trans*-ring-oxidized retinal stereoisomers showed 2.4- to 3.2-fold higher k_{cat} values with respect to the derivatives of 13-*cis*-retinal, known to be a poor substrate for several ADH enzymes studied.[17a, 30]

The k_{cat} values of ADH8 toward C_3- and C_4-hydroxyretinals are the highest among all retinoid substrates assayed for this enzyme. Similarly, C_4-oxo- and C_4-hydroxyretinoids were the most active retinoids for ADH1 and ADH4.[17b,31] In general, ADH shows low turnover numbers (k_{cat}) with retinol isomers, in comparison to those for aliphatic substrates.[17a,32] For retinoids the release of product may be a significant rate-limiting step, in contrast to the kinetics with aliphatic substrates, where the limiting step is, in general, the cofactor release.[33] For ADH8 the k_{cat} for ring-oxidized retinals (about 2000 min^{-1}, Table 1) is still lower than that for the best aliphatic aldehydes (k_{cat} for hexanal = 18600 min^{-1} and 14000 min^{-1} for *Xenopus laevis* (Table 1) and *Rana perezi* enzymes, respectively). Thus, it can be suggested that the k_{cat} for the ring-oxidized-retinals is higher than that for the parent compounds because the extra polarity facilitates the delivery to the aqueous environment of the reduced product, but still this product release is rate limiting in the reduction of hydroxy-retinals by ADH8.

The fact that ADH8 does not discriminate between the hydroxyl groups at the C_3 or C_4 positions of the cyclohexene ring, or between the C_4-hydroxyretinal enantiomers, is consistent with docking studies using several retinoids as ligands and the crystallographic ADH8 structure from *Rana perezi*[34] and other ADH structures.[35] While the functional group of the retinoid interacts with the active site Zn, buried in the inner part of the molecule, the cyclohexene ring localizes in the wide entrance of the substrate-binding pocket, accessible to the solvent, and presumably with little structural constraints.

Finally, this is the first time that ring-oxidized derivatives of the 13-*cis*-retinal isomer have been assayed for ADH activity, and they also show a great increase in their k_{cat} values when compared with 13-*cis*-retinal. The rate increase is about 10-fold, similar to that observed for the hydroxyl derivatives of all-*trans*-retinal. The 13-*cis*-isomers are among the worst retinoid substrates for ADHs because of their low k_{cat} values, being virtually inactive with ADH1.[17a] In fact, ADH8 is the most active known ADH with 13-*cis*-retinoids.[16]

Conclusions

The catalytic constant (k_{cat}) values of *Xenopus laevis* ADH8 with enantiopure C3- and C4-hydroxyretinals were about 10-fold those for the parent all-*trans*-retinal. The kinetic studies revealed neither enantiomeric discrimination (4*R* *vs* 4*S*) nor positional discrimination (3*R* *vs* 4*R*) by the enzyme, consistent with an external position of the cyclohexene ring in the ADH-retinoid complex structure. The corresponding 13-*cis* isomers of the ring oxidized derivatives, obtained as isomerization by-products in the synthetic sequence, showed from 2.4- to 3.2-fold lower k_{cat} than the *trans*-isomers.

The OH substitution at the cyclohexene ring would facilitate the study of the kinetic effect of the double bond isomerization, even using compounds, such as 13-*cis*-retinoids, with poor activity with ADHs.

Experimental

General

Solvents were dried according to published methods and distilled before use. HPLC grade solvents were used for the HPLC purification. All other reagents were commercial compounds of the highest purity available. All reactions were carried out under an argon atmosphere, and those not involving aqueous reagents were carried out in oven-dried glassware. Analytical thin layer chromatography (TLC) was performed on aluminium plates with Merck Kieselgel 60F254 and visualised by UV irradiation (254 nm) or by staining with solution of phosphomolibdic acid. Flash column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh) under pressure. High performance liquid chromatography was performed using a Waters instrument using a dualwave detector (254 and 300 nm) with a Preparative Nova Pak® HR silica, 60 Å, 19 × 300 mm and 95 : 5 hexane/ethyl acetate as eluent. Enantiomeric excess was calculated by chiral HPLC with a Waters™ 996 (Photodiode Array) detector with a Chiralcel OD–H Column 0,46 cm × 15 cm cellulose on 5 μm silica gel and 95 : 5 hexane/MeOH as eluent. UV–Vis spectra were recorded on a Cary 100 Bio spectrophotometer using MeOH as solvent. Infrared spectra were obtained on JASCO FT-IR 4200 spectrophotometer, from a thin film deposited onto a NaCl glass. Specific rotation was obtained on JASCO P-1020. Mass spectra were obtained on a Hewlett-Packard HP59970 instrument operating at 70 eV by electron ionisation. High resolution mass spectra were taken on a VG Autospec instrument. ^1H NMR spectra were recorded in $CDCl_3$, C_6D_6 and $(CD_3)_2CO$ at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz with residual protic solvent as the internal reference ($CDCl_3$, $\delta_H = 7.26$ ppm; C_6D_6, $\delta_H = 7.16$ ppm; $(CD_3)_2CO$, $\delta_H = 2.05$ ppm); chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz). The proton spectra are reported as follows: δ (multiplicity, coupling constant J, number of protons, assignment). ^{13}C NMR spectra were recorded in $CDCl_3$, C_6D_6 and $(CD_3)_2CO$ at ambient temperature on the same spectrometer at 100 MHz, with the central peak of $CDCl_3$ ($\delta_C = 77.0$ ppm), C_6D_6 ($\delta_C = 128.0$ ppm) or $(CD_3)_2CO$ ($\delta_C = 30.8$ ppm) as the internal reference. DEPT135 are used to aid in the assignment of signals in the ^{13}C NMR spectra.

(−)-(2*E*,4*E*)-5-[(*R*)-4-(*tert*-Butyldimethylsilyloxy)-2,6,6-trimethylcyclohex-1-en-1-yl]-3-methylpenta-2,4-dien-1-ol 10. General procedure for Stille cross coupling. A solution of (*R*)-5-(*tert*-butyldimethylsilyloxy)-2-[(trifluoromethanesulfonyl)oxy]-1,3,3-trimethylcyclohex-1-ene **7** (0.70 g, 1.75 mmol) in NMP (15.6 mL) was added to a solution of $Pd_2(dba)_3$ (0.04 g, 0.042 mmol), $AsPh_3$ (0.11 g, 0.35 mmol) in NMP (3.2 mL). After stirring for 10 min, a solution of (2*E*,4*E*)-3-methyl-5-(tri-*n*-butylstannyl)penta-2,4-dien-1-ol **8** (0.82 g, 2.10 mmol) in NMP (3.2 mL) and LiCl (0.22 g, 5.26 mmol) was added. The mixture was stirred for 14 h at 80 °C. An aqueous solution of KF (10 mL) was added and the mixture was stirred for 10 min and then extracted with *t*-BuOMe (3x). The combined organic layers were washed with H_2O (3x), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 90 : 10 hexane/ethyl acetate) to afford 0.42 g (68%) of a yellow oil identified as **10**. $[\alpha]_D^{18}$ − 64.9 (*c* 0.02, MeOH). ^{1}H-NMR (400.16 MHz, $CDCl_3$): δ 6.1–6.0 (m, 2H, $H_4 + H_5$), 5.61 (t, J = 6.8 Hz, 1H, H_2), 4.30 (d, J = 6.8 Hz, 2H, $2H_1$), 4.0–3.8 (m, 1H, $H_{4'}$), 2.22 (dd, J = 17.0, 5.2 Hz, 1H, $H_{3'}$), 2.1–2.0 (m, 1H, $H_{3'}$), 1.85 (s, 3H, C_3–CH_3), 1.68 (s, 3H, $C_{2'}$–CH_3), 1.6–1.5 (m, 1H, $H_{5'}$), 1.45 (t, J = 12.0 Hz, 1H, $H_{5'}$), 1.03 (s, 3H, $C_{6'}$–CH_3), 1.02 (s, 3H, $C_{6'}$–CH_3), 0.90 (s, 9H, $SiC(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$). ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 137.9 (d), 137.5 (s), 137.1 (s), 129.2 (d), 127.0 (s), 126.7 (d), 66.1 (d), 59.8 (t), 49.1 (t), 43.3 (t), 37.4 (s), 30.5 (q), 28.9 (q), 26.4 (q, 3x), 21.9 (q), 18.6 (s), 12.8 (q), −4.2 (q, 2x). MS (EI$^+$): m/z (%) 332 (M^+–H_2O, 10), 219 (100), 185 (12), 174 (35), 159 (50), 145 (11), 75 (39). HRMS (EI$^+$): Calcd. for $C_{21}H_{36}OSi$ $(M^+–H_2O)^+$, 332.2535; found, 332.2529. IR (NaCl): ν 3500–3100 (br, OH), 2955 (s, C–H), 2928 (s, C–H), 2856 (s, C–H), 1084 cm^{-1}.

(−)-(2*E*,4*E*)-5-[(*R*)-4-(*tert*-Butyldimethylsilyloxy)-2,6,6-trimethylcyclohex-1-en-1-yl]-3-methylpenta-2,4-dienal 11. To a cooled (0 °C) stirred solution of NMO (0.11 g, 0.94 mmol) in CH_2Cl_2 (3.6 mL) containing 4 Å molecular sieves was added a solution of **10** (0.22 g, 0.63 mmol) in CH_2Cl_2 (1.8 mL). After stirring for 10 min, TPAP (0.012 g, 0.032 mmol) was added and the mixture was stirred at 25 °C for 3 h. The mixture was diluted with CH_2Cl_2 (4 mL) and washed with aqueous Na_2SO_3 (3x). The organic layer was dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 93 : 4 : 3 hexane/ethyl acetate/Et_3N) to afford 0.17 g (83%) of a yellow oil identified as **11**. $[\alpha]_D^{26}$ − 130.6 (*c* 0.026, MeOH). ^{1}H-NMR (400.16 MHz, C_6D_6): δ 10.01 (d, J = 7.8 Hz, 1H, H_1), 6.38 (d, J = 16.1 Hz, 1H, H_5), 5.96 (d, J = 16.1 Hz, 1H, H_4), 5.92 (d, J = 7.8 Hz, 1H, H_2), 4.1–4.0 (m, 1H, $H_{4'}$), 2.26 (dd, J = 17.0, 5.7 Hz, 1H, $H_{3'}$), 2.15 (dd, J = 17.0, 8.9 Hz, 1H, $H_{3'}$), 1.8–1.7 (m, 1H, $H_{5'}$), 1.71 (s, 3H, C_3–CH_3), 1.7–1.6 (m, 1H, $H_{5'}$), 1.54 (s, 3H, $C_{2'}$–CH_3), 1.05 (s, 9H, $SiC(CH_3)_3$), 1.01 (s, 3H, $C_{6'}$–CH_3), 0.97 (s, 3H, $C_{6'}$–CH_3), 0.15 (s, 6H, $Si(CH_3)_2$). ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 190.4 (d), 153.8 (s), 136.6 (s), 136.4 (d), 134.0 (d), 129.2 (d), 128.9 (s), 65.1 (d), 48.6 (t), 42.9 (t), 36.6 (s), 29.5 (q), 27.9 (q), 25.3 (q, 3x), 20.8 (q), 17.7 (s), 11.9 (q), −5.3 (q, 2x). MS (EI$^+$): m/z (%) 291 (M^+-*t*-Bu, 28), 235 (90), 216 (21), 205 (23), 199 (25), 197 (21), 190 (48), 175 (59), 173 (29), 171 (33), 157 (36), 147 (38), 145 (26), 143 (38), 133 (78), 121 (29), 119 (49), 105 (41), 95 (42), 91 (25), 75 (100). HRMS (EI$^+$): Calcd. for $C_{21}H_{36}O_2Si$, 348.2485; found, 348.2487. IR (NaCl): ν 2956 (s, C–H), 2928 (s, C–H), 2856 (s, C–H), 1666 (s, C=O), 1083 cm^{-1}. UV (MeOH): λ_{max} 280 nm.

Ethyl (−)-(*R*)-all-*trans*-3-(*tert*-butyldimethylsilyloxy)-retinoate 13. A cooled (0 °C) solution of diethyl (*E*)-3-(ethoxycarbonyl)-2-methylprop-2-en-1-ylphosphonate **12** (0.09 g, 0.35 mmol) and DMPU (0.064 mL, 0.53 mmol) in THF (0.2 mL) was treated with *n*-BuLi (0.25 mL, 1.32 M in hexane, 0.33 mmol) and stirred for 30 min. The mixture was cooled down to −78 °C, and a solution of **11** (0.07 g, 0.19 mmol) in THF (0.2 mL) was added. The resulting mixture was allowed to warm to 25 °C for 14 h, and H_2O (1 mL) was added. The reaction was extracted with Et_2O (3x) and the organic layers were washed with H_2O (3x) and brine (3x), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 91 : 6 : 3 hexane/ethyl acetate/Et_3N) to afford 81 mg (99%) of a yellow oil identified as **13**. $[\alpha]_D^{25}$ −; 94.0 (*c* 0.034, MeOH). ^{1}H-NMR (400.16 MHz, $(CD_3)_2CO$): δ 7.13 (dd, J = 15.1, 11.4 Hz, 1H, H_{11}), 6.42 (d, J = 15.1 Hz, 1H, H_{12}), 6.25 (d, J = 11.4 Hz, 1H, H_{10}), 6.3–6.2 (m, 2H, $H_7 + H_8$), 5.82 (s, 1H, H_{14}), 4.13 (q, J = 7.1 Hz, OCH_2–CH_3), 4.1–4.0 (m, 1H, H_3), 2.36 (s, 3H, C_{13}–CH_3), 2.4–2.3 (m, 1H, H_4), 2.1 (d, J = 11.1 Hz, 1H, H_4), 2.05 (s, 3H, C_9–CH_3), 1.75 (s, 3H, C_5–CH_3), 1.7–1.6 (m, 1H, H_2), 1.48 (t, J = 11.9 Hz, 1H, H_2), 1.25 (t, J = 7.1 Hz, 3H, OCH_2–CH_3), 1.11 (s, 3H, C_1–CH_3), 1.09 (s, 3H, C_1–CH_3), 0.93 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 6H, $Si(CH_3)_2$). ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 167.6 (s), 153.7 (s), 140.4 (s), 139.3 (d), 138.6 (s), 136.8 (d), 132.3 (d), 131.4 (d), 128.8 (d), 128.4 (s), 119.9 (d), 66.6 (d), 60.4 (t), 50.1 (t), 44.3 (t), 38.0 (s), 31.1 (q), 29.4 (q), 26.7 (q, 3x), 22.3 (q), 19.1 (s), 15.1 (q), 14.2 (q), 13.3 (q), −3.9 (q, 2x). **MS** (EI$^+$): m/z (%) 459 ($M^+ + 1$, 37), 458 (M^+, 100), 326 (23), 285 (91), 133 (16), 131 (15), 121 (27), 105 (19), 91(13), 74 (31), 73 (35). HRMS (EI$^+$): Calcd. for $C_{28}H_{46}O_3Si$, 458.3216; found, 458.3216. **IR** (NaCl): ν 2955 (s, C–H), 2927 (s, C–H), 2856 (s, C–H), 1708 (s, C=O), 1149 cm^{-1}. **UV** (MeOH): λ_{max} 348 nm (ε = 26800).

Ethyl (−)-(*R*)-all-*trans*-3-hydroxyretinoate 14. A cooled (0 °C) solution of **13** (0.078 g, 0.17 mmol) in THF (4 mL) was treated with *n*-Bu_4NF (0.21 mL, 1 M in THF, 0.21 mmol) and stirred for 7 h. The mixture was diluted with Et_2O (2 mL) and washed with an aqueous solution of $NaHCO_3$ (1x). The aqueous layer was extracted with AcOEt (3x) and the combinated organic layers were washed with brine (3x), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (silica gel, 60 : 40 hexane/ethyl acetate) to afford 53 mg (58%) of a yellow oil identified as **14**. $[\alpha]_D^{26}$ − 114.1 (*c* 0.028, MeOH). ^{1}H-NMR (400.16 MHz, $(CD_3)_2CO$): δ 7.15 (dd, J = 15.1, 11.2 Hz, 1H, H_{11}), 6.43 (d, J = 15.1 Hz, 1H, H_{12}), 6.26 (d, J = 11.2 Hz, 1H, H_{10}), 6.3–6.2 (m, 2H, $H_7 + H_8$), 5.82 (s, 1H, H_{14}), 4.13 (q, J = 7.1 Hz, OCH_2–CH_3), 4.0–3.9 (m, 1H, H_3), 2.36 (s, 3H, C_{13}–CH_3), 2.3–2.2 (m, 1H, H_4), 2.07 (s, 3H, C_9–CH_3), 2.1–2.0 (m, 1H, H_4), 1.8–1.7 (m, 1H, H_2), 1.74 (s, 3H, C_5–CH_3), 1.43 (t, J = 11.9 Hz, 1H, H_2), 1.26 (t, J = 7.1 Hz, 3H, OCH_2–CH_3), 1.10 (s, 3H, C_1–CH_3), 1.07 (s, 3H, C_1–CH_3). ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 166.2 (s), 152.4 (s), 139.0 (s), 137.7 (d), 137.2 (s), 135.3 (d), 130.9 (d), 129.9 (d), 127.6 (d), 127.1 (s), 118.4 (d), 63.3 (d), 58.9 (t), 48.5 (t), 42.5 (t), 36.5 (s), 29.7 (q), 27.9 (q), 20.8 (q), 13.6 (q), 12.8 (q), 11.9 (q). MS (EI$^+$): m/z (%) 345 ($M^+ + 1$, 24), 344 (M^+, 100), 285 (19), 271 (35), 197 (37), 192 (22), 191 (25), 173 (33), 171 (45), 159 (25), 157 (30), 131 (30), 119 (39), 107 (38), 105 (39), 91 (42). HRMS (EI$^+$): Calcd. for $C_{22}H_{32}O_3$, 344.2351; found, 344.2350. IR (NaCl): ν 3500–3150 (br, OH), 2957 (s, C-H), 2921 (s, C–H), 1706 (s, C=O), 1151 cm^{-1}. UV (MeOH): λ_{max} 353 nm (ε = 47000).

(−)-(*R*)-All-*trans*-3-hydroxyretinal 1. Dibal-H (0.85 mL, 1 M in hexane, 0.85 mmol) was added to a solution of **14** (0.074 g, 0.21 mmol) in THF (2 mL), at −78 °C, and the resulting suspension was stirred for 1 h. After careful addition of H_2O, the mixture was extracted with Et_2O (3x) and the organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (silica gel, 97:3 hexane/Et_3N) to afford 43 mg (66%) of a yellow oil that was oxidized immediately.

To a solution of this compound (0.04 g, 0.14 mmol) in CH_2Cl_2 (2.6 mL) was added MnO_2 (0.22 g, 2.54 mmol) and Na_2CO_3 (0.27 g, 2.54 mmol), and the suspension was stirred for 5 h. The mixture was filtered throught Celite and the solvents were removed. The residue was purified by column chromatography (silica gel, 97 : 3 hexane/ethyl acetate) to afford 0.022 g (52%) as a yellow oil identified as a mixture of **1** and **2** in a 3 : 1 ratio, which were separated by HPLC.

Data for (−)-(*R*)-all-*trans*-3-hydroxyretinal **1**: $[\alpha]_D^{20}$ − 68.6 (*c* 0.022, MeOH). ^{1}H-NMR (400.16 MHz, $(CD_3)_2CO$): δ 9.81 (d, *J* = 8.0 Hz, 1H, H_{15}), 6.98 (dd, *J* = 15.1, 11.4 Hz, 1H, H_{11}), 6.17 (d, *J* = 15.1 Hz, 1H, H_{12}), 6.03 (d, *J* = 16.1 Hz, 1H, H_7), 5.98 (d, *J* = 11.5 Hz, 1H, H_{10}), 5.90 (d, *J* = 16.1 Hz, 1H, H_8), 5.59 (d, *J* = 8.0 Hz, 1H, H_{14}), 3.7–3.5 (m, 1H, H_3), 2.49 (s, 3H, C_{13}–CH_3), 2.1–2.0 (m, 1H, H_4), 1.74 (s, 3H, C_9–CH_3), 1.7–1.6 (m, 1H, H_4), 1.4–1.3 (m, 1H, H_2), 1.41 (s, 3H, C_5–CH_3), 1.09 (t, *J* = 11.9 Hz, 1H, H_2), 0.75 (s, 3H, C_1–CH_3), 0.74 (s, 3H, C_1–CH_3). ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 190.3 (d), 154.2 (s), 140.3 (s), 137.7 (d), 137.1 (s), 135.0 (d), 132.2 (d), 129.9 (d), 128.9 (d), 128.3 (d), 127.4 (s), 63.3 (d), 48.5 (t), 42.5 (t), 36.5 (s), 29.7 (q), 27.9 (q), 20.8 (q), 12.0 (q), 11.9 (q). MS (EI$^+$): *m*/*z* (%) 300 (M$^+$, 72), 171 (63), 159 (25), 157 (26), 147 (25), 145 (27), 133 (25), 131 (26), 119 (43), 105 (39), 95 (36), 91 (40), 69 (100). HRMS (EI$^+$): Calcd. for $C_{20}H_{28}O_2$, 300.2089; found, 300.2086. IR (NaCl): *ν* 3550–3150 (br, OH), 2957 (s, C–H), 2918 (s, C–H), 2850 (s, C–H), 1658 (s, C=O), 1574 cm^{-1}. UV (MeOH): λ_{max} 378 nm (ε = 28000).

Data for (−)-(*R*)-13-*cis*-3-hydroxyretinal **2**: $[\alpha]_D^{20}$ − 64.8 (*c* 0.012, MeOH). ^{1}H-NMR (400.16 MHz, $(CD_3)_2CO$): δ 10.25 (d, *J* = 7.9 Hz, 1H, H_{15}), 7.50 (d, *J* = 15.0 Hz, 1H, H_{12}), 7.20 (dd, *J* = 15.0, 11.5 Hz, 1H, H_{11}), 6.37 (d, *J* = 15.9 Hz, 1H, H_7), 6.35 (d, *J* = 11.1 Hz, 1H, H_{10}), 6.23 (d, *J* = 15.9 Hz, 1H, H_8), 5.80 (d, *J* = 7.9 Hz, 1H, H_{14}), 3.9–3.8 (m, 1H, H_3), 2.4–2.3 (m, 1H, H_2), 2.17 (s, 3H, C_{13}–CH_3), 2.04 (s, 3H, C_9–CH_3), 2.0–1.9 (m, 1H, H_2), 1.8–1.7 (m, 1H, H_4), 1.74 (s, 3H, C_5–CH_3), 1.42 (t, *J* = 12.0 Hz, 1H, H_4), 1.08 (s, 3H, C_1–CH_3), 1.07 (s, 3H, C_1–CH_3). MS (EI$^+$): *m*/*z* (%) 300 (M$^+$, 100), 147 (22), 121 (24), 119 (40), 105 (25), 95 (24), 91 (29), 77 (19). HRMS (EI$^+$): Calcd. for $C_{20}H_{28}O_2$, 300.2089; found, 300.2086. IR (NaCl): *ν* 3580–3150 (br, OH), 2956 (s, C–H), 2927 (s, C–H), 2855 (s, C–H), 1658 (s, C=O), 1575 cm^{-1}. UV (MeOH): λ_{max} 374 nm (ε = 22000).

(−)-(*R*)-All-*trans*-3-(*tert*-butyldimethylsilyloxy)retinol 15. Following the general procedure for Dibal-H reduction, the reaction of **13** (0.08 g, 0.17 mmol) with Dibal-H (0.7 mL, 1 M in hexane, 0.7 mmol) in THF (1.6 mL) at −78 °C for 1.5 h, afforded, after purification by column chromatography (silica gel, 85 : 15 hexane/ethyl acetate) 0.064 g (89%) of a yellow oil identified as **15**. $[\alpha]_D^{25}$ − 89.9 (*c* 0.03, MeOH). ^{1}H-NMR (400.16 MHz, $(CD_3)_2CO$): δ 6.65 (dd, *J* = 15.1, 11.3 Hz, 1H, H_{11}), 6.34 (d, *J* = 15.1 Hz, 1H, H_{12}), 6.3–6.2 (m, 3H, H_7 + H_8 + H_{10}), 5.70 (t, *J* = 5.8 Hz, 1H, H_{14}), 4.25 (t, *J* = 5.8 Hz, 2H, $2H_{15}$), 4.1–4.0 (m, 1H, H_3), 2.8–2.7 (m, 1H, H_2), 2.4–2.3 (m, 1H, H_2), 1.97 (s, 3H, C_{13}–CH_3), 1.83 (s, 3H, C_9–CH_3), 1.73 (s, 3H, C_5–CH_3), 1.8–1.7 (m, 1H, H_4), 1.5–1.4 (m, 1H, H_4), 1.10 (s, 3H, C_1–CH_3), 1.07 (s, 3H, C_1–CH_3), 0.92 (s, 9H, $SiC(CH_3)_3$), 0.11 (s, 6H, $Si(CH_3)_2$). ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 139.7 (d), 138.7 (s), 138.6 (d), 136.2 (s), 135.9 (s), 134.2 (d), 132.3 (d), 127.6 (s), 126.6 (d), 125.4 (d), 66.7 (d), 59.7 (t), 50.1 (t), 44.2 (t), 38.0 (s), 31.0 (q), 29.4 (q), 26.7 (q, 3x), 22.2 (q), 19.1 (s), 13.1 (q), 13.0 (q), −3.9 (q, 2x). **MS** (EI$^+$): *m*/*z* (%) 253 (44), 209 (13), 201 (20), 143 (24), 123 (24), 121 (25), 75 (100). **HRMS** (EI$^+$): Calcd. for $C_{26}H_{44}O_2Si$, 416.3126; found, 416.3111. **IR** (NaCl): *ν* 3550–3150 (br, OH), 2929 (s, C–H) cm^{-1}. **UV** (MeOH): λ_{max} 323 nm (ε = 19000).

(−)-(*R*)-All-*trans*-3-hydroxyretinal 1. Following the general procedure for TBAF deprotection, the reaction of **15** (0.036 g, 0.087 mmol) with *n*-Bu_4NF (0.35 mL, 1 M in THF, 0.35 mmol) in THF (2 mL) afforded, after purification by column chromatography (silica gel, 60 : 40 hexane/ethyl acetate) 18 mg (68%) of a yellow oil that was used immediately. Following the general procedure for MnO_2 oxidation, the reaction of the above alcohol (0.018 g, 0.14 mmol) with MnO_2 (0.093 g, 1.06 mmol) and anhydrous Na_2CO_3 (0.11 g, 1.06 mmol) in CH_2Cl_2 (1.2 mL) afforded, after purification by column chromatography (silica gel, 80 : 20 hexane/ethyl acetate), 7 mg (42%) of a yellow mixture containing **1** and **2** in a 2 : 1 ratio.

(−)-(*S*)-2,4,4-Trimethyl-3-(trimethylsilylethynyl)cyclohex-2-en-1-ol (*S*)-19. General procedure for enantioselective reduction of ketones. To a cooled (−78 °C) solution of 2,4,4-trimethyl-3-(trimethylsilylethynyl)cyclohex-2-en-1-one **18** (1.0 g, 4.26 mmol) in THF (50 mL) were sequentially added a solution of (*R*)-2-methyl-CBS-oxazaborolidine (0.43 mL, 1 M in toluene, 0.43 mmol) and $BH_3·SMe_2$ (0.43 mL, 4.26 mmol). After stirring for 21 h at −30 °C, H_2O (30 mL) was added and the mixture was extracted with *t*-BuOMe (4x). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 93 : 7 hexane/ethyl acetate) to afford 0.98 g (97%) of a white solid identified as (*S*)-**19**. MP: 57–59 °C (*t*-BuOMe). $[\alpha]_D^{22}$ − 38.4 (*c* 0.026, MeOH).^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 4.01 (t, *J* = 5.0 Hz, 1H, H_1), 2.00 (s, 3H, C_2–CH_3), 1.9–1.8 (m, 1H, H_6), 1.7–1.6 (m, 1H, H_6), 1.6–1.5 (m, 1H, H_5), 1.4–1.3 (m, 1H, H_5), 1.14 (s, 3H, C_4–CH_3), 1.08 (s, 3H, C_4–CH_3), 0.2 (s, 9H, Si–$(CH_3)_3$) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 141.7 (s), 127.7 (s), 103.3 (s), 99.4 (s), 68.8 (d), 34.1 (s), 33.0 (t), 28.8 (q), 28.1 (t), 27.6 (q), 19.3 (q), 0.0 (q, 3x) ppm. MS (FAB$^+$): *m*/*z* (%) 237 (M$^+$ + 1, 10), 236 (M$^+$, 22), 219 (M$^+$–OH, 100), 180 (33), 165 (13). HRMS (FAB$^+$): Calcd. for $C_{14}H_{25}OSi$ (M + 1)+, 237.1675; found, 237.1671. IR (NaCl):*ν* 3600–3100 (br, OH), 2961 (m, C–H), 2938 (m, C–H), 2866 (m, C–H), 2136 (w, C≡C), 1249 cm^{-1}. Elemental analysis: calcd. for $C_{14}H_{25}OSi$: C, 71.12; H, 10.23; found: C, 71.12; H, 10.27.

(+)-(*R*)-2,4,4-Trimethyl-3-(trimethylsilylethynyl)cyclohex-2-en-1-ol (*R*)-19. Following the general prodedure for enantioselective reduction of ketones, the reaction of **18** (1.0 g, 4.26 mmol) in THF (50 mL) with (*S*)-2-methyl-CBS-oxazaborolidine (0.43 mL, 1 M in toluene, 0.43 mmol) and $BH_3·SMe_2$ (0.43 mL, 4.26 mmol) afforded, after purification by column chromatography (silica gel, 93 : 7 hexane/ethyl acetate), 0.95 g (94%) of a white solid identified as (*R*)-**19**. $[\alpha]_D^{26}$ + 37.6 (*c* 0.027, MeOH). Elemental analysis: calcd. for $C_{14}H_{25}OSi$: C, 71.12; H, 10.23; found: C, 71.09; H, 10.26.

(−)-(*S*)-3-Ethynyl-2,4,4-trimethylcyclohex-2-en-1-ol (*S*)-20. General procedure for deprotection with K_2CO_3. To a cooled (0 °C) solution of (*S*)-**19** (0.34 g, 1.45 mmol) in MeOH (6.5 mL) was added K_2CO_3 (0.38 g, 2.91 mmol) and the mixture was stirred at 25 °C for 3.5 h. H_2O was added (4 mL) and the mixture was extracted with Et_2O (4x). The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 90:10 hexane/ethyl acetate) to afford 0.22 g (91%) of a white solid identified as (*S*)-**20**. MP : 47–49 °C (*t*-BuOMe). $[\alpha]_D^{22}$ − 77.6 (*c* 0.036, MeOH).^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 4.02 (t, $J = 4.8$ Hz, 1H, H_1), 3.13 (s, 1H, $H_{2'}$), 2.00 (s, 3H, C_2–CH_3), 1.7–1.5 (m, 2H, $2H_6$), 1.5–1.4 (m, 2H, $2H_5$), 1.15 (s, 3H, C_4–CH_3), 1.08 (s, 3H, C_4–CH_3) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 142.3 (s), 126.9 (s), 82.2 (d), 81.6 (s), 69.0 (d), 34.2 (s), 33.2 (t), 28.8 (q), 28.2 (t), 27.6 (q), 19.2 (q) ppm. MS (EI$^+$): *m/z* (%) 164 (M$^+$, 47), 149 (28), 109 (10), 108 (100), 107 (46), 91 (18), 90 (13), 79 (18), 77 (12). HRMS (EI$^+$): Calcd. for $C_{11}H_{16}O$, 164.1201; found, 164.1206. IR (NaCl): ν 3600–3100 (br, OH), 2961 (s, C–H), 2937 (s, C–H), 2866 (m, C–H), 2087 (w, C≡C) cm^{-1}.

(+)-(*R*)-3-Ethynyl-2,4,4-trimethylcyclohex-2-en-1-ol (*R*)-20. Following the general procedure for deprotection with K_2CO_3, the reaction of (*R*)-**19** (0.16 g, 0.66 mmol) in MeOH (3 mL) with K_2CO_3 (0.17 g, 1.32 mmol) afforded, after purification by column chromatography (silica gel, 90 : 10 hexane/ethyl acetate), 0.11 g (98%) of a white solid identified as (*R*)-**20**. $[\alpha]_D^{22}$ + 79.87 (c 0.03, MeOH).

(−)-(*S*)-3-Ethynyl-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*S*)-21. General procedure for protection of alcohols as acetates. To a solution of (*S*)-**20** (0.04 g, 0.24 mmol) in CH_2Cl_2 (2.5 mL) were sequentially added Et_3N (0.11 mL, 0.86 mmol), DMAP (1.5 mg, 0.12 mmol) and Ac_2O (37.4 mg, 0.37 mmol). After stirring for 4 h, a saturated aqueous $NaHCO_3$ solution (3 mL) was added. The mixture was extracted with AcOEt (3x) and the organic layers were washed with brine (3x), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 95 : 5 hexane/ethyl acetate) to afford 0.048 g (96%) of a colorless oil identified as (*S*)-**21**. $[\alpha]_D^{24}$ − 104.4 (*c* 0.022, MeOH).^{1}H-NMR (600.13 MHz, $CDCl_3$): δ 5.24 (t, $J = 4.1$ Hz, 1H, H_1), 3.16 (s, 1H, $H_{2'}$), 2.05 (s, 3H, C_2–CH_3), 1.9–1.8 (m, 1H, H_6), 1.86 (s, 3H, $COOCH_3$), 1.7–1.6 (m, 1H, H_6), 1.6–1.5 (m, 1H, H_5), 1.5–1.4 (m, 1H, H_5), 1.16 (s, 3H, C_4–CH_3), 1.08 (s, 3H, C_4–CH_3) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 170.8 (s), 138.6 (s), 129.1 (s), 82.9 (d), 81.2 (s), 70.8 (d), 34.1 (s), 33.2 (t), 28.7 (q), 27.4 (q), 25.0 (t), 21.2 (q), 19.1 (q) ppm. **IR** (NaCl): ν 3287 (m, C–H), 2961 (s, C–H), 2938 (s, C–H), 2861 (m, C–H), 2087 (w, C≡C), 1735 (s, C=O), 1236 cm^{-1}. **MS** (EI$^+$): *m/z* (%) 206 (M$^+$, 8), 147 (31), 146 (56), 132 (43), 131 (100), 129 (59), 128 (53), 116 (75), 115 (75), 108 (44), 91 (76), 77 (41), 65 (12), 63 (12). **HRMS** (EI$^+$): Calcd. for $C_{13}H_{18}O_2$, 206.1307; found, 206.1310.

(+)-(*R*)-3-Ethynyl-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*R*)-21. Following the general procedure for protection of alcohols as acetates, the reaction of (*R*)-**20** (0.86 g, 5.24 mmol) in CH_2Cl_2 (52.5 mL) with Et_3N (2.38 mL, 18.34 mmol), DMAP (32 mg, 0.26 mmol) and Ac_2O (0.74 mL, 7.86 mmol) afforded, after purification by column chromatography (silica gel, 95 : 5 hexane/ethyl acetate) 1.02 g (94%) of a colorless oil identified as (*R*)-**21**. $[\alpha]_D^{24}$ + 99.8 (*c* 0.016, MeOH).

(−)-(S)-3-[2-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-ethen-1-yl]-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*S*)-22. General procedure for the preparation of pinacol boronates. $BH_3{\cdot}SMe_2$ (0.35 mL, 3.77 mmol) was slowly added to a cooled (0 °C) solution of pinacol (0.45 g, 3.77 mmol) in CH_2Cl_2 (0.4 mL). The mixture was stirred at this temperature for 1h and at 25 °C for an additional 1h. A solution of (*S*)-**21** (0.131 g, 0.63 mmol) in CH_2Cl_2 (0.1 mL) was slowly added at 0 °C, and the reaction mixture was stirred at 25 °C for 1 h and at 50 °C for an additional 5 h. After cooling down to 25 °C, Et_2O (1 mL) and a saturated aqueous NH_4Cl (1 mL) were added. The organic layer was washed with saturated aqueous NH_4Cl (3x), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 95 : 5 hexane/ethyl acetate) to afford 0.086 g (54% based on recovered starting alkyne (*S*)-**21**) of a colorless oil identified as (*S*)-**22**. $[\alpha]_D^{24}$ − 87.4 (*c* 0.024, MeOH).^{1}H-NMR (600.13 MHz, $CDCl_3$): δ 6.94 (d, $J = 18.6$ Hz, 1H, $H_{1'}$), 5.46 (d, $J = 18.6$ Hz, 1H, $H_{2'}$), 5.21 (t, $J = 4.6$ Hz, 1H, H_1), 2.07 (s, 3H, $COOCH_3$), 1.9–1.8 (m, 1H, H_6), 1.8–1.7 (m, 1H, H_6), 1.67 (s, 3H, C_2–CH_3), 1.6–1.5 (m, 1H, H_5), 1.5–1.4 (m, 1H, H_5), 1.29 (s, 12H, $-OC(CH_3)_2C(CH_3)_2O-$), 1.07 (s, 3H, C_4–CH_3), 1.02 (s, 3H, C_4–CH_3) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 171.4 (s), 148.9 (d, 2x), 145.8 (s), 127.0 (s), 83.6 (s, 2x), 72.8 (d), 35.2 (t), 34.6 (s), 29.2 (q), 27.6 (q), 25.6 (t), 25.2 (q, 4x), 21.8 (q), 18.5 (q) ppm. MS (EI$^+$): *m/z* (%) 274 (M$^+$–OAc, 45), 259 (82), 172 (25), 159 (100), 158 (18), 146 (29), 145 (15), 133 (22), 131 (67), 101 (76), 84 (15), 83 (40). HRMS (EI$^+$): Calcd. for $C_{17}H_{28}BO_2$, 274.2219; found, 274.2230. IR (NaCl): ν 2975 (s, C–H), 2864 (m, C–H), 1736 (s, C=O), 1619, 1349, 1242, 1142 cm^{-1}.

(+)-(*R*)-3-[2-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-ethen-1-yl]-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*R*)-22. Following the general procedure for the preparation of pinacol boronates, the reaction of (*R*)-**21** (0.17 g, 0.81 mmol) with $BH_3{\cdot}SMe_2$ (0.45 mL, 4.8 mmol) and pinacol (0.57 g, 4.80 mmol) in CH_2Cl_2 (0.6 mL) afforded, after purification by column chromatography (silica gel, 95 : 5 hexane/ethyl acetate), 0.074 g (57% based on recovered starting alkyne (*R*)-**21**) of a colorless oil identified as (*R*)-**22**. $[\alpha]_D^{24}$ + 74.9 (*c* 0.012, MeOH).

(*R*)-3-[1-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-ethen-1-yl]-2,4,4-trimethylcyclohex-2-en-1-yl Acetate. ^{1}H-NMR (600.13 MHz, $CDCl_3$): δ 6.05 (d, $J = 3.9$ Hz, 1H, $H_{2'}$), 5.45 (d, $J = 3.9$ Hz, 1H, $H_{2'}$), 5.28 (t, $J = 5.3$ Hz, 1H, H_1), 2.10 (s, 3H, $COOCH_3$), 1.9–1.8 (m, 1H, H_6), 1.8–1.7 (m, 1H, H_6), 1.6–1.5 (m, 1H, H_5), 1.5–1.4 (m, 1H, H_5), 1.46 (s, 3H, C_2–CH_3), 1.27 (s, 12H, $OC(CH_3)_2$), 1.00 (s, 3H, C_4–CH_3), 0.94 (s, 3H, C_4–CH_3) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 171.6 (s, $COOCH_3$), 147.2 (s, C_3), 132.5 (t, $C_{2'}$), 132.5 (s, $C_{1'}$), 123.7 (s, C_2), 83.8 (s, $C(CH_3)_2$), 73.0 (d, C_1), 35.4 (t, C_5), 34.8 (s, C_4), 28.4 (q, C_4–CH_3), 27.6 (q, C_4–CH_3), 25.9 (t, C_6), 25.0 (q, 4x, $C(CH_3)_2$), 21.8 (q, $COOCH_3$), 17.7 (q, C_2–CH_3) ppm. MS (EI$^+$): *m/z* (%) 334 (M$^+$, 2), 292 (41), 274 (93), 259 (M$^+$–OAc, 100), 258 (25), 236 (42), 218 (28), 191 (22), 159 (35), 136 (26), 101 (60), 91 (26), 83 (33). HRMS (EI$^+$): Calcd. for $C_{19}H_{31}BO_4$, 334.2315; found, 334.2308.

(−)-(*S*)-3-(2-Iodoethen-1-yl)-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*S*)-17. General procedure for the boron/iodine exchange reaction. A solution of (−)-(S)-3-[2-(4,4,5,5-tetramethyl-[1,3,2]-dioxaborolan-2-yl)-ethen-1-yl]-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*S*)-**22** (0.32 g, 0.97 mmol) in THF (15 mL) was cooled down to −78 °C and treated with a suspension of MeONa (0.11 g, 2.12 mmol) in MeOH (1 mL). After stirring for 30 min, ICl (1.02 mL, 1 M in CH_2Cl_2, 1.02 mmol) was slowly added and the mixture was stirred at −78 °C for an additional 2 h. Et_2O (15 mL) was added, the organic layer was separated and washed with a saturated aqueous $Na_2S_2O_3$ (2x), H_2O (2x) and brine (2x), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 97 : 3 hexane/ethyl acetate) to afford 0.20 g (81%) of a colorless oil identified as (*S*)-**17**. $[\alpha]_D^{24}$ − 84.2 (*c* 0.032, MeOH).^{1}H-NMR (400.16 MHz, $CDCl_3$): δ 6.95 (d, J = 14.8 Hz, 1H, $H_{1'}$), 6.07 (d, J = 14.8 Hz, 1H, $H_{2'}$), 5.18 (t, J = 4.6 Hz, 1H, H_1), 2.07 (s, 3H, $COOCH_3$), 1.9–1.8 (m, 1H, H_6), 1.7–1.6 (m, 1H, H_6), 1.64 (s, 3H, C_2–CH_3), 1.6–1.5 (m, 1H, H_5), 1.5–1.4 (m, 1H, H_5), 1.04 (s, 3H, C_4–CH_3), 0.99 (s, 3H, C_4–CH_3) ppm. ^{13}C-NMR (100.62 MHz, C_6D_6): δ 170.3 (s), 144.9 (s), 143.6 (d), 128.7 (s), 80.6 (d), 72.0 (d), 35.0 (t), 34.5 (s), 28.8 (q), 27.3 (q), 25.9 (t), 21.2 (q), 18.5 (q) ppm. **MS** (EI$^+$): m/z (%) 275 (M$^+$–OAc, 1), 274 (24), 259 (47), 147 (53), 131 (100), 117 (40), 105 (32), 91 (54). HRMS (EI$^+$): Calcd. for $C_{11}H_{16}I$, 275.0297; found, 275.0298. **IR** (NaCl): ν 2929 (s, C–H), 2926 (s, C–H), 2864 (m, C–H), 1735 (s, C=O), 1241 cm^{-1}.

(+)-(*R*)-3-(2-Iodoethen-1-yl)-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*R*)-17. Following the general procedure for the boron/iodine exchange reaction, the reaction of (*R*)-**22** (0.38 g, 1.15 mmol) in THF (18 mL) with MeONa (0.14 g, 2.53 mmol) in MeOH (1.15 mL) and ICl (1.21 mL, 1 M in CH_2Cl_2, 1.21 mmol) afforded, after purification by column chromatography (silica gel, 96 : 4 hexane/ethyl acetate), 0.31 g (86%) of a colorless oil identified as (*R*)-**17**. $[\alpha]_D^{24}$ + 81.7 (*c* 0.022, MeOH).

(−)-(*S*)-All-*trans*-4-acetoxyretinol (*S*)-23. Following the general procedure for Stille cross-coupling, the reaction of (−)-(*S*)-3-(2-iodoethen-1-yl)-2,4,4-trimethylcyclohex-2-en-1-yl acetate (*S*)-**17** (0.063 g, 0.19 mmol) with (2*E*,4*E*,6*E*)-3,7-dimethyl-7-(tri-*n*-butylstannyl)hepta-2,4,6-trien-1-ol **16** (0.09 g, 0.20 mmol), $Pd_2(dba)_3$ (4.3 mg, 0.0047 mmol) and $AsPh_3$ (11.9 mg, 0.038 mmol) in NMP (3.5 mL) at 40 °C for 6 h, afforded, after purification by column chromatography (silica gel, 85 : 15 hexane/ethyl acetate), 0.046 g (70%) of a yellow oil identified as (*S*)-**23**. $[\alpha]_D^{24}$ − 44.4 (*c* 0.036, MeOH).^{1}H-NMR (400.13 MHz, $(CD_3)_2CO$): δ 6.65 (dd, J = 15.1, 11.2 Hz, 1H, H_{11}), 6.13 (d, J = 15.1 Hz, 1H, H_{12}), 6.3–6.2 (m, 3H, $H_7 + H_8 + H_{10}$), 5.70 (t, J = 6.4 Hz, 1H, H_{14}), 5.20 (t, J = 4.6 Hz, 1H, H_4), 4.32 (t, J = 6.4 Hz, 2H, $2H_{15}$), 2.05 (s, 3H, $COOCH_3$), 2.02 (s, 3H, C_{13}–CH_3), 1.9–1.8 (m, 1H, H_3), 1.85 (s, 3H, C_9–CH_3), 1.8–1.7 (m, 1H, H_3), 1.69 (s, 3H, C_5–CH_3), 1.7–1.6 (m, 1H, H_2), 1.5–1.4 (m, 1H, H_2), 1.07 (s, 3H, C_1–CH_3), 1.04 (s, 3H, C_1–CH_3) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 171.9 (s), 145.6 (s), 141.0 (d), 139.6 (d), 136.6 (s), 136.5 (s), 135.1 (d), 133.6 (d), 127.9 (s), 126.7 (d), 125.9 (d), 73.6 (d), 60.3 (t), 36.4 (t), 36.3 (s), 30.3 (q), 28.6 (q), 27.0 (t), 22.1 (q), 19.6 (q), 13.7 (q), 13.6 (q). MS (EI$^+$): m/z (%) 284 (M$^+$–OAc, 2), 278 (25), 262 (54), 247 (54), 245 (49), 232 (46), 207 (53), 195 (45), 179 (43), 171 (50), 165 (51), 157 (55), 143 (55), 141 (58), 133 (64), 131 (45), 129 (64), 128 (65), 119 (59), 117 (39), 115 (78), 105 (71), 95 (54), 91 (100), 77 (39). HRMS (EI$^+$): Calcd. for $C_{20}H_{28}O$, 284.2140; found, 284.2143. IR (NaCl): δ 3550–3100 (br, OH), 2956 (s, C–H), 2926 (s, C–H), 1731 (s, C=O), 1241 cm^{-1}. UV (MeOH): λ 323 nm (ε = 37000).

(+)-(*R*)-All-*trans*-4-acetoxyretinol (*R*)-23. Following the general procedure for Stille cross-coupling, the reaction of (*R*)-**17** (0.047 g, 0.14 mmol) with (2*E*,4*E*,6*E*)-3,7-dimethyl-7-(tri-*n*-butylstannyl)hepta-2,4,6-trien-1-ol **16** (0.064 g, 0.15 mmol), $Pd_2(dba)_3$ (3.2 mg, 0.0035 mmol) and $AsPh_3$ (8.7 mg, 0.028 mmol) in NMP (3.5 mL) at 40 °C for 7 h afforded, after purification by column chromatography (silica gel, 90 : 10 hexane/ethyl) 31.2 mg (65%) of a yellow oil identified as (*R*)-**23**. $[\alpha]_D^{24}$ + 42.8 (*c* 0.014, MeOH).

(−)-(*S*)-All-*trans*-4-acetoxyretinal (*S*)-24. General procedure for Dess–Martin oxidation. To a solution of (−)-(*S*)-all-*trans*-3-acetoxyretinol (*S*)-**23** (0.055 g, 0.16 mmol) in CH_2Cl_2 (7.5 mL) were sequentially added pyridine (0.145 mL) and Dess–Martin periodinane (0.09 g, 0.21 mmol). After stirring for 6 h, a saturated aqueous $NaHCO_3$ solution (3 mL) was added. The mixture was extracted with CH_2Cl_2 (3x) and the organic layers were washed with $NaHCO_3$ (3x) and $Na_2S_2O_3$ (3x), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 93 : 7 hexane/ethyl acetate) to afford 0.04 g (71%) of a mixture of (*S*)-**24** and (*S*, 13*Z*)-**24** in a 8.5 : 1 ratio.

*Data for (−)-(*S*)-all-*trans-*4-acetoxyretinal (*S*)-24.* $[\alpha]_D^{24}$ − 19.4 (*c* 0.036, MeOH).^{1}H-NMR (400.16 MHz, C_6D_6): δ 10.01 (d, J = 7.8 Hz, 1H, H_{15}), 6.80 (dd, J = 15.0, 11.5 Hz, 1H, H_{11}), 6.20 (s, 2H, $H_7 + H_8$), 6.04 (d, J = 15.1 Hz, 1H, H_{12}), 6.1–5.9 (m, 2H, $H_{10} + H_{14}$), 5.50 (t, J = 4.6 Hz, 1H, H_4), 1.83 (s, 3H, $COOCH_3$), 1.78 (s, 3H, C–CH_3), 1.73 (s, 3H, C–CH_3), 1.72 (s, 3H, C–CH_3), 1.7–1.6 (m, 2H, $2H_3$), 1.4–1.2 (m, 2H, $2H_2$), 1.05 (s, 3H, C_1–CH_3), 0.96 (s, 3H, C_1–CH_3) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 190.3 (d), 170.1 (s), 155.1 (s), 143.5 (s), 139.9 (s), 138.5 (d), 135.5 (d), 132.0 (d), 130.7 (d), 129.0 (d), 127.6 (d), 127.2 (s), 71.5 (d), 34.4 (t), 32.5 (s), 28.6 (q), 26.6 (q), 24.9 (t), 20.1 (q), 17.6 (q), 12.0 (q), 11.9 (q). MS (EI$^+$): m/z (%) 342 (M$^+$, 62), 300 (50), 283 (37), 282 (M$^+$–OAc, 100), 187 (34), 119 (51), 105 (44), 95 (45), 91 (45), 77 (24). HRMS (EI$^+$): Calcd. for $C_{22}H_{30}O_3$, 342.2195; found, 342.2193. IR (NaCl): δ 2953 (s, C–H), 2924 (s, C–H), 2854 (m, C–H), 1735 (m, C=O), 1661, 1457, 1240 cm^{-1}. UV (MeOH): λ 368 nm (ε = 23300).

*Data for (*S*)-13-*cis-*4-acetoxyretinal (*S*, 13*Z*)-24.* ^{1}H-NMR (400.13 MHz, C_6D_6): δ 10.13 (d, J = 7.3 Hz, 1H, H_{15}), 7.10 (d, J = 15.2 Hz, 1H, H_{12}), 6.71 (dd, J = 15.2, 11.5 Hz, 1H, H_{11}), 6.21 (s, 2H, $H_7 + H_8$), 6.00 (d, J = 11.5 Hz, 1H, H_{10}), 5.75 (d, J = 7.3 Hz, 1H, H_{14}), 5.49 (t, J = 4.5 Hz, 1H, H_4), 1.84 (s, 3H, $COOCH_3$), 1.77 (s, 3H, C–CH_3), 1.71 (s, 3H, C–CH_3), 1.58 (s, 3H, C–CH_3), 1.6–1.2 (m, 4H, $2H_3 + 2H_2$), 1.05 (s, 3H, C_1–CH_3), 0.96 (s, 3H, C_1–CH_3) ppm.

(+)-(*R*)-All-*trans*-4-acetoxyretinal (*R*)-24. Following the general procedure for Dess–Martin oxidation, the reaction of (*R*)-**23** (0.055 g, 0.16 mmol) with pyridine (0.143 mL) and Dess–Martin periodinane (0.088 g, 0.21 mmol) in CH_2Cl_2 (7.3 mL) afforded, after purification by column chromatography (silica gel, 96 : 4 hexane/ethyl acetate), 0.034 g (63%) of a yellow oil identified as a mixture of (*R*)-**24** and (*R*, 13*Z*)-**24** in a 6.5:1 ratio.

*Data for (*R*)-24.* $[\alpha]^{24}$ + 17.6 (*c* 0.034, MeOH)

(−)-(*S*)-All-*trans*-4-hydroxyretinal 3. Following the general procedure for deprotection with K_2CO_3, the reaction of (−)-(*S*)-all-*trans*-4-acetoxyretinal (*S*)-**24** (0.022 g, 0.064 mmol) in MeOH (0.6 mL) with K_2CO_3 (8.9 mg, 0.064 mmol) afforded, after purification by column chromatography (silica gel, 85 : 15 hexane/ethyl acetate), 0.014 g (73%) of a yellow oil identified as a mixture of **3** and **4** in a 3 : 1 ratio, which were separated by HPLC.

*Data for (−)-(*S*)-all-*trans-*4-hydroxyretinal 3.* $[\alpha]_D^{24}$ − 89.0 (*c* 0.016, MeOH).^{1}H-NMR (400.13 MHz, $(CD_3)_2CO$): δ 10.13 (d, J = 8.0 Hz, 1H, H_{15}), 7.31 (dd, J = 15.1, 11.5 Hz, 1H, H_{11}), 6.51 (d, $J = 15.1$ Hz, 1H, H_{12}), 6.37 (d, $J = 16.1$ Hz, 1H, H_7), 6.32 (d, $J = 11.5$ Hz, 1H, H_{10}), 6.24 (d, $J = 16.1$ Hz, 1H, H_8), 5.92 (d, $J = 8.0$ Hz, 1H, H_{14}), 3.94 (t, $J = 4.5$ Hz, 1H, H_4), 2.38 (s, 3H, C_{13}–CH_3), 2.07 (s, 3H, C_9–CH_3), 1.9–1.8 (m, 1H, H_3), 1.82 (s, 3H, C_5–CH_3), 1.7–1.6 (m, 2H, $H_3 + H_2$), 1.4–1.3 (m, 1H, H_2), 1.04 (s, 3H, C_1–CH_3), 1.03 (s, 3H, C_1–CH_3) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 190.3 (d), 154.2 (s), 140.2 (s), 139.7 (s), 137.9 (d), 135.1 (d), 132.1 (d), 131.9 (s), 130.1 (d), 128.9 (d), 128.5 (d), 68.9 (d), 34.7 (t), 34.2 (s), 28.5 (t), 28.2 (q), 27.1 (q), 17.9 (q), 12.0 (q), 11.9 (q). MS (EI$^+$): *m/z* (%) 300 (M$^+$, 72), 203 (15), 190 (14), 175 (15), 161 (26), 119 (43), 105 (37), 91 (37), 86 (69). HRMS (EI$^+$): Calcd. for $C_{20}H_{28}O_2$, 300.2089; found, 300.2086. IR (NaCl): δ 3500–3100 (br, OH), 2918 (s, C–H), 2850 (m, C–H), 1656 (m, C=O), 1575, 1164, 996 cm^{-1}. UV (MeOH): λ_{max} 375 nm ($\varepsilon = 31900$).

*Data for (−)-(*S*)-13-*cis*-4-hydroxyretinal* **4**. $[\alpha]_D^{24}$ − 75.8 (*c* 0.018, MeOH). ^{1}H-NMR (400.13 MHz, $(CD_3)_2CO$): δ 10.25 (d, $J = 7.8$ Hz, 1H, H_{15}), 7.52 (d, $J = 15.0$ Hz, 1H, H_{12}), 7.20 (dd, $J = 15.0, 11.3$ Hz, 1H, H_{11}), 6.38 (d, $J = 16.2$ Hz, 1H, H_7), 6.36 (d, $J = 11.3$ Hz, 1H, H_{10}), 6.25 (d, $J = 16.2$ Hz, 1H, H_8), 5.80 (d, $J = 8.0$ Hz, 1H, H_{14}), 3.94 (t, $J = 4.5$ Hz, 1H, H_4), 2.18 (d, $J = 0.9$ Hz, 3H, C_{13}–CH_3), 2.07 (s, 3H, C_9–CH_3), 1.9–1.8 (m, 1H, H_3), 1.82 (s, 3H, C_5–CH_3), 1.7–1.6 (m, 2H, $H_3 + H_2$), 1.4–1.3 (m, 1H, H_2), 1.05 (s, 3H, C_1–CH_3), 1.03 (s, 3H, C_1–CH_3) ppm. MS (EI$^+$): *m/z* (%) 300 (M$^+$, 100), 161 (25), 135 (25), 119 (38), 107 (25), 105 (33), 95 (25), 91 (33), 69 (31). HRMS (EI$^+$): Calcd. for $C_{20}H_{28}O_2$, 300.2089; found, 300.2087. IR (NaCl): ν 3580–3150 (br, OH), 2956 (s, C–H), 2927 (s, C–H), 2855 (s, C–H), 1658 (s, C=O), 1575 cm^{-1}. UV (MeOH): λ_{max} 368 nm ($\varepsilon = 28500$).

(+)-(*R*)-All-*trans*-4-hydroxyretinal 5. Following the general procedure for deprotection with K_2CO_3, the reaction of (*R*)-**24** (0.026 g, 0.08 mmol) in MeOH (0.7 mL) with K_2CO_3 (0.011 g, 0.07 mmol) afforded, after purification by column chromatography (silica gel, 85 : 15 hexane/ethyl acetate), 0.015 g (67%) of a yellow oil identified as a mixture of **5** and **6** in a 3 : 1 ratio, which were separated by HPLC.

Data for (+)-(*R*)-all-*trans*-4-hydroxyretinal **5**: $[\alpha]_D^{24}$ + 87.0 (*c* 0.018, MeOH).

Data for (+)-(*R*)-13-*cis*-4-hydroxyretinal **6**: $[\alpha]_D^{24}$ + 73.2 (*c* 0.012, MeOH).

Cloning, expression and purification of *Xenopus laevis* ADH8

ADH8 cDNA sequence from *Xenopus laevis* was obtained by nested-PCR, using two sets of degenerated primers based on the sequence of the orthologous enzyme from *Rana perezi*,[16] followed by 3′-end RACE-PCR amplification. The sequence was deposited in the GenBank data base under the accession no. AJ566764. The full-length cDNA was then generated by PCR amplification, cloned in the expression vector pGEX-4T-2 (Amersham Biosciences) and used to transform *E. coli* BL21 cells, as described previously.[17a] Expression, cell lysis and purification of *Xenopus* ADH8 as a fusion protein with glutathione-*S*-transferase (GST) were conducted as described for the *Rana perezi* enzyme.[36] Finally, the homogeneity of the purified protein was assessed by SDS-PAGE followed by Coomassie Brilliant Blue (Sigma) staining.

Enzyme kinetics

Enzymatic activities of purified *Xenopus* ADH8 were determined in a Varian Cary 400 spectrophotometer, at 25 °C. Standard activity was measured with 1.92 mM octanol (Merck) and 2.4 mM NADP$^+$ (Roche) in 0.1 M glycine, pH 10.0, at 340 nm, in 1-cm pathlength cuvettes. A specific activity of 34.02 U mg^{-1} for octanol was considered. One unit (U) of ADH activity is defined as the amount of enzyme required to transform 1 μmol of substrate or cofactor per min at 25 °C. Activities for retinal reduction kinetics were determined at 400 nm, with 0.6 mM NADPH (Roche), and 0.1 M sodium phosphate, pH 7.5, 0.02% Tween 80 (assay buffer), in 0.2-cm pathlength cuvettes. Retinoid concentration ranged from $0.1 \times K_m$ to $10 \times K_m$ and each individual rate measurement was run in duplicate. Kinetic constants were calculated using the GraFit 5.0 program (Erithacus Software Limited), and the results were expressed as the mean value ± SEM. A molecular weight of 80,000 for ADH dimer was used to calculate k_{cat} values.

Molar absorption coefficients in the assay buffer, used to calculate retinoid concentration, were $\varepsilon_{400} = 29500$ M^{-1} cm^{-1} for all-*trans*-retinal (Sigma) and $\varepsilon_{370} = 27000$ M^{-1} cm^{-1} for 13-*cis*-retinal (Sigma). Since the absorption coefficients for the ring-oxidized retinals were not known, these values were first estimated in methanol, and then calculated in the assay buffer: $\varepsilon_{375} = 30300$ M^{-1} cm^{-1} for 4-hydroxyretinal, $\varepsilon_{378} = 26600$ M^{-1} cm^{-1} for 3-hydroxyretinal, $\varepsilon_{368} = 28500$ M^{-1} cm^{-1} for 4-hydroxy-13-*cis*-retinal, and $\varepsilon_{374} = 25400$ M^{-1} cm^{-1} for 13-*cis*-3-hydroxyretinal. Substrate solutions were prepared by diluting 1 mg retinal, dissolved in 150–500 μL methanol, with the appropriate volume of the assay buffer to have a final concentration of approximately 200 μM, at 4 °C and under dim red light. The stability of the retinoid was checked spectrophotometrically.

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