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Synthesis of ring-oxidized retinoids as substrates of mouse class I alcohol dehydrogenase (ADH1)

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Ring-oxidized retinoids have been synthesized stereoselectively using the Stille cross-coupling reaction. Kinetic constants of mouse class I alcohol dehydrogenase (ADH1) with these retinoids were determined.

Introduction

Biochemical modifications of the prohormone vitamin A (retinol, **1**)[1] generate a diversity of metabolites that play key roles in fundamental physiological processes, such as vision,[2] cell differentiation, cell proliferation and apoptosis, development and immunity.[3] Many of these cellular processes are mediated by all-*trans*-retinoic acid and its 9-*cis* stereoisomer, which bind to and activate the retinoid receptors (RARs, RXRs), members of a nuclear receptor superfamily.[4] Retinoic acid is synthesized from retinol **1** by two consecutive oxidation reactions, with the intermediate formation of retinal **2**. Members of three enzymatic superfamilies have been implicated in the conversion of retinol **1** to retinal **2**: alcohol dehydrogenases (ADH) of the medium-chain dehydrogenases/reductases (MDR), retinol dehydrogenases of the short-chain dehydrogenases/reductases (SDR)[5] and several aldo–keto reductases (AKR).[6] In mammals five ADH classes (numbered 1 to 5) are known from biochemical and genomic analyses.[7] Relevant knowledge on the role of ADHs during *in vivo* retinoid metabolism has been obtained from experiments with knockout mice.[8] Although mice with null mutations against ADH1 or ADH4 show a normal development, they oxidize retinol **1** at reduced rates, indicating that the synthesis of retinoic acid is supported, in part, by ADHs. As a continuation of our work on the chemistry and biology of retinoids,[9] we have recently determined the kinetic constants of ADH1 and ADH4 from both human and mouse towards 11-*cis*-retinol/11-*cis*-retinal and the entire series of mono-*cis* analogs (7-*cis*, 9-*cis* and 13-*cis*).[10] In order to better understand the substrate specificity of human[11] and mouse ADH towards retinoids, we considered performing skeletal modifications other than double bond isomerization. In this regard, modifications in the cyclohexenyl ring can provide insights into the structure–activity relationships of the cyclic region, while keeping the polyene side chain intact. Within the biologically significant modifications, we have focused on the dehydrogenation at the C3–C4 bond and the oxidation at C4, since 3,4-didehydroretinoic acid has been reported as a RAR agonist,[12] and both 4-oxoretinol and 4-oxoretinal **6** are considered *bona fide* RAR ligands at physiological concentrations.[13] We report herein the synthesis of the 3,4-didehydroretinol/al (**3/4**) and 4-hydroxyretinol/4-oxoretinal (**5/6**) pairs, as well as the kinetic constants determined upon incubation of these retinoids with mouse ADH1. A kinetic analysis of human ADH1 and ADH4 with these compounds has recently been reported.[11] The present determination of the kinetic constants with mouse ADH1 is relevant for comparison with the human enzymes owing to the use of mice as the species for retinoid metabolism studies in knockout animals.

Straightforward syntheses of these ring-oxidized retinoids have been traditionally based on the direct oxidation of retinal **2** or other commercial analogs (*e.g.* ethyl retinoate) at C4 followed by either dehydration or reduction/oxidation to retinals.[14] In addition, synthesis of 3,4-didehydroretinal **4** has been achieved through double bond formation using either a Wittig/Horner–Wadsworth–Emmons reaction (for C11=C12 formation) or an ester dienolate aldol reaction followed by dehydration.[15] These sequences often suffer from low yields (the direct oxidation processes) and/or require separation of olefin isomers.

The last twenty years have witnessed an explosion of interest regarding the application of metal-catalyzed cross-coupling reactions for C–C bond formation involving unsaturated species.[16] In particular, palladium-catalyzed cross-coupling reactions have been found in general to be chemo- and stereoselective and to take place with retention of the configuration of the coupling partners. We have carried out a comprehensive study on the scope and limitations of the Stille cross-coupling reaction, with particular emphasis on its application to the synthesis of retinoids,[17] and highlighted the advantages and disadvantages of each formal single C–C bond disconnection. The synthetic approach to targets **3**–**6** benefits from previous knowledge, since it was conceived that C6–C7 (**3/4**) and C8–C9 (**5/6**) bond disconnections, two of the most efficient in this regard, could lead convergently to the desired retinoids (Fig. 1).

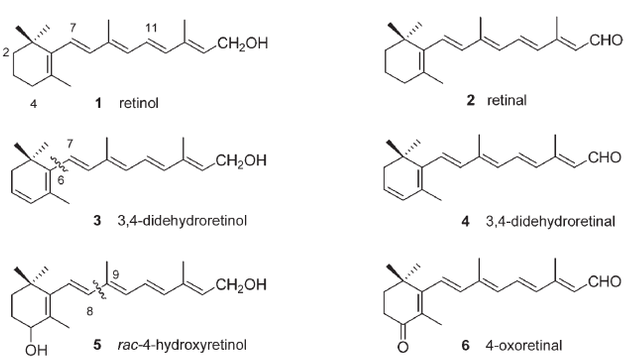

Fig. 1 Ring-oxidized vitamin A analogues and the corresponding carbonyl derivatives.

Results and discussion

Synthesis of 3,4-didehydroretinoids 3/4

The fragments required for the C6–C7 coupling to convergently afford **3** and **4** are cyclohexadienyl triflate **8** and previously described[17] tetraenylstannane **9** (Scheme 1). Dienyl triflate **8** was prepared by deprotonation of known[18] 2,6,6-trimethylcyclohex-2-en-1-one **7** with LDA, and trapping of the lithium dienolate with *N*-phenyltriflimide. The Stille cross-coupling[19] using Farina and Krishnan's conditions[20] required heating to 70 °C for 4 h, due to the low reactivity of the hindered alkenyl triflates. The

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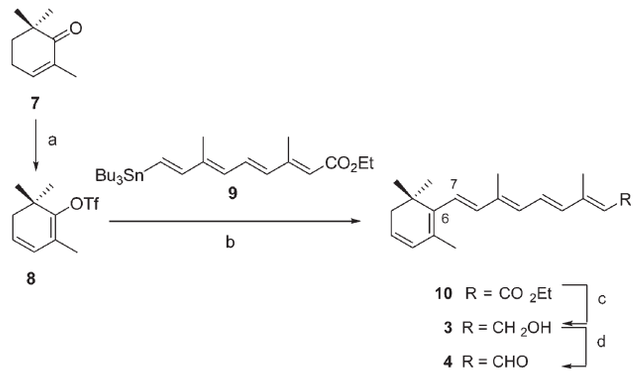
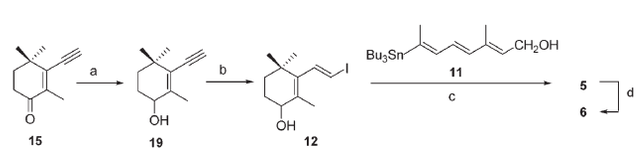
pentaenyl ester **10** was obtained in high yield after purification (88%), and no significant quantities of the homodimer derived from stannane **9** were isolated, contrary to our findings in the synthesis of ethyl retinoate using the alkenyl triflate analog.[17] Ethyl 3,4-didehydroretinoate **10** was uneventfully reduced to 3,4-didehydroretinol **3** with DIBAL and the latter oxidized to 3,4-didehydroretinal **4** using MnO_2 in a 50% combined yield.[14]



Scheme 1 *Reagents and conditions*: (a) LDA, THF, 0 °C; then Tf_2NPh, THF–HMPA, −78 °C, 4.5 h (60%); (b) tetraenylstannane **9**, $Pd_2(dba)_3$, $AsPh_3$, NMP, 70 °C, 4 h (88%); (c) DIBAL, THF, −78 °C; (d) MnO_2, CH_2Cl_2, 25 °C, 75 min (50% combined yield).

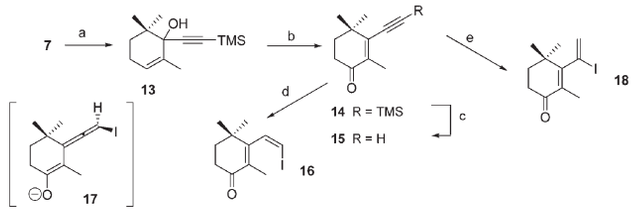
Synthesis of 4-hydroxyretinol 5 and 4-oxoretinal 6

The C8–C9 disconnection envisaged for the preparation of **5/6** required the condensation of known[17] trienylstannane **11** and dienyliodide **12**, both having stereochemically homogeneous *trans* geometries (Scheme 2). The most straightforward synthesis of alkenyliodides involves tin–iodine exchange of alkenylstannanes, themselves obtained from the terminal alkynes by a variety of methods.[21] Ketone **7** was therefore proposed to serve again as the ultimate precursor of the electrophilic component of the Stille cross-coupling (iodide **12** in this case), following alkynyllithium addition and PCC-induced rearrangement of the tertiary propargyl alcohol.[22] Indeed, addition of the lithium anion of TMS-acetylene (*n*-BuLi, THF, −78 °C to −30 °C, 4 h) to ketone **7** afforded propargylic alcohol **13** in 70% yield. Allylic transposition of the intermediate chromate ester, obtained upon treatment of **13** with PCC in silica gel and subsequent oxidation of the secondary alcohol, provided enynone **14** in 67% yield. Deprotection with TBAF afforded, in 70% yield, an unstable alkyne **15**; the precursor of the alkenyltin fragment. Upon stannylcupration[23] of **15** using the mixed cyanocuprate $(Bu_3Sn)(Bu)CuLi/LiCN$ in THF at −78 °C, followed by *in situ* trapping of the alkenylcopper species with iodine, alkenyliodide **16** with *Z* geometry (J = 8.6 Hz for the vinyl hydrogens in the ^{1}H-NMR spectrum) was obtained in 51% overall yield.



Scheme 2 *Reagents and conditions*: (a) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, Et_2O, 0 °C, 1 h (93%); (b) *n*-Bu_3SnH, AIBN, toluene, 80 °C, 2.5 h; then I_2, CH_2Cl_2, 25 °C, 10 min (71%); (c) trienylstannane **11**, $Pd_2(dba)_3$, $AsPh_3$, NMP, 30 °C, 3 h (67%); (d) Dess–Martin periodinane, CH_2Cl_2, pyridine, 30 °C, 1.5 h (76%).

Instead of a net *syn*-stannylcupration, the stereochemical outcome of the *cis*-iodide **16** was interpreted as the result of an extended[23] 1,6-addition of the cuprate to the enynone **15**, to provide a tinallene enolate, followed by tin–iodine exchange and protonation on work-up. Protonation is the stereochemically determining step, and occurs on the least hindered face of iodoallene enolate **17** (*syn* to the H, Scheme 3). On the other hand, hydrostannation of alkyne **15**, catalyzed by palladium,[24] also took place regioselectively but provided the internal



Scheme 3 *Reagents and conditions*: (a) *n*-BuLi, TMS–acetylene, THF, −78 to −30 °C, 4 h (70%); (b) PCC, silica gel, CH_2Cl_2, 25 °C (67%); (c) TBAF, THF, 0 °C (70%); (d) CuCN, *n*-BuLi, *n*-Bu_3SnH, THF, −78 to −30 °C, 4 h; then I_2, CH_2Cl_2, 25 °C, 30 min (51%); (e) $PdCl_2(PPh_3)_2$, *n*-Bu_3SnH, THF, 25 °C, 10 min; then I_2, CH_2Cl_2, 25 °C, 1.5 h (73%).

stannane, as assessed by the presence of signals in the ^{1}H-NMR spectrum of iodide **18** corresponding to the terminal vinyl hydrogens (J = 1.6 Hz).

An alternative procedure for the required transformation is the addition of tin radicals to alkynes,[25] which was most conveniently carried out on alcohol **19**, itself obtained from ketone **15** by treatment with Luche' reagent ($NaBH_4$–$CeCl_3$).[26] Addition of Bu_3SnH and AIBN to a solution of **19** in toluene at 80 °C and *in situ* exchange with iodine, afforded the stereochemically homogeneous dienyliodide **12** having *E* geometry (J = 14.8 Hz for the vinyl hydrogens in the ^{1}H-NMR spectrum).

Under Farina conditions, coupling of **11** and **12** required 3 h at 30 °C and afforded 4-hydroxyretinol **5** in 67% yield. Oxidation of diol **5** was carried out with the Dess–Martin reagent in CH_2Cl_2–pyridine to minimize isomerization at the terminal double bond. Despite the precautions, a 6:1 mixture of the *trans*:13-*cis* isomers of **6** was obtained in 76% yield, which was separated by HPLC. Spectroscopic data for **5** and **6** matched those described previously.[15]

Kinetics of mouse ADH1 with retinoids

Comparison of the kinetic data of the ring oxidized retinoids **3**–**6** with those of the parent system **1**, **2** (Table 1) reveals interesting trends. First, all compounds are good substrates for mouse ADH1, judging from the low and similar K_m values measured in all cases. Second, whereas the catalytic efficiency (k_{cat}/K_m) of the enzyme with 3,4-didehydroretinol **3** is twice that shown with retinol **1**, 4-hydroxyretinol **5** is the best substrate for the enzyme, showing a catalytic efficiency about 30 times greater than that of **1**. Third, the two directions of the reaction have distinct k_{cat} values and catalytic efficiency, where the oxidation is faster than the reduction. This seems to be a feature specific to retinoids,[10] since with other substrates, ADHs are generally more active in the reduction than the oxidation direction at neutral pH.[27] The extra unsaturation at the C3–C4 bond results in enzyme specificity and efficiency similar to the parent retinoid, which may support a role of the enzyme in the biosynthesis of 3,4-didehydroretinoic acid, a retinoid receptor modulator.[12] The catalytic activity of the enzyme in the presence of the oxidized substrates **5** and **6** was considerably greater as compared to that of the parent compounds **1** and **2**, particularly the oxidation process to provide 4-oxoretinal **6**, another ligand for retinoid nuclear receptors.[13] This also supports the involvement of the enzyme in the redox metabolism of the C4-oxidized retinoids. The general kinetic pattern of mouse ADH1 with ring-oxidized retinoids is similar to that obtained with the human ADH1B2 isozyme, while human ADH1B1 exhibits lower k_{cat} values.[11] The present results are consistent with the conclusion reached following ADH1 knockout studies in mice, for a significant contribution of this enzyme in retinoid metabolism.[8]

In summary, the Stille reaction has been used in the stereocontrolled synthesis of ring-modified retinoids, the C3–C4 dehydro- and the C4-oxygenated series, by coupling the fragments **8**–**9** and **12**–**11**, respectively. As a bonus of the synthetic effort, alternative procedures can yield regio- and stereoselectively the dienyliodides **16** and **18** (or the corresponding dienylstannanes)

Table 1 Kinetic constants of mouse ADH1 with retinoids **1**–**6**[a]

	K_m/μM	k_{cat}/min^{-1}	(k_{cat} / K_m)/mM^{-1} min^{-1}
1	31 ± 5	55 ± 3	1775 ± 300
3	32 ± 4	108 ± 4	3375 ± 450
5	2.6 ± 0.2	138 ± 3	53080 ± 4245
2	9.3 ± 1.1	19 ± 1	2045 ± 265
4	16 ± 4	23 ± 2	1440 ± 380
6	6.8 ± 1.4	119 ± 11	17500 ± 3950

[a] Activities were determined in 0.1 M sodium phosphate, pH 7.5, 0.02% Tween 80, using 0.3 mM NAD^+ for retinol oxidation, or 1 mM NADH for retinal reduction, at 25 °C.

that can be valuable fragments for other cross-coupling processes. Whereas the activity of ADH1 with 3,4-dehydroretinoids shows efficiency and specificity similar to the parent systems, greater efficiency was obtained for the C4-oxidized retinoids.

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. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel (60 F-254) plates (0.25 mm) precoated with a fluorescent indicator. Column chromatography was performed using Merck silica gel 60 particle size (0.040–0.063 μm). Proton (^{1}H) and carbon (^{13}C) magnetic resonance spectra (NMR) were recorded on a Bruker AMX-400 [400 MHz (100 MHz for ^{13}C)] Fourier transform spectrometer and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane (TMS, 0 ppm) or chloroform ($CHCl_3$, 7.24 ppm for ^{1}H and 77.00 ppm for ^{13}C) as an internal reference. ^{13}C multiplicities (s, singlet; d, doublet; t, triplet; q, quartet) were assigned with the aid of the DEPT pulse sequence. Infrared spectra (IR) were obtained on a MIDAC Prospect FT-IR spectrophotometer. Absorptions are recorded in wavenumbers (cm^{-1}). UV spectra were determined using a HP5989A spectrophotometer using MeOH as the solvent. Absorption maxima are reported in nm. Low-resolution mass spectra were obtained using a HP59970 instrument operating at 70 eV. High-resolution mass spectra were recorded using a VG Autospec M instrument.

1-(Trifluoromethanesulfonyl)oxy-2,6,6-trimethylcyclohexa-1,3-diene (8)

A cooled (0 °C) solution of diisopropylamine (0.116 mL, 0.825 mmol) in THF (1 mL) was treated with *n*BuLi (0.527 mL, 1.51 M in hexane, 0.796 mmol) and stirred for 30 min. The mixture was cooled down to −78 °C and a solution of 2,6,6-trimethylcyclohex-2-en-1-one **7** (100 mg, 0.723 mmol) in THF (1 mL) was added. After stirring for 1 h, HMPA (0.75 mL, 4.34 mmol) and a solution of *N*-phenyltriflimide (0.276 g, 0.774 mmol) in THF (1 mL) were added. The resulting mixture was stirred for 3 h at −78 °C and then an aqueous solution of NaCl (10 mL) was added. The reaction was extracted with *tert*-butyl methyl ether (TBME) (3×) and the combined organic layers were washed with 10% citric acid (3×) and aqueous $NaHCO_3$ (3×). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 98 : 2 hexane : EtOAc) to afford 117 mg (60%) of **8** as a pale yellow oil. ^{1}H-NMR (400.16 MHz, $CDCl_3$): δ 5.8–5.7 (m, 2H, H_3 + H_4), 2.27 (d, J = 2.6 Hz, 2H, $2H_5$), 1.81 (s, 3H, C_2–CH_3), 1.11 (s, 6H, C_6–$2CH_3$) ppm. ^{13}C-NMR (100.63 MHz, $CDCl_3$): δ 150.3 (s), 127.1 (d), 126.0 (d), 122.8 (s), 118.6 (s, $^1J_{C-F}$ = 320 Hz), 41.7 (t), 34.9 (s), 24.3 (q, 2×), 15.9 (q) ppm. FTIR (NaCl): ν 2969 (m, C–H), 1406 (s, S=O) cm^{-1}. MS (EI$^+$): *m*/*z* (%) 270 (M$^+$, 40), 221 (10), 219 (16), 205 (23), 203 (35), 193 (63), 191 (100), 155 (49), 137 (71), 121 (31), 119 (46), 109 (87), 105 (35), 99 (41), 93 (22), 91 (32), 81 (17). HRMS (EI$^+$): calcd for $C_{10}H_{13}F_3O_3S$, 270.0538; found, 270.0536.

Ethyl 3,4-didehydroretinoate (10)

A solution of 1-(trifluoromethanesulfonyl)oxy-2,6,6-trimethylcyclohexa-1,3-diene **8** (261 mg, 0.966 mmol) in NMP (2 mL) was added to a solution of $Pd_2(dba)_3$ (22 mg, 0.024 mmol) and $AsPh_3$ (61 mg, 0.199 mmol) in NMP (10 mL). After stirring for 10 min, a solution of ethyl (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(tri-*n*-butylstannyl)nona-2,4,6,8-tetraenoate **9** in NMP (2 mL) was added and the mixture was stirred for 4 h at 70 °C. An aqueous solution of KF (2 mL) was added and the mixture was stirred for 30 min and then extracted with TBME (3×). The combined organic layers were washed with H_2O (3×), dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 97 : 3 hexane : EtOAc) to afford 277 mg (88%) of **10** as a yellow oil. ^{1}H-NMR (400.16 MHz, C_6D_6): δ 6.94 (dd, J = 15.0, 11.5 Hz, 1H, H_{11}), 6.43 (d, J = 16.1 Hz, 1H, H_7), 6.35 (d, J = 16.1 Hz, 1H, H_8), 6.22 (d, J = 15.0 Hz, 1H, H_{12}), 6.11 (d, J = 11.5 Hz, 1H, H_{10}), 6.01 (s, 1H, H_{14}), 5.95 (d, J = 9.4 Hz, 1H, H_4), 5.76 (dt, J = 9.4, 4.4 Hz, 1H, H_3), 4.13 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 2.51 (s, 3H, CH_3), 1.96 (s, 3H, CH_3), 1.93 (d, J = 4.4 Hz, 2H, $2H_2$), 1.83 (s, 3H, CH_3), 1.16 (s, 6H, C_1–$2CH_3$), 1.11 (t, J = 7.1 Hz, 3 H, OCH_2CH_3) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 168.2 (s, C=O), 154.4 (s), 141.2 (s), 140.3 (s), 138.8 (d), 137.4 (d), 136.8 (s), 133.0 (d), 132.3 (d), 131.7 (d), 129.1 (d), 126.9 (d), 120.6 (d), 61.0 (t), 41.4 (t), 35.6 (s), 27.9 (q, 2×), 21.5 (q), 15.7 (q), 14.8 (q), 13.8 (q) ppm. FTIR (NaCl): ν 3033 (m, C–H), 2956 (m, C–H), 1710 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} 301, 373 (ε = 13 300) nm. MS (EI$^+$): *m*/*z* (%) 326 (M$^+$, 100), 265 (12), 253 (16), 237 (13), 197 (17), 175 (23), 135 (18), 131 (17), 119 (26), 91 (14). HRMS (EI$^+$): calcd for $C_{22}H_{30}O_2$, 326.2246; found, 326.2244.

3,4-Didehydroretinal (4)

DIBAL (3.85 mL, 1 M in hexanes, 3.85 mmol) was added to a solution of ethyl 3,4-didehydroretinoate **10** (315 mg, 0.97 mmol) in THF (10 mL) at −78 °C, and the resulting suspension was stirred for 2 h. After careful addition of H_2O (5 mL), the mixture was extracted with TBME (3×). The aqueous layer was saturated with NaCl and then extracted with TBME (3×). The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (activated alumina grade I, 90 : 10 hexane : EtOAc) to afford 274 mg (99%) of a yellow oil that was immediately used in the next step. MnO_2 (1.512 g, 17.39 mmol) and anhydrous Na_2CO_3 (0.512 g, 4.831 mmol) were added to a solution of this compound (274 mg, 0.97 mmol) in CH_2Cl_2 (15 mL), and the suspension was stirred for 1.5 h. The mixture was filtered through Celite® and the solvents were removed. The residue was purified by column chromatography (deactivated alumina, 95 : 5 hexane : EtOAc) to afford 135 mg (50%) of **4** as an orange oil. ^{1}H-NMR (400.16 MHz, $CDCl_3$): δ 10.06 (d, J = 7.8 Hz, 1H, H_{15}), 6.89 (dd, J = 15.0, 11.5 Hz, 1H, H_{11}), 6.44 (d, J = 16.2 Hz, 1H, H_7), 6.39 (d, J = 16.2 Hz, 1H, H_8), 6.09 (d, J = 15.0 Hz, 1H, H_{12}), 6.07 (d, J = 11.5 Hz, 1H, H_{10}), 6.03 (d, J = 7.8 Hz, 1H, H_{14}), 5.94 (d, J = 9.5 Hz, 1H, H_4), 5.8–5.7 (m, 1H, H_3), 2.10 (d, J = 2.8 Hz, 2H, $2H_2$), 1.95 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 1.80 (s, 3H, CH_3), 1.17 (s, 6H, C_1–$2CH_3$) ppm. ^{13}C-NMR (400.16 MHz, C_6D_6): δ 10.08 (d, J = 8.1 Hz, 1H, H_{15}), 7.11 (dd, J = 15.0, 11.6 Hz, 1H, H_{11}), 6.36 (d, J = 15.0 Hz, 1H, H_{12}), 6.3–6.2 (m, 2H, H_7 + H_8), 6.20 (d, J = 11.6 Hz, 1H, H_{10}), 5.95 (d, J = 8.1 Hz, 1H, H_{14}), 5.84 (d, J = 9.6 Hz, 1H, H_4), 5.8–5.7 (m, 1H, H_3), 2.33 (s, 3H, CH_3), 2.1–2.0 (m, 2H, $2H_2$), 1.97 (s, 3H, CH_3), 1.86 (s, 3H, CH_3), 1.02 (s, 6H, C_1–$2CH_3$) ppm. FTIR (NaCl): ν 2923 (m, C–H), 2857 (m, C–H), 1659 (s, C=O), 1573 (s) cm^{-1}. UV (MeOH): λ_{max} 313, 388 (ε = 18 200) nm. MS (EI$^+$): *m*/*z* (%) 282 (M$^+$, 100), 267 (12), 173 (16), 171 (39), 159 (16), 157 (22), 145 (16), 143 (20), 133

(19), 131 (16), 119 (32), 105 (21), 91 (23). HRMS (EI^+): calcd for $C_{20}H_{26}O_2$, 282.1984; found, 282.1979.

rac-2,6,6-Trimethyl-1-(trimethylsilylethynyl)cyclohex-2-en-1-ol (13)

A cooled (0 °C) solution of trimethylsilylacetylene (1.38 mL, 9.77 mmol) in THF (10 mL) was treated with *n*BuLi (7.02 mL, 1.34 M in hexane, 9.41 mmol) and stirred for 30 min. The mixture was cooled down to −78 °C and a solution of 2,6,6-trimethylcyclohex-2-en-1-one **7** (1.0 g, 7.23 mmol) in THF (20 mL) was added. The resulting mixture was allowed to warm up to 0 °C and H_2O (5 mL) was added. An aqueous solution of 10% AcOH was added until pH = 7. The reaction was extracted with TBME (3×). The aqueous layer was saturated with brine and then extracted with TBME (3×). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 95 : 5 hexane : EtOAc) to afford 1.19 g (70%) of **13** as a white solid (mp 37–39 °C, TBME). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 5.5–5.4 (m, 1H, H_3), 2.1–2.0 (m, 2H, $2H_4$), 1.85 (s, 3H, C_2–CH_3), 1.6–1.5 (m, 1H, H_5), 1.4–1.5 (m, 1H, H_5), 1.03 (s, 6H, C_6–$2CH_3$), 0.14 (s, 9H, Si–$(CH_3)_3$) ppm. ^{13}C-NMR (100.61 MHz, $CDCl_3$): δ 134.3 (s), 124.1 (d), 107.1 (s), 89.7 (s), 74.5 (s), 37.0 (s), 31.4 (t), 24.0 (q), 22.9 (q), 22.6 (t), 19.4 (q), −0.2 (q, Si–$(CH_3)_3$) ppm. FTIR (NaCl): ν 3600–3300 (br, OH), 2964 (s, C–H), 2923 (s, C–H), 2164 (m, C≡C), 1250 (s) cm^{-1}. MS (FAB^+): *m*/*z* (%) 236 (M^+, 8), 235 (23), 226 (12), 221 (16), 220 (24), 219 (100), 217 (13), 167 (19), 165 (20). HRMS (FAB^+): calcd for $C_{14}H_{25}OSi$ $(M + 1)^+$, 237.1675; found, 237.1670.

2,4,4-Trimethyl-3-(trimethylsilylethynyl)cyclohex-2-en-1-one (14)

A solution of 2,6,6-trimethyl-1-(trimethylsilylethynyl)cyclohex-2-en-1-ol **13** (111.5 mg, 0.472 mmol) in CH_2Cl_2 (1 mL) was added to a suspension of PCC (0.218 g, 0.99 mmol) and silica gel (0.218 g, 3.63 mmol) in CH_2Cl_2 (2 mL). The resulting suspension was stirred for 4 h. The mixture was filtered through Celite® and the solvents were removed. The residue was purified by column chromatography (silica gel, 95 : 5 hexane : EtOAc) to afford 74.6 mg (67%) of **14** as a white solid (mp 38–42 °C, TBME). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 2.48 (t, J = 6.7 Hz, 2H, $2H_6$), 1.95 (s, 3H, C_2–CH_3), 1.85 (t, J = 6.7 Hz, 2H, $2H_5$), 1.24 (s, 6H, C_4–$2CH_3$), 0.25 (s, 9H, Si–$(CH_3)_3$) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$): δ 198.3 (s), 150.0 (s), 138.2 (s), 111.2 (s), 101.8 (s), 36.3 (t), 35.2 (s), 34.2 (t), 27.7 (q, 2×), 14.5 (q), −0.3 (q, Si–$(CH_3)_3$) ppm. FTIR (NaCl): ν 2962 (m, C–H), 2135 (w, C≡C), 1673 (s, C=O) cm^{-1}. MS (FAB^+): *m*/*z* (%) 235 ($M + 1^+$, 100), 234 (M^+, 15), 223 (19), 222 (17), 221 (71), 219 (22), 208 (18), 207 (81), 193 (29), 191 (23), 155 (17), 154 (47). HRMS (FAB^+): calcd for $C_{14}H_{23}OSi$ $(M + 1)^+$, 235.1518; found, 235.1513.

3-Ethynyl-2,4,4-trimethylcyclohex-2-en-1-one 15

A cooled (0 °C) solution of 2,4,4-trimethyl-3-(trimethylsilylethynyl)cyclohex-2-en-1-one **14** (95.7 mg, 0.402 mmol) in THF (1.5 mL) was treated with nBu$_4$NF (0.5 mL, 1 M in THF, 0.5 mmol) and stirred for 15 min. The mixture was diluted with TBME (4 mL) and washed with aqueous $NaHCO_3$ (1 × 5 mL). The aqueous layer was extracted with TBME (3×), the combined organic layers were washed with brine (3×), dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 95 : 5 hexane : EtOAc) to afford 46.5 mg (70%) of a white solid (mp 77–79 °C, TBME). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 3.73 (s, 1H, $H_{2'}$), 2.48 (t, J = 7.1 Hz, 2H, $2H_6$), 1.94 (s, 3H, C_2–CH_3), 1.85 (t, J = 7.1 Hz, 2H, $2H_5$), 1.24 (s, 6H, C_4–$2CH_3$) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 198.3 (s), 146.2 (s), 140.2 (s), 94.9 (d), 82.1 (s), 37.9 (t), 36.8 (s), 35.6 (t), 28.8 (q, 2×), 15.6 (q) ppm. FTIR (NaCl): ν 3200 (s, C≡H), 2969 (m, C–H), 2082 (m, C≡C), 1654 (s, C=O) cm^{-1}. MS (FAB^+): *m*/*z* (%) 163 ($M + 1^+$, 7), 161 (3), 156 (4), 155 (26), 154 (100). HRMS (FAB^+): calcd for $C_{11}H_{15}O$ $(M + 1)^+$, 163.1123; found, 163.1125.

(*Z*)-3-(2-Iodoethen-1-yl)-2,4,4-trimethylcyclohex-2-en-1-one (16)

*n*BuLi (0.2 mL, 0.67 M in hexane, 0.13 mmol) was added dropwise to a suspension of CuCN (5.5 mg, 0.062 mmol) in THF (0.5 mL), at −78 °C. After stirring for 10 min, Bu_3SnH (0.034 mL, 0.13 mmol) and a solution of 3-ethynyl-2,4,4-trimethylcyclohex-2-en-1-one **15** (10 mg, 0.062 mmol) in THF (1 mL) were added and the mixture was stirred for 3 h at −78 °C and for 1 h at −30 °C. After addition of NH_4Cl : NH_3 1 : 5 (1 mL), it was extracted with TBME (3×). The organic layers were dried (Na_2SO_4) and the solvent was evaporated. The residue was disolved in CH_2Cl_2 (3 mL) and a solution of I_2 (61.2 mg, 0.24 mmol) in CH_2Cl_2 (5 mL) was added over 25 min. An aqueous $Na_2S_2O_3$ solution (2 mL) was added, and the organic layer was washed with an aqueous $Na_2S_2O_3$ solution (3×), dried and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 95 : 5 hexane : EtOAc) to afford 9.1 mg (51%) of **16** a colorless oil. ^{1}H-NMR (400.13 MHz, $(CD_3)_2CO$): δ 7.27 (d, J = 8. 6 Hz, 1H, $H_{1'}$), 6.86 (d, J = 8.6 Hz, 1H, $H_{2'}$), 2.46 (t, J = 6.8 Hz, 2H, $2H_6$), 1.88 (t, J = 6.8 Hz, 2H, $2H_5$), 1.71 (s, 3H, CH_3), 1.17 (s, 6H, C_4–$2CH_3$ ppm. MS (EI^+): *m*/*z* (%) 290 (M^+,100), 153 (49), 234 (36), 233 (33), 199 (26), 163 (95), 155 (63), 135 (22), 121 (97), 120 (40), 119 (46), 105 (66), 103 (52), 91 (97), 79 (52), 77 (86), 75 (30), 69 (27), 67 (22), 65 (48). HRMS (EI^+): calcd for $C_{11}H_{15}IO$, 290.0168; found, 290.0179.

3-(1-Iodoethen-1-yl)-2,4,4-trimethylcyclohex-2-en-1-one (18)

Bu_3SnH (0.029 mL, 0.11 mmol) was added to a solution of 3-ethynyl-2,4,4-trimethylcyclohex-2-en-1-one **15** (17 mg, 0.105 mmol) and $PdCl_2(PPh_3)_2$ (1.5 mg, 0.0021 mmol) in THF (1.5 mL). After stirring for 10 min, CH_2Cl_2 (1 mL) and a solution of I_2 (40 mg, 0.157 mmol) in CH_2Cl_2 (4 mL) were added. The reaction mixture was stirred for 1 h and an aqueous $Na_2S_2O_3$ solution (2 mL) was added. The organic layer was washed with an aqueous $Na_2S_2O_3$ solution (3×), dried and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 95 : 5 hexane : EtOAc) to afford 22.3 mg (73%) of a yellow solid (mp 72–75 °C, TBME). ^{1}H-NMR (400.13 MHz, CD_2Cl_2): δ 6.09 (d, J = 1.6 Hz, 1H, $H_{2'}$), 5.91 (d, J = 1.6 Hz, 1H, $H_{2'}$), 2.6–2.4 (m, 2H, $2H_6$), 1.9–1.8 (m, 2H, $2H_5$), 1.70 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.20 (s, 3H, CH_3) ppm. ^{13}C-NMR (100.62 MHz, $(CD_3)_2CO$): δ 198.9 (s), 162.7 (s), 131.3 (s), 128.7 (t), 100.2 (s), 38.7 (t), 36.2 (s), 34.6 (t), 29.2 (q), 26.6 (q), 13.2 (q) ppm. FTIR (NaCl): ν 2962 (m, C–H), 2927 (m, C–H), 1667 (s, C=O) cm^{-1}. MS (EI^+): *m*/*z* (%) 290 (M^+, 10), 164 (12), 163 (100), 148 (12), 135 (25), 121 (11), 107 (13), 105 (11), 93 (11), 91 (19), 79 (10), 65 (13). HRMS (EI^+): calcd for $C_{11}H_{15}IO$, 290.0168; found, 290.0176.

rac-3-Ethynyl-2,4,4-trimethylcyclohex-2-en-1-ol (19)

$NaBH_4$ (7.6 mg, 0.201 mmol) was added carefully to a cooled solution (0 °C) of 3-ethynyl-2,4,4-trimethylcyclohex-2-en-1-one **15** (32.6 mg, 0.201 mmol) and $CeCl_3 \cdot 7H_2O$ (75 mg, 0.249 mmol) in absolute EtOH (2 mL). After stirring for 1 h, an aqueous NH_4Cl solution (2 mL) was added and the mixture was extracted with TBME (3×). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 80 : 20 hexane : EtOAc) to afford 30.6 mg (93%) of a white solid (mp 47–49 °C, TBME). ^{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, CH_3), 1.08 (s, 3H, 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. FTIR (NaCl): ν 3600–3100 (br, OH), 2961 (s, C–H), 2937 (s, C–H), 2866 (m, C–H), 2087

(w, C=C) cm^{-1}. 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.

rac-(*E*)-3-(2-Iodoethen-1-yl)-2,4,4-trimethylcyclohex-2-en-1-ol (12)

Bu_3SnH (0.018 mL, 0.067 mmol) was added to a solution of 3-ethynyl-2,4,4-trimethylcyclohex-2-en-1-ol **19** (9.1 mg, 0.055 mmol) and AIBN (0.9 mg, 0.0055 mmol) in toluene (1 mL), and the mixture was stirred for 2.5 h at 80 °C. The solvent was evaporated and the residue was used in the next step without further purification. A solution of I_2 (21 mg, 0.083 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a solution of the compound obtained before in CH_2Cl_2 (1 mL), and the mixture was stirred for 10 min. After washing with aqueous $Na_2S_2O_3$ (4×), the organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 80 : 20 hexane : EtOAc) to afford 11.5 mg (71%) of **12** as a colorless oil. ^{1}H-NMR (400.13 MHz, C_6D_6): δ 6.94 (d, J = 14.8 Hz, 1H, $H_{1'}$), 5.88 (d, J = 14.8 Hz, 1H, $H_{2'}$), 3.65 (t, J = 4.5 Hz, 1H, H_1), 1.67 (s, 3H, CH_3), 1.6–1.5 (m, 2H, $2H_6$), 1.2–1.1 (m, 2H, $2H_5$), 0.87 (s, 3H, CH_3), 0.81 (s, 3H, CH_3) ppm. ^{13}C-NMR (100.62 MHz, (C_6D_6): δ 143.7 (d), 132.3 (s), 121.9 (s), 79.7 (d), 69.6 (d), 34.6 (t), 34.2 (s), 28.7 (t), 28.6 (q), 27.3 (q), 18.3 (q) ppm. FTIR (NaCl): ν 3600–3100 (br, OH), 2959 (s, C–H), 2933 (s, C–H), 2932 (m, C–H), 2864 (m, C–H) cm^{-1}. MS (EI^+): *m/z* (%) 292 (29), 277 (37), 236 (100), 165 (10), 109 (45), 107 (15), 91 (16), 79 (10). HRMS (EI^+): calcd for $C_{11}H_{17}IO$, 292.0324; found, 292.0337.

rac-4-Hydroxyretinol (5)

A solution of (*E*)-3-(2-iodoethen-1-yl)-2,4,4-trimethylcyclohex-2-en-1-ol **12** (11.8 mg, 0.04 mmol) in NMP (2.25 mL) was added to a solution of $Pd_2(dba)_3$ (0.9 mg, 0.001 mmol) and $AsPh_3$ (2.6 mg, 0.0081 mmol) in NMP (4.5 mL). After stirring for 10 min, a solution of (2*E*,4*E*,6*E*)-3,7-dimethyl-7-(tri-*n*-butylstannyl)hepta-2,4,6-trienol **11** (19.6 mg, 0.044 mmol) in NMP (2.25 mL) was added and the mixture was stirred for 3 h at 30 °C. An aqueous KF solution (2 mL) was added and the mixture was stirred for 10 min and then extracted with TBME (3×). The combined organic layers were washed H_2O (3×), dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 47 : 50 : 33 hexane : EtOAc : Et_3N) to afford 8 mg (67%) of **5** as a yellow oil. ^{1}H-NMR (400.13 MHz, C_6D_6): δ 6.62 (dd, J = 15.1, 11.2 Hz, 1H, H_{11}), 6.31 (d, J = 15.2 Hz, 1H, H_{12}), 6.15 (m, 3H, H_7 + H_8 + H_{10}), 5.65 (t, J = 6.4 Hz, 1H, H_{14}), 4.21 (t, J = 5.8 Hz, 1H, H_4), 3.57 (d, J = 6.4 Hz, 2H, $2H_{15}$), 1.94 (s, 3H, CH_3), 1.93 (s, 3H, CH_3), 1.78 (s, 3H, CH_3), 1.7–1.6 (m, 2H, $2H_5$), 1.4–1.3 (m, 2H, H_6), 1.01 (s, 3H, CH_3), 0.99 (s, 3H, CH_3) ppm. FTIR (NaCl): δ 3600–3100 (br, OH), 2955 (s, C–H), 2926 (s, C–H), 2855 (m, C–H) cm^{-1}. UV (MeOH): λ_{max} 311, 324 (ε = 24 900) nm. MS (FAB^+): *m/z* (%) 303 (M + 1^+, 13), 302 (M^+, 23), 301 (20), 295 (31), 291 (100), 290 (35), 289 (95), 288 (26), 287 (48), 285 (47), 283 (22), 281 (66), 279 (20), 271 (21), 267 (30), 265 (19), 289 (24). HRMS (FAB^+): calcd for $C_{20}H_{31}O_2$ (M + $1)^+$, 303.2324; found, 303.2312.

4-Oxoretinal (6)

Pyridine (0.043 mL) and Dess–Martin periodinane (27 mg, 0.063 mmol) were added to a solution of 4-hydroxyretinol **5** (8 mg, 0.026 mmol) in CH_2Cl_2 (1.5 mL). The mixture was stirred for 1.5 h at 25 °C and aqueous $NaHCO_3$ (1 mL) was then added. The mixture was stirred for 10 min and then extracted with CH_2Cl_2 (3×). The combined organic layers were washed with $NaHCO_3$ (3×) and $Na_2S_2O_3$ (3×). The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (silica gel, 72 : 25 : 3 hexane : AcOEt : Et_3N) to afford 6.1 mg (76%) of a yellow oil which was shown to be a mixture of 4-oxo-retinal **6** and its 13*Z* isomer (6 : 1 13*E* : 13*Z* ratio). The mixture was separated by HPLC (Waters Spherisorb® silica 5 µm, 10 × 250 mm, 82 : 18 hexane : EtOAc, t_R (13*Z*) = 18 min, t_R (13*E*) = 22 min).

4-oxoretinal (**6**). ^{1}H-NMR (400.13 MHz, C_6D_6): δ 9.98 (d, J = 7.7 Hz, 1H, H_{15}), 6.74 (dd, J = 15.2, 11.5 Hz, 1H, H_{11}), 6.21 (d, J = 16.2 Hz, 1H, H_7), 6.13 (d, J = 16.2 Hz, 1H, H_8), 6.04 (d, J = 15.2 Hz, 1H, H_{12}), 6.0–5.9 (m, 2H, H_{10} + H_{14}), 2.39 (t, J = 6.8 Hz, 2H, $2H_3$), 2.09 (s, 3H, CH_3), 1.71 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.46 (t, J = 6.8 Hz, 2H, $2H_2$), 0.91 (s, 6H, C_1–$2CH_3$) ppm. FTIR (NaCl): δ 2957 (m, C–H), 2924 (m, C–H), 2855 (m, C–H), 1657 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} 293, 377 (ε = 41 700) nm. MS (EI^+): *m/z* (%) 299 (22), 298 (M^+, 100), 269 (12), 203 (23), 201 (12), 187 (11), 147 (15), 133 (20), 128 (10), 119 (16), 115 (11), 105 (18), 95 (17), 91 (26), 79 (11), 77 (15), 69 (17). HRMS (EI^+): calcd for $C_{20}H_{26}O$, 298.1932; found, 298.1939.

(13*Z*)-4-oxoretinal. ^{1}H-NMR (400.13 MHz, C_6D_6): δ 10.11 (d, J = 7.2 Hz, 1H, H_{15}), 7.13 (m, 1H, H_{12}), 6.66 (dd, J = 14.8, 11.3 Hz, 1H, H_{11}), 6.24 (d, J = 16.4 Hz, 1H, H_7), 6.14 (d, J = 16.1 Hz, 1H, H_8), 5.97 (d, J = 11.4 Hz, 1H, H_{10}), 5.74 (d, J = 7.5 Hz, 1H, H_{14}), 2.39 (d, J = 6.9 Hz, 2H, $2H_3$), 2.11 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.47 (m, 2H, $2H_2$), 0.92 (s, 6H, C_1–$2CH_3$) ppm. FTIR (NaCl): ν 2956 (m, C–H), 2924 (m, C–H), 2854 (m, C–H), 1660 (s, C=O) cm^{-1}. UV (MeOH): λ_{max} 293, 373 (ε = 35 600) nm. MS (FAB^+): *m/z* (%) 299 (M^+ + 1, 57), 290 (100), 273 (63). HRMS (FAB^+): calcd for $C_{20}H_{27}O$ (M^+ + $1)^+$, 299.2011; found, 299.1992.

Expression and purification of ADH proteins

E. coli BL21 cells were transformed with the vector pGEX-4T-2 containing mouse ADH1 cDNA.[28] Cells were grown in 2 L of 2 × YT medium until reaching stationary phase, at 25 °C, prior to $ZnSO_4$ (10 µM) addition and induction with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside (Roche Molecular Biochemicals), at 22 °C, for 15 h. Cells were centrifuged at 2800 × g and 4 °C for 15 min, and pellets were frozen at −80 °C to enhance cell lysis. ADH fused to glutathione-S-transferase (GST) was purified using the affinity resin Glutathione-Sepharose 4B (Amersham Pharmacia Biotech). After incubation with the resin, and washing with 100 mM Tris–HCl, pH 7.0, 2.5 mM dithiotheritol (Sigma), 10% glycerol, 0.2 M NaCl, 10 µM $ZnSO_4$, ADH was eluted by thrombin digestion (10 U per mg protein, Amersham Pharmacia Biotech) in the same buffer, for 15 h at room temperature. Electrophoresis on SDS-polyacrylamide gel, followed by the Coomassie® Brilliant Blue (Sigma) stain technique, was used to check protein homogeneity.

Enzyme kinetics. Standard activity of purified mouse ADH1 was determined with ethanol as described previously.[10]

Retinoid activities were determined by measuring the change in absorbance at 400 nm (450 nm for 3,4-didehydroretinal **4**) using a Varian Cary 400 spectrophotometer, at 25 °C. 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. Retinol oxidation activities were determined in 0.1 M sodium phosphate, pH 7.5, 0.02% Tween 80, with 0.3 mM NAD^+ (Sigma), in 1 cm pathlength cuvettes. Retinal reduction activities were measured in the same buffer, using 1 mM NADH (Sigma) in 0.2 cm pathlength cuvettes. Retinoid solutions were prepared as described previously.[10] Molar absorption coefficients, determined in 0.1 M sodium phosphate, pH 7.5, 0.02% Tween 80, used to calculate retinoid concentrations, were: ε_{328} = 39 500 $M^{-1} \times cm^{-1}$ for all-*trans*-retinol **1** (Sigma), ε_{400} = 29 500 $M^{-1} \times cm^{-1}$ for all-*trans*-retinal **2** (Sigma), ε_{329} = 28 512 $M^{-1} \times cm^{-1}$ for 4-hydroxyretinol **5**, ε_{356} = 29 210 $M^{-1} \times cm^{-1}$ for 3,4-didehydro-retinol **3**, ε_{400} = 37 874 $M^{-1} \times cm^{-1}$ for 4-oxoretinal **6**, and ε_{450} = 12 534 $M^{-1} \times cm^{-1}$ for 3,4-didehydroretinal **4**.

Kinetic constants were calculated with the GraFit 5.0 program (Erithacus Software Limited), and results are reported as the mean ± S.E.M. of at least three independent determinations.

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References

1 For details on the nomenclature and numbering of retinoids, see: IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), G. P. Moss, *Eur. J. Biochem.*, 1982, **129**, 1.
2 (*a*) R. R. Rando, *Chem. Rev.*, 2001, **101**, 1881; (*b*) J. K. McBee, K. Palczewski, W. Baehr and D. R. Pepperberg, *Prog. Retinal Eye Res.*, 2001, **20**, 469.
3 J. L. Napoli, *Biochim. Biophys. Acta*, 1999, **1440**, 139.
4 (*a*) R. V. Weatherman, R. J. Fletterick and T. S. Scanlan, *Annu. Rev. Biochem.*, 1999, **68**, 559; (*b*) W. Bourquet, P. Germain and H. Gronemeyer, *Trends Pharmacol. Sci.*, 2000, **21**, 381.
5 G. Duester, *Biochemistry*, 1996, **35**, 12221.
6 B. Crosas, D. J. Hyndman, O. Gallego, S. Martras, X. Parés, T. G. Flynn and J. Farrés, *Biochem. J.*, 2003, **373**, 973.
7 G. Duester, *Eur. J. Biochem.*, 2000, **267**, 4315.
8 (*a*) L. Deltour, M. H. Foglio and G. Duester, *J. Biol. Chem.*, 1999, **274**, 16796; (*b*) A. Molotkov, L. Deltour, M. H. Foglio, A. E. Cuenca and G. Duester, *J. Biol Chem.*, 2002, **277**, 13804.
9 (*a*) A. R. de Lera, B. Domínguez and B. Iglesias, *J. Org. Chem.*, 1998, **63**, 4135; (*b*) R. Alvarez, B. Iglesias, S. López and A. R. de Lera, *Tetrahedron Lett.*, 1998, **39**, 5659; (*c*) B. Domínguez, Y. Pazos and A. R. de Lera, *J. Org. Chem.*, 2000, **65**, 5917; (*d*) Y. Pazos, B. Iglesias and A. R. de Lera, *J. Org. Chem.*, 2001, **66**, 8483; (*e*) M. P. Otero, A. Torrado, Y. Pazos, F. Sussman and A. R. de Lera, *J. Org. Chem.*, 2002, **67**, 5876; (*f*) B. Domínguez, M. J. Vega, F. Sussman and A. R. de Lera, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2607.
10 S. Martras, R. Alvarez, S. E. Martínez, D. Torres, O. Gallego, G. Duester, J. Farrés, A. R. de Lera and X. Parés, *Eur. J. Biochem.*, 2004, **271**, 1660.
11 S. Martras, R. Alvarez, O. Gallego, M. Domínguez, A. R. de Lera, J. Farrés and X. Parés, *Arch. Biochem. Biophys.,* 2004, **430**, 210.
12 C. Thaller and G. Eichele, *Nature*, 1990, **345**, 815.
13 C. C. Achkar, F. Derguini, B. Blumberg, A. Langston, A. A. Levin, J. Speck, R. M. Evans, J. Bolado, K. Nakanishi, J. Buck and L. Gudas, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 4879.
14 (*a*) M. F. Boehm, M. A. Gawinowicz, A. Foucault, F. Derguini and K. Nakanishi, *J. Am. Chem. Soc.*, 1990, **112**, 7779; (*b*) C. J. Beischel, V. Mani, R. Govindjee, T. G. Ebrey, D. R. Knapp and R. K. Crougch, *Photochem. Photobiol.*, 1991, **54**, 977; (*c*) B. Borhan, R. Kunz, A. Y. Wang and K. Nakanishi, *J. Am. Chem. Soc.*, 1997, **119**, 5758; (*d*) M. Hashimoto and Y. Fujimoto, *Synth. Commun.*, 1999, **29**, 3793; (*e*) A. A. C. van Wijk, M. B. van der Weerd and J. Lugtenburg, *Eur. J. Org. Chem.*, 2003, 863.
15 (*a*) H. B. Henbest, E. R. H. Jones, T. C. Owen and V. Thaller, *J. Chem. Soc.*, 1955, 2763; (*b*) K. Eiter; H. Oediger; E. Trusscheit; *Farbenfabriken Bayer A-G. Ger. 1, 110, 633. C. A.* 1962, **56**, p. 3522; (*c*) V. Schwieter, G. Saucy, M. Montavon, C. Von Planta, R. Rüegg and O. Isler, *Helv. Chim. Acta*, 1968, **45**, 517.
16 *Metal-catalyzed Cross-coupling Reactions*, eds. F. Diederich and P. J. Stang,Wiley-VCH, Weinheim, 1998.
17 B. Domínguez, B. Iglesias and A. R. de Lera, *Tetrahedron*, 1999, **55**, 15071.
18 M. Baumann, W. Hoffmann and A. Nürrenbach, *Liebigs Ann. Chem.*, 1979, 1945.
19 (*a*) J. K. Stille, *Pure Appl. Chem.*, 1985, **57**, 1771–1780; (*b*) J. K. Stille, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 508–524; (*c*) V. Farina, in *Comprehensive Organometallic Chemistry II*, eds. E. W. Abel, F. G. A. Stone and G. Wilkinson, Elsevier, Oxford, 1995; vol. 12, chapter 3.4, pp 161–241; (*d*) V. Farina and G. P. Roth, in *Advances in Metal-Organic Chemistry*, ed. L. S. Liebeskind, JAI Press, New York, 1996, vol. 5, pp 1–53; (*e*) V. Farina, V. Krishnamurthy and W. J. Scott, *Org. Reactions. N. Y.*, ed. L. A. Paquette, John Wiley & Sons, New York, 1997; vol. 50, chapter 1, pp 1–652.
20 V. Farina and B. Krishnan, *J. Am. Chem. Soc.*, 1991, **113**, 9585.
21 N. D. Smith, J. Mancuso and M. Lautens, *Chem. Rev.*, 2000, **100**, 3257.
22 W. G. Dauben and D. M. Michno, *J. Org. Chem.*, 1977, **42**, 682.
23 (*a*) N. Krause, *J. Org. Chem.*, 1992, **57**, 3509; (*b*) A. Haubrich, M. van Klaveren, G. van Koten, G. Handke and N. Krause, *J. Org. Chem.*, 1993, **58**, 5849; (*c*) N. Krause, R. Wagner and A. Gerold, *J. Am. Chem. Soc.*, 1994, **116**, 381; (*d*) N. Krause and A. Gerold, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 186; (*e*) J. Canisius, A. Gerold and N. Krause, *Angew. Chem., Int. Ed.*, 1999, **38**, 1644.
24 (*a*) M. E. Jung and L. A. Light, *Tetrahedron Lett.*, 1982, **23**, 3851; (*b*) K. Kikukawa, H. Umekawa, F. Wada and T. Matsuda, *Chem. Lett.*, 1988, 881; (*c*) H. Miyake and K. Yamamura, *Chem. Lett.*, 1989, 981; (*d*) H. X. Zhang, F. Guibé and G. Balavoine, *J. Org. Chem.*, 1990, **55**, 1857; (*e*) J.-F. Betzer, F. Delaloge, B. Muller, A. Pancrazi and J. Prunet, *J. Org. Chem.*, 1997, **62**, 7768.
25 S.-M. L. Chen, R. E. Schaub and C. V. Grudzinskas, *J. Org. Chem.*, 1978, **43**, 3450.
26 A. L. Gemal and J.-L. Luche, *J. Am. Chem. Soc.*, 1981, **103**, 5454.
27 P. Julià, J. Farrés and X. Parés, *Eur. J. Biochem.*, 1987, **162**, 179.
28 L. Deltour, R. J. Haselbeck, H. L. Ang and G. Duester, *Biol. Reprod.*, 1997, **56**, 102–109.