Retinoids and related compounds. Part 19.[1] Syntheses of 9*E*- and 9*Z*-locked retinoic acid analogues and their transcriptional activities as ligands for retinoic acid receptors and retinoid X receptors

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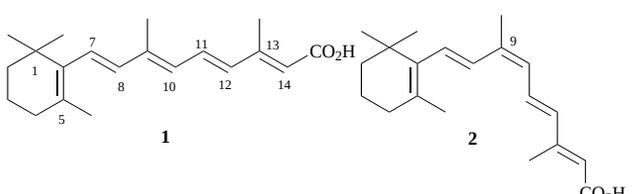
Novel retinoic acid (RA) analogues, 9*E*-locked-RA 3 and 9*Z*-locked RA 4, have been synthesized in order to prohibit geometrical isomerization at the C(9)–C(10) double bond when these ligands interact with the retinoic acid receptor (RAR) and retinoid X receptor (RXR). Transcriptional activities of these analogues for RAR and RXR are also described.

Introduction

Retinoic acid (RA) is a derivative of vitamin A which plays a key role in vertebrate vitamin A actions including proliferation, differentiation, growth and development, apart from those actions associated with vision.[2] Recently, receptors for RA have been identified as important members of the steroid/thyroid nuclear receptor superfamily, which act as ligand-dependent transcription factors. These receptors have been classified into two types, the retinoic acid receptors (RARs)[3] and the retinoid X receptors (RXRs),[4] and both have three kinds of subtypes (α, β and γ) respectively. The difference in these two receptors is due to the stereochemistry of the signalling molecules. Thus, the ligand of RARs is all-*E*-RA **1** and that of RXRs is 9*Z*-RA **2**,[5] respectively. Interestingly, 9*Z*-RA **2** is also capable of binding directly to RARs, and their complexes have also been active in the regulation of gene transcription. This implies that, unlike all-*E*-RA **1**, 9*Z*-RA **2** is a biologically active ligand for both members of the RAR and RXR subfamilies. In addition, it is suggested that the isomerization of retinoids is involved in controlling signal-transduction pathways. A number of derivatives of RA have hitherto been synthesized and their transcriptional activities have been investigated. The structure of most analogues prepared previously was aromatic and seemed to be rather different from RA; therefore, a few of them behaved as agonists or antagonists only for RARs.[6] Further, RA is known to isomerize around the double bonds rapidly *in vivo*. Hence we prepared locked RA analogues **3** and **4**, whose C(9)–C(10) double bonds are prevented from isomerizing, to investigate whether these enzyme analogues bind the receptors and exhibit the transcriptional activities. Here we describe a full account of the syntheses of the RA analogues **3** and **4** which are reported briefly in the previous communication.[7]



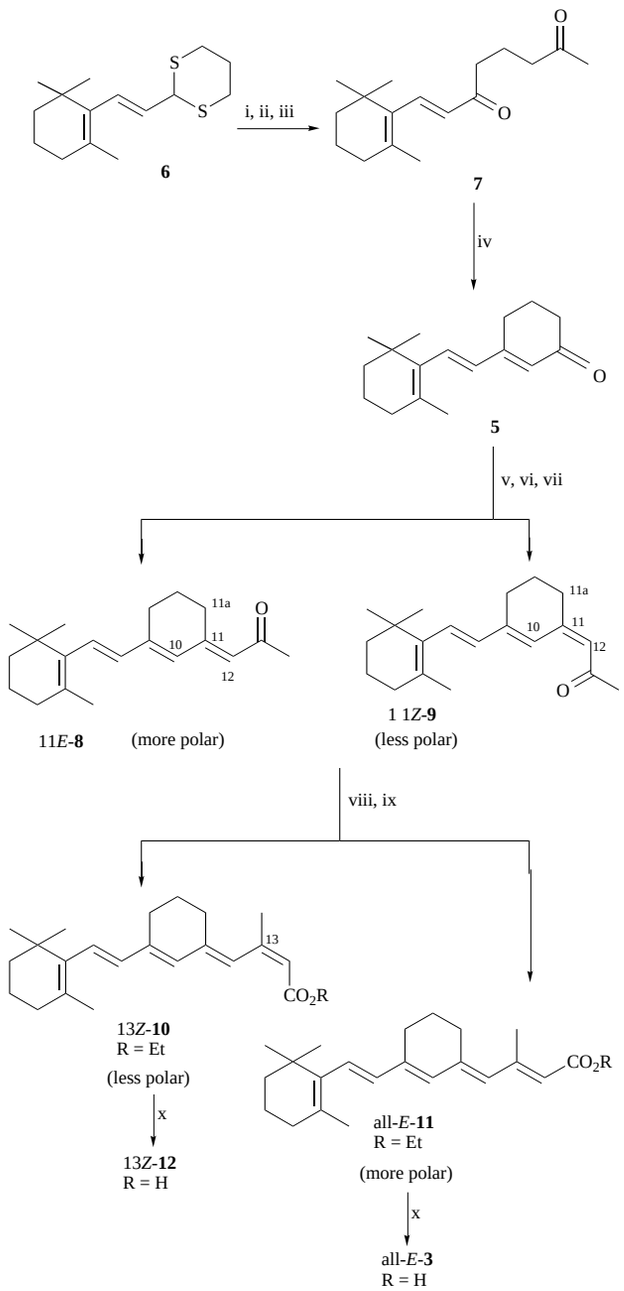
Results and discussion

Synthesis of 9*E*-locked RA 3 (Scheme 1)

Our first attempt to prepare 9*E*-locked trienone **5** according to Albeck's method[8] was unsuccessful due to the low yield in the Wittig reaction between the triphenylphosphonium bromide of β-cyclocitrol and 3-formylcyclohex-1-enone. Hence trienone **5** was synthesized by our original procedure as shown in Scheme 1. Dione **7**, derived from the reaction of the lithium salt of dithiane **6**[9] with 5-chloropentan-2-one ethylene ketal (Aldrich) and subsequent deprotection of the thioketal and ketal groups (57% in 3 steps), was easily cyclized[10] in the presence of MeONa to afford trienone **5** (100%). Condensation of the trienone **5** with the C_5-phosphonate did not proceed and the desired retinoate analogue was not obtained. Therefore, trienone **5** was converted to the retinoate analogue by stepwise elongation of the side-chain. Condensation of the trienone **5** with propan-2-one *N*,*N*-dimethylhydrazone in the presence of lithium diisopropylamide (LDA) afforded a mixture (1:1 ratio) of tetraenones **8** and **9** in 72% yield after mild deprotection.[11] The mixture was separated by low-pressure column chromatography (LPCC) and these structures were confirmed by ^{1}H NMR spectroscopy; the 11*Z*-geometry was identified from the downfield shift of the 10-H signal (δ 7.55) in compound **9** in comparison with that (δ 5.98 or 6.02) in isomer **8** and the 11*E*-geometry from the downfield shift of the 11a-H_2 signal (δ 2.94) in compound **8** compared with that (δ 2.38) in isomer **9**, both owing to anisotropic effects of the ketone groups. Peterson olefination (92%) of 11*E*-isomer **8** with ethyl trimethylsilyl acetate followed by preparative high-pressure liquid chromatography (PHPLC) gave 13*Z*-isomer **10** and all-*E*-isomer **11** of the retinoate analogues in a 1:1 ratio. These structures were confirmed on the basis of ^{1}H NMR spectroscopy data; the 12-H signal (δ 6.93) in compound **10** was observed downfield in comparison with that (δ 5.79) in isomer **11**. Assignment of the ^{13}C NMR data of isomers **10** and **11** was performed by comparison with those of the known ethyl retinoates[12] and by ^{1}H–^{13}C heteronuclear shift-correlation (HETCOR) experiments, and complete assignment of these ^{1}H NMR data was obtained by 2D homonuclear chemical-shift-correlation (COSY) experiments. These retinoate analogues were carefully hydrolysed to RA analogues **12** and **3**, respectively. δ-Values of the olefinic protons in the retinoates and the RAs (Table 1) show that no geometrical isomerization occurred during the final hydrolysis.

Synthesis of 9*Z*-locked RA 4 (Scheme 2)

9*Z*-Locked trienone **16** was synthesized by a modified procedure used for the preparation of 11*Z*-locked retinal.[13] Aldol



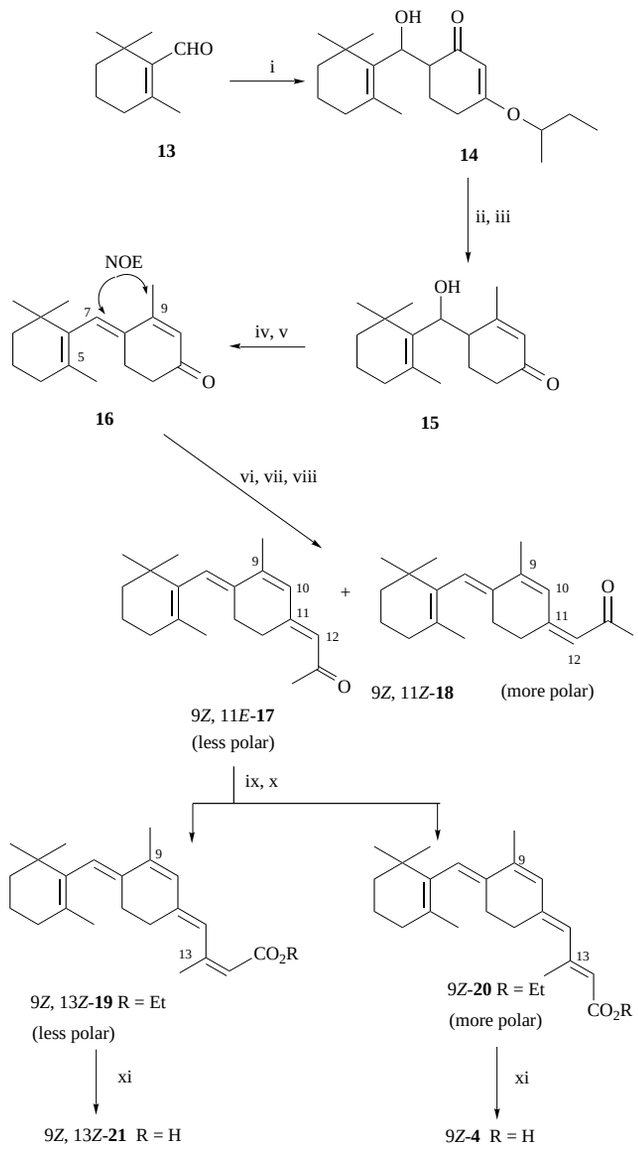
Scheme 1 *Reagents and conditions:* i, BuLi, 5-chloropentan-2-one ethylene ketal, THF, −78 °C; ii, HgO, $HgCl_2$, 97% MeOH, rt; iii, *p*-TsOH, acetone, rt (57%); iv, MeONa, THF, rt (100%); v, LDA, Me_2N-N=CMe_2, THF, rt; vi, AcOH–THF–water–NaOAc (5:2:2:1), rt (72% [94%]); vii, LPCC; viii, LDA, $Me_3SiCH_2CO_2Et$, THF, −78 °C (92%); ix, PHPLC (in the dark); x, 25% NaOH, EtOH, 50 °C (92%, 81%)

Table 1 UV–VIS and ^{1}H NMR data for 9*E*-locked retinoids

		13*Z*-**10**	All-*E*-**11**	13*Z*-**12**[a]	All-*E*-**3**[a]
UV–VIS	λ_{max}/nm	358	355	345, 246	353
^{1}H NMR (200 MHz) (δ, $CDCl_3$)	1,1-Me_2	1.00	1.01	1.01	1.01
	5-Me	1.69	1.70	1.70	1.70
	13-Me	2.10	2.29	2.13	2.31
	7-H	6.21	6.24	6.23	6.26
	8-H	6.07	6.07	6.10	6.09
	10-H	6.17	6.04	6.18	6.05
	12-H	6.93	5.79	6.90	5.82
	14-H	5.61	5.72	5.64	5.75

[a] No signal for the carboxy proton was observed.

condensation between β-cyclocitral **13**[14] and easily prepared 3-(1-methylpropoxy)cyclohex-2-enone[15] afforded hydroxy ketone **14**. After addition of methyllithium to ketone **14**, the



Scheme 2 *Reagents and conditions:* i, 3-(1-methylpropoxy)cyclohex-2-enone, LDA, THF, −78 °C (61%); ii, MeLi, THF, −78 to 0 °C; iii, 15% H_2SO_4, rt (60%); iv, Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt (83%); v, DBU, toluene, reflux (83%); vi, LDA, Me_2NN=CMe_2, THF, rt; vii, AcOH–THF–water–AcONa (5:2:2:1), rt (41% [57%]); viii, PHPLC; ix, LDA, $Me_3SiCH_2CO_2Et$, THF, −78 °C (100%); x, PHPLC (in the dark); xi, 25% NaOH, EtOH, 50 °C (92%, 100%)

Table 2 UV–VIS and ^{1}H NMR data for 9*Z*-locked retinoids

		9*Z*,13*Z*-**19**	9*Z*-**20**	9*Z*,13*Z*-**21**[a]	9*Z*-**4**[a]
UV–VIS	λ_{max}/nm	348	342	335	334, 250
^{1}H NMR (200 MHz) (δ, $CDCl_3$)	1,1-Me_2	0.95	0.96	0.95	0.96
	5-Me	1.46	1.46	1.46	1.47
	9-Me	1.96	1.97	1.97	1.98
	13-Me	2.07	2.28	2.11	2.30
	7-H	6.02	6.04	6.05	6.06
	10-H	6.11	5.97	6.11	5.98
	12-H	6.89	5.76	6.87	5.79
	14-H	5.61	5.68	5.63	5.72

[a] No signal for the carboxy proton was observed.

resulting diol was treated with acid to give compound **15** which, by acetylation and subsequent elimination (1,8-diazabicyclo-[5.4.0]undec-7-ene, DBU) was converted to 7*E*-trienone **16** exclusively. The stereostructure of trienone **16** was confirmed by a nuclear Overhauser and exchange spectroscopy (NOESY)

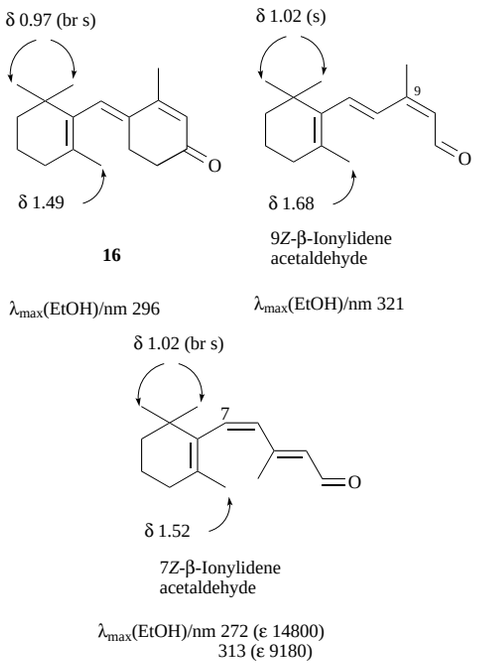


Fig. 1

experiment (observed crosspeak between 7-H and 9-Me). The broad singlet at δ 0.97 (1,1-Me_2) indicates that rotational barriers exist in the two rings in trienone **16** and hence the twisted 6*s*-*cis* conformation is consistent with the unusual high-field shift of the 5-Me group. In such a conformation, the 5-Me group is in the shielding cone of the C(7)–C(8) double bond and similar phenomena were found in 7*Z*-retinoids[16] (Fig. 1). The significantly shorter absorption maximum (296 nm) of trienone **16** than that of 9*Z*-β-ionylideneacetaldehyde (321 nm) also revealed this highly twisted 6*s*-*cis* conformation (Fig. 1). Final transformation of trienone **16** into 9*Z*-locked RAs was achieved by means of the route used for 9*E*-locked RAs **12** and **3**. Their ^{1}H NMR data in Table 2 show that no geometrical isomerization occurred during the final hydrolysis.

Transcriptional activities of 9*E*- and 9*Z*-locked RAs for RAR and RXR

Transcriptional activities of synthesized RA analogues **3**, **12**, **4** and **21** were compared with those of all-*E*-RA **1** and 9*Z*-RA **2** by chloramphenicol acetyltransferase (CAT) assay. All analogues bound to both mRARα and mRXRα, and had activities which were significant but weaker than those of all-*E*-RA **1** and 9*Z*-RA **2** (**3**; 1/10 of **1** or **2**: **12**, **4** and **21**; 1/100 of **1** or **2**). The results suggest that the synthesized analogues exhibited agonistic actions towards both mRARα and mRXRα receptors, but it was supposed that the artificial 6-membered ring hindered the interaction between ligands and receptors.

Experimental

Mps are uncorrected. Ether refers to diethyl ether and hexane to *n*-hexane. BuLi was used as a solution in hexane. UV–VIS spectra were recorded on a JASCO Ubest-55 instrument and IR or FT-IR spectra on a Shimadzu IR-27G or Shimadzu FT-IR-4200 spectrometer. ^{1}H NMR spectra at 60, 200, 300 or 500 MHz were measured on a JOEL JNM-PMX 60, a Varian Gemini-200, a Varian Gemini-300 or a Varian VXR-500 superconducting FT-NMR spectrometer using chloroform (δ 7.25) as internal reference and ^{13}C NMR spectra at 75 MHz or 125 MHz were measured on a Varian Gemini-300 or a Varian VXR-500 spectrometer. Mass spectra were determined on a Hitachi M-4100 double-focusing GC mass spectrometer. Column chromatography (CC) was performed on silica gel Merck Art. 7734. LPCC was conducted on a Yamazen Low Pressure Liquid Chromatography System using a Lobar Merck LiChroprep Si-60 column. Preparative HPLC was conducted on a Shimadzu LC-10AS instrument with a Shimadzu UV–VIS detector, SPD-10A, using a Merck LiChrosorb Si-60 (7 μm), 1.0 × 25 cm column. Unless otherwise stated, solvent extracts were dried over anhydrous sodium sulfate and all operations were carried out under nitrogen or argon. The extract or the filtrate was concentrated under reduced pressure at <30 °C using a rotary evaporator. NMR assignments follow the retinoic acid numbering system.

Synthesis of 9*E*-locked RA 3. (*E*)-8-(2,6,6-Trimethylcyclohex-1-enyl)oct-7-ene-2,6-dione 7

To a solution of the dithiane **6** (2.0 g, 7.46 mmol) in dry tetrahydrofuran (THF) (20 cm^3) was added a solution of BuLi (1.70 mol dm^{-3}; 4.83 cm^3, 8.21 mmol) in hexane at −78 °C. The mixture was stirred at room temperature (rt) for 1 h and 5-chloropentan-2-one ethylene ketal (1.35 cm^3, 8.96 mmol) was then added dropwise at −78 °C. After being stirred at rt for 3 h, the reaction mixture was quenched by addition of water. After evaporation off of the THF, the residue was extracted with ether. The extract was washed with brine, dried and evaporated to give an oil, which was dissolved with 97% methanol (100 cm^3). $HgCl_2$ (8.12 g, 29.9 mmol) and HgO (4.85 g, 22.4 mmol) were added to this solution and the reaction mixture was stirred at rt for 15 min. Evaporation off of the methanol gave a residue, which was dissolved in ether and the solution was filtered through Celite. The filtrate was washed with brine, dried and evaporated to afford a residue, which was dissolved with acetone (50 cm^3). To the solution was added toluene-*p*-sulfonic acid ($TsOH\cdot 2H_2O$) (142 mg, 0.75 mmol) at 0 °C and the reaction mixture was stirred at rt for 1 h before being neutralized by the addition of saturated aq. $NaHCO_3$ and the acetone was evaporated off to give a residue, which was extracted with ether. The extract was washed with brine, dried and evaporated to give an oil, which was purified by CC (AcOEt–hexane, 1:9). This afforded the *title compound* **7** (1.11 g, 57%) as an oil (Found: C, 77.66; H, 9.71%; M$^+$, 262.1915. $C_{17}H_{26}O_2$ requires C, 77.82; H, 9.99%; M, 262.1934); $\nu_{max}(CHCl_3)$/cm^{-1} 1712 (nonconj. C=O), 1657 (conj. C=O) and 1603 (C=C); λ_{max}(EtOH)/nm 295 and 219sh; δ_H(200 MHz; $CDCl_3$) 1.04 (6 H, s, *gem*-Me), 1.74 (3 H, s, 5-Me), 1.89 (2 H, quin, *J* 7, 9b-H_2), 2.12 (3 H, s, COMe), 2.50 and 2.59 (each 2 H, each t, *J* 7, CH_2CH_2CH_2COMe), 6.09 (1 H, d, *J* 16.5, 8-H) and 7.30 (1 H, d, *J* 16.5, 7-H).

(*E*)-3-[2-(2,6,6-Trimethylcyclohex-1-enyl)ethenyl]cyclohex-2-enone 5

To a solution of dione **7** (1.10 g, 4.20 mmol) in dry CH_2Cl_2 (10 cm^3) was added dropwise a solution of NaOMe (261 mg, 4.83 mmol) in methanol (2 cm^3) at 0 °C. After being stirred at rt for 3 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The extract was washed with brine, dried and evaporated. The residue was purified by CC (AcOEt–hexane, 1:4) to give the *title compound* **5** (1.02 g, 100%) as an oil, $\nu_{max}(CHCl_3)$/cm^{-1} 1651 (C=O) and 1610 (C=C); λ_{max}(EtOH)/nm 322 and 271; δ_H(200 MHz; $CDCl_3$) 1.02 (6 H, s, *gem*-Me), 1.70 (3 H, s, 5-Me), 2.41 and 2.51 (each 2 H, each t-like, *J* 6.5 and 6, 9a-H_2 and 11a-H_2), 5.90 (1 H, s, 10-H), 6.16 (1 H, d, *J* 16, 8-H) and 6.64 (1 H, d, *J* 16, 7-H) (Found: M$^+$, 244.1833. $C_{17}H_{24}O$ requires *M*, 244.1826).

Propan-2-one *N*,*N*-dimethylhydrazone[17]

A mixture of acetone (19.7 g, 0.34 mol), *N*,*N*-dimethylhydrazine (10 g, 0.17 mol) and benzene (100 cm^3) was refluxed for 1 h using a Dean–Stark separator and was then distilled to give a solution of the title compound in benzene (50% w/w, the concentration was determined by ^{1}H NMR); δ_H(60 MHz; $CDCl_3$) 1.92 (6 H, d, *J* 2, CMe_2), 2.41 (6 H, s, NMe_2) and 7.25 (6 H, s, benzene).

(*E,E/Z*)-3-{3-[2-(2,6,6-Trimethylcyclohex-2-enyl)ethenyl]cyclohex-2-enylidene}propan-2-one 8 and 9

To a solution of LDA (8.20 mmol, prepared from 1.15 cm^3 of diisopropylamine and 4.82 cm^3 of 1.70 mol dm^{-3} BuLi) in dry THF (8 cm^3) was added a solution of propan-2-one *N*,*N*-dimethylhydrazone (50% w/w; 1.46 g, 8.20 mmol) in benzene at 0 °C. After the reaction mixture had been stirred at rt for 30 min, a solution of trienone **5** (400 mg, 1.64 mmol) in dry THF (4 cm^3) was added at 0 °C and the mixture was stirred at rt for 2 h. The reaction was quenched by the addition of water and the THF was evaporated off to give a residue, which was extracted with AcOEt. The extract was washed with brine, dried and evaporated off to afford a crude oil, which was dissolved with a mixture of AcOH–THF–water–NaOAc (5:2:2:1) and the reaction mixture was stirred at rt for 5 h and extracted with AcOEt. The extract was washed with brine, dried and evaporated to give a residue, which was purified by CC (AcOEt–hexane, 1:9). This afforded a mixture of geometrical isomers **8** and **9** (335 mg, 72%) as a pale yellow oil, together with recovered starting material **5** (95 mg, 24%). The isomers were separated by LPCC (AcOEt–hexane, 1:19) to give the more polar compound **8** and the less polar compound **9** each in a pure state, in the ratio ~1:1.

11E-*Isomer* **8**: $\nu_{max}(CHCl_3)/cm^{-1}$ 1658 (C=O) and 1554 (C=C); λ_{max}(EtOH)/nm 346; δ_H(200 MHz; $CDCl_3$) 1.01 (6 H, s, *gem*-Me), 1.70 (3 H, s, 5-Me), 2.19 (3 H, s, 13-Me), 2.36 (2 H, t-like, *J* 6, 9a-H_2), 2.94 (2 H, m, 11a-H_2), 5.98 and 6.02 (each 1 H, each s, 10- and 12-H), 6.12 (1 H, d, *J* 16, 8-H) and 6.39 (1 H, d, *J* 16, 7-H) (Found: M^+, 284.2133. $C_{20}H_{28}O$ requires *M*, 284.2138).

11Z-*Isomer* **9**: $\nu_{max}(CHCl_3)/cm^{-1}$ 1660 (C=O) and 1560 (C=C); λ_{max}(EtOH)/nm 349; δ_H(200 MHz; $CDCl_3$) 1.01 (6 H, s, *gem*-Me), 1.71 (3 H, s, 5-Me), 2.18 (3 H, s, 13-Me), 2.38 (4 H, m, 9a- and 11a-H_2), 5.86 (1 H, s, 12-H), 6.22 (1 H, d, *J* 16, 8-H), 6.40 (1 H, d, *J* 16, 7-H) and 7.55 (1 H, s, 10-H) (Found: M^+, 284.2142).

Ethyl (*E,E,Z/E*)-3-methyl-4-{3-[2-(2,6,6-trimethylcyclohex-1-enyl)ethenyl]cyclohex-2-enylidene}but-2-enoate 10 and 11

To a solution of LDA (0.35 mmol, prepared from 0.049 cm^3 of diisopropylamine and 0.219 cm^3 of 1.60 mol dm^{-3} BuLi) in dry THF (1 cm^3) was added a solution of ethyl trimethylsilylacetate (0.064 cm^3, 0.35 mmol) in dry THF (1 cm^3) at −78 °C. After the reaction mixture had been stirred at −78 °C for 15 min, a solution of tetraenone **8** (20 mg, 0.070 mmol) in dry THF (2 cm^3) was added at −78 °C and the mixture was stirred for 30 min at rt. The whole was concentrated to give a residue, which was purified by CC (AcOEt–hexane, 1:19) to afford a mixture of geometrical isomers **10** and **11** (23 mg, 92%). The isomers were separated by PHPLC [LiChrosorb Si-60 (7 μm), 1 × 25 cm, Et_2O–hexane, 3:97] to give the less polar compound **10** and the more polar compound **11**, each in a pure state and each as a pale yellow oil, in the ratio ~1:1.

All-E-*isomer* **11**: ν_{max}(KBr)/cm^{-1} 1709 (CO_2Et) and 1571 (C=C); λ_{max}(EtOH)/nm 355; δ_H(200 MHz; $CDCl_3$) 1.01 (6 H, s, *gem*-Me), 1.28 (3 H, t, *J* 7, $CO_2CH_2CH_3$), 1.70 (3 H, s, 5-Me), 2.29 (3 H, s, 13-Me), 2.33 and 2.60 (each 2 H, t-like and m, *J* 6, 9a- and 11a-H_2), 4.15 (2 H, q, *J* 7, $CO_2CH_2CH_3$), 5.72 (1 H, s, 14-H), 5.79 (1 H, s, 12-H), 6.04 (1 H, s, 10-H), 6.07 (1 H, d, *J* 16.5, 8-H) and 6.24 (1 H, d, *J* 16.5, 7-H); δ_H(500 MHz; $CDCl_3$) 1.01 (6 H, s, *gem*-Me), 1.28 (3 H, t, *J* 7, $CO_2CH_2CH_3$), 1.45 (2 H, t, *J* 6, 2-H_2), 1.59 (2 H, quintet, *J* 6.5, 3-H_2), 1.70 (3 H, s, 5-Me), 1.77 (2 H, quintet, *J* 6, 9b-H_2), 2.00 (2 H, t, *J* 6, 4-H_2), 2.29 (3 H, s, 13-Me), 2.33 (2 H, t, *J* 6, 9a-H_2), 2.58 (2 H, m, 11a-H_2), 4.15 (2 H, q, *J* 7, $CO_2CH_2CH_3$), 5.72 (1 H, s, 14-H), 5.78 (1 H, s, 12-H), 6.04 (1 H, s, 10-H), 6.08 (1 H, d, *J* 16, 8-H) and 6.23 (1 H, d, *J* 16, 7-H); δ_C(125 MHz; $CDCl_3$) 14.39 ($CO_2CH_2CH_3$), 19.22 (C-3), 19.82 (C-20), 21.73 (C-18), 22.41 (C-21), 24.50 (C-19), 27.59 (C-22), 28.94 (1,1-*gem*-Me), 33.09 (C-4), 34.24 (C-1), 39.61 (C-2), 59.55 ($CH_2CH_2CH_3$), 117.65 (C-14), 127.48 (C-7), 129.73 (C-12), 129.86 (C-5), 131.25 (C-10), 135.33 (C-8), 137.68 (C-6), 141.14 and 142.04 (C-9 and -11), 153.35 (C-13) and 167.21 (C-15) (Found: M^+, 354.2559. $C_{24}H_{34}O_2$ requires *M*, 354.2557).

13Z-*Isomer* **10**: ν_{max}(KBr)/cm^{-1} 1707 (CO_2Et) and 1597 and 1570 (C=C); λ_{max}(EtOH)/nm 358; δ_H(200 MHz; $CDCl_3$) 1.00 (6 H, s, *gem*-Me), 1.25 (3 H, t, *J* 7, $CO_2CH_2CH_3$), 1.69 (3 H, s, 5-Me), 2.10 (3 H, s, 13-Me), 2.31 and 2.52 (each 2 H, each t-like, *J* 5.5, 9a- and 11a-H_2), 4.13 (2 H, q, *J* 7, $CO_2CH_2CH_3$), 5.61 (1 H, s, 14-H), 6.07 (1 H, d, *J* 16, 8-H), 6.17 (1 H, s, 10-H), 6.21 (1 H, d, *J* 16, 7-H) and 6.93 (1 H, s, 12-H); δ_H(500 MHz; $CDCl_3$) 1.01 (6 H, s, *gem*-Me), 1.25 (3 H, t, *J* 7, $CO_2CH_2CH_3$), 1.69 (3 H, d, *J* 1, 5-Me), 1.77 (2 H, quintet, *J* 6, 9b-H_2), 2.10 (3 H, d, *J* 1, 13-Me), 2.31 (2 H, t, *J* 6, 9a-H_2), 2.52 (2 H, m, 11a-H_2), 4.13 (2 H, q, *J* 7, $CO_2CH_2CH_3$), 5.61 (1 H, s, 14-H), 6.08 (1 H, d, *J* 16, 8-H), 6.17 (1 H, s, 10-H), 6.19 (1 H, d, *J* 16, 7-H) and 6.93 (1 H, s, 12-H) (Found: M^+, 354.2556).

(*E,E,E*)-3-Methyl-4-{3-[2-(2,6,6-trimethylcyclohex-1-enyl)-ethenyl]cyclohex-2-enylidene}but-2-enoic acid 3

A mixture of all-*E*-retinoate analogue **11** (6.6 mg, 0.019 mmol), ethanol (0.5 cm^3) and aq. NaOH (25% w/w; 0.15 cm^3) was stirred at rt for 3 h and then at 50 °C for 30 min. The reaction mixture was acidified by the addition of dil. HCl and extracted with AcOEt. The extract was washed with brine, dried and evaporated to give a residue, which was purified by CC (AcOEt–hexane, 1:4) to afford *all*-E-*RA analogue* **3** (274.9 mg, 81%) as a pale yellow solid (mp 121–123 °C), ν_{max}(KBr)/cm^{-1} 3200–2500, 1678 (CO_2H) and 1564 (C=C); λ_{max}(EtOH)/nm 353; δ_H(200 MHz; $CDCl_3$) 1.01 (6 H, s, *gem*-Me), 1.70 (3 H, s, 5-Me), 2.31 (3 H, s, 13-Me), 2.34 and 2.61 (each 2 H, each t-like, *J* 5, 9a- and 11a-H_2), 5.75 (1 H, s, 14-H), 5.82 (1 H, s, 12-H), 6.05 (1 H, s, 10-H), 6.09 (1 H, d, *J* 16, 8-H) and 6.26 (1 H, d, *J* 16, 7-H) (Found: M^+, 326.2237. $C_{22}H_{30}O_2$ requires *M*, 326.2244).

(*E,E,Z*)-3-Methyl-4-{3-[2-(2,6,6-trimethylcyclohex-1-enyl)-ethenyl]cyclohex-2-enylidene}but-2-enoic acid 12

In the same manner as described for the preparation of all-*E*-RA analogue **3** from all-*E*-retinoate analogue **11**, hydrolysis of 13*Z*-retinoate analogue **10** (7.7 mg, 0.022 mmol) by NaOH gave 13*Z*-*RA analogue* **12** (6.5 mg, 92%) as a pale yellow amorphous solid, ν_{max}(KBr)/cm^{-1} 3200–2500, 1674 (CO_2H) and 1593 and 1564 (C=C); λ_{max}(EtOH)/nm 345 and 246; δ_H(200 MHz; $CDCl_3$) 1.01 (6 H, s, *gem*-Me), 1.70 (3 H, s, 5-Me), 2.13 (3 H, s, 13-Me), 2.32 and 2.54 (each 2 H, each t-like, *J* 6 and 5, 9a-H_2 and 11a-H_2), 5.64 (1 H, s, 14-H), 6.10 (1 H, d, *J* 16.5, 8-H), 6.18 (1 H, s, 10-H), 6.23 (1 H, d, *J* 16.5, 7-H) and 6.90 (1 H, s, 12-H) (Found: M^+, 326.2245).

Synthesis of 9*Z*-locked RA 4. 3-(1-Methylpropoxy)-6-[hydroxy-(2,6,6-trimethylcyclohex-1-enyl)methyl]cyclohex-2-enone 14

To a solution of LDA (32.9 mmol, prepared from 4.60 cm^3 of diisopropylamine and 20.2 cm^3 of 1.63 mol dm^{-3} BuLi) in dry THF (14 cm^3) was added a solution of 3-(1-methylpropoxy)-cyclohex-2-enone (6.15 g, 36.2 mmol) in dry THF (14 cm^3) at −78 °C. After the reaction mixture had been stirred at −78 °C for 30 min, a solution of β-cyclocitral **13** (5.0 g, 32.9 mmol) in dry THF (10 cm^3) was added at −78 °C and the mixture was stirred at −78 °C for 1 h. The reaction mixture was quenched by addition of water. After evaporation off of the THF, the residue was extracted with ether. The extract was washed with brine, dried and evaporated to give a residue, which was purified by CC (AcOEt–hexane, 1:9) to afford the *title compound* **14** (6.42 g, 61%) as a pale yellow oil (Found: C, 74.80; H, 9.82%; M, 320.2369. $C_{20}H_{32}O_3$ requires C, 74.96; H, 10.17%; *M*, 320.2350); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420 (OH), 1617sh (C=O) and 1598 (C=C); ν_{max}(KBr)/cm^{-1} 3440 (OH), 1630 (C=O) and 1601 (C=C); λ_{max}(EtOH)/nm 253; δ_H(500 MHz; $CDCl_3$) 0.91 [6 H, m, 1-Me and $OCH(CH_3)CH_2CH_3$], 1.13 (3 H, s, 1-Me), 1.23 and

1.26 [total 3 H, each d, *J* 6, OCH(C*H*$_3$)CH$_2$CH$_3$], 1.83 (3 H, s, 5-Me), 2.39 (1 H, m, 8a-H), 2.84 (1 H, octet, *J* 5, 8-H), 4.19 [1 H, m, OC*H*(CH$_3$)CH$_2$CH$_3$], 4.49 (1 H, br s, 7-H), 4.69 (1 H, s, OH) and 5.34 (1 H, s, 10-H) (Found: M$^+$, 320.2369. $C_{20}H_{32}O_3$ requires M, 320.2350).

4-[Hydroxy-(2,6,6-trimethylcyclohex-1-enyl)methyl]-3-methylcyclohex-2-enone 15

To a solution of keto alcohol **14** (2.83 g, 8.85 mmol) in dry THF (10 cm^3) was added a solution of methyllithium (1.5 mol dm^{-3}; 17.7 cm^3, 26.6 mmol) in THF at −78 °C. After the reaction mixture had been stirred at −78 °C for 70 min, −50 °C for 20 min and at 0 °C for 20 min, it was treated with sulfuric acid (15% w/v; 10 cm^3) and the reaction mixture was stirred at 0 °C for a further 20 min before being extracted with AcOEt. The extract was washed with brine, dried and evaporated to give a residue, which was purified by CC (AcOEt–hexane, 1:4) to provide the *title compound* **15** (1.69 g, 60%) as an oil (Found: C, 77.52; H, 9.82%; M$^+$, 262.1931. $C_{17}H_{26}O_2$ requires C, 77.82; H, 9.99%; *M*, 262.1931); ν_{max}(CHCl$_3$)/cm^{-1} 3600 and 3440 (OH) and 1658 (C=O); λ_{max}(EtOH)/nm 240; δ_H(200 MHz; CDCl$_3$) 1.01 and 1.11 (each 3 H, each s, *gem*-Me), 1.85 (3 H, s, 5-Me), 2.19 (3 H, s, 9-Me), 2.51 (1 H, dq, *J* 17.5 and 5, 8′-H), 2.95 (1 H, dt, *J* 9 and 5, 8-H), 4.43 (1 H, br d, *J* 9, 7-H) and 5.91 (1 H, br s, 10-H).

(*E*)-3-Methyl-4-[(2,6,6-trimethylcyclohex-1-enyl)methylene]-cyclohex-2-enone 16

Triethylamine (11.1 cm^3, 79.6 mmol), 4-(dimethylamino)-pyridine (DMAP) (3.88 g, 31.8 mmol) and acetic anhydride (3.0 cm^3, 31.8 mmol) were added to a solution of keto alcohol **15** (4.17 g, 15.9 mmol) in dry CH_2Cl_2 (30 cm^3) at 0 °C and the mixture was stirred at rt for 4 h. After being poured into water, the reaction mixture was extracted with CH_2Cl_2. The extract was washed with brine, dried and evaporated to give a residue, which was purified by CC (AcOEt–hexane, 1:6) to provide the acetate of compound **15** (4.02 g, 83%) as an oil, ν_{max}(CHCl$_3$)/cm^{-1} 1728 (OAc) and 1660 (C=O); λ_{max}(EtOH)/nm 235; δ_H(200 MHz; CDCl$_3$) 1.10 and 1.14 (each 3 H, each s, *gem*-Me), 1.80 (3 H, s, 5-Me), 2.01 (3 H, s, OAc), 2.05 (3 H, s, 9-Me), 2.27 and 2.54 (each 1 H, each m, 8′-H$_2$), 2.99 (1 H, dt, *J* 9.5, 5, 8-H), 5.89 (1 H, s, 10-H) and 5.93 (1 H, d, *J* 9.5, 7-H) (Found: M$^+$ − OAc, 245.1921. $C_{17}H_{25}O$ requires *m/z*, 245.1905).

A mixture of a solution of the acetate (3.98 g, 13.1 mmol) in dry toluene (5 cm^3) and a solution of DBU (3.98 g, 26.2 mmol) in dry toluene (5 cm^3) was refluxed for 1 h. After being cooled to rt, the mixture was evaporated to remove toluene to give a residue, which was purified by CC (AcOEt–hexane, 1:6) to afford the *title compound* **16** (2.65 g, 83%) as an oil (Found: C, 83.27; H, 10.17%; M$^+$, 244.1837. $C_{17}H_{24}O$ requires C, 83.55; H, 9.90%; *M*, 244.1826); ν_{max}(CHCl$_3$)/cm^{-1} 1654 (C=O); λ_{max}(EtOH)/nm 296; δ_H(300 MHz; CDCl$_3$) 0.97 (6 H, br s, *gem*-Me), 1.49 (3 H, s, 5-Me), 2.10 (3 H, s, 9-Me), 2.42 and 2.52 (each 2 H, each m, 8a- and 8b-H$_2$), 5.87 (1 H, s, 10-H) and 6.40 (1 H, s, 7-H); NOESY, see Scheme 2; δ_C(75 MHz; CDCl$_3$) 19.8 (C-3), 21.1 (9-Me), 21.9 (5-Me), 27.3 (C-8a), 29.0 (*gem*-Me), 32.4 (C-4), 35.3 (C-1), 37.6 (C-8b), 39.5 (C-2), 127.1 (C-8), 129.9 (C-10), 131.3 (C-7), 135.7 and 136.7 (C-5 and -6), 156.3 (C-9) and 200.4 (C-11).

(*E,E/Z*)-3-{3-Methyl-4-[(2,6,6-trimethylcyclohex-1-enyl)methylene]cyclohex-2-enylidene}propan-2-one 17 and 18

In the same manner as described for the synthesis of tetraenones **8** and **9** from trienone **5**, trienone **16** (410 mg, 1.68 mmol) was converted into a mixture of the title compounds **17** and **18** (197 mg, 41%) as a pale yellow oil and some starting material **16** (113 mg, 28%) was recovered. The mixture of geometrical isomers was separated by PHPLC [LiChrosorb Si-60 (7 μm), 1 × 25 cm, AcOEt–hexane, 3:197] to give the less polar compound **17** and the more polar compound **18** each in a pure state, in the ratio ~1:1.

11E-*Isomer* **17**: ν_{max}(CHCl$_3$)/cm^{-1} 1659 (C=O) and 1562 (C=C); λ_{max}(EtOH)/nm 339 and 239; δ_H(200 MHz; CDCl$_3$) 0.95 (6 H, br s, *gem*-Me), 1.46 (3 H, s, 5-Me), 2.01 (3 H, s, 9-Me), 2.18 (3 H, s, 13-Me), 5.95 and 5.97 (each 1 H, each s, 10- and 12-H) and 6.14 (1 H, s, 7-H) (Found: M$^+$, 284.2137. $C_{20}H_{28}O$ requires *M*, 284.2138).

11Z-*Isomer* **18**: ν_{max}(CHCl$_3$)/cm^{-1} 1662 (C=O) and 1579 (C=C); λ_{max}(EtOH)/nm 341; δ_H(200 MHz; CDCl$_3$) 0.95 (6 H, br s, *gem*-Me), 1.45 (3 H, s, 5-Me), 2.04 (3 H, s, 9-Me), 2.17 (3 H, s, 13-Me), 5.82 (1 H, s, 12-H), 6.18 (1 H, s, 7-H) and 7.42 (1 H, s, 10-H) (Found: M$^+$, 284.2130).

Ethyl (*E,E,Z/E*)-3-methyl-4-{3-methyl-4-[(2,6,6-trimethylcyclohex-1-enyl)methylene]cyclohex-2-enylidene}but-2-enoate 19 and 20

In the same manner as described for the preparation of retinoate analogues **10** and **11** from tetraenone **8**, tetraenone **17** (71 mg, 0.25 mmol) was converted to a mixture of geometrical isomers **19** and **20** (88 mg, 100%), which was separated by PHPLC [LiChrosorb Si-60 (7 μm), 1 × 25 cm, Et$_2$O–hexane, 3:97] to give the less polar compound **19** and the more polar compound **20**, each in a pure state and each as a pale yellow oil, in the ratio ~1:1.

9Z,13Z-*Isomer* **19**: ν_{max}(KBr)/cm^{-1} 1709 (CO$_2$Et) and 1597 and 1579 (C=C); λ_{max}(EtOH)/nm 348; δ_H(300 MHz; CDCl$_3$) 0.95 (6 H, br s, *gem*-Me), 1.25 (3 H, t, *J* 7, CO$_2$CH$_2$C*H*$_3$), 1.46 (3 H, s, 5-Me), 1.96 (3 H, s, 9-Me), 2.08 (3 H, s, 13-Me), 4.13 (2 H, q, *J* 7, CO$_2$C*H*$_2$CH$_3$), 5.61 (1 H, s, 14-H), 6.02 (1 H, s, 7-H), 6.11 (1 H, s, 10-H) and 6.89 (1 H, s, 12-H); δ_H(200 MHz; CDCl$_3$) 0.95 (6 H, br s, *gem*-Me), 1.25 (3 H, t, *J* 7, CO$_2$CH$_2$C*H*$_3$), 1.46 (3 H, s, 5-Me), 1.96 (3 H, s, 9-Me), 2.07 (3 H, s, 13-Me), 4.12 (2 H, q, *J* 7, CO$_2$C*H*$_2$CH$_3$), 5.61 (1 H, s, 14-H), 6.02 (1 H, s, 7-H), 6.11 (1 H, s, 10-H) and 6.89 (1 H, s, 12-H) (Found: M$^+$, 354.2567. $C_{24}H_{34}O_2$ requires *M*, 354.2557).

9Z,13E-*Isomer* **20**: ν_{max}(KBr)/cm^{-1} 1711 (CO$_2$Et) and 1578 (C=C); λ_{max}(EtOH)/nm 342; δ_H(300 MHz; CDCl$_3$) 0.94 (6 H, br s, *gem*-Me), 1.27 (3 H, t, *J* 7, CO$_2$CH$_2$C*H*$_3$), 1.47 (3 H, s, 5-Me), 1.97 (3 H, s, 9-Me), 2.28 (3 H, s, 13-Me), 4.15 (2 H, q, *J* 7, CO$_2$C*H*$_2$CH$_3$), 5.68 (1 H, s, 14-H), 5.76 (1 H, s, 12-H), 5.97 (1 H, s, 10-H) and 6.04 (1 H, s, 7-H); δ_H(200 MHz; CDCl$_3$) 0.96 (6 H, br s, *gem*-Me), 1.27 (3 H, t, *J* 7, CO$_2$CH$_2$C*H*$_3$), 1.46 (3 H, s, 5-Me), 1.97 (3 H, s, 9-Me), 2.28 (3 H, s, 13-Me), 4.15 (2 H, q, *J* 7, CO$_2$C*H*$_2$CH$_3$), 5.68 (1 H, s, 14-H), 5.76 (1 H, s, 12-H), 5.97 (1 H, s, 10-H) and 6.04 (1 H, s, 7-H) (Found: M$^+$, 354.2549).

(*E,E,E*)-3-Methyl-4-{3-methyl-4-[(2,6,6-trimethylcyclohex-1-enyl)methylene]cyclohex-2-enylidene}but-2-enoic acid 4

In the same manner as described for the preparation of all-*E*-RA analogue **3** from all-*E*-retinoate analogue **11**, hydrolysis of 9*Z*,13*E*-retinoate analogue **20** (3.5 mg, 0.010 mmol) by NaOH yielded 9Z,13E-*RA analogue* **4** (3.3 mg, 100%) as a pale yellow solid, mp 140–141 °C; ν_{max}(KBr)/cm^{-1} 3250–2500, 1680 (CO$_2$H) and 1572 (C=C); λ_{max}(EtOH)/nm 334 and 250; δ_H(200 MHz; CDCl$_3$) 0.96 (6 H, br s, *gem*-Me), 1.47 (3 H, s, 5-Me), 1.98 (3 H, s, 9-Me), 2.30 (3 H, s, 13-Me), 5.72 (1 H, s, 14-H), 5.79 (1 H, s, 12-H), 5.98 (1 H, s, 10-H) and 6.06 (1 H, s, 7-H) (Found: M$^+$, 326.2240. $C_{22}H_{30}O_2$ requires *M*, 326.2244).

(*E,E,Z*)-3-Methyl-4-{3-methyl-4-[(2,6,6-trimethylcyclohex-1-enyl)methylene]cyclohex-2-enylidene}but-2-enoic acid 21

In the same manner as described for the preparation of all-*E*-RA analogue **3** from all-*E*-retinoate analogue **11**, hydrolysis of 9*Z*,13*Z*-retinoate analogue **19** (5 mg, 0.014 mmol) by NaOH provided 9*Z*,13*Z*-RA analogue **21** (4.2 mg, 92%) as a pale yellow solid, mp 128–130 °C; ν_{max}(KBr)/cm^{-1} 3300–2400, 1680 (CO$_2$H) and 1580 (C=C); λ_{max}(EtOH)/nm 335; δ_H(200 MHz; CDCl$_3$) 0.95 (6 H, br s, *gem*-Me), 1.46 (3 H, s, 5-Me), 1.97 (3 H, s, 9-Me), 2.11 (3 H, s, 13-Me), 5.63 (1 H, s, 14-H), 6.05 (1 H, s, 10-H), 6.11 (1 H, s, 7-H) and 6.87 (1 H, s, 12-H) (Found: M$^+$, 326.2236).

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