Synthesis and biological activity of tetralone abscisic acid analogues†

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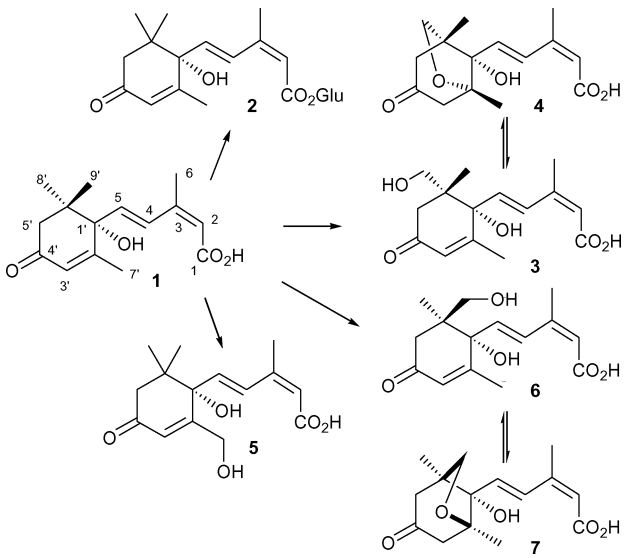
Bicyclic analogues of the plant hormone abscisic acid (ABA) were designed to incorporate the structural elements and functional groups of the parent molecule that are required for biological activity. The resulting tetralone analogues were predicted to have enhanced biological activity in plants, in part because oxidized products would not cyclize to forms corresponding to the inactive catabolite phaseic acid. The tetralone analogues were synthesized in seven steps from 1-tetralone and a range of analogues were accessible through a second route starting with 2-methyl-1-naphthol. Tetralone ABA **8** was found to have greater activity than ABA in two bioassays. The absolute configuration of (+)-**8** was established by X-ray crystallography of a RAMP hydrazone derivative. The hydroxymethyl compounds **10** and **11**, analogues for studying the roles of 8′- and 9′-hydroxy ABA **3** and **6**, were also synthesized and found to be active.

Introduction

The plant hormone abscisic acid (ABA, **1**, Scheme 1) regulates many aspects of plant growth and development as well as responses to environmental stress.[1] For example, in seed development ABA induces synthesis of storage products, prevents germination of immature embryos and is involved in desiccation tolerance and germination of mature seed.[1,2] ABA levels in plants increase transiently in response to environmental stress and trigger a set of rapid responses including closure of the stomata, reducing transpiration.

Numerous studies have been conducted to probe the structural requirements of ABA responses to develop analogues that are effective plant growth regulators.[3–7] Some features of the ABA molecule appear to be required for activity, particularly the carboxyl and ketone groups, the six-membered ring, the 7′-methyl group, and the 2-*Z* configuration of the double bond in the side chain. Other parts of the molecule can be modified without loss of activity. The ring double bond, both the 8′- and 9′-methyl groups, and the 4-*E* double bond of the side chain each can be altered and the resultant analogue retains activity.

These structure–activity results are also important for developing analogues to assess the bioactivity of metabolites, and to produce probes for identifying ABA binding proteins. As shown in Scheme 1, ABA is catabolized predominantly through oxidation of the ring methyl groups or alternatively by conjugation to the glucose ester **2**.[7–11] The principal pathway of oxidation is through P450 monooxygenase-mediated hydroxylation of the 8′-methyl group affording 8′-hydroxy ABA **3** which is in equilibrium with the closed form phaseic acid **4**.[9] Alternative pathways, through hydroxylation of the 7′-methyl group affording 7′-hydroxy ABA **5** and the 9′-methyl group to give 9′-hydroxy ABA **6**, which can also rearrange to the closed form *neo*-phaseic acid **7**, have also been observed and contribute to ABA catabolism.[10,11] Rapid catabolism by plant enzymes limits the practical application of ABA itself as a plant growth regulator.[7] Metabolism resistant analogues of ABA altered at the 8′ carbon atom have proved to be more persistent and more active than ABA.[7]



Scheme 1 Metabolism pathways of ABA (**1**).

We considered that the tetralone ABA analogue **8**, (Fig. 1) in which the planar vinyl methyl portion of ABA has been replaced with an aromatic ring, had potential to have strong biological activity as it would be unable cyclize to forms corresponding to the inactive catabolite phaseic acid **4**. The essential features of the ABA molecule are maintained, with preservation of the ABA side chain, the C-4′ ketone group, the stereochemistry at C-1′ and the

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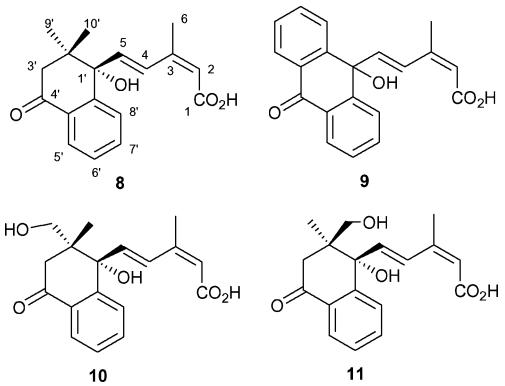


Fig. 1 ABA analogues.

planar geometry of C-3′ and C-7′. We predicted that the tetralone ABA analogue **8** would have activity as it had been shown in a previous study that a related tricyclic analogue ABA **9** had weak ABA-like response in a growth inhibition assay.[12]

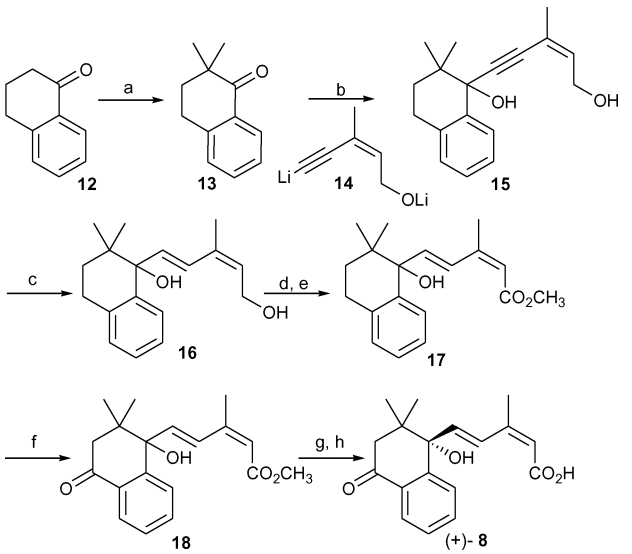
Tetralone ABA **8** and related hydroxylated compounds **10** and **11** are novel ABA analogues for probing the biological activity of ABA and its labile catabolites. We considered that a P450 monooxygenase in corn cells that hydroxylates ABA might accept the tetralone ABA analogue **8** as a substrate and generate a hydroxymethyl compound **10** (analogous to 8′-hydroxy ABA **3**). It was also predicted that the hydroxymethyl derivative, would not cyclize to a phaseic acid- like compound, as conjugate addition of the hydroxyl oxygen to the enone would be prevented, preserving the aromaticity of the fused ring, and that **10** could be employed in bioassays as a robust analogue to probe the role of 8′-hydroxy ABA. Similarly **11**, the analogue of 9′-hydroxy ABA, would be useful for probing the activity of the natural catabolite **6**.

Here we report syntheses of tetralone ABA analogues **8**, **10** and **11**. An alternate synthetic route for the production of tetralone ABA analogues with a wide range of 9′-substituents (analogous to 8′-substituted ABAs) is also discussed. The biological activity of the tetralone ABA analogue **8** is compared to that of ABA in two assays and found to be more potent. The tetralone ABA analogue can be used to probe biological activity of ABA catabolites and for affinity probes for identifying ABA binding proteins.[13] The bioactivity of hydroxylated compounds **10** and **11** is also reported.

Results and discussion

Synthesis of tetralone ABA analogue 8

Racemic tetralone methyl abscisate **18** was synthesized from commercially available 1-tetralone **12** (Scheme 2). The geminal methyl groups were introduced adjacent to the carbonyl carbon by treatment of 1-tetralone with methyl iodide in the presence of sodium hydride to give the dimethyl tetralone **13** in 83% yield.[14] The side chain was introduced by standard methods.[15] Alkylation of the dimethyl tetralone **13** with dilithium salt **14**, gave the key intermediate **15** in 64% yield. Reduction of the triple bond using RedAl® yielded the allylic alcohol **16**. Successive oxidations with manganese dioxide to the aldehyde, confirmed by the appearance of an aldehyde doublet in crude ^{1}H NMR and then with a combination of manganese dioxide, sodium cyanide, acetic acid in methanol, gave the ester **17** in 19% yield over three steps. Benzylic



Scheme 2 (a) CH_3I, NaH, THF; (b) **14**, THF; (c) RedAl, THF; (d) MnO_2, acetone; (e) MnO_2, NaCN, HOAc, MeOH; (f) PDC, *tert*-BuOOH, PhH; (g) chiral HPLC; (h) KOH, MeOH(aq).

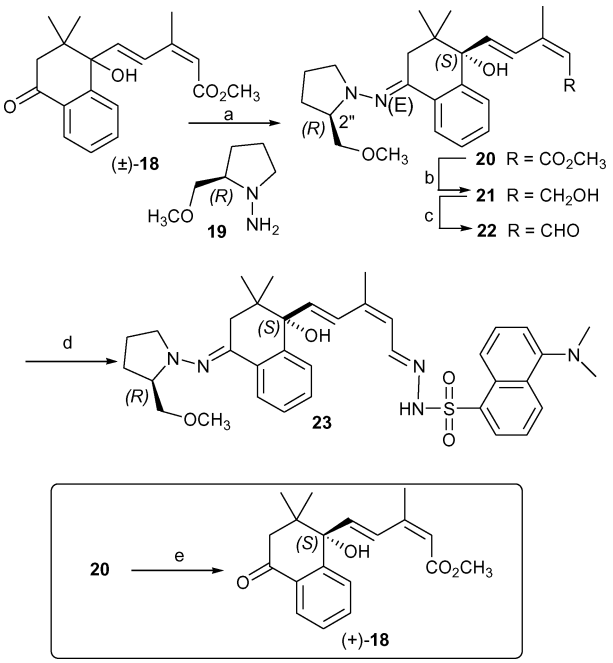
oxidation of **17** using a combination of pyridinium dichromate and *tert*-butyl hydroperoxide yielded racemic methyl ester **18**.[16] The ester **18** was resolved by preparative HPLC using a column with a chiral ligand. Base hydrolysis of the enantiomers yielded the respective enantiopure (+)- and (−)-isomers of tetralone ABA acid **8**. The absolute stereochemistry was assigned from X-ray crystallographic analysis of a derivative of the (+)-enantiomer (described below).

Conformational mobility of tetralone analogues

The ^{1}H NMR spectrum of the methyl ester **18** shows broadening of peaks, especially the H-4 (δ 7.82 ppm) of the side chain as well as the α-methylene protons at C-3′ of the ring (δ 2.5–2.9 ppm), typically a sign of restricted rotation around C-1′. This phenomenon had been observed previously with a C-1′ methyl ether ABA analogue.[5] Through variable temperature ^{1}H NMR, the peak broadening for the C-1′ methyl ether ABA had been attributed to the barrier to interconversion between conformations with sidechain–axial and sidechain–equatorial.

Absolute configuration of analogue (+)-8

The absolute stereochemistry at C-1′ of the tetralone analogue (+)-**8** was established by X-ray crystallography of a tetralone ABA derivative, which was synthesized from the tetralone ABA **18** (Scheme 3). The condensation of racemic **18** with commercially available (*R*)-1-amino-2-methoxymethyl-pyrrolidine (RAMP) **19** in the presence of *para*-toluenesulfonic acid (PTSA) gave a mixture of two diastereomers of the hydrazone **20**,[17] which were separable by column chromatography. One of the diastereomers was characterized as follows: reduction of the ester group, followed by allylic oxidation of the resulting alcohol **21**, afforded the aldehyde **22**. Condensation of the aldehyde **22** with dansyl hydrazine in the presence of trichloroacetic acid gave the derivative **23**, which gave crystals suitable for X-ray analysis. As the absolute configuration of one of the stereogenic centers (2″*R*) was known, the absolute



Scheme 3 (a) PTSA; (b) $LiAlH_4$; (c) MnO_2; (d) CCl_3CO_2H–dansyl hydrazine; (e) oxalic acid.

configuration at C-1′ was determined to be (*S*), as shown from the crystal structure (Fig. 2). The hydrazone **20** was hydrolyzed in the presence of oxalic acid to afford (+)-**18**, as determined by HPLC using the chiral column. The observed biological assay results for the tetralone analogue (+)-**8** are consistent with the biological activity in similar assays observed for natural (+)-ABA which also has the C-1′ (*S*) absolute stereochemistry.[18]

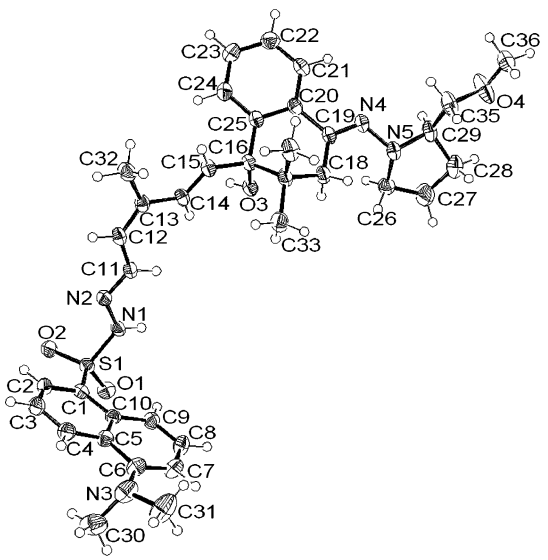
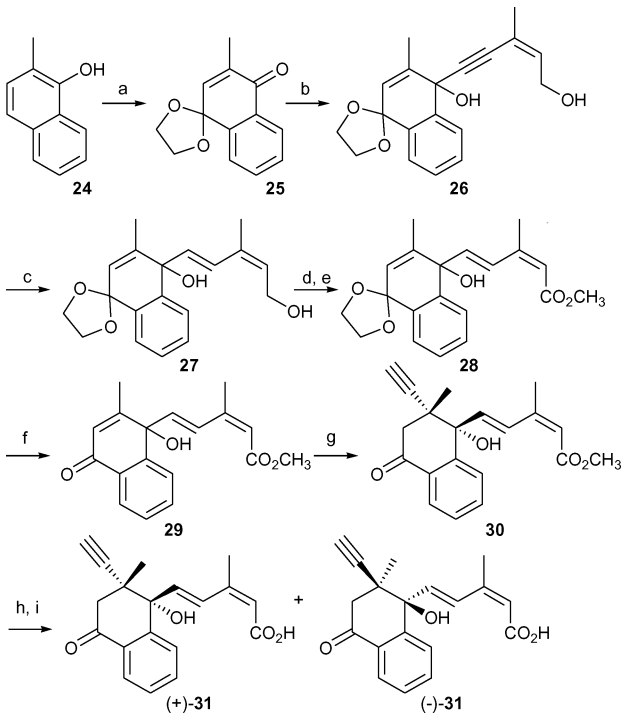


Fig. 2 Crystal structure of compound **23**.

Synthesis of methyl modified tetralone ABA analogues

An alternate synthetic route (Scheme 4) was developed to produce tetralone analogues that could have a wide range of substituents at the C-9′ (8′-substituted following ABA numbering), such as



Scheme 4 (a) $PhI(OAc)_2$–ethylene glycol; (b) **14**, THF; (c) RedAl; (d) MnO_2; (e) MnO_2–NaCN–AcOH–MeOH; (f) 10% HCl (aq); (g) HC_2MgBr; (h) chiral HPLC; (i) KOH, MeOH(aq).

analogues resistant to metabolism and the putative metabolite of (+)-**8**. More persistent ABA analogues with 8′-methylene and 8′-acetylene substituents have been synthesized and shown to have strong biological activities.[6] For example, with commercially available 2-methyl-1-naphthol (**24**) as starting material, the methyl substituted ketal **25**, was obtained through oxidation using iodobenzene diacetate. Alkylation with dilithium salt **14**, followed by triple bond reduction to intermediate **27**, two successive oxidations and deprotection of ketal **28** leads to the enone **29**. The 9′-acetylene group was introduced by the conjugate addition of ethynylmagnesium bromide to the enone **29** to afford methyl 9′-acetylene tetralone ABA **30**. Resolution of **30** by chiral HPLC followed by base hydrolysis gave enantiomers (+)-**31** and (−)-**31**. It has previously been shown that such conjugate additions afforded the product with the alkyl group adding to the same face of the molecule as the hydroxyl group at C-1′.[6,19] As well, irradiation of the 10′-methyl group of **30** (1.41 ppm) in the ^1H NMR gave a positive NOE of the proton on the side chain (H-5 doublet at 7.70 ppm), which is consistent with structure **30**.

Metabolism of (+)-8

We conducted metabolism studies to determine if the bicyclic ABA analogue **8** would be a substrate of P450 monooxygenases in corn suspension-cultured cells. Metabolism studies have been conducted in maize (*Zea mays* L. CV. Black Mexican Sweet) suspension-cultured cells, which had previously been shown to rapidly metabolize (+)-ABA to (−)-PA by hydroxylation of the 8′-methyl group.[20] The substrate ABA and metabolites were found to accumulate in the medium of the cell suspension culture. We therefore anticipated that the enantiomer of **8** like (+)-ABA would be converted to the hydroxylated compound analogous

to 8′-hydroxyABA. We also predicted that the enantiomer of **8** similar to (−)-ABA would not be converted to a compound analogous to (−)-7′-hydroxyABA, the metabolite of (−)-ABA due to the presence of the aromatic ring in compound **8**.[6] Suspension-cultured corn cells were incubated with the (+) and the (−)-enantiomers of tetralone ABA analogue **8** for 4 d (Fig. 3). The (+)-isomer was rapidly consumed (only 20% remained after 45 h), at a rate similar to that observed for (+)-ABA.[21] A single metabolite was isolated from the culture medium. Proton NMR of the isolated metabolite showed the disappearance of the 9′-methyl group at δ 1.05 ppm and the appearance of a doublet centered at δ 3.65 ppm. On the basis of the known mode of action of P450 monooxygenase in corn cells and other spectral data (mass, ^{1}H NMR, optical rotation and IR), the metabolite was confirmed to be the bicyclic 9′-hydroxy tetralone ABA (+)-**10**. The P450 monooxygenase in corn cells that metabolize ABA to 8′-hydroxyABA is likely also responsible for hydroxylation of the bicyclic ABA analogue. The analogue (+)-**10** accumulates in the corn cell medium (Fig. 3), unlike 8′-hydroxyABA **2**, which in corn cell culture, the equilibrium favors PA **4**.[21]

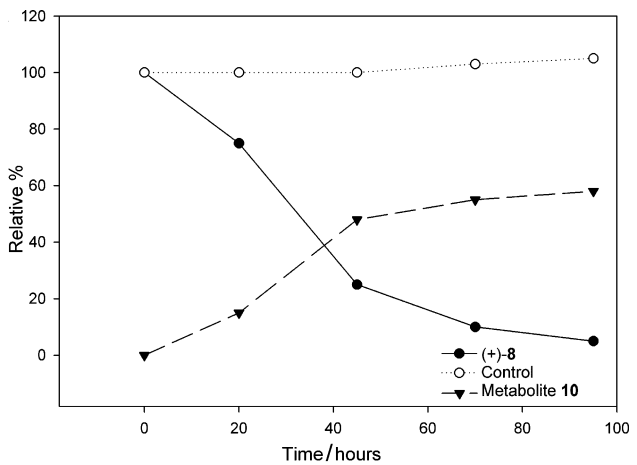


Fig. 3 Metabolism in maize suspension culture of enantiomer (+)-(*S*)-**8**.

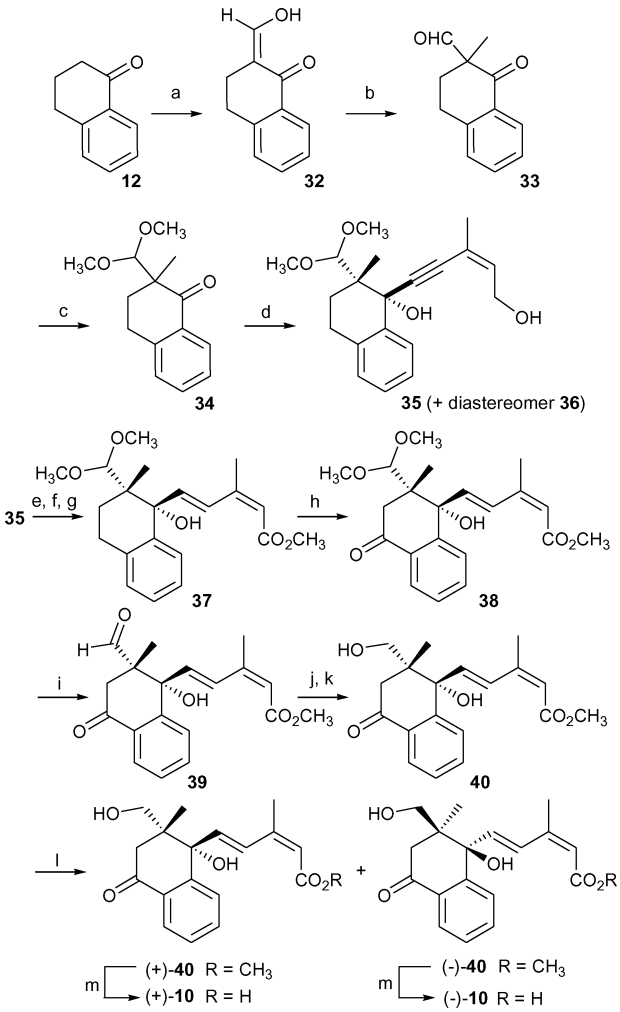
The concentration of the (−)-enantiomer of the bicyclic analogue **8**, under the same biotransformation conditions slowly decreased so that at 96 h, 50% remained and no hydroxylated product was observed. In previous studies (−)-ABA was found to be slowly transformed principally to (−)-7′-hydroxy ABA.[21]

Although these studies were performed with intact corn cells with multiple P450 monooxygenases, we are able to demonstrate that the monooxygenases present can accommodate the steric bulk of the bicyclic analogue (+)-**8**.

Encouraged by these results, we undertook to synthesize bicyclic 9′- and 10′-hydroxylated analogues to be used for probing the biological activities of the more labile 8′- and 9′-hydroxy ABA in intact plants.

Synthesis of 9′- and 10′-hydroxytetralone ABA analogues

A number of routes were attempted for the synthesis of the bicyclic 9′- and 10′-hydroxylated ABA analogues. The most efficient in our hands had as a key intermediate, the protected aldehyde **34** (Scheme 5). The major advantage of this synthetic route is that having the racemic mixture of **34**, allows us to produce the



Scheme 5 (a) $NaOCH_3$–ethyl formate; (b) CH_3I–KOH; (c) MeOH–NH_4Cl; (d) **14**, THF; (e) RedAl; (f) MnO_2; (g) MnO_2–NaCN–AcOH–MeOH; (h) PDC–*tert*-BuOOH; (i) 50% TFA–$CHCl_3$; (j) $NaBH_4$; (k) MnO_2; (l) chiral HPLC; (m) KOH, MeOH(aq).

two diastereomeric allylic alcohols (precursors for the 9′- and 10′-hydroxylated ABA analogues) from the same intermediate **34** after side chain alkylation.

Acetal **34** was obtained from readily available 1-tetralone **12**, by first introducing the hydroxymethylene group at the α-position of 1-tetralone using ethyl formate and sodium methoxide to give the hydroxymethylene tetralone **32**.[22] Methylation of **32** was achieved using methyl iodide in the presence of potassium hydroxide, which gave the required aldehyde **33** as well as a by-product formed by direct methylation of the hydroxyl group of **32**, which could be de-methylated and re-used to produce more of the required aldehyde **33**.[23] The dimethyl acetal **34** was obtained by refluxing **33** in methanol in the presence of a catalytic amount of ammonium chloride, in quantitative yield.[24] Alkylation of the keto dimethyl acetal **34** with dilithium salt **14** gave a mixture of two diastereomeric allylic alcohols, **35** and **36**, in 1.7 : 1 ratio, which were readily separable by column chromatography.

RedAl® reduction of the triple bond in **35** and successive oxidations of the resulting allylic alcohol with manganese oxide to the aldehyde, and then with manganese oxide in the presence of sodium cyanide and acetic acid affording the corresponding

aldehyde, gave the ester **37** in 37% yield. Benzylic oxidation of the ester **38**, followed by de-acetalisation with 50% aqueous TFA in chloroform mixture yielded the aldehyde **39**.[16] Reduction of the aldehyde **39** with sodium borohydride and subsequent oxidation of the crude trihydroxy ester with manganese oxide gave the dihydroxy ester **40**, which was resolved on a chiral HPLC column to afford the corresponding (+)-**40** and (−)-**40** enantiomers. Base hydrolysis of the respective enantiomers of **40** using KOH in methanol gave the corresponding enantiomeric bicyclic 9′-hydroxy ABA acids (61% yield) whose ^{1}H NMR spectra were identical to that of (+)-**10** obtained from metabolism studies of (+)-**8** (*vide supra*).

The allylic alcohol **36** was treated the same way as compound **35**, to give the corresponding enantiomeric 10′-hydroxytetralone ABA acids (+)-**11** and (−)-**11**.

Biological activity studies of tetralone ABA analogues

Growth inhibition assays. The growth inhibition assay using suspension-cultured corn cells is a well characterized experimental system that has been very useful for comparing the biological activity and metabolism of ABA and ABA analogues.[9] In this study, the tetralone ABA analogue (+)-**8**, like (+)-ABA, inhibited the growth of suspension-cultured cells of maize (Black Mexican Sweet) in a dose-dependent manner over a concentration range of 0.1–10.0 μM. As shown in Fig. 4, the analogue (−)-**8** showed inhibitory activity that is significantly higher than that of (+)-ABA. Both enantiomers of 9′-acetylene tetralone ABA **31** also had higher activity than ABA (+)-**1** in this assay.

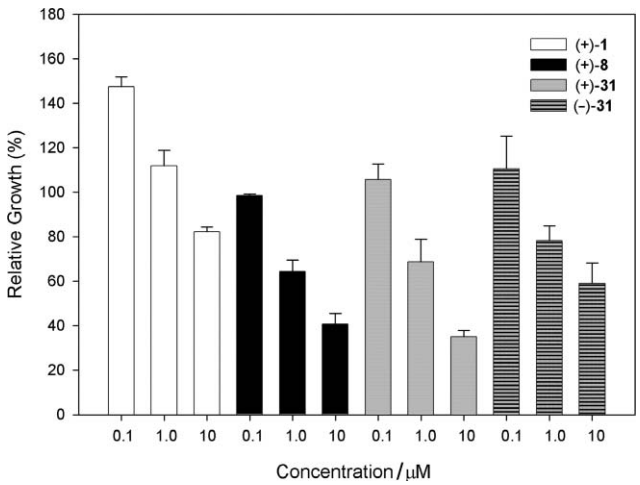


Fig. 4 Maize cell growth inhibition assay for (+)-ABA **1**, (+)-**8**, (+)-**31** and (−)-**31**.

As observed in other studies with the maize suspension culture, low concentrations of **1** were found to promote and higher concentrations inhibit growth (Fig. 5).[3] Concentrations of 1.0 micromolar or greater of (+)-**8** and both (+)- and (−)-**31** showed significant stronger inhibitory activity than (+)-**1**. In a separate experiment, at lower concentrations (+)-**10** and (+)-**11** exhibited slightly weaker activity than the natural hormone (+)-ABA. However, at the highest concentration tested (100 μM), both analogues were slightly more potent inhibitors of corn cell growth than (+)-ABA. The inhibitory activity of (+)-**10** and (+)-**11** were comparable at 1 and 10 μM, but (+)-**10** was apparently less effective at 10 μM than (+)-**11**.

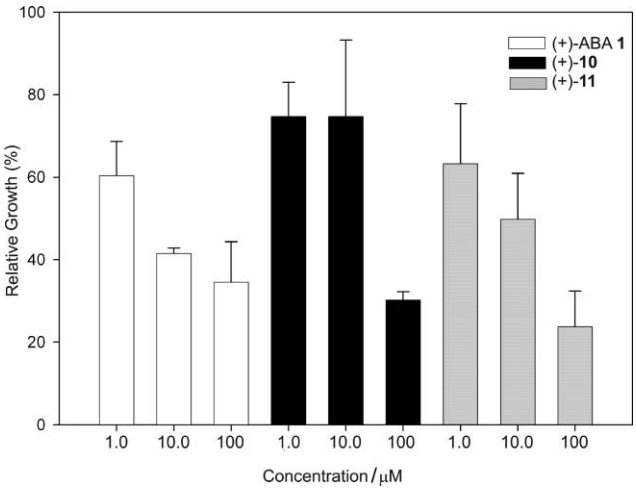


Fig. 5 Maize cell growth inhibition assay for (+)-ABA **1**, (+)-**10**, and (+)-**11**.

Arabidopsis seed germination assays. The tetralone ABA (+)-**8** was also studied in a germination assay of *Arabidopsis thaliana* (Columbia wild type) seeds over a range of concentrations (0.33–33 μM) (Fig. 6). Similar treatments were performed for ABA **1** (both enantiomers) to allow for a direct comparison between ABA and the tetralone ABA **8**. The results are expressed in terms of germination indices, which summarize the rate and extent of germination over the time of the experiment at a given concentration. As shown in Fig. 6, the (+)-enantiomer of tetralone ABA **8** was highly effective in inhibiting the germination of the seeds over the 7-day test period at all concentrations. The (+)-enantiomer of **8** is a more effective germination inhibitor than (+)-ABA. At the lowest concentration of 0.33 μM, the germination index for (+)-ABA (**1**) was almost 0.4, compared with less than 0.1 in the case of (+)-tetralone ABA **8**. As expected, the (−)-enantiomer of the tetralone ABA analogue **8** was less effective than the corresponding (+)-enantiomer. It was only active at concentrations of ≥1 μM. A similar pattern was observed

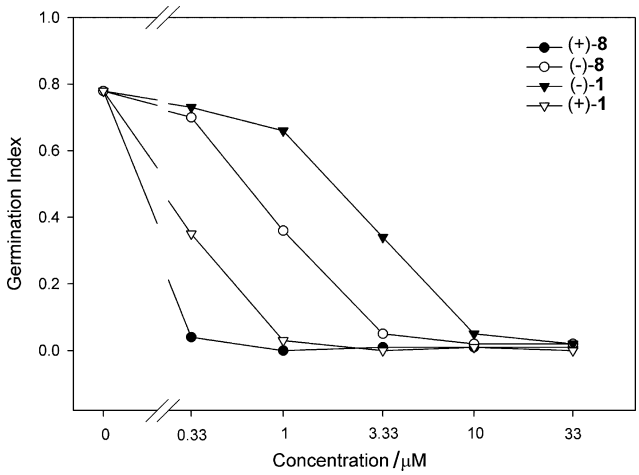


Fig. 6 *Arabidopsis* seed germination assay for (+)-**8**, (−)-**8**, (−)-**1** and (+)-**1**.

for the (−)-enantiomer of ABA **1**, which was only effective at concentrations ≥3.33 μM.

All three compounds also inhibited *Arabidopsis thaliana* seed germination (Fig. 7). Interestingly, bicyclic ABA analogue (+)-**11** and (+)-ABA produced comparable effects although (+)-**11** appeared to produce a slightly stronger inhibition of germination than (+)-ABA at the lowest concentration tested (0.33 μM). The weakest compound tested was (+)-**10**. This indicated that the 9′-derivative [ABA numbering] ((+)-**11**) possesses substantially higher activity than the 8′-derivative ((+)-**10**). This unexpected result is consistent to previous observations of very strong inhibition of ABA 8′-hydroxylase enzyme activity by 9′-derivatives.[21] Until recently, 9′-metabolites of (+)-ABA were not thought to occur naturally, however (+)-9′-hydroxy ABA **6** has recently been documented as a new ABA metabolite.[11] Metabolite **6** has been shown to be important in developing seeds of *Brassica napus*.[25] The results described here suggest that natural and synthetic 9′-ABA derivatives may have significant biological activity; even more activity than 8′-ABA derivatives.

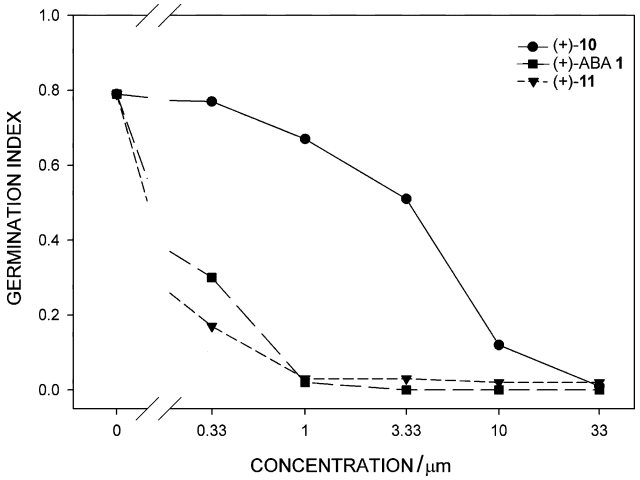


Fig. 7 *Arabidopsis* seed germination assay for (+)-**10**, (+)-ABA **1** and (+)-**11**.

Conclusion

We have successfully synthesized a novel tetralone ABA analogue **8** through an efficient 7-step synthetic scheme using readily available starting materials. This tetralone ABA analogue is significantly more active than ABA in the two assays in which the compound has been tested. The additional carbon atoms linking the C-3′ and C-7′ of ABA in the tetralone analogue do not appear to affect adversely the biological activity in either the seed germination or the corn cell growth inhibition assays. It appears that the binding sites in proteins that perceive or metabolize ABA can accommodate the extra steric bulk of the tetralone analogue. In a separate study on the structural requirements of an ABA glycosyltransferase, (+)-**8** has been found to be a better substrate than (+)-ABA **1**.[26] The presence of the aromatic moiety also has provided opportunities for the synthesis of tethered tetralone ABA analogues, which are being used in identification of ABA binding proteins.[27] The tetralone analogue has potential as a plant growth regulator for agriculture and horticulture applications. The hydroxylated hormone analogues **10** and **11** show activity in two physiological assays and will be valuable at the molecular biological level for probing the roles of ABA catabolites in processes regulated by ABA.

Experimental

General

FTIR spectra were recorded using KBr cells on a Perkin Elmer Paragon 1000 instrument. ^{1}H NMR and ^{13}C were recorded on a Bruker AM 500 MHz spectrometer. All NMR spectra were obtained using $CDCl_3$ as the solvent unless otherwise noted. Chemical shifts (δ) and coupling constants (J) are reported as if they are first order. All peak assignments refer to the numbering in structure **8**. High-resolution mass spectra (HRMS) were recorded in either electron impact (EI) mode, chemical ionization (CI) mode or in negative ion electrospray mode using capillary voltage of 2.75 KV, counter electrode 35 V, collision energy (ELAB) of 14 V and cell pressure of 1.0×10^{-3} mBar with argon. Mass spectra data are reported in mass to charge units (m/z). Optical rotations were obtained from a Perkin Elmer 141 polarimeter. Melting points were measured on an Electrothermal 9300 melting point apparatus and are not corrected.

Crystallographic data was collected at −100 °C on a Nonius Kappa CCD diffractometer, using the COLLECT program.[28] Cell refinement and data reductions used the programs DENZO and SCALEPACK.[29] SIR97[30] was used to solve the structure and SHELXL97[31] was used to refine the structure. XTAL3.7[32] was used for molecular graphics. H atoms were placed in calculated positions with U_{iso} constrained to be 1.2 times U_{eq} of the carrier atom for methine, methylene and aromatic protons and 1.5 times U_{eq} of the carrier atoms for methyl, N–H and O–H hydrogen atoms.

Seed germination test

Arabidopsis thaliana (Columbia wild type) seed germination inhibition studies were performed as described by Cutler *et al.*[21] The treatments were performed in duplicate with 50 seeds per plate and incubated at 24 °C with 16 h days and 8 h nights for the duration of the test (7 d).

Growth inhibition assay

Maize cell cultures were treated as described by Balsevich *et al.*[9] The cultures were incubated on a rotary shaker for 4 d, and then the cells were separated from the medium by vacuum filtration and weighed immediately. The effect of ABA and the tetralone ABA analogues on cell growth was determined at various concentrations (0–10 μM) by calculating the percentage increase in fresh weight [(final weight × 100/initial weight) − 100)]. Measurements were performed in triplicate and average values were normalized to a control (untreated) value of 100%.

Metabolism studies

Suspension cultures of maize (*Zea mays* L. CV. Black Mexican Sweet) were maintained as described previously by Ludwig *et al.*[20]

Isolation of hydroxylated tetralone ABA (+)-10. For each treatment, twelve 250 mL flasks containing 100 mL of medium were prepared. Eleven of these flasks contained medium to which the relevant bicyclic ABA analogue had been introduced from a 95 μM ethanolic solution to give a final concentration of 50–100 μM. The remaining flask contained an equivalent amount of ethanol to serve as an analogue-free control (C1). Ten of the flasks containing analogue-infused media, along with C1, were inoculated with cells that were 1 day post sub-culture. Typically, 2.0–3.0 g of cells was added to each flask. The remaining flask served as a control to check for compound stability over the course of the experiment (C2). Thus a control was in place to differentiate between degradation products resulting from simple chemical breakdown of the analogues (C2) and also to identify metabolites of the cells which were not attributed to the metabolism of the ABA analogues being studied (C1). The cell cultures were incubated in the dark on a rotary shaker at 25 °C for 4 d. The media from each control flask and from three of the test flasks (T1–T3) were samples for analysis at 12 h intervals.

At the indicated times, 115 mL samples were removed from C1, C2, T1, T2 and T3. From each of these samples, 100 mL was applied to a Sep Pack™ C18 cartridge that had been pre-conditioned with 1 mL methanol, followed by 1 mL H_2O. After the sample was adsorbed to the cartridge, it was washed with 5% methanol to remove salts and polar media components. Finally, adsorbed metabolites were eluted with 1 mL methanol and collected in microcentrifuge vials. The samples were then dried at 45 °C in an Eppendorf Vacufuge™ over a period of 3–4 h. The samples were re-dissolved in 50 mL methanol and diluted to 100 mL with H_2O. Samples were kept in the dark and analyzed on a Supelco (Bellefonte, PA) Supelcosil™ LC-18 column (3.3 cm × 4.6 mm i.d., 3 mm packing preceded by a Supelco C-18, 2 cm × 4.6 mm guard column). The column was eluted at 1.5 mL min^{-1} with 1% aqueous HOAc–methanol (7 : 3) using an isocratic method at 45 °C. The eluent was monitored at 262 nm using a Hewlett Packard variable wavelength detector and diode array detector referenced at 450 nm. The samples (10 μL each) were injected with an autosampler. When possible, peaks in the chromatograms were identified by comparing their retention times with those of authentic standards. At the end of the culture period, the cells were removed from all test flasks by filtration (Whatman #1) and the filtrate frozen and stored (−20 °C) until processed for metabolite and analogue isolation. After thawing, the filtrate was adjusted to pH 2.3 prior to the extraction.

The filtrate was extracted with hexanes (3 × 250 mL) then EtOAc (3 × 500 mL). The EtOAc fractions were pooled and extracted with 5% $NaHCO_3$ (2 × 330 mL). The pH of the aqueous layer was adjusted to 2.4 and then extracted with EtOAc (3 × 330 mL). The combined organic fractions were washed with saturated NaCl (150 mL), dried over anhydrous Na_2SO_4, filtered and the solvent removed *in vacuo* to give a crude extract. The extract was dissolved in 1 mL methanol then further purified using preparative HPLC (Partisil™ 10 ODS-2 M9/25 column preceded by a Superguard™ C18 2 cm × 4.6 i.d. guard column) with 1% aqueous HOAc–acetonitrile (4 : 6). Combined fractions of each peak from successive runs were pooled and the solvent removed *in vacuo*.

The methyl ester derivatives of each of the samples were made by treatment with CH_2N_2–etherate. Further purification was performed with semi-preparative Chiracel™ AS column (Daicel Chemical Industries, Ltd) with the products eluting in *iso*-PrOH-hexane (20:80). Spectroscopic characterization of metabolite from the culture filtrate confirmed it as the bicyclic methyl ester (+)-**40**.

Chemical synthesis

2,2-Dimethyl-3,4-dihydro-2*H*-naphthalen-1-one (13)[14]. To a suspension of NaH (8.2 g, 0.34 mol) in THF (250 mL) in a one-liter round bottomed flask, was added, 1-tetralone (10.0 g, 0.690 mol) dissolved in dry THF (25 mL). After stirring the mixture for 10 min at rt, methyl iodide (11.1 mL, 178 mmol) was added *via* a syringe. The mixture was then heated on an oil bath to 40 °C for 30 min, and stirring continued at rt until the starting material disappeared. The reaction was monitored by TLC using ethyl acetate–hexane (1 : 6) solvent mixture. The reaction was quenched by addition of water (slowly and dropwise) to destroy excess sodium hydride. The mixture was then extracted with ethyl acetate, washed with water and dried over sodium sulfate. Evaporation of the solvent yielded a brown oil. Column chromatography using silica gel with EtOAc–hexane (1 : 6) afforded clean 2,2-dimethyl-1-tetralone **13** (10.8 g, 83%). IR (ν_{max}): 2956, 1682, 1601 cm^{-1}. ^1H NMR: δ 8.03 (d, 1H, $J = 7.5$ Hz, ArH-8), 7.45 (t, 1H, $J = 7.5$ Hz, ArH-7), 7.28 (t, 1H, $J = 7.5$ Hz, ArH-6), 7.21 (d, 1H, $J = 7.5$ Hz, ArH-5), 2.97 (t, 2*H*, $J = 6.5$ Hz, 2 H-4), 1.97 (t, 2*H*, $J = 6.5$ Hz, 2 H-3), 1.20 (s, 6H, 2 × CH_3). HRMS (*m*/*z*) $C_{12}H_{14}O$ requires: 174.1045; found: 174.1031.

(*Z*)-1-(5′-Hydroxy-3′-methylpent-3′-en-1′-ynyl)-2,2-dimethyl-1,2,3,4-tetrahydronaphthalen-1-ol (15). (*Z*)-3-Methylpent-2-en-4-yn-1-ol (5.0 g, 52 mmol) in dry THF (300 mL) was cooled to −78 °C under an atmosphere of argon. *n*-Butyl lithium (70.0 mL, 1.6 M in hexanes, 112 mmol) was then added slowly, *via* syringe. The mixture was allowed to stir at −78 °C for 45 min, after which, 2,2-dimethyl-1-tetralone **13** (7.5 g, 43 mmol), dissolved in dry THF (50 mL), was added. The mixture was stirred for a further 15 min at −78 °C and then the ice bath was removed. The reaction mixture was stirred at rt for a further 3 h, by which point, the starting material had disappeared. The reaction was quenched by addition of a saturated solution of ammonium chloride. The mixture was stirred for 10 min and extracted with ethyl acetate (3 × 150 mL), washed with water (2 × 200 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded the desired alcohol as a brown oil. Column chromatography of the brown oil using silica gel with ethyl acetate–hexane (1 : 2) gave allylic alcohol **15** (6.1 g, 78%). IR (ν_{max}): 3376 (br), 2921, 2358 (w), 2211 (w), 1694 (m), 1633 (m), 1602 (w) cm^{-1}. ^1H NMR: δ 7.77 (m, ArH-8′), 7.20 (m, 2 ArH-6′ and 7′), 7.08 (m, ArH-5′), 5.84 (t, $J = 6.5$ Hz, 1 H-2), 4.27 (d, $J = 6.5$ Hz, 2 H-1), 2.84 (m, 2 H-4′), 2.00 (ddd, $J =$ 13.5, 2.0, 7.0 Hz, 1 H-3′), 1.88 (s, 3 H-6), 1.66 (ddd, $J = 13.5$, 1.0, 5.0 Hz, 1 H-3′), 1.15 (s, 3 H-9′/10′), 1.07 (s, 3 H-9′/10′). ^{13}C NMR: δ 138.8, 135.7, 134.9, 129.0, 128.2, 127.9, 126.4, 120.7, 96.5, 84.4, 75.1, 61.4, 37.5, 31.2, 25.7, 23.9, 23.6, 23.2. HRMS (*m*/*z*) $C_{18}H_{22}O_2$ requires: 269.1542 $[M-1]^-$; found 269.1536.

Methyl (2*Z*,4*E*)-(1′*RS*)-5-(2′,2′-dimethyl-1′-hydroxy-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl)-3-methylpenta-2,4-dienoate (17). The allylic alcohol **15** (6.0 g, 22.1 mmol) in dry THF was cooled to −78 °C and RedAl® (13.7 ml, 44.2 mmol) added dropwise *via* syringe. The reaction mixture was stirred at −78 °C for 1 h and

the allowed to warm up to 0 °C and stirred for a further 2 h. The reaction was quenched by slow addition of water (100 mL) and extracted with diethyl ether (2 × 200 mL). The organic phase was washed with water (2 × 200 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent left a crude brown oil of the allylic alcohol **16** (6.05 g), which was carried through to the next stage without any further purification. The crude allylic alcohol **16** (6.05 g, 22.2 mmol) was dissolved in dry acetone (250 mL) and manganese dioxide (38.7 g, 445 mmol) was added. The mixture was stirred at rt for 3 h, after which all the starting material had disappeared. The black suspension was then filtered through a bed on Celite®. Evaporation of solvent left a clear brown oil of the aldehyde (4.29 g), which was carried through to the next stage without any further purification. ^{1}H NMR of the crude mixture showed the presence of an aldehyde proton. To the aldehyde (4.29 g, 15.9 mmol), dissolved in methanol (150 mL), were added, manganese dioxide (27.7 g, 318.0 mmol), sodium cyanide (2.80 g, 57.2 mmol) and glacial acetic acid (1.05 g, 17.5 mmol). The mixture was stirred at rt for 4 h, after which all the starting material had disappeared. The suspension was filtered over a bed of Celite® and washed with methanol (3 × 100 mL). The combined filtrate was then concentrated under vacuum to yield a light brown solid. Water (150 mL) was then added to the crude solid and then extracted with ethyl acetate (3 × 200 mL). The organic phase was washed with water (3 × 100 mL) and dried over anhydrous Na_2SO_4. Evaporation of solvent yielded a brown oil. Column chromatography using silica gel and 25% ethyl acetate in hexane gave methyl ester **17** (3.3 g, 49%) over the three steps. IR (ν_{max}): 3500, 2924, 1715, 1633, 1601 cm^{-1}. ^{1}H NMR: δ 7.79 (d, J = 16.0 Hz, 1 H-4), 7.36 (dd, J = 6.5 and 1.0 Hz, ArH-8′), 7.16 (m, 2 ArH), 7.11 (m, 1 ArH), 6.30 (d, J = 16.0 Hz, 1 H-5), 5.68 (s, 1 H-2), 3.67 (s, 3 H, CO_2CH_3), 2.86 (t, J = 7.0 Hz, 2 H-4′), 1.98 (s, 3 H-6), 1.89 (dt, J = 14.0, 7.0 Hz, 1 H-3′), 1.68 (dt, J = 14.0, 7.0 Hz, 1 H-3′), 1.00 (s, 3 H-9′/10′), 0.96 (s, 3 H-9′/10′). ^{13}C NMR: δ 166.7, 150.3, 141.4, 140.4, 135.7, 128.9, 128.2, 127.3, 126.6, 126.4, 117.0, 78.1, 51.0, 37.2, 33.0, 25.9, 24.1, 23.1, 21.3. HRMS (m/z) $C_{19}H_{24}O_3$ requires: 300.1725; found: 300.1721.

Methyl (2*Z*,4*E*)-(1′*S*)-5-(2′,2′-dimethyl-1′-hydroxy-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl)-3-methylpenta-2,4-dienoate ((+)-18). To the ester **17** (3.0 g, 10 mmol) dissolved in benzene (100 mL), were added, pyridinium dichromate (5.64 g, 30 mmol) and *tert*-butyl hydroperoxide (1.35 g, 15 mmol). The mixture was stirred at rt for 4 h. Diethyl ether (50 mL) was added to the reaction mixture and stirring continued for a further 30 min. The mixture was then filtered through a bed of Celite® and washed with diethyl ether (3 × 25 mL). The combined organic filtrate was then concentrated *in vacuo*, leaving a brown oil. Column chromatography using silica gel with 25% ethyl acetate in hexane afforded the unreacted starting material (1.20 g) with R_f 0.5 and the desired ester **18** (1.48 g, 78%, based on amount of starting material consumed). IR (ν_{max}): 3457, 3067, 2962, 1722, 1682, and 1599 cm^{-1}. ^{1}H NMR: δ 8.03 (dd, J = 1.0, 8.0 Hz, ArH-5′), 7.82 (d, J = 16.0 Hz, 1 H-4), 7.56 (m, 2 ArH-6′ and 8′), 7.42 (t, J = 7.0 Hz, ArH-7′), 6.35 (d, J = 16.0 Hz, 1 H-5), 5.72 (s, 1 H-2), 3.66 (s, 3 H, CO_2CH_3), 2.80 (d, J = 17.0 Hz, 1 H-3′), 2.56 (d, J = 17.0 Hz, 1 H-3′), 1.98 (s, 3 H-6), 1.07 (s, 3 H-9′/10′), 1.06 (s, 3 H-9′/10′). ^{13}C NMR: δ 197.3, 166.4, 149.4, 145.7, 138.5, 134.4, 130.9, 128.1 (2), 127.2, 126.7, 118.1, 78.2, 51.1, 49.7, 41.0, 24.3, 23.4, 21.7. HRMS (m/z) $C_{19}H_{22}O_4$ requires: 314.1518; found: 314.1521. The enantiomers of ester **18** were resolved by chiral HPLC (Chiralcel™ AS column (10 × 250 mm; Daicel Chemical Industries, Ltd., *iso*-PrOH–hexane, 3 : 97) and had the following optical rotations: $[a]_D^{25}$ +247 (c 1.2, $CHCl_3$) (retention time 12.5 min) and −243 (c 1.0, $CHCl_3$) (retention time 15.8 min) for (+)-**18** and (−)-**18**, respectively.

(2*Z*,4*E*)-(1′*S*)-5-(2′,2′-Dimethyl-1′-hydroxy-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl)-3-methylpenta-2,4-dienoic acid (+)-8). A mixture of ester (+)-**18** (0.05 g, 0.159 mmol) in MeOH (4 mL) and 1.0 M KOH (4 mL) was stirred at 45 °C for 2 h, by which point, all the starting material had disappeared. The solvent was evaporated at reduced pressure, the aqueous layer acidified to pH 3 with 10% Cl and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated to provide acid (+)-**8** (0.037 g, 76%). $[a]_D^{25}$ +193 (c 1.0, MeOH). IR (ν_{max}): 3606–2488, 3453,1685, 1598 cm^{-1}. ^{1}H NMR: δ 8.02 (dd, J = 7.8, 1.2 Hz, ArH-5′), 7.74 (d, J = 16.0 Hz, 1 H-4), 7.52–7.59 (m, 2 ArH-7′ and 8′), 7.38–7.41 (m, ArH-6′/7′), 6.42 (d, J = 16.0 Hz, 1 H-5), 5.72 (s, 1 H-2), 2.80 (d, J = 17.0 Hz, 1 H-3′), 2.56 (d, J = 17.0 Hz, 1 H-3′), 2.02 (s, 3 H-6), 1.08 (s, 3 H-9′/10′), 1.06 (s, 3 H-9′/10′). ^{13}C NMR: δ 197.4, 171.0, 151.8, 145.6, 139.2, 134.5, 130.9, 128.4, 128.2, 127.2, 126.7, 117.7, 78.4, 60.4, 49.7, 41.1, 24.3, 23.4, 21.4. HRMS (m/z) $C_{18}H_{20}O_4$ requires: 300.1362; found: 300.1351.

Methyl (2*Z*,4*E*,4′*E*)-(1′*S*,2″*R*)-5-[2′,2′-dimethyl-1′-hydroxy-4′-(2″-methoxymethylpyrrolidin-1″-ylimino)-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (20). To a mixture of ester **18** (42 mg, 0.13 mmol), RAMP (20 μL, 0.14 mmol), PTSA (9.2 mg, 0.048 mmol) in dry toluene (2 mL) was heated at 110–112 °C for 1 d. The reaction mixture was diluted with CH_2Cl_2 after it was cooled to rt. The organic layer was washed with sat. $NaHCO_3$, dried, and evaporated to give a residue. The residue was separated by flash column chromatography (10–40 μ silica gel was used with 20% ethyl acetate–hexane) to provide hydrazone **20** (22.2 mg, 40%), $[a]_D^{25}$ −425 (c 1.3, CH_2Cl_2) and an inseparable mixture of starting material and the other diastereomeric product (25.3 mg). IR (ν_{max}): 3457, 2965, 1714, 1634, 1601 cm^{-1}. ^{1}H NMR: δ 8.11 (m, 1H, Ar–H), 7.96 (d, 1H, J = 16.0 Hz, H-4), 7.23 (m, 3H, Ar–H), 6.31 (d, 1H, J = 16.0 Hz, H-5), 5.69 (s, 1H, H-2), 3.50 (m, 2H, CH_2OCH_3), 3.35 (m, 1H, H-5″), 3.32 (s, 3H, OCH_3), 3.29 (m, 1H, H-2″), 2.72 (d, 1H, J = 16.0 Hz, H-3′), 2.65 (d, 1H, J = 16.0 Hz, H-3′), 2.51 (q, 1H, J = 8.5 Hz, H-5″), 2.03 (m, 1H, H-3″), 2.00 (s, 3H, 6-CH_3), 1.85 (m, 2H, H-4″), 1.71 (m, 1H, H-3″), 0.99 (s, 3H, 10′or 9′-CH_3), 0.88 (s, 3H, 9′or 10′-CH_3). ^{13}C NMR: δ 166.6, 154.9, 150.0, 140.3, 139.7, 132.4, 129.4, 127.9, 127.8, 127.2, 124.8, 117.2, 77.7, 75.6, 67.0, 59.2, 54.6, 51.1, 39.1, 38.6, 26.8, 24.2, 24.0, 22.9, 21.3. HRMS (m/z) $C_{25}H_{34}N_2O_4$ requires: 427.2596 [M + 1]$^+$; found 427.2597.

Hydrolysis of hydrazone 20

A mixture of RAMP–hydrazone **20** (12.0 mg, 0.028 mmol) in hexane (2 mL) and CH_2Cl_2 (0.1 mL) saturated oxalic acid (0.5 mL) was stirred at rt for 3 d. The reaction mixture was extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, dried and evaporated to give a residue. The residue was purified by flash column chromatography (25% ethyl acetate–hexane) to afford a product

which from ^{1}H NMR and the retention time from chiral HPLC (Chiralpak AS column, 250 × 10 mm, Diacel Chemical Industries Ltd, Japan) was confirmed as the ester (+)-**17** (5.3 mg, 61%), $[\alpha]_D^{25}$ + 255 (*c* 0.53, $CHCl_3$).

(4*E*,1′*E*,3′*Z*)-(1*S*,2″*R*)-1-(5′-Hydroxy-3′-methylpenta-1′,3′-dienyl)-4-(2″-methoxymethylpyrrolidin-1″-ylimino)-2,2-dimethyl-1,2,3,4-tetrahydronaphthalen-1-ol (21). To a suspension of $LiAlH_4$ (63.7 mg, 1.68 mmol) in anhydrous ether (15 mL) was added hydrazone **20** (113 mg, 0.28 mmol) at rt and the mixture stirred at rt for 3 h. The reaction was quenched with a drop of water and then more water added. The mixture was acidified with 3 N HCl to pH 4.0 and EtOAc added. The mixture was then stirred for 20 min and then extracted with EtOAc, dried, and concentrated to give a crude product which was purified by column chromatography on silica gel, using 30% ethyl acetate–hexane followed by 50% ethyl acetate–hexane) to provide the pure hydrazone alcohol **21** (86.9 mg, 78%). IR (ν_{max}) 3418, 2965, 2871 cm^{-1}. ^{1}H NMR: δ 8.11 (m, 1H, Ar–H), 7.25 (m, 3H, Ar–H), 6.83 (d, 1H, J = 15.5 Hz, H-4), 5.96 (d, 1H, J = 15.5 Hz, H-5), 5.56 (t, 1H, J = 7.0 Hz, H-2), 4.17 (m, 2*H*, H-1), 3.52 (m, 2*H*, CH_2OCH_3), 3.35 (m, 1H, H-5″), 3.31 (s, 3H, OCH_3), 3.29 (m, 1H, H-2″), 2.67 (s, 2*H*, H-3′), 2.51 (m, 1H, H-5″), 2.03 (m, 1H, H-3″), 1.86 (m, 2*H*, H-4″), 1.86 (s, 3H, 6-CH_3), 1.72 (m, 1H, H-3″), 0.97 (s, 3H, 10′or 9′-CH_3), 0.88 (s, 3H, 9′or 10′-CH_3). ^{13}C NMR: δ 155.1, 140.7, 134.4, 133.5, 132.5, 129.3, 128.4, 127.8, 127.1, 126.8, 124.8, 77.8, 75.6, 66.9, 59.2, 58.4, 54.6, 39.2, 38.4, 26.8, 24.2, 24.0, 22.9, 20.8. HRMS (*m*/*z*) $C_{24}H_{35}N_2O_3$ requires: 399.2647 $[M + 1]^+$; found: 399.2656.

(2*Z*,4*E*,4′*E*)-(1′*S*,2″*R*)-5-[1′-Hydroxy-4′-(2″-methoxymethylpyrrolidin-1″-ylimino)-2′,2′-dimethyl-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienal (22). A mixture of hydrazone alcohol **21** (69 mg, 0.17 mmol) and MnO_2 (300.8 mg, 3.46 mmol) in acetone (5 mL) was stirred at RT for 16 h. The reaction mixture was filtered over a bed of Celite® and washed with acetone. The combined filtrates and washings were evaporated to give a residue, which was purified by column chromatography on silica using 30% ethyl acetate–hexane to provide the hydrazone aldehyde **22** (54.4 mg, 79%) and $[\alpha]_D^{25}$ −533 (*c* 0.43, CH_2Cl_2). IR (ν_{max}): 3429, 3060, 2965, 2873, 1666, 1632 cm^{-1}. ^{1}H NMR: δ 10.2 (d, 1H, J = 8.0 Hz, H-1), 8.16 (m, 1H, Ar–H), 7.58 (d, 1H, J = 15.5 Hz, H-4), 7.27 (m, 3H, Ar–H), 6.38 (d, 1H, J = 15.5 Hz, H-5), 5.90 (d, 1H, J = 8.0 Hz, H-2), 3.54 (m, 2*H*, CH_2OCH_3), 3.40 (m, 1H, H-5″), 3.34 (s, 3H, OCH_3), 3.32 (m, 1H, H-2″), 2.71 (m, 2*H*, H-3′), 2.55 (m, 1H, H-5″), 2.10 (s, 3H, 6-CH_3), 2.07 (m, 1H, H-3″), 1.89 (m, 2*H*, H-4″), 1.75 (m, 1H, H-3″), 1.03 (s, 3H, 10′or 9′-CH_3), 0.94 (s, 3H, 9′or 10′-CH_3). ^{13}C NMR: δ 190.4, 154.0, 153.6, 140.8, 139.7, 132.6, 129.5, 128.9, 128.3, 127.0, 125.7, 125.1, 78.0, 75.6, 67.0, 59.2, 54.8, 39.2, 38.7, 26.8, 24.3, 24.0, 23.0, 21.6. HRMS (*m*/*z*) $C_{24}H_{33}N_2O_3$ requires: 397.2458 $[M + 1]^+$; found: 397.2490.

(1*E*,2′*Z*,4′*E*,4″*E*)-(1″*S*,2‴*R*)-5-(Dimethylamino)-*N*′-{5′-[1″-hydroxy-4″-(2‴-(methoxymethyl)pyrrolidin-1‴-ylimino)-2″,2″-dimethyl-1″,2″,3″,4″-tetrahydronaphthalen-1″-yl]-3′-methylpenta-2′,4′-dienylidene}-naphthalene-1-sulfonohydrazide (23). A mixture of the hydrazone aldehyde **22** (48.9 mg, 0.12 mmol), dansyl hydrazine (32.8 mg, 0.12 mmol) and trichloroacetic acid (8.6 mg, 0.053 mmol) in ethanol (2 mL) was heated at 75 °C for 5 min. The reaction was quenched by addition of several drops of sat. $NaHCO_3$. The ethanol was then removed *in vacuo* to give a residue, which was diluted with CH_2Cl_2 and washed with water, dried and concentrated to provide a crude product. The crude product was purified by column chromatography on silica gel (10–40 μ) using 30% ethyl acetate in hexane to give the pure dansyl hydrazone **23** (62.4 mg, 79%) as a yellow powder. The yellow powder was then recrystallized from hexane–ethyl acetate (4 : 1) to give the crystalline product: Mp: 110.1–114.9 °C (decomposition), $[\alpha]_D^{25}$ −306 (*c* 0.38, CH_2Cl_2). IR (ν_{max}): 3513, 3214, 3059, 2960, 2871, 1689 (w), 1610, 1574 cm^{-1}. ^{1}H NMR: δ 8.54 (d, 1H, J = 7.5 Hz, Ar–H), 8.35 (m, 2*H*, Ar–H), 8.24 (br s,1H, NH), 8.12 (m, 1H, Ar–H), 7.91 (d, 1H, J = 10.0 Hz, H-1), 7.53 (m, 2*H*, Ar–H), 7.25 (m, 3H, Ar–H), 7.15 (d, 1H, J = 7.5 Hz, Ar–H), 6.86 (d, 1H, J = 15.0 Hz, H-4), 6.04 (d, 1H, J = 15.0 Hz, H-5), 5.93 (d, 1H, J = 10.0 Hz, H-2), 3.52 (m, 2*H*, CH_2OCH_3), 3.38 (m, 1H, H-5″), 3.32 (m, 1H, H-2″), 3.32 (s, 3H, OCH_3), 2.86 (s, 6H, $N(CH_3)_2$), 2.66 (m, 2*H*, H-3′), 2.54 (m, 1H, H-5″), 2.02 (m, 1H, H-3‴), 1.88 (m, 2*H*, H-4″), 1.88 (s, 3H, 6-CH_3), 1.70 (m, 1H, H-3″), 0.95 (s, 3H, 10′or 9′-CH_3), 0.88 (s, 3H, 9′or 10′-CH_3). ^{13}C NMR: δ 154.4, 151.9, 145.0, 141.0, 140.3, 136.1, 133.6, 132.5, 131.14, 131.08, 129.82, 129.75, 129.4, 128.4, 128.1, 127.0, 126.2, 124.8, 124.4, 123.3, 118.9, 115.2, 78.0, 75.6, 66.7, 59.0, 54.8, 45.4, 39.2, 38.6, 26.7, 24.2, 24.0, 22.9, 21.0. HRMS (*m*/*z*) $C_{36}H_{46}N_5O_4S$ requires: 644.3270 $[M + 1]^+$; found: 644.3265. Single crystals of $C_{36}H_{45}N_5O_4S$ **23** were recrystalized from methanol, mounted in inert oil and transferred to the cold gas stream of the diffractometer.

Crystal structure determination of compound 23§

Crystal data. $C_{38}H_{53}N_5O_6S$, M = 707.91, orthorhombic, a = 11.2340(2), b = 16.3940(2), c = 20.9930(3) Å, U = 3866.28(10) Å^3, T = 173(2) K, space group $P2_12_12_1$, Z = 4, μ = 0.134 mm^{-1}, 32824 reflections measured, 4913 unique (R_{int} = 0.0637) which were used in all calculations. The final wR_2 was 0.0992 (all data). The absolute configuration was known from the derivatization and was not independently determined. There are two solvent methanol molecules per asymmetric unit in the crystal. Both are almost as well ordered as the major molecule and both are involve in strong H-bonding schemes with one another and the major molecule. Two atoms are showing asymmetric thermal ellipsoids (O4 and C31), one being an ether oxygen and the other a terminal methyl group. The disorder is not sufficiently severe so as to require modelling of the disorder. Both groups would be expected to have high vibrational motion. Most of the checkCIF errors are related to the fact that the absolute configuration was not determined using Friedel mates, because the configuration of the derivative was known.

2-Methyl-4,4-ethylenedioxynaphthalen-1-one (25). 2-Methyl-1-naphthol **24** (5.0 g, 31.6 mmol) dissolved in ethylene glycol (100 mL) was added to a round bottomed flask (500 mL), containing iodobenzene diacetate (21.4 g, 66.4 mmol) dissolved in ethylene glycol (100 mL) and stirred at rt for 4 h. Reaction was quenched by addition of H_2O (50 mL) followed by extraction with diethyl ether (3 × 150 mL). The organic phase was washed with saturated NaCl solution (2 × 200 mL), dried over anhydrous Na_2SO_4 and dried *in vacuo.* Flash chromatography using silica gel

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with 50% ether in hexane yielded **25** (4.4 g, 64%). Mp: 125.1–125.9 °C IR (ν_{max}): 3290, 3074, 2984, 2910, 1658 cm^{-1}. ^{1}H NMR: δ 8.05 (dt, 1H, J = 8.0, 1.0 Hz, Ar–H), 7.57 (m, 2*H*, Ar–H), 7.46 (ddd, 1H, J = 3.0, 5.5, 8.0 Hz, Ar–H), 6.62 (q, 1H, J = 1.5 Hz, H-3), 4.33 (m, 2*H*), 4.25 (m, 2*H*), 1.99 (d, 3H, J = 1.5 Hz)). ^{13}C NMR: δ 184.6, 141.1, 138.4, 135.7, 132.9, 130.9, 129.4, 126.5, 126.3, 100.2, 65.8, 16.0. HRMS (*m*/*z*) $C_{13}H_{12}O_3$ requires: 216.0786; found: 216.0790.

Methyl (2*Z*,4*E*)-(1′*RS*)-5-(1′-hydroxy-2′-methyl-4′-oxo-1′,4′-dihydronaphthalen-1′-yl)-3-methylpenta-2,4-dienoate (29). Compound **25** (2.0 g, 9.3 mmol) converted to ketal ester **28** in the same manner as compound **13** was converted methyl ester **17**. To the above brown oil (ketal ester **28**, 1.4 g) in THF (50 mL) in an ice bath, was added 10% HCl (2 mL) and mixture stirred for 1 h, after which all starting material had disappeared. Water (20 mL) was added to mixture and extracted with diethyl ether (3 × 100 mL). The organic phase was washed with saturated NaCl (100 mL) and dried over anhydrous Na_2SO_4 and dried *in vacuo*. Flash chromatography using silica gel with 3 : 1 (diethyl ether–hexane) mixture afforded enone **29** (1.2 g, 75% over five steps) as white crystals. Mp = 147–148 °C (EtOAc). IR (ν_{max}): 3402, 3070, 2951, 1710, 1657, 1599 cm^{-1}. ^{1}H NMR: δ 8.09 (d, 1H, J = 15.9 Hz), 8.01 (d, 1H, J = 7.8 Hz), 7.64 (d, 1H, J = 7.8 Hz), 7.54 (t, 1H, J = 7.8 Hz), 7.39 (t, 1H, J = 7.8 Hz), 6.21 (s, 1H), 5.75 (d, 1H, J = 15.9 Hz), 5.69 (s, 1H), 3.67 (s, 3H), 2.08 (s, 3H), 1.87 (s, 3H). ^{13}C NMR: δ 183.9, 166.4, 160.6, 149.6, 145.8, 138.4, 133.1, 129.4, 128.2, 127.5, 126.8, 126.4, 126.1, 118.2, 73.4, 51.2, 20.9, 18.6. HRMS (*m*/*z*) $C_{18}H_{18}O_4$ requires: 298.1205; found: 298.1190.

Methyl (2*Z*,4*E*)-(1′*S*,2′*R*/1′*R*,2′*S*)-5-(2′-ethynyl-1′-hydroxy-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl)-3-methylpenta-2,4-dienoate (30). Enone **29** (1.00 g, 3.35 mmol) in dry THF (15 mL) was added dropwise to a cold (0 °C) solution of ethynylmagnesium bromide (33.6 mL, 0.5 M in diethyl ether) in THF (15 mL). After stirring for 6 h, the reaction mixture was quenched by the addition of saturated NH_4Cl (25 mL), followed by extraction with diethyl ether (3 × 100 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered through Celite and dried *in vacuo*, leaving a yellow oil (1.18 g). Flash chromatography using silica gel with 20% ethyl acetate in hexane yielded methyl 8′-acetylene tetralone ABA **30** (0.633 g, 58%) as a light yellow solid. IR (ν_{max}): 3481, 3291, 2080, 1981, 1691, 1636, 1601 cm^{-1}. ^{1}H NMR: δ 8.03 (dd, 1H, J = 1.0, 8.0 Hz, H-5′), 7.70 (d, 1H, J = 8.0 Hz, H-8′), 7.62 (d, 1H, J = 16.0 Hz, H-4), 7.61 (dt, 1H, J = 1.0, 8.0 Hz, H-7′), 7.42 (dt, 1H, J = 1.0, 8.0 Hz, H-6′), 6.15 (d, 1H, J = 16.0 Hz, H-5), 5.68 (s, 1H, H-2), 3.61 (s, 3H, OCH_3), 2.93 (d, 1H, J = 17.1 Hz, H-3′), 2.82 (s, 1H, OH), 2.77 (d, 1H, J = 17.1 Hz, H-3′), 2.11 (s, 1H, alkyne), 1.93 (s, 3H, H-6), 1.42 (s, 3H, H-10′). ^{13}C NMR: δ 194.6, 166.1, 148.8, 146.5, 134.7, 134.5, 130.9, 130.1, 128.2, 127.4, 126.6, 118.9, 86.1, 77.2, 73.7, 51.1, 48.2, 43.9, 22.9, 20.9. HRMS (FAB$^+$, *m*/*z*) $C_{20}H_{21}O_4$ requires 325.1440 [M + 1]$^+$; found: 325.1401. The enantiomers of ester **30** were resolved by chiral HPLC (Chiralcel™ OD column (10 × 250 mm; Daicel Chemical Industries, Ltd., *iso*-PrOH–hexane, 20 : 80, 2.0 mL min^{-1}) and had the following optical rotations: $[\alpha]_D^{25}$ + 265 (*c* 0.88, $CHCl_3$) (retention time 18 min) and −288 (*c* 1.8, $CHCl_3$) (retention time 26 min) for (+)-**30** and (−)-**30**, respectively.

(2*Z*,4*E*)-(1′*S*,2′*R*)-5-(2′-Ethynyl-1′-hydroxy-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl)-3-methylpenta-2,4-dienoic acid ((+)-31). A mixture of ester (+)-**30** (0.013 g, 0.0401 mmol) in MeOH (1 mL) and 2.0 M KOH (1 mL) was stirred at 25 °C for 2 h, by which point, all the starting material had disappeared. The solvent was evaporated at reduced pressure, the aqueous layer acidified to pH 3 with 10% HCl and extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated to provide acid (+)-**31** (0.0101 g, 81%) as a white powder (was not re-crystallized). $[\alpha]_D^{25}$ + 200 (*c* 1.0, MeOH). IR (ν_{max}): 3600–2400, 3500, 3284, 2979, 1682, 1598 cm^{-1}. ^{1}H NMR (CD_3OD): δ 7.96 (dd, 1H, J = 1.5, 8.0 Hz, Ar–H), 7.70 (d, 1H, J = 7.5 Hz, Ar–H), 7.65 (dt, 1H, J = 1.5, 7.5 Hz, Ar–H), 7.52 (d, 1H, J = 16.0 Hz, H-4), 7.43 (dt, 1H, J = 1.5, 8.0 Hz, Ar–H), 6.37 (d, 1H, J = 16.0 Hz, H-5), 5.69 (s, 1H, H-2), 2.90 (d, 1H, J = 17.0 Hz, H-3′), 2.81 (d, 1H, J = 17.0 Hz, H-3′), 2.39 (s, 1H, alkyne), 1.98 (s, 3H, H-6), 1.43 (s, 3H, H-10′). ^{13}C NMR (CD_3OD): δ 197.4, 169.3, 150.6, 148.7, 137.8, 135.5, 132.5, 131.0, 128.9, 128.7, 127.2, 120.1, 88.0, 78.7, 73.5, 44.7, 34.3, 23.4, 21.1. HRMS (*m*/*z*) $C_{19}H_{17}O_4$ requires: 309.1127 [M-1]$^-$; found: 309.1117.

2-Hydroxymethylene-3,4-dihydro-2*H*-naphthalen-1-one (32)[22]. Hydroxymethylene-1-tetralone **32** (18.3 g, 92%) was prepared from α-tetralone **12** (16.6 g, 114 mmol). IR (ν_{max}): 3644–3200, 3025, 2846, 1602 cm^{-1}. ^{1}H NMR: δ 14.61 (s, 1H), 8.20 (s, 1H), 7.93 (d, 1H, J = 7.7 Hz, Ar–H), 7.41 (t, 1H, J = 7.5 Hz, Ar–H), 7.31 (t, 1H, J = 7.5 Hz, Ar–H), 7.20 (d, 1H, J = 7.5 Hz, Ar–H), 2.86 (t, 3H, J = 7.4 Hz, CH_3), 2.54 (t, 3H, J = 7.4 Hz, CH_3). HRMS (*m*/*z*) $C_{11}H_{10}O_2$ requires: 174.0681; found: 174.0685.

2-Methyl-1-oxo-1,2,3,4-tetrahydro-naphthalene-2-carbaldehyde (33)[23]. A mixture of 2-hydroxymethylene-1-tetralone **32** (18.0 g, 102 mmol), CH_3I (7.0 mL, 113 mmol) and KOH (6.3 g, 113 mmol) in anhydrous 1,2-dimethoxyethane (DME) (200 mL) was stirred at rt under argon atmosphere for 12 h. The precipitate (KI) was filtered off and the filtrate concentrated *in vacuo*. The residue was redissolved in diethyl ether (500 mL), washed with H_2O (2 × 150 mL) and dried over anhydrous Na_2SO_4. Concentration of the ethereal solution and column chromatography of the residue using silica gel and 15% ethyl acetate in hexane afforded the aldehyde **33** (8.2 g, 44%) and 2-methoxymethylene-3,4-dihydro-2*H*-naphthalen-1-one as a by-product (8.9 g, 46%). IR (ν_{max}): 1732, 1681, and 1602 cm^{-1}. ^{1}H NMR: δ 9.64 (s, 1H, CHO), 7.94 (d, 1H, J = 7.8 Hz, Ar–H), 7.41 (t, 1H, J = 7.4 Hz, Ar–H), 7.22 (t, 1H, J = 7.4 Hz, Ar–H), 7.15 (d, 1H, J = 7.8 Hz, Ar–H), 2.93 (m, 2*H*, CH_2), 2.40 (m, 1H, CH_2), 1.92 (m, 1H, CH_2), 1.31 (s, 3H, CH_3),. HRMS (*m*/*z*) $C_{12}H_{12}O_2$ requires: 188.0837; found: 188.0825.

2-Methoxymethylene-3,4-dihydro-2*H*-naphthalen-1-one. ^{1}H NMR: δ 8.01 (d, 1H, J = 7.8 Hz, Ar–H), 7.50 (s, 1H, CH_3), 7.39 (t, 1H, J = 7.3 Hz, Ar–H), 7.28 (t, 1H, J = 7.3 Hz, Ar–H), 7.19 (d, 1H, J = 7.5 Hz, Ar–H), 3.87 (s, 3H, CH_3), 2.85 (t, 2*H*, J = 6.5 Hz, CH_2), 2.68 (t, 2*H*, J = 6.5 Hz, CH_2). HRMS (*m*/*z*) $C_{12}H_{12}O_2$ requires: 188.0837; found 188.0839.

2-Dimethoxymethyl-2-methyl-3,4-dihydro-2*H*-naphthalen-1-one (34). To a solution of aldehyde **33** (4.0 g, 21.3 mmol) in reagent grade MeOH (200 mL), was added, 0.25 g NH_4Cl and reaction mixture stirred at 65 °C for 3 h. The solvent was evaporated

in vacuo and the resulting residue taken up in ethyl acetate (350 mL), washed with H_2O (2 × 100 mL) and dried over anhydrous Na_2SO_4. Column chromatography using silica gel with 14% ethyl acetate in hexane afforded the keto acetal **34** (3.64 g, 73%) as a yellow oil. IR (ν_{max}): 3340, 2934, 2830, 1679, 1601 cm^{-1}. ^1H NMR: δ 7.98 (d, 1H, J = 7.8 Hz, Ar–H), 7.39 (t, 1H, J = 7.3 Hz, Ar–H), 7.23 (t, 1H, J = 7.3 Hz, Ar–H), 7.17 (d, 1H, J = 7.8 Hz, Ar–H), 4.67 (s, 1H, CH), 3.51 (s, 3H, OCH_3), 3.43 (s, 3H, OCH_3), 2.91 (m, 2*H*, CH_2), 2.34 (m, 1H, CH_2), 1.88 (m, 1H, CH_2), 1.14 (s, 3H, CH_3). ^{13}C NMR: δ 200.1, 143.6, 133.0, 131.7, 127.5, 127.1, 126.3, 110.4, 58.6, 58.5, 50.4, 26.6, 24.9 and 19.0. HRMS (m/z) $C_{14}H_{18}O_3$ requires: 234.1256; found: 234.1252.

(3′*Z*)-(1*R*,2*S*/1*S*,2*R*)-2-Dimethoxymethyl-1-[5′-hydroxy-3′-methylpent-3′-en-1′-ynyl]-2-methyl-1,2,3,4-tetrahydronaphthalen-1-ol (35) and (3′*Z*)-(1*R*,2*R*/1*S*,2*S*)-2-Dimethoxymethyl-1-[5′-hydroxy-3′-methylpent-3′-en-1′-ynyl]-2-methyl-1,2,3,4-tetra-hydronaphthalen-1-ol (36). The side chain was added to **34** (24.4 g, 15.4 mmol) in the same manner as for compound **13**. Column chromatography using silica gel with hexane–EtOAc (6 : 1 to 4 : 1) afforded two diastereomeric products **35** (R_f = 0.13 hexane–EtOAc 1 : 1, 18.1 g, 52.8%) and **36** (R_f = 0.17 hexane–EtOAc 1 : 1, 10.6 g, 30.8%), both as light yellow solids. IR (ν_{max}); 3380, 3055, 2940, 2278, 1602 cm^{-1}.

Compound 35. ^1H NMR: δ 7.76 (m, 1H, Ar–H), 7.17 (m, 2*H*, Ar–H), 7.07 (m, 1H, Ar–H), 5.85 (t, 1H, J = 6.8 Hz, CH), 4.53 (s, 1H, CH), 4.27 (d, 2*H*, J = 6.8 Hz, CH_2), 3.56 (s, 6H, OCH_3), 2.86 (m, 2*H*, CH_2), 2.23 (m, 1H, CH_2), 1.91 (s, 3H, CH_3), 1.83 (m, 1H, CH_2), 1.04 (s, 3H, CH_3),. ^{13}C NMR: δ 144.3, 144.6, 135.5, 133.0, 132.8, 128.5, 127.1, 124.4, 110.3, 89.6, 75.8, 58.6, 58.7, 57.8, 50.5, 29.7, 26.7, 24.9, 22.8, and 17.8. HRMS (m/z) $C_{22}H_{29}O_6$ requires: 389.1969 $[M + O_2CCH_3]^-$; found: 389.1955.

Compound 36. ^1H NMR: δ 7.72 (d, 1H, J = 7.8 Hz, Ar–H), 7.19 (t, 1H, J = 7.3 Hz, Ar–H), 7.13 (t, 1H, J = 7.3 Hz, Ar–H), 7.02 (d, 1H, J = 7.7 Hz, Ar–H), 5.77 (t, 1H, J = 6.7 Hz, CH), 4.58 (s, 1H, CH), 4.13 (d, 2*H*, J = 6.7 Hz, CH_2), 3.64 (s, 3H, CH_3), 3.61 (s, 3H, CH_3), 2.75–2.87 (m, 2*H*, CH_2), 1.81–1.83 (m, 2*H*, CH_2), 1.80 (s, 3H, CH_3), 0.95 (s, 3H, CH_3). ^{13}C NMR: δ 143.3, 143.3, 135.5, 133.8, 132.8, 129.8, 128.3, 124.4, 111.3, 89.6, 76.8, 58.8, 58.7, 57.6, 51.5, 29.7, 27.7, 24.9, 21.8, and 18.8. HRMS (m/z) $C_{22}H_{29}O_6$ requires: 389.1969 $[M + C_2H_3O_2]^-$; found: 389.1971.

Methyl (2*Z*,4*E*)-(1′*R*,2′*S*/1′*S*,2′*R*)-5-[2′-dimethoxymethyl-1′-hydroxy-2′-methyl-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (37). Allylic alcohol **35** (1.30 g, 3.94 mmol) was converted to ester **37** (0.65 g, 37% over three steps) in the same manner as the conversion of **15** to **17**. IR (ν_{max}): 3434, 2946, 1714, 1631, 1600 cm^{-1}. ^1H NMR: δ 7.62 (d, 1H, J = 16 Hz, CH), 7.42 (d, 1H, J = 7.3 Hz, Ar–H), 7.16 (m, 3H, Ar–H), 6.32 (d, 1H, J = 16 Hz, CH), 5.66 (s, 1H, CH), 4.23 (s, 1H, CH), 3.64 (s, 3H, CH_3), 3.56 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 2.86 (m, 2*H*, CH_2), 2.12 (m, 1H, CH), 1.99 (s, 3H, CH_3), 1.76 (m, 1H, CH), 1.02 (s, 3H, CH_3),. ^{13}C NMR: δ 166.5, 150.1, 142.2, 140.6, 135.1, 128.5, 127.8, 127.1, 126.1, 117.2, 110.8, 77.3, 60.4, 58.7, 51.0, 45.0, 25.8, 25.1, 21.3, 16.2. HRMS (m/z) $C_{21}H_{28}O_5$ requires: 360.1937; found: 360.1935.

Methyl (2*Z*,4*E*)-(1′*R*,2′*S*/1′*S*,2′*R*)-5-[2′-dimethoxymethyl-1′-hydroxy-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (38)[16]. Benzylic oxidation of ester **37** (0.566 g, 1.57 mmol) yielded the desired compound **38** (0.260 g, 44%) and recovered starting material **37** (0.18 g) using same procedure as for the conversion of **17** to **18**. IR (ν_{max}): 3420, 2945, 1713, 1683, 1634, 1599 cm^{-1}. ^1H NMR: δ 7.99 (d, 1H, J = 7.8 Hz, Ar–H), 7.64 (d, 1H, J = 16 Hz, CH), 7.60 (d, 1H, J = 7.3 Hz, Ar–H), 7.56 (t, 1H, J = 6.6 Hz, Ar–H), 7.39 (t, 1H, J = 6.6 Hz, Ar–H), 6.27 (d, 1H, J = 16 Hz, CH), 5.67 (s, 1H, CH), 4.16 (s, 1H, CH), 3.62 (s, 3H, CH_3), 3.46 (s, 3H, CH_3), 3.34 (s, 3H, CH_3), 2.89 (d, 1H, J = 18 Hz, CH_2), 2.58 (d, 1H, J = 18 Hz, CH_2), 1.95 (s, 3H, CH_3), 1.11 (s, 3H, CH_3). ^{13}C NMR: δ 196.7, 166.3, 149.3, 145.6, 138.5, 134.6, 131.2, 128.9, 127.9, 127.0, 126.4, 118.3, 110.6, 77.1, 59.5, 57.9, 51.1, 48.5, 44.5, 21.2, 16.2. HRMS (m/z) $C_{21}H_{26}O_6$ requires: 374.1729; found: 374.1736.

Methyl (2*Z*,4*E*)-(1′*R*,2′*S*/1′*S*,2′*R*)-5-[2′-formyl-1′-hydroxy-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (39). The ester **38** (0.250 g, 0.688 mmol) was dissolved in $CHCl_3$ (8 mL) to which 50% aq. TFA (4 mL) was then added. The reaction mixture was refluxed for 4 h and then allowed to cool to rt. The $CHCl_3$ layer was washed with H_2O (25 mL) followed by a saturated solution of $NaHCO_3$ (25 mL) and then dried over anhydrous Na_2SO_4. PrepTLC on silica gel coated glass plate (20 × 20 cm) using 33% ethyl acetate in hexane yielded aldehyde **39** (0.180 g, 82%). IR (ν_{max}): 3432, 2949, 2845, 1717, 1686, 1636, 1599 cm^{-1}. ^1H NMR: δ 9.66 (s, 1H, CHO), 8.00 (d, 1H, J = 7.7 Hz, Ar–H), 7.72 (d, 1H, J = 16 Hz, CH),7.59 (m, 2*H*, 2 × Ar–H), 7.40 (m, 1H, Ar–H), 6.19 (d, 1H, J = 16 Hz, CH), 5.72 (s, 1H, CH), 3.63 (s, 3H, CH_3), 3.09 (d, 1H, J = 18 Hz, CH), 2.67 (d, 1H, J = 18 Hz, CH), 1.95 (s, 3H, CH_3), 1.27 (s, 3H, CH_3). ^{13}C NMR: δ 205.0, 194.3, 166.3, 148.7, 145.7, 136.2, 135.0, 130.6, 129.3, 128.6, 127.1, 126.5, 119.1, 54.8, 51.2, 43.4, 21.1, 16.7. HRMS (m/z) $C_{19}H_{20}O_5$ requires: 328.1311; found: 328.1314.

Methyl (2*Z*,4*E*)-(1′*R*,2′*R*/1′*S*,2′*S*)-5-[1′-hydroxy-2′-hydroxymethyl-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (40). To the aldehyde **39** (0.100 g, 0.305 mmol) in CH_3OH (5 mL) was added 0.1 g of $NaBH_4$ and mixture stirred at rt for 20 min. H_2O (5 mL) was then added and the mixture stirred for a further 30 min. The reaction mixture was then extracted with ethyl acetate (3 × 25 mL), washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, leaving a residue of the trihydroxy product (0.077 g), which was carried through to the next stage without further purification. To a stirred solution of the trihydroxy intermediate (0.077 g, 0.233 mmol) in reagent grade acetone (20 mL) was added MnO_2 (0.406 g, 4.67 mmol). The reaction mixture was stirred at rt for 2 h, filtered over a Celite® bed and the filtrate concentrated *in vacuo*. The resulting residue was purified by PrepTLC on silica gel coated plate (20 × 20 cm) using EtOAc–hexane (2 : 3) to give the desired hydroxyester **40** (0.074 g, 74%) over the two steps. IR (ν_{max}): 3477, 3060, 2987, 1704, 1686, and 1594 cm^{-1}. ^1H NMR: δ 8.02 (dd, 1H, J = 7.8 and 1.2 Hz, Ar–H), 7.87 (d, 1H, J = 16.0 Hz, CH), 7.55 (dt, 1H, J = 7.3 and 1.3 Hz, Ar–H), 7.50 (d, 1H, J = 7.1 Hz, Ar–H), 7.39 (dt, 1H, J = 7.3 and 1.3 Hz, Ar–H), 6.31 (d, 1H, J = 16.0 Hz, CH), 5.74 (s, 1H, CH), 3.71 (d, 1H, J = 11.3 Hz, CH_2), 3.65 (s, 3H, CH_3), 3.57 (d, 1H, J = 11.2 Hz, CH_2), 3.08 (d, 1H, J = 18.0 Hz, CH), 2.47 (d, 1H, J = 18.0 Hz, CH), 2.01 (s, 3H, CH_3), 1.03 (s, 3H, CH_3). ^{13}C NMR: δ 197.3, 166.6, 149.7, 145.3, 137.9, 134.6, 130.8, 129.1, 128.4, 127.3, 126.8, 118.1, 79.3, 69.8, 51.2,

45.4, 43.8, 21.3, 19.8. HRMS (m/z) $C_{19}H_{22}O_5$ requires: 330.1467; found: 330.1467.

(2*Z*,4*E*)-(1′*S*,2′*S*)-5-[1′-Hydroxy-2′-hydroxymethyl-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoic acid [(+)-10].

*Resolving the dihydroxyester **40** by chiral HPLC.* Dihydroxyester **40** was resolved on a chiral HPLC column (250 × 10 mm, Whelk-01, Kromasil, Regis Technologies Inc., USA) with 6% IPA in hexane at 7.5 mL min^{-1} and UV detection at 262 nm, to afford (+)-**40** $[\alpha]_D^{25}$ +189 (*c* 1.57, $CHCl_3$) and (−)-**40** $[\alpha]_D^{25}$ −191 (*c* 1.43, $CHCl_3$) with retention times of 20.5 and 24.2 min, respectively.

The resolved esters were hydrolyzed to the corresponding acids by using esterase from porcine liver (Sigma Cat. No. E-3019) as follows: The ester (+)-**40** (15.7 mg, 4.76×10^{-5}mmol) was dissolved in MeOH (10 drops) to which a phosphate buffer at pH 8.0 (1.5 mL) was added. The pH of the mixture was maintained at 8.8 using 1.0 M KOH. The reaction mixture was stirred at rt for 24 h after which all the starting material had disappeared. MeOH was evaporated *in vacuo* and reaction mixture acidified with 10% HCl. The reaction mixture was then extracted with EtOAc, washed with saturated NaCl, dried over anhydrous Na_2SO_4 and solvent evaporated *in vacuo* to afford the (+)-enantiomer of the acid (+)-**10** (9.2 mg, 61%), as an oil. $[\alpha]_D^{25}$ +163 (*c* 0.92, $CHCl_3$). IR (ν_{max}): 3610–2457, 3452, 1684, 1601 cm^{-1}. ^1H NMR: δ 8.00 (d, 1H, J = 7.7 Hz, Ar–H), 7.82 (d, 1H, J = 16.0 Hz, CH), 7.56 (t, 1H, J = 7.5 Hz, Ar–H), 7.48 (d, 1H, J = 7.2 Hz, Ar–H), 7.40 (t, 1H, J = 7.5 Hz, Ar–H), 6.31 (d, 1H, J = 16.0 Hz, CH), 5.74 (s, 1H, CH), 3.72 (d, 1H, J = 11.2 Hz, CH), 3.55 (d, 1H, J = 11.2 Hz, CH), 3.07 (d, 1H, J = 17.3 Hz, CH), 2.48 (d, 1H, J = 17.3 Hz, CH), 2.04 (s, 3H, CH_3), 1.03 (s, 3H, CH_3). ^{13}C NMR: δ 197.7, 170.1, 151.4, 144.9, 138.4, 134.6, 130.7, 129.3, 128.5, 127.4, 126.8, 118.0, 79.5, 69.6, 45.2, 43.7, 21.6, 19.8. HRMS (m/z) $C_{18}H_{19}O_5$ requires: 315.1237 $[M - 1]^-$; found: 315.1232. The ester (−)-**40** (14.3 mg, 4.33×10^{-5} mmol) was treated the same way as above and afforded (−)-**10** (8.6 mg, 63%), as an oil. $[\alpha]_D^{25}$ −160 (*c* 1.34, $CHCl_3$).

Diastereomer **36** was transformed and resovled to give (+)-**11** and (−)-**11** using the same procedure for the conversion of **35** to (+)-**10** and (−)-**10**.

Methyl (2*Z*,4*E*)-(1′*R*,2′*R*/1′*S*,2′*S*)-5-[2′-dimethoxymethyl-1′-hydroxy-2′-methyl-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (diastereomer of 37). IR (ν_{max}): 3476, 2944, 1713, 1632, 1599 cm^{-1}. ^1H NMR: δ 7.76 (d, 1H, J = 16 Hz, CH), 7.48 (d, 1H, J = 7.5 Hz, Ar–H), 7.03–7.14 (m, 3H, Ar–H), 6.38 (d, 1H, J = 16 Hz, CH), 5.65 (s, 1H, CH), 4.03 (s, 1H, CH), 3.68 (s, 3H, CH_3), 3.58 (s, 3H, CH_3), 3.48 (s, 3H, CH_3), 2.82–2.97 (m, 2*H*, CH_2), 1.92 (s, 3H, CH_3), 1.80 (m, 2*H*, CH_2), 1.04 (s, 3H, CH_3). ^{13}C NMR: δ 166.5, 150.2, 142.5, 139.3, 133.7, 128.0, 127.9, 126.7, 126.6, 124.4, 117.1, 113.1, 60.7, 57.3, 51.0, 45.7, 27.7, 24.6, 21.4, 12.3. $C_{21}H_{28}O_5Na$ requires: 383.1828 $[M + Na]^{+\bullet}$; found: 383.1833.

Methyl (2*Z*,4*E*)-(1′*R*,2′*R*/1′*S*,2′*S*)-5-[2′-dimethoxymethyl-1′-hydroxy-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (diastereomer of 38). IR (ν_{max}): 3465, 2946, 1712, 1682, 1632, 1599 cm^{-1}. ^1H NMR: δ 7.96 (dd, 1H, J = 7.9 and 1.2 Hz, Ar–H), 7.84 (d, 1H, J = 16 Hz, CH), 7.67 (dd, 1H, J = 7.3 and 0.7 Hz, Ar–H), 7.57 (dt, 1H, J = 6.6 and 1.2 Hz, Ar–H), 7.34 (dt, 1H, J = 6.7 and 1.2 Hz, Ar–H), 6.33 (d, 1H, J = 16 Hz, CH), 5.68 (s, 1H, CH), 4.20 (s, 1H, CH), 3.67 (s, 3H, CH_3), 3.59 (s, 3H, CH_3), 3.53 (s, 3H, CH_3), 2.67 (d, 1H, J = 18 Hz, CH), 2.62 (d, 1H, J = 18 Hz, CH), 1.91 (s, 3H, CH_3), 1.07 (s, 3H, CH_3). ^{13}C NMR: δ 195.8, 166.3, 149.4, 145.8, 139.3, 134.9, 129.5, 127.5, 127.1, 126.4, 126.0, 118.1, 111.4, 76.7, 60.8, 57.2, 51.1, 48.3, 44.8, 21.3, 13.9. HRMS (m/z) $C_{22}H_{27}O_8$ requires: 419.1711 $[M + CHO_2]^-$; found: 419.1718.

Methyl (2*Z*,4*E*)-(1′*R*,2′*R*/1′*S*,2′*S*)-5-[2′-formyl-1′-hydroxy-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoate (diastereomer of 39). IR (ν_{max}): 3455, 2930, 1714, 1685, 1636, 1600 cm^{-1}. ^1H NMR: δ 9.73 (s, 1H, CHO), 8.06 (d, 1H, J = 7.8 Hz, Ar–H), 7.74 (d, 1H, J = 16 Hz, CH), 7.61 (t, 1H, J = 7.3 Hz, Ar–H), 7.57 (d, 1H, J = 7.1 Hz, Ar–H), 7.45 (t, 1H, J = 7.3 Hz, Ar–H), 6.29 (d, 1H, J = 16 Hz, CH), 5.72 (s, 1H, CH), 3.63 (s, 3H, CH_3), 2.95 (d, 1H, J = 18 Hz, CH), 2.83 (d, 1H, J = 18 Hz, CH), 1.93 (s, 3H, CH_3), 1.26 (s, 3H, CH_3). ^{13}C NMR: δ 203.5, 194.9, 166.3, 148.8, 143.9, 136.8, 134.9, 130.5, 129.3, 128.7, 127.0, 126.6, 119.1, 76.5 55.1, 51.2, 42.4, 21.1, 16.7. HRMS (m/z) $C_{20}H_{21}O_7$ requires: 373.1292 $[M + CHO_2]^-$; found: 373.1299.

Methyl (2*Z*,4*E*)-(1′*R*,2′*S*/1′*S*,2′*R*)-5-[1′-hydroxy-2′-hydroxymethyl-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta - 2,4 - dienoate (diastereomer of 40). IR (ν_{max}): 3437, 2948, 1686, 1633, 1599 cm^{-1}. ^1H NMR: δ 7.99 (dd, 1H, J = 7.8 and 1.2 Hz, Ar–H), 7.75 (d, 1H, J = 15.9 Hz, CH), 7.60 (dd, 1H, J = 7.2 and 1.5 Hz, Ar–H), 7.58 (dt, 1H, J = 7.3 and 1.1 Hz, Ar–H), 7.36 (dt, 1H, J = 7.3 and 1.1 Hz, Ar–H), 6.32 (d, 1H, J = 15.9 Hz, CH), 5.68 (s, 1H, CH), 3.89 (d, 1H, J = 10.5 Hz, CH_2), 3.64 (s, 3H, CH_3), 3.44 (d, 1H, J = 10.6 Hz, CH_2), 2.50 (d, 1H, J = 17.5 Hz, CH), 2.34 (d, 1H, J = 17.5 Hz, CH), 1.92 (s, 3H, CH_3), 1.18 (s, 3H, CH_3). ^{13}C NMR: δ 195.8, 166.8, 150.4, 146.3, 138.8, 134.8, 129.7, 127.7, 127.5, 126.6, 126.3, 117.8, 78.4, 69.8, 51.3, 45.0, 44.0, 21.5, 18.6. HRMS (m/z) $C_{19}H_{21}O_5$ requires: 329.1394 $[M - H]^-$; found: 329.1394.

(2*Z*,4*E*)-(1′*R*,2′*S*)-5-[1′-Hydroxy-2′-hydroxymethyl-2′-methyl-4′-oxo-1′,2′,3′,4′-tetrahydronaphthalen-1′-yl]-3-methylpenta-2,4-dienoic acid [(+)-11]. The diastereomer of **40** was resolved as described earlier to afford (+)-(1′*R*,2′*S*)-ester, $[\alpha]_D^{25}$ +272 (*c* 0.87, $CHCl_3$) and (−)-(1′*S*,2′*R*)-ester, $[\alpha]_D$ −277 (*c* 1.01, $CHCl_3$). The retention times were 17.7 and 20.2 min, respectively.

The resolved esters were hydrolyzed using esterase as described for **40** above. The (+)-(1′*R*,2′*S*)-ester (15.0 mg, 4.55×10^{-5} mmol) afforded the corresponding acid (+)-**11** (9.0 mg, 63%), $[\alpha]_D^{25}$ + 256 (*c* 0.18, $CHCl_3$) while the (−)-(1′*S*,2′*R*)-ester (16.0 mg. 4.85×10^{-5} mmol) yielded the acid (−)-**11** (9.4 mg, 61%), $[\alpha]_D^{25}$ −259 (*c* 0.10, $CHCl_3$). IR (ν_{max}): 3652–2455, 3400, 1684, 1599 cm^{-1}. ^1H NMR: δ 8.00 (d, 1H, J = 7.8 Hz, Ar–H), 7.70 (d, 1H, J = 16.0 Hz, CH), 7.63 (d, 1H, J = 7.8 Hz, Ar–H), 7.60 (t, 1H, J = 7.2 Hz, Ar–H), 7.40 (t, 1H, J = 7.2 Hz, Ar–H), 6.37 (d, 1H, J = 16.0 Hz, CH), 5.70 (s, 1H, CH), 3.91 (d, 1H, J = 10.5 Hz, CH), 3.45 (d, 1H, J = 10.5 Hz, CH), 2.53 (d, 1H, J = 17.5 Hz, CH), 2.33 (d, 1H, J = 17.5 Hz, CH), 1.96 (s, 3H, CH_3), 1.20 (s, 3H, CH_3). ^{13}C NMR: δ 196.7, 169.0, 149.7, 146.4, 138.9, 134.7, 129.7, 127.5, 127.0, 126.4, 126.2, 118.7, 78.5, 68.9, 44.9, 43.8, 21.2, 18.4. HRMS (m/z) $C_{18}H_{19}O_5$ requires: 315.1237 $[M - 1]^-$; found: 315.1242.

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