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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1′-DEOXY-1′-FLUORO- AND 8′-FLUOROABSCISIC ACIDS

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Key Word Index—seed germination; seedling elongation; induction of α-amylase; stomatal opening; abscisic acid; 8′-hydroxyabscisic acid; 1′-deoxy-1′-fluoroabscisic acid; 8′-fluoroabscisic acid; 1′-deoxyabscisic acid.

Abstract—Resolved analogues of abscisic acid (ABA) were tested in rice seedling elongation, lettuce seed germination, barley α-amylase induction and stomatal opening in spiderwort epidermal strip bioassays. (1′*S*)-(+)-1′-Deoxy-1′-fluoroabscisic acid showed $\frac{1}{10}$ to $\frac{1}{20}$ of the activity of (1′*S*)-(+)-ABA and was almost equal to (1′*R*)-(+)-1′-deoxyabscisic acid in these assays. These results suggest that the fluorine atom cannot mimic the C-1′ hydroxyl group. (1′*R*,6′*S*)-(+)-8′-fluoroabscisic acid was as effective as (+)-ABA, and the effect on the plants tested was indistinguishable from that of (+)-ABA. This result suggests that the activity of highly labile 8′-hydroxyabscisic acid is as strong as that of (+)-ABA. The activities of (−)-enantiomers were equivalent to or less than those of (−)-ABA.

INTRODUCTION

The plant hormone abscisic acid [(1′*S*)-(+)-ABA, **1**] is involved in many physiological processes, in particular the expression of the protective response to environmental stresses [1, 2]. ABA possesses a tertiary hydroxyl group at C-1′ and another can be introduced at C-8′ by a monooxygenase to give 8′-hydroxyabscisic acid (8′-HOABA, **2**) (Fig. 1) [3, 4]; 8′-HOABA is so unstable that it is spontaneously or enzymatically cyclized to inactive phaseic acid (**3**) [4, 5].

We have described highly potent, long-lasting tri- and difluoro analogues of ABA which resist hydroxylation at C-8′ of ABA [6]. Fluorination of biologically active compounds is useful not only for developing highly active analogues, but also for studying interactions of the hydroxyl groups in the compounds with binding molecules, e.g. carriers, receptors and metabolic enzymes [7]. Fluorine, the van der Waals radius of which is intermediate between that of hydrogen and oxygen, is electronically equivalent to the oxygen of the hydroxyl group, and the C–F bond is physicochemically similar to the C–OH bond, rather than the C–H bond [7, 8]. The distinct difference between the monofluoro and hydroxyl groups is the capability for the hydrogen bonding. The hydroxyl group can be both a donor and an acceptor, whereas fluorine can act only as an acceptor [7, 8]. These properties of fluorine make the monofluorinated analogue a valuable probe with which to investigate the role of the hydroxyl group in the parent compound. Additionally, monofluoro analogues occasionally bind enzymes irreversibly due to high electronegativity, and have a fatal effect on the bioorganism [7, 9]. Therefore, monofluoro analogues of ABA and 8′-HOABA should help identify the function of the hydroxyl groups of these compounds in interactions with the binding molecules involved in the expression of the activity. The latter analogue might be an enzyme inhibitor. In this report, we describe the synthesis and optical resolution to obtain (1′*S*)-(+)-1′-deoxy-1′-fluoroabscisic acid [(+)-**4**] and (1′*R*,6′*S*)-(+)-8′-fluoroabscisic acid [(+)-**6**] as mimics of ABA and 8′-HOABA, respectively, along with (1′*R*)-(+)-1′-deoxyabscisic acid [(+)-**5**] for evaluation of the activity of (+)-**4**, and discuss their activities in relation to the function of the hydroxyl groups.

RESULTS AND DISCUSSION

Synthesis and identification

Racemic **4** was synthesized by direct replacement of the C-1′ hydroxyl group with fluorine (Fig. 2). The methyl ester (**7**) of (±)-ABA was fluorinated using dimethylaminosulphur trifluoride (DAST) to afford compound **8**. Saponification of **8** with alkali resulted in several unidentified compounds, which were probably formed by elim-

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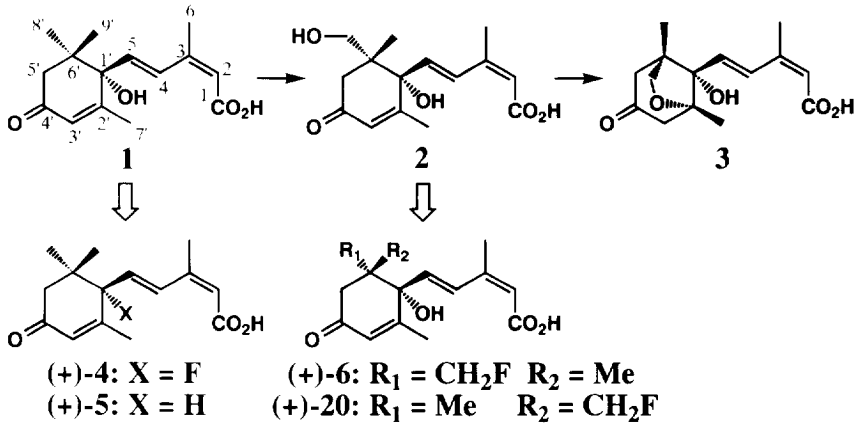


Fig. 1. Pathway for the deactivation of ABA in plants, and the design of new fluorine-containing ABA analogues.

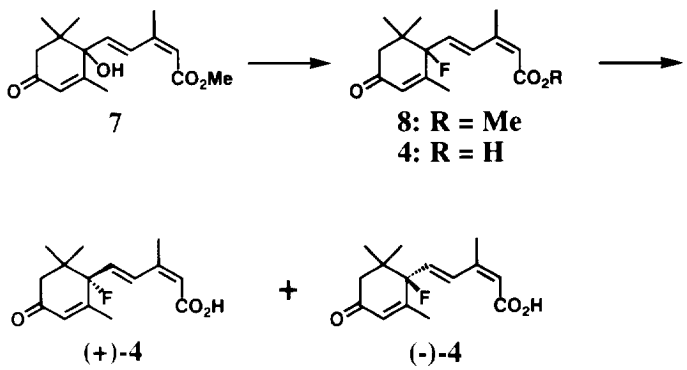


Fig. 2. Synthesis of 1′-deoxy-1′-fluoroabscisic acid (**4**).

ination of the fluorine. Hydrolysis with porcine liver esterase [10] yielded **4**. The presence of fluorine at C-1′ was revealed by the spectral data. In the ^1H and ^{13}C NMR spectra, the ^1H signal of the C-5 proton and the ^{13}C signal of C-1′ showed vicinal ^1H–F and ^{13}C F coupling, respectively. The IR spectrum showed no absorption peak corresponding to the hydroxyl group at *ca* 3600 cm^{-1}.

It has been reported that the fluorinating reactions using DAST proceed through an S_N1 or S_N2 mechanism [11–13]. To examine the mechanism of this reaction in the case of the methyl ester of ABA, the ester of (*S*)-(+)-ABA was fluorinated with DAST, followed by hydrolysis. Analysis of the product by HPLC on a chiral column revealed that the *R*/*S* ratio of the product at C-1′ was 1:4 (optical resolution of **4** and determination of the configuration at C-1′ of each enantiomer are described below). This result indicated that the reaction proceeded mainly with retention of the C-1′ configuration, probably through the S_Ni mechanism, because the steric effect in the S_N1 mechanism, or neighbouring-group participation in the S_N2 mechanism, would not occur [14]. Figure 3 shows the supposed intermediate formed between the methyl ester of ABA and DAST in the S_Ni reaction.

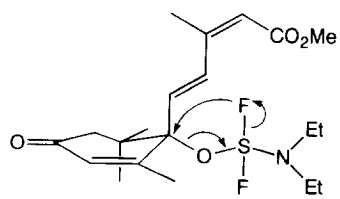


Fig. 3. Supposed intermediate in the fluorination of the methyl ester of ABA by DAST.

Racemic **6** was synthesized using a modification of the method reported for the synthesis of 8′-methoxyabscisic acid [15] (Fig. 4). Each diastereomer of 2-hydroxymethyl-2,6-dimethyl-1-cyclohexanone (**9a** and **9b**) [15] was fluorinated using DAST. The *cis*-dimethyl form, **9a**, gave the fluorinated compound **10a** in 27% yield, whereas the *trans*-dimethyl form, **9b**, afforded **10b** in only 2% yield. This difference may be caused by the intramolecular hydrogen bond, which **9b** would form more easily than **9a**, because the hydroxymethyl group of **9b** located at the equatorial position is closer to the carbonyl group than that of **9a** located at the axial position.

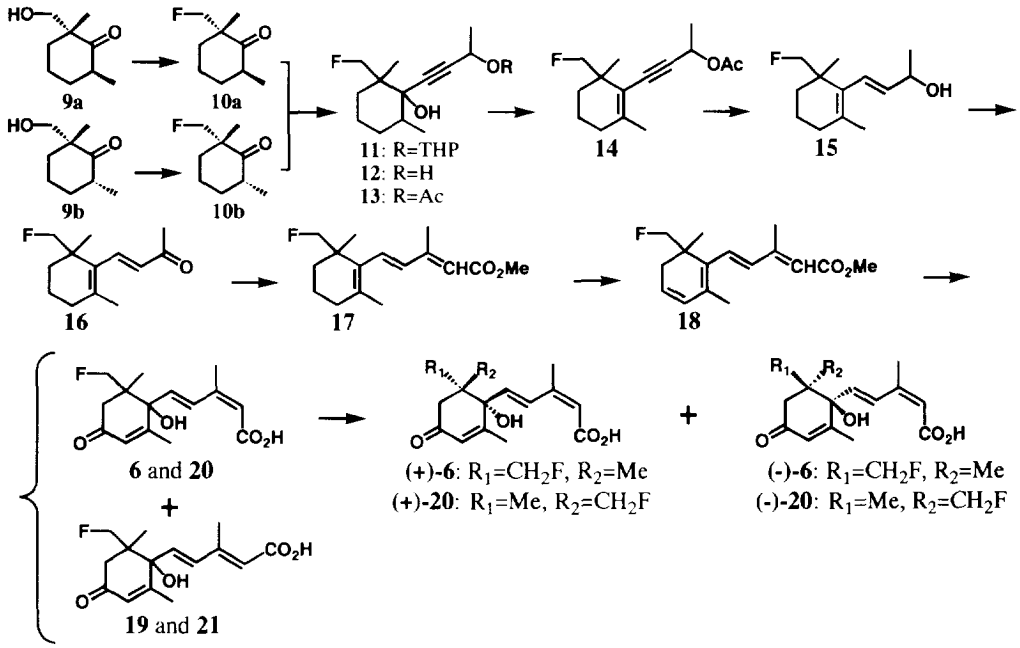
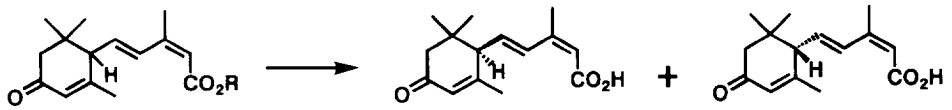


Fig. 4. Synthesis of 8′- and 9′-fluoroabscisic acids (**6** and **20**).

However, the effect of the intramolecular hydrogen bond on the reaction remains unknown. The reaction of a mixture of **10a** and **10b** with alkynyl lithium gave the tetrahydropyranyl ether **11**, which on deprotection gave the acetylenic diol **12**, which was then acetylated to afford the acetylenic acetate **13**. Dehydration of **13** gave the enyne acetate **14**, which was then reduced to the dienol **15**, which was oxidized to obtain the dienone **16**. Wittig reaction of **16** gave the methyl ester **17** as a mixture of (2*Z*)- and (2*E*)-isomers. Bromination of **17**, then dehydrobromination, formed the didehydro compound **18**, which on photosensitized oxygenation and subsequent adsorption with basic alumina afforded the methyl esters as an isomeric mixture. Saponification of this mixture with alkali gave 5′α,8′- and 5′β,9′-cycloabscisic acids by the elimination of fluorine to form the cyclopropyl group (identification and biological activity of cycloabscisic acids will be reported elsewhere). Hydrolysis with porcine liver esterase gave the free acids as an isomeric mixture of racemic **6** and its (2*E*)-isomer (**19**), and of racemic 9′-fluoroabscisic acid (**20**) and its (2*E*)-isomer (**21**) in a ratio of *ca* 1:2:2:4, as determined by HPLC on a silica gel column. This mixture was separated into its components by chromatography on silica gel and on Sephadex LH-20. The presence of fluorine at C-8′ of **6** and C-9′ of **20** was ascertained by the chemical shifts and the ^{1}H–F and ^{13}C–F couplings. In the ^{1}H NMR spectra, the signal of each proton of the fluoromethyl group appeared as a double doublet split by the geminal ^{1}H–F coupling besides geminal ^{1}H–^{1}H coupling at δ4.2–4.5 in both **6** and **20**. In the ^{13}C NMR spectra, the signals of C-8′ of **6** and C-9′ of **20** appeared as doublets at δ88.1 and 88.0, respectively, according to ^{13}C–F coupling. The distinction between 8′- and 9′-fluoroabscisic acids was made by the chemical shift and ^{1}H–F coupling of the C-5 proton. When the side-chain is pseudo-axial [16, 17], C-9′ is spatially closer to the C-5 proton than C-8′, so the influence of the C-9′ fluorine on the C-5 proton must be greater than that of the C-8′ fluorine. The signal of the C-5 proton of **20** appeared at δ6.24 as a double doublet split by ^{1}H–F coupling as well as vicinal coupling with the C-4 proton. That of **6** was observed at δ6.20 as a doublet split only by the vicinal coupling, indicating that the C-5 proton was too far away from the fluorine for coupling. This finding showed that **6** was 8′-fluoroabscisic acid and **20** was 9′-fluoroabscisic acid.

As described above, this synthetic route resulted in a **6**:**20** ratio of 1:2. This diastereomeric ratio was brought about during the photosensitized oxygenation of **18** [15], and hence showed that the oxygenation of the 6′-monofluoromethyl-didehydro compound had lower diastereoselectivity than that of the 6′-di- and trifluoromethyl-didehydro compounds (8′:9′ = 1:4) [6] and of the 6′-methoxymethyl-didehydro compound (8′:9′ = 1:3) [15]. This finding meant that the hindrance effect of the monofluoromethyl group on the approach of a singlet oxygen was the smallest in these four groups.

Racemic **5** was also synthesized to compare its activity with that of **4**. This analogue has been synthesized by two groups [18, 19], but its biological activity has not been precisely examined. The methyl ester **22** was synthesized from α-ionone [18] (Fig. 5). Saponification of **22** with alkali resulted in a mixture of **5** and some unidentified compounds which were formed, probably through deprotonation at C-1′ by the base. The methyl ester **22** was hydrolysed by the esterase to afford **5**.



22: R = Me
5: R = H

(+)-5 (-)-5

Fig. 5. Preparation of 1′-deoxyabscisic acid (**5**).

Table 1. The IC_{50} values for ABA, **4**, **5**, **6** and **20** in four bioassays

Compound	IC_{50} in assay			
	Germination* (μM)	Elongation† (μM)	α-Amylase‡ (μM)	Stomata§ (nM)
(+)-ABA	4.2	2.0	3.1	2.6
(+)-**4**	50	48	62	33
(+)-**5**	9.0	18	35	32
(+)-**6**	4.0	2.5	9.0	3.0
(+)-**20**	8.3	1.9	9.0	3.3
(−)-ABA	12	2.4	8.9	35
(−)-**4**	58	50	80	120
(−)-**5**	25	18	68	48
(−)-**6**	13	2.5	36	24
(−)-**20**	12	12	36	38

*Lettuce seed germination.

†Elongation of the second leaf sheath of rice seedlings.

‡α-Amylase induction by gibberellin A_3 (10^{-7} M) in half-seeds of barley without embryos.

§Stomatal opening of spiderwort.

Racemic **4**, **5**, **6** and **20** were optically resolved by HPLC on chiral columns to afford the (+)- and (−)-enantiomers. The Cotton effects in the CD spectra of the (+)-enantiomers of **4**, **5**, **6** and **20** were similar to those of (+)-ABA [15, 20], so their absolute configurations at C-1′ were assumed to be the same as that of (+)-ABA. The CD spectra of the respective (−)-enantiomers were also found to be similar to that of (−)-ABA. Application of the Cahn–Ingold–Prelog notation to these molecules gives (+)-**4** as 1′*S* whereas (+)-**5**, (+)-**6** and (+)-**20** are 1′*R*. The notation for the configuration at C-6′ for (+)-**6** is 6′*S* and for (+)-**20** is 6′*R*.

Biological activity

The biological activities of optically active ABA, **4**, **5**, **6** and **20** were evaluated in terms of their inhibitory activity on lettuce seed germination, elongation of the second leaf sheath of rice seedlings, α-amylase induction by gibberellin A_3 in barley half-seeds and stomatal opening of the epidermal strips of spiderwort. The concentrations giving half-maximal inhibition (IC_{50}) (Table 1) were compared. (2*E*)-Isomers of racemic **5**, **6** and **20** were inactive in the assays (data not shown).

The activity of (+)-**4** was $\frac{1}{10}$ to $\frac{1}{20}$ that of (+)-ABA in all the assays and was almost equal to that of (+)-**5** except in the lettuce assay. This suggested that fluorine could not act as a mimic of the hydroxyl group. If the value of $\frac{1}{10}$–$\frac{1}{20}$ is assumed to correspond to the ratio (K) between the dissociation constant of (+)-ABA in binding with the receptor and that of (+)-**4** or (+)-**5**, the difference (ΔG^0) in the free energy of their binding to the receptor can be estimated to be 1.4–1.8 kcal mol^{-1} at 300 K (27°), an average temperature for the assays, by using the equation $\Delta G^0 = -RT\ln K$ [21]. This implied that the contribution of the C-1′ hydroxyl group to the binding energy between ABA and the receptor was about 1.4–1.8 kcal mol^{-1}, which agreed with the contribution of an uncharged hydrogen bond (0.5–1.8 kcal mol^{-1}) [21, 22]. Thus the C-1′ hydroxyl group of ABA may interact with the receptor by means of an uncharged hydrogen bond. The lower activity of (+)-**4** compared to (+)-ABA suggests that the role of the C-1′ hydroxyl group in this interaction is that of a hydrogen donor. Only in the lettuce assay did (+)-**5** show a relatively high activity, which was half that of (+)-ABA, whereas (+)-**4** exhibited very low activity as in the other assays. This difference may result from the difference in the rate of permeation or uptake, or in the metabolic processing. The compound (+)-**5** might be converted more slowly than (+)-**4** to the glucose ester, which is the major metabolite in lettuce seed [23], owing to the lack of the hydrogen acceptor at C-1′, or be oxidized to ABA [24, 25].

The (+)-enantiomers of **6** and **20** were as effective as, or slightly less effective than (+)-ABA in all the assays. This suggested that (+)-**6** and (+)-**20** had a similar affinity for the receptor to (+)-ABA. The activity of (+)-**6** as a mimic of 8′-HOABA also suggested that the activity of 8′-HOABA, which cannot be tested due to its extreme instability, was as strong as that of (+)-ABA. The hydrogen of the C-8′ hydroxyl group in the 8′-HOABA molecule would not be essential for activity since the activity of (+)-8′-methoxyabscisic acid is comparable to that of (+)-ABA [15]. Furthermore, the effect of (+)-**6** and that of (+)-**20** on the outward appearance of the seedlings, seeds and tissues tested were very similar to that of (+)-ABA, i.e., reversible and non-toxic as far as we could ascertain during the assays. If the 8′-fluoro analogue (+)-**6** had acted as a suicide inhibitor to inactivate the C-8′ monooxygenase then it would be expected to be more potent than (+)-ABA. The usual activity and non-toxicity of (+)-**6** suggested that, contrary to our expectation, this analogue did not act as a lethal inhibitor of the monooxygenase and cyclase. The analogue (+)-**6** may be metabolized to 8′-fluorophaseic acid, or to phaseic acid after the release of fluorine, without binding irreversibly to the catalytic site of the enzymes.

The relative intensity of the activities of the (−)-enantiomers of **4**, **5**, **6** and **20** to (−)-ABA in the four assays was similar to that of the (+)-enantiomers to (+)-ABA. This finding was consistent with the hypothesis that (+)- and (−)-ABAs bind the same receptor due to their relatively symmetrical structure [26].

EXPERIMENTAL

^{1}H and ^{13}C NMR: 400 or 500 and 125 MHz, respectively, TMS as int. standard. For clarity, the atoms of all the compounds with the carbon skeleton of ABA are numbered as in ABA for the assignment of peaks in the ^{1}H and ^{13}C NMR spectra. Mass spectra were obtained with a Jeol JMS-DX300/DA5000 mass spectrometer. GC-MS was conducted with a 1% OV-17 column (1 m × 3 mm) in the EI mode.

(±)-*1′-Deoxy-1′-fluoroabscisic acid* (**4**). DAST (120 mg) was added to a stirred soln of (±)-methyl abscisate (**7**) (75 mg) in Et_2O (5 ml) cooled to −78° under N_2. The mixt. was then warmed to room temp. and stirred for 3 hr. After quenching with H_2O, the mixt. was extracted with Et_2O. The organic layer was washed with aq. $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concd. The residual oil was chromatographed on silica gel (2 g) with hexane–EtOAc (9:1) to give the methyl ester **8** (43 mg, 57% yield). The methyl ester **8** (13 mg) was dissolved in MeOH (0.8 ml) and K-Pi buffer (0.1 M, pH 8.0, 3 ml), and porcine liver esterase (EC 3.1.1.1, Sigma E-3128, 1270 units in 0.5 ml of 3.2 M $(NH_4)_2SO_4$, pH 8) was added. The soln was left at 30° overnight, then diluted with H_2O (40 ml), acidified with 1 M HCl and extracted with EtOAc. The organic layer was washed with satd aq. NaCl, dried over Na_2SO_4 and concd. The residue was chromatographed on silica gel (4.5 g) with hexane–EtOAc–HOAc (90:10:1) to give **4** (10 mg, 81% yield) as an oil. ^{1}H NMR (400 MHz, CD_3OD): δ1.07 (3H, *s*, H-9′), 1.13 (3H, *d*, $^4J_{H-F}$ = 1.2 Hz, H-8′), 1.94 (3H, *dd*, *J* = 1.5 Hz and $^4J_{H-F}$ = 2.1 Hz, H-7′), 2.04 (3H, *d*, *J* = 1.2 Hz, H-6), 2.25 (1H, *ddd*, *J* = 17.1 and 1.2 Hz, and $^4J_{H-F}$ = 5.2 Hz, H-5′-*pro-R*), 2.60 (1H, *d*, *J* = 17.1 Hz, H-5′-*pro-S*), 5.80 (1H, *br s*, H-2), 5.94 (1H, *dq*, *J* = 1.5 and 1.2 Hz, H-3′), 6.23 (1H, *ddd*, *J* = 16.2 and 0.6 Hz, and $^3J_{H-F}$ = 19.2 Hz, H-5), 7.85 (1H, *d*, *J* = 16.2 Hz, H-4); ^{13}C NMR (125 MHz, acetone-d_6): δ16.8 (*d*, $^3J_{C-F}$ = 6.4 Hz, C-7′), 20.1 (C-6), 22.1 (*d*, $^3J_{C-F}$ = 6.3 Hz, C-8′), 23.5 (C-9′), 40.7 (*d*, $^2J_{C-F}$ = 21.5 Hz, C-6′), 49.0 (*d*, $^3J_{C-F}$ = 6.2 Hz, C-5′), 99.8 (*d*, $^1J_{C-F}$ = 184.4 Hz, C-1′), 118.9 (C-2), 127.1 (*d*, $^3J_{C-F}$ = 5.0 Hz, C-3′), 128.5 (*d*, $^3J_{C-F}$ = 12.1 Hz, C-4), 132.2 (*d*, $^2J_{C-F}$ = 23.6 Hz, C-5), 149.4 (C-3), 157.5 (*d*, $^2J_{C-F}$ = 24.0 Hz, C-2′), 166.2 (C-1), 205.3 (*d*, $^4J_{C-F}$ = 39.2 Hz, C-4′); UV λ_{max}^{MeOH} nm (log ε): 238 (4.32); UV of the methyl ester, λ_{max}^{MeOH} nm (log ε): 263.5 (4.42); IR of the methyl ester, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2950, 1710, 1660, 1630, 1600, 1450, 1380, 1240, 1160; EIMS (probe) 70 eV, *m/z* (rel. int.): 266 [M]$^+$ (1), 246 (4), 210 (4), 192 (30), 164 (100), 156 (48), 136 (21), 111 (34); HREIMS: [M]$^+$ at *m/z* 266.1349 ($C_{15}H_{19}O_3F$ requires 266.1319).

The reaction mechanism of DAST with (+)-ABA. A methyl ester of (+)-ABA (50 mg), provided by Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan, was fluorinated with DAST (80 mg) and then hydrolysed by the same method used for **4**. The enantiomeric composition of the product 1′-deoxy-1′-fluoroabscisic acid was analysed by HPLC on a Chiralcel OF column, as well as (±)-**4** as described in the section titled. Optical resolution.

(±)-*2-Fluoromethyl-2,6-dimethyl-1-cyclohexanone* (**10a** *and* **10b**). The synthesis of **9** (a mixture of two diastereomers) was as reported [15]. Chromatography of **9** (25 g) on silica gel (970 g) with hexane-EtOAc (17:3–4:1) gave the *cis*-dimethyl form **9a** (11.1 g) and the *trans*-dimethyl form **9b** (13.5 g). Isomer **9a** (0.25 g) in Et_2O (5 ml) was treated with DAST (0.52 g) in Et_2O (7 ml) in the same manner as for **4**. The product was chromatographed on silica gel (6 g) with hexane–EtOAc (24:1) to give **10a** (67 mg, 27% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ1.04 (3H, *d*, *J* = 6.4 Hz, Me-6), 1.11 (3H, *d*, $^4J_{H-F}$ = 1.2 Hz, Me-2), 1.35–2.14 (6H, *m*, H-3, H-4 and H-5), 2.62 (1H, *m*, H-6), 4.41 (1H, *dd*, *J* = 9.2 Hz and $^2J_{H-F}$ = 47.3 Hz, CH_2F), 4.58 (1H, *dd*, *J* = 9.2 Hz and $^2J_{H-F}$ = 47.9 Hz, CH_2F); GC-MS 70 eV, *m/z* (rel. int.): 158 [M]$^+$ (30), 100 (20), 95 (14), 82 (43), 74 (18), 69 (100). In the same manner as **9a**, isomer **9b** (0.5 g) gave **10b** (10.2 mg, 2% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ1.01 (3H, *d*, *J* = 6.4 Hz, Me-6), 1.21 (3H, *d*, $^4J_{H-F}$ = 1.8 Hz, Me-2), 1.29–2.10 (6H, *m*, H-3, H-4 and H-5), 2.63 (1H, *m*, H-6), 4.30 (1H, *dd*, *J* = 9.2 Hz and $^2J_{H-F}$ = 47.6 Hz, CH_2F), 4.52 (1H, *dd*, *J* = 9.2 Hz and $^2J_{H-F}$ = 47.6 Hz, CH_2F); GC-MS 70 eV, *m/z* (rel. int.): 158 [M]$^+$ (44), 110 (24), 100 (42), 83 (32), 82 (64), 81 (40), 70 (55), 69 (100). The mixt. of **9a** and **9b** (240 g) was added dropwise to a soln of DAST (180 g) in Et_2O (500 ml) cooled to −78° under N_2. The mixt. was then warmed to room temp. and stirred for 20 hr. After quenching with H_2O, the mixt. was extracted with Et_2O. The organic

layer was washed with aq. $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concd. Vacuum distillation of the residual oil gave a mixt. of **10a** and **10b** (44.6 g, 18% yield) as an oil, bp 80–88° (13 mmHg).

(±)-4-(1′-*Hydroxy*-2′-*fluoromethyl*-2′,6′-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol*-*tetrahydropyranyl ether* (**11**). A 1.6 M soln of *n*-butyl lithium in hexane (350 ml) was added dropwise to a stirred soln of 1-methyl-2-propynyl tetrahydropyranyl ether (90 g) in THF (300 ml) over 1 hr at −78° under N_2. After stirring for 1 hr, the reaction mixt. was warmed to −25°, and a mixt. of **10a** and **10b** (30 g) in THF (50 ml) was added dropwise. The mixt. was stirred for 2 hr at −25° to −10°, then warmed to room temp. After quenching with 0.1 M NH_4Cl (700 ml), the mixt. was extracted with Et_2O, and the organic layer was successively washed with 0.1 M NH_4Cl and H_2O, dried over Na_2SO_4, and concd to give a crude oil. Purification by chromatography on silica gel (1.2 kg) with hexane–EtOAc (7:3) gave **11** (49 g, 83% yield) as a mixt. of diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$) of the major diastereomer: δ1.05 (3H, *d*, J = 6.7 Hz, Me-6′), 1.18 (3H, *s*, Me-2′), 1.22–1.90 (12H, *m*, H-3′, H-4′, H-5′, H-2″, H-3″ and H-4″), 1.49 (3H, *d*, J = 6.7 Hz, H-1), 3.53 (2H, *m*, H-5″), 4.49 (1H, *dd*, J = 9.2 Hz and $^2J_{H-F}$ = 47.7 Hz, CH_2F), 4.70 (1H, *dd*, J = 9.2 Hz and $^2J_{H-F}$ = 47.9 Hz, CH_2F), 4.58 (1H, *m*, H-2), 4.92 (1H, *m*, H-1″); EIMS (probe) 70 eV, *m/z* (rel. int.): 312 $[M]^+$ (1), 228 (3), 210 (9), 190 (10), 175 (12), 147 (13), 133 (15), 121 (26), 109 (27), 93 (29), 85 (100).

(±)-4-(1′-*Hydroxy*-2′-*fluoromethyl*-2′,6′-*dimethylcyclohexyl*)-*but*-3-*yn*-2-*ol*. (**12**). To a stirred soln of **11** (49 g) in EtOH (600 ml) was added pyridinium *p*-toluenesulphonate (4 g), and the mixt. was stirred at 55° for 4 hr. The soln was concd and the residue diluted with Et_2O (1 l), successively washed with aq. $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concd. Chromatography of the residual oil on silica gel (900 g) with hexane–EtOAc (7:3) gave **12** (30 g, 84% yield) as a mixt. of diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$) of the major diastereomer: δ1.04 (3H, *d*, J = 6.5 Hz, Me-6′), 1.19 (3H, *s*, Me-2′), 1.25–1.90 (6H, *m*, H-3′, H-4′ and H-5′), 1.49 (3H, *d*, J = 6.5 Hz, H-1), 4.52 (1H, *dd*, J = 9.2 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F), 4.62 (1H, *q*, J = 6.5 Hz, H-2), 4.69 (1H, *dd*, J = 9.2 Hz and $^2J_{H-F}$ = 47.9 Hz, CH_2F); EIMS (probe) 70 eV, *m/z* (rel. int.): 228 $[M]^+$ (16), 196 (25), 175 (29), 148 (30), 139 (58), 121 (100), 111 (38).

(±)-3-(1′-*Hydroxy*-2′-*fluoromethyl*-2′,6′-*dimethylcyclohexyl*)-1-*methyl*-2-*propynyl acetate* (**13**). A soln of **12** (30 g) and Ac_2O (110 ml) in pyridine (200 ml) was stirred at room temp. for 15 hr. The soln was poured into ice-cooled H_2O and extracted with Et_2O. The organic layer was successively washed with 0.1 M HCl, aq. $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concd. The residual oil was chromatographed on silica gel (900 g) with hexane-EtOAc (9:1) to give **13** (33 g, 93% yield) as a mixt. of diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$) of the major diastereomer: δ1.02 (3H, *d*, J = 6.4 Hz, Me-6′), 1.17 (3H, *s*, Me-2′), 1.24–1.90 (6H, *m*, H-3′, H-4′ and H-5′), 1.51 (3H, *d*, J = 6.7 Hz, Me-1), 2.07 (3H, *s*, OAc), 4.49 (1H, *dd*, J = 9.2 Hz and $^2J_{H-F}$ = 47.7 Hz, CH_2F), 4.69 (1H, *dd*, J = 9.2 Hz and $^2J_{H-F}$ = 47.9 Hz, CH_2F), 5.47 (1H, *q*, J = 6.7 Hz, H-1); EI-MS (probe) 70 eV, *m/z* (rel. int.): 270 $[M]^+$ (1), 208 (14), 190 (7), 175 (10), 147 (11), 121 (20), 109 (24), 91 (25), 80 (100).

(±)-3-(2′-*Fluoromethyl*-2′,6′-*dimethyl*-1′-*cyclohexen*-1′-*yl*)-1-*methyl*-2-*propynyl acetate* (**14**). To a stirred soln of **13** (56 g) in pyridine (200 ml), a mixt. of $POCl_3$ (115 ml) and pyridine (200 ml) was added dropwise at 0°, then the soln was heated at 100° for 3 hr. The soln was poured into ice-cooled H_2O and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4 and concd to give a crude oil. Purification by chromatography on silica gel (330 g) with hexane–EtOAc (97:3) gave **14** (11 g, 21% yield) as a mixt. of two diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.08 (3/2H, *d*, $^4J_{H-F}$ = 5.6 Hz, Me-2′), 1.09 (3/2H, *d*, $^4J_{H-F}$ = 5.6 Hz, Me-2′), 1.37–2.06 (6H, *m*, H-3′, H-4′ and H-5′), 1.52 (3H, *d*, J = 6.6 Hz, Me-1), 1.89 (3H, *s*, Me-6′), 2.07 (3H, *s*, OAc), 4.18 (1/2H, *dd*, J = 5.7 Hz and $^2J_{H-F}$ = 47.7 Hz, CH_2F), 4.23 (1/2H, *dd*, J = 5.7 Hz and $^2J_{H-F}$ = 47.7 Hz, CH_2F), 4.37 (1/2H, *dd*, J = 8.9 Hz and $^2J_{H-F}$ = 48.3 Hz, CH_2F), 4.40 (1/2H, *dd*, J = 8.9 Hz and $^2J_{H-F}$ = 48.3 Hz, CH_2F), 5.59 (1H, *q*, J = 6.6 Hz, H-1): EIMS (probe) 70 eV, *m/z* (rel. int.): 252 $[M]^+$ (11), 208 (22), 185 (26), 175 (39), 159 (55), 137 (47), 115 (54), 105 (75), 91 (100).

(±)-(E)-4-(2′-*Fluoromethyl*-2′,6′-*dimethyl*-1′-*cyclohexen*-1′-*yl*)-3-*buten*-2-*ol* (**15**). To a stirred soln of **14** (4.5 g) in THF (80 ml), a mixt. of Red-Al (3.4 M in toluene, 85 ml) and THF (60 ml) was added dropwise at 0° over 30 min under N_2. The soln was refluxed for 2 hr. Satd NH_4Cl was added to quench the reaction, and the mixt. was filtered and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4 and concd. The residual oil was chromatographed on silica gel (120 g) with hexane–EtOAc (19:1) to give **15** (3.3 g, 87% yield) as a mixt. of two diastereomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.01 (3H, *d*, $^4J_{H-F}$ = 2.1 Hz, Me-2′), 1.31 (3H, *d*, J = 6.4 Hz, H-1), 1.35–2.03 (6H, *m*, H-3′, H-4′ and H-5′), 1.69 (3H, *s*, Me-6′), 4.08 (1/2H, *dd*, J = 5.6 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F), 4.10 (1/2H, *dd*, J = 5.6 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F), 4.292 (1/2H, *dd*, J = 8.9 Hz and $^2J_{H-F}$ = 48.4 Hz, CH_2F), 4.294 (1/2H, *dd*, J = 8.9 Hz and $^2J_{H-F}$ = 48.4 Hz, CH_2F), 4.37 (1H, *dq*, J = 6.5 and 6.4 Hz, H-2), 5.50 (1H, *dd*, J = 15.9 and 6.5 Hz, H-3), 6.01 (1H, *d*, J = 15.9 Hz, H-4); EIMS (probe) 70 eV, *m/z* (rel. int.): 212 $[M]^+$ (4), 194 (21), 179 (30), 161 (53), 154 (24), 121 (100), 105 (34).

(±)-(E)-4-(6′-*fluromethyl*-2′,6′-*dimethyl*-1′-*cyclohexen*-1′-*yl*)-3-*buten*-2-*one* (**16**). A mixt. of active MnO_2 (30 g) and **15** (3.3 g) was stirred in CH_2Cl_2 (200 ml) at room temp. for 4 hr. The suspension was filtered, and the resulting cake of MnO_2 was washed with CH_2Cl_2. After being concd, the residual oil was purified by chromatography on silica gel (80 g) with hexane–EtOAc (19:1) to give **16** (3.1 g, 93% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.09 (3H, *d*, $^4J_{H-F}$ = 2.0 Hz, Me-6′), 1.39–2.13 (6H, *m*, H-3′, H-4′ and H-5′), 1.79 (3H, *s*, Me-2′), 2.30 (3H, *s*, H-1), 4.16 (1H, *dd*, J = 9.0 Hz and $^2J_{H-F}$ = 47.6 Hz, CH_2F), 4.31 (1H, *dd*, J = 9.0 Hz and $^2J_{H-F}$ = 48.1 Hz, CH_2F), 6.09 (1H, *d*, J = 16.4 Hz, H-3), 7.22 (1H, *d*, J = 16.4 Hz,

H-4); EIMS (probe) 70 eV, *m/z* (rel. int.): 210 $[M]^+$ (5), 195 (73), 181 (11), 149 (8), 131 (17), 105 (15), 91 (21), 77 (15), 69 (100).

(±)-(2Z,4E *and* 2E,4E)-*Methyl* 5-(6′-*fluoromethyl*-2′,6′-*dimethyl*-1′-*cyclohexen*-1′-*yl*)-3-*methyl*-2,4-*pentadienoate* (**17**). A mixt. of **16** (3.1 g) and methyl (triphenylphosphoranylidene) acetate (10.5 g) was stirred at 175° for 2 hr, then dissolved in EtOAc (50 ml). The soln was concd and the residue chromatographed on silica gel (80 g) with hexane–EtOAc (99:1) to give **17** (2.7 g, 69% yield) as a mixt. of two geometrical isomers (2Z:2E = 3:7, determined by integrating the C-6′ methyl singlets in the ^{1}H NMR spectrum). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.04 (3H, *d*, $^4J_{H-F}$ = 2.1 Hz, Me-6′-2*E*), 1.09 (3H, *d*, $^4J_{H-F}$ = 2.0 Hz, Me-6′-2*Z*), 1.37–2.10 (12H, *m*, H-3′, H-4′ and H-5′), 1.72 (3H, *s*, Me-2′-2*E*), 1.80 (3H, *s*, Me-2′-2*Z*), 2.04 (3H, *d*, *J* = 1.2 Hz, H-6-2*Z*), 2.33 (3H, *d*, *J* = 1.1 Hz, H-6-2*E*), 3.69 (3H, *s*, CO_2Me-2*Z*), 3.71 (3H, *s*, CO_2Me-2*E*), 4.10 (1H, *dd*, *J* = 8.9 Hz and $^2J_{H-F}$ = 47.7 Hz, CH_2F-2*E*), 4.15 (1H, *dd*, *J* = 8.9 Hz and $^2J_{H-F}$ = 47.7 Hz, CH_2F-2*Z*), 4.29 (1H, *dd*, *J* = 8.9 Hz and $^2J_{H-F}$ = 48.3 Hz, CH_2F-2*E*), 4.35 (1H, *dd*, *J* = 8.9 Hz and $^2J_{H-F}$ = 48.4 Hz, CH_2F-2*Z*), 5.67 (1H, *s*, H-2-2*Z*), 5.75 (1H, *s*, H-2-2*E*), 6.08 (1H, *d*, *J* = 16.1 Hz, H-4-2*E*), 6.50 (1H, *d*, *J* = 16.1 Hz, H-5-2*E*), 6.53 (1H, *d*, *J* = 16.4 Hz, H-5-2*Z*), 7.61 (1H, *d*, *J* = 16.4 Hz, H-4-2*Z*); EIMS (probe) 70 eV, *m/z* (rel. int.): 266 $[M]^+$ (56), 251 (7), 234 (17), 219 (4), 207 (18), 199 (21), 187 (12), 178 (38), 159 (45), 145 (34), 133 (47), 125 (82), 119 (100), 112 (30), 105 (36).

(±)-(2Z,4E *and* 2E,4E)-*Methyl* 5-(6′-*fluoromethyl*-2′,6′-*dimethyl*-3′,4′-*didehydro*-1′-*cyclohexene*-1′-*yl*)-3-*methyl*-2,4-*pentadienoate* (**18**). *N*-Bromosuccinimide (4.2 g) and benzoyl peroxide (40 mg) were added to a soln of **17** (3.7 g) in CCl_4 (40 ml), and the mixt. was then refluxed for 2 hr under N_2. After cooling the mixt. to room temp., it was filtered and quinoline (13.5 ml) added to the filtrate. The mixt. was concd and the residue heated at 100° for 1 hr under N_2. After cooling to room temp., the reaction mixt. was poured into 1% H_2SO_4 (400 ml) and extracted with Et_2O. The organic layer was successively washed with satd $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concd. The residual oil was purified by chromatography on silica gel (60 g) with hexane–EtOAc (39:1) to give the didehydro compound **18** (2.0 g, 55% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ1.11 (3H, *d*, $^4J_{H-F}$ = 1.5 Hz, Me-6′-2*E*), 1.15 (3H, *d*, $^4J_{H-F}$ = 1.5 Hz, Me-6′-2*Z*), 1.87 (3H, *s*, Me-2′-2*E*), 1.95 (3H, *s*, Me-2′-2*Z*), 2.06 (3H, *d*, *J* = 1.1 Hz, H-6-2*Z*), 2.26–2.42 (4H, *m*, H-5′), 2.34 (3H, *d*, *J* = 1.0 Hz, H-6-2*E*), 3.70 (3H, *s*, CO_2Me-2*Z*), 3.72 (3H, *s*, CO_2Me-2*E*), 4.14 (1H, *dd*, *J* = 8.8 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F-2*E*), 4.17 (1H, *dd*, *J* = 8.7 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F-2*Z*), 4.32 (1H, *dd*, *J* = 8.8 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F-2*E*), 4.35 (1H, *dd*, *J* = 8.7 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F-2*Z*), 5.69 (1H, *s*, H-2-2*Z*), 5.78 (1H, *s*, H-2-2*E*), 5.79–5.90 (4H, *m*, H-3′ and H-4′), 6.21 (1H, *d*, *J* = 16.2 Hz, H-4-2*E*), 6.52 (1H, *d*, *J* = 16.2 Hz, H-5-2*E*), 6.55 (1H, *d*, *J* = 16.5 Hz, H-5-2*Z*), 7.76 (1H, *d*, *J* = 16.5 Hz, H-4-2*Z*); EIMS (probe) 70 eV, *m/z* (rel. int.): 264 $[M]^+$ (63), 249 (17), 231 (15), 205 (42), 199 (100), 185 (22), 171 (56), 157 (52), 143 (32), 119 (56).

(±)-8′-*Fluoroabscisic acid* (**6**), *its* (2E)-*isomer* (**19**), (±)-9′-*fluoroabscisic acid* (**20**), *and its* (2E)-*isomer* (**21**). A soln of **18** (2.0 g) and rose bengal (0.35 g) in MeOH (250 ml) was stirred under O_2 under fluorescent irradiation at 30° for 12 hr. After being concd, the residue was dissolved in MeOH (20 ml), and Al_2O_3 (active basic, 15 g) was added to the soln. After evaporating the MeOH, hexane (15 ml) was added to the mixt., and the suspension was stirred at room temp. for 2 hr before being chromatographed on Al_2O_3 (80 g). Elution with 10–100% EtOAc in hexane afforded the crude ester as an oil. The crude ester was purified by chromatography on silica gel (40 g) with hexane EtOAc (4:1) to give 790 mg (35% yield) of a mixt. of four isomers. This mixt. (300 mg) was dissolved in MeOH (5 ml) and K-Pi buffer (0.1 M, pH 8.0, 25 ml) and porcine liver esterase (12400 units in 4.9 ml) was added. The soln was left at 30° overnight, then diluted with H_2O (200 ml), acidified with 1 M HCl and extracted with EtOAc. The organic layer was washed with satd aq. NaCl, dried over Na_2SO_4 and concd. The residue was chromatographed on silica gel (30 g) with 4% HOAc in CH_2Cl_2 to give 106 mg of a mixt. of (2*E*)-isomers and 115 mg of a mixt. of (2*Z*)-isomers. The (2*Z*)-isomers (70 mg) were sepd again by chromatography on silica gel (30 g) with 1% TFA in CH_2Cl_2 to give 42 mg of **6** and 22 mg of **20** as white amorphous powders. (2*E*)-Isomers (100 mg) were sepd by chromatography on Sephadex LH-20 (25 g) with 4% HOAc in CH_2Cl_2 to give 6.1 mg of **19** and 8.4 mg of **21** as white amorphous powders. Compound **6**. ^{1}H NMR (500 MHz, CD_3OD): δ1.07 (3H, *d*, $^4J_{H-F}$ = 1.4 Hz, H-9′), 1.92 (3H, *d*, *J* = 1.3 Hz, H-7′), 2.05 (3H, *d*, *J* = 0.8 Hz, H-6), 2.44 (1H, *dd*, *J* = 17.4 and 1.4 Hz, H-5′-*pro-R*), 2.51 (1H, *d*, *J* = 17.4 Hz, H-5′-*pro-S*), 4.38 (1H, *dd*, *J* = 9.4 Hz and $^2J_{H-F}$ = 48.0 Hz, CH_2F), 4.55 (1H, *dd*, *J* = 9.4 Hz and $^2J_{H-F}$ = 48.2 Hz, CH_2F), 5.78 (1H, *br s*, H-2), 5.97 (1H, *br s*, H-3′), 6.20 (1H, *d*, *J* = 16.2 Hz, H-5), 7.76 (1H, *d*, *J* = 16.2 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD): δ19.1 (*d*, $^3J_{C-F}$ = 4.3 Hz, C-9′), 19.4 (C-7′), 21.2 (C-6), 44.4 (*d*, $^3J_{C-F}$ = 5.1 Hz, C-5′), 46.9 (*d*, $^2J_{C-F}$ = 15.5 Hz, C-6′), 79.2 (C-1′), 88.1 (*d*, $^1J_{C-F}$ = 170.8 Hz, C-8′), 120.1 (C-2), 128.3 (C-3′), 129.8 (C-4), 136.9 (C-5), 150.6 (C-3), 165.7 (C-2′), 169.6 (C-1), 199.6 (C-4′), UV λ_{max}^{MeOH} nm (log ε):244 (4.28); UV of the methyl ester, λ_{max}^{MeOH} nm (log ε): 264 (4.28); IR of the methyl ester, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3000, 2950, 1700, 1660, 1630, 1600, 1430, 1375, 1235, 1160; EIMS (probe) 70 eV, *m/z* (rel. int.): 282 $[M]^+$ (4), 264 (10), 231 (11), 223 (22), 203 (7), 190 (100), 172 (22), 162 (54), 147 (16), 134 (60), 119 (15), 111 (49); HREIMS: $[M]^+$ at *m/z* 282.1255 ($C_{15}H_{19}O_4F$ requires 282.1268). Compound **20**. ^{1}H NMR (500 MHz, CD_3OD): δ1.06 (3H, *d*, $^4J_{H-F}$ = 2.1 Hz, H-8′), 1.93 (3H, *d*, *J* = 1.3 Hz, H-7′), 2.02 (3H, *d*, *J* = 1.0 Hz, H-6), 2.27 (1H, *d*, *J* = 16.9 Hz, H-5′-*pro-R*), 2.75 (1H, *d*, *J* = 16.9 Hz, H-5′-*pro-S*), 4.22 (1H, *dd*, *J* = 9.5 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F), 4.53 (1H, *dd*, *J* = 9.5 Hz and $^2J_{H-F}$ = 48.0 Hz, CH_2F), 5.74 (1H, *br s*, H-2), 5.96 (1H, *br s*, H-3′), 6.24 (1H, *dd*, *J* = 16.0 Hz and $^5J_{H-F}$ = 3.3 Hz, H-5), 7.75 (1H, *d*, *J* = 16.0 Hz, H-4);

^{13}C NMR (125 MHz, CD_3OD): δ17.9 (*d*, $^3J_{C-F}$ = 6.1 Hz, C-8′), 19.1 (C-7′), 21.2 (C-6), 44.9 (*d*, $^3J_{C-F}$ = 4.0 Hz, C-5′), 47.3 (*d*, $^2J_{C-F}$ = 15.3 Hz, C-6′), 79.1 (C-1′), 88.0 (*d*, $^1J_{C-F}$ = 173.0 Hz, C-9′), 119.5 (C-2), 127.5 (C-3′), 129.0 (C-4), 137.2 (C-5), 151.2 (C-3), 165.4 (C-2′), 169.4 (C-1), 199.9 (C-4′); UV λ_{max}^{MeOH} nm (log ε): 244 (4.32); UV of the methyl ester, λ_{max}^{MeOH} nm (log ε): 264 (4.28); IR of the methyl ester, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3000, 2950, 1700, 1660, 1630, 1600, 1430, 1375, 1235, 1160; EIMS (probe) 70 eV, *m/z* (rel. int.): 282 [M]$^+$ (3), 264 (13), 231 (10), 223 (20), 203 (6), 190 (100), 172 (12), 162 (58), 147 (17), 134 (64), 119 (16), 111 (60); HREIMS: [M]$^+$ at *m/z* 282.1263 ($C_{15}H_{19}O_4F$ requires 282.1268). Compound **19**. ^{1}H NMR (500 MHz, CD_3OD): δ1.06 (3H, *d*, $^4J_{H-F}$ = 1.4 Hz, H-9′), 1.90 (3H, *d*, *J* = 1.3 Hz, H-7′), 2.27 (3H, *d*, *J* = 0.7 Hz, H-6), 2.47 (1H, *dd*, *J* = 17.4 and 1.3 Hz, H-5′-*pro-R*), 2.54 (1H, *d*, *J* = 17.4 Hz, H-5′-*pro-S*), 4.37 (1H, *dd*, *J* = 9.3 Hz and $^2J_{H-F}$ = 48.0 Hz, CH_2F), 4.54 (1H, *dd*, *J* = 9.3 Hz and $^2J_{H-F}$ = 48.1 Hz, CH_2F), 5.86 (1H, *br s*, H-2), 5.95 (1H, *br s*, H-3′), 6.22 (1H, *d*, *J* = 15.7 Hz, H-5), 6.45 (1H, *d*, *J* = 15.7 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 246 (4.34); UV of the methyl ester, λ_{max}^{MeOH} nm (log ε): 264 (4.36); IR of the methyl ester, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3000, 2950, 1705, 1660, 1630, 1610, 1430, 1350, 1230, 1160, EIMS (probe) 70 eV, *m/z* (rel. int.): 282 [M]$^+$ (4), 264 (7), 231 (10), 223 (18), 208 (7), 190 (100), 172 (12), 162 (48), 147 (14), 134 (61), 119 (14), 111 (27); HREIMS: [M]$^+$ at *m/z* 282.1266 ($C_{15}H_{19}O_4F$ requires 282.1268). Compound **21**. ^{1}H NMR (500 MHz, CD_3OD): δ1.06 (3H, *d*, $^4J_{H-F}$ = 2.0 Hz, H-8′), 1.90 (3H, *d*, *J* = 1.2 Hz, H-7′), 2.25 (3H, *d*, *J* = 1.1 Hz, H-6), 2.30 (1H, *dd*, *J* = 17.1 and 0.7 Hz, H-5′-*pro-R*), 2.77 (1H, *d*, *J* = 17.1 Hz, H-5′-*pro-S*), 4.21 (1H, *dd*, *J* = 9.5 Hz and $^2J_{H-F}$ = 47.8 Hz, CH_2F), 4.49 (1H, *dd*, *J* = 9.5 Hz and $^2J_{H-F}$ = 48.0 Hz, CH_2F), 5.83 (1H, *br s*, H-2), 5.94 (1H, *br s*, H-3′), 6.27 (1H, *dd*, *J* = 15.7 Hz and $^5J_{H-F}$ = 2.9 Hz, H-5), 6.45 (1H, *d*, *J* = 15.7 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 246 (4.37); UV of the methyl ester, λ_{max}^{MeOH} nm (log ε): 264 (4.34); IR of the methyl ester, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560, 3000, 2950, 1705, 1660, 1630, 1610, 1430, 1360, 1230, 1160; EIMS (probe) 70 eV, *m/z* (rel. int.): 282 [M]$^+$ (5), 264 (8), 231 (9), 223 (18), 208 (7), 190 (100), 172 (14), 162 (41), 147 (12), 134 (59), 119 (12), 111 (25); HREIMS: [M]$^+$ at *m/z* 282.1265 ($C_{15}H_{19}O_4F$ requires 282.1268).

(±)-1′-Deoxyabscisic acid (**5**) *and its* (2E)*-isomer* (**24**). The mixt. of (±)-methyl 1′-deoxyabscisate (**22**) and its (2*E*)-isomer (**23**) was prepared as reported by Roberts *et al* [18]. This mixt. (300 mg) was hydrolysed by porcine liver esterase (2530 units in 1 ml) in the same manner as **4**. The hydrolysate was chromatographed on silica gel (5.6 g) with 10–25% EtOAc in hexane containing 1% HOAc to give a mixt. of **5** and **24** (20 mg, 35% yield). The mixt. (20 mg) was sepd by HPLC on a silica gel column (YMC A023, 250 mm × 10 mm; solvent, 1% HOAc in $CHCl_3$; flow rate, 3.0 ml min^{-1}; detection, 254 nm) to give 10.4 mg of **5** and 7.5 mg of **24** as oils. Compound **5**. ^{1}H NMR (500 MHz, $CDCl_3$): δ0.99 (3H, *s*, H-9′), 1.07 (3H, *s*, H-8′), 1.93 (3H, *d*, *J* = 0.7 Hz, H-7′), 2.04 (3H, *d*, *J* = 0.7 Hz, H-6), 2.16 (1H, *d*, *J* = 1.69 Hz, H-5′), 2.38 (1H, *d*, *J* = 16.9 Hz, H-5′), 2.75 (1H, *d*, *J* = 9.6 Hz, H-1′), 5.74 (1H, *s*, H-2), 5.95 (1H, *s*, H-3′), 5.98 (1H, *dd*, *J* = 15.7 and 9.6 Hz, H-5), 7.70 (1H, *d*, *J* = 15.7 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 264 (4.33); UV of the methyl ester, λ_{max}^{MeOH} nm (log ε): 266 (4.27); IR of the methyl ester, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2950, 1710, 1660, 1625, 1610, 1430; EIMS of the methyl ester, (probe) 70 eV, *m/z* (rel. int.): 262 [M]$^+$ (2), 247 (1), 231 (7), 215 (4), 189 (4), 174 (40), 146 (100), 125 (>100), 119 (24), 112 (19); HREIMS of the methyl ester: [M]$^+$ at *m/z* 262.1559 ($C_{16}H_{22}O_3$ requires 262.1569). Compound **24**. ^{1}H NMR (500 MHz, $CDCl_3$): δ0.98 (3H, *s*, H-9′), 1.07 (3H, *s*, H-8′), 1.90 (3H, *d*, *J* = 0.9 Hz, H-7′), 2.14 (1H, *d*, *J* = 16.8 Hz, H-5′), 2.29 (3H, *s*, H-6), 2.36 (1H, *d*, *J* = 16.8 Hz, H-5′), 2.67 (1H, *d*, *J* = 9.5 Hz, H-1′), 5.81 (1H, *s*, H-2), 5.96 (1H, *s*, H-3′), 6.02 (1H, *dd*, *J* = 15.5 and 9.4 Hz, H-5), 6.25 (1H, *d*, *J* = 15.5 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 257 (4.47); UV of the methyl ester, λ_{max}^{MeOH} nm (log ε): 266 (4.45); IR of the methyl ester, $\nu_{max}^{CHCL_3}$ cm^{-1}: 3000, 2950, 1710, 1660, 1625, 1610, 1430; EIMS of the methyl ester, (probe) 70 eV, *m/z* (rel. int.): 262 [M]$^+$ (3), 231 (7), 206 (14), 189 (4), 174 (63), 146 (100), 125 (93), 119 (21), 112 (15); HREIMS of the methyl ester: [M]$^+$ at *m/z* 262.1571 ($C_{16}H_{22}O_3$ requires 262.1569).

Optical resolution. When the solns collected from the columns in this section were concd, toluene was added to evaporate TFA as an azeatrope. Racemic **4** (7 mg) was injected into a Chiralcel OF HPLC column (250 mm × 10 mm, Diacel; solvent, 8% isopropanol in hexane containing 0.1% TFA; flow rate, 3.5 ml min^{-1}; detection, 254 nm). The materials at R_t 16.4 and 18.2 min were collected to give (−)- and (+)-**4** (1.8 and 3.2 mg) with an optical purity of 99.9 and 99.2%, respectively, measured by HPLC on the same column. (−)-**4**: $[\alpha]_D^{15}$ − 358° (MeOH; *c*. 0.379); CD: $\Delta\varepsilon_{223}$ + 13.3, $\Delta\varepsilon_{257}$ − 18.2, $\Delta\varepsilon_{319}$ + 1.8 (MeOH; *c* 0.000994). (+)-**4**: $[\alpha]_D^{15}$ + 357° (MeOH; *c* 0.212); CD: $\Delta\varepsilon_{222}$ − 17.2, $\Delta\varepsilon_{254}$ + 19.5, $\Delta\varepsilon_{315}$ − 1.6 (MeOH; *c* 0.000989). Racemic **6** (21 mg) was injected into a Chiralcel OD HPLC column (250 mm × 4.6 mm, Daicel; solvent, 11% isopropanol in hexane containing 0.1% TFA; flow rate, 1.2 ml min^{-1}; detection, 254 nm). The materials at R_t 8.0 and 15.0 min were collected to give (+)- and (−)-**6** (10.2 and 10.3 mg) with an optical purity of 99.9 and 99.3%, respectively, measured by HPLC on the same column. Racemic **20** (15 mg) was injected into a Chiralcel OD column under the same conditions as described for **6**, and the materials at R_t 8.0 and 16.0 min were collected to give (+)- and (−)-**20** (7.4 and 7.4 mg) with optical purity of 99.9 and 99.5%, respectively, measured by HPLC on the same column. (+)-**6**: $[\alpha]_D^{28}$ + 351° (MeOH; *c* 0.667); CD: *c* 0.00083). (−)-**6**: $[\alpha]_D^{28}$ − 345° (MeOH; *c* 0.667); CD: $\Delta\varepsilon_{229}$ + 27.5, $\Delta\varepsilon_{260}$ − 34.3, $\Delta\varepsilon_{319}$ + 2.5 (MeOH; *c* 0.00083). (+)-**20**: $[\alpha]_D^{28}$ + 368° (MeOH; *c* 0.493); CD: $\Delta\varepsilon_{230}$ − 27.8, $\Delta\varepsilon_{261}$ + 34.5, $\Delta\varepsilon_{323}$ − 2.9 (MeOH; *c* 0.00093). (−)-**20**: $[\alpha]_D^{28}$ − 375° (MeOH; *c* 0.493); CD: $\Delta\varepsilon_{228}$ + 28.1, $\Delta\varepsilon_{260}$ − 35.0, $\Delta\varepsilon_{322}$ + 2.9 (MeOH; *c* 0.00093). Racemic **5** (9.4 mg) was injected into a Chiralcel OD HPLC column (solvent, 8% isopropanol in hexane containing 0.1% TFA; flow rate, 1.0 ml min^{-1}; detection, 254 nm). The materials at R_t 11.4 and 14.4 min

were collected to give (+)- and (−)-**5** (4.8 and 2.0 mg) with an optical purity of 93 and 97%, respectively, measured by HPLC on the same column. (+)-**5**: $[\alpha]_D^{29}$ + 344 (MeOH; *c* 0.2); CD: $\Delta\varepsilon_{216}$ − 11.4, $\Delta\varepsilon_{265}$ + 23.7, $\Delta\varepsilon_{313}$ − 2.3 (MeOH; *c* 0.001). (−)-**5**: $[\alpha]_D^{29}$ − 334 (MeOH; *c* 0.2); CD: $\Delta\varepsilon_{216}$ + 11.7, $\Delta\varepsilon_{264}$ − 24.2, $\Delta\varepsilon_{316}$ + 2.4 (MeOH; *c* 0.001). Racemic ABA was optically resolved as described [15].

Bioassays. Details of the four bioassays have been reported [15]. For the germination assay, the number of germinated lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds was counted after incubation with the test soln at 25° for 48 hr. For the elongation assay, the length of the second leaf sheath of rice (*Oryza sativa* L. cv. Nihonbare) seedlings was measured after incubation with the test soln in continuous light at 30° for 7 days. For the α-amylase assay, after incubating barley (*Hordeum vulgare* L. cv. Himalaya) half-seeds without embryos in the test soln at 30° for 48 hr in the dark, the absorbance of the test soln at 660 nm was measured by the Somogyi–Nelson method [27]. For the stomata assay, the width of stomatal apertures on epidermal strips of spiderwort (*Tradescantia reflexa* Rafin) was measured after incubation with the test soln in continuous light at 25° for 3 hr.

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