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8′,8′-DIFLUORO- AND 8′,8′,8′-TRIFLUOROABSCISIC ACIDS AS HIGHLY POTENT, LONG-LASTING ANALOGUES OF ABSCISIC ACID

YASUSHI TODOROKI, NOBUHIRO HIRAI* and KOICHI KOSHIMIZU

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606-01, Japan

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Key Word Index—Inhibition of lettuce seed germination, rice seedling elongation, induction of α-amylase and stomatal opening; long-lasting analogues; abscisic acid; 8′,8′-difluoroabscisic acid; 8′,8′,8′-trifluoroabscisic acid.

Abstract—Racemic 8′,8′-difluoroabscisic acid (difluoro-ABA) and 8′,8′,8′-trifluoroabscisic acid (trifluoro-ABA) were synthesized as highly potent, long-lasting analogues of abscisic acid (ABA). The individual optical isomers were obtained by optical resolution of the racemic mixture by HPLC with a chiral column. (+)-8′,8′-Difluoro-ABA and (+)-8′,8′,8′-trifluoro-ABA inhibited the elongation of rice seedlings six and 30 times more strongly, respectively, than (+)-ABA. These analogues also showed double the (+)-ABA-induced inhibition of lettuce seed germination. In causing stomatal closure and inhibiting the induction of α-amylase by gibberellin A_3, these analogues were equally as effective as (+)-ABA. The high activity in the assays over a long period suggested that the metabolism of difluoro- and trifluoro-ABAs was delayed. (−)-Enantiomers were equal to, or weaker than (−)-ABA in the assays.

INTRODUCTION

The plant hormone abscisic acid (ABA, **1**) regulates various physiological processes in higher plants [1]. The agricultural uses of ABA as a plant-growth regulator have been restricted owing to its rapid metabolism to the inactive phaseic acid (PA, **3**) via 8′-hydroxy-ABA (**2**) as well as photoisomerization to the inactive 2-*E* isomer [2]. It should be possible to enhance the effectiveness of applied ABA by supplying it in the form of an analogue which has an extended half-life through reduced metabolism without diminishing its biological activity. Previously, we synthesized 8′-methoxy-ABA (**4**) which because it is resistant to cyclization to phaseic acid is a highly potent, long-lasting analogue of ABA [3], and found that it inhibits the elongation of rice seedlings four times more strongly than ABA. In the present report, we describe the synthesis and biological activity of 8′,8′-difluoro-ABA (**6**) and 8′,8′,8′-trifluoro-ABA (**8**) as new long-lasting analogues of ABA (Fig. 1).

The steric size of fluorine is very similar to that of hydrogen, and the energy of the C–F bond is higher than that of the C–H bond [4]. The replacement of hydrogen by fluorine at C-8′ of ABA, therefore, should block hydroxylation at C-8′ without reducing the affinity of the active site. 24,24-Difluoro-25-hydroxy-vitamin D_3 is a long-lasting analogue of 25-hydroxy-vitamin D_3 which owing to its resistance to metabolic hydroxylation at C-24 has a higher and longer-lasting activity than the parent molecule [5].

Recently, 7′,7′-difluoro-ABA has been synthesized as the first fluorine-containing ABA analogue [6]. However, 7′,7′-difluoro-ABA was shown to be less active than ABA probably because C-7′ of ABA is the minor site available for oxidation [7]. We report here the first fluorination of the major site available for oxidation in the ABA molecule.

RESULTS AND DISCUSSION

Synthesis and identification

Difluoro-ABAs were synthesized by a modification of the method reported for the synthesis of 8′-methoxy-ABA [3] (Fig. 2). Oxidation of **10** gave the formyl ketone **11**. Compound **11** was fluorinated using diethylaminosulphur trifluoride (DAST) to give the difluoromethyl ketone **12** in a 45% yield. The reaction of **12** with alkynyl lithium gave the tetrahydropyranyl ether **13**. Deprotection of **13** gave the acetylenic diol **14**, which was then acetylated to afford the acetylenic acetate **15**. Dehydration of **15** gave the enyne acetate **16**. Reduction of **16** gave the dienol **17**, which was then oxidized yielding the dienone **18**. A Wittig reaction with **18** gave the methyl ester **19** as a mixture of (2*Z*)- and (2*E*)-isomers. Bromination of **19** and then dehydrobromination formed the didehydro compound **20**, which on photosensitized oxygenation and subsequent treatment with basic alumina gave the methyl esters of difluoro-ABAs: four stereoisomers resulting from 2*Z*/2*E* isomerism and from the *cis*

*Author to whom correspondence should be addressed.

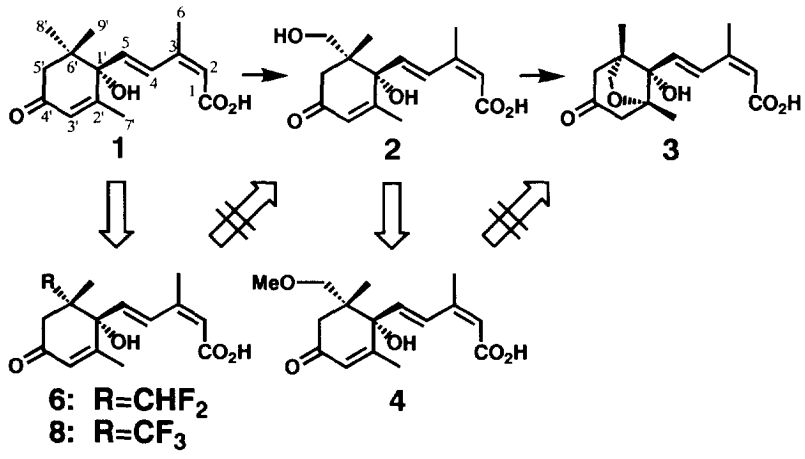

Fig. 1. Deactivation pathway for ABA in plants, and design of long-lasting analogues.

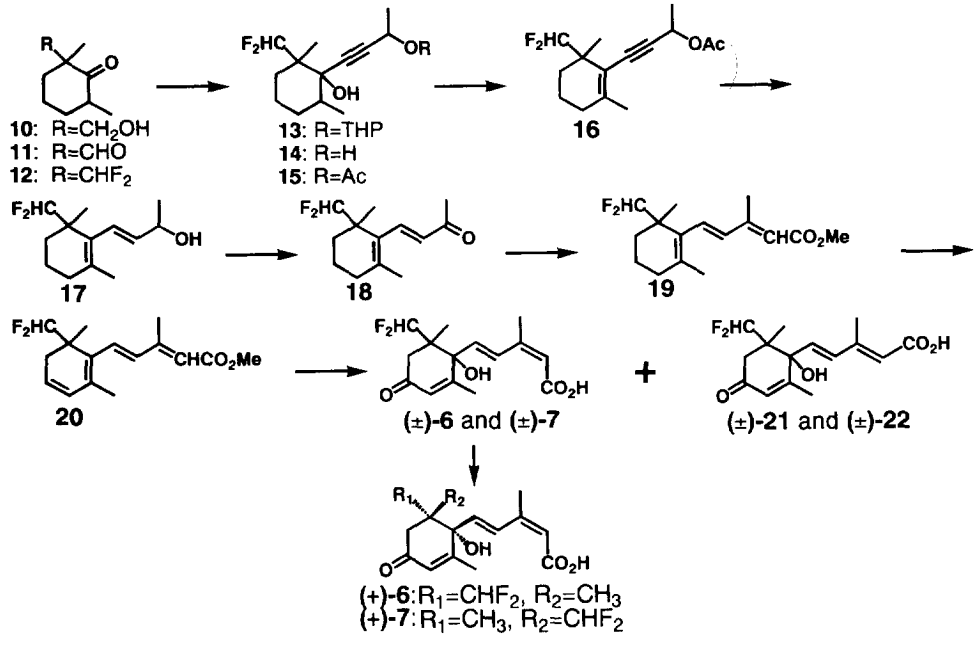

Fig. 2. Synthesis of 8′,8′- and 9′,9′-difluoro-ABAs (**6** and **7**).

or *trans* relationship of the 6′-difluoromethyl group to the 1′-hydroxyl group. Hydrolysis of these methyl esters gave an isomeric mixture of (±)-8′,8′-difluoro-ABA (**6**) and its (±)-(2*E*)-isomer (**21**), and of (±)-9′,9′-difluoro-ABA (**7**) and its (±)-(2*E*)-isomer (**22**) (*ca* 1:2:4:8, as determined by HPLC). This mixture was separated into its components by HPLC on an ODS column.

Trifluoro-ABAs were synthesized from compound **23** prepared by the method reported for the synthesis of 16,16,16-trifluororetinal [8] (Fig. 3). In the same manner as for **19**, compound **23** gave an isomeric mixture of (±)-8′,8′,8′-trifluoro-ABA (**8**) and its (±)-(2*E*)-isomer (**25**), and of (±)-9′,9′,9′-trifluoro-ABA (**9**) and its (±)-(2*E*)-isomer (**26**) (*ca* 1:2:4:8, as determined by HPLC). This mixture was separated into its components by HPLC on an ODS column.

The ratio of the C-8′ fluorinated analogue to C-9′ fluorinated analogue in difluoro- and trifluoro-ABAs was 1:4, and was brought about during the photosensitized oxygenation of the didehydro compounds (**20** and **24**). Thus, the photosensitized oxygenation of **20** and **24** showed higher diastereoselectivity than in the case of the synthesis of methoxy-ABAs where the corresponding ratio was 1:3 [3]. A singlet oxygen will add from the sterically or electrically less-hindered site, that is, from the opposite side to the C-6′ substituent group. The steric sizes of the di- and trifluoromethyl groups are smaller in the direction parallel to the C-6′/C-8′ or the C-6′/C-9′

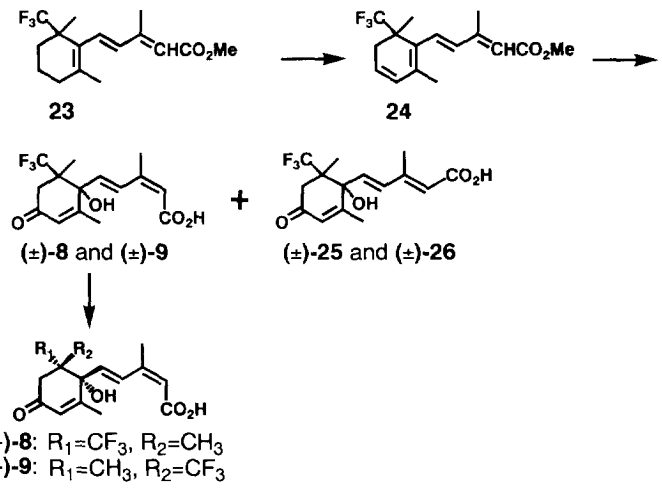

Fig. 3. Synthesis of 8′,8′,8′- and 9′,9′,9′-trifluoro-ABAs (**8** and **9**).

bond than that of the methoxymethyl group and may be a little larger perpendicularly to the bond [9]. The electrical effects of two or three fluorines will be higher than that of one oxygen [4]. This suggests that the higher diastereoselectivity observed in the synthesis of difluoro- and trifluoro-ABAs may be attributable to electrical repulsion between fluorine and oxygen rather than steric hindrance.

Identification of difluoro- and trifluoro-ABAs was accomplished by analysis of ^{1}H and ^{13}C NMR spectral data. In the ^{1}H NMR spectra, the C-6′ methyl groups of **6** and **8** (δ1.10 and 1.26) appeared at higher field than those of **7** and **9** (δ1.18 and 1.33), respectively. The C-9′ proton of ABA (δ1.01) appeared at higher field than the C-8′ proton (δ1.11) [10], so the spectra of **6** and **8** both lacked a methyl singlet corresponding to the C-8′ proton of ABA, and those of **7** and **9** lacked a methyl singlet corresponding to the C-9′ proton. Analogues **6** and **7** both showed a triplet signal of one proton at δ6.03 and 5.92, respectively, which were assigned to the proton at C-8′ and at C-9′, respectively, bonded to two fluorine atoms. These findings showed that **6** was 8′,8′-difluoro-ABA and **8** was 8′,8′,8′-trifluoro-ABA and that **7** was 9′,9′-difluoro-ABA and **9** was 9′,9′,9′-trifluoro-ABA. The ^{13}C NMR spectra confirmed the above identification. The signals of C-8′ of **6** and C-9′ of **7** appeared as triplets at δ119.9 and 119.2, respectively, by coupling with two fluorine atoms, and the ^{13}C signals of C-8′ of **8** and C-9′ of **9** appeared as quartets at δ130.3 and 129.1, respectively, by coupling with three fluorine atoms.

(±)-Difluoro- and (±)-trifluoro-ABAs were optically resolved by HPLC on a Chiralcel OD column to afford the (+)- and (−)-enantiomers with an optical purity of more than 99%. The CD spectra of the (+)-enantiomers showed the same positive first and negative second Cotton effects, i.e. the positive exciton chirality, as those of (*S*)-(+)-ABA [11]. Therefore, the absolute configuration at C-1′ of (+)-difluoro- and (+)-trifluoro-ABAs was *R*.

Biological activity

The optically active analogues were compared with the (+)- and (−)-ABAs and (−)-PA for their inhibitory activity in four bioassays: lettuce seed germination [12]; elongation of the second leaf sheath of rice seedlings [13]; α-amylase induction by gibberellin A_3 in barley half-seeds without embryos [14]; and stomatal opening of the epidermal strips of spiderwort [15]. Values for the concentration giving half-maximal inhibition (IC_{50}) from the assays are summarized in Table 1. The 2-*E* isomers of recemic **6**–**9** were inactive in the assays (data not shown).

The C-8′ trifluorinated analogue (+)-**8** caused 50% inhibition of germination at a concentration of 1.9 μM, and (+)-ABA caused the same degree of inhibition at 5 μM; i.e. the activity of (+)-**8** was 2.6 times that of (+)-ABA. In the elongation assay, (+)-**8** was more powerful than in the germination assay, and its IC_{50} value was only 0.082 μM, while that for (+)-ABA was 2.6 μM; i.e. (+)-**8** was more than 30 times as effective as (+)-ABA. The C-8′ difluorinated analogue (+)-**6** also showed strong activity in the germination and elongation assays; *ca* two and six times those of ABA, respectively. Investigation of the changes with time in the effects of (+)-**6** and (+)-**8** in these two assays showed that (+)-**6** and (+)-**8** were superior in stability to (+)-ABA (Fig. 4); as time passed, the ratio of the activity of (+)-**6** and (+)-**8** to that of (+)-ABA increased, becoming 2/1 on day 2 and 4/1 on day 5 for both analogues in the germination assay, and 6/1 on day 5 and 10/1 on day 9 for (+)-**6**, and 25/1 on day 5 and 40/1 on day 9 for (+)-**8** in the elongation assays. These results suggested that the high activity of (+)-**6** and (+)-**8** observed in these two assays was a result of a slower inactivation than ABA.

In the α-amylase and stomata assays, the activities of (+)-**6** and (+)-**8** were as effective as those of (+)-ABA. In the stomata assay which is a short-term assay (3 hr), the activity of a test compound will be unaffected by the

Table 1. IC_{50} values for ABA, difluoro-ABAs, trifluoro-ABAs and PA in four bioassays

Compound	IC_{50} in assay			
	Germination* (μM)	Elongation† (μM)	α-Amylase‡ (μM)	Stomata§ (nM)
(+)-ABA	5.0	2.6	2.9	5.0
(+)-**6**	2.3	0.45	3.3	5.8
(+)-**7**	5.7	1.5	4.0	4.3
(+)-**8**	1.9	0.082	2.0	4.6
(+)-**9**	7.9	2.4	6.4	4.2
(−)-ABA	9.7	3.5	7.3	67
(−)-**6**	10	4.0	7.5	68
(−)-**7**	12	12	26	62
(−)-**8**	11	2.8	24	330
(−)-**9**	7.9	27	20	60
(−)-PA	> 300	> 300	9.2	> 1000

*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.

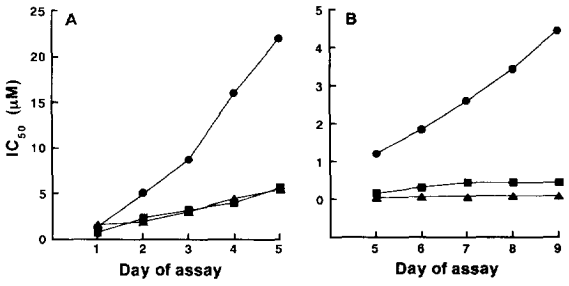

Fig. 4. Changes with time in the inhibitory activity of (+)-ABA (●), (+)-**6** (■) and (+)-**8** (▲) in lettuce seed germination (A) and rice seedling elongation (B) bioassays.

speed of its metabolism [16]. In this assay, therefore, blockage of metabolic inactivation will have little or no influence on the activity. In the α-amylase assay which takes place over a rather longer period (2 days), (−)-PA showed activity *ca* 1/3 that of (+)-ABA. This result agrees with those reported by Lin and Ho [17]. This finding implies that blockage of conversion to (−)-PA via 8′-hydroxy-ABA does not contribute to the enhancement of the activity, thus explaining the lower activity of (+)-**6** and (+)-**8** in the α-amylase assay than in the germination and the elongation assays. Consequently, the results obtained in these four assays suggests that (+)-**6** and (+)-**8** act as long-lasting analogues, as expected.

The activities of the C-9′ difluorinated analogue (+)-**7** and the C-9′ trifluorinated analogue (+)-**9** were almost equal to that of (+)-ABA in all the bioassays. This result suggests that di- and trifluorination of C-9′ of (+)-ABA did not influence the affinity for the active site and had no effect on inhibiting the approach or action of the hydroxylation enzyme.

The activity of (−)-ABA was slightly lower than that of (+)-ABA in the four assays used in the present study, as also reported previously [3]. The (−)-enantiomers of the di- and trifluoro analogues showed activity which was the same as or less than that of (−)-ABA; compound (−)-**6** was equivalent to (−)-ABA in all the assays, (−)-**8** was less active in the α-amylase and stomata assays, and (−)-**7** and (−)-**9** were less effective in the elongation and α-amylase assays. The cyclohexenone ring of ABA is relatively symmetrical, so (−)-ABA may bind to the same site that (+)-ABA binds. In this case, C-8′ or C-9′ of (−)-ABA occupies the site normally filled by C-7′ of (+)-ABA which is essential for activity [18, 19]. The van der Waals radius of fluorine is a little larger than that of hydrogen. The slightly bulkier C-8′ or C-9′ of the (−)-enantiomers of the long-lasting analogues would cause a weakening of the affinity for the active site and hence reduced activity as observed. This implies that the steric requirement around C-2′ is much stricter than that around C-6′. The differences in the effects of the individual (−)-enantiomers among the four assays suggests that steric tolerance of the binding site for the equatorial direction and for the lower axial direction at C-2′ differs with species or tissue.

EXPERIMENTAL

^1H and ^{13}C NMR: TMS as int. standard using a Jeol GX400 (400 MHz), Jeol GSX270J (270 MHz) or JNM GSX-500 (500 MHz). For clarity, the conventional ABA numbering system is used in the assignment of peaks in the ^1H and ^{13}C NMR spectra. Mass spectra: Jeol JMS-DX300/DA5000 mass spectrometer; GC-MS: 1% OV-17 column (1 m × 3 mm) in the EI mode.

(±)-2-*Formyl*-2,6-*dimethyl*-1-*cyclohexanone* (**11**). The synthesis of **10** was previously reported [3]. A mixture of

10 (66 g) and pyridinium chlorochromate (110 g) in CH_2Cl_2 (200 ml) was stirred at room temp. for 2 hr. The suspension was filtered and the filtrate concd. The residual oil was chromatographed on silica gel with hexane–EtOAc (9:1) to give **11** (33 g, 51% yield) as a mixture of two diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ1.03 (3/2H, *d*, *J* = 6.7 Hz, Me-6), 1.06 (3/2H, *d*, *J* = 6.4 Hz, Me-6), 1.21 (3/2H, *s*, Me-2), 1.35 (3/2H, *s*, Me-2), 1.35–2.46 (6H, *m*, H-3, H-4, and H-5), 2.52 (1/2H, *m*, H-6), 2.65 (1/2H, *m*, H-6), 9.45 (1/2H, *d*, *J* = 0.6 Hz, CHO), 9.72 (1/2H, *s*, CHO); GC-MS 70 eV, *m/z* (rel. int.): 154 $[M]^+$ (4), 139 (4), 126 (57), 111 (35), 97 (34), 84 (32), 71 (100).

(±)-2-*Difluoromethyl-2,6-dimethyl-1-cyclohexanone* (**12**). Compound **11** (33 g) was added dropwise to a soln of DAST (57.4 g) in CH_2Cl_2 (300 ml) cooled to − 78° under N_2. The mixture was then warmed to room temp. and stirred for 4 hr. After being quenched with aq. $NaHCO_3$ and H_2O, the mixture was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over Na_2SO_4 and concd. The residual oil was chromatographed on silica gel with hexane–EtOAc (97:3) to give **12** (17.1 g, 45% yield) as a mixture of two diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01 (3/2H, *d*, *J* = 6.4 Hz, Me-6), 1.07 (3/2H, *d*, *J* = 6.4 Hz, Me-6), 1.13 (3/2H, *t*, $^4J_{H-F}$ = 1.2 Hz, Me-2), 1.29 (3/2H, *t*, $^4J_{H-F}$ = 1.2 Hz, Me-2), 1.34–2.27 (6H, *m*, H-3, H-4, and H-5), 2.58 (1/2H, *m*, H-6), 2.61 (1/2H, *m*, H-6), 5.98 (1/2H, *t*, $^2J_{H-F}$ = 56.2 Hz, CHF_2), 6.03 (1/2H, *t*, $^2J_{H-F}$ = 55.5 Hz, CHF_2); GC-MS 70 e V, *m/z* (rel. int.): 176 $[M]^+$ (30), 128 (11), 113 (8), 109 (8), 98 (37), 86 (25), 81 (11), 73 (17), 69 (100).

(±)-4-(1′-*Hydroxy-2′-difluoromethyl-2′,6′-dimethylcyclohexyl*)-*but-3-yn-2-ol-tetrahydropyranyl ether* (**13**). A 1.6 M soln of *n*-butyl lithium in hexane (100 ml) was added dropwise to a stirred soln of 1-methyl-2-propynyl tetrahydropyranyl ether (24 g) in THF (100 ml) over 30 min at − 78° under N_2. After being stirred for 1 hr, the reaction mixture was warmed to − 25°, and **12** (17.1 g) in THF (50 ml) was added dropwise to the stirred mixture. The mixture was stirred for 2 hr at − 25° to − 10° and then warmed to room temp. After being quenched with 0.1 M NH_4Cl (250 ml), the mixture was extracted with Et_2O, and the organic layer successively washed with 0.1 M NH_4Cl and H_2O, dried over Na_2SO_4, and concd. The residual oil was chromatographed on silica gel with hexane–EtOAc (5:1) to give **13** (24.6 g, 77% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) of the major diastereomer: δ1.06 (3H, *d*, *J* = 6.4 Hz, Me-6′), 1.18 (3H, *s*, Me-2′), 1.48 (3H, *d*, *J* = 6.7 Hz, H-1), 1.51–2.11 (12H, *m*, H-3′, H-4′, H-5′, H-2″, H-3″ and H-4″), 4.62 (1H, *q*, *J* = 6.7 Hz, H-2), 4.94 (1H, *dd*, *J* = 4.3 and 2.7 Hz, H-1″), 6.06 (1H, *t*, $^2J_{H-F}$ = 56.2 Hz, CHF_2); EI-MS (probe) 70 eV, *m/z* (rel. int.): 330 $[M]^+$ (3), 246 (21), 228 (30), 210 (31), 177 (15), 159 (48), 139 (23), 121 (66), 109 (41), 91 (43), 84 (100).

(±)-4-(1′-*Hydroxy-2′-difluoromethyl-2′,6′-dimethylcyclohexyl*)-*but-3-yn-2-ol* (**14**). To a stirred soln of **13** (24.6 g) in EtOH (400 ml) was added pyridinium *p*-toluenesulphonate (2 g), and the mixture was stirred at 55° for 5 hr. The soln was concd and the residue was diluted with Et_2O (1 litre), successively washed with aq. $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concd. Chromatography of the residual oil on silica gel with hexane–EtOAc (5:1) gave **14** (17.8 g, 97% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) of the major diastereomer: δ1.05 (3H, *d*, *J* = 6.4 Hz, Me-6′), 1.13 (3H, *s*, Me-2′), 1.22–2.10 (6H, *m*, H-3′, H-4′, and H-5′), 1.49 (3H, *d*, *J* = 6.7 Hz, H-1), 4.61 (1H, *q*, *J* = 6.7 Hz, H-2), 6.07 (1H, *t*, $^2J_{H-F}$ = 56.2 Hz, CHF_2); EI-MS (probe) 70 eV, *m/z* (rel. int.): 246 $[M]^+$ (60), 228 (4), 213 (7), 193 (8), 177 (6), 166 (28), 149 (15), 139 (81), 121 (100), 109 (80).

(±)-3-(1′-*Hydroxy-2′-difluoromethyl-2′,6′-dimethylcyclohexyl*)-1-*methyl-2-propynyl acetate* (**15**). A soln of **14** (17.8 g) and Ac_2O (40 ml) in pyridine (100 ml) was stirred at room temp. for 13 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 with hexane–EtOAc (9:1) to give **5** (17.8 g, 85% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) of the major diastereomer: δ1.04 (3H, *d*, *J* = 6.4 Hz, Me-6′), 1.09–1.95 (6H, *m*, H-3′, H-4′, and H-5′), 1.12 (3H, *s*, Me-2′), 1.52 (3H, *d*, *J* = 6.7 Hz, Me-1), 2.07 (3H, *s*, OAc), 5.45 (1H, *q*, *J* = 6.7 Hz, H-1), 6.06 (1H, *t*, $^2J_{H-F}$ = 57.2 Hz, CHF_2); EI-MS (probe) 70 eV, *m/z* (rel. int.): 288 $[M]^+$ (13), 270 (3), 246 (11), 228 (63), 213 (13), 193 (11), 177 (16), 166 (21), 135 (35), 121 (29), 109 (37), 93 (18), 80 (100).

(±)-3-(2′-*Difluoromethyl-2′,6′-dimethyl-1′-cyclohexen-1′-yl*)-1-*methyl-2-propynyl acetate* (**16**). To a stirred soln of **15** (17.3 g) in pyridine (150 ml), a mixture of $POCl_3$ (37.3 ml) and pyridine (40 ml) was added dropwise at 0°, and the soln was then heated at 100° for 23 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. The residual oil was chromatographed on silica gel with hexane–EtOAc (19:1) to give **16** (5.8 g, 30% yield) as a mixture of two diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ1.16 (3/2H, *t*, $^4J_{H-F}$ = 1.2 Hz, Me-2′), 1.17 (3/2H, *t*, $^4J_{H-F}$ = 1.2 Hz, Me-2′), 1.37–2.14 (6H, *m*, H-3′, H-4′, and H-5′), 1.52 (3H, *d*, *J* = 6.7 Hz, Me-1), 1.90 (3H, *s*, Me-6′), 2.07 (3H, *s*, OAc), 5.57 (1H, *q*, *J* = 6.7 Hz, H-1), 5.84 (1/2H, *t*, $^2J_{H-F}$ = 57.1 Hz, CHF_2), 5.87 (1/2H, *t*, $^2J_{H-F}$ = 57.1 Hz, CHF_2); EI-MS (probe) 70 eV, *m/z* (rel. int.): 270 $[M]^+$ (10), 242 (19), 226 (21), 213 (85), 203 (66), 185 (33), 175 (54), 159 (78), 149 (40), 142 (46), 129 (55), 115 (75), 105 (61), 91 (100).

(±)-(E)-4-(2′-*Difluoromethyl-2′,6′-dimethyl-1′-cyclohexen-1′-yl*)-3-*buten-2-ol* (**17**). To a stirred soln of **16** (4.9 g) in THF (50 ml) a mixture of Red-Al (3.4 M in toluene, 60 ml) and THF (40 ml) was added dropwise at 0° over 40 min under N_2. The soln was refluxed for 3 hr. A satd NH_4Cl soln was added to quench the reaction, and the mixture 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 with hexane–EtOAc (19:1–9:1) to give **17** (3.4 g, 81% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ1.09 (3/2H, *s*, Me-2′), 1.09 (3/2H, *s*,

Me-2'), 1.31 (3/2H, *d*, $^4J_{H-F}$ = 56.4 Hz, H-1), 1.31 (3/2H, *d*, $^4J_{H-F}$ = 6.4 Hz, H-1), 1.40–2.12 (6H, *m*, H-3', H-4', and H-5'), 1.71 (3H, *s*, Me-6'), 4.37 (1H, *dq*, *J* = 6.4 and 6.4 Hz, H-2), 5.52 (1H, *dd*, *J* = 16.2 and 6.4 Hz, H-3), 5.66 (1/2H, *t*, $^2J_{H-F}$ = 56.8 Hz, CHF_2), 5.66 (1/2H, *t*, $^2J_{H-F}$ = 56.8 Hz, CHF_2), 5.99 (1H, *d*, *J* = 16.2 Hz, H-4); EI-MS (probe) 70 eV, *m/z* (rel. int.): 230 $[M]^+$ 212 (43), 197 (12), 172 (34), 161 (81), 145 (10), 133 (15), 121 (100), 105 (46).

(±)-(E)-4-(6'-*Difluoromethyl*-2', 6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*buten*-2-*one* (**18**). A mixture of active MnO_2 (27 g) and **17** (3.3 g) was stirred in CH_2Cl_2 (130 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 chromatographed on silica gel with hexane–EtOAc (19:1) to give **18** (2.9 g, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ1.16 (3H, *s*, Me-6'), 1.45–2.16 (6H, *m*, H-3', H-4', and H-5'), 1.80 (3H, *s*, Me-2'), 2.30 (3H, *s*, H-1), 5.68 (1H, *t*, $^2J_{H-F}$ = 56.8 Hz, CHF_2), 6.07 (1H, *d*, *J* = 16.2 Hz, H-3), 7.17 (1H, *d*, *J* = 16.2 Hz, H-4); EI-MS (probe) 70 eV, *m/z* (rel. int.): 228 $[M]^+$ (4), 213 (100), 199 (6), 185 (5), 177 (5), 162 (4), 159 (5), 135 (2).

(±)-(2Z,4E *and* 2E,4E)-*Methyl* 5-(6'-*difluoromethyl*-2',6'-*dimethyl*-1'-*cyclohexen*-1'-*yl*)-3-*methyl*-2,4-*pentadienoate* (**19**). A mixture of **18** (2.9 g) and methyl (triphenylphosphoranylidene)acetate (10 g) was stirred at 175° for 2 hr, and then dissolved in EtOAc (50 ml). The soln was chromatographed on silica gel with hexane–EtOAc (49:1) to give **19** (3.1 g, 87% yield) as a mixture of two geometrical isomers (2*Z*:2*E* = 45:55, determined by integrating the C-6' methyl singlets in the ^{1}H NMR spectrum). ^{1}H NMR (400 MHz, $CDCl_3$): δ1.12 (3H, *s*, Me-6'-*E*), 1.16 (3H, *s*, Me-6'-*Z*), 1.44–2.28 (12H, *m*, H-3', H-4', and H-5'), 1.73 (3H, *d*, *J* = 0.6 Hz, Me-2'-*E*), 1.81 (3H, *s*, Me-2'-*Z*), 2.04 (3H, *d*, *J* = 1.2 Hz, H-6-*Z*), 2.32 (3H, *d*, *J* = 1.2 Hz, H-6-*E*), 3.69 (3H, *s*, CO_2Me-*Z*), 3.72 (3H, *s*, CO_2Me-*E*), 5.64 (1H, *t*, $^2J_{H-F}$ = 56.8 Hz, CHF_2-*E*), 5.68 (1H, *s*, H-2-*Z*), 5.73 (1H, *t*, $^2J_{H-F}$ = 56.8 Hz, CHF_2-*Z*), 5.76 (1H, *s*, H-2-*E*), 6.08 (1H, *d*, *J* = 15.9 Hz, H-4-*E*), 6.46 (1H, *d*, *J* = 15.9 Hz, H-5-*E*), 6.50 (1H, *d*, *J* = 16.5 Hz, H-5-*Z*), 7.61 (1H, *d*, *J* = 16.5 Hz, H-4-*Z*); EI-MS (probe) 70 eV, *m/z* (rel. int.): 284 $[M]^+$ (100), 269 (4), 225 (56), 209 (52), 183 (12), 173 (21), 159 (81), 145 (16), 131 (20), 119 (58), 105 (33).

(±)-8',8'-*Difluoro-ABA* (**6**) and *its* (2E)-*isomer* (**21**), and 9',9'-*difluoro-ABA* (**7**) *and its* (2E)-*isomer* (**22**). *N*-bromosuccinimide (2.5 g) and benzoyl peroxide (26 mg) were added to a soln of **19** (3.1 g) in CCl_4 (20 ml), and the mixture was then refluxed for 2.5 hr under N_2. After cooling the mixture to room temp., it was filtered, and quinoline (8 ml) was added to the filtrate. The mixture was concd, and the residue was heated at 100° for 1 hr under N_2. After being cooled to room temp., the reaction mixture 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 chromatographed on silica gel with hexane–EtOAc (99:1–49:1) to give the didehydro compound (**20**, 1.31 g) as a crude oil. A soln of **20** (1.3 g) and rose bengal (0.2 g) in MeOH (200 ml) was stirred under O_2 while being irradiated with a fluorescent lamp at 25° for 15 hr. After being concd, the residue was dissolved in MeOH (20 ml), and alumina (active basic, 15 g) was added to the soln. After evaporating the MeOH, hexane (15 ml) was added to the mixture, and the suspension was stirred at room temp. for 2.5 hr before being chromatographed on Al_2O_3. Elution with 40–100% EtOAc in hexane afforded the crude ester as an oil. The crude ester was purified by chromatography on silica gel with hexane–EtOAc (17:3) to give 600 mg (42% yield) of a mixture of four isomers. To a soln of this mixture (600 mg) in MeOH was added 1 N NaOH (10 ml). The mixture was stirred at room temp. for 6 hr, then diluted with H_2O (150 ml) and washed with hexane. The organic layer was discarded, and the aq. layer was acidified with 1 N HCl and extracted with EtOAc. The organic layer was washed with H_2O, dried over Na_2SO_4, and concd. The residue was separated by HPLC on μBondasphere 5 μ C18-100Å (150 × 19 mm, Waters; solvent, 52% MeOH in 1% HOAc; flow rate, 4.8 ml min^{-1}; detection, 254 nm) to give as amorphous powders 39 mg of (±)-8',8'-difluoro-ABA, 57 mg of its (2*E*)-isomer, 156 mg of 9',9'-difluoro-ABA, and 243 mg of its (2*E*)-isomer. (±)-8',8'-Difluoro-ABA (**6**). ^{1}H NMR (400 MHz, CD_3OD): δ1.10 (3H, *s*, H-9'), 1.93 (3H, *d*, *J* = 1.5 Hz, H-7'), 2.04 (3H, *d*, *J* = 1.2 Hz, H-6), 2.46 (1H, *d*, *J* = 17.7 Hz, H-5'-*pro*-*R*), 2.64 (1H, *d*, *J* = 17.7 Hz, H-5'-*pro*-*S*), 5.78 (1H, *s*, H-2), 5.94 (1H, *s*, H-3'), 6.03 (1H, *t*, $^2J_{H-F}$ = 56.2 Hz, H-8'), 6.17 (1H, *d*, *J* = 15.9 Hz, H-5), 7.79 (1H, *d*, *J* = 15.9 Hz, H-4); ^{13}C NMR (67.5 MHz, CD_3OD): δ17.5 (C-9'), 19.3 (C-7'), 21.2 (C-6), 41.7 (C-5'), 78.4 (C-1'), 119.9 (*t*, J_{C-F} = 244.2 Hz, C-8'), 120.6 (C-2), 128.0 (C-3'), 129.9 (C-4), 136.7 (C-5), 150.2 (C-3), 165.2 (C-2'), 169.7 (C-1), 198.6 (C-4'); UV λ_{max}^{MeOH} nm (log ε): 249.5 (4.27); IR of the methyl ester $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3000, 2950, 1700, 1665, 1630, 1600, 1433, 1373, 1240, 1160, 1127, 1090, 1050; EI-MS (probe) 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (2), 282 (11), 241 (25), 231 (12), 213 (5), 203 (37), 190 (100), 175 (13), 162 (58), 147 (21), 134 (83), 119 (23), 111 (84); HR-EI-MS: $[M]^+$ at *m/z* 300.1165 ($C_{15}H_{18}O_4F_2$ requires 300.1173). (±)-9',9'-Difluoro-ABA (**7**). ^{1}H NMR (400 MHz, CD_3OD): δ1.18 (3H, *d*, $^4J_{H-F}$ = 1.0 Hz, H-8'), 1.92 (3H, *d*, *J* = 1.5 Hz, H-7'), 2.03 (3H, *d*, *J* = 1.0 Hz, H-6), 2.28 (1H, *dd*, *J* = 17.1 and 1.0 Hz, H-5'-*pro*-*R*), 2.79 (1H, *d*, *J* = 17.1 Hz, H-5'-*pro*-*S*), 5.76 (1H, *s*, H-2), 5.92 (1H, *t*, $^2J_{H-F}$ = 56.2 Hz, H-9'), 5.95 (1H, *s*, H-3'), 6.23 (1H, *dd*, *J* = 15.9 Hz and $^4J_{H-F}$ = 4.9 Hz, H-5), 7.79 (1H, *d*, *J* = 15.9 Hz, H-4); ^{13}C NMR (67.5 MHz, CD_3OD): δ16.3 (C-8'), 19.1 (C-7'), 21.5 (C-6), 41.6 (C-5'), 50.6 (C-6'), 78.8 (C-1'), 119.2 (*t*, J_{C-F} = 244.1 Hz, C-9'), 120.4 (C-2), 127.6 (C-3'), 129.6 (C-4), 136.7 (C-5), 151.1 (C-3), 165.1 (C-2'), 169.8 (C-1), 198.6 (C-4'); UV λ_{max}^{MeOH} nm (log ε): 245.0 (4.31); IR of the methyl ester $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3000, 2940, 1700, 1665, 1630, 1600, 1433, 1373, 1238, 1160, 1120, 1085, 1050; EI-MS (probe) 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (1), 282 (6), 241 (19), 231 (9), 203 (54), 190 (100), 175 (13), 162 (54), 147 (18), 134 (77), 119 (22), 111 (93); HR-EI-MS: $[M]^+$ at *m/z* 300.1163 ($C_{15}H_{18}O_4F_2$ requires 300.1173). (±)-(2*E*)-8',8'-Difluoro-ABA (**21**). ^{1}H NMR (400 MHz, CD_3OD): δ1.08 (3H, *s*, H-9'), 1.91 (3H, *d*, *J* = 1.5 Hz, H-7'), 2.27 (3H, *d*, *J*

= 1.2 Hz, H-6), 2.48 (1H, *d*, *J* = 17.7 Hz, H-5′-*pro-R*), 2.67 (1H, *d*, *J* = 17.7 Hz, H-5′-*pro-S*), 5.87 (1H, *s*, H-2), 5.93 (1H, *br s*, H-3′), 6.02 (1H, *t*, $^2J_{H-F}$ = 56.5 Hz, H-8′), 6.20 (1H, *d*, *J* = 15.9 Hz, H-5), 6.49 (1H, *d*, *J* = 15.9 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 248.5 (4.29); IR of the methyl ester $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3000, 2950, 1710, 1667, 1630, 1613, 1433, 1358, 1256, 1162, 1090, 1055; EI-MS (probe) 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (3), 282 (11), 241 (25), 231 (12), 213 (4), 203 (17), 190 (100), 175 (11), 162 (55), 147 (20), 134 (83), 119 (21), 111 (50); HR-EI-MS: $[M]^+$ at *m/z* 300.1171 ($C_{15}H_{18}O_4F_2$ requires 300.1173). (±)-(2*E*)-9′,9′-Difluoro-ABA (**22**). ^{1}H NMR (400 MHz, CD_3OD): δ1.17 (3H, *s*, H-8′), 1.89 (3H, *d*, *J* = 1.2 Hz, H-7′), 2.26 (3H, *d*, *J* = 1.2 Hz, H-6), 2.31 (1H, *dd*, *J* = 17.1 and 1.2 Hz, H-5′-*pro-R*), 2.83 (1H, *d*, *J* = 17.1 Hz, H-5′-*pro-S*), 5.85 (1H, *s*, H-2), 5.89 (1H, *t*, $^2J_{H-F}$ = 55.9 Hz, H-9′), 5.94 (1H, *qd*, *J* = 1.2 and 1.2 Hz, H-3′), 6.26 (1H, *dd*, *J* = 15.6 Hz and $^4J_{H-F}$ = 4.0 Hz, H-5), 6.50 (1H, *d*, *J* = 15.6 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 246.0 (4.33); IR of the methyl ester $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3000, 2950, 1710, 1670, 1632, 1613, 1433, 1358, 1255, 1162, 1120, 1085, 1053; EI-MS (probe) 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (4), 282 (10), 241 (27), 231 (12), 213 (4), 203 (17), 190 (100), 175 (11), 162 (55), 147 (20), 134 (83), 119 (21), 111 (50); HR-EI-MS: $[M]^+$ at *m/z* 300.1178 ($C_{15}H_{18}O_4F_2$ requires 300.1173).

(±)-8′,8′,8′-Trifluoro-ABA (**8**) *and its* (2E)-*isomer* (**25**), *and 9′,9′,9′-trifluoro-ABA* (**9**) *and its* (2E)-*isomer* (**26**). In the same manner as for **19**, (±)-(2*Z*,4*E* and 2*E*, 4*E*)-methyl 5-(6′-difluoromethyl-2′,6′-dimethyl-1′cyclohexen-1′-yl)-3-methyl-2,4-pentadienoate (215 mg, **23**) gave didehydro compound **24** (75 mg) as a crude oil. In the same manner as for **20**, the didehydro compound **24** (74 mg) gave 0.5 mg of (±)-8′,8′,8′-trifluoro-ABA as an amorphous powder, 1.1 mg of its (2*E*)-isomer as an oil, 2.1 mg of 9′,9′,9′-trifluoro-ABA as an oil, and 3.4 mg of its (2*E*)-isomer as an oil. (±)-8′,8′,8′-Trifluoro-ABA (**8**). ^{1}H NMR (400 MHz, CD_3OD): δ1.26 (3H, *s*, H-9′), 1.95 (6H, *d*, *J* = 1.2 Hz, H-6 and H-7′), 2.67 (1H, *d*, *J* = 19.6 Hz, H-5′-*pro-R*), 2.72 (1H, *d*, *J* = 19.6 Hz, H-5′-*pro-S*), 5.85 (1H, *s*, H-2), 5.92 (1H, *br s*, H-3′), 5.98 (1H, *d*, *J* = 15.9 Hz, H-5), 7.69 (1H, *d*, *J* = 15.9 Hz, H-4); ^{13}C NMR (125 MHz, CD_3OD): δ19.1 (C-9′), 20.1 (C-7′), 21.4 (C-6), 45.1 (C-5′), 79.2 (C-1′), 118.5 (C-2), 128.2 (C-3′), 129.1 (C-4), 130.3 (*q*, J_{C-F} = 276.3 Hz, C-8′), 133.1 (C-5), 167.3 (C-2′), 169.8 (C-1), 198.2 (C-4′); UV λ_{max}^{MeOH} nm (log ε): 242.5 (4.36); EI-MS of the methyl ester (probe) 70 eV, *m/z* (rel. int.): 332 $[M]^+$ (3), 314 (4), 301 (6), 300 (5), 272 (3), 259 (7), 203 (2), 190 (35), 162 (16), 149 (6), 134 (26), 125 (100), 112 (26); HR-EI-MS of the methyl ester: $[M]^+$ at *m/z* 332.1221 ($C_{16}H_{19}O_4F_3$ requires 332.1236). (±)-9′,9′,9′-Trifluoro-ABA (**9**). ^{1}H NMR (400 MHz, CD_3OD): δ1.33 (3H, *s*, H-8′), 1.90 (3H, *s*, H-7′), 1.94 (3H, *d*, *J* = 1.2 Hz, H-6), 2.38 (1H, *dd*, *J* = 16.8 and 0.9 Hz, H-5′-*pro-R*), 2.91 (1H, *d*, *J* = 16.8 Hz, H-5′-*pro-S*), 5.86 (1H, *s*, H-2), 5.94 (1H, *br s*, H-3′), 6.02 (1H, *dq*, *J* = 16.2 Hz and $^4J_{H-F}$ = 2.8 Hz, H-5), 7.58 (1H, *d*, *J* = 16.2 Hz, H-4); ^{13}C NMR (67.5 MHz, CD_3OD): δ16.5 (C-8′), 19.1 (C-7′), 21.0 (C-6), 42.8 (C-5′), 52.2 (C-6′), 78.5 (C-1′), 122.0 (C-2), 127.2 (C-3′), 129.1 (*q*, J_{C-F} = 284.0 Hz, C-9′), 129.5 (C-4), 135.0 (C-5), 148.7 (C-3), 164.9 (C-2′), 196.7 (C-4′); UV λ_{max}^{MeOH} nm (log ε): 239.5 (4.39); EI-MS of the methyl ester (probe) 70 eV, *m/z* (rel. int.): 332 $[M]^+$ (6), 314 (4), 301 (13), 300 (17), 273 (6), 272 (6), 259 (20), 203 (4), 190 (67), 162 (48), 149 (17), 134 (52), 125 (100), 112 (17); HR-EI-MS of the methyl ester: $[M]^+$ at *m/z* 332.1208 ($C_{16}H_{19}O_4F_3$ requires 332.1236). (±)-(2*E*)-8′,8′,8′-Trifluoro-ABA (**25**). ^{1}H NMR (400 MHz, CD_3OD): δ1.24 (3H, *s*, H-9′), 1.92 (3H, *d*, *J* = 1.2 Hz, H-7′), 2.23 (3H, *s*, H-6), 2.73 (2H, *s*, H-5′), 5.90 (1H, *s*, H-2), 5.94 (1H, *br s*, H-3′), 6.17 (1H, *d*, *J* = 15.6 Hz, H-5), 6.53 (1H, *d*, *J* = 15.6 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 242.5 (4.37); EI-MS of the methyl ester (probe) 70 eV, *m/z* (rel. int.): 332 $[M]^+$ (6), 314 (4), 301 (13), 300 (13), 273 (6), 272 (6), 259 (20), 203 (5), 190 (75), 162 (35), 149 (10), 134 (51), 125 (100), 112 (21); HR-EI-MS of the methyl ester: $[M]^+$ at *m/z* 332.1197 ($C_{16}H_{19}O_4F_3$ requires 332.1236). (±)-(2*E*)-9′,9′,9′-Trifluoro-ABA (**26**). ^{1}H NMR (400 MHz, CD_3OD): δ1.34 (3H, *s*, H-8′), 1.92 (3H, *d*, *J* = 1.2 Hz, H-7′), 2.25 (3H, *d*, *J* = 1.2 Hz, H-6), 2.44 (1H, *dd*, *J* = 17.1 and 1.2 Hz, H-5′-*pro-R*), 2.94 (1H, *d*, *J* = 17.1 Hz, H-5′-*pro-S*), 5.86 (1H, *s*, H-2), 5.97 (1H, *dq*, *J* = 1.2 and 1.2 Hz, H-3′), 6.31 (1H, *dq*, *J* = 15.6 Hz and $^4J_{H-F}$ = 1.4 Hz, H-5), 6.49 (1H, *d*, *J* = 15.6 Hz, H-4); UV λ_{max}^{MeOH} nm (log ε): 241.5 (4.34); EI-MS of the methyl ester (probe) 70 eV, *m/z* (rel. int.): 332 $[M]^+$ (6), 314 (4), 301 (14), 300 (14), 273 (6), 272 (5), 259 (20), 203 (4), 190 (70), 162 (35), 149 (14), 134 (52), 125 (100), 112 (21); HR-EI-MS of the methyl ester: $[M]^+$ at *m/z* 332.1180 ($C_{16}H_{19}O_4F_3$ requires 332.1236).

Optical resolution of (±)-8′,8′- *and* (±)-9′,9′-*difluoro-ABAs* (**6** *and* **7**), (±)-8′,8′,8′- *and* (±)-9′,9′,9′-*trifluoro-ABAs* (**8** *and* **9**) *and ABA*. Racemic mixtures of **6** and **7** were separated into enantiomers by HPLC on Chiralpak OD (250 × 4.6 mm, Daicel; solvent, 13% isopropanol in hexane containing 0.1% TFA; flow rate, 0.7 ml min^{-1}; detection, 254 nm). The materials at R_t 10.6 and 17.2 min of **6** (26 mg) were collected to give (+)-and (−)-**6** (12.3 and 13.2 mg) with optical purity of 99.6%, and the materials at R_t 9.4 and 17.4 min of **7** (57 mg) were collected to give (+)- and (−)-**7** (28.0 and 28.2 mg) with optical purity of 99.9 and 99.8%, respectively. (+)-8′,8′-Difluoro-ABA: $[\alpha]_D^{27}$ + 236.6° (MeOH; *c* 0.410); CD: $\Delta\varepsilon_{229}$ − 11.7, $\Delta\varepsilon_{263}$ + 13.0, $\Delta\varepsilon_{320}$ − 1.5 (MeOH; *c* 0.001256). (−)-8′,8′-Difluoro-ABA: $[\alpha]_D^{27}$ − 240.9° (MeOH; *c* 0.440); CD: $\Delta\varepsilon_{225}$ + 12.2, $\Delta\varepsilon_{255}$ − 11.9, $\Delta\varepsilon_{317}$ + 1.6 (MeOH; *c* 0.001329). (+)-9′,9′-Difluoro-ABA: $[\alpha]_D^{27}$ + 336.4° (MeOH; *c* 0.933); CD: $\Delta\varepsilon_{226}$ − 15.8, $\Delta\varepsilon_{262}$ + 17.4, $\Delta\varepsilon_{319}$ − 2.3 (MeOH; *c* 0.001003). (−)-9′,9′-Difluoro-ABA: $[\alpha]_D^{27}$ − 343.6° (MeOH; *c* 0.940); CD: $\Delta\varepsilon_{225}$ − 16.0, $\Delta\varepsilon_{258}$ − 16.7, $\Delta\varepsilon_{317}$ + 2.2 (MeOH; *c* 0.00102). Racemic mixtures of **8** and **9** were separated into enantiomers by HPLC on Chiralpak OD (solvent, 11 and 8% isopropanol, respectively, in hexane containing 0.1% TFA; flow rate, 1.0 ml min^{-1}; detection, 254 nm). The materials at R_t 8.0 and 15.7 min of **8** (0.5 mg) were collected to give (+)- and (−)-**8** (0.2 and 0.2 mg) with optical purity of 99.8 and 99.7%, respectively, and the materials at R_t 11.1 and 16.3 min of **9** (2 mg) were collected to give (+)-and (−)-**9** (0.9 and 0.9 mg) with optical purity of 99.6 and 99.7%, respectively. (+)-8′,8′,8′-Trifluoro-ABA: $[\alpha]_D^{17}$ + 283.3° (MeOH; *c* 0.012); CD: $\Delta\varepsilon_{227}$ − 17.5, $\Delta\varepsilon_{257}$ + 18.9, $\Delta\varepsilon_{317}$ − 1.7 (MeOH;

c 0.000567). (−)-8′,8′,8′-Trifluoro-ABA: $[\alpha]_D^{17}$ − 290.0° (MeOH; *c* 0.020); CD: $\Delta\varepsilon_{228}$ + 15.7, $\Delta\varepsilon_{259}$ − 18.1, $\Delta\varepsilon_{321}$ + 2.5 (MeOH; *c* 0.000567). (+)-9′,9′,9′-Trifluoro-ABA: $[\alpha]_D^{17}$ + 390.6° (MeOH; *c* 0.064); CD: $\Delta\varepsilon_{229}$ − 19.9, $\Delta\varepsilon_{258}$ + 22.3, $\Delta\varepsilon_{315}$ − 2.1 (MeOH; *c* 0.0009). (−)-9′,9′,9′-Trifluoro-ABA: $[\alpha]_D^{17}$ − 391.5° (MeOH; *c* 0.071); CD: $\Delta\varepsilon_{227}$ + 20.9, $\Delta\varepsilon_{256}$ − 20.4, $\Delta\varepsilon_{322}$ + 2.3 (MeOH; *c* 0.0009). Optical resolution of ABA was reported previously [3].

(−)-*PA*. (−)-PA was prepared by hydrolysis of the β-hydroxy-β-methylglutaryl ester of 8′-hydroxyabscisic acid [20].

Bioassays. The details of the four bioassays were reported previously [3]. 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 assays, 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 [21]. 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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