



## CAGED PLANT HORMONES

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**Key Word Index**—Caged plant hormones; gibberellin; abscisic acid; jasmonic acid; 1-naphthylacetic acid; salicylic acid.

**Abstract**—The syntheses of 2-nitrobenzyl and 1-(2-nitrophenyl)ethyl esters of the plant hormones, gibberellin A<sub>4</sub>, abscisic acid, jasmonic acid, 1-naphthylacetic acid and salicylic acid are described. These esters are readily photolysed to the corresponding free acids and, thus, are suitable for the photo-release of these hormones after loading into plant cells.

### INTRODUCTION

The use of light-activated caged molecules is well-established for the study of animal cell biochemistry [1-3]. Caged compounds are biologically inactive, but photolabile, derivatives of intracellularly active molecules. These neutral derivatives can be readily loaded into cells by diffusion, or by recently developed microinjection methods. Subsequent photolysis yields a burst of the free active species, within the cell, thereby allowing study of intracellular processes mediated by the ligand concerned. Methods for the caging of molecules of interest have been developed for phosphoinositides, ATP and other nucleotides; they have been applied to ions, e.g.  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  [1-3]. Usually, caged compounds are 2-nitrobenzyl esters or derivatives thereof. These esters are readily photolysed by pulsed lasers or xenon arc flashlamps to release the parent free acid.

As the majority of the classes of plant hormones are carboxylic acids, which presumably mainly enter plant cells by passive diffusion of the unionized species [4], it may be possible to apply the caged molecule approach to the study of plant hormone perception and control of intracellular events. Although several different types of photocleavable carboxylic acid protecting groups are known [5], 2-nitrobenzyl esters and derivatives have been found to have suitable properties for the preparation of caged compounds. In this paper, we describe the synthesis of both 2-nitrobenzyl and 1-(2-nitrophenyl)ethyl esters of gibberellin A<sub>4</sub> (GA<sub>4</sub>), abscisic acid (ABA), 1-naphthylacetic acid (NAA), jasmonic acid (JA) and salicylic acid (SA) and report on their photolytic characteristics. In addition, we describe the corresponding 4-nitrobenzyl esters of the same hormones which should find use as structurally similar, but non-photocleavable

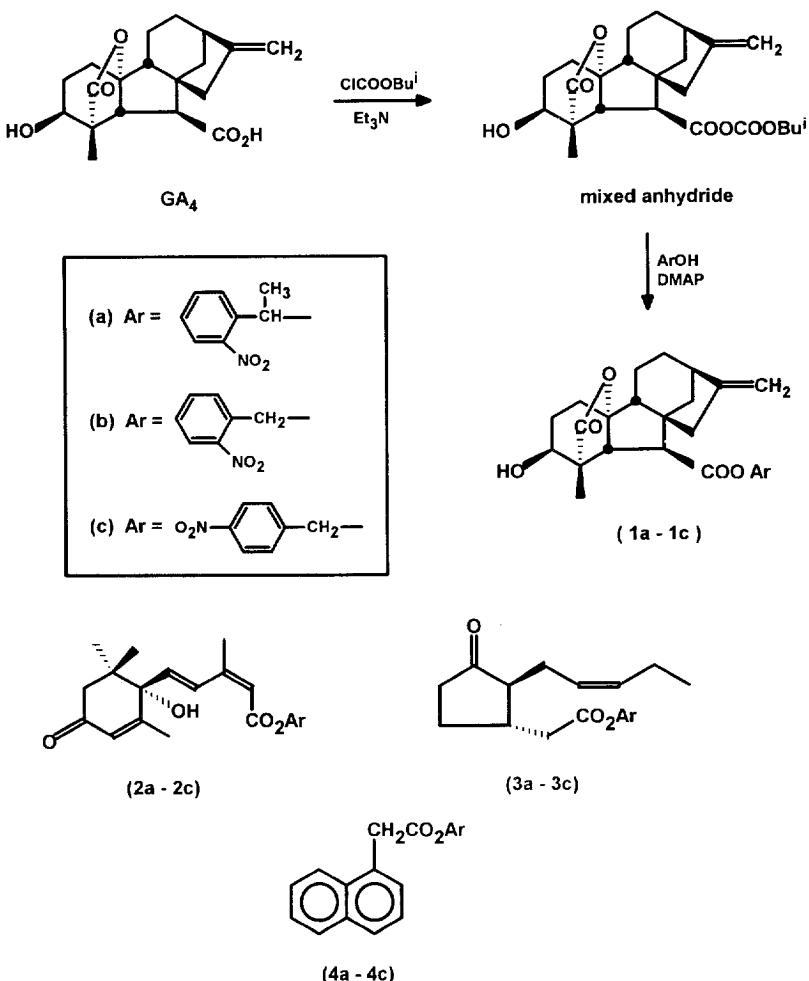
control compounds, in biological experiments using the caged hormones.

### RESULTS AND DISCUSSION

#### *Synthesis of caged hormones*

Of the many methods available for the synthesis of esters by condensation of carboxylic acids with alcohols, we found that the mixed anhydride method was convenient, as it required no protection of the other functional groups present on GA<sub>4</sub>, ABA, JA or NAA. The route that we have developed is illustrated for GA<sub>4</sub> in Scheme 1. Dry acetonitrile was found to be the best solvent for both the preparation of mixed anhydrides using isobutyl-chloroformate and triethylamine, and for the reaction with the corresponding alcohol catalysed by 4-dimethylaminopyridine (DMAP). It was found that the mixed anhydrides had to be isolated before reaction with the alcohol, otherwise the esterification reaction failed. The intermediate anhydrides were reasonably stable. This was especially so for ABA where the mixed anhydride can be chromatographed, recrystallized and stored for subsequent use. The anhydrides gave characteristic <sup>1</sup>H NMR spectra containing a six proton doublet ( $\delta$  ca 0.9), a one proton multiplet ( $\delta$  ca 1.9) and a two proton doublet ( $\delta$  ca 3.9) for the isobutoxy group, in addition to the signals of the hormone concerned. Reactions of these mixed anhydrides with 1-(2-nitrophenyl)ethanol, itself prepared by sodium borohydride reduction of *o*-nitroacetophenone [6], or with either 2-nitro- or 4-nitrobenzyl alcohol were rapid in the presence of DMAP. The resultant 1-(2-nitrophenyl)ethyl esters (**1a-4a**), 2-nitrobenzyl esters (**1b-4b**) and 4-nitrobenzyl esters (**1c-4c**) were formed in moderate yield together with some unreacted anhydride and hormone free acid. The required derivatives were readily purified by flash chromatography and gave

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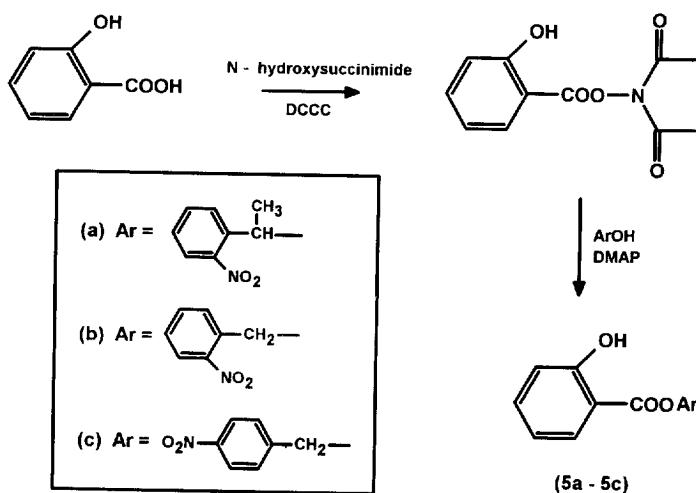
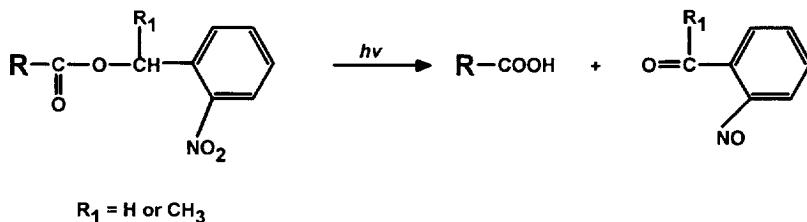
Scheme 1. Synthesis of caged hormones by the mixed anhydride route.

<sup>1</sup>H NMR spectra totally consistent with their structures; the 1-(2-nitrophenyl)ethyl ester of GA<sub>4</sub> (**1a**) displayed doubling of some signals due to the presence of a 4:3 mixture of diastereomers as a result of the addition of a new chiral centre. The effect on the chemical shift of some of the signals in the <sup>1</sup>H NMR spectrum of the diastereomers is unusually large. For example, the 18-methyl occurs at  $\delta$ 0.92 in the major isomer (cf.  $\delta$ 1.15 in the minor isomer, a normal GA<sub>4</sub> position). Similarly, the minor isomer shows an upfield shift of H<sub>2</sub>-17 ( $\delta$ 4.67 and 4.90) relative to the major isomer at  $\delta$ 4.86 and 5.0. These shifts reflect the orientation of the nitrophenyl group in the two diastereomers.

The mixed anhydride method is not applicable for the synthesis of salicylates due to the presence of the phenolic hydroxy group. For caged SA, the coupling was carried out by activation of the acid as the *N*-hydroxysuccinimidyl ester (SA-NHS), formed by reaction with *N*-hydroxysuccinimidyl and dicyclohexylcarbodiimide. Reaction of SA-NHS with the relevant benzyl alcohol yielded the desired esters (**5a-e**) [Scheme 2].

#### Photolytic properties

Photolysis of 2-nitrobenzylesters yields the corresponding acids and 2-nitrosobenzaldehyde as shown in Scheme 3. Similarly, 1-(2-nitrophenyl)ethyl esters give rise to the acids and 2-nitrosoacetophenone. Techniques for the efficient photolysis of caged compounds in the m.sec. time frame using filtered light from xenon arc lamps or pulsed lasers are well-established in this area of cell biochemistry. In this work, we have not examined the rapid photolysis using this specialist equipment, but we needed to demonstrate that the compounds could be cleanly photolysed to give back the parent plant hormone. For this purpose, solutions of the caged hormones in cuvettes were exposed to unfocused light from a greenhouse mercury lamp and the photolysis reaction monitored by UV spectroscopy. Using these conditions, the 1-(2-nitrophenyl)ethyl esters (**1a-5a**) were readily cleaved within 5 min. This was demonstrated by the disappearance of the characteristic UV absorbance of the nitrobenzyl group at  $\lambda$  253 nm and the appearance of

Scheme 2. Synthesis of caged salicylate via *N*-hydroxysuccinimide.

Scheme 3. Photolysis of caged compounds.

the spectrum of nitrosoacetophenone ( $\lambda$  280 nm with a shoulder at 315 nm). This is illustrated for GA<sub>4</sub>-1-(2-nitrophenyl)ethyl ester (**1c**) in Fig. 1. Similar results were obtained for the other hormone 1-(2-nitrophenyl)ethyl esters. The 2-nitrobenzyl esters (**1b**-**5b**) were more difficult to photolysis, with some ester remaining after 30 min of photolysis, while the 4-nitrobenzyl esters (**1c**-**5c**) were not photocleavable. As well as UV analysis of the extent of reaction, the products were also extracted and analysed by TLC against ester and free hormone standards. Esters (**1a**, **3a**-**5a**) were photolysed to give the parent acid as the sole hormone-derived product. ABA-1-(2-nitrophenyl)ethyl ester (**2a**), however, gave a 1:1 mixture of ABA and the biologically inactive 2-*trans*-isomer (**6**) as revealed by GC-mass spectrometry (Scheme 4). This was not unexpected as the photoisomerization of ABA is a well-known reaction [7]. This effect does not preclude the use of caged ABA in cell biological experiments as the photoisomerization is reversible and equilibrates to the observed 1:1 mixture of active and inactive forms.

In conclusion, we have developed methods to prepare caged derivatives of plant hormones. Hormone 1-(2-nitrophenyl)ethyl esters were shown to be suitable derivatives for photorelease of free hormone within plant cells. The corresponding 4-nitrobenzyl esters were prepared as photo-inactive, but chemically similar, derivatives for use in control experiments. Used together, these compounds

promise to be of value in the study of intracellular action of plant hormones. Indeed, this technology has recently been successfully applied to the study of ABA action in stomatal guard cells [8].

## EXPERIMENTAL

**1-(2-nitrophenyl)ethanol.** This alcohol was prep'd by the method of ref. [6]. 2-Nitroacetophenone (5.2 g) in dioxane-MeOH (2:3, 75 ml) at 0° was treated with NaBH<sub>4</sub> (3.5 g). The soln was allowed to warm to room temp. and then stirred for 1.5 hr. The MeOH-dioxane was concd *in vacuo* and the residue treated with H<sub>2</sub>O and acidified with dilute HCl. Extraction with CH<sub>2</sub>Cl<sub>2</sub>, followed by drying with MgSO<sub>4</sub>, yielded the required 1-(2-nitrophenyl)ethanol (4.9 g). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.92 (1H, *d*, *J* = 8 Hz), 7.88 (1H, *d*, *J* = 8 Hz), 7.68 (1H, *t*, *J* = 8 Hz) and 7.45 (1H, *t*, *J* = 8 Hz), 5.42 [1H, *q*, *J* = 7 Hz, CH (Me)OH] and 1.58 (3H, *d*, *J* = 7 Hz, Me). This material was suitable for use in subsequent coupling reactions without further purification.

**GA<sub>4</sub>-1-(2-nitrophenyl)ethyl ester (1a).** GA<sub>4</sub> (200 mg) in dry MeCN (5 ml) was treated with Et<sub>3</sub>N (92  $\mu$ l) and isobutylchloroformate (860  $\mu$ l). After stirring for 10 min, TLC analysis indicated complete conversion to a less polar compound. EtOAc (50 ml) was added and the soln washed with 5% aq. citric acid (50 ml). Evapn gave the

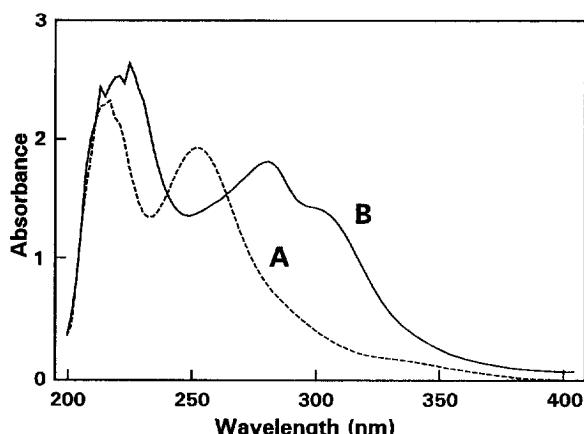


Fig. 1. Photolysis of caged GA<sub>4</sub>. UV spectra of GA<sub>4</sub>-1-(2-nitrophenyl)ethyl ester (**1a**). (A) Before photolysis; (B) after photolysis.

mixed anhydride. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 4.98 and 4.90 (2H, each *br*, H<sub>2</sub>-17), 4.06 [2H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>], 3.82 (1H, *br*, H-3), 3.21 (1H, *d*, *J* = 11 Hz, H-5), 2.87 (1H, *d*, *J* = 11 Hz, H-6), 1.21 (3H, *s*, H<sub>3</sub>-18), 0.98 [6H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>]. The anhydride was dissolved in dry MeCN (5 ml), 1-(2-nitrophenyl)ethanol (110 mg) and DMAP (30 mg) added, and the resulting bright yellow soln stirred for 1 hr. EtOAc (50 ml) and 5% aq. citric acid (50 ml) were added and the product recovered from the organic layer was purified by flash CC using hexane-EtOAc mixts to give, in sequence, unchanged 1-(2-nitrophenyl)ethanol (16 mg), residual anhydride (36 mg), the required GA<sub>4</sub>-1-(2-nitrophenyl)ethyl ester (**1a** 52 mg) and GA<sub>4</sub>. Data for **1a**: <sup>1</sup>H NMR (CDCl<sub>3</sub>) [4:3 mixt. of diastereomers]: δ 7.93 (1H, *m*, ArH), 7.61 (2H, *m*, ArH<sub>2</sub>) and 7.42 (1H, *m*, ArH), 6.38 and 6.32 [1H, *2q*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>].

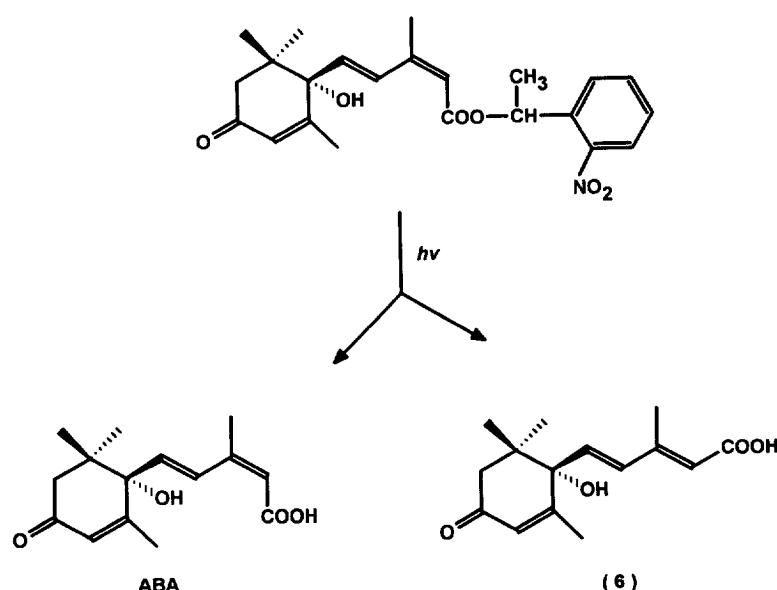
= 7 Hz, OCH(Me)Ar], 5.00, 4.90, 4.86 and 4.67 (2H, 4 × *br* *s*, H<sub>2</sub>-17), 3.82 and 3.77 (1H, 2 × *br* *s*, H-3), 3.17 and 3.08 (1H, 2*d*, *J* = 11 Hz, H-5), 2.73 and 2.69 (1H, 2*d*, *J* = 11 Hz, H-6), 2.66 and 2.59 (1H, 2 × *br* *t*, H-13), 1.65 (3H, *d*, *J* = 7 Hz, CHMeAr), 1.15 and 0.92 (3H, 2*s*, H<sub>3</sub>-18). MS *m/z* (rel. int.): 481 [M<sup>+</sup>] (3), 331 (3), 314 (9), 270(49), 269(39), 225(19), 224(20), 151(92), 150(100), 134(69), 105(48), 91(98), 77(80).

The above method was used to prepare all the GA<sub>4</sub>, ABA, 1-NAA and JA esters, via the corresponding mixed anhydride, using the appropriate nitrobenzyl alcohol.

*GA*<sub>4</sub>-2-nitrobenzyl ester (**1b**). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.09 (1H, *dd*, *J* = 8 and 1 Hz, ArH), 7.66 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 7.59 (1H, *dd*, *J* = 8 and 1 Hz, ArH), 7.52 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 5.58 and 5.48 (2H, 2*d*, *J* = 12 Hz, OCH<sub>2</sub>Ar), 4.97 and 4.82 (2H, 2 × *br* *s*, H<sub>2</sub>-17), 3.85 (1H, *br* *s*, H-3), 3.22 (1H, *d*, *J* = 11 Hz, H-5), 2.79 (1H, *d*, *J* = 11 Hz, H-6), 2.62 (1H, *br* *t*, H-13), 1.15 (3H, *s*, H<sub>3</sub>-18). MS *m/z* (rel. int.): 467 [M<sup>+</sup>] (10), 331(24), 314(9), 270(43), 269(82), 225(20), 224(28), 136(42), 106(100), 91(77), 78(68).

*GA*<sub>4</sub>-4-nitrobenzyl ester (**1c**). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 8.22 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 7.50 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 5.28 and 5.19 (2H, 2*d*, *J* = 12 Hz, OCH<sub>2</sub>Ar), 4.97 and 4.80 (2H, 2 × *br* *s*, H<sub>2</sub>-17), 3.85 (1H, *br* *s*, H-3), 3.22 (1H, *d*, *J* = 11 Hz, H-5), 2.75 (1H, *d*, *J* = 11 Hz, H-6), 2.62 (1H, *br* *t*, H-13), 1.10 (3H, *s*, H<sub>3</sub>-18). MS *m/z* (rel. int.): 467 [M<sup>+</sup>] (5), 331(12), 314(28), 270(58), 269(100), 225(24), 224(33), 137(53), 106(100), 91(98), 78(61).

*ABA*-isobutyloxycarbonyl anhydride. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.82 (1H, *d*, *J* = 16 Hz, H-5), 6.32 (1H, *d*, *J* = 16 Hz, OH-4), 5.95 (1H, *s*, H-3'), 5.76 (1H, *s*, H-2), 4.06 [2H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>], 2.48 and 2.32 (2H, 2*d*, *J* = 17 Hz, H<sub>2</sub>-5'), 2.07 and 1.92 (6H, 2*s*, H<sub>3</sub>-7' and H<sub>3</sub>-6), 2.05 [1H, *m*, CH<sub>2</sub>CH(Me)<sub>2</sub>], 1.12 and 1.04 (6H, 2*s*, H<sub>3</sub>-8' and H<sub>3</sub>-9'), 0.99 [6H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>].



Scheme 4. Photolysis of caged ABA.

*ABA-1-(2-nitrophenyl)ethyl ester (2a).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.98 (1H, *dd*, *J* = 8 and 1 Hz, ArH), 7.80 (1H, *d*, *J* = 16 Hz, H-5), 7.65 (2H, *m*, ArH<sub>2</sub>), 7.45 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 6.40 [1H, *q*, *J* = 7 Hz, OCH(Me)Ar], 6.18 (1H, *d*, *J* = 16 Hz, H-4), 5.90 (1H, *br s*, H-3'), 5.80 (1H, *br s*, H-2), 2.45 and 2.29 (2H, *2d*, *J* = 17 Hz, H<sub>2</sub>-5'), 2.10 and 2.02 (6H, 2s, H<sub>3</sub>-7' and H<sub>3</sub>-6), 1.67 (3H, *d*, *J* = 7 Hz, CHMeAr), 1.10 and 0.98 (6H, 2s, H<sub>3</sub>-8' and H<sub>3</sub>-9'). MS *m/z* (rel. int.): 413 [M]<sup>+</sup> (0.4), 383(1), 263(5), 190(49), 162(19), 150(100), 135(33), 120(43), 91(65).

*ABA-2-nitrobenzyl ester (2b).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.10 (1H, *dd*, *J* = 8 and 1 Hz, ArH), 7.86 (1H, *d*, *J* = 16 Hz, H-5), 7.65 (2H, *m*, ArH<sub>2</sub>), 7.50 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 6.21 (1H, *d*, *J* = 16 Hz, H-4), 5.95 (1H, *s*, H-3'), 5.85 (1H, *s*, H-2), 5.58 (2H, *s*, OCH<sub>2</sub>Ar), 2.50 and 2.30 (2H, *2d*, *J* = 17 Hz, H<sub>2</sub>-5'), 2.07 and 1.93 (6H, 2s, H<sub>3</sub>-7' and H<sub>3</sub>-6), 1.12 and 1.01 (6H, 2s, H<sub>3</sub>-8' and H<sub>3</sub>-9'). MS *m/z* (rel. int.): 399 [M]<sup>+</sup> (1), 369(5), 263(10), 207(23), 190(100), 161(33), 136(100), 121(32), 106(60), 91(70).

*ABA-4-nitrobenzyl ester (2c).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.20 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 7.84 (1H, *d*, *J* = 16 Hz, H-5), 7.55 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 6.21 (1H, *d*, *J* = 16 Hz, H-4), 5.95 (1H, *s*, H-3'), 5.85 (1H, *s*, H-2), 5.23 (2H, *s*, OCH<sub>2</sub>Ar), 2.50 and 2.30 (2H, *2d*, *J* = 17 Hz, H<sub>2</sub>-5'), 2.07 and 1.95 (6H, 2s, H<sub>3</sub>-7' and H<sub>3</sub>-6), 1.12 and 1.01 (6H, 2s, H<sub>3</sub>-8' and H<sub>3</sub>-9'). MS *m/z* (rel. int.): 399 [M]<sup>+</sup> (3), 343(8), 263(16), 246(29), 207(50), 190(100), 161(50), 136(39), 121(45), 106(91), 91(92).

*Jasmonic acid-isobutyloxycarbonyl anhydride.*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.48 (1H, *m*, H-10), 5.25 (1H, *m*, H-9), 4.06 [2H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>], 2.90 (1H, *dd*, *J* = 11 and 3 Hz, H-2), 2.43–2.00 (10H, *m*), 1.92 (1H, *m*, H-7), 1.55 (1H, *m*, H-4), 0.99 [6H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>], 0.96 (3H, *t*, *J* = 7 Hz, H<sub>3</sub>-12).

*JA-1-(2-nitrophenyl)ethyl ester (3a).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.94 (1H, *d*, *J* = 8 Hz, ArH), 7.62 (2H, *m*, ArH<sub>2</sub>), 7.45 (1H, *m*, ArH), 6.35 [1H, *q*, *J* = 7 Hz, OCH(Me)Ar], 5.42 (1H, *m*, H-10), 5.22 (1H, *m*, H-9), 2.69 (1H, *d*, *J* = 11 Hz, H-2), 2.35–2.00 (9H, *m*), 1.86 (1H, *m*, H-7), 1.66 (3H, *d*, *J* = 7 Hz, CHMeAr), 1.50 (1H, *m*, H-4), 0.91 (3H, *t*, *J* = 7 Hz, H<sub>3</sub>-12). MS *m/z* (rel. int.): 359 [M]<sup>+</sup> (11), 312(2), 209(100), 191(14), 150(79), 141(36), 134(44), 120(65), 105(37), 91(79), 83(82).

*JA-2-nitrobenzyl ester (3b).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.11 (1H, *dd*, *J* = 8 and 1 Hz, ArH), 7.66 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 7.57 (1H, *d*, *J* = 8 Hz, ArH), 7.51 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 5.53 (2H, *s*, OCH<sub>2</sub>Ar), 5.45 (1H, *m*, H-10), 5.25 (1H, *m*, H-9), 2.82 (1H, *dd*, *J* = 11 and 3 Hz, H-2), 2.43–2.00 (9H, *m*), 1.90 (1H, *m*, H-7), 1.50 (1H, *m*, H-4), 0.94 (3H, *t*, *J* = 7 Hz, H<sub>3</sub>-12). MS *m/z* (rel. int.): 345 [M]<sup>+</sup> (16), 327(1), 277(4), 209(75), 191(16), 151(51), 141(36), 136(59), 121(74), 106(81), 95(45), 83(80).

*JA-4-nitrobenzyl ester (3c).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.24 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 7.53 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 5.46 (1H, *m*, H-10), 5.26 (1H, *m*, H-9), 5.24 (2H, *s*, OCH<sub>2</sub>Ar), 2.80 (1H, *dd*, *J* = 11 and 3 Hz, H-2), 2.43–2.00 (9H, *m*), 1.91 (1H, *m*, H-7), 1.50 (1H, *m*, H-4), 0.94 (3H, *t*, *J* = 7 Hz, H<sub>3</sub>-12). MS *m/z* (rel. int.): 345 [M]<sup>+</sup> (11), 327(3), 277(5), 209(73), 191(15), 151(46), 141(80), 121(37), 106(100), 95(40), 83(74).

*Naphthyl-1-acetic acid-isobutyloxycarbonyl anhydride.*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.02 (1H, *d*, *J* = 8 Hz, ArH), 7.89 (1H, *d*, *J* = 8 Hz, ArH), 7.78 (1H, *m*, ArH), 7.52 (2H, *m*, ArH<sub>2</sub>), 7.42 (2H, *m*, ArH<sub>2</sub>), 4.09 (2H, *s*, naphthyl CH<sub>2</sub>CO), 3.89 [2H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)], 1.87 [1H, *m*, CH<sub>2</sub>CH(Me)<sub>2</sub>], 0.82 [6H, *d*, *J* = 7 Hz, OCH<sub>2</sub>CH(Me)<sub>2</sub>].

*NAA-1-(2-nitrophenyl)ethyl ester (4a).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.91 (1H, *d*, ArH), 7.85 (2H, *m*, ArH<sub>2</sub>), 7.79 (1H, *d*, ArH), 7.44 (4H, *m*, ArH<sub>4</sub>), 7.28 (2H, *m*, ArH<sub>2</sub>), 7.14 (1H, *m*, ArH), 6.33 [1H, *q*, *J* = 7 Hz, OCH(Me)Ar], 4.09 (2H, *s*, naphthyl CH<sub>2</sub>CO), 1.59 (3H, *d*, *J* = 7 Hz, CHMeAr). MS *m/z* (rel. int.): 335 [M]<sup>+</sup> (17), 186(41), 141(100), 115(52), 91(19).

*NAA-2-nitrobenzyl ester (4b).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.00 (2H, *t*, ArH<sub>2</sub>), 7.88 (1H, *d*, ArH), 7.80 (1H, *m*, ArH), 7.50 (2H, *m*, ArH<sub>2</sub>), 7.42 (2H, *m*, ArH<sub>2</sub>), 7.38 (2H, *m*, ArH<sub>2</sub>) and 7.16 (1H, *m*, ArH), 5.50 (2H, *s*, OCH<sub>2</sub>Ar), 4.18 (2H, *s*, naphthyl CH<sub>2</sub>CO). MS *m/z* (rel. int.): 321 [M]<sup>+</sup> (6) 186(41), 168(4), 141(100), 115(45), 106(20).

*NAA-4-nitrobenzyl ester (4c).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.07 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 7.94 (1H, *m*, ArH), 7.88 (1H, *m*, ArH), 7.81 (1H, *m*, ArH), 7.50 (2H, *m*, ArH<sub>2</sub>), 7.43 (2H, *m*, ArH<sub>2</sub>), 7.25 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 5.18 (2H, *s*, OCH<sub>2</sub>Ar), 4.15 (2H, *s*, naphthyl CH<sub>2</sub>CO). MS *m/z* (rel. int.): 321 [M]<sup>+</sup> (35), 186(23), 141(100), 115(44), 106(19).

*SA-NHS ester.* Salicylic acid (2.5 g) in Me<sub>2</sub>CO (75 ml) was treated with *N*-hydroxysuccinimide (2.08 g). Dicyclohexylcarbodiimide (3.73 g) was added slowly and the mixt. stirred for 3 hr giving a ppt. The mixt. was filtered and the filtrate evapd to yield salicylic acid–NHS ester (4.4 g), which was used in subsequent coupling reactions without further purification.

*SA-1-(2-nitrophenyl)ethyl ester (5a).* SA–NHS ester (100 mg) in dry MeCN (10 ml) was treated with 1-(2-nitrophenyl)ethanol (71 mg) and DMAP (30 mg). The mixt. was stirred under N<sub>2</sub> at room temp. for 1 hr. EtOAc (50 ml) and 5% aq. citric acid (50 ml) were added and the product, recovered from the organic layer, was purified by flash CC using hexane–EtOAc mixts to give salicylic acid–1-(2-nitrophenyl)ethyl ester (5a, 92 mg).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  7.99 (1H, *dd*, *J* = 8 and 1 Hz, ArH), 7.95 (1H, *dd*, *J* = 8 and 1.5 Hz, ArH), 7.71 (1H, *dd*, *J* = 8 and 1.5 Hz, ArH), 7.64 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 7.46 (2H, *m*, ArH<sub>2</sub>), 6.96 (1H, *dd*, *J* = 8 and 0.7 Hz, ArH), 6.92 (1H, *dt*, *J* = 7 and 0.8 Hz, ArH), 6.61 [1H, *q*, *J* = 7 Hz, OCH(Me)<sub>3</sub>Ar], 1.80 (3H, *d*, *J* = 7 Hz, CHMeAr). MS *m/z* (rel. int.): 287 [M]<sup>+</sup> (3), 219(3), 151(11), 150(100), 138(31), 120(82), 103(17), 92(64).

The above method was used to prepare the other nitrobenzylsalicylates **5b** and **5c**.

*SA-2-nitrobenzyl ester (5b).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.16 (1H, *d*, *J* = 8 Hz, ArH), 7.91 (1H, *dd*, *J* = 9 and 1.5 Hz, ArH), 7.69 (2H, *m*, ArH<sub>2</sub>), 7.54 (2H, *m*, ArH<sub>2</sub>), 7.00 (1H, *d*, *J* = 8 Hz, ArH), 6.92 (1H, *t*, *J* = 8 Hz, ArH), 5.81 (2H, *s*, OCH<sub>2</sub>Ar). MS *m/z* (rel. int.): 273 [M]<sup>+</sup> (9), 245(28), 164(15), 136(100), 120(66), 106(15), 92(53).

*SA-4-nitrobenzyl ester (5c).*  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.25 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 7.89 (1H, *dd*, *J* = 8 and 1 Hz,

ArH), 7.60 (2H, *d*, *J* = 9 Hz, ArH<sub>2</sub>), 7.46 (1H, *dt*, *J* = 8 and 1 Hz, ArH), 7.00 (1H, *dd*, *J* = 8 and 1 Hz, ArH), 6.90 (1H, *dt*, *J* = 8 and 1 Hz), 5.48 (2H, *s*, OCH<sub>2</sub>Ar). MS *m/z* (rel. int.): 273 [M]<sup>+</sup> (48), 164(44), 136(74), 120(100), 106(40), 92(48).

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