An Efficient and Scalable Synthesis of a Persistent Abscisic Acid Analog (+)-Tetralone ABA

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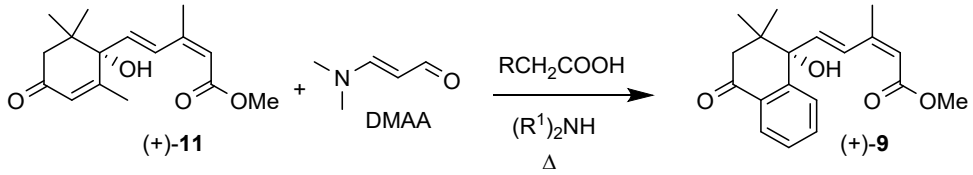
Supporting Information

I. Chemistry

1. General methods

Concentration refers to removal of volatiles at water aspirator pressure on a rotary evaporator. Evacuation at ca. 0.1 torr with a vacuum pump generally followed rotary evaporation. Flash column chromatography (FCC) was performed according to Still et al.[1] with Merck Silica Gel 60 (40-63 mm). All mixed solvent eluents are reported as v/v solutions. Unless otherwise noted, all reported compounds were homogeneous by thin layer chromatography (TLC) and by 1H NMR spectroscopy. The NMR solvent $CDCl_3$ was passed through small plug of basic alumina prior to use. Unless otherwise noted, NMR spectra were measured in $CDCl_3$ solution at 500 or 600 MHz (Bruker Avance) for ^1H. Signals due to the solvent or residual protonated solvent (1H NMR) served as the internal standard: $CDCl_3$ (7.26 δH). The 1H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), ap (apparent); the list of couplings constants (*J*) corresponds to the order of the multiplicity assignment. Coupling constants are reported to the nearest 0.5 Hz (consistent with the digital resolution of ca. 0.2 Hz/pt). High resolution mass spectra (HRMS) were obtained on a JEOL AccuTOF 4G GCv Mass spectrometer using field desorption (FD) ionization or a QSTAR XL MS/MS Mass spectrometer using electrospray ionization (ESI) method; only partial data are reported. Alternatively, HRMS was obtained on a LC-MS/MS time-of-flight high resolution spectrometer with electrospray ionization (ESI) from methanol solution. All other reagents were commercially available and, unless otherwise noted, were used as received.

2. Reaction Optimization



The following sequence of reactions were carried out to optimize the conversion of (+)-**11** to (+)-**9**. Reaction of ABA methyl ester (**11**) and 3-dimethylaminoacrolein (DMAA) in presence of acetic acid and piperidine, produced the known tetralone ABA methyl ester (+)-**9** in 5 % yield

(Table 1, Entry 1). Increasing the amount of aldehyde in the reaction further improved the yield (entry 2). We observed that the low boiling reagents (acetic acid, bp 118 °C and piperidine, bp 106 °C) were escaping from the reaction flask (refluxing at 125-135 °C) and trapped in the Dean Stark apparatus (confirmed by ^{1}H NMR). Using a directly fitted cold finger or condenser (125 °C, oil bath temp., no Dean Stark apparatus) to the reaction flask further improved the yields (entries 3 and 4). Addition of water scavengers (acetic anhydride or trimethyl orthoformate) or excess acid and base resulted in either no reaction or poor yields (entries 5-8). Treatment of **11** (1 equiv) with DMAA (1.8 equiv) in presence of 4:1 ratio of AcOH (0.4 equiv) and piperidine (0.1 equiv) in toluene at 135 °C (Dean-Stark) afforded the desired (+)-**9** in 80 % yield (entry 9). We found that the amount of aldehyde and the ratio of acid and base were critical in this reaction to obtain optimal results. Using an alternative acid (propionic acid) and base (2,2,6,6-tetramethylpiperidine) those with higher boiling points of 141.2 °C and 152 °C, respectively, did not improve the yield (entries 10 and 11); however, the reaction with acetic acid and morpholine (boiling point 129 °C) afforded (+)-**9** in 87 % yield by^{1}H NMR (entry 12). Although good yields were observed, the starting material (+)-**11** was never completely consumed. Portion wise addition of the reagents (acid, base, and aldehyde) to Me ABA ((+)-**11**) led the reaction proceeding to completion over 48 h (entries 14-16). These are the optimized conditions to convert (+)-**11** to (+)-**9** via the cascade sequence. The crude product (+)-**9** from the 27 mmol scale reaction (entry 16) was carried to the hydrolysis step without further purification. Saponification of the crude tetralone ester (+)-**9** afforded the tetralone acid (+)-**5** in 91 % yield starting from Me ABA (+)-**11** (Scheme 1). Notably, no column chromatography was used in either step to obtain the pure (>95 % by 1H NMR) tetralone ABA (+)-**5**.

Table 1: Optimization study for the conversion of (+)-**11** to (+)-**9**.

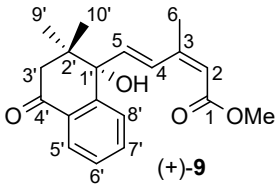
Entry	AcOH (equiv)	piperidine (equiv)	DMAA (equiv)	rxn conc. [M]	Temp. (°C)	% Yield[a]	% SM[a]
1	0.2	0.05	0.9	0.5	135	6 (5)	94 (91)
2	0.2	0.05	1.8	0.2	135	40	60
3[b]	0.2	0.05	0.9	0.5	125	63	36
4[b]	0.4	0.1	1.8	0.2	125	63	26
5[b, c]	0.2	0.3	1.8	0.2	125	0	100

6[b, d]	0.4	0.1	1.8	0.2	125	25	52
7	1.0	0.25	1.8	0.2	135	50	50
8	0.4	0	1.8	0.2	135	14	85
9	0.4	0.1	1.8	0.2	135	83 (80)	17 (15)
10	0.4[e]	0.1	1.8	0.2	135	27	70
11	0.4[e]	0.1[f]	1.8	0.2	135	0	100
12	0.4	0.1[g]	1.8	0.2	135	87	8
13	0.4	0.1	2.0	0.2	135	87	9
14[h]	0.4	0.1	2.0	0.2	135	95 (88)	-----
15[h, i]	0.4	0.1	2.0	0.2	135	96	-----
16[j]	0.4	0.1	2.0	0.2	135	98	-----

All the reactions were performed on 1 mmol scale using Dean-Stark condenser for 24 h unless otherwise noted. [a]yield by 1H NMR & isolated yield given in parenthesis. [b]cold finger or condenser was used. [c]0.5 equiv of Ac_2O was used as an additive. [d]trimethyl orthoformate (excess) was used as an additive. [e]propionic acid was used. [f]2,2,6,6-tetramethylpiperidine was used. [g]morpholine was used. [h]reagents (acetic acid, piperidine and aldehyde) were added in 3 portions over 48 h. [i]reaction on 10 mmol scale. [j]reaction on 27 mmol scale, reagents were added in 2 portions over 48h.

3. Experimental procedures and spectral data for compounds

Small scale two-step synthesis of (+)-5:

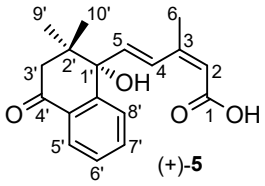


(+)-**9**

Synthesis of (+)-**9**: DMAA (0.11 mL, 1.1 mmol) was added to a stirring solution of (+)-ABA methyl ester (278 mg, 1.0 mmol) in 5 mL of premade toluene solution (0.12 mL of AcOH and 0.05 mL of piperidine were dissolved in 20 mL of toluene) at room temperature. A Dean-Stark apparatus (filled with the above premade toluene solution) was attached to the flask, which was quickly lowered into a preheated oil batch (135 °C, oil temp.), and was stirred for 24 h at the same temperature. More DMAA (0.05 mL, 0.5 mmol), AcOH (0.015 mL) and piperidine (0.007 mL) was added to the reaction mixture and was stirred for another 24 h at 135 °C. More DMAA (0.04 mL, 0.4 mmol), AcOH (0.008 mL) and piperidine (0.004 mL) were added to the reaction mixture and was stirred for another 5 h at 135 °C. The reaction mixture was cooled to room temperature, quenched with sat. $NaHCO_3$ solution and was diluted with water, extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated to obtain the crude as a dark brown syrup. Fractionation of the crude by FCC (20 %-30 % EtOAc in hexanes)

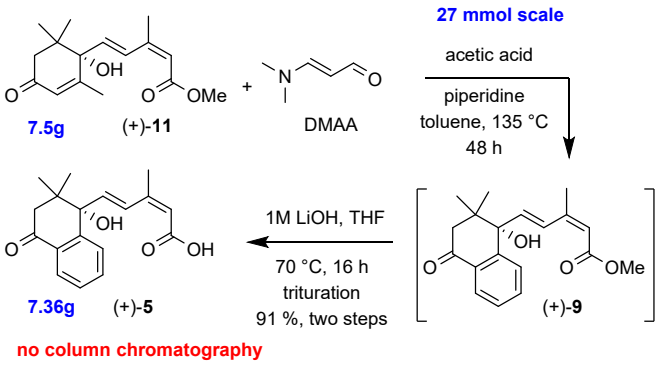
gave the tetralone ABA methyl ester (+)-**9** (275 mg, 88 %) as a pale-yellow syrup. ^{1}H NMR data for (+)-**9** closely matched those previously reported.[2]

TLC (EtOAc:Hexane, 40:60 v/v): R_f = 0.35; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05 (dd, J = 8.0, 1 Hz, 1H, ArH-5'), 7.86 (d, J = 16.0 Hz, 1H, H-4), 7.61–7.54 (m, 2H, ArH-6' & H-8'), 7.42 (t, J = 7.5, 1H, ArH-7'), 6.38 (d, J = 16.0 Hz, 1H, H-5), 5.74 (s, 1H, H-2), 3.68 (s, 3 H, CO_2CH_3), 2.83 (d, J = 17.0 Hz, 1H, H-3'), 2.60 (d, J = 17.0 Hz, 1H, H-3'), 2.00 (s, 3H, H-6), 1.09 (s, 3H, H-9'/H-10'), 1.07 (s, 3H, H-9'/H-10').



Synthesis of (+)-**5** from (+)-**9**: The ester (+)-**9** (275 mg, 0.88 mmol) was dissolved in THF (9 mL), added 1M LiOH solution (9 mL), water (2 mL), and was heated to 70 °C for 16 h. The reaction mixture was cooled to 0 °C, diluted with hexane (10 mL), 1M LiOH solution (5 mL), water (5 mL) and separated the layers. The aqueous layer was further washed with diethyl ether (2 X 20 mL), cooled to 0 °C acidified with 6N HCl solution, and extracted with dichloromethane (3 X 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to obtain the tetralone ABA (+)-**5** (240 mg, 91 %) as an off-white powder. ^{1}H NMR data for (+)-**5** closely matched those previously reported.[2] For 1H NMR data, please see under gram scale telescope synthesis of (+)-**5**.

Gram scale (27 mmol) telescope synthesis of (+)-**5:**



DMAA (3.0 mL, 30.0 mmol) was added to a stirring solution of (+)-ABA methyl ester (7.5 g, 27.0 mmol) in 180 mL of premade toluene solution (0.84 mL of AcOH and 0.36 mL of piperidine were dissolved in 180 mL of toluene) at room temperature. A Dean-Stark apparatus (filled with the above premade toluene solution) was attached to the flask, which was quickly lowered into in a preheated oil batch (135 °C, oil temp.), and was stirred for 24 h at the same temperature (68 % conversion was observed by ^{1}H NMR). More DMAA (2.4 mL, 24.0 mmol), AcOH (0.20 mL) and

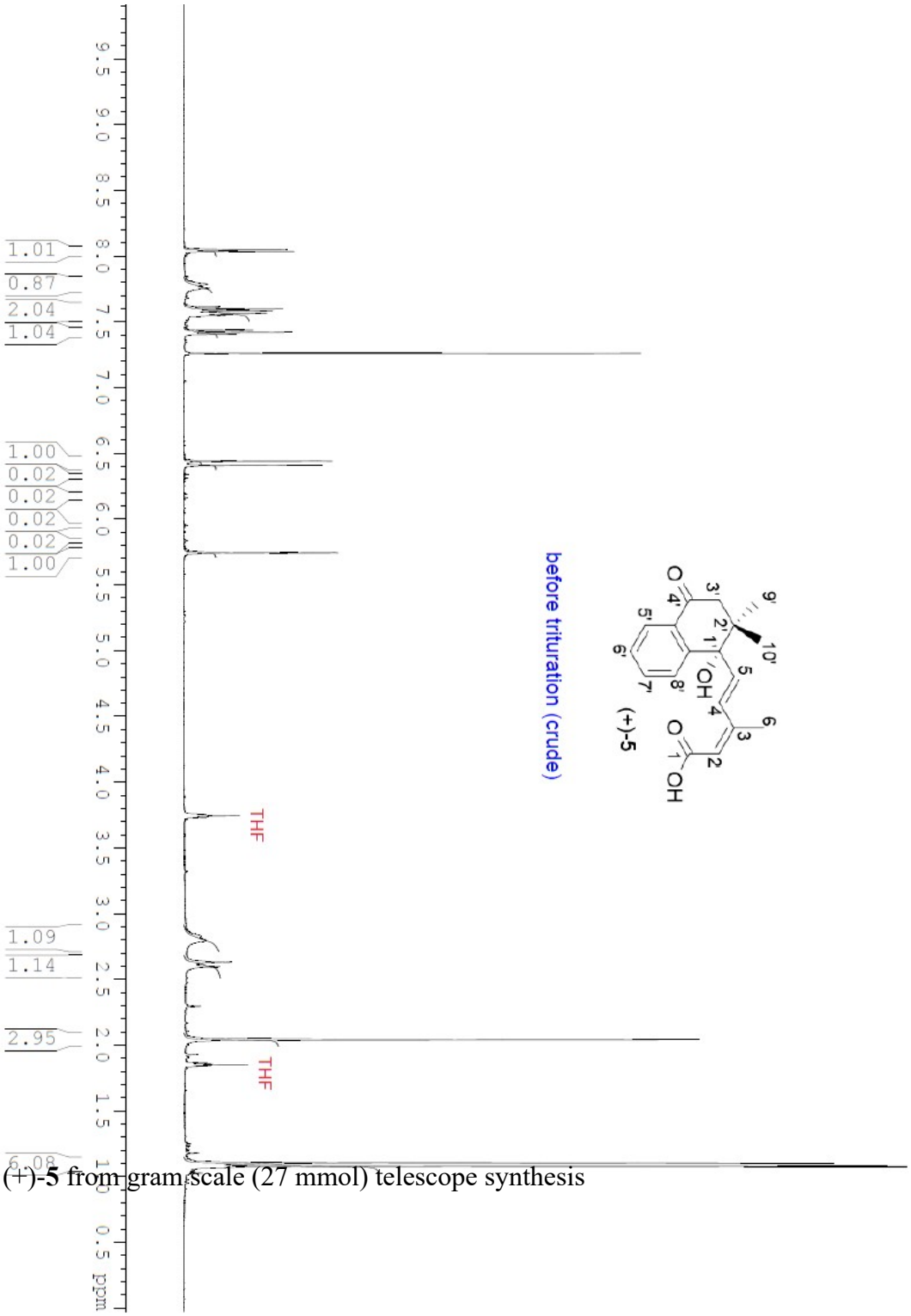
piperidine (0.09 mL) were added to the reaction mixture and was stirred for another 24 h at 135 °C (98 % conversion was observed by ^{1}H NMR). The reaction mixture was cooled to room temperature, quenched with sat. $NaHCO_3$ solution and was diluted with water, EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated to obtain the crude as a dark brown syrup, which was used in the next step without further purification. The crude ester was dissolved in THF (240 mL), added 1M LiOH solution (240 mL), water (50 mL), and was heated to 70 °C for 16 h. The reaction mixture was cooled to 0 °C, diluted with diethyl ether (250 mL), 1M LiOH solution (100 mL), water (100 mL) and separated the layers. The aqueous layer was further washed with diethyl ether (250 mL), cooled to 0 °C, acidified with 6N HCl solution, and extracted with dichloromethane (3 X 250 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to obtain the crude as a pale yellow foamy solid. The crude product was triturated with 10 % EtOAc in hexane (100 mL), filtered, and the solid was dried under vacuum to give the tetralone ABA (+)-**5** (7.36 g, 91 % from (+)-**11**) as a pale brown powder. ^{1}H NMR data for (+)-**5** closely matched those previously reported.[2]

mp:* 170–173 °C; TLC (EtOAc:Hexane:AcOH, 40:55:5 v/v): R_f = 0.35; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (dd, J = 7.8, 1.2 Hz, 1H, ArH-5'), 7.78 (d, J = 16.0 Hz, 1H, H-4), 7.62–7.55 (m, 2H, ArH-7' & H-8'), 7.45–7.41 (m, 1H, ArH-6'), 6.43 (d, J = 16.0 Hz, 1H, H-5), 5.75 (s, 1H, H-2), 2.83 (d, J = 17.0 Hz, 1H, H-3'), 2.62 (d, J = 17.0 Hz, 1H, H-3'), 2.04 (s, 3H, H-6), 1.1 (s, 3H,H-9'/H-10'), 1.08 (s, 3H, H-9'/H-10'); $UV_{\lambda max}$ (MeOH) nm (log ε): 205 (4.4), 250 (4.4).

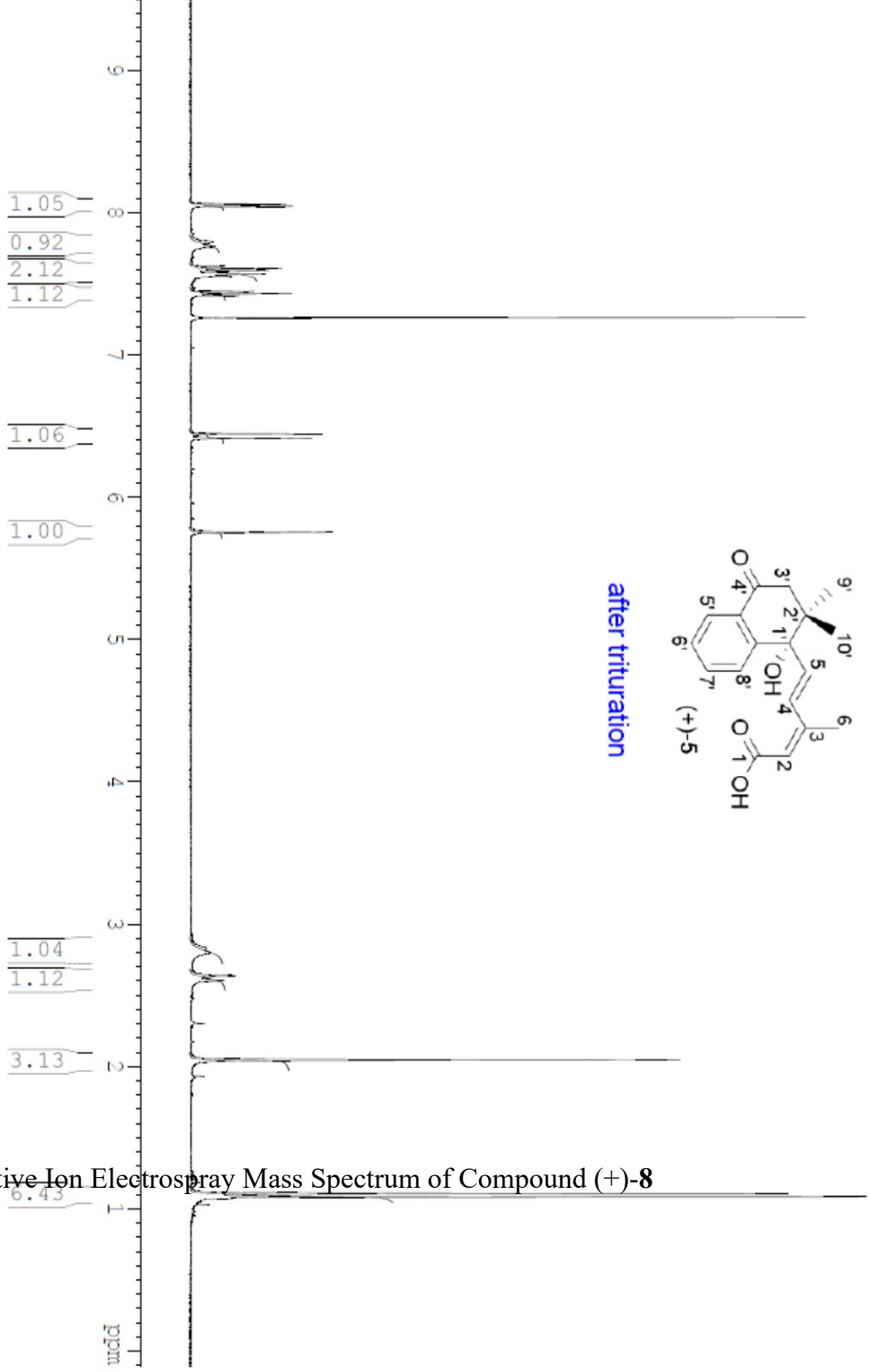
*A small amount of the tetralone ABA (+)-**5** was crystallized from ethyl acetate to obtain melting point.

4. Spectra for t

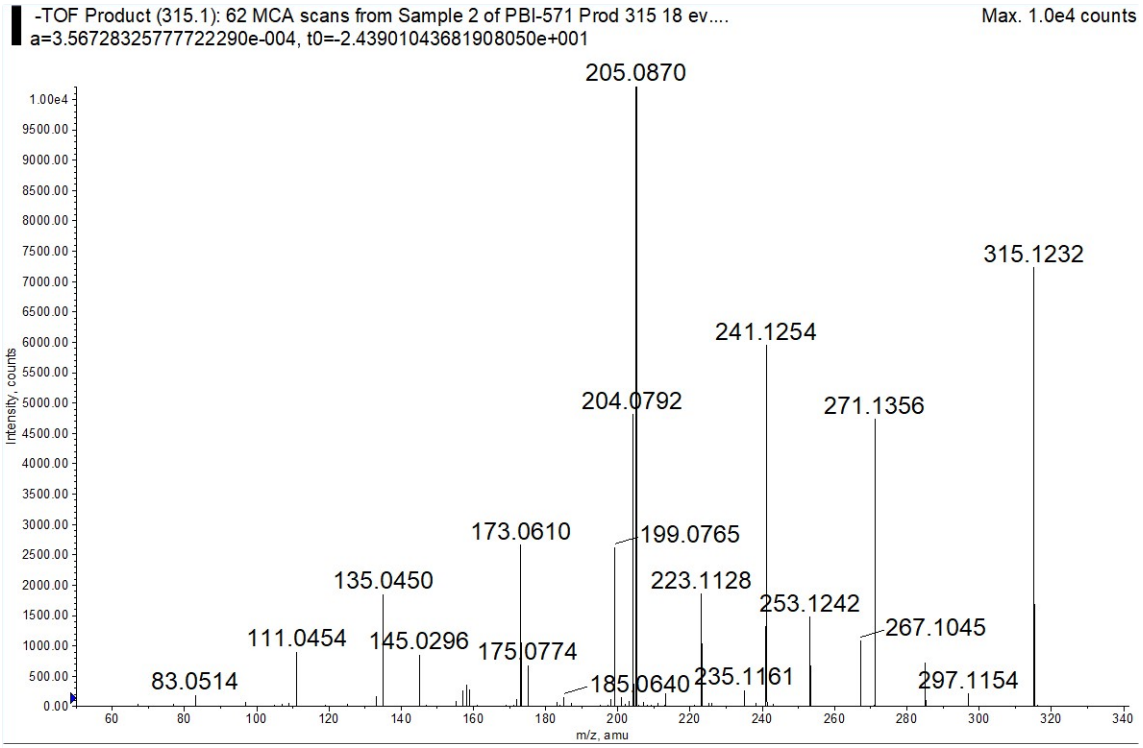
Spectrum of crud



Spectrum of pure (+)-**5** from gram scale (27 mmol) telescope synthesis



Product Ion Scan Negative Ion Electrospray Mass Spectrum of Compound (+)-**8**


-TOF Product (315.1): 62 MCA scans from Sample 2 of PBI-571 Prod 315 18 ev....
a=3.56728325777722290e-004, t0=-2.43901043681908050e+001
Max. 1.0e4 counts
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m/z, amu
205.0870
315.1232
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253.1242
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111.0454
145.0296
175.0774
83.0514
185.0640
235.1161
297.1154

II. Biology

1. Persistence assays of (+)-**5** and (+)-**8** in Arabidopsis plants

Arabidopsis wild-type (Columbia-0) seeds were surface-sterilized with chlorine gas for 2-4 hours, as described in Lindey et al. 2017.[3] The sterilized seeds were stratified in water for 3-4 days at 4°C in the dark. The seedlings were grown vertically on Petri dishes containing 0.5X Murashige and Skoog (MS) medium and 0.8 % plant agar for 7 days, then transferred to a new plate (NuncTM OmniTrayTM Single-Well plate) until 10 days. These seedlings were treated with a mixture of 25 µM (+)-d_6-ABA ((3',5',5',7',7','7-d_6 ABA) Olchemim) and 25 µM (+)-**5** containing 0.5 % Tween-20 by applying a 2-µL drop at the abaxial side of the true leaf. Over the course of 3 days, sampling events were taken with 8 replicates at indicated periods post application. The whole plant was measured in its fresh weight and homogenized in a 1.5-mL centrifuge tube with 1 mL methanol containing 1 % (v/v) glacial acetic acid (MeOH/1 % AcOH) via TissueLyser (QIAGEN). Compounds 100 pg (±)-8',8',8'-d_3-ABA, 50 pg (-)-7',7',7'-d_3-PA (National Research Council of Canada) and 200 pg (±)-9',9',9'-d_3-tetralone ABA (prepared according to Nyangulu et al 2006)[2] were added as internal standards. Samples were centrifuged at 12,000 rpm for 5 minutes at 4 °C. The supernatant was evaporated in a SpeedVac (LABCONCOTM), and the dried pellet was suspended in 1mL of water with 1 % (v/v) acetic acid. The samples were purified by solid-phase extraction using IRIS-N cartridge columns (IRISTM Polymeric SPE). The samples were eluted with MeOH/1 % AcOH, dried using SpeedVac, and subjected to a liquid chromatography-electron spray ionization-tandem mass spectrometry (LC-ESI-MS/MS, Agilent 6410 Triple quad LC-MS) equipped with a ZORBAX Eclipse XDB-C18 column (Agilent) for quantification. MSMS settings are as follows: m/z =302.3/258.2 for d_3-(+)-**5**, 299.3/255.2 for (+)-**5**; Collision Energy, 10V; Fragmentor Voltage, 100V; Cell Accelerator Voltage 7V. For (+)-**8**, m/z =315.3/205.2; Collision Energy, 4V; Fragmentor Voltage, 100V; Cell Accelerator Voltage 7V. MSMS settings for other chemicals were described in Yan et al. 2016.[4] Standard curves were made with corresponding deuterated compounds, except for (+)-**8** in which d_3-PA and PA were used to make the standard curve. An average with a standard error (SE) is shown in Fig. 4a and 4b (n=8) (see main article).

To measure the conjugated forms of (+)-**5** and (+)-**8**, alkaline hydrolysis was performed as described in Neill et al.[5] with minor modifications. Briefly, a half volume of purified extracts (aqueous solution) was treated with 0.05 N potassium hydroxide at 60 °C for 30 min. The reactants

were neutralized with an equal volume of 0.05 N hydrogen chloride and purified by IRIS-N cartridge columns. After drying under vacuum, samples were reconstituted with water containing 1% acetic acid and subjected to the LC-ESI-MSMS analysis as described above. The contents of (+)-**5** and (+)-**8** were normalized by d_3-(+)-**5** and d_3-PA, respectively, obtained from samples without alkaline hydrolysis because the alkaline treatment eliminated some deuterium atoms from d_6-PA converted from fed d_6-ABA in planta. Conjugated (+)-**5** and (+)-**8** contents were determined from data after alkaline hydrolysis minus those without alkaline treatment.

2. Chemical complementation of an ABA-deficient mutant by (+)-**5**

Arabidopsis wild-type Col and the *aba2-2* mutant seeds were surface-sterilized, stratified for 4 days, and sown on the soil Sunshine® Mix #1 (Sun Gro® Horticulture). Plants were grown for 5 weeks at 22 °C in a 16-h/8-h light/dark cycle under white light irradiation (~100 μmol m^{-2} s^{-1}). Ten μl of indicated concentrations of (+)-ABA or (+)-**5** was administered once to the leaf surface with a needle scratch (~5 mm in length). The same volume of water containing 0.1 % methanol and 0.5 % Tween-20 was applied as a mock treatment. After the solution was absorbed from the leaf surface, plants were grown in the same chamber for 14 days, then fresh weights were measured. An average of FW with an SD is shown in Fig. 5 (see main article) for Col and *aba2-2* (n = 5~6).

III. References

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