JOC The Journal of Organic Chemistry

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J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.8b00876 • Publication Date (Web): 22 May 2018

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Syntheses of Gibberellins A_{15} and A_{24}, the Key Metabolites in Gibberellin Biosynthesis

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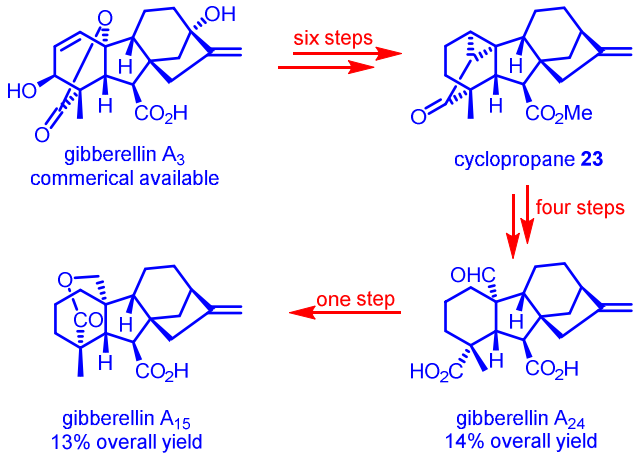
ABSTRACT



Gibberellins (GAs) are essential phytohormones involved in numerous aspects of plant growth and development. Notably, the biochemistry and genetics of GA biosynthesis which is associated with their endogenous regulation has been largely resolved, however, a crucial unsolved question remains: the precise mechanism of the stepwise oxidation and subsequent removal of C-20 from C_{20}-precursors leading to bioactive C_{19} gibberellins is still unresolved. In order to satisfy numerous requests from biologists, practical preparations of certain GAs that were isolated in miniscule quantities are highly demanded. Herein we report the first practical syntheses of GA_{15} and GA_{24}, the key C_{20} metabolites in gibberellin biosynthesis, from commercially available GA_3. The protocols are robust and offer the capacity to produce GA_{24} and GA_{15} under gram scales in high overall yields and thus aid in further biological and related studies.

INTRODUCTION

The gibberellins (GAs) are a large class of highly functionalized diterpenoids that are commonly found throughout the plant kingdom where they serve as plant hormones.[1,2] These hormones regulate a variety of plant developmental processes, notably – the germination of seeds, trichome formation, pollen maturation, and play a role in the commencement of flowering.[3] For instance, mutant plants that express lower levels of GA exhibit a dwarf and late-flowering phenotype, when this deficiency is supplemented with exogenous GA, normal plant growth is restored.[4] The profound effects of GAs on plants make them a valuable tool in modern agriculture to increase crop yields.[5,6] As a consequence, GAs are of considerable agricultural significance, not surprisingly, they have been widely accepted throughout the world as a useful agricultural chemical for the treatment of increasing numbers of fruit and vegetable crops including grapes, citrus, apples, artichokes, tomatoes, rice and grains.[7-9]

Since the first discovery of GA in the pathogenic fungus *Gibberella fujikuroi*, the compound responsible for the "silly seedling" disease of rice,[10] more than 136 GAs have been identified from plants, fungi and bacteria.[11] Among the known GAs, only a small number of them, such as GA_1 (**1**), GA_3 (**2**), GA_4 (**3**) and GA_7 (**4**) are biologically active, and they are analogous possessing a pentacyclic diterpenoid framework with a C-6 carboxylic acid, C-3 hydroxyl functionality and exocyclic olefin (Figure 1).[4] Meanwhile, many nonbioactive GAs are present in plants as "dormant" precursors for the bioactive forms or are in turn deactivated metabolites.[12] The regulation of the GA levels is believed to be complex given the numbers of GAs isolated from nature, which has received a great deal of attention.[3] Figure 2 summarizes the brief biosynthesis of major bioactive GAs.[12] It was accepted early on that the biosynthesis of GA begins from *ent*-kaurenoic acid (**5**) and through a hydroxylation at C-7 (leading to *ent*-7α-hydroxykaurenoic acid **6**) and subsequent ring contraction with expulsion of C-7 the GA progenitor, GA_{12} aldehyde (**7**) is formed.[12] In both

processes, *ent*-kaurenoic acid oxidase (KAO) plays a crucial role, in fact, this KAO also converts the GA_{12} aldehyde into GA_{12} (**8**). The biosynthesis then diverges based on the organism involved (plant, fungi or bacteria) however the pathways are analogous overall, varying mainly by the stage, position and level of oxidation.[2] Thus, GA_{12} is sequentially converted to GA_{15} then to GA_{24} and GA_9, by GA 20-oxidase (GA20ox), and upon installation of the C-3 hydroxyl group by GA 3-oxidase (GA3ox), the bioactive GA_4 is generated. In parallel, GA_{12} is also a substrate for GA 13-oxidase (GA13ox) for the production of GA_{53}, which is a precursor for GA_1 (through the $GA_{44}\rightarrow GA_{19}\rightarrow GA_{20}$ pathway) and GA_3 (via the $GA_{20}\rightarrow GA_5$ pathway). In particular, GA biosynthesis is tightly regulated by those enzymes, especially the GA20ox and GA3ox that catalyse the crucial steps in the biogenesis of active GAs.[13,14]

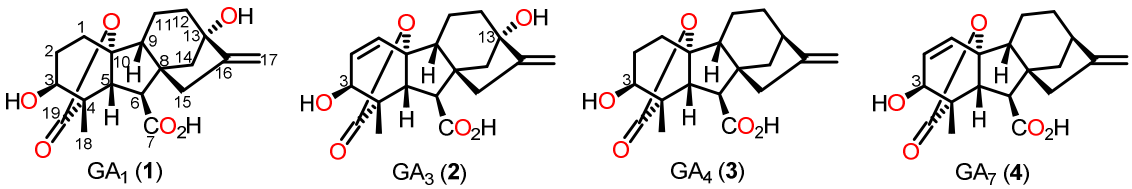


Figure 1. Chemical structures of bioactive gibberellins A_1, A_3, A_4 and A_7.

Although the chemistry, biochemistry and genetics of GA biogenesis has been largely resolved,[4,10,12,13] a crucial unsolved question remains: the precise mechanism of the stepwise oxidation and subsequent removal of C-20 from C_{20}-precursors (i.e., GA_{15} and GA_{44}) leading to bioactive C_{19} gibberellins is still unresolved, despite that these bio-transformations have been observed in many plants as well as cell-free systems.[15-17] Numerous biologists having taken up this challenge were struggling to gain access to several C_{20}-precursors, preferably with incorporated isotopes. GA_{15} and GA_{24}, the key C_{20}-precursors to GA_4, are of particular interest to biologists. They have been isolated from various higher plants, though often in miniscule quantities.[1] Manifold efforts have been made to prepare those metabolites via chemical synthesis. The first total synthesis of GA_{15} was accomplished by Nagata *et al.* in

1971,[18] albeit in a racemic form, moreover, this synthesis suffered from multi-step procedure and the starting materials were not readily available. Somei *et al.*[19] reported the chemical interconversion of enmein to GA_{15} in 1970, however, this method was low-yielding and the final purification was complex. The interconversions of gibberellins seem to be advantageous, for instance, Ward *et al.*[15] prepared the labelled GA_{15} from GA_{13} trimethyl ester via an eight-steps sequence, nevertheless, the GA_{13} has to be isolated from plants after tedious procedures.

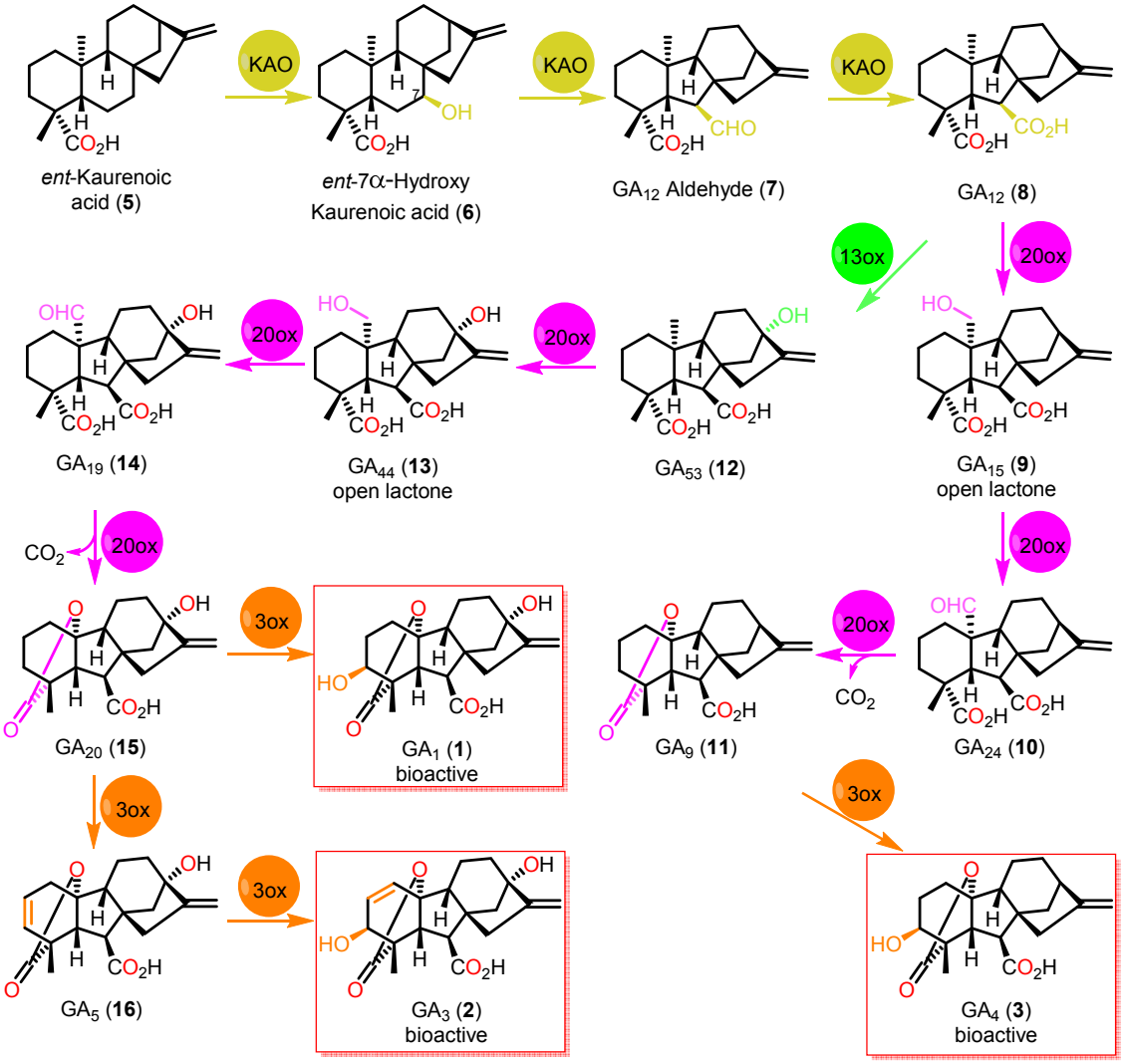
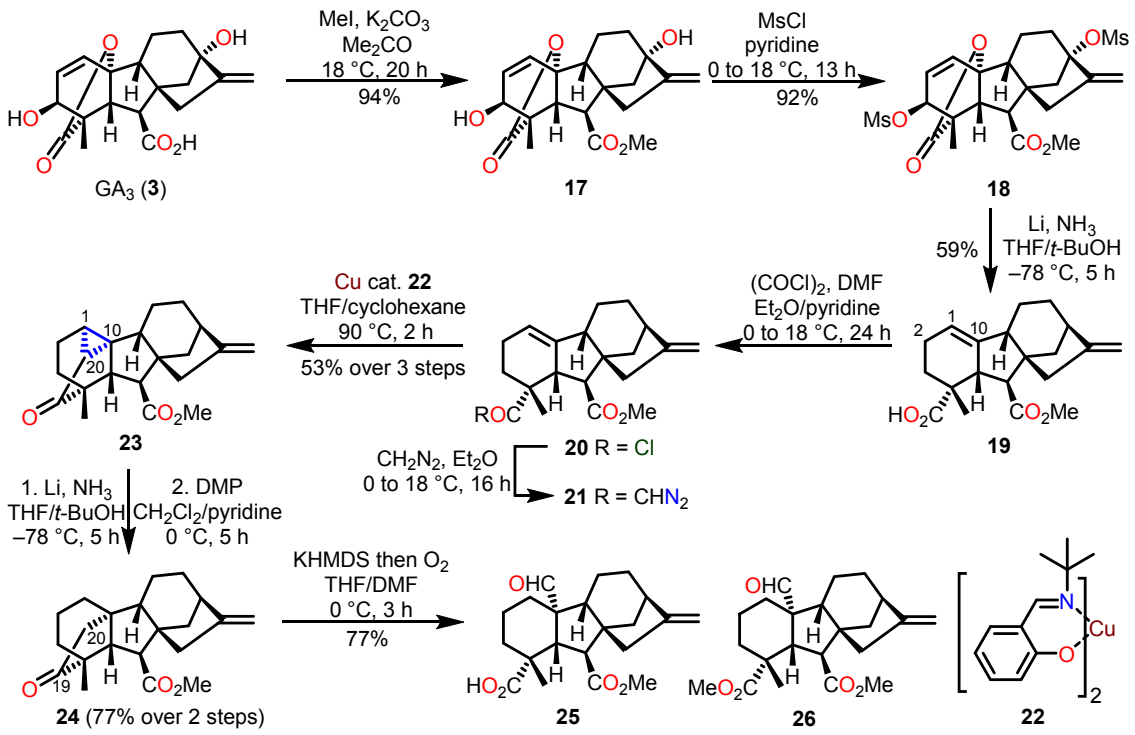


Figure 2. Biosynthetic route of bioactive gibberellins A_1, A_3 and A_4 from *ent*-kaurenoic acid. KAO: *ent*-kaurenoic acid oxidase; 20ox: GA 20-oxidase; 3ox: GA 3-oxidase; 13ox: GA 13-oxidase.

In order to satisfy numerous requests for biosynthetic and related studies, herein we report the first practical syntheses of GA_{15} and GA_{24}, from GA_3 which is now produced by industrial fermentation at ton scale. The conversion of the C_{19} member GA_3 to C_{20} ones GA_{15} and GA_{24} must contend with several difficulties: the introduction of C-20 into the very hindered C-10 position on the concave α-face of the gibberellin molecule, and the elaboration of an aldehyde group in a 1,3-syn-diaxial relationship to the C-19 carboxylic acid. By utilizing the methodologies established over the past thirty years,[2] we are now able to complete the concise practical syntheses of GA_{15} and GA_{24}.

Scheme 1. Establishment of the C_{20} Framework from GA_3.



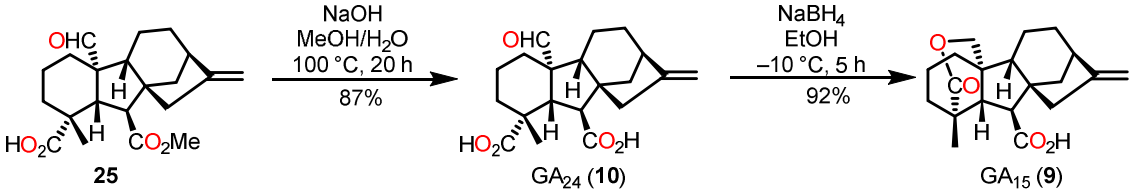
RESULTS AND DISCUSSION

As shown in Scheme 1, the synthesis begins with the use of GA_3 (**3**), a commercially available and biosynthetically abundant compound obtained as a microbial metabolite.[20] From here standard esterification with methyl iodide under basic conditions, methyl

gibberellate (**17**) was achieved in excellent yield (94%). At this point, for the synthesis of the nor-hydroxy gibberellins A_{15} and A_{24}, both of the C-3 and C-13 hydroxyl groups of **17** have to be removed. This was achieved by di-mesylation of the hydroxyl functionality leading to dimesyl methyl gibberellate (**18**) followed by dissolving metal reduction.[21] This dissolving metal reduction is a synthetically powerful reaction serving to not only affect the 13-hydroxyl functionality but also deletion of the superfluous 3-hydroxyl with isomerisation of the olefin, expelling the lactone functionality to provide acid (**19**) in a respectable yield of 59%. The rearrangement of the olefin is crucial for insertion of the extra carbon to the concave, sterically hindered and pre-quaternary carbon centre (C-10) and thus generation of C_{20} GAs from their C_{19} congeners. Thus, the newly-generated proximal carboxylic acid (**19**) was converted into the diazoketone (**21**) via formation of the acid chloride (**20**) followed by treatment of the latter with diazomethane. Upon treatment with the commercially available copper (II) catalyst (**22**), the final carbon was added through carbene addition to the olefin providing the corresponding cyclopropane (**23**) in 53% over 3 steps.[22] Salicylate-derived copper catalyst (**22**) was found to produce the most satisfactory results wherein minimal formation of the C-H insertion side product at the C-2 position was observed. Employment of another dissolving metal reduction[23,24] exclusively resulted in cleavage of the less hindered C1-C20 bond in cyclopropane (**23**) to provide the desired ketone (**24**) with some over-reduced alcohols which were recycled through a subsequent oxidation step with Dess-Martin periodinane, thus affording the ketone (**24**) in 77% yield. Functionalization of the extremely hindered C-20 position in ketone (**24**) as a prelude to oxidative fission had previously proved to be very difficult.[22] Herein we report a new and effective method for the oxidative cleavage of the C19-C20 bond and required formation of an enolate intermediate by using potassium hexamethyldisilazide (KHMDS) in THF/DMF. The fission of this bond was then achieved through treatment of the generated enolate with a steady stream of oxygen gas into the

reaction mixture, yielding half-acid (**25**) in a satisfactory yield (77%). Implementing the base KHMDS to initiate the enolate formation was found to be safe and efficient even under an atmosphere of oxygen. Due to serious lactol resonances, further confirmation of compound (**25**) was achieved through derivatization with diazomethane so providing the methyl ester (**26**) which was not susceptible to lactol tautomer equilibrium effects,[25,26] providing clear ^{1}H and ^{13}C NMR spectra data.[27]

Scheme 2. Completion of the Syntheses of GA_{24} and GA_{15}.



The elaboration of compound (**25**) to target GA_{24} (**10**) and GA_{15} (**9**) is outlined in Scheme 2. Saponification of methyl ester (**25**) provided GA_{24} (**10**) in 87% yield, which again, was treated with diazomethane so as to afford the dimethyl ester (**26**) for further structural determination. With GA_{24} in hand, clean reduction with sodium borohydride delivered the lactone GA_{15} (**9**) in high yield (92%). Surprisingly, although GA_{15} has been isolated from various higher plants,[1,28-32] as well as been prepared by some synthetic work,[15,18,19] the full NMR spectra data of this compound has not been reported. In current work, we are now able to provide a complete set of characterization data for GA_{15} (**9**) by undertaking IR, MS spectrometric and NMR spectroscopic analyses.

CONCLUSION

The protocols defined here are robust and offer the capacity to produce GA_{24} and GA_{15} under gram scales in high overall yields (13% and 14%, respectively). The strategies could also be applied to pursue certain labelled GAs by using some labelled reagents (i.e., ^{14}C diazomethane) or by incorporating the terminal olefin protons at C-17 with deuterons.[33] Given

the versatile applications of GA_3 in construction of structurally diverse compounds[34] as well as bioactive agents,[35] the chemistry developed in this work provides valuable insights into the reactivity of GA_3.

EXPERIMENTAL SECTION

Materials. Gibberellin A_3 (>90%), methyl iodide (>99%), methanesulfonly chloride (>99%), *tert*-butyl alcohol (>99%), lithium metal (>99%), oxalyl chloride (>99%), bis(*N*-*tert*-butylsalicylaldiminato)copper(II) [96%], potassium hexamethyldisilazide solution, Dess-Martin periodinane (97%), sodium borohydride (>99%), were all purchased from Sigma-Aldrich (St. Louis, MO). Aluminum-backed 0.2 mm thick silica gel 60 F_{254} plates for analytical thin layer chromatography (TLC) as well as silica gel 60 (40–63 μm) for flash chromatographic separations were supplied by Merck (Darmstadt, Germany).

General Experimental Protocols. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded at 25 °C on a Bruker spectrometer (Billerica, MA) operating at 400 MHz for proton and 100 MHz for carbon nuclei. For ^{1}H NMR spectra, the residual protio-solvent signals were used as internal standards. Infrared spectra (ν_{max}) were recorded on a Perkin–Elmer 1800 Series FTIR Spectrometer (Waltham, MA). Samples were applied to KBr plates as $CDCl_3$ solutions followed by generous air drying. Low-resolution ESI mass spectra were recorded on a single quadrupole liquid chromatograph-mass spectrometer, while high-resolution measurements were conducted on a time-of-flight instrument. Melting points were measured on an Optimelt automated melting point system (Praxair Inc., Danbury, CT) and are uncorrected. Analytical thin layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F_{254} plates as supplied by Merck (Darmstadt, Germany). Visualization was accomplished with potassium permanganate solution. Flash chromatographic separations were carried out with silica gel 60 (40–63 μm) as the stationary

phase and using the analytical reagent grade solvents indicated. Where necessary, reactions were performed under an nitrogen atmosphere.

Specific Chemical Transformations

Methyl (1*S*,2*S*,4a*R*,4b*R*,7*S*,9a*S*,10*S*,10a*R*)-2,7-dihydroxy-1-methyl-8-methylene-13-oxo-1,2,4b,5,6,7,8,9,10,10a-decahydro-4a,1-(epoxymethano)-7,9a-methanobenzo[*a*]azulene-10-carboxylate (17). To a stirred solution of gibberellin A_3 (**3**, 10.2 g, 29.38 mmol) in acetone (800 mL) was added anhydrous potassium carbonate (8.93 g, 44.1 mmol) and methyl iodide (11 mL, 176 mmol). The reaction mixture was stirred at 18 °C for 20 h. The resulting suspension was filtered through Celite™, the filter cake rinsed with acetone (300 mL) and concentrated under reduced pressure. The resulting white solid was subjected to chromatography (silica, 2:3 v/v acetone/hexane elution) to afford, after concentration of the relevant fractions (R_f = 0.1 in hexane/EtOAc 1:1), methyl gibberellate **17** (10 g, 94%) as a white solid. The spectroscopic data was in good comparison with that previously described in the literature.[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.32 (d, *J* = 9.2 Hz, 1H), 5.91 (dd, *J* = 9.2, 3.9 Hz, 1H), 5.28 (dd, *J* = 3.1, 1.9 Hz, 1H), 4.97 (d, *J* = 2.4 Hz, 1H), 4.16 (dd, *J* = 3.9, 0.9 Hz, 1H), 3.73 (s, 3H), 3.21 (d, *J* = 10.8 Hz, 1H), 2.79 (d, *J* = 10.8 Hz, 1H), 2.20 (m, 1H), 2.11−2.03 (complex m, 2H), 1.96−1.90 (complex m, 2H), 1.81−1.67 (complex m, 4H), 1.24 (s, 3H).

Methyl (1*S*,2*S*,4a*R*,4b*R*,7*S*,9a*S*,10*S*,10a*R*)-1-methyl-8-methylene-2,7-bis((methylsulfonyl)oxy)-13-oxo-1,2,4b,5,6,7,8,9,10,10a-decahydro-4a,1-(epoxymethano)-7,9a-methanobenzo[*a*]azulene-10-carboxylate (18). To a magnetically stirred mixture of methyl gibberellate **17** (10.0 g, 27.7 mmol) in pyridine (18 mL) was added, dropwise, methanesulfonly chloride (5.37 mL, 69.4 mmol) at 0 °C. The ensuing yellow solution was then stirred at 18 °C for 13 h before being poured into aqueous 1M HCl (200

mL) and extracted with ethyl acetate (3 x 250 mL). The combined organic extracts were washed with 1 M HCl (100 mL), brine (75 mL), then dried (Na_2SO_4), filtered and concentrated under reduced pressure. The ensuing yellow residue was subjected to flash chromatography (silica, 1:15 v/v ethyl acetate/dichloromethane elution) to afford, after concentration of the relevant fractions (R_f = 0.7), dimesyl methyl gibberellate **18** (13.2 g, 92%) as an amorphous, white solid. The spectroscopic data was in good comparison with that previously described in the literature.[36] ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.46 (d, *J* = 9.2 Hz, 1H), 6.03 (dd, *J* = 9.2, 3.8 Hz, 1H), 538 (m, 1H), 5.15 (m, 1H), 5.07 (d, *J* = 3.8 Hz, 1H), 3.76 (s, 3H), 3.31 (d, *J* = 10.7 Hz, 1H), 3.10 (s, 3H), 3.04 (s, 3H), 2.81 (d, *J* = 10.7 Hz, 1H), 2.60 (m, 1H), 2.51−2.43 (complex m, 2H), 2.25−2.17 (complex m, 2H), 2.04−1.94 (complex m, 3H), 1.76 (m, 1H), 1.28 (s, 3H).

(1*R*,4b*R*,7*R*,9a*R*,10*S*,10a*S*)-10-(methoxycarbonyl)-1-methyl-8-methylene-2,3,4b,5,6,7,8,9,10,10a-decahydro-1H-7,9a-methanobenzo[*a*]azulene-1-carboxylic acid (19). To a magnetically stirred mixture of dimesyl methyl gibberellate **18** (3.31 g, 6.41 mmol) in dry THF (26 mL), *tert*-butyl alcohol (2.7 mL, 37.8 mmol) and liquid ammonia (100 mL, distilled with a piece of lithium) at –78 °C was added lithium metal (289 mg, 41.7 mmol) in small pieces over a period of 5 hours. The reaction was monitored via TLC by taking small aliquots of the reaction mixture and quenching with 1M HCl and diluting with ethyl acetate. Upon reaction completion, solid NH_4Cl (10 g) was added and the ammonia was left to evaporate overnight. The resulting residue was brought to pH 4 with 1M HCl and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with 1M NaH_2PO_4 solution (100 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The ensuing yellow residue obtained from four parallel reactions were combined and subjected to flash chromatography (silica, 1:9:0.1 v/v/v ethyl acetate/hexane/AcOH elution) to afford, after concentration of the relevant fractions (R_f = 0.8), acid **19** (5.0 g, 59%)

as an amorphous, white solid. The spectroscopic data was in good comparison with that previously described in the literature.[21] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (m, 1H), 4.87 (broad s, 2H), 3.70 (s, 3H), 3.03 (d, J = 5.5 Hz, 1H), 2.88 (m, 1H), 2.55−2.50 (m, 2H), 2.43−2.38 (m, 2H), 2.22−2.08 (complex m, 4H), 1.76−1.61 (complex m, 2H), 1.58−1.43 (complex m, 4H), 1.24 (s, 3H), 1.20 (m, 2H).

Methyl (1*S*,1a*R*,4*R*,4a*S*,5*S*,5a*R*,8*R*,10a*R*,10b*R*)-4-methyl-7-methylene-12-oxododecahydro-1H-1,4:5a,8-dimethanocyclopropa[1,6]benzo[1,2-a]azulene-5-carboxylate (23). *Step i:* To a magnetically stirred mixture of oxalyl chloride (10.2 mL, 120.8 mmol) in dry Et_2O (60 mL) at 0 °C was added, dropwise a solution of acid **19** (5.0 g, 15.1 mmol) in dry Et_2O (80 mL) and pyridine (20 mL). Upon completion of addition, the reaction was allowed to warm to 18 °C, dry DMF (1 mL) was added and the reaction was left to stir for 24 h. The resulting suspension was filtered through Celite™ under a stream of nitrogen and the filter cake was rinsed thoroughly with Et_2O (6 × 100 mL). The combined filtrates were concentrated under reduced pressure and the higher boiling point components azeotrophed with dry toluene (100 mL) to provide the crude acyl chloride **20** as a yellow oil, which was subjected to next step directly. *Step ii:* The crude acyl chloride **20** obtained was dissolved in dry Et_2O (50 mL) and added, dropwise, to a stirred solution of diazomethane in ether (170 mL of a 0.9 M solution in diethyl ether, prepared from the known literature procudure[37]) at 0 °C. Upon completion of addition the solution was allowed to warm to 18 °C over 16 h. Careful evaporation of the excess diazomethane and removal of Et_2O provided the crude diazoketone **21** as a yellow oil, which was subjected to next step directly. *Step iii:* The obtained crude diazoketone **21** was dissolved in dry THF and cyclohexane (80 mL, 1:1 v/v) and added, dropwise to a refluxing solution of bis(*N*-*tert*-butylsalicylaldiminato)copper(II) **22** (586 mg, 1.4 mmol) in cyclohexane (100 mL). The ensuing dark red solution was heated at reflux 2 h before being allowed to cool to room temperature and concentrated under reduced

pressure. The dark red residue obtained was diluted with Et_2O (200 mL) and washed with 10% aqueous solution of ammonia (200 mL), the aqueous layer was further extracted by Et_2O (3 × 100 mL), and the combined organic extracts washed with 1M NaH_2PO_4 (100 mL), brine (100 mL), dried with anhydrous $NaSO_4$, filtered and concentrated under reduced pressure. The ensuing residue was subjected to flash chromatography (silica, 2:98 v/v ethyl acetate/dichloromethane elution) to afford, after concentration of the relevant fractions (R_f = 0.5), cyclopropane **23** (2.6 g, 53% over 3 steps) as a colourless oil, $[\alpha]_D^{20}$ = −90.4 (*c* 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (broad s, 1H), 4.87 (broad s, 1H), 3.68 (s, 3H), 2.70 (d, *J* = 10.4 Hz, 1H), 2.59 (t, *J* = 6.4 Hz, 1H), 2.36 (d, *J* = 10.4 Hz, 1H), 2.22−2.16 (complex m, 2H), 2.03−1.96 (complex m, 2H),1.95−1.87 (complex m, 2H), 1.82 (d, *J* = 8.2 Hz, 1H), 1.76−1.72 (complex m, 1H), 1.67−1.61 (complex m, 2H), 1.60−1.52 (complex m, 2H), 1.39−1.29 (complex m, 1H), 1.27−1.17 (complex m, 1H), 0.91 (complex m, 1H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 215.0, 173.9, 156.9, 107.3, 53.0, 52.1, 51.7, 50.0, 47.8, 46.5, 46.3, 44.1, 38.9, 37.8, 36.9, 31.8, 31.5, 30.9, 18.9, 17.6, 15.5. IR (KBr): ν_{max} 2933, 2863, 1723, 1658, 1435, 1375, 1260, 1191, 1165, 1029, 991, 881 cm^{-1}. MS (ESI, +ve): *m/z* 349 ($[M+Na]^+$, 100%). HRMS $[M+Na]^+$ Calcd for $C_{21}H_{26}O_3{}^{23}Na$: 349.1780. Found: 349.1780.

Methyl (1*R*,4a*R*,4b*S*,7*R*,9a*R*,10*S*,10a*S*)-1-methyl-8-methylene-13-oxododecahydro-1,4a-ethano-7,9a-methanobenzo[*a*]azulene-10-carboxylate (24). *Step i:* To a magnetically stirred mixture of cyclopropane **23** (2.6 g, 8.0 mmol) in dry THF (100 mL), *tert*-butyl alcohol (2.2 mL, 24.0 mmol) and liquid ammonia (200 mL, distilled with a piece of lithium) at −78 °C was added lithium metal (0.14 g, 20.0 mmol) in small pieces over a period of 5 hours. The reaction was monitored via TLC by taking small aliquots of the reaction mixture and quenching with 1M HCl and diluting with ethyl acetate. Upon reaction completion, solid NH_4Cl (10 g) was added and the ammonia was left to evaporate overnight. The resulting

residue was brought to pH 4 with 1M HCl and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with 1M NaH_2PO_4 solution (100 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The ensuing yellow residue was subjected to next step directly. *Step ii*: A magnetically stirred mixture of the above-mentioned crude material in dry dichloromethane (50 mL) and pyridine (0.27 mL, 3.4 mmol) at 0 °C was treated with Dess-Martin Periodinane (1.7 g, 4.0 mmol) and the reaction mixture was left to stir for 5 h. Removal of the solvent and dilution with diethyl ether produced a white precipitate which was filtered through Celite™, the filter cake was rinsed with additional diethyl ether (50 mL× 3), and the filtrate was concentrated under reduced pressure to afford a yellow oil. Of which was then subjected to flash chromatography (silica, 2:98 v/v ethyl acetate/dichloromethane elution) to afford, after concentration of the relevant fractions (R_f = 0.5), ketone **24** (2.0 g, 77% over 2 steps) as an amorphous, white powder, $[\alpha]_D^{20} = -30.0$ (*c* 1.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.95 (broad s, 1H), 4.83 (broad s, 1H), 3.67 (s, 3H), 2.62 (m, 1H), 2.42 (d, *J* = 11.9 Hz, 1H), 2.34 (d, *J* = 11.9 Hz, 1H), 2.16−2.02 (complex m, 3H), 1.94−1.89 (complex m, 1H), 1.86−1.78 (complex m, 2H), 1.71−1.62 (complex m, 3H), 1.58−1.49 (complex m, 4H), 1.48−1.33 (complex m, 3H), 1.21−1.12 (complex m, 1H), 0.90 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 220.8, 173.8, 157.6, 106.7, 59.0, 54.8, 53.5, 52.4, 51.6, 51.0, 49.2, 45.4, 43.7, 39.0, 38.1, 36.6, 36.3, 31.8, 19.9, 19.0, 17.1. IR (KBr): ν_{max} 2933, 2867, 1736, 1656, 1439, 1375, 1285, 1194, 1171, 1087, 1016, 878 cm^{-1}. MS (ESI, +ve): *m/z* 351 ([M+Na]$^+$, 19%), 301 (100). HRMS [M+Na]$^+$ Calcd for $C_{21}H_{28}O_3{}^{23}Na$: 351.1936. Found: 351.1937.

(1*R*,4a*R*,4b*R*,7*R*,9a*R*,10*S*,10a*S*)-4a-Formyl-10-(methoxycarbonyl)-1-methyl-8-methylenedodecahydro-1H-7,9a-methanobenzo[*a*]azulene-1-carboxylic acid (25). A magnetically stirred mixture of ketone **24** (2.0 g, 6.1 mmol) in tetrahydrofuran (50 mL) and dimethylformamide (50 mL) at 0 °C was treated with potassium hexamethyldisilazide (14.6

mL of a 0.5 M solution in toluene, 7.3 mmol). The mixture was stirred at 0 °C for 1 h before being bubbled with oxygen gas. After a further 2 h, the reaction was quenched with water (1 mL). The solvent was removed under reduced pressure and the resulting solid mixed with a small amount of silica, dissolved in dichloromethane and dried to a fine powder which was subjected to flash chromatography (silica, 1:4:0.05 v/v/v ethyl acetate/hexane/acetic acid elution) to afford, after concentration of the relevant fractions (R_f = 0.3), lactol **25** (1.7 g, 77%) as a white crystalline solid (m.p. 165−168 °C), $[\alpha]_D^{20}$ = −76.0 (*c* 0.5, $CHCl_3$). This compound is not suitable for NMR analysis due to lactol resonances. IR (KBr): ν_{max} 3377, 2940, 2877, 1732, 1436, 1381, 1266, 1237, 1195, 1169, 1123, 983, 916, 807 cm^{-1}. MS (ESI, +ve): *m/z* 383 ($[M+Na]^+$, 23%), 744 ($[2M+Na]^+$, 100). HRMS $[M+Na]^+$ Calcd for $C_{21}H_{28}O_5{}^{23}Na$: 383.1834. Found: 383.1834.

Further structural determination of lactol **25** was achieved on the corresponding methyl ester, which was prepared by treating compound **25** (10 mg) with a solution of diazomethane in ether until the yellow colour persisted. The solution was concentrated under reduced pressure. The resulting methyl ester **26** was then submitted to structural analysis. ^1H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 4.90 (broad s, 1H), 4.82 (broad s, 1H), 3.86 (d, *J* = 12.6 Hz, 1H), 3.70 (s, 3H), 3.62 (s, 3H), 2.60 (t, *J* = 6.1 Hz, 1H), 2.37 (dd, *J* = 13.0, 3.4 Hz, 1H), 2.22 (d, *J* = 12.6 Hz, 1H), 2.18−2.09 (complex m, 2H), 1.98 (dt, *J* = 15.9, 2.9 Hz, 1H), 1.83 (m, 1H), 1.69−1.61 (complex m, 1H), 1.62−1.58 (complex m, 2H), 1.57−1.50 (complex m, 3H), 1.40−1.31 (complex m, 1H), 1.25−1.15 (complex m, 1H), 1.12 (s, 3H), 1.11−1.08 (complex m, 1H), 0.95 (td, *J* = 13.0, 4.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.5, 176.6, 175.1, 155.8, 106.5, 60.4, 56.6, 56.0, 51.6, 51.6, 50.3, 50.0, 45.8, 45.2, 39.5, 38.3, 37.8, 33.0, 31.9, 28.1, 20.8, 17.7. IR (KBr): ν_{max} 2939, 2871, 1733, 1435, 1376, 1262, 1231, 1196, 1169, 1168, 1147 cm^{-1}. MS (ESI, +ve): *m/z* 397 ($[M+Na]^+$, 100%), 375 ($[M+H]^+$, 33). HRMS $[M+Na]^+$ Calcd for $C_{22}H_{30}O_5{}^{23}Na$: 397.1991. Found: 397.1996.

***GA_{24}* (10)**. To a magnetically stirred mixture of lactol **25** (1.7 g, 4.72 mmol) in methanol (12 mL) was added NaOH aqueous solution (2M, 60 mL) and the mixture was heated at 100 °C for 20 h. The volatile solvent was then removed, the pH was adjusted to 4 with 1 M HCl, and the aqueous layer was extracted with ethyl acetate (3 × 75 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The ensuing residue was subjected to flash chromatography (silica, 1:2:0.05 v/v/v ethyl acetate/hexane/acetic acid elution) to afford, after concentration of the relevant fractions (R_f = 0.45), GA_{24} **10** (1.5 g, 87%) as a white, crystalline solid, m.p. 203−205 °C (MeOH/EtOAc) {lit[38]. 198−203 °C}, $[\alpha]_D^{20}$ = −105.0 (*c* 0.8, EtOH) {lit[38]. −88 (*c* 0.6, EtOH)}.GA_{24} is not suitable for NMR analysis due to lactol resonances. IR (KBr): ν_{max} 3336, 2939, 2875, 1705, 1407, 1287, 1267, 1194, 1147, 1126, 977, 950, 876 cm^{-1}. MS (ESI, +eV): *m/z* 369 ($[M+Na]^+$, 95%), 715 ($[2M+Na]^+$, 100). HRMS $[M+Na]^+$ Calcd for $C_{20}H_{26}O_5{}^{23}Na$: 369.1678. Found: 369.1678.

Further structural determination of GA_{24} was achieved on the corresponding di-methyl ester **26**, which was prepared by treating GA_{24} (10 mg) with a solution of diazomethane in ether until the yellow colour persisted. The solution was concentrated under reduced pressure. The ensuing di-methyl ester showed the same structural data, in all respects, to compound **26** obtained previously.

***GA_{15}* (9)**. To a magnetically stirred mixture of GA_{24} **10** (1.0 g, 2.9 mmol) in ethanol (20 mL) at −10 °C was added, portion-wise, sodium borohydride (378 mg, 10.0 mmol). The mixture was stirred at −10 °C for 5 h. The excess sodium borohydride was quenched with acetone (3 mL) followed by 1M HCl (20 mL), and the mixture was left to stir for 5 min before the volatile solvent was removed under reduced pressure. The solution was then extracted with ethyl acetate (3 × 75 mL), the combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The ensuing residue was subjected to flash

chromatography (silica, 1:3:0.05 v/v/v ethyl acetate/hexane/acetic acid elution) to afford, after concentration of the relevant fractions (R_f = 0.8), GA_{15} **9** (0.87 g, 92%) as a white, crystalline solid, m.p. 266−270 °C (MeOH/EtOAc) {lit[39] 274−276 °C}, $[\alpha]_D^{20}$ = −83.6 (*c* 0.55, $CHCl_3$) {lit[39] +5.0 (no concentration and solvent indicated)}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (broad s, 1H), 4.82 (broad s, 1H), 4.40 (d, *J* = 12.1 Hz, 1H), 4.10 (d, *J* = 12.1 Hz, 1H), 2.79 (d, *J* = 12.7 Hz, 1H), 2.65 (m, 1H), 2.19−2.10 (complex m, 2H), 2.08−1.99 (complex m, 3H), 1.86−1.82 (complex m, 1H), 1.75−1.71 (complex m, 2H), 1.64−1.54 (complex m, 3H), 1.51−1.41 (complex m, 2H), 1.40−1.31 (complex m, 2H), 1.20 (s, 3H), 1.11−1.02 (complex m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ178.2, 175.3, 156.4, 106.9, 74.4, 55.9, 52.7, 52.0, 49.8, 46.1, 42.8, 41.9, 40.0, 39.4, 38.4, 36.5, 31.4, 23.3, 20.8, 16.0. IR (KBr): ν_{max} 3196, 2935, 2872, 1724, 1422, 1262, 1194, 1156, 1036, 912, 879, 732 cm^{-1}. MS (ESI, +ve): *m/z* 353 ($[M+Na]^+$, 21%), 683 ($[2M+Na]^+$, 100). HRMS $[M+Na]^+$ Calcd for $C_{20}H_{26}O_4{}^{23}Na$: 353.1729. Found: 353.1728.

ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra of compounds **17-19**, ^{1}H and ^{13}C NMR spectra of compounds **9**, **23**, **24** and **26**. This material is available free of charge via the internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Fundamental Research Funds for the Central Universities (grant 21617327), China and the Chinese National Natural Science Foundation (grant 31700670), the Guangdong Natural Science Foundation (grant 2017A030313202), as well as the Department of Science and Technology of Guangdong Province under grant 2016A010105010 for financial support.

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