Synthesis and structure determination of three new 12β-hydroxy C_{20} gibberellins (GA_{127}, GA_{128} and GA_{129})

Sarah K. Leitch,[a] Patrick S. Blake,[b] and Lewis N. Mander*[c]

[a] Discipline of Chemistry, School of Biological and Chemical Sciences, University of Newcastle, Callaghan, NSW 2308, Australia; [b] Horticulture Research International, East Malling, West Malling, Kent ME19 6BJ, UK; and [c] Research School of Chemistry, Institute of Advanced Studies, Australian National University, Canberra ACT 0200, Australia
E-mail: mander@rsc.anu.edu.au

Dedicated to Professor Tony McKervey on the occasion of his 65th birthday

(received 24 Feb 03; accepted 11 Apr 03; published on the web 11 May 03)

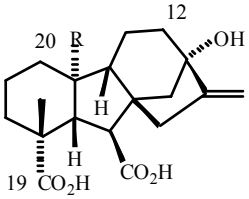
Abstract

Three new 12β-hydroxylated C_{20} gibberellins have been isolated from strawberry fruitlets and their structures confirmed by synthesis from gibberellic acid. The key stages in the synthesis involved transannular hydroxylation using diacetoxy iodobenzene and oxidative cleavage of an enolate function by molecular oxygen.

Keywords: Gibberellin, strawberry, transannular hydroxylation, diacetoxy iodobenzene, enolate, oxidative cleavage

Introduction

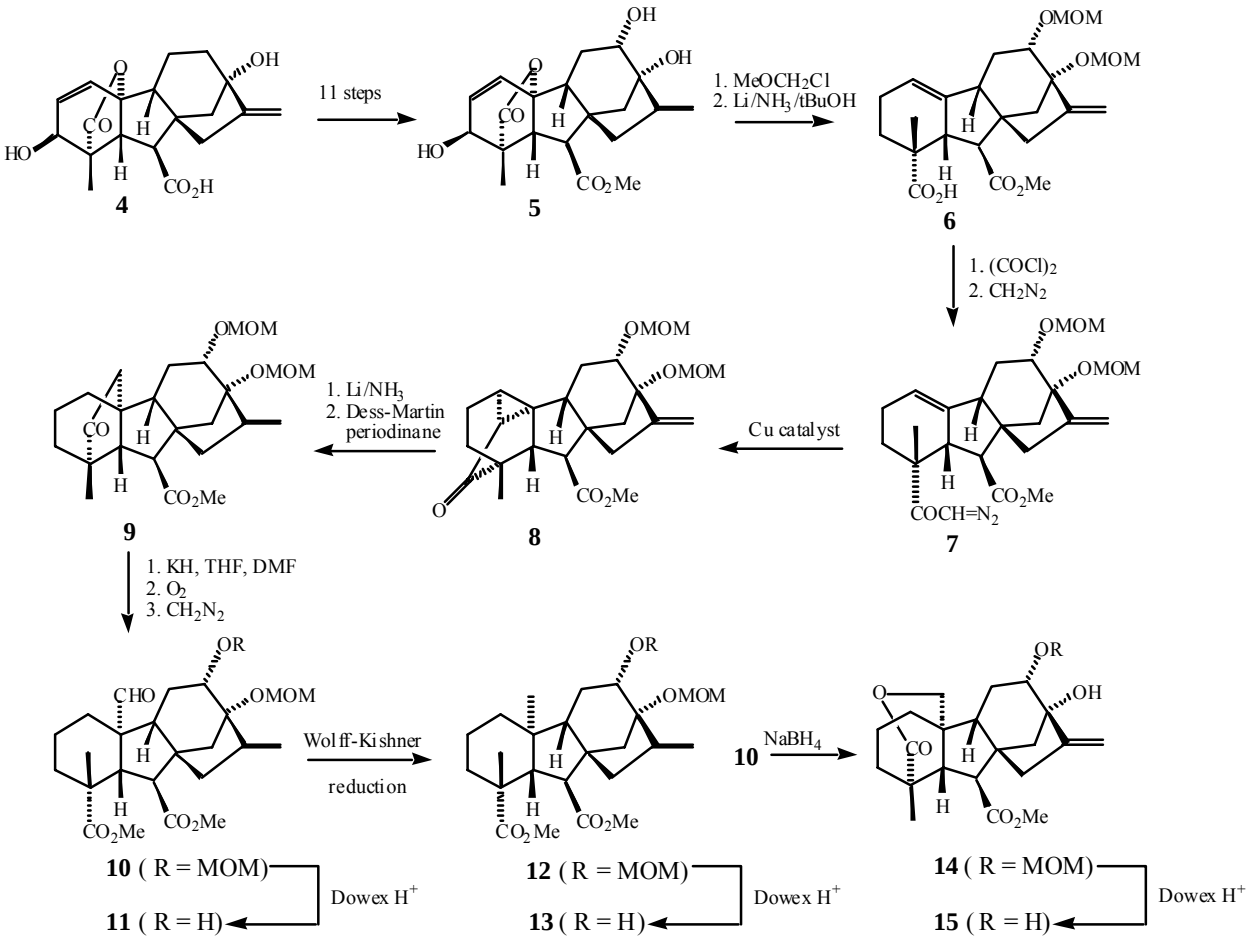
In a recent study of naturally occurring endogenous gibberellins ("GAs") in strawberry fruitlets, we discovered a family of 12,13-dihydroxylated C_{20} GAs.[1] Three of these were identified as the 12α–hydroxy derivatives of GA_{53} **1**, GA_{44} **2** and GA_{19} **3** (Figure 1) by direct comparison with authentic samples and assigned as GA_{123}, GA_{124} and GA_{125}, respectively. Three further GAs were tentatively identified as the corresponding 12β-epimers since they afforded mass spectra that were identical with the equivalent 12α-epimers, but possessed different retention times on GCMS.



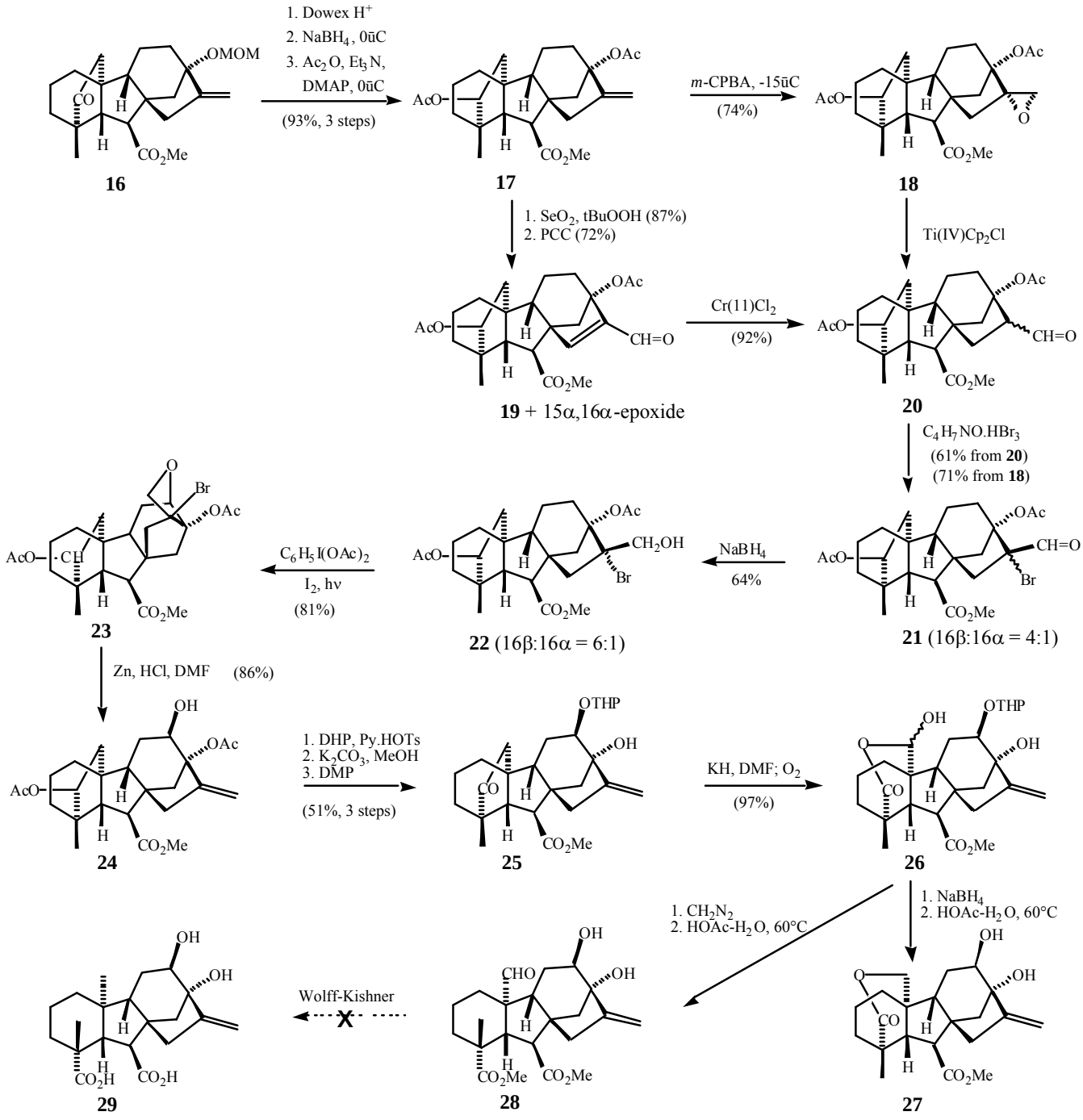
1 R = Me
2 R = CH_2OH (19,20-lactone)
3 R = CH=O

Figure 1

In order to confirm the putative structures of the 12α-epimers, we had successfully undertaken their synthesis[2] from gibberellic acid (GA_3) **4** *via* GA_{87} methyl ester **5** as indicated briefly in Scheme 1, *i.e.* the key stages were transannular hydroxylation of C–12[3] followed by introduction of C–20 by means of a copper catalyzed intramolecular cyclopropanation by a 19-diazoacetyl substituent.[4] We had also applied the same strategy to the preparation of the 12β-epimers,[2] and although the GA_{19} analogue was successfully prepared this way, an unexpected Wagner–Meerwein rearrangement occurred during the last deprotection step for the GA_{44} and GA_{53} derivatives. In this paper we describe our efforts to prepare the 12β-hydroxy series by an alternative route that re-orders the major transformations and postpones the 12–hydroxylation step to a later stage of the synthetic sequence (Scheme 2).



Scheme 1

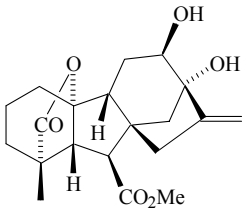

1. Dowex H+
2. NaBH4, 0ūC
3. Ac2O, Et3N,
DMAP, 0ūC
(93%, 3 steps)
m-CPBA, -15ūC
(74%)
1. SeO2, tBuOOH (87%)
2. PCC (72%)
Ti(IV)Cp2Cl
Cr(11)Cl2
(92%)
C4H7NO.HBr3
(61% from 20)
(71% from 18)
NaBH4
64%
C6H5I(OAc)2
I2, hν
(81%)
Zn, HCl, DMF (86%)
1. DHP, Py.HOTs
2. K2CO3, MeOH
3. DMP
(51%, 3 steps)
KH, DMF; O2
(97%)
1. CH2N2
2. HOAc-H2O, 60°C
1. NaBH4
2. HOAc-H2O, 60°C
Wolff-Kishner
16
17
18
19 + 15α,16α-epoxide
20
21 (16β:16α = 4:1)
22 (16β:16α = 6:1)
23
24
25
26
27
28
29

Scheme 2

Results and Discussion

Ketone **16** is reliably available[4] in only 6 steps from GA_3 **4** using the same cyclopropane-based methodology outlined in Scheme 1 and was selected as the substrate for the introduction of the 12β–hydroxyl. Attempts to mask the C-19 carbonyl function as an acetal required fairly vigorous conditions that resulted in partial Wagner–Meerwein rearrangement of the C/D ring system, so instead, the methoxymethyl group was removed from **16**, the product reduced with $NaBH_4$ and acetylated. The stereochemistry at C-19 was established as *S* from nOe experiments on the intermediate diol, *i.e.* irradiation of 19-CH (at 4.19 ppm) afforded enhancement of H-6 (doublet, J = 12.0 Hz, at 2.64 ppm). Next, in preparation for the transannular oxidation step (**22**→**23**), oxygenation at C-17 was effected by two different approaches: allylic oxidation at C-15 with SeO_2[5] followed by pyridinium chlorochromate to aldehyde **19** (obtained as a 2:1 mixture with the 15,16-epoxy derivative) and then reduction with $Cr(II)Cl_2$ to give aldehyde **20**;[6] or by treatment of epoxide **18** with dicyclopentadienyl titanium (III) chloride.[7] There was little to choose between the two routes. Bromination of aldehyde **20** afforded a 4:1 mixture of *endo* and *exo* aldehydes, and the mixture reduced with $NaBH_4$, then treated with diacetoxy iodobenzene and iodine while irradiating with a 250W tungsten lamp. Ether **23** was obtained in excellent yield and subjected to a reductive elimination with zinc–HCl to afford the 12β-ol **24**. Again, the yield was excellent. After protection of the 12-hydroxyl as a THP ether, and hydrolysis of the two acetate functions, the C-19 carbonyl group was re-established (Dess–Martin periodinane)[8] and oxidative cleavage of the C19–C20 bridge effected by oxygenation of the derived potassium enolate.[9]

The product from this last step is a carboxy aldehyde in equilibrium with the hydroxy lactone **26** with a rate constant similar to that of the NMR time scale, so NMR spectra were poor (compounded by the diastereomeric THP groups). A sample was therefore methylated with diazomethane for characterization purposes. Removal of the THP group then revealed the dimethyl ester of 12β-hydroxy GA_{19} (now assigned as GA_{129}),[10] identical with the sample prepared previously.[2] Reduction of **26** with $NaBH_4$ readily furnished the GA_{44} analogue (= GA_{128}), but Wolff–Kishner reduction of **26**, **28** or the corresponding dicarboxylic acid completely failed under conditions that have been successfully employed on GA_{19} and several of its derivatives.[11] 12β-hydroxy-GA_{53} (GA_{127}) was subsequently prepared by applying the 12β-hydroxylation protocol to GA_{53}, however.[12] With these three reference samples in hand, we were able to confirm by direct comparison (GCMS) the identity of the new strawberry GAs as tentatively assigned. 12-*epi*-GA_{77} **30** was also identified as an endogenous GA by direct comparison with an authentic sample[3] and is assigned as GA_{130}. It is tempting to speculate, as with the 12α,13-dihydroxy GA series, that this collection of 12β-hydroxy GAs constitutes an early 12β,13-dihydroxylation biosynthetic pathway, but we suspect that in both cases it is more likely a consequence of indiscriminate hydroxylation by a C-12 dioxygenase.[13]



30

With these four GAs, the total of GAs isolated from strawberry fruit has reached 26, of which seven are new. Further novel GAs have also been isolated from this source and we will report on progress towards the determination of their structures shortly. This alternative approach to the synthesis of 12–hydroxy C_{20} GAs is somewhat more efficient in terms of individual yields, but the sequence is similar in length to those of the earlier efforts.[2]

Experimental Section

General Procedures. Infrared spectra (ν_{max} cm^{-1}) were recorded on a Perkin–Elmer 1800 Fourier Transform Infrared spectrometer or a Perkin–Elmer 683 Infrared Spectrometer using NaCl or KI plates. ^{1}H NMR were recorded on either a Varian Gemini 300 MHz or Varian VXR500 MHz instrument and ^{13}C NMR were recorded on either a Varian Gemini 300 MHz or Varian VXR500 MHz instrument. Chemical shifts are reported as values in parts per million (δ ppm). For proton spectra recorded in chloroform, the residual peak of $CHCl_3$ was used as the internal reference (7.26 ppm) while the central peak of $CDCl_3$ (77.0 ppm) was used as the reference for ^{13}C spectra. Multiplicities are abbreviated: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants are recorded in Hertz (Hz). When THP protecting groups have been utilized integrations relate to individual diastereomers; where peaks are superimposed integrations indicate the sum of both diastereomers. Thus a single methyl peak for the mixture integrates to 6H. Assignment has been made where possible with the anomeric protons of the diastereomers and designated as: THP-H1 and THP-H1' in ^{1}H NMR. Similarly, in the ^{13}C NMR, where possible, the anomeric carbon is assigned as: THP-C1 and THP-C1'. The remaining protons associated with the THP protecting group have been designated as 'THP' where possible. Distortionless enhancement by polarisation transfer (DEPT) and attached proton test (APT) experiments were used in the assignment of carbon spectra. Low resolution EI mass spectra (EIMS, 70 eV) were recorded on a VG Micromass 7070F double focussing mass spectrometer. Analytical thin layer chromatography (tlc) was conducted on Merck aluminium backed tlc sheets with silica gel 60 F_{254} or Merck glass backed tlc plates coated with 0.2 mm thick silica gel GF_{254}. The developed plates were visualized under shortwave ultraviolet light and exposed to an ammonium molybdate dip combined with heat. All flash chromatography was carried out using the flash technique as reported by Still[14] using Merck Kieselgel 60 and analytical reagent (AR) grade solvents as indicated.

Methyl *ent*-13-hydroxy-19-oxo-19,20-cyclogibberell-16-en-7-oate. A mixture of ketone **16** (4.12 g, 0.011 mmol) and wet Dowex H^+/50W (12.4 g) in MeOH/H_2O (120:24 mL) was heated at reflux, 84°C, for 2.5 hours. By this time, all of the starting material had reacted by TLC and the reaction mixture was filtered through a sintered funnel, diluted with EtOAc (100 mL) and NaOAc was added until the pH was ~ 7. The solvent was removed under reduced pressure and the residue was taken up in EtOAc (200 mL). The aqueous phase was extracted with EtOAc (2 × 100 mL) and the combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to afford the title alcohol (3.73 g, 98%) as a white amorphous solid. Purification of the product was not necessary. R_f 0.3 (EtOAc/petrol 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (3H, s, H18), 1.38–1.74 (10H, m), 1.81–1.90 (3H, m), 1.91–2.11 (4H, m), 2.22 (1H, dd, J_{gem} = 15.8 Hz, J = 2.2 Hz, H15), 2.37 (1H, dd, $J_{5,6}$ = 12.0, 1.3 Hz, H5), 2.43 (1H, d, $J_{6,5}$ = 11.9 Hz, H6), 3.60 (3H, s, $CO_2\underline{CH_3}$), 4.93 (1H, s, H17), 5.24 (1H, d, J = 3.1 Hz, H'17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.6 (CH_3, C18), 19.4, 19.5 (CH_2, C2, C11), 36.1 (CH_2, C20), 37.6, 38.5 (CH_2, C3, C12), 43.1 (CH_2, C1), 43.7 (CH_2 × 2, C14, C15), 48.4, 48.5 (C, C4, C10), 51.3 (CH_3, $CO_2\underline{CH_3}$), 51.7 (CH, C6), 53.1 (C, C8), 54.1 (CH, C9), 59.0 (CH, C5), 77.7 (C, C13), 106.2 (CH_2, C17), 157.4 (C, C16), 173.0 (C, C7), 220.0 (C, C19). IR ν_{max} (cm^{-1}): 3453 (br), 2933, 2869, 1732, 1659. MS (EI) *m/z* 344 (M^+, 100%), 312 (69), 285 (74), 135 (62), 91 (43). HRMS (EI) *m/z* calcd for M^+, $C_{21}H_{28}O_4$: 344.1988; found: 344.1990.

Methyl *ent*-13,19-dihydroxy-19,20-cyclogibberell-16-en-7-oate. A solution of the ketone prepared above (2.11 g, 6.12 mmol) in MeOH (anhydrous, 61 mL) at 0°C was treated with finely ground $NaBH_4$ (347 mg, 9.19 mmol). The reaction was monitored by TLC analysis and was complete in 10 minutes. After diluting the reaction mixture with EtOAc (100 mL) the entire mixture was washed with K_2HPO_4 (20% aqueous solution, 50 mL). The aqueous layer was further extracted with EtOAc (2 × 100 mL) and the combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The residue was chromatographed (silica gel, EtOAc/petrol 3:1) to afford the title alcohol quantitatively (2.15 g) as a white amorphous solid. R_f 0.4 (EtOAc/petrol 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, s, H18), 1.18–1.33 (2H, m), 1.45–1.83 (10H, m), 1.70 (1H, d, J = 9.7 Hz), 1.89–2.23 (7H, m), 2.64 (1H, d, $J_{6,5}$ = 12.0 Hz, H6), 3.68 (3H, s, $CO_2\underline{CH_3}$), 4.10 (1H, m, H19), 4.88 (1H, s, H17), 5.20 (1H, d, J = 3.0 Hz, H'17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.6, 19.9 (CH_2, C2, C11), 21.9 (CH_3, C18), 35.0 (CH_2, C20), 38.3, 38.9, 39.0 (CH_2, C3, C12, C14), 44.1, 44.9 (CH_2, C1, C15), 45.3, 48.9 (C, C4, C10), 50.8 (CH, C6), 51.5 (CH_3, $CO_2\underline{CH_3}$), 52.0 (C, C8), 55.2 (CH, C9), 61.7 (CH, C5), 77.8 (C, C13), 78.4 (CH, C19), 106.2 (CH_2, C17), 158.3 (C, C16), 174.2 (C, C7). IR ν_{max} (cm^{-1}): 3292 (br), 2941, 1740, 1658. MS (EI) *m/z* 346 (M^+, 100%), 314 (91), 286 (73), 166 (36), 135 (40), 91 (35). HRMS (EI) *m/z* calcd for M^+, $C_{21}H_{30}O_4$: 346.2144; found: 346.2147.

Methyl *ent*-13,19-diacetoxy-19,20-cyclogibberell-16-en-7-oate (17). The diol prepared above (2.15 g, 6.2 mmol) was taken up in DCM (62 mL). After the addition of DMAP (catalytic) the solution was cooled to 0°C then Et_3N (4.32 mL, 0.031 mmol) was added followed by dropwise addition of Ac_2O (2.93 mL, 0.031 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight, by which time the reaction was complete. Ice was then added

to the reaction mixture and after stirring for 30 minutes the mixture was diluted with EtOAc (100 mL) and HCl (1M aqueous solution, 80 mL) was added. The aqueous phase was extracted with EtOAc (3 × 100 mL) and the combined organic extracts were washed with H_2O (100 mL) and brine. After being dried over $MgSO_4$ and filtered, the solvent was removed under reduced pressure to give the desired diacetate **17** (2.59 g, 97%) as a white amorphous solid. R_f 0.5 (EtOAc/petrol 1:3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (3H, s, H18), 1.11–1.31 (3H, m), 1.42–1.71 (8H, m), 1.89–2.06 (4H, m), 1.97 (3H, s, OCO$\underline{CH_3}$), 2.01 (3H, s, OCO$\underline{CH_3}$), 2.30 (3H, m), 2.65 (1H, d, $J_{6,5}$ = 12.0 Hz, H6), 3.63 (3H, s, $CO_2\underline{CH_3}$), 4.85 (1H, dd, J = 14.3, 3.9 Hz, H19), 4.88 (1H, s, H17), 5.09 (1H, s, H'17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 19.5 (CH_2, C2, C11), 20.8, 21.7 (× 2) (CH_3, C18, OCO$\underline{CH_3}$ × 2), 35.4 (CH_2, C20), 36.4, 36.8, 37.9 (CH_2, C1, C3, C12), 40.0 (CH_2, C14), 43.2 (CH_2, C15), 44.6, 49.4 (C, C4, C10), 50.3 (CH, C6), 51.3 (CH_3, $CO_2\underline{CH_3}$), 51.9 (C, C8), 54.9 (CH, C9), 61.0 (CH, C5), 79.4 (CH, C19), 84.5 (C, C13), 106.9 (CH_2, C17), 154.5 (C, C16), 169.6, 170.8 (C, O$\underline{C}OCH_3$), 173.5 (C, C7). IR ν_{max} (cm^{-1}): 2948, 2871, 1737, 1662, 1243. MS (EI) *m/z* 430 (M^+, 9%), 388 (74), 370 (100), 328 (38), 310 (73), 283 (60), 268 (34), 251 (32). HRMS (EI) *m/z* calcd for M^+, $C_{25}H_{34}O_6$: 430.2355; found: 430.2354.

Methyl *ent*-13,19-diacetoxy-16β,17-epoxy-19,20-cyclogibberellane-7-oate (18). A solution of diacetate **17** (4.36 g, 0.010 mmol) in DCM (150 mL) cooled to –15°C was treated with a solution of *m*–CPBA (4.36 g of 60%, 0.015 mmol) in DCM (100 mL). The mixture was stirred at –15°C for 30 minutes and the allowed to warm to 4°C and stirred overnight. $NaHCO_3$ (saturated aqueous solution, 100 mL) was added and the reaction mixture stirred for a further 30 minutes. The aqueous phase was extracted with DCM (2 × 100 mL) and the combined organic extracts were washed with brine, dried over $MgSO_4$ filtered, and the solvent removed under reduced pressure. The residue was chromatographed (silica gel, EtOAc/petrol 1:3) to afford α-epoxide **18** (3.29 g, 74%). R_f 0.3 (EtOAc/petrol 1:3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (3H, s, H18), 1.14–1.31 (4H, m), 1.46–1.74 (8H, m), 1.87–2.18 (4H, m), 1.96 (3H, s, OCO$\underline{CH_3}$), 2.03 (3H, s, OCO$\underline{CH_3}$), 2.29 (1H, dd, J_{gem} = 14.7 Hz, J = 12.1 Hz), 2.41 (1H, d, $J_{5,6}$ = 11.0 Hz, H5), 2.70 (1H, d, $J_{6,5}$ = 11.7 Hz, H6), 2.70 (1H, d, J_{gem} = 5.4 Hz, H'17), 3.01 (1H, d, J_{gem} = 5.3 Hz, H17), 3.64 (3H, s, $CO_2\underline{CH_3}$), 4.88 (1H, dd, J = 10.4, 3.4 Hz, H19). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.4, 19.7 (CH_2, C2, C11), 20.7, 21.3, 21.6 (CH_3, C18, OCO$\underline{CH_3}$ × 2), 33.3, 35.4, 36.6, 37.9 (CH_2, C1, C3, C12, C20), 40.8 (CH_2, C14), 43.7 (CH_2, C15), 44.6, 47.9 (C, C4, C10), 50.4 (CH_2, C17), 51.1 (CH, C6), 51.5 (CH_3, $CO_2\underline{CH_3}$), 51.9 (C, C8), 56.2 (CH, C9), 61.0 (CH, C5), 67.1 (C, C16), 79.4 (CH, C19), 80.5 (C, C13), 169.8, 171.1 (C, O$\underline{C}OCH_3$), 173.4 (C, C7). IR ν_{max} (cm^{-1}): 1737, 1243.

Methyl *ent*-13,19-diacetoxy-15β-hydroxy-19,20-cyclogibberell-16-en-7-oate. A solution of diacetate **17** (1.33 g, 3.09 mmol) in DCM (44 mL) with ^{t}BuOOH (70% aqueous solution, 2 mL) and SeO_2 (1.03 g, 9.26 mmol) was sonicated for 3 hours. The reaction mixture was then diluted with EtOAc (50 mL), washed with H_2O (20 mL), brine and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was chromatographed (silica gel, EtOAc/petrol 1:2) to afford the desired title alcohol (1.20 g, 87%) as a white amorphous solid. R_f 0.6 (EtOAc/petrol 1:3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (3H, s, H18), 1.16 (1H, dd,

J_{gem} = 16.9 Hz, J = 2.9 Hz), 1.23–1.35 (2H, m), 1.40 (1H, d, J = 8.8 Hz), 1.50–1.89 (8H, m), 2.02 (3H, s, OCO$\underline{CH_3}$), 2.05 (3H, s, OCO$\underline{CH_3}$), 2.15 (1H, d, J = 11.3 Hz, H20), 2.14–2.34 (2H, m), 2.57 (1H, d, $J_{6,5}$ = 12.0 Hz, H6), 2.88 (1H, d, J = 11.2 Hz, H'20), 3.64 (3H, s, $CO_2\underline{CH_3}$), 3.75 (1H, d, $J_{OH,15\beta}$ = 7.5 Hz, 15–O$\underline{H}$), 3.95 (1H, d, $J_{15\beta,OH}$ = 8.3 Hz, H15), 4.88 (1H, dd, J = 10.7, 3.9 Hz, H19), 5.14 (1H, s, H17),5.34 (1H, s, H'17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7, 19.7 (CH_2, C2, C11), 21.1, 21.7, 22.1 (CH_3, C18, OCO$\underline{CH_3}$ × 2), 35.6, 35.8, 37.6 (× 2), 38.1 (CH_2, C1, C3, C12, C14, C20), 45.1 (C), 48.2 (CH, C6), 51.2 (CH, C9), 51.8 (CH_3, $CO_2\underline{CH_3}$), 52.4 (C), 56.4 (C, C8), 61.5 (CH, C5), 78.0 (CH, C15), 79.8 (CH, C19), 83.1 (C, C13), 112.3 (CH_2, C17), 156.2 (C, C16), 170.9, 171.4 (C, O$\underline{C}OCH_3$), 174.7 (C, C7). IR ν_{max} (cm^{-1}): 3487, 1729, 1664, 1250. MS (EI) *m/z* 446 (M^+, 9%), 414 (23), 326 (100), 266 (84). HRMS (EI) *m/z* calcd for M^+, $C_{25}H_{34}O_7$: 446.2305; found: 446.2309.

Methyl *ent*-13,19-diacetoxy-17-oxo-19,20-cyclogibberell-15-en-7-oate (19) and methyl *ent*-13,19-diacetoxy-15α,16α-epoxy-17-oxo-19,20-cyclogibberellan-7-oate. A finely ground mixture of PCC (3.02 g, 0.014 mmol) and SiO_2 (3.02 g, mass equivalent to PCC) was added to a solution of the allylic alcohol prepared above (2.09 g, 4.68 mmol) in DCM (100 mL) and the suspension was sonicated for 9 hours. Ether (100 mL) was added to the reaction mixture and the mixture was then filtered through a pad of celite which was washed thoroughly with ether (100 mL). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, EtOAc/petrol 1:2) to afford a mixture (1.51 g, 72%) of aldehyde **19** with its 15,16 epoxide in a 2:1 ratio. The mixture was not separable by flash chromatography. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (3H, s, 6H), 1.10–2.38 (m), 1.98, 1.99, 2.02 (12H, s, OCO$\underline{CH_3}$), 2.48–2.70 (m), 3.45 (1H, d, J = 6 Hz), 3.65 (3H, s, $CO_2\underline{CH_3}$[¶]), 3.70 (3H, s, $CO_2\underline{CH_3}$[§]), 4.89 (2H, m, H19), 6.85 (1H, s, H15[¶]), 9.23 (1H, s, H17[§]), 9.57 (1H, s, H17[¶]). ^{13}C NMR (75 MHz, $CDCl_3$) δ (diagnostic peaks only) 62.6 (CH, C15[§]), 66.5 (C, C16[§]), 83.2 (CH, C13[§]), 84.2 (CH, C13[¶]), 150.1 (C, C16[¶]), 156.1 (C, C15[¶]), 187.5 (C, C17[¶]), 193.3 (C, C17[§]). MS (EI) *m/z* 460 (M^+, 36%)[§], 442 (M^+, 17)[¶], 400 (41), 384 (46), 372 (100), 356 (34), 346 (46), 330 (42), 312 (47), 301 (71), 283 (54), 269 (62), 259 (56), 241 (72), 225 (64), 213 (38).

Methyl *ent*-13,19-diacetoxy-17-oxo-19,20-cyclogibberellan-7-oate (20). $CrCl_2$ (60 mL) was freshly prepared by shaking a mixture of zinc dust (88 g) with $HgCl_2$ (8.25 g) in H_2O (36 mL) and HCl (conc., 1.8 mL) for 10 minutes to form an amalgam. A green solution of $CrCl_3$ (21.5 g) in H_2O (72 mL) and HCl (conc., 14.4 mL) was then added to the amalgam and CO_2 (g) was bubbled through the mixture. When the supernatant turned a vibrant blue color it was ready for use. The blue supernatant of the $CrCl_2$ solution (60 mL), prepared above, was added to a solution of the aldehydes prepared earlier (2.3 g, 5.16 mmol) in acetone (100 mL) which was then left to stir overnight. The acetone was evaporated under nitrogen and EtOAc (200 mL) and H_2O (200 mL) were added. The aqueous phase was extracted with EtOAc (3 × 200 mL) and the combined organic phases were washed with $NaHCO_3$ (saturated aqueous solution, 100 mL), brine and dried over $MgSO_4$. After filtration the solvent was removed under reduced pressure to afford the desired saturated aldehyde **20** (2.06 g, 92%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (3H, s, H18), 1.14–1.48 (6H, m), 1.51–1.79 (6H, m), 2.06 (3H, s,

OCO$\underline{CH_3}$), 2.07 (3H, s, OCO$\underline{CH_3}$), 1.84–2.25 (3H, m), 1.88 (1H, d, J_{gem} = 11.0 Hz, H14α), 2.28 (2H, d, J_{gem} = 11.0 Hz, H14β), 2.69 (1H, d, $J_{6,5}$ = 12.1 Hz, H6), 3.02 (1H, dd, J = 12.0, 3.8 Hz), 3.69 (3H, s, $CO_2\underline{CH_3}$), 4.88 (1H, dd, J = 10.9, 4.3 Hz, H19), 9.98 (1H, s, H17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.0, 19.5 (CH_2, C2, C11), 20.9, 21.5. 21.8 (CH_3, C18, OCO$\underline{CH_3}$ × 2), 30.1 (CH_2, C12), 35.3, 35.5, 36.7, 37.9 (CH_2, C1, C3, C14, C20), 43.3 (CH_2, C15), 44.7, 49.7 (C, C4, C10), 51.0, 51.4 (CH, C6, C9), 52.0 (C, C8), 55.7 (CH, C5), 57.9 (CH_3, $CO_2\underline{CH_3}$), 60.7 (CH, C16), 79.4 (CH, C19), 85.5 (C, C13), 170.2, 171.0 (C, O$\underline{C}OCH_3$), 173.4 (C, C7), 201.9 (CH, C17). IR ν_{max} (cm^{-1}): 1730 (br), 1240. MS (EI) *m/z* 445 (M–H$^+$, 9%), 415 ($M^{+\bullet}$–CH_3O, 15), 386 (87), 358 (98), 326 (87), 298 (100), 271 (46), 256 (62), 227 (70), 185 (33), 145 (35), 131 (32), 105 (44), 81 (83). HRMS (EI) *m/z* calcd for M^+–CH_3O^-, $C_{24}H_{31}O_6$: 415.2121; found: 415.2118.

Methyl 16-bromo-13,19-diacetoxy-17-oxo-19,20-cyclogibberellan-7-oate (21). (a) A solution of aldehyde **20** (3.07 g, 7.32 mmol) in THF (146 mL) was treated with pyrrolidone.HBr_3 (10.9 g, 0.022 mmol) and stirred overnight, by which time the reaction was complete by TLC analysis. EtOAc (200 mL) was added and the entire mixture was washed with $Na_2S_2O_3$ (saturated aqueous solution, 100 mL), $NaHCO_3$ (saturated aqueous solution, 100 mL) and brine. The organic phase was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Chromatography of the residue (silica gel, EtOAc/petrol 1:2) afforded the desired bromo aldehyde **21** as a 4:1 mixture (2.33 g, 61%) with its 16β–epimer. R_f 0.7 (EtOAc/petrol 1:4). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.78 (CH_3, s, H18†), 0.79 (CH_3, s, H18*), 1.10–1.68 (m), 1.86–2.22 (m), 1.98, 2.01, 2.05 (12H, OCO$\underline{CH_3}$), 2.44 (1H, d, $J_{6,5}$ = 11.5 Hz, H6*), 2.56 (1H, d, $J_{6,5}$ = 12.1 Hz, H6†), 2.68 (m), 3.67 (3H, s, $CO_2\underline{CH_3}^*$), 3.68 (3H, s, $CO_2\underline{CH_3}^\dagger$), 4.83 (2H, dd, J = 10.4, 3.4 Hz, H19), 9.48 (s, H17†), 9.49 (s, H17*). ^{13}C NMR$^\#$ (75 MHz, $CDCl_3$) δ 19.1, 19.5 (CH_2, C2, C11), 21.0, 21.3, 21.8 (CH_3, C18, OCO$\underline{CH_3}$ ×°2), 30.3 (CH_2, C12), 35.5 (CH_2, C20), 36.5, 37.9 (CH_2, C1, C3), 41.4 (CH_2, C14), 44.7, 47.4 (C, C4, C10), 50.1 (CH_2, C15), 51.5 (CH, C6), 51.7 (CH_3, $CO_2\underline{CH_3}$), 52.0 (C, C8), 57.0 (CH, C9), 60.7 (CH, C5), 79.4 (C, C16), 79.5 (CH, C19), 84.0 (C, C13), 170.2, 171.2 (C, O$\underline{C}OCH_3$), 173.3 (C, C7), 193.6 (C, C17). IR ν_{max} (cm^{-1}): 2950, 2874, 1732, 1240. MS (EI) *m/z* 524 (M^+–1, 6%), 445 (66), 436 (60), 416 (30), 385 (100), 374 (32), 357 (40), 347 (98), 325 (58), 287 (84), 265 (54), 237 (42), 227 (52), 105 (32), 91 (36). HRMS (EI) *m/z* calcd for M^+–Br, $C_{25}H_{33}O_7$: 445.2226; found: 445.2231. (b) A solution of Ti(IV)Cp_2Cl_2 (246 mg, 0.987 mmol) and zinc (77.4 mg, 1.18 mmol) in THF (3.1 mL) was stirred at room temperature for 2 hours and then cooled to –10°C and stirred for 30 minutes. This was then transferred *via* cannula into a solution of the α-epoxide (176 mg, 0.394 mmol) in THF (3.9 mL) also at –10°C, and the reaction was monitored by TLC analysis. After 20 minutes PHT (585 mg, 1.18 mmol) was added. The reaction mixture was then allowed to warm to room temperature and stirred for 30 minutes. By this time the reaction was complete by TLC analysis and the reaction mixture was poured onto ice cold HCl (2M aqueous solution, 10 mL) layered with EtOAc (10 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL) and the combined organic extracts were washed with H_2O (20 mL), $Na_2S_2O_3$ (saturated aqueous solution, 20 mL), $NaHCO_3$ (saturated aqueous solution, 10 mL) and brine. After being dried over $MgSO_4$ and filtered, the solvent was removed under reduced pressure and the residue chromatographed

(silica gel, EtOAc/petrol 1:2) which afforded the desired mixture of bromo aldehydes **21** and 16-epi-**21** (4:1, 148.0 mg, 71%) followed by the alcohol (11.4 mg, 6%) corresponding to aldehyde **20**. R_f 0.4 (EtOAc/petrol 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (3H, s, H18), 0.92 (1H, dq, J = 12.9, 2.3 Hz, H11), 1.13 (1H, dd, J_{gem} = 14.8 Hz, J = 2.6 Hz, H15), 1.19–1.30 (2H, m), 1.44–1.72 (8H, m), 1.81 (1H, d, $J_{5,6}$ = 11.2 Hz, H5), 1.93–2.08 (3H, m), 1.98 (3H, s, OCO$\underline{CH_3}$), 2.02 (3H, s, OCO$\underline{CH_3}$), 2.20–2.36 (3H, m, H16 + 2), 2.62 (1H, d, $J_{6,5}$ = 12.0 Hz, H6), 3.52 (1H, dd, J_{gem} = 11.2 Hz, $J_{17,16}$ = 6.0 Hz, H17), 3.65 (3H, s, $CO_2\underline{CH_3}$), 3.77 (1H, dd, J_{gem} = 12.0 Hz, $J_{17,16}$ = 9.3 Hz, H'17), 4.7 (1H, br s, O$\underline{H}$), 4.85 (1H, dd, J = 10.7, 3.8 Hz, H19). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 19.6 (CH_2, C2, C11), 21.0, 21.9, 22.1 (CH_3, C18, OCO$\underline{CH_3}$ × 2), 27.6 (CH_2, C12), 35.6 (CH_2, C20), 36.8, 38.2 (CH_2, C1, C3), 39.6 (CH_2, C14), 43.6 (CH_2, C15), 44.8 (C), 48.2 (CH, C16), 49.8 (C), 51.3 (CH, C6), 51.6 (CH_3, $CO_2\underline{CH_3}$), 52.3 (C, C8), 57.2 (CH, C9), 60.9 (CH, C5), 63.2 (CH_2, C17), 79.6 (CH, C19), 86.6 (C, C13), 171.1, 171.3 (C, O$\underline{C}OCH_3$), 173.9 (C, C7). IR ν_{max} (cm^{-1}): 3451, 2950, 1731, 1574. MS (EI) *m/z* 448 (M^+, 2%), 388 (90), 347 (61), 328 (71), 287 (100), 269 (36), 227 (71). HRMS (EI) *m/z* calcd for M^+–60, $C_{23}H_{32}O_5$: 388.2250; found: 388.2250.

Methyl *ent*-16β-bromo-13,19-diacetoxy-17-hydroxy-19,20-cyclogibberellan-7-oate (22). Bromo aldehyde mixture **21** plus 16-epi-**21** (1.04 g, 1.98 mmol) was taken up in DME (50 mL) and the solution cooled to 0°C. Finely ground $NaBH_4$ (112.3 mg, 2.97 mmol) was added and the suspension stirred for 30 minutes by which time TLC analysis indicated that all of the starting material had been consumed. The reaction mixture was diluted with EtOAc (50 mL) and HCl (1M aqueous solution) was added in a dropwise fashion until all of the remaining $NaBH_4$ was quenched. The aqueous phase was re-extracted with EtOAc (2 × 100 mL) and the combined organic phases were washed with H_2O (3 × 100 mL), brine and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the crude matarial was chromatographed (silica gel, EtOAc/petrol 1:2) which afforded a 6:1 mixture of the bromo alcohol **22** with its 16-epimer (0.92 g, 64%). R_f 0.4 (EtOAc/petrol 1:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81 (3H, s, H18), 1.27–1.31 (5H, m), 1.44–1.71 (7H, m), 1.84–2.10 (4H, m), 2.03 (3H, s, OCO$\underline{CH_3}$), 2.05 (3H, s, OCO$\underline{CH_3}$), 2.16–2.29 (2H, m), 2.35 (1H, d, $J_{5,6}$ = 12.3 Hz, H5), 2.45 (1H, d, J_{gem} = 11.4 Hz, H14), 2.68 (1H, d, $J_{6,5}$ = 12.0 Hz, H6), 3.70 (4H, s, $CO_2\underline{CH_3}$ + H17 partly obscured), 3.99 (1H, d, J_{gem} = 12.9 Hz, H'17), 4.87 (1H, dd, J = 10.4, 3.9 Hz, H19). IR ν_{max} (cm^{-1}): 3450, 1730, 1574.

Methyl *ent*-16β-bromo-13,19-diacetoxy-12α,17-epoxy-19,20-cyclogibberellan-7-oate (23). The mixture of bromo alcohols **22** (662.9 mg, 1.26 mmol) was taken up in DCM (10 mL) and benzene (30 mL) followed by the addition of $PhI(OAc)_2$ (1.22 g, 3.77 mmol) and I_2 (0.894 g, 3.52 mmol), then subjected to photolysis (250W tungsten lamp) for 45 minutes. The reaction mixture was diluted with EtOAc (50 mL) and washed with $Na_2S_2O_3$ (saturated aqueous solution, 50 mL). The aqueous phase was re-extracted with EtOAc (2 × 50 mL) and the combined organic extracts were washed with H_2O (100 mL), brine, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure and any residual benzene was azeotroped with MeOH. The residue was chromatographed (silica gel, EtOAc/petrol 1:2) to afford the desired cyclic bromo

ether **23** (447.1 mg, 81% yield based on pure **22**) as a solid. R_f 0.8 (EtOAc/petrol 1:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (3H, s, H18), 1.12–2.18 (11H, m), 1.92 (1H, d, J_{gem} = 14.7 Hz, H15), 2.05 (3H, s, OCO$\underline{CH_3}$), 2.09 (3H, s, OCO$\underline{CH_3}$), 2.23–2.35 (3H, m), 2.54 (1H, J_{gem} = 11.5 Hz, H14), 2.68 (1H, d, $J_{6,5}$ = 11.6 Hz, H6), 3.70 (3H, s, $CO_2\underline{CH_3}$), 3.86 (2H, s, H17, H'17), 4.38 (1H, d, J = 2.6 Hz, H12), 4.93 (1H, dd, J = 10.6, 3.8 Hz, H19). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.4 (CH_2, C2), 21.0, 21.3, 21.6 (CH_3, C18, OCO$\underline{CH_3}$ × 2), 26.2 (CH_2, C11), 35.6, 36.6, 37.8, 38.3 (CH_2, C1, C3, C14, C20), 44.8, 48.9 (C, C4, C10), 51.6 (C, C16), 51.6 (CH_3, $CO_2\underline{CH_3}$), 51.7, 52.4 (CH, C6, C9), 55.0 (CH_2, C15), 61.5 (CH, C5), 65.6 (C, C8), 75.9 (CH_2, C17), 79.5 (CH, C19), 83.2 (CH, C12), 87.9 (C, C13), 170.2, 171.0 (C, O$\underline{C}OCH_3$), 173.6 (C, C7). IR ν_{max} (cm^{-1}): 1730. MS (EI) *m/z* 464 (M^+–60, 64%), 445 (M–Br^+, 10), 385 (100), 265 (30). HRMS (EI) *m/z* calcd for M^+–Br, $C_{25}H_{33}O_7$: 445.2226; found: 445.2226.

Methyl *ent*-13,19-diacetoxy-12α-hydroxy-19,20-cyclogibberell-16-en-7-oate (24). A suspension of activated zinc (1.02 g, 15.7 mmol) in a solution of the cyclic bromo ether **23** (349.9 mg, 0.666 mmol) in DMF (6 mL) was cooled to 0°C. This was followed by dropwise addition of HCl (5M aqueous solution, 9 mL) and the mixture stirred for 70 minutes. The reaction mixture was then diluted with EtOAc (50 mL) and washed with H_2O (50 mL). The aqueous phase was re-extracted with EtOAc (2 × 50 mL) and the combined organic extracts were washed with H_2O (20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, EtOAc/petrol 1:2) to afford the desired alcohol **24** (255.5 mg, 86%) as a white amorphous solid. R_f 0.2 (EtOAc/petrol 1:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (3H, s, H18), 1.17 (1H, dm, J_{gem} = 13.2 Hz, H14), 1.20–1.34 (2H, m), 1.53–1.96 (9H, m), 1.97–2.16 (3H, m), 2.04 (6H, s, OCO$\underline{CH_3}$ × 2), 2.20–2.28 (2H, m), 2.68 (1H, d, $J_{6,5}$ = 12.1 Hz, H6), 3.68 (3H, s, CO$\underline{CH_3}$), 4.38 (1H, d, J = 6.6 Hz, H12), 4.87 (1H, dd, J = 10.6, 3.9 Hz, H19), 5.21 (1H, br s, H17), 5.24 (1H, br s, H'17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.6 (CH_2, C2), 21.0, 21.8, 21.9 (CH_3, C18, OCO$\underline{CH_3}$ × 2), 30.5 (CH_2, C11), 35.5, 36.9, 38.0 (CH_2, C1, C3, C20), 41.8 (CH_2, C14), 43.9 (CH_2, C15), 44.9, 49.3 (C, C4, C10), 50.4 (CH, C6), 51.6 (CH_3, $CO_2\underline{CH_3}$), 51.8 (CH, C9), 51.9 (C, C8), 61.4 (CH, C5), 71.9 (CH, C12), 79.5 (CH, C19), 89.5 (C, C13), 112.8 (CH_2, C17), 145.8 (C, C16), 170.7, 171.2 (C, O$\underline{C}OCH_3$), 173.5 (C, C7). IR ν_{max} (cm^{-1}): 3515, 2950, 1732, 1660, 1240. MS (EI) *m/z* 446 (M^+, 3%), 404 (100), 386 (60), 326 (30). HRMS (EI) *m/z* calcd for M^+, $C_{25}H_{34}O_7$: 446.2305; found: 446.2303.

Methyl *ent*-13,19-diacetoxy-12α-tetrahydropyranyloxy-19,20-cyclogibberell-16-en-7-oate. A solution of alcohol **24** (163 mg, 0.365 mmol) in DCM (5 mL) was treated with dihydropyran (100 μL, 1.09 mmol) and pyridinium *p*-toluene sulfonate (a few crystals). The mixture was stirred at room temperature overnight. Although TLC analysis indicated that some starting material was still present the reaction mixture was diluted with ether (50 mL) and washed with brine. The aqueous phase was extracted with ether (2 × 10 mL) and the combined organic phases were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The residue was chromatographed (silica gel, EtOAc/petrol 1:3) with the desired protected alcohol eluting first (141.3 mg, 90%) as a white amorphous solid, followed by recovered starting material

(26 mg). R_f 0.4 (EtOAc/petrol 1:3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (6H, s, H18), 1.11–2.19 (37H, m), 1.99 (6H, s, OCO$\underline{CH_3}$), 2.03 (6H, s, OCO$\underline{CH_3}$), 2.45 (1H, t, J = 7.3 Hz), 2.65 (1H, d, $J_{6,5}$ = 11.8 Hz, H6), 2.66 (1H, $J_{6,5}$ = 12.1 Hz, H6), 3.65 (6H, s, $CO_2\underline{CH_3}$), 3.33–4.00 (10H, m, THP), 4.54 (2H, d, J = 6.0 Hz, H12), 4.67 (1H, br s, THP-H1), 4.72 (1H, br s, THP-H1'), 4.87 (2H, dd, J = 10.4, 3.4 Hz, H19), 5.03 (1H, s, H17), 5.12 (1H, s, H'17), 5.18 (1H, s, H17), 5.32 (1H, s, H'17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7 (CH_2, C2), 18.9 (CH_2, C2), 19.4 (CH_2), 19.5 (CH_2, × 2), 20.9 (CH_3 × 2), 21.8 (CH_3 × 2), 21.9 (CH_3), 22.0 (CH_3), 25.3 (CH_2, C11), 25.5 (CH_2, C11), 27.6 (CH_2), 29.4 (CH_2), 30.4 (CH_2), 30.5 (CH_2), 35.5 (CH_2), 36.8 (CH_2), 37.0 (CH_2), 38.0 (CH_2), 41.9 (CH_2), 43.3 (CH_2), 43.4 (CH_2), 44.9 (CH_2), 49.6 (C), 49.8 (C), 50.2 (CH), 50.4 (CH), 51.5 (CH_3, CO_2CH_3 × 2), 51.9 (C), 52.0 (CH), 52.1 (C), 52.4 (CH), 60.7 (CH_2), 61.3 (CH), 61.4 (CH), 62.1 (CH_2, THP), 62.6 (CH_2, THP), 66.8 (CH_2), 73.8 (CH, C12), 76.5 (CH, C12), 79.5 (CH, C19 × 2), 87.5 (C), 88.9 (C), 94.5 (CH, THP-C1), 99.3 (CH, THP-C1'), 111.5 (CH2, C17), 112.5 (CH_2, C17), 145.3 (C, C16), 169.6 (C, $OCOCH_3$), 169.8 (C, $OCOCH_3$), 171.0 (C, $OCOCH_3$ × 2), 173.5 (C, C7), 173.6 (C, C7). IR ν_{max} (cm^{-1}): 2945, 1737, 1663, 1239, 1044, 1032, 1021, 755. MS (EI) *m/z* 530 (M^+, 4%), 488 (20), 460 (24), 386 (28), 85 (100). HRMS (EI) *m/z* calcd for M^+, $C_{30}H_{42}O_8$: 530.2880; found: 530.2888.

Methyl *ent*-13,19-dihydroxy-12α-tetrahydropyranyloxy-19,20-cyclogibberell-16-en-7-oate. A suspension of the THP ether prepared above (141.3 mg, 0.266 mmol) and K_2CO_3 (anhydrous, 438 mg, 3.17 mmol) in MeOH (anhydrous, 6 mL) was stirred at room temperature for 48 hours. The reaction mixture was diluted with EtOAc (10 mL) and HCl (1M aqueous solution, 3 mL) was added and the mixture stirred for 30 minutes. This was followed by extraction with EtOAc (3 × 30 mL) and the combined organic phases were washed with brine and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was then subjected to chromatography (silica gel, EtOAc/petrol 3:1) which afforded the title diol (82.6 mg, 70%) as a white amorphous solid. R_f 0.4 (EtOAc/petrol 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (6H, s, H18), 1.15–1.41 (5H, m), 1.48–1.91 (28H, m), 1.94–2.23 (10H, m), 2.60 (1H, d, $J_{6,5}$ = 11.8 Hz, H6), 2.61 (1H, d, $J_{6,5}$ = 11.8 Hz, H6), 3.46–3.52 (3H, m, THP), 3.65 (6H, s, $CO_2\underline{CH_3}$), 3.83–3.89 (2H, m, THP), 3.98–4.12 (6H, H19 + THP), 4.21 (1H, d, J = 5.7 Hz, H12), 4.38 (1H, d, J = 5.6 Hz, H12), 4.85 (2H, m, THP-H1, THP-H1'), 5.12 (2H, s, H17, H'17), 5.16 (1H, s, H17), 5.24 (1H, s, H'17). IR ν_{max} (cm^{-1}): 3500, 1730, 1660. MS (ES) *m/z* 468 ($M+Na^+$, 100%), 446 (M^+, 100). HRMS (EI) *m/z* calcd for M^+, $C_{26}H_{38}O_6$: 446.2668; found: 446.2669.

Methyl *ent*-13-hydroxy-12α-tetrahydropyranyloxy-19-oxo-19,20-cyclogibberell-16-en-7-oate. A solution of the diol prepared above (82.6 mg, 0.191 mmol) in DCM (4 mL) was treated with Dess–Martin periodinane (89 mg, 0.23 mmol). After 1 hour the reaction was complete, so the reaction mixture was diluted with ether (10 mL) and treated with NaOH (1M aqueous solution, 5 mL) and stirred for a further 10 minutes. The entire mixture was extracted with ether (3 × 10 mL) and the combined organic extracts were washed with NaOH (1M aqueous solution, 10 mL), H_2O (10 mL) and brine (10 mL). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to afford ketone **25** (66.7 mg, 81%) as a white amorphous solid. R_f 0.6 (EtOAc/petrol 3:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (6H, s, H18),

1.31–2.09 (38H, m), 2.20–2.38 (7H, m), 3.41–3.51 (4H, m, THP), 3.65 (6H, s, $CO_2\underline{CH_3}$), 3.83–3.89 (1H, m, THP), 4.00–4.08 (2H, m, THP), 4.23 (1H, t, *J* = 4.4 Hz, H12), 4.38 (1H, d, *J* = 5.9 Hz, H12), 4.82 (1H, m, THP-H1), 4.95 (1H, s, THP-H1'), 5.15 (2H, s, H17, H'17), 5.20 (1H, s, H17), 5.27 (1H, s, H'17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.0 (CH_3, C18 × 2), 19.7 (CH_2 × 2), 20.3 (CH_2), 21.9 (CH_2), 24.9 (CH_2, C11), 25.3 (CH_2, C11), 29.9 (CH_2), 30.0 (CH_2), 30.6 (CH_2), 31.3 (CH_2), 36.4 (CH_2 × 2), 37.9 (CH_2 × 2), 43.4 (CH_2 × 2), 43.9 (CH_2), 44.2 (CH_2), 44.6 (CH_2), 45.8 (CH_2), 48.9 (C), 49.0 (C), 49.1 (C), 51.5 (C), 51.7 (CH; CH_3, $CO_2\underline{CH_3}$), 51.8 × 2 (CH; CH_3, $CO_2\underline{CH_3}$), 51.9 (CH), 52.0 (CH), 53.7 (C × 2), 59.5 (CH), 59.6 (CH), 63.6 (CH_2, THP), 66.0 (CH_2, THP), 79.2 (C, C13), 79.7 (CH, C12), 82.2 (C, C13), 84.8 (CH, C12), 100.5 (CH, THP-C1), 101.9 (CH, THP-C1'), 111.8 (CH_2, C17), 113.2 (CH_2, C17), 148.8 (C, C16), 149.5 (C, C16), 173.2 (C, C7), 173.3 (C, C7), 220.0 (C, C19), 220.1 (C, C19). IR ν_{max} (cm^{-1}): 3540, 1750, 1730, 1660. MS (ES) *m/z* 483 ($M+K^+$, 10%), 467 ($M+Na^+$, 73), 445 ($M+H^+$, 52). HRMS (EI) *m/z* calcd for M^+, $C_{26}H_{36}O_6$: 444.2512; found: 444.2529.

***ent*-12α-Tetrahydropyranyloxy-13,20,20-trihydroxygibberell-16-en-7,19-dioic acid 19,20-lactone 7-methyl ester (26).** A solution of ketone **25** (46.8 mg, 1.05 mmol) and dry KH (large excess, prepared by washing 3 × with petrol 60–80°C while under nitrogen and then putting under vacuum for 10 minutes) in DMF (dry, 2.7 mL) was degassed by evacuating under high vacuum and re-filling with N_2 (3×). THF (2.7 mL) was added and the mixture was cooled to 0°C and stirred for 2 hours. The mixture was then degassed by evacuating under high vacuum and re-filling with N_2 (3×) before O_2 was bubbled through the reaction mixture for 1 hour. The reaction was purged with N_2, then quenched with MeOH (5 mL) and the solvent was removed under reduced pressure. The residue was taken up in EtOAc (10 ml), washed with H_2O (2 × 10 mL) and the aqueous layer then re-extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with K_2PO_4 (20% aqueous solution, 10 mL), brine, and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure to afford hydroxy lactone **26** (48.3 mg, 97% yield, crude) which was characterized as the dimethyl ester of the tautomeric aldehyde. A portion of **26** (20 mg) was taken up in MeOH and treated with CH_2N_2 (1 mL) for 30 minutes. The excess CH_2N_2 was evaporated under a stream of N_2 and the residue was chromatographed (silica gel, EtOAc/petrol 1:1) which afforded the desired dimethyl ester 181 (9.0 mg). R_f 0.66, 0.60 (EtOAc/petrol 1:1). 1H NMR (300 MHz, CDCl3) δ 1.10 (3H, s, H18), 1.12 (3H, s, H18), 0.85–2.43 (36, m), 3.51 (3H, m, THP), 3.60–3.78 (5H, m, THP), 3.68 (3H, s, $COCH_3$), 3.69 (3H, s, CO_2CH_3), 3.73 (6H, s, CO_2CH_3), 3.89 (3H, m, THP), 4.02 (2H, dm, J = 11.7 Hz, THP), 4.35 (1H, d, J = 7.6 Hz, H12), 4.71 (2H, m, THP-H1, THP-H1'), 5.10 (3H, s, 2 × H17, H'17), 5.17 (1H, s, H'17), 9.75 (1H, s, CHO), 9.78 (1H, s, CHO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.0 (CH_2), 20.7 (CH_2), 20.8 (CH_2), 21.8 (CH_2), 24.8 (CH_2), 25.2 (CH_2), 26.9 (CH_2), 27.5 (CH_3, C18), 27.6 (CH_2), 27.7 (CH_3, C18), 30.7 (CH_2), 31.6 (CH_2), 33.1 (CH_2), 33.3 (CH_2), 37.5 (CH_2), 37.6 (CH_2), 43.3 (C, C4), 43.5 (C, C4), 44.6 (CH_2), 44.8 (CH_2), 48.1 (CH_2), 48.3 (CH_2), 48.4 (C), 48.5 (C), 49.0 (CH), 49.1 (CH), 51.7 (CH_3, $CO_2\underline{CH_3}$ × 2), 51.8 (CH_3, $CO_2\underline{CH_3}$ × 2), 56.9 (CH), 57.2 (CH), 57.2 (CH), 57.4 (CH), 60.0 (C), 60.3 (C), 63.9 (CH_2, THP), 66.1 (CH_2, THP), 77.8 (C, C13), 79.2 (C, C13), 80.9 (CH, C12), 84.7 (CH, C12), 100.7 (CH, THP-C1),

102.0 (CH, THP-C1'), 109.9 (CH_2, C17), 110.6 (CH_2, C17), 148.7 (C, C16), 149.0 (C, C16), 175.3 (C, O<u>C</u>OCH_3 × 2), 176.3 (C, C7), 176.4 (C, C7), 205.1 (CH, C20), 205.3 (CH, C20). IR ν_{max} (cm^{-1}): 3500, 1730, 1660. MS (EI) *m/z* 492 (M^+, 6%), 462 (17), 434 (53), 419 (63), 402 (30), 372 (55), 344 (65), 300 (51), 97 (100), 62 (38). HRMS (EI) *m/z* calcd for M^+–CH_3OH, $C_{26}H_{36}O_7$: 460.2461; found: 460.2461.

***ent*-13,20-Dihydroxy-12α-tetrahydropyranyloxygibberell-16-ene-7,19-dioic acid 19,20-lactone 7–methyl ester.** A solution of hydroxy lactone **26** (10 mg, 0.0210 mmol) in anhydrous dimethoxyethane (0.5 mL, distilled from CaH_2) was cooled to 0°C. Finely ground $NaBH_4$ (7.0 mg, 0.071 mmol) was added and the mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (10 mL), washed with KH_2PO_4 (20% aqueous solution, 5 mL), brine and dried over $MgSO_4$. Following filtration, the solvent was removed under reduced pressure and the residue was chromatographed (silica gel, EtOAc/petrol 2:1) to afford the 12-THP-ether of lactone **27** (5.4 mg, 56%). R_f 0.3 (EtOAc/petrol 2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (6H, s, H18), 1.18–2.30 (36H, m), 2.74 (1H, d, $J_{6,5}$ = 12.6 Hz, H6), 2.76 (1H, d, $J_{6,5}$ = 12.5 Hz, H6), 3.52 (3H, t, J = 4.1 Hz, THP), 3.69 (6H, s, CO_2<u>CH_3</u>), 3.88 (3H, m, THP), 3.98 (2H, m, THP), 4.08 (1H, d, J_{gem} = 12.2 Hz, H20-pro*S*), 4.09 (1H, d, J_{gem} = 12.2 Hz, H20 pro-*S*), 4.17 (2H, m, THP), 4.35 (2H, d, J_{gem} = 12.1 Hz, H20-pro*R*), 4.39 (2H, t, J = 6.1 Hz, H12), 4.80 (1H, m, THP-H1), 5.00 (1H, s, THP-H1'), 5.16 (2H, s, H17, H'17), 5.21 (1H, s, H17), 5.28 (1H, s, H'17). IR ν_{max} (cm^{-1}): 3500, 1730, 1720, 1660. MS (EI) *m/z* 460 (M^+, 3%), 149 (22), 85 (44), 62 (100). HRMS (EI) *m/z* calcd for M^+, $C_{26}H_{36}O_7$: 460.2461; found: 460.2468.

***ent*-12α,13,20-Trihydroxygibberell-16-ene-7,19-dioic acid 19,20-lactone 7-methyl ester (27).** The lactone prepared above (4.1 mg, 0.010 mmol) was dissolved in THF (667 μL), H_2O (167 μL), AcOH (167 μL) and the solution was heated at 62°C for 7 hours. Ether (5 mL) was added and the entire mixture was washed with brine. The aqueous phase was re-extracted with ether (2 × 5 mL) and the combined organic extracts were washed with $NaHCO_3$ (saturated aqueous solution, 5 mL) and brine. The organic phase was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure and the residue was chromatographed (silica gel, EtOAc/petrol 2:1) to afford the desired diol **27** (0.2 mg). R_f 0.1 (EtOAc/petrol 2:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.12 (3H, s, H18), 1.27–1.34 (2H, m), 1.46–1.79 (6H, m), 1.81 (1H, dd, J = 12.0, 2.7 Hz, H11), 1.85 (1H, dm, J_{gem} = 11.6 Hz, H14α), 1.92 (1H, d, J_{gem} = 11.6 Hz, H14β), 1.93–2.10 (3H, m), 2.13 (1H, dt, J_{gem} = 16.2 Hz, J = 2.9 Hz, H15α), 2.20 (1H, d, $J_{5,6}$ = 12.3 Hz, H5), 2.27 (1H, dm, J_{gem} = 16.0 Hz, H15β), 2.77 (1H, d, $J_{6,5}$ = 12.3 Hz, H6), 3.71 (6H, s, CO_2<u>CH_3</u>), 4.10 (2H, m, H12; d, J_{gem} = 11.3 Hz, H20-pro*S*), 4.35 (1H, d, J_{gem} = 12.3 Hz, $J_{20,1\beta}$ = 2.4 Hz, H20-pro*R*), 5.27 (1H, d, J = 1.9 Hz, H17), 5.28 (1H, d, J = 1.3 Hz, H'17). IR ν_{max} (cm^{-1}): 3500, 1730, 1660. MS (EI) *m/z* 376 (M^+, 70%), 344 (56), 316 (68), 286 (51), 253 (35), 167 (45), 121 (89), 91 (100). HRMS (EI) *m/z* calcd for M^+, $C_{21}H_{28}O_6$: 376.1886; found: 376.1882.

***ent*-12α,13-Dihydroxy-20-oxogibberell-16-ene-7,19-dioic acid 7,19-dimethyl ester (28).** The dimethyl ester from **26** (3.1 mg, 6.33 mmol) was dissolved in THF/H_2O/AcOH (400 μL:100 μL:100 μL) and the solution was heated at 62°C for 6 hours. The reaction mixture

was diluted with EtOAc (5 mL) and the entire mixture was washed with H_2O (5 mL). The aqueous phase was re-extracted with EtOAc (2 × 5 mL) and the combined organic extracts were washed with H_2O (2 × 5 mL) and brine. The organic phase was dried over $MgSO_4$, filtered and most of the solvent removed under reduced pressure upon which H_2O (5 mL) was added and the remainder of the organic solvent removed under reduced pressure and the resulting solution was freeze dried. The desired aldehyde **28** was obtained as a white solid (3.5 mg) and the ^{1}H NMR spectrum was identical to that previously reported. MS (EI) *m/z* 406 (M^+, 43%), 328 (22), 300 (24), 121 (25). HRMS (EI) *m/z* calcd for M^+, $C_{22}H_{30}O_7$: 406.1992; found: 406.1987.

Acknowledgements

We are grateful to Bruce Twitchin for technical support.

References and Notes

¶ Unsaturated Aldehyde 19
§ Epoxy Aldehyde
* Major Diastereomer
† Minor Diastereomer
# Peaks associated with the Major Diastereomer

1. Blake, P. S.; Taylor, D. R.; Crisp, C. M.; Mander, L. N.; Owen, D. J. *Phytochemistry* **2000**, *55*, 887.
2. Mander, L. N.; Owen, D. J. *Tetrahedron* **1997**, *53*, 2137.
3. Chu, A.; Mander, L. N. *Tetrahedron Lett.* **1998**, *29*, 2727.
4. Dawe R. D., Mander L. N.; Turner J. V. *Tetrahedron Lett.* **1985**, *26,* 363.
5. Umbreit, M. A.; Sharpless, K. B. *J. Am. Chem. Soc.* **1977**, *99*, 5526.
6. Bhaskar, K. V.; Chu, W.-L. A.; Gaskin, P.; Mander, L. N.; Murofushi, N.; Pearce, D. R.; Pharis, R. P.; Takahashi, N.; Yamaguchi, I. *Tetrahedron Lett.* **1991**, *32*, 6203.
7. Bhaskar, K. V.; Mander, L. N. *Tetrahedron Lett.* **1996**, *37*, 719.
8. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
9. Dawe, R. D.; Mander, L. N.; Turner, J. V.; Xinfu, P. *Tetrahedron Lett.* **1985**, *26*, 5725.
10. Gibberellins are assigned a number corresponding to their order of discovery according to the protocol established by MacMillan, J.; Takahashi, N. *Nature* **1968**, *217*, 170. See also Mander, L. N. *Nat. Prod. Rep.* **2003**, *20*, 49 and www.plant-hormones.bbsrc.ac.uk/gainplants/gibberellin_nomenclature.htm. Details of the isolation and structure determination for GA_{127} are reported in Nakayama, M.; Koshioka, M.; Matsui, H.; Ohara, H.; Mander, L. N.; Leitch,

S. K.; Twitchin, B; Kraft-Klaunzer, P.; Pharis, R. P.; Yokota, T. *Phytochemistry* **2001**, *57*, 749.
11. Mander L. N.; Owen D. J.; Twitchin, B. *Aust. J. Chem.* **1996**, *49*, 249. Owen, D. J.; Mander, L. N.; Gaskin, P.; MacMillan, J. *Phytochemistry* **1996**, *42*, 921. Mander, L. N.; Owen, D. J.; Croker, S. J.; Gage, D. A.; Gaskin, P.; Hedden, P.; Lewis, M. J.; Talon, M.; Zeevaart, J. A. D.; Brenner, M. L.; Sheng, C. *Phytochemistry* **1996**, *43*, 23.
12. Pearce, D. W.; Hutt, O. E.; Rood, S. B.; Mander, L. N. *Phytochemistry* **2002**, *59*, 679.
13. MacMillan, J. *Nat. Prod. Rep.* **1997**, *14*, 221.
14. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.