Synthesis and bioactivity of the gibberellin, 18-hydroxy-GA_1 (GA_{132})†

Ellen Morrison,[a] Peter M. Chandler,[b] Regan J. Thomson[a] and Lewis N. Mander*[a]

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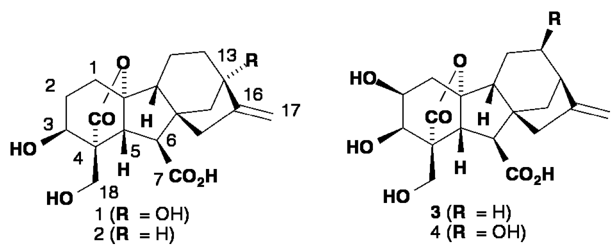
As part of a study to confirm putative structural assignments to new gibberellins and to furnish sufficient quantities for biological investigations, a twenty step synthesis of 18-hydroxy GA_1 from gibberellic acid (GA_3) is described, allowing the confirmation of structure for a new gibberellin, GA_{132}, that occurs in developing grains of barley (*Hordeum vulgare*). The early part of the sequence involved cleavage of the C(3)–C(4) bond in the A-ring of a 3-oxo intermediate. The ring was then reformed as part of a "domino" process involving the conjugate addition of alkoxide to an α-methylene lactone moiety followed by an intramolecular aldol reaction. The bioactivities of the new GA, and its 18-hydroxy-GA_4 relative, have been confirmed in dwarf barley growth and α-amylase induction assays.

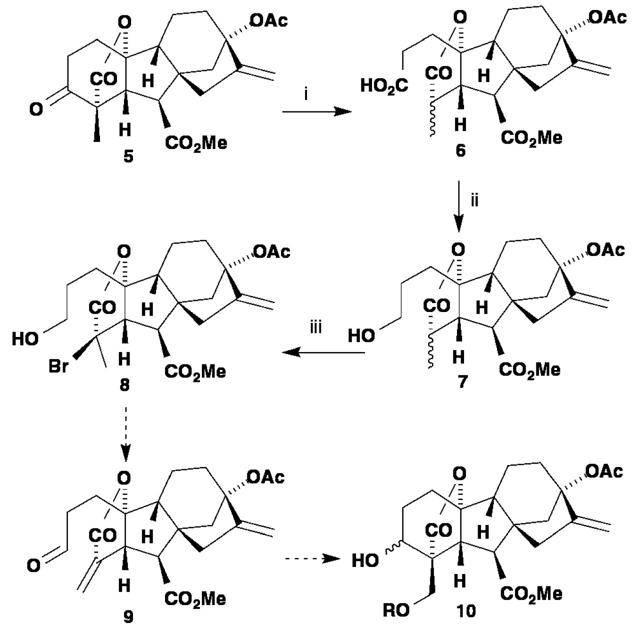
Introduction

Gibberellins ("GAs") in which the 18-methyl group has undergone oxidation have been isolated from immature seeds of the sword bean (*Canavalia gladiata*)[1] and from both immature and germinated barley grain (*Hordeum vulgare*). GAs from the latter species were identified as putative 18-OH derivatives of GA_1 (**1**), GA_4 (**2**), GA_{34} (**3**) and GA_{48} (**4**)[2,3] on the basis of GC-MS spectral comparisons and KRIs[4] of reference compounds that were derived from metabolic feeds of 18-hydroxy GA_{12} to the fungus *Gibberella fujikuroi* B1–41a.[5] We have recently confirmed the identity of 18-hydroxy GA_4 by synthesis[6] and have now embarked upon the preparation of 18-hydroxy GA_1 with a view to confirming both the identity of the endogenous material and providing sufficient material to assess its bioactivity.



Results and discussion

Our first attempt to prepare 18-hydroxy-GA_1 followed the same strategy that we had employed in the synthesis of 18-hydroxy-GA_4[6] and is outlined in Scheme 1. Initially, we employed ketone **5**,[7] and although the preparation of **6** proceeded smoothly, yields for subsequent steps en route to the ene-lactone **9** were less than satisfactory (for details see experimental section).



Scheme 1 *Reagents*: i, NaOH, H_2O–THF; ii, EtOCOCl, Et_3N; $NaBH_4$; iii, LDA; CBr_4.

We suspected that the problems arose from our choice of acetate for the protection of the 13-hydroxyl and accordingly employed a MOM ether group instead. The preparation of an advanced 18-substituted intermediate then proceeded uneventfully and mostly in excellent yield as outlined in Scheme 2, although, as in the GA_4 series, reduction of acid **14** proved to be problematical. Thus, the A-ring double bond in **12** was removed by conjugate hydride addition following Hanson's protocol[8] and after reconstitution of the 3-oxo function to give ketone **13**, cleavage of the A-ring was readily achieved by means of a retro-Claisen reaction induced by brief treatment (3 minutes) with NaOH in aqueous THF.[9] Under these conditions, a 5 : 1 mixture of C4 epimers was obtained with the *endo*-methyl isomer predominating.[10] Attempts to reduce the 3-carboxyl by the standard treatment with $NaBH_4$ of the mixed anhydride formed from ethyl chloroformate[11] that had been

[a]*Research School of Chemistry, Institute of Advanced Studies, The Australian National University, Canberra, ACT 0200, Australia*
[b]*CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia. E-mail: mander@rsc.anu.edu.au; Fax: 61-2-61258114*

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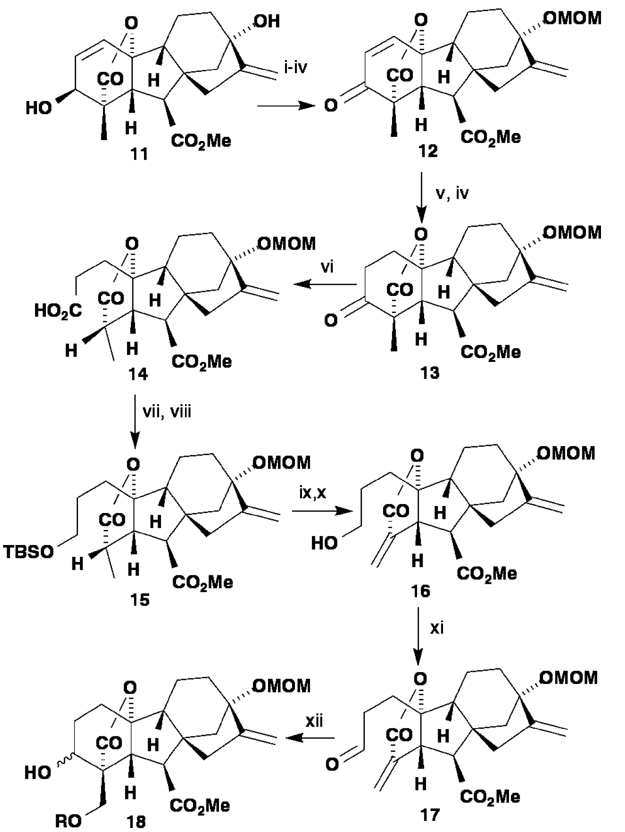
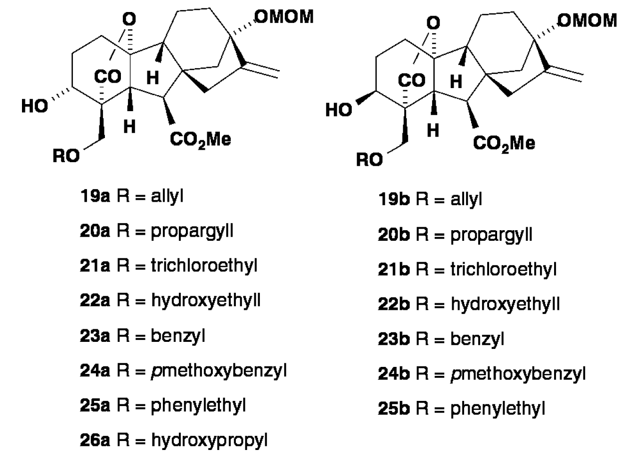


Scheme 2 *Reagents*: i, Ac_2O, Et_3N; ii, MOMCl, *i*Pr_2NEt; iii, K_2CO_3, MeOH; iv, H_2CrO_4; v, $NaBH_4$, CuCl; vi, NaOH, H_2O–THF; vii, BOP, *i*Pr_2NEt; $NaBH_4$; viii, TBSCl, imidazole, Et_3N; ix, LDA; CBr_4; x, TBAF; xi, Dess–Martin periodinane; xii, ROH, DBU.

reasonably effective in the GA_4 series proved to be unsatisfactory, as did numerous other methods; only a procedure utilizing benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate ("BOP")[12] proved to be acceptable.

To prepare **16**, it was thought to be necessary to protect the free C-3 hydroxyl, and accordingly the *tert*-butyldimethylsilyl ether **15** was formed in quantitative yield using standard conditions. Subsequent treatment with LDA followed by tetrabromomethane[13] afforded a bromolactone in excellent yield (99%) which, when treated with TBAF, afforded carbinol **16** in 62% yield, the reagent effecting both elimination of HBr and liberation of the 3-hydroxyl. Then, formation of aldehyde **17** was smoothly achieved by Dess–Martin periodinane oxidation[14] as a prelude to carrying out the desired domino transformation to **18** ($R = CH_2CH{=}CH_2$) by means of the conjugate addition of allyl alcohol followed by an aldol reaction. This process resulted in a 62% yield of a 2 : 1 mixture of the 3α-epimer with the 3β-diastereomer. Unfortunately, we could not remove the allyl group[15] from the 3β-epimer and achieved only a 40% yield with the 3α-epimer. We therefore examined a number of other alcohols that might generate a C-18 protecting group that would be more amenable to removal (Scheme 3).

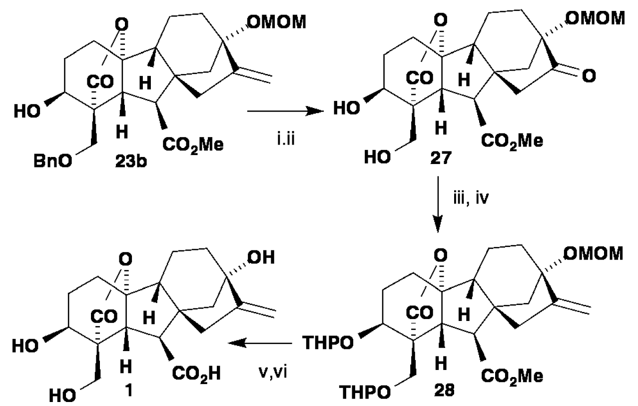
We also hoped to improve the total yield and proportion of the 3β-epimer. The outcomes for small scale experiments are displayed in Table 1. Once reproducibility and yields on a larger scale were taken into account, we decided in favour of the benzyloxy adduct,



Scheme 3 Products from alkoxide additions to aldehyde **15**.

even though the 3β : 3α ratio was unfavourable and the 16-ene function would complicate removal of the benzyl group.

We dealt with the first issue through base catalysed equilibration of the 3α-epimer which afforded a 2 : 1 mixture favouring the 3α-epimer; separation, and recycling was effected with 92% recovery for each cycle.[16] Then, as outlined in Scheme 4, **23b** was oxidised first to the 17-nor-ketone with OsO_4–$NaIO_4$[17] to allow hydrogenolytic removal of the benzyl group,[18] resulting in diol **27**.



Scheme 4 *Reagents*: i, OsO_4, $NaIO_4$; ii, H_2, $Pd(OH)_2$-C; iii, CH_2Br_2, Zn, TiCl4; iv, DHP, Py.HOTs; v, nBuSLi, HMPA; vi, Dowex (H^+).

The Δ^{16} double bond was restored by means of the Lombardo procedure,[19] the product protected as bis-tetrahydropyranyl adduct **28**, and then the ester function demethylated with lithium propanethiolate.[20] Finally, the target GA (**1**) was liberated by acidic hydrolysis with Dowex resin. Confirmation of structure for the putative 18-hydroxy-GA_1 was then established by GCMS comparison of the silylated methyl ester with that of the synthetic material and has been assigned as GA_{132}.[21]

The bioactivities of the new GA and the previously prepared 18-hydroxy-GA_4,[6] as well as their parents, GA_1 and GA_4, were compared in dwarf barley growth assays[22] and are shown in Table 2. GA_4 was about 10-fold less active than GA_1 in this bioassay, and the 18-hydroxy derivatives each had about one quarter of the activity of the parent compound.

18-OH GA_1 and 18-OH-GA_4 were tested at two concentrations in the α-amylase induction bioassay: 10^{-8} M and 10^{-9} M for GA_1 and 18-OH GA_1, and at 10^{-7} M and 10^{-8} M for the less active GA_4

Table 1 Yields of adducts from treatment of aldehyde **17** with various alkoxides

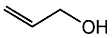
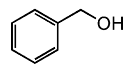
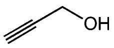
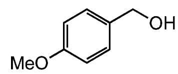
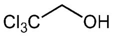
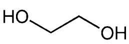
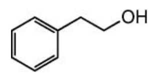
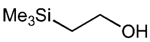
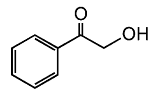
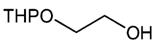
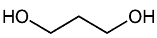
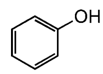
Alcohol	Yield (%)	3α : β epimer ratio	Alcohol	Yield (%)	3α : β epimer ratio
	63	2 : 1		72	2 : 1
	72	3 : 1		24	2 : 1
	57	1 : 1		58	1 : 1
	31	10 : 1			
	—				
	—				
	25	>10 : 1			

Table 2 Estimates of relative activities of different GAs in a dwarf barley leaf growth assay

Compound	$[H]_{50}$[a] (M)
GA_1	3.1×10^{-7}
GA_4	3.1×10^{-6}
18-OH GA_1	1.3×10^{-6}
18-OH GA_4	1.2×10^{-5}

[a] $[H]_{50}$ is the concentration of GA required for half-maximal stimulation of leaf elongation rate.

and 18-OH GA_4 (Fig. 1). In this assay, α-amylase is produced in the interval 2–4 days following the commencement of GA treatment.[23] At 2 d the highest α-amylase activities occurred at the highest concentrations of GA_1 or GA_4, with the 18-OH derivatives of these GAs being less active than the parent GA. There was a difference of about 10-fold in the relative activities of GA_1 and GA_4, with the former being the more active. In both cases the 18-OH derivatives have less activity than the parent compounds. At later times the 18-OH GAs had either lower or approximately equivalent activity to the parent GAs. The α-amylase induction assay is less suited than the growth assay to determining relative activities of different GAs because it requires destructive sampling and is not a linear response.

Conclusion

The methodology for introducing the 18-hydroxyl into the gibberellin skeleton has been more thoroughly explored and has allowed the structure of putative **1** to be confirmed. Also, sufficient material has been obtained to allow a preliminary examination of its biological activity. In the barley grain development mutant described by Green *et al.*[3] there were elevated levels of GA_1 and of 18-OH GA_1, associated with the production of α-amylase in the mutant grains. The probability that 18-OH GA_1 might have biological activity has now been confirmed in both leaf growth and α-amylase induction bioassays. It was not possible to make an accurate estimate of the relative amounts of GA_1 and of 18-OH GA_1 in the original study[3] because a deuterated standard is not yet available for 18-OH GA_1. However, based on its relative abundance to ^{2}H GA_8, 18-OH GA_1 may be present at up to 6 times the content of GA_1, and even though it is not as active as GA_1 in α-amylase induction, it might still constitute the major bioactive GA in such grains. GA_4 and 18-OH GA_4 were not examined in the original study, but these GAs are considerably less active than their 13-hydroxylated counterparts in both the growth assay and the α-amylase assay.

Experimental section

Bioassays

The dwarf barley growth assay is based on the maximal elongation rate of the first leaf of a GA-deficient dwarf mutant (M489) of Himalaya barley.[22] This mutant contains a single amino acid substitution in the GA 3-oxidase enzyme predominant in vegetative parts of the plant. Grains were placed in filter paper envelopes, and imbibed and germinated in 1 mM potassium phosphate buffer, pH 5.5, either without GA (control), or containing GA_3 (10 µM, which gives a near-maximal growth response), or the GAs to be tested (a range of concentrations). The concentration of GA required for half-maximal stimulation of elongation rate ($[H]_{50}$) was estimated by re-arrangement of equation [3] of Weyers *et al.*,[24] assuming that $p = 1$:

$$[H]_{50} = \frac{R_{amp} \times [H] - [H]}{R - R_{min}} \quad (1)$$

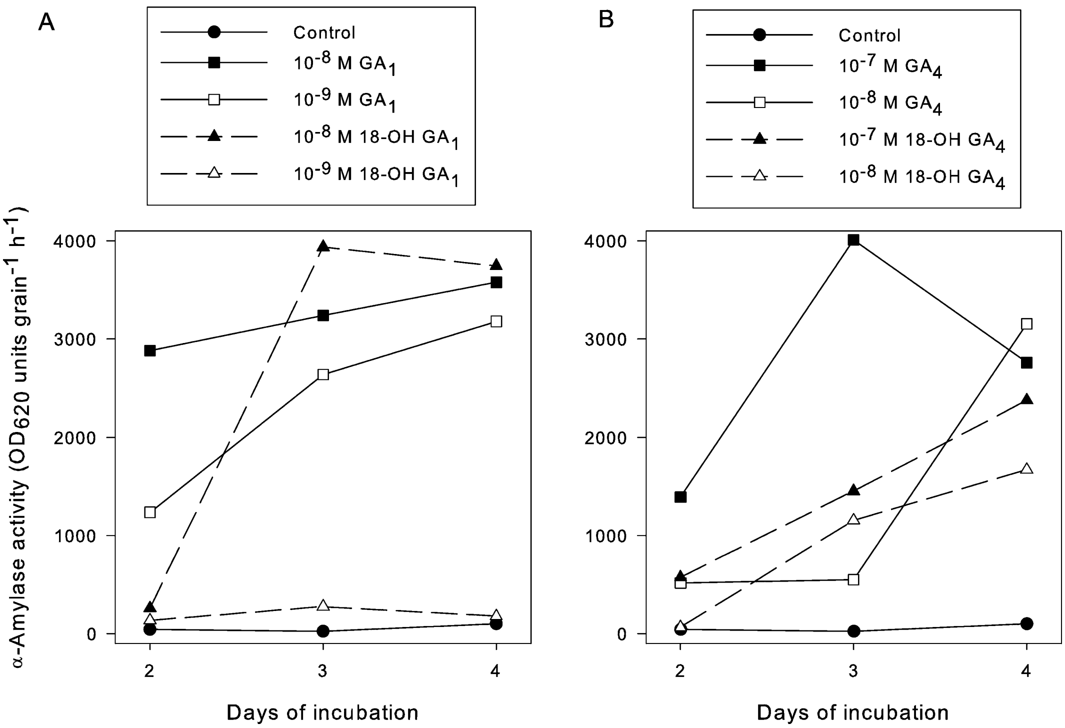


Fig. 1 Production of α-amylase by endosperm half-grains incubated with GAs at different concentrations. **A**. GA_1 and 18-OH GA_1. **B**. GA_4 and 18-OH GA_4. At the indicated time the half-grains were homogenised with the medium, and α-amylase activity assayed as described in Methods.

where R_{amp} is the amplitude of the response (response in the presence of saturating GA_3 minus the response in the absence of GA_3), R is the response for the compound being assessed for activity, R_{min} is the response on control medium, and [H] is the concentration of the compound being assessed.

The α-amylase assay using barley endosperm half-grains was utilized as previously described.[23]

Synthetic procedures

For general directions see ref. 6.

ent-13-Acetoxy-3,4-seco-20-norgibberell-16-ene-3,7-dioic acid 7-methyl ester 19,10-lactone 4-epimers (6)

To a stirred solution of **5** (2.0 g, 5.0 mmol) in THF (40.0 mL) and water (10.0 mL), was added NaOH (1.2 g, 6 equiv.). After vigorous stirring for 3 min, HCl (1 M) was added and the reaction mixture poured into a bilayer of water and EtOAc. The aqueous layer was separated, back extracted in EtOAc and the combined organic layers extracted several times with aqueous sodium bicarbonate. The combined alkaline layers were acidified with HCl (1 M) and extracted several times with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and the solvent removed *in vacuo* to afford, without further purification, a 7 : 1 (*exo*-H : *endo*-H) mixture of the C-4 epimers of **6** (1.7 g, 85%); ν_{max} (cm^{-1}): 3058, 2951, 1767, 1731, 1665, 1437, 1369, 1311, 1265, 1241, 1173, 1099, 1043, 962, 893. δ_H ($CDCl_3$) 1.21 (2.5 H, d, *J* 7.0, H-18 major epimer), 1.40 (0.5 H, d, *J* 7.6, H-18 minor epimer), 2.00 (3 H, s, OAc), 2.81 (1 H, d, *J* 2.1, H-6), 2.98 (1 H, dq, *J* 11.0, $J_{4,5}$ 11.0, *J* 7.0, H-4), 3.07 (1 H, dd, $J_{5,4}$ 11.0, $J_{5,6}$ 2.1, H-5), 3.69 (3 H, s, CO_2CH_3), 4.93 (1 H, d, *J* 1.8, H-17), 4.99 (1 H, s, H-17); *m*/*z* (EI): 420.1784 (M^+, 38% $C_{22}H_{28}O_8$ requires 420.1784), 378 (85), 360 (100), 346 (33), 332 (66), 305 (78), 290 (51), 272 (42), 227 (44), 199 (27), 171 (31), 129 (34), 105 (34), 91 (60), 79 (30).

ent-13-Acetoxy-3-hydroxy-3,4-seco-20-norgibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone 4-epimers 7

To a stirred solution of **6** (150 mg, 0.36 mmol) in THF (4.0 mL) at 0 °C, was added Et_3N (109 µL, 2.2 equiv.) and ethyl chloroformate (88 µL, 2.0 equiv.) dropwise. After 1.5 h, $NaBH_4$ (54 mg, 4.0 equiv.) was added and the mixture stirred a further 3 h after which $NaBH_4$ (92 mg, 6.85 equiv.) was added. After stirring a further 2 h, the mixture was acidified with HCl (1 M) and the reaction mixture poured into a bilayer of HCl (1 M) and EtOAc. The aqueous layer was separated and the organic layer washed several times with 1.0 M HCl. The aqueous layers were back extracted into EtOAc, and the combined organic layers washed several times with aqueous sodium bicarbonate. The alkaline aqueous layers were back extracted into EtOAc, the combined organic layers washed with brine and dried (Na_2SO_4). Concentration *in vacuo* and column chromatography on silica (50% EtOAc 50% light petroleum, increasing to 100% EtOAc) gave a 7 : 1 (*exo*-H : *endo*-H) ratio of the C-4 epimers **7** (44 mg, 30%); ν_{max} (cm^{-1}): 3443, 3058, 2949, 1764, 1732, 1664, 1437, 1369, 1311, 1241, 1202, 1173, 1100, 1050, 964, 894. δ_H ($CDCl_3$) 1.19 (2.6 H, d, *J* 7.3, H-18 major epimer), 1.38 (0.4 H, d, *J* 7.6, H-18 minor epimer), 1.99 (3 H, s, OAc), 2.79 (1 H, d, *J* 2.3, H-6), 2.98 (1 H, m, H-4), 3.08 (1 H, dd, *J* 2.4, *J* 10.9, H-5), 3.66 (2 H, t, *J* 6.3, H-3), 3.67 (3 H, s, CO_2CH_3), 4.92 (1 H, d, *J* 1.9, H-17), 4.97 (1 H, s, H-17). *m*/*z* (EI): 406.1988 (M^+, 38% $C_{22}H_{30}O_7$ requires 406.1992, M^+, 65%), 388 (10), 364

(52), 346 (93), 332 (62), 318 (88), 305 (100), 286 (67), 259 (67), 231 (67), 213 (45), 171 (53), 129 (52), 105 (57), 91 (92), 69 (70).

ent-13-Acetoxy-4α-bromo-3-hydroxy-3,4-seco-norgibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone 8

To a stirred solution of the alcohol mixture prepared above (50 mg, 0.12 mmol) in THF (2.0 mL), at −78 °C, was added LDA (0.25 M, 1.25 mL, 2.5 equiv.). The solution was stirred for 15 min before adding a THF solution (0.5 mL) of CBr_4 (102 mg, 2.5 equiv.), the reaction mixture was then stirred a further 40 min before quenching with aqueous ammonium chloride. The solution was allowed to warm to room temp and poured into a bilayer of EtOAc and water. The organic layer was separated and washed several times with water. The aqueous layers were back extracted into EtOAc, and the combined organic layers washed with brine and dried (Na_2SO_4). Concentration *in vacuo* and column chromatography on silica (20% EtOAc and 80% light petroleum, increasing to 80% EtOAc) gave the bromide as a brown oil (16 mg, 27%); ν_{max} (cm^{-1}): 3380, 2952, 2870, 1772, 1733, 1665, 1592, 1452, 1437, 1171, 1045, 984, 894. δ_H ($CDCl_3$): 1.92 (3 H, s, H-18), 2.00 (3 H, s, OAc), 2.81 (1 H, t, *J* 2.2, J_{gem} 15.5, H-15), 2.87 (1 H, d, *J* 1.9, H-6), 3.61 (1 H, d, *J* 2.0, H-5), 3.70 (2 H, t, *J* 6.3, H-3), 3.72 (3 H, s, CO_2CH_3), 4.92 (m, 1 H, H-17), 4.99 (1 H,s, H-17); *m*/*z* (EI): 484.1097 (M^+, 38% $C_{22}H_{29}O_7{}^{79}Br$ requires 406.1992, M^+, 4%), 424/426 (21), 405 (26), 387 (48), 373 (44), 359 (28), 345 (90), 331 (48), 303/301 (70), 285 (92), 273 (100), 231 (50), 213 (49), 171/169 (42), 129 (57), 105 (48), 91 (97), 79 (49), 69 (62).

ent-3α-Acetoxy-13-hydroxy-gibberella-1,16-dien-7-oic acid 7-methyl ester 19,10-lactone

To a solution of GA_3 methyl ester (**11**) (41.6 g, 0.12 mol) in CH_2Cl_2 (250 mL) cooled to 0 °C was added Et_3N (80.5 mL, 0.58 mol, 5 eq) and acetic anhydride (54.5 mL, 0.58 mol, 5 eq). The reaction was stirred overnight at room temp and then cooled to 0 °C. Water (5 mL) was added and the mixture was stirred for 15 min at 0 °C. An additional 20 mL of water was added, the ice bath removed and the reaction was stirred for 20 min. The layers were then separated and the aqueous phase was extracted with CH_2Cl_2 (×3). The organic phases were combined and washed with 1 M HCl, water, K_2CO_3 solution and brine, dried ($MgSO_4$), filtered, and the solvent evaporated. Purification by recrystallisation from ether and hexane furnished a white solid (40.4 g, 86%); mp 175–177 °C (Found: C, 65.35, H, 6.54%. $C_{22}H_{26}O_7$ requires C, 65.66; H, 6.51%); ν_{max} (cm^{-1}): 3513, 2939, 2873, 1777, 1735, 1450, 1437, 1373, 1331, 1228, 1159, 1100, 1022, 974, 896, 735; δ_H ($CDCl_3$): 1.15 (3 H, s, H-18), 1.69–2.21 (9 H, m), 2.13 (3 H, s, OAc), 2.78 (1 H, d, *J* 10.9, H-6), 3.33 (1 H, d, *J* 10.9, H-5), 3.75 (3 H, s, CO_2CH_3), 4.98 (1 H, m, H-17), 5.29 (1 H, d, *J* 1.9, H-17), 5.34 (1 H, d, *J* 3.7, H-3), 5.88 (1 H, dd, *J* 3.7, 9.3, H-2), 6.39 (1 H, dd, *J* 0.69, 9.3, H-1); δ_C ($CDCl_3$): 14.5, 17.2, 21.1, 38.4, 43.3, 44.9, 50.6, 50.7, 51.2, 52.3, 52.4, 53.6, 70.4, 78.4, 90.4, 108.0, 129.4, 134.4, 157.1, 170.3, 172.6, 177.4. *m*/*z* (EI) 402.1680 (M^+ $C_{22}H_{26}O_7$ requires 406.1679).

ent-3α-Acetoxy-13-methoxymethoxy-gibberella-1,16-dien-7-oic acid 7-methyl ester 19,10-lactone

A solution of GA_3 methyl ester 3-acetate (44.55 g, 0.11 mol) in CH_2Cl_2 (300 mL) was cooled to 0 °C. Diisopropylethylamine (58 mL, 0.33 mol, 3 eq) was added followed by dropwise addition of MOMCl (25.2 mL, 0.33 mol, 3eq). The reaction was allowed to warm to room temp and then DMAP (100 mg) was added and the reaction was stirred for 2 days at room temp. After cooling to 0 °C, sat. $NaHCO_3$ (20 mL) was added and the mixture was stirred for 15 min. The organic phase was then washed with water, cold 2 M HCl, sat. $NaHCO_3$ and brine, dried ($MgSO_4$) and the solvent removed *in vacuo* to furnish a colourless oil. No further purification was carried out. ν_{max} (cm^{-1}): 2940, 2884, 1780, 1736, 1449, 1437, 1372, 1332, 1269, 1227, 1158, 1101, 1039, 976, 895; δ_H ($CDCl_3$) 1.15 (3 H, s, H-18), 1.72–2.21 (9 H, m), 2.13 (3 H, s, OAc), 2.78 (1 H, d, *J* 10.7, H-6), 3.32 (1 H, d, *J* 10.7, H-5), 3.38 (3 H, s, MOM), 3.75 (3 H, s, CO_2CH_3), 4.55, 4.77 (2 H, 2 × ABd, *J* 7.1, MOM), 5.04 (1 H, m, H-17), 5.18 (1 H, dd, *J* 1.5, 2.7, H-17), 5.34 (1 H, dd, *J* 0.69, 3.7, H-3), 5.87 (1 H, dd, *J* 3.8, 9.3, H-2), 6.39 (1 H, dd, *J* 0.69, 9.3, H-1); δ_C NMR ($CDCl_3$) 14.5, 16.9, 21.1, 38.0, 41.2, 43.8, 50.4, 50.8, 51.0, 52.3, 52.5, 53.7, 55.6, 70.5, 83.4, 90.5, 92.2, 108.8, 129.3, 134.4, 153.4, 170.3, 172.7, 177.4; *m*/*z* (EI): 446.1943 (M^+ $C_{24}H_{30}O_8$ requires 446.1941).

ent-3α-Hydroxy-13-methoxymethoxy-gibberella-1,16-dien-7-oic acid 7-methyl ester 19,10-lactone

To a solution of the acetate prepared above (0.11 mol) in methanol (400 mL) was added a K_2CO_3–$KHCO_3$ solution (1 : 1, 110 mL, 0.22 mol, 2 eq) in 3 portions—7 min apart—and then the reaction was stirred until complete (1 h). The reaction was quenched and brought to pH 5 with cold 2 M HCl. The solvent was removed and the residue was taken up in EtOAc and water. The aqueous phase was extracted with EtOAc (×5). The combined organic phase was then washed with 10% K_2CO_3 solution and brine, dried ($MgSO_4$) and the solvent removed to furnish a white solid (43.21 g, 97% over two steps); mp 124–126 °C (Found: C, 65.20, H, 6.94%. $C_{22}H_{28}O_7$ requires C, 65.33; H, 6.98%); ν_{max} (cm^{-1}): 3457, 2949, 2884, 1775, 1734, 1451, 1377, 1331, 1269, 1251, 1197, 1160, 1102, 1039, 894, 747; δ_H ($CDCl_3$) 1.24 (3 H, s, H-18), 1.60–2.24 (9 H, m), 2.44 (1 H, br, OH), 2.80 (1 H, d, *J* 10.6, H-6), 3.22 (1 H, d, *J* 10.6, H-5), 3.37 (3 H, s, MOM), 3.74 (3 H, s, CO_2CH_3), 4.16 (1 H, d, *J* 3.7, H-3), 4.55, 4.77 (2 H, 2 × ABd, *J* 7.1, MOM), 5.04 (1 H, m, H-17), 5.17 (1 H, m, H′-17), 5.91 (1 H, dd, *J* 3.7, 9.3, H-2), 6.32 (1 H, dd, *J* 0.69, 9.3, H-1); δ_C ($CDCl_3$) 14.6, 17.0, 38.0, 41.3, 43.8, 50.6, 50.9, 51.0, 52.5, 53.1, 53.7, 55.6, 70.0, 83.5, 90.8, 92.2, 108.8, 132.7, 133.0, 153.4, 173.0, 178.8; *m*/*z* (EI): 404.1833 (M^+ $C_{22}H_{28}O_7$ requires 404.1835).

ent-13-Methoxymethoxy-3-oxo-gibberella-1,16-dien-7-oic acid 7-methyl ester 19,10-lactone (12)

To a cooled solution of alcohol prepared above (25.7 g, 0.063 mol) in acetone (300 mL) was added Jones reagent until an orange colour persisted. The excess Jones reagent was then quenched with propan-2-ol and the reaction was diluted with EtOAc. The reaction was washed with water, sat. $NaHCO_3$ (×3) and brine, dried ($MgSO_4$) and the solvent removed to furnish the ketone **12** as a white solid (24.71 g, 97%); mp 134–136 °C (Found: C, 65.28; H, 6.53%. $C_{22}H_{26}O_7$ requires C, 65.66; H, 6.51%); ν_{max} (cm^{-1}): 2950, 2883, 1782, 1733, 1696, 1439, 1380, 1318, 1269, 1260, 1197, 1149, 1093, 1037, 916, 713; δ_H ($CDCl_3$): 1.28 (3 H, s, H-18), 1.77–2.26 (9 H, m), 2.90 (1 H, d, *J* 10.3, H-6), 3.38 (3 H, s, MOM), 3.52

(1 H, d, *J* 10.3, H-5), 3.74 (3 H, s, CO_2CH_3), 4.56, 4.78 (2 H, 2 × ABd, *J* 7.1, MOM), 5.06 (1 H, m, H-17), 5.19 (1 H, dd, *J* 1.7, 3.0, H-17), 6.05 (1 H, d, *J* 9.5, H-2), 7.26 (1 H, d, *J* 9.5, H-1); δ_C ($CDCl_3$): 11.8, 17.1, 38.0, 41.7, 43.9, 50.2, 51.4, 51.8, 52.8, 55.7, 62.5, 65.2, 83.3, 89.8, 92.3, 109.1, 129.4, 147.3, 152.6, 171.8, 173.1, 191.7; *m/z* (EI) 402.1682 (M^+ $C_{22}H_{26}O_7$ requires 402.1679).

ent-3-Hydroxy-13-methoxymethoxy-gibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone 3-epimers

To a cooled solution of enone **12** (20.5 g, 0.051 mol) in methanol (1.2 L) was added CuCl (25.2 g, 0.25 mol, 5 eq) and then $NaBH_4$ (19.2 g, 0.51 mol, 10 eq) carefully in several portions (bubbles vigorously!). After $NaBH_4$ addition was complete the reaction was stirred for 20 min more and then quenched with acetic acid and filtered through Celite. The solvent was then removed *in vacuo* and the residue was taken up in EtOAc and water. The aqueous phase was extracted with EtOAc (×3) and the combined organic phase was washed with water, sat. $NaHCO_3$ (×3) and brine, dried ($MgSO_4$) and the solvent removed to furnish the alcohol as a white solid (19.96 g, 97%) which was used directly in the next step. Column chromatography on silica (EtOAc–light petroleum, 1 : 1) of a small portion (170 mg) gave the 3β-alcohol (12 mg) as a gum followed by the crystalline 3α-epimer (145 mg).

3β-epimer: δ_H ($CDCl_3$): 1.15 (3 H, s, H-18), 1.60–2.23 (13 H, m), 2.69 (1 H, d, *J* 10.1, H-6), 3.20 (1 H, d, *J* 10.1, H-5), 3.37 (3 H, s, MOM), 3.71 (3 H, s, CO_2CH_3), 3.84 (1 H, m, H-3), 4.55, 4.77 (2 H, 2 × ABd, *J* 7.2, MOM), 5.02 (1 H, br s, H-17), 5.14 (1 H, t, *J* 2.5, H-17); δ_C ($CDCl_3$): 15.0, 17.4, 27.5, 28.4, 38.2, 42.0, 43.9, 49.8, 51.6, 52.1, 52.5, 53.0, 54.8, 55.7, 70.5, 83.7, 92.2, 94.0, 108.5, 153.3, 173.2, 178.2; *m/z* (EI) 406.1991 ($C_{22}H_{30}O_7$ M^+ requires 406.1992).

3α-epimer: mp 120–122 °C; Found: C, 64.75, H, 7.59%. $C_{22}H_{30}O_7$ requires C, 65.01; H, 7.44%; ν_{max} (cm^{-1}): 3462, 2948, 2885, 1772, 1733, 1439, 1380, 1345, 1285, 1271, 1255, 1194, 1148, 1077, 1040, 899, 735; δ_H ($CDCl_3$): 1.19 (3 H, s, H-18), 1.40–2.30 (13 H, m,), 2.54 (1 H, d, *J* 9.5, H-6), 2.76 (1 H, d, *J* 9.5, H-5), 3.37 (3 H, s, MOM), 3.66 (1 H, m, H-3), 3.73 (3 H, s, CO_2CH_3), 4.54, 4.77 (2 H, 2 × ABd, *J* 7.2, MOM), 5.02 (1 H, br s, H-17), 5.13 (1 H, t, *J* 2.3, H-17); δ_C ($CDCl_3$): 13.1, 17.5, 28.0, 29.9, 30.26, 38.1, 42.3, 43.9, 51.0, 51.5, 52.6, 54.7, 55.7, 57.6, 73.4, 83.6, 92.2, 92.8, 108.5, 153.0, 173.2, 177.3;%. *m/z* (EI) 406.1995 ($C_{22}H_{30}O_7$ M^+ requires 406.1992).

ent-13-Methoxymethoxy-3-oxo-gibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone (13)

To a cooled solution of alcohols prepared above (17.9 g, 0.044 mol) in acetone (200 mL) was added Jones reagent until an orange colour persisted. The excess Jones reagent was then quenched with propan-2-ol and the reaction was diluted with EtOAc and water. The reaction was washed with water, sat. $NaHCO_3$ (×3) and brine, dried ($MgSO_4$) and the solvent removed to furnish the ketone **13** as a white solid (13.34 g, 75%). mp 79–80 °C; Found: C, 64.86, H, 6.77%. $C_{22}H_{28}O_7$ requires C, 65.33; H, 6.98%; ν_{max} (cm^{-1}): 2941, 2890, 1781, 1726, 1448, 1382, 1325, 1289, 1199, 1148, 1038, 958, 935, 916, 898, 732; δ_H ($CDCl_3$): 1.20 (3 H, s, H-18), 1.70–2.21 (10 H, m), 2.46–2.66 (3 H, m), 2.81 (1 H, d, *J* 9.1, H-6), 3.06 (1 H, d, *J* 9.1, H-5), 3.38 (3 H, s, MOM), 3.72 (3 H, s, CO_2CH_3), 4.56, 4.78 (2 H, 2 × ABd, *J* 7.2, MOM), 5.05 (1 H, br s, *J* 2.1, H-17), 5.15 (1 H, t, *J* 2.2, H-17); δ_C ($CDCl_3$): 10.7, 17.7, 28.0, 31.0, 35.0, 38.0, 42.7, 43.7, 51.6, 52.3, 52.7, 55.8, 57.8, 63.3, 83.4, 92.2, 92.8, 108.8, 152.3, 172.4, 174.0, 200.2; *m/z* (EI) 404.1838 ($C_{22}H_{28}O_7$ M^+ requires 406.1835).

ent-13-Methoxymethoxy-3,4-seco-20-norgibberell-16-ene-3,7-dioic acid 7-methyl ester 19,10-lactone (14)

To a solution of ketone **13** (0.281 g, 0.69 mmol) in THF (12 mL) was added NaOH (0.083 g, 2.08 mmol, 3 eq) in water (3 mL). The reaction was stirred vigorously for 3 min and then quenched with 1 M HCl. The reaction mixture was poured into EtOAc and water and the layers separated. The aqueous phase was extracted with EtOAc (×3) and then the combined organic phase was extracted with sat. $NaHCO_3$ (×4). The aqueous extract was acidified with 1 M HCl and back-extracted with EtOAc (×5). The organic phase was then washed with brine, dried ($MgSO_4$) and the solvent removed to furnish the carboxylic acid **14** as a white solid (0.293 g, 100%). No further purification was necessary; mp 139–141 °C; Found: C, 62.35, H, 7.01%. $C_{22}H_{30}O_8$ requires C, 62.55; H, 7.16%; ν_{max} (cm^{-1}): 3518, 3188, 2950, 1767, 1729, 1439, 1367, 1310, 1244, 1172, 1117, 1099, 1039, 960, 895, 800; δ_H ($CDCl_3$): 1.24 (3 H, d, *J* 7.1, H-18), 1.60–2.68 (13 H, m), 2.78 (1 H, d, $J_{6,5}$, H-6), 2.99 (1 H, dq, $J_{4,5}$ 11.0, *J* 7.1, H-4), 3.08 (1 H, dd, $J_{5,4}$ 11.0, $J_{5,6}$ 2.1, H-5), 3.35 (3 H, s, MOM), 3.69 (3 H, s, CO_2CH_3), 4.56, 4.80 (2 H, 2 × ABd, *J* 7.3, MOM), 5.06 (2 H br t, H-17); δ_C ($CDCl_3$): 12.3, 19.0, 28.0, 29.2, 32.5, 38.1, 38.3, 41.5, 44.4, 49.3, 49.4, 51.0, 51.1, 52.3, 55.7, 84.2, 91.9, 95.3, 107.9, 149.7, 174.8, 178.1, 179.0; *m/z* (EI) 422.1937 ($C_{22}H_{30}O_8$ M^+ requires 422.1941).

ent-3-Hydroxy-13-methoxymethoxy-3,4-seco-20-norgibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone

Diisopropylethylamine (0.32 mL, 1.8 mmol, 1.2 eq) was added to a mixture of the carboxylic acid (0.65 g, 1.5 mmol) and BOP reagent (0.75 g, 1.7 mmol, 1.1 eq) in THF (15 mL) and stirred at room temp for 45 min. $NaBH_4$ (0.102 g, 2.7 mmol, 1.75 eq) was added and the reaction was stirred for 30 min at room temp. A second portion of $NaBH_4$ (0.102 g, 2.7 mmol, 1.75 eq) was added and the reaction was again stirred for 30 min. The reaction was then quenched with 1 M HCl (5 mL), stirred for 20 min and then poured into EtOAc–5% 2-butanol and water. The organic phase was washed with water (×2) and the combined aqueous phases were extracted with EtOAc–5% 2-butanol (×3). The combined organic phase was then washed with brine, dried ($MgSO_4$) and the solvent removed *in vacuo.* Purification by silica gel chromatography (EtOAc) furnished the alcohol (0.529 g, 84%) as a colourless oil; ν_{max} (cm^{-1}): 3452, 2945, 2879, 1764, 1729, 1448, 1436, 1369, 1310, 1239, 1191, 1169, 1040, 960, 916; δ_H ($CDCl_3$): 1.23 (3 H, d, *J* 7.3, H-18), 1.58–2.05 (12 H, m), 2.58 (1 H, dt, *J* 15.8, 3.1, H-15), 2.78 (1 H, d, *J* 2.2, H-6), 2.94–3.12 (2 H, m, H-4, H-5), 3.35 (3 H, s, MOM), 3.69 (3 H, s, CO_2CH_3), 3.69 (2 H, overlapped, H-3), 4.56, 4.81 (2 H, 2 × ABd, *J* 7.0, MOM), 5.06 (2 H, m, H-17); δ_C ($CDCl_3$): 12.3, 19.0, 27.4, 34.2, 38.2, 38.6, 41.6, 44.4, 49.5, 51.0, 51.1 (2 × C), 52.2, 55.7, 62.8, 84.2, 91.9, 96.3, 107.7, 149.9, 174.9, 179.4; *m/z* (EI) 408.2150 ($C_{22}H_{32}O_7$ M^+ requires 408.2148).

***ent*-3-*tert*-Butyldimethylsilyloxy-13-methoxymethoxy-3,4-seco-20-norgibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone (15)**

To a cooled solution of alcohol prepared above (0.429 g, 1.1 mmol) and imidazole (14 mg, 0.21 mmol, 0.2 eq) in DMF (15 mL) was added Et_3N (0.29 mL, 2.1 mmol, 2 eq) and then *tert*-butyldimethylsilyl chloride (0.32 g, 2.1 mmol, 2 eq). The reaction was stirred overnight at room temp and then poured into EtOAc and water. The aqueous phase was extracted with EtOAc (×3). The combined organic phase was washed with water and brine, dried ($MgSO_4$) and the solvent evaporated. Purification by silica gel chromatography (4 : 1 hexane–EtOAc–2 : 1 hexane–EtOAc) furnished **15** (0.494 g, 90%) as a colourless oil; ν_{max} (cm^{-1}): 2951, 2857, 1769, 1733, 1436, 1361, 1251, 1195, 1150, 1099, 1042, 961, 836, 776; δ_H ($CDCl_3$): 0.04 (6 H, s, Si-Me_2), 0.88 (9 H, s, Si-*t*Bu), 1.23 (3 H, d, *J* 7.2, H-18), 1.50–2.10 (12 H, m), 2.55 (1 H, dt, *J* 15.8, 2.9, H-15), 2.77 (1 H, d, *J* 2.2, H-6), 2.95–3.10 (2 H, m, H-4, H-5), 3.35 (3 H, s, MOM), 3.62 (2 H, t, *J* 5.1, H-3), 3.68 (3 H, s, CO_2CH_3), 4.56, 4.80 (2 H, 2 × ABd, *J* 7.2, MOM), 5.05 (2 H, m, H-17); δ_C ($CDCl_3$) : −4.9, 12.3, 18.7, 19.0, 26.3, 27.6, 28.0, 34.1, 38.2, 38.6, 41.6, 44.4, 49.5, 51.00, 51.04, 52.2, 55.7, 63.1, 84.2, 91.9, 96.4, 107.6, 150.1, 174.8, 179.3; *m*/*z* (EI) 522.3013 ($C_{28}H_{46}O_7Si$ M^+ requires 522.3013).

***ent*-4α-Bromo-3-*tert*-butyldimethylsilyloxy-13-methoxymethoxy-3,4-seco-20-norgibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone**

A solution of *n*-butyllithium (1.6 M in hexanes, 0.25 mL, 0.40 mmol, 1.5 eq) was added to a solution of diisopropylamine (0.056 mL, 0.40 mmol, 1.5 eq) in THF (3 mL) at 0 °C and the reaction was stirred for 20 min. After cooling to −78 °C, **15** (0.14 g, 0.27 mmol) in THF (3 mL) was added and the reaction was stirred for 25 min at −78 °C. CBr_4 (0.22 g, 0.66 mmol, 2.5 eq) in THF (3 mL) was added and the reaction was stirred for 40 min at −78 °C. The reaction was then quenched with NH_4Cl and allowed to warm to room temp (60 min) before pouring into EtOAc and water. The organic phase was washed with water (×2). The aqueous phase was extracted with EtOAc (×2). The combined organic phase was then washed with brine, dried ($MgSO_4$) and the solvent removed. Purification by silica gel chromatography (4 : 1 hexane–EtOAc) furnished the bromide (0.143 g, 88%) as a colourless oil; ν_{max} (cm^{-1}): 2951, 2857, 1775, 1734, 1436, 1359, 1280, 1246, 1213, 1169, 1149, 1107, 1040, 960, 835, 775; δ_H ($CDCl_3$) : 0.05 (6 H, s, $SiMe_2$), 0.89 (9 H, s, Si-*t*Bu), 1.50–2.24 (12 H, m), 1.95 (3 H, s, H-18), 2.57 (1 H, dt, *J* 15.8, 3.1, H-15), 2.83 (1 H, d, *J* 2.1, H-6), 3.33 (3 H, s, 3H, MOM), 3.60 (1 H, d, *J* 2.1, H-5), 3.65 (2 H, t, *J* 6.3, H-3), 3.72 (3 H, s, CO_2CH_3), 4.54, 4.79 (2 H, 2 × ABd, *J* 7.3, MOM), 5.06 (2 H, m, H-17); δ_C ($CDCl_3$) : −4.9, 18.6, 18.7, 26.1, 26.3, 28.2, 33.4, 38.1, 41.3, 44.5, 47.0, 50.9, 52.4, 53.3, 55.6, 55.7, 59.2, 63.0, 84.1, 91.9, 97.2, 107.8, 149.3, 173.4, 174.6; *m*/*z* (EI) 600.2120 ($C_{28}H_{45}O_7Si^{79}Br$ M^+ requires 600.2118); 602.2104 ($C_{28}H_{45}O_7Si^{81}Br$ M^+ requires 602.2097).

***ent*-3-Hydroxy-13-methoxymethoxy-3,4-seco-20-norgibberella-4(18),16-dien-7-oic acid 7-methyl ester 19,10-lactone (16)**

To a solution of the bromide prepared above (0.034 g, 0.057 mmol) in THF (3 mL) was added 1 M TBAF solution in THF (0.17 mL, 0.17 mmol, 3eq) and the reaction stirred for 4 h at room temp. The reaction was poured into EtOAc–5% 2-butanol and water. The organic phase was washed with water (×2) and the combined aqueous phases were extracted with EtOAc–5% 2-butanol (×3). The combined organic phase was then washed with brine, dried ($MgSO_4$) and the solvent removed *in vacuo.* Purification by silica gel chromatography (2 : 1 EtOAc–hexane) furnished **16** (0.017 g, 74%) as a colourless oil; ν_{max} (cm^{-1}): 3494, 2947, 1759, 1732, 1659, 1438, 1402, 1351, 1329, 1274, 1196, 1169, 1115, 1041, 975, 895, 818; δ_H ($CDCl_3$) : 1.50–2.10 (12 H, m), 2.61 (1 H, dt, *J* 16.1, 3.1, H-15), 2.65 (1 H, d, *J* 0.88, H-6), 3.33 (3 H, s, MOM), 3.51 (1 H, d, *J* 1.3, H-5), 3.65 (2 H, t, *J* 6.4, H-3), 3.72 (3 H, s, CO_2CH_3), 4.54, 4.78 (2 H, 2 × ABd, *J* 7.3, MOM), 5.06 (2 H, m, H-17), 5.72 (1 H, d, *J* 2.2, H-18), 6.31 (1 H, d, *J* 2.6, H-18); δ_C ($CDCl_3$): 19.0, 27.2, 35.2, 38.2, 41.2, 44.6, 48.9, 50.2, 51.8, 52.3, 55.8, 60.5, 62.8, 84.2, 91.9, 95.2, 107.8, 124.3, 140.6, 149.4, 170.2, 174.1; *m*/*z* (EI) 406.1992 ($C_{22}H_{30}O_7$ M^+ requires 406.1992).

***ent*-13-Methoxymethoxy-3-oxo-3,4-seco-20-norgibberell-4(18),16-dien-7-oic acid 7-methyl ester 19,10-lactone (17)**

Dess–Martin periodinane (0.060 g, 0.16 mmol, 1.5 eq) was added to **14** (0.043 g, 0.11 mmol) in CH_2Cl_2 (5 mL) and stirred at room temp for 2 h. $Na_2S_2O_3$ (2.5 mL) and sat. $NaHCO_3$ (2.5 mL) were then added and the reaction was stirred vigorously just until both layers were clear. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (×3). The combined organic phase was washed with water and brine, dried ($MgSO_4$) and the solvent removed *in vacuo.* Purification by silica gel chromatography (1 : 1 EtOAc–hexane) furnished aldehyde **15** (0.039 g, 91%) as a colourless oil. ν_{max} (cm^{-1}): 2948, 1761, 1725, 1659, 1439, 1274, 1169, 1114, 1041, 895; δ_H ($CDCl_3$): 1.50–2.09 (9 H, m), 2.26–2.64 (4 H, m), 2.65 (1 H, d, *J* 1.1, H-6), 3.33 (3 H, s, MOM), 3.46 (1 H. d, *J* 1.1, H-5), 3.72 (3 H, s, CO_2CH_3), 4.54, 4.78 (2 H, 2 × ABd, *J* 7.3, MOM), 5.06 (2 H, m, H-17), 5.74 (1 H, d, *J* 2.2, H-18), 6.34 (1 H, d, *J* 2.6, H-18), 9.76 (1 H, t, *J* 1.1, CHO); δ_H ($CDCl_3$): 19.0, 30.9, 38.2, 38.8, 41.2, 44.6, 48.7, 50.2, 51.9, 52.4, 55.8, 60.5, 84.1, 91.9, 94.2, 107.9, 125.0, 140.0, 149.2, 169.8, 174.0, 200.5; *m*/*z* (EI) 404.1834 ($C_{22}H_{28}O_7$ M^+ requires 406.1835).

***ent*-18-Benzyloxy-3-hydroxy-13-methoxymethoxy-gibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone 3-epimers (18, R = CH_2Ph)**

DBU (4.5 mL, 30 mmol, 10 eq) was added to a solution of aldehyde **17** (1.22 g, 3.0 mmol) in 10 mL of benzyl alcohol and the reaction was stirred at room temp for 18 h. After quenching with 1 M HCl, the reaction mixture was poured into EtOAc and water. The organic phase was washed with water (×2). The combined aqueous phase was extracted with EtOAc (×3). The combined organic phase was washed with brine, dried over $MgSO_4$ and the solvent removed. Purification by silica gel chromatography (5 : 1 hexane–EtOAc–1 : 1 EtOAc–hexane) furnished in sequence, the 3β-epimer (0.717 g) followed by the 3α-isomer (0.396 g), 72% total yield, both as colourless oils.

3β-epimer: ν_{max} (cm^{-1}): 3480, 2947, 2879, 1772, 1734, 1439, 1270, 1196, 1158, 1111, 1073, 1040, 918, 893, 863, 737, 699; δ_H ($CDCl_3$): 1.65–2.35 (13 H, m), 2.76 (1 H, d, *J* 9.7, H-6), 3.36 (3 H, s, OCH_3-MOM), 3.51 (1 H, d, *J* 1.3, 3-OH), 3.51 (1 H, d, *J* 9.7, H-5), 3.60, 3.96 (2 H, 2 × ABd, *J* 9.8, H-18), 3.62 (3 H, s, CO_2CH_3), 4.16 (1 H, br d, *J* 2.3, H-3), 4.45, 4.47 (2 H, 2 × ABd, *J* 11.6, $PhCH_2$), 4.54, 4.76 (2 H, 2 × ABd, *J* 7.2, –MOM), 5.03 (1 H, br s, H-17), 5.13 (1 H, t, *J* 1.9, H-17), 7.31 (5 H, m, C_6H_5); δ_C ($CDCl_3$): 17.5, 27.3, 27.5, 38.1, 42.8, 44.0, 50.3, 50.8, 51.3, 52.2, 52.4, 55.7, 57.9, 70.8, 71.9, 74.4, 83.7, 92.1, 95.7, 108.3, 127.8, 128.3, 128.8, 136.8, 152.9, 173.0, 175.0.

3α epimer: Found: C, 67.78, H, 6.77%; $C_{29}H_{36}O_8$ requires C, 67.95, H, 7.08%; ν_{max} (cm^{-1}): 3435, 2948, 2879, 1771, 1733, 1439, 1286, 1254, 1196, 1148, 1115, 1075, 1040, 914, 899, 735, 698; δ_H ($CDCl_3$): 1.45–2.30 (13 H, m), 2.69 (1 H, d, *J* 9.1, H-6), 2.90 (1 H, d, *J* 9.1, H-5), 3.36 (3 H, s, MOM), 3.55 (3 H, s, CO_2CH_3), 3.58, 3.97 (2 H, 2 × ABd, *J* 10.6, H-18), 4.01 (1 H, m, H-3), 4.42, 4.47 (2 H, 2 × ABd, *J* 11.9, $PhCH_2$), 4.54, 4.77 (2 H, 2 × ABd, *J* 7.0, MOM), 5.01 (1 H, br s, H-17), 5.11 (1 H, m, H-17), 7.31 (5 H, m, C_6H_5); δ_C ($CDCl_3$): 17.7, 29.2, 30.0, 38.1, 43.1, 43.8, 51.26, 51.30, 51.9, 52.2, 55.7, 56.1, 58.7, 66.4, 70.1, 73.8, 83.6, 92.2, 94.1, 108.3, 127.6, 128.0, 128.6, 137.8, 152.6, 173.2, 175.0; *m/z* (FAB) 535.2308 ($C_{29}H_{36}O_8Na$ $[M + Na]^+$ requires 535.2308).

DBU (1.1 mL, 7.2 mmol, 10 eq) was added to a solution of the 3α-epimer (0.367 g, 0.72 mmol) in 5 mL of benzyl alcohol and the reaction was stirred at room temp for 2 h. After quenching with 1 M HCl, the reaction mixture was poured into EtOAc and water. The organic phase was washed with water (×2). The aqueous phase was extracted with EtOAc (×3). The combined organic phase was washed with brine, dried over $MgSO_4$ and the solvent removed. Purification by silica gel chromatography (5 : 1 hexane–EtOAc–1 : 1 EtOAc–hexane) furnished the products as a mixture of the 3α-epimer (0.230 g) with the 3β isomer (0.108 g); 92% total recovery, both as colourless oils.

ent-3α,18-Dihydroxy-13-methoxymethoxy-16-oxo-17-norgibberellan-7-oic acid 7-methyl ester 19,10-lactone (27)

To a solution of **23b** (0.037 g, 0.072 mmol) in dioxane and water (3 : 1, 5 mL) was added osmium tetraoxide (1 mol%) and the reaction was stirred for 5 min at room temp. Then sodium periodate (0.046 g, 0.22 mmol, 3 eq) was added and the reaction was stirred overnight at room temp. The following day, more sodium periodate (0.046 g, 0.22 mmol, 3 eq) was added and the reaction was again stirred overnight at room temp. Upon completion, the reaction was diluted with EtOAc and then washed with 1 M $Na_2S_2O_3$ (×2), water and brine, dried over $MgSO_4$, and the solvent removed *in vacuo*. Purification by silica gel chromatography (2 : 1 EtOAc–hexane) furnished ketone **27** (0.022 g, 59% yield) as a colourless oil; ν_{max} (cm^{-1}): 3492, 2950, 2893, 1753, 1453, 1366, 1284, 1203, 1153, 1113, 1049, 1032, 990, 916, 735; δ_H ($CDCl_3$): 1.66–2.40 (13 H, m), 2.85 (1 H, d, *J* 10.0, H-6), 3.34 (3 H, s, MOM), 3.46 (1 H, d, *J* 0.9, C-3 OH), 3.55 (1 H, d, *J* 10.0, H-5), 3.62 (3 H, s, CO_2CH_3), 3.62, 3.97 (2 H, 2 × ABd, *J* 9.8, H-18), 4.16 (1 H, d, *J* 2.9, H-3), 4.46 (2H, s, $PhCH_2$), 4.62, 4.78 (2 H, 2 × ABd, *J* 7.5, MOM), 7.31 (5 H, m, C_6H_5); δ_C ($CDCl_3$): 17.4, 27.2, 27.4, 32.0, 39.4, 47.4, 49.4, 50.9, 52.5, 52.8, 53.0, 56.2, 58.2, 70.7, 71.8, 74.5, 83.2, 92.9, 95.3, 127.8, 128.4, 128.9, 136.7, 172.3, 174.6, 215.5.

Benzyl ether (0.070 g, 0.14 mmol) and palladium hydroxide on carbon (10 mg) were combined in EtOAc (5 mL) and hydrogen gas was introduced to the flask *via* a balloon. The reaction was stirred at room temp for 2 h and then filtered through Celite and the solvent removed *in vacuo*. Ketone **27** was obtained as a white solid after removal of the solvent (0.052 g, 90% yield); mp 170–172 °C; Found C, 59.13; H, 6.60%; $C_{21}H_{28}O_9$: C, 59.43; H, 6.65%; ν_{max} (cm^{-1}): 3480, 2951, 1752, 1439, 1284, 1203, 1154, 1118, 1034, 989, 918, 733; δ_H ($CDCl_3$): 1.60–2.45 (13 H, m), 2.91 (1 H, d, *J* 11.0, H-6), 2.98 (1 H, dd, *J* 4.8, 8.8, 18-OH), 3.35 (3 H, s, MOM), 3.42 (1 H, d, *J* 10.8, H-5), 3.66 (1 H, m, 3-OH), 3.71 (1 H dd, *J* 4.8, 12.5, H-18), 3.80 (3 H, s, CO_2CH_3), 4.03 (1 H, dd, *J* 8.8, 12.5, H-18), 4.30 (1 H, m, H-3), 4.64, 4.81 (2 H, 2 × ABd, *J* 7.5, MOM); δ_C ($CDCl_3$): 17.5, 27.5, 27.6, 31.8, 38.4, 46.6, 48.8, 49.3, 52.3, 53.3, 53.8, 56.3, 58.7, 64.2, 70.3, 82.9, 93.0, 94.1, 173.8, 175.0, 215.7; *m/z* (EI) 424.1738 ($C_{21}H_{28}O_9$ M^+ requires 424.1733).

ent-3α,18-Dihydroxy-13-methoxymethoxy-gibberell-16-en-7-oic acid 7-methyl ester 19,10-lactone

Lombardo reagent prepared from CH_2Br_2 (0.75 mL)[19] was added as a suspension dropwise *via* a pipette to a solution of ketone (0.046 g, 0.11 mmol) in CH_2Cl_2 (2 mL) at room temp. The reaction was monitored by TLC and when complete was poured into a slurry of $NaHCO_3$ (solid) and water (2 : 1) and ether. The mixture was shaken until a clear organic layer was obtained and then the layers were separated. The organic layer was dried over $MgSO_4$ and the solvent removed. The crude 16-alkene (0.020 g, 43%) was obtained as an oil and carried through to the next reaction; ν_{max} (cm^{-1}): 3440, 2930, 1764, 1733, 1455, 1435, 1283, 1247, 1199, 1159, 1112, 1039, 918, 892; δ_H ($CDCl_3$) : 1.59–2.22 (13 H, m), 2.85 (1 H, d, *J* 11.0, H-6), 3.01 (1 H, dd, *J* 4.8, 9.2, 18-OH), 3.38 (1 H, d, *J* 11.0, H-5), 3.38 (3 H, s, MOM), 3.67 (1 H, dd, *J* 4.8, 12.5, H-18), 3.71 (1 H, m, 3-OH), 3.78 (3 H, s, CO_2CH_3), 4.00 (1 H, dd, *J* 9.1, 12.6, H-18), 4.28 (1 H, m, H-3), 4.56, 4.76 (2 H, 2 × ABd, *J* 7.2, MOM), 5.05 (1 H, m, H-17), 5.20 (1 H, m, H-17); δ_C ($CDCl_3$): 17.5, 27.67, 27.73, 38.3, 41.0, 43.4, 49.0, 49.3, 50.9, 53.0, 53.5, 55.7, 58.5, 64.6, 70.7, 83.4, 92.3, 94.3, 109.2, 153.7, 174.8, 175.2; *m/z* (EI) 378.1678 (M^+ 50%), HRMS: $C_{20}H_{26}O_7$ (M+) requires 378.1684.

ent-3α,13,18-Trihydroxy-gibberell-16-en-7-oic acid 19,10-lactone (1)

Dihydropyran (0.1 mL, 1.1 mmol, 10 eq) was added to a solution of the diol (0.11 mmol from the previous reaction) and PPTS (5 mg) in 4 mL CH_2Cl_2 and stirred at room temp overnight. The reaction was then washed with water, sat. $NaHCO_3$, and brine, dried over $MgSO_4$ and the solvent removed *in vacuo*. No further purification was carried out. The crude mixture of diastereomers **28** was obtained as an oil and carried through to the next reaction. δ_H (crude, $CDCl_3$): 1.0–2.30 (25 H, m), 2.75–2.82 (1 H, d × 2, *J* 9.5, H-6), 3.36 (3 H, s, MOM), 3.36–3.75 (5 H, m), 3.67 (3 H, s, CO_2CH_3), 3.69–3.93 (2 H, 2 × ABd, H-18), 4.21 [1 H, m, OCHO (THP)], 4.53–4.78 (2 H, 2 × ABd, MOM), 5.03, 5.13 (2 H, m, H-17).

A solution of lithium *n*-butanethiolate in HMPA (0.26 mL,1.5 M, 0.39 mmol) was added to the methyl ester (0.11 mmol) in HMPA (0.5 mL) and stirred overnight at room temp. The reaction mixture was then diluted with water, and acidified with cold 2 M HCl to pH 3. The mixture was extracted with EtOAc (×3) and the combined organic phase was washed with KH_2PO_4 (to pH 4.5), $CuCl_2$ solution, and brine. The organic layer was dried over $MgSO_4$ and the solvent removed *in vacuo*.

The crude product was dissolved in methanol (1 mL) and water (0.2 mL), Dowex resin (H^+) added, and the mixture heated for 3 h. The reaction was then cooled to room temp and filtered. The resin was rinsed with methanol and the solvent was evaporated. Purification on reverse-phase HPLC furnished a colourless gum, (0.003 g, 8% over 4 steps); δ_H (MeOH-d$_4$): 1.64–2.01 (11 H, m), 2.19 (1 H, d, J 15.6, H-15), 2.39 (1 H, dt, J 2.89, 15.6, H-15), 2.93 (1 H, d, J 10.3, H-6), 3.30 (1 H, d, J 10.3, H-5), 3.80, 3.84 (2 H, 2 × ABd, J 11.2, H-18), 3.97 (1 H, d, J 3.5, H-3), 4.94 (1 H, m, H-17), 5.20 (1 H, m, H-17); δ_H (MeOH-d$_4$): 17.1, 27.3, 27.8, 38.7, 42.9, 44.8, 49.4, 50.1, 51.4, 52.4, 59.1, 61.4, 67.2, 77.5, 94.7, 106.2, 157.3, 175.0, 177.4; m/z (EI) 364 (5%), 346.1415 (55%, M^+-18. $C_{19}H_{22}O_6$ requires 346.1416), 328 (40), 300 (35), 284 (30), 270 (32), 255 (25), 242 (30), 227 (19), 213 (24), 199 (48), 121 (48), 105 (80), 91 (96), 69 (100); m/z (Me-TMS, EI) 594 (M^+,%), 579 (21), 562 (10), 536 (9), 429 (15), 401 (21), 385 (37), 374 (46), 355 (44); KRI 2745.

Preparation of 18-alkoxy derivatives 19a–26a and 19b–25b

General procedure. DBU (0.18 mL,) was added to a solution of aldehyde **17** (50 mg) in 3 mL of alcohol and the reaction was stirred at room temp for 18 h. After quenching with 1 M HCl, the reaction mixture was poured into EtOAc and water. The organic phase was washed with water (×2). The combined aqueous phase was extracted with EtOAc (×3). The combined organic phase was washed with brine, dried over $MgSO_4$ and the solvent removed. Purification by silica gel chromatography (5 : 1 hexane–EtOAc–1 : 1 EtOAc–hexane) furnished in sequence, the 3β-epimer followed by the 3α-isomer, both as colourless oils. Yields and ratios are summarized in Table 1. Selected NMR data are available in the ESI (Table 3).†

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Notes and references

1 N. Murofushi, N. Takahashi, T. Yokata and S. Tamura, *Agric. Biol. Chem.*, 1969, **33**, 592; N. Murofushi, N. Takahashi, T. Yokata and S. Tamura, *Agric. Biol. Chem.*, 1969, **33**, 598.
2 P. Gaskin, S. J. Gilmour, J. R. Lenton, J. MacMillan and V. M. Sponsel, *J. Plant Growth Regul.*, 1984, **2**, 229.
3 L. S. Green, E. M. Færgestard, A. Poole and P. M. Chandler, *Plant Physiol.*, 1997, **114**, 203.
4 E. Kovats, *Helv. Chim. Acta*, 1958, **41**, 1951.
5 S. J. Gilmour, P. Gaskin, V. M. Sponsel and J. MacMillan, *Planta*, 1984, **161**, 186; L. J. Beeley, PhD Thesis, University of Bristol, 1975.
6 J. R. Crow, R. J. Thomson and L. N. Mander, *Org. Biomol. Chem.*, 2006, **4**, 2532.
7 L. Lombardo, J. V. Turner and L. N. Mander, *Aust. J. Chem.*, 1981, **34**, 745.
8 Z. J. Duri, B. M. Fraga and J. R. Hanson, *J. Chem. Soc., Perkin Trans. 1*, 1981, 161.
9 An analogous retro-Claisen reaction had previously been carried out on methyl gibberellate; see G. Stork and J. Singh, *J. Am. Chem. Soc.*, 1979, **101**, 7109. *cf.*; I. A. Gurvich, I. M. Milstein and V. F. Kucherov, *Tetrahedron Lett.*, 1967, 4293.
10 In ^{1}H-NMR spectra, 3,4-*seco*-gibberellins typically show a doublet at *ca.* 1.2 ppm for the *endo*-methyl epimers (as determined by NOE experiments in the GA_4 series), while the *exo*-epimers afford a doublet at *ca.* 1.4 ppm.
11 J. S. Bindra, A. Grodski, T. K. Schaaf and E. J. Corey, *J. Am. Chem. Soc.*, 1973, **95**, 7522.
12 R. P. McGeary, *Tetrahedron Lett.*, 1998, **39**, 3319.
13 R. T. Arnold and S. T. Kulenovic, *J. Org. Chem.*, 1978, **43**, 3687.
14 D. B. Dess and J. C. Martin, *J. Org. Chem.*, 1983, **48**, 4155.
15 E. J. Corey and W. J. Suggs, *J. Org. Chem.*, 1973, **38**, 3224.
16 *Cf.*J. MacMillan and R. J. Pryce, *J. Chem. Soc.*, 1967, 740.
17 R. Pappo, D. S. Allen, R. U. Lemieux and W. S. Johnson, *J. Org. Chem.*, 1956, **21**, 478.
18 W. M. Pearlman, *Tetrahedron Lett.*, 1967, **8**, 1663.
19 L. Lombardo, *Tetrahedron Lett.*, 1982, **23**, 4293.
20 P. A. Bartlett and W. S. Johnson, *Tetrahedron Lett.*, 1970, **21**, 4459.
21 P. Gaskin and J. MacMillan, *GC-MS of Gibberellins and Related Compounds: Methodology and a Library of Reference Spectra*, Cantock Enterprises, Bristol, U.K., 1992; http://www.plant-hormones.info/gibberellin_nomenclature.htm.
22 P. M. Chandler and M. Robertson, *Plant Physiol.*, 1999, **120**, 623.
23 P. M. Chandler, A. Marion-Poll, M. Ellis and F. Gubler, *Plant Physiol.*, 2002, **129**, 181.
24 J. D. B. Weyers, N. W. Paterson, R. A'Brook and Z.-Y. Peng, *Physiol. Plant*, 1995, **95**, 486.