Synthesis and bioactivity of the gibberellin, 18-hydroxy-GA\textsubscript{1} (GA\textsubscript{132})†

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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\textsubscript{1} from gibberellic acid (GA\textsubscript{3}) is described, allowing the confirmation of structure for a new gibberellin, GA\textsubscript{132}, that occurs in developing grains of barley (\textit{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 \(\alpha\)-methylene lactone moiety followed by an intramolecular aldol reaction. The bioactivities of the new GA, and its 18-hydroxy-GA\textsubscript{4} relative, have been confirmed in dwarf barley growth and \(\alpha\)-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 (\textit{Canavalia gladiata}) and from both immature and germinated barley grain (\textit{Hordeum vulgare}). GAs from the latter species were identified as putative 18-OH derivatives of GA\textsubscript{1} (1), GA\textsubscript{4} (2), GA\textsubscript{34} (3) and GA\textsubscript{48} (4) on the basis of GC-MS spectral comparisons and KRIs of reference compounds that were derived from metabolic feeds of 18-hydroxy GA\textsubscript{1} to the fungus \textit{Gibberella fujikuroi} B1–41a.\textsuperscript{5} We have recently confirmed the identity of 18-hydroxy GA\textsubscript{4} by synthesis\textsuperscript{6} and have now embarked upon the preparation of 18-hydroxy GA\textsubscript{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\textsubscript{1} followed the same strategy that we had employed in the synthesis of 18-hydroxy-GA\textsubscript{4}\textsuperscript{6} and is outlined in Scheme 1. Initially, we employed ketone 5,\textsuperscript{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).

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\textsubscript{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\textsuperscript{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. Under these conditions, a 5 : 1 mixture of C4 epimers was obtained with the \textit{endo}-methyl isomer predominating.\textsuperscript{9} Attempts to reduce the 3-carboxyl by the standard treatment with NaBH\textsubscript{4} of the mixed anhydride formed from ethyl chloroformate\textsuperscript{10} that had been...

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\textsuperscript{†} Electronic supplementary information (ESI) available: Selected NMR data for 18-alkoxy gibberellin derivatives. See DOI: 10.1039/b800728d
reasonably effective in the GA4 series proved to be unsatisfactory, as did numerous other methods; only a procedure utilizing benzotriazol-1-yl oxytris(dimethyl am ino)phosphonium hexafluorophosphate (“BOP”) 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 was formed in quantitative yield using standard conditions. Subsequent treatment with LDA followed by tetrabromomethane 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 as a prelude to carrying out the desired domino transformation to 18 (R = CH2 CH=CH2) 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-diastereomer. Unfortunately, we could not remove the allyl group from the 3β-diastereomer and achieved only a 40% yield with the 3α-diastereomer. 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-diastereomer. 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, 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. Then, as outlined in Scheme 4, 23b was oxidised first to the 17-nor-ketone with OsO4–NaIO4 to allow hydrogenolytic removal of the benzyl group, resulting in diol 27.

The Δ16 double bond was restored by means of the Lombardo procedure, the product protected as bis-tetrahydropyranyl adduct 28, and then the ester function demethylated with lithium propanethiolate. Finally, the target GA (1) was liberated by acidic hydrolysis with Dowex resin. Confirmation of structure for the putative 18-hydroxy-GA1 was then established by GCMS comparison of the silylated methyl ester with that of the synthetic material and has been assigned as GA132.

The bioactivities of the new GA and the previously prepared 18-hydroxy-GA4, as well as their parents, GA1 and GA4, were compared in dwarf barley growth assays and are shown in Table 2. GA1 was about 10-fold less active than GA4 in this bioassay, and the 18-hydroxy derivatives each had about one quarter of the activity of the parent compound. 18-OH GA1 and 18-OH-GA4 were tested at two concentrations in the α-amylase induction bioassay: 10⁻⁸ M and 10⁻⁹ M for GA1 and 18-OH GA1, and at 10⁻⁷ M and 10⁻⁸ M for the less active GA,
Table 1  Yields of adducts from treatment of aldehyde 17 with various alkoxides

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>3α : β epimer ratio</th>
<th>Alcohol</th>
<th>Yield (%)</th>
<th>3α : β epimer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>=CH₂OH</td>
<td>63</td>
<td>2 : 1</td>
<td>=CH₂OH</td>
<td>72</td>
<td>2 : 1</td>
</tr>
<tr>
<td>=C(H)OH</td>
<td>72</td>
<td>3 : 1</td>
<td>=CH₂OH</td>
<td>24</td>
<td>2 : 1</td>
</tr>
<tr>
<td>Cl₂C =CH₂OH</td>
<td>57</td>
<td>1 : 1</td>
<td>Cl₂C =CH₂OH</td>
<td>58</td>
<td>1 : 1</td>
</tr>
<tr>
<td>HO =CHHO</td>
<td>31</td>
<td>10 : 1</td>
<td>HO =CHHO</td>
<td>25</td>
<td>&gt;10 : 1</td>
</tr>
<tr>
<td>Me₂Si =CH₂OH</td>
<td>—</td>
<td></td>
<td>Me₂Si =CH₂OH</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>THPO =CH₂OH</td>
<td>—</td>
<td></td>
<td>THPO =CH₂OH</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>HO =CHOOH</td>
<td>25</td>
<td>&gt;10 : 1</td>
<td>HO =CHOOH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>[H]₅₀° (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA₁</td>
<td>3.1 × 10⁻⁷</td>
</tr>
<tr>
<td>GA₄</td>
<td>3.1 × 10⁻⁸</td>
</tr>
<tr>
<td>18-OH GA₁</td>
<td>1.3 × 10⁻⁸</td>
</tr>
<tr>
<td>18-OH GA₄</td>
<td>1.2 × 10⁻⁵</td>
</tr>
</tbody>
</table>

* [H]₅₀ is the concentration of GA required for half-maximal stimulation of leaf elongation rate.

and 18-OH GA₁ (Fig. 1). In this assay, α-amylase is produced in the interval 2–4 days following the commencement of GA treatment. At 2 d the highest α-amylase activities occurred at the highest concentrations of GA₁ or GA₄, 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 GA₁ and GA₄, 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-hydroxylation 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., there were elevated levels of GA₁ and of 18-OH GA₁, associated with the production of α-amylase in the mutant grains. The probability that 18-OH GA₁ 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 GA₁ and of 18-OH GA₁ in the original study because a deuterated standard is not yet available for 18-OH GA₁. However, based on its relative abundance to ¹H GA₁, 18-OH GA₁ may be present at up to 6 times the content of GA₁, and even though it is not as active as GA₁ in α-amylase induction, it might still constitute the major bioactive GA in such grains. GA₁ and 18-OH GA₁ 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. 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 GA₃ (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., assuming that p = 1:

\[
[H]_{50} = \frac{R_{max} \times [H] - [H]}{R - R_{min}}
\]
Fig. 1  Production of α-amylase by endosperm half-grains incubated with GAs at different concentrations. A. GA1 and 18-OH GA1. B. GA4 and 18-OH GA4. 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 GA3 minus the response in the absence of GA3), $R$ 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.

Synthetic procedures

For general directions see ref. 6.

**ent-13-Acetoxy-3,4-seco-20-norgibberell-16-en-7-oic 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 the reaction mixture poured into a bilayer of water and EtOAc. The aqueous layer was separated and the organic layer washed several times with EtOAc. The combined organic layers were washed with brine, dried (Na2SO4) 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%); $\nu_{max}$ (cm$^{-1}$): 3058, 2951, 1767, 1731, 1665, 1437, 1369, 1311, 1265, 1241, 1173, 1099, 1043, 962, 893, $\delta_{q}$ (CDCl3) 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, d, J 1.0, J11, J10, J 7.0, H-4), 3.07 (1 H, dd, J11, J10, J11, J12, 2.1, H-5), 3.69 (3 H, s, CO2CH3), 4.93 (1 H, d, J 1.8, H-17), 4.99 (1 H, s, H-17); m/z (EI): 420.1784 (M+, 38% C22H28O8 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).

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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 CBr₂ (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 (Na₂SO₄). Concentration in vacuo and column chromatography on silica (20% EtOAc and 80% light petroleum, increasing to 80% EtOAc) gave the bromide as a brown solid (0.16 g, 96%).

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

To a solution of GA₃ methyl ester (44.55 g, 0.11 mol) in CH₂Cl₂ (300 mL) cooled to 0 °C, was added Et₃N (80.5 mL, 0.58 mol, 5 eq) and MOMCl (25.2 mL, 0.33 mol, 3 eq). The reaction was washed with water, sat. NaHCO₃, and brine, dried (MgSO₄) and the solvent removed to furnish a colourless oil. No further purification was carried out. νmax (cm⁻¹): 2940, 2884, 2870, 1736, 1449, 1437, 1372, 1332, 1269, 1227, 1158, 1101, 1039, 976, 895; δH (CDCl₃): 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, CO₂CH₃), 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); δC (NMR) (CDCl₃): 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, 177.4; m/z (EI): 446.1943 (M⁺ C₂₄H₂₉O₇ 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 K₂CO₃–KHCO₃ 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 (x 3). The combined organic phase was then washed with 10% K₂CO₃ solution and brine (2 × 100 mL), dried (MgSO₄) and the solvent removed to furnish a colourless oil. No further purification was carried out. νmax (cm⁻¹): 1416–1424 This journal is © 2008 The Royal Society of Chemistry.

A solution of GA₃ methyl ester 3-acetate (44.55 g, 0.11 mol) in CH₂Cl₂ (300 mL) was cooled to 0 °C. Disopropylethylamine (67 mL, 0.40 mol, 4 eq) was added followed by dropwise addition of MOMCl (25.2 mL, 0.33 mol, 3 eq). 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. NaHCO₃ (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. NaHCO₃ and brine, dried (MgSO₄) and the solvent removed in vacuo to furnish a colourless oil. No further purification was carried out. νmax (cm⁻¹): 2940, 2884, 2870, 1736, 1449, 1437, 1372, 1332, 1269, 1227, 1158, 1101, 1039, 976, 895; δH (CDCl₃): 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, CO₂CH₃), 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); δC (NMR) (CDCl₃): 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, 177.4; m/z (EI): 446.1943 (M⁺ C₂₄H₂₉O₇ requires 446.1941).
(1 H, d, J 10.3, H-5), 3.74 (3 H, s, CO₂CH₃), 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); δc (CDCl₃): 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, 124.9, 147.3, 152.6, 171.8, 173.1, 191.7; m/z (EI) 402.1682 (M⁺C₂₂H₂₆O₇ 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 NaBH₄ (19.2 g, 0.51 mol, 10 eq) carefully in several portions (bubbles vigorously!). After NaBH₄ 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 × 3) and the combined organic phase was washed with water, sat. NaHCO₃ (3 × 3) and brine, dried (MgSO₄) and the solvent removed to furnish the alcohol as a white solid (19.6 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-epimer (12 mg) as a gum followed by the crystalline 3α-epimer (145 mg).

3β-epimer: δH (CDCl₃): 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, CO₂CH₃), 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); δc (CDCl₃): 15.0, 17.4, 27.5, 28.4, 38.2, 42.0, 43.9, 49.8, 51.6, 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 (C₂₂H₂₆O₇ requires 406.1992).

3α-epimer: mp 120–122 °C; Found: C, 64.75, H, 7.59%. C₂₂H₂₆O₇ requires C, 62.55; H, 7.16%; C₂₂H₃₀O₈ requires C, 62.35; H, 7.01%. C₂₂H₂₆O₇ requires C, 62.55; H, 7.16%; C₂₂H₃₀O₈ requires C, 62.35; H, 7.01%.

**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 × 3) and then the combined organic phase was extracted with sat. NaHCO₃ (4 × 3). The aqueous extract was acidified with 1 M HCl and back-extracted with EtOAc (5 × 5). The organic phase was then washed with brine, dried (MgSO₄) 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%. C₂₂H₂₆O₇ requires C, 62.55; H, 7.16%; C₂₂H₃₀O₈ requires C, 62.35; H, 7.01%.
ent-3-tert-Butyldimethylsilylxy-13-methoxymethoxy-3,4-seco-20-norgibberell-16-en-7-oxoic 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 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 (MgSO4) 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: νmax (cm⁻¹): 3494, 2947, 1759, 1732, 1659, 1438, 1402, 1351, 1329, 1274, 1196, 1169, 1115, 1041, 975, 895, 818; δH (CDCl₃): 1.50–2.10 (12 H, m), 2.61 (1 H, d, 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, CO₂CH₃), 4.54, 4.78 (2 H, 2 × Abd, J 7.3, MOM), 5.06 (2 H, m, H-17); δC (CDCl₃): 19.0, 27.2, 35.2, 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 (C₁₆H₁₆O₆Si M⁺ requires 406.2131).

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

Dee–Martin periodinanone (0.060 g, 0.16 mmol, 1.5 eq) was added to 14 (0.043 g, 0.11 mmol) in CH₂Cl₂ (5 mL) and stirred at room temp for 2 h. Na₂S₂O₃ (0.25 mL, 2.5 eq) and NaHCO₃ (2.5 mL) were added and the reaction was stirred vigorously just until both layers were clear. The layers were separated and the aqueous phase was extracted with CH₂Cl₂ (×3). The combined organic phase was washed with water and brine, dried (MgSO₄) 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. νmax (cm⁻¹): 2948, 1761, 1725, 1659, 1439, 1274, 1169, 1114, 1041, 895; δH (CDCl₃): 1.50–2.09 (9 H, m), 2.26–2.64 (4 H, m), 2.65 (1 H, d, J 1.1, H-6), 3.37 (3 H, s, MOM), 3.46 (1 H, d, J 1.1, H-5), 3.72 (3 H, s, CO₂CH₃), 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); δC (CDCl₃): 19.0, 30.9, 38.2, 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 (C₁₆H₁₆O₆Si M⁺ requires 406.1835).

ent-18-Benzylxy-3-hydroxy-13-methoxymethoxy-gibberell-16-en-7-oxoic acid 7-methyl ester 19,10-lactone 3-epimers (18, R = CH₂Ph)

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 MgSO₄ and the solvent removed. Purification by silica gel chromatography (5 : 1 hexane–EtOAc–1 : 1 EtOAc–hexane–hexane) furnished sequence, the 3β-epimer (0.717 g) followed by the 3α-isomer (0.396 g), 72% total yield, both as colourless oils.

3β-epimer: νmax (cm⁻¹): 3480, 2947, 2879, 1772, 1734, 1439, 1270, 1196, 1158, 1111, 1073, 1040, 918, 893, 863, 737, 699; δH (CDCl₃): 1.65–2.35 (13 H, m), 2.76 (1 H, d, J 9.7, H-6), 3.36 (3 H, s, OCH₃-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, CO2CH3), 4.16 (1 H, br d, J 2.3, H-3), 4.45, 4.47 (2 H, 2 × ABd, J 11.6, PhCH2), 4.54, 4.76 (2 H, 2 × ABd, J 7.2, -MOM), 5.03 (1 H, br s, H-17), 5.13 (1 H, J 11.9, H-17), 7.31 (5 H, m, C6H5); δc (CDCl3): 17.5, 27.3, 27.5, 38.1, 42.4, 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%; C29H36O8Na (M + Na)+ requires 535.2308.

δC (CDCl3): 17.5, 27.6, 27.7, 38.3, 41.3, 43.8, 51.6, 51.3, 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, 173.0, 175.0; m/z (EI): 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 extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with 1 M Na2SO3 (2 × 20 mL) and stirred overnight at room temp. Upon completion, the reaction mixture was then diluted with water, and the solvent was removed in vacuo.

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

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 CH3Cl; and stirred at room temp overnight. The reaction was then washed with water, sat. NaHCO3, and brine, dried over MgSO4 and the solvent removed in vacuo. No further purification was carried out. The crude mixture of diastereomers was obtained as an oil and carried through to the next reaction. δc (CDCl3): 17.5, 27.6, 27.7, 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: C29H36O8 (M+) requires 378.1684.

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

Lombardo reagent prepared from CH3Br2 (0.75 mL) was added as a suspension dropwise via a pipette to a solution of ketone (0.046 g, 0.11 mmol) in CH2Cl2 (2 mL) at room temp. The reaction was monitored by TLC and when complete was poured into a slurry of NaHCO3 (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 MgSO4 and the solvent removed. The crude 16-alkene (0.020 g, 43%) was obtained as an oil and carried through to the next reaction. δc (CDCl3): 3440, 2930, 1764, 1733, 1455, 1453, 1283, 1247, 1199, 1159, 1112, 1039, 918, 892; δH (CDCl3): 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, 18-0H), 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, CO2CH3), 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-7), 5.20 (1 H, m, H-17), 5.71 (1 H, m, C6H5); δc (CDCl3): 17.5, 27.6, 27.7, 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: C29H36O8 (M+) requires 378.1684.

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

No further purification was carried out. The crude mixture of diastereomers 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%; C29H36O8Na; C, 59.43; H, 6.65%; δc (CDCl3): 3480, 2951, 1752, 1439, 1284, 1203, 1154, 1118, 1034, 989, 918, 733; δH (CDCl3): 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, 18-0H), 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, CO2CH3), 4.03 (1 H, dd, J 8.8, 12.5, H-18), 4.30 (1 H, m, H-3), 4.60, 4.81 (2 H, 2 × Abd, J 7.5, MOM), δc (CDCl3): 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 (C21H28O9 M+) requires 424.1733.
The crude product was dissolved in methanol (1 mL) and water (0.2 mL), Dowex resin (H\textsuperscript{+}) 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); \( \delta_{H} \) (MeOH-d\textsubscript{4}): 1.64–2.01 (11 H, m), 2.19 (1 H, d, J 15.6, H\textsubscript{15}), 2.39 (1 H, dt, J 2.89, 15.6, H\textsubscript{15}), 2.93 (1 H, d, J 10.3, H\textsubscript{6}), 3.30 (1 H, d, J 10.3, H\textsubscript{5}), 3.80, 3.84 (2 H, 2 \times ABd, J 11.2, H\textsubscript{18}), 3.97 (1 H, d, J 3.5, H\textsubscript{3}), 4.94 (1 H, m, H\textsubscript{17}), 5.20 (1 H, m, H\textsubscript{17}); \( \delta_{H} \) (MeOH-d\textsubscript{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\textsuperscript{+} -18. C\textsubscript{19} H\textsubscript{22} O\textsubscript{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\textsuperscript{+} ,%), 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 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 brine, dried over MgSO\textsubscript{4} and the solvent removed. Purification by silica gel chromatography (5:1 hexane–EtOAc), furnished in sequence, the 3\textsubscript{a},4-s\textsubscript{h} epimer followed by the 3\textsubscript{a}-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**

10. In 1H-NMR spectra, 3,4-s\textsubscript{h} epimers typically show a doublet at ca. 1.2 ppm for the endo-methyl epimer (as determined by NOE experiments in the GA\textsubscript{4} series), while the exo-epimers afford a doublet at ca. 1.4 ppm.