The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/91570

Please be advised that this information was generated on 2018-02-18 and may be subject to change.
Aromatic A-ring analogues of orobanchol, new germination stimulants for seeds of parasitic weeds†

Heetika Malik,a Wouter Kohlen,b Muhammad Jamil,b Floris P. J. T. Rutjes** and Binne Zwanenburg*b

Received 17th September 2010, Accepted 4th November 2010
DOI: 10.1039/c0ob00735h

Strigolactones are signaling compounds in plants of increasing importance. In this paper the focus is on their activity as germinating agents for seeds of parasitic weeds. The syntheses of aromatic A-ring analogues of the germination stimulant orobanchol have been described. Starting substrate is the ABC unit of the stimulant GR24. Oxidation at the C-4 position gives a 4-oxo derivative which on subsequent reduction produces two C-4 epimeric alcohols, syn and anti in a ratio of 82 : 3. For practical access of the C-4 anti alcohol, the predominant syn epimer is inverted by a Mitsunobu procedure. The anti C-4 alcohol is then coupled with the D-ring in a one-pot two-step process involving a formylation and a reaction with bromobutenolide to give a mixture of the diastereomeric aromatic A-ring analogues of orobanchol. In contrast, the syn C-4 alcohol cannot be coupled directly with the D-ring. Protection of the C-4 syn OH is a prerequisite. The best protecting function is the SEM group as deprotection after coupling with the D-ring can then readily be achieved. The structures of these new analogues have been ascertained by X-ray analyses. Both diastereomers of the C-4 syn as well as the C-4 anti orobanchol analogues have been tested as germination agents of seeds of Striga hermonthica and Orobanche ramosa. In addition, the acetates of both epimeric C-4 alcohols have been prepared and tested. Both diastereomers of the 4-oxo derivative have been prepared and bioassayed as well. The bioassays reveal that the diastereomers having the natural relative configuration are most active. The data also suggest that hydrogen bonding is not an important factor in the binding of the stimulant molecules in the receptor.

Introduction

Strigolactones constitute an important family of bioactive terpenoids that are present in root exudates of several plants and that were initially identified as germinating agent for seeds of root parasites such as Striga and Orobanche spp.†,‡ Interestingly, recent findings show that strigolactones also serve as the branching factor for arbuscular mycorrhizal (AM) fungi.§,¶ and as inhibitor of shoot branching and bud outgrowth in various plants.†,‡ Currently, strigolactones are considered as a new type of plant hormones.†,‡ Several recent reviews demonstrate the current interest in these compounds.†,‡,§,¶ The isolation of natural strigolactones is extremely difficult due to the very small quantities that are present in root exudates.†,‡ The estimated production is ca. 15 pg per day per plant. Strigol (see Fig. 1) was the first natural strigolactone that was isolated in 1966 from cotton roots.†,‡ The details of its chemical structure were elucidated many years later.†,‡ At present several natural strigolactones are known,†,‡ of which typical examples are depicted in Fig. 1. All strigolactones known thus far consist of an annelated three ring ABC skeleton invariably attached to an aromatic ring.‡ The structural differences of strigolactones are mainly present in the AB part of these molecules. The total syntheses of natural strigolactones involve multistep sequences; especially installing the correct stereochemistry has been proven very demanding.†,‡ There has been a continuous quest for structurally simplified strigolactones in which the bioactivity is predominantly retained.†,‡ The most notable synthetic analogue is GR24 wherein the A-ring of strigolactones is replaced by an aromatic ring.‡ This analogue has a high germination activity towards seeds of parasitic weeds and is widely used as a standard positive control in germination experiments.†,‡ By systematically simplifying the strigolactone structure the bioactiphore in these biocompounds has been established and found to be residing in the CD-part of these molecules.¶,§ Substituents, especially a hydroxy group, can considerably influence, in most cases enhance,
Fig. 1 Natural strigolactones and synthetic analogue GR24.

With the aim to a better understanding of the structure–activity relationship, the synthesis of the aromatic A-ring analogue of orobanchol, viz. GR24 having a hydroxy group in the B-ring, was undertaken. The synthetic route should thereby be chosen in such a manner that both C-4-OH epimers are accessible, as the spatial position of the OH group or its acetate may provide relevant information about the structure–activity requirements of the germination stimulatory activity of strigolactone analogues.

Interestingly, a strigolactone having an aromatic A-ring has recently been isolated from root exudates of tobacco. The structure of this solanacol 6 was first incorrectly assigned. The methyl groups in the A-ring are positioned ortho instead of para. The occurrence of a natural aromatic strigolactone is an extra stimulus for the present study.

Results and discussion

Synthesis

For the synthetic strategy of an aromatic A-ring analogue of orobanchol, the ABC skeleton of GR24 is a logical starting material. This compound (viz. rac. 7) is readily available from either indan-1-one22 or benzaldehyde.23 Selective oxidation at the C-4 position could only be achieved after considerable experimentation (Scheme 1). It was found that the use of FeCl3 with tert-butylhydroperoxide (TBHP) in pyridine24 is most adequate resulting in 60% yield and 90% based on recovered starting material. Subsequent reduction of the thus obtained 4-oxo compound 8 with sodium borohydride under Luche conditions delivered a mixture of the cis and trans C-4-OH products, 9a and 9b, respectively, in a ratio of 82 : 3. The structure of the cis product 9a was ascertained by X-ray diffraction analysis (Fig. 2). This analysis showed that the OH group was oriented cis to the C-ring, thereby confirming that hydride attack takes place primarily from the least hindered convex side.

Scheme 1 Reagents and conditions: (i) FeCl3 (2 mol%), aq TBHP (70%, 3 equiv) pyridine, 82 °C, 24 h, (ii) NaBH4, CeCl3·7H2O, EtOH, (iii) PPh3, PhCO2H, EtO2CNCO2Et, toluene, (iv) K2CO3, MeOH.

In orobanchol, however, the OH has a trans orientation with respect to the lactone unit. In order to obtain the desired trans

Fig. 2 Crystal structures of compounds 9a, 11αβ and 16βα.
product 9b in better quantities, the cis compound 9a was subjected to a Mitsunobu reaction, which afforded the trans product in 96% yield. Having both C-4-OH epimers 9a and 9b in hand, the next step was coupling with the D-ring.

To this end, the trans C-4-OH compound was formylated with ethyl formate in the presence of potassium tert-butoxide, followed by reaction with bromobutenolide 10 (Scheme 2).22,23 This two-step one-pot process resulted in a mixture of two diastereomeric aromatic A-ring analogues of orobanchol, 11aα and 11aβ (ratio ca 1 : 1), in 78% overall yield. These diastereoisomers were readily separated by column chromatography, after which both structures were confirmed by X-ray diffraction analysis (Fig. 2).

Much to our surprise, direct coupling of cis alcohol 9a with the D-ring using the one-pot two-step sequence did not meet with success, probably due to the fact that the spatial position of the free C-4 hydroxyl is in close proximity of the enolate anion. Initially, we tried to solve this problem by using the methoxymethyl (MOM) group as a protecting group, resulting in a smooth coupling with butenolide 10 in 78% yield (Scheme 3). However, deprotection required prolonged treatment with ZnBr₂, leading to a relatively low yield (ca. 60%) of the desired alcohols 16aα and 16ββ due to unreacted starting material and to decomposition. Next, we turned to the 2-trimethylsilylethoxymethyl (SEM) protecting group giving 15b in 93% yield.

The subsequent coupling proceeded smoothly, after which deprotection was conveniently achieved by direct treatment of the reaction mixture with 1 M HCl. Via the latter pathway the cis aromatic A-ring analogues 16aα and 16ββ were obtained in a ca. 1 : 1 ratio in 74% yield starting from 15b and 68% overall yield starting from 9a. The structure of alcohol 16aα was also confirmed by X-ray crystallographic analysis (Fig. 2).

The corresponding acetates 12aα and β and 17bα and β of all four diastereomeric aromatic A-ring analogues were prepared by straightforward acetylation of the hydroxyl groups in very good yields (Schemes 2 and 3). These aromatic A-ring orobanchyl acetates are in fact aromatic analogues of alcol. The structure of this strigolactone was under discussion for quite a while,16,25–27 but recently shown to be orobanchyl acetate.28

Furthermore, the trans alcohols 11aα and β were also subjected to oxidation conditions using PDC in CH₂Cl₂, providing the corresponding ketones 18aα and β in 81% and 84% yield, respectively (Scheme 4). These analogues are also of biological interest as little is known about the activity of oxo containing strigolactones.

The results reveal that the oxidation route to introduce a hydroxyl group at the C-4 position of the B-ring in GR24 is quite effective. Takikawa et al.21 used an entirely different approach to install a hydroxyl group in the B-ring in their synthesis of solanacol with the aim to prove its structure. These authors used a Diels–Alder reaction of a furanone as the first step following a literature procedure.29 Our route is more direct.

Bioactivity

The newly prepared aromatic A-ring analogues of orobanchol were bioassayed against seeds of Striga hermonthica and Orobanche ramosa using a standard protocol (see Fig. 3–6).29 In all cases GR24 was used as the standard and water as the blank control. The bioassays for 11aα, 11aβ, 16aα and 16ββ on seeds of O. ramosa clearly reveal that 11aα is the most active one. In this analogue the relative position of the C-4-OH and the D-ring with respect to the C-ring lactone is the same as in natural (+)-orobanchol (3). The same holds for the bioassays of this series of analogues for seeds of S. hermonthica. When the C-4-OH is positioned cis with respect to the C-ring lactone, the activities are markedly lower. This difference may be due to a favorable effect of the trans-C-4-OH on binding the stimulants in the receptor. The results suggest that S. hermonthica is more sensitive to structural changes than...
Fig. 3  Bioassay: germination activity towards seeds of O. ramosa.

Fig. 4  Bioassay: germination activity towards seeds of O. ramosa.

Fig. 5  Bioassay: germination activity towards seeds of S. hermonthica.

**O. Ramosa**. In the literature only a few examples are known in which all possible diastereoisomers of a particular strigolactone have been tested for germination of parasitic weed seeds.\(^{14a,16-12}\) In case of strigolactone 2, it was shown that the natural stereochemistry of the stimulant corresponds with the highest activity.\(^{14a}\) The same was observed for strigol (1),\(^{11}\) GR24\(^{12}\) and demethylsorgolactone\(^*\) (compound 2, lacking the methyl group in the A-ring).

The bioactivity of all acetates is very high for O. ramosa. It seems that the spatial arrangement of the acetate group hardly influences the germination seeds of O. ramosa. This finding is remarkable as in natural strigolactones C-4-O-acetates score lower than the corresponding hydroxy compounds.\(^{2,20}\) In the case of S. hermonthica seeds the C-4-O-acetate 12aa, which has the same relative *syn* stereochemistry of the C-4-O-acetate and the D-ring as in natural orobanchyl acetate (alectrol), is considerably more active than 12ab having the *anti* stereochemistry. The activities of the stereoisomers 17ba and 17bf which have the C-4-O-acetate in the unnatural position are remarkably high. Here the O-acetylation enhances the activity (compare the activities of hydroxy compounds 16ba and 16bf with those of the acetates 17ba and 17bf).

The two oxo compounds 18a and 18b both are remarkably active in inducing germination of both seed types, but the isomers with the D-ring in the natural configuration perform best. These observations for the C-4-O-acetates and the C-4-oxo compounds may be an indication that hydrogen bonding of the C-4-OH is not an important aspect for the binding of the stimulant molecules in the receptor.

In summary, it may be concluded that the diasteromers of the newly prepared aromatic A-ring analogues of orobanchol possessing the natural relative configuration both at C-4 and at the D-ring are the most active ones. S. hermonthica seeds are more sensitive to structural changes than seeds of O. ramosa.

**Experimental section**

**General remarks**

All glass apparatus were oven dried prior to use. Solvents were distilled from appropriate drying agents prior to use and stored under nitrogen. Standard syringe techniques were applied for the transfer of dry solvents and air or moisture-sensitive reagents. All chemicals were obtained from commercial sources and used without further purification. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer, or on a Bruker Tensor 27 FTIR spectrometer. Melting points were analyzed with a Buchi melting point apparatus B-545. \(^1H\) NMR and \(^13C\) NMR spectra were recorded on a Bruker DRX-300 (operating at 300 MHz for \(^1H\) and at 75 MHz for \(^13C\) spectra) spectrometer using deuterated solvents. Tetramethylsilane (0.00 ppm) served as an internal standard in \(^1H\) NMR and CDCl\(_3\) (77.0 ppm) in \(^13C\) NMR spectra. Coupling constants are reported as *J*-values in Hz. Multiplicities are reported as: s (singlet), d (doublet), t (triplet), m (multiplet).
Reactions were monitored using thin layer chromatography (TLC) on silica gel-coated plates (Merck 60 F254) with the indicated solvent mixture. Detection was performed with UV-light, and/or by charring at ~150 °C after dipping in to a solution of either 2% anisaldehyde in ethanol/H2SO4 or (NH4)2MoO4·4H2O (25 g L-1) or KMnO4. High resolution mass spectra were recorded on a JEOL AccuTOF (ESI), or a MAT900 (EI, Cl, and ESI). Column chromatography was performed over silica gel (0.035–0.070 mm) using freshly distilled solvents. Air and moisture sensitive reactions were carried out under an inert atmosphere of dry nitrogen or argon.

To a stirred solution of ketone 5 (3.00 g, 15.9 mmol) in ethanol (50 mL) was added CeCl3·7 H2O (5.94 g, 15.9 mmol) followed by slow addition of NaBH4 (0.60 g, 15.9 mmol). After stirring for 10 min, the reaction mixture was quenched by dropwise addition of 1 M HCl and then extracted with dichloromethane (2×100 mL). The organic layer was washed with brine and dried (MgSO4). Evaporation of the solvent gave a crude product, which was chromatographed on silica gel (EtOAc/n-heptane 1:3) giving a mixture of isomers (75% based on recovered 7). Mg 113.5–114.5 °C. FT-IR (solid) cm-1: 1757, 1713. H NMR (300 MHz, CDCl3): δ 7.84–7.82 (m, 1H), 7.78–7.76 (m, 2H), 7.63–7.58 (m, 1H), 6.10 (d, 1H, J = 6.6 Hz), 3.61–3.53 (m, 1H), 2.79 (dd, 1H, J = 19.2, 4.5 Hz). 13C NMR (75 MHz, CDCl3): δ 202.0 (s), 174.3 (s), 149.2 (2 d with dichloromethane (2 ¥ wk). To a stirred mixture of alcohol 1b (3a) and solid (EtOAc/n-heptane 1 : 1) was added 5-Bromo-3-methylfuran-2(5H)-one (0.20 g, 2.06 mmol) in dry CCl4 (20 mL) was added NBS (0.40 g, 2.26 mmol) and AIBN (5 mg) and the resulting reaction mixture was heated at reflux for 2 h while irradiating with a 250 W lamp. The mixture was cooled to 0°C and solid succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.

To a stirred mixture of ketone 8 (3.24 g, 60% (90% based on recovered 7)) and K2CO3 (0.99 g, 7.18 mmol) in MeOH (20 mL) was added Succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.

To a stirred mixture of alcohol 1b (3a) and solid (EtOAc/n-heptane 1 : 1) was added 5-Bromo-3-methylfuran-2(5H)-one (0.20 g, 2.06 mmol) in dry CCl4 (20 mL) was added NBS (0.40 g, 2.26 mmol) and AIBN (5 mg) and the resulting reaction mixture was heated at reflux for 2 h while irradiating with a 250 W lamp. The mixture was cooled to 0°C and solid succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.

To a stirred mixture of ketone 8 (3.24 g, 60% (90% based on recovered 7)) and K2CO3 (0.99 g, 7.18 mmol) in MeOH (20 mL) was added Succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.

To a stirred mixture of ketone 8 (3.24 g, 60% (90% based on recovered 7)) and K2CO3 (0.99 g, 7.18 mmol) in MeOH (20 mL) was added Succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.

To a stirred mixture of ketone 8 (3.24 g, 60% (90% based on recovered 7)) and K2CO3 (0.99 g, 7.18 mmol) in MeOH (20 mL) was added Succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.

To a stirred mixture of ketone 8 (3.24 g, 60% (90% based on recovered 7)) and K2CO3 (0.99 g, 7.18 mmol) in MeOH (20 mL) was added Succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.

To a stirred mixture of ketone 8 (3.24 g, 60% (90% based on recovered 7)) and K2CO3 (0.99 g, 7.18 mmol) in MeOH (20 mL) was added Succinimide was filtered off. The solvent was removed in vacuo to give bromobutenolide 10, which was used as such in the coupling step.
1H), 1.96 (t, 3H, J = 1.5 Hz), 13C NMR (75 MHz, CDCl3): δ 170.3 (s), 170.1 (s), 152.0 (d), 144.6 (s), 141.6 (d), 139.1 (s), 134.4 (s), 129.0 (d), 129.8 (d), 125.6 (d), 125.4 (d), 109.1 (s), 100.8 (d), 83.6 (d), 78.1 (d), 49.7 (d), 9.3 (s). HRMS (ESI) m/z calc for C11H10O2 (M+Na)+: 337.0681, found: 337.0693.

(3aS*,4R*,8bS*,E)-4-((3aR*,4R*,5aR*,6aS*,7aS*,8bR*)-4-methyl-5-oxo-2,5-dihydropyran-2-yl)methene)-3a,4,8b-tetrahydro-2H-indeno[1,2-b][furan-4-yl acetate (12aa)]. To a solution of 11aa (0.10 g, 0.31 mmol) in dichloromethane (5 mL) was added pyridine (1 mL), DMAP (catalytic) and acetic anhydride (0.5 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Solvent was removed in vacuo and the residue was purified by silica gel flash chromatography (EtOAc/n-hexane 1:2) to afford 12aa (0.10 g, 95%) as a white solid. Mp 190.8–191.3 °C. FT-IR (solid) cm⁻¹: 3457, 1781, 1738, 1676. 1H NMR (300 MHz, CDCl3): δ 7.59 (d, 1H, J = 2.7 Hz), 7.55–7.53 (m, 1H), 7.47–7.40 (m, 3H), 7.01–7.00 (m, 1H), 6.44 (bs, 1H), 6.18–6.17 (m, 1H), 6.10 (d, 1H, J = 7.5 Hz), 3.84–3.88 (m, 1H), 2.05 (s, 3H), 2.02 (t, 3H, J = 2.7 Hz). 13C NMR (75 MHz, CDCl3): δ 169.8 (s), 169.7 (s), 169.6 (s), 152.5 (d), 140.6 (d), 140.1 (s), 135.1 (s), 130.1 (d), 130.0 (d), 126.0 (d), 125.9 (d), 108.2 (s), 100.1 (d), 83.2 (d), 78.4 (d), 46.7 (d), 20.6 (q), 10.2 (q). HRMS (ESI) m/z calc for C19H14O6 (M+Na)+: 379.0737, found: 379.0731.

Compounds 12aβ, 17ba and 17bb were prepared following the same procedure.

(3aS*,4R*,8bS*,E)-3-((3R*,4R*,5aR*,6aS*,7aS*,8bR*)-4-methyl-5-oxo-2,5-dihydropyran-2-yl)methene)-2-oxo-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b][furan-4-yl acetate (12aβ). Yield: 95%. Mp 167.3–167.8 °C. FT-IR (solid) cm⁻¹: 3442, 1769, 1729, 1660. 1H NMR (300 MHz, CDCl3): δ 7.54–7.50 (m, 2H), 7.46–7.39 (m, 3H), 6.98–6.96 (m, 1H), 6.37 (bs, 1H), 6.21–6.20 (m, 1H), 6.10 (d, 1H, J = 7.2 Hz), 3.85–3.89 (m, 1H), 2.03–2.02 (m, 6H). 13C NMR (75 MHz, CDCl3): δ 169.7 (s), 169.6 (s), 169.4 (s), 151.3 (d), 140.3 (d), 140.0 (2×s), 135.7 (s), 130.1 (d), 130.0 (d), 126.2 (d), 125.8 (d), 108.5 (s), 99.5 (d), 83.1 (d), 78.5 (d), 46.8 (d), 20.6 (q), 10.3 (q). HRMS (ESI) m/z calc for C18H12O5 (M+Na)+: 379.0739, found: 379.0745.

(3aS*,4S*,8bS*,E)-3-(((3S*,4R*,5aR*,6aS*,7aS*,8bR*)-4-methyl-5-oxo-2,5-dihydropyr an-2-yl)methene)-2-oxo-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b][furan-4-yl acetate (12b). Yield: 93%. Mp 160.4–160.9 °C. FT-IR (solid) cm⁻¹: 2958, 1782, 1731, 1687. 1H NMR (300 MHz, CDCl3): δ 7.58–7.56 (m, 2H), 7.46–7.37 (m, 3H), 6.95–6.93 (m, 1H), 6.54 (d, 1H, J = 8.1 Hz), 6.16–6.14 (m, 1H), 5.70 (d, 1H, J = 7.2 Hz), 4.19–4.13 (m, 1H), 2.03 (s, 3H), 2.01 (t, 3H, J = 1.5 Hz). 13C NMR (75 MHz, CDCl3): δ 170.1 (s), 170.0 (s), 169.5 (s), 150.7 (d), 140.8 (d), 140.4 (s), 138.5 (s), 135.7 (s), 130.4 (d), 129.7 (d), 126.1 (d), 125.7 (d), 107.8 (s), 99.6 (d), 82.6 (d), 73.6 (d), 43.2 (d), 20.7 (q), 10.3 (q). HRMS (ESI) m/z calc for C18H12O5 (M+Na)+: 379.0739, found: 379.0780.

(3aS*,4R*,8bS*,E)-3-(((3S*,4R*,5aR*,6aS*,7aS*,8bR*)-4-methyl-5-oxo-2,5-dihydropyran-2-yl)methene)-2-oxo-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b][furan-4-yl acetate (17b). Yield: 94%. Mp 158.1–158.6 °C. FT-IR (solid) cm⁻¹: 2958, 1774, 1735, 1683. 1H NMR (300 MHz, CDCl3): δ 7.64 (d, 1H, J = 2.4 Hz), 7.59–7.56 (m, 1H), 7.47–7.37 (m, 3H), 6.94–6.92 (m, 1H), 6.53 (d, 1H, J = 8.4 Hz), 6.15–6.14 (m, 1H), 5.72 (d, 1H, J = 7.5 Hz), 4.20–4.14 (m, 1H), 2.03 (t, 3H, J = 1.5 Hz), 2.00 (bs, 3H). 13C NMR (75 MHz, CDCl3): δ 170.1 (s), 169.7 (s), 169.5 (s), 151.8 (d), 140.9 (s), 140.1 (d), 138.4 (s), 135.3 (s), 130.4 (d), 129.7 (d), 126.0 (d), 125.7 (d), 107.2 (s), 100.0 (d), 82.5 (d), 73.2 (d), 42.9 (d), 20.4 (q), 10.3 (q). HRMS (ESI) m/z calc for C18H12O5 (M+Na)+: 379.0793, found: 379.0797.

(3aS*,4S*,8bS*,E)-3-(((3S*,4R*,5aR*,6aS*,7aS*,8bR*)-4-methyl-5-oxo-2,5-dihydropyr an-2-yl)methene)-2-oxo-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b][furan-4-yl acetate (13b). To a solution of compound 9a (0.51 g, 2.68 mmol) in dichloromethane (15 mL) was added N,N-diisopropylethylamine (0.7 mL, 4.01 mmol) and MOMCl (0.3 mL, 3.85 mmol) at 25°C for 2 h. Solvent was removed in vacuo and the residue was purified by silica gel column chromatography (EtOAc/n-hexane 1:4) to afford 13b (0.59 g, 95%) as a white solid.
solid. Mp 84.3–84.8 °C. FT-IR (solid) cm⁻¹: 2954, 1757. ¹H NMR (300 MHz, CDCl₃): δ 7.49–7.35 (m, 4H), 5.68 (d, 1H, J = 7.2 Hz), 5.13 (d, 1H, J = 6.9 Hz), 4.82–4.75 (m, 2H), 3.64–3.54 (m, 1H), 3.44 (s, 3H), 2.76–2.52 (m, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 176.3 (s), 141.6 (s), 137.5 (s), 129.6 (d), 129.0 (d), 125.6 (d), 125.1 (d), 96.1 (t), 83.2 (d), 78.1 (d), 55.6 (q), 42.4 (t), 28.7 (d). HRMS (ESI) m/z calcd for C₁₉H₂₃O₇ (M+Na⁺): 375.1336, found: 375.1334.

(3aS*,4R*,8bS*)-4-(((2-Trimethylsilyl)ethoxy)ethoxy)ethoxy)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (18b). To a solution of compound 11a (0.51 g, 2.68 mmol) in anhydrous dichloromethane (15 mL) was added N,N-disopropylethylamine (2.74 mL, 15.72 mmol) and [β-(trimethylsilyl)ethoxy]methyl chloroide (1 mL, 5.65 mmol), and the mixture was stirred under a nitrogen atmosphere for 22 h. The solution was diluted with dichloromethane and washed successively with 0.5 N aqueous HCl (2 × 20 mL) and water (2 × 20 mL). The organic layer was dried (Na₂SO₄), evaporated, and the resulting yellow syrup was purified by silica gel column chromatography (EtOAc/n-heptane 1:4) to afford 18b (0.79 g, 93%) as a white solid. Mp 84.3–84.8 °C. FT-IR (solid) cm⁻¹: 2954, 1756. ¹H NMR (300 MHz, CDCl₃): δ 7.48–7.42 (m, 4H), 5.71–5.69 (m, 1H), 5.18–5.16 (m, 1H), 4.88–4.80 (m, 2H), 3.75–3.58 (m, 3H), 2.76–2.50 (m, 2H), 1.01–0.89 (m, 2H), 0.03 (m, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 176.3 (s), 141.6 (s), 137.5 (s), 129.6 (d), 128.9 (d), 125.6 (d), 125.0 (d), 94.2 (t), 83.2 (d), 77.8 (d), 65.4 (t), 42.4 (d), 28.7 (t), 17.7 (t), −1.8 (3 x q). HRMS (ESI) m/z calcd for C₁₉H₂₃O₇Si (M+Na⁺): 373.1345, found: 373.1341.

Bioassay

The germination bioassays were conducted as reported earlier, with the number of germinated seeds being counted under a microscope. All tests were carried out in triplicate. The bar diagrams in Fig. 3–6 show the average values with the standard deviation.

Notes and references

