An expeditious preparation of all enantiopure diastereoisomers of aromatic A-ring analogues of strigolactones, germination stimulants for seeds of the parasitic weeds *Striga* and *Orobanche*

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An expeditious manner to prepare all enantiopure diastereomers of aromatic A-ring strigolactone analogues is described. The racemic diastereoisomers of 8-methyl GR 24 and of its regioisomer 6-methyl GR 24 were prepared and separated, and subsequently chromatographed to give the pure enantiomers, using a Chiralcel OD® HPLC column. The biological activity of all enantiopure strigolactone analogues towards seeds of *Striga hermonthica* and *Orobanche crenata* was determined. The presence of a methyl group on position 8 of GR 24 did not result in increased biological activity, whereas a 6-methyl substituent on GR 24 resulted in higher percentages of germinated *O. crenata* seeds, when compared with GR 24.

Introduction

The angiosperms *Striga*, *Alectra* (Scrophulariaceae) and *Orobanche* (Orobanchaceae) are root parasitic plants, which can only survive when attached to the roots of an appropriate host plant. Host plants for *Striga* include cereals such as maize and sorghum, whereas *Orobanche* mainly parasitizes legumes *e.g.* tomato and eggplant. Parasitic weeds deprive their host plants from nutrients, minerals and water and have an extremely devastating effect on the host crop yield.[1,2]

The lifecycles of *Striga*, *Alectra* and *Orobanche* are closely adapted to those of their host plants. The seeds only germinate after exposure to a specific germination stimulant, which is exuded by the roots of suitable host plants and also by some non-hosts. Thus far, four naturally occurring germination stimulants, all belonging to the class of strigolactones[3] (Fig. 1), have been identified, *viz.* strigol (**1**),[4] sorgolactone (**2**),[5] alectrol[6] and orobanchol.[7]

Several synthetic strigol analogues, *e.g.* GR 24 (**3**) have been prepared in order to gain more insight into the germination process at the molecular level.[8,9] Considerable attention has been given to examining the influence of the stereochemistry of the stimulants on the biological activity.[10–13] In the case of GR 24, only the stereoisomer possessing the same stereochemical configuration as the naturally occurring stimulants, exhibited appreciable activity at low concentrations, whereas the other three stereoisomers were virtually inactive at sensitive concentrations.[11] The synthesis and biological testing of all eight diastereomers of sorgolactone, **2**, showed the same trend.[13] In the latter case, the methyl substituent on the sorgolactone A-ring also clearly influences the biological activity. It would therefore be interesting to study the biological activity of all diastereomers of 8-methyl GR 24 (**4**), which can be considered as the most genuine aromatic A-ring analogue of sorgolactone.

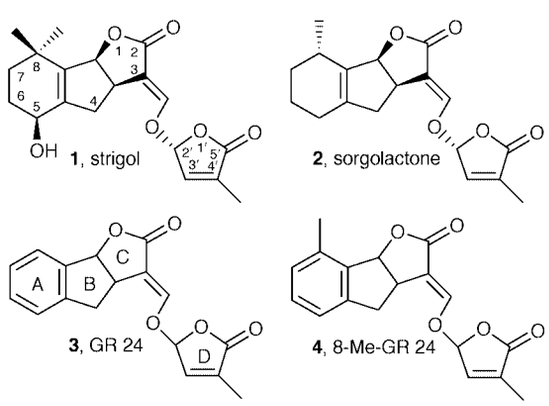


Fig. 1 Structures of strigol, sorgolactone and the synthetic germination stimulants GR 24 and 8-methyl GR 24.

Enantiopure samples of strigol, and several of its analogues have been obtained both by resolution using a chiral auxiliary, and by asymmetric synthesis.[14–17] Usually, these methods are relatively time consuming. To evaluate the influence of the stereochemistry of the stimulants on the germination of *Striga* and *Orobanche* seeds, a more expeditious route to obtain enantiopure strigolactone analogues would be desirable. In this paper, a facile route to the enantiopure aromatic strigolactone analogues 8-methyl GR 24 (**4**), and its regioisomer 6-methyl GR 24 (**12**), is presented. The biological activity of all compounds towards *S. hermonthica* and *O. crenata* was determined.

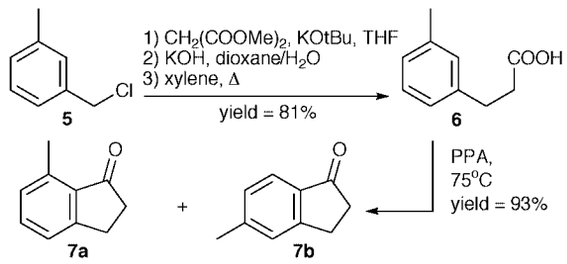
Results and discussion

Synthesis

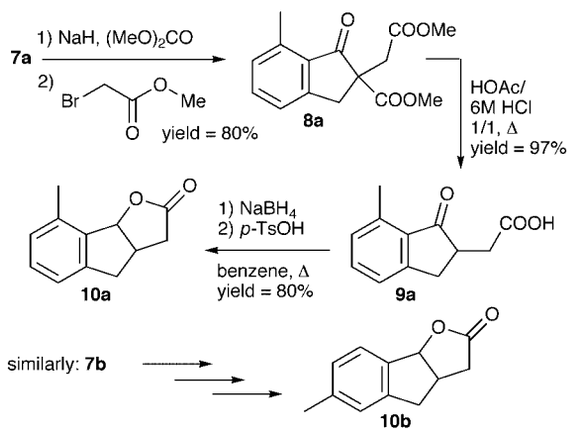
The strigolactones 8-methyl GR 24 (**4**) and 6-methyl GR 24 (**12**) were prepared following the same strategy as used for the synthesis of GR 24, namely first construction of the ABC-part followed by coupling with the D-ring. The ABC-part of the target compounds was synthesized starting from 1-(chloromethyl)-3-methylbenzene (**5**), as outlined in Scheme 1. Treatment with dimethyl malonate and subsequent decarboxylation in the usual manner, followed by ring closure using polyphosphoric acid[18] gave a 1 : 1 mixture of **7a** and **7b** in good yields.

The annelation of ring C was performed as described previously[9] (see Scheme 2) to give the ABC lactones **10a** and **10b**, respectively.

Before the coupling with the D-ring, **10a** and **10b** were formylated using ethyl formate and metallic sodium in the rather short reaction time of 1.5 h. It should be noted that usually the formylation of strigolactone ABC-parts is performed in THF or diethyl ether with 10 equivalents of ethyl formate employing KOtBu or NaH as the base in an overnight



Scheme 1 Synthesis of the AB-part of methyl-GR 24.



Scheme 2 Construction of the tricyclic lactones **10a** and **10b**.

reaction. The actual coupling with chlorobutenolide **11** was most conveniently conducted in THF, instead of the usual solvent DMF. In the present case, the initially obtained sodium enolate is a suspension in THF, which smoothly reacts with butenolide **11**. The thus obtained 1 : 1 mixture of diastereomers **4a** and **4b** was separated by flash chromatography over silica gel. Similarly **12a** and **12b** were acquired starting from **10b** (Scheme 3).

The racemic diastereomers **4a** and **4b** were both subjected to chromatographic separation using a semi-preparative cellulose carbamate Chiralcel OD® HPLC column, to give enantiomerically pure compounds **(+)-4a** and **(−)-4a**, and **(+)-4b** and **(−)-4b**, respectively. Similarly, all four diastereomers of **12** were obtained as single enantiomers. A semi-preparative chiral HPLC system was preferred over a preparative Chiralcel OD® column, because of the very high costs of large scale chiral HPLC material. In a single run the semi-preparative method allowed the separation of 1 mg of racemate in 30–90 min runtime. Repetitive chromatography readily afforded sufficient material for analysis and biological testing (5 mg). Also with racemic mixtures of sorgolactone, dehydroxy strigol and several simpler strigol analogues, good separation was achieved on this type of cellulose carbamate column, demonstrating the scope of this method.[19]

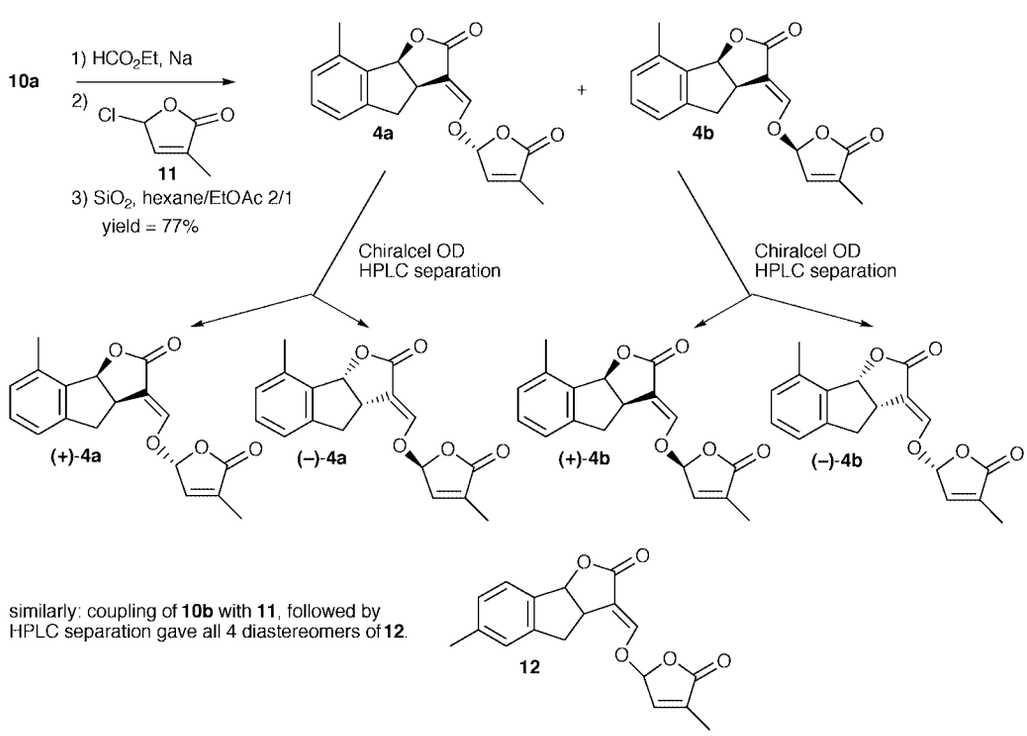
This method, involving the separation of enantiomers on a chiral semi-preparative HPLC column, is very useful when only small amounts of material are required for biological evaluation. Thus far, only (+)-strigol and its enantiomer have been separated on a column of cellulose triacetate.[20] Analogously, the ABC-parts of strigol[21] and GR 24[11] were separated on the aforementioned type of column.

The absolute configuration of the enantiopure compounds **4** and **12** was determined by comparing their optical rotation with the $[\alpha]_D^{20}$ values reported for all diastereomers of GR 24.[11] Clearly, the influence of the methyl group at the A-ring does not affect the optical rotation. For chiral strigol analogues containing the same chromophores as strigol and sorgolactone (*i.e.* the α,β unsaturated system of the CD-moiety) and no other chromophoric systems, circular dichroism can be used to determine their absolute stereochemistry. If no reference data are available, X-ray crystallography must be applied.

Biological activity

All enantiopure stereoisomers of **4** and **12** respectively, as well as their racemic mixtures, were assayed for their capacity to induce germination of *Striga hermonthica* and *Orobanche crenata* seeds. In all germination assays an aqueous solution of acetone (0.1% v/v) was included as a negative control and a diastereomeric 1 : 1 mixture of GR 24 as a positive control. This procedure enables comparison between results obtained in different test series.

In previous research, it was established that the concentration



Scheme 3 Coupling reactions of **10a** and **10b** with chlorobutenolide **11** and subsequent separation of the products in enantiopure diastereomers.

Table 1 Percentages of germinated seeds of *S. hermonthica* after exposure to solutions (5×10^{-6} and 5×10^{-9} mol L^{-1}) of 8-methyl GR 24 diastereoisomers **4** and the control GR 24[a]

Entry	Compound	% germination ± SE at a concentration of 5×10^{-6} mol L^{-1}	% germination ± SE at a concentration of 5×10^{-9} mol L^{-1}
1	**(+)-4a**	41.9 ± 3.3	31.3 ± 1.1
2	**(−)-4a**	38.9 ± 3.1	8.8 ± 0.3
3	**(+)-4b**	34.0 ± 1.5	13.1 ± 1.9
4	**(−)-4b**	40.4 ± 3.0	5.1 ± 0.7
5	**racemic 4**[c]	40.1 ± 3.2	26.1 ± 3.0
6	**GR 24**[c]	36.4 ± 3.8	25.8 ± 3.1

[a] Data presented are the mean ± SE of one representative experiment. [b] Not significantly different from aqueous control (without stimulant). [c] Equimolar mixture of two racemic diastereomers.

Table 2 Percentages of germinated seeds of *S. hermonthica* after exposure to solutions (5×10^{-6} and 5×10^{-9} mol L^{-1}) of 6-methyl GR 24 diastereoisomers **12** and the control GR 24[a]

Entry	Compound	% germination ± SE at a concentration of 5×10^{-6} mol L^{-1}	% germination ± SE at a concentration of 5×10^{-9} mol L^{-1}
1	**(+)-12a**	48.8 ± 1.5	33.3 ± 2.5
2	**(−)-12a**	23.3 ± 3.3	5.6 ± 0.8[b]
3	**(+)-12b**	32.5 ± 2.5	20.6 ± 1.7
4	**(−)-12b**	29.9 ± 2.7	4.6 ± 1.1[b]
5	**racemic 12**[c]	42.0 ± 1.7	32.0 ± 1.0
6	**GR 24**[c]	36.4 ± 3.8	25.8 ± 3.1

[a] Data presented are the mean ± SE of one representative experiment. [b] Not significantly different from aqueous control (without stimulant). [c] Equimolar mixture of two racemic diastereomers.

Table 3 Percentages of germinated seeds of *O. crenata* after exposure to solutions (5×10^{-5} and 2×10^{-7} mol L^{-1}) of 6-methyl GR 24 diastereoisomers **4** and the control GR 24[a]

Entry	Compound	% germination ± SE at a concentration of 5×10^{-5} mol L^{-1}	% germination ± SE at a concentration of 2×10^{-7} mol L^{-1}
1	**(+)-4a**	47.3 ± 3.4	20.2 ± 2.7
2	**(−)-4a**	32.5 ± 1.6	3.1 ± 0.9
3	**(+)-4b**	42.3 ± 3.9	12.8 ± 1.4
4	**(−)-4b**	41.1 ± 2.5	15.2 ± 2.6
5	**racemic 4**[c]	42.2 ± 2.9	17.9 ± 1.9
6	**GR 24**[c]	44.0 ± 1.3	15.7 ± 0.9

[a] Data presented are the mean ± SE of one representative experiment. [b] Not significantly different from aqueous control (without stimulant). [c] Equimolar mixture of two racemic diastereomers.

that induces half-maximal response (the ED_{50} value) of *S. hermonthica* seeds toward GR 24 is 5×10^{-9} M. Maximum germination is induced at concentrations $\geq 5 \times 10^{-6}$ M. For *O. crenata*, the ED_{50} value was established to be 2×10^{-7} M in the case of GR 24, whereas concentrations $\geq 5 \times 10^{-5}$ M cause maximum germination.[22] Because compounds **4** and **12** are very similar to GR 24, these concentrations were also used in the present bioassay.

The results of the bioassays with *Striga hermonthica* are collected in Tables 1 and 2, and a bar chart representation of this data is given in Fig. 2. The percentages of germinated *Orobanche crenata* seeds are presented in Tables 3 and 4 and in Fig. 3.

Biological testing of all four diastereomers of GR 24 revealed a very marked difference in germinating activity, between the diastereomer possessing the 'natural' absolute

Table 4 Percentages of germinated seeds of *O. crenata* after exposure to solutions (5×10^{-5} and 2×10^{-7} mol L^{-1}) of 6-methyl GR 24 diastereoisomers **12** and the control GR 24[a]

Entry	Compound	% germination ± SE at a concentration of 5×10^{-5} mol L^{-1}	% germination ± SE at a concentration of 2×10^{-7} mol L^{-1}
1	**(+)-12a**	48.2 ± 2.2	28.9 ± 0.9
2	**(−)-12a**	14.1 ± 2.1	0.7 ± 0.3[b]
3	**(+)-12b**	30.9 ± 1.4	17.1 ± 2.4
4	**(−)-12b**	29.6 ± 1.3	4.8 ± 0.3
5	**racemic 12**[c]	44.0 ± 2.3	26.1 ± 2.2
6	**GR 24**[c]	44.0 ± 1.3	15.7 ± 0.9

[a] Data presented are the mean ± SE of one representative experiment. [b] Not significantly different from aqueous control (without stimulant). [c] Equimolar mixture of two racemic diastereomers.

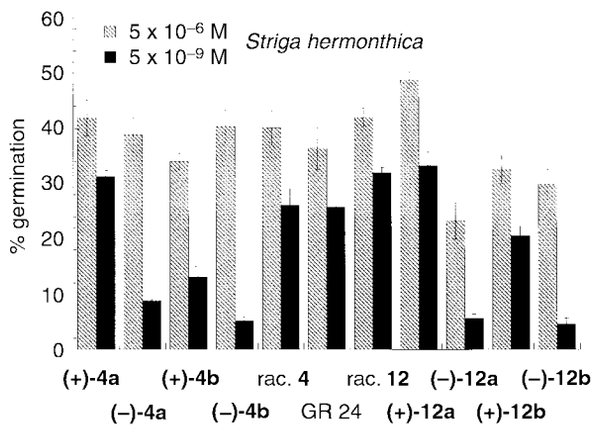


Fig. 2 Bar chart representation of the biological activities of methyl-GR 24 stereoisomers **4** and **12**, their racemates and the positive control GR 24 toward seeds of *Striga hermonthica*.

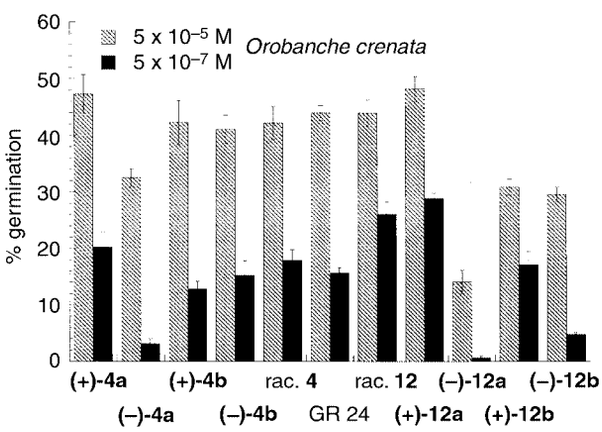


Fig. 3 Bar chart representation of the biological activities of methyl-GR 24 stereoisomers **4** and **12**, their racemates and the positive control GR 24 toward seeds of *Orobanche crenata.*

stereochemistry and its optical antipode. The 'natural' diastereomer was the most active one, whereas its mirror image was virtually inactive, both at the high and at the low concentrations that were tested (concentrations that are similar to the ones used in the present assay).[11] In contrast, in the case of sorgolactone, only at sensitive concentrations were clear differences in activity between the eight diastereomers observed. At higher concentrations, all sorgolactone diastereomers exhibited rather high activity.[13]

The results for compound **4** (Tables 1 and 3) are, in this respect, similar to those of sorgolactone: only at low stimulant concentrations, were there significant differences in activity

between the diastereomers; the 'natural' diastereomer, **(+)-4a**, was the most active one. For 6-methyl GR 24 (**12**), the results are more similar to those of GR 24 itself: even at the higher stimulant concentration, there are significant differences in activity between the 'natural' analogue **(+)-12a** and its antipode **(−)-12a**, especially in the case of *Orobanche*.

Racemic GR 24 induced equally high percentages of germinated seeds as the same concentration of the enantiopure 'natural' GR 24 isomer.[11] The same feature was observed for 8-methyl GR 24 and 6-methyl GR 24.

The bioassays reveal, that 8-methyl GR 24 (**4**) and 6-methyl GR 24 (**12**) are equally good synthetic germination stimulants as GR 24 itself. Only in the case of *O. crenata* did 6-methyl GR 24 show increased activity at the lower concentration in comparison with GR24. Therefore, it may be concluded that a small hydrophobic moiety on the 6-position of the A-ring leads to a somewhat more favourable interaction with the strigolactone receptor on *Orobanche crenata* seeds, resulting in a higher germination inducing activity.

In conclusion, the activity of all diastereomers of 8-methyl GR 24 and 6-methyl GR 24 followed the expected trend: the 'natural' diastereomer is the most active one, whereas its optical antipode is the least active. A methyl group at C-8 did not significantly increase the activity of the molecule toward *S. hermonthica* or *O. crenata* compared with GR 24, and the results of the germination assay are similar to those of the bioassay for all sorgolactone stereoisomers. When an extra methyl group is located on the 6-position, the results of the bioassay are similar to those of all diastereomers of GR 24, with the exception of the more active diastereomer **(+)-12b**. Especially for *Orobanche crenata* seeds, racemic **12** is a very active germination stimulant.

Experimental

Synthesis

General remarks. ^{1}H NMR (300 MHz) and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer, using Me_4Si as internal standard. All coupling constants are given as ^3J in Hz, unless indicated otherwise. Melting points were measured with a Reichert thermopan microscope and are uncorrected. IR spectra were recorded on a Bio-Rad FTS-25 instrument. For mass spectra a double focusing VG7070E mass spectrometer was used. GC-MS spectra were run on a Varian Saturn 2 GC-MS ion-trap system. Separation was carried out on a fused-silica capillary column (DB-5, 30 m × 0.25 mm). Helium was used as the carrier gas and electron impact (EI) was used as ionization mode. Elemental analyses were conducted on a Carlo Erba Instruments CHNSO EA 1108 element analyser. For the determination of optical rotations a Perkin-Elmer 241 polarimeter was used. Solvents were dried using the following methods: dichloromethane was distilled from P_2O_5; ethyl acetate was distilled from K_2CO_3; diethyl ether was distilled from NaH; hexane was distilled from CaH_2; tetrahydrofuran was distilled from lithium aluminium hydride just before use. All other solvents were of analytical grade and used as purchased. Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F254 plates (0.25 mm). Spots were visualized with UV or using a molybdate spray. Flash chromatography was carried out at a pressure of *ca.* 1.5 bar, using Merck Kieselgel 60H. Column chromatography at atmospheric pressure was performed with Merck Kieselgel 60. Preparative HPLC separation of racemates **4** and **12** was carried out on a semi-preparative Chiralcel OD® (10 μm) cellulose carbamate column (Daicel Chemical Industries Ltd., 1 × 25 cm) using mixtures of hexane and propan-2-ol or ethanol as the eluent. Products were detected with a Merck-Hitachi L-4000 UV detector at 254 nm. Enantiomeric excess and purities of enantiopure isomers of **4** and **12** were determined by analytical HPLC using a Chiralcel OD® (10 μm) cellulose carbamate column (Baker, 250 × 4.6 mm) with hexane–ethanol 85 : 15 (**12a**, **12b**) and 90 : 10 (**4a**, **4b**) as the eluent.

8-Methyl-3-{(*E*)-1-[(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)-oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one 4

To a cooled (0 °C) and stirred solution of tricyclic lactone **10a** (200 mg, 1.06 mmol) in ethyl formate (10 mL) was added, under a continuous stream of nitrogen, 1.1 equiv. of metallic sodium (27 mg, 1.17 mmol). The mixture was allowed to warm to room temperature and stirred for 1.5 hours. When TLC analysis indicated complete formylation, excess ethyl formate was removed by evaporation *in vacuo*. The thus obtained sodium salt of formylated **10a** was suspended in THF (10 mL) and cooled to 0 °C. Upon addition of chlorobutenolide **11** (212 mg, 1.6 mmol) the reaction mixture became clear. After 2 hours of stirring at room temperature, another 0.5 equiv. of **11** (70 mg, 0.53 mmol) was added and the mixture was stirred overnight. Then THF was removed *in vacuo*. The residue was dissolved in a mixture of brine and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2 ×) and the combined organic layers were washed with saturated NH_4Cl (1 ×), dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified using flash chromatography (SiO_2, hexane–ethyl acetate 2 : 1) to afford two diastereomeric products. Fast moving diastereomer **4a** (129 mg, 39%) and slow moving diastereomer **4b** (126 mg, 38%) were obtained as white crystalline solids. **4a**: mp: 199–200 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H, CH_3 D-ring), 2.44 (s, 3H, CH_3 A-ring), 3.08 (dd, 1H, $J_{4,3a,cis}$ = 3.7 Hz, ^2J = 16.9 Hz, H4), 3.43 (dd, 1H, $J_{4,3a,trans}$ = 9.5 Hz, ^2J = 16.9 Hz, H4), 3.93 (m, 1H, H3a), 6.00 (d, 1H, J = 7.9 Hz, H8b), 6.17 (m, 1H, H2′), 6.97 (m, 1H, H3′), 7.06 (m, 2H, H5 + H7), 7.24 (m, 1H, H6), 7.48 (d, 1H, $^4J_{3a,6'}$ = 2.5 Hz, H6′). ^{13}C NMR ($CDCl_3$): δ 11.4 (CH_3 D-ring), 19.2 (CH_3 A-ring), 38.4, 39.1 (CH_2, CH3a), 86.2 (CH8b), 101.2 (CH2′), 114.2 (Cq4′), 123.0, 129.1, 130.9, (3 × CH_{arom}), 136.6, 137.6, 138.2, 143.4 (3 × Cq_{arom}, Cq-C ring), 141.6 (CH3′), 151.5 (CH6′), 170.9, 172.0 (2 × C=O). IR (KBr): ν (cm^{-1}) 1787, 1733, 1681 (C=O, C=C), 1182 (lactone). MS [EI *m*/*z*, rel. intensity (%)]: 312 ([M]$^+$, 2.0), 215 ([$C_{13}H_{11}O_3$]$^+$, 39.7), 97 ([$C_5H_5O_2$]$^+$, 100). Anal. calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.19; H, 5.20%. **4b**: mp: 178–179 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 3H, CH_3 D-ring), 2.44 (s, 3H, CH_3 A-ring), 3.07 (dd, 1H, $J_{4,3a,cis}$ = 3.6 Hz, ^2J = 17.0 Hz, H4), 3.42 (dd, 1H, $J_{4,3a,trans}$ = 9.5 Hz, ^2J = 17.0 Hz, H4), 3.92 (m, 1H, H3a), 6.00 (d, 1H, J = 7.9 Hz, H8b), 6.18 (m, 1H, H2′), 6.97 (m, 1H, H3′), 7.06 (m, 2H, H5 + H7), 7.24 (m, 1H, H6), 7.48 (d, 1H, $^4J_{3a,6'}$ = 2.5 Hz, H6′). ^{13}C NMR ($CDCl_3$): δ 11.4 (CH_3 D-ring), 19.2 (CH_3 A-ring), 38.4, 39.0 (CH_2, CH3a), 86.2 (CH8b), 101.3 (CH2′), 114.3 (Cq4′), 123.0, 129.0, 131.0, (3 × CH_{arom}), 136.6, 137.5, 138.1, 143.4 (3 × Cq_{arom}, Cq-C ring), 141.7 (CH3′), 151.5 (CH6′), 170.9, 172.1 (2 × C=O). IR (KBr): ν (cm^{-1}) 1785, 1738, 1678 (C=O, C=C), 1181 (lactone). MS [EI *m*/*z*, rel. intensity (%)]: 312 ([M]$^+$, 3.0), 215 ([$C_{13}H_{11}O_3$]$^+$, 52.8), 97 ([$C_5H_5O_2$]$^+$, 100). Anal. calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.26; H, 5.19%.

(3a*R*,8b*S*)-8-Methyl-3-{(*E*)-1-[(2′R)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one (+)-4a and its enantiomer (3a*S*,8b*R*)-8-methyl-3-{(*E*)-1-[(2′*S*)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]-furan-2-one (−)-4a

Fast moving 8-Me GR24 diastereomer **4a** was separated into its enantiomers **(+)-4a** and **(−)-4a** using a semi-preparative Chiralcel OD® HPLC column and hexane–propan-2-ol 90 : 10 as the eluent. Flow: 3.3 mL min^{-1}. Recrystallization from hexane–dichloromethane afforded **(+)-4a** and **(−)-4a** as colour-

less needles. **(+)-4a**: mp: 156–157.5 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = +435.4$ ($c = 0.02$, CH_2Cl_2). **(−)-4a**: mp: 157–158 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = -430.0$ ($c = 0.04$, CH_2Cl_2). All other analytical data were identical with those of the racemate **4a**.

(3a*R*,8b*S*)-8-Methyl-3-{(*E*)-1-[(2′*S*)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one (+)-4b and its enantiomer (3a*S*,8b*R*)-8-methyl-3-{(*E*)-1-[(2′*R*)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]-furan-2-one (−)-4b

Slow moving 8-Me GR24 diastereomer **4b** was separated into its enantiomers **(+)-4b** and **(−)-4b** using a semi-preparative Chiralcel OD® HPLC column and hexane–ethanol 90 : 10 as the eluent. Flow: 3.3 mL min^{-1}. Recrystallization from hexane–dichloromethane gave **(+)-4b** and **(−)-4b** as colourless needles. **(+)-4b**: mp: 159–160 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = +288.2$ ($c = 0.03$, CH_2Cl_2). **(−)-4b**: mp: 159–160 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = -291.6$ ($c = 0.04$, CH_2Cl_2). All other analytical data were identical with those of the racemate **4b**.

Dimethyl 2-(3-methylbenzyl)malonate

Dimethyl malonate (17.2 mL, 150 mmol) was dissolved in tetrahydrofuran (100 mL). Potassium *tert*-butoxide (16.8 g, 150 mmol) was added gradually and the resulting slurry was stirred at room temperature for 30 minutes. 1-(Chloromethyl)-3-methylbenzene **5** (13.2 mL, 100 mmol) was added dropwise and the mixture was stirred overnight. After 24 hours, TLC analysis indicated complete conversion. THF was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (100 mL) and washed with water (3 ×). Drying ($MgSO_4$) and concentration *in vacuo* gave the desired diester as a colourless oil, which was used in the next reaction without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.29 (s, 3H, CH_3), 3.17 (d, 2H, $J = 7.8$ Hz, CH_2), 3.66 (s, 6H, 2 × OCH_3), 3.66 (t, 1H, $J = 7.8$ Hz, CH), 6.95–7.16 (m, 4H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 21.8 (CH_3), 35.2 (CH_2), 52.9 (CH), 54.0, 54.1 (2 × OCH_3), 126.1, 127.5, 128.9, 129.9 (4 × CH_{arom}), 138.1, 138.2 (2 × Cq_{arom}), 169.7 (2 × C=O). IR (KBr): ν (cm^{-1}) 1755, 1737 (C=O). GC-MS [EI *m*/*z*, rel. intensity (%)]: 236 ($[M]^+$, 50.8), 176 ($[C_{11}H_{12}O_2]^+$, 100), 145 ($[C_{10}H_9O]^+$, 88.3), 105 ($[C_8H_9]^+$, 31.4), 91 ($[C_7H_7]^+$, 27.5), 77 ($[C_5H_6]^+$, 18.5).

2-(3-Methylbenzyl)malonic acid

Crude dimethyl 2-(3-methylbenzyl)malonate (~150 mmol) was hydrolysed in a mixture of dioxane (50 mL) and 5 M aqueous potassium hydroxide (100 mL). The solution was stirred overnight at room temperature and then concentrated to a volume of approximately 75 mL. Non-ionic species were removed by extraction with ethyl acetate. The organic layer was discarded and the aqueous phase was acidified with sulfuric acid until the pH < 5. The acidic solution was extracted with ethyl acetate (3 ×). The combined organic extracts were washed with water (1 ×), dried ($MgSO_4$) and concentrated *in vacuo* to yield 2-(3-methylbenzyl)malonic acid as a white solid (28.7 g, 92% 2 steps). An analytical sample was obtained by recrystallization from hexane–ethyl acetate. Mp: 136–138 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, CH_3), 3.21 (d, 2H, $J = 7.6$ Hz, CH_2), 3.75 (t, 1H, $J = 7.6$ Hz, CH), 6.99–7.25 (m, 4H, H_{arom}), 9.63 (br s, 2H, 2 × COOH). ^{13}C NMR ($CDCl_3$): δ 22.0 (CH_3), 35.0 (CH_2), 54.1 (CH), 126.4, 128.5, 129.3, 130.1 (4 × CH_{arom}), 137.6, 139.0 (2 × Cq_{arom}), 175.0 (2 × C=O). IR (KBr): ν (cm^{-1}) 3019 (broad, OH), 1709 (C=O). MS [EI *m*/*z*, rel. intensity (%)]: 208 ($[M]^+$, 9.8), 164 ($[C_{10}H_{12}O_2]^+$, 37.8), 105 ($[C_8H_9]^+$, 100), 91 ($[C_7H_7]^+$, 19.9), 77 ($[C_5H_6]^+$, 15.3). Anal. calcd. for $C_{11}H_{12}O_4$: C, 63.46; H, 5.77. Found: C, 63.39; H, 5.70%.

3-(3-Methylphenyl)propanoic acid 6

2-(3-Methylbenzyl)malonic acid (20.8 g, 100 mmol) was dissolved in xylene (150 mL) and heated to reflux for 4 hours and then cooled. Evaporation of the solvent afforded acid **6** as a colourless oil that was sufficiently pure for use in the following cyclization reaction. Yield: 88%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, CH_3), 2.65 (t, 2H, $J = 7.8$ Hz, ArCH_2), 2.90 (t, 2H, $J = 7.8$ Hz, CH_2COOH), 6.98–7.19 (m, 4H, H_{arom}), 8.9 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ 22.0 (CH_3), 31.1, 36.3 (2 × CH_2), 125.9, 127.8, 129.1, 129.7 (4 × CH_{arom}), 138.1, 140.7 (2 × Cq_{arom}), 180.3 (C=O). IR (KBr): ν (cm^{-1}) 3024 (broad OH), 1706 (C=O). GC-MS [EI *m*/*z*, rel. intensity (%)]: 119 ($[C_9H_{11}]^+$, 31.0), 105 ($[C_8H_9]^+$, 9.9), 91 ($[C_7H_7]^+$, 55.3), 77 ($[C_5H_6]^+$, 7.0).

7-Methylindan-1-one 7a and 5-methylindan-1-one 7b

Polyphosphoric acid (PPA, 75 g) was stirred with an efficient mechanical stirrer and heated to 75 °C. 3-(3-Methylphenyl)-propanoic acid (**6**) (9.84 g, 60 mmol) was added at once and the mixture was stirred at 75 °C during 1 hour. The appearance of a persistent bright red colour indicates completion of the reaction.[23] The mixture was cooled to room temperature and ice water (100 mL) was added to decompose the PPA. The acidic solution was extracted with diethyl ether (3 ×) and the combined ether fractions were subsequently washed with water, saturated $NaHCO_3$ and water. Drying ($MgSO_4$), followed by concentration *in vacuo* resulted in an oily mixture of 7-methylindan-1-one (**7a**) and 5-methylindan-1-one (**7b**) (8.15 g, 93%) in a ratio of 1 : 1 (determined by NMR).[24] Separation was achieved by column chromatography (SiO_2, dichloromethane–hexane 1 : 1). Recrystallization from dichloromethane–hexane afforded pure **7a** (3.15 g, 36%) and **7b** (4.0 g, 46%) both as white needles. **7a**: mp: 54–55 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.62 (s, 3H, CH_3), 2.63 (t, 2H, $J = 6.0$ Hz, ArCH_2), 3.05 (t, 2H, $J = 6.0$ Hz, CH_2C=O), 7.07 (d, 1H, $J = 7.3$ Hz, H6), 7.25 (d, 1H, $J = 7.3$ Hz, H4), 7.41 (m, 1H, H5). ^{13}C NMR ($CDCl_3$): δ 18.9 (CH_3), 25.9, 37.3 (2 × CH_2), 124.5, 129.6, 134.5 (3 × CH_{arom}), 135.0, 139.3, 156.4 (3 × Cq_{arom}), 208.4 (C=O). IR (KBr): ν (cm^{-1}) 2916 (arom. C-H), 1702 (C=O). GC-MS [EI *m*/*z*, rel. intensity (%)]: 146 ($[M]^+$, 90.1), 117 ($[C_9H_9]^+$, 100), 91 ($[C_7H_7]^+$, 15.3). Anal. calcd. for $C_{10}H_{10}O$: C, 82.16; H, 6.89. Found: C, 81.63; H, 6.76%. **7b**: mp: 70–71 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 3H, CH_3), 2.67 (t, 2H, $J = 5.9$ Hz, ArCH_2), 3.09 (t, 2H, $J = 5.9$ Hz, CH_2C=O), 7.17 (d, 1H, $J = 7.8$ Hz, H6), 7.27 (s, 1H, H4), 7.65 (d, 1H, $J = 7.8$ Hz, H7). ^{13}C NMR ($CDCl_3$): δ 22.7 (CH_3), 26.3, 37.0 (2 × CH_2), 124.1, 127.6, 129.2 (3 × CH_{arom}), 135.5, 146.4, 156.3 (3 × Cq_{arom}), 207.2 (C=O). IR (KBr): ν (cm^{-1}) 2919 (arom. C-H), 1699 (C=O). GC-MS [EI *m*/*z*, rel. intensity (%)]: 146 ($[M]^+$, 80.1), 117 ($[C_9H_9]^+$, 100), 91 ($[C_7H_7]^+$, 18.6). Anal. calcd. for $C_{10}H_{10}O$: C, 82.16; H, 6.89. Found: C, 82.23; H, 6.85%.

Methyl 2-(2-methoxy-2-oxoethyl)-7-methyl-1-oxoindane-2-carboxylate 8a

7-Methylindan-1-one was alkylated according to the method of Mangnus *et al.*[9] Yield: 80%. Mp: 59–60 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.62 (s, 3H, CH_3), 2.74 and 3.36 (AB, 2H, $J = 17.3$ Hz, CH_2COOMe), 3.14 and 3.86 (AB, 2H, $J = 17.6$ Hz, ArCH_2), 3.66 and 3.68 (2 × s, 2 × 3H, 2 × OCH_3), 7.14 (d, 1H, $J = 7.4$ Hz, H6), 7.30 (d, 1H, $J = 7.6$ Hz, H4), 7.47 (m, 1H, H5). ^{13}C NMR ($CDCl_3$): δ 19.0 (CH_3), 37.9, 39.4 (2 × CH_2), 52.5, 53.6 (2 × OCH_3), 58.8 (Cq_{aliph}), 124.3, 130.3, 135.5 (3 × CH_{arom}), 132.8, 140.6, 154.7 (3 × Cq_{arom}), 171.1, 172.1 (2 × *C*OOMe), 207.2 (C=O). IR (KBr): ν (cm^{-1}) 2956 (arom. C-H), 1737 (C=O, ester), 1701 (C=O, ketone). GC-MS [EI *m*/*z*, rel. intensity (%)]: 276 ($[M]^+$, 16.4), 245 ($[C_{14}H_{13}O_4]^+$, 57.5), 216 ($[C_{13}H_{12}O_3]^+$, 100), 157 ($[C_{11}H_9O]^+$, 80.5), 77 ($[C_6H_5]^+$, 11.4). Anal. calcd. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 65.47; H, 5.65%.

Methyl 2-(2-methoxy-2-oxoethyl)-5-methyl-1-oxoindane-2-carboxylate 8b

Diester **8b** was prepared in 79% yield starting from ketone **7b** analogous to the procedure described by Mangnus *et al.*[9] Mp: 94 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (s, 3H, CH_3), 2.76 and 3.35 (AB, 2H, J = 17.4 Hz, CH_2COOMe), 3.15 and 3.85 (AB, 2H, J = 17.6 Hz, ArCH_2), 3.66 and 3.67 (2 × s, 2 × 3H, 2 × OCH_3), 7.21 (d, 1H, J = 7.9 Hz, H6), 7.29 (s, 1H, H4), 7.67 (d, 1H, J = 7.9 Hz, H7). ^{13}C NMR ($CDCl_3$): δ 22.8 (CH_3), 38.2, 39.2 (2 × CH_2), 52.5, 53.6 (2 × OCH_3), 58.8 (Cq_{aliph}), 125.4, 127.4, 129.8 (3 × CH_{arom}), 133.0, 147.7, 154.5 (3 × Cq_{arom}), 171.0, 172.1 (2 × *C*OOMe), 201.0 (C=O). IR (KBr): ν (cm^{-1}) 2958 (arom. C-H), 1747, 1734 (C=O, ester), 1703 (C=O, ketone). GC-MS [EI *m*/*z*, rel. intensity (%)]: 276 ([M]$^+$, 16.5), 245 ([$C_{14}H_{13}O_4$]$^+$, 58.5), 216 ([$C_{13}H_{12}O_3$]$^+$, 100), 157 ([$C_{11}H_9O$]$^+$, 67.0), 77 ([C_6H_5]$^+$, 9.0). Anal. calcd. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 65.25; H, 5.75%.

2-(7-Methyl-1-oxo-2,3-dihydro-1*H*-inden-2-yl)acetic acid 9a

Carboxylic acid **9a** was obtained from diester **8a** in 97% yield using the procedure reported by Mangnus *et al.*[9] Mp: 146–148 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.63 (s, 3H, CH_3), 2.59 (dd, 1H, $J_{vic,trans}$ = 10.0 Hz, ^2J = 17.9 Hz, CH_2COOH), 2.85 (dd, 1H, $J_{vic,cis}$ = 4.6 Hz, ^2J = 17.0 Hz, ArCH_2), 3.00 (m, 1H, CH), 3.03 (dd, 1H, $J_{vic,cis}$ = 4.4 Hz, ^2J = 17.9 Hz, CH_2COOH), 3.42 (dd, 1H, $J_{vic,trans}$ = 7.8 Hz, ^2J = 17.0 Hz, ArCH_2), 7.11 (d, 1H, J = 7.4 Hz, H6), 7.26 (d, 1H, J = 7.6 Hz, H4), 7.46 (m, 1H, H5), 9.30 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ 19.0 (CH_3), 33.2, 35.8 (2 × CH_2), 44.3 (CH), 124.4, 130.0, 135.0 (3 × CH_{arom}), 134.2, 139.8, 154.5 (3 × Cq_{arom}), 179.0 (COOH), 208.1 (C=O). IR (KBr): ν (cm^{-1}) 3041 (broad, OH), 1696 (C=O), 1596 (arom. ring). MS [EI *m*/*z*, rel. intensity (%)]: 204 ([M]$^+$, 100), 159 ([$C_{11}H_{11}O$]$^+$, 81.4), 91 ([C_7H_7]$^+$, 26.9), 77 ([C_6H_5]$^+$, 14.5). Anal. calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.57; H, 5.82%.

2-(5-Methyl-1-oxo-2,3-dihydro-1*H*-inden-2-yl)acetic acid 9b

Acid **9b** was prepared in the same way as described for **9a**. Yield: 98%. Mp: 115–117 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (s, 3H, CH_3), 2.60 (dd, 1H, $J_{vic,trans}$ = 9.9 Hz, ^2J = 17.9 Hz, CH_2COOH), 2.85 (dd, 1H, $J_{vic,cis}$ = 4.3 Hz, ^2J = 17.1 Hz, Ar-CH_2), 3.01 (m, 1H, CH), 3.03 (dd, 1H, $J_{vic,cis}$ = 4.3 Hz, ^2J = 17.9 Hz, CH_2COOH), 3.42 (dd, 1H, $J_{vic,trans}$ = 7.6 Hz, ^2J = 17.1 Hz, ArCH_2), 7.19 (d, 1H, J = 7.9 Hz, H6), 7.26 (s, 1H, H4), 7.66 (d, 1H, J = 7.9 Hz, H7), 9.50 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$): δ 22.8 (CH_3), 33.6, 35.8 (2 × CH_2), 44.2 (CH), 124.6, 127.5, 129.6 (3 × CH_{arom}), 134.4, 147.1, 154.5 (3 × Cq_{arom}), 178.4 (COOH), 206.9 (C=O). IR (KBr): ν (cm^{-1}) 3023 (broad, OH), 1713, 1697 (C=O), 1612 (arom. ring). MS [EI *m*/*z*, rel. intensity (%)]: 204 ([M]$^+$, 76.0), 159 ([$C_{11}H_{11}O$]$^+$, 100), 91 ([C_7H_7]$^+$, 17.7), 77 ([C_6H_5]$^+$, 8.9). Anal. calcd. for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.42; H, 5.85%.

8-Methyl-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one 10a

Cyclization of carboxylic acid **9a** was effected by the method of House *et al.*[25] Tricyclic lactone **10a** was obtained as colourless needles in 80% yield. Mp: 71–73 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, CH_3), 2.43 (dd, 1H, $J_{3,3a,cis}$ = 4.9 Hz, ^2J = 18.1 Hz, CH_2C=O), 2.90 (m, 1H, ArCH_2), 2.92 (dd, 1H, $J_{3,3a,trans}$ = 6.6 Hz, ^2J = 18.1 Hz, CH_2C=O), 3.32 (dd, 1H, $J_{4,3a,trans}$ = 8.6 Hz, ^2J = 15.5 Hz, ArCH_2), 3.34 (m, 1H, H3a), 5.95 (d, 1H, J = 7.0 Hz, H8b), 7.08 (m, 2H, H5 + H7), 7.26 (m, 1H, H6). ^{13}C NMR ($CDCl_3$): δ 19.0 (CH_3), 36.4, 38.9 (2 × CH_2), 37.4 (CH3a), 87.9 (CH8b), 123.1, 129.1, 130.8 (3 × CH_{arom}), 137.4, 138.0, 143.4 (3 × Cq_{arom}), 177.7 (C=O). IR (KBr): ν (cm^{-1}) 1757 (C=O), 1180 (lactone). GC-MS [EI *m*/*z*, rel. intensity (%)]: 189 ([M]$^+$, 18.1), 144 ([$C_{11}H_{12}$]$^+$, 57.3), 129 ([$C_{10}H_9$]$^+$, 100), 77 ([C_6H_5]$^+$, 5.8). Anal. calcd. for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.71; H, 6.34%.

6-Methyl-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one 10b

Lactone **10b** was prepared in the same manner as reported for **10a**. Yield: 82%. Mp: 62–63 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 2.36 (dd, 1H, $J_{3,3a,cis}$ = 5.4 Hz, ^2J = 18.0 Hz, CH_2C=O), 2.82 (dd, 1H, $J_{4,3a,cis}$ = 2.8 Hz, ^2J = 16.0 Hz, ArCH_2), 2.88 (dd, 1H, $J_{3,3a,trans}$ = 9.5 Hz, ^2J = 18.0 Hz, CH_2C=O), 3.27 (dd, 1H, $J_{4,3a,trans}$ = 8.4 Hz, ^2J = 16.0 Hz, ArCH_2), 3.33 (m, 1H, H3a), 5.84 (d, 1H, J = 6.9 Hz, H8b), 7.07 (s, 1H, H5), 7.09 (d, 1H, J = 7.6 Hz, H7), 7.35 (d, 1H, J = 7.6 Hz, H8). ^{13}C NMR ($CDCl_3$): δ 22.1 (CH_3), 36.4, 38.5 (2 × CH_2), 38.2 (CH3a), 88.2 (CH8b), 126.4, 126.6, 129.1 (3 × CH_{arom}), 136.6, 140.7, 143.4 (3 × Cq_{arom}), 177.6 (C=O). IR (KBr): ν (cm^{-1}) 1765 (C=O), 1170 (lactone). GC-MS [EI *m*/*z*, rel. intensity (%)]: 189 ([M]$^+$, 18.6), 144 ([$C_{11}H_{12}$]$^+$, 57.3), 129 ([$C_{10}H_9$]$^+$, 100), 77 ([C_6H_5]$^+$, 5.1). Anal. calcd. for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.64; H, 6.26%.

6-Methyl-3-{(*E*)-1-[(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]-furan-2-one 12

6-Methyl GR 24 (**12**) was prepared in the same way as described for its isomer **4**. Yield: fast moving diastereomer **12a** (130 mg, 39%), slow moving diastereomer **12b** (122 mg, 37%). **12a**: Mp: 205 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 3H, CH_3 D-ring), 2.35 (s, 3H, CH_3 A-ring), 3.05 (dd, 1H, $J_{4,3a,cis}$ = 3.3 Hz, ^2J = 16.9 Hz, H4), 3.39 (dd, 1H, $J_{4,3a,trans}$ = 9.3 Hz, ^2J = 16.9 Hz, H4), 3.93 (m, 1H, H3a), 5.92 (d, 1H, J = 7.8 Hz, H8b), 6.17 (m, 1H, H2′), 6.96 (m, 1H, H3′), 7.04 (s, 1H, H5), 7.10 (d, 1H, J = 7.8 Hz, H7), 7.38 (d, 1H, J = 7.8 Hz, H8), 7.46 (d, 1H, $^4J_{3a,6'}$ = 2.5 Hz, H6′). ^{13}C NMR ($CDCl_3$): δ 11.5 (CH_3 D-ring), 22.1 (CH_3 A-ring), 37.9, 39.8 (CH_2, CH3a), 86.5 (CH8b), 101.2 (CH2′), 114.3 (Cq4′), 126.2, 126.8, 129.2 (3 × CH_{arom}), 136.7, 136.8, 140.8, 143.5 (3 × Cq_{arom}, Cq-C ring), 141.5 (CH3′), 151.4 (CH6′), 170.8, 172.0 (2 × C=O). IR (KBr): ν (cm^{-1}) 1793, 1784, 1748, 1678 (C=O, C=C), 1186 (lactone). MS [EI *m*/*z*, rel. intensity (%)]: 312 ([M]$^+$, 3.6), 215 ([$C_{13}H_{11}O_3$]$^+$, 39.1), 97 ([$C_5H_5O_2$]$^+$, 100). Anal. calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.09; H, 5.09%. **12b**: Mp: 166 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (s, 3H, CH_3 D-ring), 2.35 (s, 3H, CH_3 A-ring), 3.05 (dd, 1H, $J_{4,3a,cis}$ = 3.3 Hz, ^2J = 16.9 Hz, H4), 3.37 (dd, 1H, $J_{4,3a,trans}$ = 9.3 Hz, ^2J = 16.9 Hz, H4), 3.92 (m, 1H, H3a), 5.92 (d, 1H, J = 7.8 Hz, H8b), 6.17 (m, 1H, H2′), 6.96 (m, 1H, H3′), 7.04 (s, 1H, H5), 7.09 (d, 1H, J = 7.8 Hz, H7), 7.38 (d, 1H, J = 7.8 Hz, H8), 7.47 (d, 1H, $^4J_{3a,6'}$ = 2.5 Hz, H6′). ^{13}C NMR ($CDCl_3$): δ 11.5 (CH_3 D-ring), 22.1 (CH_3 A-ring), 37.9, 39.7 (CH_2, CH3a), 86.5 (CH8b), 101.3 (CH2′), 114.4 (Cq4′), 126.3, 126.7, 129.2 (3 × CH_{arom}), 136.6, 136.7, 140.9, 143.5 (3 × Cq_{arom}, Cq-C ring), 141.6 (CH3′), 151.5 (CH6′), 170.8, 171.9 (2 × C=O). IR (KBr): ν (cm^{-1}) 1792, 1783, 1738, 1680 (C=O, C=C), 1203 (lactone). MS [EI *m*/*z*, rel. intensity (%)]: 312 ([M]$^+$, 5.0), 215 ([$C_{13}H_{11}O_3$]$^+$, 47.8), 97 ([$C_5H_5O_2$]$^+$, 100). Anal. calcd. for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.03; H, 5.10%.

(3a*R*,8b*S*)-6-Methyl-3-{(*E*)-1-[(2′*R*)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)oxy]methylidene-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one (+)-12a and its enantiomer (3a*S*,8b*R*)-6-methyl-3-{(*E*)-1-[(2′*S*)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′yl)-oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]-furan-2-one (−)-12a

Fast moving 6-Me GR24 diastereomer **12a** was separated into its enantiomers **(+)-12a** and **(−)-12a** using a semi-preparative Chiralcel OD® HPLC column and hexane–propan-2-ol 80 : 20 as the eluent. Flow: 3.0 mL min^{-1}. Recrystallization from hexane–dichloromethane yielded **(+)-12a** and **(−)-12a** as

colourless needles. **(+)-12a**: mp: 162–163 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = +417.2$ ($c = 0.03$, CH_2Cl_2). **(−)-12a**: mp: 160–161 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = -428.4$ ($c = 0.03$, CH_2Cl_2). All other analytical data were identical with those of the racemate **12a**.

(3a*R*,8b*S*)-6-Methyl-3-{(*E*)-1-[(2′*S*)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]furan-2-one (+)-12b and its enantiomer (3a*S*,8b*R*)-6-methyl-3-{(*E*)-1-[(2′*R*)-(4′-methyl-5′-oxo-2′,5′-dihydrofuran-2′-yl)-oxy]methylidene}-3,3a,4,8b-tetrahydro-2*H*-indeno[1,2-*b*]-furan-2-one (−)-12b

Slow moving 6-Me GR24 diastereomer **12b** was separated into its enantiomers **(+)-12b** and **(−)-12b** using a semi-preparative Chiralcel OD® HPLC column with hexane–ethanol 85:15 as the eluent. Flow: 3.0 mL min^{-1}. Recrystallization from hexane–dichloromethane furnished **(+)-12b** and **(−)-12b** as colourless needles. **(+)-12b**: mp: 159–161 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = +275.6$ ($c = 0.04$, CH_2Cl_2). **(−)-12b**: mp: 158–160 °C. Ee >99% (determined by HPLC). $[\alpha]_D^{20} = -267.9$ ($c = 0.04$, CH_2Cl_2). All other analytical data were identical with those of the racemate **12b**.

Biological activity

Plant material. Seeds of *Striga hermonthica* (Del.) Benth. were collected from Sorghum (*Sorghum bicolor* (L.) Moench) on Gezira Research Station, Sudan in 1994. *Orobanche crenata* Forsk. Seeds were harvested from faba bean (*Vicia faba* L.) in Beheira, Egypt in 1993. The seeds were stored in glass vials in the dark at room temperature until use in germination tests.

Preparation of the test solutions. A compound to be tested was weighed out very accurately to the amount of 1.0 mg, dissolved in 5 mL acetone p.a. and diluted with demineralized water to 50 mL. These stock solutions of approximately 10^{-4} mol L^{-1} (the exact concentration depending on the molecular mass of the compound used) were further diluted with demineralized water to obtain test solutions with concentrations ranging from 5×10^{-5} to 5×10^{-9} mol L^{-1}. All solutions were prepared directly before use.

Bioassays. For surface sterilization all seeds were exposed for 5 minutes to a 50% (v/v) aqueous solution of commercial bleach (2% hypochlorite). Subsequently, the seeds were thoroughly rinsed with demineralized water and air-dried. For conditioning the seeds were spread on glass fibre filter paper disks (8 mm diameter, approximately 60–100 seeds per disk) in Petri dishes, wetted with demineralized water and stored in the dark at 20 °C for *Orobanche* seeds and at 30 °C for *Striga* seeds. Thereafter the conditioning water was removed and conditioned seeds were placed in new Petri dishes and exposed to test solution. After incubation for 24 h (*Striga*) and 7 days (*Orobanche*) in the dark at the indicated temperatures the percentages of germinated seeds were determined under a microscope. Seeds were considered to be germinated if the radicle protruded through the seed coat. In each test series an aqueous solution of 0.1% (v/v) acetone was included as a negative control. For full details of the bioassay, see Mangnus *et al.*[26]

References

1 J. Sauerborn, in *Proceedings of the Fifth International Symposium on Parasitic Weeds*, eds. J. K. Ransom, L. J. Musselman, A. D. Worsham and C. Parker, CIMMYT, Nairobi, Kenya, 1987, p. 137.
2 C. Parker and C. R. Riches, *Parasitic Weeds of the World*: *Biology and Control*, CAB International Press: Wallingford, Oxon, UK, 1993.
3 L. G. Butler, *ACS Symposium Series*, 1995, **582**, 158.
4 C. E. Cook, L. P. Whichard, B. Turner, M. E. Wall and G. H. Egley, *Science*, 1966, 1189.
5 C. Hauck, S. Müller and H. Schildknecht, *J. Plant Physiol.*, 1992, **139**, 474.
6 S. Müller, C. Hauck and H. Schildknecht, *J. Plant Growth Regul.*, 1992, **11**, 77.
7 T. Yokota, H. Sakai, K. Okuno, K. Yoneyama and Y. Takeuchi, *Phytochemistry*, 1998, **49**, 1967.
8 A. W. Johnson, G. Gowda, A. Hassanali, J. Knox, S. Monaco, Z. Razawi and G. Roseberry, *J. Chem. Soc.*, *Perkin Trans. 1*, 1981, 1734.
9 E. M. Mangnus, F. J. Dommerholt, R. L. P. de Jong and B. Zwanenburg, *J. Agric. Food Chem.*, 1992, **40**, 1230.
10 E. M. Mangnus and B. Zwanenburg, *J. Agric. Food Chem.*, 1992, **40**, 697.
11 J. W. J. F. Thuring, G. H. L. Nefkens and B. Zwanenburg, *J. Agric. Food Chem.*, 1997, **45**, 2278.
12 J. W. J. F. Thuring, N. W. J. T. Heinsman, R. W. A. W. M. Jacobs, G. H. L. Nefkens and B. Zwanenburg. *J. Agric. Food Chem.*, 1997, **45**, 507.
13 Y. Sugimoto, S. C. M. Wigchert, J. W. J. F. Thuring and B. Zwanenburg, *J. Org. Chem.*, 1998, **63**, 1259.
14 J. B. Heather, R. S. D. Mittal and C. J. Sih, *J. Am. Chem. Soc.*, 1976, **98**, 3661.
15 D. W. Brooks, H. S. Bevinakatti and D. R. Powell, *J. Org. Chem.*, 1985, **50**, 3779.
16 J. W. J. F. Thuring, G. H. L. Nefkens, R. Schaafstra and B. Zwanenburg, *Tetrahedron*, 1995, **51**, 5047.
17 S. Röhrig, L. Hennig, M. Findeisen and P. Welzel, *Tetrahedron Lett.*, 1997, **38**, 5489.
18 H. O. House and M. Schellenbaum, *J. Org. Chem.*, 1963, **28**, 31.
19 Unpublished results from the authors' laboratory.
20 C. Hauck and H. Schildknecht, *J. Plant Physiol.*, 1990, **136**, 126.
21 E. Samson, K. Frischmuth, U. Berlage, U. Heinz, K. Hobert and P. Welzel, *Tetrahedron*, 1991, **47**, 1411.
22 S. C. M. Wigchert, E. Kuiper, G. J. Boelhouwer, G. H. L. Nefkens, J. A. C. Verkleij and B. Zwanenburg, *J. Agric. Food Chem.*, 1999, **47**, 1705.
23 H. R. Snyder and F. X. Werber, *J. Am. Chem. Soc.*, 1950, **72**, 2965.
24 J. A. Elvidge and R. G. Foster, *J. Chem. Soc.*, 1963, 590.
25 H. O. House, H. Badad, R. B. Toothill and A. W. Noltes, *J. Org. Chem.*, 1962, **27**, 4141.
26 E. M. Mangnus, P. L. A. Stommen and B. Zwanenburg, *J. Plant Growth Regul.*, 1992b, **11**, 91.

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