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New Synthesis of A-Ring Aromatic Strigolactone Analogues and Their Evaluation as Plant Hormones in Pea (*Pisum sativum*)

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Abstract: A new general access to A-ring aromatic strigolactones, a new class of plant hormones, has been developed. The key transformations include in sequence ring-closing metathesis, enzymatic kinetic resolution and a radical cyclization with atom transfer to install the tricyclic ABC-ring system. The activity as plant hormones for the inhibition of shoot branching in pea of various analogues synthesized by this strategy is reported.

Introduction

Strigolactones (SLs), the most recent class of hormones identified in plants,^[1] are especially studied in pea, *Arabidopsis*, petunia and rice. Formed mainly in the lower parts of the stem and roots and transported presumably to the aerial parts,^[2] they suppress shoot branching^[3] and are involved in nodule formation,^[4] root architecture^[5] and the stimulation of cambium activity.^[6] Their production would be inversely correlated with the concentration of phosphate and nitrogen available for the plants.^[7] SLs belong to a class of compounds first identified in 1966 as stimulants of the seed germination of parasitic weeds *Orobanche* and *Striga*.^[8] They are produced in trace concentrations and are partly excreted in the rhizosphere. These molecules, recently identified as stimulants for spore germination and hyphal proliferation of arbuscular mycorrhizal fungi (AMF),^[9] were also

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found to have an effect on phytopathogenic fungi.^[10] In these AMF symbioses, plants receive water and mineral nutrients from their fungal partners, hence promoting optimal plant growth conditions. SLs belong to a class of compounds, the γ -butyrolactone, known as pheromones or allelochemicals:^[11] Recently, one of us^[12] demonstrated that SLs regulate protonema branching and act as a quorum sensing-like signal in the moss *Physcomitrella patens* emphasizing their roles in both plant development and communications between organisms. The structural core of SLs is a tricyclic lactone (ABC rings) with various substitution patterns on AB rings. It is connected via an enol ether linkage to an invariable α , β -unsaturated furanone moiety (D-ring) (Scheme 1).

To date, at least 15 naturally occurring SLs have been completely identified and characterized in root exudates. It is expected that many other SLs or derivatives will be identified in the future.^[1] They are derived from the carotenoid pathway, involving isomerization of β -carotene 1 by a β -carotene isomerase (D27), and cleavage at the C9',C10' position by Carotenoid Cleavage Dioxygenases 7 (CCD7) to form 9-cis-\beta-apo-10'-carotenal. Introduction of oxygens and intramolecular rearrangement by CCD8 lead to carlactone 2.^[13] Bioactive carlactone 2, very recently isolated, is central for understanding the biosynthesis of SLs but also for conceiving SL analogues. It implies that the BC rings are formed after the D ring to give 5-deoxystrigol (3) contrary to a previous hypothesis.^[14] This required cyclization was recently rationalized^[15] by synthetic studies based on a simple linear precursor by an acid-catalyzed double cyclization. Further hydroxylations, epoxidation/oxidation and methyl transfer or acetylation from 3 would lead to hydroxy-SLs or acetylated-SLs: strigol (4), strigyl acetate (4-Ac), orobanchol (5),^[16] orobanchyl acetate (5-Ac), solanacol (6) and fabacyl acetate (7), six major hydroxylated and acetylated-SLs found in Nature. Although the synthetic aromatic SL analogue GR24 has been known for a long time^[17] as a reference compound in bioassays on seed germination of parasitic weeds, solanacol (6), the first natural SL containing a

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- 4849



Scheme 1. Biosynthetic precursors of SLs, selected natural SLs and synthetic analogue GR24. $D27 = Dwarf 27 = \beta$ -carotene isomerase); CCD = Carotenoid Cleavage Dioxygenase.

phenyl ring was recently isolated from tobacco and tomato root exudates.^[18,19] GR24 presents high germination activity and increased stability compared to natural SLs, which are sensitive in a natural media resulting from a Michael addition of water on the enol ether linkage. The bioactiphore for the germination of parasitic weeds has been extensively studied since its discovery in the 1970s and was found reside in the CD part.^[20] Essential structural features for AM fungi branching appear to be similar, but important SL structure variations seem to affect bioactivity.^[21] Contrary to the germination of parasitic weeds, the oxygenation pattern of the SL and the replacement of the enol ether link between rings C and D by an alkoxy or an imino ether have little effect in activity. Also, the presence of the ABC-rings is essential.

The great importance of SLs in many plant chemical biology areas with opposite effects (repression of cell division in axillary bud and stimulation of cambial activity or hyphal proliferation) depending on the target, their intriguing origin in green lineage,^[22] and their extremely low bio-availability prompted us to develop a new strategy for the synthesis of this class of compounds to perform structure-activity relationship studies as a plant hormone. Indeed, contrary to the germination activity,^[1] few studies have been described for the hormonal function of SLs.^[3b,23] Due to the fact that compounds active on the parasitic seed germination^[24] possess a hydroxyl group at the C4 position and are the most difficult to synthesize, we focused on developing a ChemPubSoc Europe

method to access SL analogues bearing substituents at this position. We^[25] recently reported the first total synthesis of (-)-solanacol and the first SAR study on pea as a plant hormone.^[26] We established that the presence of a Michael acceptor and a methylbutenolide or a dimethylbutenolide motif in the same molecule is essential. We demonstrated that SLs show potent activity for the control of shoot branching in a structure dependent manner but with low specificity. Herein, we present a full report concerning our strategy for the enantioselective synthesis of the natural aromatic SL solanacol and epimers useful for our very recently published SAR study. We also report a detailed diastereoselective synthesis for A-ring aromatic derivatives and helpful complementary information on our SAR study in pea,^[26] confirming our first findings. This class of SL derivatives bearing a hydroxy group at C4 were only tested for the germination of parasitic weeds^[27] for which they showed great differences in bioactivity from one species to another.

Results and Discussion

Our retrosynthetic analysis is outlined in Scheme 2. Construction of aromatic SLs **8** was envisioned from tricyclic lactones **10a–b** with the correct relative stereochemistry by coupling with bromofuranone **9** as the D-ring precursor, using well-known procedures.^[28] The stereoselective introduction of a C4-hydroxyl group^[29] could be achieved from trichlorides **11a–b** by a substitution with retention of configuration at this benzylic position. Compounds **10a–b** could in turn be derived from enantiopure esters **12a–b** by an atom transfer radical cyclization (ATRC),^[30] which would originate from a ring closing metathesis (RCM)/kinetic resolution sequence on dienes **13a–b**. These aromatic dienes would be prepared from inexpensive and commercially available 2-bromostyrene and 3,4-dimethyl phenol.

Alcohol **13a** was easily prepared in two steps from 2-bromostyrene by formylation and alkylation with vinylmagnesium bromide in an 89% overall yield (Scheme 3). Because of the aromatic *o*-dimethyl substitution, the preparation of



Scheme 2. Retrosynthesis of A-ring aromatic SLs for SAR studies targeted in this study.

4850

diene 13b needed a more elaborated route requiring nine steps from the starting 3,4-dimethylphenol. It started by a selective o-bromation at position 6 of 3,4-dimethylphenol. This step suitably established a temporary protection at this position in the following sequence. Thus, alkylation of the phenol with allyl bromide and Lewis acid catalyzed Claisen rearrangement were completely regioselective providing bromide 14 in a 90% overall yield. Isomerization of the terminal C-C double bond in 14 (90% yield) and ozonolysis furnished aldehyde 15 (79% yield). It was quantitatively debrominated by catalytic hydrogenolysis using hydrogen and Pd/ C in the presence of an excess of triethylamine. The vinylation conditions of the trisubstituted phenol were obtained by triflation under standard conditions to triflate 16, and cross-coupling with vinyl trifluoroborate^[31] under Suzuki-Miyaura conditions to furnish aldehyde 17 (98% yield). Vinylation with the vinylic Grignard reagent completed the sequence to diene 13b in an 89% overall yield from 16.

Elaboration of the B- and C-ring was studied using ruthenium-catalyzed RCM and ATRC reactions on dienes **13a** and **13b** as shown in Table 1. Not surprisingly, direct trichloroacetylation of alcohol **13a** led to the quantitative formation of **19** (entry 1, Table 1) via a [3,3]-sigmatropic rearrangement. This result impeded a possible RCM/ATRC tandem version to the ABC-tricyclic structure.

Elaboration of the B-ring was performed using a rutheniumcatalyzed RCM reaction on dienes **13a**,**b** furnishing racemic indenols (\pm) -**18a**,**b** in high yields (entries 2–3, Table 1).

RCM followed by trichloroacetylation of indenol could be done in a one-pot procedure (entry 4, Table 1) but any at-



Scheme 3. Formation of the B-ring precursors **13a** and **13b**. a) *n*BuLi, *N*-formylpiperidine, THF, -78 °C to RT, 18 h, 94%; b) CH₂=CHMgBr, THF, RT, 3 h, 95%; c) 1) Br₂, CH₂Cl₂, 0 °C, 1 h, 90%; d) allyl bromide, K₂CO₃, acetone, reflux, 3 h, quant.; e) Et₂AlCl, hexane, RT, 6 h, quant.; f) *t*BuOK, THF, RT, 48 h, 90%; g) O₃, CH₂Cl₂, -78 °C; Me₂S, -78 °C to RT, 12 h, 79%; h) Pd/C, H₂, NEt₃, MeOH, RT, 2 h, quant.; i) Pyr, Tf₂O, CH₂Cl₂, 0 °C to RT, 1 h, 74%; j) CH₂=CHMgBr, THF, RT, 3 h, 91%.

FULL PAPER

Table 1. Synthesis of the tricyclic lactones 20 a,b.



Entry	Starting material	Conditions	Product (yield [%])
1	13 a	CCl ₃ COCl ^[a]	19 (78)
2	13 a	Grubbs ^[b]	18a (84)
3	13b	Grubbs ^[b]	18b (88)
4	13b	1) Grubbs, 2) (CCl ₃ CO) ₂ O ^[c]	12b (88)
5	13b	1) Grubbs, 2) (CCl ₃ CO) ₂ O, 3) Grubbs ^[d]	12a (50)
6	13b	1) Grubbs, 2) (CCl ₃ CO) ₂ O, 3) CuCl; one-pot procedure ^[e]	12a (34)
7	13b	1) Grubbs, 2) (CCl ₃ CO) ₂ O, one-pot procedure, ^[c] 3) CuCl ^[f]	11a (65)
8	13 a	1) Grubbs, 2) (CCl ₃ CO) ₂ O, one-pot procedure, ^[c] 3) CuCl ^[f]	11b (66)

[a] CCl₃COCl, Et₃N, Et₂O, 0°C, 15 min. [b] Grubbs I catalyst, 5 mol%, CH₂Cl₂, RT, 12 h. [c] 1) Grubbs I catalyst, 5 mol%, toluene, RT, 12 h, 2) (CCl₃CO)₂O, pyr, 0°C, one-pot procedure. [d] 1) Grubbs I catalyst, 5 mol%, toluene, RT, 12 h, 2) (CCl₃CO)₂O, pyr, 0°C, 3) Grubbs I catalyst, 5 mol%, one-pot procedure. [e] 1) Grubbs I catalyst, 5 mol%, one-pot procedure. [e] 1) Grubbs I catalyst, 5 mol%, toluene, RT, 12 h, 2) (CCl₃CO)₂O, pyr, 0°C, 3) CuCl/ dHBipy catalyst, 5 mol%, one-pot procedure. [f] CuCl/dHBipy catalyst, 5 mol%, DCE, 90°C, 12 h.

tempt of ATRC by Grubbs I^[30] or copper(I) catalysts in the same medium failed to furnish the tricyclic lactone **11a** (entries 5–6, Table 1). The best way to synthesize **11a**, **11b** was ultimately to proceed via isolation of trichloroesters **12a**, **12b** and cyclization to lactones **11a**, **11b** in a next step using catalytic copper(I) coordinated to 4,4'-di-*n*-heptylbipyridine $(dHbipy)^{[32]}$ (entries 7–8, Table 1). The stereoselective lactonization to the ABC-tricyclic system **11a** (**11b**) was best obtained in a 78% (77%) yield, for the ATRC step.^[30,32] Sterically-controlled halogen transfer to benzylic radical **20** from the Cu^{II} complex generated in the catalytic process, proceeded stereoselectively *anti* to ring C. The stereochemistry was unambiguously established by X-ray analysis of **11b**.^[25]

Access to enantiomerically pure indenol derivatives was conveniently performed by enzymatic kinetic resolution. Alcohols (\pm)-**18a** and **18b** were acetylated to esters (\pm)-**21a** and (\pm)-**21b**, respectively (Scheme 4). Their kinetic resolution with immobilized *Candida antarctica* lipase^[33] was particularly efficient in producing both enantiomerically pure alcohols (*R*)-**18a,b** (>99% *ee*), and the enantiomeric esters (*S*)-**21 a,b** (>99% *ee*).^[33] Direct kinetic resolution of allylic trichloroester (\pm)-**12b** unfortunately failed as the *Candida antarctica* lipase was completely inactive on this substrate. The enantiomerically pure ABC-tricyclic trichlorides (+)-**11a,b** and (-)-**11a,b** were elaborated from (+)-**18a,b** and (-)-**18a,b** following the steps (RCM, trichloroacetyla-

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Scheme 4. Enzymatic kinetic resolution of (\pm) -18a,b. a) Ac₂O, pyr, THF, 0°C, 15 min, 21a: 78% from 13a, 21b: 95%; b) immobilized Candida antarctica lipase, CH₃CN, H₂O, 24 h, 50% of (R)-18a,b, 50% of (S)-21 a,b.



F.-D. Boyer, J.-M. Beau et al.



Scheme 6. Presumed intermediates in the formation of the C4-hydroxy ABC tricyclic compounds 23-25. HFIP = hexafluoroisopropanol.

Table 2. Formation of the C4-hydroxy ABC tricyclic compounds 23-25.

tion and ATRC) reported above (Table 1) for the racemic substrates.

No enantioselective synthesis of GR24 has been reported until now and the only preparation of the (+)- and (-)-GR24 we are aware of was established by chromatographic resolution of lactone 22.[34] With (+)- and (-)-11a in hand, a novel access to (+)- and (-)-GR24 was easily achieved by dechlorination leading to (+)- and (-)-22 with Bu₃SnH in high yield (97%). Unfortunately, dechlorination with zinc dust was not compatible with the presence of a chlorine atom at C4. Enantiopure (+)and (-)-22 are the precursors to (+)- and (-)-GR24 via formylation and coupling with D-ring precursor 9 (Scheme 5) as was early described.^[34]



Scheme 5. Enantioselective access to GR24. a) Bu₃SnH, AIBN, toluene, 90°C, 15 min, 22: 97%; b) see reference [34].

The following substitution of the benzylic chlorine atom in 11a and 11b by a hydroxyl group was achieved with good stereocontrol under conditions varying according to the substrate (Table 2). Under basic conditions (NaOH generated from MeONa in a mixture of MeOH/H₂O), chloride 11a led to alcohol 10a with inversion of configuration at C4 (entry 1, Table 2). Under the same conditions **11b** gave an equimolar mixture of 25b and 24 (entry 2, Table 2). This indicated that, in both cases, the displacement of the chlorine atom was followed by the opening of the lactone and re-closure on either hydroxyl groups through undetected diol 27 (Scheme 6). This event was hidden with **11a**, which only gave product 23a because of the symmetry in the open intermediate **27** ($\mathbf{R}^1 = \mathbf{R}^2 = \mathbf{H}$).

11a 11b ^{or}					
	23a : R ¹ = 23b: R ¹ =	$R^2 = H$ 24 $R^2 = Me$	25a : R ¹ = R ² = H 25b : R ¹ = R ² = Me		
Entry	Starting material	Conditions	Product (yield [%])		
1	11a	MeONa (1.1 equiv), MeOH/H ₂ O 9:1, RT, 4 h	23 a (82)		
2	11b	MeONa (1.1 equiv), MeOH/H ₂ O 9:1, RT, 4 h	23 b/24 ^[a] 1:1 (64)		
3	11b	H ₂ O/HFIP 1:1, 90 °C, 1 h	25 b/23 b ^[a] 9:1 (quant.)		
4	(+)-11 a	H ₂ O/HFIP 1:1, 90 °C, 4 h	(±)- 23 a (82)		
a) Incenarable mixture					

eparable mixture [a]

> The substitution with retention of configuration of the benzylic chlorine atom in (+)-, (-)- and (\pm)-11b (entry 3, Table 2) by a hydroxyl group was achieved with good stereocontrol (9:1, anti/syn to ring C) under S_N1 conditions, by heating a solution of 11b in a 1:1 mixture of water/hexafluoroisopropanol.^[35] The same conditions applied on (+)-11a, however, gave racemic (\pm) -23a because of the above opening/closure of the lactone precluding the use of this strategy to prepare enantiopure 4-hydroxy-GR24 derivatives (entry 4, Table 2). This last result emphasizes the importance of the aromatic dimethyl substitution in 11b to facilitate the formation of the presumed benzylic cationic intermediate 26, trapped by water anti to ring C in a S_N1 fashion. This provided an enantioselective access to (+)- or (-)solanacol starting from (+)-25b or (-)-25b (Scheme 6).

> At this stage, the synthesis was pursued with (\pm) -23a and (\pm) -25b as well as with the enantiopure compounds (+)and (-)-25b. Dechlorination of the gem-dichloro motif with zinc dust afforded tricyclic lactone 28a (10b) in a 95% (91%) overall yield from 23a (25b/23b) (Scheme 7).

> To allow^[28c] coupling with the D-ring, inversion of the stereochemistry of the hydroxy group at C4 (28a to 10a) was performed in high yield (90%) under Mitsunobu conditions. Derivative 29a bearing a fluorine atom at C4 could easily be prepared from alcohol 28a using Deoxo-Fluor at -78°C. Careful formylation, by controlling the temperature, of 10a, 10b, and 29a followed by coupling (in the same pot in the case of 10b) with D-ring precursor 9 completed the synthesis to provide the same proportions of compound 6 identified as (-)-solanacol^[25] and (-)-2'-epi-solanacol 30b

FULL PAPER



Scheme 7. Final steps in the synthesis of aromatic SL analogues. a) Zn dust, NH₄Cl, MeOH, 0°C to reflux, 2 h, (95% for 28a (two steps), 91% for **10b** (two steps)); b) Deoxo-Fluor, CH₂Cl₂, -78 °C, 2 h, 91 %; c) PPh₃, DEAD, PhCO₂H, THF, RT, 12 h; d) K₂CO₃, MeOH; RT, 2 h, 90% (2 steps), e1) 1) tBuOK, ethyl formate, THF, -10°C, 12 h, 83%; 2) 9, K₂CO₂, NMP, RT, 3 h, 36% for **30 a**; 34% for **31 a**; 52% for the inseparable mixture 36/37 1:1; e1) Ac2O, pyr, RT, 12 h, 92%. e2) tBuOK, ethyl formate, THF, -78 to -40 °C, 6 h; 9, -60 °C to RT, 12 h, 38% for 3; 38% for 43; f) Ac₂O, pyr, RT, 12 h, 50% for 32 and 40% for 33 from a mixture (1:1) of 30/31; 37% for 32b and 38% for 33b from a 1:1 mixture of 30b/6; g) DCC, DMAP, n-butyric acid, CH2Cl2, 0°C to RT, 3 h, 38% for 34 and 28% for 35 from a 1:1 mixture of 30 a/31 a; h) DCC, DMAP, octanoic acid, CH₂Cl₂, 0 °C to RT, 3 h, 43 % for 36 and 37 % for 37 from a 1:1 mixture of 30 a/31 a; i) DCC, DMAP, undecanoic acid, CH2Cl2, 0°C to RT, 3 h, 35% for 38 and 26% for 39 from a 1:1 mixture of 30a/31a; j) DCC, DMAP, Boc-Ala-OH, CH2Cl2, 0°C to RT, 3 h, 72% for 40 from 31 a.

separated by chromatography on silica gel in a 76% combined yield. The same sequence furnished 4-OH-GR24 30a, 4-OH-2'-epi-GR24 31a and 4-F-GR24 (41/42) as an inseparable mixture of diastereoisomers in the latter case. The stereochemistry at C2' relative to the other asymmetric centers was unambiguously assessed by X-ray analysis of compounds $6^{[25]}$ and **30b** showing an *R* configuration at C2' in 6 and an S configuration at this center in 30b. We clearly established, in our previous communication, that the natural product corresponded to the more polar synthetic diastereoisomer (-)- $6^{[25]}$ by comparing the CD and NMR spectra of (-)-6 with that of the natural product.^[17] This first total synthesis of solanacol (6) and 2'-epi-solanacol (30b) involved 15 linear steps with a 21 % overall yield. Our synthesis of 4-OH-GR24 30a and 4-OH-2'-epi-GR24 31a is more rapid (10 linear steps) but with a similar overall yield (24%). We previously^[25,26] demonstrated that solanacyl acetate 33b is a more active compared with 6 to inhibit the outgrowth of axillary buds into branches. These results prompted us to examine the effect of the substitution at C4 on the biological activity by synthesis of esters 34/35, 36/37, 38/39 and 40 from alcohols 30 a/31 a using standard procedures (Scheme 7).

Pea (*Pisum sativum*) is an excellent model for physiological studies, and in particular, to study the control of branching. Its simple architecture is particularly suitable for precise exogenous hormone applications directly onto the bud at the axil of leaf. One major advantage of our bioassay with direct bud application is the small quantity (less than 1 mg) of molecule needed contrary to the relatively large amount of samples required in rice where a hydroponic culture has to be used.^[3b,23] The evaluation of hormonal activity in inhibiting bud outgrowth with our synthesized compounds was performed and compared to the active synthetic SL analogue (\pm)-GR24 commonly used for this bioassay.^[3a] Application of the solution to be tested (10 µL per plant) was carried out directly onto the axillary bud at node 3 or 4 of 10 day-old SL-deficient *rms1/ccd8* plants.^[3a]

At a concentration of 1 µm, a significant effect in bud outgrowth repression was observed for the new compounds of the solanacol series in comparison with control 0 (Figure 1). Hydroxy-SLs were already found less active than their corresponding acetates,^[26] which was also observed here with 2'-epimer 30b being less active than 33b and GR24 (Figure 1B). At a concentration of 100 nm, a significant effect for branching inhibition was observed for GR24 and acetates 32b and 33b (Figure 1A and B). Bud outgrowth activity was found barely significant at 10 nm for acetate **33b** (P =0.12, Kruskal-Wallis rank sum test) (Figure 1A).^[26] The difference of activity of hydroxyl-SL when compared with the corresponding acetate, may be attributed to its instability in an aqueous solution or to its lower lipophilicity making it difficult for 6 to reach the receptor. This last hypothesis was reinforced by a molecular bioassay using transcript levels in axillary buds of the transcription factor PsBRC1, 6 h after SL application. PsBRC1 is the homologue from pea of the Arabidopsis BRANCHED1 (BRC1) and TEOSINTE BRANCHED1 (TB1) from Maize and is transcriptionally regulated by SL. Transcript levels were found significantly higher for 33b compared to solanacol 6, 6 h after application.^[26,36] In contrast to the importance of the absolute configuration of SLs for their germination stimulation activities in root parasitic weeds^[1a,27] and stimulation of hyphal branching in AM fungi,^[21] we observed no significant activity difference for the (+) and (-)-enantiomers (Figure 1).

At a 1 μ M concentration, a significant effect in bud outgrowth repression was observed for the new compounds of the 4-R-GR24 series except for compound **40** (Figure 2A). As for the solanacol series, the activity of acetates **32a** and **33a** is higher than that of the corresponding alcohols **30a** and **31a**, especially at low concentrations <1 μ M (Figure 2B). Similar activity was found by replacing the hydroxy group by a fluorine atom (fluorides **41/42**) compared with the acetates **32a** or **33a** (Figure 2A). However the esterification of 4-OH by N-Boc alanine (**40**) led to loss of bioactivity (Figure 2A). Surprisingly, the esters 4-OR-2'-*epi*-GR24 (**33a**,

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Figure 1. Comparative activity in the solanacol series. A) Length of axillary bud, 10 d after direct application at node 4 for compounds GR24, (+)-**30b**, (-)-**30b**, (±)-**33b** at 1 μ M to 10 nM concentrations. B) Length of axillary bud, 8 d after direct application at node 3 for compounds GR24, (+)-**32b**, (-)-**32b**, (+)-**33b**, (-)-**33b**, (±)-**33b** at 1 μ M to 100 nM concentrations. Data are means ±SE (n=24). * P < 0.05, *** P < 0.001 indicate significant differences with GR24 at the same concentration (Kruskal–Wallis rank sum test). CTL 0=control 0.

35, **37** and **39**) bearing a linear alkyl chain from 1 to 10 carbons presented similar dose dependent activity (Figure 3). This result underlines the fact that the ABC-part is not important for the hormonal activity.

Conclusion

In summary, we have exploited an efficient ring closing metathesis/enzymatic kinetic resolution/atom transfer radical cyclization sequence of key transformations to construct the key ABC-ring system in the first synthesis of the natural aromatic SL solanacol (6). This firmly established its complete structure. We have demonstrated that this strategy can be applied to other SL analogues. We have further established that solanacyl acetate **33b**, a natural SL found in tobacco,^[37] showed in our bioassay high hormonal activity for shoot branching but not better than GR24.^[26] The hydrophobic analogues tested were more active in our bioassays than the hydroxyl analogues. Our SAR studies of SLs as plant hormones confirmed that SLs show potent activity in a structure dependent manner but with low specificity concerning the stereochemistry or the substitution at C4 posi-



Figure 2. Comparative activity in the 4-R-GR24 series. A) Comparative activity of compounds **32a-33a** and **40-42** at 1 μ M concentration. B) Comparative activity of compounds **30a-33a** and GR24 at 1 μ M to 10 nM concentrations. Length of axillary bud, 8 days after direct application of a solution of the tested compound at node 3. Data are means \pm SE (n= 24). * P < 0.05, ** P < 0.01, *** P < 0.001 indicate significant differences with GR24 at the same concentration (Kruskal–Wallis rank sum test). P < 0.001 indicates significant differences with CTL 0 (Kruskal–Wallis rank sum test). CTL 0=control 0.



Figure 3. Comparative activity of more active 4-OR-2'-epi-GR24 (33a, 35, 37, 39). Length of axillary bud, 10 d after direct application at node 3. Data are means \pm SE (n=24). P < 0.001 indicates significant differences with CTL 0 (Kruskal–Wallis rank sum test). CTL 0=control 0.

tion. These results confirm our previous ones demonstrating the low specificity of the SL receptor^[38] for shoot branching inhibition^[26] contrary to other models (AM fungi^[21] and parasitic plants^[20]). A mechanism for the SL mode of action involving the hydrolysis of the butenolide D ring,^[39] very recently proposed, is in accordance with our last SAR data.^[26]

Experimental Section

All non-aqueous reactions were run under an inert atmosphere (argon), by using standard techniques for manipulating air-sensitive compounds. All glassware was stored in the oven and/or was flame-dried prior to use. THF was purified by distillation, under nitrogen, from sodium/benzophenone. CH2Cl2 was dried by distillation under nitrogen from P2O5. All reagents and solvents were commercially available and were used without further purification. Chiral HPLC analyses were performed using a Chiralcel AD-H column (4.6×250 mm) with UV detection at 254 nm. Analytical thin-layer chromatography (TLC) was performed on plates precoated with silica gel layers (PLC silica gel 60 F254, 0.5 mm). Visualization of the developed chromatogram was followed by UV absorbance and/or by staining with vanillin or 5% ethanolic phosphomolybdic acid and heat as developing agent. Flash column chromatography was performed using 35-70 mesh silica. NMR spectra (1H; 13C) were recorded respectively at (300; 75) MHz. Chemical shifts are reported in parts per million relative to an internal standard of residual chloroform (δ = 7.24 ppm for ¹H NMR and 77.23 ppm for ¹³C NMR). For the ¹H spectra, data were reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q= quartet, m=multiplet, brs=broad singulet), coupling constant in Hz, integration, and assignments. Assignments were obtained using DEPT 135, ¹H-¹H COSY, ¹H-¹³C HMQC and ¹H, ¹³C HMBC experiments. IR spectra were reported in reciprocal centimeters (cm⁻¹). Mass spectra (MS) were determined either by electrospray ionization (ESI) or chemical ionization with ammonia (CI, NH₃) and high-resolution mass spectra (HRMS) were determined by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Optical rotations were determined using a cell of 1 dm-length path. Data are reported as follows: $[\alpha]_{D}^{T}$, concentration (c in g per 100 mL) and solvent.

(3a*R**,4*S**,8b*S**)-3,3,4-Trichloro-3,3a,4,8b-tetrahydro-2*H*-indeno-[1,2-

b]furan-2-one (11a): A solution of commercial CuCl (54 mg, 0.54 mmol, 0.05 equiv) and dHbipy (191 mg, 0.54 mmol, 0.05 equiv) in degassed DCE (10 mL) was prepared and stirred at room temperature under argon. The solution turned into dark brown after 10 min and a solution of trichloroester 12a (3.00 g, 10.81 mmol, 1 equiv) in degassed DCE (30 mL) was added to it. The mixture was heated at 90 °C for 12 h. The solvent was evaporated and the crude product was directly purified on silica gel (heptane/EtOAc 95:5). The product 11a (2.34 g, 8.43 mmol, 78%) was obtained as a very pale yellow solid. (+)-(3aR,4S,8bS)-11a: $[\alpha]_{\rm D}^{24} = +113.9 \ (c = 1, \text{CHCl}_3); \ (-)-(3aS,4R,8bR)-11a: \ [\alpha]_{\rm D}^{26} = -115.2 \ (c$ = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ = 7.58–7.42 (M, 4H, Har), 5.96 (d, 1H, $J_{8b,3a} = 6.0$ Hz, H8b), 5.43 (d, 1H, $J_{4,3a} = 6.0$ Hz, H4), 4.02 ppm (t, 1H, $J_{3a,4} = J_{3a,8b} = 6.0$ Hz, H3a); ¹³C NMR (75 MHz, CDCl₃): $\delta = 167$ (C2), 143 (C8), 135 (C4a), 132 (Car), 131 (Car), 127 (Car), 126 (Car), 83 (C8b), 79 (C3), 66 (C3a), 60 ppm (C4); IR (film): $\nu = 2929$, 1798, 1466, 1249, 1162, 1151, 955, 909, 726 cm⁻¹; MS (IE): m/z: 278 [M+2H]⁺, 276.0 [M]⁺, 241 [M-Cl]⁺; elemental analysis (%) calcd for $C_{11}H_{13}BrO:\ C$ 47.60, H 2.54, O 11.53; found: C 47.46, H 2.50, O 11.61.

(-)-(3a*R*,4*R*,8b*R*)-7,8-Dimethyl-3,3,4-trichloro-3,3a,4,8b-

tetrahydroindeno[1,2-b]furan-2-one (11b): A solution of commercial CuCl (5.0 mg, 0.05 mmol, 0.05 equiv) and dHbipy (19.0 mg, 0.05 mmol, 0.05 equiv) in degassed dichloroethane (2 mL) was prepared and stirred at room temperature under argon. The solution turned to dark brown after 10 min and a solution of trichloroester **12b** (328.0 mg, 1.07 mmol, 1 equiv) in degassed dichloroethane (4 mL) was added. The mixture was heated at 90 °C for 6 h, cooled to RT and the solvent evaporated under reduced pressure. The crude product was directly purified on silica gel (heptane/ethyl acetate 95:5). Product 11b (271 mg, 0.89 mmol, 83%) was obtained as a white solid. M.p. 135–137 °C; $[\alpha]_{D}^{26} = -130.5$ (c = 1.03, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ = 7.31 (d, $J_{6,5}$ = 7.9 Hz, 1 H, H6), 7.20 (d, $J_{5,6} = 7.9$ Hz, 1 H, H5), 6.03 (d, $J_{8b,3a} = 5.8$ Hz, 1 H, H8b), 5.40 (d, $J_{4,3a} = 5.8$ Hz, 1 H, H4), 3.98 (t, $J_{3a,4} = 5.8$, $J_{3a,8b} = 5.8$ Hz, 1 H, H1), 2.34 (s, 3H, H10), 2.31 ppm (s, 3H, H9); ¹³C NMR (75 MHz, CDCl₃): δ = 167.1 (C2), 140.3 (C4a), 139.1 (C7), 135.2 (C8), 133.9 (C8a), 133.8 (C6), 122.7 (C5), 82.3 (C8b), 78.9 (C3), 66.0 (C3a), 60.6 (C4), 19.6 (C9), 15.4 ppm (C10); IR (film): $\nu = 1794$, 1481, 1161, 955, 825 cm⁻¹; elemental analysis (%) calcd for $C_{13}H_{11}Cl_3O\colon C$ 51.10, H 3.63, O 10.47; found: C 51.05, H 3.56, O 10.32.

(3aS*,4S*,8bS*,E)-4-Hydroxy-3-((((R*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno-[1,2-b]furan-2one (30a) and (3aS*,4S*,8bS*,E)-4-hydroxy-3-((((S*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno-[1,2-b]furan-2-one (31a): To a solution of lactone 10a (312 mg, 1.64 mmol, 1 equiv) and freshly distilled ethyl formate (1.33 mL, 16.40 mmol, 10 equiv) in THF (25 mL) was added tBuOK (405 mg, 3.61 mmol, 2.2 equiv) in small portions at -10 °C under argon. After 6 h, the reaction was guenched with 1N HCl and the product was extracted with EtOAc. The crude product was then purified by flash chromatography (heptane/EtOAc 100:0, then 50:50). The pure enol was obtained as a white solid (295 mg, 1.35 mmol, 83%) in a mixture of diastereoisomers. Major diastereoisomer: ¹H NMR (300 MHz, [D₆]acetone): $\delta = 9.99$ (brs, 1 H, 9OH), 7.65 (d, 1 H, $J_{9,3a}$ =2.4 Hz, H9), 7.50–7.39 (m, 4 H, Har), 6.02 (d, 1H, J_{8b,3a}=7.5 Hz, H8b), 5.36 (s, 1H, H4), 3.81 (m, 1H, H3), 2.93 ppm (br s, 1 H, 4OH); ¹³C NMR (75 MHz, [D₆]acetone): $\delta = 172.3$ (C2), 154.5 (C9), 146.5 (C4a or C8a), 141.5 (C4a or C8a), 130.7 (Car), 130.0 (Car), 126.9 (2 Car), 105.6 (C3), 84.1 (C8b), 79.4 (C4), 51.1 ppm (C3a); IR (film): $\nu = 3176$, 2948, 1712, 1651, 1462, 1196, 986 cm⁻¹; MS (ESI): m/z: 273.1 [M+Na+MeOH]+, 241.1 [M+Na]+; HRMS (ESI): m/z: calcd for $C_{12}H_{10}O_4Na \ [M+Na]^+: 241.0477; found: 241.0470.$

To a solution of enol (100.0 mg, 0.46 mmol, 1 equiv) in freshly distilled *N*-methylpyrrolidone (2 mL) was added K₂CO₃ (129.9 mg, 0.94 mmol, 2.05 equiv) at room temperature under argon. To this mixture was added a solution of $9^{[28]}$ (166.4 mg, 0.94 mmol, 2.05 equiv) in *N*-methylpyrrolidone (2 mL). After 3 h, the reaction was quenched with a solution of 1 m HCl and the product was extracted with EtOAc. The organic layer was washed with water, brine and dried on MgSO₄. The crude product was purified by preparative TLC (heptane/EtOAc 70:30) to afford the two diastereoisomers as two pure fractions (F1=**30a**: 63.6 mg, 0.20 mmol, 44%; F2=**31a**: 59.3 mg, 0.19 mmol, 41 %) as amorphous white solids.

30a: ¹H NMR (300 MHz, [D₆]acetone): δ = 7.52–7.49 (m, 2H, Har, H6'), 7.43–7.39 (m, 3H, Har), 6.97 (t, 1H, $J_{3'2'}$ = $J_{3',7'}$ =1.5 Hz, H3'), 6.21 (t, 1H, $J_{2'3'}$ =1.5 Hz, H2'), 6.05 (d, 1H, $J_{3b,3a}$ =7.3 Hz, H8b), 5.29 (brs, 1H, OH), 5.27 (s, 1H, H4), 3.79 (dt, 1H, $J_{3a,8b}$ =7.3, $J_{3a,6'}$ =2.6 Hz, H3a), 2.01 ppm (t, 3H, $J_{7'3'}$ =1.5 Hz, H7'); ¹³C NMR (75 MHz, [D₆]acetone): δ = 170.9 (C2), 170.3 (C5'), 151.9 (C6'), 144.0 (C8a), 141.0 (C3'), 139.4 (C4a), 136.5 (C4'), 130.9 (Car), 130.4 (Car), 126.8 (Car), 125.8 (Car), 110.4 (C3), 100.7 (C2'), 84.4 (C8b), 79.9 (C4), 50.7 (C3a), 11.0 ppm (C7'); IR (film): ν = 3417, 3076, 2927, 1778, 1745, 1674, 1330, 1183, 733 cm⁻¹; MS (ESI): *m/z*: 337.1 [*M*+Na]⁺; HRMS (ESI): *m/z*: calcd for C₁₇H₁₄O₆Na [*M*+Na]⁺: 337.0688; found: 337.0685.

31a: ¹H NMR (300 MHz, $[D_6]$ acetone): $\delta = 7.53-7.47$ (m, 2H, Har, H6'), 7.42–7.38 (m, 3H, Har), 6.97 (t, 1H, $J_{3',2'} = J_{3',7'} = 1.5$ Hz, H3'), 6.20 (t, 1H, $J_{2',3'} = 1.5$ Hz, H2'), 6.05 (d, 1H, $J_{8b,3a} = 7.3$ Hz, H8b), 5.27 (s, 1H, H4), 3.79 (dt, 1H, $J_{3a,8b} = 7.3$, $J_{3a,6'} = 2.6$ Hz, H3a), 2.01 ppm (t, 3H, $J_{7',3'} =$ 1.5 Hz, H7'); ¹³C NMR (75 MHz, $[D_6]$ acetone): $\delta = 171.0$ (C2), 170.4 (C5'), 152.3 (C6'), 144.0 (C8a), 141.2 (C3'), 139.4 (C4a), 136.3 (C4'), 131.0 (Car), 130.3 (Car), 126.7 (Car), 125.9 (Car), 110.3 (C3), 101.0 (C2'), 84.4 (C8b), 79.8 (C4), 50.6 (C3a), 11.0 ppm (C7'); IR (film): $\nu = 3415$, 3078, 2925, 1776, 1747, 1672, 1332, 1181, 733 cm⁻¹; MS (ESI): m/z: 337.1 [*M*+Na]⁺; HRMS (ESI): m/z: calcd for $C_{17}H_{14}O_6Na$ [*M*+Na]⁺: 337.0688; found: 337.0680.

 $(3aS^*,4S^*,8bS^*,E)-3-((((R^*)-4-Methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)-methylene)-3,3a,4,8b-tetrahydro-2H-indeno-[1,2-b]furan-4-yl acetate (32 a) and (3aS^*,4S^*,8bS^*,E)-3-((((S^*)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno-[1,2-b]furan-4-yl$

acetate (33a): Pyridine (500 μ L, excess) was added to a equimolar solution of 30a and 31a (30.0 mg, 0.10 mmol, 1 equiv) in acetic anhydride (500 μ L, excess) at room temperature under argon. After 12 h, the reaction mixture was evaporated to dryness and the obtained residue was purified on silica gel (heptane/EtOAc 70:30) to afford the two diastereoisomers as two pure fractions (F1=32a: 17.7 mg, 0.05 mmol, 52%; F2=33a: 13.6 mg, 0.04 mmol, 40%) as amorphous white solids.

32a: ¹H NMR (300 MHz, CDCl₃): δ = 7.59–7.39 (m, 5H, Har and H6'), 6.99 (t, 1H, $J_{3,2}$ = $J_{3,7}$ =1.5 Hz, H3'), 6.43 (s, 1H, H4), 6.16 (t, 1H, $J_{2,3}$ =

FULL PAPER

A EUROPEAN JOURNAL

1.5 Hz, H2'), 6.10 (d, 1 H, $J_{8b,3a}$ =7.5 Hz, H8b), 3.85 (dq, 1 H, $J_{3a,8b}$ =7.5, $J_{3a,6'}$ =2.6 Hz, H3a), 2.05 (s, 3 H, H4c), 2.01 ppm (t, 3 H, $J_{7,3'}$ =1.5 Hz, H7'); ¹³C NMR (75 MHz, CDCl₃): δ = 170.4 (C2 or C4b or C5'), 170.3 (C2 or C4b or C5'), 170.2 (C2 or C4b or C5'), 153.1 (C6'), 141.2 (C3'), 140.7 (C4a or 8a), 140.6 (C4a or C8a), 136.0 (C4'), 130.9 (Car), 130.8 (Car), 126.8 (Car), 126.7 (Car), 109.0 (C3), 100.8 (C2'), 83.9 (C8b), 79.1 (C4), 47.5 (C3a), 21.4 (C4c), 11.0 ppm (C7'); IR (film): ν = 2972, 2899, 1781, 1748, 1679, 1372, 1329, 1228, 1080, 863, 744 cm⁻¹; MS (ESI): *m/z*: 379.1 [*M*+Na]⁺; HRMS (ESI): *m/z*: calcd for C₁₉H₁₆O₇Na [*M*+Na]⁺: 379.0794; found: 379.0806.

33a: ¹H NMR (300 MHz, CDCl₃): δ = 7.53–7.37 (m, 5 H, Har, H6'), 6.96 (s, 1 H, H3'), 6.37 (s, 1 H, H4), 6.19 (s, 1 H, H2'), 6.09 (d, 1 H, $J_{8b,3a}$ = 7.3 Hz, H8b), 3.86 (brd, 1 H, $J_{3a,8b}$ = 7.3 Hz, H3a), 2.03 ppm (s, 6 H, H4c, H7'); ¹³C NMR (75 MHz, CDCl₃): δ = 170.4 (C2 or C4b or C5'), 170.2 (C2 or C4b or C5'), 170.1 (C2 or C4b or C5'), 151.9 (C6'), 141.0 (C3'), 140.7 (C4a or 8a), 140.6 (C4a or C8a), 136.6 (C4'), 130.8 (Car), 130.7 (Car), 126.9 (Car), 126.5 (Car), 109.2 (C3), 100.2 (C2'), 83.9 (C8b), 79.1 (C4), 47.6 (C3a), 21.3 (C4c), 11.0 ppm (C7'); IR (film): ν = 2970, 2900, 1780, 1747, 1680, 1371, 1330, 1227, 1081, 862, 746 cm⁻¹; MS (ESI): *m/z*: 379.1 [*M*+Na]⁺; 379.0794; found: 379.0802.

Solanacol (6) and 2'-epi-solanacol (30b): Potassium tert-butoxide (67.9 mg, 0.61 mmol, 2.2 equiv) was added to a mixture of lactone 10b (60.0 mg, 0.28 mmol, 1 equiv) and ethyl formate (0.23 mL, 2.80 mmol, 10 equiv) in THF (1 mL) at -78 °C under argon. It was then warmed to -40°C and was stirred for 6 h at this temperature. The mixture was then cooled to -60 °C and $9^{[28]}$ (99.8 mg, 0.56 mmol, 2.05 equiv) was gradually added. The mixture was then warmed to room temperature. The reaction was quenched with AcOH (1 mL) after 12 h at this temperature. The solvent was evaporated and the crude product was purified on preparative TLC (heptane/ethyl acetate 50:50) to afford the two diastereomers as two pure fractions (F1=30b: 36.0 mg, 0.11 mmol, 38%; F2=6: 36.0 mg, 0.11 mmol, 38%) as colorless oils. (-)-(2'S,3aR,4R,8bR,E)-**30b**: $[\alpha]_{D}^{26} =$ $-176.4 \ (c = 1.7, \text{ CHCl}_3); \ (+)-(2'R,3aS,4S,8bS,E)-30b: \ [\alpha]_D^{26} = +178.6 \ (c$ = 1, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ = 7.54 (d, $J_{9,3a}$ = 2.6 Hz, 1 H, H9), 7.25 (d, *J*_{6,5}=7.7 Hz, 1 H, H6), 7.17 (d, *J*_{5,6}=7.7 Hz, 1 H, H5), 7.00 (t, $J_{3',2'} = 1.5, J_{3',7'} = 1.5$ Hz, 1H, H3'), 6.24 (t, $J_{2',3'} = 1.5, J_{2',7'} = 1.5$ Hz, 1H, H2'), 6.15 (d, $J_{8b,3a}$ =7.5 Hz, 1 H, H8b), 5.27 (d, $J_{4,3a}$ =5.8 Hz, 1 H, H4), 3.81 (ddd, J_{3a.8b}=7.5, J_{3a.4}=5.8, J_{3a.9}=2.6 Hz, 1 H, H3a), 2.37 (s, 3 H, H10), 2.31 (s, 3H, H9), 2.08 (d, 1H, OH), 2.05 ppm (t, $J_{7,2}$ = 1.5, $J_{7,3}$ = 1.5 Hz, 3H, H7'); ¹³C NMR (75 MHz, CDCl₃): δ = 171.0 (C2), 170.2 (C5'), 151.5 (C6'), 141.9 (C4a or C8), 141.0 (C3'), 139.0 (C7 or C8a), 138.4 (C7 or C8a), 136.6 (C4'), 135.7 (C4), 132.9 (C6), 122.5 (C5), 110.9 (C3), 100.7 (C2'), 84.2 (C8b), 80.3 (C4), 50.8 (C3a), 19.8 (C9), 15.8 (C10), 11.1 ppm (C7'); IR (film): $\nu = 3437$, 1780, 1740,1677, 1334, 1186, 1092, 1016, 957 cm⁻¹; MS: m/z (%): 365 (100) [M+Na]⁺, 343 (75) [M+H]⁺; HRMS (ESI): m/z: calcd for C₁₉H₁₈NaO₆ [*M*+Na]⁺: 365.1001; found: 365.1010. $(-)-(2'R,3aR,4R,8bR,E)-6: [\alpha]_{D}^{26} = -164.2 \ (c = 2.2, CHCl_3); \ (-)-$ (2'S,3aS,4S,8bS,E)-3: $[\alpha]_{D}^{26} = +162.7 \ (c = 1, \text{CHCl}_3); {}^{1}\text{H NMR} \ (300 \text{ MHz},$ CDCl₃): $\delta = 7.55$ (d, $J_{9,3a} = 2.6$ Hz, 1H, H6'), 7.23 (d, $J_{6,5} = 7.7$ Hz, 1H, H6), 7.16 (d, $J_{5,6}=7.7$ Hz, 1H, H5), 6.99 (t, $J_{3',2'}=1.5$, $J_{3',7'}=1.5$ Hz, 1H, H3'), 6.22 (t, *J*_{2',3'}=1.5, *J*_{2',7'}=1.5 Hz, 1 H, H2'), 6.15 (d, *J*_{8b,3a}=7.5 Hz, 1 H, H8b), 5.25 (d, $J_{4,3a} = 5.8$ Hz, 1H, H4), 3.81 (ddd, $J_{3a,8b} = 7.5$, $J_{3a,4} = 5.8$, J_{3a,9}=2.6 Hz, 1H, H3a), 2.37 (s, 3H, H10), 2.30 (s, 3H, H9), 2.06 (d, 1H, OH), 2.05 ppm (t, $J_{7',2'}=1.5$, $J_{7',3'}=1.5$ Hz, 3H, H6'); ¹³C NMR (75 MHz, CDCl₃): $\delta = 171.0$ (C2), 170.3 (C5'), 151.8 (C6'), 141.9 (C4a or C8), 141.1 (C3'), 138.9 (C7 or C8a), 138.3 (C7 or C8a), 136.4 (C4'), 135.6 (C4), 132.9 (C6), 122.6 (C5), 110.8 (C3), 100.9 (C2'), 84.3 (C8b), 80.2 (C4), 50.6 (C3a), 19.8 (C9), 15.8 (C10), 11.0 ppm (C7'); IR (film): $\nu = 3333$, 1781, 1737,1675, 1329, 1183, 953 cm⁻¹; MS: m/z (%): 365 (100) $[M+Na]^+$, 343 (75) $[M+H]^+$; HRMS (ESI): m/z: calcd for $C_{19}H_{18}NaO_6$ $[M+Na]^+$: 365.1001; found: 365.1015.

(+)-(3aS,4S,8bS,E)-7,8-Dimethyl-3-((((R)-4-methyl-5-oxo-2,5-dihydrofuran-2-yl)oxy)methylene)-3,3a,4,8b-tetrahydro-2H-indeno-[1,2-b]furan-4-yl acetate (32b) and (+)-(3aS,4S,8bS,E)-7,8-dimethyl-3-((((S)-4-methyl-5oxo-2,5-dihydrofuran-2-yl)oxy)methylene)-3,3a,4,8b-tetrahydro-2Hindeno-[1,2-b]furan-4-yl acetate (33b): To a mixture of solanacol 6 and

2'-epi-solanacol **30b** (50.0 mg, 0.15 mmol, 1 equiv) in dry pyridine (1 mL)

was added acetic anhydride (0.5 mL). The mixture was stirred overnight. After 12 h, the solvent was evaporated and the crude product was purified on silica gel (heptane/EtOAc 70:30). Two fractions corresponding to the 2 epimers were collected in pure form (F1=32b: 27.0 mg, 0.07 mmol, 48%; F2=33b: 25.3 mg, 0.07 mmol, 45%) as amorphous white solids. (+)-32b: $[\alpha]_{D}^{26} = +99.6$ (c = 2.34, CHCl₃); (-)-32b: $[\alpha]_{D}^{26} = -88.4$ (c = 0.7, CHCl₃); ¹H NMR (300 MHz, CDCl₃): $\delta = 7.59$ (d, 1 H, $J_{6',3a} = 2.6$ Hz, H6'), 7.20 (d, 1H, J_{ar,ar}=7.7 Hz, Har), 7.11 (d, 1H, J_{ar,ar}=7.7 Hz, Har), 6.99 (t, 1H, $J_{3',2'}=J_{3',7'}=1.3$ Hz, H3'), 6.40 (brs, 1H, H4), 6.16 (d, 1H, $J_{8b,3a} = 7.5$ Hz, H8b), 6.15 (d, 1H, $J_{2',3'} = 1.3$ Hz, H2'), 3.84 (dt, 1H, $J_{3a,8b} =$ 7.5 Hz, $J_{3a,4} = J_{3a,6'} = 2.6$ Hz, H3a), 2.35 (s, 3 H, H7a or H8c), 2.28 (s, 3 H, H7a or H8c), 2.03 (brs, 3H, H4c), 2.02 ppm (d, 3H, J_{7',3'}=1.3 Hz, H7'); ¹³C NMR (75 MHz, CDCl₃): $\delta = 170.4$ (C2, C4b or C5'), 170.3 (C2, C4b or C5'), 170.1 (C2, C4b or C5'), 152.8 (C6'), 141.2 (C3'), 139.6 (C4', C4a, C7, C8 or C8a), 139.5 (C4', C4a, C7, C8 or C8a), 138.6 (C4', C4a, C7, C8 or C8a), 135.9 (C4', C4a, C7, C8 or C8a), 135.7 (C4', C4a, C7, C8 or C8a), 132.8 (C5 or C6), 123.5 (C5 or C6), 109.5 (C3), 100.8 (C2'), 83.9 (C8b), 79.6 (C4), 47.4 (C3a), 21.5 (C4c), 19.8 (C7a or C8c), 15.9 (C7a or C8c), 10.9 ppm (C7'); IR (film): $\nu = 2926$, 1785, 1745, 1681, 1372, 1233, 1014, 751 cm⁻¹; MS (ESI): *m/z*: 385.1 [*M*+H]⁺; HRMS (ESI): *m/z*: calcd for C₂₁H₂₁O₇ [*M*+H]⁺: 385.1287; found: 385.1287;

(+)-33b: $[\alpha]_{D}^{27} = +52.1 \ (c = 3.11, CHCl_3); (-)-33b: <math>[\alpha]_{D}^{27} = -52.8 \ (c = 1.1, CHCl_3); {}^{1}H NMR (300 MHz, CDCl_3): \delta = 7.48 \ (d, 1H, J_{6;3a}=2.4 Hz, H6'), 7.17 \ (d, 1H, J_{ar,ar}=7.7 Hz, Har), 7.10 \ (d, 1H, J_{ar,ar}=7.7 Hz, Har), 6.96 \ (s, 1H, H3'), 6.32 \ (s, 1H, H4), 6.19 \ (s, 1H, H2'), 6.14 \ (d, 1H, J_{8b,3a}= 7.5 Hz, H8b), 3.84 \ (d, 1H, J_{3a,8b}=7.5 Hz, H3a), 2.33 \ (s, 3H, H7a \ or H8c), 2.01 \ (brs, 3H, H7'), 2.00 \ ppm \ (s, 3H, H4c); 1{}^{3}C NMR \ (75 MHz, CDCl_3): \delta = 170.6 \ (C2, C4b \ or C5'), 170.3 \ (C2, C4b \ or C5'), 170.1 \ (C2, C4b \ or C5'), 151.5 \ (C6'), 141.1 \ (C3'), 139.4 \ (C4', C4a, C7, C8 \ or C8a), 136.4 \ (C4', C4a, C7, C8 \ or C8a), 132.7 \ (C5 \ or C6), 123.6 \ (C5 \ or C6), 109.7 \ (C3), 100.1 \ (C2'), 83.9 \ (C8b), 79.6 \ (C4), 47.5 \ (C3a), 21.3 \ (C4c), 19.8 \ (C7a \ or C8c), 15.8 \ (C7a \ or C8c), 15.9 \ (C4'); 18 \ (ESI): m/z: calcd for C_{21}H_{21}O_7 \ [M+H]^+; 385.1287; found: 385.1289.$

Plant assays: Pea *rms1* mutant plants (allele *rms1–10* identified in the line Térèse)^[40] deficient in SLs were used for the bioassay. The compound to be tested was applied directly to the bud with a micropipette as 10 μ L of solution containing 0.1% acetone with 2% polyethylene glycol 1450, 50% ethanol and 0.4% DMSO. 24 plants were sown per treatment in trays. The treatment was generally done 10 days after sowing, on the axillary bud at node 4 (or 3). The branches at nodes 1 to 2 were removed to encourage the outgrowth of axillary buds at nodes above. Nodes were numbered acropetally from the first scale leaf as node 1 and cotyledonary node as node 0. Bud growth at node 4 (node 3) was measured 8 to 10 days after treatment with an electronic calliper.

Statistical analyses: Because deviations from normality were observed for axillary bud length after SL treatment, the Kruskal-Wallis test was used to assess the significance of treatment in comparison to the control treatment (0 nm) or to GR24 treatment at the same concentration using R Commander version 1.7-3.^[41]

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