

Novel non-canonical strigolactone analogs highlight selectivity for stimulating germination in two Phelipanche ramosa populations

Suzanne Daignan Fornier, Alexandre de Saint Germain, Pascal Retailleau, Jean-Paul Pillot, Quentin Taulera, Lucile Andna, Laurence Miesch, Soizic Rochange, Jean-Bernard Pouvreau, François-Didier Boyer

▶ To cite this version:

Suzanne Daignan Fornier, Alexandre de Saint Germain, Pascal Retailleau, Jean-Paul Pillot, Quentin Taulera, et al.. Novel non-canonical strigolactone analogs highlight selectivity for stimulating germination in two Phelipanche ramosa populations. 2022. hal-03616875

HAL Id: hal-03616875 https://hal.science/hal-03616875

Preprint submitted on 23 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Novel non-canonical strigolactone analogs highlight selectivi-1 ty for stimulating germination in two Phelipanche ramosa 2

populations 3

Suzanne Daignan Fornier¹, Alexandre de Saint Germain², Pascal Retailleau¹, Jean-Paul Pillot², 4

Quentin Taulera³, Lucile Andna⁴, Laurence Miesch⁴, Soizic Rochange³, Jean-Bernard Pouvreau⁵, 5

- François-Didier Boyer^{1*} 6
- ¹ Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France 7
- ² Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000, Versailles, France 8
- ³ Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, UPS, Toulouse INP, 31320 Auzeville-9 **Tolosane**, France 10
- ⁴ Université de Strasbourg, Institut de Chimie, UMR 7177, Équipe Synthèse Organique et Phytochimie, 4 rue Blaise 11
- Pascal CS 90032, 67081 Strasbourg Cedex, France 12
- ⁵ Nantes Université, CNRS, US2BN, UMR 6286, F-44000 Nantes, France 13
- KEY WORDS: Phelipanche ramosa, Parasitic weeds, Germination stimulants, Strigolactones, Synthetic analogs, Struc-14

15 ture-activity relationship, Plant hormone, Pisum sativum, Arabidopsis thaliana, Rhizophagus irregularis.

16

ABSTRACT: Strigolactones (SLs) are plant hormones exuded in the rhizosphere with a signaling role for the development of 17 arbuscular mycorrhizal (AM) fungi and as stimulants of seed germination of the parasitic weeds Orobanche, Phelipanche and 18 Striga, the most threatening weeds of major crops worldwide. Phelipanche ramosa is present mainly on rape, hemp and 19 tobacco. P. ramosa 2a preferentially attacks hemp while P. ramosa 1 attacks rapeseed. The recently isolated Cannalactone 20 14 from hemp root exudates has been characterized as a non-canonical SL that selectively stimulates the germination of P. 21 ramosa 2a seeds in comparison with *P. ramosa* 1. In the present work, we established that (–)-solanacol 5, a canonical oro-22 banchol-type SL exuded by tobacco and tomato, possesses a remarkable selective germination stimulant activity for *P. ra*-23 mosa 2a seeds. We synthesized cannalactone analogs, named (±)-SdL19 and (±)-SdL118 which have an unsaturated acyclic 24 carbon chain with a tertiary hydroxyl group and a methyl or a cyclopropyl group instead of a cyclohexane A-ring, respec-25 tively. (±)-SdL analogs are able to selectively stimulate P. ramosa 2a revealing that these minimal structural elements are 26 key for this selective bioactivity. In addition, we showed that (±)-SdL19 is able to inhibit shoot branching in *Pisum sativum* 27 and Arabidopsis thaliana, and induces hyphal branching in AM fungus R. irregularis, like SLs. 28

INTRODUCTION 29

Strigolactones (SLs) are a class of compounds first identi-30 fied in 1966¹ as stimulants of seed germination of the root 31 parasitic weeds Orobanche, Phelipanche and Striga. More 32 recently, SLs have been discovered as the 9th class of plant 33 hormone that control plant architecture including shoot 34 branching stem secondary growth, plant height, root ar-35 chitecture, and adventitious roots²⁻⁵. They are synthesized 36 37 in plant roots in trace concentrations, and are partly excreted into the rhizosphere. They belong to the apocarot-38 enoid family (Scheme 1). The structural core of SLs is a 39 tricyclic lactone (ABC part, canonical SLs) or an unclosed 40 BC-ring (non-canonical SLs) connected via an enol ether 41 42 bridge to an invariant α_{β} -unsaturated furanone moiety (D ring). All natural SLs have the same *R*-configuration at the 43 C-2' position. Canonical SLs are divided into strigol (1) 44

and orobanchol (2) -types corresponding to a β - (e.g., 5-45 deoxystrigol 4, sorgolactone 7) or an α -oriented C-ring 46 (e.g., orobanchyl acetate 3, solanacol 5, solanacyl acetate 47 6, fabacyl acetate 8), respectively and with A and B cycles 48 bearing various substituents (Chart 1A)⁶. Non-canonical 49 SLs (e.g., avenaol 11, zealactone 12, bryosymbiol 13 and 50 cannalactone 14) can be structurally more diverse than 51 canonical SLs (Chart 1B). The common biosynthetic pre-52 cursors for canonical and non-canonical SLs are carlac-53 tonoic acid 18 (CLA) and hydroxy-carlactonoic acids (HO-54 CLAs)⁷⁻⁹ biosynthesized from all-trans-β-carotene 17 via 55 carlactone 9 (CL) and hydroxy-carlactones (HO-CLs) 56 (Scheme 1)^{10,11}. In addition, methyl carlactonoate (MeCLA 57 10) and HO-MeCLAs^{12,13} have been identified as biosyn-58 thetic precursors for numerous non-canonical SLs. 59

To date, more than 23 canonical SLs and more than 10
non-canonical SLs have been identified in plant root exudates and plant tissues¹⁴. Natural SLs are difficult to obtain by organic synthesis due to long multistep
syntheses¹⁵⁻¹⁸. Numerous SL analogs and mimics have
been developed for plant chemical biology^{19,20}. GR24,
invented by Gerald Rosbery, is a canonical strigol-type SL

analog with an aromatic A-ring²¹. GR24, in most cases as
racemic mixture, is the worldwide reference compound
(Chart 1C) in all assays investigating the role of SL in biological processes. The simplified GR-type compound
GR5²¹, possessing only C and D rings (Chart 1C) has been
also developed as a germination stimulant of root parasitic plant seeds and proved later to



74

Chart 1. Natural strigolactones (SLs), canonical (A), non-canonical (B) and artificial SL analogs and mimic (C). For
 bryosymbiol, the absolute stereochemistry at C-4 and C-5 could be interchangeable²².

R = Ac: (±)-7,8-desmethyl solanacyl acetate 16



78 Scheme 1. Strigolactone biosynthetic scheme. CL=

79 carlactone ; CLA = carlactonoic acid ; MeCLA = methyl

carlactonoate ; SL strigolactone. * Isolated and identi fied to date.

be bioactive as plant hormone in the control of shoot
branching²³⁻²⁵. More recently, CL²⁶ and MeCLA noncanonical SL synthetic analogs^{27,28} have been developed to
study the bioactivity of structures close to highly unstable
CL 9 and MeCLA 10¹⁴.

The parasitic plants Orobanche, Phelipanche and Striga are 87 major agricultural pests around the Mediterranean Sea 88 and in Sub-Saharan Africa, respectively. They tragically 89 constitute there a major cause of crop damage²⁹ in inten-90 sive crop systems that is expected to expand to new terri-91 tories in the near future³⁰. They are obligate parasites 92 93 producing numerous and extremely small seeds that can remain viable in soil for decades before germination³¹. 94

The induction of germination by host plant SLs is a critical 95

step in the development cycle of these weeds³². SL synthe-96

sis and signaling are therefore important targets for crop 97

protection^{20,33}. 98

SLs have been shown to play an additional rhizospheric 99 signaling role by stimulating spore germination and hy-100 phal proliferation of AM fungi^{34,35}. The hypothesis is that 101 plant SLs boost fungal metabolism prior to root infec-102 tion³⁶. The AM symbiosis arose very early in land plant 103 evolution and is thought to have been instrumental in 104 allowing successful plant conquest of terrestrial environ-105 ments³⁷. It has been proposed that an ancestral role of SLs 106 107 was to attract these highly beneficial fungal symbionts²², root parasitic plants having highjacked this system much 108 later to detect their host²⁵. Biological activities of SLs 109 could be detected at concentrations as low as 10⁻¹³ M on 110 AM fungi, 10⁻¹² M on seeds of parasitic weeds and 10⁻⁸ M 111 on lateral buds as plant hormone. 112

Phelipanche ramosa seeds recognize allelochemical signals 113 and interact with their hosts depending on their genetic 114 structure as reported recently^{38,39}. Three main genotypic 115 groups were described in European *P. ramosa* populations 116 from the Mediterranean basin. P. ramosa of genetic group 117 1 (P. ramosa 1) is present exclusively in western France. It 118 parasites essentially oilseed rape but also melon, tobacco, 119 and sunflower. P. ramosa 2a attacks preferentially hemp 120 but also tobacco and tomato in France and Italy. P. ramosa 121 2b is widespread in in Europe and Turkey and attacks 122 mainly tobacco but also various crops like oilseed rape, 123 hemp and tomato. Seed sensitivity to germination stimu-124 lants as SLs emitted by host plants is proposed as marker 125 of genetic groups. In oilseed rape no SL has been yet iden-126 tified⁴⁰. Tobacco plants exude canonical strigol-type and 127 orobanchol-type SLs^{41,42} and tomato plants exude oroban-128 chol-type SLs⁴³. The non-canonical SL cannalactone **14** has 129 been recently characterized from hemp (Cannabis 130 sativa)44. Differential sensitivities to synthetic GR24 enan-131 tiomers and cannalactone 14 for germination of *P. ramosa* 132 seeds of different genetic groups have been report-133 ed^{39,44,45}. 134

The SL receptor in vascular plants DWARF14 (D14) has 135 been identified as a member of the superfamily of the α/β -136 hydrolases^{4,46}. In Arabidopsis, a paralog of AtD14, 137 KARRIKIN INSENSITIVE2/HYPOSENSITIVE TO LIGHT 138 (AtKAI2 /AtHTL), has also been described. It encodes a 139 protein that shows a global structure very similar to 140 AtD14 and has conserved the catalytic triad (Ser, His, 141 142 Asp). AtKAI2 has been shown to regulate *Arabidopsis* seed germination⁴⁷. Interestingly, the KAI2 gene family has 143 expanded during the evolution of parasitic plant genomes. 144 Some members of these expanded families in witchweeds 145 and broomrapes have evolved the capacity to bind SLs, 146 making them good candidates for SL receptors. Six of the 147 11 S. hermonthica KAI2 paralogs, belonging to the diver-148 gent clade, were identified as highly sensitive to SLs⁴⁸⁻⁵⁰. 149 These results indicated the involvment of one or several 150 α/β -hydrolases in SL perception in parasitic plants. In *P*. 151 ramosa 1, among the 5 KAI2 paralogs, we recently estab-152 lished that PrKAI2d3 encodes a SL receptor which is also 153 able to interact with isothiocyanates (ITCs), other germi-154 nation stimulants of *P. ramosa* seeds⁵¹. 155

The objective of the present work is to analyze the biological activity of various cannalactone analogs in comparison with natural cannalactone 14, natural SLs and espe-158 cially SLs produced by tobacco41,42 and tomato43 ((-)orobanchol 2, (-)-orobanchyl acetate 3, 5-deoxystrigol 4, (-)-solanacol 5 and (-)-solanacyl acetate 6), the SL mimic contalactone⁴⁵ and well-known GR SL analogs. Through a structure-activity relationship (SAR) study, we decipher key minimal structural elements for selective germination activity of P. ramosa seeds focused on P. ramosa 1 versus 165 2a³⁸ which attack selectively major crops as rapeseed and 166 hemp, respectively. Moreover, we show that these novel 167 non-canonical SL analogs possess hormonal activity in pea and induce hyphal branching in arbuscular mycorrhizal 169 (AM) fungi. 170

RESULT AND DISCUSSION

156

157

159

160

161

162

163

164

168

171

172

180

Synthesis of cannalactone analogs. We designed two 173 types of cannalactone analogs (Chart 2). Type I possesses 174 an unsaturated acyclic chain instead of the cyclohexane A-175 ring of cannalactone 14. Type II bears an unsaturated 176 acyclic carbon chain with a tertiary hydroxyl group and a 177 methyl or a cyclopropyl group (R² group) replacing the 178 cyclohexane A-ring. 179



Chart 2. Cannalactone analogs Type I and II designed 181 in this work. Numbering for cannalactone analogs 182 (SdL) and chemicals synthesized here. 183

The starting material for type I and type II analogs is (±)-184 4-vinyldihydrofuran-2(3H)-one 19 or Taniguchi lactone⁵² 185 prepared at multigram-scale from the commercially avail-186 able cis-2-butene-1,4-diol via formation of a ketene acetal 187 and its [3,3]-sigmatropic rearrangement. Formylation of 188 (±)-Taniguchi lactone and one-pot coupling with D-Br 20 189 at -78 °C led to the formation of diastereomers Type-I 190 cannalactone analogs (±)-SdL4F1 and (±)-SdL4F2 easily 191 192 separable by silica gel chromatography in 41% overall yield (Scheme 2). SdL7, another Type-I cannalactone ana-193 log was obtained by cross metathesis (CM) of (±)-SdL4F1 194 with a large excess of methyl acrylate at 50 °C with Hov-195 eyda-Grubbs II (HG II) initiator in 57% yield. The E-196 geometry of the α,β -unsaturated ester group and the E-197 geometry of the enol function in SdL7 were proven by 198 NMR analysis (3 /_{H6-H7} = 15.5 Hz, 3 /_{C2-H6'} = 2.0 Hz). 199



Scheme 2. Preparation of Type-I cannalactone ana-201 logs: (±)-SdL4F1, (±)-SdL4F2 and (±)-SdL7. 202

Preparation of Type-II analogs with R¹ = methyl or cyclo-203 propyl group involved the sequence detailed in Scheme 3. 204 Taniguchi lactone 19 was engaged in CM with butane-3-205 one in presence of HG II and afforded enone 21 in 68% 206 yield. The selective addition of methyl and cyclopropyl 207 group on the ketone group of enone 21 was accomplished 208 using Grignard reagents with cerium(III) chloride leading 209 to alcohol 22a and 22b in near quantitative yields^{53,54}. 210 Cerium(III) chloride plays an important role in increasing the 211 nucleophilicity and decreasing the basicity of the Grignard 212 239

reagents. The direct formylation on alcohols 22a,b was un-213 successful. However, silvlation of alcohols 22a.b in neat 214 trimethylsilyl imidazole furnished protected derivatives 23a.b 215 in 70% yield, which led by formylation and coupling with D-216 Br 20 to a separable mixture of diastereomers in moderate 217 vields (34-54%) $((\pm)-SdL18F1/(\pm)-SdL18F2,$ 218 (±)-219 SdL118F1/(±)-SdL118F2). A mild deprotection of separated silvlated derivatives ((±)-SdL18F1, (±)-SdL18F2) with cata-220 lytic Lewis acid (Sc(OTf)₃) and H_2O^{55} led to both alcohols 221 (±)-SdL19 and (±)-SdL21 in non optimized 53 and 89% 222 yields, respectively. However, these conditions for the silvlat-223 ed cyclopropyl derivative (±)-SdL118F1 were inefficient but 224 the targeted alcohol (±)-SdL127 was produced with scandi-225 um(III) triflate and Ac₂O⁵⁶ in poor 19% yield and without 226 detection of the acetylated product. Tertiary alcohols (±)-227 SdL19 and (±)-SdL21 were acylated by standard conditions 228 furnish acetylated derivative (±)-SdL51 and 4-229 to nitrobenzoates (±)-SdL51 and (±)-SdL50. In addition, for-230 mation of 3,5-nitrobenzoate (±)-SdL50 from (±)-SdL21 fur-231 nished a crystalline compound whose X-ray diffraction prop-232 erties were sufficiently exploited to establish unambiguously 233 the relative configurations of stereogenic centers C2' and C4 234 of (\pm) -SdL50 as 2'R and 4S, respectively (Figure 1) and by 235 consequence the relative configurations of stereogenic centers 236 for the other SdL analogs. With these putative SL analogs in 237 our hands, their biological activity was studied. 238



241

- Scheme 3. Preparation of Type-II cannalactone analogs (±)-SdL18F1, (±)-SdL18F2, (±)-SdL19, (±)-SdL21, (±)-SdL33, 242 (±)-SdL34, (±)-SdL50, (±)-SdL51, (±)-SdL118 and (±)-SdL127. a) Ac₂O, 4-DMAP, pyridine, rt ; b) ClCO-Ph-4-NO₂, 4-
- 243 DMAP, pyridine, 40 °C ; c) ClCO-Ph-3,5-NO₂, 4-DMAP, pyridine, 40 °C. 244



Figure 1. ORTEP view of the asymmetric unit of (±)-SdL50
(solvent molecule omitted) with selected atom numbering.
Ellipsoids are represented with 30% of probability.

Ellipsoids are represented with 30% of probability.
 Most CdL derivatives recess similar bioactions

Most SdL derivatives possess similar bioactivity and 249 selectivity as cannalactone toward Phelipanche ra-250 mosa 1 versus 2a. The germination stimulant activities of 251 SdL analogs on *P. ramosa* 1 and 2a seeds were determined 252 and compared to (±)-GR24, (±)-GR5 and natural cannalac-253 tone 14 by measuring the maximum of germination 254 stimulant activity as well as half maximal effective concen-255 trations (EC₅₀) using a concentration range from 10^{-13} to 256 10⁻⁶ mol.L⁻¹ (Chart 3, Figure 2A)⁵⁷. Germination stimulant 257 activities of all SdL analogs and the putative cannalactone 258 biosynthetic precursor, (±)-MeCLA, reached the maximum 259 induced by (±)-GR24 which makes them germination 260 stimulants as expected for SL analogues (Figure S1A). We 261 compared the bioactivities of these chemicals based on 262 EC₅₀ values. 263

We previously reported that cannalactone 14, a non-264 canonical SL, possesses a stronger germination stimula-265 tion activity on P. ramosa 2a than on P. ramosa 1. On con-266 trary, (±)-GR24, an artificial canonical SL possesses a 267 268 stronger germination stimulation activity on P. ramosa 1 rather than on P. ramosa 2a44. These results led us to pro-269 pose that *P. ramosa* 1 could be more sensitive to canonical 270 SLs and P. ramosa 2a to non-canonical SLs. To illustrate 271 the selectivity of seed germination between P. ramosa 1 272 and P. ramosa 2a, the ratio of EC₅₀ (P. ramosa 1/ P. ramosa 273 2a) (rEC50) of each molecule was calculated (Figure 2B); for 274 example, $r_{EC50}((\pm)-GR24) = 0.014$ and $r_{EC50}(14) = 410$. 275

We studied a set of various canonical SLs belonging to 276 strigol- $((\pm)$ -1, (\pm) -4, (\pm) -7) and orobanchol-types $((\pm)$ -2, 277 (±)-3, (–)-5, (–)-6, (±)-8) and (±)-contalactone (Chart 1).45 278 a recent SL mimic discovered during the preparation of 279 (\pm) -GR24. All canonical SLs (1-4, 7-8) and (\pm) -280 281 contalactone⁴⁵ were found less active than (\pm) -GR24, (+)-GR24 and (-)-2'-epi-GR24,45 possessing stereochemistries 282 found in natural canonical SLs (strigol-type and oroban-283 chol-type, respectively). Their r_{EC50} (< 1) reflect selectivity 284 for P. ramosa 1, except for the cases of (-)-solanacol 5 and 285 (-)-solanacyl acetate 6 where high germination activity 286 and selectivity for P. ramosa 2a were recorded (recso ((-)-287 5) = 164) (Figure 3, Figure S1B). Though high germination 288 activities were observed with (-)-solanacyl acetate 6 and 289

7.8-desmethyl solanacol derivatives 15 and 16, selectivity 290 was reduced, highlighting the importance of the hydroxyl 291 group but also the methyl group on the A aromatic ring for 292 selectivity. High levels of (-)-solanacol 5 and (-)-solanacyl 293 acetate 6 were detected in tobacco root exudates⁴¹ and in 294 tomato root extracts⁴³. To note, tobacco and tomato plants 295 are also hosts for *P. ramosa* 2a^{38,39}, in accordance with the 296 sensitivity of P. ramosa 2a seeds to (-)-solanacol 5 and 297 (-)-solanacyl acetate 6 shown here. 298

We studied the germination stimulation activities of the 299 SL biosynthetic intermediates (Scheme 1). For all-trans-β-300 carotene 17, a SL biosynthetic precursor without D-ring, 301 no germination was recorded, demonstrating that this 302 compound is not bioactive and/or that no enzyme of the 303 SL biosynthesis pathway was sufficiently present in plant 304 parasitic seeds (Figure S1). For racemic MeCLA 10 and CL 305 9, the SL biosynthetic precursors possessing the D-ring 306 but no C ring and A ring decorated with a tertiary hydrox-307 yl group, germination stimulation activities were drasti-308 cally reduced in comparison with 14 and (±)-GR24. A 309 small difference was observed between P. ramosa 1 and P. 310 ramosa 2a for MeCLA 10 ($r_{EC50} = 0.47$), the putative bio-311 312 synthetic precursor of cannalactone **14**⁴⁴ (Figure 3**B**). The specificity of biological response would appear from the 313 compounds downstream of MeCLA in the biosynthetic 314 pathway. Isothiocyanates (ITCs) as glucosinolate-315 breakdown products are germination stimulants of P. 316 ramosa seeds secreted in the soil by host plants as Brassi-317 318 caceae^{31,40,58}. As for (±)-MeCLA 10, a small difference of 319 germination was observed between P. ramosa 1 and P. 320 ramosa 2a ($0.1 < r_{EC50} < 1.1$ for 2-phenylethyl isothiocyanate)39,45. 321



323 Chart 3. Cannalactone analogs synthetized for thisstudy.



Figure 2. Bioactivity on Phelipanche ramosa 1 versus Phelipanche ramosa 2a seed germination of SdL analogs (see Chart 3). (A) EC₅₀ (half maximal effective concentration) (mol L-1) of SdL analogs, (±)-GR5, natural cannalactone 14 and (±)-GR24 toward P. ramosa 1 and P. ramosa 2a. EC_{50} are presented ± SE, ($6 \le n \le 12$). (B) $r_{EC_{50}}$ (P. ramosa 1)/ EC_{50} (P. ramosa 2a)] for each chem-ical.



Figure 3. Bioactivity on Phelipanche ramosa 1 versus Phelipanche ramosa 2a seed germination of canonical SLs, SL biosynthetic 339 precursors in comparison with (+)-GR24⁴⁵ and (-)-2'-epi-GR24⁴⁵. (A) EC₅₀ (half maximal effective concentration) (mol L⁻¹) of ca-340 nonical SLs and (\pm) -GR24 toward *P. ramosa* 1 and *P. ramosa* 2a. EC₅₀ are presented \pm SE, $(6 \le n \le 12)$. (B) $r_{EC50} = EC_{50} (P. ramosa 1)/2$ 341 EC_{50} (*P. ramosa* 2a) for each SL and (±)-contalactone⁴⁵. ND = not determined. 342

Comparing the germination stimulation activities of (±)-344 GR5 to that of (±)-GR24, loss of cycles A and B led to bio-345 activity reductions but also dampened the difference be-346 tween P. ramosa 1 and P. ramosa 2a $(r_{EC50} ((\pm)-GR5) =$ 347 0.10) (Figure 2B). All (±)-SdL analogs are bioactive but 354 348 less than (±)-GR24 for P. ramosa 1. (±)-SdL118F1 and (±)- 355 349

SdL127F2 proved to be more bioactive toward P. ramosa 350 2a while all the other SdLs were less bioactive than can-351 nalactone even if they have high activity for this popula-352 353 tion. SdL analogs possess EC₅₀ values varying between 5.6×10-10-4.8×10-8 M for P. ramosa 1, and 6.4×10-12-2.1×10⁻⁷ M for P. ramosa 2a. Removal of AB rings and

replacement by an unsaturated acyclic carbon chain at C-356 3a position induced a loss of bioactivity toward *P. ramosa* 357 1 for all SdL analogs compared to (±)-GR24. Moreover, no 358 significant difference was observed between "cis" 359 $(2'R^*,4R^*)$ and "trans" $(2'R^*,4S^*)$ SdL analogs toward P. 360 ramosa 1. On the other hand, on P. ramosa 2a seeds, SdL 361 analogs with an acyclic unsaturated carbon chain have a 362 bioactivity similar to (±)-GR24. For the genetic group 2a, it 363 was also interesting to find that the (±)-trans-SdL analogs 364 were more active than the (±)-cis-SdL analogs. It was 365 therefore possible to classify SdL analog bioactivity using 366 the r_{EC50} diagram (Figure 2B). The most active and selec-367 tive molecules toward P. ramosa 2a are thus (±)-SdL118F1 368 $(EC_{50} = 5.6 \times 10^{-10} \text{ M}, r_{EC50} = 87.5), (\pm) - SdL19 (EC_{50} =$ 369 1.5×10^{-10} M, $r_{EC50} = 18.7$) and (±)-SdL7 (EC₅₀ = 2.4×10^{-10} 370 M, $r_{EC50} = 10.8$). The analog (±)-SdL19 showed a similar 371 selectivity for the germination of P. ramosa 2a versus P. 372 ramosa 1 as cannalactone 14, and a similar bioactivity 373 toward *P. ramosa* 2a (EC₅₀ 1.5×10⁻¹⁰ M *versus* 1.0×10⁻¹⁰ M) 374 (Figure 2A). Subsequently, due to the ease of preparation 375 of the compound (±)-SdL19 and its specificity, we focused 376 on this compound in the rest of this study. 377



Figure 4. Stability of (±)-SdL19. Chemical hydrolysis of (±)-SdL19 and (±)-GR24 in ethanol/PBS at pH 6.8. Data are means \pm SE (n = 3).

SLs and mimics are sensitive to hydrolysis giving for (\pm) -GR24 poorly active or inactive 5-hydroxy-3-methylbutenolide $(D-OH)^{51}$ and inactive ABC=CHOH tricycle⁵⁹. Their sensitivity to hydrolysis has been tested routinely in aqueous solutions and compared to that of (\pm) -GR24^{23,60}, even though the assay was not performed at biologically active concentrations. Therefore, the chemical hydrolysis of (\pm) -SdL19 in comparison with (\pm) -GR24 was evaluated in a mixture of ethanol/water at pH 6.8, as previously reported for other SL analogs and mimics^{23,60}. Under these conditions, a slightly higher stability of (\pm) -SdL19 t_{1/2} \approx 120 h, (\pm) -GR24 t_{1/2} \approx 100 h) (Figure 4).

382

383

384

385

386

387

388

389

390

391

392

393

394

395 (±)-SdL19 cannalactone analog weakly interacts with the SL receptor PrKAI2d3. We have recently character-396 397 ized the PrKAI2d3 protein as a SL receptor from P. ramosa 398 1 among five PrKAI2 candidates, and we have demon-399 strated its high interaction with (±)-GR24⁵¹. We tested the 400 interaction between (±)-SdL19 and PrKAI2d3 using nano 401 differential scanning fluorimetry (nanoDSF). NanoDSF 402 allows to evaluate the interaction between a receptor protein and a ligand by monitoring the melting tempera-403 ture of the receptor protein. In nanoDSF, changes in the 404 protein fluorescence (ratio 350 nm/330 nm) are recorded 405 and do not require a dye. This technique can highlight 406 interactions that induce minor conformational changes. 407 Compared to (±)-GR24, incubation with (±)-SdL19 at low 408 concentrations did not affect the melting temperature of 409 PrKAI2d3. However, the use of high concentrations of (±)-410 SdL19 lowered the inflection point of the melting curve 411 indicating the binding of the ligand to the protein (Figure 412 5). We studied also the binding affinity of (±)-SdL19 to-413 wards PrKAI2d3 by intrinsic fluorescence but no Ka value 414 was measurable in comparison with (±)-GR24 (Figure 6). 415 The lower affinity of (±)-SdL19 for PrKAI2d3 in compari-416 son with (±)-GR24 confirmed the nanoDSF data. These 417 results are in accordance with the lower bioactivity of (±)-418 SdL19 on seeds of P. ramosa 1 in comparison with (±)-419 GR24. The characterization of SL receptor(s) from P. ra-420 mosa 2a is awaiting and would be appreciable to discuss 421 the SAR results presented here in the light of ligand-422 protein interactions. 423



428

429

430

Figure 5. Biochemical analysis of the interaction between the PrKAI2d3 with (±)-GR24 and (±)-SdL19 by nanoDSF. **(A, B)** Thermostability of PrKAI2d3 at 5 μM in the absence of ligand or in the presence of various ligand concentrations. Panels **(A, B)** show the changes in fluorescence (ratio F_{350nm}/F_{330nm}) with temperature for (±)-SdL19 and (±)-GR24, respectively. The panels **(C, D)** show the first derivatives for the F_{350nm}/F_{330nm} curve against the temperature gradient from which the apparent melting temperatures (T_m) were determined for (±)-SdL19 and (±)-GR24, respectively. The experiment was carried out twice.



431

Figure 6. Biochemical analysis of the interaction between the
PrKAI2d3, RMS3 and AtD14 proteins with (±)-GR24 (A) and
(±)-SdL19 (B) by intrinsic tryptophan fluorescence. Plots of
fluorescence intensity *versus* (±)-SdL19 or (±)-GR24 concen-

trations were used to determine the apparent K_d values. The plots represent the mean of two replicates and the experiments were repeated at least three times.

Cannalactone analogs efficiently inhibit bud out-439 growth in pea and Arabidopsis, and interact with SL 440 receptor D14 proteins. The biological activities of (±)-441 SdL analogs as plant hormones were evaluated using a 442 pea branching assay with the highly branched SL-deficient 443 rms1-10 mutant⁶¹. All (±)-SdL analogs showed activity at a 444 concentration of 1 µM but were found to be notably less 445 active than (±)-GR24, since they were not significantly 446 bioactive at 10 nM or below (Figure 7, Table S2). This 447 confirms the low specificity of SL reception in pea for 448 which a wide range of SLs, analogs and mimics are bioac-449 tive^{23,60}. Moreover, (±)-SdL analogs were inactive on the 450 branching of the pea rms3-4 perception mutant (Figure 451 7A), Table S3). These results suggest that (±)-SdL analogs, 452 as (±)-GR24, are bioactive SL analogs and inhibit bud 453 outgrowth via the RMS3 receptor, an ortholog of D14 in 454 pea, and not because of toxicity. To investigate potential 455 456 species differences, we examined the effect of (±)-SdL19 on Arabidopsis shoot branching. We applied (±)-SdL19 457 and (±)-GR24 to the hydroponically grown max3-11 458 plants. max3-11 is a SL-deficient mutant⁶². Treatment with 459 all concentrations of (±)-SdL19 inhibited outgrowth of 460 axillary buds in *max3-11*. However, a significant difference 461 was observed with (±)-GR24 for low concentrations test-462 ed (< 3000 nM) (Figure 7B). These results indicated that 463 (±)-SdL19 is less active than (±)-GR24 in Arabidopsis for 464 shoot branching inhibition when applied to the roots in 465 466 hydroponic culture.





Figure 7. Bioactivity in pea and Arabidopsis. Bioactivity in pea of (±)-SdL7, (±)-SdL19 and (±)-SdL118F1 versus (±)-GR24 (rms1, 469 rms3) (A). Length of the axillary buds of rms1-10 and rms3-5 pea plants, 8 d after direct application of the various analogs; CTL0 = 470 DMSO treatment ; WT Térèse plants were used as controls without treatment. Data are means \pm SE (\geq 20 plants) ***P < 0.001, *P < 471 0.5, Kruskal-Wallis rank sum test, compared to CTL0 value. See also supplementary data in the Supporting Information. Bioactivity 472 in Arabidopsis of probes versus (±)-GR24 (max3-11). Bioactivity in Arabidopsis of (±)-SdL19 versus (±)-GR24 (B). Number of rosette 473 branches of mutant plants max3-11 grown in long-day conditions. These data were obtained from means ± SE (n = 16 plants). *** P 474 < 0.001 indicate significant differences with the control treatment (0 nM) (CTL0) (Shapiro-Wilkinson normality test), + P < 0.05, ++ 475 P < 0.01 indicate significant differences with (±)-GR24 treatment (Shapiro-Wilkinson normality test). ns = not significant. CTL0 = 476 control 0. 477

In order to validate that (±)-SdL analogs are perceived by 478 the pea SL receptor RMS3, we performed differential 479 scanning fluorimetry (DSF) and revealed a shift in RMS3 480 melting temperature in the presence of (±)-SdL19, corre-481 sponding to a protein destabilization as for the other SL 482 bioactive analogs⁴ (Figure 8A-B). We showed that the 483 destabilization of RMS3 by (±)-SdL19 was recorded at 484 higher ligand concentrations in comparison with (\pm) -485 GR24 suggesting lower affinity. For AtD14, an ortholog of 486 D14 in Arabidopsis, DSF recordings revealed an increased 487 sensitivity for (±)-SdL19, inversely for (±)-GR24 as previ-488 ously reported for cannalactone 1444 and in contrast to 489 RMS3 (Figure 8C-D). These results are in accordance with 490

491 the fact that in pea, the main SLs detected are canonical 492 SLs⁶³, while in Arabidopsis, no canonical SL has been de-493 tected but only non-canonical SLs or their biosynthetic precursors such as methyl carlactonoate ((+)-MeCLA 10) 494 and HO-MeCLAs^{12,13}. Then, we estimated the binding affin-495 ity of (±)-SdL19 towards RMS3 and AtD14 by intrinsic 496 fluorescence and found a measurable K_d value (98.65 ± 497 10.86 μ M) only for AtD14 (Figure 6**B**). The lower affinity 498 of (±)-SdL19 for RMS3 in comparison with AtD14 high-499 lights differences between biochemical and in planta re-500 sults where in both Arabidopsis and pea, (±)-SdL19 is less 501 bioactive than (±)-GR24 for bud outgrowth inhibition. 502



Figure 8. Biochemical analysis of the interaction RMS3/PsD14, AtD14 proteins with (±)-GR24 and (±)-SdL19. The melting temperature curves of RMS3 (**A**, **B**) and AtD14 (**C**, **D**) proteins with varying indicated concentrations of (±)-GR24 and (±)-SdL19, as assessed by DSF, are shown. Each line represents the average protein melt curve for three technical replicates and the experiment was carried out three times.

563

(±)-SdL19 cannalactone analog promotes hyphal 509 branching in the AM fungus Rhizophagus irregularis. 510 The SL analogs produced in the present study can also 511 help to address SAR in AM symbiosis. SLs are known to 512 induce a number of developmental, cellular and metabolic 513 responses in AM fungi³⁴⁻³⁶. In particular, the stimulation of 514 hyphal branching is commonly used in bioassays to detect 515 the bioactivity of SLs on these fungi. Using this approach, 516 a number of SLs and related compounds have been tested 517 for their activity on the AM fungus Gigaspora margarita. 518 Taken together, these studies highlighted the complexity 519 of structural requirements in the ABC moiety. GR5 was 520 shown to be inactive, and early studies proposed that a 521 minimal structure would contain the C-D moiety fused to 522 at least one ring system^{35,64}. This proposal is consistent 523 with the recent discovery of bioactive bryosymbiol 13 524 carrying A, C and D rings²². However, results obtained 525 with CL derivatives later indicated that some compounds 526 without a C ring could retain activity²⁶. Combined with 527 reports on natural non-canonical SLs bearing variations 528 on the A or C rings⁶, these studies indicate that require-529 ments for bioactivity on AM fungi can be fulfilled in sever-530 al divergent ways. 531

In the present study, we first used an in vitro assay which 532 allows a quantification of hyphal branching65 in the AM 533 fungus Rhizophagus irregularis. As shown in Figure 9A, 534 (±)-SdL19 stimulated hyphal branching (*i.e.* the formation 535 of first-order and higher-order branches) in a similar 536 fashion to (\pm) -GR24. This indicates that a C-D moiety 537 combined with an acyclic carbon chain is sufficient for 538 539 bioactivity on this AM fungus.

The hyphal branching response triggered by SL analogs is 540 commonly used as a proxy measure for the stimulation of 541 the fungal symbiont, but this response does not always 542 reflect the ability of these compounds to facilitate the 543 initiation of symbiosis⁶⁵. To further document the ability 544 of (±)-SdL19 to stimulate *R. irregularis* in a true symbiotic 545 context, we used an additional test. This assay involves 546 Medicago truncatula plants mutated in the SL biosynthe-547 sis gene CCD8. These mutants are strongly affected in 548

their ability to initiate symbiosis with R. irregularis, and 549 550 this phenotype can be rescued by exogenous application 551 of SL analogs⁶⁵. Like other bioassays involving SL biosyn-552 thesis plant mutants and designed to investigate the hormonal activities of SL analogs, this test records the ability 553 of these analogs to "replace" natural SLs in a physiological 554 context. Using this assay, we have previously shown the 555 556 bioactivity of cannalactone 14 in AM symbiosis⁴⁴, indicating that the B ring is dispensable. In the present study, we 557 found that (\pm) -SdL19 exhibited an activity similar to (\pm) -558 GR24 (Figure 9B). This extends our previous observations 559 by showing that symbiotic activity can be conserved when 560 both A and B rings are absent and replaced by an unsatu-561 rated acyclic carbon chain. 562



Figure 9. Activity of (±)-SdL19 on the development of R. 564 irregularis and its symbiotic ability. (A) R. irregularis was 565 grown from spores for 12 days on medium containing 10⁻⁷ M 566 (±)-GR24 or (±)-SdL19, or the solvent alone (mock), then the 567 number of branches of first order (black bars) and higher 568 order (grey bars) were measured. Bars represent the mean ± 569 SE, n = 6 to 8 plates per condition, representing an average of 570 200 spores. (B) R. irregularis spores were used to inoculate 571 M. truncatula ccd8-1 SL-deficient plants. 10-7 M (±)-GR24 or 572 (±)-SdL19, or the solvent alone (mock) were supplied in the 573 nutrient solution. The ability of (±)-SdL19 to restore symbi-574 otic ability in R. irregularis was assessed by examining root 575 colonization, 23 days post-inoculation. Bars represent the 576 mean number of infection units per plant \pm s.e.m. n = 14 to 16 577 578 plants per condition. Bars topped with the same letter do not differ significantly by Mann-Whitney U test (P > 0.05). 579

To conclude, we provide with this study comprehensive 580 SAR data in two genetic groups of *P. ramosa*, comprising 581 582 routes for synthesis of 14 analogs (SdL) of the noncanonical SL cannalactone 14. The SdL analogues with the 583 best specificity towards the population 2a are the com-584 pounds with a polar part on the side carbon chain. In 585 addition to non-canonical SL cannalactone 14 recently 586 587 identified from hemp in our group⁴⁴, natural canonical SLs (-)-solanacol 5 and (-)-solanacyl acetate 6 exuded by 588 tobacco^{41,42} and tomato⁶⁶, host plants for *P. ramosa* 2a^{38,39}, 589 are reported here as remarkable selective germination 590 stimulants for P. ramosa 2a seeds, contrary to other SLs 591 such as orobanchol 2, orobanchyl acetate 3 and 5-592 deoxystrigol 4, also detected in tobacco root exudates⁴². 593 Our data further extend common knowledge on SL ana-594 logs that are involved in plant and AM fungi development. 595 Further studies on the interaction of those substances 596 with specific receptors in AM fungi, key players for a sus-597 tainable agriculture, and in *P. ramosa* 2a, will help to fully 598 599 resolve the structure-activity relationships in these species⁶⁵. Moreover, these highly active non-canonical SLs 600 (e.g., (\pm) -SdL19) could be useful for the study of other 601 organisms which putatively produce only non-canonical 602 SLs such as the moss Physcomitrium patens^{6,67,68} and Ara-603 bidopsis thaliana¹³. 604

605

606 EXPERIMENTAL SECTION

Synthesis: General experimental procedures. All non-607 aqueous reactions were run under an inert atmosphere 608 (argon), by using standard techniques for manipulating 609 air-sensitive compounds. All glassware was stored in the 610 oven and/or was flame-dried prior to use. Anhydrous 611 solvents were obtained by filtration through drying col-612 umns. Analytical thin-layer chromatographies (TLC) were 613 performed on plates precoated with silica gel layers. 614 Compounds were visualized by one or more of the follow-615 ing methods: (1) illumination with a short wavelength UV 616 lamp (i.e., $\lambda = 254$ nm), (2) spray with a 1% (w/v) KMnO₄ 617 solution in H₂O. All separations were carried out under 618 flash-chromatographic conditions on silica gel (prepacked 619 column, 230 - 400 mesh) at medium pressure (20 psi) 620 with Armen fraction collector and pump. Nuclear magnet-621 ic resonance spectra (1H; 13C NMR) were recorded respec-622 tively at [500 or 300; 175, 125 or 75] MHz on recorded on 623 Brucker Avance spectrometers at 298 K. For the ¹H spec-624

tra, data are reported as follows: chemical shift, multiplic-625 626 ity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad, coupling constant in Hz and integra-627 tion. The numbering for signal assignment was done ac-628 cording to Chart 2. IR spectra are reported in reciprocal 629 centimeters (cm⁻¹). Buffers and aqueous mobile-phases 630 for UPLC were prepared using water purified with a Milli-631 Q system. Mass spectra (MS) and high-resolution mass 632 spectra (HRMS) were determined by electrospray ioniza-633 tion (ESI) coupled to a time-of-flight analyzer (Waters 634 LCT Premier XE, Waters, USA) or with an Ultra Perfor-635 mance Liquid Chromatography system equipped with a 636 Triple Quadrupole mass spectrometer Detector (Acquity 637 UPLC-TQD, Waters, USA). 638

639 All-*trans*- β -carotene **17** is commercially available. (±)-GR24 was prepared according to known procedures⁶⁹ and 640 641 careful purification (HPLC)⁴⁵. (±)-GR5 was synthesized by known procedures²¹. (±)-MeCLA was prepared by an 642 unpublished method (Lucile Andna, Laurence Miesch). 643 (±)-Carlactone was synthesized by Adrian Scaffidi⁷⁰. Natu-644 ral cannalactone 14 was isolated as described in 645 Hamzaoui et al44. (±)-Strigol 1, (±)-5-deoxystrigol 4, (±)-646 sorgolactone 5, (±)-orobanchol 2 are commercially avail-647 able. Orobanchyl acetate 3 was synthesized from (±)-648 orobanchol by acetylation with acetic anhydride in pyri-649 dine as described in Boutet et al⁷¹. (-)-Solanacol 5 and 650 (-)-solanacyl acetate 6 are obtained as described in Boyer 651 et al²³ and (±)-7,8-desmethyl solanacol 15 and (±)-7,8-652 653 desmethyl solanacyl acetate 16 as described in Chen et al⁷². (±)-Fabacyl acetate 7 was kindly furnished by Koichi 654 Yoneyama. (±)-4-Vinyldihydrofuran-2(3H)-one 19 was 655 prepared by a known procedure⁵². 656

(2'R*,4R*)-SdL4F1 and (2'R*,4S*)-SdL4F2. A mixture of 657 alkene 19 (200 mg, 1.78 mmol), ethyl formate (144 µL, 658 1.96 mmol) in THF (4 mL) was placed at -78 °C. Potassi-659 um tert-butoxide was added (240 mg, 2.14 mmol). The 660 mixture was stirred for 2 h at -78 °C. A solution of bro-661 mide derivative 2073 (350 mg, 1.96 mmol) in THF (3.2 662 mL) was added. The mixture was stirred at room temper-663 ature during 12 h. The mixture was diluted with EtOAc 664 and saturated NH₄Cl aqueous solution and then separat-665 ed. The organic phase was washed with water and brine. 666 The combined organic layers were dried with Na₂SO₄, 667 filtered and evaporated under reduced pressure. The 668 mixture was purified by chromatography on silica gel 669 (heptane/EtOAc 80: 20 to 60: 40 during 30 min) to afford 670 pure (±)-SdL4F1 (87.5 mg, 21%) and (±)-SdL4F2 (85.7 671 mg, 20%) as a brown oils. (±)-SdL4F1: ¹H NMR (300 MHz, 672 CDCl₃) δ : 7.51 (d, J = 2.0 Hz, H10), 6.88 (t, J = 1.5 Hz, H6'), 673 6.12 (t, / = 1.5 Hz, H2'), 5.81-5.70 (m, H6), 5.12 (dt, / = 1.0, 674 6.0 Hz, H7a), 5.08 (d, J = 1.0 Hz, H7b), 4.41 (t, J = 9.0 Hz, 675 H5a), 4.07 (q, / = 4.5 Hz, H5b), 3.83-3.74 (m, H4), 1.97 (t, / 676 = 1.5 Hz, 3 H7'). ¹³C NMR (75 MHz, CDCl₃) δ: 171.5 (C5'), 677 170.4 (C2), 151.7 (C6'), 141.1 (C3'), 135.9 (C4'), 135.4 678 (C6), 116.7 (C7), 109.9 (C3), 100.7 (C2'), 71.1 (C5), 40.6 679 (C4), 10.9 (C7'). IR (cm⁻¹): 3092, 2983, 2919, 2850, 1781, 680 1752, 1683, 1344, 1207, 1184, 1083, 1026, 955, 866, 681 801 745. HRMS (ESI): Calcd for C₁₂H₁₃O₅ [M + H]⁺: 682 683 237.0763, found: 237.0762. (±)-SdL4F2: ¹H NMR (300 MHz, CDCl₃) δ : 7.48 (d, I = 2.0 Hz, H10), 6.88 (t, I = 1.5 Hz, 684 H6'), 6.12 (t, J = 1.5 Hz, H2'), 5.79-5.68 (m, H6), 5.10 (dt, J 685

= 1.0, 6.0 Hz, H7a), 5.06 (d, / = 1.0 Hz, H7b), 4.40 (t, / = 9.0 686 Hz, H5a), 4.05 (q, J = 4.5 Hz, H5b), 3.82-3.74 (m, H4), 1.96 687 (t, J = 1.5 Hz, 3 H7'). ¹³C NMR (75 MHz, CDCl3) δ: 171.5 688 (C5'), 170.4 (C2), 151.4 (C6'), 141.3 (C3'), 136.0 (C4'), 689 135.1 (C6), 116.9 (C7), 110.0 (C3), 100.6 (C2'), 71.0 (C5), 690 40.5 (C4), 10.9 (C7'). IR (cm⁻¹): 3089, 2917, 2850, 1775, 691 1747, 1679, 1342, 1181, 1082, 1003, 950. HRMS (ESI): 692 Calcd for C₁₂H₁₃O₅ [M + H]⁺: 237.0768, found: 237.0762. 693

(2'R*,4R*)-SdL7. To a solution of (±)-SdL4F1 (76.5 mg, 694 0.32 mmol, 1 equiv) in CH₂Cl₂ (0.36 mL), was added me-695 thyl acrylate (0.294 mL, 3.24 mmol, 10 equiv) and Hov-696 eyda-Grubbs II metathesis initiator (HGII) (10.9 mg, 0.016 697 698 mmol, 5 mol%) under argon. The mixture was stirred for 24 h at 50 °C in a closed flask with rodavis cap. The mix-699 ture was purified by chromatography on silica gel (hep-700 tane/EtOAc 40: 60) to afford pure (±)-SdL7 (54.4 mg, 701 702 57%, brown oil). ¹H NMR (300 MHz, CDCl₃) δ: 7.58 (d, J = 703 2.0 Hz, H6'), 6.87 (t, J = 2.0 Hz, H3'), 6.80 (dd, J = 8.0, 15.5 Hz, H6), 6.12 (t, J = 2.0 Hz, H2'), 5.88 (dd, J = 1.0, 15.5 Hz, 704 H7), 4.46 (t, / = 8.5 Hz, H5a), 4.13 (dd, / = 3.5, 9.5 Hz, H5b), 705 4.00-3.90 (m, H4), 3.73 (s, 3 H9), 1.99 (s, 3 H7'). ¹³C NMR 706 (75 MHz, CDCl₃) δ: 170.6 (C2), 170.3 (C5'), 166.5 (C8), 707 152.7 (C6'), 144.3 (C6), 140.9 (C3'), 136.1 (C4'), 122.9 708 (C7), 108.5 (C3), 100.8 (C2'), 69.9 (C5), 52.0 (C9), 39.1 709 (C4), 10.9 (C7'). $J_{C2-H6'} = 2.0$ Hz. IR (cm⁻¹): 3096, 2959, 710 2919, 2850, 1782, 1756, 1721, 1683, 1437, 1348, 1280, 711 1186, 1091, 1033, 1010, 956, 867, 748. HRMS (ESI): Calcd 712 for C₁₆H₁₇NO₇Na [M + CH₃CN + Na]⁺: 358.0903, found: 713 358.0903. 714

4-(3-Oxobut-1-en-1-yl)dihydrofuran-2(3H)-one (21). 715 To a solution of 4-vinyldihydrofuran-2(3H)-one 19 (400.4 716 mg. 3.57 mmol. 1 equiv) in CH₂Cl₂ (4 mL), was added 717 buten-3-one (2.98 mL, 35.70 mmol, 10 equiv) and HGII 718 (110.4 mg, 0.17 mmol, 5 mol%) under argon. The mixture 719 was stirred for 24 h at 50 °C in a closed flask with rodavis 720 cap. The mixture was purified by chromatography on 721 silica gel (heptane/EtOAc 70 : 30 to 50 : 50 during 30 722 min) to afford pure ketone 21 as a brown oil (375.9 mg, 723 68%). ¹H NMR (300 MHz, CDCl₃) δ: 6.66 (dd, *J* = 7.5, 15.5 724 Hz, H6), 6.17 (dd, / = 1.0, 16.0 Hz, H7), 4.48 (dd, / = 7.5, 725 11.5 Hz, H5a), 4.08 (dd, / = 7.5, 11.5 Hz, H5b), 3.38 (sext, / 726 =8.0 Hz, H4), 2.74 (dd, J = 7.5, 16.0 Hz, H3a), 2.44 (dd, J = 727 7.5, 16.0 Hz, H3b), 2.25 (s, 3 H9). ¹³C NMR (75 MHz, 728 CDCl₃) δ: 197.5 (C2), 175.4 (C8), 142.9 (C6) 132.5 (C7), 729 71.4 (C5), 38.6 (C4), 34.0 (C3), 27.8 (C9). IR (cm⁻¹): 3000, 730 2913, 1771, 1672, 1629, 1360, 1257, 1162, 1015, 979, 731 890, 838. UPLC-TQD (MS): *m/z* 155.2 ([M + H] +, 100%). 732

733 (E)-4-(3-Hydroxy-3-methylbut-1-en-1-

vl)dihvdrofuran-2(3H)-one (21a). THF (20 mL) was 734 added to anhydrous CeCl₃ (800.0 mg, 3.24 mmol, 1.4 735 equiv), dried by heating at 140 °C/30 rpm for 2 h, 0 °C. 736 The mixture was stirred overnight under argon at room 737 temperature. At this temperature, a solution of ketone **21** 738 (357.3 mg, 2.32 mmol) in THF (11.5 mL) was added and 739 stirred for 1 h. Then it was cooled to -70 °C and a solution 740 of methylmagnesium chloride in THF (1.1 mL (3 M), 3.24 741 mmol, 1.4 equiv) was added by syringe. The reaction 742 mixture was stirred until TLC indicated disappearance of 743 starting material. The reaction was quenched with an 744 aqueous 10% acetic acid solution (20 mL) and extracted 745 with EtOAc (3×10 mL). The organic layer was washed 746

747 with water $(2 \times 10 \text{ mL})$ and brine (10 mL). The combined organic layers were dried with Na₂SO₄, filtered and evap-748 orated under reduced pressure to afford crude product 749 22a as a brown oil (381.8 mg, 96%) used in the next step 750 without further purification but which can be also puri-751 fied by chromatography on silica gel (heptane/EtOAc 40: 752 60) to afford pure alcohol 22a. ¹H NMR (300 MHz, CDCl3) 753 δ: 5.75 (dd, / = 1.0, 15.5 Hz, H6), 5.59 (dd, / = 8.0, 15.5 Hz, 754 H7), 4.41 (dd, J = 8.0, 10.0 Hz, H5a), 3.97 (t, J = 8.5 Hz, 755 H5b), 3.19 (sext, J = 8.5, H4), 2.65 (dd, J = 8.5, 17.5 Hz, 756 H3a), 2.34 (dd, / = 9.5, 17.5 Hz, H3b), 1.30 (s, 3 H9 and 3 757 H10). ¹³C NMR (75 MHz, CDCl₃) δ: 176.7 (C2), 141.3 (C7), 758 124.5 (C6), 72.8 (C8), 70.7 (C5), 38.8 (C4), 34.8 (C3), 30.0 759 (C9 or C10), 20.4 (C9 or C10). IR (cm⁻¹): 3446, 2973, 2921, 760 1774, 1677, 1464, 1420, 1374, 1170, 1043, 1015, 975, 761 908, 839, 796, 690. UPLC-TQD (MS): m/z 93.1 (100%), 762 153.2 ([M – H₂O]⁺, 90%). 763

764 Preparation of cyclopropylmagnesium bromide⁷⁴. To a stirred suspension of magnesium turnings (300 mg. 765 12.30 mmol) in dry THF (2.0 mL) under argon was added 766 commercially available cyclopropyl bromide (0.800 mL, 767 9.99 mmol) in dry THF (12.2 mL) dropwise at room tem-768 perature over 30 min. During this operation, the reaction 769 continued refluxing without heating. After the addition of 770 the halide was completed, the mixture was stirred at 771 room temperature for 5 h. The titration was performed as 772 described in Sugano et al74. 773

774 (E)-4-(3-cyclopropyl-3-hydroxybut-1-en-1-

yl)dihydrofuran-2(3H)-one (22b). Ground CeCl3 (455.5 775 mg, 1.80 mmol, 1.4 equiv) was dried by heating at 776 140 °C/30 rpm for 2 h. Dry THF (11 mL) was added at 777 778 0°C. The mixture was stirred overnight under argon at room temperature. At this temperature, a solution of 779 780 ketone **21** (204.8 mg, 1.32 mmol) in 4 mL of dry THF was added and left to stir for 1 h. Then it was cooled to -70 °C 781 and cyclopropylmagnesium bromide (5.6 mL, 0.32 M, 1.80 782 mmol, 1.4 equiv) was added by syringe. The reaction 783 mixture was left to stir until TLC indicated that no start-784 ing material remained. The reaction was quenched with 785 an aqueous 10% acetic acid solution (10 mL) and extract-786 ed with EtOAc (3 × 10 mL). The organic layer was washed 787 with water (2 × 10 mL) and brine (10 mL). The combined 788 organic layers were dried (Na₂SO₄). Solvent was removed 789 to afford crude product 22b (266.4 mg, quantitative) used 790 791 in the next step without further purification but which can be also purified by chromatography on silica gel (hep-792 tane/EtOAc 40: 60) to afford pure alcohol 22b. ¹H NMR 793 794 (300 MHz, CDCl₃) δ: 5.64 (s, H7), 5.62 (s, H6), 4.40 (t, J =8.5 Hz, H5a), 3.97 (t, J = 8.0 Hz, H5b), 3.19 (sext, J = 8.0 795 Hz, H4), 2.64 (dd, / = 8.5, 17.5 Hz, H3a), 2.33 (dd, / = 9.0, 796 17.5 Hz, H3b), 1.23 (s, 3H10), 1.01-0.92 (m, H9), 0.40 (d, J 797 = 8.0 Hz, 2H11 or 2H12), 0.28 (d, J = 5.5 Hz, 2H11 or 798 2H12). ¹³C NMR (75 MHz, CDCl₃) δ: 176.6 (C2), 139.0 (C7), 799 125.8 (C6), 72.8 (C5), 71.6 (C8), 38.9 (C4), 34.9 (C3), 27.5 800 (C10), 21.6 (C9), 1.1 (C11 or C12), 0.97 (C11 or C12). IR 801 (cm⁻¹): 3446, 2921, 2851, 1777, 1462, 1423, 1373, 1171, 802 1045, 1016, 977, 737. HRMS (ESI): Calcd for C₁₁H₁₇O₃ [M + 803 H]+: 197.1178, found: 197.1180. 804

805 (E)-4-(3-Methyl-3-((trimethylsilyl)oxy)but-1-en-1-

806 yl)dihydrofuran-2(*3H*)-one (23a). A mixture of alcohol
 807 22a (137 mg, 0.80 mmol) and TMS-imidazole (7.4 mL,

50.39 mmol, 62.6 equiv) were stirred at 50 °C under ar-808 gon for 1 h. The mixture was stirred at room temperature 809 for 1 h and diluted with heptane (20 mL). The organic 810 layer was washed with brine $(2 \times 10 \text{ mL})$, dried with 811 Na₂SO₄, filtered and evaporated under reduced pressure 812 to afford pure product 23a (136.2 mg, 70%). ¹H NMR 813 (300 MHz, CDCl₃) δ: 5.66 (dd, / = 1.0, 15.5 Hz, H6), 5.46 814 (dd, / = 8.0, 15.5 Hz, H7), 4.38 (dd, / = 8.0, 9.0 Hz, H5a), 815 3.95 (t, J = 9.0 Hz, H5b), 3.16 (sext, J = 8.5, H4), 2.62 (dd, J 816 = 8.0, 17.5 Hz, H3a), 2.31 (dd, / = 9.5, 17.5 Hz, H3b), 1.26 817 (s, 3 H9 and 3 H10), 0.07 (s, 9 H TMS). ¹³C NMR (75 MHz, 818 CDCl₃) δ: 176.7 (C2), 142.2 (C7), 123.8 (C6), 73.2 (C8), 819 72.8 (C5), 38.9 (C4), 34.7 (C3), 30.5 (C9 and C10), 2.7 (C 820 TMS). IR (cm⁻¹): 2966, 2918, 1783, 1377, 1362, 1250, 821 1165, 1038, 1019, 972, 839, 755. UPLC-TOD (MS): m/z 822 153.3 ([M - OTMS]⁺, 100%), 93.2 (90%). 823

(E)-4-(3-cyclopropyl-3-((trimethylsilyl)oxy)but-1-en-824 825 1-yl)dihydrofuran-2(3H)-one (23b). A mixture of alcohol 22b (240.7 mg, 1.23 mmol) and TMS-imidazole (7.22 826 mL, 49.20 mmol, 40 equiv) were stirred at 50 °C under 827 argon during 1 h. The mixture was stirred at room tem-828 perature during 1 h and diluted with 20 mL of heptane. 829 The organic layer was washed with brine $(2 \times 10 \text{ mL})$, 830 dried over Na₂SO₄ and evaporated. The mixture was puri-831 fied by chromatography on silica gel (4 g, heptane/EtOAc 832 80:20) to afford pure product 23b (222.4 mg, 67%). ¹H 833 NMR (300 MHz, CDCl₃) δ : 5.62 (d, I = 15.5 Hz, H6), 5.52 834 (dd, J = 7.5, 15.5, H7), 4.40 (dd, J = 8.0, 9.0, H5a), 3.97 (t, J 835 = 8.5, H5b), 3.18 (sext, / = 8.0, H4), 2.64 (dd, / = 8.5, 17.5 836 Hz, H3a), 2.33 (dd, J = 9.5, 17.5 Hz, H3b), 1.25 (s, 3 H10), 837 0.88-0.78 (m, H9), 0.35-0.25 (m, 2 H11 and 2 H12), 0.06 838 (s, 9 HTMS). ¹³C NMR (75 MHz, CDCl₃) δ: 176.7 (C2), 140.4 839 (C7), 125.0 (C6), 74.0 (C8), 72.8 (C5), 38.9 (C4), 34.8 (C3), 840 27.3 (C10), 22.2 (C9), 2.7 (C11 or C12), 1.3 (C11 or C12). 841 IR (cm⁻¹): 3007, 2958, 2894, 1785, 1702, 1420, 1372, 842 1250, 1209, 1165, 1095, 1065, 1018, 975, 887, 839, 754, 843 685. HRMS (ESI): Calcd for C14H24O3Si [M - OTMS]+: 844 179.1072, found: 179.1070. 845

```
(2'R*,4R*)-SdL18F1 and (2'R*,4S*)-SdL18F2. A mixture
846
847
    of compound 23a (363.4 mg, 1.5 mmol), ethyl formate
    (0.12 mL, 1.50 mmol, 1.0 equiv) in THF (3 mL) was placed
848
    at -78 °C. Potassium tert-butoxide was added (190.7 mg,
849
    1.70 mmol, 1.13 equiv). The mixture was stirred for 2 h. A
850
    solution of bromide derivative 20 (286.7 mg, 1.60 mmol,
851
    1.1 equiv) in THF (3 mL) was added. The mixture was
852
853
    stirred at room temperature for 12 h. The mixture was
    diluted with EtOAc and saturated NH<sub>4</sub>Cl aqueous solution.
854
855
    The organic phase was washed with water and brine. The
    combined organic layers were dried with Na<sub>2</sub>SO<sub>4</sub>, filtered
856
    and evaporated under reduced pressure. The mixture was
857
    purified by chromatography on silica gel (heptane/EtOAc
858
    80: 20 to 70: 30 in 20 min) to afford products (±)-
859
    SdL18F1 and (±)-SdL18F1 as brown oils ((±)-SdL18F1:
860
    100.7 mg (18%) and (±)-SdL18F2: 86.0 mg (16%), total:
861
    34%). (±)-SdL18F1: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 7.50 (d, J
862
    = 2.5 Hz, H6'), 6.82 (t, / = 1.5 Hz, H3'), 6.08 (t, / = 1.5 Hz,
863
    H2'), 5.64 (d, / = 15.5 Hz, H7), 5.48 (dd, / = 7.5, 15.5 Hz,
864
    H6), 4.43 (t, I = 9.0 Hz, H5a), 4.06 (q, I = 4.0 Hz, H5b), 3.82-
865
    3.74 (m, H4), 1.99 (t, J = 1.5 Hz, 3 H7'), 1.27 (s, 3 H9 and 3
866
    H10), 0.09 (s, 9 HTMS). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ: 171.6
867
    (C2), 170.3 (C5'), 151.6 (C6'), 141.4 (C3'), 141.0 (C7),
868
```

136.0 (C4'), 123.6 (C6), 110.6 (C3), 100.7 (C2'), 73.4 (C8), 869 71.5 (C5), 39.5 (C4), 30.7 (C9 or C10), 30.6 (C9 or C10), 870 10.9 (C7'), 2.8 (3C TMS). IR (cm⁻¹): 2967, 2925, 1785, 871 1754, 1683, 1380, 1344, 1250, 1183, 1082, 1031, 956, 872 840, 752. HRMS (ESI): Calcd for C₁₅H₁₇O₅Na [M + Na -873 OTMS]+: 317.1001, found: 317.1004. (±)-SdL18F2: 1H 874 NMR (300 MHz, CDCl₃) δ: 7.50 (d, J = 2.5 Hz, H6'), 6.88 (t, J 875 = 1.5 Hz, H3'), 6.11 (t, / = 1.5 Hz, H2'), 5.63 (d, / = 15.5 Hz, 876 H7), 5.45 (dd, J = 7.5, 15.5 Hz, H6), 4.42 (t, J = 9.0 Hz, H5a), 877 4.03 (q, J = 4.5 Hz, H5b), 3.81-3.72 (m, H4), 1.96 (t, J = 1.5 878 Hz, 3 H7'), 1.22 (s, 3 H9 or 3 H10), 1.20 (s, 3 H9 or 3 H10), 879 0.05 (s, 9 HTMS). ¹³C NMR (75 MHz, CDCl₃) δ: 171.7 (C2), 880 170.3 (C5'), 151.6 (C6'), 141.5 (C3'), 141.1 (C7), 136.0 881 (C4'), 123.3 (C6), 110.5 (C3), 100.6 (C2'), 73.4 (C8), 71.5 882 (C5), 39.5 (C4), 30.7 (C9 or C10), 30.4 (C9 or C10) 10.8 883 (C7'), 2.7 (3C TMS). IR (cm⁻¹): 2967, 2925, 1786, 1755, 884 1683, 1380, 1344, 1264, 1183, 1084, 1032, 956, 840, 735. 885 HRMS (ESI): Calcd for C₁₅H₁₇O₅Na [M + Na - OTMS]⁺: 886 317.1001, found: 317.0998. 887

(2'R*,4R*)-SdL19. To a solution of scandium trifluoro-888 methanesulfonate (0.66 mg, 1.3 µmol) in CH₃CN (1.5 mL) 889 was added silvl derivative (±)-SdL18F1 (98 mg, 0.27 890 mmol) in CH₃CN (1.0 ml) and water (2.5 μ L, 0.14 μ mol) at 891 room temperature. The resulting mixture was stirred for 892 893 1 h at room temperature and quenched with a phosphate buffer (pH 7). The organic materials were extracted with 894 dichloromethane (3 × 10 mL), the combined extracts 895 were washed with brine, dried with Na₂SO₄, filtered and 896 897 evaporated under reduced pressure to afford pure (±)-SdL19 (87.7 mg, quantitative). ¹H NMR (300 MHz, CDCl₃) 898 δ: 7.50 (d, / = 2.5 Hz, H6'), 6.85 (t, / = 1.5 Hz, H3'), 6.10 (t, / 899 = 1.5 Hz, H2'), 5.71 (d, J = 15.5 Hz, H7), 5.58 (dd, J = 7.5, 900 15.5 Hz, H6), 4.43 (t, / = 9.0 Hz, H5a), 4.07 (dd, / = 4.5, 9.0 901 Hz, H5b), 3.83-3.76 (m, H4), 1.99 (t, / = 1.5 Hz, 3 H7'), 1.29 902 (s, 3 H9 and 3 H10). ¹³C NMR (75 MHz, CDCl₃) δ: 171.4 903 (C2), 170.2 (C5'), 151.6 (C6'), 140.9 (C3'), 140.3 (C7), 904 135.8 (C4'), 123.7 (C6), 110.2 (C3), 100.6 (C2'), 71.2 (C5), 905 70.5 (C8), 39.3 (C4), 29.8 (C9 and C10), 10.7 (C7'). IR (cm⁻ 906 '): 3460, 2973, 2924, 2850, 1783, 1750, 1682, 1372, 1345, 907 1265, 1185, 1089, 1032, 1010, 956, 863, 735, 703. HRMS 908 (ESI): Calcd for C₁₅H₁₈O₆Na [M + Na]⁺: 317.1001, found: 909 317.0994. 910

(2'R*,4R*)-SdL21. To a solution of scandium trifluoro-911 methanesulfonate (0.56 mg, 1.14 µmol) in CH₃CN (1.3 912 mL) was added silyl derivative (±)-SdL18F2 (83.5 mg, 913 0.33 mmol) in CH₃CN (0.29 mL) and water (2.0 µL, 0.11 914 umol) at room temperature. The resultant mixture was 915 stirred for 1 h at room temperature and quenched with a 916 phosphate buffer (pH 7). The organic materials were 917 extracted with dichloromethane $(3 \times 5 \text{ mL})$, the combined 918 extracts were washed with brine, dried with Na₂SO₄, fil-919 tered and evaporated under reduced pressure to afford 920 pure (±)-SdL21 (59.7 mg, 89%). ¹H NMR (300 MHz, 921 $CDCl_3$) δ : 7.50 (d, l = 2.5 Hz, H6'), 6.89 (t, l = 1.5 Hz, H3'), 922 6.11 (t, / = 1.5 Hz, H2'), 5.68 (d, / = 15.5 Hz, H7), 5.54 (dd, / 923 = 7.5, 15.5 Hz, H6), 4.43 (t, / = 9.0 Hz, H5a), 4.04 (dd, / = 924 4.5, 9.0 Hz, H5b), 3.82-3.74 (m, H4), 1.97 (t, J = 1.5 Hz, 3 925 H7'), 1.22 (s, 3 H9 and 3 H10). ¹³C NMR (75 MHz, CDCl₃) δ: 926 927 171.6 (C2), 170.4 (C5'), 151.7 (C6'), 141.1 (C3'), 140.7 928 (C7), 135.9 (C4'), 123.7 (C6), 110.6 (C3), 100.7 (C2'), 71.4 (C5), 70.7 (C8), 39.4 (C4), 29.9 (C9 and C10), 10.8 (C7'). IR 929

(cm⁻¹): 3488, 2969, 2925, 1784, 1751, 1682, 1343, 1263, 930 1185, 1084, 1026, 1013, 955, 863, 802, 730, 701. HRMS 931 (ESI): Calcd for C₁₅H₁₈O₆Na [M + Na]⁺: 317.1001, found: 932 317.1000. IR (cm⁻¹): 3488, 2969, 2925, 1784, 1751, 1682, 933 1343, 1263, 1185, 1084, 1026, 1013, 955, 863, 802, 730, 934 701. HRMS (ESI): Calcd for C₁₅H₁₈O₆Na [M + Na]⁺: 935 317.1001, found: 317.1000. 936

(2'R*,4R*)-SdL33. To a solution of (±)-SdL19 (15.6 mg, 937 0.05 mmol) in 0.3 mL of CH₂Cl₂ was added pyridine (0.09 938 mL, 1.06 mmol, 20 equiv), 4-nitrobenzoylchloride (49.2 939 mg, 0.26 mmol, 5 equiv) and DMAP (tip of spatula). The 940 mixture was stirred overnight at 40 °C. The mixture was 941 942 co-evaporated with toluene, extracted with EtOAc, washed with NaHCO3 and dried with Na2SO4, filtered and 943 944 evaporated under reduced pressure. The product was purified by PTLC (heptane/EtOAc 60: 40) to afford pure 945 946 (±)-SdL33 (18.2 mg, 77%, yellow amorphous solid). ¹H 947 NMR (300 MHz, CDCl₃) δ: 8.26 (dt, *J* = 2.0, 8.5 Hz, H14 and H14'), 8.11 (dt, / = 2.0, 8.5 Hz, H13 and H13'), 7.51 (d, / = 948 2.5 Hz, H6'), 6.89 (t, J = 1.5 Hz, H3'), 6.10 (t, J = 1.5 Hz, 949 H2'), 5.98 (dd, / = 1.0, 15.5 Hz, H7), 5.63 (q, / = 8.0 Hz, H6), 950 4.45 (t, J = 8.5 Hz, H5a), 4.10 (t, J = 7.5 Hz, H5b), 3.87-3.79 951 (m, H4), 2.02 (s, 3 H7'), 1.23 (t, J = 7.0 Hz, 3 H9 and 3 952 H10). ¹³C NMR (175 MHz, CDCl₃) δ: 171.4 (C2), 170.3 953 (C5'), 163.5 (C11), 151.8 (C6'), 150.6 (C15), 141.1 (C3'), 954 136.4 (C7), 131.5 (C4'), 130.7 (C13 and C13'), 126.8 (C6), 955 123.8 (C12), 123.7 (C14 and C14'), 110.0 (C3), 100.6 956 (C2'), 82.2 (C8), 71.2 (C5), 39.6 (C4), 27.3 (C9 or C10), 957 27.1 (C9 or C10), 11.0 (C7'). IR (cm⁻¹): 2919, 2850, 1785, 958 1755, 1722, 1683, 1607, 1527, 1464, 1349, 1290, 1185, 959 1102, 1033, 1014, 956, 876, 842, 720. HRMS (ESI): Calcd 960 for C₂₂H₂₁NO₉Na [M + Na]⁺: 466.1114, found: 466.1131. 961

(2'R*,4S*)-SdL34. To a solution of (±)-SdL21 (15.6 mg, 962 0.05 mmol) in 0.3 mL of CH₂Cl₂ was added pyridine (0.09 963 mL, 1.06 mmol, 20 equiv), 4-nitrobenzoylchloride (49.17 964 mg, 0.265 mmol, 5 equiv) and DMAP (tip of spatula). The 965 mixture was stirred overnight at 40 °C. The mixture was 966 co-evaporated with toluene, extracted with EtOAc, 967 washed with NaHCO3 and dried with Na2SO4, filtered and 968 evaporated under reduced pressure. The product was 969 purified by PTLC (heptane/EtOAc 60: 40) to afford pure 970 (±)-SdL34 (6.8 mg, 25%, yellow amorphous solid). ¹H 971 NMR (300 MHz, CDCl₃) δ: 8.25 (dt, J = 2.0, 8.5 Hz, H14 and 972 973 H14'), 8.10 (dt, J = 2.0, 8.5 Hz, H13 and H13'), 7.50 (d, J = 2.5 Hz, H6'), 6.92 (t, / = 1.5 Hz, H3'), 6.11 (t, / = 1.5 Hz, 974 H2'), 5.96 (dd, / = 1.0, 15.5 Hz, H7), 5.60 (q, / = 8.0 Hz, H6), 975 4.46 (t, J = 8.5 Hz, H5a), 4.09 (t, J = 7.5 Hz, H5b), 3.87-3.79 976 (m, H4), 2.02 (s, 3 H7'), 1.24 (t, J = 7.0 Hz, 3 H9 and 3 977 H10). ¹³C NMR (175 MHz, CDCl₃) δ: 171.3 (C2), 170.3 978 (C5'), 163.5 (C11), 151.8 (C6'), 150.6 (C15), 141.2 (C3'), 979 136.6 (C7), 135.4 (C4'), 130.8 (C13 and C13'), 126.6 (C6), 980 125.2 (C12), 123.7 (C14 and C14'), 110.1 (C3), 100.7 981 (C2'), 82.2 (C8), 71.1 (C5), 39.7 (C4), 27.1 (C9 or C10), 982 27.0 (C9 or C10), 10.9 (C7'). IR (cm⁻¹): 2919, 2850, 1784, 983 1754, 1722, 1683, 1607, 1527, 1464, 1349, 1289, 1184, 984 1101, 1032, 1013, 955, 875, 842, 721. HRMS (ESI): Calcd 985 for C₂₄H₂₄N₂O₉Na [M + CH₃CN + Na]⁺: 507.1380, found: 986 507.1389. 987

(2'R*,4S*)-SdL50. To a solution of (±)-SdL21 (34.3 mg, 988 0.117 mmol) in CH₂Cl₂ (0.6 mL) was added pyridine 989 (0.189 mL, 2.34 mmol, 20 equiv), 3,5-dinitrobenzoyl chlo-990

1051

ride (134.9 mg, 0.58 mmol, 5 equiv) and DMAP (tip of 991 992 spatula). The mixture was stirred at 40 °C overnight. The mixture was co-evaporated with toluene, extracted with 993 EtOAc, washed with NaHCO₃ and dried with Na₂SO₄. The 994 product was purified by PTLC (heptane/EtOAc 45: 55) to 995 afford pure (±)-SdL50 as a white solid (17.7 mg, 31%). ¹H 996 NMR (300 MHz, CDCl₃) δ: 9.19 (t, J = 2.0 Hz, H15), 9.04 (d, 997 / = 2.0 Hz, H13 and H13'), 7.52 (d, / = 2.5 Hz, H6'), 6.94 (t, / 998 = 1.5 Hz, H3'), 6.12 (t, J = 1.5 Hz, H2'), 5.96 (dd, J = 1.0, 999 16.0 Hz, H7), 5.65 (dd, / = 7.5, 15.5 Hz, H6), 4.46 (t, / = 9.0 1000 Hz, H5a), 4.08 (t, J = 5.0 Hz, H5b), 3.89-3.81 (m, H4), 2.02 1001 (s, 3 H7'), 1.65 (s, 3 H9 or 3 H10), 1.62 (s, 3 H9 or 3 H10). 1002 ¹³C NMR (125 MHz, CDCl₃) δ: 206.3 (C11), 171.2 (C5'), 1003 170.2 (C2), 152.0 (C6'), 148.8 (C12), 141.1 (C3'), 135.8 1004 (C7), 132.3 (C14 and C14'), 129.5 (C13 and C13'), 127.4 1005 (C6), 122.4 (C15), 109.9 (C4'), 100.9 (C2'), 71.1 (C8), 67.2 1006 (C5), 39.5 (C4), 29.9 (C3), 27.2 (C9 or C10), 26.8 (C9 or 1007 C10), 10.9 (C7'). R (cm⁻¹): 2985, 1737, 1447, 1373, 1301, 1008 1233, 1098, 1043, 938, 918, 847, 786. HRMS (ESI): Calcd 1009 1010 for C₂₄H₂₃N₃O₁₁Na [M + CH₃CN + Na]⁺: 552.1230, found: 552.1226. 1011

(2'R*,4R*)-SdL51. To a mixture of (±)-SdL19 (21.8 mg, 1012 0.07 mmol) and pyridine (250 µL, 3.09 mmol, 41.7 equiv) 1013 was added acetic anhydride (250 µL, 2.64 mmol, 35.7 1014 equiv) and DMAP (tip of spatula). The mixture was stirred 1015 at room temperature under argon overnight. The product 1016 was co-evaporated with toluene and purified by PTLC 1017 1018 (heptane/EtOAc 45: 55) to afford pure (±)-SdL51 (10.2 mg, 41%). ¹H NMR (300 MHz, CDCl₃) δ: 7.51 (d, J = 2.5 Hz, 1019 H6'), 6.95 (t, J = 1.5 Hz, H3'), 6.09 (t, J = 1.4 Hz, H2'), 5.83 1020 (dd, J = 1.0, 15.5 Hz, H7), 5.48 (dd, J = 8.0, 15.5 Hz, H6), 1021 4.43 (t, / = 8.5 Hz, H5a), 4.03 (t, / = 5.0 Hz, 5Hb), 3.83-3.74 1022 (m, H4), 1.95 (s, 3 H7'), 1.46 (d, J = 4.0 Hz, 3 H9 and 3 1023 H10), 1.23 (t, J = 7.0 Hz, 3 H12). ¹³C NMR (175 MHz, 1024 CDCl₃) δ: 171.4 (C11), 170.5 (C2), 170.0 (C5') 151.9 (C6'), 1025 141.4 (C3'), 137.3 (C7), 135.8 (C4'), 125.8 (C6), 109.7 1026 (C3), 100.7 (C2'), 80.0 (C8), 71.3 (C5), 39.6 (C4), 27.3 (C9 1027 or C10), 27.1 (C9 or C10), 14.4 (C12), 10.9 (C7'). IR (cm⁻¹): 1028 2985, 1737, 1447, 1373, 1301, 1234, 1097, 1043, 938, 1029 918, 847, 786. HRMS (ESI): Calcd for C17H20O7Na [M + 1030 Na]+: 359.1107, found: 359.1109. 1031

(2'R*,4R*)-SdL118F1 and (2'R*,4S*)-SdL118F2. A mix-1032 ture of compound 23b (150.8 mg, 0.56 mmol), ethyl for-1033 1034 mate (0.09 mL, 1.12 mmol, 2 equiv) in THF (1.2 mL) was 1035 placed at -78°C. Potassium tert-butoxide was added (142.5 mg, 1.27 mmol, 2.26 equiv). The mixture was 1036 stirred during 2 h. A solution of bromide derivative 20 1037 1038 (106.2 mg, 0.60 mmol, 1.08 equiv) in THF (1.2 mL) was added. The mixture was stirred at room temperature for 1039 12 h. The mixture was diluted with EtOAc and saturated 1040 NH₄Cl aqueous solution. The organic phase was washed 1041 with water and brine. The combined organic layers were 1042 dried with Na₂SO₄, filtered and evaporated under reduced 1043 pressure. The mixture was purified by chromatography 1044 on silica gel (heptane/EtOAc 80: 20 to 70: 30 in 20 min) 1045 to afford pure products (±)-SdL118F1 and (±)-SdL118F2 1046 ((±)-SdL118F1: 52.3 mg (24%), (±)-SdL118F2: 66.3 mg 1047 (30%), total: 54%). (±)-SdL118F1: ¹H NMR (300 MHz, 1048 CDCl₃) δ : 7.51 (d, J = 2.5 Hz, H6'), 6.84 (quint, J = 1.5 Hz, 1049 H3'), 6.08 (dd, J = 1.0, 3.0 Hz, H2'), 5.52 (t, J = 5.5 Hz, H7 1050 and H6), 4.43 (td, J = 2.0, 15.5 Hz, H5a), 4.10 (q, J = 7.0 Hz,

H5b), 3.81-3.74 (m, H4), 1.99 (t, I = 1.5 Hz, 3 H7'), 1.27 (s, 1052 3 H10), 0.91-0.82 (m, H9), 0.34-0.24 (m, 2 H11 and 2 1053 H12), 0.07 (s, 9 HTMS). ¹³C NMR (75 MHz, CDCl₃) δ: 173.2 1054 (C2), 169.1 (C5'), 151.6 (C6'), 141.0 (C3'), 140.9 (C7), 1055 138.9 (C4'), 125.1 (C6), 110.1 (C3), 100.8 (C2'), 73.9 (C8), 1056 71.5 (C5), 39.7 (C4), 27.7 (C10), 22.6 (C9), 10.9 (C7'), 2.7 1057 (3C TMS), 1.5 (C11 and C12). IR (cm⁻¹): 2959, 1784, 1755, 1058 1684, 1346, 1249, 1185, 1093, 1019, 1028, 955, 863, 841, 1059 754. HRMS (ESI): Calcd for C₂₀H₂₈O₆SiNa [M + Na]⁺: 1060 415.1553, found: 415.1547. (±)-SdL118F2: ¹H NMR (300 1061 MHz, CDCl₃) δ : 7.51 (t, J = 1.5 Hz, H6'), 6.87 (sext, J = 1.0 1062 Hz, H3'), 6.09 (q, J = 1.5 Hz, H2'), 5.52-5.49 (m, H7 and 1063 H6), 4.43 (td, J = 2.0, 15.5 Hz, H5a), 4.03 (q, J = 7.0 Hz, 1064 H5b), 3.81-3.74 (m, H4), 1.97 (q, / = 1.5 Hz, 3 H7'), 1.23 (s, 1065 3 H10), 0.88-0.77 (m, H9), 0.28-0.21 (m, 2 H11 and 2 1066 H12), 0.05 (s, 9 HTMS). ¹³C NMR (125 MHz, CDCl₃) δ: 1067 171.3 (C2), 170.2 (C5'), 151.3 (C6'), 141.0 (C3'), 140.9 1068 (C7), 134.8 (C4'), 126.7 (C6), 113.6 (C3), 100.5 (C2'), 74.3 1069 (C8), 71.5 (C5), 40.2 (C4), 29.9 (C10), 21.2 (C9), 10.9 1070 (C7'), 5.7 (3C TMS), 1.5 (C11 and C12). Carbonyl reso-1071 nances are missing in ¹³C NMR spectra of (±)-SdL118F1 1072 and (±)-SdL118F2 and found by HMBC spectra. IR (cm⁻¹): 1073 1074 2969, 1784, 1760, 1685, 1346, 1248, 1185, 1086, 1030, 1007, 956, 839, 750. HRMS (ESI): Calcd for C20H28O6SiNa 1075 [M + Na]⁺: 415.1553, found: 415.1540. 1076

(2'R*,4R*)-SdL127. To a solution of silvl ether (±)-1077 SdL118F1 (23.0 mg, 0.06 mmol, 1 equiv) in CH₃CN (0.1 1078 mL), was added acetic anhydride (0.011 mL, 0.12 mmol, 2 1079 equiv) and scandium trifluoromethanesulfonate (0.29 mg, 1080 0.59 µmol) under argon. The mixture was stirred for 2 h 1081 at room temperature under argon. The mixture was puri-1082 fied by PTLC (heptane/EtOAc 40: 60) to afford pure alco-1083 hol (±)-SdL127 as a colorless oil (1.8 mg, 19%). ¹H NMR 1084 (300 MHz, CDCl₃) δ: 7.50 (t, J = 3.0 Hz, H6'), 6.87 (dt, J = 1085 1.5, 9.5 Hz, H3'), 6.09 (s, H2'), 5.62 (dq, J = 3.5, 15.5 Hz, 1086 H7), 5.51 (dd, / = 7.0, 15.5 Hz, H6), 4.43 (td, / = 2.0, 9.0 Hz, 1087 H5a), 4.05 (dd, J = 4.0, 9.0 Hz, H5b), 3.83-3.75 (m, H4), 1088 1.99 (s, 3 H7'), 1.28 (s, 3 H10), 0.89-82 (m, H9), 0.43-0.34 1089 (m, 2 H11 or 2 H12), 0.28-0.22 (m, 2 H11 or 2 H12). ¹³C 1090 NMR (125 MHz, CDCl₃) δ: 171.5 (C2), 170.3 (C5'), 151.7 1091 (C6'), 141.0 (C3'), 137.6 (C6), 136.2 (C4'), 125.9 (C7), 1092 110.5 (C3), 100.8 (C2'), 72.0 (C8), 71.4 (C5), 39.7 (C4), 1093 28.1 (C10), 22.0 (C9), 10.9 (C7'), 1.5 (C11 or C12), 0.9 1094 (C11 or C12). IR (cm⁻¹): 3468, 2958, 2925, 2856, 1780, 1095 1745, 1680, 1459, 1369, 1344, 1263, 1183, 1083, 1029, 1096 1007, 953, 864, 798, 741, 672. HRMS (ESI): Calcd for 1097 C₁₇H₂₀O₆Na [M + Na]⁺: 347.1158, found: 347.1157. 1098

X-ray Diffraction. Thin colorless needle 0.30 × 0.04 × 1099 0.03 mm was mounted on a nylon loop with protection 1100 Paratone® oil. Cell dimensions and intensities were 1101 measured at 203 K on a RIGAKU diffractometer constitut-1102 ed by a MM007 HF rotating-anode generator, delivering 1103 intense Cu(K_a) radiation ($\lambda = 1.54187$ Å) through Osmic 1104 CMF confocal optics, and a Rapid II curved Image Plate 1105 detector allowing data measurement up to 2θ max = 144°; 1106 1107 Clearly, the tiny crystals obtained by dissolving in ethyl acetate (48e)/ heptane (58e) and slow cooling of the 1108 solution turned out to be poor diffractors and a compro-1109 mise was chosen between reasonable exposure time and 1110 exploitable data for model solution. 10732 measured 1111 reflections up to the θ value matching the *iUCR* criteria, 1112

1113 4562 independent reflections (Rint = 0.138) of which only 687 had $|F_0| > 4\sigma$ (F₀) using the CrystalClear 2.0 1114 suite75. Data were corrected for Lorentz and polarization 1115 effects and for absorption ($T_{min, max}$: 0.448, 1.000). De-1116 spite the little signal detected beyond the 1.4 Å resolution limit, the structure could be readily solved either by dual 1118 methods (SHELXD)⁷⁶, or intrinsic phasing methods 1119 (SHELXT program⁷⁷; all other calculations regarding the 1120 model refinement were performed with SHELXL system⁷⁸ 1121 and PLATON79 programs, keeping meaningful X-ray dif-1122 fraction intensities. A potential solvent accessible region 1123 with disordered electron density was detected within the 1124 crystal structure. SQUEEZE⁸⁰ was used to model the unre-1125 solved electron density likely resulting from disordered 1126 crystallizing solvent molecules, representing a total of 44 1127 electrons per unit cell. This contribution was not included 1128 in the crystal data. Full-matrix least-squares refinement 1129 based on *F* using the weight of $1/[\sigma^2(F_0) + 0.2(F_0^2)]$ gave 1130 1131 final values of R1 = 0.101, $\omega R2 = 0.269$, and GOF(F) =1132 1.241 for 319 parameters, 276 restraints on the thermal 1133 parameters for all the non-H atoms with the SHELXL RIGU 1134 command and 1012 contributing reflections. Maximum 1135 shift/error = 0.0000, max/min residual electron density = 0.283/-0.210 e.Å -3. Hydrogen atoms were placed in calcu-1136 lated positions (C—H = 0.94-1.00 Å) and refined as riding 1137 on their parent atoms, with $U_{iso}(H)$ values constrained to 1138 1139 1.2 $U_{eq}(C)$ or 1.5 $U_{eq}(C_{methyl})$. See Tables S1.

1117

Plant material and growth conditions. Pea (Pisum sa-1140 tivum) branching mutants used in this study were derived 1141 from various cultivars of pea after ethyl methanesulfonate 1142 (EMS) mutagenesis and were described previously⁸¹. The 1143 rms1-10 (M3T-884) and rms3-4 (M2T-30) mutants were 1144 obtained from the dwarf cv Térèse. Plants were grown in 1145 a greenhouse under long days as described in Braun et 1146 al.⁸². 1147

1148 All A. thaliana plants used in this study originated from 1149 the Columbia (Col-0) ecotype background and have been 1150 described previously: max3-1162. Plants were grown as described in Cornet et al.83 for a hydroponic assay (see 1151 1152 also below).

Two batches of parasitic plant seeds were used in this 1153 study. A population of seeds of Phelipanche ramosa (L.) 1154 Pomel associated to genetic group 1 (P. ramosa 1) was 1155 collected from Saint Martin-de-Fraigneau, France, on 1156 broomrape parasitizing winter oilseed rape (Brassica 1157 napus L.) in 2015. Seeds of P. ramosa from genetic sub-1158 clade 2a (P. ramosa 2a) were harvested at Saint Martin-1159 1160 de-Bossenay, France, on broomrape developed on hemp (Cannabis sativa L.) in 2012³⁸. Seeds were surface steri-1161 lized and conditioned according to Pouvreau et al.⁵⁷ (dark 1162 condition; 21 °C). 1163

Pea shoot branching assay. The compounds to be tested 1164 were applied directly to the axillary bud with a micropi-1165 pette as 10 µL of a solution containing 0.1% DMSO with 1166 2% polyethylene glycol 1450, 50% ethanol and 0.4% 1167 DMSO^{23,84}. The control-0 is the treatment with 0.1% 1168 DMSO without compound. 24 plants were sown per 1169 treatment in trays (2 repetitions of 12 plants). The treat-1170 ment was generally performed 10 days after sowing, on 1171 the axillary bud at node 3. The branches at nodes 1 to 2 1172 were removed to encourage the outgrowth of axillary 1173

buds at nodes above. Nodes were numbered acropetally 1174 from the first scale leaf as node 1 and cotyledonary node 1175 as node 0. Bud growth at node 3 was measured with digi-1176 tal callipers 8 to 10 days after treatment. Plants with a 1177 damaged main shoot apex or showing a dead white treat-1178 ed-bud were excluded from the analysis. The SL-deficient 1179 rms1-10 and SL-reception rms3-4 pea mutants were used 1180 for all experiments and WT Térèse was used as control. 1181

Hydroponic assay on Arabidopsis. The hydroponic 1182 assay was adapted from Cornet et al.83. Seeds were sur-1183 face-sterilized for 8 min in a solution of ethanol (95%)-1184 Bayrochlore (Bayrol, Mundolsheim, France) (10%), and 1185 1186 were rinsed twice with ethanol (100%). Each seed was sown on top of a cut 0.5 mL Eppendorf tube filled with 1187 agar medium containing 0.65% agar and 10% nutritive 1188 solution 5 mM NO₃. Tubes were soaked in water and 1189 stored in the dark at 4 °C for 2 d. Twelve plants per pi-1190 pette tip box ($13 \times 9 \times 7$ cm) were grown and supplied 1191 with nutrient solution as in Boyer et al.60 at a concentra-1192 tion of 5 mL.L⁻¹ (750 mL of solution per box). Every week 1193 the nutrient solution was renewed and every ten days 1194 when the molecules were added into the solution. The 1195 first treatment occurred at day 27 after sowing when 1196 plants started to bolt. The number of rosette branches 1197 was performed at day 42. 1198

Germination stimulation activity assay on root para-1199 sitic plant seeds. Germination Stimulant activity (GS) of 1200 chemicals on seeds of parasitic plants were determined 1201 using a method described previously^{57,85}. Chemicals were 1202 suspended in DMSO, except all-trans-\beta-carotene 17 in 1203 THF, at 10 mmol.L⁻¹, then diluted with water at 1 mmol.L⁻¹ 1204 (water/DMSO; v/v; 9/1). Dilutions of 1×10^{-5} mol.L⁻¹ to 1205 1×10⁻¹² mol.L⁻¹ were then performed in water/DMSO 1206 (v/v; 9/1). For each compound, a range of concentrations 1207 from 10⁻¹³ to 10⁻⁶ mol.L⁻¹ (water/DMSO; 99/1) were ap-1208 plied to conditioned parasitic seeds. DMSO 1% was used 1209 as negative control (seed germination < 1%) and (±)-1210 GR24 at a concentration of 1 µmol.L⁻¹ was used as a posi-1211 tive control and induced 72-87% of seed germination for 1212 P. ramosa 1, 80-90% for P. ramosa 2a. To avoid variations 1213 related to sterilization events percentages of germination 1214 are reported as a ratio relative to the positive control 1215 ((±)-GR24, 1 µmol L-1) included in each germination as-1216 say. Each dilution and germination assay was repeated at 1217 least three times. For each compound tested, dose-1218 response curves (Germination stimulation = f(c), Germi-1219 nation Stimulant activity relative to (\pm) -GR24 1 µmol L⁻¹; 1220 c : concentration (mol. L-1), half maximal effective concen-1221 tration (EC₅₀), and maximum of germination stimulant 1222 activity were determined using a Four Parameter Logistic 1223 Curve computed with SigmaPlot[®] 10.0. 1224

Fungal material. Rhizophagus irregularis spores (strain 1225 DAOM197198) were purchased from Agronutrition 1226 (France). They were rinsed twice with sterile water be-1227 fore use. 1228

Analysis of hyphal branching in vitro. Experiments 1229 were carried out as described in Taulera et al. (2020)65. 1230 Spores were placed on plates containing M medium⁸⁶ and 1231 supplemented with SL analogs (100 nM.L⁻¹), or 0.1% 1232 DMSO for mock treatments. Plates were incubated at 1233 30 °C under 2% CO2 for 12 days. Germ tubes were then 1234

1235 identified as the longest hypha coming out of each spore. 1236 The number of hyphal branches of the 1st order (growing from the germ tube) and higher order (growing from 1storder branches) was scored using a dissecting micro-1238 scope for each germinated spore. The average number of branches of each order was calculated for all the germinated spores on a plate (typically 25 to 35 spores). For statistical analysis, each plate was treated as a replication 1242 unit (represented by the plate mean), and 6 to 8 plates were analyzed for each treatment.

1237

1239

1240

1241

1243

1244

1245

1246

1247

1248

1249

1250

1251

1252

1253

1254

1255

1256

1257

1258

1259

1260

1261

1262

1264

1265

1266

1267

1268

1269

1270

1271

1273

1281

Symbiosis initiation assay. The assay was carried out as described in Taulera et al.65. Briefly, ccd8-1 mutants of Medicago truncatula⁸⁷ were grown on a clay substrate inoculated with 150 spores of *R. irregularis*. SL analogs were added to the nutrient solution, to reach a final concentration of 100 nM.L-1. Mock treatments were performed with 0.1% DMSO. The number of infection points in each root system was recorded three weeks postinoculation, after staining fungal structures with ink.

Expression and purification of proteins. Expression and purification of proteins RMS3, AtD14, and PrKAI2d3 with a cleavable GST tag were performed in accordance with de Saint Germain et al.^{51,61}.

Differential Scanning Fluorimetry (DSF). DSF experiments were performed on a CFX384 Touch™ Real-Time PCR Detection System (Biorad) using excitation and emission wavelengths of 490 and 575 nm, respectively. Sypro Orange ($\lambda_{Ex}/\lambda_{Em}$: 490/610 nm; Life technologies) was used as the reporter dye. Samples were heat-denatured 1263 using a linear 25 to 95 °C gradient at a rate of 1.3 °C per minute after incubation at 25 °C for 30 min in the absence of light. The denaturation curve was obtained using CFX manager software. Final reaction mixtures were prepared in triplicate in 384-well white microplates, and each reaction was carried out in 20-µL scale in PB buffer pH 6.8 containing 10 µg protein, each concentration of SL derivatives (DMSO solution, final DMSO concentration was 4%), 1272 and 0.008 µL Sypro Orange. In the control reaction, DMSO was added instead of chemical solution.

NanoDSF. Proteins were diluted in PBS (100 mM Phos-1274 phate, pH 6.8, 150 mM NaCl) to a concentration of 1275 $\sim 10 \,\mu$ M. Ligands were tested at a concentration of 1276 200 µM. The intrinsic fluorescence signal was measured 1277 as a function of increasing temperature with a Prome-1278 theus NT.48 fluorimeter (NanoTemper Technologies), 1279 with 55% excitation light intensity and 1 °C/min temper-1280 ature ramp. Analyses were done on capillaries filled with 10 µL of the respective samples. Intrinsic fluorescence 1282 signals expressed by the 350 nm/330 nm emission ratio 1283 that increases as the proteins unfold, were plotted as a 1284 function of temperature (Figure 5A and Figure 5B). The 1285 plots show one of the three independent data collections 1286 performed for each protein. 1287

1288 Intrinsic tryptophan fluorescence assays and determination of the dissociation constant K_D. These exper-1289 1290 iments were performed as described previously in de Saint Germain et al.⁶¹ using a Spark Multimode Microplate 1291 1292 Reader (Tecan).

Hydrolysis of (±)-SdL19 and (±)-GR24 in aqueous 1293 solution. (±)-GR24 and (±)-SdL19 were tested for their 1294

chemical stability in an aqueous solution. Aqueous solu-1295 tions of the compound to be tested (50 μ g/mL) were 1296 incubated at 22 °C in the HPLC vials. The compounds 1297 were first dissolved in DMSO (2 mg/mL). Then, 25 µL of 1298 these solutions ((±)-GR24 and (±)-SdL19) were diluted to 1299 the final concentration with H_2O (750 µL) and EtOH (175 1300 µL) and the solution adjusted to pH 6.8. Aqueous solu-1301 tions of the compounds to be tested (50 μ g/mL) were 1302 incubated at 22 °C in the HPLC vials. (±)-1-Indanol (25 µL 1303 of a 1 mg/mL solution in DMSO) was added as internal 1304 standard to each solution. The samples were subjected to 1305 reverse-phase-ultra-performance liquid chromatography 1306 (RP-UPLC)-MS analyses by means of UPLC system 1307 equipped with a Photo Diode Array (PDA) and a Triple 1308 Quadrupole Detector (TOD) mass spectrometer (Acquity 1309 UPLC-TQD, Waters). RP-UPLC (HSS C18 column, 1.8 µm, 1310 2.1 mm \times 50 mm) with 0.1% (v/v) formic acid in CH₃CN 1311 and 0.1% (v/v) formic acid in water (aq. FA, 0.1%, v/v, pH 1312 2.8) as eluents [10% CH₃CN, followed by linear gradient 1313 from 10% to 100% of CH₃CN (4 min)] at a flow rate of 1314 0.6 mL.min⁻¹. The detection was done by PDA and with 1315 the TQD mass spectrometer operated in Electrospray 1316 ionization-positive mode at 3.2 kV capillary voltage. To 1317 maximize the signal, the cone voltage and collision energy 1318 were optimized to 20 V and 12 eV, respectively. The colli-1319 sion gas was argon at a pressure maintained near 4.5 10-3 1320 1321 mBar. The relative quantity of remaining (non degraded) product was determined by integration in comparison 1322 with the internal standard. 1323

Statistical analyses. Because deviations from normality 1324 were observed for axillary bud length after SL treatment, 1325 the Kruskal-Wallis test was used to compare treatments 1326 using R Commander version 1.7-3⁸⁸. For bioassays with 1327 Arabidopsis thaliana, data were analyzed with the 1328 Shapiro-Wilkinson normality test. For bioassays with AM 1329 fungi, data were analyzed using Statgraphics Centurion 1330 software (SigmaPlus). Non-parametric tests were used 1331 because normality or homoscedasticity criteria were not 1332 met. Datasets were analyzed using the Kruskal-Wallis 1333 test, followed by pairwise comparisons with Mann-1334 Whitney U test. 1335

ASSOCIATED CONTENT 1336

The Supporting Information is available free of charge at 1337 https://pubs.acs.org/doi/xxx. Crystallographic data of race-1338 mic SdL50 (Table S1) and CIF file, bioactivity data (supple-1339 mentary data for Figure 7, Tables S2-S3, Figure S1) and ¹H 1340 and ¹³C NMR spectra for the SdL compounds and precursors 1341 (21; 22a,b; 23a,b). 1342

AUTHOR INFORMATION 1343

Corresponding Author 1344

François-Didier Boyer - Université Paris-Saclay, CNRS, 1345 Institut de Chimie des Substances Naturelles, UPR 2301, 1346 91198, Gif-sur-Yvette, France; orcid.org/0000-0001-9855-1347 1348 7234; tel, ++33-1-69823017;Email francoisdidier.boyer@cnrs.fr 1349

Authors 1350

Suzanne Daignan Fornier – Université Paris-Saclay, CNRS, 1351 Institut de Chimie des Substances Naturelles. UPR 2301. 1352 91198, Gif-sur-Yvette, France; suzanne.daignan@cnrs.fr 1353

1354 Alexandre de Saint Germain - Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), Versailles. France: Alexandre.De-Saint-78000. Germain@inrae.fr; orcid.org/0000-0003-2814-7234

Pascal Retailleau - Université Paris-Saclay, CNRS, Institut 1358 de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-1359 pascal.retailleau@cnrs.fr; sur-Yvette, France; or-1360 cid.org/0000-0003-3995-519X

Jean-Paul Pillot - Université Paris-Saclay, INRAE, AgroPa-1362 risTech, Institut Jean-Pierre Bourgin (IJPB), 78000, Ver-1363 sailles, France; jean-paul.pillot@inrae.fr 1364

Quentin Taulera - Laboratoire de Recherche en Sciences 1365 1366 Végétales, Université de Toulouse, CNRS, UPS, Toulouse INP, 1367 31320 Auzeville-Tolosane, France; quentin.taulera@lrsv.ups-1368 tlse.fr

Lucile Andna – Université de Strasbourg, Institut de Chimie, 1369 UMR 7177, Équipe Synthèse Organique et Phytochimie, 4 rue 1370 Blaise Pascal CS 90032, 67081 Strasbourg Cedex France; 1371 ljouffro@its.jnj.com; orcid.org/0000-0002-4185-9458 1372

1373 Laurence Miesch - Université de Strasbourg, Institut de Chimie, UMR 7177, Équipe Synthèse Organique et Phytochi-1374 mie, 4 rue Blaise Pascal CS 90032, 67081 Strasbourg Cedex 1375 France; lmiesch@unistra.fr; orcid.org/0000-0002-0369-1376 9908 1377

1378 Soizic Rochange - Laboratoire de Recherche en Sciences 1379 Végétales, Université de Toulouse, CNRS, UPS, Toulouse INP, rochange@lrsv.ups-31320 Auzeville-Tolosane, France; 1380 tlse.fr; orcid.org/0000-0002-3889-7859 1381

Jean-Bernard Pouvreau – Nantes Université, CNRS, US2BN, 6286, F-44000 Nantes, UMR France: lean-Bernard.Pouvreau@univ-nantes.fr; orcid.org/0000-0002-8351-9195

Author Contributions 1386

1355

1356

1357

1361

1382

1383

1384

1385

1387

1388

1389

1390

1391

1392

1393

1394

1395

F.-D.B. designed research; F.-D.B and S.D.F. designed and synthesized the SdL analogs; L.A. and L.M. synthesized (±)-MeCLA; F.-D.B. performed the HPLC analyses; F.-D.B., J.-B.P., J.-P.P., S.R. and Q.T. designed and performed the biological experiments; A.deS.G. performed the biochemical experiments. P.R. performed the X-ray analysis. All authors analyzed the data; S.R. and F.-D.B. wrote the paper. All authors critically revised the manuscript and approved the submitted version.

Funding Sources 1396

We are grateful to Hemp *it adn* for financial support. The IJPB 1397 benefits from the support of Saclay Plant Sciences-SPS (ANR-1398 17-EUR-0007). This work has benefited from the support of 1399 1400 IJPB's Plant Observatory technological platforms. A.d.S.G. has received the support of the EU in the framework of the Ma-1401 rie-Curie FP7 COFUND People Programme, through the 1402 award of an AgreenSkills/AgreenSkills+ fellowship and the 1403 support of Saclay Plant Sciences-SPS (ANR-17-EUR-0007) 1404 1405 through the award of a fellowship. The work of S.R. and Q.T. was supported by the TULIP LabEx (ANR-10-LABX-41). The 1406 CHARM3AT LabEx program (ANR-11-LABX-39) is also 1407 1408 acknowledged for its support.

Notes

1409

1410

1411

The authors declare no competing financial interest.

ACKNOWLEDGMENT 1412

The authors thank Catherine Rameau for statistical analyses 1413 and Adrian Scaffidi for the generous gift of a sample of (±)-1414 carlactone. The authors thank Jean-Marie Beau, Catherine 1415

1416Rameau and Sandrine Bonhomme for comments on the14871417manuscript.1488

1489

1490

1491

1492

1493

1494

1495

1496

1497

1498

1499

1500

1501

1502

1503

1504

1505

1506

1507

1508

1509

1510

1511

1512

1513

1514

1515

1516

1517

1518

1519

1520

1521

1522

1523

1524

1525

1526

1527

1528

1529

1530

1531

1532

1533

1534

1535

1536

1537

1538

1539

1540

1541

1542

1543

1544

1545

1546

1547

1548

1549

1550

1551

1552

1553

1554

1555

1556

1557

1558

1559

1560

1418 **REFERENCES**

- 14191Cook, C. E., Whichard, L. P., Turner, B. & Wall, M. E.1420Germination of Witchweed (Striga Lutea Lour) Isolation1421and Properties of a Potent Stimulant. Science 154, 1189-14221190, doi:10.1126/science.154.3753.1189 (1966).
- 2 Gomez-Roldan, V., Fermas, S., Brewer, P. B., Puech-Pages, 1423 V., Dun, E. A., Pillot, J.-P., Letisse, F., Matusova, R., Danoun, 1424 S., Portais, J.-C., Bouwmeester, H., Bécard, G., Beveridge, C. 1425 1426 A., Rameau, C. & Rochange, S. F. Strigolactone inhibition of 455, shoot branching. Nature 189-194. 1427 doi:10.1038/nature07271 (2008). 1428
- Idage Strain S
- 1434
 4
 Waters, M. T., Gutjahr, C., Bennett, T. & Nelson, D. C.

 1435
 Strigolactone Signaling and Evolution. Annu. Rev. Plant

 1436
 Biol. 68, 291-322, doi:10.1146/annurev-arplant-042916

 1437
 040925 (2017).
- 14385Lopez-Obando, M., Ligerot, Y., Bonhomme, S., Boyer, F.-D.1439& Rameau, C. Strigolactone biosynthesis and signaling in1440plant development. Development 142, 3615-3619,1441doi:10.1242/dev.120006 (2015).
- Yoneyama, K., Xie, X., Yoneyama, K., Kisugi, T., Nomura, T.,
 Nakatani, Y., Akiyama, K. & McErlean, C. S. P. Which are the
 major players, canonical or non-canonical strigolactones? *J. Exp. Bot.* 69, 2231-2239, doi:10.1093/jxb/ery090
 (2018).
- Wakabayashi, T., Hamana, M., Mori, A., Akiyama, R., Ueno,
 K., Osakabe, K., Osakabe, Y., Suzuki, H., Takikawa, H.,
 Mizutani, M. & Sugimoto, Y. Direct conversion of
 carlactonoic acid to orobanchol by cytochrome P450
 CYP722C in strigolactone biosynthesis. *Sci. Adv.* 5,
 eaax9067, doi:10.1126/sciadv.aax9067 (2019).
- 14538Wakabayashi, T., Shida, K., Kitano, Y., Takikawa, H.,1454Mizutani, M. & Sugimoto, Y. CYP722C from Gossypium1455arboreum catalyzes the conversion of carlactonoic acid to14565-deoxystrigol. Planta 251, 97, doi:10.1007/s00425-020-145703390-6 (2020).
- 9 Mori, N., Sado, A., Xie, X., Yoneyama, K., Asami, K., Seto, Y., 1458 Nomura, T., Yamaguchi, S., Yoneyama, K. & Akiyama, K. 1459 1460 Chemical identification of 18-hydroxycarlactonoic acid as an LjMAX1 product and in planta conversion of its methyl 1461 ester to canonical and non-canonical strigolactones in 1462 japonicus. Phytochemistry Lotus 174. 112349. 1463 1464 doi:10.1016/j.phytochem.2020.112349 (2020).
- 146510Wang, J. Y., Lin, P.-Y. & Al-Babili, S. On the biosynthesis and
evolution of apocarotenoid plant growth regulators.1466seminars in Cell & Developmental Biology 109, 3-11,
doi:10.1016/j.semcdb.2020.07.007 (2021).
- 1469 Yoda, A., Mori, N., Akiyama, K., Kikuchi, M., Xie, X., Miura, 11 1470 K., Yoneyama, K., Sato-Izawa, K., Yamaguchi, S., Yoneyama, K., Nelson, D. C. & Nomura, T. Strigolactone biosynthesis 1471 catalyzed by cytochrome P450 and sulfotransferase in 1472 1473 232, 1999-2010, sorghum. New Phytol. doi:10.1111/nph.17737 (2021). 1474
- 12 Brewer, P. B., Yoneyama, K., Filardo, F., Meyers, E., Scaffidi, 1475 A., Frickey, T., Akiyama, K., Seto, Y., Dun, E. A., Cremer, J. E., 1476 1477 Kerr, S. C., Waters, M. T., Flematti, G. R., Mason, M. G., Weiller, G., Yamaguchi, S., Nomura, T., Smith, S. M. & 1478 Beveridge, C. A. LATERAL BRANCHING OXIDOREDUCTASE 1479 1480 acts in the final stages of strigolactone biosynthesis in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 113, 6301-6306, 1481 doi:10.1073/pnas.1601729113 (2016). 1482
- 148313Yoneyama, K., Akiyama, K., Brewer, P. B., Mori, N.,1484Kawano-Kawada, M., Haruta, S., Nishiwaki, H., Yamauchi,1485S., Xie, X., Umehara, M., Beveridge, C. A., Yoneyama, K. &1486Nomura, T. Hydroxyl carlactone derivatives are

predominant strigolactones in *Arabidopsis. Plant Direct* **4**, e00219, doi:10.1002/pld3.219 (2020).

- 14 Yoneyama, K. Recent progress in the chemistry and biochemistry of strigolactones. *J. Pestic. Sci.* **45**, 45-53, doi:10.1584/jpestics.D19-084 (2020).
- 15 Bromhead, L. J. & McErlean, C. S. P. Accessing Single Enantiomer Strigolactones: Progress and Opportunities. *Eur. J. Org. Chem.*, 5712-5723, doi:10.1002/ejoc.201700865 (2017).
- 16 Yasui, M., Ota, R., Tsukano, C. & Takemoto, Y. Total synthesis of avenaol. *Nat. Commun.* 8, 674, doi:10.1038/s41467-017-00792-1 (2017).
- 17 Yasui, M., Yamada, A., Tsukano, C., Hamza, A., Pápai, I. & Takemoto, Y. Enantioselective Acetalization by Dynamic Kinetic Resolution for the Synthesis of γ-Alkoxybutenolides by Thiourea/Quaternary Ammonium Salt Catalysts: Application to Strigolactones. *Angew. Chem. Int. Ed.* **59**, 13479-13483, doi:10.1002/anie.202002129 (2020).
- 18 Yoshimura, M., Dieckmann, M., Dakas, P.-Y., Fonné-Pfister, R., Screpanti, C., Hermann, K., Rendine, S., Quinodoz, P., Horoz, B., Catak, S. & De Mesmaeker, A. Total Synthesis and Biological Evaluation of Zealactone 1a/b. *Helv. Chim. Acta* 103, e2000017, doi:10.1002/hlca.202000017 (2020).
- 19 Takahashi, I. & Asami, T. Target-based selectivity of strigolactone agonists and antagonists in plants and their potential use in agriculture. *J. Exp. Bot.* **69**, 2241-2254, doi:10.1093/jxb/ery126 (2018).
- 20 Jamil, M., Kountche, B. A. & Al-Babili, S. Current progress in *Striga* management. *Plant Physiol.* **185**, 1339-1352, doi:10.1093/plphys/kiab040 (2021).
- 21 Johnson, A. W., Gowda, G., Hassanali, A., Knox, J., Monaco, S., Razavi, Z. & Rosebery, G. The Preparation of Synthetic Analogs of Strigol. *J. Chem. Soc., Perkin Trans.* 1, 1734-1743, doi:10.1039/P19810001734 (1981).
- 22 Kodama, K., Rich, M. K., Yoda, A., Shimazaki, S., Xie, X., Akiyama, K., Mizuno, Y., Komatsu, A., Luo, Y., Suzuki, H., Kameoka, H., Libourel, C., Keller, J., Sakakibara, K., Nishiyama, T., Nakagawa, T., Mashiguchi, K., Uchida, K., Yoneyama, K., Tanaka, Y., Yamaguchi, S., Shimamura, M., Delaux, P.-M., Nomura, T. & Kyozuka, J. An Ancestral Function of Strigolactones as Symbiotic Rhizosphere Signals. *bioRxiv*, doi:10.1101/2021.08.20.457034 (2021).
- Boyer, F.-D., de Saint Germain, A., Pillot, J.-P., Pouvreau, J.-B., Chen, V. X., Ramos, S., Stévenin, A., Simier, P., Delavault, P., Beau, J.-M. & Rameau, C. Structure-Activity Relationship Studies of Strigolactone-Related Molecules for Branching Inhibition in Garden Pea: Molecule Design for Shoot Branching. *Plant Physiol.* **159**, 1524-1544, doi:10.1104/pp.112.195826 (2012).
- Umehara, M., Cao, M., Akiyama, K., Akatsu, T., Seto, Y., Hanada, A., Li, W., Takeda-Kamiya, N., Morimoto, Y. & Yamaguchi, S. Structural Requirements of Strigolactones for Shoot Branching Inhibition in Rice and *Arabidopsis*. *Plant Cell Physiol.* 56, 1059-1072, doi:10.1093/pcp/pcv028 (2015).
- 25 Xie, X., Yoneyama, K. & Yoneyama, K. The Strigolactone Story. Annu. Rev. Phytopathol. 48, 93-117, doi:10.1146/annurev-phyto-073009-114453 (2010).
- 26 Mori, N., Nishiuma, K., Sugiyama, T., Hayashi, H. & Akiyama, K. Carlactone-type strigolactones and their synthetic analogues as inducers of hyphal branching in arbuscular mycorrhizal fungi. *Phytochemistry* **130**, 90-98, doi:10.1016/j.phytochem.2016.05.012 (2016).
- 27 Jamil, M., Kountche, B. A., Wang, J. Y., Haider, I., Jia, K. P., Takahashi, I., Ota, T., Asami, T. & Al-Babili, S. A New Series of Carlactonoic Acid Based Strigolactone Analogs for Fundamental and Applied Research. *Front. Plant Sci.* 11, 434, doi:10.3389/fpls.2020.00434 (2020).
- 28 Jamil, M., Kountche, B. A., Haider, I., Guo, X., Ntui, V. O., Jia, K.-P., Ali, S., Hameed, U. S., Nakamura, H., Lyu, Y., Jiang, K., Hirabayashi, K., Tanokura, M., Arold, S. T., Asami, T. & Al-Babili, S. Methyl phenlactonoates are efficient

 1561
 strigolactone analogs with simple structure. J. Exp. Bot. 69, 1635
 1635

 1562
 2319-2331, doi:10.1093/jxb/erx438 (2018). 1636
 1636

 1563
 29
 Parker, C. Parasitic Weeds: A World Challenge. Weed Sci. 1637

1638

1639

1640

1641

1642

1643

1644

1645

1646

1647

1648

1649

1650

1651

1652

1653

1654

1655

1656

1657

1658

1659

1660

1661

1662

1663

1664

1665

1666

1667

1668

1669

1670

1671

1672

1673

1674

1675

1676

1677

1678

1679

1680

1681

1682

1683

1684

1685

1686

1687

1688

1689

1690

1691

1692

1693

1694

1695

1696

1697

1698

1699

1700

1701

1702

1703

1704

1705

1706

1707

- 1563
 29
 Parker, C. Parasitic Weeds: A World Challenge. Weed Sci.

 1564
 60, 269-276, doi:10.1614/WS-D-11-00068.1 (2012).
- 156530Grenz, J. H. & Sauerborn, J. Mechanisms limiting the
geographical range of the parasitic weed Orobanche
crenata. Agric. Ecosyst. Environ. 122, 275-281,
doi:10.1016/j.agee.2007.01.014 (2007).
- 1569
 31
 Delavault, P., Montiel, G., Brun, G., Pouvreau, J. B., Thoiron,

 1570
 S. & Simier, P. Communication Between Host Plants and

 1571
 Parasitic Plants. Adv. Bot. Res. 82, 55-82,

 1572
 doi:10.1016/bs.abr.2016.10.006 (2017).
- 157332Brun, G., Spallek, T., Simier, P. & Delavault, P. Molecular1574actors of seed germination and haustoriogenesis in1575parasitic weeds. Plant Physiol. 185, 1270-1281,1576doi:10.1093/plphys/kiaa041 (2021).
- Fernandez-Aparicio, M., Reboud, X. & Gibot-Leclerc, S. 1577 33 Broomrape Weeds. Underground Mechanisms of 1578 Parasitism and Associated Strategies for their Control: A 1579 Plant Review. Front. Sci. 7. 135. 1580 doi:10.3389/fpls.2016.00135 (2016). 1581
- 158234Akiyama, K., Matsuzaki, K. & Hayashi, H. Plant1583sesquiterpenes induce hyphal branching in arbuscular1584mycorrhizal fungi. Nature1585doi:10.1038/nature03608 (2005).
- 158635Besserer, A., Puech-Pages, V., Kiefer, P., Gomez-Roldan, V.,1587Jauneau, A., Roy, S., Portais, J. C., Roux, C., Bécard, G. &1588Sejalon-Delmas, N. Strigolactones stimulate arbuscular1589mycorrhizal fungi by activating mitochondria. *PLoS Biol.* 4,15901239-1247, doi:e226 (2006).
- 36 Besserer, A., Bécard, G., Jauneau, A., Roux, C. & Sejalon-1591 1592 Delmas, N. GR24, a synthetic analog of strigolactones, stimulates the mitosis and growth of the arbuscular 1593 mycorrhizal fungus Gigaspora rosea by boosting its energy 1594 1595 metabolism. Plant Physiol. 148. 402-413. doi:10.1104/pp.108.121400 (2008). 1596
- Rich, M. K., Vigneron, N., Libourel, C., Keller, J., Xue, L., 1597 37 Hajheidari, M., Radhakrishnan Guru, V., Le Ru, A., Diop 1598 Seydina, I., Potente, G., Conti, E., Duijsings, D., Batut, A., Le 1599 Faouder, P., Kodama, K., Kyozuka, J., Sallet, E., Bécard, G., 1600 Rodriguez-Franco, M., Ott, T., Bertrand-Michel, J., Oldroyd 1601 1602 Giles, E. D., Szövényi, P., Bucher, M. & Delaux, P.-M. Lipid exchanges drove the evolution of mutualism during plant 1603 1604 terrestrialization. Science 372. 864-868, doi:10.1126/science.abg0929 (2021). 1605
- 160638Stojanova, B., Delourme, R., Duffé, P., Delavault, P. &1607Simier, P. Genetic differentiation and host preference1608reveal non-exclusive host races in the generalist parasitic1609weed Phelipanche ramosa. Weed Res. 59, 107-118,1610doi:10.1111/wre.12353 (2019).
- 1611 39 Huet, S., Pouvreau, J.-B., Delage, E., Delgrange, S., Marais,
 1612 C., Bahut, M., Delavault, P., Simier, P. & Poulin, L.
 1613 Populations of the Parasitic Plant *Phelipanche ramosa*1614 Influence Their Seed Microbiota. *Front. Plant Sci.* 11, 1075,
 1615 doi:10.3389/fpls.2020.01075 (2020).
- 161640Auger, B., Pouvreau, J.-B., Pouponneau, K., Yoneyama, K.,1617Montiel, G., Le Bizec, B., Yoneyama, K., Delavault, P.,1618Delourme, R. & Simier, P. Germination Stimulants of1619Phelipanche ramosa in the Rhizosphere of Brassica napus1620Are Derived from the Glucosinolate Pathway. Mol. Plant-1621Microbe Interact. 25, 993-1004, doi:10.1094/mpmi-01-162212-0006-r (2012).
- 162341Xie, X., Kusumoto, D., Takeuchi, Y., Yoneyama, K., Yamada,1624Y. & Yoneyama, K. 2 '-Epi-orobanchol and solanacol, two1625unique strigolactones, germination stimulants for root1626parasitic weeds, produced by tobacco. J. Agric. Food. Chem.162755, 8067-8072, doi:10.1021/jf0715121 (2007).
- 162842Xie, X., Yoneyama, K., Kisugi, T., Uchida, K., Ito, S., Akiyama,1629K., Hayashi, H., Yokota, T., Nomura, T. & Yoneyama, K.1630Confirming Stereochemical Structures of Strigolactones1631Produced by Rice and Tobacco. Mol. Plant 6, 153-163,1632doi:10.1093/mp/sss139 (2013).
- 163343Koltai, H., Cohen, M., Chesin, O., Mayzlish-Gati, E., Bécard,1634G., Puech, V., Ben Dor, B., Resnick, N., Wininger, S. &

Kapulnik, Y. Light is a positive regulator of strigolactone levels in tomato roots. *J. Plant Physiol.* **168**, 1993-1996, doi:10.1016/j.jplph.2011.05.022 (2011).

- Hamzaoui, O., Maciuk, A., Delgrange, S., Kerdja, K., Thouminot, C., Germain, A. d. S., Rochange, S., Boyer, F.-D., Simier, P. & Pouvreau, J.-B. Cannalactone: a new noncanonical strigolactone exudated by *Cannabis sativa* roots with a pivotal role in host specialization within French broomrape (*Phelipanche ramosa*) populations in *15th World Congress on Parasitic Plants*. (Amsterdam, 2019).
 de Saint Germain, A., Retailleau, P., Norsikian, S.,
 - de Saint Germain, A., Retailleau, P., Norsikian, S., Servajean, V., Pelissier, F., Steinmetz, V., Pillot, J.-P., Rochange, S., Pouvreau, J.-B. & Boyer, F.-D. Contalactone, a contaminant formed during chemical synthesis of the strigolactone reference GR24 is also a strigolactone mimic. *Phytochemistry* **168**, 112112, doi:10.1016/j.phytochem.2019.112112 (2019).
- 46 Hamiaux, C., Drummond, R. S. M., Janssen, B. J., Ledger, S. E., Cooney, J. M., Newcomb, R. D. & Snowden, K. C. DAD2 Is an α/β Hydrolase Likely to Be Involved in the Perception of the Plant Branching Hormone, Strigolactone. *Curr. Biol.* **22**, 2032-2036, doi:10.1016/j.cub.2012.08.007 (2012).
- Waters, M. T., Nelson, D. C., Scaffidi, A., Flematti, G. R., Sun, Y. K., Dixon, K. W. & Smith, S. M. Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in *Arabidopsis. Development* 139, 1285-1295, doi:10.1242/dev.074567 (2012).
- 48 Conn, C. E., Bythell-Douglas, R., Neumann, D., Yoshida, S., Whittington, B., Westwood, J. H., Shirasu, K., Bond, C. S., Dyer, K. A. & Nelson, D. C. Convergent evolution of strigolactone perception enabled host detection in parasitic plants. *Science* **349**, 540-543, doi:10.1126/science.aab1140 (2015).
- 49 Toh, S., Holbrook-Smith, D., Stogios, P. J., Onopriyenko, O., Lumba, S., Tsuchiya, Y., Savchenko, A. & McCourt, P. Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga. Science* **350**, 203-207, doi:10.1126/science.aac9476 (2015).
- 50 Nelson, D. C. The mechanism of host-induced germination in root parasitic plants. *Plant Physiol.* **185**, 1353-1373, doi:10.1093/plphys/kiab043 (2021).
- 51 de Saint Germain, A., Jacobs, A., Brun, G., Pouvreau, J.-B., Braem, L., Cornu, D., Clavé, G., Baudu, E., Steinmetz, V., Servajean, V., Wicke, S., Gevaert, K., Simier, P., Goormachtig, S., Delavault, P. & Boyer, F.-D. A *Phelipanche ramosa* KAI2 protein perceives strigolactones and isothiocyanates enzymatically. *Plant Commun.* **2**, 100166, doi:10.1016/j.xplc.2021.100166 (2021).
- 52 Križková, P. M., Lindner, W. & Hammerschmidt, F. Improved Synthesis of Racemate and Enantiomers of Taniguchi Lactone and Conversion of Their C-C Double Bonds into Triple Bonds. *Synthesis* **50**, 651-657, doi:10.1055/s-0036-1591516 (2018).
- 53 Ballini, R., Marcantoni, E. & Perella, S. A Two Steps Synthesis of γ -Substituted and γ , γ -Disubstituted α -(Alkylmethylene)- γ -butyrolactones. J. Org. Chem. **64**, 2954-2957, doi:10.1021/jo982131c (1999).
- 54 Bartoli, G., Marcantoni, E. & Petrini, M. CeCl3-Mediated Addition of Grignard Reagents to 1,3-Diketones. *Angew. Chem. Int. Ed.* **32**, 1061-1062, doi:10.1002/anie.199310611 (1993).
- 55 Oriyama, T., Kobayashi, Y. & Noda, K. Chemoselective and Practical Deprotection of Alkyl Trialkylsilyl Ethers in the Presence of Aryl Trialkylsilyl Ethers by a Catalytic Amount of Sc(0Tf)3. *Synlett*, 1047-1048, doi:10.1055/s-1998-1890 (1998).
- 56 Norsikian, S., Holmes, I., Lagasse, F. & Kagan, H. B. A onepot esterification of chiral *O*-trimethylsilyl-cyanohydrins with retention of stereochemistry. *Tetrahedron Lett.* **43**, 5715-5717, doi:10.1016/S0040-4039(02)01200-5 (2002).
- 57 Pouvreau, J. B., Gaudin, Z., Auger, B., Lechat, M. M., Gauthier, M., Delavault, P. & Simier, P. A high-throughput

- 1708
 seed germination assay for root parasitic plants. Plant

 1709
 Methods 9, 32, doi:10.1186/1746-4811-9-32 (2013).
- 171058Miura, H., Ochi, R., Nishiwaki, H., Yamauchi, S., Xie, X.,1711Nakamura, H., Yoneyama, K. & Yoneyama, K. Germination1712Stimulant Activity of Isothiocyanates on Phelipanche spp.1713Plants 11, 606, doi:10.3390/plants11050606 (2022).
- 59 Boyer, F.-D., de Saint Germain, A., Pillot, J. P., Pouvreau, J.-1714 1715 B., Chen, V. X., Ramos, S., Stevenin, A., Simier, P., Delavault, P., Beau, J.-M. & Rameau, C. Structure-activity relationship 1716 1717 studies of strigolactone-related molecules for branching inhibition in garden pea: molecule design for shoot 1718 1719 branching. Plant Physiol. 159, 1524-1544, doi:10.1104/pp.112.195826 (2012). 1720
- 172160Boyer, F.-D., de Saint Germain, A., Pouvreau, J.-B., Clavé, G.,1722Pillot, J.-P., Roux, A., Rasmussen, A., Depuydt, S.,1723Lauressergues, D., Frei dit Frey, N., Heugebaert, T. S. A.,1724Stevens, C. V., Geelen, D., Goormachtig, S. & Rameau, C.1725New Strigolactone Analogs as Plant Hormones with Low1726Activities in the Rhizosphere. Mol. Plant 7, 675-690,1727doi:10.1093/mp/sst163 (2014).
- 61 de Saint Germain, A., Clavé, G., Badet-Denisot, M.-A., Pillot, 1728 J.-P., Cornu, D., Le Caer, J.-P., Burger, M., Pelissier, F., 1729 Retailleau, P., Turnbull, C., Bonhomme, S., Chory, J., 1730 1731 Rameau, C. & Boyer, F.-D. An histidine covalent receptor butenolide complex and mediates strigolactone 1732 1733 perception. Nat. Chem. Biol. 12, 787-794, doi:10.1038/nchembio.2147 (2016). 1734
- 173562Auldridge, M. E., Block, A., Vogel, J. T., Dabney-Smith, C.,1736Mila, I., Bouzayen, M., Magallanes-Lundback, M.,1737DellaPenna, D., McCarty, D. R. & Klee, H. J. Characterization1738of three members of the Arabidopsis carotenoid cleavage1739dioxygenase family demonstrates the divergent roles of1740this multifunctional enzyme family. Plant J. 45, 982-993,1741doi:10.1111/j.1365-313X.2006.02666.x (2006).
- 174263Xie, X. Structural diversity of strigolactones and their1743distribution in the plant kingdom. J. Pestic. Sci. 41, 175-1744180, doi:10.1584/jpestics.J16-02 (2016).
- 174564Akiyama, K., Ogasawara, S., Ito, S. & Hayashi, H. Structural1746Requirements of Strigolactones for Hyphal Branching in1747AM Fungi. Plant Cell Physiol. 51, 1104-1117,1748doi:10.1093/pcp/pcq058 (2010).
- Taulera, Q., Lauressergues, D., Martin, K., Cadoret, M.,
 Servajean, V., Boyer, F.-D. & Rochange, S. Initiation of
 arbuscular mycorrhizal symbiosis involves a novel
 pathway independent from hyphal branching. *Mycorrhiza* **30**, 491-501, doi:10.1007/s00572-020-00965-9 (2020).
- 175466López-Ráez, J. A., Charnikhova, T., Gomez-Roldan, V.,1755Matusova, R., Kohlen, W., De Vos, R., Verstappen, F., Puech-1756Pages, V., Becard, G., Mulder, P. & Bouwmeester, H.1757Tomato strigolactones are derived from carotenoids and1758their biosynthesis is promoted by phosphate starvation.1759New Phytol. **178**, 863-874 (2008).
- 67 Decker, E. L., Alder, A., Hunn, S., Ferguson, J., Lehtonen, M. 1760 T., Scheler, B., Kerres, K. L., Wiedemann, G., Safavi-Rizi, V., 1761 1762 Nordzieke, S., Balakrishna, A., Baz, L., Avalos, J., Valkonen, J. P. T., Reski, R. & Al-Babili, S. Strigolactone biosynthesis is 1763 evolutionarily conserved, regulated by phosphate 1764 starvation and contributes to resistance against 1765 phytopathogenic fungi in a moss, Physcomitrella patens. 1766 New Phytol. 216, 455-468, doi:10.1111/nph.14506 1767 (2017). 1768
- Lopez-Obando, M., Guillory, A., Boyer, F.-D., Cornu, D.,
 Hoffmann, B., Le Bris, P., Pouvreau, J.-B., Delavault, P.,
 Rameau, C., de Saint Germain, A. & Bonhomme, S. The *Physcomitrium (Physcomitrella*) patens PpKAI2L receptors
 for strigolactones and related compounds function via
 MAX2-dependent and -independent pathways. *Plant Cell* **33**, 3487-3512, doi:10.1093/plcell/koab217 (2021).
- 177669Mangnus, E. M., Dommerholt, F. J., Dejong, R. L. P. &
Zwanenburg, B. Improved Synthesis of Strigol Analog1777GR24 and Evaluation of the Biological-Activity of Its
Diastereomers. J. Agric. Food. Chem. 40, 1230-1235,
doi:10.1021/jf00019a031 (1992).

70 Scaffidi, A., Waters, M. T., Ghisalberti, E. L., Dixon, K. W., Flematti, G. R. & Smith, S. M. Carlactone-independent seedling morphogenesis in Arabidopsis. *Plant J.* 76, 1-9, doi:10.1111/tpj.12265 (2013).

1781

1782

1783

1784

1785

1786

1787

1788

1789

1790

1791

1792

1793

1794

1795

1796

1797

1798

1799

1800

1801

1802

1803

1804

1805

1806

1807

1808

1809

1810

1811

1812

1813

1814

1815

1816

1817

1818

1819

1820

1821

1822

1823

1824

1825

1826

1827

1828

1829

1830

1831

1832

1833

1834

1835

1836

1837

1838

1839

1840

1841

1842

1843

1844

1845

1846

1847

1848

1849

1850

1851

1852

1853

- Boutet-Mercey, S., Perreau, F., Roux, A., Clavé, G., Pillot, J.-P., Schmitz-Afonso, I., Touboul, D., Mouille, G., Rameau, C. & Boyer, F.-D. Validated Method for Strigolactone Quantification by Ultra High-Performance Liquid Chromatography Electrospray Ionisation Tandem Mass Spectrometry Using Novel Deuterium Labelled Standards. *Phytochem. Anal.* 29, 59-68, doi:10.1002/pca.2714 (2018).
- 72 Chen, V. X., Boyer, F.-D., Rameau, C., Pillot, J.-P., Vors, J.-P. & Beau, J.-M. New Synthesis of A-Ring Aromatic Strigolactone Analogues and Their Evaluation as Plant Hormones in Pea (*Pisum sativum*). Chem. – Eur. J. 19, 4849-4857, doi:10.1002/chem.201203585 (2013).
- Macalpine, G. A., Raphael, R. A., Shaw, A., Taylor, A. W. & Wild, H. J. Synthesis of Germination Stimulant (±)-Strigol. J. Chem. Soc., Perkin Trans. 1, 410-416, doi:10.1039/C39740000834 (1976).
- 74 Sugano, G., Kawada, K., Shigeta, M., Hata, T. & Urabe, H. Iron-catalyzed δ-selective conjugate addition of methyl and cyclopropyl Grignard reagents to α , β , γ , δ -unsaturated esters and amides. *Tetrahedron Lett.* **60**, 885-890, doi:10.1016/j.tetlet.2018.12.043 (2019).
- 75 Rigaku, O. D. CrystalClear-SM Expert 2.0 r4 Rigaku Corporation, Tokyo, Japan. (2009).
- Schneider, T. R. & Sheldrick, G. M. Substructure solution with SHELXD. Acta Crystallogr., Sect. D: Biol. Crystallogr. 58, 1772-1779, doi:doi:10.1107/S0907444902011678 (2002).
- 77 Sheldrick, G. M. Crystal structure refinement with SHELXL. Acta Crystallogr., Sect. C: Cryst. Struct. Commun. **71**, 3-8, doi:10.1107/s2053229614024218 (2015).
- 78 Sheldrick, G. M. SHELXT Integrated space-group and crystal-structure determination. Acta Crystallogr., Sect. A: Found. Crystallogr. 71, 3-8, doi:10.1107/s2053273314026370 (2015).
- 79 Spek, A. L. Structure validation in chemical crystallography. Acta Crystallogr., Sect. D: Biol. Crystallogr.
 65, 148-155, doi:10.1107/s090744490804362x (2009).
- 80 Spek, A. L. PLATONSQUEEZE: a tool for the calculation of the disordered solvent contribution to the calculated structure factors. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **71**, 9-18, doi:10.1107/s2053229614024929 (2015).
- 81 Rameau, C., Bodelin C, Cadier D, Grandjean O, Miard F & Murfet IC. New *ramosus* mutants at loci *Rms1*, *Rms3* and *Rms4* resulting from the mutation breeding program at Versailles. *Pisum Genetics* **29**, 7-12 (1997).
- 82 Braun, N., de Saint Germain, A., Pillot, J. P., Boutet-Mercey, S., Dalmais, M., Antoniadi, I., Li, X., Maia-Grondard, A., Le Signor, C., Bouteiller, N., Luo, D., Bendahmane, A., Turnbull, C. & Rameau, C. The pea TCP transcription factor PsBRC1 acts downstream of Strigolactones to control shoot branching. *Plant Physiol.* **158**, 225-238, doi:10.1104/pp.111.182725 (2012).
- 83 Cornet, F., Pillot, J.-P., Le Bris, P., Pouvreau, J.-B., Arnaud, N., de Saint Germain, A. & Rameau, C. Strigolactones (SLs) modulate the plastochron by regulating KLUH (KLU) transcript abundance in Arabidopsis. *New Phytol.* **232**, 1909-1916, doi:10.1111/nph.17725 (2021).
- Muñoz, A., Pillot, J.-P., Cubas, P. & Rameau, C. Methods for Phenotyping Shoot Branching and Testing Strigolactone Bioactivity for Shoot Branching in Arabidopsis and Pea in *Strigolactones: Methods and Protocols* (eds Cristina Prandi & Francesca Cardinale) 115-127 (Springer US, 2021). doi:10.1007/978-1-0716-1429-7_10.
- 85 Pouvreau, J.-B., Poulin, L., Huet, S. & Delavault, P. Strigolactone-Like Bioactivity via Parasitic Plant Germination Bioassay in *Strigolactones: Methods and Protocols* (eds Cristina Prandi & Francesca Cardinale) 59-73 (Springer US, 2021). doi:10.1007/978-1-0716-1429-7_6.

1855	86	Bécard, G. & Fortin, J. A. Early events of vesicular	1863	R108. J. Exp. Bot. 66, 1237-1244, doi:10.1093/jxb/eru471
1856		arbuscular mycorrhiza formation on Ri T-DNA	1864	(2015).
1857		transformed roots. New Phytol. 108, 211-218,	1865 88	Fox, J. The R commander: A basic-statistics graphical user
1858		doi:10.1111/j.1469-8137.1988.tb03698.x (1988).	1866	interface to R. J. Stat. Softw. 14, 1-42,
1859	87	Lauressergues, D., André, O., Peng, J., Wen, J., Chen, R.,	1867	doi:10.18637/jss.v014.i09 (2005).
1860		Ratet, P., Tadege, M., Mysore, K. S. & Rochange, S. F.		
1861		Strigolactones contribute to shoot elongation and to the	1868	
1862		formation of leaf margin serrations in Medicago truncatula		

