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# Noncanonical Strigolactone Analogues Highlight Selectivity for Stimulating Germination in Two *Phelipanche ramosa* Populations

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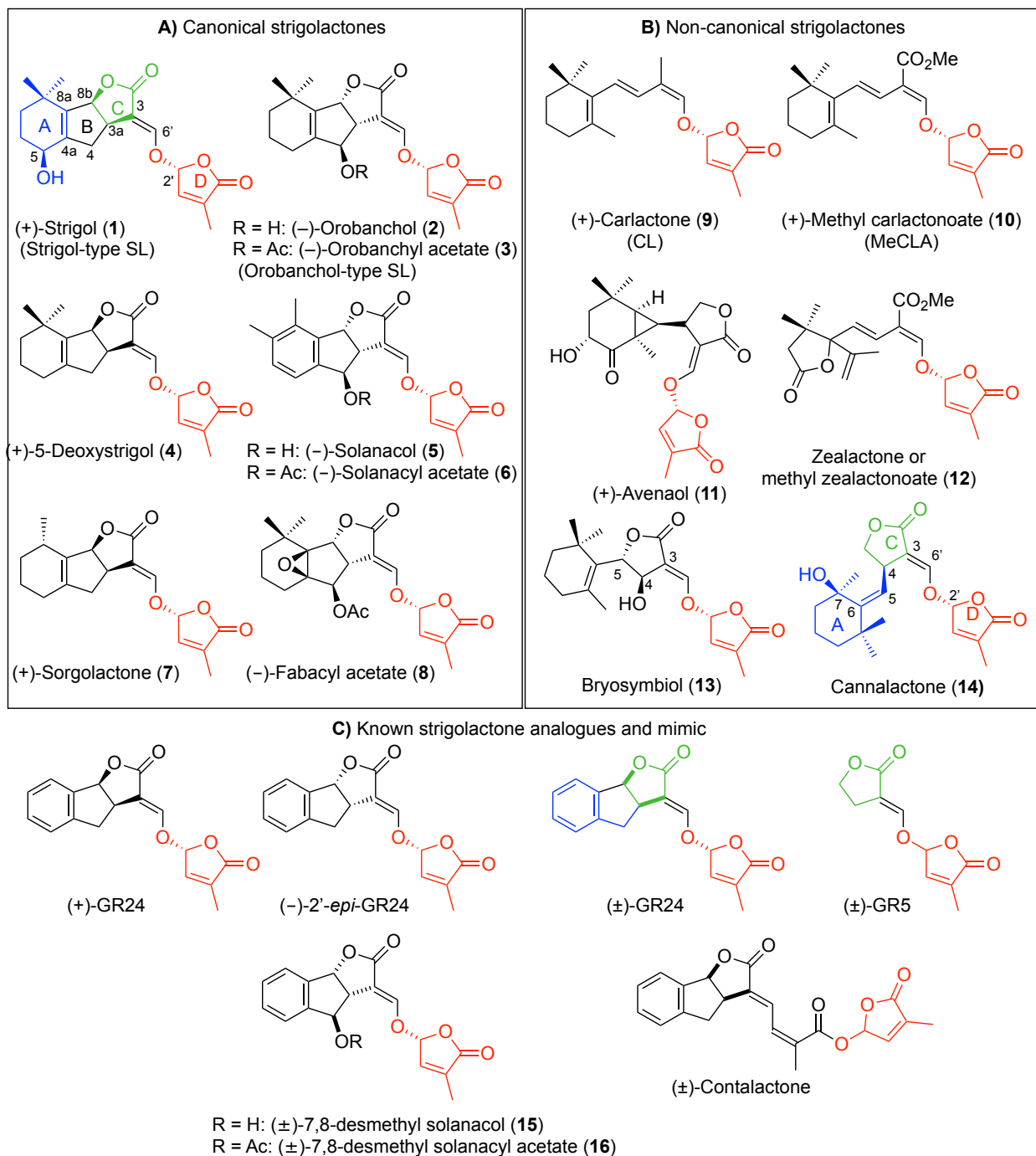
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ABSTRACT. Strigolactones (SLs) are plant hormones exuded in the rhizosphere with a signaling role for the development of arbuscular mycorrhizal (AM) fungi and as stimulants of seed germination of the parasitic weeds *Orobanche*, *Phelipanche* and *Striga*, the most threatening weeds of major crops worldwide. *Phelipanche ramosa* is present mainly on rape, hemp and tobacco in France. *P. ramosa* 2a preferentially attacks hemp while *P. ramosa* 1 attacks rapeseed. The recently isolated cannalactone (**14**) from hemp root exudates has been characterized as a non-canonical SL that selectively stimulates the germination of *P. ramosa* 2a seeds in comparison with *P. ramosa* 1. In the present work, (-)-solanacol (**5**), a canonical orobanchol-type SL exuded by tobacco and tomato, was established to possess a remarkable selective germination stimulant activity for *P. ramosa* 2a seeds. Two cannalactone analogues, named (±)-SdL19 and (±)-SdL118, have been synthesized. They have an unsaturated acyclic carbon chain with a tertiary hydroxy group and a methyl or a cyclopropyl group instead of a cyclohexane A-ring, respectively. (±)-SdL analogues are able to selectively stimulate *P. ramosa* 2a revealing that these minimal structural elements are key for this selective bioactivity. In addition, (±)-SdL19 is able to inhibit shoot branching in *Pisum sativum* and *Arabidopsis thaliana*, and induces hyphal branching in AM fungus *Rhizophagus irregularis*, like SLs.

Strigolactones (SLs) are a class of compounds first identified in 1966<sup>1</sup> as stimulants of seed germination of the root parasitic weeds *Orobanche*, *Phelipanche* and *Striga*. More recently, SLs have been discovered as the 9<sup>th</sup> class of plant hormone that control plant architecture including shoot branching stem secondary growth, plant height, root architecture, and adventitious roots.<sup>2-5</sup> They are biosynthesized in plant roots in trace concentrations, and are partly excreted into the rhizosphere. They belong to the apocarotenoid family (**Figure 1**). The structural core of SLs is a tricyclic lactone (ABC part, canonical SLs) or an unclosed BC-ring (non-canonical SLs) connected via an enol ether bridge to an invariant  $\alpha,\beta$ -unsaturated furanone moiety (D ring). All natural SLs have the same *R*-configuration at the C-2' position. Canonical SLs are divided into strigol (**1**) and orobanchol (**2**) types, corresponding to a  $\beta$ - [e.g., 5-deoxystrigol (**4**), sorgolactone (**7**)] or an  $\alpha$ -oriented C-ring [e.g., orobanchyl acetate (**3**), solanacol (**5**), solanacyl acetate (**6**), fabacyl acetate (**8**)], respectively, and with A and B rings bearing various substituents (**Figure 1A**).<sup>6</sup> Non-canonical SLs [e.g., avenaol (**11**), zealactone (**12**), bryosymbiol (**13**), and cannalactone (**14**)] can be structurally more diverse than canonical SLs (**Figure 1B**).

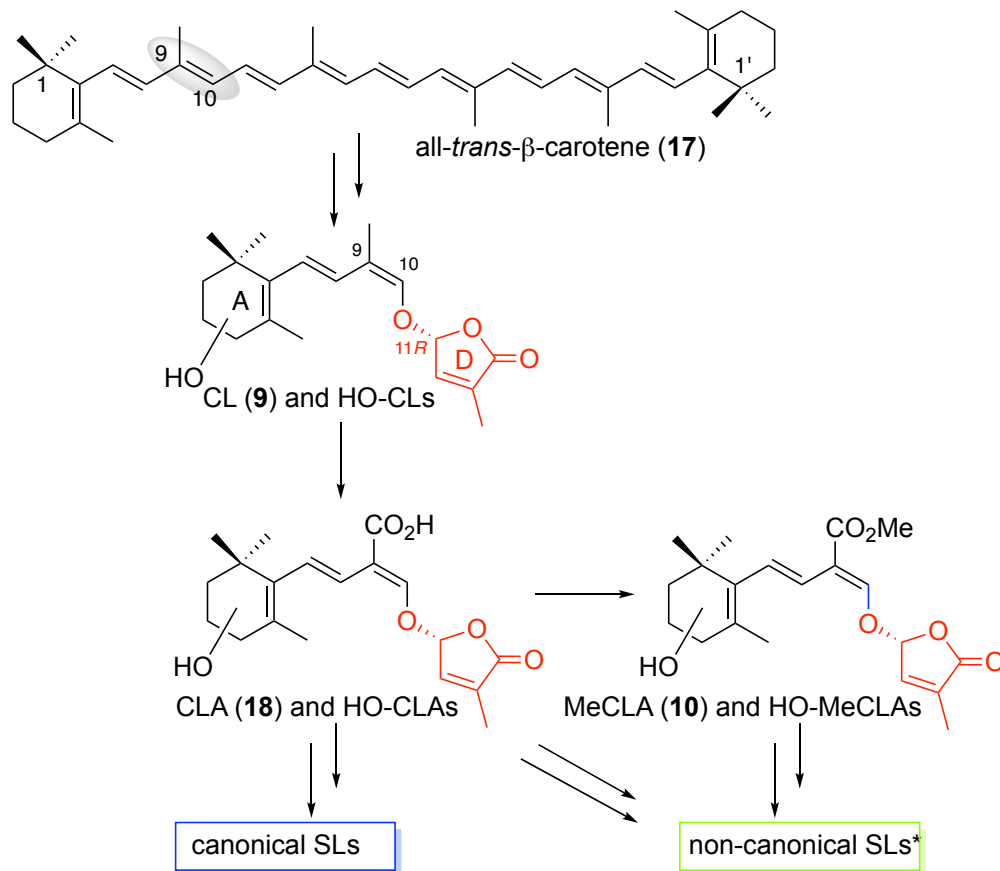


**Figure 1.** Natural strigolactones (SLs), of the canonical (A), non-canonical (B) types and artificial SL analogues and mimic (C). For bryosymbiol (13), the absolute configuration at C-4 and C-5 could be interchangeable.<sup>7</sup>

The common biosynthetic precursors for canonical and non-canonical SLs are carlactonoic acid (**18**) (CLA) and hydroxy-carlactonoic acids (HO-CLAs)<sup>8-10</sup> biosynthesized from all-*trans*- $\beta$ -carotene (**17**) via carlactone (**9**) (CL) and hydroxy-carlactones (HO-CLs) (**Scheme 1**).<sup>11,12</sup> In addition, methyl carlactonoate (**10**) (MeCLA) and HO-MeCLAs<sup>13,14</sup> have been identified as biosynthetic precursors for numerous non-canonical SLs.

To date, greater than 23 canonical SLs and more than 10 non-canonical SLs have been identified in plant root exudates and plant tissues.<sup>15</sup> Natural SLs are difficult to obtain by organic synthesis due to the long multistep syntheses necessary.<sup>16-19</sup> Numerous SL analogues and mimics have been developed for plant chemical biology.<sup>20,21</sup> GR24, invented by **Gerald Rosbery**, is a canonical strigol-type SL analogue with an aromatic A-ring.<sup>22</sup> GR24, in most cases used as a racemic mixture, is a worldwide reference compound (**Figure 1C**) in all assays investigating the role of SL in biological processes. The simplified GR-type compound GR5,<sup>22</sup> possessing only C and D rings (**Figure 1C**) has been also developed as a germination stimulant of root parasitic plant seeds and proved later to be bioactive as plant hormone in the control of shoot branching.<sup>23-25</sup> More recently, CL<sup>26</sup> and MeCLA non-canonical SL synthetic analogues<sup>27,28</sup> have been developed to study the bioactivity of structures close to the highly unstable CL (**9**) and MeCLA (**10**).<sup>15</sup>

### **Scheme 1. Strigolactone Biosynthetic Scheme**



CL = carlactone; CLA = carlactonoic acid; MeCLA = methyl carlactonoate; SL strigolactone.

\* Isolated and identified to date.

The parasitic plants *Orobanche*, *Phelipanche* and *Striga* are major agricultural pests around the Mediterranean Sea and in Sub-Saharan Africa, respectively. Tragically they cause there a major cause of crop damage<sup>29</sup> in intensive crop systems that is expected to expand to new territories in the near future.<sup>30</sup> They are obligate parasites producing numerous and extremely small seeds that can remain viable in soil for decades before germination.<sup>31</sup> The induction of germination by host plant SLs is a critical step in the development cycle of these weeds.<sup>32</sup> SL synthesis and signaling are therefore important targets for crop protection.<sup>21,33</sup>

SLs have been shown to play an additional rhizospheric signaling role by stimulating spore germination and hyphal proliferation of arbuscular mycorrhizal (AM) fungi.<sup>34,35</sup> The hypothesis is that plant SLs boost fungal metabolism prior to root infection.<sup>36</sup> The AM symbiosis arose very early in land plant evolution and is thought to have been instrumental in allowing the successful plant conquest of terrestrial environments.<sup>37</sup> It has been proposed that an ancestral role of SLs was to attract these highly beneficial fungal symbionts,<sup>7</sup> with root parasitic plants having hijacked this system much later to detect their host.<sup>25</sup> Biological activities of SLs could be detected at concentrations as low as  $10^{-13}$  M on AM fungi,  $10^{-12}$  M on seeds of parasitic weeds, and  $10^{-8}$  M on lateral buds as plant hormones.

*Phelipanche ramosa* seeds recognize allelochemical signals and interact with their hosts depending on their genetic structure, as reported recently.<sup>38,39</sup> Three main genotypic groups were described in European *P. ramosa* populations from the Mediterranean basin. *P. ramosa* of genetic group 1 (*P. ramosa* 1) is present exclusively in western France. It parasitizes essentially rapeseed but also melon, tobacco, and sunflower. *P. ramosa* 2a attacks preferentially hemp but also tobacco and tomato in France and Italy. *P. ramosa* 2b is widespread in Europe and Turkey and attacks mainly tobacco but also various crops like rapeseed, hemp and tomato. Seed sensitivity to germination stimulants as SLs emitted by host plants is proposed as a marker of genetic groups and their adaptation to hosts. In rapeseed, no SL has been yet identified.<sup>40</sup> Tobacco plants exude canonical strigol-type and orobanchol-type SLs<sup>41,42</sup> and tomato plants exude orobanchol-type SLs.<sup>43</sup> The non-canonical SL cannalactone (**14**) has been recently characterized from hemp (*Cannabis sativa*).<sup>44</sup> Differential sensitivities to synthetic GR24 enantiomers and cannalactone (**14**) for germination of *P. ramosa* seeds of different genetic groups have been reported.<sup>39,44,45</sup>

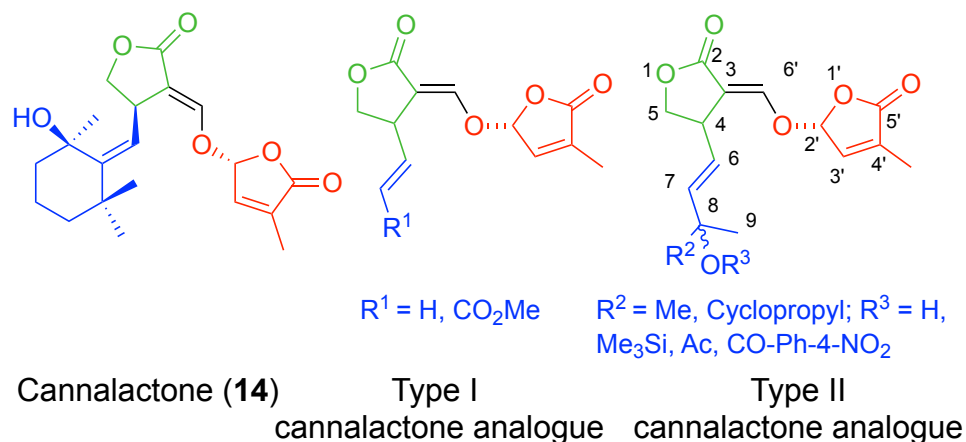


The SL receptor in seed plants DWARF14 (D14)<sup>46</sup> has been identified as a member of the superfamily of the  $\alpha/\beta$ -hydrolases.<sup>4,47</sup> In *Arabidopsis*, a paralogue of AtD14, KARRIKIN INSENSITIVE2/HYPOSENSITIVE TO LIGHT (AtKAI2 /AtHTL), has also been described. It encodes a protein that shows a global structure very similar to AtD14 and has conserved the catalytic triad (Ser, His, Asp). AtKAI2 has been shown to regulate *Arabidopsis* seed germination.<sup>48</sup> Interestingly, the *KAI2* gene family has expanded during the evolution of parasitic plant genomes. Some members of these expanded families in witchweeds and broomrapes have evolved the capacity to bind SLs, making them good candidates for SL receptors. Six of the 11 *S. hermonthica* KAI2 paralogues, belonging to the divergent clade, were identified as being highly sensitive to SLs.<sup>49-51</sup> These results indicated the involvement of one or several  $\alpha/\beta$ -hydrolases in SL perception in parasitic plants. In *P. ramosa* 1, among the 5 KAI2 paralogues, our group established recently that PrKAI2d3 encodes a SL receptor that is also able to interact with isothiocyanates (ITCs), other germination stimulants of *P. ramosa* seeds.<sup>52</sup>

The objective of the present work was to analyze the biological activity of various cannalactone analogues in comparison with natural cannalactone (**14**), natural SLs and especially SLs produced by tobacco<sup>41,42</sup> and tomato<sup>43</sup> [(–)-orobanchol (**2**), (–)-orobanchyl acetate (**3**), 5-deoxystrigol (**4**), (–)-solanacol (**5**) and (–)-solanacyl acetate (**6**)], the SL mimic conalactone<sup>45</sup> and well-known GR SL analogues. Through a structure-activity relationship (SAR) study, key minimal structural elements were deciphered for the selective germination activity of *P. ramosa* seeds focused on *P. ramosa* 1 vs. 2a,<sup>38</sup> which attack selectively major crops as rapeseed and hemp, respectively. Moreover, the results have shown that these novel non-canonical SL analogues possess hormonal activity in the pea and induce hyphal branching in arbuscular mycorrhizal (AM) fungi.

## RESULTS AND DISCUSSION

**Synthesis of Cannalactone Analogues.** Two types of cannalactone analogues were designed (**Figure 2**). Type I possesses an unsaturated acyclic chain instead of the cyclohexane A-ring of cannalactone (**14**), and type II bears an unsaturated acyclic carbon chain with a tertiary hydroxy group and a methyl or a cyclopropyl group ( $R^2$  group) replacing the cyclohexane A-ring.

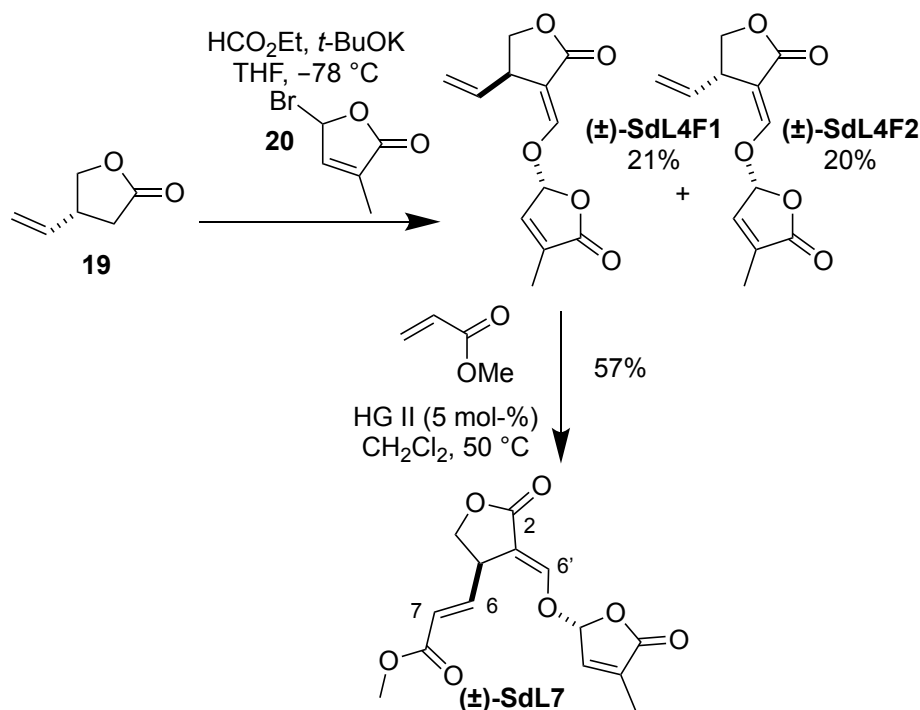


**Figure 2.** Cannalactone analogues Types I and II designed in this work. Numbering is provided for cannalactone analogues (SdL) and the chemicals synthesized herein.

The starting material for type I and type II analogues is ( $\pm$ )-4-vinyldihydrofuran-2(3*H*)-one (**19**) or Taniguchi lactone,<sup>53</sup> prepared in a multigram-scale from the commercially available *cis*-2-butene-1,4-diol via formation of a ketene acetal and its [3,3]-sigmatropic rearrangement. Formylation of ( $\pm$ )-Taniguchi lactone and one-pot coupling with D-Br **20** at  $-78$  °C led to the formation of the diastereomeric type-I cannalactone analogues ( $\pm$ )-SdL4F1 and ( $\pm$ )-SdL4F2 that are easily separable by silica gel chromatography in 41% overall yield (**Scheme 2**). SdL7, another Type-I cannalactone analogue was obtained by cross metathesis (CM) of ( $\pm$ )-SdL4F1 with a large excess of methyl acrylate at 50 °C with Hoveyda-Grubbs II (HG II) initiator in 57%

yield. The *E*-geometry of the  $\alpha,\beta$ -unsaturated ester group and the *E*-geometry of the enol function in SdL7 were proven by NMR analysis ( $^3J_{\text{H-6-H-7}} = 15.5$  Hz,  $^3J_{\text{C-2-H-6'}}$  = 2.0 Hz).

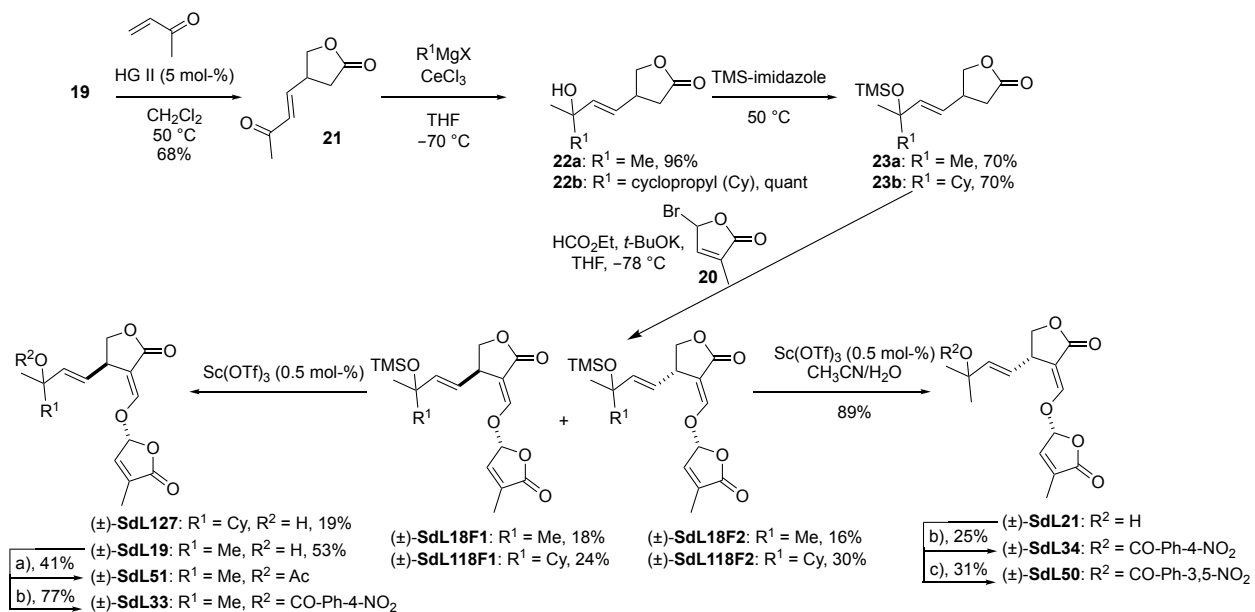
**Scheme 2. Preparation of Type-I Cannalactone Analogues: ( $\pm$ )-SdL4F1, ( $\pm$ )-SdL4F2 and ( $\pm$ )-SdL7**



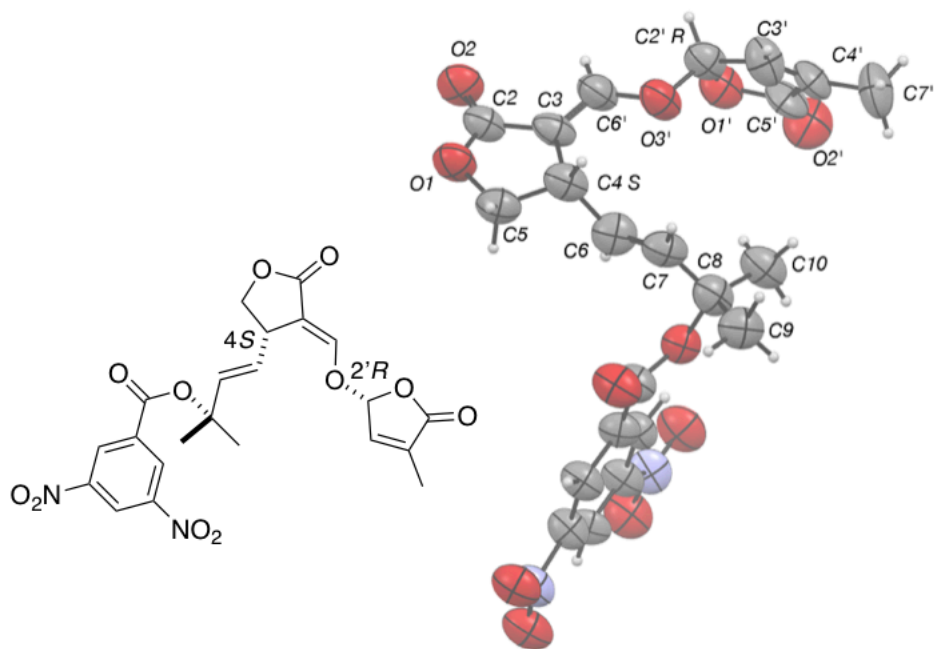
Preparation of Type-II analogues with  $R^1$  = methyl or a cyclopropyl group involved the sequence detailed in **Scheme 3**. Taniguchi lactone **19** was engaged in CM with butane-3-one in the presence of HG II and afforded enone **21** in 68% yield. The selective addition of a methyl and cyclopropyl group on the ketone group of enone **21** was accomplished using Grignard reagents with cerium(III) chloride leading to alcohol **22a** and **22b** in near quantitative yields.<sup>54,55</sup> Cerium(III) chloride plays an important role in increasing the nucleophilicity and decreasing the basicity of the Grignard reagents. The direct formylation on alcohols **22a,b** was unsuccessful.

However, silylation of alcohols **22a,b** in neat trimethylsilyl imidazole furnished the protected derivatives **23a,b** in 70% yield, which led by formylation and coupling with D-Br **20** to a separable mixture of diastereomers in moderate yields (34-54%) [(±)-SdL18F1/(±)-SdL18F2, (±)-SdL118F1/(±)-SdL118F2]. A mild deprotection of the separated silylated derivatives [(±)-SdL18F1 and (±)-SdL18F2] with catalytic Lewis acid [Sc(OTf)<sub>3</sub>] and H<sub>2</sub>O<sup>56</sup> led to both alcohols (±)-SdL19 and (±)-SdL21 in non-optimized 53 and 89% yields, respectively. However, these conditions for the silylated cyclopropyl derivative (±)-SdL118F1 were inefficient but the targeted alcohol (±)-SdL127 was produced with scandium(III) triflate and Ac<sub>2</sub>O<sup>57</sup> in poor 19% yield and without detection of the acetylated product. The tertiary alcohols (±)-SdL19 and (±)-SdL21 were acylated using standard conditions to furnish acetylated derivative (±)-SdL51 and the 4-nitrobenzoates (±)-SdL33 and (±)-SdL34. In addition, the formation of the 3,5-nitrobenzoate (±)-SdL50 from (±)-SdL21 furnished a crystalline compound for which the X-ray diffraction properties were suitable for establishing unambiguously the relative configurations of stereogenic centers C-2' and C-4 of (±)-SdL50 as 2'*R* and 4*S*, respectively (**Figure 3**) and, by consequence, the relative configurations of stereogenic centers for the other SdL analogues, where R<sup>1</sup> = Me (**Scheme 3**). For (±)-SdL127, (±)-SdL118F1, and (±)-SdL118F2 (R<sup>1</sup> = Cy) the relative configurations at C-4 and C-2' were proposed according to the order of elution on SiO<sub>2</sub> in comparison with the series where R<sup>1</sup> = Me. With these putative SL analogues in our hands, their biological activity was studied.

**Scheme 3. Preparation of Type-II Cannalactone Analogues ( $\pm$ )-SdL18F1, ( $\pm$ )-SdL18F2, ( $\pm$ )-SdL19, ( $\pm$ )-SdL21, ( $\pm$ )-SdL33, ( $\pm$ )-SdL34, ( $\pm$ )-SdL50, ( $\pm$ )-SdL51, ( $\pm$ )-SdL118 and ( $\pm$ )-SdL127<sup>a</sup>**



<sup>a</sup>Reagents and conditions: a) Ac<sub>2</sub>O, 4-DMAP, pyridine, rt ; b) ClCO-Ph-4-NO<sub>2</sub>, 4-DMAP, pyridine, 40 °C ; c) ClCO-Ph-3,5-NO<sub>2</sub>, 4-DMAP, pyridine, 40 °C.



**Figure 3.** ORTEP view of the asymmetric unit of (±)-SdL50 (solvent molecule omitted) with selected atom numbering. Ellipsoids are represented with 30% of probability.

**Bioactivity and Selectivity of SdL Derivatives toward *Phelipanche ramosa* 1 vs. 2a.** The germination stimulant activities of SdL analogues on *P. ramosa* 1 and 2a seeds were determined and compared to (±)-GR24, (±)-GR5 and natural cannalactone (**14**) by measuring the maximum of germination stimulant activity as well as half maximal effective concentrations ( $EC_{50}$ ) using a concentration range from  $10^{-13}$  to  $10^{-6}$  mol.L<sup>-1</sup> (**Figure 4A**, Figure S1, Supplementary Information).<sup>58</sup> Germination stimulant activities of all SdL analogues and the putative cannalactone biosynthetic precursor, (±)-MeCLA, reached the maximum induced by (±)-GR24, which makes them germination stimulants, as expected for SL analogues (Figure S2A,

Supplementary Information). The bioactivities of these chemicals based were compared based on their EC<sub>50</sub> values.

Our group reported previously that cannalactone (**14**), a non-canonical SL, possesses a stronger germination stimulation activity on *P. ramosa* 2a than on *P. ramosa* 1. In contrast, (±)-GR24, an artificial canonical SL, possesses a more potent germination stimulation activity for *P. ramosa* 1 rather than for *P. ramosa* 2a.<sup>44</sup> These results have led to the proposal that *P. ramosa* 1 could be more sensitive to canonical SLs and *P. ramosa* 2a to non-canonical SLs. To illustrate the selectivity of seed germination between *P. ramosa* 1 and *P. ramosa* 2a, the ratio of EC<sub>50</sub> (*P. ramosa* 1/ *P. ramosa* 2a) ( $r_{EC50}$ ) of each molecule was calculated (**Figure 4B**). For example,  $r_{EC50}$  [(±)-GR24] = 0.014 and  $r_{EC50}$  (**14**) = 410.

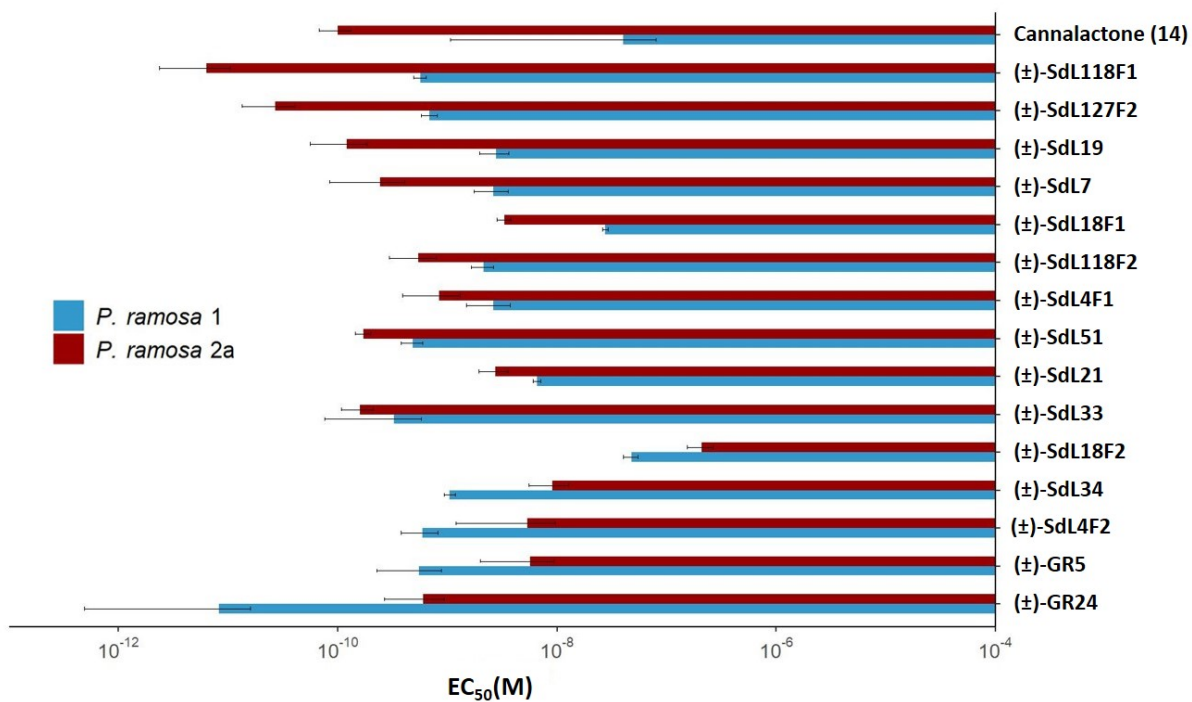
A set of various canonical SLs were studied belonging to the strigol- [(±)-**1**, (±)-**4**, (±)-**7**] and orobanchol-types [(±)-**2**, (±)-**3**, (-)-**5**, (-)-**6**, (±)-**8**] as well as (±)-contalactone (**Figure 1**),<sup>45</sup> a recent SL mimic discovered during the preparation of (±)-GR24. All canonical SLs (**1-4**, **7-8**) and (±)-contalactone<sup>45</sup> were found less active than (±)-GR24, (+)-GR24 and (-)-2'-*epi*-GR24,<sup>45</sup> possessing configurations found in natural canonical SLs (strigol-type and orobanchol-type, respectively). Their  $r_{EC50}$  (< 1) reflect selectivity for *P. ramosa* 1, except for the cases of (-)-solanacol (**5**) and (-)-solanacyl acetate (**6**) where high germination activity and selectivity for *P. ramosa* 2a were recorded ( $r_{EC50}$  [(-)-**5**] = 164) (**Figure 5**, Figure S2B, Supplementary Information). Although high germination activities were observed with (-)-solanacyl acetate (**6**) and the 7,8-desmethyl solanacol derivatives **15** and **16**, selectivity was reduced, highlighting the importance of the hydroxy group but also the methyl group on the A aromatic ring for selectivity. High levels of (-)-solanacol (**5**) and (-)-solanacyl acetate (**6**) were detected in tobacco root exudates<sup>41</sup> and in tomato root extracts.<sup>43</sup> Notably, tobacco and tomato plants are also

hosts for *P. ramosa* 2a,<sup>38,39</sup> in accordance with the sensitivity of *P. ramosa* 2a seeds to (-)-solanacol (**5**) and (-)-solanacyl acetate (**6**) shown herein.

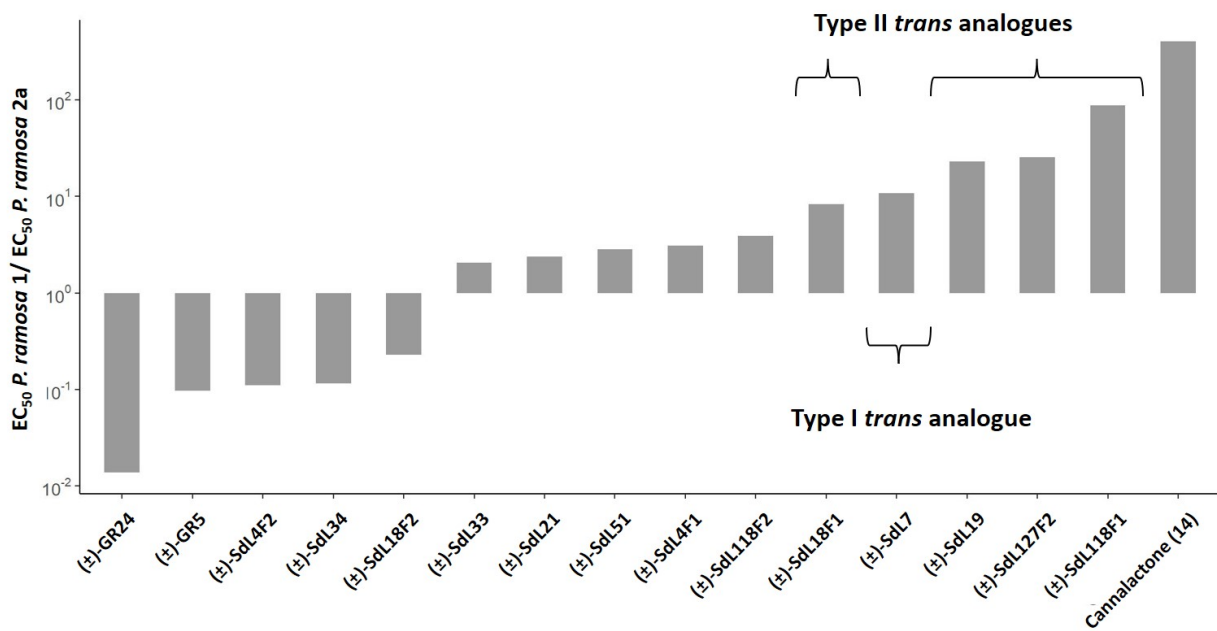
The germination stimulation activities of the SL biosynthetic intermediates were also studied (**Scheme 1**). For all-*trans*- $\beta$ -carotene (**17**), a SL biosynthetic precursor without any D-ring, no germination was recorded, demonstrating that this compound is not bioactive and/or that no enzyme of the SL biosynthesis pathway was sufficiently present in plant parasitic seeds (Figure S2, Supplementary Information). For racemic MeCLA (**10**) and CL (**9**), which are SL biosynthetic precursors possessing a D-ring but no C ring and having an A ring substituted with a tertiary hydroxy group, their germination stimulation activities were drastically reduced in comparison with **14** and ( $\pm$ )-GR24. A small difference was observed between *P. ramosa* 1 and *P. ramosa* 2a for MeCLA (**10**) ( $r_{EC50} = 0.47$ ), the putative biosynthetic precursor of cannalactone (**14**)<sup>44</sup> (**Figure 5B**). A specificity of biological response appeared from the compounds downstream of MeCLA in the biosynthetic pathway. Isothiocyanates (ITCs) as glucosinolate-breakdown products are germination stimulants of *P. ramosa* seeds secreted in the soil by host plants as Brassicaceae.<sup>31,40,59</sup> As for ( $\pm$ )-MeCLA (**10**), a small difference of germination was observed between *P. ramosa* 1 and *P. ramosa* 2a ( $0.1 < r_{EC50} < 1.1$  for 2-phenylethyl isothiocyanate).<sup>39,45</sup>



A

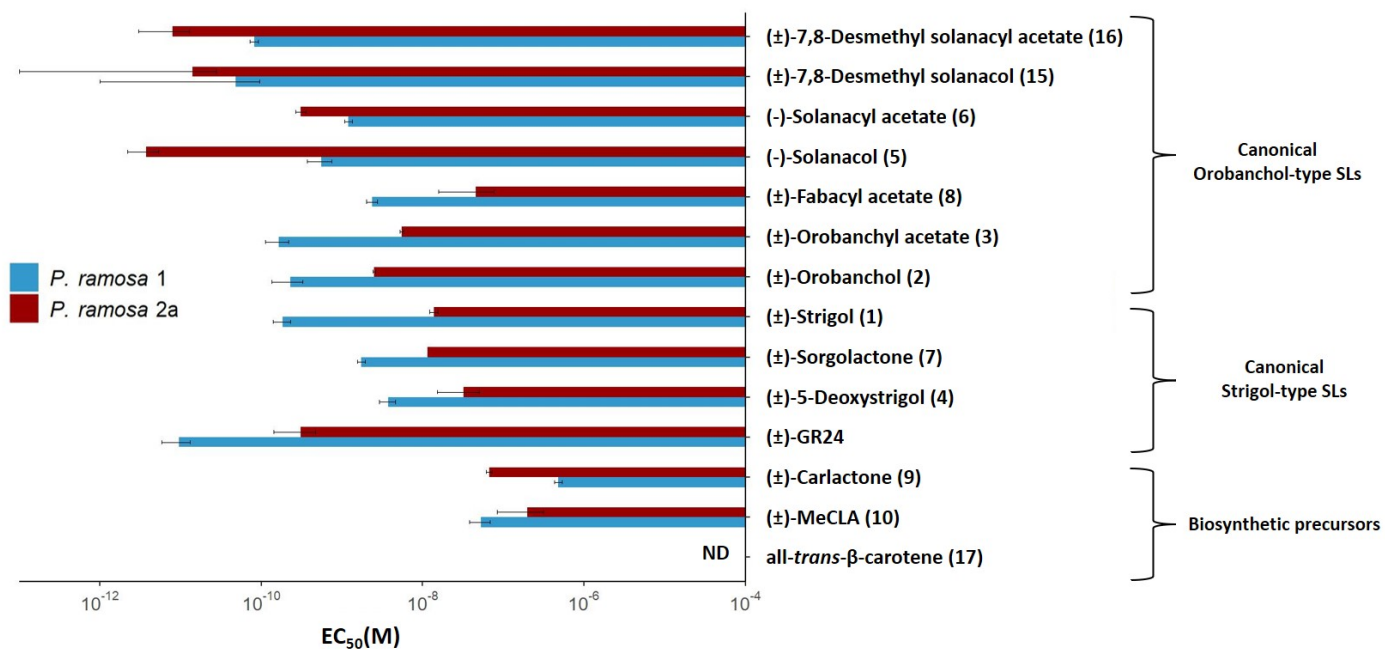


B

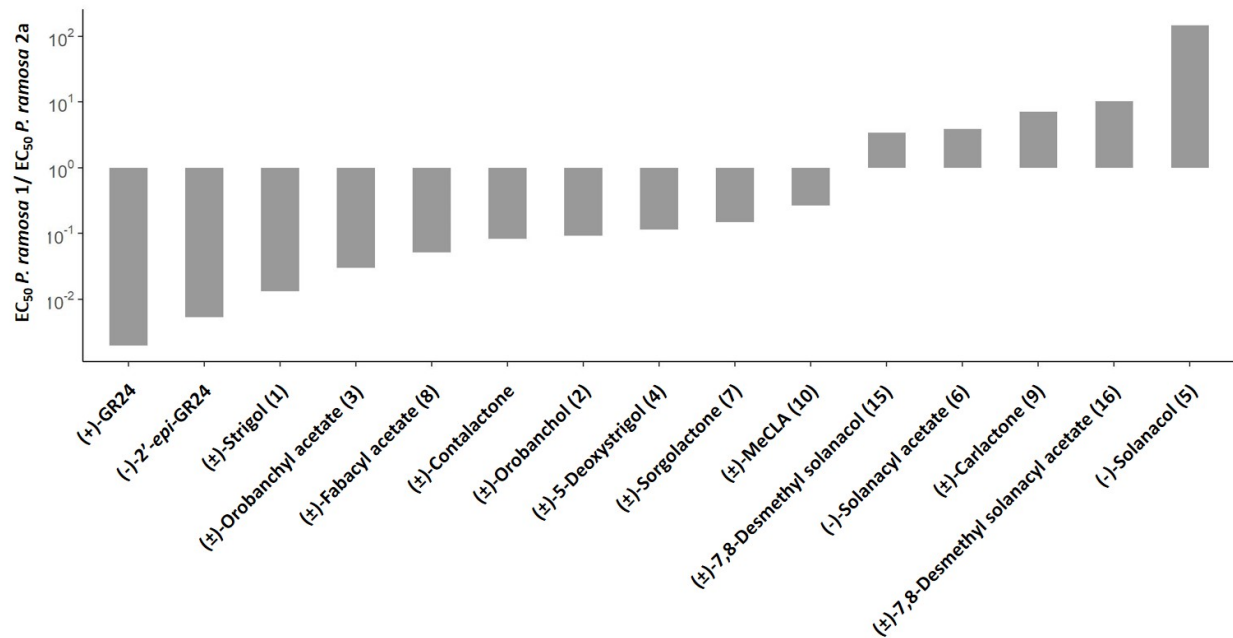


**Figure 4.** Bioactivity on *Phelipanche ramosa* 1 vs. *Phelipanche ramosa* 2a seed germination of SdL analogues (see Figure S1, Supplementary Information). **(A)**  $EC_{50}$  (half maximal effective concentration) ( $\text{mol.L}^{-1}$ ) of SdL analogues, ( $\pm$ )-GR5, natural cannalactone (**14**) and ( $\pm$ )-GR24 toward *P. ramosa* 1 and *P. ramosa* 2a.  $EC_{50}$  values are presented  $\pm$  SE, ( $6 \leq n \leq 12$ ). **(B)**  $r_{EC50} = EC_{50}(P. ramosa\ 1)/EC_{50}(P. ramosa\ 2a)$  for each test compound.

A



B



**Figure 5.** Bioactivity on *Phelipanche ramosa* 1 vs. *Phelipanche ramosa* 2a seed germination of canonical SLs, SL biosynthetic precursors in comparison with (+)-GR24<sup>45</sup> and (-)-2'-*epi*-GR24.<sup>45</sup>

(A)  $EC_{50}$  (half maximal effective concentration) ( $\text{mol}\cdot\text{L}^{-1}$ ) of canonical SLs and ( $\pm$ )-GR24 toward *P. ramosa* 1 and *P. ramosa* 2a.  $EC_{50}$  values are presented  $\pm$  SE, ( $6 \leq n \leq 12$ ). (B)  $r_{EC_{50}} = EC_{50}(P. ramosa\ 1)/EC_{50}(P. ramosa\ 2a)$  for each SL and ( $\pm$ )-cantalactone.<sup>45</sup> ND = not determined.

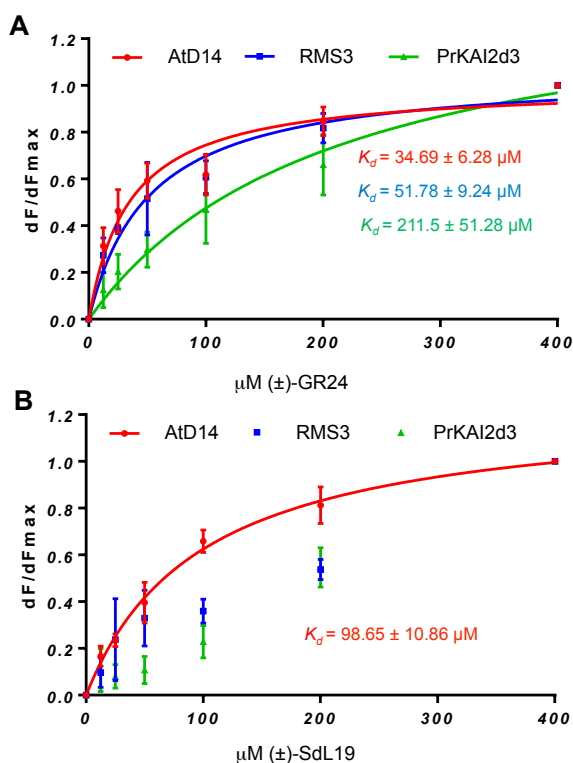
On comparing the germination stimulation activities of ( $\pm$ )-GR5 to that of ( $\pm$ )-GR24, the loss of rings A and B led to bioactivity reductions but also lessened the difference between *P. ramosa* 1 and *P. ramosa* 2a ( $r_{EC_{50}}[(\pm)\text{-GR5}] = 0.10$ ) (**Figure 4B**). All ( $\pm$ )-SdL analogues were bioactive but less than ( $\pm$ )-GR24 for *P. ramosa* 1. ( $\pm$ )-SdL118F1 and ( $\pm$ )-SdL127F2 proved to be more bioactive toward *P. ramosa* 2a while all the other SdLs were less bioactive than cannalactone even if they have high activity for this population. The SdL analogues exhibited  $EC_{50}$  values varying between  $5.6 \times 10^{-10}$ - $4.8 \times 10^{-8}$  M for *P. ramosa* 1, and  $6.4 \times 10^{-12}$ - $2.1 \times 10^{-7}$  M for *P. ramosa* 2a. Removal of the A and B rings and replacement by an unsaturated acyclic carbon chain at the C-3a position induced a loss of bioactivity toward *P. ramosa* 1 for all SdL analogues compared to ( $\pm$ )-GR24. Moreover, no significant difference was observed between “*cis*” ( $2'R^*,4R^*$ ) and “*trans*” ( $2'R^*,4S^*$ ) SdL analogues toward *P. ramosa* 1. On the other hand, for *P. ramosa* 2a seeds, SdL analogues with an acyclic unsaturated carbon chain gave bioactivities similar to that of ( $\pm$ )-GR24. For the genetic group 2a, it was also interesting to find that the ( $\pm$ )-*trans*-SdL analogues were more active than the ( $\pm$ )-*cis*-SdL analogues. It was therefore possible to classify SdL analogue bioactivity using the  $r_{EC_{50}}$  diagram (**Figure 4B**). The most active and selective molecules toward *P. ramosa* 2a are thus ( $\pm$ )-SdL118F1 ( $EC_{50} = 5.6 \times 10^{-10}$  M,  $r_{EC_{50}} = 87.5$ ), ( $\pm$ )-SdL19 ( $EC_{50} = 1.5 \times 10^{-10}$  M,  $r_{EC_{50}} = 18.7$ ) and ( $\pm$ )-SdL7 ( $EC_{50} = 2.4 \times 10^{-10}$  M,  $r_{EC_{50}} = 10.8$ ). The analogue ( $\pm$ )-SdL19 showed a similar selectivity for the germination of *P. ramosa* 2a vs. *P. ramosa* 1 as cannalactone (**14**), and a similar bioactivity toward *P. ramosa* 2a ( $EC_{50}$   $1.5 \times 10^{-10}$  M

vs.  $1.0 \times 10^{-10}$  M) (**Figure 4A**). Consequently, due to the ease of preparation of compound ( $\pm$ )-SdL19 and its specificity, the rest of this study was focused on this compound.

SLs and mimics are sensitive to hydrolysis, giving for ( $\pm$ )-GR24 poorly active or inactive 5-hydroxy-3-methylbutenolide (D-OH)<sup>52</sup> and inactive ABC=CHOH tricyclic analogues.<sup>23</sup> Their sensitivity to hydrolysis has been tested routinely in aqueous solutions and compared to that of ( $\pm$ )-GR24,<sup>23,60</sup> 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 analogues and mimics.<sup>23,60</sup> Under these conditions, a slightly higher stability of ( $\pm$ )-SdL19 was recorded in comparison with ( $\pm$ )-GR24 [( $\pm$ )-SdL19  $t_{1/2} \approx 120$  h, ( $\pm$ )-GR24  $t_{1/2} \approx 100$  h] (Figure S3, Supplementary Information).

**The Cannalactone Analogue ( $\pm$ )-SdL19 Interacts weakly with the SL Receptor PrKAI2d3.** The PrKAI2d3 protein has been characterized recently as a SL receptor from *P. ramosa* 1 among five PrKAI2 candidates, and its potent interaction with ( $\pm$ )-GR24 has been demonstrated.<sup>52</sup> The interaction between ( $\pm$ )-SdL19 and PrKAI2d3 was tested using nano differential scanning fluorimetry (nanoDSF). NanoDSF allows an evaluation of the interaction between a receptor protein and a ligand by monitoring the melting temperature of the receptor protein. In nanoDSF, changes in the protein fluorescence (ratio 350 nm/330 nm) are recorded and do not require a dye. This technique can highlight interactions that induce minor conformational changes. Compared to ( $\pm$ )-GR24, incubation with ( $\pm$ )-SdL19 at low concentrations did not affect the melting temperature of PrKAI2d3. However, the use of high concentrations of ( $\pm$ )-SdL19 lowered the inflection point of the melting curve to indicate the binding of the ligand to the protein (Figure S4, Supporting Information). The binding affinity of

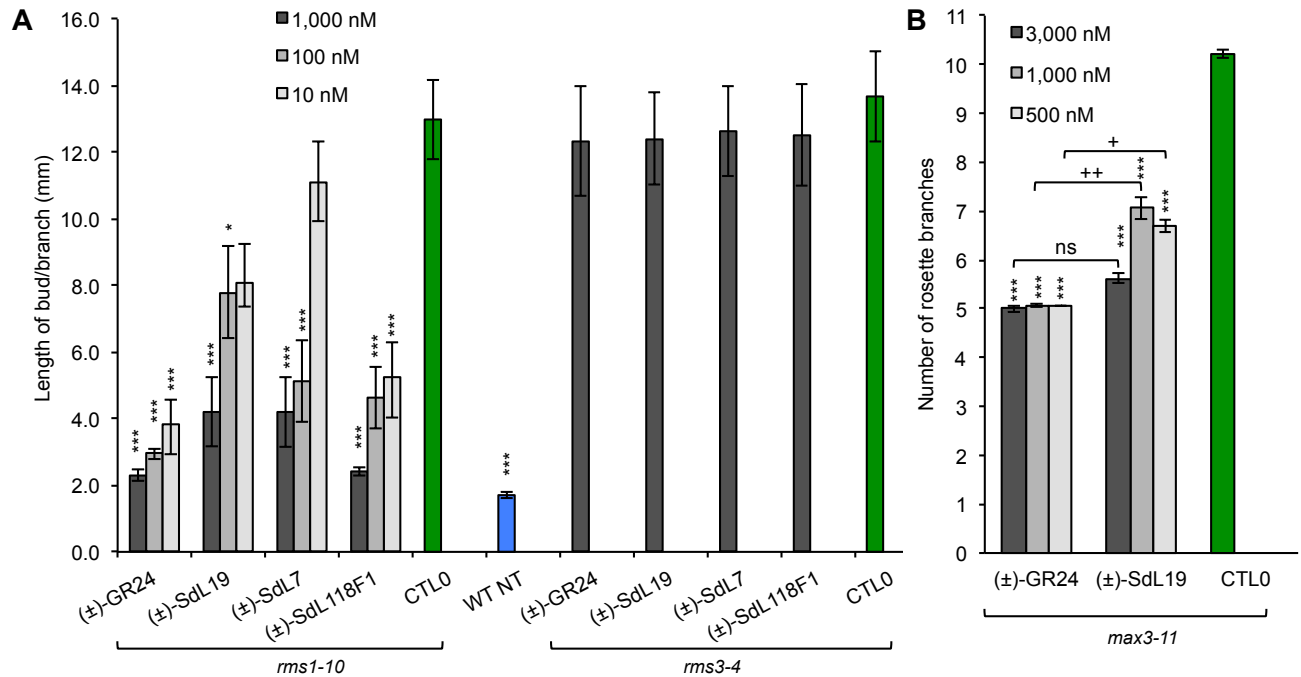
(±)-SdL19 towards PrKAI2d3 was also studied by intrinsic fluorescence but no  $K_d$  value was measurable in comparison with (±)-GR24 (**Figure 6**). The lower affinity of (±)-SdL19 for PrKAI2d3 in comparison with (±)-GR24 confirmed the nanoDSF data. These results are in accordance with the lower bioactivity of (±)-SdL19 on seeds of *P. ramosa* 1 in comparison with (±)-GR24. The characterization of SL receptor(s) from *P. ramosa* 2a is still required and would be applicable to discuss the SAR results presented herein in light of the ligand-protein interactions.



**Figure 6.** Biochemical analysis of the interaction between the PrKAI2d3, RMS3 and AtD14 proteins with (±)-GR24 (**A**) and (±)-SdL19 (**B**) using their intrinsic tryptophan fluorescence. Plots of fluorescence intensity vs. (±)-SdL19 or (±)-GR24 concentrations were used to determine

the apparent  $K_0$  values. The plots represent the mean of two replicates and the experiments were repeated at least three times.

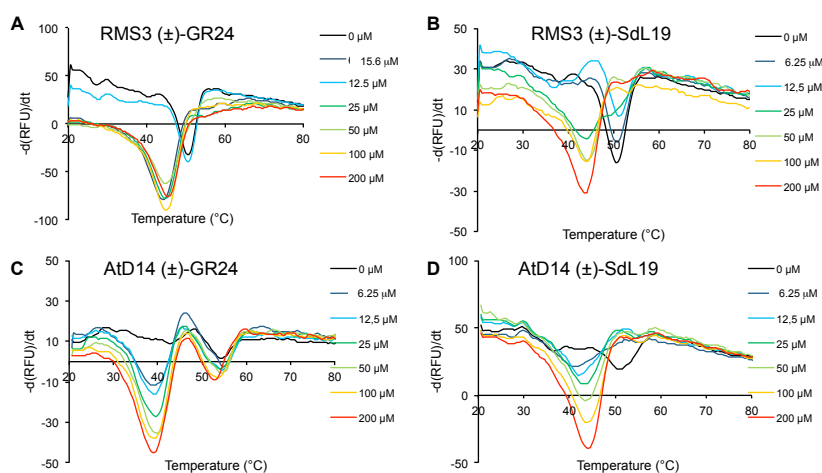
**Cannalactone Analogues Efficiently Inhibit Bud Outgrowth in Pea and *Arabidopsis*, and Interact with SL Receptor D14 Proteins.** The biological activities of (±)-SdL analogues as plant hormones were evaluated using a pea branching assay with the highly branched SL-deficient *rms1-10* mutant.<sup>61</sup> All (±)-SdL analogues showed activity at a concentration of 1  $\mu$ M but were found to be notably less active than (±)-GR24, since they were not significantly bioactive at 10 nM or below (**Figure 7, Table S1**). This confirms the low specificity of SL reception in the pea in which a wide range of SLs, and their analogues and mimics are bioactive.<sup>23,60</sup> Moreover, (±)-SdL analogues were inactive on the branching of the pea *rms3-4* perception mutant (**Figure 7A, Table S2**). These results suggest that (±)-SdL analogues, such as (±)-GR24, are bioactive SL analogues and inhibit bud outgrowth via the RMS3 receptor, an orthologue of D14 in pea, and not because of toxicity. To investigate potential species differences, the effect of (±)-SdL19 on *Arabidopsis* shoot branching was examined. Thus, (±)-SdL19 and (±)-GR24 were applied to hydroponically grown *max3-11* plants. *max3-11* is a SL-deficient mutant.<sup>62</sup> Treatment with all concentrations of (±)-SdL19 inhibited outgrowth of axillary buds in *max3-11*. However, a significant difference between (±)-SdL19 and (±)-GR24 was observed at the low concentrations tested (< 3  $\mu$ M) (**Figure 7B**). These results indicated that (±)-SdL19 is less active than (±)-GR24 in *Arabidopsis* for shoot branching inhibition when applied to the roots in a hydroponic culture.



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



In order to validate that ( $\pm$ )-SdL analogues are perceived by the pea SL receptor RMS3, differential scanning fluorimetry (DSF) was performed and this revealed a shift in RMS3 melting temperature in the presence of ( $\pm$ )-SdL19, corresponding to a protein destabilization as for the other SL bioactive analogues<sup>4</sup> (**Figure 8A-B**). It was shown that the destabilization of RMS3 by ( $\pm$ )-SdL19 was recorded at higher ligand concentrations in comparison with ( $\pm$ )-GR24, suggesting a lower affinity. For AtD14, an orthologue of D14 in *Arabidopsis*, DSF recordings revealed an increased sensitivity for ( $\pm$ )-SdL19, inversely for ( $\pm$ )-GR24, as previously reported for cannalactone (**14**)<sup>44</sup> and in contrast to RMS3 (**Figure 8C-D**). These results are in accordance with the fact that in pea, the main SLs detected are canonical SLs,<sup>63</sup> while in *Arabidopsis*, no canonical SL has been detected but only non-canonical SLs or their biosynthetic precursors such as methyl carlactonoate [(+)-MeCLA (**10**)] and HO-MeCLAs.<sup>13,14</sup> Then, the binding affinity of ( $\pm$ )-SdL19 towards RMS3 and AtD14 was estimated by intrinsic fluorescence and a measurable  $K_a$  value ( $98.65 \pm 10.86 \mu\text{M}$ ) was determined only for AtD14 (**Figure 6B**). The lower affinity of ( $\pm$ )-SdL19 for RMS3 in comparison with AtD14 highlighted differences between biochemical and in planta results where in both *Arabidopsis* and pea, ( $\pm$ )-SdL19 is less bioactive than ( $\pm$ )-GR24 for bud outgrowth inhibition.



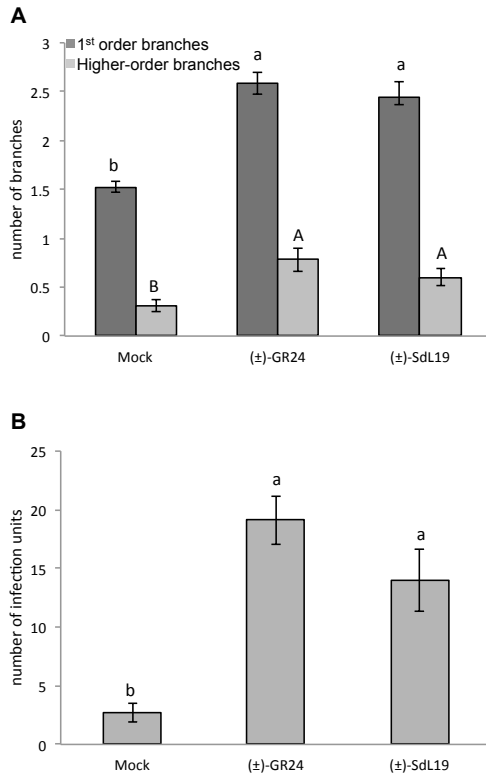
**Figure 8.** Biochemical analysis of the interaction RMS3/PsD14, AtD14 proteins with (±)-GR24 and (±)-SdL19. The melting temperature curves of RMS3 (**A, B**) and AtD14 (**C, D**) proteins with varying indicated concentrations of (±)-GR24 and (±)-SdL19, as assessed by DSF, are shown. Each line represents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

**The Cannalactone Analogue (±)-SdL19 Promotes Hyphal Branching in the Arbuscular Mycorrhizal (AM) Fungus *Rhizophagus irregularis*.** The SL analogues produced in the present study can also help to address SAR requirements in AM symbiosis. SLs are known to induce a number of developmental, cellular and metabolic responses in AM fungi.<sup>34-36</sup> In particular, the stimulation of hyphal branching is commonly used in bioassays to detect the bioactivity of SLs on these fungi. Using this approach, a number of SLs and related compounds have been tested for their activity on the AM fungus *Gigaspora margarita*. Taken together, these studies highlighted the complexity of structural requirements in the rings ABC moiety. GR5 was shown to be inactive, and early studies proposed that a minimal structure would contain the ring C-D moiety fused to at least one ring system.<sup>35,64</sup> This proposal is consistent with the recent discovery of bioactive bryosymbiol (**13**) carrying A, C and D rings.<sup>7</sup> However, the results obtained with CL derivatives later indicated that some compounds without a C ring could retain activity.<sup>26</sup> Combined with reports on natural non-canonical SLs bearing variations on the A or C rings,<sup>6</sup> these studies indicate that requirements for bioactivity on AM fungi can be fulfilled in several divergent ways.

In the present study, an in vitro assay was first used, which allowed a quantification of hyphal branching<sup>65</sup> in the AM fungus *Rhizophagus irregularis*. As shown in **Figure 9A**, (±)-SdL19 stimulated hyphal branching (i.e., the formation of first-order and higher-order branches) in a

similar fashion to (±)-GR24. This indicates that a ring C-D moiety combined with an acyclic carbon chain is sufficient for bioactivity against this AM fungus.

The hyphal branching response triggered by SL analogues is used commonly as a proxy measure for the stimulation of the fungal symbiont, but this response does not always reflect the ability of these compounds to facilitate the initiation of symbiosis.<sup>65</sup> To further document the ability of (±)-SdL19 to stimulate *R. irregularis* in a true symbiotic context, an additional test was used. This assay involves *Medicago truncatula* plants mutated in the SL biosynthesis gene *CCD8*. These mutants are strongly affected in their ability to initiate symbiosis with *R. irregularis*, and this phenotype can be rescued by exogenous application of SL analogues.<sup>65</sup> Like other bioassays involving SL biosynthesis plant mutants and designed to investigate the hormonal activities of SL analogues, this test records the ability of these analogues to “replace” natural SLs in a physiological context. Using this assay, the bioactivity of cannalactone (**14**) was previously shown in AM symbiosis,<sup>44</sup> indicating that the B ring is dispensable. In the present study, (±)-SdL19 exhibited an activity similar to (±)-GR24 (**Figure 9B**). This extends previous observations by showing that symbiotic activity can be conserved when both the A and B rings are absent and replaced by an unsaturated acyclic carbon chain.



**Figure 9.** Activity of (±)-SdL19 on the development of *R. irregularis* and its symbiotic ability. **(A)** *R. irregularis* was grown from spores for 12 days on medium containing  $10^{-7}$  mol.L<sup>-1</sup> (±)-GR24 or (±)-SdL19, or the solvent alone (mock), then the number of branches of first order (black bars) and higher order (grey bars) were measured. Bars represent the means  $\pm$  SE,  $n = 6$  to 8 plates per condition, representing an average of 200 spores. **(B)** *R. irregularis* spores were used to inoculate *M. truncatula ccd8-1* SL-deficient plants.  $10^{-7}$  mol.L<sup>-1</sup> (±)-GR24 or (±)-SdL19, or the solvent alone (mock) were supplied in the nutrient solution. The ability of (±)-SdL19 to restore symbiotic ability in *R. irregularis* was assessed by examining root colonization, 23 days post-inoculation. Bars represent the mean number of infection units per plant  $\pm$  s.e.m.  $n = 14$  to 16 plants per condition. Bars topped with the same letter did not differ significantly by Mann-Whitney U test ( $p > 0.05$ ).

In conclusion, with this study comprehensive SAR data were provided for two genetic groups of *P. ramosa*, comprising routes for synthesis of 14 analogues (SdL) of the non-canonical SL cannalactone (**14**). The SdL analogues with the best specificity towards the population 2a were found to be the compounds with a polar moiety on the side carbon chain. In addition to non-canonical SL cannalactone (**14**) recently identified from hemp by our group,<sup>44</sup> the natural canonical SLs (-)-solanacol (**5**) and (-)-solanacyl acetate (**6**) exuded by tobacco<sup>41,42</sup> and tomato,<sup>66</sup> host plants for *P. ramosa* 2a,<sup>38,39</sup> are reported here as potent selective germination stimulants for *P. ramosa* 2a seeds, contrary to other SLs such as orobanchol (**2**), orobanchyl acetate (**3**), and 5-deoxystrigol (**4**), also detected in tobacco root exudates.<sup>42</sup> The present data further extend basic knowledge on SL analogues that are involved in plant and AM fungi development. Further studies on the interaction of those substances with specific receptors in AM fungi, key players for a sustainable agriculture, and in *P. ramosa* 2a, will help to fully resolve the structure–activity relationships in these species.<sup>65</sup> Moreover, these highly active non-canonical SLs [e.g., (±)-SdL19] could be useful for the study of other organisms which putatively produce only non-canonical SLs such as the moss *Physcomitrium patens*<sup>6,67,68</sup> and *Arabidopsis thaliana*.<sup>14</sup>

## EXPERIMENTAL SECTION

**General Experimental Procedures.** IR spectra were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer, in reciprocal centimeters (cm<sup>-1</sup>). Nuclear magnetic resonance spectra (<sup>1</sup>H; <sup>13</sup>C NMR) were recorded, respectively, at [500 or 300; 175, 125 or 75] MHz on Bruker Avance spectrometers at 298 K. The numbering for signal assignment was done according to **Figure 2**. Low-resolution mass spectra were obtained on a Waters Acquity UPLC system by electrospray ionization (ESI), combined to a photodiode array detector (PDA), an evaporating light scattering detector (ELSD) and a tandem quadrupole detector (TQD). High-resolution mass

spectra were obtained with a Waters Acquity UPLC (by direct injection or with a BEH C<sub>18</sub> 2.1 Å ~50 mm, 1.7 µm column) combined with a PDA and a Waters LCT Premier XE mass instrument (electrospray ionization with a time of-flight (ToF) analyzer). All non-aqueous reactions were run under an inert atmosphere (argon), using standard techniques for manipulating air-sensitive compounds. All glassware was stored in an oven and/or was flame-dried prior to use. Anhydrous solvents were obtained by filtration through drying columns. Analytical thin-layer chromatography (TLC) was performed on plates precoated with silica gel layers. Compounds were visualized by one or more of the following methods: (1) illumination with a short wavelength UV lamp (i.e.,  $\lambda = 254$  nm), (2) spraying with a 1% (w/v) KMnO<sub>4</sub> solution in H<sub>2</sub>O. All separations were carried out under flash-chromatographic conditions on silica gel (prepacked column, 230 – 400 mesh) at medium pressure (20 psi) with an Armen fraction collector and pump. Buffers and aqueous mobile phases for UPLC were prepared using water purified with a Milli-Q system.

All-*trans*- $\beta$ -carotene (**17**) is commercially available from Sigma-Aldrich. ( $\pm$ )-GR24 was prepared according to known procedures<sup>69</sup> and careful purification (HPLC).<sup>45</sup> ( $\pm$ )-GR5 was synthesized by known procedures.<sup>22</sup> ( $\pm$ )-MeCLA (**10**) is available via the synthesis described in Abe et al.<sup>70</sup> ( $\pm$ )-Carlactone (**9**) was synthesized by Adrian Scaffidi.<sup>71</sup> Natural cannalactone (**14**) was isolated as described in Hamzaoui et al.<sup>44</sup> ( $\pm$ )-Strigol (**1**), ( $\pm$ )-5-deoxystrigol (**4**), ( $\pm$ )-sorgolactone (**5**), ( $\pm$ )-orobanchol (**2**) are commercially available from OIChemIm. Orobanchyl acetate (**3**) was synthesized from ( $\pm$ )-orobanchol by acetylation with acetic anhydride in pyridine as described in Boutet et al.<sup>72</sup> (-)-Solanaol (**5**) and (-)-solanacyl acetate (**6**) are obtained as described in Boyer et al.<sup>23</sup> and ( $\pm$ )-7,8-desmethyl solanaol (**15**) and ( $\pm$ )-7,8-desmethyl solanacyl

acetate (**16**) as described in Chen et al.<sup>73</sup> ( $\pm$ )-Fabacyl acetate (**7**) was kindly furnished by Koichi Yoneyama. ( $\pm$ )-4-Vinyldihydrofuran-2(3*H*)-one (**19**) was prepared by a known procedure.<sup>53</sup>

### General Procedures for Compound Synthesis.

(2'*R*\*,4*R*\*)-SdL4F1 and (2'*R*\*,4*S*\*)-SdL4F2. A mixture of alkene **19** (200 mg, 1.78 mmol) and ethyl formate (144  $\mu$ L, 1.96 mmol) in THF (4 mL) was placed at  $-78$  °C. Potassium *tert*-butoxide was added (240 mg, 2.14 mmol). The mixture was stirred for 2 h at  $-78$  °C. A solution of bromide derivative **20**<sup>74</sup> (350 mg, 1.96 mmol) in THF (3.2 mL) was added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with EtOAc and saturated  $\text{NH}_4\text{Cl}$  aqueous solution and then separated. The organic phase was washed with water and brine. The combined organic layers were dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The mixture was purified by chromatography on silica gel (heptane-EtOAc, 80:20 to 60:40 for 30 min) to afford pure ( $\pm$ )-SdL4F1 (87.5 mg, 21%) and ( $\pm$ )-SdL4F2 (85.7 mg, 20%) as brown oils. ( $\pm$ )-SdL4F1: IR (film)  $\nu_{\text{max}}$  3092, 2983, 2919, 2850, 1781, 1752, 1683, 1344, 1207, 1184, 1083, 1026, 955, 866, 801, 745  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.51 (1H, d,  $J = 2.0$  Hz, H-10), 6.88 (1H, t,  $J = 1.5$  Hz, H-6'), 6.12 (1H, t,  $J = 1.5$  Hz, H-2'), 5.81-5.70 (1H, m, H-6), 5.12 (1H, dt,  $J = 1.0, 6.0$  Hz, H-7a), 5.08 (1H, d,  $J = 1.0$  Hz, H-7b), 4.41 (1H, t,  $J = 9.0$  Hz, H-5a), 4.07 (1H, q,  $J = 4.5$  Hz, H-5b), 3.83-3.74 (1H, m, H-4), 1.97 (3H, t,  $J = 1.5$  Hz, H-7');  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.5 (C-5'), 170.4 (C-2), 151.7 (C-6'), 141.1 (C-3'), 135.9 (C-4'), 135.4 (C-6), 116.7 (C-7), 109.9 (C-3), 100.7 (C-2'), 71.1 (C-5), 40.6 (C-4), 10.9 (C-7'); HRESIMS  $m/z$  237.0762  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{12}\text{H}_{13}\text{O}_5$ , 237.0763). ( $\pm$ )-SdL4F2: IR (film)  $\nu_{\text{max}}$  3089, 2917, 2850, 1775, 1747, 1679, 1342, 1181, 1082, 1003, 950  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.48 (1H, d,  $J = 2.0$  Hz, H-10), 6.88 (1H, t,  $J = 1.5$  Hz, H-6'), 6.12 (1H, t,  $J = 1.5$  Hz, H-2'), 5.79-5.68 (1H, m, H-6), 5.10 (1H, dt,  $J = 1.0, 6.0$  Hz, H-7a), 5.06 (1H, d,  $J = 1.0$  Hz, H-

7b), 4.40 (1H, t,  $J = 9.0$  Hz, H-5a), 4.05 (1H, q,  $J = 4.5$  Hz, H-5b), 3.82-3.74 (1H, m, H-4), 1.96 (3H, t,  $J = 1.5$  Hz, H-7');  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.5 (C-5'), 170.4 (C-2), 151.4 (C-6'), 141.3 (C-3'), 136.0 (C-4'), 135.1 (C-6), 116.9 (C-7), 110.0 (C-3), 100.6 (C-2'), 71.0 (C-5), 40.5 (C-4), 10.9 (C-7'); HRESIMS  $m/z$  237.0762  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{12}\text{H}_{13}\text{O}_5$ , 237.0763).

(2'*R*\*,4*R*\*)-SdL7. To a solution of ( $\pm$ )-SdL4F1 (76.5 mg, 0.32 mmol, 1 equiv.) in  $\text{CH}_2\text{Cl}_2$  (0.36 mL), was added methyl acrylate (0.294 mL, 3.24 mmol, 10 equiv.) and Hoveyda-Grubbs II metathesis initiator (HGII) (10.9 mg, 0.016 mmol, 5 mol%) under argon. The mixture was stirred for 24 h at 50 °C in a closed flask with a rodavis cap. The mixture was purified by chromatography on silica gel (heptane-EtOAc, 40:60) to afford pure ( $\pm$ )-SdL7 (54.4 mg, 57%, brown oil): IR (film)  $\nu_{\text{max}}$  3096, 2959, 2919, 2850, 1782, 1756, 1721, 1683, 1437, 1348, 1280, 1186, 1091, 1033, 1010, 956, 867, 748  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.58 (1H, d,  $J = 2.0$  Hz, H-6'), 6.87 (1H, t,  $J = 2.0$  Hz, H-3'), 6.80 (1H, dd,  $J = 8.0, 15.5$  Hz, H-6), 6.12 (1H, t,  $J = 2.0$  Hz, H-2'), 5.88 (1H, dd,  $J = 1.0, 15.5$  Hz, H-7), 4.46 (1H, t,  $J = 8.5$  Hz, H-5a), 4.13 (1H, dd,  $J = 3.5, 9.5$  Hz, H-5b), 4.00-3.90 (1H, m, H-4), 3.73 (3H, s, H-9), 1.99 (3H, s, H-7');  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  170.6 (C-2), 170.3 (C-5'), 166.5 (C-8), 152.7 (C-6'), 144.3 (C-6), 140.9 (C-3'), 136.1 (C-4'), 122.9 (C-7), 108.5 (C-3), 100.8 (C-2'), 69.9 (C-5), 52.0 (C-9), 39.1 (C-4), 10.9 (C-7'); HRESIMS  $m/z$  358.0903  $[\text{M} + \text{CH}_3\text{CN} + \text{Na}]^+$  (calcd for  $\text{C}_{16}\text{H}_{17}\text{NO}_7\text{Na}$ , 358.0903).

4-(3-Oxobut-1-en-1-yl)dihydrofuran-2(3H)-one (**21**). To a solution of 4-vinyldihydrofuran-2(3H)-one **19** (400.4 mg, 3.57 mmol, 1 equiv.) in  $\text{CH}_2\text{Cl}_2$  (4 mL), was added buten-3-one (2.98 mL, 35.70 mmol, 10 equiv.) and HGII (110.4 mg, 0.17 mmol, 5 mol%) under argon. The mixture was stirred for 24 h at 50 °C in a closed flask with a rodavis cap. The mixture was purified by chromatography on silica gel (heptane-EtOAc, 70:30 to 50:50 for 30 min) to afford pure ketone **21** as a brown oil (375.9 mg, 68%): IR (film)  $\nu_{\text{max}}$  3000, 2913, 1771, 1672, 1629, 1360, 1257,



1162, 1015, 979, 890, 838  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  6.66 (1H, dd,  $J = 7.5, 15.5$  Hz, H-6), 6.17 (1H, dd,  $J = 1.0, 16.0$  Hz, H-7), 4.48 (1H, dd,  $J = 7.5, 11.5$  Hz, H-5a), 4.08 (1H, dd,  $J = 7.5, 11.5$  Hz, H-5b), 3.38 (1H, sext,  $J = 8.0$  Hz, H-4), 2.74 (1H, dd,  $J = 7.5, 16.0$  Hz, H-3a), 2.44 (1H, dd,  $J = 7.5, 16.0$  Hz, H-3b), 2.25 (3H, s, H-9);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  197.5 (C-2), 175.4 (C-8), 142.9 (C-6) 132.5 (C-7), 71.4 (C-5), 38.6 (C-4), 34.0 (C-3), 27.8 (C-9); UPLC-TQD (MS)  $m/z$  155.2 ( $[\text{M} + \text{H}]^+$ , 100%).

(*E*)-4-(3-Hydroxy-3-methylbut-1-en-1-yl)dihydrofuran-2(3H)-one (**22a**). Ground  $\text{CeCl}_3$  was dried by heating at 140  $^\circ\text{C}$ /30 rpm for 2 h. THF (20 mL) was added to anhydrous  $\text{CeCl}_3$  (800.0 mg, 3.24 mmol, 1.4 equiv.) at 0  $^\circ\text{C}$ . The mixture was stirred overnight under argon at room temperature. At this temperature, a solution of ketone **21** (357.3 mg, 2.32 mmol) in THF (11.5 mL) was added and stirred for 1 h. Then it was cooled to  $-70$   $^\circ\text{C}$  and a solution of methylmagnesium chloride in THF (1.1 mL (3 M), 3.24 mmol, 1.4 equiv.) was added by syringe. The reaction mixture was stirred until TLC indicated disappearance of starting material. The reaction was quenched with an aqueous 10% acetic acid solution (20 mL) and extracted with EtOAc (3  $\times$  10 mL). The organic layer was washed with water (2  $\times$  10 mL) and brine (10 mL). The combined organic layers were dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to afford crude product **22a** as a brown oil (381.8 mg, 96%). This was used in the next step without further purification but can also be purified by chromatography on silica gel (heptane-EtOAc, 40:60) to afford pure alcohol **22a** as an oil: IR (film)  $\nu_{\text{max}}$  3446, 2973, 2921, 1774, 1677, 1464, 1420, 1374, 1170, 1043, 1015, 975, 908, 839, 796, 690  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.75 (1H, dd,  $J = 1.0, 15.5$  Hz, H-6), 5.59 (1H, dd,  $J = 8.0, 15.5$  Hz, H-7), 4.41 (1H, dd,  $J = 8.0, 10.0$  Hz, H-5a), 3.97 (1H, t,  $J = 8.5$  Hz, H-5b), 3.19 (1H, sext,  $J = 8.5$ , H-4), 2.65 (1H, dd,  $J = 8.5, 17.5$  Hz, H-3a), 2.34 (1H, dd,  $J = 9.5, 17.5$  Hz, H-3b), 1.30 (6H, s, H-9 and

H-10);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  176.7 (C-2), 141.3 (C-7), 124.5 (C-6), 72.8 (C-8), 70.7 (C-5), 38.8 (C-4), 34.8 (C-3), 30.0 (C-9 or C-10), 20.4 (C-9 or C-10); UPLC-TQD (MS)  $m/z$  93.1 (100%), 153.2 ( $[\text{M} - \text{H}_2\text{O}]^+$ , 90%).

**Preparation of Cyclopropylmagnesium Bromide.**<sup>75</sup> To a stirred suspension of magnesium turnings (300 mg, 12.30 mmol) in dry THF (2.0 mL) under argon was added commercially available cyclopropyl bromide (0.800 mL, 9.99 mmol) in dry THF (12.2 mL) dropwise at room temperature for 30 min. During this operation, the reaction continued refluxing without heating. After the addition of the halide was completed, the mixture was stirred at room temperature for 5 h. The titration was performed as described in Sugano et al.<sup>75</sup>

*(E)*-4-(3-Cyclopropyl-3-hydroxybut-1-en-1-yl)dihydrofuran-2(3H)-one (**22b**). Ground  $\text{CeCl}_3$  (455.5 mg, 1.80 mmol, 1.4 equiv.) was dried by heating at 140 °C/30 rpm for 2 h. Dry THF (11 mL) was added at 0°C. The mixture was stirred overnight under argon at room temperature. At this temperature, a solution of 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 and cyclopropylmagnesium bromide (5.6 mL, 0.32 M, 1.80 mmol, 1.4 equiv.) was added by syringe. The reaction mixture was left to stir until TLC indicated that no starting material remained. The reaction was quenched with an aqueous 10% acetic acid solution (10 mL) and extracted with EtOAc (3 × 10 mL). The organic layer was washed with water (2 × 10 mL) and brine (10 mL). The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ). Solvent was removed to afford crude product **22b** (266.4 mg, quantitative), which was used in the next step without further purification, but also could be purified by chromatography on silica gel (heptane-EtOAc, 40:60) to afford pure alcohol **22b** as an oil: IR (film)  $\nu_{\text{max}}$  3446, 2921, 2851, 1777, 1462, 1423, 1373, 1171, 1045, 1016, 977, 737  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.64 (1H, s, H-7), 5.62 (1H, s, H-6), 4.40 (1H, t,  $J = 8.5$  Hz, H-5a), 3.97

(1H, t,  $J = 8.0$  Hz, H-5b), 3.19 (1H, sext,  $J = 8.0$  Hz, H-4), 2.64 (1H, dd,  $J = 8.5, 17.5$  Hz, H-3a), 2.33 (1H, dd,  $J = 9.0, 17.5$  Hz, H-3b), 1.23 (3H, s, H-9), 1.01-0.92 (1H, m, H-10), 0.40 (2H, d,  $J = 8.0$  Hz, H-11 or H-12), 0.28 (2H, d,  $J = 5.5$  Hz, H-11 or H-12);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  176.6 (C-2), 139.0 (C-7), 125.8 (C-6), 72.8 (C-5), 71.6 (C-8), 38.9 (C-4), 34.9 (C-3), 27.5 (C-10), 21.6 (C-9), 1.1 (C-11 or C-12), 0.97 (C-11 or C-12); HRESIMS  $m/z$  197.1180 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{11}\text{H}_{17}\text{O}_3$ , 197.1178).

*(E)*-4-(3-Methyl-3-[(trimethylsilyl)oxy]but-1-en-1-yl)dihydrofuran-2(3H)-one (**23a**). A mixture of alcohol **22a** (137 mg, 0.80 mmol) and TMS-imidazole (7.4 mL, 50.39 mmol, 62.6 equiv.) were stirred at 50 °C under argon for 1 h. The mixture was stirred at room temperature for 1 h and diluted with heptane (20 mL). The organic layer was washed with brine (2  $\times$  10 mL), dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to afford pure product **23a** as an oil (136.2 mg, 70%): IR (film)  $\nu_{\text{max}}$  2966, 2918, 1783, 1377, 1362, 1250, 1165, 1038, 1019, 972, 839, 755  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.66 (1H, dd,  $J = 1.0, 15.5$  Hz, H-6), 5.46 (1H, dd,  $J = 8.0, 15.5$  Hz, H-7), 4.38 (1H, dd,  $J = 8.0, 9.0$  Hz, H-5a), 3.95 (1H, t,  $J = 9.0$  Hz, H-5b), 3.16 (1H, sext,  $J = 8.5$  Hz, H-4), 2.62 (1H, dd,  $J = 8.0, 17.5$  Hz, H-3a), 2.31 (1H, dd,  $J = 9.5, 17.5$  Hz, H-3b), 1.26 (6H, s, H-9 and H-10), 0.07 (9H, s, TMS);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  176.7 (C-2), 142.2 (C-7), 123.8 (C-6), 73.2 (C-8), 72.8 (C-5), 38.9 (C-4), 34.7 (C-3), 30.5 (C-9 and C-10), 2.7 (TMS); UPLC-TQD (MS)  $m/z$  153.3 ([ $\text{M} - \text{OTMS}$ ] $^+$ , 100%), 93.2 (90%).

*(E)*-4-(3-Cyclopropyl-3-[(trimethylsilyl)oxy]but-1-en-1-yl)dihydrofuran-2(3H)-one (**23b**). A mixture of alcohol **22b** (240.7 mg, 1.23 mmol) and TMS-imidazole (7.22 mL, 49.20 mmol, 40 equiv.) were stirred at 50 °C under argon for 1 h. The mixture was stirred at room temperature for 1 h and diluted with 20 mL of heptane. The organic layer was washed with brine (2  $\times$  10 mL), dried over  $\text{Na}_2\text{SO}_4$  and evaporated. The mixture was purified by chromatography on silica

gel (4 g, heptane-EtOAc, 80:20) to afford pure product **23b** as an oil (222.4 mg, 67%): IR (film)  $\nu_{\max}$  3007, 2958, 2894, 1785, 1702, 1420, 1372, 1250, 1209, 1165, 1095, 1065, 1018, 975, 887, 839, 754, 685  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  5.62 (1H, d,  $J = 15.5$  Hz, H-6), 5.52 (1H, dd,  $J = 7.5, 15.5$ , H-7), 4.40 (1H, dd,  $J = 8.0, 9.0$ , H-5a), 3.97 (1H, t,  $J = 8.5$ , H-5b), 3.18 (1H, sext,  $J = 8.0$ , H-4), 2.64 (1H, dd,  $J = 8.5, 17.5$  Hz, H-3a), 2.33 (1H, dd,  $J = 9.5, 17.5$  Hz, H-3b), 1.25 (3H, s, H-9), 0.88-0.78 (1H, m, H-10), 0.35-0.25 (4H, m, H-11 and H-12), 0.06 (9H, s, TMS);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  176.7 (C-2), 140.4 (C-7), 125.0 (C-6), 74.0 (C-8), 72.8 (C-5), 38.9 (C-4), 34.8 (C-3), 27.3 (C-10), 22.2 (C-9), 2.7 (C-11 or C-12), 1.3 (C-11 or C-12); HRESIMS  $m/z$  179.1070  $[\text{M} - \text{OTMS}]^+$  (calcd for  $\text{C}_{14}\text{H}_{24}\text{O}_3\text{Si}$ , 179.1072).

(2'*R*\*,4*R*\*)-SdL18F1 and (2'*R*\*,4*S*\*)-SdL18F2. A mixture 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 stored at  $-78$  °C. Potassium *tert*-butoxide was added (190.7 mg, 1.70 mmol, 1.13 equiv.). The mixture was stirred for 2 h. A solution of bromide derivative **20** (286.7 mg, 1.60 mmol, 1.1 equiv.) in THF (3 mL) was added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with EtOAc and saturated  $\text{NH}_4\text{Cl}$  aqueous solution. The organic phase was washed with water and brine. The combined organic layers were dried with  $\text{Na}_2\text{SO}_4$ , filtered, and then evaporated under reduced pressure. The mixture was purified by chromatography on silica gel (heptane-EtOAc, 80:20 to 70:30 for 20 min) to afford the products ( $\pm$ )-SdL18F1 and ( $\pm$ )-SdL18F1 as brown oils [( $\pm$ )-SdL18F1: 100.7 mg (18%) and ( $\pm$ )-SdL18F2: 86.0 mg (16%), total: 34%]. ( $\pm$ )-SdL18F1: IR (film)  $\nu_{\max}$  2967, 2925, 1785, 1754, 1683, 1380, 1344, 1250, 1183, 1082, 1031, 956, 840, 752  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.50 (1H, d,  $J = 2.5$  Hz, H-6'), 6.82 (1H, t,  $J = 1.5$  Hz, H-3'), 6.08 (1H, t,  $J = 1.5$  Hz, H-2'), 5.64 (1H, d,  $J = 15.5$  Hz, H-7), 5.48 (1H, dd,  $J = 7.5, 15.5$  Hz, H-6), 4.43 (1H, t,  $J = 9.0$  Hz, H-5a), 4.06 (1H, q,  $J = 4.0$  Hz, H-5b), 3.82-3.74 (1H, m, H-4),

1.99 (3H, t,  $J = 1.5$  Hz, H-7'), 1.27 (6H, s, H-9 and H-10), 0.09 (9H, s, TMS);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.6 (C-2), 170.3 (C-5'), 151.6 (C-6'), 141.4 (C-3'), 141.0 (C-7), 136.0 (C-4'), 123.6 (C-6), 110.6 (C-3), 100.7 (C-2'), 73.4 (C-8), 71.5 (C-5), 39.5 (C-4), 30.7 (C-9 or C-10), 30.6 (C-9 or C-10), 10.9 (C-7'), 2.8 (TMS); HRESIMS  $m/z$  317.1004 [ $\text{M} + \text{Na} - \text{OTMS}$ ] $^+$  (calcd for  $\text{C}_{15}\text{H}_{17}\text{O}_5\text{Na}$ , 317.1001). ( $\pm$ )-SdL18F2: IR (film)  $\nu_{\text{max}}$  2967, 2925, 1786, 1755, 1683, 1380, 1344, 1264, 1183, 1084, 1032, 956, 840, 735  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.50 (1H, d,  $J = 2.5$  Hz, H-6'), 6.88 (1H, t,  $J = 1.5$  Hz, H-3'), 6.11 (1H, t,  $J = 1.5$  Hz, H-2'), 5.63 (1H, d,  $J = 15.5$  Hz, H-7), 5.45 (1H, dd,  $J = 7.5, 15.5$  Hz, H-6), 4.42 (1H, t,  $J = 9.0$  Hz, H-5a), 4.03 (1H, q,  $J = 4.5$  Hz, H-5b), 3.81-3.72 (1H, m, H-4), 1.96 (3H, t,  $J = 1.5$  Hz, H-7'), 1.22 (3H, s, H-9 or H-10), 1.20 (3H, s, H-9 or H-10), 0.05 (9H, s, TMS);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.7 (C-2), 170.3 (C-5'), 151.6 (C-6'), 141.5 (C-3'), 141.1 (C-7), 136.0 (C-4'), 123.3 (C-6), 110.5 (C-3), 100.6 (C-2'), 73.4 (C-8), 71.5 (C-5), 39.5 (C-4), 30.7 (C-9 or C-10), 30.4 (C-9 or C-10) 10.8 (C-7'), 2.7 (TMS); HRESIMS  $m/z$  317.0998 [ $\text{M} + \text{Na} - \text{OTMS}$ ] $^+$  (calcd for  $\text{C}_{15}\text{H}_{17}\text{O}_5\text{Na}$ , 317.1001).

(2*R*\*,4*R*\*)-SdL19. To a solution of scandium trifluoromethanesulfonate (0.66 mg, 1.3  $\mu\text{mol}$ ) in  $\text{CH}_3\text{CN}$  (1.5 mL) was added silyl derivative ( $\pm$ )-SdL18F1 (98 mg, 0.27 mmol) in  $\text{CH}_3\text{CN}$  (1.0 mL) and water (2.5  $\mu\text{L}$ , 0.14  $\mu\text{mol}$ ) at room temperature. The resulting mixture was stirred for 1 h at room temperature and quenched with a phosphate buffer (pH 7). The organic materials were extracted with dichloromethane ( $3 \times 10$  mL), the combined extracts were washed with brine, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to afford pure ( $\pm$ )-SdL19 as an oil (87.7 mg, quantitative): IR (film)  $\nu_{\text{max}}$  3460, 2973, 2924, 2850, 1783, 1750, 1682, 1372, 1345, 1265, 1185, 1089, 1032, 1010, 956, 863, 735, 703  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.50 (1H, d,  $J = 2.5$  Hz, H-6'), 6.85 (1H, t,  $J = 1.5$  Hz, H-3'), 6.10 (1H, t,  $J = 1.5$  Hz, H-2'), 5.71

(1H, d,  $J = 15.5$  Hz, H-7), 5.58 (1H, dd,  $J = 7.5, 15.5$  Hz, H-6), 4.43 (1H, t,  $J = 9.0$  Hz, H-5a), 4.07 (1H, dd,  $J = 4.5, 9.0$  Hz, H-5b), 3.83-3.76 (1H, m, H-4), 1.99 (3H, t,  $J = 1.5$  Hz, H-7'), 1.29 (6H, s, H-9 and H-10);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.4 (C-2), 170.2 (C-5'), 151.6 (C-6'), 140.9 (C-3'), 140.3 (C-7), 135.8 (C-4'), 123.7 (C-6), 110.2 (C-3), 100.6 (C-2'), 71.2 (C-5), 70.5 (C-8), 39.3 (C-4), 29.8 (C-9 and C-10), 10.7 (C-7'); HRESIMS  $m/z$  317.0994  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{15}\text{H}_{18}\text{O}_6\text{Na}$ , 317.1001).

(2*R*\*,4*R*\*)-SdL21. To a solution of scandium trifluoromethanesulfonate (0.56 mg, 1.14  $\mu\text{mol}$ ) in  $\text{CH}_3\text{CN}$  (1.3 mL) was added silyl derivative ( $\pm$ )-SdL18F2 (83.5 mg, 0.33 mmol) in  $\text{CH}_3\text{CN}$  (0.29 mL) and water (2.0  $\mu\text{L}$ , 0.11  $\mu\text{mol}$ ) at room temperature. The resultant mixture was stirred for 1 h at room temperature and quenched with a phosphate buffer (pH 7). The organic materials were extracted with dichloromethane (3  $\times$  5 mL), and the combined extracts were washed with brine, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to afford pure ( $\pm$ )-SdL21 as an oil (59.7 mg, 89%): IR (film)  $\nu_{\text{max}}$  3488, 2969, 2925, 1784, 1751, 1682, 1343, 1263, 1185, 1084, 1026, 1013, 955, 863, 802, 730, 701  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.50 (1H, d,  $J = 2.5$  Hz, H-6'), 6.89 (1H, t,  $J = 1.5$  Hz, H-3'), 6.11 (1H, t,  $J = 1.5$  Hz, H-2'), 5.68 (1H, d,  $J = 15.5$  Hz, H-7), 5.54 (1H, dd,  $J = 7.5, 15.5$  Hz, H-6), 4.43 (1H, t,  $J = 9.0$  Hz, H-5a), 4.04 (1H, dd,  $J = 4.5, 9.0$  Hz, H-5b), 3.82-3.74 (1H, m, H-4), 1.97 (3H, t,  $J = 1.5$  Hz, H-7'), 1.22 (6H, s, H-9 and H-10);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  171.6 (C-2), 170.4 (C-5'), 151.7 (C-6'), 141.1 (C-3'), 140.7 (C-7), 135.9 (C-4'), 123.7 (C-6), 110.6 (C-3), 100.7 (C-2'), 71.4 (C-5), 70.7 (C-8), 39.4 (C-4), 29.9 (C-9 and C-10), 10.8 (C-7'); HRESIMS  $m/z$  317.1000  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{15}\text{H}_{18}\text{O}_6\text{Na}$ , 317.1001).

(2'R\*,4R\*)-SdL33. To a solution of (±)-SdL19 (15.6 mg, 0.05 mmol) in 0.3 mL of CH<sub>2</sub>Cl<sub>2</sub> was added pyridine (0.09 mL, 1.06 mmol, 20 equiv.), 4-nitrobenzoylchloride (49.2 mg, 0.26 mmol, 5 equiv.), and DMAP (tip of spatula). The mixture was stirred overnight at 40 °C. The mixture was co-evaporated with toluene, extracted with EtOAc, washed with NaHCO<sub>3</sub> and dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The product was purified by preparative TLC (heptane-EtOAc, 60:40) to afford pure (±)-SdL33 (18.2 mg, 77%, yellow amorphous solid): IR (film)  $\nu_{\max}$  2919, 2850, 1785, 1755, 1722, 1683, 1607, 1527, 1464, 1349, 1290, 1185, 1102, 1033, 1014, 956, 876, 842, 720 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.26 (2H, dt,  $J = 2.0, 8.5$  Hz, H-14 and H-14'), 8.11 (2H, dt,  $J = 2.0, 8.5$  Hz, H-13 and H-13'), 7.51 (1H, d,  $J = 2.5$  Hz, H-6'), 6.89 (1H, t,  $J = 1.5$  Hz, H-3'), 6.10 (1H, t,  $J = 1.5$  Hz, H-2'), 5.98 (1H, dd,  $J = 1.0, 15.5$  Hz, H-7), 5.63 (1H, q,  $J = 8.0$  Hz, H-6), 4.45 (1H, t,  $J = 8.5$  Hz, H-5a), 4.10 (1H, t,  $J = 7.5$  Hz, H-5b), 3.87-3.79 (1H, m, H-4), 2.02 (3H, s, H-7'), 1.23 (6H, t,  $J = 7.0$  Hz, H-9 and H-10); <sup>13</sup>C NMR (175 MHz, CDCl<sub>3</sub>)  $\delta$  171.4 (C-2), 170.3 (C-5'), 163.5 (C-11), 151.8 (C-6'), 150.6 (C-15), 141.1 (C-3'), 136.4 (C-7), 131.5 (C-4'), 130.7 (C-13 and C-13'), 126.8 (C-6), 123.8 (C-12), 123.7 (C-14 and C-14'), 110.0 (C-3), 100.6 (C-2'), 82.2 (C-8), 71.2 (C-5), 39.6 (C-4), 27.3 (C-9 or C-10), 27.1 (C-9 or C-10), 11.0 (C-7'); HRESIMS  $m/z$  466.1131 [M + Na]<sup>+</sup> (calcd for C<sub>22</sub>H<sub>21</sub>NO<sub>9</sub>Na, 466.1114).

(2'R\*,4S\*)-SdL34. To a solution of (±)-SdL21 (15.6 mg, 0.05 mmol) in 0.3 mL of CH<sub>2</sub>Cl<sub>2</sub> was added pyridine (0.09 mL, 1.06 mmol, 20 equiv.), 4-nitrobenzoylchloride (49.17 mg, 0.265 mmol, 5 equiv.), and DMAP (tip of spatula). The mixture was stirred overnight at 40 °C. The mixture was co-evaporated with toluene, extracted with EtOAc, washed with NaHCO<sub>3</sub> and dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The product was purified by preparative TLC (heptane-EtOAc, 60:40) to afford pure (±)-SdL34 (6.8 mg, 25%, yellow

amorphous solid): IR (film)  $\nu_{\max}$  2919, 2850, 1784, 1754, 1722, 1683, 1607, 1527, 1464, 1349, 1289, 1184, 1101, 1032, 1013, 955, 875, 842, 721  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.25 (2H, dt,  $J = 2.0, 8.5$  Hz, H-14 and H-14'), 8.10 (2H, dt,  $J = 2.0, 8.5$  Hz, H-13 and H-13'), 7.50 (1H, d,  $J = 2.5$  Hz, H-6'), 6.92 (1H, t,  $J = 1.5$  Hz, H-3'), 6.11 (1H, t,  $J = 1.5$  Hz, H-2'), 5.96 (1H, dd,  $J = 1.0, 15.5$  Hz, H-7), 5.60 (1H, q,  $J = 8.0$  Hz, H-6), 4.46 (1H, t,  $J = 8.5$  Hz, H-5a), 4.09 (1H, t,  $J = 7.5$  Hz, H-5b), 3.87-3.79 (1H, m, H-4), 2.02 (3H, s, H-7'), 1.24 (6H, t,  $J = 7.0$  Hz, H-9 and H-10);  $^{13}\text{C}$  NMR (175 MHz,  $\text{CDCl}_3$ )  $\delta$  171.3 (C-2), 170.3 (C-5'), 163.5 (C-11), 151.8 (C-6'), 150.6 (C-15), 141.2 (C-3'), 136.6 (C-7), 135.4 (C-4'), 130.8 (C-13 and C-13'), 126.6 (C-6), 125.2 (C-12), 123.7 (C-14 and C-14'), 110.1 (C-3), 100.7 (C-2'), 82.2 (C-8), 71.1 (C-5), 39.7 (C-4), 27.1 (C-9 or C-10), 27.0 (C-9 or C-10), 10.9 (C-7'); HRESIMS  $m/z$  507.1389 [ $\text{M} + \text{CH}_3\text{CN} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_9\text{Na}$ , 507.1380).

(2'*R*\*,4*S*')-SdL50. To a solution of ( $\pm$ )-SdL21 (34.3 mg, 0.117 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.6 mL) was added pyridine (0.189 mL, 2.34 mmol, 20 equiv.), 3,5-dinitrobenzoyl chloride (134.9 mg, 0.58 mmol, 5 equiv.), and DMAP (tip of spatula). The mixture was stirred at 40 °C overnight. The mixture was co-evaporated with toluene, extracted with EtOAc, washed with  $\text{NaHCO}_3$  and dried with  $\text{Na}_2\text{SO}_4$ . The product was purified by preparative TLC (heptane-EtOAc, 45:55) to afford pure ( $\pm$ )-SdL50 as a white solid (17.7 mg, 31%): IR (film)  $\nu_{\max}$  2985, 1737, 1447, 1373, 1301, 1233, 1098, 1043, 938, 918, 847, 786  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  9.19 (1H, t,  $J = 2.0$  Hz, H-15), 9.04 (2H, d,  $J = 2.0$  Hz, H-13 and H-13'), 7.52 (1H, d,  $J = 2.5$  Hz, H-6'), 6.94 (1H, t,  $J = 1.5$  Hz, H-3'), 6.12 (1H, t,  $J = 1.5$  Hz, H-2'), 5.96 (1H, dd,  $J = 1.0, 16.0$  Hz, H-7), 5.65 (1H, dd,  $J = 7.5, 15.5$  Hz, H-6), 4.46 (1H, t,  $J = 9.0$  Hz, H-5a), 4.08 (1H, t,  $J = 5.0$  Hz, H-5b), 3.89-3.81 (1H, m, H-4), 2.02 (3H, s, H-7'), 1.65 (3H, s, H-9 or H-10), 1.62 (3H, s, H-9 or H-10);  $^{13}\text{C}$



NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  206.3 (C-11), 171.2 (C-5'), 170.2 (C-2), 152.0 (C-6'), 148.8 (C-12), 141.1 (C-3'), 135.8 (C-7), 132.3 (C-14 and C-14'), 129.5 (C-13 and C-13'), 127.4 (C-6), 122.4 (C-15), 109.9 (C-4'), 100.9 (C-2'), 71.1 (C-8), 67.2 (C-5), 39.5 (C-4), 29.9 (C-3), 27.2 (C-9 or C-10), 26.8 (C-9 or C-10), 10.9 (C-7'); HRESIMS  $m/z$  552.1226 [M + CH<sub>3</sub>CN + Na]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>23</sub>N<sub>3</sub>O<sub>11</sub>Na, 552.1230).

(2'R\*,4R\*)-SdL51. To a mixture of ( $\pm$ )-SdL19 (21.8 mg, 0.07 mmol) and pyridine (250  $\mu$ L, 3.09 mmol, 41.7 equiv.) was added acetic anhydride (250  $\mu$ L, 2.64 mmol, 35.7 equiv.), and DMAP (tip of spatula). The mixture was stirred at room temperature under argon overnight. The product was co-evaporated with toluene and purified by preparative TLC (heptane-EtOAc, 45:55) to afford pure ( $\pm$ )-SdL51 as an oil (10.2 mg, 41%): IR (film)  $\nu_{\max}$  2985, 1737, 1447, 1373, 1301, 1234, 1097, 1043, 938, 918, 847, 786 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.51 (1H, d,  $J$  = 2.5 Hz, H-6'), 6.95 (1H, t,  $J$  = 1.5 Hz, H-3'), 6.09 (1H, t,  $J$  = 1.4 Hz, H-2'), 5.83 (1H, dd,  $J$  = 1.0, 15.5 Hz, H-7), 5.48 (1H, dd,  $J$  = 8.0, 15.5 Hz, H-6), 4.43 (1H, t,  $J$  = 8.5 Hz, H-5a), 4.03 (1H, t,  $J$  = 5.0 Hz, H-5b), 3.83-3.74 (1H, m, H-4), 2.02 (3H, s, H-7'), 1.95 (3H, s, H-12), 1.47 (3H, s, H-9 or H-10), 1.45 (3H, s, H-9 or H-10); <sup>13</sup>C NMR (175 MHz, CDCl<sub>3</sub>)  $\delta$  171.4 (C-11), 170.5 (C-2), 170.0 (C-5') 151.9 (C-6'), 141.4 (C-3'), 137.3 (C-7), 135.8 (C-4'), 125.8 (C-6), 109.7 (C-3), 100.7 (C-2'), 80.0 (C-8), 71.3 (C-5), 39.6 (C-4), 27.3 (C-9 or C-10), 27.1 (C-9 or C-10), 14.4 (C-12), 10.9 (C-7'); HRESIMS  $m/z$  359.1109 [M + Na]<sup>+</sup> (calcd for C<sub>17</sub>H<sub>20</sub>O<sub>7</sub>Na, 359.1107).

(2'R\*,4R\*)-SdL118F1 and (2'R\*,4S\*)-SdL118F2. A mixture of compound **23b** (150.8 mg, 0.56 mmol), ethyl formate (0.09 mL, 1.12 mmol, 2 equiv.) in THF (1.2 mL) was placed at -78°C. Potassium *tert*-butoxide was added (142.5 mg, 1.27 mmol, 2.26 equiv.). The mixture was stirred for 2 h. A solution of bromide derivative **20** (106.2 mg, 0.60 mmol, 1.08 equiv.) in THF

(1.2 mL) was added. The mixture was stirred at room temperature for 12 h. The mixture was diluted with EtOAc and saturated NH<sub>4</sub>Cl aqueous solution. The organic phase was washed with water and brine, and the combined organic layers were dried with Na<sub>2</sub>SO<sub>4</sub>, and filtered, and evaporated under reduced pressure. The mixture was purified by chromatography on silica gel (heptane-EtOAc, 80:20 to 70:30 for 20 min) to afford the pure products (±)-SdL118F1 and (±)-SdL118F2 as oils [(±)-SdL118F1: 52.3 mg (24%), (±)-SdL118F2: 66.3 mg (30%), total: 54%].

(±)-SdL118F1: IR (film)  $\nu_{\max}$  2959, 1784, 1755, 1684, 1346, 1249, 1185, 1093, 1019, 1028, 955, 863, 841, 754 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.51 (1H, d, *J* = 2.5 Hz, H-6'), 6.84 (1H, quint, *J* = 1.5 Hz, H-3'), 6.08 (1H, dd, *J* = 1.0, 3.0 Hz, H-2'), 5.52 (1H, t, *J* = 5.5 Hz, H-7 and H-6), 4.43 (1H, td, *J* = 2.0, 15.5 Hz, H-5a), 4.10 (1H, q, *J* = 7.0 Hz, H-5b), 3.81-3.74 (1H, m, H-4), 1.99 (3H, t, *J* = 1.5 Hz, H-7'), 1.27 (3H, s, H-9), 0.91-0.82 (1H, m, H-10), 0.34-0.24 (4H, m, H-11 and H-12), 0.07 (9H, s, TMS); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  173.2 (C-2), 169.1 (C-5'), 151.6 (C-6'), 141.0 (C-3'), 140.9 (C-7), 138.9 (C-4'), 125.1 (C-6), 110.1 (C-3), 100.8 (C-2'), 73.9 (C-8), 71.5 (C-5), 39.7 (C-4), 27.7 (C-10), 22.6 (C-9), 10.9 (C-7'), 2.7 (TMS), 1.5 (C-11 and C-12); HRESIMS *m/z* 415.1547 [M + Na]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>28</sub>O<sub>6</sub>SiNa, 415.1553).

(±)-SdL118F2: IR (film)  $\nu_{\max}$  2969, 1784, 1760, 1685, 1346, 1248, 1185, 1086, 1030, 1007, 956, 839, 750 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.51 (1H, t, *J* = 1.5 Hz, H-6'), 6.87 (1H, sext, *J* = 1.0 Hz, H-3'), 6.09 (1H, q, *J* = 1.5 Hz, H-2'), 5.52-5.49 (2H, m, H-7 and H-6), 4.43 (1H, td, *J* = 2.0, 15.5 Hz, H-5a), 4.03 (1H, q, *J* = 7.0 Hz, H-5b), 3.81-3.74 (1H, m, H-4), 1.97 (3H, q, *J* = 1.5 Hz, H-7'), 1.23 (3H, s, H-9), 0.88-0.77 (1H, m, H-10), 0.28-0.21 (4H, m, H-11 and H-12), 0.05 (9H, s, TMS); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  171.3 (C-2), 170.2 (C-5'), 151.3 (C-6'), 141.0 (C-3'), 140.9 (C-7), 134.8 (C-4'), 126.7 (C-6), 113.6 (C-3), 100.5 (C-2'), 74.3 (C-8), 71.5 (C-5), 40.2 (C-4), 29.9 (C-

10), 21.2 (C-9), 10.9 (C-7'), 5.7 (TMS), 1.5 (C-11 and C-12); Carbonyl resonances were missing in the  $^{13}\text{C}$  NMR spectra of ( $\pm$ )-SdL118F1 and ( $\pm$ )-SdL118F2 but were present in the HMBC spectra; HRESIMS  $m/z$  415.1540 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{20}\text{H}_{28}\text{O}_6\text{SiNa}$ , 415.1553).

(2*R*\*,4*R*\*)-SdL127. To a solution of silyl ether ( $\pm$ )-SdL118F1 (23.0 mg, 0.06 mmol, 1 equiv.) in  $\text{CH}_3\text{CN}$  (0.1 mL), was added acetic anhydride (0.011 mL, 0.12 mmol, 2 equiv.) and scandium trifluoromethanesulfonate (0.29 mg, 0.59  $\mu\text{mol}$ ) under argon. The mixture was stirred for 2 h at room temperature under argon. The mixture was purified by preparative TLC (heptane-EtOAc, 40:60) to afford the pure alcohol ( $\pm$ )-SdL127 as a colorless oil (1.8 mg, 19%): IR (film)  $\nu_{\text{max}}$  3468, 2958, 2925, 2856, 1780, 1745, 1680, 1459, 1369, 1344, 1263, 1183, 1083, 1029, 1007, 953, 864, 798, 741, 672  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.50 (1H, t,  $J = 3.0$  Hz, H-6'), 6.87 (1H, dt,  $J = 1.5, 9.5$  Hz, H-3'), 6.09 (1H, s, H-2'), 5.62 (1H, dq,  $J = 3.5, 15.5$  Hz, H-7), 5.51 (1H, dd,  $J = 7.0, 15.5$  Hz, H-6), 4.43 (1H, td,  $J = 2.0, 9.0$  Hz, H-5a), 4.05 (1H, dd,  $J = 4.0, 9.0$  Hz, H-5b), 3.83-3.75 (1H, m, H-4), 1.99 (3H, s, H-7'), 1.28 (3H, s, H-9), 0.89-82 (1H, m, H-10), 0.43-0.34 (2H, m, H-11 or H-12), 0.28-0.22 (2H, m, H-11 or H-12);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  171.5 (C-2), 170.3 (C-5'), 151.7 (C-6'), 141.0 (C-3'), 137.6 (C-6), 136.2 (C-4'), 125.9 (C-7), 110.5 (C-3), 100.8 (C-2'), 72.0 (C-8), 71.4 (C-5), 39.7 (C-4), 28.1 (C-10), 22.0 (C-9), 10.9 (C-7'), 1.5 (C-11 or C-12), 0.9 (C-11 or C-12); HRESIMS  $m/z$  347.1157 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{17}\text{H}_{20}\text{O}_6\text{Na}$ , 347.1158).

**X-ray Crystal Structure Analysis.** A thin colorless needle (0.30  $\times$  0.04  $\times$  0.03 mm) of ( $\pm$ )-SdL50 was mounted on a nylon loop with protective Paratone® oil. Cell dimensions and intensities were measured at 203 K on a Rigaku diffractometer constituted by a MM007 HF rotating-anode generator, delivering intense  $\text{Cu}(\text{K}\alpha)$  radiation ( $\lambda = 1.54187$  Å) through Osmic CMF confocal optics, and a Rapid II curved Image Plate detector allowing data measurement up

to  $2\theta_{\max} = 144^\circ$ . However, the tiny crystals obtained by dissolving in ethyl acetate (48 electrons)/ heptane (58 electrons), and slow cooling of the solution turned out to be poor diffractors, so a compromise was chosen between reasonable exposure time and exploitable data for model solution. 10732 measured reflections up to the  $\theta$  value matching the *iUCR* criteria, and 4562 independent reflections ( $R_{\text{int}} = 0.138$ ), of which only 687 had  $|F_o| > 4\sigma(F_o)$ , using the *CrystalClear 2.0* suite. Data were corrected for Lorentz and polarization effects, and also for absorption ( $T_{\text{min}\rightarrow\text{max}} : 0.448, 1.000$ ). Despite the small signal detected beyond the 1.4 Å resolution limit, the structure could be readily solved either by dual methods (SHELXD),<sup>76</sup> or intrinsic phasing methods (*SHELXT* program);<sup>77</sup> all other calculations regarding the model refinement based on  $F$  using full-matrix least-squares methods with the weight scheme of  $1/[\sigma^2(F_o) + 0.2(F_o^2)]$  were performed with *SHELXL* system<sup>77</sup> and *PLATON*<sup>78</sup> programs, keeping meaningful X-ray diffraction. A potential solvent accessible region with disordered electron density was detected within the crystal structure. *SQUEEZE*<sup>79</sup> was used to model the unresolved electron density likely resulting from disordered crystallizing solvent molecules, representing a total of 44 electrons per unit cell. This contribution was not included in the crystal data. All the non-hydrogen atom displacements were refined in anisotropic manner applying *RIGU(sd 0.02)* restraints throughout the structure, while hydrogen atoms were placed in calculated positions ( $\text{C}-\text{H} = 0.94\text{-}1.00$  Å) and refined as riding on their parent atoms, with  $U_{\text{iso}}(\text{H})$  values constrained to  $1.2 U_{\text{eq}}(\text{C})$  or  $1.5 U_{\text{eq}}(\text{C}_{\text{methyl}})$ . Molecular graphics were computed with *MERCURY*.<sup>80</sup> Crystallographic data for the reported structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 2082797. Copies of the data can be obtained free of charge by request to CCDC, 12 Union Road, Cambridge CB 1EZ, U.K. [fax: int. +44(0) (1223) 336 033]; e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)].

*Crystal data for SdL50: C<sub>22</sub>H<sub>20</sub>N<sub>2</sub>O<sub>11</sub> [+solvent], M = 488.40, a = 15.593(5) Å, b = 5.9014(18) Å, c = 29.216(9) Å, α = 90°, β = 94.304(9)°, γ = 90°, V = 2680.8(14) Å<sup>3</sup>, T = 203 (2) K, space group P2<sub>1</sub>/n, Z = 4, μ(Cu Kα) = 0.848 mm<sup>-1</sup>, 4848 reflections measured, 1013 independent reflections (R<sub>int</sub> = 0.1141). The final R<sub>I</sub> values were 0.1010 (I > 2σ(I)). The final wR(F<sup>2</sup>) values were 0.2686 (I > 2σ(I)). The final R<sub>1</sub> values were 0.1317 (all data). The final wR(F<sup>2</sup>) values were 0.3086 (all data). The goodness of fit on F<sup>2</sup> was 1.238.*

**Plant Material.** Pea (*Pisum sativum*) branching mutants used in this study were derived from various cultivars of pea after ethyl methanesulfonate (EMS) mutagenesis, as described previously.<sup>81</sup> The *rms1-10* (M3T-884) and *rms3-4* (M2T-30) mutants were obtained from the dwarf cv Tèrese. Plants were grown in a greenhouse under long days as described in Braun et al.<sup>82</sup>

All *Arabidopsis thaliana* plants used in this study originated from the Columbia (Col-0) ecotype background and have been described previously: *max3-11*.<sup>62</sup> Plants were grown as described in Cornet et al.<sup>83</sup> for a hydroponic assay (see also below).

Two batches of parasitic plant seeds were used in this study. A population of seeds of *Phelipanche ramosa* associated to genetic group 1 (*P. ramosa* 1) was collected from Saint Martin-de-Fraigneau, France, on broomrape parasitizing winter rapeseed (*Brassica napus*) in June 2015. Seeds of *P. ramosa* from genetic subclade 2a (*P. ramosa* 2a) were harvested at Saint Martin-de-Bossenay, France, on broomrape developed on hemp (*Cannabis sativa*) in August 2012.<sup>38</sup> Seeds were surface sterilized and conditioned according to Pouvreau et al.<sup>58</sup> (dark condition; 21 °C).

**Pea Shoot Branching Assay.** The compounds to be tested were applied directly to the axillary bud with a micropipette as 10 μL of a solution containing 0.1% DMSO with 2% polyethylene

glycol 1450, 50% ethanol and 0.4% DMSO.<sup>23,84</sup> The control-0 is the treatment with 0.1% DMSO without compound. Altogether, 24 plants were sown per treatment in trays (2 repetitions of 12 plants). The treatment was generally performed 10 days after sowing, on the axillary bud at node 3. The branches at nodes 1 to 2 were removed to encourage the outgrowth of axillary buds at the nodes above. Nodes were numbered acropetally from the first scale leaf as node 1 and cotyledonary node as node 0. Bud growth at node 3 was measured with digital callipers 8 to 10 days after treatment. Plants with a damaged main shoot apex or showing a dead white treated-bud were excluded from the analysis. The SL-deficient *rms1-10* and SL-reception *rms3-4* pea mutants were used for all experiments and WT T r se was used as control.

**Hydroponic Assay on *Arabidopsis*.** The hydroponic assay was adapted from Cornet et al.<sup>83</sup> Seeds were surface-sterilized for 8 min in a solution of ethanol (95%)-Bayrochlore (Bayrol, Mundolsheim, France) (10%), and were rinsed twice with ethanol (100%). Each seed was sown on top of a cut 0.5 mL Eppendorf tube filled with agar medium containing 0.65% agar and 10% nutritive solution 5 mM NO<sub>3</sub>. Tubes were soaked in water and stored in the dark at 4  C for 2 days. Twelve plants per pipette tip box (13   9   7 cm) were grown and supplied with nutrient solution as in Boyer et al.<sup>60</sup> at a concentration of 5 mL.L<sup>-1</sup> (750 mL of solution per box). Each week the nutrient solution was renewed and every ten days when the molecules were added into the solution. The first treatment occurred at day 27 after sowing when plants started to bolt. The number of rosette branches was performed at day 42.

**Germination Stimulation Activity Assay on Root Parasitic Plant Seeds.** Germination stimulant activity (GS) of chemicals on seeds of parasitic plants was determined using a method described previously.<sup>58,85</sup> Chemicals were suspended in DMSO, except all-*trans*- -carotene (**17**) in THF, at 10 mmol.L<sup>-1</sup>, then diluted with water at 1 mmol.L<sup>-1</sup> (water/DMSO; v/v; 9/1). Dilutions

of  $1 \times 10^{-5}$  mol.L<sup>-1</sup> to  $1 \times 10^{-12}$  mol.L<sup>-1</sup> were then performed in water/DMSO (v/v; 9/1). For each compound, a range of concentrations from  $10^{-13}$  to  $10^{-6}$  mol.L<sup>-1</sup> (water/DMSO; 99/1) was applied to the conditioned parasitic seeds. DMSO (1%) was used as a negative control (seed germination < 1%) and (±)-GR24 at a concentration of 1 μmol.L<sup>-1</sup> was used as a positive control and induced 72-87% of seed germination for *P. ramosa* 1 and 80–90% for *P. ramosa* 2a. To avoid variations related to sterilization events percentages of germination are reported as a ratio relative to the positive control [(±)-GR24, 1 μmol L<sup>-1</sup>] included in each germination assay. Each dilution and germination assay was repeated at least three times. For each compound tested, dose-response curves [Germination Stimulation = f(c), Germination Stimulant activity relative to (±)-GR24 1 μmol.L<sup>-1</sup>; c : concentration (mol.L<sup>-1</sup>)], half maximal effective concentration (EC<sub>50</sub>), and maximum of germination stimulant activity were determined using a Four Parameter Logistic Curve computed with SigmaPlot® 10.0.

**Fungal Material.** *Rhizophagus irregularis* spores (strain DAOM197198) were purchased from Agronutrition (France). They were rinsed twice with sterile water before use.

**Analysis of Hyphal Branching in Vitro.** Experiments were carried out as described in Taulera et al.<sup>65</sup> Spores were placed on plates containing M medium<sup>86</sup> and supplemented with SL analogues (100 nM.L<sup>-1</sup>), or 0.1% DMSO for mock treatments. Plates were incubated at 30 °C under 2% CO<sub>2</sub> for 12 days. Germ tubes were then identified as the longest hypha coming out of each spore. The number of hyphal branches of the 1<sup>st</sup> order (growing from the germ tube) and higher order (growing from 1<sup>st</sup>-order branches) was scored using a dissecting microscope 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 unit (represented by the plate mean), and 6 to 8 plates were analyzed for each treatment.

**Symbiosis Initiation Assay.** The assay was carried out as described in Taulera et al.<sup>65</sup> Briefly, *ccd8-1* mutants of *Medicago truncatula*<sup>87</sup> were grown on a clay substrate inoculated with 150 spores of *R. irregularis*. SL analogues were added to the nutrient solution, to reach a final concentration of 100 nM.L<sup>-1</sup>. Mock treatments were performed with 0.1% DMSO. The number of infection points in each root system was recorded three weeks post-inoculation, after staining fungal structures with Schaeffer black ink.<sup>65,88</sup>

**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.<sup>52,61</sup>

**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 ( $\lambda_{\text{Ex}}/\lambda_{\text{Em}}$ : 490/610 nm; Life Technologies) was used as the reporter dye. Samples were heat-denatured 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- $\mu$ L scale in PB buffer pH 6.8 containing 10  $\mu$ g protein. Each concentration of the SL derivatives was used with a DMSO concentration of 4% with 0.008  $\mu$ L Sypro Orange. In the control reaction, DMSO was added instead of a test compound solution.

**NanoDSF.** Proteins were diluted in PBS (100 mM phosphate, pH 6.8, 150 mM NaCl) to a concentration of ~10  $\mu$ M. Ligands were tested at a concentration of 200  $\mu$ M. The intrinsic



fluorescence signal was measured as a function of increasing temperature with a Prometheus NT.48 fluorimeter (NanoTemper Technologies), with 55% excitation light intensity and 1 °C/min temperature ramp. Analyses were done on capillaries filled with 10  $\mu$ L of the respective samples. Intrinsic fluorescence signals expressed by the 350 nm/330 nm emission ratio that increased as the proteins unfolded, were plotted as a function of temperature (Figure S4A and Figure S4B, Supplementary Information). The plots showed one of three independent data collections performed for each protein.

**Intrinsic Tryptophan Fluorescence Assays and Determination of the Dissociation Constant  $K_D$ .** These experiments were performed as described previously in de Saint Germain et al.<sup>61</sup> using a Spark Multimode Microplate Reader (Tecan).

**Hydrolysis of ( $\pm$ )-SdL19 and ( $\pm$ )-GR24 in Aqueous Solution.** ( $\pm$ )-GR24 and ( $\pm$ )-SdL19 were tested for their chemical stability in an aqueous solution. Aqueous solutions of the compound to be tested (50  $\mu$ g/mL) were incubated at 22 °C in the HPLC vials. The compounds were first dissolved in DMSO (2 mg/mL). Then, 25  $\mu$ L of these solutions [( $\pm$ )-GR24 and ( $\pm$ )-SdL19] were diluted to the final concentration with H<sub>2</sub>O (750  $\mu$ L) and EtOH (175  $\mu$ L) and the solution adjusted to pH 6.8. Aqueous solutions of the compounds to be tested (50  $\mu$ g/mL) were incubated at 22 °C in the HPLC vials. ( $\pm$ )-1-Indanol (25  $\mu$ L of a 1 mg/mL solution in DMSO) was added as internal standard to each solution. The samples were subjected to reverse-phase-ultra-performance liquid chromatography (RP-UPLC)-MS analyses by means of UPLC system equipped with a PDA and a TQD mass spectrometer (Acquity UPLC-TQD, Waters). RP-UPLC (HSS C<sub>18</sub> column, 1.8  $\mu$ m, 2.1 mm  $\times$  50 mm) involved 0.1% (v/v) formic acid in CH<sub>3</sub>CN and 0.1% (v/v) formic acid in water (aq. FA, 0.1%, v/v, pH 2.8) as eluents [10% CH<sub>3</sub>CN, followed by linear gradient from 10% to 100% of CH<sub>3</sub>CN (4 min)] at a flow rate of 0.6 mL.min<sup>-1</sup>. The

detection was done using a PDA and with the TQD mass spectrometer operated in an electrospray ionization-positive mode at 3.2 kV capillary voltage. To maximize the signal, the cone voltage and collision energy were optimized to 20 V and 12 eV, respectively. The collision gas was argon at a pressure maintained near  $4.5 \times 10^{-3}$  mBar. The relative quantity of remaining (non degraded) product was determined by integration in comparison with the internal standard.

**Statistical Analyses.** Since deviations from normality were observed for axillary bud length after SL treatment, the Kruskal–Wallis test was used to compare treatments using R Commander version 1.7–3.<sup>89</sup> For bioassays with *Arabidopsis thaliana*, data were analyzed with the Shapiro-Wilkinson normality test. For bioassays with AM fungi, data were analyzed using Statgraphics Centurion software (SigmaPlus). Non-parametric tests were used because normality or homoscedasticity criteria were not met. Datasets were analyzed using the Kruskal-Wallis test, followed by pairwise comparisons with Mann-Whitney U test.

## ASSOCIATED CONTENT

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/xxx>. Bioactivity data (supplementary data for **Figure 7**, Tables S1-S2, Figure S1-S2), stability of SdL19 (Figure S3), nanoDSF data (Figure S4) and <sup>1</sup>H and <sup>13</sup>C NMR spectra for the SdL compounds and precursors (**21**; **22a,b**; **23a,b**).

## AUTHOR INFORMATION

### Notes

The authors declare no competing financial interest.

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