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Modular and scalable synthesis of nematode pheromone ascarosides: implications in eliciting plant defense response†

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A highly efficient and modular synthesis of nematode pheromone ascarosides was developed, which highlights a 4-step scalable synthesis of the common intermediate **10** in 23% yield from commercially available L-rhamnose by using orthoesterification/benzylation/orthoester rearrangement as the key step. Six diverse ascarosides were synthesized accordingly. Notably, biological investigations revealed that ascr#1 and ascr#18 treatment resulted in enhanced callose accumulation in *Arabidopsis* leaves. And ascr#18 also increased the expression of defense-related genes such as PR1, PDF1.2, LOX2 and AOS, which might contribute to the enhanced plant defense responses. This study not only allows a facile access to 1-*O*, 2-*O*, and 4-*O* substituted ascarosides, but also provides valuable insights into their biological activities in inducing plant defense response, as well as their mode of action.

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Introduction

Nematodes are ubiquitous and parasitize most plants and animals. Plant-parasitic nematodes cause agricultural damage of more than $100 billion annually worldwide.[1] The recognition of specific small molecular patterns has been shown to play a central role in plant immune response.[2,3] Plants possess pattern recognition receptors that serve to detect microbe-associated molecular patterns (MAMPs), which thereby elicit defense reactions known as MAMP-triggered immunity (MTI).[4] Identification of MAMPs represents a sustainable crop disease management approach. However, investigations on conserved nematode-associated molecular patterns that are recognized by plants remain scarce.[5]

Ascarosides represent an evolutionarily conserved family of glycolipid pheromones derived from nematodes. Since the first ascaroside ascr#1 was isolated and characterized from *C. elegans* by Jeong *et al.* in 2005,[6] more than 200 ascarosides have been identified from over 20 different nematode species, demonstrating their wide distribution in the nematode phylum, including plant-parasitic nematodes (Fig. 1).[7] Structurally, ascarosides are a family of glycosides of 3,6-dideoxysugar L-ascaryloses with hydroxyl fatty acids of varying lengths attached at the penultimate (ω-1) or terminal (ω) carbon, which was labelled according to the small molecule identifier (SMID) as promoted by Schroeder's lab.[8] Additional modifications can occur at 2-*O* and 4-*O* of the sugar, including indole-3-carbonyl,[9] 4-hydroxybenzoyl,[10,11] (*E*)-2-methyl-2-butenoyl,[10,11] glucosyl,[12] *etc.* Ascarosides serve essential

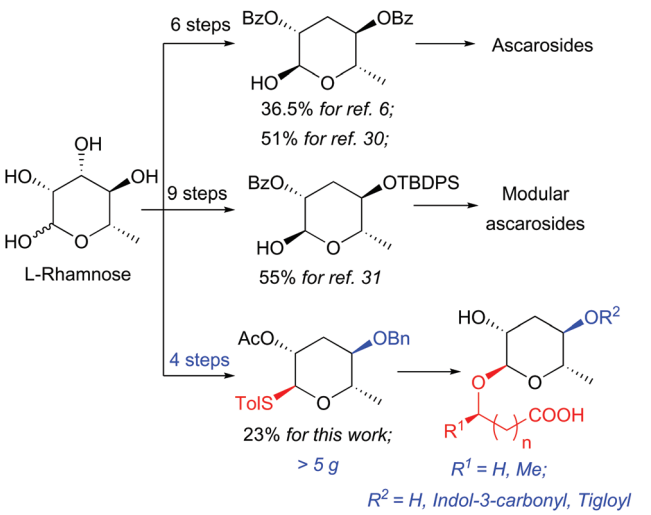

Fig. 1 Synthetic approaches towards ascarosides and the key intermediates.

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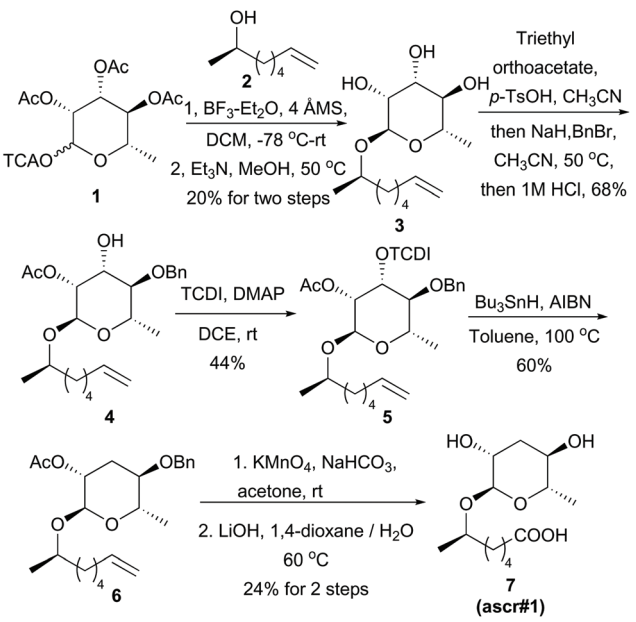
functions in chemical signalling of nematodes and regulate development,[6,13–15] lifespan,[16,17] morphology[18] and social behaviour,[11,14,15,19–21] which are highly sex- and structure-dependent.[7,22–24] Strikingly, ascarosides can be active at extremely low concentrations (1.0 fmol) and subtle modifications in the chemical structure can exert significant impacts on their biological functions.[10,11] Recently, Manosalva *et al.* showed that ascr#18, the most abundant ascaroside in plant-parasitic nematodes, triggered plant hallmark defense responses, including activation of MAMP-triggered immunity, as well as salicylic acid- and jasmonic acid-mediated defense signalling pathways, which thereby increased resistance to pathogen infections in plants.[25,26] This result suggests that ascarosides represent a nematode-associated molecular pattern (NAMP). Additionally, ascr#18 secreted by plant-parasitic nematodes can be metabolized by plants to shorter side-chain ascarosides, which confer nematode repellence.[27] These results suggest that ascarosides not only elicit plant-defense response by conventional pattern-triggered immunity, but can also be edited by plants to manipulate nematode chemical signalling, which demonstrates their potential utility to improve economic and environmental sustainability of agriculture. However, their structure–activity relationships (SARs), as well as their mode of action, remain largely unexplored due to their limited access.

To this end, several elegant synthetic strategies towards ascarosides have been developed (Fig. 1). For instance, Jeong *et al.*, as well as several other groups, developed a synthetic strategy of ascarosides featuring a 6-step synthesis of the key 2,4-di-*O*-benzoylascarylose in 36.5% yield from L-rhamnose.[6,28–30] Similarly, Martin *et al.* made an improvement in efficiency to 51% overall yield in 6 steps from L-rhamnose.[31] However, lack of versatility renders this route highly restricted in the regioselective synthesis or modifications of 2′- or 4′-substituted ascarosides. A significant synthetic advancement was achieved by Zhang *et al.*, which highlights a 9-step regioselective manipulation of L-rhamnose to afford 4-*O*-TBDPS-2-*O*-Bz-ascarylose in 55% overall yield. It thereby allowed the versatile synthesis of several structurally diverse ascarosides.[32] Besides, Guo *et al.* developed a *de novo* asymmetric synthetic strategy evolving through the Noyori reduction of an acylfuran and a propargyl ketone, the Achmatowicz rearrangement and diastereoselective palladium-catalyzed glycosylation and epoxidation/ring-opening sequence.[33,34] Since these processes require more than 6 synthetic steps and tedious operation, further improvements are still required. Herein, we developed an efficient and modular synthetic route to a diverse set of ascarosides with 1-tolylthio-2-*O*-acetyl-4-*O*-Bn-ascaroside **10** as a common intermediate, which can be easily scaled up in 4 steps, with 23% total yield from L-rhamnose by using the orthoesterification/benzylation/orthoester rearrangement sequence and the Barton–McCombie deoxygenation as the key steps. Furthermore, the abilities of ascarosides in eliciting defense responses, including callose deposition and defense-associated gene expression in *Arabidopsis*, were also studied.

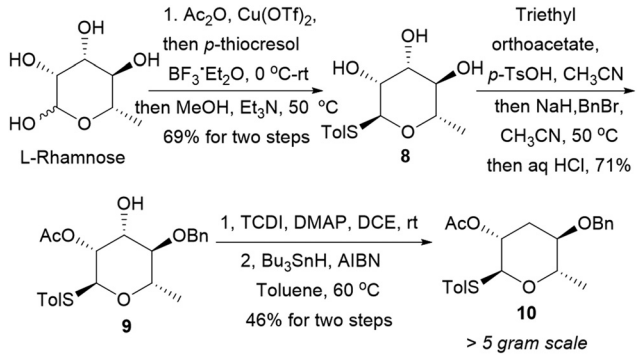
Results and discussion

Initially, we envisaged to avoid tedious anomeric protection/deprotection by introducing the fatty acid side chain at the early stage. As shown in Scheme 1, the synthetic endeavours commenced with Lewis acid-catalyzed glycosylation of aliphatic alcohol **2** with L-rhamnose derivative **1**.[32] Unexpectedly, this reaction was quite inefficient even after extensive screening and optimizations, which gave the corresponding coupling products as a 1 : 1 mixture of diastereoisomers. The desired product **3** was obtained in only 20% yield upon removal of the acetyl group (Table S1†). We then proceeded to protect C2 and C4 with distinct masking groups and remove the C3-oxygen group. Inspired by Mukhopadhyay and Field's work which described a one-pot orthoester formation/benzylation/regioselective hydrolysis sequence of sugars to prepare partially protected glycosyl acceptors,[35] we applied the established protocol to precursor **3** to afford the desired intermediate **4** in 68% yield. Subsequent deoxygenation at C3 was not straightforward. Initial nucleophilic reduction of 3-*O*-mesylate with $NaBH_4$ proved unsuccessful,[32] and afforded the C2-deacetylated side product instead. The Barton–McCombie deoxygenation reaction was proved to be effective for this transformation.[36] Specifically, activation of C3-OH with TCDI followed by radical reduction provided the desired C3-deoxygenated product **6** in 26.4% yield. The final oxidative cleavage of the double bond was also inefficient, only delivering 7 (ascr#1) in 24% yield after saponification.

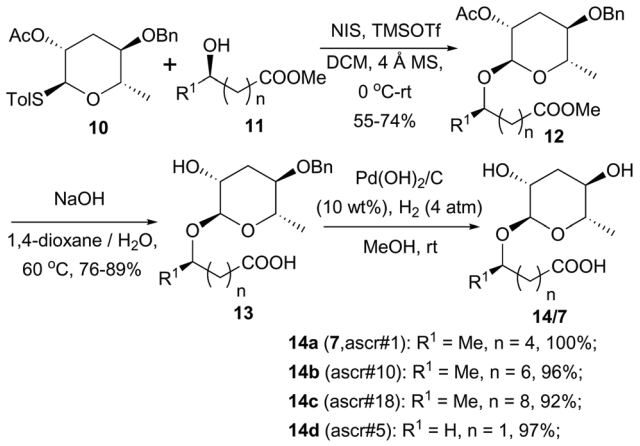
Although ascarosides can be obtained *via* intermediate **6** with fewer steps, the overall efficiency and scalability remains far from satisfactory. Besides, modifications at the anomeric position require synthesis from the very early stage. To address these problems, 2nd generation synthesis was further devel-



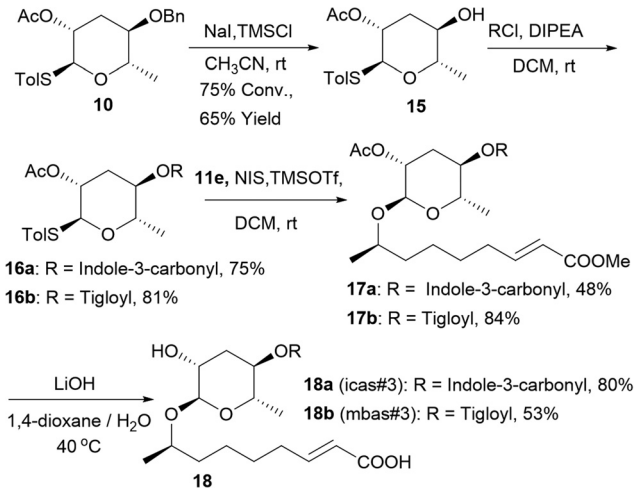
Scheme 1 Synthesis of ascr#1.



Scheme 2 Synthesis of the common intermediate **10**.



Scheme 3 Synthesis of 1-*O*-substituted ascarosides.



Scheme 4 Synthesis of 4-*O*-substituted ascarosides.

oped. As shown in Scheme 2, a one-pot acetylation/thionylation/hydrolysis sequence of L-rhamnose delivered the TolS-activated rhamnose **8** in 69% overall yield,[37] which was followed by a one-pot orthoester formation/benzylation/regioselective hydrolysis sequence to give intermediate **9** in 71% yield.[38] Subsequent Barton–McCombie deoxygenation provided intermediate **10** in 42% yield. Remarkably, the common precursor **10** can be easily prepared on a scale of more than 5 gram.

With enough amount of **10** in hand, we then proceeded to attach it with different substituents to access diverse ascarosides. For 1-*O*-substituted ascarosides, glycosylation of **10** with freshly prepared (ω-1)-hydroxyacyl esters **11** upon treatment of NIS in the presence of TMSOTf gave the coupling products **12** in 55–74% yields. Remarkably, only the desired α-anomer was detected for all the substrates examined, which was attributed to the anchimeric assistance of the 2-acetoxy group. This is the first example of using a tolyl thioascaroside as the glycosyl donor for the glycosylation reaction, wherein the efficiency is comparable or slightly inferior to that of the previously reported Lewis acid-activated glycosylation of 2,4-di-*O*-benzoyl ascarylose,[6,9] 4-*O*-TBDPS-2-*O*-Bz ascarylose[32] and their 1-*O*-trichloroacetimidate derivatives *via* a two-step process[31,32] or Pd-catalyzed allylic glycosylation.[33,34] Subsequent saponification with NaOH in 1,4-dioxane/H_2O delivered **13**, which was selectively deprotected with catalytic hydrogenation to give the desired ascarosides in moderate to good overall yields. By varying the fatty acid chain at the anomeric carbon, several representative ascarosides **14a–14d**, namely ascr#1, ascr#5, ascr#10, and ascr#18, were synthesized accordingly (Scheme 3).

For 4-*O*-substituted ascarosides, a slightly modified synthetic route was developed. As shown in Scheme 4, an acid-mediated debenzylation of the common intermediate **10** gave the alcohol **15** in good yield, which was followed by installation of an ester group (*e.g.* indole-3-carbonyl or senecioyl group) at 4-*O* to give the corresponding esters **16a** and **16b** uneventfully. Subsequent glycosylation with the fatty acid **11e** delivered the coupling products **17a** and **17b** in 48% and 84% yields respectively. The final selective hydrolysis of the acetyl esters afforded the desired 4-*O*-substituted ascarosides **18a** (icas#3) and **18b** (mbas#3) in 80% and 53% yields respectively.

Perception of pathogen associated molecular patterns (PAMPs) activates the first line of innate immunity to fend off pathogen infection. Various defense responses in plant cells have been well characterized after pathogen recognition, including reactive oxygen species (ROS) production, callose deposition, and immune gene activation.[39–42] Although ascr#18 has been shown to activate the defense signalling pathways[26] and can be further metabolized into ascarosides with a shorter side chain in plants,[27] the immunity-related activity of other ascarosides is unclear. Whether callose deposition and ROS production are influenced by ascarosides including ascr#18 is unknown. In order to investigate the immune activation ability of different ascarosides, the biological activities of several ascarosides in inducing plant defense responses were further explored in *Arabidopsis thaliana*. As shown in Fig. 2, significant callose accumulation in *Arabidopsis* leaves was detected upon treatment with ascr#1 (**14a**/**7**, 1 μM) and ascr#18 (**14c**, 1 μM), but not with ascr#5 (**14d**) and ascr#10 (**14b**) (see Fig. S1A in the ESI†). This

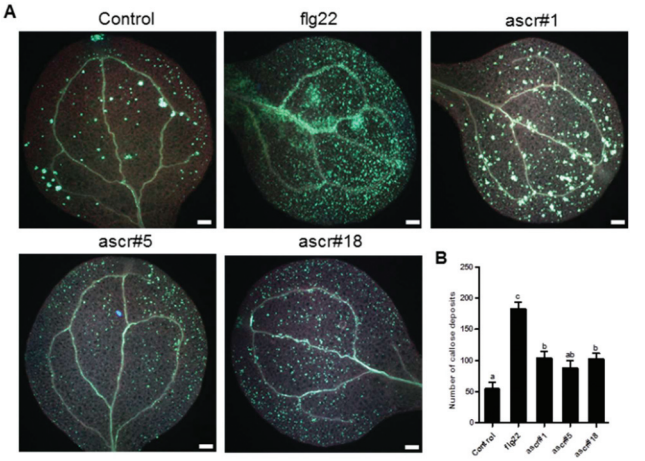


Fig. 2 Ascaroside treatment induces callose deposition in *Arabidopsis thaliana* Col-0. (A) Ten-day-old seedlings were treated with 1 μM flg22 and 1 μM ascarosides (ascr#1 (**14a**/**7**), ascr#5 (**14d**) and ascr#18 (**14c**)) for 24 h and callose was detected by aniline blue staining. (B) Water treatment serves as the control. ImageJ software was used to quantify the number of callose deposits. Data are average ± SE ($n \geq 9$). Different letters denote significant differences by the one-way ANOVA test ($p < 0.05$). Bar = 200 μm.

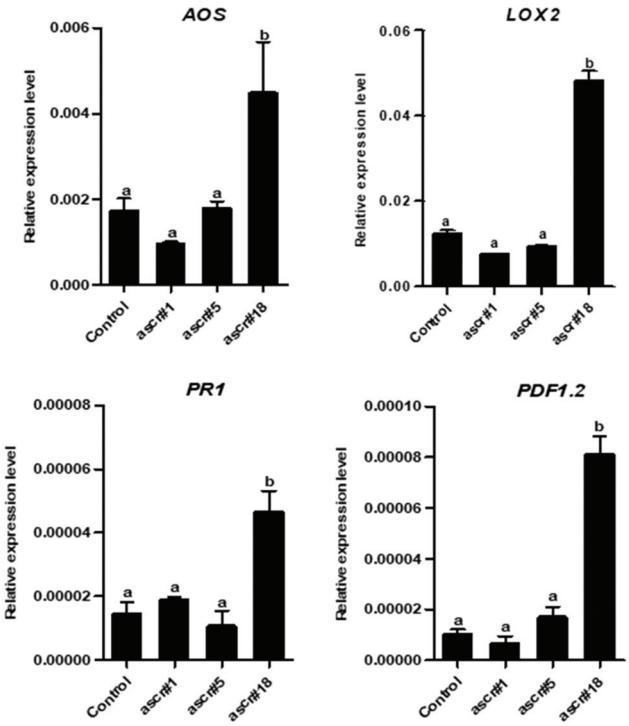


Fig. 3 Immune gene expression in *Arabidopsis thaliana* Col-0 is induced upon ascaroside treatment. Ten-day-old seedlings were treated with ddH_2O and 1 μM ascarosides for 60 min, and total RNA was extracted for qRT-PCR analysis. Data represent the mean ± SE of two biological replicates. Different letters denote significant differences by the one-way ANOVA test ($n = 2$, $p < 0.05$).

means that only specific side chains at the anomeric carbon will trigger the optimal activity, which indicates that early recognition by host PAMP receptors might be specific for certain ascarosides. The stable activity obtained for ascr#18 is consistent with the fact that ascr#18 is the most abundant ascaroside excreted by plant-parasitic nematodes. However, callose deposition is still considerably less pronounced with ascaroside treatment compared with that triggered by the positive control flg22. It is worth noting that no significant ROS burst was detected (see Fig. S1B in the ESI†), which might be rationalized that ROS burst is mediated by distinct signalling pathways or the signal is too weak to be measured since the ROS burst in the apoplast is often fast and transient.

Using whole seedlings, we also examined the transcript levels of genes involved in biosynthetic or responsive pathways of the two major defense hormones, salicylic acid (SA) and jasmonic acid (JA) (Fig. 3). qRT-PCR analysis revealed that ascr#18 pretreatment increased the expression levels of the JA-biosynthesis genes *Lipoxygenase 2* (*LOX2*) and *Allene Oxide Synthase* (*AOS*), and the JA responsive gene *PDF1.2* in whole seedlings, whereas neither ascr#1 nor ascr#5 shows any effect under the conditions tested. In addition, the expression of the SA-responsive marker *PR1* is also induced by ascr#18, suggesting the important role of ascarosides in the elicitation of JA and SA-mediated plant defense responses in a structure-dependent manner.[26,43] These results provide a rationale for the previous results that JA and SA are involved in plant-parasitic nematode-induced resistance.[44] The fact that JA and SA responsive pathways are activated upon ascaroside treatment suggests potential application of ascarosides as plant immunity inducers.

Conclusions

In summary, we developed an improved synthetic strategy to enable efficient and modular synthesis of diverse ascarosides, which features a 4-step scalable synthesis of the key intermediate **10** from commercially available L-rhamnose and uses a tolyl thioascaroside as the glycosyl donor for the glycosylation reaction. Furthermore, the biological activities of ascarosides in eliciting plant defense response were also investigated. This study not only allows a facile access to 1-*O*, 2-*O*, and 4-*O* substituted ascarosides, but also provides valuable insights into their structure–activity relationships, as well as their mode of action. Although variable immune responses present a challenge for future mechanistic study, further investigations for the discovery of more potent elicitors and characterization of the detailed mechanism as well as the receptors that are responsible for plant immune response would be prominent for better management of the pathogen.

Experimental

The procedures for the synthesis of the compounds mentioned in this article and their characterization data are presented in the ESI.†

Plant materials and growth conditions. The *Arabidopsis thaliana* Col-0 accession was obtained from the *Arabidopsis* Biological Resources Centre (ABRC). Seedlings were germinated on half-strength Murashige and Skoog (MS) plates containing 0.5% (w/v) agar, grown at 23 °C and under 75 μE m^{-2} s^{-1} light with a 12 h-light/12 h-dark photoperiod for 10 days.

Callose deposition assays. Ten-day-old seedlings were transferred to a 12-well tissue culture plate with 2 mL ddH_2O for 12 h for recovering, and treated with and without 1 μM flg22 or 1 μM ascarosides for 24 h. Then the seedlings were transferred into FAA solution (10% formaldehyde, 5% acetic acid and 50% ethanol) for 12 h and de-stained in 95%, 70% and 50% ethanol for 6 h. After washing two times with ddH_2O, the cleared seedlings were stained with 0.01% aniline blue solution (150 mM KH_2PO_4, pH 9.5) for 30 min. Callose deposits were visualized using a fluorescence microscope under UV excitation and counted using ImageJ 1.52a software (http://rsb.info.nih.gov/ij/).

RNA isolation and qRT-PCR analysis. For RNA isolation, 10-day-old *Arabidopsis thaliana* Col-0 seedlings grown on half-strength MS plates were transferred to a 12-well culture plate and treated with and without 1 μM ascarosides for 60 min. RNA was extracted using TRIzol reagent (Invitrogen, USA) and quantified using a NanoDrop spectrophotometer. Total RNA was reverse transcribed with HiScript II Q RT SuperMix (Vazyme, China). Real-time PCR was performed using AceQ qPCR SYBR Green Master Mix (Vazyme, China) and a Bio-Rad FX-96 Real-Time PCR System (Bio-Rad, USA). UBQ10 (AT4G05320) was used as an endogenous control. Relative gene expression levels were determined using the ΔΔCt method compared with the internal control. The primers used for qRT-PCR are listed in Table S2.†

Statistical analysis. All statistical analysis was conducted using GraphPad Prism 8. Statistically significant differences were determined by one-way ANOVA, and P values less than 0.05 were considered statistically significant in all cases.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

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Notes and references

1 N. A. Mitkowski and G. S. Abawi, *Nematology*, 2003, **5**, 77–83.
2 T. Boller and G. Felix, *Annu. Rev. Plant Biol.*, 2009, **60**, 379–406.
3 C. M. J. Pieterse, D. Van der Does, C. Zamioudis, A. Leon-Reyes and S. C. M. Van Wees, *Annu. Rev. Cell Dev. Biol.*, 2012, **28**, 489–521.
4 P. Bittel and S. Robatzek, *Curr. Opin. Plant Biol.*, 2007, **10**, 335–341.
5 E. A. Schmelz, J. Engelberth, H. T. Alborn, J. H. Tumlinson and P. E. A. Teal, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 653–657.
6 P.-Y. Jeong, M. Jung, Y.-H. Yim, H. Kim, M. Park, E. Hong, W. Lee, Y. H. Kim, K. Kim and Y.-K. Paik, *Nature*, 2005, **433**, 541–545.
7 F. C. Schroeder, *Nematology*, 2015, **22**, 7–16.
8 C. S. Frank, *C. elegans Small Molecule Identifier Database (SMID DB)*, Boyce Thompson Institute, Cornell University, 2011, http://smid-db.org, accessed on 27 Oct. 2011.
9 R. A. Butcher, J. R. Ragains and J. Clardy, *Org. Lett.*, 2009, **11**, 3100–3103.
10 S. H. von Reuss, N. Bose, J. Srinivasan, J. J. Yim, J. C. Judkins, P. W. Sternberg and F. C. Schroeder, *J. Am. Chem. Soc.*, 2012, **134**, 1817–1824.
11 J. Srinivasan, S. H. von Reuss, N. Bose, A. Zaslaver, P. Mahanti, M. C. Ho, O. G. O'Doherty, A. S. Edison, P. W. Sternberg and F. C. Schroeder, *PLoS Biol.*, 2012, **10**, e1001237.
12 T. Boller and G. Felix, *Annu. Rev. Plant Biol.*, 2009, **60**, 379–406.
13 R. A. Butcher, M. Fujita, F. C. Schroeder and J. Clardy, *Nat. Chem. Biol.*, 2007, **3**, 420–422.
14 C. Pungaliya, J. Srinivasan, B. W. Fox, R. U. Malik, A. H. Ludewig, P. W. Sternberg and F. C. Schroeder, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 7708–7713.
15 J. Srinivasan, F. Kaplan, R. Ajredini, C. Zachariah, H. T. Alborn, P. E. A. Teal, R. U. Malik, A. S. Edison, P. W. Sternberg and F. C. Schroeder, *Nature*, 2008, **454**, 1115–1118.
16 A. H. Ludewig, Y. Izrayelit, D. Park, R. U. Malik, A. Zimmermann, P. Mahanti, B. W. Fox, A. Bethke, F. Doering, D. L. Riddle and F. C. Schroeder, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 5522–5527.
17 E. Z. Aprison and I. Ruvinsky, *Curr. Biol.*, 2016, **26**, 2827–2833.
18 N. Bose, A. Ogawa, R. S. H. von, J. J. Yim, E. J. Ragsdale, R. J. Sommer and F. C. Schroeder, *Angew. Chem., Int. Ed.*, 2012, **51**, 12438–12443.
19 A. B. Artyukhin, J. J. Yim, J. Srinivasan, Y. Izrayelit, N. Bose, S. H. von Reuss, Y. Jo, J. M. Jordan, L. R. Baugh,

M. Cheong, P. W. Sternberg, L. Avery and F. C. Schroeder, *J. Biol. Chem.*, 2013, **288**, 18778–18783.
20 Y. Izrayelit, J. Srinivasan, S. L. Campbell, Y. Jo, S. H. von Reuss, M. C. Genoff, P. W. Sternberg and F. C. Schroeder, *ACS Chem. Biol.*, 2012, **7**, 1321–1325.
21 C. Dong, F. Dolke and S. H. von Reuss, *Org. Biomol. Chem.*, 2016, **14**, 7217–7225.
22 C. D. Chute and J. Srinivasan, *Semin. Cell Dev. Biol.*, 2014, **33**, 18–24.
23 S. H. von Reuss and F. C. Schroeder, *Nat. Prod. Rep.*, 2015, **32**, 994–1006.
24 R. A. Butcher, *Nat. Prod. Rep.*, 2017, **34**, 472–477.
25 G. B. Jagdale, S. Kamoun and P. S. Grewal, *Biol. Control*, 2009, **51**, 102–109.
26 P. Manosalva, M. Manohar, R. S. H. von, R. J. Micikas, F. C. Schroeder, D. F. Klessig, S. Chen, A. Koch, K.-H. Kogel, F. Kaplan, A. Choe, P. W. Sternberg, X. Wang and V. M. Williamson, *Nat. Commun.*, 2015, **6**, 7795.
27 M. Manohar, F. Tenjo-Castano, S. Chen, Y. K. Zhang, A. Kumari, V. M. Williamson, X. Wang, D. F. Klessig and F. C. Schroeder, *Nat. Commun.*, 2020, **11**, 208–218.
28 O. J. Varela, A. Fernandez Cirelli and R. M. De Lederkremer, *Carbohydr. Res.*, 1979, **70**, 27–35.
29 M. Jung, Y. Lee, H.-I. Moon, Y. Jung, H. Jung and M. Oh, *Eur. J. Med. Chem.*, 2009, **44**, 3120–3129.
30 K. A. Hollister, E. S. Conner, X. Zhang, M. Spell, G. M. Bernard, P. Patel, A. C. G. V. de Carvalho, R. A. Butcher and J. R. Ragains, *Bioorg. Med. Chem.*, 2013, **21**, 5754–5769.
31 R. Martin, T. Schäfer, G. Theumer, E. V. Entchev, T. V. Kurzchalia and H.-J. Knölker, *Synthesis*, 2009, 3488–3492.
32 Y. K. Zhang, M. A. Sanchez-Ayala, P. W. Sternberg, J. Srinivasan and F. C. Schroeder, *Org. Lett.*, 2017, **19**, 2837–2840.
33 H. Guo and G. A. O'Doherty, *Org. Lett.*, 2005, **7**, 3921–3924.
34 H. Guo, J. J. La Clair, E. P. Masler, G. A. O'Doherty and Y. Xing, *Tetrahedron*, 2016, **72**, 2280–2286.
35 B. Mukhopadhyay and R. A. Field, *Carbohydr. Res.*, 2003, **338**, 2149–2152.
36 S. C. Dolan and J. Macmillan, *J. Chem. Soc., Chem. Commun.*, 1985, 1588–1589.
37 C.-A. Tai, S. S. Kulkarni and S.-C. Hung, *J. Org. Chem.*, 2003, **68**, 8719–8722.
38 A. K. Ghosh, G. Gong, V. Grum-Tokars, D. C. Mulhearn, S. C. Baker, M. Coughlin, B. S. Prabhakar, K. Sleeman, M. E. Johnson and A. D. Mesecar, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5684–5688.
39 A. Robert-Seilaniantz, M. Grant and J. D. G. Jones, *Annu. Rev. Phytopathol.*, 2011, **49**, 317–343.
40 S. Lehmann, M. Serrano, F. L'Haridon, S. E. Tjamos and J.-P. Metraux, *Phytochemistry*, 2015, **112**, 54–62.
41 C. A. Voigt, *Front. Plant Sci.*, 2014, **5**, 168.
42 A. Averyanov, *Front. Biosci., Elite Ed.*, 2009, **E1**, 142–152.
43 C. Wasternack and B. Hause, *Ann. Bot.*, 2013, **111**, 1021–1058.
44 K. Nahar, T. Kyndt, D. De Vleesschauwer, M. Höfte and G. Gheysen, *Plant Physiol.*, 2011, **157**, 305–316.