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Synthesis of Caeliferins, Elicitors of Plant Immune Responses: Accessing Lipophilic Natural Products via Cross Metathesis

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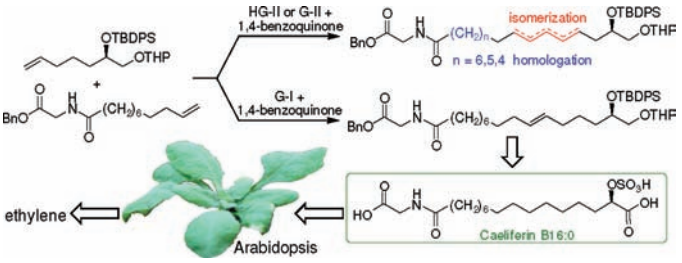
ABSTRACT



A cross metathesis (CM)-based synthesis of the caeliferins, a family of sulfooxy fatty acids that elicit plant immune responses, is reported. Unexpectedly, detailed NMR spectroscopic and mass spectrometric analyses of CM reaction mixtures revealed extensive isomerization and homologation of starting materials and products. It is shown that the degree of isomerization and homologation in CM strongly correlates with substrate chain length and lipophilicity. Side-product suppression requires appropriate catalyst selection and use of 1,4-benzoquinone as a hydride scavenger.

The caeliferins (**1**–**4**) are a family of insect-derived small molecule signals that play an important role in plant defenses. Originally isolated from grasshopper saliva, these molecules elicit immune responses in corn and *Arabidopsis* that attract natural predators of the feeding herbivores.[1]

Despite their interesting role in insect–plant interactions, a lack of synthetic caeliferin samples has prevented further study of their mode of action, including the identification of specific receptors in plants that activate immune responses. We envisioned that a cross metathesis (CM)-based approach would provide us with the flexibility to create all known caeliferins **1**–**4** from chiral synthon **9** via variation of its long-chained metathesis partners, **7** and **8** (Scheme 1). Using a large excess of inexpensive **7** or **8** in the metathesis reaction should allow suppression of competing dimerization of **9**.

The enantiomers of **9** were obtained from THP-protected glycidol via a Cu(I) catalyzed Grignard reaction with 4-bromo-1-butene (Scheme 2).[2] Alkenes **7** and **8** were each prepared in one step from commercially available precursors.[3]

Next we investigated conditions for CM of **9** with **7** or **8**.[4] Strikingly, CM on substrates **9** and **8** using Grubbs first generation (G-I), Grubbs second generation (G-II), or Hoveyda–Grubbs second generation (HG-II) catalysts resulted in complex product mixtures, containing only

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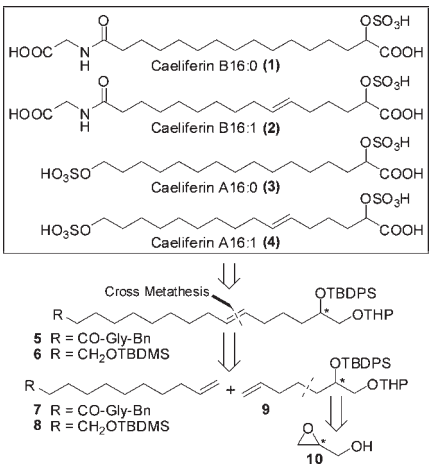
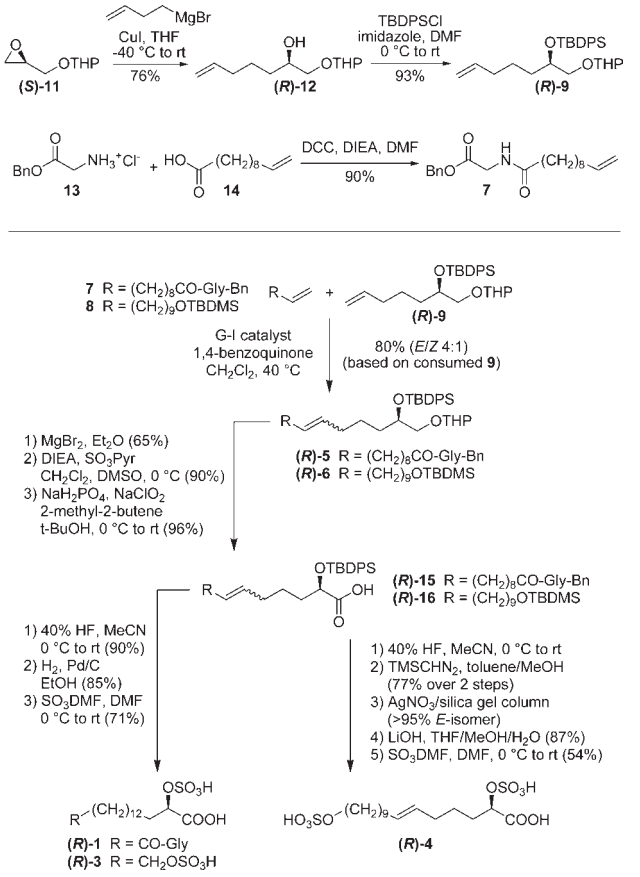
Scheme 1. Caeliferin Structures and Retrosynthesis



Scheme 2. Synthesis of Caeliferins **1**, **3**, and **4**



20–50% of desired **6** in addition to significant quantities of side products. ESI$^+$-MS analysis of CM reactions revealed CH_2 insertions and deletions for substrate **9** as well as CH_2 deletions for product **6** (Figures 1, S3). Similar results were obtained in the CM of **9** with **7** (Figure S4). Using the more active G-II or HG-II catalysts, significant amounts of chain shortened starting materials and products were already observed after 2 h, at which time less than 40% of starting material **9** had been consumed. Previous studies have shown that isomerization and CH_2 insertion and deletion during metathesis reactions are likely the result of ruthenium hydride formation,[5,6] which can be suppressed by the addition of hydride scavengers, e.g., 1,4-benzoquinone (BQ).[7] We found that, even with the addition of BQ, the use of either G-II or HG-II resulted in the formation of significant quantities of side products (Figures S3, S4).

Our ESI$^+$-MS-based analyses revealed significant homologation; however, the extent of CM-induced product and starting material isomerization remained unclear. Building on recent experience using 2D NMR spectroscopy for the analysis of mixtures,[8] we used high-resolution dqfCOSY spectra to further characterize CM reaction outcomes. To simplify NMR spectroscopic analysis, we isolated the mixture of residual **9** as well as its isomers and homologues from G-II-catalyzed CM of **8** and **9** and subsequently removed the stereogenic THP group, producing a corresponding mixture of **17** and derivatives (Figure 2). dqfCOSY spectra[8] of this mixture enabled identification

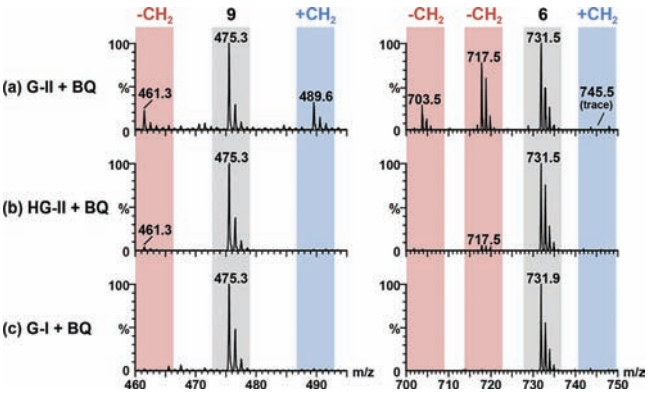


Figure 1. ESI$^+$-MS analysis of CM reaction mixtures showing [M+Na$^+$] for starting material **9** and product **6**. Ion signals corresponding to chain shortened and chain extended homologues are shown in red and blue, respectively. Conditions: (a) G-II (0.05 equiv), BQ (0.1 equiv), 40 °C, 20 h; (b) HG-II (0.05 equiv), BQ (0.1 equiv), 40 °C, 20 h; (c) G-I (0.05 equiv), BQ (0.1 equiv), 40 °C, 20 h.

of all significant isomerization and homologation products (**17a–i**), revealing extensive double-bond isomerization and confirming CH_2 deletions in **9** during CM, which

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explains the formation of nor-homologues of metathesis product **6**. Analysis of the dqfCOSY spectra further revealed large quantities of the chain-extended compounds **17d** and **17e**. However, only trace amounts of the chain-extended terminal alkene **17c** were found. This is consistent with the hypothesis that CH_2-extended variants of **9** form mostly via ethylidene transfer during CM of isomerized starting materials, without significant contribution from isomerized product **6** (isomerization and homologation pathways are shown in Figure S2). The low abundance of the CH_2-extended terminal alkene **17c** also explains that only trace amounts of CH_2 insertion products of **6** were observed (m/z 745.5 in Figure 1), as their formation in the absence of chain-extended terminal alkenes would require multiple isomerization and metathesis steps (see Figure S2). In contrast to G-II and HG-II, use of the G-I catalyst in the presence of BQ did not result in homologation (Figure 1c) or isomerization (as confirmed by dqfCOSY), even when using as much as 0.1 equiv of catalyst and reaction times of up to 48 h. Using the G-I catalyst, **6** was obtained in 80% yield (E/Z = 4:1), based on consumed **9**.

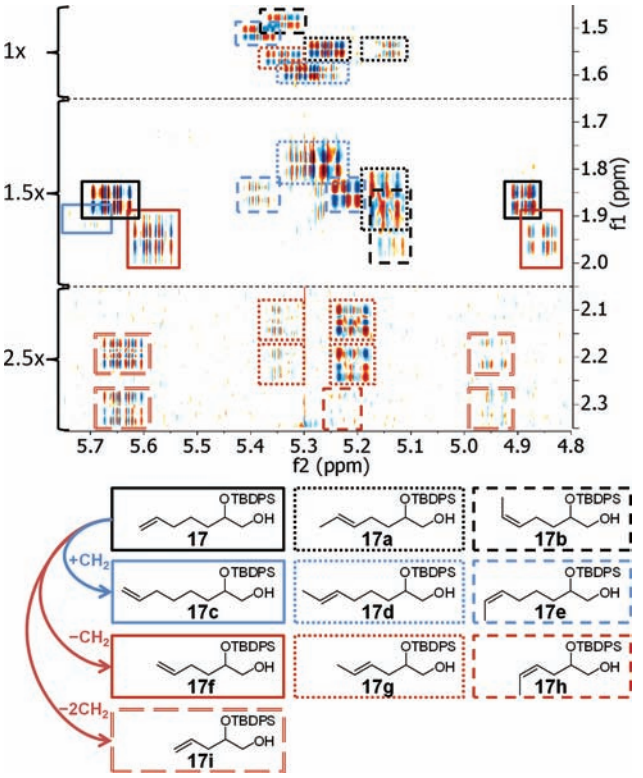

Figure 2. Section of dqfCOSY spectrum of the mixture of **17** and its isomers and homologues, as derived from the corresponding mixture of isomers and homologues of **9** isolated from CM of **8** with **9** using G-II and BQ (600 MHz, $CDCl_3$, see Figure S1 for full spectrum). Intensity of parts of the spectrum was scaled (1.5x, 2.5x).

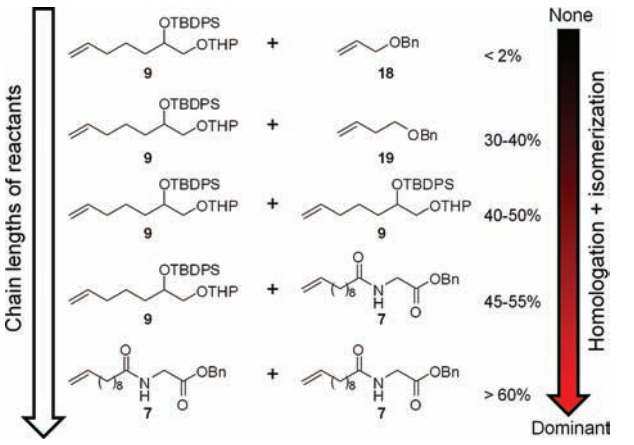

Figure 3. Amounts of detected homologation and isomerization products correlate with chain lengths of CM reactants. Percentages refer to amounts of homologated products formed in G-II-catalyzed CM without BQ, as determined by ESI^+-MS (see Supporting Information for conditions). Additional side product formation occurs due to product isomerization.

Given that both G-II and HG-II have been employed successfully in many CM reactions,[9] we investigated whether the degree of homologation and isomerization depends on specific properties of the reactants **7**–**9** (Figure 3). Using the G-II catalyst without BQ, we found that CM of **9** with allyl benzyl ether proceeded cleanly without formation of isomerized products or substrates, whereas CM of **9** and homoallyl benzyl ether and homodimerization of **9** consistently yielded significant amounts of isomerized and homologated products (Figure S6). Even larger quantities of isomerized and homologized products were obtained in G-II-catalyzed homodimerization reactions of **7** and **8** (Figures S7, S8), similar to amounts of side products produced in G-II-catalyzed CM reactions of **9** with **7** or **8** (Figures S3, S4). Thus it appears that the severity of isomerization in our metathesis reactions generally correlates with the chain lengths of the reaction partners and products (Figures 3, S3, S4, S6–S8). That long-chain, lipophilic substrates are particularly susceptible to homologation and isomerization during CM is also suggested by recent acyclic diene metathesis (ADMET) studies.[6,10] Avoiding homologation and isomerization is of importance for both ADMET and natural product synthesis, as physical or biological properties may be affected greatly by small amounts of structurally similar impurities. Our results show that among tested catalysts, G-I with added BQ is most suitable for CM involving long-chain substrates.

To complete the synthesis of the caeliferins, CM products **5** and **6** were deprotected[11] and converted into acids **15** and **16** via sequential Swern and Pinnick oxidation.[12]

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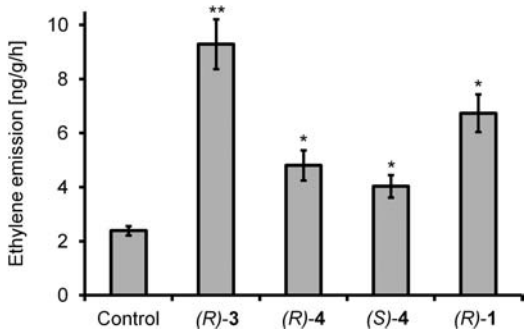

Figure 4. Ethylene emission of *Arabidopsis* seedlings after treatment with synthetic caeliferin solutions; errors bars, s.d.; two-tailed Student's *t* test; $*P < 0.05$, $**P < 0.005$.

Desilylation followed by hydrogenation and sulfation produced **1** and **3**. For the preparation of pure (*E*)-**4**, desilylated **16** was methylated and freed from the contaminating (*Z*)-isomer using $AgNO_3$-impregnated silica gel.[13] Samples of synthetic caeliferins were tested in *Arabidopsis*[1] for their activity to elicit immune responses and thus mimic the effects of a feeding herbivore's saliva. When delivered at concentrations corresponding to those found in grasshopper saliva,[1] the tested caeliferins strongly induced ethylene production (Figure 4), revealing differences in the relative potency of different compounds.[1] The ethylene-inducing activity of synthetic caeliferin A16:0 (**3**) is similar to that previously reported.[1]

In summary, we report the first enantioselective syntheses of (*R*)-**1**, (*R*)-**3**, (*R*)-**4**, and (*S*)-**4**, using CM conditions suitable for long-chained substrates. Our CM-based caeliferin synthesis is flexible and provides access to related compounds, which will enable studies aimed at identifying molecular targets of these elicitors of plant immune responses. A better understanding of the caeliferins' biological mode of action may facilitate the development of sustainable pesticides that take advantage of natural plant defense responses.

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Supporting Information Available. Supporting figures, experimental procedures, and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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