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Synthesis of (+)-crocacin D and simplified bioactive analogues

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ABSTRACT

The total synthesis of (+)-crocacin D has been achieved in 15 steps (9 isolated intermediates) and 14% overall yield from commercially available starting materials and using (+)-crocacin C as a key intermediate. A number of simplified analogues and their biological activities are also reported.

1. Introduction

The crocacins are a family of four antifungal and highly cytotoxic metabolites extracted from myxobacteria of the genus *Chondromyces*. Crocacins A **1**, B **2** and C **3** were isolated from the acetone extract of wet cell mass of *C. crocatus*. Crocacin D **4** was obtained from shaken cultures of *C. pediculatus*, strain Cm p17.[1]

Structurally, crocacins A, B and D are unusual dipeptides consisting of glycine and a 6-aminohexenoic or -hexadienoic acid, *N*-substituted by a complex polyketide-derived acyl residue. Crocacin C on the other hand, is the primary amide (Fig. 1).

Biologically, the crocacins display fungicidal activity against a number of important plant pathogens including *Blumeria graminis*, *Mycosphaerella graminicola*, *Phytophthora infestans*, *Plasmopara viticola*, *Puccinia triticina*, *Puccinia recondita* and *Septoria nodorum*. Significantly, in all cases, (+)-crocacin D **4** proved to be the most active and promising compound of the family, while (+)-crocacin C **3** was essentially inactive. In 2008, Crowley et al. showed that the potent activity of the crocacins was due to the inhibition of the electron flow within the cytochrome bc1 segment (complex III) of the respiratory chain.[2–4]

The biological data may point to a hypothesis that the (*Z*)-enamide moiety, present in the lateral chain of the crocacins is responsible for the observed biological activity. Speculative proposals on the mechanistic mode of action of enamides have been made suggesting that protonation occurs at the enamide moiety, followed by nucleophilic attack onto the resulting *N*-acyliminium ion.[5]

The total synthesis of (+)-crocacin D **4** was first achieved by Rizzacasa and co-workers through the use of isocyanate chemistry to generate the key enamide unit. Rizzacasa's breakthrough synthesis was completed in 2% yield and in 18 steps in the longest linear sequence.[6]

In the same year, Chakraborty and Laxman reported the total synthesis of (+)-crocacin D **4**, taking advantage of a one-pot desilylation/Peterson elimination to install the *Z*-enamide. Chakraborty's total synthesis of (+)-crocacin D **4** was achieved in 24 steps and 1% overall yield.[7]

Dias later reported a convergent approach to the total synthesis of (+)-crocacin D **4**, in which the enamide unit was introduced via Buchwald's copper-catalysed coupling of amides with vinyl halides. Dias was able to complete the total synthesis of (+)-crocacin D **4** in 6% overall yield and 17 steps in the longest linear sequence.[8]

As part of our efforts into the synthesis of the crocacins, we have recently reported the total synthesis of (+)-crocacin C **3**. The synthetic route developed is able to generate significant amounts of (+)-crocacin C **3** quickly and efficiently.[9] We now report the total synthesis of (+)-crocacin D **4** as well as a number of simplified analogues.

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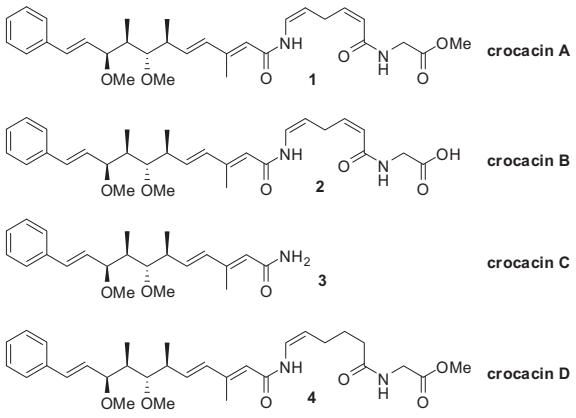


Figure 1. Crocacins A–D.

2. Results and discussion

As part of our convergent synthetic approach to (+)-crocacin D, in our first disconnection we envisioned (+)-crocacin D **4** directly originating from (+)-crocacin C **3** through the ruthenium-catalysed *anti*-Markovnikov hydroamidation of terminal alkynes reported by Gooβen et al.[10]

The Gooβen hydroamidation is an atom-economic process in which the double bond geometry of the product depends on the nature of the catalyst's ligands.[11] Importantly, it opened the possibility of generating (+)-crocacin D **4** as well as novel (+)–(*E*)-crocacin D **5** analogues in a stereocontrolled fashion from (+)-crocacin C **3** and terminal alkyne **6** (Scheme 1).

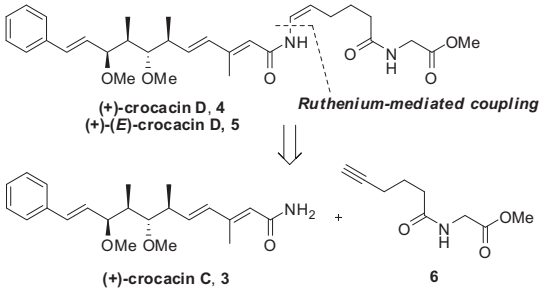
The alkyne building block that would be the coupling partner with crocacin C, **6**, was accessed in excellent yield in a straightforward manner by the HBTU-mediated peptide coupling between carboxylic acid **7** and glycine methyl ester **8** (Scheme 2).

Unfortunately, despite extensive experimentation, alkyne **6** failed to react either with (+)-crocacin C **3** (Scheme 3) or with simpler model amides (not shown). It is possible that the presence of the amide moiety proximal to the alkyne unit hindered any catalytic activity.

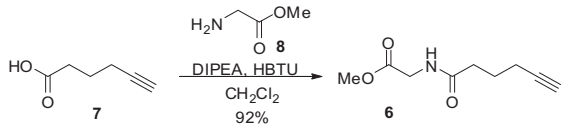
Rather than pursuing a protection-deprotection strategy, a different endgame approach was then pursued for the completion of (+)-crocacin D **4**. In order to ensure as much convergence as possible, the revised synthetic strategy envisioned (+)-crocacin D **4** as being directly derived from (+)-crocacin C **3** via a Buchwald copper(I)-mediated coupling with the (*Z*)-vinyl iodide **9** in an approach analogous to that of Dias (Scheme 4).[12,7]

In our revised approach, alkyne **6** was quantitatively converted to iodo-alkyne **10**. Hydroboration-protonolysis of iodo-alkyne **10** completed the synthesis of the iodo-alkene unit **9**. Thus, the (+)-crocacin D lateral chain was completed in 3 steps and in 83% overall yield (Scheme 5).

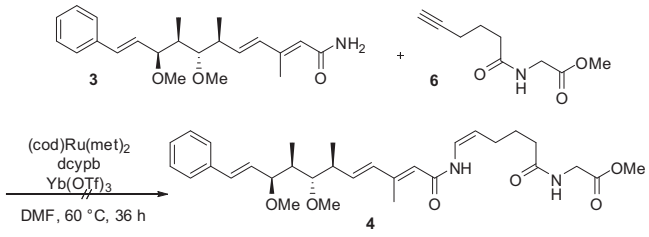
With (+)-crocacin C **3** and (*Z*)-iodo-olefin **9** in hand, the copper-mediated coupling was explored. Gratifyingly, the reaction directly afforded (+)-crocacin D **4** in good semi-crude yield (70%) as determined by ^{1}H NMR, IR and MS analysis. Unfortunately (+)-crocacin D **4**, due to its inherent instability, decomposed during HPLC purification. This total synthesis of (+)-crocacin D (15 steps with 9 isolated intermediates on the longest linear sequence) is the shortest synthesis reported to date from commercially available starting materials.



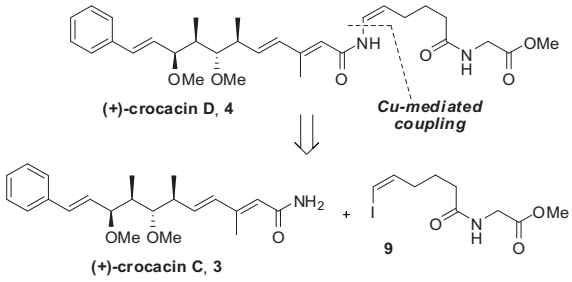
Scheme 1.



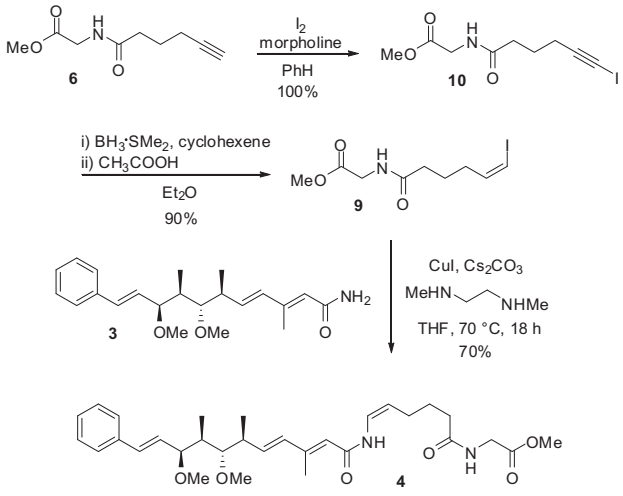
Scheme 2.



Scheme 3.

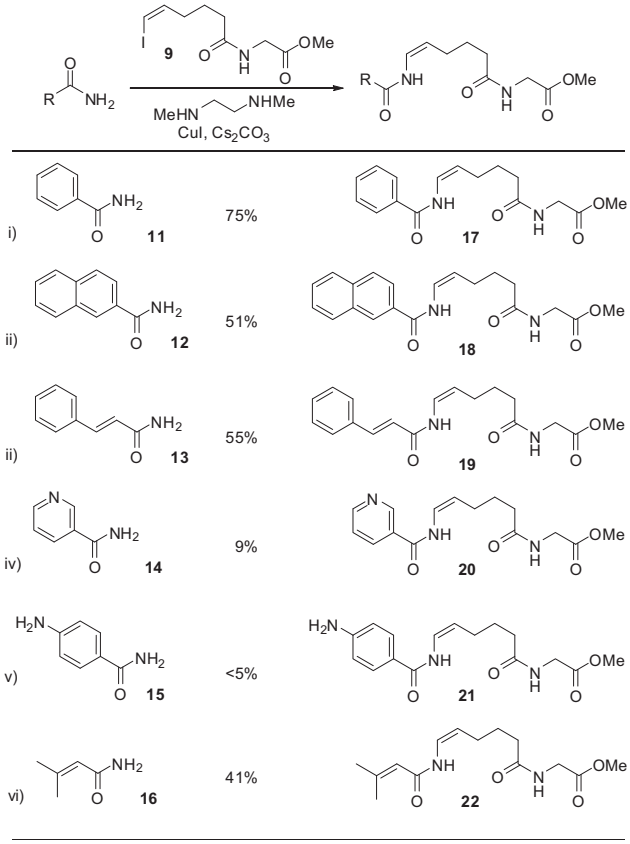


Scheme 4.



Scheme 5.

Table 1
Simplified crocacin D analogues

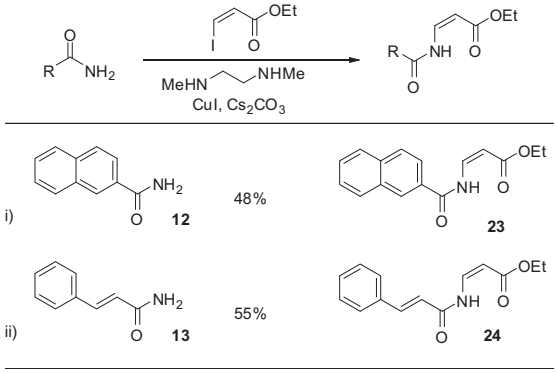


The decomposition of (+)-crocacin D **4** during purification is consistent with the instability that has hampered its development in the agrochemical industry. In simulated sunlight tests, (+)-crocacin D **4** showed very poor photo-stability, with 50% decomposition within 37 min.[2]

Given the success of the copper-mediated coupling for the synthesis of (+)-crocacin D **4**, it was reasoned to use the same strategy for the preparation of a focused library of unnatural analogues of (+)-crocacin D **4**. Since it is the lateral chain containing the (*Z*)-enamide moiety that is believed to be responsible for the biological activity of the crocacins, our rationale was that this should remain unaltered, while the complex and lipophilic western portion was simplified.

Following our hypothesis, a number of simplified (+)-crocacin D analogues were generated incorporating various curtailed lipophilic cores in good yield (Table 1, entries i-iii). Nicotinamide **14** and 4-aminobenzamide **15**, proved to be poorly reactive under the copper-mediated coupling conditions with the custom lateral chain (entries iv and v).

Table 2
Conjugated crocacin D analogues



The smaller non-aromatic 3-methylbut-2-enamide **16** was also considered as an interesting framework (entry vi). Coupling of amide **16** to the lateral chain effectively generated the entire C1–C14 fragment of crocacin D, and would test the need for the C15–C27 polypropionate fragment. The Cu(I) mediated coupling afforded the desired enamide analogue **22** in a moderate 41% yield. However, as seen with crocacin D, the product suffered from significant instability, leading to decomposition during characterisation. This result suggests that the C1–C14 fragment of (+)-crocacin D, even if not strictly required for the biological activity of the molecule, does not exhibit the required characteristics of stability necessary to be considered an analogue of interest.

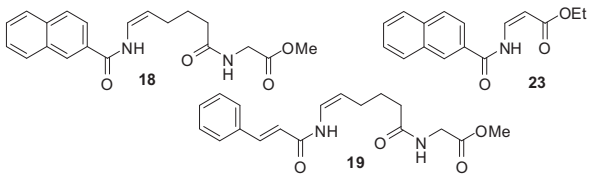
Finally, two further-simplified analogues based on the naphthyl- and *trans*-cinnamyl-frameworks were prepared in which the (*Z*)-enamide unit was shortened significantly to just an ethyl ester functionality (Table 2).

In summary, 9 unnatural analogues of (+)-crocacin D were synthesised. Among these, 5 compounds (analogues **17**, **18**, **19**, **23**, **24**) were stable enough to be evaluated at the Syngenta Discovery Early Screen (DES) platform.

In all the biological assays, none of the analogues showed activity as weed control agents when tested for activity against *Arabidopsis thaliana* and *Poa annua*.

In fungicidal assays, naphthyl derivative **18**, showed moderate activity against *Uromyces viciae-fabae* at 100 ppm scored using a 3 band system (0, 55 and 99 where 99 is total inhibition of hyphal growth/disease development, 55 is partial inhibition and 0 is no inhibition). Enamide **19** showed partial inhibition against *Uromyces viciae-fabae* and total inhibition of *Phytophthora infestans* at 60 and 100 ppm, respectively (Table 3).

Table 3
Biological screening results



Compd	*Uromyces viciae-fabae* (60 ppm)	*Phytophthora infestans* (100 ppm)	*Caenorhabditis elegans* (10 ppm)
18	55, 55, 55	0, 0, 0	0, 0, 0
19	0, 55, 27	99, 0, 49	0, 0, 0
23	0, 0, 0	0, 0, 0	99, 99, 99

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In insecticidal assays napthyl compound **23** proved to be highly active against the nematode species *Caenorhabditis elegans* at 10 ppm using a 2 band system (0 or 99 where 99 is significant mortality and 0 is no significant effect).

These results further corroborate the proposal that the enamide unit is responsible for the biological activity of the crocacin family of compounds, and of the lipophilic role of the crocacin C framework. Enamide **23** also demonstrates that it is possible to achieve biological selectivity by fine-tuning the enamide substituents.

In conclusion, the total synthesis of the bioactive (+)-natural product crocacin D **4** has been completed in 15 steps (9 isolated intermediates) and 14% overall yield. The synthesis hinges on a Buchwald copper-mediated coupling between crocacin C **3** and vinyl iodide **9** to introduce the desired enamide functionality, and is the shortest synthesis of (+)-crocacin D **4** reported to date. A number of unnatural analogues of (+)-crocacin D with interesting biological activities have also been identified, and are being currently investigated as leads in crop protection.

3. Experimental

All reactions were performed in oven-dried glassware under an inert argon atmosphere unless otherwise stated. Tetrahydrofuran (THF), diethyl ether, and dichloromethane (DCM) were purified through a Pure Solv 400-5MD solvent purification system (Innovative Technology, Inc). All reagents were used as received, unless otherwise stated. Solvents were evaporated under reduced pressure at 40 °C.

IR spectra were recorded using a JASCO FT/IR410 Fourier Transform spectrometer using a diamond gate. Only significant absorptions (ν_{max}) are reported in wavenumbers (cm^{-1}).

Proton magnetic resonance spectra (^1H NMR) were recorded either at 400 MHz or 500 MHz using either a Bruker DPX Avance400 instrument or a Bruker DPX Avance500 instrument. Carbon magnetic resonance spectra (^{13}C NMR) were recorded either at 100 MHz or 125 MHz using either a Bruker DPX Avance400 instrument or a Bruker DPX Avance500 instrument. Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to the residual solvent peak. The order of citation in parentheses is (1) number of equivalent nuclei (by integration), (2) multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, sept = septet, m = multiplet, b = broad), (3) and coupling constant (J) quoted in Hertz to the nearest 0.1 Hz. High resolution mass spectra were recorded on a JEOL JMS-700 spectrometer by electrospray (ESI) chemical ionisation (CI) mass spectrometry operating at a resolution of 15,000 full widths at half height.

Flash chromatography was performed using silica gel (Merck Silica Gel 60, 40–63 micron) as the stationary phase. TLC was performed on aluminium sheets pre-coated with silica (Merck Silica Gel 60 F_{254}). The plates were visualised by the quenching of UV fluorescence (λ_{max} 254 nm) and/or by staining with anisaldehyde, potassium permanganate, iodine or cerium ammonium molybdate followed by heating.

3.1. Methyl 2-hex-5-ynamidoacetate, 6

A solution of hex-5-ynoic acid **7** (1.0 g, 8.9 mmol) and glycine methyl ester chlorohydrate (1.35 g, 10.7 mmol) in dichloromethane (25 mL) under argon was treated with HBTU (4.07 g, 10.7 mmol) followed by the dropwise addition of DIPEA (3.9 mL, 22.3 mmol). The reaction mixture was stirred at rt for 16 h, quenched with sat'd aq NH_4Cl (25 mL) and extracted with diethyl-ether (3 × 25 mL). The combined organic phases were collected, dried over Na_2SO_4, filtered and concentrated under vacuum to afford the crude product as a yellow oil. The crude product was purified by flash column chromatography (silica gel, EtOAc/PE elution gradient 0:1 to 3:7) to afford 1.5 g (92%) of the desired alkyne **6** as a colourless oil. R_f 0.55 (EtOAc); ^1H NMR (500 MHz, $CDCl_3$): δ 6.37 (1H, br s), 3.97 (2H, d, J = 5.2 Hz), 3.69 (3H, s), 2.34 (2H, t, J = 7.3 Hz), 2.21 (2H, td, J = 6.8 Hz, 2.6 Hz), 1.94 (1H, t, J = 2.7 Hz), 1.81 (2H, qn, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.7, 170.5, 83.5, 69.2, 52.3, 41.2, 34.6, 24.1, 17.8; ν_{max} (film) 3284, 2954, 1743, 1652, 1539, 1436, 1409, 1370, 1205, 1181, 1038, 1010, 982, 639 cm^{-1}; HRMS (CI+/ISO) calcd for $C_9H_{13}NO_3$ $[M]^+$: 183.0895. Found: 183.0894.

3.2. Methyl 2-(6-iodohex-5*Z*-enamido)acetate, 9

A 0 °C solution of $BH_3{\cdot}SMe_2$ (160 μL, 0.32 mmol) in diethyl ether (5 mL) was treated dropwise with cyclohexene (70 μL, 0.64 mmol) and the resulting mixture was allowed to warm up to room temperature and was stirred for 1 h. The resulting white suspension was cooled back down to 0 °C and a solution of methyl 2-(6-iodohex-5-ynamido)acetate **10** (100 mg, 0.32 mmol) in dry diethyl ether (3 mL) was added dropwise. The reaction mixture was then allowed to warm up to room temperature and to stir for 1 h. The reaction mixture was cooled once again to 0 °C, and it was quenched with glacial acetic acid (0.2 mL). The mixture was stirred at room temperature for 2 h, washed with H_2O (10 mL), dried over Na_2SO_4, filtered and concentrated under vacuum to afford the crude product as an orange oil. The crude product was purified by flash column chromatography (silica gel, elution gradient EtOAc/hexane, 0:1 to 4:6) to afford 91 mg (90%) of the desired vinyl iodide **9** as a colourless oil. R_f 0.21 (PE/EtOAc, 1:1); ^1H NMR (500 MHz, $CDCl_3$): δ 6.25 (1H, dt, J = 7.4, 1.2 Hz,), 6.16 (1H, q, J = 7.0 Hz), 5.98 (1H, br s), 4.06 (2H, d, J = 4.4 Hz), 3.77 (3H, s), 2.28 (2H, t, J = 7.6 Hz), 2.21 (2H, qd, J = 7.3, 1.0 Hz,), 1.81 (2H, qn, J = 7.4 Hz); ^1H NMR (500 MHz, C_6D_6): δ 5.85 (1H, dt, J = 7.3, 1.3 Hz), 5.63 (1H, q, J = 6.9 Hz), 5.11 (1H, br s), 3.73 (2H, d, J = 5.0 Hz), 3.20 (3H, s), 1.97 (2H, qd, J = 7.3, 1.3 Hz), 1.69 (2H, t, J = 7.6 Hz), 1.60–1.54 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.7, 170.6, 140.4, 83.5, 52.5, 41.4, 35.5, 34.2, 23.8; ν_{max} (film) 3287, 3082, 2949, 1748, 1649, 1537, 1456, 1437, 1406, 1370, 1288, 1269, 1202, 1179, 1152, 1036, 982, 692 cm^{-1}; HRMS (CI+/ISO) calcd for $C_9H_{15}INO_3$ $[M+H]^+$: 312.0097. Found: 312.0099.

3.3. Methyl 2-(6-iodohex-5-ynamido)acetate, 10

A solution of methyl 2-hex-5-ynamidoacetate **6** (0.10 g, 0.54 mmol) and iodine (208 mg, 0.82 mmol) in benzene (5 mL) under argon was treated dropwise with morpholine (0.24 mL, 2.7 mmol). The reaction was then stirred at 45 °C for 12 h, before being cooled down to room temperature. The mixture was filtered, and extracted with diethyl ether (3 × 5 mL). The combined organic phases were collected, washed with brine (10 mL), satd aq $NaHCO_3$ (10 mL), satd aq $Na_2S_2O_3$ (10 mL), dried over Na_2SO_4, filtered and concentrated under vacuum to afford the crude product as a yellow thick oil in a quantitative yield (0.169 g, 0.54 mmol). The crude product was carried through without any further purification. R_f 0.32 (PE/EtOAc, 1:1); ^1H NMR (500 MHz, $CDCl_3$): δ 5.91 (1H, br s), 4.05 (2H, d, J = 5.4 Hz), 3.77 (3H, s), 2.47 (2H, t, J = 7.0 Hz), 2.37 (2H, t, J = 7.0 Hz), 1.88 (2H, qn, J = 7.0 Hz).

3.4. Methyl 2-((*Z*)-6-((2*E*,4*E*,6*S*,7*S*,8*R*,9*S*,10*E*)-7,9-dimethoxy-3,6,8-trimethyl-11-phenylundeca-2,4,10-trienamido)hex-5-enamido)acetate, (+)-crocacin D, 4

A suspension of (+)-crocacin C **3** (8.50 mg, 24 μmol), cesium carbonate (7.40 mg, 23 μmol), CuI (1.00 mg, 5 μmol) and *N*,*N*′-methylenediamine (1 μL, 9 μmol) in degassed and dry THF

(0.5 mL) in a 2.0–5.0 mL microwave vial, was treated dropwise with a solution of (*Z*)-methyl 2-(6-iodohex-5-enamido)acetate **9** (6.60 mg, 21 μmol) in anhydrous, degassed THF (0.5 mL). The resulting reaction mixture was stirred at 70 °C for 24 h. The resulting blue-purple suspension was diluted with ethyl acetate (1 mL) and filtered through a short pad of silica gel (previously deactivated with Et_3N) using EtOAc (10 mL) as eluent. The filtrate was then concentrated under vacuum to afford a crude green-yellow oil. The crude oil was purified by flash column chromatography on silica gel previously deactivated with triethylamine (EtOAc/DCM/Et_3N, 2.9:7.0:0.1) to afford semi-crude (+)-crocacin D **4** as a yellow gum in 70% yield (8.00 mg, 15 μmol). R_f 0.38 (EtOAc/PE/Et_3N, 4.9:5.0:0.1); ^{1}H NMR (500 MHz, acetone-d_6): δ_H 9.21 (1H, bd, J = 10.5 Hz), 7.61–7.52 (1H, m), 7.51 (2H, d, J = 7.3 Hz), 7.35 (2H, dd, J = 7.4, 7.4 Hz), 7.27 (1H, dd, J = 7.4, 7.4 Hz), 6.80 (1H, dd, J = 10.4, 9.0 Hz), 6.62 (1H, d, J = 16.0 Hz), 6.29 (1H, dd, J = 16.1, 7.3 Hz), 6.15–6.10 (2H, m), 5.95 (1H, d, J = 1.0 Hz), 4.71 (1H, dt, J = 9.0, 7.3 Hz), 4.12 (1H, dd, J = 7.4, 1.6 Hz), 3.98 (2H, d, J = 6.0 Hz), 3.71 (3H, s), 3.56 (3H, s), 3.30 (3H, s), 3.22 (1H, dd, J = 9.5, 2.4 Hz), 2.65–2.57 (1H, m), 2.31 (3H, d, J = 1.0 Hz), 2.29 (2H, t, J = 7.1 Hz), 2.11 (2H, dt, J = 7.2, 6.8 Hz), 1.67 (2H, tt, J = 7.2, 6.8 Hz), 1.56–1.55 (1H, m), 1.22 (3H, d, J = 6.8 Hz), 0.91 (3H, d, J = 7.1 Hz); ν_{max} (film) 3302, 2924, 1744, 1653, 1603, 1514, 1262, 1089 and 972 cm^{-1}; LRMS (ESI): $[M+H]^+$: 541.8; $[M+Na]^+$: 563.8.

3.5. Methyl 2-(6-benzamidohex-5*Z*-enamido)acetate, 17

A suspension of benzamide **11** (5.3 mg, 44 μmol), cesium carbonate (14 mg, 44 μmol), CuI (1 mg, 5 μmol) and *N*,*N*′-methylenediamine (1 μL, 9 μmol) in anhydrous, degassed THF (0.5 mL) in a 2.0–5.0 mL microwave vial, was treated dropwise with a solution of (*Z*)-methyl 2-(6-iodohex-5-enamido)acetate **9** (10 mg, 38 μmol) in anhydrous degassed THF (0.5 mL). The resulting reaction mixture was stirred at 70 °C for 24 h and then allowed to cool down to rt. The blue-purple suspension was diluted with EtOAc (1 mL), filtered through a short pad of silica (previously deactivated with Et_3N) using EtOAc (10 mL) as eluent. The filtrate was concentrated under vacuum to afford a crude green-yellow oil which was purified by flash column chromatography (silica gel, EtOAc/DCM/Et_3N, 2.9:7.0:0.1) to afford the desired enamide **17** as a white gum in 75% yield (10 mg, 33 μmol). R_f 0.42 (EtOAc/PE/Et_3N, 2.9:7.0:0.1); ^{1}H NMR (500 MHz, acetone-d_6): δ 9.67 (1H, br s), 8.13–8.08 (2H, m), 7.75 (1H, br s), 7.60–7.57 (1H, m), 7.53–7.47 (2H, m), 7.02–6.98 (1H, m), 4.89 (1H, q, J = 8.2 Hz), 4.03 (2H, d, J = 5.8 Hz), 3.66 (3H, s), 2.39–2.29 (4H, m), 1.82–1.75 (2H, qn, J = 6.6 Hz); ^{13}C NMR (125 MHz, acetone-d_6): δ 175.7, 171.8, 166.0, 135.8, 133.1, 129.8, 129.5, 125.2, 112.4, 52.9, 42.4, 34.8, 26.9, 26.1; ν_{max} (film) 3304, 2953, 2923, 2854, 1745, 1646, 1515, 1484, 1457, 1437, 1375, 1281, 1207, 1030 and 708 cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{20}N_2O_4$ $[M]^+$: 304.1423. Found: 304.1417.

3.6. Methyl 2-(6-(2-naphthamido)hex-5*Z*-enamido)acetate, 18

A suspension of 2-naphtamide **12** (30 mg, 175 μmol), cesium carbonate (57 mg, 175 μmol), CuI (1.7 mg, 8 μmol) and *N*,*N*′-methylenediamine (1.8 μL, 16 μmol) in degassed and dry THF (1 mL) in a 2.0–5.0 mL microwave vial, was treated dropwise with a solution of (*Z*)-methyl 2-(6-iodohex-5-enamido)acetate **9** (48 mg, 160 μmol) in degassed and dry THF (2 mL). The reaction mixture was stirred at 70 °C for 18 h and then allowed to cool down to rt. The light-green suspension was diluted with EtOAc (3 mL), filtered through a short pad of silica (previously deactivated with Et_3N) using EtOAc (15 mL) as eluent. The filtrate was concentrated under vacuum to afford a crude green-yellow oil which was purified by flash column chromatography (silica gel, EtOAc/DCM/Et_3N, 2.9:7.0:0.1) to afford the desired enamide **18** as a white gum in 51% yield (29 mg, 82 μmol). R_f 0.46 (EtOAc/PE/Et_3N, 2.9:7.0:0.1); ^{1}H NMR (500 MHz, acetone-d_6): δ 9.90 (1H, bd, J = 7.2 Hz), 8.78 (1H, s), 8.17 (1H, dd, J = 8.6, 1.7 Hz), 8.09 (1H, d, J = 7.7 Hz), 8.02 (2H, d, J = 9.0 Hz), 7.81 (1H, br s), 7.65 (2H, dqn, J = 6.9, 1.5 Hz), 7.07 (1H, t, J = 9.0 Hz), 4.93 (1H, q, J = 8.6 Hz), 4.08 (2H, d, J = 5.5 Hz), 3.59 (3H, s), 2.44–2.34 (4H, m), 1.82 (2H, qn, J = 6.9 Hz); ^{13}C NMR (125 MHz, acetone-d_6): δ 175.8, 171.8, 166.0, 136.6, 134.4, 132.9, 130.7, 129.9, 129.5, 129.3, 129.2, 128.2, 126.3, 125.4, 112.3, 52.8, 42.5, 34.9, 26.9, 26.1; ν_{max} (film) 3300, 3071, 2951, 2928, 2855, 1748, 1647, 1520, 1499, 1437, 1370, 1294, 1204 and 1180 cm^{-1}; HRMS (CI+/ISO) calcd for $C_{20}H_{22}N_2O_4$ $[M]^+$: 354.1580. Found: 354.1581.

3.7. Methyl 2-(6-cinnamamidohex-5*Z*-enamido)acetate, 19

A suspension of cinnamamide **13** (30 mg, 204 μmol), cesium carbonate (66 mg, 204 μmol), CuI (2 mg, 10 μmol) and *N*,*N*′-methylenediamine (2.2 μL, 204 μmol) in degassed and dry THF (1 mL) in a 2.0–5.0 mL microwave vial, was treated dropwise with a solution of (*Z*)-methyl 2-(6-iodohex-5-enamido)acetate **9** (55 mg, 186 μmol) in degassed and dry THF (2 mL). The reaction mixture was stirred at 70 °C for 18 h and then allowed to cool down to rt. The light-green suspension was diluted with EtOAc (3 mL), filtered through a short pad of silica (previously deactivated with Et_3N) using EtOAc (15 mL) as eluent. The filtrate was concentrated under vacuum to afford a crude green-yellow oil which was purified by flash column chromatography (silica gel, EtOAc/DCM/Et_3N, 2.9:7.0:0.1) to afford the desired enamide **19** as a white gum in 55% yield (34 mg, 103 μmol). R_f 0.21 (EtOAc/DCM/Et_3N, 2.9:7.0:0.1); ^{1}H NMR (500 MHz, acetone-d_6): δ 9.59 (1H, bd, J = 9.5 Hz), 7.77 (1H, br s), 7.66 (1H, d, J = 15.9 Hz), 7.65–7.63 (2H, m), 7.49–7.40 (3H, m), 6.91 (1H, t, J = 8.9 Hz), 6.89 (1H, d, J = 15.9 Hz), 4.84 (1H, q, J = 8.6 Hz), 4.07 (2H, d, J = 5.6 Hz), 3.71 (3H, s), 2.36 (2H, t, J = 6.6 Hz), 2.22 (2H, q, J = 6.7 Hz), 1.77 (2H, qn, J = 6.7 Hz); ^{13}C NMR (125 MHz, acetone-d_6): δ 175.6, 171.9, 164.2, 142.1, 137.0, 131.2, 130.5, 129.3, 124.8, 123.1, 111.5, 52.9, 42.5, 35.2, 26.8, 26.2; ν_{max} (film) 3297, 2951, 2361, 1750, 1651, 1520, 1206, 1182, 980, 766 and 682 cm^{-1}; HRMS (CI+/ISO) calcd for $C_{18}H_{22}N_2O_4$ $[M]^+$: 330.1580. Found: 330.1577.

3.8. Ethyl *cis*-3-iodoacrylate

A solution of ethyl propiolate (1.0 mL, 9.8 mmol) in glacial acetic acid (5 mL) was treated with sodium iodide (1.5 g, 10 mmol) and the reaction mixture was warmed up to 70 °C and stirred for 16 h. The reaction was quenched with H_2O (5 mL) followed by aq. NaOH (1 N, 5 mL) and extracted with diethyl ether (3 × 5 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under vacuum to afford the desired iodoacrylate as orange oil in a quantitative yield (2.23 g, 9.8 mmol). The product was used without any further purification. R_f 0.59 (Et_2O/PE, 2.5:7.5); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31 (1H, dd, J = 8.8, 1.0 Hz), 6.74 (1H, dd, J = 8.9, 1.3 Hz), 4.06 (2H, qd, J = 7.2, 1.7 Hz), 1.14 (3H, td, J = 7.2, 1.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0, 129.6, 94.7, 60.4, 13.9; ν_{max} (film) 1721, 1597, 1321, 1192, 1159, 1024 and 804 cm^{-1}; HRMS (ESI) calcd for $C_5H_7IO_2$ $[M]^+$: 225.9491. Found: 225.9494.

3.9. Ethyl 3-(2-naphthamido)-*Z*-acrylate, 23

A suspension of 2-naphtamide **12** (50 mg, 292 μmol), cesium carbonate (95 mg, 292 μmol), CuI (3 mg, 15 μmol) and *N*,*N*′-methylenediamine (3.2 μL, 30 μmol) in degassed and dry THF (2 mL) in a

2.0–5.0 mL microwave vial, was treated dropwise with a solution of (*Z*)-ethyl 3-iodoacrylate (60 mg, 266 μmol) in degassed and dry THF (2 mL). The reaction mixture was stirred at 70 °C for 18 h and then allowed to cool down to rt. The light-green suspension was diluted with EtOAc (4 mL), filtered through a short pad of silica (previously deactivated with Et_3N) using EtOAc (20 mL) as eluent. The filtrate was concentrated under vacuum to afford a crude green-yellow oil which was purified by flash column chromatography (silica gel, elution gradient EtOAc/PE, from 0:10 to 0.5:9.5) to afford the desired enamide **23** as a pale yellow gum in 48% yield (34.3 mg, 128 μmol). R_f 0.16 (EtOAc/PE, 0.4:9.6); ^{1}H NMR (500 MHz, acetone-d_6): δ 11.69 (1H, bd, J = 8.8 Hz), 8.59 (1H, s), 8.20 (1H, d, J = 7.9 Hz), 8.17 (1H, d, J = 8.9 Hz), 8.09 (1H, d, J = 7.6 Hz), 8.03 (1H, dd, J = 8.5, 1.9 Hz), 7.86 (1H, dd, J = 11.1, 8.9 Hz), 7.72 (2H, dqn, J = 7.0 1.6 Hz), 5.38 (1H, d, J = 8.9 Hz), 4.31 (2H, q, J = 7.1 Hz), 1.36 (3H, t, J = 7.1 Hz); ^{13}C NMR (125 MHz, acetone-d_6): δ 170.9, 165.5, 140.5, 140.3, 137.1, 134.4, 131.4, 130.9, 130.7, 130.2, 129.5, 128.9, 125.0, 98.5, 61.7, 15.3; ν_{max} (film) 3327, 1678, 1622, 1487, 1397, 1381 and 1199 cm^{-1}; HRMS (CI+/ISO) calcd for $C_{16}H_{16}O_3N$ [M+H]$^+$: 270.1130. Found: 270.1136.

3.10. Ethyl 3-cinnamamido-*Z*-acrylate, 24

A suspension of cinnamamide **13** (50 mg, 340 μmol), cesium carbonate (111 mg, 340 μmol), CuI (3.1 mg, 16 μmol) and *N*,*N*′-methylenediamine (3.4 μL, 32 μmol) in degassed and dry THF (2 mL) in a 2.0–5.0 mL microwave vial, was treated dropwise with a solution of (*Z*)-ethyl 3-iodoacrylate (70 mg, 310 μmol) in degassed and dry THF (2 mL). The reaction mixture was stirred at 70 °C for 18 h and then allowed to cool down to rt. The light-green suspension was diluted with EtOAc (4 mL), filtered through a short pad of silica (previously deactivated with Et_3N) using EtOAc (20 mL) as eluent. The filtrate was concentrated under vacuum to afford a crude green-yellow oil which was purified by flash column chromatography (silica gel, elution gradient EtOAc/PE, from 0:10 to 0.5:9.5) to afford the desired enamide **24** as a white gum in 55% yield (36 mg, 147 μmol).R_f 0.1 (EtOAc/PE, 0.4:9.6); ^{1}H NMR (500 MHz, acetone-d_6): δ 10.65 (1H, bd, J = 8.3 Hz), 7.83–7.78 (2H, m), 7.79 (1H, d, J = 16.0 Hz), 7.69 (1H, dd, J = 11.4, 9.0 Hz), 7.54–7.46 (3H, m), 7.10 (1H, d, J = 16.0 Hz), 5.24 (1H, d, J = 9.2 Hz), 4.23 (2H, q, J = 6.9 Hz), 1.31 (3H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, acetone-d_6): δ 170.2, 164.9, 145.4, 139.9, 136.4, 131.9, 130.6, 129.9, 121.7, 97.7, 61.4, 15.3; ν_{max} (film) 3333, 2983, 1713, 1676, 1620, 1379, 1260, 1198 and 1138 cm^{-1}; HRMS (CI+/ISO) calcd for $C_{14}H_{16}O_3N$ [M+H]$^+$: 246.1130. Found: 246.1130.

3.11. Biological assessment

In all the biological assays, mycelial growth or disease inhibition was assessed visually and scored using a 3 band system (0, 55 and 99 where 99 is total inhibition of hyphal growth/disease development, 55 is partial inhibition and 0 is no inhibition), 4–14 days after inoculation depending on the assay. Positive control compounds were included in each test as appropriate: Azoxystrobin and/or Prochloraz for fungicide assays, Thiamethoxam and Indoxacarb for the insecticide assays and Norflurazon for herbicide assays.

3.12. Herbicidal activity

In herbicide assays the compounds were tested for activity against *Arabidopsis thaliana* at 10 ppm and *Poa annua* at 32 ppm through pre emergence treatment. Test plates were stored for seven days in a controlled environment cabinet. Scores were given as 0 or 99, where 99 is any herbicidal effect, and 0 is no effect.

Compound	*Arabidopsis thaliana*	*Poa annua*
Analogue **17**	0, 0, 0	0, 0, 0
Analogue **18**	0, 0, 0	0, 0, 0
Analogue **19**	0, 0, 0	0, 0, 0
Analogue **23**	0, 0, 0	0, 0, 0
Analogue **24**	0, 0, 0	0, 0, 0

3.13. Insecticidal activity

In insecticide assays the compounds were tested for activity against the aphid species, *Aphis gossypii*, in a leaf-disc assay at 1000 ppm. The compounds were also evaluated at a rate of 5000 ppm on *Plutella maculipennis* in an artificial diet assay and against the nematode species *Caenorhabditis elegans* in liquid culture at 10 ppm. The analogues were applied to feeding aphids, prior to infestation with *Plutella xylostella* larvae, or diluted into the *C. elegans* culture. Mortality was assessed relative to control wells using a 2 band system (0 or 99 where 99 is significant mortality and 0 is no significant effect), 5–9 days after the treatment depending on the assay. *C. elegans* were also assessed for symptomology.

Compound	*Aphis gossypii*	*Plutella maculipennis*	*Caenorhabditis elegans*
Analogue **17**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **18**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **19**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **23**	0, 0, 0	0, 0, 0	99, 99, 99
Analogue **24**	0, 0, 0	0, 0, 0	0, 0, 0

3.14. Fungicidal activity

In fungicidal assays, the compounds were evaluated in mycelial growth tests in artificial media against *Pythium dissimile*, *Alternaria solani*, *Botryotini cinerea* and *Gibberella zeae* at rates of 20 ppm and 2 ppm. Leaf-piece assays were also conducted. The compounds were evaluated at 200 ppm and 60 ppm against *Phytophthora infestans* on tomato and 100 ppm for *Uromyces viciae-fabae* on bean. The analogues were applied prior to inoculation in the leaf-piece assays.

Compound	*Gibberella zeae*	*Botryotinia cinerea*	*Alternaria solani*
Analogue **17**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **18**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **19**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **23**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **24**	0, 0, 0	0, 0, 0	0, 0, 0

Compound	*Pythium dissimile*	*Uromyces viciae-fabae*	*Phytophthora infestans*
Analogue **17**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **18**	0, 0, 0	55, 55, 55	0, 0, 0
Analogue **19**	0, 0, 0	0, 55, 27	99, 0, 49
Analogue **23**	0, 0, 0	0, 0, 0	0, 0, 0
Analogue **24**	0, 0, 0	0, 0, 0	0, 0, 0

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.01.008.

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