Efficient synthesis of brussalexin A, a remarkable phytoalexin from Brussels sprouts

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Experimental Section

General

All solvents were HPLC grade and used as such, except for THF and Et_2O (dried over sodium) and chloroform (glass redistilled). Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Flash column chromatography (FCC): silica gel, grade 60, 230-400 μm. Organic extracts were dried over Na_2SO_4 and the solvents were removed using a rotary evaporator. Melting points are uncorrected.

Analytical HPLC was performed with a liquid chromatograph equipped (Agilent 1100 series HPLC system, Agilent Technologies, USA) with a quaternary pump, an automatic injector, a photodiode array detector, a degasser, and a Hypersil ODS column (5 µm particle size silica, 4.6 i.d. × 200 mm), equipped with an in-line filter. HPLC retention times were obtained under the following conditions: mobile phase, H_2O-CH_3CN (75% : 25%) to CH_3CN (100%) for 35 min, linear gradient and a flow rate of 1.0 ml/min. For LC-ESI-MS, an Agilent 1100 series HPLC system equipped with an autosampler, binary pump, degasser, a photodiode array detector connected directly to a mass detector (Agilent G2440A MSD-Trap-XCT ion trap mass spectrometer) with an ESI source was used. Chromatographic separation was carried out at room temperature using an Eclipse XSB C-18 column (5 μm particle size silica, 150 mm × 4.6 mm I.D.). The mobile phase consisted of a gradient of 0.2% formic acid in water (A) and 0.2% formic acid in acetonitrile (B) (75% A to 75% B in 35 min, to 100% B in 5 min) and a flow rate of 1.0 ml/min. The ion mode was set as positive and negative. The interface and MSD parameters were as follows: nebuliser pressure, 70.0 psi (N_2); dry gas, N_2 (12.0 L/min); dry gas temperature, 350 °C; spray capillary voltage 3500 V; skimmer voltage, 40.0 V; ion transfer capillary exit, 100 V; scan range, *m*/*z* 100-500. Ultrahigh pure He was used as

the collision gas. Mass spectral (MS) [high resolution (HR), electron impact (EI)] data were obtained on a mass spectrometer using a solids probe.

NMR spectra were recorded on 500 MHz spectrometers. For ^{1}H NMR spectra (500 MHz) the chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. The δ values were referenced to $CDCl_3$ ($CHCl_3$ at 7.28 ppm), CD_3CN (CD_2HCN at 1.94 ppm). For ^{13}C NMR (125.8 MHz) the chemical shifts (δ) were referenced to $CDCl_3$ (77.4 ppm). The multiplicities of the ^{13}C signals refer to the number of attached protons: s = C, d = CH, t = CH_2, q = CH_3. Fourier transform infrared (FT-IR) data were recorded on a spectrometer and spectra were measured by the diffuse reflectance method on samples dispersed in KBr. Ultraviolet (UV) spectra were recorded on a spectrophotometer using a 1 cm path length quartz cell.

Elicitation and isolation of brussalexin A

Plant material: Brussels sprouts were purchased from local markets.

Brussels sprouts were cut into two symmetrical pieces and were incubated for 24 h in a closed chamber, 100% humidity. The slices were irradiated with a UV light (250 nm) for 20 min. Control tissues were not UV elicited but otherwise were treated similarly. Control and elicited tissues were incubated further at 20 °C in darkness; one of the control slices and one of the elicited slices were collected at 24 h intervals for five days. Each slice was ground in a blender and the resulting material was stirred in EtOAc (150 ml) for 12 h. The macerates were filtered, the filtrates were dried and concentrated. The residues were dissolved in CH_3CN and were analysed by HPLC.

To isolate phytoalexin, Brussels sprouts (3.9 kg) were treated as described above. After four days of incubation, slices were ground in a blender and the resulting material was stirred in EtOAc (6 l) for 12 h. The macerates were filtered, the filtrates were dried and were concentrated. The residue (6.7 g) was fractionated by FCC (CH_2Cl_2–hexane and CH_2Cl_2–MeOH gradient elution).

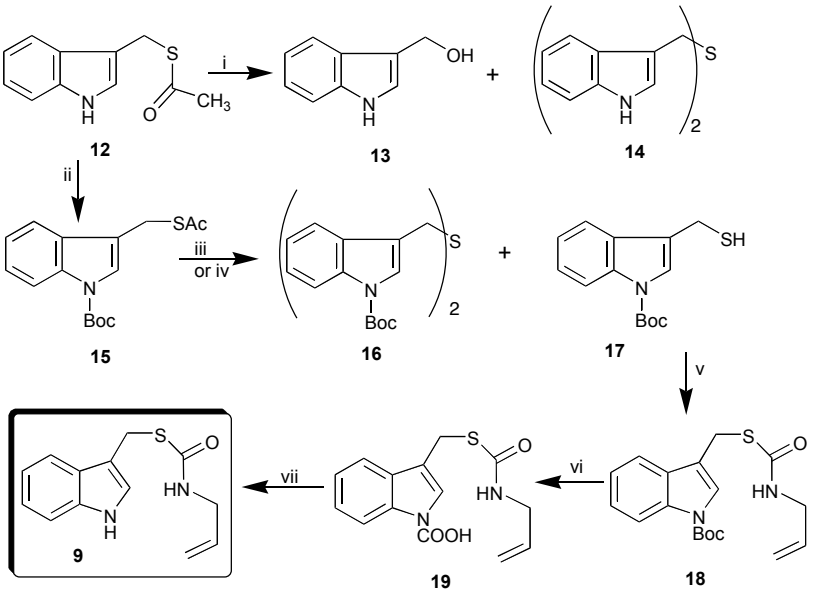
Fractions containing elicited metabolites (HPLC analysis) were combined and were further fractionated using FCC, preparative TLC and reversed phase chromatography to obtain brussalexin A (**9**, 2.0 mg).

Fungal cultures and antifungal bioassays

L. maculans (isolates BJ-125 and Laird-2), *R. solani* AG 2-1, *S. sclerotiorum*, *A. brassicicola* were obtained from AAFC, Saskatoon, Canada. Spores of *A. brassicicola*, *L. maculans*, sclerotia of *S. sclerotiorum*, mycelia of *R. solani* were grown on potato dextrose agar (PDA) plates at 23 ± 2 ºC, under constant light.

The antifungal activity of brussalexin A (**9**) was investigated using the following mycelia radial growth bioassay. Solutions of **9** in DMSO were used to prepare sterile assay plates (6 wells per plate, 36 mm diameter, 2 ml per well) in PDA media (5.0×10^{-4} M, 2.5×10^{-4} M, 5.0×10^{-5} M). Control plates contained 1% DMSO in PDA. Plates containing test solutions and the control solution were inoculated with mycelia plugs (4 mm diameter) placed upside down on the centre of each plate, the plates were sealed with parafilm, and incubated at 23 ± 2 ºC under constant light for 24 hr for *S. sclerotiorum*, 72 hr for *R. solani*, 120 hr for *L. maculans* (isolates BJ-125 and Laird -2) and *A. brassicicola*. The radial growth of mycelia was measured and compared with control plates containing only DMSO. Each experiment was conducted in triplicate and repeated three times.

Synthesis



Scheme 2 Synthesis of brussalexin A (**9**). Reagents and conditions: i, KOH, THF, argon, rt; ii, (*t*-Boc)$_2$O, 4-DMAP, THF, 60 min, 99% yield; iii, KOH, THF, argon, rt, 4 h, 88% yield; iv, KOH, THF, argon, 70 ^{o}C, 4 h, 44% yield; v, allyl isocyanate (**11**), KOH, 70 ^{o}C, 20 min, 88% yield; vi, TFA, rt; vii, 50 ^{o}C, 2 h, 91%.

1-Bocindolyl-3-methyl thioacetate (15). Di-*tert*-butyl dicarbonate (135.0 mg, 0.600 mmol) in THF (1 ml) was added in one portion to a solution of indolyl-3-methyl thioacetate (**12**, 105.3 mg, 0.5 mmol) in THF (4 ml). The mixture was cooled to 0 ºC and 4-DMAP (7.6 mg) was added. The reaction mixture was allowed to rise to room temperature with stirring and allowed to proceed for 1 h. The solvent was removed under reduced pressure, the residue was dissolved in EtOAc (20 ml) and washed with 1N HCl (20 ml) and brine (20 ml) respectively. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield quantitatively pure 1-Bocindolyl-3-methyl thioacetate (**15**) as white crystals. HPLC t_R = 35.3 min. HR-EI-MS: calc. for $C_{16}H_{19}NO_3S$ (M^+) *m/z* 305.1086, found 305.1095; EI-MS (*m/z*, % relative int.): 305 (10), 249 (25), 174 (37), 162 (7), 130 (100). ^{1}H-NMR (500.3 MHz, $CDCl_3$): δ 8.15 (d, *J* = 6.5 Hz, 1H), 7.57 (s, 1H), 7.55 (d, *J* = 8 Hz, 1H),

7.35 (dd, *J* = 8, 8 Hz, 1H), 7.28 (dd, *J* = 8, 8 Hz, 1H), 4.28 (brs, 2H), 2.39 (s, 3H), 1.69 (s, 9H). ^{13}C-NMR ($CDCl_3$, 125.8 MHz): δ 195.9 (s), 150.1 (s), 136.2 (s), 129.9 (s), 125.3 (d), 125.0 (d), 123.2 (d), 119.6 (d), 116.7 (s), 116.0 (d), 84.4 (s), 31.1 (t), 28.8 (q, 3C), 24.5 (q). υ_{max}(KBr)/cm^{-1} : 2980, 1733, 1691, 1452, 1363, 1257, 1156, 1081, 765, 745. λ_{max} (CH_2Cl_2)/nm: 230 (log ε, 4.51), 266 (log ε, 4.03), 294 (log ε, 3.86).

1-Bocindolyl-3-methanethiol (17). Potassium hydroxide aqueous solution (0.5 N, 1 ml) was added drop wise to a solution of 1-Bocindolyl-3-methyl thioacetate (**15**, 106.0 mg, 0.35 mmol) in THF (10 ml) under argon atmosphere. The reaction mixture was stirred at room temperature for 4 h under argon atmosphere. The reaction mixture was diluted with water and extracted with EtOAc (2 × 20 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was separated by FCC to yield 1-Bocindolyl-3-methanethiol as a white powder (**17,** 80.8 mg, 88%). When the hydrolysis was carried out at higher temperature (> 50 ºC), di-(1-Boc-indolyl-3-methyl) sulphide (**16**) was also formed. 1-Bocindolyl-3-methanethiol (**17**): HPLC t_R = 34.4 min. HR-EI-MS: calc. for $C_{14}H_{17}NO_2S$ (M^+) *m/z* 263.0980, found 263.0979; EI-MS (*m/z*, % relative int.): 263 (17), 207 (29), 190 (5), 174 (32), 162 (5), 130 (90), 57 (100). ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.17 (brs, 1H), 7.66 (d, *J* = 8 Hz, 1H), 7.57 (s, 1H), 7.37 (dd, *J* = 8, 8 Hz, 1H), 7.30 (dd, *J* = 8, 8 Hz, 1H), 3.92 (d, *J* = 7 Hz, 2H), 1.84 (t, *J* = 7 Hz, 1H, -S<u>H</u>), 1.70 (s, 9H). ^{13}C-NMR ($CDCl_3$, 125.8 MHz): δ 149.6 (s), 135.8 (s), 129.1 (s), 124.7 (d), 123.3 (d), 122.6 (d), 120.2 (s), 119.2 (d), 115.3 (d), 83.7 (s), 28.2 (q, 3C), 19.2 (t). υ_{max}(KBr)/cm^{-1}: 2977, 2929, 1731, 1452, 1367, 1257, 1156, 1083, 764, 744. λ_{max} (CH_2Cl_2)/nm: 230 (log ε, 4.26), 266 (log ε, 3.86), 294 (log ε, 3.67). Di-(1-Boc indole-3-methyl) sulfide (**16**): HPLC t_R = 35.3 min. HR-EI-MS: calc. for $C_{28}H_{32}N_2O_4S$ (M^+) *m/z* 492.2082, found 492.2076; EI-MS (*m/z*, % relative int.): 492 (0.7), 460 (1), 262 (5), 230 (20), 174 (38), 130 (100). ^{1}H-NMR (500.1 MHz, $CDCl_3$): 8.16 (d, *J* = 6 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.49 (brs, 2H), 7.36 (t, *J* = 7.5 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 3.85 (brs, 4H), 1.70 (s, 18 H).

1-Boc-brussalexin A (18). A mixture of 1-Bocindolyl-3-methanethiol (**17**, 27.8 mg, 0.11 mmol) in THF (10 ml) and KOH (0.5 N, 0.5 ml) was heated to 70 ºC under argon atmosphere. Allyl isocyanate (20 μl, 0.23 mmol) was added drop wise to the mixture. The reaction was continued for 20 min. The reaction mixture was diluted with water (20 ml) and extracted with EtOAc (2 × 20 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield pure 1-Boc-brussalexin A (**18**, 32.2 mg, 88%). HPLC t_R = 31.7 min. HR-EI-MS: calc. for $C_{18}H_{22}N_2O_3S$ (M^+) *m/z* 346.1351, found 346.1352; EI-MS (*m/z*, % relative int.): 346 (9), 290 (15), 207 (23), 174 (23), 130 (100). ESI-MS (positive): 369.3 [M^++23], 347.3 [M^++1], 174.2, 130.3. ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.16 (d, *J* = 6.5 Hz, 1H), 7.62 (d, *J* = 8 Hz, 1H), 7.59 (s, 1H), 7.35 (dd, *J* = 8, 8 Hz, 1H), 7.28 (dd, *J* = 8, 8 Hz, 1H), 5.86 (m, 1H), 5.43 (brs, 1H), 5.24 (d, *J* = 17 Hz, 1H), 5.18 (d, *J* = 10 Hz, 1H), 4.34 (brs, 2H), 3.97 (brs, 2H), 1.69 (s, 9H). ^{13}C-NMR ($CDCl_3$, 125.8 MHz): δ 166.7 (s), 149.6 (s), 135.7 (s), 133.6 (d), 129.4 (s), 124.6 (d), 124.4 (d), 122.6 (d), 119.2 (d), 116.9 (t), 116.8 (s), 115.4 (d), 83.7 (s), 43.8 (t), 28.2 (q, 3C), 24.9 (t). υ_{max}(KBr)/cm^{-1} : 3309, 2979, 1733, 1642, 1563, 1452, 1363, 1256, 1156, 1082, 766, 747. λ_{max} (CH_2Cl_2)/nm: 230 (log ε, 3.70), 266 (log ε, 3.27), 294 (log ε, 3.10).

Brussalexin A (**9**). 1-Boc-brussalexin (**18**, 29.6 mg, 0.086 mmol) was dissolved in TFA (200 μl) and the mixture was stirred at room temperature for 10 min. Then, the solvent was removed under reduced pressure to yield a white powder which was characterized in the reaction mixture as brussalexin A 1-carboxylic acid (**19**) and brussalexin A (**9**) in a 4:1 ratio based on ^{1}H-NMR and ESI/MS analysis. The powder was heated at 50 ºC for 2 h and the residue was separated by FCC on silica gel to yield brussalexin (**9**, 19.1 mg, 0.078 mmol, 91% yield) as white crystals. M.p. 122-123 ºC. HPLC t_R = 17.1 min. HR-EI-MS: calc. for $C_{13}H_{14}N_2OS$. (M^+) *m/z* 246.0826, found 246.0824; EI-MS (*m/z*, % relative int.): 246 (9), 130 (100). ^{1}H-NMR (500.1 MHz, CD_3CN): 9.17 (brs, 1H), 7.62 (d, *J* = 8 Hz, 1H), 7.42 (d, *J* = 8 Hz, 1H), 7.23 (d, *J* = 2 Hz, 1H), 7.18 (dd, *J* = 8 Hz, 1H), 7.09 (dd, *J*

= 8 Hz, 1H), 6.48 (brs, 1H), 5.87 (m, 1H), 5,17 (dd, *J* = 1.5, 17 Hz, 1H), 5.11 (dd, *J* = 1.5, 11 Hz, 1H), 4.36 (brs, 2H), 3.86 (brs, 2H). ^{1}H-NMR (500.1 MHz, $CDCl_3$): δ 8.05 (brs, 1H), 7.69 (d, *J* = 8 Hz, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.23 (brs, 1H), 7.24 (dd, *J* = 8, 8 Hz, 1H), 7.18 (dd, *J* = 8, 8 Hz, 1H), 5.85 (m, 1H), 5.36 (brs, 1H), 5.23 (d, *J* = 17 Hz, 1H), 5.17 (d, *J* = 10 Hz, 1H), 4.44 (brs, 2H), 3.96 (brs, 2H). ^{13}C-NMR ($CDCl_3$, 125.8 MHz): δ 167.5 (s), 136.3 (s), 133.7 (d), 126.6 (s), 123.4 (d), 122.4 (d), 119.8 (d), 119.0 (d), 116.9 (t), 112.3 (s), 111.3 (d), 43.7 (t), 25.5 (t). υ_{max}(KBr)/cm^{-1}: 3395, 3315, 3046, 2922, 1652, 1497, 1420, 1340, 1203, 743. λ_{max} (CH_2Cl_2)/nm: 228 (log ε, 4.39), 279 (log ε, 3.85).