Electronic Supplementary Information

Unveiling the first indole-fused thiazepine: structure, synthesis and biosynthesis of cyclonasturlexin, a remarkable cruciferous phytoalexin

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1 Materials and general experimental

All solvents were HPLC grade and used as such or treated as specified for each case. Chemicals were purchased from Sigma-Aldrich Canada Ltd., Oakville, ON. Labeled (*S*)-Trp containing U-$^{13}C_{11}$, 99%; U-$^{15}N_2$, 99% was purchased from Cambridge Isotope Laboratories, Inc., Andover, MA, USA.

Flash column chromatography (FCC) was carried out using silica gel grade 60, mesh size 230–400 Å or WP C18 prep-scale bulk packing 275 Å (J.T. Baker, NJ, USA). Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 500 MHz Avance spectrometers (for ^{1}H, 500.3 MHz and for ^{13}C, 125.8 MHz) and Bruker 600 MHz Advance III HD 600 MHz spectrometer (for ^{1}H, 600.2 MHz and for ^{13}C, 150.9 MHz); chemical shifts (δ) are reported in parts per million (ppm) relative to TMS; spectra were calibrated using solvent peaks; spin coupling constants (*J*) are reported to the nearest 0.5 Hz. Fourier transform infrared (FTIR) data were recorded on a spectrometer Bio-Rad FTS-40 and spectra were measured by the diffuse reflectance method on samples dispersed in KBr. MS data [high resolution (HR), electron impact (EI)] were obtained on a VG 70 SE mass spectrometer employing a solids probe, or on a Jeol AccuToF GCv 4G mass spectrometer [field desorption (FD)] by direct insertion. HPLC-DAD analysis was carried out with Agilent 1100 and 1200 series systems equipped with quaternary pumps, autosamplers, diode array detectors (DAD, wavelength range 190–600 nm, bandwidth 4 nm), degassers and Zorbax Eclipse XDB-C18 columns (5 µL particle size silica, 150 x 4.6 mm I.D.), equipped with an in-line filter. The mobile phase used H_2O–CH_3CN from 75:25 to 25:75, linear gradient for 35 min, and a flow rate of 1.0 mL/min. HPLC-DAD-ESI-MS analysis was carried out with an Agilent 1100 series HPLC system equipped with an autosampler, binary pump, degasser, and a diode array detector connected directly to a mass detector (Agilent G2440A MSD-Trap-XCT ion trap mass spectrometer) with an electrospray ionization (ESI) source. Chromatographic separations were carried out at room temperature using an Eclipse XDB-C-18 column (5 µL particle size silica, 150 mm x 4.6 mm I.D.). The mobile phase consisted of a linear gradient of H_2O (0.2% HCO_2H) – CH_3CN (0.2% HCO_2H) from 75:25 to 25:75 in 25 min and a flow rate of 1.0 mL/min. Data acquisition was carried out in positive and negative polarity modes in a single LC run, and data processing was carried out with Agilent Chemstation Software.

Seeds of watercress were obtained from a commercial source (Sand Mountain Herbs, www.sandmountainherbs.com) and sown in a perlite and nutrient free LG-3 soil (Sun Gro Horticulture Canada) in small pots in a growth chamber at 16 h of light/8 h of dark, 20 °C day/18 °C night, 350–360 µE $m^{-2}s^{-1}$, and with ambient humidity.

2 Time-course analysis of phytoalexin production in watercress and isolation of cyclonasturlexin (15)

2.1 Time-course analysis of phytoalexins elicited with $CuCl_2$ and UV radiation

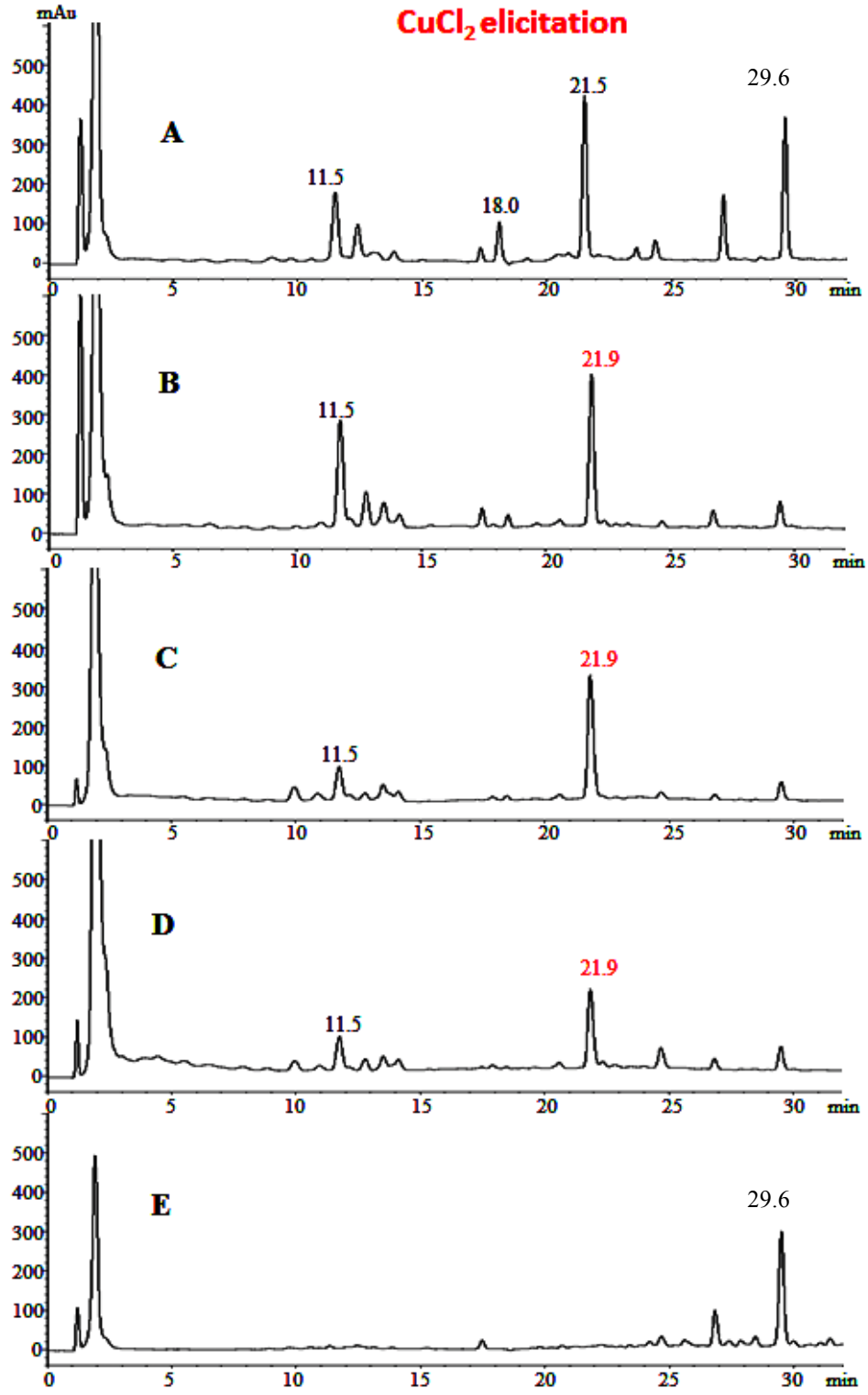
Elicited with $CuCl_2$: 4-week-old plants were sprayed with an aq. $CuCl_2$ solution (2 mM) and control plants were sprayed with H_2O. The leaves (ca. 1.5 g fresh weight per sample) were excised 1, 2, 4 and 6 days after elicitation, frozen in liquid nitrogen, ground and the resulting leaf materials were extracted with MeOH (5 mL). The extracts were concentrated and rinsed with CH_2Cl_2, the CH_2Cl_2 extracts were concentrated, dissolved in CH_3CN-MeOH (1:1) and analysed by HPLC-DAD. Quantification of cyclonasturlexin was carried out using a calibration curve built from an authentic synthetic sample (Fig. S1; Table S1).

Elicited with UV radiation: 4-week-old plants were exposed to UV light (Philips TUV G30T8 30W) for 90 min, incubated, treated and analysed as above (Fig. S2; Table S1).

Table S1. Concentration[a] of cyclonasturlexin (**15**) in leaves of watercress sprayed with $CuCl_2$ or irradiated with UV light.

Elicitation method	nmol/g fresh weight ± standard deviation			
	24 h post elicitation	48 h post elicitation	96 h post elicitation	144 h post elicitation
$CuCl_2$ spray	not detected	166 ± 42	141 ± 23	94 ± 9
UV irradiation	113 ± 53	305 ± 87	237 ± 95	550 ± 203

[a]Values represent the mean and standard deviation of triplicate samples.


CuCl2 elicitation
mAu
A
11.5
18.0
21.5
29.6
B
11.5
21.9
C
11.5
21.9
D
11.5
21.9
E
29.6
min

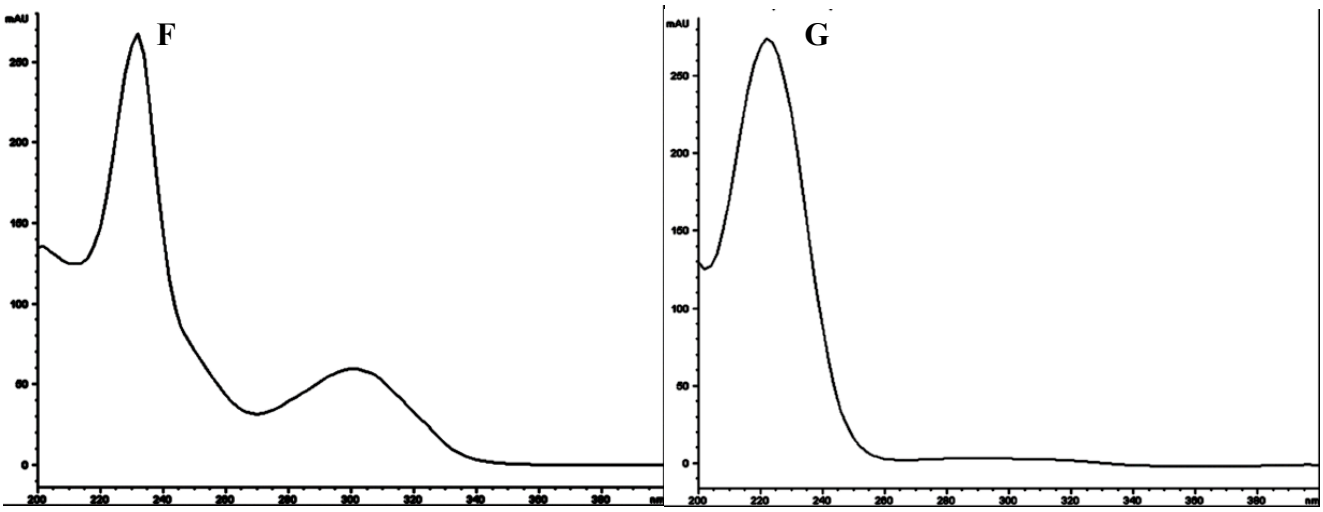


Fig. S1. HPLC-DAD chromatograms (detection at 220 nm) of extracts of leaves of watercress after elicitation with $CuCl_2$: (A) 24 h post elicitation; (B) 48 h post elicitation; (C) 96 h post elicitation; (D) 144 h post elicitation; (E) control; (F) UV spectrum of t_R = 21.9 min; (G) UV spectrum of t_R = 29.6 min; elicited metabolites: t_R = 11.5 min, nasturlexin B (**10**); t_R = 18.0 min, brassinin (**1**); t_R = 21.5 min, nasturlexin A (**9**); t_R = 21.9 min, cyclonasturlexin (**15**); t_R = 29.6, unknown compound, decomposed during column chromatography.

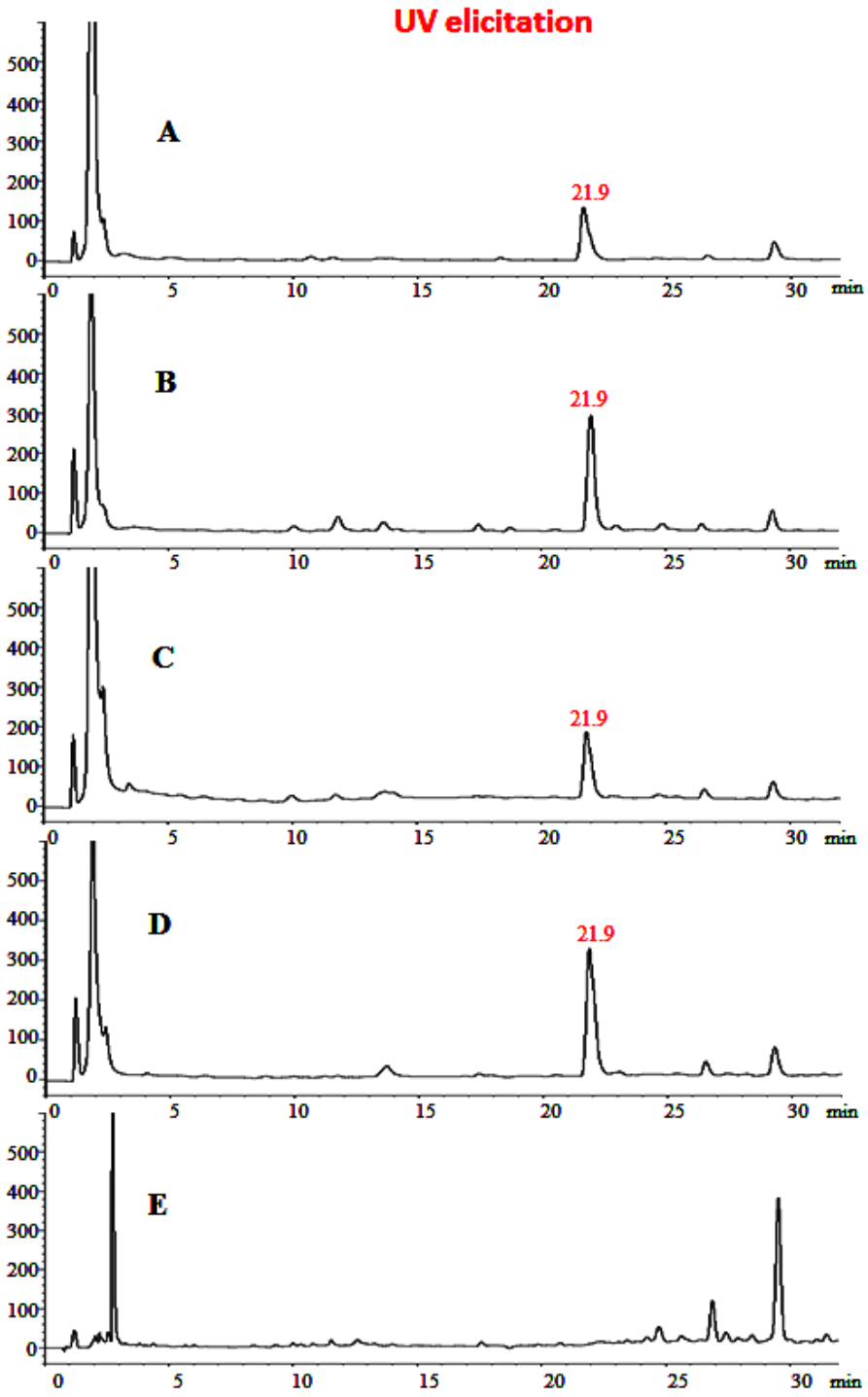


Fig. S2. HPLC-DAD chromatograms (detection at 220 nm) of extracts of leaves of watercress after elicitation with UV radiation: (A) 24 h post elicitation; (B) 48 h post elicitation; (C) 96 h post elicitation; (D) 144 h post elicitation; (E) control; elicited metabolites: t_R = 21.9 min, cyclonasturlexin (**15**).

2.2 Isolation of cyclonasturlexin (15) from leaves of watercress and X-ray structure

Watercress plants 4-week old (40 pots) were sprayed with aq. $CuCl_2$ solution (2 mM). Leaves were collected 48 h after spraying (ca. 300 g fresh weight), frozen in liquid nitrogen, and ground. Cyclonasturlexin (**15**) was isolated from ca. 1.7 g of extract as summarized in Fig. S3.

Crystals of cyclonasturlexin (**15**) suitable for X-ray diffraction analysis were obtained by slow evaporation of a solution (ca. 3 mg) in CH_2Cl_2 (0.5 mL) stored at rt; a single crystal was analysed by X-ray crystallography (Fig. S4) (CCDC 1456343).

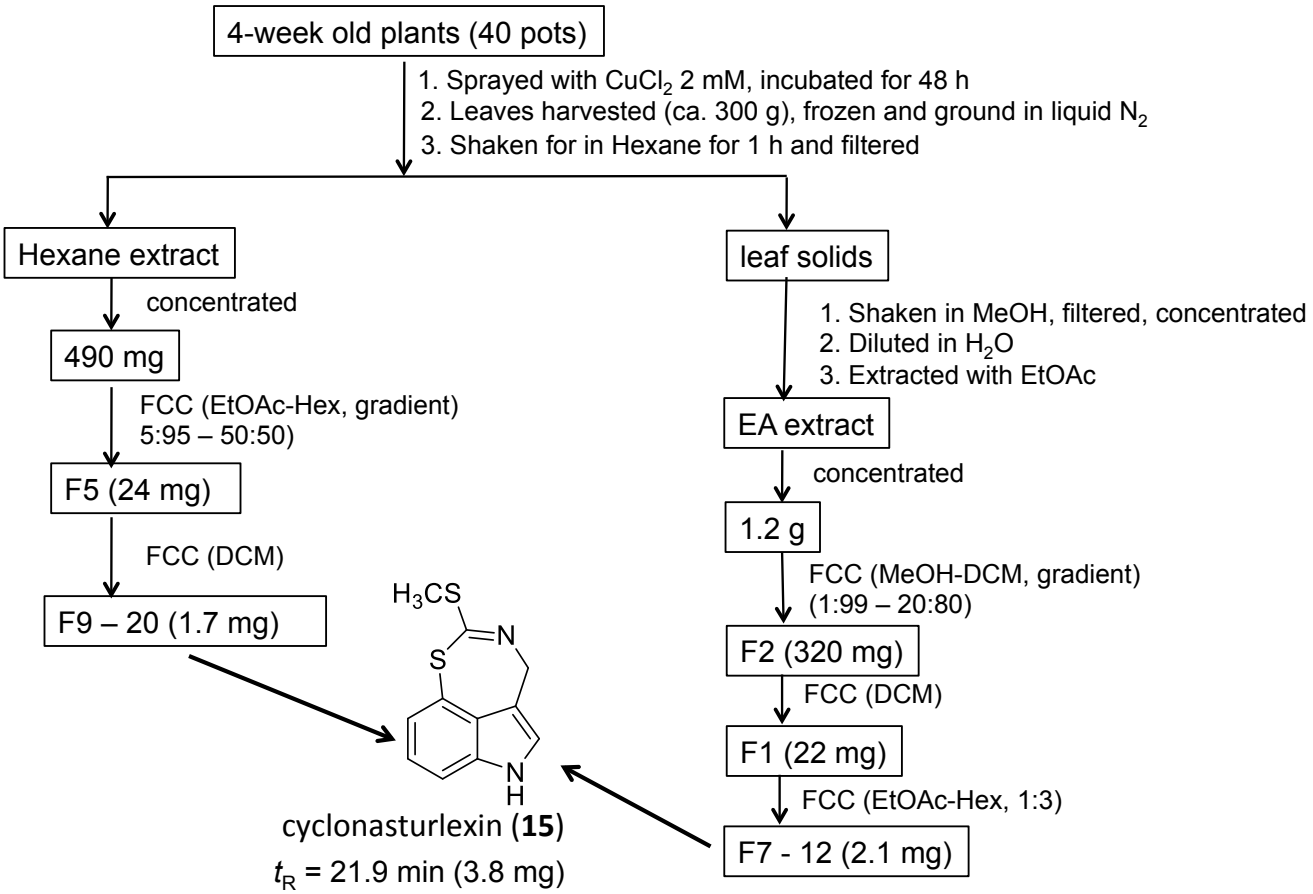


Fig. S3. Isolation of cyclonasturlexin (**15**).

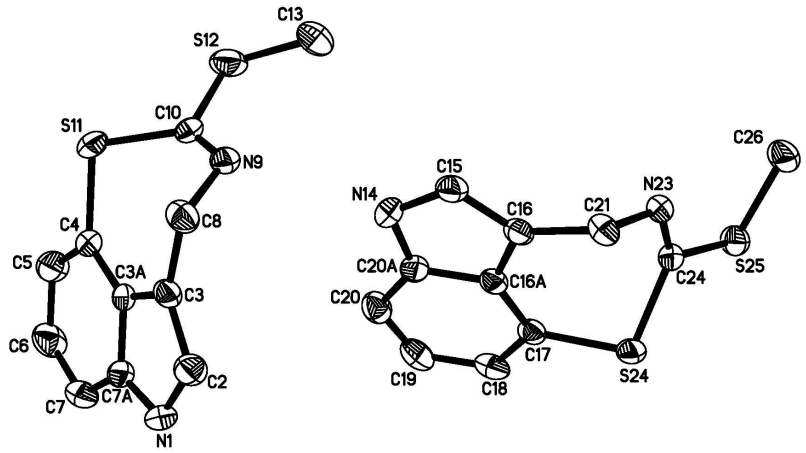
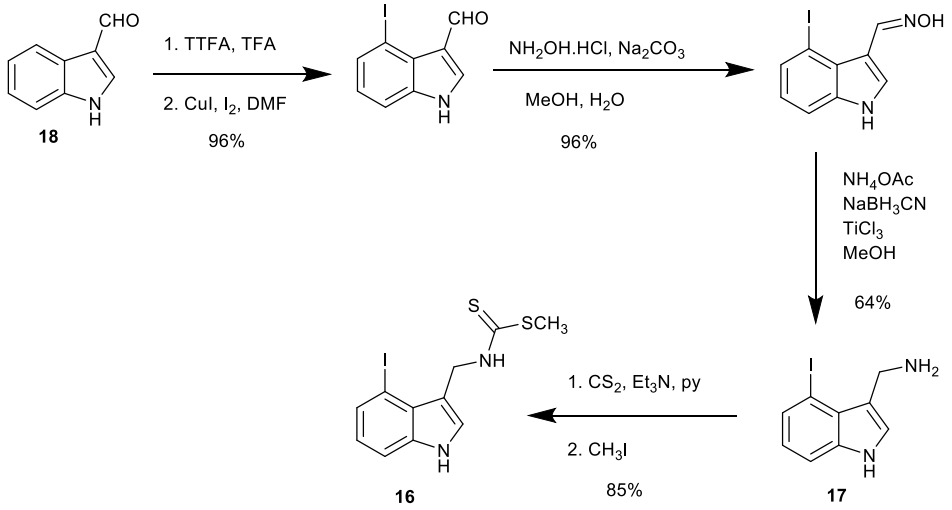


Fig. S4. Single crystal X-ray structure of cyclonasturlexin (**15**).

3 Synthesis and spectroscopic characterization of new compounds

3.1 4-Iodobrassinin (16)



Scheme S1. Synthesis of 4-iodobrassinin (**16**).

A suspension of thallium (III) trifluroacetate (1,350 mg, 2.48 mmol) in TFA (4 mL) was added to a solution of indole-3-carboxaldehyde (300 mg, 2.07 mmol) in TFA (2 mL). After stirring at 30 ºC for 2 h, the mixture was concentrated and a suspension of CuI (1,573 mg, 8.26 mmol) and I_2 (1,577 mg, 6.21

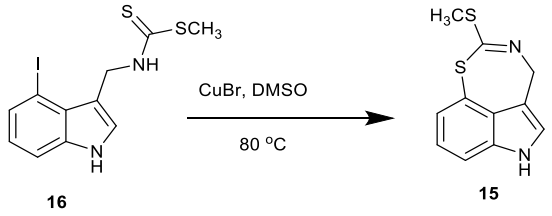
mmol) in DMF (10 mL) was added. After stirring at rt for 3 h, EtOAc was added and the mixture was filtered through celite. The filtrate was washed 3 times with saturated aq. $Na_2S_2O_3$ solution. The organic extract was dried over Na_2SO_4 and concentrated to give 4-iodoindole-3-carboxaldehyde as brown solid (539 mg, 2.00 mmol, 96%).

A solution of $NH_2OH.HCl$ (102 mg, 1.48 mmol) and Na_2CO_3 (78 mg, 0.74 mmol) in H_2O (2 ml) was added to a solution of 4-iodoindole-3-carboxaldehyde (200 mg, 0.74 mmol) in EtOH at 60 ^oC. After stirring at 80 ^oC for 2 h, the mixture was concentrated, diluted with H_2O, and extracted with EtOAc. The organic extract was dried over Na_2SO_4 and concentrated to give 4-iodoindole-3-oxime as a brown solid (215 mg, 0.72 mmol, 96%).

$NaCNBH_3$ (164 mg, 2.6 mmol) was added to a mixture of oxime (150 mg, 0.52 mmol) and NH_4OAc (400 mg, 5.2 mmol) in MeOH (5 mL) at rt. To this suspension, a solution of $TiCl_3$ (in 3% HCl, 20% wt.) was added dropwise over 2 min. The reaction was immediately poured into ice cold water, basified with NH_4OH and extracted with $CHCl_3$. The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (MeOH-DCM-NH_4OH, 20:80:1) to give 4-iodoindole-3-methylamine (90 mg, 0.33 mmol, 64%).

CS_2 (40 µL, 0.66 mmol) was added to a solution of 4-iodoindole-3-methylamine (90 mg, 0.33 mmol) and Et_3N (140 µL, 0.99 mmol) in pyridine (1.0 mL) at rt. After stirring for 15 min, CH_3I (40 µL, 0.66 mmol) was added and stirring was continued for additional 15 min. The mixture was acidified with H_2SO_4 5% and extracted with ethyl acetate. The organic extract was dried over Na_2SO_4 and separated by EtOAc-Hex (1:3) to give compound 4-iodobrassinin (102 mg, 0.28 mmol, 85%) as a white solid, mp123 – 124 °C. HPLC: t_R = 23.4 min. ^1H NMR (500 MHz, $CDCl_3$): δ 8.36 (1H, br), 7.61 (1H, d, J = 7.5 Hz), 7.45 (1H, br), 7.38 – 7.37 (2H, m), 6.90 (1H, t, J = 8.0 Hz), 5.16 (2H, d, J = 5.0 Hz), 2.61 (3H, s) and peaks of rotamer at δ 8.45 (br), 7.89 (br), 7.57 (d), 7.24 (d), 4.91 (d), 2,72 (s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 197.3, 136.7, 131.6, 127.9, 127.6, 124.2, 112.0, 111.7, 84.5, 42.0, 18.2 and rotamer peaks at δ 200.4, 131.7, 126.7, 124.2, 111.1, 41.6, 19.2. HREI-MS *m/z* $[M]^+$: calc. for $C_{11}H_{11}IN_2S_2$: 361.9408, found 361.9405 (3), 255.96 (100), 235.04 (57), 106.99 (97). UV (HPLC, CH_3CN – H_2O) λ_{max} (nm): 223, 250, 275. FTIR (KBr, cm^{-1}) ν_{max} 3314, 1505, 1418, 1339, 1247, 1091, 923.

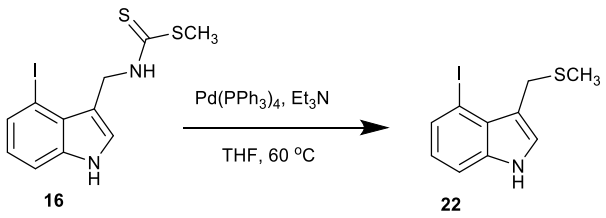
3.2 Cyclonasturlexin (15)



Scheme S2. Synthesis of cyclonasturlexin (**15**).

A mixture of 4-iodobrassinin (40 mg, 0.11 mmol) and CuBr (47 mg, 0.33 mmol) in DMSO (0.8 mL) was stirred at 80 °C for 10 min. The reaction was immediately poured into cold H_2O and extracted with EtOAc. The organic extract was washed 3 times with H_2O, dried over Na_2SO_4 and separated by FCC using DCM to give cyclonasturlexin (9.0 mg, 0.038 mmol, 35%) as a white solid, mp 178 – 180 °C . HPLC: t_R = 21.9 min. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.93 (1H, br), 7.13 (1H, d, J = 8.0 Hz, H-7), 7.04 – 7.01 (2H, m, H-6,2), 6.89 (1H, d, J = 7.5 Hz, H-5), 5.13 (2H, s, CH_2), 2.36 (3H, s, SCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 158.7 (br, C=N), 136.7 (C-4), 128.0 (C-3a), 124.7 (C-7a), 123.4 (C-6), 121.0 (C-2), 119.2 (C-5), 113.2 (C-3), 110.7 (C-7), 49.9 (CH_2), 16.1 (SCH_3). HREI-MS m/z $[M]^+$: calc. for $C_{11}H_{10}N_2S_2$: 234.0285, found 234.0282 (17), 161.03 (100). UV (HPLC, CH_3CN-H_2O) λ_{max} (nm): 215, 300. FTIR (KBr, cm^{-1}) ν_{max} 3171, 1570, 1426, 1347, 1194, 977.

3.3 4-Iodo-3-[(methylthio)methyl]indole (22)

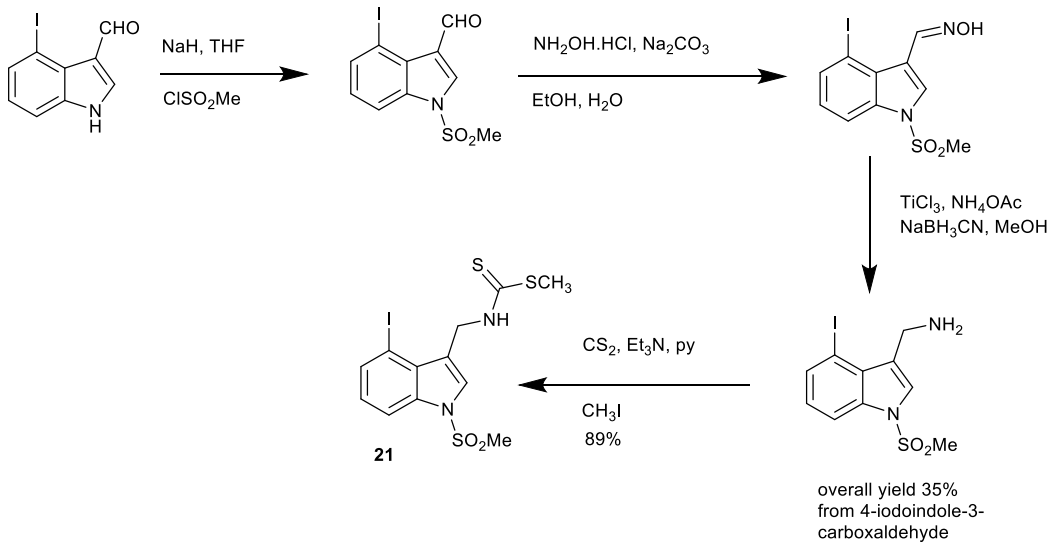


Scheme S3. Synthesis of 4-iodo-3-[(methylthio)methyl]indole (**22**).

$Pd(PPh_3)_4$ (2.0 mg, 0.0014 mmol) was added to a solution of 4-iodobrassinin (10 mg, 0.028 mmol) and Et_3N (20 μL, 0.14 mmol) in THF (0.3 mL). The mixture was stirred at 60°C in a sealed vial

for 2 h. Solvent was removed and the crude was separated by FCC using DCM to give compound **22** (7.0 mg, 0.023 mmol, 83%) as a white solid, mp 106 – 107 °C. HPLC: t_R = 23.2 min. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.08 (1H, br), 7.61 (1H, d, *J* = 7.0 Hz), 7.33 (1H, d, *J* = 8.0 Hz), 7.20 (1H, d, *J* = 2.5 Hz), 6.86 (1H, t, *J* = 8.0 Hz), 4.15 (2H, s), 2.11 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 137.3, 131.9, 127.5, 125.1, 123.8, 113.9, 111.7, 85.1, 29.5, 15.3. HREI-MS *m/z* $[M]^+$: calc. for $C_{10}H_{10}NSI$: 302.9579, found 302.9559 (23), 255.99 (100), 129.06 (24). UV (HPLC, CH_3CN-H_2O) λ_{max} (nm): 228, 286. FTIR (KBr, cm^{-1}) ν_{max} 3412, 1316, 1333, 744.

3.4 1-SO_2Me-4-iodobrassinin (21)



Scheme S4. Synthesis of 1-SO_2Me-4-iodobrassinin (**21**).

A solution of 4-iodoindole-3-carboxaldehyde (230 mg, 0.85 mmol) was added to a suspension of NaH (170 mg, 4.25 mmol) in THF (3.0 mL) at 0 °C. After stirring at rt for 15 min, $ClSO_2Me$ (330 µL, 4.25 mmol) was added. The reaction was stirred for additional 15 min and concentrated. H_2O was added and the mixture was extracted with DCM. The organic extract was dried over Na_2SO_4 and concentrated to give crude 1-SO_2Me-4-iodoindole-3-carboxaldehyde (297 mg, 0.85 mmol).

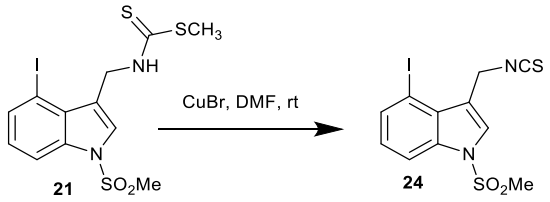
A solution of $NH_2OH.HCl$ (117 mg, 1.70 mmol) and Na_2CO_3 (90 mg, 0.85 mmol) in H_2O (4 mL) was added to a solution of 1-SO_2Me-4-iodoindole-3-carboxaldehyde (297 mg, 0.85 mmol). After stirring at 60 °C for 1 h, H_2O was added and the mixture was extracted with EtOAc. The organic

extract was dried over Na_2SO_4 and concentrated to give the crude oxime (300 mg, 0.82 mmol).

$NaBH_3CN$ (268 mg, 4.25 mmol) was added to a solution of oxime (300 mg, 0.82 mmol) at rt, followed by addition of $TiCl_3$ solution (20% in HCl 3%, 5.5 mL, 8.5 mmol). The mixture was immediately poured into iced cold H_2O, basified with NH_4OH and extracted with $CHCl_3$. The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (MeOH-DCM:NH_4OH, 20:80:1) to give 1-SO_2Me-4-iodoindole-4-methylamine as a white solid (104 mg, 0.28 mmol, 35% from 4-iodoindole-3-carboxaldehyde .

CS_2 (34 µL, 0.56 mmol) was added to a solution of amine (104 mg, 0.28 mmol) in pyridine (2.0 mL), followed by Et_3N (117 µL, 0.84 mmol). After stirring at rt for 15 min, CH_3I (35 µL, 0.56 mmol) was added and stirring was continued for additional 15 min. The mixture was diluted with EtOAc and washed with H_2SO_4 5%. The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (EtOAc-hexane, 1:3) to give **21** as a white solid, mp 154 – 155 °C. HPLC: t_R = 26.1 min. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95 (1H, dd, *J* = 7.0, 0.5 Hz), 7.79 (1H, d, *J* = 6.5 Hz), 7.67 (1H, s), 7.43 (1H, br), 7.08 (1H, t, *J* = 6.5 Hz), 5.20 (2H, d, *J* = 4.5 Hz), 3.13 (3H, s), 2.64 (3H, s).^{13}C NMR (125 MHz, $CDCl_3$): δ 198.7, 135.9, 135.3, 130.9, 128.6, 126.6, 117.2, 113.5, 85.0, 41.7, 41.4, 18.4. HRFD-MS *m/z* $[M]^+$: calc. for $C_{12}H_{13}IN_2O_2S_3$: 439.9184, found 439.9192. UV (HPLC, CH_3CN-H_2O) λ_{max} (nm): 222, 266. FTIR (KBr, cm^{-1}) ν_{max} 3175, 1506, 1369, 1169, 938, 782, 543.

3.5 1-SO_2Me-4-iodoindole-3-methylisothiocyanate (24)



Scheme S5. Synthesis of 1-SO_2Me-4-iodoindole-3-methylisothiocyanate (**24**).

A mixture of 1-SO_2Me-4-iodobrassinin (**21**) (10 mg, 0.023 mmol) and CuBr (10 mg, 0.069 mmol) in DMF (0.2 mL) was stirred at rt for 24 h. The mixture was diluted with EtOAc and washed with H_2O. The organic extract was dried over Na_2SO_4 and concentrated to give **24** (7.0 mg, 0.018 mmol, 78%) as a white solid.

White solid, mp 120 – 122 °C. HPLC: t_R = 28.6 min. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94 (1H,

d, J = 8.5 Hz), 7.79 (1H, d, J = 7.5 Hz), 7.61 (1H, s), 7.09 (1H, t, J = 8.0 Hz), 5.19 (2H, s), 3.17 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 135.9, 135.4, 134.0, 130.2, 127.0, 126.5, 116.9, 113.4, 84.7, 41.5, 41.4. HRFD-MS *m/z* $[M]^+$: calc. for $C_{11}H_9IN_2O_2S_2$: 391.9150, found 391.9164. UV (HPLC, CH_3CN-H_2O) λ_{max} (nm): 222, 264. . FTIR (KBr, cm^{-1}) ν_{max}. 2928, 2054, 1549, 1365, 1173, 984, 755, 542.

4 Antifungal bioassays

The biological activity of cyclonasturlexin (**15**) toward the fungal phytopathogen *Alternaria brassicicola* was evaluated using a mycelial radial growth bioassay (PDA media and DMSO solutions 0.15, 0.10 and 0.05 mM; control solutions contained 1% DMSO in PDA media). *A. brassicicola* isolate UAMH 7474 was obtained from the University of Alberta Microfungus Collection and Herbarium. Spores of *A. brassicicola* were spotted onto potato dextrose agar plates (PDA) and allowed to grow for seven days under constant light at 23 ± 1 ^{o}C. Plugs (4 mm) were cut from the edges of mycelia and placed inverted onto 12-well plates. Plates were allowed to grow under constant light/dark at 23 ± 1 ^{o}C for 72 h; the diameter of the mycelial mat was measured and compared to control mycelia grown on plates containing DMSO, using the formula in Table S2 (the diameter of the mycelial plug was subtracted from the total mycelial diameter).

Table S2. Antifungal activity of cyclonasturlexin (**15**) and biosynthetically related phytoalexins against *Alternaria brassicicola* (72 h of incubation). Camalexin is a phytoalexin not biosynthetically related used as a positive control.

Phytoalexin		% Inhibition[a] ± standard deviation
Cyclonasturlexin (**15**)		
	0.15 mM[b]	87 ± 2
	0.10 mM	77 ± 5
	0.05 mM	54 ± 5
Brassinin (**1**)[c]		
	0.50 mM	39 ± 2
	0.20 mM	31 ± 2
	0.10 mM	22± 1
Cyclobrassinin (**4**)[c]		
	0.50 mM[d]	61 ± 8
	0.20 mM	34 ± 4
	0.10 mM	9 ± 4
Brassilexin (**6**)[c]		
	0.50 mM	85 ± 2
	0.20 mM	59 ± 2
	0.10 mM	50 ± 3
Camalexin[c]		
	0.50 mM	100 ± 0
	0.20 mM	85 ± 1
	0.05 mM	53 ± 1

[a] Percentage of inhibition = 100 – [(growth on medium containing compound/ growth on control medium) × 100)] ± standard deviation (average of six replicate samples).

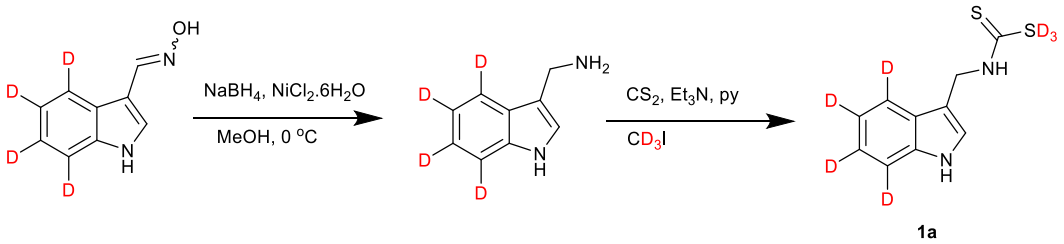
[b] Not completely soluble in the medium at higher concentration.

[c] For comparison, data taken from: M. S. C. Pedras, P. B. Chumala, W. Jin, M. S. Islam and D. W. Hauck, *Phytochemistry*, 2009, **70**, 394.

[d] Not completely soluble in the medium at 0.50 mM.

5 Biosynthetic experiments

5.1 Synthesis of [$^2H_3$C-S 4',5',6',7'-$^2H_4$]brassinin (1a) and [4',5',6',7'-$^2H_4$]brassinin (1b)



Scheme S6. Synthesis of [$^2H_3$-S 4′,5′,6′,7′-$^2H_7$]brassinin (**1a**).

[4′,5′,6′,7′-$^2H_4$]Indole-3-oxime was obtained from [4,5,6,7-$^2H_4$]indole as previously reported.[1] $NaBH_4$ (23 mg, 0.60 mmol) was added in portions to a mixture of [4′,5′,6′,7′-$^2H_4$]indole-3-oxime (20 mg, 0.12 mmol) and $NiCl_2.6H_2O$ (29 mg, 0.12 mmol) in MeOH (1.0 mL) at 0 °C. After stirring for 10 min, the mixture was diluted with H_2O, basified with NH_4OH, and extracted with EtOAc. The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (MeOH-DCM-NH_4OH, 20:80:1) to give [4′,5′,6′,7′-$^2H_4$]indole-3-methylamine (17 mg, 0.11 mmol).

CS_2 (11 µL, 0.18 mmol) was added to a solution of [4′,5′,6′,7′-$^2H_4$]indole-3-methylamine (17 mg, 0.11 mmol) and Et_3N (50 µL, 0.36 mmol) in pyridine (0.5 mL) at rt. After stirring for 15 min, C^2H_3I (11 µL, 0.18 mmol) was added and stirring was continued for additional 10 min. The mixture was diluted with H_2SO_4 5% and extracted with EtOAc. The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (EtOAc-hexane, 1:4) to give **1a** as a white solid (15 mg, 0.062 mmol, 52% yield from oxime).

[4',5',6',7'-$^2H_4$]brassinin (**1b**) was synthesised similarly but replacing C^2H_3I with CH_3I.

[1] E. E. Yaya, Ph.D. thesis, University of Saskatchewan, 2013.

5.2 Administration of isotopically labelled compounds

Four-week old plants were sprayed with aq. $CuCl_2$ solution (2 mM) and incubated under fluorescent light for 24 h. Stems with 3-5 leaves were cut and immediately immersed in Falcon tubes (6.0 mL) containing an aqueous solution of the labelled compound (5 mL, 5×10^{-4} M dissolved in H_2O for [$^{13}C_{11}$,$^{15}N_2$]Trp or H_2O-MeOH, 1:9, v/v, for brassinin). After uptake of each solution, the tubes were refilled with H_2O and leaves were further incubated for another 24 h under continuous fluorescent light. The leaves were frozen in liquid N_2, and extracted with MeOH under shaking for 1 h. The MeOH extract was filtered, concentrated and rinsed with CH_2Cl_2. The CH_2Cl_2 extract was concentrated and analyzed by HPLC-DAD-ESI-MS. Elicited leaves fed with non-labelled compounds or H_2O were used as controls. Experiments with non-elicited leaves were carried out in a similar way, but omitting spraying with $CuCl_2$ solution. All experiments were conducted in triplicate. All extracts were analysed by HPLC-DAD and HPLC-ESI-MS as described below.

5.3 Analysis and quantification of brassinin (1) and cyclonasturlexin (15) in leaf extracts by HPLC-DAD and HPLC-ESI-MS

The concentrations of brassinin (**1**, **1a**, **1b**) and cyclonasturlexin (**15**, **15a**, **15b**, **15c**) per g of leaf fresh weight in each independent experiment were determined from calibration curves built with authentic synthetic compounds. Slopes of calibration curves built with deuterated brassinins (**1a**, **1b**) were identical to those of non-deuterated brassinin (**1**).

The percentages of isotopically labelled compounds (%) ± SD (standard deviation of triplicate samples) present in extracts of leaves of watercress treated with (*S*)-[$^{13}C_{11}$,$^{15}N_2$]Trp, [$^2H_3$C-S, 4',5',6',7'-$^2H_7$]brassinin (**1a**) and [4',5',6',7'-$^2H_4$]brassinin (**1b**) were calculated according from HPLC-ESI-MS (normalized peak intensities) data according to the following equations:

For brassinin (**1a**) and (**1b**) – negative ion mode: % of ^{2}H incorporation = $\{[M - 1 + n]^-/([M - 1]^- + [M - 1 + n]^-)\} \times 100$ ± SD (standard deviation), where n=number of ^{2}H atoms.

For cyclonasturlexin (**15a**), (**15b**) and (**15c**), – positive ion mode: % of $^{13}C^{15}N$ or ^{2}H incorporation = $\{[M + 1 + n]^+/([M + 1]^+ + [M + 1 + n]^+)\} \times 100$ ± SD (standard deviation), where n=number of ^{13}C plus ^{15}N (**15c**) atoms or ^{2}H (**15a** or **15b**) atoms.

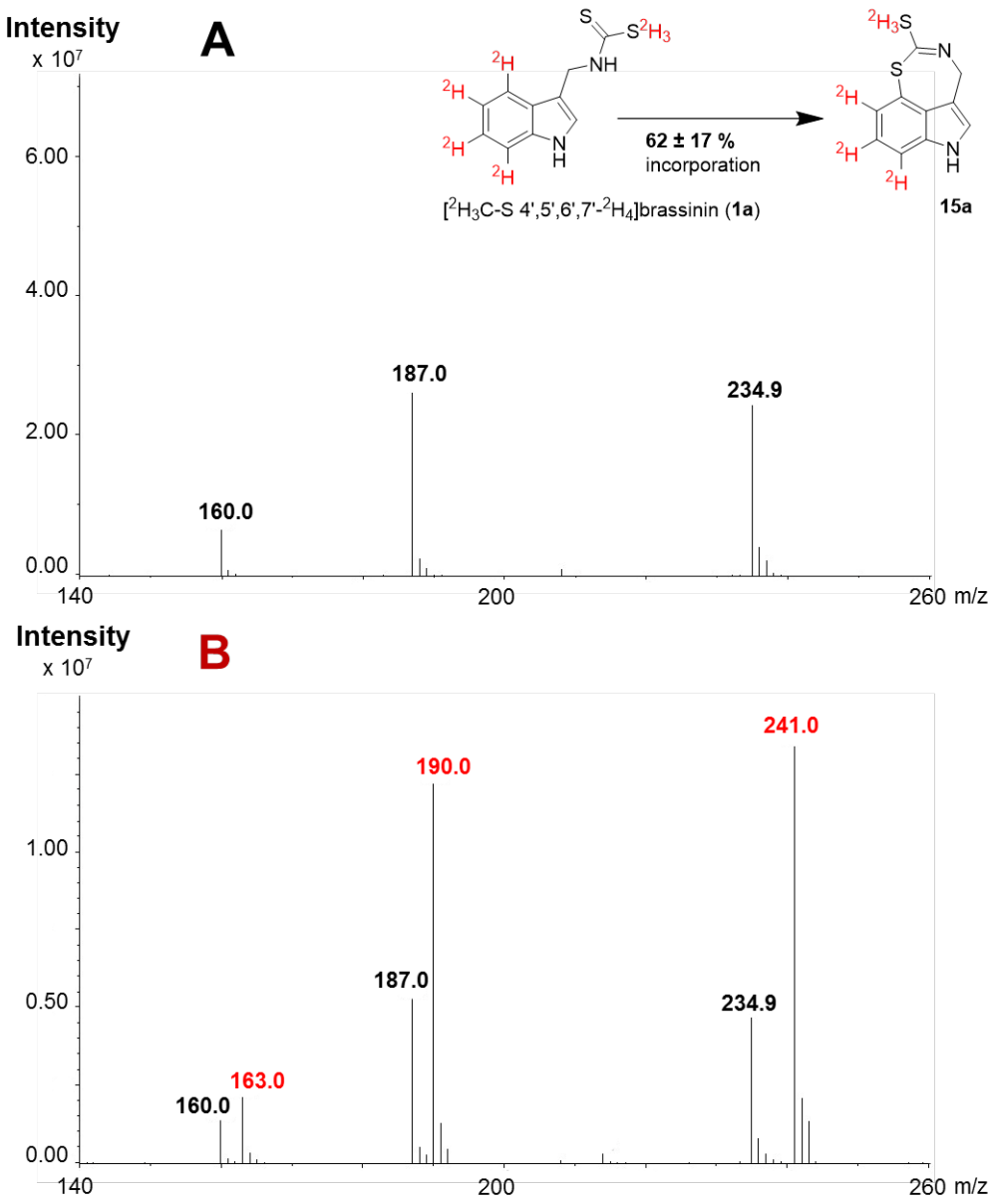


Fig. S5. ESI spectra (positive mode) of cyclonasturlexin (**15**): A, natural abundance, $[M+1]^+$ = 234.9, $[M\text{-}SCH_3]^+$ = 187.0, $[M\text{-}HNCSCH_3]^+$ = 160.0; B, elicited – resulting from incorporation of [$^2H_3$C-S 4',5',6',7'-$^2H_4$]brassinin (**1a**), $[M+1+6]^+$ = 241.0 and $[M+1]^+$ = 234.9, $[M+6\text{-}SC^2H_3]^+$ = 190.0 and $[M\text{-}SCH_3]^+$ = 187.0, $[M+6\text{-}HNCSC^2H_3]^+$ = 163.0 and $[M\text{-}HNCSCH_3]^+$ = 160.0.

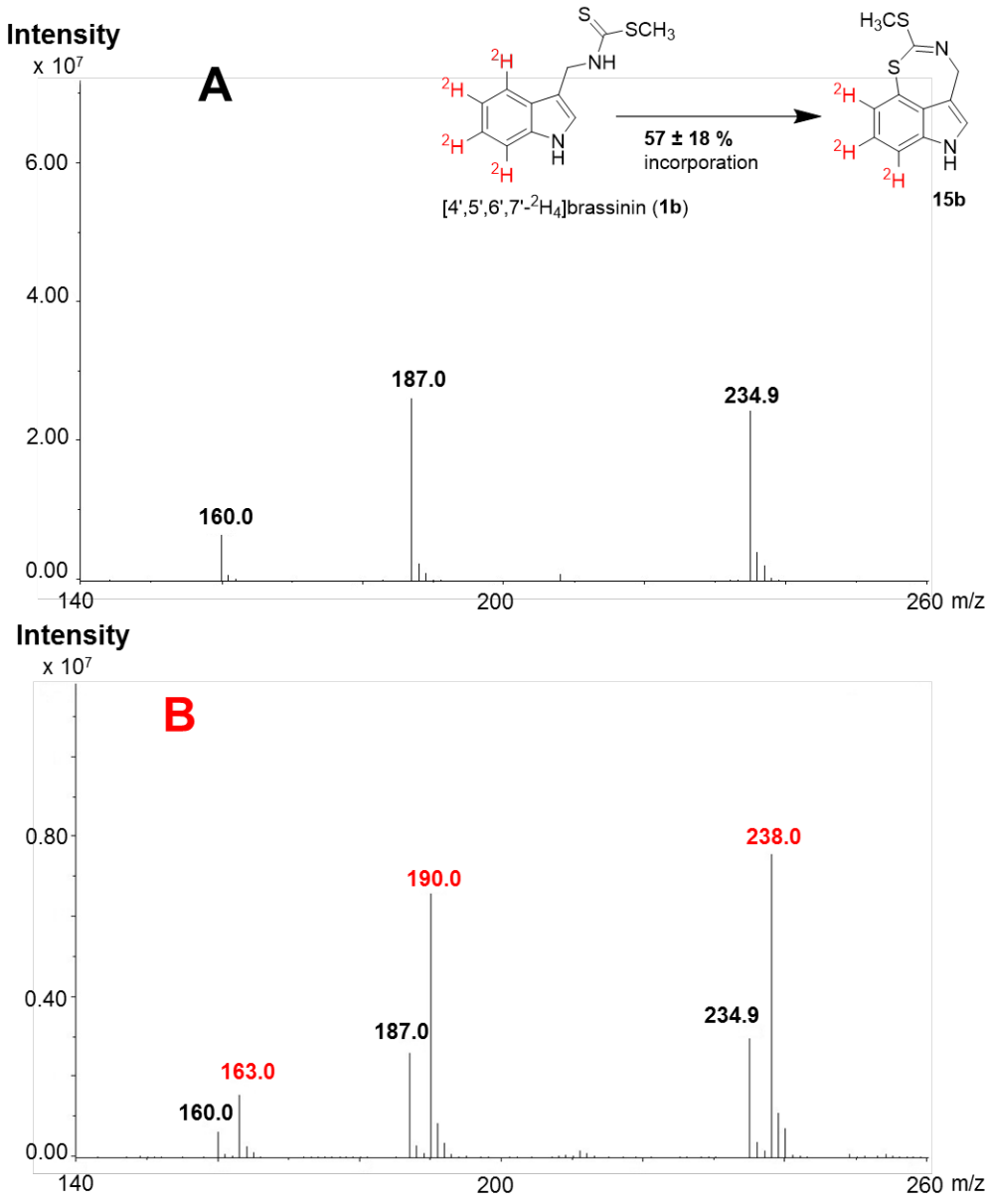


Fig. S6. ESI spectra (positive mode) of cyclonasturlexin (**15**): A, natural abundance, $[M+1]^+$ = 234.9, $[M\text{-}SCH_3]^+$ = 187.0, $[M\text{-}HNCSCH_3]^+$ = 160.0; B, elicited – resulting from incorporation of [4',5',6',7' -$^2H_4$]brassinin (**1b**), $[M+1+3]^+$ = 238.0 and $[M+1]^+$ = 234.9, $[M+3\text{-}SCH_3]^+$ = 190.0 and $[M\text{-}SCH_3]^+$ = 187.0, $[M+3\text{-}HNCSCH_3]^+$ = 163.0 and $[M\text{-}HNCSCH_3]^+$ = 160.0.

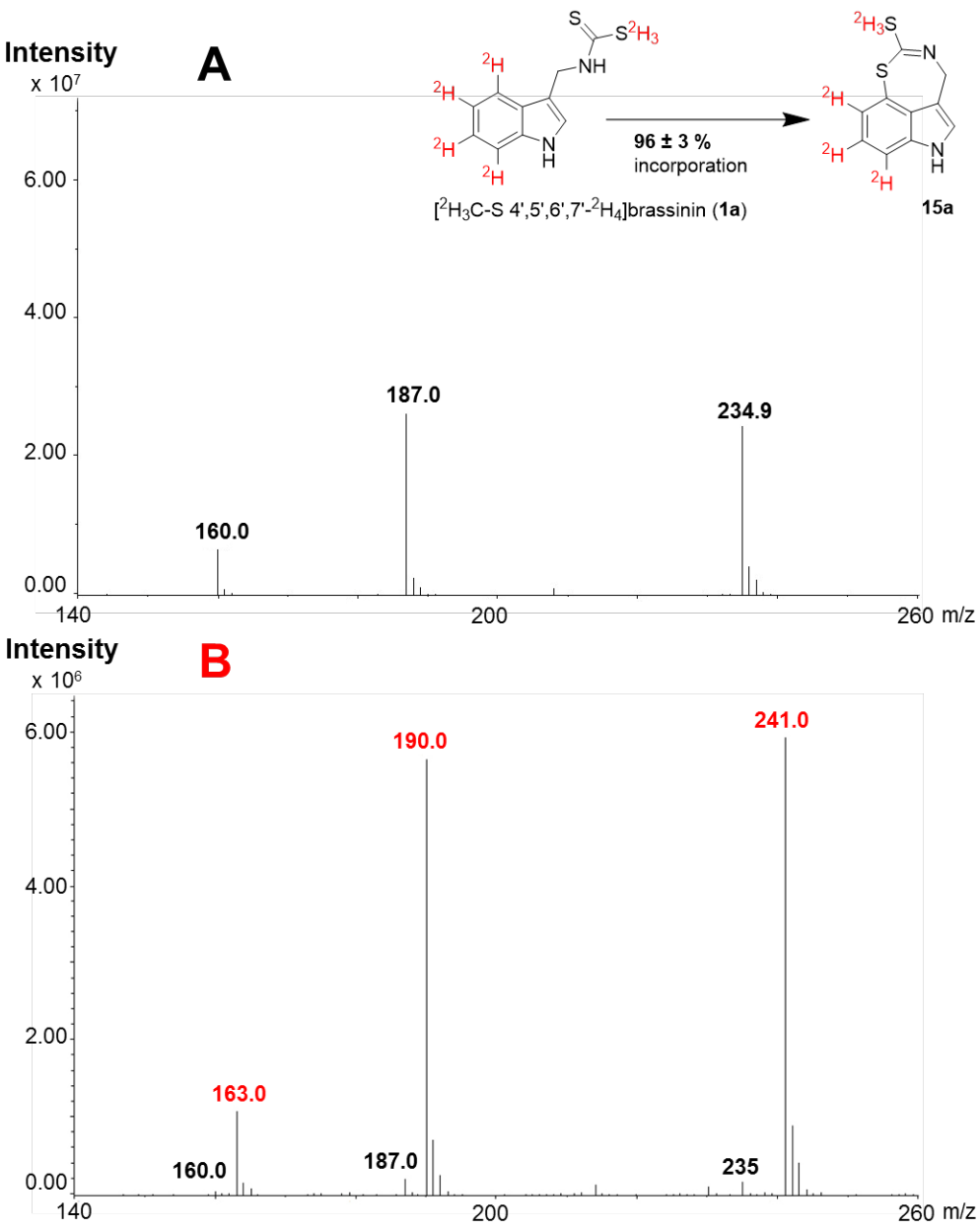


Fig. S7. ESI spectra (positive mode) of cyclonasturlexin (**15**): A, natural abundance, $[M+1]^+$ = 234.9, $[M\text{-}SCH_3]^+$ = 187.0, $[M\text{-}HNCSCH_3]^+$ = 160.0; B, non-elicited – resulting from incorporation of [$^2H_3$C-S 4',5',6',7'-$^2H_4$]brassinin (**1a**), $[M+1+6]^+$ = 241.0 and $[M+1]^+$ = 234.9, $[M+6\text{-}SC^2H_3]^+$ = 190.0 and $[M\text{-}SCH_3]^+$ = 187.0, $[M+6\text{-}HNCSC^2H_3]^+$ = 163.0 and $[M\text{-}HNCSCH_3]^+$ = 160.0.

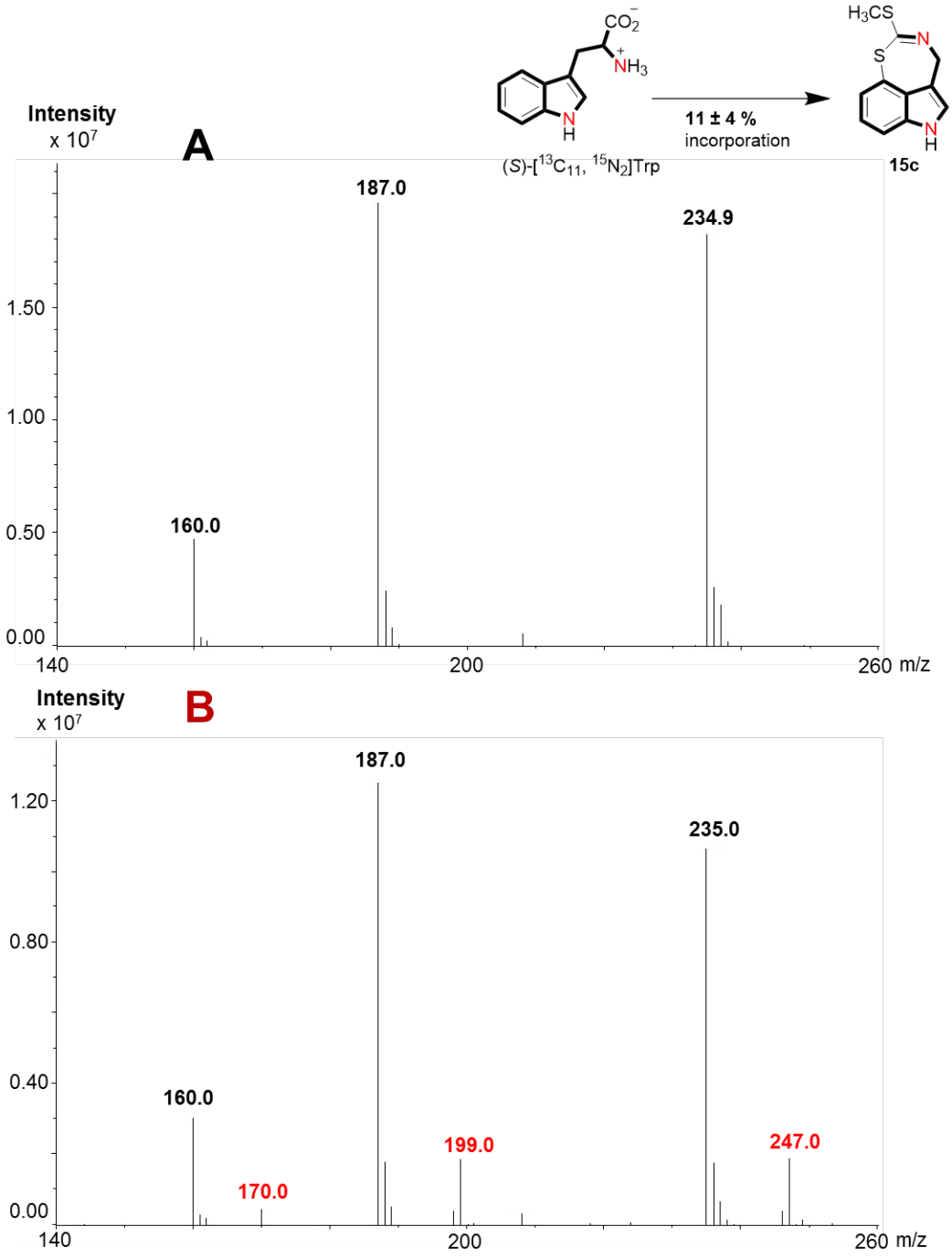


Fig. S8. ESI spectra (positive mode) of cyclonasturlexin (**15**): A, natural abundance, $[M+1]^+$ = 234.9, $[M\text{-}SCH_3]^+$ = 187.0, $[M\text{-}HNCSCH_3]^+$ = 160.0; B, elicited – resulting from incorporation of (*S*)-[$^{13}C_{11}$, $^{15}N_2$]Trp, $[M+1+12]^+$ = 247.0 and $[M+1]^+$ = 234.9, $[M+12\text{-}SCH_3]^+$ = 199.0 and $[M\text{-}SCH_3]^+$ = 187.0, $[M+12\text{-}H^{15}N^{13}CSCH_3]^+$ = 170.0 and $[M\text{-}HNCSCH_3]^+$ = 160.0.

6 NMR Spectra of new compounds

15,16, 21, 22, 24