ELECTRONIC SUPPLEMENTARY MATERIAL INFORMATION

Interrogation of biosynthetic pathways of the cruciferous phytoalexins nasturlexins with isotopically labelled compounds

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1 Electronic supplementary information (ESI) available: tables of isotope incorporation data for compounds 18a, 20a, 21a, 5a
and Phe; synthesis of new compounds 18a, 21a, 26c, incorporation schemes with ESI-HR-MS data and ESI spectra;
references; 1H and 13C NMR spectra of new compounds.
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Table S1. Metabolism of [2,3,4,5,6,7,8-D₇]-(E)-styril glucosinolate (18a) in elicited and non-elicited leaves of upland cress plants (Barbara verna).

<table>
<thead>
<tr>
<th>Metabolites detected in leaf extracts</th>
<th>Elicited leaves</th>
<th>Non-elicited leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of Deuterium (Conc., µmol/100 g)ᵃ</td>
<td>% Deuterium (Conc., µmol/100 g)ᵇ</td>
</tr>
<tr>
<td>[2,3,4,5,6,7,8-D₇]-(E)-styril glucosinolate (18a)</td>
<td>ca. 99ᵇ (156 ± 40)</td>
<td>ca. 99%ᵇ (157 ± 54)</td>
</tr>
<tr>
<td>Gluconasturtiin (8)</td>
<td>NIᵇ (2,819 ± 507)</td>
<td>NIᵇ (6,171 ± 334)</td>
</tr>
<tr>
<td>Dihydronasturlexin C (23)</td>
<td>NIᵇ (≤ 0.3)</td>
<td>NIᵇ (ND)ᶜ</td>
</tr>
<tr>
<td>Nasturlexin C (11)</td>
<td>NIᵇ (16 ± 5)</td>
<td>NIᵇ (ND)ᶜ</td>
</tr>
<tr>
<td>Nasturlexin D (13)</td>
<td>NIᵇ (28 ± 10)</td>
<td>NIᵇ (ND)ᶜ</td>
</tr>
</tbody>
</table>

ᵃ Conc. = total concentration of non-labelled and labelled metabolites (µmol/100 g of fresh tissue, quantified by HPLC-DAD); values represent the mean and standard deviation of two independent experiments conducted in triplicate.
ᵇ Isotope incorporations calculated from HPLC-ESI-MS (peak intensities in negative mode); % of incorporation = \( \frac{[M – 1 + n]}{[M – 1] + [M – 1 + n]} \times 100 \) where n = number of D atoms; values represent the mean and standard deviation of two independent experiments conducted in triplicate.
ᶜ NI = no incorporation means D% ≤ 0.1, ESI-MS.
ᶜ ND = not detected (HPLC-DAD).

Table S2. Metabolism of [2,3,4,5,6-D₅]-(E,Z)-styril isothiocyanate (20a) in elicited leaves of upland cress plants (Barbara verna).

<table>
<thead>
<tr>
<th>Metabolites detected in leaf extracts</th>
<th>% of Deuterium (Conc., µmol/100 g)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2,3,4,5,6-D₅]-(E,Z)-Styril isothiocyanate (20a)</td>
<td>NIᵇ (ND)ᶜ</td>
</tr>
<tr>
<td>Dihydronasturlexin C (23)</td>
<td>NIᵇ (11 ± 13)</td>
</tr>
<tr>
<td>Nasturlexin C (11)</td>
<td>NIᵇ (12 ± 9)</td>
</tr>
<tr>
<td>Nasturlexin D (13)</td>
<td>NIᵇ (3 ± 2)</td>
</tr>
</tbody>
</table>

ᵃ Conc. = total concentration of non-labelled and labelled metabolites (µmol/100 g of fresh tissue, quantified by HPLC-DAD); values represent the mean and standard deviation of two independent experiments conducted in triplicate.
ᵇ NI = no incorporation means D% ≤ 0.1, ESI-MS.
ᶜ ND = not detected (HPLC-DAD).
**Table S3.** Metabolism of $[\text{D}_3\text{CS}]$methyl (2-hydroxy-2-[2,3,4,5,6-$\text{D}_5$]phenylethyl) dithiocarbamate (21a) in elicited leaves of upland cress plants (*Barbarea verna*).

<table>
<thead>
<tr>
<th>Metabolites detected in leaf extracts</th>
<th>% of Deuterium (Conc., $\mu$mol/100 g)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[D$_3$CS]methyl (2-hydroxy-2-[2,3,4,5,6-$\text{D}_5$]phenylethyl) dithiocarbamate (21a)</td>
<td>NI$^b$ (ND)$^c$</td>
</tr>
<tr>
<td>Dihydrornasturlexin C (23)</td>
<td>NI$^b$ (37 ± 8)</td>
</tr>
<tr>
<td>Nasturlexin C (11)</td>
<td>NI$^b$ (52 ± 28)</td>
</tr>
<tr>
<td>Nasturlexin D (13)</td>
<td>NI$^b$ (99 ± 25)</td>
</tr>
</tbody>
</table>

$^a$ Conc. = total concentration of non-labelled and labelled metabolites ($\mu$mol/100 g of fresh tissue, quantified by HPLC-DAD); values represent the mean and standard deviation of two independent experiments conducted in triplicate.

$^b$ NI = no incorporation means D% ≤ 0.1, ESI-MS.

$^c$ ND = not detected (HPLC-DAD).

**Table S4.** Metabolism of [2,3,4,5,6-$\text{D}_5$]phenylalanine in elicited leaves of watercress (*Nasturtium officinale*).

<table>
<thead>
<tr>
<th>Metabolites detected in leaf extracts</th>
<th>% of Deuterium (Conc., $\mu$mol/100 g)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[2,3,4,5,6-$\text{D}_5$]Phenylalanine</td>
<td>52 ± 14$^b$ (369 ± 147)</td>
</tr>
<tr>
<td>Gluconasturtiin (8)</td>
<td>NI$^c$ (467 ± 132)</td>
</tr>
<tr>
<td>Nasturlexin B (2)</td>
<td>NI$^c$ (8 ± 6)</td>
</tr>
<tr>
<td>[2,3,5,6-$\text{D}_4$]Tridentatol C (3)</td>
<td>NI$^c$ (≤2)</td>
</tr>
<tr>
<td>Cyclonasturlexin (4)</td>
<td>NI$^c$ (≤5)</td>
</tr>
</tbody>
</table>

$^a$ Conc. = total concentration of natural abundance and labelled metabolites (nmol/g of fresh tissue, quantified by HPLC-DAD); values represent the mean and standard deviation of triplicate samples.

$^b$ Isotope incorporations calculated from HPLC-ESI-MS (peak intensities in positive mode); % of incorporation = $\{[M + 1 + n]^+ /[M + 1]^+ + [M + 1 + n]^+]\} \times 100$, where n = number of D atoms; values represent the mean and standard deviation of triplicate samples.

$^c$ NI = no incorporation means D% ≤ 0.1, ESI-MS.
### Table S5. Metabolism of [D\textsubscript{3}C-S 4,5,6,7-D\textsubscript{4}]brassinin (5a) in elicited and non-elicited leaves of watercress (Nasturtium officinale).

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Elicited leaves</th>
<th>Non-elicited leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of Deuterium</td>
<td>% of Deuterium</td>
</tr>
<tr>
<td></td>
<td>(Conc., µmol/100 g)\textsuperscript{a}</td>
<td>(Conc., µmol/100 g)\textsuperscript{a}</td>
</tr>
<tr>
<td>[D\textsubscript{3}CS, 4,5,6,7-D\textsubscript{4}]Brassinin (5a)</td>
<td>≥ 97\textsuperscript{b} (9 ± 6)</td>
<td>≥ 97\textsuperscript{b} (39 ± 8)</td>
</tr>
<tr>
<td>[D\textsubscript{3}SC, 5,6,7-D\textsubscript{3}]Cyclonasturlexin (4b)</td>
<td>62 ± 17\textsuperscript{b} (74 ± 26)</td>
<td>96 ± 3\textsuperscript{b} (≤ 5)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Conc. = total concentration of non-labelled and labelled metabolites (nmol/g of fresh tissue, quantified by HPLC-DAD). Values represent the mean and standard deviation of triplicate samples.

\textsuperscript{b} Isotope incorporations calculated from HPLC-ESI-MS (peak intensities in positive mode); % of incorporation = \(\frac{([M + 1 + n])}{([M + 1] + [M + 1 + n])} \times 100\), where \(n\) = number of D atoms, values represent the mean and S.D. of triplicate samples.

### Experimental

#### Synthesis of new compounds

**[2,3,4,5,6,7,8-D\textsubscript{7}]-**(E)-Styryl glucosinolate (18a)

![Diagram of the synthesis of [2,3,4,5,6,7,8-D\textsubscript{7}]-**(E)-Styryl glucosinolate (18a).](image)

**Scheme S1.** Synthesis of [2,3,4,5,6,7,8-D\textsubscript{7}]-**(E)-Styryl glucosinolate (18a).

A suspension of LiAlH\textsubscript{4} in dry THF (0.75 mL) was added dropwise to a solution of \textit{trans-}[2,3,4,5,6,7,8-D\textsubscript{7}]cinnamic acid (42a, 98\%D, 150 mg, 0.97 mmol) in dry THF (0.75 mL) at 0 °C.\textsuperscript{1} After stirring at rt for 30 min, the reaction mixture was added dropwise to a vigorously stirred suspension of PCC (420 mg,
1.94 mmol) in dry DCM (2.5 mL). The mixture was stirred for 2 h and filtered through celite. The filtrate was concentrated and fractionated by FCC (EtOAc-hexane, 1:9) to give trans-[2,3,4,5,6,7,8-D7]cinnamaldehyde (43a) (55 mg, 0.40 mmol, 41%). A solution of NH2OH-HCl (27 mg, 0.38 mmol) and NaOAc (31 mg, 0.38 mmol) in H2O (1.0 mL) was added to a solution aldehyde 43a (35 mg, 0.25 mmol) in EtOH (1.0 mL) at rt. After stirring for 1 h, the mixture was concentrated, diluted with H2O and extracted with EtOAc. The organic extract was dried over Na2SO4 and concentrated to give oxime 44a (39 mg, 0.25 mmol, 100%). NCS (68 mg, 0.51 mmol) was added in portions to a solution of oxime 44a (39 mg, 0.25 mmol) and pyridine (0.10 mL) in DCM (1.0 mL) at 0 °C. After stirring at rt for 30 min, a solution of 1-β-D-thioglucose tetraacetate (82 mg, 0.22 mmol) and Et3N (105 µL, 0.75 mmol) in DCM (1.0 mL) was added and stirring was continued for 4 h. The mixture was diluted with 1 M H2SO4 and extracted with DCM. The organic extract was washed with EtOH (1.0 mL) at rt. After stirring for 1 h, the mixture was concentrated, diluted with MeOH and extracted with EtOAc. The organic extract was dried over Na2SO4 and concentrated to give thioglucoame 45b (101 mg, 0.20 mmol, 91%) as a yellowish solid.

**Compound 45a:** ¹H NMR (500 MHz, CDCl₃): δ 8.06 (1H, s), 5.22 (1H, t, J = 9.0 Hz), 5.15-5.08 (2H, m), 5.05 (1H, d, J = 10.0 Hz), 4.25 (1H, dd, J = 12.0, 5.5 Hz), 4.12 (1H, dd, J = 12.0, 2.0 Hz), 3.74-3.72 (1H, m), 2.06 (3H, s), 2.02 (4H, m). HR-ESI-MS m/z [M + H]+: calc. for C₂₃H₂₧O₂N₄O₆S₂: 517.1868, found 517.1880 (28%), 331.10 (100%). PySO₃ (80 mg, 0.60 mmol) was added to a solution of thioglucoame 45a (64 mg, 0.12 mmol) in dry DCM (3.0 mL) and the mixture was stirred for 40 °C for 18 h. Solvent was concentrated, H₂O was added, and the mixture was extracted with MeOH-CHCl₃ (1:4). The organic extract was dried over Na₂SO₄, concentrated and separated by FCC (MeOH-DCM, 1:9) to give compound 46a (64 mg, 0.11 mmol, 89%) as a solid.

**Compound 46a:** ¹H NMR (500 MHz, CD₂OD): δ 5.40-5.34 (2H, m), 5.09-5.04 (2H, m), 4.25 (1H, dd, J = 12.5, 6.0 Hz), 4.12 (1H, dd, J = 12.5, 2.0 Hz), 4.02-3.98 (1H, m), 2.04 (3H, s), 2.01 (3H, s), 1.97 (3H, s), 1.92 (3H, s). HR-ESI-MS m/z [M-H]: calc. for C₂₃H₂₧O₂N₄O₆S₂: 595.1279, found 595.1301 (100%). K₂CO₃ (28 mg, 0.20 mmol) was added to a solution of 46a (40 mg, 0.067 mmol) in MeOH (2.0 mL) at rt. After stirring for 1 h, the mixture was neutralized with acetic acid (ca. 1 drop) and filtered. The filtrate was concentrated to give [2,3,4,5,6,7,8-D7]-{(E)-styryl glucosinolate (18a) in a quantitative yield. The spectroscopic data of the non-labelled compound 18 was in agreement with reported literature.²

**Compound 18a:** HPLC tᵣ = 12.1 min (method B). ¹H NMR (500 MHz, D₂O): δ 5.04 (1H, d, J = 9.5 Hz), 3.88 (1H, dd, J = 12.5, 2.0 Hz), 3.70 (1H, dd, J = 12.5, 5.0 Hz), 3.56-3.44 (4H, m). ¹³C NMR (125 MHz, D₂O): δ 161.1, 134.6, 82.7, 80.5, 77.1, 72.0, 69.2, 60.6. HR-ESI-MS m/z [M-K]: calc. for C₁₅H₁₈O₂N₂S₂: 427.0857, found 427.0868 (100%). UV (HPLC, CH₃CN–H₂O) λₘₐₓ (nm): 220, 280.
[D$_3$CS]Methyl [2,3,4,5,6-D$_5$]-2-hydroxy-2-phenylethyl)dithiocarbamate (21a)

Scheme S2. Synthesis of [D$_3$CS]Methyl [2,3,4,5,6-D$_5$]-2-hydroxy-2-phenylethyl)dithiocarbamate (21a)

Compound 47a was synthesized as previously reported.$^3$ CS$_2$ (9 µL, 0.15 mmol) was added to a solution of 47a (20 mg, 0.14 mmol) in pyridine (0.50 mL) at 0 °C, followed by Et$_3$N (60 µL, 0.42 mmol). After stirring for 15 min, CD$_3$I (9 µL, 0.15 mmol) was added and stirring was continued for 30 min. The mixture was diluted with EtOAc and washed with 1 M H$_2$SO$_4$. The organic extract was dried over Na$_2$SO$_4$ and concentrated to give compound 21a (30 mg, 0.13 mmol, 93%, D ≥ 99%, determined by HR-FD-MS).

Compound 21a: HPLC $t_R = 12.4$ min (method A). $^1$H NMR (600 MHz, CDCl$_3$): δ 7.48 (1H, br), 5.05 (1H, dd, $J = 8.5$, 3.5 Hz), 4.36-4.32 (1H, m), 3.68-3.63 (1H, m), 2.52 (1H, br), and a rotamer at 8.15 (br), 4.98 (br), 3.78 (br), 3.54 (br). HR-FD-MS $m/z$: calc. for C$_{10}$H$_{12}$H$_8$NOS$_2$: 235.0941, found 235.0934. UV (HPLC, CH$_3$CN – H$_2$O) $\lambda_{max}$ (nm): 250, 270.

Compound 21: HPLC $t_R = 12.4$ min (method A). $^1$H NMR (600 MHz, CDCl$_3$): δ 7.56 (1H, br), 7.40-7.31 (5H, m), 5.04 (1H, dd, $J = 9.0$, 3.5 Hz), 4.34-4.30 (1H, m), 3.67-3.63 (1H, m), 2.67 (1H, s), 2.63 (3H, s), and a rotamer at 8.23 (br), 4.96 (br), 3.77 (br), 3.53 (br). $^{13}$C NMR (150 MHz, CDCl$_3$): δ 200.4, 141.2, 128.9, 128.5, 126.0, 72.5, 53.9, 18.5. HR-FD-MS $m/z$: calc. for C$_{10}$H$_{13}$NOS$_2$: 227.0439, found 227.0441. UV (HPLC, CH$_3$CN – H$_2$O) $\lambda_{max}$ (nm): 250, 270.

[D$_3$CS]Dihydronasturlexin D (26c)

Scheme S3. Synthesis of [D$_3$CS]dihydronasturlexin D (26c)
A solution of SOCl₂ (362 µL, 5.0 mmol) and DMF (10 µL) in CHCl₃ (1.0 mL) was added dropwise to a suspension of norphenylephrine hydrochloride (48) (95 mg, 0.50 mmol) in CHCl₃ (2.0 mL) at rt. After stirring for 24 h, solvent was removed and the mixture was washed with Et₂O and concentrated to give compound 49. CS₂ (60 µL, 1.0 mmol) was added to a suspension of 49 in CHCl₃ (3.0 mL) at rt, followed by Et₃N (210 µL, 1.5 mmol). After stirring for 15 min, CD₃I (60 µL, 1.0 mmol) was added and stirring was continued for 1 h. H₂O was added and the mixture was extracted with CHCl₃. The organic extract was dried over Na₂SO₄, concentrated and separated by FCC (EtOAc-hexane, 1:3) to give compound [D₃CS]Dihydronasturlexin D (26c) (52 mg, 0.23 mmol, 46%; D ≥ 99%, determined by HR-FD-MS) as a solid.

Compound 26c: HPLC tᵣ = 11.5 min (method A). ¹H NMR (600 MHz, CD₃CN): δ 7.15 (1H, t, J = 8.0 Hz), 6.79 (1H, d, J = 8.0 Hz), 6.75 (1H, t, J = 2.0 Hz), 6.72-6.70 (1H, m), 5.08 (1H, dd, J = 8.5, 5.0 Hz), 4.44 (1H, dd, J = 15.0, 8.5 Hz), 4.29 (1H, dd, J = 15.0, 5.0 Hz). HR-FD-MS m/z [M]⁺ calc. for C₁₀H₈D₃NOS₂: 228.0470, found 228.0461 (100%). UV (HPLC, CH₃CN-H₂O) λ_max (nm): 220, 280.

Compound 26: HPLC tᵣ = 11.5 min (method A). ¹H NMR (600 MHz, CD₃CN): δ 7.16 (1H, t, J = 8.0 Hz), 6.79 (1H, d, J = 8.0 Hz), 6.75 (1H, s), 6.72-6.70 (1H, m), 5.09 (1H, dd, J = 8.5, 5.5 Hz), 4.45 (1H, dd, J = 15.0, 8.5 Hz), 4.30 (1H, dd, J = 15.0, 5.0 Hz), 2.55 (3H, s). ¹³C NMR (150 MHz, CD₃CN): δ 166.1, 158.6, 144.8, 131.4, 119.7, 116.1, 115.0, 73.0, 57.4, 16.0. HR-FD-MS m/z [M]⁺ calc. for C₁₀H₁₁NOS₂: 225.0282, found 225.0277 (100%). UV (HPLC, CH₃CN-H₂O) λ_max (nm): 220, 280.
ESI-MS spectra of incorporation experiments in upland cress (*Barbarea verna*)

Incorporation of [2,3,4,5,6-D₅]gluconasturtiin (8a)

[2,3,4,5,6-D₅]Nasturlexin C (11a): HR-ESI-MS *m/z* [M + H]⁺, calculated for C₁₀H₅₂H₂N₂S₂, 213.0569, found 213.0578.

[2,3,5,6-D₄]Nasturlexin D (13b): HR-ESI-MS *m/z* [M + H]⁺, calculated for C₁₀H₆₂H₄NOS₂, 228.0455, found 228.0459.

**Figure S1** ESI-MS spectra (positive mode) of nasturlexins C (11/11a) and D (13/13b) in extracts of elicited leaves (A and B) fed with [2,3,4,5,6-D₅]gluconasturtiin (8a) and control leaves.
Incorporation of [2,4,6-D$_3$,$^{15}$N]-3-hydroxygluconasturtiin (9a)

[2,4,6-D$_3$,$^{15}$N]Dihyronasturlexin D (26a): HR-ESI-MS $m/z$ [M + H]$^+$, calculated for C$_{10}$H$_9$D$_3$H$_3$$^{15}$NS$_2$: 230.0519, found 230.0512.

[2,4,6-D$_3$,$^{15}$N]Nasturlexin D (13a): HR-ESI-MS $m/z$ [M + H]$^+$, calculated for C$_{10}$H$_7$D$_3$$^{15}$NS$_2$: 228.0362, found 228.0355.
Figure S2  ESI-MS spectra (positive mode) of 3-hydroxyphenylethyl isothiocyanate (24/24a), dihydronasturlexin D (26/26a), and nasturlexin D (13/13a) in extracts of elicited leaves (A, B and C) fed with [2,4,6-D₃,¹⁵N]-3-hydroxyphenylethyl glucosinolate (9a) and control leaves.

Incorporation of [2,3,4,5,6-D₅]phenylethyl isothiocyanate (19a)

[2,3,4,5,6-D₅]Nasturlexin C (11a): HR-ESI-MS m/z [M + H]⁺, calculated for C₁₀H₅₂H₅NS₂, 213.0569, found 213.0557.

[2,3,5,6-D₄]Nasturlexin D (13b): HR-ESI-MS m/z [M + H]⁺, calculated for C₁₀H₆₂H₆NOS₂, 228.0455, found 228.0446.
Figure S3 ESI-MS spectra (positive mode) of dihydrornasturlexin C (23/23a), nasturlexins C (11/11a) and D (13/13b) in extracts of elicited leaves (A, B and C) fed with [2,3,4,5,6-D₅]phenylethyl isothiocyanate (19a) and control leaves.
Incorporation of [2,4,6-D_3,^{15}N]-3-hydroxyphenylethyl isothiocyanate (24a)

[2,4,6-D_3,^{15}N]Dihydronasturlexin D (26a): HR-ESI-MS m/z [M + H]^+, calculated for C_{10}H_{12}H_{3}^{15}NS_{2}, 230.0519, found 230.0536.

[2,4,6-D_3,^{15}N]Nasturlexin D (13a): HR-ESI-MS m/z [M + H]^+, calculated for C_{10}H_{7}H_{3}^{15}NS_{2}, 228.0362, found 228.0399.

Figure S4 ESI-MS spectra (positive mode) of dihydronasturlexin D (26/26a) and nasturlexin D (13/13a) in extracts of elicited leaves (A and B) fed with [2,4,6-D_3,^{15}N]-3-hydroxyphenylethyl isothiocyanate (24a) and control leaves.
[2,3,4,5,6-D_5]Dihyronasturlexin C (23a): HR-ESI-MS m/z [M + H]^+, calculated for C_{10}H_{7}H_2NS_2, 215.0725, found 215.0737.

[2,3,4,5,6-D_5]Nasturlexin C (11a): HR-ESI-MS m/z [M + H]^+, calculated for C_{10}H_5H_5NS_2, 213.0569, found 213.0557.

[2,3,5,6-D_4]Nasturlexin D (13b): HR-ESI-MS m/z [M + H]^+, calculated for C_{10}H_6^2H_4NOS_2, 228.0455, found 228.0446.

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**Intensity**

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**Figure S5** ESI-MS spectra (positive mode) of dihydronasturlexin C (23/23a), nasturlexins C (11/11a) and D (13/13b) in extracts of elicited leaves (A, B and C) fed with [2,3,4,5,6-D$_5$]nasturlexin A (1a) and control leaves.

**Incorporation of [D$_3$CS, 2,4,6-D$_3$,$^{15}$N]-3-hydroxynasturlexin A (25b)**

[D$_3$CS, 2,4,6-D$_3$,$^{15}$N]Dihydronasturlexin D (26b): HR-ESI-MS $m/z$ [M + H]$^+$, calculated for C$_{10}$H$_6^2$H$_6^{15}$NS$_2$, 233.0707, found 233.0698.

[D$_3$CS, 2,4,6-D$_3$,$^{15}$N]Nasturlexin D (13c): HR-ESI-MS $m/z$ [M + H]$^+$, calculated for C$_{10}$H$_4^2$H$_6^{15}$NS$_2$, 231.0551, found 231.0538.
Figure S6 ESI-MS spectra (positive mode) of dihydronasturlexin D (26/26b) and nasturlexin D (13/13c) in extracts of elicited leaves (A and B) fed with [D$_3$CS, 2,4,6-D$_3$,$^{15}$N]-3-hydroxynasturlexin A (25b) and control leaves.

Incorporation of methyl [2,3,4,5,6-D$_5$]-(E)-styryl dithiocarbamate (22a)

[2,3,4,5,6-D$_5$]Nasturlexin C (11a): GC-FI-MS m/z [M]$^+$, calculated for C$_{10}$H$_9$H$_2$NS$_2$, 212.0490, found 212.0487.
Figure S7 ESI-MS spectra (positive mode) of nasturlexin C (11/11a) in extracts of elicited leaves (A) fed with methyl [2,3,4,5,6-D$_5$]-(E)-styryl dithiocarbamate (22a) and control leaves.

Incorporation of [2,3,4,5,6-D$_5$]dihyronasturlexin C (23a)

[2,3,4,5,6-D$_5$]Nasturlexin C (11a): HR-ESI-MS $m/z$ [M + H]$^+$, calculated for C$_{10}$H$_7$H$_5$NS$_2$, 213.0569, found 213.0567.

[2,3,4,5,6-D$_5$]Nasturlexin C sulfoxide (12a): HR-ESI-MS $m/z$ [M + H]$^+$, calculated for C$_{10}$H$_7$H$_5$NOS$_2$, 229.0533, found 229.0546.

[2,3,5,6-D$_4$]Nasturlexin D (13b): HR-ESI-MS $m/z$ [M + H]$^+$, calculated for C$_{10}$H$_6$H$_4$NOS$_2$, 228.0455, found 228.0443.
Figure S8 ESI-MS spectra (positive mode) of nasturlexins C (11/11a), C sulfoxide (12/12a), and D (13/13b) in extracts of elicited leaves (A, B, and C) fed with [2,3,4,5,6-D5]dihydonasturlexin C (23a) and control leaves.
Incorporation of \([\text{D}_3\text{CS}]\text{dihydrornasturlexin D (26c)}\)

\([\text{D}_3\text{CS}]\text{Nasturlexin D (13d): HR-ESI-MS } m/z [M + H]^+, \text{ calculated for } C_{10}H_7^2H_3NOS_2, 227.0392, \text{ found } 227.0389.\]

Figure S9 ESI-MS spectra (positive mode) of nasturlexin D (13/13d) in extracts of elicited leaves (A) fed with \([\text{D}_3\text{CS}]\text{dihydrornasturlexin D (26c)}\) and control leaves.

Incorporation of \([2,3,4,5,6-\text{D}_5]\text{nasturlexin C (11a)}\)

\([2,3,4,5,6-\text{D}_5]\text{Nasturlexin C sulfoxide (12a): HR-ESI-MS } m/z [M + H]^+, \text{ calculated for } C_{10}H_7^2H_3NOS_2, 229.0533, \text{ found } 229.0540.\]

\([2,3,5,6-\text{D}_4]\text{Nasturlexin D (13b): HR-ESI-MS } m/z [M + H]^+, \text{ calculated for } C_{10}H_6^2H_4NOS_2, 228.0455, \text{ found } 228.0460.\]
Figure S10 ESI-MS spectra (positive mode) of nasturlexin C sulfoxide (12/12a) and nasturlexin D (13/13b) in extracts of elicited leaves (A and B) fed with [2,3,4,5,6-D₅]nasturlexin C (11a) and control leaves.

ESI-MS spectra of incorporation experiments in watercress (*Nasturtium officinale*)

Incorporation of [D₃CS 4,5,6,7-D₄]brassinin (5a)

[D₃CS 4,5,6,7-D₄]cyclonasturlexin (4a): HR-ESI-MS \( m/z \) \([M + H]^+\), calculated for C₁₁H₅₅H₆N₂S₂, 241.0735, found 241.0739.
Figure S11 ESI-MS spectra (positive mode) of cyclonasturlexin (4/4a) in extracts of elicited leaves (A) fed with [D₃CS 4,5,6,7-D₄]brassinin (5a) and control leaves.

Incorporation of [2,3,4,5,6-D₅]gluconasturtiin (8a)


[2,3,5,6-D₄]Tridentatol C (3a): HR-ESI-MS m/z [M + H]⁺, calculated for C₁₀H₆₂H₄NOS₂, 228.0449, found 228.0459.
Figure S12 ESI-MS spectra (positive mode) of nasturlexin B (2/2a) and tridentatol C (3/3a) in extracts of elicited leaves (A and B) fed with [2,3,4,5,6-D$_5$]gluconasturtiin (8a) and control leaves.

References


NMR spectra of new compounds 18a, 21, 21a, 26 and 26a
Compound 18a - $^1$H NMR Spectrum

D$_2$O
Compound 21a - $^1$H NMR Spectrum

CDCl$_3$
Compound 21 - $^{13}$C NMR Spectrum

CDCl$_3$
Compound 26c - $^1$H NMR Spectrum

CD$_3$CN
Compound 26 - $^1$H NMR Spectrum

CD$_3$CN
Compound 26 - $^{13}$C NMR Spectrum

CD$_3$CN