ELECTRONIC SUPPLEMENTARY MATERIAL INFORMATION

Interrogation of biosynthetic pathways of the cruciferous phytoalexins nasturlexins with isotopically labelled compounds[1]

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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; ^{1}H and ^{13}C NMR spectra of new compounds.

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Table S1. Metabolism of [2,3,4,5,6,7,8-D_7]-(*E*)-styryl glucosinolate (**18a**) in elicited and non-elicited leaves of upland cress plants (*Barbarea verna*).

Metabolites detected in leaf extracts	Elicited leaves	Non-elicited leaves
	% of Deuterium (Conc., μmol/100 g)[a]	% Deuterium (Conc., μmol/100 g)[a]
[2,3,4,5,6,7,8-D_7]-(*E*)-styryl glucosinolate (**18a**)	ca. 99[b] (156 ± 40)	ca. 99%[b] (157 ± 54)
Gluconasturtiin (**8**)	NI[c] (2,819 ± 507)	NI[c] (6,171 ± 334)
Dihydronasturlexin C (**23**)	NI[c] (≤ 0.3)	NI[c] (ND)[d]
Nasturlexin C (**11**)	NI[c] (16 ± 5)	NI[c] (ND)[d]
Nasturlexin D (**13**)	NI[c] (28 ± 10)	NI[c] (ND)[d]

[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 = $\{[M - 1 + n]^-/([M - 1]^- + [M - 1 + n]^-)\} \times 100$ 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.
[d] ND = not detected (HPLC-DAD).

Table S2. Metabolism of [2,3,4,5,6-D_5]-(*E,Z*)-styryl isothiocyanate (**20a**) in elicited leaves of upland cress plants (*Barbarea verna*).

Metabolites detected in leaf extracts	% of Deuterium (Conc., μmol/100 g)[a]
[2,3,4,5,6-D_5]-(*E,Z*)-Styryl isothiocyanate (**20a**)	NI[b] (ND)[c]
Dihydronasturlexin C (**23**)	NI[b] (11 ± 13)
Nasturlexin C (**11**)	NI[b] (12 ± 9)
Nasturlexin D (**13**)	NI[b] (3 ± 2)

[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 [D_3CS]methyl (2-hydroxy-2-[2,3,4,5,6-D_5]phenylethyl) dithiocarbamate (**21a**) in elicited leaves of upland cress plants (*Barbarea verna*).

Metabolites detected in leaf extracts	% of Deuterium (Conc., μmol/100 g)[a]
[D_3CS]methyl (2-hydroxy-2-[2,3,4,5,6-D_5]phenylethyl) dithiocarbamate (**21a**)	NI[b] (ND)[c]
Dihydronasturlexin C (**23**)	NI[b] (37 ± 8)
Nasturlexin C (**11**)	NI[b] (52 ± 28)
Nasturlexin D (**13**)	NI[b] (99 ± 25)

[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 S4. Metabolism of [2,3,4,5,6-D_5]phenylalanine in elicited leaves of watercress (*Nasturtium officinale*).

Metabolites detected in leaf extracts	% of Deuterium (Conc., μmol/100 g)[a]
[2,3,4,5,6-D_5]Phenylalanine	52 ± 14[b] (369 ± 147)
Gluconasturtiin (**8**)	NI[c] (467 ± 132)
Nasturlexin B (**2**)	NI[c] (8 ± 6)
[2,3,5,6-D_4]Tridentatol C (**3**)	NI[c] (≤2)
Cyclonasturlexin (**4**)	NI[c] (≤5)

[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_3C-S 4,5,6,7-D_4]brassinin (**5a**) in elicited and non-elicited leaves of watercress (*Nasturtium officinale*).

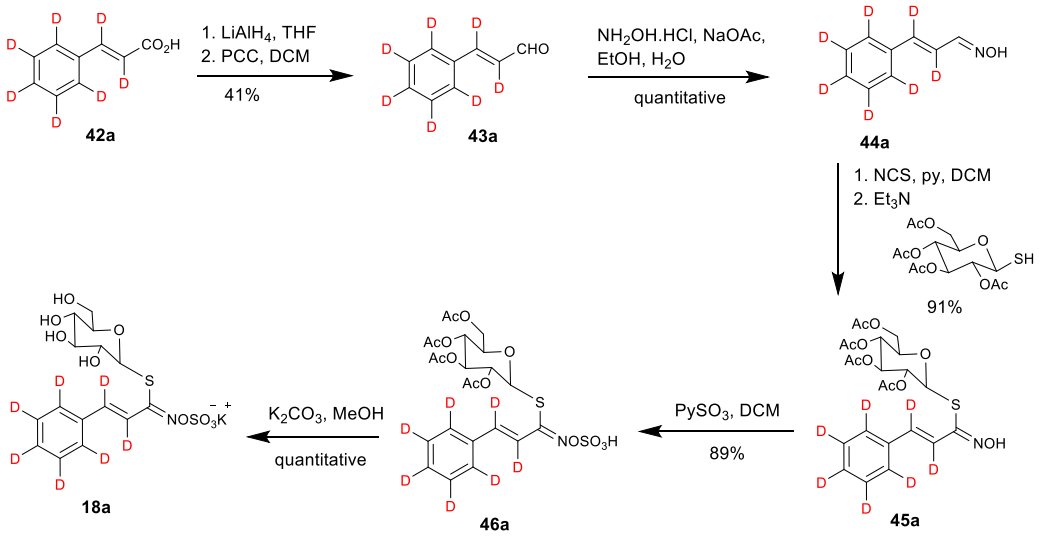
Metabolites	Elicited leaves	Non-elicited leaves
	% of Deuterium (Conc., μmol/100 g)[a]	% of Deuterium (Conc., μmol/100 g)[a]
[D_3CS, 4,5,6,7- D_4]Brassinin (**5a**)	≥ 97[b] (9 ± 6)	≥ 97[b] (39 ± 8)
[D_3SC, 5,6,7-D_3]Cyclonasturlexin (**4b**)	62 ± 17[b] (74 ± 26)	96 ± 3[b] (≤ 5)

[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.
[b] Isotope incorporations calculated from HPLC-ESI-MS (peak intensities in positive mode); % of incorporation = {[M + 1 + n]$^+$/([M + 1]$^+$ + [M + 1 + n]$^+$)} × 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_7]-(*E*)-Styryl glucosinolate (18a)



Scheme S1. Synthesis of [2,3,4,5,6,7,8-D_7]-(*E*)-styryl glucosinolate (**18a**).

A suspension of $LiAlH_4$ in dry THF (0.75 mL) was added dropwise to a solution of *trans*-[2,3,4,5,6,7,8-D_7]cinnamic acid (**42a**, 98%D, 150 mg, 0.97 mmol) in dry THF (0.75 mL) at 0 °C.[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-D_7]cinnamaldehyde (**43a**) (55 mg, 0.40 mmol, 41%). A solution of $NH_2OH{\cdot}HCl$ (27mg, 0.38 mmol) and NaOAc (31 mg, 0.38 mmol) in H_2O (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 H_2O and extracted with EtOAc. The organic extract was dried over Na_2SO_4 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 Et_3N (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 H_2SO_4 and extracted with DCM. The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (EtOAc-hexane, 1:1) to give thiohydroximate **45a** (101 mg, 0.20 mmol, 91%) as a yellowish solid.

Compound **45a**: ^{1}H NMR (500 MHz, $CDCl_3$): 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 (3H, s), 2.02 (3H, s), 2.00 (3H, s). HR-ESI-MS *m/z* $[M + H]^+$: calc. for $C_{23}H_{21}{}^2H_7NO_{10}S$: 517.1868, found 517.1880 (28%), 331.10 (100%).

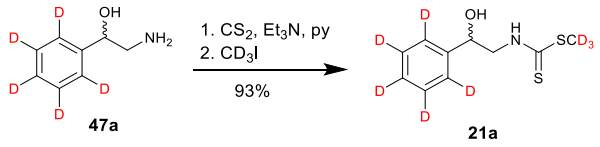
$PySO_3$ (80 mg, 0.60 mmol) was added to a solution of thiohydroximate **45a** (64 mg, 0.12 mmol) in dry DCM (3.0 mL) and the mixture was stirred at 40 °C for 18 h. Solvent was concentrated, H_2O was added, and the mixture was extracted with MeOH-$CHCl_3$ (1:4). The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (MeOH-DCM, 1:9) to give compound **46a** (64 mg, 0.11 mmol, 89%) as a solid.

Compound **46a**: ^{1}H NMR (500 MHz, CD_3OD): 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 $C_{23}H_{19}{}^2H_7NO_{13}S_2$: 595.1279, found 595.1301 (100%).

K_2CO_3 (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-D_7]-(*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 t_R = 12.1 min (method B). ^{1}H NMR (500 MHz, D_2O): 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). ^{13}C NMR (125 MHz, D_2O): 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 $C_{15}H_{11}{}^2H_7NO_9S_2$: 427.0857, found 427.0868 (100%). UV (HPLC, CH_3CN–H_2O) λ_{max} (nm): 220, 280.

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



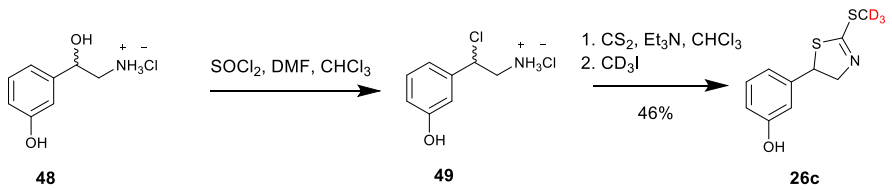
Scheme S2. Synthesis of [D$_3$CS]Methyl [2,3,4,5,6-D$_5$]-(2-hydroxy-2-phenylethyl) dithiocarbamate (**21a**)

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_3N (60 μL, 0.42 mmol). After stirring for 15 min, CD_3I (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_2SO_4. The organic extract was dried over Na_2SO_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 $C_{10}H_5{}^2H_8NOS_2$: 235.0941, found 235.0934. UV (HPLC, CH_3CN – H_2O) λ_{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_3CN – H_2O) λ_{max} (nm): 250, 270.

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



Scheme S3. Synthesis of [D$_3$CS]dihydronasturlexin D (**26c**)

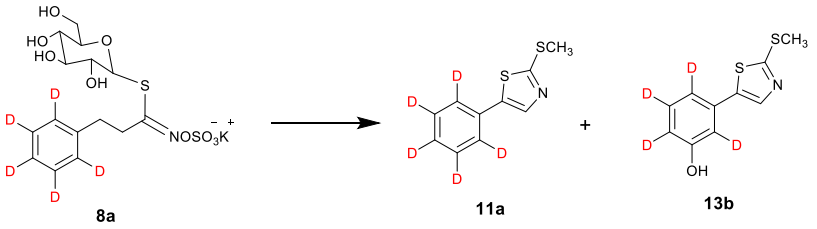
A solution of $SOCl_2$ (362 μL, 5.0 mmol) and DMF (10 μL) in $CHCl_3$ (1.0 mL) was added dropwise to a suspension of norphenylephrine hydrochloride (**48**) (95 mg, 0.50 mmol) in $CHCl_3$ (2.0 mL) at rt.[3] After stirring for 24 h, solvent was removed and the mixture was washed with Et_2O and concentrated to give compound **49**. CS_2 (60 μL, 1.0 mmol) was added to a suspension of **49** in $CHCl_3$ (3.0 mL) at rt, followed by Et_3N (210 μL, 1.5 mmol). After stirring for 15 min, CD_3I (60 μL, 1.0 mmol) was added and stirring was continued for 1 h. H_2O was added and the mixture was extracted with $CHCl_3$. The organic extract was dried over Na_2SO_4, concentrated and separated by FCC (EtOAc-hexane, 1:3) to give compound [D_3CS]Dihydronasturlexin D (**26c**) (52 mg, 0.23 mmol, 46%; D ≥ 99%, determined by HR-FD-MS) as a solid.

Compound **26c**: HPLC t_R = 11.5 min (method A). ^{1}H NMR (600 MHz, CD_3CN): 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 $C_{10}H_8{}^2H_3NOS_2$: 228.0470, found 228.0461 (100%). UV (HPLC, CH_3CN-H_2O) λ_{max} (nm): 220, 280.

Compound **26**: HPLC t_R = 11.5 min (method A). ^{1}H NMR (600 MHz, CD_3CN): 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). ^{13}C NMR (150 MHz, CD_3CN): 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 $C_{10}H_{11}NOS_2$: 225.0282, found 225.0277 (100%). UV (HPLC, CH_3CN-H_2O) λ_{max} (nm): 220, 280.

ESI-MS spectra of incorporation experiments in upland cress (*Barbarea verna)*

Incorporation of [2,3,4,5,6-D$_5$]gluconasturtiin (8a)



[2,3,4,5,6-D$_5$]Nasturlexin C (**11a**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_5{}^2H_5NS_2$, 213.0569, found 213.0578.

[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 228.0459.

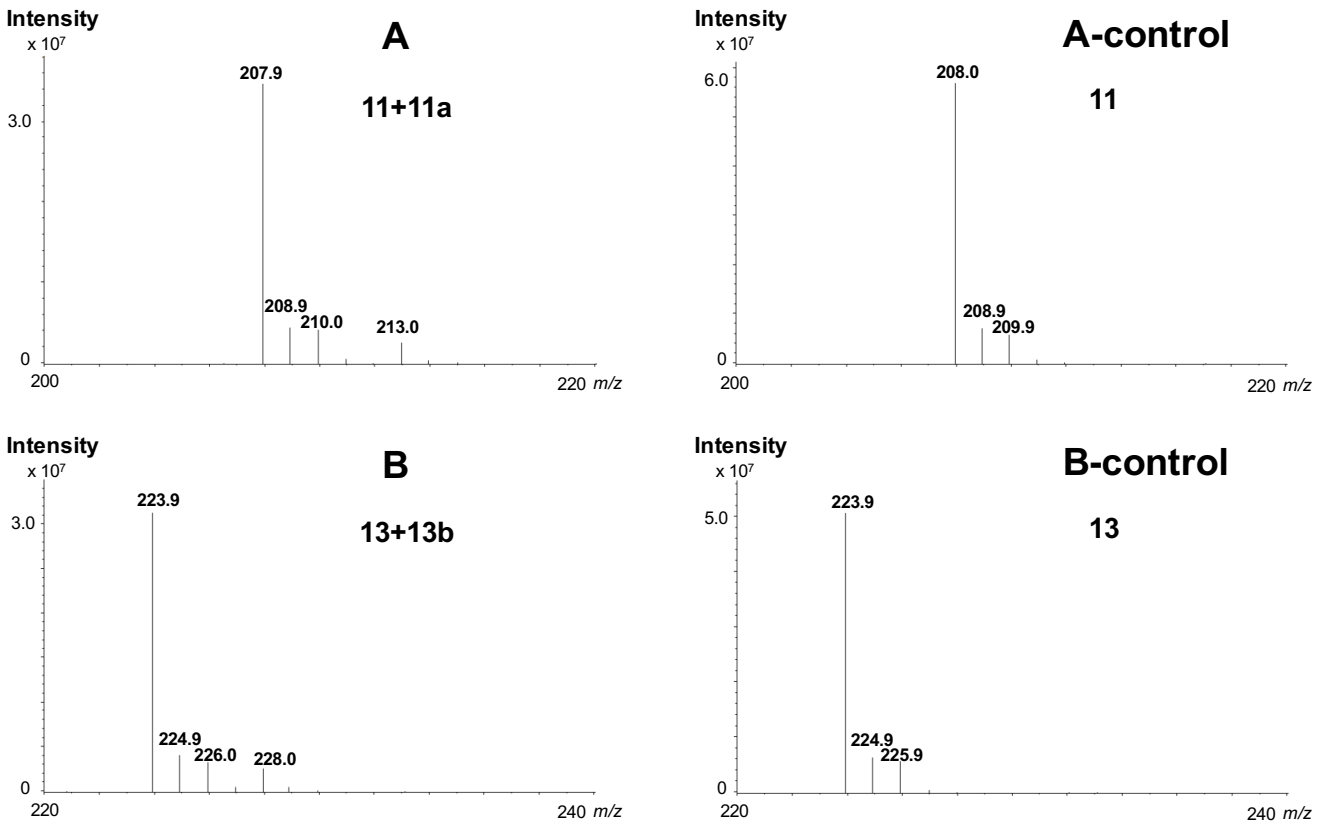
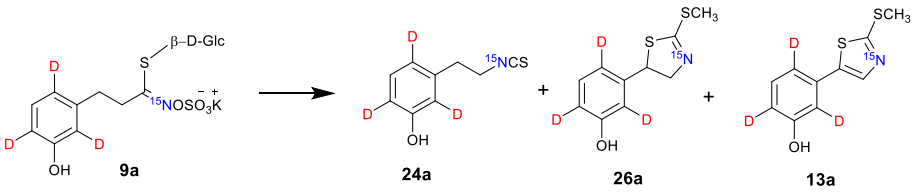


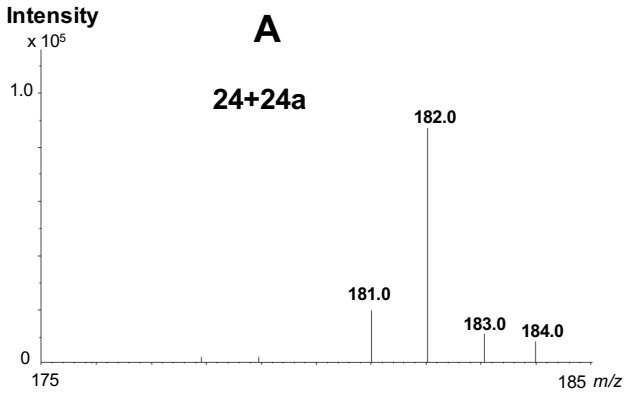
Figure S1 ESI-MS spectra (positive mode) of nasturlexins C (**11/11a**) and D (**13/13b**) in extracts of elicited leaves (A and B) fed with [2,3,4,5,6-D$_5$]gluconasturtiin (**8a**) and control leaves.

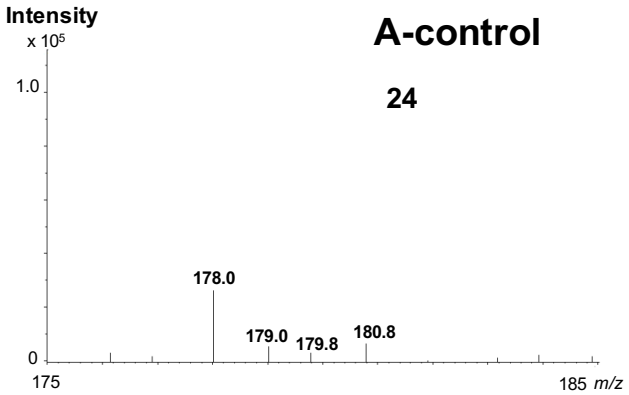
Incorporation of [2,4,6-D_3,^{15}N]-3-hydroxygluconasturtiin (9a)

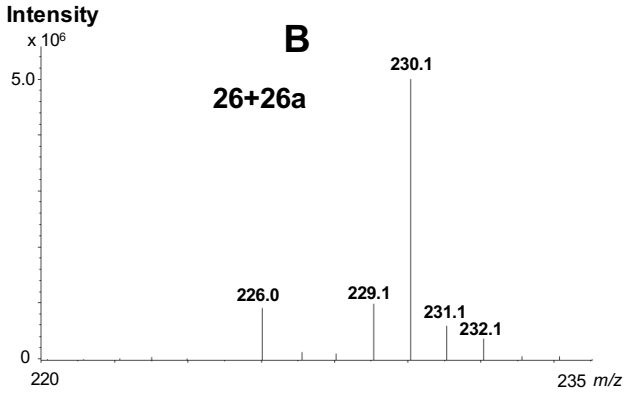


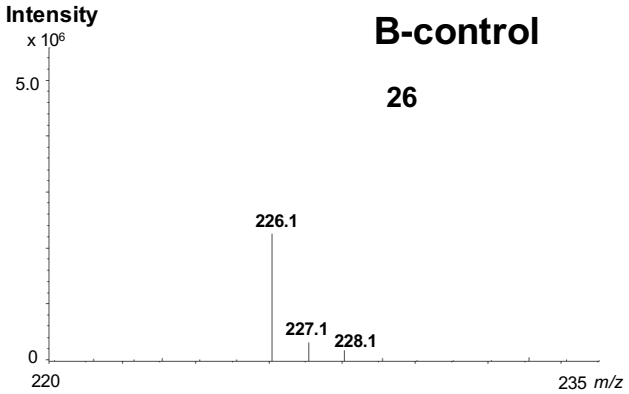
[2,4,6-D_3,^{15}N]Dihydronasturlexin D (**26a**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_9{}^2H_3{}^{15}NS_2$, 230.0519, found 230.0512.

[2,4,6-D_3,^{15}N]Nasturlexin D (**13a**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_7{}^2H_3{}^{15}NS_2$: 228.0362, found 228.0355.









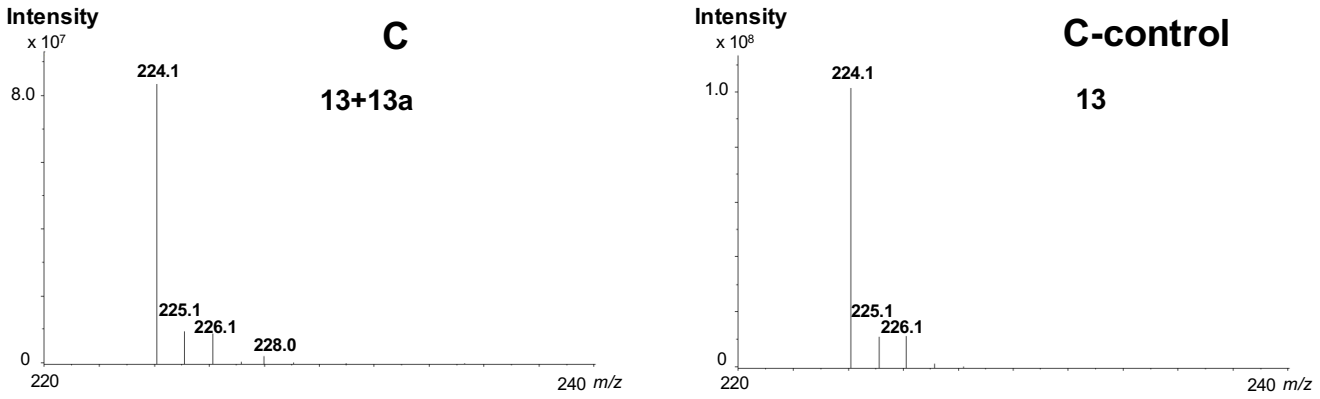
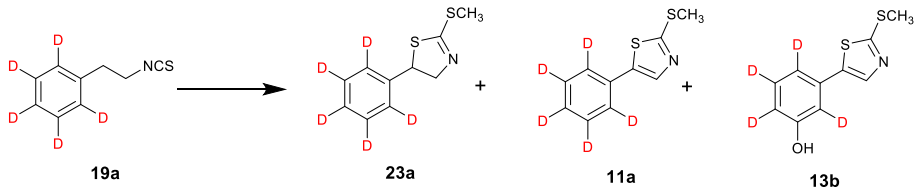


Figure S2 ESI-MS spectra (positive mode) of 3-hydroxyphenylethyl isothiocyanate (**24/24a**), dihydronasturlexin D **(26/26a**), and nasturlexin D (**13/13a**) in extracts of elicited leaves (A, B and C) fed with [2,4,6-D_3,^{15}N]-3-hydroxyphenylethyl glucosinolate (**9a**) and control leaves.

Incorporation of [2,3,4,5,6-D_5]phenylethyl isothiocyanate (19a)



[2,3,4,5,6-D_5]Nasturlexin C (**11a**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_5{}^2H_5NS_2$, 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.

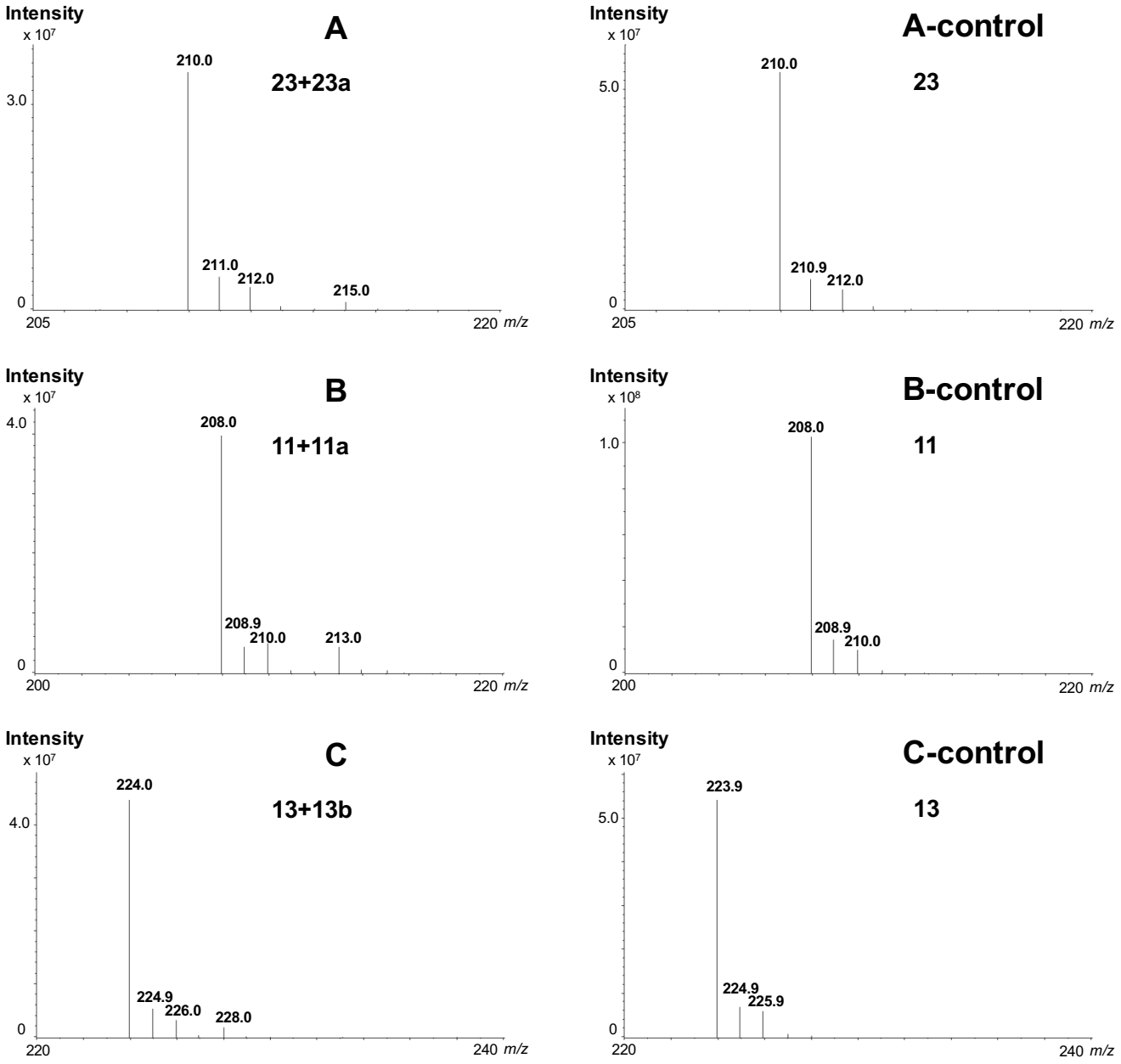
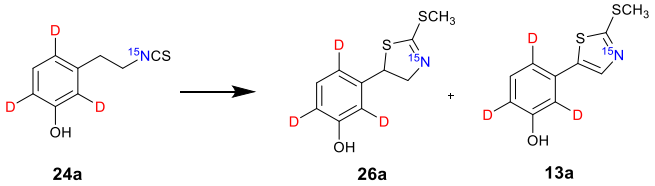


Figure S3 ESI-MS spectra (positive mode) of dihydronasturlexin C (**23/23a**), nasturlexins C (**11/11a**) and D (**13/13b**) in extracts of elicited leaves (A, B and C) fed with [2,3,4,5,6-D_5]phenylethyl isothiocyanate (**19a**) and control leaves.

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 $C_{10}H_9{}^2H_3{}^{15}NS_2$, 230.0519, found 230.0536.

[2,4,6-D$_3$,^{15}N]Nasturlexin D (**13a**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_7{}^2H_3{}^{15}NS_2$, 228.0362, found 228.0399.

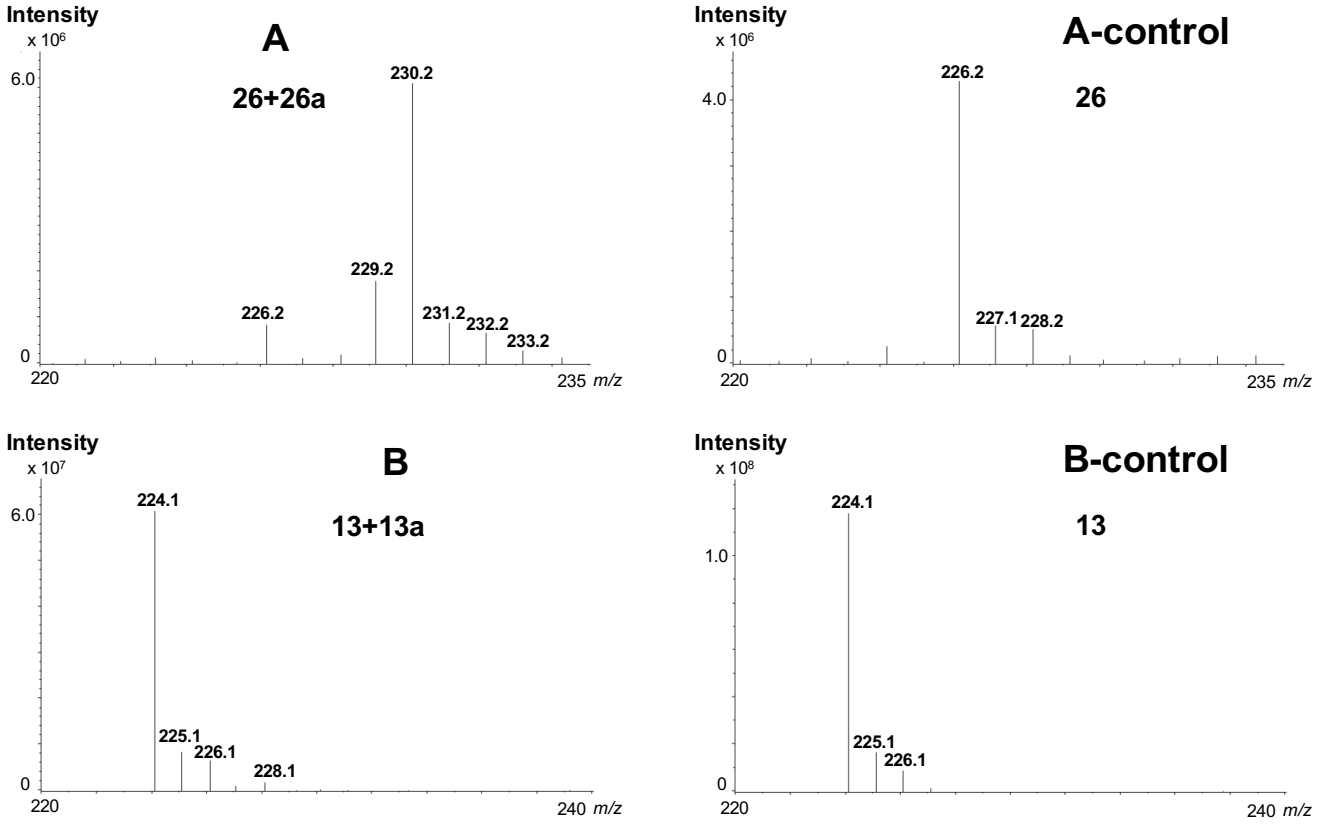
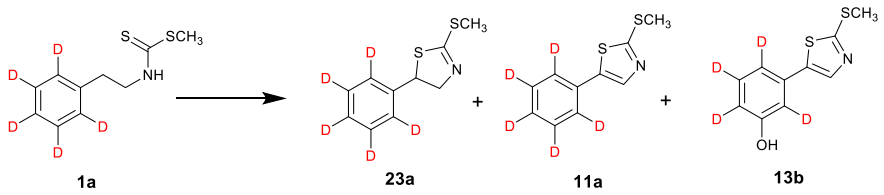


Figure S4 ESI-MS spectra (positive mode) of dihydronasturlexin D (**26/26a**) and nasturlexin D (**13/13a**) in extracts of elicited leaves (A and B) fed with [2,4,6-D$_3$,^{15}N]-3-hydroxyphenylethyl isothiocyanate (**24a**) and control leaves.

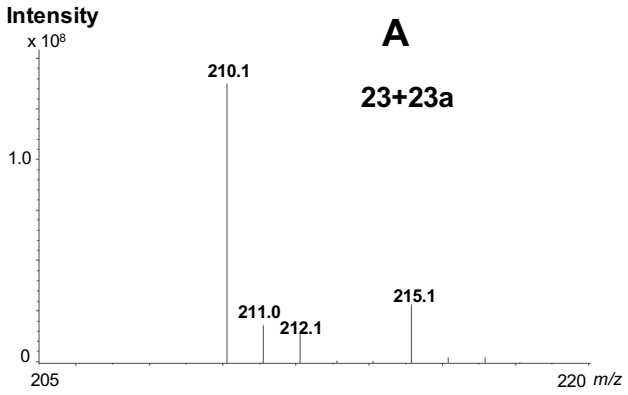
Incorporation of [2,3,4,5,6-D$_5$]nasturlexin A (1a)

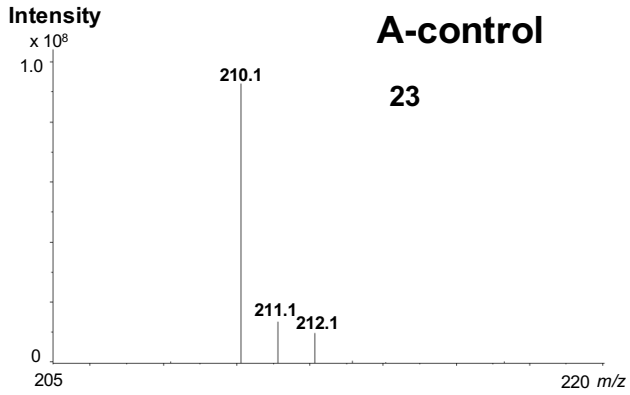


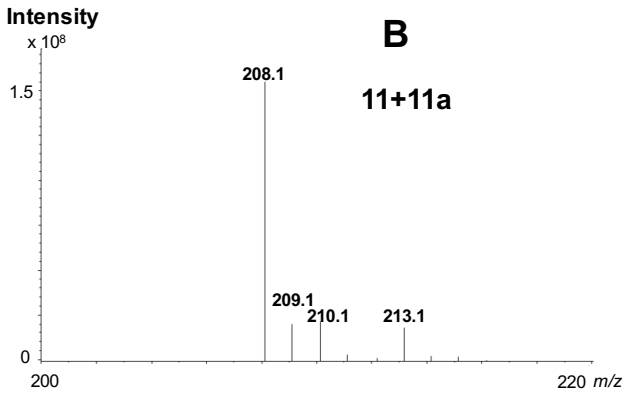
[2,3,4,5,6-D$_5$]Dihydronasturlexin C (**23a**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_7{}^2H_5NS_2$, 215.0725, found 215.0737.

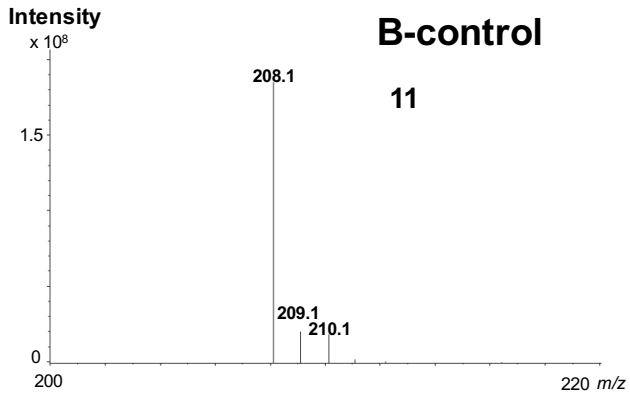
[2,3,4,5,6-D$_5$]Nasturlexin C (**11a**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_5{}^2H_5NS_2$, 213.0569, found 213.0557.

[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.









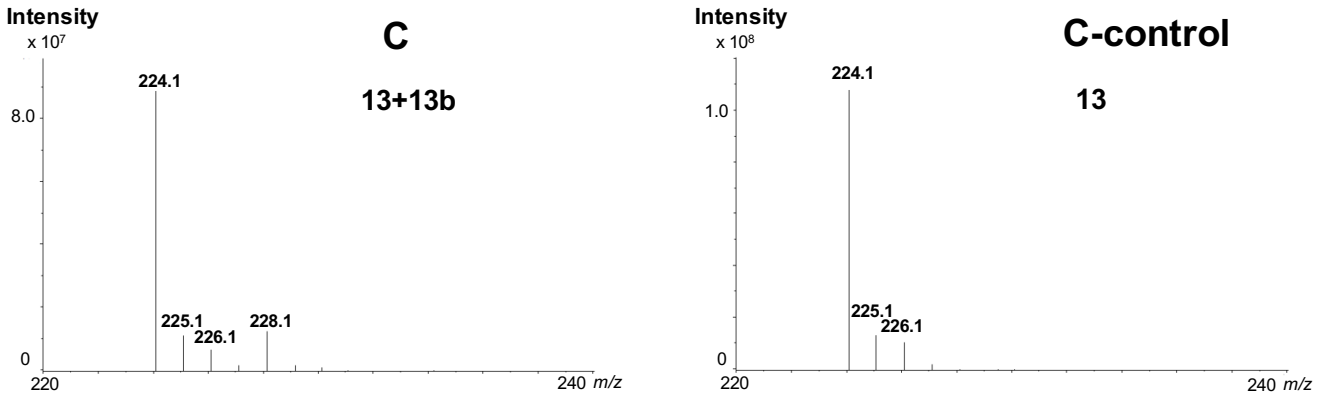
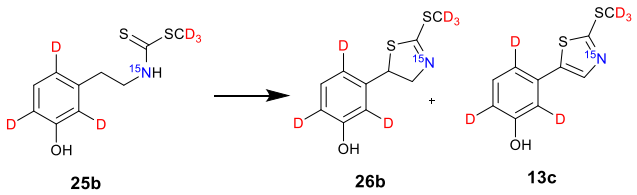


Figure S5 ESI-MS spectra (positive mode) of dihydronasturlexin C (**23/23a**), nasturlexins C (**11/11a**) and D (**13/13b**) in extracts of elicited leaves (A, B and C) fed with [2,3,4,5,6-D_5]nasturlexin A (**1a**) and control leaves.

Incorporation of [D_3CS, 2,4,6-D_3,^{15}N]-3-hydroxynasturlexin A (25b)



[D_3CS, 2,4,6-D_3,^{15}N]Dihydronasturlexin D (**26b**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_6{}^2H_6{}^{15}NS_2$, 233.0707, found 233.0698.

[D_3CS, 2,4,6-D_3,^{15}N]Nasturlexin D (**13c**): HR-ESI-MS *m/z* [M + H]$^+$, calculated for $C_{10}H_4{}^2H_6{}^{15}NS_2$, 231.0551, found 231.0538.

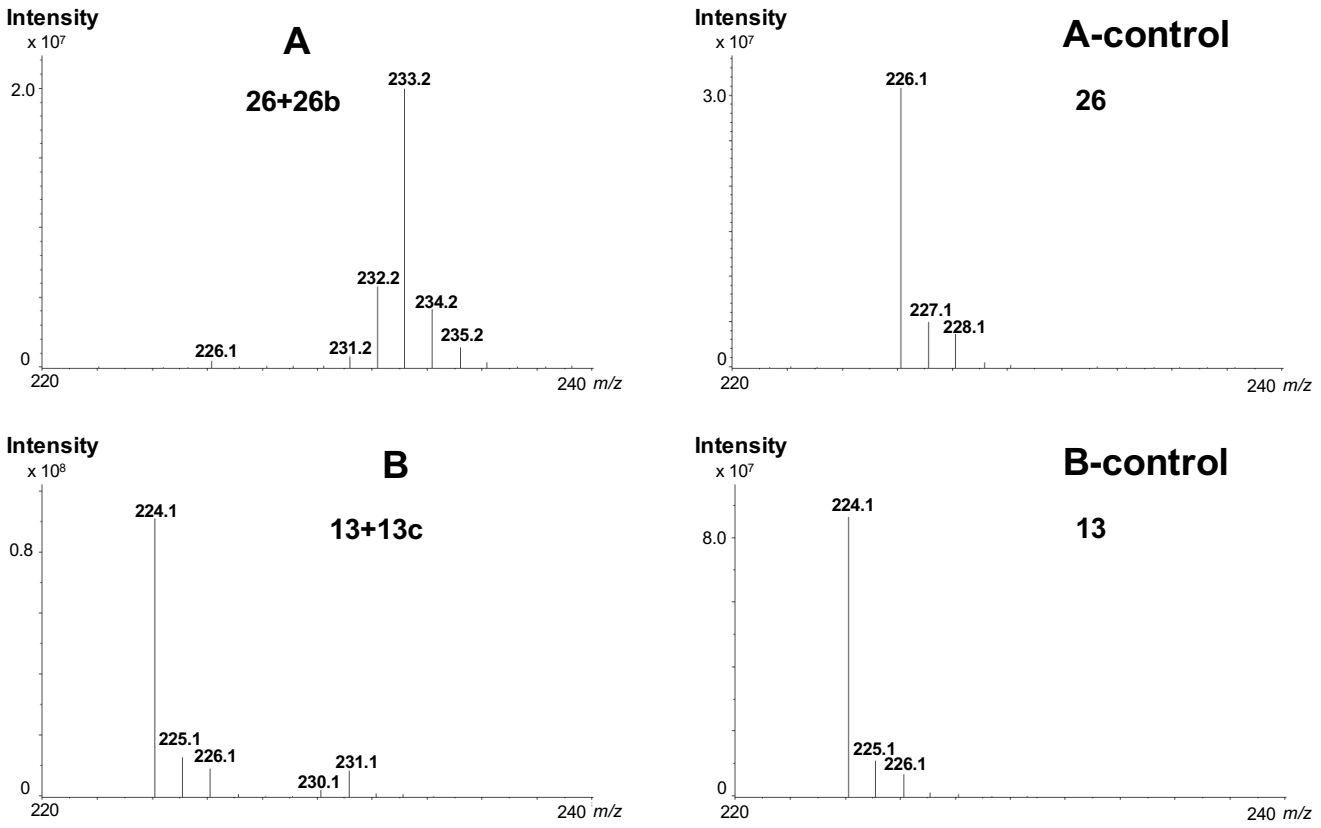
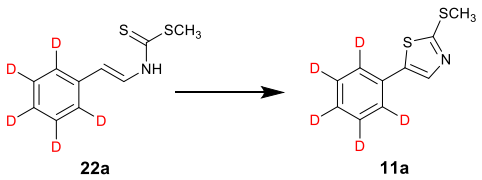


Figure S6 ESI-MS spectra (positive mode) of dihydronasturlexin D (**26/26b**) and nasturlexin D (**13/13c**) in extracts of elicited leaves (A and B) fed with [D_3CS, 2,4,6-D_3,^{15}N]-3-hydroxynasturlexin A (**25b**) and control leaves.

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 $C_{10}H_4{}^2H_5NS_2$, 212.0490, found 212.0487.

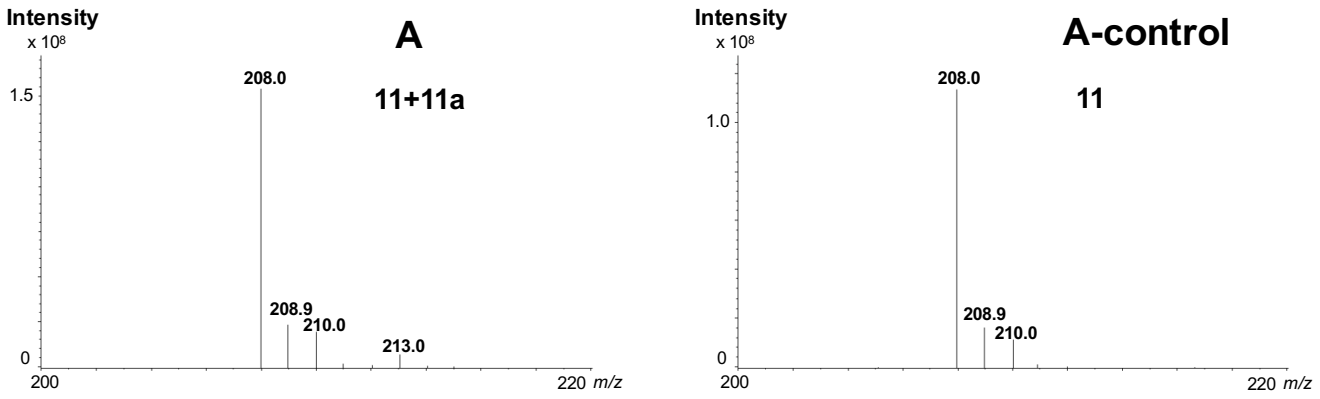
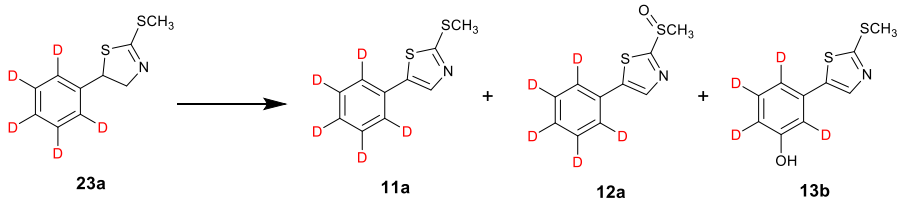


Figure S7 ESI-MS spectra (positive mode) of nasturlexin C (**11/11a**) in extracts of elicited leaves (A) fed with methyl [2,3,4,5,6-D_5]-(*E*)-styryl dithiocarbamate (**22a**) and control leaves.

Incorporation of [2,3,4,5,6-D_5]dihydronasturlexin C (23a)



[2,3,4,5,6-D_5]Nasturlexin C (**11a**): HR-ESI-MS *m/z* $[M + H]^+$, calculated for $C_{10}H_5{}^2H_5NS_2$, 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 $C_{10}H_7{}^2H_5NOS_2$, 229.0533, found 229.0546.

[2,3,5,6-D_4]Nasturlexin D (**13b**): HR-ESI-MS *m/z* $[M + H]^+$, calculated for $C_{10}H_6{}^2H_4NOS_2$, 228.0455, found 228.0443.

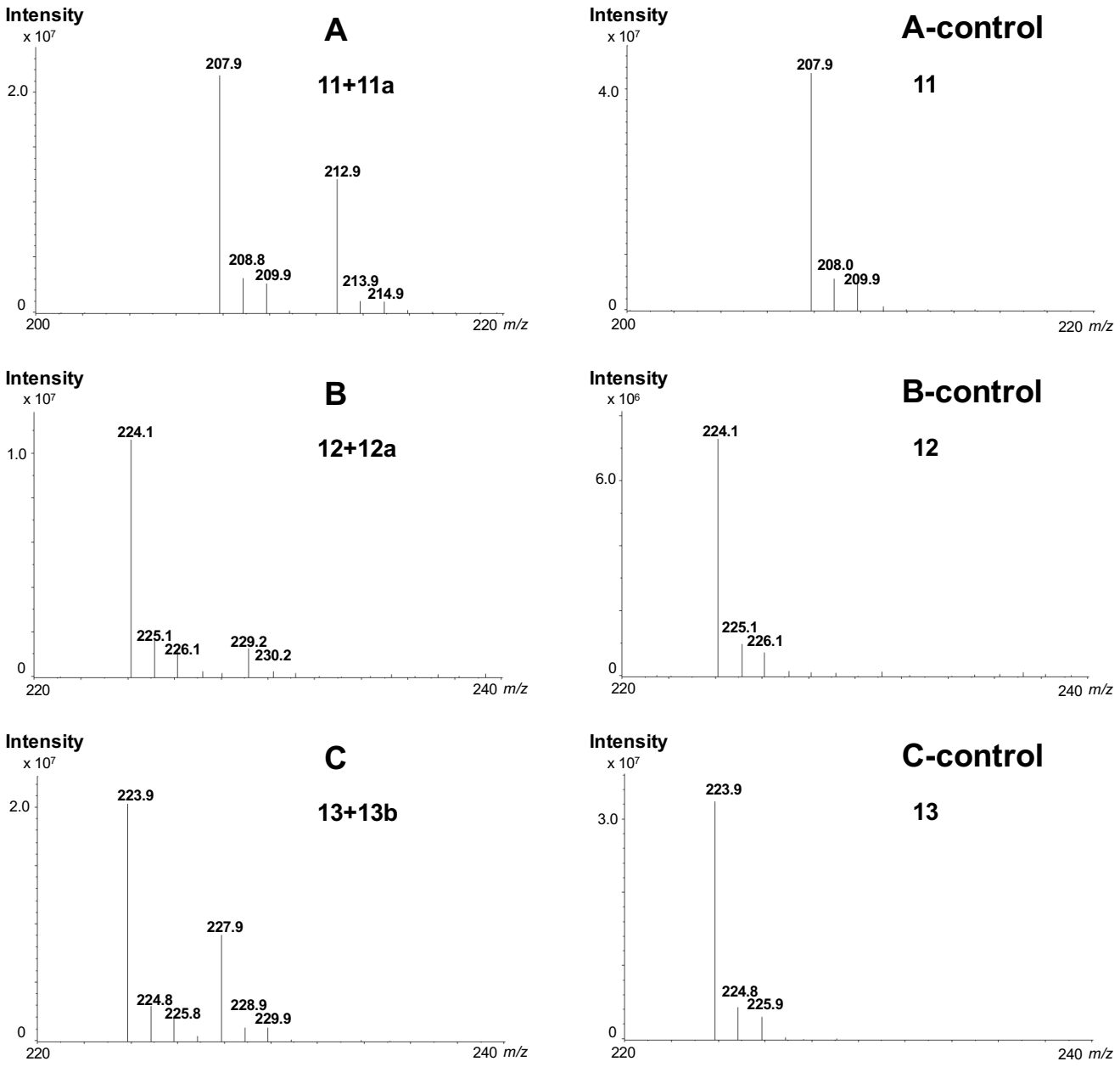
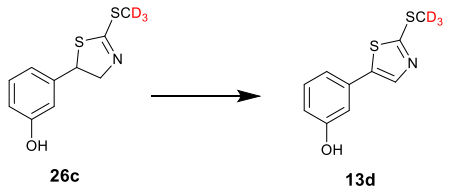


Figure S8 ESI-MS spectra (positive mode) of nasturlexins C (**11**/**11a**), C sulfoxide (**12**/**12a**), and D (**13**/**13b**) in extracts of elicited leaves (A, B, and C) fed with [2,3,4,5,6-D_5]dihydronasturlexin C (**23a**) and control leaves.

Incorporation of [D_3CS]dihydronasturlexin D (26c)



[D_3CS]Nasturlexin D (**13d**): HR-ESI-MS *m/z* $[M + H]^+$, calculated for $C_{10}H_7{}^2H_3NOS_2$, 227.0392, found 227.0389.

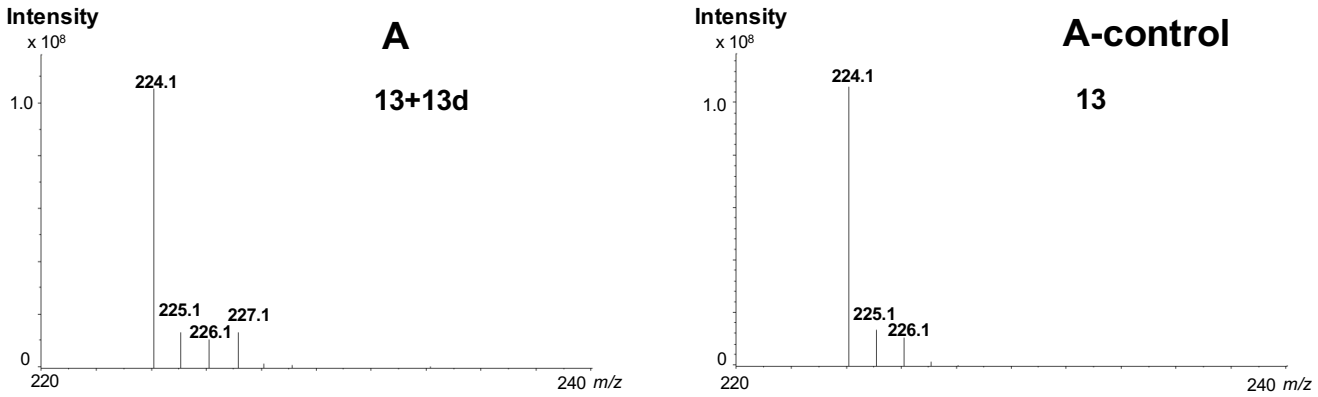
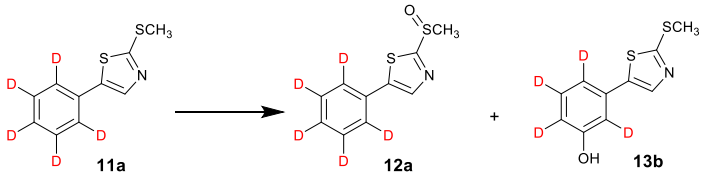


Figure S9 ESI-MS spectra (positive mode) of nasturlexin D (**13/13d**) in extracts of elicited leaves (A) fed with [D_3CS]dihydronasturlexin D (**26c**) and control leaves.

Incorporation of [2,3,4,5,6-D_5]nasturlexin C (11a)



[2,3,4,5,6-D_5]Nasturlexin C sulfoxide (**12a**): HR-ESI-MS *m/z* $[M + H]^+$, calculated for $C_{10}H_7{}^2H_5NOS_2$, 229.0533, found 229.0540.

[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 228.0460.

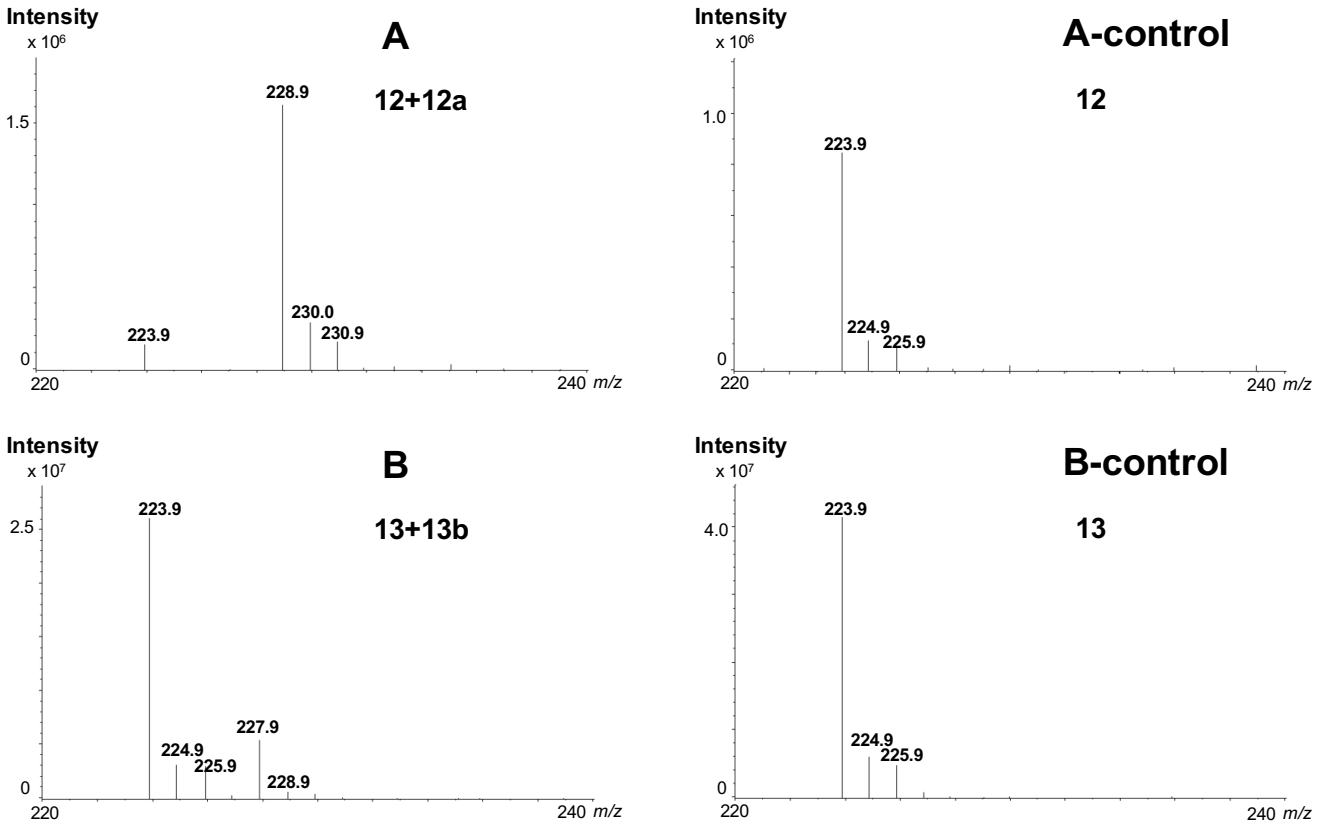
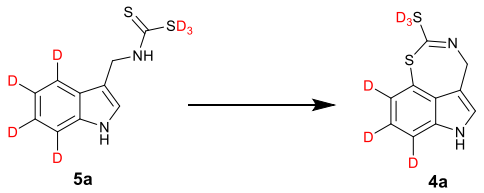


Figure S10 ESI-MS spectra (positive mode) of nasturlexin C sulfoxide (**12/12a**) and nasturlexin D (**13/13b**) in extracts of elicited leaves (A and B) fed with [2,3,4,5,6-D_5]nasturlexin C (**11a**) and control leaves.

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

Incorporation of [D_3CS 4,5,6,7-D_4]brassinin (5a)



[D_3CS 4,5,6,7-D_4]cyclonasturlexin (**4a**): HR-ESI-MS *m/z* $[M + H]^+$, calculated for $C_{11}H_5{}^2H_6N_2S_2$, 241.0735, found 241.0739.

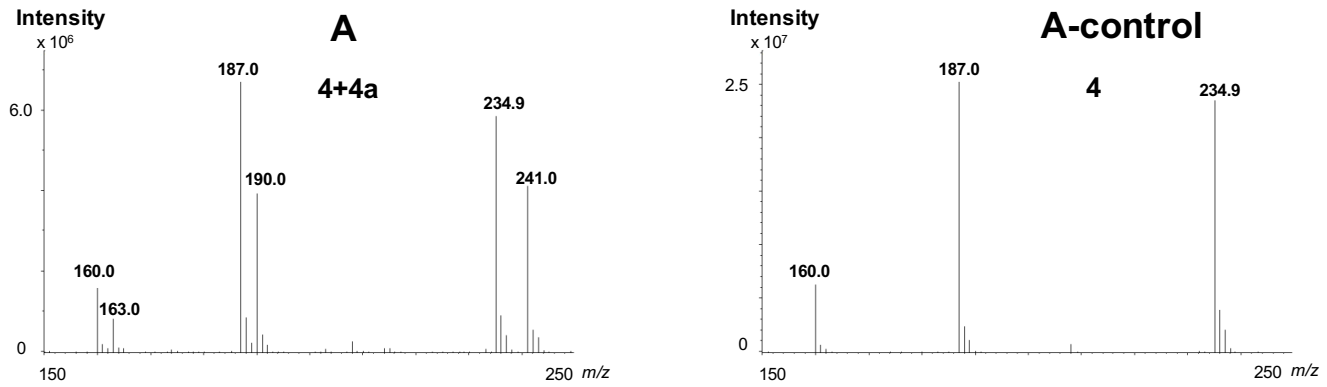
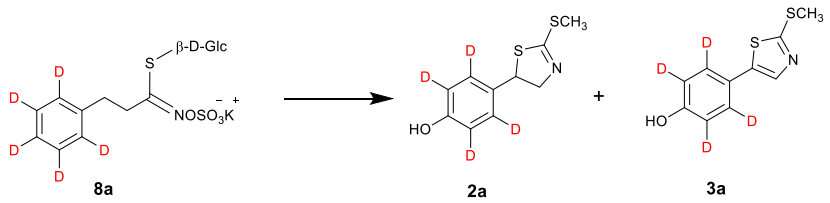


Figure S11 ESI-MS spectra (positive mode) of cyclonasturlexin (**4/4a**) in extracts of elicited leaves (A) fed with [D_3CS 4,5,6,7-D_4]brassinin (**5a**) and control leaves.

Incorporation of [2,3,4,5,6-D_5]gluconasturtiin (8a)



[2,3,5,6-D_4]Nasturlexin B (**2a**): HR-ESI-MS *m/z* $[M + H]^+$, calculated for $C_{10}H_8{}^2H_4NOS_2$, 230.0606, found 230.0635.

[2,3,5,6-D_4]Tridentatol C (**3a**): HR-ESI-MS *m/z* $[M + H]^+$, calculated for $C_{10}H_6{}^2H_4NOS_2$, 228.0449, found 228.0459.

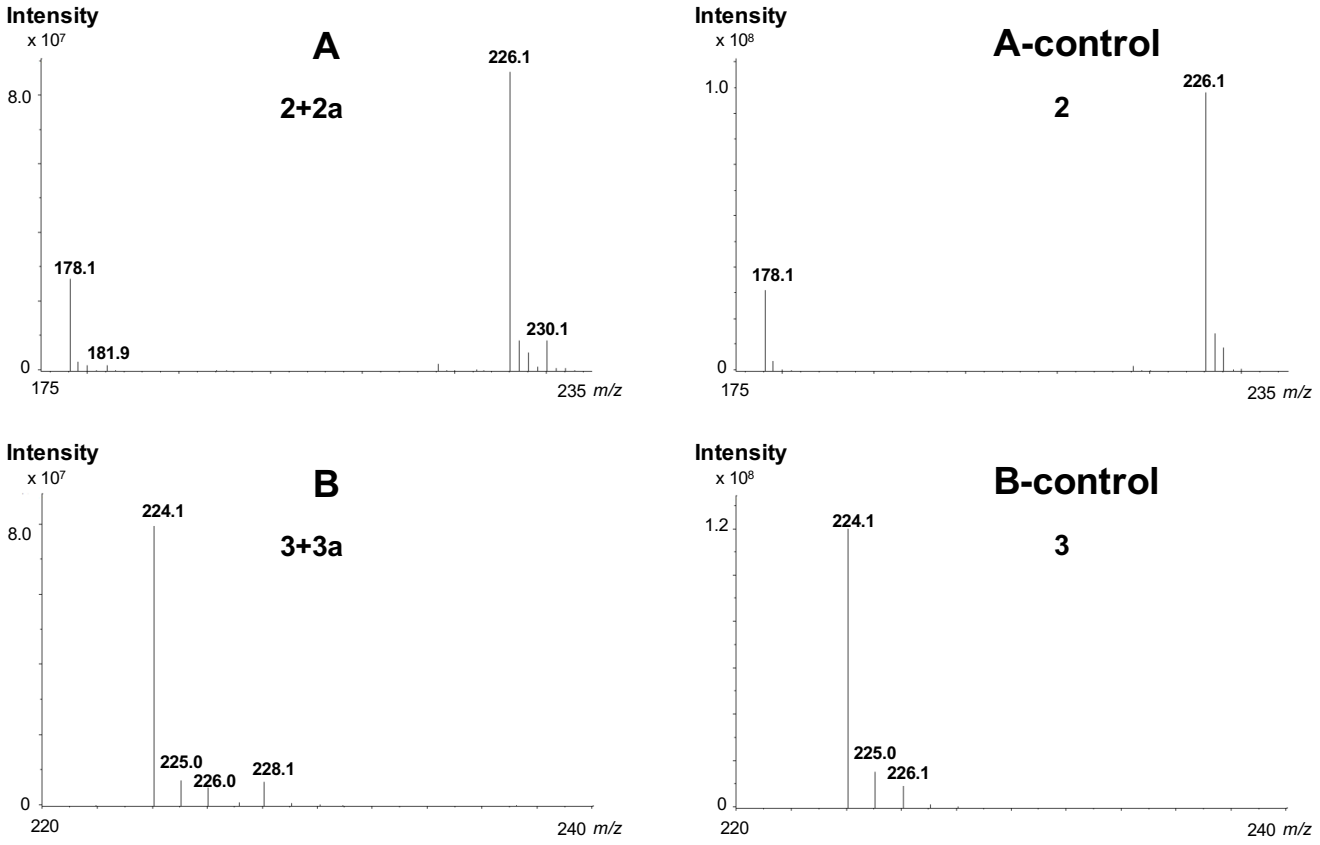


Figure S12 ESI-MS spectra (positive mode) of nasturlexin B (**2/2a**) and tridentatol C (**3/3a**) in extracts of elicited leaves (A and B) fed with [2,3,4,5,6-D_5]gluconasturtiin (**8a**) and control leaves.

References

1 J. S. Cha, J. H. Chun, J. M. Kim, O. O. Kwon, S. Y. Kwon and J. C. Lee, *Bull. Korean Chem. Soc.*, 1999, **20**, 400–402.

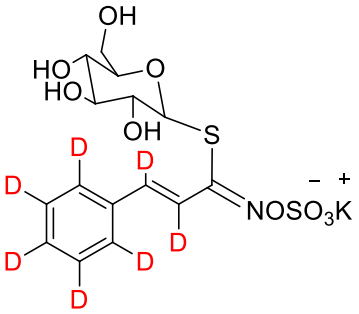
2 Q. V Vo, C. Trenerry, S. Rochfort, J. Wadeson, C. Leyton and A. B. Hughes, *Bioorg. Med. Chem.*, 2013, **21**, 5945–5954.

3 M. S. C. Pedras and Q. H. To, *J. Label. Compd. Radiopharm.*, 2018, **61**, 94–106.

NMR spectra of new compounds 18a, 21, 21a,26 and 26a

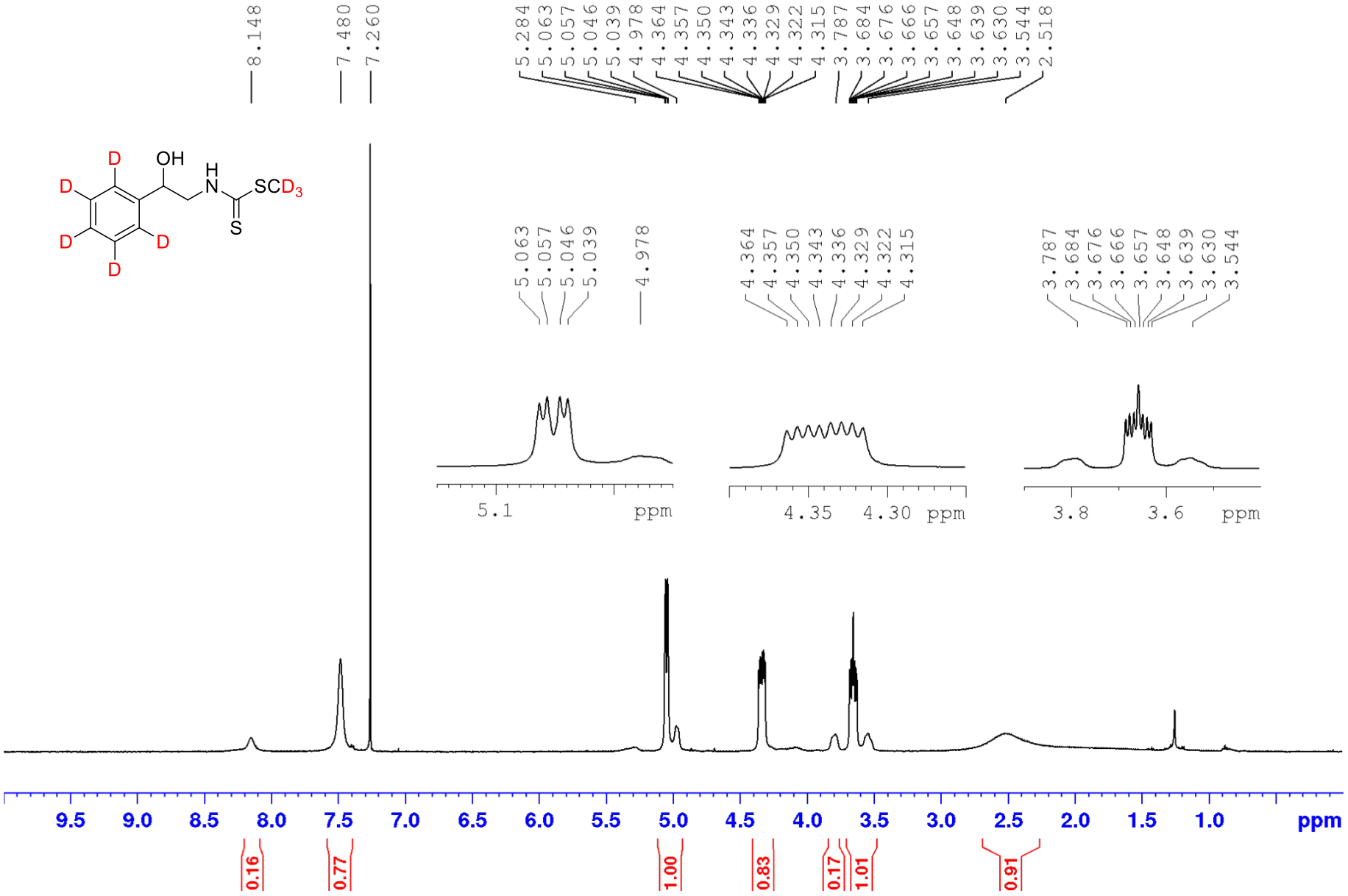
Compound **18a** - ^{1}H NMR Spectrum

D_2O



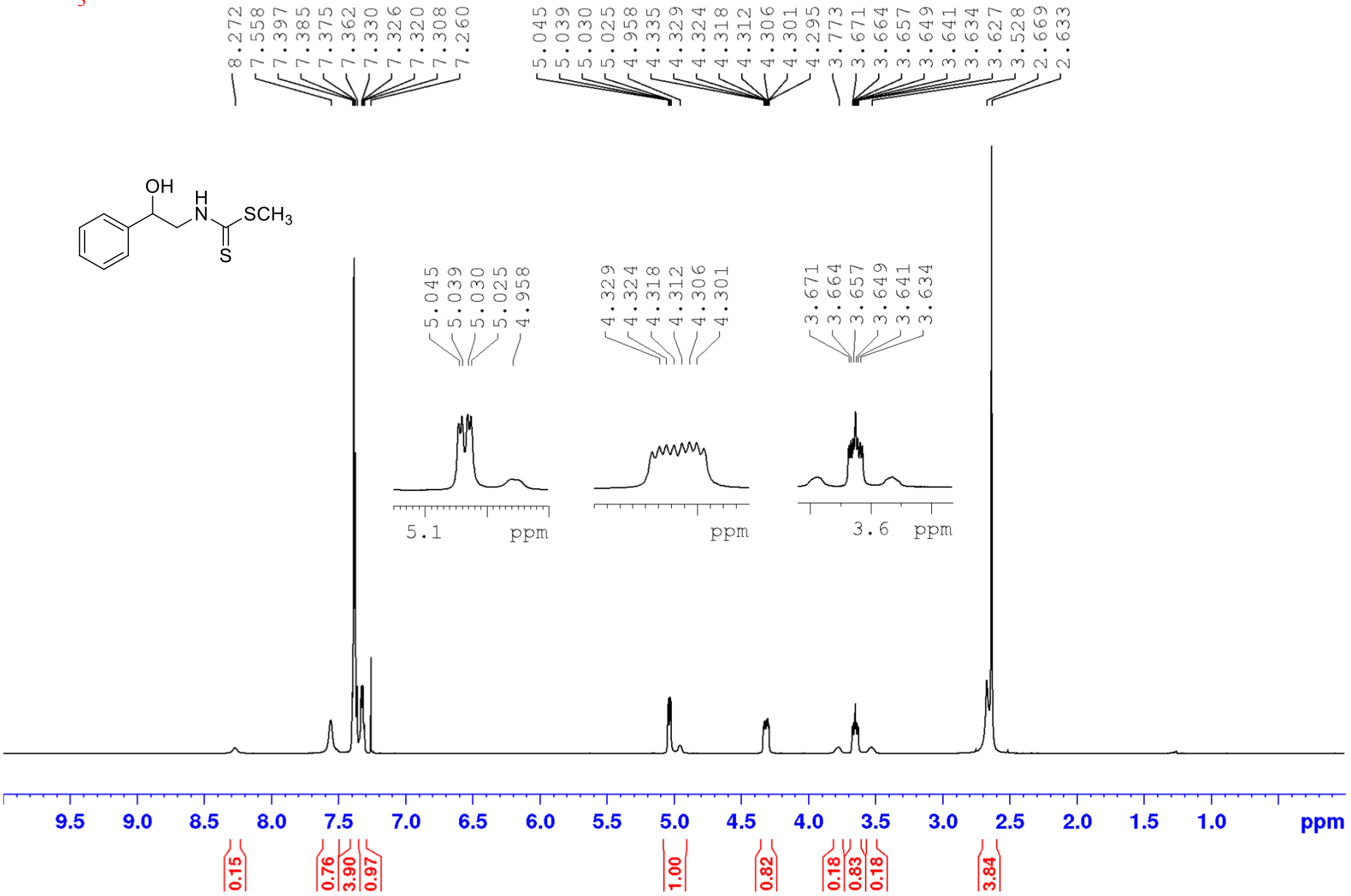
Compound **21a** - ^{1}H NMR Spectrum

$CDCl_3$



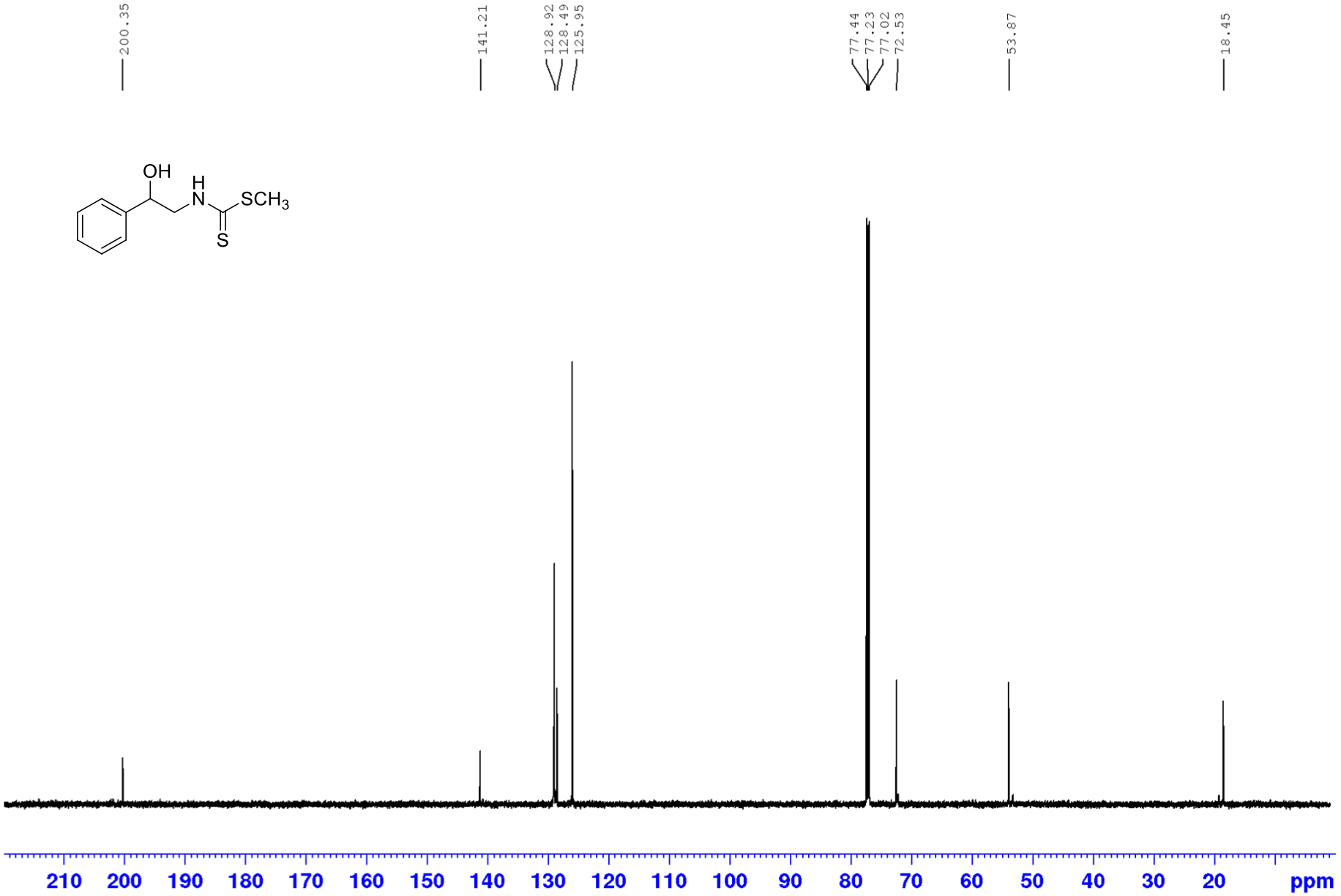
Compound **21** - ^{1}H NMR Spectrum

$CDCl_3$



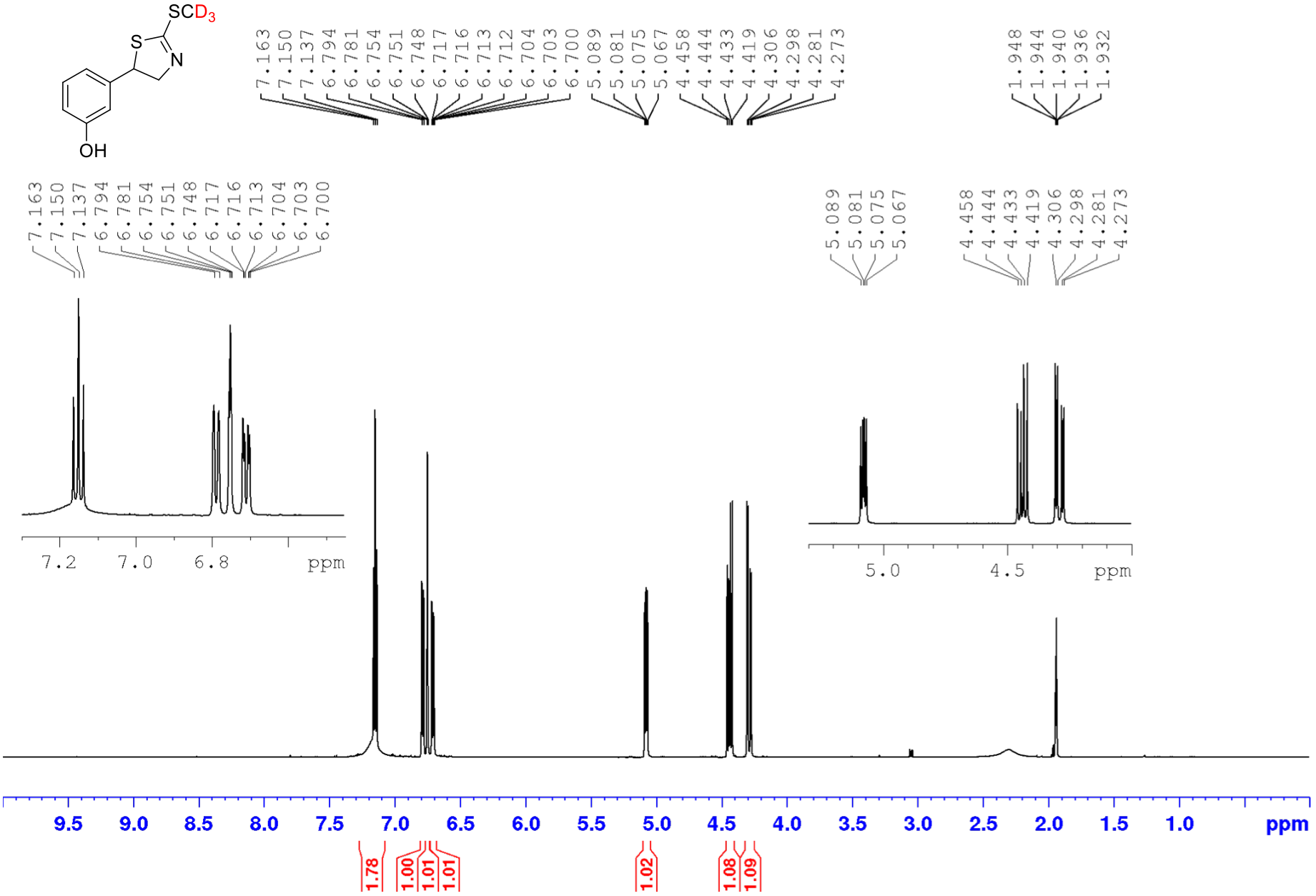
Compound **21** - ^{13}C NMR Spectrum

$CDCl_3$



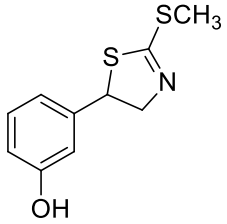
Compound **26c** - ^{1}H NMR Spectrum

CD_3CN



Compound **26** - ^{1}H NMR Spectrum

CD_3CN



Compound **26** - ^{13}C NMR Spectrum

CD_3CN