

Supplementary material as noted in the text.

Supplemental Material: Figures S1-S6 and Tables S1-S6

**Collision-Induced Dissociation of the A+2 Isotope Ion Facilitates Glucosinolates Structure
Elucidation by ESI-Tandem Mass Spectrometry with a Linear Quadrupole Ion Trap**

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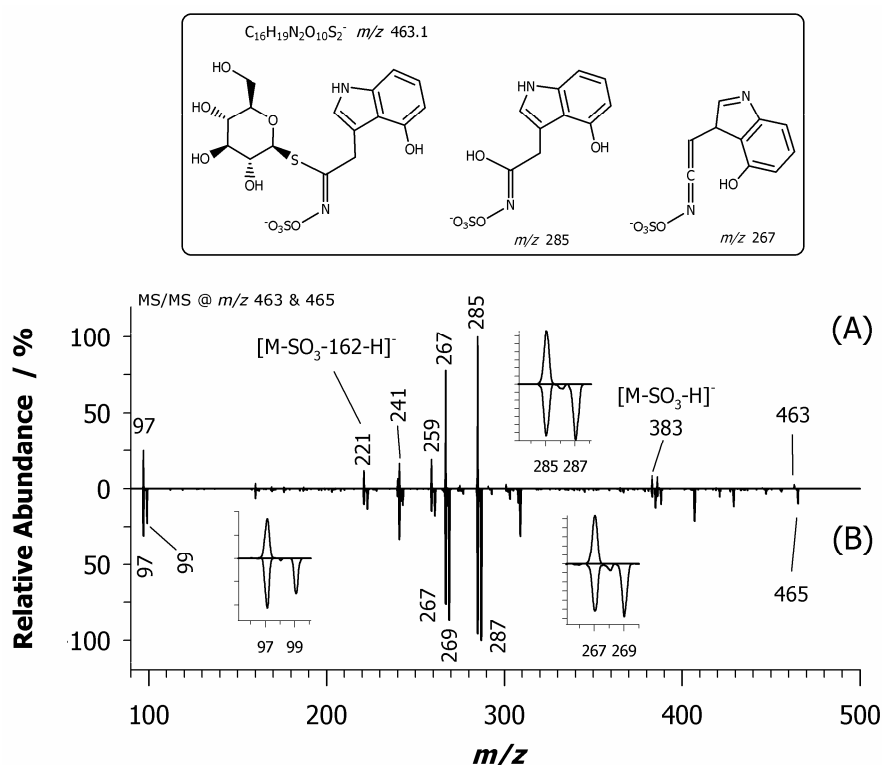


Figure S1. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of (A) 4-hydroxy-glucobrassicin [1S] at m/z 463 and 20% collision energy. (B) The A+2 isotope peak at m/z 408 was fragmented at the same collision energy. See data reported in Table S1.

Table S1. Experimental and calculated ratios (I_A/I_{A+2}) for the isotope abundances of the fragment ions as observed by CID of the +2 Da isotopomer of deprotonated 4-hydroxy-glucobrassicin $[M-H]^-$ at m/z 465 ($C_{16}H_{18}N_2O_{10}S_2^- + 2$).

Ions A and A+2 (m/z)	Molecular formula (A)	Expected ratio	Expected ratio counting only the contribution of ^{34}S	Measured ratio ($n=5$)
97-99	HSO_4^-	1.08	1 S : 1 S	1.21 ± 0.08
259-261	$C_6H_{11}O_9S^-$	0.80	1 S : 1 S	0.82 ± 0.04
267-269	$C_{10}H_7N_2O_5S^-$	0.94	1 S : 1 S	0.91 ± 0.05
285-287	$C_{10}H_9N_2O_6S^-$	0.88	1 S : 1 S	0.95 ± 0.06
383-385	$C_{16}H_{18}N_2O_7S^-$	0.70	1 S : 1 S	0.62 ± 0.10

[1S] 4-hydroxy-glucobrassicin is an indole glucosinolate (see Agerbirk N, De Vos M, Kim JH, Jander G. Indole glucosinolate breakdown and its biological effects *Phytochem Rev.* 2009; 8: 101).

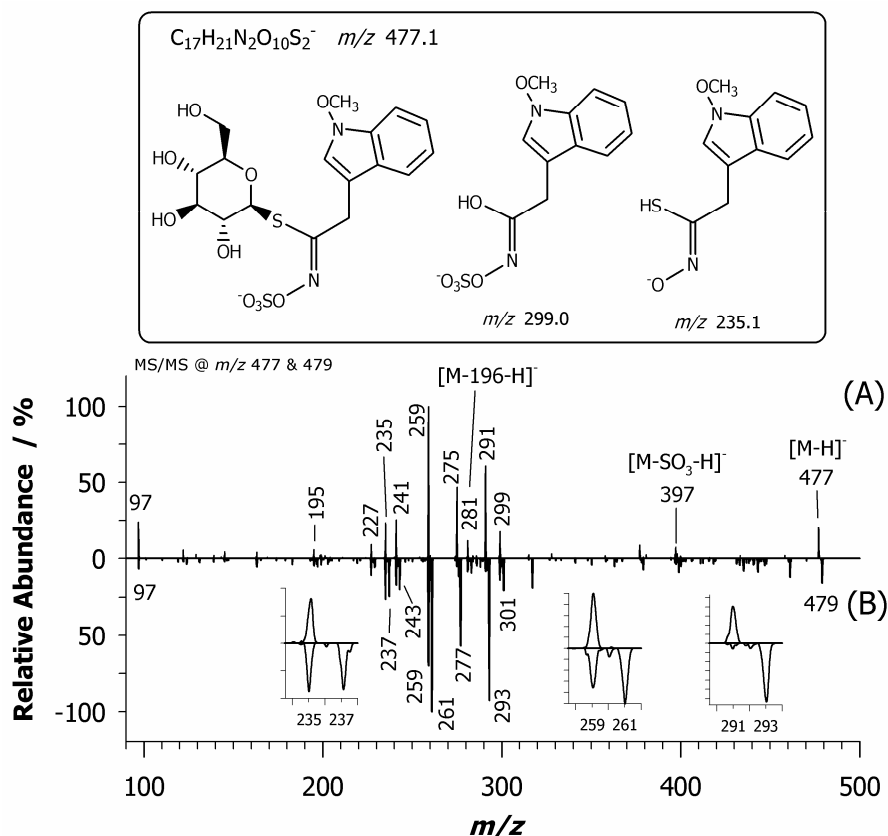


Figure S2. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of (A) neoglucobrassicin (1-methoxy-3-indolylmethyl glucosinolate) $[M-H]^-$ at m/z 477 and 20% collision energy. (B) The A+2 isotope peaks at m/z 479 (A+2) was fragmented at the same collision energy. See data reported in Table S2.

Table S2. Experimental and calculated ratios (I_A/I_{A+2}) for the isotope abundances of the fragment ions as observed by CID of the +2 Da isotopomer of deprotonated neoglucobrassicin $[M-H]^-$ at m/z 479 ($C_{17}H_{21}N_2O_{10}S_2^- + 2$).

Ions A and A+2 (m/z)	Molecular formula (A)	Expected ratio	Expected ratio counting only the contribution of ^{34}S	Measured ratio ($n=5$)
97-99	HSO_4^-	1.08	1 S : 1 S	LSs
235-237	$C_{11}H_{11}N_2O_2S^-$	1.11	1 S : 1 S	1.07 ± 0.04
241-243	$C_6H_9O_8S^-$	0.90	1 S : 1 S	0.88 ± 0.04
259-261	$C_6H_{11}O_9S^-$	0.84	1 S : 1 S	0.80 ± 0.04
275-277	$C_6H_{11}O_8S_2^-$	0.11	0 S : 1 S	0.10 ± 0.02
291-293	$C_6H_{11}O_9S_2^-$	0.09	0 S : 1 S	0.09 ± 0.02
299-301	$C_{11}H_{11}N_2O_6S^-$	0.84	1 S : 1 S	0.80 ± 0.03

LSs= low signals.

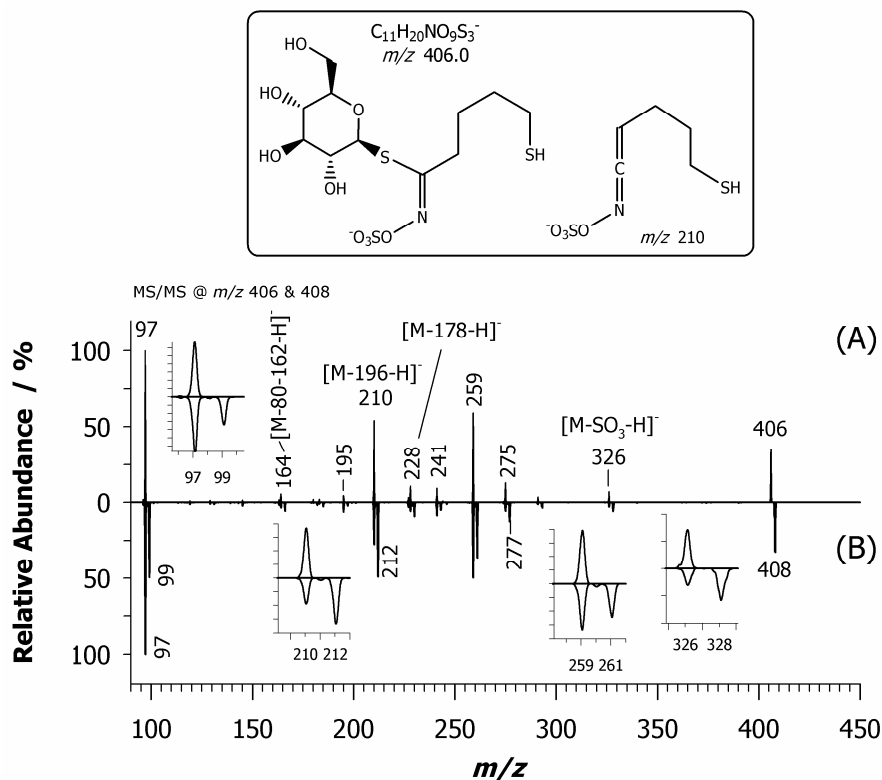


Figure S3. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of (A) 4-mercaptobutyl-GLS $[M-H]^-$ at m/z 406 and 25% collision energy. (B) The A+2 isotope peak at m/z 408 was fragmented at the same collision energy. See data reported in Table S3.

Table S3. Experimental and calculated ratios (I_A/I_{A+2}) for the isotope abundances of the fragment ions as observed by CID of the +2 Da isotopomer of deprotonated 4-mercaptobutyl-GLS at m/z 408 ($C_{11}H_{20}NO_9S_3^- + 2$).

Ions A and A+2 (m/z)	Molecular formula (A)	Expected ratio	Expected ratio counting only the contribution of ^{34}S	Measured ratio ($n=5$)
97-99	HSO_4^-	1.89	2 S : 1 S	1.85 ± 0.04
210-212	$C_5H_8NO_4S_2^-$	0.57	1 S : 2 S	0.55 ± 0.05
259-261	$C_6H_{11}O_9S^-$	1.40	2 S : 1 S	1.42 ± 0.06
275-277	$C_6H_{11}O_8S_2^-$	0.44	1 S : 2 S	0.46 ± 0.06
326-328	$C_{11}H_{20}NO_6S_2^-$	0.47	1 S : 2 S	0.46 ± 0.03

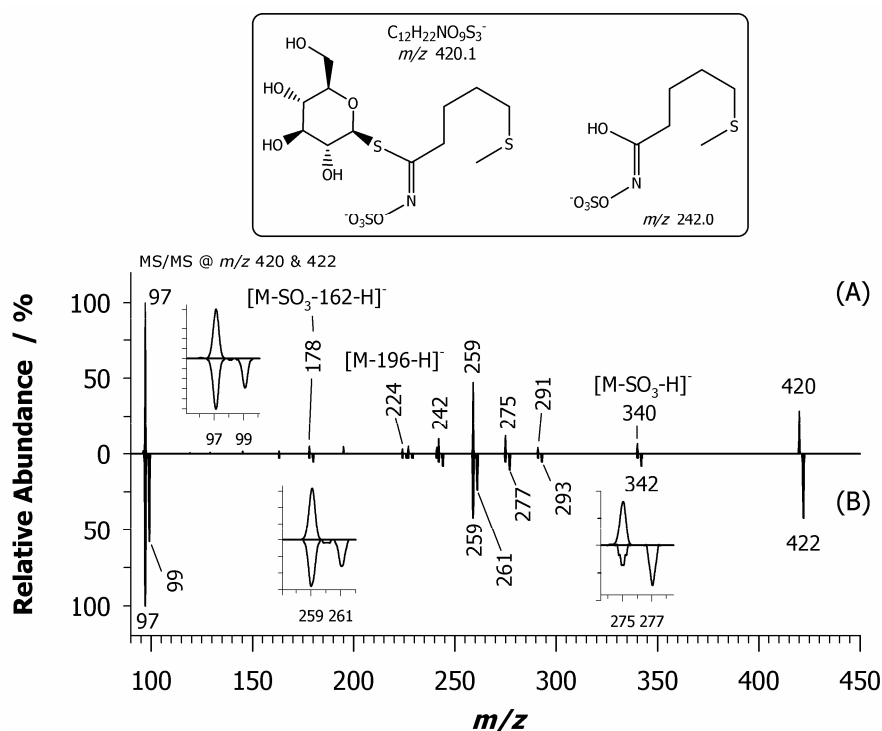


Figure S4. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of (A) glucorucin (4-methylthiobutyl glucosinolate) $[M-H]^-$ at m/z 420 and 20% collision energy. (B) The A+2 isotope peak at m/z 422 was fragmented at the same collision energy. See data reported in Table S4.

Table S4. Experimental and calculated ratios (I_A/I_{A+2}) for the isotope abundances of the fragment ions as observed by CID of the +2 Da isotopomer of deprotonated glucorucin $[M-H]^-$ at m/z 422 ($C_{12}H_{21}NO_9S_3^- + 2$).

Ions A and A+2 (m/z)	Molecular formula (A)	Expected ratio	Expected ratio counting only the contribution of ^{34}S	Measured ratio ($n=5$)
97-99	HSO_4^-	1.89	2 S : 1 S	1.86 ± 0.05
178-180	$C_6H_{12}NOS_2^-$	0.67	1 S : 2 S	0.63 ± 0.08
242-244	$C_6H_{12}NO_5S_2^-$	0.54	1 S : 2 S	0.60 ± 0.04
259-261	$C_6H_{11}O_9S^-$	1.41	2 S : 1 S	1.46 ± 0.05
275-277	$C_6H_{11}O_8S_2^-$	0.45	1 S : 2 S	0.48 ± 0.04
340-342	$C_{12}H_{21}NO_6S_2^-$	0.46	1 S : 2 S	0.41 ± 0.05

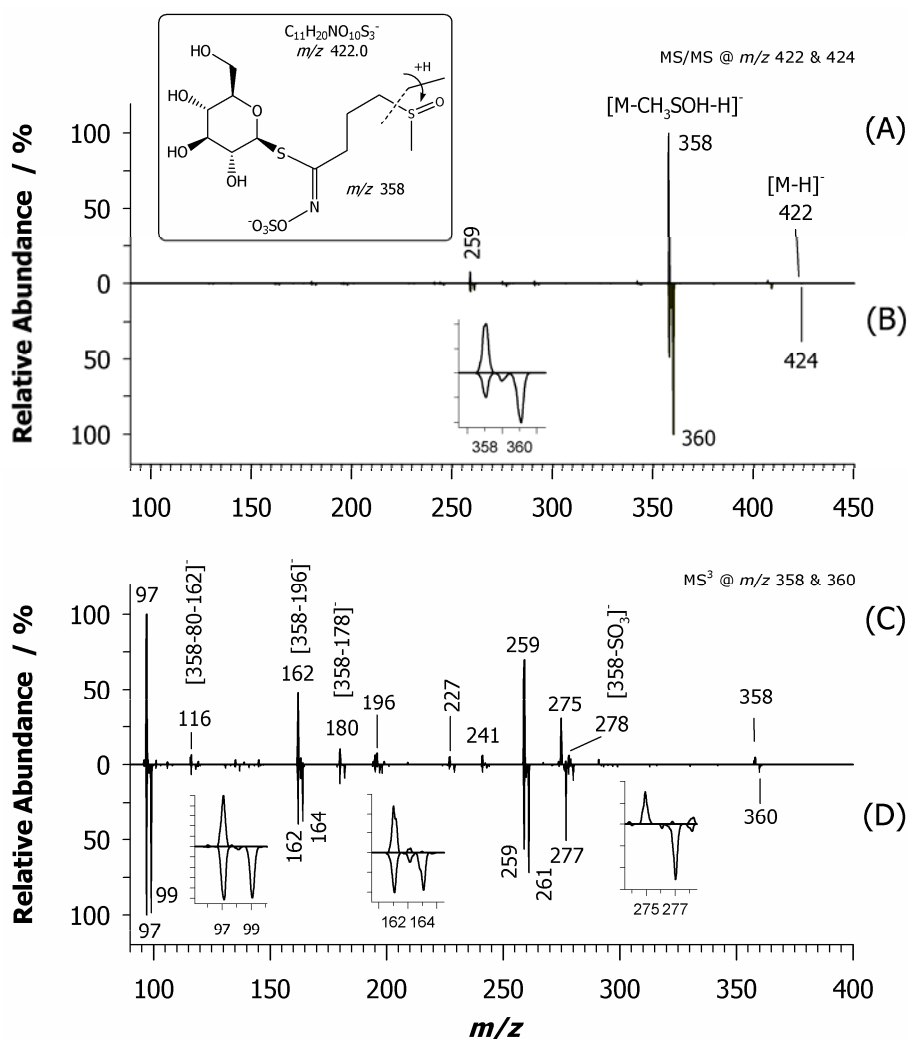


Figure S5. Product ion spectra obtained by LC-ESI-LTQ MS/MS in negative ion mode of (A) glucoiberin (3-methylsulfinylpropyl glucosinolate) $[M-H]^-$ at m/z 422 (20% collision energy). The main product ion is due to the characteristic loss of methanesulfenic acid (CH_3SOH , 64 Da) from the side chain of glucoiberin. (B) The A+2 isotopomer peak at m/z 424 was fragmented at the same collision energy. Fragment ions (25% collision energy) at m/z 414 and m/z 416 were selected as precursor ions for a further stage of fragmentation (MS³) and the resulting mass spectra are illustrated in plots (C) and (D), respectively. See data reported in Table S5.

Table S5. Experimental and calculated ratios (I_A/I_{A+2}) for the isotope abundances of the fragment ions as observed by CID of the +2 Da isotopomer of deprotonated glucoiberin $[M-H]^-$ at m/z 424 ($C_{11}H_{20}NO_{10}S_3^- + 2$) and subsequent fragmentation of the prominent A+2 ion at m/z 360.

Ions A and A+2 (m/z)	Molecular formula (A)	Expected ratio	Expected ratio counting only the contribution of ^{34}S	Measured ratio ($n=5$)
358-360	$C_{10}H_{16}NO_9S_2^-$	0.41	1 S : 2 S	0.43±0.05
MS ³ at m/z 360 ($C_{10}H_{16}NO_9S_2^- + 2$)				
97-99	HSO_4^-	1.04	1 S : 1 S	1.01±0.03
162-164	$C_4H_4NO_4S^-$	1.06	1 S : 1 S	1.03±0.05
259-261	$C_6H_{11}O_9S^-$	0.70	1 S : 1 S	0.73±0.05
275-277	$C_6H_{11}O_8S_2^-$	0.03	0 S : 1 S	0.02±0.02

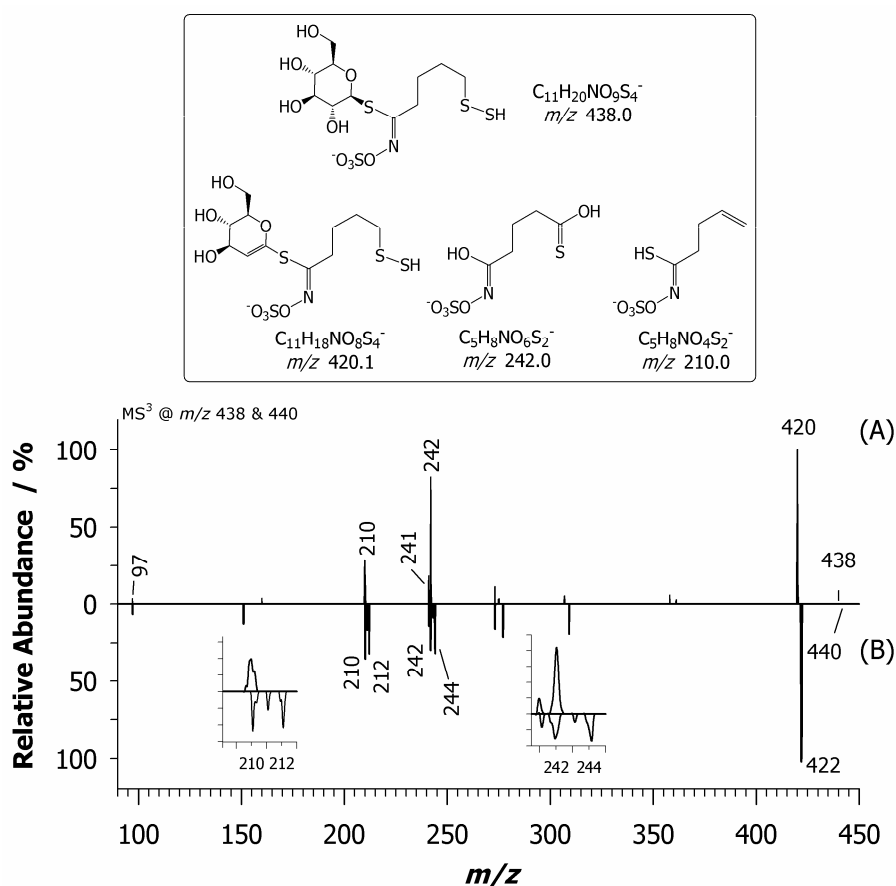


Figure S6. Product ion spectra (MS^3) of deprotonated 4-(β -D-glucopyranosyl-disulfanyl)butyl-GLS at 30% collision energy. Fragment ions at m/z 438 and m/z 440 were selected as precursor ions, plots (A) and (B), respectively. See data reported in Table S6.

Table S6. Experimental and calculated ratios (I_A/I_{A+2}) for the isotope abundances of the fragment ions as observed by CID of the +2 Da isotopomer of the fragment ion at m/z 440 ($C_{11}H_{20}NO_9S_4^- + 2$) which is a product ion (MS^3) of deprotonated 4-(β -D-glucopyranosyl-disulfanyl)butyl-GLS $[M-H]^-$ (see Figure 6).

Ions A and A+2 (m/z)	Molecular formula (A)	Expected ratio	Expected ratio counting only the contribution of ^{34}S	Measured ratio ($n=5$)
97-99	HSO_4^-	2.93	3 S : 1 S	LSs
210-212	$C_5H_8NO_4S_2^-$	1.03	1 S : 1 S	1.04 \pm 0.04
242-244	$C_5H_8NO_6S_2^-$	0.95	1 S : 1 S	0.96 \pm 0.05
420-422	$C_{11}H_{18}NO_8S_4^-$	0.01	0 S : 1 S	0.01 \pm 0.02

LSs= low signals.