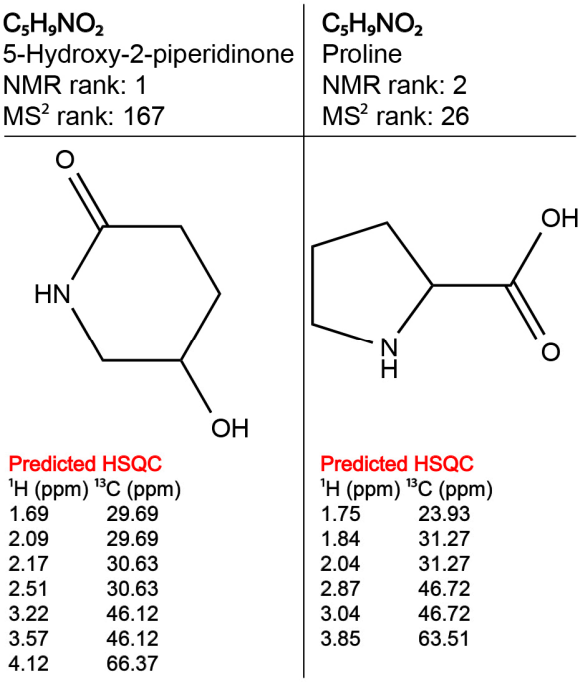



**Figure S1.** Chemical structure of the best and second best match when HSQC predictions of all $C_5H_9NO_2$ isomers were queried against experimental HSQC of proline. HSQC prediction of 5-hydroxy-2-piperidinone was the best match (false positive) and proline was the second best match. However, when $MS^2$ predictions of all $C_5H_9NO_2$ isomers were queried against experimental $MS^2$ of proline, 5-hydroxy-2-piperidinone was the 167$^{th}$ match, much higher than proline prediction (26$^{th}$ match). Therefore, combining NMR with $MS^2$ helped discrimination of the false positive.

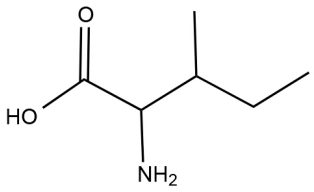
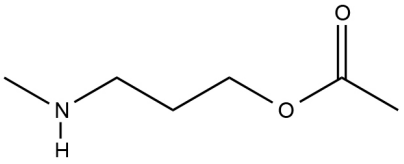
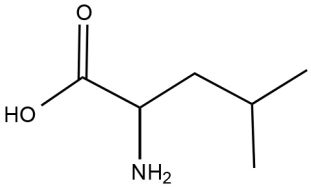
| $C_6H_{13}NO_2$<br>Isoleucine<br>NMR rank: 1 | $C_6H_{13}NO_2$<br>3-methylaminopropyl acetate<br>NMR rank: 2 | $C_6H_{13}NO_2$<br>Leucine<br>NMR rank: 3 |
|---|---|---|
|  |  |  |
| Predicted HSQC<br>$^1$H (ppm) $^{13}$C (ppm)<br>0.99 11.60<br>1.10 14.98<br>1.31 24.17<br>1.78 24.17<br>1.65 36.67<br>3.06 61.17 | Predicted HSQC<br>$^1$H (ppm) $^{13}$C (ppm)<br>2.02 20.55<br>1.88 29.61<br>2.42 33.39<br>2.93 49.62<br>4.15 62.95 | Predicted HSQC<br>$^1$H (ppm) $^{13}$C (ppm)<br>0.95 22.46<br>1.64 24.55<br>1.60 42.20<br>2.56 42.20<br>3.27 53.60 |

**Figure S2.** Chemical structure of the best, second and third best match when HSQC predictions of all $C_6H_{13}NO_2$ isomers were queried against deconvoluted experimental HSQCs of thymidine, proline, phenylalanine, pantothenate, nicotinate, methionine, isoleucine, glutamine, leucine and valine. Isoleucine HSQC prediction found its experimental HSQC as the best match, 3-methylaminopropyl acetate HSQC prediction found experimental HSQC of proline as the second best match (false positive). Finally, leucine HSQC prediction found experimental HSQC of leucine as the third best match.

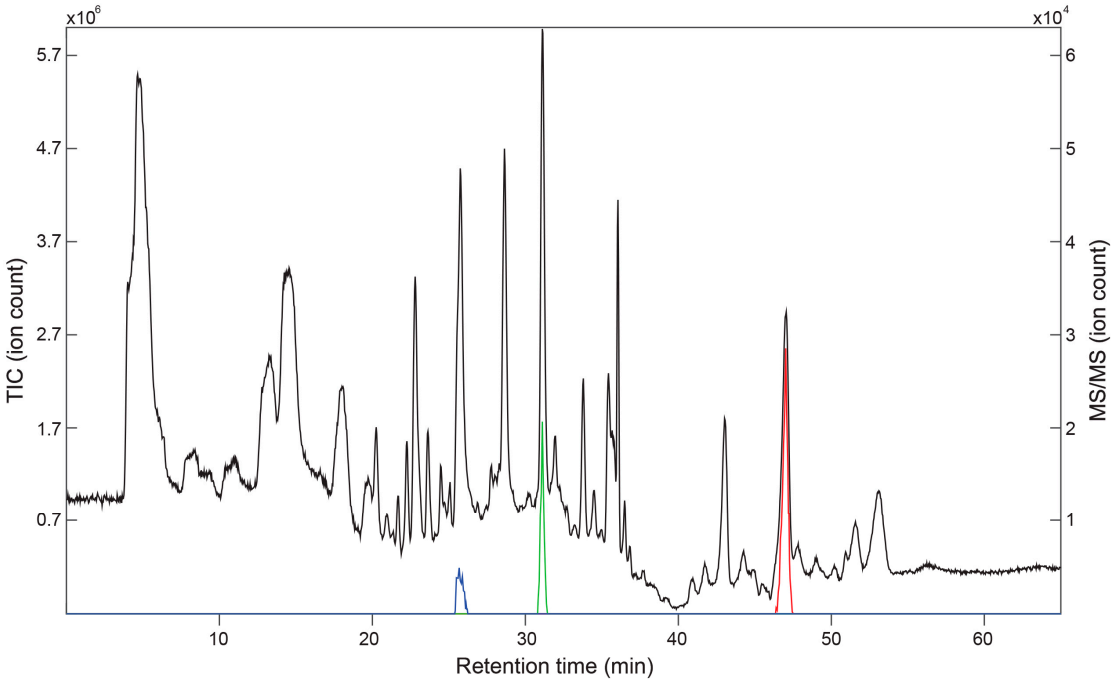



**Figure S3.** Total ion chromatogram (black) of *A. thaliana* metabolite extract. Overlaid LC-MS/MS chromatogram of three compounds selected based on precursor and daughter ions; blue (precursor *m*/z: 367.0976, daughter *m*/z: 115.0171, retention time: 25.8 min), green (precursor *m*/z: 341.1032, daughter *m*/z: 59.0114, retention time: 31.1 min) and red (precursor *m*/z: 436.0310, daughter *m*/z: 96.9567, retention time: 47.0 min). The red chromatogram belongs to glucoraphanin (see Figure 3).

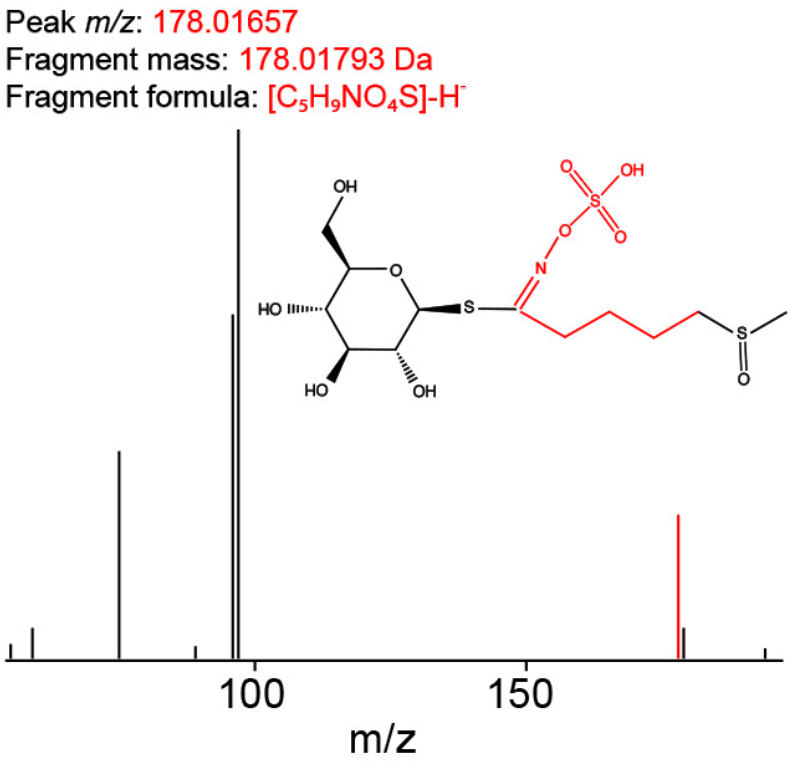



**Figure S4.** The MS/MS peak (red) differentiating MS/MS prediction of structure **c** from structure **a** and **b** in Figure 3. The peak experimentally appeared at ~*m*/*z* 178.0166. It matched to the theoretical mass of the fragment formula of $[C_5H_9NO_4S]$-$H^-$ including the $SO_4$ group and part of the aliphatic chain of glucoraphanin (red).

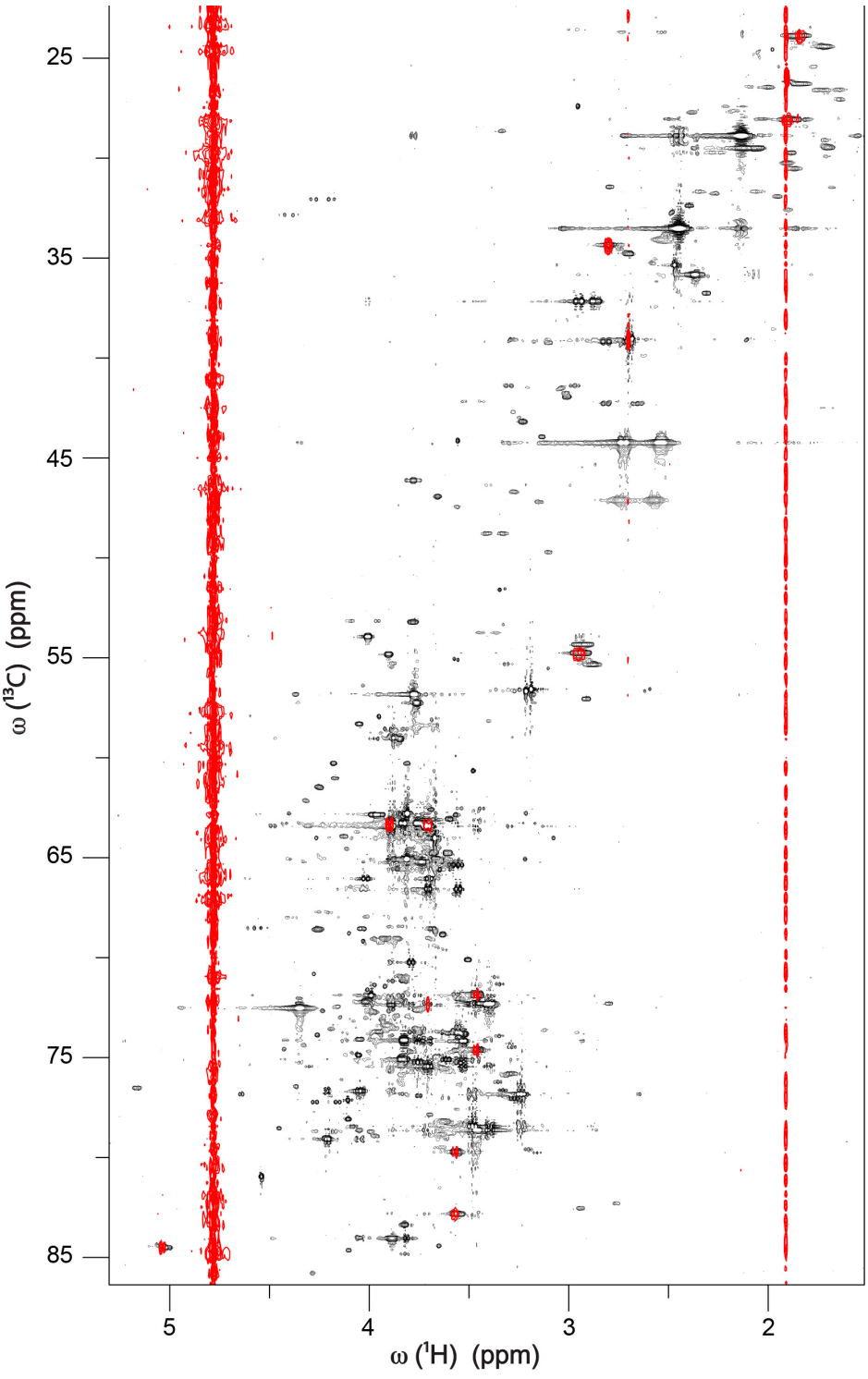



**Figure S5.** Overlay of the experimental 2D $^{13}$C-$^{1}$H HSQC spectra of the unfractionated *A. thaliana* extract (black) and LC-fractionated *A. thaliana* extract at the retention time 47 min (red).

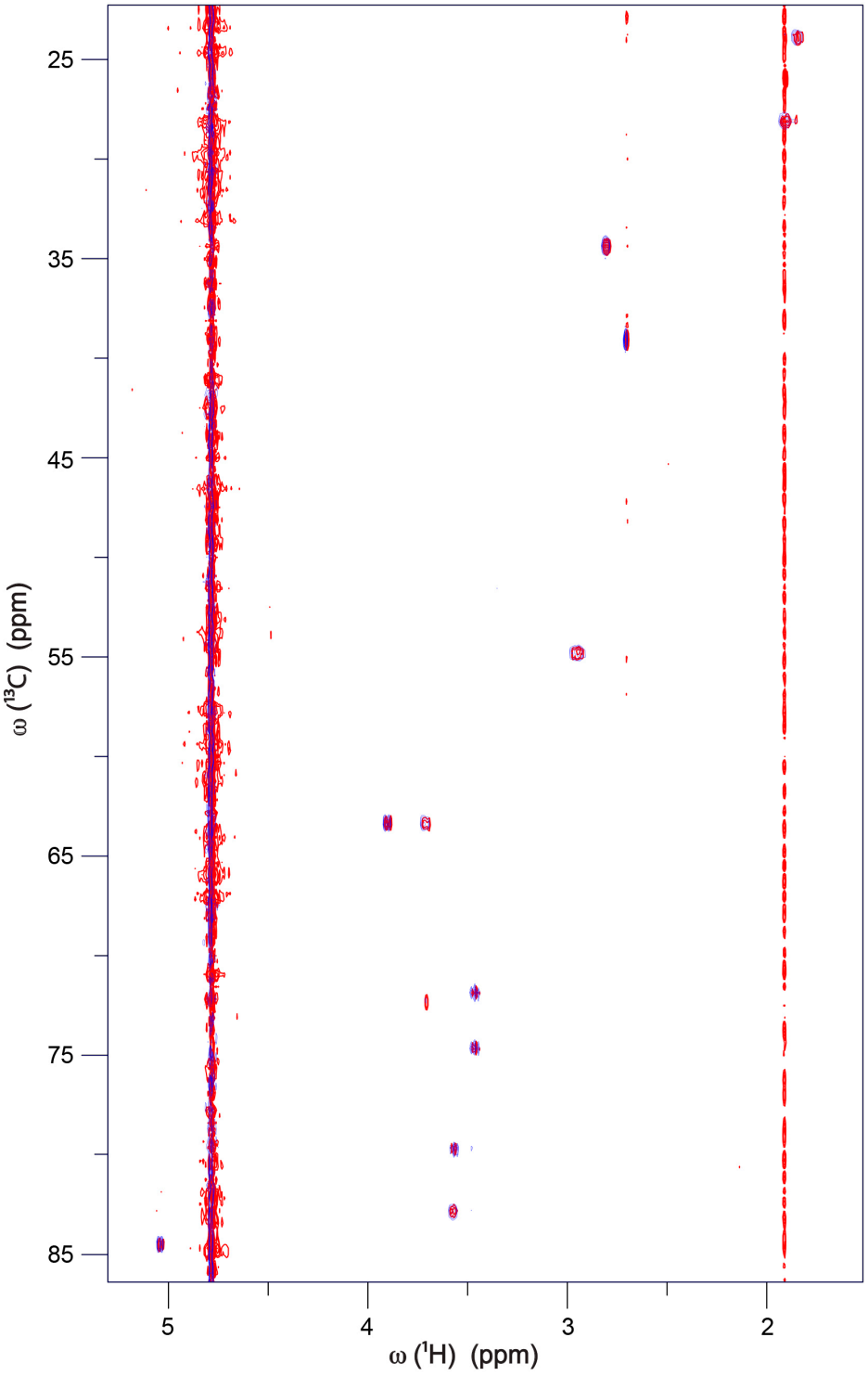



**Figure S6.** Overlay of the experimental 2D $^{13}C$-$^{1}H$ HSQC spectra of the LC-fractionated *A. thaliana* extract at the retention time 47 min (red) and glucoraphanin standard (blue).

**Table S1.** The list of precursor ions that were selected for fragmentation in this study

| metabolite | precursor *m/z* | retention time (min) | collision energy (V) | ion mode | daughter *m/z* list[a] |
|---|---|---|---|---|---|
| Thymidine | 243.0967 | 12.2 | 30 | + | 127.0499 |
| | | | | | 110.0235 |
| | | | | | 69.0336 |
| | | | | | 84.0444 |
| | | | | | 109.0398 |
| Proline | 116.0701 | 30.8 | 30 | + | 70.0651 |
| | | | | | 71.0683 |
| | | | | | 68.0489 |
| | | | | | 57.0566 |
| | | | | | 70.0908 |
| Phenylalanine | 166.0857 | 31.9 | 30 | + | 103.0538 |
| | | | | | 120.0803 |
| | | | | | 77.0385 |
| | | | | | 79.0540 |
| | | | | | 93.0689 |
| Pantothenate | 220.1170 | 45.4 | 30 | + | 70.0292 |
| | | | | | 67.0543 |
| | | | | | 57.0703 |
| | | | | | 90.0548 |
| | | | | | 98.0231 |
| Nicotinate | 124.0390 | 47.1 | 30 | + | 78.0335 |
| | | | | | 80.0491 |
| | | | | | 53.0384 |
| | | | | | 79.0403 |
| | | | | | 52.0186 |
| Methionine | 150.0575 | 32.1 | 30 | + | 61.0108 |
| | | | | | 56.0496 |
| | | | | | 59.0727 |
| | | | | | 58.0653 |
| | | | | | 63.0063 |
| Isoleucine | 132.1015 | 29.0 | 30 | + | 69.0698 |
| | | | | | 58.0661 |
| | | | | | 71.0718 |
| | | | | | 57.0565 |
| | | | | | 70.0729 |
| Glutamine | 147.0746 | 35.3 | 30 | + | 84.0431 |
| | | | | | 56.0488 |
| | | | | | 85.0273 |
| | | | | | 105.0321 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | 56.4253 |
| Leucine | 132.1010 | 28.3 | 30 | + | 55.0540 |
| | | | | | 86.0963 |
| | | | | | 53.0380 |
| | | | | | 55.0173 |
| | | | | | 69.0691 |
| Valine | 118.0850 | 30.5 | 30 | + | 55.0540 |
| | | | | | 57.0569 |
| | | | | | 72.0798 |
| | | | | | 56.0496 |
| | | | | | 58.0642 |
| Glucoraphanin | 436.0310 | 47.0 | 30 | - | 96.9567 |
| | | | | | 95.9484 |
| | | | | | 74.9883 |
| | | | | | 98.9526 |
| | | | | | 178.0166 |

[a] Only the highest intensity (top five) daughter ions were included in the table.