Combining stable isotope labeling and molecular networking for biosynthetic pathway characterization

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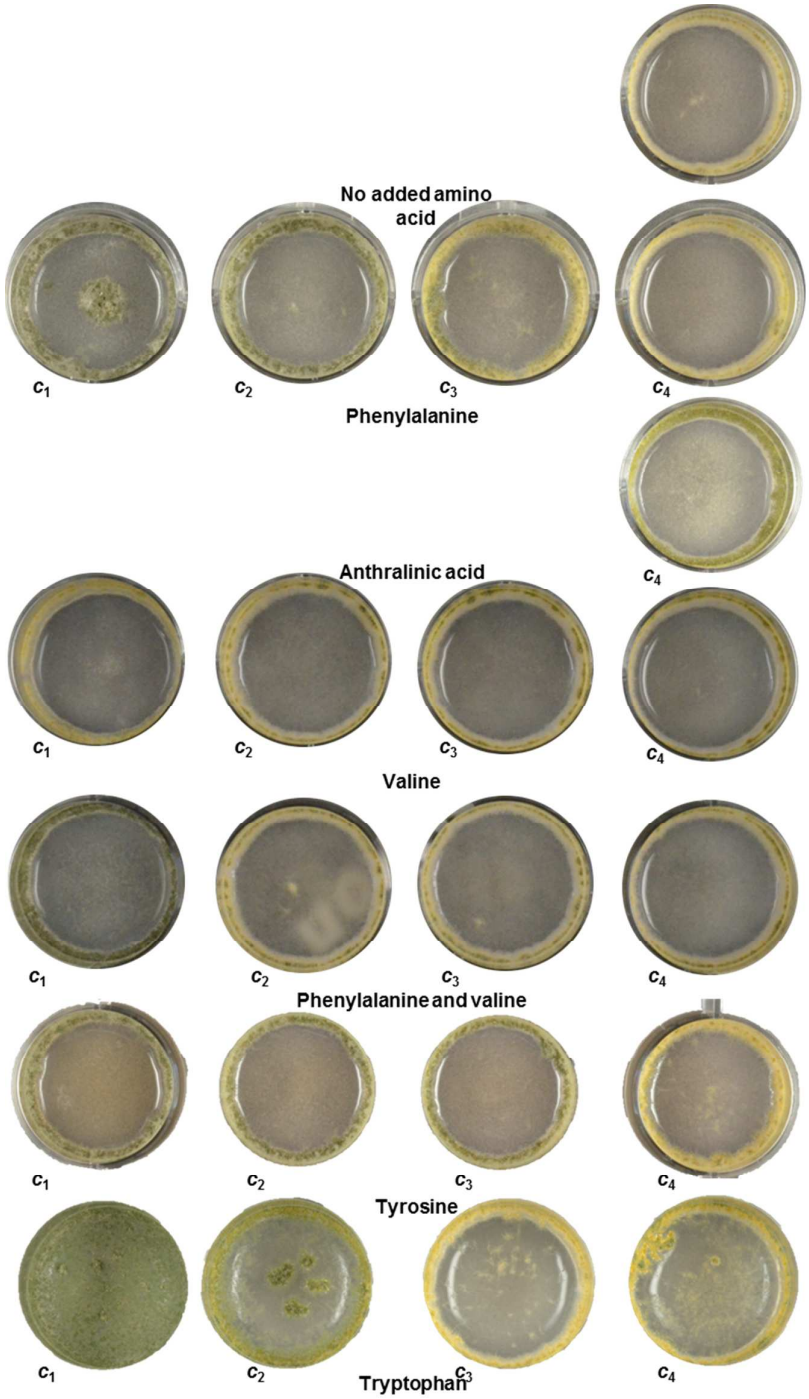


Figure S1. Photographs of *Aspergiullus nidulans* IBT4887 used in the study. The top row is a photo of *A. nidulans* cultivated without the addition of any amino acids. The other rows are photographs of *A. nidulans* cultivated with the addition of the noted amino acids at the indicated concentrations. The addition of anthranilic acid only resulted in growth of the fungus at the lowest tested concentration. The fungi were kept stationary while being incubated at 25 °C in darkness for 7 days in MM without any added amino acids.

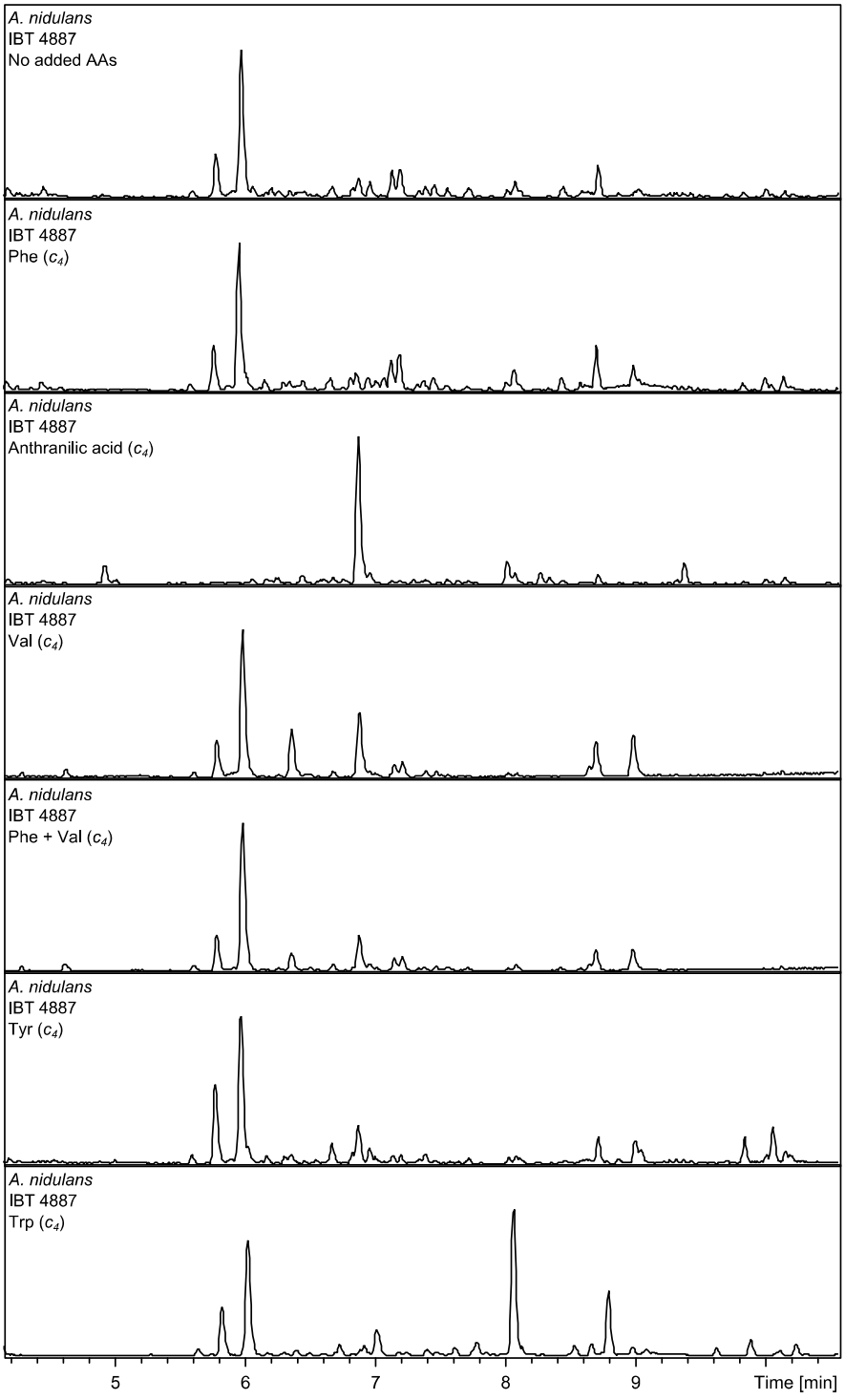


Figure S2. Top is a BPC from *A.* nidulans IBT4887 cultivated without any added amino acids, while the other BPC are from fungi where AAs have been added in the denoted concentration. The chromatograms showed a difference in intensity of several peaks, including peaks at RT 5.8 min (austinol), 6.0 min (dehydroaustinol), and 6.9 min (sterigmatocystin). However, a close inspection of the data showed that no signs of incorporation of labeled AAs in any of the corresponding compounds. The chromatograms have been scaled to the highest signal. The extract from the sample with added Trp showed a strong signal at 8.1 min, which corresponded to a known impurity (tributyrin).

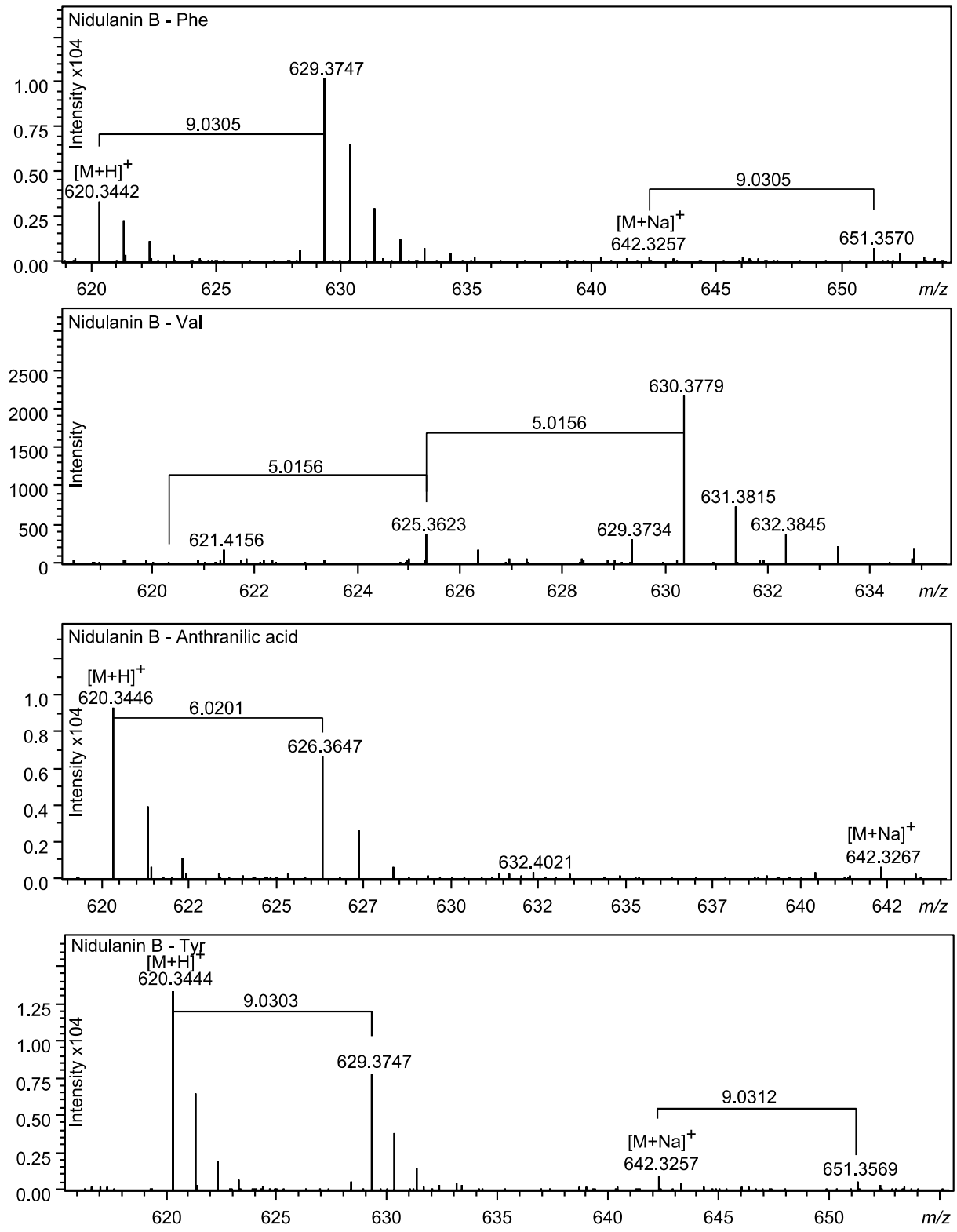


Figure S3. Labeling of nidulanin B. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 7.70-7.75 min and have been scale to the highest signal.

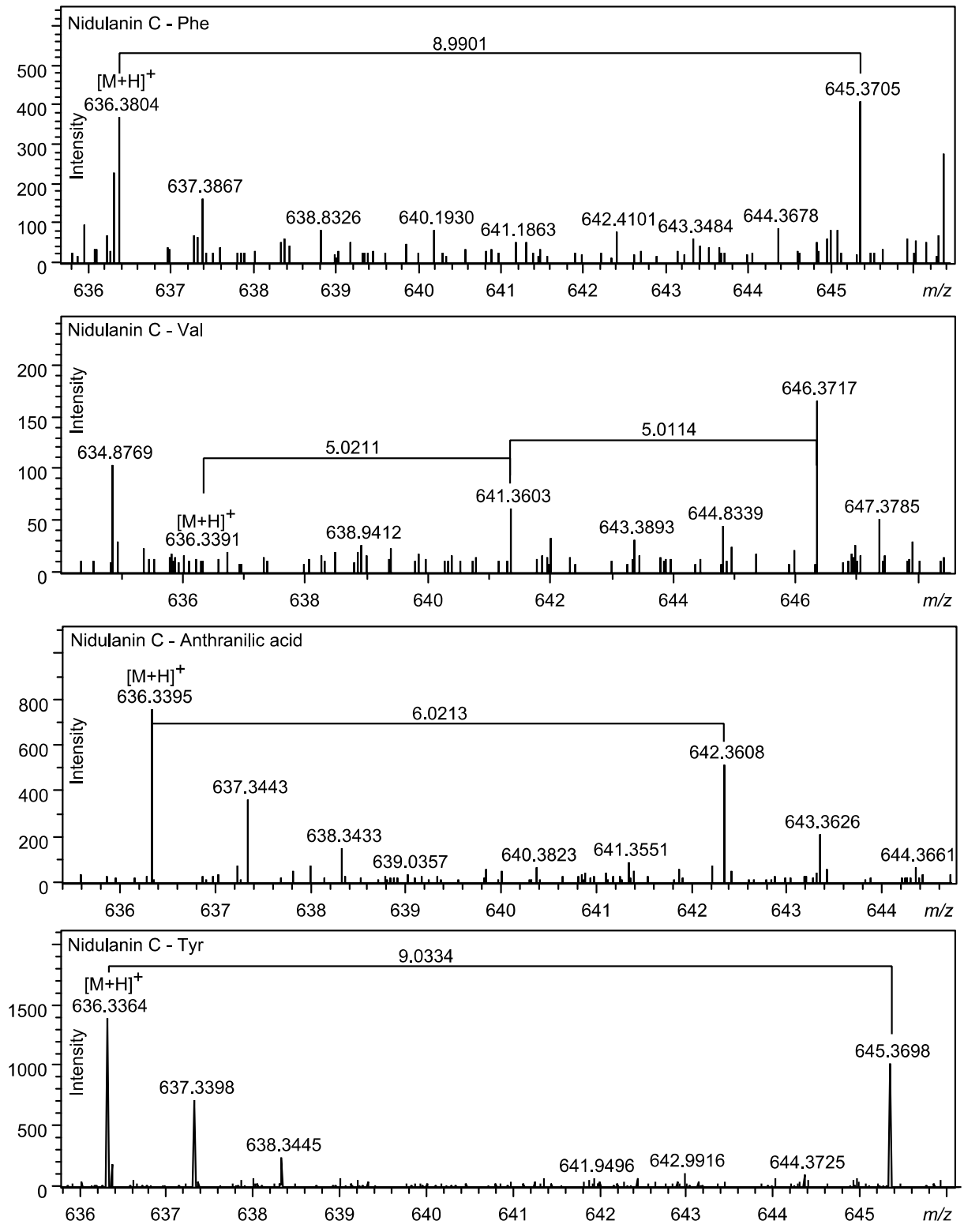


Figure S4. Labeling of nidulanin C. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 7.27-7.32 min and have been scale to the highest signal.

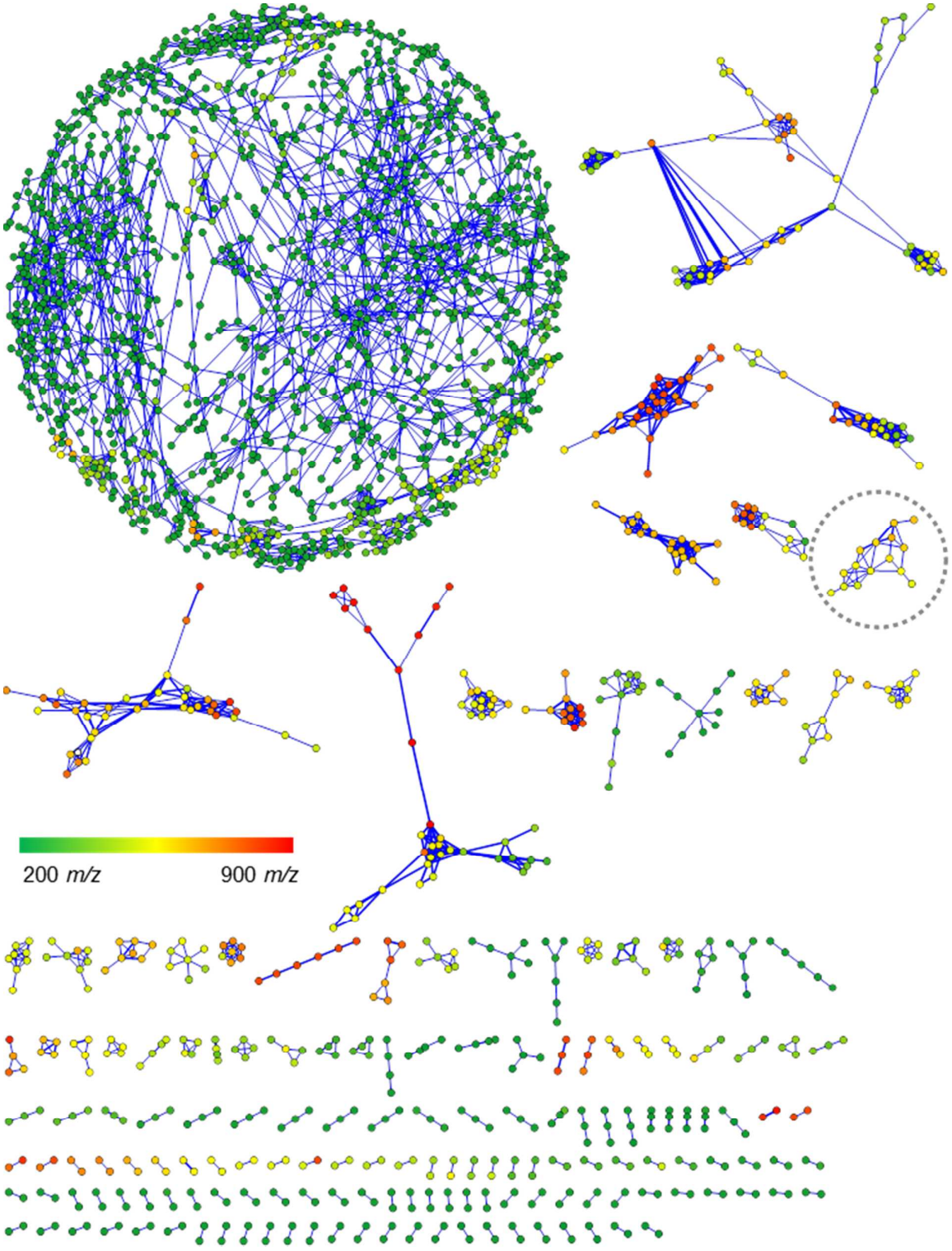


Figure S5. Molecular network generated from analysis of samples from *A. nidulans.* Each circle represents the precursor ion of a given compound where as the color of the circle represents the *m/z*-ratio. The thickness of the blue lines connecting the nodes (circles) indicates the similarity of the MS/MS spectra for the connected nodes, as scored by the networking algorithm. The network was constructed based on samples from experiments with and without addition of stable isotope labeled AAs. The sub-network marked with the dotted ring contains a node corresponding to NA.

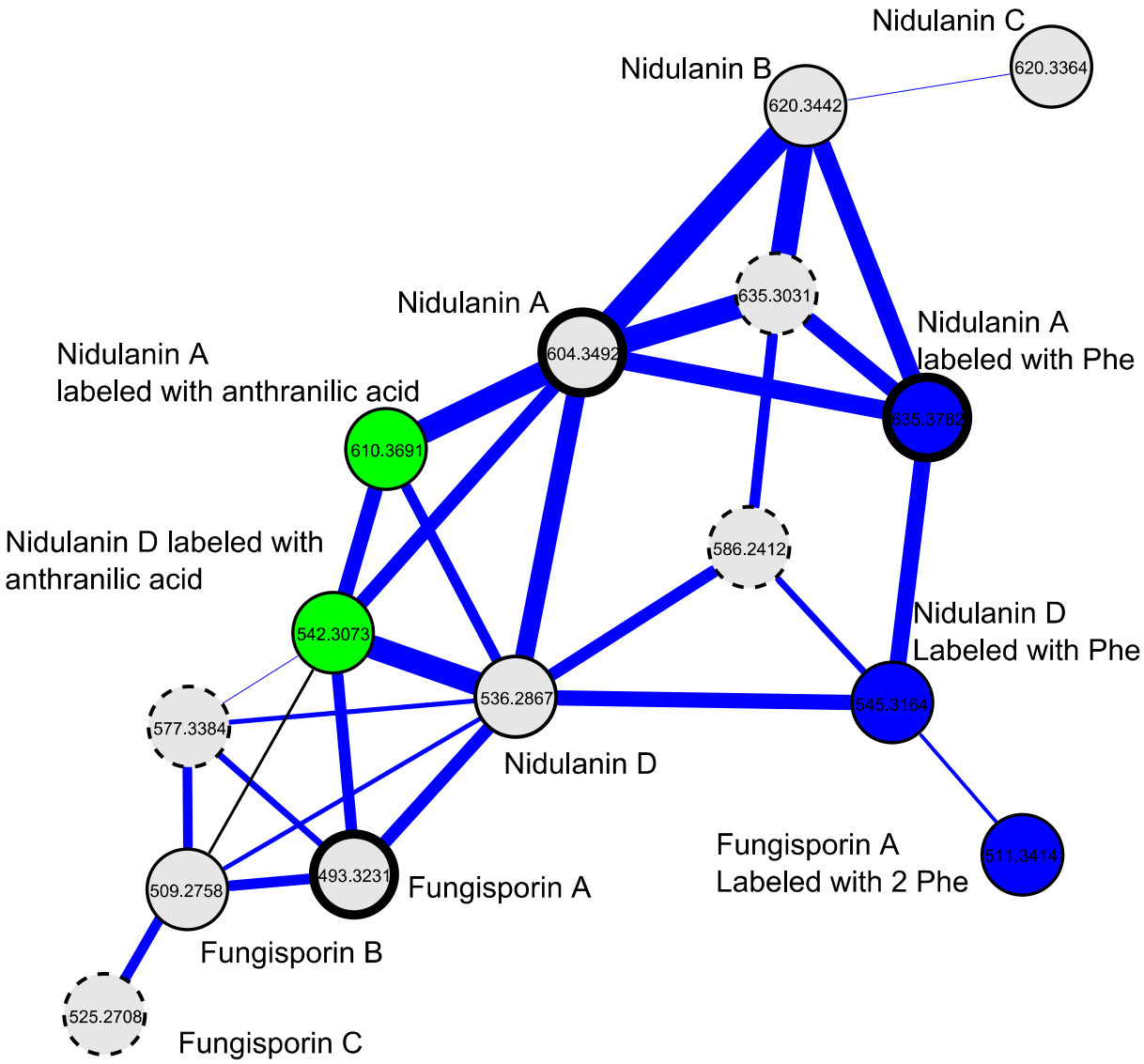


Figure S6 – Sub-cluster containing a node corresponding to nidulanin A and several previously described analogues. The circles represent the consensus MS/MS spectrum for a given parent. The thickness of the blue lines connecting the nodes (circles) indicates the similarity of the MS/MS spectra for the connected nodes, as scored by the networking algorithm. Previously undescribed compounds are marked with a dashed outline.

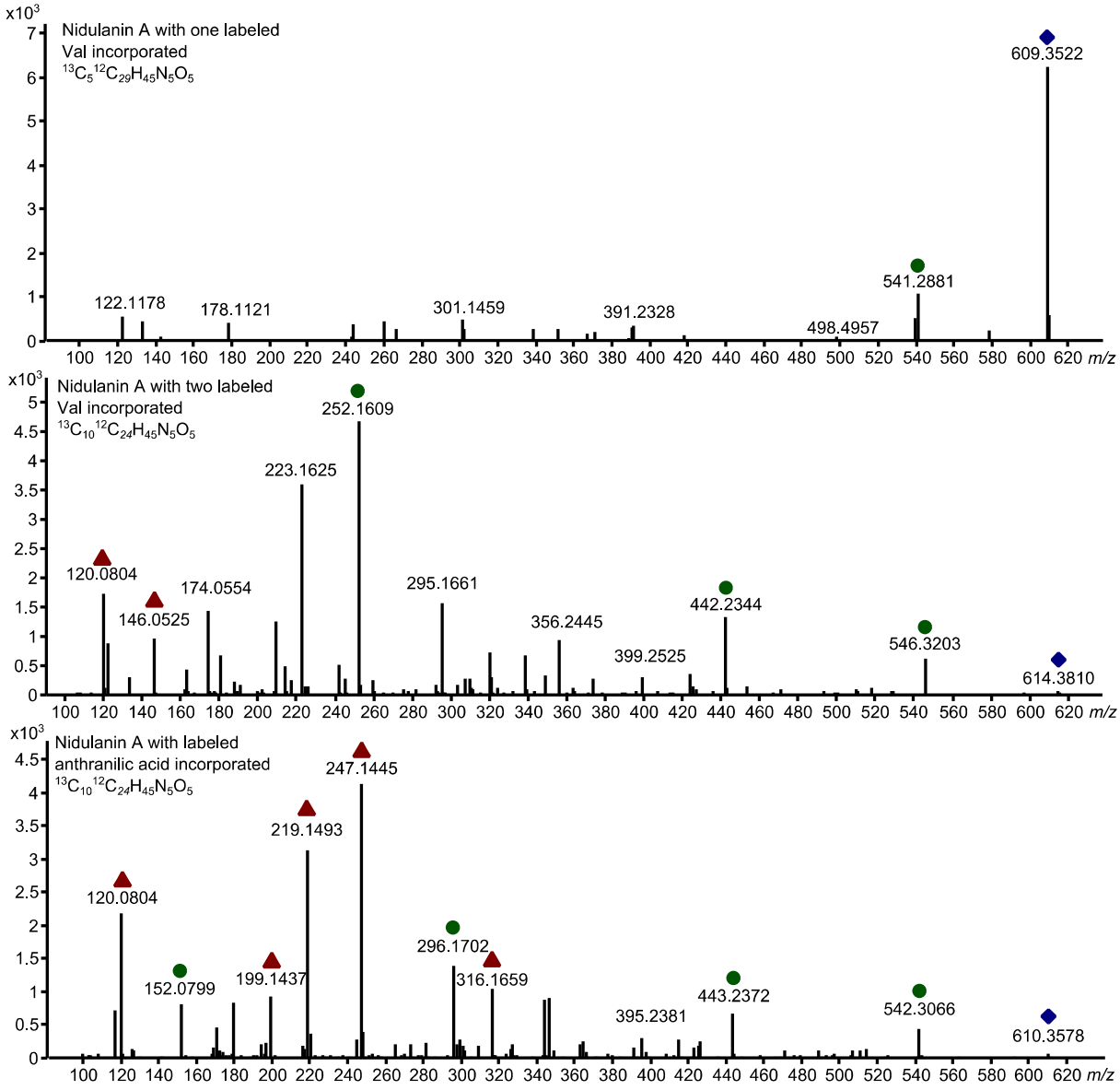
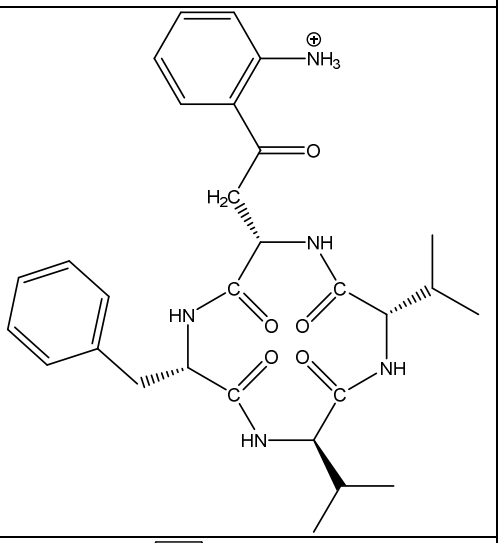
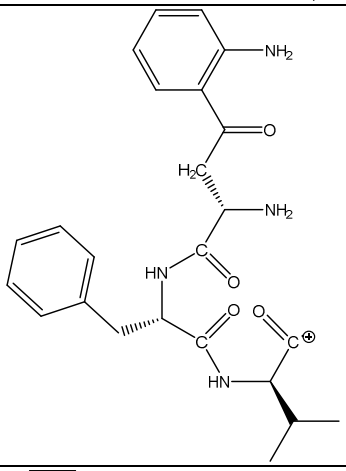
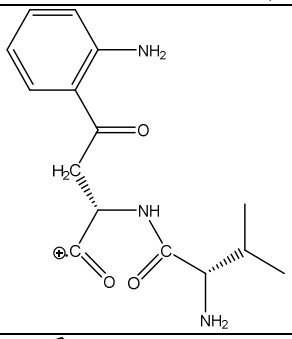
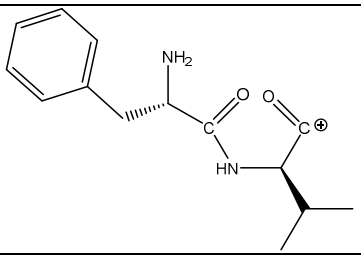
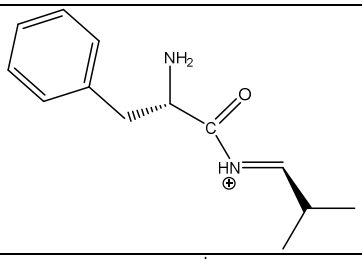
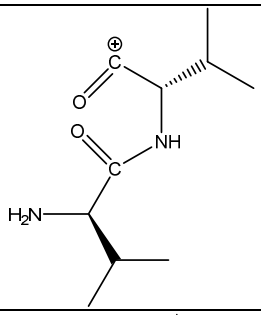
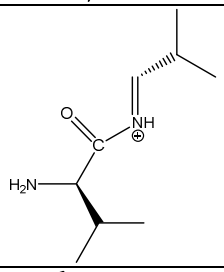
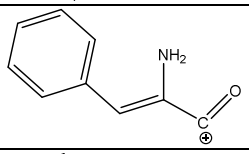
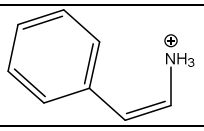


Figure S7. MS/MS spectra obtained from analysis of NA labeled with anthranilic acid as well as one and two Val residues respectively. The blue diamonds denote to the product ion of the compounds, red triangles denote fragments formed by the unlabeled NA, while the green circles denote fragments found in NA that now contain labeled atoms.

Table S1. Fragment ions formed by fragmentation of nidulanin A

Fragment [*m/z*]	Chemical formula	Structure
536	$C_{29}H_{37}N_5O_5$	
437	$C_{24}H_{29}N_4O_4$	
290	$C_{15}H_{20}N_3O_3$	
247	$C_{14}H_{19}N_2O_2$	

219	$C_{13}H_{19}N_2O$	
199	$C_{10}H_{19}N_2O_2$	
171	$C_9H_{18}N_2O$	
146	C_9H_8NO	
120	$C_8H_{10}N$	

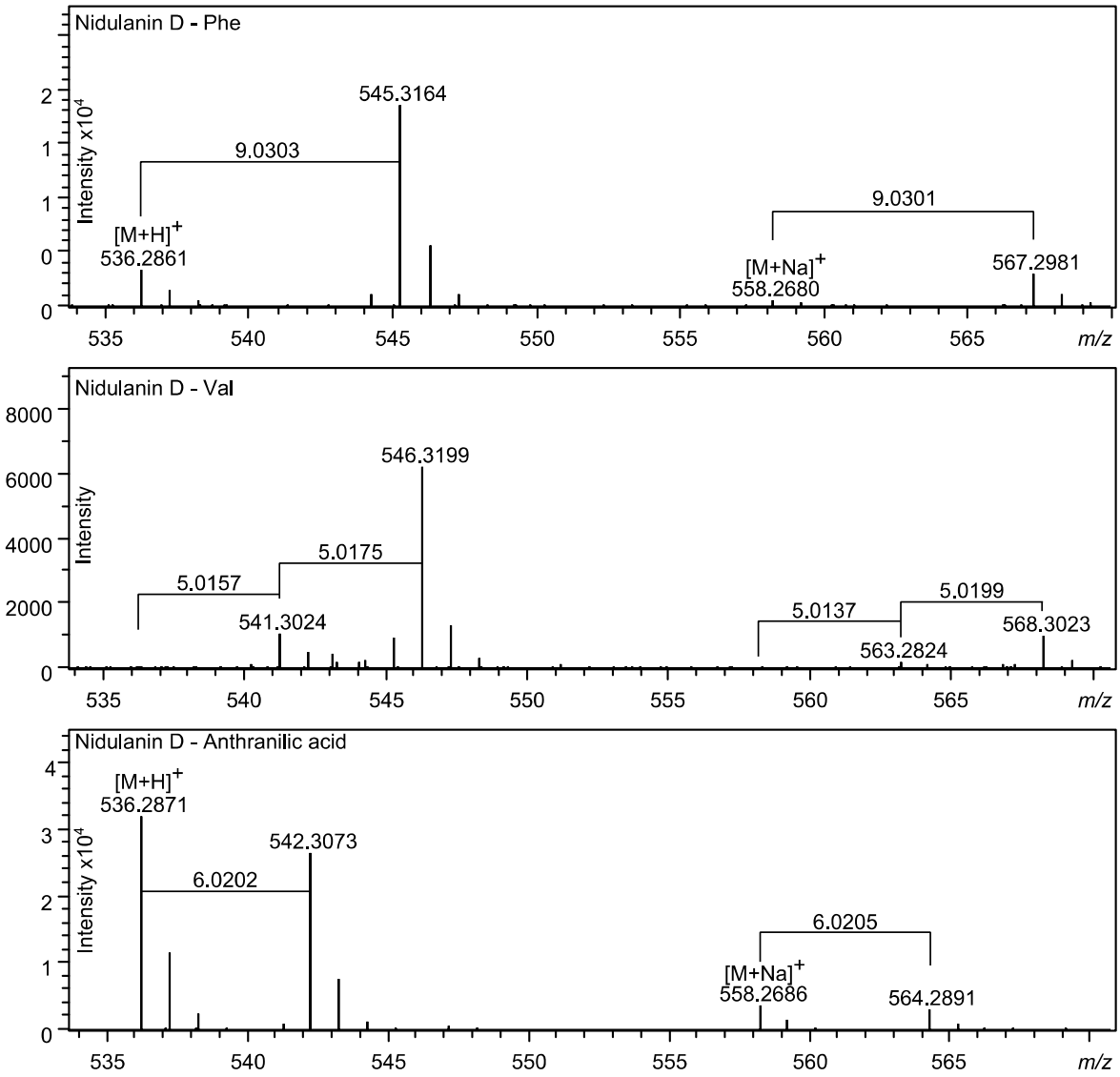


Figure S8. Labeling of nidulanin D. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 6.95-7.00 min and have been scale to the highest signal.

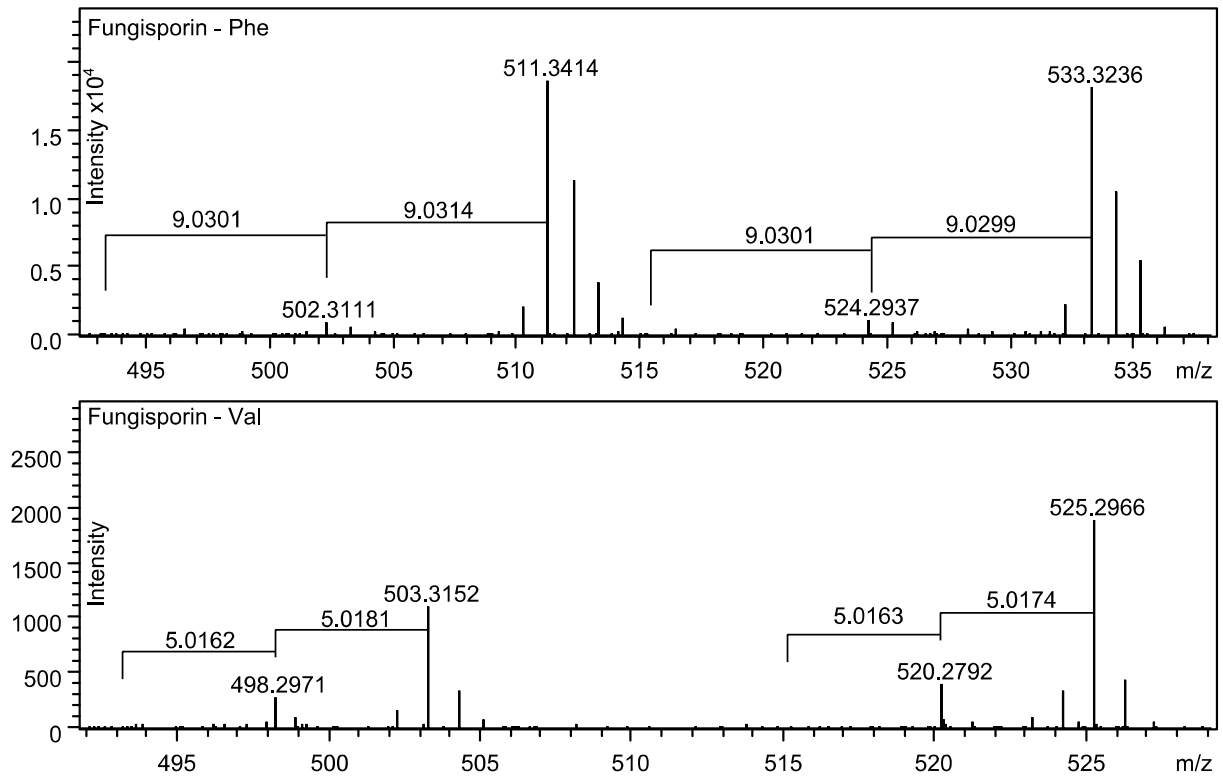
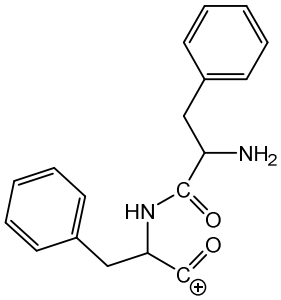
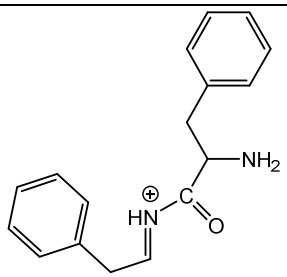
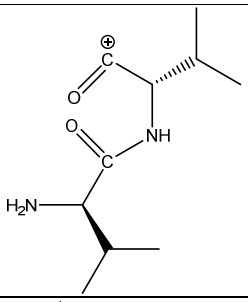
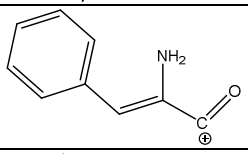
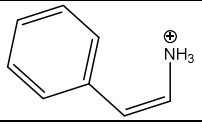


Figure S9. Labeling of fungisporin. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 7.34-7.40 min and have been scale to the highest signal.

Table S2. Fragment ions formed by fragmentation of nidulanin fungisporin A

Fragment [*m/z*]	Chemical formula	Structure
295	$C_{18}H_{18}N_2O_2$	
267	$C_{17}H_{18}N_2O$	
199	$C_{10}H_{19}N_2O_2$	
171	$C_9H_{18}N_2O$	
120	$C_8H_{10}N$	

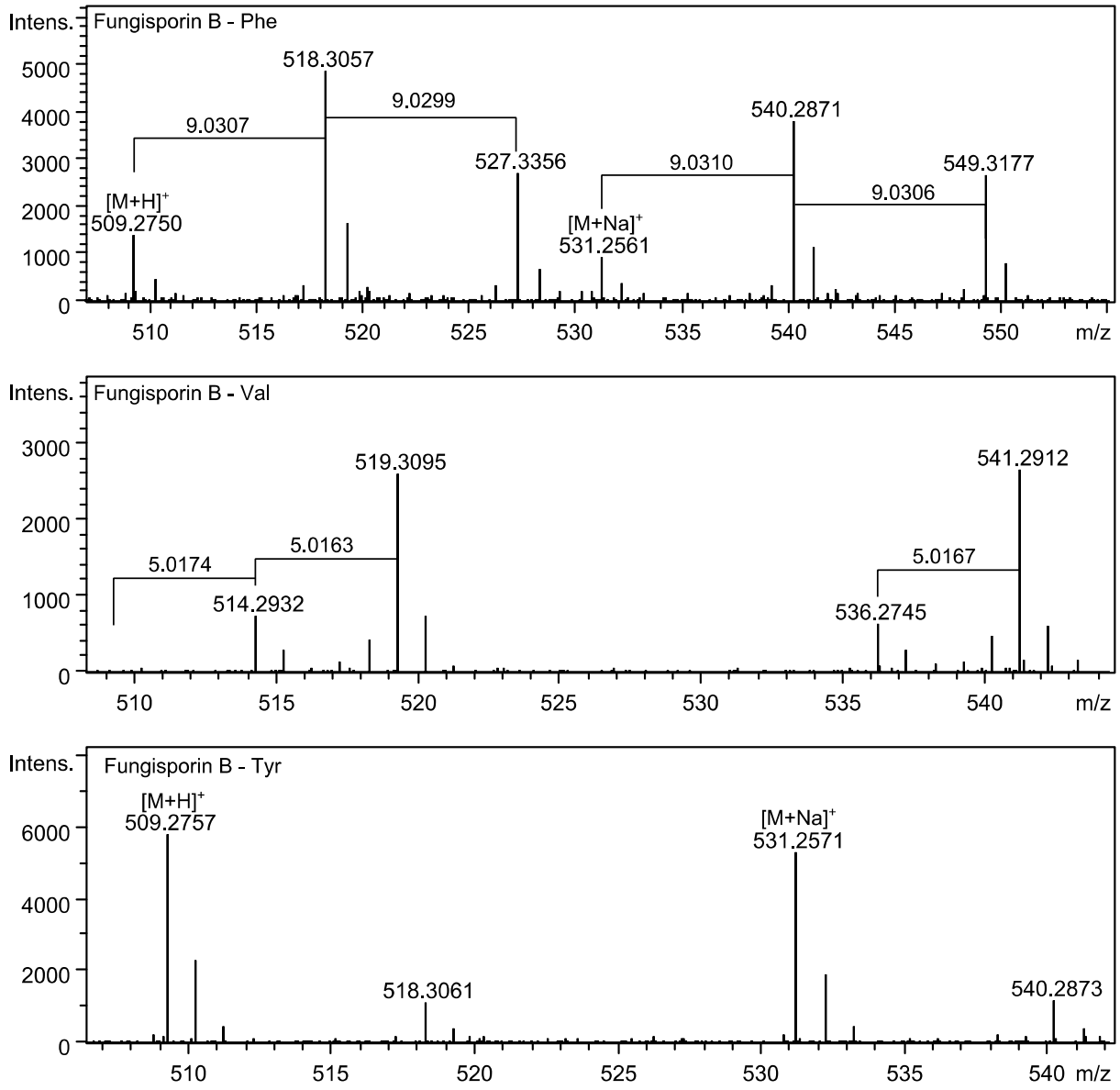


Figure S10. Labeling of fungisporin B. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 6.25-6.29 min and have been scale to the highest signal.

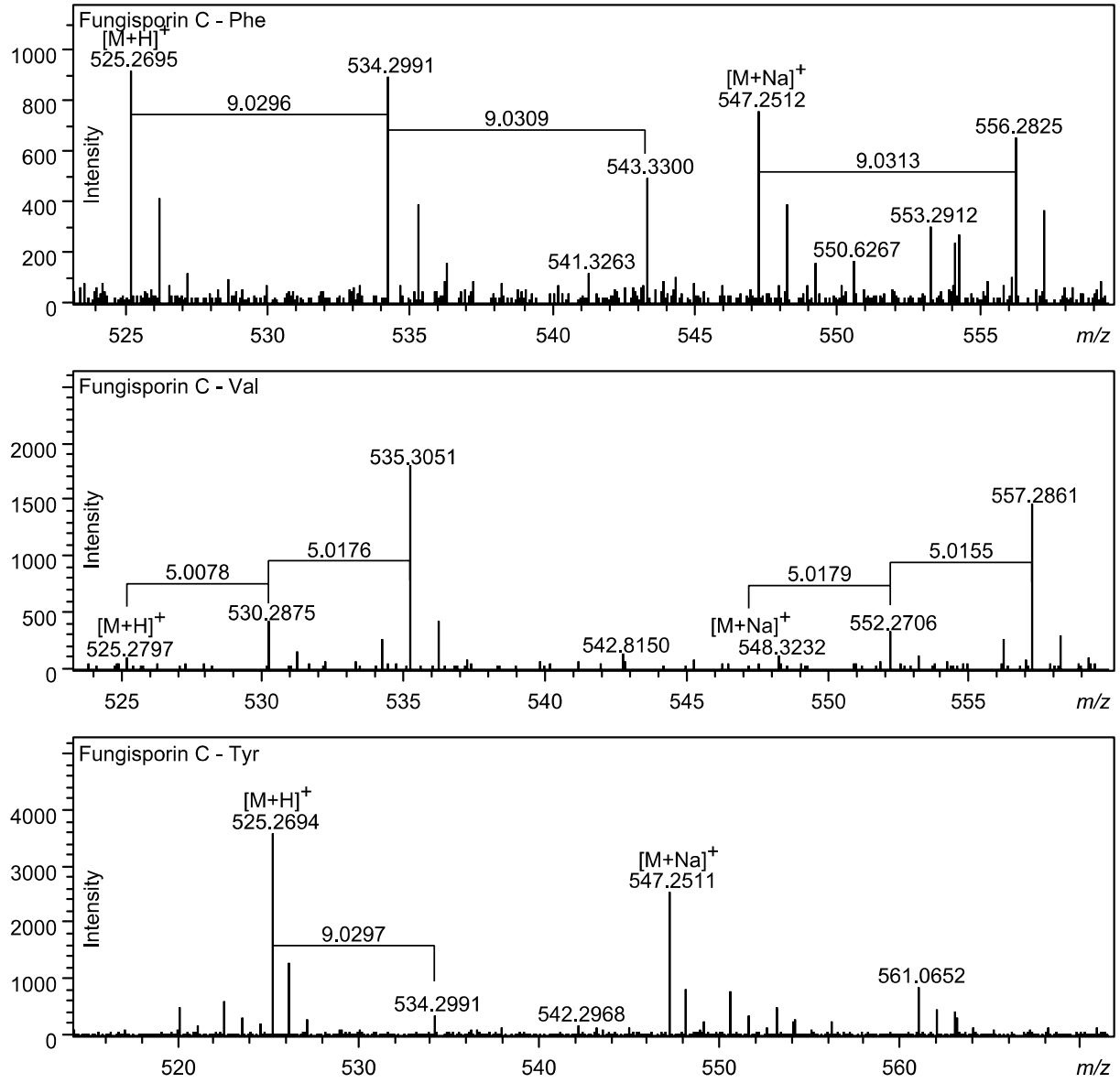


Figure S11. Labeling of fungisporin C. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 5.40-5.45 min and have been scale to the highest signal.

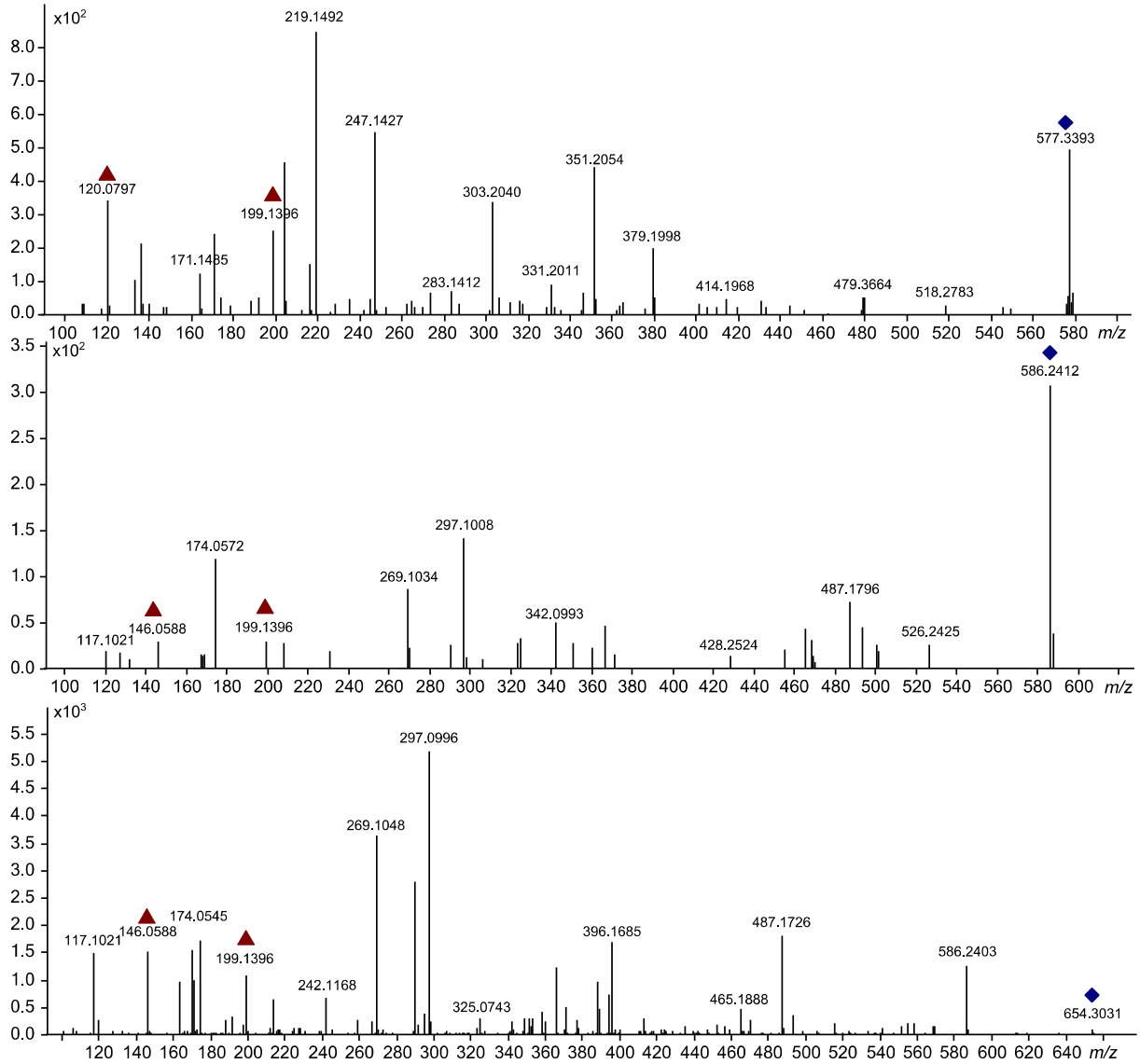


Figure S12 MS/MS spectra obtained from analysis of three unknown. The blue diamonds denote to the product ion of the compounds while the red triangles denote fragments formed by the unlabeled NA. The MS/MS obtained from fragmentation of the ion 654 exhibits many of the same ions as the one obtained from 586. The mass difference between the two ions indicate that they could be a prenylated and unprenylated form of the same compound.

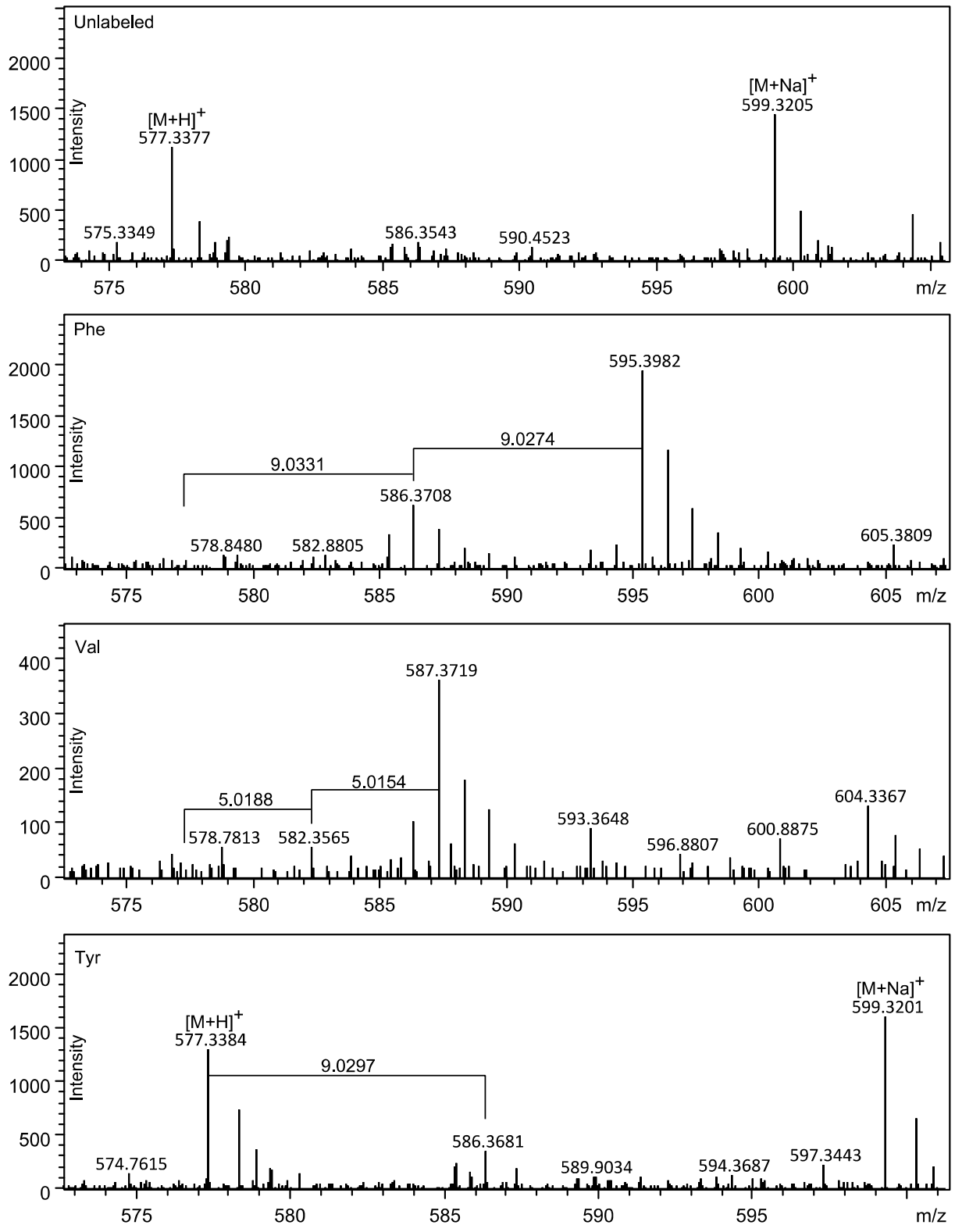


Figure S13. Labeling of new compound with the molecular formula $C_{33}H_{45}N_4O_5$. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 7.3-7.4 min and have been scale to the highest signal.

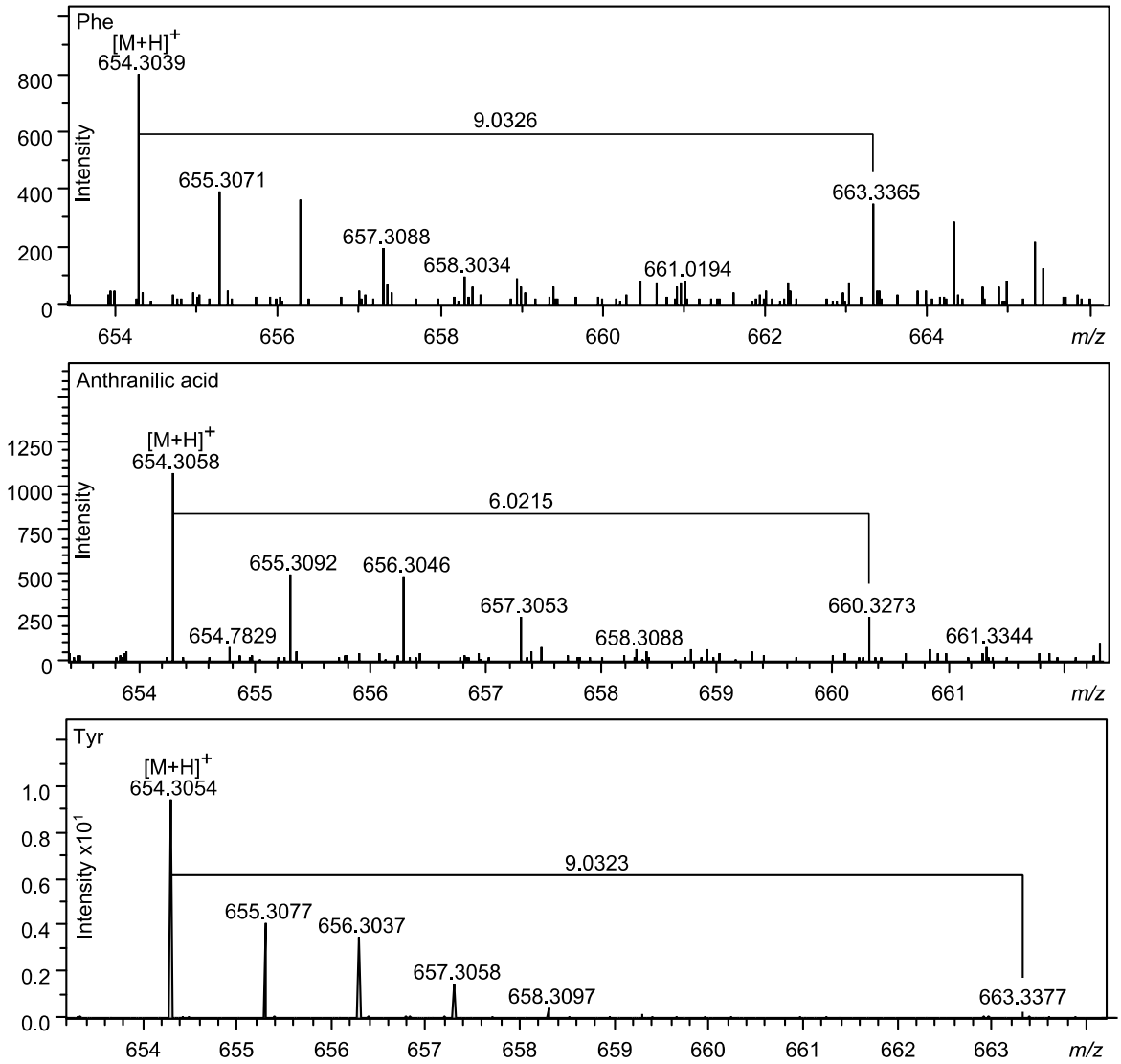


Figure S14. Labeling of new compound with the molecular formula $C_{34}H_{40}N_5O_7$. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 7.93-7.99 min and have been scale to the highest signal.

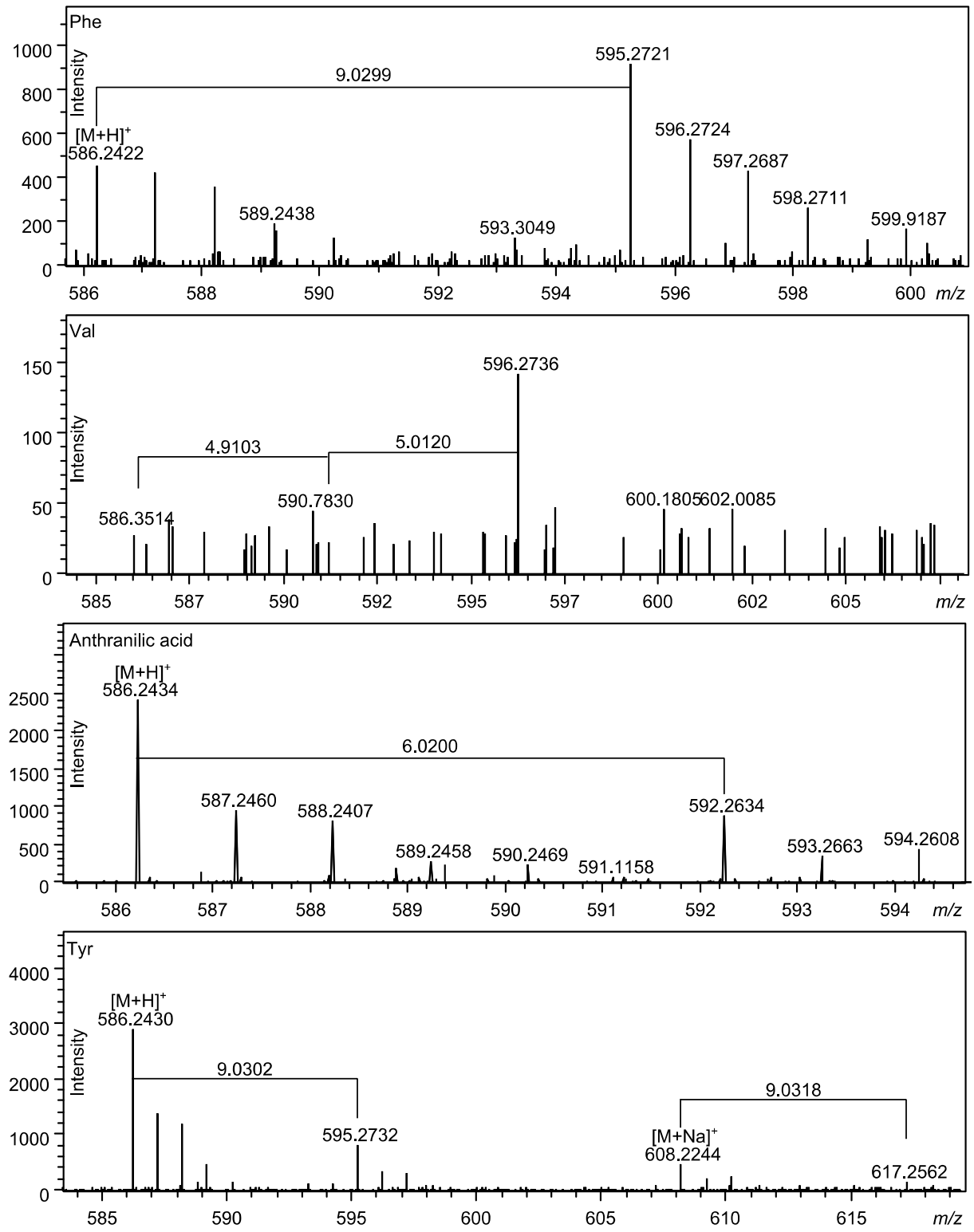


Figure S15. Labeling of new compound with the molecular formula $C_{35}H_{32}N_5O_4$. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 6.50-7.00 min and have been scale to the highest signal.

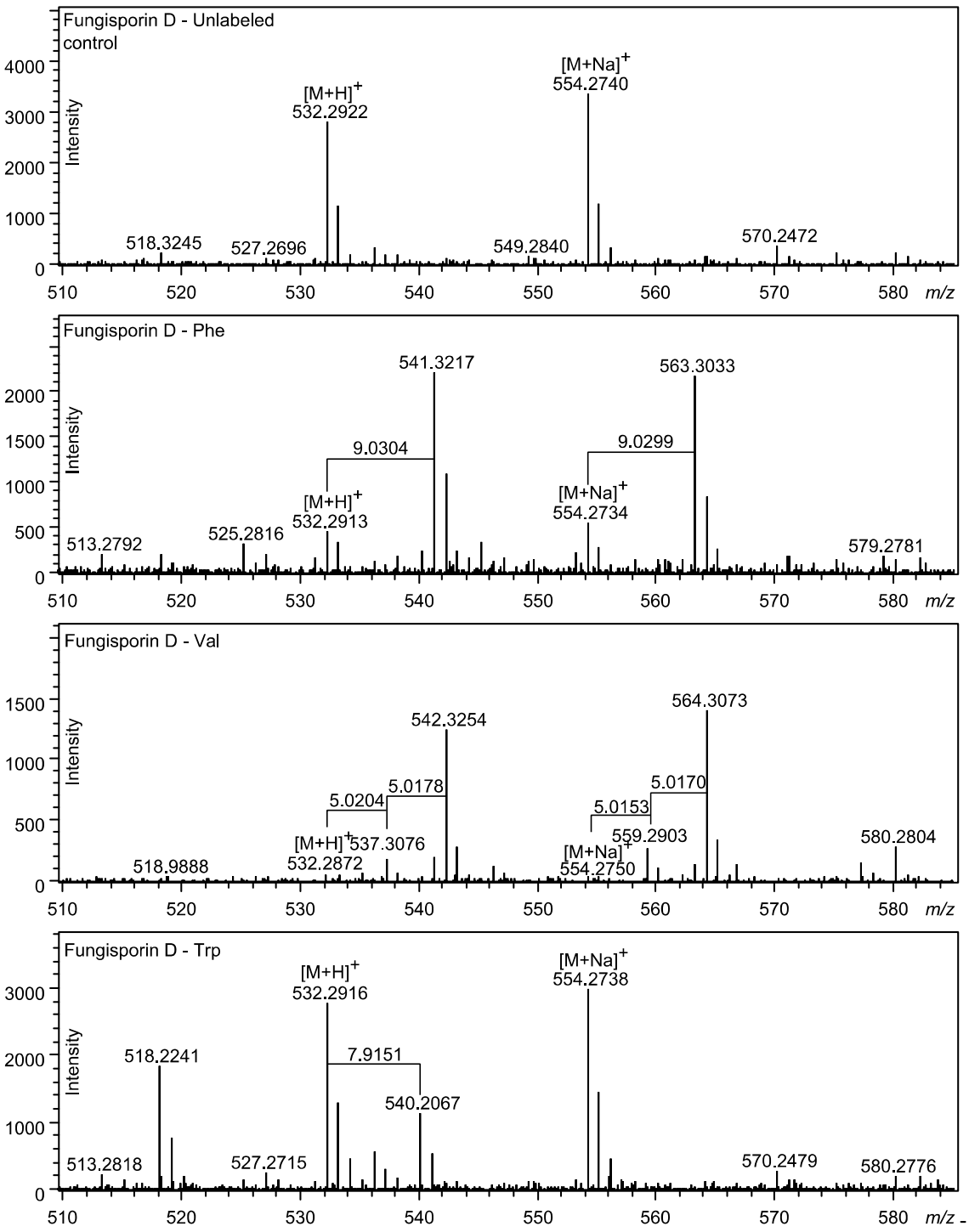


Figure S16. Labeling of fungisporin D. The mass spectra are from *A. nidulans* IBT 4887, cultivated at 25 °C in darkness for 7 days on MM. The mass spectra were extracted at RT 7.15-7.20 min and have been scale to the highest signal.