Supplementary Materials

Miniaturized Bioaffinity Assessment Coupled to Mass Spectrometry for Guided Purification of Bioactives from Toad and Cone Snail

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Received: 9 December 2013; in revised form: 23 January 2014 / Accepted: 26 January 2014 / Published: 13 February 2014

Figure S1. On-line bioassay analysis of three consecutive 500 nL sample injections containing 0.1, 0.5 and 2.5 µg crude *Conus textile* venom. The 0.1 and 0.5 µg venom injections also contained 40 µM nicotine to align the MS and bioassay trace.

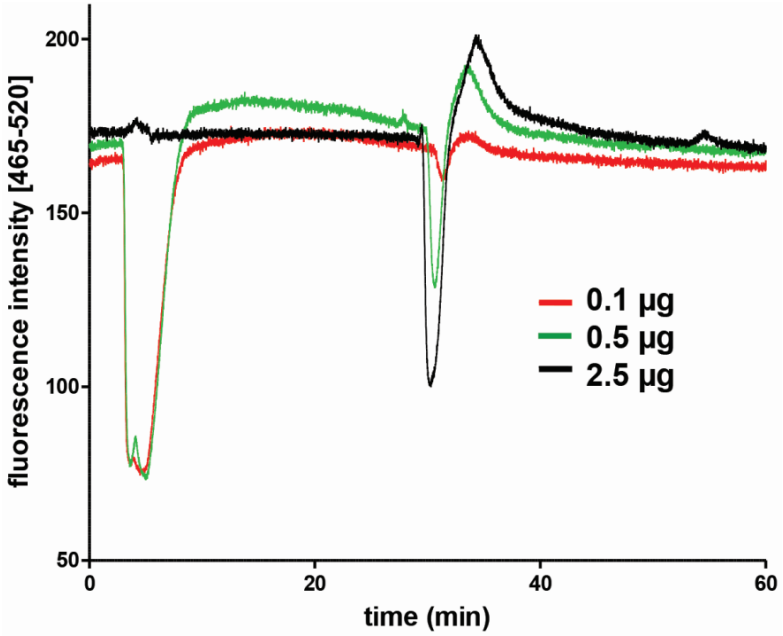


Figure S2. An analysis of a 500 nL sample containing 5 µg *Bufo alvarius* skin secretion extract obtained identical binding signals and attributed masses as the *Bufo marinus* extract analysis.

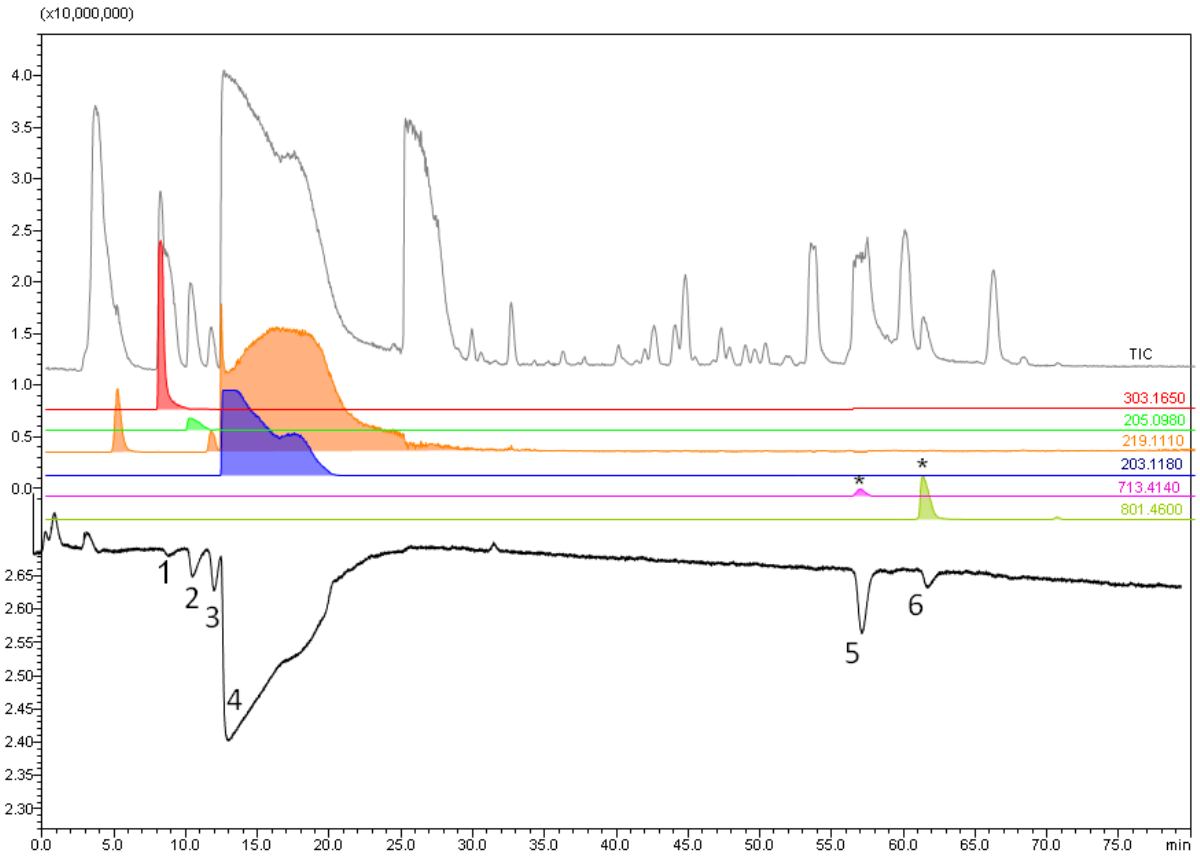
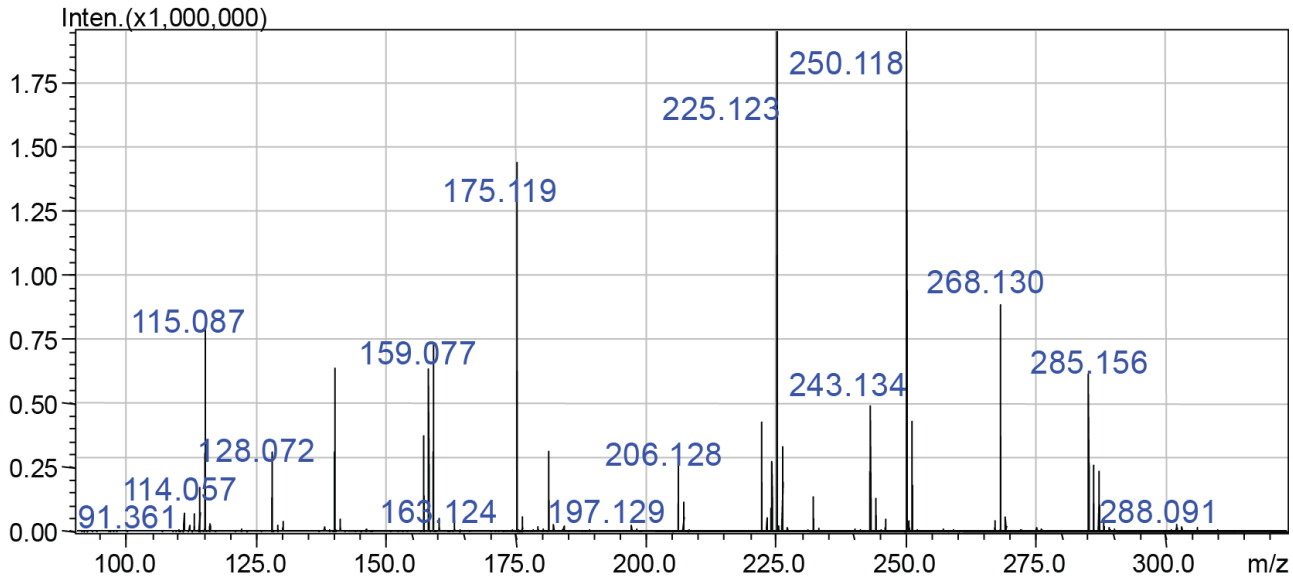
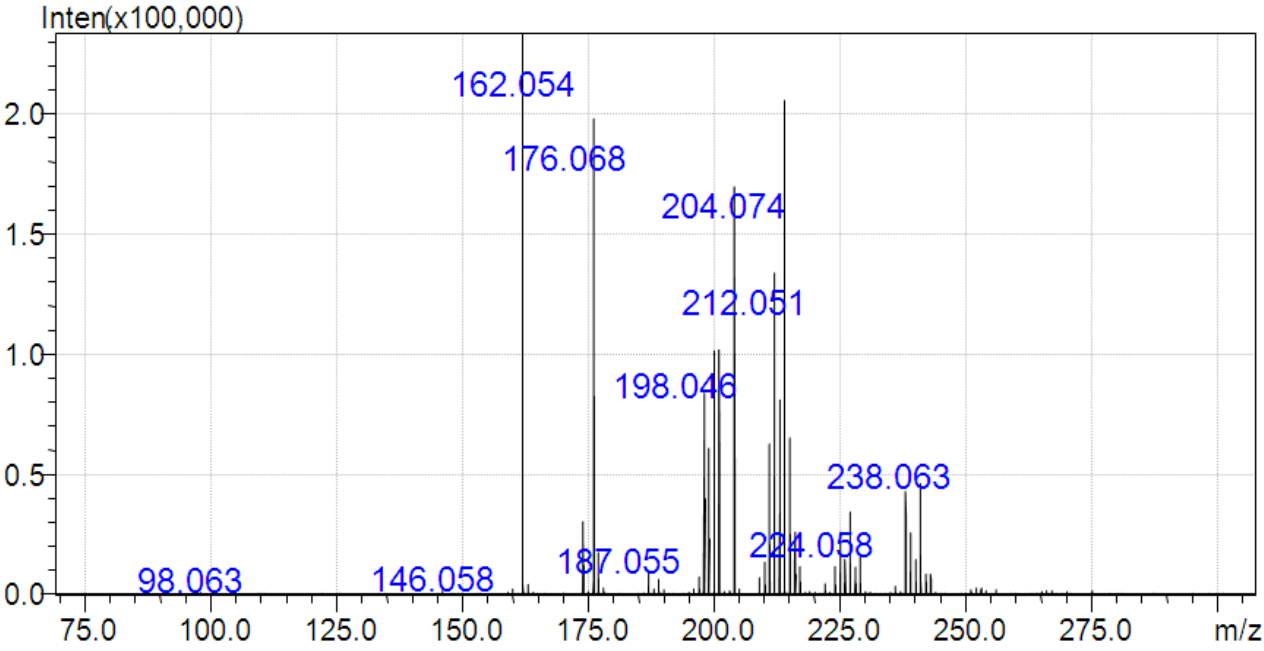


Figure S3. MS^2 spectrum of the unknown bioactive compound with *m/z* 303.168, eluting at 8.5 minutes. In the table below Figure S3, the proposed molecular formula, and proposed fragmentation of the compound is shown.

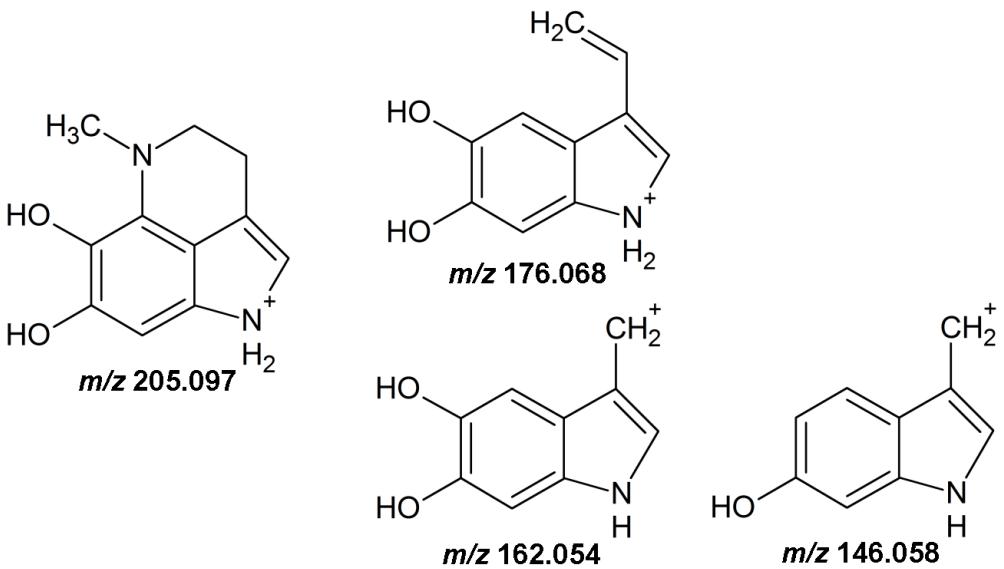


m/z	Formula	Interpretation
303.168	$C_{12}H_{23}N_4O_5^+$	$[M+H]^+$
285.156	$C_{12}H_{21}N_4O_4$	Loss of H_2O
268.130	$C_{12}H_{18}N_3O_4$	Loss of H_2O and NH_3
250.118	$C_{12}H_{16}N_3O_3$	Loss of $2\times H_2O$ and NH_3
243.134	$C_{11}H_{19}N_2O_4$	Loss of CH_4N_2O, e.g., NH_3 and HNCO
225.123	$C_{11}H_{17}N_2O_3$	Loss of H_2O from *m/z* 243
181.133	$C_{10}H_{17}N_2O$	Loss of $C_2H_6N_2O_4$, e.g., $2xH_2O$ and 2xHNCO
175.119	$C_6H_{15}N_4O_2$	Loss of $C_6H_8O_3$
159.077	$C_6H_{11}N_2O_3$	Loss of $C_6H_{12}N_2O_2$
140.082	$C_6H_{10}N_3O$	Loss of $C_6H_{13}NO_4$
128.072	$C_6H_{10}NO_2$	Loss of $C_6H_{13}N_3O_3$
115.087	$C_5H_{11}N_2O$	Loss of $C_7H_{12}N_2O_4$ or $C_6H_{12}N_2O_2$ and CO_2
91.361		Not consistent, too big mass defect

Figure S4. (**A**) MS^2 spectra of the bioactive compound with *m/z* 205.098, eluting at 10.0 min. (**B**) The proposed structure, and proposed fragmentation scheme of the bioactive compound with *m/z* 205.098, eluting at 10.0 min.

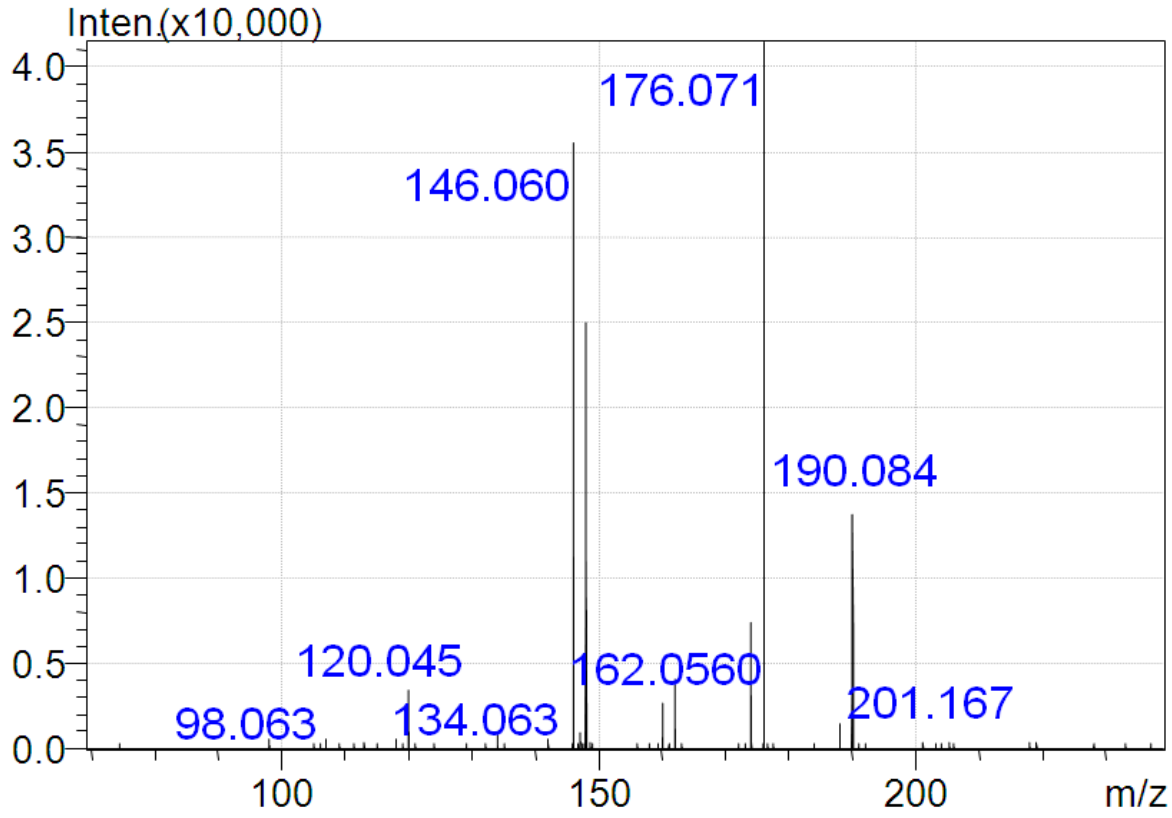


(**A**)

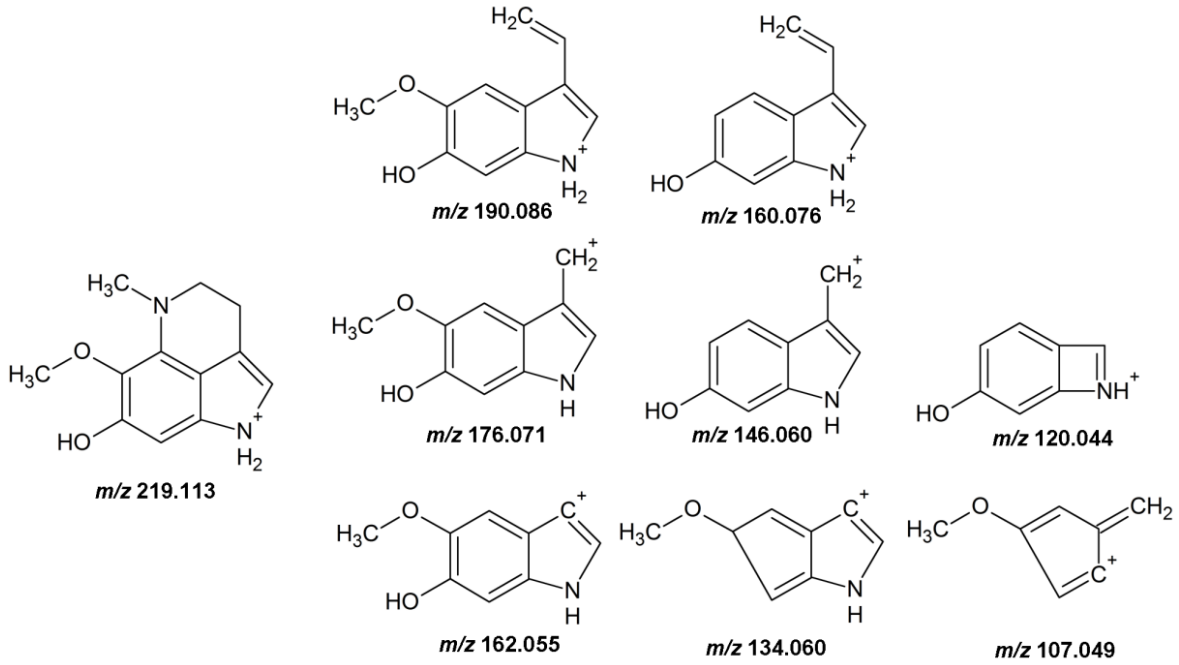


(**B**)

Figure S5. (**A**) MS^2 spectrum, proposed structure, and proposed fragmentation scheme of the bioactive compound with *m/z* 219.112, eluting at 10.5 min. (**B**) Proposed structure and proposed fragmentation scheme of the bioactive compound with *m/z* 219.112, eluting at 10.5 min.

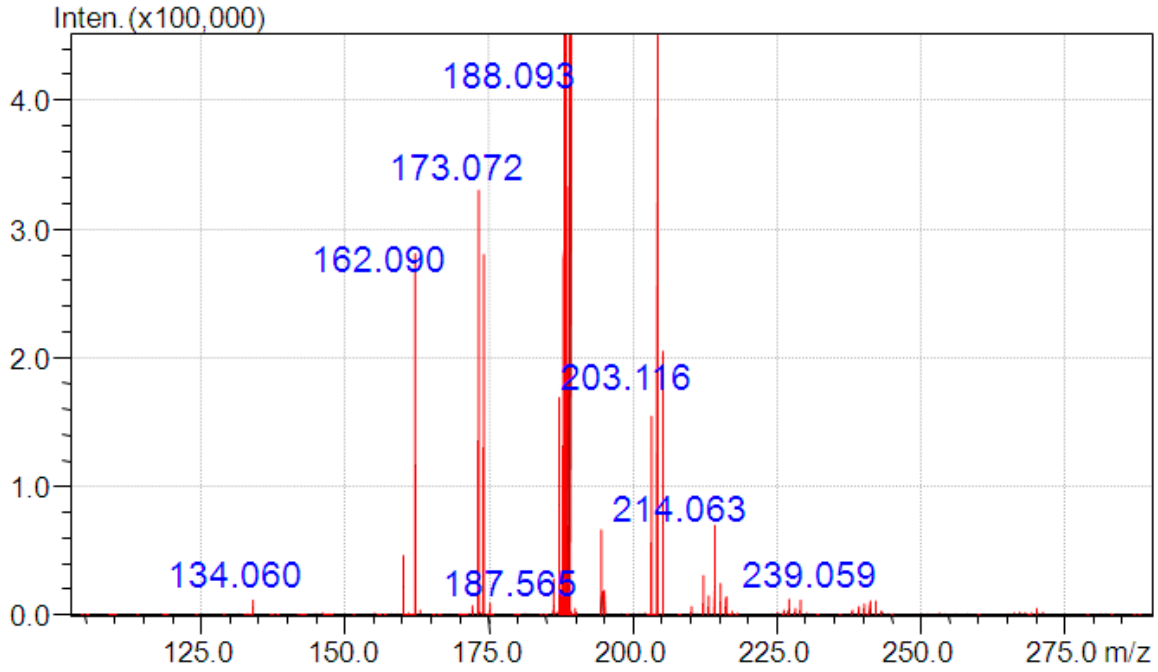


(**A**)

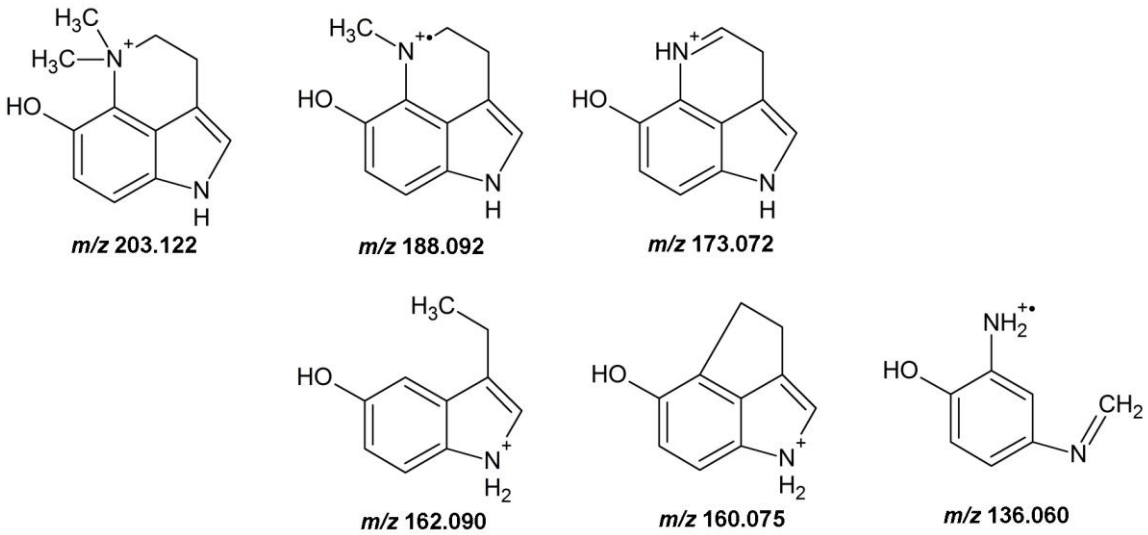


(**B**)

Figure S6. (**A**) MS^2 spectrum, proposed structure, and proposed fragmentation scheme of the bioactive compound with *m/z* 203.118, eluting at 10.5 min. (**B**) Proposed structure, and proposed fragmentation scheme of the bioactive compound with *m/z* 203.118, eluting at 10.5 min.

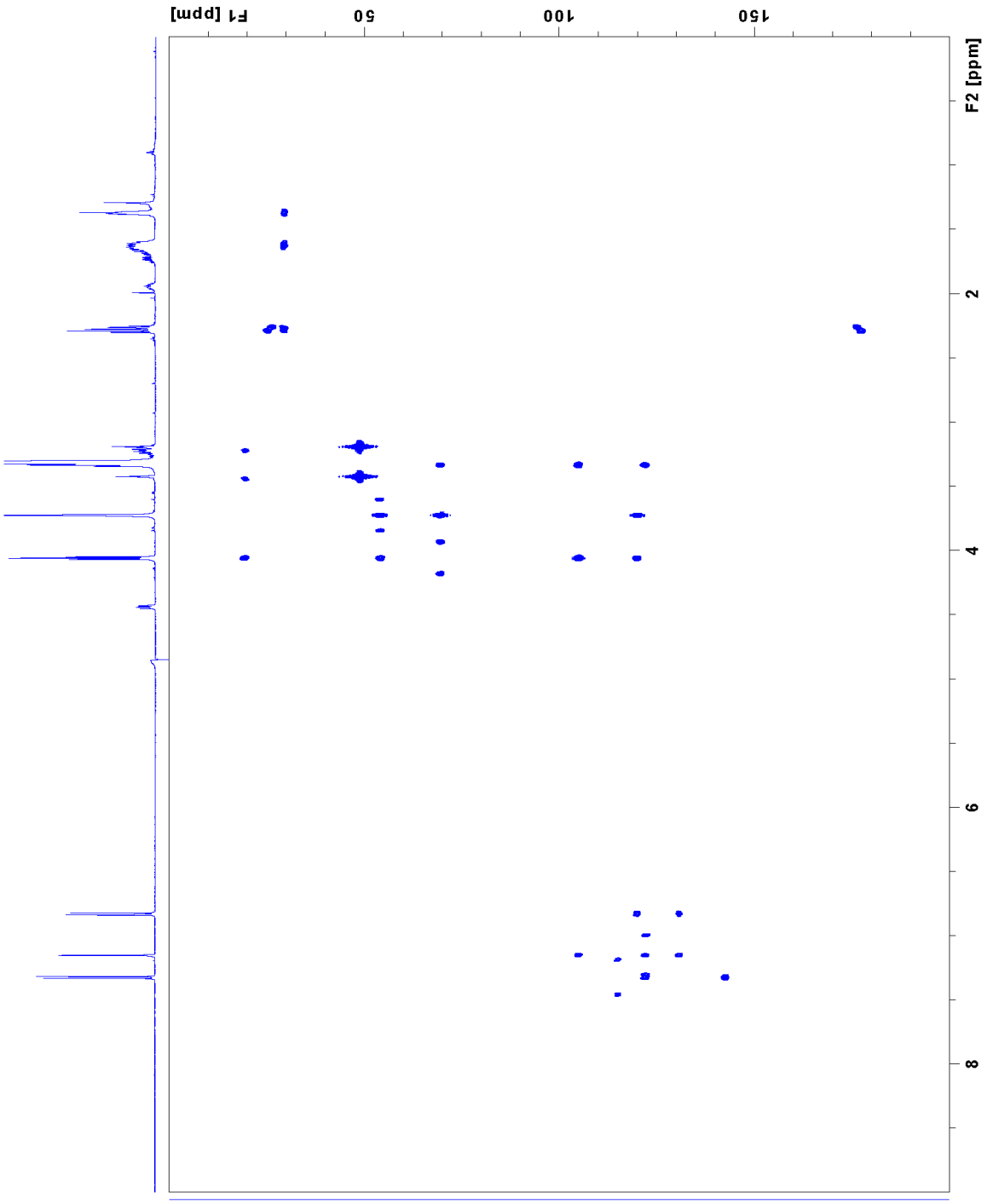


(**A**)

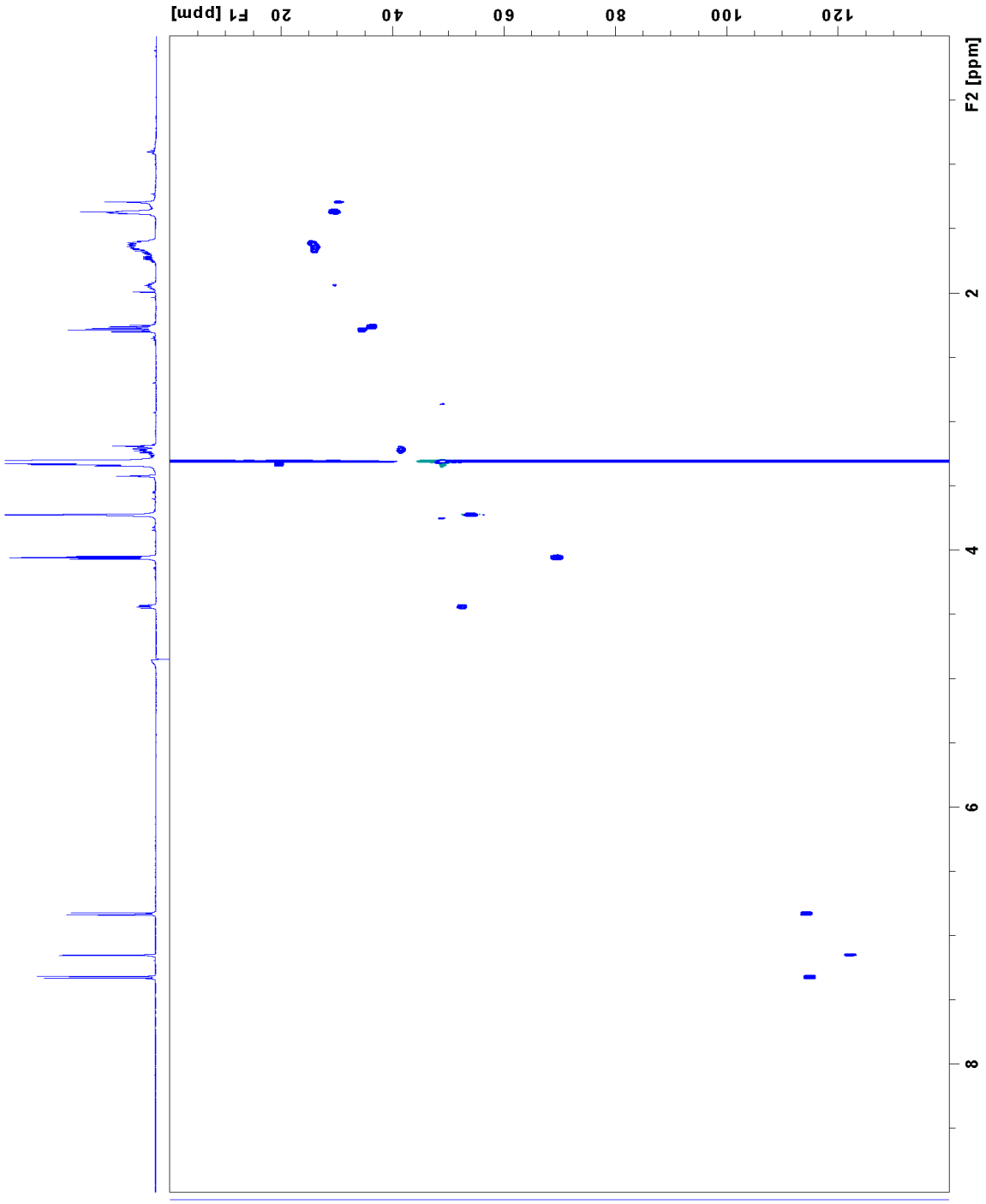


(**B**)

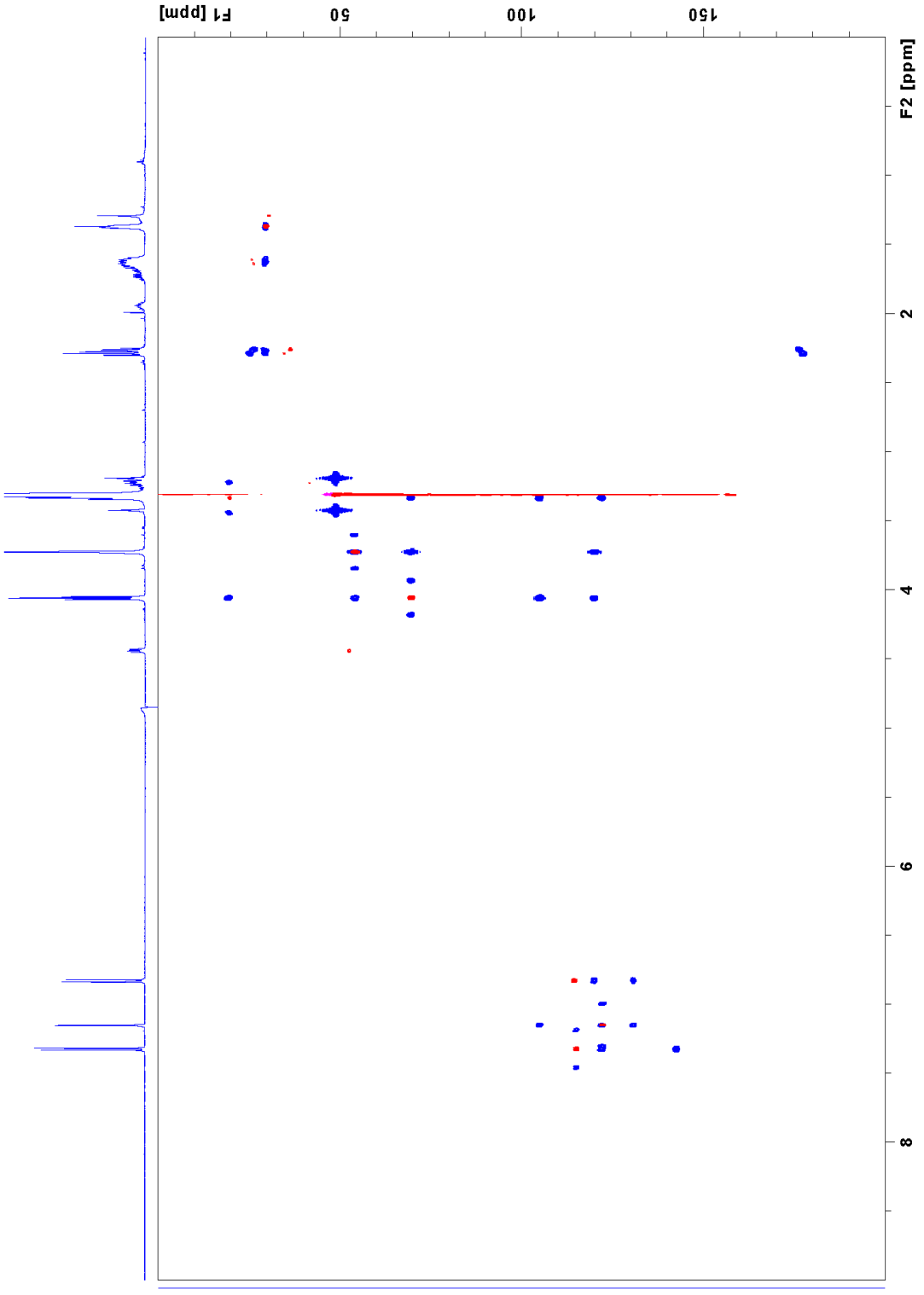
Figure S7. (**A**) HMBC-NMR spectrum of the bioactive compound with *m/z* 203.118, eluting at 10.5 min. (**B**) HSQC-NMR spectrum of the bioactive compound with *m/z* 203.118, eluting at 10.5 min. (**C**) HSQCHMBC-NMR spectrum of the bioactive compound with *m/z* 203.118, eluting at 10.5 min.



(**A**)

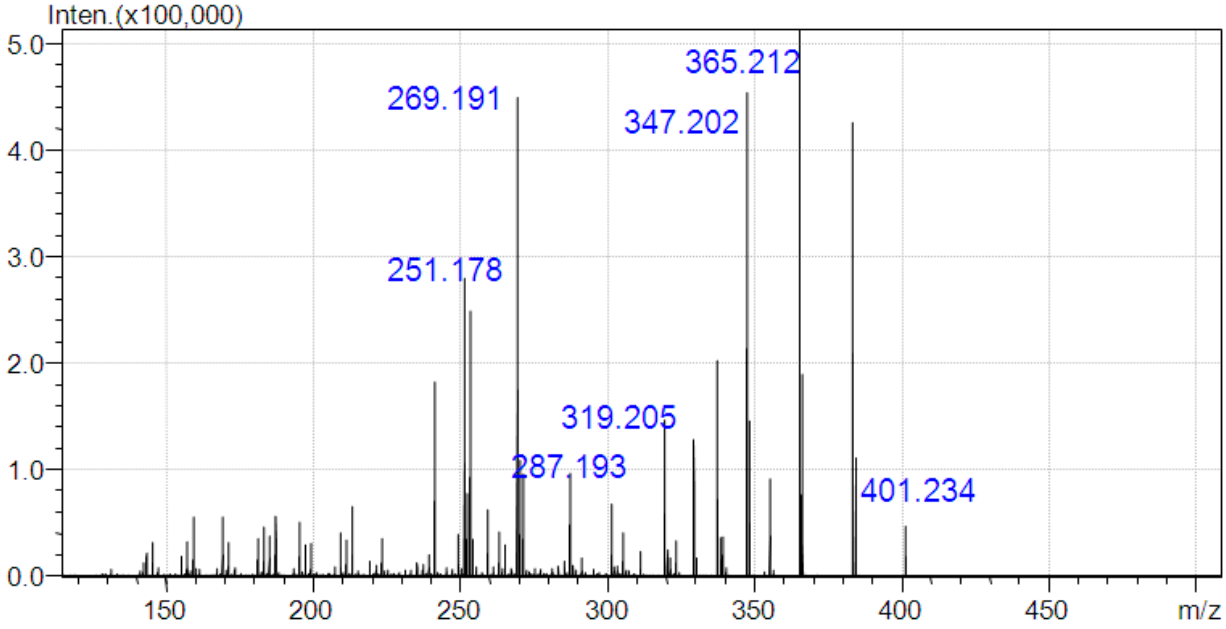


(**B**)



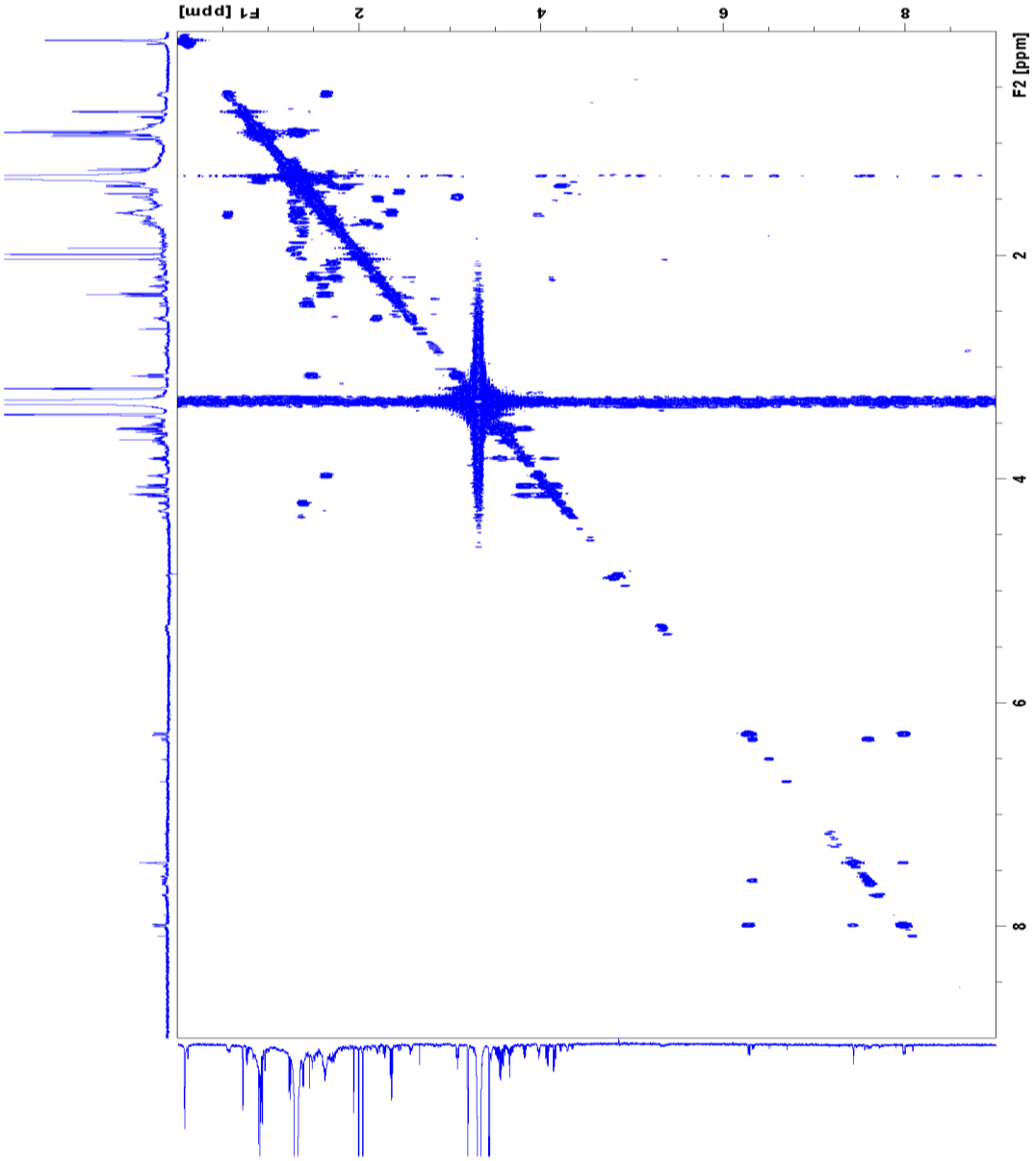
(**C**)

Figure S8. MS^2 spectrum, proposed structure, and proposed fragmentation scheme of the bioactive compound with *m/z* 401.235, eluting at 58.0 min. Most of the peaks in the MS^2 spectrum can be readily interpreted, e.g., in terms of water and CO losses, although it is generally unclear where exactly these losses occur.

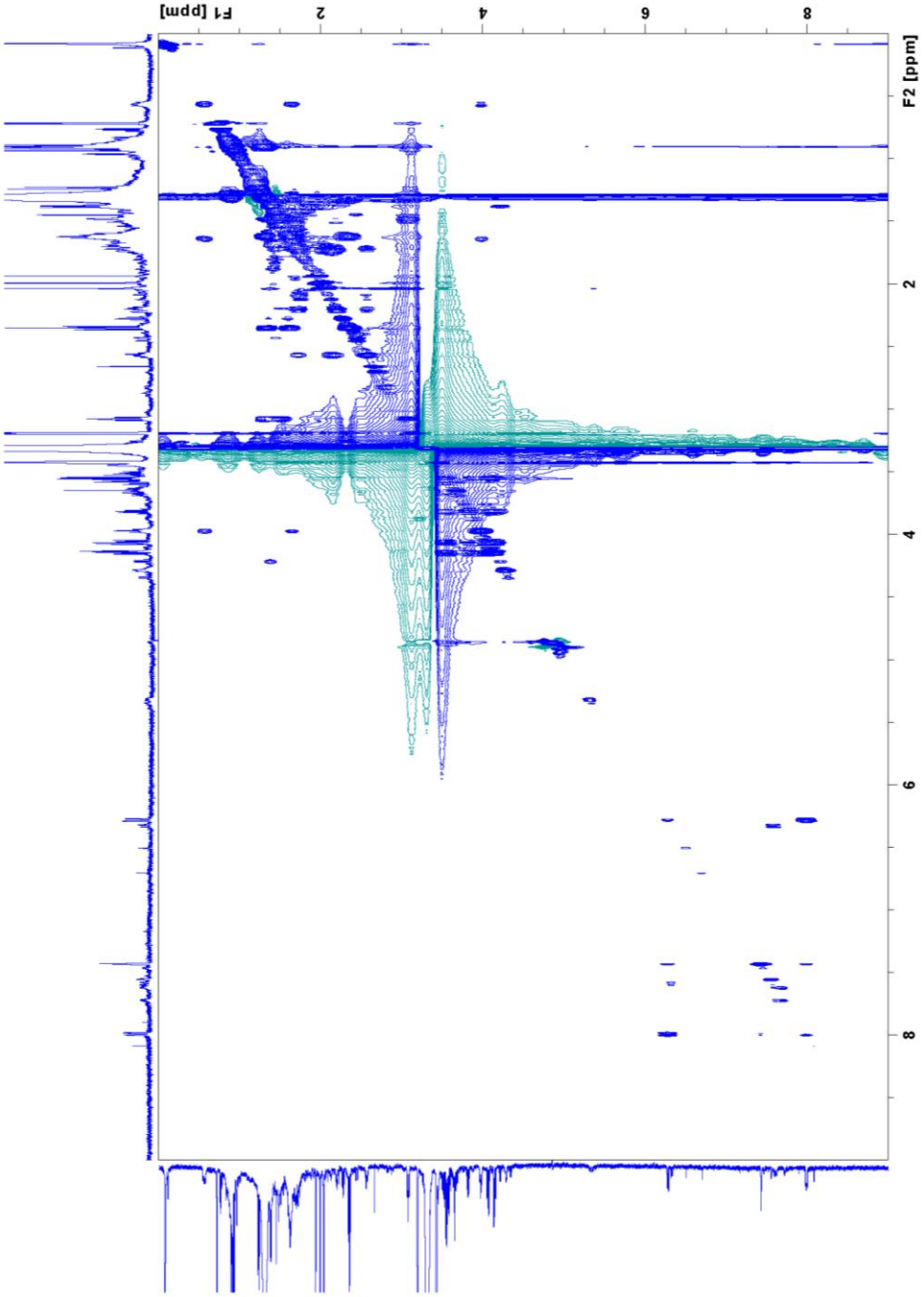


m/z	Formula	Interpretation
401.234	$C_{24}H_{33}O_5^+$	$[M+H]^+$
383.222	$C_{24}H_{31}O_4^+$	Loss of H_2O
365.212	$C_{24}H_{29}O_3^+$	Loss of 2 × H_2O; Loss of H_2O from *m/z* 383
355.228	$C_{23}H_{31}O_5^+$	Loss of HCOOH or CO and H_2O; Loss of CO from *m/z* 383
347.202	$C_{24}H_{27}O_2^+$	Loss of 3 × H_2O; Loss of H_2O from *m/z* 365
337.218	$C_{23}H_{29}O_2^+$	Loss of 2 × H_2O and CO; Loss of H_2O from *m/z* 355
329.191	$C_{24}H_{25}O^+$	Loss of 4 × H_2O; Loss of H_2O from *m/z* 347
319.205	$C_{23}H_{27}O^+$	Loss of 3 × H_2O and CO; Loss of H_2O from *m/z* 337
301.195	$C_{23}H_{25}^+$	Loss of 4 × H_2O and CO; Loss of H_2O from *m/z* 319
287.193		No matches within 5 mDa
269.191	$C_{19}H_{25}O^+$	Loss of $C_5H_8O_4$; Loss of $C_5H_4O_2$ from *m/z* 365
253.196	$C_{19}H_{25}^+$	Loss of $C_4H_4O_2$ from *m/z* 337
251.178	$C_{19}H_{23}^+$	Loss of H_2O from *m/z* 269
241.159	$C_{17}H_{21}O^+$	Loss of C_2H_4 from *m/z* 269
213.164	$C_{16}H_{21}^+$	Loss of CO from *m/z* 241

Figure S9. (**A**) COSY-NMR spectrum of the bioactive compound with *m/z* 401.235, eluting at 58.0 min. (**B**) TOCSY-NMR spectrum of the bioactive compound with *m/z* 401.235, eluting at 58.0 min.

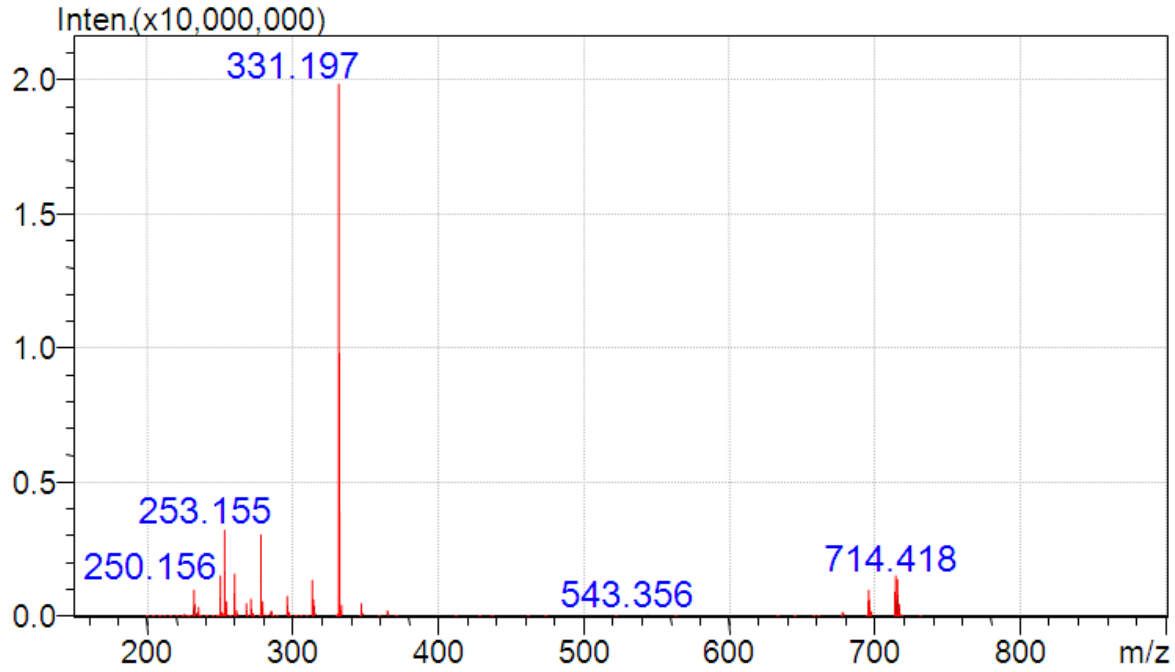


(**A**)

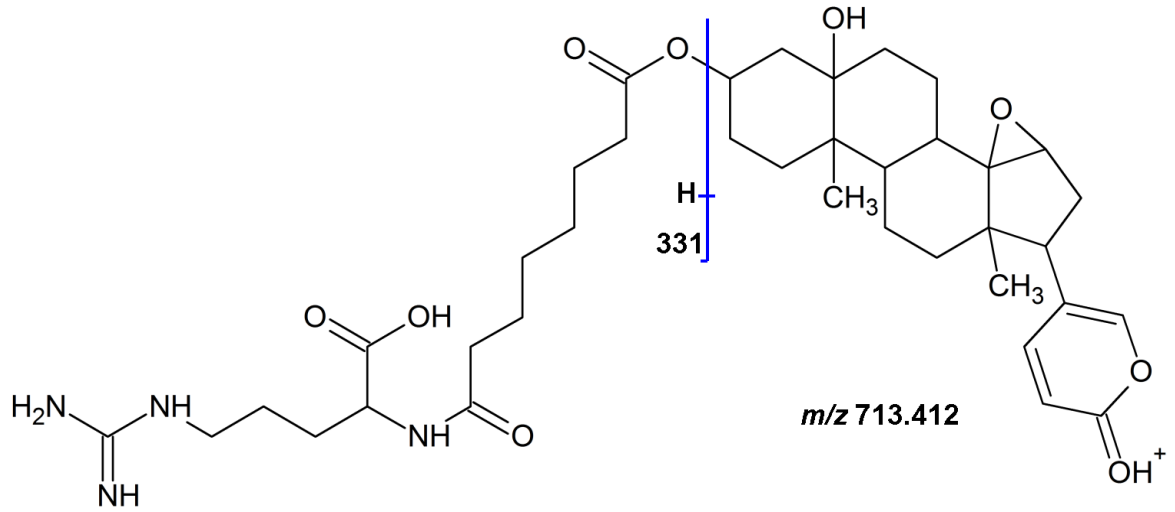


(**B**)

Figure S10. (**A**) MS^2 spectrum of the bioactive compound with *m/z* 713.412, eluting at 62.0 min. (**B**) Proposed structure and proposed fragmentation scheme of the bioactive compound with *m/z* 713.412, eluting at 62.0 min. The minor fragments can be considered as secondary fragments of the ion with *m/z* 331 and are consistent with subsequent losses of H_2O, CO and/or NH_3, e.g., loss of water to the ion with *m/z* 313, loss of NH_3 to *m/z* 296, loss of H_2O to *m/z* 278, and either loss of H_2O to *m/z* 260 or CO to *m/z* 250. The ion with *m/z* 253 is consistent with the loss of HN=C=CH and 2 × H_2O from the fragment ion with *m/z* 331.



(**A**)



(**B**)