Electronic Supplementary Material (ESI) for Analyst.

General Rules of Fragmentation Evidencing Lasso Structures in CID and ETD

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Supporting Information

Table S1. Conditions for the production of lasso peptides.

Peptide	Medium	Conditions for production	Producing Strain	Ref
Anantin	GYM	30°C for 2 days	*Streptomyces coerulescens*	1
Astexin-1(19)	M9	37°C for 1 day	*E. coli* BL21 (DE3) transformed with pET41a construct	2
BI-32169	GYM	28°C for 5 days	*Streptomyces* sp.	3
Capistruin	M20	37°C for 2 days	*Burkholderia thailandensis* E264	4
Caulonodin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Caulonodin II	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Caulonodin III	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Caulosegnin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	6
Caulosegnin II	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	6
Caulosegnin III	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	6
MccJ25	M63	37°C for 1 day	*E. coli* MC4100 transformed with pTUC202	7
Propeptin	*	27°C for 9 days	*Microbispora* sp.	8
Rubrivinodin	M9	37°C for 1 day	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Siamycin I	GYM	28°C for 4 days	*Streptomyces* sp.	9
Sphingonodin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Sphingopyxin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Sphingopyxin II	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Sviceucin	GYM	28°C for 5 days	*S. coelicolor* transformed with the p4H7 cosmid	10
Syanodin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	5
Xanthomonin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	11
Xanthomonin II	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct	11

* Medium composed of glucose 2%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, soybean flour 2.5%, NaCl 0.2% and K_2HPO_4 0.005%, (pH 6.7).

Table S2. Data collection and refinement statistics for the lasso peptide rubrivinodin.

	Rubrivinodin
Data collection	
Space group	$P2_1$
X-ray source	BESSY, BL14.1
Cell dimensions	
a, *b*, *c* (Å)	15.03, 27.24, 15.17
....α, β, γ (°)	90.00, 116.76, 90.00
Monomers per AU	1
Wavelength (Å)	0.7999
Resolution (Å)	50.00-0.80 (0.85-0.80)*
R_{merge}	0.019 (0.097)
Average $I/\sigma(I)$	35.85 (6.76)
$CC_{1/2}$	1.00 (0.971)
No. unique reflections	9545 (536)
Completeness (%)	81.8 (28.9)
Redundancy	3.00 (1.71)
Refinement	
Resolution (Å)	13.622-0.806 (0.827-0.806)
No. unique reflections	9038 (175)
R_{work} / R_{free}	0.0844/0.0913 (0.121/0.141)
No. atoms (non-H) †	154
Protein	138
Water	16
Average *B*-factor (Å²)	
Protein	3.77
Water	8.96
R.m.s deviations	
Bond lengths (Å)	0.014
Bond angles (°)	2.147
Ramachandran	
Favored (%)	100.0
PDB code	5OQZ

Dataset was acquired from a single crystal.

*Values in parentheses refer to the highest-resolution shell.

†Includes atoms from residues in alternate conformations.

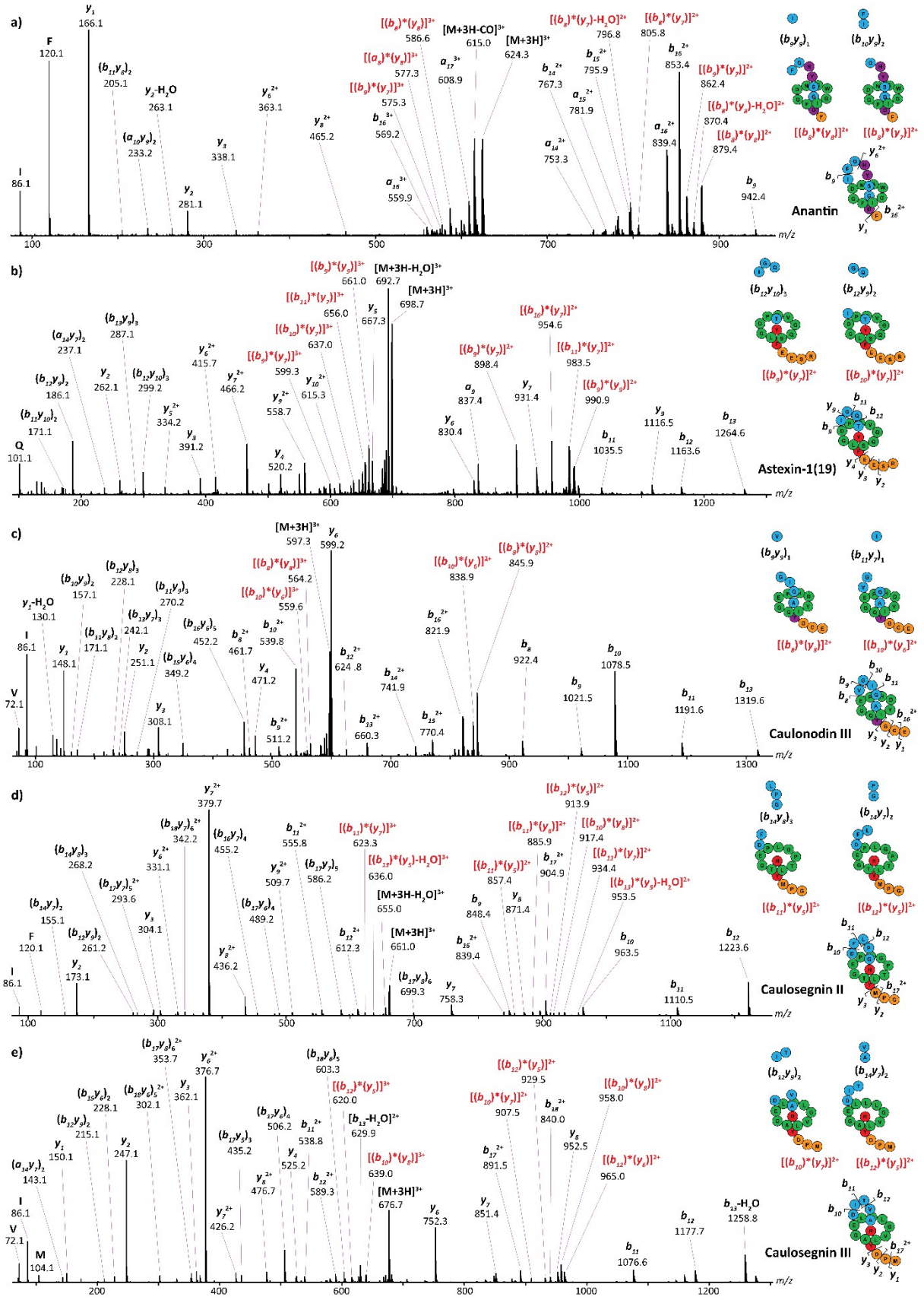

Figure S1a. CID spectra of the $[M+3H]^{3+}$ species of a) anantin (*m/z* 623.3), b) astexin-1(19) (*m/z* 698.7), c) caulonodin III (*m/z* 597.3), d) caulosegnin II (*m/z* 661.0), and e) caulosegnin III (*m/z* 676.7). Typical lasso-specific interlocked species are highlighted in red and labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

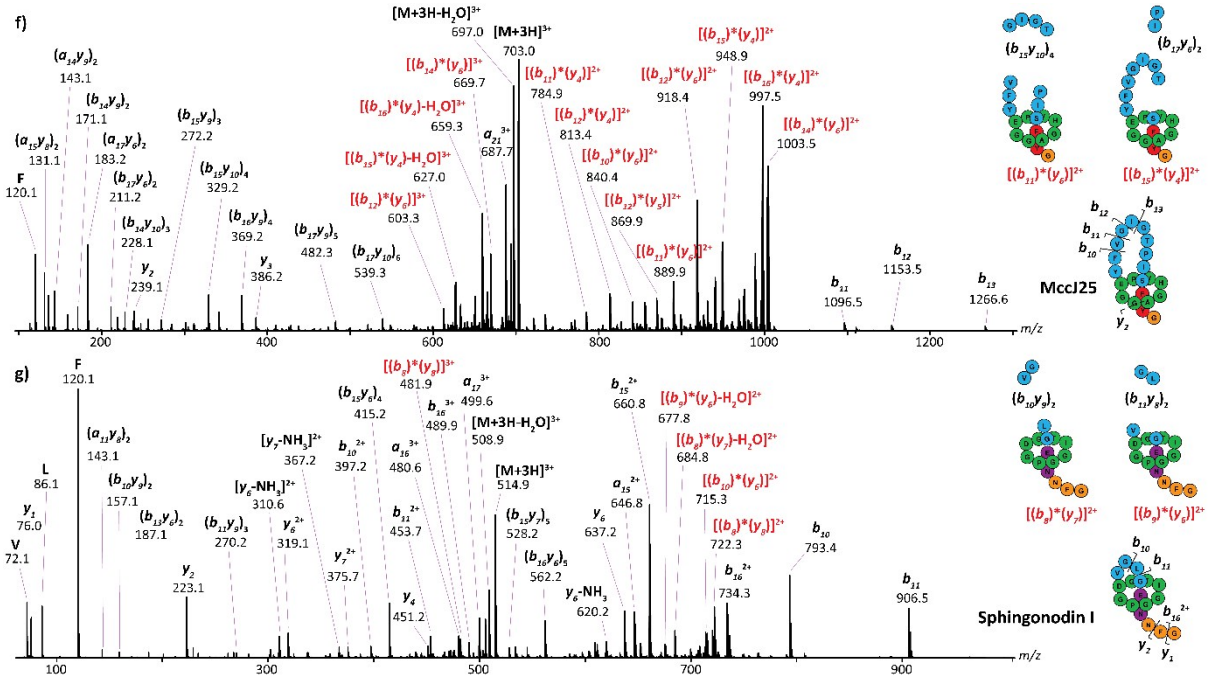

Figure S1b. CID spectra of the $[M+3H]^{3+}$ species of f) MccJ25 (*m/z* 703.0), and g) sphingonodin I (*m/z* 514.9). Typical lasso-specific interlocked species are highlighted in red and labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

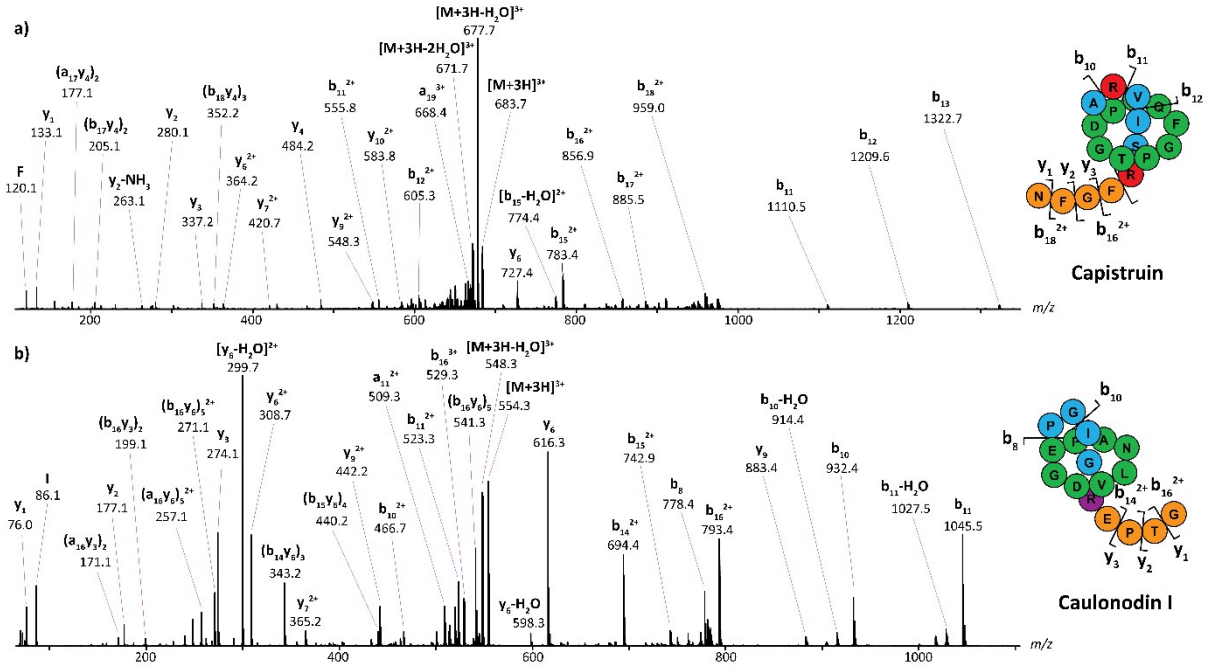

Figure S2a. CID spectra of the $[M+3H]^{3+}$ species of a) capistruin (*m/z* 683.7), and b) caulonodin I (*m/z* 554.3). Typical product ions are labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

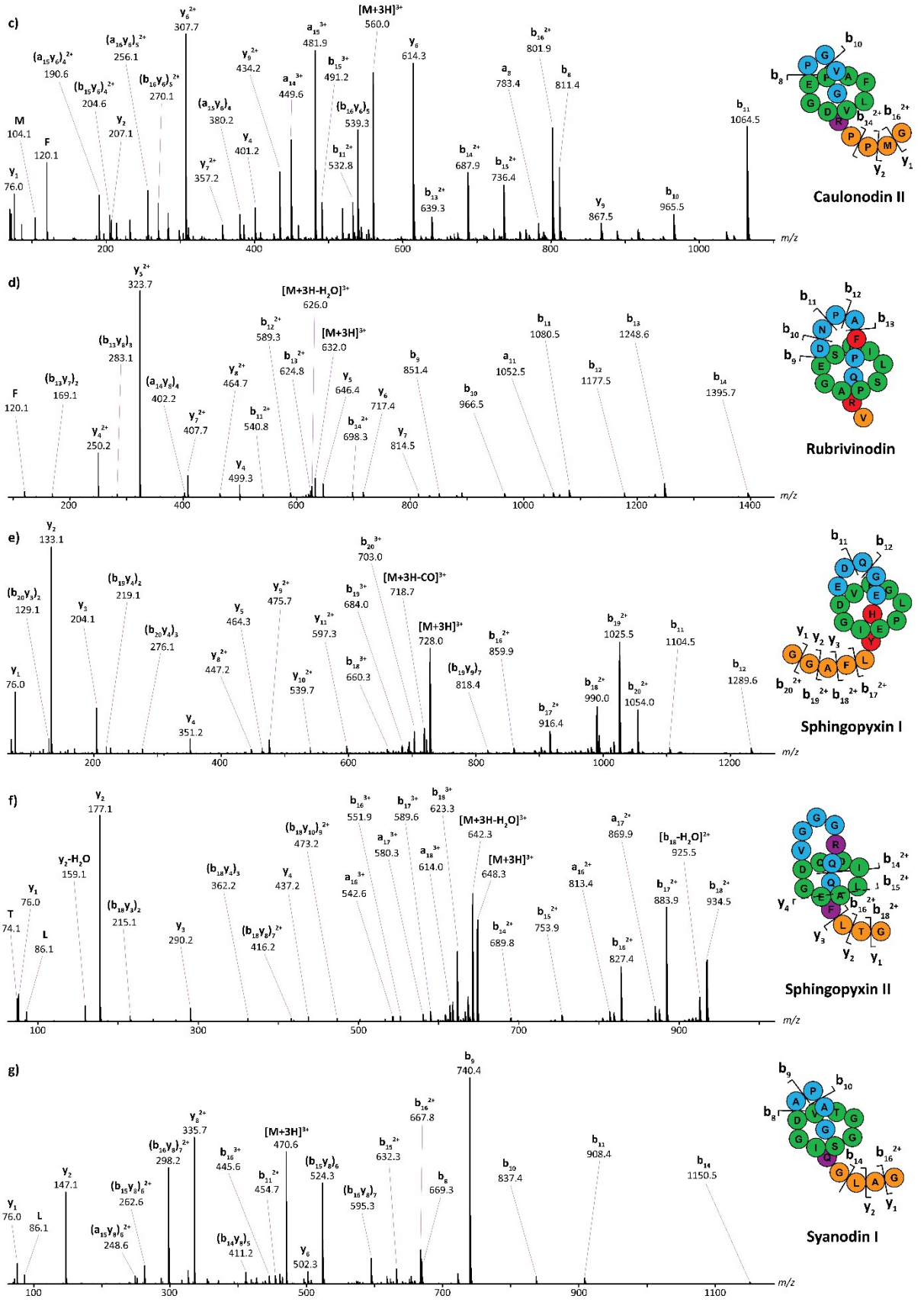

Figure S2b. CID spectra of the $[M+3H]^{3+}$ species of c) caulonodin II (*m/z* 560.0), d) rubrivinodin (*m/z* 632.0), e) sphingopyxin I (*m/z* 728.0), f) sphingopyxin II (*m/z* 648.3), and g) syanodin I (*m/z* 470.6). Typical product ions are labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

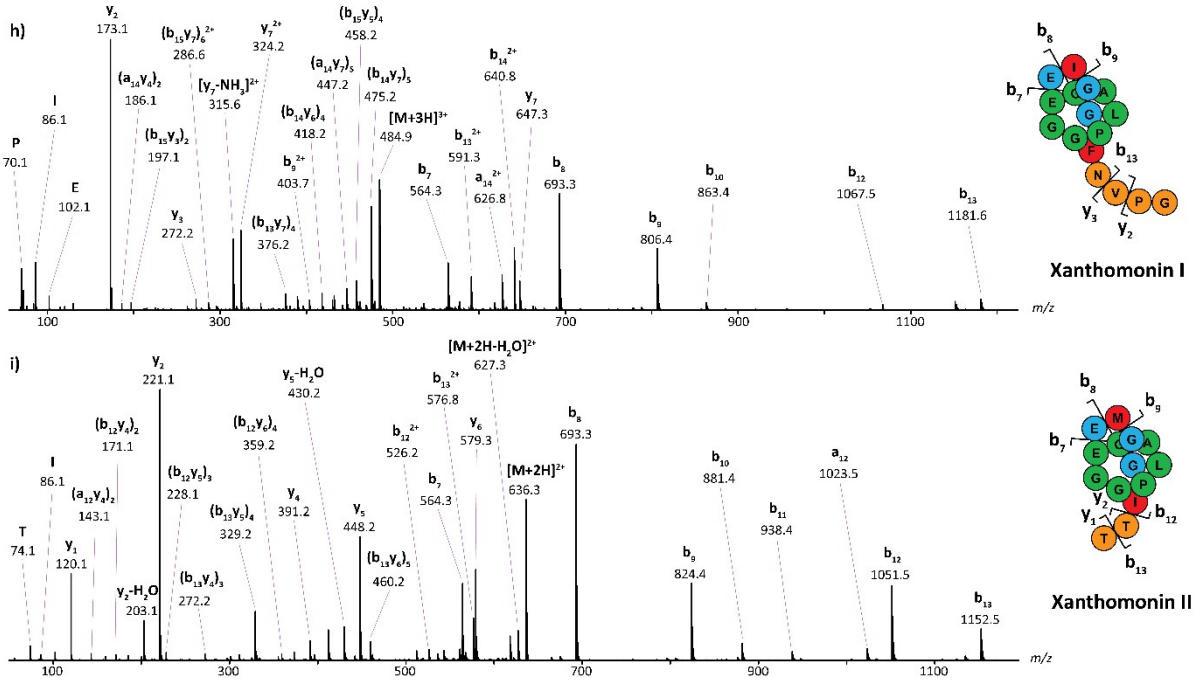

Figure S2c. CID spectra of h) the $[M+3H]^{3+}$ species of xanthomonin I (*m/z* 484.9), and i) the $[M+2H]^{2+}$ species of xanthomonin II (*m/z* 636.3). Typical product ions are labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs and the C-terminal tails are highlighted in green, blue, red and orange, respectively.

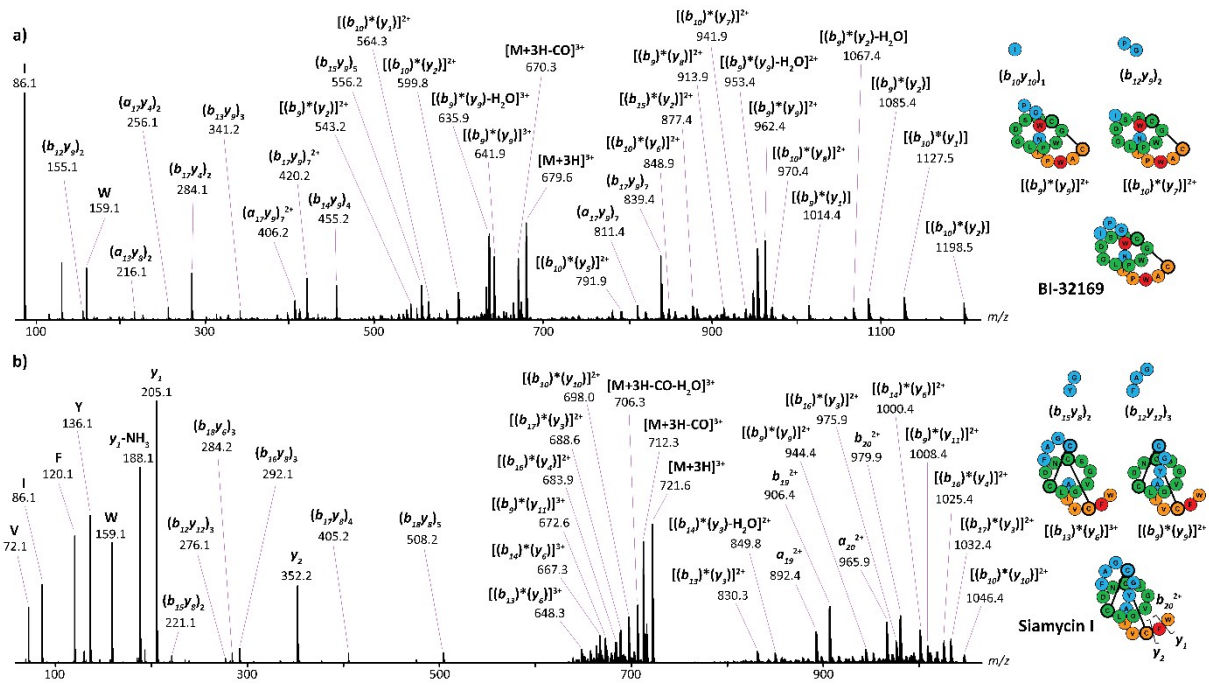

Figure S3. CID spectra of the $[M+3H]^{3+}$ species of a) BI-32169 (*m/z* 679.6), and b) siamycin I (*m/z* 721.6). Typical crosslinked product ions are labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the disulfide bonds are highlighted in green, blue, red, orange and black, respectively.

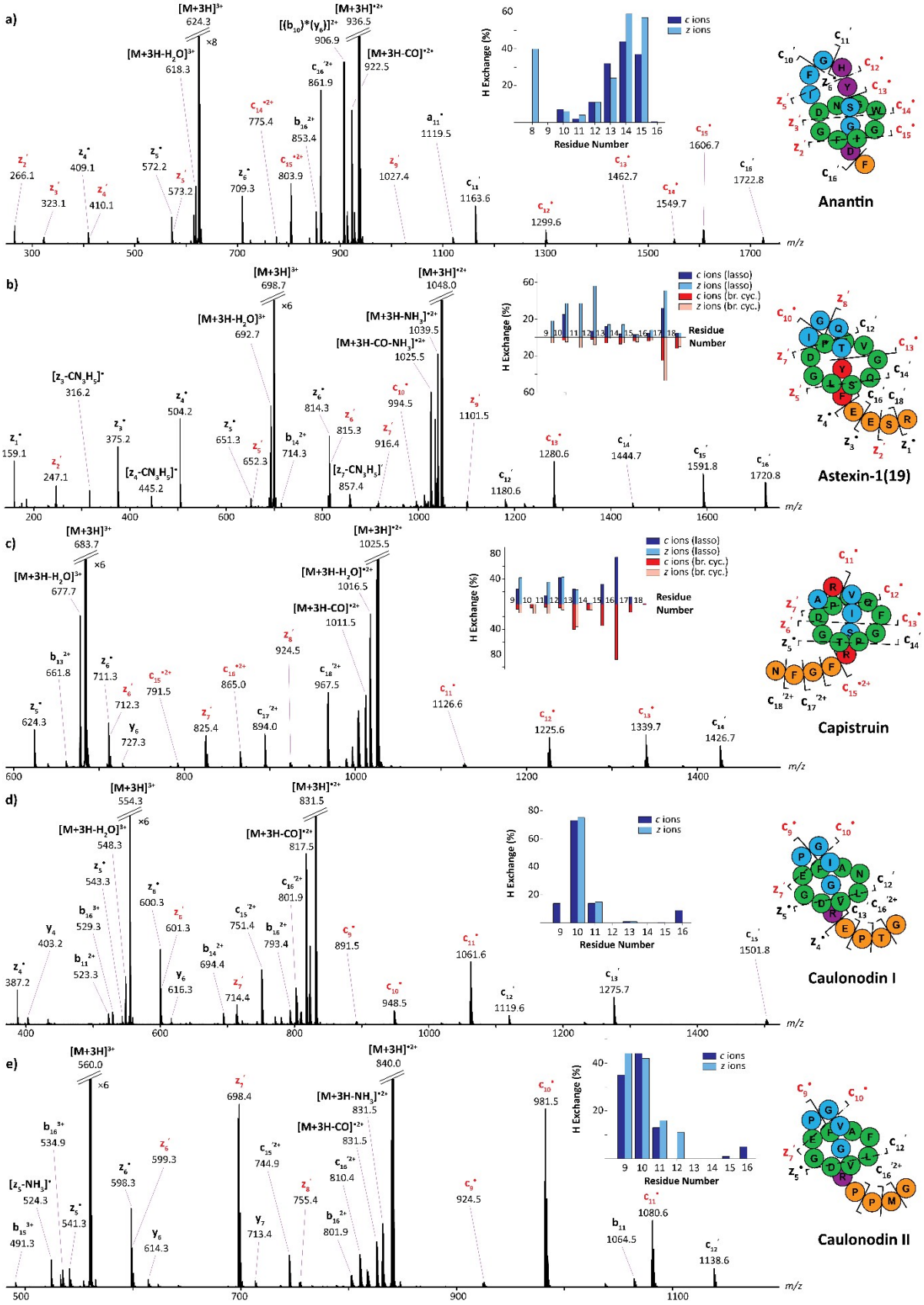

Figure S4a. ETD spectra of the $[M+3H]^{3+}$ species of a) anantin (*m/z* 624.3), b) astexin-1(19) (*m/z* 698.7), c) capistruin (*m/z* 683.7), d) caulonodin I (*m/z* 554.3) and e) caulonodin II (*m/z* 560.0). Typical hydrogen migration product ions are highlighted in red and labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

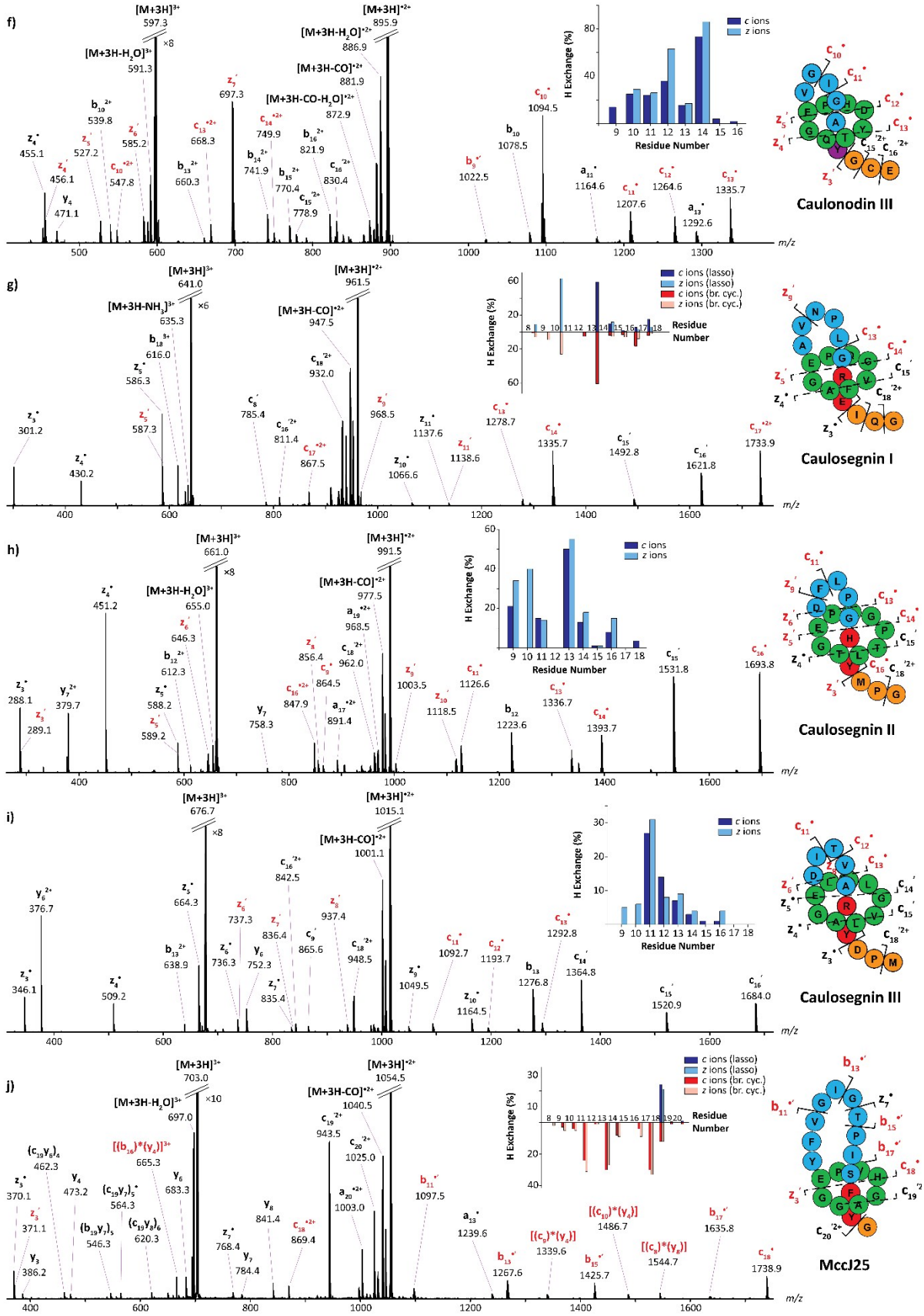

Figure S4b. ETD spectra of the $[M+3H]^{3+}$ species of f) caulonodin III (*m/z* 597.3), g) caulosegnin I (*m/z* 641.0), h) caulosegnin II (*m/z* 661.0), i) caulosegnin III (*m/z* 676.7) and j) MccJ25 (*m/z* 703.0). Typical hydrogen migration product ions are highlighted in red and labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

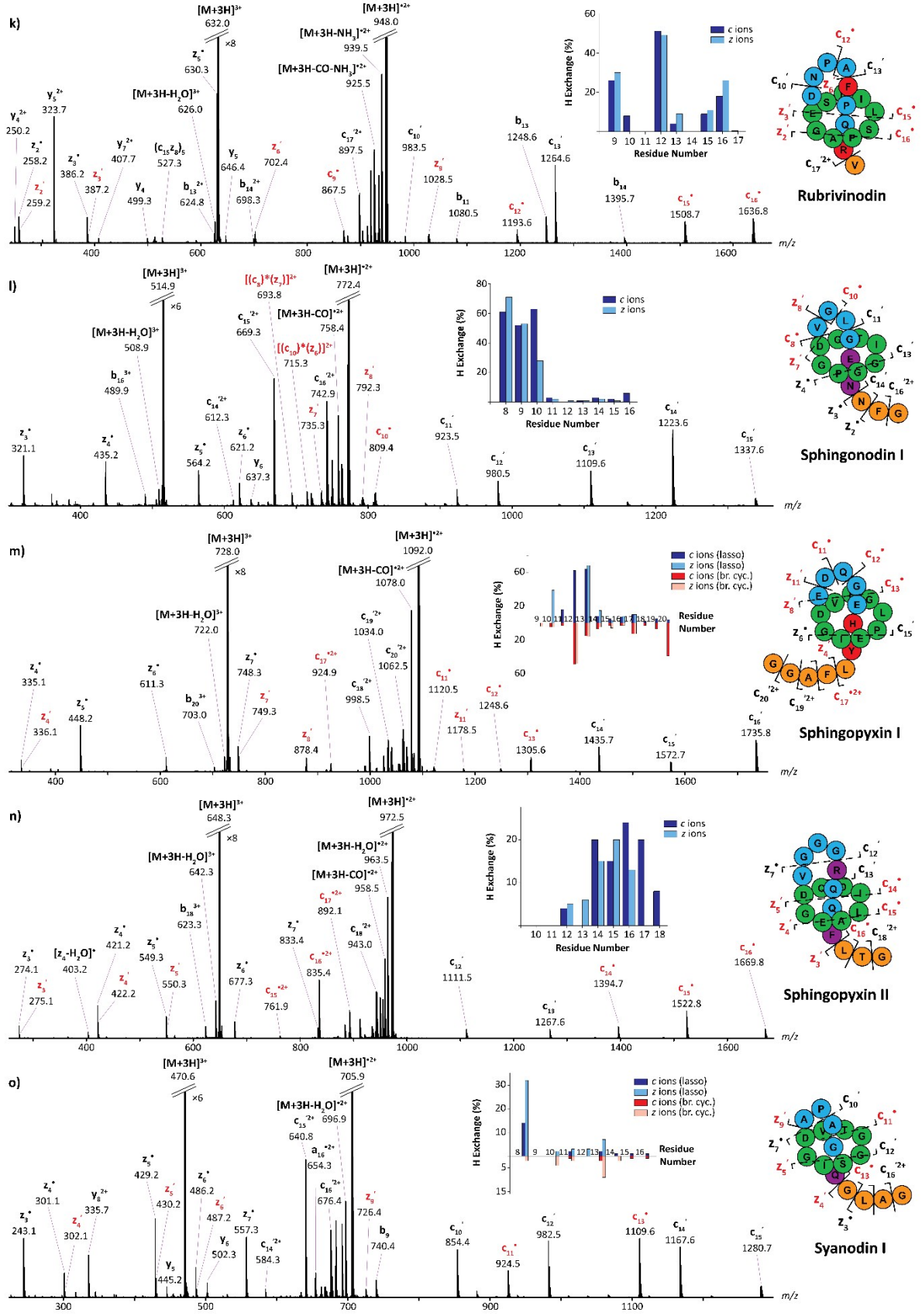

Figure S4c. ETD spectra of the $[M+3H]^{3+}$ species of k) rubrivinodin (*m/z* 632.0), l) sphingonodin I (*m/z* 514.1), m) sphingopyxin I (*m/z* 728.0), n) sphingopyxin II (*m/z* 648.3) and o) syanodin I (*m/z* 470.6). Typical hydrogen migration product ions are highlighted in red and labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

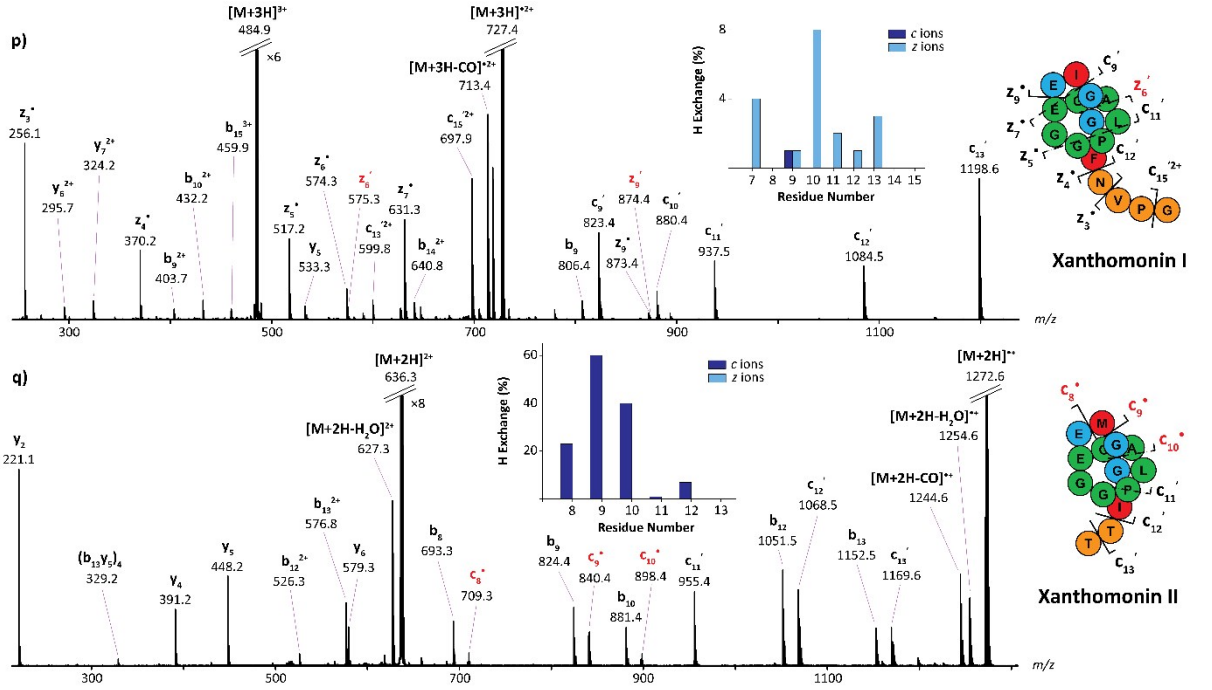

Figure S4d. ETD spectra of the $[M+3H]^{3+}$ species of p) xanthomonin I (*m/z* 484.9) and of the $[M+2H]^{2+}$ species of q) xanthomonin II (*m/z* 636.3). Typical hydrogen migration product ions are highlighted in red and labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the proposed plugs are highlighted in green, blue, red, orange and purple, respectively.

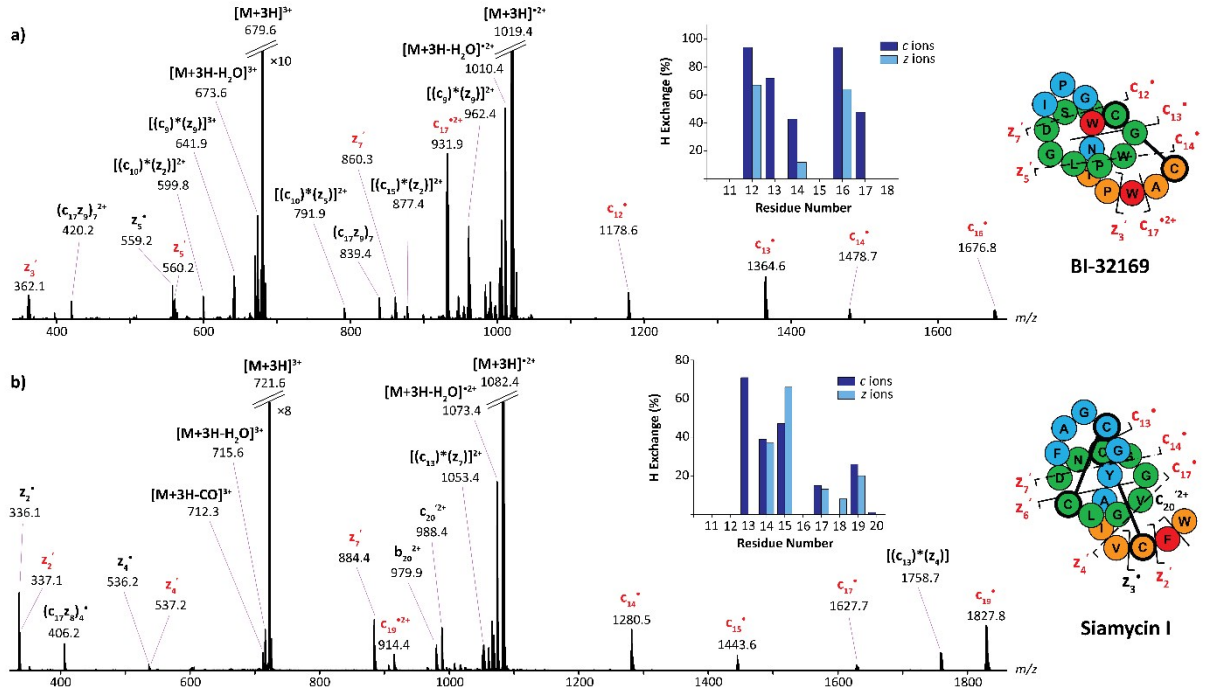

Figure S5. ETD spectra of the $[M+3H]^{3+}$ species of a) BI-32169 (*m/z* 679.6), and b) siamycin I (*m/z* 721.6). Typical hydrogen migration product ions are highlighted in red and labeled on the peptide cartoons (right of each panel). The macrolactam rings, the loops, the plugs, the C-terminal tails and the disulfide bonds are highlighted in green, blue, red, orange and black, respectively.

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