Supplementary Information

Metal Ions Induced Secondary Structure Rearrangements: Mechanically Interlocked Lasso vs Unthreaded Branched-Cyclic Topoisomers

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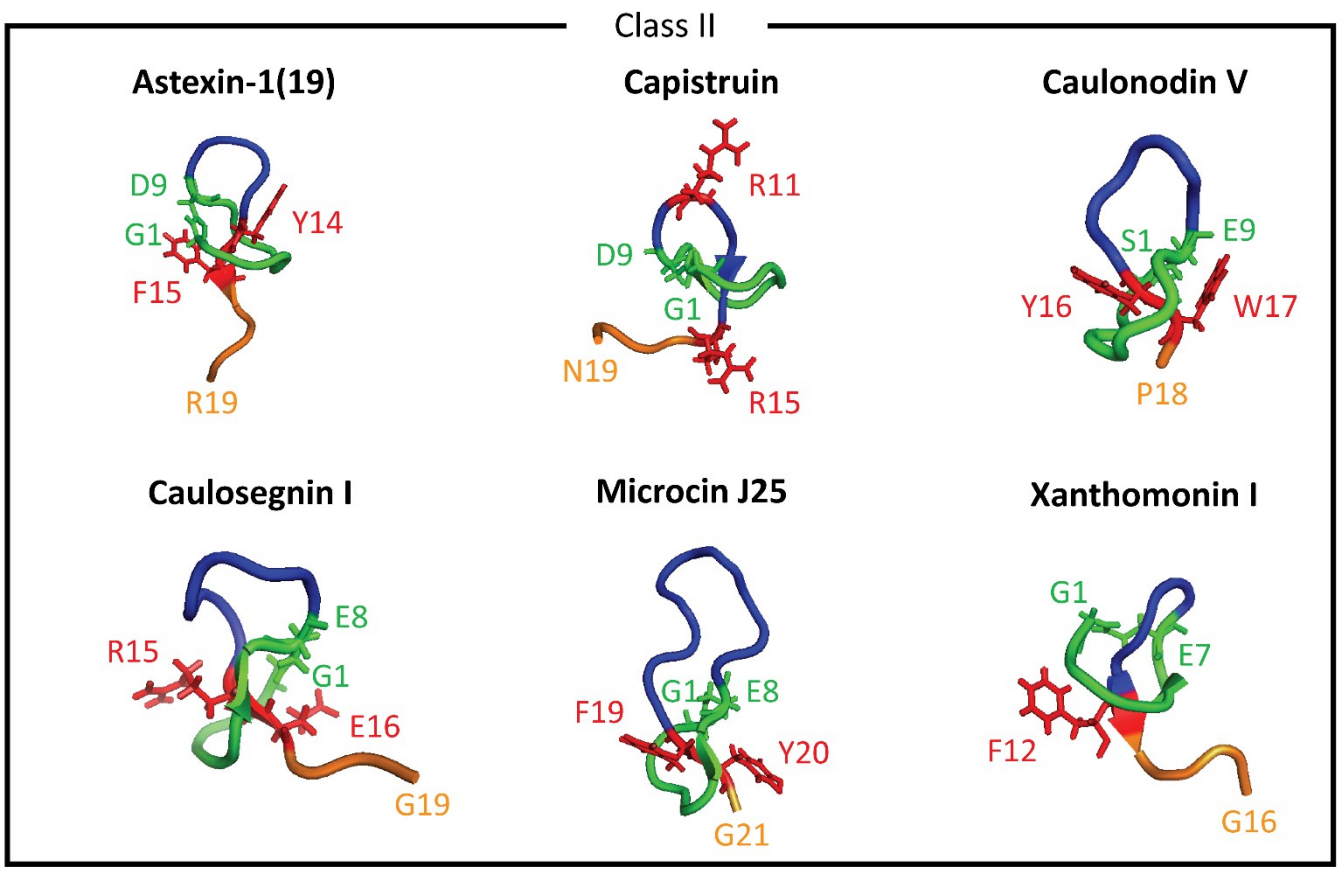

Figure S1. Three-dimensional representations of class II lasso peptides. The lasso topology of astexin-1 (19) (PDB 2M37),[1] capistruin,[2] caulonodin V (PDB 2MLJ),[3] caulosegnin I (PDB 2LX6),[4] microcin J25 (PDB 1Q71),[5] and xanthomonin I (PDB 4NAG)[6] are shown. In the 3D lasso structures, the macrolactam rings, loops, plugs and C-terminal tails are displayed in green, blue, red and orange, respectively.

Table S1. Conditions for the production of the studied class II lasso peptides.

Peptide	Medium	Conditions for production	Producing Strain
Caulonodin III	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct
Microcin J25	M63	37°C for 1 day	*E. coli* MC4100 transformed with pTUC202
Sphingonodin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct
Syanodin I	M9	20°C for 3 days	*E. coli* BL21 (DE3) transformed with pET41a construct

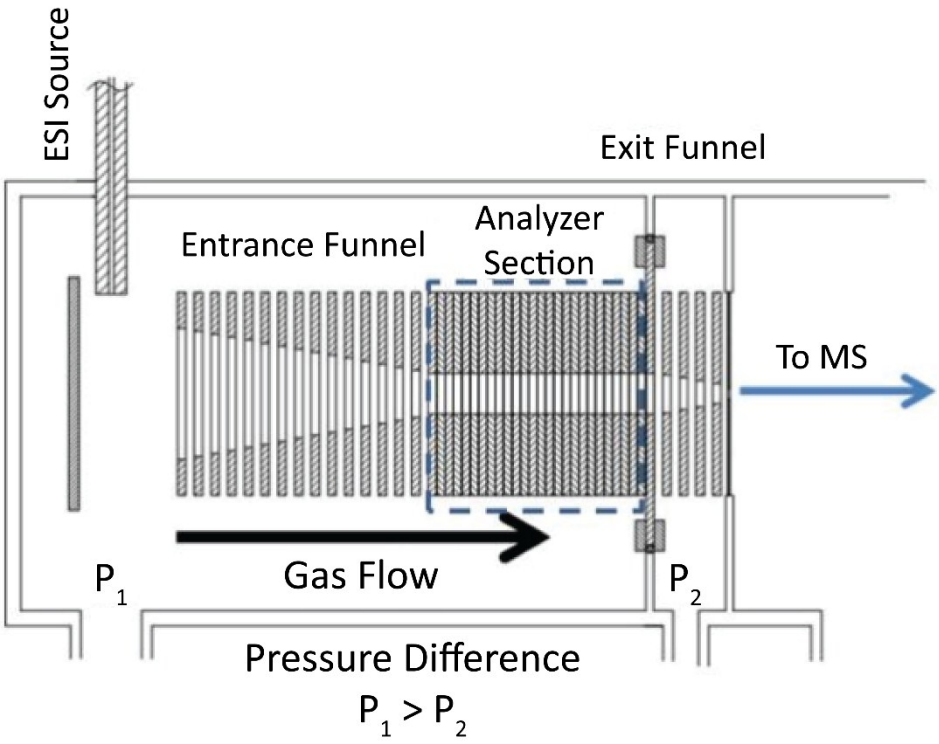

Figure S2. Scheme of the TIMS cell

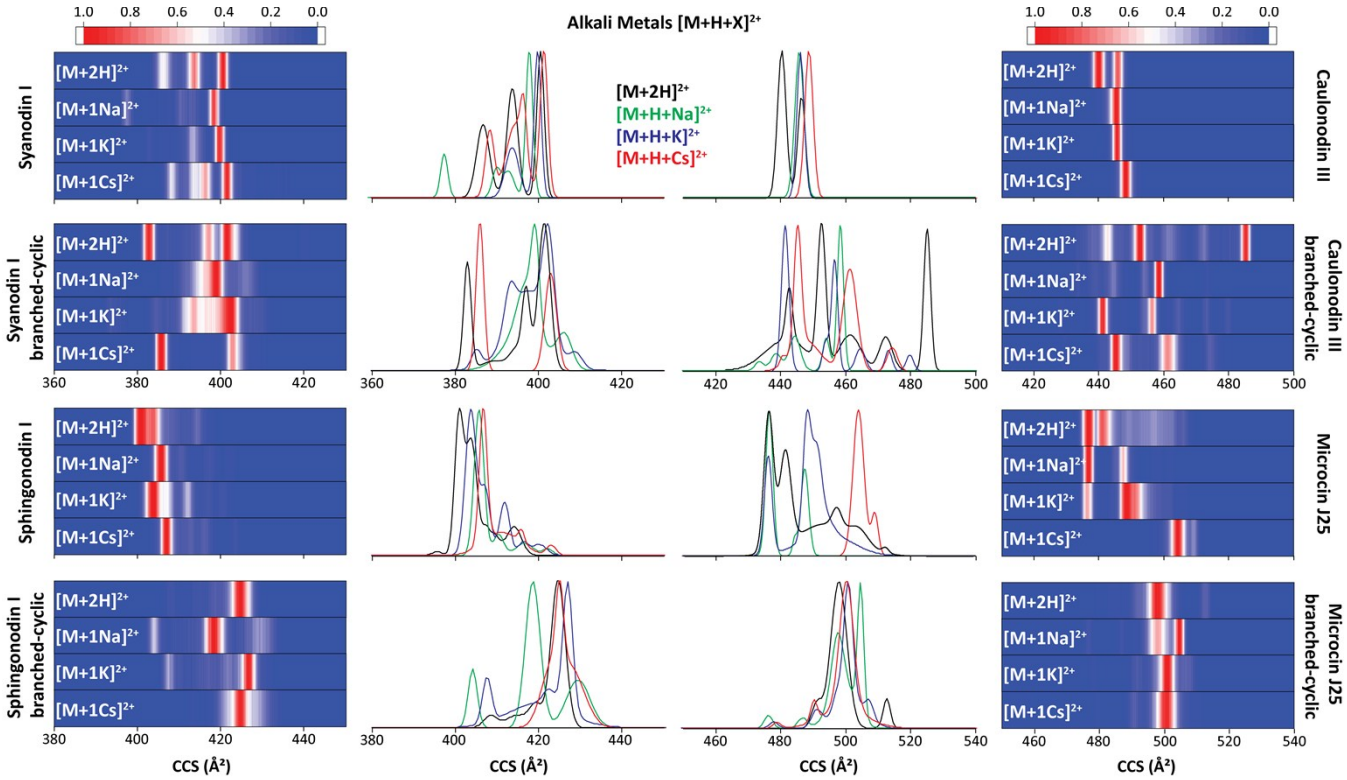

Figure S3. Typical TIMS spectra of lasso and branched-cyclic topoisomers of the protonated (black), sodiated (dark green), potassiated (dark blue) and cesiated (red) species. Heat maps representing the TIMS spectra are illustrated. A typical *Sr* of 0.56 V/ms was used.

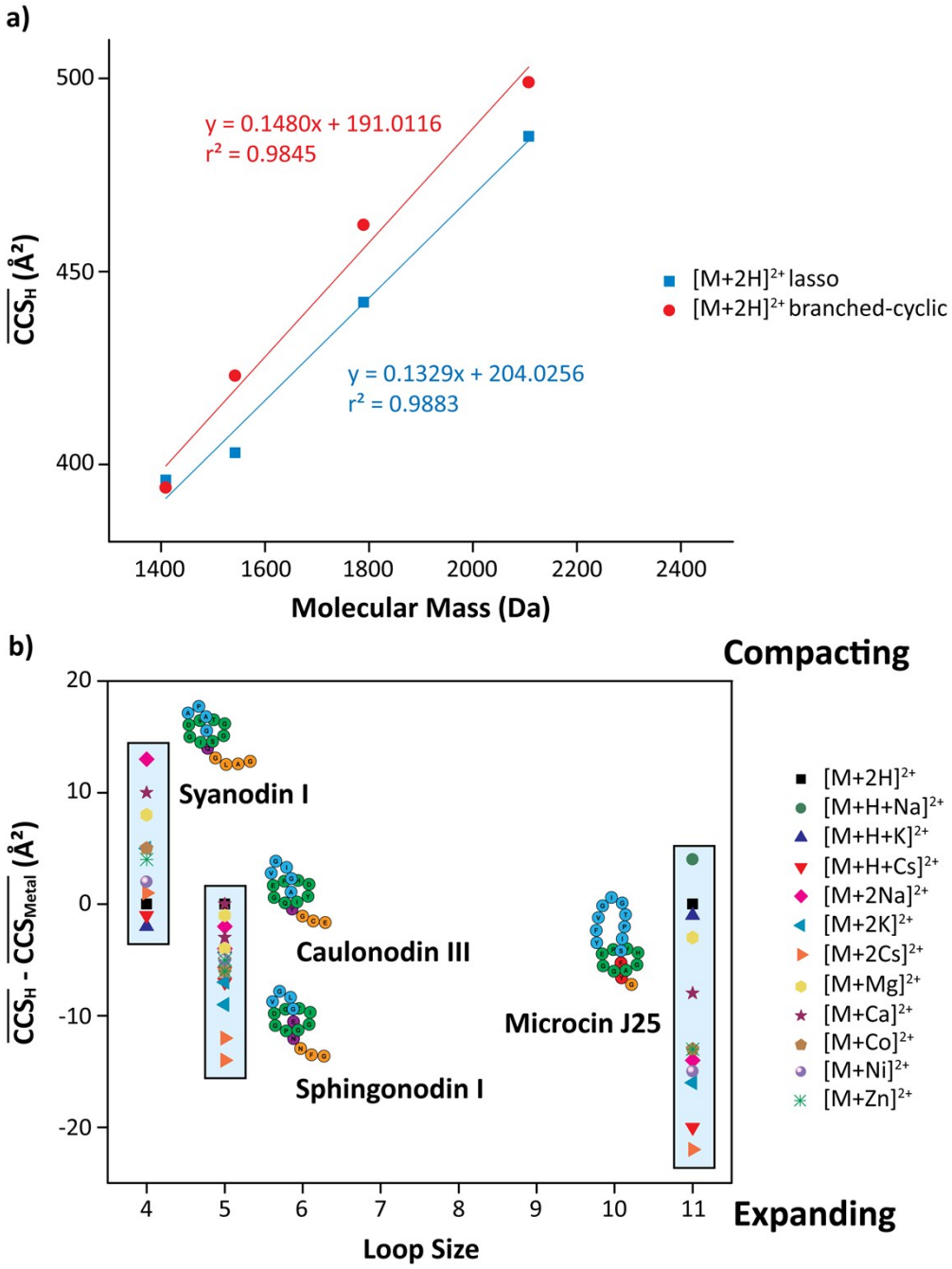

Figure S4. **(a)** Correlation of the $\overline{CCS}$ of the doubly protonated species of lasso (blue trace) and branched-cyclic (red trace) peptides as a function of the molecular mass. **(b)** Effect of the metalated species on the lasso conformational spaces as a function of the loop size.

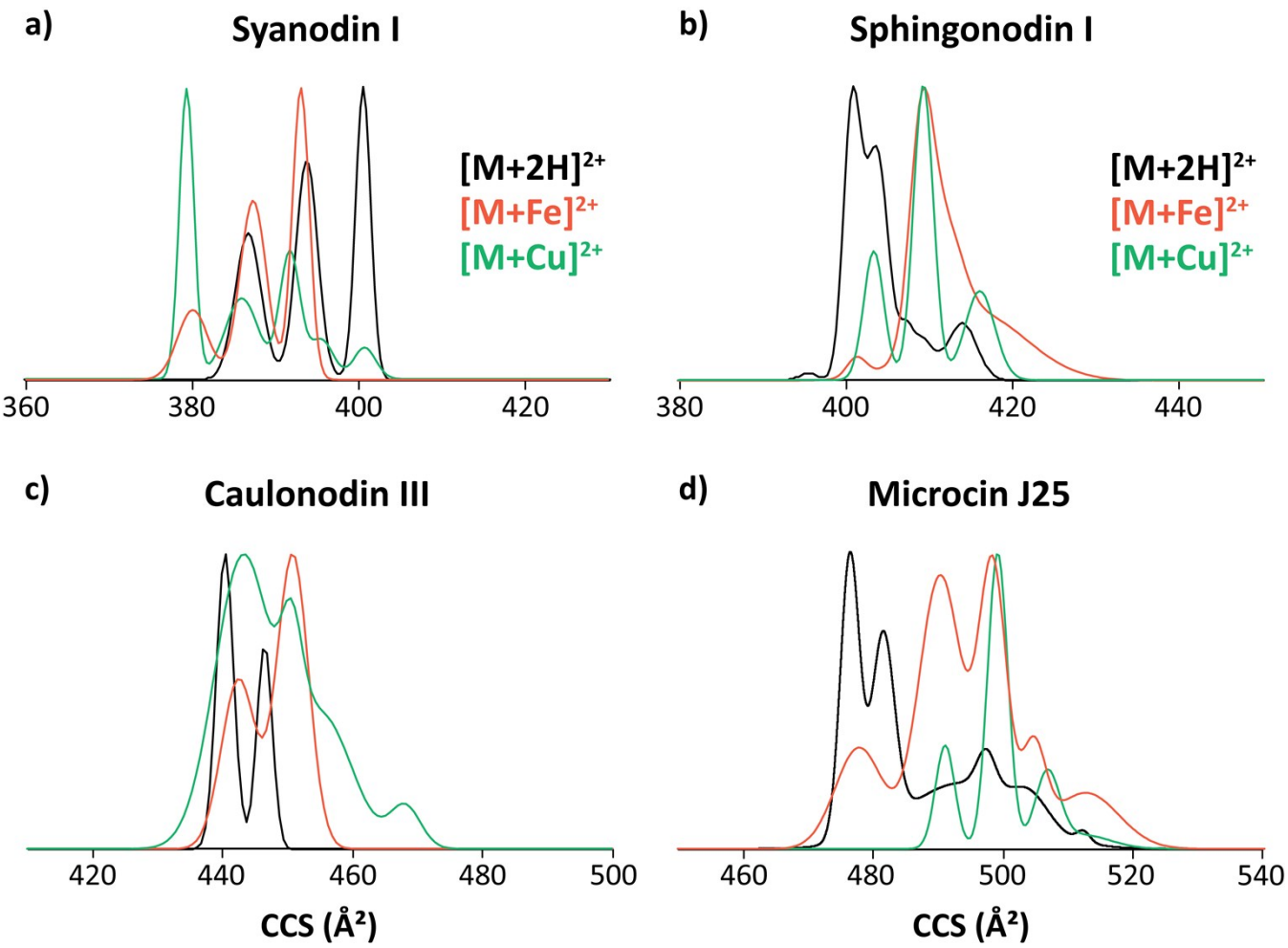

Figure S5. Typical TIMS spectra for a) syanodin I, b) sphingonodin I, c) caulonodin III and d) microcin J25 cationized by protonated (black), ironated (red) and coppiated (green) species. A typical *Sr* of 0.56 V/ms was used.

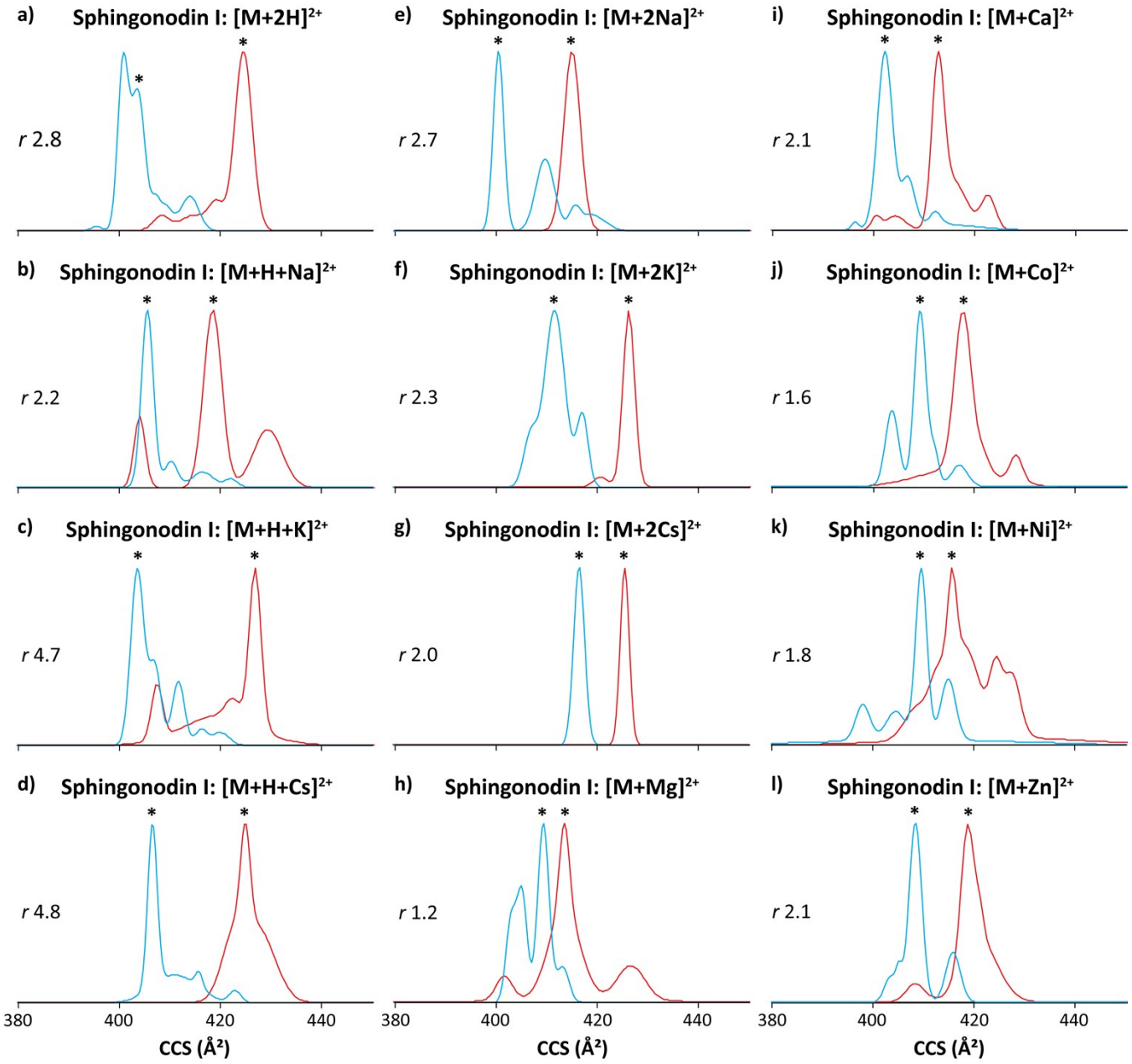

Figure S6. Typical TIMS spectra for sphingonodin I (blue traces) and its branched-cyclic (red traces) topoisomers cationized by (a) protonated, (b, e) sodiated, (c, f) potassiated, (d, g) cesiated, (h) magnesiated, (i) calciated, (j) cobaltiated, (k) nickelated and (l) zincated species. A typical *Sr* of 0.56 V/ms was used and the resolution (*r*) values are given. The peaks highlighted by * symbols were taken to calculate the resolution.

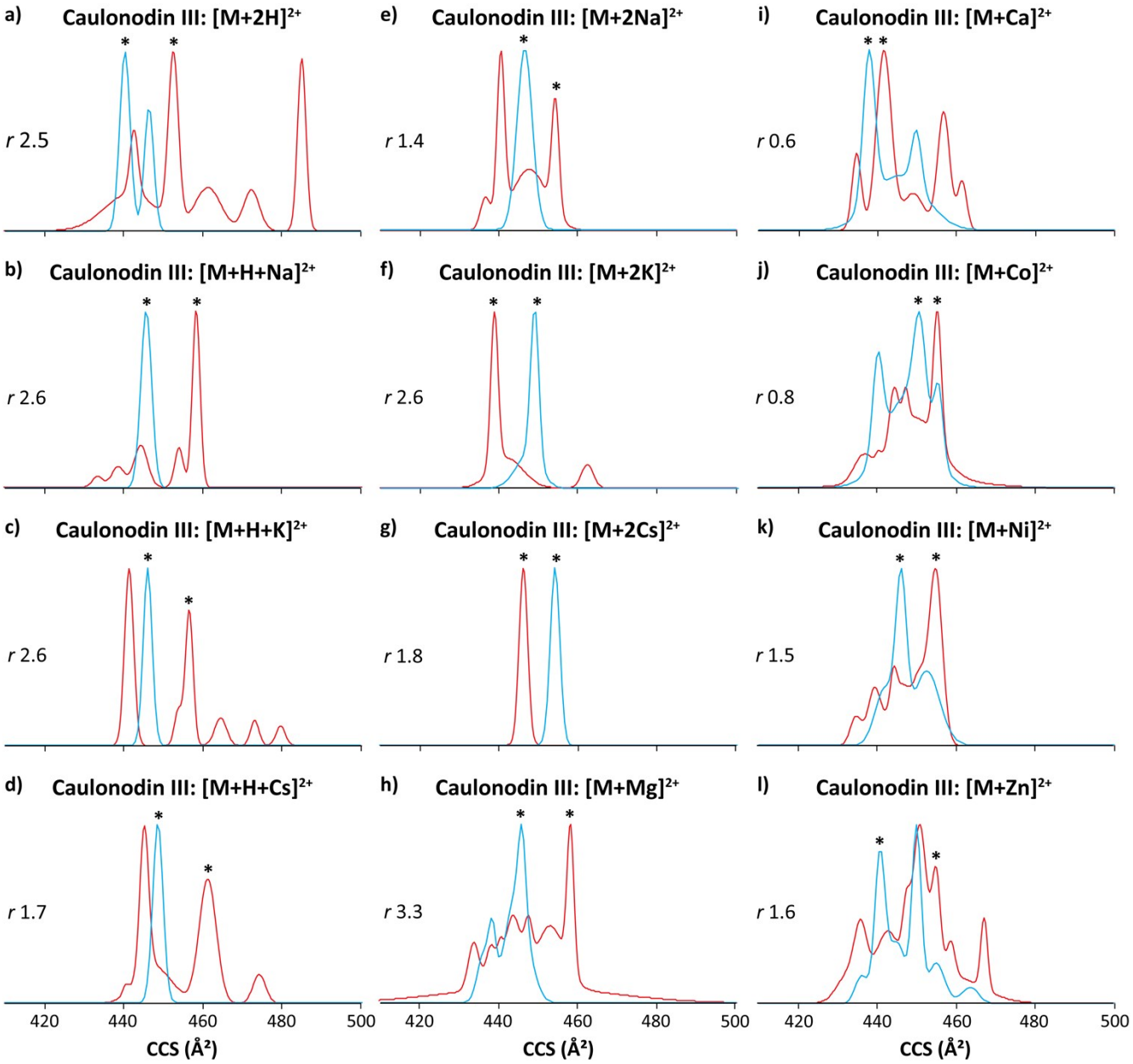

Figure S7. Typical TIMS spectra for caulonodin III (blue traces) and its branched-cyclic (red traces) topoisomers cationized by (a) protonated, (b, e) sodiated, (c, f) potassiated, (d, g) cesiated, (h) magnesiated, (i) calciated, (j) cobaltiated, (k) nickelated and (l) zincated species. A typical *Sr* of 0.56 V/ms was used and the resolution (*r*) values are given. The peaks highlighted by * symbols were taken to calculate the resolution.

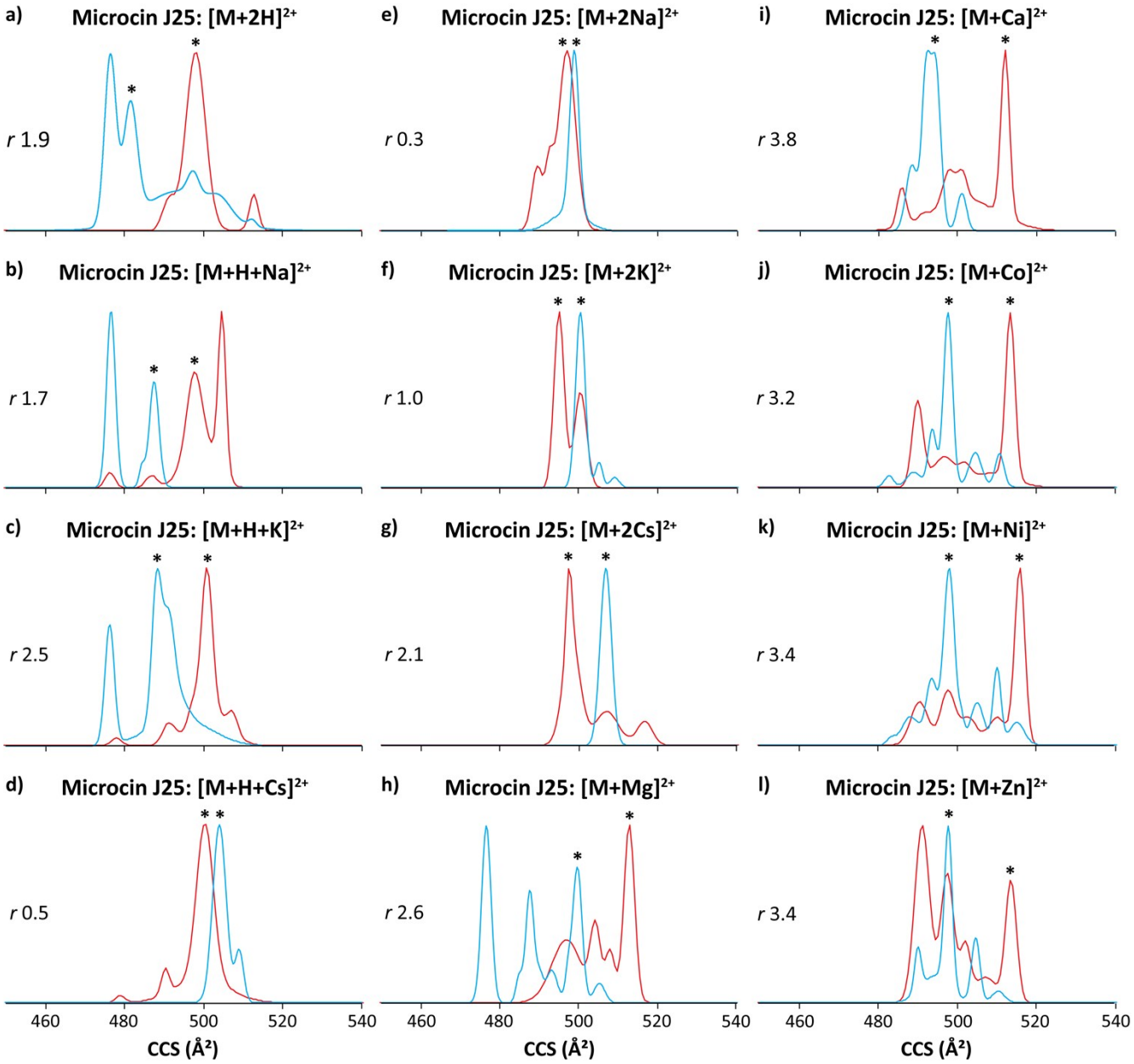

Figure S8. Typical TIMS spectra for microcin J25 (blue traces) and its branched-cyclic (red traces) topoisomers cationized by (a) protonated, (b, e) sodiated, (c, f) potassiated, (d, g) cesiated, (h) magnesiated, (i) calciated, (j) cobaltiated, (k) nickelated and (l) zincated species. A typical *Sr* of 0.56 V/ms was used and the resolution (*r*) values are given. The peaks highlighted by * symbols were taken to calculate the resolution.

Table S2. TIMS experimental ion-neutral collision cross sections (CCS, Å²) for the doubly cationized species of syanodin I and its branched-cyclic topoisomer. The values involved in the resolution calculation are in bold characters.

Peptide	Ion	CCS (Å²), std. error of mean: ± 0.04%	Resolution (*r*)
Syanodin I	$[M+2H]^{2+}$	387/**394**/401	0.7 (2H)
	$[M+H+Na]^{2+}$	378/391/394/**399**	
	$[M+H+K]^{2+}$	394/**400**	
	$[M+H+Cs]^{2+}$	388/**395**/397/401	0.2 (1Na)
	$[M+2Na]^{2+}$	377/**390**	
	$[M+2K]^{2+}$	384/**394**	0.5 (1K)
	$[M+2Cs]^{2+}$	**395**	
	$[M+Mg]^{2+}$	379/**384**/391/395/400	1.7 (1Cs)
	$[M+Ca]^{2+}$	380/**384**/390/398	
	$[M+Fe]^{2+}$	380/387/393	3.4 (2Na)
	$[M+Co]^{2+}$	378/382/390/**392**	
	$[M+Ni]^{2+}$	**394**	1.8 (2K)
	$[M+Cu]^{2+}$	379/386/392/396/401	
	$[M+Zn]^{2+}$	378/385/389/**393**	1.5 (2Cs)
Syanodin I branched-cyclic	$[M+2H]^{2+}$	383/389/394/**397**/401	
	$[M+H+Na]^{2+}$	397/**399**/406	2.2 (Mg)
	$[M+H+K]^{2+}$	385/393/397/**402**/409	
	$[M+H+Cs]^{2+}$	**386**/403	1.3 (Ca)
	$[M+2Na]^{2+}$	**404**/411	
	$[M+2K]^{2+}$	390/396/**401**/409/417	2.2 (Co)
	$[M+2Cs]^{2+}$	**404**/407	
	$[M+Mg]^{2+}$	385/390/**395**/399/408	1.6 (Ni)
	$[M+Ca]^{2+}$	376/386/**392**/397/404/408	
	$[M+Co]^{2+}$	380/385/392/396/**401**/407/410	1.8 (Zn)
	$[M+Ni]^{2+}$	387/390/394/**400**/405/408	
	$[M+Zn]^{2+}$	380/384/390/393/396/**400**/404/410	

Table S3. TIMS experimental ion-neutral collision cross sections (CCS, Å²) for the doubly cationized species of sphingonodin I and its branched-cyclic topoisomer. The values involved in the resolution calculation are in bold characters.

Peptide	Ion	CCS (Å²), std. error of mean: ± 0.04%	Resolution (*r*)
Sphingonodin I	$[M+2H]^{2+}$	396/401/**404**/414	2.8 (2H)
	$[M+H+Na]^{2+}$	**406**/410/417/422	
	$[M+H+K]^{2+}$	**404**/407/412/416/420	2.2 (1Na)
	$[M+H+Cs]^{2+}$	**407**/411/416/423	
	$[M+2Na]^{2+}$	**401**/410/416/419	4.7 (1K)
	$[M+2K]^{2+}$	407/**412**/417	
	$[M+2Cs]^{2+}$	**417**	4.8 (1Cs)
	$[M+Mg]^{2+}$	403/405/**409**/413	
	$[M+Ca]^{2+}$	396/**402**/407/412	2.7 (2Na)
	$[M+Fe]^{2+}$	401/409/412/418	
	$[M+Co]^{2+}$	404/**409**/417	2.3 (2K)
	$[M+Ni]^{2+}$	398/404/**409**/415	
	$[M+Cu]^{2+}$	403/409/416	2.0 (2Cs)
	$[M+Zn]^{2+}$	403/405/**408**/416	
Sphingonodin I branched-cyclic	$[M+2H]^{2+}$	408/415/419/**425**	1.2 (Mg)
	$[M+H+Na]^{2+}$	404/**418**/429	
	$[M+H+K]^{2+}$	407/422/**427**	2.1 (Ca)
	$[M+H+Cs]^{2+}$	423/**425**/428	
	$[M+2Na]^{2+}$	**415**	1.6 (Co)
	$[M+2K]^{2+}$	421/**426**	
	$[M+2Cs]^{2+}$	**426**	1.8 (Ni)
	$[M+Mg]^{2+}$	402/**414**/427	
	$[M+Ca]^{2+}$	400/404/**413**/423	2.1 (Zn)
	$[M+Co]^{2+}$	408/**418**/428	
	$[M+Ni]^{2+}$	409/413/**416**/418/424/427	
	$[M+Zn]^{2+}$	408/**419**	

Table S4. TIMS experimental ion-neutral collision cross sections (CCS, Å²) for the doubly cationized species of caulonodin III and its branched-cyclic topoisomer. The values involved in the resolution calculation are in bold characters.

Peptide	Ion	CCS (Å²), std. error of mean: ± 0.04%	Resolution (*r*)
Caulonodin III	$[M+2H]^{2+}$	**440**/446	2.5 (2H)
	$[M+H+Na]^{2+}$	**446**	
	$[M+H+K]^{2+}$	**446**	
	$[M+H+Cs]^{2+}$	**449**	2.6 (1Na)
	$[M+2Na]^{2+}$	**446**	
	$[M+2K]^{2+}$	447/**449**	2.6 (1K)
	$[M+2Cs]^{2+}$	**454**	
	$[M+Mg]^{2+}$	436/438/444/**446**	1.7 (1Cs)
	$[M+Ca]^{2+}$	**438**/445/450	
	$[M+Fe]^{2+}$	442/451	1.4 (2Na)
	$[M+Co]^{2+}$	440/447/**451**/455	
	$[M+Ni]^{2+}$	442/**446**/452	2.6 (2K)
	$[M+Cu]^{2+}$	443/451/455/468	
	$[M+Zn]^{2+}$	436/**441**/445/450/455/463	1.8 (2Cs)
Caulonodin III branched-cyclic	$[M+2H]^{2+}$	438/443/**453**/461/472/485	
	$[M+H+Na]^{2+}$	433/439/444/454/**458**	3.3 (Mg)
	$[M+H+K]^{2+}$	441/454/**457**/465/473/480	
	$[M+H+Cs]^{2+}$	441/445/448/**461**/474	0.6 (Ca)
	$[M+2Na]^{2+}$	437/441/448/**454**	
	$[M+2K]^{2+}$	**439**/444/462	0.8 (Co)
	$[M+2Cs]^{2+}$	**446**	
	$[M+Mg]^{2+}$	434/438/440/444/448/453/**458**	1.5 (Ni)
	$[M+Ca]^{2+}$	435/**442**/449/457/462	
	$[M+Co]^{2+}$	436/440/444/447/451/**455**	1.6 (Zn)
	$[M+Ni]^{2+}$	435/439/444/451/**455**	
	$[M+Zn]^{2+}$	436/443/447/451/**455**/459/467	

Table S5. TIMS experimental ion-neutral collision cross sections (CCS, Å²) for the doubly cationized species of microcin J25 and its branched-cyclic topoisomer. The values involved in the resolution calculation are in bold characters.

Peptide	Ion	CCS (Å²), std. error of mean: ± 0.04%	Resolution (*r*)
Microcin J25	$[M+2H]^{2+}$	476/**481**/491/497/505/512	1.9 (2H)
	$[M+H+Na]^{2+}$	477/484/**487**	
	$[M+H+K]^{2+}$	476/**488**/491	
	$[M+H+Cs]^{2+}$	**504**/509	1.7 (1Na)
	$[M+2Na]^{2+}$	497/**499**	
	$[M+2K]^{2+}$	**500**/505/509	2.5 (1K)
	$[M+2Cs]^{2+}$	**507**	
	$[M+Mg]^{2+}$	476/485/487/493/**500**/505	0.5 (1Cs)
	$[M+Ca]^{2+}$	488/492/**494**/501	
	$[M+Fe]^{2+}$	478/490/498/505/513	0.3 (2Na)
	$[M+Co]^{2+}$	483/489/494/**498**/504/511	
	$[M+Ni]^{2+}$	483/488/493/**498**/505/510/515	1.0 (2K)
	$[M+Cu]^{2+}$	491/499/507/512	
	$[M+Zn]^{2+}$	490/494/**498**/505/510	2.1 (2Cs)
Microcin J25 branched-cyclic	$[M+2H]^{2+}$	491/**498**/513	
	$[M+H+Na]^{2+}$	476/487/**498**/505	2.6 (Mg)
	$[M+H+K]^{2+}$	478/491/**501**/507	
	$[M+H+Cs]^{2+}$	479/490/**500**	3.8 (Ca)
	$[M+2Na]^{2+}$	489/493/**497**	
	$[M+2K]^{2+}$	**495**/500	3.2 (Co)
	$[M+2Cs]^{2+}$	**498**/507/517	
	$[M+Mg]^{2+}$	497/504/508/**513**	3.4 (Ni)
	$[M+Ca]^{2+}$	486/491/498/501/**512**	
	$[M+Co]^{2+}$	490/497/502/**513**	3.4 (Zn)
	$[M+Ni]^{2+}$	490/498/503/510/**516**	
	$[M+Zn]^{2+}$	491/497/502/507/**514**	

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