

# LETHAL AND SUBLETHAL EFFECTS TOWARDS ZEBRAFISH LARVAE OF MICROCYSTINS AND OTHER CYANOPEPTIDES PRODUCED BY CYANOBACTERIA

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## *Supporting Information*

**Table SI1.** ASM-1 culture medium composition (GORHAM et al., 1964).

Stock solution	Components	Concentration in the stock solution (mg/L)	Concentration in the culture medium (mg/L)
A	NaNO <sub>3</sub>	8.5	170
	MgSO <sub>4</sub> .7H <sub>2</sub> O	2.45	49
	MgCl <sub>2</sub> .6H <sub>2</sub> O	2.05	41
	CaCl <sub>2</sub> .2H <sub>2</sub> O	1.45	29
B	KH <sub>2</sub> PO <sub>4</sub>	8.7	43.5
	Na <sub>2</sub> HPO <sub>4</sub> .12H <sub>2</sub> O	7.06	35.3
	H <sub>3</sub> BO <sub>3</sub>	28.4	2.84
	MnCl <sub>2</sub> .4H <sub>2</sub> O	13.9	1.39
C	FeCl <sub>2</sub> .6H <sub>2</sub> O	9.14	0.914
	ZnCl <sub>2</sub>	3.35	0.335
	CoCl <sub>2</sub> .6H <sub>2</sub> O	0.19	0.019
	CuCl <sub>2</sub> .2H <sub>2</sub> O	0.014	0.0014
D	Titriplex® III (EDTA)	18.6	7.44

**Table S12.** Concentration (mg/L) of cyanopeptides as MC-LR equivalents in the FET test exposure solutions at 0h in the experiments with the pooled and single fractions of both MIRS-04 and NPCD-01 strains.

<i>Microcystis panniformis</i> (MIRS-04)			
Cyanopeptide	Dilution of FET exposure solution	Cyanopeptide concentration (mg/L, MC-LR equivalents)	
		Pooled fractions	Fraction #7 (RT 16 to 18 min)
Micropeptin K139	C1	24.716	18.324
	C2	9.886	7.329
	C3	3.955	2.932
	C4	1.582	1.173
	C5	0.633	0.469
Microcystin-LR	C1	6.182	0.00340
	C2	2.473	0.00144
	C3	0.989	0.00077
	C4	0.396	0.00061
	C5	0.158	0.00060
Cyanopeptolin 972	C1	3.769	0.105
	C2	1.508	0.050
	C3	0.603	0.027
	C4	0.241	0.002
	C5	0.081	0.001
Cyanopeptolin 958	C1	2.906	0.004
	C2	1.162	0.002
	C3	0.465	0.001
	C4	0.186	0.001
	C5	0.070	0.001
[D-Asp <sup>3</sup> ]MC-LR	C1	0.847	0.001
	C2	0.339	0.001
	C3	0.135	<LOQ
	C4	0.047	<LOQ
	C5	0.027	<LOQ
Cyanopeptolin 1014	C1	0.764	0.004
	C2	0.254	0.002
	C3	0.076	0.001
	C4	0.036	0.001
	C5	0.005	0.001
[D-Asp <sup>3</sup> ,ADMAAdda <sup>6</sup> ]MC-LHar	C1	0.749	0.001
	C2	0.311	0.001
	C3	0.110	0.001
	C4	0.044	<LOQ
	C5	0.026	<LOQ
MC-LY	C1	0.377	<LOQ
	C2	0.151	<LOQ
	C3	0.065	<LOQ
	C4	0.035	<LOQ
	C5	0.006	<LOQ

LOD 0.0027 mg/L; LOQ 0.0083 mg/L.

**Table S12.** Concentration (mg/L) of cyanopeptides as MC-LR equivalents in the FET test exposure solutions at 0h in the experiments with the pooled and single fractions of both MIRS-04 and NPCD-01 strains (continued).

<i>Microcystis aeruginosa</i> (NPCD-01)				
Cyanopeptide	Dilution	Cyanopeptide concentration (mg/L, MC-LR equivalents)		
		Pooled fractions	Fraction #1 (RT 4 to 6 min)	Fraction #3 (RT 8 to10 min)
<b>Nostoginin BN741</b>	C1	2.316	0.012	2.910
	C2	0.927	0.005	1.164
	C3	0.371	0.001	0.466
	C4	0.148	<LOQ	0.186
	C5	0.060	<LOQ	0.076
<b>Microginin FR6</b>	C1	0.283	0.003	0.165
	C2	0.113	0.002	0.059
	C3	0.043	0.001	0.031
	C4	0.007	<LOQ	0.005
	C5	0.003	<LOQ	0.002
<b>Cyanopeptolin 959</b>	C1	0.201	0.089	0.031
	C2	0.088	0.052	0.005
	C3	0.042	0.034	0.002
	C4	0.027	0.010	0.001
	C5	0.003	0.005	0.001
<b>Microginin SD755</b>	C1	0.155	<LOQ	0.051
	C2	0.052	<LOQ	0.027
	C3	0.026	<LOQ	0.002
	C4	0.001	<LOQ	0.001
	C5	0.002	<LOQ	0.001

LOD 0.0027 mg/L; LOQ 0.0083 mg/L.

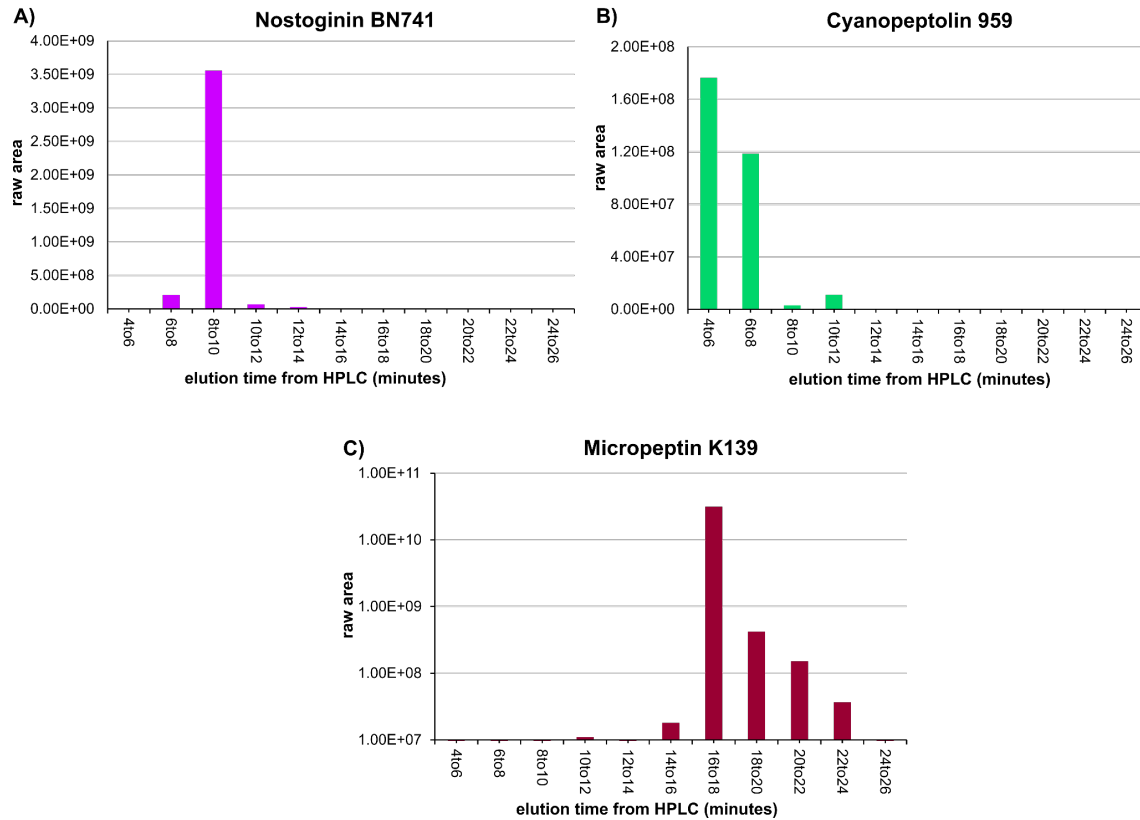
**Table SI3.** Molecular networks generated on the Global Natural Products Social Molecular Networking (GNPS) platform (available on <http://gnps.ucsd.edu>)

Sample	GNPS link
Molecular network of NPCD-01 fractions #9 + #10 (RT 22 to 26 min)	<a href="https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=b824403b3b774a27acda0f57a033ed1f">https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=b824403b3b774a27acda0f57a033ed1f</a>
MolNetEnhancer analysis of NPCD-01 fractions #9 + #10 (RT 22 to 26 min)	<a href="https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=8bf6ebc2f98144b382d1acff5f2f5c95">https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=8bf6ebc2f98144b382d1acff5f2f5c95</a>
Molecular network of MIRS-04 fractions #9 + #10 (22 – 26 min)	<a href="https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=dfe4df6fa354cdb9bf8c5a3c406de33">https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=dfe4df6fa354cdb9bf8c5a3c406de33</a>
MolNetEnhancer analysis of MIRS-04 fractions #9 + #10 (RT 22 to 26 min)	<a href="https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=f06f839360cc4fa9a76824cbab9e0e98">https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=f06f839360cc4fa9a76824cbab9e0e98</a>

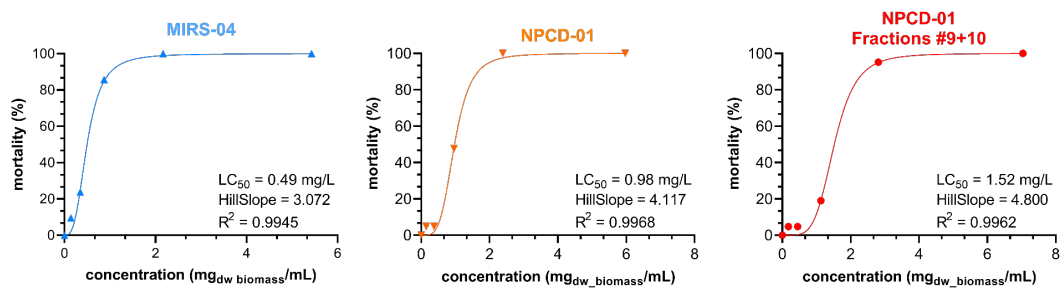
**Table SI4.** Concentrations of the exposure groups of the FET tests in terms of dry weight of cyanobacterial biomass per mL (mg<sub>dw\_biomass</sub>/mL)

Exposure group	Exposure concentrations (mg <sub>dw_biomass</sub> /mL)					
	NPCD-01 (non-producer)			MIRS-04 (MC-producer)		
	Pooled fractions	NG BN741-containing fraction (#3)	CP 959-containing fraction (#1)	Apolar fractions (#9+10)	Pooled fractions	MP K139-containing fraction (#7)
C1	5.97	12.48	11.27	7.04	5.43	8.74
C2	2.39	4.99	4.51	2.81	2.17	3.49
C3	0.96	1.99	1.80	1.13	0.87	1.40
C4	0.38	0.80	0.72	0.45	0.35	0.56
C5	0.15	0.32	0.29	0.18	0.14	0.22

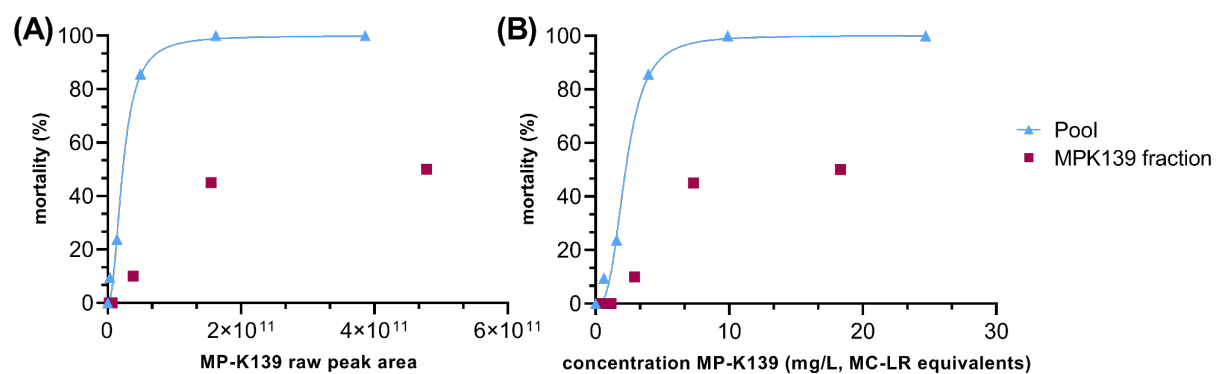




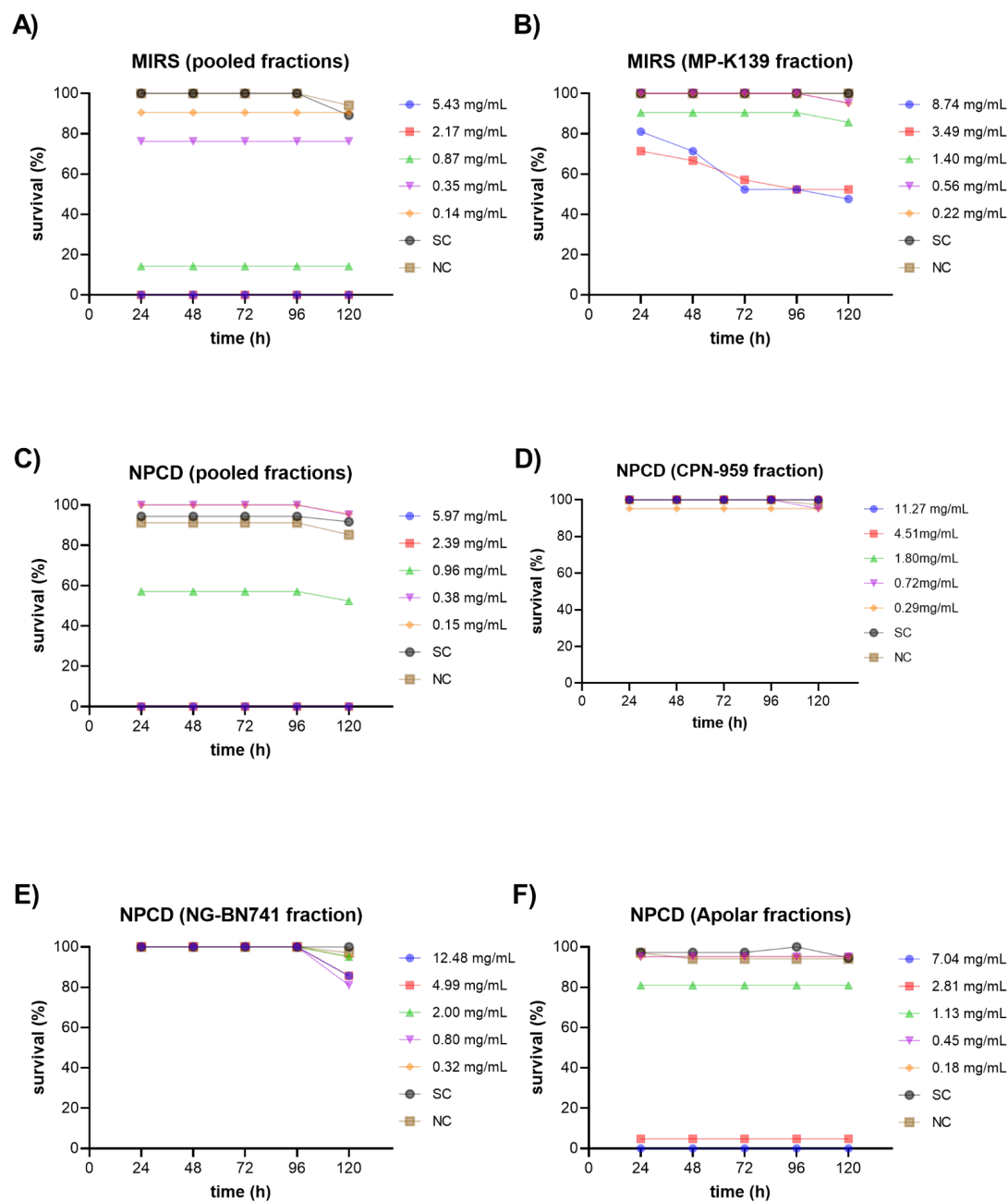
**Figure S11.** Raw area distribution of nostoginin BN741 (A) and cyanopeptolin-959 (B) over the different fractions from the NPCD-01 extract; and raw area distribution of micropeptin K139 over the fractions of the MIRS-01 strain (C).



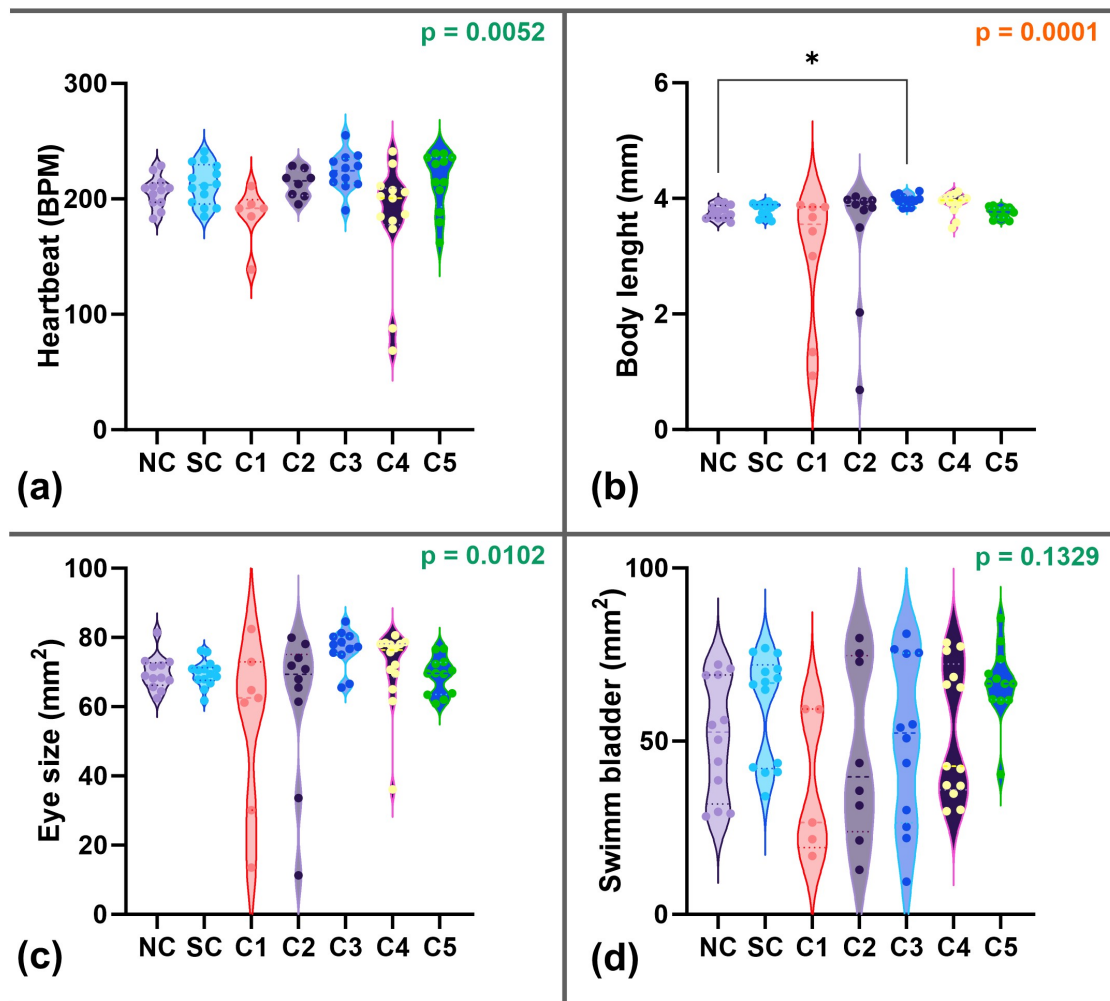
**Figure S12.** Mortality curves after 120h exposure of zebrafish larvae to MIRS-04 pooled fractions (a), NPCD-01 pooled fractions (b), and NPCD-01's fractions #9+10 (c). Fitted lines and  $LC_{50}$  values were obtained using a non-linear regression model, and the slope factor (HillSlope) and goodness of fit ( $R^2$ ) of each model are presented in the plots ( $p < 0.05$ ).



**Figure S13.** Mortality curves after 120h exposure of zebrafish larvae to MIRS-04 pooled fractions (blue triangles) and MPK139-containing fraction (dark-red squares) against MPK139 raw peak area (a), and MPK139 concentration in MC-LR equivalents (b).

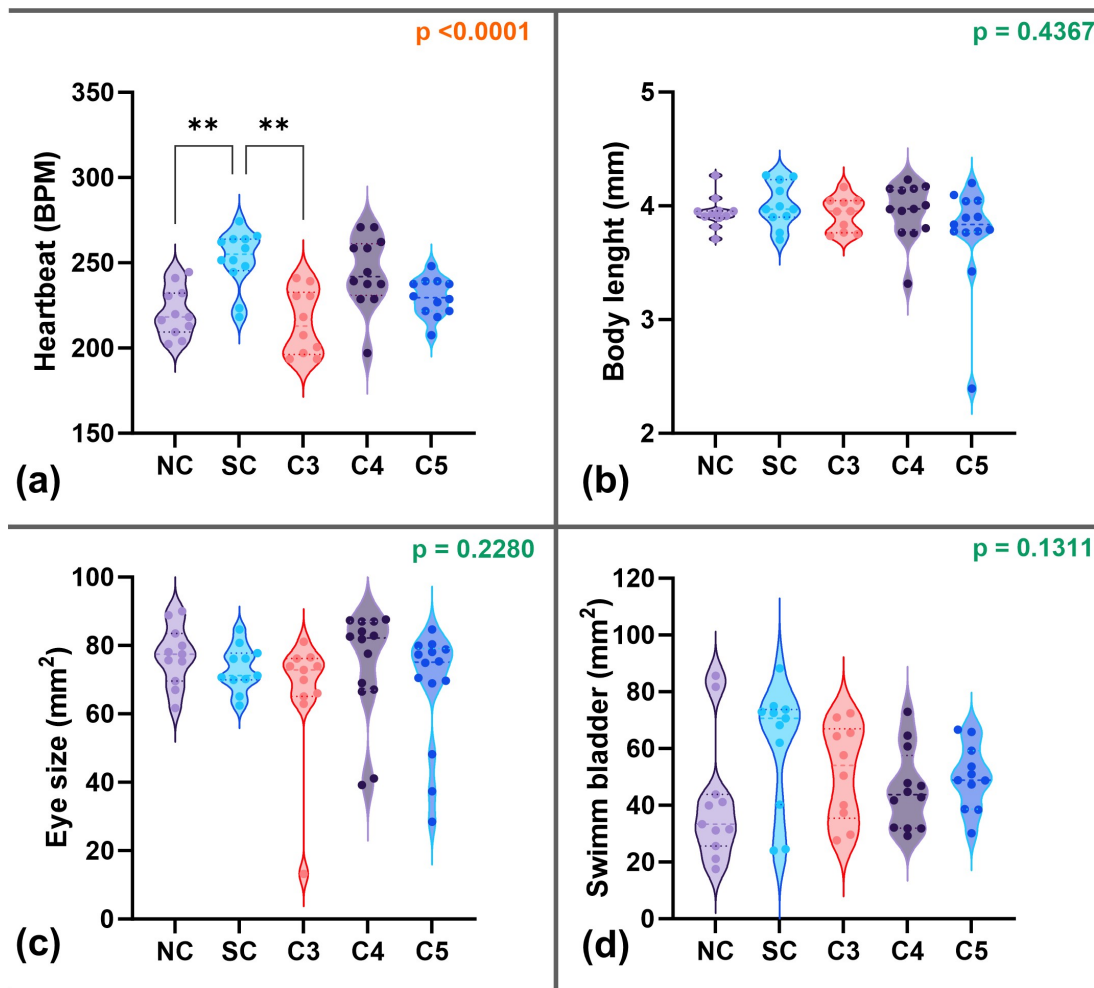


**Figure SI4.** Survival (%) over 120 hour exposure of zebrafish larvae to (A) MIRS-04 pooled fractions; (B) MIRS-04 fraction #7 containing predominantly micropeptin K-139; (C) NPCD-01 pooled fractions; (D) NPCD-01 fraction #1 containing predominantly cyanopeptolin 959; (E) NPCD-01 fraction #3 containing predominantly nostoginin BN741; and (F) NPCD-01 apolar fractions #9+10.



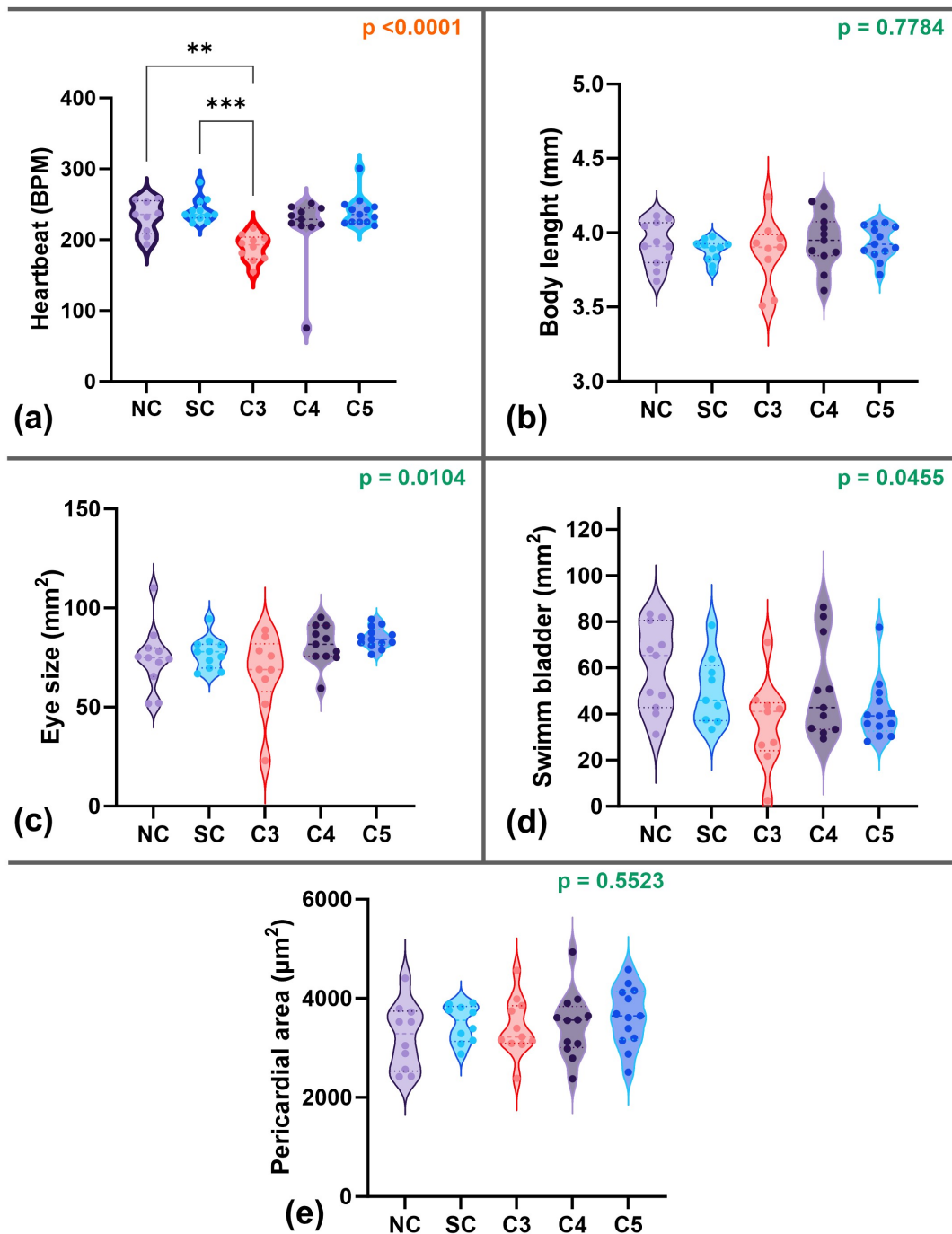
The p values reported refer to the Kruskal–Wallis one-way ANOVA. Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\* $p < 0.05$ ); NC: Negative control; SC: Solvent control.

**Figure SI5.** Heartbeat (a), body length (b), eye size (c), and swim bladder size (d) of larvae exposed (120 hour) to MIRS-04 micropeptin-K139-containing fraction (fraction #7).



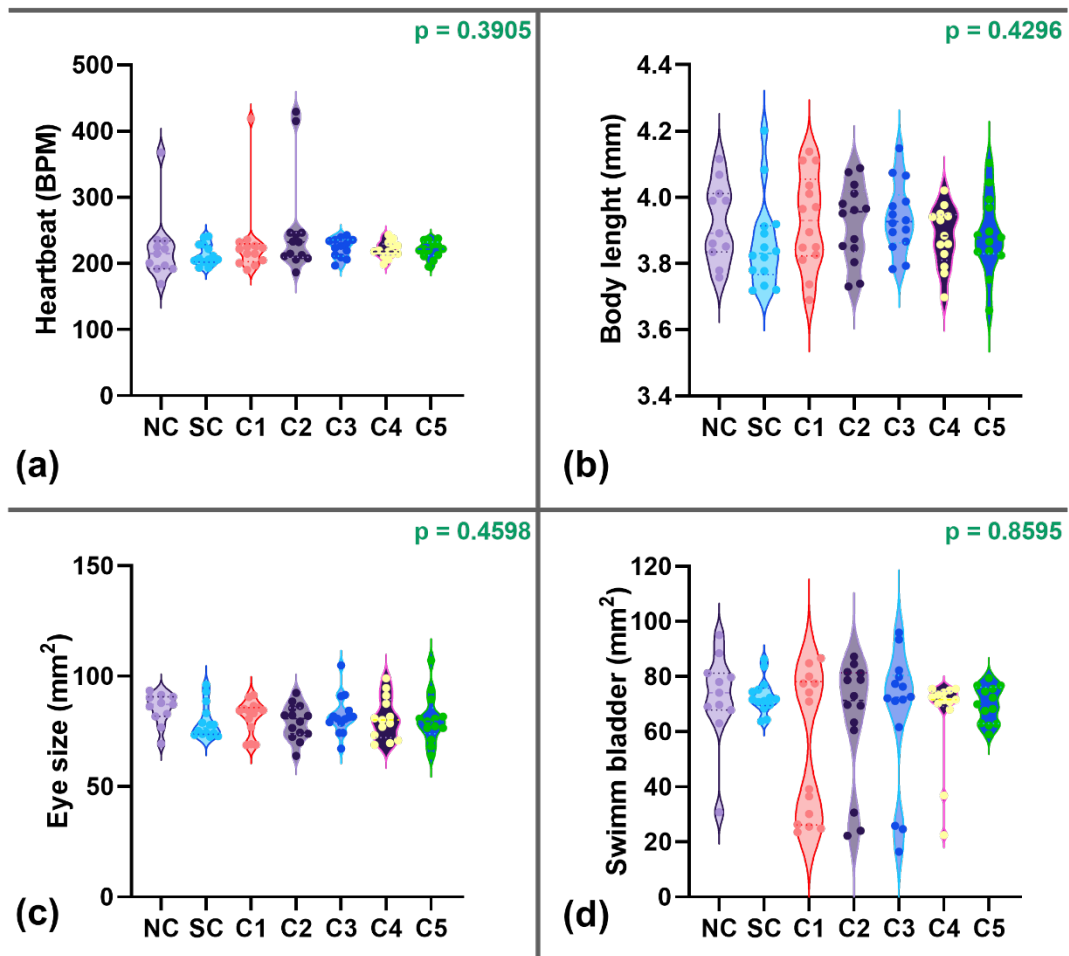
The p values reported refer to the Kruskal–Wallis one-way ANOVA. Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\* $p < 0.05$ ; \*\* $p < 0.01$ ); NC: Negative control; SC: Solvent control.

**Figure S16.** Heartbeat (a), body length (b), eye size (c), and swim bladder size (d) of larvae exposed (120 hours) to NPCD-01 pooled fractions.



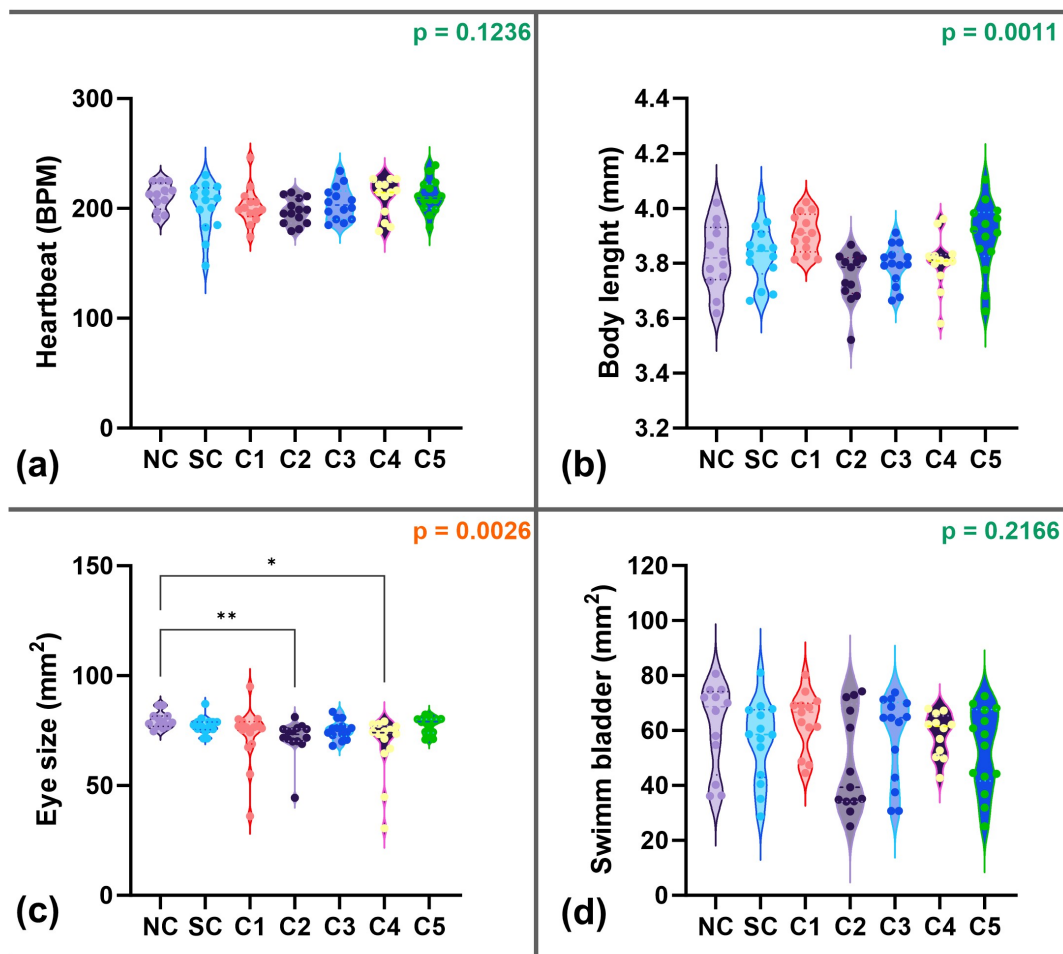
The p values reported refer to the Kruskal–Wallis one-way ANOVA. Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001); NC: Negative control; SC: Solvent control.

**Figure SI7.** Heartbeat (a), body length (b), eye size (c), swim bladder size (d), and pericardial area (e) of larvae exposed (120 hours) to the NPCD-01 apolar fractions #9+10.



The p values reported refer to the Kruskal-Wallis one-way ANOVA. NC: Negative control; SC: Solvent control.  
 NC: Negative control; SC: Solvent control.

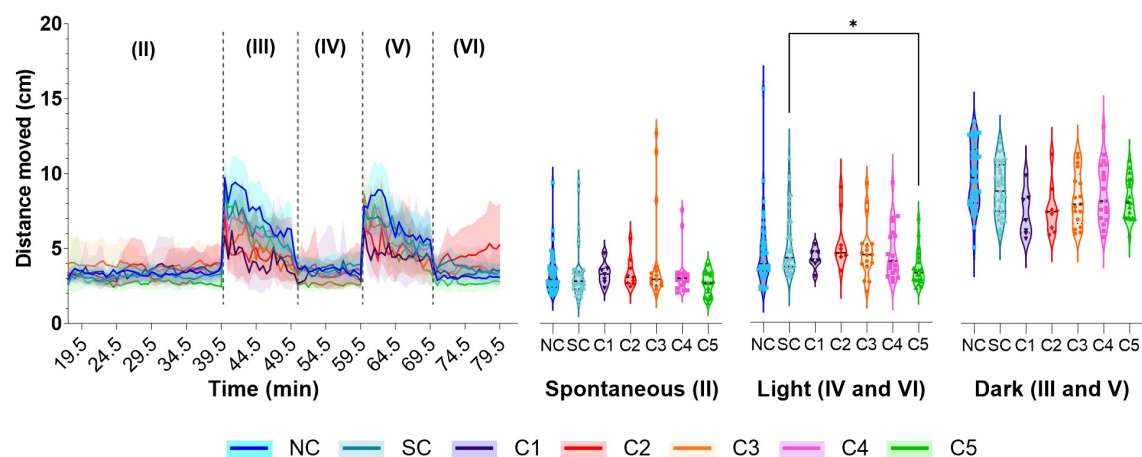
**Figure S18.** Heartbeat (a), body length (b), eye size (c), and swim bladder size (d) of larvae exposed (120 hours) to NPCD-01 cyanopeptolin-959-containing fraction (fraction #1).



The p values reported refer to the Kruskal–Wallis one-way ANOVA. Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\* $p < 0.05$ ; \*\* $p < 0.01$ ); NC: Negative control; SC: Solvent control.

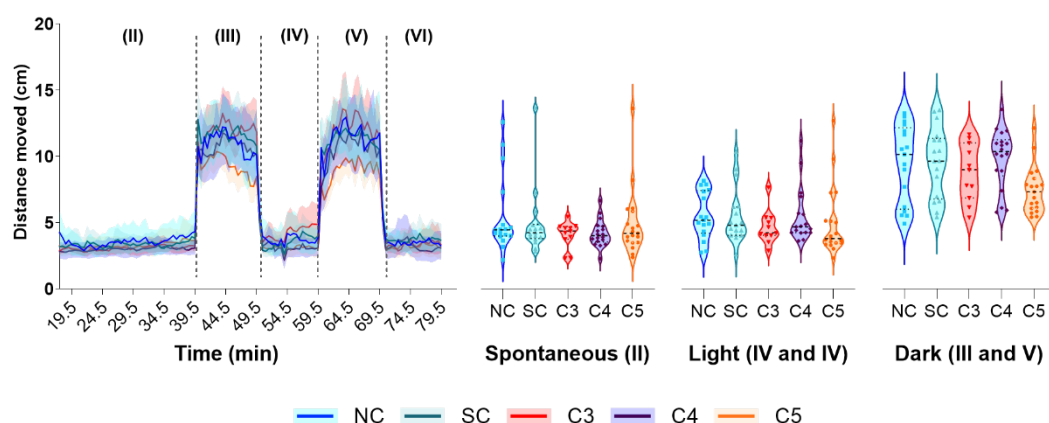
**Figure S19.** Heartbeat (a), body length (b), eye size (c), and swim bladder size (d) of larvae exposed (120 hours) to NPCD-01 nostoginin BN741-containing fraction (fraction #3).





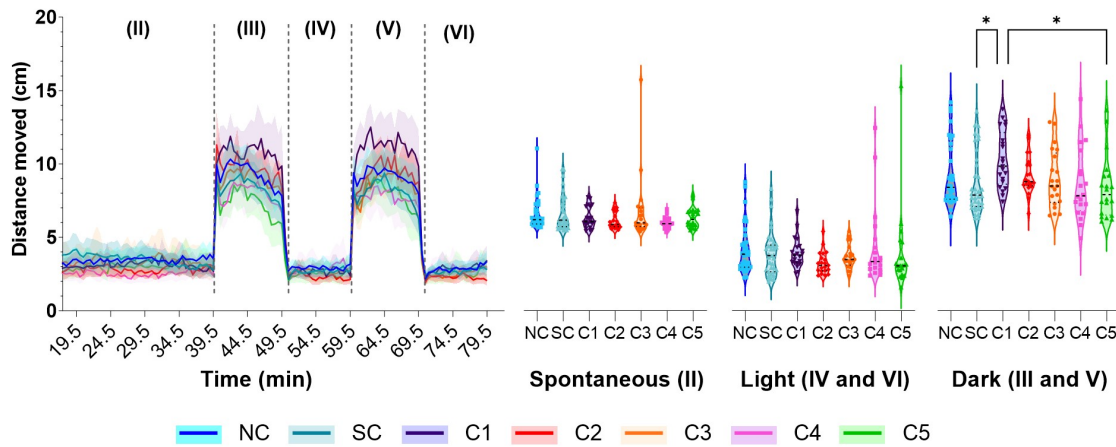
Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\*p < 0.05). NC: Negative control; SC: Solvent control. Data from dead and affected embryos was removed to perform the statistical analyses and plots.

**Figure SI10.** Time series of the accumulated distance moved (recorded every 30 seconds), and violin plots representing the average distance moved in each phase of the behaviour assay - spontaneous, light intervals, and dark intervals - of larvae exposed to different concentrations of MIRS-04 micropeptin-K139-containing fraction (#7) and the controls.



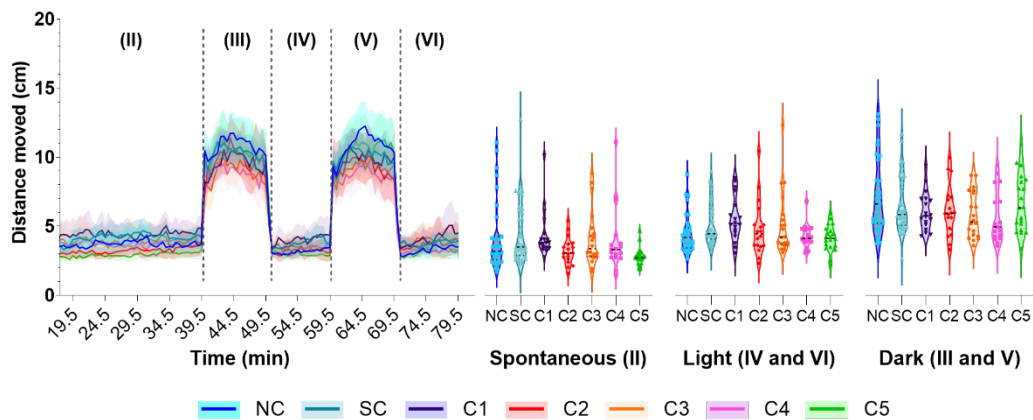
Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.0001). NC: Negative control; SC: Solvent control. Data from dead and affected embryos was removed to perform the statistical analyses and plots.

**Figure SI11.** Time series of the accumulated distance moved (recorded every 30 seconds), and violin plots representing the average distance moved in each phase of the behaviour assay - spontaneous, light intervals, and dark intervals - of larvae exposed to different concentrations of NPCD-01 pooled fractions and the controls.



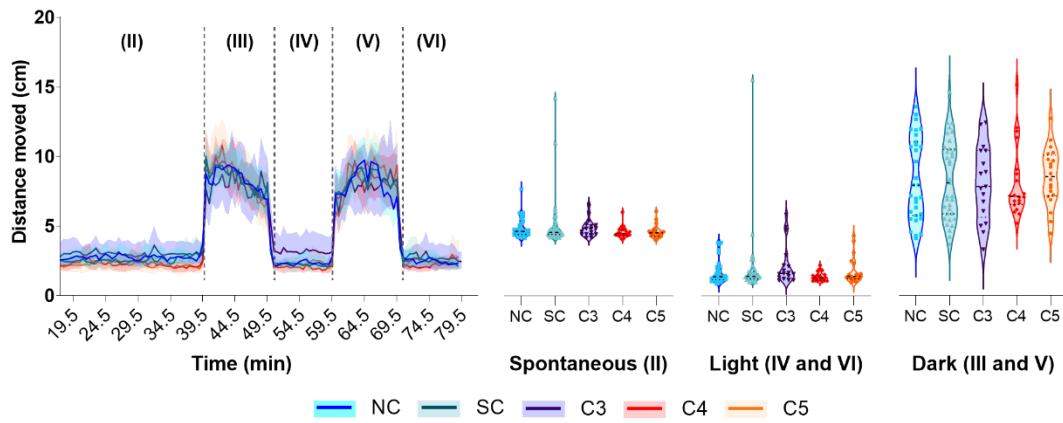
Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\*p < 0.05). NC: Negative control; SC: Solvent control. Data from dead and affected embryos was removed to perform the statistical analyses and plots.

**Figure SI12.** Time series of the accumulated distance moved (recorded every 30 seconds), and violin plots representing the average distance moved in each phase of the behaviour assay - spontaneous, light intervals, and dark intervals - of larvae exposed to different concentrations of the cyanopeptolin-dominated fraction from the NPCD-01 strain.



Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001; \*\*\*\*p < 0.0001). NC: Negative control; SC: Solvent control. Data from dead and affected embryos was removed to perform the statistical analyses and plots.

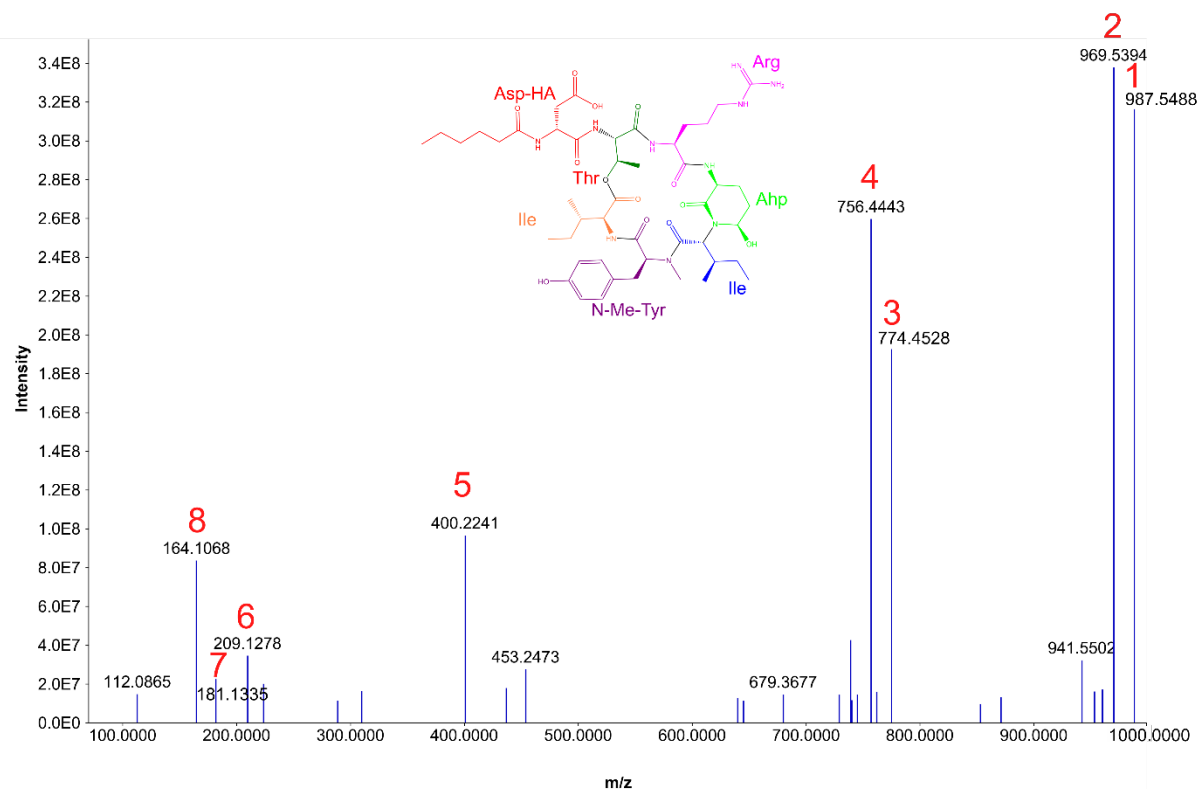
**Figure SI13.** Time series of the accumulated distance moved (recorded every 30 seconds), and violin plots representing the average distance moved in each phase of the behaviour assay - spontaneous, light intervals, and dark intervals - of larvae exposed to different concentrations of NPCD-01 nostoginin BN741-containing fraction (#3) and the controls.



Significant alterations between groups in the post-hoc Dunn's for multiple comparisons are indicated by asterisks (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ). NC: Negative control; SC: Solvent control. Data from dead and affected embryos was removed to perform the statistical analyses and plots.

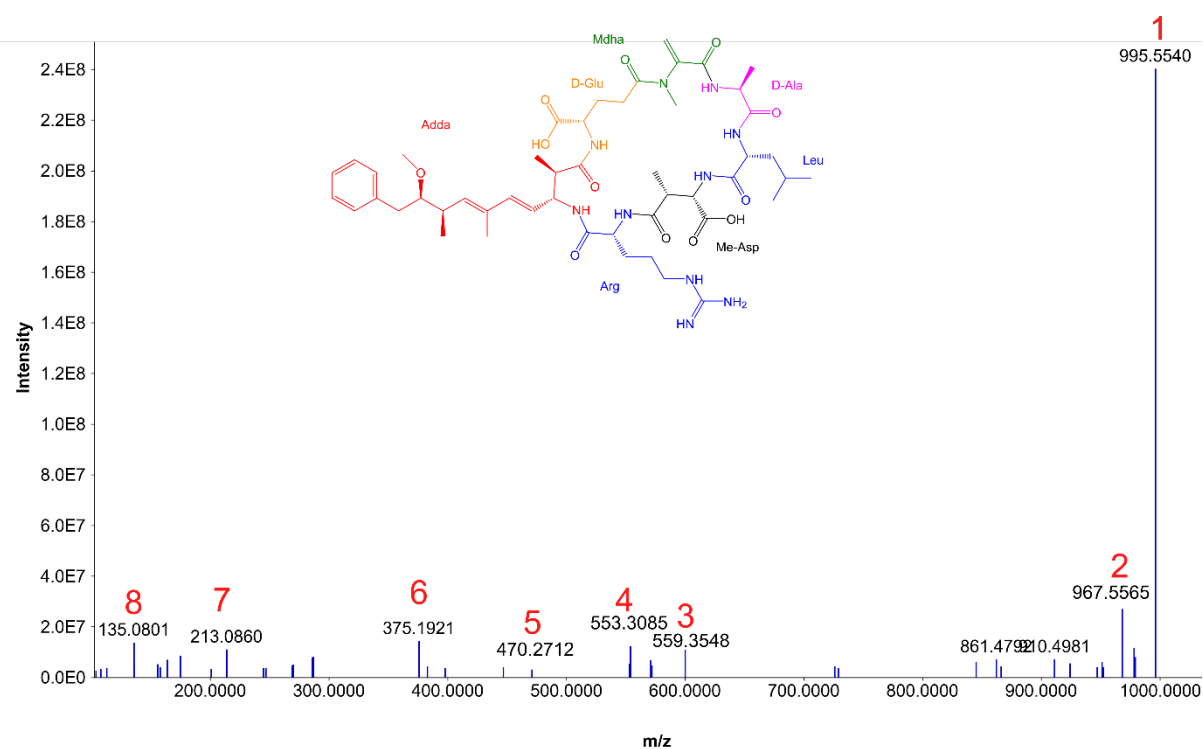
**Figure SI14.** Time series of the accumulated distance moved (recorded every 30 seconds), and violin plots representing the average distance moved in each phase of the behaviour assay - spontaneous, light intervals, and dark intervals - of larvae exposed to different concentrations of fractions 9+10 (NPCD-01) and controls.

**Micropeptin K139**  
m/z 987.5488 | RT 12.21 | HCD 30 | [Arg-Ahp-Ile-N-Me-Tyr-Ile-O-Thr-Asp-HA]



**Figure SI15.** MS/MS annotation at HCD30 for the *m/z* 987.5488 and RT 12.21, matching with micropeptin K139 (*m/z* 987.5510, C<sub>47</sub>H<sub>74</sub>N<sub>10</sub>O<sub>13</sub>, confidence level 2b based on MS<sup>2</sup> annotation) from the MIRS-04 strain.

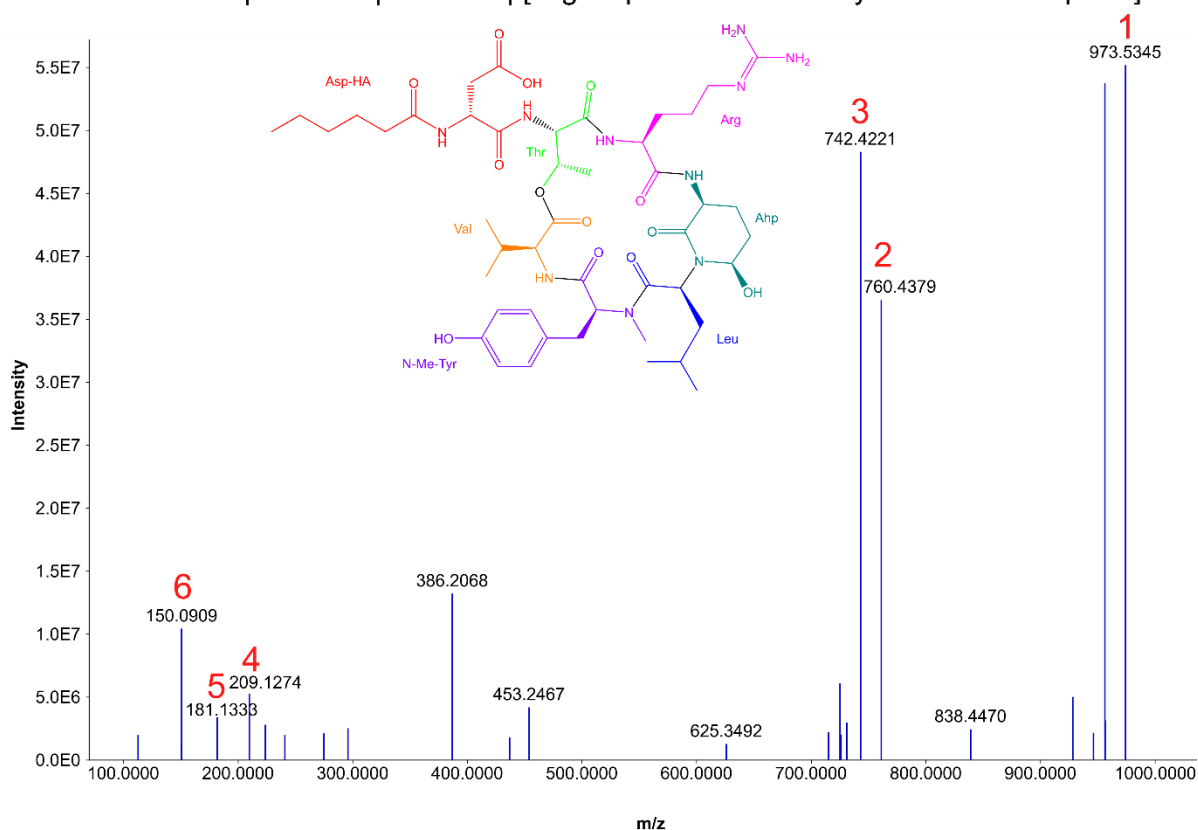
**MC-LR**  
m/z 995.5544 | RT 9.91 | HCD 30 | cyclo[D-Ala-Leu-D-bMe-Asp-Arg-Adda-D-Glu-Mdha]



Fragment number	Calculated m/z	Peak m/z MIRS-04 pooled fractions	Peak m/z MC-LR analytical standard (0.5mg/L)	Fragment
1	995.5560	995.5540	995.5533	M+H
2	967.5611	967.5565	967.5590	[M - CO] <sup>+</sup> H <sup>+</sup>
3	599.3552	599.3548	599.3566	[M - Glu-Mdha-Ala-Arg] + H <sup>+</sup>
4	553.3093	553.3085	553.3093	[M - Adda-Glu] + H <sup>+</sup>
5	470.2727	470.2712	470.2680	[M - Adda-Glu-Mdha]+H <sup>+</sup>
6	375.1914	375.1921	375.1924	[C <sub>11</sub> H <sub>15</sub> O (Adda fragment)-Glu-Mdha] <sup>+</sup>
7	213.0875	213.0860	213.0872	[Glu-Mdha] + H <sup>+</sup>
8	135.0804	135.0801	135.0804	Adda fragment

**Figure SI16.** MS/MS annotation at HCD30 for *m/z* 995.5544 and RT 9.91, matching with microcystin-LR (*m/z* 995.5560, C<sub>49</sub>H<sub>74</sub>N<sub>10</sub>O<sub>12</sub>, confidence level 1 confirmed by reference standard) from the MIRS-04 extract and analytical standard.

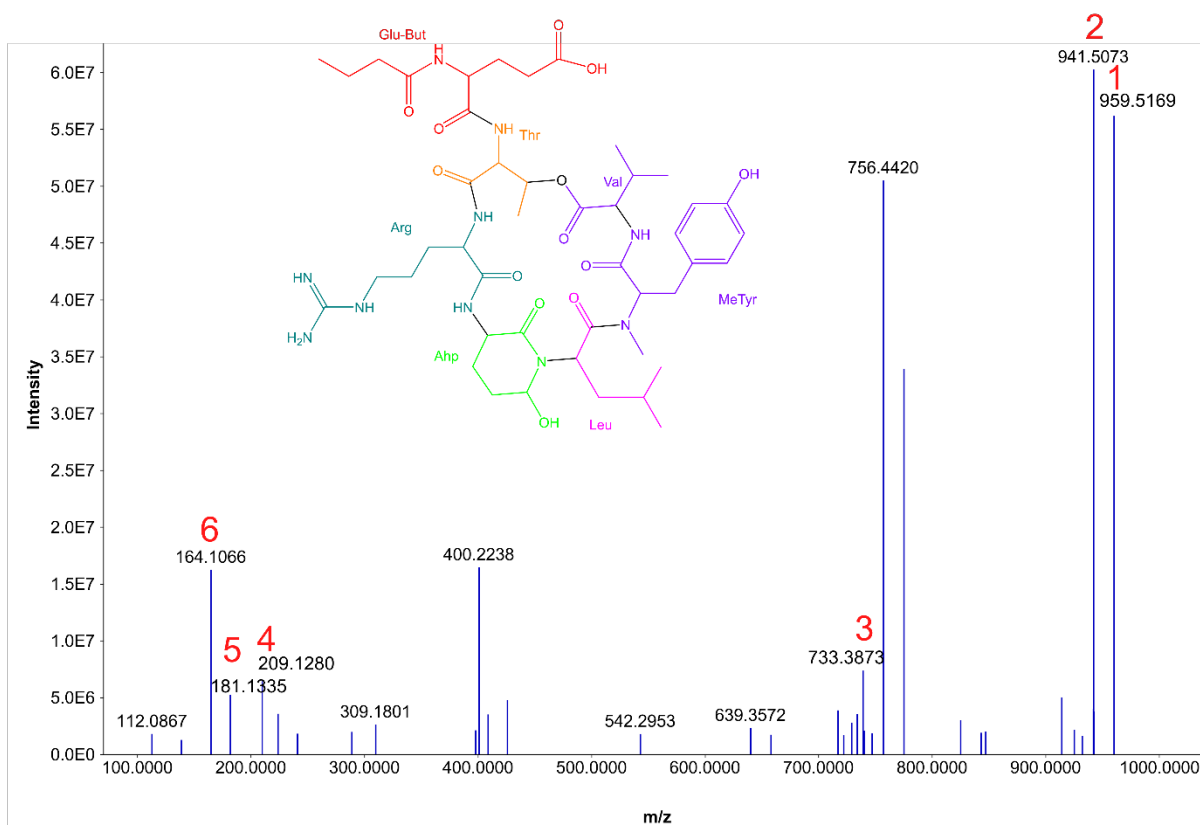
**Cyanopeptolin 972**  
 m/z 973.5345 | RT 8.59 | HCD 30 | [Arg-Ahp-Leu/Ile-N-Me-Tyr-Val-O-Thr-Asp-HA]



Fragment number	Calculated m/z	Peak m/z	Fragment
1	973.5353	973.5345	M+H
2	760.4358	760.4379	[M - Asp-HA] + H+
3	742.4252	742.4221	[M - Asp-HA - H <sub>2</sub> O] + H+
4	209.1274	209.1274	[Leu/Ile-Ahp - H <sub>2</sub> O] + H+
5	181.1336	181.1333	[Leu/Ile-Ahp - H <sub>2</sub> O - CO] + H+
6	150.0909	150.0909	Tyr fragment

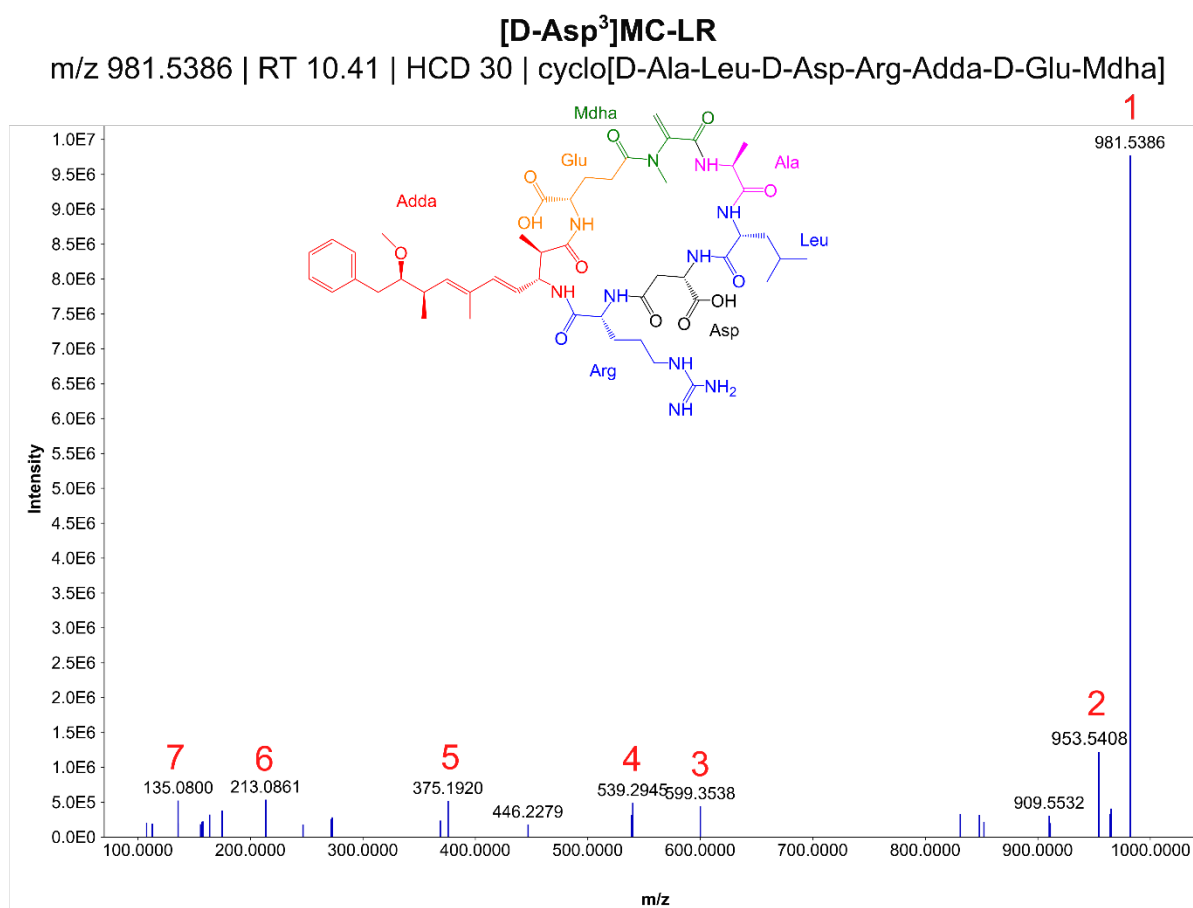
**Figure SI17.** MS/MS annotation at HCD30 for *m/z* 973.5345 and RT 8.59, matching with cyanopeptolin 872 (*m/z* 973.5353, C<sub>46</sub>H<sub>72</sub>N<sub>10</sub>O<sub>13</sub>, confidence level 2b based on MS<sup>2</sup> annotation) from the MIRS-04 extract.

**Cyanopeptolin 958**  
m/z 959.5169 | RT 8.82 | HCD 30 | [Arg-Ahp-Leu-N-Me-Tyr-Val-O-Thr-Glu-But]



Fragment number	Calculated m/z	Peak m/z	Fragment
1	959.5197	959.5169	M+H
2	941.5096	941.5073	[M - H <sub>2</sub> O] <sup>+</sup>
3	733.3885	733.3873	[M - Leu-Ahp] <sup>+</sup>
4	209.1274	209.1280	[Leu/Ile-Ahp - H <sub>2</sub> O] + H <sup>+</sup>
5	181.1336	181.1335	[Leu/Ile -Ahp - H <sub>2</sub> O - CO] + H <sup>+</sup>
6	164.1070	164.1066	MeHTyr immonium ion

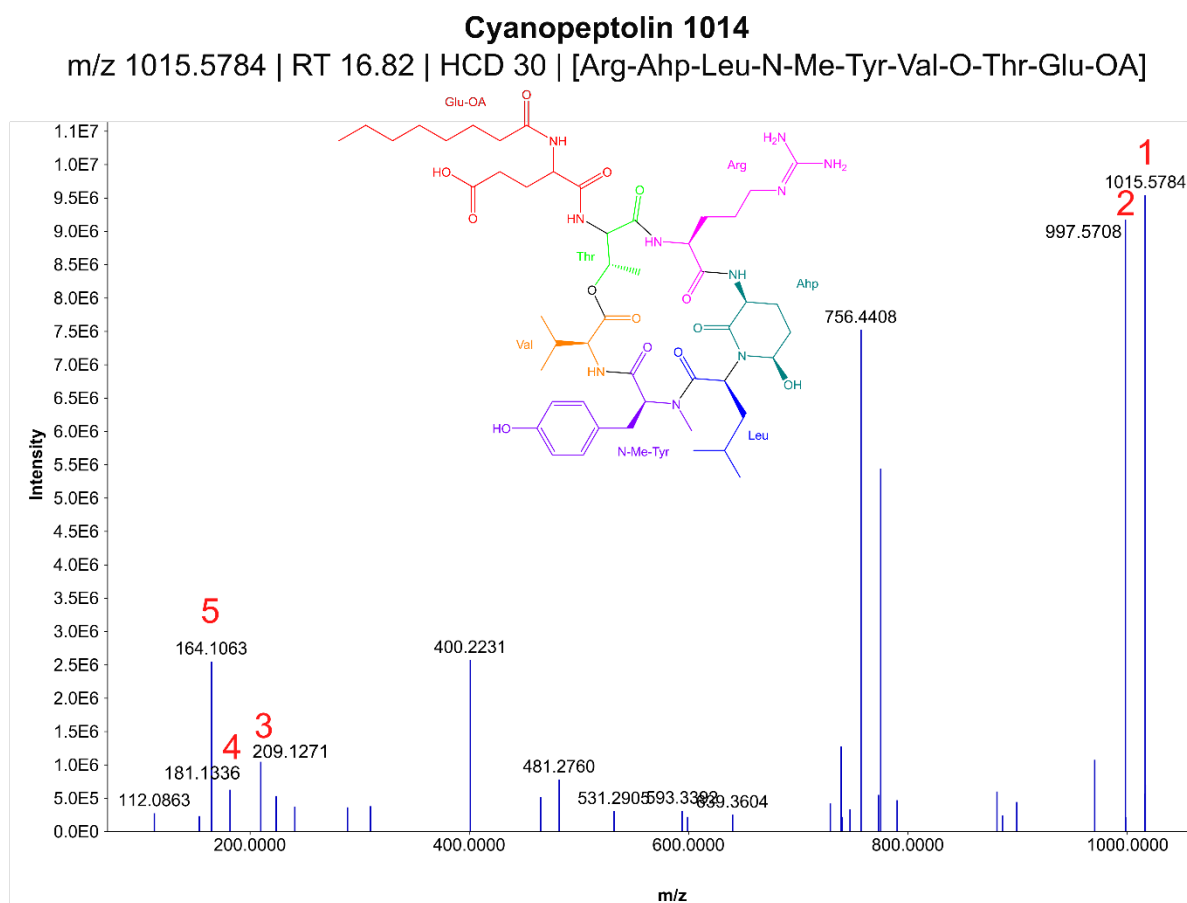
**Figure SI18.** MS/MS annotation at HCD30 for m/z 959.5169 and RT 8.82, matching with cyanopeptolin 958 (m/z 959.5197, C<sub>45</sub>H<sub>70</sub>N<sub>10</sub>O<sub>13</sub>, confidence level 2b based on MS<sup>2</sup> annotation) from the MIRS-04 extract.



Fragment number	Calculated m/z	Peak m/z MIRS-04 pooled fractions	Peak m/z MC-LR analytical standard (0.5mg/L)	Fragment
1	981.5404	981.5386	981.5408	[M + H] <sup>+</sup>
2	953.5455	953.5408	953.5413	M+ H -CO
3	599.3557	599.3538	599.3574	[M - D-Ala - Leu - D-Asp -Mdha]
4	539.2942	539.2945	539.2950	[M - Adda - D-Glu - Mdha]
5	375.1914	375.1920	375.1925	[C <sub>11</sub> H <sub>15</sub> O (Adda fragment)–Glu–Mdha] <sup>+</sup>
6	213.0875	213.0861	213.0869	[Glu–Mdha] + H <sup>+</sup>
7	135.0804	135.0800	135.0804	Adda fragment

**Figure SI19.** MS/MS annotation at HCD30 for *m/z* 981.5386 and RT 10.41, matching with [D-Asp<sup>3</sup>]microcystin-LR (*m/z* 981.5404, C<sub>48</sub>H<sub>72</sub>N<sub>10</sub>O<sub>12</sub>, confidence level 1 confirmed by reference standard) from the MIRS-04 extract and analytical standard.

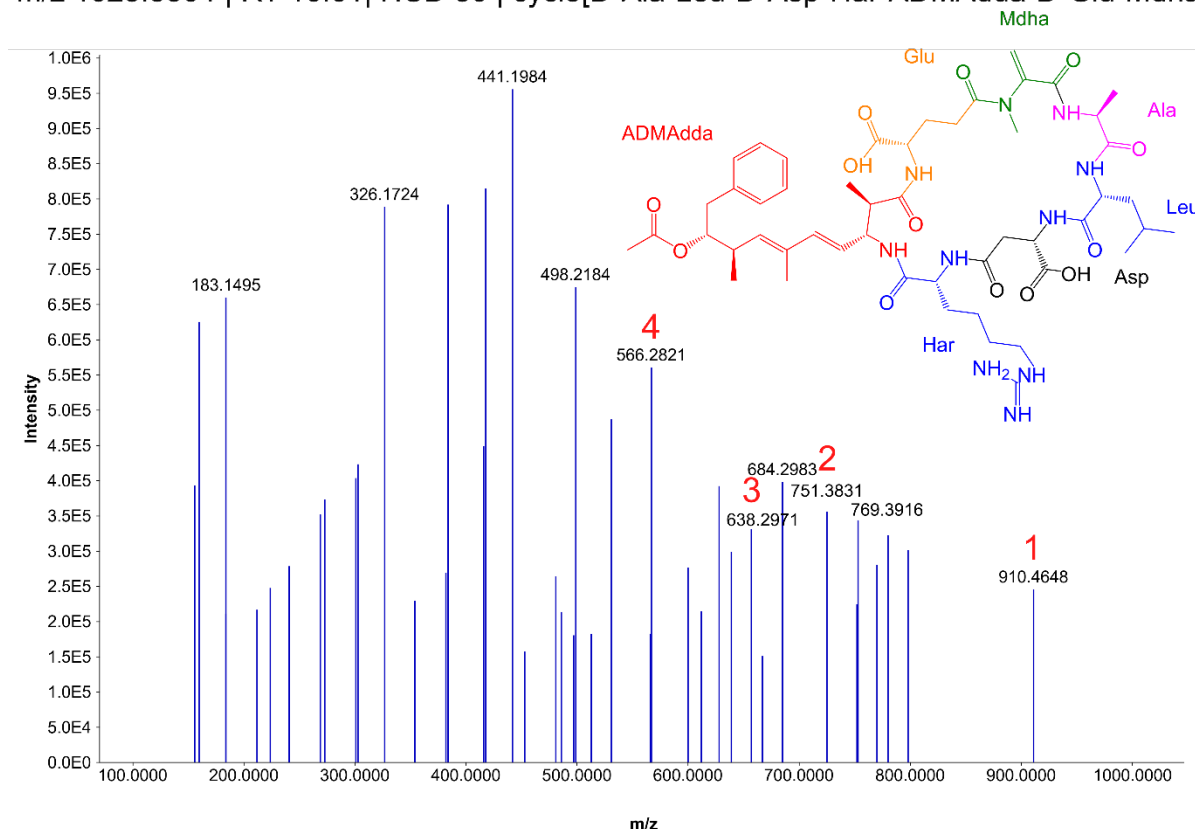




Fragment number	Calculated m/z	Peak m/z	Fragment
1	1015.5823	1015.5784	M+H
2	997.5717	997.5708	[M - H <sub>2</sub> O] <sup>+</sup>
3	209.1274	209.1271	[Leu/Ile-Ahp - H <sub>2</sub> O] + H <sup>+</sup>
4	181.1336	181.1336	[Leu/Ile -Ahp - H <sub>2</sub> O - CO] + H <sup>+</sup>
5	164.1070	164.1063	MeHTyr immonium ion

**Figure SI20.** MS/MS annotation at HCD30 for *m/z* 1015.5784 and RT 16.82, matching with cyanopeptolin 1014 (*m/z* 1015.5823, C<sub>49</sub>H<sub>78</sub>N<sub>10</sub>O<sub>13</sub>, confidence level 2b based on MS<sup>2</sup> annotation) from the MIRS-04 extract.

**[D-Asp<sup>3</sup>,ADMAdda<sup>5</sup>]MC-LHar**  
 m/z 1023.5504 | RT 16.61 | HCD 30 | cyclo[D-Ala-Leu-D-Asp-Har-ADMAdda-D-Glu-Mdha]

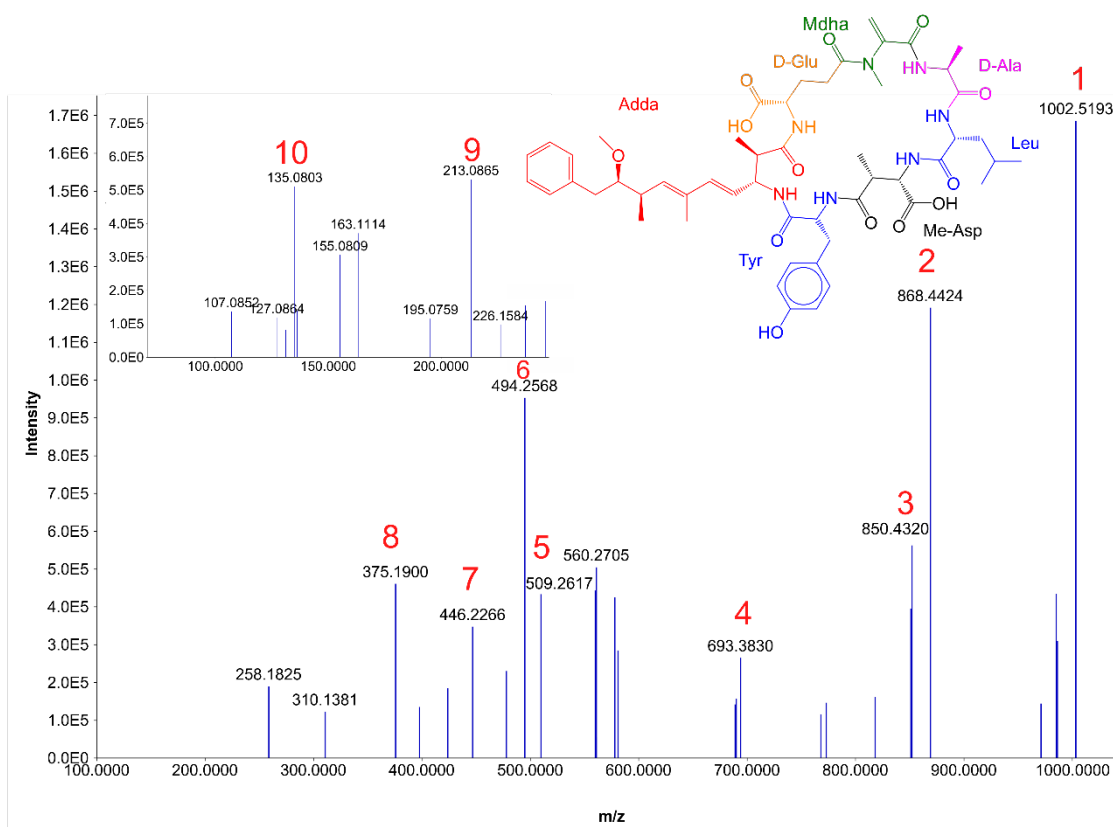


Fragment number	Calculated m/z	Peak m/z	Fragment
*	1023.5509	1023.5504	M+H
1	910.4674	910.4648	[M- Leu]
2	751.3865	751.3831	[M - Part of ADMAdda (C <sub>17</sub> H <sub>21</sub> O <sub>2</sub> ) - NH <sub>2</sub> ]
3	638.2952	638.2971	[M - Har - Asp - Leu]
4	566.2813	566.2821	[M - ADMAdda - Glu]

**Figure SI21.** MS/MS annotation at HCD30 for *m/z* 1023.5504 and RT 16.61, matching with [D-Asp<sup>3</sup>,ADMAdda<sup>5</sup>]MC-LHar (*m/z* 1023.5509, C<sub>50</sub>H<sub>74</sub>N<sub>10</sub>O<sub>13</sub>, confidence level 3 as only few fragments could be annotated) from the MIRS-04 extract. (\*) precursor ion peak not shown in the spectrum but visible with HCD15..

# MC-LY

$m/z$  1002.5193 | RT 15.50 | HCD 15&30 | cyclo[D-Ala-Leu-D-MeAsp-Tyr-Adda-D-Glu-Mdha]

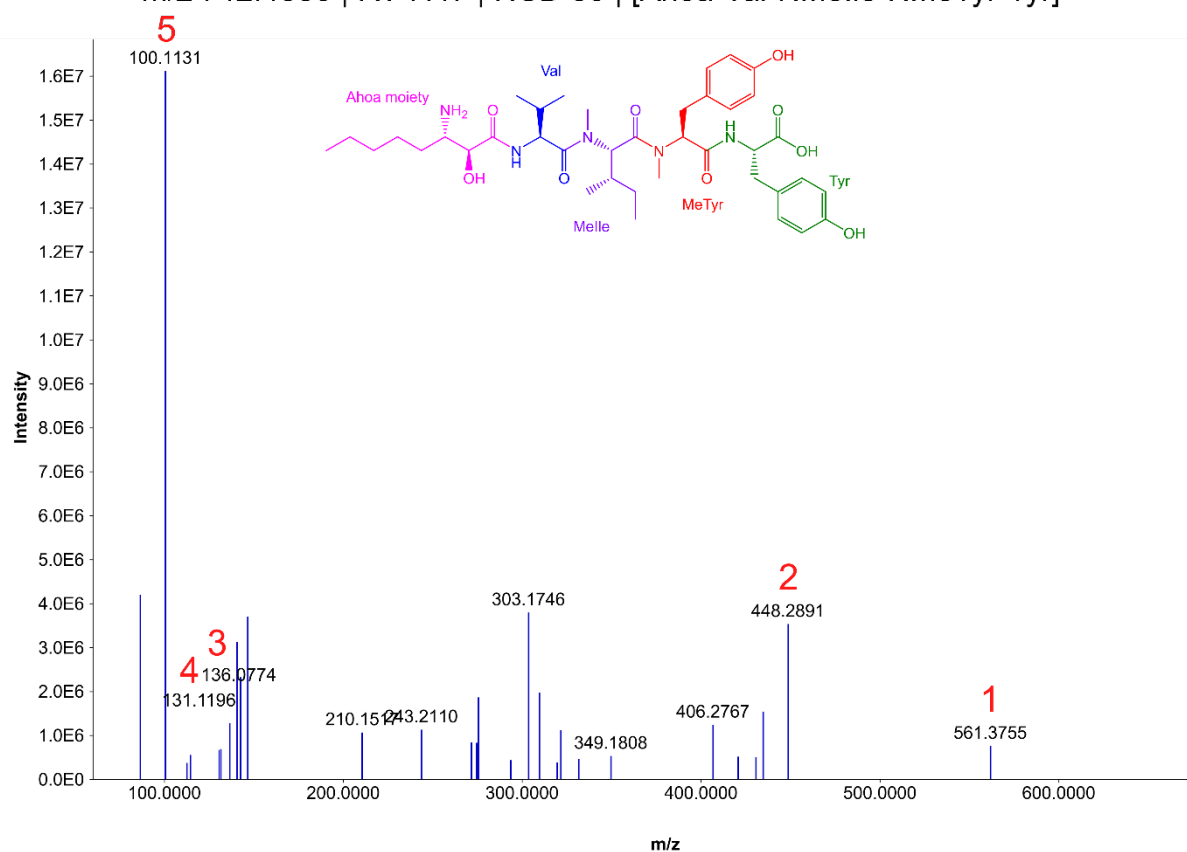


Fragment number	Calculated $m/z$	Peak $m/z$ MIRS-04 pooled fractions	Peak $m/z$ MC-LR analytical standard (0.5mg/L)	Fragment
1	1002.5183	1002.5193	1002.5165	[M + H] <sup>+</sup>
2	868.4456	868.4424	868.4462	[M - Adda fragment] + H <sup>+</sup>
3	850.4351	850.4320	850.4350	[M - Adda fragment - H <sub>2</sub> O] +
4	693.3863	693.3830	693.3828	[M - Leu - D-MeAsp - Tyr - NH <sub>3</sub> ] +
5	509.2652	509.2617	509.2639	[M - Ala - Leu - D-MeAsp - Tyr - NH <sub>3</sub> ] +
6	494.2615	494.2568	494.2604	[M - Adda - Glu - Mdha - NH <sub>2</sub> ] + H <sup>+</sup>
7	446.2291	446.2266	446.2287	[M - Leu - D-MeAsp - Tyr - NH <sub>3</sub> - C <sub>9</sub> H <sub>11</sub> O (Part of Adda)] +
8	375.1914	375.1900	375.1925	[C <sub>11</sub> H <sub>15</sub> O (Adda fragment)–Glu–Mdha] <sup>+</sup>
9	213.0875	213.0865	213.0869	[Glu–Mdha] + H <sup>+</sup>
10	135.0804	135.0803	135.0804	Adda fragment

**Figure SI22.** MS/MS annotation at HCD 15 and HCD30 (see inset) for  $m/z$  1002.5193 and RT 15.50, matching with microcystin-LY ( $m/z$  1002.5183, C<sub>50</sub>H<sub>74</sub>N<sub>10</sub>O<sub>13</sub>, confidence level 1 confirmed by reference standard) from the MIRS-04 extract and analytical standard.

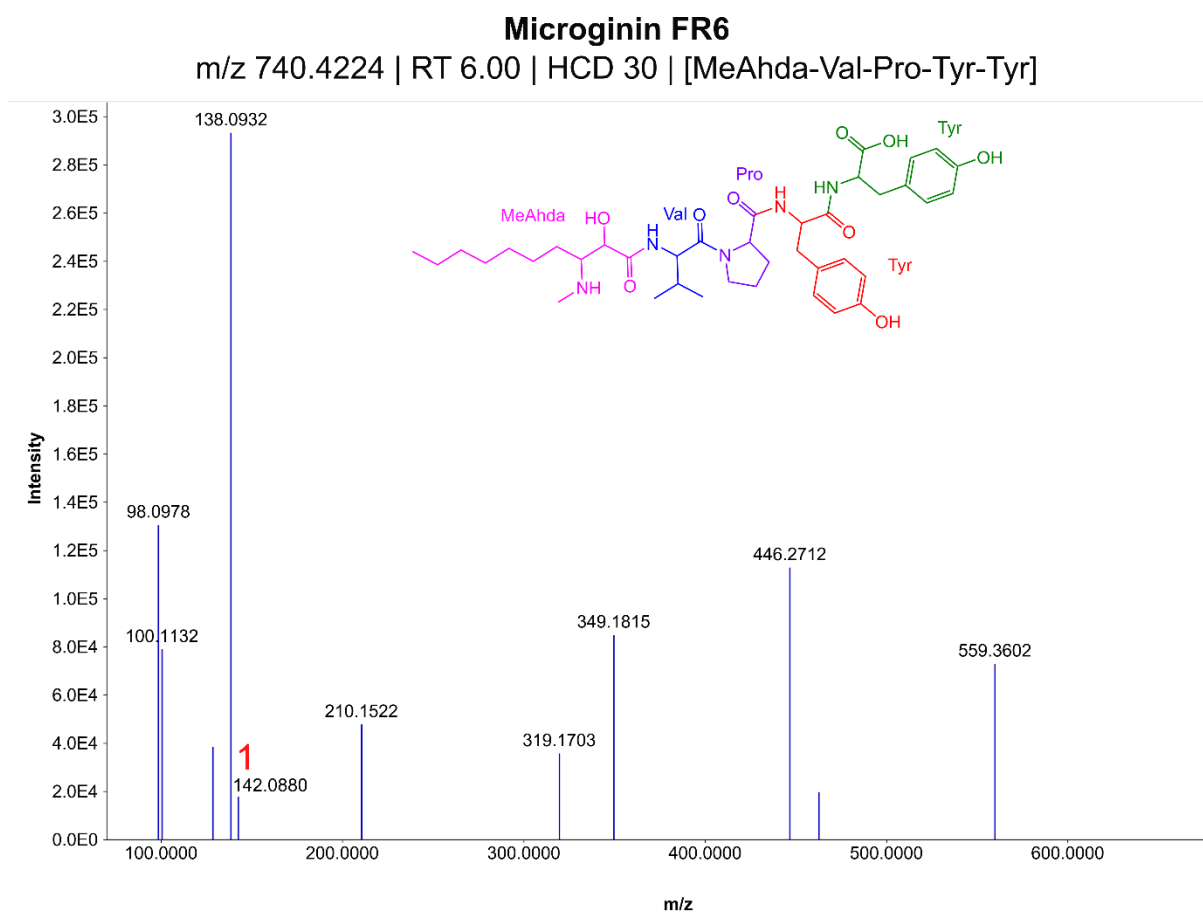
## Nostoginin BN741

m/z 742.4386 | RT 7.47 | HCD 30 | [Ahoa-Val-NMelle-NMeTyr-Tyr]



Fragment number	Calculated m/z	Peak m/z	Fragment
*	742.4373	742.4386	M+H
1	561.3652	561.3755	[M-Tyr] <sup>+</sup>
2	448.2811	448.2891	[Part of Ahoa (C <sub>2</sub> H <sub>2</sub> O <sub>2</sub> )-Val-MeLeu-MeTyr] <sup>+</sup>
3	136.0762	136.0774	Tyr immonium ion
4	131.1184	131.1196	Valine residue
5	100.1126	100.1131	Ahoa fragment or Melle immonium ion

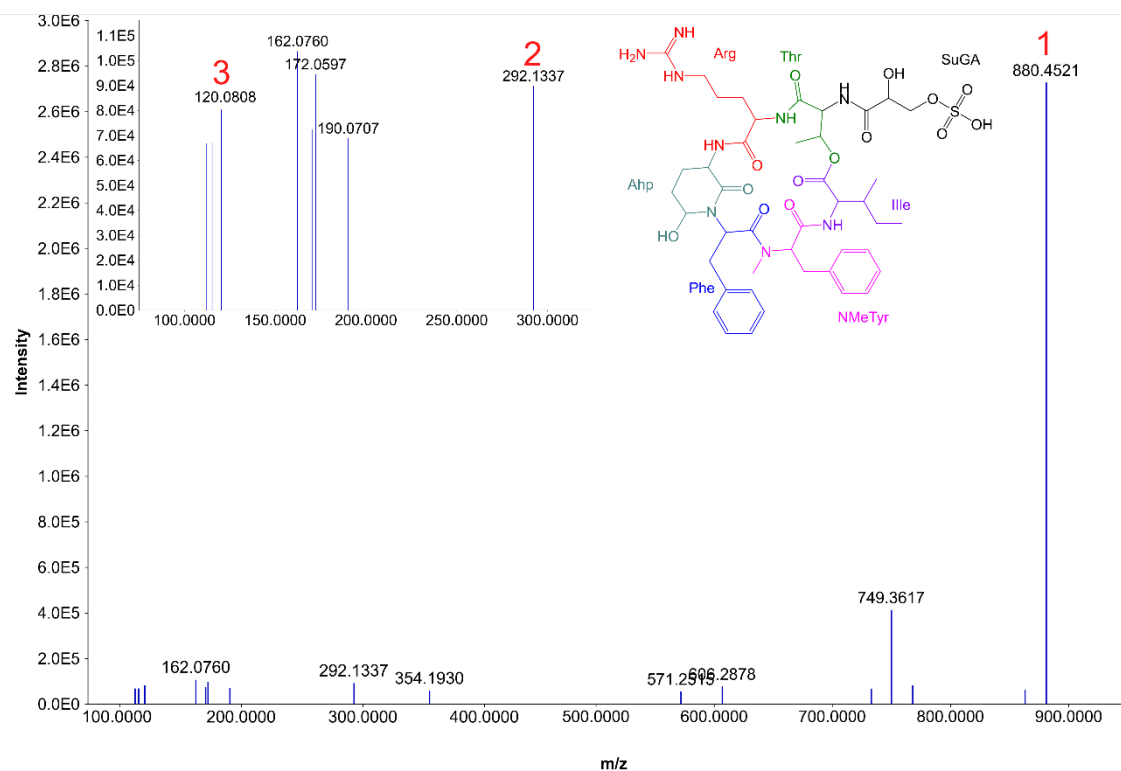
**Figure SI23.** MS/MS annotation at HCD30 for m/z 742.4386 and RT 7.47, matching with microginin variant Nostoginin BN741 (m/z 742.4373, C<sub>39</sub>H<sub>59</sub>N<sub>5</sub>O<sub>9</sub>, confidence level 2b based on MS<sup>2</sup> annotation) from the NPCD-01 extract. (\*) precursor ion peak not shown in the spectrum but visible with HCD15.



Fragment number	Calculated m/z	Peak m/z	Fragment
*	740.4229	740.4224	M+H
1	142.0882	142.0880	[MeAhda] + H+

**Figure SI24.** MS/MS annotation at HCD30 for *m/z* 740.4224 and RT 6.00, matching with microginin FR6 (*m/z* 740.4229, C<sub>39</sub>H<sub>57</sub>N<sub>5</sub>O<sub>9</sub>, level 3 as only few fragments could be annotated) from the NPCD-01 extract. (\*) precursor ion peak not shown in the spectrum but visible with HCD15.

**Cyanopeptolin 959**  
 $m/z$  960.4119 | RT 9.30 | HCD 30 & 45 | [Arg-Ahp-Phe-NMe-Tyr-Ile-Thr]-SuGA

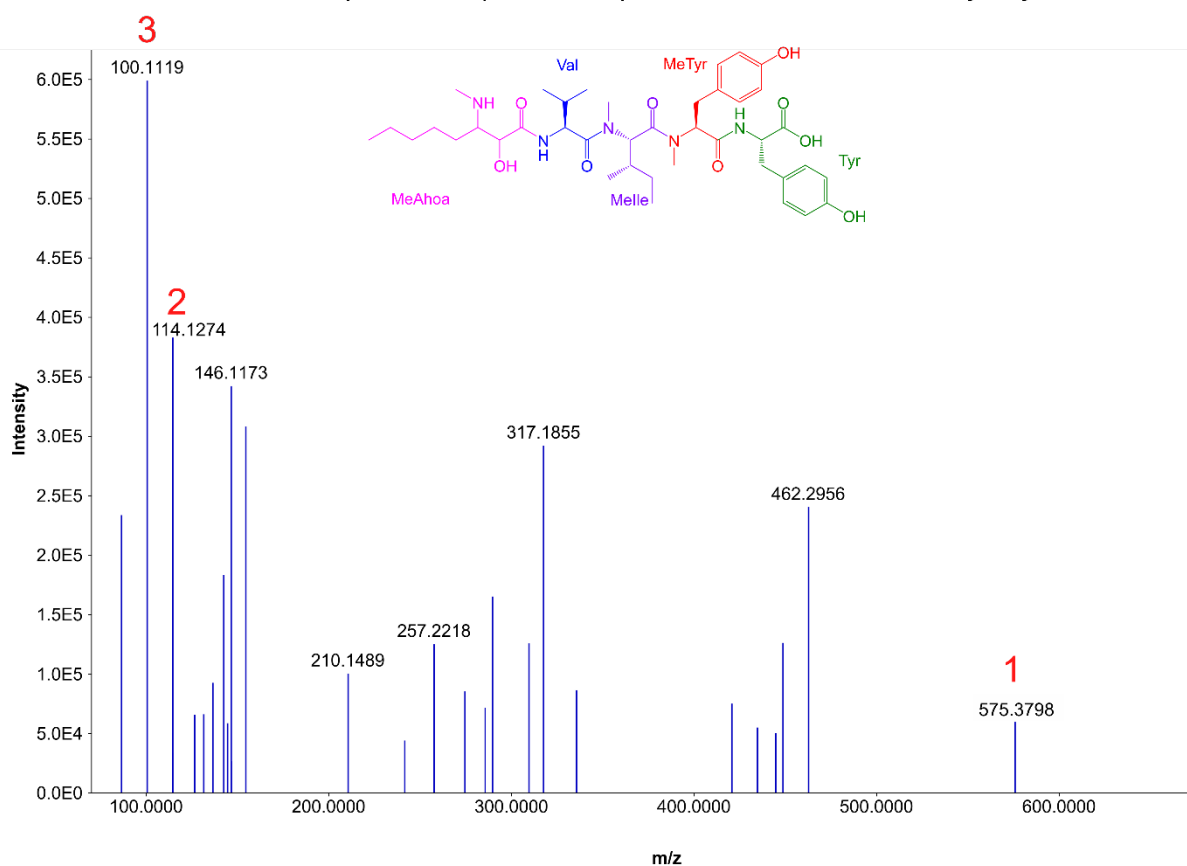


Fragment number	Calculated $m/z$	Peak $m/z$	Fragment
*	960.4131	960.4119	M+H
1	880.4563	880.4521	[M+H <sup>+</sup> ] - SO <sub>3</sub>
2	292.1333	292.1337	Phe-MeTyr - H <sub>2</sub> O
3	120.0808	120.0808	Phe immonium ion

**Figure SI25.** MS/MS annotation at HCD 30 and HCD45 (inset) for  $m/z$  960.4119 and RT 9.30, matching with cyanopeptolin 959 ( $m/z$  960.4131, C<sub>43</sub>H<sub>61</sub>N<sub>9</sub>O<sub>14</sub>S, level 2b based on MS<sup>2</sup> annotation) from the NPCD-01 extract. (\*) precursor ion peak not shown in the spectrum but visible with HCD15.

# Microginin SD755

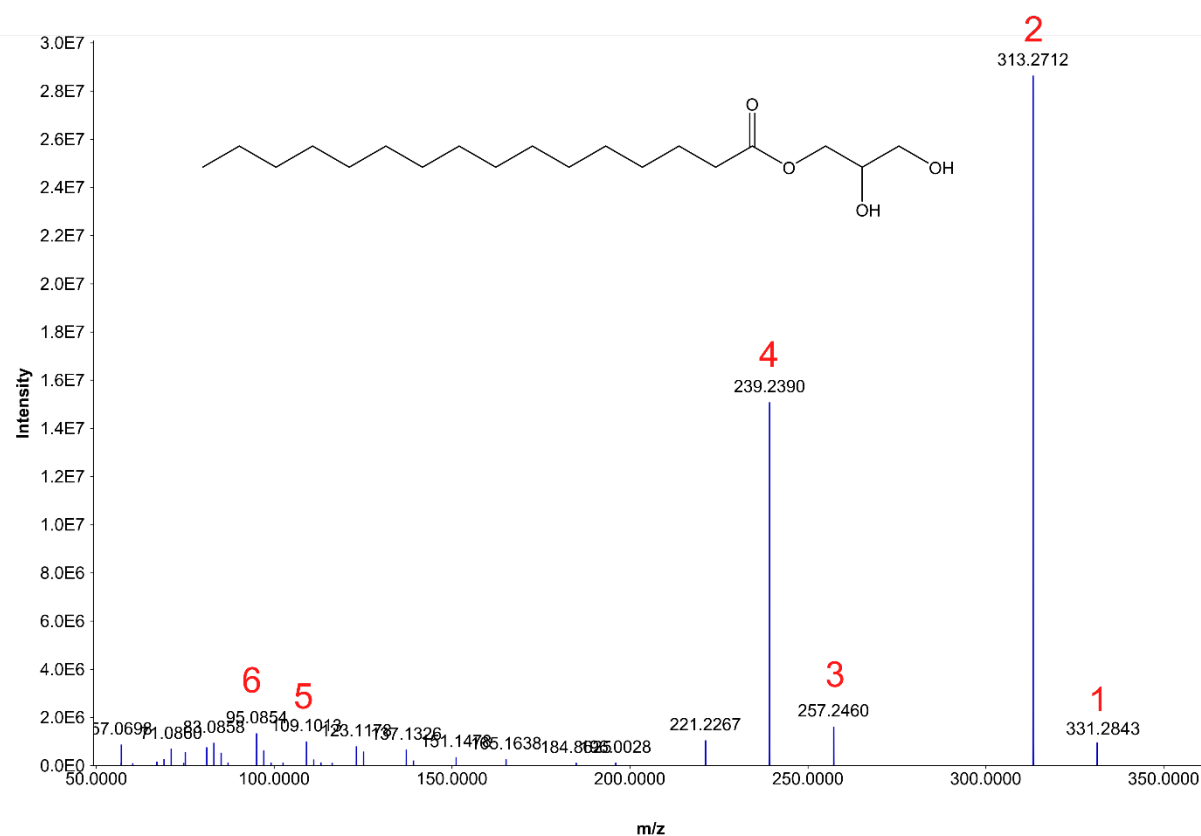
m/z 756.4528 | RT 9.24 | HCD 30 | MeAhoa-Val-Melle-MeTyr-Tyr



Fragment number	Calculated m/z	Peak m/z	Fragment
*	756.4542	756.4528	M+H
1	575.3809	575.3798	[M-Tyr] <sup>+</sup>
2	114.1278	114.1274	MeAhoa fragment
3	100.1120	100.1119	MeLeu immonium

**Figure SI26.** MS/MS annotation at HCD30 for *m/z* 756.4528 and RT 9.24, matching with microginin SD755 (*m/z* 756.4542, C<sub>40</sub>H<sub>61</sub>N<sub>5</sub>O<sub>9</sub>, confidence level 2b based on MS<sup>2</sup> annotation) from the NPCD-01 extract. (\*) precursor ion peak not shown in the spectrum but visible with HCD15.

**Glyceryl palmitate**  
 $m/z$  331.2843 | RT 8.13 | HCD 15



Fragment number	Calculated $m/z$	Peak $m/z$
1	331.2843	331.2843
2	313.2743	313.2712
3	257.2445	257.2460
4	239.2373	239.2390
5	109.1008	109.1009
6	95.0851	95.0851

**Figure S127.** MS/MS annotation at HCD15 for  $m/z$  331.2843 and RT 8.13, detected in the HPLC fractions #9 and # 10 (22 – 26 min) of the NPCD-01 extract, matching with glyceryl palmitate ( $m/z$  331.2843,  $C_{19}H_{38}O_4$ , confidence level 2a based on MS<sup>2</sup> annotation. Compound and spectra information were obtained in the MassBank of North America (MoNA ID CCMSLIB00000849055).