Supplementary information

Supplementary table 1

Chromatographic and exact mass spectrometric characterization of isolated cyclolinopeptides used for compound identification

Cyclolinopeptide	RT min	*m/z* found ($[M+H]^+$ / $[M+Na]^+$)	*m/z* calculated ($[M+H]^+$ / $[M+Na]^+$)	Δ mDa	Sum formula
CL1	13.767	1040.6544 / 1062.6355	1040.6549 / 1062.6368	-0.5 / -0.7	$C_{57}H_{86}N_9O_9$ / $C_{57}H_{85}N_9O_9Na$
1-Met-CL2	13.488	1058.6108 / 1080.5927	1058.6113 / 1080.5932	-0.5 / -0.5	$C_{56}H_{84}N_9O_9S$ / $C_{56}H_{83}N_9O_9SNa$
1-Mso-CL2	12.658	1074.6063 / 1096.5879	1074.6062 / 1096.5881	0.1 / -0.2	$C_{56}H_{84}N_9O_{10}S$ / $C_{56}H_{83}N_9O_{10}SNa$
1-Msn-CL2	12.865	1090.5998 / 1112.5823	1090.6011 / 1112.5830	-0.5 / -0.7	$C_{56}H_{84}N_9O_{11}S$ / $C_{56}H_{83}N_9O_{11}SNa$
1-Abu-CL3	13.817	1012.6234 / 1034.6052	1012.6236 / 1034.6055	-0.2 / -0.3	$C_{55}H_{82}N_9O_9$ / $C_{55}H_{81}N_9O_9Na$
1-Met-CL3	13.886	1048.5680 / 1070.5509	1048.5694 / 1070.5514	-1.4 / -0.5	$C_{57}H_{78}N_9O_8S$ / $C_{57}H_{77}N_9O_8SNa$
1-Mso-CL3	13.481	1064.5647 / 1086.5461	1064.5643 / ----------	0.4 / ----	$C_{57}H_{78}N_9O_9S$ / ----------
1-Met-CL4	13.680	961.5575 / 983.5388	961.5585 / 983.5405	-1.0 / -1.7	$C_{51}H_{77}N_8O_8S$ / $C_{51}H_{76}N_8O_8SNa$
1-Mso-CL4	12.792	977.5538 / 999.5355	977.5534 / 999.5354	0.4 / 0.1	$C_{51}H_{77}N_8O_9S$ / $C_{51}H_{76}N_8O_9SNa$
1-Msn-CL4	13.174	993.5482 / 1015.5298	993.5483 / 1015.5303	0.1 / -0.5	$C_{51}H_{77}N_8O_{10}S$ / $C_{51}H_{76}N_8O_{10}SNa$
1-Met,3-Met-CL5	13.981	1052.5114 / 1074.4926	1052.5102 / 1074.4921	1.2 / 0.5	$C_{55}H_{74}N_9O_8S_2$ / $C_{55}H_{73}N_9O_9S_2Na$
1-Met,3-Mso-CL5	13.270	1068.5060 / 1090.4869	1068.5051 / 1090.4870	0.9 / -0.1	$C_{55}H_{74}N_9O_9S_2$ / $C_{55}H_{73}N_9O_8S_2Na$
1-Mso,3-Mso-CL5	12.295	1084.4991 / 1106.4814	1084.5000 / 1106.4820	-0.9 / -0.6	$C_{55}H_{74}N_9O_{10}S_2$ / $C_{55}H_{73}N_9O_{10}S_2Na$
1-Met,3-Met-CL6	13.767	1066.5247 / 1088.5067	1066.5258 / 1088.5078	-1.1 / -1.1	$C_{56}H_{76}N_9O_8S_2$ / $C_{51}H_{76}N_8O_8S_2Na$
1-Mso,3-Met-CL6	13.460	1082.5210 / 1104.5029	1082.5207 / 1104.5027	0.3 / 0.2	$C_{56}H_{76}N_9O_9S_2$ / $C_{51}H_{76}N_8O_9S_2Na$
1-Mso,3-Mso-CL6	12.544	1098.5151 / 1120.4972	1098.5157 / 1120.4970	-0.6 / -0.4	$C_{56}H_{76}N_9O_{10}S_2$ / $C_{51}H_{76}N_8O_{10}S_2Na$

Supplementary table 2

LC-MS/MS properties of cyclolinopeptides in targeted quantification method. Retention time, mass transitions, linear range, R2, precision and accuracy values.

Cyclolinopeptide	Rt. (min)	Q1/Q3 (m/z)[a]	Linear range (µM)		R^2	Precision RSD (%)[b]	accuracy (%)[b]
CL1	6.13	1040.62→120.2/<u>70.2</u>[c]/245.2/217.2	0.09–5.6	y=1.11x-0.00048	0.996	4.3–8.2	88 – 101
1-Met-CL2	5.37	1058.53→120.1/<u>70.1</u>[c]/217.2/245.2	0.08–5.4	y=1.07x-0.00609	0.994	4.3–7.8	96 – 105
1-Mso-CL2	2.11	1074.55→120.2/<u>70.1</u>[c]/217.2/245.2	0.12–8.0	y=0.73x-0.0142	0.995	1.8–11.6	92 – 104
1-Msn-CL2	2.88	1090.55→120.2/<u>70.1</u>[c]/217.0/245.1	0.6–16.0	y=0.713x-0.0558	0.996	3.2–12.1	85 – 104
1-Met-CL3	7.09	1048.55→245.2/217.2/<u>70.1</u>[c]/120.1	0.05–3.1	y=0.0.689x+0.017	0.994	2.3–17.3	86 – 105
1-Mso-CL3	3.35	1064.66→217.2/245.2/<u>70.2</u>[c]/120.2	0.09–5.6	y=0.102x-0.00031	0.997	3.9–11.9	90 – 104
1-Met-CL4	5.97	961.48→<u>70.1</u>[c]/211.1/358.2/183.2	0.05–3.4	y=0.953x+0.00023	0.996	2.1–8.8	92 – 107
1-Mso-CL4	2.63	977.54→<u>70.1</u>[c]/120.2/183.2/211.2	0.09–5.6	y=1.42x-0.00784	0.996	2.2–11.1	90 – 114
1-Msn-CL4	3.64	993.57→<u>70.0</u>[c]/120.1/183.2/211.1	0.2–16.0	y=0.477x+0.00248	0.995	1.4–10.8	90 – 110
1-Met,3-Met-CL5[d]	5.33	1052.57→<u>336.2</u>[c]/352.3/70.0/189.2					
1-Met,3-Mso-CL5[d]	3.18	1068.35→<u>217.2</u>[c]/245.3/120.1/70.1	0.15–2.3	y=0.175x-0.066	0.974	0.7–15.7	97 – 103
1-Mso,3-Mso-CL5[d]	1.43	1084.63→217.3/245.1/<u>70.2</u>[c]/120.0	0.02–0.3	y=0.549x-0.0334	0.965	6.7–13.9	89 – 118
1-Met,3-Met-CL6	6.66	1066.50→217.2/245.1/<u>70.1</u>[c]/120.0	0.07–4.4	y=0.401x+0.000057	0.992	2.0–19.0	94 – 110
1-Mso,3-Met-CL6	3.84	1082.65→245.2/217.2/<u>70.1</u>[c]/120.2	0.1–6.6	y=0.262x+0.00056	0.995	2.0–19.1	97 - 112
1-Mso,3-Mso-CL6	1.63	1098.67→217.2/245.2/<u>70.2</u>[c]/120.2	0.2–2.7	y=0.478x+0.00725	0.997	1.3–20.0	98 - 108
IS: 1-Abu-CL2	4.35	1012.62→<u>70.0</u>[c]/120.0/217.3/245.3	-	-	-	-	-

[a] dwell time 15 msec. [b] linear range: <20% RSD. 80-120% accuracy. [c] Quantifier. [d] quantified using 1-Met-3-Met-CL6

Supplementary table 3

Precision of the targeted LC-MS/MS method in authentic linseed oil sample.

Cyclolinopeptide	Precision in linseed oil (% RSD)	
	Fresh	aged
CL1	7.4	7.4
1-Met-CL2	5.2	-
1-Mso-CL2	2.9	3.2
1-Msn-CL2	3.3	5.8
1-Met-CL3	5.9	-
1-Mso-CL3	16.8	8.1
1-Met-CL4	8.7	-
1-Mso-CL4	3.1	5.0
1-Msn-CL4	3.4	8.5

1-Met,3-Met-CL5	8.0	-
1-Met,3-Mso-CL5	13.0	-
1-Mso,3-Mso-CL5	22.5	-
1-Met,3-Met-CL6	3.8	-
1-Mso,3-Met-CL6	10.2	-
1-Mso,3-Mso-CL6	25.6	7.1

[a] n=3

Supplementary table 4

1H/ 13C NMR shifts of 1-Met, 3-Met-CL6 (signal assignment was performed based on 2D H,H correlated experiment COSY, TOCSY and ROESY, 2D H,C correlated experiments HSQC and HMBC as well as a 2D H,N correlated experiment HSQC.

	^{1}H-NMR (COSY, TOCSY, ROESY)				^{13}C- NMR (HSQC,HMBC)		^{1}H-NMR (COSY, TOCSY, ROESY)				^{13}C- NMR (HSQC,HMBC)
	δ [ppm]	I	M	*J* [Hz]	δ [ppm]		δ [ppm]	I	M	*J* [Hz]	δ [ppm]
Pro[1]						Ile[5]					
α	4.16	1	t	7.8	62.6	α	4.27	1	m		58.3
β	1.96	1	m		29.4	β	1.80	1	m		37.3
	1.67	1	m			γ	1.40	1	m		25.1
γ	1.93	1	m		25.1		1.12	1	m		
δ	3.63	1	m		47.6	γ_{CH3}	0.87	3	d	7.6	15.9
	3.57	1	m			δ_{CH3}	0.85	3	m		11.8
C=O					173.7	NH	7.58	1	bs		
						C=O					172.21
Phe[2]											
α	4.10	1	m		56.8	Met[6]					
β	2.98	1	dd	14.0/10.1	35.8	α	4.09	1	m		53.9
	2.88	1	dd	14.0/4.8		β	2.06	1	m		30.2
γ					138.6		1.84	1	m		
δ	7.24	1	m		129.1	γ	2.36	2	m		30.6
ε	7.23-7.29		m		128.6	ε_{CH3}	2.00	3	s		15.0
ζ	7.16-7.23		m		126.7	NH	7.94	1	bs		
NH	8.23	1	d	6.4		C=O					171.9
C=O					170.8						
						Leu[7]					
Phe[3]						α	3.72	1	m		53.4
α	4.60	1	m		54.1	β	1.88	1	m		38.1
β	3.29	1	m		37.0		1.60	1	m		
	2.76	1	m			γ	1.55	1	m		25.0
γ					138.8	δ_{CH3}	0.87	3	d	6.4	23.5
δ	7.00	1	d	7.4	129.2		0.82	3	d	6.4	21.5
ε	7.23-7.29	1	m		128.6	NH	8.05	1	d	6.8	
ζ	7.16-7.23	1	m		126.7	C=O					171.7
NH	7.87	1	d	8.6							
C=O					171.6	Met[8]					
						α	4.79	1	dt	9.2/3.7	50.2
Trp[4]						β	2.08	1	m		32.3
α	4.17	1	m		56.5		1.86	1	m		
β	3.29	2	m		25.0	γ	2.48	2	m		30.3
^{1}NH	10.57	1	s			ε_{CH3}	2.02	3	s		15.5
2	7.23	1	s		123.5	NH	7.34	1	bs		
3					110.9	C=O					171.4
4	7.44	1	d	7.9	118.3						
5	6.95	1	t	7.9	118.7						
6	7.04	1	t	7.9	121.3						
7	7.32	1	d	7.9	111.8						
8					136.5						
9					127.9						
NH	8.33	1	bs								
C=O					172.8						