Electronic Supplementary Material (ESI) for ChemComm.
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Supporting information

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List of the toxins

Table S1. List of the toxins used in the study and their characteristics

N°	Name	Sequence	Cysteine pattern	Cysteine pairing	M ($g.mol^{-1}$)	pI	GRAVY Index
1	Alpha-conotoxin GI	ECCNPACGRHYSC*	CC-C-C	1-3, 2-4	1437.63	7.59	-0.54
2	Alpha-conotoxin MI	GRCCHPACGKNYSC*	CC-C-C	1-3, 2-4	1493.74	8.36	-0.56
3	Alpha-conotoxin SI	ICCNPACGPKYSC*	CC-C-C	1-3, 2-4	1353.63	8.01	0.25
4	Alpha-conotoxin BuIA	GCCSTPPCAVLYC*	CC-C-C	1-3, 2-4	1311.59	7.58	1.03
5	Alpha-conotoxin PnIB	GCCSLPPCALSNPDYC*	CC-C-C	1-3, 2-4	1637.90	5.69	0.27
6	Rho-conotoxin TIA	FNWRCCLIPACRRNHKKFC*	CC-C-C	1-3, 2-4	2390.92	10.53	-0.44
7	Alpha-conotoxin-EI	RDOCCYHPTCNMSNPQIC*	CC-C-C	1-3, 2-4	2093.41	7.59	-0.72
8	Alpha-conotoxin CnIB	CCHPACGKYYSC*	CC-C-C	1-3, 2-4	1329.57	8.01	-0.06
9	Alpha-conotoxin GIA	ECCNPACGRHYSCGK*	CC-C-C	1-3, 2-4	1622.85	8.01	-0.75
10	Alpha-conotoxin-like MIA	DGRCCHPACAKHFNC*	CC-C-C	1-3, 2-4	1656.92	8.01	-0.49
11	Alpha-conotoxin-like MIB	NGRCCHPACARKYNC*	CC-C-C	1-3, 2-4	1690.98	8.77	-0.85
12	Alpha-conotoxin GII	ECCHPACGKHFSC*	CC-C-C	1-3, 2-4	1416.65	7.61	-0.15
13	Alpha-conotoxin PIB	ZSOGCCWNPACVKNRC*	CC-C-C	1-3, 2-4	1760.04	8.18	-0.51
14	Alpha-conotoxin-like Reg2a	GCCSHPACNVNNPHIC*	CC-C-C	1-3, 2-4	1663.91	7.61	-0.05
15	Alpha-conotoxin CnIA	GRCCHPACGKYYSC*	CC-C-C	1-3, 2-4	1542.81	8.36	-0.40
16	Chi-conotoxin MrIA	NGVCCGYKLCHOC	CC-C-C	1-4, 2-3	1408.67	7.59	0.28
17	Chi-conotoxin MrIB	VGVCCGYKLCHOC	CC-C-C	1-4, 2-3	1396.58	7.59	0.88
18	XEN2174	ZGVCCGYKLCHOC*	CC-C-C	1-4, 2-3	1404.68	7.71	0.73
19	Chi-conotoxin CMrX	GICCGVSFCYOC	CC-C-C	1-4, 2-3	1266.45	5.56	1.42
20	Chi-conotoxin-like 2	ZTCCGYRMCVOC*	CC-C-C	1-4, 2-3	1360.49	7.70	0.34
21	Chi-conotoxin-like Ar1248	GVCCGVSFCYOC	CC-C-C	1-4, 2-3	1252.44	5.56	1.39
22	Chi-conotoxin-like Ar1311	RCCGYKMCHOC	CC-C-C	1-4, 2-3	1315.48	8.01	-0.27
23	Chi-conotoxin CMrVIA	VCCGYKLCHOC	CC-C-C	1-4, 2-3	1240.49	7.59	0.69
24	Conotoxin mr1e	CCHSSWCKHLC	CC-C-C	1-4, 2-3	1305.49	7.61	0.09
25	Apamin	CNCKAPETALCARRCQQH*	C-C-C-C	1-3, 2-4	2027.37	8.36	-0.73
26	Mast cell degranulating peptide	IKCNCKRHVIKPHICRKICGKN*	C-C-C-C	1-3, 2-4	2587.25	10.71	-0.53
27	Tertiapin	ALCNCNRIIIPHMCWKKCGKK*	C-C-C-C	1-3, 2-4	2455.11	10.09	-0.10
28	Alpha-conotoxin Pu14,1	DCPPHPVPGMHKCVCLKTC	C-C-C-C	1-3, 2-4	2061.53	7.61	-0.06
29	Alpha-conotoxin-like ts14a	DGCPPHPVPGMHPCMCTNTC	C-C-C-C	1-3, 2-4	2093.47	6.02	-0.28
30	Alpha/kappa-conotoxin pl14a	FPRPRICNLACRAGIGHKYPFCHCR*	C-C-C-C	1-3, 2-4	2911.51	10.49	-0.27
31	Sarafotoxin-D	CTCKDMTDKECLYFCHQDIIW	C-C-C-C	1-4, 2-3	2594.08	4.48	-0.22
32	Sarafotoxin-C	CTCNDMTDEECLNFCHQDVIW	C-C-C-C	1-4, 2-3	2517.94	3.61	-0.30
33	Sarafotoxin-A, Thr-isoform	CSCKDMTDKECLNFCHQDVIW	C-C-C-C	1-4, 2-3	2517.03	4.48	-0.34
34	Sarafotoxin-A, Ser-isoform	CSCKDMSDKECLNFCHQDVIW	C-C-C-C	1-4, 2-3	2503.01	4.48	-0.35
35	Bibrotoxin	CSCADMTDKECLYFCHQDVIW	C-C-C-C	1-4, 2-3	2508.99	4.06	0.03
36	Potassium channel toxin κ-KTx 1,1	GHACYRNCWREGNDEETCKERC*	C-C-C-C	1-4, 2-3	2657.07	6.75	-1.68

* C-terminal amidation

Solid-phase peptide synthesis

Fmoc protected amino acids and HCTU were purchased at Activotec (Cambridge, UK). Wang-PS functionalized resins preloaded with protected amino acid were purchased from Merck (Darmstadt, Germany). N-methylmorpholine (NMM) and acetic anhydride (Ac_2O) were obtained from Sigma Aldrich (St Quentin en Fallavier, France). N-methyl-2-pyrrolidone (NMP)

The toxins were synthesized on a 50 µmol scale using Fmoc chemistry on a Prelude synthesizer (Protein Technologies®) using Wang-PS resin functionalized with the appropriate protected amino acid (AA) or a ChemMatrix® Rink Amide. Each coupling step was carried out twice 3 min using a mixture 5 eq./5 eq./10 eq. Fmoc-AA/HCTU/NMM in NMP (0.1 M final) followed by a capping step of 5 min with a solution of Ac_2O/NMM 1/1 v/v in NMP (2.5 mL). Fmoc deprotection was performed twice 2 min using 2 mL of a 20% piperidine solution in NMP.

After concomitant cleavage from the resin and amino acid side chains deprotection using TFA/H_2O/TIS 90/5/5 solution, the crude peptide was precipitated in cold Et_2O followed by a centrifugation (2000 rpm) and the removal of the supernatant. The precipitate was washed twice following the same procedure and lyophilized from 15 mL of 10% acetic acid solution.

Linear peptide purities were analysed using a Waters Alliance e2695 system on a reverse phase column Waters XBridge C18 BEH300Å using a gradient from 95/5 A/B (A=H_2O, 0.1% TFA and B=CH_3CN, 0.1% TFA) to 50/50 in 20 minutes. An example of chromatogram obtained is represented in Fig. S1. Purities and MALDI TOF MS results are reported in Table S2.

Table S2. Results obtained for the crude linear peptide. The method used for the HPLC analyses is 95/5 to 50/50 A/B in 20 min.

N°	HPLC purity	MALDI TOF MS $[M+H]^+$ obtained	MALDI TOF MS $[M+H]^+$ theoretical	N°	HPLC Purity	MALDI TOF MS $[M+H]^+$ obtained	MALDI TOF MS $[M+H]^+$ theoretical
1	86%	1441.48	1441.52	**19**	84%	1267.23	1267.46
2	81%	1497.58	1497.60	**20**	80%	1361.22	1361.49
3	92%	1357.54	1357.55	**21**	75%	1253.41	1253.45
4	88%	1315.58	1315.53	**22**	84%	1316.47	1316.48
5	76%	1641.76	1641.65	**23**	85%	1241.47	1241.49
6	82%	2394.17	2394.18	**24**	94%	1306.50	1306.49
7	78%	2096.84	2096.82	**25**	88%	2030.92	2030.92
8	75%	1333.48	1333.49	**26**	86%	2490.31	2590.43
9	79%	1626.64	1626.64	**27**	81%	2459.15	2459.26
10	80%	1660.67	1660.67	**28**	83%	2064.78	2064.93
11	80%	1694.68	1694.72	**29**	85%	2096.66	2096.79
12	76%	1420.54	1420.54	**30**	93%	2915.36	2914.46
13	81%	1763.78	1763.72	**31**	81%	2594.98	2595.08
14	76%	1667.67	1667.67	**32**	80%	2518.91	2518.94
15	75%	1546.66	1546.62	**33**	75%	2517.98	2518.03
16	88%	1412.55	1412.56	**34**	82%	2503.93	2504.02
17	83%	1397.58	1397.58	**35**	78%	2509.94	2509.99
18	89%	1408.56	1408.56	**36**	76%	2657.97	2658.08

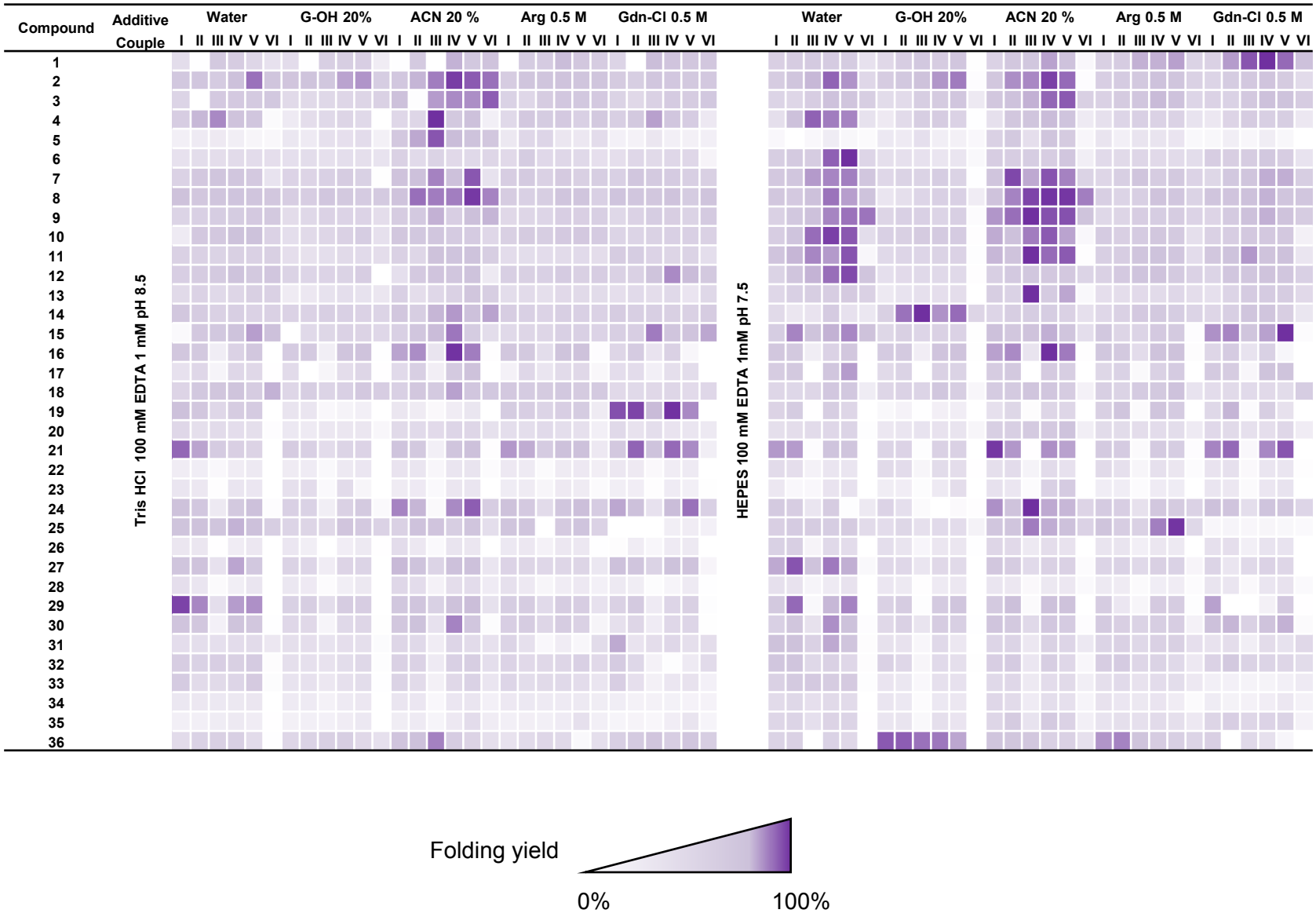
Screening of folding conditions

Redox couples / Additives	GSH/GSSG 1 mM/0.1 mM	GSH/GSSG 1 mM/1 mM	GSH/GSSG 0.1 mM/1 mM	Cys/C-C 1 mM/0.1 mM	Cys/C-C 0.1 mM/1 mM	Water	GSH/GSSG 1 mM/0.1 mM	GSH/GSSG 1 mM /1 mM	GSH/GSSG 0.1 mM/1 mM	Cys/C-C 1 mM/0.1 mM	Cys/C-C 0.1 mM/1 mM	Water
Water	78.3	71.5	74.5	75.5	74.6	71.5	71.9	68.8	79.9	90.8	84.8	77.3
GOH 20 %	64.0	64.1	72.4	69.6	66.4	67.5	33.8	41.6	53.8	60.3	60.2	24.8
ACN 20%	76.4	91.3	89.5	89.2	98.9	88.5	76.7	88.6	97.2	99.6	99.1	89.3
Arg 0,5M	65.0	59.8	63.3	74.6	74.7	59.9	61.5	55.6	61.6	76.4	72.5	56.8
Gdn-Cl 0,5M	75.6	72.7	72.1	76.6	65.9	75.8	73.7	75.0	79.3	82.0	74.5	72.7
Buffer	TRIS HCl 100 mM EDTA 1mM pH 8.5						HEPES 100 mM EDTA 1mM pH 7.5					

Fig. S1 Well-plate scheme including the different conditions analysed and the yields obtained for the α-conotoxin S1 (**3**) as example.

For each peptide, 60 folding conditions were screened, each in a volume of 1.2 mL in a 96 well-plate including different buffers, additives and redox couples at 4°C during 48 hours. All the combinations are reported in the well-plate scheme in Fig. S1. The final peptide concentration was of 20µM. After acidification of each well using 120 µL of a 20% TFA solution, 200 µL of each folding solution were analysed using HPLC with adapted methods of 10 min (see table S3). The folding yields were determined by comparison of the area of the folded peak and with the reference reduced peptide at a wavelength of 210 nm.

Table S3. Results obtained for the folding conditions screening (Redox couples: I=GSH/GSSG 1/0.1 mM, II= GSH/GSSG 1/1 mM, III= GSH/GSSG 0.1/1 mM, IV=Cysteine/Cystine 1/0.1 mM, V= Cysteine/Cystine 0.1/1 mM, VI= no redox couple)



Statistical analysis

Hierarchical Ascending Analysis (HCA)

The analysis was carried out on the different folding conditions using XLSTAT (Addinsoft®). The conditions were classified using dissimilarity (Euclidian distance) and the Ward method for the aggregation. Results are summarized in fig. S2 and constitution of the three groups A, B and C in the tables S4, S5 and S6 respectively.

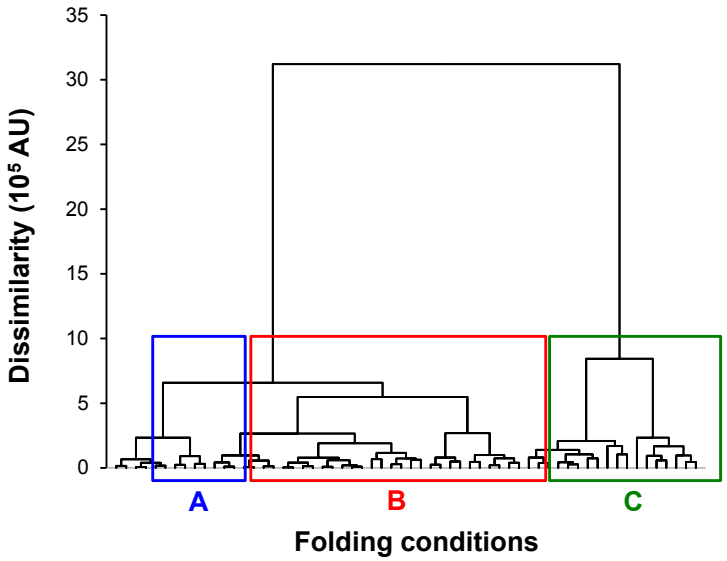


Fig. S2 Dendogram representing the HCA results.

Table S4. Constitution of the group A determined by HCA.

Buffer	Additive	Redox couple
Tris-Cl 100 mM EDTA 1 mM pH 8.5	ACN 20%	Cys/C-C 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	ACN 20%	Cys/C-C 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	ACN 20%	GSH/GSSG 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	ACN 20%	GSH/GSSG 1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	ACN 20%	Cys/C-C 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	ACN 20%	Cys/C-C 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	No (water)	GSH/GSSG 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	No (water)	GSH/GSSG 1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	No (water)	Cys/C-C 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	No (water)	Cys/C-C 0.1 mM/1 mM

Table S5. Constitution of the group B determined by HCA.

Buffer	Additive	Redox couple
Tris-Cl 100 mM EDTA 1 mM pH 8.5	ACN 20%	GSH/GSSG 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	ACN 20%	GSH/GSSG 1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Arg 0.5 M	GSH/GSSG 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Arg 0.5 M	GSH/GSSG 1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Arg 0.5 M	GSH/GSSG 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Arg 0.5 M	Cys/C-C 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Arg 0.5 M	Cys/C-C 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Arg 0.5 M	GSH/GSSG 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Arg 0.5 M	GSH/GSSG 1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Arg 0.5 M	Cys/C-C 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Arg 0.5 M	Cys/C-C 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Gdn-Cl 0.5 M	GSH/GSSG 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Gdn-Cl 0.5 M	GSH/GSSG 1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Gdn-Cl 0.5 M	GSH/GSSG 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Gdn-Cl 0.5 M	Cys/C-C 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Gdn-Cl 0.5 M	Cys/C-C 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Gdn-Cl 0.5 M	GSH/GSSG 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Gdn-Cl 0.5 M	GSH/GSSG 1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Gdn-Cl 0.5 M	Cys/C-C 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Gdn-Cl 0.5 M	Cys/C-C 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	G-OH 20%	GSH/GSSG 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	G-OH 20%	GSH/GSSG 1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	G-OH 20%	Cys/C-C 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	G-OH 20%	Cys/C-C 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	G-OH 20%	GSH/GSSG 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	G-OH 20%	GSH/GSSG 1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	G-OH 20%	Cys/C-C 1 mM/0.1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	G-OH 20%	Cys/C-C 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	No (water)	GSH/GSSG 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	No (water)	GSH/GSSG 1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	No (water)	Cys/C-C 1 mM/0.1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	No (water)	Cys/C-C 0.1 mM/1 mM

Table S6. Constitution of the group C determined by HCA.

Buffer	Additive	Redox couple
Tris-Cl 100 mM EDTA 1 mM pH 8.5	ACN 20%	GSH/GSSG 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	ACN 20%	No (water)
HEPES 100 mM EDTA 1 mM pH 7.5	ACN 20%	GSH/GSSG 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	ACN 20%	No (water)
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Arg 0.5 M	No (water)
HEPES 100 mM EDTA 1 mM pH 7.5	Arg 0.5 M	GSH/GSSG 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Arg 0.5 M	No (water)
Tris-Cl 100 mM EDTA 1 mM pH 8.5	Gdn-Cl 0.5 M	No (water)
HEPES 100 mM EDTA 1 mM pH 7.5	Gdn-Cl 0.5 M	GSH/GSSG 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	Gdn-Cl 0.5 M	No (water)
Tris-Cl 100 mM EDTA 1 mM pH 8.5	G-OH 20%	GSH/GSSG 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	G-OH 20%	No (water)
HEPES 100 mM EDTA 1 mM pH 7.5	G-OH 20%	GSH/GSSG 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	G-OH 20%	No (water)
Tris-Cl 100 mM EDTA 1 mM pH 8.5	No (water)	GSH/GSSG 0.1 mM/1 mM
Tris-Cl 100 mM EDTA 1 mM pH 8.5	No (water)	No (water)
HEPES 100 mM EDTA 1 mM pH 7.5	No (water)	GSH/GSSG 0.1 mM/1 mM
HEPES 100 mM EDTA 1 mM pH 7.5	No (water)	No (water)

Yields distribution per group

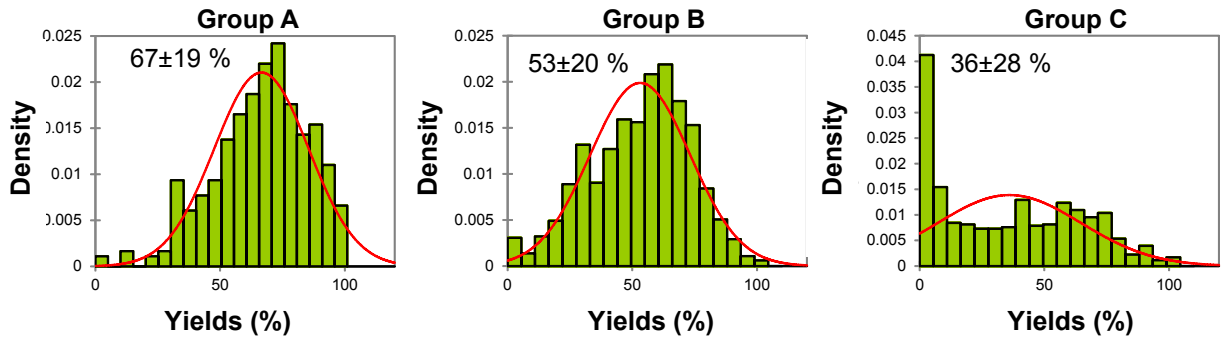


Figure S3. Yields distribution within each group. The dispersion was determined using a XLSTAT (Addinsoft®)

Protocol for the folding of the toxins using optimal condition

At 4°C, 2 mL of a solution of 300 mM HEPES with 3 mM EDTA, 2 mL of a solution of 60 % ACN and 1 ml of a freshly prepared solution of 6 mM / 0.6 mM Cysteine/Cystine (1mL) were mixed with 1 mL of the linear peptide solution in water at 120 µM. All the solutions used were previously degased using Argon bubbling during 30 minutes. The folding solution was conserved at 4°C for 48 h and then acidified using 600 µL of 20% TFA solution. The resulted solution was diluted to 15 mL and loaded on a homemade C18 SPE cartridge containing 2 mL of C18 silica (Sigma Aldrich, Saint Quentin en Fallavier, France). The SPE cartdridge was washed with 6 mL of water 0.1% TFA, twice 6 mL of milliQ water and the compound was eluted using 6 mL of a solution of 50 % CAN. The resulted solution was lyophilized and dissolved in milliQ water for HPLC and MS analyses. The final concentration and therefore the yield were determined either by UV spectroscopy or amino acid composition analysis (Table S7).

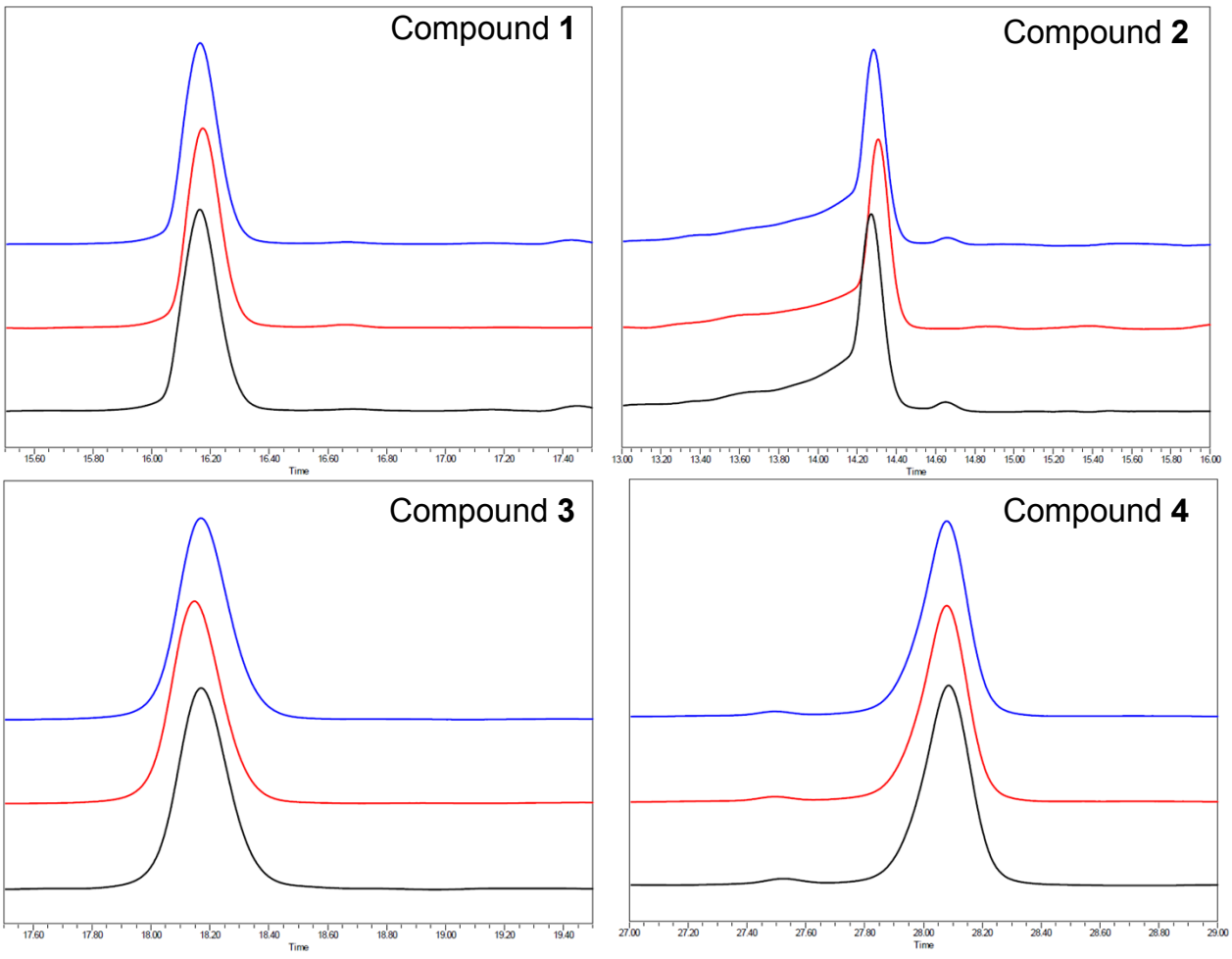
Table S7. Results obtained for the different toxins including retention times of the reduced and oxidized forms, the yields obtained for the optimal condition (100 mM HEPES, 1 mM EDTA pH 7.5, 20% ACN and 1 mM/0.1 mM Cysteine/Cystine) and the MALDI TOF MS.

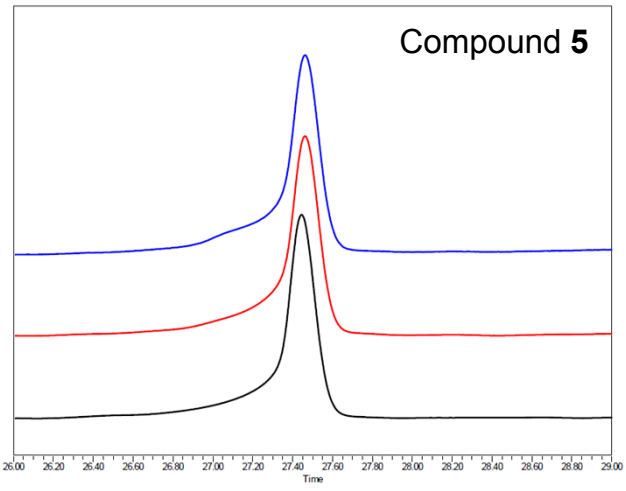
N°	t_R reduced (min)	t_R oxidized (min)	Method	Yield (optimal condition)	MALDI TOF MS obtained	MALDI TOF MS theoretical
1	8.6	9.2	90/10 to 80/20 A/B in 10 min	84%	1437.51	1437.49
2	6.4	7.8	90/10 to 80/20 A/B in 10 min	98%	1493.57	1493.57
3	5.8	6.5	85/15 to 75/25 A/B in 10 min	92%	1353.47	1353.52
4	4.5	3.4	70/30 to 60/40 A/B in 10 min	80%	1311.52	1311.50
5	6.6	9.9	80/20 to 70/30 A/B in 10 min	75%	1637.58	1637.62
6	5.8	6.7	80/20 to 70/30 A/B in 10 min	64%	2390.19	2390.15
7	4.5	6.7	85/15 to 75/25 A/B in 10 min	95%	2092.70	2092.79
8	3.7	4.1	85/15 to 75/25 A/B in 10 min	99%	1329.50	1329.46
9	3.2	4.1	85/15 to 75/25 A/B in 10 min	95%	1622.65	1622.60
10	6.1	8.0	85/15 to 75/25 A/B in 10 min	94%	1656.66	1656.64
11	6.9	7.5	90/10 to 80/20 A/B in 10 min	92%	1690.72	1690.69
12	4.2	6.9	85/15 to 75/25 A/B in 10 min	70%	1416.50	1416.51
13	9.2	7.6	85/15 to 75/25 A/B in 10 min	66%	1759.71	1759.69
14	7.0	4.2	85/15 to 75/25 A/B in 10 min	65%	2663.63	1663.63
15	6.7	7.0	85/15 to 75/25 A/B in 10 min	82%	1542.63	1542.59
16	8.0	6.8	85/15 to 75/25 A/B in 10 min	97%	1408.62	1408.53
17	8.7	7.5	85/15 to 75/25 A/B in 10 min	67%	1393.44	1393.55
18	8.3	7.2	85/15 to 75/25 A/B in 10 min	73%	1404.59	1404.53
19	6.1	4.0	75/25 to 65/35 A/B in 10 min	53%	1263.37	1263.43
20	5.1	4.6	80/20 to 70/30 A/B in 10 min	69%	1357.38	1357.46
21	6.4	4.6	77/23 to 67/33 A/B in 10 min	87%	1249.39	1249.41
22	7.6	6.6	88/12 to 78/22 A/B in 10 min	47%	1312.38	1312.45
23	7.0	7.2	85/15 to 75/25 A/B in 10 min	60%	1237.38	1237.46
24	5.2	6.3	75/25 to 65/35 A/B in 10 min	83%	1302.37	1302.46
25	6.5	7.5	90/10 to 80/20 A/B in 10 min	83%	2026.76	2026.89
26	6.8	6.0	85/15 to 75/25 A/B in 10 min	44%	2586.32	2586.40
27	5.7	3.7	77/23 to 67/33 A/B in 10 min	59%	2454.11	2454.23
28	6.4	4.4	83/17 to 73/27 A/B in 10 min	30%	2060.75	2060.90
29	6.6	5.9	83/17 to 73/27 A/B in 10 min	79%	2092.61	2092.76
30	8.9	9.4	80/20 to 70/30 A/B in 10 min	71%	2910.34	2910.43
31	10.0	7.0	75/25 to 65/35 A/B in 10 min	58%	2590.93	2591.05
32	7.4	4.6	75/25 to 65/35 A/B in 10 min	62%	2514.86	2514.91
33	7.1	4.1	75/25 to 65/35 A/B in 10 min	44%	2513.87	2514.00
34	7.1	4.1	75/25 to 65/35 A/B in 10 min	43%	2499.87	2499.97
35	4.9	3.2	70/30 to 60/40 A/B in 10 min	68%	2505.24	2505.96
36	5.6	3.8	85/15 to 75/25 A/B in 10 min	59%	2653.91	2654.05

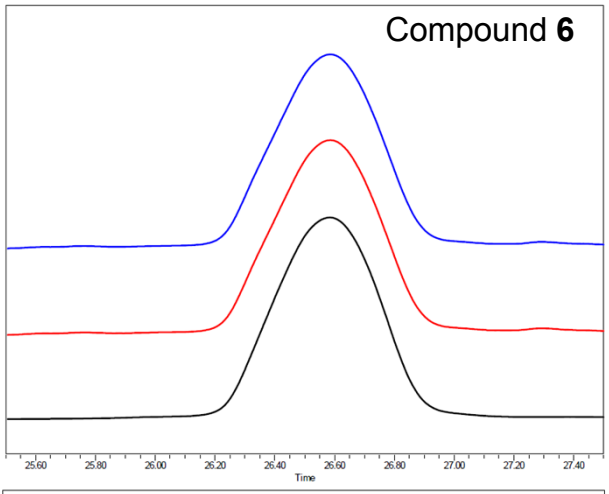
Characterization of the toxins

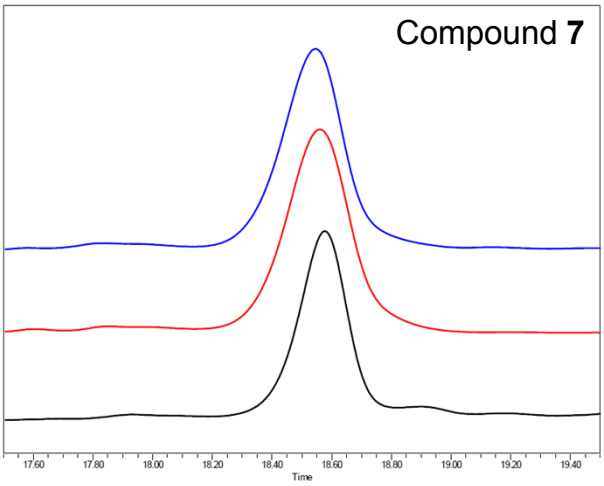
HPLC co-elution

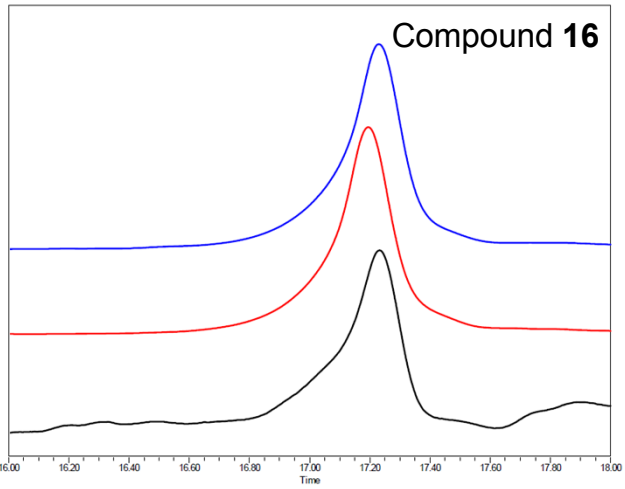
Method. 100 µL of a solution of the synthesized toxin (black curve), its commercial form (red curve) and the mix of both of them all at a 20 µM concentration were analysed in HPLC using a gradient 95/5 to 50/50 A/B in 45 min using the same instrument and the same column presented above.

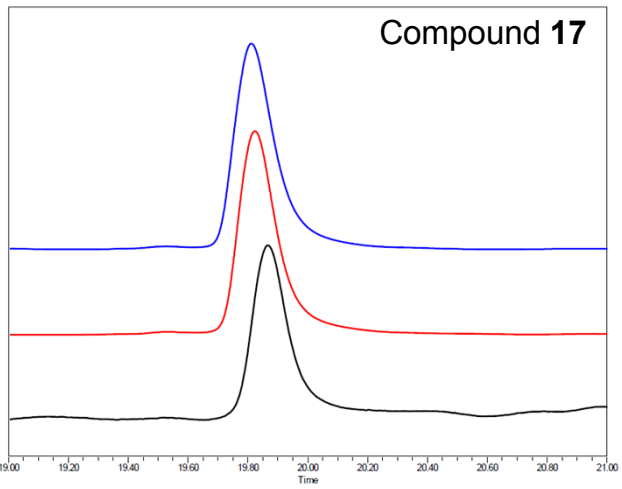


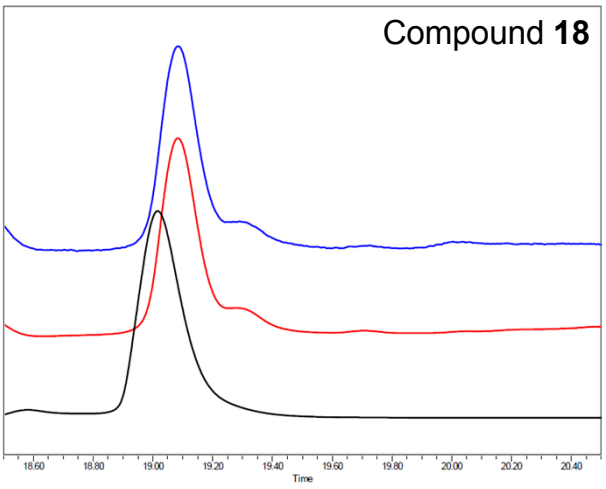

Compound **5**
Time

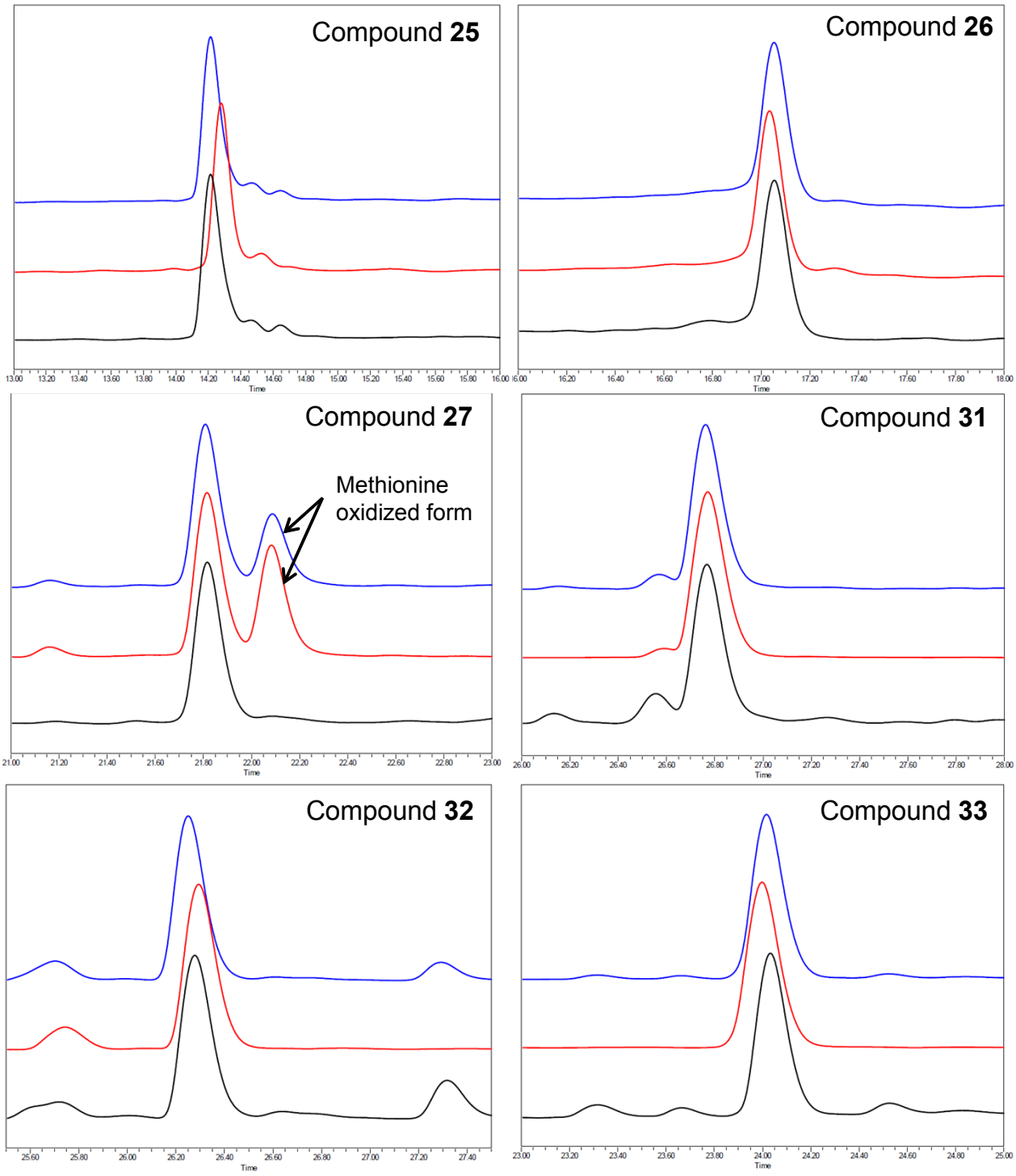

Compound **6**
Time


Compound **7**
Time


Compound **16**
Time

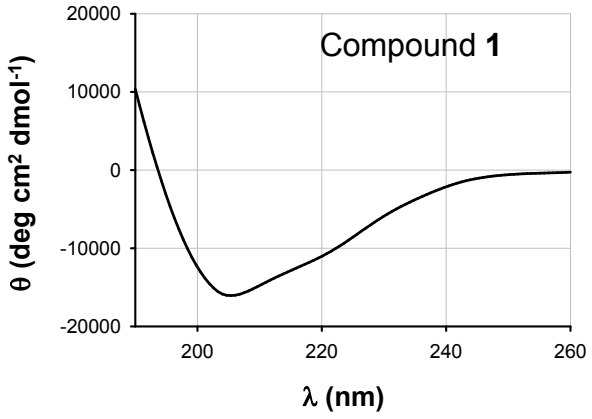

Compound **17**
Time

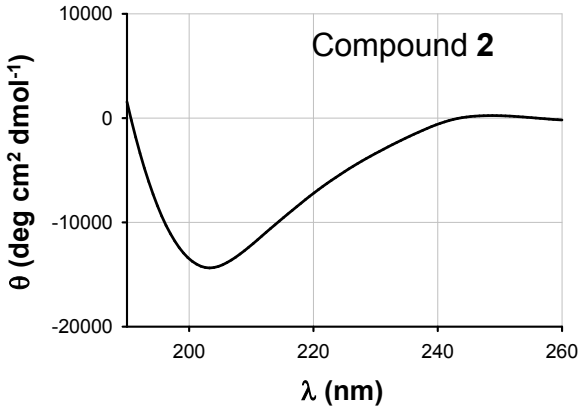

Compound **18**
Time

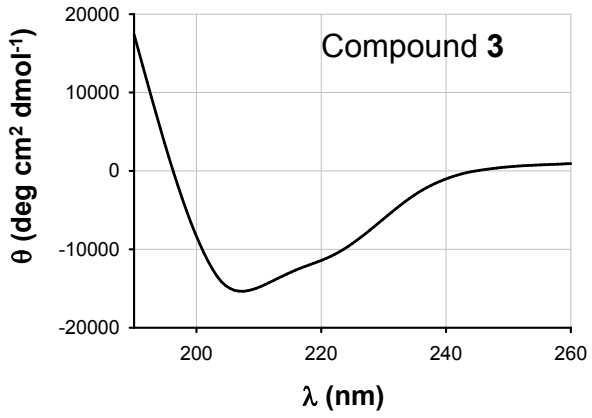

Compound 25
Compound 26
Compound 27
Methionine
oxidized form
Compound 31
Compound 32
Compound 33
Time

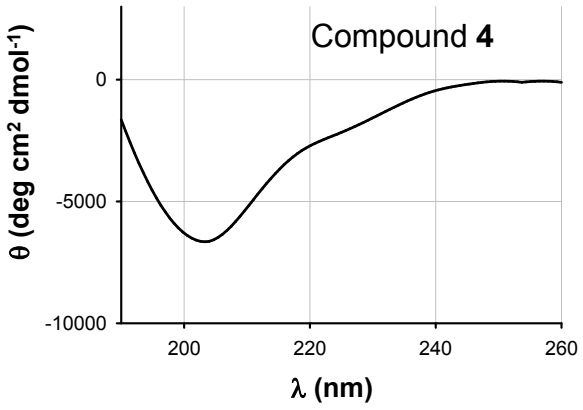
Circular dichroism

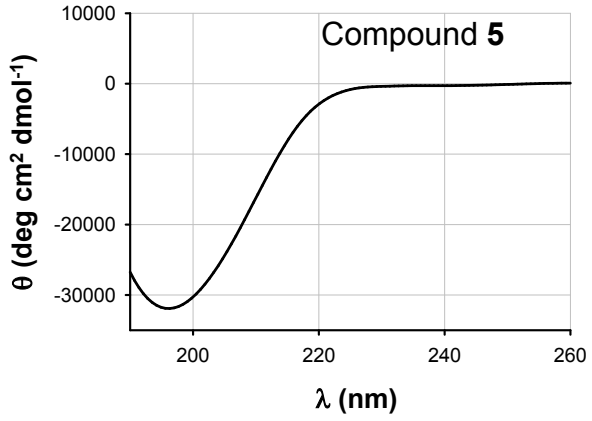
Circular dichroism spectra were obtained on a JASCO CD J-815 apparatus using solutions of each toxin with a 20 µM concentration in milliQ water in 1 mm path cuvettes from Hellma.

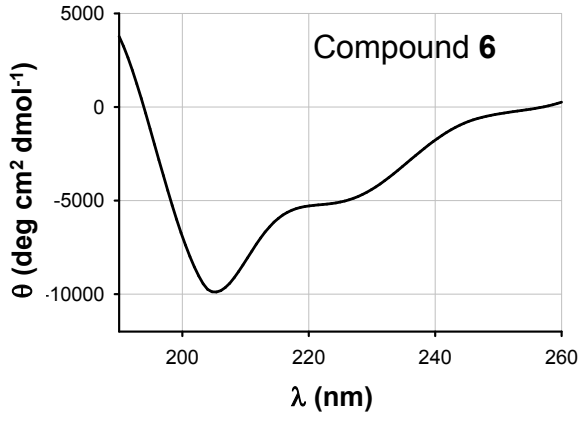


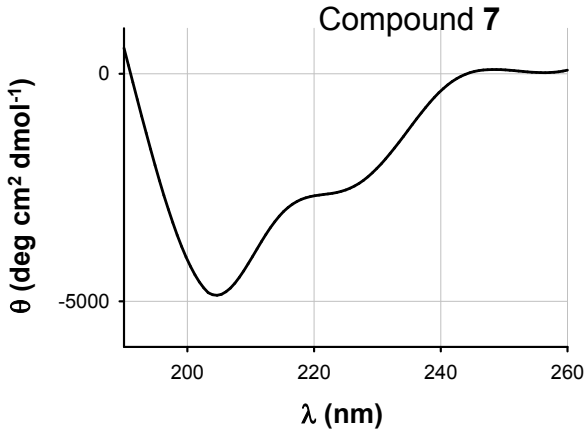


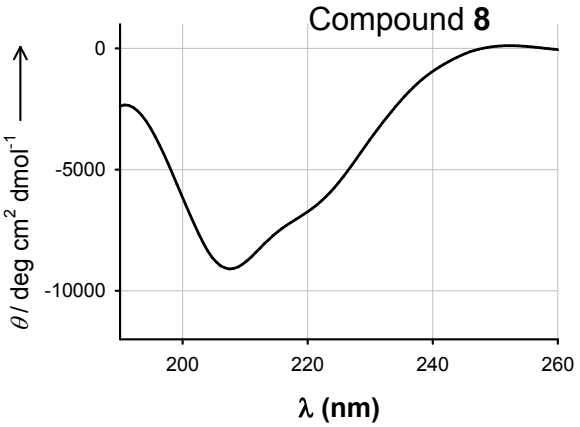


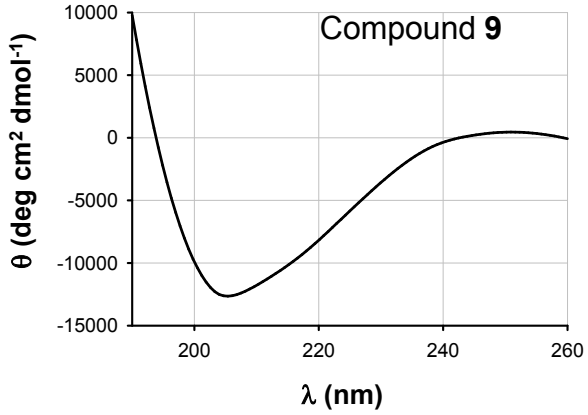


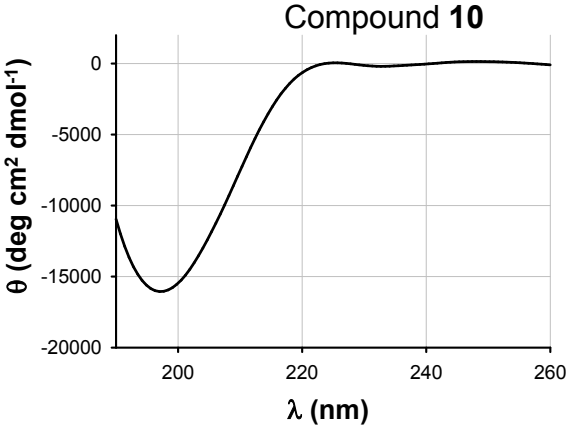


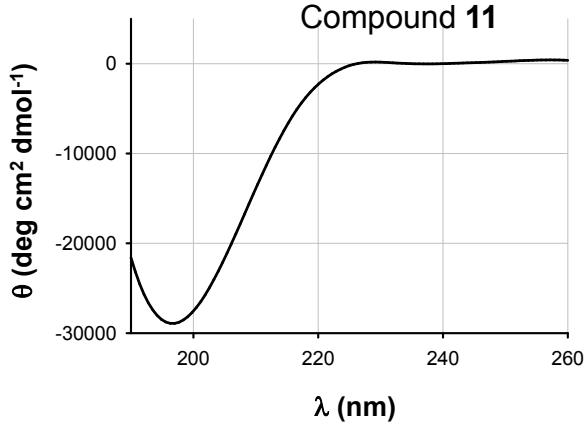


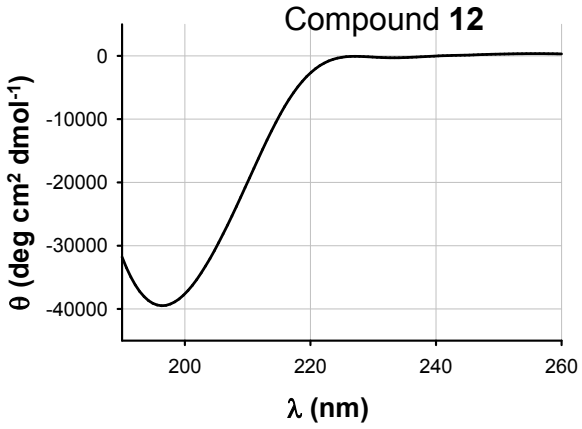

Compound 7
θ (deg cm² dmol⁻¹)
0
-5000
200
220
240
260
λ (nm)

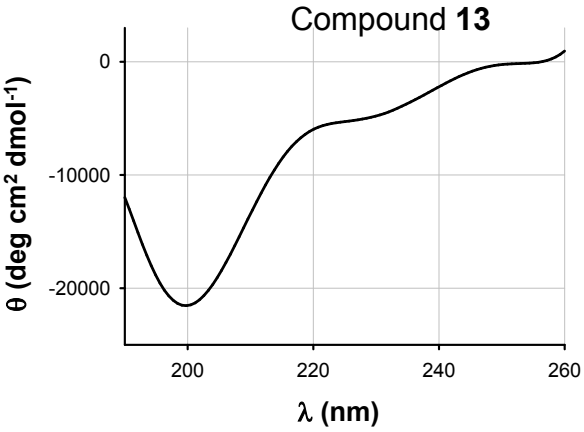

Compound 8
θ / deg cm² dmol⁻¹
0
-5000
-10000
200
220
240
260
λ (nm)

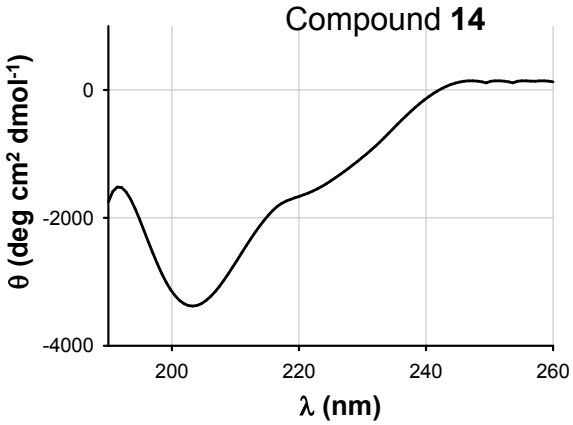

Compound 9
θ (deg cm² dmol⁻¹)
10000
5000
0
-5000
-10000
-15000
200
220
240
260
λ (nm)

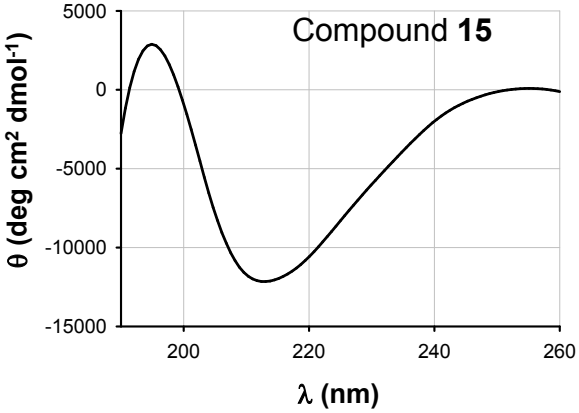

Compound 10
θ (deg cm² dmol⁻¹)
0
-5000
-10000
-15000
-20000
200
220
240
260
λ (nm)

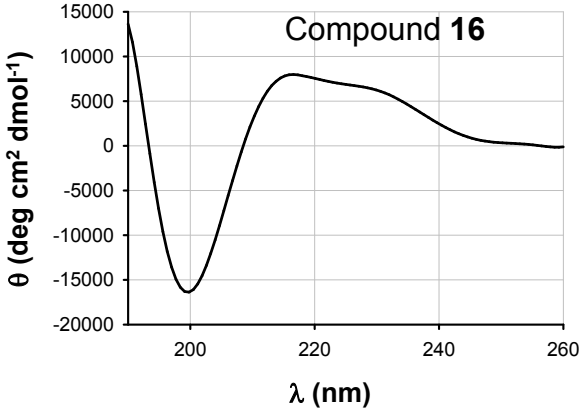

Compound 11
θ (deg cm² dmol⁻¹)
0
-10000
-20000
-30000
200
220
240
260
λ (nm)

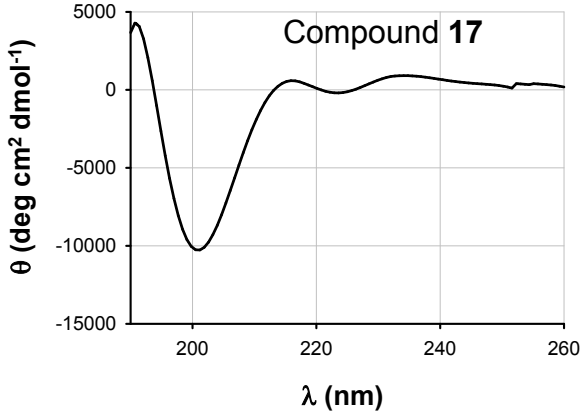

Compound 12
θ (deg cm² dmol⁻¹)
0
-10000
-20000
-30000
-40000
200
220
240
260
λ (nm)

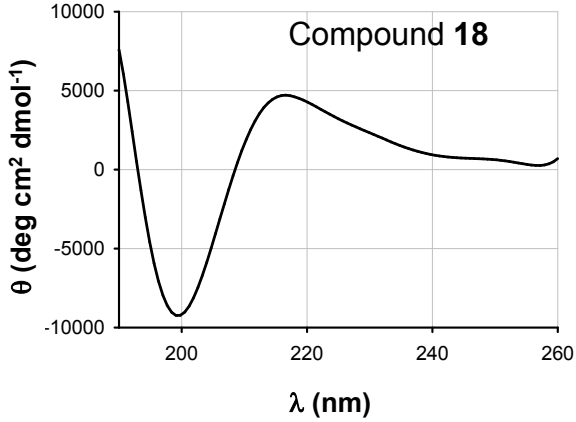

Compound 13
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)

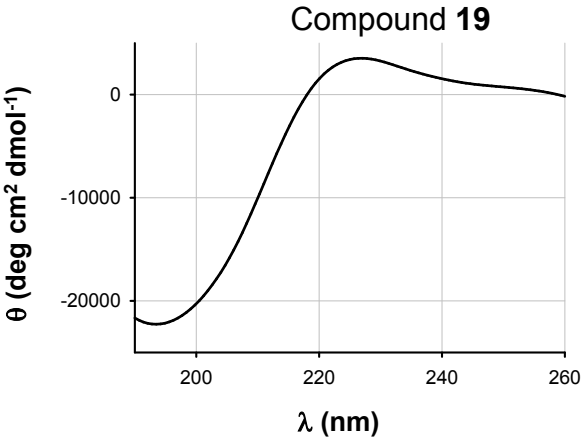

Compound 14
θ (deg cm² dmol⁻¹)
0
-2000
-4000
200
220
240
260
λ (nm)

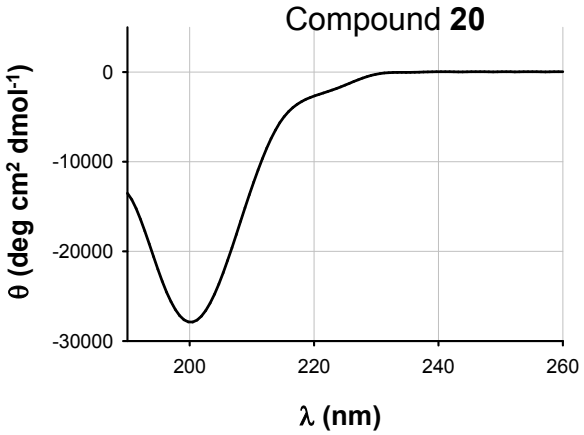

Compound 15
θ (deg cm² dmol⁻¹)
5000
0
-5000
-10000
-15000
200
220
240
260
λ (nm)

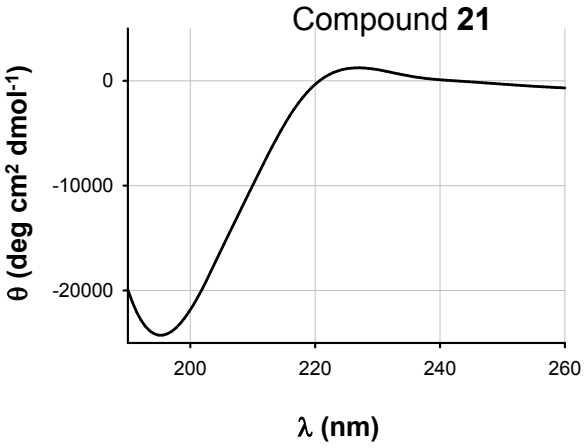

Compound 16
θ (deg cm² dmol⁻¹)
15000
10000
5000
0
-5000
-10000
-15000
-20000
200
220
240
260
λ (nm)

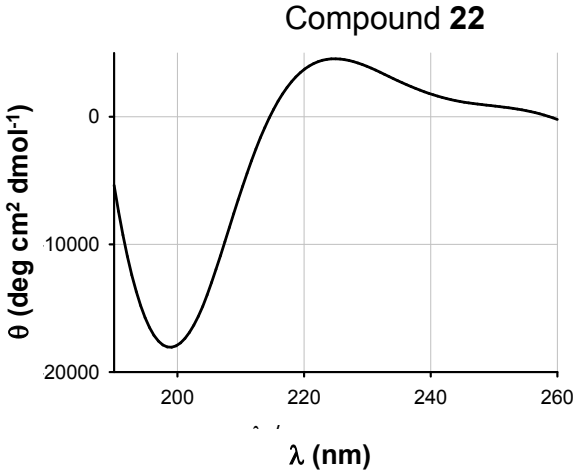

Compound 17
θ (deg cm² dmol⁻¹)
5000
0
-5000
-10000
-15000
200
220
240
260
λ (nm)

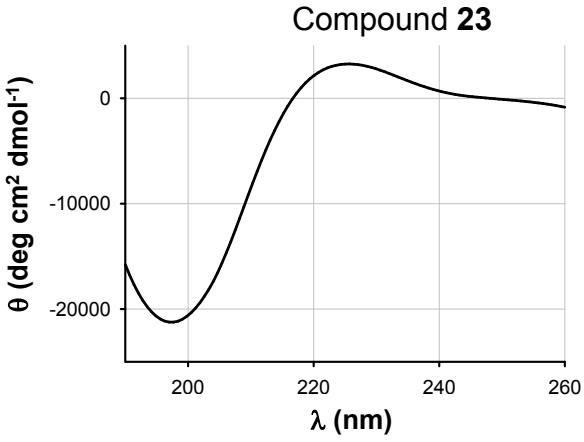

Compound 18
θ (deg cm² dmol⁻¹)
10000
5000
0
-5000
-10000
200
220
240
260
λ (nm)

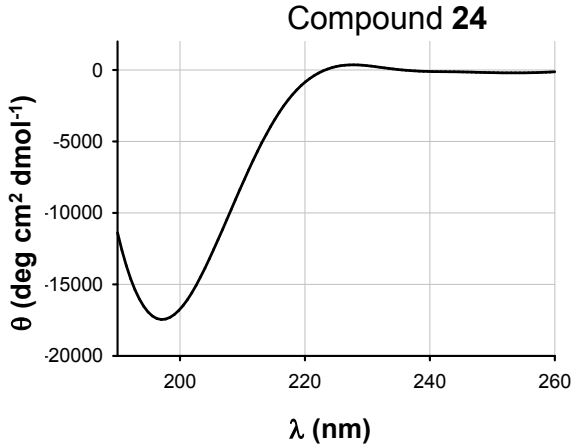

Compound **19**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)

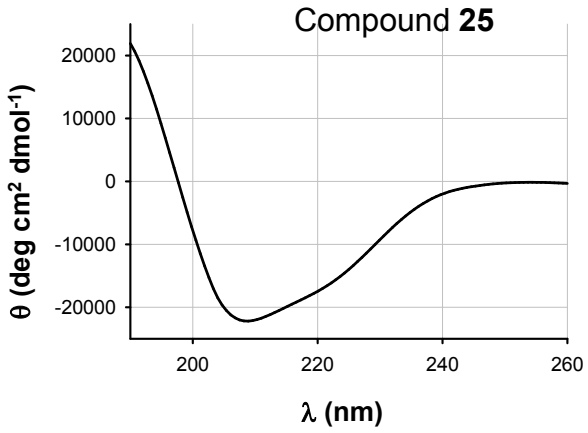

Compound **20**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
-30000
200
220
240
260
λ (nm)

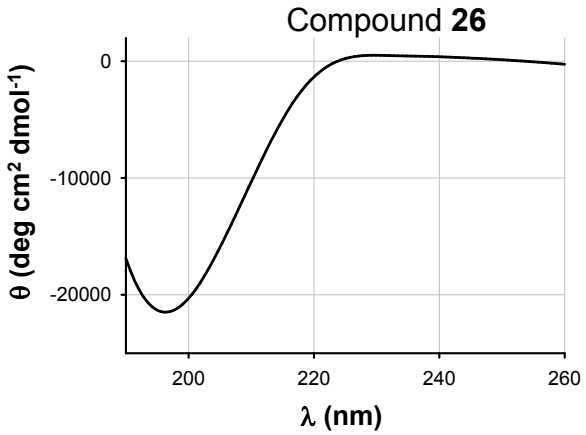

Compound **21**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)

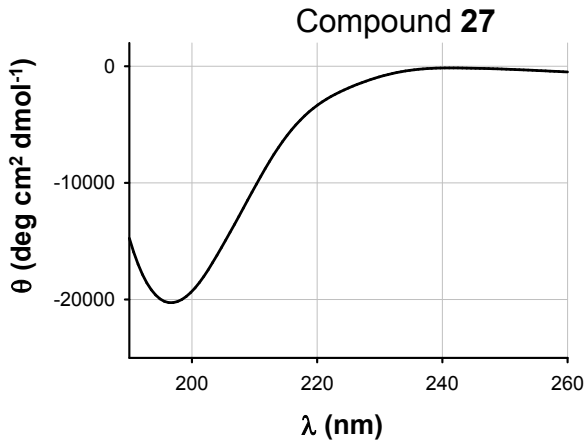

Compound **22**
θ (deg cm² dmol⁻¹)
0
10000
20000
200
220
240
260
λ (nm)

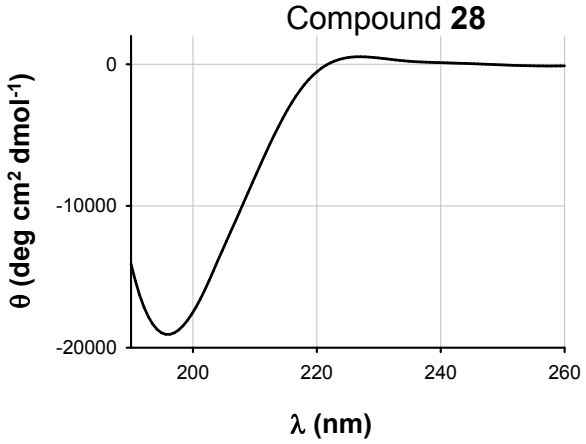

Compound **23**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)


Compound **24**
θ (deg cm² dmol⁻¹)
0
-5000
-10000
-15000
-20000
200
220
240
260
λ (nm)


Compound **25**
θ (deg cm² dmol⁻¹)
20000
10000
0
-10000
-20000
200
220
240
260
λ (nm)


Compound **26**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)


Compound **27**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)


Compound **28**
θ (deg cm² dmol⁻¹)
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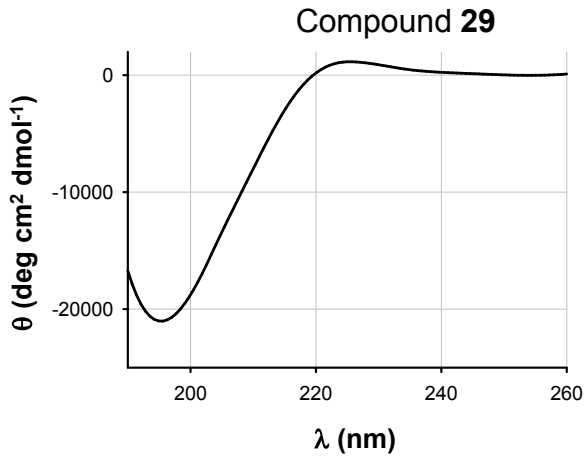
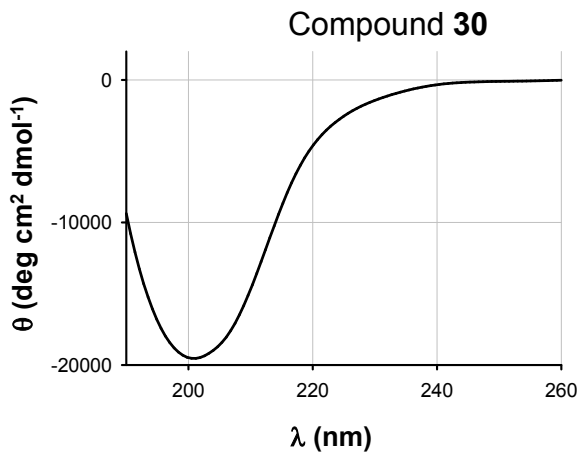
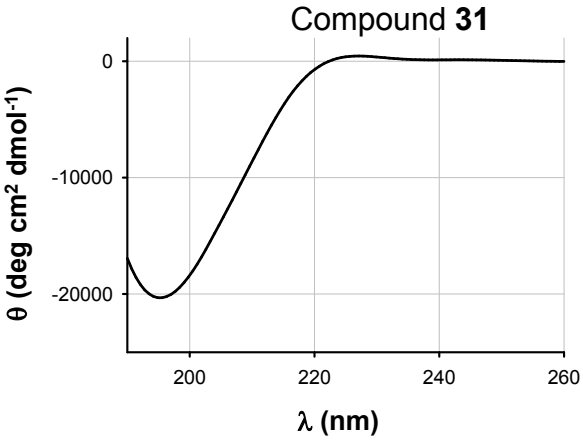
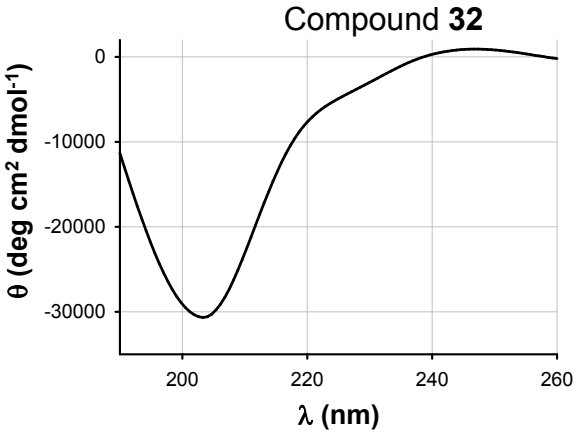
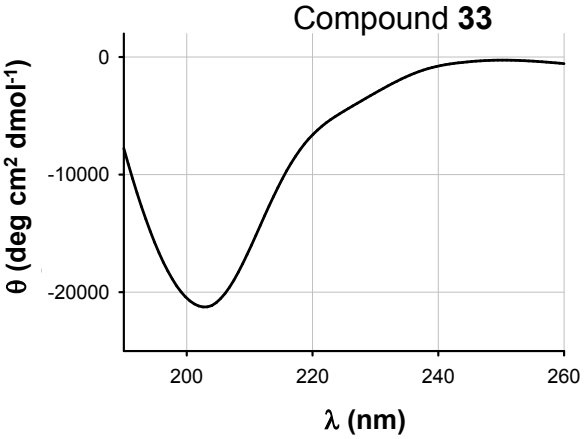
-10000
-20000
200
220
240
260
λ (nm)

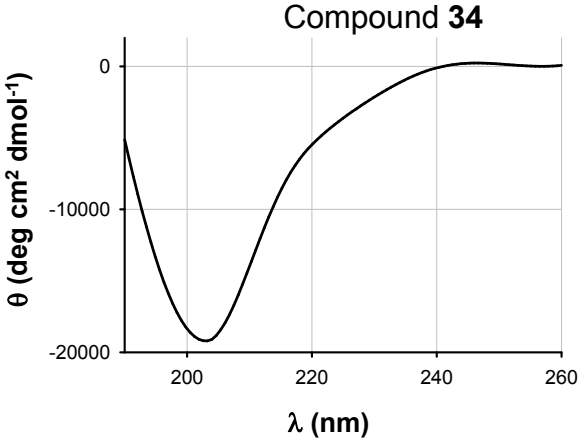

Compound **29**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)

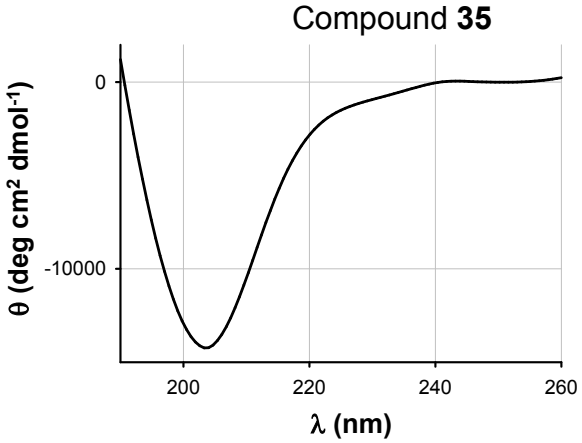

Compound **30**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)

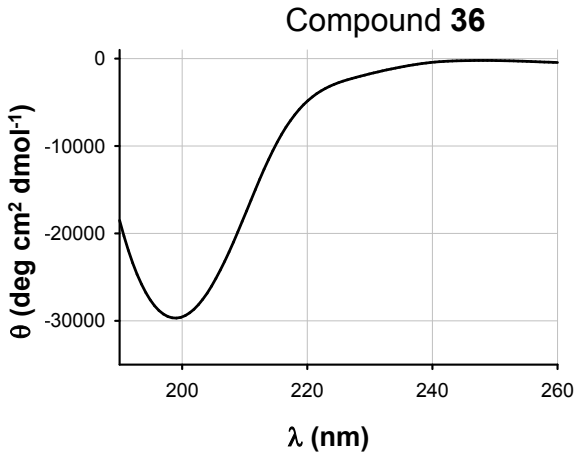

Compound **31**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)


Compound **32**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
-30000
200
220
240
260
λ (nm)


Compound **33**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)


Compound **34**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
200
220
240
260
λ (nm)


Compound **35**
θ (deg cm² dmol⁻¹)
0
-10000
200
220
240
260
λ (nm)


Compound **36**
θ (deg cm² dmol⁻¹)
0
-10000
-20000
-30000
200
220
240
260
λ (nm)

Pharmacology

Pharmacological characterization of the interaction of toxins with nicotinic receptors

Materials and Methods

Materials – cDNA coding for chick α7-5HT$_3$, human α3β2 and human α4β2 neuronal receptors were kindly provided by the laboratory of Pr. J.P. Changeux, Pasteur institute, Paris, France and Pr. O. Steinlein, Institute of Human Genetics. Bonn, Germany, respectively. [125I]-Bungarotoxin and (±)-[3H]-Epibatidine were purchased from PerkinElmer.

Expression of nicotinic receptors in Human Embryonal Kidney (HEK) Cells - A chimeric cDNA of the neuronal type α7-5HT$_3$ nAChR was transfected into HEK-293 cells by calcium phosphate precipitation, as previously described (Servent, et al., 1997; Eiselé et al., 1993). Briefly, the chick cDNA (15 µg of α7-5HT$_3$) was transfected by calcium precipitation with a careful control of the pH (6.95). The cells were placed at 37°C under 5% CO_2 and 2 days after transfection, cells were harvested in a phosphate-buffered saline (PBS) with 5 mM EDTA, and resuspended in 3 ml/plate of this buffer for binding experiments. For human α3β2 and α4β2 receptor subtypes, 24 h after the calcium phosphate transfection (7 µg α3 or α4 and 7 µg β2) the cells were placed two days at 30°C, 5% CO_2 before being collected for binding assays, that used a cell density adjusted to specifically bind ≤10% of radioligand.

Binding Assays - All binding experiments were done in 96-well plates in a total volume of 100µl. Competition experiments with *Torpedo* and α7-5HT$_3$ receptor subtypes were performed at equilibrium by incubating *Torpedo* or HEK-293 cells membrane overnight with different concentrations of toxins and ^{125}I-Bgtx (0.5 - 1nM). The mixture was filtered, rinsed and counted as previously described for the α7 receptor. Equilibrium binding experiments on α4β2 and α3β2 subtypes used [3H]-Epibatidine as radioactive tracer. Cells expressing this receptor were incubated with 0.5 to 1 nM [3H]-Epibatidine and various concentrations of toxins for 4 hours. The reaction was stopped by filtration of the 96-well simultaneously through a GF/C plate presoaked in 0.5% polyethylenimine, using a FilterMate harvester (PerkinElmer, Courtaboeuf,

France). The filters were washed twice with ice-cold buffer, dried and the bound radioactivity was counted, after the addition of 25 µl of MicroScint per well, by scintillation spectrometry on a TopCount beta counter (PerkinElmer, Courtaboeuf, France). Each experiment was done at least two times in duplicate. In equilibrium competition experiments, IC_{50} values were determined by fitting the competition data by the empirical Hill equation and converted to Ki constants using the Cheng-Prussoff equation :

Ki = IC_{50}/(1+L*/Kd), with Kd Bgtx on muscular and α7-$5HT_3$ receptors of 50pM and 5nM, respectively and Kd for epibatidine on hα4β2 and hα3β2 equal to 20pM and 35 pM, respectively.

Pharmacological characterization of the interaction of toxins with Norepinephrine transporter

Materials and Methods

Binding reactions were set up in duplicate wells of 96-well plates. 2 µl of human NET membranes (PerkinElmer, Courtaboeuf, France) were incubated with [3H]-nisoxetine (2nM) in the absence or presence of χ-conotoxins (1 nM–200 µM) in buffer containing 20 mM Tris_HCl, 75 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% bovine serum albumin, pH 7.4, for 1 h at room temperature. The final assay volume was 100 µl. The amount of nonspecific binding was determined by the addition of desipramine (10 µM) in the reaction. Bound and free radioactivity were separated by rapid vacuum filtration onto GF/C filtrers presoaked in 0.5% polyethylenimine, using a FilterMate harvester (PerkinElmer, Courtaboeuf, France). Filters were washed two times with ice-cold buffer and allowed to dry. Filter-retained radioactivity was quantified by liquid scintillation counting on a TopCount beta counter (PerkinElmer, Courtaboeuf, France) after the addition of 25 µl of MicroScint per well.

IC_{50} values were determined by fitting the competition data by the empirical Hill equation and converted to Ki constants using the Cheng-Prussoff equation :

Ki = IC_{50}/(1+L*/Kd), with Kd [3H]-nisoxetine on hNET equal to 2nM.

Results

Table S8. Affinity constants of synthetic conotoxins interacting with nicotinic acetylcholine receptors (nAChRs) or norepinephrine transporter (NET). Affinity constants values are similar to those previously described in the literature, even if we cannot strictly compared Ki from binding experiments and IC50 from electrophysiological (ephy) studies.

Toxins	Affinity constants (Ki in binding experiments, nM)	Reference affinity (nM)
1	5.7 ± 1.1 (*Torpedo* nAChR)	4.5 – 87 (IC_{50} on *Torpedo* nAChR-binding)[1]
2	3.8 ± 1 (*Torpedo* nAChR)	1.4 (Kd on *M.musculus* nAChR-ephy)[2]
3	106 ± 5.8 (*Torpedo* nAChR)	170 (IC_{50} on *M.musculus* nAChR-binding)[3]
7	3.2 ± 0.7 (*Torpedo* nAChR)	0.37 (IC50 on muscle nAChR-ephy)[4]
13	0.25 ± 0.08 - 79 ± 49 (*Torpedo* nAChR)	45 (IC_{50} on *M.musculus* nAChR-ephy)[5]
15	1.3 ± 0.2 (*Torpedo* nAChR)	4.8 (Ki on *Torpedo* nAChR-binding)[1]
16	4700 ± 950 (hNET)	1800 (IC_{50} on hNET-binding)[6]
18	5600 ± 170 (hNET)	2000 (IC_{50} on hNET-binding)[7]
28	76.7 ± 15.9 (*Torpedo* nAChR)	<1000 (IC_{50} on *M.musculus* nAChR-ephy)[8]
30	91.5 ± 10.5 (*Torpedo* nAChR)	540 (IC_{50} on *M.musculus* nAChR-ephy)[9]

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