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Supplementary Materials for

Somatostatin venom analogs evolved by fish-hunting cone snails: From prey capture behavior to identifying drug leads

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

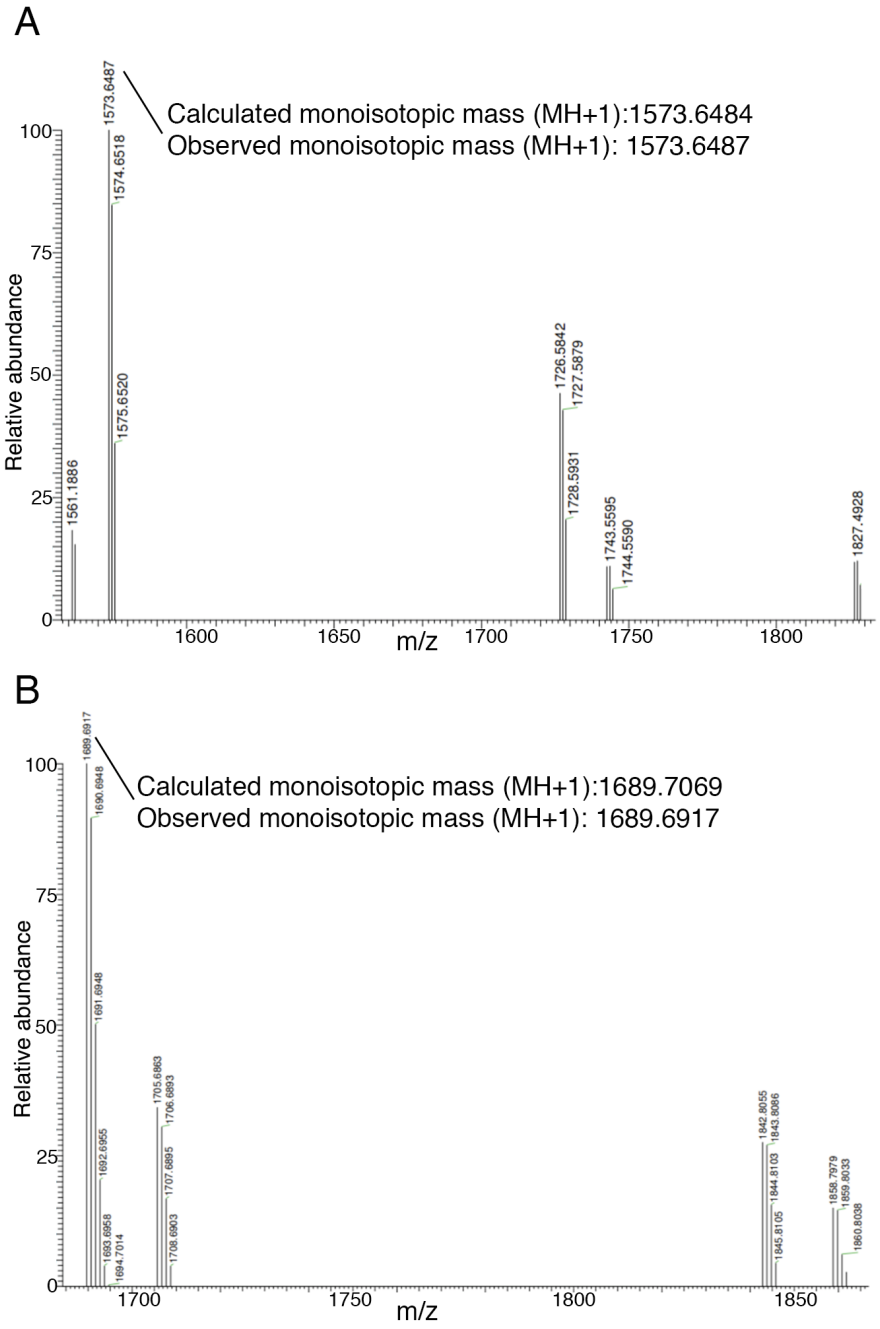

Fig. S1. Mass determination of compounds in fraction #16-12 confirming the molecular mass of Consomatin Ro1 sequenced by Edman and transcriptome analysis. **A.** Nonderivatized fraction 16-12. **B.** Fraction derivatized with dithiothreitol (DTT) and iodoacetamide (IAA).

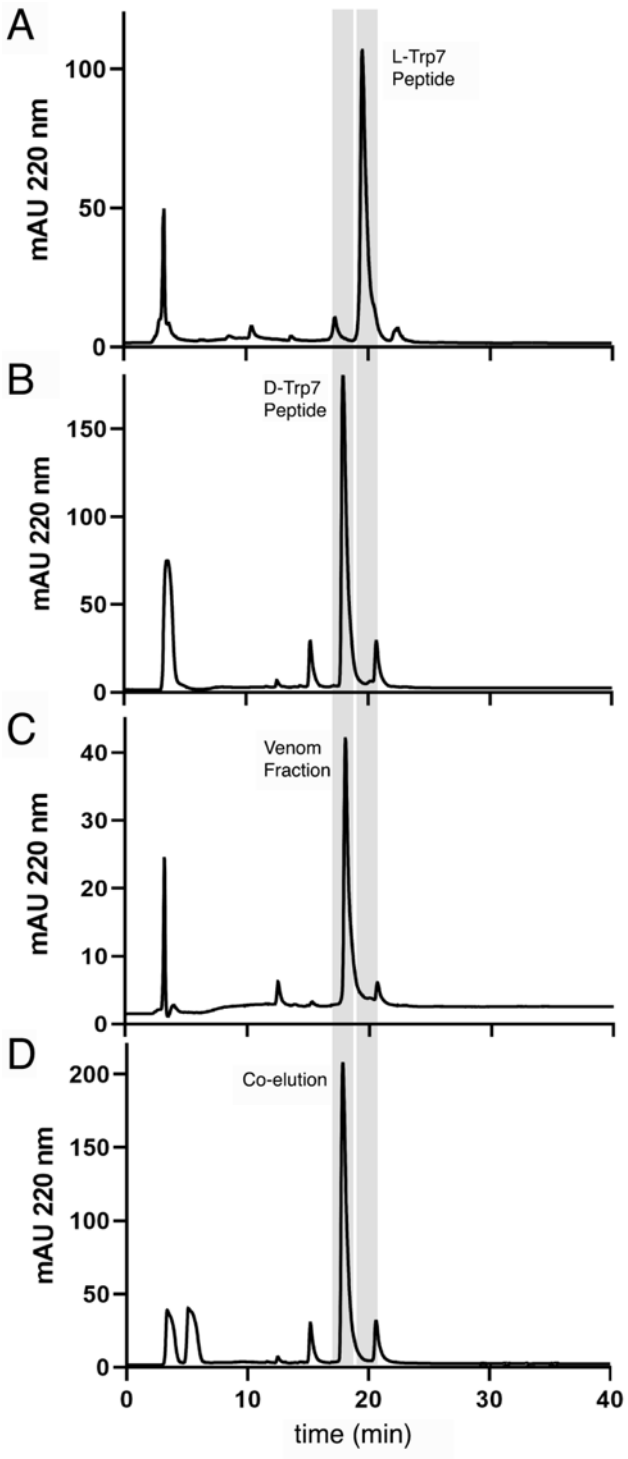

Fig. S2. Reverse-phase elution profile of **A**. Consomatin Ro1 containing L-Trp7, **B**. Consomatin Ro1 containing D-Trp7, **C**. the venom fraction #16-12, and **D**. co-elution of Consomatin Ro1 (D-Trp7) with the venom fraction.

Fig. S3. Alignment of the precursor sequences of Consomatin Ro1 and other consomatins identified in the venom gland transcriptome of *Conus rolani* (Ro), *Conus neocostatus* (Nc), *Conus geographus* (G), *Conus grahami* (Gh), *Conus maioensis* (Ma), *Conus cuneolus* (Cu), *Conus boavistensis* (Bv), *Conus galeao* (Go), *Conus infinitus* (It), *Conus verdensis* (Vd), *Conus mercator* (Mrc), *Conus raulsilvai* (Rs1). Precursor regions encoding the predicted mature toxins are shown in red (signal peptide in gray and pro-and post-peptides in blue). Amino acids with sequence similarity to SS are shown in bold. Identical amino acids are denoted by an asterisk (*). Full stops (.) and colons (:) represent a low and high degree of similarity, respectively. Names of toxins that were synthesized and functionally characterized here are shown in bold. Signal peptides were predicted using SignalP online software (version 5.0, Technical University of Denmark (DTU) Health Tech). The mature toxin regions were predicted using the PeptideCutter online software (Expasy, SIB Swiss Institute of Bioinformatics) with enzyme selection based on the processing of Consomatin Ro1 (a trypsin-like protease cleavage site at the N-terminus and a Proteinase K-like cleavage site following the cysteine loop region). Regions preceding and proceeding the mature toxins were designated as pro-peptide and post-peptide regions, respectively. SRA accession numbers of all sequencing datasets used in this study are provided in Table S5.

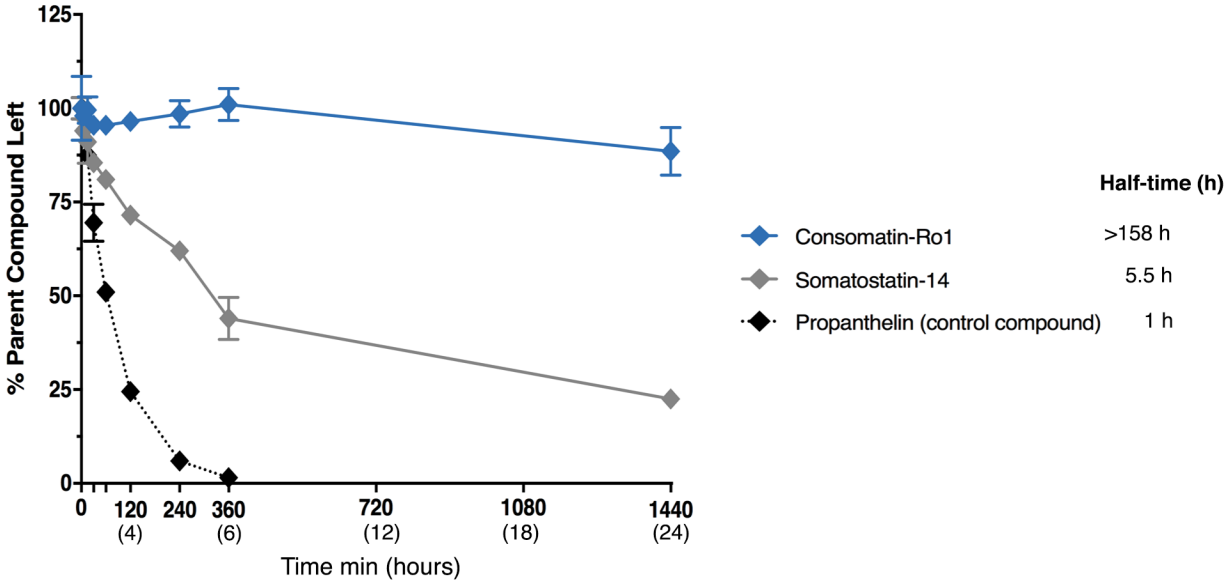

Fig. S4. Stability profiles of human SS-14, Consomatin Ro1, and an assay control compound, Porpenthelin, measured over 24 hours. Parent compound disappearance was based on relative LC/MS peak area (0 min = 100%). The values represent the mean ± S.D. of duplicate samples.

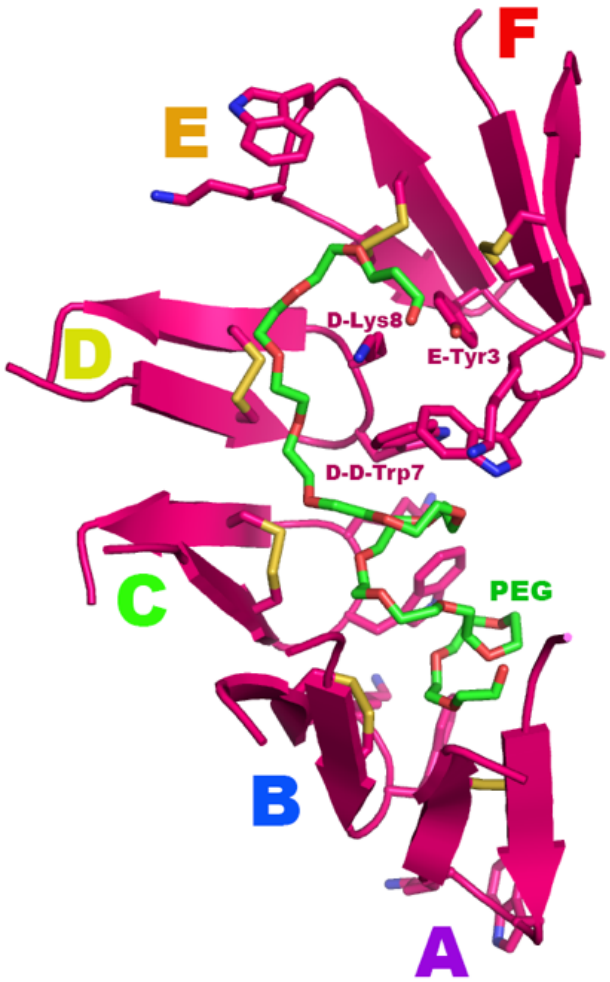

Fig. S5. Packing of molecules in the crystal. Six Consomatin Ro1 molecules (pink ribbons), and one large PEG molecule (green sticks with red oxygen atoms) form the asymmetric unit in the crystal. The origin of the PEG molecule in the crystal is not apparent, but it provided a good description of the electron density. Chain identifiers, A,B,C,D,E,F, are spectrum-coded to match the overlapped models in figure S6. The C5-C10 Cys-Cys disulfide bond of each molecule is shown as sticks (yellow). The two residues at the apex of the cyclic core (cysteine loop) (D-Trp7 and Lys8) are shown as sticks with blue nitrogen atoms. The six copies of Ro1 form a curving anti-parallel beta sheet with the apex of the turn of each copy of Ro1 oriented to the same side of the sheet except for copy E which is oriented opposite the others. Although copy E is oriented opposite the others, the same "top side" of the molecule faces the inner curve of the arc where the disulfide bond of all six molecules interacts with the PEG molecule. The PEG molecule facilitates crystallization primarily through hydrophobic interactions with the disulfide bonds and the D-Trp side chains. One end of the PEG wraps around D-Lys8. D-Lys8 adopts a different conformation compared to Lys8 in the other 5 copies of Ro1 because of the favorable interactions with the PEG, and, because E-Tyr3 packs against D-Lys8, constraining it an orientation different than Lys8 of the other copies of Ro1. D-D-Trp7 adjusts to the unique orientation of D-Lys8 in order to maintain a stacking arrangement with D-Lys8, despite there being no significant constraints on the side chain orientation of D-D-Trp7, suggesting that the D-Trp7-Lys8 side-by-side stacking arrangement at the apex of the cyclic loop is a strongly favored interaction inherent to Ro1. Figures generated with the program Pymol (version 1.8.4.1).

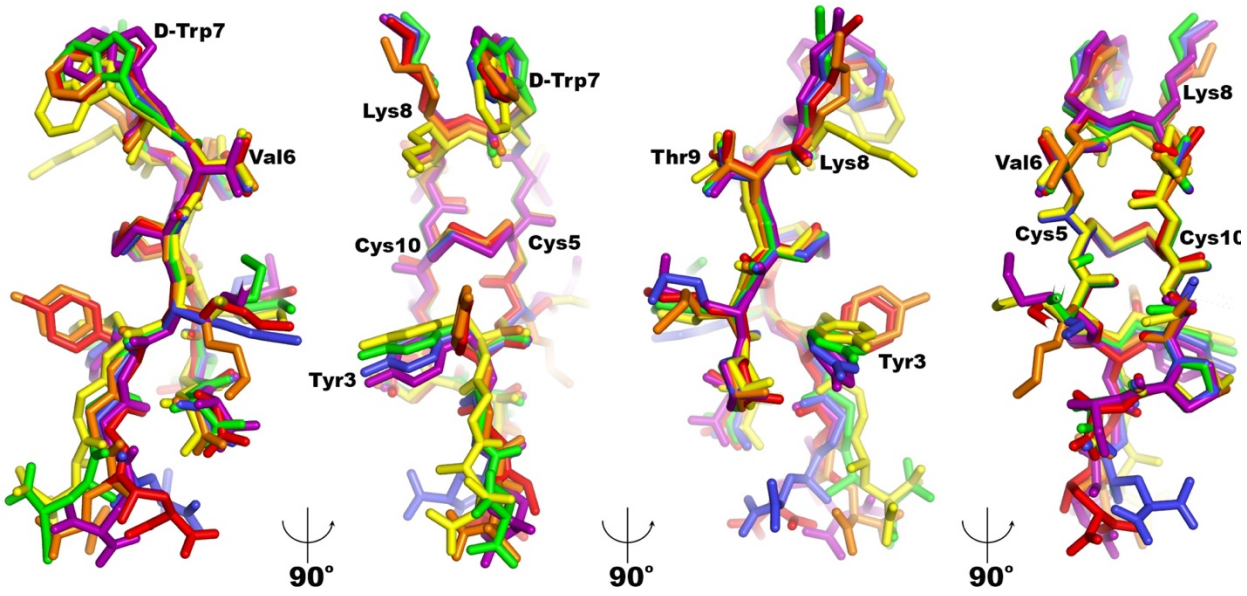

Fig. S6. Four views of the overlap of all copies of Consomatin Ro1 on copy **E** (colored orange). Alignment of the six copies of the molecule was performed with the program LSQKAB (75). Some of the residues are labeled in black. The side chain orientations of D-D-Trp7 and D-Lys8 differ from the other five copies yet the side-by-side stacking arrangement of D-Trp7 and Lys8 is a distinctive feature in all 6 copies of Ro1 that form the asymmetric unit of the crystal.

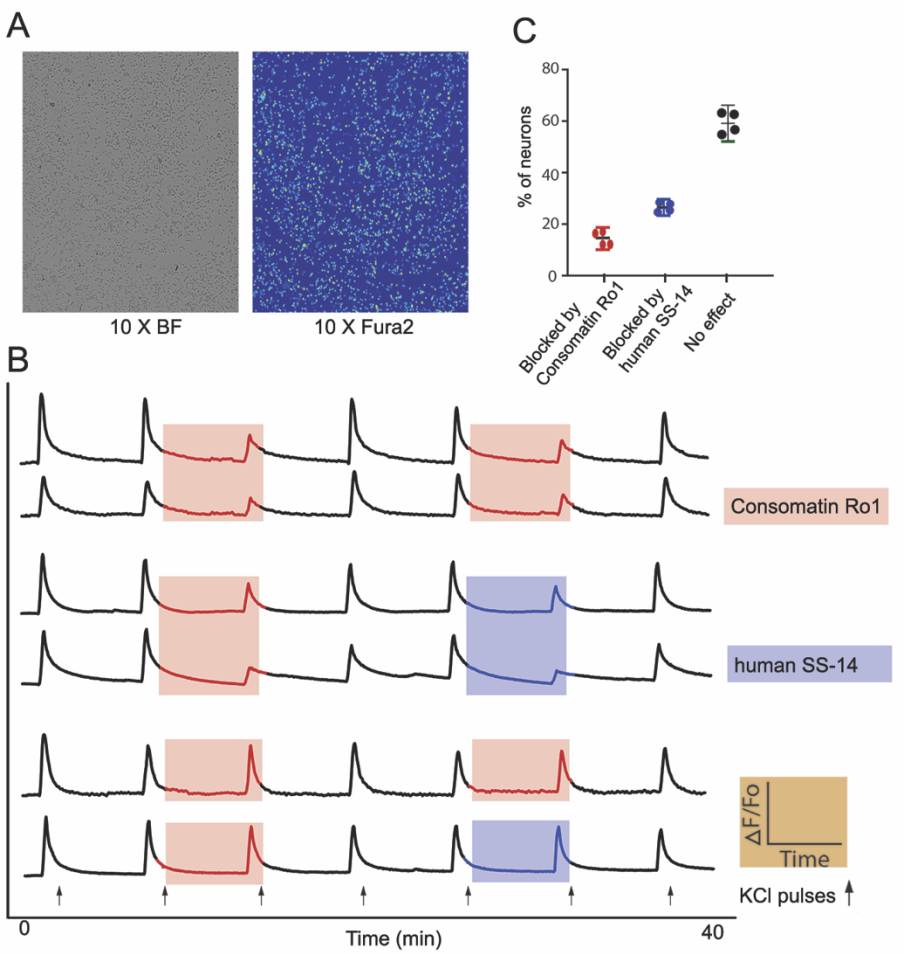

Fig. S7. Block of potassium-evoked calcium signals by Consomatin Ro1 and somatostatin in zebrafish larvae CNS neurons. **A.** Bright-field (BF, left) and fluorescence image (right) of dissociated CNS neurons from larvae zebrafish at 10 X magnification. Fluorescence images were acquired with 380-nm excitation and 510-nm emission filters after loading cells with Fura2-AM dye. **B.** Example calcium-imaging traces of neurons responding with a partial block to applications of 5 µM Consomatin Ro1 (top two traces, Consomatin Ro1 application highlighted in red), neurons responding with a partial block to one application of 5 µM Consomatin Ro1 (highlighted red) and one application of 100 nM of somatostatin (highlighted blue, middle two traces), and example traces of neurons that did not respond to either peptide (bottom two traces). **C.** Total percentage of neurons that responded to Consomatin Ro1 (14.14 ± 2.64% of cells), somatostatin (26.44 ± 1.54 % of cells), or did not respond to either compound (59.17 ± 4.36% of cells). Values represent means ± CI95 of 4 independent repeats). KCl was applied at 30 mM.

Table S1. Timeline of predation events for the 3 movie files provided for *Conus neocostatus* (Movies S3-S5).

Time (h:min:sec)	Events
Movie S3	*Conus neocostatus* catches a fang blenny, single strike
00:00:40	Snail envenomates fish
00:15:00	Fish loses motor control; abnormal swimming behavior
00:16:30	Fish comes to rest on substrate
01:15:00	Snail starts tracking down fish
01:22:00	Fish appears to have died
01:23:00	Snail eats dead fish
Movie S4	*Conus neocostatus* envenomates a goby, night camera, two strikes
00:00:00	*Conus neocostatus* approaches fish from distance and strikes the first time
00:16:00	Goby starts climbing the wall and shows beginning of loss of balance
00:35:00	Snail becomes active, presumably stalking the fish
01:02:00	Fish appears to have recovered and is correctly balanced
03:17:00	Snail strikes a second time
03:18:00	Snail starts ingesting the fish shortly after the second strike
03:19:00	Final twitch from live fish while being ingested
Movie S5	*Conus neocostatus* catches a blenny with two strikes
00:00:00	Proboscis emerges
00:02:02	Snail envenomates fish (first sting)
00:13:50	Proboscis emerges again
00:23:02	Snail envenomates fish (second sting with brief tether)
00:23:40	Fish begins to climb wall
00:24:20	Fish falls and remains immobile
01:06:00	Fish appears motionless, possibly dead; snail engulfs the fish

Table S2. Summarized activity of select *C. rolani* venom fractions and synthetic Consomatin Ro1 in mice [intracranial] and fish. 15 fractions (*C. rolani* fractions # 10 to 24) out of a total of 38 HPLC fractions were tested for bioactivity in mice.

***Conus rolani* venom fractions (concentration unknown) – Mice injections**				
Age of mice (days)	Weight of mice (g)	Number of mice tested (n)	Venom fraction #	Observed Behavior (time = min post injection)
15	7.33	1	Control	normal: moving, responsive to prodding || grooming, walking, rearing
	7.84	1	*C. rolani* fraction # 16	loss of balance at 3 min; unresponsive to prodding at 17 min and lasts for at least 3.5 h, recovered the next day
16	8.38 ± 1.79	3	Control	normal: moving, responsive to prodding || grooming, walking, rearing
	8.00 ± 0.42	2	*C. rolani* 16-12 (Consomatin Ro1 native)	initially normal: moving, responsive to prodding hypoactive: less responsive to prodding 37 min - 1 h 5 min heavy body, moves in place only, leans left unresponsive: no movement when prodded except the tail 1h 8 min - 3 h hypoactive: less responsive to prodding 3 h 18 min - 4 h 43 min stretches only when prodded, moves in place, reacts sluggishly mouse 1: checked the next day, recovered mouse 2: observation ended at ~5h
Synthetic Consomatin Ro1 – Mice injections				
Age (days)	Weight (g)	n	Dose (nmol)	Observed Behavior (time = min post injection)
12	8.20 ± 0.11	2	0	normal: moving, responsive to prodding || grooming, walking, rearing
	7.57± 0.14	2	10	less responsive to non-responsive to prodding from 1 h 15 min and lasted for 3 h 5 min
	8.09	1	25.75	less responsive to non-responsive to prodding from 31 min and lasted for 4 h 15 min
16	7.33 ± 0.38	6	0	normal: moving, responsive to prodding || grooming, walking, rearing
	7.72 ± 0.21	2	2.5	(only one out of 2 mice tested showed activity)

				less responsive to prodding from 2 h and lasted for 2 h 6 min, recovered at 4 h
	7.25 ± 0.06	2	5	less responsive to non-responsive to prodding from 3 h 11 min and lasted for 1 h 11 min, recovered the next day
	8.14 ± 0.18	2	10	less responsive to non-responsive to prodding from 2 h 4 min and lasted for 1.5 h
	7.58 ± 0.81	2	20	less responsive to non-responsive to prodding from 1 h 4 min and lasted for 3 h 37 min, recovered the next day, checked at ~17 h
	7.25	1	30	less responsive to prodding from 1 h 24 min and lasted for 2 h 43 min
17	9.10 ± 0.70	3	0	normal: moving, responsive to prodding || grooming, walking, rearing
	7.39	1	1	normal: grooming, moving, responsive to prodding
	7.54 ± 0.16	2	2.5	less responsive to prodding from 2 h 2 min and lasted for 2 h
	8.97 ± 0.73	2	10	less responsive to prodding from 1 h 25 min and lasted for 1 h 39 min
	9.13 ± 0.18	2	12.38	less responsive to non-responsive to prodding from 1h 45 min and lasted for 2 h 19 min
	9.17	1	25.75	less responsive to non-responsive to prodding from 2 h 7 min and lasted for 1 h 53 min
19	10.15 ± 0.84	5	0	normal: moving, responsive to prodding || grooming, walking, rearing
	9.45 ± 0.57	2	5	less responsive to non-responsive to prodding from 2 h 12 min and lasted for 2 h 30 min
	10.27	1	30	less responsive to non-responsive to prodding from 2 h, assay cut short at 2h 30 min
22	14.53 ± 0.57	2	0	normal: moving, responsive to prodding || grooming, walking, rearing
	13.61	1	20	less responsive to prodding from 4 h and lasted for 3 h

Table S3. Consomatin Ro1 crystallographic data and refinement statistics

Data		
Crystal	WT	K_2PtCl_4-soak
Source/Wavelength/Date of data collection	SSRL 9-2 / 1.7711/ 05/12/2019	SSRL 14-1 / 1.0716/ 07/16/2019
Space Group (unit cell dimensions, Å)	$P6_522$ (50.28, 50.28, 135.01)	$P6_522$ (50.32, 50.32, 134.60)
Resolution (Å)	36.59 – 1.95	36.58 – 2.40
Resolution (Å) (high-resolution shell)	(2.00 – 1.95)	(2.49 – 2.40)
# Reflections measured	1,125,051	615,190
# Unique reflections	8,016	4,434
Redundancy (high-resolution shell)	140 (103)	139 (144)
Completeness (%)(high-resolution shell)	99.9 (98.2)	100.0 (99.7)
Anomalous redundancy (high-resolution shell)		82 (80)
Anomalous completeness (%)(high-resolution shell)		99.9 (99.6)
<I/σI> (high-resolution shell)	24.3 (1.4)	24.8 (3.7)
<CC1/2>	1.000 (0.700)	0.999 (0.906)
Rpim (high-resolution shell)	0.019 (0.744)	0.023 (0.389)
Mosaicity (°)	0.11	0.13
Refinement		
Program	Phenix.refine	
Resolution (Å)	36.62 – 1.95	
Resolution (Å) – (high-resolution shell)	(2.01 – 1.95)	
# Reflections used for refinement	6,796	
# Reflections in Rfree set	1,167	
Rcryst	0.239 (0.304)	
Rfree	0.283 (0.379)	
RMSD: bonds (Å) / angles (°)	0.011 / 2.068	
<B> ($Å^2$): all atoms / # atoms	61.0 / 723	
<B> ($Å^2$): water molecules / #water	54.2 / 34	
<B> ($Å^2$): PEG molecule / #non-hydrogen atoms ($C_{34}O_{17}H_{69}$)	62.7 / 51	
ϕ/ψ most favored (%) / additionally allowed (%)	100.0/0.0	

Table S4. EC_{50} values for human SS-14, Consomatin Ro1, Consomatin G1 at the human SST_{1-5} using the PRESTO-Tango

	Human SS-14	**Consomatin Ro1**	**Consomatin G1**
SST_1			
EC_{50}	3.72E-09	2.88E-06	
pEC_{50}	8.43	5.54	
+/-CI95	0.39	0.77	
+/- SEM	0.16	0.18	
n	7	3	
SST_2			
EC_{50}	1.26E-08		2.58E-09
pEC_{50}	7.90		8.59
+/-CI95	0.22		0.58
+/- SEM	0.09		0.13
n	7		3
SST_3			
EC_{50}	4.58E-08		
pEC_{50}	7.34		
+/-CI95	0.29		
+/- SEM	0.12		
n	7		
SST_4			
EC_{50}	4.54E-09	5.05E-06	
pEC_{50}	8.34	5.30	
+/-CI95	0.52	0.28	
+/- SEM	0.21	0.06	
n	7	3	
SST_5			
EC_{50}	3.00E-08		
pEC_{50}	7.52		
+/-CI95	0.36		
+/- SEM	0.15		
n	7		

Table S5. Venom gland transcriptome datasets interrogated in this study for the bioinformatic identification of SS-like sequences

Conus species	Subgenera	No of assembled contigs	Accession numbers
Conus rolani	*Asprella*	32,957	SRA: SAMN22417925
Conus neocostatus	*Asprella*	10,919	SRA: SAMN22417934
Conus geographus	*Gastridium*	24,643	SRA: SAMN22417935
Conus ermineus	*Chelyconus*	7,543	SRA: SRR6983169
Conus marmoreus	*Conus*	18,596	SRA: SRX5015020, SRX1323884
Conus gloriamaris	*Cylinder*	15,437, 14,178	SRA: SRX2779517, SRR5499408
Conus textile	*Cylinder*	19,161	SRA: SRX5015023
Conus episcopatus	*Darioconus*	5,767	DDBJ: SAMD00029746
Conus betulinus	*Dendroconus*	32,400	SRA: SRR2124881
Conus geographus	*Gastridium*	9,191	SRA: SRX151242, SRX151241, SRX151240, SRX151239
Conus sponsalis	*Harmoniconus*	3,700	SRA: SRX1323890
Conus trochulus	*Kalloconus*	18,552	SRA: SRR11807506
Conus antoniomonteiroi	*Lautoconus/Africonus*	26,034	SRA: SRR11807494
Conus boavistensis	*Lautoconus/Africonus*	6,882	SRA: SRR11807497
Conus cuneolus	*Lautoconus/Africonus*	4,220	SRA: SRR11807496
Conus galeao	*Lautoconus/Africonus*	8,962	SRA: SRR11807500
Conus grahami	*Lautoconus/Africonus*	10,682	SRA: SRR11807507
Conus infinitus	*Lautoconus/Africonus*	12,104	SRA: SRR11807493
Conus maioensis	*Lautoconus/Africonus*	45,991	SRA: SRR11807501, SRR11807499
Conus miruchae	*Lautoconus/Africonus*	12,992	SRA: SRR11807495
Conus raulsilvai	*Lautoconus/Africonus*	18,028	SRA: SRR11807492
Conus verdensis	*Lautoconus/Africonus*	14,942	SRA: SRR11807498
Conus guanche	*Lautoconus/Varioconus*	17,586	SRA: SRR11807502
Conus mercator	*Lautoconus/Varioconus*	27,651	SRA: SRR11807503, SRR11807505
Conus reticulatus	*Lautoconus/Varioconus*	11,242	SRA: SRR11807504
Conus lividus	*Lividoconus*	3,951	SRA: SRX1323888
Conus quercinus	*Lividoconus*	13,002	CNGB: CNS0048932
Conus magus	*Pionoconus*	45,382	SRA: SRX5015024, SRR9831255
Conus striatus	*Pionoconus*	29,445	SRA: SRX5015022
Conus arenatus	*Puncticulis*	3,581	SRA: SRX1323893
Conus caracteristicus	*Puncticulis*	20,037	CNGB: CNS0048931
Conus rattus	*Rhizoconus*	3,982	SRA: SRX1323889
Conus bayani	*Splinoconus*	43,997	SRA: SRR13781584
Conus imperialis	*Stephanoconus*	2,690	SRA: SRX1323891
Conus generalis	*Strategoconus*	25,594	CNGB: CNS0048933
Conus varius	*Strategoconus*	5,606	SRA: SRX1323892
Conus terebra	*Virgiconus*	11,520	SRA: SRX5015025
Conus virgo	*Virgiconus*	25,178	SRA: SRX1323883, SRX5015021
Conus coronatus	*Virroconus*	2,715	SRA: SRX1323894
Conus ebraeus	*Virroconus*	3,856	SRA: SRX1323887

Movie S1: The taser-and-tether hunting strategy. *Conus bullatus* (type species of the *Textilia* clade) catching a blenny.

Movie S2: The net hunting strategy. *Conus geographus* (type species of the *Gastridium* clade) chasing after and catching a group of fish.

Movie S3: The ambush-and-assess hunter *Conus neocostatus* envenomates a fang blenny, *Meiacanthus grammistes*.

Movie S4: The ambush-and-assess hunter *Conus neocostatus* envenomates a fish (genus Amblyeleotris) with two strikes, caught on night camera.

Movie S5: The ambush-and-assess hunter *Conus neocostatus* catches a blenny with two strikes.

Movie S6: *Conus bullatus* being attacked by an aggressive fish (type species of the *Textilia* clade).