

SUPPORTING INFORMATION

Design and synthesis of cyclic lipidated peptides derived from the C-terminus of Cx43 for hemichannel inhibition and cardiac endothelium targeting

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Content

1.	Circular Dichroism (CD)	
	spectra.....	2
2.	Biological activity.....	2
3.	Peptide characterization.....	4
	<i>3a. HPLC chromatograms of</i>	
	<i>peptides.....</i>	<i>4</i>
	<i>3b. Characterization.....</i>	<i>15</i>

1. Circular Dichroism (CD) spectra

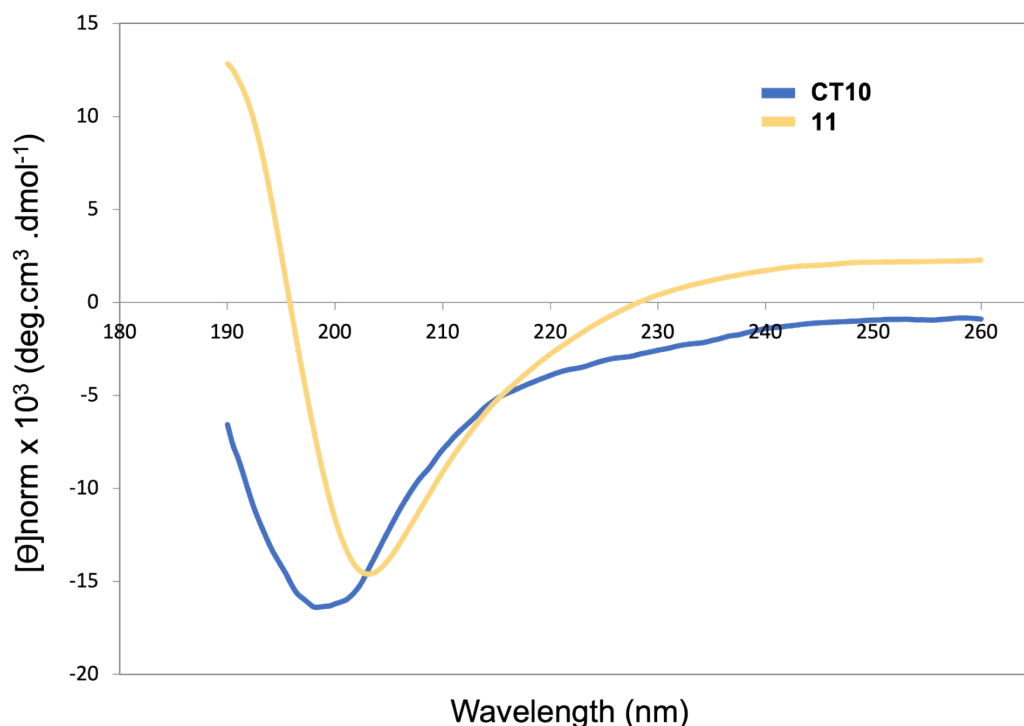


Figure S1. CD spectra of linear peptide **CT10** (blue) and cyclic analogue **11** (yellow) at 200 μM concentration in mQ water. A minimum at 200 nm indicates a random coil conformation.

2. Biological activity

Table S1. ATP release data shown in **Figure 5a**.

	ATP release ^a	SEM
Control	38.4228	3.8230
Calcium-free	100	/
Cbx	43.7932	5.5259
Triton	248.6069	26.2110
TAT-Gap19 100 μM	215.7692	16.4991
TAT-Gap19 50 μM	127.3083	5.5652
TAT-Gap19 10 μM	59.2634	2.5058
TAT-Gap19 5 μM	47.4886	1.8262
TAT-Gap19 1 μM	47.6801	6.1592
TAT-Gap19 0.1 μM	54.7775	8.0668
Gap19 100 μM	82.8115	4.2932
Gap19 5 μM	75.4661	2.7612
Gap27 100 μM	81.9131	4.8462
Gap27 5 μM	67.1248	12.4100
αCT1 100 μM	106.6684	26.5628
αCT1 5 μM	38.9928	2.9283

^aMean normalized to calcium-free condition.

Table S2. ATP release data shown in **Figure 5b**. All compounds are used at 5 μM concentration unless otherwise stated.

	ATP release ^a	SEM
Control	49.3877	3.5875
Calcium-free	100	/

Cbx 25 μM	60.4766	7.7089
Gap19	53.6525	6.9376
Gap27	58.4393	5.0960
TAT-Gap19	39.8281	3.6046
αCT1	41.1484	2.3267
CT10	55.4854	3.5503
1	56.0797	4.7121
2	53.0107	4.3760
3	58.4702	4.6215
4	66.2402	8.3886
5	55.5167	5.0309
6	53.7892	5.7785
7	54.2125	4.6657
8	47.8943	6.1749
9	46.7801	5.8877
10	54.7488	7.8867
11	43.9668	4.0310
12	46.6075	5.0229
13	43.8298	4.8075
14	46.2794	4.9171
Palm-CT10	44.6669	4.0904
Palm-Gap19	54.6035	5.4062
15	133.7852	28.8214
16	67.2242	7.8381
17	72.5860	15.9468

^aMean normalized to calcium-free condition.

Table S3. ATP release data shown in **Figure 6b**.

	ATP release^a	SEM
Control	54.5982	4.6247
Calcium-free	100	/
Cbx 25 μM	78.1965	8.6963
Gap27 5 μM	57.8875	5.2452
Gap27 0.1 μM	55.6856	1.3460
TAT-Gap19 5 μM	37.8190	4.6963
TAT-Gap19 0.1 μM	44.7697	3.7317
αCT1 5 μM	42.2267	3.1507
αCT1 0.1 μM	50.5858	5.4295
11 5 μM	43.9424	5.5982
11 0.1 μM	52.4234	4.9713
12 5 μM	62.4101	6.1084
12 0.1 μM	57.9562	9.9702
13 5 μM	51.4148	7.3095
13 0.1 μM	79.7386	20.1216
14 5 μM	54.5492	7.2228
14 0.1 μM	70.4794	12.3845
Palm-CT10 5 μM	58.4669	12.1414
Palm-CT10 0.1 μM	50.8713	4.5227
Palm-Gap19 5 μM	49.4210	6.9054
Palm-Gap19 0.1 μM	55.9035	7.7251
15 5 μM	133.7852	28.8214
15 0.1 μM	81.9773	5.9136
16 5 μM	77.0091	12.0120
16 0.1 μM	44.8448	3.5623

17 5 μM	72.5860	15.9468
17 0.1 μM	54.4498	7.2777
18 5 μM	61.6970	5.0018
18 0.1 μM	52.2265	7.4227

^aMean normalized to calcium-free condition.

Table S4. ATP release data shown in **Figure 8b**. All compounds are used at 5 μ M concentration unless otherwise stated.

	Parental HeLa		HeLa-Cx43	
	ATP release ^a	SEM	ATP release ^a	SEM
Control	1.7647	0.1682	4.0067	0.2481
Calcium-free	1.7919	0.4219	18.2058	1.2244
Cbx 25 μM	0.9207	0.0167	2.1867	0.5339
Gap27	0.9529	0.0983	9.3690	0.9358
TAT-Gap19	1.3701	0.2847	10.9629	0.7689
αCT1	1.6218	0.4958	9.6282	0.1351
11	1.5371	0.4916	8.7419	0.9250

^aMean (in nM).

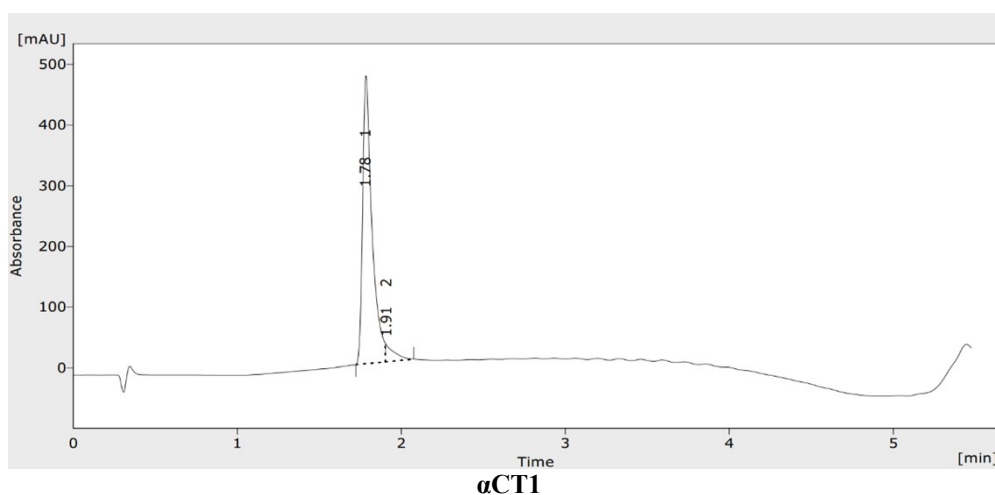
Table S5. ATP release data shown in **Figure 8c**. All compounds are used at 5 μ M concentration unless otherwise stated.

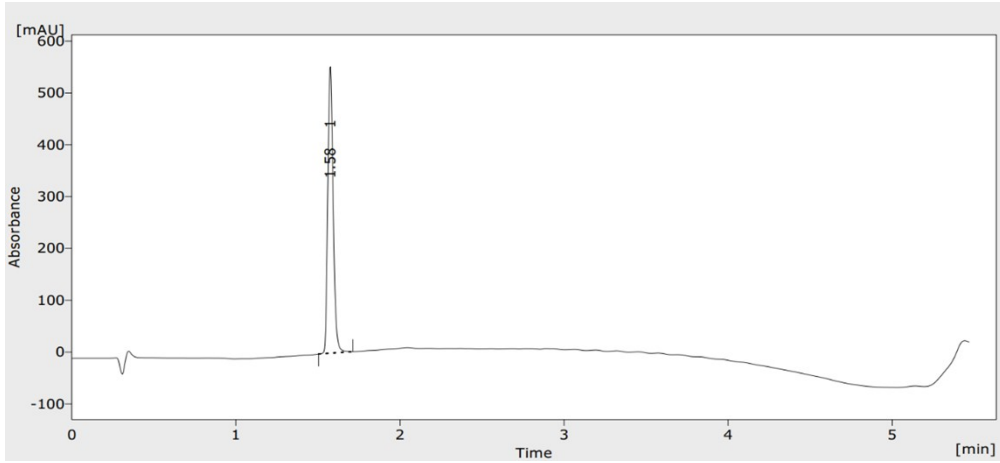
	Parental SK-HEP-1		SK-HEP-1-Cx43	
	ATP release ^a	SEM	ATP release ^a	SEM
Control	10.0176	1.5382	30.0127	3.2453
Calcium-free	28.7505	4.9305	92.3906	17.9121
Cbx 25 μM	13.9931	2.4139	26.4826	3.7740
Gap27	8.5784	0.9461	36.9304	1.2387
TAT-Gap19	15.3639	2.1802	55.5511	7.7043
αCT1	6.6804	1.0241	39.5655	4.5254
11	7.9522	0.3945	38.4712	1.9023

^aMean (in nM).

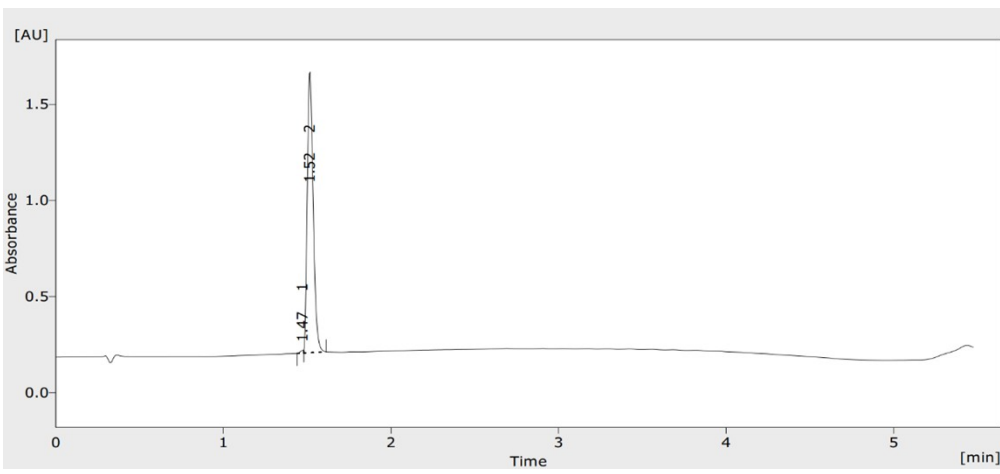
3. Peptide characterization

3a. HPLC chromatograms of peptides

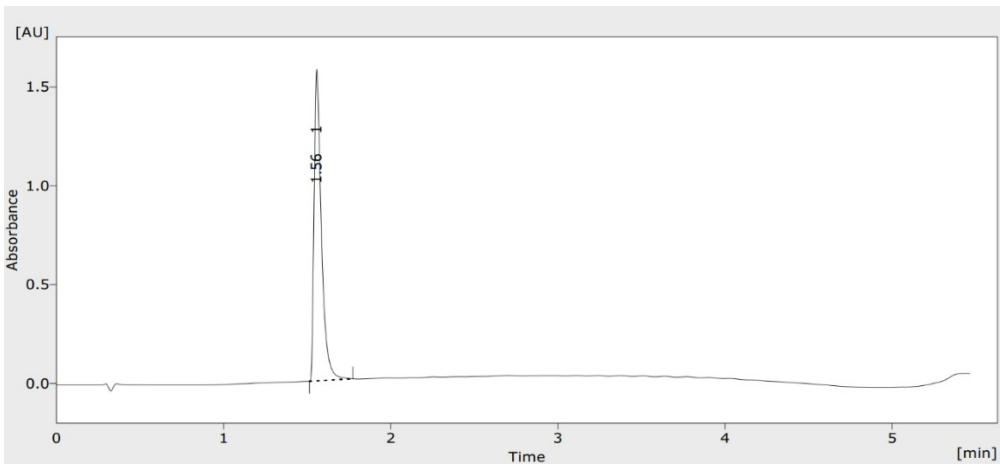




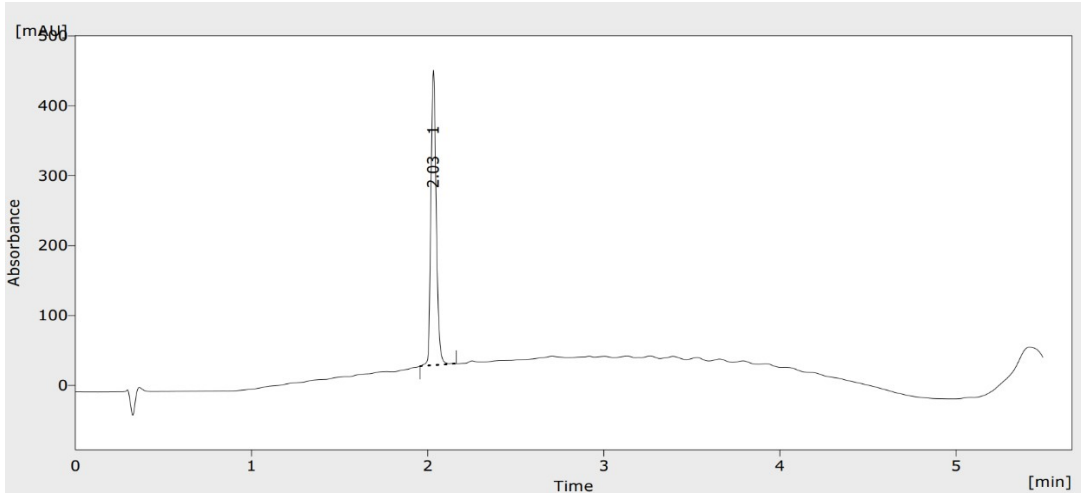
CT10



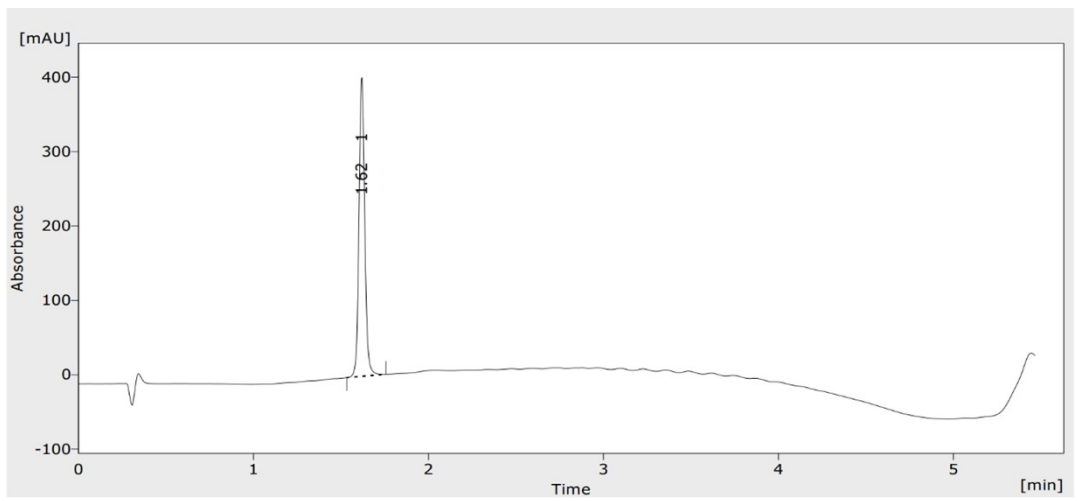
Gap19



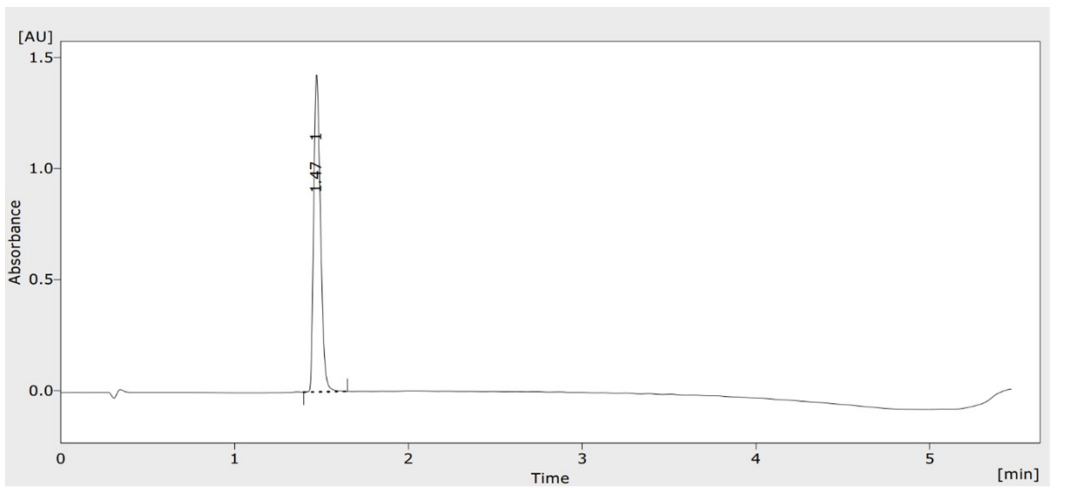
TAT-Gap19



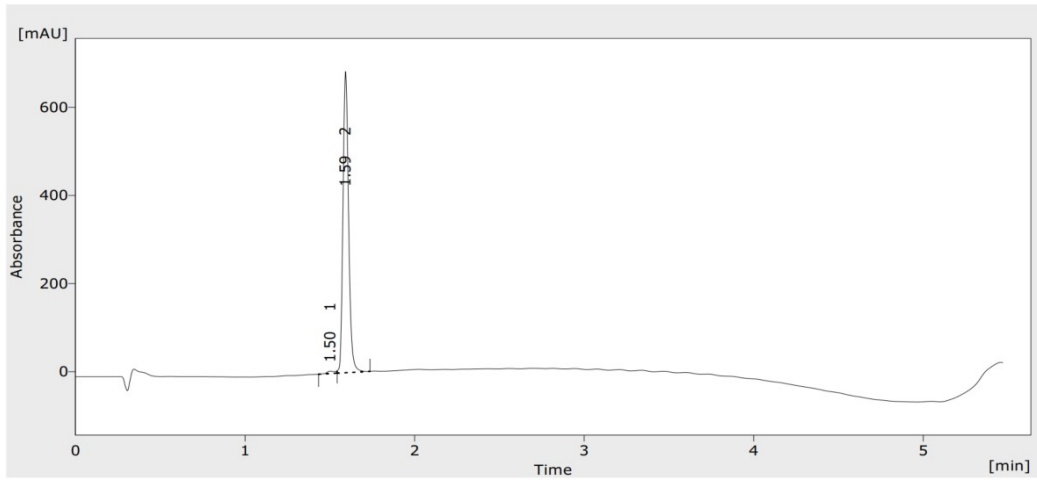
Gap27



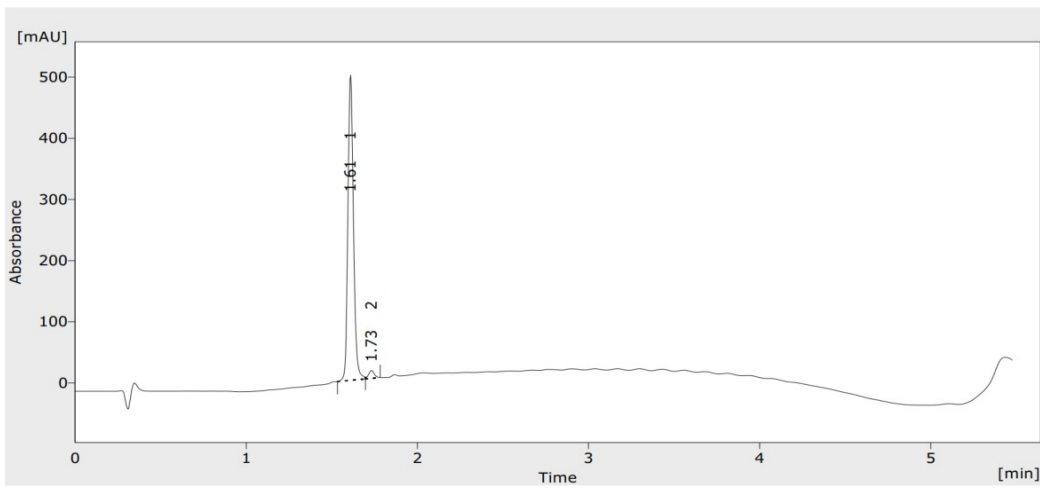
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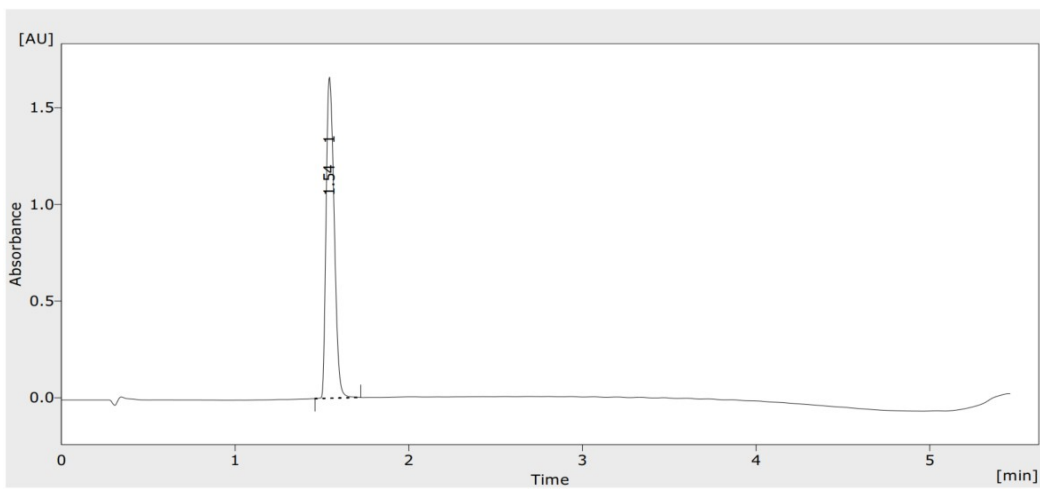
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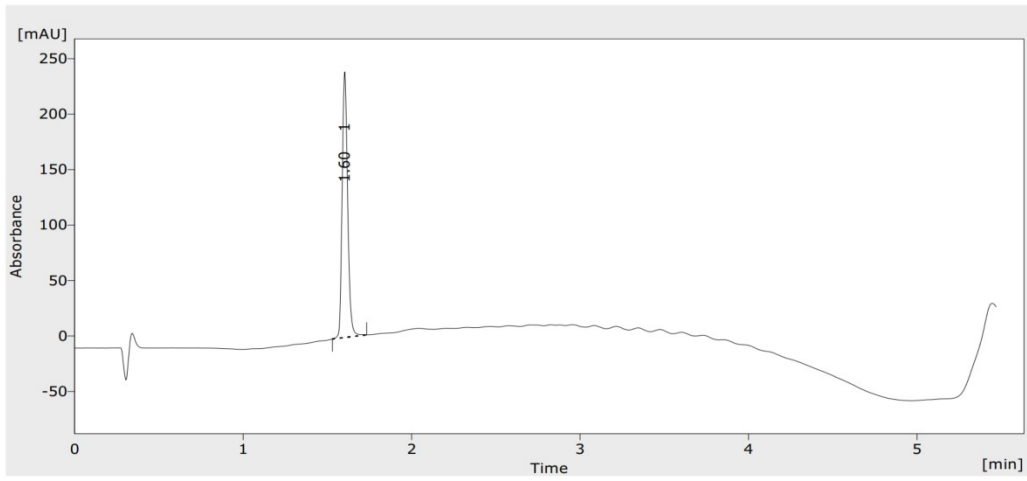
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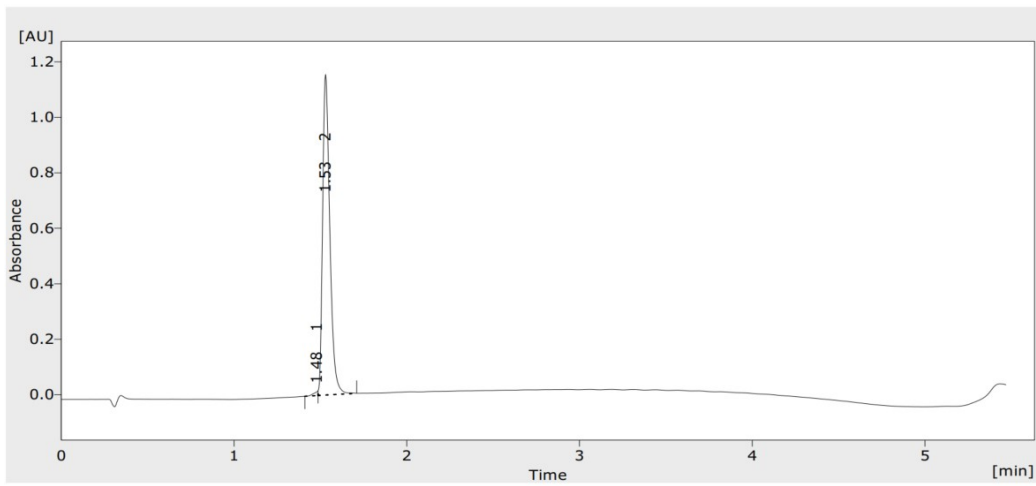
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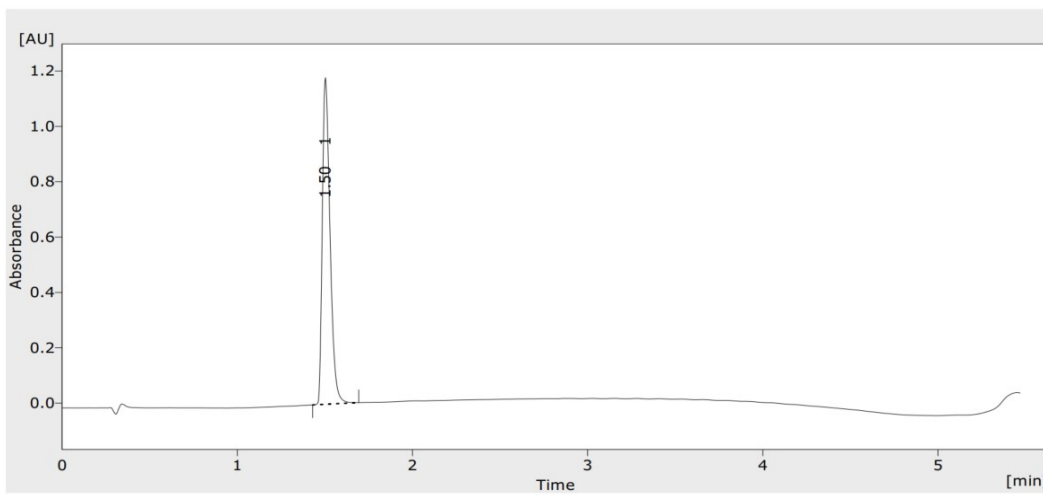
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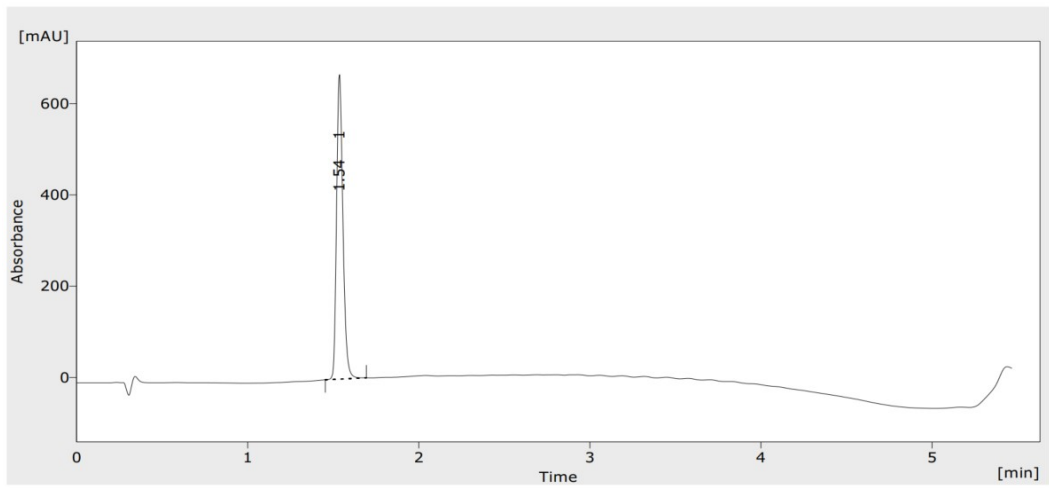
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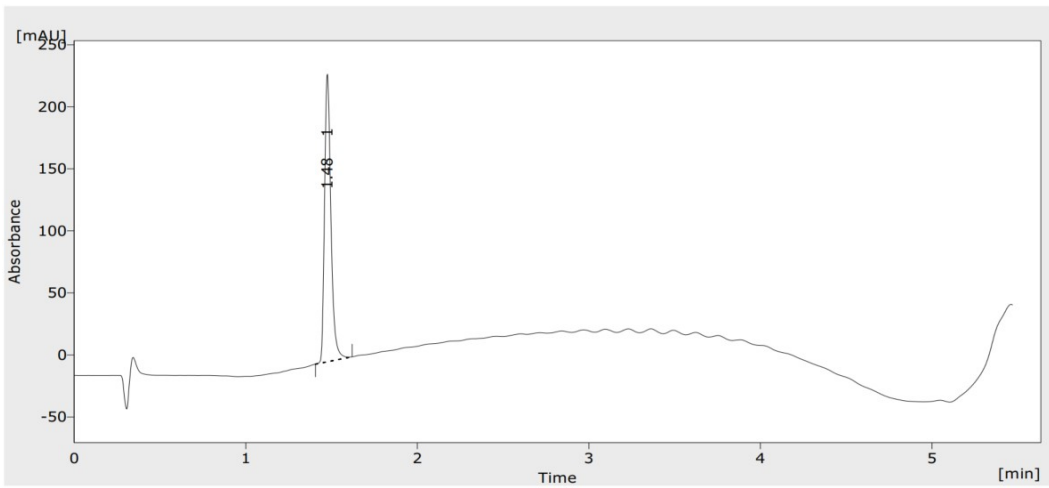
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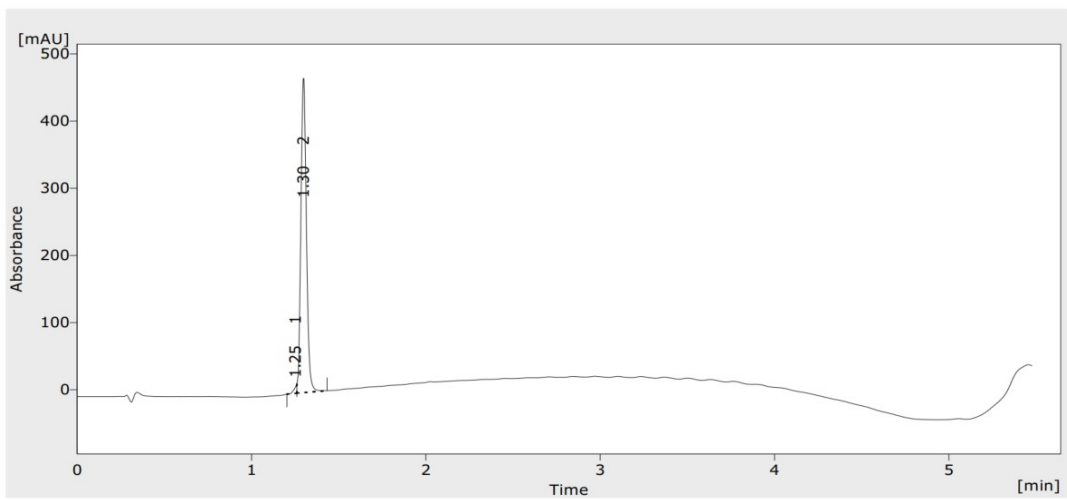
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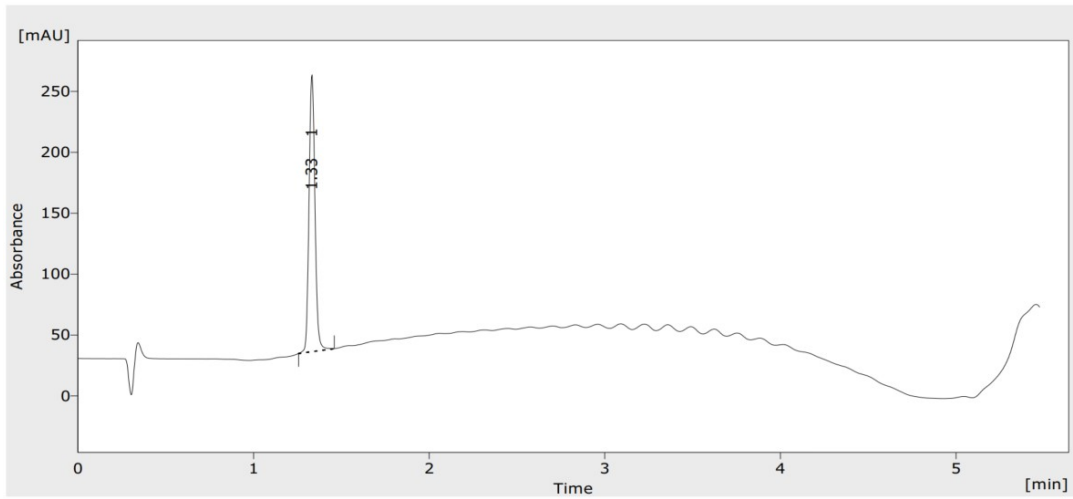
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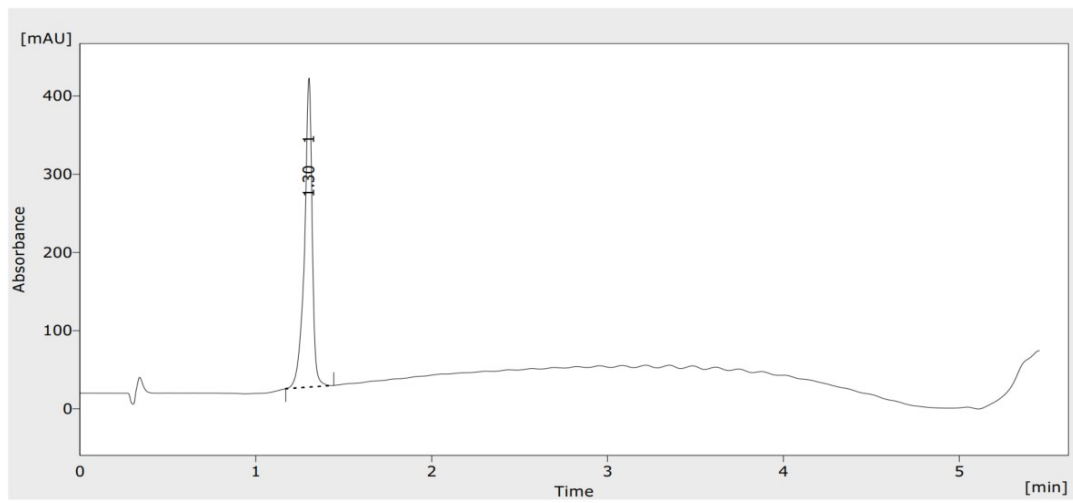
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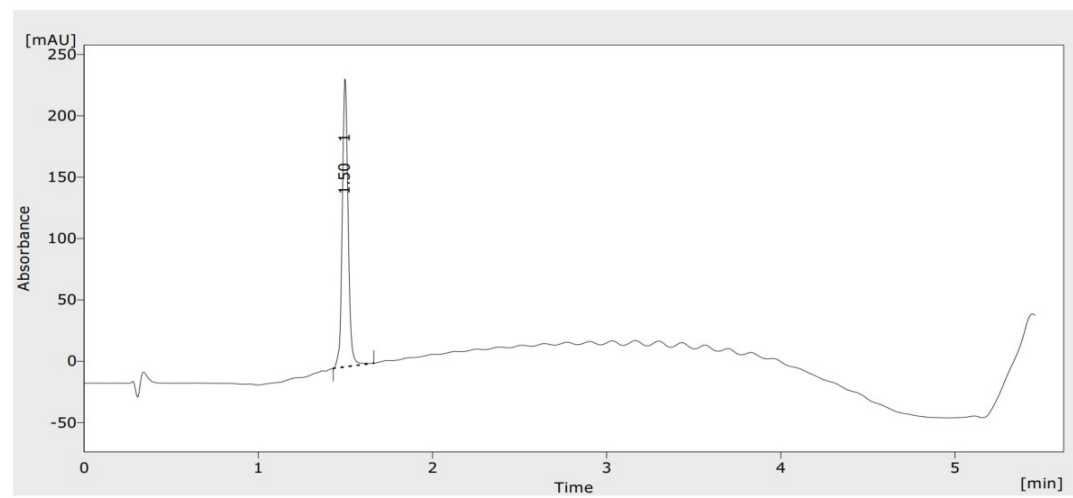
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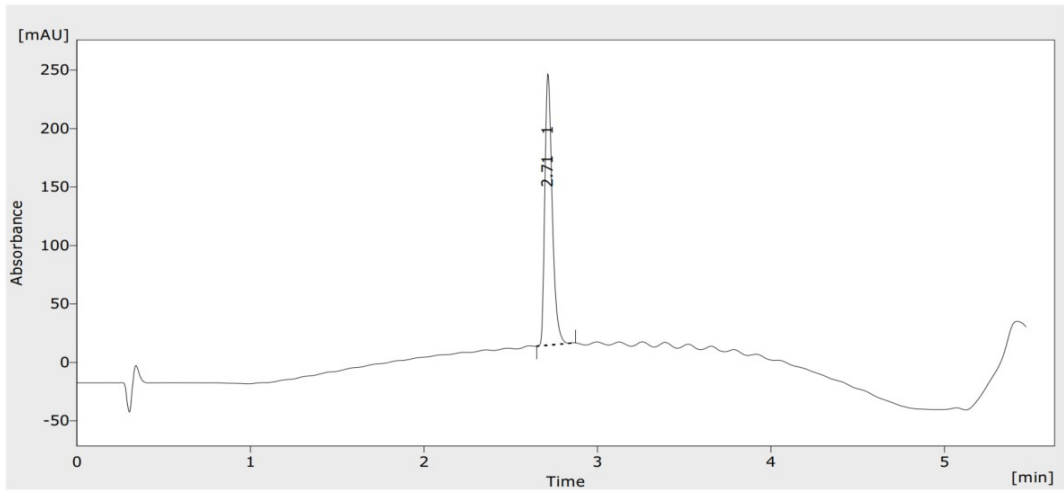
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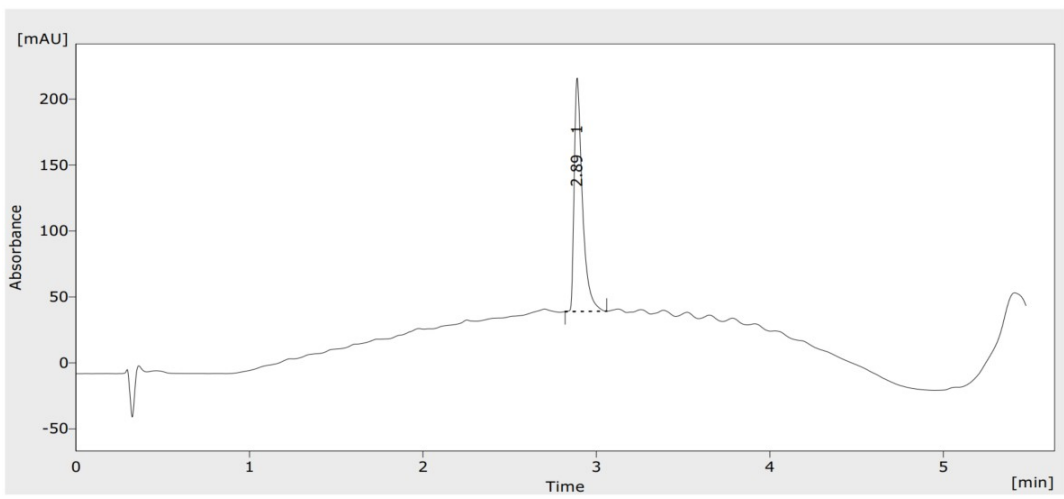
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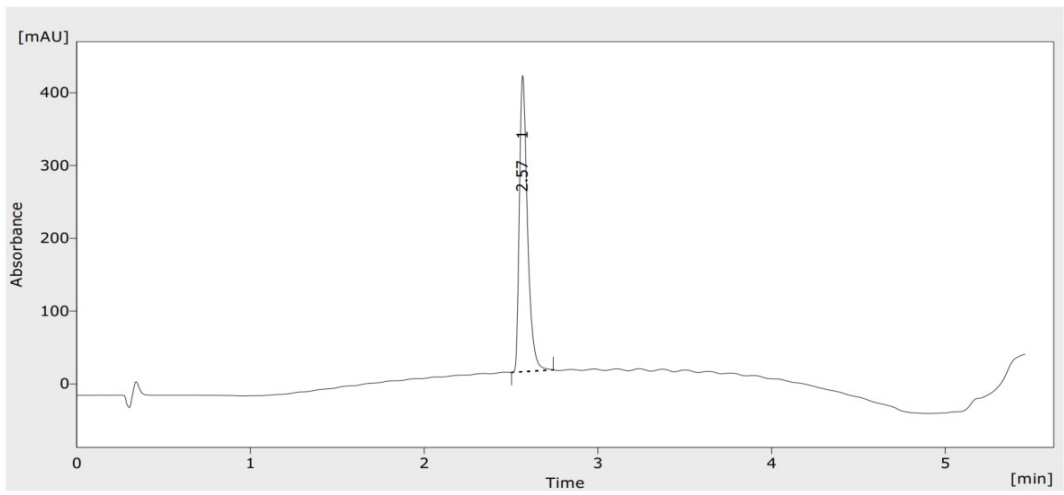
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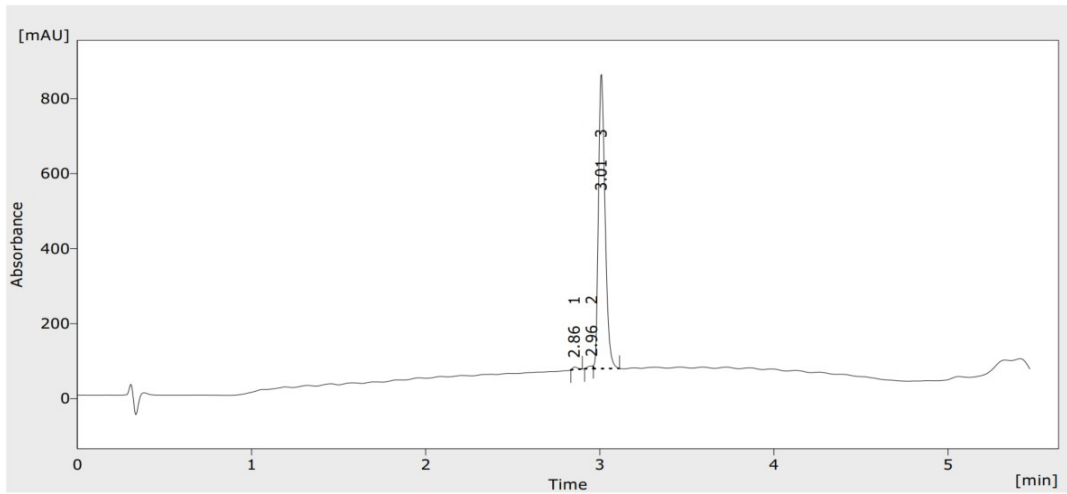
Tat-CT10



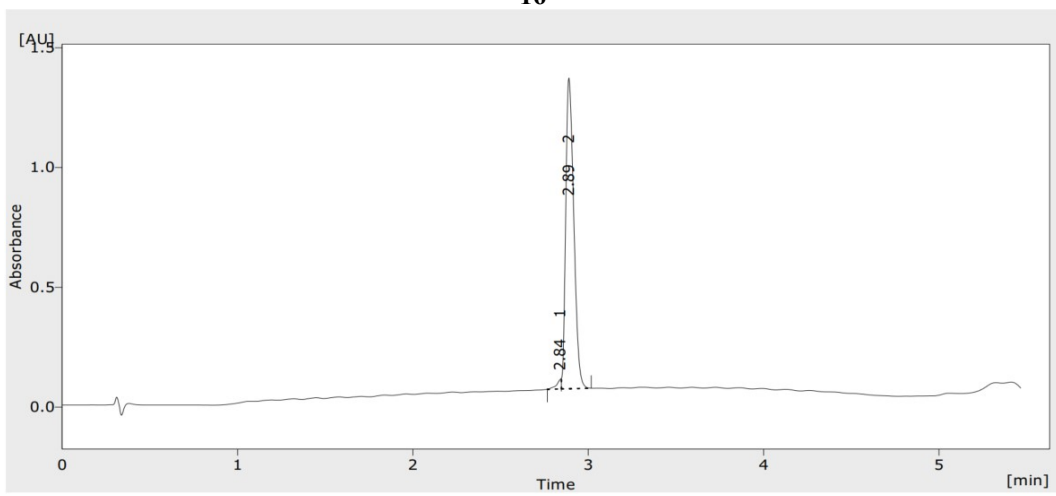
TAT-Gap19



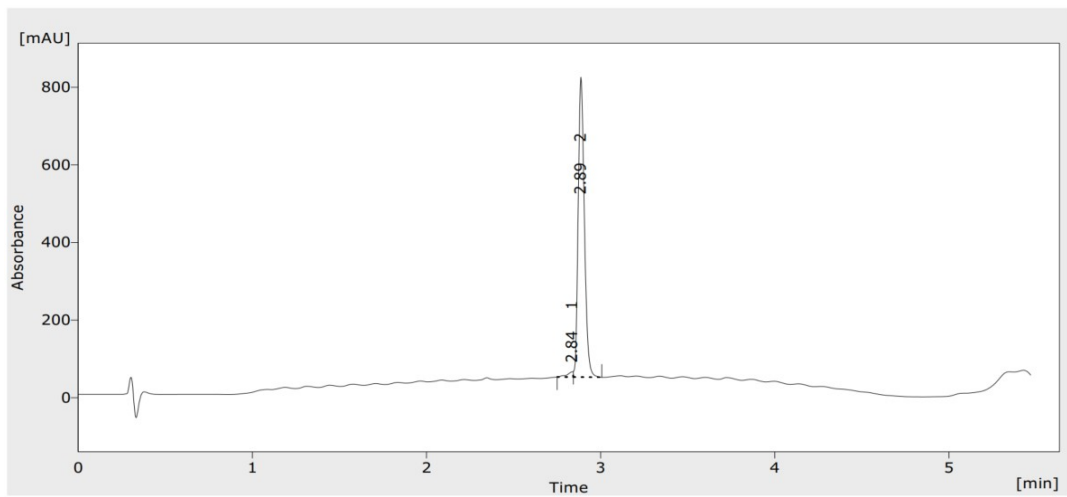
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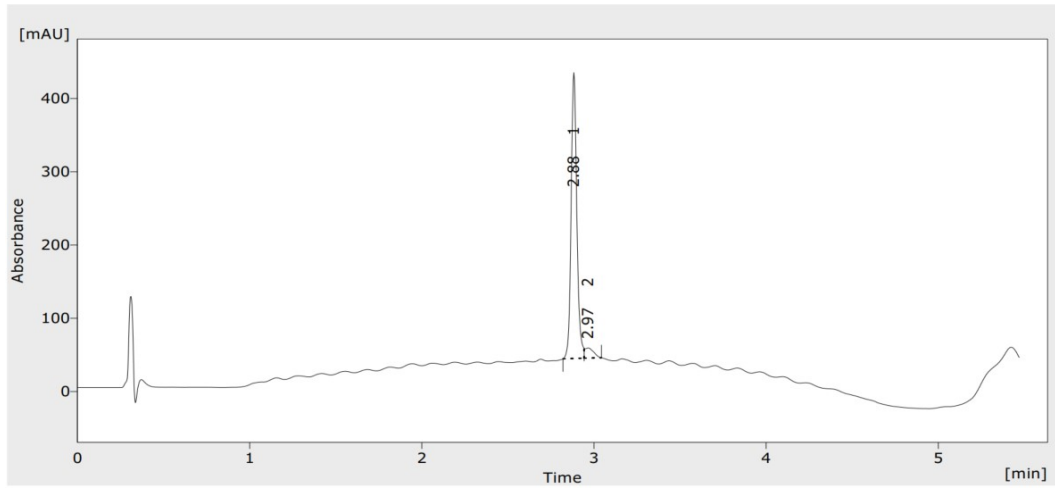
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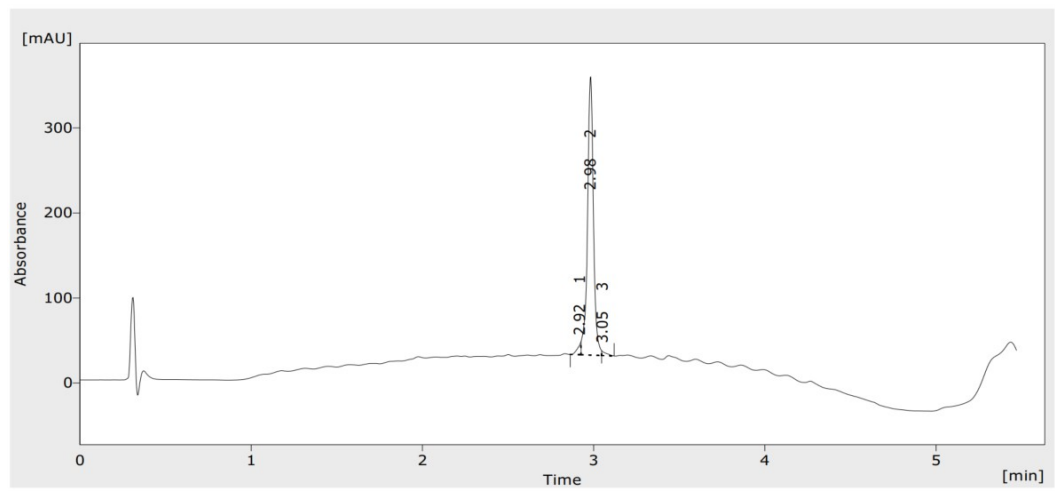
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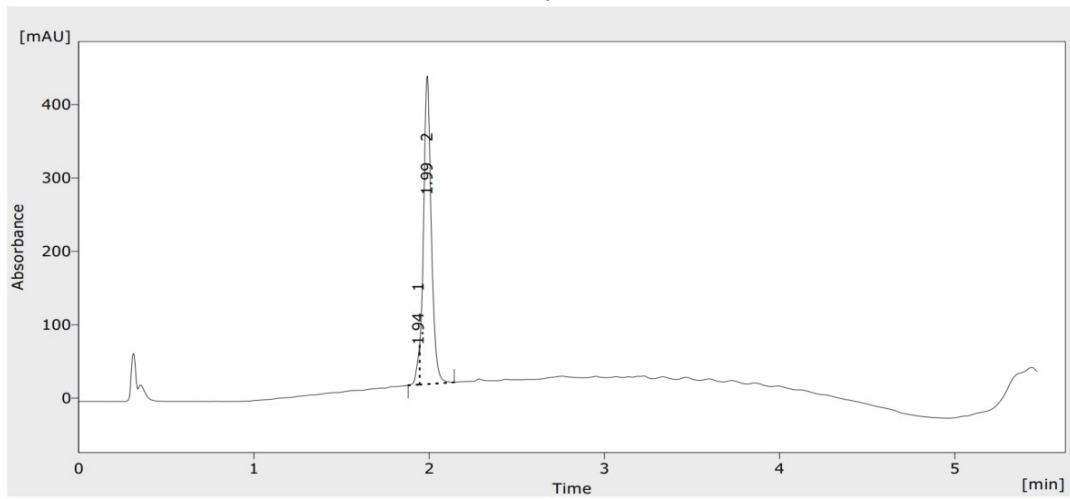
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3b. Characterization

Table S6. a) [M+H]⁺; (b) as determined by RP-HPLC; (c) [M+2H]⁺⁺. Azp = (4S)-4-azidoproline, Pra = propargylglycine, Azk = azidolysine, Palm = palmitic acid, Ahx = 6-aminohexanoic acid. Residues between squares? are part of the peptide macrocycle.

Peptide	Sequence	MW (TFA salt)	HRMS calculated ^(a)	HRMS found ^(a)	Rt ^(b) (min)	Yield (%)	Purity (%)	Formula
αCT1	H-Arg-Gln-Pro-Lys-Ile-Trp-Phe-Pro-Asn-Arg-Arg-Lys-Pro-Trp-Lys-Lys-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	4398.09	1629.4277 ^(c)	1629.4271 ^(c)	1.78	17	95	C150H238N48O34
CT10	H-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	1539.37	1197.6228	1197.6255	1.58	55	>99	C50H84N16O18
Gap19	H-Lys-Gln-Glu-Ile-Lys-Lys-Phe-Lys-OH	1731.57	1161.7360	1161.7383	1.52	49	99	C55H96N14O13
TAT-Gap19	H-Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Lys-Gln-Ile-Glu-Ile-Lys-Lys-Phe-Lys-OH	4185.60	1351.8419 ^(c)	1351.8451 ^(c)	1.56	34	>99	C119H212N46O26
Gap27	H-Ser-Arg-Pro-Thr-Glu-Lys-Thr-Ile-Phe-Ile-Ile-OH	1646.63	1304.7578	1304.7594	2.03	49	>99	C49H85N13O12
1 (SBL-CX43-106)	Ac-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	1467.38	1239.6333	1239.6327	1.62	77	>99	C52H86N16O19
2 (SBL-CX43-107)	H-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-NH ₂	1538.37	1196.6388	1196.6404	1.47	16	99	C50H85N17O17
3 (SBL-CX43-108)	H-Ser-Ala-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	1340.25	1112.5588	1112.5626	1.59	22	99	C47H77N13O18
4 (SBL-CX43-109)	H-Ser-Arg-Pro-Ala-Pro-Asp-Asp-Leu-Glu-Ile-OH	1340.25	1112.5588	1112.5614	1.61	44	98	C47H77N13O18
5 (SBL-CX43-110)	H-Ser-Arg-Pro-Arg-Pro-Ala-Asp-Leu-Glu-Ile-OH	1495.38	1153.6329	1153.6359	1.54	23	>99	C49H84N16O16
6 (SBL-CX43-111)	H-Ser-Arg-Pro-Arg-Pro-Asp-Ala-Leu-Glu-Ile-OH	1495.38	1153.6329	1153.6375	1.60	42	>99	C49H84N16O16
7 (SBL-CX43-112)	H-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Ala-Ile-OH	1481.35	1139.6173	1139.6201	1.53	13	99	C48H82N16O16
8 (SBL-CX43-113)	H-Ser-Arg-Ala-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	1513.35	1171.6072	1171.6097	1.50	20	99	C48H82N16O18
9 (SBL-CX43-114)	H-Ser-Arg-Pro-Arg-Ala-Asp-Asp-Leu-Glu-Ile-OH	1513.35	1171.6072	1171.6073	1.54	48	>99	C48H82N16O18
10 (SBL-CX43-147)	H-Ser-Arg-Ala-Arg-Ala-Asp-Asp-Leu-Glu-Ile-OH	1487.31	1145.5914	1145.5938	1.48	19	>99	C46H80N16O18
11 (SBL-CX43-125)	Ac-Ser-Arg-Pro-Arg- [Azp] -Asp-Asp-Leu-Glu- [Pra] -NH ₂	1489.37	1261.6038	1261.6045	1.30	14	99	C51H80N20O18
12 (SBL-CX43-138)	Ac-Ser-Arg-Pro-Arg- c[Pra] -Asp-Asp-Leu-Glu- [Azk] -NH ₂	1505.41	1277.6351	1277.6340	1.33	9	>99	C52H84N20O18
13 (SBL-CX43-139)	Ac-Ser-Arg-Pro-Arg- c[Azp] -Asp-Asp- [Pra] -Glu-Ile-NH ₂	1489.37	1261.6038	1261.6038	1.30	9	>99	C51H80N20O18
14 (SBL-CX43-140)	Ac- c[Pra] -Arg-Pro-Arg- [Azk] -Asp-Asp-Leu-Glu-Ile-NH ₂	1531.49	1303.6871	1303.6846	1.50	4	95	C55H90N20O17
Palm-CT10 (SBL-CX43-135)	Palm-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	1663.78	1435.8524	1435.8545	2.71	26	99	C66H114N16O19
Palm-Gap19 (SBL-CX43-137)	Palm-Lys-Gln-Ile-Glu-Ile-Lys-Lys-Phe-Lys-OH	1855.97	1399.9656	1399.9698	2.89	31	>99	C71H126N14O14
15 (SBL-CX43-152)	Palm-εLys-PEG-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	2051.13	1709.0212	1709.0217	2.57	6	98	C78H137N19O23
16 (SBL-CX43-153)	Palm-εLys-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	1905.98	1563.9474	1563.9469	3.01	11	98	C72H126N18O20
17 (SBL-CX43-154)	Palm-Lys-Ser-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH	1905.98	1563.9474	1563.9492	2.89	24	98	C72H126N18O20
18 (SBL-CX43-157)	Palm-εLys-Ser-Arg-Pro-Arg- c[Azp] -Asp-Asp-Leu-Glu- [Pra] -NH ₂	1927.95	1585.9178	1585.9183	2.89	4	98	C71H120N22O19
19 (SBL-CX43-158)	Palm-εLys-Ser-Arg-Pro-Arg- c[Azp] -Asp-Asp-Leu-Glu- [Pra] -Ahx-Lys(Fluorescein)-Ahx-Cys-Arg-Pro-Pro-Arg-NH ₂	3476.57	1454.2767 ^c	1454.2802 ^c	2.88	2	95	C135H207N37O33S
20 (SBL-CX43-159)	Palm-εLys-Ser-Arg-Pro-Arg- c[Azp] -Asp-Asp-Leu-Glu- [Pra] -Ahx-Lys(Fluorescein)-NH ₂	2525.61	1093.0762 ^c	1093.0773 ^c	2.98	2	96	C104H153N25O27
21 (SBL-CX43-160)	Ac-Ser-Arg-Pro-Arg- c[Azp] -Asp-Asp-Leu-Glu- [Pra] -Ahx-Lys(Fluorescein)-Ahx-Cys-Arg-Pro-Pro-Arg-NH ₂	3037.99	1292.1196 ^c	1292.1194 ^c	1.99	1	95	C115H167N35O32S