Supplementary Information

Difatty Acyl-Conjugated Linear and Cyclic peptides for siRNA Delivery

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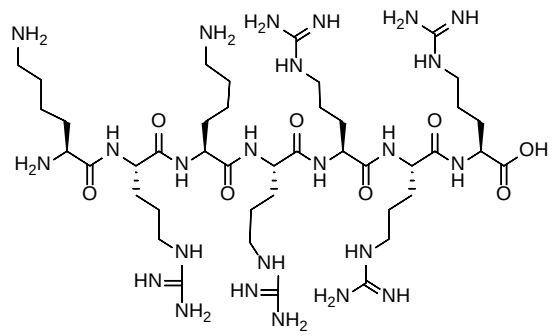
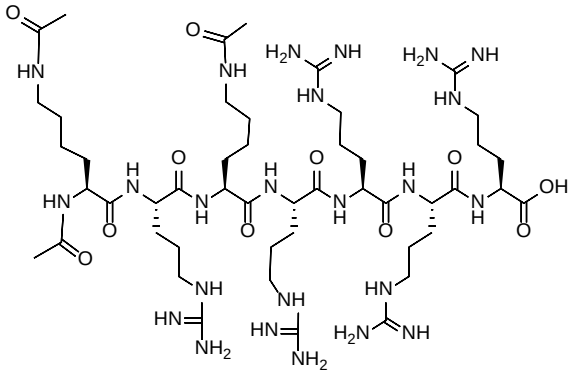


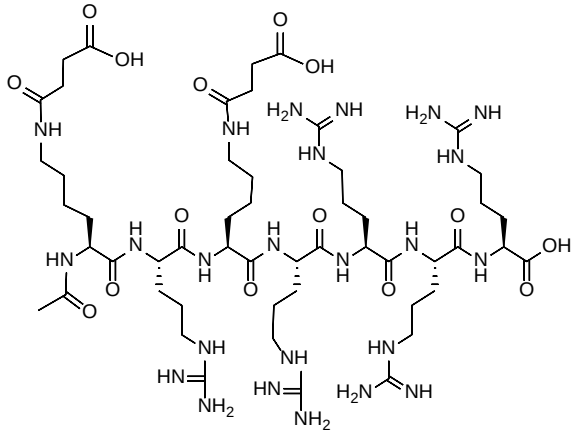
Chemical Formula: $C_{42}H_{86}N_{24}O_8$
Exact Mass: 1054.7060

LP



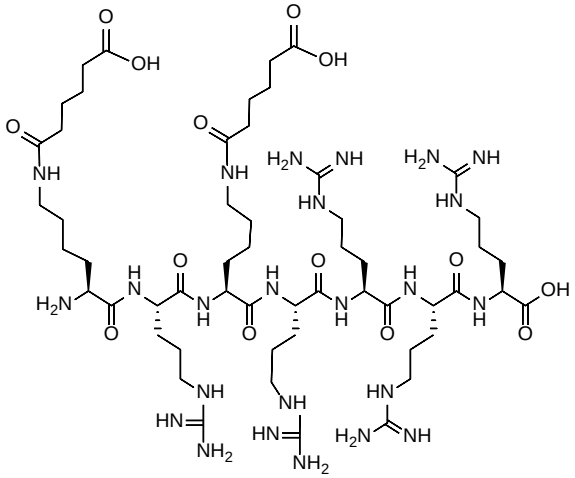
Chemical Formula: $C_{48}H_{92}N_{24}O_{11}$
Exact Mass: 1180.7377

LP-C2



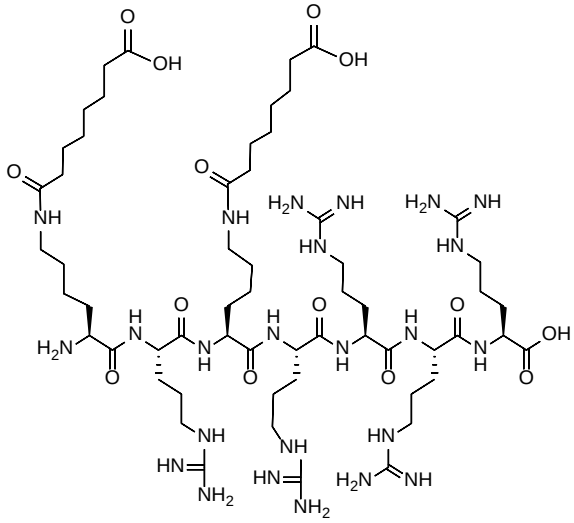
Chemical Formula: $C_{52}H_{96}N_{24}O_{15}$
Exact Mass: 1296.7487

LP-C4



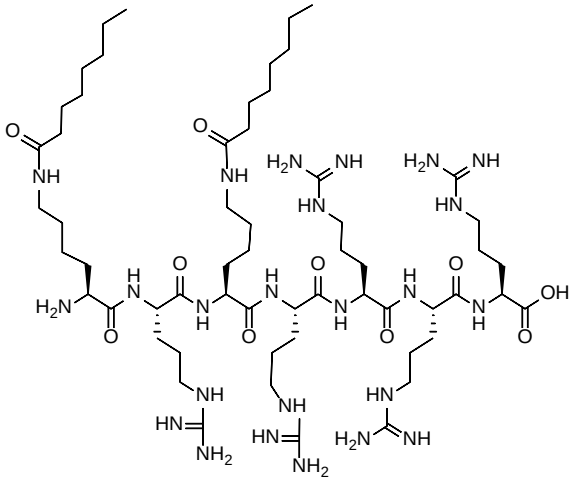
Chemical Formula: $C_{54}H_{102}N_{24}O_{14}$
Exact Mass: 1310.8007

LP-C6



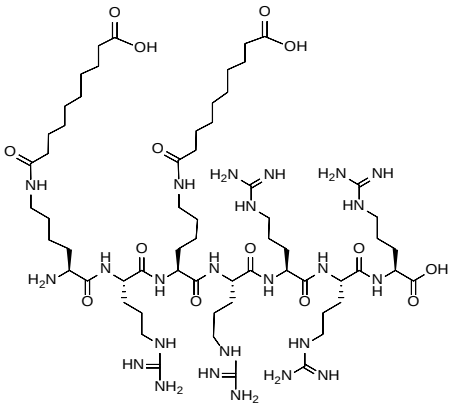
Chemical Formula: $C_{58}H_{110}N_{24}O_{14}$
Exact Mass: 1366.8633

LP-C8



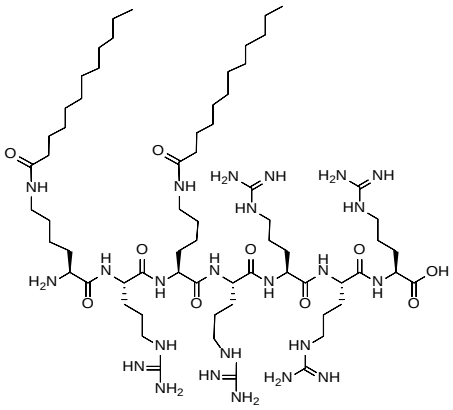
Chemical Formula: $C_{58}H_{114}N_{24}O_{10}$
Exact Mass: 1306.9150

LP-C8*



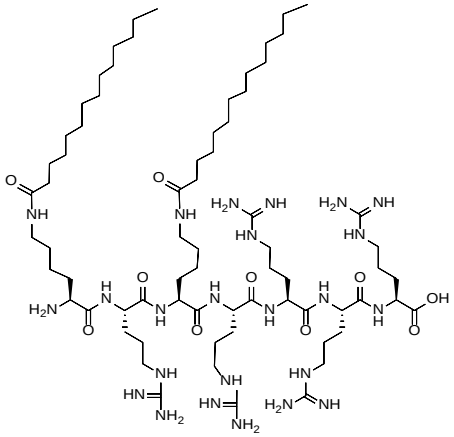
Chemical Formula: $C_{62}H_{118}N_{24}O_{14}$
Exact Mass: 1422.9259

LP-C10



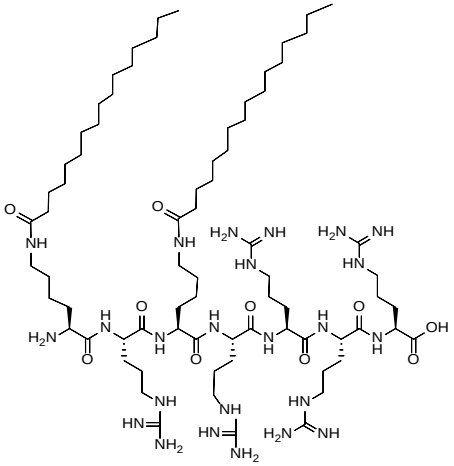
Chemical Formula: $C_{66}H_{130}N_{24}O_{10}$
Exact Mass: 1419.0402

LP-C12



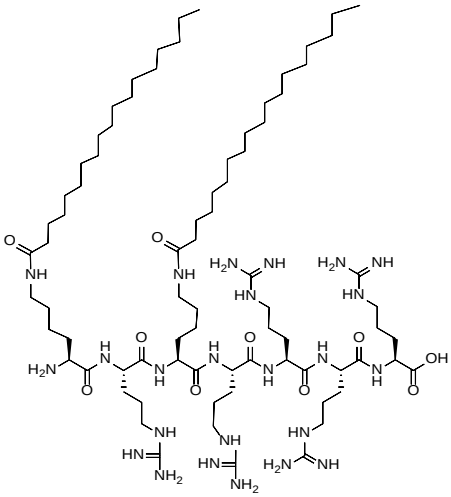
Chemical Formula: $C_{70}H_{138}N_{24}O_{10}$
Exact Mass: 1475.1028

LP-C14



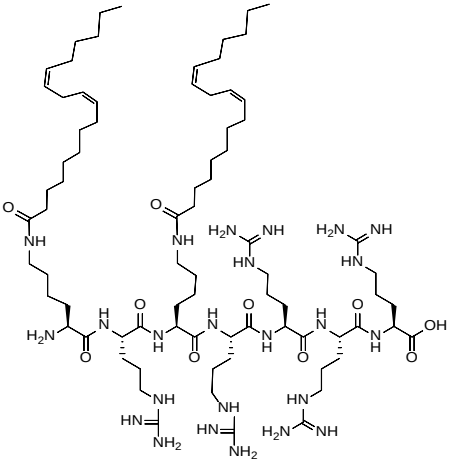
Chemical Formula: $C_{74}H_{146}N_{24}O_{10}$
Exact Mass: 1531.1654

LP-C16



Chemical Formula: $C_{78}H_{154}N_{24}O_{10}$
Exact Mass: 1587.2280

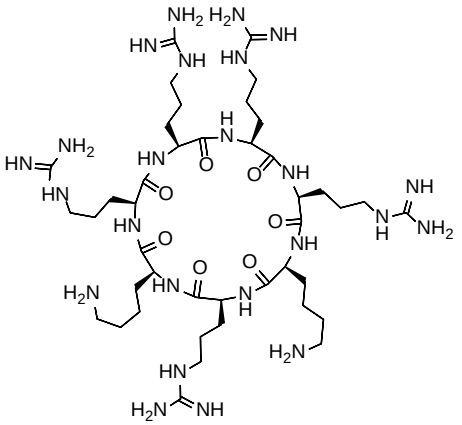
LP-C18



Chemical Formula: $C_{78}H_{146}N_{24}O_{10}$
Exact Mass: 1579.1654

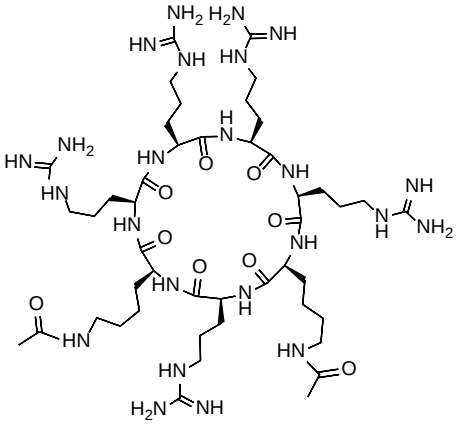
LP-C18*

Figure S1. Chemical structures of linear peptide-fatty acid conjugates



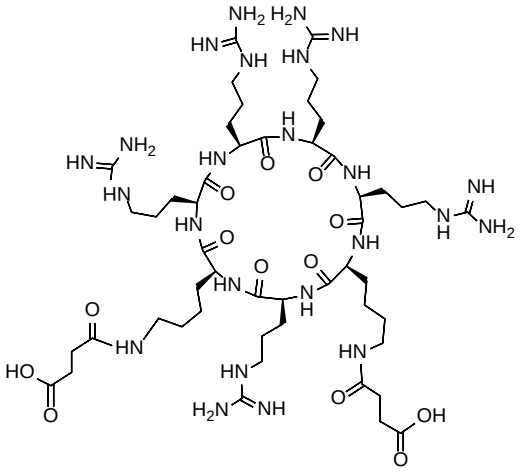
Chemical Formula: $C_{42}H_{84}N_{24}O_7$
Exact Mass: 1036.6955

CP



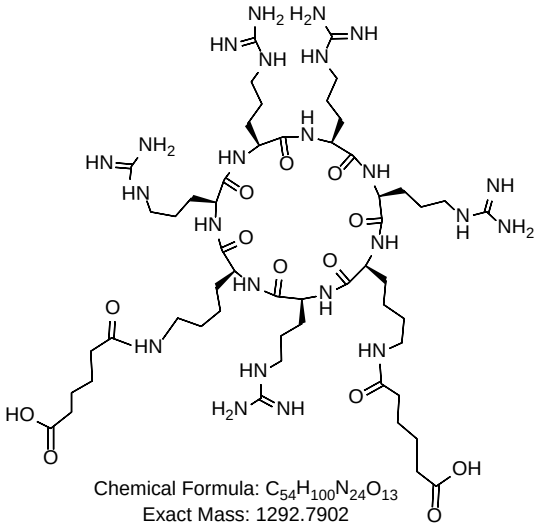
Chemical Formula: $C_{46}H_{88}N_{24}O_9$
Exact Mass: 1120.7166

CP-C2



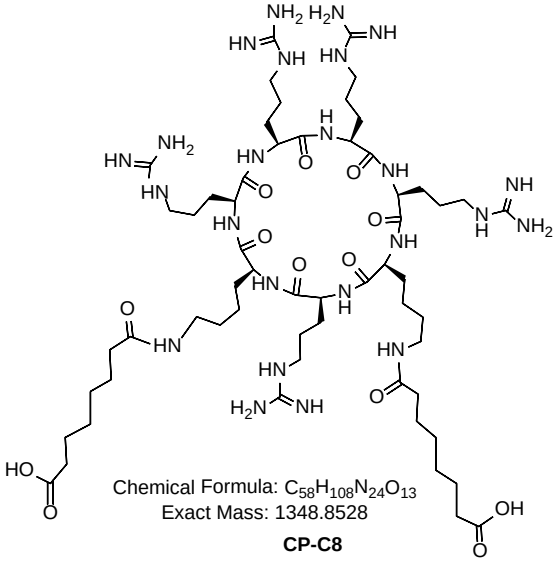
Chemical Formula: $C_{50}H_{92}N_{24}O_{13}$
Exact Mass: 1236.7276

CP-C4



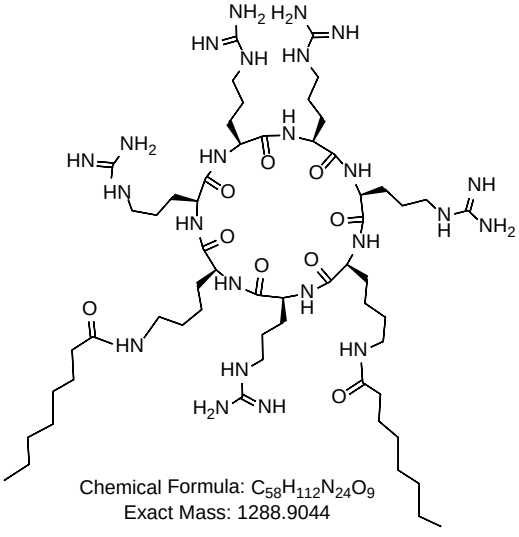
Chemical Formula: $C_{54}H_{100}N_{24}O_{13}$
Exact Mass: 1292.7902

CP-C6



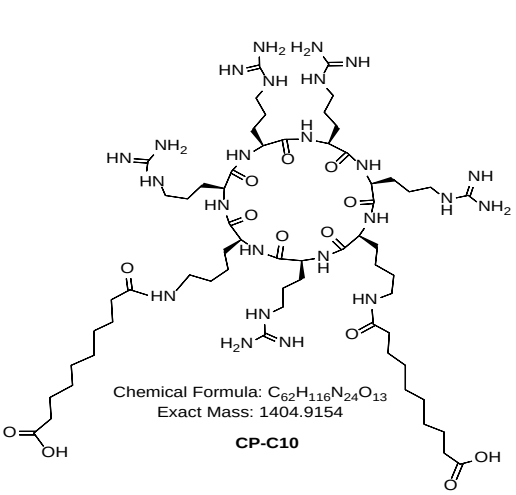
Chemical Formula: $C_{58}H_{108}N_{24}O_{13}$
Exact Mass: 1348.8528

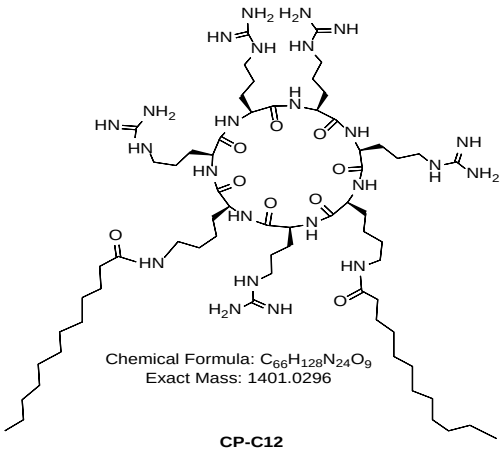
CP-C8

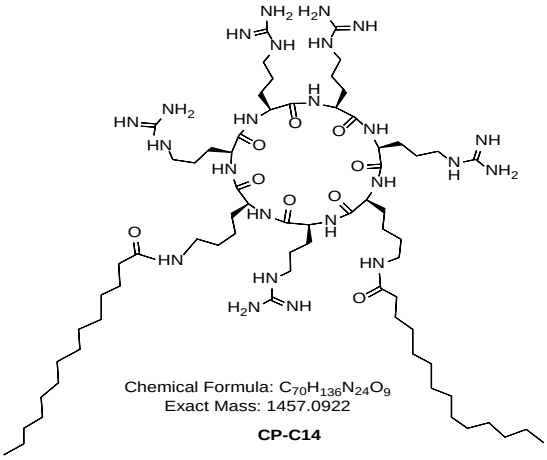


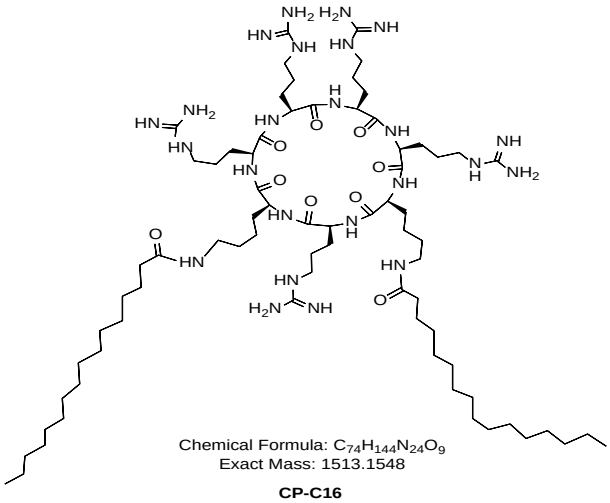
Chemical Formula: $C_{58}H_{112}N_{24}O_9$
Exact Mass: 1288.9044

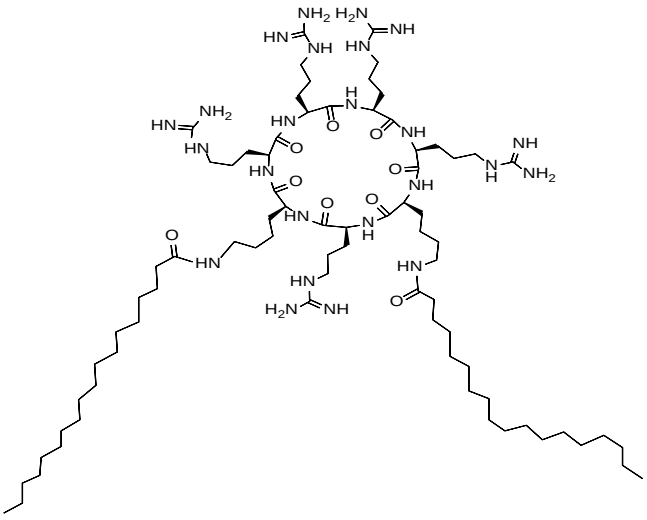
CP-C8*











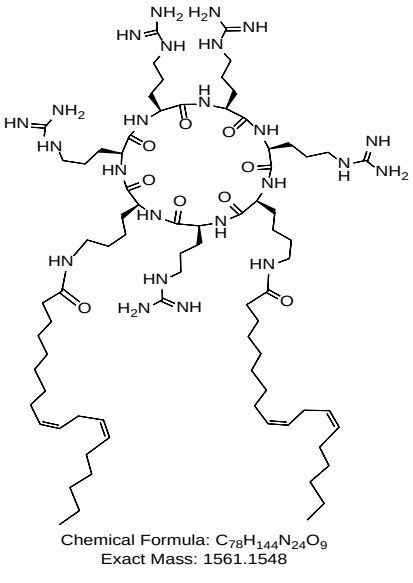


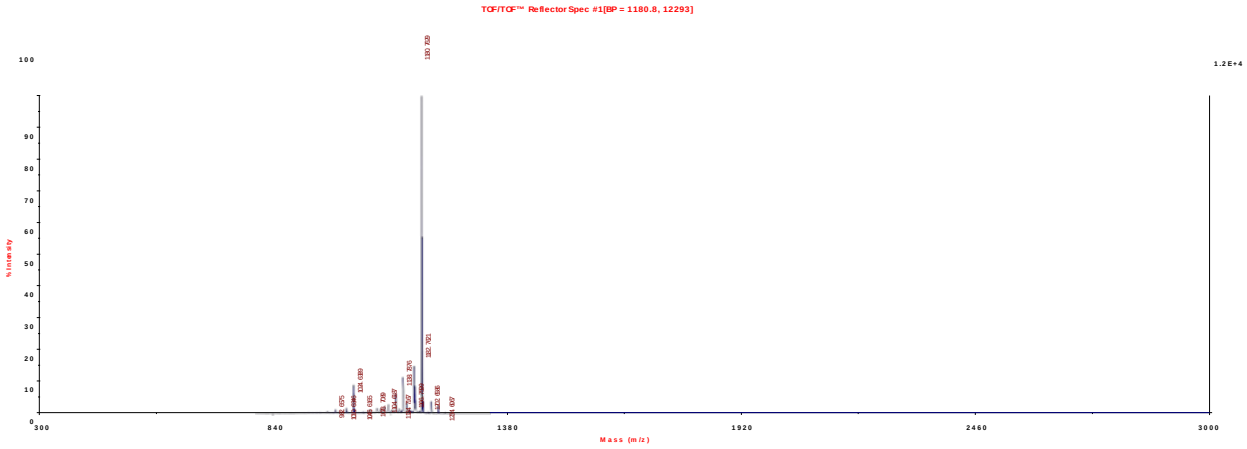
Figure S2. Chemical structures of cyclic peptide-fatty acid conjugates.

Table S1. MALDI-TOF Mass Spectrometer Data for Difatty Acyl Linear and Cyclic Peptides

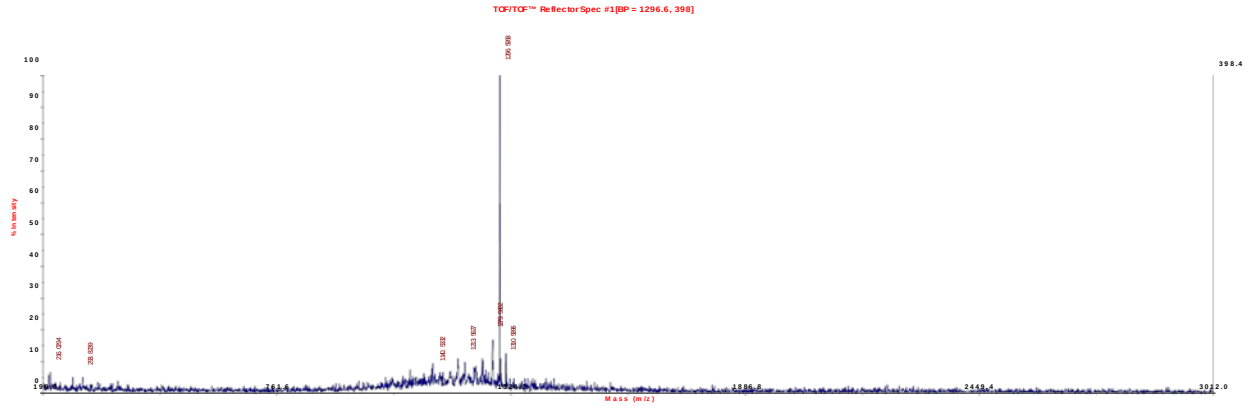
Compound	Molecular Formula	Calculated MW	MALDI-TOF (m/z): found
R_5K_2 (Linear Peptide, LP)	$C_{42}H_{86}N_{24}O_8$	1054.7060	1055.5539 $[M+H]^+$
R_5K_2-C_2 (LP-C2, with *N*-acetyl cap)	$C_{48}H_{92}N_{24}O_{11}$	1180.7377	1180.7629 $[M]^+$
R_5K_2-C_4 (LP-C4, with *N*-acetyl cap)	$C_{52}H_{96}N_{24}O_{15}$	1296.7478	1296.5958 $[M]^+$
R_5K_2-C_6 (LP-C6)	$C_{54}H_{102}N_{24}O_{14}$	1310.8007	1311.4558 $[M+H]^+$
R_5K_2-C_8 (LP-C8)	$C_{58}H_{110}N_{24}O_{14}$	1366.8633	1367.4758 $[M+H]^+$
R_5K_2-C_{8*} (LP-C8*, Caprilic Acid)	$C_{58}H_{114}N_{24}O_{14}$	1306.9150	1307.3541 $[M+H]^+$
R_5K_2-C_{10} (LP-C10, Sebacic Acid)	$C_{62}H_{118}N_{24}O_{14}$	1422.9259	1423.5748 $[M+H]^+$
R_5K_2-C_{12} (LP-C12)	$C_{66}H_{130}N_{24}O_{10}$	1419.0402	1419.8375 $[M+H]^+$
R_5K_2-C_{14} (LP-C14)	$C_{70}H_{138}N_{24}O_{10}$	1475.1028	1475.8762 $[M+H]^+$
R_5K_2-C_{16} (LP-C16)	$C_{74}H_{146}N_{24}O_{10}$	1531.1654	1531.9497 $[M+H]^+$
R_5K_2-C_{18} (LP-C18, Steric Acid)	$C_{78}H_{154}N_{24}O_{10}$	1587.2280	1588.0333 $[M+H]^+$
R_5K_2-C_{18*} (LP-C18*, Linoleic Acid)	$C_{78}H_{146}N_{24}O_{10}$	1579.1654	1582.0409 $[M+3]^+$.
[R_5K_2] (Cyclic Peptide, CP)	$C_{42}H_{84}N_{24}O_7$	1036.6955	1037.9739 $[M+H]^+$
[R_5K_2]-C_2 (CP-2)	$C_{46}H_{88}N_{24}O_9$	1120.7166	1121.8856 $[M+H]^+$
[R_5K_2]-C_4 (CP-4)	$C_{50}H_{92}N_{24}O_{13}$	1236.7276	1296.8387 [M + Na + K -2H]$^+$
[R_5K_2]-C_6 (CP-6)	$C_{54}H_{100}N_{24}O_{13}$	1292.7902	1293.2880 $[M+H]^+$
[R_5K_2]-C_8 (CP-8, Suberic Acid)	$C_{58}H_{108}N_{24}O_{13}$	1348.8528	1349.9468 $[M+H]^+$
[R_5K_2]-C_{8*} (CP-8*, Caprilic Acid)	$C_{58}H_{112}N_{24}O_9$	1289.9044	1311.4558 $[M+Na-H]^+$
[R_5K_2]-C_{10} (CP-10)	$C_{62}H_{116}N_{24}O_{13}$	1404.9154	1405.9323 $[M+H]^+$
[R_5K_2]-C_{12} (CP-12)	$C_{66}H_{128}N_{24}O_9$	1401.0296	1403.3227 $[M+2]^+$
[R_5K_2]-C_{14} (CP-14)	$C_{70}H_{136}N_{24}O_9$	1457.0922	1460.8668 $[M+3]^+$
[R_5K_2]-C_{16} (CP-16)	$C_{74}H_{144}N_{24}O_9$	1513.1548	1514.3129 $[M+H]^+$
[R_5K_2]-C_{18} (CP-18, Steric Acid)	$C_{78}H_{152}N_{24}O_9$	1569.2174	1570.0027 $[M+H]^+$
[R_5K_2]-C_{18*} (CP-18*, Linoleic Acid)	$C_{78}H_{144}N_{24}O_9$	1561.1548	1563.0090 $[M+2]^+$

Table S2 – The N/P ratio for selected w/w peptide:siRNA ratios used in this project

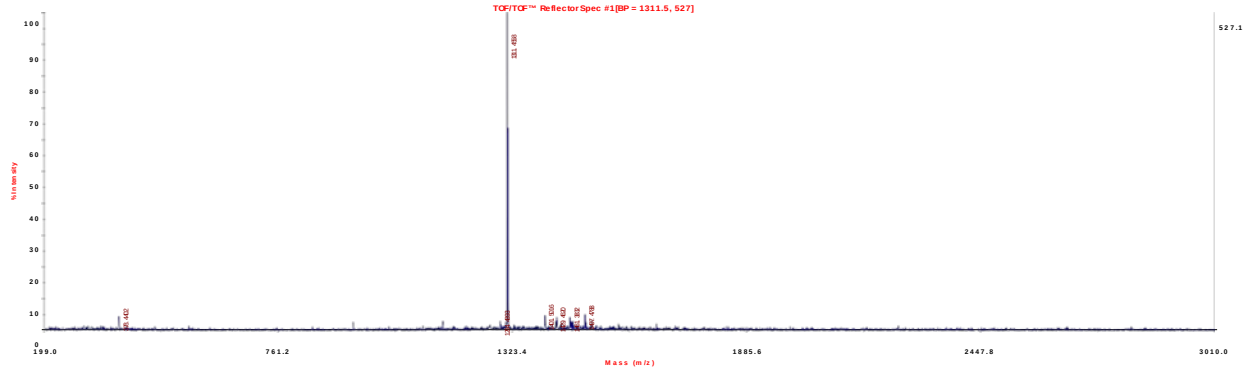
			N/P ratio		
Peptide	Molecular weight	Net Positive Charge	1:1 w/w	1:20 w/w	1:80 w/w
LP	1055	7	2.194749	43.89498	175.5799
LP-C2	1181	4	1.120275	22.40551	89.62203
LP-C4	1297	2	0.510026	10.20053	40.80212
LP-C6	1311	3	0.756838	15.13676	60.54702
LP-C8	1367	3	0.725796	14.51591	58.06366
LP-C8*	1307	5	1.265147	25.30293	101.2117
LP-C10	1423	3	0.6972	13.94399	55.77597
LP-C12	1419	5	1.165181	23.30363	93.21451
LP-C14	1475	5	1.120898	22.41795	89.67181
LP-C16	1531	5	1.079857	21.59713	86.38853
LP-C18	1587	5	1.041715	20.8343	83.3372
LP-C18*	1579	5	1.047034	20.94067	83.76268
CP	1037	7	2.232878	44.65756	178.6303
CP-C2	1121	5	1.475341	29.50682	118.0273
CP-C4	1237	3	0.802168	16.04336	64.17345
CP-C6	1293	3	0.767382	15.34763	61.39053
CP-C8	1349	3	0.735487	14.70974	58.83895
CP-C8*	1290	5	1.281831	25.63662	102.5465
CP-C10	1405	3	0.706138	14.12275	56.491
CP-C12	1401	5	1.18016	23.6032	94.4128
CP-C14	1457	5	1.134753	22.69505	90.78021
CP-C16	1513	5	1.09271	21.8542	87.41679
CP-C18	1569	5	1.053671	21.07342	84.29369
CP-C18*	1561	5	1.059113	21.18226	84.72903



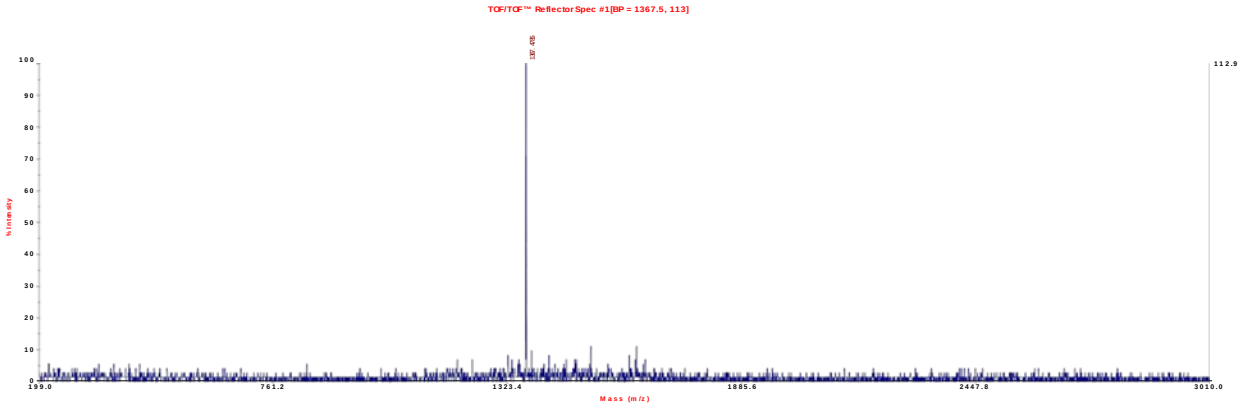
R_5K_2-C_2 (LP-C2, with *N*-acetyl cap): MALDI-TOF (m/z): $C_{48}H_{92}N_{24}O_{11}$ calc: 1180.7377; found: 1180.7629 $[M]^+$.



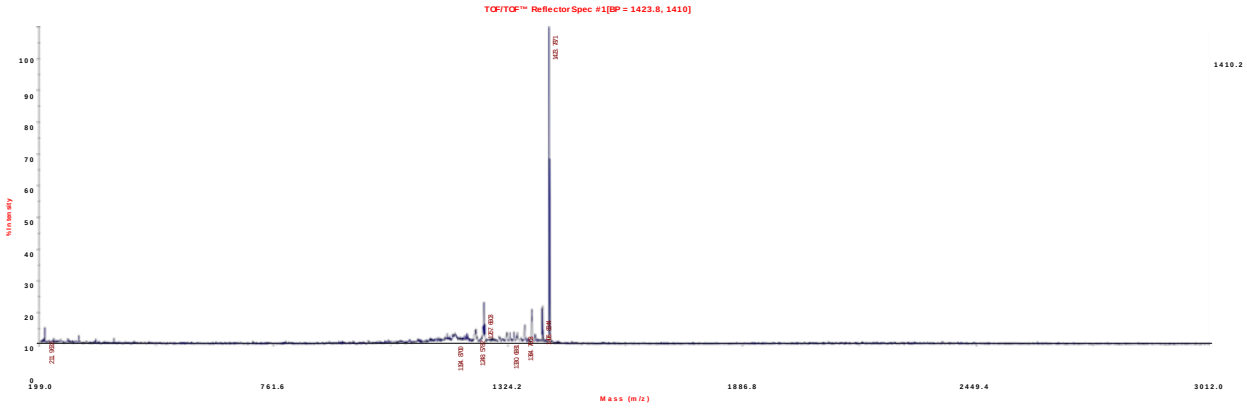
R_5K_2-C_4 (LP-C4 with *N*-acetyl cap): MALDI-TOF (m/z): $C_{52}H_{96}N_{24}O_{15}$ calc: 1296.7478; found: 1296.5958 $[M]^+$.



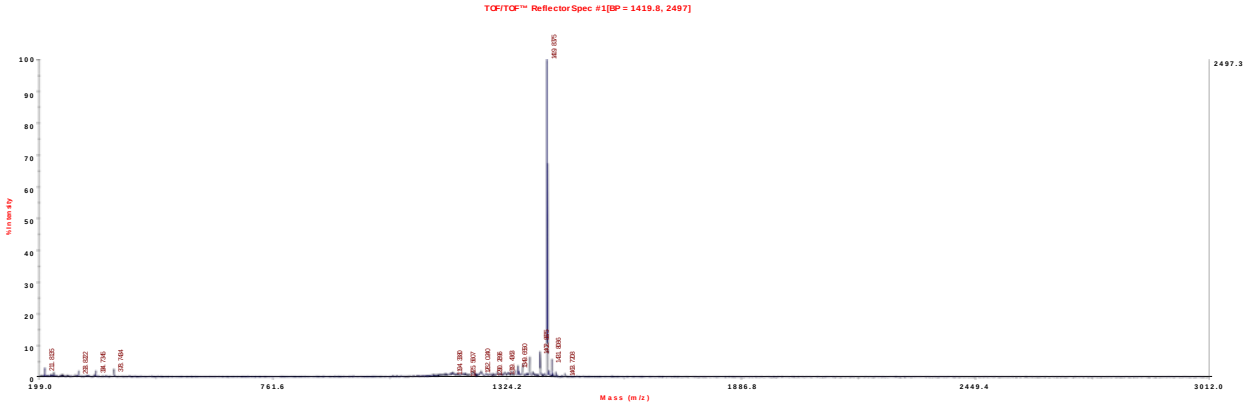
R_5K_2-C_6 (LP-C6): MALDI-TOF (m/z): $C_{54}H_{102}N_{24}O_{14}$ calc: 1310.8007; found: 1311.4558 $[M+H]^+$.



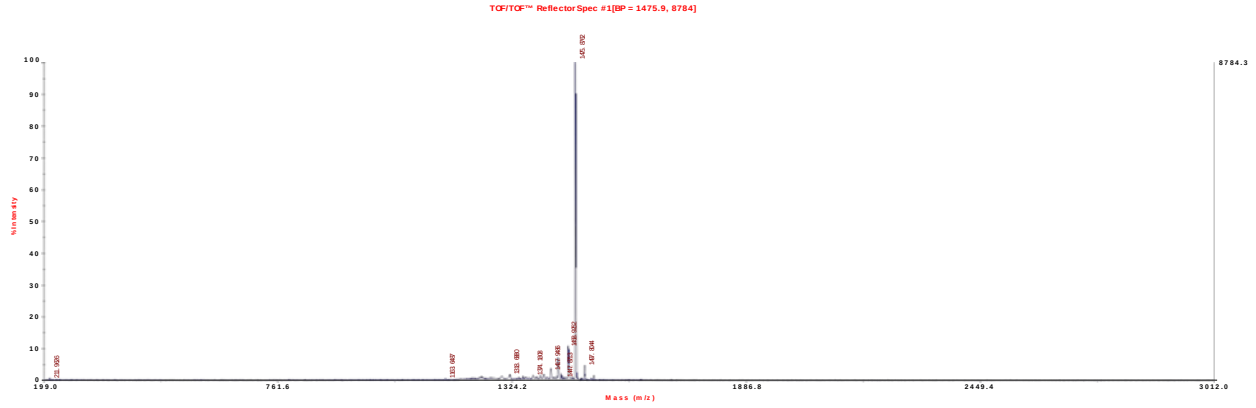
R_5K_2-C_8 (LP-C8): MALDI-TOF (m/z): $C_{58}H_{110}N_{24}O_{14}$ calc: 1366.8633; found: 1367.4758 $[M+H]^+$.



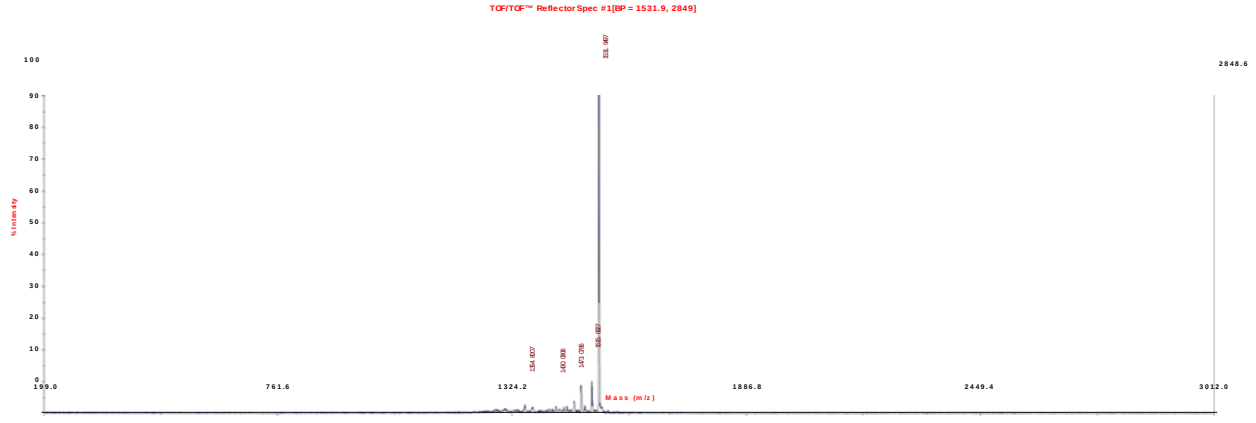
R_5K_2-C_{10} (LP-C10): MALDI-TOF (m/z): $C_{62}H_{118}N_{24}O_{14}$ calc: 1422.9259; found: 1423.5748 $[M+H]^+$.



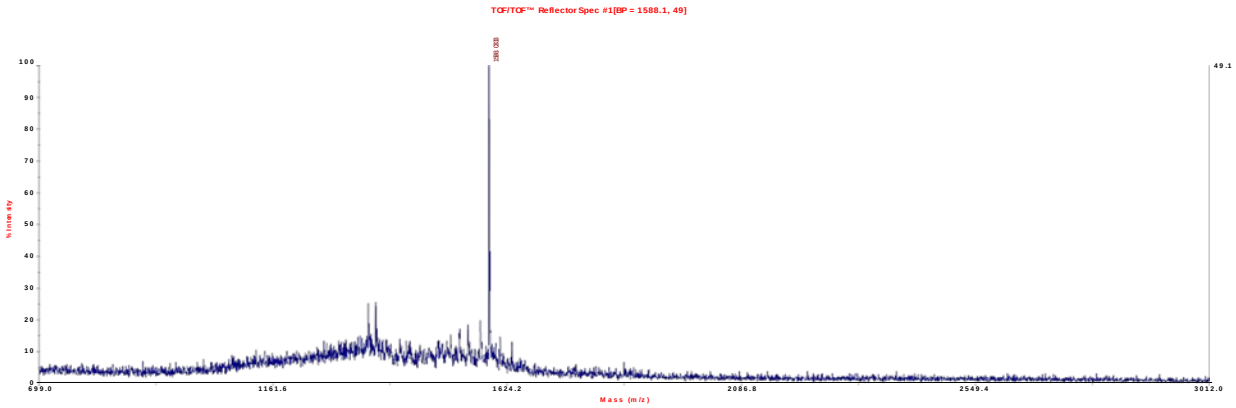
R_5K_2-C_{12} (LP-C12): MALDI-TOF (m/z): $C_{66}H_{130}N_{24}O_{10}$ calc: 1419.0402; found: 1419.8375 $[M+H]^+$.



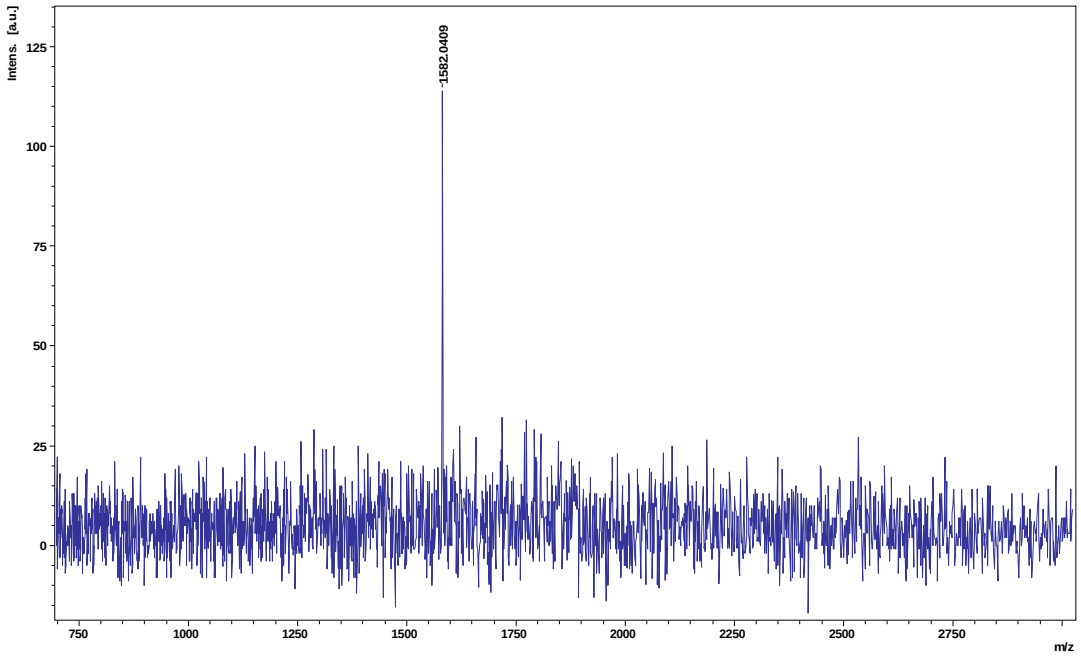
R_5K_2-C_{14} (LP-C14): MALDI-TOF (m/z): $C_{70}H_{138}N_{24}O_{10}$ calc: 1475.1028; found: 1475.8762 $[M+H]^+$.



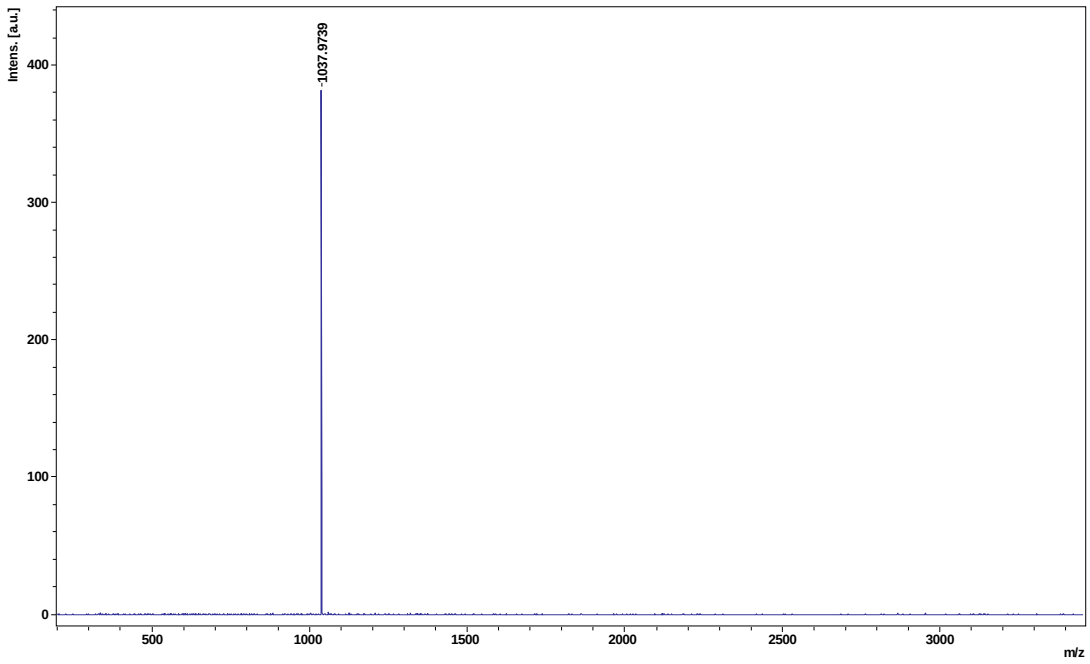
R_5K_2-C_{16} (LP-C16): MALDI-TOF (m/z): $C_{74}H_{146}N_{24}O_{10}$ calc: 1531.1654; found: 1531.9497 $[M+H]^+$.



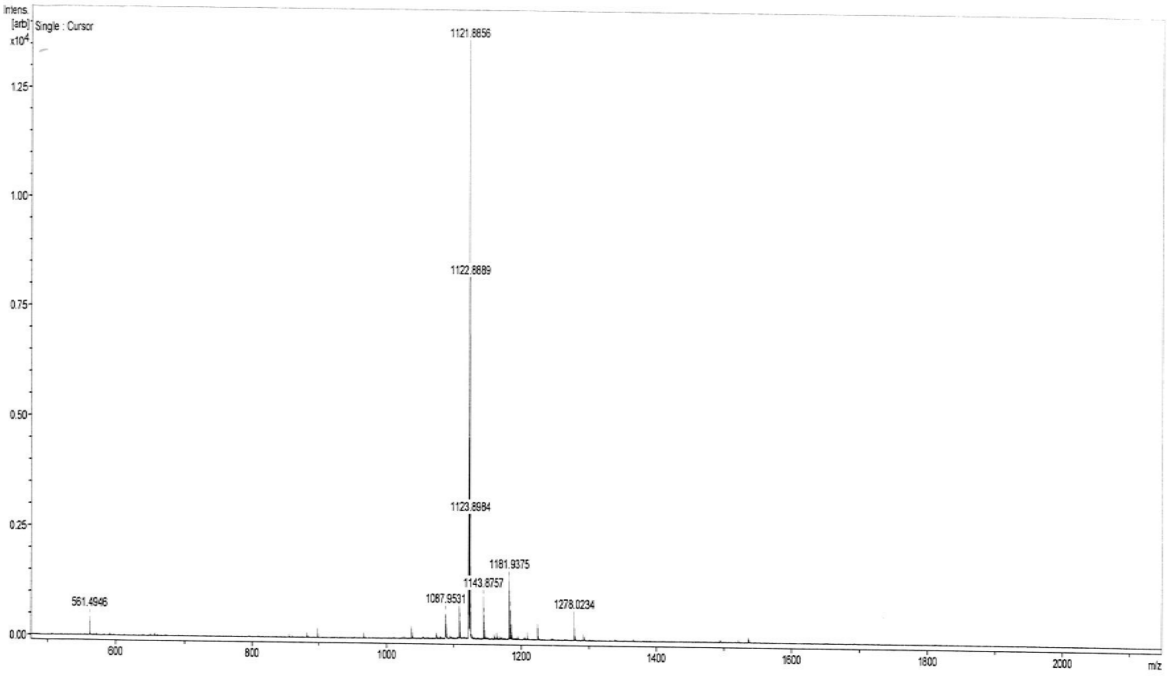
R_5K_2-C_{18} (LP-C18): MALDI-TOF (m/z): $C_{78}H_{154}N_{24}O_{10}$ calc: 1587.2280; found: 1588.0333 $[M+H]^+$.



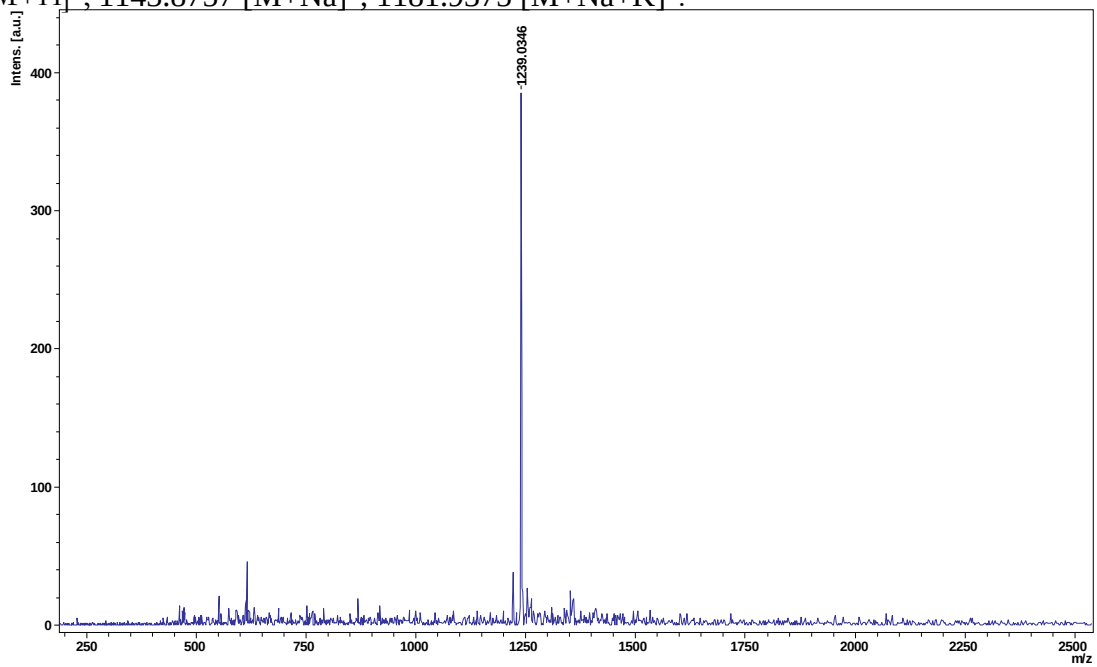
R_5K_2-C_{18} (LP-C18*): MALDI-TOF (m/z): $C_{78}H_{146}N_{24}O_{10}$ calc: 1579.1654; found: 1582.0409 $[M+3]^+$.



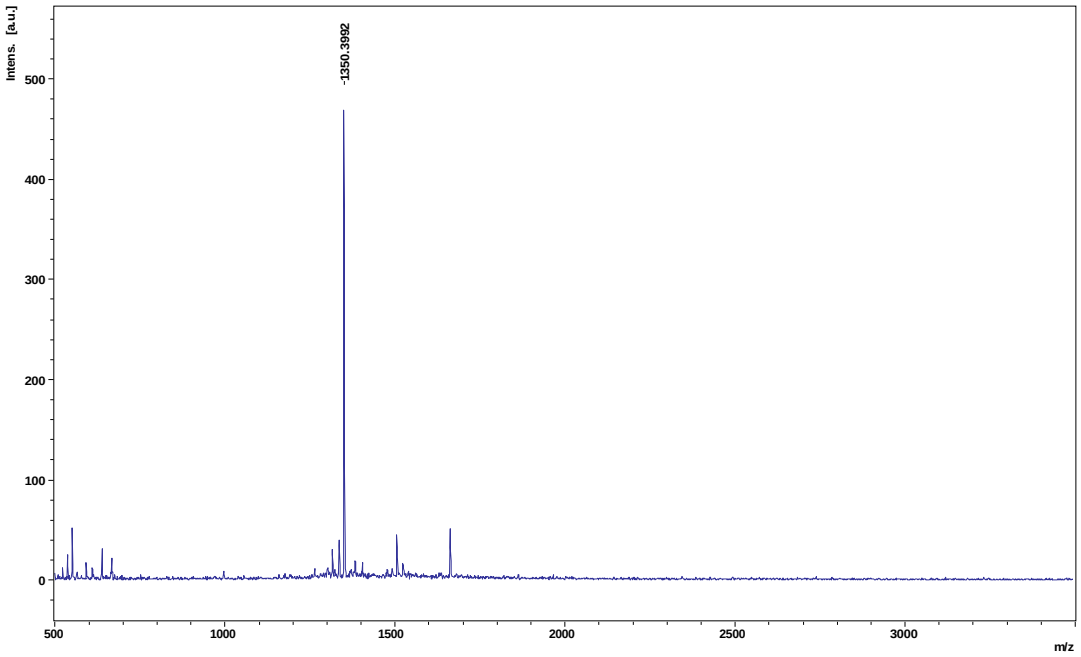
[R_5K_2] (Cyclic Peptide): MALDI-TOF (m/z): $C_{42}H_{84}N_{24}O_7$ calc: 1036.6955; found: 1037.9739 $[M+H]^+$.



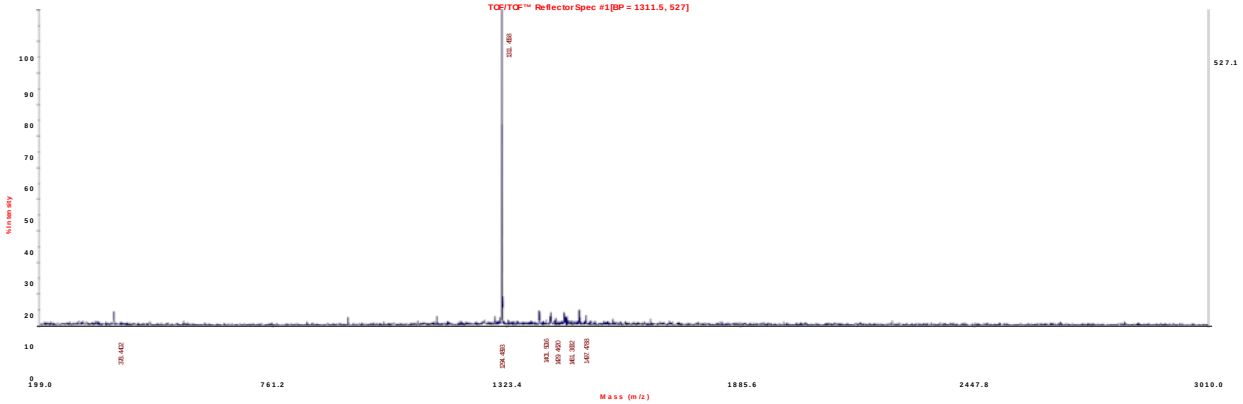
$[R_5K_2]$-C_2 (CP-C2): MALDI-TOF (m/z): $C_{46}H_{88}N_{24}O_9$ calc: 1120.7166; found: 1121.8856 $[M+H]^+$, 1143.8757 $[M+Na]^+$, 1181.9375 $[M+Na+K]^+$.



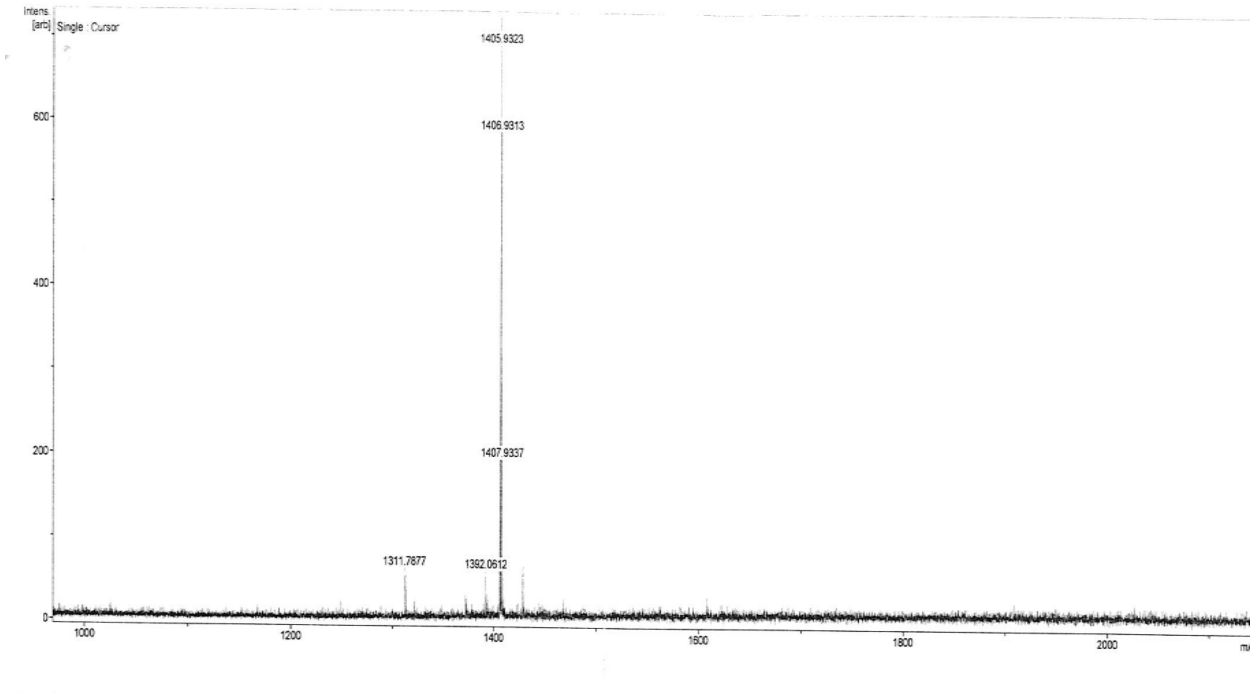
$[R_5K_2]$-C_4 (CP-C4): MALDI-TOF (m/z): $C_{50}H_{92}N_{24}O_{13}$ calc: 1236.7276; found: 1239.0346 $[M+3]^+$.



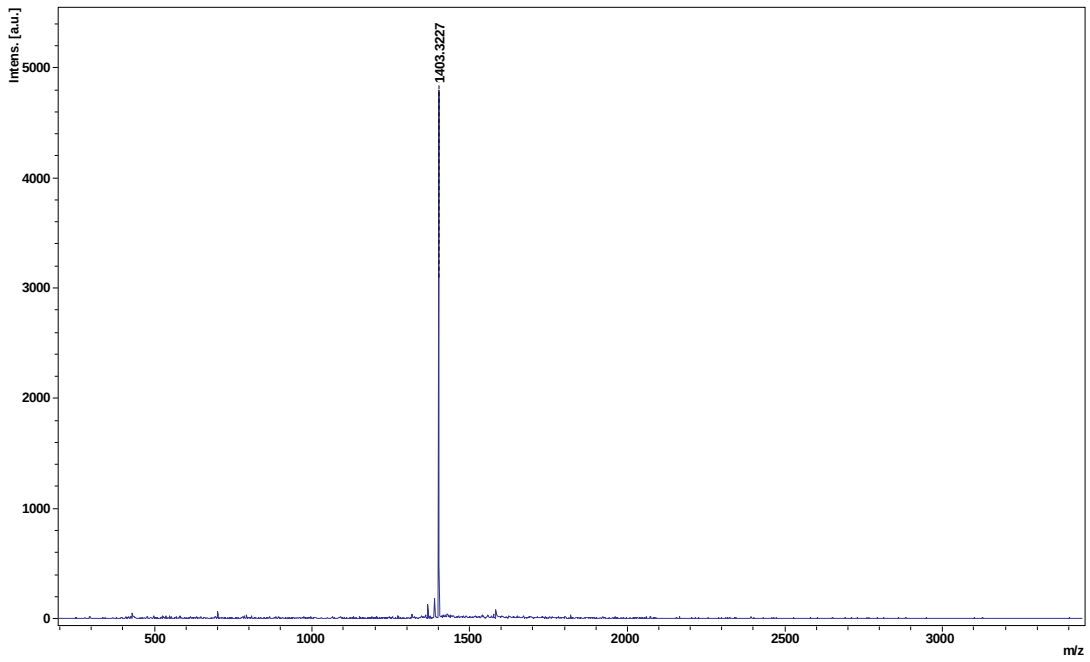
$[R_5K_2]$-C_8 (CP-C8) MALDI-TOF (m/z): $C_{58}H_{108}N_{24}O_{13}$ calc: 1348.8528; found: 1350.3992 $[M+H]^+$.



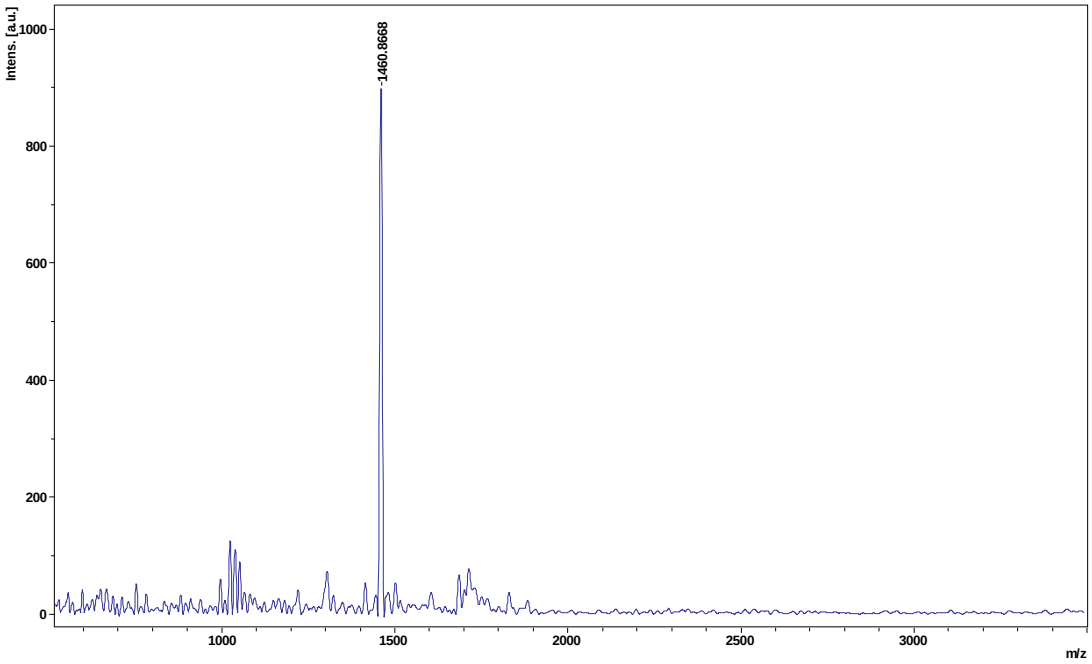
$[R_5K_2]$-C_{8*} (CP-C8*): MALDI-TOF (m/z): $C_{58}H_{112}N_{24}O_9$ calc: 1289.9044; found: 1311.4558 $[M+Na-H]^+$.



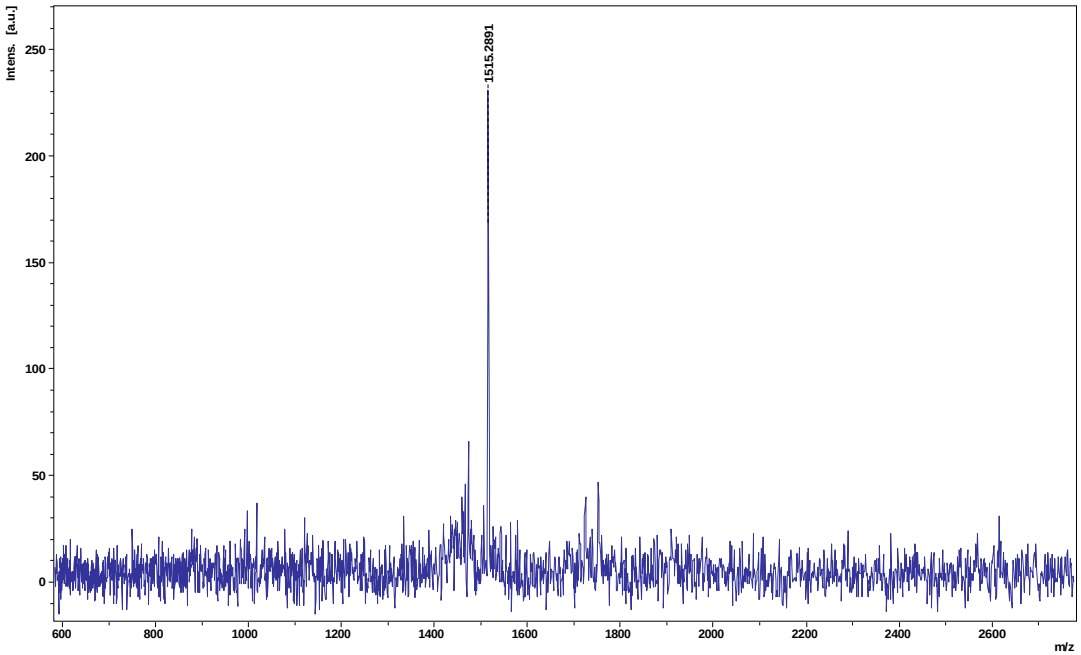
$[R_5K_2]$-C_{10} (CP-C10): MALDI-TOF (m/z): $C_{62}H_{116}N_{24}O_{13}$ calc: 1404.9154; found: 1405.9323 $[M+H]^+$.



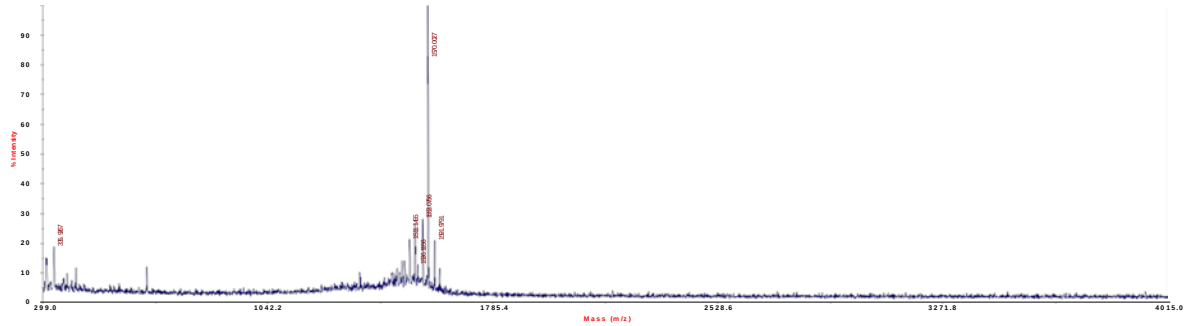
$[R_5K_2]$-C_{12} (CP-C12): MALDI-TOF (m/z): $C_{66}H_{128}N_{24}O_9$ calc: 1401.0296; found: 1403.3227 $[M+2]^+$.



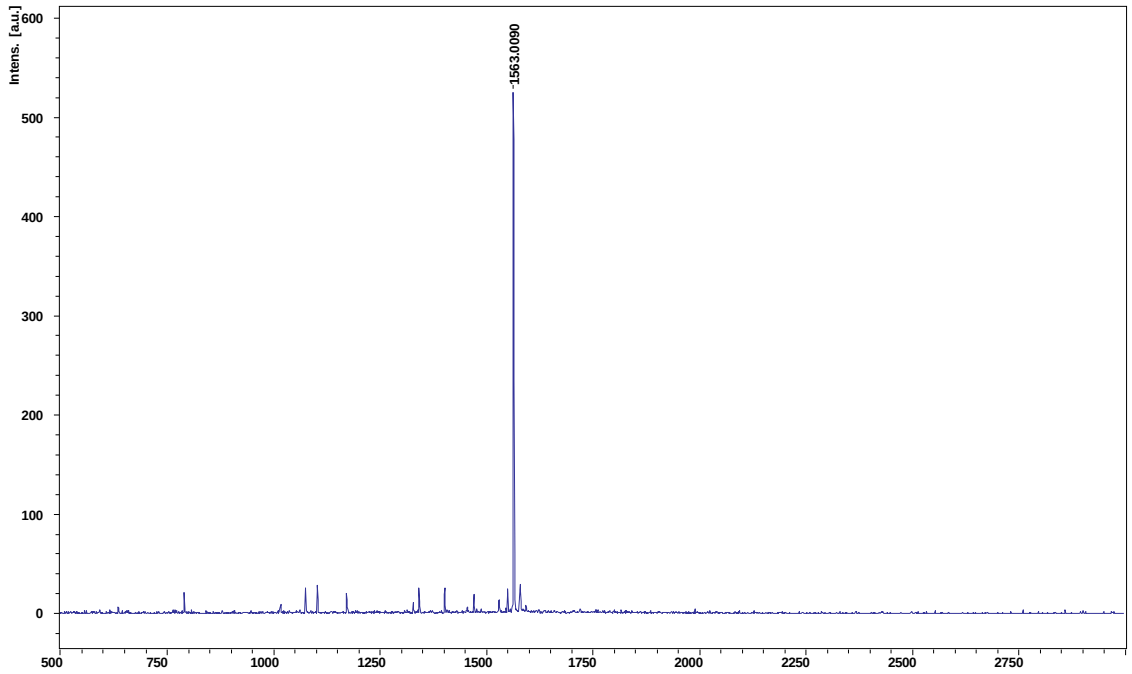
$[R_5K_2]$-C_{14} (CP-C14): MALDI-TOF (m/z): $C_{70}H_{136}N_{24}O_9$ calc: 1457.0922; found: 1460.8668 $[M+3]^+$.



$[R_5K_2]$-C_{16} (CP-C16): MALDI-TOF (m/z): $C_{74}H_{144}N_{24}O_9$ calc: 1513.1548; found: 1515.2891 $[M+2]^+$.



$[R_5K_2]$-C_{18} (CP-C18): MALDI-TOF (m/z): $C_{78}H_{152}N_{24}O_9$ calc: 1569.2174; found: 1570.0027 $[M+H]^+$.



$[R_5K_2]$-C_{18^*} (CP-C18*): MALDI-TOF (m/z): $C_{78}H_{144}N_{24}O_9$ calc: 1561.1548; found: 1563.0090 $[M+2]^+$.

Figure S2. MALDI mass spectra for the designed modified peptides

Materials

2-Chlorotrityl chloride arginine resin, Arg-Wang Resin, fluorenylmetholoxycarbonyl (Fmoc)-Arg(Pbf)-OH, Fmoc-Lys(Dde)-OH), Fmoc-Lys(Boc)-OH, and Fmoc-Arg(Pbf)-OH were purchased from AAPPTec (Louisville, KY, USA). Acetic anhydride, succinic acid, adipic acid, suberic acid, caprylic acid, sebacic acid, lauric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, piperdine, *N*,*N*-diisopropylethylamine (DIPEA), hydrazine monohydrate, anisole, thioanisole, dithiothreiitol (DTT), *N*-methylmorpholine, trifluoroacetic acid, acetonitrile, dichloromethane, α-cyano-4-hydroxycinnamic acid (α-cyano), and trifluoroethanol (TFE) were supplied by Sigma-Aldrich (St. Louis, MO, USA). XBridge BEH130 Prep C18 and XBridge BEH130 C18 columns were obtained from Waters Corporation (Milford, MA, USA). LC-2030C 3D and LC-20AP HPLC from Shimadzu (Columbia, MD, USA) were used for the purification of the peptides. High-resolution Autoflex speed MALDI-TOF used for mass spectrometry was from Bruker Inc. (Bremen, Germany). Tribute Automatic peptide synthesizer (Protein Technologies, Inc.) was used for the peptide synthesis, and all the accompanying accessories and supplies were manufactured by Protein Technologies Inc. (Tucson, AZ, USA). Hanks Balanced Salt Solution (HBSS), Dulbecco's modified Eagle medium (DMEM; low glucose with L-glutamine), RPMI Medium 1640 with L-glutamine, Fetal bovine serum (FBS), SYBR Green II, penicillin (10000U/mL), and streptomycin (10mg/mL) were provided by Life Technologies (Grand Island, NY, USA). Scrambled negative control siRNA (Catalogue # AM4635; Molecular weight: 13300), 5′-carboxyfluorescein (FAM)-labeled negative control siRNA (Catalogue # AM4620; Molecular weight: 13676), and the siRNA targeting kinesin spindle protein (KSP; Catalogue # AM16704; Molecular weight: 13842) were obtained from Ambion (Austin, TX). The siRNA targeting Janus kinase 2 (JAK2) was purchased from Qiagen (Catalogue # SI02659657, Valencia, CA, USA; Molecular Weight: 13255; Sense: 5'-CCAUCAUACGAGAUCUUAATT-3'; Antisense:

5’UUAAGAUCUCGUAUGAUGGCT-3’). All the primers were designed and ordered from Integrated DNA Technologies (IDT; Coralville, IA, USA). Heparin sodium salt from porcine intestinal mucosa was provided by Alfa Aesar (Ward Hill, MA). 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). PCR master mixes iScriptTM Reverse transcription Supermix and iTaq Universal SYBR Green Supermix were supplied by Bio-Rad (Hercules, CA). Ethylenediaminetetraacetic acid (EDTA) and all other materials were obtained from Fisher Scientific (Carlsbad, CA).