Electronic Supporting Information

Solution-phase and solid-phase sequential, selective modification of side chains in KDYWEC and KDYWE as models for usage in single-molecule protein sequencing

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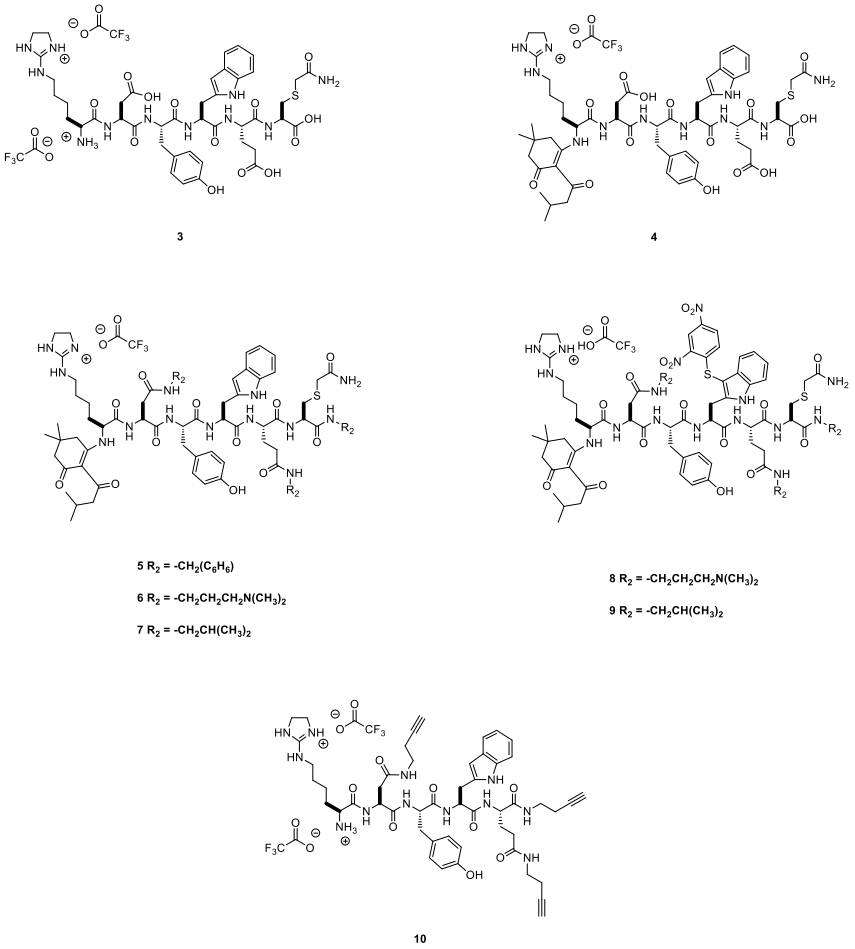


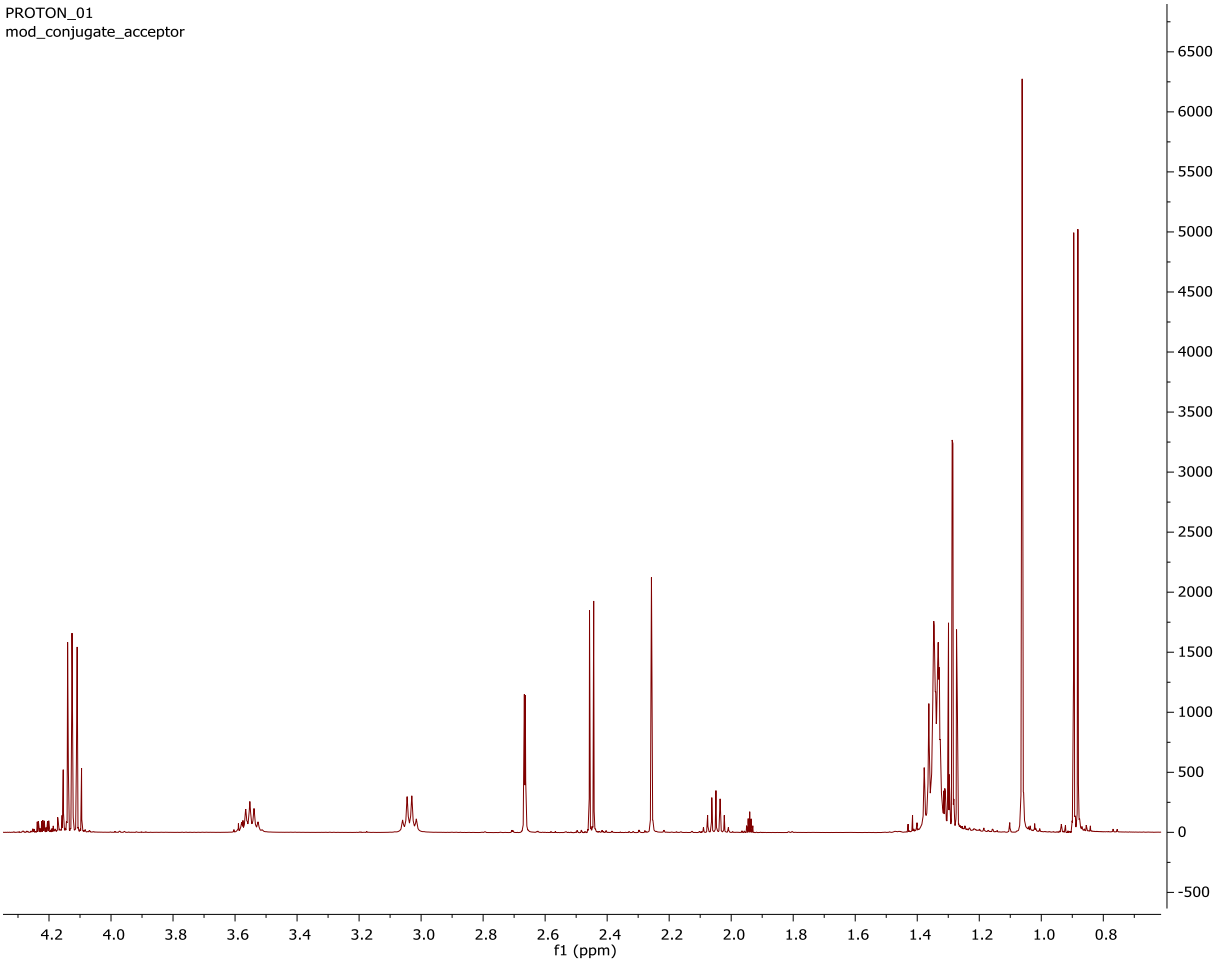
(SI-Figure 1). KDYWEC and KDYWE derivatives.

Preparation of Modifiying Reagents

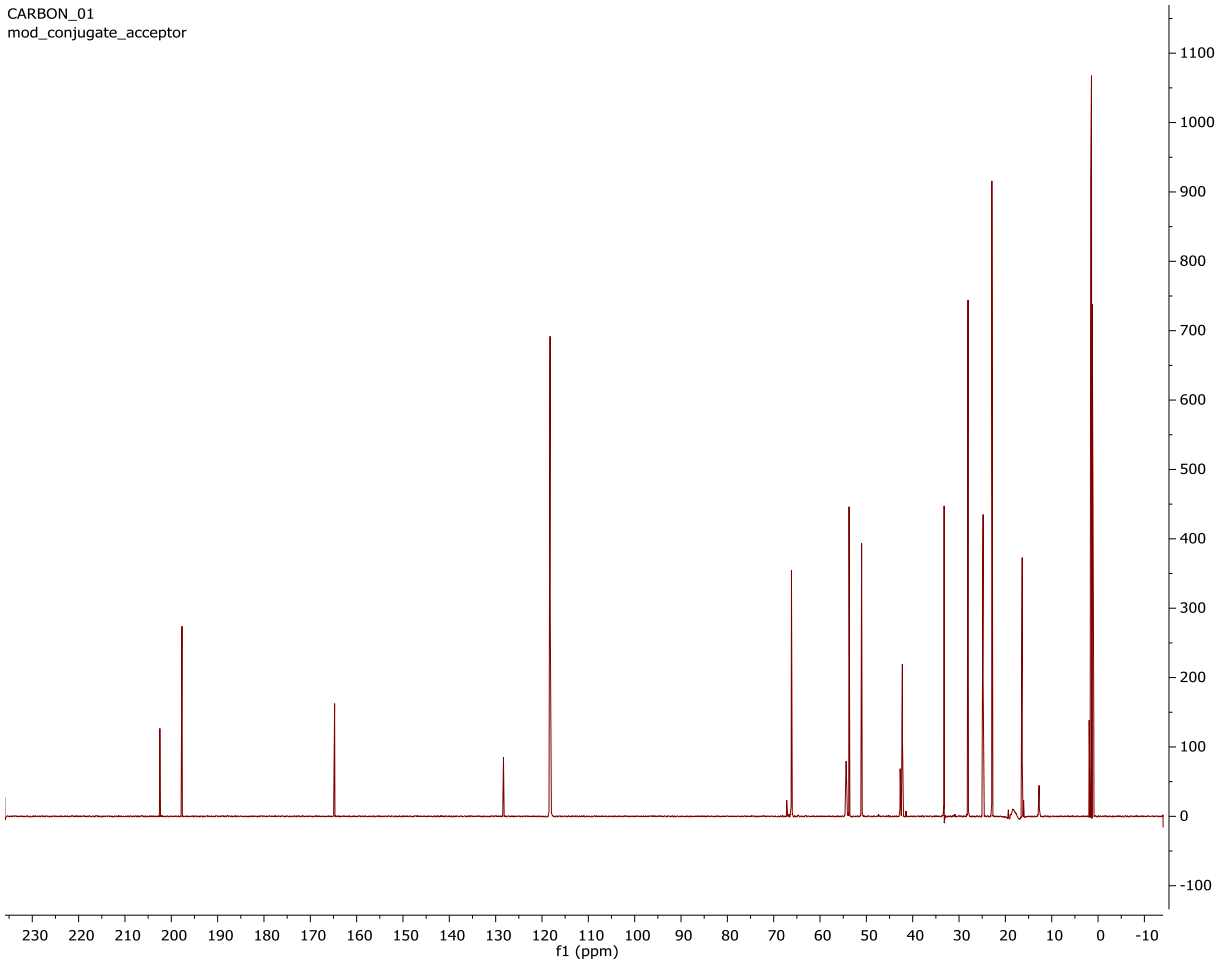
2-Methoxy-4,5-dihydro-1H-imidazole was prepared following a literature protocol. (Peters EC, Horn DM, Tully DC, Brock A. A novel multifunctional modifiying reagent for enhanced protein characterization with mass spectrometry. *Rapid Commun. Mass Spectrom*. 2001; **15**: 2387-2392.)

1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl diethyl phosphate was prepared by dissolving of 2-(3-methylbutyryl)-5,5-dimethyl-1,3-cyclohexandione (17 µmole) in 0.5 ml of dry MeCN under argon. Solution was placed in ice bath. DIPEA (20 µmole) was introduced, followed by slow introduction of diethylchlorophosphate (22 µmole). Reaction was stirred

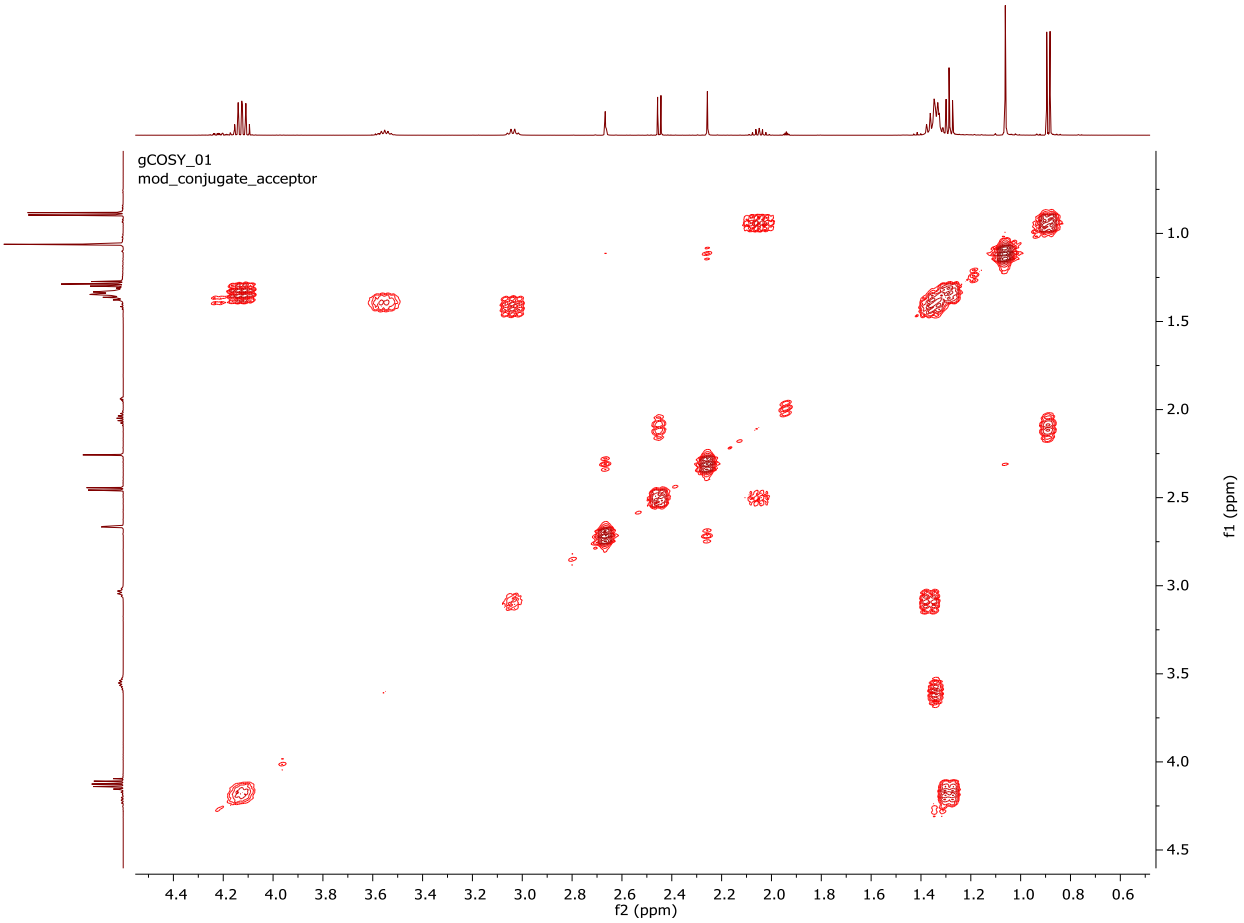
overnight at RT. Yield: quantitative. 1H NMR (500 MHz, Acetonitrile-d3) δ 4.12 (dq, J = 8.3, 7.1 Hz, 4H), 2.67 (d, J = 1.6 Hz, 2H), 2.45 (d, J = 6.8 Hz, 2H), 2.26 (s, 2H), 2.09 – 2.00 (m, 1H), 1.30 – 1.27 (m, 6H), 1.06 (s, 6H), 0.89 (d, J = 6.7 Hz, 6H). 13C NMR (126 MHz, cd3cn) δ 197.70, 164.75, 66.22, 66.17, 53.74, 51.03, 42.23, 33.22, 28.06, 24.81, 22.92, 16.42, 16.37. HR-res MS: found m/z 383.15970, calcd. 383.15940 $(M+Na^{+})^{+}$; found m/z 359.16290, calcd. 359.16290 $(M-H)^{-}$ (Adapted from Zhang, H. A process for the preparation of the intermediate of β-methyl carbapenem. WO 2007104219 A1, September 20, 2007.)



SI-Figure 2. 1 Proton NMR of 5,5-dimethyl-2-(3-methylbutanoyl)-3-oxocyclohex-1-en-1-yl diethyl phosphate (Phos-DOD).

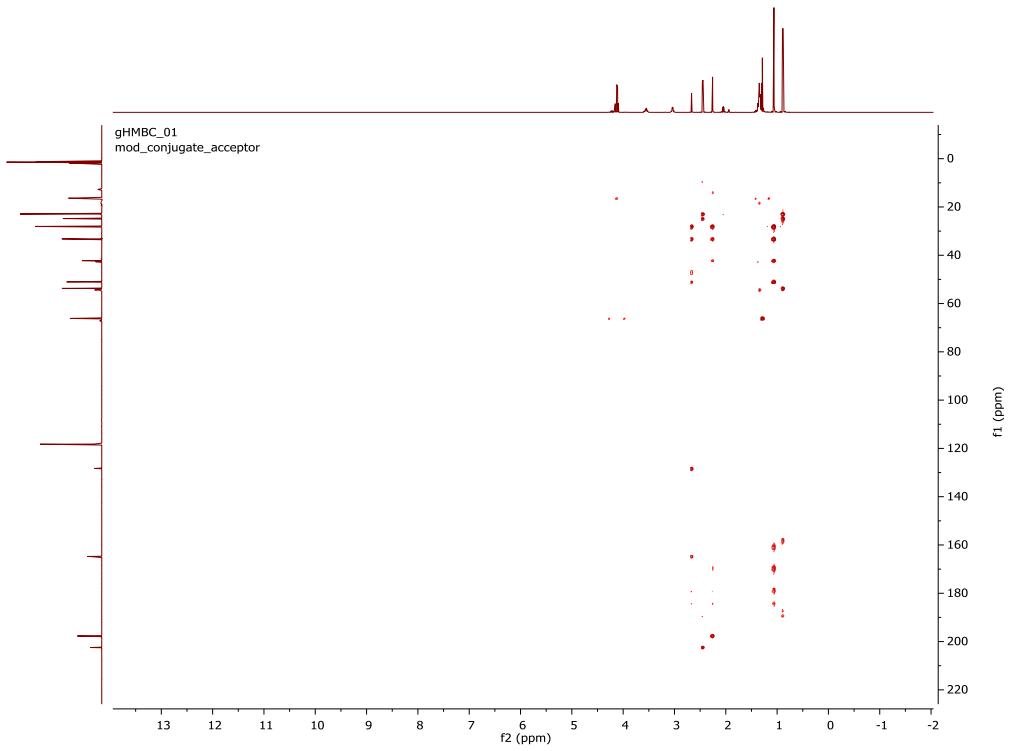


SI-Figure 3. 13 Carbon NMR of phos-DOD.



SI-Figure 4. COSY NMR of phos-DOD.

(a)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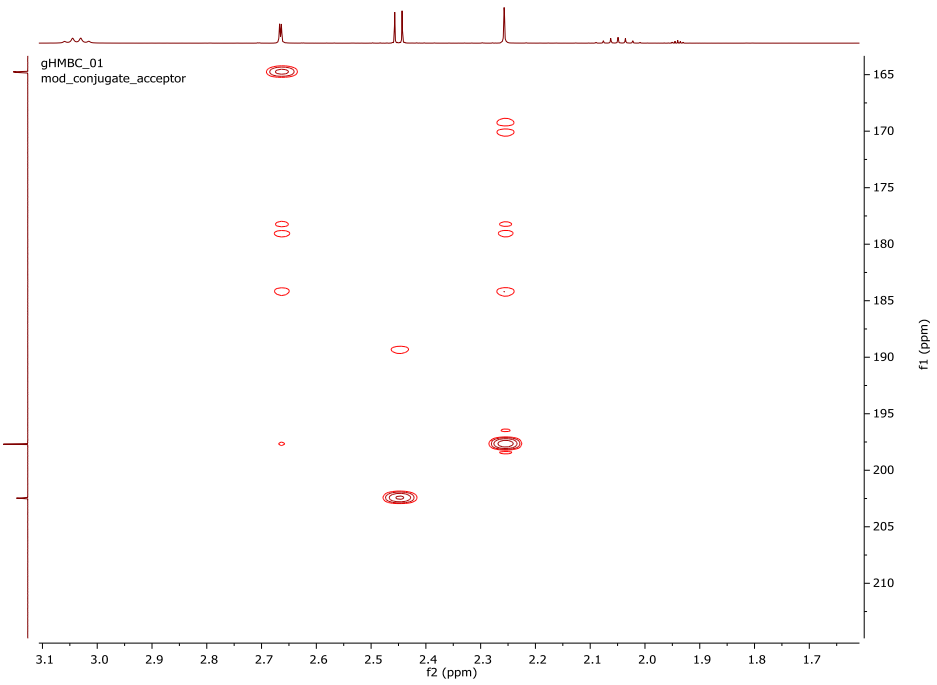
f2 (ppm)
f1 (ppm)

(b)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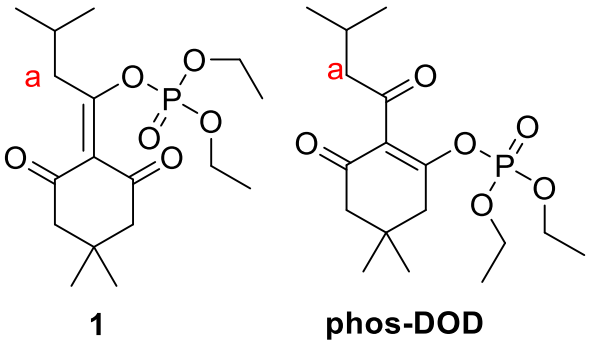
f2 (ppm)
f1 (ppm)

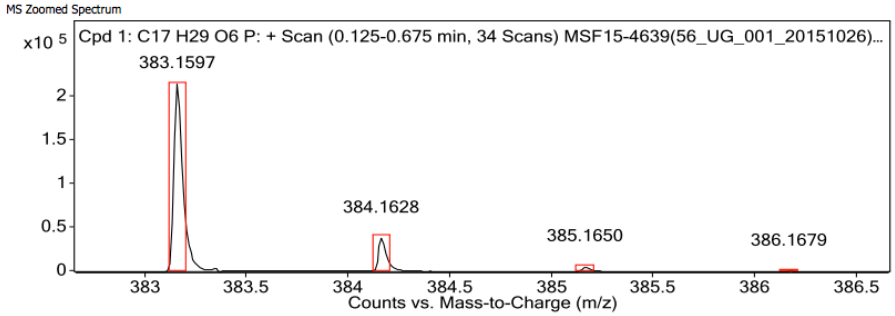


SI-Figure 5.(a) HMBC NMR of phos-DOD. (b) Region shown demonstrated doublet attributed to position **a** in phos-DOD correlated with a carbon in the carbonyl region, instead of an alkene as shown in **SI-1**.

Target Compound Screening Report

Data File	MSF15-4639(56_UG_001_20151026)_hrESIpos1.d	**Sample Name**	4639(56_UG_001_20151026)	**Comment**	4639(56_UG_001_20151026)
Position	P1-B4	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	10/27/2015 2:34:51 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

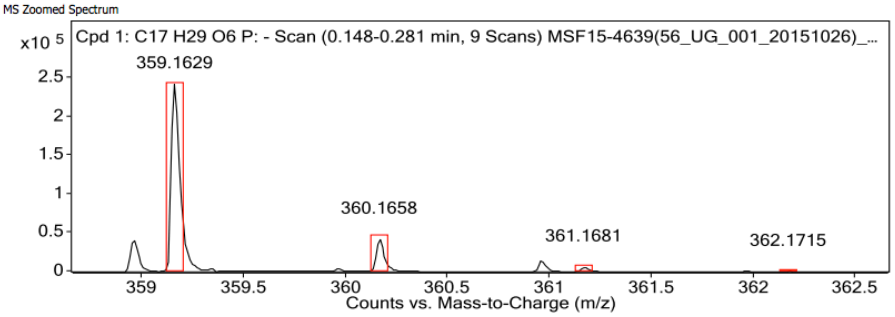
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
130.15910			2418499.71			
383.15970	383.15940	1	216028.95	C17H29O6P	(M+Na)+	-0.88
384.16280	384.16280	1	38420.76	C17H29O6P	(M+Na)+	-0.04
385.16500	385.16510	1	5741.21	C17H29O6P	(M+Na)+	0.45
386.16790	386.16780	1	640.61	C17H29O6P	(M+Na)+	-0.22

--- End Of Report ---

Target Compound Screening Report

Data File	MSF15-4639(56_UG_001_20151026)_hrESIneg1.d	**Sample Name**	4639(56_UG_001_20151026)	**Comment**	4639(56_UG_001_20151026)
Position	P1-B4	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	10/27/2015 2:49:13 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
331.13190			613191.49			
359.16290	359.16290	1	242412.53	C17H29O6P	(M-H)-	0.09
360.16580	360.16630	1	42336.24	C17H29O6P	(M-H)-	1.43
361.16810	361.16860	1	6543.88	C17H29O6P	(M-H)-	1.49
362.17150	362.17130	1	676.43	C17H29O6P	(M-H)-	-0.48

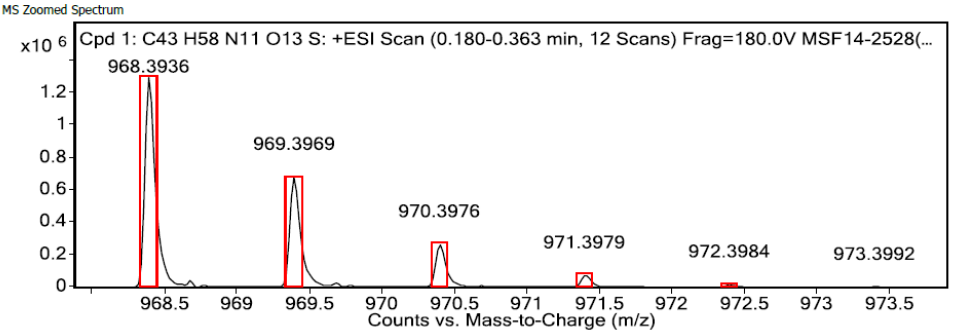
--- End Of Report ---

SI-Figure 6. High-resolution mass spectrometry data for phos-DOD.

Target Compound Screening Report

Data File	MSF14-2528(CK_KDYWEC)_hrESIpos2.d	**Sample Name**	2528	**Comment**	CK_KDYWEC
Position	P1-B6	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	5/13/2014 4:41:31 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

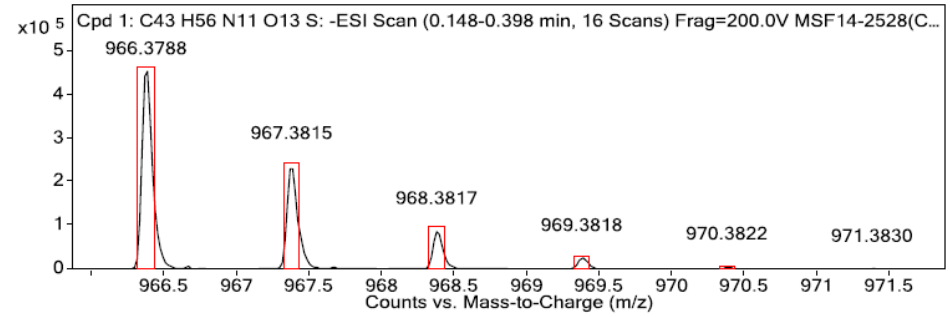
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
968.39360	968.39310	1	1301931.29	C43H58N11O13S	M+	-0.57
969.39690	969.39590	1	685577.67	C43H58N11O13S	M+	-1.02
970.39760	970.39640	1	267939.09	C43H58N11O13S	M+	-1.16
971.39790	971.39750	1	75230.14	C43H58N11O13S	M+	-0.39
972.39840	972.39880	1	19090.08	C43H58N11O13S	M+	0.4
973.39920	973.40040	1	3462.31	C43H58N11O13S	M+	1.16
974.40160	974.40210	1	857.75	C43H58N11O13S	M+	0.51

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-2528(CK_KDYWEC)_hrESIneg1.d	**Sample Name**	2528	**Comment**	CK_KDYWEC
Position	P1-B6	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	5/13/2014 4:46:23 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
268.95430			679714.55			
966.37880	966.37850	1	463032.74	C43H56N11O13S	M-	-0.27
967.38150	967.38140	1	237486.19	C43H56N11O13S	M-	-0.15
968.38170	968.38190	1	88069.86	C43H56N11O13S	M-	0.24
969.38180	969.38290	1	26133.49	C43H56N11O13S	M-	1.16
970.38220	970.38420	1	6741.97	C43H56N11O13S	M-	2.05
971.38300	971.38580	1	1626.65	C43H56N11O13S	M-	2.89
972.38350	972.38750	1	542.09	C43H56N11O13S	M-	4.18

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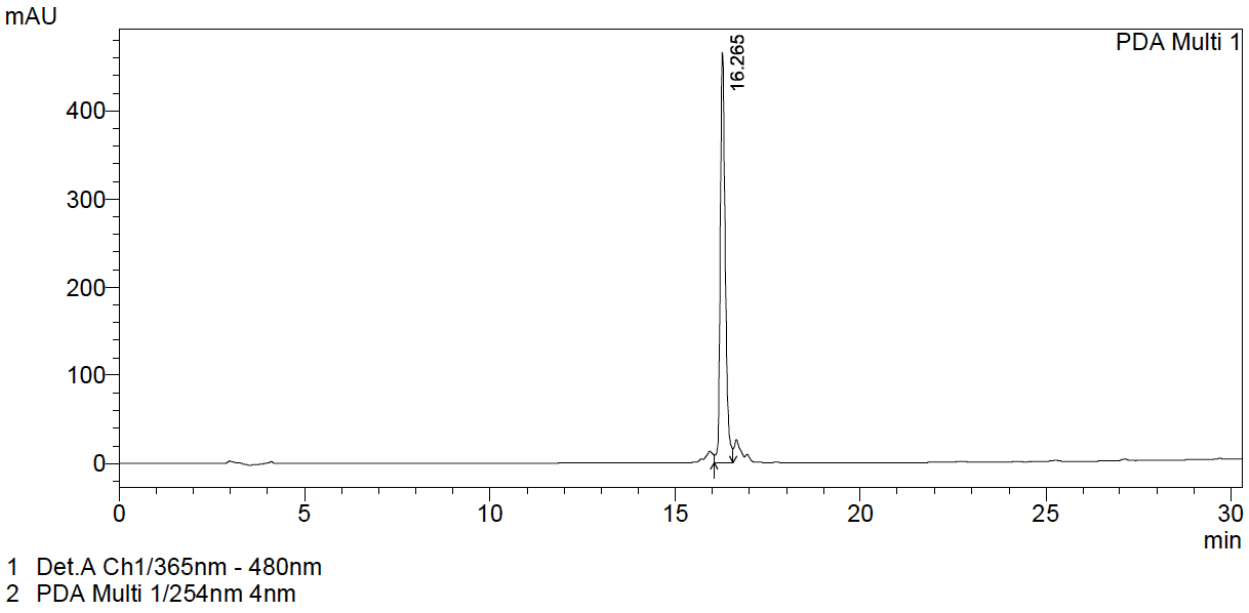
SI-Figure 7. High resolution mass spectrometry data for peptide **3**.



SI-Figure 8. HPLC trace for purified peptide **3**.

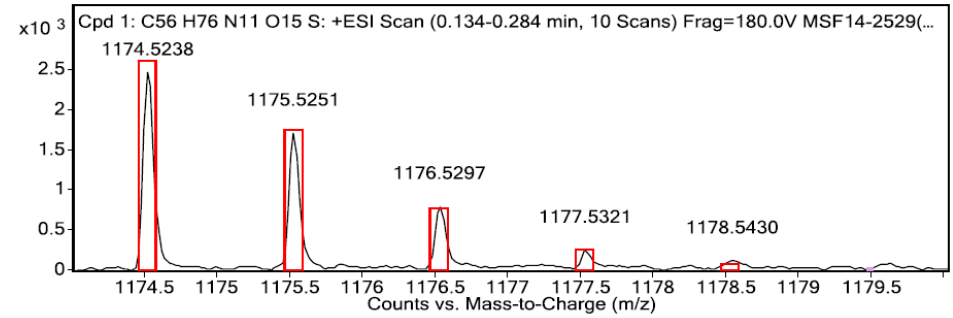
Desalting of peptide 4

Crude peptide was prepared for preparative HPLC using an Extract Clean^{TM} C_{18} 500 mg /4 ml solid phase extraction column. Column was flushed with 6 ml of 90/10 MeOH/H_2O with 0.1% TFA (v/v/v) at a flow rate of 1 drop sec^{-1} (RT), followed by equilibration with 3 ml of 0.1% TFA in water (v/v) at a flow rate of 1 drop sec^{-1}. Acidified peptide solution was loaded on the column 1 drop sec^{-1} (RT). Peptide was eluted with 1 ml 5% MeOH/Water with 0.1% TFA (v/v/v). Residually bound peptide was eluted with 50/50 MeCN/Water with 0.1% TFA (v/v/v).

Target Compound Screening Report

Data File	MSF14-2529(nCK_KDYWEC)_hrESIpos1.d	**Sample Name**	2529	**Comment**	nCK_KDYWEC
Position	P1-B7	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	5/13/2014 5:03:41 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

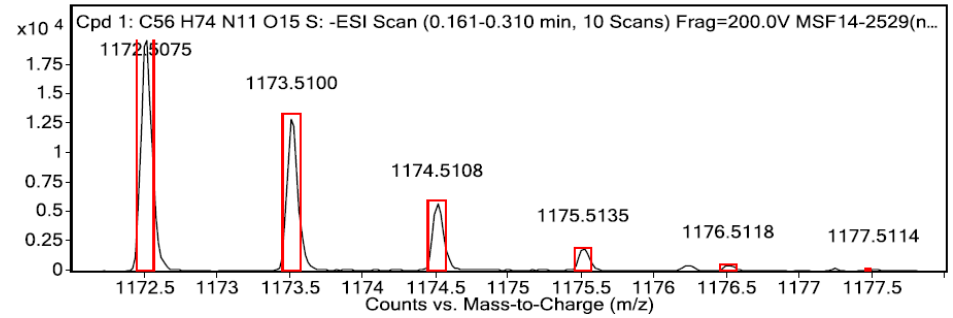
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
313.05800			819276.72			
1174.52380	1174.52380	1	2515.13	C56H76N11O15S	M+	-0.01
1175.52510	1175.52670	1	1723.84	C56H76N11O15S	M+	1.4
1176.52970	1176.52800	1	810.08	C56H76N11O15S	M+	-1.48
1177.53210	1177.52930	1	264.74	C56H76N11O15S	M+	-2.4
1178.54300	1178.53070	1	131.04	C56H76N11O15S	M+	-10.46

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-2529(nCK_KDYWEC)_hrESIneg1.d	**Sample Name**	2529	**Comment**	nCK_KDYWEC
Position	P1-B7	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	5/13/2014 5:10:44 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
585.75120			654506.35			
1172.50750	1172.50920	1	20019.71	C56H74N11O15S	M-	1.46
1173.51000	1173.51220	1	13205.15	C56H74N11O15S	M-	1.84
1174.51080	1174.51340	1	5758.11	C56H74N11O15S	M-	2.2
1175.51350	1175.51470	1	1930.16	C56H74N11O15S	M-	1.04
1176.51180	1176.51610	1	546.85	C56H74N11O15S	M-	3.69
1177.51140	1177.51780	1	198.53	C56H74N11O15S	M-	5.43

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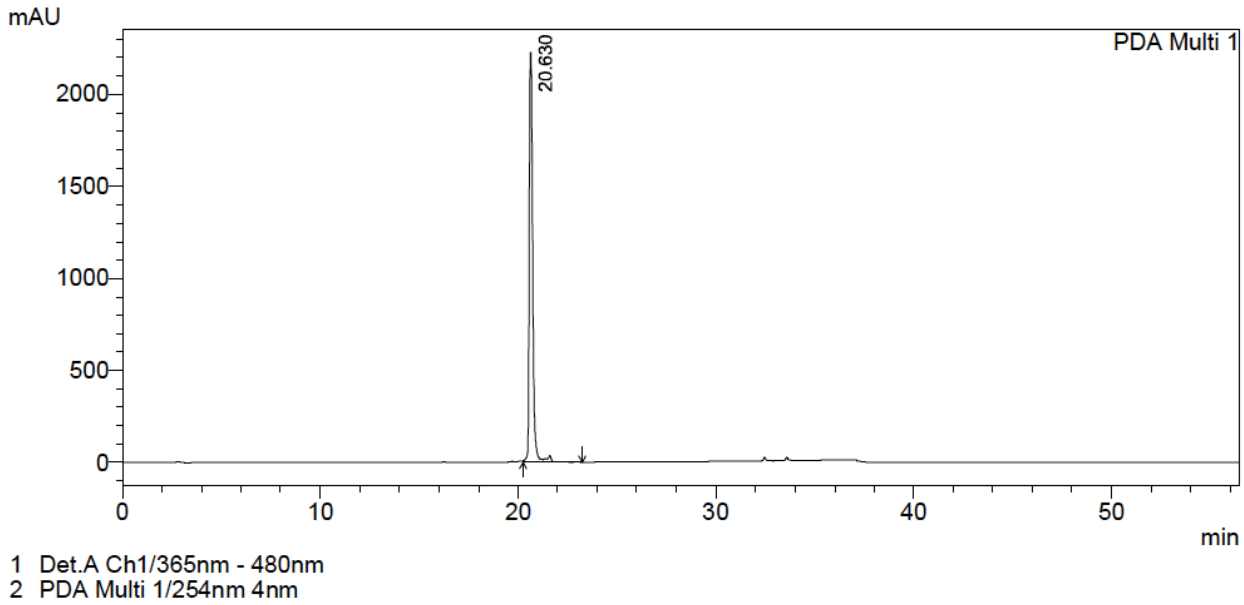
SI-Figure 9. High resolution mass spectrometry data for peptide **4**.

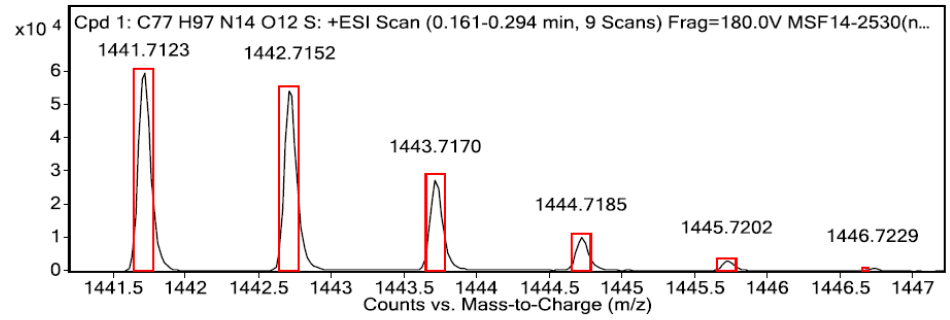


SI-Figure 10. HPLC trace for purified peptide **4**.

Target Compound Screening Report

Data File	MSF14-2530(nCKDE_KDYWECc)_hrESIpos1.d	**Sample Name**	2530	**Comment**	nCKDE_KDYWECc
Position	P1-B8	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	5/13/2014 5:22:11 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

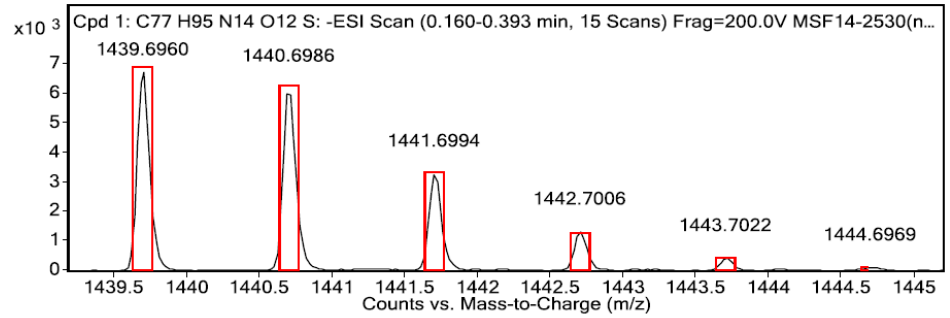
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
732.35260			923156.33			
1441.71230	1441.71260	1	61023.95	C77H97N14O12S	M+	0.19
1442.71520	1442.71560	1	55993.15	C77H97N14O12S	M+	0.23
1443.71700	1443.71750	1	27812.39	C77H97N14O12S	M+	0.33
1444.71850	1444.71910	1	10276.71	C77H97N14O12S	M+	0.43
1445.72020	1445.72070	1	3357.35	C77H97N14O12S	M+	0.33
1446.72290	1446.72240	1	809.52	C77H97N14O12S	M+	-0.34
1447.71640	1447.72430	1	250.93	C77H97N14O12S	M+	5.4
1448.72660	1448.72620	1	91.34	C77H97N14O12S	M+	-0.28

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-2530(nCKDE_KDYWECc)_hrESIneg1.d	**Sample Name**	2530	**Comment**	nCKDE_KDYWECc
Position	P1-B8	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	5/13/2014 5:26:03 PM	**DA Method**	Ian.m

MS Zoomed Spectrum

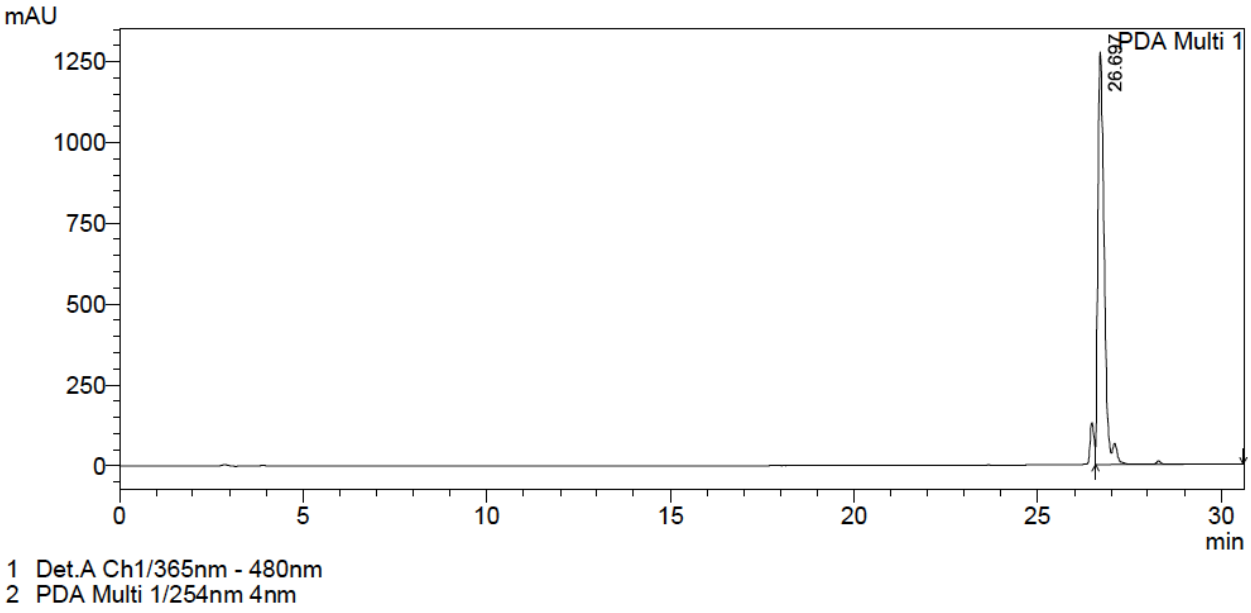


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
248.96040			90929.08			
1439.69600	1439.69800	1	6802.97	C77H95N14O12S	M-	1.39
1440.69860	1440.70100	1	6190.92	C77H95N14O12S	M-	1.67
1441.69940	1441.70300	1	3303.65	C77H95N14O12S	M-	2.5
1442.70060	1442.70460	1	1334.91	C77H95N14O12S	M-	2.75
1443.70220	1443.70620	1	434.08	C77H95N14O12S	M-	2.74
1444.69690	1444.70790	1	119.82	C77H95N14O12S	M-	7.57
1445.72040	1445.70970	1	35.77	C77H95N14O12S	M-	-7.43

--- End Of Report ---

SI-Figure 11. High resolution mass spectrometry data for peptide **5**.

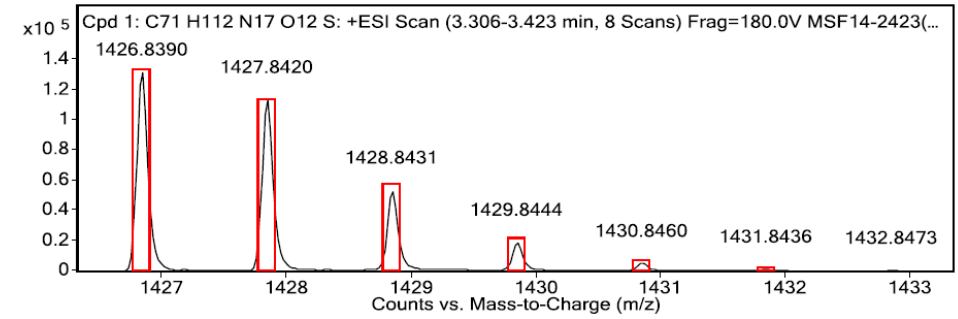


SI-Figure 12. HPLC trace for purified peptide **5**.

Target Compound Screening Report

Data File	MSF14-2423(nCKDE_KDYWECc_b)_hrESIpos2.d	**Sample Name**	2423(nCKDE_KDYWECc_b)	**Comment**	2423(nCKDE_KDYWECc_b)
Position	P1-F1	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos_column_20min.m	**Acquired Time**	6/25/2014 11:55:56 AM	**DA Method**	Ian.m

MS Zoomed Spectrum

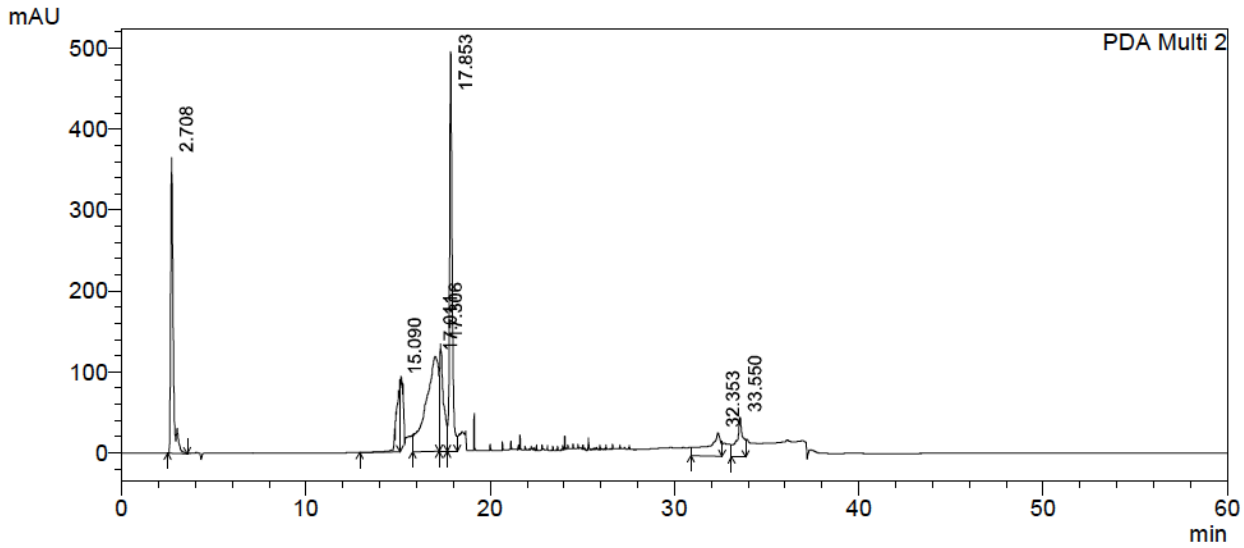


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
357.46650			2350276.17			
1426.83900	1426.83920	1	132784.34	C71H112N17O12S	M+	0.1
1427.84200	1427.84210	1	113609.25	C71H112N17O12S	M+	0.05
1428.84310	1428.84380	1	53501.04	C71H112N17O12S	M+	0.53
1429.84440	1429.84530	1	18949.68	C71H112N17O12S	M+	0.63
1430.84600	1430.84680	1	4918.58	C71H112N17O12S	M+	0.58
1431.84360	1431.84840	1	1160.52	C71H112N17O12S	M+	3.34
1432.84730	1432.85020	1	271.57	C71H112N17O12S	M+	2
1433.85390	1433.85210	1	86.89	C71H112N17O12S	M+	-1.24

--- End Of Report ---

SI-Figure 13. High resolution mass spectrometry data for peptide **6**.

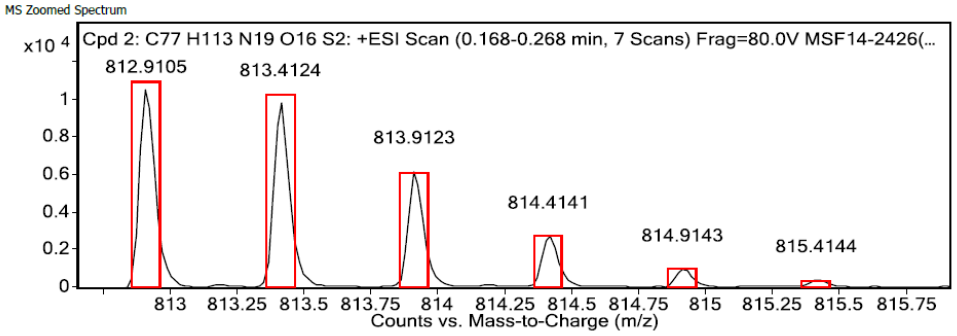


SI-Figure 14. HPLC trace for purified peptide **6**.

Target Compound Screening Report

Data File	MSF14-2426(nCKDEW_KDYWECc)_hrESIpos3.d	**Sample Name**	2426(nCKDEW_KDYWECc)	**Comment**	2426(nCKDEW_KDYWECc)
Position	P1-E1	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	9/11/2014 4:06:51 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

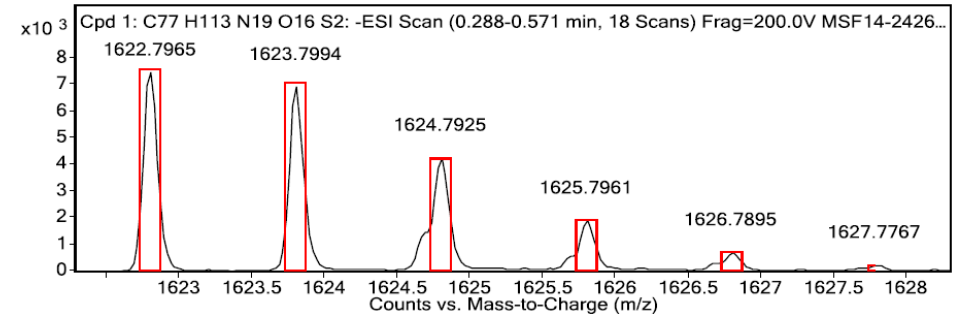
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
342.27890			487108.04			
812.91050	812.91000	2	10702.85	C77H113N19O16S2	(M+2H)+2	-0.65
813.41240	813.41140	2	9851.45	C77H113N19O16S2	(M+2H)+2	-1.2
813.91230	813.91200	2	6261.34	C77H113N19O16S2	(M+2H)+2	-0.39
814.41410	814.41250	2	2794.08	C77H113N19O16S2	(M+2H)+2	-1.9
814.91430	814.91300	2	1043.97	C77H113N19O16S2	(M+2H)+2	-1.58
815.41440	815.41350	2	468.78	C77H113N19O16S2	(M+2H)+2	-1.06

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-2426(nCKDEW_KDYWECc)_hrESIneg1.d	**Sample Name**	2426(nCKDEW_KDYWECc)	**Comment**	2426(nCKDEW_KDYWECc)
Position	P1-E1	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	9/11/2014 4:12:30 PM	**DA Method**	Ian.m

MS Zoomed Spectrum

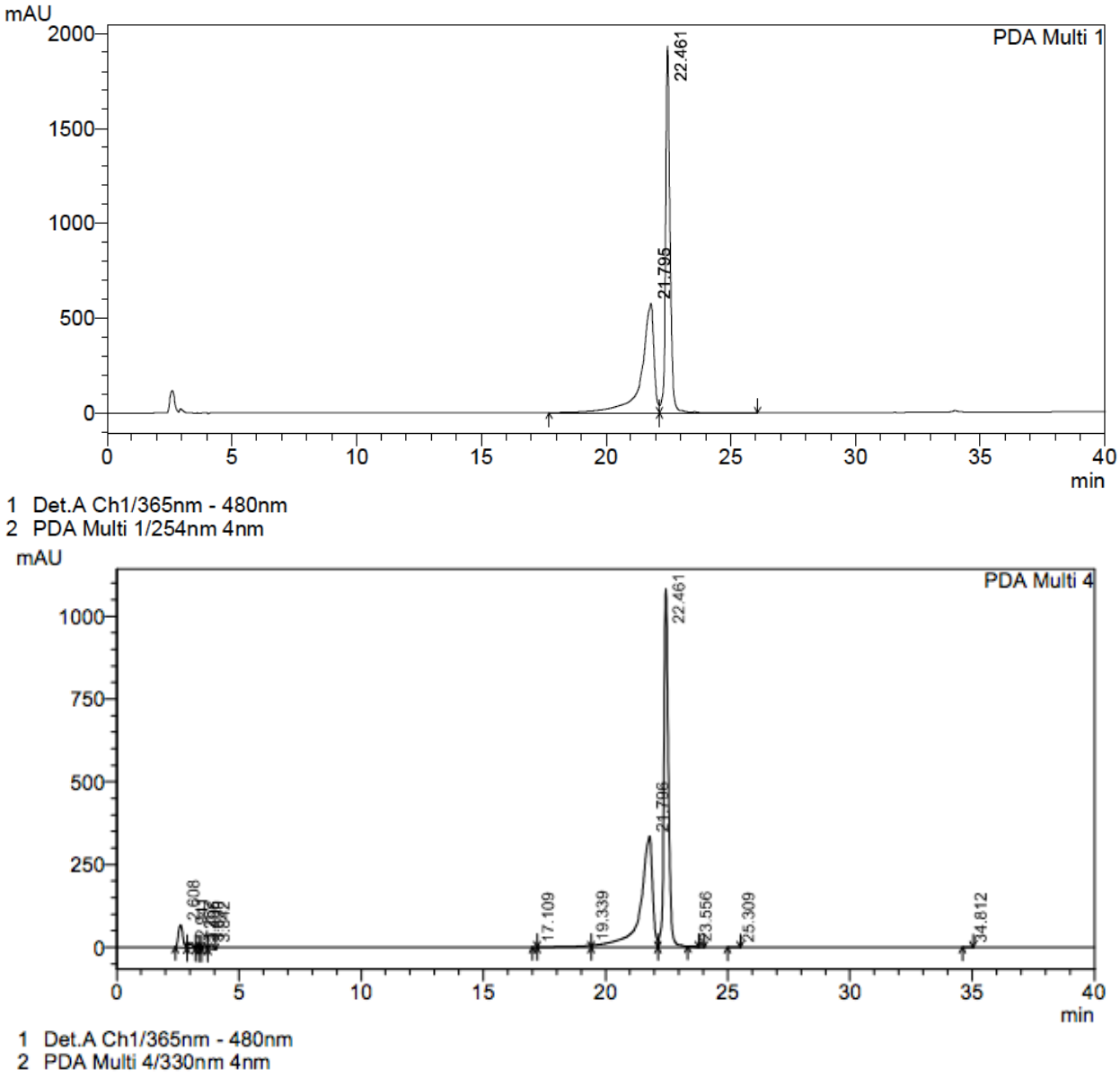


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
248.96060			234543.08			
1622.79650	1622.79810	1	7509.6	C77H113N19O16S2	(M-H)-	1.01
1623.79940	1623.80100	1	6927.6	C77H113N19O16S2	(M-H)-	0.96
1624.79250	1624.80220	1	4266.76	C77H113N19O16S2	(M-H)-	5.93
1625.79610	1625.80320	1	1905.57	C77H113N19O16S2	(M-H)-	4.36
1626.78950	1626.80420	1	710.54	C77H113N19O16S2	(M-H)-	9.05
1627.77670	1627.80520	1	235.21	C77H113N19O16S2	(M-H)-	17.53

--- End Of Report ---

SI-Figure 15. High resolution mass spectrometry data for peptide **8**.

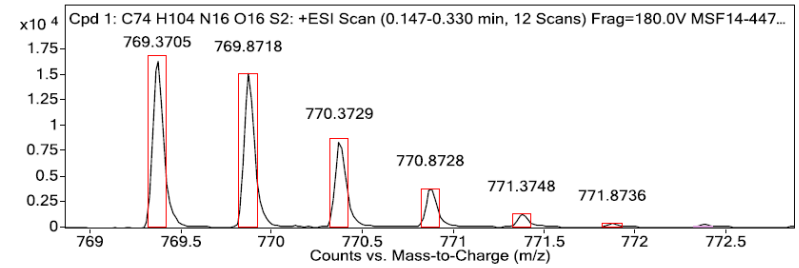


SI-Figure 16. HPLC trace for purified peptide **8**.

Target Compound Screening Report

Data File	MSF14-4477(nCKDEW_KDYWECc_isobutylamine)_hrESIpos1.d	**Sample Name**	4477(nCKDEW_KDYWECc_isobutylamine)	**Comment**	4477(nCKDEW_KDYWECc_isobutylamine)
Position	P1-E4	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	12/17/2014 1:17:46 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

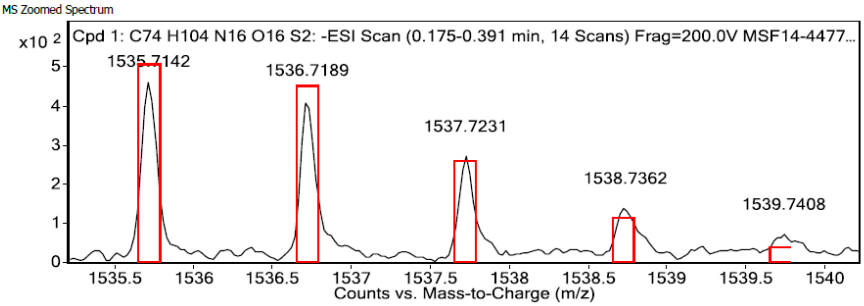
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
769.37050	769.37020	2	16614.5	C74H104N16O16S2	(M+2H)+2	-0.49
769.87180	769.87160	2	15109.76	C74H104N16O16S2	(M+2H)+2	-0.23
770.37290	770.37220	2	8503.04	C74H104N16O16S2	(M+2H)+2	-0.97
770.87280	770.87260	2	3884.32	C74H104N16O16S2	(M+2H)+2	-0.18
771.37480	771.37310	2	1382.74	C74H104N16O16S2	(M+2H)+2	-2.14
771.87360	771.87360	2	462.97	C74H104N16O16S2	(M+2H)+2	0.05
780.36040			37856.99			

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-4477(nCKDEW_KDYWECc_isobutylamine)_hrESIneg1.d	**Sample Name**	4477(nCKDEW_KDYWECc_isobutylamine)	**Comment**	4477(nCKDEW_KDYWECc_isobutylamine)
Position	P1-E4	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	12/17/2014 1:25:47 PM	**DA Method**	Ian.m

MS Zoomed Spectrum

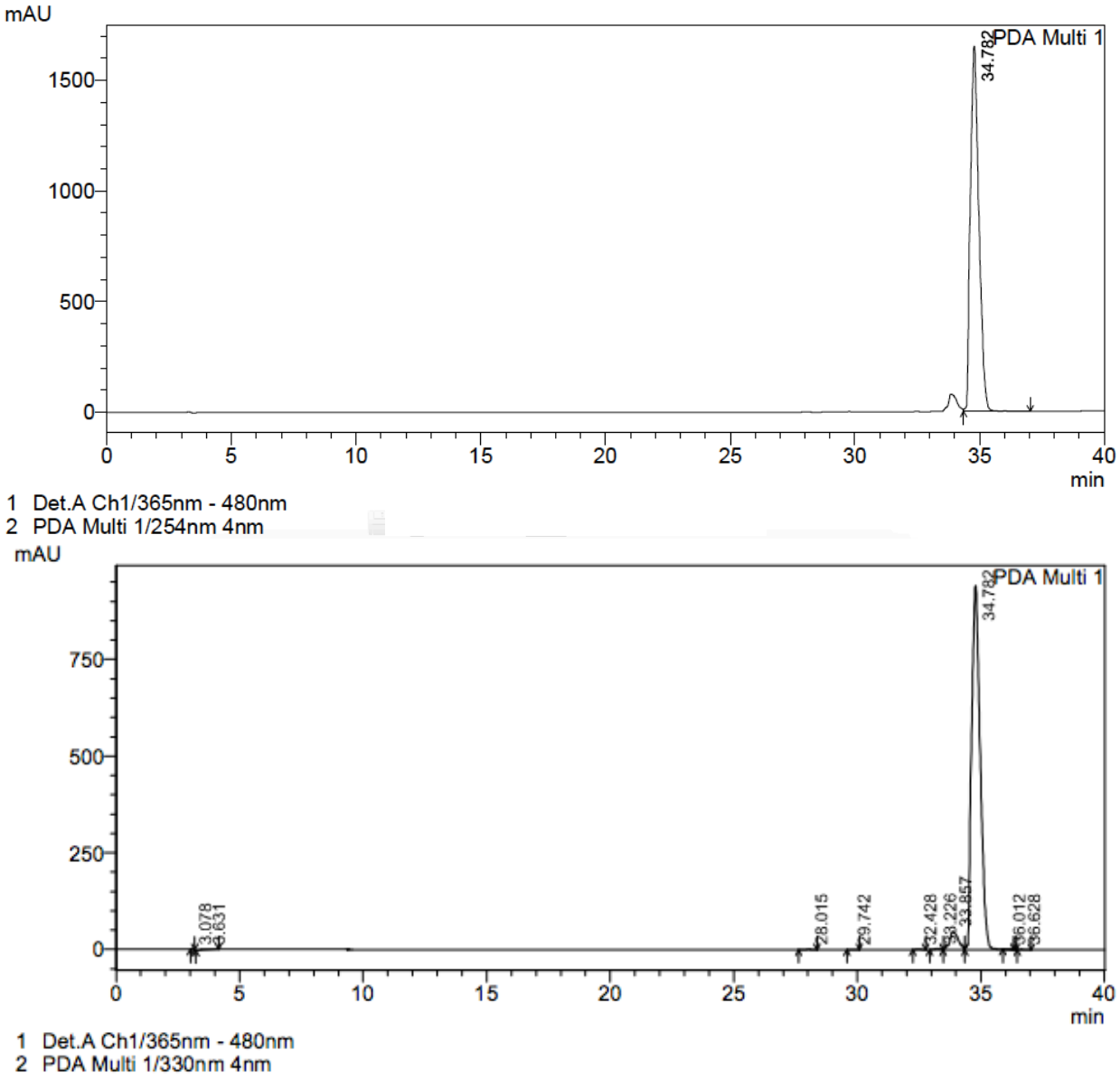


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
248.96000			64305.2			
1535.71420	1535.71850	1	463.88	C74H104N16O16S2	(M-H)-	2.82
1536.71890	1536.72140	1	417.47	C74H104N16O16S2	(M-H)-	1.61
1537.72310	1537.72250	1	273.98	C74H104N16O16S2	(M-H)-	-0.38
1538.73620	1538.72350	1	140.73	C74H104N16O16S2	(M-H)-	-8.25
1539.74080	1539.72440	1	74.22	C74H104N16O16S2	(M-H)-	-10.66

--- End Of Report ---

SI-Figure 17. High resolution mass spectrometry data for peptide **9**.

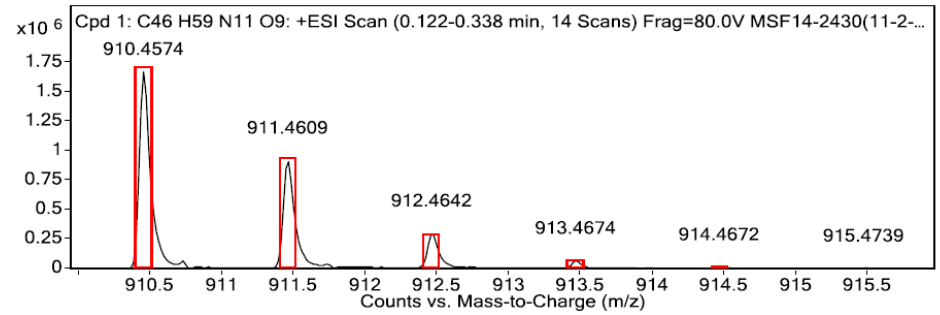


SI-Figure 18. HPLC trace for purified peptide **9**.

Target Compound Screening Report

Data File	MSF14-2430(11-2-14_KDE_KDYWE)_hrESIpos3.d	**Sample Name**	2430(11-2-14_KDE_KDYWE)	**Comment**	2430(11-2-14_KDE_KDYWE)
Position	P1-F6	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	11/13/2014 3:04:18 PM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

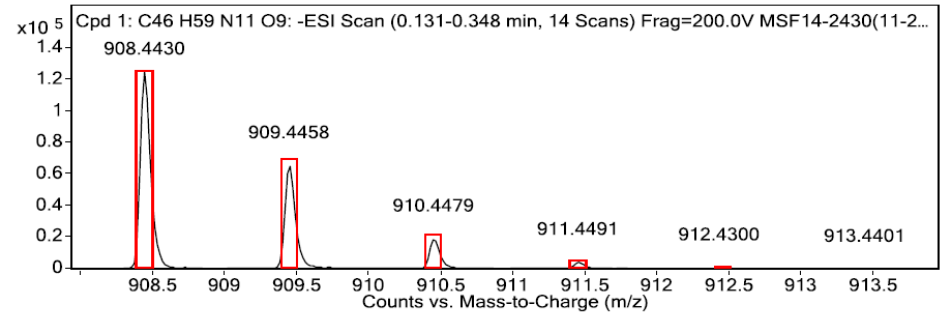
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
455.73310			2158894.04			
910.45740	910.45700	1	1675021.78	C46H59N11O9	(M+H)+	-0.41
911.46090	911.45990	1	921286.98	C46H59N11O9	(M+H)+	-1.04
912.46420	912.46270	1	301557.87	C46H59N11O9	(M+H)+	-1.68
913.46740	913.46530	1	68797.4	C46H59N11O9	(M+H)+	-2.31
914.46720	914.46790	1	14291.77	C46H59N11O9	(M+H)+	0.73
915.47390	915.47050	1	3706.55	C46H59N11O9	(M+H)+	-3.76

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-2430(11-2-14_KDE_KDYWE)_hrESIneg2.d	**Sample Name**	2430(11-2-14_KDE_KDYWE)	**Comment**	2430(11-2-14_KDE_KDYWE)
Position	P1-F6	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	11/13/2014 3:08:03 PM	**DA Method**	Ian.m

MS Zoomed Spectrum

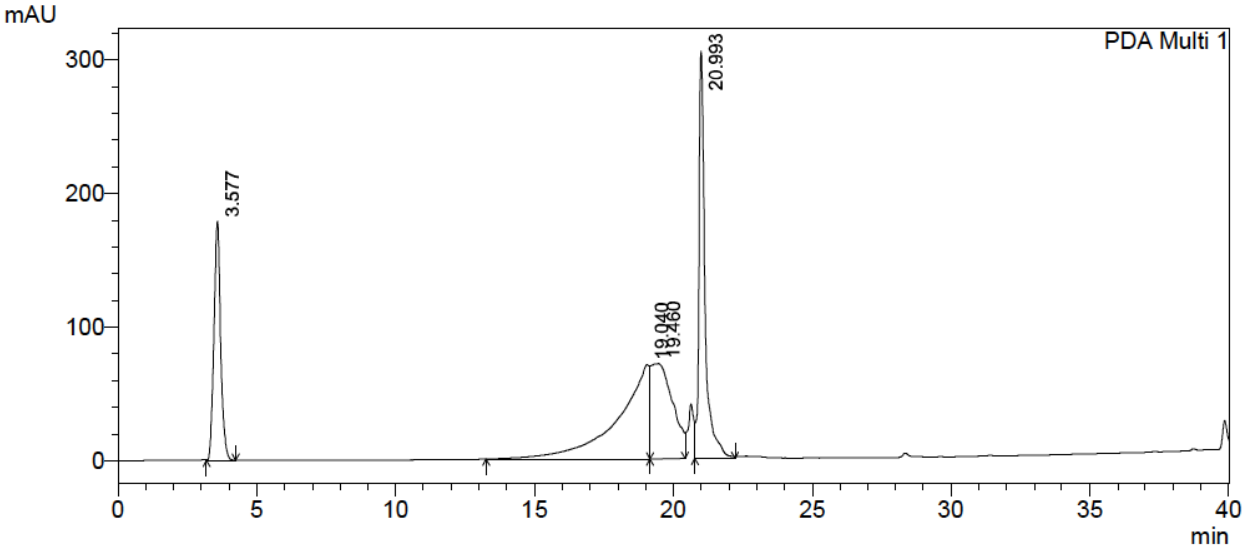


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
908.44300	908.44240	1	125450.88	C46H59N11O9	(M-H)-	-0.56
909.44580	909.44540	1	65993.42	C46H59N11O9	(M-H)-	-0.47
910.44790	910.44810	1	19498.3	C46H59N11O9	(M-H)-	0.23
911.44910	911.45080	1	4609.84	C46H59N11O9	(M-H)-	1.82
912.43000	912.45340	1	1120.5	C46H59N11O9	(M-H)-	25.63
913.44010	913.45590	1	261.95	C46H59N11O9	(M-H)-	17.34
1022.43610			159301.06			

--- End Of Report ---

SI-Figure 19. High resolution mass spectrometry data for peptide **10**.

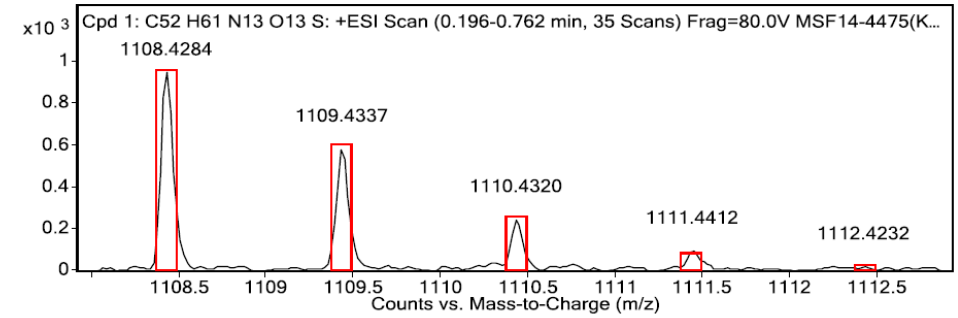


SI-Figure 20. HPLC trace for purified peptide **10**.

Target Compound Screening Report

Data File	MSF14-4475(KDEW_KDYWE)_hrESIpos2.d	**Sample Name**	4475(KDEW_KDYWE)	**Comment**	4475(KDEW_KDYWE)
Position	P1-B5	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	11/18/2014 9:26:15 AM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

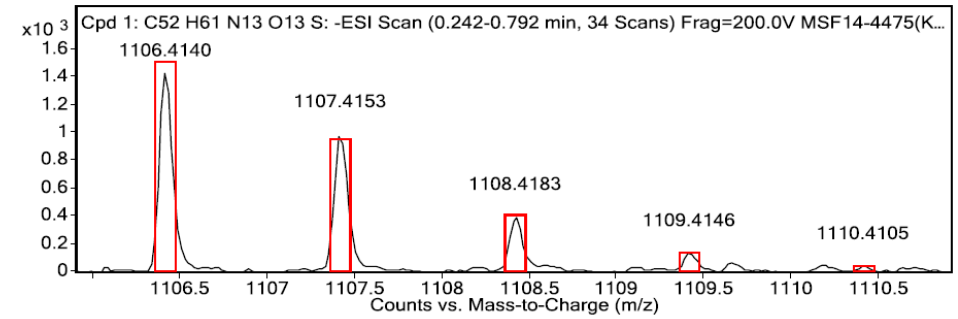
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
554.71870			6263.03			
1108.42840	1108.43050	1	953.45	C52H61N13O13S	(M+H)+	1.94
1109.43370	1109.43340	1	586.36	C52H61N13O13S	(M+H)+	-0.24
1110.43200	1110.43440	1	248.59	C52H61N13O13S	(M+H)+	2.2
1111.44120	1111.43560	1	97.3	C52H61N13O13S	(M+H)+	-5.1
1112.42320	1112.43690	1	21.73	C52H61N13O13S	(M+H)+	12.36

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-4475(KDEW_KDYWE)_hrESIneg2.d	**Sample Name**	4475(KDEW_KDYWE)	**Comment**	4475(KDEW_KDYWE)
Position	P1-B5	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	11/18/2014 9:23:11 AM	**DA Method**	Ian.m

MS Zoomed Spectrum

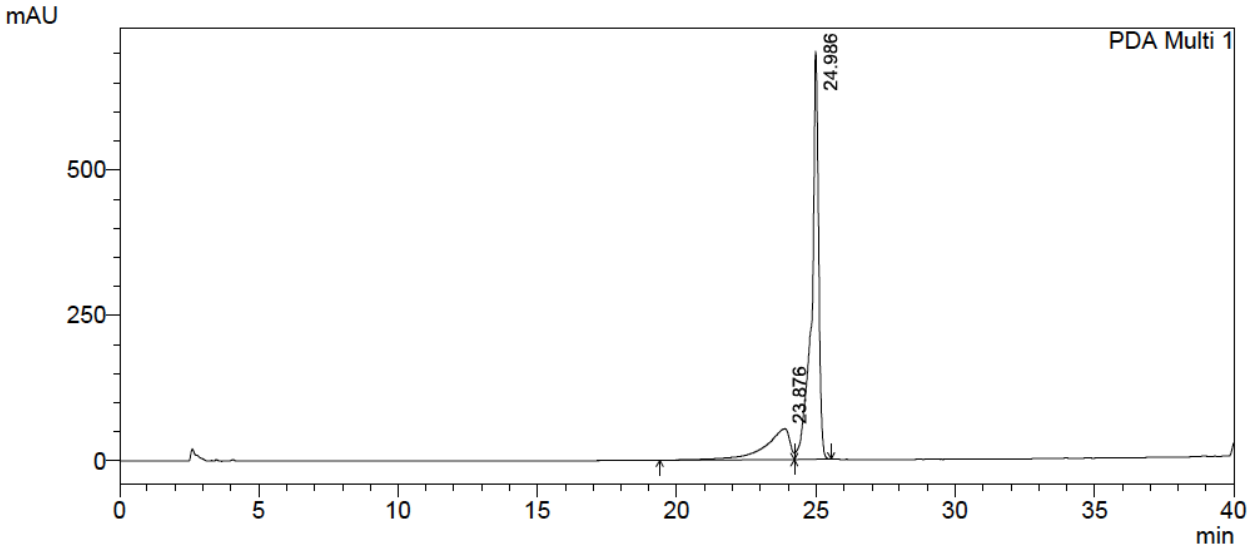


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
979.96560			1505.41			
1106.41400	1106.41600	1	1434.94	C52H61N13O13S	(M-H)-	1.76
1107.41530	1107.41880	1	991.33	C52H61N13O13S	(M-H)-	3.17
1108.41830	1108.41990	1	396.96	C52H61N13O13S	(M-H)-	1.44
1109.41460	1109.42100	1	135.62	C52H61N13O13S	(M-H)-	5.74
1110.41050	1110.42240	1	42.51	C52H61N13O13S	(M-H)-	10.67

--- End Of Report ---

SI-Figure 21. High resolution mass spectrometry data for peptide **11**.

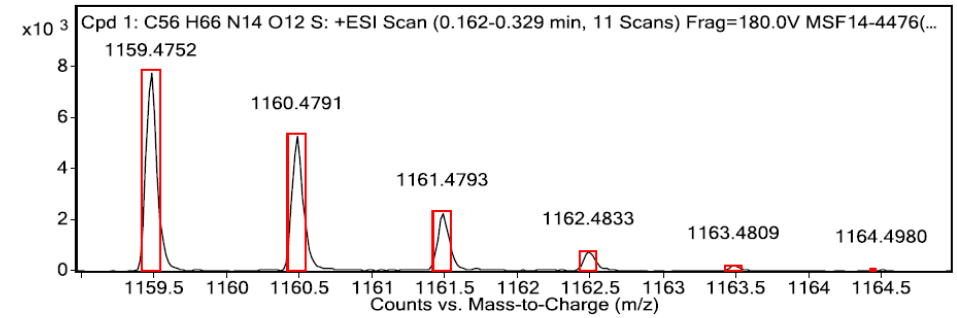


SI-Figure 22. HPLC trace for purified peptide **11**.

Target Compound Screening Report

Data File	MSF14-4476(KDEW_KDYWEc)_hrESIpos1.d	**Sample Name**	4476(KDEW_KDYWEc)	**Comment**	4476(KDEW_KDYWEc)
Position	P1-B5	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	pos.m	**Acquired Time**	12/16/2014 8:02:31 AM	**DA Method**	Ian.m

MS Zoomed Spectrum



MS Spectrum Peak List

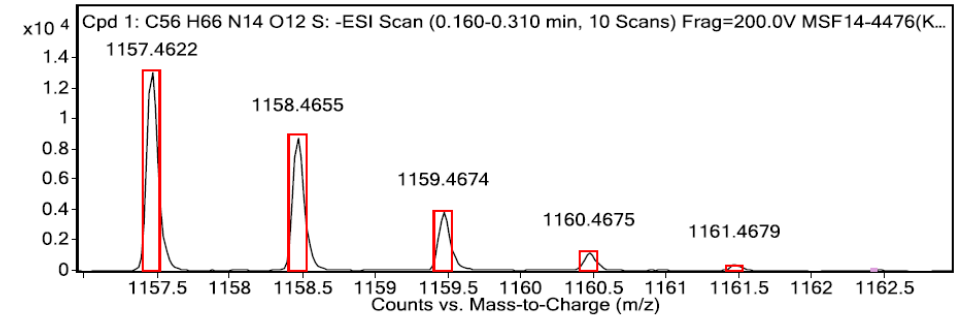
Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
580.24270			21918.24			
1159.47520	1159.47780	1	7795.29	C56H66N14O12S	(M+H)+	2.24
1160.47910	1160.48070	1	5294.49	C56H66N14O12S	(M+H)+	1.38
1161.47930	1161.48190	1	2299.1	C56H66N14O12S	(M+H)+	2.27
1162.48330	1162.48310	1	780.91	C56H66N14O12S	(M+H)+	-0.18
1163.48090	1163.48440	1	221.77	C56H66N14O12S	(M+H)+	3.06
1164.49800	1164.48600	1	78.18	C56H66N14O12S	(M+H)+	-10.3

--- End Of Report ---

Target Compound Screening Report

Data File	MSF14-4476(KDEW_KDYWEc)_hrESIneg1.d	**Sample Name**	4476(KDEW_KDYWEc)	**Comment**	4476(KDEW_KDYWEc)
Position	P1-B5	**Instrument Name**	Instrument 1	**User Name**	
Acq Method	neg.m	**Acquired Time**	12/16/2014 8:05:08 AM	**DA Method**	Ian.m

MS Zoomed Spectrum

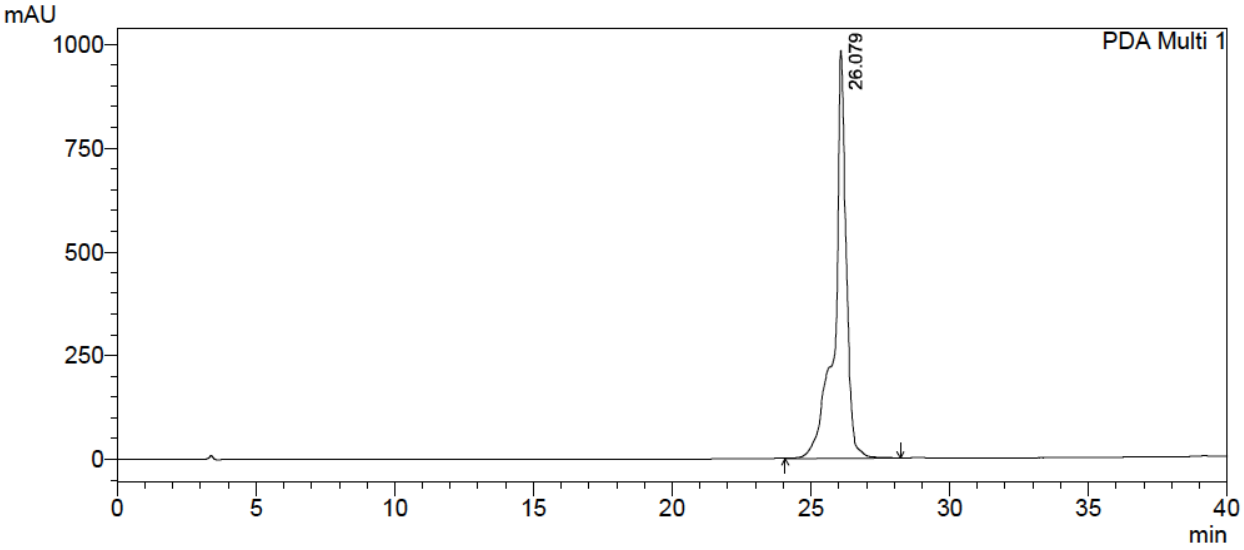


MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abund	Formula	Ion/Isotope	Tgt Mass Error (ppm)
112.98560			19732.4			
1157.46220	1157.46330	1	13140.28	C56H66N14O12S	(M-H)-	0.9
1158.46550	1158.46610	1	8800.68	C56H66N14O12S	(M-H)-	0.56
1159.46740	1159.46740	1	3897.37	C56H66N14O12S	(M-H)-	0
1160.46750	1160.46850	1	1237.52	C56H66N14O12S	(M-H)-	0.9
1161.46790	1161.46990	1	464.1	C56H66N14O12S	(M-H)-	1.66

--- End Of Report ---

SI-Figure 23. High resolution mass spectrometry data for peptide **12**.



SI-Figure 24. HPLC trace for purified peptide **12**.