Supplemental Material

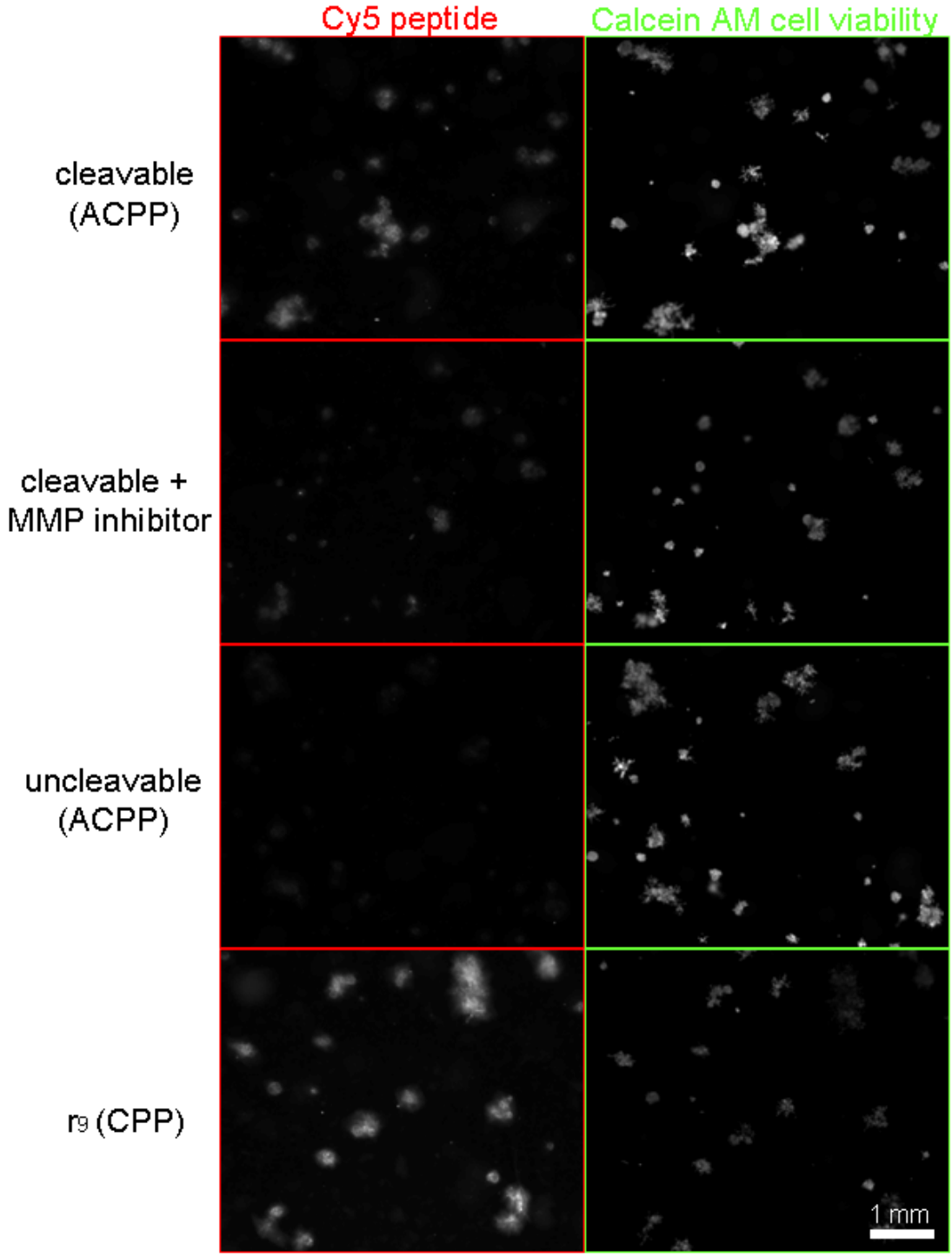
Supplemental Figure Legends

Supp. Fig. 1. Fluorescence images of 3-D clusters of MDA-MB-231 cells after treatment with MMP cleavable ACPP, ACPP + MMP inhibitor (100μM), uncleavable d-amino acid ACPP, and r_9 CPP positive control. These are representative images by which quantitative data is derived in Fig. 2E. Images on left measure Cy5 peptide uptake and calcein green AM images stain for cell viability (right). Scale bar 1mm.

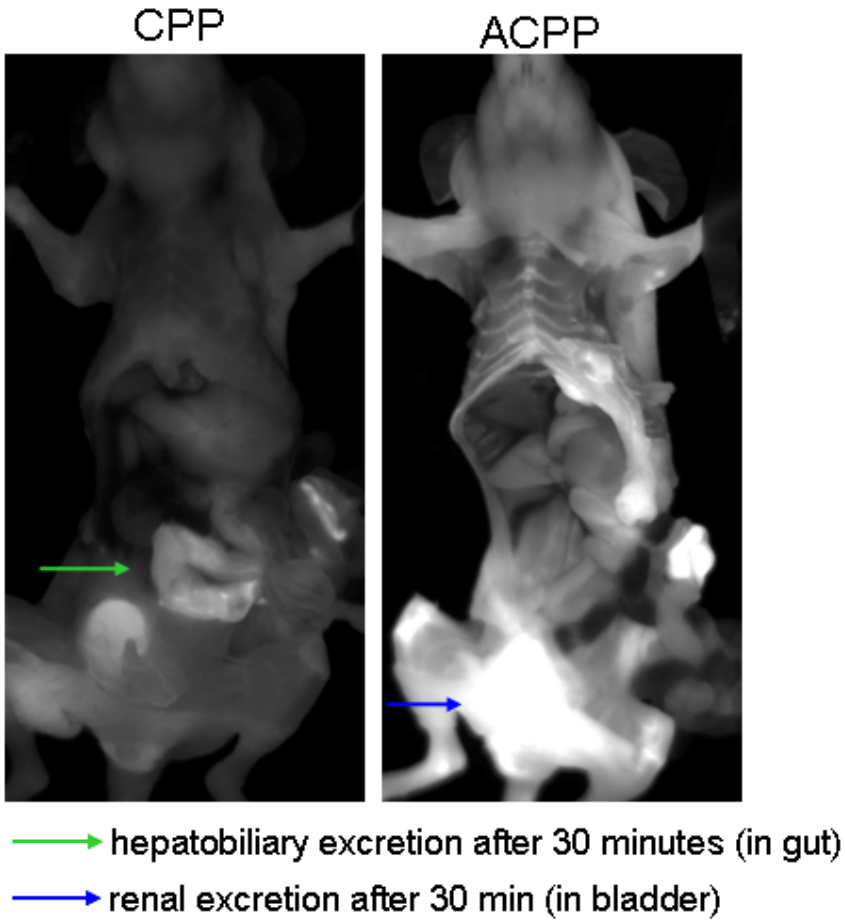
Supp. Fig. 2. 30 minute image of dissected mice injected with CPP and ACPP. The green arrow shows early hepatic excretion of peptide for CPP supporting rapid uptake and excretion via liver after IV injection. The blue arrow shows high level of renal excretion of ACPP supporting rapid renal filtration and excretion after IV injection.

Supp. Fig. 3. Confocal slices of kidneys approximately 10-20 minutes (fresh) after mice were sacrificed and approximately 40-60 minutes dead-dried out. These data highlight the redistribution of CPP subcellular puncta to a more diffuse and nuclear uptake showing redistribution artifact in live tissues as they sit around post mortem (arrows pointing to uptake in nuclei). White arrows point to cells with nuclear uptake. Red is r_9Cy5 CPP, blue indicates Hoechst labeled nuclei, and green is rhodamine dextran blood pool. Scale bar is 20 μm.

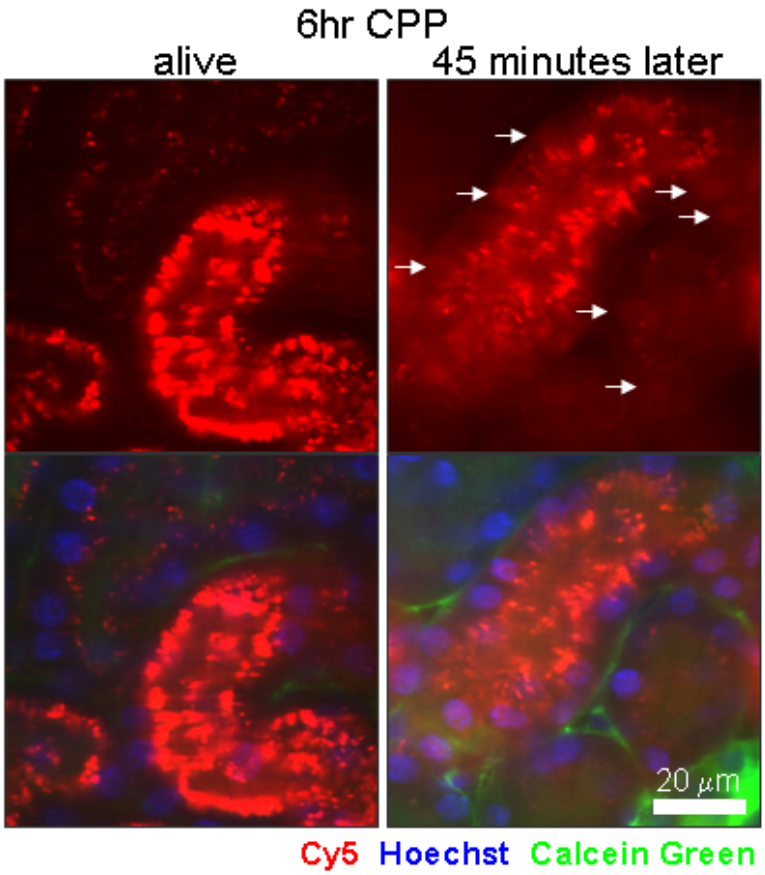
Supplemental Figures



Supplemental figure 1



Supplemental figure 2



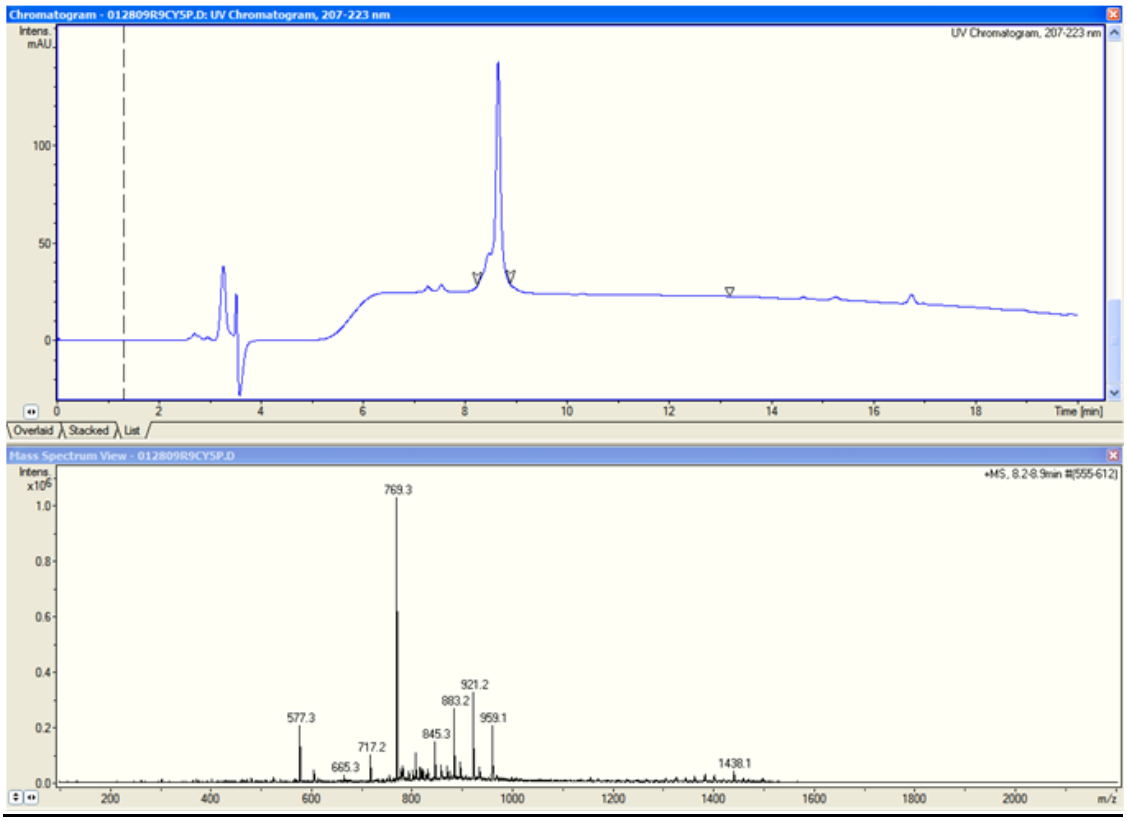
Supplemental figure 3

LC Mass Spectra for peptides used in the study

The upper panel in each figure is the chromatogram monitored by UV absorbance at 207-223 nm. The mass spectrum of the peak bracketed by the arrowheads is shown in the lower panel.

r9-c(Cy5)
Found mass 2304

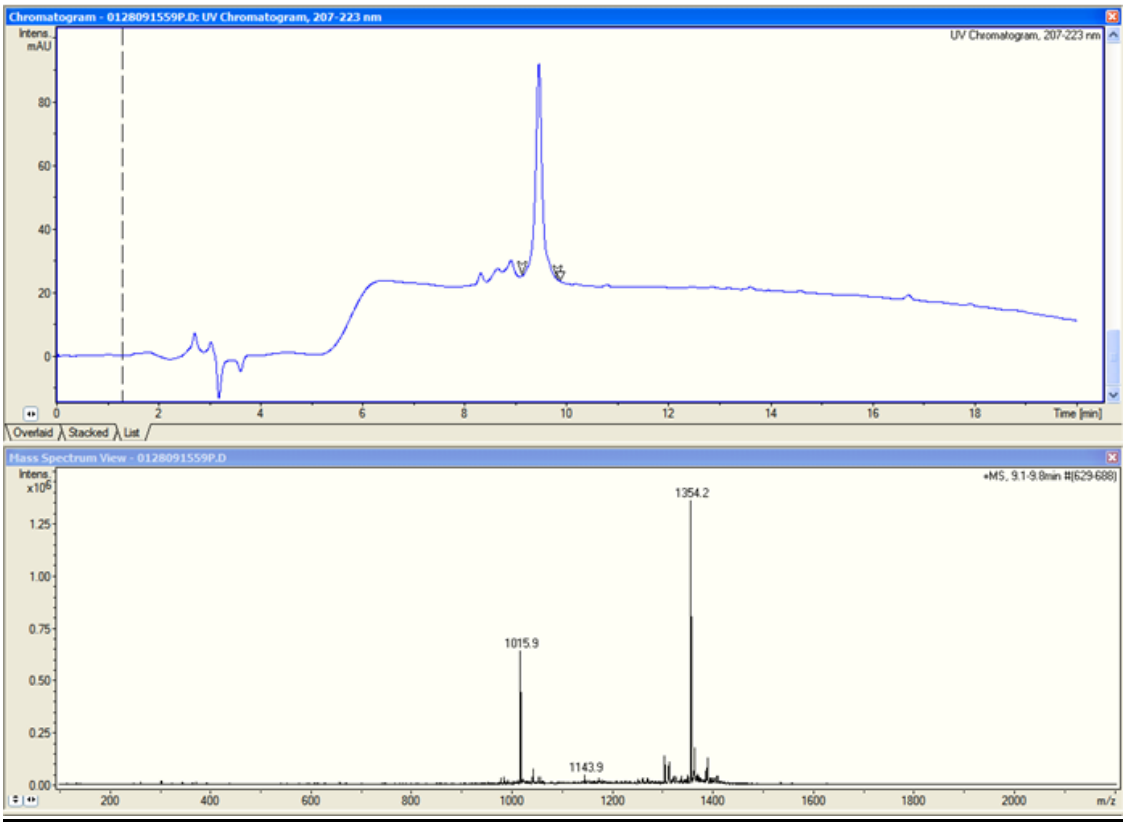
Chemical Formula: $C_{96}H_{161}N_{42}O_{19}S_3$
Exact Mass: 2302.21
Molecular Weight: 2303.77



The m/z peaks at 769.3 and 577.3 Da represent the molecule plus 3 or 4 protons ($M + 3H^+$ and $M + 4H^+$) respectively.

Suc-e8-xPLGLAG-r9-c(Cy5)
Found mass 4060

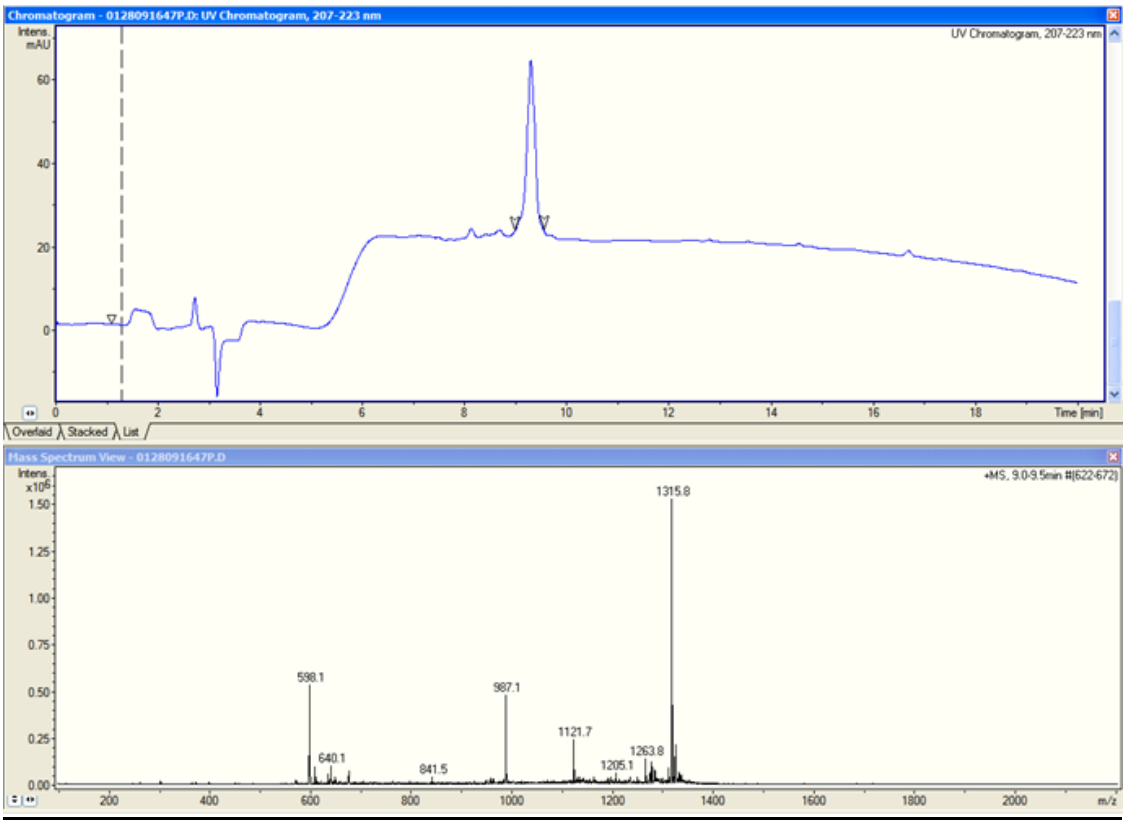
Chemical Formula: $C_{170}H_{273}N_{57}O_{53}S_3$
Exact Mass: 4056.96
Molecular Weight: 4059.53



The m/z peaks at 1354.2 and 1015.9 Da represent the molecule plus 3 or 4 protons ($M + 3H^+$ and $M + 4H^+$) respectively.

Suc-e8-xplglag-r9-k(Cy5)
Found mass 3943

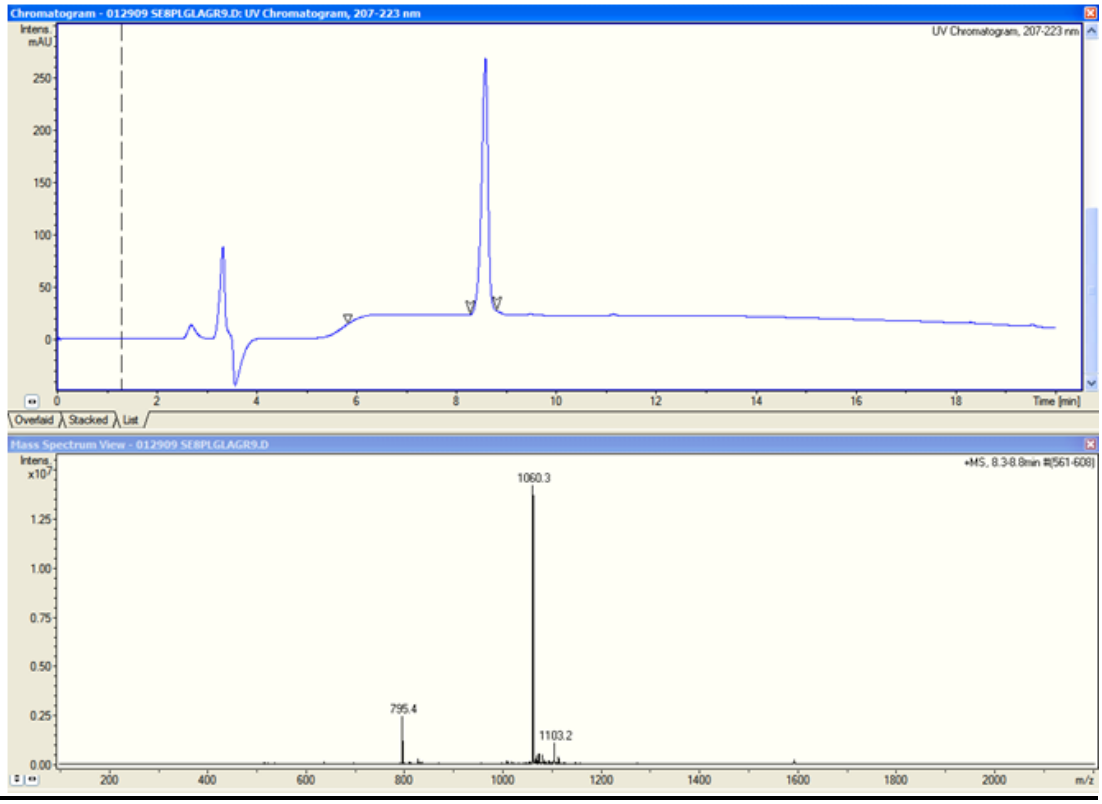
Chemical Formula: $C_{167}H_{273}N_{57}O_{50}S_2$
Exact Mass: 3941.00
Molecular Weight: 3943.44



The m/z peaks at 1315.8 and 987.1 Da represent the molecule plus 3 or 4 protons ($M + 3H^+$ and $M + 4H^+$) respectively.

Suc-e8-xPLGLAG-r9
Found mass 3177

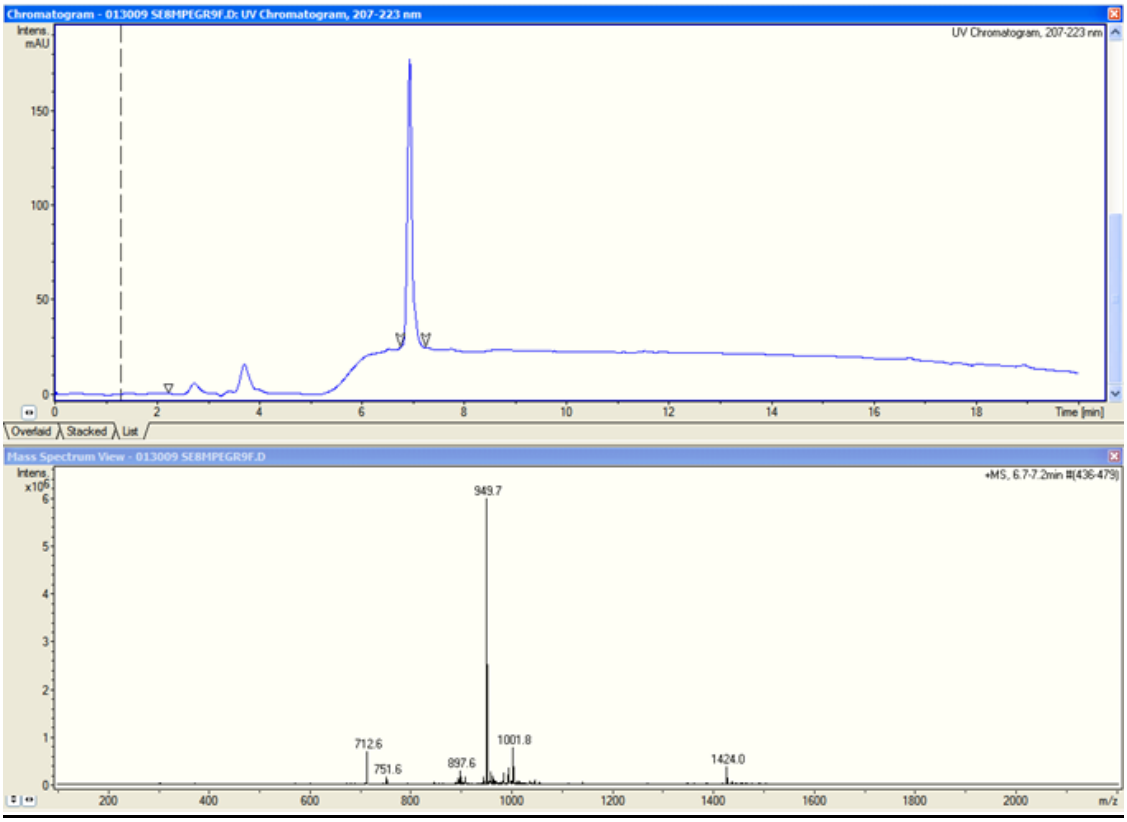
Chemical Formula: $C_{128}H_{222}N_{52}O_{43}$
Exact Mass: 3175.68
Molecular Weight: 3177.45



The m/z peaks at 1060.3 and 795.4 Da represent the molecule plus 3 or 4 protons ($M + 3H^+$ and $M + 4H^+$) respectively.

Suc-e8-(mPeg2)$_2$-r9
Found mass 2846

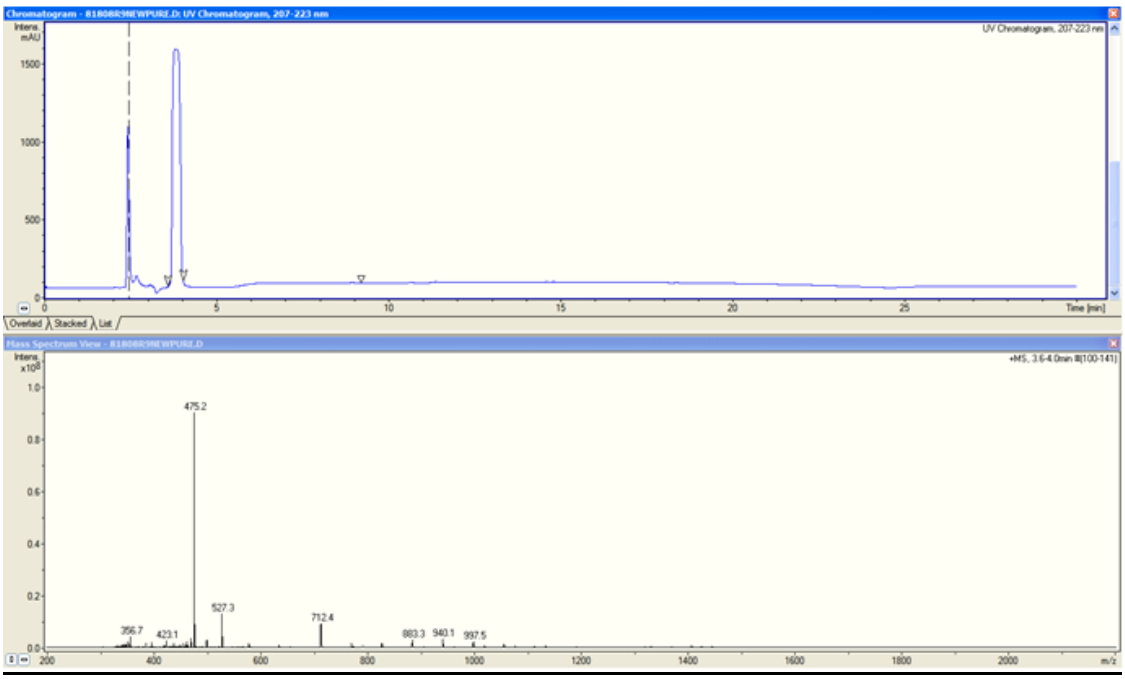
Chemical Formula: $C_{110}H_{193}N_{47}O_{42}$
Exact Mass: 2844.44
Molecular Weight: 2846.00



The m/z peaks at 949.7 and 712.6 Da represent the molecule plus 3 or 4 protons ($M + 3H^+$ and $M + 4H^+$) respectively.

r9
Found mass 1422.6

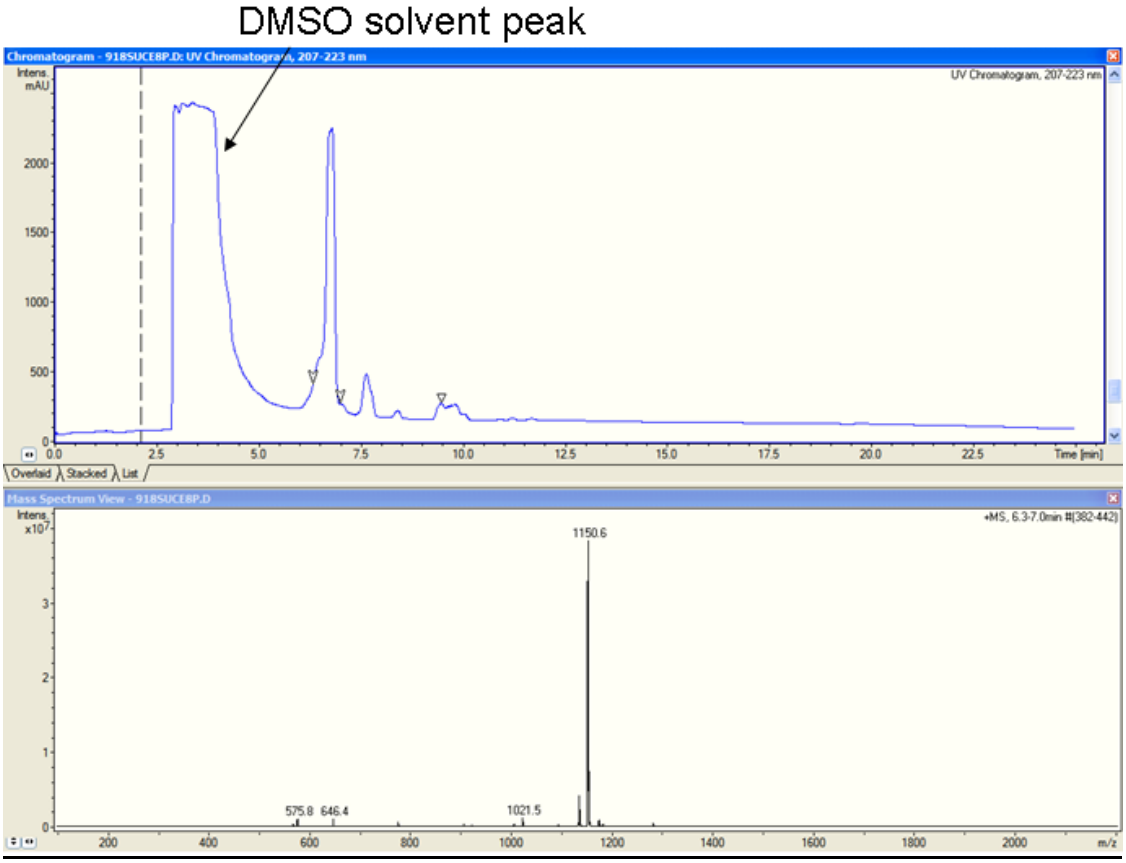
Chemical Formula: $C_{54}H_{111}N_{37}O_9$
Exact Mass: 1421.94
Molecular Weight: 1422.70



The m/z peaks at 712.4, 475.2, and 356.7 Da represent the molecule plus 2, 3, or 4 protons ($M + 2H^+$, $M + 3H^+$ or $M + 4H^+$) respectively.

Suc-e8
Found mass 1149

Chemical Formula: $C_{44}H_{62}N_9O_{27}$
Exact Mass: 1148.38
Molecular Weight: 1149.01



The m/z peaks at 1150.6 and 575.8 Da represent the molecule plus 1 or 2 protons ($M + H^+$ and $M + 2H^+$) respectively.