

Results

Mass spectrometry

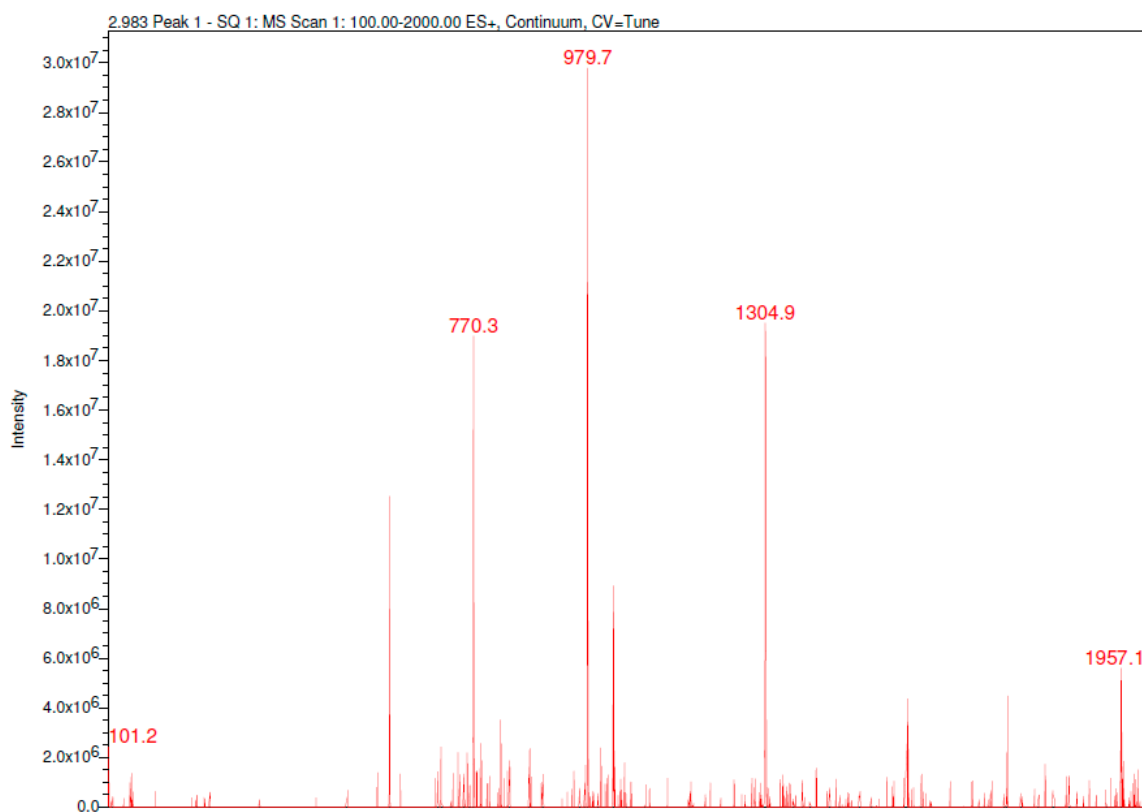


Figure S1. Mass spectrum of Cy5-C-P0₁₀₁₋₁₂₅, m/z [M+3H – 979.7].

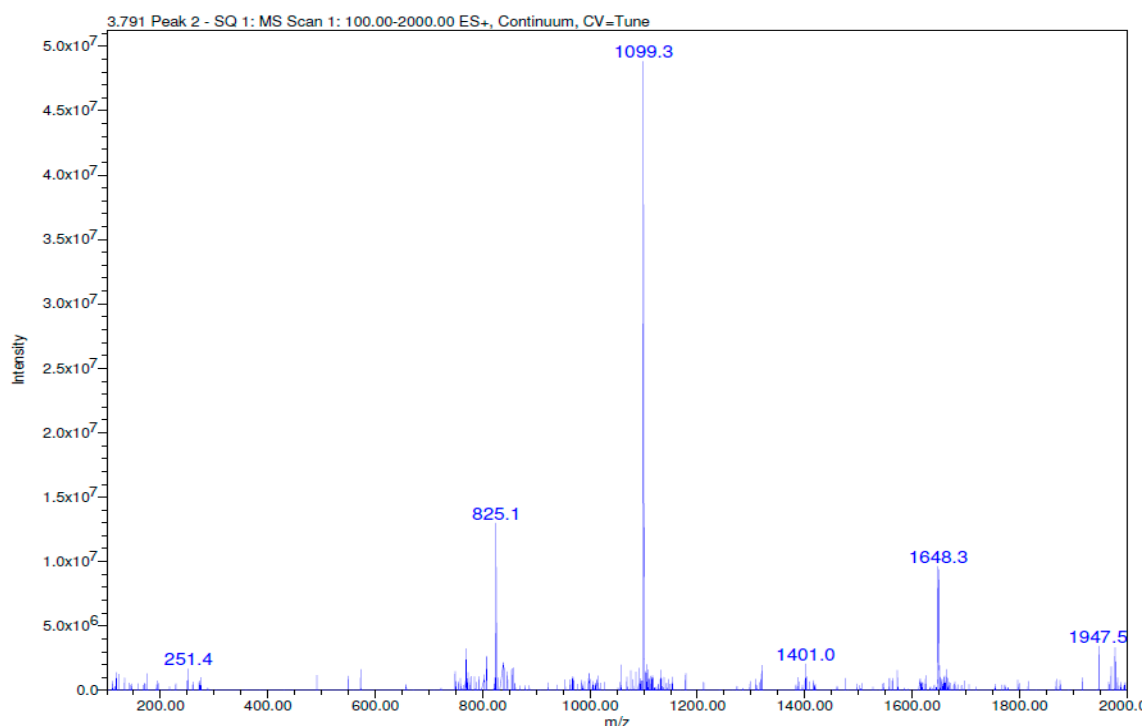


Figure S2. Mass spectrum of Cy5-C-P0₁₀₁₋₁₂₀, m/z [M+3H – 1999.3].

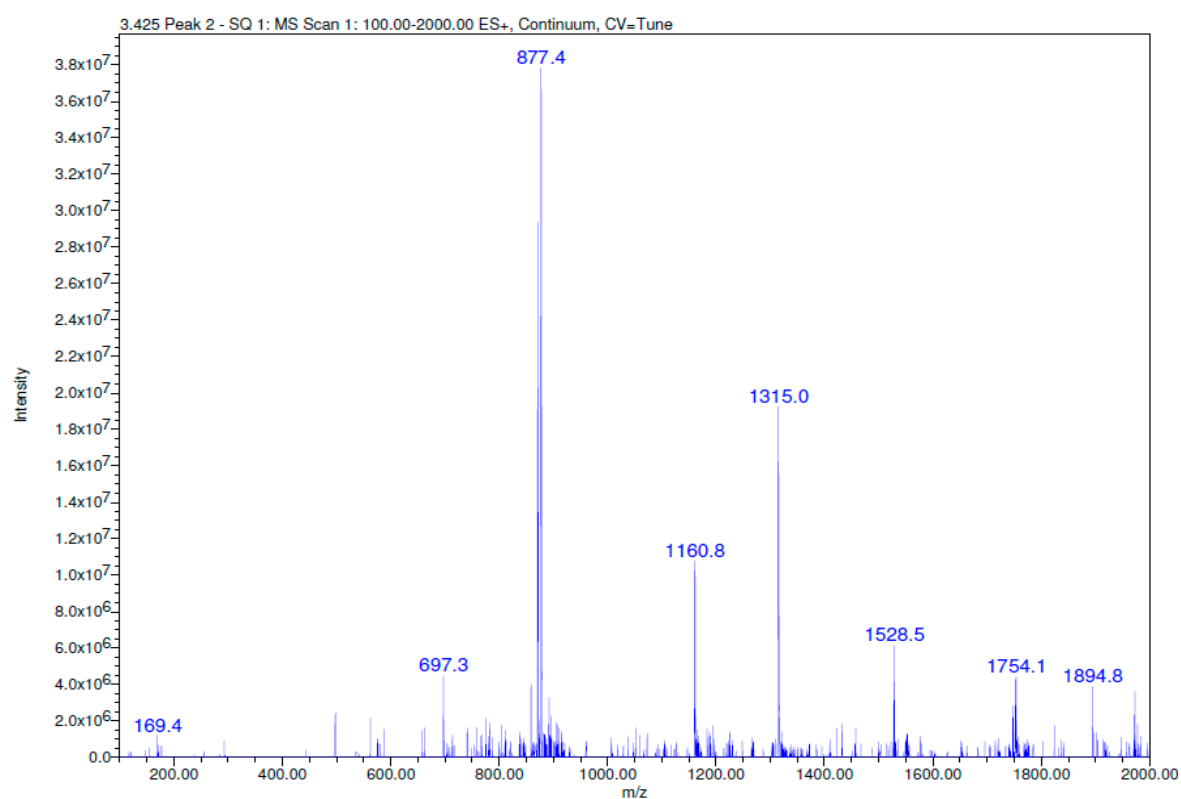


Figure S3. Mass spectrum of Cy5-C-P0₁₀₁₋₁₁₅. m/z [M+3H – 877.4].

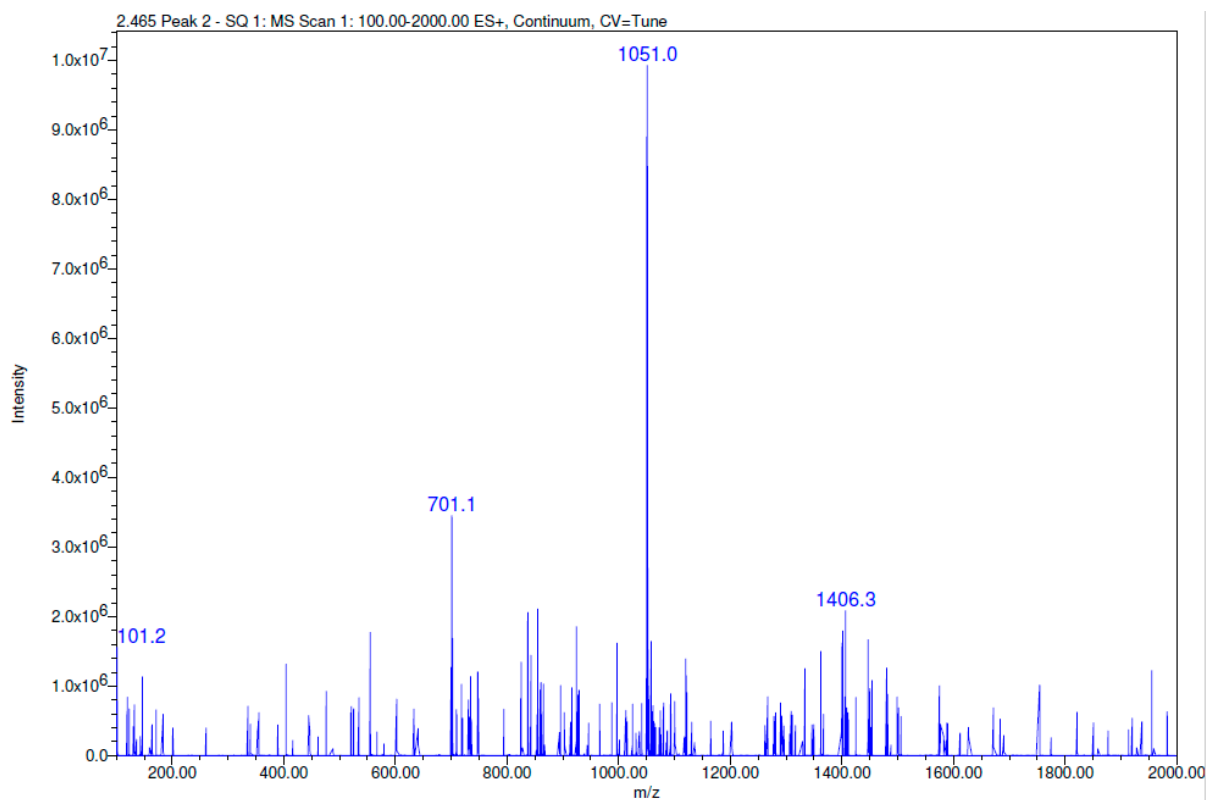


Figure S4. Mass spectrum of Cy5-C-P0₁₀₁₋₁₁₀. m/z [M+2H – 1051.0].

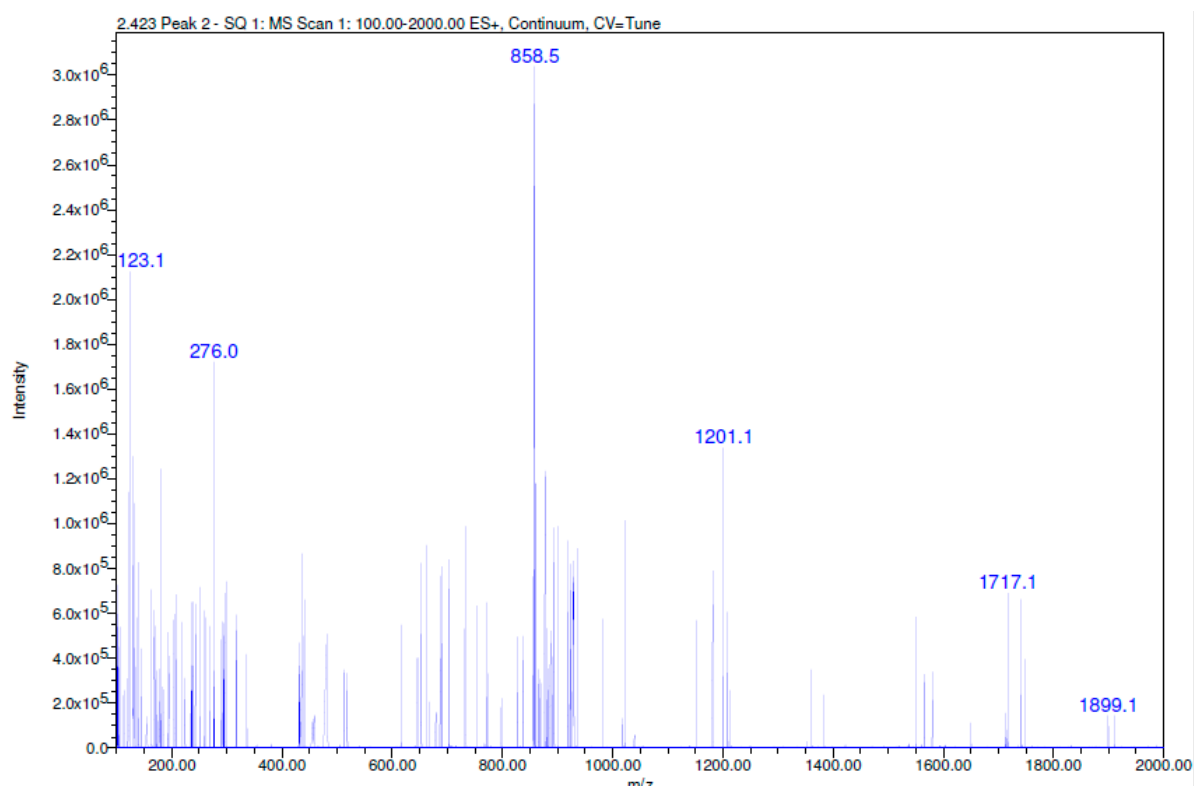


Figure S5. Mass spectrum of Cy5-C-P0101-106. m/z [$M+2H - 858.5$].

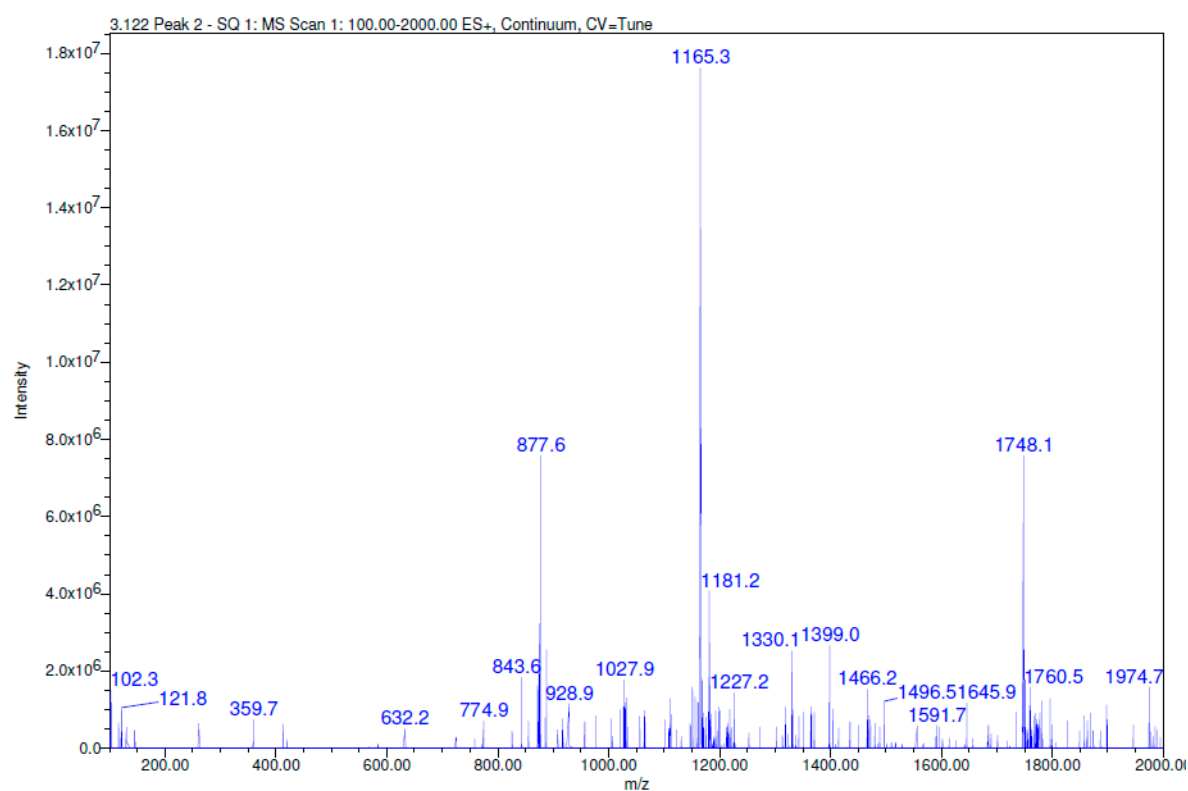


Figure S6. Mass spectrum of Cy5-C-P0105-125. m/z [$M+3H+Na^+ - 1165.3$].

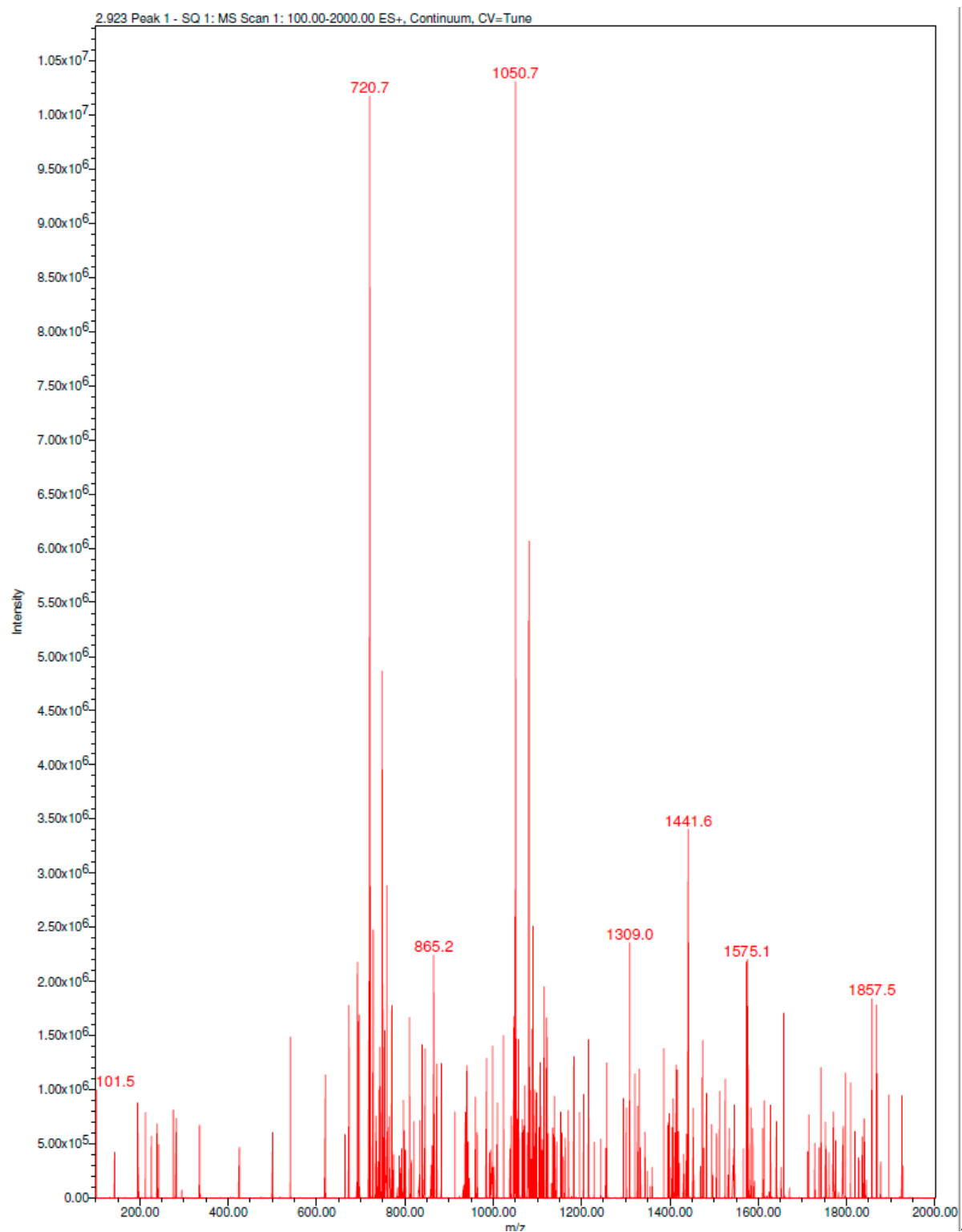


Figure S7. Mass spectrum of Cy5-C-P0108-125. m/z [M+2H- 1050.7].

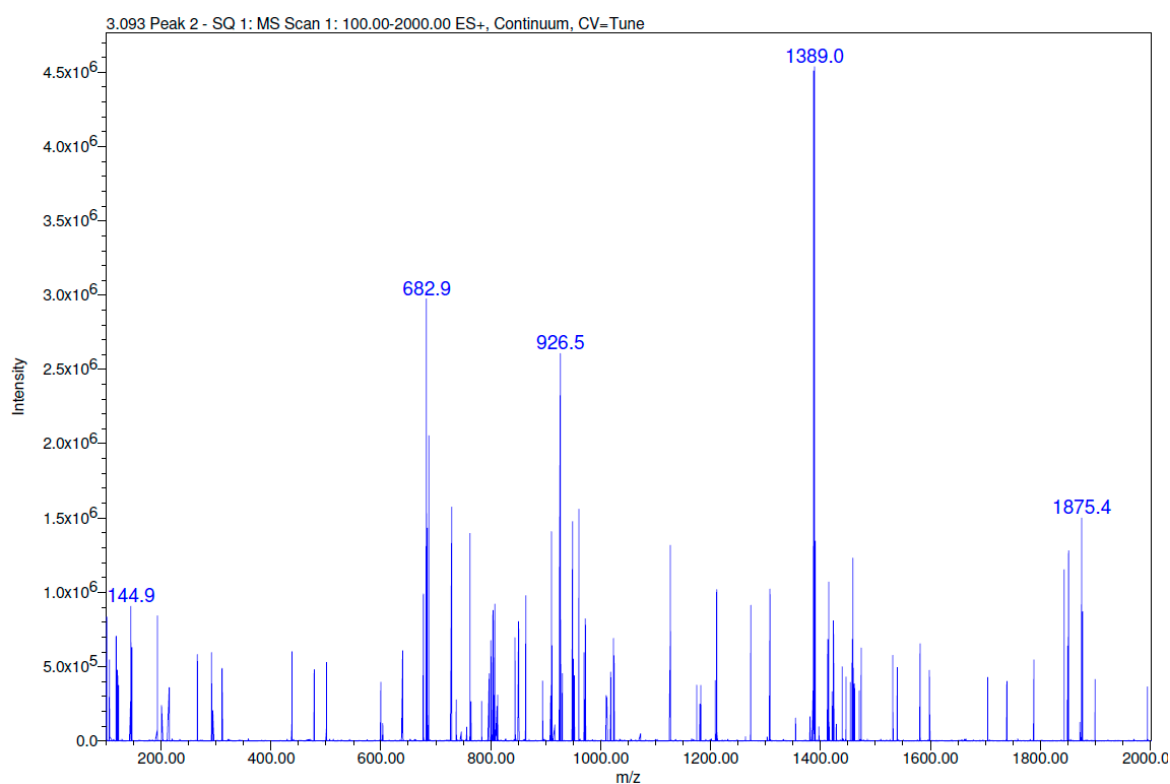


Figure S8. Mass spectrum of Cy5-C-P0_{112–125}. m/z [M+2H – 1389.0].

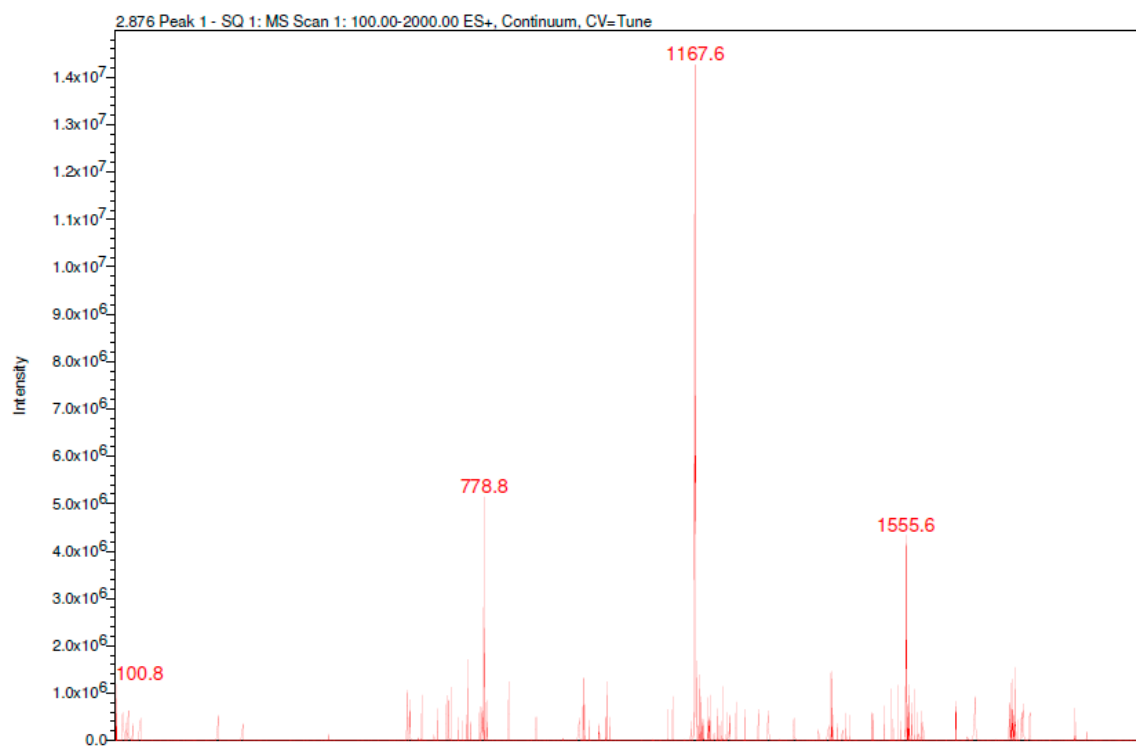


Figure S9. Mass spectrum of Cy5-C-P0_{116–125}. m/z [M+2H – 1167.6].

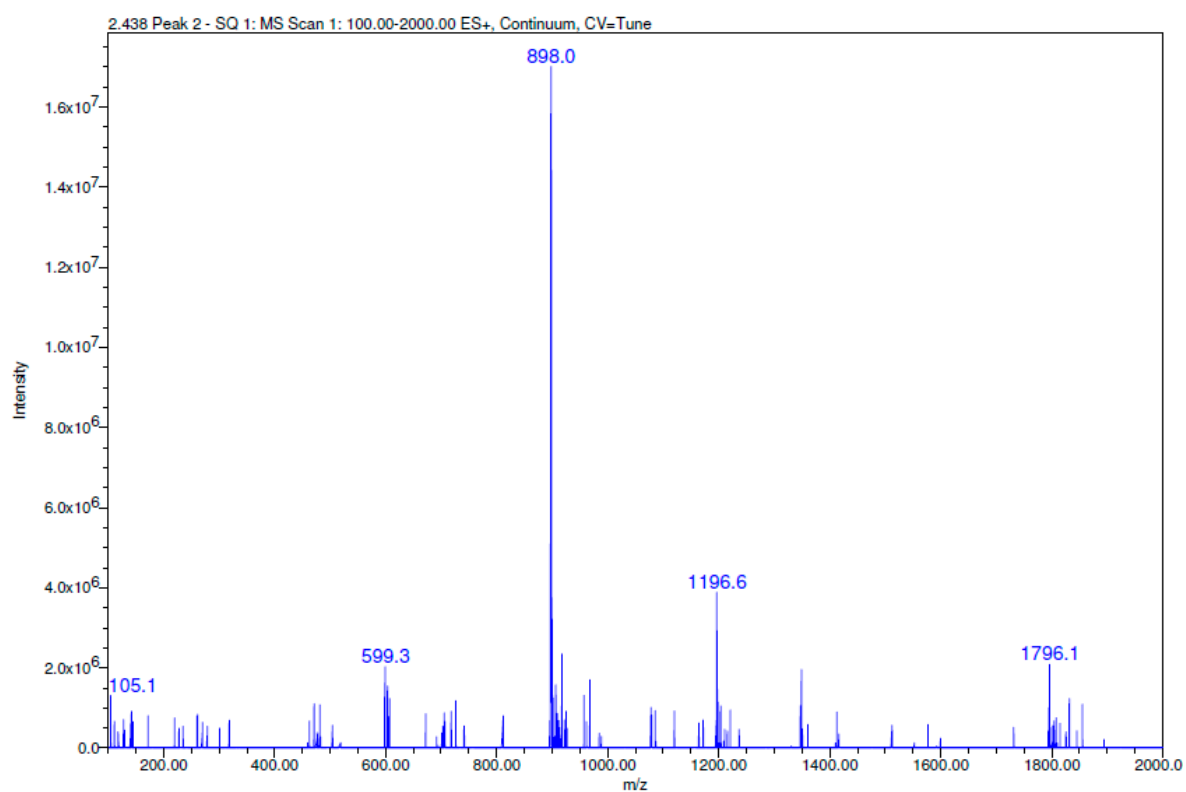


Figure S10. Mass spectrum of Cy5-C-P0₁₂₀₋₁₂₅. m/z [M+2H – 898.0].

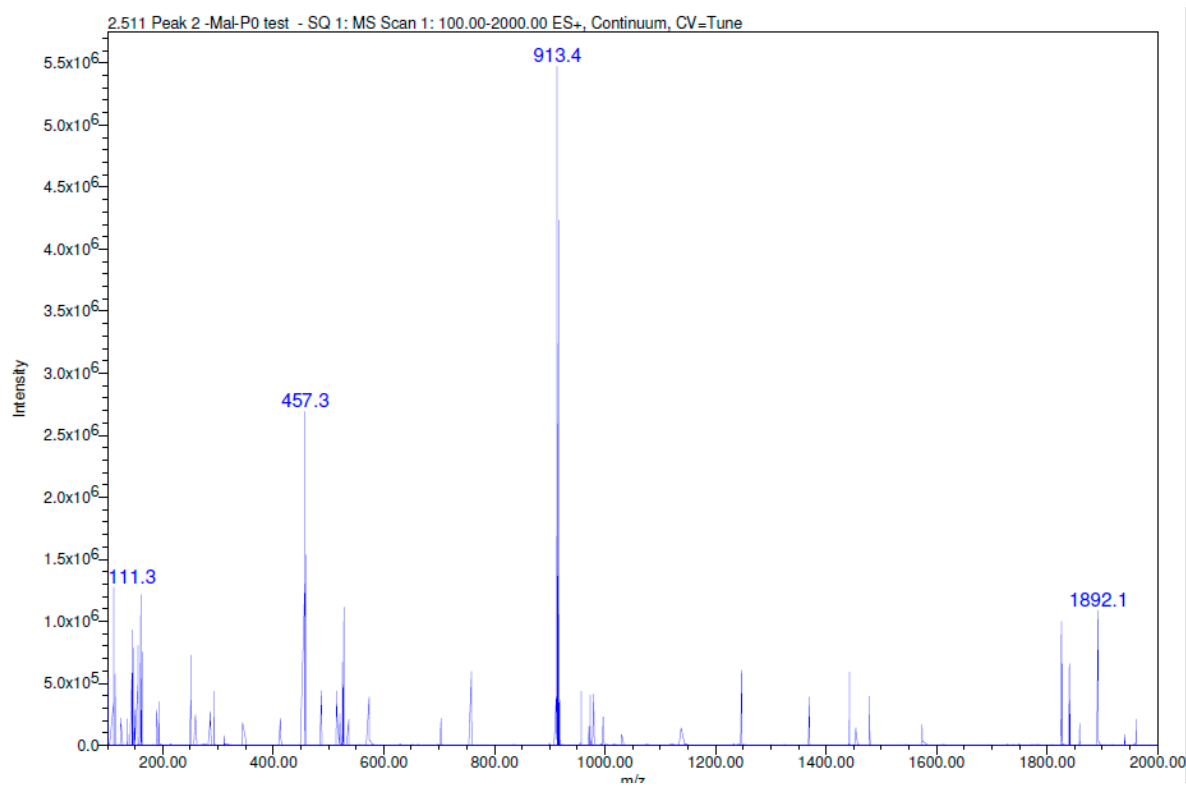


Figure S11. Mass spectrum of Maleimide-(SO₃)Cy5(SO₃)Sulfonate. m/z [M – 913.4].

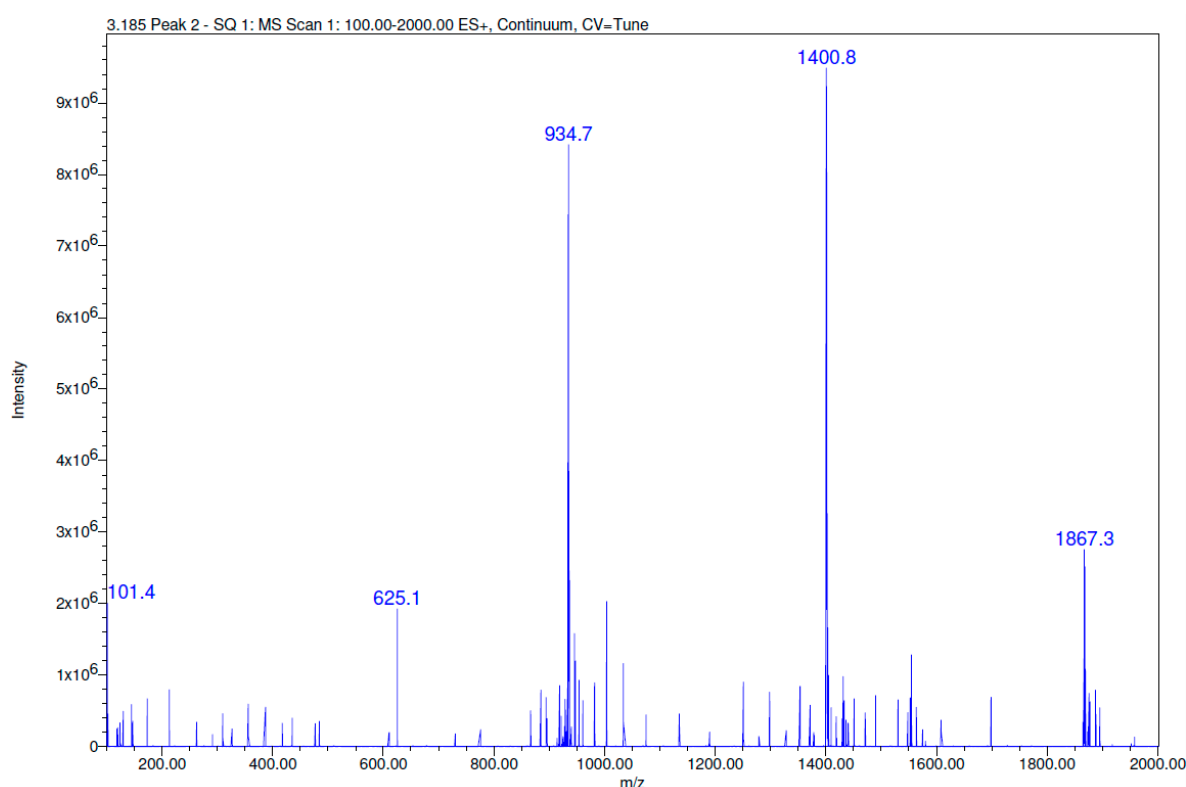


Figure S12. Mass spectrum of Cy7-C-P0_{112–125}. m/z [$M+2H - 1400.8$].

Co-localization P0 staining and lysosomal and nuclear control staining

(3D) surface plot analysis was used to evaluate the localization of the P0-specific staining further (Figure S13). For peptides with a nanomolar affinity (Cy5-P0_{101–125}, Cy5-P0_{105–125}, Cy5-P0_{112–125} and Cy5-P0_{116–125}) distribution over the cellular outgrowths was confirmed, with limited overlay with the intracellular lysosomal control staining (overlay Cy5/lysosomes in yellow). No significant co-localization with the nuclear control staining (in blue) was seen. Moreover, increasing affinity (in particular for Cy5-P0_{112–125} and Cy5-P0_{116–125}) resulted in increased fluorescence peak intensities within the Cy5-related staining pattern.

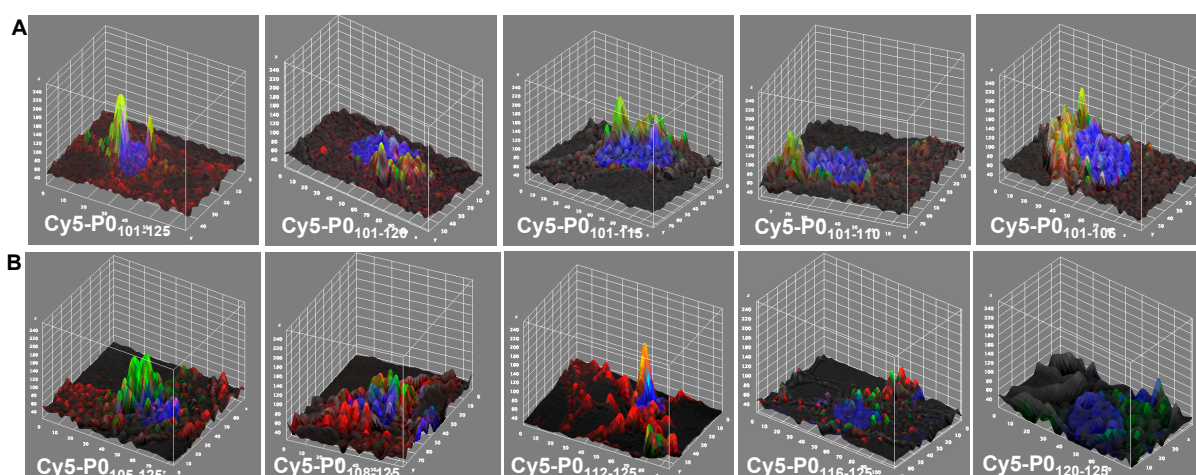


Figure S13. Surface plot analysis fluorescence confocal images Surface plot analysis of the staining pattern throughout the cells for A) the C-terminal matrix and B) the N-terminal matrix. Peptide-related staining (Cy5) in red, lysosomes in green and nuclear staining in blue. Yellow line = orientation 3D analysis.

Semi-quantitative assessment of the level of co-staining within the fluorescence confocal images (Table S1) revealed an increasing trend of co-localization of lysosomal intracellular staining with decreasing affinity (Figure 3). Co-localization with the nuclear control staining was comparably low for all compounds.

Semi-quantitative assessment of the co-localization of the P0-related staining of Cy5-P0_{101–125} and the intracellular lysosomal (green) was shown to be low for this high affinity peptide (co-localization factor: 0.29; Table S1), while for Cy5-P0_{101–120}, Cy5-P0_{101–115}, Cy5-P0_{101–110} and Cy5-P0_{101–106} co-localization of signal was more prominent (co-localization factor of respectively 0.42, 0.59, 0.78 and 0.77). Co-localization with the nuclear (blue) control staining was low for all compounds. The absence of staining of outgrowths combined with the higher level of lysosomal co-localization would therefore suggest that the relatively low Cy5-related fluorescence signal obtained after C-terminal truncation can be contributed to non-specific staining.

Similar to the results obtained C-terminal matrix, the level of staining of the outgrowths was shown to correspond to the level of co-localization with the intracellular control staining/non-specific staining (Table S1). The lysosomal co-localization factor for Cy5-P0_{105–125} (0.32), Cy5-P0_{108–125} (0.27) and Cy5-P0_{112–125} (0.21) was comparable or lower than the original lead compound, whereas a loss in P0-related staining resulted in increased co-localization for Cy5-P0_{116–125} (0.40) and Cy5-P0_{120–125} (0.39).

Table S1. Semi-quantitative assessment of co-localization staining P0 tracers and lysosomal and nuclear control staining.

Peptide	Co-localization factor (scale: 0-1)	
	Cy5 vs Lysosomes	Cy5 vs nucleus
Cy5-P0 _{101–125}	0.29 ± 0.03	0.14 ± 0.03
Cy5-P0 _{101–120}	0.42 ± 0.01	0.06 ± 0.02
Cy5-P0 _{101–115}	0.59 ± 0.20	0.78 ± 0.13
Cy5-P0 _{101–110}	0.77 ± 0.15	0.15 ± 0.06
Cy5-P0 _{101–106}	0.77 ± 0.12	0.15 ± 0.06
Cy5-P0 _{105–125}	0.32 ± 0.04	0.07 ± 0.04
Cy5-P0 _{108–125}	0.27 ± 0.11	0.05 ± 0.04
Cy5-P0 _{112–125}	0.21 ± 0.06	0.11 ± 0.01
Cy5-P0 _{116–125}	0.40 ± 0.01	0.04 ± 0.02
Cy5-P0 _{120–125}	0.39 ± 0.06	0.07 ± 0.02