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# Targeted degradation via direct 26S proteasome recruitment

In the format provided by the authors and unedited

# Supplementary Information

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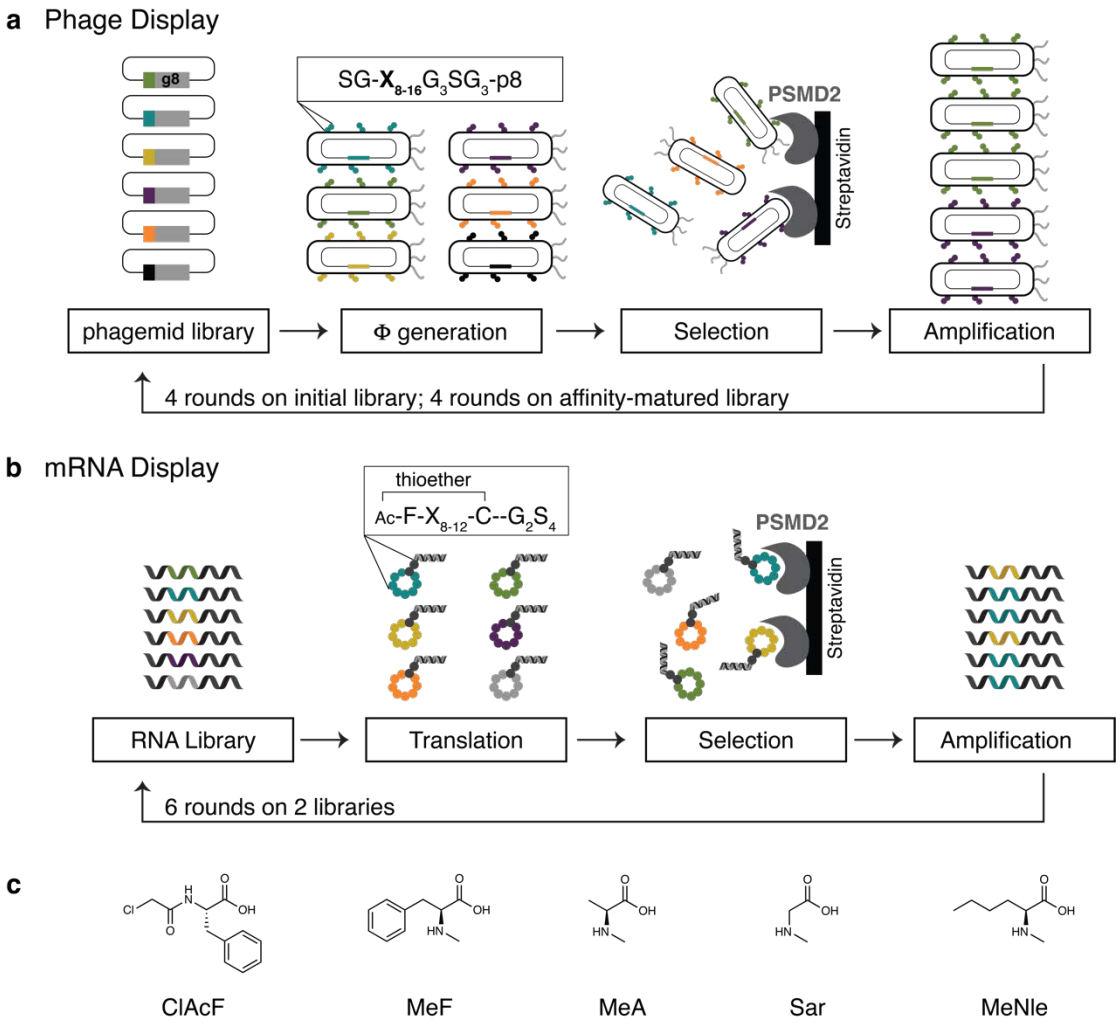
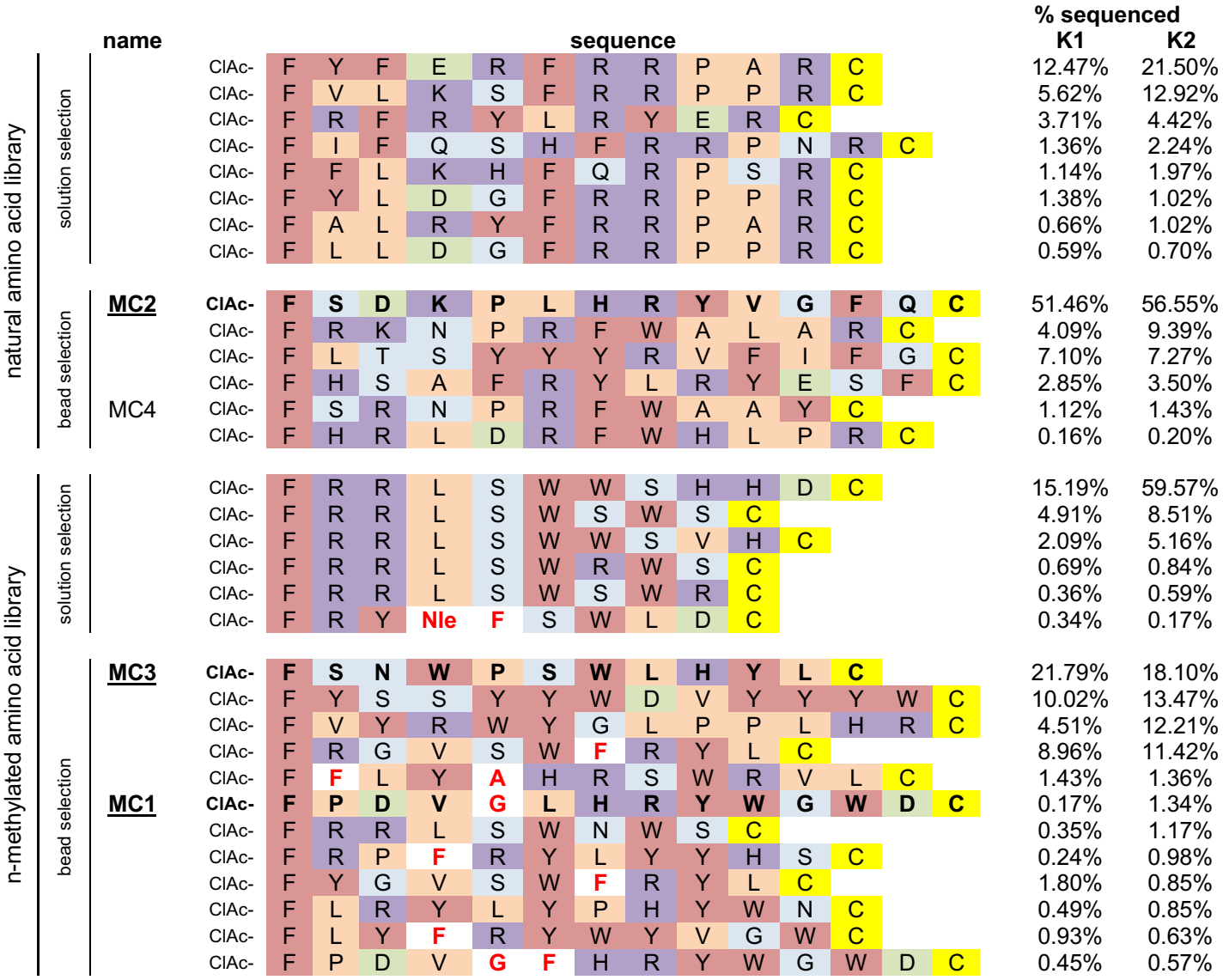
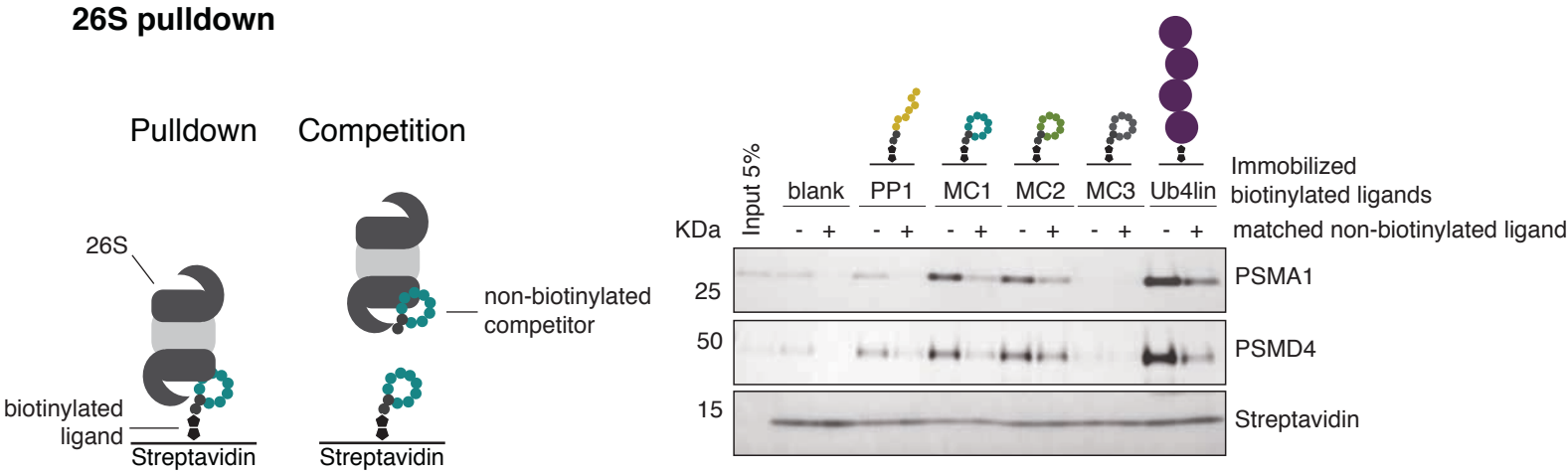


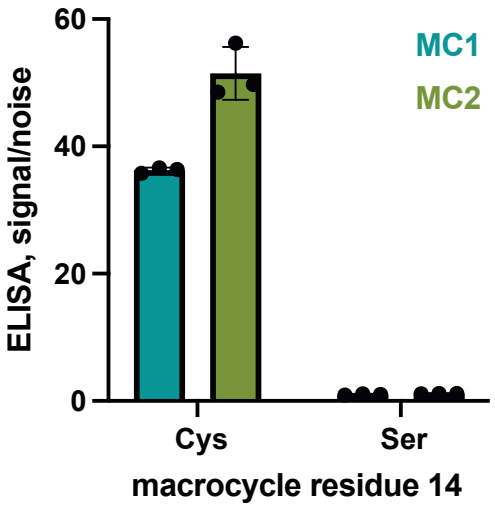

**Supplementary Fig. 1: Ligands discovered from display efforts.**
**a**, Representation of phage display selection. Libraries containing variable peptide sequences fused to gene 8 were selected against PSMD2 through iterative rounds of phage generation, selection, and amplification. Top candidates were then used to generate soft-randomized library for affinity maturation, resulting in the best linear peptide hit, PP1. **b**, Representation of mRNA display selection. Macrocycle peptide libraries varying from 8-16 amino acids in length are encoded in an mRNA library, and sequences that bind to PSMD2 are enriched over iterative cycles of in-vitro translation/transcription, selection, and sequence amplification. **c**, Structures of 5 non-natural amino acids used in libraries, including the initiator ClAcF (chloroacetyl-phenylalanine), MeF (N-methyl-L-phenylalanine), Sar (Sarcosine or N-methyl glycine), MeAla (N-methyl-L-alanine), and MeNle (N-methyl-L-norleucine).



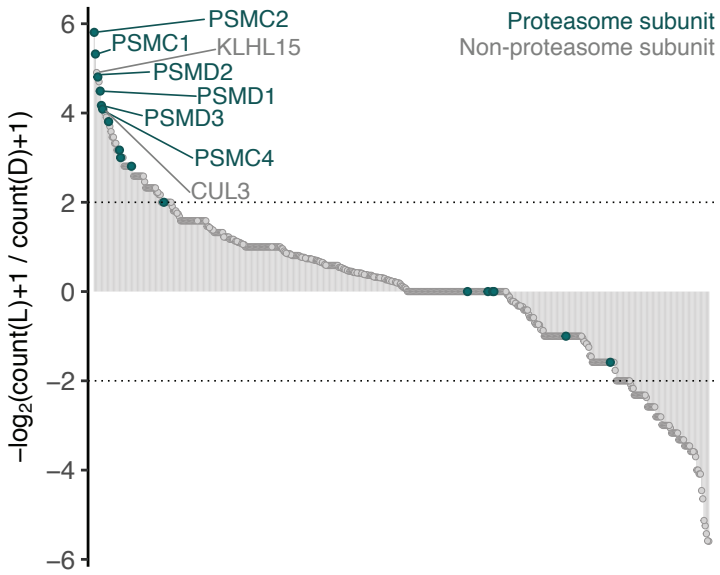

**Supplementary Fig. 2: Sequences from mRNA display.**
Sequencing results from 6 rounds of selections. Two libraries, one with natural amino acids and one including 4 n-methylated amino acids were selected against PSMD2 in solution or on beads. Sequence enrichment after the final two rounds of most stringent washing (K1 and K2) are shown on the right. Top candidates are labeled in bold underlined text. Red text represents an n-methylated amino acid: MeF (N-methyl-L-phenylalanine), Sar (Sarcosine or N-methyl glycine), MeAla (N-methyl-L-alanine), and MeNle (N-methyl-L-norleucine). All sequences begin with ClAcF (chloroacetyl-phenylalanine) and end with C (cysteine) to enable cyclization.




**Supplementary Fig. 3: Biotinylated macrocycles can pulldown 26S proteasome.** Biotinylated versions of MC1, MC2, MC3, and PP1 ($MC1_{biotin}$ $MC2_{biotin}$, $MC3_{biotin}$, $PP1_{biotin}$) were incubated with 26S proteasome *in vitro* in the presence or absence of excess corresponding non-biotinylated ligand. Linear ubiquitin was included as a positive control. Both PSMD4 (19S) and PSMA1 (20S) subunits were pulled down to some extent by 3 of the 4 ligands. Data were reproduced in 2 independent experiments.

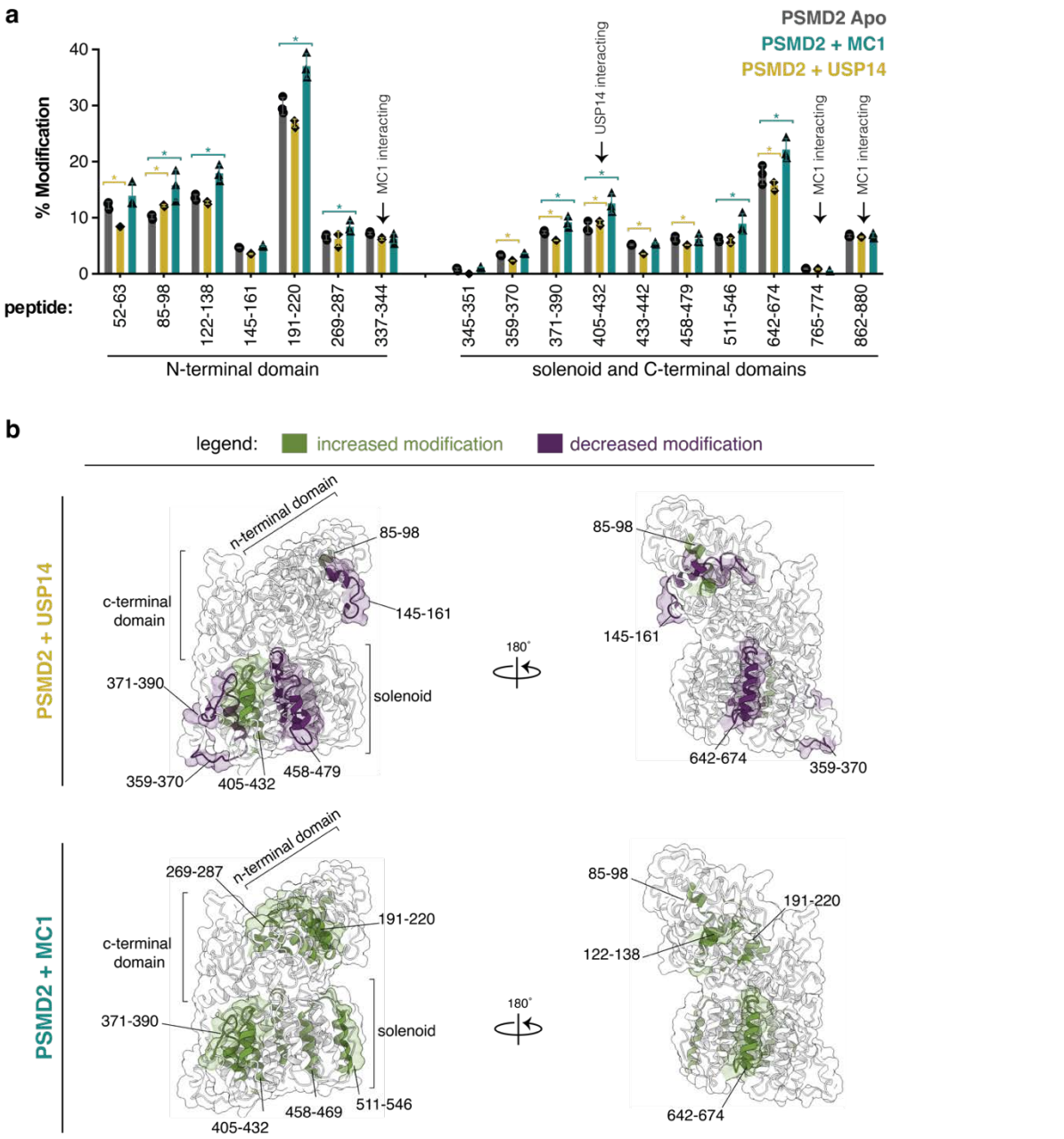



**Supplementary Fig. 4: Cyclization is required for binding to PSMD2.**
ELISA signal to noise for MC1 and MC2 containing the original cysteine residue or a serine mutation at position 14. Data are shown as the ratio of PSMD2 signal to Streptavidin signal alone. Bars represent the mean ± standard deviation of 3 independent experiments.

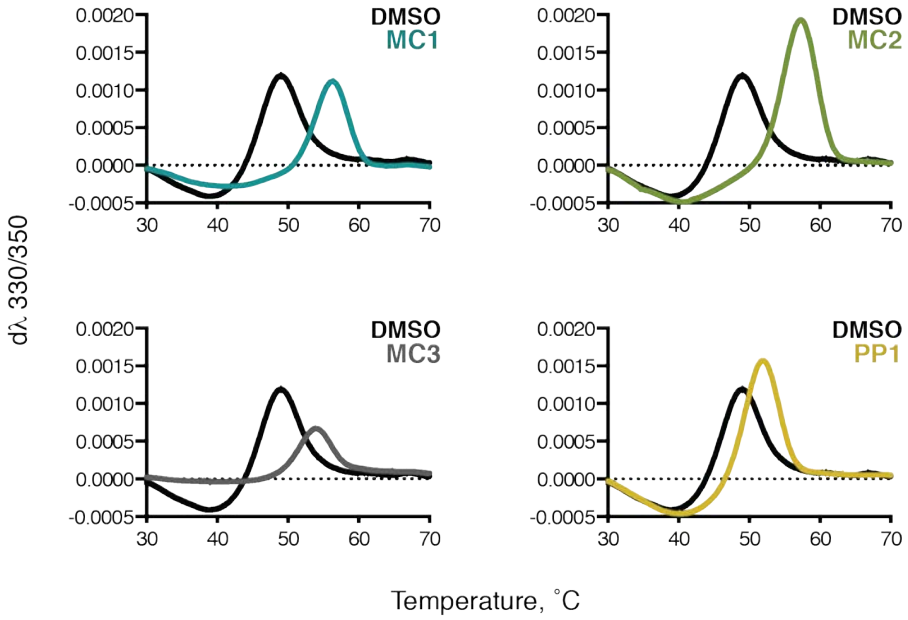



**Supplementary Fig. 5: Identification of KLHL15: macrocycle interaction from label-free AP-MS**.

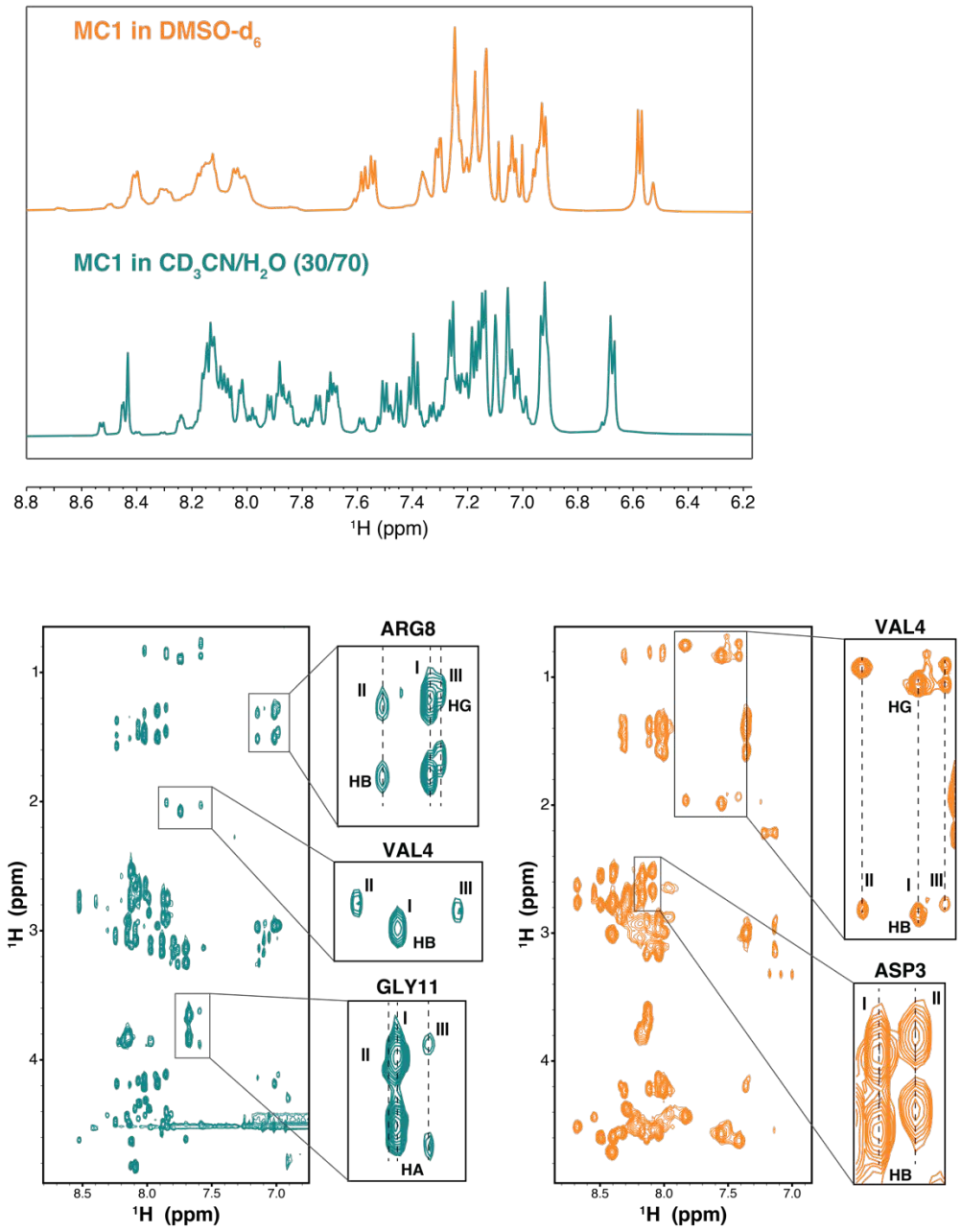
$MC1_{biotin}$ and D-$MC1_{biotin}$, synthesized from L- and D- amino acids respectively, were incubated with lysates from HEK 293 cells, and co-purifying proteins were identified by mass spectrometry. As expected, the active L- version did co-purify with several subunits of the 26S proteasome, while the inactive D-version did not. Non proteasomal interacting partners include CUL3 and adapter KLHL15. Data represent a single experiment.



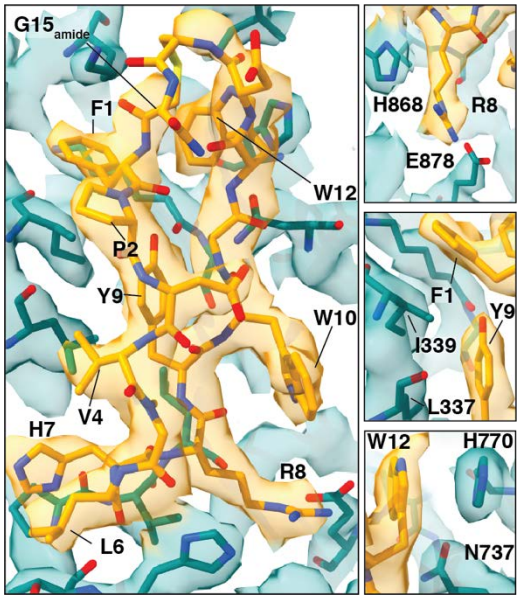

**Supplementary Fig. 6: Macrocyclic ligand MC1 shows differential impact on PSMD2 dynamics compared to USP14 by hydroxyl-radical footprinting**.
**a**, Oxidation profiles for selected PSMD2 tryptic peptides shown for apo PSMD2 (gray) and PSMD2 bound to MC1 (teal) or USP14 (gold). Sequences with color-coded asterisks were classified as having different modification levels compared to apo (* = notable difference based on heuristic classification using individual-sample percent oxidation means and confidence intervals). Percent oxidation is presented as the mean of triplicate runs after subtraction of the "no laser" background oxidation control. Error bars for each mean represent a 95% confidence interval for that mean based on the three replicates. The tryptic peptide that contains the homologous sequence to the published USP14 binding site, as well as regions that interact with MC1 in the EM structure are labeled with an arrow. **b**, Regions showing increased or decreased oxidation are mapped onto PSMD2 model PDB 6MSE chain f.



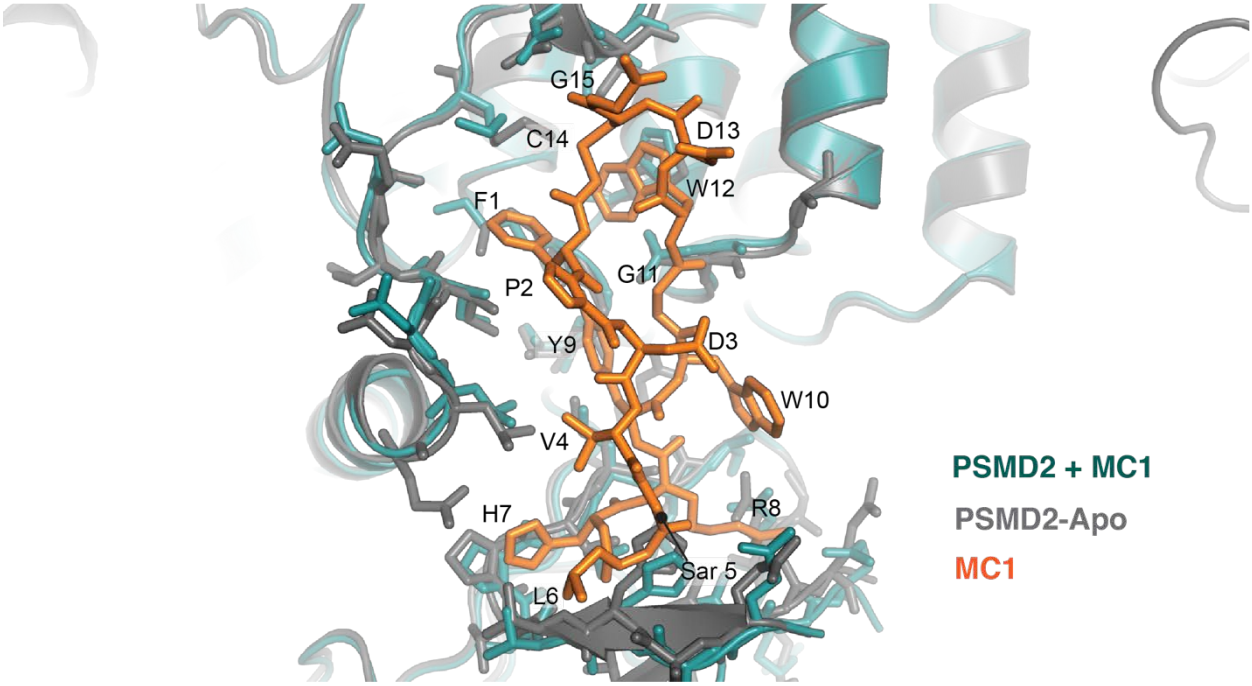

**Supplementary Fig. 7: Macrocycles differentially stabilize PSMD2.**
PSMD2 treated with DMSO (black), or a 10-fold excess of MC1 (teal), MC2 (green), MC3 (gray), or PP1 (gold) and thermal stability was assayed by nanoDSF. Folded state was measured by tryptophan fluorescence at 330 and 350 nm; the ratio each is plotted as a function of temperature. Curves represent mean of three technical replicates.



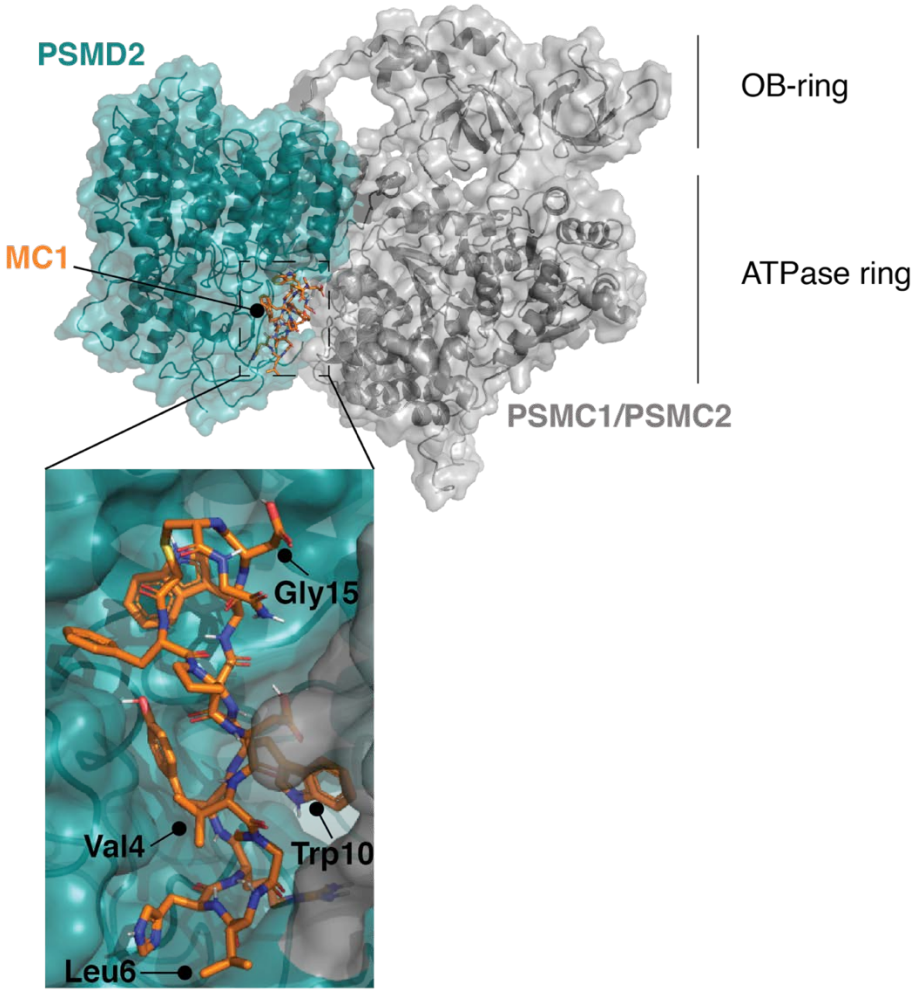

**Supplementary Fig. 8: Solution NMR analysis shows that MC1 macrocyclic peptide exists in multiple conformations.**
Top: aromatic and amide regions of 1D $^1$H NMR spectra of macrocyclic peptide in DMSO-$d_6$ (orange) and $CD_3CN/H_2O$ in (30/70) (teal) at 298K. Multiple conformers were identified in both solvents. Significant signal broadening was also observed in DMSO-$d_6$ suggesting conformational exchange in the intermediate exchange regime. Bottom: select regions of 2D TOCSY spectra in $CD_3CN/H_2O$ (30/70) (left panel) and DMSO-$d_6$ (right panel) highlighting the distinct populations of conformers observed for several amino acids in MC1. One major population (I) and two minor populations (II and III, respectively) were observed in $CD_3CN/H_2O$ (left panel).



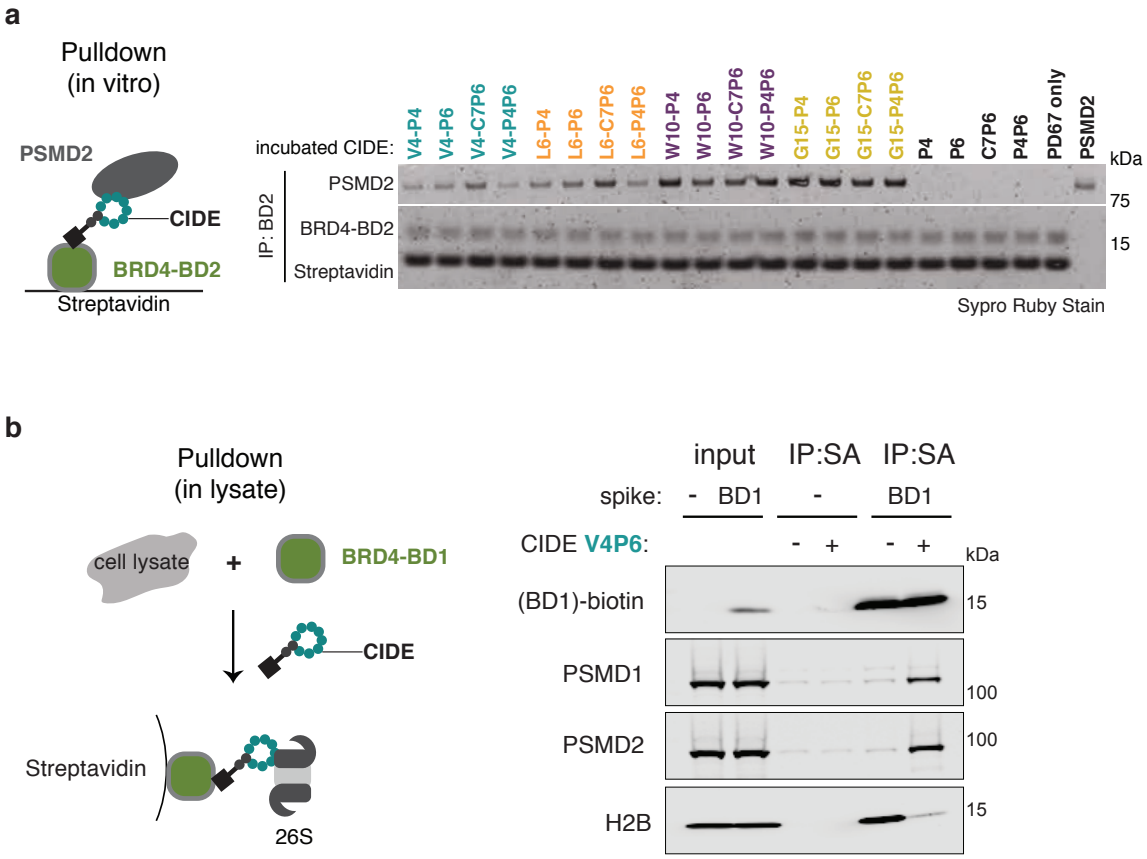

**Supplementary Fig. 9: PSMD2-MC1 binding site.**
Structure of PSMD2-MC1 (sticks, PSMD2 in teal and MC1 in orange) fit into the electron density map (transparent surface in corresponding colors). Images show residues within 4 Å and density within 7 Å of MC1 ligand, with nearby PSMD2 residues that do not make any contacts hidden for visual clarity. Main panel shows overview of MC1 ligand with close-ups highlighting various interactions on the right.



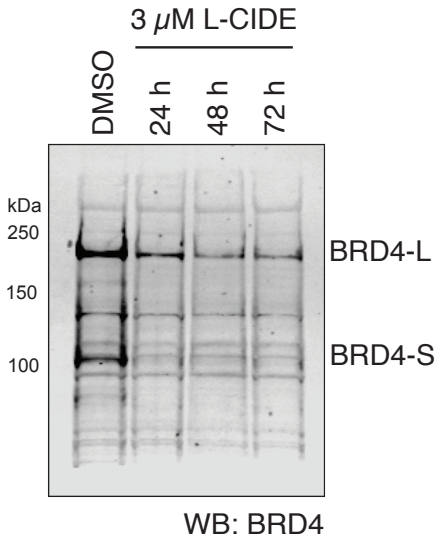

**Supplementary Fig. 10: MC1 binding does not affect PSMD2 conformation at binding site.**
Comparison between PSMD2-MC1 complex (in teal) and PSMD2 apo (in gray) show minimal differences in PSMD2 upon MC1 binding.




**Supplementary Fig. 11: MC1 has solvent-accessible residues ideal for conjugation.** Top panel: PSMD2:MC1 structure aligned to 6MSJ showing PSMD2 (teal) and the nearest AAA+ subunits PSMC1 and PSMC2 (gray). MC1 is between PSMD2 and PSMC1/2 AAA+ domains. Bottom panel: Zoom-in of binding site shows several solvent exposed residues for potential conjugation to make heterobifunctional degraders.




**Supplementary Fig. 12: Ternary complex formation between PSMD2 and BRD4.** **a**, PSMD2 pulls down with biotinylated BRD4 BD2 domain in the presence of all 16 CIDEs, but not in the presence of the BET-L+linker compounds alone, or macrocycle alone as shown by SDS-PAGE. Lanes are color coded by CIDE conjugation point as described in Figure 3a. **b**, HEK293 lysates supplemented with bortezomib, ATPγS and recombinant biotinylated BD1 interact with 26S subunits PSMD2 and PSMD1 when incubated with CIDE V4-P6 from (a), as shown my western blot. Note biotinylated BD1 protein is functional and interacts with histone protein H2B. Data in (**a**) and (**b**) were reproduced in two and three independent experiments, respectively.

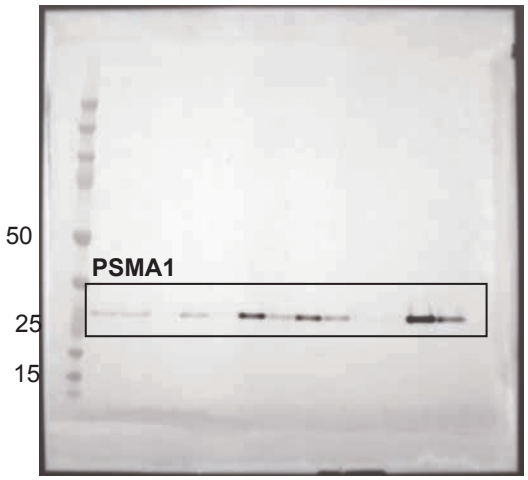



**Supplementary Fig. 13: BRD4 degradation is sustained over 72 hours.**
HEK293 cells treated with L-CIDE show increasing degradation of both long and short (BRD4-L and BRD4-S) forms of BRD4 over the course of 72 hours. Data were reproduced in 2 independent experiments.

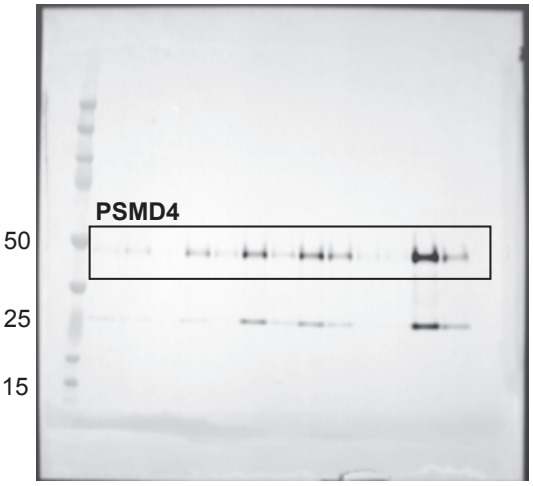
**Supplementary Figures 14-17**
Unprocessed gels and blots from Supplementary Figures 1-13

**Supplementary Fig. 14**
(unprocessed blots from Supplementary Fig. 3)

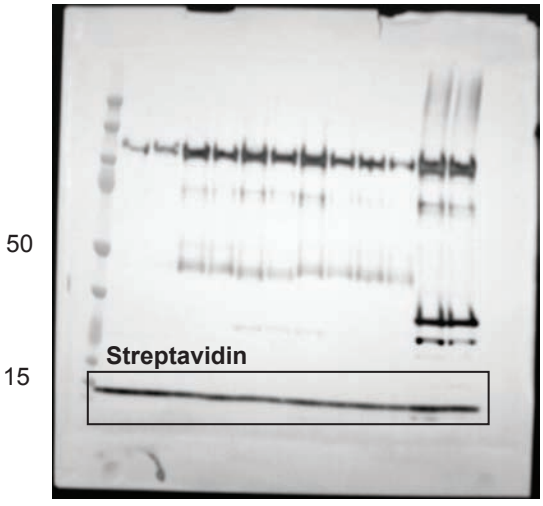
**Blot 1**




**Blot 2 (Blot 1 stripped and re-probed)**

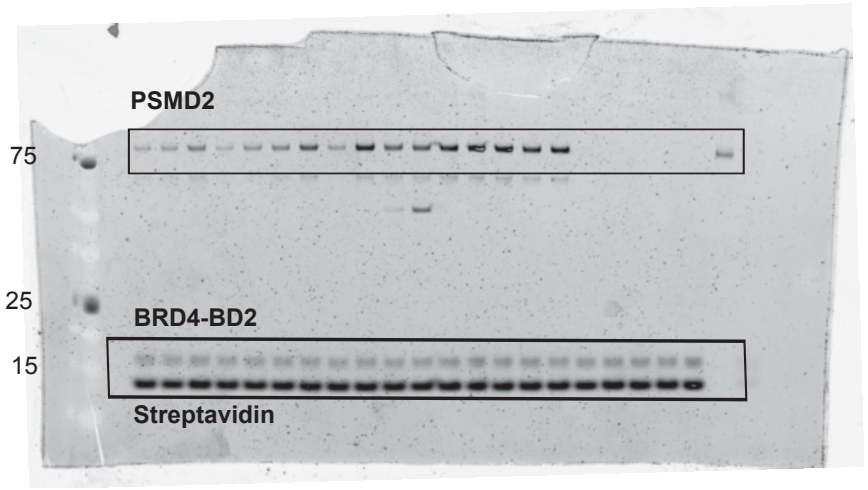



**Blot 3 (Blot 2 stripped and re-probed)**



**Supplementary Fig. 15**
(unprocessed gel from Supplementary Fig. 12a)

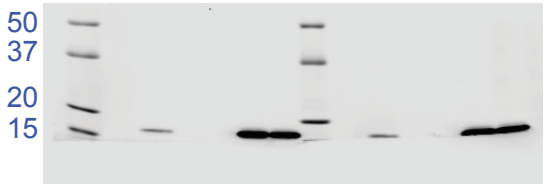
**Gel**



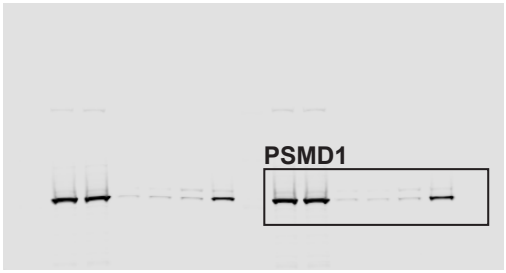
**Supplementary Fig. 16**
(unprocessed blots from Supplementary Fig. 12b)

**Blot 1**

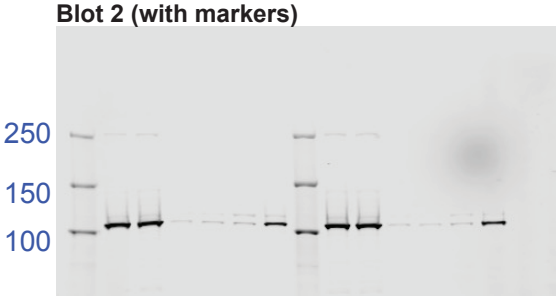

**Blot 1 (with markers)**



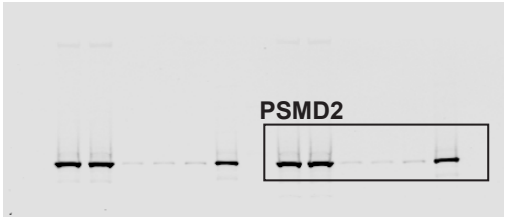

**Blot 2**



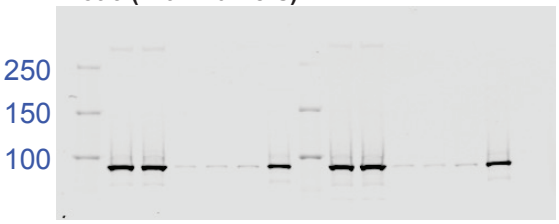

**Blot 2 (with markers)**



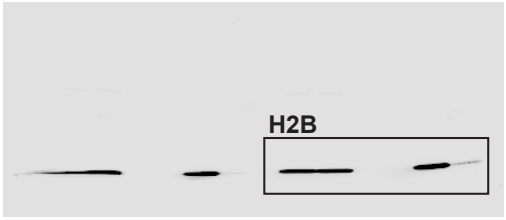

**Blot 3**



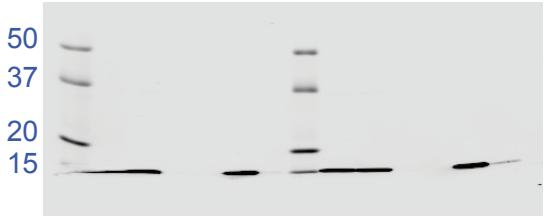

**Blot 3 (with markers)**




**Blot 4**



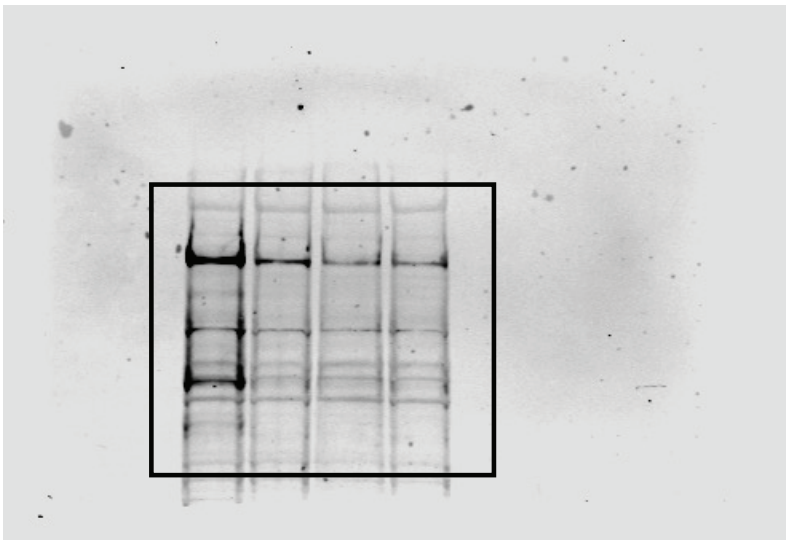

**Blot 4 (with markers)**



**Supplementary Fig. 17**
(unprocessed blots from Supplementary Fig. 13)

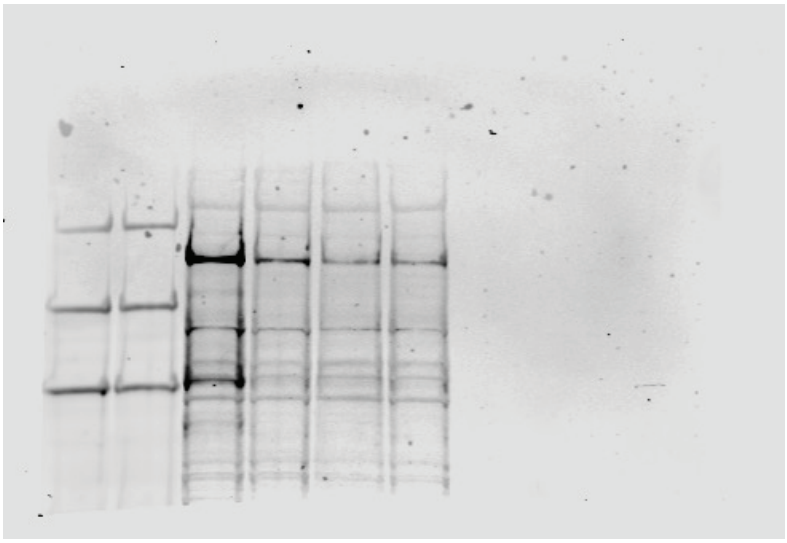
**Blot**

BRD4



**Blot (with markers)**

250
150
100



**Peptides used in this study**

| full peptide name | nickname | peptide type | c-term modification | amino acid stereochemistry |
|---|---|---|---|---|
| PSMD2-MC1 | MC1 | macrocycle | | L |
| PSMD2-MC2 | MC2 | macrocycle | | L |
| PSMD2-MC3 | MC3 | macrocycle | | L |
| PSMD2-MC4 | MC4 | macrocycle | | L |
| PSMD2-PP1 | PP1 | linear peptide | | L |
| PSMD2-MC1$_{biotin}$ | MC1$_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-MC2$_{biotin}$ | MC2$_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-MC3$_{biotin}$ | MC3$_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-PP1$_{biotin}$ | PP1$_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-D-MC$_{biotin}$ | D-MC1$_{biotin}$ | macrocycle | PEG3-Lys biotin | D |

**Supplementary Table 1: List of peptides used in this study.**
Peptides that were ordered and custom synthesized commercially for use in this study. Molecules sequences are described in Figure 1a. Macrocycles are cyclized via thioether bond as described in text and in Supplementary Figure 1. Peptides contain an N-acetyl and C-amide groups.

| Sample | ka (1/Ms) | kd (1/s) | KD (M) | Rmax (RU) | Chi² (RU²) | MW | Model |
|---|---|---|---|---|---|---|---|
| MC1 | 60800 | 0.00008 | 1.32E-09 | 34.2 | 0.186 | 1500 | 1:1 Binding |
| MC1 | 62200 | 8.22E-05 | 1.32E-09 | 34.1 | 0.259 | 1500 | 1:1 Binding |
| MC1 | 60000 | 8.31E-05 | 1.39E-09 | 32.3 | 0.32 | 1500 | 1:1 Binding |
| MC2 | 85200 | 0.000132 | 1.55E-09 | 33.1 | 0.103 | 1500 | 1:1 Binding |
| MC2 | 85100 | 0.000139 | 1.63E-09 | 33.9 | 0.214 | 1500 | 1:1 Binding |
| MC2 | 83200 | 0.000146 | 1.75E-09 | 32.2 | 0.102 | 1500 | 1:1 Binding |
| MC3 | 92200 | 0.00481 | 5.22E-08 | 17.7 | 0.916 | 1100 | 1:1 Binding |
| MC3 | 95400 | 0.00452 | 4.73E-08 | 17.4 | 1.47 | 1100 | 1:1 Binding |
| MC3 | 90900 | 0.00498 | 5.48E-08 | 17.1 | 1.37 | 1100 | 1:1 Binding |
| PP1 | 73600 | 0.00917 | 1.24E-07 | 21.1 | 2.27 | 1293 | 1:1 Binding |
| PP1 | 78000 | 0.00975 | 1.25E-07 | 21.9 | 2.56 | 1293 | 1:1 Binding |
| PP1 | 70400 | 0.00772 | 1.1E-07 | 20.1 | 2.91 | 1293 | 1:1 Binding |

**Supplementary Table 2: SPR measurements and kinetic fit parameters of macrocycle and peptide ligands binding to PSMD2.**

| | PSMD2-PSMD2-MC1-Fabs | PSMD2-Fabs |
|---|---|---|
| **Data Collection** | | |
| Magnification | 165,000x | 165,000x |
| Voltage (kV) | 300 | 300 |
| Electron exposure (e/Å$^2$) | 58 | 64 |
| Defocus range (μm) | 0.5-1.5 | 0.5-1.5 |
| Pixel size (Å) | 0.824 | 0.824 |
| Symmetry imposed | C1 | C1 |
| Initial particle images | 1,311,869 | 737,190 |
| Final particle images | 105,705 | 82,738 |
| Map resolution (Å) overall | 2.5 | 3.3 |
| FSC threshold | 0.143 | 0.143 |
| Map resolution range (Å) | 2.7-47.6 | 3.1-50.0 |
| **Refinement** | | |
| Initial models used (PDB code) | PSMD2: 6MSJ | PSMD2: 6MSJ |
| Model resolution (Å) | 2.9 | 3.5 |
| FSC threshold | 0.5 | 0.5 |
| Model resolution range (Å) | 2.5-47.6 | 3.3-50.0 |
| Map-sharpening B-factors (Å$^2$) | -90 | -90 |
| Model composition | | |
| Non-hydrogen atoms | 5269 | 5114 |
| Protein residues | 665 | 662 |
| Waters | 0 | 0 |
| Ligands | 1 | 0 |
| B factors (Å$^2$) | | |
| Protein | 35.36 | 83.38 |
| Ligand | 31.87 | 0 |
| Validation | | |
| MolProbity score | 1.46 | 1.56 |
| Clashscore | 4.44 | 6.38 |
| Poor rotamers (%) | 0.00 | 0.00 |
| Ramachandran Plot | | |
| Favored (%) | 96.37 | 96.67 |
| Allowed (%) | 3.63 | 3.33 |
| Disallowed (%) | 0.00 | 0.00 |
| **EMDB accession #** | 24742 | 24743 |
| **PDB accession #** | 7UJD | 7UIH |

**Supplementary Table 3: Cryo-EM data collection, refinement and validation statistics.**

**MC1 - derived conjugates used in this study**

| Compound name | Nickname | Cmpd type | Conjugation point | Linker* | amino acid stereo-chemistry |
|---|---|---|---|---|---|
| BETcide1 G15-P4 | G15-P4 | CIDE | Gly15 | P4 | L |
| BETcide2 G15-P6 | G15-P6 | CIDE | Gly15 | P6 | L |
| BETcide3 G15-C7P6 | G15-C7P6 | CIDE | Gly15 | C7P6 | L |
| BETcide4 G15-P4P6 | G15-P4P6 | CIDE | Gly15 | P4P6 | L |
| BETcide5 V4-P4 | V4-P4 | CIDE | Val4 | P4 | L |
| BETcide6 V4-C7P6 | V4-C7P6 | CIDE | Val4 | C7P6 | L |
| BETcide7 V4-P4P6 | V4-P4P6 | CIDE | Val4 | P4P6 | L |
| BETcide8 V4-P6 | L-CIDE | CIDE | Val4 | P6 | L |
| BETcide9 L6-P4 | L6-P4 | CIDE | Leu6 | P4 | L |
| BETcide10 L6-C7P6 | L6-C7P6 | CIDE | Leu6 | C7P6 | L |
| BETcide11 L6-P4P6 | L6-P4P6 | CIDE | Leu6 | P4P6 | L |
| BETcide12 L6-P6 | L6-P6 | CIDE | Leu6 | P6 | L |
| BETcide13 W10-P4 | W10-P4 | CIDE | Trp10 | P4 | L |
| BETcide14 W10-C7P6 | W10-C7P6 | CIDE | Trp10 | C7P6 | L |
| BETcide15 W10-P4P6 | W10-P4P6 | CIDE | Trp10 | P4P6 | L |
| BETcide16 W10-P6 | W10-P6 | CIDE | Trp10 | P6 | L |
| D-BETcide8 V4-P6 | D-CIDE | CIDE | val4 | P6 | D |
| PSMD2-MC1$_{Cy5.5}$ | MC1$_{Cy5.5}$ | Fluorescent macrocycle | Val4 | | L |
| BETi P4 | P4 | BET ligand | | P4 | |
| BETi P6 | P6 | BET ligand | | P6 | |
| BETi C7P6 | C7P6 | BET ligand | | C7P6 | |
| BETi P4P6 | P4P6 | BET ligand | | P4P6 | |

* P4 = PEG4, P6 = PEG6, C7P6 = -$(CH_2)_6$CONH- (C7) + PEG6, P4P6 = PEG4+PEG6. See Supporting Information for more details

**Supplementary Table 4: Summary of conjugated macrocycles synthesized by click chemistry.**

MC1 was conjugated to BETi ligand or Cy5.5 fluorophore as described above and in the Supporting Information.

| [Compound] uM | BRD4L | BRD4S | BRD3 | BRD2 | Treatment |
|---|---|---|---|---|---|
| 0 | 1 | 1 | 1 | 1 | DMSO |
| 0.1 | 0.92 | 0.77 | 0.97 | 1.31 | L-CIDE |
| 0.3 | 0.65 | 0.66 | 0.93 | 1.32 | L-CIDE |
| 0.6 | 0.38 | 0.55 | 0.77 | 1.45 | L-CIDE |
| 1.25 | 0.25 | 0.34 | 0.73 | 1.35 | L-CIDE |
| 2.5 | 0.22 | 0.27 | 0.55 | 1.59 | L-CIDE |
| 5 | 0.21 | 0.21 | 0.49 | 1.84 | L-CIDE |
| 0.1 | 1.14 | 0.91 | 1.15 | 1.32 | D-CIDE |
| 0.3 | 1.17 | 0.90 | 1.05 | 1.54 | D-CIDE |
| 0.6 | 1.55 | 0.98 | 1.10 | 1.28 | D-CIDE |
| 1.25 | 1.34 | 0.87 | 0.93 | 1.26 | D-CIDE |
| 2.5 | 0.93 | 0.61 | 0.78 | 1.15 | D-CIDE |
| 5 | 0.63 | 0.50 | 0.58 | 1.00 | D-CIDE |

**Supplementary Table 5: Quantification of bands in Figure 6d western blots.**
Tubulin background-corrected signal intensity was used to normalize for loading and reported band intensities normalized to DMSO control.

| Treatment | BRD4L | BRD4S | BRD3 | BRD2 |
|---|---|---|---|---|
| DMSO | 1 | 1 | 1 | 1 |
| L-CIDE | 0.28 | 0.25 | 0.49 | 1.03 |
| D-CIDE | 0.93 | 0.67 | 0.58 | 0.86 |
| DMSO + Btz | 1.99 | 0.93 | 0.79 | 1.30 |
| L-CIDE + Btz | 2.05 | 0.98 | 0.80 | 1.45 |
| D-CIDE + Btz | 2.38 | 1.07 | 0.92 | 1.39 |

**Supplementary Table 6: Quantification of bands in Figure 6e western blots**.
Tubulin background-corrected signal intensity was used to normalize for loading and reported band intensities normalized to DMSO control.

| [BETi], uM | BRD4L | BRD4S | BRD3 | BRD2 | Treatment |
|---|---|---|---|---|---|
| 0 | 1 | 1 | 1 | 1 | DMSO |
| 0.1 | 1.55 | 1.58 | 0.99 | 1.97 | DMSO |
| 1 | 1.37 | 1.38 | 1.18 | 2.10 | DMSO |
| 10 | 1.51 | 1.47 | 1.04 | 1.76 | DMSO |
| 0 | 0.24 | 0.23 | 0.40 | 0.96 | L-CIDE |
| 0.1 | 0.67 | 0.98 | 0.62 | 1.55 | L-CIDE |
| 1 | 0.79 | 1.06 | 0.68 | 1.93 | L-CIDE |
| 10 | 1.25 | 1.48 | 0.60 | 1.62 | L-CIDE |
| 0 | 1.25 | 0.83 | 0.76 | 0.75 | D-CIDE |
| 0.1 | 2.26 | 2.18 | 0.97 | 1.55 | D-CIDE |
| 1 | 2.08 | 2.04 | 0.86 | 1.56 | D-CIDE |
| 10 | 1.94 | 2.10 | 0.86 | 1.32 | D-CIDE |

**Supplementary Table 7: Quantification of bands in Figure 6f western blots.**
Tubulin background-corrected signal intensity was used to normalize for loading and reported band intensities normalized to DMSO control.

| cell line | BRD4L | PSMD2 | Treatment |
|---|---|---|---|
| NT-gRNA | 1 | 1 | D-CIDE |
| NT-gRNA | 0.53 | 0.95 | L-CIDE |
| KLHL15 KO #1 | 1 | 1 | D-CIDE |
| KLHL15 KO #1 | 0.36 | 0.82 | L-CIDE |
| KLHL15 KO #2 | 1 | 1 | D-CIDE |
| KLHL15 KO #2 | 0.46 | 1.01 | L-CIDE |

**Supplementary Table 8. Quantification of bands in Figure 6g western blots.**
Histone H2B background-corrected signal intensity was used to normalize for loading and reported band intensities normalized to DMSO control.

**Supplementary Tables 9-11**
KLHL15 KO reagents

| sgRNA | Sequence |
|---|---|
| hKLHL15_sg1 | TTCATGGCAGGGGACGTGGA |
| hKLHL15_sg2 | GACACTGGTGTCGTGGATGG |
| hKLHL15_sg3 | GATATTCTGAATGATGGTAT |
| control guide RNA | GCTTATGGATTAGCCAATC |
| | |
| **Donor** | **Sequence** |
| mScarletKI long ssDNA donor | TCCCAAAGGACTTGTACATTTTGGACTTAGTCAGTAGAATTGAGATAATAGCAAAAATTGTTAAAACTCTAA<br>GTTAATATTTTCTTCTGAATGCATGGAAGTGTTTAACATTGTTGTCTTTGATTTTTTTCCAGGTGATTCATGGC<br>AGGGgctccggtgcccgtcagtgggcagagcgcacatcgcccacagtccccgagaagttgggggggaggggtcggcaattgaaccggtgcc<br>tagagaaggtggcgcggggtaaactgggaaagtgatgtcgtgtactggctccgccttttcccgagggtgggggagaaccgtatataagtgc<br>agtagtcgccgtgaacgttctttttcgcaacgggtttgccgccagaacacaggtaagtgccgtgtgtggttcccgcgggcctggcctctttacggg<br>ttatggcccttgcgtgccttgaattacttccacgcccctggctgcagtacgtgattcttgatcccgagcttcgggttggaagtgggtgggagagttc<br>gaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcttgggcgctggggccgccgcgtgcgaatctggtggcaccttc<br>gcgcctgtctcgctgctttcgataagtctctagccatttaaaatttttgatgacctgctgcgacgctttttttctggcaagatagtcttgtaaatgcgg<br>gccaagatctgcacactggtatttcggttttggggccgcgggcggcgacggggcccgtgcgtcccagcgcacatgttcggcgaggcggggcc<br>tgcgagcgcggccaccgagaatcggacgggggtagtctcaagctggccggcctgctctggtgcctggcctcgcgccgccgtgtatcgccccgcc<br>ctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatggagg<br>acgcggcgctcgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccgtcctcagccgtcgcttcatgtgactccacgga<br>gtaccgggcgccgtccaggcacctcgattagttctcaagcttttggagtacgtcgtctttaggttgggggagggggttttatgcgatggagtttcc<br>ccacactgagtgggtggagactgaagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggatcttggttcattct<br>caagcctcagacagtggttcaaagttttttttcttccatttcaggtgtcgtgaccctagcgctacctctagagccaccatggtgagcaaaggcgaag<br>cggtgattaaagaatttatgcgctttaaagtgcatatggaaggcagcatgaacggccatgaatttgaaattgaaggcgaaggcgaaggccg<br>cccgtatgaaggcacccagaccgcgaaactgaaagtgaccaaaggcggcccgctgccgtttagctgggatattctgagcccgcagtttatgta<br>tggcagccgcgcgtttaccaaacatccggcggatattccggattattataaacagagctttccggaaggctttaaatgggaacgcgtgatgaac<br>tttgaagatggcggcgcggtgaccgtgacccaggataccagcctggaagatggcaccctgatttataaagtgaaactgcgcggcaccaacttt<br>ccgccggatggcccggtgatgcagaaaaaaaccatgggctgggaagcgagcaccgaacgcctgtatccggaagatggcgtgctgaaaggc<br>gatattaaaatggcgctgcgcctgaaagatggcggccgctatctggcggattttaaaaccacctataaagcgaaaaaaccggtgcagatgcc<br>gggcgcgtataacgtggatcgcaaactggatattaccagccataacgaagattataccgtggtggaacagtatgaacgcagcgaaggccgc<br>catagcaccggcggcatggatgaactgtataaataaGCTAGCaacttgtttattgcagcttataatggttacaaataaagcaatagcatcac<br>aaatttcacaaataaagcatttttttcactgcattctagttgtggtttgtccaaactcatcaatgtatcttaAGCGTTTTTGAGAAGGTTAG<br>TGCATTTGAAAGCAATAGACAGTACTCATCATTTAGTTAAGGCAGTGTATGTCTTTATAGATAAGATTCACAG<br>ACTTAAGAGCCAAGAATGAATAACTTCATTTTGTTATTTCTTATGTTATTCAAGAAT |

**Supplementary Table 9. sgRNA and donors**

| Primer | Sequence |
|---|---|
| hKLHL15_genF | TCCTTAGATAGGGAAGCCAGAAA |
| hKLHL15_genR | ACGTGGTTTATCTTTCACCCACA |

**Supplementary Table 10. Genomic PCR oligos**

| Primer | Sequence | Notes |
|---|---|---|
| hKLHL15-ddF1 | GATAGGGAAGCCAGAAATATTAGTC | control, sg1, sg2 and mScarlet knock-in assays |
| hKLHL15_conddR | ACACTTCCATGCATTCAGAAGAA | control assay |
| hKLHL15-ddR1 | AAGCAATCCCTCCTCATACA | sg1 and sg2 assays |
| hKLHL15KI-ddR1 | CGACATCACTTTCCCAGTTTAC | mScarlet knock-in assay |
| hKLHL15-ddF2 | AGCAGGTTCCCAGAGATAGA | sg3 assay |
| hKLHL15-ddR2 | TAAATGCACAACAAATCAGGCT | sg3 assay |
| | | |
| **Probe** | **Sequence** | |
| hKLHL15-WTcon-Hex | AA+C+CTTT+A+G+ATT+GTCT | control assay probe |
| hKLHL15-sg1FAM | CTT+C+C+A+CGT+CC | sg1 cutsite probe |
| hKLHL15-sg2FAM | CTC+C+A+T+CC+A+CGA | sg2 cutsite probe |
| hKLHL15-sg3FAM | CC+GA+T+A+C+CA+TCA | sg3 cutsite probe |
| hKLHL15-KI-FAM | CG+GT+T+CAA+T+T+GC | HDR knock-in probe |
| | | |
| **Centromeric copy number reference assay** | **Assay ID** | |
| PLEKHF1 FAM | dHsaCP2506315 | Bio-Rad |
| PLEKHF1 HEX | dHsaCP2506723 | Bio-Rad |

**Supplementary Table 11. ddPCR sequences**

# Supplementary Note

(synthesis of MC1-Cy5.5 and CIDEs)

## General Methods

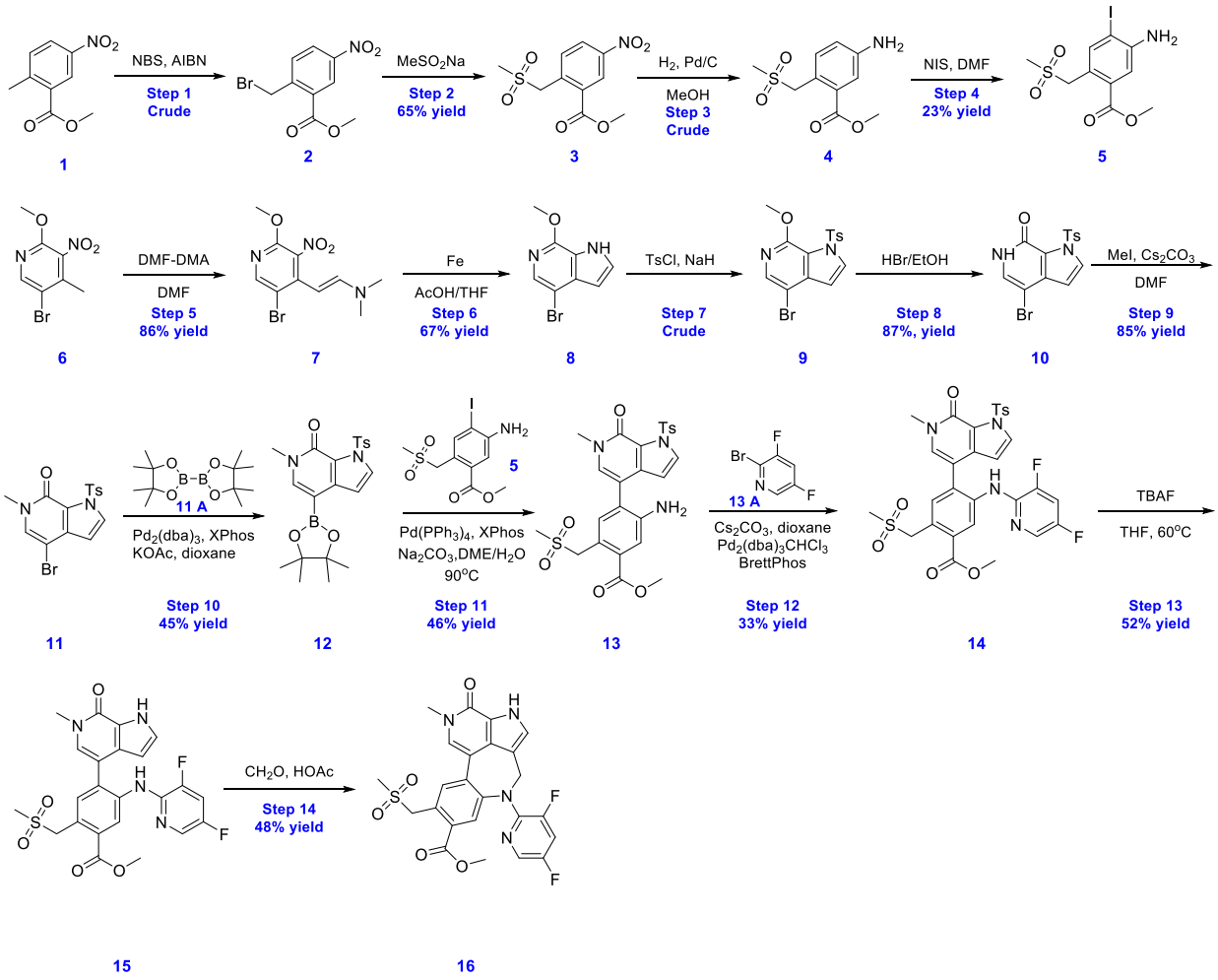
A. Materials

All chemical reagents for the synthesis of BET ligands were purchased form Sigma Aldrich. Fmoc amino acids where purchased from commercial sources namely, EMD Millipore, Chem-Impex, CEM and Bachem. OxymaPure® (ethyl (2*E*)-2-cyano-2-hydroxyiminoacetate) and Rink amide ProTide™ resin were purchased from CEM. *N*,*N*-Diisopropylcarbodiimide (DIC), 4-methylpiperidine and Chelex 100 resin were purchased from Chem-Impex, Beantown Chemical and Bio-Rad, respectively. All solvents and other chemical reagents for peptide synthesis were purchased from Millipore Sigma.

B. Methods and General experimental procedures

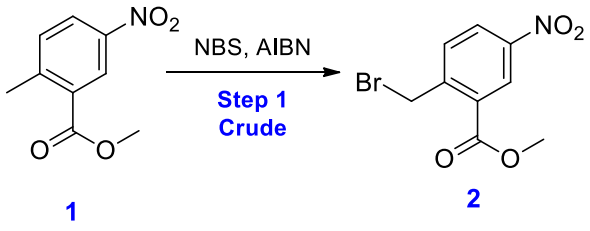
## I. Synthesis of BET Ligands

### Synthetic Scheme for compound 16




***Experimental Procedure for BET Ligands:***

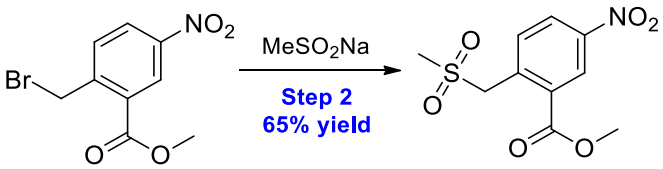
**1. *Preparation of Compound 2***




To a solution of Compound **1** (370 g, 1.90 mol) in ACN (2.50 L) was added AIBN (156 g, 948 mmol) at 25°C, and the reaction was heated to 80°C. NBS (506 g, 2.84 mol) was added in batches at 80°C and the resulting solution was stirred for 1 hour at 80°C. The reaction mixture

was concentrated to remove solvent and dissolved in ethyl acetate (2.00 L). The mixture was then washed with water (500 mL x 3). The aqueous was extracted with ethyl acetate (500 mL x 3)and the combined the organics were washed with brine (200 mL) and concentrated to give a residue. The crude product, compound **2** (550 g, crude), was obtained as a brown solid and used without purification.

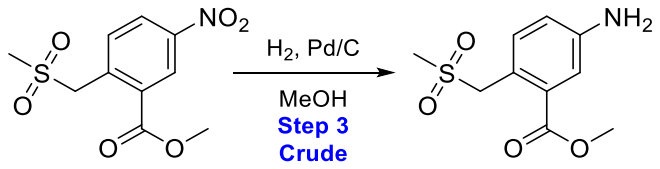
### 2. *Preparation of Compound 3*




2 3

A solution of **2** (277 g, 1.01 mol) in DMF (1.50 L) was treated with $MeSO_2Na$ (155 g, 1.52 mol) at 25°C. The resulting solution was heated to 60°C and stirred for 2 hours. Two reactions of the same scale were combined for work-up. The combined reaction mixtures were diluted with 15.0 L of water and filtered. The resultant solid was dissolved in ethyl acetate (8.00 L), extracted with water (2.00 L x 3) and brine (2.00 L) and the organic dried over $Na_2SO_4$. Concentration and trituration with Petroleum ether/ethyl acetate = 5/1 (3.00 L) delivered compound **3** (380 g, 1.32 mol, 65.2% yield, 94.7% purity) as a brown solid. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.88 (s, 1 H), 8.41 (dd, *J* = 8, 2 Hz, 1 H), 7.78 (d, *J* = 8 Hz, 1 H), 5.03 (s, 1 H), 4.00 (s, 3 H), 2.91 (s, 3 H). LCMS (ESI): *m/z* 273 $(M+H)^+$.

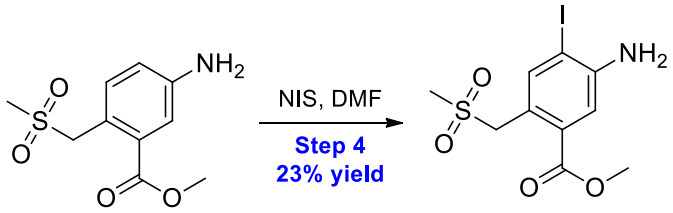
### 3. *Preparation of Compound 4*




3 4

A solution of **3** (98.0 g, 359 mmol) in MeOH (1.00 L) was treated with Pd/C (20.0 g, 10% purity) under $N_2$. The suspension was degassed under vacuum and purged with $H_2$ several times. The mixture was stirred under $H_2$ (50 psi) at 30°C for 2 hours. Five reaction mixtures of this scale were combined for work-up. The combined reaction mixtures were filtered and concentrated. The crude product was triturated with MTBE/MeOH = 3/1 (1 L) to give **4** (448 g) as a white crude solid that was used without further purification.

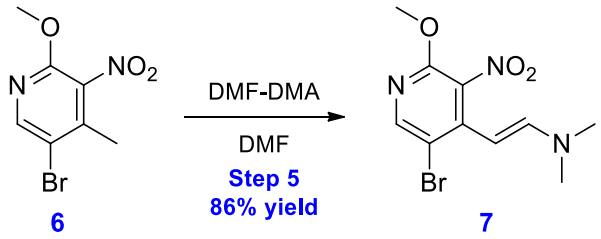
### *4. General procedure for preparation of Compound 5*




4 5

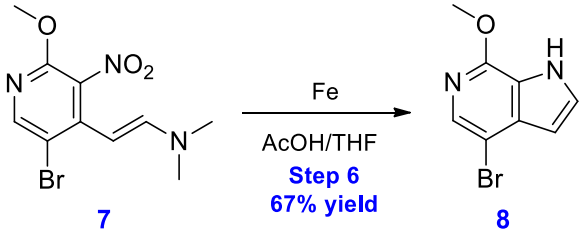
A solution of **4** (308 g, 1.27 mol) in DMF (2.10 L) was treated with NIS (308 g, 1.37 mol) at 25°C and stirred for 2 hours. The reaction mixture was diluted with 5.00 L of saturated $Na_2SO_3$ solution and 5.00 L of saturated $NaHCO_3$ solution. The solid was collected by filtration and purified by column chromatography ($SiO_2$, Petroleum ether/Ethyl acetate = 100/1 to 0/1) followed by trituration with ethyl acetate (1.00 L) to give **5** (110 g, 298 mmol, 23.5% yield) as a brown solid. $^1$H NMR (400 MHz, DMSO-d6): δ 7.72 (s, 1 H), 7.25 (s, 1 H), 5.69 (s, 2H), 4.70 (s, 2H), 3.78 (s, 3 H), 2.81 (s, 3 H).

### *5. Preparation of Compound 7*




A solution of **6** (150 g, 607 mmol) in DMF (1.20 L) at 25°C and was treated with DMF-DMA (606 g, 5.08 mol, 675 mL) at 80° C in portions over 30 min. The reaction mixture was stirred at 95° C for 2.5 hours. Three reaction of the same scale were combined for work-up. The combined reaction mixtures were dissolved in ethyl acetate (10.0 L), washed with brine (200 mL x 3), dried over $Na_2SO_4$ and concentrated give crude **7** (530 g, 1.75 mol, 96.3% yield) reddish solid that was used without further purification. $^1$H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 1 H), 6.99 (d, *J* = 14 Hz, 1 H), 4.92 (d, *J* = 14 Hz, 1 H), 3.94 (s, 3 H), 2.92 (s, 6H).

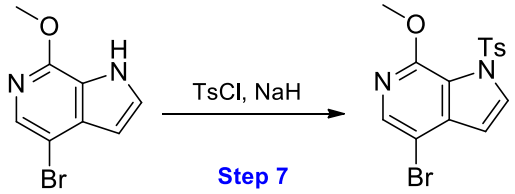
### *6. Preparation of Compound 8*




A solution of **7** (167 g, 554 mmol) in THF (1.67 L) was treated with AcOH (800 mL) at 25°C. The mixture was heated to 60° C, treated with iron powder (170 g, 3.05 mol) and stirred for 8 hours at 60°C. Two reactions were combined for work-up. The combined reaction mixtures were concentrated, dissolved in ethyl acetate (5.00 L), washed with saturated $NaHCO_3$ solution

(1.00 L x 3), water (400 mL x 3) and brine (500 mL). The organic was concentrated and purified by column chromatography ($SiO_2$, Petroleum ether/Ethyl acetate = 100/1 to 0/1) to deliver **8** (230g, 1.01 mol, 67.3% yield) as light yellow solid. $^1$H NMR (400 MHz, DMSO- d6): δ 12.16 (s, 1 H), 7.75 (s, 1 H), 7.56-7.55 (m, 1 H), 6.43-6.42 (m, 1 H), 4.00 (s, 3 H).

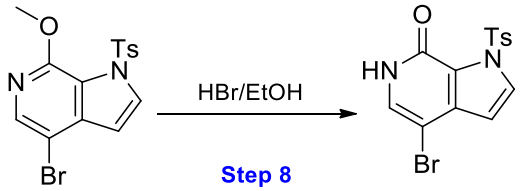
### *7. Preparation of Compound 9*




8 9

A 5.00 L 3-necked round-bottom flask purged with nitrogen and charged with NaH (41.66 g, 1.04 mol, 60% purity) and THF (1.00 L). Compound **8** (215 g, 947 mmol) was dissolved in THF (400 mL) and added dropwise with stirring at 0 °C. TsCl (217 g, 1.14 mol) was added in portions. The resulting solution was stirred for 1 hour at 0 °C. Two reactions were combined for workup. The mixture was diluted with $NH_4Cl$ solution (500 mL), extracted with 3 x 500 mL of ethyl acetate. The organic layers were combined and washed with brine (100 mL x 2), dried over $Na_2SO_4$ and concentrated under vacuum to deliver **9** (380 g, crude) as a white solid. $^1$H NMR (400 MHz, DMSO- d6): δ 8.18 (d, *J* = 4 Hz, 1 H), 7.99 (s,1 H), 7.85 (d, *J* = 8 Hz, 2 H), 7.46 (d, *J* = 8 Hz, 2 H), 6.81 (d, *J* = 4 Hz, 1 H), 3.82 (s, 3 H), 2.38 (s, 3 H).

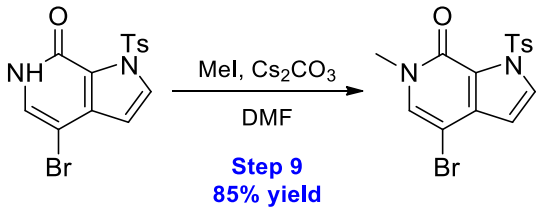
### *8. Preparation of Compound 10*




9 10

A solution of **9** (380 g, 997 mmol) in EtOH (1.00 L) was treated with HBr (1.79 kg, 8.84 mol, 1.20 L, 40% purity) at 25°C then warmed to 90°C to stir for 2 hoursThe reaction mixture was adjusted to pH = 7-8 with saturated $NaHCO_3$ solution and extracted with 2-Me-THF (2.00 L x 4). The layers were separated and the organic layer was washed with water (1.00 L x 3) and brine (1.00 L), dried over $Na_2SO_4$ and concentrated to give a residue. The crude product was triturated with MTBE (600 mL), and filtration delivered **10** (320 g, 871 mmol, 87.4% yield) as a light brown solid. $^1$H NMR (400 MHz, DMSO- d6): δ 11.50 (s, 1 H), 8.04 (d, *J* = 3 Hz, 1 H), 7.93 (d, *J* = 8 Hz, 2 H), 7.41 (d, *J* = 8 Hz, 2 H), 7.36 (s, 1 H), 6.60 (d, *J* = 3 Hz, 1 H), 2.37 (s, 3 H).

### *9. Preparation of Compound 11*

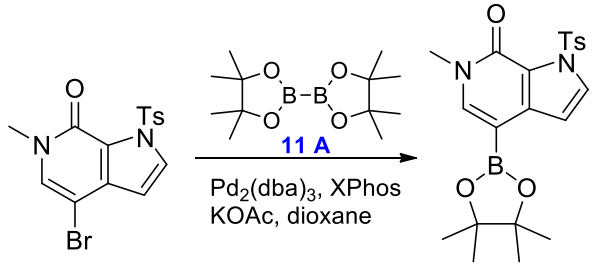



10 11

A solution of **10** (225 g, 613 mmol) in DMF (1.57 L) was treated with $Cs_2CO_3$ (240 g, 735 mmol) and MeI (153 g, 1.08 mol, 67.2 mL and stirred at 25°C for 3 hours. Trituration of the reaction mixture with petroleum ether/ethyl acetate (5/1, 300mL) gave **11** (204 g, 525 mmol, 85.7%

yield, 98.1% purity) as a light brown solid. $^1$H NMR (400 MHz, DMSO- d6): δ 8.06 (d, *J* = 4 Hz, 1 H), 7.95 (d, *J* = 8 Hz, 2 H), 7.80 (s, 1 H), 7.42 (d, *J* = 8 Hz, 2 H), 6.60 (d, *J* = 4 Hz, 1 H), 3.39 (s, 3 H), 2.38 (s, 3 H). LCMS (ESI): *m/z* 382 $(M+H)^+$.

***10. Preparation of Compound 12***

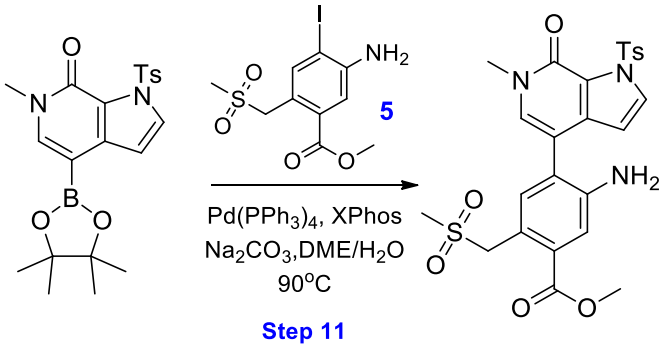



**Step 10**
**45% yield**

**11** **12**

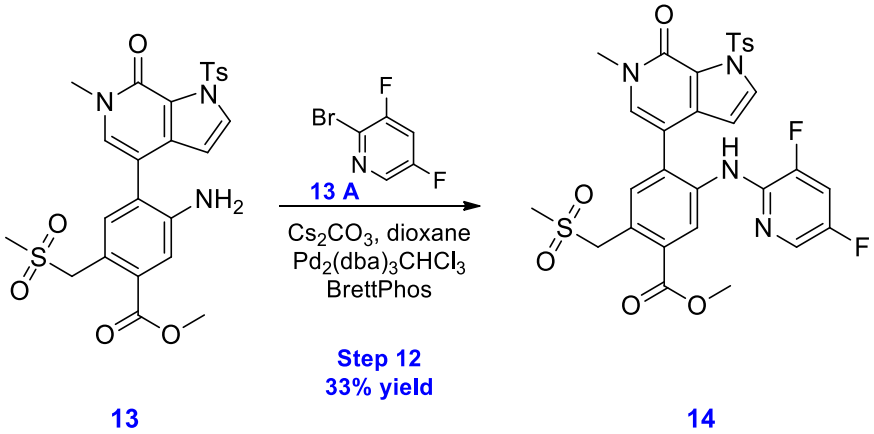
Compound **11** (84.0 g, 220 mmol) and KOAc (54.1 g, 551 mmol) were dissolved in dioxane (1.30 L) under $N_2$ atmosphere. Then the mixture was treated with (112 g, 441 mmol), $Pd_2(dba)_3$ (10.1 g, 11.0 mmol) and XPhos (10.5 g, 22.0 mmol). The reaction mixture was purged with argon and stirred at 90°C for 2 hours. Three reactions were combined for work-up. The combined reaction mixtures were concentrated, dissolved in ethyl acetate (1.00 L) and washed with $H_2O$ (200 mL x 3). The aqueous phase was extracted with ethyl acetate (200 mL x 2), then the combined organics were washed with brine (100 mL), dried over $Na_2SO_4$, concentrated and the resultant solid purified by column chromatography (ether/Ethyl acetate = 100/1 to 0/1). Trituration with n-heptane (200 mL) gave **12** (140 g, 299 mmol, 45.3% yield, 91.5% purity) as a grey solid. NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J* = 8 Hz, 2 H), 7.88 (d, *J* = 3 Hz, 1 H), 7.51 (s, 1 H), 7.29~7.26 (m, 2 H), 6.90 (d, *J* = 3 Hz, 1 H), 3.52 (s, 3 H), 2.39 (s, 3 H), 1.33 (s, 12H). LCMS (ESI): *m/z* 429 $(M+H)^+$.

***11. Preparation of Compound 13***



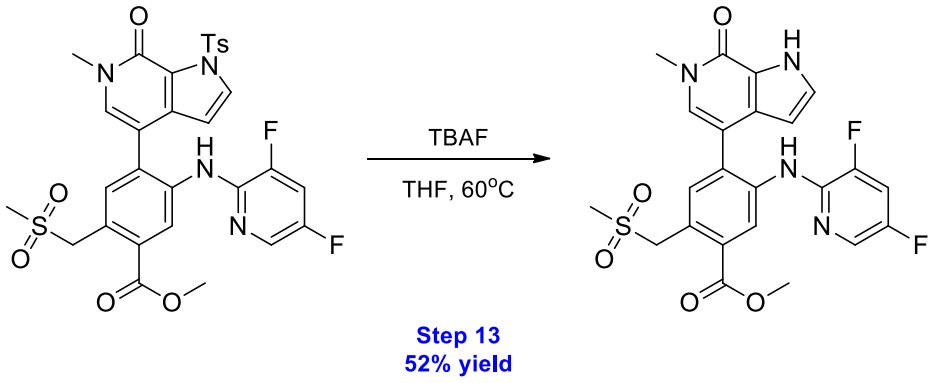

A 3.00 L 3-necked round-bottom was purged with argon and charged with **12** (70.0 g, 163 mmol), **5** (60.3 g, 163 mmol), XPhos (15.6 g, 32.7 mmol), DME (700 mL), $Na_2CO_3$ (34.6 g, 327 mmol), $Pd(PPh_3)_4$ (18.9 g, 16.3 mmol) and $H_2O$ (700 mL). The resulting solution was stirred for 4 hours at 90 °C. Two reactions of this scale were combined for work-up. The mixtures were concentrated, and the resultant solid triturated with DCM (200 mL) to give **13** (90.0 g, 151 mmol, 46.2% yield, 91.2% purity) was obtained as yellow solid. NMR (400 MHz, $CDCl_3$): δ 8.05~8.03 (d, *J* = 8 Hz, 2 H), 7.91 (d, *J* = 4 Hz, 1 H), 7.42 (s, 2 H), 7.33 (d, *J* = 8 Hz, 2 H), 7.31 (s, 1 H), 7.00 (s, 1 H), 6.34 (d, *J* = 4 Hz, 1 H), 4.77 (s, 2 H), 3.92 (s, 3 H), 3.55 (s, 3 H), 2.82 (s, 3 H), 2.42 (s, 3 H). LCMS (ESI): *m/z* 544 $(M+H)^+$.

***12. Preparation of Compound 14***



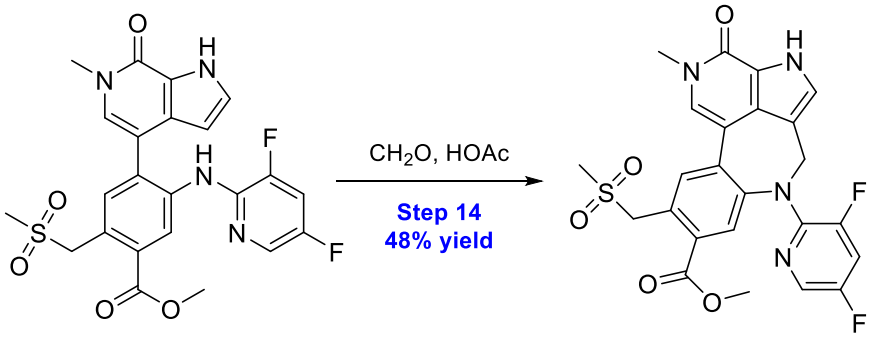
A 2.00 L 3-necked round-bottom was purged with nitrogen and charged with **13** (84.5 g, 155 mmol), **13 A** (60.3 g, 311 mmol), $Cs_2CO_3$ (101 g, 311mmol), dioxane (720 mL), BrettPhos (66.8 g, 124 mmol) and $Pd_2(dba)_3CHCl_3$ (56.9 g, 62.2 mmol). The reaction mixture was heated to 90°C for 4 hours, cooled to 25°C and dissolved into DCM (500 mL). The mixture was filtered through celite (DCM wash) and the combined organics were washed with water (200 mL x 3), brine (100 mL) and then dried over $Na_2SO_4$. Concentration followed by trituration with ethyl acetate (300 mL) at yielded **14** (35.0 g, 51.9 mmol, 33.3% yield, 97.3% purity) as a pale green solid. $^1$H NMR (400 MHz, DMSO- d6): δ 8.29 (s, 1 H), 8.15 (s, 1 H), 7.91~7.89 (m, 3 H), 7.85~7.84 (m, 1 H), 7.73~7.70 (m, 1 H), 7.48 (d, *J* = 8 Hz, 2 H), 7.43 (d, *J* = 8 Hz, 2 H), 6.31 (d, *J* = 3 Hz, 1 H), 4.93 (s, 2 H), 3.84 (s, 3 H), 3.40 (s, 3 H), 2.93 (s, 3 H), 2.39 (s, 3 H). LCMS (ESI): *m/z* 657 $(M+H)^+$.

***13. Preparation of Compound 15***




A solution of **14** (30.0 g, 45.7 mmol) in THF (100 mL) was treated with TBAF (1 M, 137 mL) at 25°C and the mixture was stirred to 60°C for 3 hours. The reaction mixture was diluted with DCM (100 mL), filtered through a pad of celite and the filtrate was washed with $H_2O$ (50.0 mL x 3) and brine (50.0 mL). Trituration of the concentrated organics with ethyl acetate (50.0 mL) at gave **15** (13.0 g, 24.0 mmol, 52.6% yield, 92.8% purity) a pale green solid. $^1$H NMR (400 MHz, DMSO- d6: δ 12.08 (s, 1 H), 8.40 (s, 1 H), 7.96~7.93 (m, 1 H), 7.74~7.73 (m, 1 H), 7.73~7.71 (m, 1 H), 7.55 (s, 1 H), 7.33 (s, 1 H), 7.26~7.25 (m, 1 H), 6.06 (t, *J* = 2 Hz, 1 H), 4.95 (s, 2 H), 3.85 (s, 3 H), 3.53 (s, 3 H), 2.94 (s, 3 H). LCMS (ESI): *m/z* 503 $(M+H)^+$.

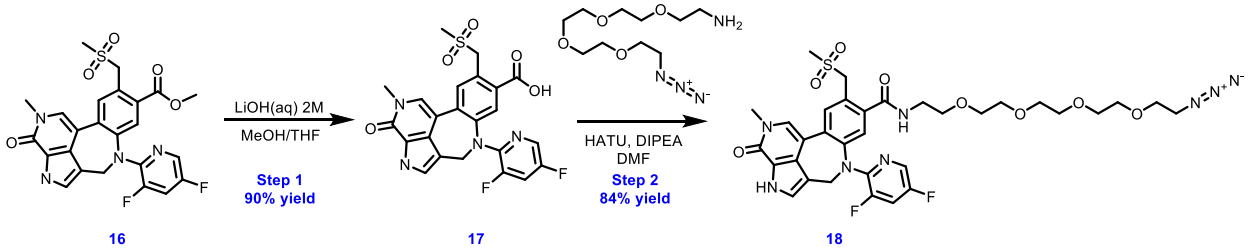
***14. Preparation of Compound 16***




**15** **16**

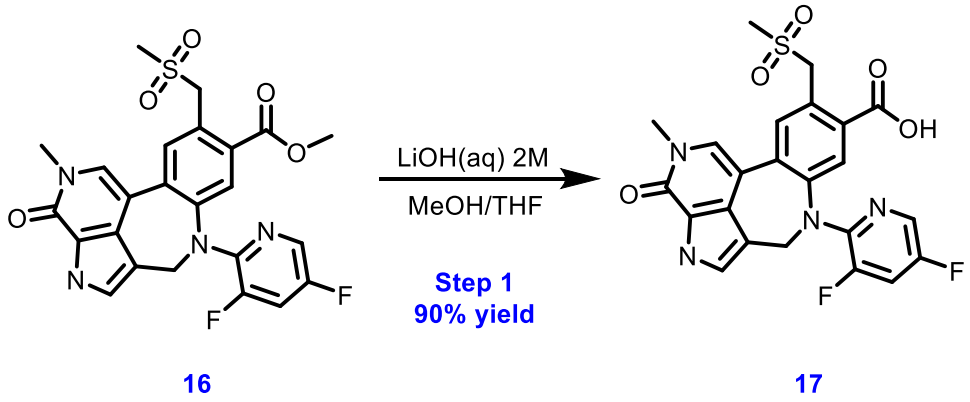
A 500-mL 3-necked round-bottom flask was purged with nitrogen and charged with **15** (13.0 g, 25.9 mmol), AcOH (130 mL) and HCHO (6.30 g, 77.6 mmol, 5.78 mL, 37% purity). The resulting solution was stirred for 2 h at 75 $^{o}$C. The reaction was filtered and the solid washed with petroleum ether. The solid was dissolved in DCM (200 mL) and the pH was adjusted to pH = 8 with saturated $NaHCO_3$ solution. The organic phase was washed with $H_2O$ (100 mL) and brine (50.0 mL), dried over $Na_2SO_4$, filtered and concentrated to give a residue. Two reactions were combined and the crude solid was triturated with MeOH (200 mL) to give **16** (10.5 g, 0.02 mol, 48.8% yield, 98.2% purity) as a pale yellow solid. $^{1}$H NMR (400 MHz, DMSO- d6: δ 11.96 (d, *J* = 2 Hz, 1 H), 8.09 (d, *J* = 2 Hz, 1 H), 8.00 (s, 1 H), 7.83 (s, 1 H), 7.68~7.62 (m, 1 H), 7.57 (s, 1 H), 7.28 (d, *J* = 2 Hz, 1 H), 5.92 (br, 1 H), 5.12~4.86 (m, 2 H), 4.29 (br, 1 H), 3.78 (s, 3H), 3.63 (s, 3 H), 2.95 (s, 3 H). LCMS (ESI): *m/z* 515 $(M+H)^{+}$.

**Synthetic scheme for preparation of compound 18 (BETi P4)**



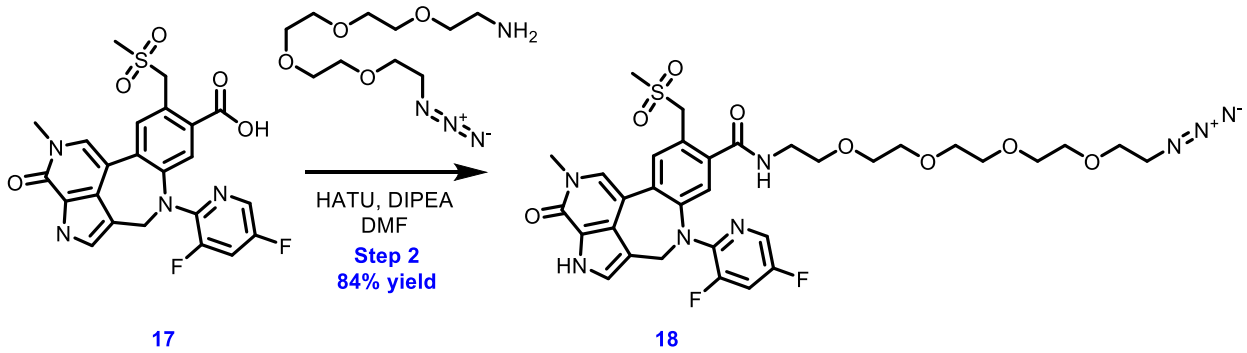

***Experimental Procedure:***

***1. Preparation of Compound 17***




A mixture of compound **16** (950 mg, 1.85 mmol) and LiOH 2M (3.7 mL, 7.38 mmol, 4 equiv.) in THF (18 mL, 0.1 M) / MeOH (6.2 mL, 0.3 M) was stirred at room temperature for 4h. The volatiles were removed under vacuum. The residue was taken up in THF and HCl was added (2M, 3.7 mL, 7.38 mmol, 4 equiv.). The mixture was concentrated under vacuum and purified by reverse phase chromatography (20-80% ACN/0.05% TFA in water). The fractions containing the desired product were combined, concentrated and lyophilized to obtained **17** (831 mg, 1.66 mmol, 90% Yield) as a yellow solid. 1H NMR (400 MHz, DMSO-d6) δ 11.93 (s, 1H), 8.09 (d, J = 2.6 Hz, 1H), 7.97 (s, 1H), 7.81 (s, 1H), 7.70 – 7.60 (m, 1H), 7.58 (s, 1H), 7.28 (d, J = 2.6 Hz, 1H), 3.63 (s, 3H), 2.93 (s, 3H). LCMS (ESI): *m/z* 501 $(M+H)^+$.

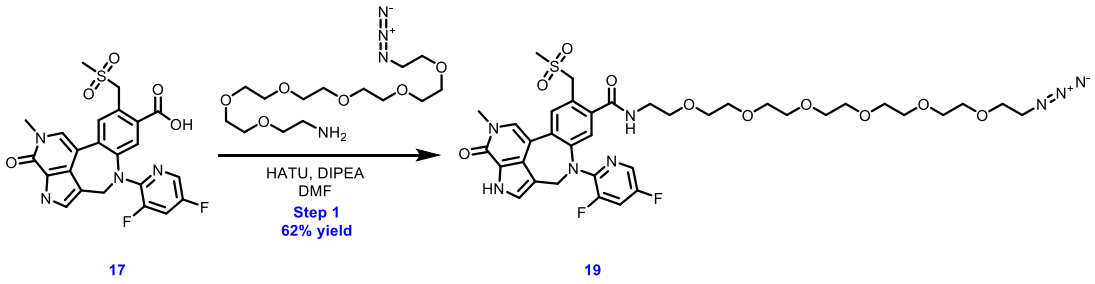
***Preparation of compound 18***




17 18

A mixture of compound 17 (150 mg, 0.299 mmol), 14-azido-3,6,9,12-tetraoxatetradecan-1-amine (113 µL, 0.449 mmol, 1.5 equiv.), HATU (141 mg, 0.359 mmol, 1.2 equiv.) and DIPEA (105 µL, 0.599 mmol, 2 equiv.) in DMF (1.5 mL, 0.2 M) was stirred at room temperature for 45 min. The mixture was purified by silica gel chromatography using 0-10% MeOH/DCM. Lyophilization gave **18** (187 mg, 0.251 mmol, 84% Yield) as an off-white solid. 1H NMR (400 MHz, DMSO-d6) δ 11.92 (d, J = 2.7 Hz, 1H), 8.46 (t, J = 5.7 Hz, 1H), 8.07 (d, J = 2.5 Hz, 1H), 7.92 (s, 1H), 7.75 (s, 1H), 7.61 (ddd, J = 12.1, 8.2, 2.5 Hz, 1H), 7.31 – 7.24 (m, 2H), 5.94 (s, 1H), 5.16 (s, 1H), 4.61 (s, 1H), 4.26 (s, 1H), 3.63 (s, 3H), 3.61 – 3.57 (m, 2H), 3.56 – 3.45 (m, 16H), 3.37 (dd, J = 5.6, 4.4 Hz, 2H), 2.90 (s, 3H), 1.25 (d, J = 6.9 Hz, 1H). LCMS (ESI): *m/z* 745 $(M+H)^+$.

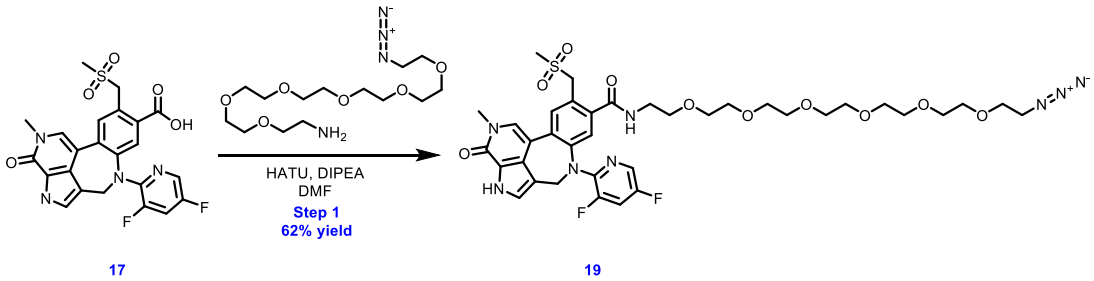
## Synthetic scheme for preparation of compound 19 (BETi P6)




17 19

***Experimental Procedure:***

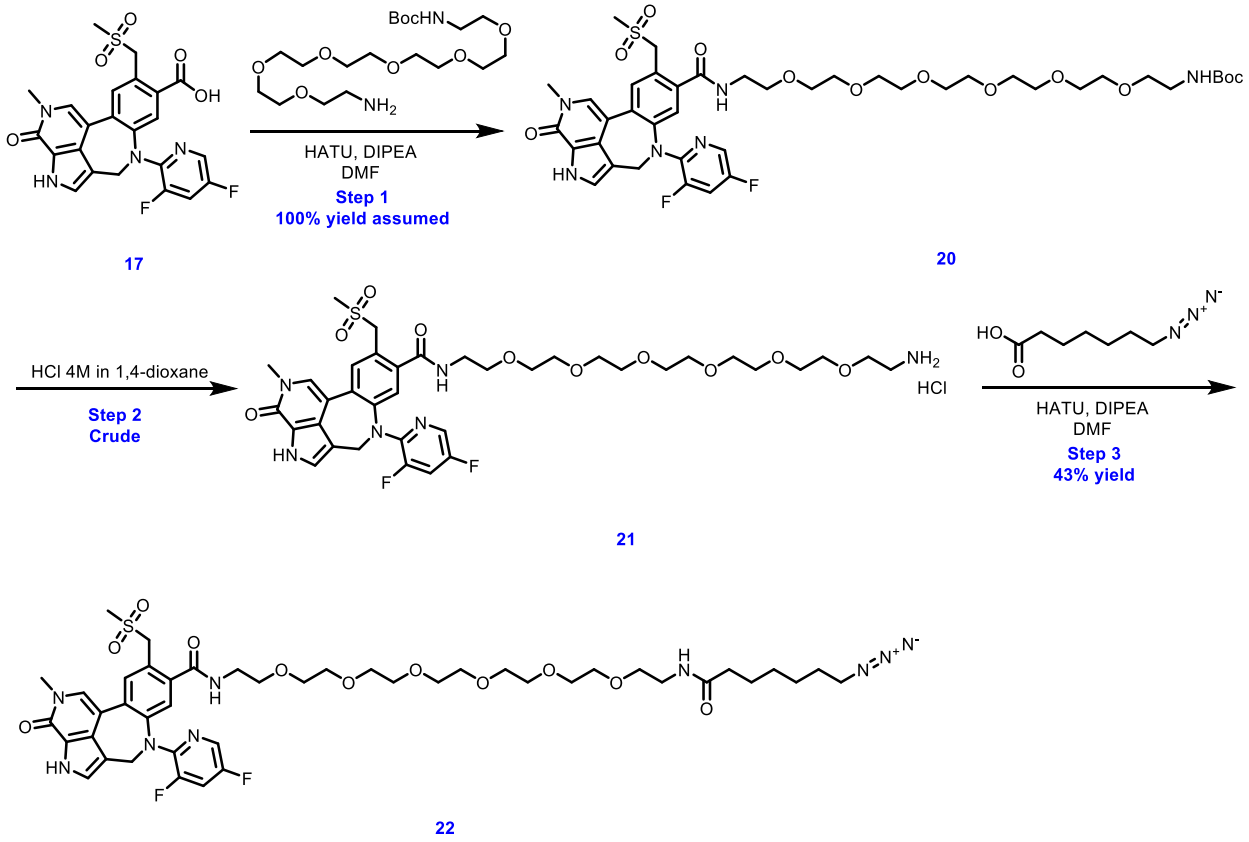
### *1. Preparation of compound 19 (BETi P6)*




17 19

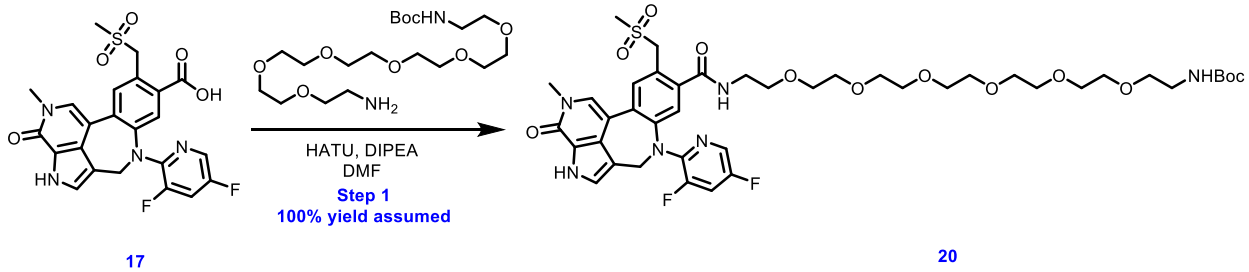
A mixture of compound **17** (150 mg, 0.299 mmol), 20-azido-3,6,9,12,15,18-hexaoxaicosan-1-amine (175.0 mg, 0.449 mmol, 1.5 equiv.), HATU (141.0 mg, 0.359 mmol, 1.2 equiv.) and DIPEA (105 µL, 0.599 mmol, 2 equiv.) in DMF (1.5 mL, 0.2 M) was stirred at room temperature for 4h. The mixture was purified by silica gel chromatography using 0-10% MeOH/DCM. Lyophilization gave **19** (155 mg, 0.186 mmol, 62% yield) as a yellow solid. 1H NMR (400 MHz, DMSO-d6) δ 11.92 (d, J = 2.8 Hz, 1H), 8.46 (t, J = 5.7 Hz, 1H), 8.07 (d, J = 2.5 Hz, 1H), 7.92 (s, 1H), 7.75 (s, 1H), 7.61 (ddd, J = 12.5, 8.3, 2.6 Hz, 1H), 7.31 – 7.24 (m, 2H), 6.06 – 5.83 (m, 1H), 5.16 (s, 1H), 4.59 (s, 1H), 4.26 (s, 1H), 3.63 (d, J = 3.5 Hz, 3H), 3.59 (dd, J = 5.6, 4.3 Hz, 2H), 3.56 – 3.45 (m, 24H), 3.41 – 3.35 (m, 2H), 2.91 (d, J = 9.5 Hz, 3H). LCMS (ESI): *m/z* 833 $(M+H)^+$.

**Synthetic scheme for compound 22 (BETi C7P6)**




***Experimental Procedure:***

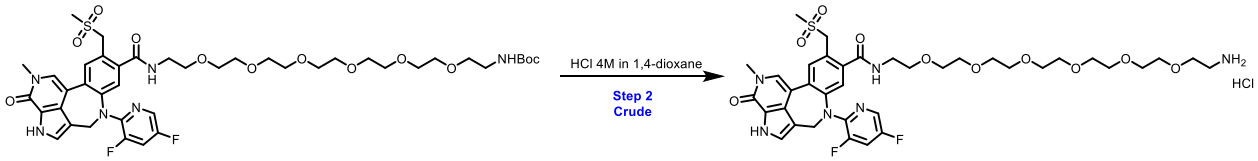
### *1. Preparation of compound 20*




A mixture of compound **17** (400 mg, 0.799 mmol), Boc-N-amino-PEG6-amine (480 mg, 1.12 mmol, 1.4 equiv.), HATU (407 mg, 1.04 mmol, 1.3 equiv.) and DIPEA (279 µL, 1.60 mmol, 2 equiv.) in DMF (4.0 mL, 0.2 M) was stirred at room temperature for 20h. The reaction mixture was partitioned in water/DCM and extracted with DCM (3x). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude mixture was

adsorbed on silica gel and purified by silica gel chromatography using 0-10% MeOH/DCM to give **20** (1.02 g, 1.12 mmol) as a yellow residue. NMR showed residual DMF. 1H NMR (400 MHz, DMSO-*d*6) d 11.91 (s, 1H), 8.46 (t, *J* = 5.6 Hz, 1H), 8.06 (d, *J* = 2.7 Hz, 1H), 7.92 (s, 1H), 7.75 (s, 1H), 7.67 – 7.54 (m, 1H), 7.30 – 7.24 (m, 2H), 6.73 (s, 2H), 3.62 (s, 3H), 3.49 (dq, *J* = 5.1, 2.4 Hz, 37H), 3.06 (t, *J* = 5.9 Hz, 3H), 2.90 (s, 4H), 2.69 (s, 2H), 1.37 (d, *J* = 3.6 Hz, 17H), 1.24 (s, 12H). LCMS (ESI): *m/z* 907 $(M+H)^+$.

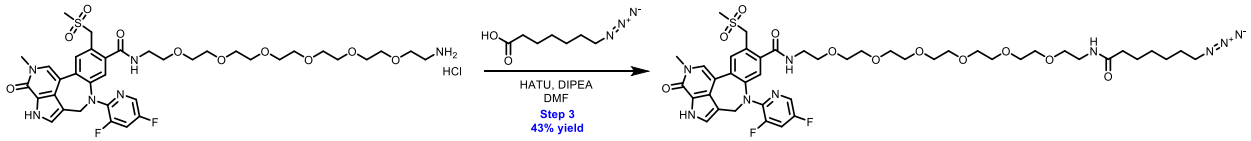
### *2. Preparation of Compound 21*




20 21

Compound **20** (725 mg, 0.799 mmol) was stirred with HCl (4 M in 1,4-dioxane) (4.0 mL, 16.0 mmol, 0.2 M) at room temperature for 4h. The reaction mixture was concentrated to dryness and diluted with toluene and concentrated (2x). Crude product **21** was carried over to the next step without purification.

### *3. Preparation of compound 22 (BETi C7P6)*

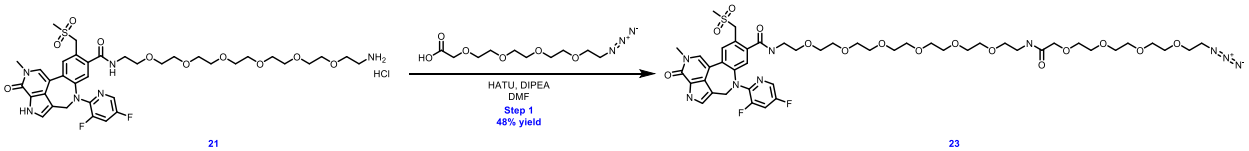



21 22

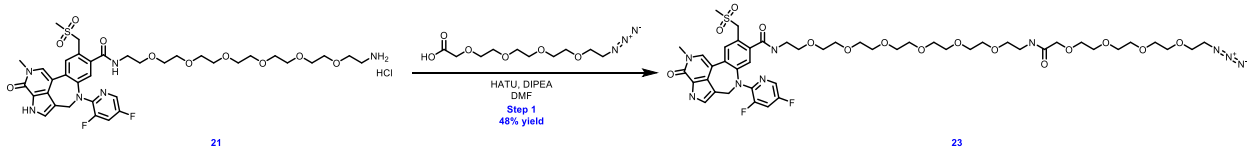
A mixture of compound **21** (337 mg, 0.399 mmol), 7-azidoheptanoic acid (103 mg, 0.599 mmol, 1.5 equiv.), HATU (313 mg, 0.799 mmol, 2 equiv.) and DIPEA (698 µL, 3.99 mmol, 10 equiv.) in DMF (2.0 mL, 0.2 M) was stirred at room temperature for 3h. The crude product was adsorbed on silica gel and purified by silica gel chromatography using 0-10% MeOH/DCM. The compound was further purified by prep-HPLC (20-60 CAN/0.1% $NH_4OH$ in water, XSelect CSH Prep C18, 50 x 30mm, 5µm, run time: 10 min) to afford compound **22** (164 mg, 0.171 mmol, 43% Yield) as a

yellow residue. 1H NMR (400 MHz, DMSO-d6) δ 11.91 (d, J = 2.8 Hz, 1H), 8.46 (t, J = 5.6 Hz, 1H), 8.07 (d, J = 2.5 Hz, 1H), 7.92 (s, 1H), 7.80 (t, J = 5.7 Hz, 1H), 7.75 (s, 1H), 7.61 (ddd, J = 12.3, 8.3, 2.5 Hz, 1H), 7.29 (s, 1H), 7.27 (d, J = 2.7 Hz, 1H), 5.95 (s, 1H), 5.16 (s, 1H), 4.61 (s, 1H), 4.26 (s, 1H), 3.62 (s, 3H), 3.49 (d, J = 2.5 Hz, 22H), 3.42 – 3.33 (m, 4H), 3.28 (d, J = 6.8 Hz, 2H), 3.17 (q, J = 5.9 Hz, 2H), 2.90 (s, 3H), 2.05 (t, J = 7.4 Hz, 2H), 1.49 (tq, J = 14.4, 7.4, 7.0 Hz, 4H), 1.35 – 1.17 (m, 4H). LCMS (ESI): *m/z* 960 $(M+H)^+$.

**Synthetic scheme for preparation of compound 23 (BETi P4P6)**




***1. Preparation of compound 23 (BETi P4P6)***




Compound **21** (337 mg, 0.399 mmol), 2-[2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]ethoxy]acetic acid 0.5M in MTBE (1 mL, 0.599 mmol, 1.5 equiv.), HATU (313 mg, 0.799 mmol, 2 equiv.) and DIPEA (698 µL, 3.99 mmol, 10 equiv.) in DMF (2.0 mL, 0.2 M) was stirred at room temperature for 3h. The crude product was adsorbed on silica gel and purified by silica gel chromatography using 0-10% MeOH/DCM. The compound was further purified by prep-HPLC (20-60 CAN/0.1% Ammonium hydroxide in water, XSelect CSH Prep C18, 50 x 30mm, 5µm, run time: 10 min) to afford compound **23** (199 mg, 0.187 mmol, 48% Yield) as a yellow residue. 1H NMR (400 MHz, DMSO-d6) δ 11.92 (s, 1H), 8.46 (t, J = 5.6 Hz, 1H), 8.07 (d, J = 2.5 Hz, 1H), 7.92 (s, 1H), 7.75 (s, 1H), 7.66 – 7.56 (m, 2H), 7.29 (s, 1H), 7.27 (s, 1H), 5.94 (s, 1H), 5.16 (s, 1H), 4.61 (s, 1H), 4.26 (s, 1H), 3.87 (s, 2H), 3.62 (s, 3H), 3.61 – 3.57 (m, 2H), 3.57 – 3.52 (m, 11H), 3.49 (d, J = 3.2 Hz, 21H), 3.46 – 3.34 (m, 8H), 3.27 (d, J = 6.7 Hz, 2H), 2.90 (s, 3H). LCMS (ESI): *m/z* 1066 $(M+H)^+$.

### *1. General Experimental Procedure for Synthesis of BETcides*

**II. Peptide Synthesis**

All peptides were synthesized using standard Fmoc (9-fluorenylmethoxycarbonyl) solid-phase peptide synthesis (SPPS) [1]. A combination of both manual and automated synthesis was employed where appropriate. Automated microwave-assisted SPPS was performed on a Liberty Blue™ Automated Microwave Peptide Synthesizer (CEM) using a 0.5 mmol-scale protocol. Rink amide ProTide™ resin (loading 0.52 mmol/g) was used as the solid support, and DIC/ OxymaPure® as the activating system. In the coupling steps, a solution of an Fmoc-protected amino acid (0.4 M, 10 mL) in *N*,*N*-dimethylformamide (DMF) was added to the resin (0.7 g, 0.36 mmol), followed by the addition of OxymaPure® solution (1 M, 4 mL) and DIC solution (1 M, 4 mL) in DMF. The resulting mixture was purged with nitrogen under microwave irradiation at 90 °C for 2 min with the exceptions of L-and D-cysteine (75 °C, 2 min), and L- and D-histidine (50 °C, 10 min). The supernatant was drained, and the process was repeated once for all amino acids except L- and D-histidine. The supernatant was drained, and the resin was washed with DMF (20 mL × 3). For Fmoc removal, a 20% solution of 4-methylpiperidine in DMF (10 mL) was added to the resin. The resulting mixture was purged with nitrogen under microwave irradiation at 90 °C for 1 min. The supernatant was drained, and the process was repeated once. The resin was then washed thoroughly with DMF (10 mL × 5). The coupling-deprotection cycle was repeated until the peptide assembly was complete.

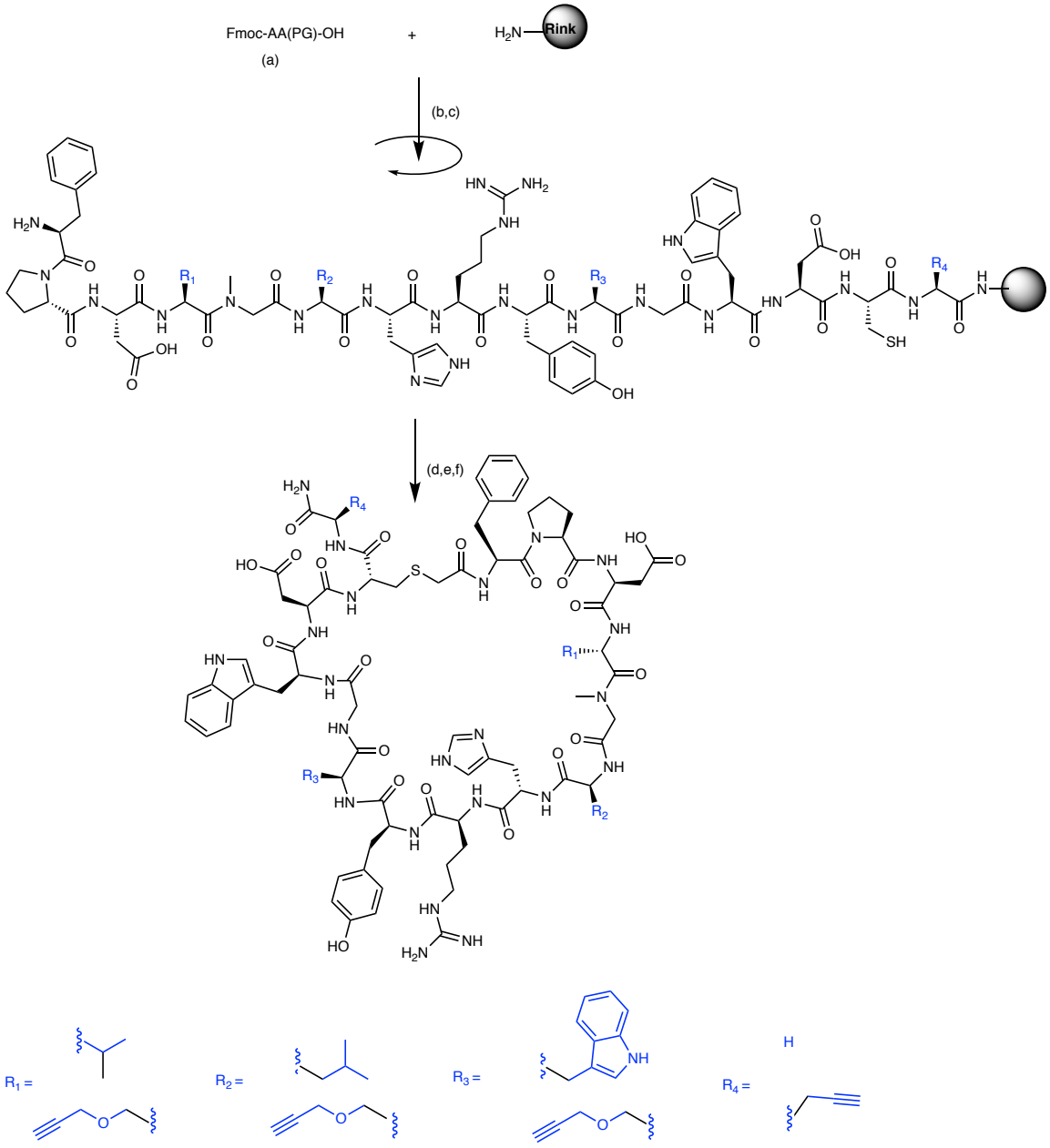
The coupling of Fmoc-Ser(propargyl)-OH was done manually. A solution of Fmoc-Ser(Propargyl)-OH (0.4 g, 1.08 mmol, 3.0 equiv) in DMF was added to the resin (0.7 g, 0.36 mmol, 1.0 equiv), followed by the addition of 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU, 411 mg, 1.08 mmol, 3 equiv) and *N*,*N*-diisopropylethylamine (DIEA, 0.188 mL, 1.08 mmol, 3 equiv). The resulting mixture was agitated at room temperature overnight. Ninhydrin test indicated completion of reaction. The supernatant was drained, and the resin was washed with DMF (20 mL × 3).

For the synthesis of thioether-cyclic peptides, the N-terminus of the peptide was capped with chloroacetyl by agitating the resin (0.7 g, 0.36 mmol, 1.0 equiv) with a solution of chloroacetic anhydride (0.65 g, 3.6 mmol, 10.0 equiv) and DIEA (0.63 mL, 3.6 mmol, 10.0 equiv) in 20 mL DMF at room temperature overnight. Ninhydrin test indicated complete reaction. The supernatant was drained, and the resin was washed with DMF (20 mL × 3).

For peptide cleavage, the resin (0.7 g, 0.36 mmol) was washed thoroughly with (DMF ,15 mL × 3) and dichloromethane (DCM, 20 mL × 3), and then dried in vacuo. A mixture of trifluoroacetic acid/phenol/water/thioanisole/triisopropylsilane (v/w/v/v/v 90:2.5:1.5:3.5:2.5, 15 mL) was added to the resin at 0 °C. The resulting mixture was slowly warmed to room temperature and allowed to mix for 3 h. The supernatant was collected by filtration, and the resin was washed with the cleavage mixture (8 mL × 3). The combined filtrate was concentrated under reduced pressure to a small volume, which was diluted with anhydrous diethyl ether (90 mL). The resulting mixture was allowed to stand at 0 °C for 30 min and then centrifuged. The precipitate was washed with anhydrous diethyl ether (90 mL × 3) and dried in vacuo to afford the crude linear peptide as a white powder. Crude yield 45-58%.

For cyclization via a thioether bond, the crude linear peptide was dissolved in 50% acetonitrile (ACN) in water at a concentration of 10 mg/mL, and the pH was adjusted to 8 – 9 by dropwise addition of DIEA. The reaction was stirred overnight and was monitored with liquid chromatography-mass spectrometry (LC-MS). Upon completion of cyclization, the solution was lyophilized to dryness. The obtained crude cyclic peptides were purified by reverse-phase high-performance chromatography (RP-HPLC, C18 column, 21 mm × 250 mm, 5 μm, 100 Å, 10 mL/min, 60-min gradient from 30 to 60% ACN/water containing 0.1% TFA). Purification yield 10-15%.

**General Synthetic Scheme for Microwave assisted and Manual SPPS**



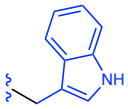
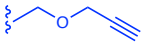
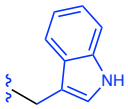
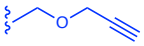
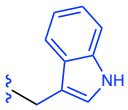
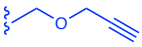
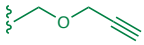
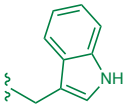




Peptide Sequence modified accordingly with alkyne structures represented above at the Gly, Val, Leu and Trp positions: cyc(Ac) FPDV(Sar)LHRYWGWDCG amide

**Scheme for SPPS**: a) 0.25 mmol Fmoc amino acid/DMF b) 1M DIC/ 1M Oxyma pure activating reagent/ DMF c) 20% Piperidine /DMF d) Chlroacetyl anhydride/DMF, DIEA e) TFA cocktail f) DIEA

**Table (1) Representation of Macrocyclic Peptide Alkynes**

| Side chain / Compound Number | R1 | R2 | R3 | R4 | Yield % |
|---|---|---|---|---|---|
| 24 |  |  |  |  | 15 |
| 25 |  |  |  | H | 12 |
| 26 |  |  |  | H | 14 |
| 27 |  |  |  | H | 15 |
| 28D* |  |  |  | H | 10 |

D* = D isomer macrocyclic peptide, where all amino acids in the macrocycle are Fmco-D-AA-OH

**Conjugation of Peptides to BRD4 Ligands**

The alkyne-bearing peptides were conjugated to BRD4 ligands containing an azido group on a polyethylene glycol (PEG) linker via click chemistry. In a typical procedure, the alkyne-bearing peptide (40 mg, 1.0 equiv) was dissolved in degassed DMF (3 mL) under nitrogen atmosphere, and the pH was adjusted to 8 with DIEA by adding excess DIEA.

Tris(hydroxypropyltriazolylmethyl)amine

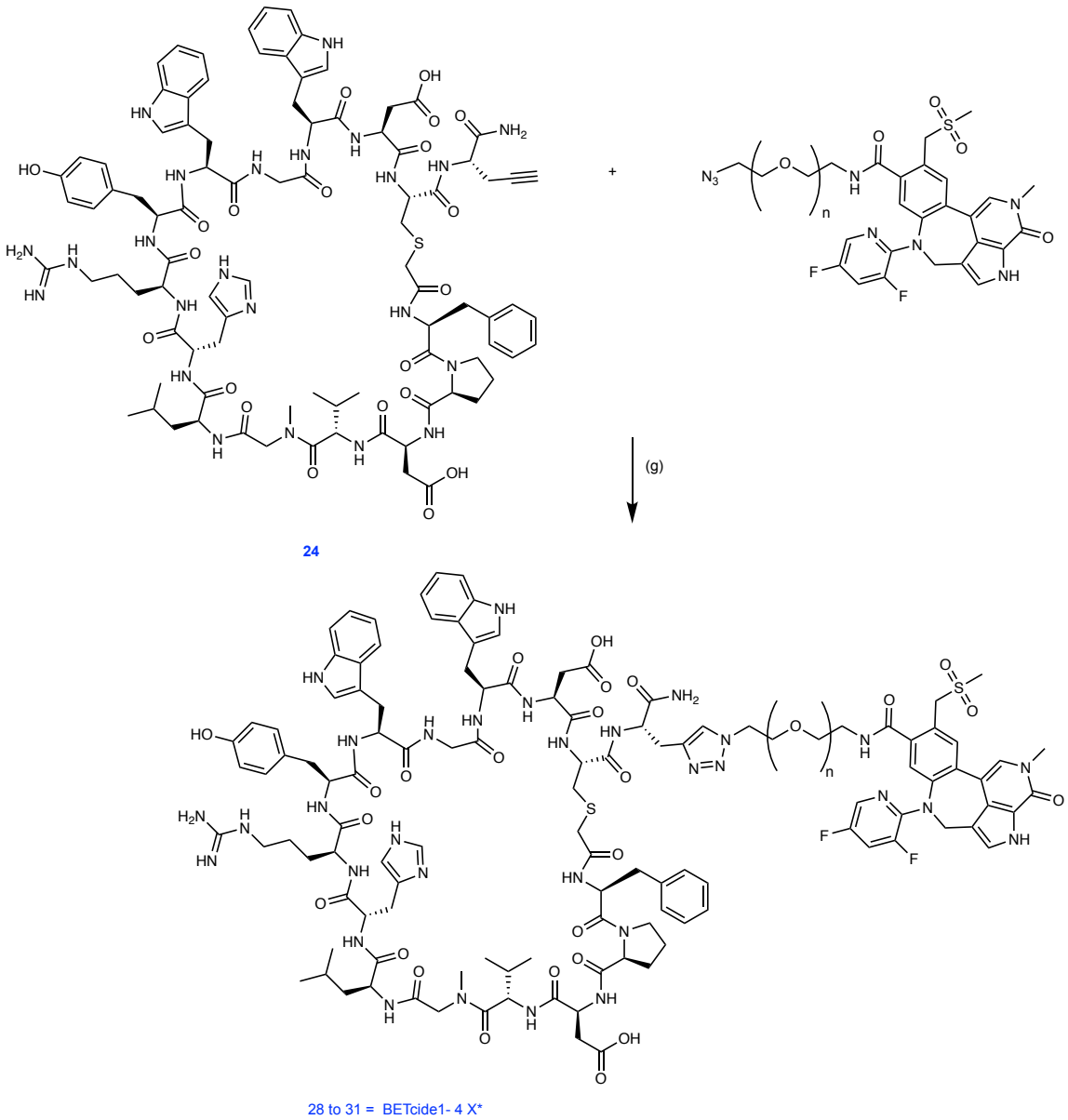
(THPTA) ligand (10.0 equiv) was added. The resulting mixture was agitated. A stochiometric amount of tetrakis(acetonitrile)copper(I) hexafluorophosphate (Cu (I)) (3.0 equiv) was added to the solution under nitrogen atmosphere, followed by addition of the azide-derivatized BRD4 ligand (4.0 equiv). The resulting mixture was purged with nitrogen at room temperature overnight, and the reaction was monitored by LC-MS. Upon completion of reaction, Chelex 100 resin (1.0 g) was added. The resulting mixture was agitated for 5 min and then filtered. The

filtrate was used directly for RP-HPLC purification (C18 column, 21 mm × 250 mm, 5 µm, 100 Å, 10 mL/min, 60-min gradient from 30 to 60% ACN/water containing 0.1% TFA). BETCIDEs were obtained as white powder with purification yields 10-20%.

**Fluorescent labeling of peptides with Cyanine 5.5**

The fluorescently labeled PSMD2 Macrocyclic peptide PSMD2-MC1$_{Cy5.5,}$ was obtained following the click chemistry protocol under similar reaction conditions as described above for BRD4 conjugation. The PSMD2-MC1$_{Cy5.5}$ was obtained as a blue powder with purification yield of 23%. The control compound triazole capped Cyanine5.5 (**55**) dye was commercially sourced from Lumiprobe Corporation.

**General Synthetic Scheme for C-Terminal Conjugation Compounds (28 to 31) (BETcide1-4 X*)**



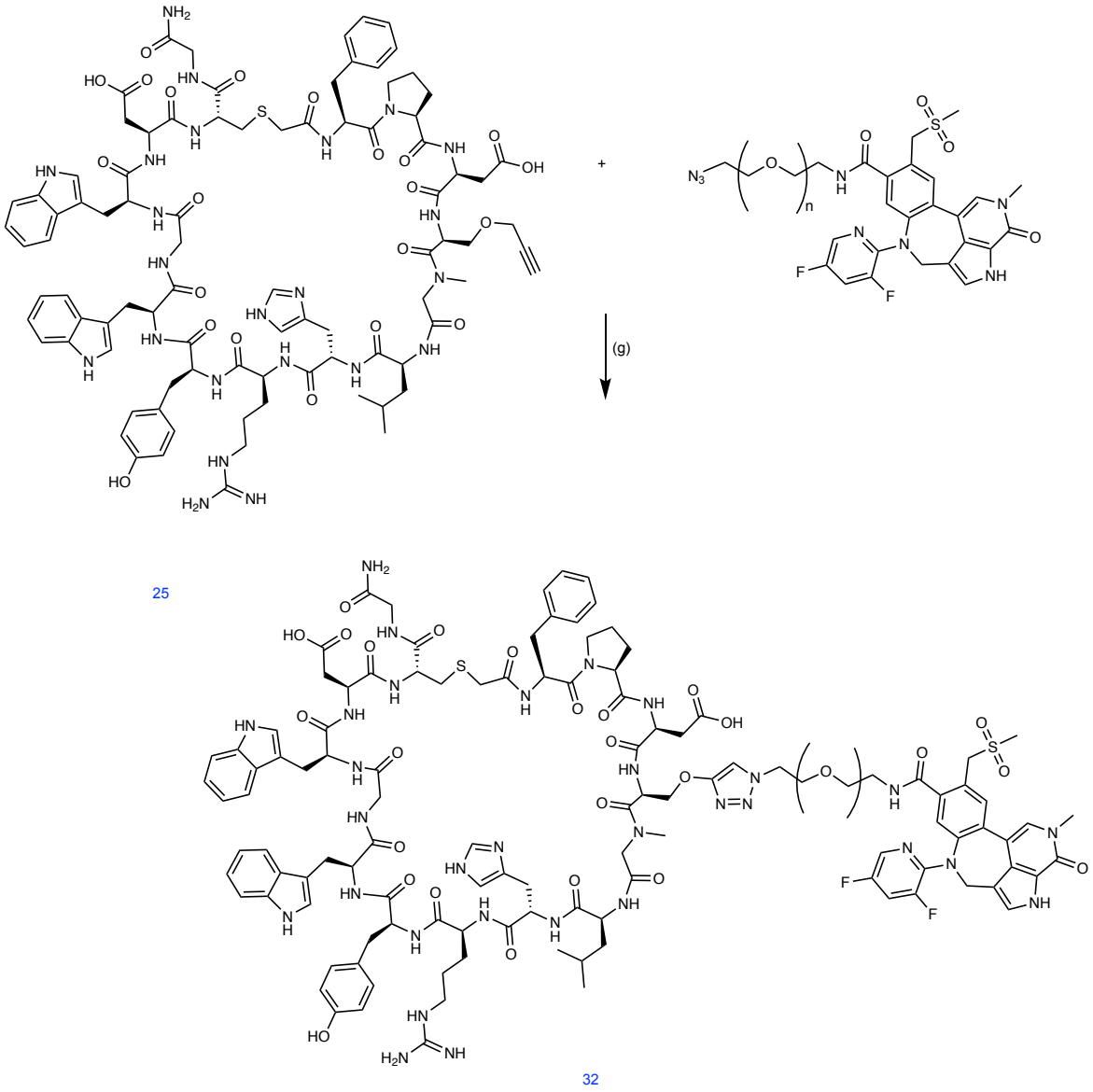

24

28 to 31 = BETcide1- 4 X*

**Scheme for Conjugation:** g) THPTA, Cu(I), DIEA, DMF

*X represents linkers P4, P6, C7P6, P4P6 refer to table.

**General Synthetic Scheme for on-the-ring Macrocycle Conjugation, Compounds (32 to 35 ) (BETcide5 -8 X*)**



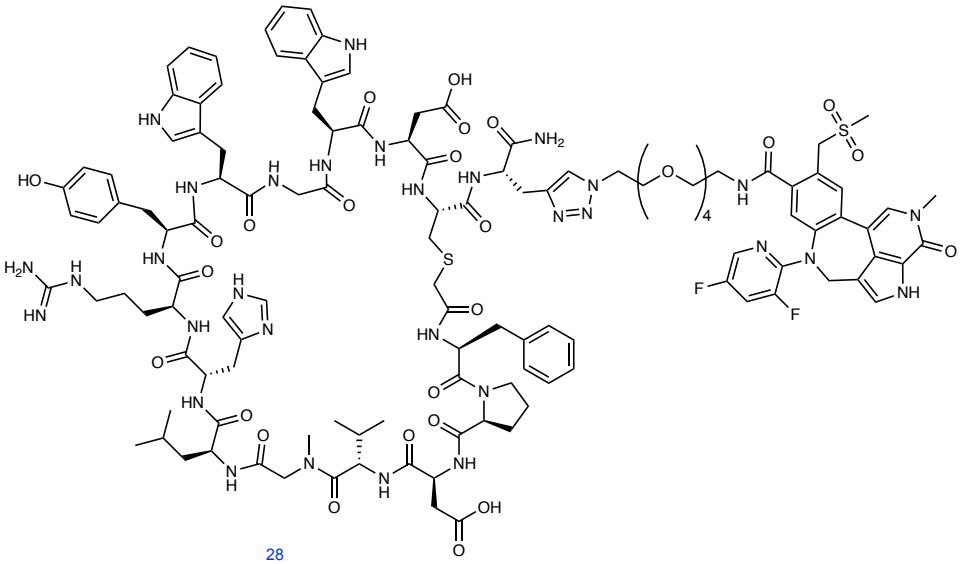

**Scheme for Conjugation:** g) THPTA, Cu(I), DIEA, DMF

**Table (2)** Representation of BETcide Peptide Macrocyclic conjugates with their Respective Abbreviations of Compound Names and Numbers

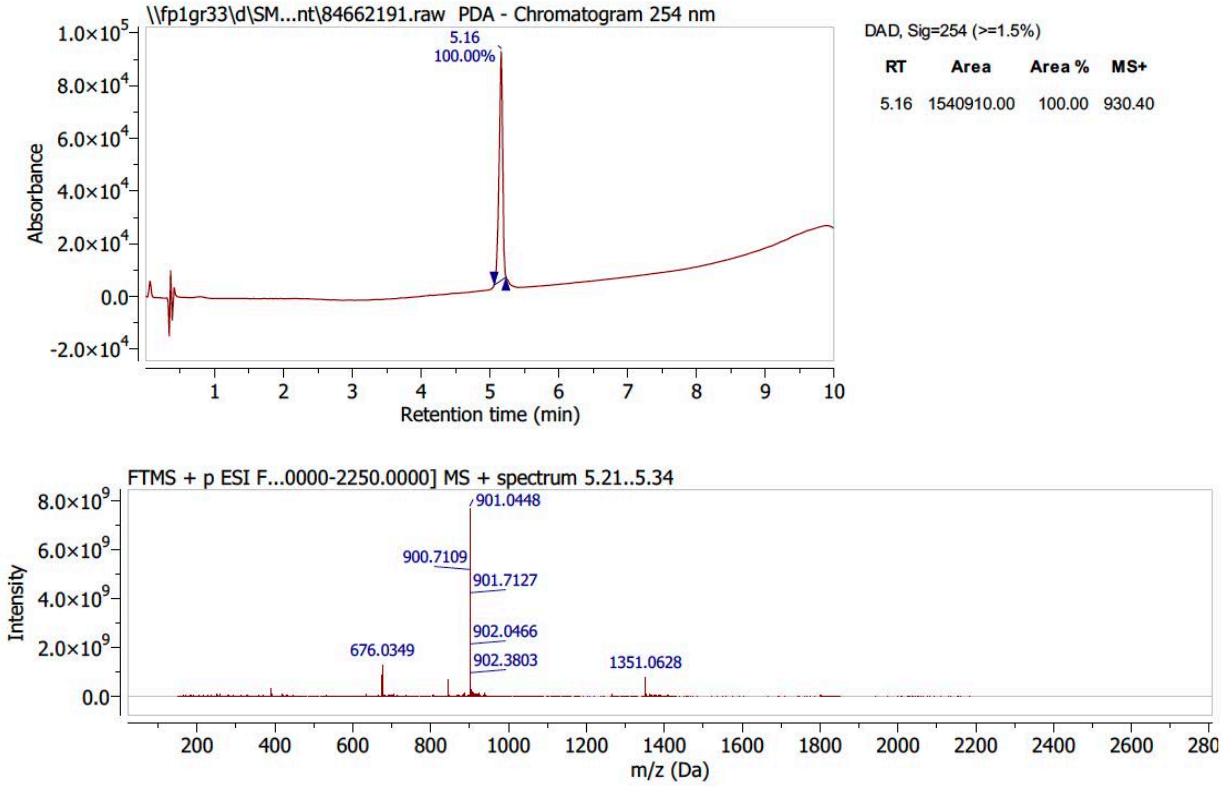
| PSMD2-MC1 - derived CIDEs used in this study | | | | | | |
|---|---|---|---|---|---|---|
| **Compound name/ or Number** | **Yeild %** | **Nickname** | **Compound type** | **Conjugation point** | **X= Linker*** | **amino acid stereochemistry** |
| (28) BETcide1 G15-P4 | 18 | G15-P4 | CIDE | Gly15 | P4 | L |
| (29) BETcide2 G15-P6 | 20 | G15-P6 | CIDE | Gly15 | P6 | L |
| (30) BETcide3 G15-C7P6 | 10 | G15-C7P6 | CIDE | Gly15 | C7P6 | L |
| (31) BETcide4 G15-P4P6 | 15 | G15-P4P6 | CIDE | Gly15 | P4P6 | L |
| (32) BETcide5 V4-P4 | 20 | V4-P4 | CIDE | Val4 | P4 | L |
| (33) BETcide6 V4-C7P6 | 10 | V4-C7P6 | CIDE | Val4 | C7P6 | L |
| (34) BETcide7 V4-P4P6 | 16 | V4-P4P6 | CIDE | Val4 | P4P6 | L |
| ( 35) BETcide8 V4-P6 | 17 | L-CIDE | CIDE | Val4 | P6 | L |
| (36) BETcide9 L6-P4 | 19 | L6-P4 | CIDE | Leu6 | P4 | L |
| (37) BETcide10 L6-C7P6 | 12 | L6-C7P6 | CIDE | Leu6 | C7P6 | L |
| (38) BETcide11 L6-P4P6 | 12 | L6-P4P6 | CIDE | Leu6 | P4P6 | L |
| (39) BETcide12 L6-P6 | 18 | L6-P6 | CIDE | Leu6 | P6 | L |
| ( 40) BETcide13 W10-P4 | 20 | W10-P4 | CIDE | Trp10 | P4 | L |
| ( 41) BETcide14 W10-C7P6 | 14 | W10-C7P6 | CIDE | Trp10 | C7P6 | L |
| (42) BETcide15 W10-P4P6 | 18 | W10-P4P6 | CIDE | Trp10 | P4P6 | L |
| (43) BETcide16 W10-P6 | 15 | W10-P6 | CIDE | Trp10 | P6 | L |
| (44) D-BETcide8 V4-P6 | 20 | D-CIDE | CIDE | val4 | P6 | D |
| *P4 = PEG4, P6 = PEG6, C7P6= 7alkyl-PEG6, P4P6 = PEG4+PEG6 | | | | | | |

1. Chemical Structures and LC-MS Data for BRD4 Conjugates

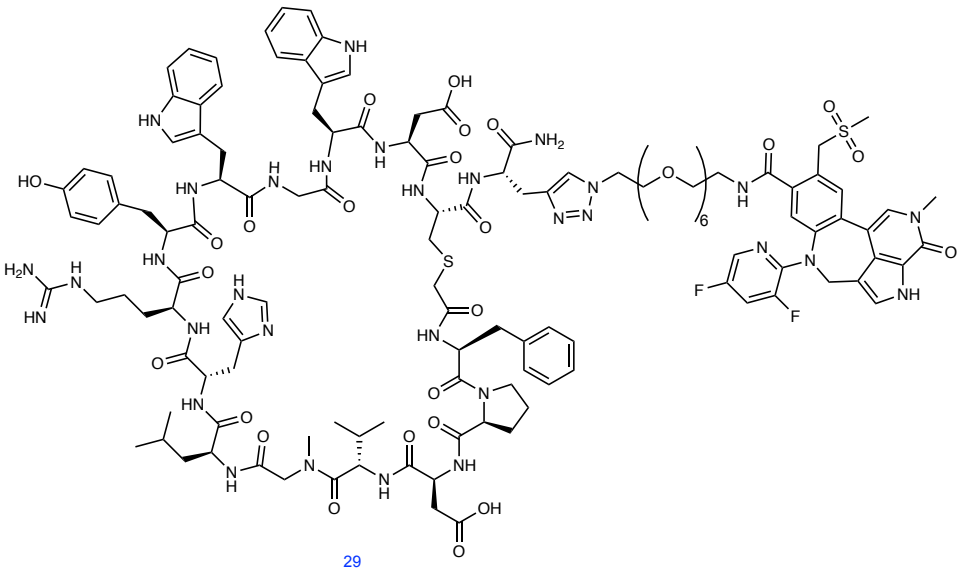
## LC-MS data for compound (28) BETcide1 G15-P4 - G03428399

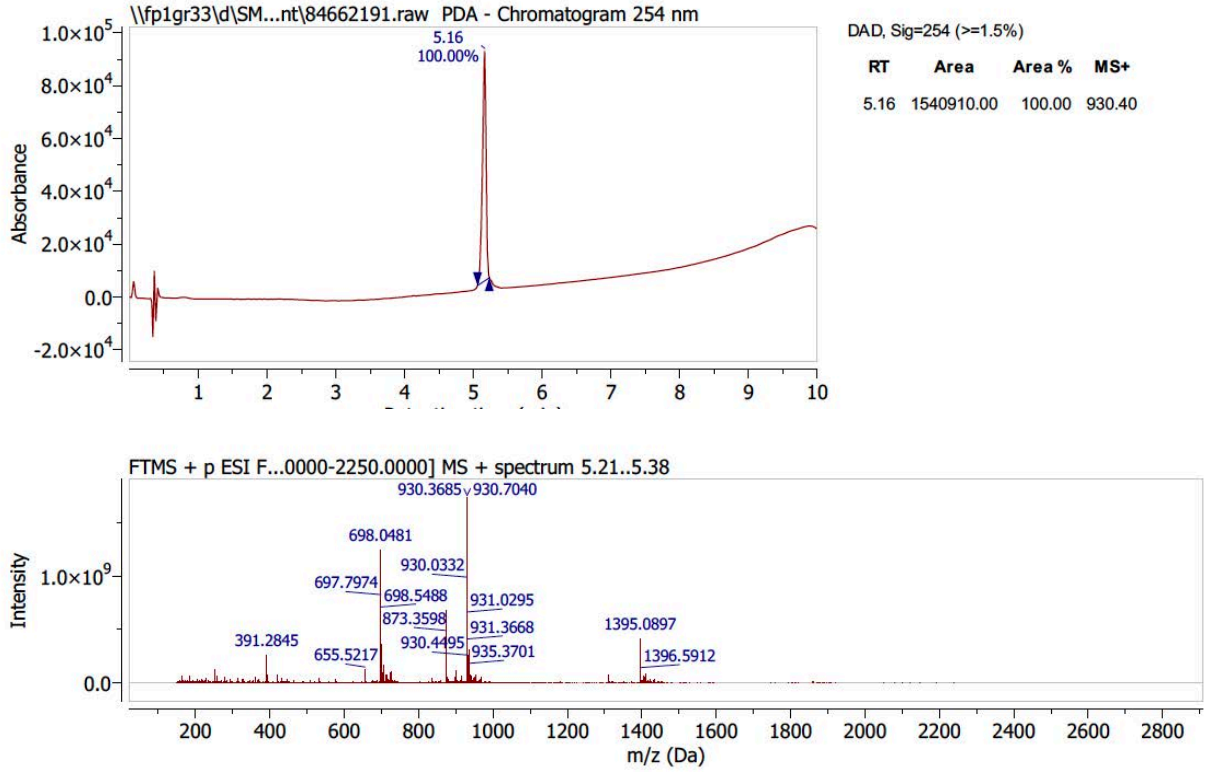


28

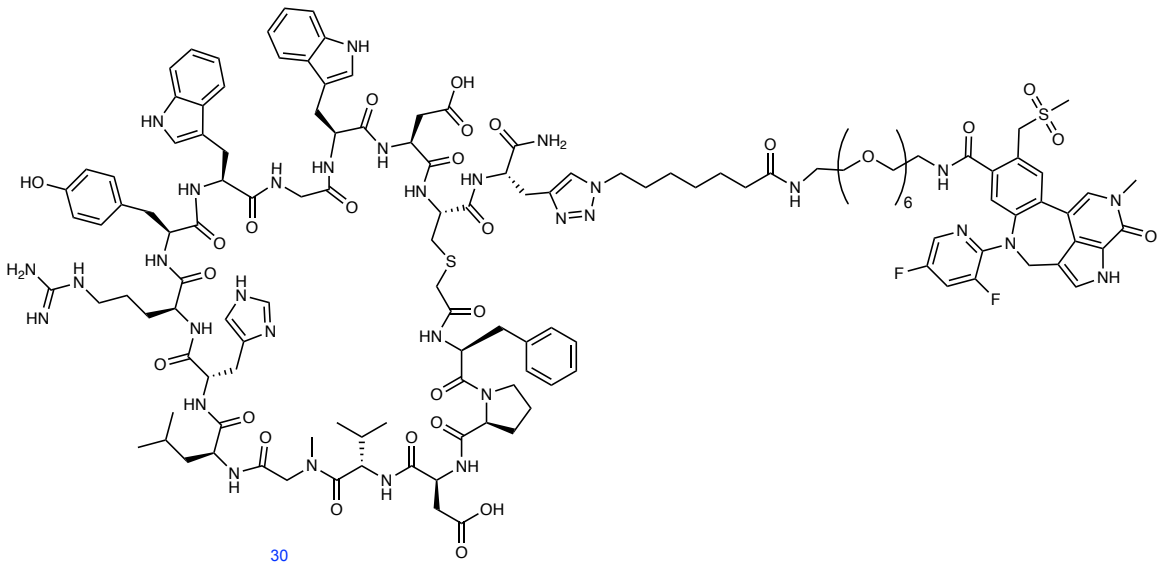


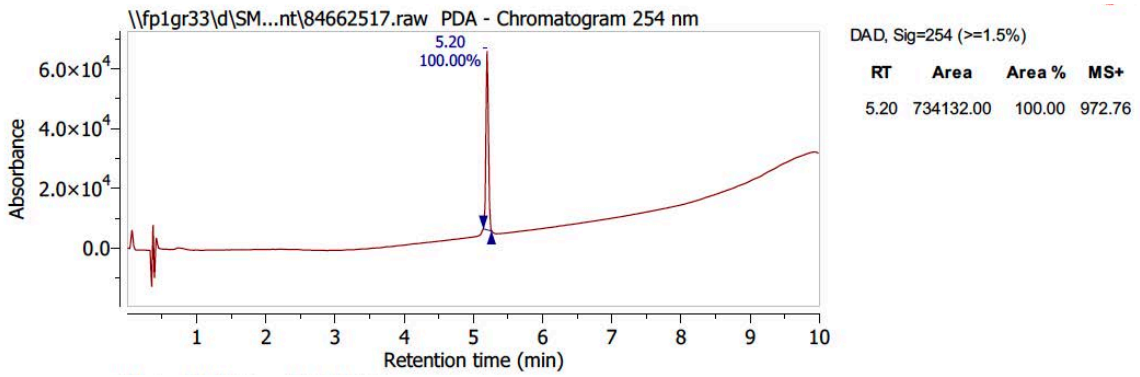

LC-MS data for compound (29) BETcide2 G15-P6 - G03428400





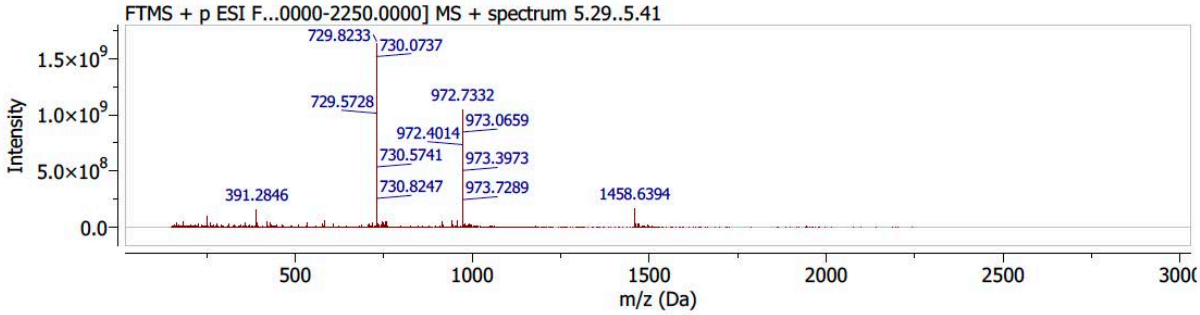

LC-MS data for compound (30) BETcide3 G15-C7P6 - G03428401



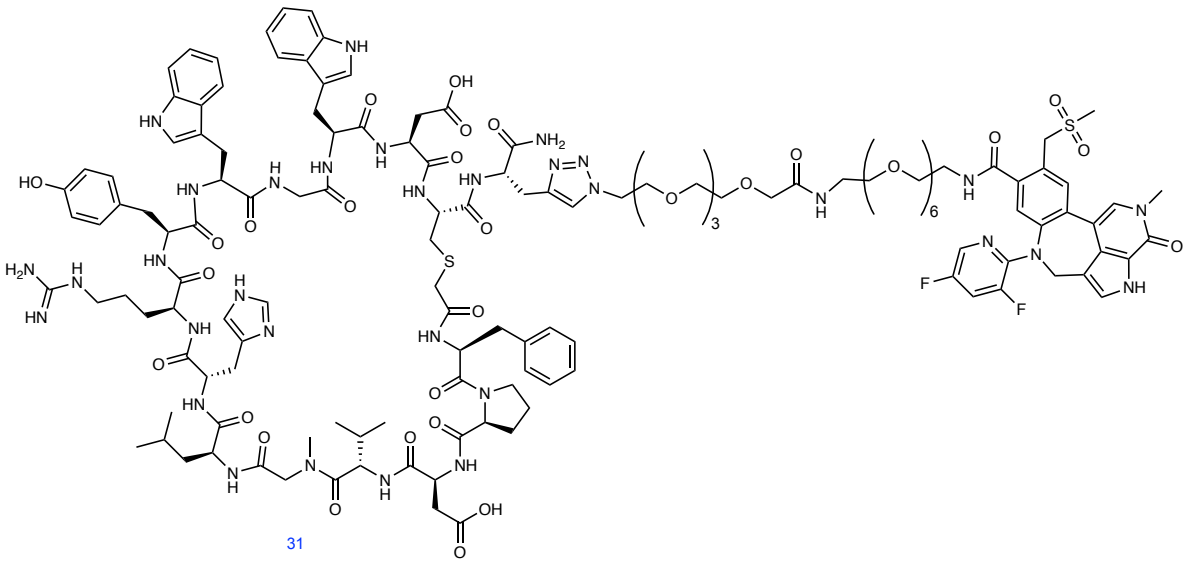



DAD, Sig=254 (>=1.5%)

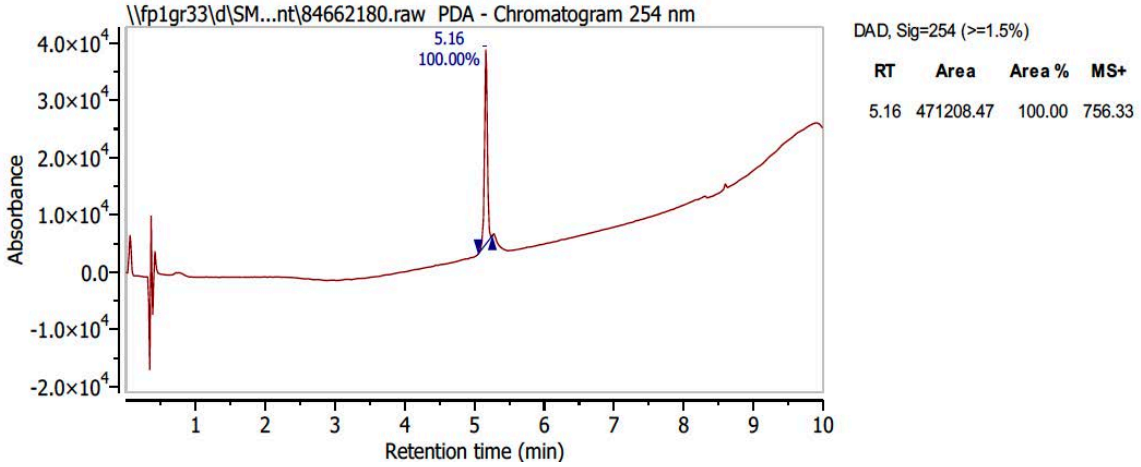
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 5.20 | 734132.00 | 100.00 | 972.76 |




LC-MS data for compound (31) BETcide4 G15-P4P6 - G03428402



31




DAD, Sig=254 (>=1.5%)

| RT | Area | Area % | MS+ |
|---|---|---|---|
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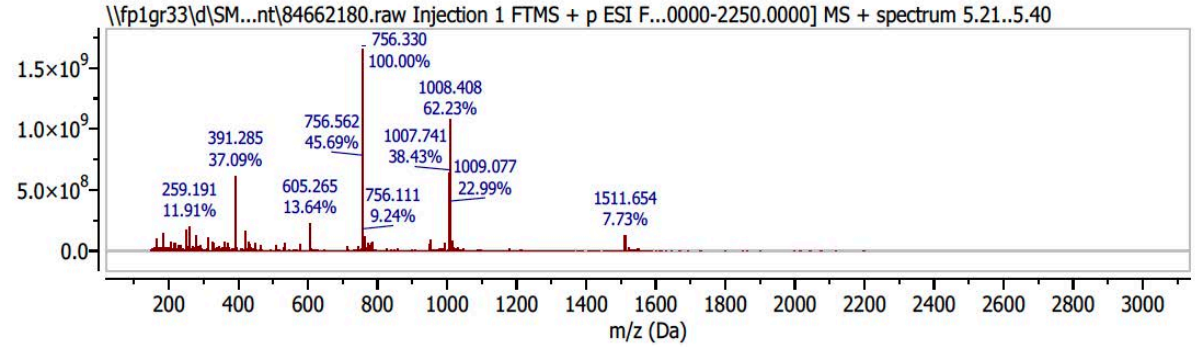
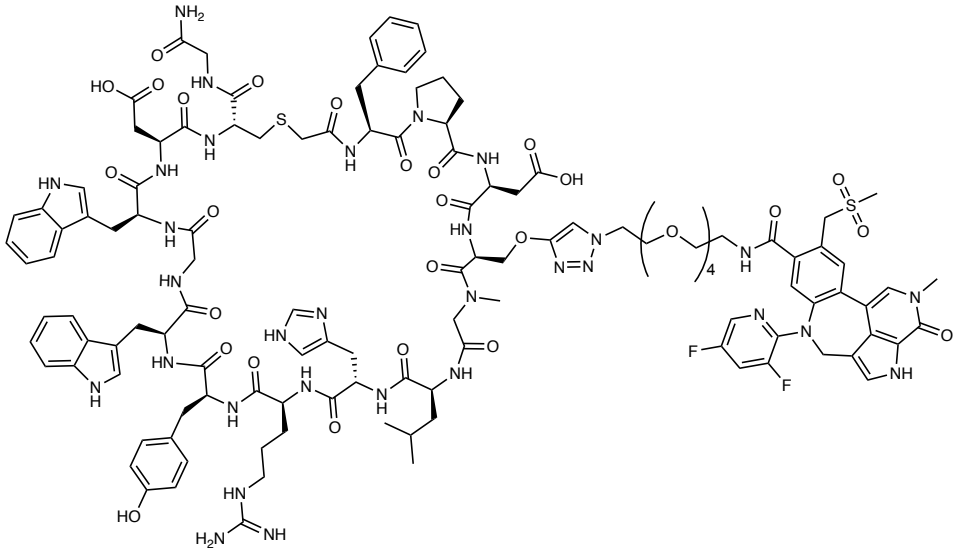
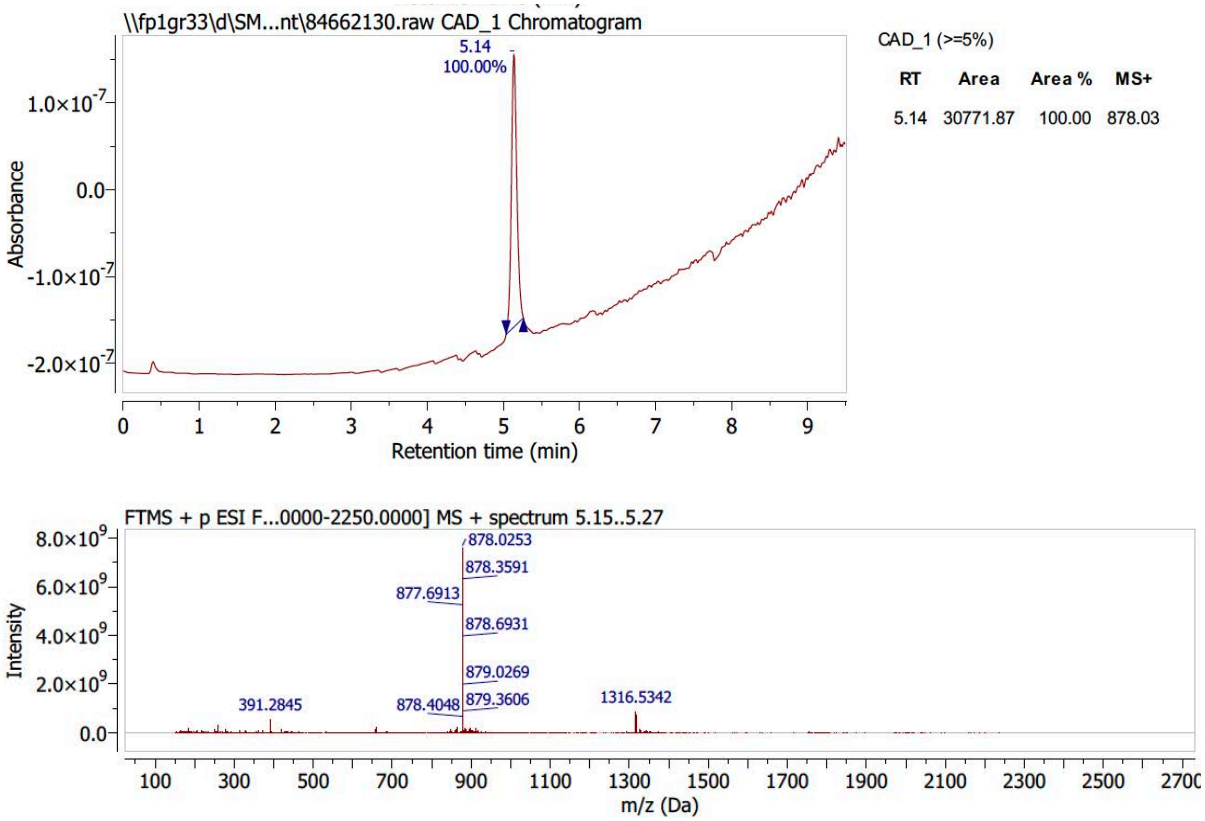


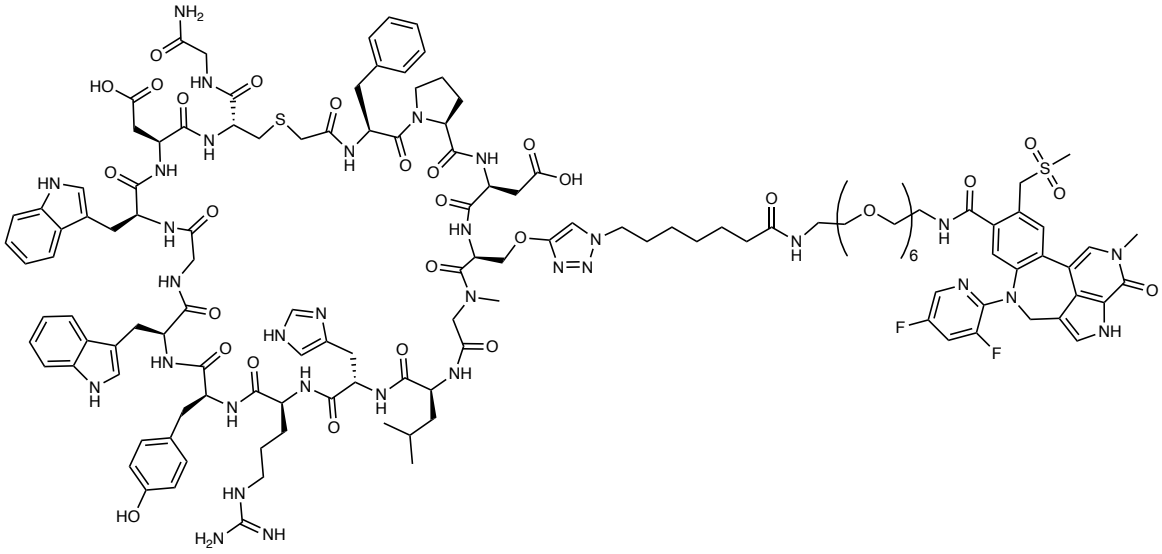
LC-MS data for compound (32) BETcide5 V4-P4 - G03409111

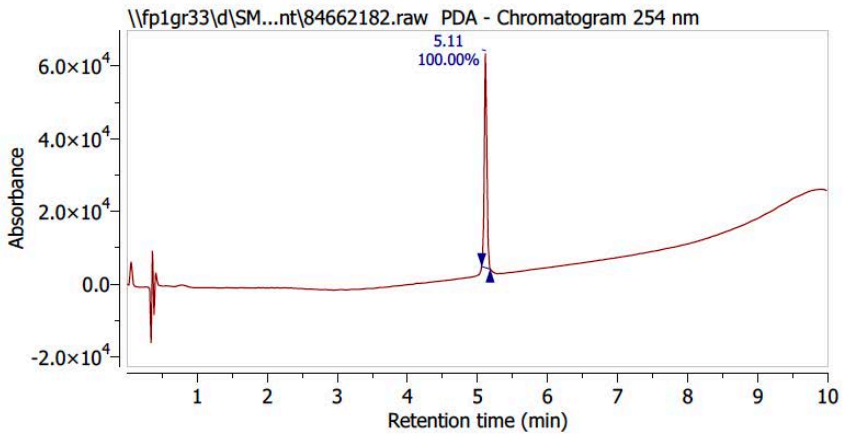


32




LC-MS data for compound (33) BETcide6 V4-C7P6 - G03409112






DAD, Sig=254 (>=1.5%)

| RT | Area | Area % | MS+ |
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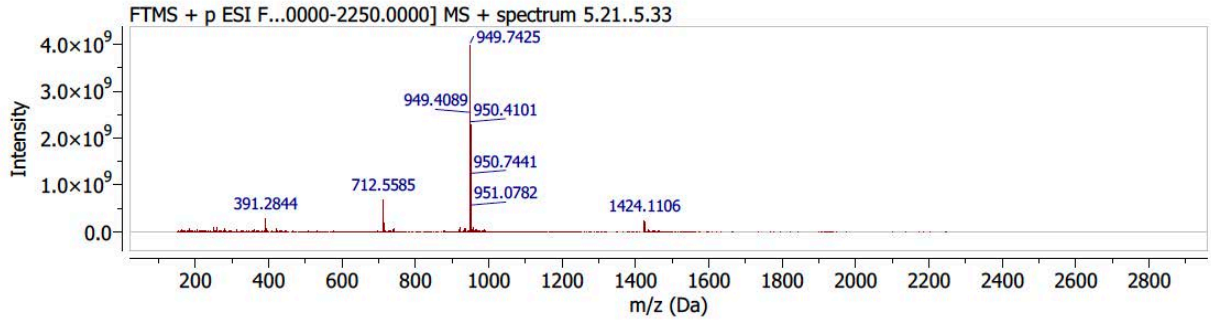
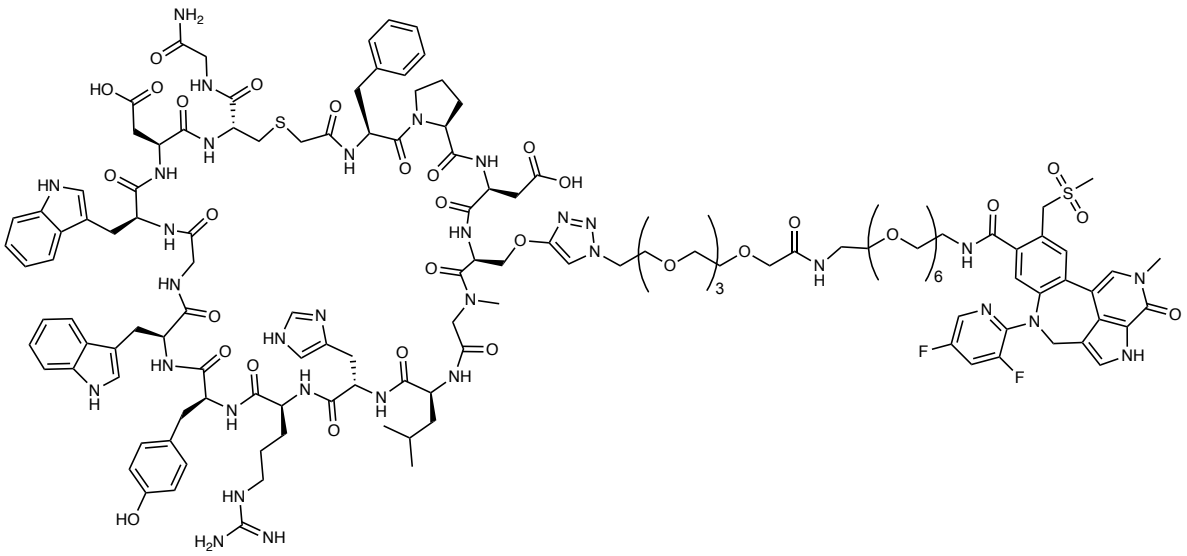
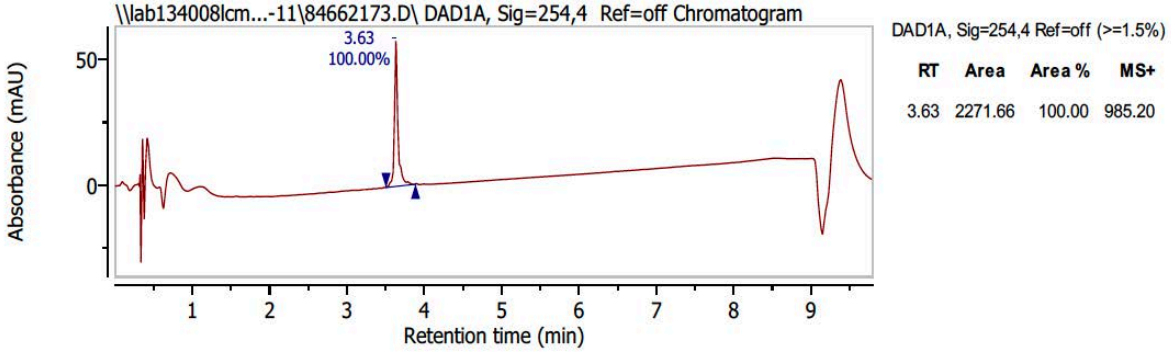


LC-MS data for compound (34) BETcide7 V4-P4P6 - G03409113

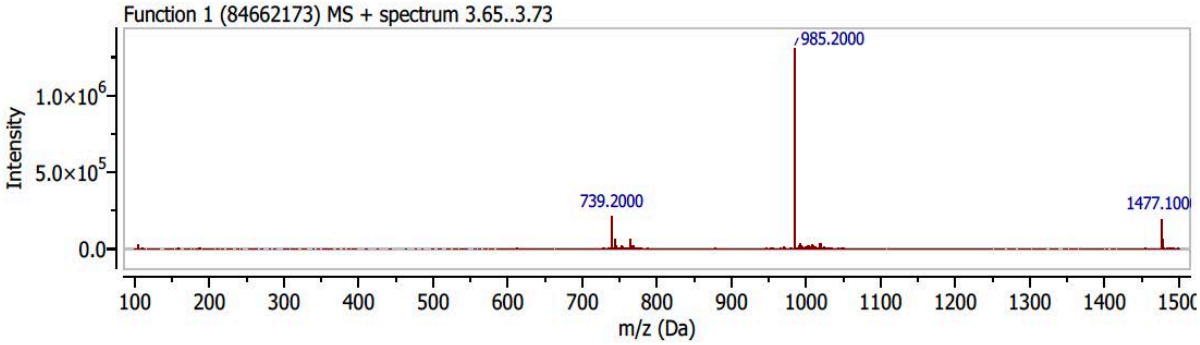


34

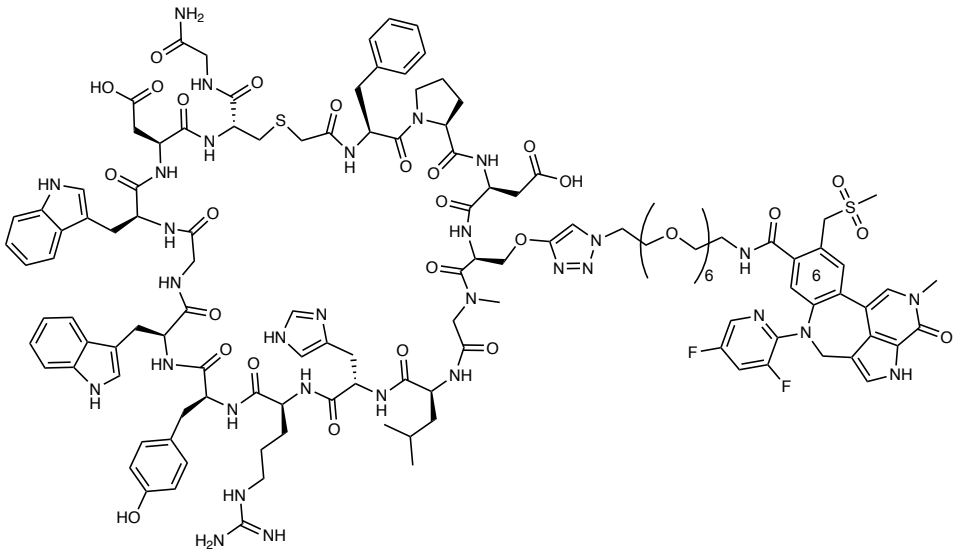



DAD1A, Sig=254,4 Ref=off (>=1.5%)

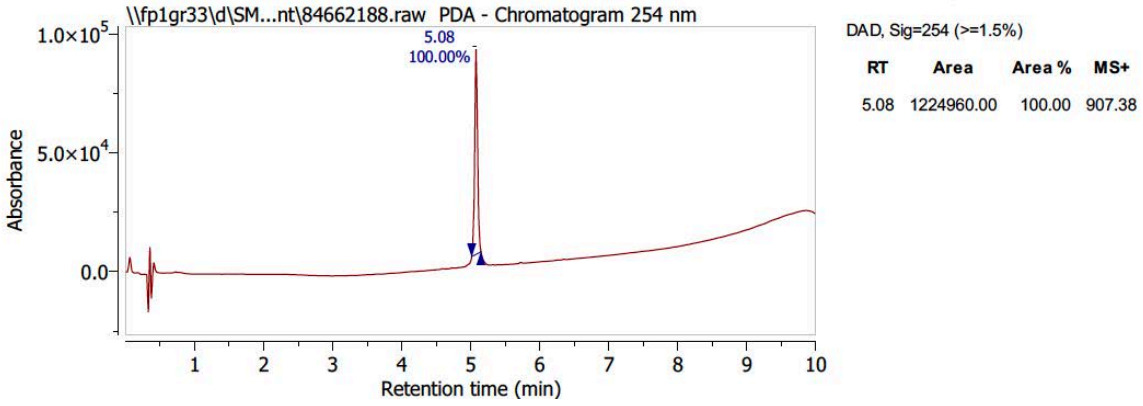
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 3.63 | 2271.66 | 100.00 | 985.20 |



LC-MS data for compound (35) BETcide8 V4-P6 - G03409115

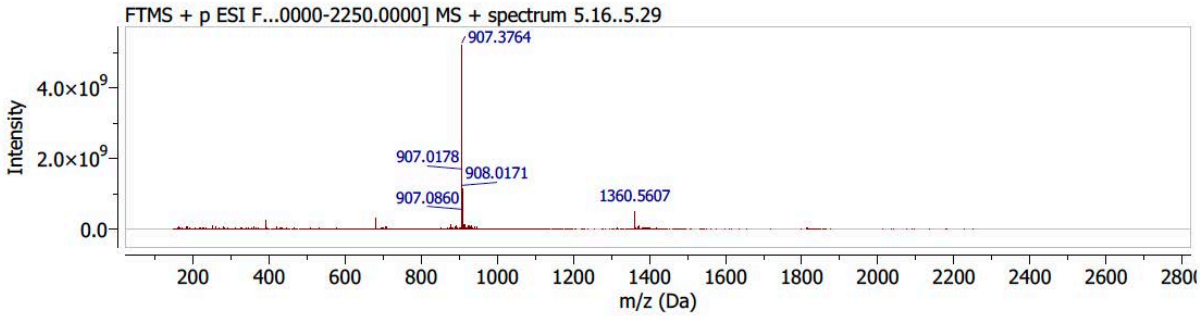


35

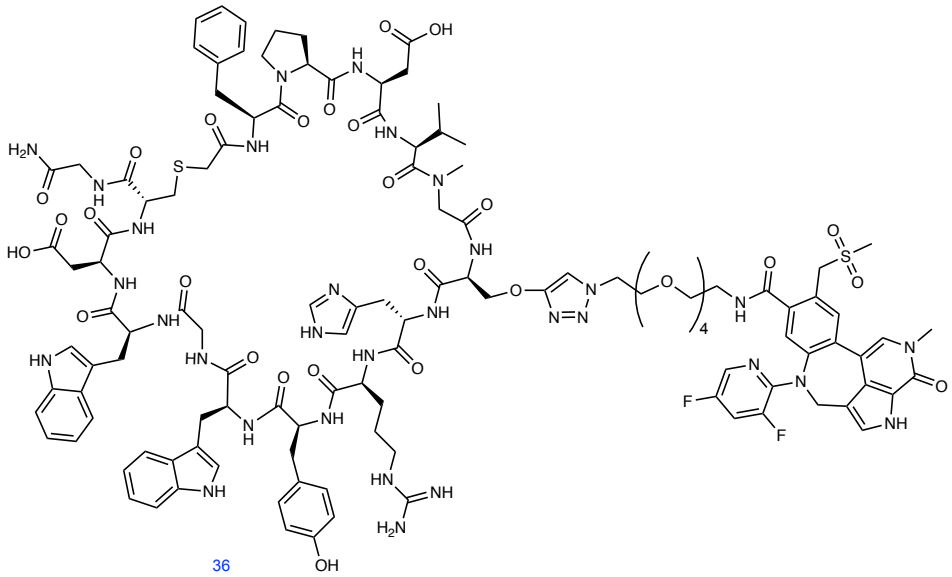



DAD, Sig=254 (>=1.5%)

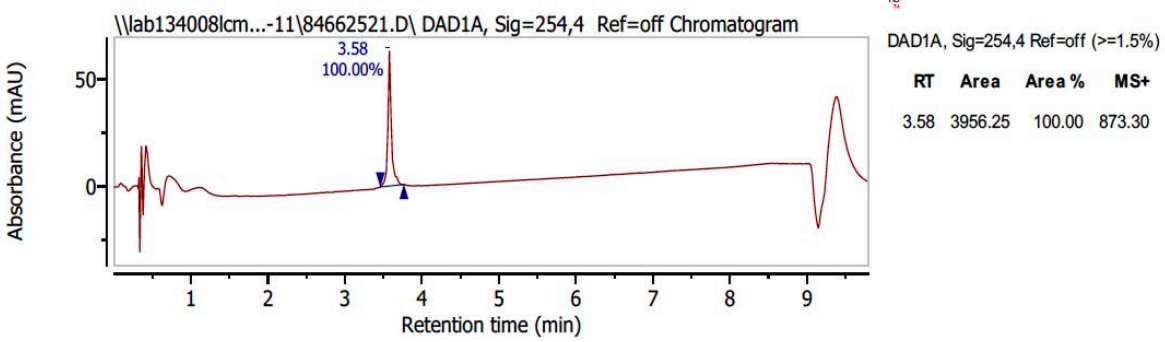
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 5.08 | 1224960.00 | 100.00 | 907.38 |



LC-MS data for compound (36) BETcide9 L6-P4 - G03409124

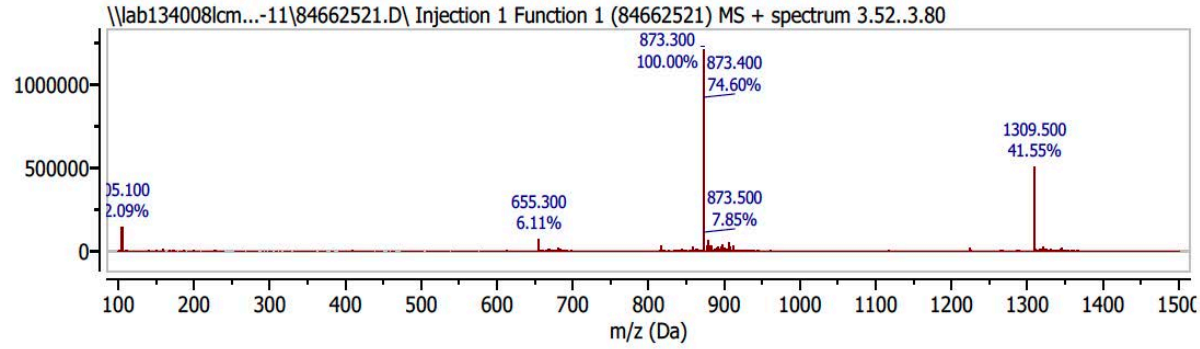


36

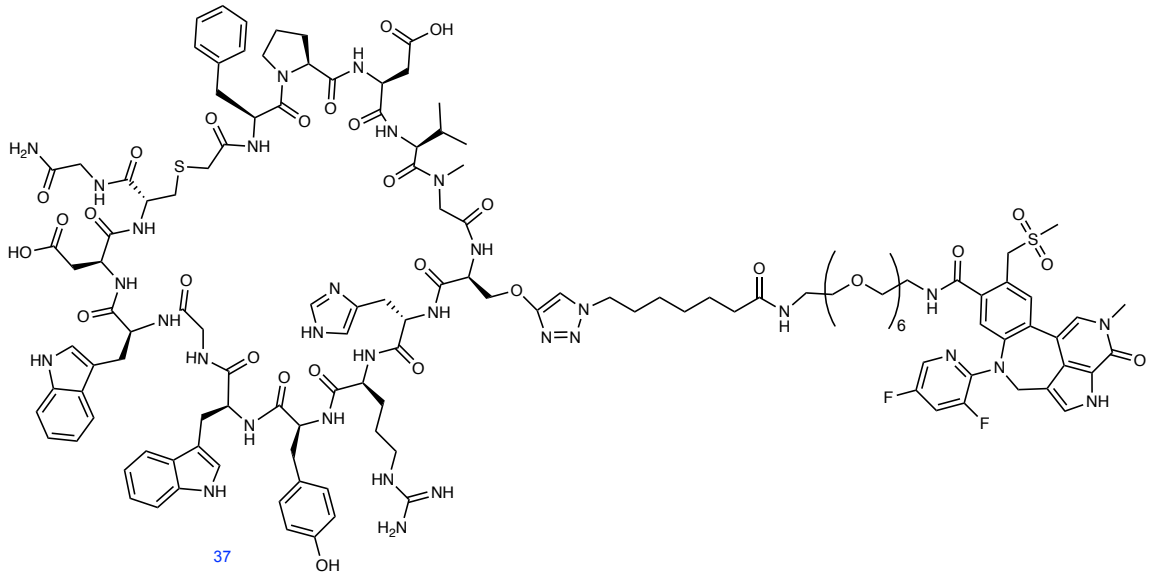



DAD1A, Sig=254,4 Ref=off (>=1.5%)

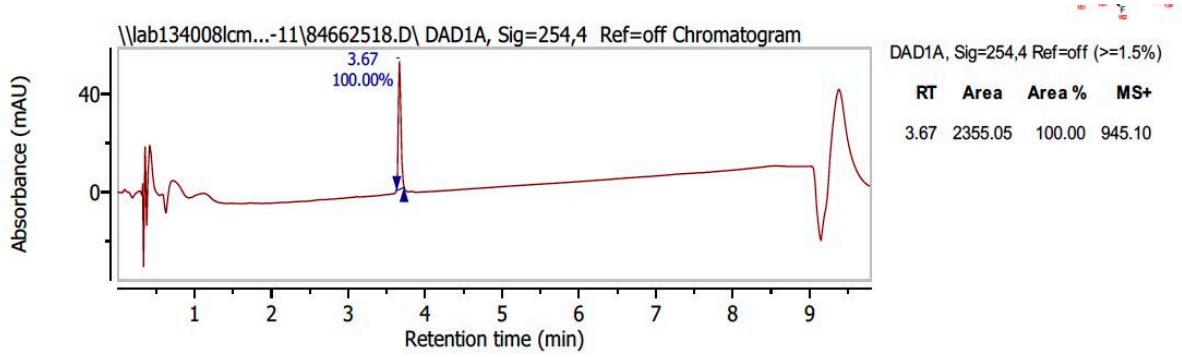
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 3.58 | 3956.25 | 100.00 | 873.30 |



LC-MS data for compound (37) BETcide10 L6-C7P6 - G03409125

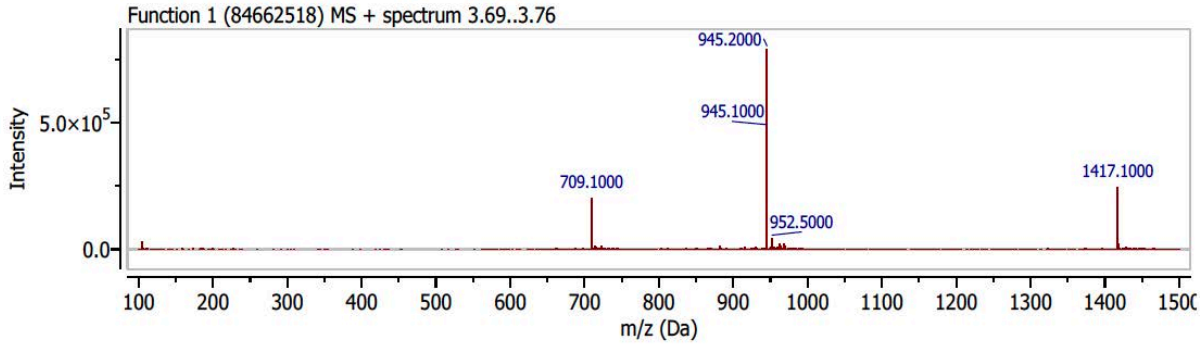


37

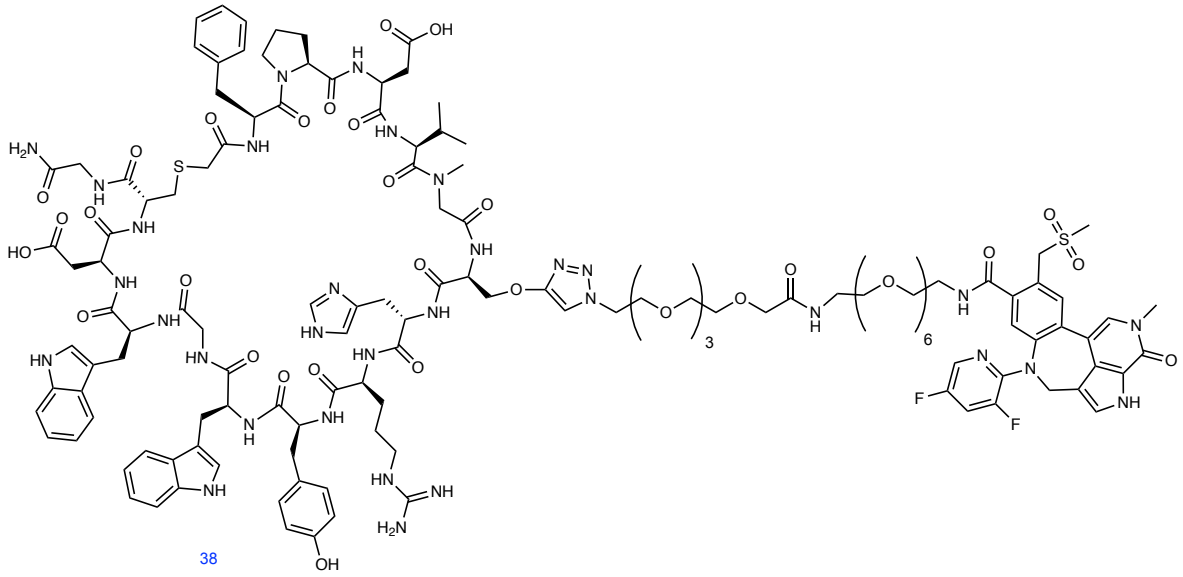



DAD1A, Sig=254,4 Ref=off (>=1.5%)

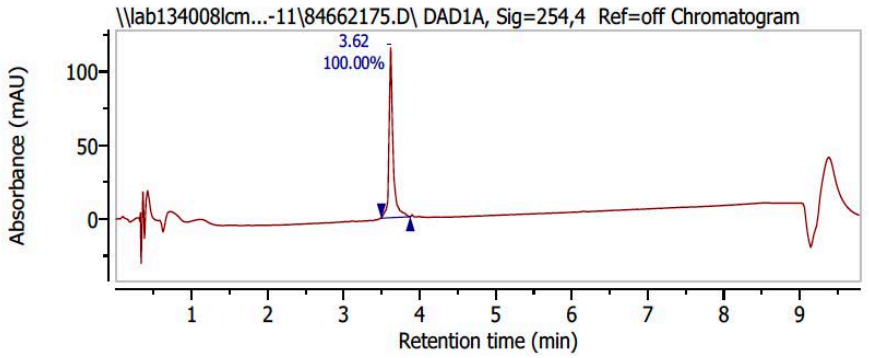
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 3.67 | 2355.05 | 100.00 | 945.10 |



LC-MS data for compound (38) BETcide11 L6-P4P6 - G03409126

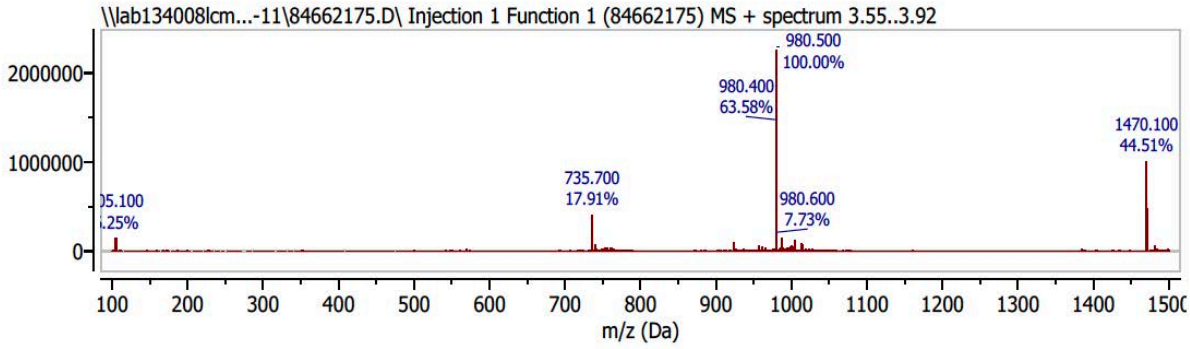


38

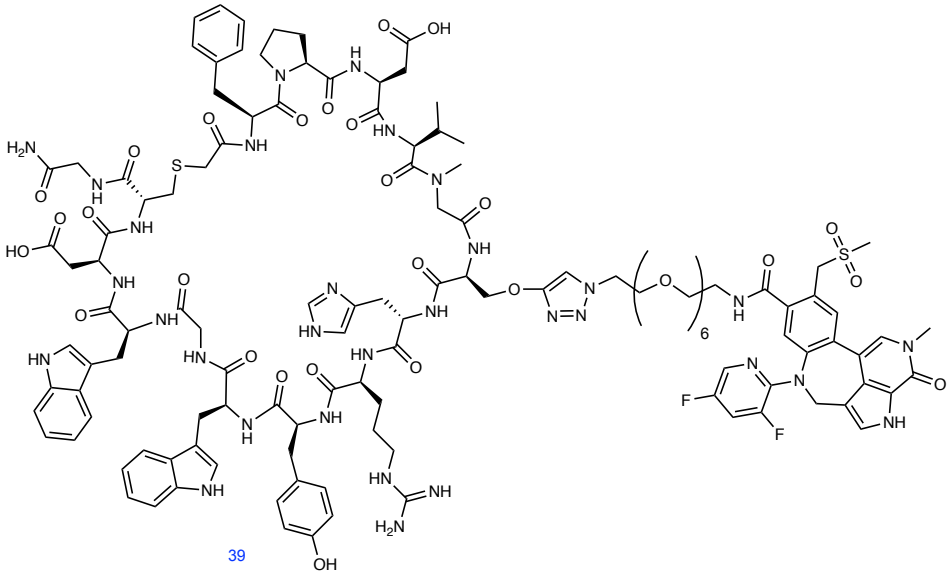



DAD1A, Sig=254,4 Ref=off (>=1.5%)

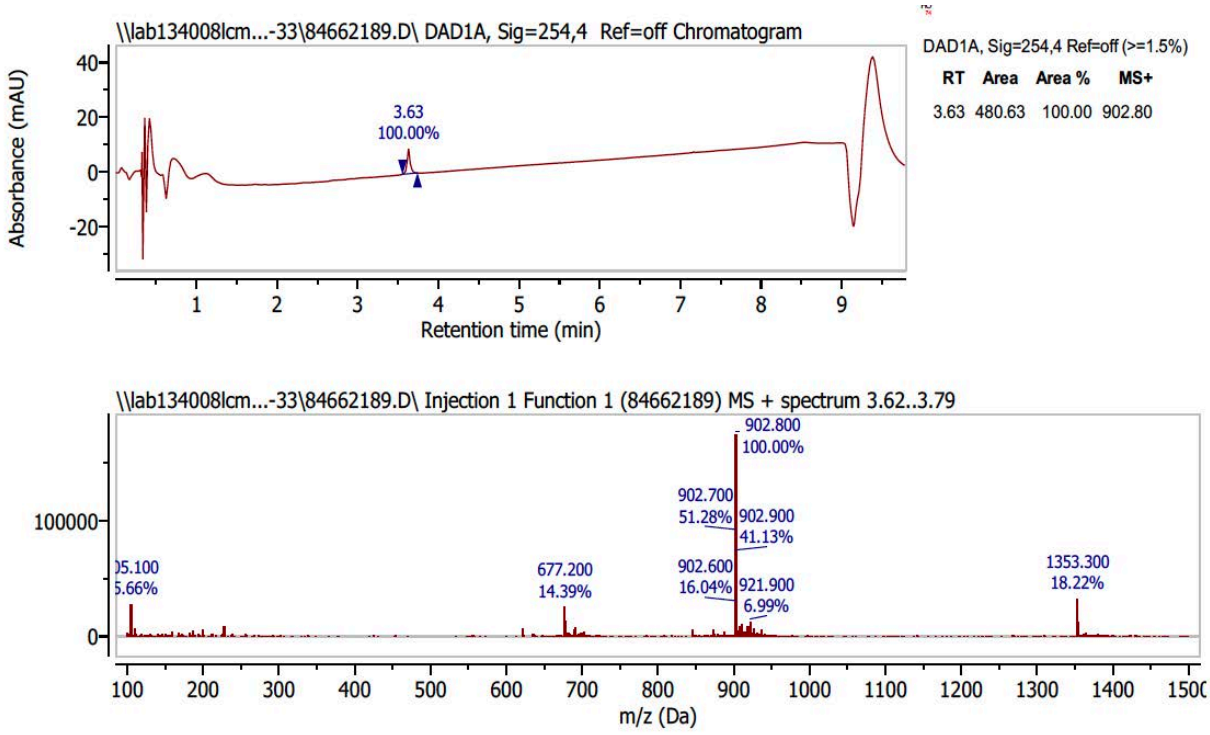
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 3.62 | 8293.42 | 100.00 | 980.50 |



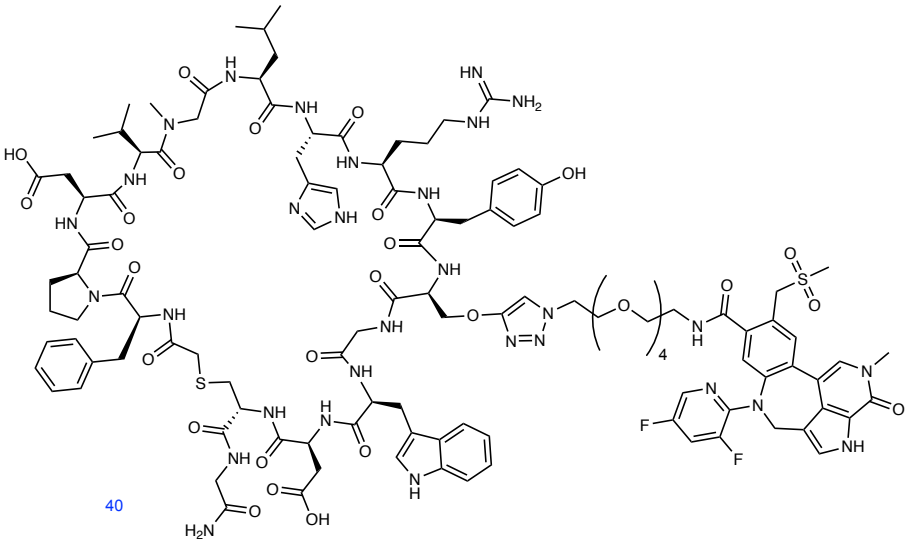

LC-MS data for compound (39) BETcide12 L6-P6 - G03409127



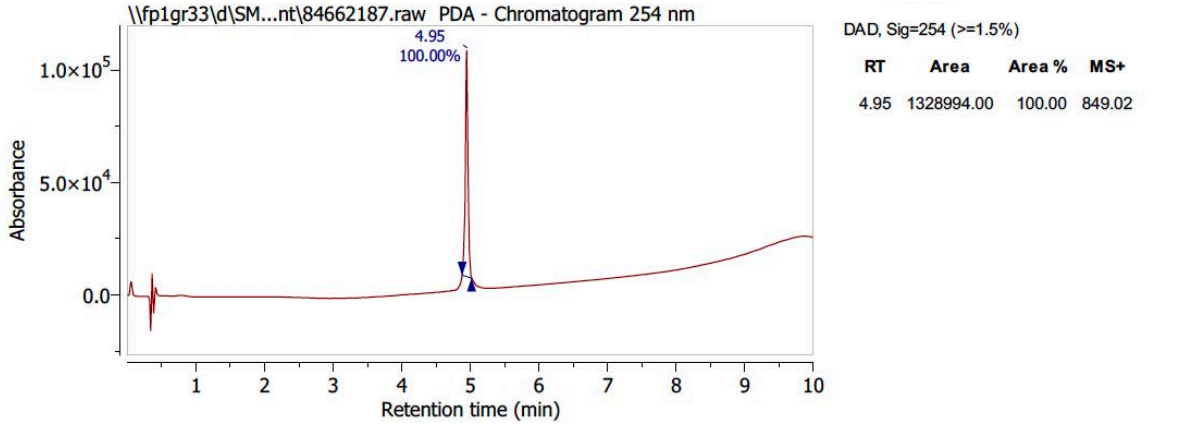
39

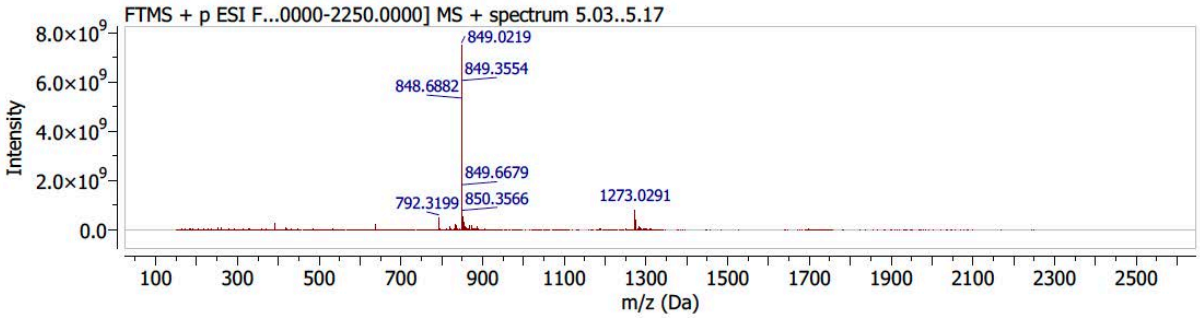



LC-MS data for compound (40) BETcide13 W10-P4 - G03409128

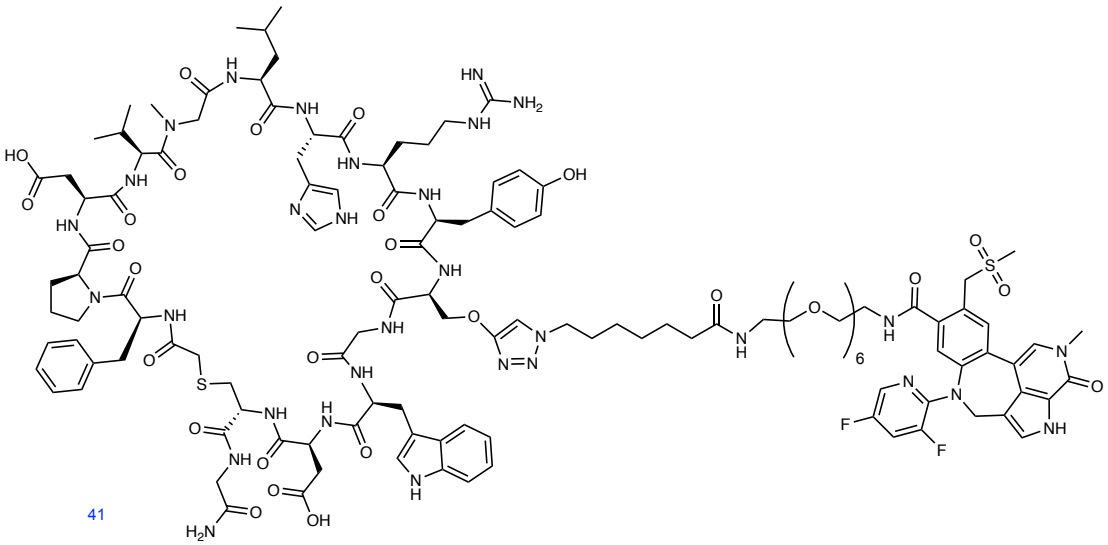


40

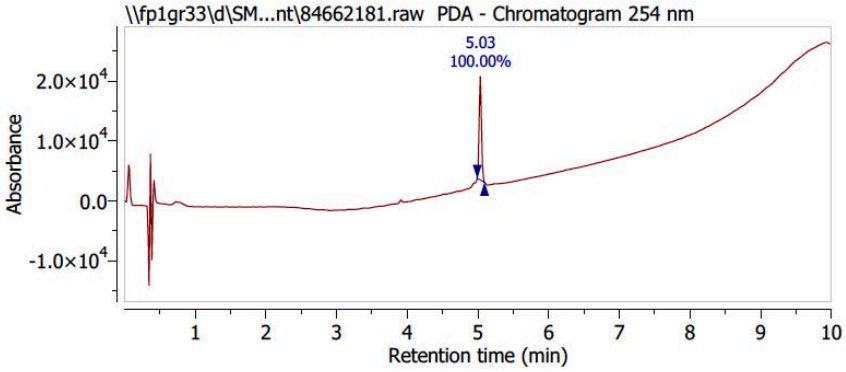



LC-MS data for compound (41) BETcide14 W10-C7P6 - G03409129

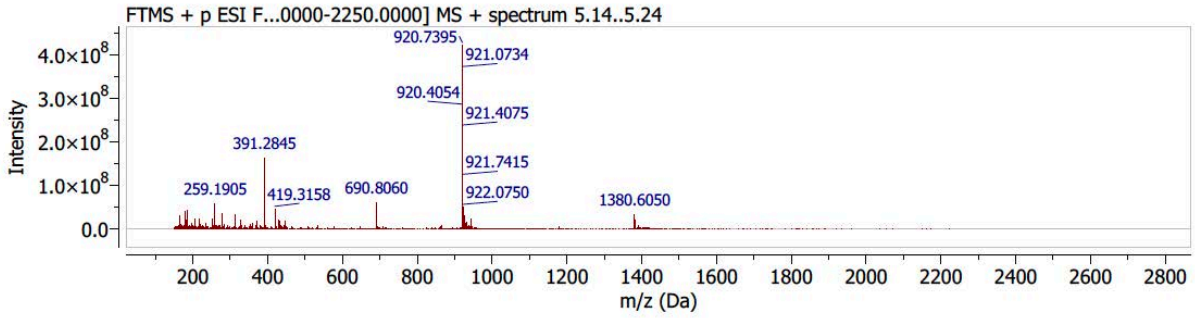


41

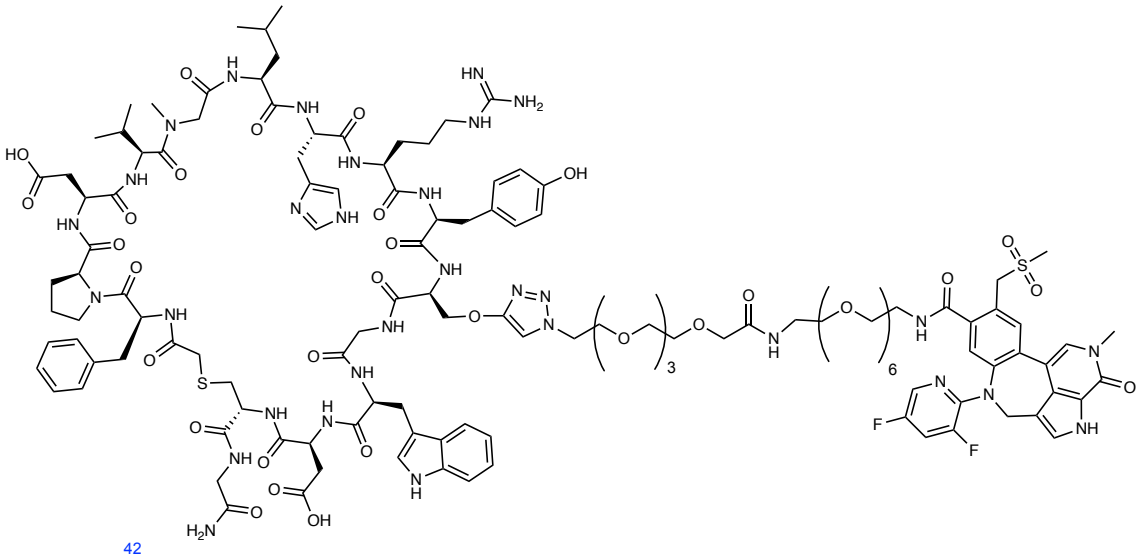



DAD, Sig=254 (>=1.5%)

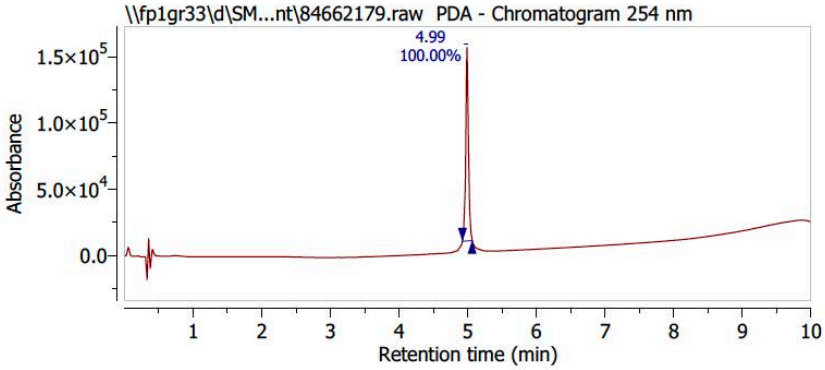
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 5.03 | 195567.00 | 100.00 | 920.74 |



LC-MS data for compound (42) BETcide15 W10-P4P6 - G03409130

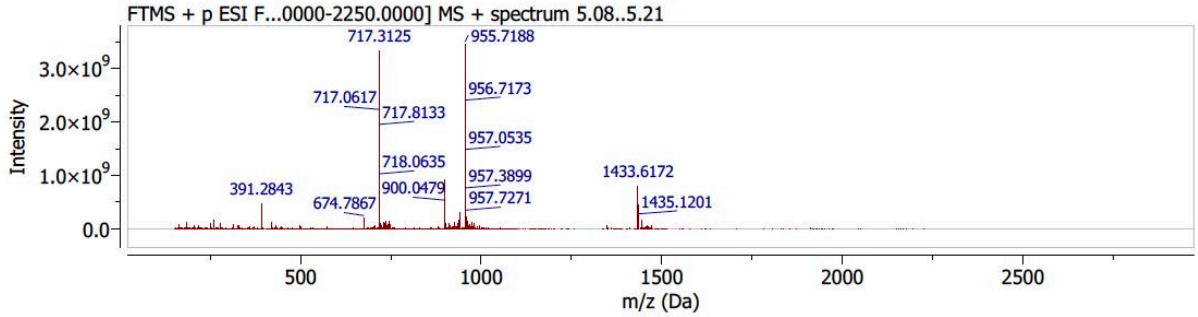


42

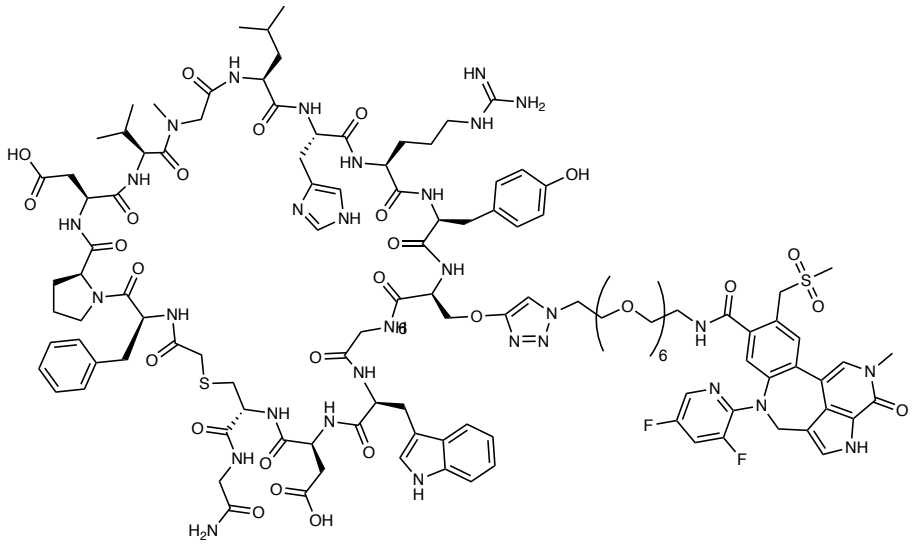



DAD, Sig=254 (>=1.5%)

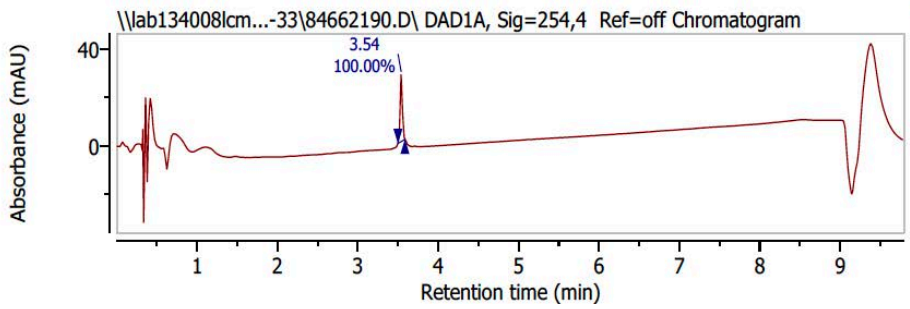
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 4.99 | 1997127.50 | 100.00 | 956.08 |



LC-MS data for compound (43) BETcide16 W10-P6 - G03409131

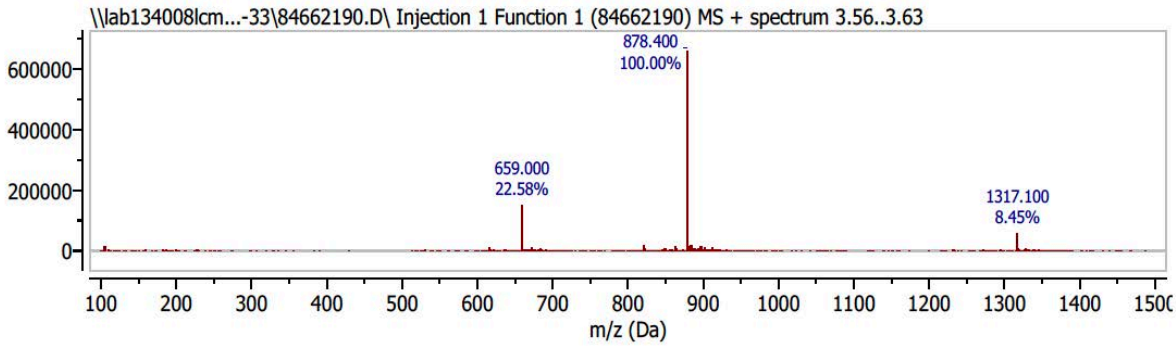


43

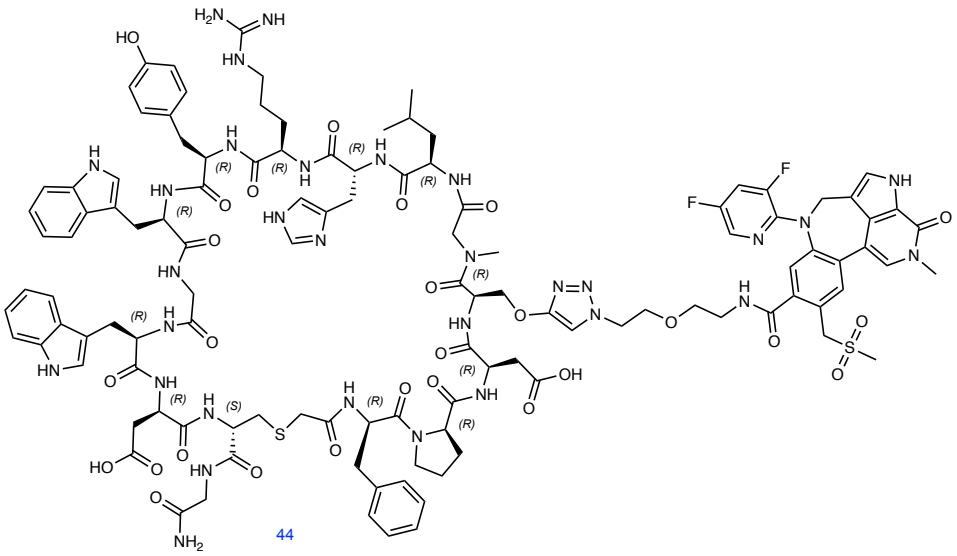



DAD1A, Sig=254,4 Ref=off (>=1.5%)

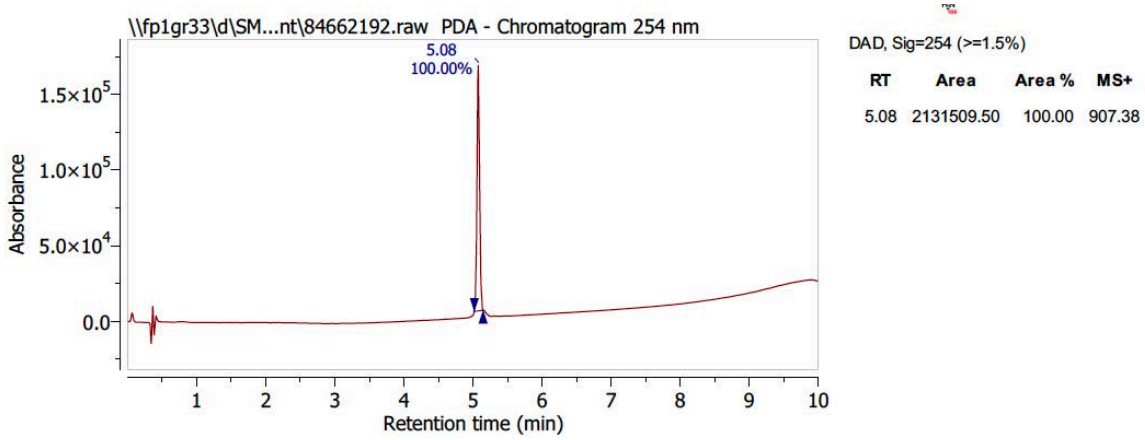
| RT | Area | Area % | MS+ |
|---|---|---|---|
| 3.54 | 1096.57 | 100.00 | 878.40 |



LC-MS data for compound (44) D-BETcide8 V4-P6 - G03438264

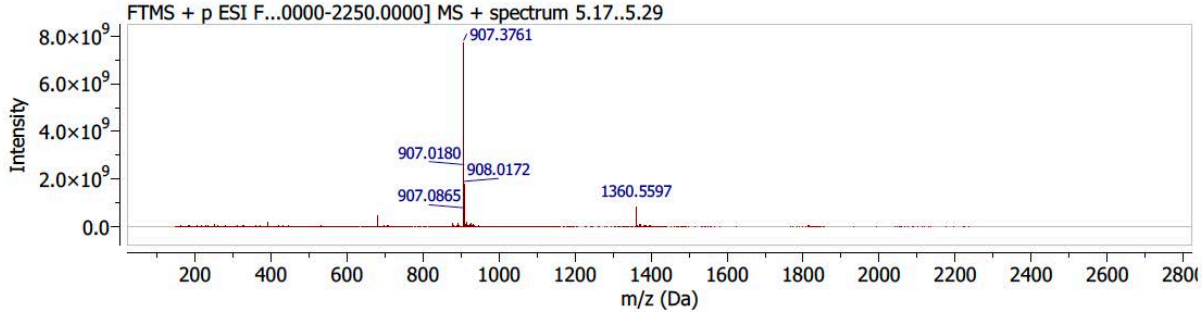


44




DAD, Sig=254 (>=1.5%)

| RT | Area | Area % | MS+ |
|---|---|---|---|
| 5.08 | 2131509.50 | 100.00 | 907.38 |

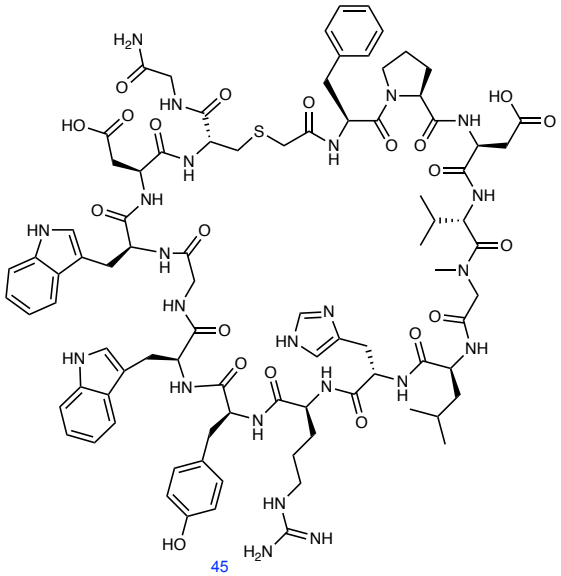


2. Chemical structures and LC-Ms Data for Biotin Conjugates

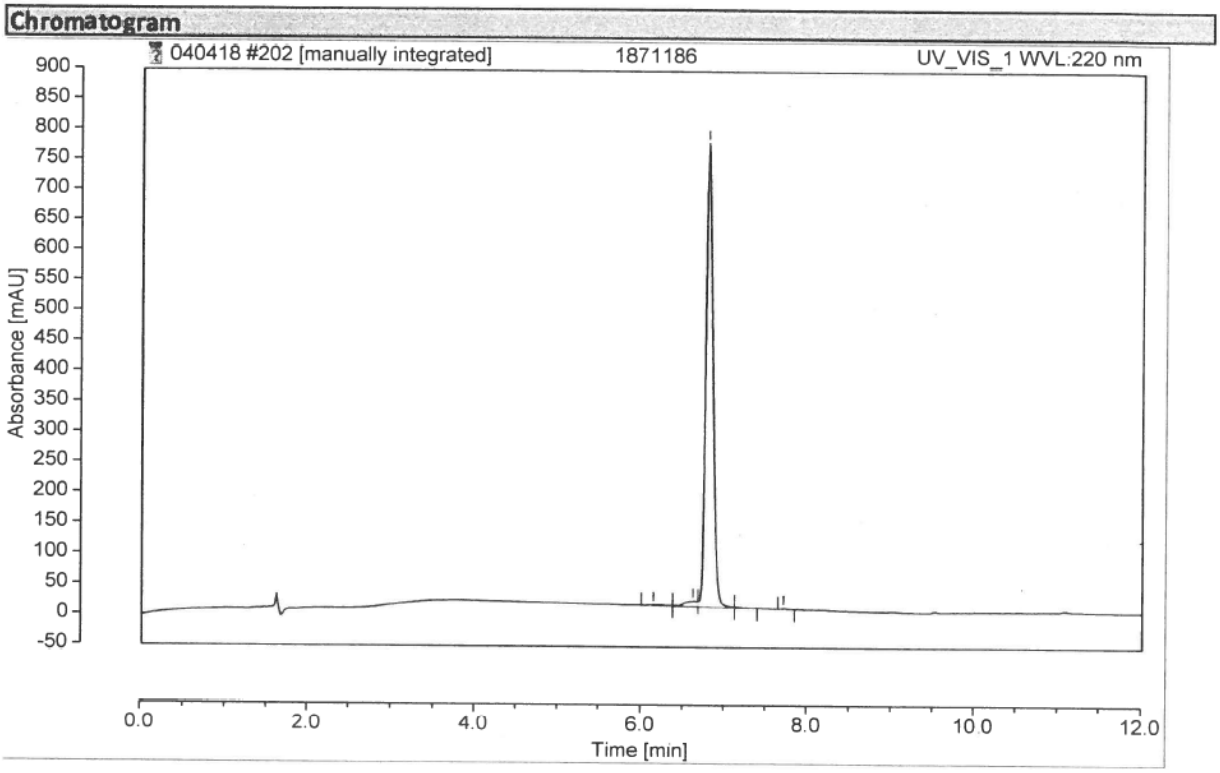
**Table (3)** Representation of Macrocyclic Peptides and their respective Biotin conjugates showing Abbreviations of Compound Names

| Peptides used in this study | | | | | |
|---|---|---|---|---|---|
| **peptide name** | **Peptide Sequence** | **nickname** | **peptide type** | **c-term modification** | **amino acid stereochemistry** |
| PSMD2-MC1 | cyc(Ac) FPDV(Sar)LHRYWGWDCG amide | MC1 | macrocycle | - | L |
| PSMD2-MC2 | cyc(Ac) FSDKPLHRYVGFQCG amide | MC2 | macrocycle | - | L |
| PSMD2-MC3 | cyc(Ac) FSNWPSWLHYLCG amide | MC3 | macrocycle | - | L |
| PSMD2-pp1 | Ac SHHSQVLAFAR amide | PP1 | linear peptide | - | L |
| PSMD2-$MC1_{biotin}$ | cyc(Ac) FPDV(Sar)LHRYWGWDCG(Peg3)(K(B)) amide | $MC1_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-$MC2_{biotin}$ | cyc(Ac) FSDKPLHRYVGFQCG(Peg3)(K(B)) amide | $MC2_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-$MC3_{biotin}$ | cyc(Ac) FSNWPSWLHYLCG(Peg3)(K(B)) amide | $MC3_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-$PP1_{biotin}$ | Ac SHHSQVLAFARG(Peg3)(K(B)) amide | $PP1_{biotin}$ | macrocycle | PEG3-Lys biotin | L |
| PSMD2-D-$MC_{biotin}$ | cyc(Ac) fpdv(Sar)lhrywGwdcG(Peg3)(K(B)) amide | D-$MC1_{biotin}$ | macrocycle | PEG3-Lys biotin | D |

LC-MS data for compound (45) PSMD2-MC1

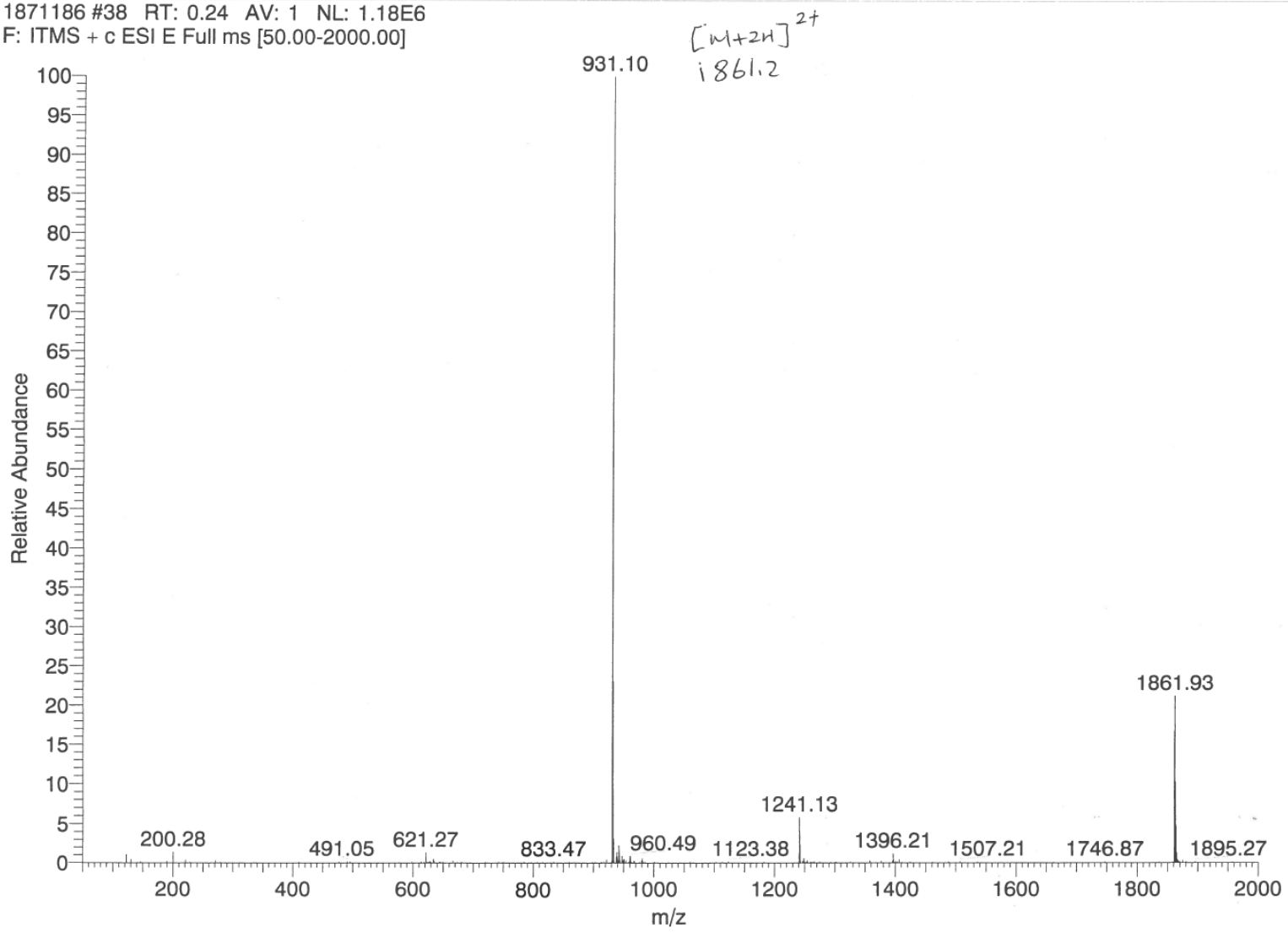


45

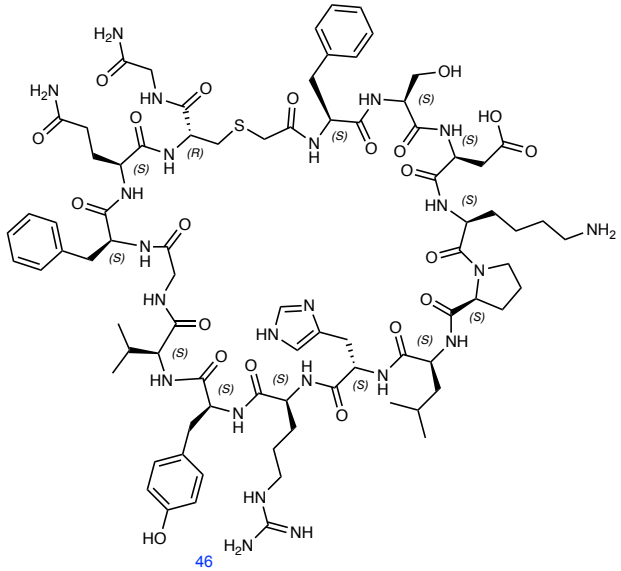

Chromatogram
040418 #202 [manually integrated]
1871186
UV_VIS_1 WVL:220 nm
Absorbance [mAU]
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Time [min]

C:\Xcalibur\Data\1871186 04/13/18 09:46:12

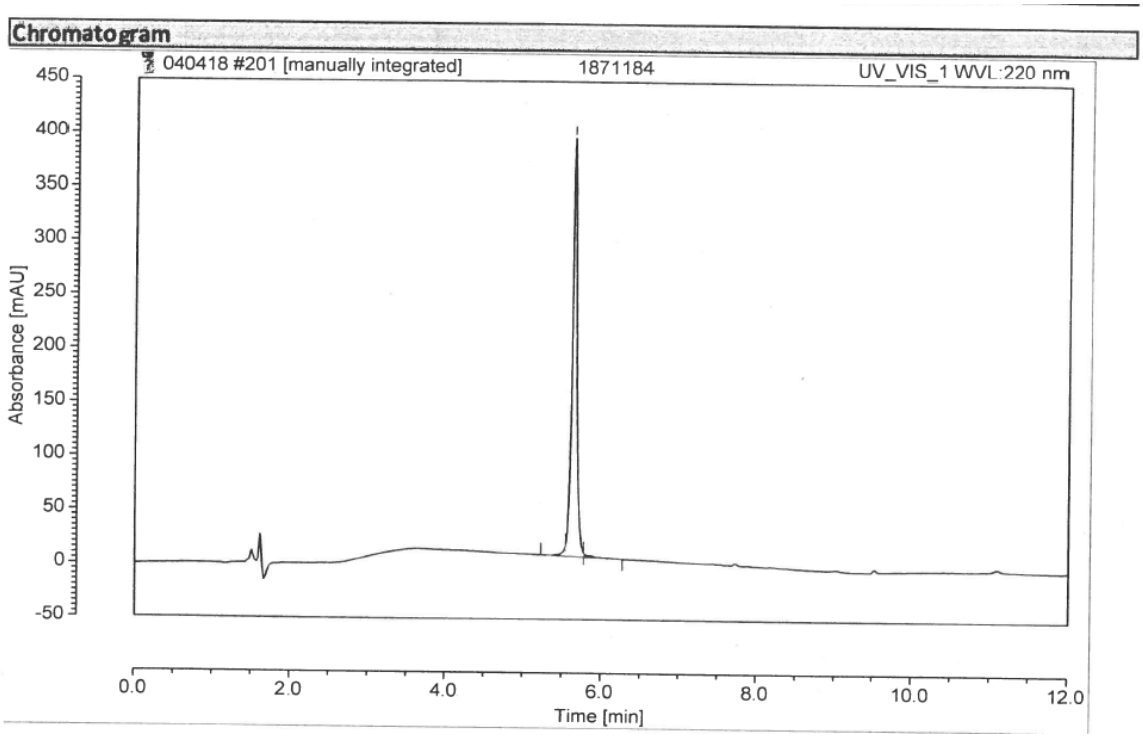
1871186 #38 RT: 0.24 AV: 1 NL: 1.18E6
F: ITMS + c ESI E Full ms [50.00-2000.00]



LC-MS data for compound (46) PSMD2-MC2

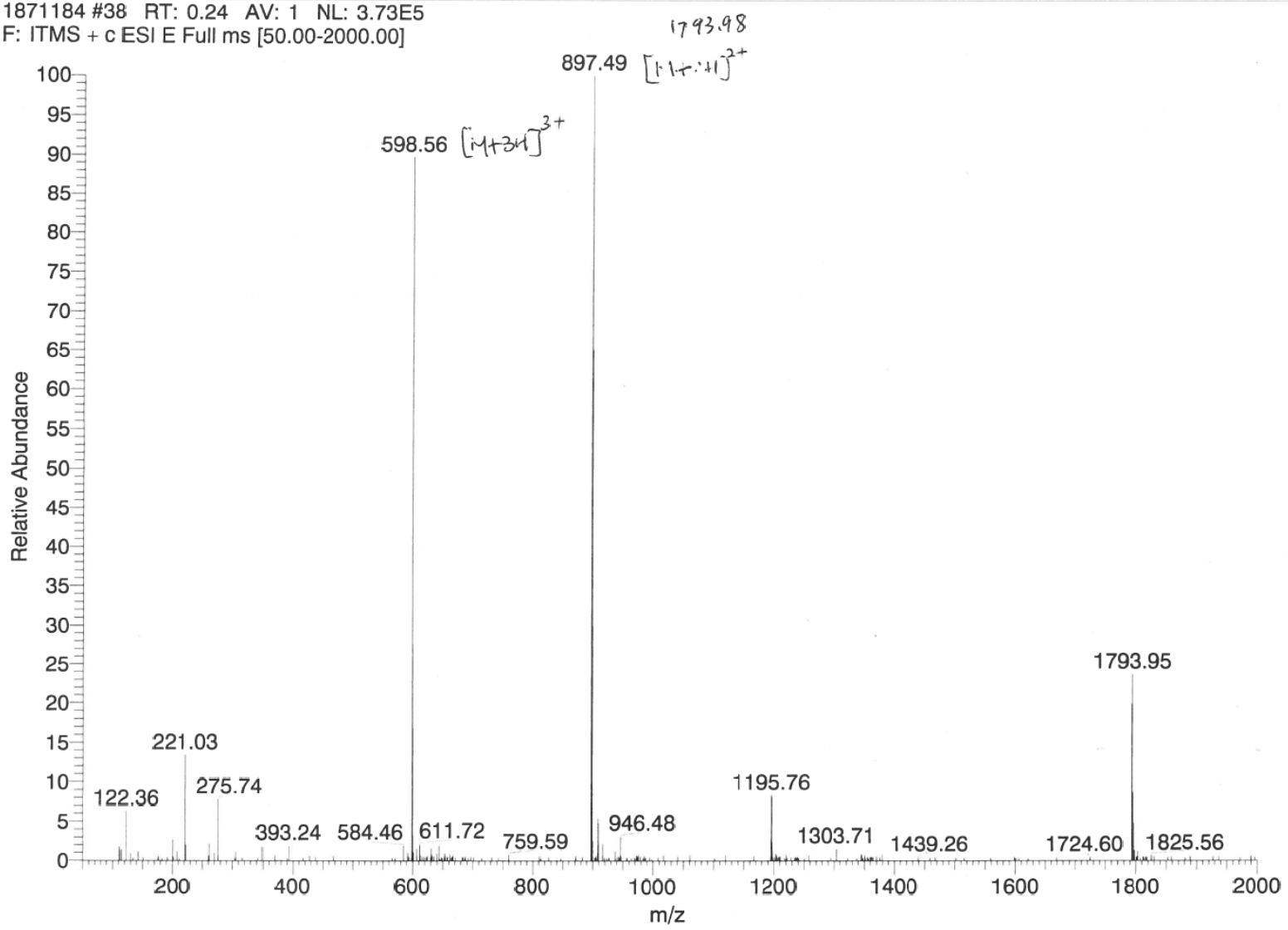


46

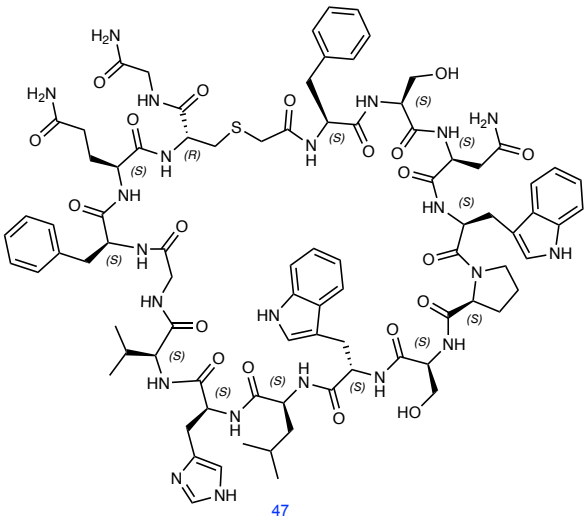


C:\Xcalibur\Data\1871184 04/13/18 09:43:17

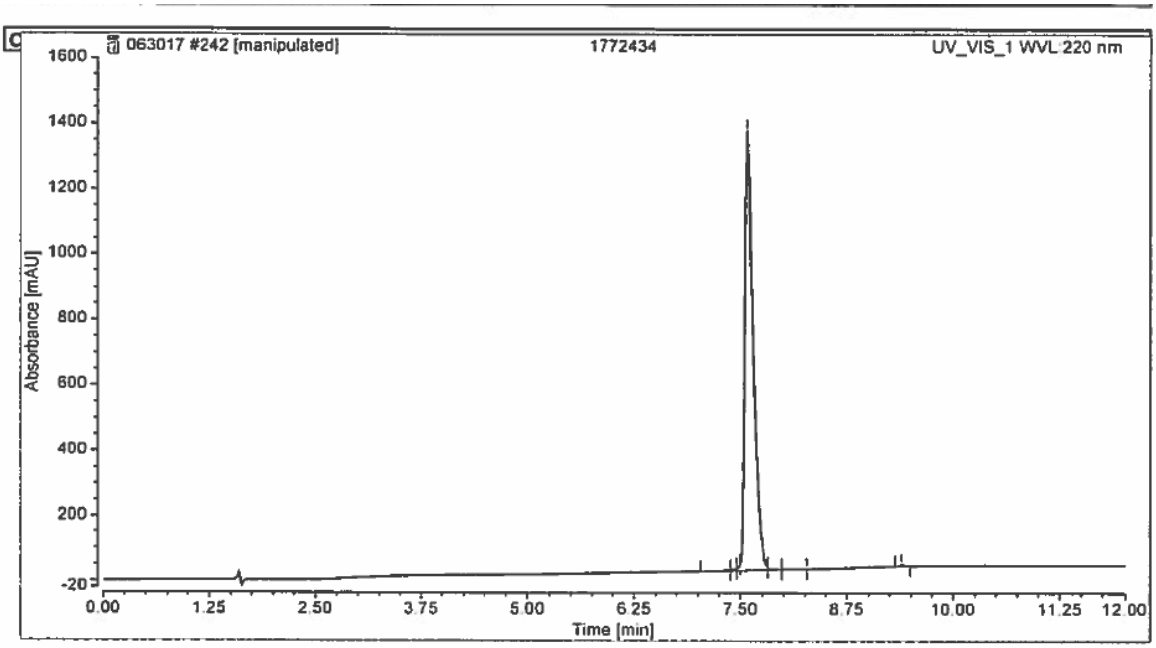
1871184 #38 RT: 0.24 AV: 1 NL: 3.73E5
F: ITMS + c ESI E Full ms [50.00-2000.00]



LC-MS data for compound (47) PSMD2-MC3



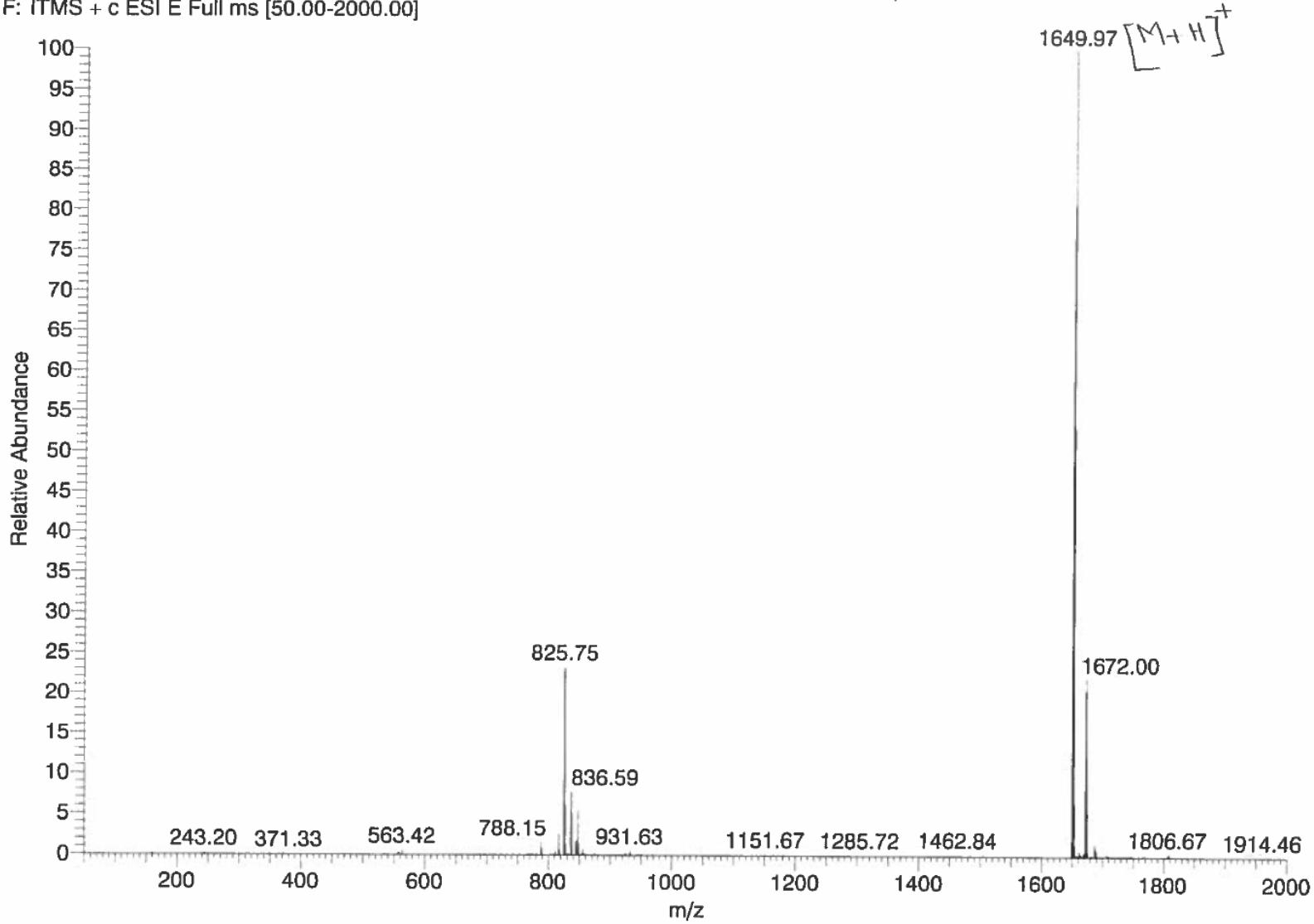
47



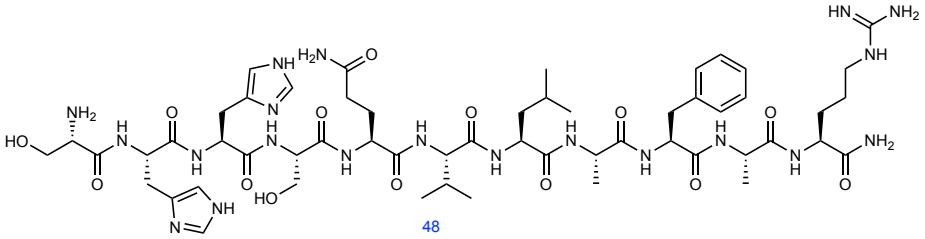
C:\Xcalibur\data\1772434_170721143208 7/21/2017 2:32:08 PM

07/21/17 ✍

1772434_170721143208 #27-55 RT: 0.18-0.33 AV: 9 NL: 3.47E6
F: ITMS + c ESI E Full ms [50.00-2000.00]

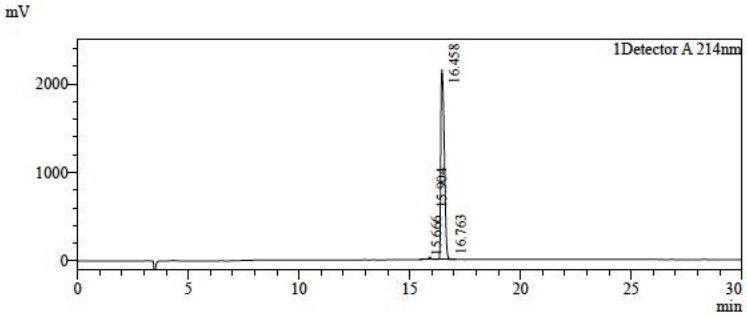


LC-MS data for compound (48) PSMD2-pp1

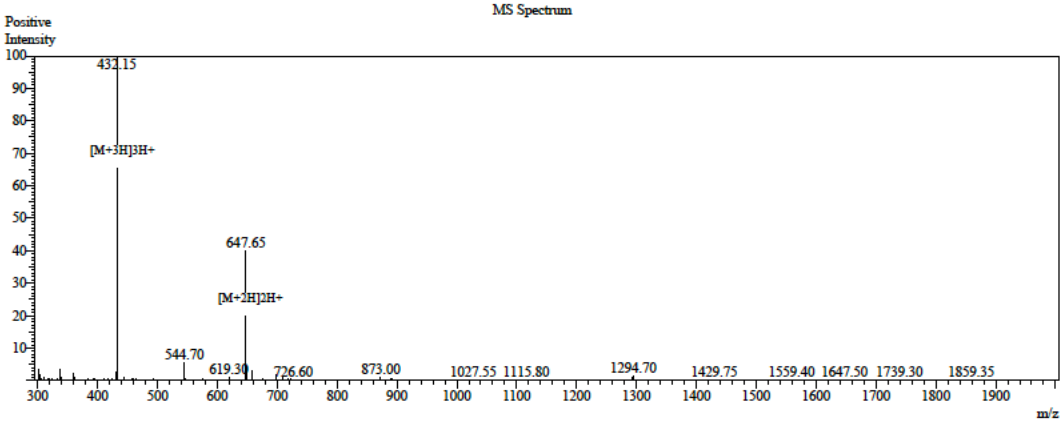


48

Chromatogram

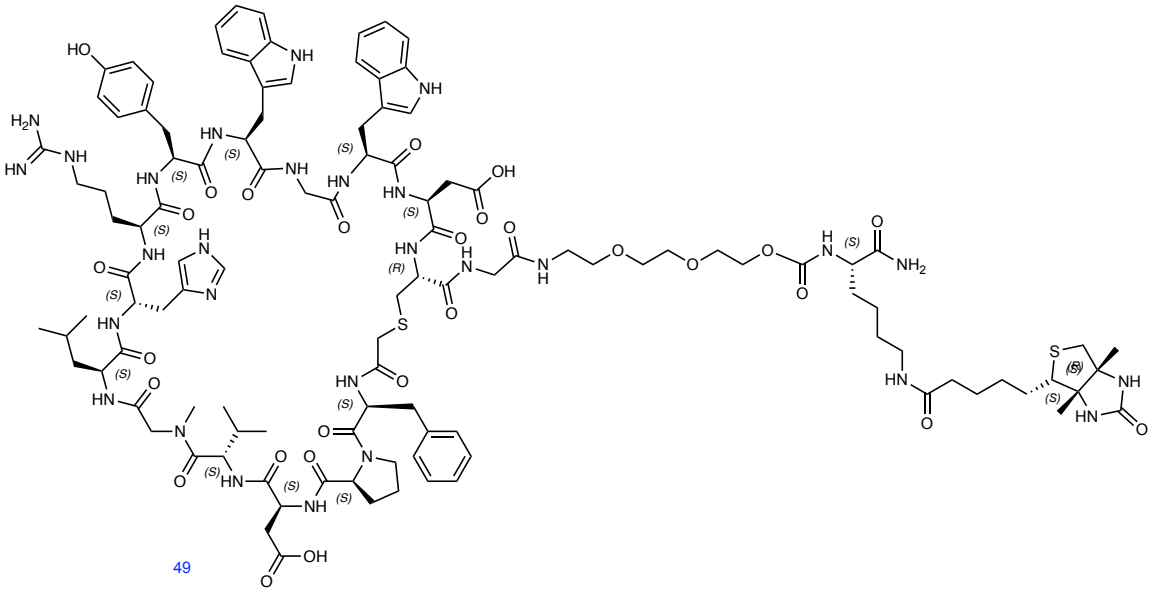



MS Spectrum

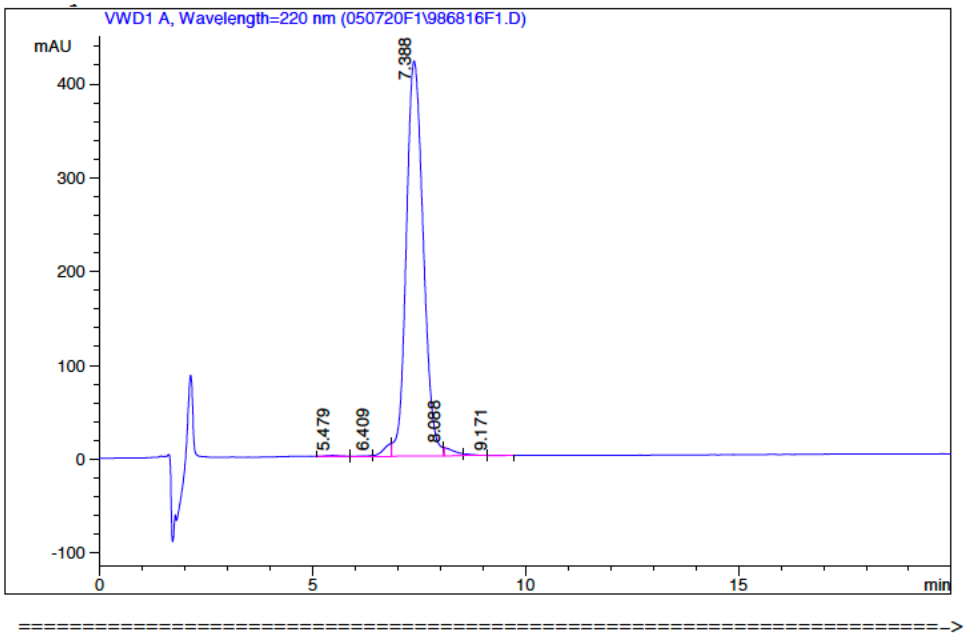



Sample Information

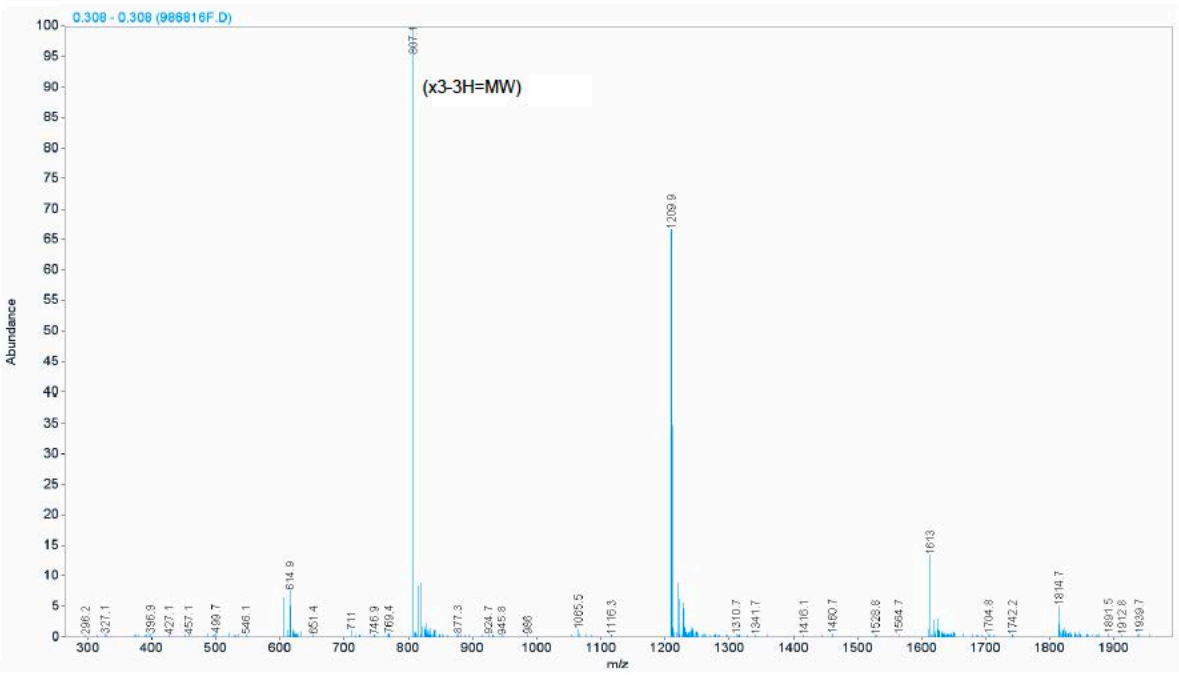
LC-MS data for compound (49) PSMD2-MC1biotin



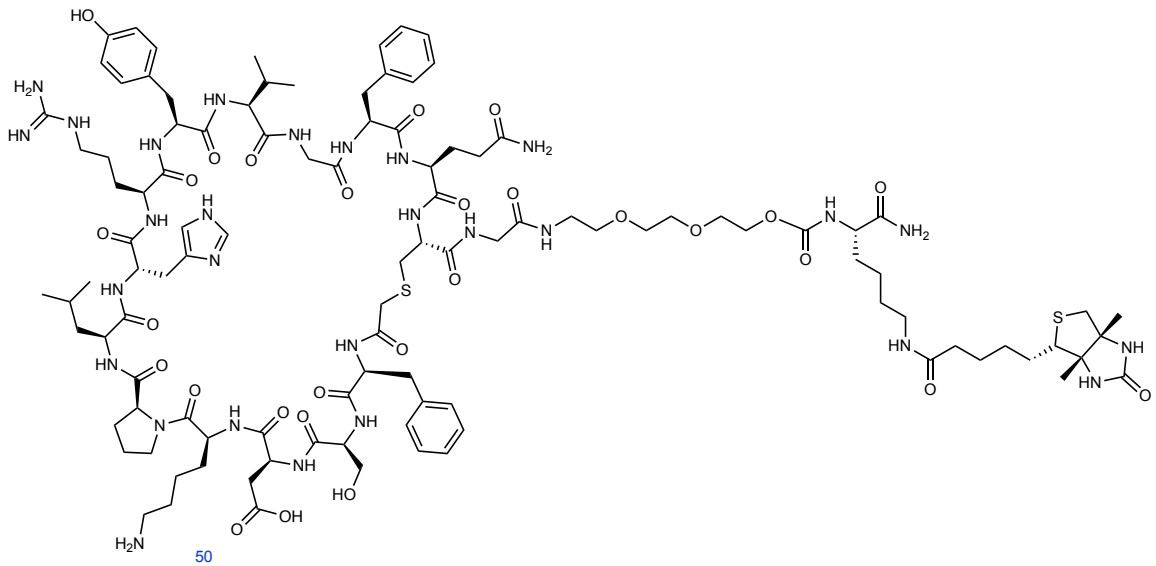
49



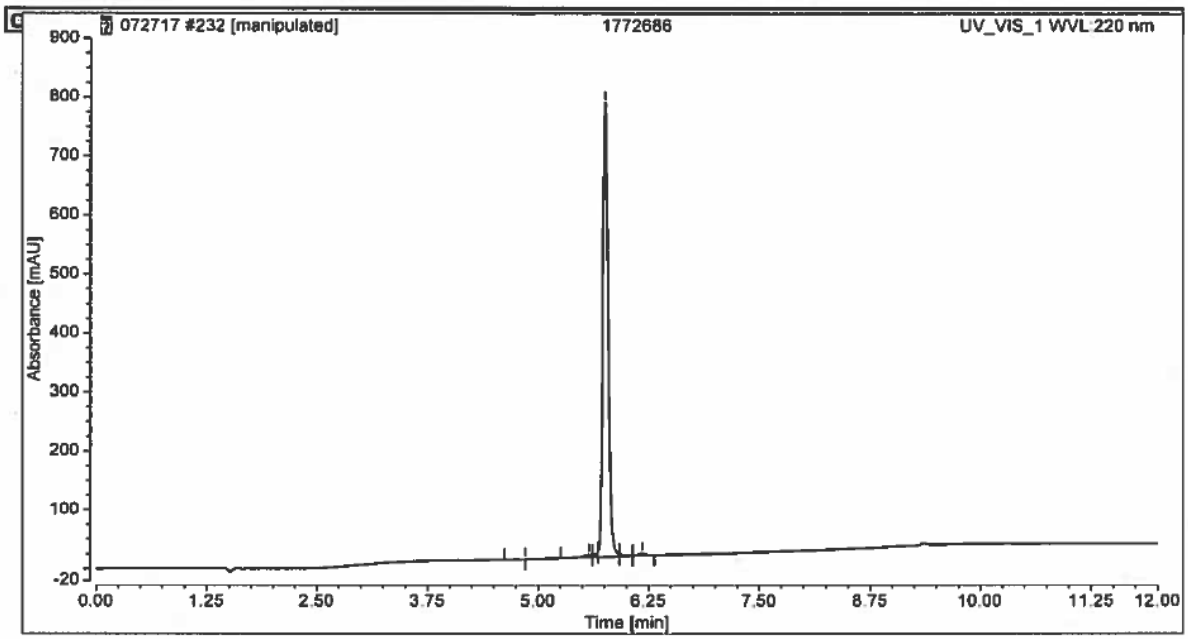

Signal 1:VWD1 A, Wavelength=220 nm




LC-MS data for compound (50) PSMD2-MC2biotin



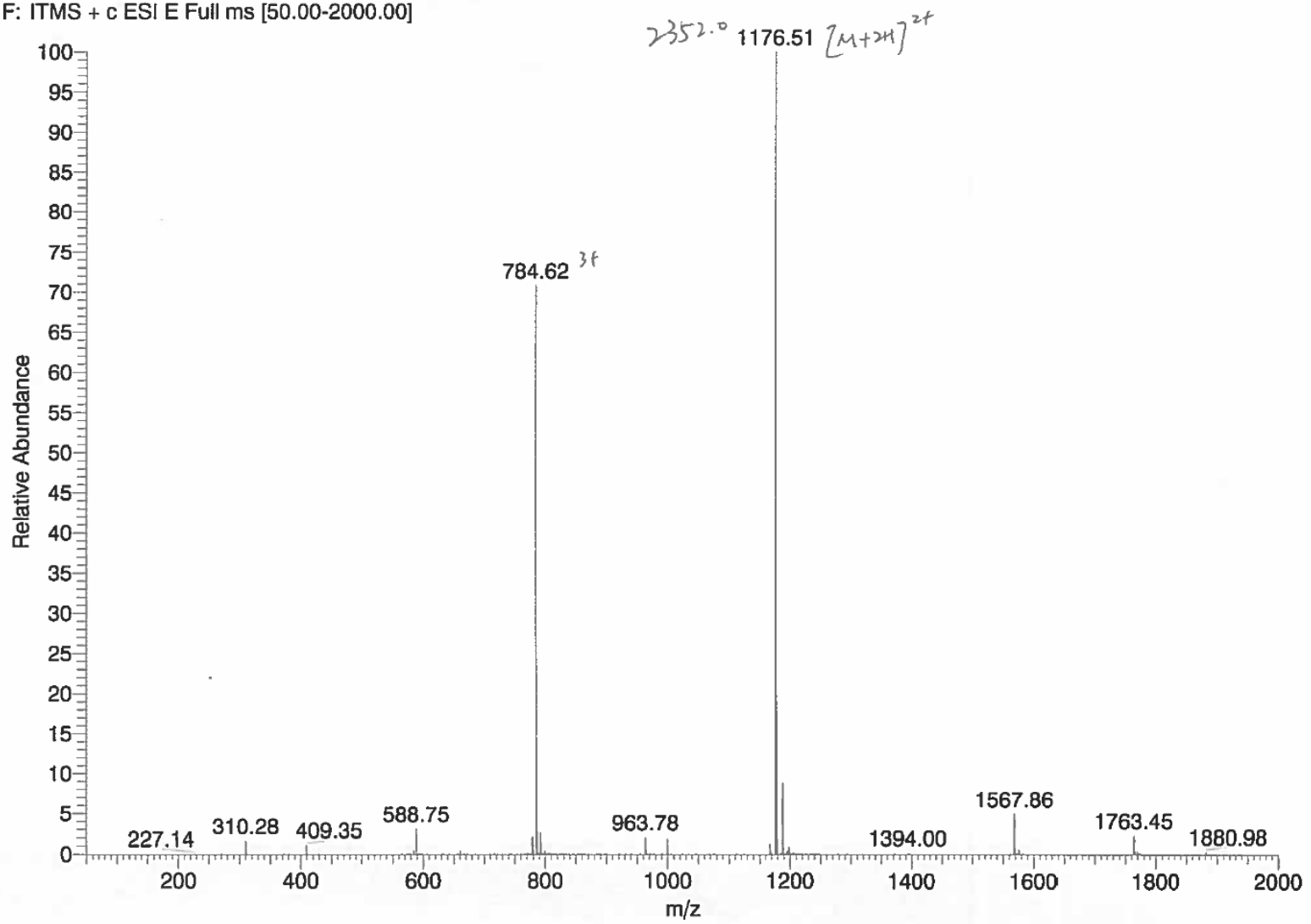
50


072717 #232 [manipulated]
1772686
UV_VIS_1 WVL:220 nm
Absorbance [mAU]
900
800
700
600
500
400
300
200
100
-20
0.00
1.25
2.50
3.75
5.00
6.25
7.50
8.75
10.00
11.25
12.00
Time [min]

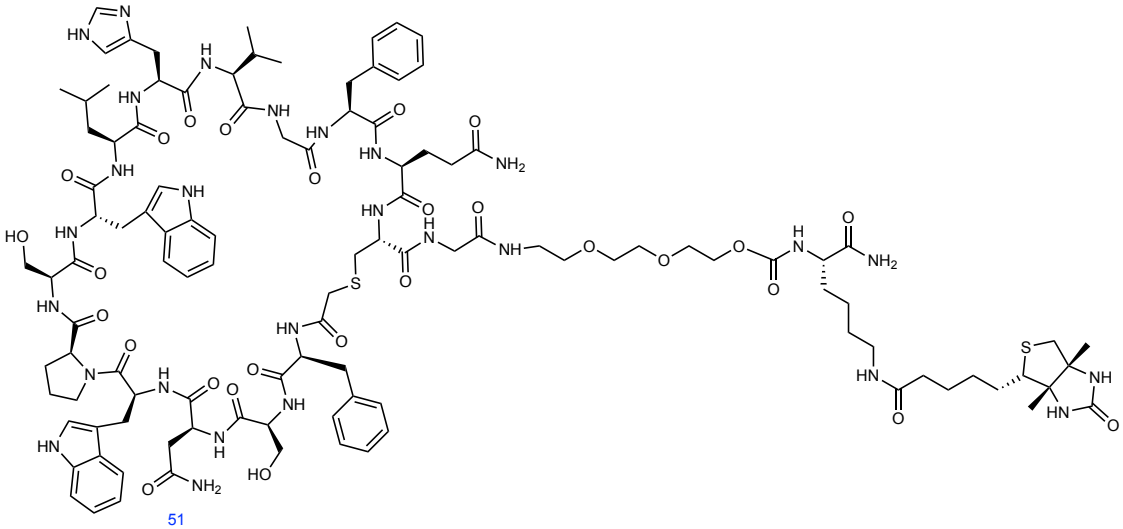
C:\Xcalibur\data\1772686 8/11/2017 10:12:41 AM

8/11/17

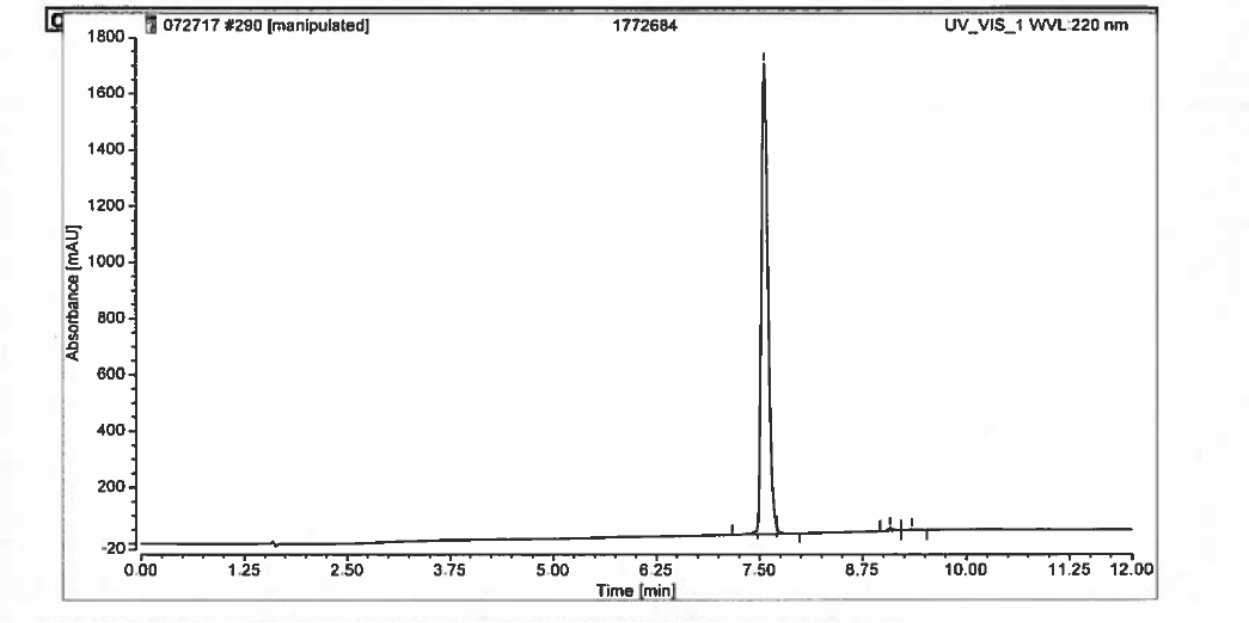
1772686 #23-57 RT: 0.14-0.35 AV: 12 NL: 2.88E6
F: ITMS + c ESI E Full ms [50.00-2000.00]



LC-MS data for compound (51) PSMD2-MC3biotin



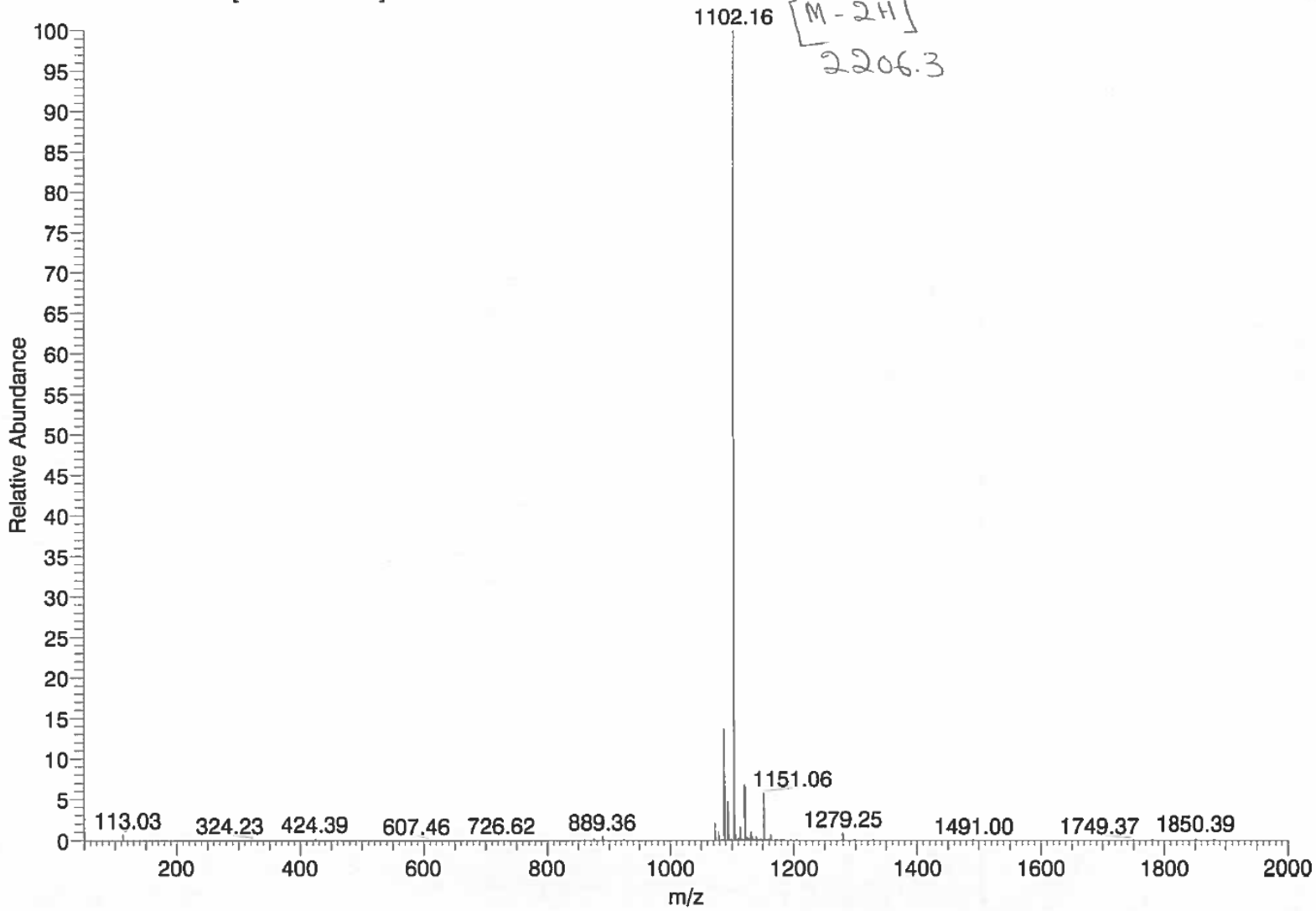
51



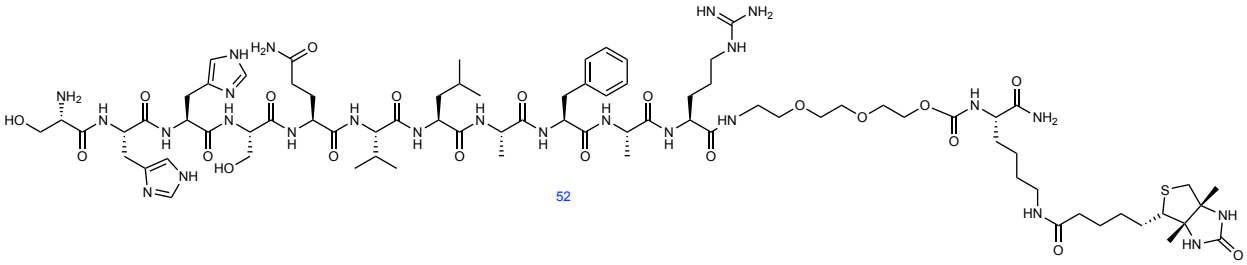
C:\Xcalibur\Data\1772684 8/16/2017 1:45:00 PM

08/16/17 dR

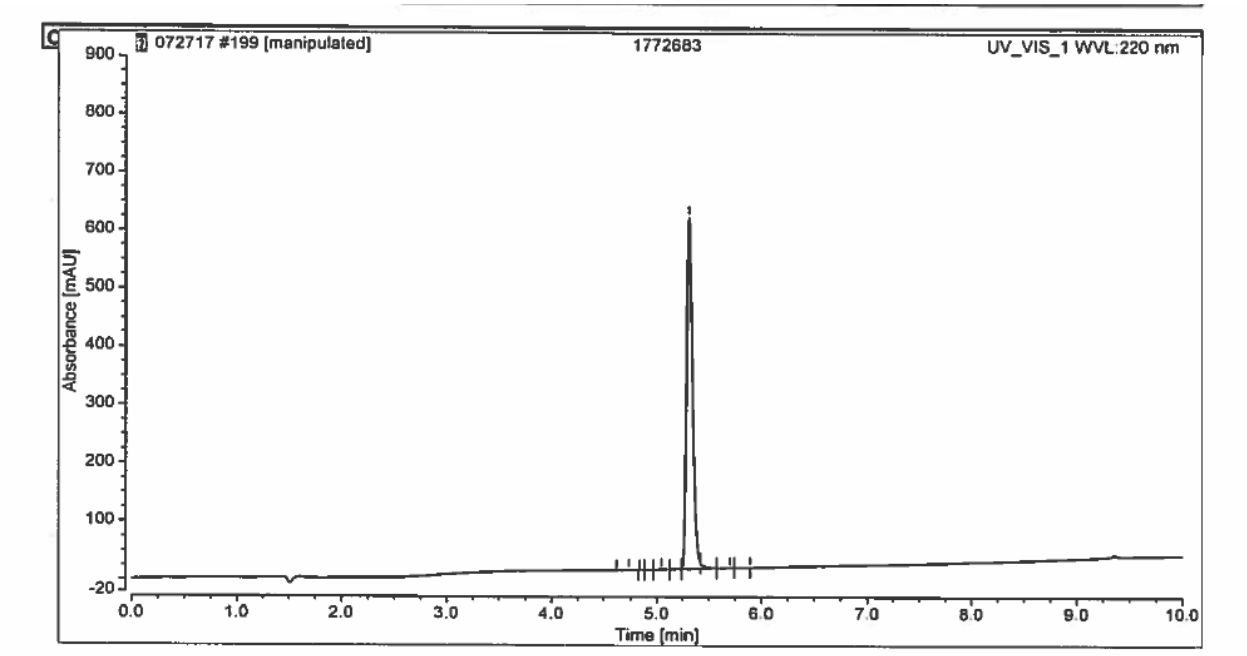
1772684 #19-54 RT: 0.17-0.46 AV: 12 NL: 6.41E5
F: ITMS - c ESI Full ms [50.00-2000.00]



LC-MS data for compound (52) PSMD2-PP1biotin



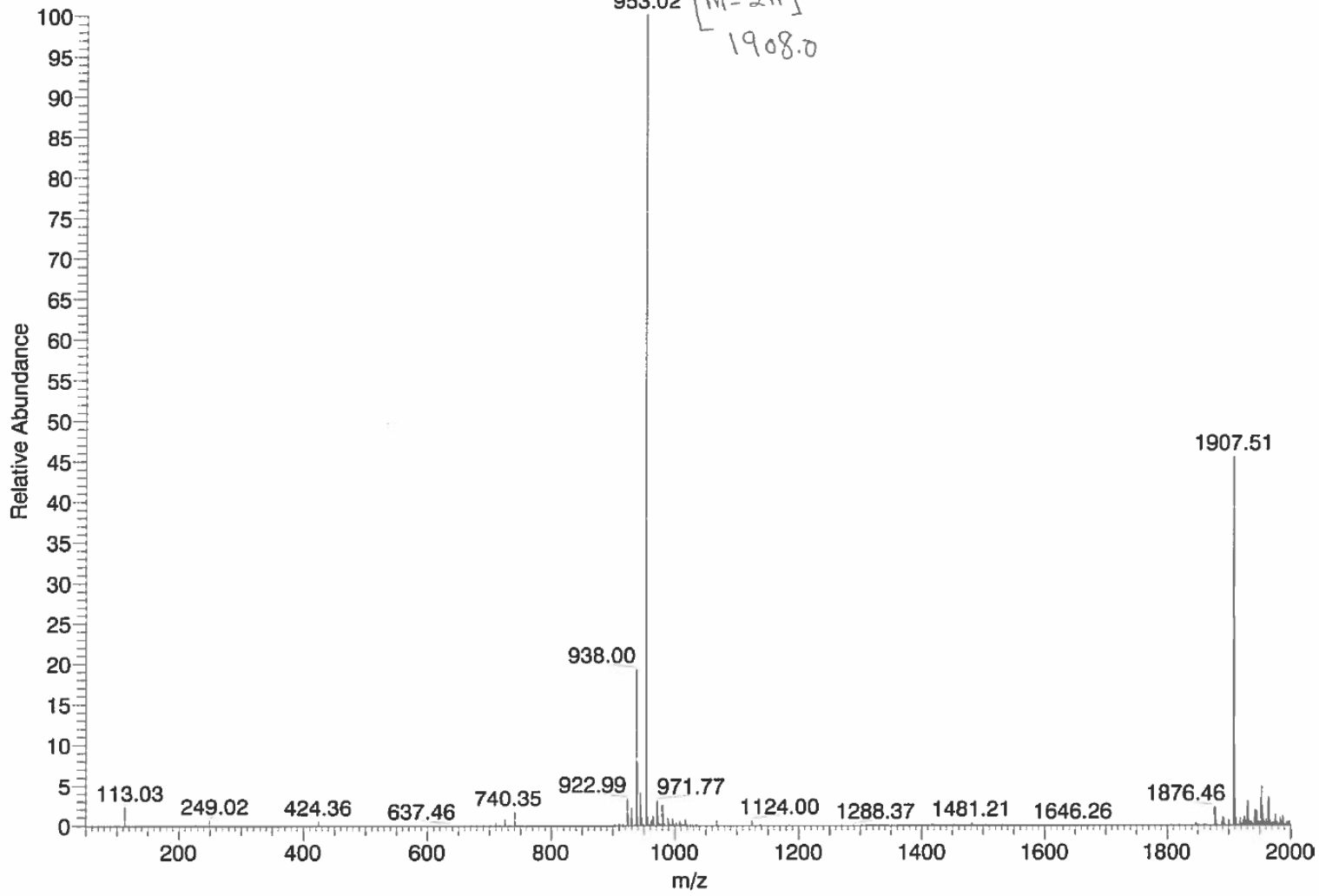
52



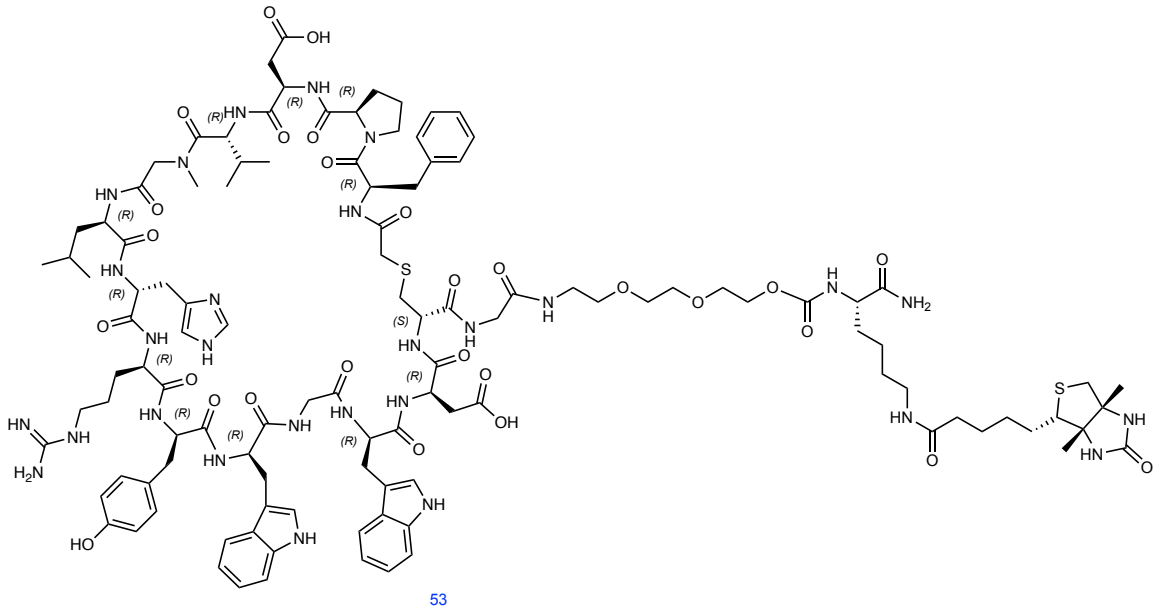
C:\Xcalibur\data\1772683_170809151405 8/9/2017 3:14:05 PM

08/09/17

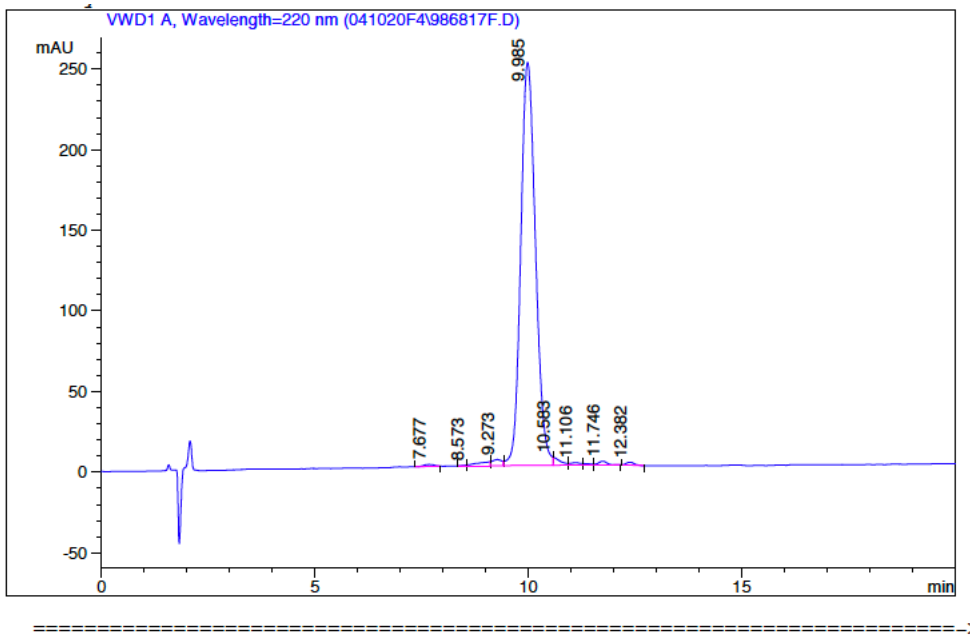
1772683_170809151405 #19-55 RT: 0.17-0.48 AV: 13 NL: 3.21E5
F: ITMS - c ESI Full ms [50.00-2000.00]



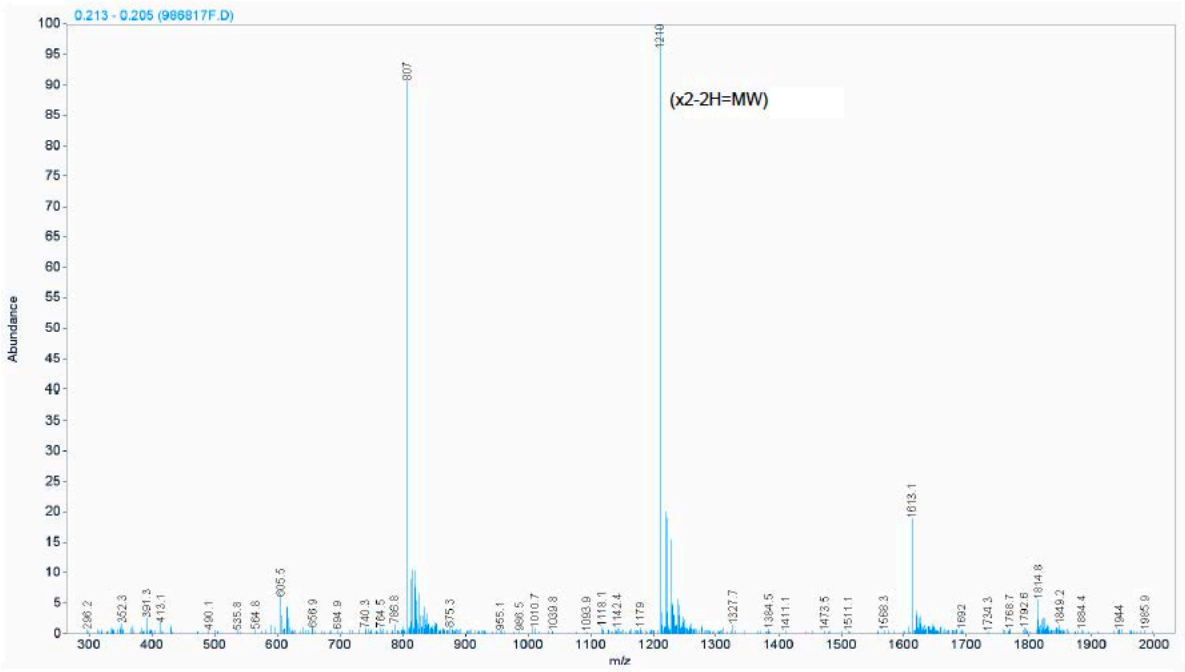
LC-MS data for compound (53) PSMD2-D-MCbiotin



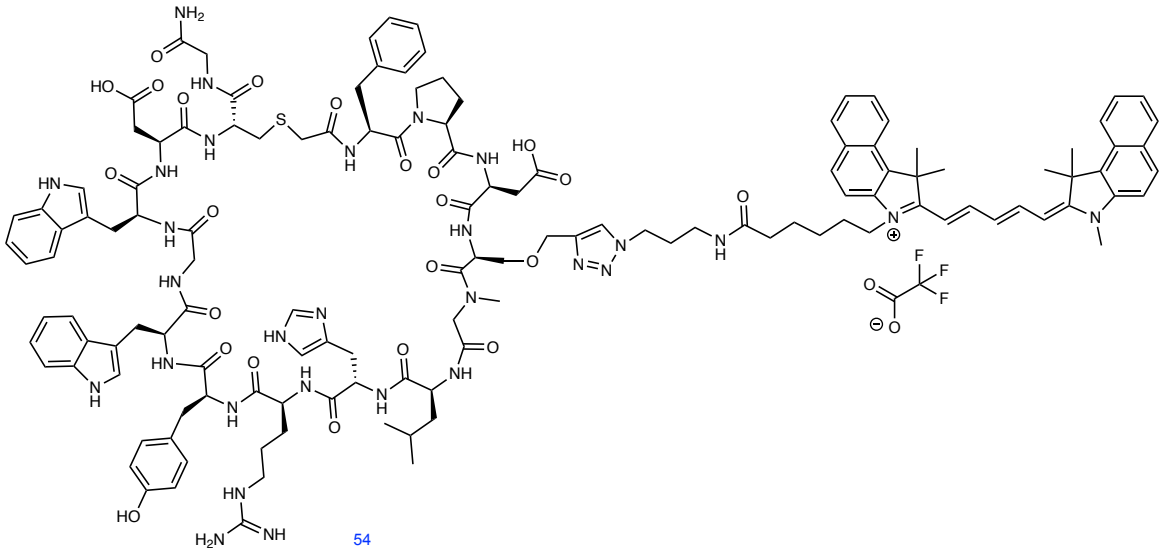
53

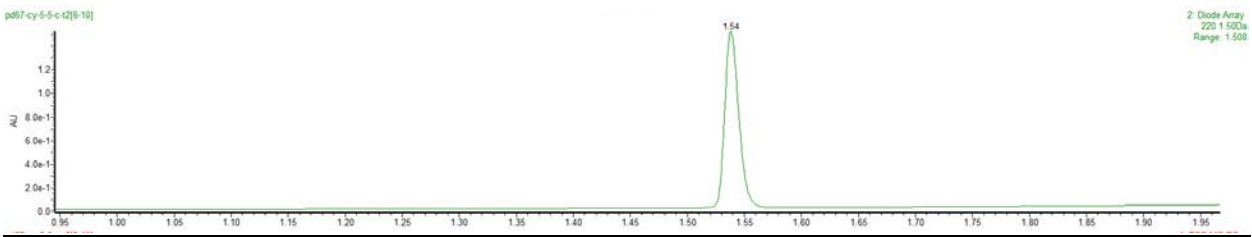


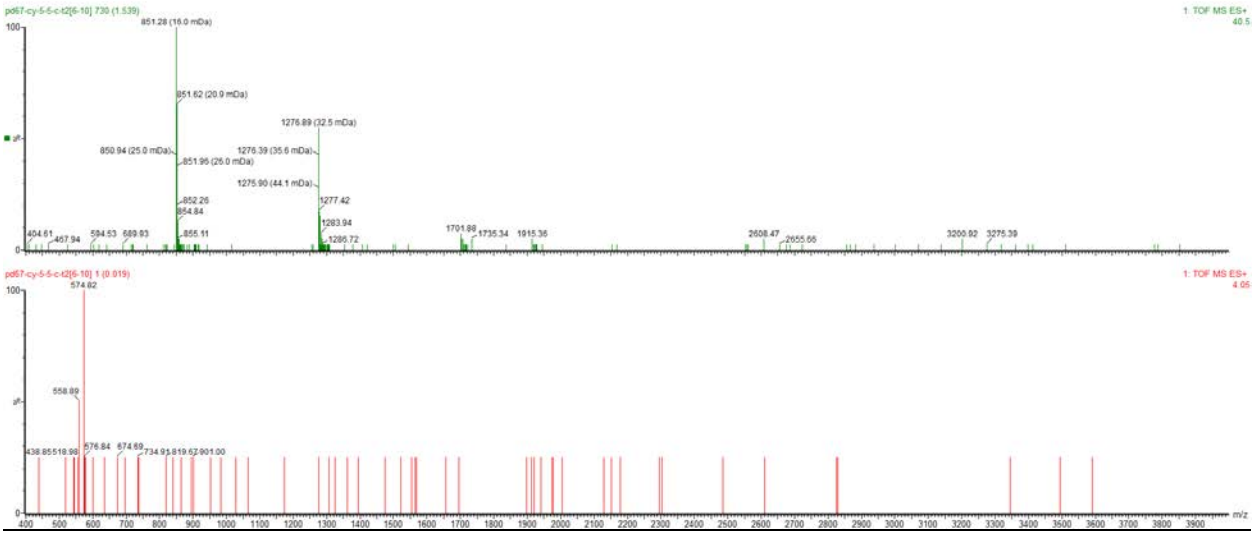

Signal 1:VWD1 A, Wavelength=220 nm




LC-MS data for compound (54) fluorescently labeled peptide $MC1_{Cy5.5}$




pd67-cy-5-5-c-t2[6-10] 730 (1.539)
1: TOF MS ES+
40.5
851.28 (16.0 mDa)
851.62 (20.9 mDa)
1276.89 (32.5 mDa)
850.94 (25.0 mDa)
1276.39 (35.6 mDa)
851.96 (26.0 mDa)
1275.90 (44.1 mDa)
852.26
854.84
1277.42
1283.94
404.61
457.94
584.53
689.93
855.11
1285.72
1701.98
1735.34
1915.36
2608.47
2655.66
3200.92
3275.39
pd67-cy-5-5-c-t2[6-10] 1 (0.019)
1: TOF MS ES+
4.05
574.82
558.89
438.85
518.98
576.84
674.69
734.95
819.62
901.00
m/z