# Chemically synthesized molecules with the targeting and effector functions of antibodies

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**Supporting Information**

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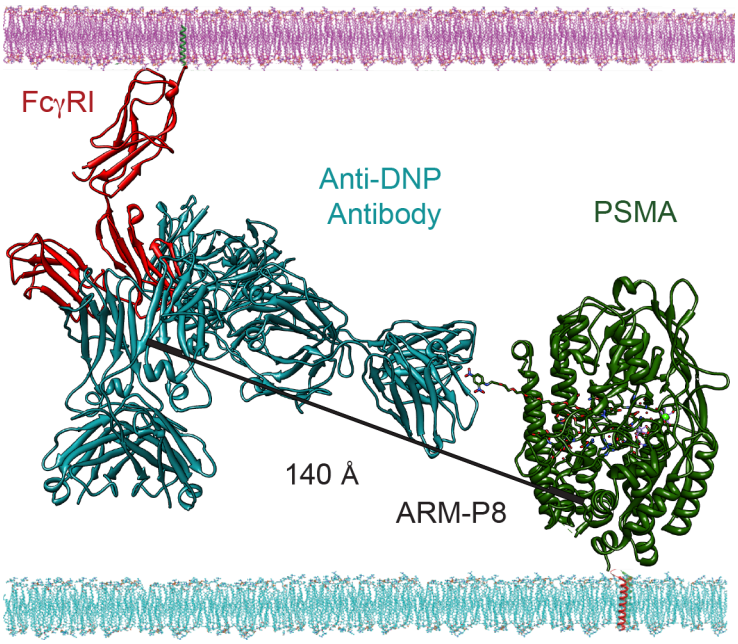
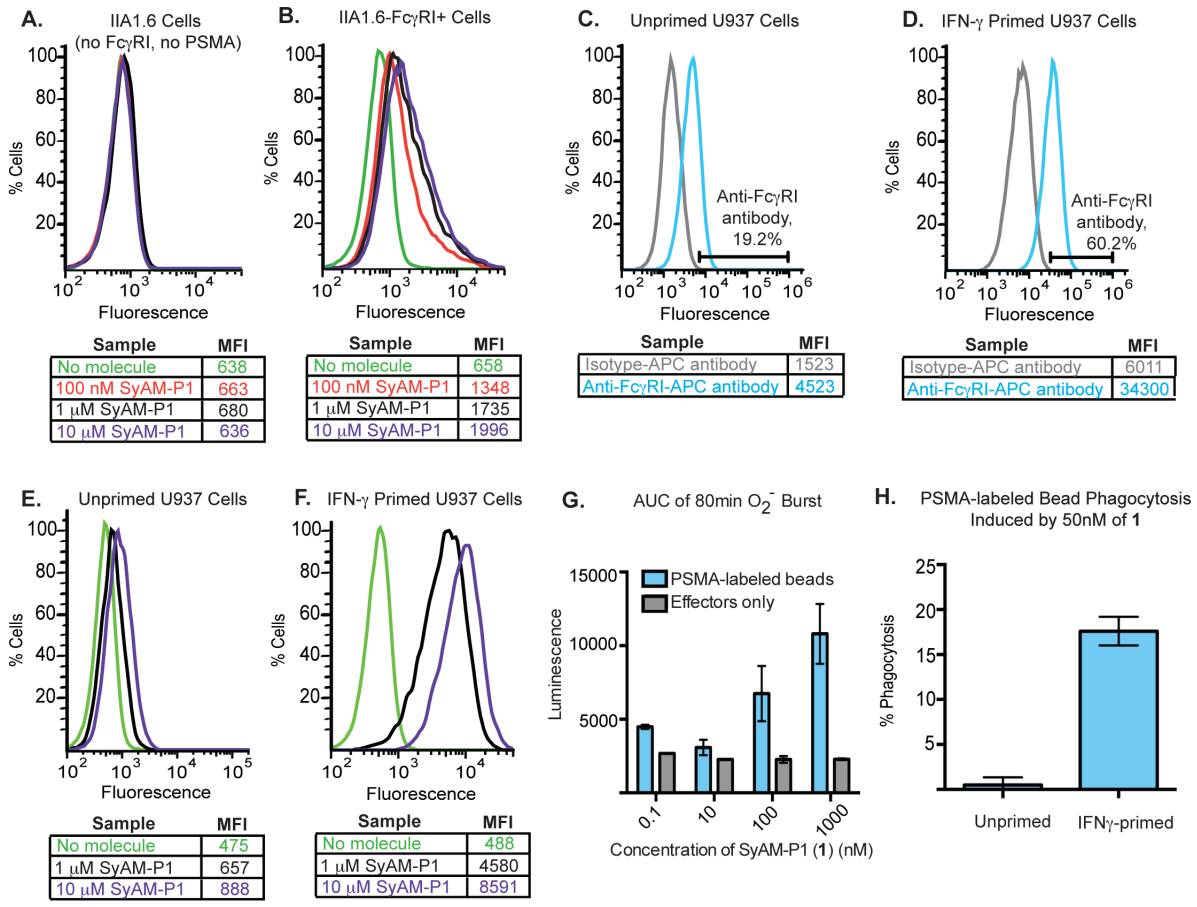
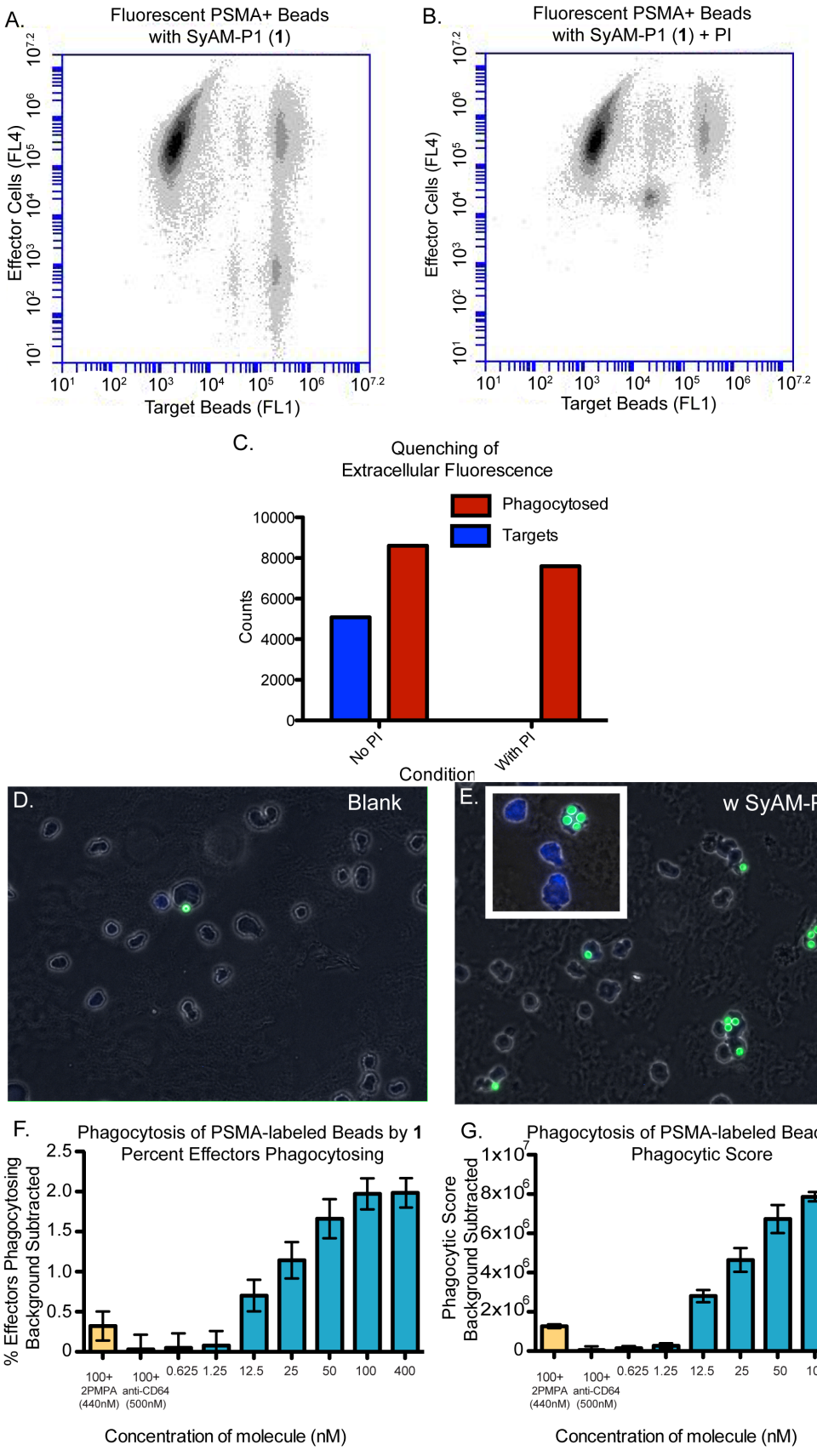


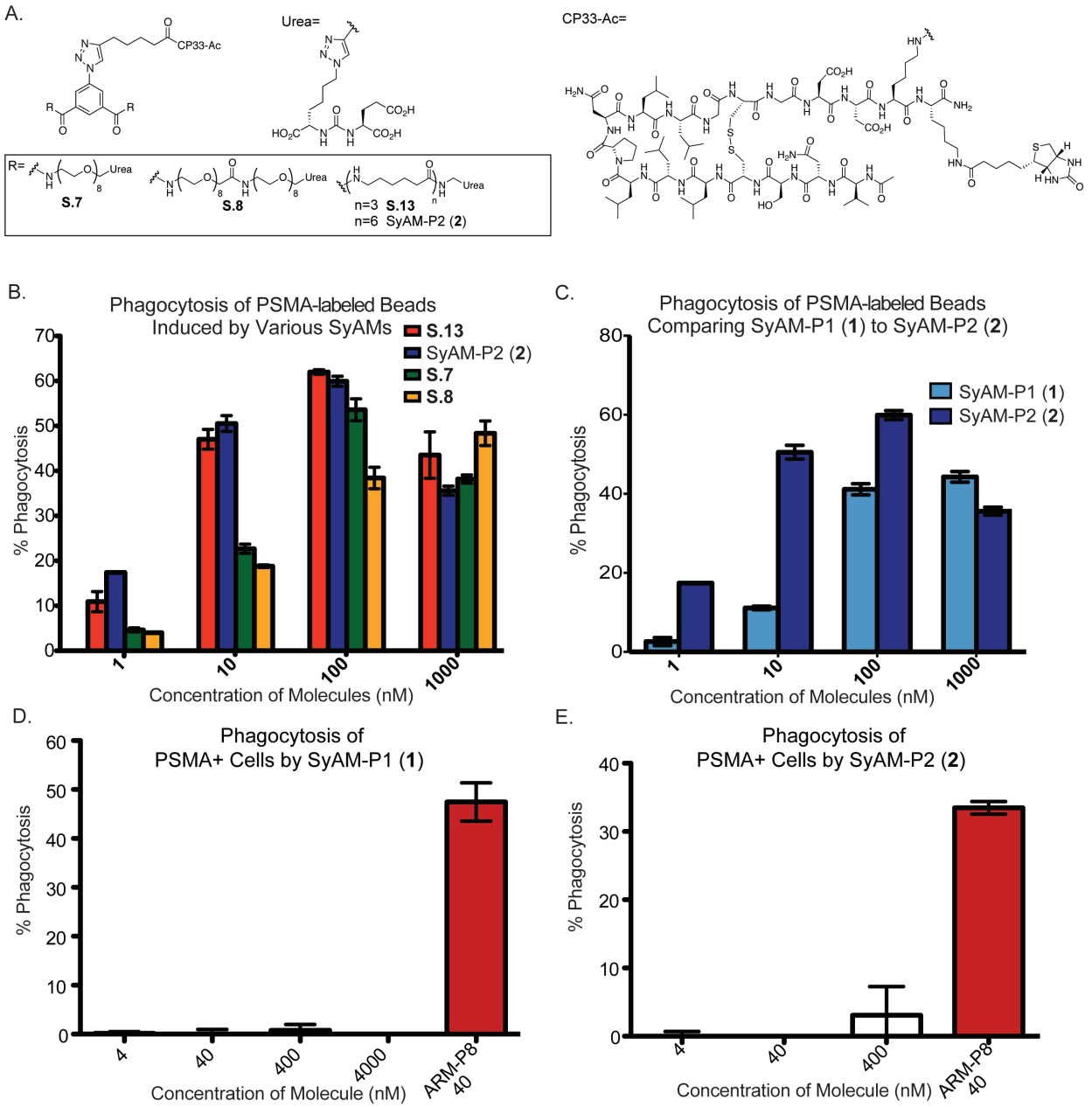

**Supplementary Figure S1**: Docking of ARM-P8 into the PSMA binding pocket in complex with IgG also binding to FcγRI for determination of distances in the quaternary complex. Approximate distance between binding pocket and FcγRI is 140 Å.



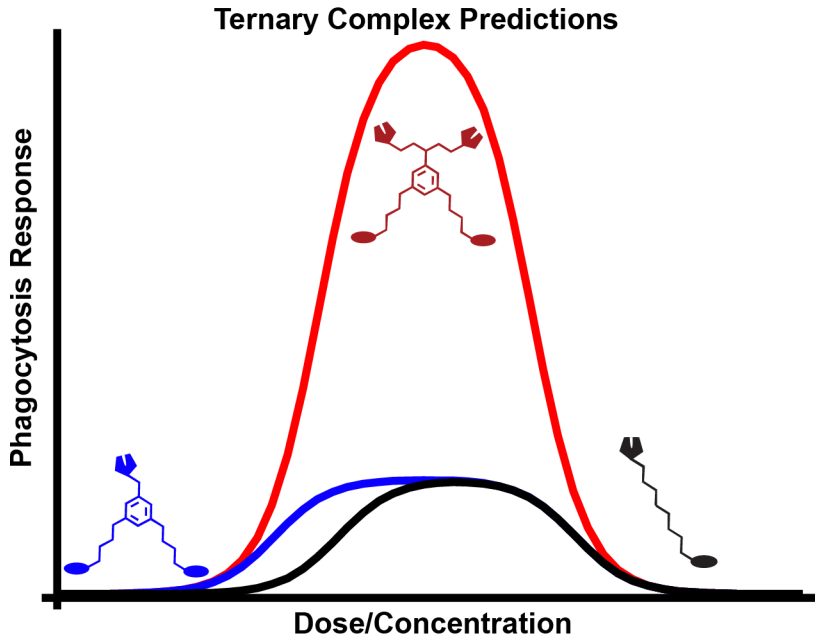

**Supplementary Figure S2**: Control experiments for the binding and effector functions of SyAM-P1 (**1**). **A)** Concentrations of up to 10 μM of **1** do not bind to IIA1.6 cells that lack FcγRI and PSMA expression, as shown by lack of change in the mean fluorescence intensity (MFI). Streptavidin-Alexafluor647 was used to detect binding of the biotinylated SyAM-P1. **B**) SyAM-P1 binds in a concentration-dependent manner to IIA1.6 cells stably transfected with FcγRI. **C**) Staining of unprimed U937 cells with an anti-FcγRI antibody shows low-to-moderate expression of FcγRI. **D**) Staining of interferon-gamma- (IFN-γ-) primed U937 cells with an anti-FcγRI antibody shows a significant increase in expression of FcγRI. **E**) SyAM-P1 binds weakly to unprimed U937 monocytic cells. **F**) SyAM-P1 binds strongly to U937 monocytic cells that have been primed for 72hr with IFN–γ. **G**) The area under the curve (AUC) for the complete 80 minutes of the superoxide burst assay in the presence of various concentrations of **1**, +/- target PSMA-labeled beads. Superoxides were detected by reaction with the chemiluminescent probe lucigenin. Little accumulation of superoxide is seen in the absence of targets compared to the presence of PSMA+ targets. **H**) Unprimed U937 monocytes are unable to mediate SyAM-P1-induced phagocytosis. Phagocytosis was calculated by the formula % targets phagocytosed = [(double-positive cells) / (remaining target cells + double-positive cells) × 100%] – background phagocytosis.


A.
Fluorescent PSMA+ Beads
with SyAM-P1 (1)
Effector Cells (FL4)
Target Beads (FL1)
B.
Fluorescent PSMA+ Beads
with SyAM-P1 (1) + PI
Effector Cells (FL4)
Target Beads (FL1)
C.
Quenching of
Extracellular Fluorescence
Phagocytosed
Targets
Counts
No PI
With PI
Condition
D.
Blank
E.
w SyAM-P1 (1)
F.
Phagocytosis of PSMA-labeled Beads by 1
Percent Effectors Phagocytosing
% Effectors Phagocytosing
Background Subtracted
100+ 2PMPA (440nM)
100+ anti-CD64 (500nM)
Concentration of molecule (nM)
G.
Phagocytosis of PSMA-labeled Beads by 1
Phagocytic Score
Phagocytic Score
Background Subtracted
Concentration of molecule (nM)

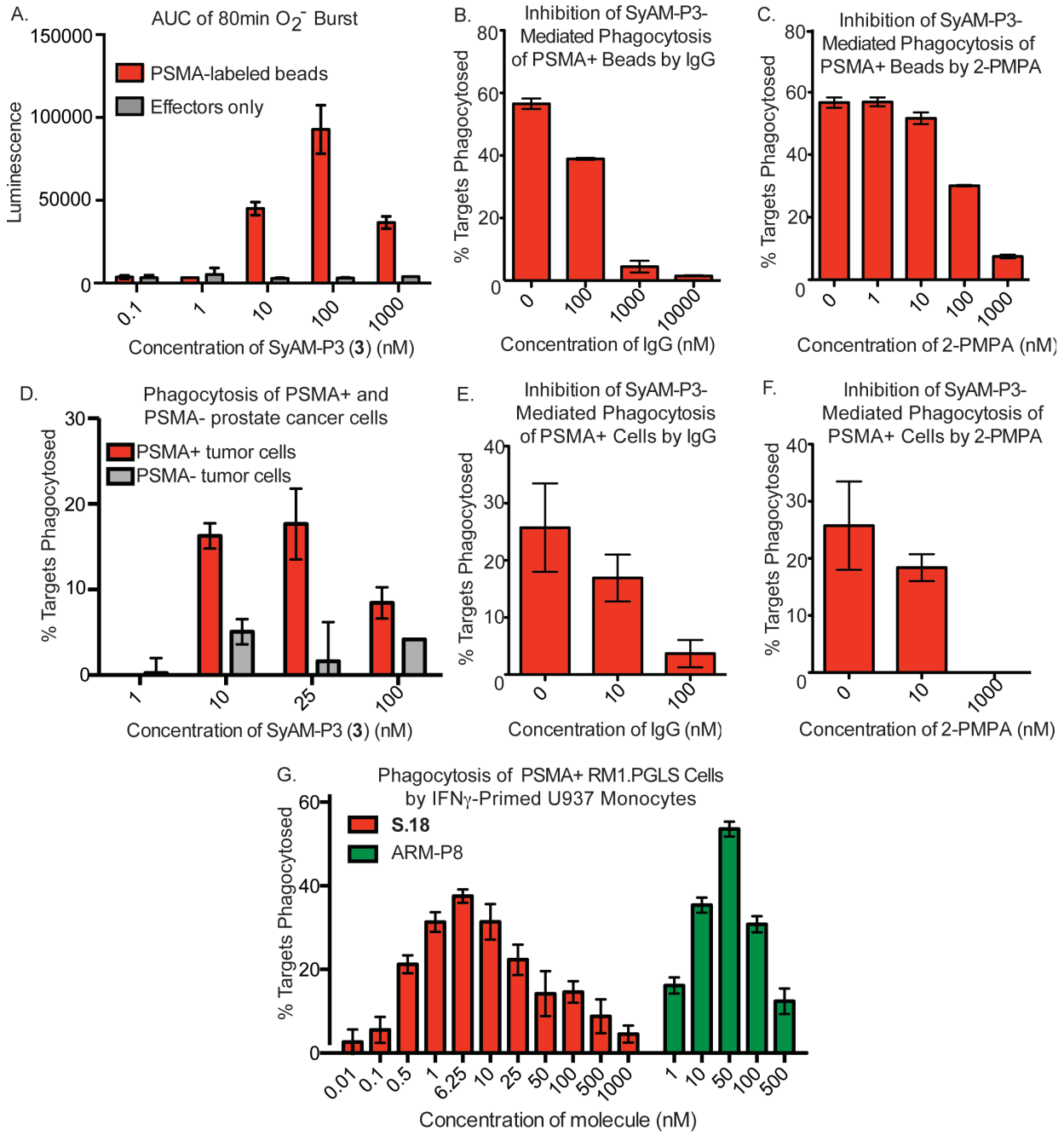
**Supplementary Figure S3**: Confirmation of SyAM-induced phagocytosis of PSMA-labeled beads by IFN-γ primed U937 monocytic cells. **A)** Representative two color flow-cytometry dot plot with Alexafluor-488-labeled PSMA+ beads incubated in the presence of 50 nM of SyAM-P1 (**1**) and FcγRI+ U937 cells in the absence of propidium iodide (PI) quencher. **B)** Same sample as in (A) incubated with PI, with loss of fluorescence of unphagocytosed target beads observed. Double-positive (FL-4+, FL-1+) signals do not lose FL-1 fluorescence, indicating that beads have been internalized by the U937 cells. **C)** Graph indicating cell counts of targets (blue bars) and phagocytosed (red bars) beads in the presence, or absence, of PI. **D)** Representative microscopy image of beads incubated with FcγRI+ U937 cells in the absence of SyAM-P1 (**1**). Only bead-cell aggregates noted. **E)** Representative microscopy image of PSMA-labeled beads incubated in the presence of 50 nM of SyAM-P1 (**1**) and FcγRI+ U937 cells. Inset, magnified view of same conditions: a U937 cell phagocytosing multiple beads when incubated with SyAM-P1 (**1**). **F**) Phagocytosis of PSMA-labeled beads induced by **1** as shown in Figure 2F, with % phagocytosis calculated by the alternate formula (% effectors phagocytosing) = [(double-positive cells) / (non-participating effectors + double-positive cells) × 100%] - background phagocytosis. **G**) Phagocytic score of PSMA-labeled beads induced by **1** as shown in Figure 2F, as calculated by the formula (% effectors phagocytosing × MFI of phagocytosing effectors) - background phagocytic score in the absence of compound. For G and H, error bars represent the standard deviation of at least duplicates, with trends repeated on at least three separate occasions.




**Supplementary Figure S4**: Analysis of linker composition on the functional phagocytic response against PSMA-labeled targets. **A)** Structures of different linkers, with varying length and hydrophobicity, connecting the PSMA binding moieties. **B)** Comparison of phagocytosis induced by various concentrations of these molecules against PSMA-labeled beads by primed FcγRI+ effector cells. **C)** Comparison of phagocytosis of PSMA-labeled beads in the presence of SyAM-P1 (**1**) or SyAM-P2 (**2**). **D)** Comparison of phagocytosis of PSMA+ target cells (RM1.PGLS) by SyAM-P1 (**1**) and by a positive control ARM-P8. **E)** Comparison of phagocytosis of PSMA+ target cells (RM1.PGLS) by SyAM-P2 (**2**) and by a positive control ARM-P8. Phagocytosis was calculated by the formula % targets phagocytosed = [(double-positive cells) / (remaining target cells + double-positive cells) × 100%] – background phagocytosis.

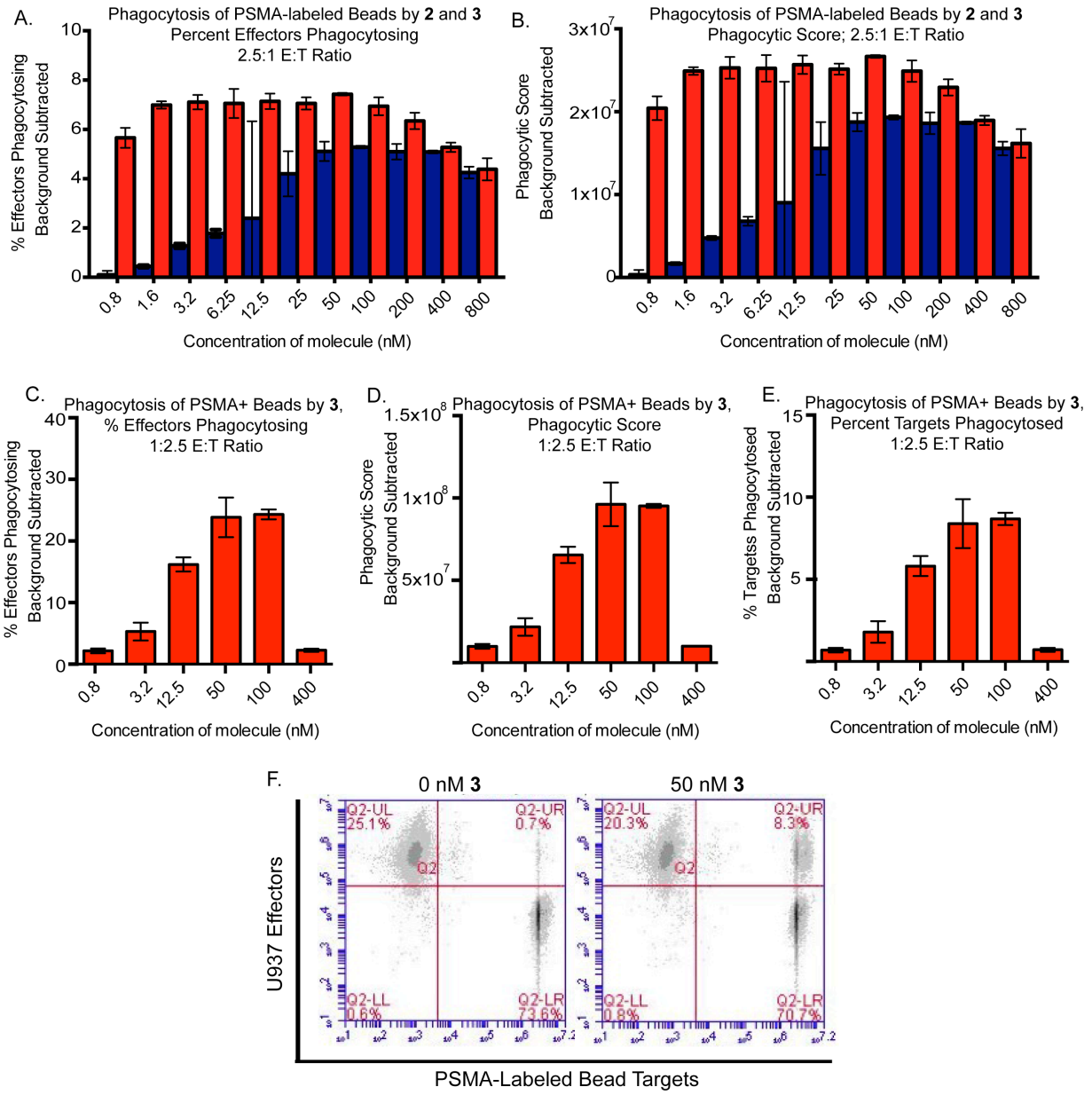



**Supplementary Figure S5**: Mechanistic explanation of the different avidity enhancements observed for SyAM-P2 (**2**) (in blue) and SyAM-P3 (**3**) (in red) over SyAM-P1 (**1**) (in black). Briefly, improving the weaker binding affinity (here $K_{Fc\gamma RI}$) has a much greater effect than improving the stronger binding affinity (here $K_{PSMA}$). As shown here, improving the stronger binding affinity by a factor of 10 only improves the width/potency of the dose-response curve (explaining the experimental improvement observed between SyAM-P1 (**1**) and SyAM-P2 (**2**)). Conversely, improving the weaker binding affinity by a factor of 5 results in a more pronounced improvement in the magnitude/efficacy of the dose-response curve. Ternary complex equilibria simulations were performed in Mathematica using a mathematical model for ternary complex developed in our laboratory.



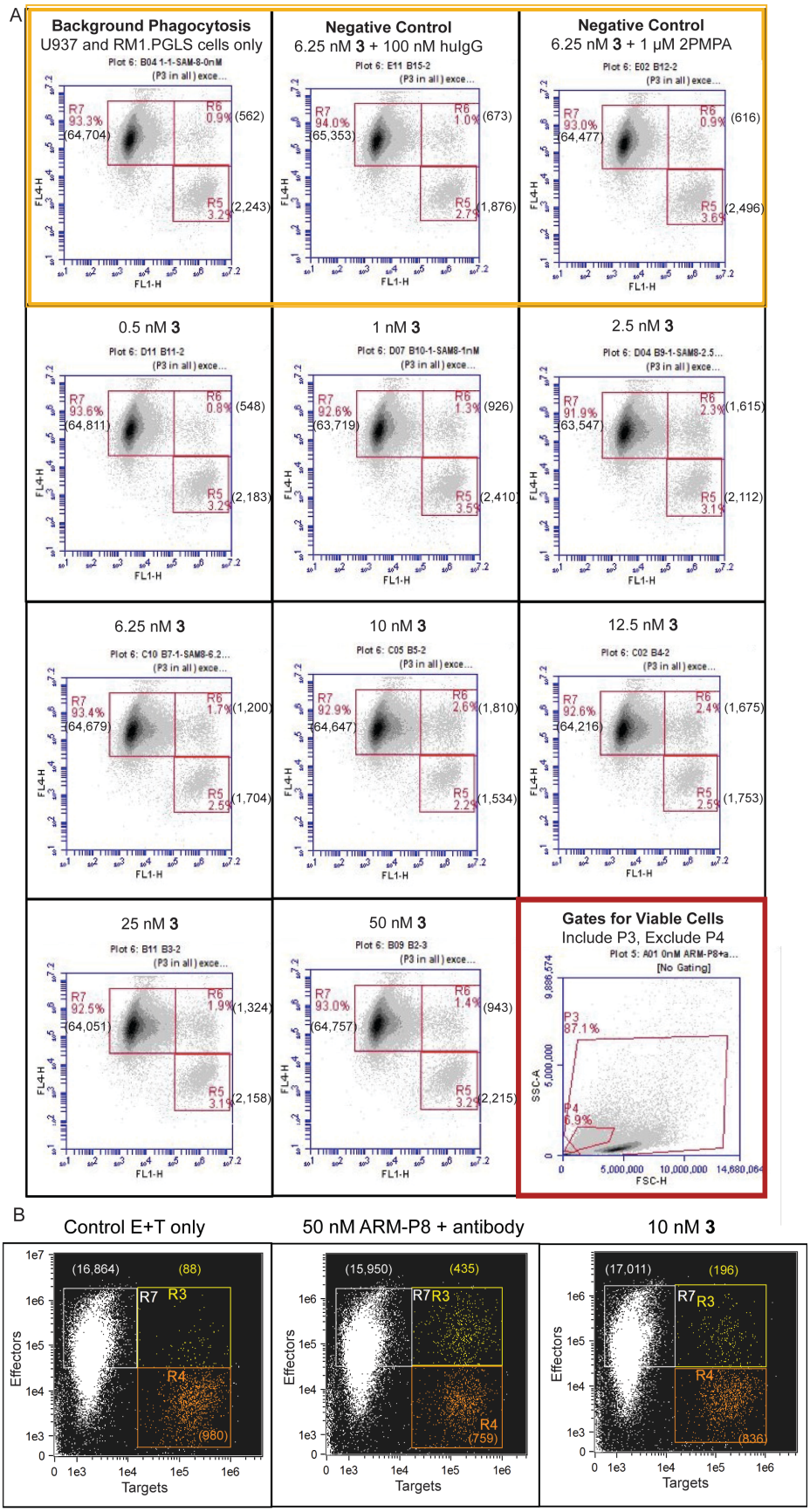

**Supplementary Figure S6**: Control experiments for superoxide burst and phagocytosis, showing their dependence on PSMA binding, FcγR binding, and SyAM concentration. **A)** The area under the curve (AUC) for the complete 80 minutes of the superoxide burst assay in the presence of various concentrations of SyAM-P3 (**3**), +/- target PSMA-labeled beads. Superoxides were detected by reaction with the chemiluminescent probe lucigenin. Little to no accumulation of superoxide is seen in the absence of targets. **B)** Phagocytosis of PSMA-labeled beads by primed FcγRI+ cells in the presence 50 nM of **3** SyAM-P3 (**3**). Phagocytosis is inhibited by increasing concentrations of human IgG, which block the interaction of the molecule with FcγRI. Phagocytosis in this panel and all subsequent panels of this figure is calculated by

(% targets phagocytosed) = [(double-positive cells) / (remaining target cells + double-positive cells) × 100%] - background phagocytosis. **C)** Inhibition of bead phagocytosis by increasing concentrations of 2-PMPA, which blocks the interaction of 50 nM SyAM with PSMA. **D)** Comparison of U937-mediated phagocytosis of PSMA+ tumor cells or PSMA- isogenic control cells in the presence of SyAM-P3 (**3**). The PSMA+ cells were a murine RM1 prostate tumor line stably transfected with human PSMA (RM1.PGLS); the PSMA- cells were the parent RM1 line. **E)** Concentration-dependent, human IgG-mediated inhibition of PSMA+ cell phagocytosis through blocking the interaction of SyAM-P3 (6.25 nM) with FcγRI. **F)** Phagocytosis of PSMA+ cells by primed FcγRI+ effector cells in the presence 6.25 nM of SyAM-P3 (**3**), with phagocytosis inhibited in a concentration-dependent manner by the PSMA-competitive ligand 2-PMPA. **G**) Comparison of the phagocytosis of RM1.PGLS cells by IFN-γ-primed U937 cells induced by either ARM-P8 + 133nM anti-DNP antibody or the closely related SyAM-P3 derivative **S.18**.




**Supplementary Figure S7**: Alternative metrics of assessing SyAM-P3-dependent phagocytosis of PSMA-labeled beads. **A**) Phagocytosis of PSMA-labeled beads induced by **3** as shown in Figure 3B, with % phagocytosis calculated by the alternate formula (% effectors phagocytosing) = [(double-positive cells) / (non-participating effectors + double-positive cells) × 100%] - background phagocytosis in the absence of compound. An effector-to-target (E:T) ratio of 2.5:1 was used. **B**) Phagocytic score of PSMA-labeled beads induced by **3** as shown in Figure 3B, as calculated by the formula (% effectors phagocytosing x MFI of phagocytosing effectors) - background phagocytic score. Data shown in A and B represent the mean of at least duplicate samples plus/minus standard deviation, and the reported trends were reproduced on at least three separate occasions. **C**) SyAM-P3 increases the percent of IFN-γ-primed U937 monocytic cells engaged in phagocytosis at a 1:2.5 E:T ratio (targets now in excess of U937 monocytes, unlike in panels A and B). $2 \times 10^4$ effector cells were mixed with $5 \times 10^4$ targets, and phagocytosis was

performed as described in the Materials and Methods section. Percent effector cells engaged in phagocytosis was calculated by the formula (% effectors phagocytosing) = [(double-positive cells) / (non-participating effectors + double-positive cells) × 100%] – background phagocytosis. **D**) Phagocytic score of PSMA-labeled beads induced by **3** at a 1:2.5 E:T ratio as in C, as calculated by the formula (% effectors phagocytosing x MFI of phagocytosing effectors) - background phagocytic score. **E**) Phagocytosis of PSMA-labeled beads induced by **3** by U937 cells at an E:T ratio of 1:2.5 as in C and D, with phagocytosis calculated by the formula (% targets phagocytosed) = [(double-positive cells) / (remaining target cells + double-positive cells) × 100%] - background phagocytosis. For C, D, and E samples were run in duplicate and error bars reflect standard deviation of duplicates. **F**) Representative raw dot plots for Figure S7C-E, showing U937 cells stained with DiD (FL-4 channel, y-axis) phagocytosing PSMA-labeled beads functionalized with an AlexaFluor-488 derivative (FL-1 channel, x-axis). The E:T ratio was 1:2.5.


A
Background Phagocytosis
U937 and RM1.PGLS cells only
Negative Control
6.25 nM 3 + 100 nM huIgG
Negative Control
6.25 nM 3 + 1 µM 2PMPA
0.5 nM 3
1 nM 3
2.5 nM 3
6.25 nM 3
10 nM 3
12.5 nM 3
25 nM 3
50 nM 3
Gates for Viable Cells
Include P3, Exclude P4
B
Control E+T only
50 nM ARM-P8 + antibody
10 nM 3
Effectors
Targets

**Supplementary Figure S8**: Representative example of raw dot plot data used in creating Figure 3. A) For Figure 3C, primed U937 effector cells were stained with DiD (an FL-4 membrane dye), and phagocytosed PSMA+ RM1.PGLS tumor targets were stained with DiO (an FL-1 membrane dye). Numbers in parentheses represent the absolute cell counts in each gate. Plots were first gated to exclude dead cells (bottom right panel). The starting effector-to-target (E:T) ratio was 8:1. As reported previously,[1] more target cells than effectors died during the course of the assay, yielding a increased final E:T ratio. Data was acquired using an Accuri C6 flow cytometer. **B)** Raw dot plot data used in preparing Figure 3D, in which primed U937 effector cells stained with anti-CD11b-APC, anti-CD14-APC, and DiD dye phagocytosed PSMA+ RM1.PGLS tumor targets stained with DiO dye. Data was acquired from an Amnis Imagestream flow cytometer.

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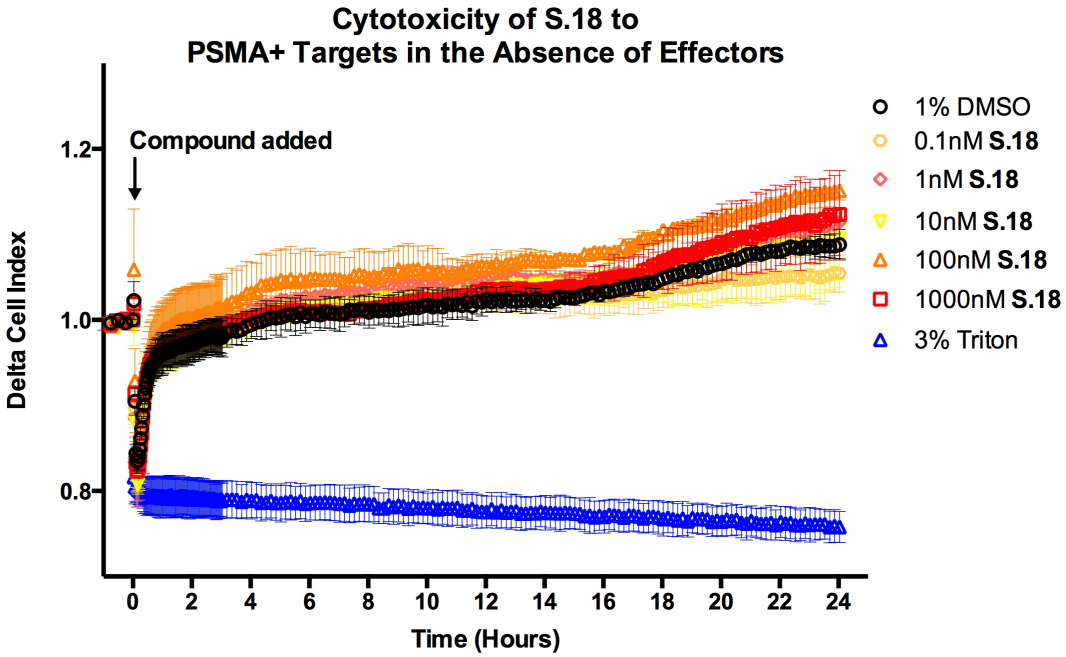
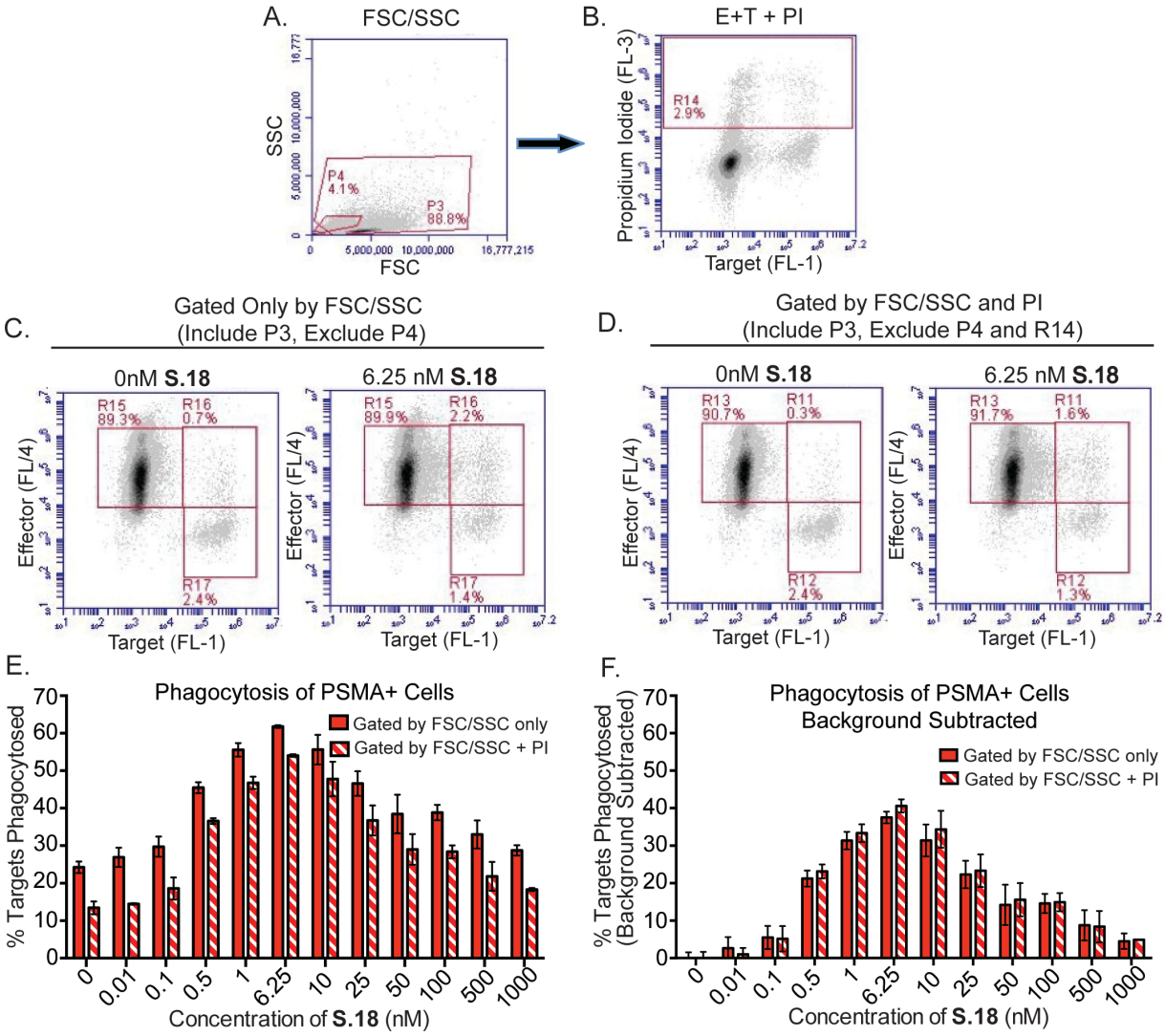
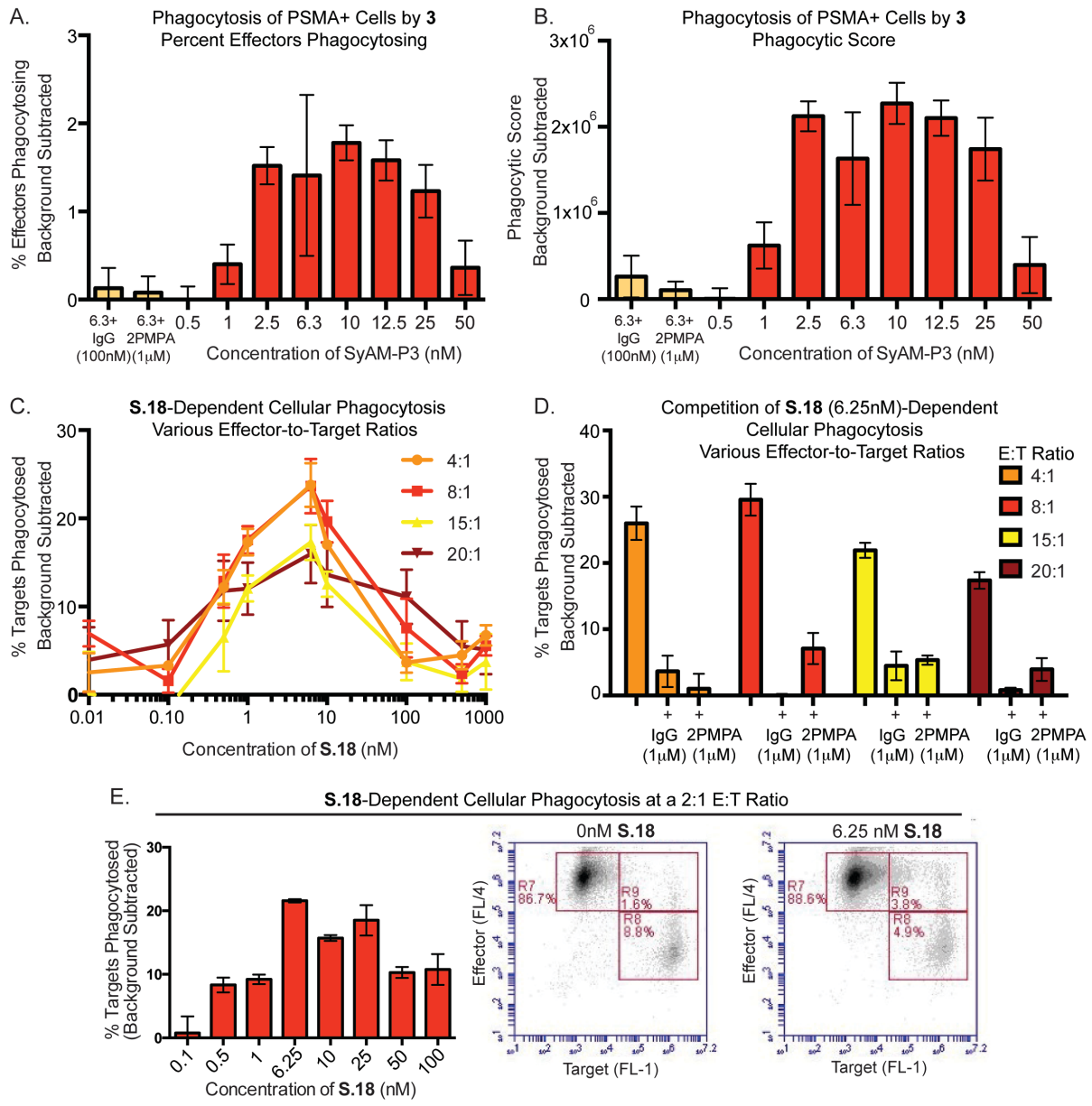



**Supplementary Figure S9:** Evaluation of SyAM-P3 cytotoxicity in the absence of effector white blood cells. $1.25 \times 10^4$ RM1-hPSMA+ targets were seeded on an Xcelligence electrode plate 36 hours before the start of the experiment. At time 0, old media was removed from the wells and fresh media containing various concentrations of the closely related SyAM-P3 derivative **S.18**, vehicle only (1% DMSO; negative control), or 3% Triton detergent (positive control) was added to the wells. Cell index (a measure of electric resistance on the plate, which corresponds to target cell adherence) was monitored over the next 24 hours. A range of **S.18** concentrations spanning 5 orders of magnitude failed to cause cell death or impede cell growth compared to the negative control of 1% DMSO alone. 3% Triton detergent caused significant cell death.




**Supplementary Figure S10:** Measured SyAM-induced phagocytosis corresponds only to viable events. Phagocytosis was performed as described in the Materials and Methods section, with a starting E:T ratio of 8:1 (100,000 IFN-γ-primed U937 cells and 12,500 PSMA+ RM1 murine prostate cancer cells). Effector cells were stained with DiD (an FL-4 dye) and targets were stained with DiO (an FL-1 dye). Propidium iodide was added at a 2 μg/mL concentration after 1 hour of phagocytosis. **A**) Forward scatter (FSC) and side scatter (SSC) were used to define the original gates for viable, non-debris events. All events included in P3 but excluding P4 were analyzed for phagocytosis. P4 was excluded based on previous studies that showed dying cells displayed a decreased FSC and increased SSC profile. **B**) The events gated from Panel A (include P3, exclude P4) were stained with propidium iodide (PI, detected in FL-3) to show viability. A small percentage of cells not already excluded by gate P4 stained PI+ (gate R14). **C**) Sample raw dot plots to determine effector-only (upper left gate, R15), target-only (lower right gate, R17), and double-positive (upper right gate, R16) events, with cells gated only by the FSC/SSC gates in Panel A. **D**) Sample raw dot plots to determine effector-only (upper left gate, R13), target-only (lower right gate, R12), and double-positive (upper right gate, R11) events, with cells gated by the FSC/SSC gates in Panel A as well as excluding the PI+ events in gate

R14 in Panel B. **E)** The percent of targets phagocytosed as gated either by FSC/SSC only or FSC/SSC + PI, as calculated by the formula % targets phagocytosed = [(double-positive cells) / (remaining target cells + double-positive cells) × 100%]. Background phagocytosis with 0 nM of compound and thus overall phagocytosis for all conditions is higher when PI+ events are not excluded (solid bars). **F**) The percent of targets phagocytosed as described in Panel E, now with background phagocytosis at 0 nM compound subtracted from SyAM-dependent phagocytosis as done for all other figures in the paper. Excluding PI+ events now makes no difference the measurement of SyAM-induced phagocytosis, indicating that the additional double-positive events that occur in the presence of SyAMs are phagocytosis of and with viable cells.




**Supplementary Figure S11:** The influence of effector-to-target (E:T) ratio on SyAM-P3-dependent cellular phagocytosis. **A**) Phagocytosis of RM1.PGLS cells induced by **3** as shown in Figure 3C, with % phagocytosis calculated by the alternate formula (% effectors phagocytosing) = [(double-positive cells) / (non-participating effectors + double-positive cells) × 100%] - background phagocytosis in the absence of compound. E:T ratio used was 8:1. **B**) Phagocytic score of RM1.PGLS cells induced by **3** as shown in Figure 3C, as calculated by the formula (% effectors phagocytosing x MFI of phagocytosing effectors) - background phagocytic score. E:T ratio used was 8:1. Data shown in A and B represent the mean of at least triplicate experiments plus/minus standard deviation, and the reported trends were reproduced on at least three separate occasions. **C)** SyAM-P3 induces phagocytosis of RM1.PGLS PSMA+ prostate cancer cells by IFN-γ primed U937 monocytes over a range of E:T ratios. $10^5$ effector cells were mixed with 2.5 × $10^4$, 1.25 × $10^4$, 6.66 × $10^3$, or 5.0 × $10^3$ target cells, and phagocytosis was performed as

described in the Materials and Methods section. **D)** Competition of SyAM-P3 induced phagocytosis of RM1.PGLS PSMA+ prostate cancer cells at all E:T ratios by IgG (a competitive ligand for FcγR) and by 2-PMPA (a competitive ligand for PSMA). C and D were performed with **S.18**, a version of SyAM-P3 that lacks a biotin handle. Error bars represent the standard deviation of duplicates, with trends repeated on two separate occasions. For C and D, phagocytosis was calculated by the formula (% targets phagocytosed) = [(double-positive cells) / (remaining target cells + double-positive cells) × 100%] - background phagocytosis. **E**) The SyAM-P3 derivative **S.18** was also able to induce similar levels of phagocytosis when 100,000 IFN-γ-primed U937 monocytes were mixed with 50,000 PSMA+ RM1.PGLS cells at a 2:1 E:T ratio. Raw 2D dot plots of the background at 0 nM compound and the maximal response at 6.25 nM compound are shown.

| Molecule | $K_d$ of PSMA Binding (nM) | $K_d$ of FcγRI Binding (nM) |
|---|---|---|
| SyAM-P1 (**1**) | 40.7 | 248.7 |
| SyAM-P2 (**2**) | 26 | ND |
| SyAM-P3 (**3**) | 23.2 | 57.7 |

**Supplementary Table S1**: Measurement of the binding Kd of the SyAMs to either PSMA or FcγRI. PSMA binding was measured by incubating various concentrations of molecules with PSMA-labeled beads and probing with fluorescently labeled-streptavidin. FcγRI binding was measured by incubating various concentrations of molecules with FcγRI-expressing cells and probing with fluorescently-labeled streptavidin. SyAM-P2 (**2**) binding to FcγRI was not determined (ND).

| Target | PSMA/$\mu m^2$ | % Phagocytosis (50 nM, 1) |
|---|---|---|
| Bead high-loading | 2375 † (± 250) | 46.2 (+/- 3) |
| Bead medium-loading | 1651†† (± 174) | 24.0 (+/- 1) |
| Bead low-loading | 840 †† (± 88) | 10.1 (+/- 1) |
| RM1.PGLS | 720 † (+/- 139) | 0.2 (+/- 3) |

**Supplementary Table S2**: Effects of PSMA level per area unit ($\mu m^2$) on immune response. PSMA per $\mu m^2$ was measured using a phycoerythrin labeled anti-PSMA antibody for PSMA+ RM1.PGLS cells and high loading beads. PSMA density was calculated for beads with other loading capacities by multiplying the number of PSMA proteins found on 2.0 mg/mL (high) loading capacity beads by the ratio of medium:high loading capacity (5.7 pmol/bead:8.2 pmol/bead) or low:high loading capacity (2.9 pmol/bead:8.2 pmol/bead). Phagocytosis of targets was performed with primed FcγRI+ effector cells and 50 nM of SyAM-P1 (**1**).

† indicates measured PSMA level. †† indicates calculated value based on loading with error calculated from measured beads.

| | no treatment | ARM-P8 (50 nM) + anti-DNP ab | 3 (10 nM) |
|---|---|---|---|
| **Image-based FACS analysis** | | | |
| % Early phagocytosis (attached double positives) | 3.0 | 23.4 | 10.7 |
| % Intermediate phagocytosis (phagocytic cup) | 1.2 | 8.4 | 5.3 |
| % Completed phagocytosis (engulfment) | 4.0 | 4.6 | 3.0 |
| % Total phagocytosis (early + intermediate + completed) | 8.2 | 36.4 | 19.0 |
| *Early as % of total phagocytosis* | *36.6* | *64.3* | *56.3* |
| *Intermediate as % of total phagocytosis* | *14.6* | *23.1* | *27.9* |
| *Completed as % of total phagocytosis* | *48.8* | *12.6* | *15.8* |

**Supplementary Table S3**: Analysis and comparison of phagocytosis as assessed by Amnis imaging flow cytometry (Image-Based). We visually inspected all events that were positive on both the fluorescent channel for DiO dye (target stain) and the channel for DiD dye/allophycocyanin (effector stains). All such double-positive events were then classified as "early" phagocytosis (in which two cells were attached and clearly making contact), "intermediate" phagocytosis (in which a clear phagocytic cup had formed between macrophage and target), or "completed" phagocytosis (where a target was entirely engulfed by a macrophage). Percentages are calculated with respect to total targets collected. The three stages of phagocytosis were then expressed as fractions of the total phagocytosis (italicized).

**Fitting procedure for SyAM-P induced bead phagocytosis dose-response curves**

Bead phagocytosis data was fit to a variation on the ternary complex equilibrium model published by Alan Perelson[7] which was chosen for its applicability to our system ($[PSMA]_t$ & $[FcgRI]_t$ <<$[SyAM\text{-}P]_t$) and its ease of implementation in GraphPad Prism. Concentration parameters were constrained to: $[PSMA]_t$ = 80 pM $[Fc\gamma RI]_t$ = 800 pM which were determined experimentally by multiplying expression level by cell count and dividing by experimental volume. Binding constants and cooperativity factors were allowed to vary but were constrained between SyAM-P1, SyAM-P2 and SyAM-P3 as follows: 1) To account for the change in valency/binding constant for PSMA, binding ($K_{PSMA}$) between SyAM-P1 and SyAM-P2 $K_{PSMA}$ was allowed to vary between these data sets while $K_{Fc\gamma RI}$ was constrained to be consistent between these data sets. 2) To account for the change in valency/binding constant for FcγRI binding between SyAM-P2 and SyAM-P3 $K_{Fc\gamma RI}$ was allowed to vary between these data sets while $K_{PSMA}$ was constrained between these data sets. Finally, the fit for the cooperativity factor α was constrained to be consistent between all three SyAM-P data sets. Final fit parameters are as follows:

SyAM-P1: $[PSMA]_t$ = 80 pM $[Fc\gamma RI]_t$ = 800 pM $\boldsymbol{K_{PSMA}}$ **= 150 nM** $K_{Fc\gamma RI}$ = 300 nM α = 792
SyAM-P2: $[PSMA]_t$ = 80 pM $[Fc\gamma RI]_t$ = 800 pM $\boldsymbol{K_{PSMA}}$ **= 50 nM** $\boldsymbol{K_{Fc\gamma RI}}$ **= 300 nM** α = 792
SyAM-P3: $[PSMA]_t$ = 80 pM $[Fc\gamma RI]_t$ = 800 pM $K_{PSMA}$ = 50 nM $\boldsymbol{K_{Fc\gamma RI}}$ **= 10 nM** α = 792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**Abbreviations:**

Ac = Acetyl

AcOH = Acetic acid

Ahx = Aminocaproic acid

AMC = 7-amino-4-methylcoumarin

Boc = *tert*-Butoxycarbonyl

BSA = Bovine Serum Albumin

Cbz = Benzyloxycarbonyl

DCM = Dichloromethane

DIPEA = *N*,*N*-Diisopropylethylamine

DMF = *N*,*N*-Dimethylformamide

DMSO = Dimethylsulfoxide

DPBS = Dulbecco's Phosphate-Buffered Saline

EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

EDTA = Ethylenediaminetetraacetic acid, disodium salt

EGTA = Ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid

$Et_2O$ = Diethyl ether

EtOAc = Ethyl acetate

Fmoc = 9-Fluorenylmethyloxycarbonyl

HBTU = *N*,*N*,*N'*,*N'*-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate, *O*-(Benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate

HI-FBS = Heat Inactivated Fetal Bovine Serum

HOBt = Hydroxybenzotriazole

*iPr*OH = Isopropyl alcohol

MeCN = Acetonitrile

MeOH = Methanol

Mtt = 4-Methyltrityl

NHS = *N*-hydroxysuccinimide

NMP = *N*-Methyl-2-pyrrolidone

Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl

Pd/C = Palladium on carbon 10 wt %

TBS = Tris-buffered saline

tBu = *tert*-Butyl

TEA = Triethylamine

TFA = Trifluoroacetic acid

TFAA = Trifluoroacetic anhydride

THF = Tetrahydrofuran

TIPS = Triisopropylsilane

Trt = Trityl

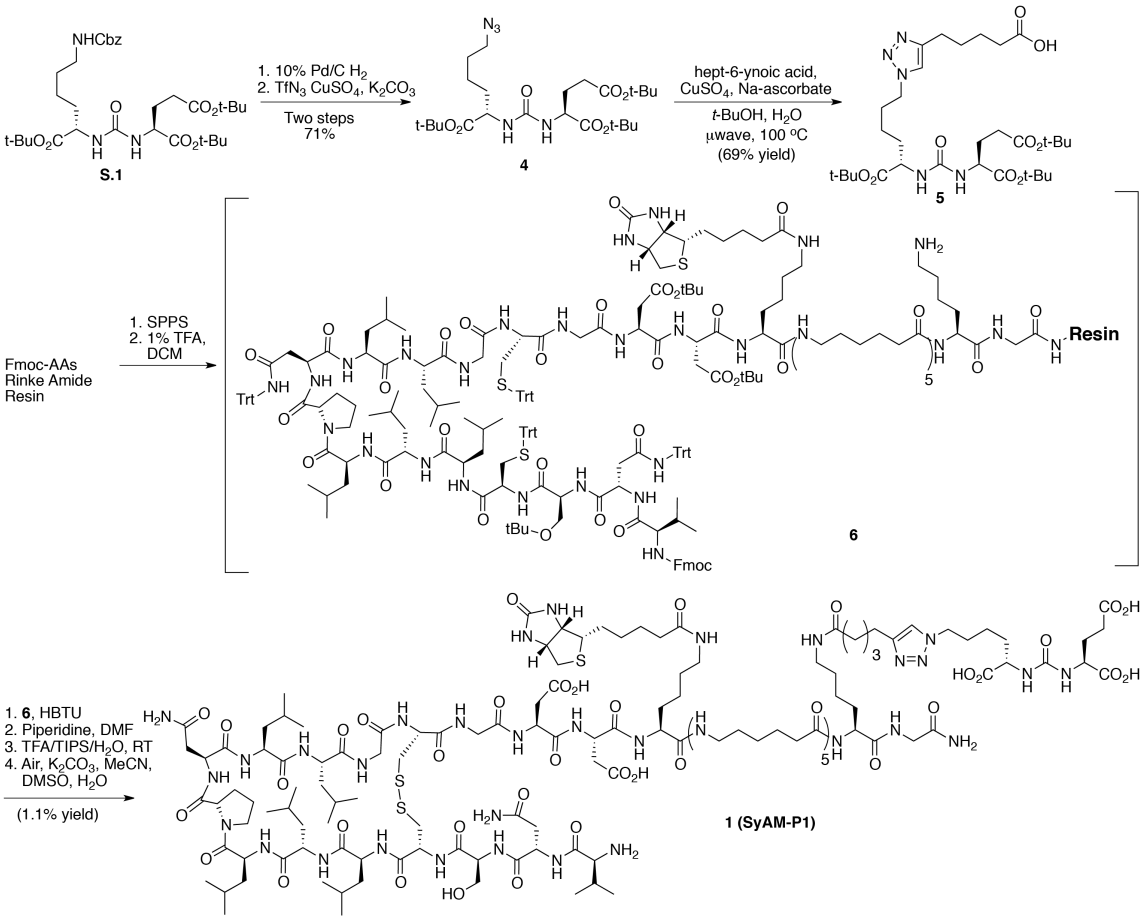
**General Synthetic Procedures:**

**Synthesis:** All starting materials and reagents were purchased from commercially available sources and used without further purification. Solvents for reactions (MeCN, DMF, DCM, $Et_2O$, THF and toluene) were dried using a Glass Contour purification system. Other solvents were used as received. $^1$H NMR spectra were recorded on either a 500 or 400 MHz Bruker spectrometer. $^1$H NMR shifts are measured using the solvent residual peak as the internal standard ($CDCl_3$ δ 7.26, MeOD δ 3.31), and reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, dt = doublet of triplet, q = quartet, m = multiplet), coupling constant (Hz), integration. $^{13}$C NMR spectra were recorded on a 500 MHz (125 MHz) Bruker spectrometer with complete proton decoupling. $^{13}$C NMR shifts are measured using the solvent residual peak as the internal standard ($CDCl_3$ δ 77.20, MeOD δ 49.00, or DMSO δ 39.52), and reported as chemical shifts. Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w). HPLC purification was performed with a Waters 2767 sample manager equipped with the 2545 binary gradient module and detection with a 2998 photodiode array equipped with a Sunfire™ Prep C18 column (10 x 150 mm). HRMS (ESI-TOF) analyses were performed on either a Waters Xevo QToF equipped with Z-spray electrospray ionization source or a 9.4T Bruker Qe FT-ICR MS.

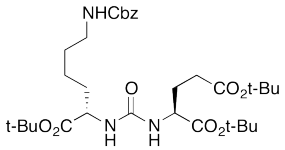
**Solid Phase Peptide Synthesis General Procedure:**

Solid-Phase Peptide Synthesis (SPPS) was performed in a CEM Discover Liberty Microwave Peptide Synthesizer. The amount of resin, (Rink amide resin 100-200 mesh, EMD Millipore, Cat. #855001, unless otherwise stated) amino acids, and reagents used in the synthesis were calculated using manufacture suggested protocols based on 0.1 mmol scale synthesis. Upon completion of SPPS, the resin was collected through vacuum filtration and rinsed several times with DCM. After drying in open air, the resin was then added to a flask containing the cleavage cocktail mixture (92:4:4 mixture of TFA:TIPS:$H_2O$) and stirred for the time specified below. Subsequently, the resin was filtered through a cotton-plugged pipette, and the filtrate was directly collected into a 50 mL conical tube containing 35 mL of cold (-78 °C) $Et_2O$, at which point a white precipitate formed. The suspension was centrifuged at 3000 RCF for 5 minutes and the supernatant decanted. The residual pellet was taken up in 50% MeCN/$H_2O$ and purified using reverse-phase HPLC. The desired HPLC fractions were combined and lyophilized to give the corresponding peptides as white powder.

**Synthesis**



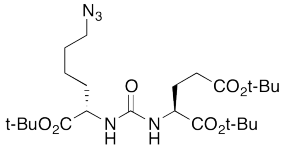

**Supplementary Scheme 1:** Synthesis of **1 (SyAM-P1)**




**S.1**

**(9S,13S)-tri-tert-butyl 3,11-dioxo-1-phenyl-2-oxa-4,10,12-triazapentadecane-9,13,15-tricarboxylate (S.1)**[1]

L-glutamic acid di-tertbutyl ester hydrochloride (1.0 g, 3.38 mmol, 1.0 equiv.) and TEA (1.54 mL, 11.09 mmol, 3.28 equiv.) were dissolved in DCM (30 mL) and cooled to -78 °C. Triphosgene (341 mg, 1.15 mmol, 0.34 equiv.) in DCM (10 mL) was added dropwise to the reaction mixture. Upon complete addition, the reaction was allowed to warm to room temperature (RT) and stir for 30 minutes. H-Lys(Z)-O*t*-Bu hydrochloride (757 mg, 2.03 mmol, 0.6 equiv.) was added, followed by TEA (283 μL, 2.03 mmol, 0.6 equiv.). The reaction was stirred at RT overnight (16 h). The reaction was then diluted with DCM (50 mL), and washed with $H_2O$ (2 x 100 mL). The crude mixture was dried over $Na_2SO_4$ and concentrated under reduced pressure. Column chromatography (3 x 25 cm Silica gel, 1.5:1 Hexanes:EtOAc) yielded **S.1** as a colorless oil (1.09g, 86%). **IR (thin film/KBr)** 3342 (br), 2976 (m), 1731 (s), 1650 (m), 1552 (s), 1454 (w), 1368 (m), 1255 (s), and 1153 (s) $cm^{-1}$; **$^1$H NMR (500 MHz, $CDCl_3$)** δ 7.35 (d, *J* = 3.8 Hz, 4H), 7.33-7.30 (m, 1H), 5.10 (d, *J* = 4.6 Hz, 2H), 5.06-5.01 (m, 2H), 4.99 (s, 1H), 4.34-4.31 (m, 2H), 3.20-3.18 (m, 2H), 2.36-2.23 (m, 2H), 2.10-2.03 (m, 1H), 1.88- 1.75 (m, 2H), 1.65-1.57 (m, 1H), 1.57-1.45 (m, 2H), 1.453 (s, 9H), 1.446 (s, 9H), 1.43 (s, 9H), 1.40-1.30 (m, 2H); **$^{13}$C NMR (125 MHz, $CDCl_3$)** δ 172.6, 172.5, 172.2, 136.8, 128.6, 128.5, 128.2, 82.2, 82.0, 80.7, 66.7, 53.4, 53.2, 40.7, 32.8, 31.7, 29.4, 28.5, 28.2, 28.1, 22.3; **HRMS (ES+)** calc'd for $C_{32}H_{52}N_3O_9$ (M+H) *m/z* 622.3625. Found (M+H) 622.3695.

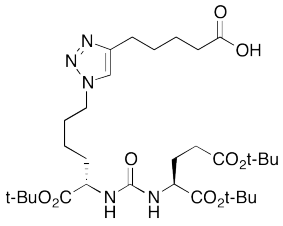



**4**

**(S)-di-tert-butyl 2-(3-((S)-6-azido-1-tert-butoxy-1-oxohexan-2- yl)ureido)pentanedioate (4)**[1]

**S.1** (2.35 g, 3.78 mmol, 1.0 equiv.) was dissolved in MeOH (40 ml) and was added dropwise to a vigorously stirred reaction flask containing dry 10% Pd/C (475 mg, 20% by mass). $H_2$ was bubbled through the solution for 1-2 min, and then ran for 13 h under a balloon of $H_2$. The reaction was deemed complete by TLC (Rf = 0.48 in 10% MeOH/DCM), plugged through celite, and concentrated to give yield (*S*)-di-*tert*-butyl 2-(3-((*S*)-6-amino-1-(*tert*-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate as a viscous oil, which was carried on without further purification. Sodium azide (2.629 g, 40.75 mmol, 10.0 equiv.) was dissolved in $H_2O$ (8 mL), and DCM (13 mL) was added. The reaction mixture was cooled to 0°C and triflic anhydride (1.4 mL, 8.09 mmol, 2.0 equiv.) was added. The solution was stirred for 3 h at RT, and the organic layer was separated from the aqueous layer.[2] The aqueous layer was extracted with DCM (3 x 4 mL). The organic layers were combined and washed with $Na_2CO_3$(aq) (10 mL) to give 25 ml of a solution of $TfN_3$ (theoretical concentration of 0.391 M). (*S*)-di-*tert*-butyl 2-(3-((*S*)-6-amino-1-(*tert*-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate (1.97 g, 4.04 mmol, 1.0 equiv.) was dissolved in $H_2O$ (14 mL) and MeOH (29 mL). To this solution were added $CuSO_4{\cdot}5H_2O$ (10.1 mg, 0.04 mmol, 0.01 equiv.) and $K_2CO_3$ (837.5 mg,

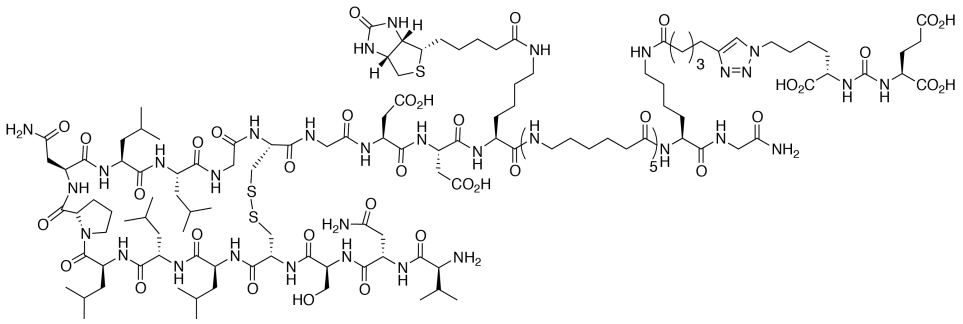
6.06 mmol, 1.5 equiv.). The $TfN_3$ solution (25 ml, 8.09 mmol, 2.0 equiv.) was added rapidly to the stirring solution of (*S*)-di-*tert*-butyl 2-(3-((*S*)-6-amino-1-(*tert*-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate, and the reaction stirred for 19 h at RT. The organic layer was separated from the aqueous layer, and the $H_2O$/MeOH layer was extracted once with DCM. The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure, and purified by column chromatography (3 x 25 cm Silica, 10% MeOH in DCM) to yield **4** as a white solid (1.440 g, 71%). Rf =0.68 in 10% MeOH:DCM. **IR (Thin film / NaCl)** 3335 (br), 2980 (m), 2933 (m), 2868 (w), 2097 (s), 1733 (s), 1635 (s), 1560 (m), 1368 (s), 1257 (m), and 1155 (s) $cm^{-1}$; **$^1$H NMR (500 MHz, $CDCl_3$)** δ 5.01 (d, *J* = 8.25 Hz, 2H), 4.34 (m, 2H), 3.26 (t, *J* = 7.4 Hz, 2H), 2.35-2.25 (m, 2H), 2.09-2.05 (m, 1H), 1.87-1.76 (m, 2H), 1.66-1.55 (m, 3H), 1.46 (s, 18H), 1.43 (s, 9H), 1.45-1.35 (m, 2H) ppm; **$^{13}$C NMR (125 MHz, $CDCl_3$)** δ 172.6, 172.4, 172.2, 156.8, 82.3, 82.1, 80.7, 53.4, 53.2, 51.3, 33.0, 31.7, 28.6, 28.5, 28.2, 28.1, 22.4 ppm; **$[\alpha]_D$** = -0.03o (*c* = 0.88, MeOH). **HRMS (ES+)** calc'd for $C_{24}H_{44}N_5O_7$ (M+H) *m/z* 514.3235. Found (M+H) 514.3225.




**5**

**5-(1-((*R*)-6-(*tert*-butoxy)-5-(3-((*S*)-1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)ureido)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)pentanoic acid (5)**

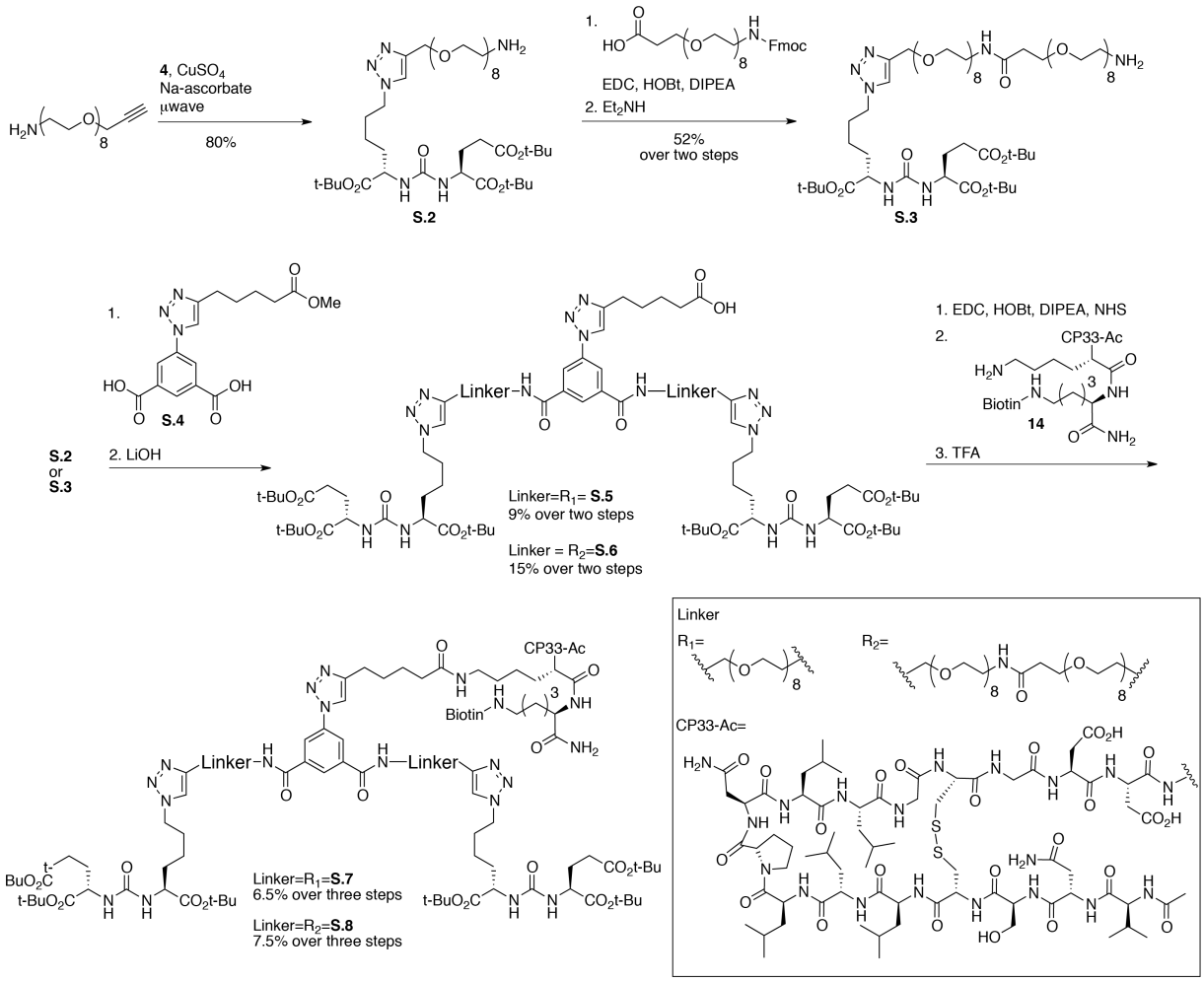
A mixture of **4** (98 mg, 0.191 mmol, 1.0 equiv.) and heptynoic acid (120 mg, 0.954 mmol, 5.0 equiv.) was dissolved in a mixture of $H_2O$ (1.25 mL) and *t*-BuOH (1.25 mL) in a 5 mL microwave (μwave) reaction tube. To this solution were added 0.1 M sodium ascorbate (0.059 mmol, 0.2 equiv.) and 0.1 M $CuSO_4$ (0.012 mmol, 0.04 equiv.).[3] The tube was capped, and subjected to μwave irradiation for 10 minutes at 110 °C. The reaction was then concentrated under reduced pressure, and chromatographed (1 x 15 cm silica gel, 20% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$ + 1% TFA) to yield **5** as a colorless oil (84 mg, 69%) **$^1$H NMR (500 MHz, MeOH)** δ 7.77 (s, 1H), 4.39 (t, *J* = 6.9 Hz, 2H), 4.20 (ddd, *J* = 22.7, 8.2, 5.2 Hz, 2H), 2.73 (t, *J* = 7.3 Hz, 2H), 2.40 – 2.27 (m, 5H), 2.26 – 2.18 (m, 1H), 2.07 (dddd, *J* = 13.8, 8.7, 7.0, 5.0 Hz, 1H), 1.94 (ddd, *J* = 8.9, 6.8, 4.6 Hz, 2H), 1.88 – 1.63 (m, 9H), 1.62 – 1.52 (m, 1H), 1.49 (s, 9H), 1.46 (s, 9H), 1.46 (s, 9H), 1.44 – 1.31 (m, 3H). **$^{13}$C NMR (126 MHz, MeOH)** δ 177.62, 174.15, 174.07, 173.85, 160.27, 123.58, 85.06, 83.22, 83.10, 82.16, 70.22, 55.10, 54.63, 51.45, 35.03, 33.37, 32.96, 31.20, 30.38, 29.52, 29.45, 28.85, 28.78, 26.45, 25.96, 25.60, 23.89, 19.21. **HRMS (ES+)** calc'd for $C_{31}H_{53}N_5O_9$ (M+H) *m/z* 640.7806. Found (M+H) 640.7809



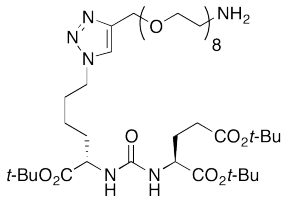
**1 (SyAM-P1)**

**SyAM-P1 (1)**

Standard solid phase peptide synthesis (SPPS) was performed on a 0.1 mmol resin (rink amide resin 100-200 mesh, EMD Millipore, Cat. #855001) scale to yield fully protected CP33[4] with linker (Fmoc-VN(Trt)S(tBu)C(Trt)LLLPN(Trt)LLGC(Trt)GD(tBu)D(tBu)K(biotin)-Ahx$_5$-Lys(Mtt)-G-Resin). While on resin the Mtt group was removed by washing with 5 mL of 1% TFA in DCM three times. Resin was then neutralized by washing with DMF (3x, approx. 10 mL per wash) with 0.1 mM DIPEA. **5** (160 mg, 0.25 mmol, 2.5 equiv.), dissolved in 3 mL of DMF, was added to the resin with HBTU (95 mg, 0.25 mmol, 2.5 equiv.) and 86 μL of DIPEA (64mg, 5 mmol, 5 equiv.). The mixture was subjected to μwave irradiation for 10 minutes at 75 °C. Resin was then washed with DMF (3x, approx. 10 mL per wash), and the Fmoc group was removed with 20% piperidine in DMF for 20 minutes at RT. Global deprotection and cleavage from solid support was achieved using standard conditions of 92:4:4 mixture of TFA:$H_2O$:TIPS stirring for 90 minutes at RT. The cleavage mixture was filtered through a cotton-plugged pipette to remove resin, and the filtrate was collected into a 50 mL conical tube containing 35 mL of cold (-78 °C) $Et_2O$, forming a white precipitate. The suspension was centrifuged at 3000 RCF for 5 minutes and the supernatant decanted. The residual pellet was dissolved in 25 mL of 20% MeCN in $H_2O$ containing 1 mL of DMSO and 40 mg of potassium carbonate. This solution was stirred open to air for 48 hours to form the disulfide bond.[5] After oxidation to form the disulfide bond, the peptide was purified using reverse-phase HPLC (C18 reverse phase, 5 mL/min, 25%-70% MeCN in $H_2O$ with 0.1% TFA over 40 minutes) to yield **1** as a white powder after lyophilization (5.1 mg, 1.1%). **HRMS (ES+)** calc'd for $C_{142}H_{238}N_{36}O_{41}S_3K$ $(M+K)^+$ *m/z* 3238.6439. Found $(M+K)^+$ 3238.6549




**Supplementary Scheme 2:** Synthesis of **S.7** and **S.8**

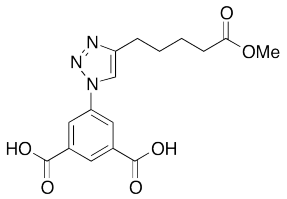


**S.2**

**(S)-di-tert-butyl 2-(3-((S)-6-(4-(25-amino-2,5,8,11,14,17,20,23-octaoxapentacosyl)-1H-1,2,3-triazol-1-yl)-1-(tert-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate (S.2)**

A mixture of **S.2** (152 mg, 0.295 mmol, 1.0 equiv.) and 3,6,9,12,15,18,21,24-octaoxaheptacos-26-yn-1-amine[1] (120 mg, 0.295 mmol, 1.0 equiv.) was dissolved in a mixture of $H_2O$ (1.1 mL) and *t*-butanol (1.1 mL) in a 5 mL μwave reaction tube. To this mixture was added 0.1 M sodium ascorbate (0.6 mL, 0.2 equiv.) and 0.1 M copper (II) sulfate (0.12 mL, 0.04 equiv.). The tube was capped, and subject to μwave radiation for 2.5 minutes at 110 °C. The reaction was then concentrated under reduced pressure, and chromatographed (1 x 15 cm silica gel, 10% MeOH in DCM, then 10% MeOH in DCM + 2.5% $Et_3N$) to yield **S.2** (254 mg, 80%) as a brown oil. **IR (thin film)** 2869 (m), 1729 (s), 1680 (w), 1534 (m), 1456 (w), 1367 (m), 1252 (w), 1152 (s), 1113 (s) $cm^{-1}$. **$^1$H NMR (400 MHz, $CDCl_3$)** δ

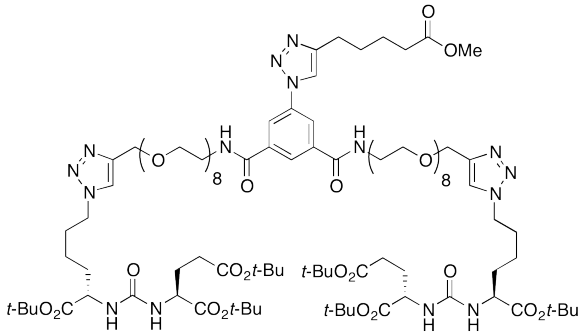
7.71 (s, 1H), 5.32 (d, *J* = 8 Hz, 1H), 5.25 (d, *J* = 8 Hz, 1H), 4.72-4.64 (dd, *J* = 8 Hz, 2H), 4.38-4.28 (m, 4H), 3.68-3.64 (m, 30 H), 2.98-2.93 (m, 2H), 2.35-2.28 (m, 2H), 2.08-2.05 (m, 1H), 1.97-1.77 (m, 4H), 1.65-1.59 (m, 1H), 1.46 (s, 9H), 1.43 (s, 18H), 1.49-1.27 (m, 2H). **$^{13}$C NMR (100 MHz, $CDCl_3$)** δ 172.5, 172.1, 156.9, 145.0, 123.0, 81.9, 81.9, 90.5, 70.6, 70.5, 70.5, 70.5, 70.4, 70.4, 70.4, 70.2, 69.6, 64.6, 53.1, 53.0, 49.9, 32.3, 31.7, 29.6, 28.3, 28.1, 28.1, 28.0, 21.9. **HRMS (ES+)** calc'd for $C_{43}H_{81}N_6O_{15}$ (M+H) *m/z* 921.5754. Found (M+H) 921.5754.




**S.4**

**5-(4-(5-methoxy-5-oxopentyl)-1*H*-1,2,3-triazol-1-yl)isophthalic acid (S.4)**

A mixture of azidoisophthalic acid (100 mg, 0.483 mmol, 1.0 equiv.) and methyl hept-6-ynoate (100 mg, 0.714 mmol, 1.45 equiv.) was dissolved in a mixture of $H_2O$ (1.7 mL) and *t*-BuOH (1.7 mL) in a 5 mL μwave reaction tube. To this mixture 0.1 M sodium ascorbate (0.059 mmol, 0.2 equiv.) and 0.1 M copper (II) sulfate (0.012 mmol, 0.04 equiv.) was added. The tube was capped, and subjected to μwave irradiation for 2.5 minutes at 110 °C. The reaction was then concentrated under reduced pressure, and chromatographed (1 x 15 cm silica gel, 20% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$ + 1% TFA) to yield **S.4** as a beige colored solid. **IR (thin film)** 3151 (w), 2951 (w), 1721 (s), 1604 (w), 1463 (w), 1291 (m), 1248 (m), 1071 (m) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 8.70 (s, 1H), 8.66 (s, 2H), 8.51 (s, 1H), 3.66 (s, 3H), 2.83 (t, *J* = 7.4 Hz, 2H), 2.41 (t, *J* = 6.8 Hz, 2H), 1.81-1.70 (m, 4H). **$^{13}$C NMR (100 MHz, DMSO-$d_6$)** δ 173.3, 165.9, 148.3, 137.2, 133.5, 129.1, 123.7, 120.6, 118.0, 51.2, 33.0, 27.9, 24.7, 24.0. **HRMS (ES+)** calc'd for $C_{16}H_{18}N_3O_6$ (M+H) *m/z* 348.1190. Found (M+H) 348.1184.

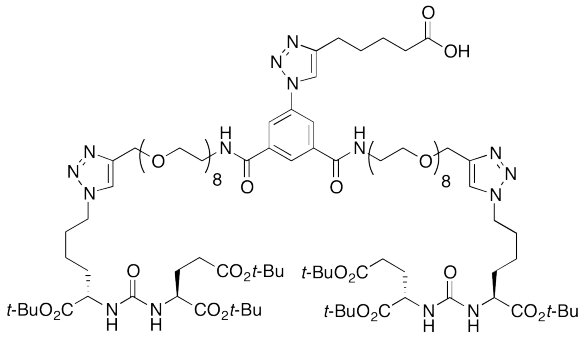



**S.5a**

**(2*S*,2'*S*)-tetra-*tert*-butyl 2,2'-(((((2*S*,2'*S*)-(4,4'-(1,1'-(5-(4-(5-methoxy-5-oxopentyl)-1*H*-1,2,3-triazol-1-yl)-1,3-phenylene)bis(1-oxo-5,8,11,14,17,20,23,26-octaoxa-2-azaheptacosane-27,1-diyl))bis(1*H*-1,2,3-triazole-4,1-diyl))bis(1-*tert*-butyl-1-oxohexane-6,2-diyl))bis(azanediyl))bis(carbonyl))bis(azanediyl))dipentanedioate (S.5a)**

In a flame dried round bottom flask **S.4** (20 mg, 0.06 mmol, 1 equiv.) was dissolved in 1 mL of dry DCM and to this solution was added EDC (29 mg, 0.15 mmol, 2.5 equiv.) and HOBt (23 mg, 0.15 mmol, 2.5 equiv.), followed by a solution of **S.2** (122 mg, 0.133 mmol, 2.2 equiv.) in 1 mL of dry DCM and pyridine (15 μL, 0.181 mmol, 3

equiv.). The reaction was stirred at RT for 13 hours, after which it was concentrated under reduced pressure at 37 °C and chromatographed (silica gel, 2 x 15 cm, 5% MeOH in $CHCl_3$) to give **S.5a** as a pale brown oil (90 mg, 73%). **IR (thin film)** 2867 (w), 2405 (br), 1728 (s), 1661 (m), 1456 (s), 1367 (m), 1250 (w), 1149 (s), 1098 (s), 846 (w) $cm^{-1}$. **$^1$H NMR (500 MHz, MeOD)** δ 8.50 (s, 1H), 8.50 (s, 1H), 8.42 (t, *J* = 1.5 Hz, 1H), 7.96 (s, 2H), 6.36-6.33 (dd, *J* = 4, 4.5 Hz, 3H), 4.60 (s, 4H), 4.40 (t, *J* = 7.5 Hz, 4H), 4.22-4.11 (m, 4H), 3.70 (t, *J* = 5.5 Hz, 4H), 3.67-3.54 (m, 66 H), 2.83 (t, *J* = 7 Hz, 2H), 2.40 (t, *J* = 7 Hz, 2H), 2.37-2.27 (m, 4H), 2.07-2.00 (m, 2H), 1.96-1.89 (m, 4H), 1.84-1.70 (m, 8H), 1.69-1.61 (m, 2H), 1.47 (s, 19H), 1.44 (s, 21H), 1.41 (s, 17H), 1.40-1.34 (m, 4H). **$^{13}$C NMR (125 MHz, MeOD)** δ 175.6, 173.7, 173,6, 173.4, 167.9, 159.9, 150.1, 146.0, 138.7, 138.0, 127.4, 125.0, 122.8, 121.8, 82.8, 82.6, 81.7, 71.6, 71.6, 71.5, 71.3, 70.8, 70.4, 65.0, 54.7, 54.1, 52.1, 51.1, 41.3, 34.4, 32.9, 32.5, 30.8, 29.8, 29.0, 28.4, 28.3, 26.0, 23.5. **HRMS (ES+)** calc'd for $C_{102}H_{175}N_{15}O_{34}$ $(M+2H)^{+2}$ *m/z* 1077.1185. Found $(M+2H)^{+2}$ 1077.1271.

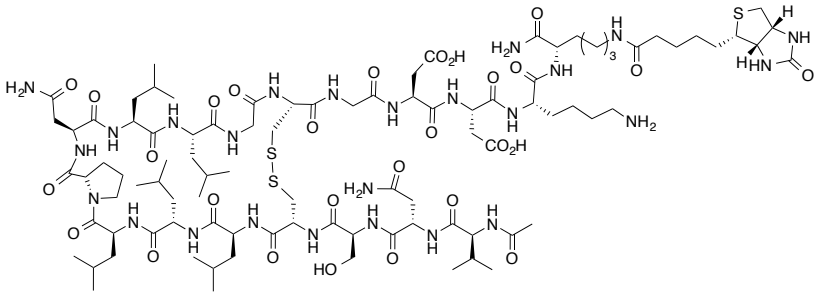


**S.5**

**5-(1-(3,5-bis((1-(1-((*S*)-5-(3-((*S*)-1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)ureido)-6-*tert*-butyl-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)-2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl)carbamoyl)phenyl)-1*H*-1,2,3-triazol-4-yl)pentanoic acid (S.5)**

**S.5a** (90 mg, 0.042 mmol, 1 equiv.) was dissolved in MeOH (1 mL). To this solution was added 1 M LiOH in $H_2O$ (4 equiv.). The reaction was allowed to stir at RT for 2 hours, after which another 4 equiv. of 1 M LiOH in $H_2O$ was added. The reaction was allowed to stir at RT for another 2 hours, after which it was neutralized with 6 equiv. of 1 M HCl in $H_2O$. The reaction was concentrated and chromatographed (1 x 15 cm silica gel, 20% MeOH in $CHCl_3$). The fractions containing product were concentrated and taken up with 80% MeCN in $H_2O$ with 0.1% TFA and purified using reverse phase HPLC (C18, 5 mL/min, 50%-75% MeCN in $H_2O$ with 0.1% TFA over 40 minutes). The product was isolated and lyophilized to give **S.5** as a white powder (10 mg, 12%). **IR (thin film)** 3341 (br), 3108 (w), 2931 (m), 1729 (s), 1667 (s), 1556 (w), 1457 (w), 1151 (s) $cm^{-1}$. **$^1$H NMR (500 MHz, MeOD)** δ 8.73 (t, *J* = 5.5 Hz, 1H), 8.51 (s, 3H), 8.42 (s, 1H), 7.97 (s, 2H), 4.64 (s, 1H), 4.60 (s, 4H), 4.41 (t, *J* = 7.2 Hz, 4H), 4.18 (dd, *J* = 5, 9 Hz, 2H), 4.13 (dd, *J* = 5, 8 Hz, 2H), 4.06 (s, 1H), 4.01 (s, 1H), 3.99-3.96 (m, 1H), 3.74-3.55 (m, 80H), 3.48-3.44 (m, 1H), 3.17-3.13 (m, 1H), 2.84 (t, *J* = 7.2 Hz, 2H), 2.66 (s, 1H), 2.39-2.29 (m, 6H), 2.07-2.00 (m, 2H), 1.96-1.89 (m, 4H), 1.84-1.76 (m, 6H), 1.75-1.70 (m, 2H), 1.69-1.61 (m, 2H), 1.60-1.52 (m, 2H), 1.48-1.41 (m, 4H), 1.47 (s, 18H), 1.44 (s, 18H), 1.43 (s, 18H), 1.40-1.30 (m, 6H). **$^{13}$C NMR (125 MHz, MeOD)** δ 177.2, 173.7, 173.7, 173.4, 168.0, 159.9, 138.8, 138.0, 127.4, 125.0, 122.7, 121.8, 82.8, 81.8, 71.5, 71.4, 71.4, 71.4, 71.3, 70.7, 70.5,

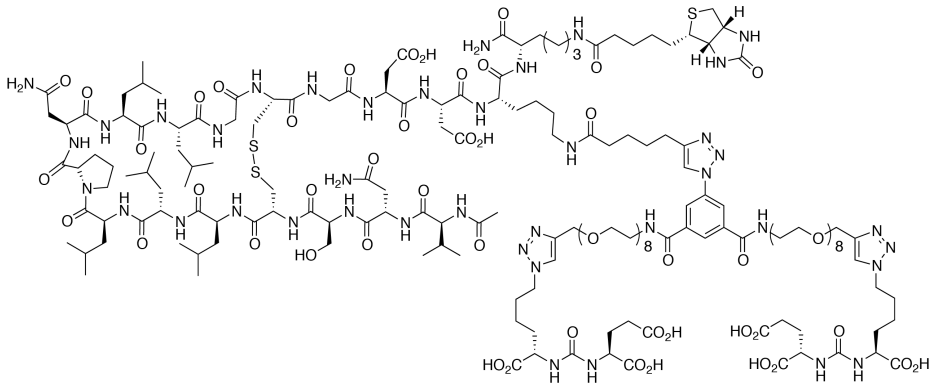
65.0, 54.7, 54.2, 41.3, 34.6, 32.9, 32.5, 30.8, 29.8, 29.0, 28.4, 28.3, 26.1, 25.5, 23.5. **HRMS (ES+)** calc'd for $C_{101}H_{173}N_{15}O_{34}$ $(M+2H)^{+2}$ *m/z* 1070.1107. Found $(M+2H)^{+2}$1070.1426.



**14**

**(3*S*,6*S*,9*S*,12*S*)-3-(2-((3*S*,6*S*,9*S*,15*R*,20*R*,23*S*,26*S*,29*S*,34a*S*)-20-((*S*)-2-((*S*)-2-((*S*)-2-acetamido-3-methylbutanamido)-4-amino-4-oxobutanamido)-3-hydroxypropanamido)-3-(2-amino-2-oxoethyl)-6,9,23,26,29-pentaisobutyl-1,4,7,10,13,21,24,27,30-nonaoxodotriacontahydropyrrolo[2,1-*s*][1,2,5,8,11,14,17,20,23,26,29]dithianonaazacyclodotriacontine-15-carboxamido)acetamido)-9-(4-aminobutyl)-12-carbamoyl-6-(carboxymethyl)-4,7,10,18-tetraoxo-22-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-5,8,11,17-tetraazadocosan-1-oic acid (14)**

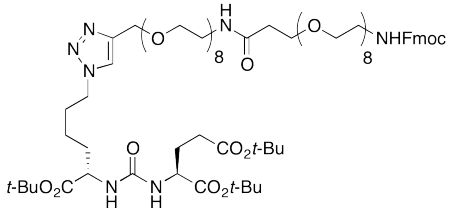
**14** was synthesized using standard SPPS as previously described on a 0.1 mmol scale to generate Ac-VN(Trt)S(tBu)C(Trt)LLLPN(Trt)LLGC(Trt)GD(tBu)D(tBu)K(Boc)K(biotin)-Resin. Global deprotection and cleavage from solid support was performed with 92:4:4 mixture of TFA:$H_2O$:TIPS for 90 minutes, stirring at RT. The suspension was centrifuged at 3000 RCF for 5 minutes and the supernatant decanted. The cleavage mixture was filtered through a cotton-plugged pipette to remove resin, and the filtrate was collected into a 50 mL conical tube containing 35 mL of cold (-78 °C) $Et_2O$, forming a white precipitate. The suspension was centrifuged at 3000 RCF for 5 minutes and the supernatant decanted. The residual pellet was dissolved in 25 mL of 20% MeCN in $H_2O$, containing 1 mL of DMSO and 40 mg of potassium carbonate. This solution was stirred open to air for 48 hours to form the disulfide bond. After oxidation, the peptide was purified using reverse-phase HPLC (C18 reverse phase, 5 mL/min, 20%-55% MeCN in $H_2O$ with 0.1% TFA over 30 minutes). To yield **14** as a white powder after lyophilization (53 mg, 25%)**. HRMS (ES+)** calc'd for $C_{93}H_{157}N_{25}O_{28}S_3$ $(M+2H)^{+2}$ *m/z* 1084.0318. Found $(M+2H)^{+2}$ 1083.9846.



**S.7**

**(2*S*,2'*S*)-2,2'-(((((1*S*,1'*S*)-(4,4'-(1,1'-(5-(4-((6*S*,9*S*,12*S*)-1-((3*S*,6*S*,9*S*,15*R*,20*R*,23*S*,26*S*,29*S*,34a*S*)-20-((*S*)-2-((*S*)-2-acetamido-4-amino-4-oxobutanamido)-3-hydroxypropanamido)-3-(2-amino-2-oxoethyl)-6,9,23,26,29-pentaisobutyl-1,4,7,10,13,21,24,27,30-nonaoxodotriacontahydropyrrolo[2,1-*s*][1,2,5,8,11,14,17,20,23,26,29]dithianonaazacyclodotriacontin-15-yl)-12-(((*S*)-1-amino-1-oxo-6-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexan-2-yl)carbamoyl)-6,9-bis(carboxymethyl)-1,4,7,10,18-pentaoxo-2,5,8,11,17-pentaazadocosan-22-yl)-1*H*-1,2,3-triazol-1-yl)-1,3-phenylene)bis(1-oxo-5,8,11,14,17,20,23,26-octaoxa-2-azaheptacosane-27,1-diyl))bis(1*H*-1,2,3-triazole-4,1-diyl))bis(1-carboxypentane-5,1-diyl))bis(azanediyl))bis(carbonyl))bis(azanediyl))dipentanedioic acid (S.7)**

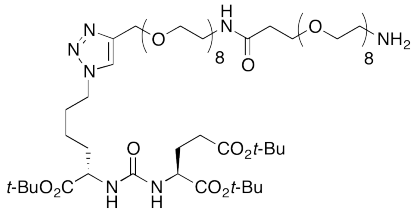
**S.5** (45 mg, 0.021 mmol, 1.0 equiv.) was dissolved in DCM. EDC·HCl (4.4 mg, 0.023 mmol, 1.1 equiv.), HOBt (3.5 mg, 0.023 mmol, 1.1 equiv.), and NHS (3.5 mg, 0.023 mmol, 1.1 equiv.) were added sequentially, followed by the addition of 17 μL of DIPEA (13.5 mg, 0.105 mmol, 5.0 equiv.), and the reaction stirred at RT for 6 hours, with conversion monitored via LC-MS. The reaction was concentrated under reduced pressure, and a mixture of 95% TFA, 2.5% PBS, 2.5% TIPS was added. The reaction was allowed to stir for 30 minutes, after which it was concentrated under reduced pressure. To the flask was added 1 mL of PBS and 1.5 mL of a saturated sodium bicarbonate solution, followed by **14** (16 mg, 0.007 mmol, 0.3 equiv.). The reaction was allowed to stir at RT for 12 hours, and subsequently purified by reverse phase HPLC (C18, 5 mL/min, 30%-42% MeCN in $H_2O$ 0.1% TFA over 66 minutes). The fractions containing product were collected and lyophilized to give **S.7** as a white powder (1.2 mg, 6.5%). **HRMS (ES+)** calc'd for $C_{170}H_{275}N_{40}O_{61}S_3NaK$ $(M\text{-}H+NaK)^+$ *m/z* 4010.8343. Found $(M\text{-}H+NaK)^+$ 4010.8295.



**S.3a**

**(*S*)-di-*tert*-butyl 2-(3-((*S*)-6-(4-(1-(9*H*-fluoren-9-yl)-3,31-dioxo-2,7,10,13,16,19,22,25,28,35,38,41,44,47,50,53,56-heptadecaoxa-4,32-diazaheptapentacontan-57-yl)-1*H*-1,2,3-triazol-1-yl)-1-(*tert*-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate (S.3a)**

**S.2** (90 mg, 0.135 mmol, 1 equiv.) was dissolved in 1 mL of dry DCM in a flame-dried flask, and EDC·HCl (29 mg, 0.15 mmol, 1.1 equiv.) and HOBt·$H_2O$ (23 mg, 0.15 mmol, 1.1 equiv.) were sequentially added. To this a solution of Fmoc-N-amido-dPEG8-acid (Cat# 10273 Quanta Biodesign Ltd. 138 mg, 0.15 mmol, 1.1 equiv.) in 1 mL of dry DCM and DIPEA (28 μL, 0.162 mmol, 1.2 equiv.) was also added. The reaction was allowed to stir for 2.5 hours, after which 1 mL of MeOH was added. The reaction was then concentrated under reduced pressure and chromatographed (silica gel, 1 x 15 cm, 2.5% MeOH in $CHCl_3$, then 5% MeOH in $CHCl_3$, then 10% MeOH in $CHCl_3$) to yield **S.3a** as a clear oil (149 mg, 70%). **IR (thin film)** 3332 (br), 2868 (m), 1727 (m), 1672 (w), 1547 (m), 1451 (w), 1367 (w), 1251 (m), 1149 (s), 1110 (s) $cm^{-1}$. **$^1$H NMR (500 MHz, $CDCl_3$)** δ 7.75 (d, *J* = 7.5 Hz, 2H) 7.62 (s, 1H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 6.63 (s, 1H), 5.42 (s, 1H), 5.24 (d, J = 8 Hz, 1H), 5.15 (d, *J* = 8 Hz, 1H), 4.67 (dd, *J* = 10 Hz, 25 Hz, 2H), 4.40 (d, *J* = 7 Hz, 2H), 4.37-4.27 (m, 4H), 4.22 (t, *J* = 7 Hz, 1H), 3.72 (t, *J* = 6 Hz, 2H), 3.68-3.55 (m, 66H), 3.54 (t, *J* = 5 Hz, 2H), 3.43 (dd, *J* = 5.5, 12 Hz, 2H), 3.39 (dd, *J* = 5.5, 12 Hz, 2H), 2.46 (t, *J* = 6 Hz, 2H), 2.37-2.24 (m, 2H) 2.10-2.03 (m, 2H), 1.95-1.76 (m, 5H), 1.64-1.57 (m, 1H), 1.46 (s, 9H), 1.43 (s, 18H), 1.39-1.29 (m, 2H). **$^{13}$C NMR (125 MHz, $CDCl_3$)** δ 172.4, 172.1, 172.1, 171.4, 156.8, 145.1, 144.0, 141.3, 127.7, 127.0, 125.1, 122.9, 120.0, 81.9, 81.9, 80.6, 70.6, 70.6, 70.6, 70.5, 70.4, 70.4, 70.3, 70.3. 70.1, 69.9, 69.6, 67.3, 66.6, 64.6, 53.1, 53.0, 49.9, 47.3, 41.0, 39.2, 37.0, 32.4, 31.7, 29.6, 28.3, 28.1, 28.1, 28.0, 21.8. **HRMS (ES+)** calc'd for $C_{77}H_{128}N_7O_{26}$ (M+H) *m/z* 1566.8904. Found (M+H) 1566.8892.

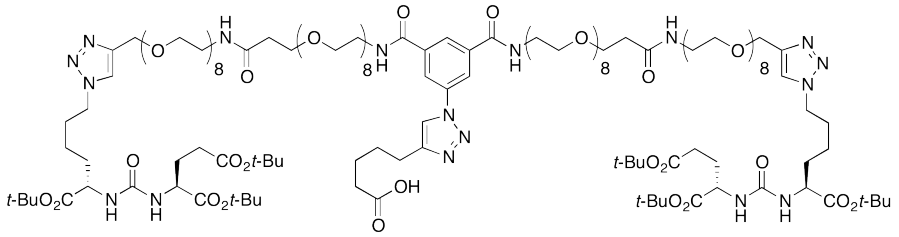


**S.5**

**(*S*)-di-*tert*-butyl 2-(3-((*S*)-6-(4-(53-amino-27-oxo-2,5,8,11,14,17,20,23,30,33,36,39,42,45,48,51-hexadecaoxa-26-azatripentacontyl)-1*H*-1,2,3-triazol-1-yl)-1-(*tert*-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate (S.3)**

**S.3a** (135 mg) was dissolved in 3 mL of $Et_2NH$ and 3 mL of DCM. The reaction was allowed to stir at RT for 3 hours, after which it was concentrated under reduced pressure and chromatographed (silica gel, 2 x 15 cm, 2.5% MeOH in $CHCl_3$, then 5% MeOH in $CHCl_3$, then 10% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$ + 2.5% $Et_3N$) to yield **S.3** as a clear oil (86 mg, 75%). **$^1$H NMR (400 MHz, MeOD)** δ 8.01 (s, 1H), 4.64 (s, 2H), 4.42 (t, *J* = 7.2 Hz, 3H), 4.20-4.17 (m, 1H), 4.15-4.11 (m, 1H), 3.77-3.63 (m, 72H), 3.54 (t, *J* = 5.6 Hz, 2H), 3.37 (t, *J* = 5.6 Hz, 2H), 3.14 (t, *J* = 5.2 Hz, 2H), 3.00 (dd, *J* = 7.2, 15.2 Hz, 1H), 2.61 (t, *J* = 6 Hz, 1H), 2.48 (t, *J* = 6 Hz, 2H), 2.38-2.25 (m, 2H), 2.08-2.00 (m, 1H), 1.98-1.90 (m, 2H), 1.85-1.74 (m, 2H), 1.70-1.60 (m, 1H), 1.48 (s, 9H), 1.44 (s, 18H), 1.40-1.36 (m, 2H), 1.29 (t, *J* = 7.2 Hz, 2H). **$^{13}$C NMR (100 MHz, MeOD)** δ 173.9, 173.8, 173.7, 173.5, 159.9, 146.0, 125.1, 82.8, 82.7, 81.8, 71.6, 71.5, 71.5, 71.4, 71.4, 71.3, 71.3, 71.2, 71.2, 71.1, 71.1,

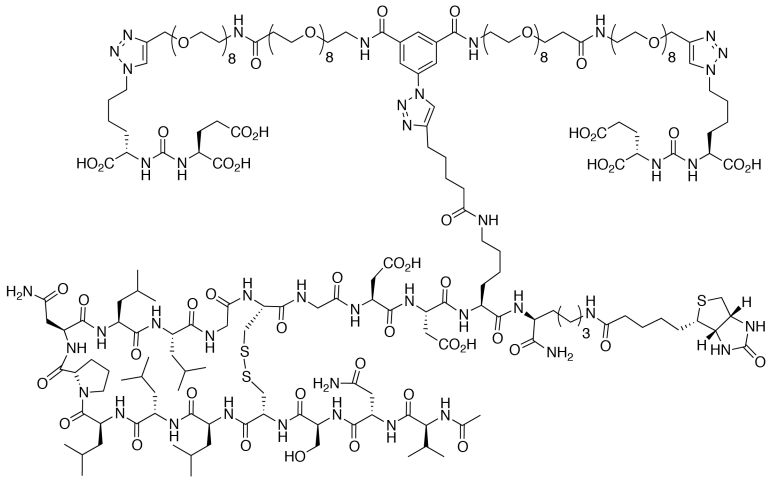
70.8, 70.7, 70.6, 68.5, 68.3, 67.6, 65.0, 54.7, 54.2, 52.2, 51.1, 43.7, 40.8, 40.5, 37.5, 35.7, 32.9, 32.5, 30.9, 29.0, 28.4, 28.4, 23.5. **HRMS (ES+)** calc'd for $C_{62}H_{118}N_7O_{24}$ (M+H) *m/z* 1344.8150. Found (M+H) 1344.8251.



**S.6**

**5-(1-(3,5-bis((1-(1-((*S*)-5-(3-((*S*)-1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)ureido)-6-*tert*-butoxy -6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)-27-oxo-2,5,8,11,14,17,20,23,30,33,36,39,42,45,48,51-hexadecaoxa-26-azatripentacontan-53-yl)carbamoyl)phenyl)-1*H*-1,2,3-triazol-4-yl)pentanoic acid (S.6)**

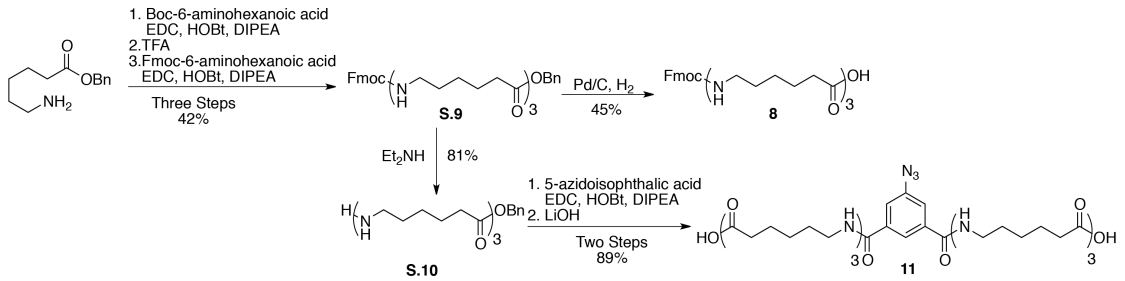
**S.4** (8 mg, 0.023 mmol, 1.0 equiv.) was dissolved in DCM (1 mL). To this solution were added EDC·HCl (15 mg, 0.076 mmol, 3.3 equiv.), HOBt·$H_2O$ (12 mg, 0.076 mmol, 3.3 equiv.), and a solution of **S.3** (102 mg, 0.076 mmol, 3.3 equiv.) in DCM (4 mL). Pyridine (5.5 mg, 5.7 μL, 0.7 mmol, 3.0 equiv.) was added to the reaction, which was allowed to proceed at RT for 16 hours. At this point, the reaction was concentrated and partially purified (1 x 15 cm, silica gel, 10% MeOH in $CHCl_3$). The fractions containing product were concentrated and immediately taken up with MeOH (1 mL). 1 M LiOH in $H_2O$ (92 μL, 0.092 mmol, 4 equiv.) was added to the solution, and the reaction was allowed to stir at RT for 3 hours, after which 1 M LiOH in $H_2O$ (92 μL, 0.092 mmol, 4 equiv.) was added. The reaction was allowed to stir at RT for an additional 2 hours, at which point it was neutralized with 1 M HCl in $H_2O$ (138 mL, 0.138 mmol, 6.0 equiv.). The reaction was concentrated, taken up with 80% MeCN in $H_2O$ with 0.1% TFA, and purified by HPLC (reverse phase, C18, 5 mL/min, 50%-75% MeCN in $H_2O$ with 0.1% TFA over 40 minutes). The product was isolated and lyophilized to yield **S.6** as a white powder (8 mg, 20%). **IR (thin film)** 2873 (m), 1729 (m), 1666 (s), 1555 (w), 1456 (w), 1368 (w), 1140 (s) 950 (w), 846 (w) $cm^{-1}$. **$^1$H NMR (500 MHz, MeOD)** δ 8.72 (t, *J* = 5.2 Hz, 1H), 8.51 (s), 3H), 8.42 (s, 1H), 7.99 (s, 2H), 7.90 (s), 3H), 4.62 (s, 4H), 4.41 (t, *J* = 7 Hz, 4H), 4.18 (dd, *J* = 5, 7.2 Hz, 2H), 4.13 (dd, *J* = 5, 6 Hz, 2H), 3.71-3.69 (m, 10H), 3.66-3.57 (m, 118H), 3.52 (t, *J* = 5.5 Hz, 4H), 3.36-3.34 (m, 8H), 2.84 (t, *J* = 6 Hz, 2H), 2.44 (t, *J* = 6.2 Hz, 4H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.33-2.28 (m, 4H), 2.06-2.00 (m, 2H), 1.96-1.90 (m, 4H), 1.83-1.77 (m, 6H), 1.75-1.68 (m, 2H), 1.67-1.63 (m, 2H), 1.46 (s, 18H), 1.44 (s, 18H), 1.43 (s, 18H), 1.39-1.34 (m, 4H). **$^{13}$C NMR (125 MHz, MeOD)** δ 177.2, 174.0, 173.7, 173.7, 173.5, 167.9, 159.9, 150.2, 146.0, 138.8, 138.0, 127.4, 125.1, 122.8, 121.8, 82.8, 82.7, 81.8, 79.5, 71.5, 71.5, 71.5, 71.4, 71.4, 71.4, 71.3, 71.3, 71.3, 70.7, 70.6, 70.5, 68.3, 65.0, 54.7, 54.2, 51.1, 41.3, 40.4, 37.5, 34.6, 32.9, 32.5, 30.8, 29.8, 29.0, 28.4, 28.3, 28.3, 26.1, 25.5, 25.3, 23.5. **HRMS (ES+)** calc'd for $C_{139}H_{247}N_{17}O_{52}$ $(M+2H)^{+2}$ *m/z* 1493.3525. Found $(M+2H)^{+2}$ m/z 1493.3458.



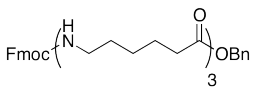
**S.8**

**S.8**

**S.6** (22 mg, 0.0074 mmol, 1.0 equiv.) was dissolved in 1 mL of DMF. To this solution was added EDC (14.2 mg, 0.074 mmol, 10.0 equiv.), HOBt (11.3 mg, 0.074 mmol, 10.0 equiv.), and NHS (8.5 mg, 0.074 mmol, 10.0 equiv.) sequentially, followed by DIPEA (13.0 μL, 0.074 mmol, 10.0 equiv.). The reaction was allowed to stir at RT for 13 hours, after which the DMF was removed by passing a steady stream of $N_2$ over the reaction. A mixture of 97.5% TFA / 2.5% TIPS was added to the flask, and the reaction was allowed to stir for 1 hour, after which the TFA was removed under reduced pressure. The crude reaction mixture was purified by HPLC (Sunfire™ Prep C18 column (10 x 150 mm) using a 50% MeCN to 80% MeCN in $H_2O$ with 0.1% TFA gradient over 66 min at 5 mL/min) to give the NHS-ester of **S.6** as a white powder (2.5 mg) which was immediately carried on. The NHS-ester of **S.6** (2.5 mg, 0.0009 mmol, 1.0 equiv.) was dissolved in 2 mL of PBS and 1 mL of saturated sodium bicarbonate in water. **14** (2.5 mg, 0.0011 mmol, 1.2 equiv.) was added, and the reaction was allowed to stir at RT for 4 hours, after which it was injected directly onto the reverse phase HPLC (Sunfire™ Prep C18 column (10 x 150 mm) using a 30% MeCN to 42% MeCN in $H_2O$ with 0.1% TFA gradient over 39 min at 5 mL/min). The fractions containing the product, **S.8**, were isolated and lyophilized to give a white powder (1.4 mg, 7.5% over three steps). **HRMS (ES+)** calc'd for $C_{208}H_{349}N_{42}O_{79}S_3NaK$ $(M-H+NaK)^+$ *m/z* 4857.3280. Found $(M-H+NaK)^+$ 4857.3329.



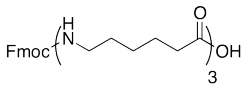

**Supplementary Scheme 3:** Synthesis of **S.9**, **8**, and **11**



**S.9**

**benzyl 1-(9H-fluoren-9-yl)-3,10,17-trioxo-2-oxa-4,11,18-triazatetracosan-24-oate (S.9)**

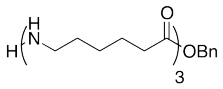
6-((*tert*-butoxycarbonyl)amino)hexanoic acid (2.58 g, 11.15 mmol, 1.1 equiv.) was dissolved in 50 mL of DCM. To the solution were added EDC·HCl (2.14 g, 11.15 mmol, 1.1 equiv.), HOBt·$H_2O$ (1.71 g, 11.15 mmol, 1.1 equiv.), and a solution of benzyl 6-aminohexanoate (2.24 g, 10.14 mmol, 1.0 equiv.) in DCM (50 mL). DIPEA (2.0 mL, 11.15 mmol, 1.1 equiv.) was added, and the solution was allowed to stir at RT for 90 min, after which the reaction was washed with 10% citric acid (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL). The organic layer was collected, dried with $MgSO_4$, and concentrated to give benzyl 6-(6-((*tert*-butoxycarbonyl)amino)hexanamido)hexanoate[6] (3.17 g, 73% crude) as a white powder. The benzyl 6-(6-((*tert*-butoxycarbonyl)amino)hexanamido)hexanoate (3.10 g) was dissolved in 15 mL of TFA and stirred at RT for 1 hour, after which the TFA was partially removed under reduced pressure. The resulting oil was washed with 150 mL of $Et_2O$, and the $Et_2O$ was carefully decanted into 50 mL centrifuge tubes. The centrifuge tubes were spun down at 3000 RCF for 10 minutes, resulting in the product settling to the bottom, and the ethereal layer was carefully removed. The products were combined with methanol, concentrated down under reduced pressure, and azeotroped with chloroform to yield benzyl 6-(6-aminohexanamido)hexanoate[6] as a green oil (2.27 g, 92% crude). 6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)hexanoic acid (0.187 g, 0.529 mmol 1 equiv.) was dissolved in DMF (2.5 mL) and benzyl 6-(6-aminohexanamido)hexanoate (0.177 g, 0.529 mmol, 1 equiv.) was dissolved in DMF (2.5 mL) EDC·HCl (0.608 g, 3.175 mmol, 6 equiv.), and HOBt·$H_2O$ (0.486 g, 3.175 mmol, 6 equiv.) and DIPEA (0.544 mL, 3.175 mmol, 6 equiv.) was added. The solutions were combined in a μwave vial and were heated to 70 °C under μwave conditions for 45 minutes. The solution was diluted with 50 mL of DCM and washed with 10% citric acid (50 mL), 10% sodium bicarbonate (50 mL), saturated $NH_4Cl$ (50mL) and saturated brine (50 mL). The organic layer was collected, dried with $NaSO_4$, concentrated under reduced pressure, and purified (silica gel, 5% MeOH in DCM, then 10% MeOH in DCM). The fractions containing the product were collected and dried yielding **S.9** a single spot. (white solid 0.224 g 63%). **IR (thin film)** 3306 (br), 2936 (m), 2861 (w), 1719 (s), 1647 (s), 1544 (s), 1450 (m), 1254 (s), 1160 (m), 741 (m) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 7.80 (d, *J* = 8 Hz, 2H), 7.64 (d, *J* = 8 Hz, 2H), 7.39 (t, *J* = 8 Hz, 2H), 7.35-7.32 (m, 5H), 7.30 (t, *J* = 8 Hz, 2H), 5.10 (s, 2H), 4.33 (d, *J* = 6.4 Hz, 2H), 4.21 (t, *J* = 7.2 Hz, 1H), 3.16-3.08 (m, 6 H), 2.36 (t, *J* = 7.2 Hz, 2H), 2.19-2.13 (m, 4H), 1.65-1.58 (m, 6H), 1.53-1.46 (m, 6H), 1.36-1.30 (m, 6H). **$^{13}$C NMR (100 MHz, MeOD)** δ 176.0, 175.0, 158.9, 145.3, 142.6, 137.7, 129.5, 129.2, 128.8, 128.1, 126.2, 120.9, 67.6, 67.1, 41.6, 40.2, 40.1, 37.0, 37.0, 34.9, 30.6, 30.0, 27.5, 27.4, 27.4, 26.7, 25.7. **HRMS (ES+)** calc'd for $C_{40}H_{52}N_3O_6$ (M+H) *m/z* 670.3850. Found (M+H) 670.3847.



**8**

**1-(9*H*-fluoren-9-yl)-3,10,17-trioxo-2-oxa-4,11,18-triazatetracosan-24-oic acid (8)**

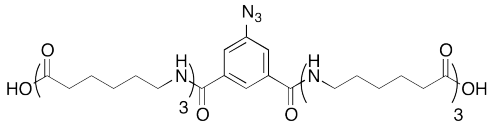
**S.9** was taken up with 90% *iPr*OH / 10% MeOH (30 mL) and added to a slurry of 10% Pd/C in 10 mL of 90% *iPr*OH / 10% MeOH. 40 mL of 90% *iPr*OH / 10% MeOH was used to add the remaining starting material, and the flask was purged with $N_2$ for 15 minutes. $H_2$ gas was then bubbled through the slurry for 5 minutes, and the reaction was allowed to stir for 2 hours under $H_2$ atmosphere, at RT, after which it was filtered with Celite and chromatographed (3 x 25 cm silica gel, 10% MeOH in $CHCl_3$) to yield **8** as a white solid (580 mg, 45%). **IR (thin film)** 3312 (br), 2935 (m), 2861 (w), 1705 (s), 1647 (s), 1546 (s), 1450 (w), 1255 (m) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 7.97 (s, 2H), 7.83 (d, *J* = 8 Hz, 2H), 7.67 (d, *J* = 8 Hz, 2H), 7.62 (t, *J* = 8 Hz, 2H), 7.35-7.32 (t, *J* = 8 Hz, 2H), 7.13 (s, 1H), 4.37 (d, *J* = 6.4 Hz, 2H), 4.22 (t, *J* = 7.2 Hz, 1H), 3.20-3.12 (m, 6H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.22-2.16 (m, 4H), 1.66-1.59 (m, 6H), 1.56-1.49 (m, 6H), 1.42-1.33 (m, 6H). **$^{13}$C NMR (100 MHz, MeOD)** δ 177.6, 176.1, 158.9, 145.4, 142.6, 128.8, 128.2, 126.2, 121.0, 67.6, 41.6, 40.2, 37.0, 37.0, 30.6, 30.1, 27.6, 27.5, 27.4, 26.8, 26.7, 25.8. **HRMS (ES+)** calc'd for $C_{33}H_{46}N_3O_6$ (M+H) *m/z* 580.3308. Found (M+H) 580.3377.




**S.10**

**benzyl 6-(6-(6-aminohexanamido)hexanamido)hexanoate (S.10)**

**S.9** (1.17 g) was dissolved in a mixture of 1:1 $Et_2NH$/DCM (37 mL), and the reaction was stirred at RT for 8 hours, after which it was concentrated under reduced pressure and chromatographed (3 x 25 cm silica gel, 20% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$ + 2.5% $Et_3N$) to yield **S.10** as a white solid (630 mg, 81%). **IR (thin film)** 3221 (w), 3277 (w), 2941 (m), 2862 (w), 1727 (m), 1633 (s), 1542 (m), 1477 (w), 1262 (w), 1184 (w) $cm^{-1}$. **$^1$H NMR (400 MHz, $CDCl_3$)** δ 7.38-7.30 (m, 5H), 5.89 (s, 1H), 5.67 (s, 1H), 5.11 (s, 2H), 3.26-3.20 (m, 6H), 2.27 (t, *J* = 7.2, 2H), 2.19-2.14 (m, 4H), 1.69-1.62 (m, 6H), 1.55-1.48 (m, 6H), 1.40-1.32 (m, 6H). **$^{13}$C NMR (100 MHz, $CDCl_3$)** δ 173.5, 173.0, 172.9, 136.0, 128.4, 128.3, 128.2, 66.2, 41.7, 39.2, 39.1, 36.6, 36.5, 34.1, 32.5, 29.3, 29.2, 26.4, 25.4, 25.1, 24.5. **HRMS (ES+)** calc'd for $C_{25}H_{42}N_3O_4$ (M+H) *m/z* 448.3097. Found (M+H) 448.3150.

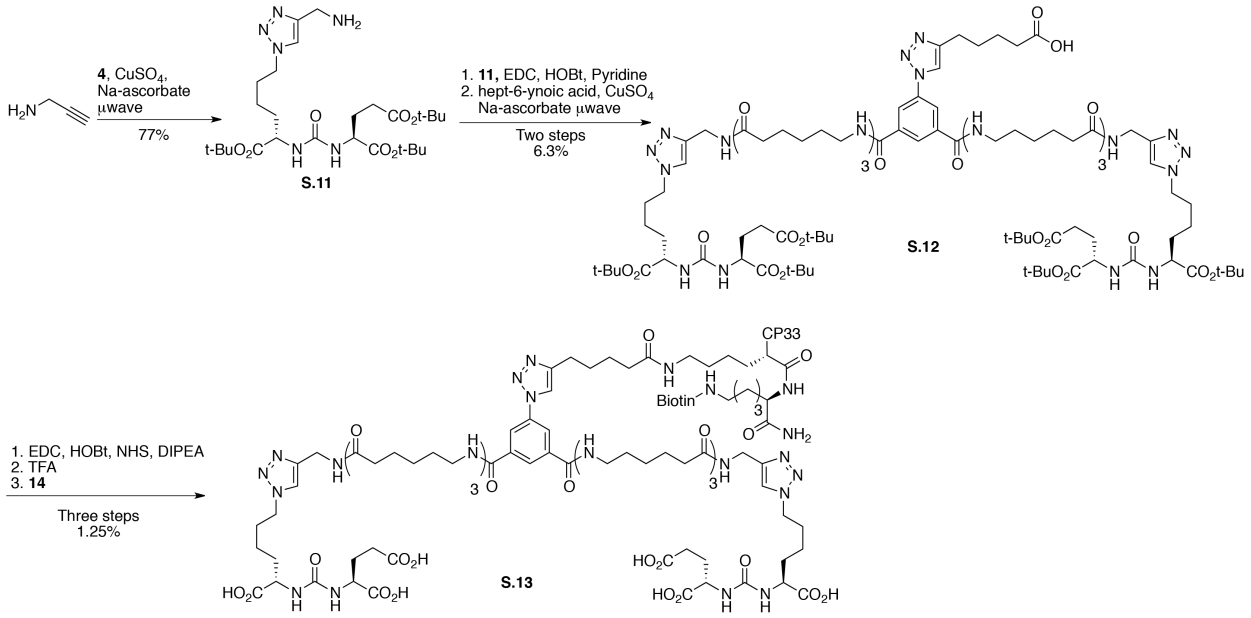



**11**

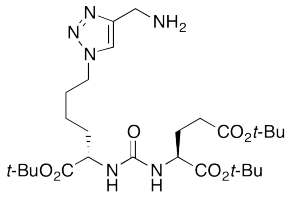
**6,6'-((6,6'-((6,6'-((5-azidoisophthaloyl)bis(azanediyl))bis(hexanoyl))bis(azanediyl))bis(hexanoyl))bis(azanediyl))dihexanoic acid (11)**

A mixture of azidoisophthalic acid (100 mg, 0.483 mmol, 1 equiv.) in 5 mL of dry DCM was prepared in a flame-dried flask. To this mixture were sequentially added EDC·HCl (233 mg, 1.21 mmol, 2.5 equiv.), HOBt·$H_2O$ (183 mg, 1.21 mmol, 2.5 equiv.), **S.10** (476 mg, 1.06 mmol, 2.2 equiv.), and pyridine (0.117 mL, 1.45 mmol, 3 equiv.). The reaction was allowed to stir at RT for 18 hours, after which it was directly loaded onto a silica gel column and partially purified to remove the coupling agents (silica gel, 2 x 15 cm, 10% MeOH in $CHCl_3$). The crude material was dissolved in 5 mL of MeOH and 5 mL of THF. A 1 M solution of LiOH in $H_2O$ was added (1.9 mL, 4 equiv.), and the reaction was allowed to stir at RT for 24 hours. A solution of 1 M HCl was added (0.95 mL,

2 equiv.), and the reaction was concentrated under reduced pressure. The crude mixture was then chromatographed (silica gel, 2 x 15 cm, 20% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$ + 1% TFA), and fractions containing the product were collected and concentrated under reduced pressure to obtain an light brown oil. 50 mL of 95% $Et_2O$ / 5% MeOH was then added to the oil. The $Et_2O$/MeOH mixture was decanted, and the oil dried under reduced pressure to obtain **11** as a white solid (383 mg, 89%, 2 steps). **IR (thin film)** 3310 (br), 2937 (m), 2865 (w), 2112 (m), 1650 (s), 1553 (m), 1439 (w), 1202 (m), 1139 (m) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 8.05 (s, 1H), 7.65 (s, 2H), 3.39 (t, *J* = 5.6 Hz, 4H), 3.15 (dd, *J* = 3.2, 6.6 Hz, 8H), 2.29 (t, *J* = 7.2 Hz, 4H), 2.22-2.15 (m, 8H), 1.68-1.58 (m, 16H), 1.51-1.47 (m, 8H), 1.42-1.32 (m, 12H). **$^{13}$C NMR (100 MHz, MeOD)** δ 177.4, 176.0, 168.3, 138.1, 123.6, 121.5, 41.0, 40.2, 37.0, 34.8, 30.1, 30.1, 27.6, 27.5, 26.7, 25.7. **HRMS (ES+)** calc'd for $C_{44}H_{72}N_9O_{10}$ (M+H) *m/z* 886.5324. Found (M+H) 886.5408.




**Supplementary Scheme 4:** Synthesis of **S.13**

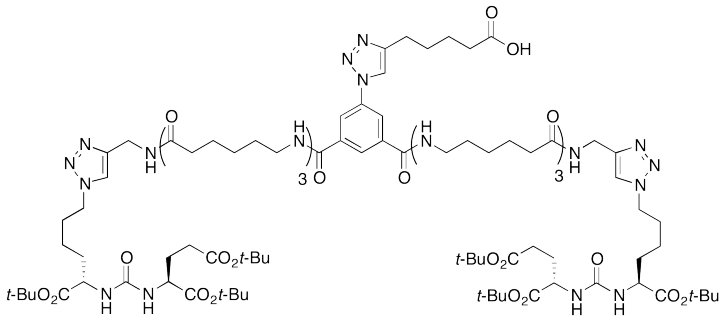


**S.11**

**(S)-di-tert-butyl 2-(3-((S)-6-(4-(aminomethyl)-1H-1,2,3-triazol-1-yl)-1-(tert-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate (S.11)**

A mixture of **4** (180 mg, 0.35 mmol, 1 equiv.) and propargylamine (117 μL, 1.75 mmol, 5 equiv.) was dissolved in a mixture of $H_2O$ (1.25 mL) and *t*-butanol (1.25 mL) in a 5 mL microwave reaction tube. To the mixture was added 0.1 M sodium ascorbate (0.7 mL, 0.2 equiv.) and 0.1 M $CuSO_4$ (0.14 mL, 0.04 equiv.). The tube was capped, and

subject to microwave irradiation for 2.5 minutes at 110 °C. The reaction was then concentrated under reduced pressure at 60 °C, and chromatographed (silica gel, 1 x 15 cm, 10% MeOH in $CHCl_3$, then 10% MeOH in $CHCl_3$ + 2.5% $Et_3N$) to obtain **S.11** as a pale, green oil (152 mg, 77%). **IR (thin film)** 2978 (m), 2933 (w), 1730 (s), 1647 (m), 1559 (m), 1456 (w), 1368 (m), 1255 (w), 1154 (s) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 7.90 (s, 1H), 4.90 (t, *J* = 7.2 Hz, 2H), 4.20-4.16 (m, 1H), 4.14-4.12 (m, 1H), 3.96 (s, 2H), 2.36-2.26 (m, 2H), 2.08-2.00 (m, 1H), 1.95-1.89 (m, 2H), 1.84-1.73 (m, 2H), 1.69-1.59 (m, 1H), 1.46 (s, 9H), 1.44 (s, 18H), 1.39-1.31 (m, 2H). **$^{13}$C NMR (100 MHz, MeOD)** δ 173.7, 173.7, 173.5, 159.9, 147.7, 123.8, 82.8, 82.7, 81.8, 54.2, 51.1, 37.1, 32.9, 32.5, 30.8, 29.0, 28.4, 28.3, 23.5. **HRMS (ES+)** calc'd for $C_{27}H_{49}N_6O_7$ (M+H) *m/z* 568.3585. Found (M+H) 568.3637.

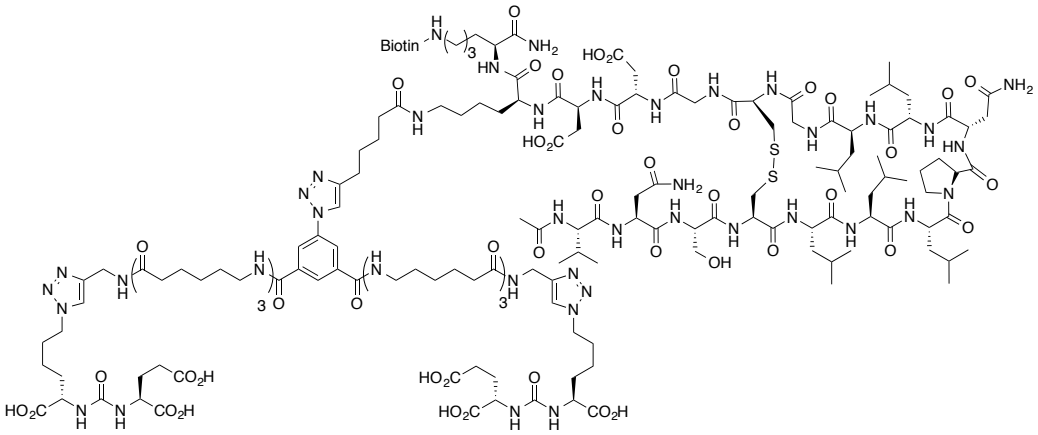


**S.12**

**5-(1-(3,5-bis((6-((6-((6-(((1-((*S*)-6-(*tert*-butoxy)-5-(3-((*S*)-1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)ureido)-6-oxohexyl)-1*H*-1,2,3-triazol-4-yl)methyl)amino)-6-oxohexyl)amino)-6-oxohexyl)amino)-6-oxohexyl)carbamoyl)phenyl)-1*H*-1,2,3-triazol-4-yl)pentanoic acid (S.12)**

To a solution of **11** (150 mg, 0.169 mmol, 1.0 equiv.) in DCM (1.5 mL) were added EDC·HCl (81 mg, 0.423 mmol, 2.5 equiv.), HOBt·$H_2O$ (65 mg, 0.423 mmol, 2.5 equiv.), followed by a solution of **S.11** (212 mg, 0.373 mmol, 2.2 equiv.) in DCM (3.5 mL). Pyridine (82 μL, 0.507 mmol, 3 equiv.) was added to the resulting solution, and the reaction was allowed to stir at RT for 20 hours, after which it was concentrated under reduced pressure. The compound was partially purified by silica gel plug (1 x 15 cm, silica gel, 5% MeOH in $CHCl_3$, then 10% MeOH in $CHCl_3$) to yield the azide-intermediate a dark red oil (190 mg, 57% crude) that was carried onto the next step without further purification. Partially pure azide-intermediate (100 mg, 0.05 mmol, 1.0 equiv.) was dissolved in $H_2O$ (1.25 mL) and *t*-BuOH (1.25 mL) in a 5 mL microwave vial. To this solution were added 6-heptynoic acid (6.4 μL, 0.05 mmol, 1.0 equiv.), 0.1 M sodium ascorbate (0.5 mL, 0.05 mmol, 1.0 equiv.), and 0.1 M $CuSO_4$ (0.25 mL, 0.025 mmol, 0.5 equiv.). The vial was sealed and subjected to microwave irradiation for 1 hour at 110 °C, after which the reaction was concentrated and purified by HPLC (Sunfire™ Prep C18 column (10 x 150 mm) using a 50% MeCN to 80% MeCN in $H_2O$ with 0.1% TFA gradient over 66 min at 5 mL/min). The product, **S.12**, was isolated and lyophilized to yield a white powder (11 mg, 6.3% over two steps). **IR (thin film)** 2938 (w), 1634 (s), 1553 (m), 1461 (m), 1369 (w), 1141 (s), 975 (w), 841 (w), 800 (w), 722 (w) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 8.78 (t, *J* = 5.4 Hz, 1H), 8.48 (s, 1H), 8.45 (d, *J* = 1.6 Hz, 2H), 8.36 (s, 1H), 7.88 (s, 2H), 4.41-4.37 (m, 8H), 4.18 (dd, *J* = 5, 8.8 Hz, 2H), 4.12 (dd, *J* = 5.2, 8 Hz, 2H), 3.67-3.64 (m, 1H), 3.43 (t, *J* = 6.8 Hz, 4H), 3.14 (t, *J* = 6.4 Hz, 8H), 2.84 (t, *J* = 7 Hz, 2H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.34-2.29 (m, 4H), 2.26-2.13 (m, 12H), 2.08-1.99 (m, 2H),

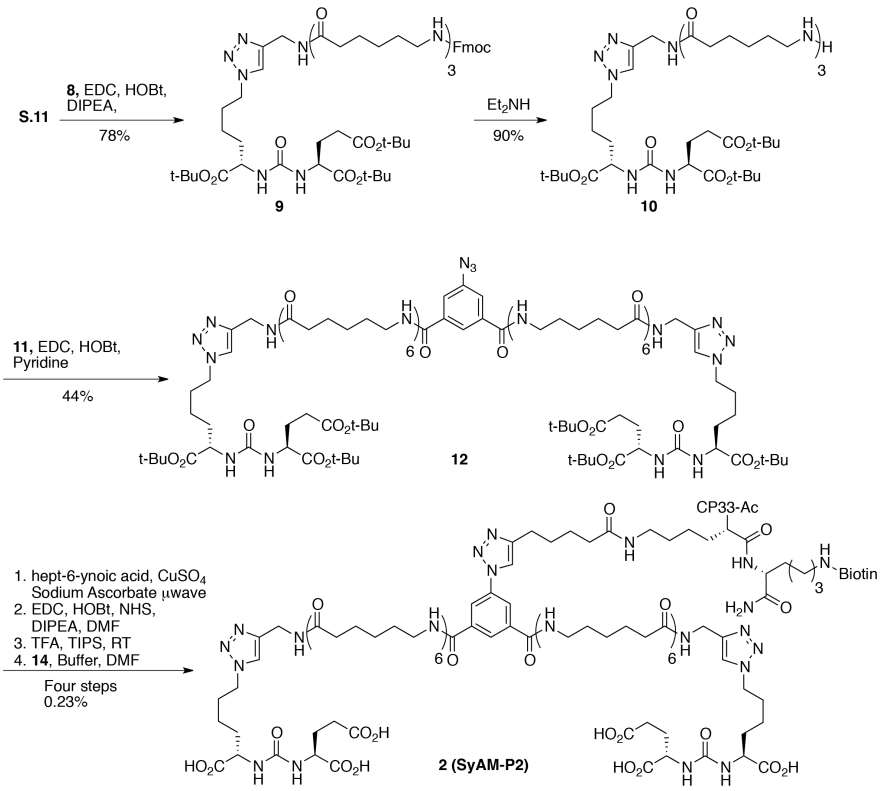
1.95-1.87 (m, 4H), 1.84-1.71 (m, 6H), 1.69-1.54 (m, 20H), 1.50-1.42 (m, 10H), 1.46 (s, 18H), 1.44 (s, 18H), 1.43 (s, 18H), 1.38-1.26 (m, 12H). **$^{13}$C NMR (100 MHz, MeOD)** δ 176.0, 173.7, 173.7, 173.5, 167.9, 159.9, 138.2, 122.6, 82.8, 82.7, 81.7, 54.6, 54.2, 40.2, 37.0, 32.9, 32.5, 30.1, 29.0, 28.4, 28.3, 27.6, 26.7, 23.4. **HRMS (ES+)** calc'd for $C_{105}H_{175}N_{21}O_{24}$ $(M+2H)^{+2}$ *m/z* 1057.1481. Found $(M+2H)^{+2}$ 1057.1462.



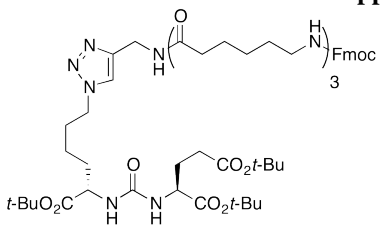

**S.13**

**S.13**

**S.12** (35 mg, 0.018 mmol, 1.0 equiv.) was dissolved in THF. To this solution were added the N-hydroxysuccinimidyl ester of TFA (19 mg, 0.09 mmol, 5.0 equiv.) and pyridine (10 mg, 0.125 mmol, 7.0 equiv.). The reaction was allowed to stir at RT for 1.5 hours, after which it was quenched with 0.5 mL of PBS and concentrated under reduced pressure. To the resulting mixture was added 2 mL of 95% TFA / 5% TIPS, and the reaction was allowed to stir at RT for 1 hour, after which the TFA was removed under reduced pressure. The reaction mixture was purified by HPLC (Sunfire™ Prep C18 column (10 x 150 mm) using a 0% MeCN to 80% MeCN in $H_2O$ with 0.1% TFA gradient over 51 min at 5 mL/min) and fractions containing the product was isolated and lyophilized to give the NHS-ester intermediate (1.9 mg), which was used immediately in the next reaction. The NHS-ester intermediate was dissolved in 975 μL of PBS and 325 μL of saturated sodium bicarbonate in water. **S.9** (2.4 mg, 0.0011 mmol, 1.1 equiv.) was added, and the reaction was allowed to stir at RT for 7 hours, after which the reaction mixture was loaded directly onto the HPLC and purified (Sunfire™ Prep C18 column (10 x 150 mm) using a 0% MeCN to 80% MeCN in $H_2O$ with 0.1% TFA gradient over 51 min at 5 mL/min). The fractions containing the product was collected and lyophilized to yield **S.13** as a white powder (1.1 mg, 1.6% over two steps). **HRMS (ES+)** calc'd for $C_{174}H_{277}N_{46}O_{51}S_3K_2$ $(M-H+2K)^+$ *m/z* 4000.8932. Found $(M-H+2K)^+$ 4000.8921




**Supplementary Scheme 5:** Synthesis of **2 (SyAM-P2)**

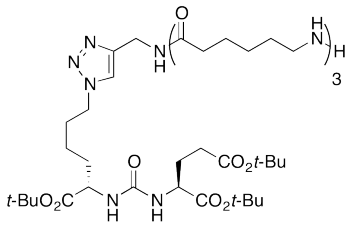


**9**

**(*S*)-di-*tert*-butyl 2-(3-((*S*)-6-(4-(1-(9*H*-fluoren-9-yl)-3,10,17,24-tetraoxo-2-oxa-4,11,18,25-tetraazahexacosan-26-yl)-1*H*-1,2,3-triazol-1-yl)-1-(*tert*-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate (9)**

A mixture of **8** (156 mg, 0.267 mmol, 1 equiv.) was prepared in 1.5 mL of dry DCM. To this slurry were added EDC·HCl (56 mg, 0.294 mmol, 1.1 equiv.) and HOBt·$H_2O$ (45 mg, 0.294 mmol, 1.1 equiv.), followed by addition of a solution of **S.11** (152 mg, 0.267 mmol, 1 equiv.) in 3 mL of dry DCM, and DIPEA (32 μL, 0.294 mmol, 1.1 equiv.). The reaction was allowed to stir at RT for 2 hours, after which EDC·HCl (28 mg, 0.247 mmol, 0.5 equiv.), HOBt·$H_2O$ (23 mg, 0.247 mmol, 0.5 equiv.), and DIPEA (32 μL, 0.294 mmol, 1.1 equiv.) were added. The reaction was allowed to stir at RT for an additional 2 hours, after which the reaction was concentrated under reduced pressure and chromatographed (silica gel, 2 x 15 cm, 10% MeOH in $CHCl_3$) to give **9** as a viscous, pale green oil (235 mg, 78%). **IR (thin film)** 2933 (m), 2862 (w), 2413 (br), 1729 (s), 1630 (s), 1453 (s), 1367 (m), 1251 (w), 1153 (s), 742 (w) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 7.84 (s, 1H), 7.80 (d, *J* = 7.2 Hz, 2H), 7.64 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 8 Hz, 2H), 4.40 (d, *J* = 2 Hz, 2H), 4.37 (t, *J* = 6.8 Hz, 2H), 4.33 (d, J = 6.8 Hz, 2H), 4.21-4.17 (m, 2H), 4.13 (dd, *J* = 5.2, 8 Hz, 1H), 3.17-3.08 (m, 6H), 2.34-2.29 (m, 2H), 2.23-2.13 (m, 6H), 2.08-2.00 (m, 1H), 1.94-1.86 (m, 2H),

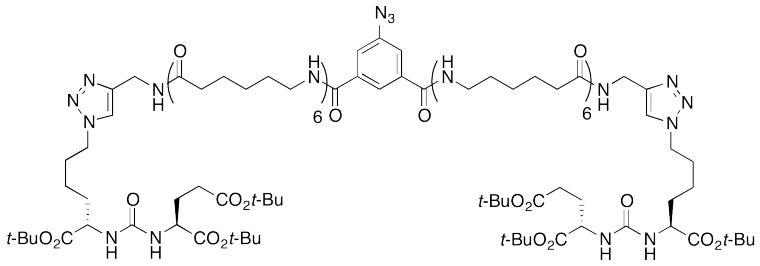
1.84-1.73 (m, 2H), 1.64-1.57 (m, 7H), 1.52-1.43 (m, 5H), 1.46 (s, 9H), 1.43 (s, 18H), 1.39-1.29 (m, 9H). **$^{13}$C NMR (100 MHz, MeOD)** δ 176.0, 175.9, 173.7, 173.6, 173.4, 159.9, 158.8, 146.2, 145.3, 142.6, 128.8, 128.1, 126.2, 124.1, 120.9, 82.8, 82.6, 81.7, 67.6, 54.6, 54.1, 51.3, 41.6, 40.2, 37.0, 37.0, 36.7, 35.6, 32.9, 30.8, 30.6, 30.1, 29.0, 28.4, 28.3, 27.5, 27.4, 26.7, 26.7, 26.5, 23.4. **HRMS (ES+)** calc'd for $C_{60}H_{92}N_9O_{12}$ (M+H) *m/z* 1130.6860. Found (M+H) 1130.6848.



**10**

**(S)-di-tert-butyl 2-(3-((S)-6-(4-((6-(6-(6-aminohexanamido)hexanamido)hexanamido)methyl)-1H-1,2,3-triazol-1-yl)-1-(tert-butoxy)-1-oxohexan-2-yl)ureido)pentanedioate (10)**

**9** (195 mg, 0.172 mmol) was dissolved in 2.5 mL of $Et_2NH$ and 2.5 mL of DCM. The reaction was allowed to stir at RT for 26 hours, after which it was concentrated under reduced pressure and chromatographed (silica gel, 2 x 15 cm, 20% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$ + 2.5% $Et_3N$) to obtain **10** as a sticky, pale yellow solid (137 mg, 90%). **IR (thin film)** 3271 (br), 2934 (m), 2864 (w), 1731 (s), 1643 (s), 1556 (s), 1457 (w), 1367 (m), 1256 (w), 1154 (s) cm$^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 7.99 (s, 1H), 7.88 (s, 1H), 6.40 (dd, *J* = 12, 14 Hz, 1H), 4.42 (s, 2H), 4.41 (t, *J* = 9 Hz, 2H), 4.21-4.11 (m, 2H), 3.22-3.14 (m, 4H), 2.93 (t, *J* = 8 Hz, 2H), 2.35-2.30 (m, 2H), 2.25-2.16 (m, 6H), 2.08-2.01 (m, 1H), 1.95-1.90 (m, 1H), 1.83-1.74 (m, 2H), 1.71-1.58 (m, 9H), 1.55-148 (m, 4H), 1.48 (s, 9H), 1.45 (s, 18H), 1.42-1.29 (m, 10H). **$^{13}$C NMR (100 MHz, MeOD)** δ 176.0, 175.7, 173.8, 173.7, 173.5, 159.9, 146.3, 124.2, 82.8, 82.7, 81.8, 81.7, 40.6, 40.2, 40.2, 37.0, 36.8, 36.6, 35.6, 32.9, 32.5, 30.8, 30.6, 30.2, 29.0, 28.4, 28.3, 27.6, 27.0, 26.8, 26.5, 26.4, 23.5. **HRMS (ES+)** calc'd for $C_{45}H_{82}N_9O_{10}$ (M+H) *m/z* 908.6179. Found 908.6165.

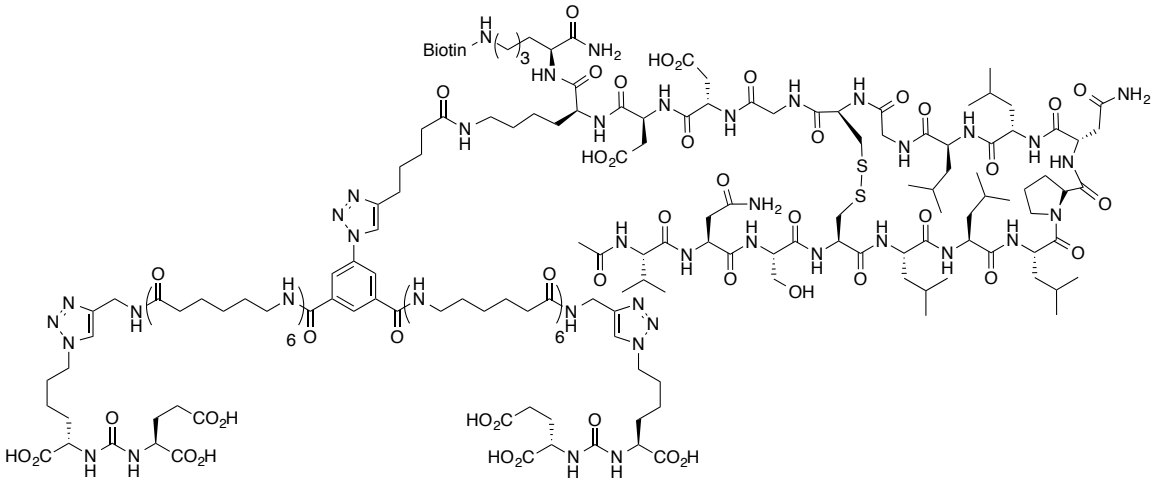


**12**

**12**

A solution of **10** (190 mg, 0.214 mmol, 1.0 equiv.) was prepared in DCM (5 mL). EDC·HCl (103 mg, 0.535 mmol, 2.5 equiv.) and HOBt·$H_2O$ (82 mg, 0.535 mmol, 2.5 equiv.) were added to the solution, which was followed by a solution of **11** (428 mg, 0.472 mmol, 2.2 equiv.) in DCM (5 mL). Pyridine (52 μL, 0.642 mmol, 3.0 equiv.) was added to this and the reaction was allowed to stir at RT for 24 hours, after which it was concentrated under reduced

pressure and chromatographed (Silica gel, 1 x 15 cm, 10% MeOH in $CHCl_3$, then 15% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$) to yield **12** as a pale yellow solid (250 mg, 44%). **HRMS (ES+)** calc'd for $C_{134}H_{232}N_{27}O_{28}$ $(M+3H)^{+3}$ *m/z* 889.2515. Found $(M+3H)^{+3}$ 889.2538

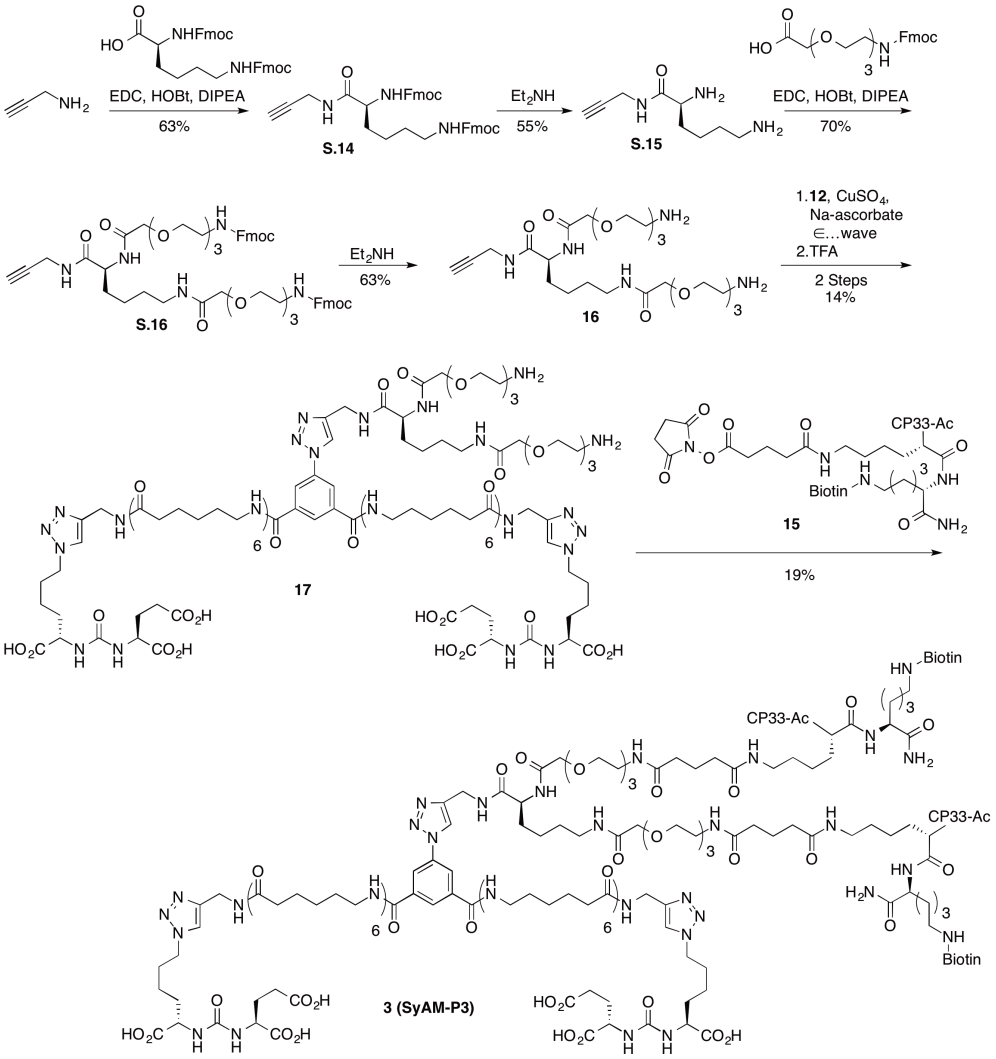


**2 (SyAM-P2)**

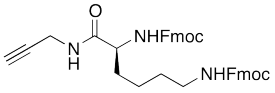
**2 (SyAM-P2)**

**12** (50 mg, 0.019 mmol, 1.0 equiv.) was dissolved in $H_2O$ (0.5 mL) and *t*-BuOH (0.5 mL) in a 2 mL microwave vial. To this solution were added hept-6-ynoic acid (2.5 μL, 0.019 mmol, 1.0 equiv.), 0.1 M sodium ascorbate (0.2 mL, 1.0 equiv.), and 0.1 M $CuSO_4$ (0.1 mL, 0.5 equiv.). The vial was sealed and subjected to microwave irradiation for 1 hour at 110 °C, after which the reaction was concentrated and run through a silica plug (1 x 15 cm, silica gel, 20% MeOH in $CHCl_3$, then 20% MeOH in $CHCl_3$ + 1% TFA). The fractions containing the product were concentrated, washed with $Et_2O$ (50 mL) to give a partially pure green solid (37 mg) that was carried forward without further purification. The partially pure triazole-acid intermediate (37 mg, 0.013 mmol, 1.0 equiv.) was dissolved in DMF (0.5 mL). EDC (7.7 mg, 0.04 mmol, 3.0 equiv.), HOBt (6.2 mg, 0.04 mmol, 3.0 equiv.), and NHS (4.6 mg, 0.04 mmol, 3.0 equiv.) were added sequentially, followed by DIPEA (7.1 μL, 0.04 mmol, 3.0 equiv.). The reaction was allowed to stir for 1 hour, after which EDC (7.7 mg, 0.04 mmol, 3.0 equiv.) and DIPEA (7.1 μL, 0.04 mmol, 3.0 equiv.) were added. The reaction was allowed to stir for another 2 hours, after which the DMF was removed by passing a steady stream of $N_2$ over the reaction. 1 mL of a mixture of 98% TFA / 2% TIPS was added to the flask, and the reaction was allowed to stir at RT for 30 minutes, after which the TFA was removed under reduced pressure. The reaction was purified by HPLC (Sunfire™ Prep C18 column (10 x 150 mm) using a 0% MeCN to 80% MeCN in $H_2O$ with 0.1% TFA gradient over 51 min at 5 mL/min). Fractions containing the product were collected and lyophilized to give the NHS-ester intermediate as a white powder (2.5 mg), which was used immediately in the next step to minimize degradation. NHS-ester intermediate (2.5 mg, 0.86 μmol, 1.0 equiv.) was dissolved in 0.9 mL of PBS and 0.4 mL of a 7.5% solution of sodium bicarbonate in water. **14** (2 mg, 0.65 μmol, 0.75 equiv.) was added, and the reaction was allowed to stir at RT for 1 hour, after which 1 mL of DMF was added. The reaction was allowed to stir for an additional 6 hours at RT, after which the reaction mixture was injected directly onto a reverse

phase HPLC and purified (Sunfire™ Prep C18 column (10 x 150 mm) using a 10% MeCN to 65% MeCN in $H_2O$ with 0.1% TFA gradient over 66 min at 5 mL/min). Fractions containing the product were collected and lyophilized to give **2 (SyAM-P2)** as a white powder (0.2 mg, 0.23% over four steps). **HRMS (ES+)** calc'd for $C_{210}H_{347}N_{52}O_{57}S_3$ $(M+3H)^{+3}$ *m/z* 1536.1650. Found $(M+3H)^{+3}$ 1536.1517




**Supplementary Scheme 6:** Synthesis of **3 (SyAM-P3)**

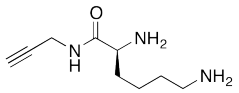


**S.14**

**(*S*)-bis((9*H*-fluoren-9-yl)methyl) (6-oxo-6-(prop-2-yn-1-ylamino)hexane-1,5-diyl)dicarbamate (S.14)**

To a solution of Fmoc-Lys(Fmoc)-OH (0.5 g, 0.846 mmol, 1.0 equiv.) in DMF (2 mL) was added HBTU (385 mg, 1.015 mmol, 1.2 equiv.), followed by DIPEA (503 mg, 0.678 mL, 2.03 mmol, 3 equiv.) and propargylamine (65.3 mg, 0.075 mL, 1.185 mmol, 1.4 equiv.). The reaction was allowed to stir at RT for 2 hours. It was taken up with DCM (50 mL), washed with 10% citric acid (50 mL), 10% saturated sodium bicarbonate (50 mL), saturated

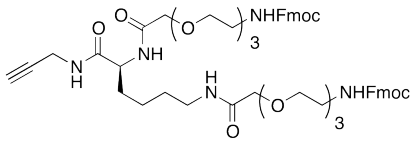
ammonium chloride (50mL) and brine (50 mL). The organic layer was collected, dried with $NaSO_4$, and concentrated under reduced pressure to give **S.14** as a white solid (331 mg, 63.6%) **IR (thin film)** 3297 (s), 3068 (br), 2937 (br), 1687 (s), 1650 (s), 1539 (m), 1450 (w), 1264 (w) cm-1. **$^1$H NMR (500 MHz, $CDCl_3$)** δ 7.75 (t, *J* = 7.8 Hz, 4H), 7.56 (d, *J* = 7.4 Hz, 4H), 7.39 (q, *J* = 7.5 Hz, 4H), 7.30 (q, *J* = 7.5 Hz, 4H), 6.28 (bs, 1H), 5.44 (bs, 1H), 4.85 (bs, 1H), 4.43-4.35 (m, 4H), 4.21-4.30 (m, 3H), 4.02 (s, 2H), 3.25-3.15 (m, 2H), 2.18 (t, *J* = 2.4 Hz, 1H), 1.95-1.85 (m, 1H), 1.75-1.65 (m, 1H), 1.60-1.50 (m, 2H), 1.42-1.32 (m, 2H). **$^{13}$C NMR (125 MHz, $CDCl_3$)** δ 171.4, 156.9, 144.1, 144.0, 143.9, 143.8, 141.5, 141.4, 127.9, 127.8, 127.2, 127.2, 125.1, 125.1, 120.2, 120.1, 79.3, 72.0, 67.2, 66.8, 47.4, 40.3, 31.7, 29.6, 29.4, 22.3. **HRMS (ES+)** calc'd for $C_{39}H_{38}N_3O_5$ (M+H) *m/z* 628.2806. Found (M+H) 628.2802.




**S.15**

**(*S*)-2,6-diamino-*N*-(prop-2-yn-1-yl)hexanamide (S.15)**

**S.14** (300 mg, 0.478 mmol) was dissolved in 10 mL of $Et_2NH$ and 10 mL of DCM. The reaction was allowed to stir for 24 hours, after which it was concentrated and purified with column chromatography (25% MeOH in DCM, then 25% MeOH in DCM + 2% $NH_4OH$) to yield **S.15** as a white solid (48 mg, 55%). **IR (thin film)** 3278 (br), 2931 (m), 2861 (w), 1649 (s), 1554 (s), 1345 (w), 1262 (w), 920 (w) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 3.96 (dd, *J* = 2.4, 7.2 Hz, 2H), 3.26 (t, *J* = 7 Hz, 1H), 2.71 (t, *J* = 7.6 Hz, 2H), 2.59 (m, 1H), 1.69-1.61 (m, 1H), 1.58-1.47 (m, 3H), 1.46-1.34 (m, 2H). **$^{13}$C NMR (100 MHz, MeOD)** δ 177.3, 80.1, 72.3, 55.9, 41.7, 36.0, 31.8, 29.3, 23.8. **HRMS (ES+)** calc'd for $C_9H_{18}N_3O$ (M+H) *m/z* 184.1444. Found (M+H) 184.1441.

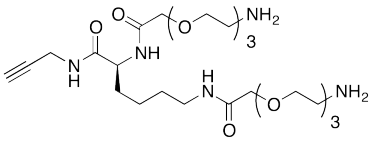



**S.16**

**(*S*)-bis((9*H*-fluoren-9-yl)methyl) (11,19-dioxo-13-(prop-2-yn-1-ylcarbamoyl)-3,6,9,21,24,27-hexaoxa-12,18-diazanonacosane-1,29-diyl)dicarbamate (S.16)**

9-Fluorenylmethoxycarbonyl-11-Amino-3,6,9-Trioxaundecanoic Acid (164 mg, 0.38 mmol, 2.2 equiv.) was dissolved in DCM (5 mL). To this solution were added EDC·HCl (84 mg, 0.44 mmol, 2.5 equiv.) and HOBt·$H_2O$ (67 mg, 0.44 mmol, 2.5 equiv.). The resulting solution was transferred to a flask containing **S.15** (32 mg, 0.174 mmol, 1.0 equiv.). To this reaction, DIPEA (68 mg, 92 µL, 0.52 mmol, 3.0 equiv.) was added, followed by 1 mL of DMF. The reaction was allowed to stir at RT for 6 hours, after which it was concentrated and chromatographed (Silica gel, 1 x 15 cm, 5% MeOH in $CHCl_3$) to yield **S.16** as a clear oil (122 mg, 70%). **IR (thin film)** 3307 (br), 3063 (w), 2918 (s), 1712 (m), 1659 (m), 1535 (s), 1450 (w), 1253 (m), 1105 (m) $cm^{-1}$. **$^1$H NMR (500 MHz, $CDCl_3$)** δ 7.76 (d, *J* = 7.5 Hz, 4H), 7.60 (d, *J* = 7.5 Hz, 4H), 7.39 (t, *J* = 7.5 Hz, 4H), 7.30 (t, *J* = 7.5 Hz, 4H), 6.97 (bs, 1H), 6.84 (bs, 1H), 5.74 (bs, 1H), 5.63 (bs, 1H), 4.44-4.38 (m, 5H), 4.21 (t, *J* = 7 Hz, 2H), 4.05-3.96 (m, 6H), 3.65-3.61 (m, 16H), 3.57-3.55 (m, 4H), 3.42-3.35 (m, 4H), 3.28-3.21 (m, 3H), 2.15 (t, *J* = 2 Hz, 1H), 1.93-1.87 (m, 1H), 1.72-

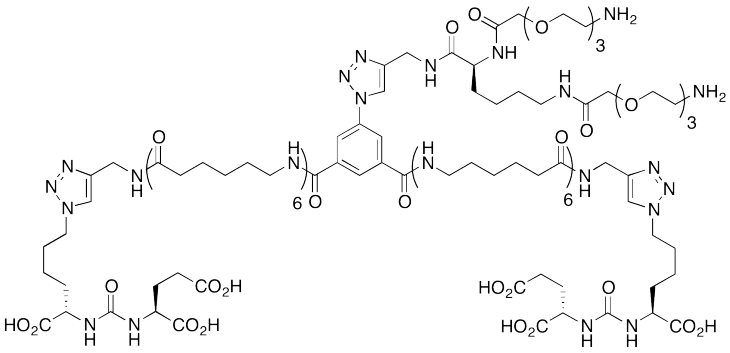
1.66 (m, 1H), 1.56-1.50 (m, 2H), 1.39-1.34 (m, 2H). **$^{13}$C NMR (125 MHz, $CDCl_3$)** δ 171.2, 170.6, 170.1, 156.8, 144.2, 144.1, 141.5, 127.8, 127.8, 127.2, 125.3, 125.2, 120.1, 71.7, 71.1, 71.0, 70.6, 70.5, 70.5, 70.4, 70.4, 70.2, 70.2, 66.7, 66.7, 52.5, 47.4, 47.4, 41.1, 38.6, 31.5, 29.2, 23.1. **HRMS (ES+)** calc'd for $C_{55}H_{68}N_5O_{13}$ (M+H) *m/z* 1007.4840. Found (M+H) 1007.4839.



**16**

**(*S*)-*N*,*N*'-(6-oxo-6-(prop-2-yn-1-ylamino)hexane-1,5-diyl)bis(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)acetamide) (16)**

**S.16** (120 mg, 0.119 mmol) was dissolved in 5 mL of DCM and 5 mL of $Et_2NH$. The reaction was allowed to stir at RT for 24 hours, after which it was concentrated and chromatographed (1 x 15 cm silica gel, 25% MeOH in DCM, then 25% MeOH in DCM + 2% $NH_4OH$) to yield **16** as a colorless oil (42 mg, 63%). **IR (thin film)** 3289 (br), 2865 (m), 1655 (s), 1532 (m), 1457 (w), 1103 (m) $cm^{-1}$. **$^1$H NMR (400 MHz, MeOD)** δ 4.40 (dd, *J* = 5.4, 8.8 Hz, 1H), 4.04 (s, 2H), 3.98-3.95 (m, 4H), 3.73-3.61 (m, 16H), 3.53 (dt, *J* = 2, 5.2 Hz, 4H), 3.24 (t, *J* = 7 Hz, 2H), 2.62 (t, *J* = 1.5 Hz, 1H), 1.89-1.80 (m, 1H), 1.76-1.68 (m, 1H), 1.59-1.52 (m, 2H), 1.43-1.33 (m, 2H). **$^{13}$C NMR (100 MHz, MeOD)**, δ 173.5, 172.6, 172.6, 80.5, 73.3, 73.2, 72.4, 72.0, 71.9, 71.6, 71.5, 71.4, 71.4, 71.2, 71.2, 71.2, 53.9, 42.1, 42.1, 39.7, 33.1, 30.1, 29.5, 24.1. **HRMS (ES+)** calc'd for $C_{25}H_{48}N_5O_9$ (M+H) *m/z* 562.3447 Found (M+H) 562.3448.

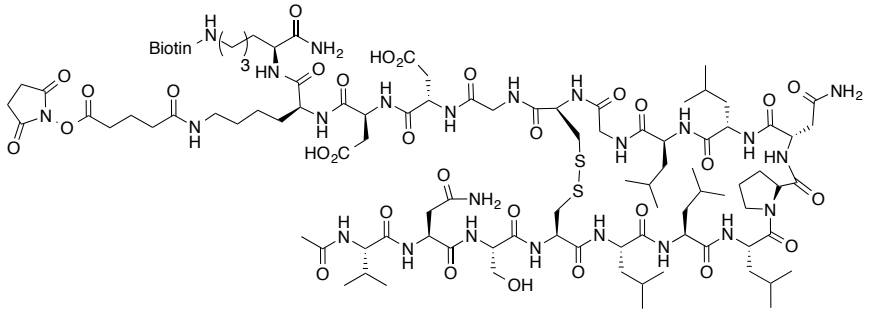


**17**

**(2*S*,2'*S*)-2,2'-(((((1*S*,1'*S*)-(((5-(4-((*S*)-16-amino-4-(4-(2-(2-aminoethoxy)acetamido)butyl)-3,6-dioxo-8,11,14-trioxa-2,5-diazahexadecyl)-1*H*-1,2,3-triazol-1-yl)-1,3-phenylene)bis(3,10,17,24,31,38,45-heptaoxo-2,9,16,23,30,37,44-heptaazapentatetracontane-45,1-diyl))bis(1*H*-1,2,3-triazole-4,1-diyl))bis(1-carboxypentane-5,1-diyl))bis(azanediyl))bis(carbonyl))bis(azanediyl))diglutaric acid (17)**

**12** (162 mg, 0.091 mmol, 1 equiv.) and **16** (34 mg, 0.091 mmol, 1 equiv.) were dissolved in $H_2O$ (1.5 mL) and *t*-BuOH (1.5 mL) in a 5 mL microwave vial. To this solution were added 0.1 M sodium ascorbate (0.6 mL, 1 equiv.) and 0.1 M $CuSO_4$ (0.3 mL, 0.5 equiv.). The vial was sealed and subjected to microwave irradiation for 1 hour at 110

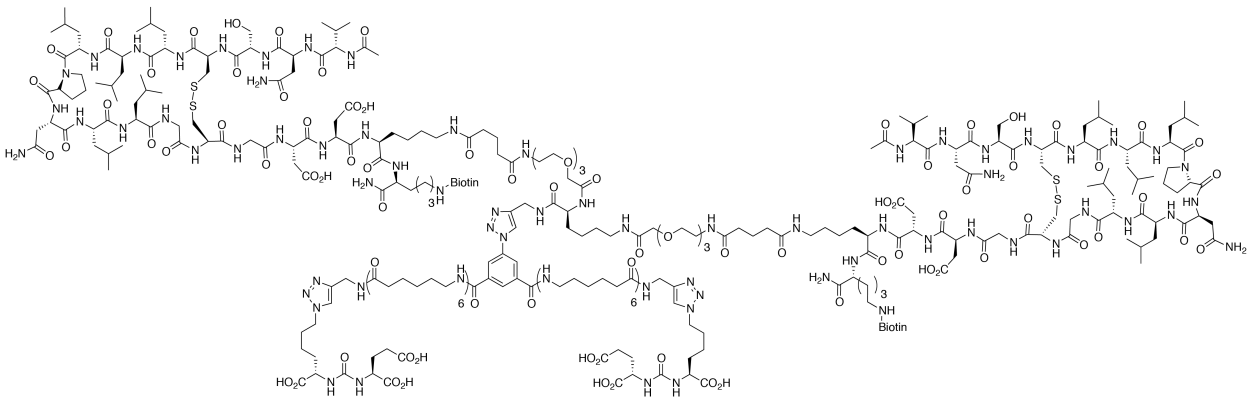
°C, after which the reaction was concentrated under reduced pressure to yield a brown oil. The crude oil was taken up with 67% TFA in DCM (3 mL) in a 5 mL microwave vial. The vial was sealed and subjected to microwave irradiation for 2 minutes at 70 °C, after which the reaction was concentrated under reduced pressure and purified using HPLC (Sunfire™ Prep C18 column (10 x 150 mm) using a 50% MeCN to 80% MeCN in $H_2O$ with 0.1% TFA gradient over 66 min at 5 mL/min). The product was isolated and lyophilized to yield **17** as a white powder (25 mg, 14%, 2 steps). **IR (thin film)** 3300 (br), 3093 (w), 2935 (m), 2866 (w), 1640 (s), 1552 (m), 1459 (w), 1201 (w), 1135 (w) cm-1. **$^1$H NMR (500 MHz, MeOD)** δ 8.56 (s, 1H), 8.45 (d, *J* = 1.6 Hz, 2H), 8.37 (t, *J* = 1.5 Hz, 2H), 7.86 (s, 2H), 5.49 (s, 2H), 4.58 (s, 2H), 4.45-4.40 (m, 6H), 4.39 (t, *J* = 7 Hz, 4H), 4.30 (dd, *J* = 5, 9 Hz, 2H), 4.26 (dd, *J* = 5, 9 Hz, 2H), 4.07 (s, 2H), 4.00 (s, 2H), 3.73-3.66 (m, 24H), 3.43 (t, *J* = 7 Hz, 4H), 3.23 (dt, *J* = 3.1, 7.5 Hz, 2H), 3.17-3.13 (m, 26H), 2.43-2.39 (m, 4H), 2.24-2.13 (m, 28H), 1.96-1.82 (m, 10H), 1.78-1.71 (m, 2H), 1.71-1.63 (m, 12H), 1.63-1.54 (m, 24H), 1.53-1.46 (m, 24H), 1.44-1.37 (m, 12H), 1.35-1.29 (m, 22H), 1.04 (d, *J* = 5.6 Hz, 2H). **$^{13}$C NMR (125 MHz, MeOD)** δ 176.3, 176.1, 175.9, 175.8, 174.1, 172.6, 172.5, 171.1, 167.8, 161.5, 161.2, 160.1, 147.4, 138.6, 138.3, 127.9, 124.3, 122.7, 122.6, 118.4, 116.1, 71.8, 71.7, 71.4, 71.2, 71.2, 71.1, 67.9, 67.9, 54.8, 54.2, 53.7, 53.5, 51.1, 41.1, 40.7, 40.6, 40.2, 39.6, 37.0, 36.8, 35.6, 32.9, 32.8, 31.1, 30.7, 30.1, 30.1, 28.8, 27.6, 27.5, 26.7, 26.5, 25.7, 24.1, 23.4, 17.6, 12.9. **HRMS (ES+)** calc'd for $C_{135}H_{231}N_{32}O_{37}$ $(M+3H)^{+3}$ *m/z* 964.2314. Found $(M+3H)^{+3}$ 964.2380.




**15**

**(3*S*,6*S*,9*S*,12*S*)-3-(2-((3*S*,6*S*,9*S*,15*R*,20*R*,23*S*,26*S*,29*S*,34a*S*)-20-((*S*)-2-((*S*)-2-((*S*)-2-acetamido-3-methylbutanamido)-4-amino-4-oxobutanamido)-3-hydroxypropanamido)-3-(2-amino-2-oxoethyl)-6,9,23,26,29-pentaisobutyl-1,4,7,10,13,21,24,27,30-nonaoxodotriacontahydropyrrolo[2,1-*s*][1,2,5,8,11,14,17,20,23,26,29]dithianonaazacyclodotriacontine-15-carboxamido)acetamido)-12-carbamoyl-6-(carboxymethyl)-9-(4-(5-((2,5-dioxopyrrolidin-1-yl)oxy)-5-oxopentanamido)butyl)-4,7,10,18-tetraoxo-22-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-5,8,11,17-tetraazadocosan-1-oic acid (15)**

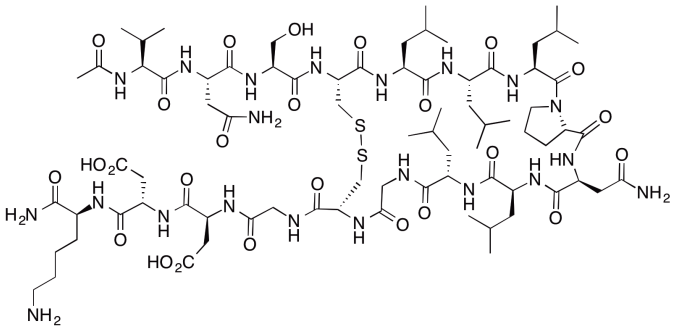
**14** (11.8mg, 0.0065mmol, 1 equiv.) was dissolved in 100 µL of DMF with 100 µL of THF. To this solution was added disuccinimidyl gluturate (8.5 mg, 0.026 mmol, 4 equiv.), followed by addition of DIPEA (5.7 µL, 0.0325 mmol, 5 equiv.) and stirred for 2 hours at RT. Reaction quenched with 40 µL of TFA, and THF was removed under reduced pressure and the sample was diluted with 0.1% TFA $H_2O$/MeCN (70:30) and purified on reverse phase HPLC (C18 Sunfire™ column, 5 mL/min 10% MeCN to 50% MeCN over 20 minutes). To yield **15** as a white powder after lyophilization (8.9 mg 74%). **HRMS (ES+)** calc'd for $C_{86}H_{140}N_{22}O_{30}S_2$ $(M+2H)^{+2}$ *m/z* 1012.4695. Found $(M+2H)^{+2}$ 1012.3970.



**3 (SyAM-P3)**

**3 (SyAM-P3)**

**17** (1.0 mg, 0.31 μmol, 1.0 equiv.) was dissolved in 0.15 mL of DMF at room temperature. To this solution was added a solution of **15** (1.48 mg, 0.62 μmol, 2.0 equiv.) in 0.15 mL of DMF. DIPEA (0.4 mg, 0.5 μL, 10 equiv.) was subsequently added, and the reaction was allowed to stir at RT for 12 hours. The reaction was quenched with 1 mL of water and injected directly onto the HPLC and purified (Sunfire™ Prep C18 column (10 x 150 mm) using a 20% MeCN to 60% MeCN in $H_2O$ with 0.1% TFA gradient over 30 min at 5 mL/min) to yield **3** as a white powder after lyophilization (0.4 mg, 19%). **HRMS (ES+)** calc'd for $C_{331}H_{550}N_{82}O_{97}S_6$ $(M+4H)^{+4}$ *m/z* 1855.6925. Found $(M+4H)^{+4}$ 1855.6819.

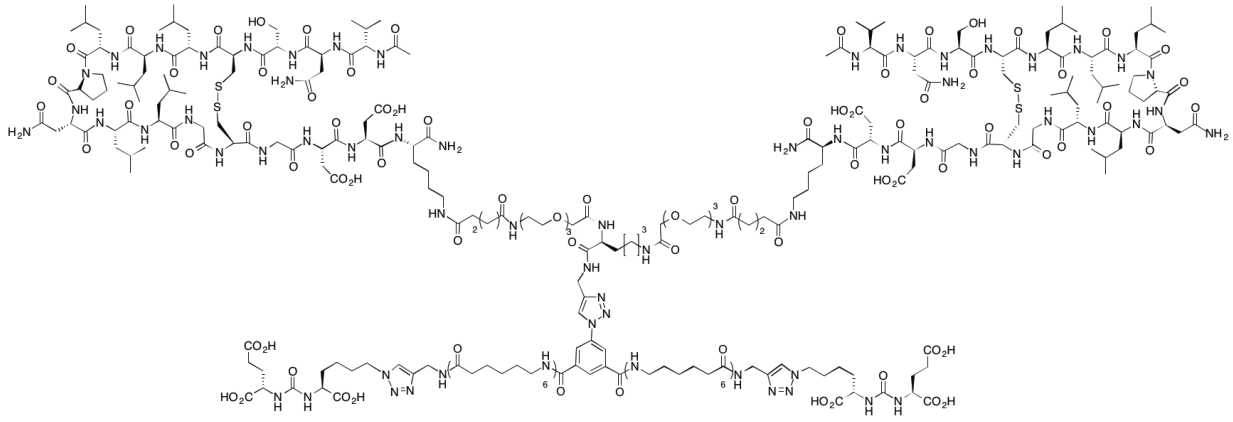


**S.17**

**(S)-3-(2-((3S,6S,9S,15R,20R,23S,26S,29S,34aS)-20-((S)-2-((S)-2-((S)-2-acetamido-3-methylbutanamido)-4-amino-4-oxobutanamido)-3-hydroxypropanamido)-3-(2-amino-2-oxoethyl)-6,9,23,26,29-pentaisobutyl-1,4,7,10,13,21,24,27,30-nonaoxodotriacontahydropyrrolo[2,1-s][1,2]dithia[5,8,11,14,17,20,23,26,29]nonaazacyclodotriacontine-15-carboxamido)acetamido)-4-(((S)-3-carboxy-1-(((R)-1,6-diamino-1-oxohexan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutanoic acid (S.17)**

**S.17** was synthesized using standard SPPS as previously described on a 5 g scale to generate Ac-VN(Trt)S(tBu)C(Trt)LLLPN(Trt)LLGC(Trt)GD(tBu)D(tBu)K(Boc)-Resin. Global deprotection and cleavage from solid support was performed with 92:4:4 mixture of TFA:$H_2O$:TIPS for 90 minutes, stirring at RT. The suspension was centrifuged at 3000 RCF for 5 minutes and the supernatant decanted. The cleavage mixture was filtered through a cotton-plugged pipette to remove resin, and the filtrate was collected into a 50 mL conical tube

containing 35 mL of cold (-78 °C) $Et_2O$, forming a white precipitate. The suspension was centrifuged at 3000 RCF for 5 minutes and the supernatant decanted. The residual pellet was dissolved in 25 mL of 20% MeCN in $H_2O$, containing 1 mL of DMSO and 40 mg of potassium carbonate. This solution was stirred open to air for 48 hours to form the disulfide bond. After oxidation, the peptide was purified using reverse-phase HPLC (C18 reverse phase, 5 mL/min, 20%-55% MeCN in $H_2O$ with 0.1% TFA over 30 minutes) to yield **S.17** as a white powder after lyophilization. **HRMS (ES+)** calc'd for $C_{77}H_{131}N_{21}O_{25}S_2$ $(M+2H)^{+2}$ *m/z* 906.9528. Found 906.9514

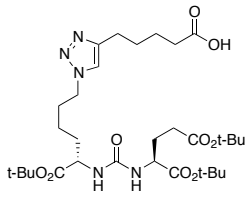


**S.18**

**S.18 (Non-biotinylated SyAM-P3)**

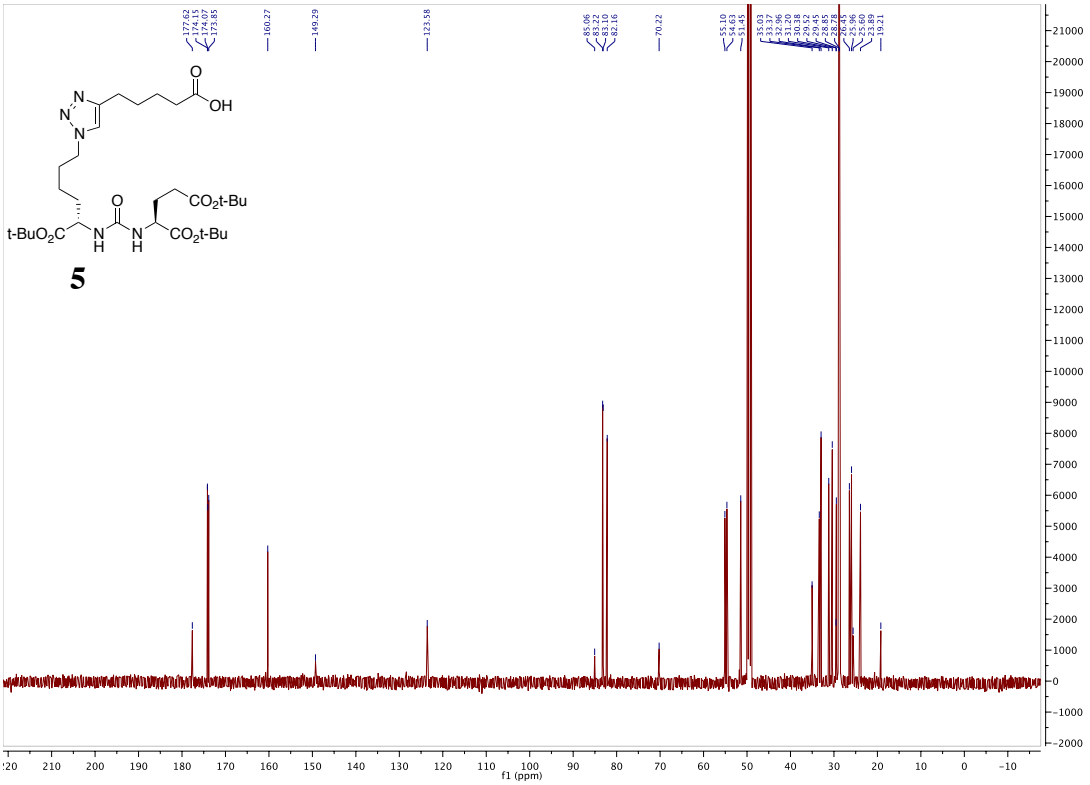
**S.17** (500 mg, 0.276 mmol, 1 equiv.) was dissolved in 4 mL of DMF with 4 mL of THF. To this solution was added disuccinimidyl gluturate (144 mg, 0.441 mmol, 1.6 equiv.), followed by addition of DIPEA (193 μL, 1.103 mmol, 4 equiv.) and stirred for 2 hours at RT. Reaction was quenched with 1.6 mL of TFA, and THF was removed under reduced pressure and the sample was diluted with 0.1% TFA $H_2O$/MeCN (70:30) and purified on reverse phase HPLC (C18 Sunfire™ column, 5 mL/min 10% MeCN to 50% MeCN over 20 minutes) to obtain the corresponding non-biotinylated CP33 NHS ester (270 mg, 0.133 mmol, 48%). This NHS ester (96 mg, 0.048 mmol, 1 equiv) was then dissolved in 15 mL of DMF at room temperature. To this solution was added a solution of **17** (51 mg, 0.018 mmol, 0.4 equiv.) in 15 mL of DMF. DIPEA (62 μL, 0.353 mmol, 7.4 equiv.) was subsequently added, and the reaction was allowed to stir at RT for 12 hours. The reaction was quenched with 10 mL of water and injected directly onto the HPLC and purified (Sunfire™ Prep C18 column (10 x 150 mm) using a 20% MeCN to 60% MeCN in $H_2O$ with 0.1% TFA gradient over 30 min at 5 mL/min) to yield **S.18** as a white powder after lyophilization (49 mg, 6.94 μmol, 39%). **HRMS (ES+)** calc'd for $C_{299}H_{499}N_{74}O_{91}S_4$ $(M+5H)^{+5}$ *m/z* 1342.1110. Found 1342.1158.

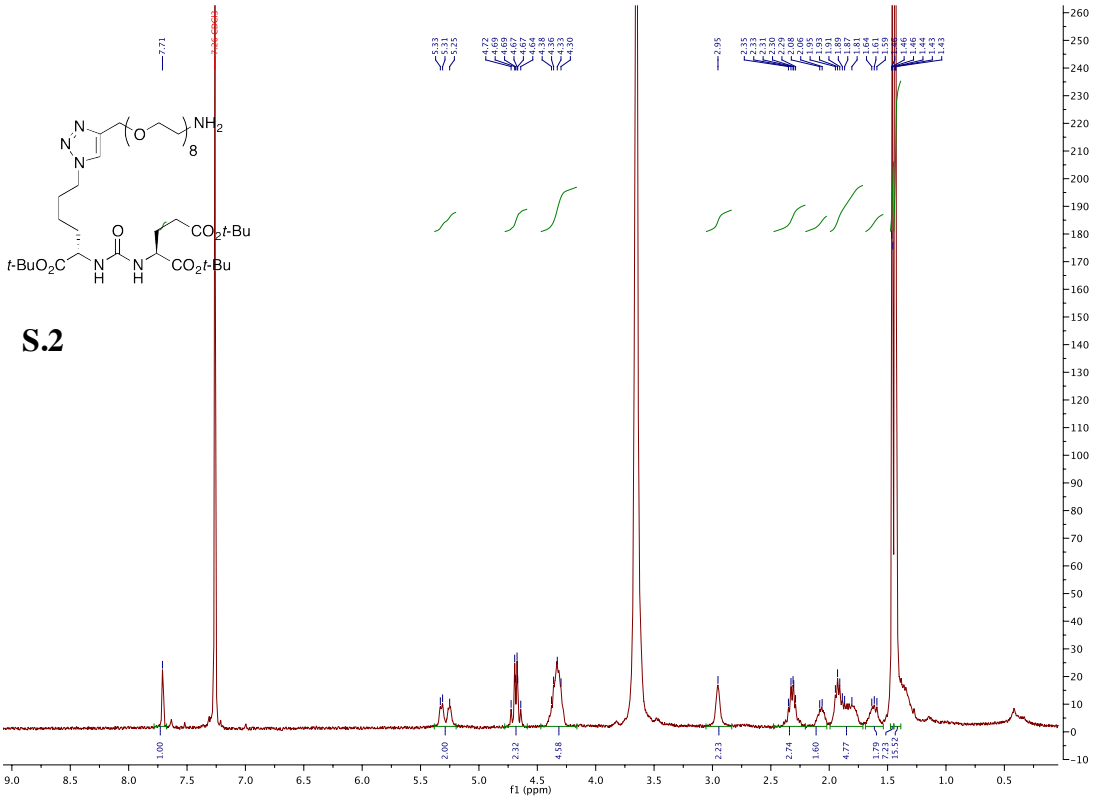
**Catalog of $^{1}H$ and $^{13}C$ Spectra**




**5**

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7.26 CDCl3
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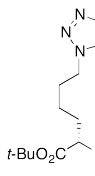
5.31
5.25
4.72
4.69
4.67
4.67
4.64
4.38
4.36
4.33
4.30
2.95
2.35
2.33
2.31
2.30
2.29
2.08
2.06
1.95
1.93
1.91
1.89
1.87
1.81
1.64
1.61
1.59
1.46
1.46
1.44
1.43
1.43
1.00
2.00
2.32
4.58
2.23
2.74
1.60
4.77
1.79
7.23
15.52
f1 (ppm)



**S.2**


172.46
172.13
156.89
144.98
123.04
81.88
81.65
80.54
70.57
70.54
70.48
70.44
70.40
70.36
70.18
69.56
64.60
53.08
53.03
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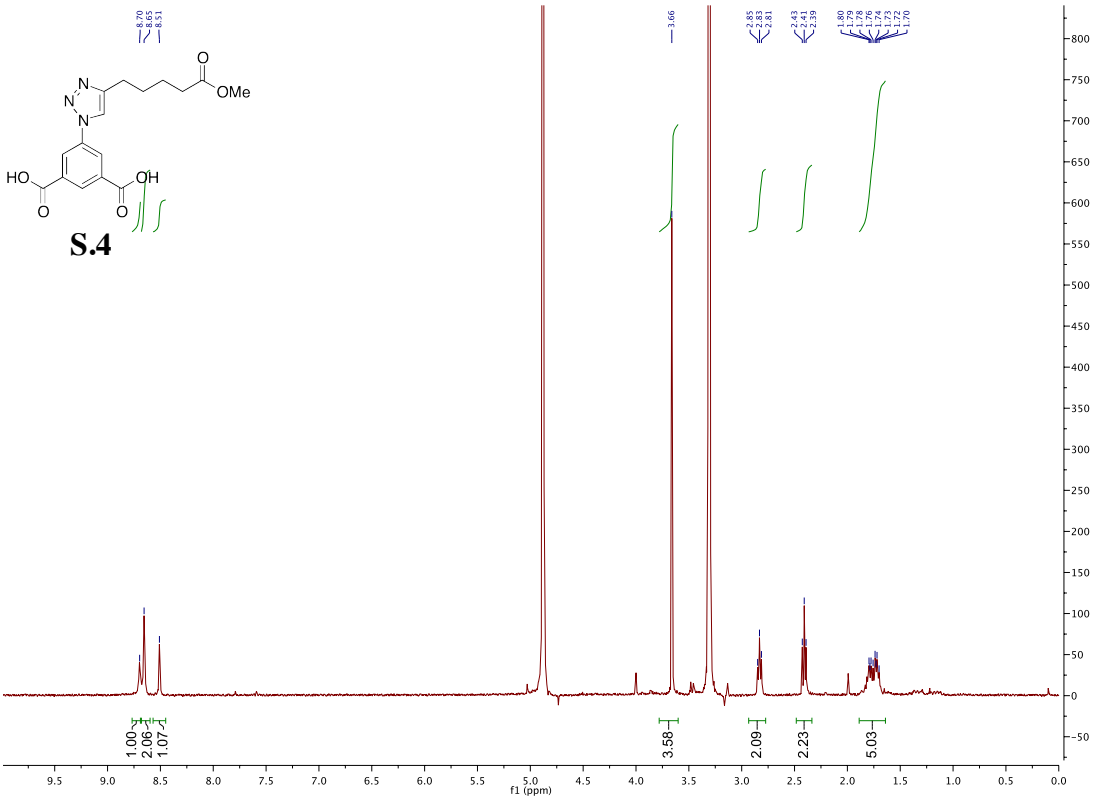
28.27
28.11
28.06
28.03
21.86
f1 (ppm)



**S.2**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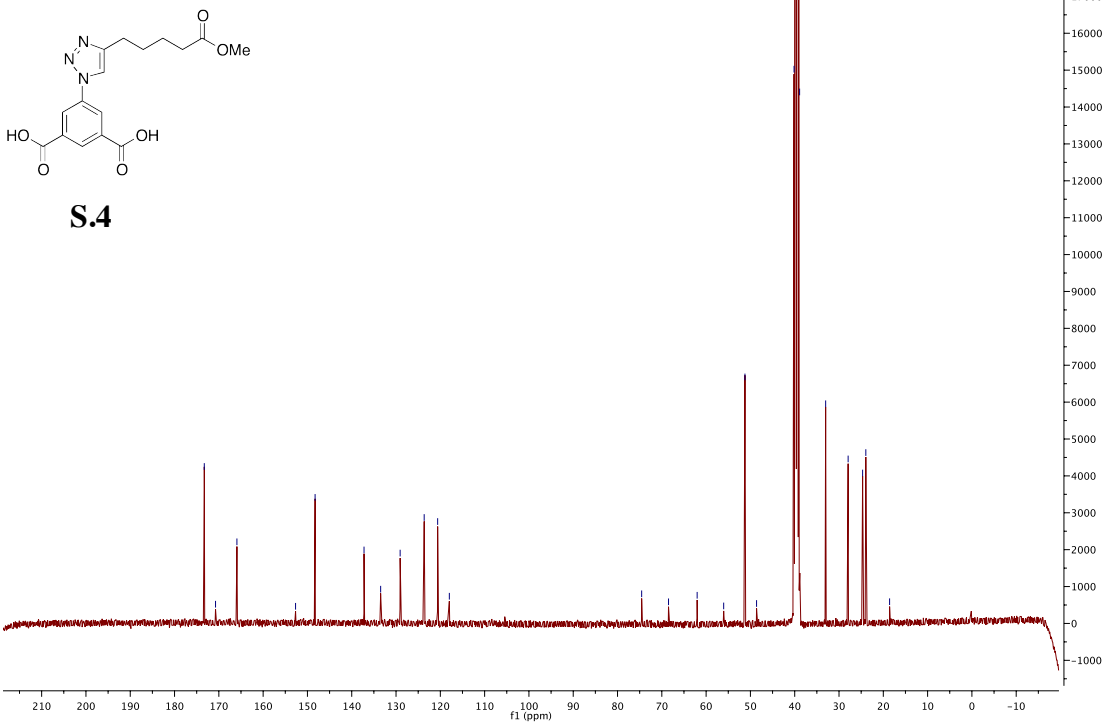
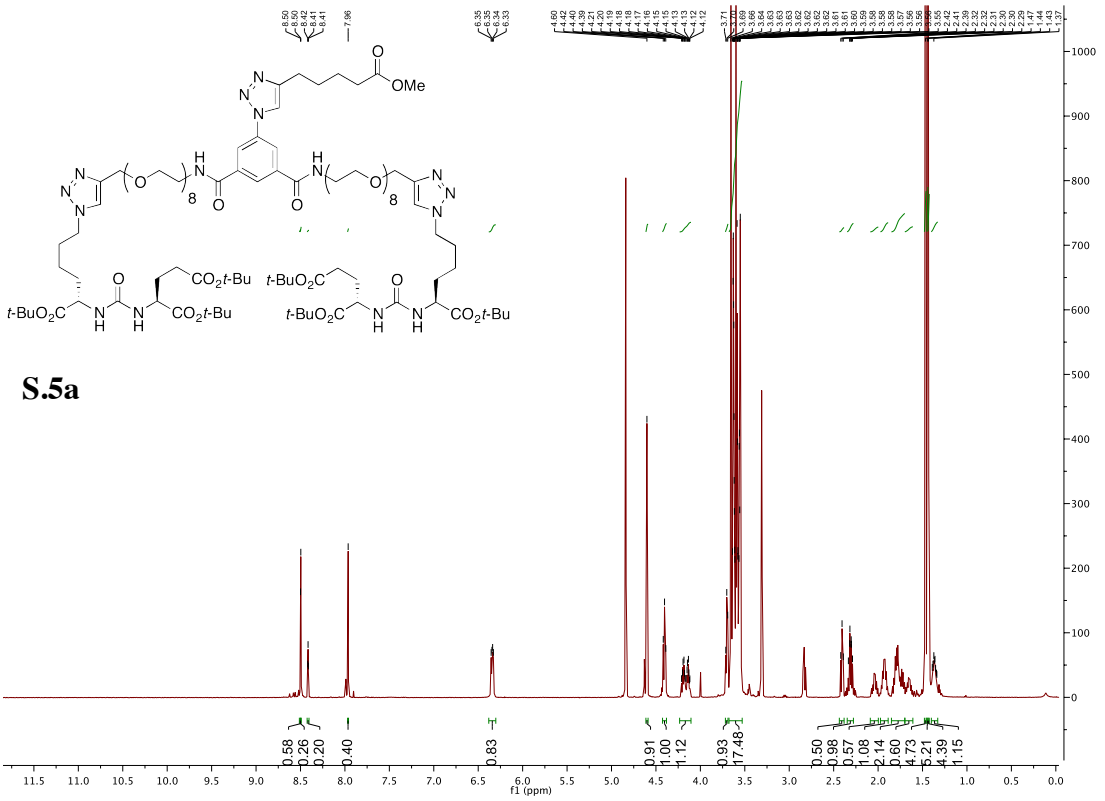
8.61
8.51
3.66
2.85
2.83
2.81
2.43
2.41
2.39
1.80
1.79
1.78
1.76
1.74
1.73
1.72
1.70
1.00
2.06
1.07
3.58
2.09
2.23
5.03
f1 (ppm)


173.28
170.75
165.92
152.68
148.27
137.22
133.47
129.07
123.64
120.61
117.97
74.55
68.49
62.02
56.04
53.23
48.61
40.15
39.94
39.73
39.52
39.31
39.10
38.89
33.01
27.94
24.67
23.95
18.58
f1 (ppm)

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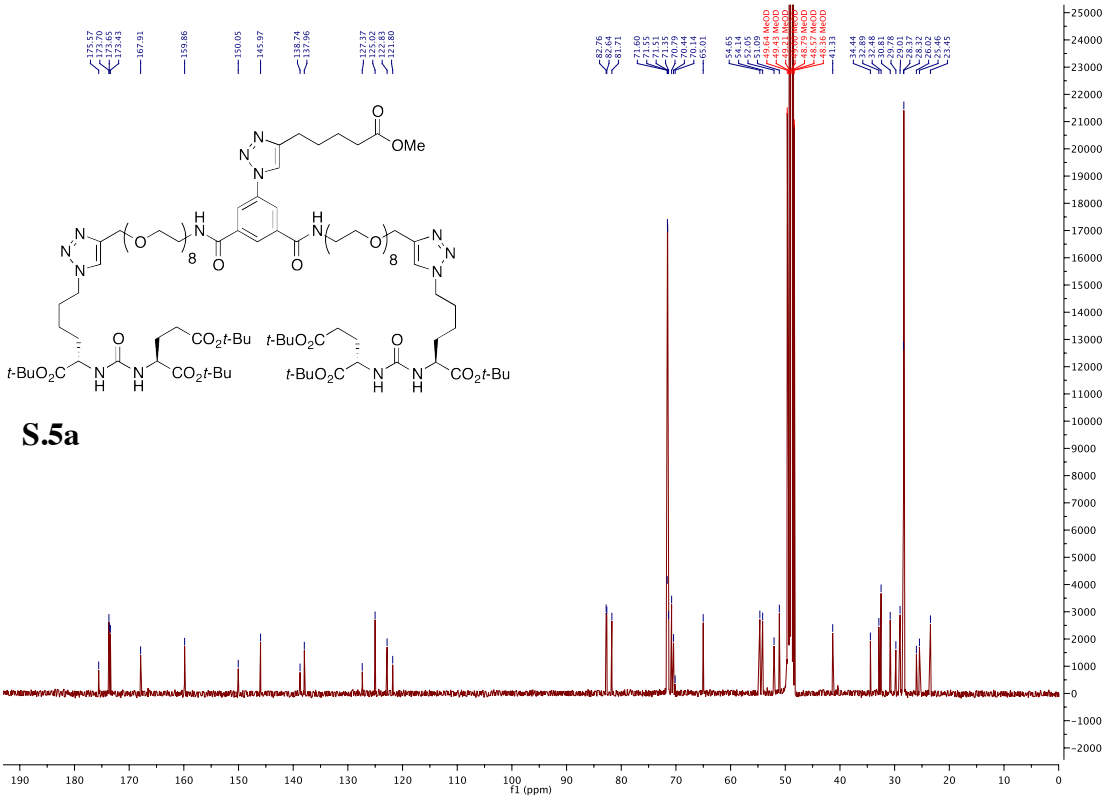
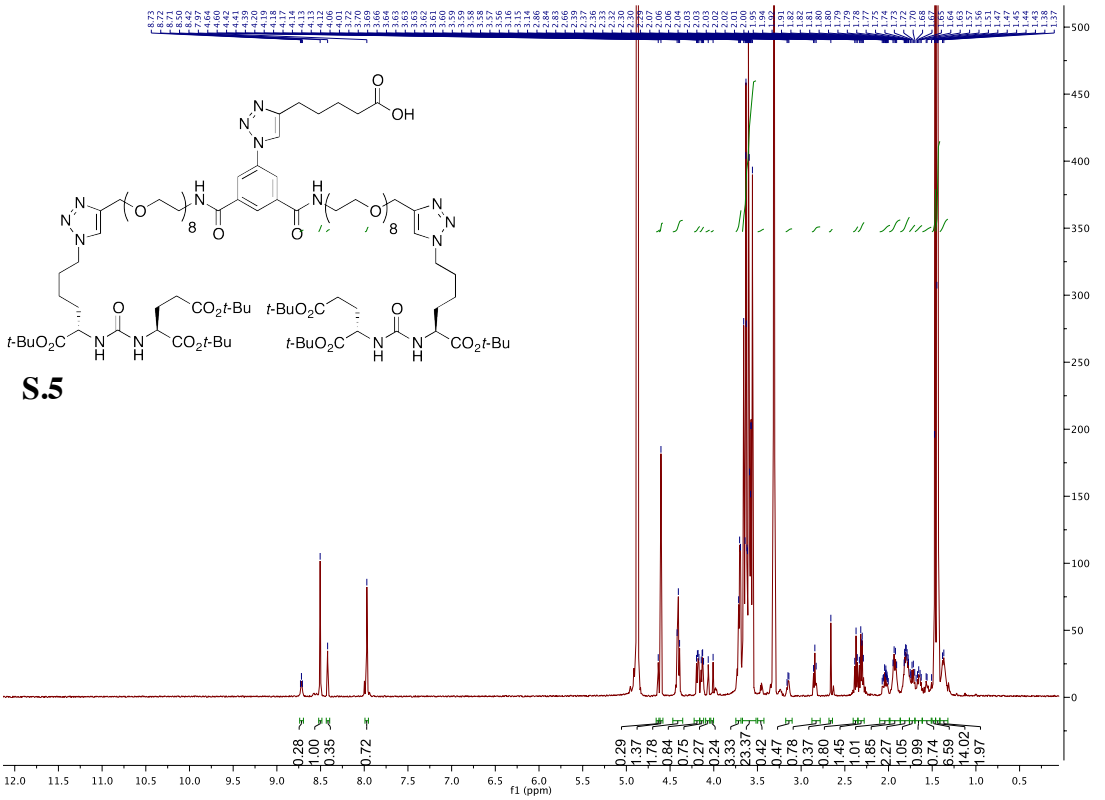
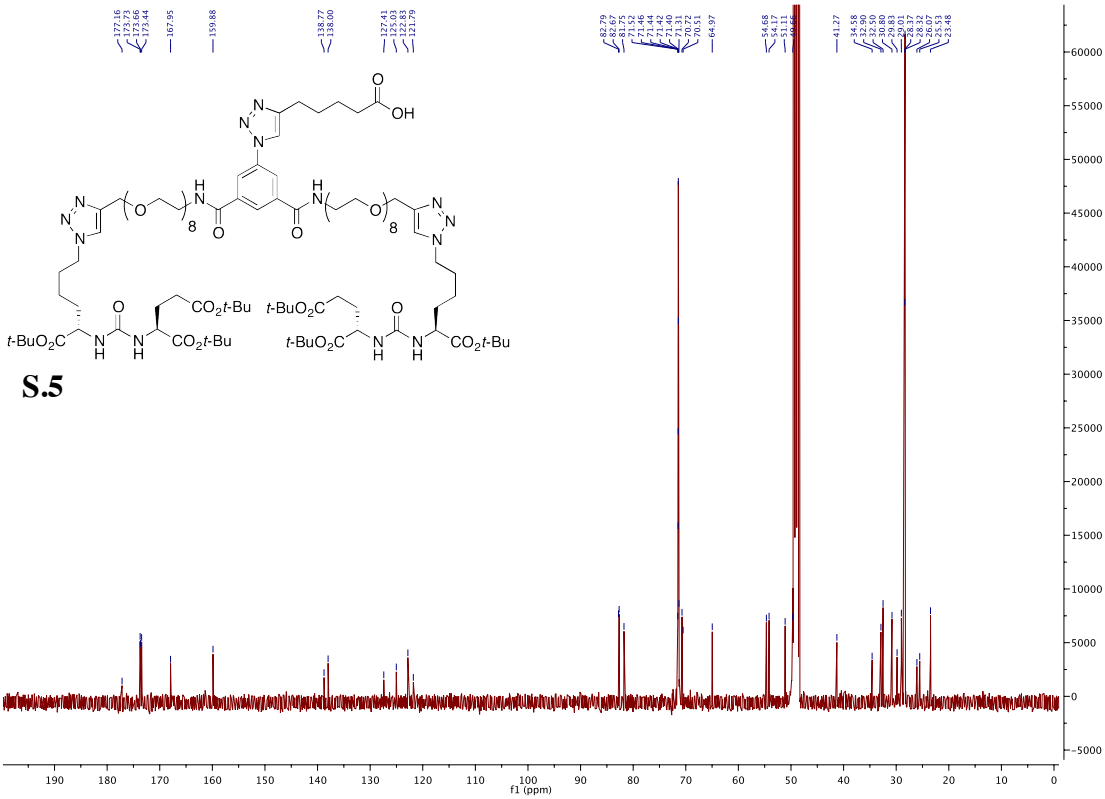
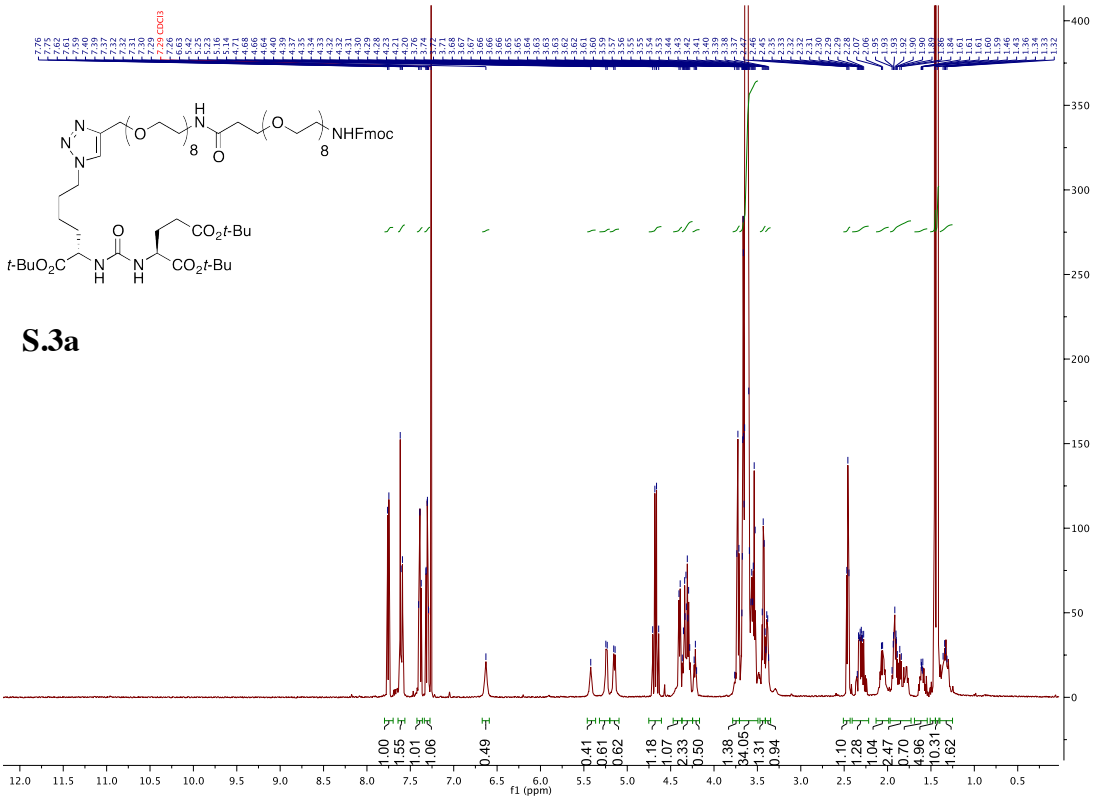


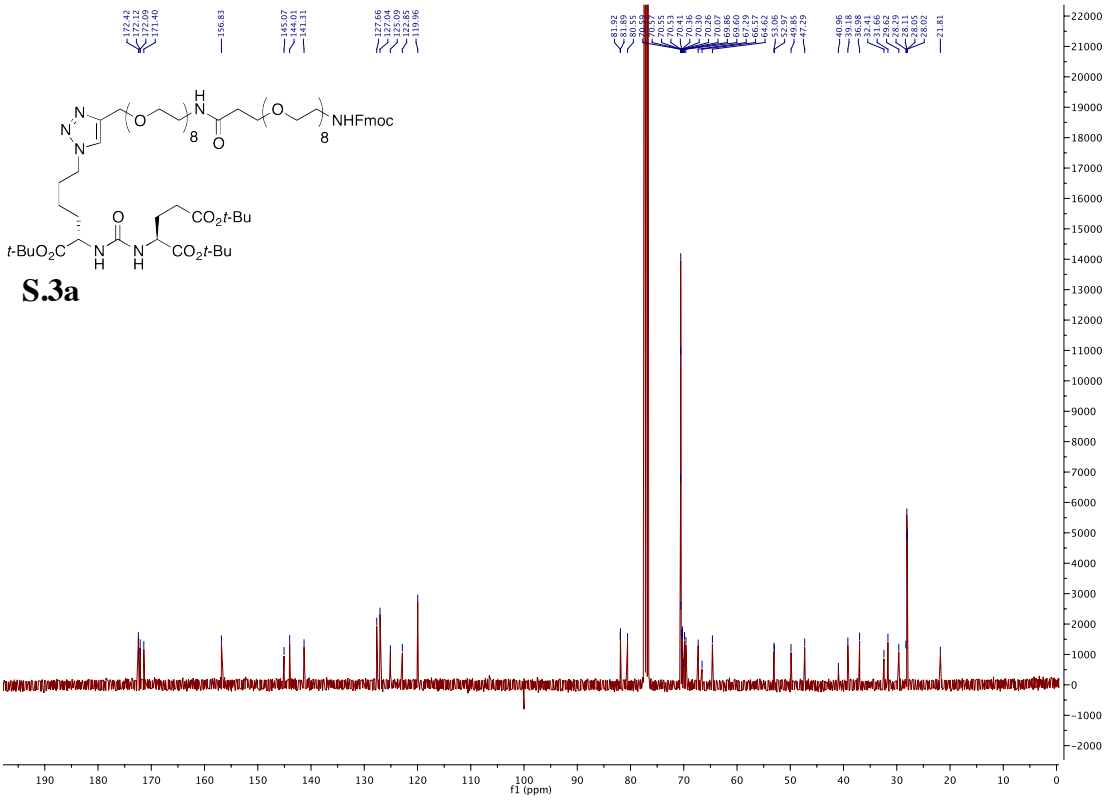

**S.5a**


S.5
f1 (ppm)

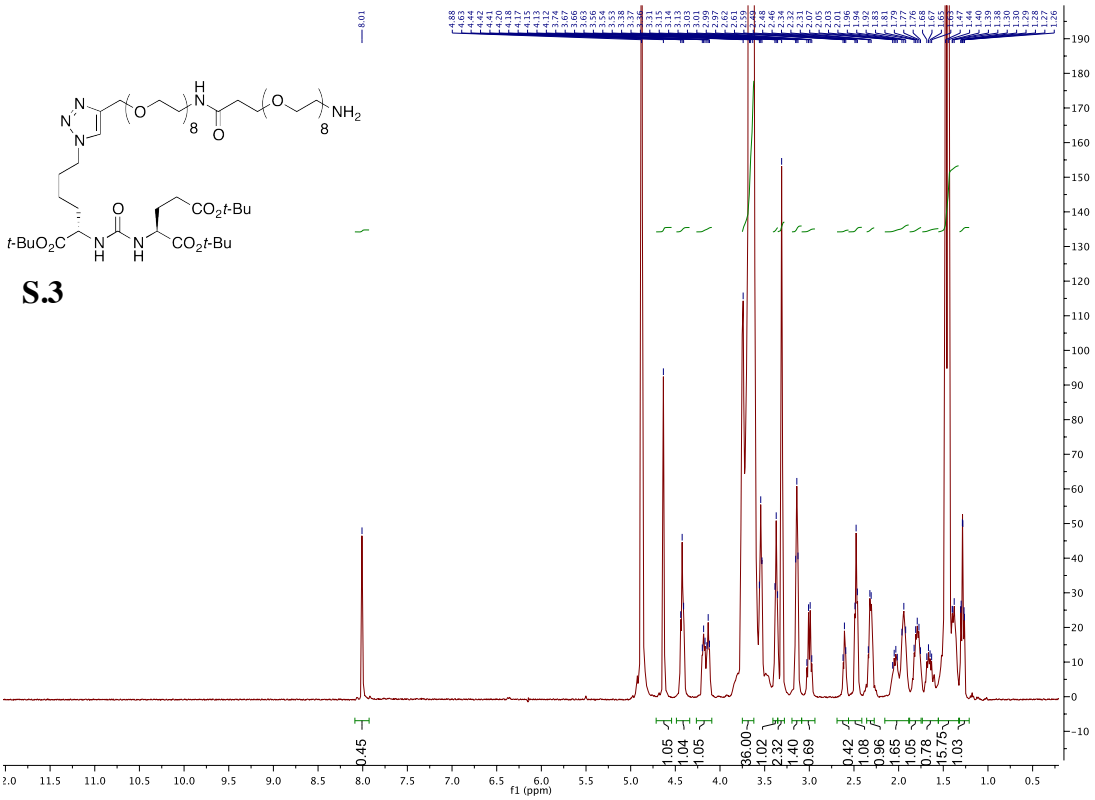

S.5
f1 (ppm)

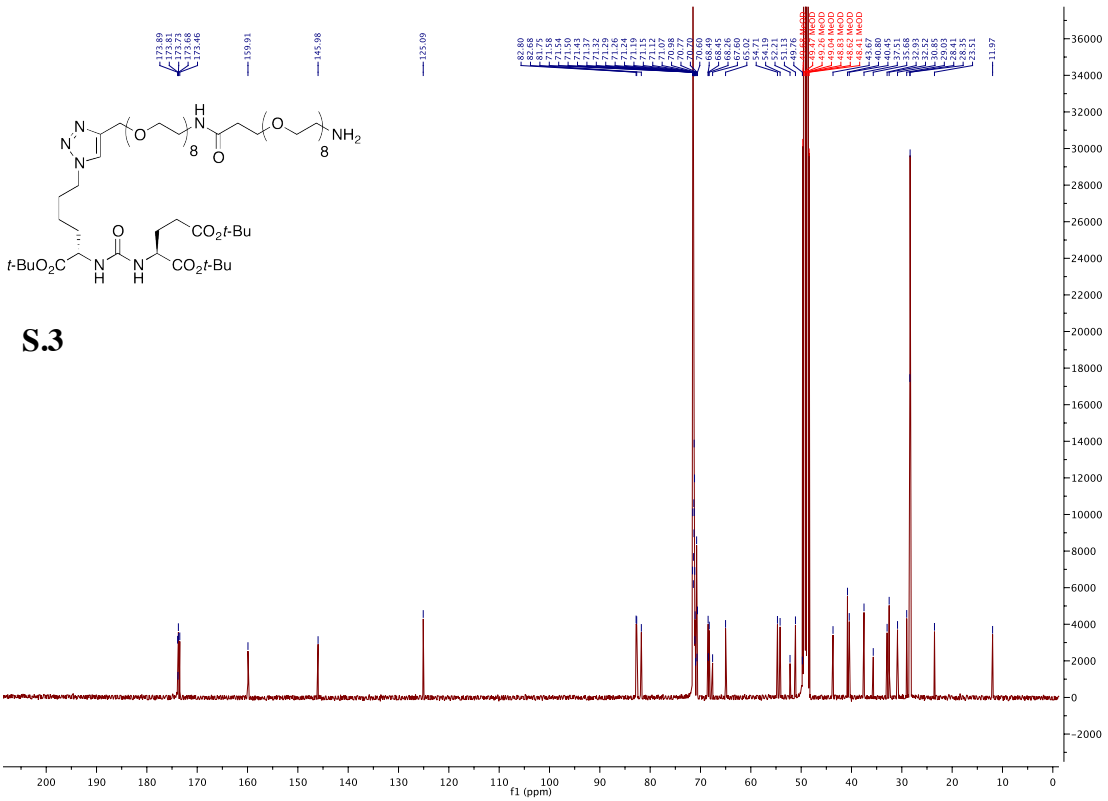

NHFmoc
CO2t-Bu
t-BuO2C
CO2t-Bu
f1 (ppm)

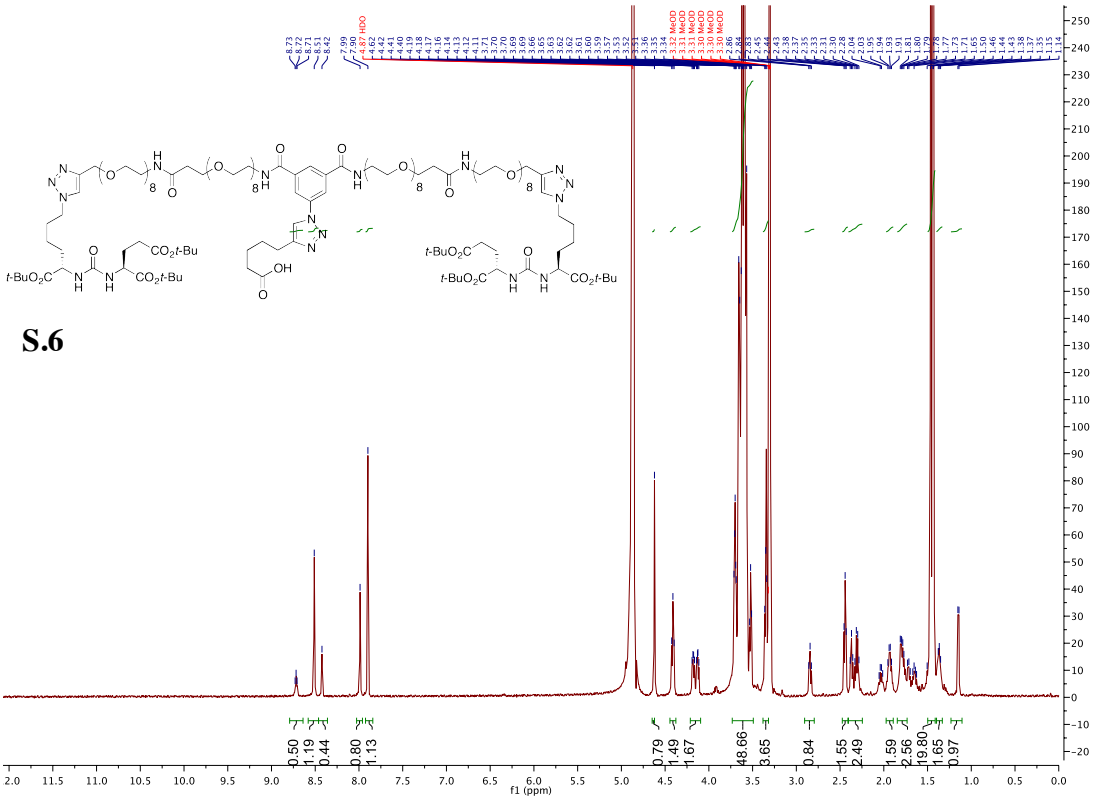
**S.3a**


NHFmoc
CO2t-Bu
t-BuO2C
CO2t-Bu
f1 (ppm)

**S.3a**


S.3
f1 (ppm)


S.3
f1 (ppm)



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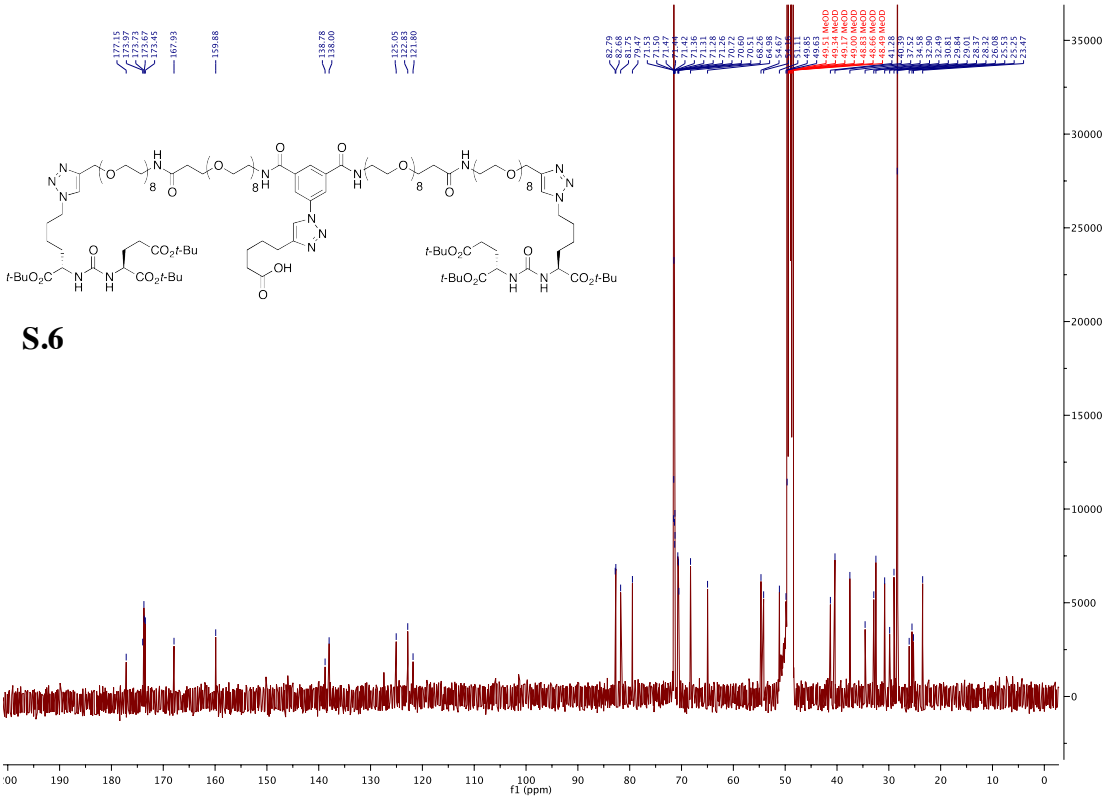
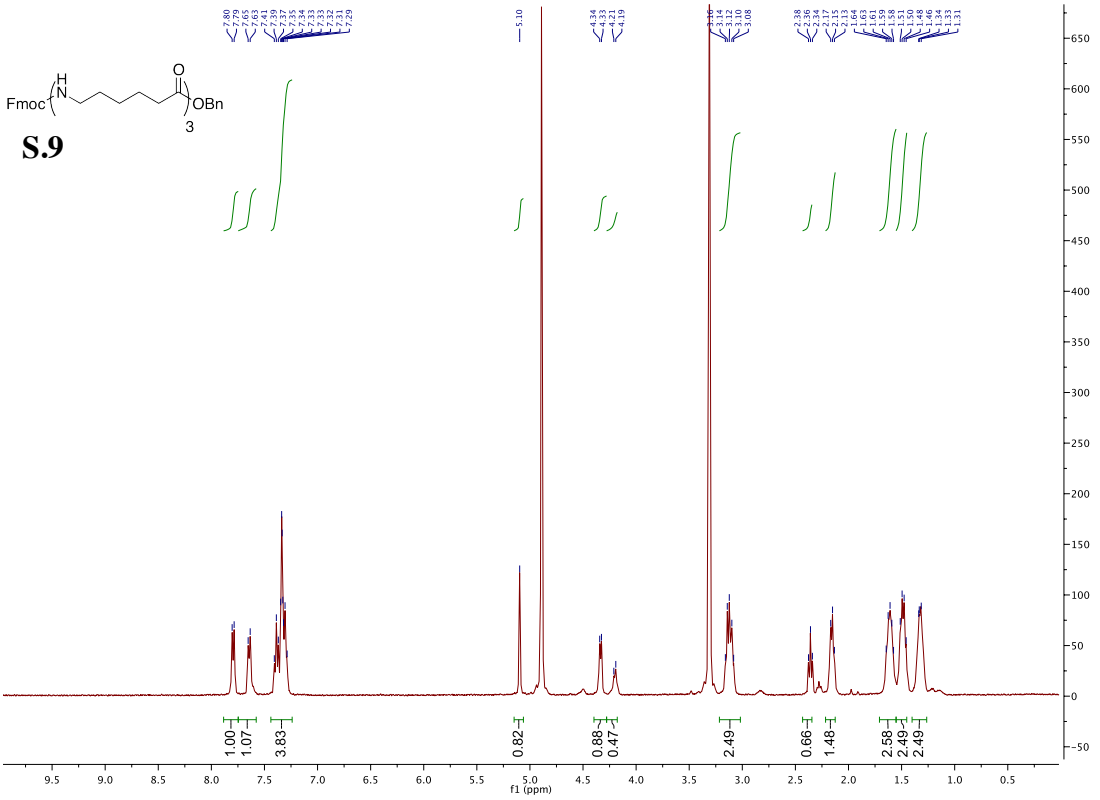
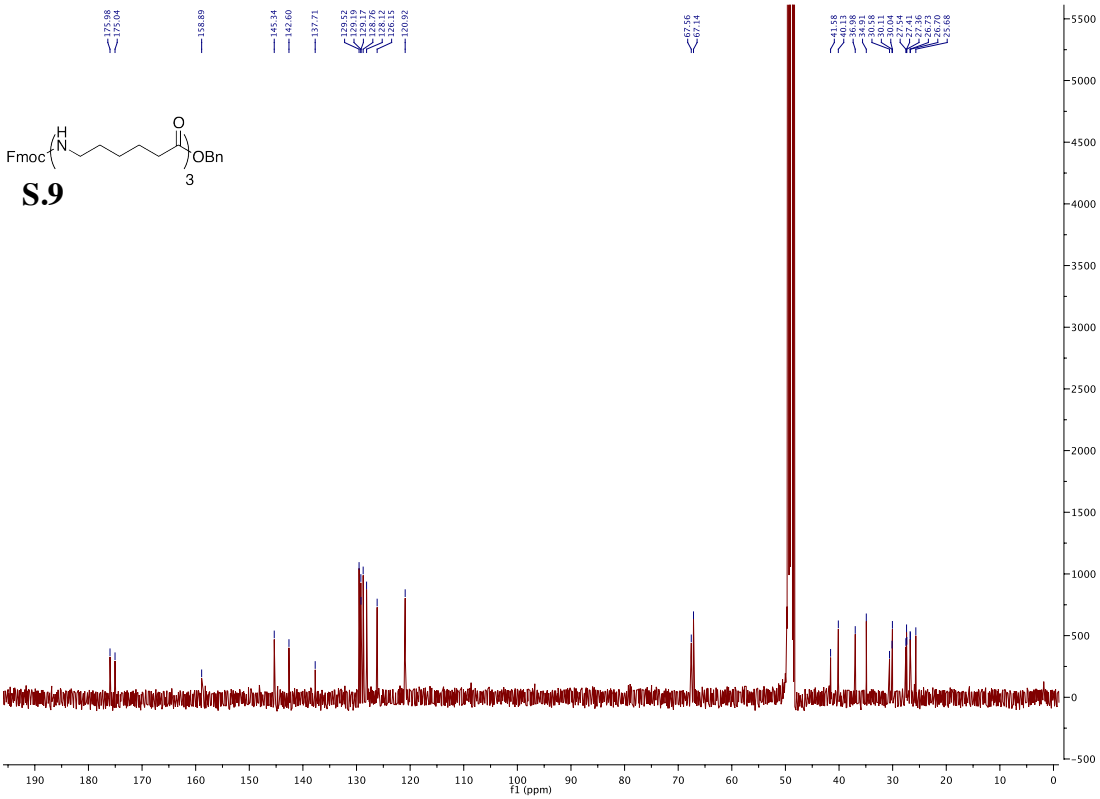
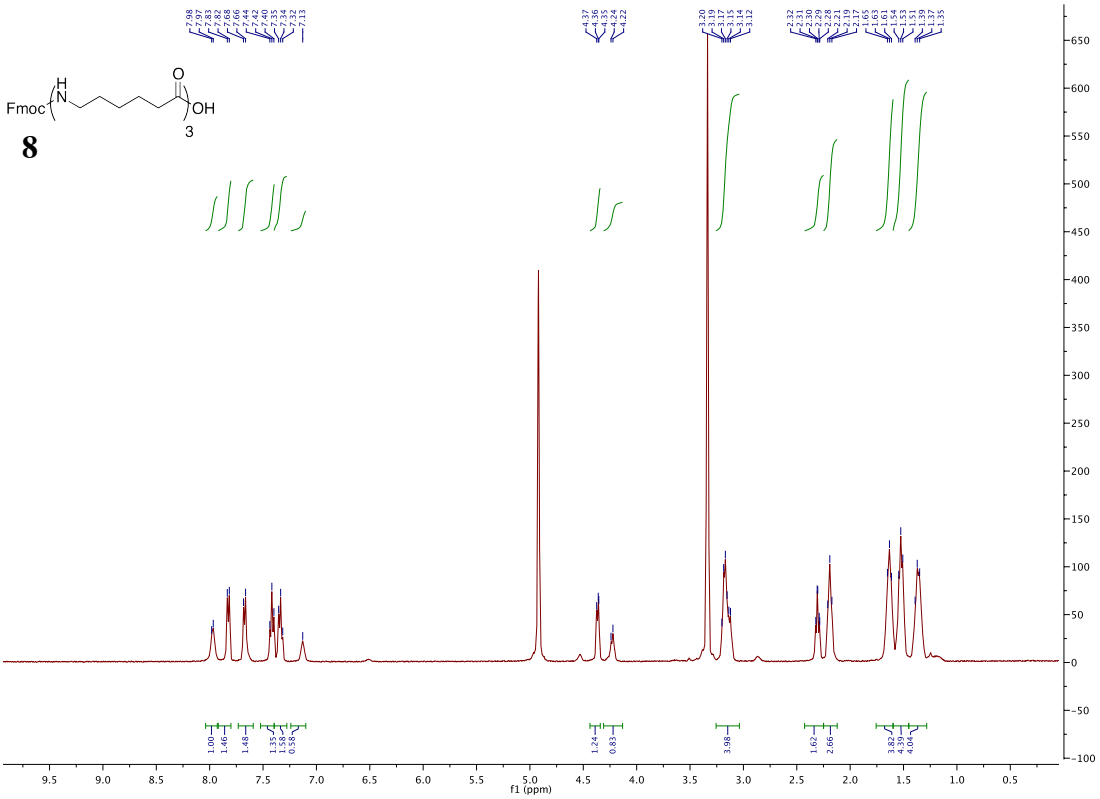
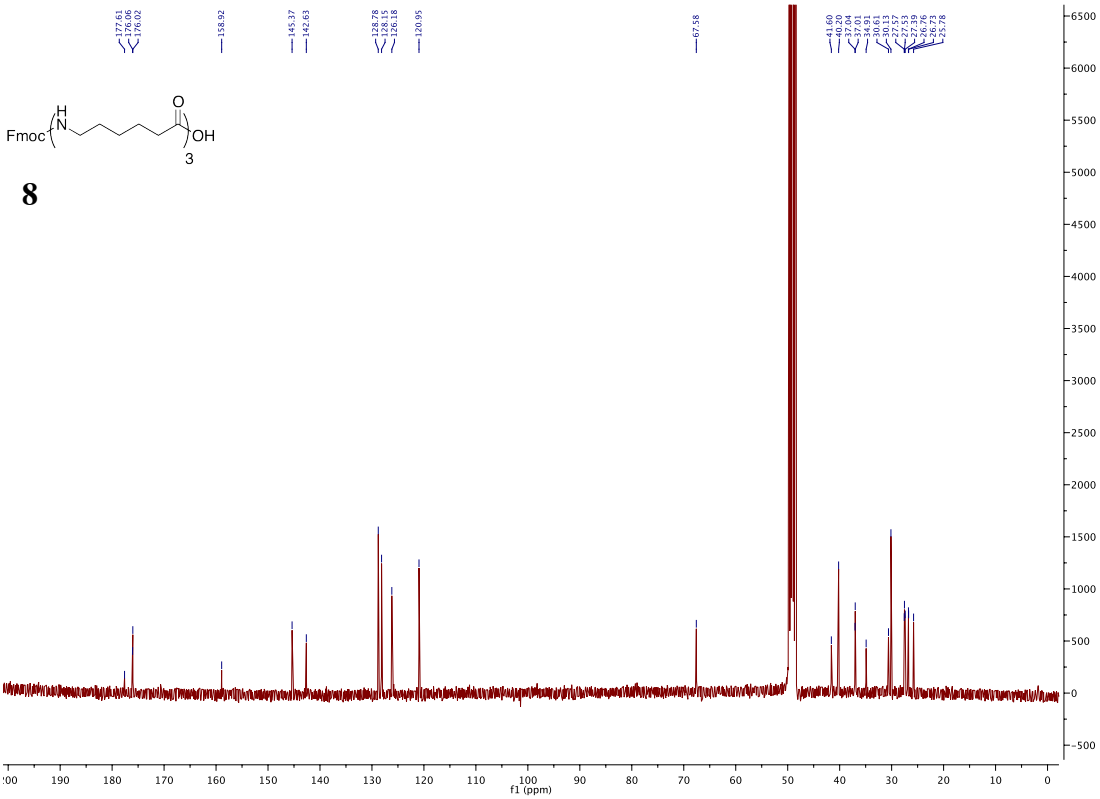


**S.6**


Fmoc
N
H
O
OBn
3
S.9


Fmoc
N
H
O
OBn
3
S.9


Fmoc
N
H
O
OH
3
8
f1 (ppm)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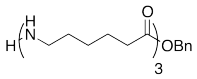
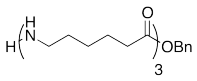
H
O
OH
3
8
f1 (ppm)



**S.10**





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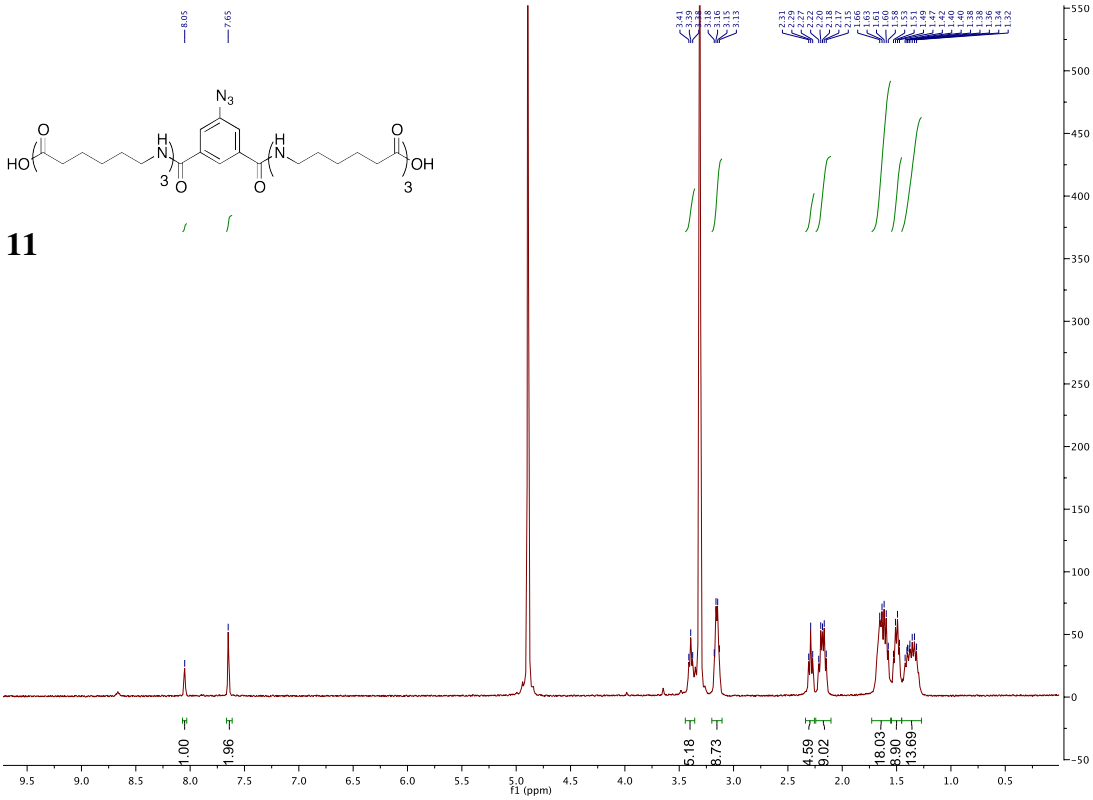
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3.39
3.38
3.18
3.16
3.15
3.13
2.31
2.29
2.27
2.22
2.20
2.18
2.17
2.15
1.66
1.63
1.61
1.60
1.58
1.53
1.51
1.49
1.47
1.42
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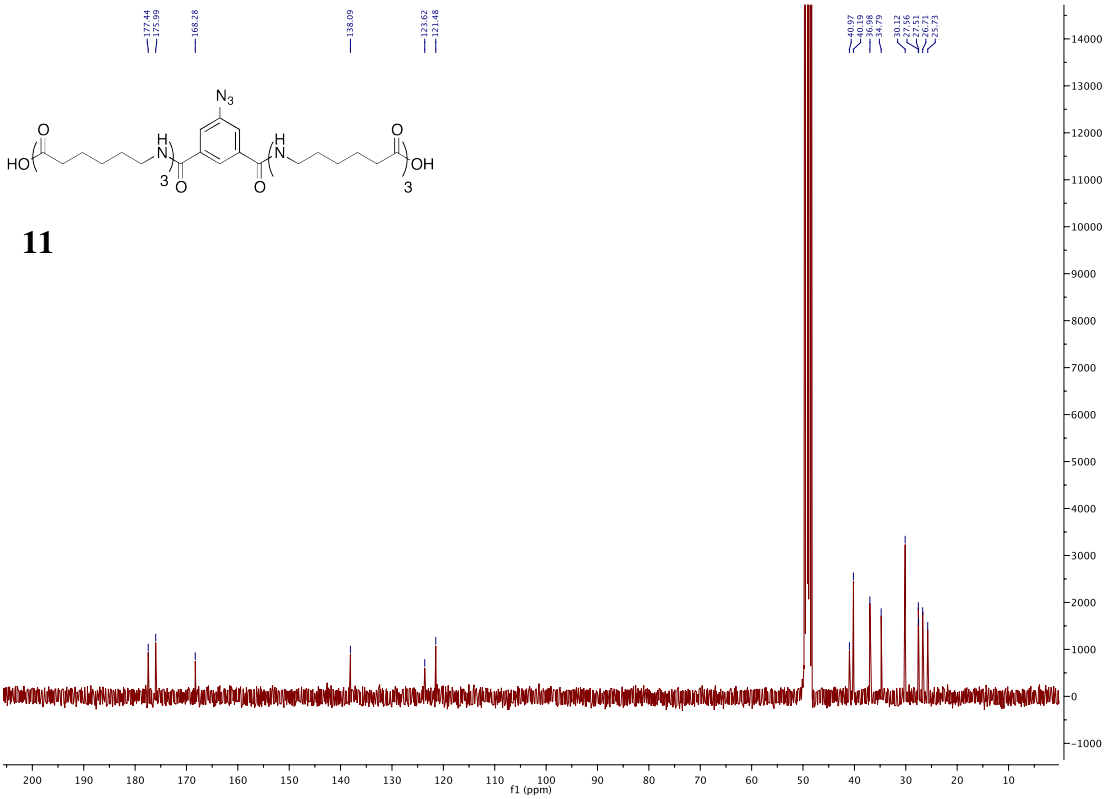
18.03
8.90
13.69
f1 (ppm)

**11**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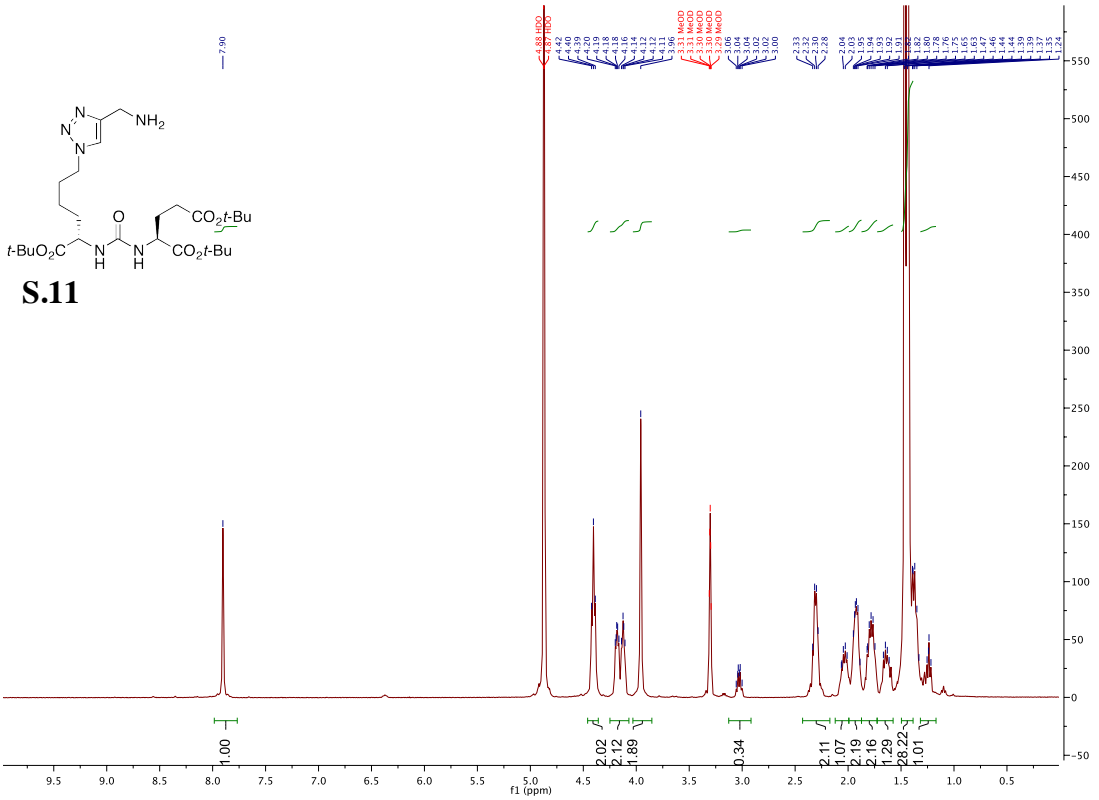
34.79
30.12
27.56
27.31
26.71
25.73
f1 (ppm)

**11**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CO2t-Bu
t-BuO2C
CO2t-Bu
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2.02
2.12
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2.11
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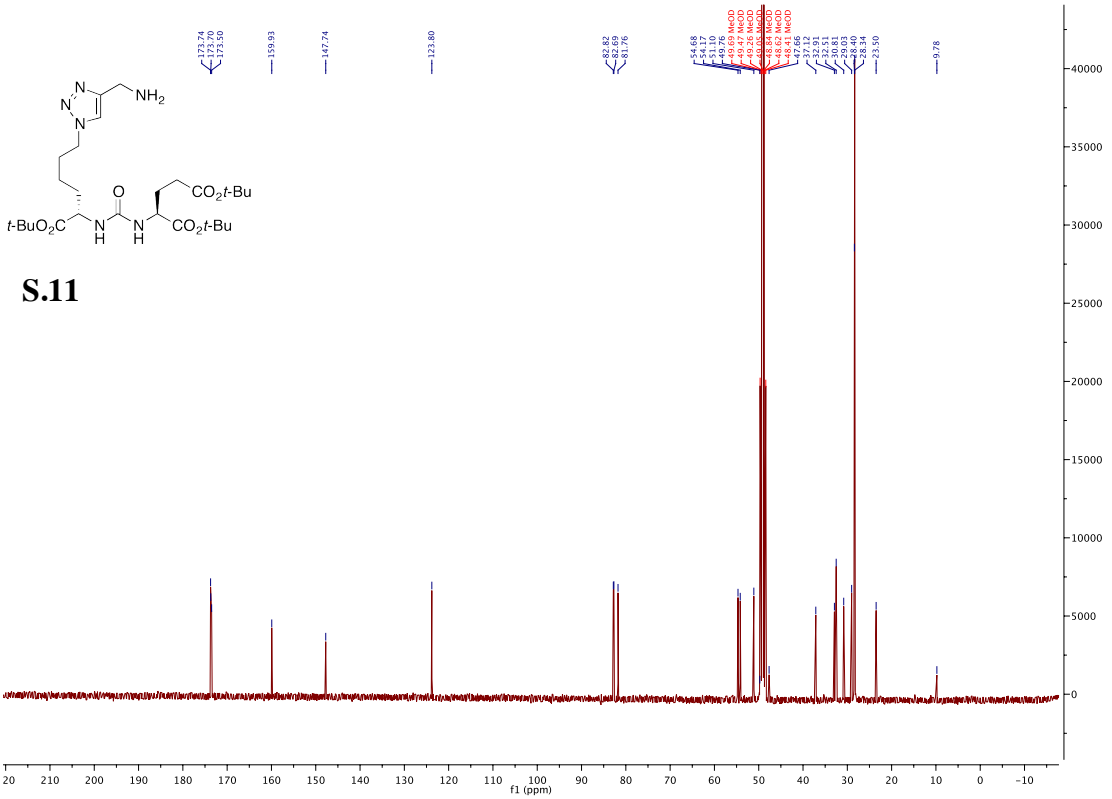
2.19
2.16
1.29
28.22
1.01
f1 (ppm)

**S.11**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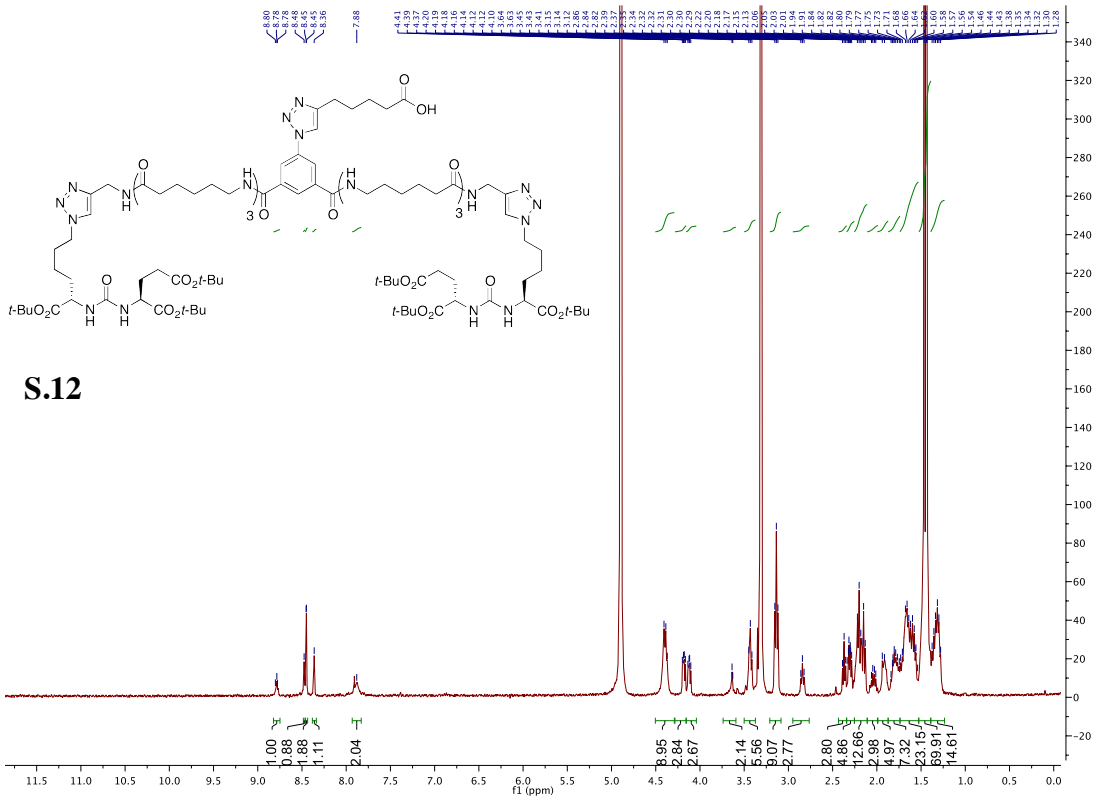
CO2t-Bu
t-BuO2C
CO2t-Bu
f1 (ppm)

**S.11**



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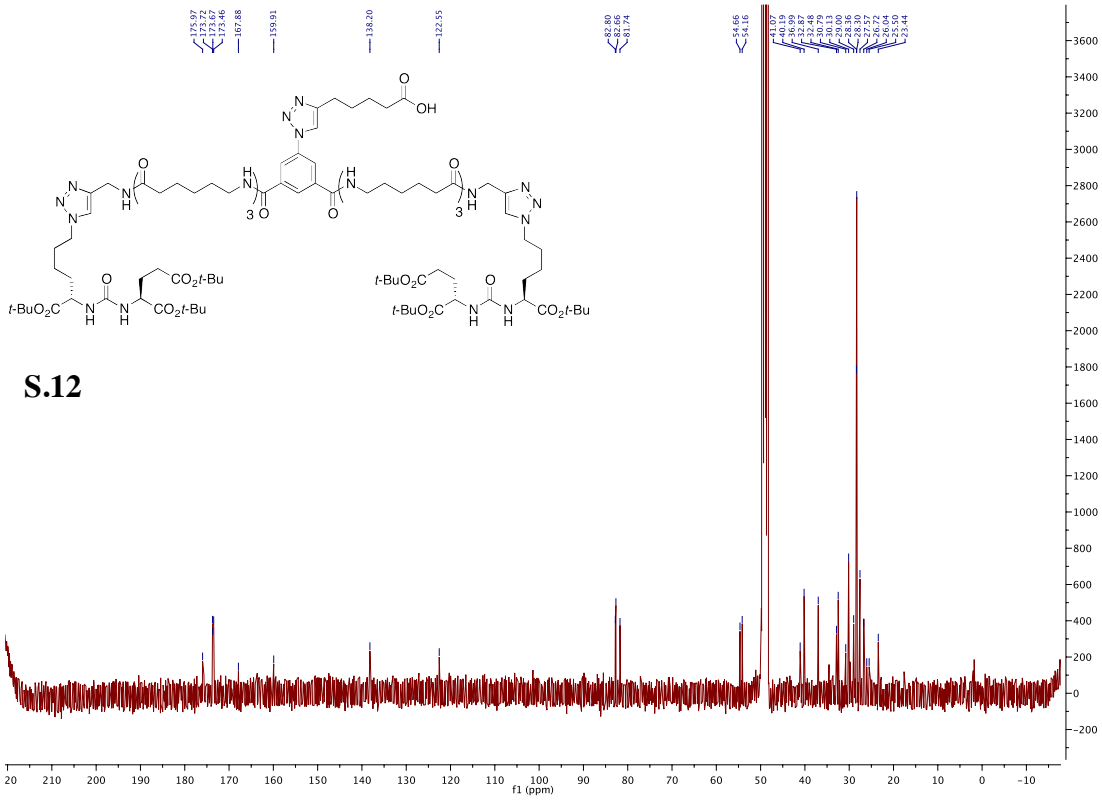

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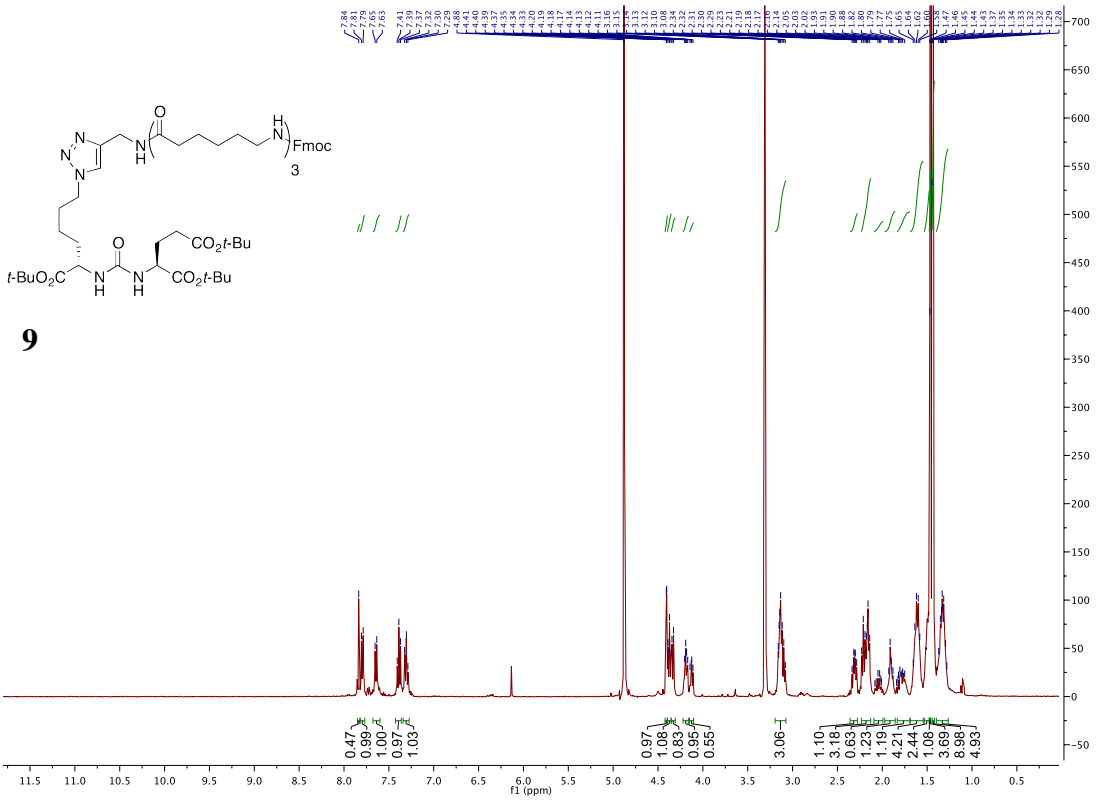
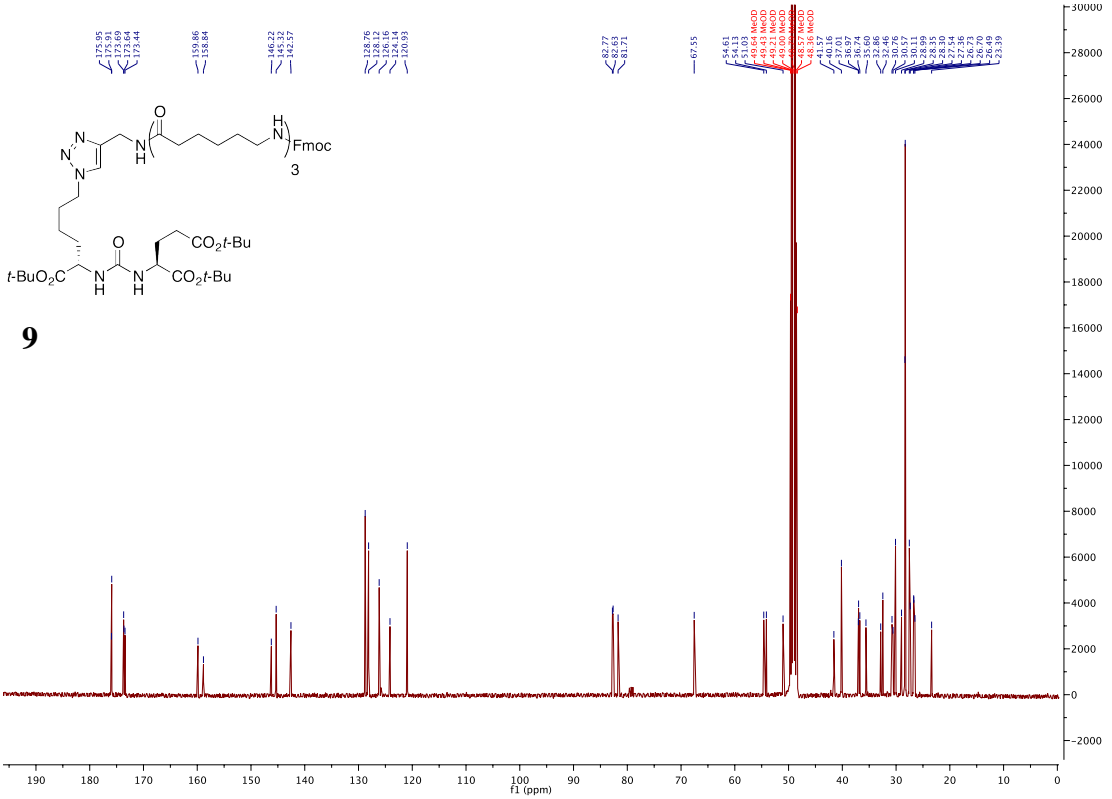

Fmoc
3
CO2t-Bu
t-BuO2C
CO2t-Bu
f1 (ppm)

**9**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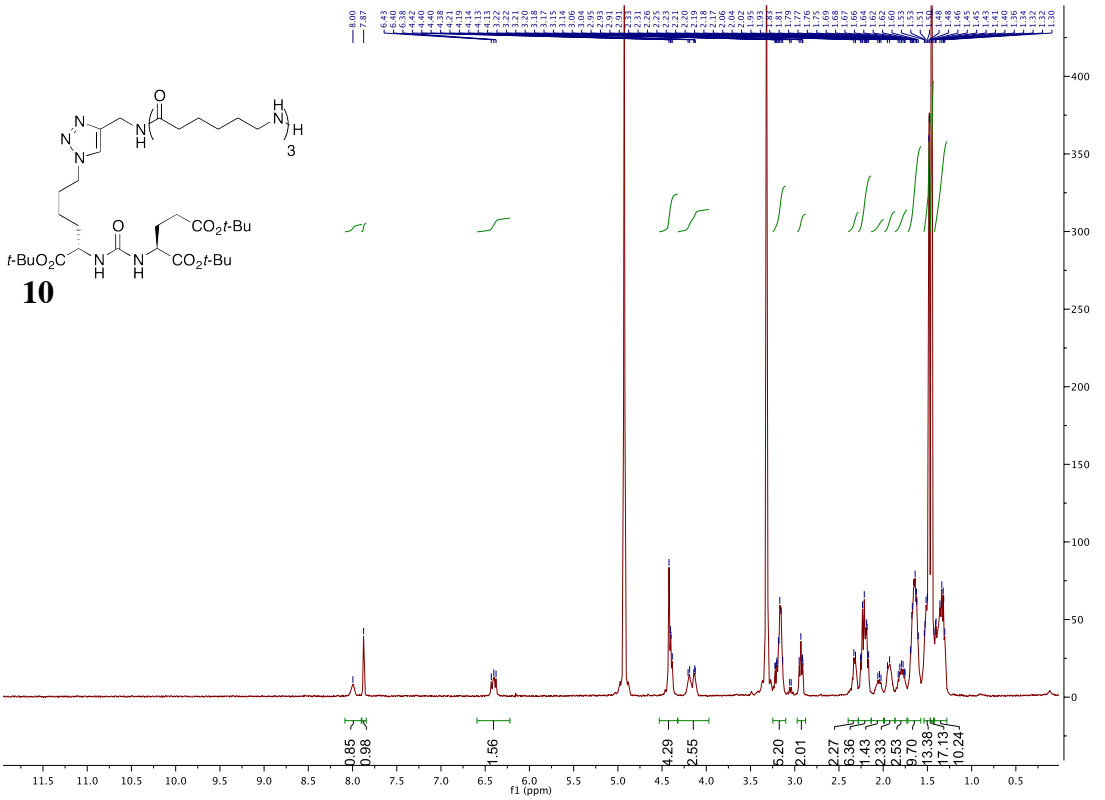
3
CO2t-Bu
t-BuO2C
CO2t-Bu
f1 (ppm)

**9**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6.36
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2.33
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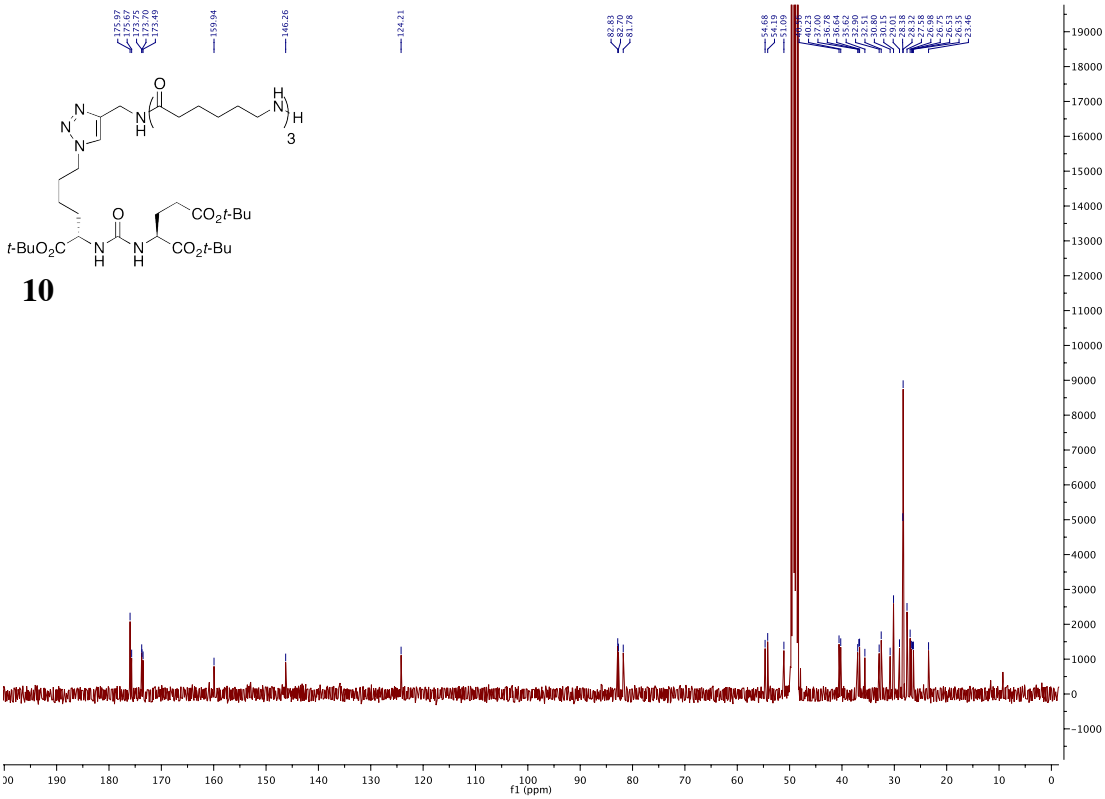
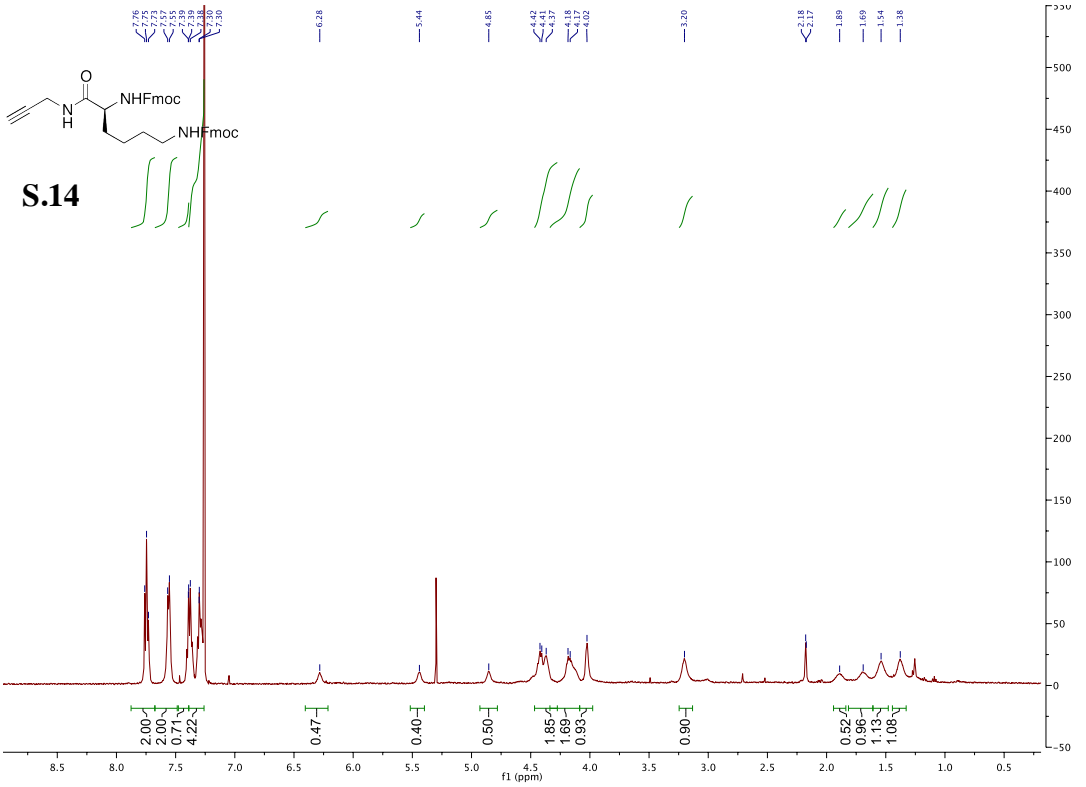
9.70
13.38
17.13
10.24
f1 (ppm)


10
f1 (ppm)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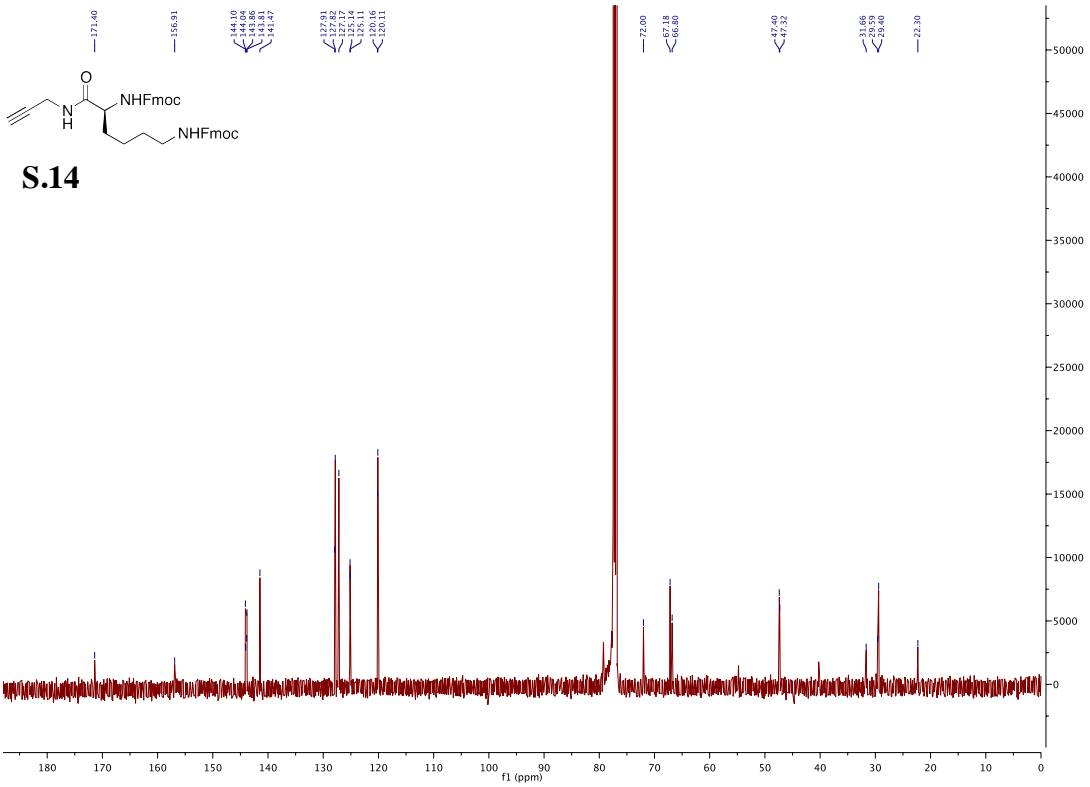
NHFmoc
S.14
f1 (ppm)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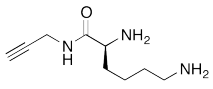
NHFmoc
S.14
f1 (ppm)



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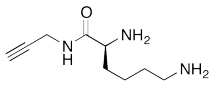

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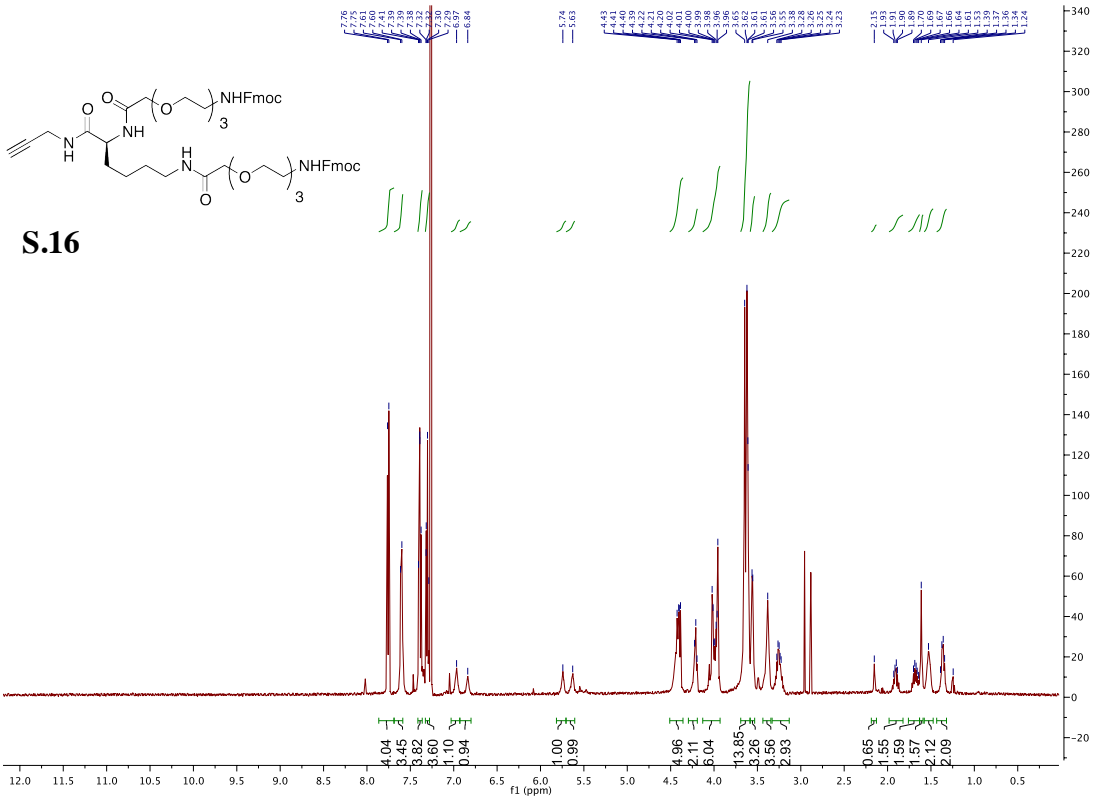
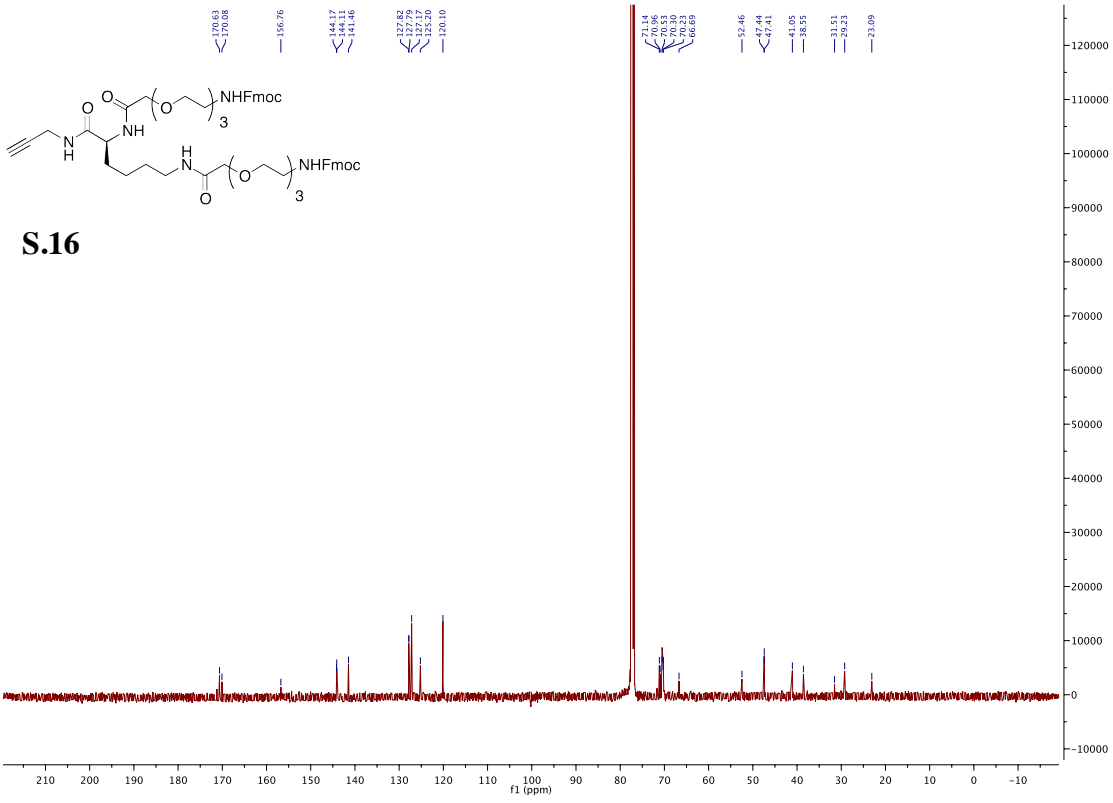
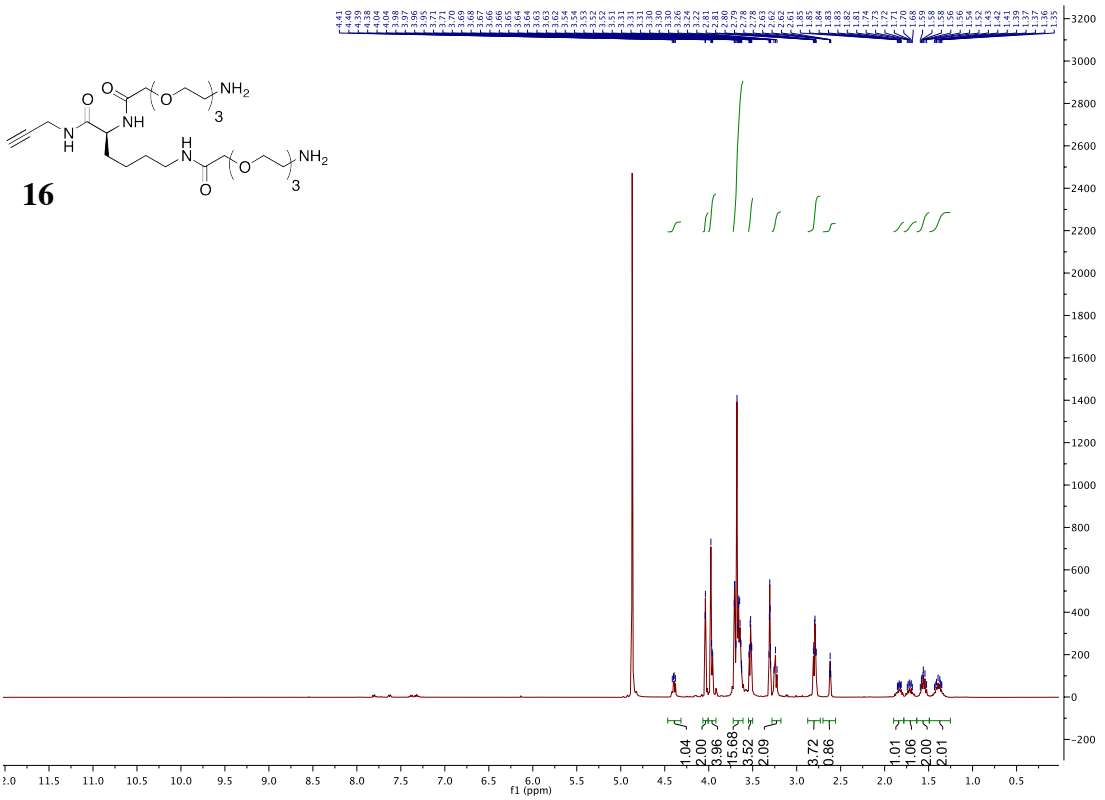
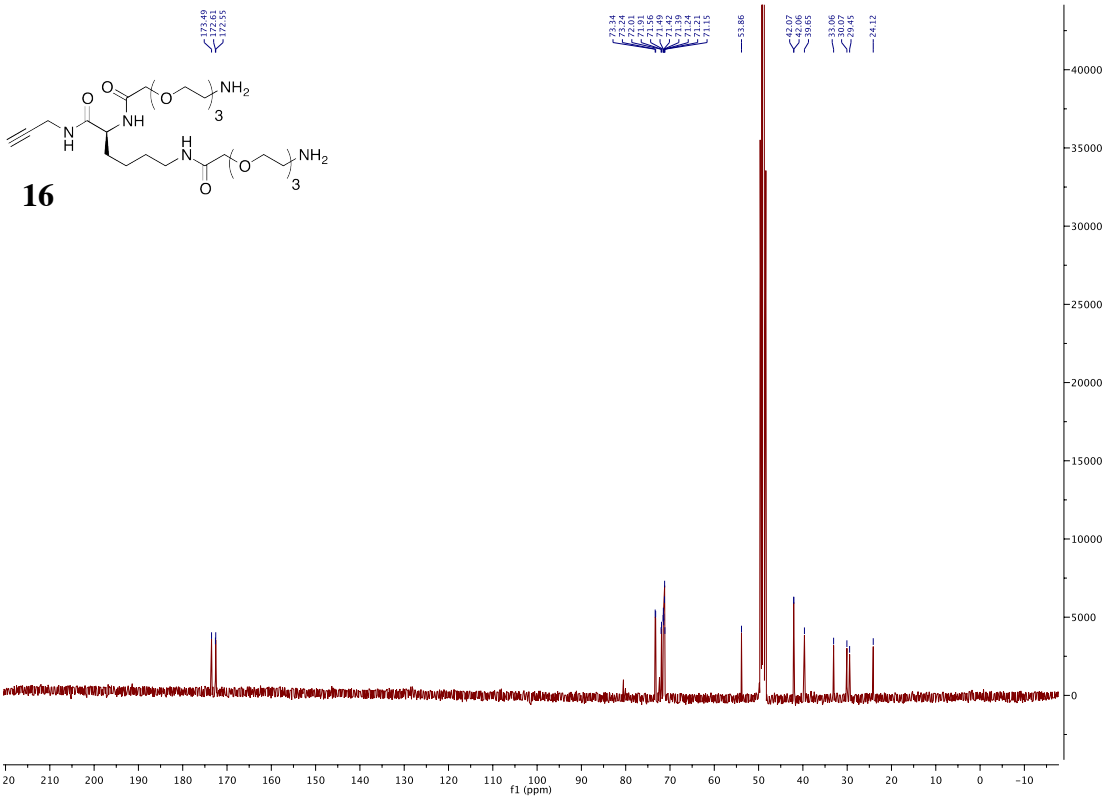


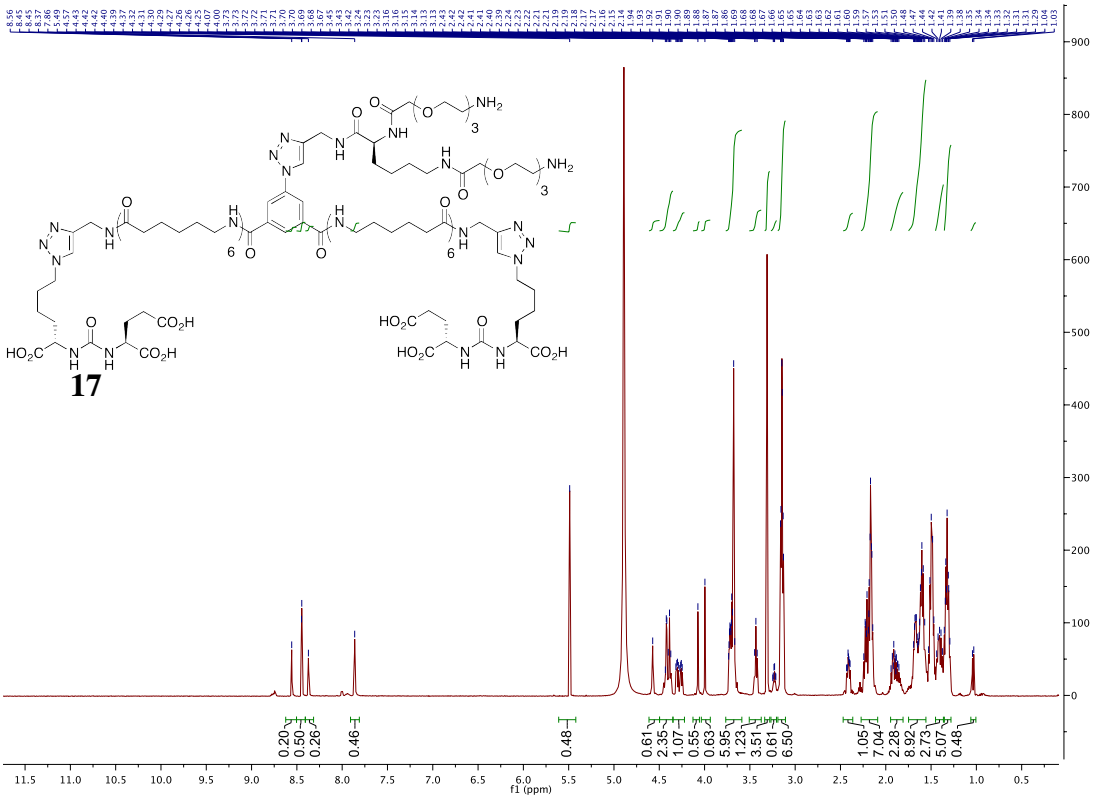

**S.16**



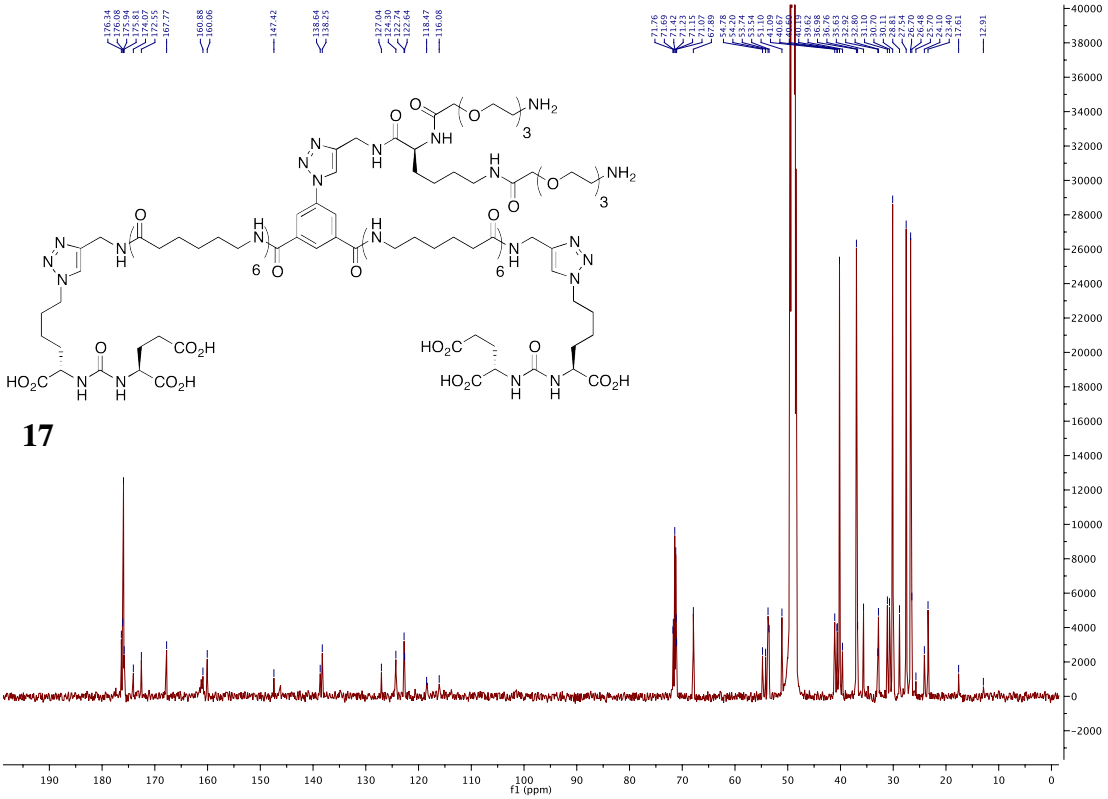

**S.16**


16
f1 (ppm)


16
f1 (ppm)



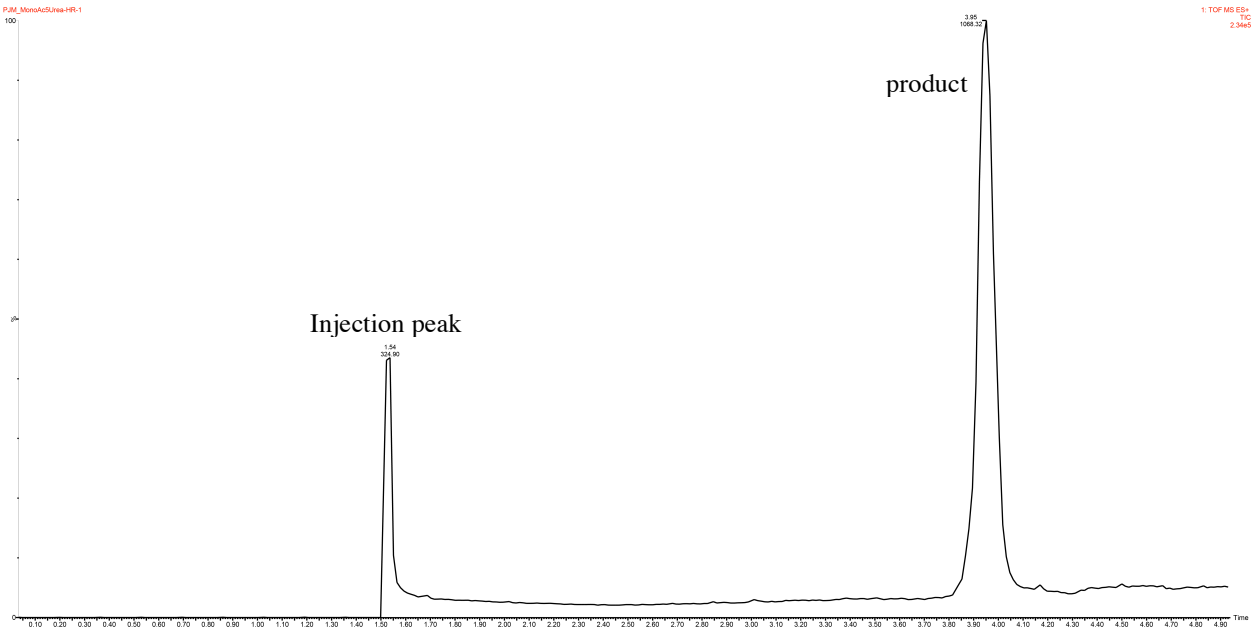
**17**



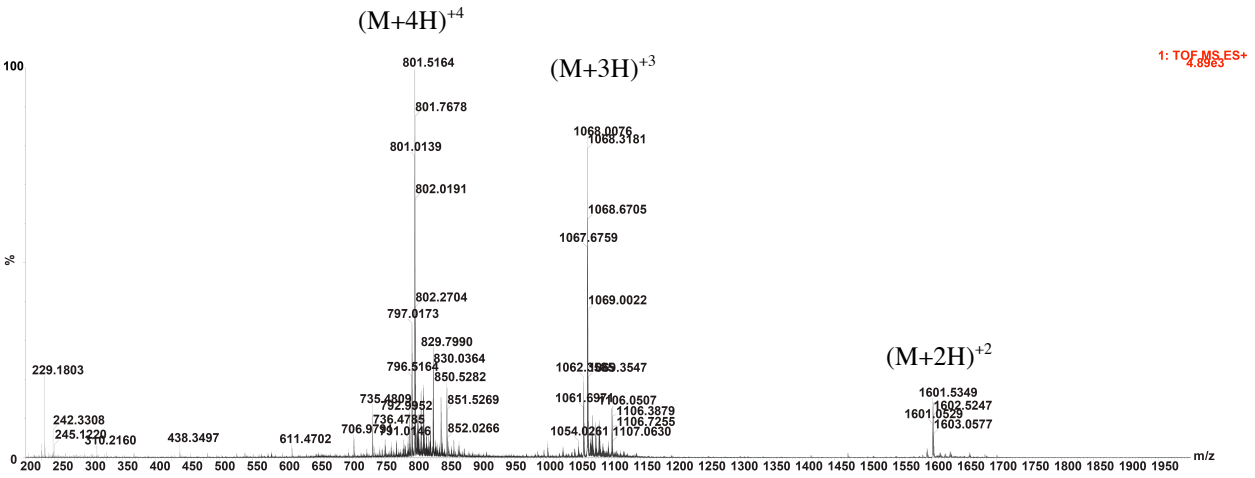
**17**

**Catalog of Liquid Chromatography traces and Mass Spectra**

**1 (SyAM-P1)**

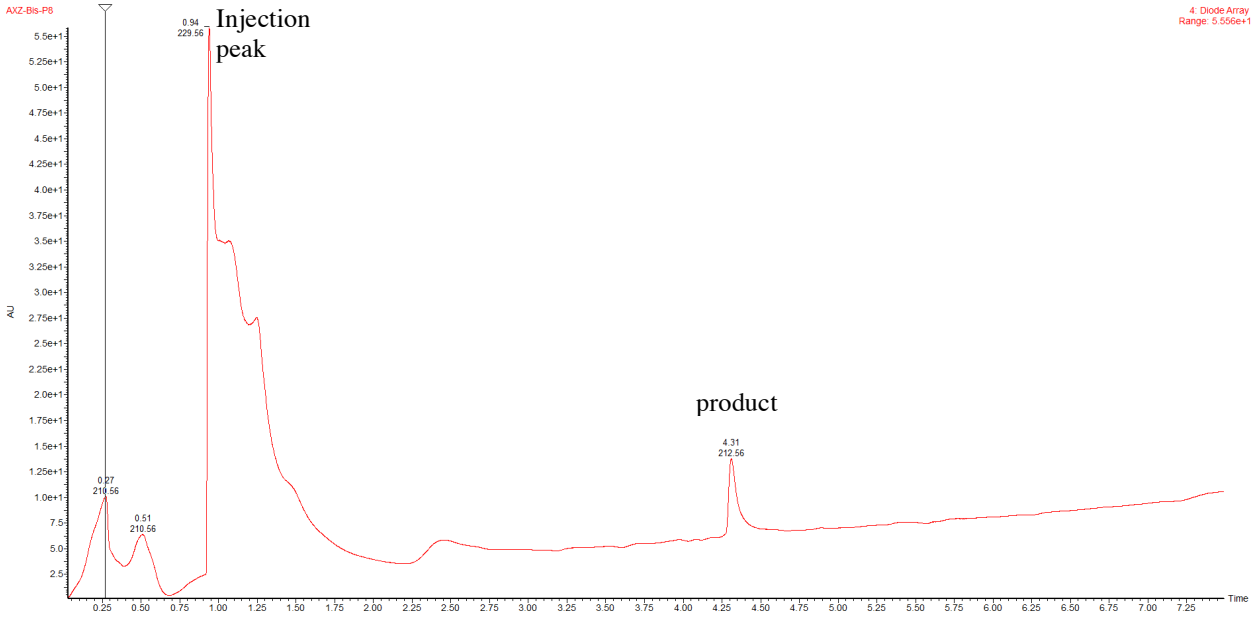



**1 (SyAM-P1)** total ion count

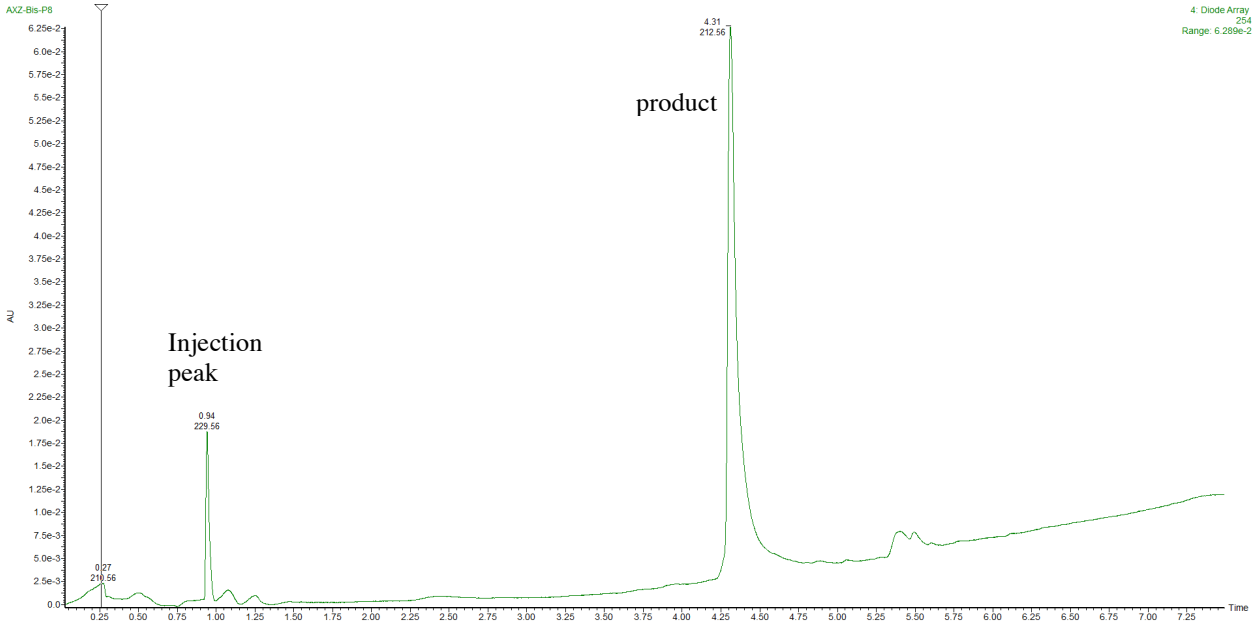



**1 (SyAM-P1)** mass trace

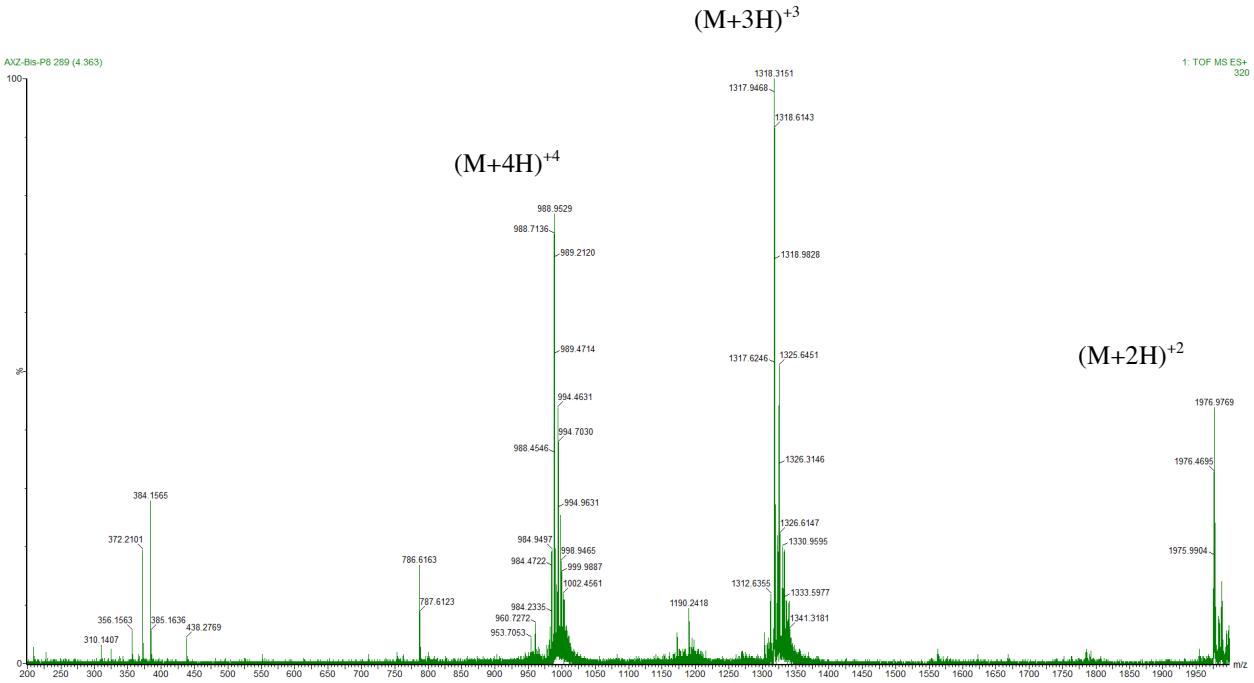
**(S.7)**




**S.7** diode array

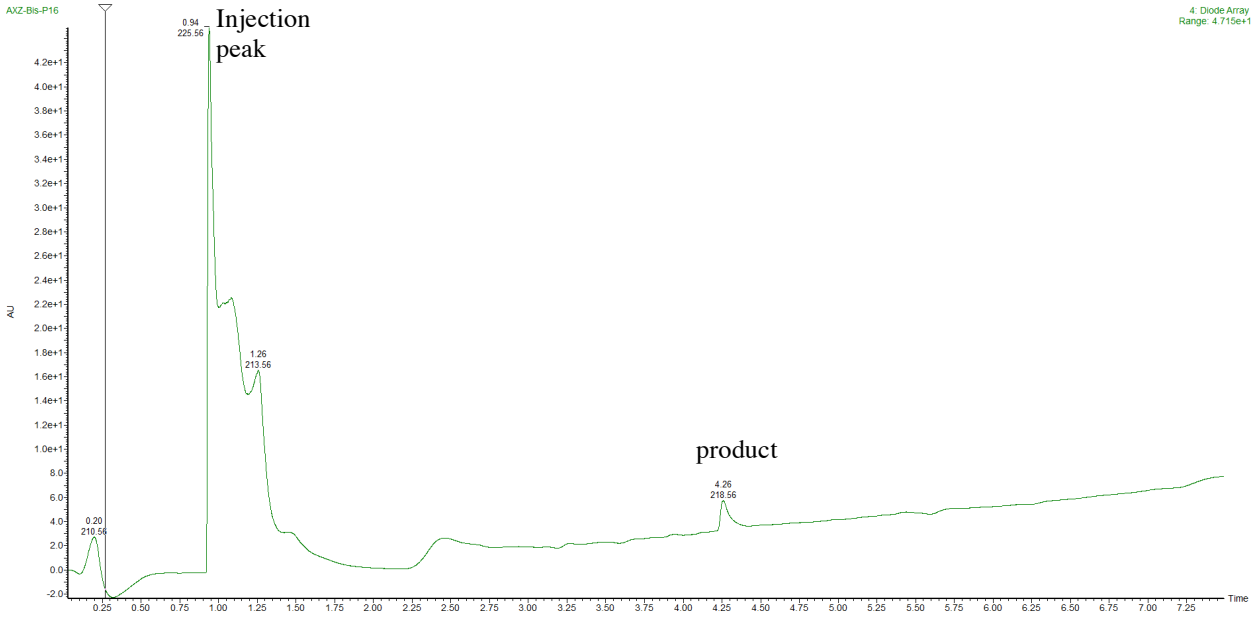



**S.7** 254 nm absorbance

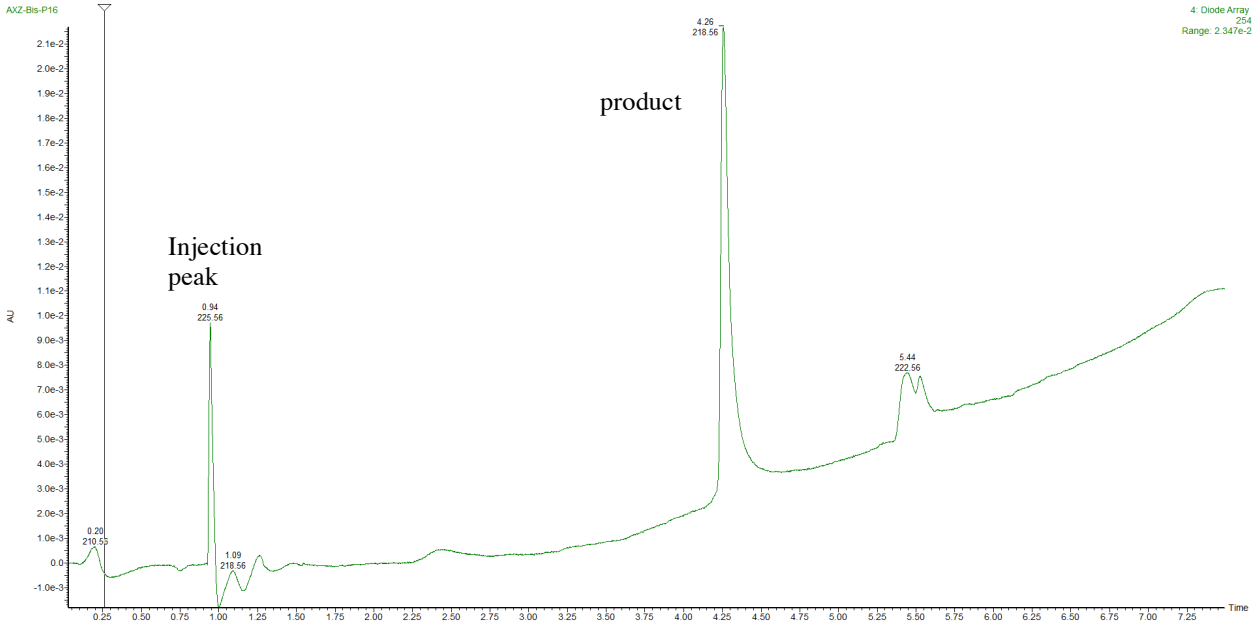



**S.7** mass trace

**(S.8)**

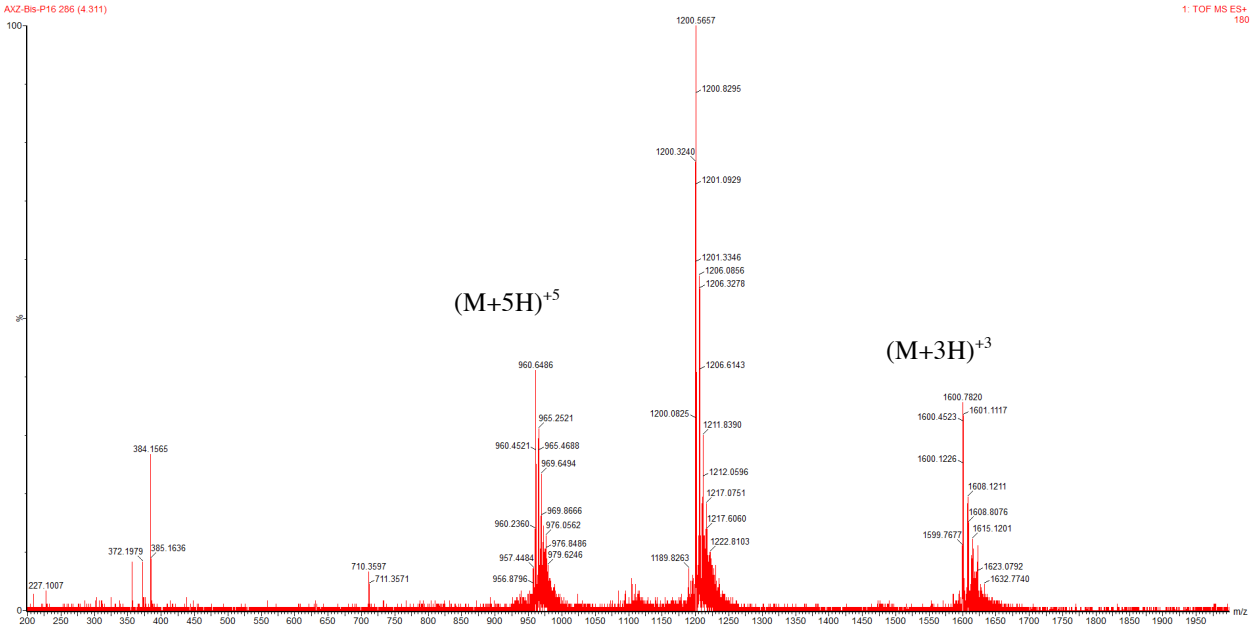



**S.8** diode array



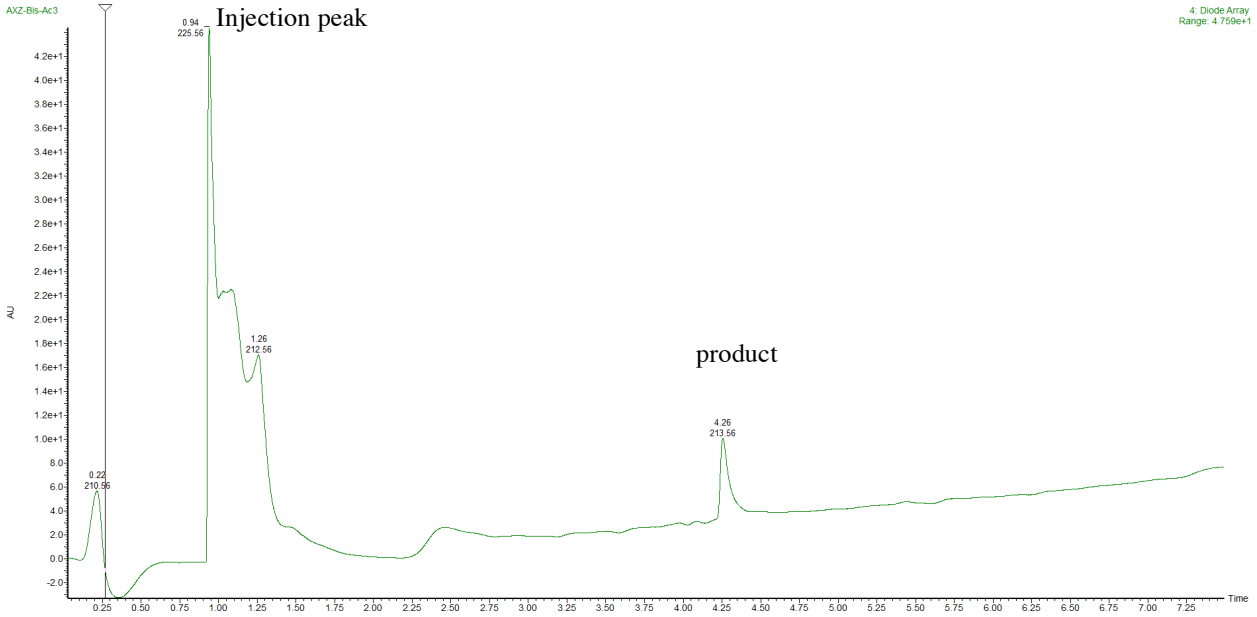

**S.8** 254nM absorbance

$(M+4H)^{+4}$

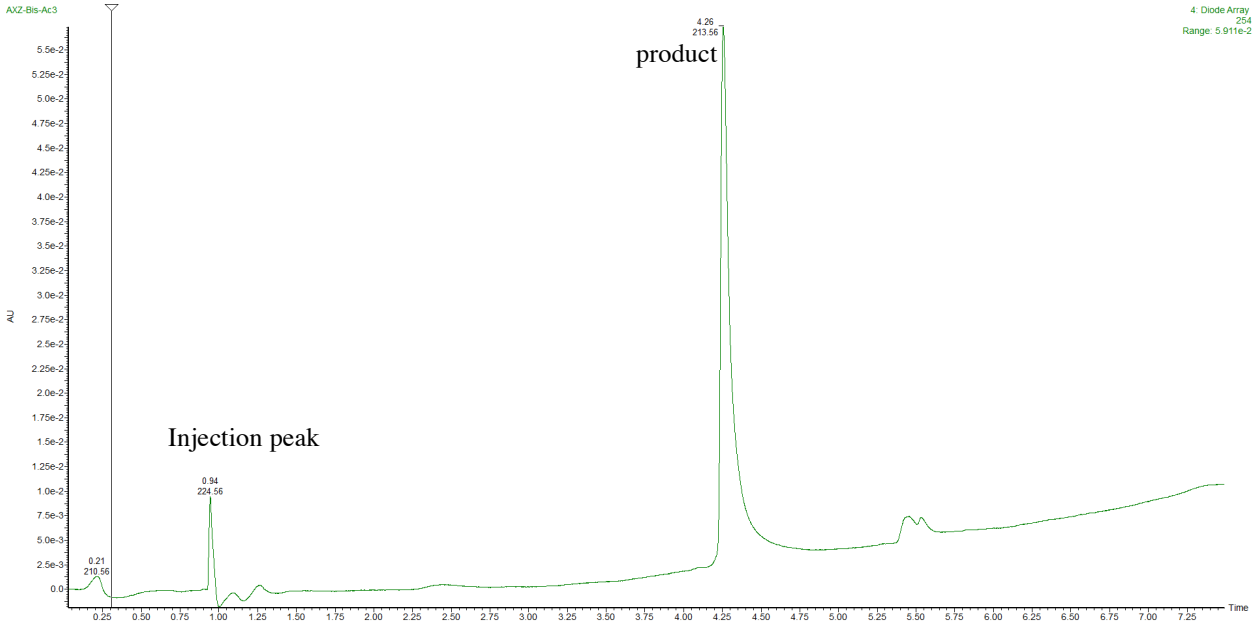



**S.8** mass trace

**(S.13)**

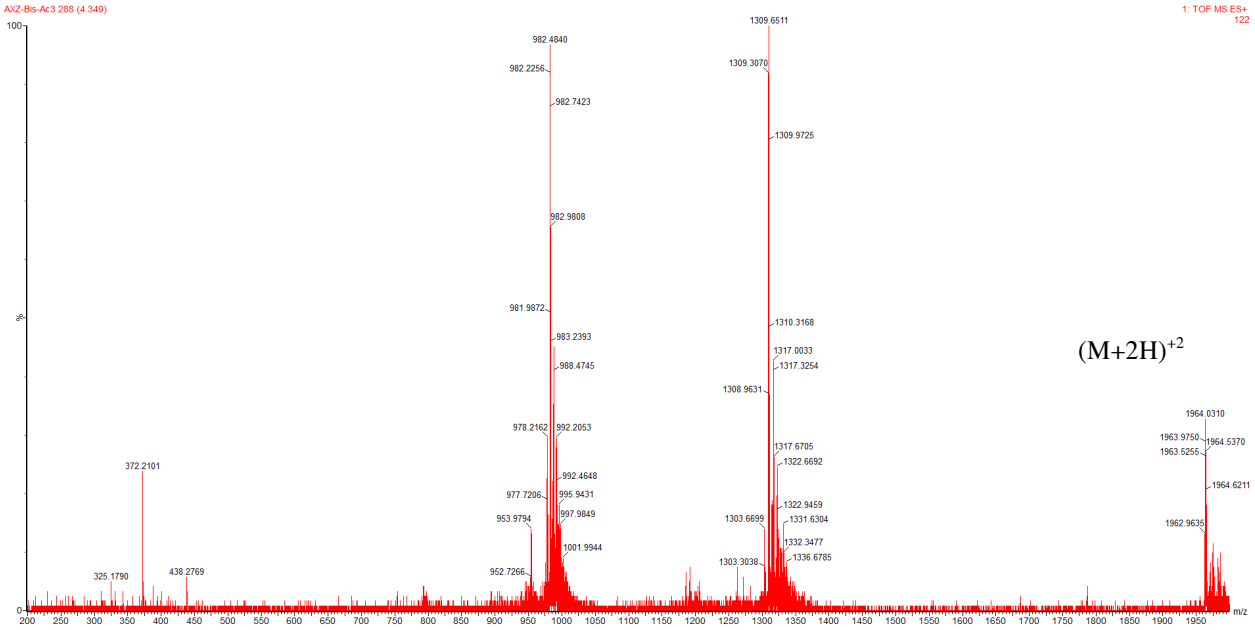



**S.13** diode array



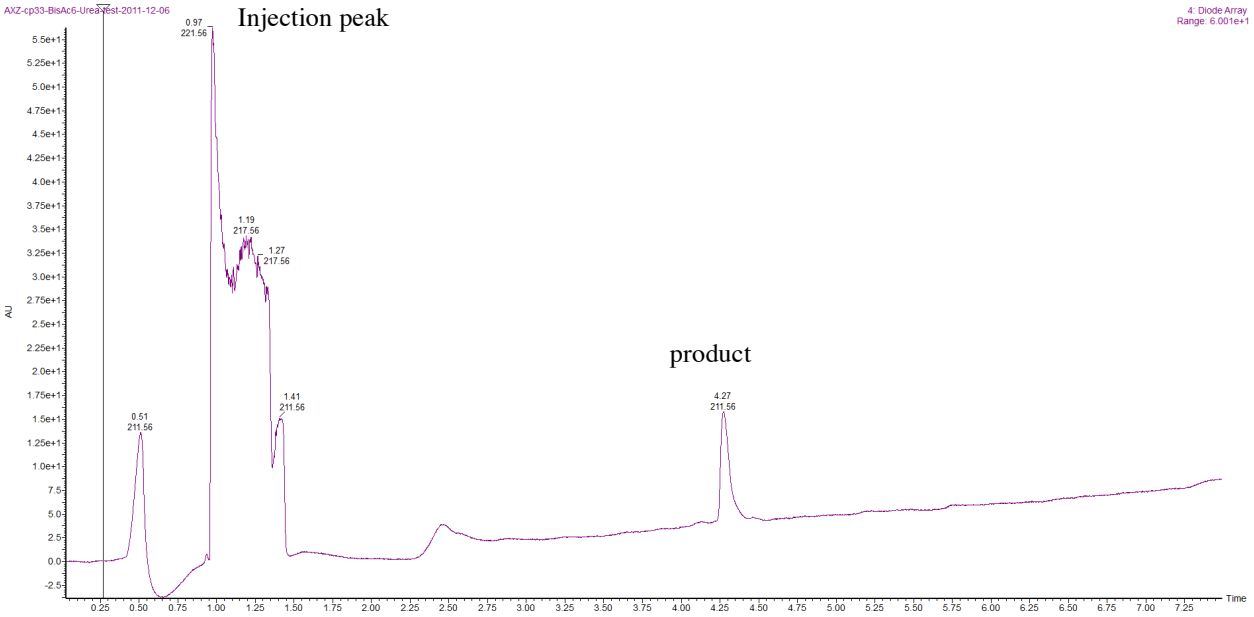

**S.13** 254 nM absorbance

$(M+4H)^{+4}$ $(M+3H)^{+3}$

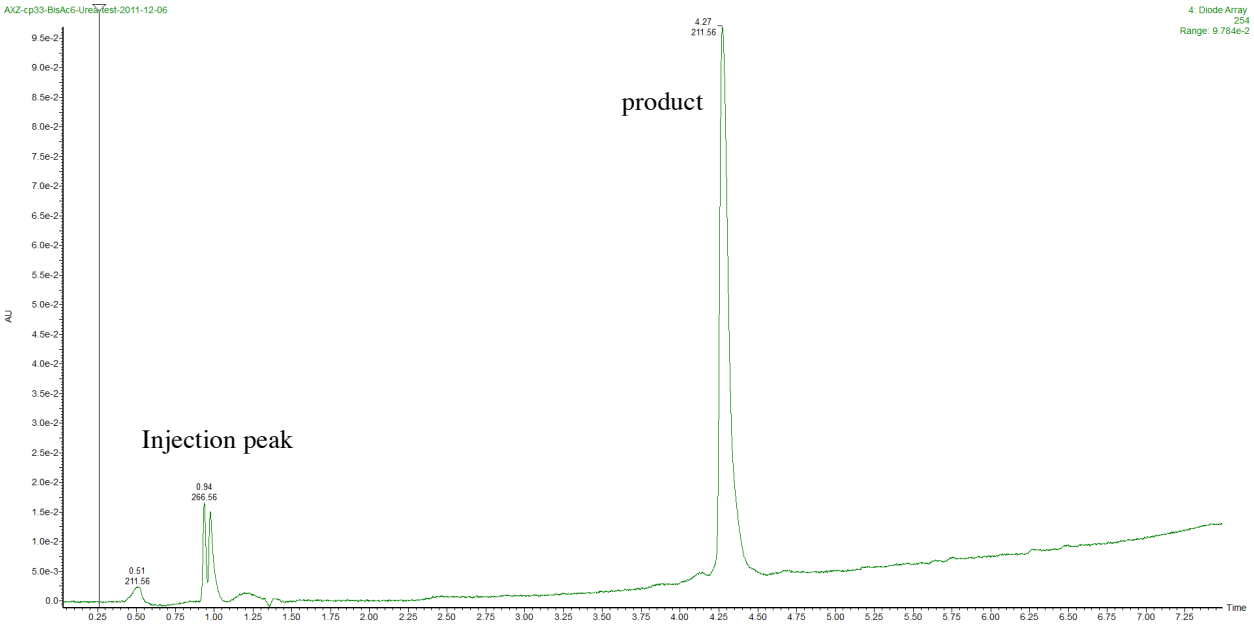



**S.13** mass trace

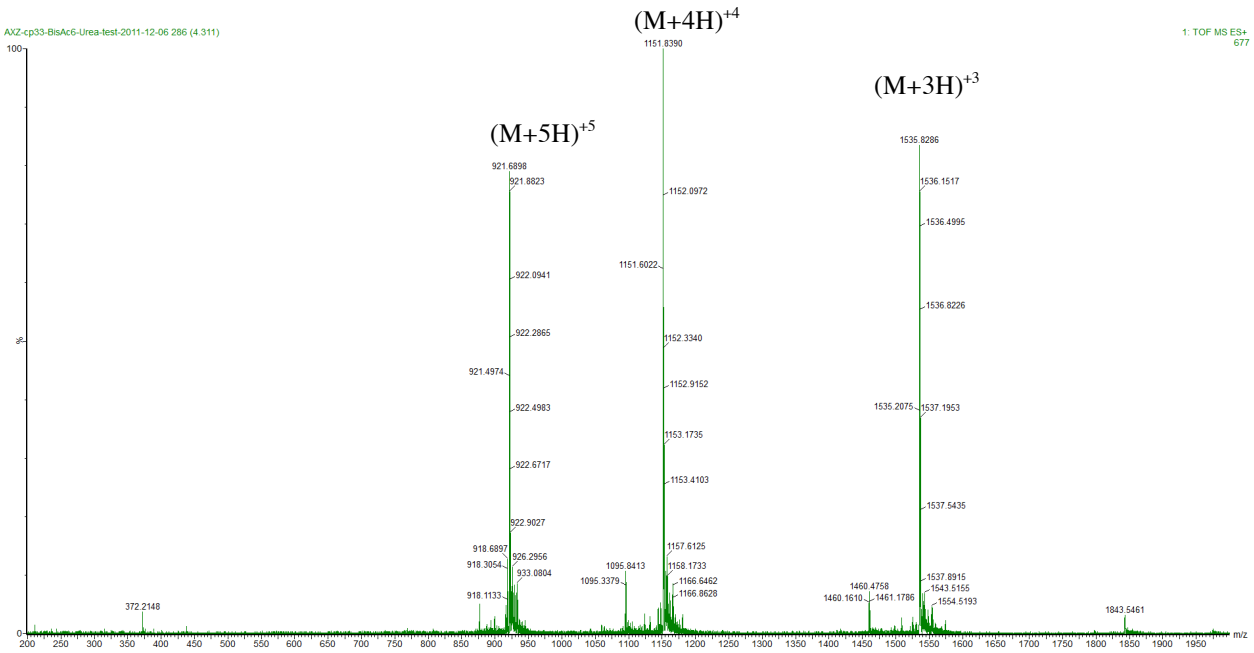
**2 (SyAM-P2)**




**2 (SyAM-P2)** diode array

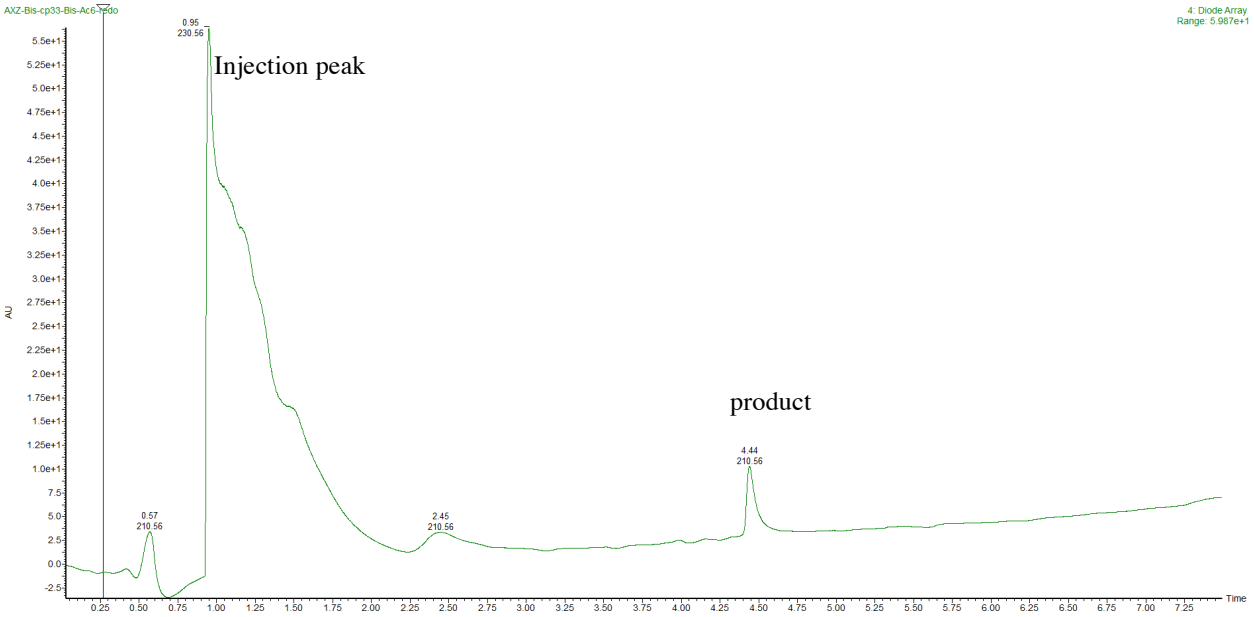



**2 (SyAM-P2)** 254 nm absorbance

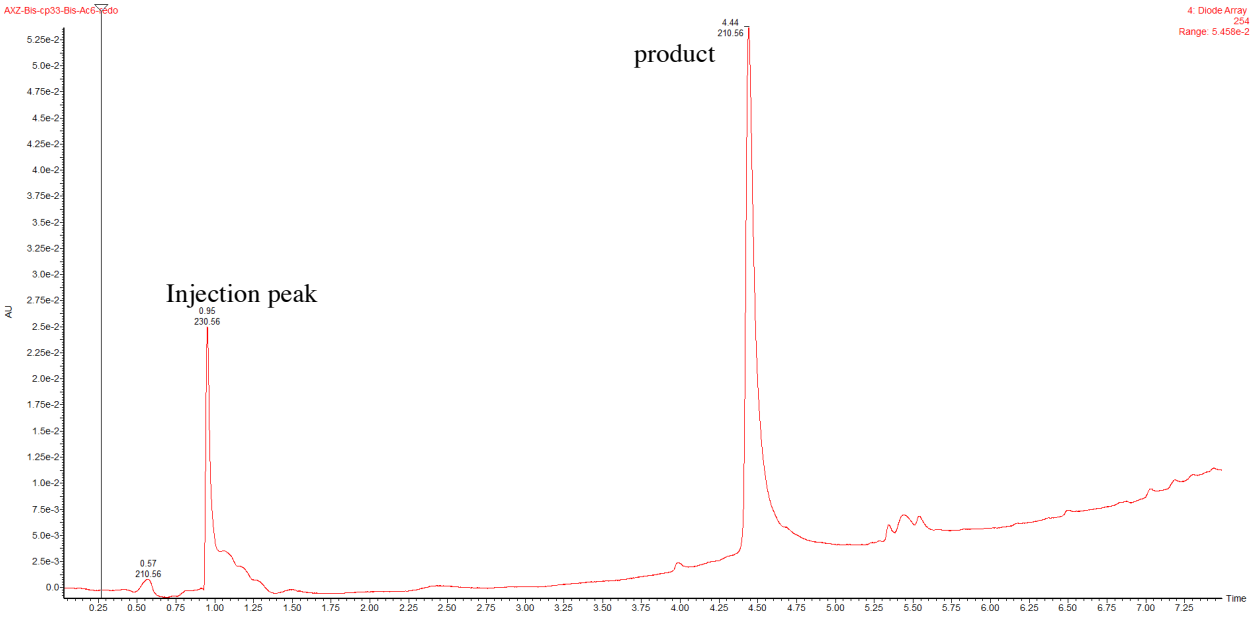



**2 (SyAM-P2)** mass trace

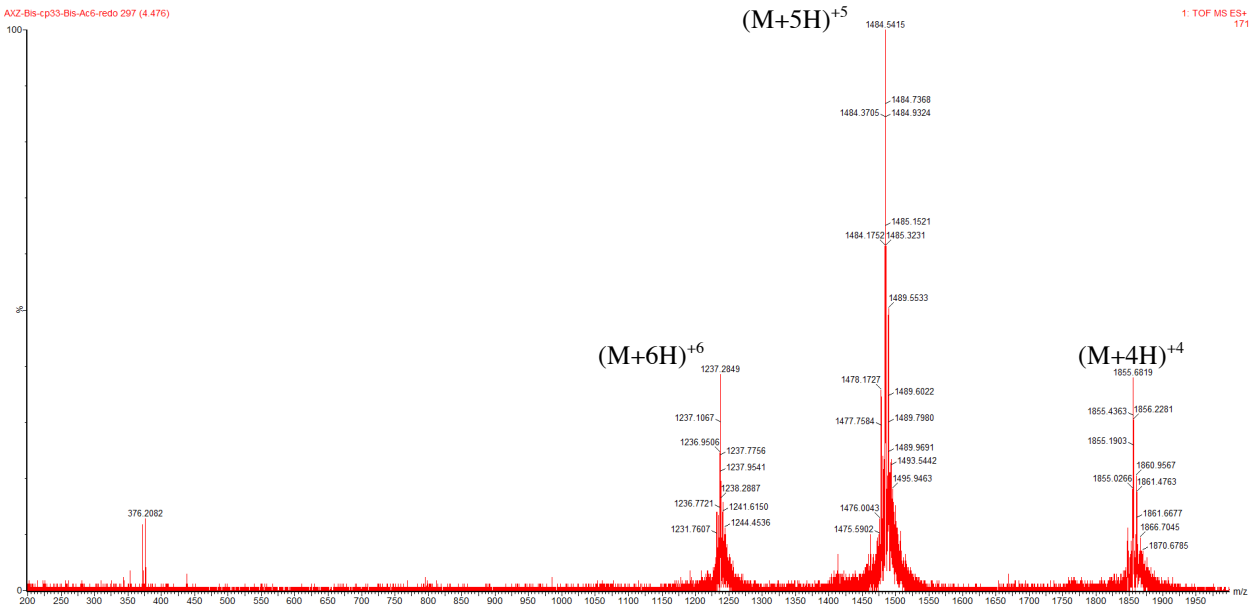
**3 (SyAM-P3)**



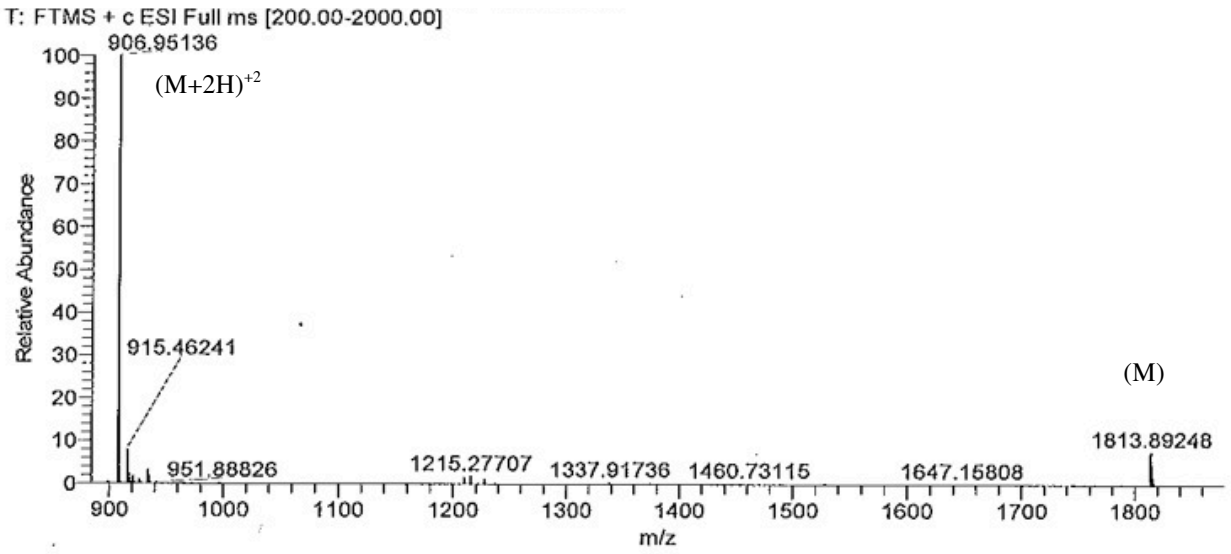

**3 (SyAM-P3)** diode array



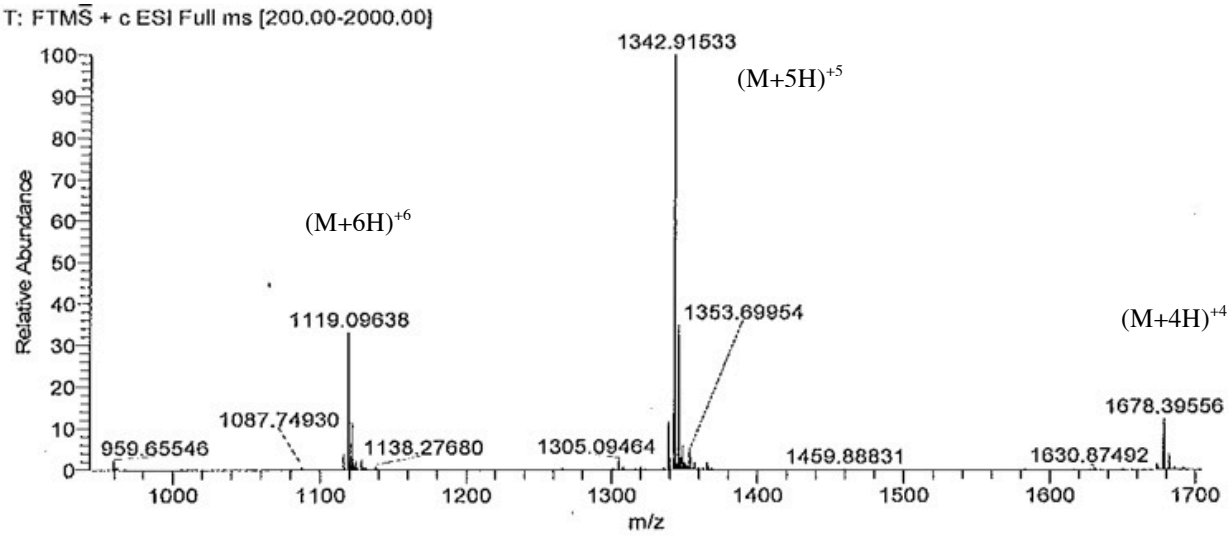

**3 (SyAM-P3)** 254 nm absorbance




**3 (SyAM-P3)** mass trace




**S.17** mass trace




**S.18** (non-biotinylated **SyAM-P3**) mass trace