*Supporting Information for*

# Tailored Peptide Nanomaterials for Receptor Targeted Prostate Cancer Imaging

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## 1. Characterization

**High performance liquid chromatography (HPLC):** Analytical and preparative HPLC-MS was performed on a system containing a Waters 600 controller, Waters prep degasser, and Waters MassLynx software. Purification of peptides was carried out using preparative reverse-phase HPLC-MS using an Agilent Zorbax PrepHT SB-C18 column (21.2 x 150 mm, 5 µm). The purity of peptides was assessed with analytical HPLC-MS using an Agilent Zorbax SB-C18 column (4.6 x 150 mm, 5 µm). The UV absorbance was detected using a Waters 2998 photodiode array detector, and the solvent system ran gradients of 0.1% TFA in acetonitrile (solvent A) and 0.1% TFA in ultrapure water (solvent B).

**Nuclear Magnetic Resonance (NMR) spectroscopy:** $^{1}$H NMR and $^{13}$C NMR spectra were measured using a Bruker Neo 600 MHz spectrometer. The solvent used was MeOD (Sigma Aldrich). Chemical shifts are reported in parts per million (ppm).

**Mass Spectrometry (ESI-TOF):** High resolution mass spectrum of peptides and PSMA-based compounds (except **CP4** and compound **8**) were measured on a Bruker micrOTOF-II spectrometer in ESI+ mode. Peptide samples were dissolved in acetonitrile and water, and small molecule samples were dissolved in methanol. Peptide **CP4** and compound **8** were measured on Agilent 6550 QToF mass spectrometer in ESI+ mode.

**Circular Dichroism (CD) spectroscopy:** An aliquot of **CP1** nanoassemblies was placed on a 1 mm quartz cuvette. Circular dichroism spectra were recorded on a Jasco J-810 CD spectrometer at 20 ºC and 37 ºC between 190 nm and 270 nm. Both spectra were the average of ten measurements.

**Energy dispersive X-ray mapping:** The elemental composition was determined using a Bruker Quantax FlatQUAD SDD detector. The maps were acquired after 10 min, with the microscope operating at 3kV with a current intensity of 20 µA.

**Low-voltage transmission electron microscopy:** 4 µL of the sample was drop-casted on the carbon-coated grid. Once dry, the samples were measured without washing or staining. LV-TEM micrographs were acquired with a LVEM5 benchtop transmission electron microscope operating at 5 kV. All the LV-TEM images are representative of different batches of cyclic peptide nanotubes (n=3).

**Column chromatography:** Column chromatography of the small molecules was performed under systems of EtOAc/hexanes and MeOH/DCM using SilicaFlash 60 irregular silica gel (40 – 63 µm, 60 Å).

## 2. Abbreviations

Alloc: allyloxycarbonyl, Boc: tert-butyloxycarbonyl, CP: cyclic peptide, DCM: dichloromethane, DIPEA: N,N-Diisopropylethylamine DMF: N,N-dimethylformamide, EtOAc: ethyl acetate, Fmoc: 9-fluorenylmetoxycarbonyl, HATU: Hexafluorophosphate

Azabenzotriazole Tetramethyl Uronium, HBTU: O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, HCTU: O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HFIP: 1,1,1,3,3,3-hexafluoro-isopropanol, HOBt: 1-hydroxybenzotriazole, TFA: trifluoroacetic acid, TIPS: triisopropylsilane, Trt: trityl.

## 3. Fmoc solid phase peptide synthesis

Automated peptide synthesis was carried out with a Biotage Syrowave synthesizer at a 0.1 mmol scale using a pre-loaded resin. The resin was swelled with dichloromethane (DCM) before use, followed by two cycles of Fmoc deprotection using 2 mL of 40% piperidine in N,N-dimethylformamide (DMF). Amino acids were coupled by combining Fmoc-protected amino acid (4 eq), HCTU (4 eq), and DIPEA (8 eq) in DMF/N-methylpyrrolidinone (NMP). The resin was vortexed for 1 h, and the cycles were repeated until all the amino acids were coupled.

Allyloxycarbonyl (alloc) deprotection was performed using DCM (2 mL). Phenylsilane (296 μL, 24 eq) was then added to the peptide resin and shaken for 5 min. After that, tetrakis(triphenylphosphine)-palladium(0) ($Pd(PPh_3)_4$) (17 mg, 0.1 eq) was added and allowed to react for 1 h. The resin was washed with DMF, MeOH, and DCM (4 x 2 mL).On resin-PEGylation of lysine residues was performed with a solution containing $mPEG_8$-COOH (2.2 eq) and HCTU (2.2 eq) in DMF (1.5 mL). Then, DIPEA (6 eq) was added, and the peptide vessel was vortexed for 5 h. The resin was thoroughly washed with DMF and DCM (3 x 2 mL). Dye labeling was performed on resin. On 0.01 mmol resin, a solution with fluorescein-NHS (2 eq) and DIPEA (12 eq) in DMF (1.5 mL) was added. The peptide vessel was shaken in the dark for 24 h. Then, the resin was washed with DMF, isopropanol, and DCM (2 x 3 mL). Coupling of PSMA-ligand was performed with a solution containing $PEG_4$-PSMA (1.4 eq) and HCTU (1.4 eq) in DMF (1.5 mL). After that, DIPEA (6 eq) was added, and the reaction mixture was left overnight. The resin was washed with DMF and DCM (3 x 2 mL).

To cleave the peptides from the solid support, 2 mL of 20% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in DCM was added, and the mixture was vortexed for 30 min. The resulting filtrate was collected, and the solvent was then concentrated under reduced pressure, yielding the linear peptide. This procedure was repeated two more times.

## 4. Peptide cyclization

Cyclization of peptides was carried out under inert atmospheric $N_2$ conditions and following to the protocol reported by Chatterjee *et al.*[1] Linear peptide (1 eq) was dissolved in DMF to make a 0.5 – 1 mM solution. A solution containing HATU (1.5 eq), HOBt (1.5 eq), and 2,4,6-trimethylpyridine (1.5 eq) in DMF was added dropwise to let it stir overnight. The solvent was evaporated under reduced pressure to yield the protected cyclic peptide.

Protected cyclic peptides were treated with a cleavage cocktail consisting of 95% TFA, 2.5% TIPS and 2.5% $H_2O$ (4 mL), and allowed to react for 3 – 5 h. The deprotected peptides were

isolated by precipitation in cold tert-butyl methyl ether (TBME) and centrifuged two times (1450 g, 10 min, -10 °C). Preparative reverse-phase HPLC-MS was used to purify peptides over gradients of acetonitrile (0.1% TFA) and water (0.1% TFA).

## 5. PSMA ligand synthesis

### (9S,13S)-Tri-tert-butyl 3,11-dioxo-1-phenyl-2-oxa-4,10,12-triazapentadecane-9,13,15-tricarboxylate (5).

Synthesized adapting the method reported by Zhang *et al*.[2] Triphosgene (0.454 g, 1 eq) was dissolved in DCM (15 mL) at 0 ºC. Then, a solution consisting of H-Lys(Cbz)-OtBu.HCl (1.540 g, 2.7 eq), DIPEA (1.599 mL, 6 eq), and DCM (15 mL) was added with an addition funnel. After 2 h, H-Glu(OtBu)-OtBu.HCl (1.221 g, 2.7 eq), and DIPEA (1.599 mL, 6 eq) in DCM (15 mL) was added to the reaction flask and stirred for 45 min. DCM was removed under reduced pressure and the crude was taken up in EtOAc and extracted with 1M $NaHSO_4$ (2 x 30 mL) and brine (2 x 30 mL). The organic phase was dried with $MgSO_4$ and concentrated to yield a yellow oil, that underwent column chromatography (30% EtOAc/hexanes) to yield a viscous colorless oil (0.833 g, 88% yield). $^1$H NMR (600 MHz, Methanol-$d_4$) δ 7.40 – 7.33 (m, 4H), 7.30 (m, 1H), 5.07 (s, 2H), 4.20 (dd, J= 8.7, 5.1 Hz, 1H), 4.15 (dd, J= 8.1, 5.2 Hz, 1H), 3.13 (t, J = 6.9 Hz, 2H), 2.33 (m, 2H), 2.04 (m, 1H), 1.80 (m, 2H), 1.69 – 1.50 (m,3H), 1.48 (s, 9H), 1.47 (s, 9H), 1.46 (s, 9H), 1.44 – 1.38 (m, 2H). $^{13}$C NMR (151 MHz, MeOD) δ 173.67, 173.50, 173.21, 159.68, 158.64, 138.17, 129.17, 128.65, 128.49, 82.53, 82.29, 81.47, 67.05, 54.56, 53.91, 41.25, 32.94, 32.22, 30.19, 28.72, 28.07, 28.02, 27.99, 23.49. HRMS ($ESI^+$): $C_{32}H_{52}N_3O_9$ $[M+H]^+$ calcd: 622.3698, $[M+H]^+$ found: 622.3714

### (S)-di-tert-butyl 2-(3-((S)-6-amino-1-tert-butoxy-1-oxohexan-2-yl)ureido)pentanedioate (6).

The compound 6 (0.761 g, 1 eq) was dissolved in 15 mL MeOH. 10% Pd/activated wood carbon was added to the solution. The reaction mixture was stirred under $H_2$ for 18 h at room temperature and the resulting solution was filtered through celite and the filtrate was concentrated under reduced pressure to yield a yellow oil. The crude oil underwent column chromatography (10% MeOH/DCM) to yield a yellow oil (0.416 g, 70% yield). $^1$H NMR (600 MHz, Methanol-$d_4$) δ 4.20 (dd, J= 8.7, 5.1 Hz, 1H), 4.16 (dd, J= 8.5, 5.0 Hz, 1H), 2.73 (m, 2H), 2.33 (m, 2H), 2.05 (m, 1H), 1.80 (m, 2H), 1.66 – 1.52 (m, 2H), 1.48 (s, 9H), 1.47 (s, 9H), 1.46 (s, 9H), 1.45 – 1.41 (m, 2H). $^{13}$C NMR (151 MHz, MeOD) δ 174.16, 174.07, 173.98, 160.42, 83.32, 83.26, 82.99, 82.24, 54.83, 54.58, 41.02, 33.69, 33.61, 32.93, 29.39, 28.79, 28.78, 28.76, 28.73, 28.71, 28.45, 24.01. HRMS ($ESI^+$): $C_{24}H_{46}N_3O_7$ $[M+H]^+$ calcd: 488.3330, $[M+H]^+$ found: 488.3279

**1,5-di-tert-butyl(2S)-2-({[(2S)-1-(tert-butoxy)-6-[1-({[(9H-fluoren-9-yl)methoxy]carbonyl}amino)-3,6,9,12-tetraoxapentadecan-15-amido]-1-oxohexan-2-yl]carbamoyl}amino)pentanedioate (7).**

To a solution of Fmoc-*N*-Amido-dPEG$_4$ acid (0.457 g, 1.1 eq) in anhydrous DCM (6 mL), was added HBTU (0.324 g, 1 eq). DIPEA (298 uL, 2 eq) was added dropwise to the mixture. The compound 6 (0.416 g, 1 eq) was dissolved in anhydrous DCM (2 mL) and added. The reaction proceeded under inert atmospheric $N_2$ conditions for 72 h. The resulting mixture was washed with saturated $NaHCO_3$ (2 x 20 mL) and brine (2 x 20 mL). The organic phase was dried with MgSO4 and reduced under pressure. The crude was purified under column chromatography (3% MeOH/DCM) to yield a dark yellow oil (0.370 g, 45% yield). $^1$H NMR (600 MHz, Methanol-$d_4$) δ 7.81 (d, $J$ = 7.6 Hz, 2H), 7.67 (d, $J$ = 7.4 Hz, 2H), 7.41 (t, $J$ = 7.5 Hz, 2H), 7.33 (m, 2H), 4.39 (dd, $J$ = 6.9, 2.8 Hz, 2H), 4.23 (d, J= 6.9 Hz, 1H), 4.21 (dd, J= 8.7, 5.0 Hz, 1H), 4.13 (dd, $J$ = 8.3, 5.2 Hz, 1H), 3.73 – 3.52 (m, 18H), 3.18 (m, 2H), 2.42 (t, J= 6.2 Hz, 2H), 2.32 (m, 2H), 2.04 (m, 1H), 1.79 (m, 2H), 1.66 – 1.50 (m, 3H), 1.48 (s, 9H), 1.47 (s, 9H), 1.46 (s, 9H), 1.44 – 1.39 (m, 2H). $^{13}$C NMR (151 MHz, MeOD) δ 173.81, 173.74, 173.63, 173.33, 159.79, 145.22, 142.50, 128.65, 128.02, 126.04, 120.81, 82.66, 82.41, 81.61, 71.46, 71.43, 71.38, 71.30, 71.24, 71.19, 71.18, 70.80, 68.16, 67.51, 54.72, 54.02, 41.65, 39.93, 37.57, 33.00, 32.35, 29.84, 28.93, 28.21, 28.19, 28.16, 23.78. HRMS (ESI$^+$): $C_{50}H_{76}N_4NaO_{14}$ $[M+Na]^+$ calcd: 979.5250, $[M+Na]^+$ found: 979.5164

**3-[(14-{[(5S)-5-({[(2S)-1,5-bis(tert-butoxy)-1,5-dioxopentan-2-yl]carbamoyl}amino)-6-(tert-butoxy)-6-oxohexyl]carbamoyl}-3,6,9,12tetraoxatetradecan-1-yl)carbamoyl]propanoic acid (8).**

5 mL of 20% piperidine/DMF was added dropwise to a reaction flask containing compound 7 (0.240 g, 1 eq) and stirred for 20 min. The resulting solution was taken up in 1:3 water/DCM and washed with saturated copper (II) sulfate (2 x 30 mL) and brine (2 x 20 mL). The organic phase was dried over $MgSO_4$ and reduced under pressure. The crude was taken up in anhydrous DCM. DIPEA (80 µL, 1.4 eq) and succinic anhydride (0.033 g, 1.02 eq) were added, and the mixture was let it stir overnight. Then, MeOH (26 µL, 2 eq) was added to the solution and stirred for 2 h. The resulting product was extracted with 0.1 M HCl (3 x 20 mL) and brine (3 x 20 mL). The organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. The product was purified using column chromatography (10% MeOH/DCM) to yield a colorless oil (0.128 g, 46% yield). $^1$H NMR (600 MHz, Methanol-$d_4$) δ 4.23-4.19 (m,1H), 4.16 – 4.11 (m, 1H), 3.78 – 3.52 (m, 18H), 3.20 (m, 2H), 2.50 – 2.44 (m, 4H), 2.33 (m, 2H), 2.04 (m, 1H), 1.80 (m, 2H), 1.68 – 1.51 (m, 3H), 1.48 (s, 9H), 1.47 (s, 9H), 1.46 (s, 9H), 1.45 – 1.39 (m, 2H). $^{13}$C NMR (151 MHz, Methanol-d$_4$) δ 174.39, 174.20, 160.38, 83.23, 82.99, 71.93, 71.89, 71.87, 71.81, 71.73, 71.64, 71.16, 68.83, 55.31, 54.58, 40.76, 40.54, 38.00, 33.57, 32.92, 30.39, 29.49, 28.79, 28.76, 28.73, 24.38. HRMS (ESI$^+$): $C_{39}H_{70}N_4O_{15}$ $[M+H]^+$ calcd: 835.4893, found: 835.4838.

## 6. Confocal Microscopy imaging

### Cell lines and culture conditions

The human prostate cancer cell lines PC3 and LNCaP were purchased from ATCC.

LNCaP is a cell line exhibiting epithelial morphology that was isolated from a needle aspiration biopsy of the left supraclavicular lymph node of a 50-year-old, white, male (blood type B+) with confirmed diagnosis of metastatic prostate carcinoma. PC3 is a cell line initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old, white, male.

PC3 cells were cultured in F-12K medium supplemented with 10% fetal bovine serum (FBS) and 1% P/S in an incubator containing 5% $CO_2$ at 37 °C. LNCaP cells were cultured under the same conditions using RPMI-1640 medium.

### Confocal microscopy

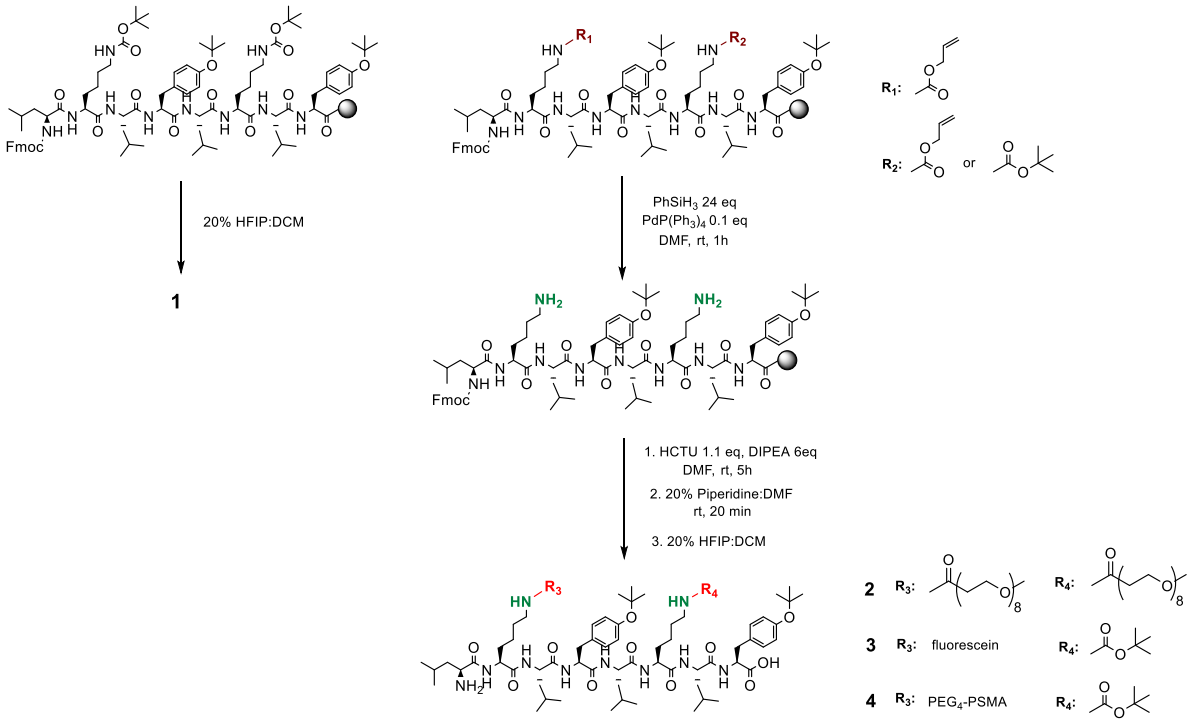
All the experiments were performed with the cells after three passages and all confocal microscopy images are representative of three replicates of experiments.

LNCaP cells were imaged on a Nikon A1R confocal microscope using a 60x/1.4 oil objective. DAPI was imaged with a 402.1 nm excitation laser and emission was recorded at 450/50. Fluorescein was imaged with a 488 nm Argon excitation laser and emission was recorded at 525/50 nm. The images were collected at 1024x1024 pixels, using the following settings:

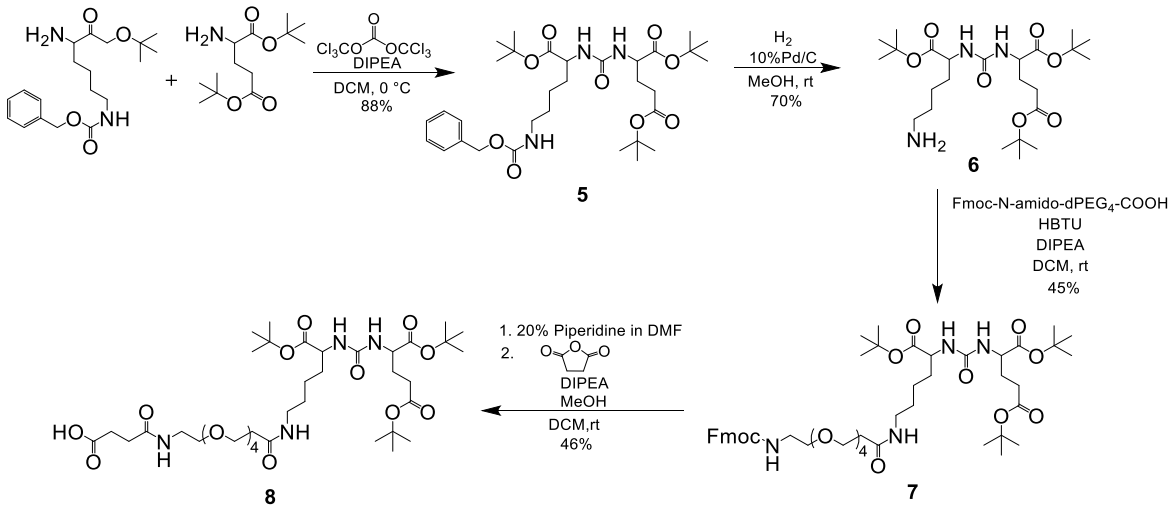
DAPI: Laser power 25%, HV: 105, offset: 0

Fluorescein: Laser power 25%, HV: 159, offset: 0

PC3 cells were imaged on a Nikon Spinning Disk Confocal microscope using a 60x/1.4 oil objective (DAPI laser power 40%, fluorescein laser power 25% exposure time 2000 ms).




**Scheme S1.** Synthesis of linear peptides **1 – 4**




**Scheme S2.** Synthesis of a PSMA-modified ligand ($PEG_4$-PSMA)

**Table S1. High resolution mass spectrometry (ESI+) analysis of purified cyclic peptides. Purity and overall yield values of final cyclic peptides.**

| CP # | Sequence | $[M+H]^+$ calcd. | $[M+H]^+$ found | Purity (%) | Yield (%) |
|---|---|---|---|---|---|
| **CP1** | cyclo-(D-L-K-D-L-Y)$_2$ | 1035.6601 | 1035.6562 | 98% | 7 |
| **CP2** | cyclo-(D-L-K(mPEG$_8$)-D-L-Y)$_2$ | 1824.1007 | 1824.1015 | 98% | 3 |
| **CP3** | cyclo-(D-L-K-D-L-Y-D-L-K(fluorescein)-D-L-Y) | 1393.7092 | 1393.7079 | 98% | 2 |
| **CP4** | cyclo-(D-L-K-D-L-Y-D-L-K(PEG$_4$-PSMA)-D-L-Y) | 1683.9455 | 1683.9458 | 95% | 4 |

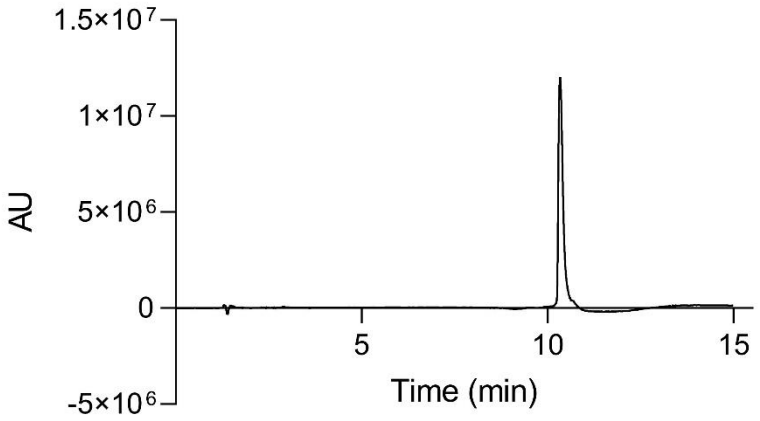



**Figure S1**. Analytical UV chromatogram from HPLC of purified peptide **CP1**

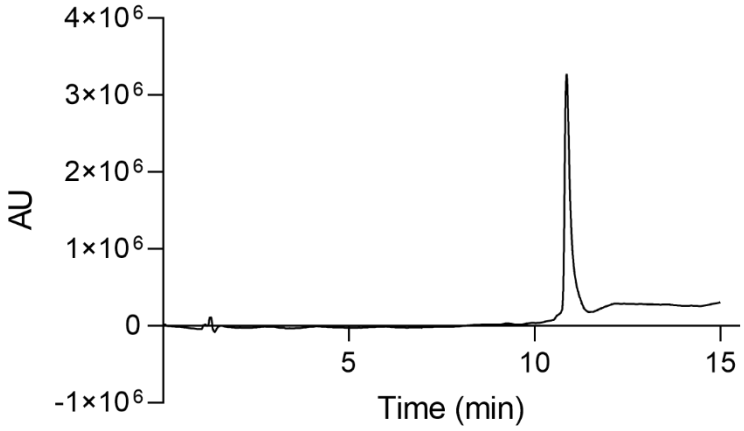



**Figure S2**. Analytical UV chromatogram from HPLC of purified peptide **CP2**

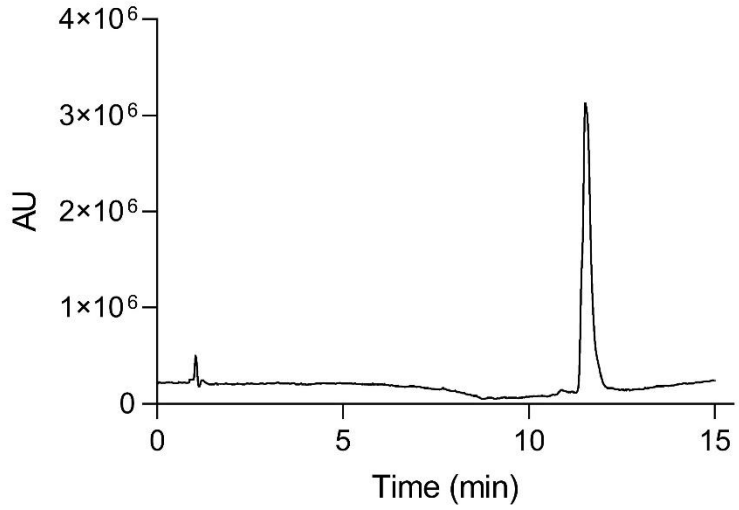



**Figure S3**. Analytical UV chromatogram from HPLC of purified peptide **CP3**

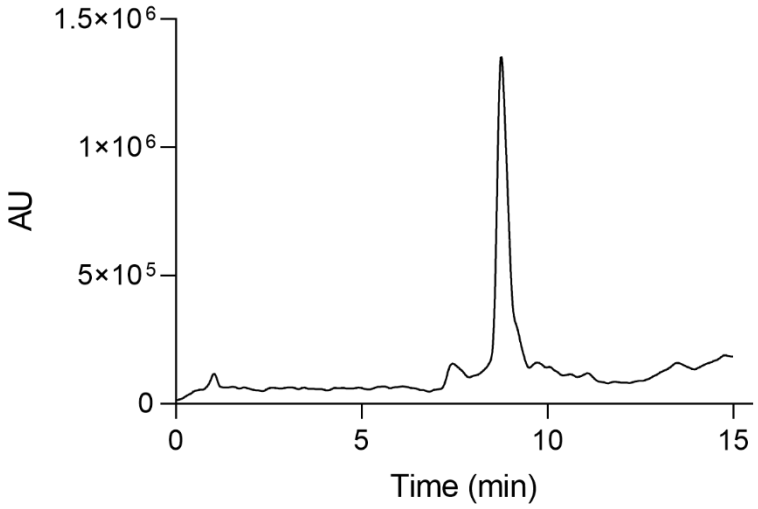



**Figure S4**. Analytical UV chromatogram from HPLC of purified peptide **CP4**

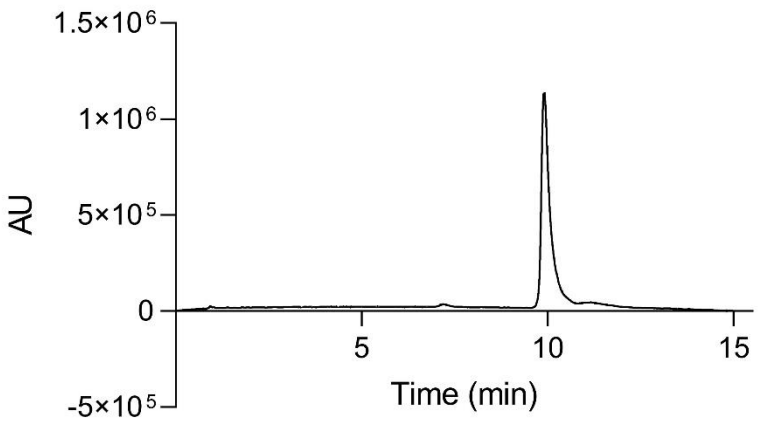



**Figure S5**. Analytical UV chromatogram from HPLC of purified compound **5**

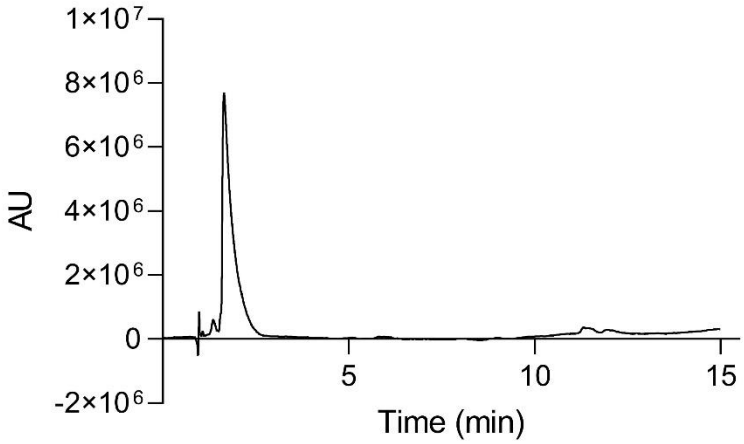



**Figure S6**. Analytical UV chromatogram from HPLC of purified compound **6**

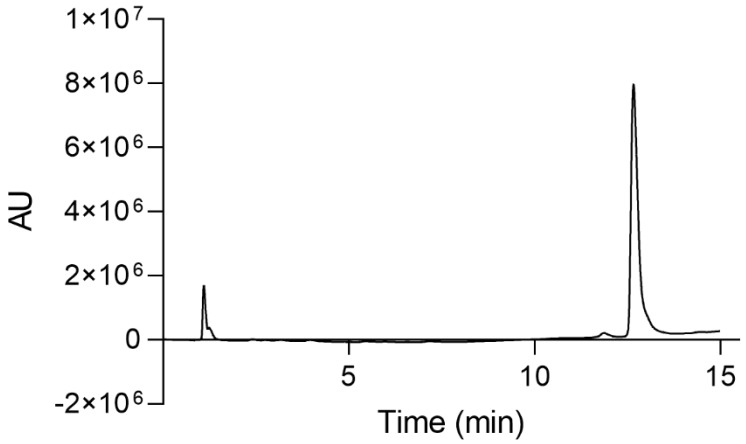



**Figure S7**. Analytical UV chromatogram from HPLC of purified compound **7**

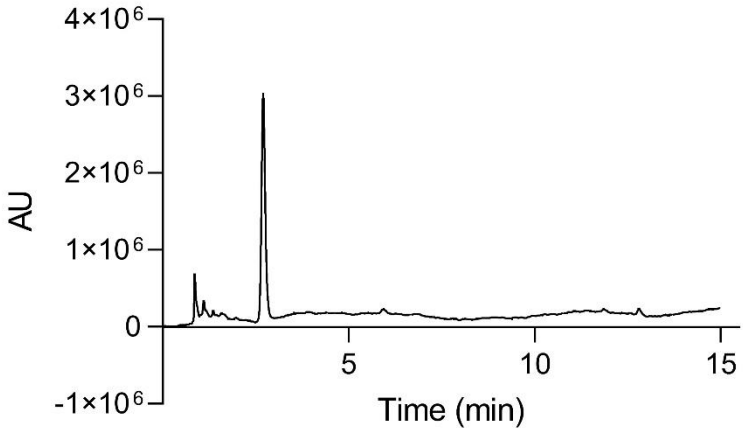



**Figure S8**. Analytical UV chromatogram from HPLC of purified compound **8**

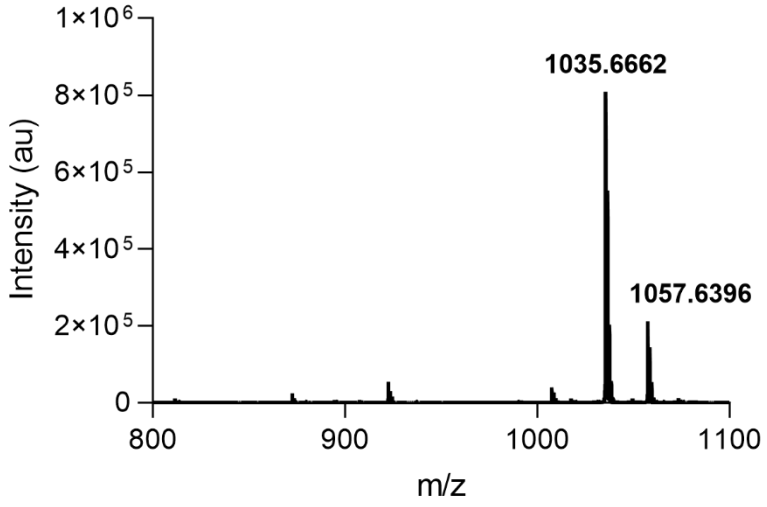



**Figure S9**. High resolution mass spectrometry (HR-MS) analysis of peptide **CP1**

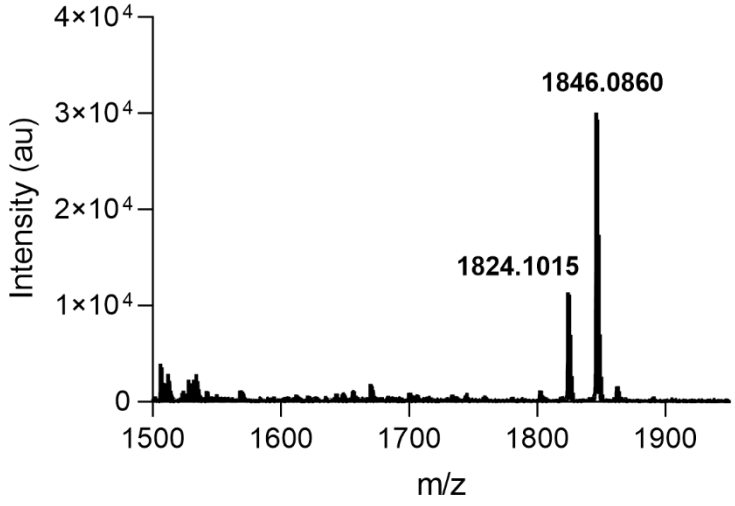



**Figure S10**. High resolution mass spectrometry (HR-MS) analysis of peptide **CP2**

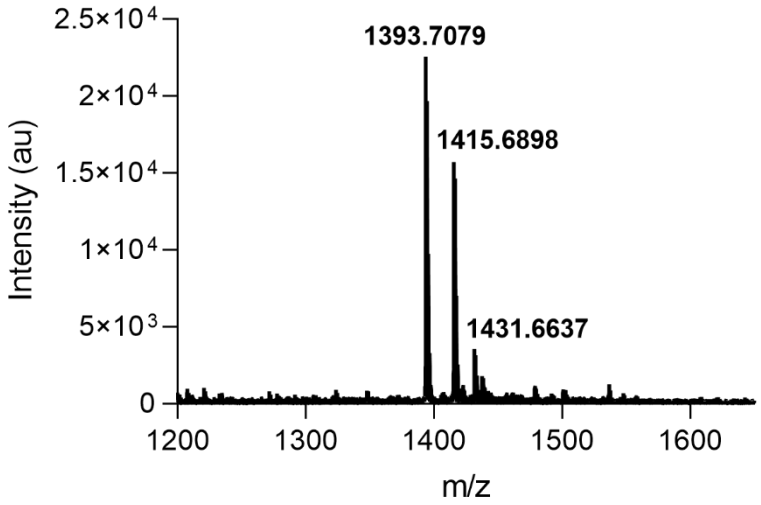



**Figure S11**. High resolution mass spectrometry (HR-MS) analysis of peptide **CP3**

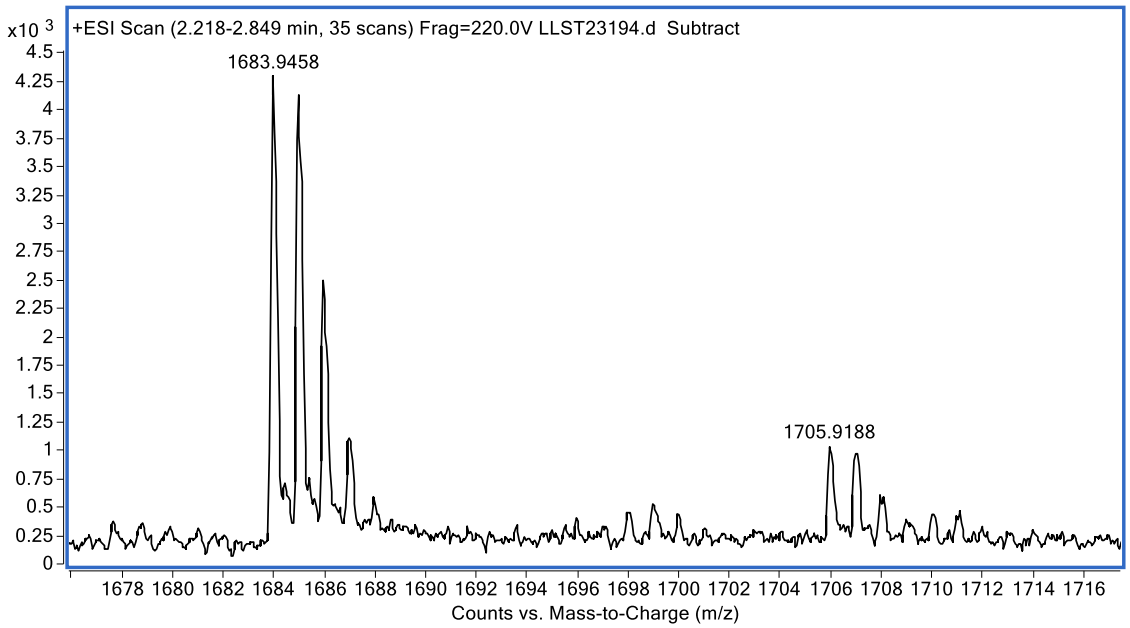



**Figure S12**. High resolution mass spectrometry (HR-MS) analysis of peptide **CP4**

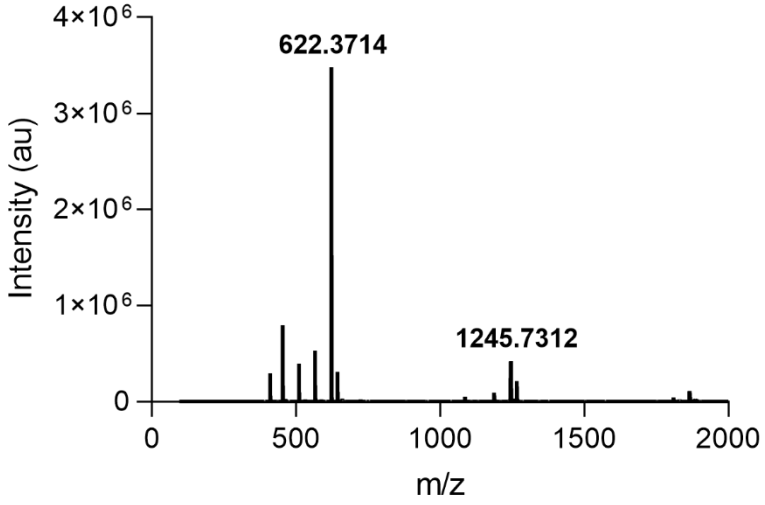



**Figure S13**. High resolution mass spectrometry (HR-MS) analysis of compound **5**

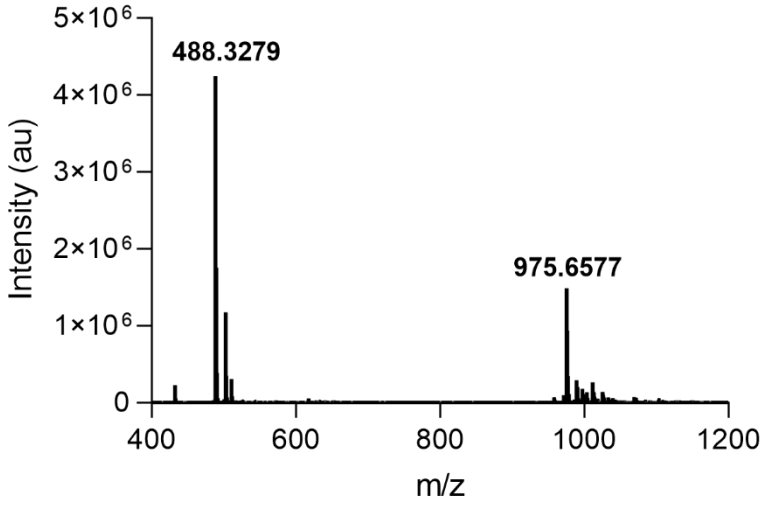



**Figure S14**. High resolution mass spectrometry (HR-MS) analysis of compound **6**

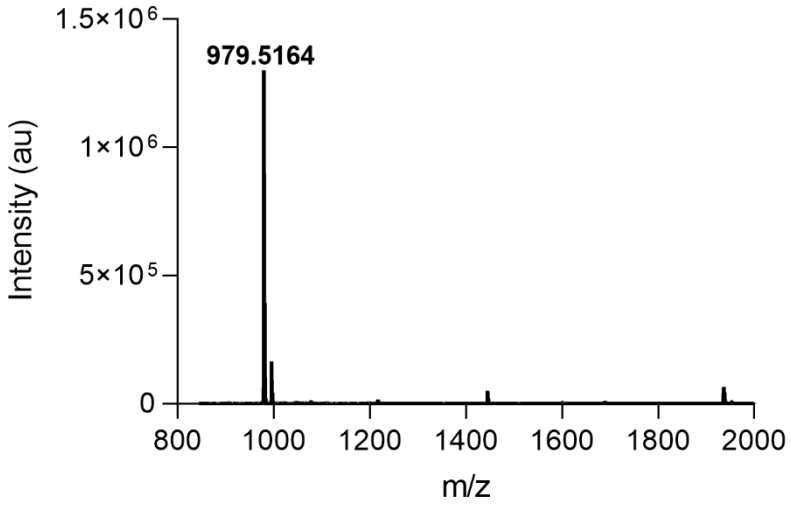



**Figure S15**. High resolution mass spectrometry (HR-MS) analysis of compound **7**

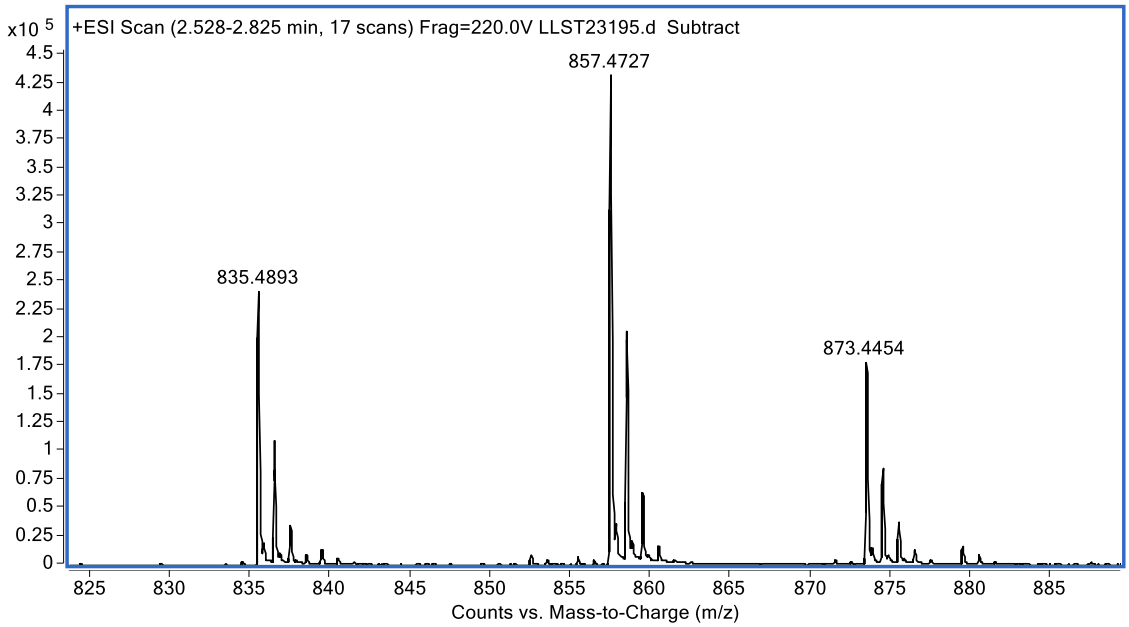



**Figure S16**. High resolution mass spectrometry (HR-MS) analysis of compound **8**

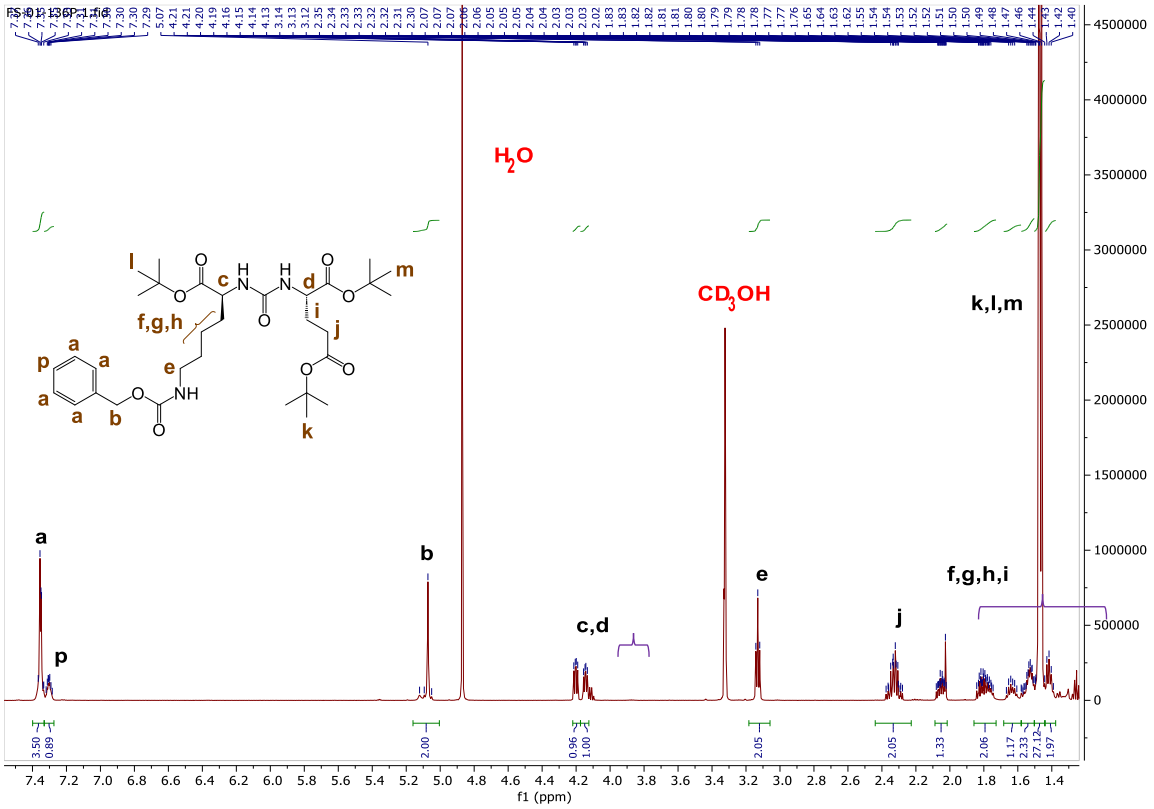



**Figure S17**. $^{1}$H NMR of compound **5** in $CD_3OD$ at room temperature (600 MHz)

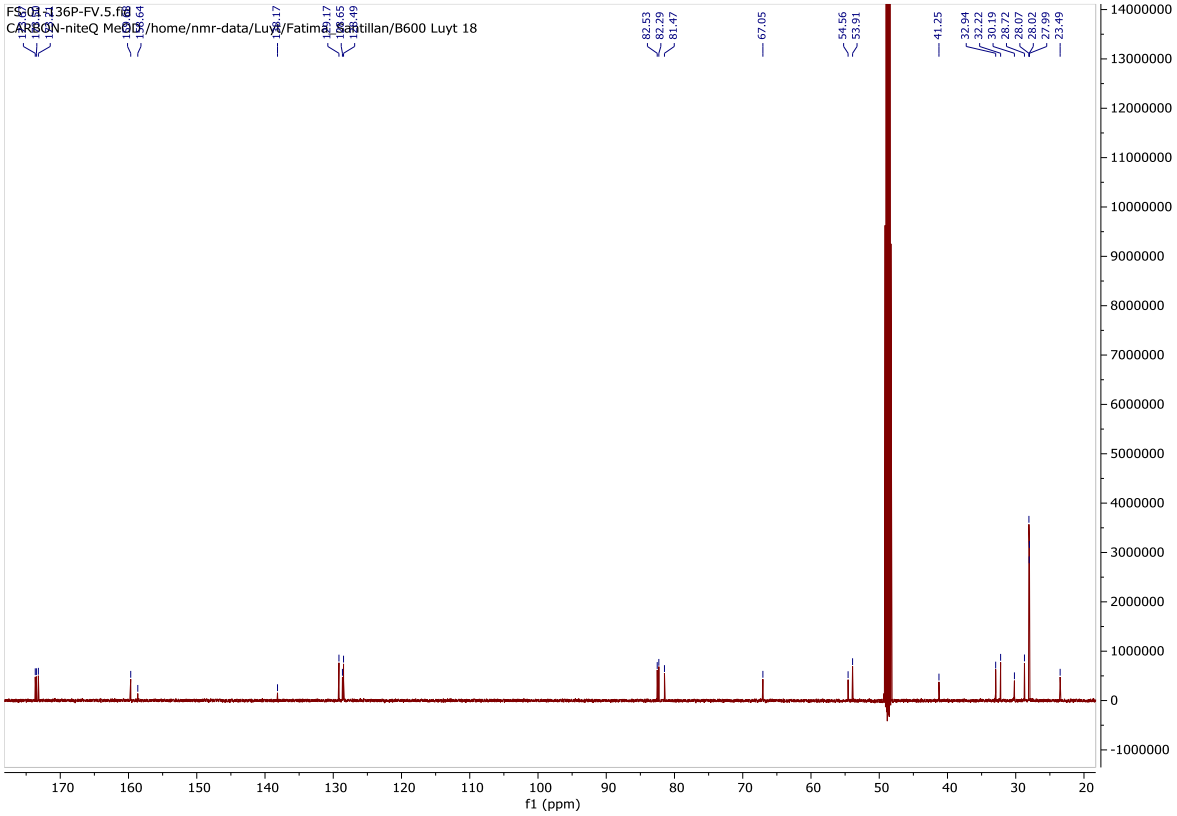



**Figure S18**. $^{13}C$ NMR of compound **5** in $CD_3OD$ at room temperature (151 MHz)

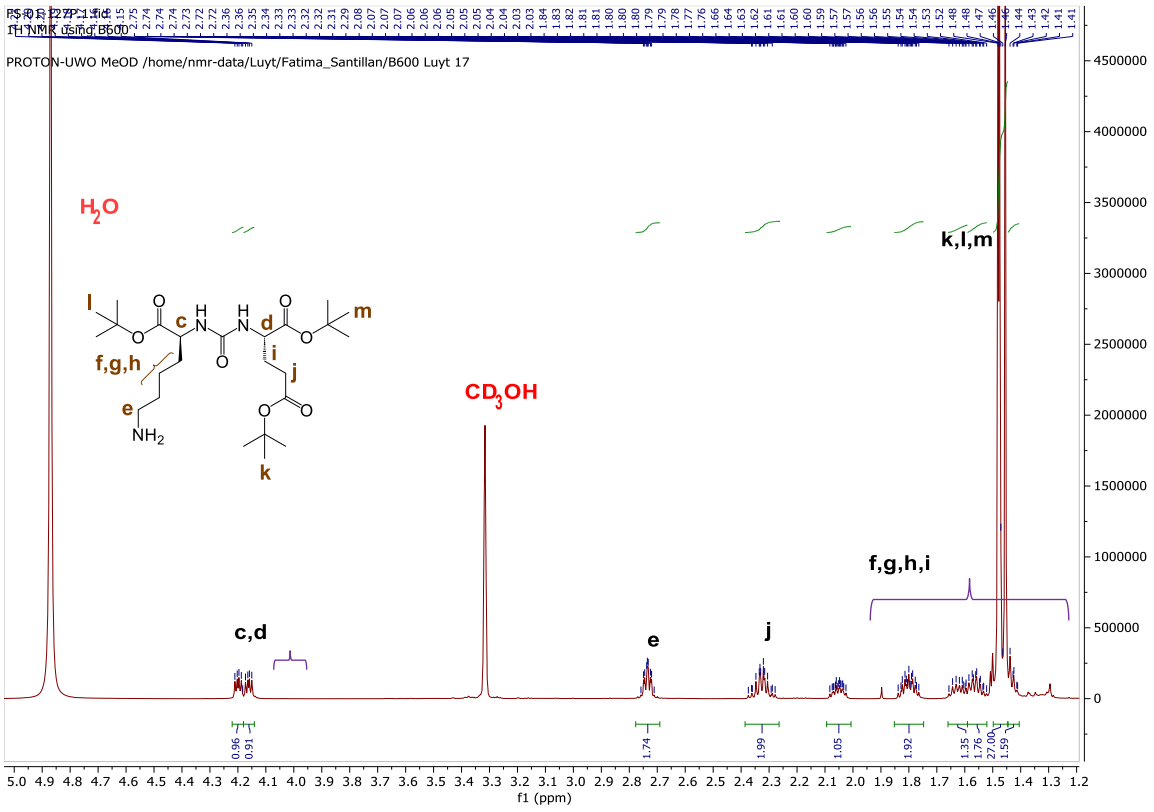



**Figure S19**. $^{1}$H NMR of compound **6** in $CD_3OD$ at room temperature (600 MHz)

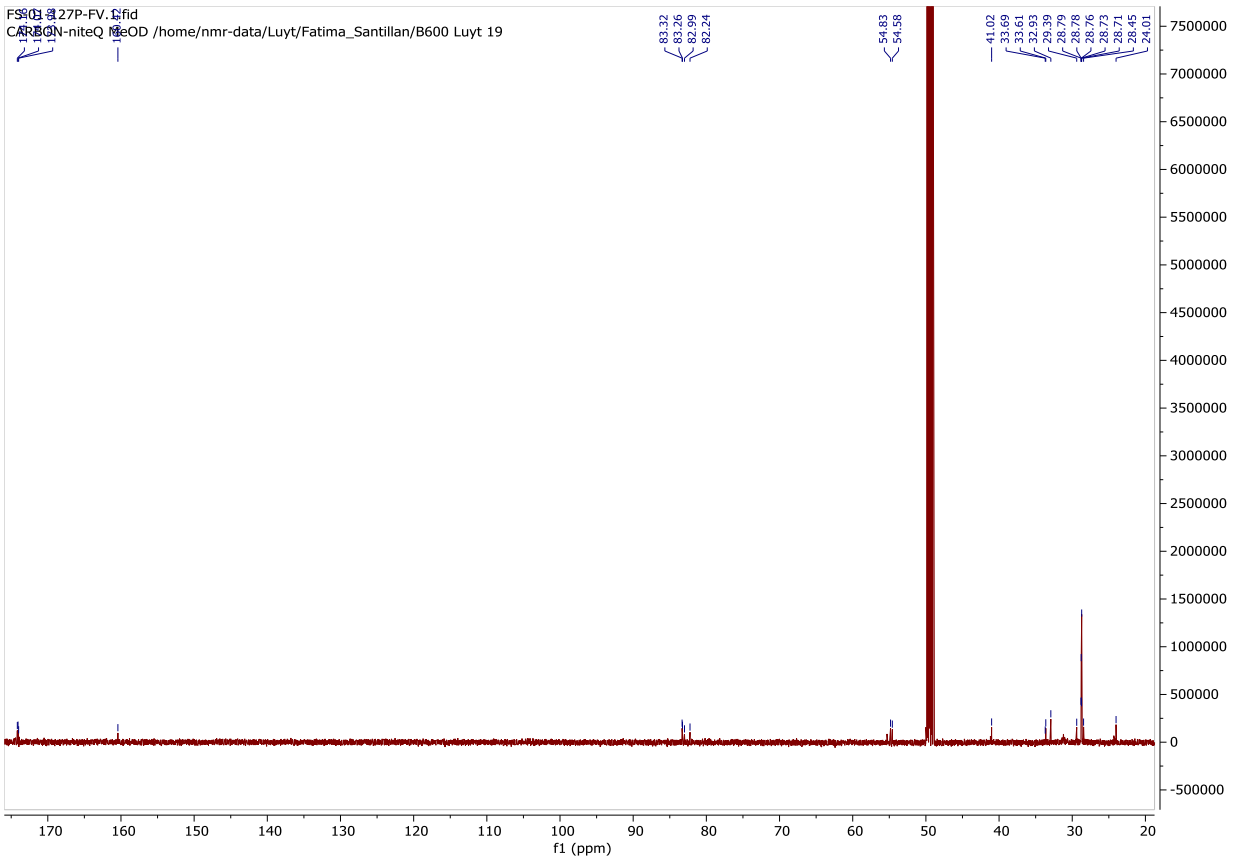



**Figure S20**. $^{13}C$ NMR of compound **6** in $CD_3OD$ at room temperature (151 MHz)

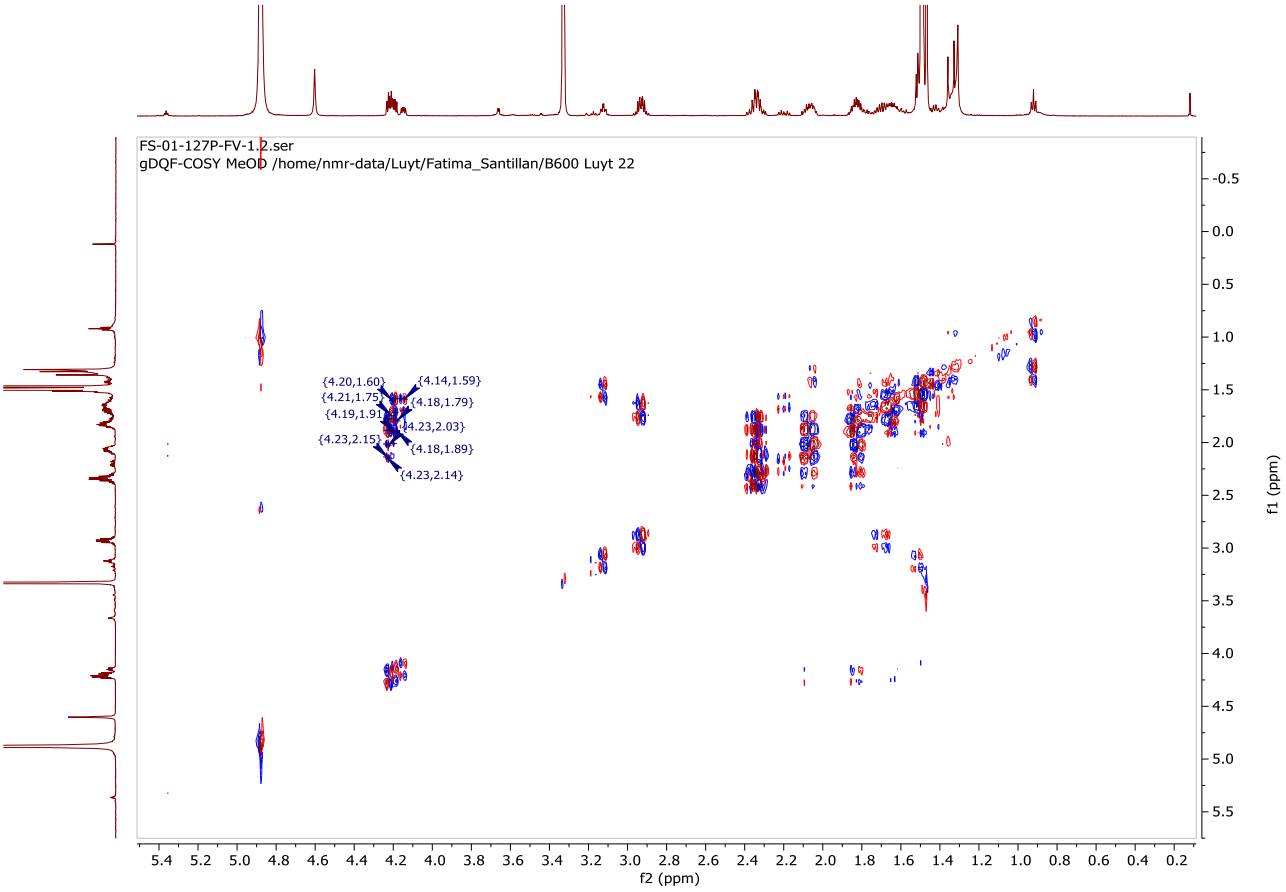



**Figure S21**. $^{1}$H-$^{1}$H NMR of compound **6** in $CD_3OD$ at room temperature (600 MHz)

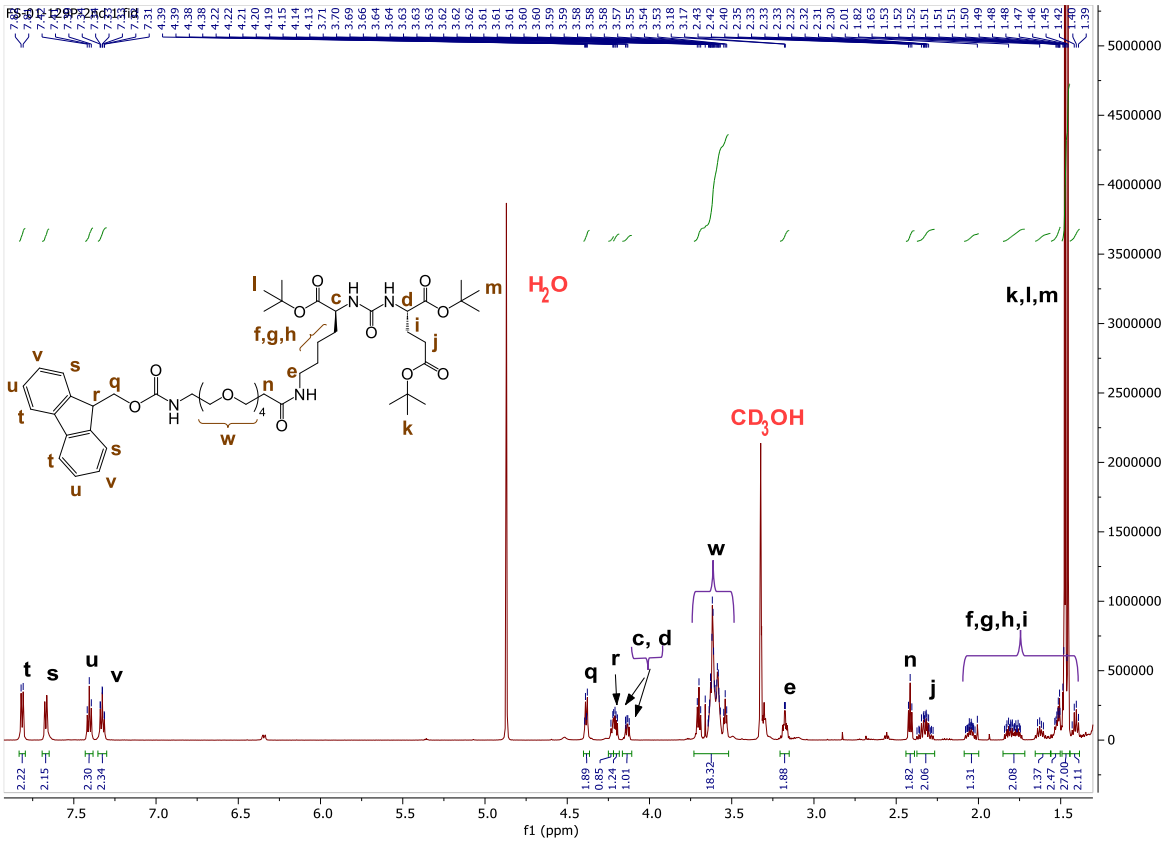



**Figure S22**. $^{1}$H NMR of compound **7** in $CD_3OD$ at room temperature (600 MHz)

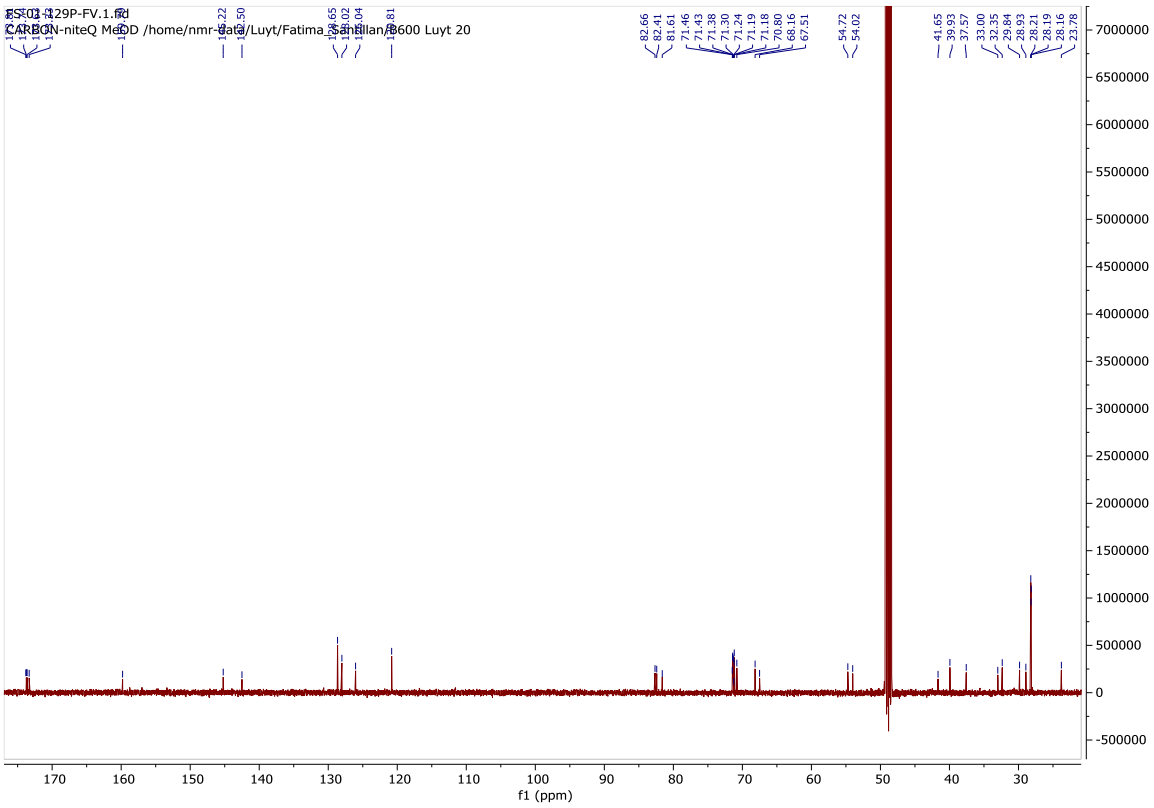



**Figure S23**. $^{13}C$ NMR of compound **7** in $CD_3OD$ at room temperature (151 MHz)

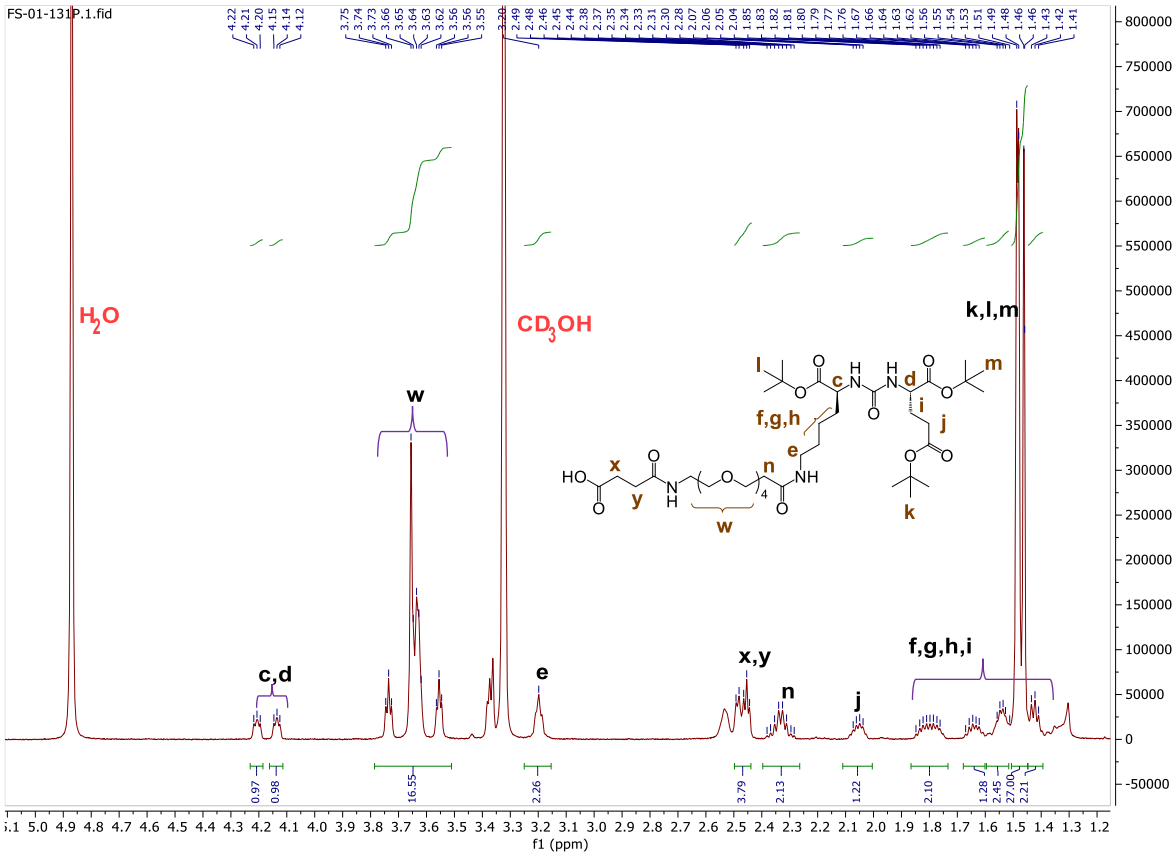



**Figure S24**. $^{1}$H NMR of compound **8** in $CD_3OD$ at room temperature (600 MHz)

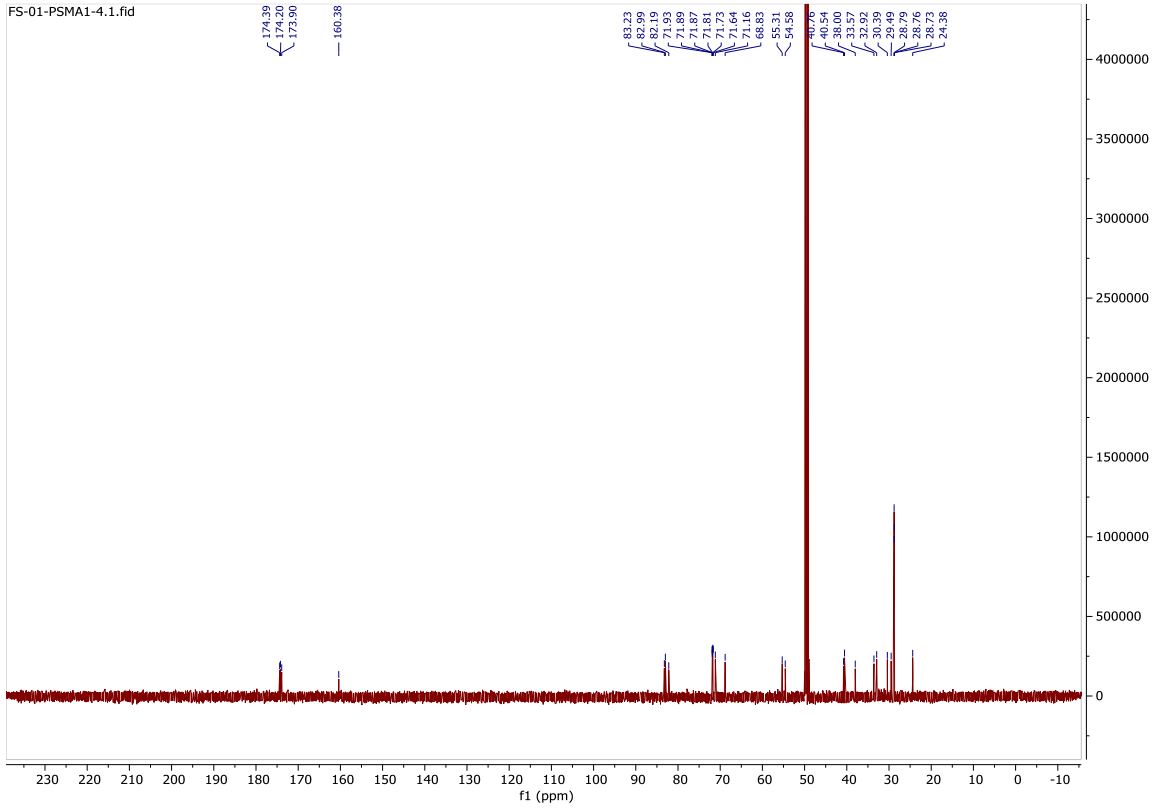



**Figure S25**. $^{13}C$ NMR of compound **8** in $CD_3OD$ at room temperature (151 MHz)

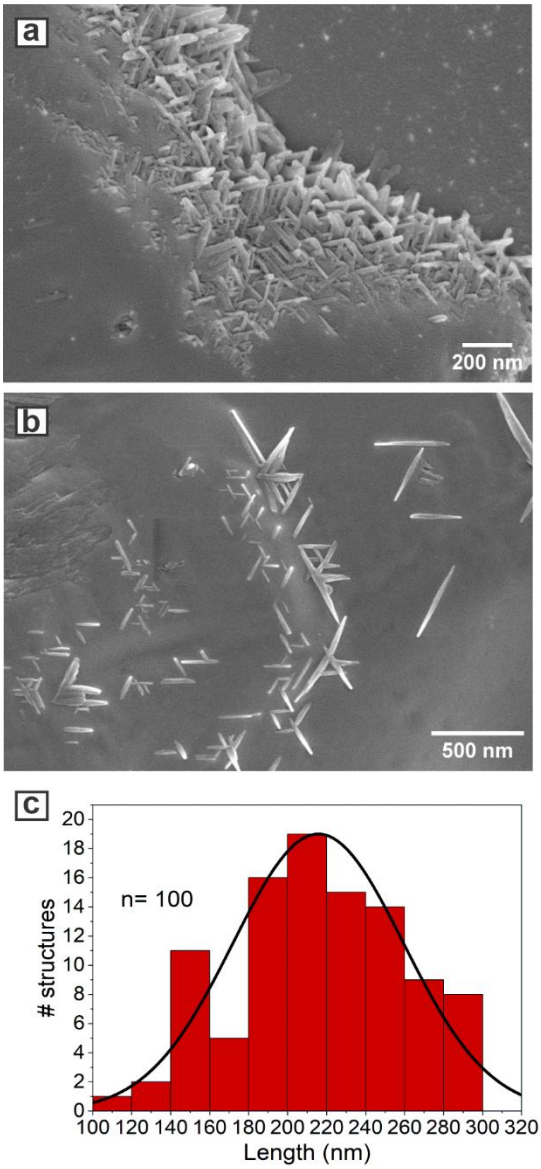



**Figure S26.** (a) Additional FESEM image of the self-assembly of **CP1**, and (b) different population of nanotubes within this sample. (c) Length distribution of the resulting nanostructures.

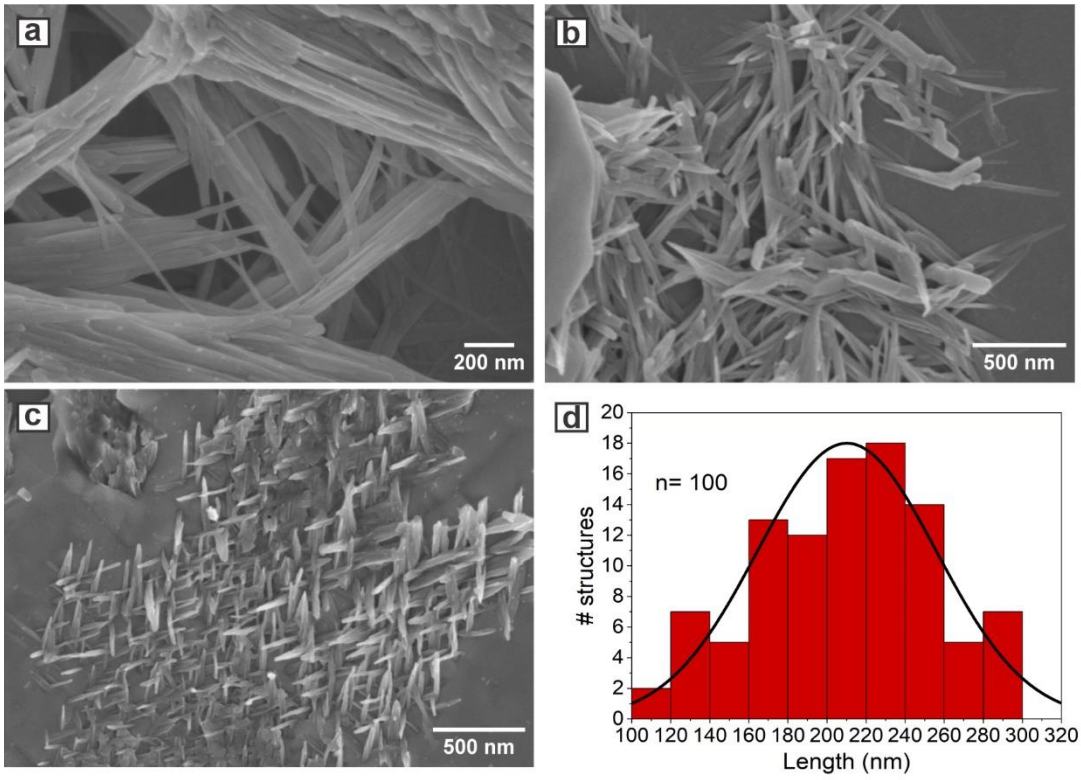



**Figure S27.** (a), (b), and (c) Additional FESEM images of the self-assembly of **CP2**. (d) Length distribution of the resulting nanostructures.

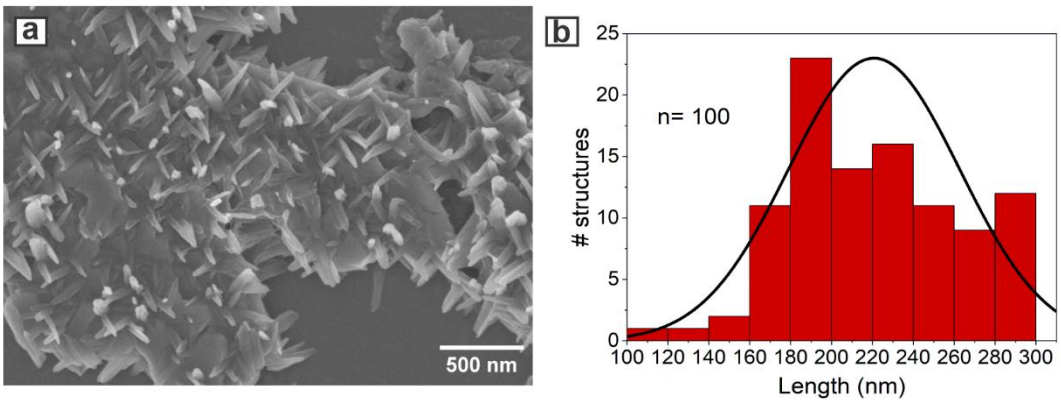



**Figure S28.** (a) Additional FESEM image of the self-assembly of **CP3.** (b) Length distribution of the resulting nanostructures.

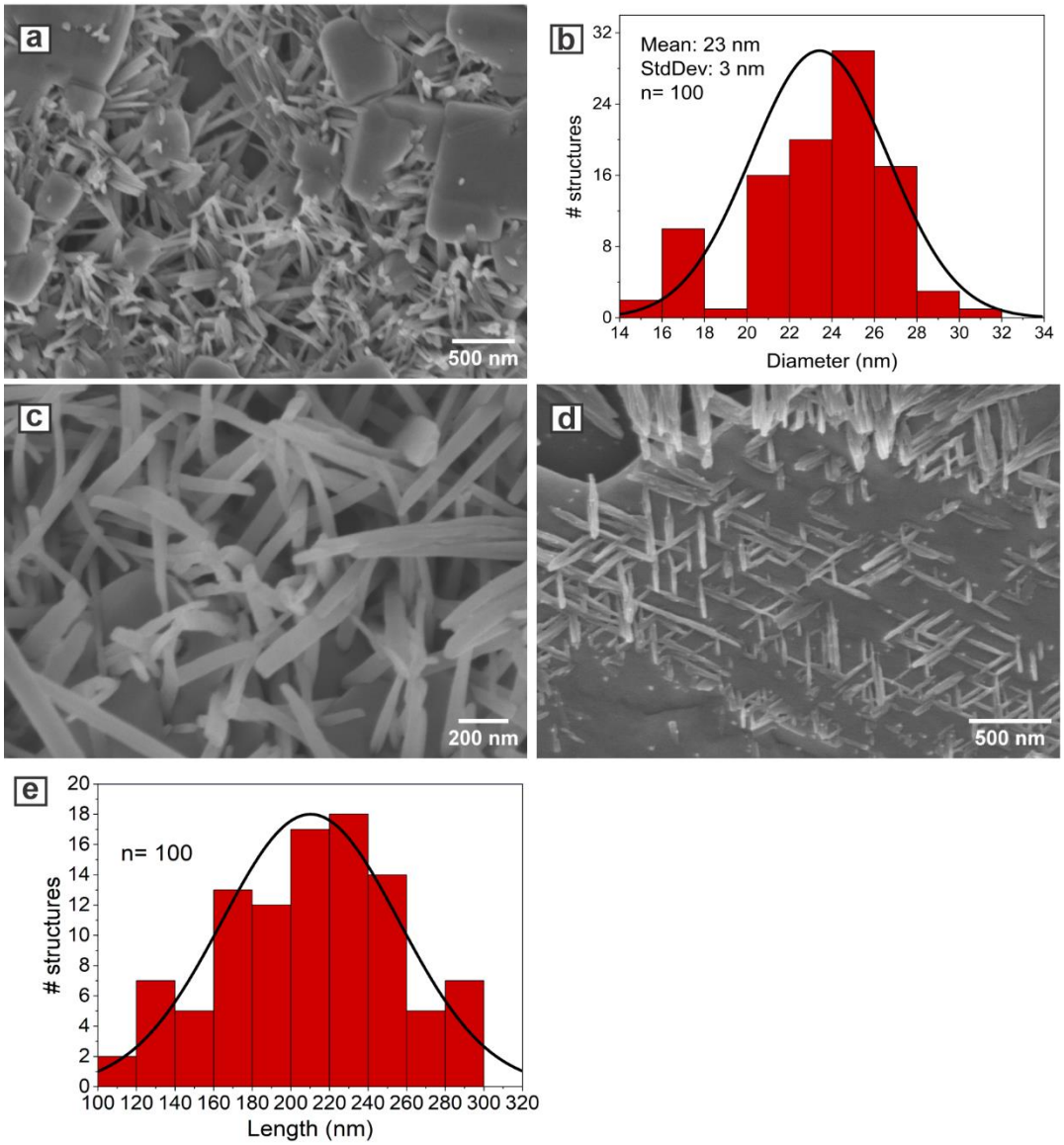



**Figure S29.** (a), (c), and (d) FESEM images of the co-assembly between **CP1 and CP2**, and (b) average diameter distribution. (e) Length distribution of the resulting nanostructures.

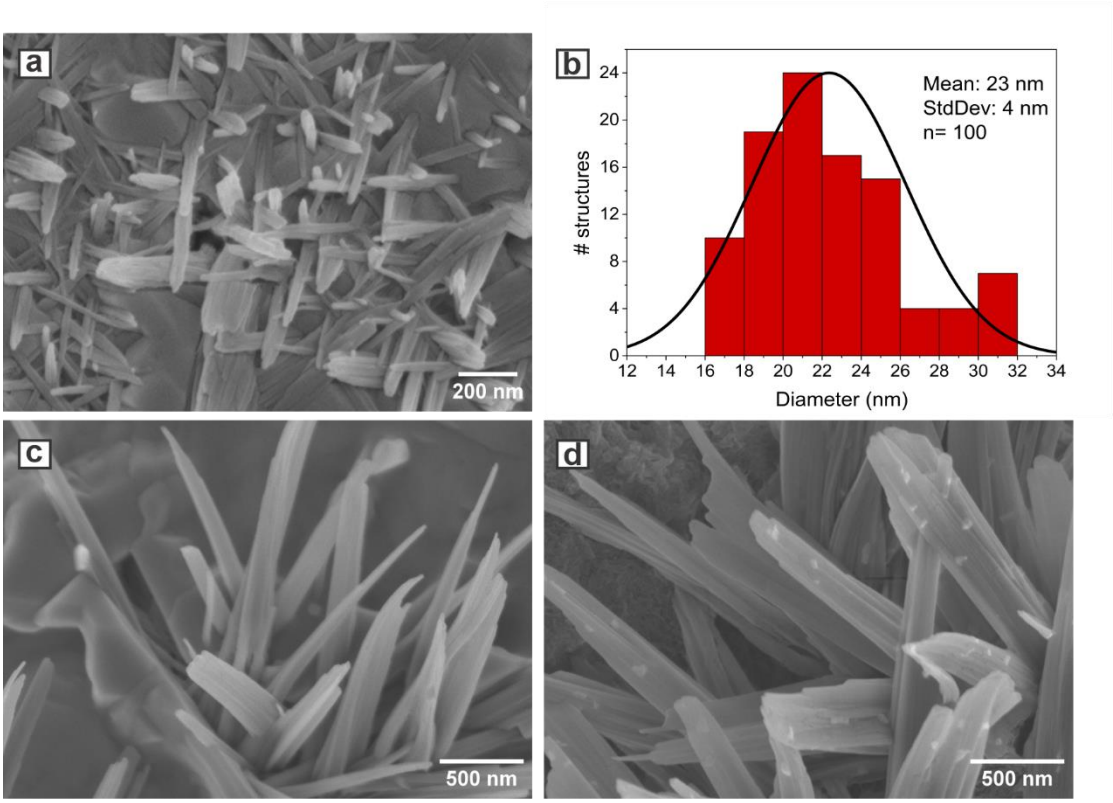



**Figure S30.** (a), (c), and (d) FESEM images of the co-assembly between **CP1 and CP3**, and (b) average diameter distribution.

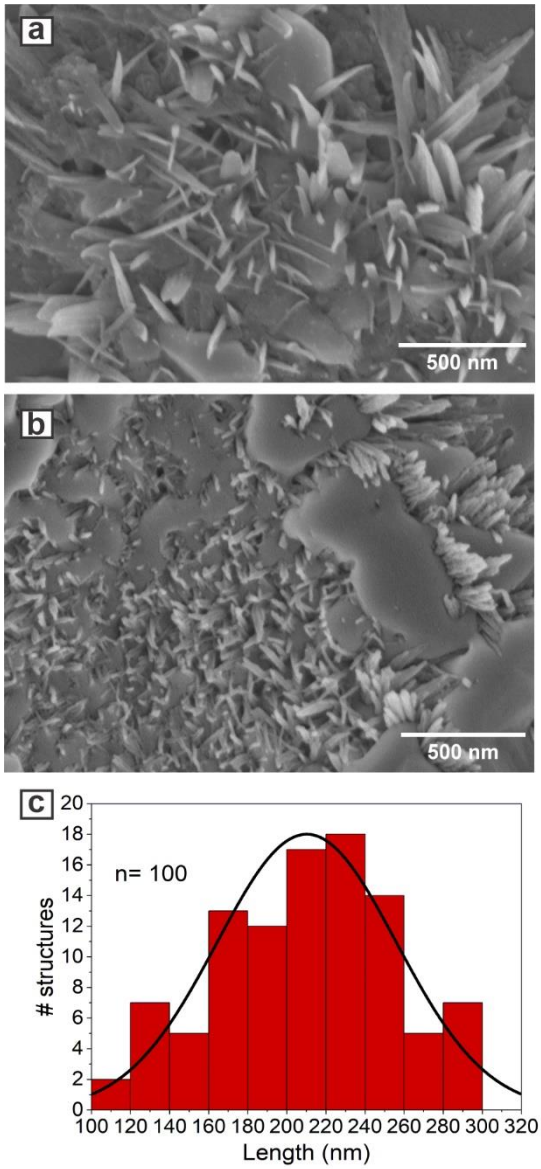



**Figure S31.** (a), and (b) Additional FESEM images of the co-assembly between **CP1**, **CP2**, **and CP3**.

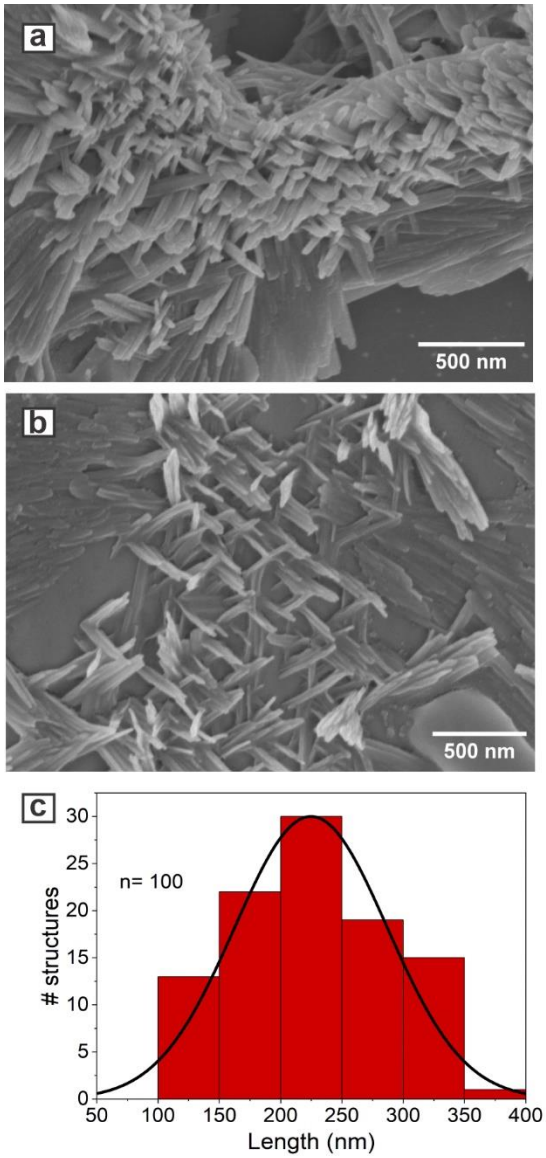



**Figure S32.** (a) and (b) Additional FESEM images of the co-assembly between **all cyclic peptides** (**CP1, CP2, CP3, and CP4**).(c) Length distribution of resulting nanostructures.

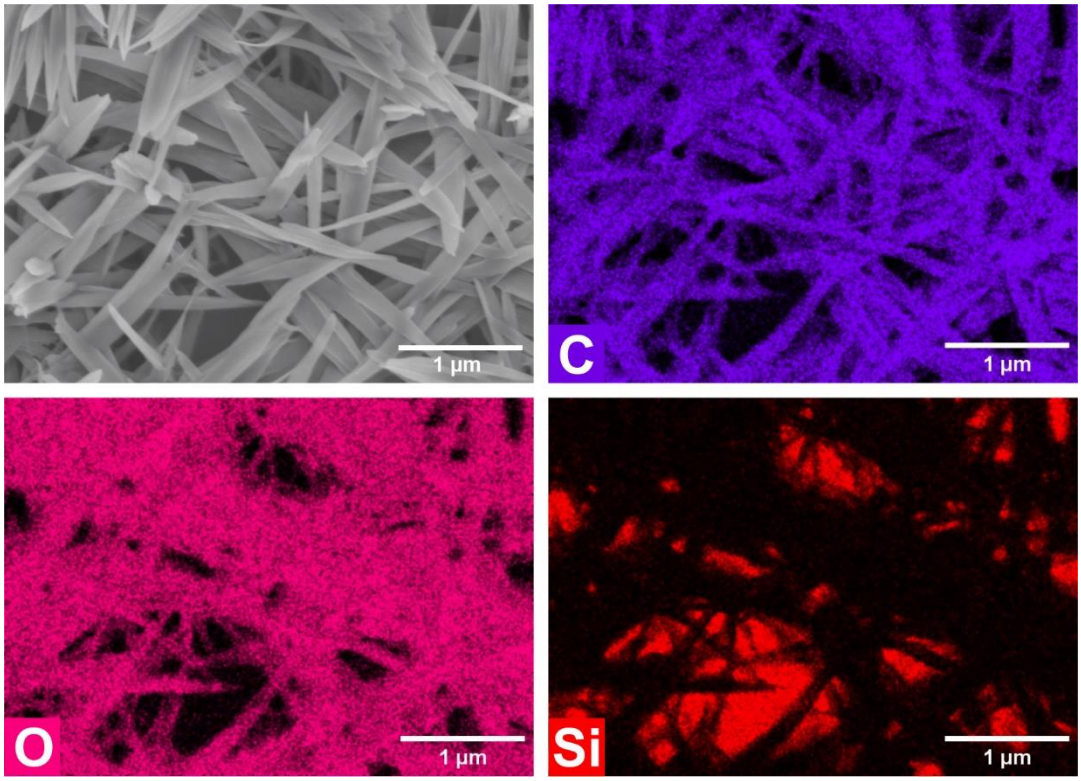



**Figure S33.** Additional EDX elemental mapping of targeting cyclic peptide nanotubes (co-assembly of **CP1, CP2, CP3, and CP4**).

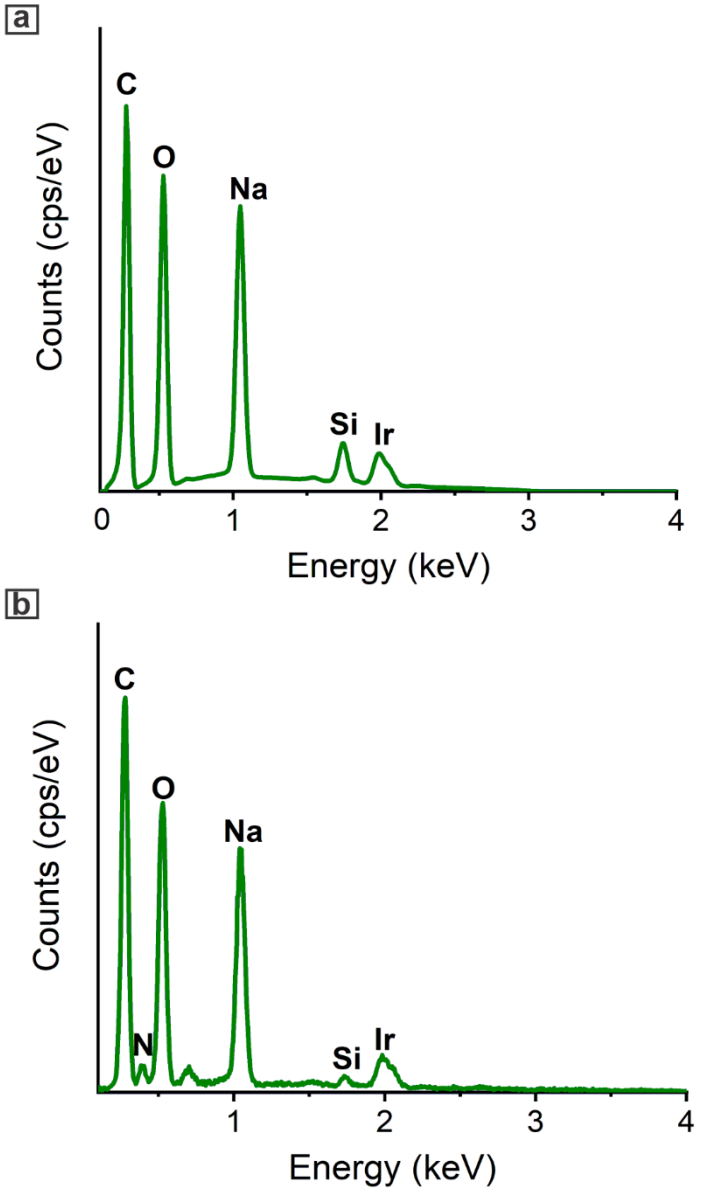



**Figure S34.** EDX spectra of targeting cyclic peptide nanotubes at pH 11 (a) and physiological pH (b). Signals of carbon (C), oxygen (O), and nitrogen (N) are derived from the peptide. The sodium (Na) signal originates from the hydroxide solution used for the self-assembly process. Silicon (Si) and iridium (Ir) signals come from the substrate and the coating, respectively.

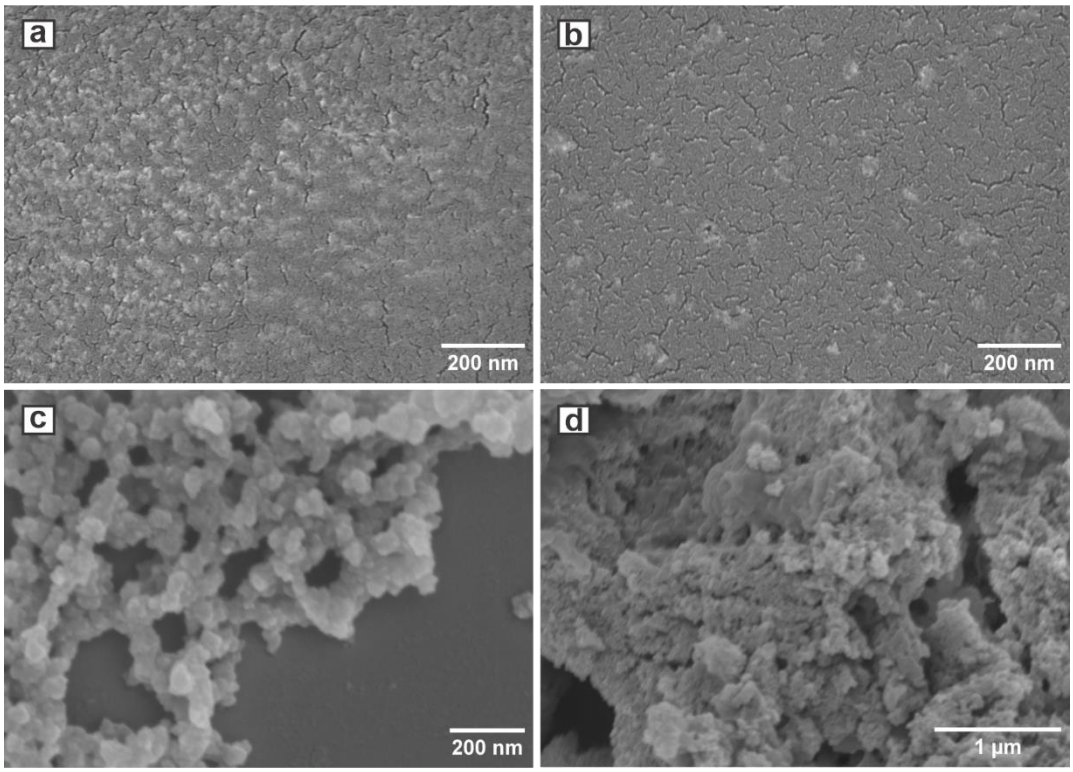
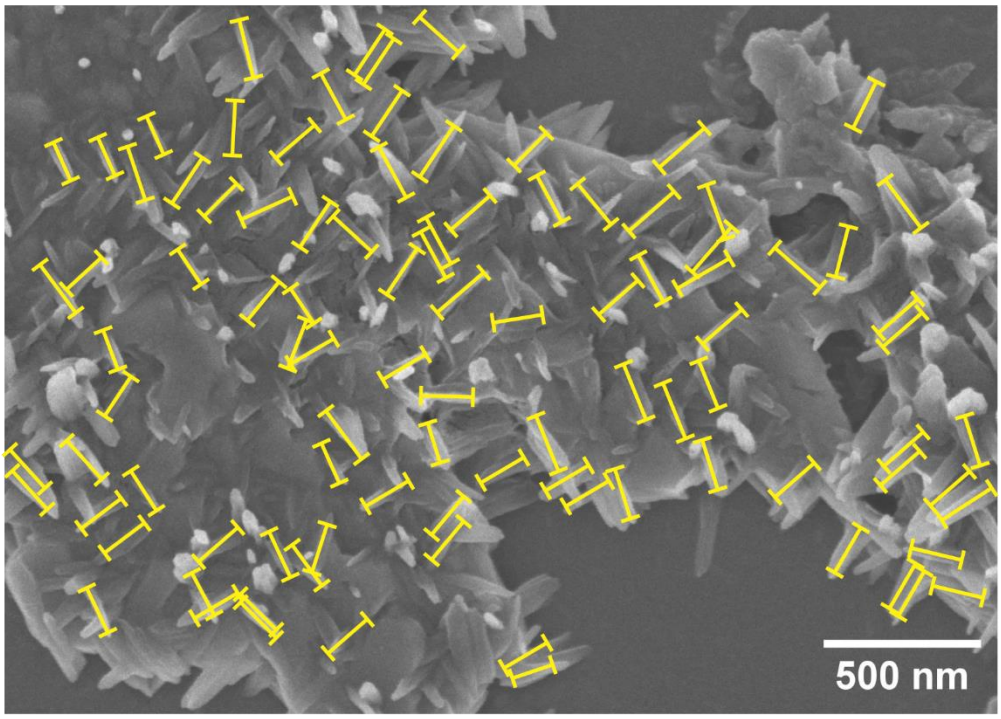



**Figure S35.** (a) and (b) FESEM images of the self-assembly of **CP2** without varying the pH, (c) and (d) co-assembly between all cyclic peptides (**CP1, CP2, CP3, and CP4**) without varying the pH.

Length




Diameter

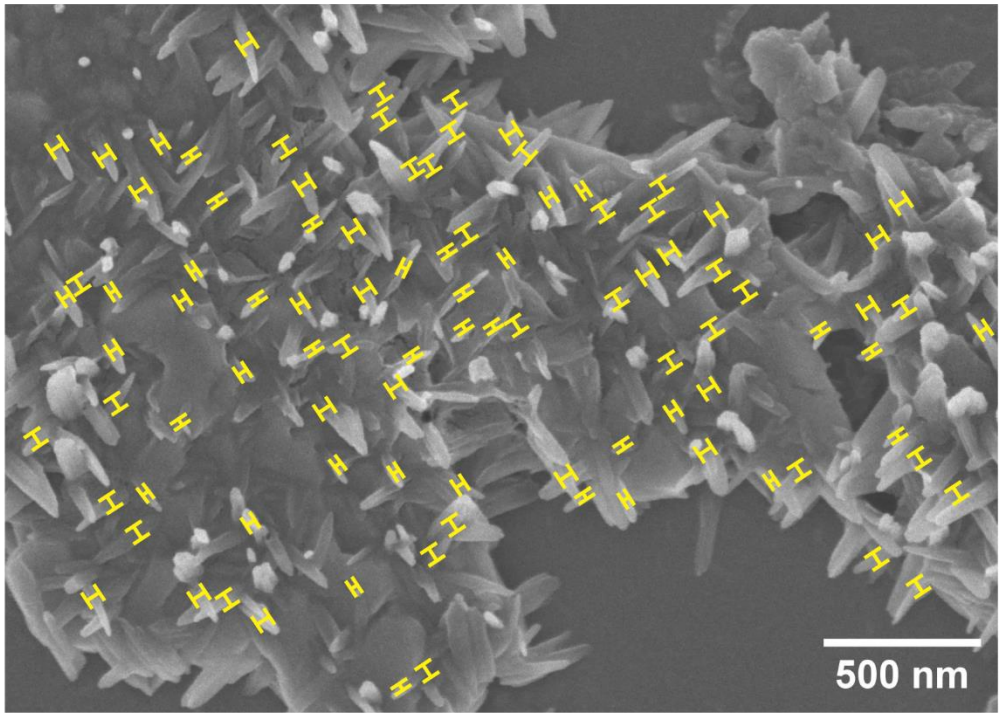


**Figure S36.** Random selection of nanostructures for the determination of (a) the length, and (b) diameter of **CP3** nanotubes using the software Image J. The yellow lines indicate the selected nanostructures.

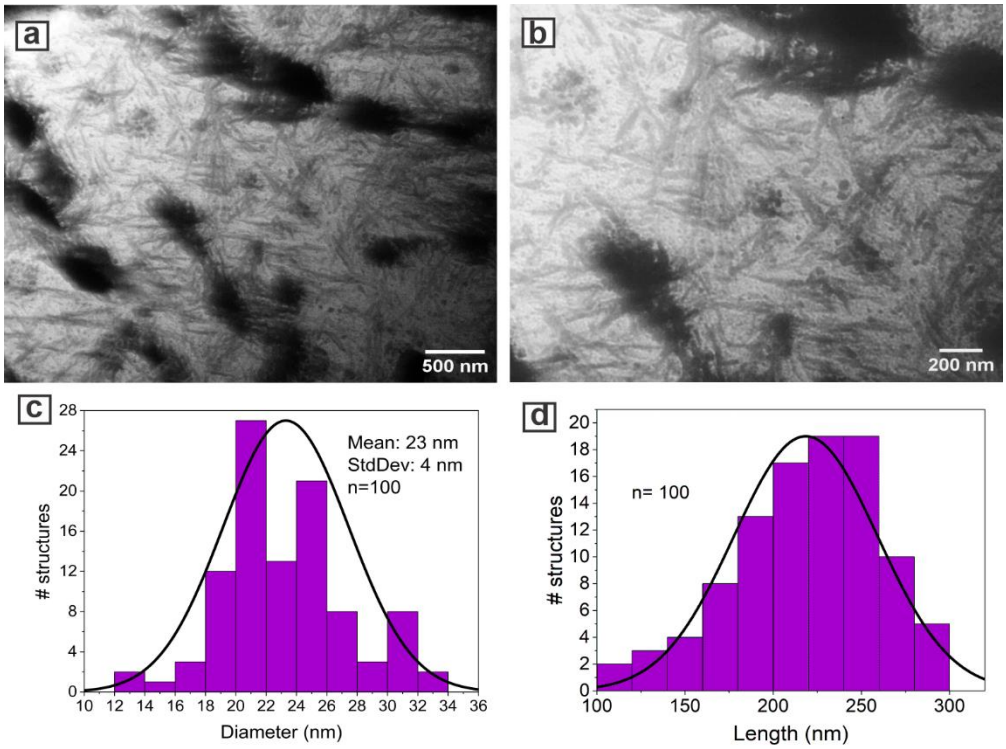



**Figure S37.** (a), and (b) LV-TEM images of the co-assembly between **CP1**, **CP2**, **and CP3**. (c) Average diameter distribution, and (d) length distribution of the resulting nanostructures.

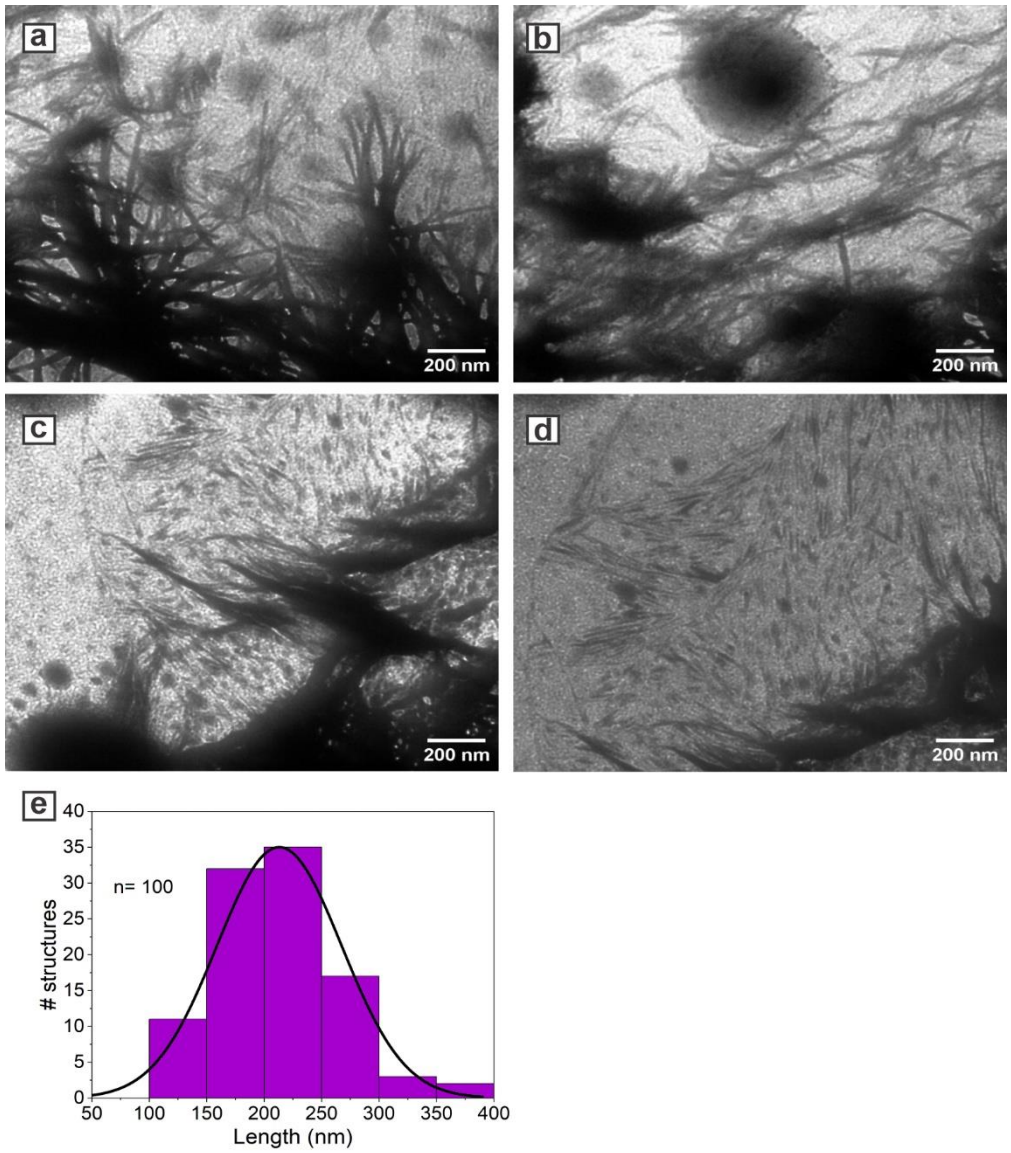



**Figure S38.** (a), (b), (c), and (d) Additional LV-TEM images of the co-assembly between **CP1**, **CP2**, **CP3**, **and CP4**. (e) Length diameter distribution of the resulting nanostructures.

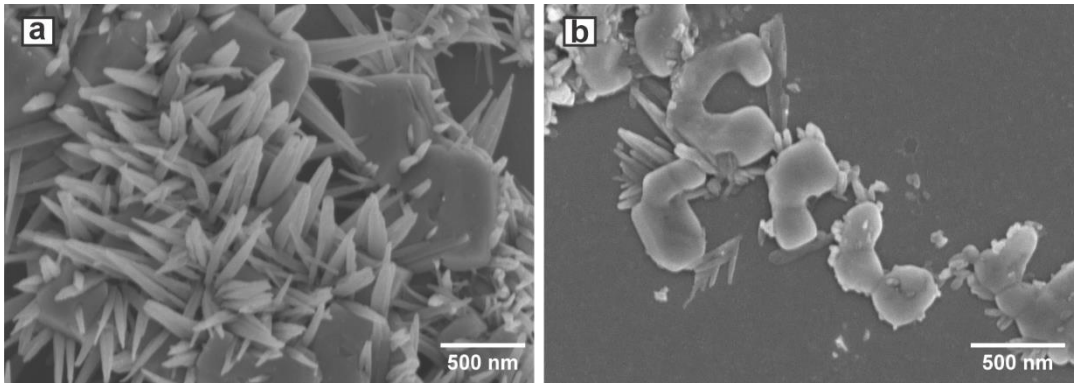



**Figure S39.** (a), and (b) FESEM images of the co-assembly between all cyclic peptides (**CP1, CP2, CP3, and CP4**) under physiological pH.

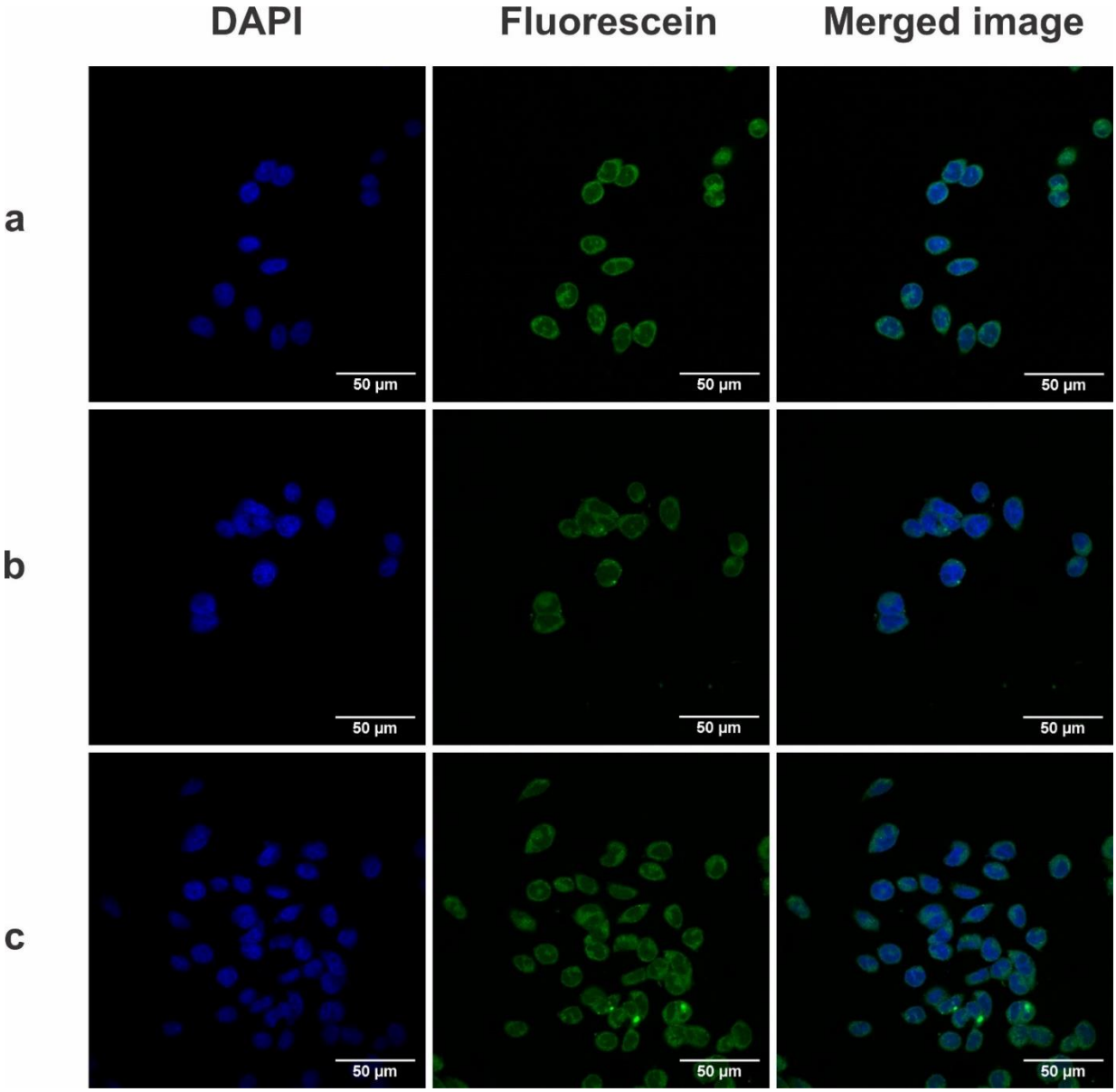



**Figure S40.** Additional confocal fluorescence microscopy images taken from different spots within the same sample. (a), (b), and (c) LNCaP cells and targeting CPNTs were incubated at 37 ºC for 2 h.

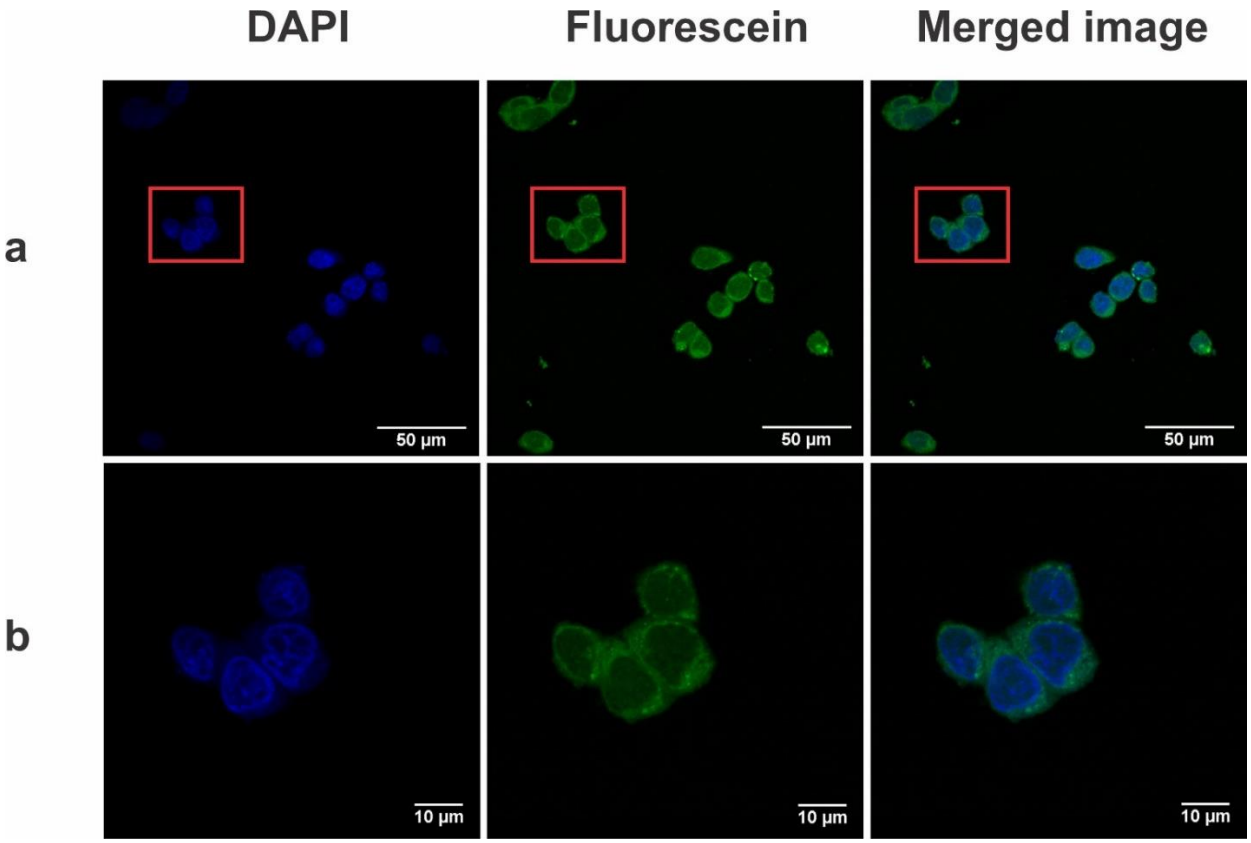



**Figure S41.** Additional confocal fluorescence microscopy images. (a) LNCaP cells and targeting CPNTs were incubated at 37 ºC for 2 h. (b) Zoomed image of (a).

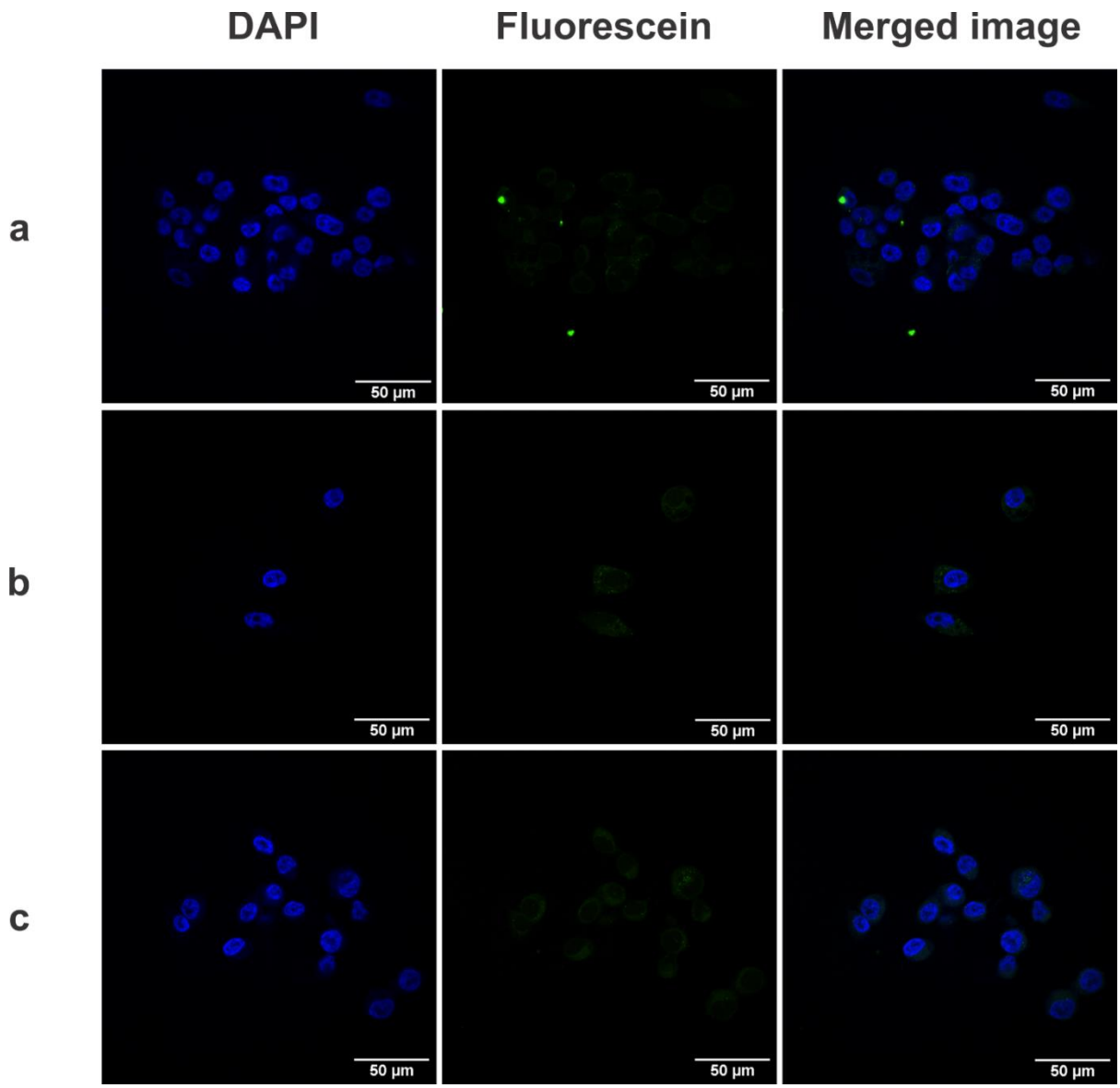



**Figure S42.** (a), (b), (c) PC3 cells and targeting CPNTs were incubated at 37 ºC for 2 h (images taken from different spots).

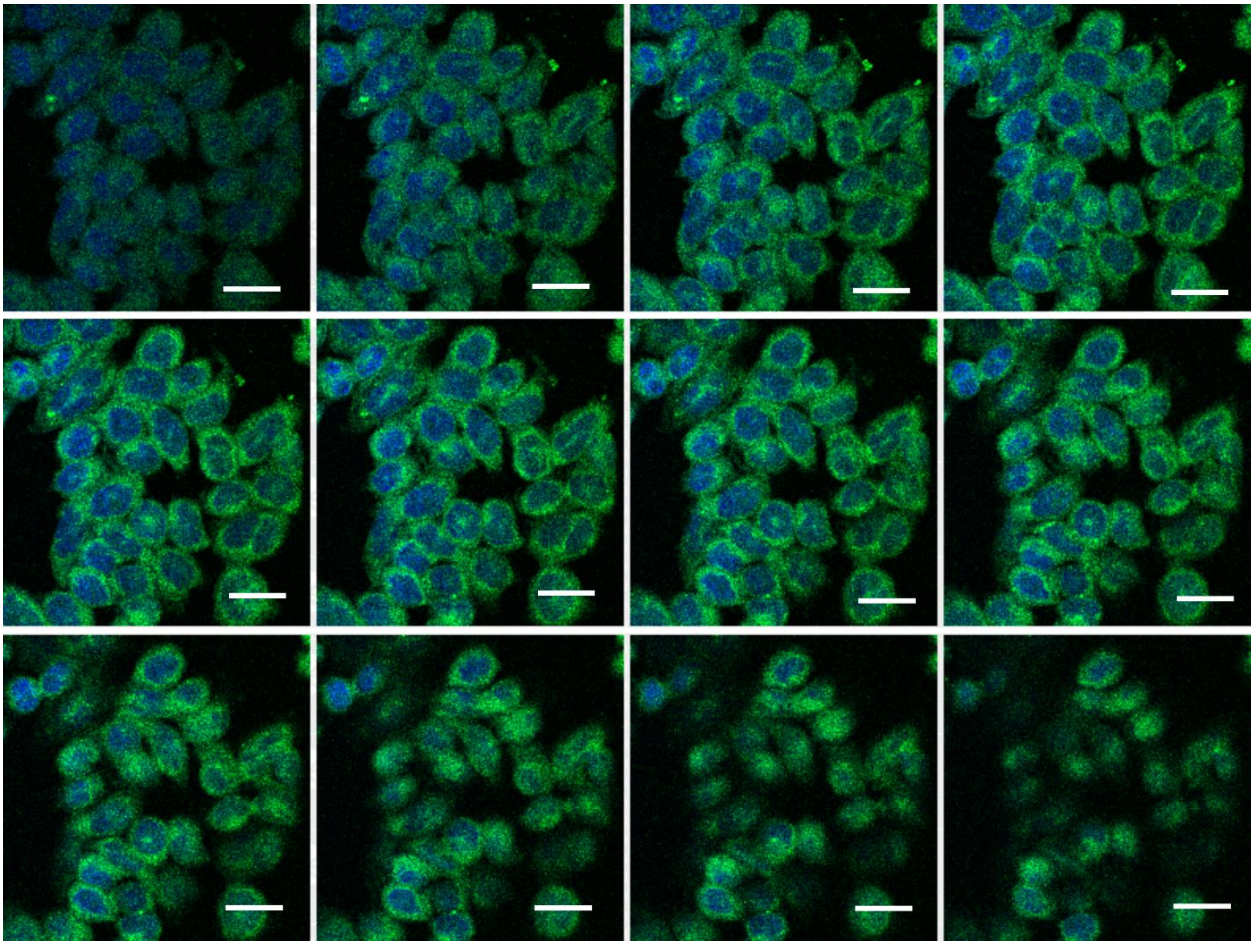


**Figure S43.** Z-stacking slices of LNCaP cells and targeting CPNTs (scale bars: 10 μm)