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Supporting information for Self-Healing Cyclic Peptide Hydrogels

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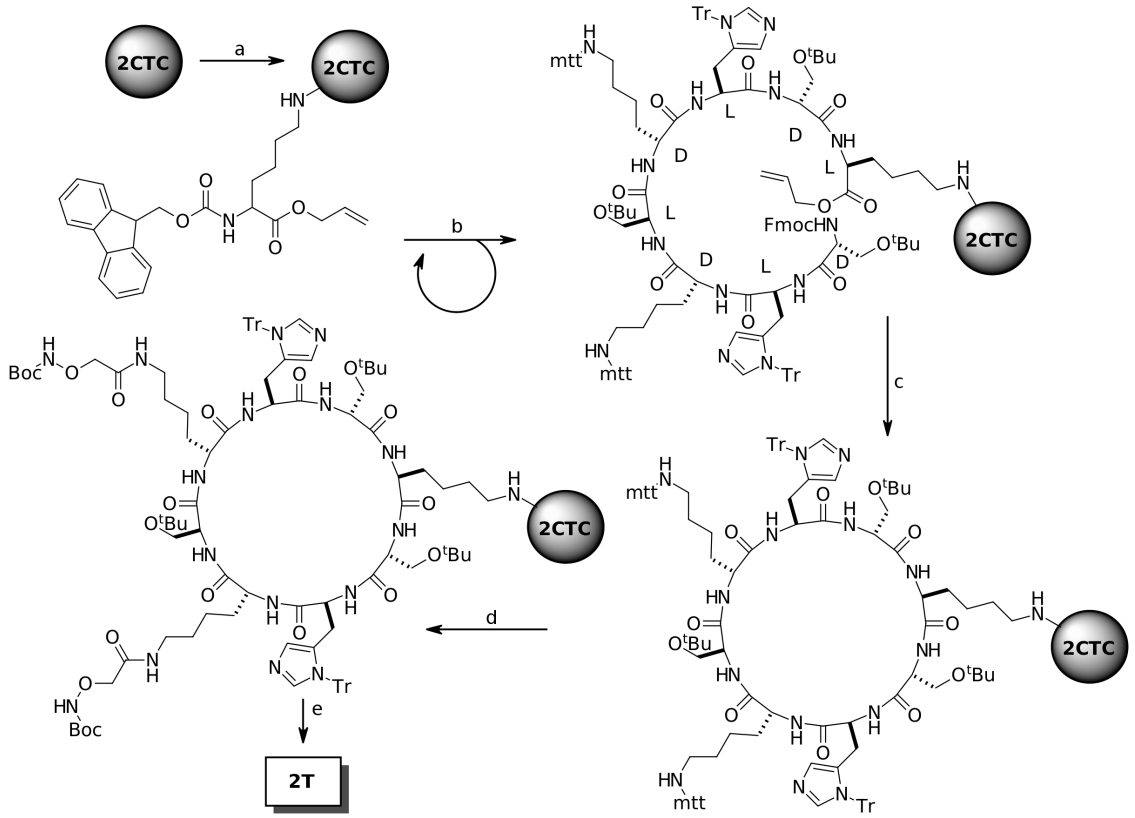
S1 Synthesis of CP derivatives

S1.1 Synthesis of CP derivatives



Scheme S1: Preparation of precursor **2T**: a) *N*-α-(9-Fluorenylmethyloxycarbonyl)-L-lysine allyl ester hydrochloride, DIEA, DCM, 2 h; b) i) piperidine/DMF (1:4), 10 min, ii) amino acid, *N*-HBTU, DIEA, DMF, 30 min, repeat 7 cycles with the corresponding aminoacid; c) i) $Pd(OAc)_2$, PPh_3, phenylsilane, 4-methylmorpholine, DCM, overnight, ii) piperidine/DMF (1:4), 10 min, iii) PyAOP, DIEA, DMF, 4 h.; d) i) DCM-TFA-TIS (97.6:1.4:1), 1 h, twice ii) [(tert-butoxycarbonyl)aminoxy]acetic acid, *N*-HATU, DIEA, DMF, 45 min; e) TFA-DCM-H_2O-TIS (9:0.5:0.25:0.25).

S2 Characterization of CP derivatives by NMR, HPLC-MS and ATR-IR

S2.1 2T

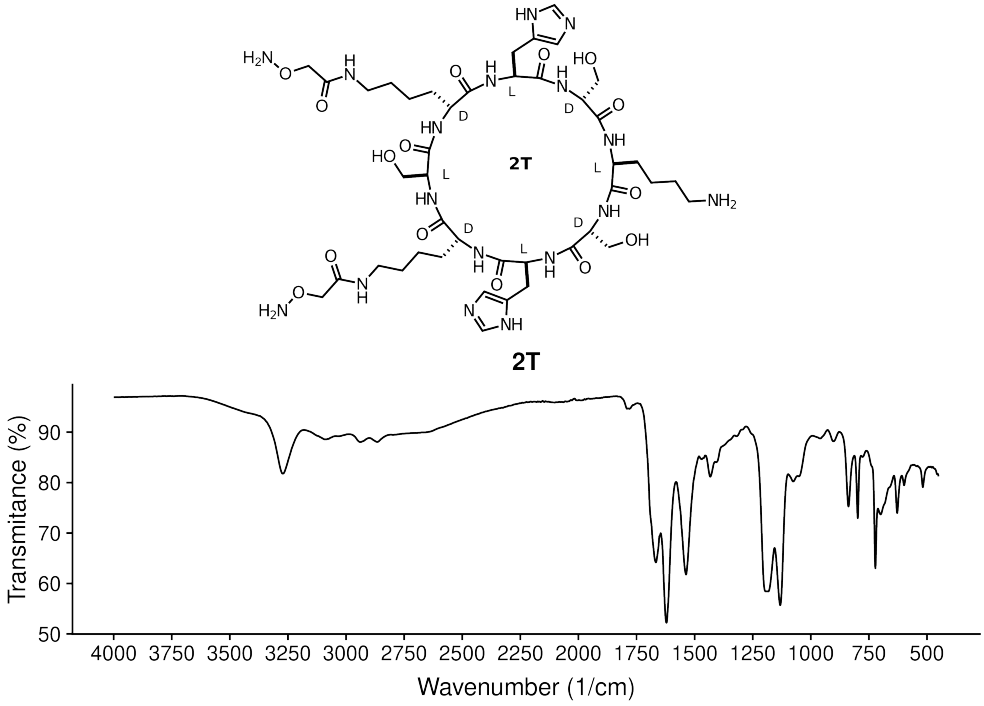


Figure S1: FTIR-ATR of **2T**.

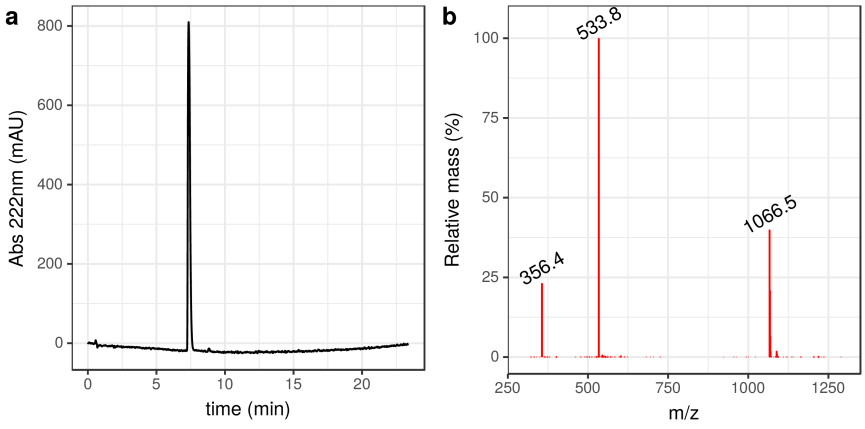


Figure S2: a) HPLC chromatogram of peptide **2T**. Gradient of 0% to 75% ACN (0.1% TFA) in 20 min; b) MS spectra of the main peak.

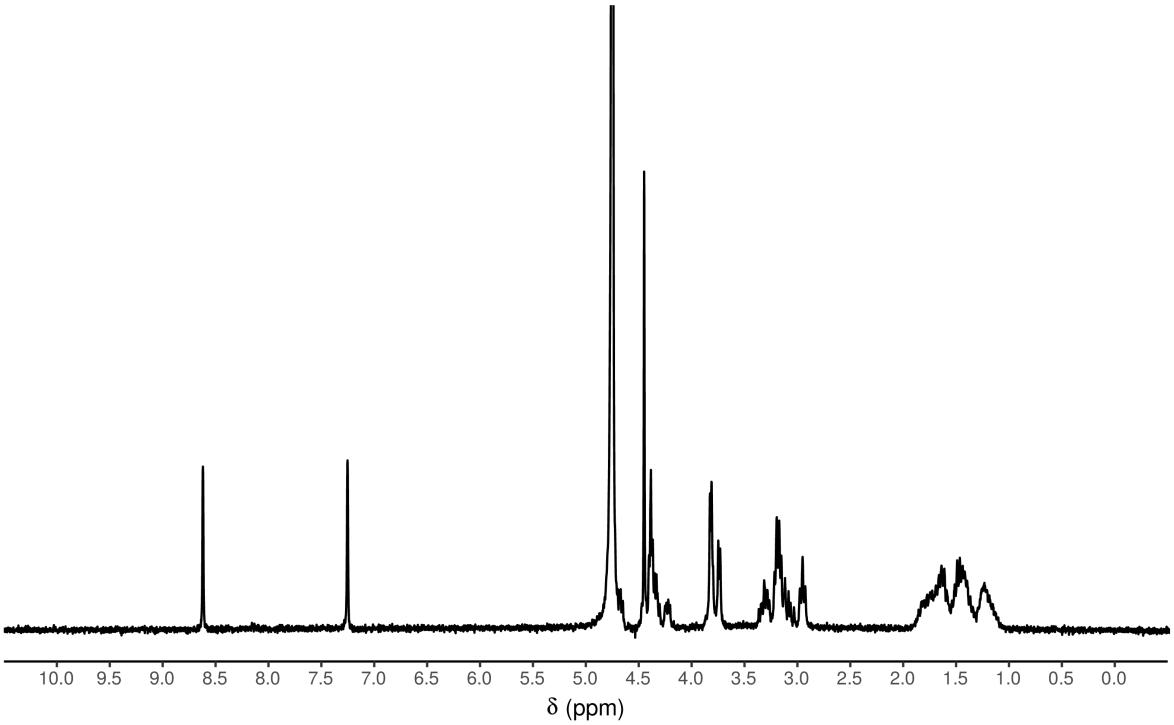


Figure S3: ^{1}H NMR spectra (300 MHz, D_2O, room temperature) of cyclic peptide **2T**.

S2.2 1TN

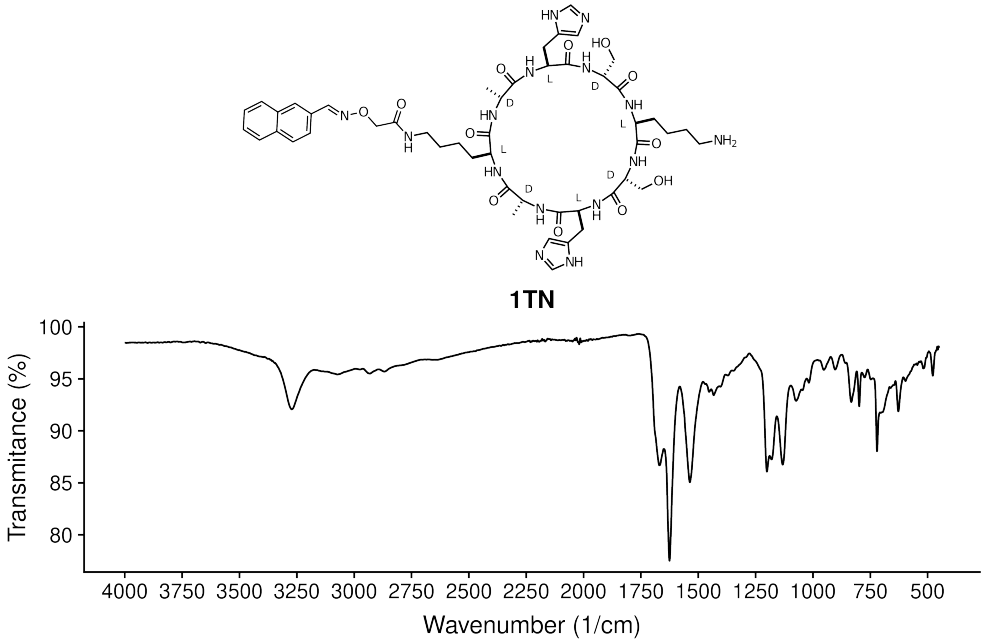


Figure S4: FTIR-ATR of **1TN**.

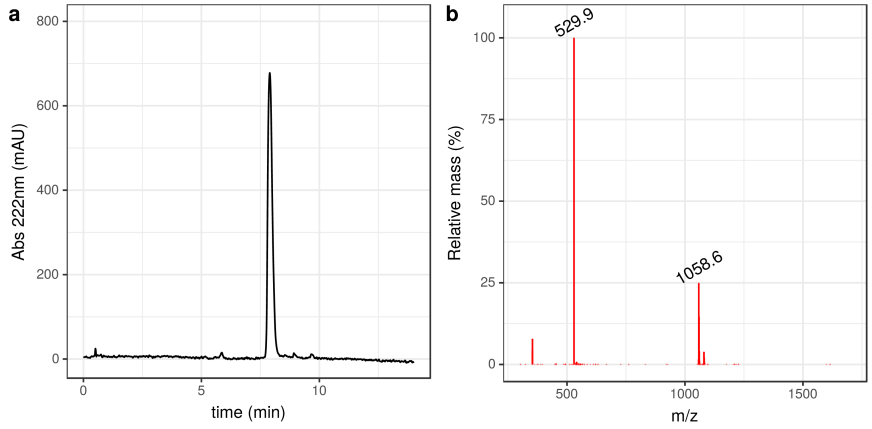


Figure S5: a) HPLC chromatogram of peptide **1TN**. Gradient of 5% to 95% ACN (0.1% TFA) in 15 min; b) MS spectra of the main peak.

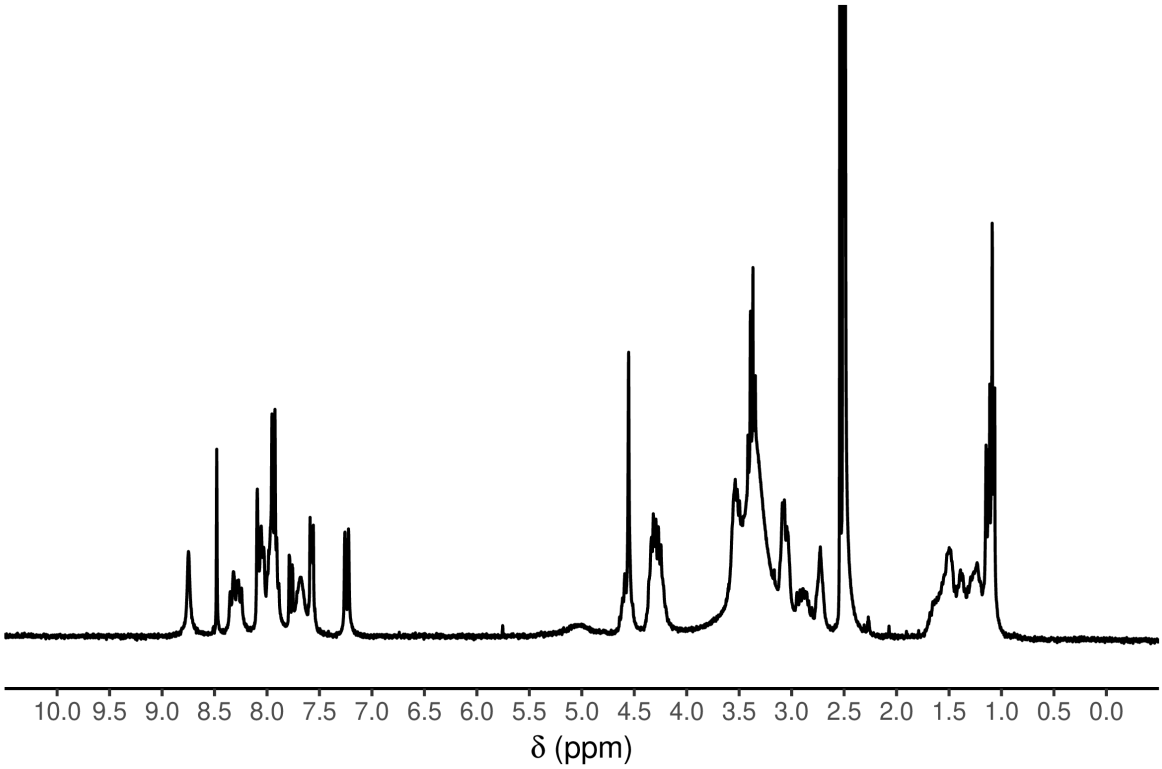


Figure S6: ^{1}H NMR spectra (300 MHz, DMSO-d_6, room temperature) of cyclic peptide **1TN**.

S2.3 1TA

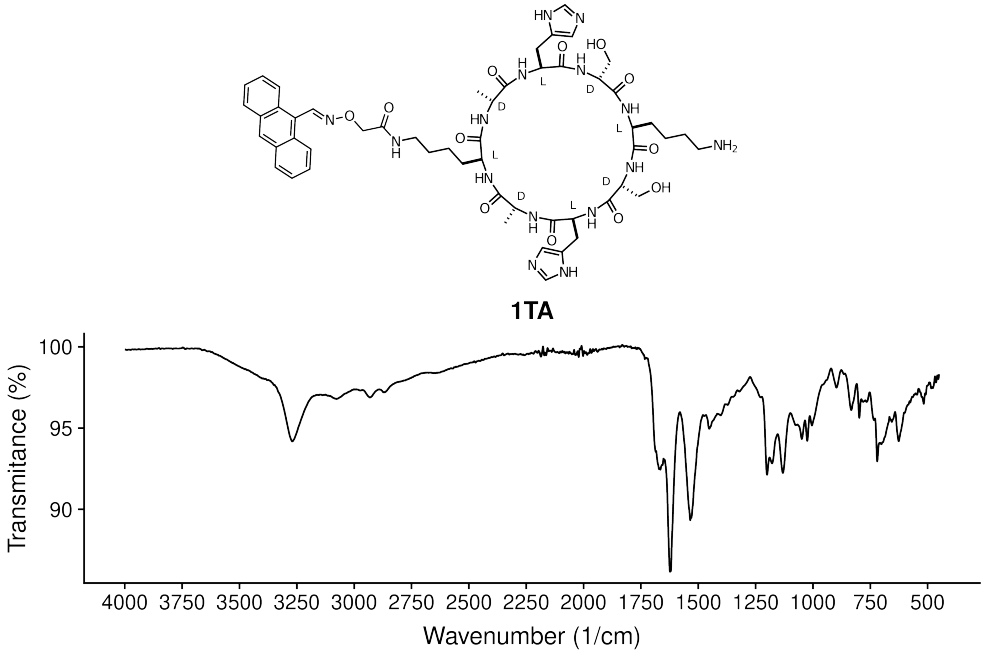


Figure S7: FTIR-ATR of **1TA**.

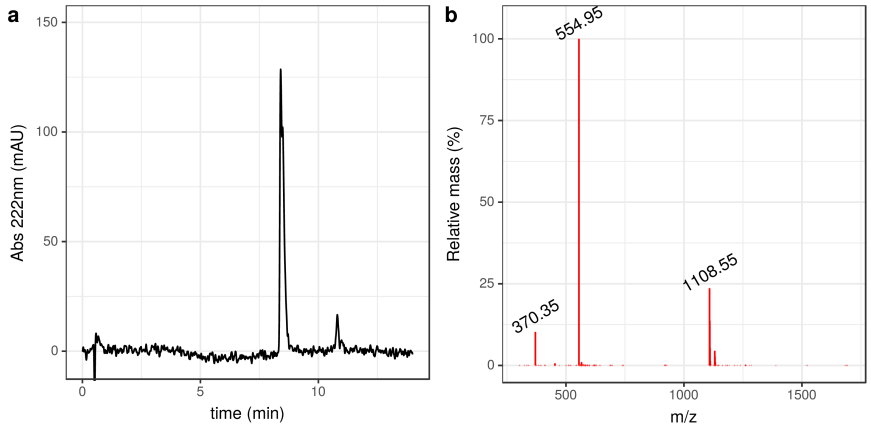


Figure S8: a) HPLC chromatogram of peptide **1TA**. Gradient of 5% to 95% ACN (0.1% TFA) in 15 min; b) MS spectra of the main peak.

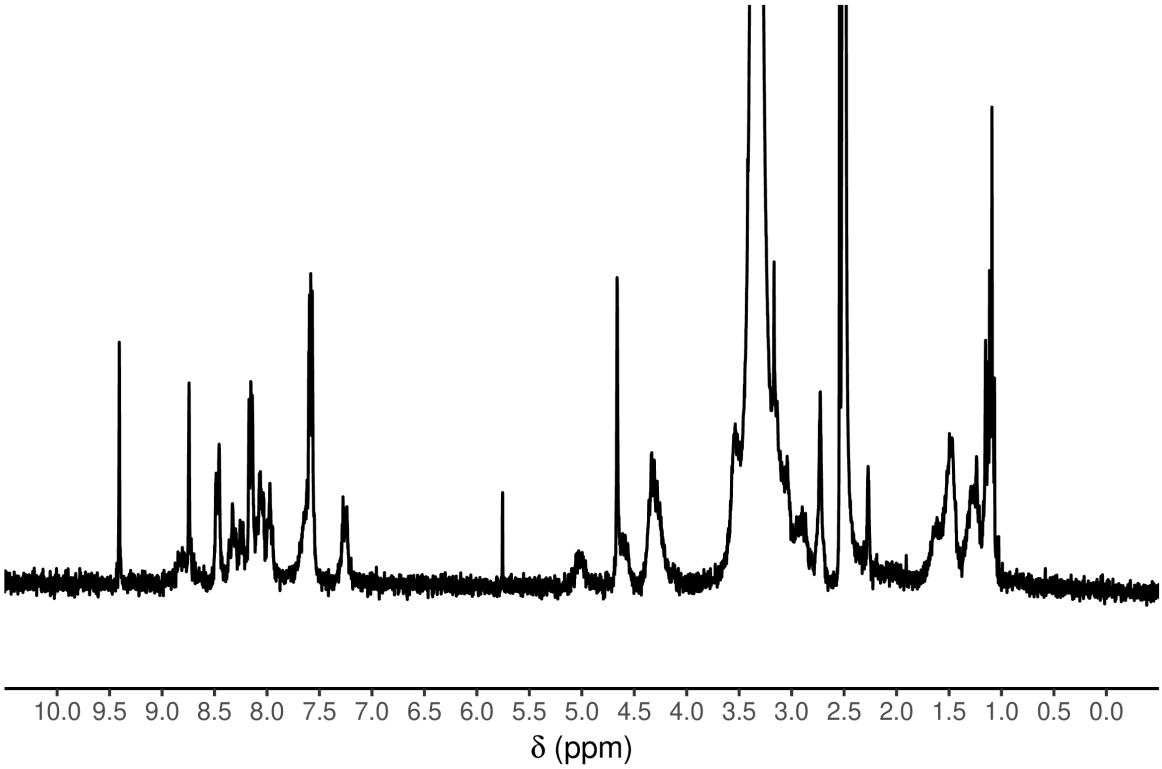


Figure S9: ^{1}H NMR spectra (300 MHz, DMSO-d_6, room temperature) of cyclic peptide **1TA**.

S2.4 2TN

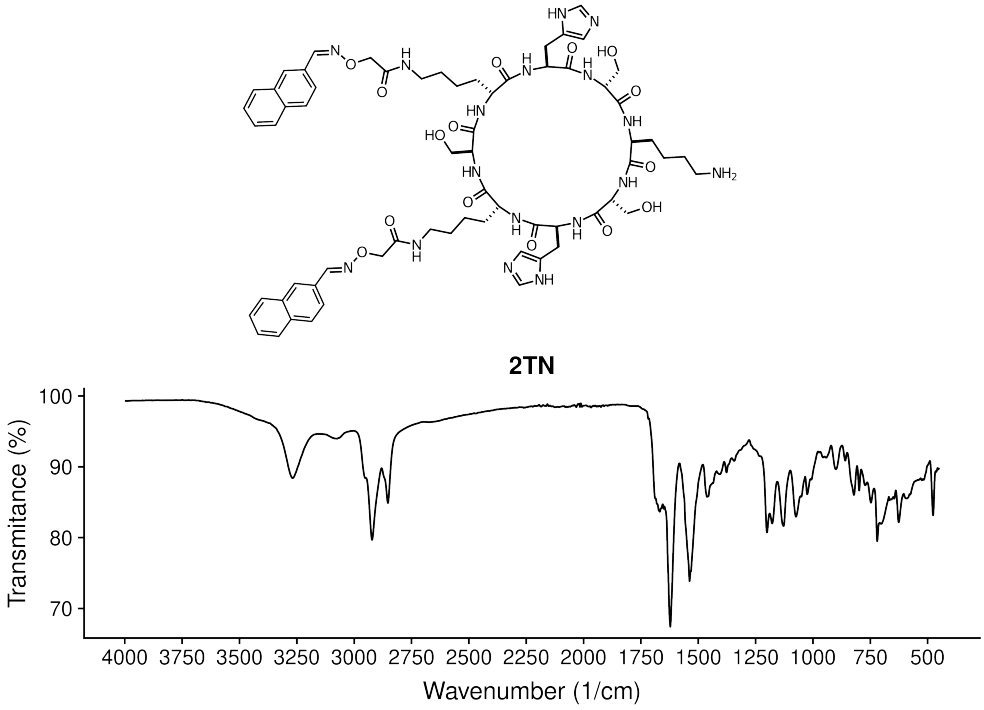


Figure S10: FTIR-ATR of **2TN**.

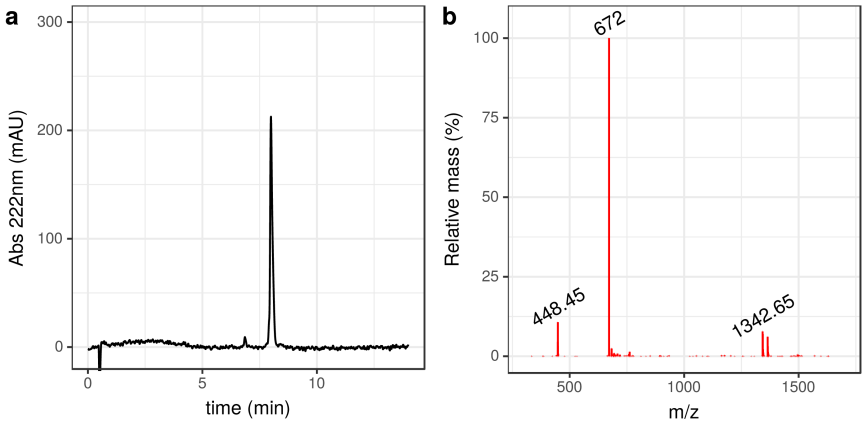


Figure S11: a) HPLC chromatogram of peptide **2TN**. Gradient of 5% to 95% ACN (0.1% TFA) in 12 min; b) MS spectra of the main peak.

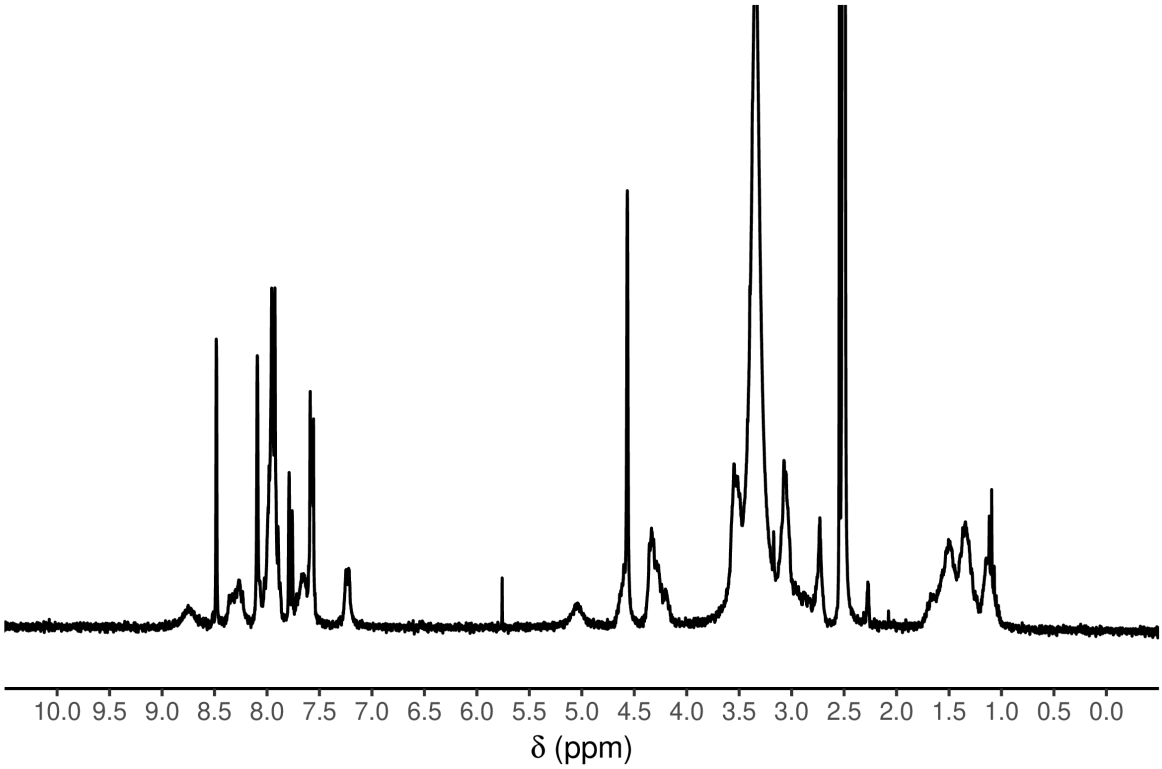


Figure S12: ^{1}H NMR spectra (300 MHz, DMSO-d_6, room temperature) of cyclic peptide **2TN**

S2.5 2TA

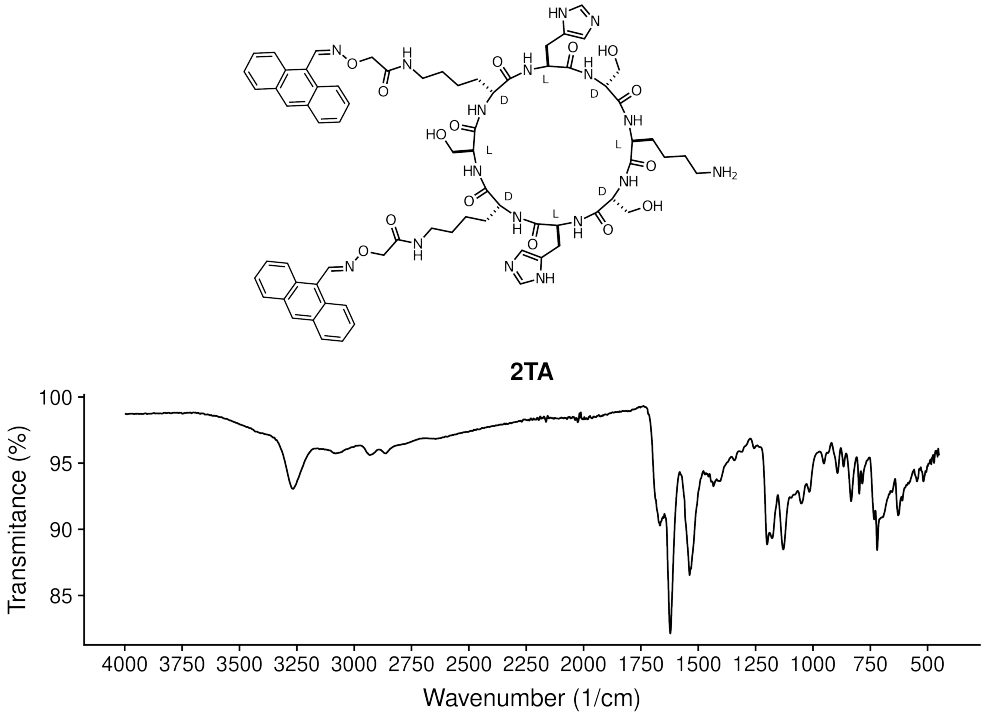


Figure S13: FTIR-ATR of **2TA**.

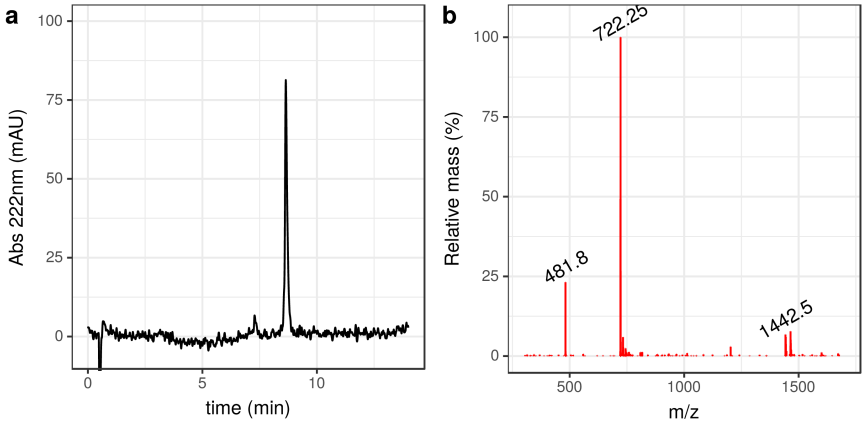


Figure S14: a) HPLC chromatogram of peptide **2TA**. Gradient of 5% to 95% ACN (0.1% TFA) in 12 min; b) MS spectra of the main peak.

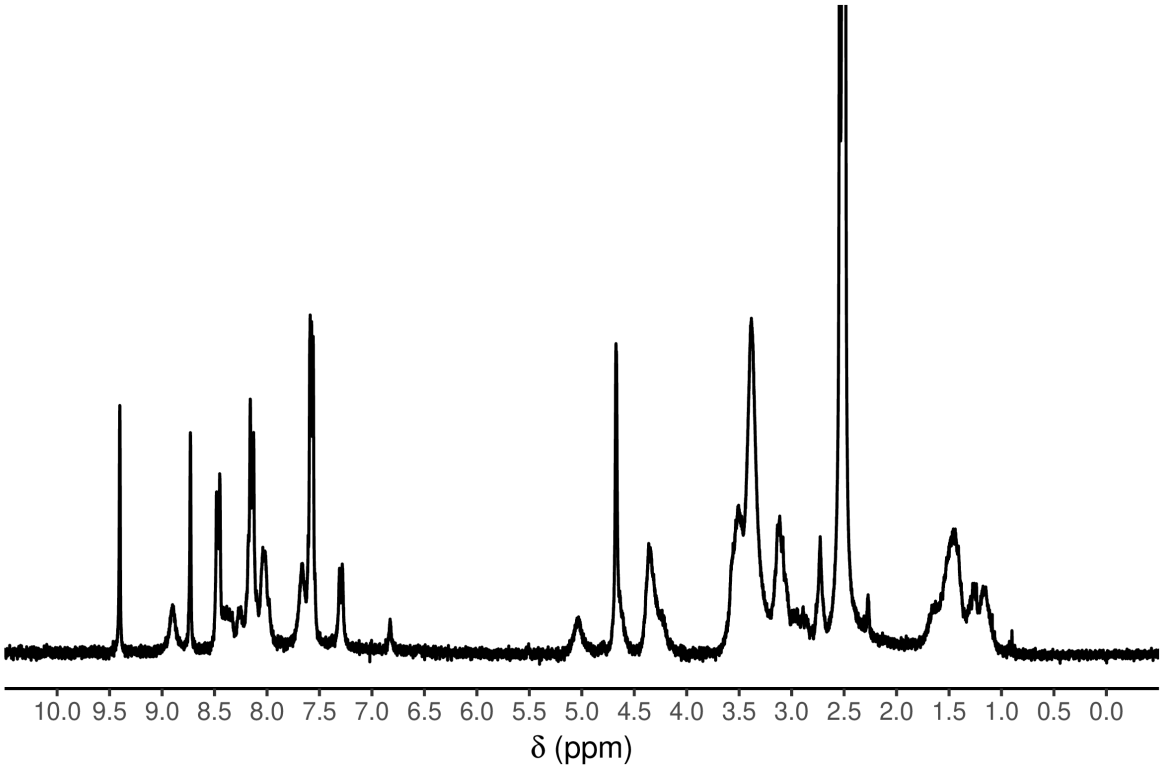


Figure S15: ^{1}H NMR spectra (300 MHz, DMSO-d_6, room temperature) of cyclic peptide **2TA**.

S2.6 2TP

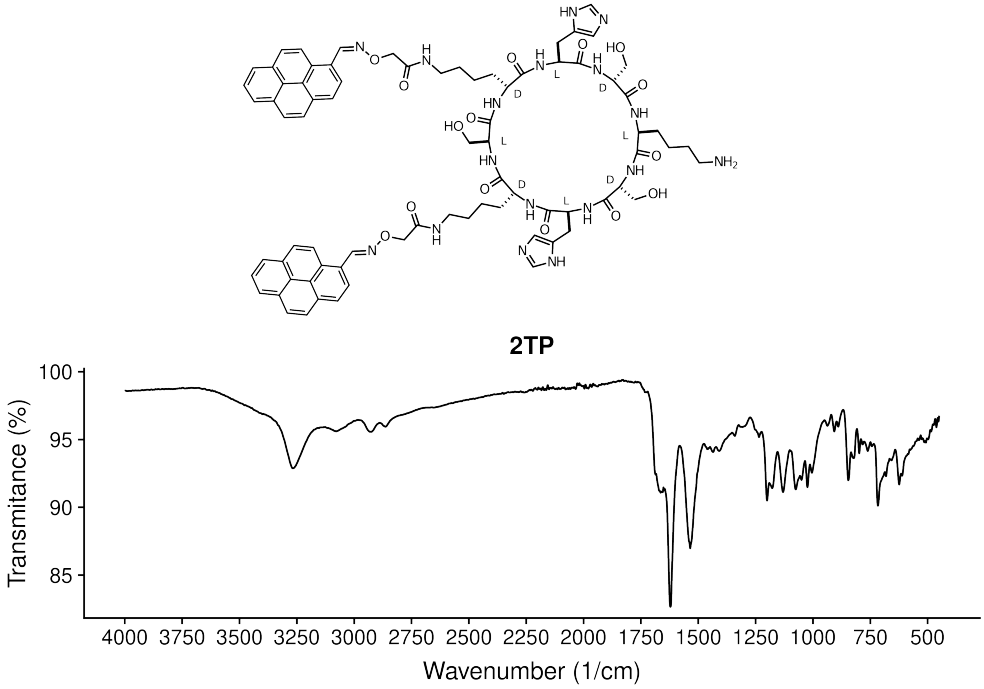


Figure S16: FTIR-ATR of **2TP**.

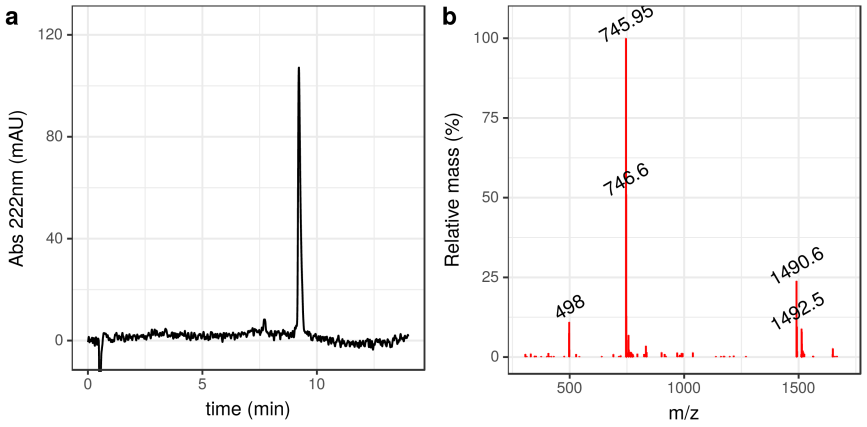


Figure S17: a) HPLC chromatogram of peptide **2TP**. Gradient of 5% to 95% ACN (0.1% TFA) in 12 min; b) MS spectra of the main peak.

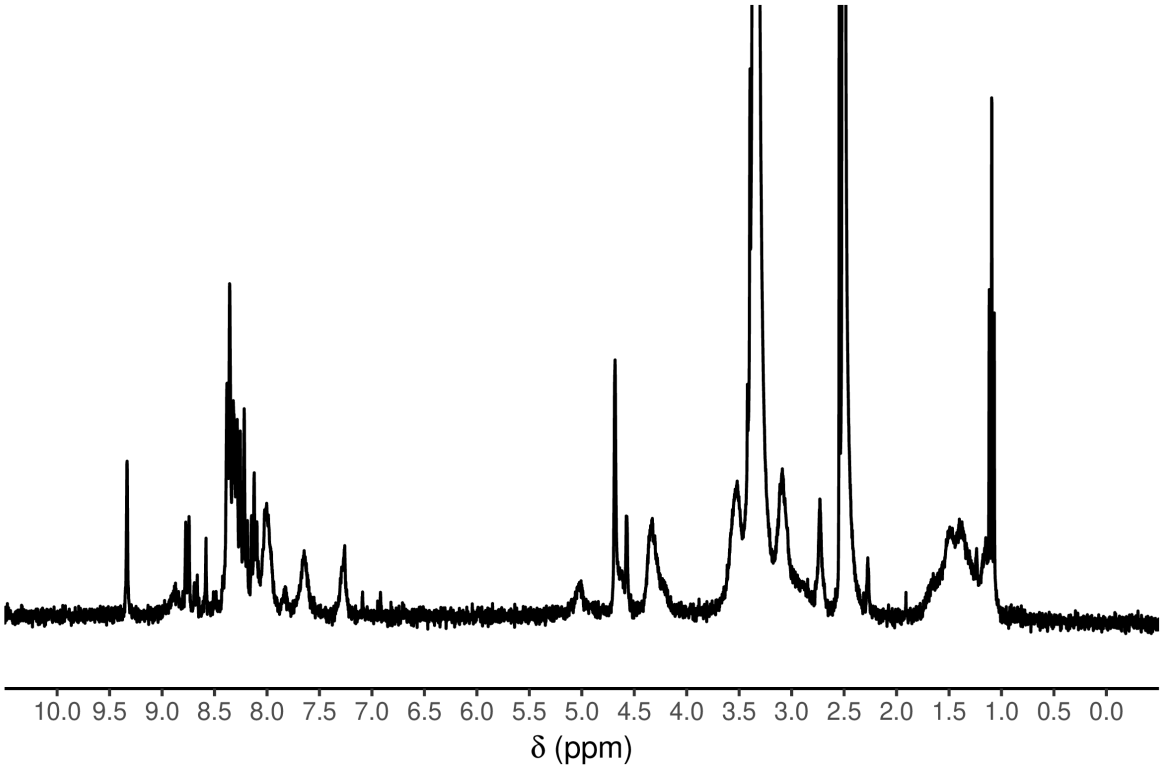


Figure S18: ^{1}H NMR spectra (300 MHz, DMSO-d_6, room temperature) of cyclic peptide **2TP**.

S3 Self-assembly of CP derivatives

Hydrogel to solution transition upon acidification

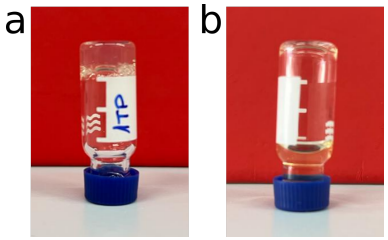


Figure S19: Reversible gel-sol transition upon acidification of **1TP** hydrogels a) Preformed hydrogel (2% w/w, pH 8-9); b) Transition to a solution state after addition of concentrated acid.

S3.1 Self-assembly monitored by spectroscopic techniques

ATR-FTIR spectra collected from assembled CP

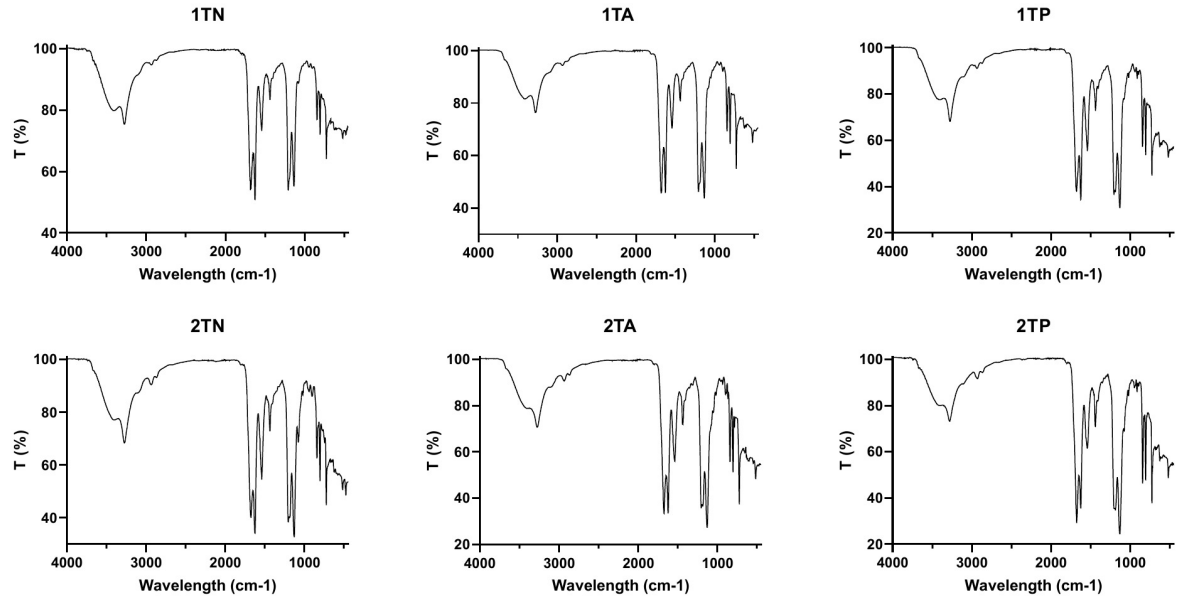


Figure S20: ATR-FTIR spectra for freeze-dried alkaline samples of **1TN**, **1TA**, **1TP**, **2TN**, **2TA** and **2TP**.

CP	Wavelength (cm^{-1})
1TN	1624, 1683
1TA	1624, 1678
1TP	1622, 1674
2TN	1622, 1673
2TA	1621, 1673
2TP	1622, 1673

Table S1: Wavenumber of maximum intensity corresponding to the Amide I stretching bands obtained from ATR-FTIR spectra in Figure S20.

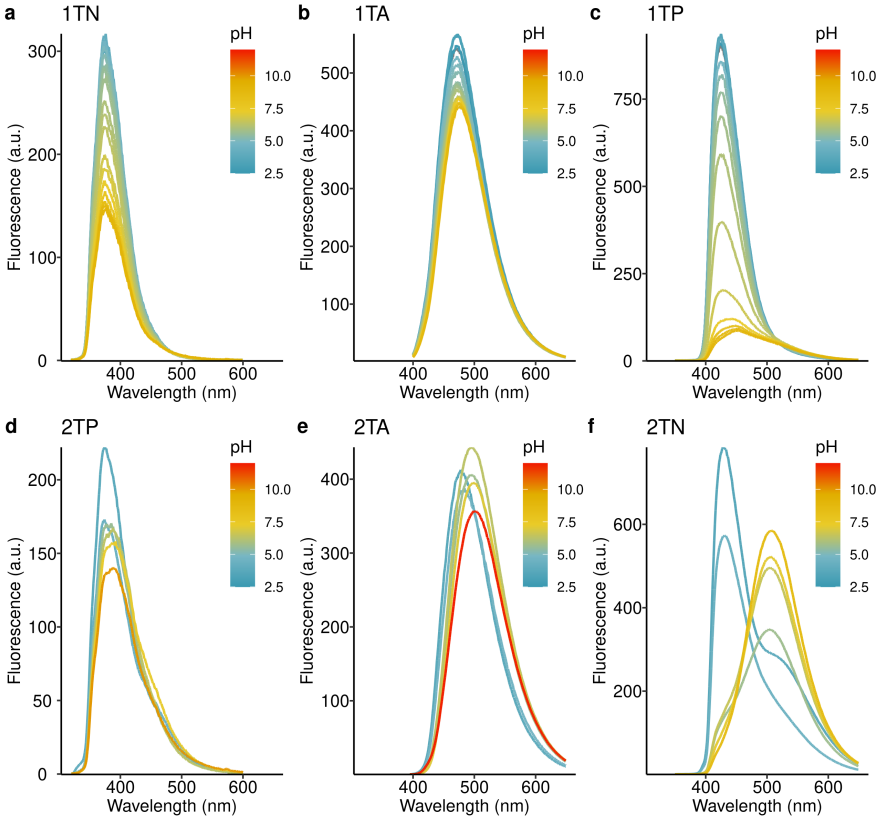


Figure S21: Fluorescence emission spectra of CPs (400 μM, water) at different pH values. The parameters are: a) **1TN**: $\lambda_{ex} = 230$ nm; b) **1TA**: $\lambda_{ex} = 385$ nm; c) **1TP**: $\lambda_{ex} = 340$ nm: d) **2TN**: $\lambda_{ex} = 230$ nm; e) **2TA**: $\lambda_{ex} = 385$ nm; f) **2TP**: $\lambda_{ex} = 340$ nm.

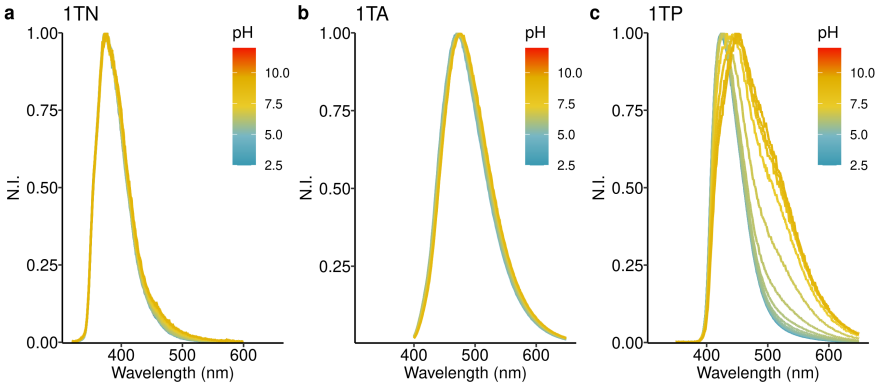


Figure S22: Fluorescence emission spectra of CPs (400 μM, water) at different pH values, normalized to the maximum fluorescence emission at each pH.

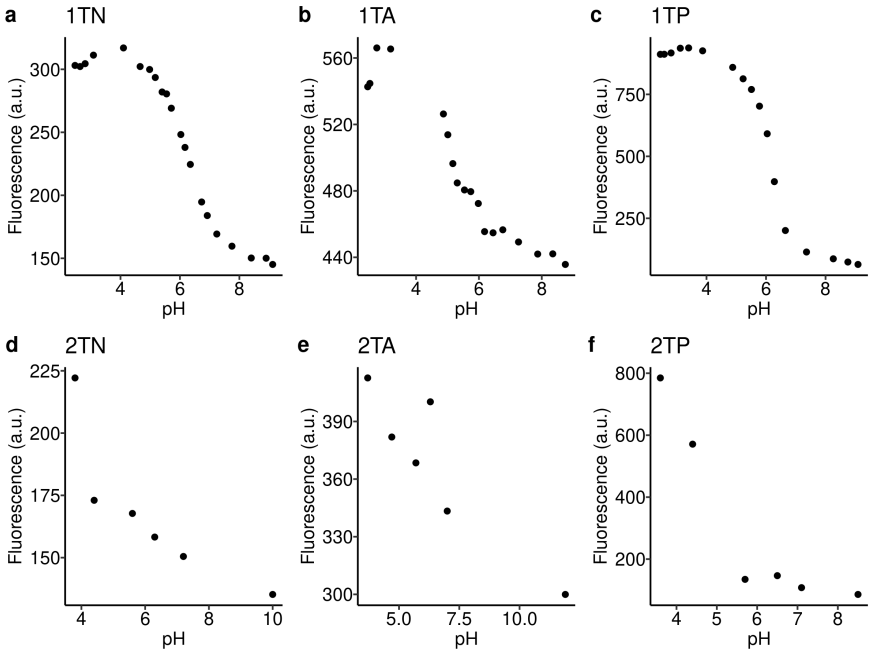


Figure S23: Aggregation-induced quenching of CPs fluorescence emission (400 μM, water) at different pH values. The wavelength showing maximum intensity at acidic pH was used as reference: a) **1TN**: $\lambda_{max} = 376$ nm; b) **1TA**: $\lambda_{max} = 471$ nm; c) **1TP**: $\lambda_{max} = 425$ nm: d) **2TN**: $\lambda_{max} = 374$ nm; e) **2TA**: $\lambda_{max} = 477$ nm; f) **2TP**: $\lambda_{max} = 428$ nm.

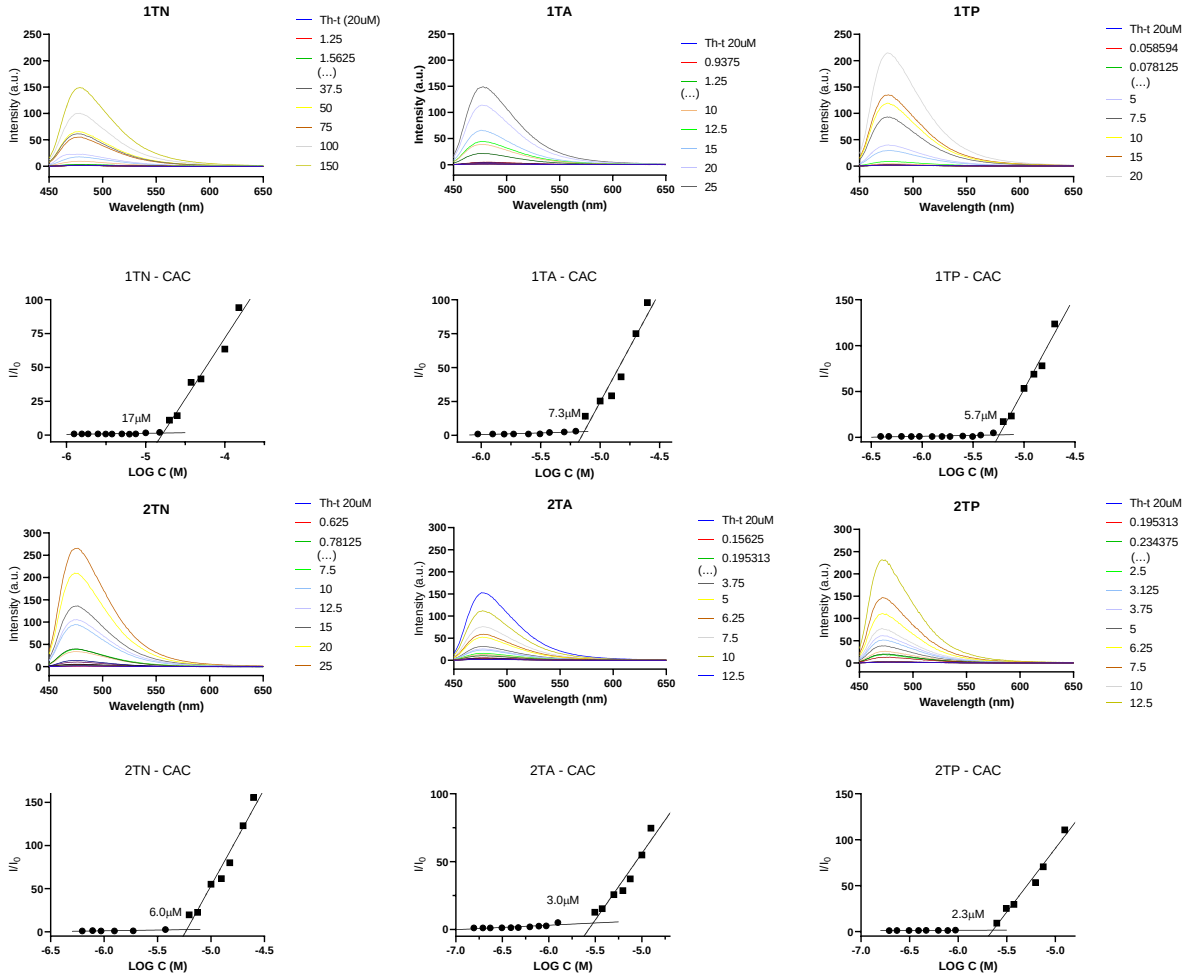


Figure S24: Determination of critical aggregation concentration (CAC) by fluorescence experiments in the presence of ThT (20 µM).

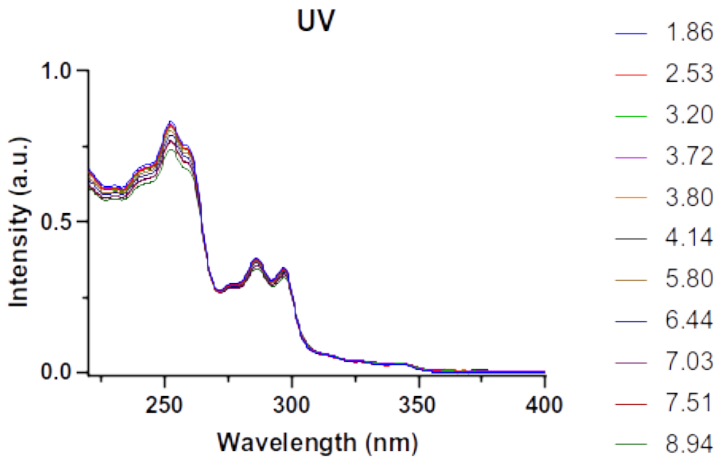


Figure S25: UV spectra at different pH values of CP **1TN** (100 μM)

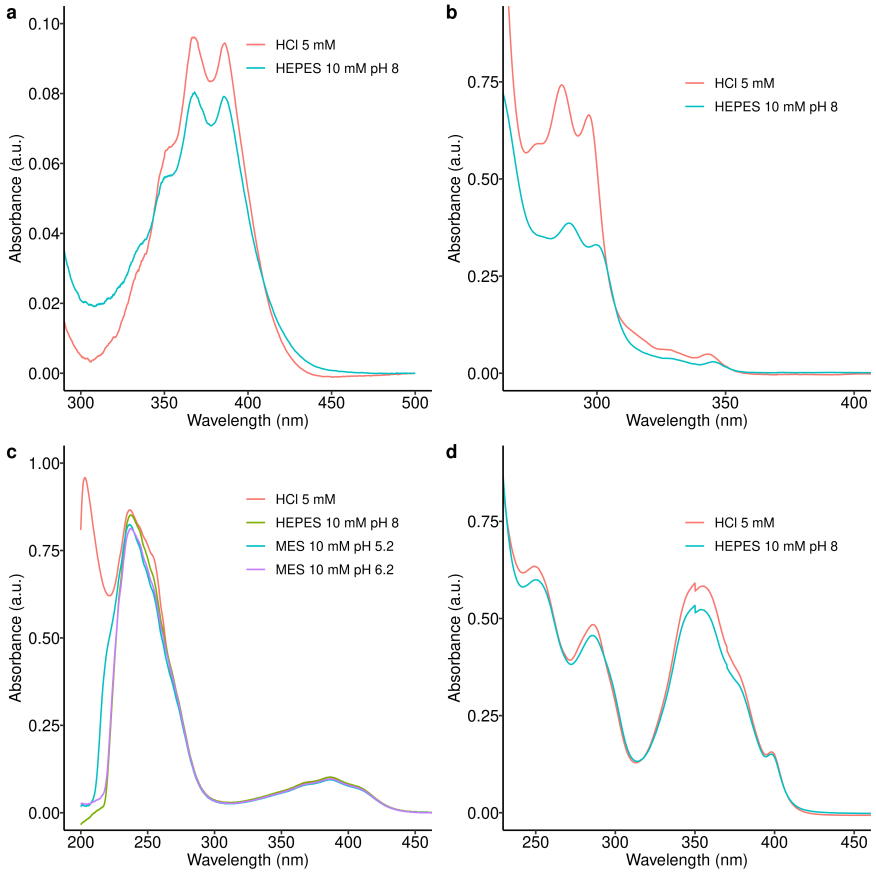


Figure S26: UV spectra of CP (3 μM) acquired in acidic pH (HCl 5 mM) or alkaline pH (HEPES 10 mM); a)**1TA**; b) **2TN**; c) **2TA**, acquired also in MES 10 mM at pH 5.2 and 6.2; d) **2TP**.

S3.2 SEM images of gels

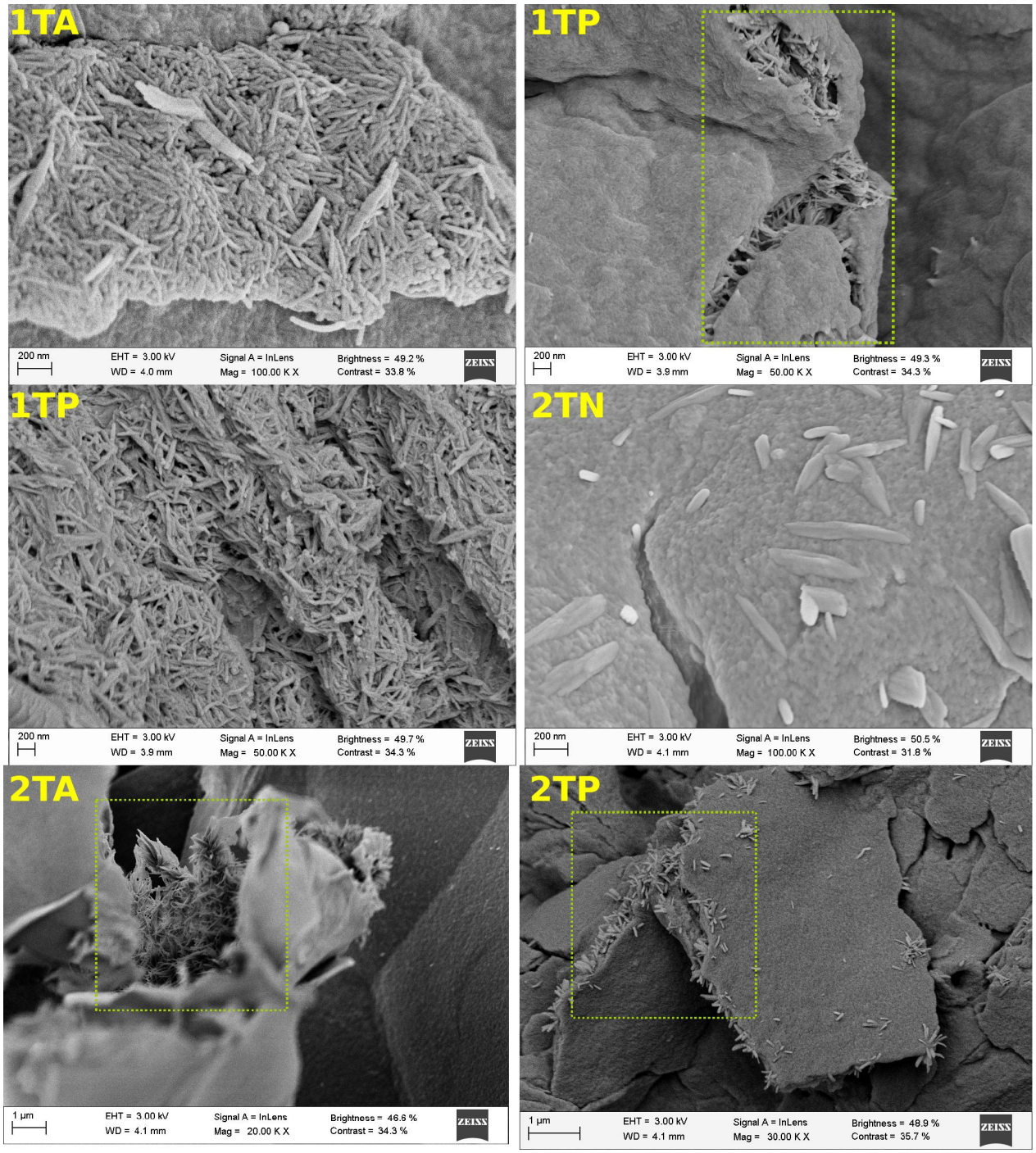


Figure S27: SEM images of **1TA**, **1TP**, **2TN**, **2TA** and **2TP** freeze-dried gels showing local regions were nanotubes are clearly visualized. The dashed boxes highlight the presence of nanotubes under the surface.

S3.3 Nanotube characterization by STEM imaging

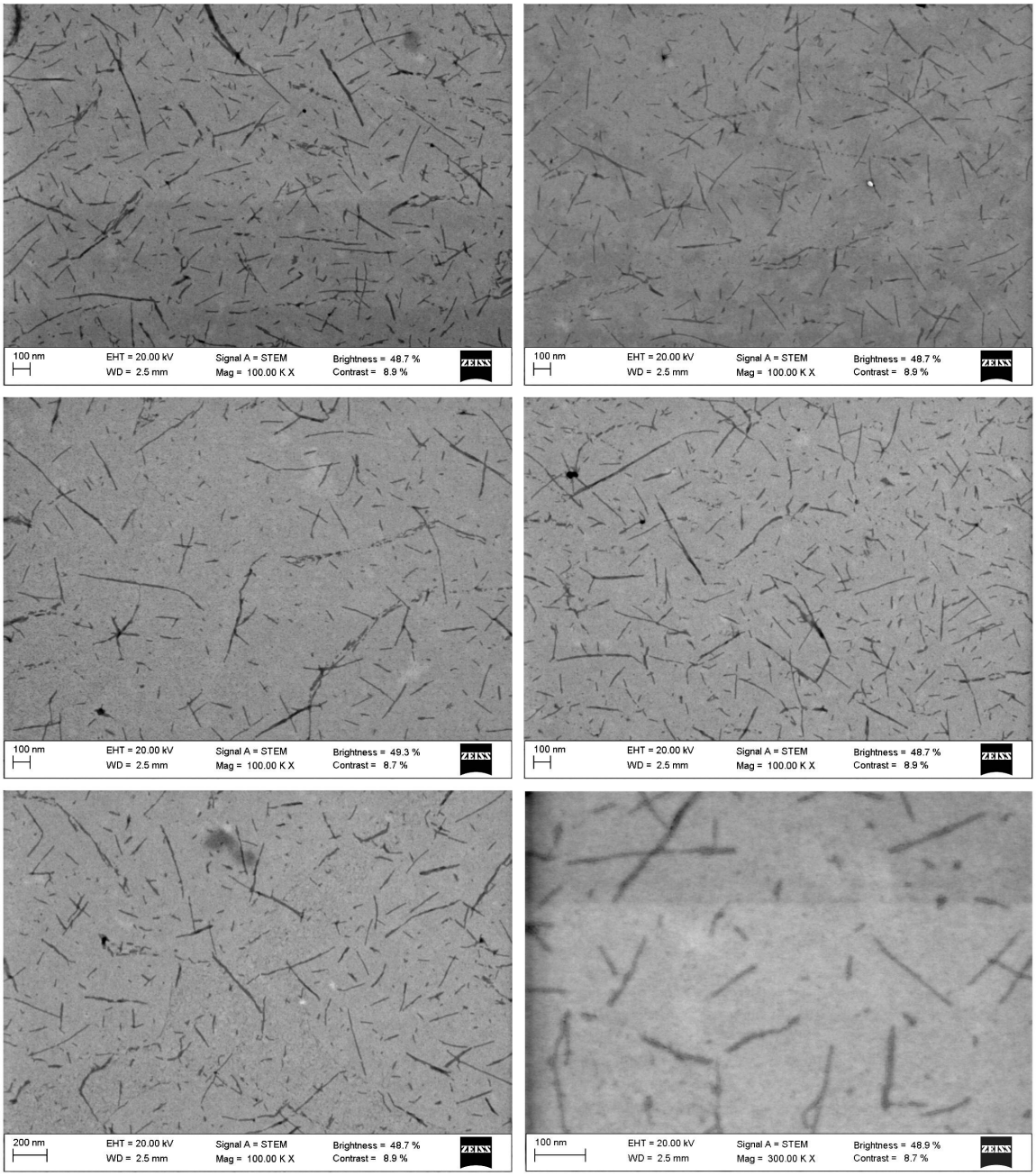


Figure S28: STEM micrographs of dilutions of **1TA** gels (final concentration 11 μM) after staining with uranyl acetate.

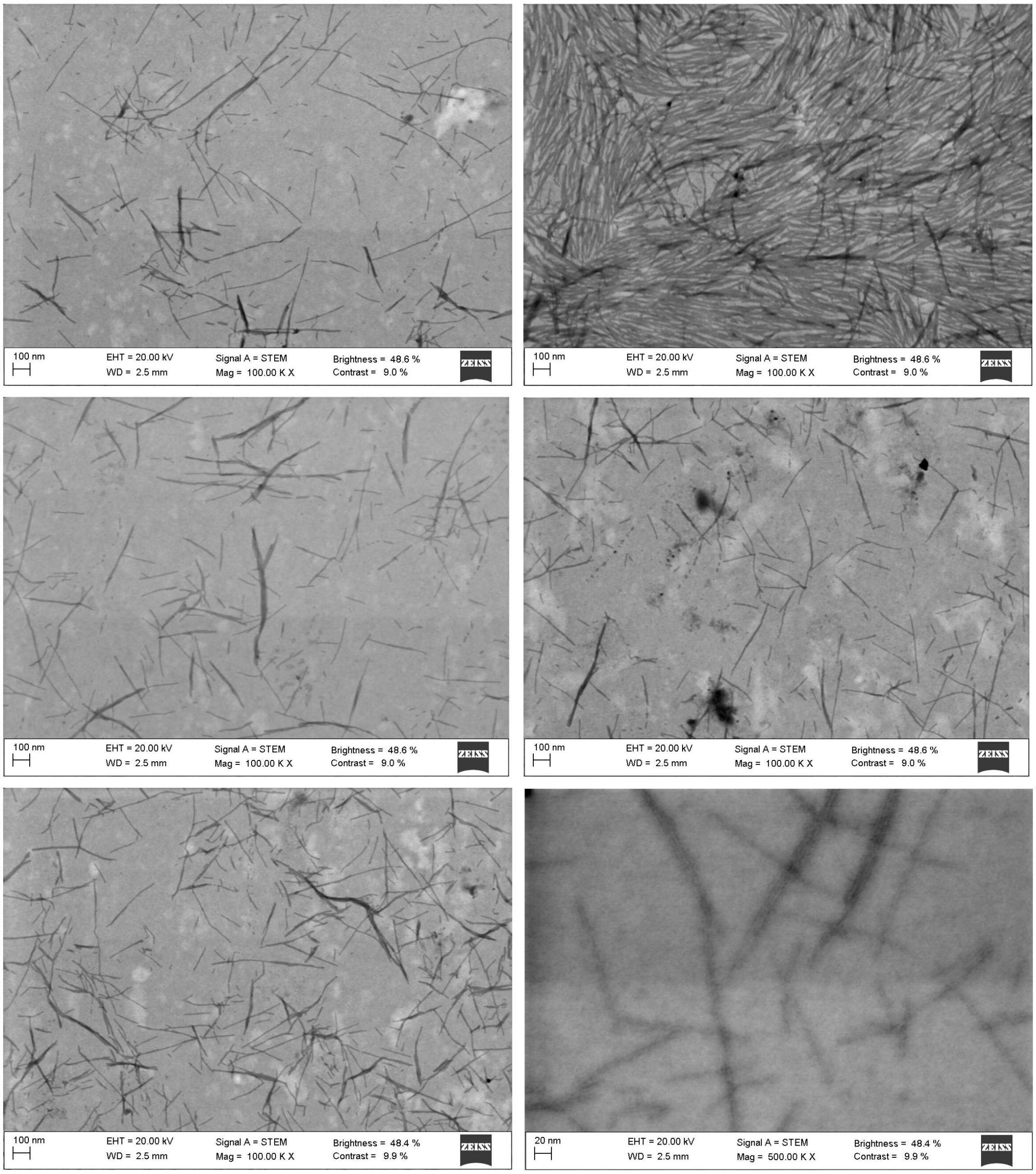


Figure S29: STEM micrographs of dilutions of **1TP** gels (final concentration 11 μM) after staining with uranyl acetate.

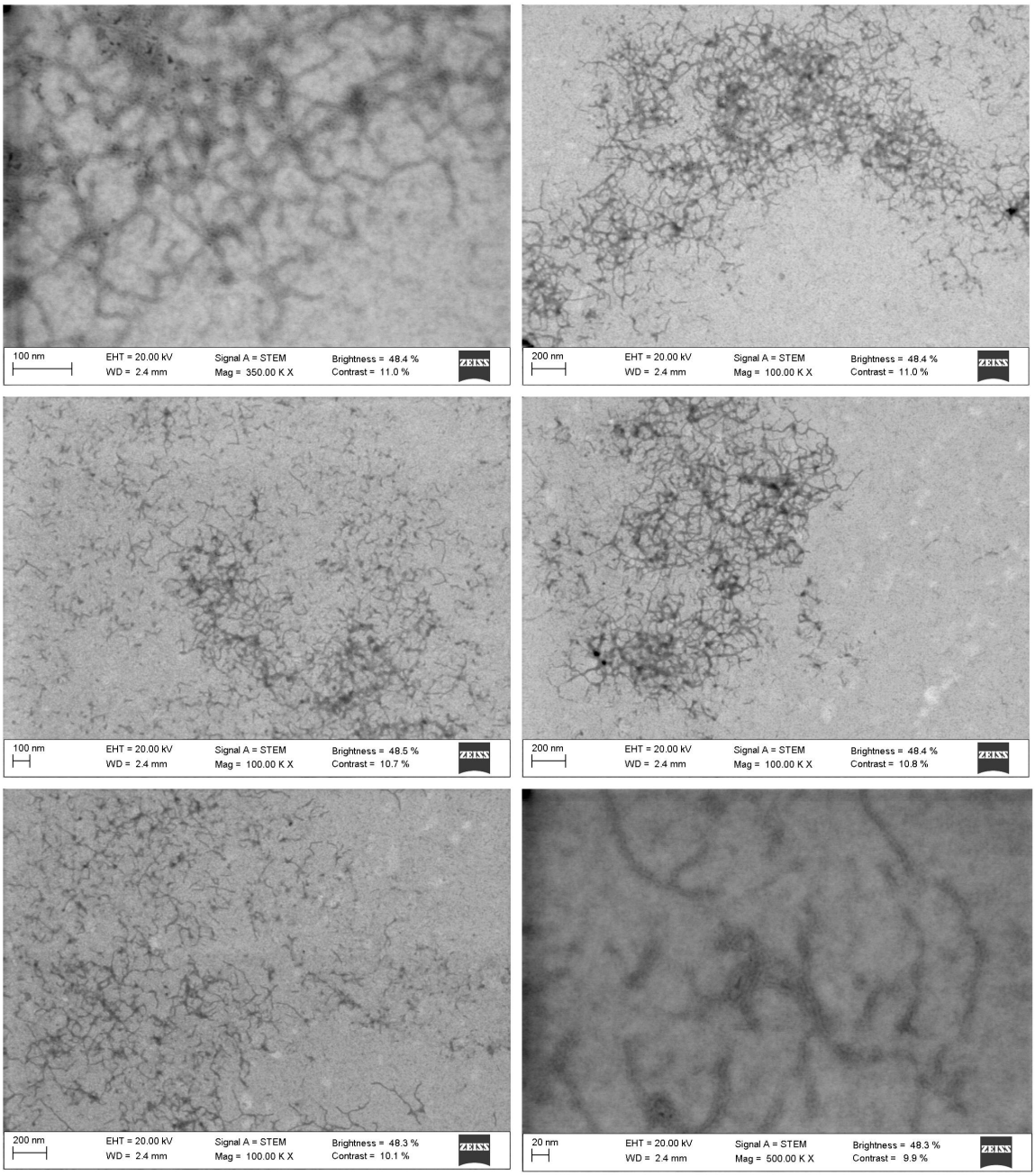


Figure S30: STEM micrographs of dilutions of **2TN** gels (final concentration 11 μM) after staining with uranyl acetate

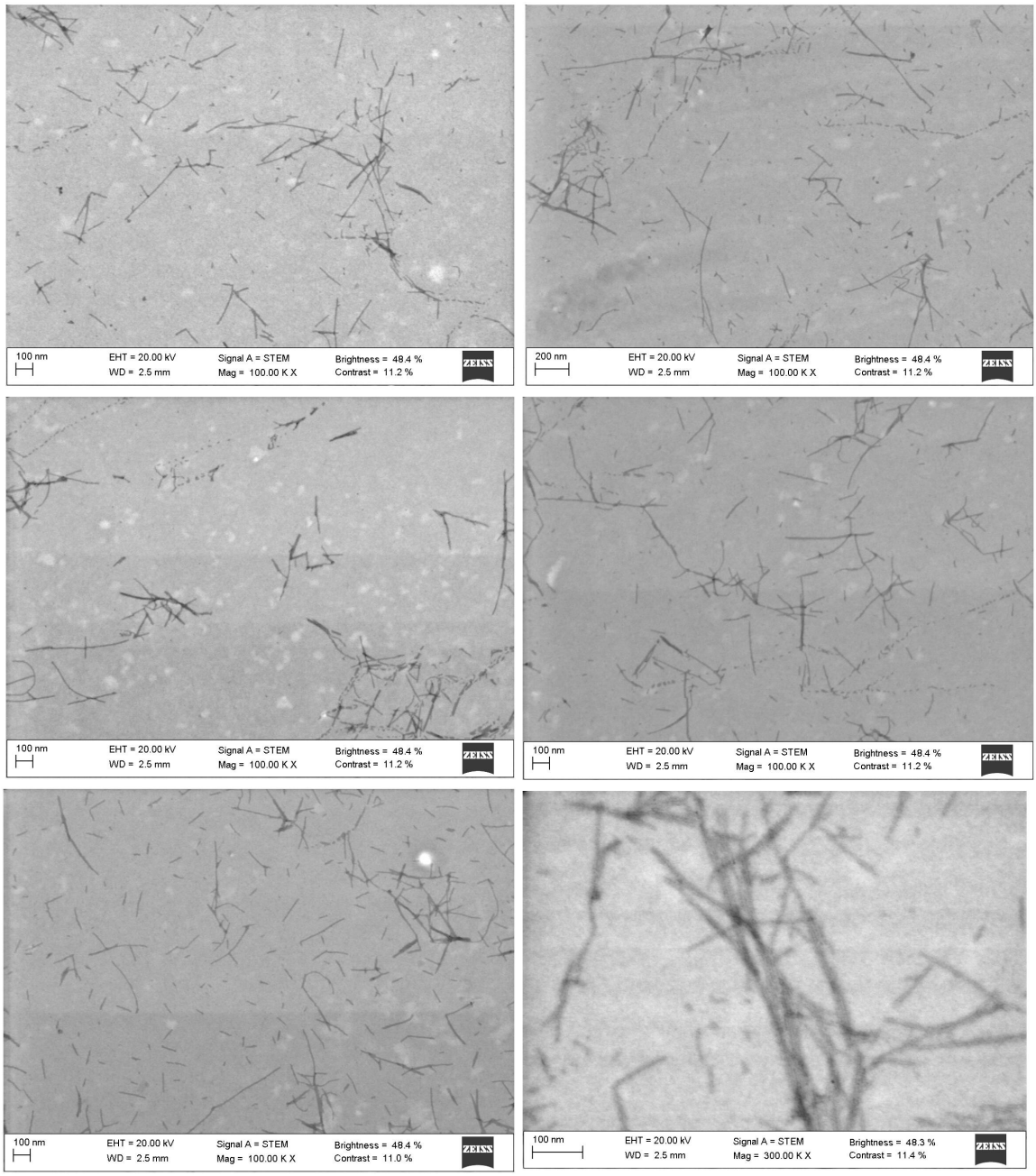


Figure S31: STEM micrographs of dilutions of **2TA** gels (final concentration 11 μM) after staining with uranyl acetate.

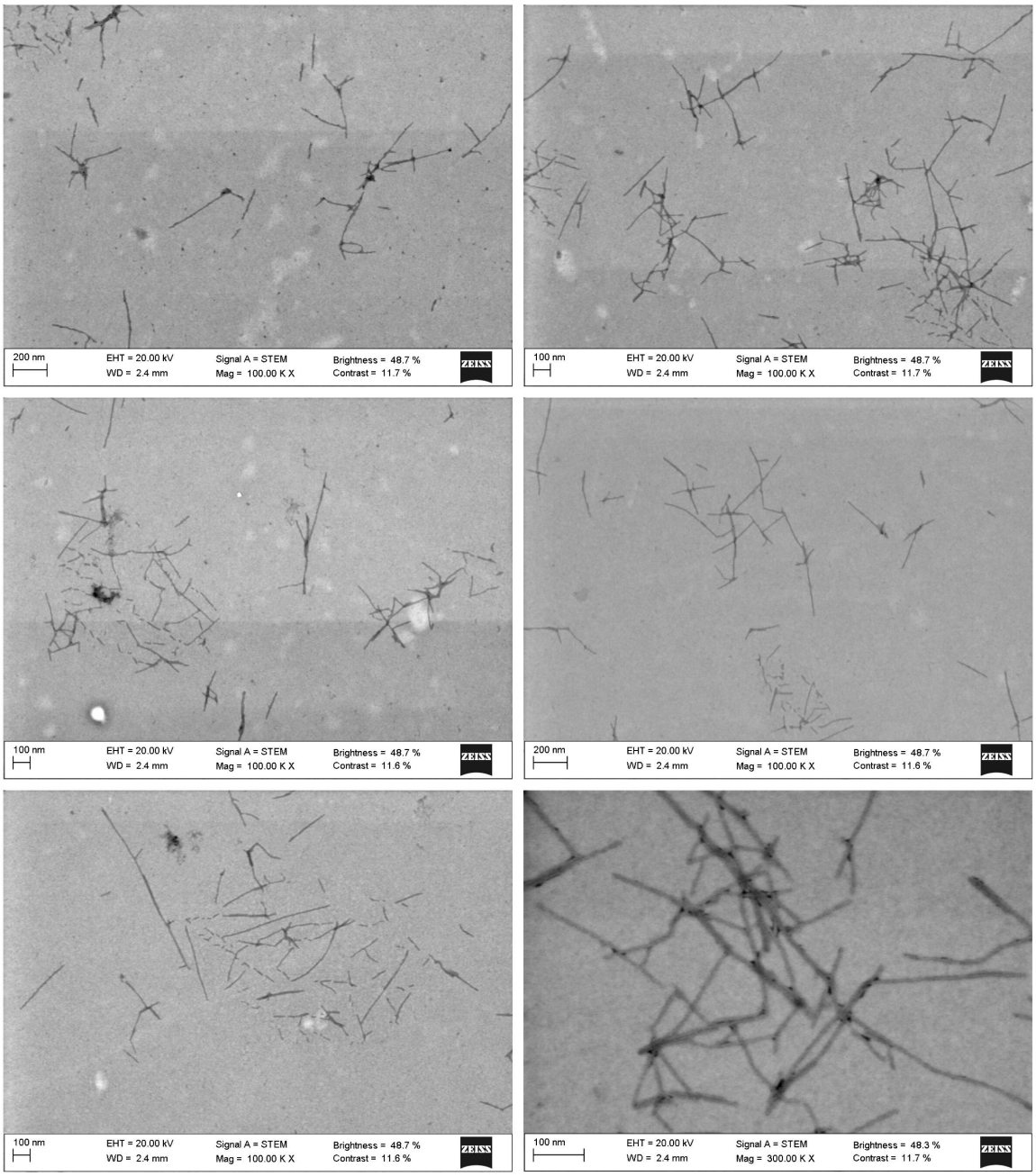


Figure S32: STEM micrographs of dilutions of **2TP** gels (final concentration 11 μM) after staining with uranyl acetate.

S4 Abbreviations

ACN: acetonitrile; CP: Cyclic peptide, 2CTC: 2-Chlorotrityl chloride, DCM: dichloromethane; DIEA: *N*,*N*-Diisopropylethylamine, DMF: *N*,*N*-Dimethylformamide, Fmoc: 9-Fluorenylmethoxycarbonyl; Mtt: 4-Methyltrityl; *N*-HATU: *N*-[(Dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-

N-methyl methanaminium hexafluorophosphate *N*-oxide; *N*-HBTU: *N*-[(1*H*-Benzotriazol-1-yl)- (dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HFIP: 1,1,1,3,3,3-Hexafluoropropan-2-ol; PyAOP: (7-Azabenzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate; TFA: Trifluoroacetic acid; TFE: 2,2,2-Trifluoroethanol; TIS: Triisopropylsilane; UV-vis: Ultraviolet-visible