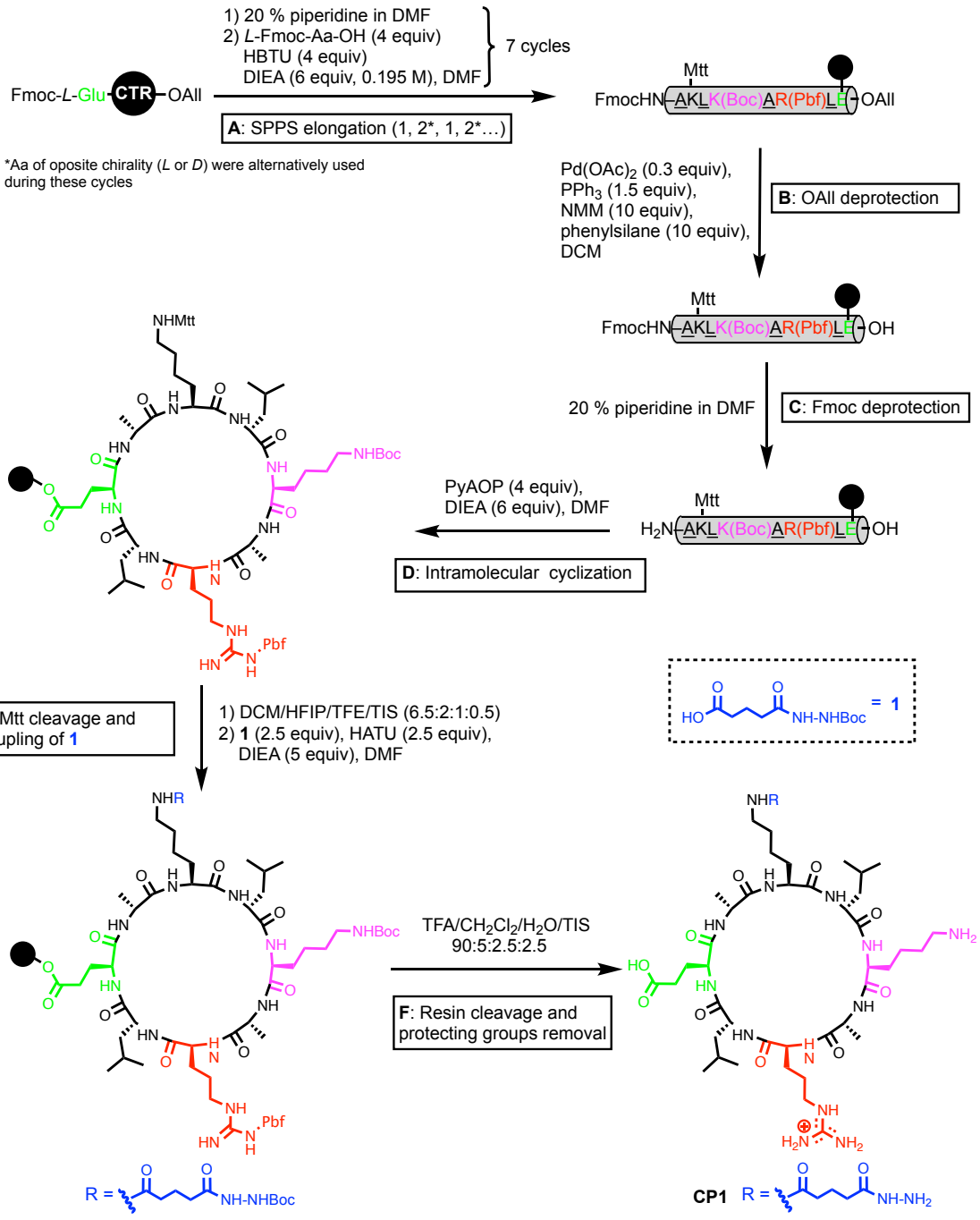
Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

1D alignment of proteins and other nanoparticles by using reversible covalent bonds on cyclic peptide nanotubes.

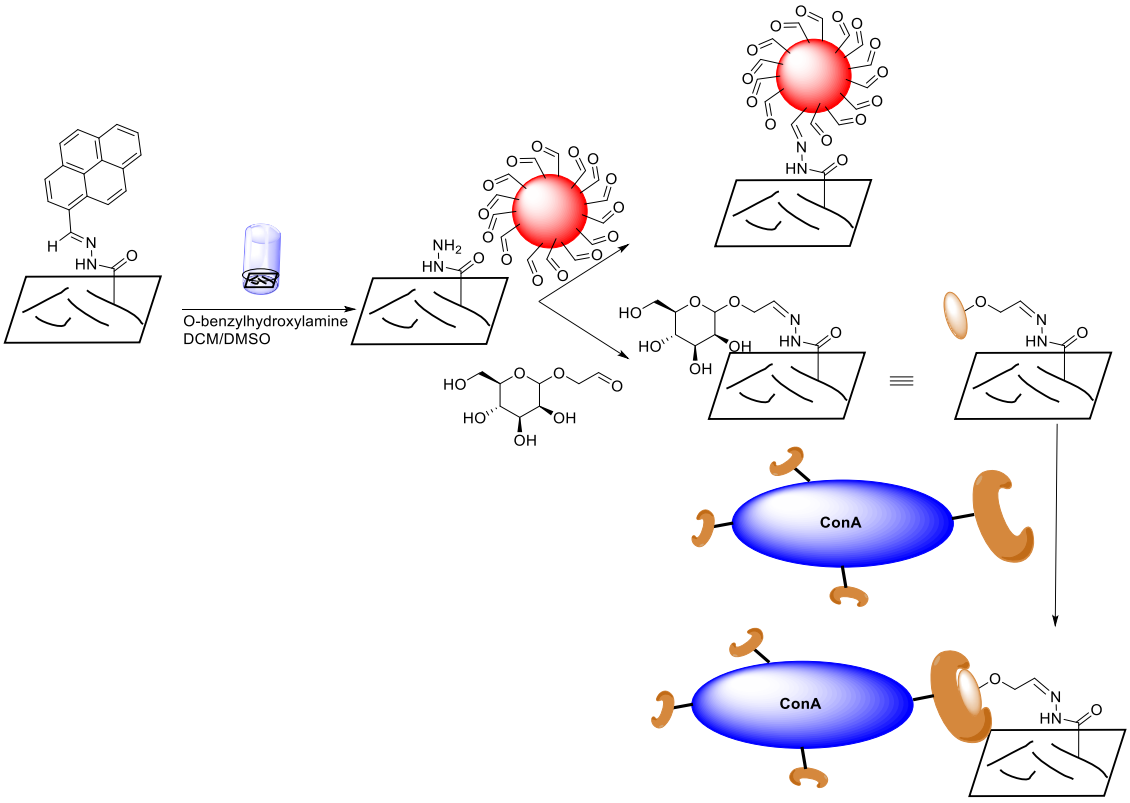
Juan M. Priegue, Iria Louzao, Iván Gallego, Javier Montenegro* Juan R. Granja*

Table of contents

Figures and schemes



Scheme S1. General synthetic scheme for the synthesis of **CP1** peptide. Underlined amino acid letters were used to denote residues of *D*-chirality.



Scheme S2. Chemical transformations carried out onto mica substrate for AFM measurements. Cartoons representing the dendrimer, the lectin Concanavalin A and its carbohydrate binding sites and mannose moiety are not scaled.

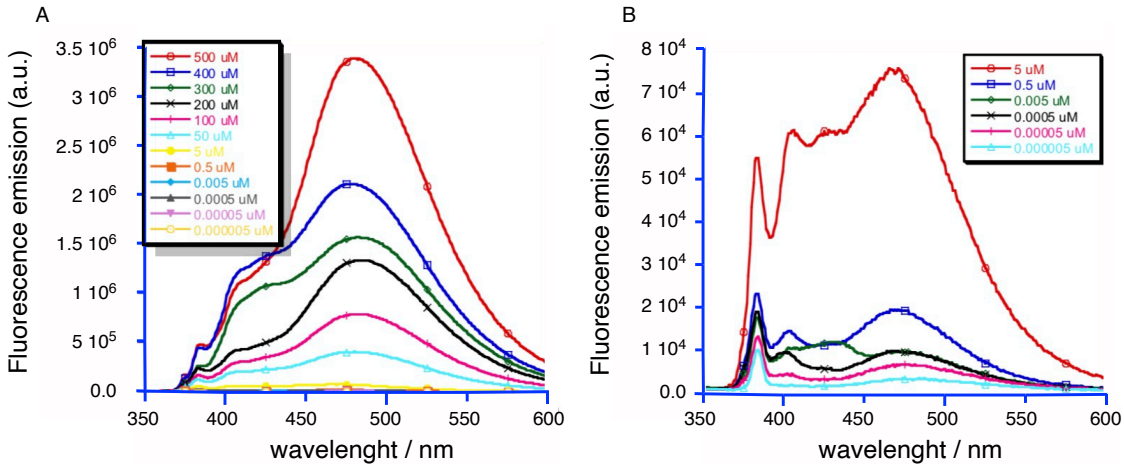


Figure S1. Fluorescence emission graph of the **CP2** solution. A) all concentrations (500 µM to 5.0 nM) and B) concentrations between 5.0 µM and 5 nM.

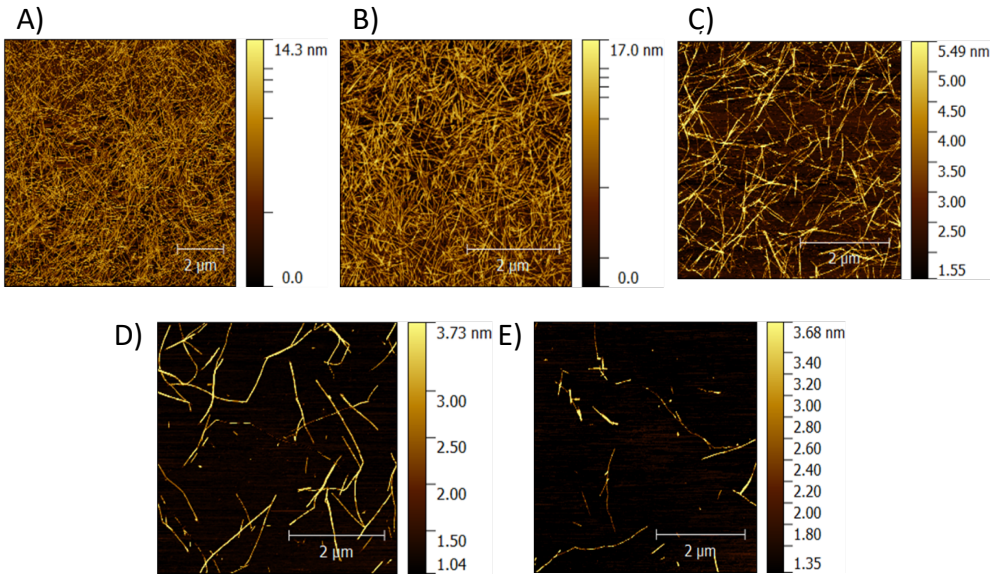


Figure S2. AFM images of **SCPN2UMica** at A) 666 µM, B) 333 µM, C) 66 µM, D) 10 µM and E) 1 µM. The modified peptide was deposited dissolved in ACN/H_2O and thoroughly washed with MilliQ water and dried under nitrogen flow.

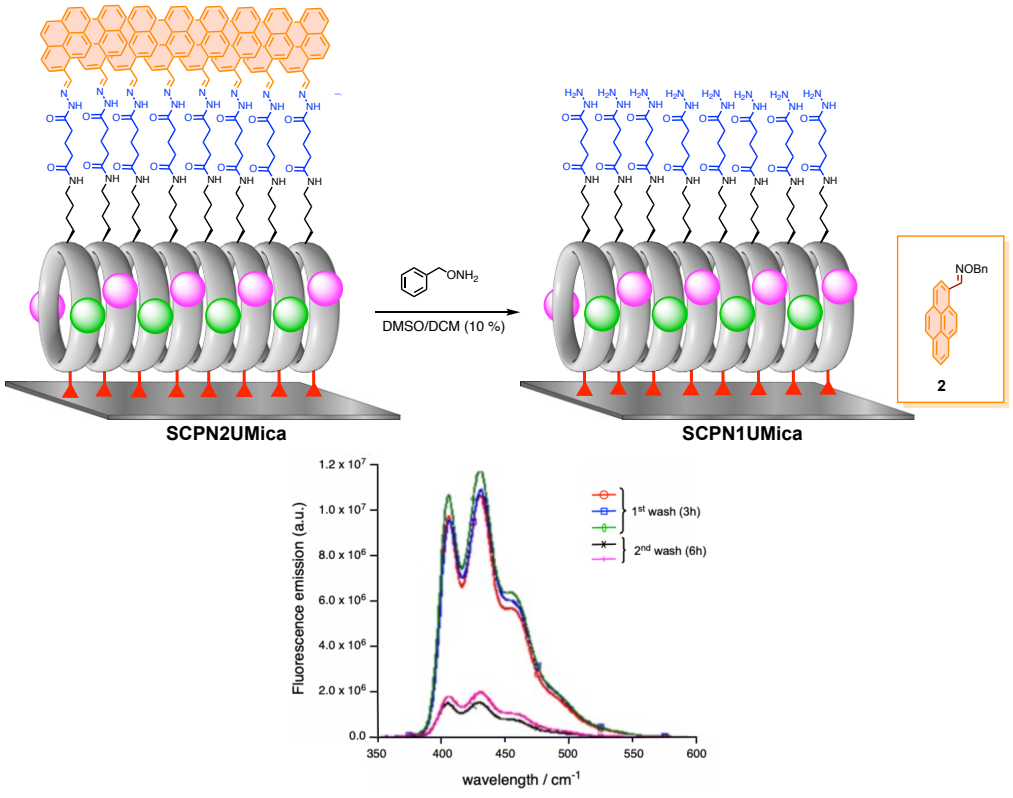


Figure S3. Top, General synthetic scheme for the hydrolysis of **SCPN2UMica**. Bottom, Fluorescence graph of compound **2** (λ = 465 nm) resulting of the reaction of benzylhydroxylamine with **SCPN2UMica**. Measurements of the release of pyrene were carried out, samples (after 3 and 6 h) were extracted from the solutions in which the micas with nanotubes were submerged.

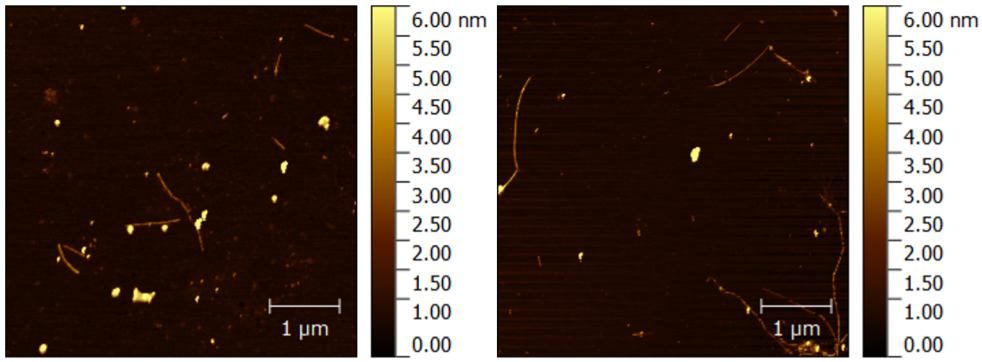


Figure S4. Additional AFM micrographs (5.0 x5.0 mm) of **SCPN1UMica**, resulting of the reaction of **SCPN2UMica** with benzylhydroxylamine.

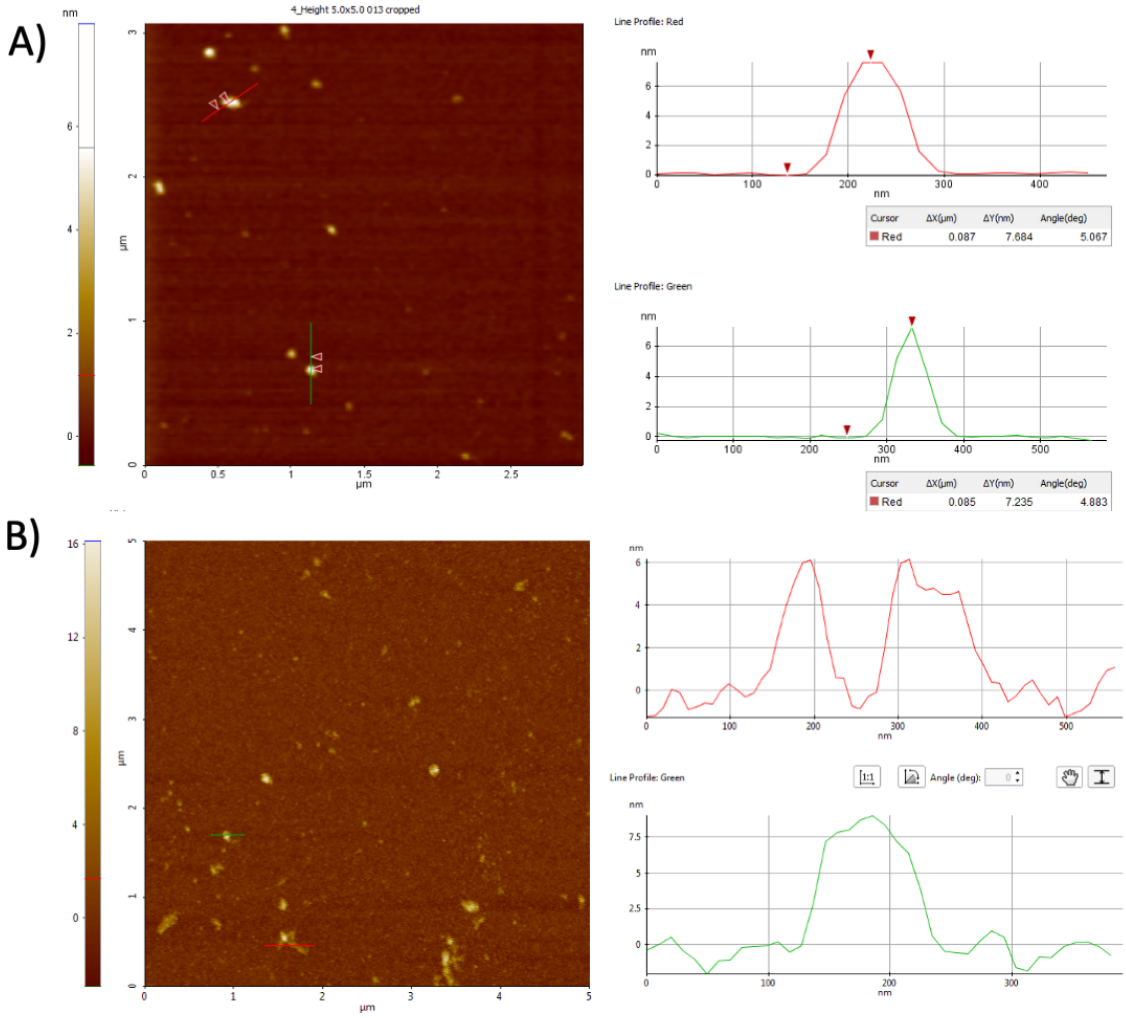


Figure S5. AFM topography micrograph of hydrazide derivative **CP1** (Grade V-I muscovite) from aqueous solutions A) 100 µM and B) 300 uM, the corresponding height profiles along the transects in different colors. The absence of fibre like structures incompatible with peptide nanotubes indicates that the nanotube formation is promoted by the aromatic interactions.

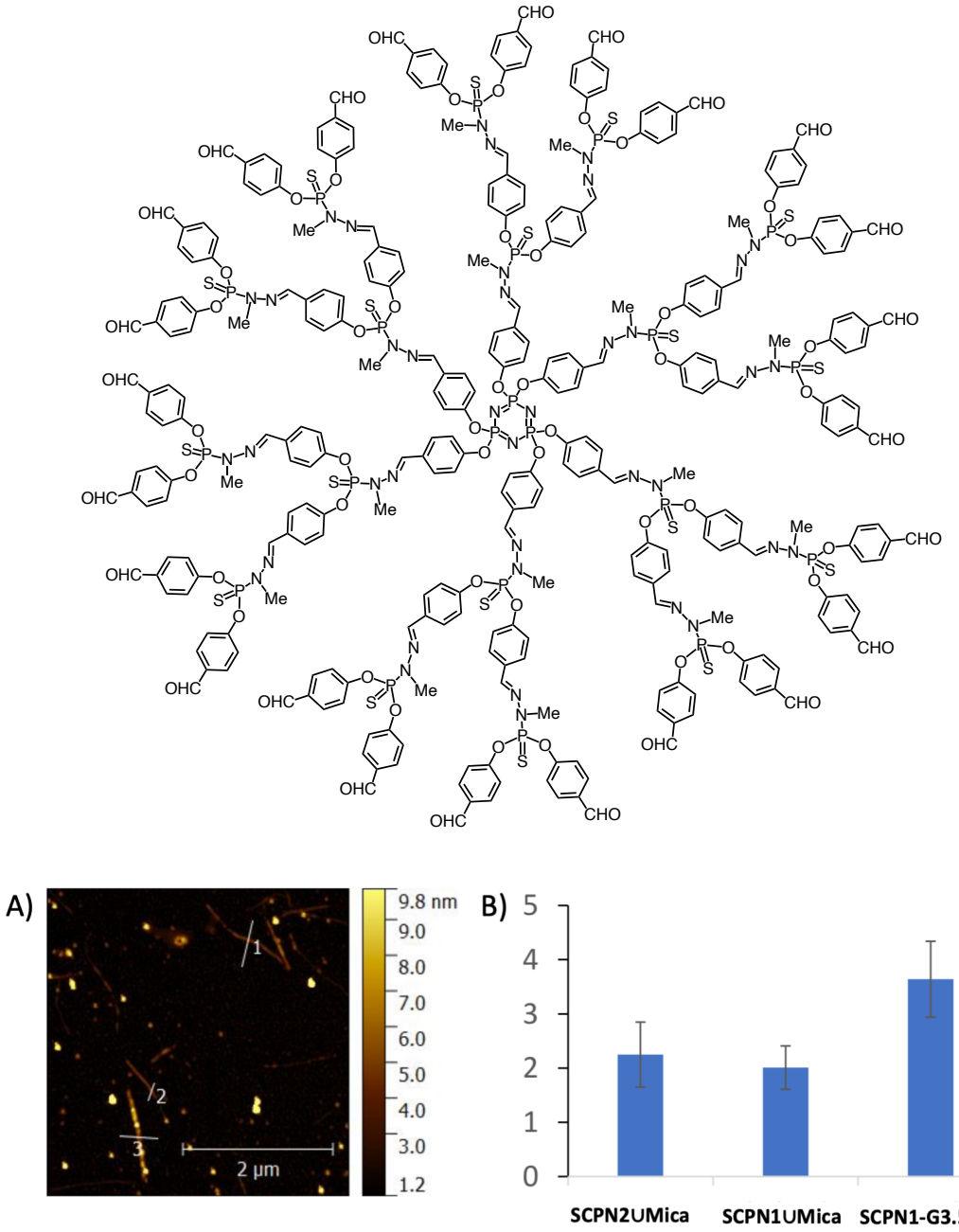


Figure S6. Structure of dendrimer **G3.5** and A) AFM topography micrograph image of **SCPN1-G3.5UMica.** The lines correspond to the profiles used to calculate the average heights. B) Comparison of the average heights of the different modified nanotubes (error bars depict standard deviation).

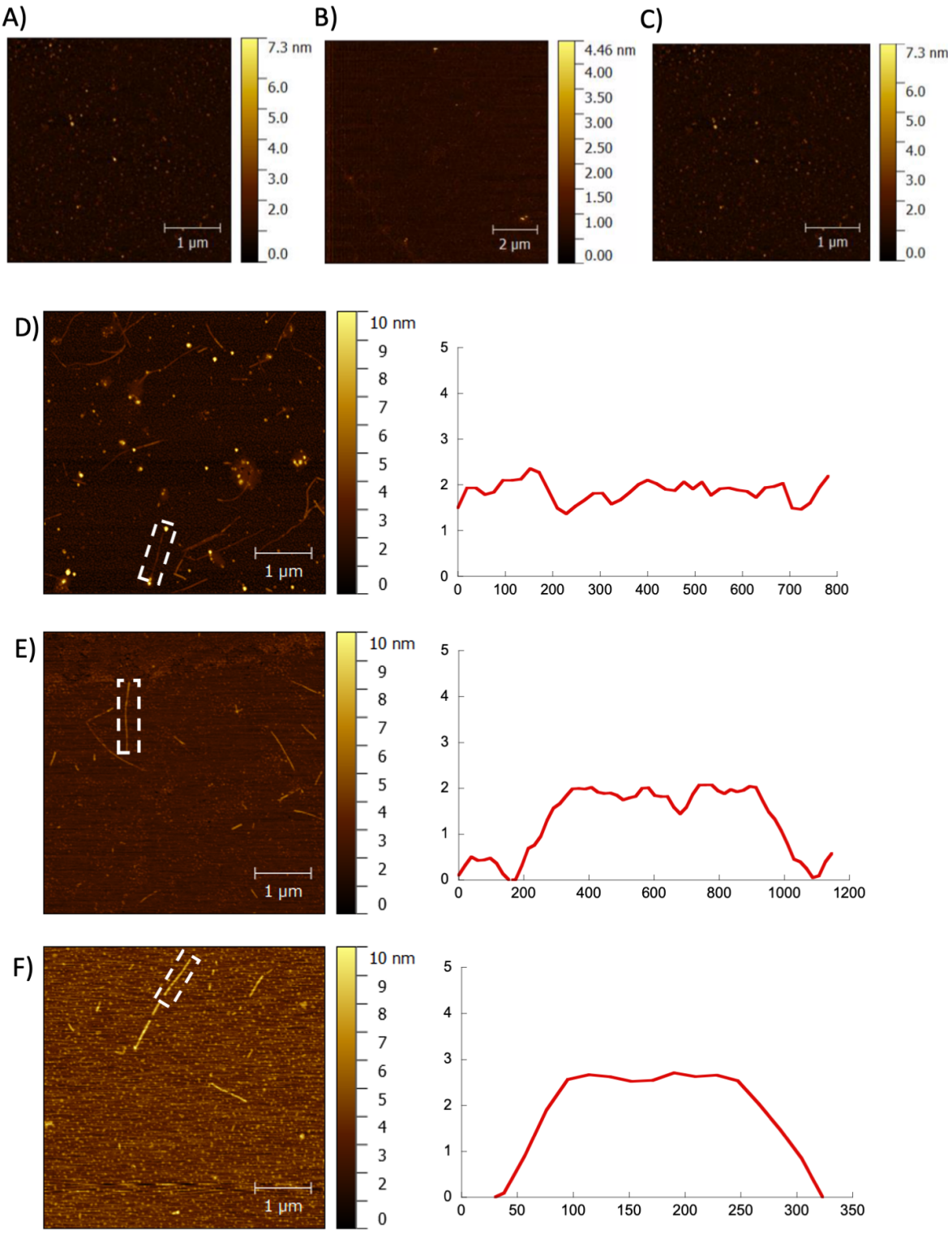


Figure S7. AFM topography micrograph (Grade V-I muscovite) of solutions of: A) **G3.5** in DCM (10 µM), B) **4** in H_2O (5 µM) and C) ConA in H_2O (20 µM). AFM topography micrograph (Grade V-I muscovite) and the corresponding height profiles of the highlighted nanotubes of D) **SCPN2UMica** incubated with **G3.5** (10 µM) in DCM, E) **SCPN2UMica** incubated with **ConA** (20 µM) in H_2O and F) **SCPN1UMica**, resulting of the reaction of benzylhydroxylamine with **SCPN2UMica**, incubated with **ConA** (20 µM) in H_2O.

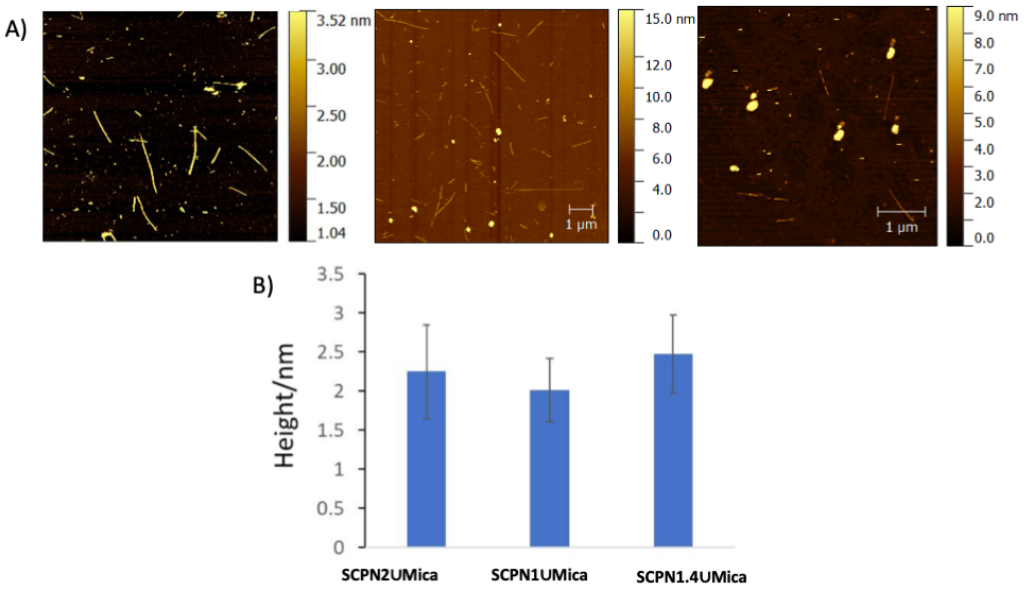


Figure S8. A) AFM topography micrographs (Grade V-I muscovite) of **SCPN1.4UMica**, resulting of the reaction of **SCPN1UMica** with 1-α-formylmethyl-mannopyranoside (**4**) in water (5 µM), and B) comparison of height averages and standard deviation (error bars) of nanotube modifications.

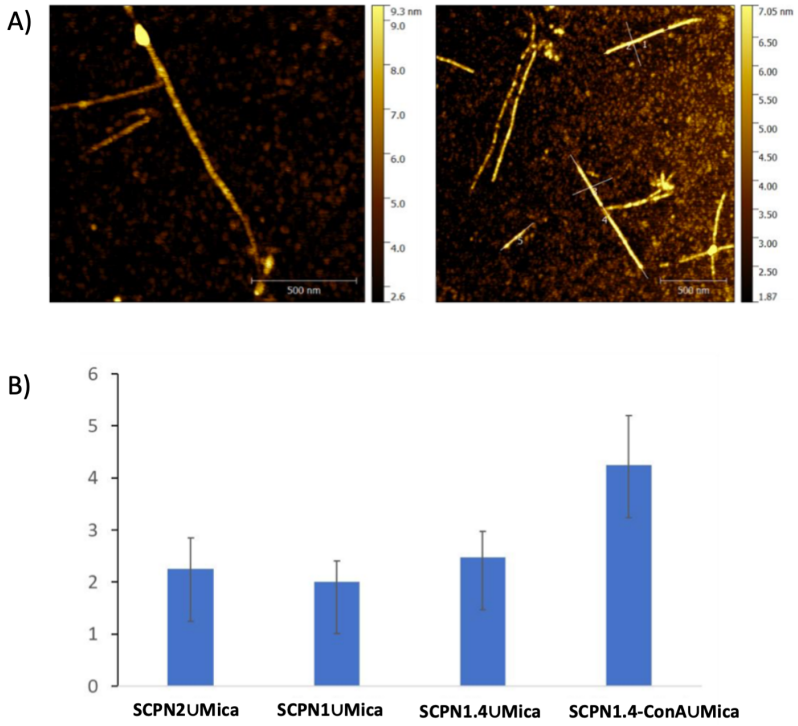


Figure S9. A) AFM topography micrographs (Grade V-I muscovite) of **SCPN1.4-ConAUMica** obtained by incubation of **SCPN1.4 UMica** with a solution of ConA (20 µM) in H_2O. B) Comparison of height averages and standard deviation (error bars) between different nanotubes functionalization. The lines correspond to the profiles used to calculate the average heights.

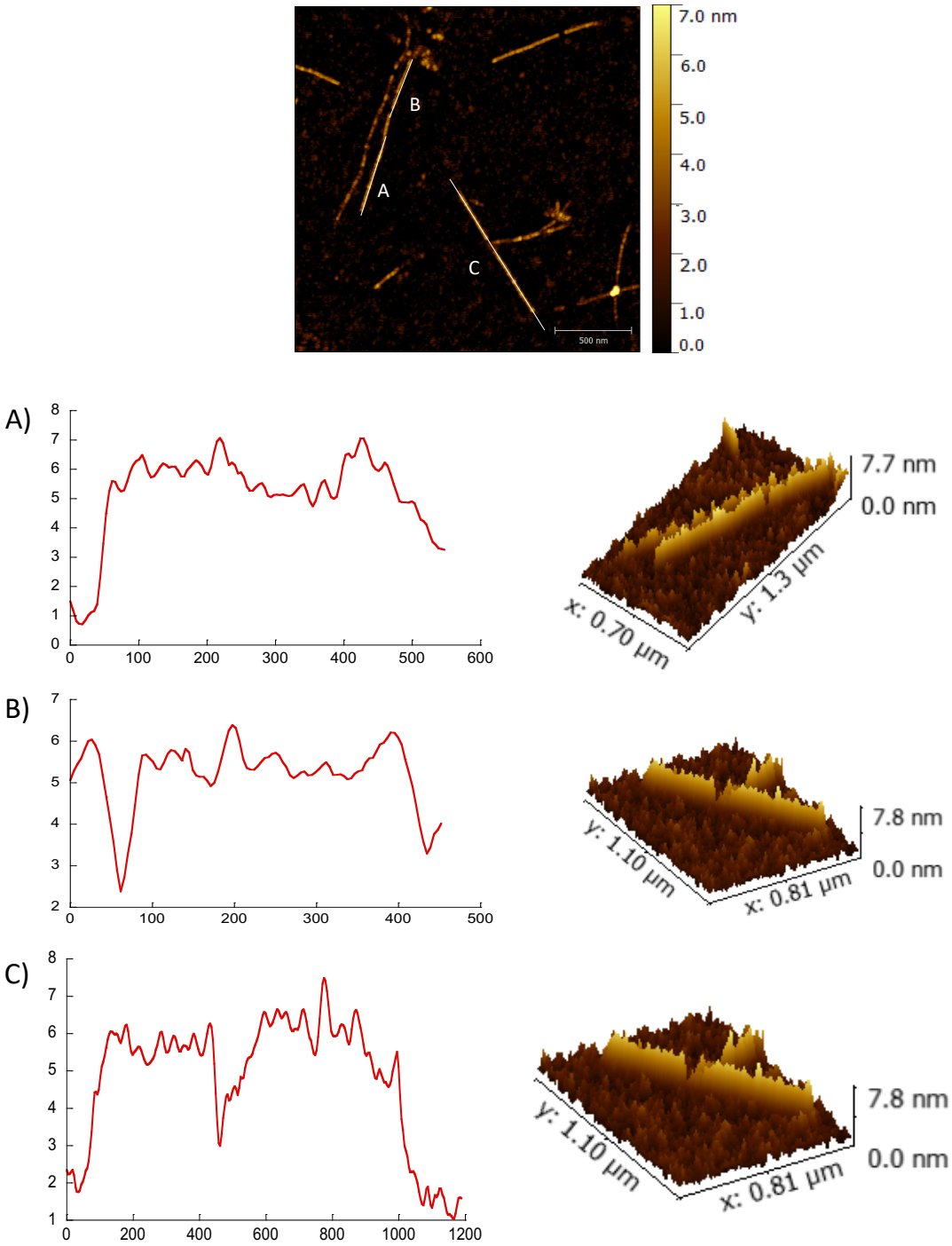


Figure S10. AFM topography micrograph (Grade V-I muscovite) of **SCPN1.4-ConAUMica** and the magnified 3D topographic images and of single nanotube (right) and, corresponding height profiles (left) along the tube longitudinal axis (A, B and C).

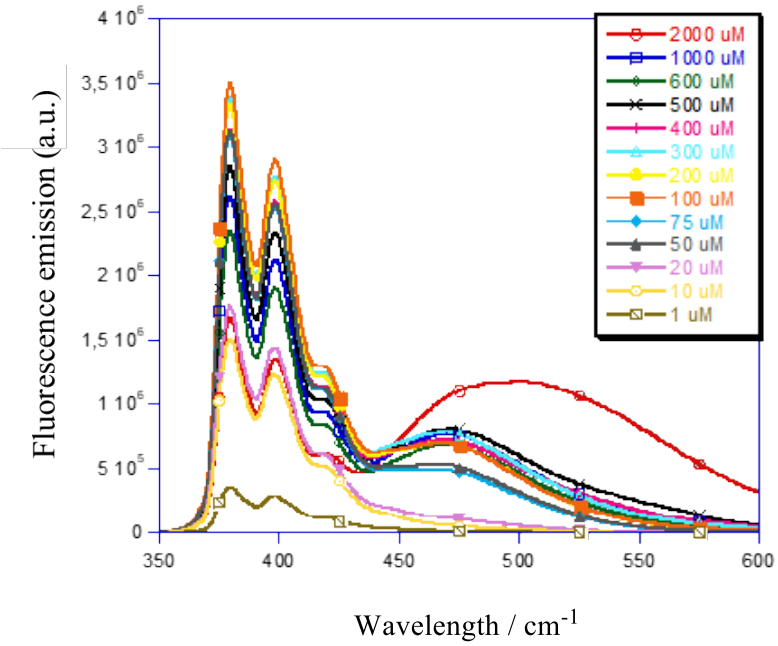


Figure S11. A) Fluorescence graph (λ = 465 nm) of the solution of 1-pyrenecarboxaldehyde at different concentrations.

Table S1. Height and length averages (n = 10) and standard deviations for different nanotubes at different steps of the synthetic modifications performed on mica surface.

	Height / nm	Height SD/ nm	Length/ um	Length SD/ um
SCPN2UMica	2.4	0.4	1.5	1.0
SCPN1UMica	2.2	0.3	1.1	0.4
SCPN1-G3.5UMica	5.8	0.5	1.8	0.7
SCPN1.4UMica	2.6	0.3	1.6	1.0
SCPN1.4-ConA U Mica	6.4	0.6	1.2	0.7

Abbreviations

Aa: Amino acid; AFM: Atomic Force Microscopy; Arg: Arginine; Boc: tert-Butoxycarbonyl; Calcd: Calculated; DEDTC: sodium diethyldithiocarbamate trihydrate; DIEA: diisopropylethylamine; DMF: N,N-Dimethylformamide; DMSO: Dimethylsulfoxide; CTR: Chlorotrityl-Resin; Fmoc: 9-fluorenylmethoxycarbonyl; HFIP: 1,1,1,3,3,3-Hexafluoro-2-propanol; HPLC: High-performance liquid chromatography; HRMS: High resolution mass spectrometry; Leu: leucine; Lys: Lysine; *N*-HBTU: N-[(1H-Benzotriazol-1-yl)4-(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate N-oxide; *N*-HATU: *N*-[(Dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-*N*-methylmethanaminiun hexafluorophosphate *N*-oxide; MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide; PyAOP: [(7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate]; RP: Reverse Phase; SPPS: Solid Phase Peptide Synthesis; TNBS: 2,4,6-trinitrobenzenesulfonic acid; TFE: Trifluoroethanol; TIS: Triisopropylsilane.

Materials and methods

Polystyrene chlorotrityl resin (**CTR**, load 0.71 mmol/g) and Fmoc-L-Lys(Mtt)-OH were purchased from Iris. Fmoc-L-Leu-OH, glutaric anhydride, *tert*-butyl carbazate, triisopropylsilane (TIS) and diisopropylethylamine (DIEA) were obtained from Sigma-Aldrich. Fmoc-L-Arg(Pbf)-OH was purchased from Carbosynth, Trifluoroethanol (TFE) and 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) were purchased from TCI. Peptide synthesis grade *N,N*-dimethylformamide (DMF) was purchased from Scharlau. All other chemicals were purchased from Sigma-Aldrich, TCI or Fisher.

1-Pyrenecarboxaldehyde and thiophosphoryl-PMMH-24 dendrimer, generation 3.5 were purchased from Sigma-Aldrich. (Boc-aminooxy)acetic acid was purchased from TCI and 5-(2-(tert-butoxycarbonyl)hydrazineyl)-5-oxopentanoic acid was prepared as previously described.[1,2] Mannose derived aldehyde, 1-α-formylmethyl-mannopyranoside was synthesised as previously described.[3,4]

A Park NX10 atomic force miscroscope was used for image acquisition (Park Systems) operating in non-contact mode by using ACTA cantilevers (AppNano, nominal spring constant=37 N/m).

High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) analyses were carried out on Agilent Technologies 1260 Infinity II connected to a 6120 Quadrupole LC-MS using an Agilent SB-C18 column with *Solvent A: Solvent B* gradients between 5:95 (*Solvent A*: H_2O with 0.1 % TFA; *Solvent B*: CH_3CN with 0.1 % TFA). High-performance liquid chromatography (HPLC) preparative purification was carried out on Waters 1525 composed by a binary pump with a dual Waters 2489 detector with a Phenomenex Luna C18(2) 100A column. An Agilent 1200 with an Agilent Eclipse XDB-C18 column was used for semi-preparative purification using gradients of 5:95 (*Solvent A*: H_2O with 0.1 % TFA; *Solvent B*: CH_3CN with 0.1 % TFA). Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Mercury 300 MHz spectrometer. Chemical shifts are reported in ppm (δ units) referenced to the following solvent signals: $CDCl_3$ δ H 7.26, D_2O δ H 4.79. Spin multiplicities are reported as a singlet (s), doublet (d), with coupling constants (*J*) given in Hz, or multiplet (m). Accurate mass determinations (HR-MS) using ESI-MS were performed on a Sciex QSTAR Pulsar mass spectrometer and are reported as mass-per-charge ratio (*m/z*).

Synthesis of CP (Scheme 1S)

Attachment of Fmoc-Glu-OAll to CTR-resin. A solution of L-Fmoc-Glu-OAll (205 mg, 0.50 mmol) in dried DCM (4 mL) and DIEA (350 µL, 2.00 mmol) was stirred at r.t. for 5 min. The mixture was added over chlorotrityl chloride (**CTR**) resin (500 mg, 0.50 mmol), previously swollen and washed (dried DCM, 4 mL). The resulting suspension was mechanically stirred for 2h. Finally, the resin was filtered and washed with DCM (3x4 mL, 3 min), DCM/MeOH/DIEA mixture (17:2:1, 3x4 mL, 10 min), again with DCM (3x4 mL, 3 min), DMF (3x4 mL, 3 min) and DCM (3x4 mL, 3 min) to give the desired L-Fmoc-Glu(CTR)-OAll. The resin loading (0.73 mmol/g) was calculated using Fmoc test.[5]

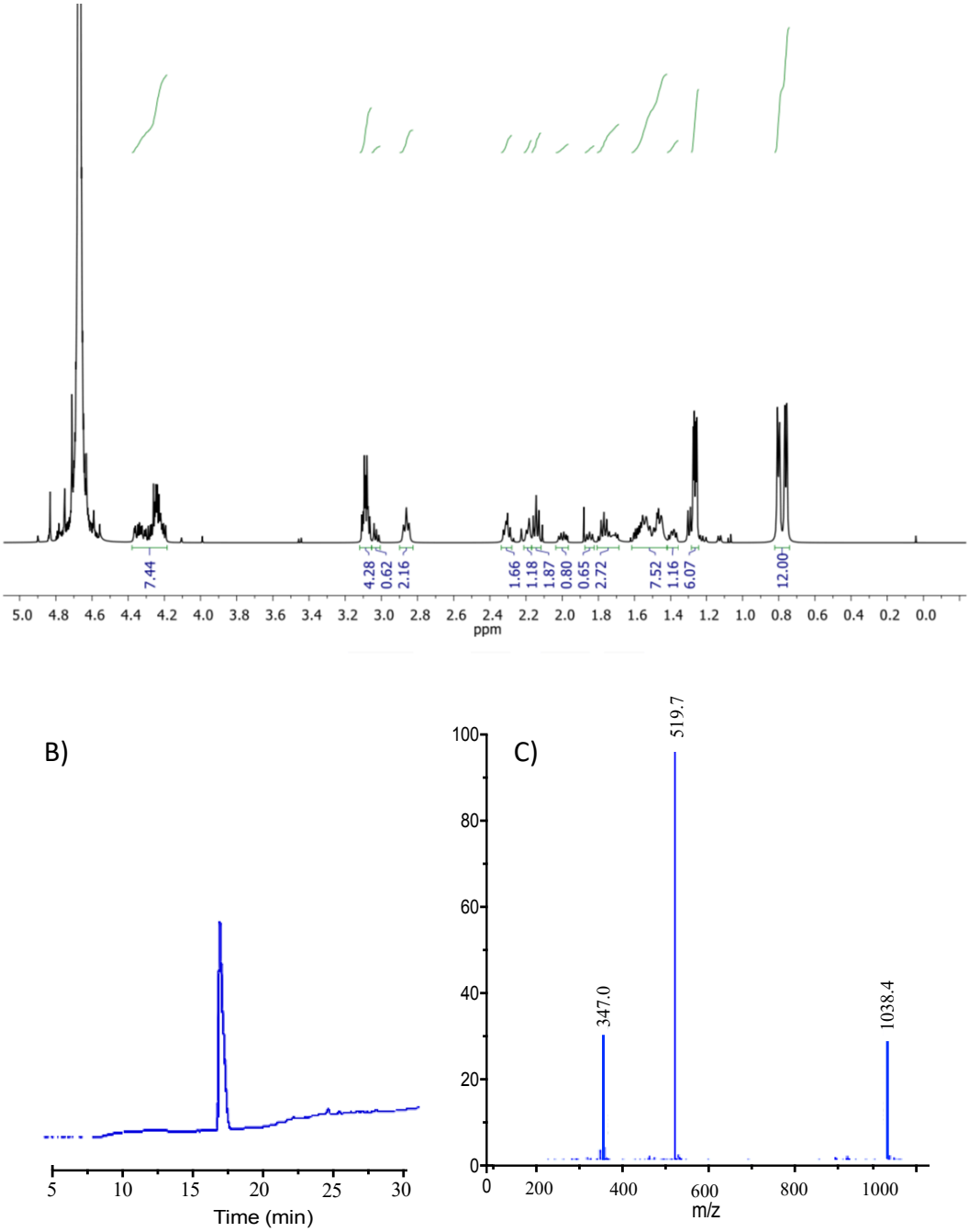
Solid phase synthesis: **CP1** was synthesized by manual Fmoc solid phase peptide synthesis from L-Fmoc-Glu(CTR)-OAll (0.73 mmol/g). The resin was weighed in a peptide synthesis vessel. Coupling cycles consisted of Fmoc group cleavage with 20% piperidine

in DMF (2x5 mL, 15 min) and DMF wash (3x3 mL, 1 min) followed by amino acid coupling with Fmoc-α-aminoacids (4 equiv.), N-HBTU (4 equiv.) and DIEA (6 equiv.) for 45 min and finally washed with DMF (3x3mL, 1 min). Each coupling and deprotection cycle was monitored by employing the 2,4,6-trinitrobenzenesulfonic acid (TNBS) test.[6] Fmoc-D-Ala-*L*-Lys(Mtt)-*D*-Leu-*L*-Lys(Boc)-*D*-Ala-*L*-Arg(Pbf)-*D*-Leu-*L*-Glu-(CTR)-OAll was swollen in DCM (4 mL, 15 min), then treated at r.t. for 12h with a deoxygenated mixture of $Pd(OAc)_2$ (0.3 equiv.), PPh_3 (1.5 equiv.), N-methylmorpholine (NMM) (10 equiv.) and phenylsilane (10 equiv.) in DCM (4 mL). Finally, the resin was filtered and washed with DCM (3x4 mL, 1 mL), DIEA in DMF (2%, 2x4 mL, 1 min), sodium diethyldithiocarbamate trihydrate (DEDTC) solution in DMF (0.5 %, 3x5 mL, 3 min), DMF (3x4 mL, 1 min) and DCM (3x4 mL, 1 min) to give the corresponding C-terminal free Fmoc-*D*-Ala-*L*-Lys(Mtt)-*D*-Leu-*L*-Lys(Boc)-*D*-Ala-*L*-Arg(Pbf)-*D*-Leu-*L*-Glu(CTR)-OH. Removal of Fmoc protecting group was performed by treatment with piperidine in DMF (20%, 4 mL, 30 min), and then washed with DMF (3x2 mL, 1 min). The resulting H_2N-*D*-Ala-*L*-Lys(Mtt)-*D*-Leu-*L*-Lys(Boc)-*D*-Ala-*L*-Arg(Pbf)-*D*-Leu-*L*-Glu(CTR)-OH was treated at r.t. for 12h with PyAOP (4 equiv.) and DIEA (6 equiv.), and then washed with DMF (3x3 mL, 1 min).

The methyltrityl (Mtt) protecting group was selectively removed by mechanically stirring the resin in a mixture of DCM/HFIP/TFE/TIS (6.5:2:1:0.5, 2x3 mL, 3h). After Mtt elimination, the resin was washed with DCM (3x3 mL, 1 min) and DMF (3x3 mL, 10 min). A solution of the hydrazide derivative **1** (2.5 equiv. per lysine residue, **Fig. S1**) and *N*-HATU (2.5 equiv. per lysine residue) dissolved in DMF (1 mL) was added to the resin followed by the dropwise addition of a solution of a DIEA (8 equiv.) in DMF (1 mL) for 30 min. The peptide was deprotected and cleaved off from the resin by standard TFA cleavage procedure at rt (TFA/DCM/H_2O/TIS, 90:5:2.5:2.5, 2h). Then, the mixture was filtered, and the peptide was precipitated in ice-cold Et_2O. The suspension was centrifuged and washed with Et_2O (2x10 mL).

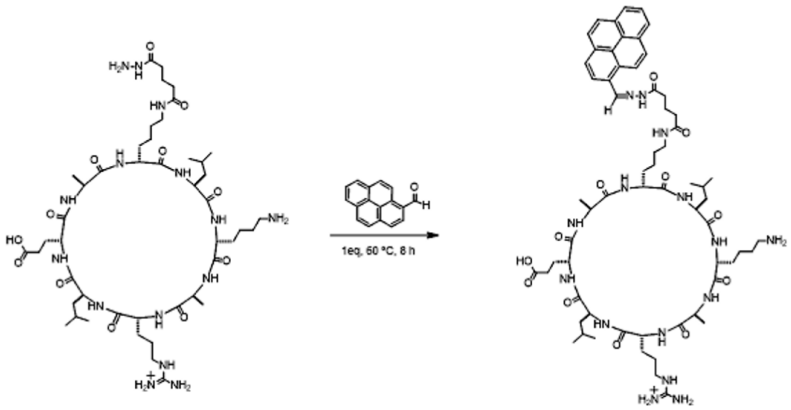
Crude peptide was dissolved in ultrapure H_2O (3 mL), purified using a C18 reverse-phase HPLC [Nucleosil 100-7 C18, H_2O (0.1% TFA)/CH_3CN (0.1% TFA) with a binary gradient of *Solvent A* and *Solvent B*, the collected fractions were lyophilized and stored at -20°C. Purity and characterization were confirmed by analytical HPLC. R_t = 16.8 min. RP-HPLC [Nucleosil 100-7 C18, H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (5→35 min), 0:100

(>35 min)]. **MS** (ESI-TOF, H_2O): 1038 (30, $[M+H]^+$); 520 (100, $[M+2H]^{2+}$); 347 (30, $[M+3H]^{3+}$); **^{1}H-NMR** (D_2O, 500 MHz), δ(ppm): 4.40-4.17 (m, 8H), 3.14-2.99 (m, 4H), 2.89-2.84 (m,2H), 2.36-2.25 (m, 2H), 2.21-2.11 (m, 3H), 2.04-1.93 (m,1H), 1.90-1.65 (m, 2H), 1.60-1.37 (m, 7H), 1.27 (dd, *J* = 7.2 and 3.0 Hz, 6H), 0.8 (d, *J* = 5.5 Hz, 6H), 0.76 (d, *J* = 5.5 Hz, 6H). **HRMS** (ESI): Calculated for $C_{46}H_{84}N_{15}O_{12}$: 1038.6421; found 1038.6475.



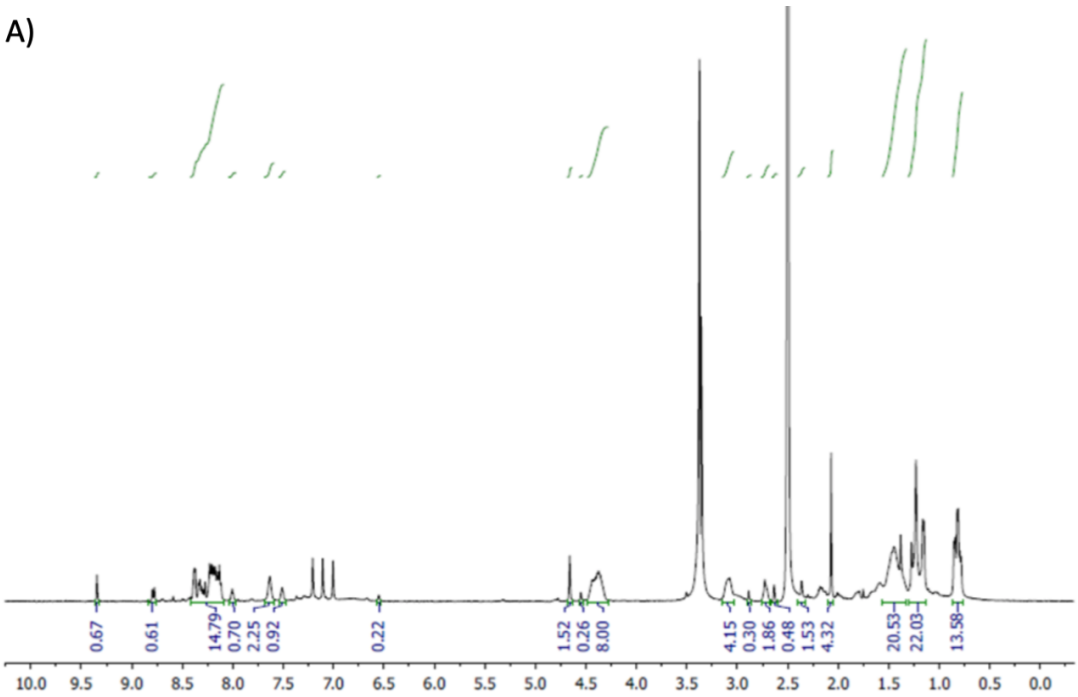
A) ^{1}H-NMR of **CP1** in D_2O, B) RP-UHPLC [SB 100-7 C18, H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (5→35 min), 0:100 (>35 min)]. R_t 16.8 min; C) ESI-MS of **CP1**.

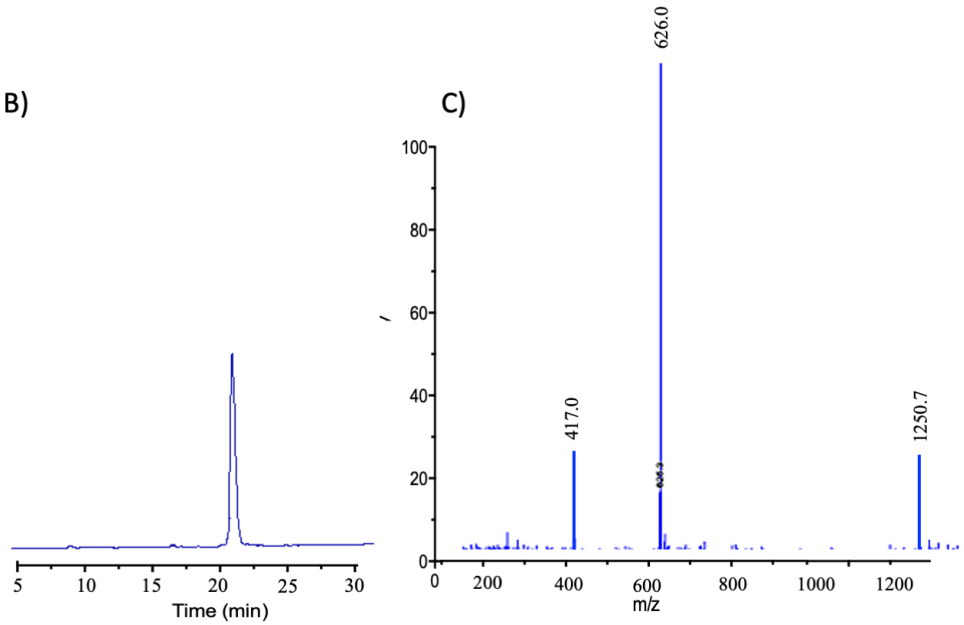
Preparation of pyrene hydrazone derivative CP2



CP1 was dissolved in a solution of AcOH (2 mM, 50 µL pH=3.5) and mixed with a solution of one equivalent of 1-pyrenecarboxaldehyde (2mM, 50 µL, 3:2 ACN:H_2O) and adjusted to a total volume of 150 µL with DMSO. The mixture was stirred at 60°C for 6h, and the resulting hydrazone was then characterised and used for further experiments. **CP2** was checked by HPLC and confirmed by ESI-MS. R_t = 20,7 min. RP-HPLC [Nucleosil 100-7 C18, H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (5→35 min), 0:100 (>35 min)]. **^{1}H-NMR** (D_2O, 500 MHz), δ(ppm): 9.3 (s, 1H), 8.78 (d, 1H, *J*=8.8), 8.42-8.10 (m, 15H), 8.05-7.95 (m, 1H), 7.70-7.60 (m, 2H), 7.55-7.47 (m, 1H), 4.65 (s, 2H), 4.50-4.30 (m, 8H), 3.2-3.0 (m, 4H), 2.77-2.68 (m, 2H), 1.30-1.10 (m, 22H), 1.55-1.30 (m, 20H), 0.90- 0.70 (m, 12H). **MS** (ESI-TOF, H_2O): 1251 (20, $[M+H]^+$); 626 (100, $[M+2H]^{2+}$); 417 (20, $[M+3H]^{3+}$); **HRMS** (ESI): $C_{63}H_{92}N_{15}O_{12}$: 1251,0925; found: 1251,0927.

A)





A) ^{1}H-NMR of **CP2** in D_2O, B) RP-UHPLC [SB 100-7 C18, H_2O (0.1% TFA)/CH_3CN (0.1% TFA) 95:5→5:95 (5→35 min), 0:100 (>35 min)]. R_t 20.7 min; C) ESI-MS of **CP2.**

Mica deposition of CP2 and Atomic Force Microscopy (AFM)

AFM measurements were conducted at ambient atmosphere at room temperature using a Park NX10 instrument (Park Systems Corporation) in non-contact mode. High-resonance non-contact cantilevers (ACTA probe, v = 330 kHz) of nominal spring constant of 37 N/m were used for imaging. Samples were prepared by pipetting 30 µL of the corresponding peptide solution (ACN/H_2O 3:2) at every stage of the synthetic modifications (**Scheme 2S**) onto freshly exfoliated mica sheet (SPI Supplies, grade V-1 Muscovite) and, after 1 min, the remaining solution was removed, thoroughly washed with Milli-Q water, and dried under nitrogen flow. Unless otherwise stated, all the AFM samples were prepared by following this procedure. **SCPN2UMica** samples prepared at different concentrations (333 µM-1 µM) are depicted in **Fig. S2**.

Hydrolysis of SCPN2UMica to obtain SCPN1UMica

A mica substrate containing **CP2** nanotubes (**SCPN2UMica**, 10 µM) was placed in a vial prior to add a solution of O-benzylhydroxylamine [100 mM, DCM:DMSO (9:1), 1mL] for 24h at r.t. under gently stirring to obtain **SCPN1UMica**. Then, the mica was washed with DCM:DMSO (9:1, 3x1 mL). The specimen was visualised and measured by AFM upon

drying as previously described. The supernatant solution containing the formed oxime was measured by fluorescence in order to check the pyrene cleavage (**Fig. S3**).

Nanotube functionalization with G3.5: SCPN1-G3.5∪Mica

A solution of thiophosphoryl-PMMH-24 dendrimer in DCM (**G3.5**, 10 µM, 1 mL) was deposited on mica containing **SCPN1∪Mica**. The free hydrazides were left to react for 2h at r.t. The mica was then washed with DCM (3x3 mL) and measured by AFM (**Fig. S6**).

Nanotube functionalization with 4: SCPN1.4∪Mica

A solution of aldehyde derived mannose (1-α-formylmethyl-mannopyranoside, 5 µM, 1 mL, H_2O) was added onto a mica substrate with deposited hydrolysed nanotubes for 6 hours. The mica was washed with H_2O (3x3 mL) and measured by AFM (**Fig. S8**).

ConA interaction: SCPN1.4-ConA∪Mica

The mica was added the solution of ConA (20 µM) in HEPES-buffered Krebs-Ringer solution (HKR, 30 µL, 5mM HEPES, 137 mM NaCl, 2,68 mM KCl, 2,05 $MgCl_2$, 1,8 mM de $CaCl_2$, pH = 7,4) for 1h and washed with H_2O (3x3 mL). The resulting experiment was imaged and measured by AFM (**Fig. S9-S10**).

Nanotubes statistical analysis of height and length

AFM images were measured by using Gwiddion 2.44 software. Data of ten nanotubes (n=10) from at least, 5 different images (typically 6-8 images) were used. Height and length averages and the corresponding standard deviations are shown in **Table S1**.

Supporting references

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