SUPPORTING INFORMATION

1. General procedures and materials

a) Reagents and instruments

All reagents and solvents were obtained from commercial sources and were used without further purification. The modified amino acids Fmoc-*L*-Lys[Boc-*L*-Ser(OtBu)]-OH,[1] Fmoc-*L*-Lys[N-4-pentynoic acid]-OH,[2] and Fmoc-*L*-Glu[NHNH(2Cl-Trt)]-OH,[3] were synthesized following the cited literature procedures.

The Fmoc-protected hydrazine resin (0.8 mmol/g) was prepared from the 2-chlorotrityl chloride resin (loading; 1.60 mmol Cl/g) as previously described.[4,5]

The non-modified peptides **A** and **H** were synthesized as previously described.[3]

EGFP mRNA, CleanCap® Enhanced Green Fluorescent Protein mRNA (capped with CleanCap AG, substituted with 5-methoxy-U) was purchased from TriLink BioTechnologies (San Diego, USA).

Nuclear magnetic resonance spectroscopy (NMR). ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz for ^{1}H and 101 or 126 MHz for ^{13}C (Bruker Avance 400/500 instruments) in deuterated solvents. Peaks were referenced in ppm with respect to the residual solvent peak. Data are reported as follows: chemical shift (δ in ppm), multiplicity (s for singlet, d for doublet, t for triplet, m for multiplet), coupling constant (*J* in Hertz), and integration.

High-performance liquid chromatography (HPLC). Analytical reverse-phase HPLC (RP-HPLC) analyses were performed on a Thermo Scientific™ - UltiMate™ 3000 UHPLC system equipped with a Thermo Scientific™ Hypersil GOLD™ aQ C18 Polar Endcapped HPLC Column 25302-052130, (1.9 µm, 2.1 x 50 mm) column and a Thermo Scientific™ Dionex™ UltiMate™ DAD 3000 detector using the following linear gradients (Solution A: 99.9% Water, 0.1% TFA; Solution B: 99.9% Acetonitrile, 0.1% TFA). Method **[HPLC]:** 95% A (5% B) to 0% A (100% B) in 5 minutes, then up to 10 min at 100% B; flow: 0.5 mL/min.

Retention times (t_R) are given in minutes.

Preparative HPLC (Prep-HPLC) was performed on a Gilson® PLC 2250 Purification System equipped with a UV-Vis Glison® DAD detector and using the following solutions (Solution A: 99.9% Water, 0.1% TFA; Solution B: 99.9% Acetonitrile, 0.1% TFA). The purification system was equipped with a:

i) **[Prep-HPLC]** WATERS™ XSelect™ CSH C18 OBD Prep Column 186005493, (130Å, 5 µm, 30 nm X 250 nm)

ii) **[Semiprep-HPLC]** Macherey-Nagel® VP HPLC Column 250/10 NUCLEODUR C18 Htec 762566.100 (110Å, 7 µm, 10 nm X 250 nm).

Liquid chromatography-mass spectrometry (LC/MS). Analyses were performed on a Shimadzu LCMS2020 (Phenomex Kenetex C18, 2.6 µm x 7.5 cm, 100Å) equipped with a SPD-M20A detector with the following linear gradient of solvent B (99.9% acetonitrile, 0.1% HCOOH) and solvent A (99.9% water and 0.1% HCOOH): 5 to 95% of solvent B in 5 min; flow 1 ml/min. Retention times (t_R) are given in minutes.

ESI/ASAP mass spectrometry. Analyses were carried out at the Laboratoire de Mesures Physiques, IBMM, Université de Montpellier.

b) Solid Phase Peptide Synthesis (SPPS)

All peptide syntheses were based on a Fmoc strategy and were carried out manually at room temperature. Scales used varies from 0.2 to 0.37 mmol.

1) C-terminal amide peptides were synthesized from AmphiSpheres™ rink amide resin (loading 0.34 mmol/g)
2) C-terminal hydrazide peptides were synthetized from modified Fmoc-hydrazine resin.

The Fmoc-hydrazine resin (0.6 - 1.00 mmol/g, depending on the yield of resin modification) was prepared from the commercial 2-chlorotrityl chloride resin (loading: 1.60 mmol Cl/g) as previously described.[4,5]

3) C-terminal carboxylic acid peptides were synthesized from 2-chlorotrityl chloride resin (loading 1.60 mmol/g)

Prior to peptide synthesis, the loading of the resin was calculated after loading of the first amino acid of the sequence as previously descibred.[6,7] Briefly, 2-chlorotrityl chloride resin (1 eq.) was suspended in a solution of DMSO/NMP (8/2), then DIEA (4 eq.) was added, then the suspension was gently stirred. Next, Fmoc-*L*-Arg(Pbf)-OH (1 eq.) was added in one portion, and the reaction was allowed to react for 72 h. Finally, resin was filtered and capped with MeOH, followed by thorough wash with DMF. The resin loading was quantified by the Fmoc absorbance measurement (loading 0.57 mmol/g).

The following conditions were used for peptide sequence construction:

- Resin deprotection (Fmoc removal): piperidine/DMF (2/8) at r.t for 2 min (twice).
- Coupling conditions: Fmoc-AA-OH 0.6 M in DMF (5 eq.), HATU 0.2 M in DMF (5 eq.), DIEA (10 eq.), stirred for 10 min. Double coupling was used except for the modified amino acid Fmoc-L-Glu[$NHNH_2$(Cl-Trt)]-OH.
- Fmoc deprotection conditions: piperidine/DMF (2/8) for 5 min (twice).
- N-terminal acetylation: Ac_2O (150 eq.)/DCM 1/1 (v/v) for 5 min (twice).
- Cleavage conditions: TFA/TIS/H_2O (95/2.5/2.5) solution for 3 hours at room temperature.
- Peptide recovery after cleavage: filtration of the resin, concentrated in vacuo, precipitated with ice cold Et_2O and supernatant removal.
- RP-HPLC purification.
- Crude product is freeze dried to obtain the final peptide as light powder.

Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC). The bishydrazide clicked peptides were synthetized by an adapting a literature protocole.[8] The protected alkyne peptide anchored to the solid support (quantities described for a 0.37 mmol scale) synthetized according to **(SPPS)** protocol was mixed with the corresponding azide compound (0.74 mmol, 2 eq.) and soaked in 2 ml of DMF. The mixture was then flushed three times with argon. On the other hand, TBTA (99 mg, 0.19 mmol, 0.5 eq.), Sodium Ascorbate (37 mg, 0.19 mmol, 0.5 eq.) and Copper(I) Iodide (18 mg, 0.09 mmol, 0.25 eq.) were mixed in 6 ml of a DMF/H_2O solution (8:2) and flushed 3 times with argon. Next, the Copper(I) Iodide solution was added to the resin

mixture and flushed again three times with argon. The reaction was then slowly stirred at room temperature for 16 h. Finally, the resin was filtered and washed thoroughly with DMF, H_2O, MeOH and finally DCM to remove the maximum amount of non-anchored residues. The washed resin was then dried over vacuum and the peptide was cleaved according to the general **(SPPS)** protocol. The solution was then concentrated, precipitated by adding ice cold Et_2O, and centrifuged. The supernatant was removed, and the crude material was freeze-dried for further purification.
When carried out in solution for the syntheses of the bisaldehyde clicked peptides, the same conditions were used, only with the starting cleaved peptide now in solution. The reaction was then stirred at room temperature for 16 h, and the crude material was purified by RP-HPLC.

Peptide titration. The final peptides were all titrated by ^{1}H NMR (in D_2O or MeOD) using *tert*-butanol (in D_2O) or toluene (in MeOD) as internal reference to determine the exact concentration. For this, the compound was solubilized in D_2O or MeOD (final concentration around 30 mM) *tert*-butyl alcohol or toluene was added (50 µL of a 30 mM solution in the corresponding deuterated solvent) in the NMR tube (total volume of 500 µL). ^{1}H NMR was recorded, and the relative peak integration was used to calculate the exact concentration of the compound. Indicated yields are therefore calculated from the titration value

c) mRNA binding and cell studies

Gel retardation assay. In sodium acetate buffer (25 mM, pH 5.5), a fixed concentration (0.06 µM) of EGFP mRNA (5moU) was mixed with the appropriate amounts of monomers to reach the different N/P ratios for a final volume of 10 µL. After 30 minutes incubation time, 2.5 µL of blue 6X loading dye (Fisher Scientific) were added to the mixture and 5 µL were loaded on the gel. Electrophoresis was carried out on a 1 % w/v agarose gel mixed with GelRed™ nucleic acid gel stain (Interchim, France) in 1X TAE buffer (Tris-acetate-EDTA, pH 8.2). The gel was run in 1X TAE buffer at 100 V for 20 minutes. A 100 bp DNA ladder from Sigma-Aldrich (Saint-Quentin-Fallavier, S4 France) was used as a reference for the gel. The GelRed-stained mRNA was visualized using a TFX-20 M model-UV transilluminator (Vilber Lourmat, Marne-la-Vallée, France) and gel photographs were obtained with a smartphone camera.

Dynamic light scattering (DLS) and ζ-potential measurements. In sodium acetate buffer (25 mM, pH 5.5), a fixed concentration (0.12 µM) of EGFP mRNA (5moU) was mixed with the appropriate amounts of monomers to reach the different N/P ratios for a final volume of 15 µL. Samples were diluted down to 6 nM mRNA before analysis using the same buffer. Measurements were performed using Zetasizer Nano-ZS instrument (Malvern, United Kingdom) with transparent ZEN0040 disposable micro-cuvette (40 µL) at 25°C. The same samples were used for ζ-potential measurements, which were performed using Zetasizer Nano-ZS instrument and DTS 1070 zeta potential cells at 25°C. The DCPs and monomers samples with mRNA were prepared at the same peptide/mRNA concentration ratio. As a consequence, experiences with DCPs at N/P 20 were compared to experience with monomers are at N/P 10.

Transmission electron microscopy (TEM). TEM images were obtained by using a JEM 1400+ electron microscopy, at Microscopie Electronique et Analytique (MEA), Université de

Montpellier. In sodium acetate buffer (25 mM, pH 5.5), mRNA complexes were prepared at 1 mM concentration of monomers and N/P 20 or the equivalent concentration in absence of mRNA. 10 µL of the sample were dropped on a carbon coated copper grid and dried at room temperature, then the samples were observed at a 120 kV acceleration voltage at 25 °C.

Cell culture. Human Breast cancer (MCF-7, ATCC® HTB-22™) cell line was purchased from ATCC (Manassas, Virginia, USA). MCF-7 cells were grown in F12/Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 $U.ml^{-1}$ penicillin and 100 $\mu g.mL^{-1}$ streptomycin. All cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

Transfection efficiency assay: Briefly, 20.000 cells were seeded into a 24 multi-well plate in complete culture media. Twenty-four hours after seeding, cell media was exchanged with DMEM (without antibiotic and without serum). Then, cells were transfected with mRNA samples prepared as follows. In sodium acetate buffer (25 mM, pH 5.5), a fixed concentration (200 µM) of monomer(s) was mixed with the needed amount of EGFP mRNA (5moU) to achieve the different desired N/P (N/P 20: 0.06 µM, N/P 10: 0.12 µM and N/P 20: 0.24 µM). The samples were then incubated for 30 minutes and diluted down to 0.06 µM concentration of mRNA in DMEM if necessary. Finally, cells were transfected by adding the necessary volume of mRNA complexes samples to achieve the different mRNA doses of 1.5 nM, 3 nM and 6 nM (or 0.5 ng/well, 1 ng/well and 2 ng/well respectively). Lipofectamine was used as positive control for the normalization of the transfection efficiency, while untreated cells were used as negative control. Twenty-four hours after transfection, EGFP Transfection efficiency was assessed by fluorescent Microscopy using EVOS M5000 Imaging System (ThermoFisher Scientific, Waltham, MA, USA), which software allows to estimate the transfection efficiency by calculating the ratio of fluorescence area (i.e., cells expressing the GFP protein) to the total cell area.

Cell viability (MTT) assay. MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to evaluate the cell viability.[9] Briefly, 20.000 cells were seeded into a 96 multi-well plate in complete culture media. Twenty-four hours after seeding, cells were treated with mRNA complexes for 24 h as described in the section "Transfection efficiency assay". Cells treated with the vehicle were considered as a control. After this incubation, cells were treated for 4 h with 0.5 $mg.ml^{-1}$ of MTT in media. The MTT/media solution was then removed, and the precipitated crystals were dissolved in EtOH/DMSO (1:1). The solution absorbance was read at 540 nm. The percentage of viable cells was calculated according to the following equation: $\%viability = A_{measured}/ A_{control}*100$.

2. Synthesis of aliphatic azides

The azide aliphatic chains were synthesized from the corresponding bromoalkanes according to a modified literature protocol,[10] with the following modifications: the equivalents of sodium azide (NaN_3) were decreased to 1.1, and the organic solvent used during crude mixture washing was changed to diethylether (Et_2O) for 1-azidohezane and 1-azidododecane synthesis to facilitate drying.

1-azidohexane. 1-bromohexane (850 µl, 6.0 mmol, 1 eq) and NaN_3 (430 mg, 6.6 mmol, 1.1 eq) were mixed in DMF (10 ml). The reaction was stirred at 60°C for 16 hours. Next, the crude mixture was diluted in Et_2O (60 ml), and the organic phase was washed with a 0.1 M HCl solution (2 x 60 ml) and brine (2 x 60 ml). The organic phase was dried over Na_2SO_4 and concentrated over vacuum at room temperature to afford **1-azidohexane** as a pale-yellow viscous oil (650 mg, 84%). The product was used in the following synthetic step without further purification. **^{1}H NMR** ($CDCl_3$) δ_H 3.25 (t, ^3J = 7.0, 2H, CH_2-N_3(H1)), 1.64 – 1.56 (m, 2H, CH_2(H2)), 1.43 – 1.25 (m, 6H, CH_2(H3, H4 and H5)), 0.90 (t, ^3J = 6.9, 3H, CH_3(H6)). **^{13}C NMR** ($CDCl_3$) δ_C 51.6, 31.5, 28.9, 26.5, 22.7, 14.1. Characterisation is in accordance with the experimental spectra described in the literature.[11]

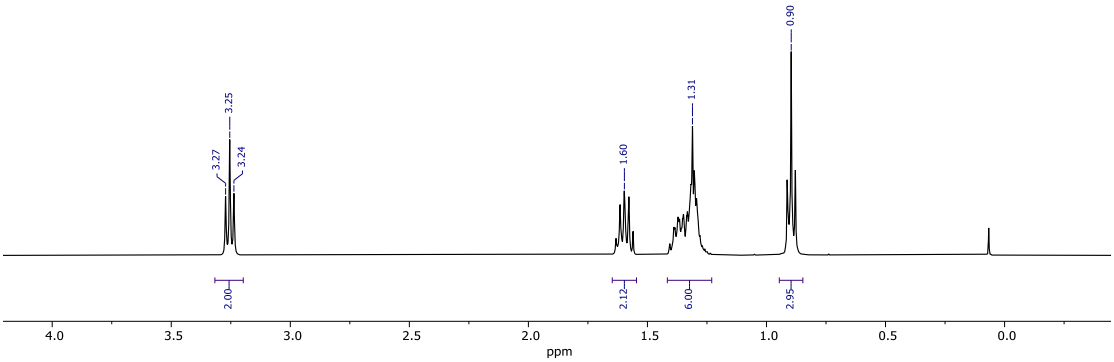


Figure 1: ^{1}H NMR ($CDCl_3$) spectrum of 1-azidohexane.

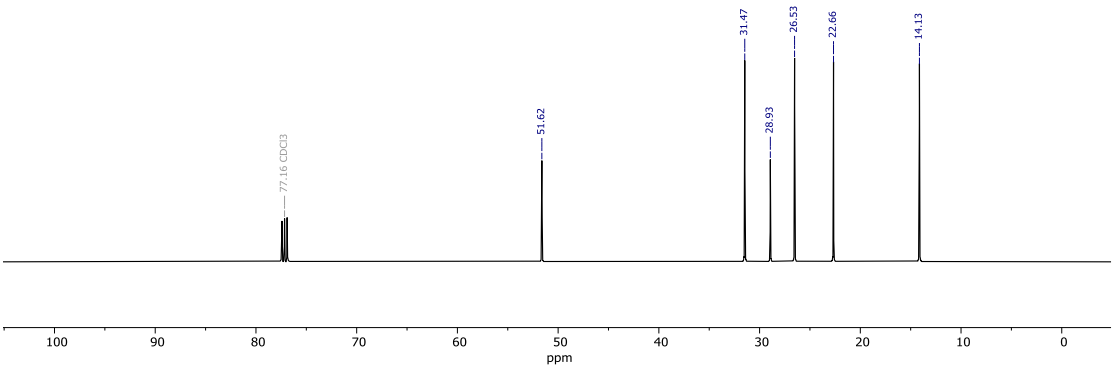


Figure 2: ^{13}C NMR ($CDCl_3$) spectrum of 1-azidohexane.

1-azidododecane. 1-bromododecane (960 µl, 4.0 mmol, 1 eq) and NaN_3 (290 mg, 4.4 mmol, 1.1 eq) were mixed in DMF (10 ml). The reaction was stirred at 60°C for 16 hours. Next, the crude mixture was diluted in Et_2O (60 ml), and the organic phase was washed with a 0.1 M HCl solution (2 x 60 ml) and brine (2 x 60 ml). The organic phase was dried over Na_2SO_4 and concentrated over vacuum at room temperature to afford **1-azidododecane** as a pale-yellow viscous oil (640 mg, 75%). The product was used in the following synthetic step without further purification. **HR-TOF-MS(ASAP)** m/z calcd for $[C_{12}H_{25}N_3\text{-}N_2\text{+}H]^+$ 184.2065, found 184.2317. **^{1}H NMR** ($CDCl_3$) δ_H: 3.25 (t, ^3J = 7.0, 2H, CH_2-N_3(H1)), 1.65 – 1.56 (m, 2H, CH_2(H2)), 1.43 – 1.17 (m, 18H, CH_2(H3 – H_{11})), 0.88 (t, ^3J = 6.9, 3H, CH_3(H12)). **^{13}C NMR** ($CDCl_3$) δ_C 51.6, 32.1, 29.8, 29.7, 29.6, 29.8, 29.5, 29.3, 29.0, 26.9, 22.8, 14.3. Characterisation is in accordance with the experimental spectra described in the literature.[12]

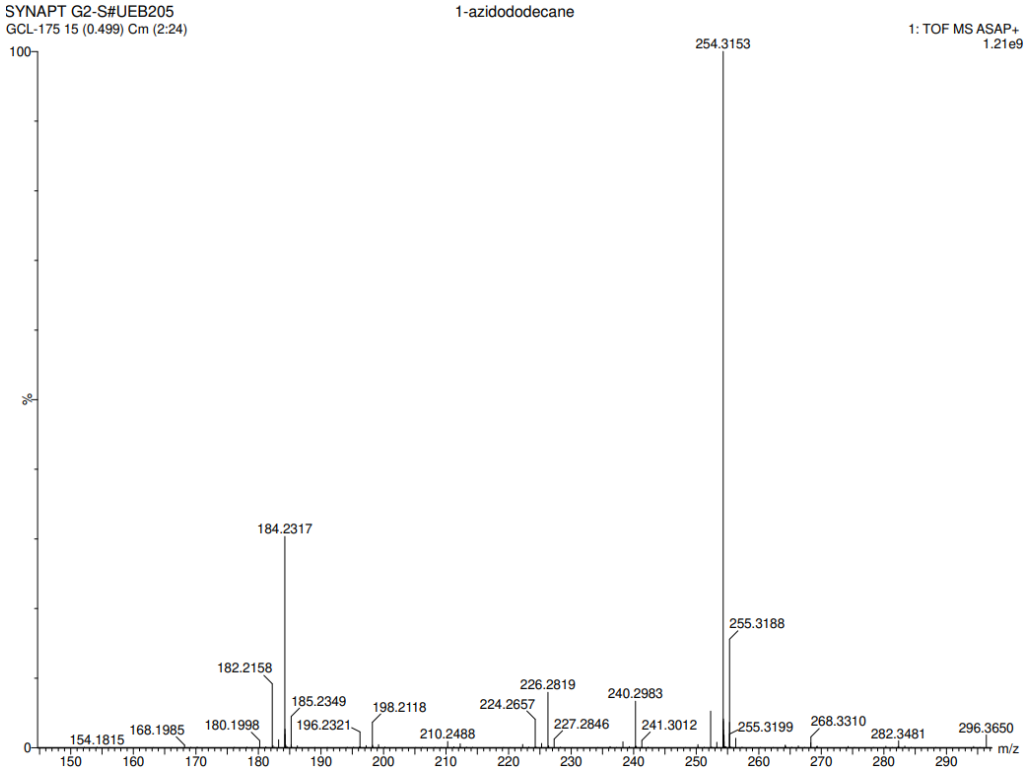


Figure 3: HR-MS(ASAP) spectra of 1-azidododecane.

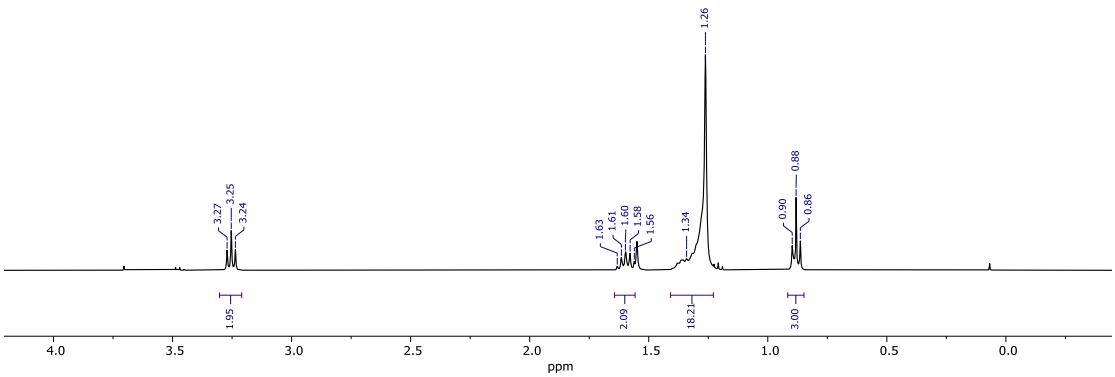


Figure 4: ^{1}H NMR ($CDCl_3$) spectrum of 1-azidododecane.

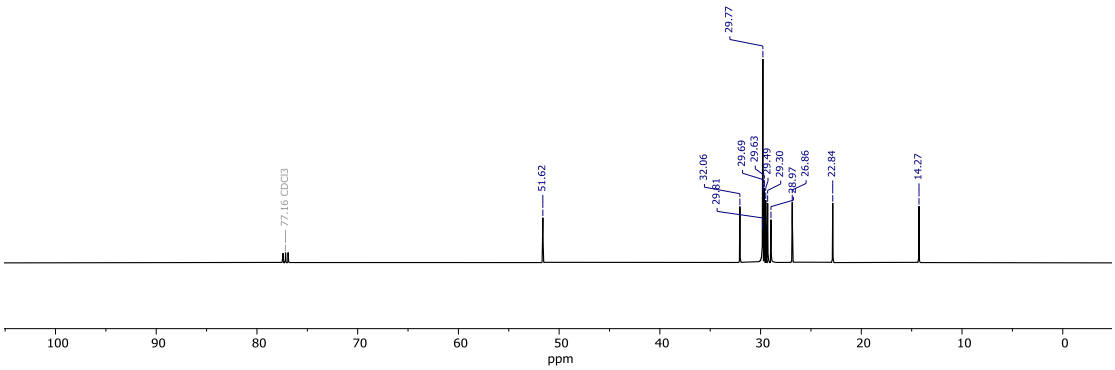


Figure 5: ^{13}C NMR ($CDCl_3$) spectrum of 1-azidododecane.

1-azidooctadecane. 1-bromooctadecane (2.00 g, 6.0 mmol, 1 eq) and NaN_3 (430 mg, 6.6 mmol, 1.1 eq) were mixed in DMF (10 ml). The reaction was stirred at 60°C for 16 hours. Next, the crude mixture was diluted in DCM (60 ml), and the organic phase was washed with a 0.1 M HCl solution (2 x 60 ml) and brine (2 x 60 ml). The organic phase was dried over Na_2SO_4 and concentrated over vacuum to afford **1-azidooctadecane** as a pale-yellow viscous oil (1.70 g, 95%). The product was used in the following synthetic step without further purification. **HR-TOF-MS(ASAP)** m/z calcd for $[C_{18}H_{38}N_3\text{-}N_2+H]^+$ 268.3004, found 268.2999. **^{1}H NMR** ($CDCl_3$) δ_H: 3.25 (t, ^3J = 7.0, 2H, CH_2-N_3(H1)), 1.65 – 1.55 (m, 2H, CH_2(H2)), 1.42 – 1.18 (m, 30H, CH_2(H3 – H_{17})), 0.88 (t, ^3J = 6.9, 3H, CH_3(H18)). **^{13}C NMR** ($CDCl_3$) δ_C 51.6, 32.1, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 29.0, 26.9, 22.9, 14.3. Characterisation is in accordance with the experimental spectra described in the literature.[13]

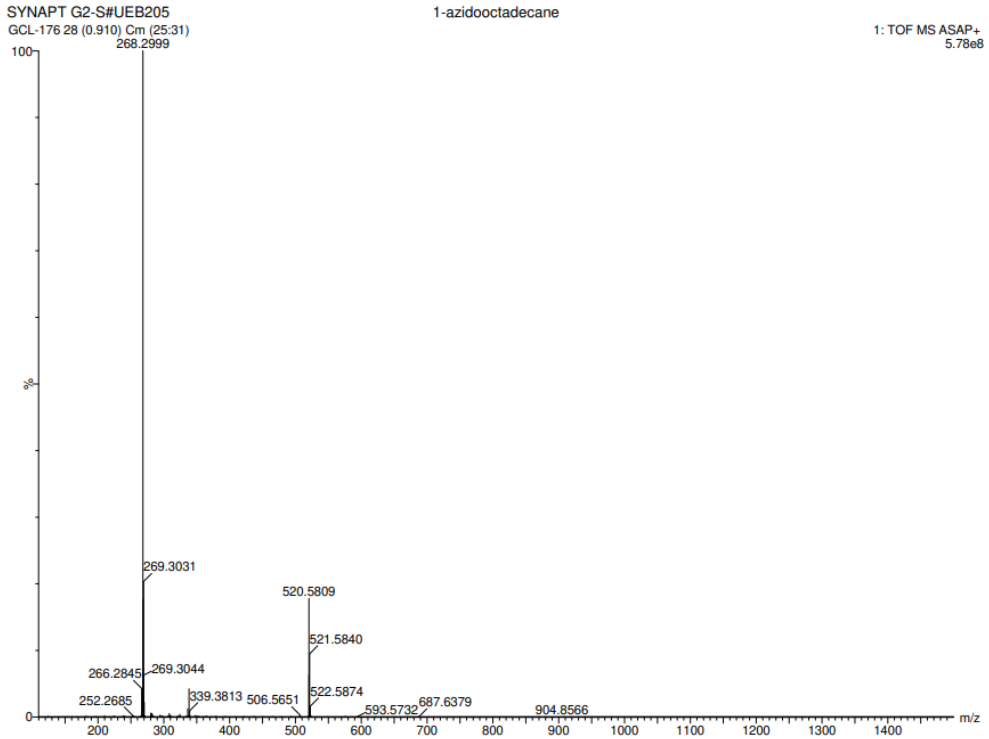


Figure 6: HR-MS(ASAP) spectra of 1-azidooctadecane.

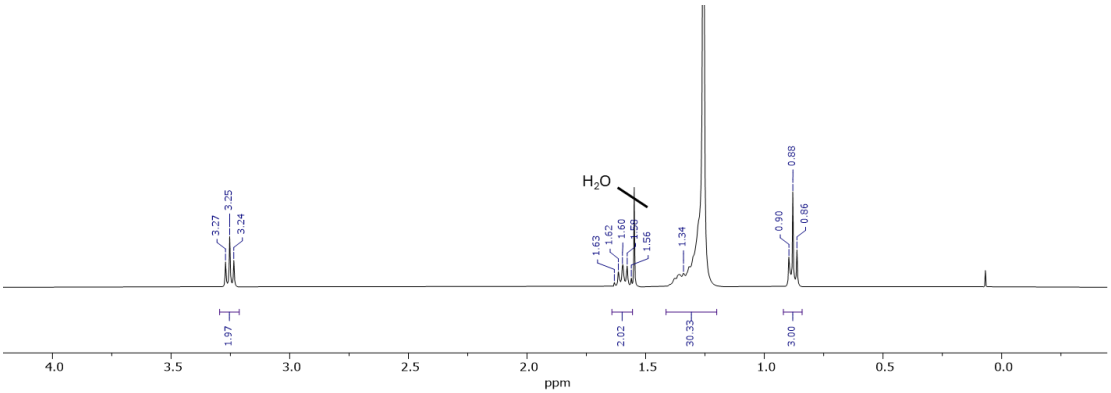


Figure 7: ^{1}H NMR ($CDCl_3$) spectrum of 1-azidooctadecane.

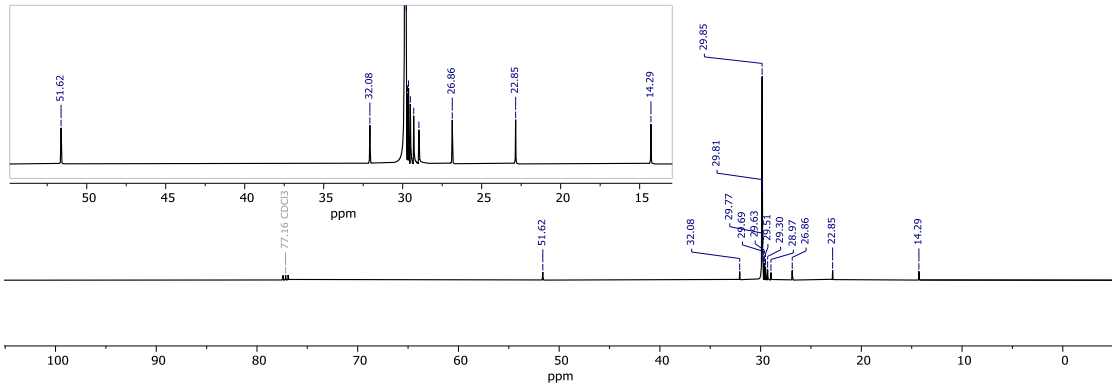


Figure 8: ^{13}C NMR ($CDCl_3$) spectrum of 1-azidooctadecane.

3. Synthesis of bisaldehyde peptides A-n

a) Compound 1

The peptide was synthesized according to the general **(SPPS)** procedure using Fmoc-*L*-Lys[*N*-4-pentynoic acid]-OH, Fmoc-*L*-Lys[Boc-*L*-Ser(O*t*Bu)]-OH and Fmoc-*L*-Arg(Pbf)-OH. Note that a scale up to 1.19 mmol was performed in this case. The compound **1** was obtained after preparative **[Prep-HPLC]** purification (gradient: 0 min, 100% A, 0% B; 5 min, 100% A, 0% B; 35 min, 60% A, 40% B). 560 mg were obtained (40%). **HPLC** t_R: 2.55 min. **HR-ESI-MS** m/z calcd for $[C_{49}H_{91}N_{21}O_{12}+2H]^{2+}$ 583.8651, found 583.8655; $[C_{49}H_{91}N_{21}O_{12}+3H]^{3+}$ 389.5791, found 389.5796. **^{1}H NMR** (D_2O) δ_H: 4.39 – 4.27 (m, 3H, Hα_R) (m, 1H, Hα_K), 4.27 – 4.16 (m, 2H, Hα_K), 4.09 (t, ^3J = 5.0, 2H, Hα_S), 3.99 (dd, ^2J = 12.3, ^3J = 4.2, 2H, Hβ_S), 3.92 (dd, ^2J = 12.3, ^3J = 5.7, 2H, Hβ'_S), 3.32 – 3.14 (m, 6H, Hδ_R) (m, 6H, Hε_K), 2.53 – 2.46 (m, 2H, CH_2(H3)), 2.46 – 2.40 (m, 2H, CH_2(H2)), 2.38 (t, ^4J = 2.5, 1H, CH(H5)), 2.04 (s, 3H, CH_3(H6)), 1.92 – 1.48 (m, 6H, Hβ_R) (m, 6H, Hγ_R) (m, 6H, Hβ_K) (m, 6H, Hδ_K), 1.48 – 1.29 (m, 6H, Hγ_K).

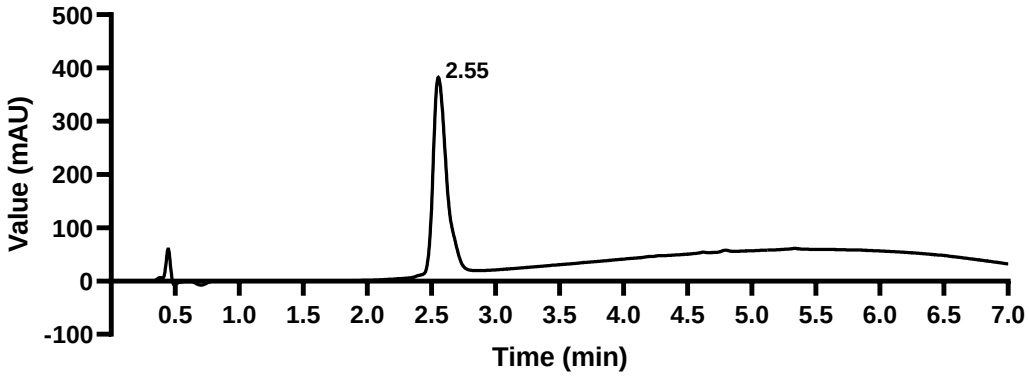


Figure 9: HPLC chromatogram of compound **1**.

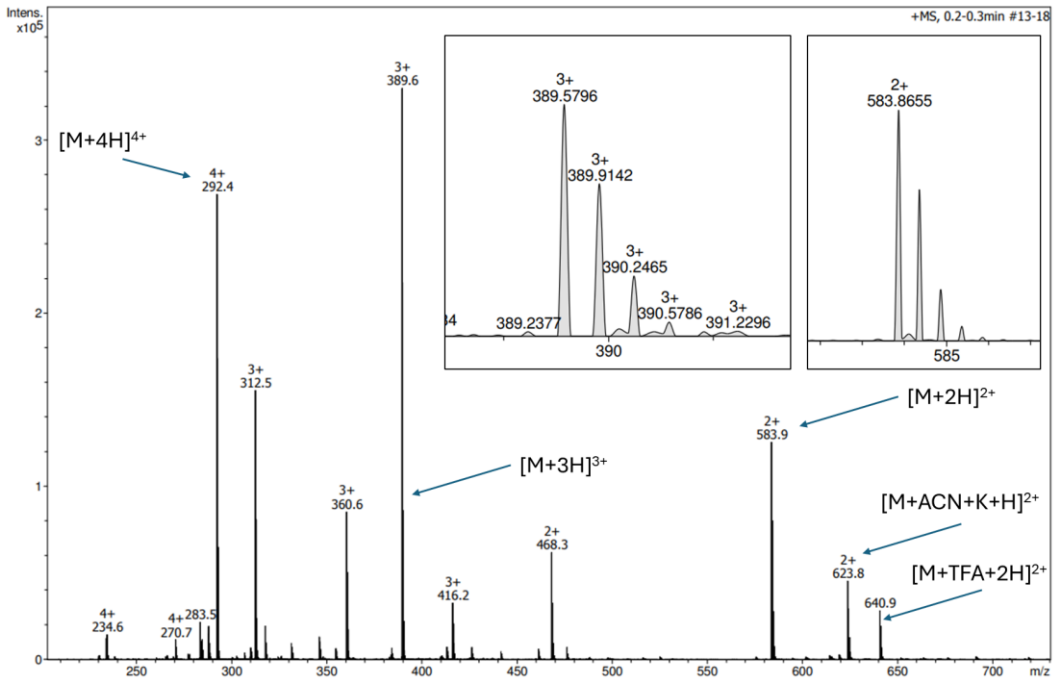


Figure 10: HR-ESI-MS spectra of compound **1**.

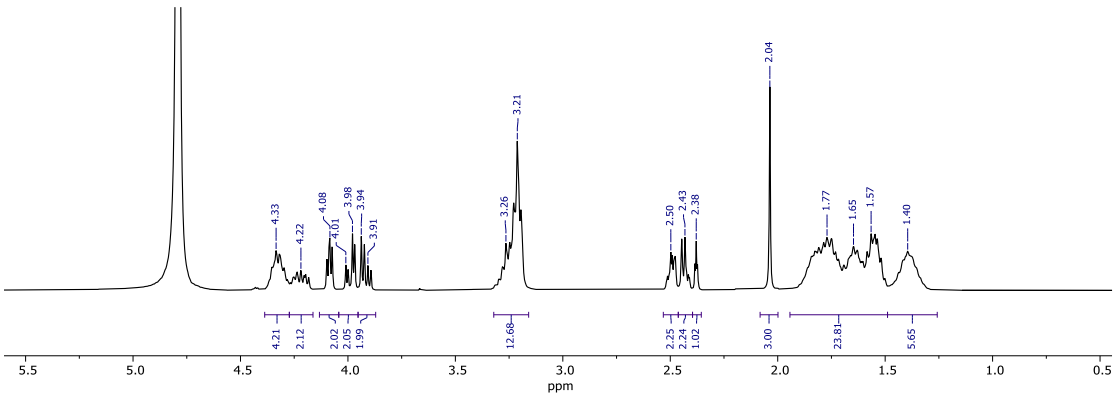


Figure 11: ^{1}H NMR (D_2O) spectrum of compound **1**.

b) Compound 2

The oxidative cleavage was carried out by dissolving compound **1** (550 mg, 0.47 mmol, 1 eq.) in H_2O (10 mM) and then adding $NaIO_4$ (1.0 g, 4.7 mmol, 10 eq.). After 2 hours stirring, the desired product was obtained after preparative **[Prep-HPLC]** purification (gradient: 0 min, 100% A, 0% B; 5 min, 100% A, 0% B; 35 min, 60% A, 40% B). 390 mg were obtained (71%). **HPLC** t_R: 2.69 min. **HR-ESI-MS** m/z calcd for $[C_{47}H_{81}N_{19}O_{12}+2H]^{2+}$ 552.8229, found 552.8229; $[C_{47}H_{81}N_{19}O_{12}+2H_2O+3H]^{3+}$ 380.8914, found 380.8914. **^{1}H NMR** (D_2O) δ_H: 5.29 (s, 2H, CH(acetals)), 4.41 – 4.23 (m, 3H,

$H\alpha_R$) (m, 2H, $H\alpha_K$), 4.20 (t, ^3J = 7.1, 1H, $H\alpha_K$), 3.32 – 3.15 (m, 6H, $H\delta_R$) (m, 6H, $H\varepsilon_K$), 2.53 – 2.46 (m, 2H, CH_2(H3)), 2.46 – 2.40 (m, 2H, CH_2(H2)), 2.38 (t, ^4J = 2.5, 1H, CH(H5)), 2.03 (s, 3H, CH_3(H6)), 1.92 – 1.48 (m, 6H, $H\beta_R$) (m, 6H, $H\gamma_R$) (m, 6H, $H\beta_K$) (m, 6H, $H\delta_K$), 1.48 – 1.27 (m, 6H, $H\gamma_K$).

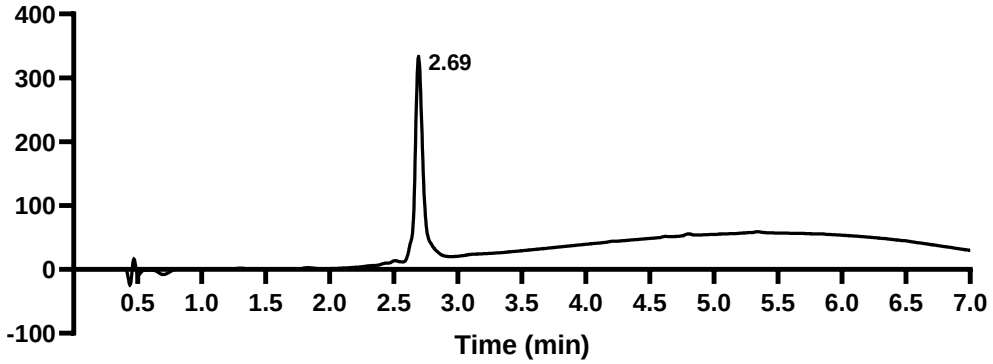


Figure 12: HPLC chromatogram of compound **2**.

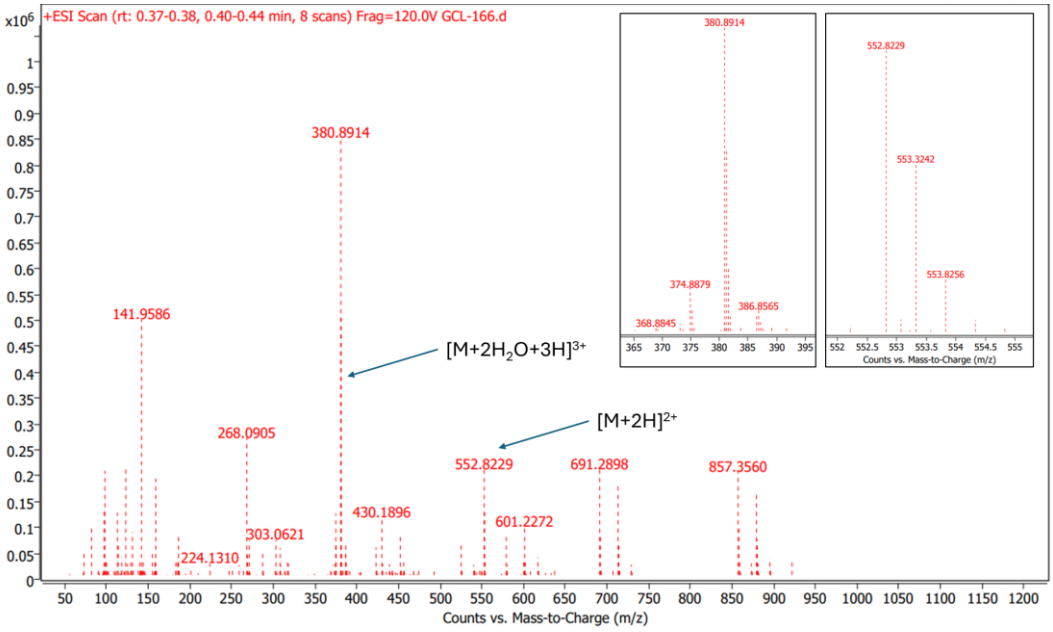


Figure 13: HR-ESI-MS spectra of compound **2**.

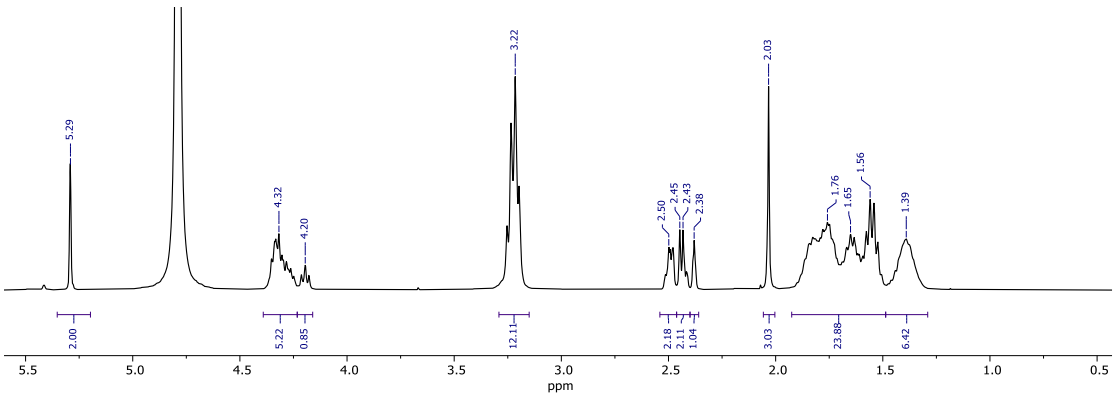


Figure 14: ^{1}H NMR (D_2O) spectrum of compound **2**.

c) A-6

The peptide was synthesized according to the general **(CuAAC)** procedure using compound **2** (50 mg, 0.042 mmol) and **1-azidohexane**. The compound **A-6** was obtained after preparative **[Semiprep-HPLC]** purification (gradient: 0 min, 100% A, 0% B; 5 min, 100% A, 0 % B; 45 min, 0% A, 100% B). 39 mg were obtained (75%). **HPLC** t_R: 3.44 min. **HR-ESI-MS** m/z calcd for $[C_{53}H_{94}N_{22}O_{12}+2H_2O+4H]^{4+}$ 317.6981, found 317.6982; $[C_{53}H_{94}N_{22}O_{12}+2H_2O+3H]^{3+}$ 423.2617, found 423.2618; $[C_{53}H_{94}N_{22}O_{12}+2H]^{2+}$ 616.3784, found 616.3784. **^{1}H NMR** (D_2O/MeOD 8/2) δ_H: 7.83 (s, 1H, CH(H5)), 5.24 (s, 1H, CH(acetals)), 4.94 (s, 1H, CH(acetals)), 4.43 – 4.00 (m, 3H, Hα_R) (m, 3H, Hα_K) (m, 2H, CH_2(H7)), 3.97 – 3.32 (m, 4H, Hε_K), 3.32 – 3.11 (m, 6H, Hδ_R), 3.11 – 3.02 (m, 2H, Hε_K), 2.98 (t, ^{3}J = 7.2, 2H, CH_2(H3)), 2.56 (t, ^{3}J = 7.1, 2H, CH_2(H3)), 2.00 (s, 3H, CH_3(H19)), 1.94 – 1.09 (m, 6H, Hβ_R) (m, 6H, Hγ_R) (m, 6H, Hβ_K) (m, 6H, Hγ_K) (m, 6H, Hδ_K), (m, 8H, CH_2(H8-17)), 0.87 – 0.73 (m, CH_3(H13)).

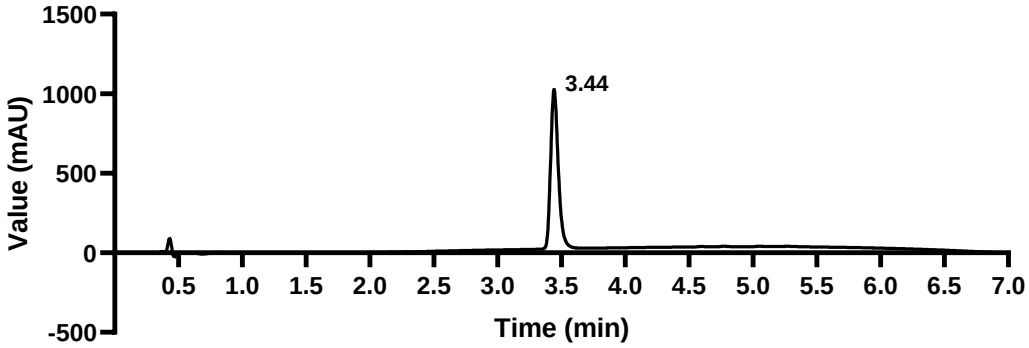


Figure 15: HPLC chromatogram of **A-6**.

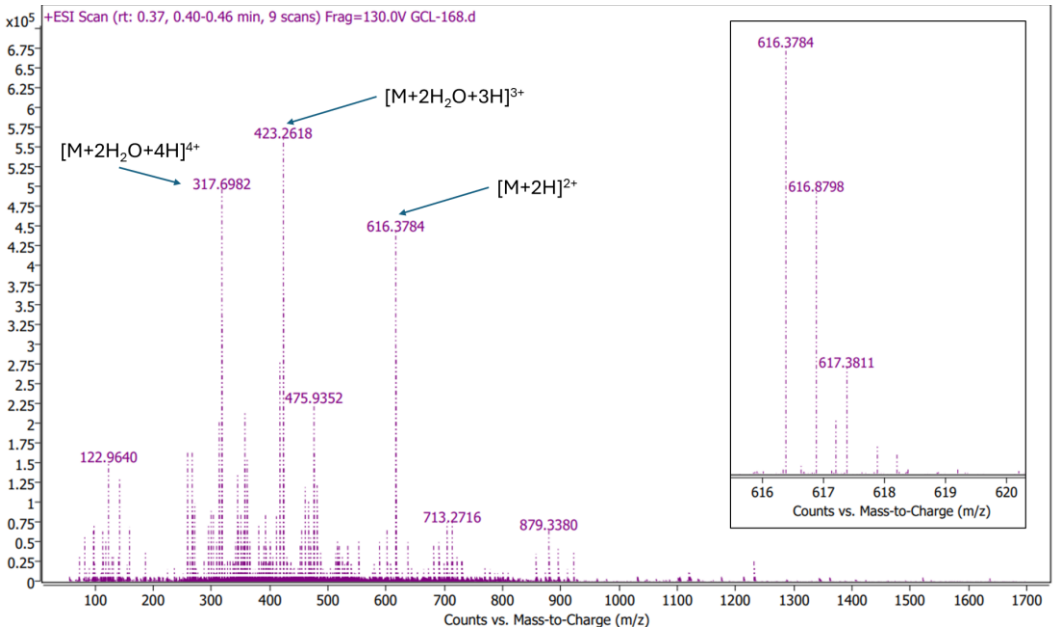


Figure 16: HR-ESI-MS spectra of **A-6**.

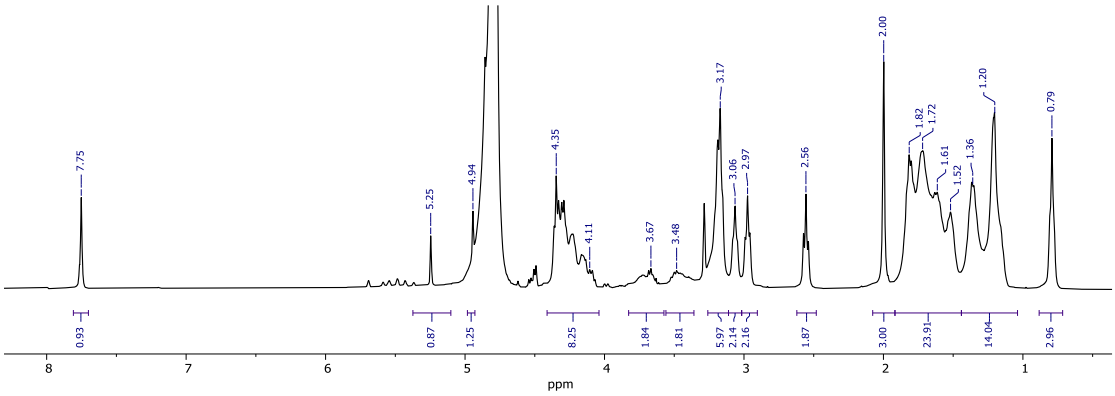
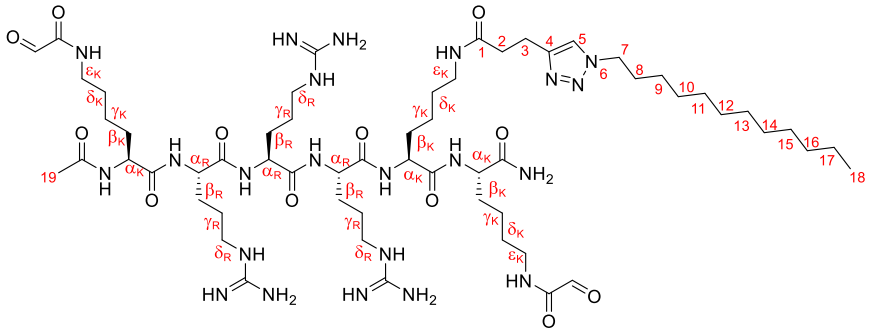


Figure 17: ^{1}H NMR (D_2O/MeOD, 8/2) spectrum of **A-6**.

d) A-12

The peptide was synthesized according to the general **(CuAAC)** procedure using



Compound 2 (50 mg, 0.042 mmol) and **1-azidododecane**. The compound **A-12** was obtained after preparative **[Semiprep-HPLC]** purification (gradient: 0 min, 70% A, 30% B; 5 min, 70% A, 30% B; 45 min, 0% A, 100% B). 10 mg were obtained (18%). **HPLC** t_R: 4.52 min. **HR-ESI-MS** m/z calcd for $[C_{59}H_{106}N_{22}O_{12}+H]^+$ 1315.8433, found 1315.8440; $[C_{59}H_{106}N_{22}O_{12}+2H]^{2+}$ 658.4253, found 658.4254. **^{1}H NMR** (MeOD/D_2O 8/2) δ_H: 7.75 (s, 1H, CH(H5)), 4.86 (s, 2H, CH(acetals)),

4.45 – 3.99 (m, 3H, $H\alpha_R$) (m, 3H, $H\alpha_K$) (m, 2H, CH_2(H7)), 3.27 – 3.08 (m, 6H, $H\delta_R$) (m, 6H, $H\varepsilon_K$), 2.99 (t, ^3J = 7.6, 2H, CH_2(H3)), 2.57 (t, ^3J = 7.6, 2H, CH_2(H3)), 2.02 (s, 3H, CH_3(H19)), 1.97 – 1.18 (m, 6H, $H\beta_R$) (m, 6H, $H\gamma_R$) (m, 6H, $H\beta_K$) (m, 6H, $H\gamma_K$) (m, 6H, $H\delta_K$), (m, 20H, CH_2(H8-17)), 0.89 (t, ^3J = 6.7, 3H, CH_3(H24)).

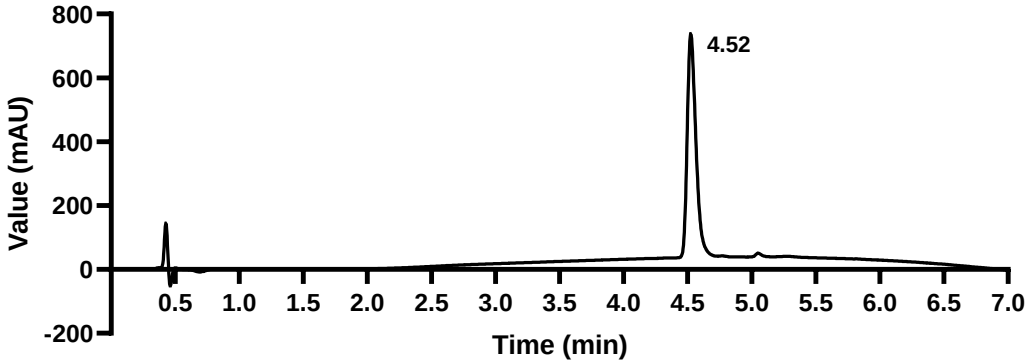


Figure 18: HPLC chromatogram of **A-12**.

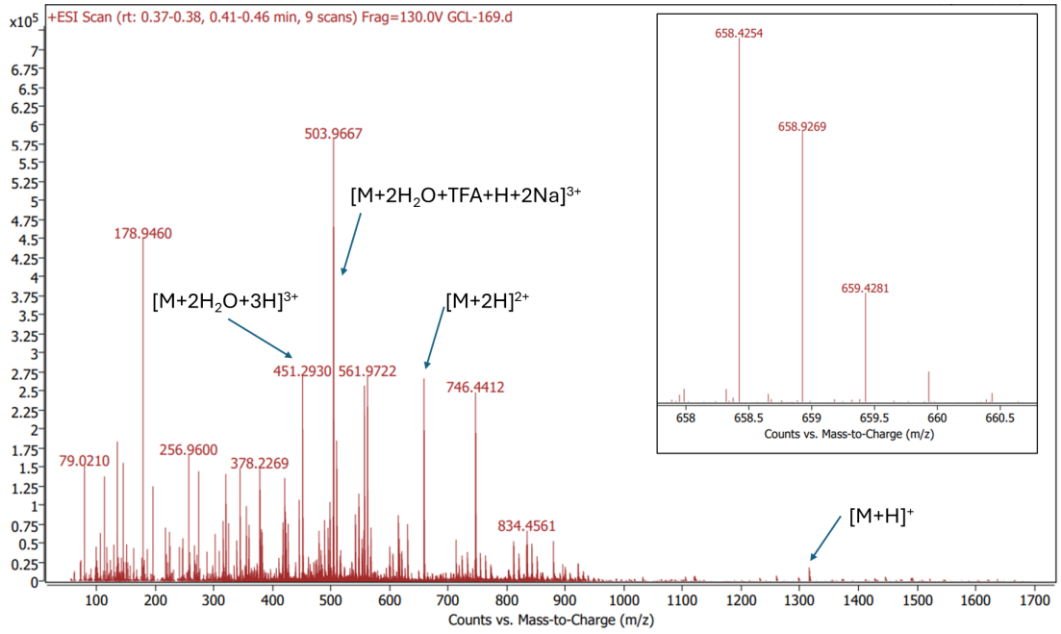


Figure 19: HR-ESI-MS spectra of **A-12**.

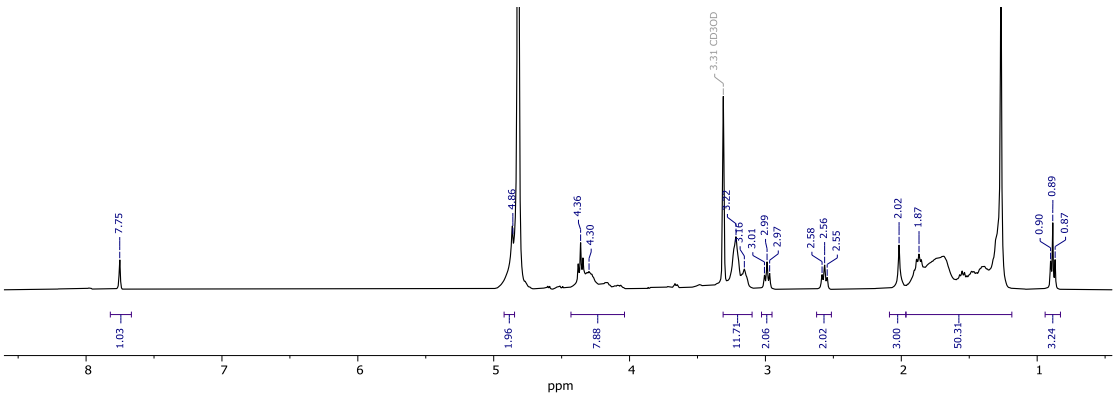
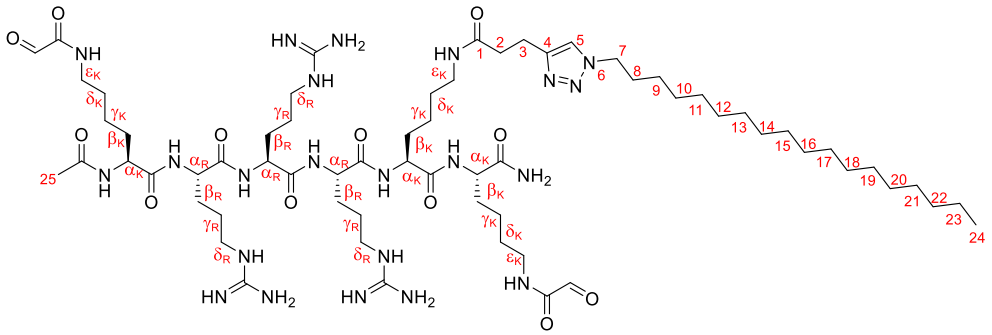


Figure 20: ^{1}H NMR (D_2O/MeOD, 2/8) spectrum of **A-12**.

e) A-18

The peptide was synthesized according to the general **(CuAAC)** procedure using



Compound 2 (25 mg, 0.022 mmol) and **1-azidooctadecane**. The compound **A-18** was obtained after preparative **[Semiprep-HPLC]** purification (gradient: 0 min, 70% A, 30% B; 5 min, 70% A, 30% B; 45 min, 0% A, 100% B). 13 mg were obtained (42%). **HPLC** t_R: 5.60 min. **HR-ESI-MS** m/z calcd for $[C_{65}H_{118}N_{22}O_{12}+2H]^{2+}$ 700.4723, found 700.4722. **^{1}H NMR** (MeOD/D_2O 9/1) δ_H: 7.75 (s, 1H, CH(H5)), 4.43 – 4.23 (m, 3H, Hα_R) (m, 2H, Hα_K) (m, 2H, CH_2(H7)), 4.16 (t, ^{3}J = 7.0, 1H, Hα_K), 3.29 – 3.10 (m, 6H, Hδ_R) (m, 6H, Hε_K), 2.99 (t, ^{3}J = 7.7, 2H, CH_2(H3)), 2.56 (t, ^{3}J = 7.7, 2H, CH_2(H3)), 2.02 (s, 3H, CH_3(H25)), 1.96 – 1.16 (m, 6H, Hβ_R) (m, 6H, Hγ_R) (m, 6H, Hβ_K) (m, 6H, Hγ_K) (m, 6H, Hδ_K), (m, 32H, CH_2(H8-23)), 0.89 (t, ^{3}J = 6.7, 3H, CH_3(H24)).

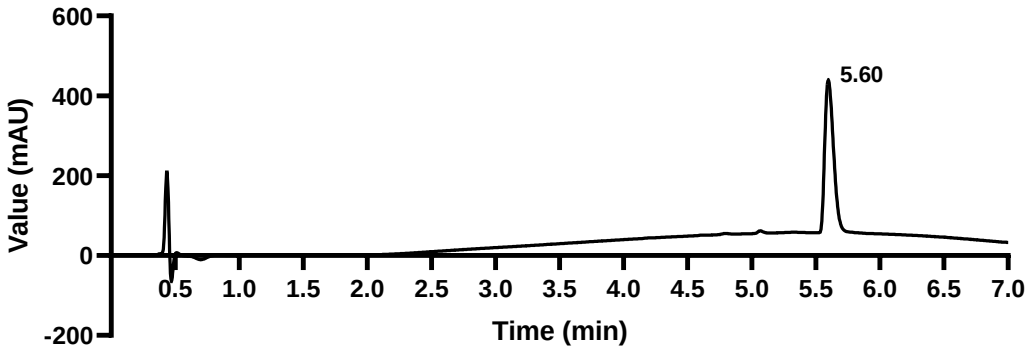


Figure 21: HPLC chromatogram of **A-18**.

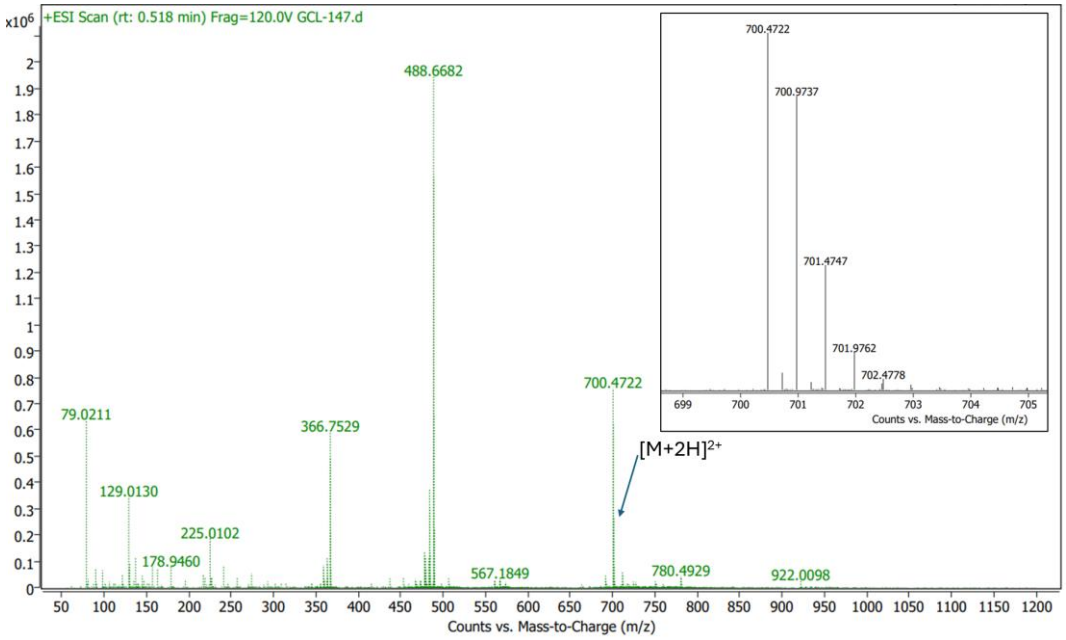


Figure 22: HR-ESI-MS spectra of **A-18**.

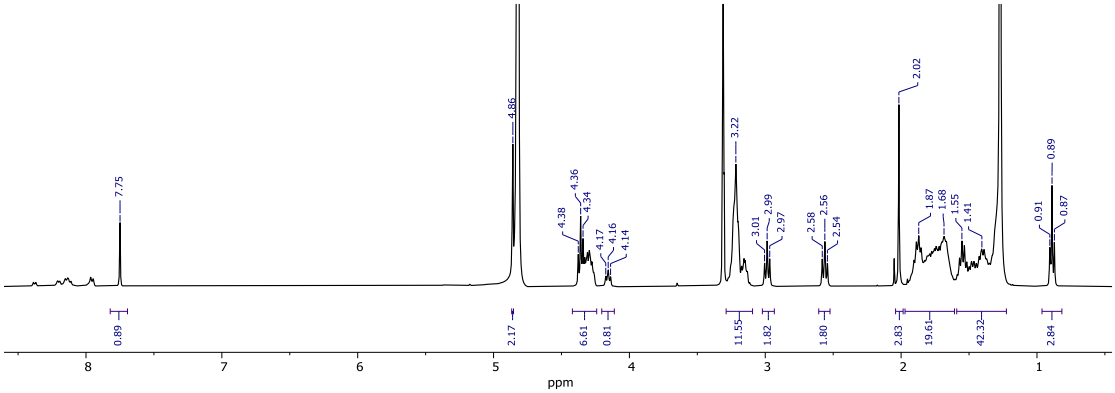


Figure 23: ^{1}H NMR (MeOD/D_2O 9/1) spectrum of **A-18**.

4. Synthesis of bishydrazide peptides H-n

a) H-6

The peptide was synthesized according to the general **(SP-CuAAC)** procedure using Fmoc-*L*-Lys[*N*-4-pentynoic acid]-OH, Fmoc-*L*-Arg(Pbf)-OH, Fmoc-*L*-Glu[NHNH(2Cl-Trt)]-OH and **1-azidohexane**. The compound **H-6** was obtained after preparative **[Prep-HPLC]** purification (gradient: 0 min, 100% A, 0% B; 5 min, 100% A, 0% B; 45 min, 0% A, 100% B). 88 mg were obtained (25%). **HPLC** t_R: 3.67 min. **HR-ESI-MS** m/z calcd for $[C_{40}H_{78}N_{22}O_7+2H]^{2+}$ 490.3285, found 490.3285; $[C_{40}H_{78}N_{22}O_7+3H]^{3+}$ 327.2214, found 327.2224. **^{1}H NMR** (D_2O) δ_H: 7.78 (s, 1H, CH(H8)), 4.48 – 4.27 (m, 3H, Hα_R) (m, 1H, Hα_K) (t, ^3J = 6.8, 2H, CH_3(H9)), 4.27 – 4.16 (m, 1H, Hα_{E*}), 3.27 – 3.15 (m, 6H, Hδ_R) (m, 2H, Hε_K), 3.09 (t, ^3J = 6.6, 2H, Hε_K), 3.01 (t, ^3J = 7.0, 2H, CH_2(H3)), 2.59 (t, ^3J = 7.1, 2H, CH_2(H2)), 2.43 (t, ^3J = 8.0, 2H, Hγ_{E*}), 2.22 – 1.96 (m, 2H, Hβ_{E*}), 1.93 – 1.52 (m, 6H, Hβ_R) (m, 6H, Hγ_R) (m, 2H, Hβ_K) (m, 2H, CH_2(H10)), 1.48 – 1.32 (m, 2H, Hδ_K), 1.32 – 1.14 (m, 2H, Hγ_K) (m, 6H, CH_2(H11 – H13)), 0.83 (t, ^3J = 6.6, 3H, CH_3(H14)).

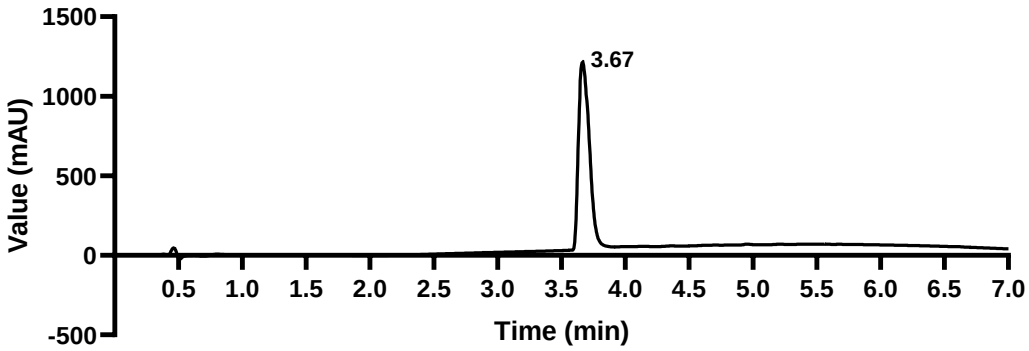


Figure 24: HPLC chromatogram of **H-6**.

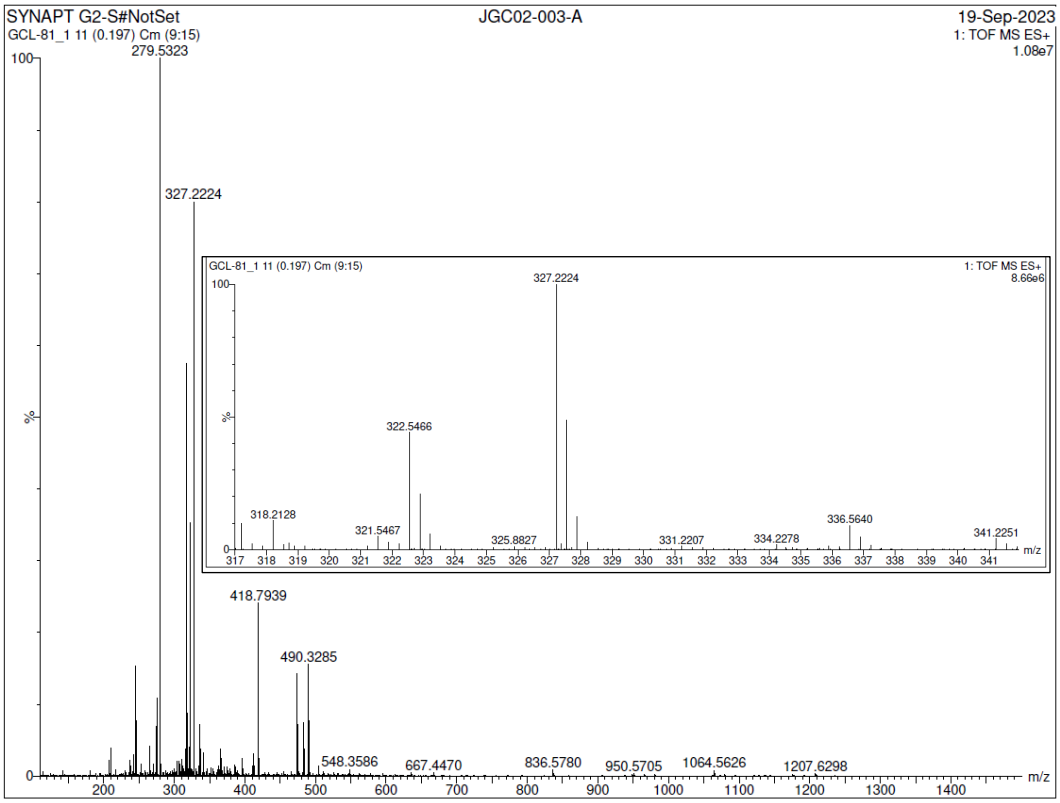


Figure 25: HR-ESI-MS spectra of **H-6**.

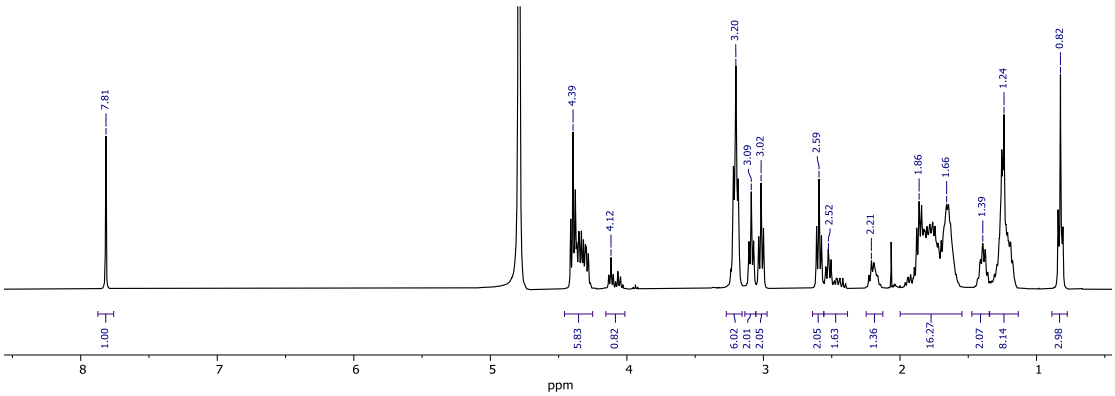
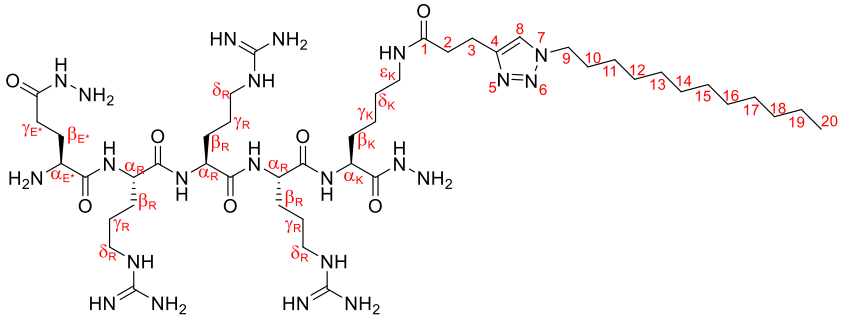


Figure 26: ^{1}H NMR (D_2O) spectrum of **H-6**.

b) H-12

The peptide was synthesized according to the general **(SP-CuAAC)** procedure using Fmoc-*L*-Lys[*N*-4-pentynoic acid]-OH, Fmoc-*L*-Arg(Pbf)-OH, Fmoc-*L*-Glu[NHNH(2Cl-Trt)]-OH and **1-azidododecane**. The compound **H-12** was obtained



after preparative **[Prep-HPLC]** purification (gradient: 0 min, 70% A, 30% B; 5 min, 70% A, 30% B; 45 min, 0% A, 100% B). 35 mg were obtained (9%). **HPLC** t_R: 4.52 min. **HR-ESI-MS** m/z calcd for $[C_{46}H_{90}N_{22}O_7+H]^+$ 1063.7436, found 1063.7479; $[C_{46}H_{90}N_{22}O_7+2H]^{2+}$ 532.3754, found 532.3752; $[C_{46}H_{90}N_{22}O_7+3H]^{3+}$ 355.2527, found 355.2527; $[C_{46}H_{90}N_{22}O_7+4H]^{4+}$ 266.6913, found 266.6914. **^{1}H NMR** (MeOD) δ_H: 7.75 (s, 1H, CH(H8)), 4.48 – 4.26 (m, 3H, Hα_R) (m, 1H, Hα_K) (t, ^3J = 7.7, 2H, CH_3(H9)), 4.04 (t, ^3J = 6.4, 1H, Hα_{E^*}), 3.27 – 3.10 (m, 6H, Hδ_R) (m, 2H, Hε_K), 2.99 (t, ^3J = 7.5, 2H, CH_2(H3)), 2.60 – 2.47 (m, 2H, CH_2(H2)) (m, 2H, Hγ_{E^*}), 2.27 – 2.11 (m, 2H, Hβ_{E^*}), 1.96 – 1.60 (m, 6H, Hβ_R) (m, 6H, Hγ_R) (m, 2H, Hβ_K) (m, 2H, CH_2(H10)), 1.55 – 1.43 (m, 2H, Hδ_K), 1.43 – 1.20 (m, 2H, Hγ_K) (m, 18H, CH_2(H11 – H19)), 0.89 (t, ^3J = 6.8, 3H, CH_3(H20)). **^{13}C NMR** (MeOD) δ_C: 174.59, 173.97, 172.82, 169.89, 169.05, 162.88, 162.53, 158.68, 147.63, 123.49, 119.35, 116.45, 54.93, 54.73, 54.52, 54.45, 54.34, 54.27, 53.44, 53.22, 52.60, 51.37, 41.94, 39.95, 36.45, 33.03, 32.34, 32.30, 31.51, 31.27, 30.70, 30.61, 30.53, 30.42, 30.06, 29.86, 29.58, 27.90, 27.63, 27.45, 26.28, 26.18, 23.97, 23.70, 22.55, 14.42.

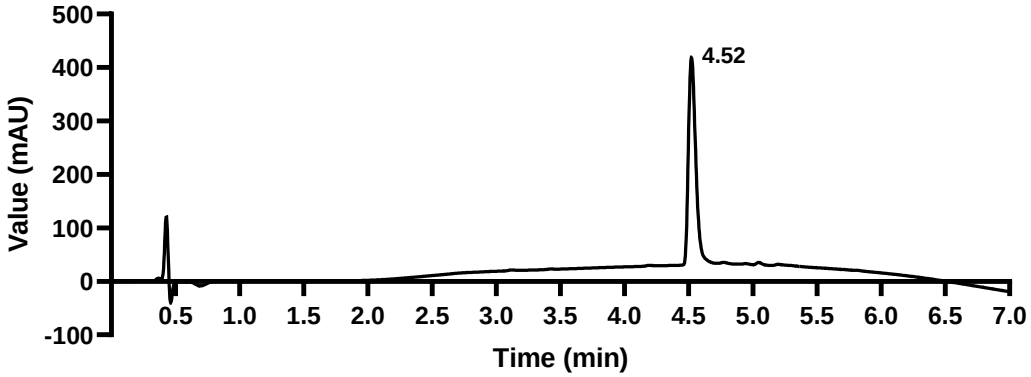


Figure 27: HPLC chromatogram of **H-12**.

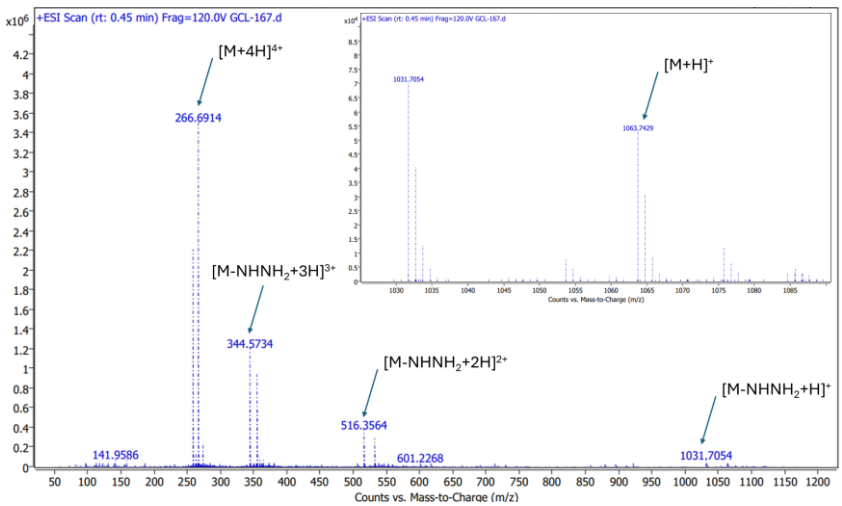


Figure 28: HR-ESI-MS spectra of **H-12**.

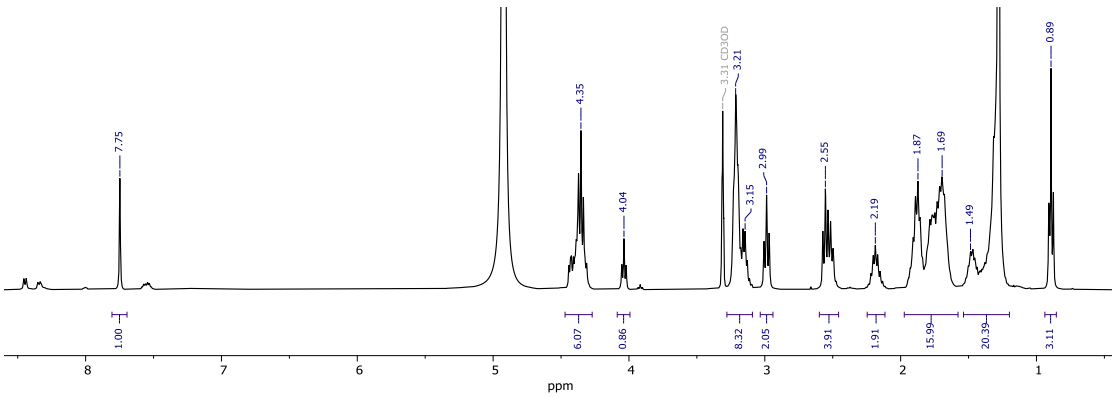


Figure 29: ^{1}H NMR (MeOD) spectrum of **H-12**.

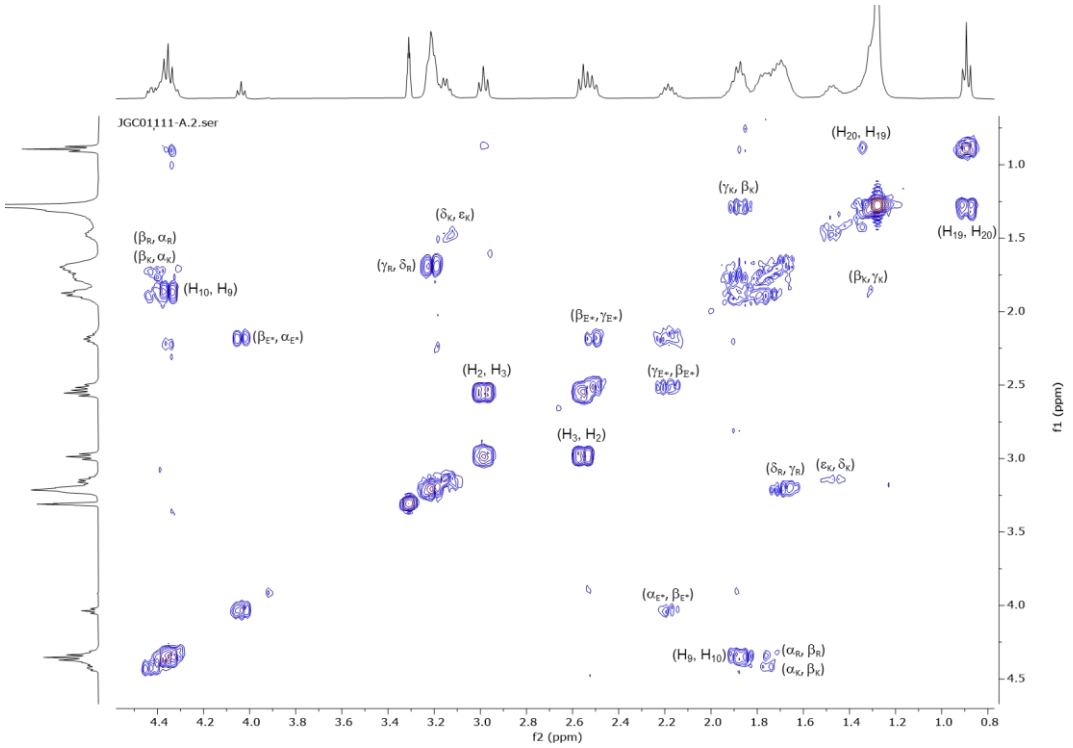


Figure 30: ^{1}H COSY NMR (400 MHz, MeOD) spectrum of compound **H-12**.

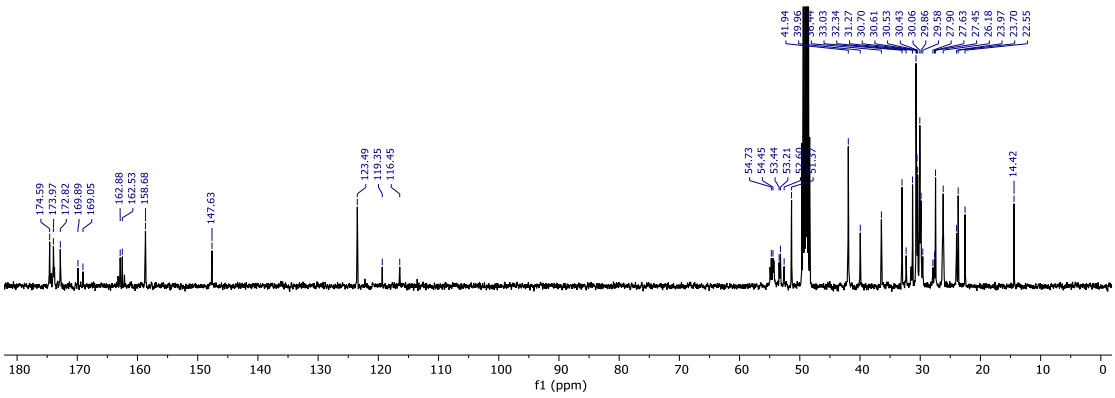
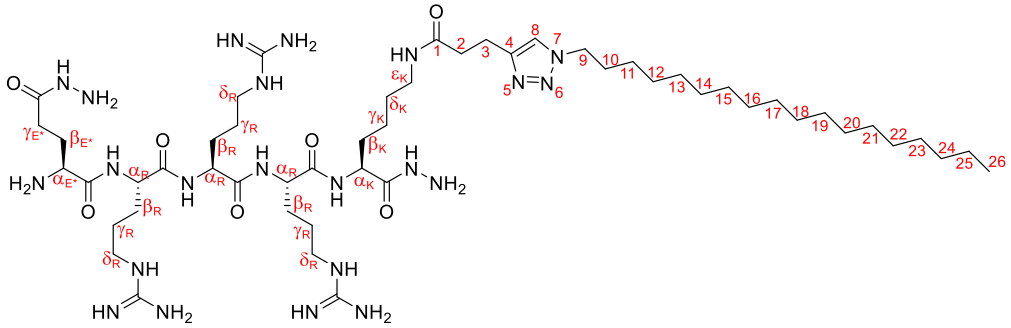


Figure 31: ^{13}C NMR (MeOD) spectrum of **H-12**.

c) H-18

The peptide was synthesized according to the general **(SP-CuAAC)** procedure



using Fmoc-*L*-Lys[*N*-4-pentynoic acid]-OH, Fmoc-*L*-Arg(Pbf)-OH, Fmoc-*L*-Glu[NHNH(2Cl-Trt)]-OH and 1-azidooctadecane. The compound **H-18** was obtained after preparative **[Prep-HPLC]** purification (gradient: 0 min, 70% A, 30% B; 5 min, 70% A, 30% B; 45 min, 0% A, 100% B). 22 mg were obtained (9%). **HPLC** t_R: 5.63 min. **HR-ESI-MS** m/z calcd for $[C_{52}H_{102}N_{22}O_7+3H]^{3+}$ 383.2840, found 383.2845; $[C_{52}H_{102}N_{22}O_7+4H]^{4+}$ 287.7148, found 287.7153. **^{1}H NMR** (MeOD) δ_H: 7.73 (s, 1H, CH(H8)), 4.49 – 4.22 (m, 3H, Hα_R) (m, 1H, Hα_{E^*}) (m, 1H, Hα_K) (t, ^3J = 7.0, 2H, CH_3(H9)), 3.26 – 3.10 (m, 6H, Hδ_R) (m, 2H, Hε_K), 2.98 (t, ^3J = 7.5, 2H, CH_2(H3)), 2.64 – 2.38 (m, 2H, CH_2(H2)) (m, 2H, Hγ_{E^*}), 2.22 – 1.98 (m, 2H, Hβ_{E^*}), 1.93 – 1.62 (m, 6H, Hβ_R) (m, 6H, Hγ_R) (m, 2H, Hβ_K) (m, 2H, CH_2(H10)), 1.54 – 1.23 (m, 2H, Hδ_K) (m, 2H, Hγ_K) (m, 30H, CH_2(H11 – H25)), 0.90 (t, ^3J = 7.2, 3H, CH_3(H26)).

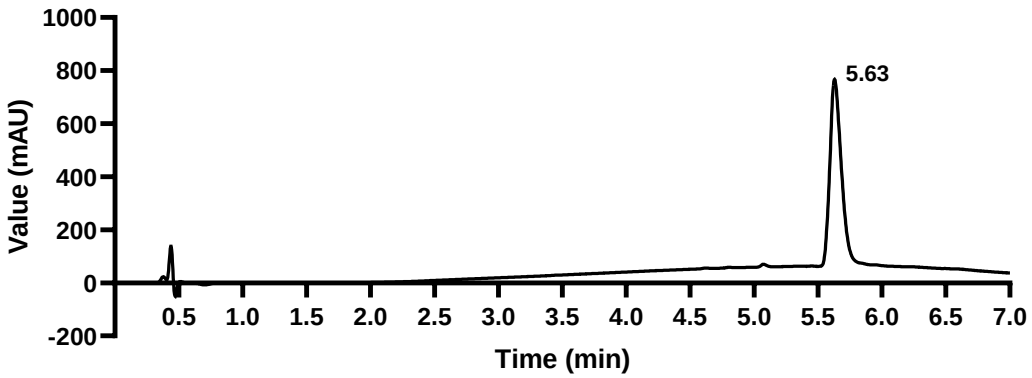


Figure 32: HPLC chromatogram of **H-18**.

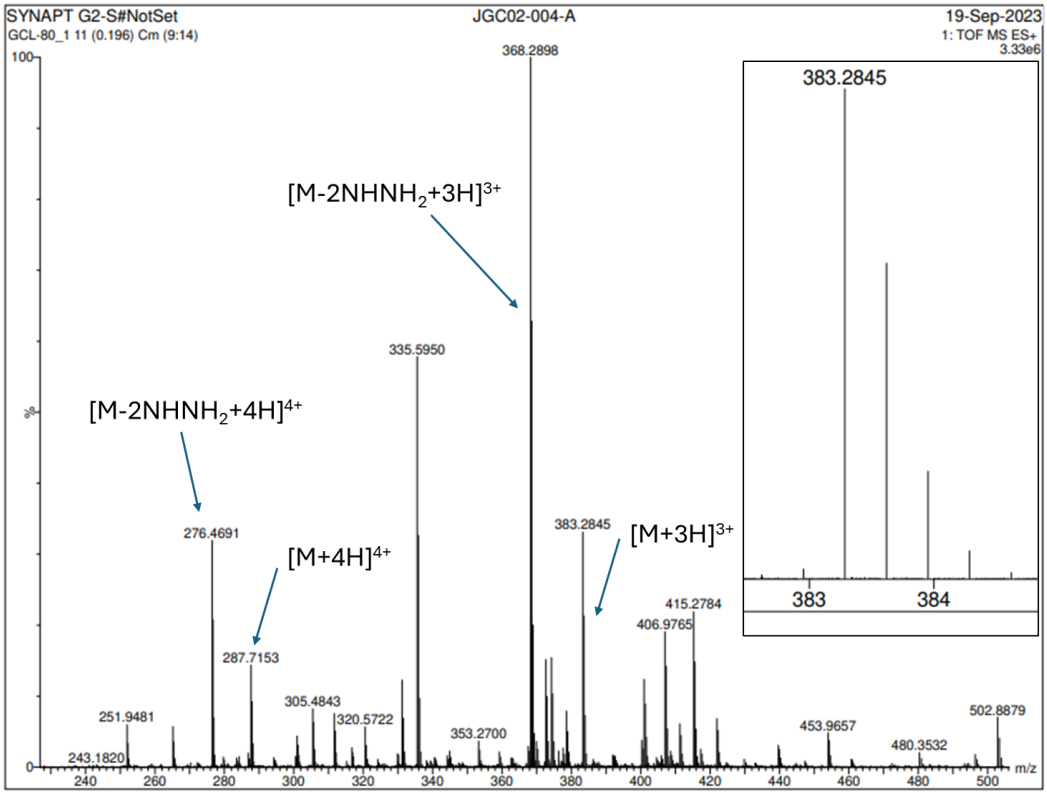


Figure 33: HR-ESI-MS spectra of **H-18**.

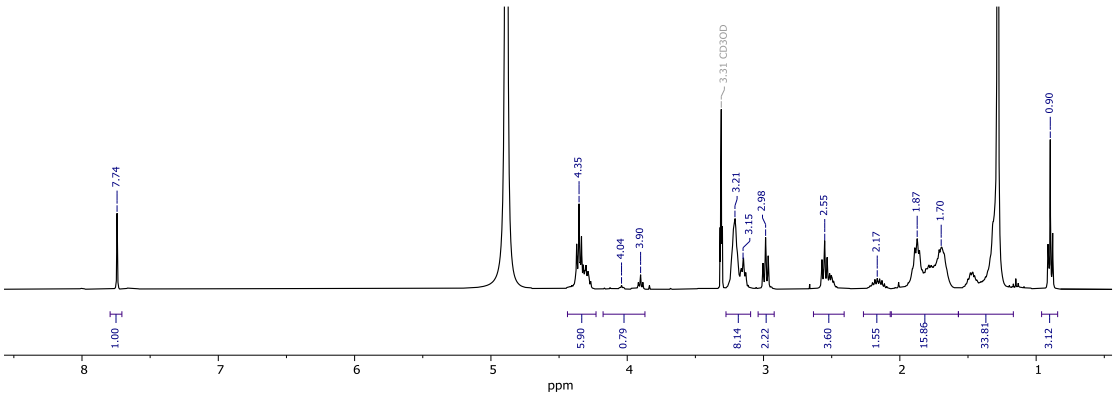
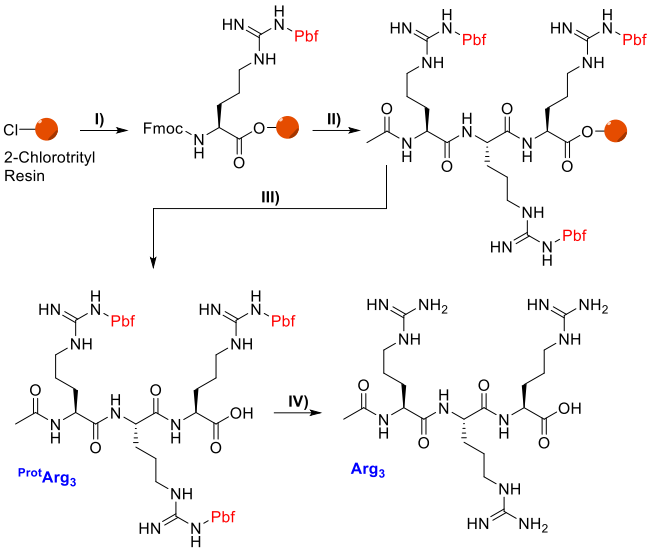
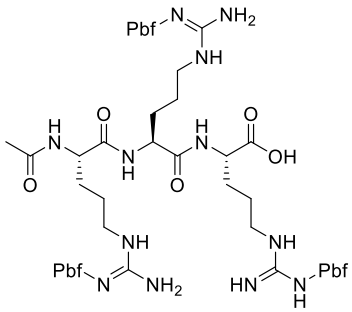


Figure 34: ^{1}H NMR (MeOD) spectrum of **H-18**.

5. Synthesis of reference peptide Arg_3



Scheme 1: Synthetic scheme for the preparation of **Arg_3** control peptide. I) First amino acid coupling and loading calculation, II) SPPS, III) Mild cleavage and IV) Deprotection.



➢ **$^{Prot}Arg_3$.** The peptide was synthesized according to the general **(SPPS)** procedure using Fmoc-*L*-Arg(Pbf)-OH. A mild cleavage using TFA/CH_2Cl_2 1/99 for 5 minutes, 4 times, then MeOH/Pyridine (8/2) was used to isolate this protected intermediate. The compound **$^{Prot}Arg_3$** was obtained after preparative **[Prep-HPLC]** purification (gradient: 0 min, 70% A, 30% B; 5 min, 70% A, 30% B; 45 min, 0% A, 100% B). 440 mg were obtained (90%). **HPLC** t_R: 5.51 min. **HR-ESI-MS** m/z calcd for $[C_{59}H_{88}N_{12}O_{14}S_3+H]^+$ 1285.5778, found 1285.5785; $[C_{59}H_{88}N_{12}O_{14}S_3+2H]^{2+}$ 643.2925, found 643.2927; $[C_{59}H_{88}N_{12}O_{14}S_3+3H]^{3+}$ 429.1974, found 429.1977.

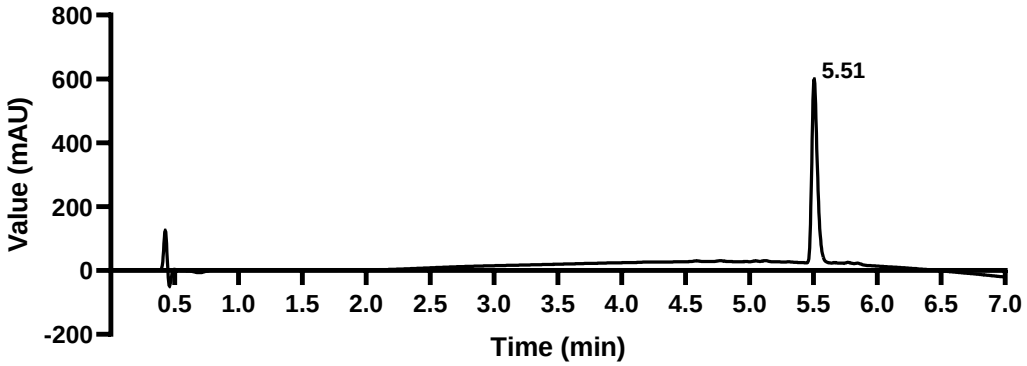


Figure 35: HPLC chromatogram of **$^{Prot}Arg_3$**.

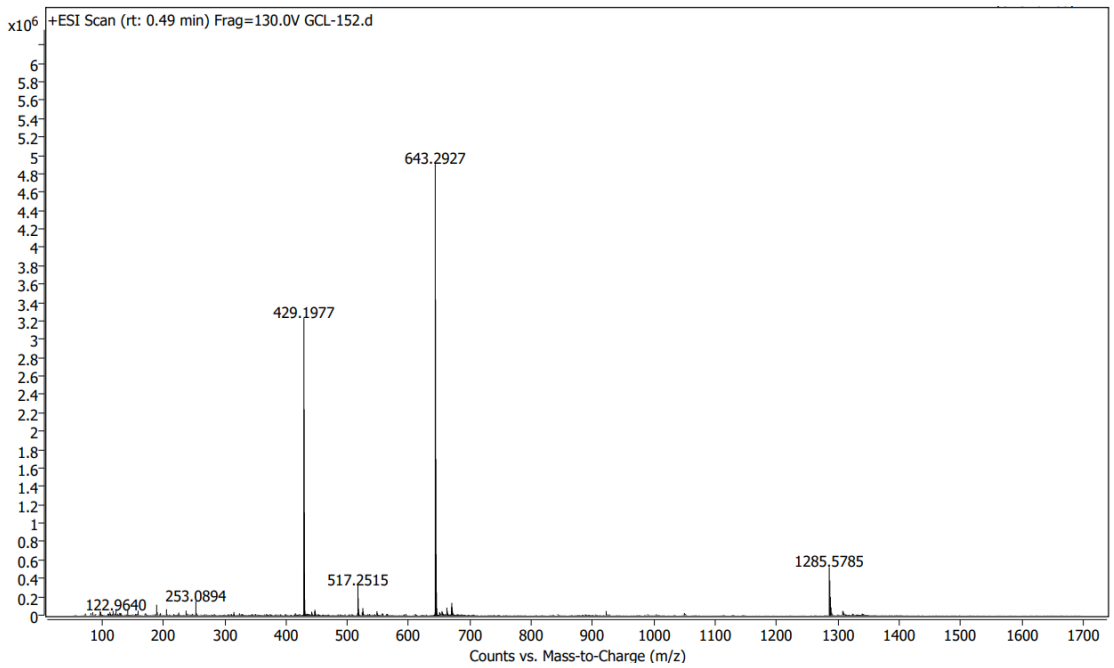
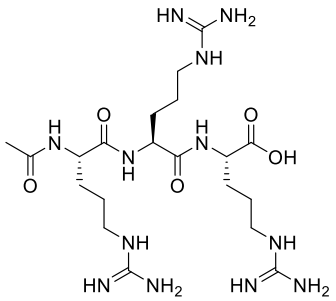


Figure 36: HR-ESI-MS spectra of ^{Prot}**Arg$_3$**.



Arg$_3$. Compound ^{Prot}**Arg$_3$** was deprotected according to the general **(SPPS)** procedure yielding 144 mg (81%) of a white solid. **HR-ESI-MS** m/z calcd for $[C_{20}H_{40}N_{12}O_5+H]^+$ 529.3317, found 529.3317; $[C_{20}H_{40}N_{12}O_5+2H]^{2+}$ 265.1695, found 265.1695; $[C_{20}H_{40}N_{12}O_5+3H]^{3+}$ 177.1154, found 177.1154. **^{1}H NMR** (D_2O) δ_H: 4.38 – 4.19 (m, 3H, Hα), 3.24 – 3.09 (m, 6H, Hδ), 1.99 (s, 3H, CH_3), 1.96 – 1.53 (m, 6H, Hβ) (m, 6H, Hγ).

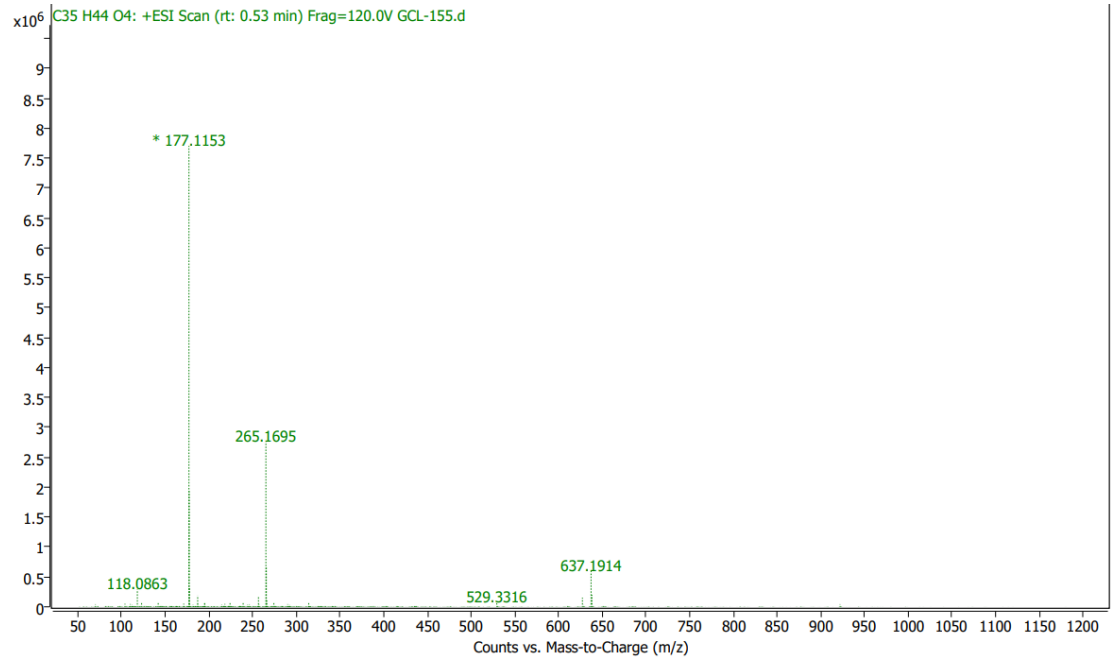


Figure 37: HR-ESI-MS spectra of **Arg$_3$**.

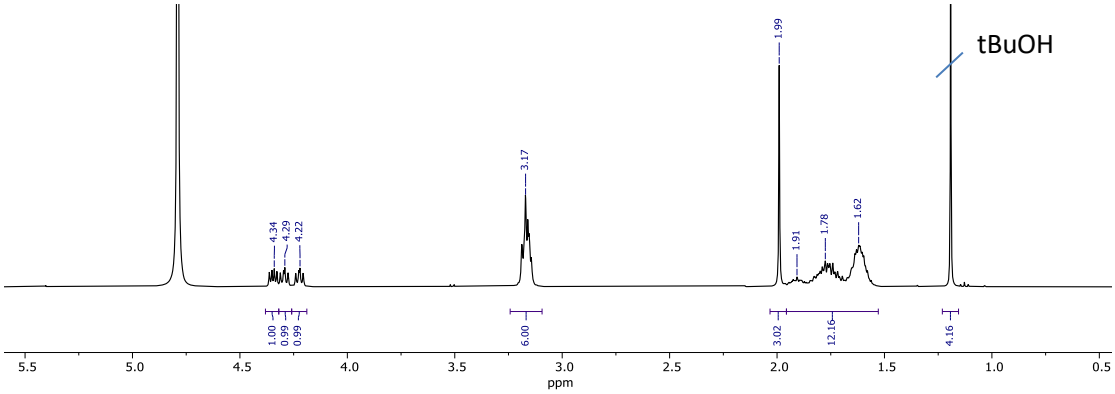


Figure 38 : ^{1}H NMR spectrum of **Arg$_3$**.

6. DLS & ζ Potential

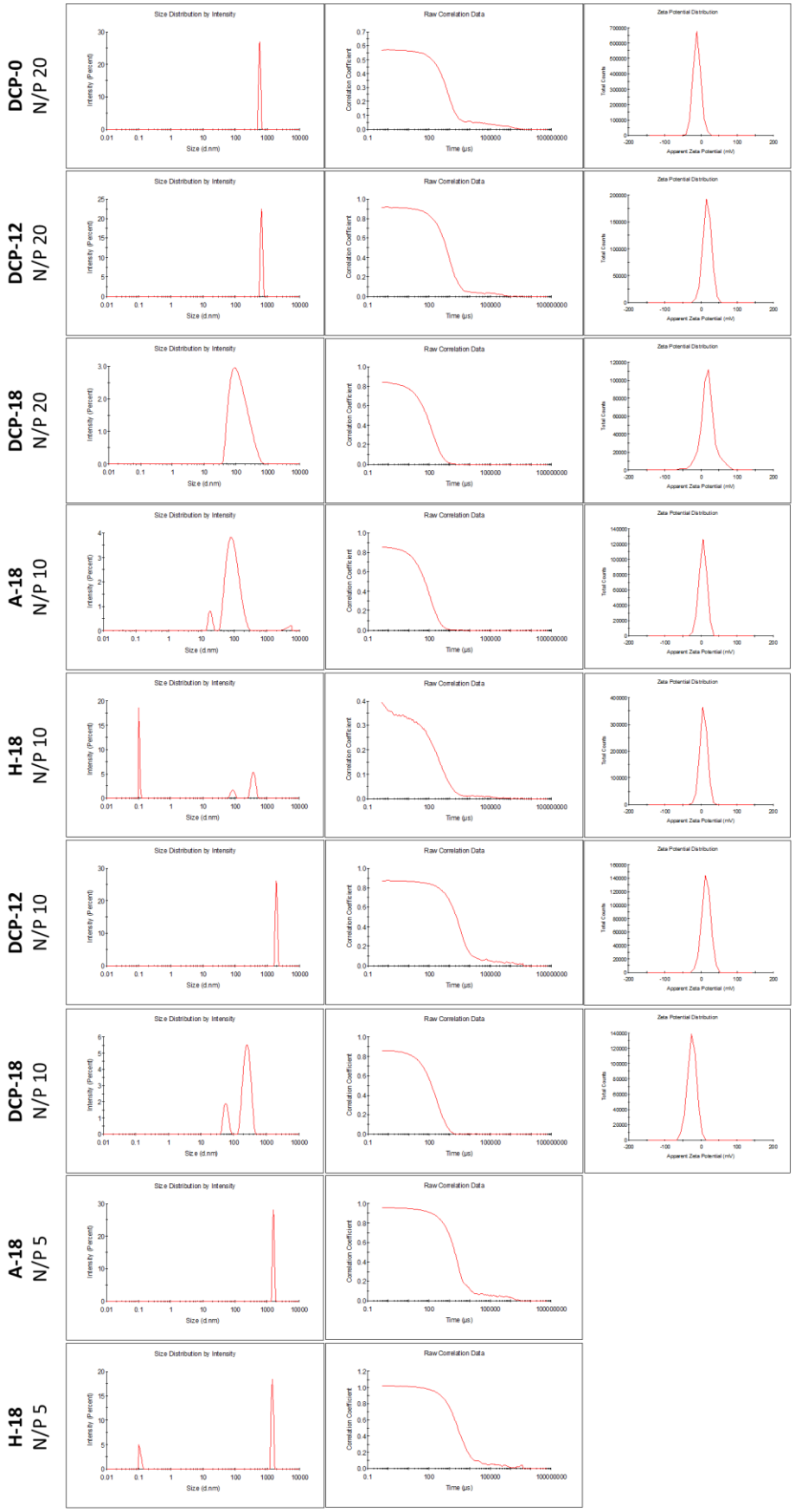


Figure 39 : DLS and ζ Potential data.

Table S1: Dynamic light scattering analyses and ζ potential measurements for samples prepared at lower charge ratio N/P 10 and 5. Error given is the standard deviation of three replicates. PDI: polydispersity index. N.d.: not determined, sample too polydisperse.

Entry	Composition	N/P	Size (nm)	PDI	ζ potential (mV)
1	**DCP-12**	10	≥ 1500	0.30	+14.4 ± 0.3
2	**A12**	5	n.d.	> 0.8	-
3	**H12**	5	n.d.	> 0.6	-
4	**DCP-18**	10	260 ± 63	0.29	-25.3 ± 0.7
5	**A18**	5	≥ 1500	0.30	-
6	**H18**	5	≥ 1400	0.40	-

7. TEM analyses

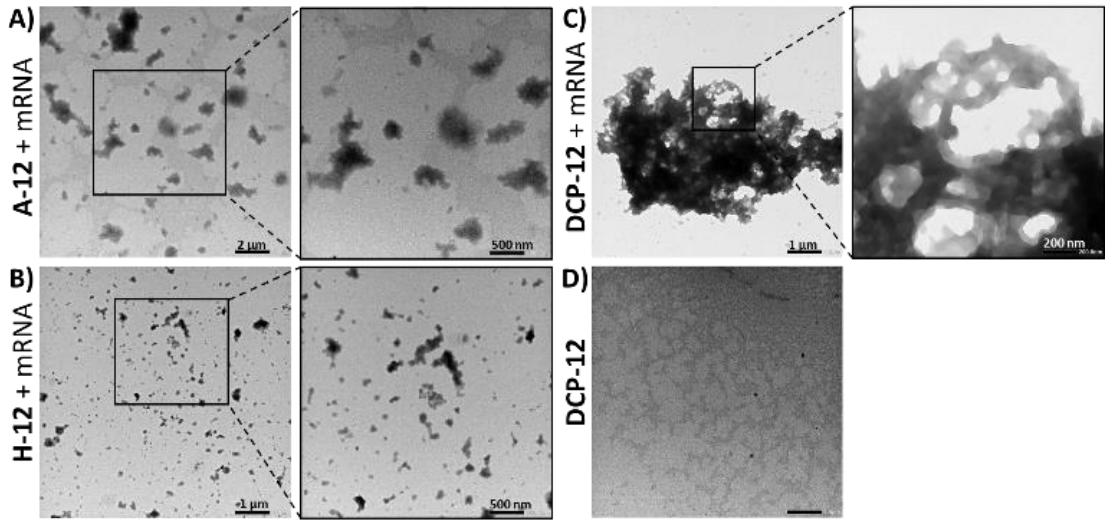
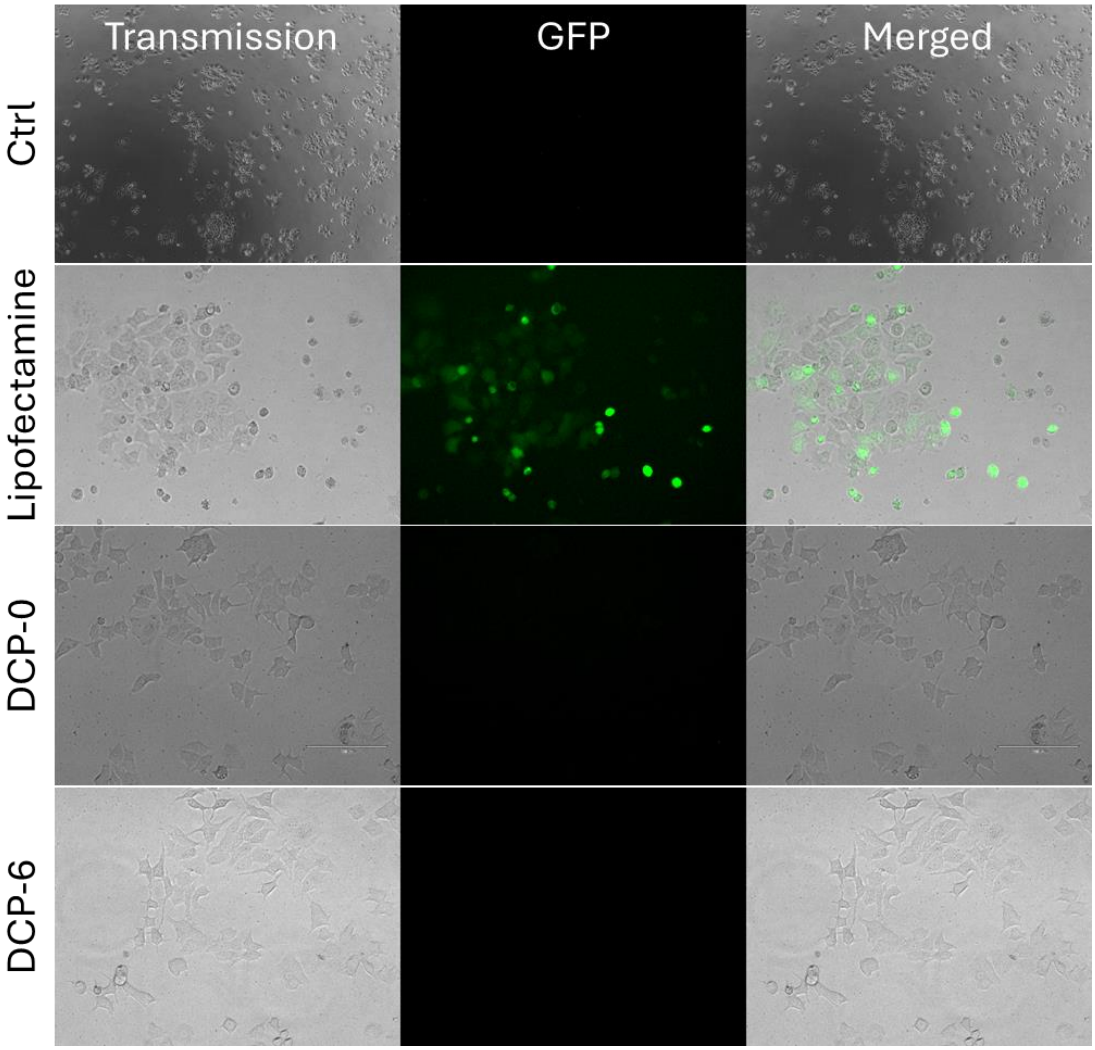
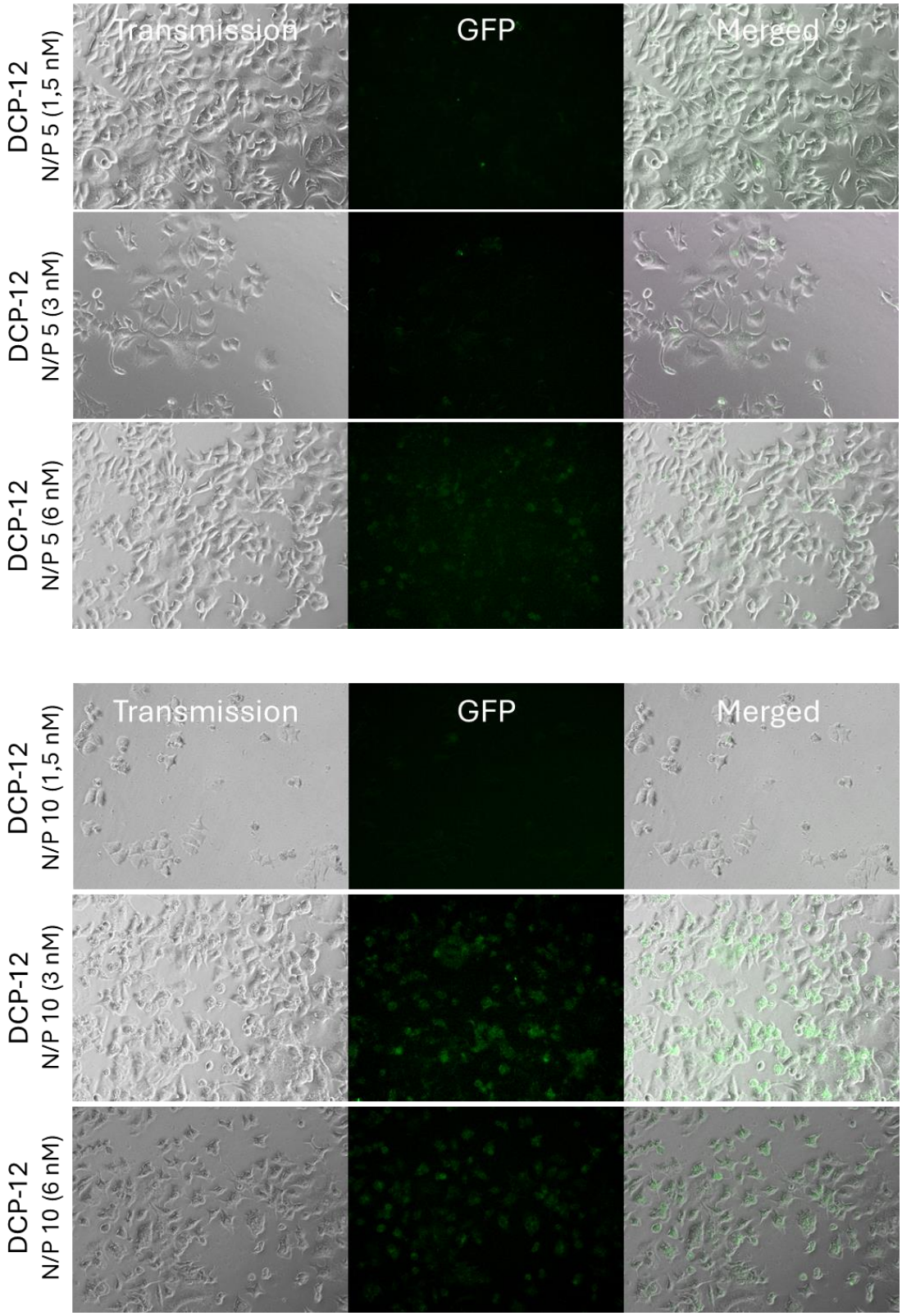
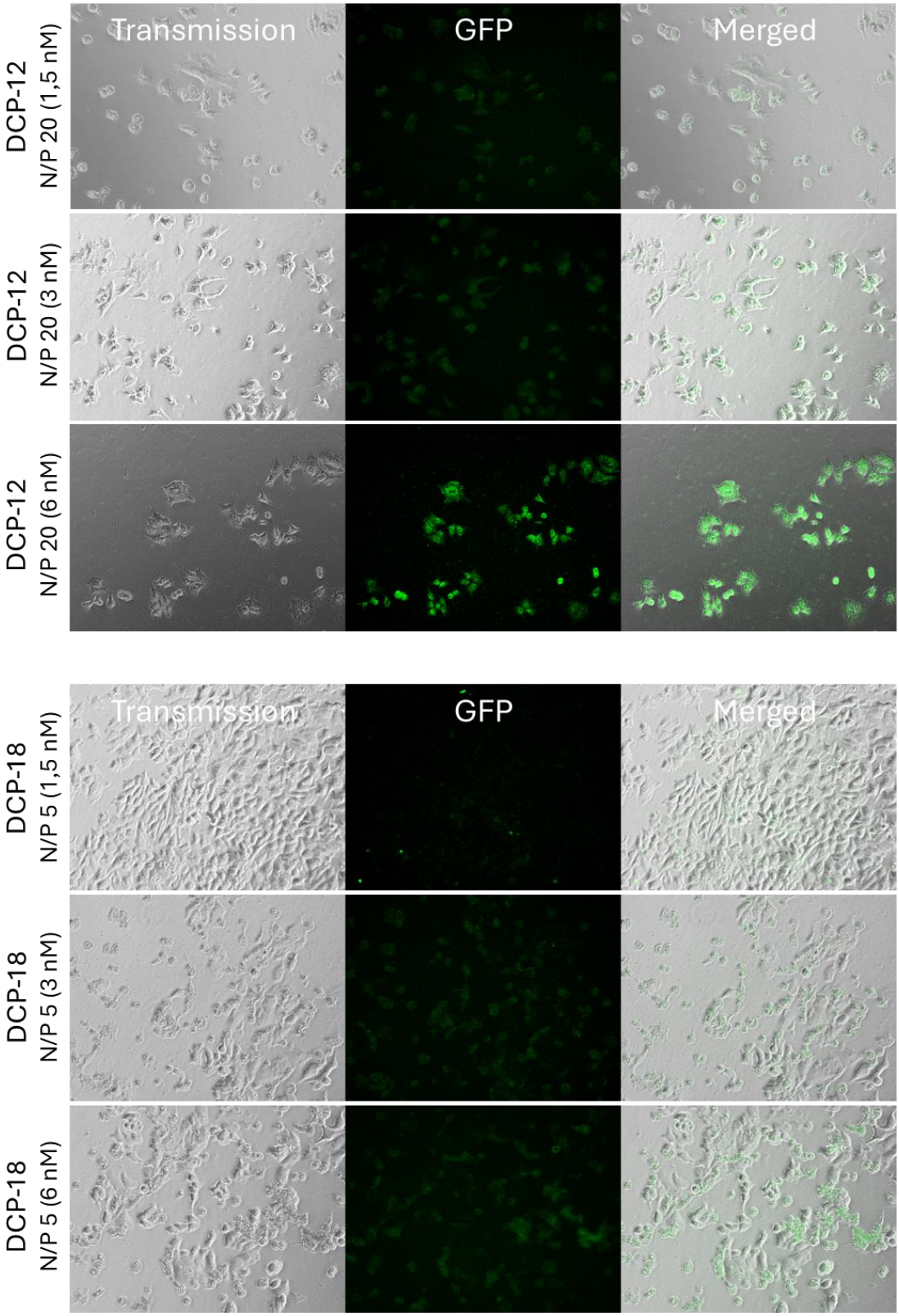


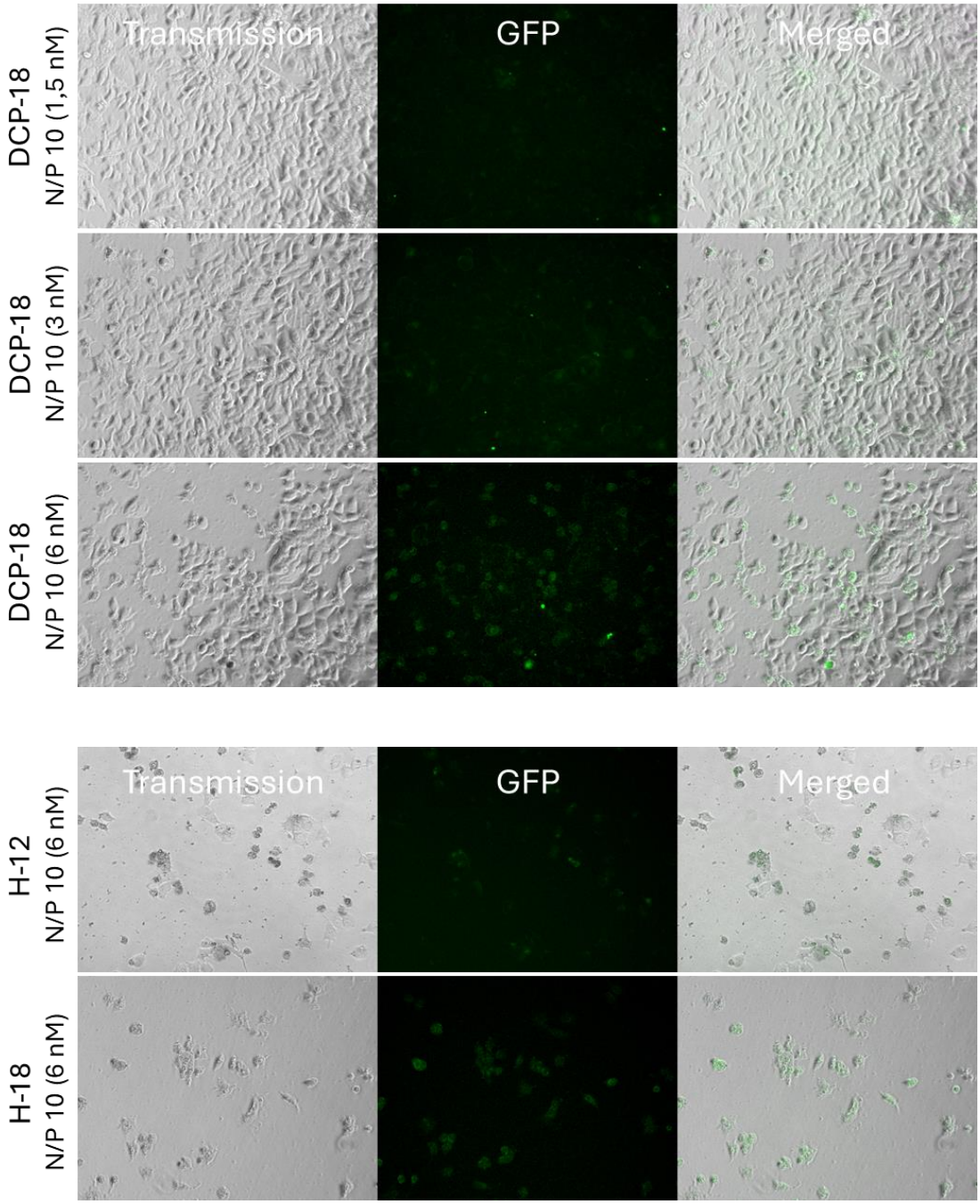
Figure 40 : TEM images of the mRNA complexes formed with **A-12** @ N/P 10 (A), **H-12** @ N/P 10 (B), **DCP-12** @ N/P 20 (C), and of the **DCP-12** alone in absence of mRNA (D)

8. mRNA delivery




Transmission
GFP
Merged
DCP-12
N/P 5 (1,5 nM)
DCP-12
N/P 5 (3 nM)
DCP-12
N/P 5 (6 nM)
Transmission
GFP
Merged
DCP-12
N/P 10 (1,5 nM)
DCP-12
N/P 10 (3 nM)
DCP-12
N/P 10 (6 nM)


Transmission
GFP
Merged
DCP-12
N/P 20 (1,5 nM)
DCP-12
N/P 20 (3 nM)
DCP-12
N/P 20 (6 nM)
Transmission
GFP
Merged
DCP-18
N/P 5 (1,5 nM)
DCP-18
N/P 5 (3 nM)
DCP-18
N/P 5 (6 nM)


Transmission
GFP
Merged
DCP-18
N/P 10 (1,5 nM)
DCP-18
N/P 10 (3 nM)
DCP-18
N/P 10 (6 nM)
Transmission
GFP
Merged
H-12
N/P 10 (6 nM)
H-18
N/P 10 (6 nM)

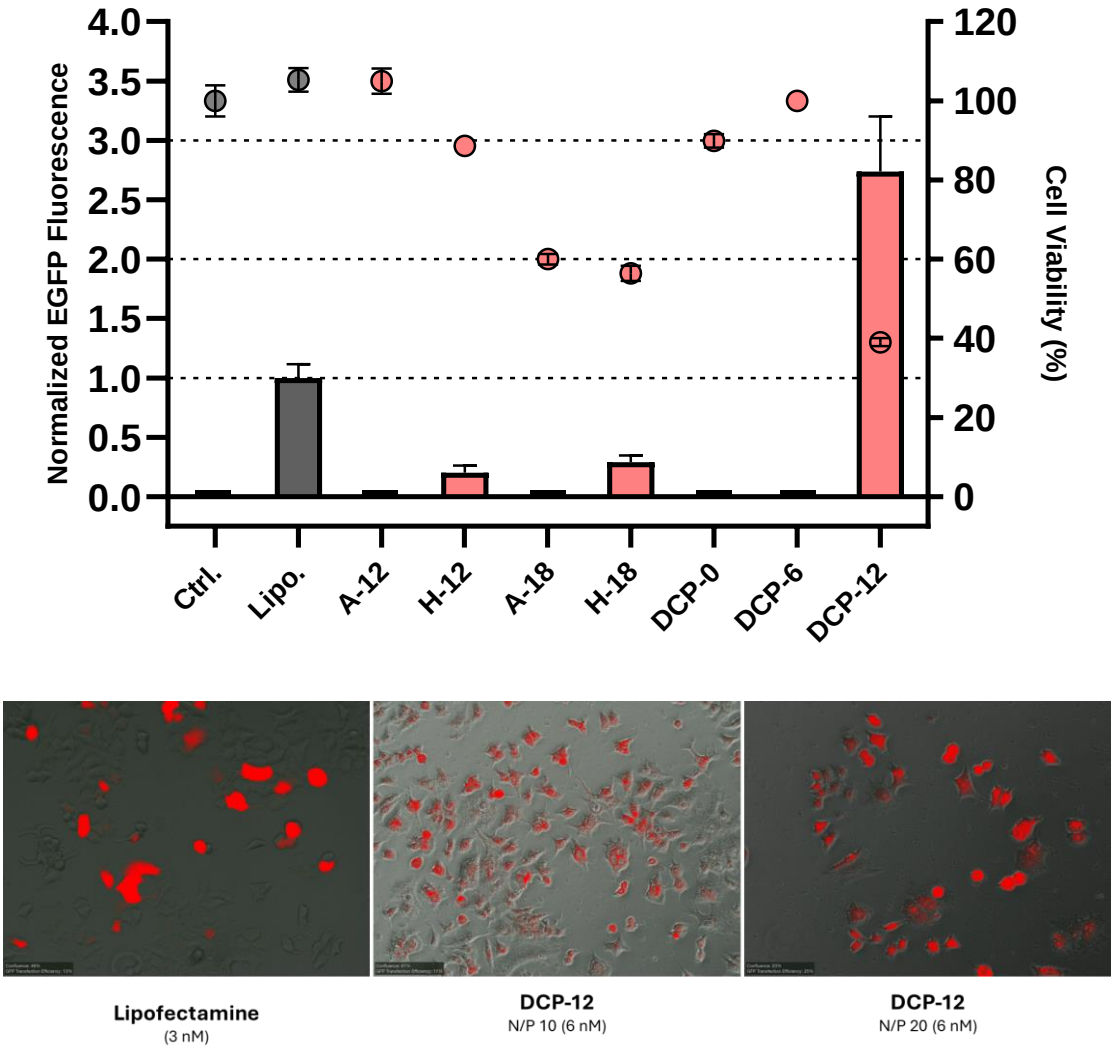


Figure 41 : top: EGFP fluorescence imaging data using monomers and DCPs at different N/P and EGFP mRNA doses, 24 hours post-transfection; graph: Quantitative analysis of EGFP fluorescence following transfection of EGFP mRNA (6 nM), normalized against lipofectamine, for the monomers and DCPs at, respectively, N/P 10 and 20 (bars), and cell viability (circles). Fluorescence measurements were performed 12 hours post-transfection. Data shows the mean ± S.E.M. of conditions performed in triplicates for activity and duplicates for cell viability; bottom: EVOS M5000 Imaging System transfection quantification threshold for Lipofectamine and **DCP-12** at N/P 10 and 20.

9. References

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