Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Assembly and optically triggered disassembly of lipid–DNA origami fibers

Sofia Julin[a], Nadine Best[a,b,c], Eduardo Anaya Plaza[a], Eeva Enlund[a], Veikko Linko[a,d] & Mauri A. Kostiainen[a,e*]

[a] Biohybrid Materials, Department of Bioproducts and Biosystems, Aalto University, P.O. Box 16100, 00076 Aalto, Finland

[b] Technische Universität Darmstadt, Katolinenpl. 5, 64289 Darmstadt, Germany

[c] Fraunhofer Institute for Microengineering and Microsystems IMM, Carl-Zeiss-Str. 18-20, 55129 Mainz, Germany

[d] Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia

[e] LIBER Center of Excellence, Aalto University, P.O. Box 16100, 00076 Aalto, Finland

[*]Correspondence and requests for materials should be addressed to mauri.kostiainen@aalto.fi

Contents

1. Materials

All chemicals were purchased from commercial suppliers and used as received unless otherwise noted. In all experiments, deionized water (Milli-Q grade) was used.

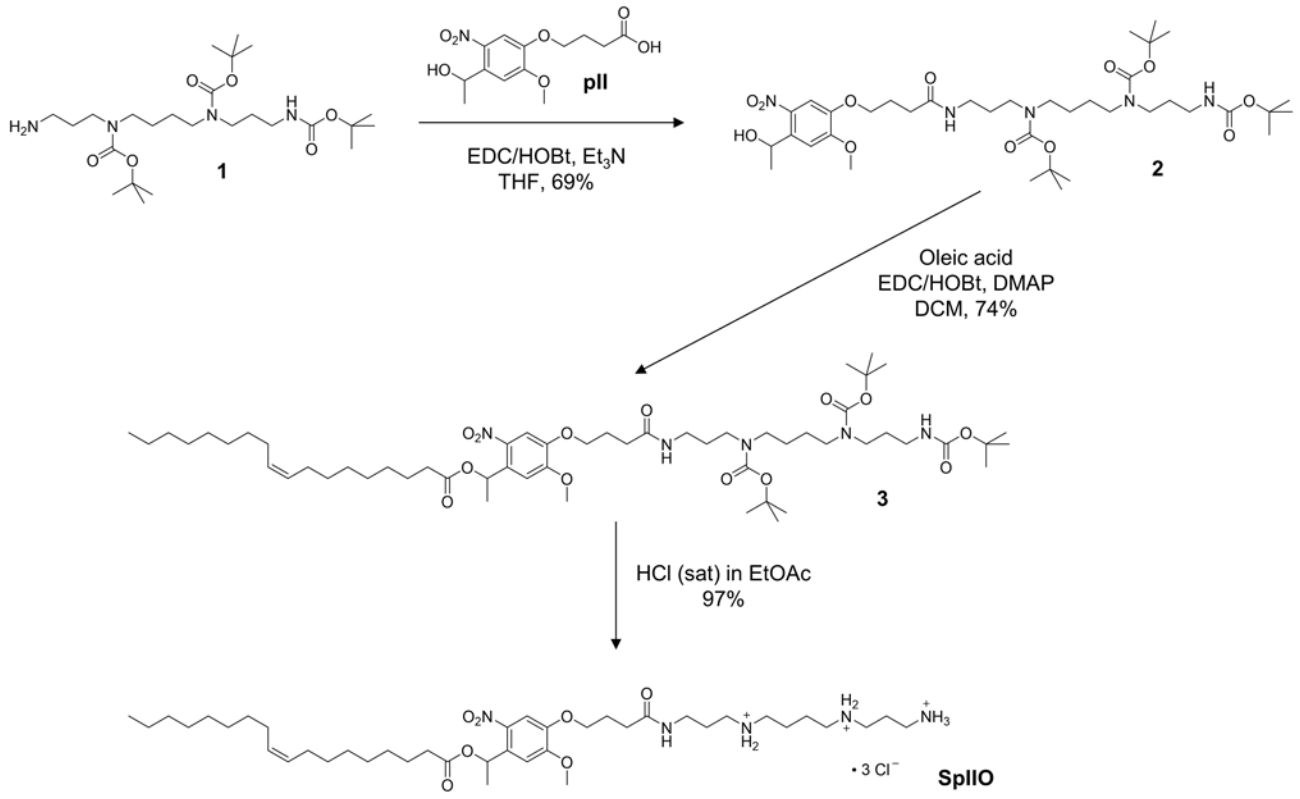
For the synthesis, spermine was purchased from MP Biomedicals, whereas 4-[4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy]butyric acid (pll), hydroxybenzotriazole (HOBt), oleic acid, and *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma Aldrich. 4-dimethylaminopyridine (DMAP) and triethylamine (Et_3N) was obtained from Acros Organics. The solvents were purchased from either Sigma Aldrich or Thermo Fischer Scientific. The reactions were monitored by thin-layer chromatography (TLC) with silica gel 60 F_{254} and the column chromatography was carried out on silcia gel 60 (40–63 μm, 230–400 mesh particle size), both purchased from Merck.

For preparation of the 6HB DNA origami, the circular single-stranded p7249 scaffold (c = 100 nM) was purchased from Tilibit Nanosystems and the single-stranded staple strands from Integrated DNA Technologies. 50× TAE buffer (2 M tris(hydroxymethyl)aminomethane (Tris), 1 M acetic acid, 50 mM ethylenediaminetetraacetic acid (EDTA), pH 8.4) was purchased from Thermo Fischer Scientific.

For the agarose gel electrophoresis (AGE), the ethidium bromide and gel loading dye solution were purchased from Sigma Aldrich, whereas the agarose was purchased from Meridian Bioscience. The gel loading dye solution (0.25% bromophenol blue, 0.25% xylene cyanol, 40% sucrose) was diluted 1:30 in 40% (w/v) sucrose before used. For staining the TEM samples, uranyl formate was purchased from Electron Microscopy Sciences. For the DNase I digestion assays, the DNase I (from bovine pancreas) was obtained from Sigma Aldrich.

2. Methods

2.1. Synthesis of Spermine-hydroxyethyl photolinker-oleic ester (SpIIO)



Scheme S1. Synthesis of spermine-hydroxyethyl photolinker-oleic ester (**SpIIO**).

BOC-spermine (1)
Compound **1** was synthesised as previously reported, and the characterization data was in full agreement with previously published data.[1]

BOC-spermine-pll (2)
Compound **2** was synthesized mainly as previously reported.[2] Compound **1** (425 mg, 0.85 mmol) and 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitropehynoxy]-butyric acid (pll, 242.6 mg, 0.81 mmol) were dissolved into 10 mL of THF in the dark, and deoxygenerated by N_2 bubbling. EDC (158.2 mg, 1.02 mmol), HOBt (156.3 mg, 1.02 mmol) and Et_3N (150 µL, 1.07 mmol) were added, and the mixture was further deoxygenated by N_2 bubbling. The mixture was left to stir at room temperature in the dark for 12 h. The progress of the reaction was monitored by TLC using ninhydrin stain. The dicyclohexylurea precipitate was removed by filtration, after which the crude product was purified by column chromatography using DCM/MeOH (97:3) as eluent. The target product **2** was obtained as a light-yellow solid (562.1 mg, 69%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): δ 7.51 (s, 1H), 7.30 (s, 1H), 5.52 (d, J = 6.1 Hz, 1H), 4.06 (t, J = 6.2 Hz, 2H), 3.92 (s, 3H), 3.14 (m, 12H), 2.38 (t, J = 7.1 Hz, 2H), 2.15 (t, J = 7.1 Hz, 3H), 1.72 – 1.53 (m, 5H), 1.49 (d, J = 6.2 Hz, 3H), 1.42 (s, 27H).

^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): δ 172.2, 154.2, 139.5, 109.1, 108.9, 79.9, 68.7, 65.6, 56.4, 46.8, 35.8, 32.8, 28.5, 28.4, 25.0, 24.6.

BOC-spermine-pll-oleic ester (3)
First, oleic acid (38 µL, 0.12 mmol) and DMAP (10 mg, 0.081 mmol) were dissolved into 10 mL of dry DCM and degassed by N_2 bubbling. EDC (60 mg, 0.39 mmol) was dissolved into 5 mL of DCM and slowly added to the reaction mixture kept under inert atmosphere at 0 °C. Next, compound **2** (101.8 mg, 0.12 mmol) was dissolved into dry DCM, deoxygenated by N_2 bubbling, and added dropwise to the reaction mixture. The reaction was stirred for 30 min at 0 °C, after which the reaction was allowed to reach room temperature and stirred for another 12 h. The reaction was all the time kept in the dark.

Reaction progress was monitored by TLC using ninhydrin stain. The EDC urea precipitate was removed by filtration, and the supernatant was washed three times with distilled water, one time with brine. The combined organic phases were dried with $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by chromatography column, using DCM/MeOH (9:1) as eluent. The target product **3** was obtained as a light-yellow solid (97.2 mg, 74%)

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): δ 7.57 (s, 1H), 6.99 (s, 1H), 6.46 (q, J = 6.4 Hz, 1H), 5.34 (dt, J = 5.8, 4.5 Hz, 2H), 4.11 (t, J = 6.3 Hz, 2H), 3.93 (d, J = 9.6 Hz, 3H), 3.72 (qd, J = 7.0, 5.3 Hz, 2H), 3.17 (m, 12H), 2.40 (m, 2H), 2.33 (td, J = 7.5, 5.1 Hz, 2H), 2.25 – 2.13 (m, 2H), 2.05 – 1.94 (m, 4H), 1.61 (t, J = 8.3 Hz, 8H), 1.45 (bs, 27H), 1.36 – 1.16 (m, 21H), 0.88 (t, J = 6.9 Hz, 3H).

Spermine-pll-oleic ester (SpllO)
Compound **3** (97.2 mg, 0.093 mmol) was dissolved in a small amount of EtOAc in the dark, after which 5 mL of HCl-saturated EtOAc was added dropwise at 0 °C. The reaction was left to warm up to room temperature and stirred for 1 h. The precipitate was filtered, and washed with abundant EtOAc, resulting in an off-white solid (67.2 mg, 97%).

^{1}H NMR (MeOH-d4, 400 MHz) 7.59 (s, 1H), 7.12 (s, 1H), 6.34 (t, J = 6.4 Hz, 1H), 5.32 (m, 2H), 4.10 (t, J = 6.0 Hz, 2H), 3.93 (s, 3H), 3.36 – 3.27 (bs, 2H), 3.09 (m, 10H), 2.45 (t, J = 7.2 Hz, 2H), 2.35 (t, J = 7.3 Hz, 2H), 2.12 (s, 4H), 2.01 (d, J = 5.8 Hz, 4H), 1.94 – 1.79 (m, 6H), 1.59 (m, 5H (3+2H)), 1.28 (s, 20H), 0.88 (t, J = 8.3, 3H).

^{13}C NMR (MeOD-d4, 100 MHz) δ (ppm): δ 176.4, 174.4, 155.5, 148.8, 141.5, 134.1, 130.9, 130.7, 110.1, 109.7, 69.8, 69.3, 57.0, 48.2, 46.5, 46.0, 37.95, 36.92, 35.18, 33.2, 33.0, 30.8, 30.7, 30.6, 30.4, 30.3, 30.2, 30.2, 30.1, 28.1, 28.1, 27.8, 26.3, 26.0, 25.4, 24.4, 24.3, 23.8, 22.0, 14.5.

ESI-MS (*m/z*) Calculated value for $C_{41}H_{73}N_5O_7$ 747.551, found 748.557 $[M+H]^+$

2.2. Folding and purification of 6HB DNA origami

The design and staple strands for the 6-helix bundle (6HB) DNA origami structure can be found in the original publication by Bui *et al.*[3] However, in the 6HB used in this work, the extended staple strands, originally used for quantum dot attachment, have been shortened to avoid undesired overhangs in the structure.

The 6HBs were folded in 50 µL quantities in a one-pot folding reaction by mixing p7249 scaffold (final concentration of 20 nM), 10× excess of staple strands (final concentration of 200 nM) in folding buffer (FOB, 1× TAE, 12.5 mM $MgCl_2$). The folding reaction was thermally annealed in a ProFlex PCR system using the following thermal ramp: (1) Cooling from 90 to 70 °C at a rate of –0.2°C / 8 s; (2) cooling from 70 to 60 °C at a rate of –0.1°C / 8 s; (3) cooling from 60 to 27 °C at a rate of –0.1°C / 2 min; (4) cooling down to 20 °C and stay at this temperature unit the program is manually stopped.

After the folding of the 6HB, the excess staple strands were removed using polyethylene glycol (PEG) precipitation.[4] First, the 6HB solution ($c \sim 20$ nM) was diluted 4-fold with 1× FOB. The diluted 6HB solution was mixed 1:1 with PEG precipitation buffer (15% PEG 8000, 1× TAE, 505 mM NaCl) before centrifuged at 14 000g for 30 min at room temperature using an Eppendorf 5424R microcentrifuge. The supernatant was carefully removed, and the 6HB pellet resuspended in 1× FOB to 0.25–1× of the initial reaction volume. To properly dissolve the pellet, the 6HB solution was incubated overnight at 30 °C under continuous shaking at 600 rpm using an Eppendorf Thermomixer C.

If needed, the 6HB solution was further upconcentrated after the PEG-purification using spin-filtration with 100 kDa molecular weight cut off (MWCO) spin-filters (0.5 mL, Amicon Ultra, Merck). Before use, the filter was washed with 200 µL of 1× FOB that was centrifuged through the filter at 12 000g for 5 min at room temperature using an Eppendorf 5424R microcentrifuge. 400 µL of PEG-purified 6HB solution (c = 55-70 nM) was added to the filter and the filter was centrifuged for 20 min at 6 000g. The 6HB solution was collected into a fresh tube by inverting the filter and centrifuging at 2 000g for 2.5 min.

The DNA origami concentration was estimated from the absorbance at 260 nm (A_{260}) using Beer-Lambert law. The absorbance was measured using a BioTek Eon Microplate Spectrophotometer and a Take3 microvolume plate. A sample size of 2 µL was used for the measurements and the concentration was obtained as the average of three measurements. The molar extinction coefficient for the 6HB was estimated to 0.98×10^8 $M^{-1}cm^{-1}$ based on the number of non-hybridized and hybridized nucleotides in the DNA origami unit.[5]

2.3. Co-assembly of 6HB and SpllO

The **SpllO**-6HB complexes were prepared by mixing PEG-purified 6HBs with the desired amount of **SpllO** (dissolved in deionized water) in 1× FOB supplemented with 0–1000 mM NaCl. The **SpllO** was always added to the mixture as the last

compound. To allow the formation of assemblies, the samples were incubated at room temperature for at least 30 min before further analysis by AGE, TEM or SAXS.

2.4. DNase I digestion assay

SpllO-6HB complexes were prepared by mixing PEG-purified 6HBs (final concentration of 7.5/50 nM) with **SpllO** (dissolved in deionzed water) at a ratio of $n_{\mathrm{SpllO}}/n_{\mathrm{6HB}} \sim 8000$ in 1× FOB supplemented with 200 mM NaCl. Similarly, also plain 6HB samples (final concentration of 7.5/50 nM) were prepared in 1× FOB supplemented with 200 mM NaCl. The samples were incubated at room temperature for 30 minutes before $CaCl_2$ was added to a final concentration of 1 mM. Next, DNase I ($c =$ 0.8 Kunitz units (KU) μL^{-1}) and deionized water were added to obtain samples with final DNase I concentrations of 0, 25, 50, 75, and 100 KU mL^{-1}. The samples were incubated at room temperature for 60 min (c_{6HB} = 7.5 nM) or 6 h 40 min (c_{6HB} = 50 nM) before the DNase I was inactivated by addition of 1% sodium dodecyl sulfate (SDS) to a final concentration of 0.04%. Immediately after the DNase I inactivation, the samples were analysed by AGE.

2.5. Photolytic degradation of SpllO

The photolytic degradation of plain **SpllO** was studied using UV/Vis spectroscopy. 750 µL of aqueous **SpllO** solution (c = 0.1 mg mL^{-1}) was placed in a quartz cuvette and irradiated for different time periods using a Rayonet RPR-200 photochemical reactor equipped with 16 RPR-3500A lamps (intensity approximately 9.2 mW cm^2, λ = 350 nm) while keeping the quartz cuvette on ice. After the UV-A irradiation. the UV/Vis spectra was measured using an Agilent Cary 5000 UV-Vis-NIR spectrophotometer.

2.6. Disassembly of SpllO-6HB complexes by UV-A light

The **SpllO**-6HB complexes were prepared by mixing PEG-purified 6HBs (final concentration of 7.5 nM or 50 nM) with **SpllO** (dissolved in deionzed water) at a ratio of $n_{\mathrm{SpllO}}/n_{\mathrm{6HB}} \sim 8000$ in 1× FOB supplemented with 200 mM NaCl. The samples were incubated at room temperature for at least 30 minutes before irradiated for different time periods using a Rayonet RPR-200 photochemical reactor equipped with 16 RPR-3500A lamps (intensity approximately 9.2 mW cm^2, λ = 350 nm). The samples were kept in PCR tubes on ice for the UV-A irradiation. After the UV-A irradiation, the samples were analyzed by AGE or TEM.

2.7. Nuclear magnetic resonance

^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker NMR Spectrometer AV III 400 using deuterated chloroform ($CDCl_3$) or methanol (MeOH-d4) as the solvent. The residual solvent peak was used as an internal standard for peak calibration.

2.8. Electrospray ionization mass spectrometry

The samples for electrospray ionization (ESI) mass spectrometry were prepared by dissolving **SpllO** in an ACN/deionized water mixture (1:1) to a final concentration of ∼1 mM. The ESI mass analysis was performed with an Agilent Technologies Accurate-Mass Q-TOF LC/MS 6530 using direct injection. Samples were detected by ITC, and ionized at 150.0 V.

2.9. Agarose gel electrophoresis

Agarose gel electrophoresis (AGE) was used to confirm the correct folding of the 6HB as well as the removal of excess staple strands by PEG precipitation. The binding of SpllO to the 6HBs, the DNase I digestion, and the release of the 6HBs from the complexes upon UV-A irradiation were studied using an agarose gel electrophoretic mobility shift assay (EMSA). A 2 % (w/v) agarose gel was prepared in 1× TAE buffer supplemented with 11 mM $MgCl_2$ and 0.46 µg mL^{-1} ethidium bromide. Before loading the samples into the gel pockets, gel loading dye solution was added to the samples (volume of 10–25 µL depending on the gel) at a ratio of 1:5. The gel was run at a constant voltage of 95 V for 45 minutes using a BioRad Wide Mini-Sub Cell GT System and a BioRad PowerPac Basic power supply. 1× TAE supplemented with 11 mM $MgCl_2$ was used as running buffer and the gel electrophoresis chamber was kept on an ice bath during the run. The gel was visualized by UV light using a BioRad Gel Doc XR+ documentation system.

2.10. Transmission electron microscopy (TEM)

The transmission electron microscopy (TEM) samples were prepared on glow-charged (20 s oxygen plasma flash) Formvar carbon-coated copper grids (FCF400-Cu, Electron Microscopy Science) mainly following the protocol previously described by Castro *et al.*[6] For all TEM samples, 3 µL of sample solution was applied onto the carbon-coated side of the gird and incubated for 1.5–3 min before excess sample solution was blotted away with filter paper. The exact incubation times for different sample solutions are given in Table S1. Subsequently, the samples were negatively stained with 2% (w/v) aqueous uranyl formate solution containing 25 mM NaOH (added to increase the pH). First, the TEM grid was immersed into a 5 µL droplet of uranyl formate solution, after which the the stain was immediately blotted away using filter paper. Next, the TEM grid was immersed into a 20 µL droplet of uranyl formate solution, and incubated for 45 s before excess stain was removed using filter paper. The TEM sample grids were left to dry under ambient conditions for at least 15 min before imaged using a FEI Tecnai Bio-Twin electron microscope operated at an acceleration voltage of 120 kV or a JEOL JEM-2800 electron microscope operated at an acceleration voltage of 100 kV. The TEM images were processed using ImageJ.

Table S1. Incubation times for different TEM sample solutions.

Sample solution and concentration	Incubation time
Plain 6HB solution, c_{6HB} = 7.5 nM	2 min
Plain **SpllO** solution, c_{SpllO} = 0.34 mg mL^{-1}	2 min
Plain **SpllO** solution, c_{SpllO} = 0.69 mg mL^{-1}	1.5 min
SpllO-6HB complex, c_{6HB} = 7.5 nM	3 min
SpllO-6HB complex, c_{6HB} = 50 nM	2 min
SpllO-6HB complex, c_{6HB} = 100 nM	1.5 min
Photocleaved **SpllO** solution, c_{SpllO} = 0.34 mg mL^{-1}	2 min
Photocleaved **SpllO**-6HB complex, c_{6HB} = 7.5 nM	3 min
Photocleaved **SpllO**-6HB complex, c_{6HB} = 50 nM	2 min

2.11. Small-angle X-ray scattering (SAXS)

For the small-angle X-ray scattering (SAXS) measurements, 10 µL of sample solution (c_{6HB} = 250 nM) was placed in glass capillaries (1.5 mm, Hilgenberg GmbH). The SAXS mesurements were performed using an Xenocs Xeuss 3.0 instrument (Cu Kα radiation, λ = 1.54 Å). To reduce background scattering from air, the sample chamber environment including the detector was in vacuum. The beam was mono-chromated and collimated for standard flux. The sample-to-detector distance was set to 600 mm, and a lanthanum hexaboride (LaB_6) standard sample was used for calibration of the length of the scattering vector q. One-dimensional SAXS data was obtained by azimuthally averaging the 2D scattering data, and the magnitude of the scattering vector, q, is given by $q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle.

3. Characterization of spermine-pll-oleic ester (SpllO)

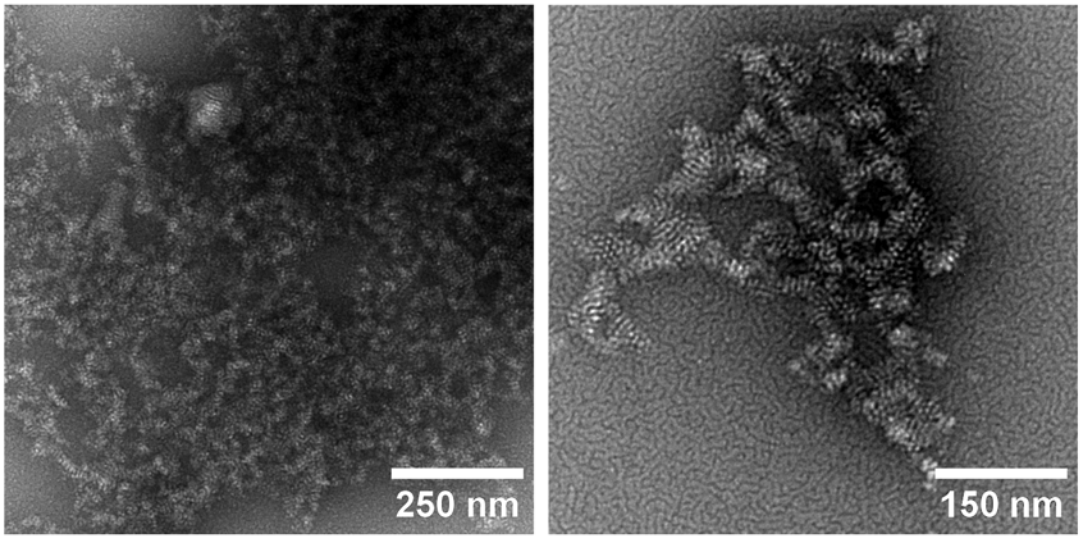


Figure S1. TEM images of spermine-pll-oleic ester (**SpllO**) (c = 0.34 mg mL^{-1}) in 1× FOB (1× TAE, 12.5 mM $MgCl_2$). The TEM samples are negatively stained with uranyl formate (2 % (w/v)).

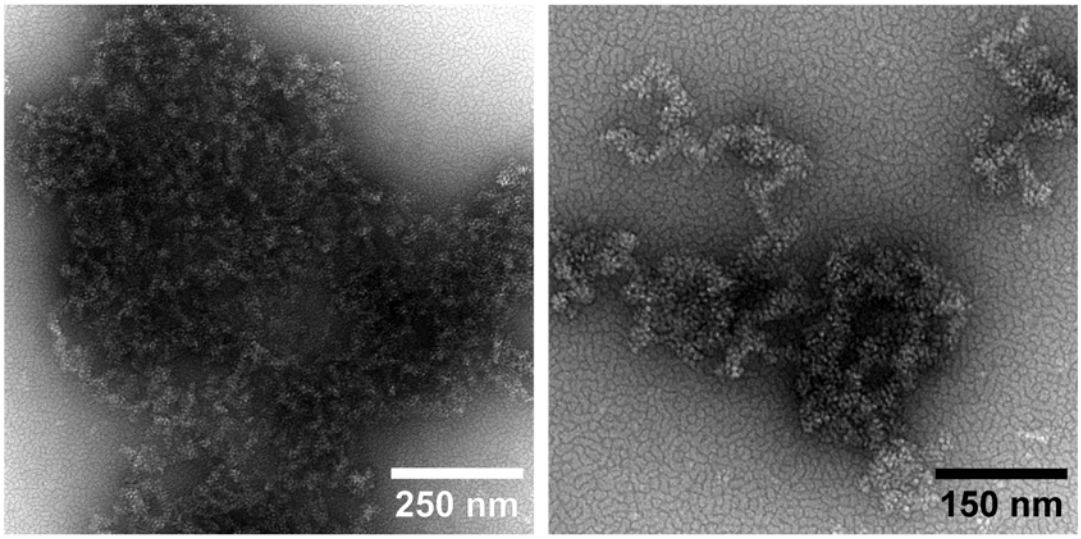


Figure S2. TEM images of **SpllO** (c = 0.69 mg mL^{-1}) in 1× FOB (1× TAE, 12.5 mM $MgCl_2$) supplemented with 200 mM NaCl. The TEM samples are negatively stained with uranyl formate (2 % (w/v)).

4. Characterization of 6HB DNA origami structure

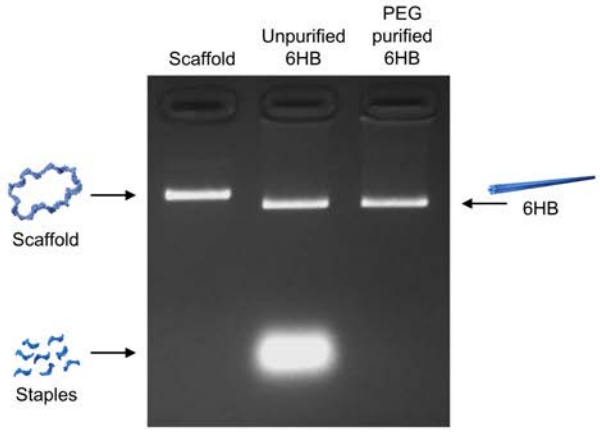


Figure S3. Characterization of the folding and PEG purification of the 6-helix bundle (6HB) DNA origami by agarose gel electrophoresis (AGE). The p7249 scaffold concentration is 30 nM and the 6HB concentration is 15 nM in the gel. The gel (2% (w/v)) was run at a constant voltage of 95 V for 45 min.

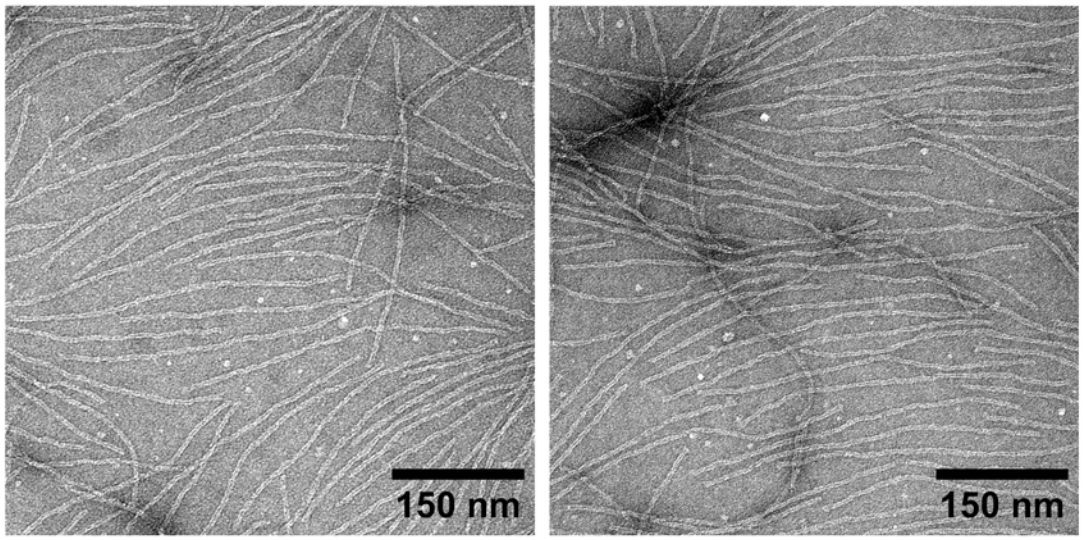


Figure S4. TEM images of the 6HB (c = 7.5 nM) in 1× FOB (1× TAE, 12.5 mM $MgCl_2$). The TEM sample is negatively stained with uranyl formate (2 % (w/v)).

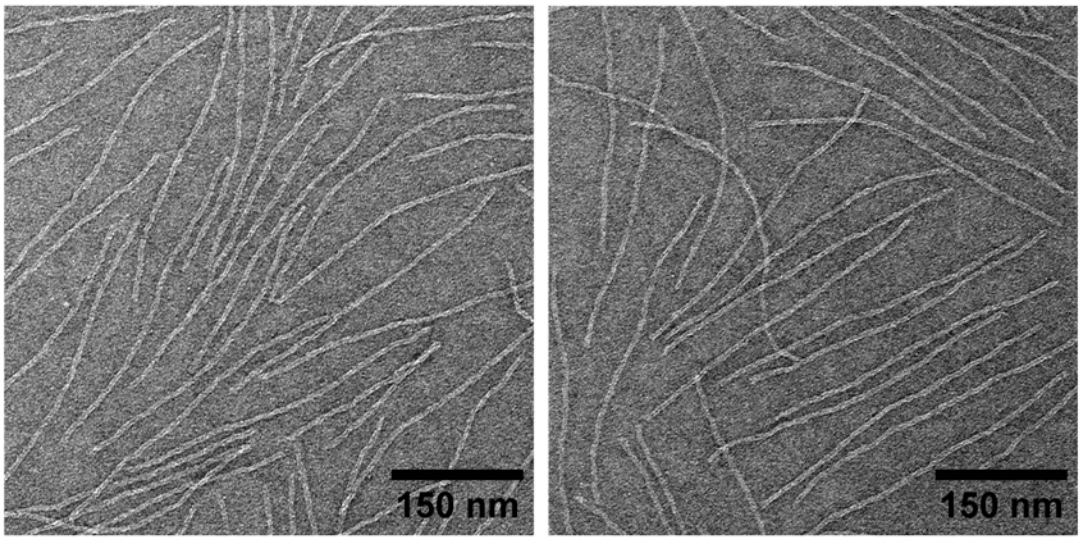


Figure S5. TEM images of the 6HB (c = 7.5 nM) in 1× FOB (1× TAE, 12.5 mM $MgCl_2$) supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 % (w/v)).

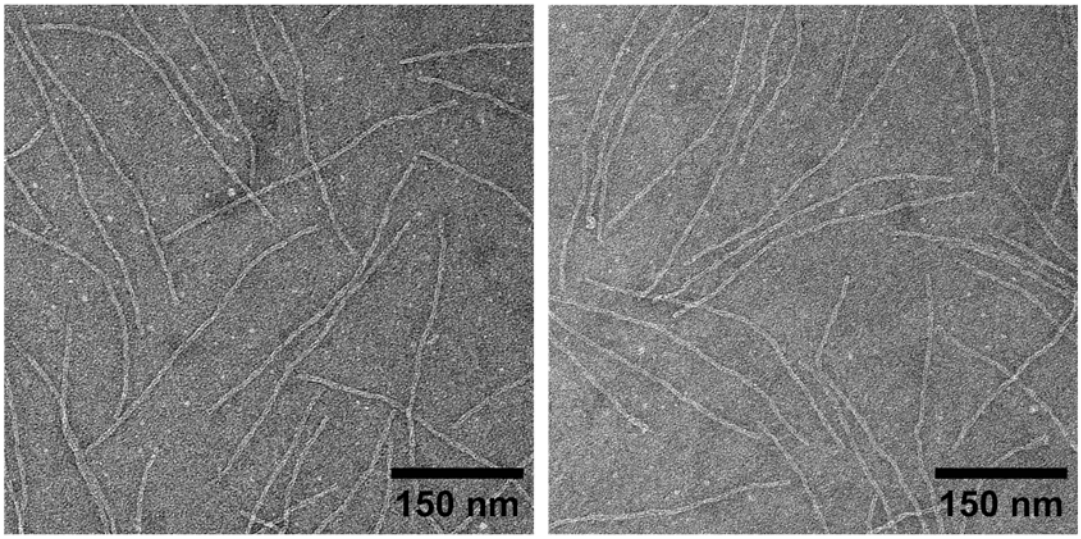


Figure S6. TEM images of the 6HB (c = 7.5 nM) in 1× FOB (1× TAE, 12.5 mM $MgCl_2$) supplemented with 500 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 % (w/v)).

5. Characterization of lipid-DNA origami assemblies

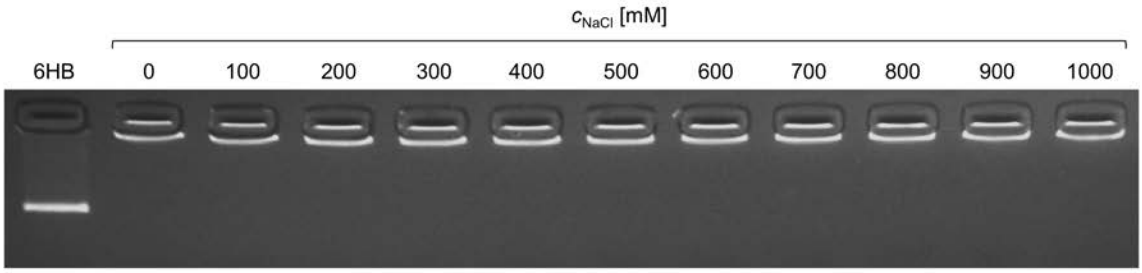


Figure S7. Agarose gel electrophoretic shift assay (EMSA) of **SpIIO**-6HB complexes (c_{6HB} = 7.5 nM, $n_{SpIIO}/n_{6HB} \sim 3000$) prepared in 1× FOB supplemented with increasing amounts of NaCl. The 6HB sample is a control sample containing only 6HB solution.The gel (2% (w/v)) was run at a constant voltage of 95 V for 45 min.

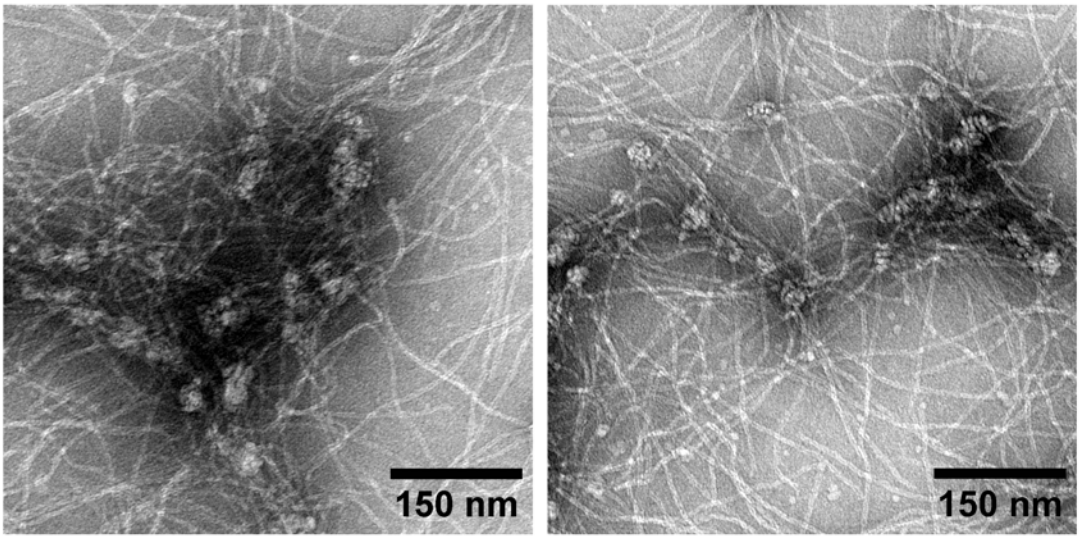


Figure S8. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 7.5 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 1000$ in 1× FOB supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 % (w/v)).

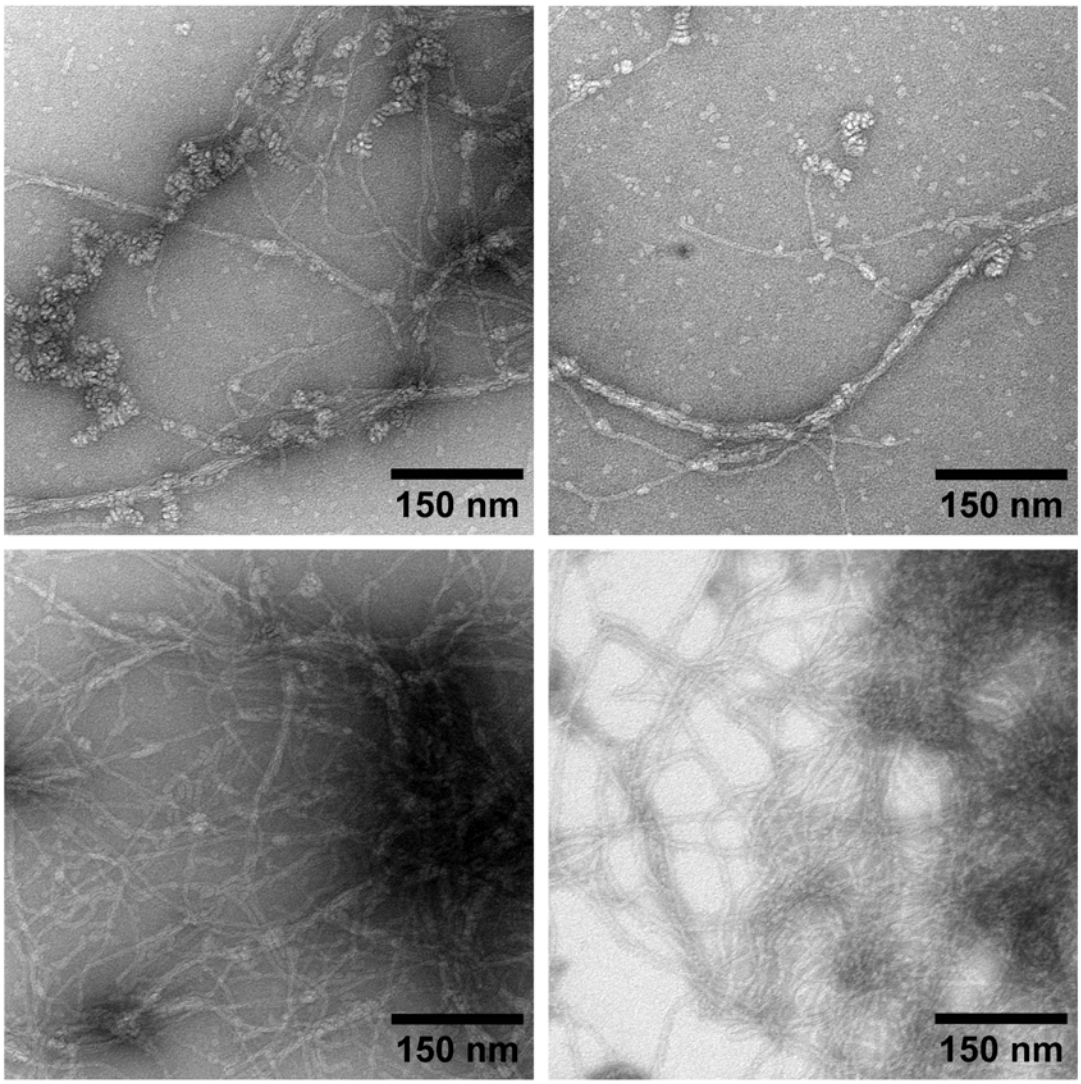


Figure S9. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 7.5 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 6000$ in 1$\times$ FOB supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 $\%$ (w/v)).

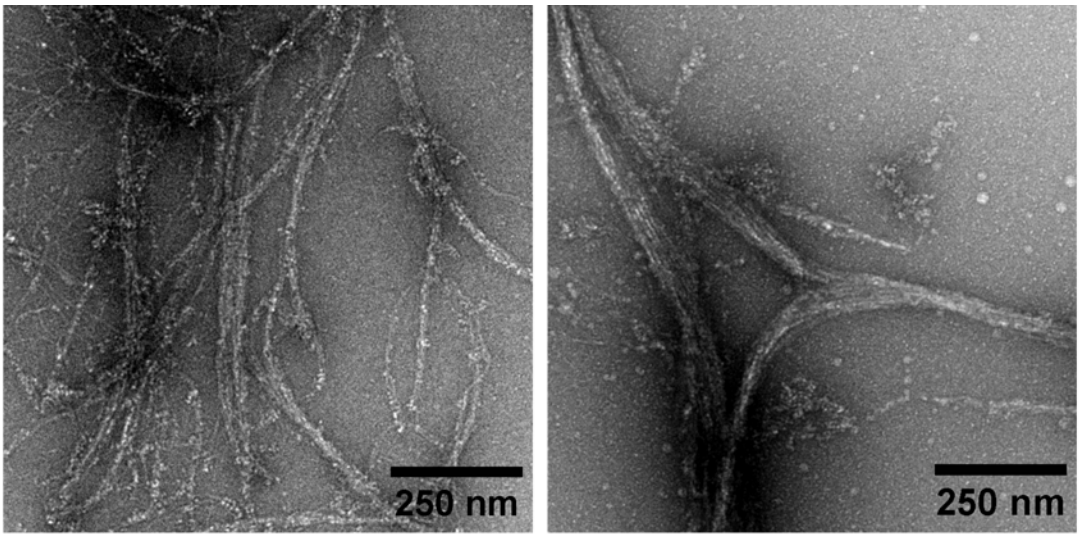


Figure S10. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 7.5 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 8000$ in 1$\times$ FOB supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 $\%$ (w/v)).

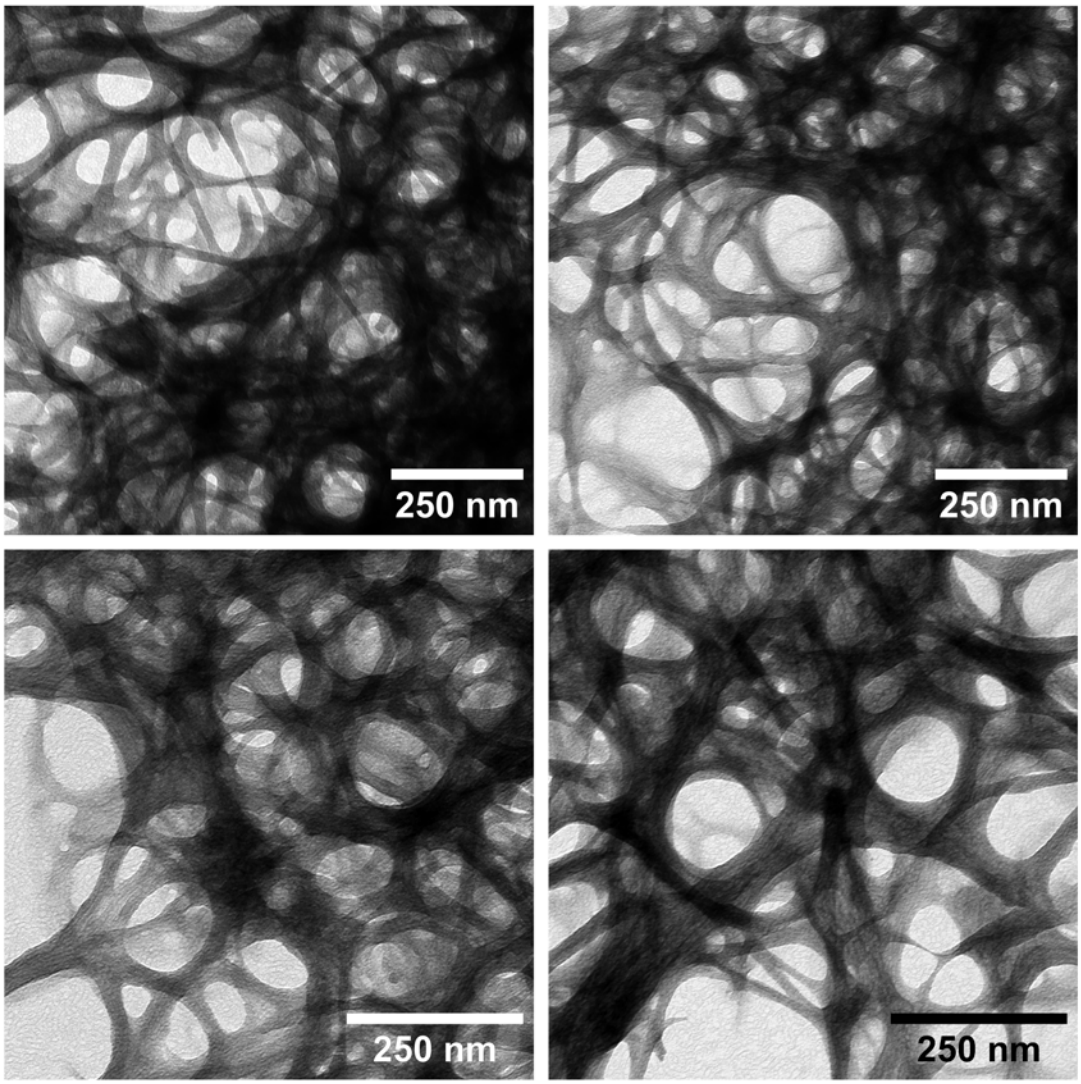


Figure S11. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 50 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 8000$ in 1$\times$ FOB. The TEM sample is negatively stained with uranyl formate (2 $\%$ (w/v)).

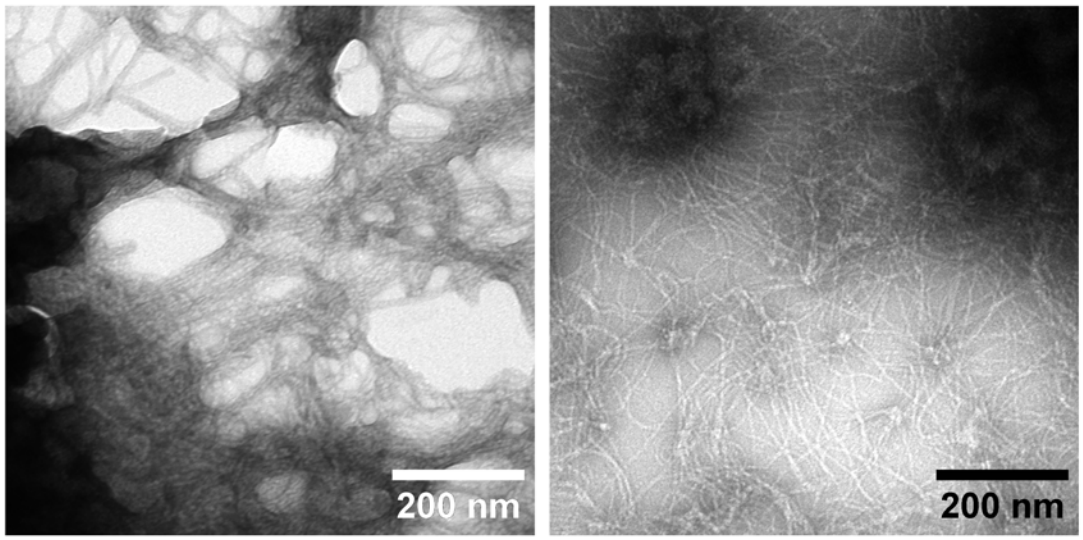


Figure S12. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 50 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 4000$ in 1$\times$ FOB supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 $\%$ (w/v)).

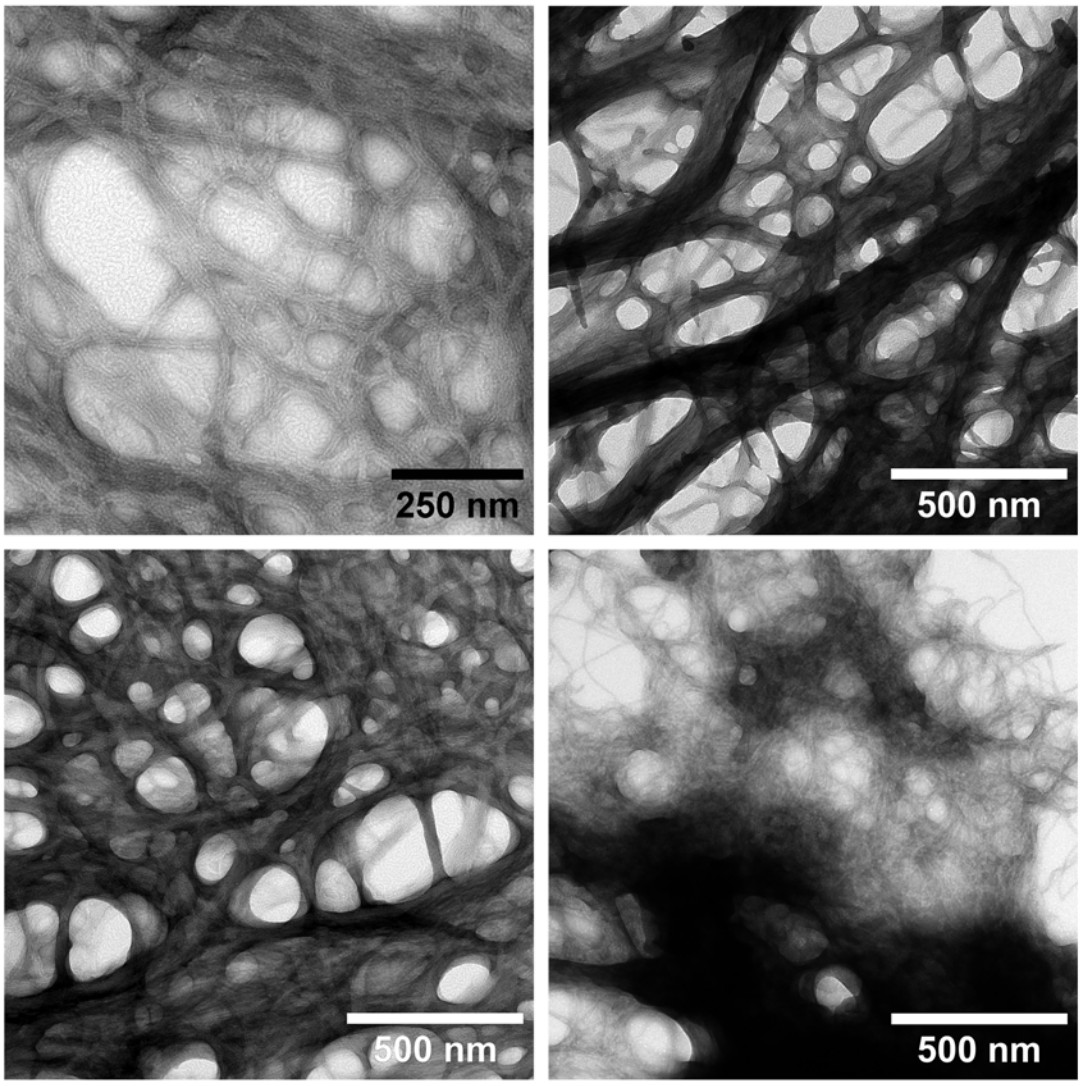


Figure S13. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 50 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 6000$ in 1× FOB supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 % (w/v)).

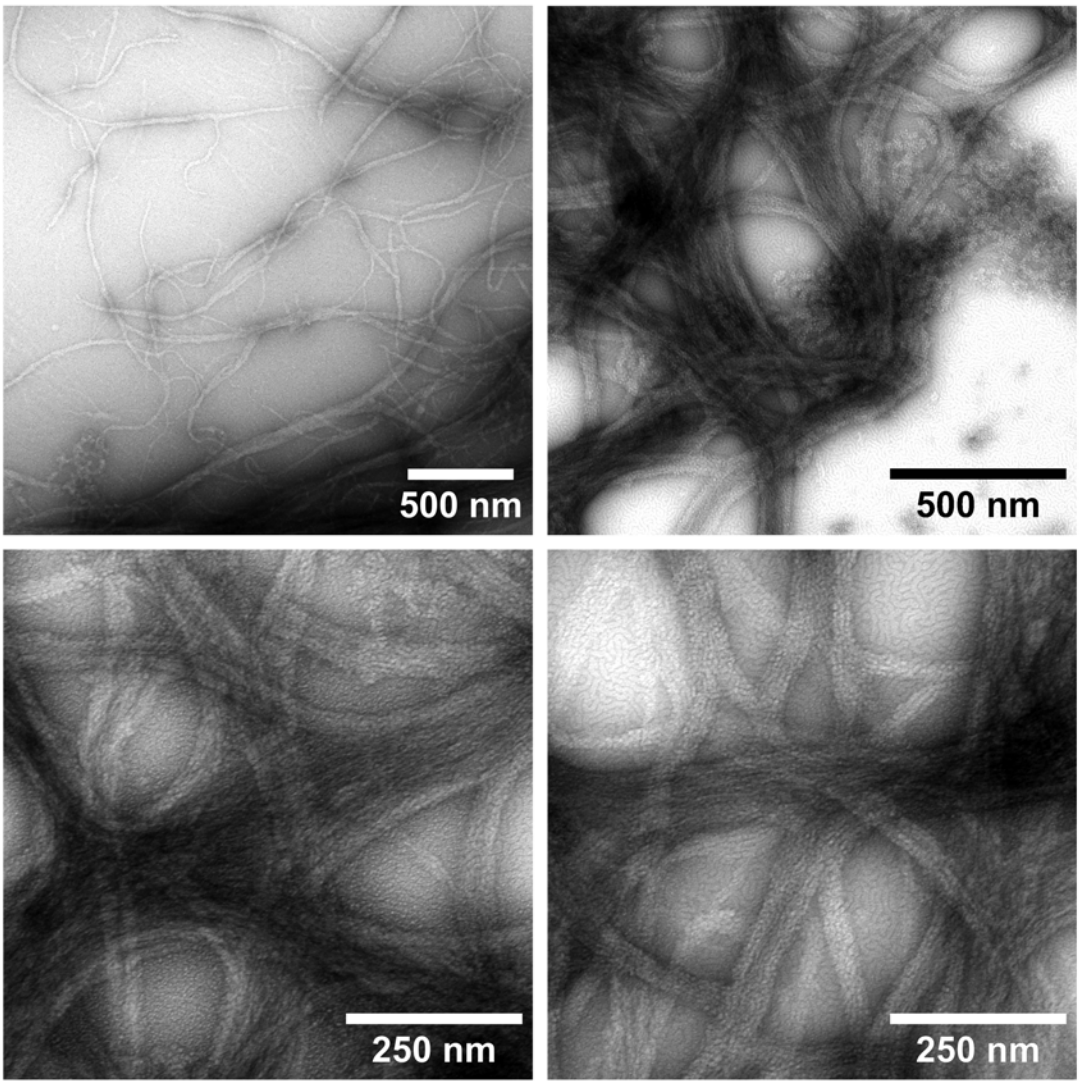


Figure S14. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 50 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 8000$ in 1$\times$ FOB supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 $\%$ (w/v)).

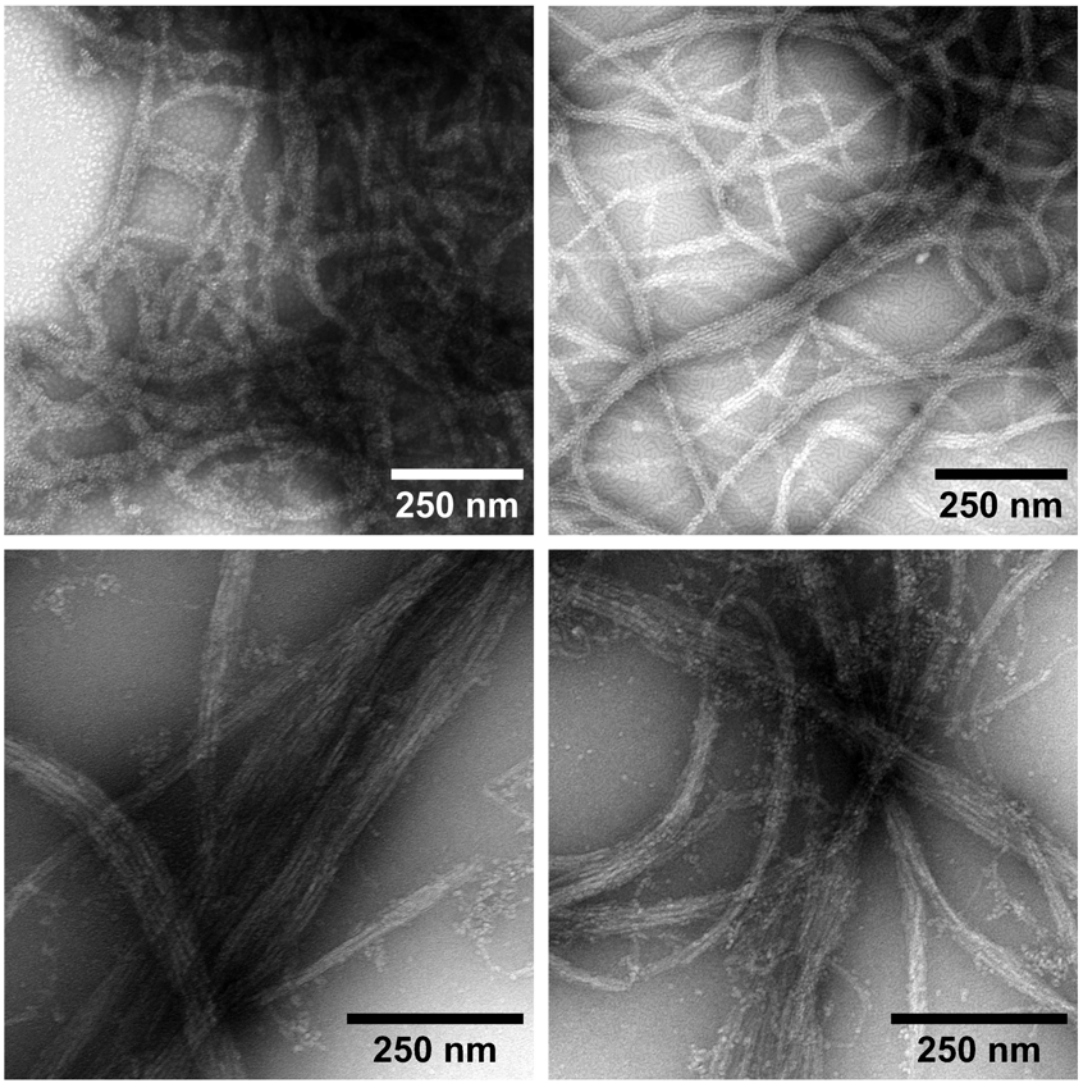


Figure S15. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 50 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 8000$ in 1× FOB supplemented with 500 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 % (w/v)).

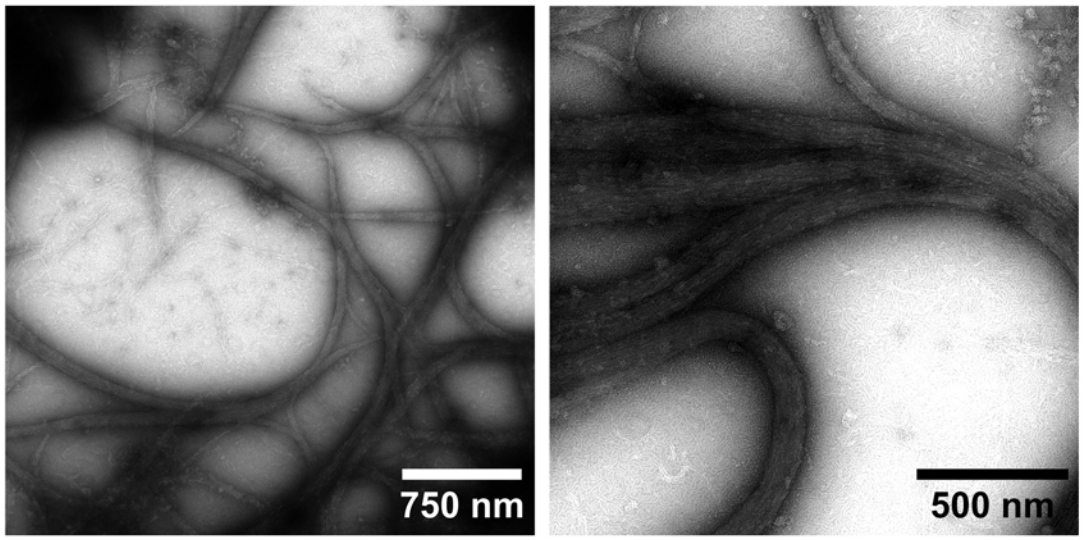


Figure S16. TEM images of the **SpIIO**-6HB complexes (c_{6HB} = 100 nM) formed at ratio of $n_{SpIIO}/n_{6HB} \sim 8000$ in 1× FOB supplemented with 200 mM NaCl. The TEM sample is negatively stained with uranyl formate (2 % (w/v)).

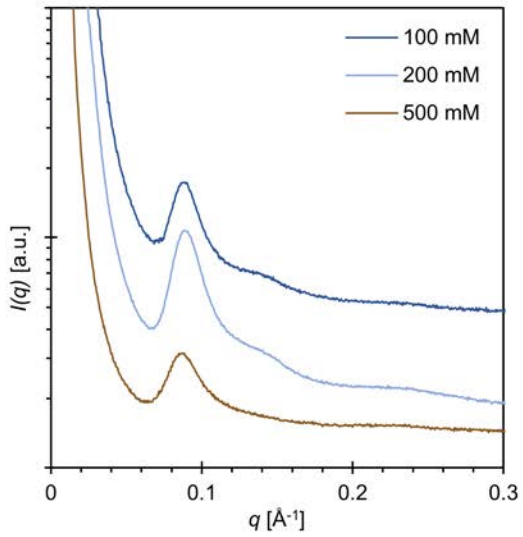


Figure S17. Small-angle X-ray scattering (SAXS) data measured from **SpIIO**-6HB complexes ($n_{SpIIO}/n_{6HB} \sim 9000$) in 1× FOB supplemented with 100-500 mM NaCl.

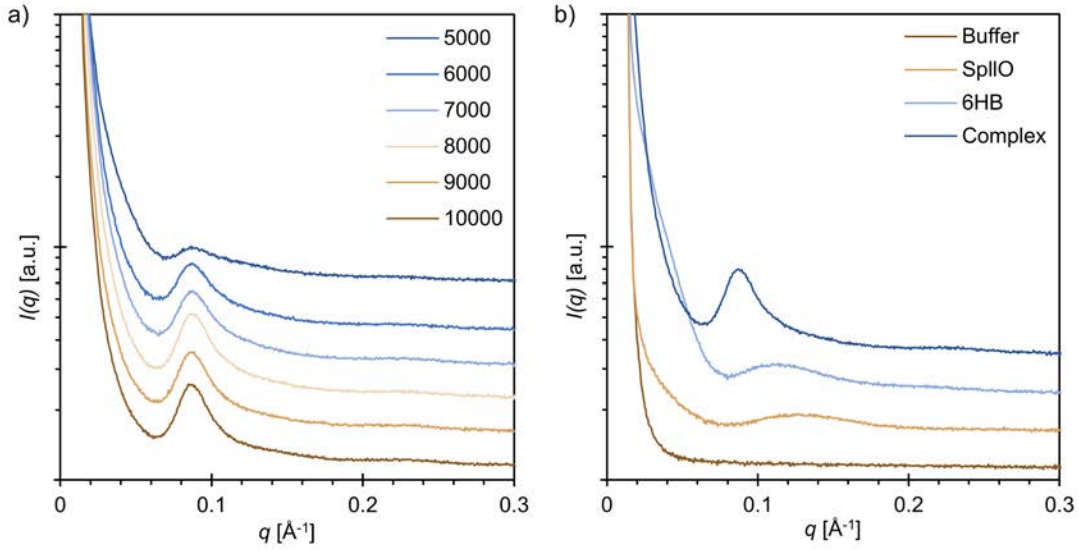


Figure S18. SAXS data measured from **SpIIO**-6HB complexes formed in 1 × FOB supplemented with 500 mM NaCl. SAXS data measured from a) **SpIIO**-6HB complexes assembled at different $n_{\mathrm{SpIIO}}/n_{\mathrm{6HB}}$. b) the individual building blocks and a **SpIIO**-6HB complex ($n_{\mathrm{SpIIO}}/n_{\mathrm{6HB}} \sim 8000$).

6. Stability of lipid-DNA origami assemblies against DNase I digestion

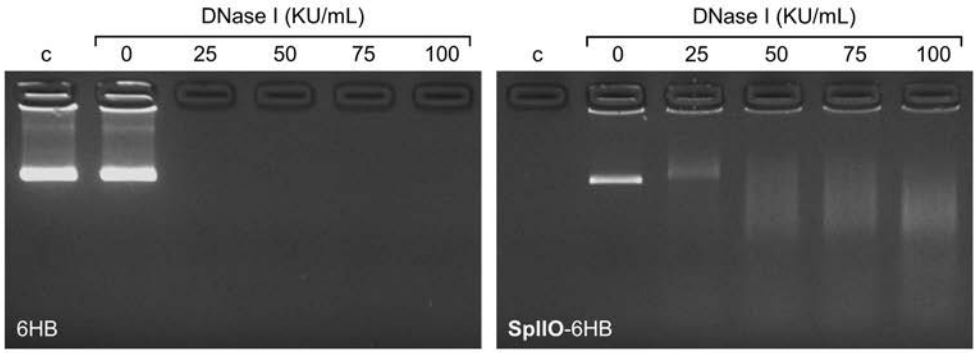


Figure S19. Agarose gel EMSA of bare 6HBs (left) and **SpIIO**-6HB complexes (right) after incubation with DNase I for 6 h 40 min. The **SpIIO**-6HB complexes are formed at c_{6HB} = 50 nM and $n_{SpIIO}/n_{6HB} \sim 8000$. The DNase I is deactivated by sodium dodecyl sulfate (SDS) before the gel run, and c is a control sample without added SDS and DNase I. The gel (2% (w/v)) was run at a constant voltage of 95 V for 45 min.

7. Photocleavage of lipid-DNA origami assemblies

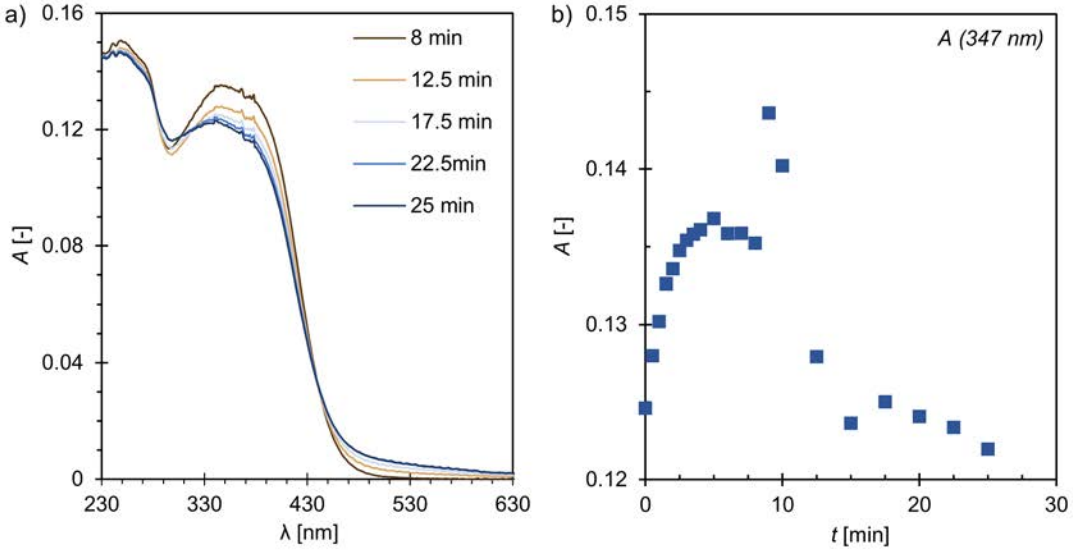


Figure S20. Photocleavage of **SpIIO**. a) UV/Vis spectra of **SpIIO** (c = 0.1 mg mL^{-1}) after longer UV-A irradiation times (λ = 350 nm). b) The absorbance at 347 nm as a function of UV exposure time.

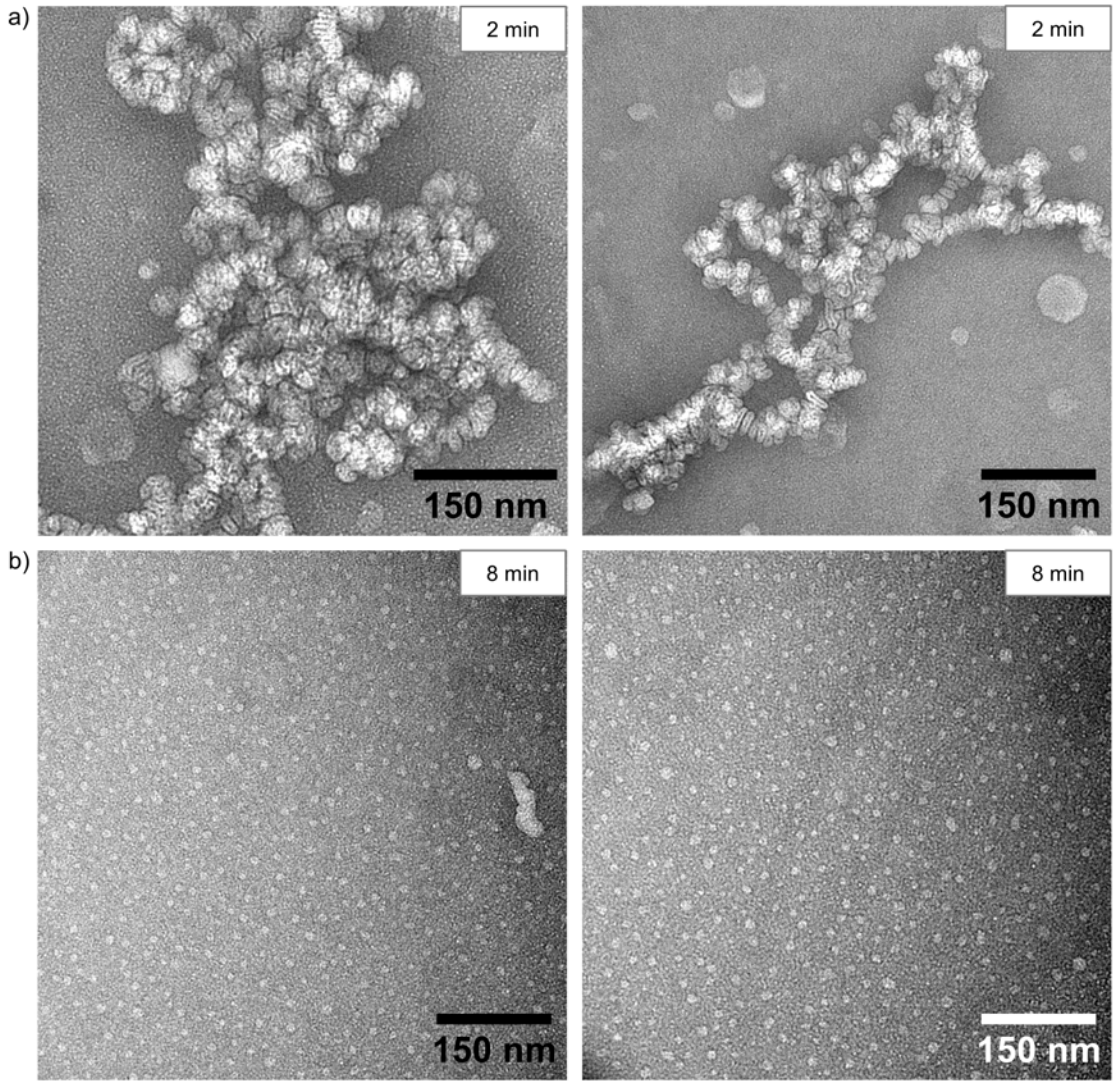


Figure S21. TEM images of **SpIIO** (c = 0.34 mg mL^{-1}) in 1× FOB (1× TAE, 12.5 mM $MgCl_2$) after a) 2 min, and b) 8 min of UV-A irradiation. The TEM samples are negatively stained with uranyl formate (2 % (w/v)).

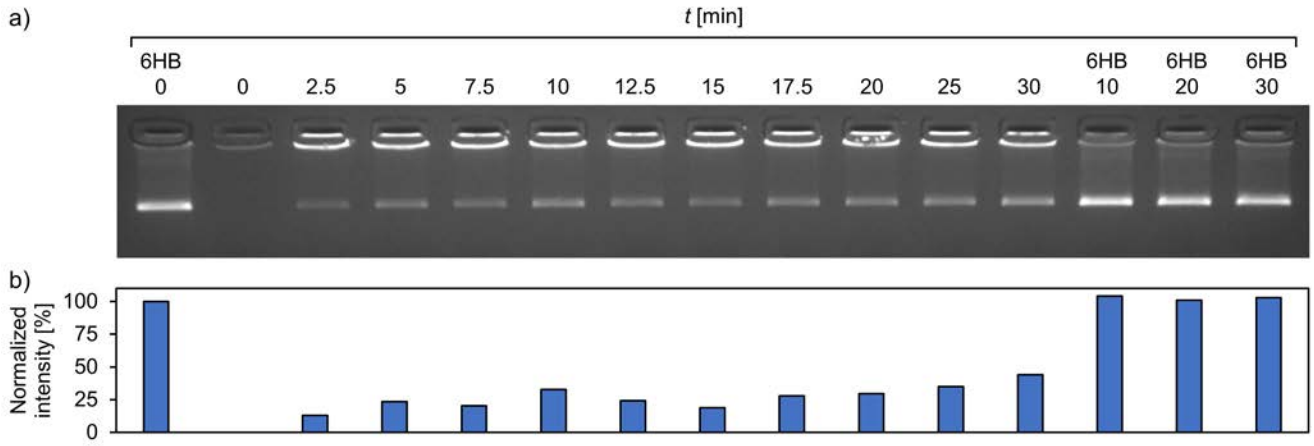


Figure S22. Optically triggered disassembly of the **SplIO**-6HB complexes. a) Agarose gel EMSA of **SplIO**-6HB complexes after different UV-A irradiation times. The samples marked with "6HB" are control samples without added **SplIO**. The gel (2% (w/v)) was run at a constant voltage of 95 V for 45 min. b) Normalized ethidium bromide intensity of the band corresponding to the plain 6HB. For the normalization, the band intensity of the control sample (6HB, 0) was set to 100%.

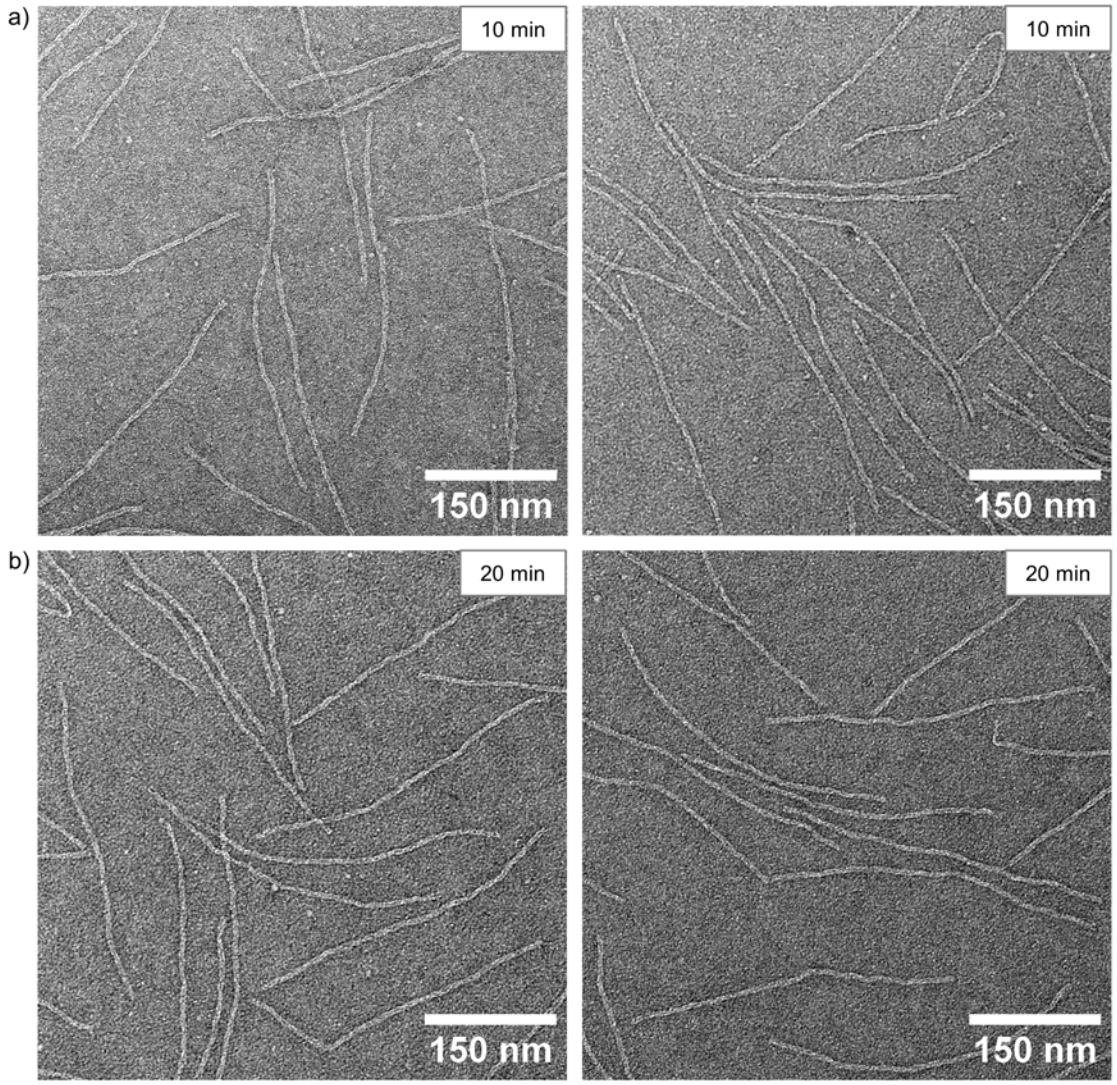


Figure S23. TEM images of **SpIIO**-6HB complexes (c_{6HB} = 7.5 nM, $n_{SpIIO}/n_{6HB} \sim 8000$) prepared in 1$\times$ FOB supplemented with 200 mM NaCl after a) 10 min, and b) 20 min of UV-A irradiation. The TEM samples are negatively stained with uranyl formate (2 $\%$ (w/v)).

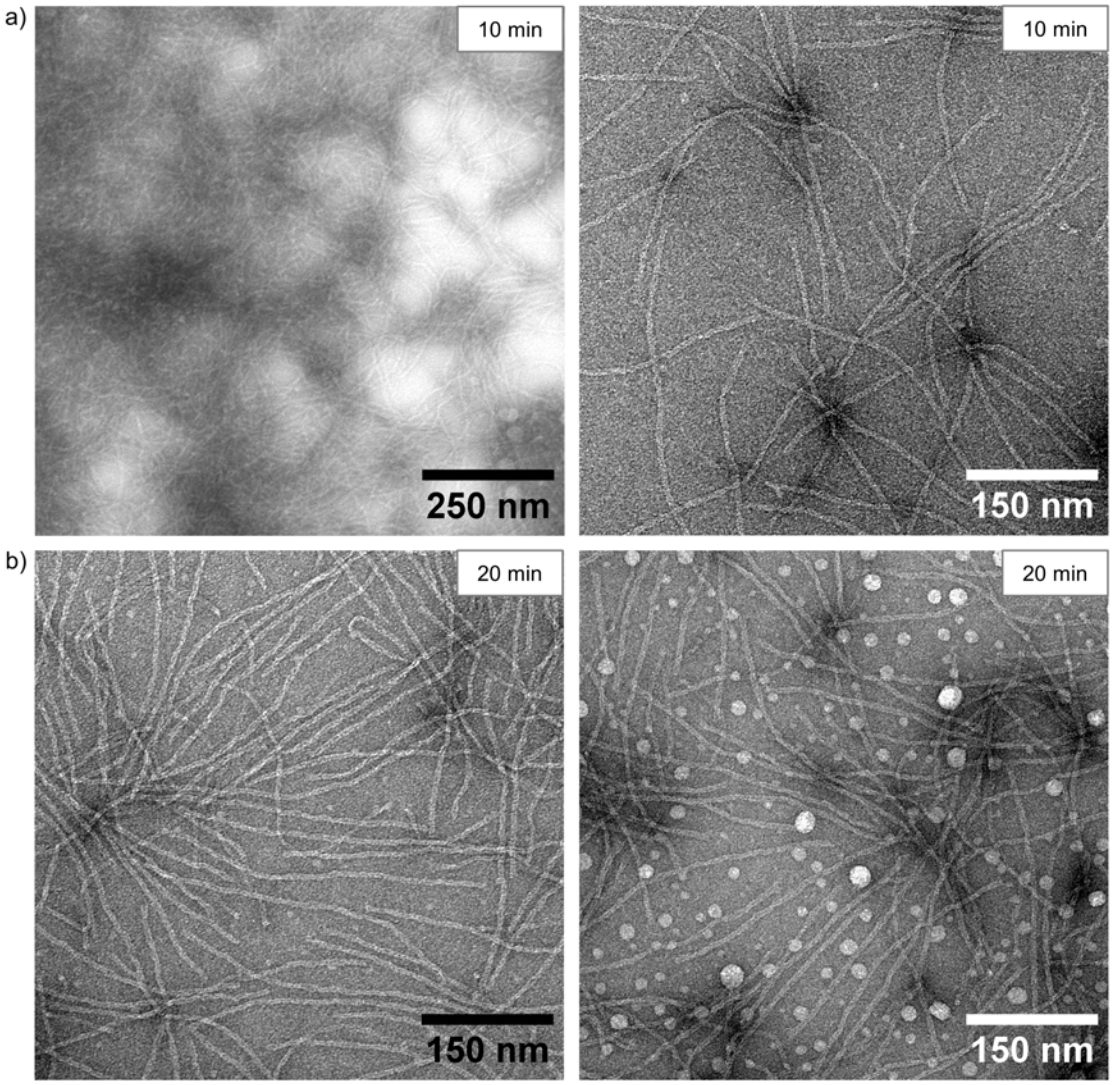


Figure S24. TEM images of **SpIIO**-6HB complexes (c_{6HB} = 50 nM, $n_{SpIIO}/n_{6HB} \sim 8000$) prepared in 1× FOB supplemented with 200 mM NaCl after a) 10 min, and b) 20 min of UV-A irradiation. The TEM samples are negatively stained with uranyl formate (2 % (w/v)).

8. Nuclear magnetic resonance (NMR) spectra

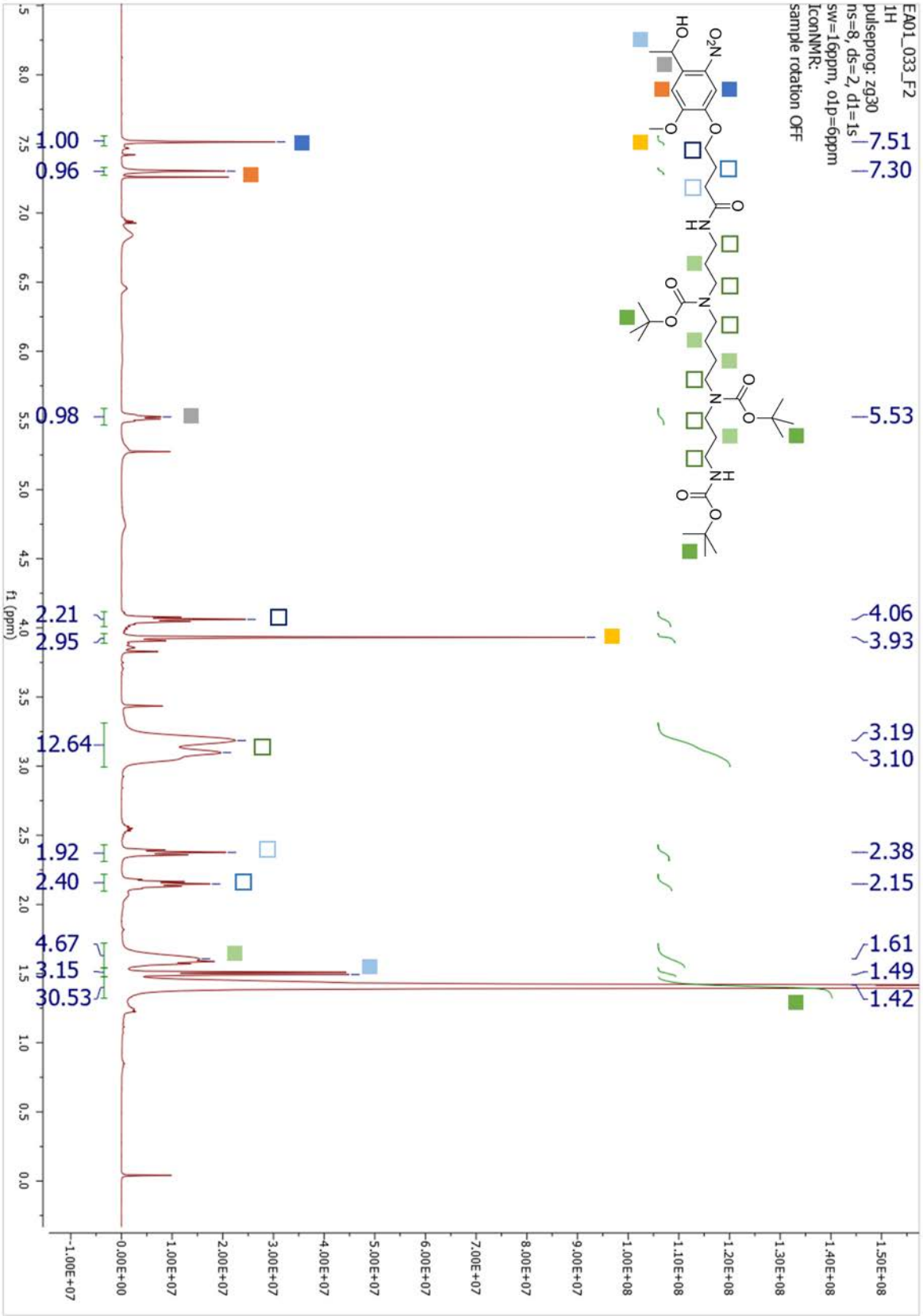


Figure S25. ^{1}H-NMR spectra ($CDCl_3$, 400 MHz) of BOC-spermine-pll (**2**). The inset shows chemical structure and peak assignment.

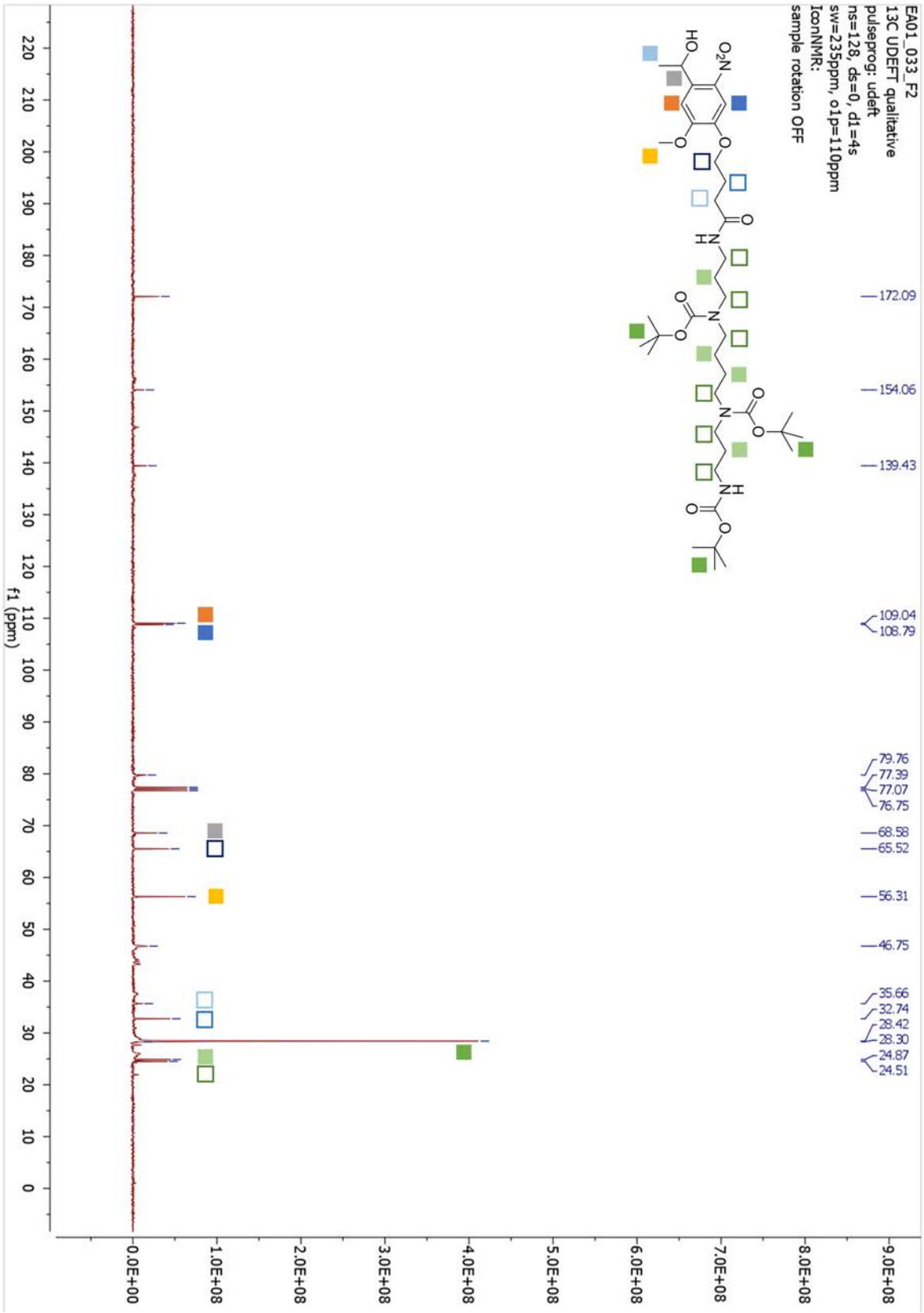


Figure S26. ^{13}C-NMR spectra (MeOD-d4, 100 MHz) of BOC-spermine-pll (**2**). The inset shows chemical structure and peak assignment.

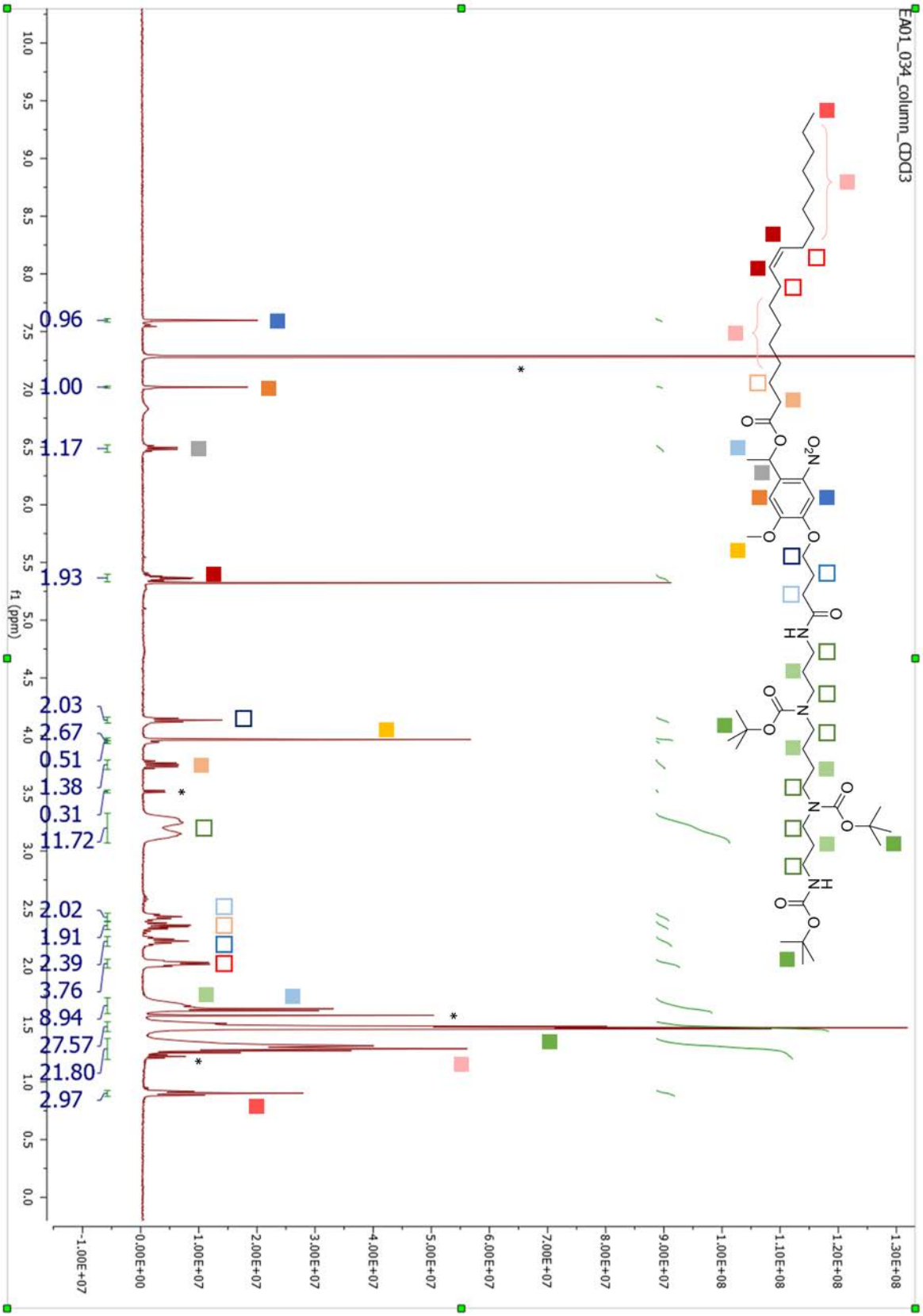


Figure S27. ^{1}H-NMR spectra ($CDCl_3$, 400 MHz) of BOC-spermine-pll-oleic ester (**3**). The inset shows chemical structure and peak assignment. Minor solvent impurities are marked with asterisks.

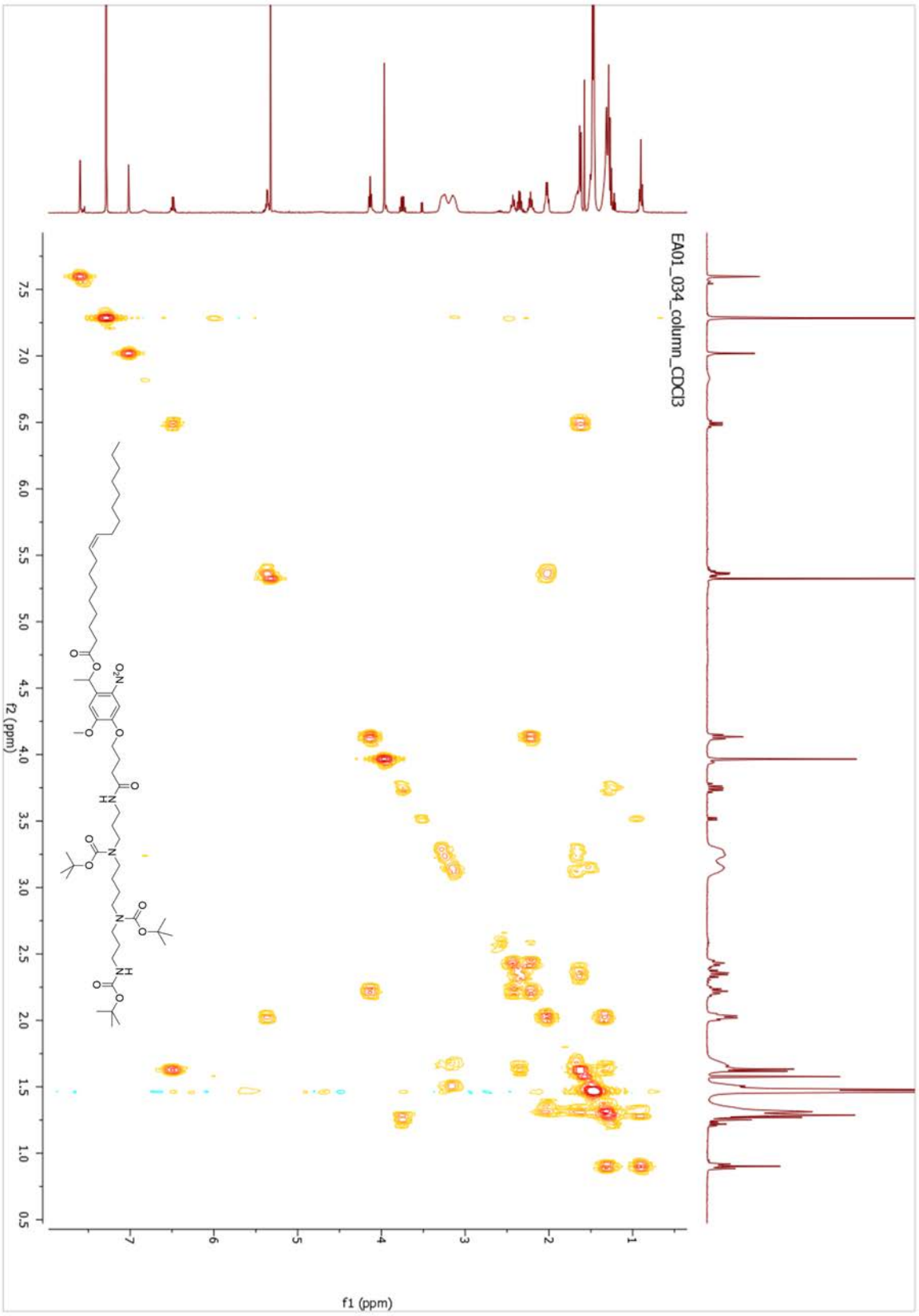


Figure S28. COSY NMR spectra ($CDCl_3$, 400 MHz) of BOC-spermine-pll-oleic ester (**3**).

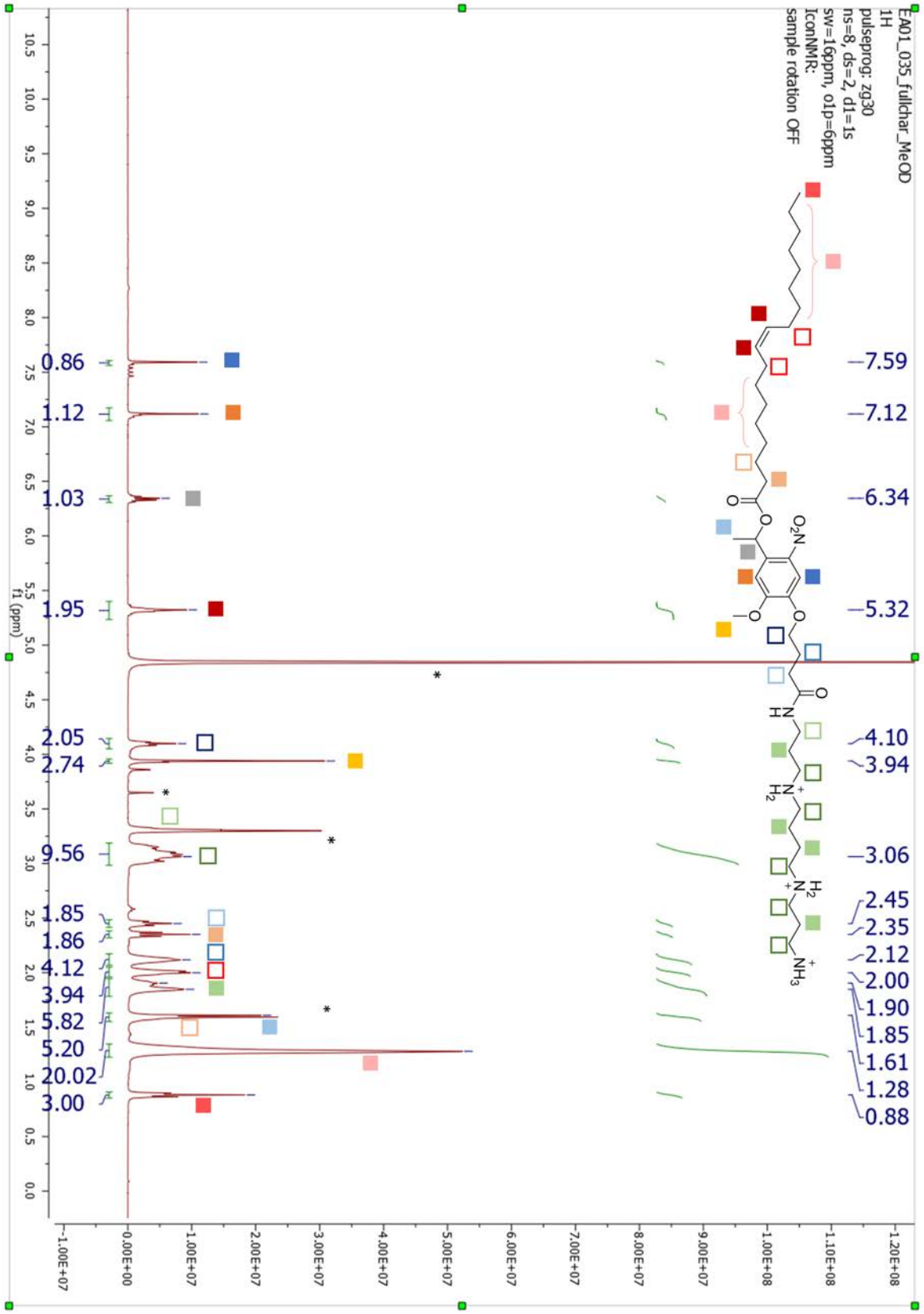


Figure S29. [1]H-NMR spectra (MeOD-d4, 400 MHz) of **SpIIO**. The inset shows chemical structure and peak assignment. Minor solvent impurities are marked with asterisks.

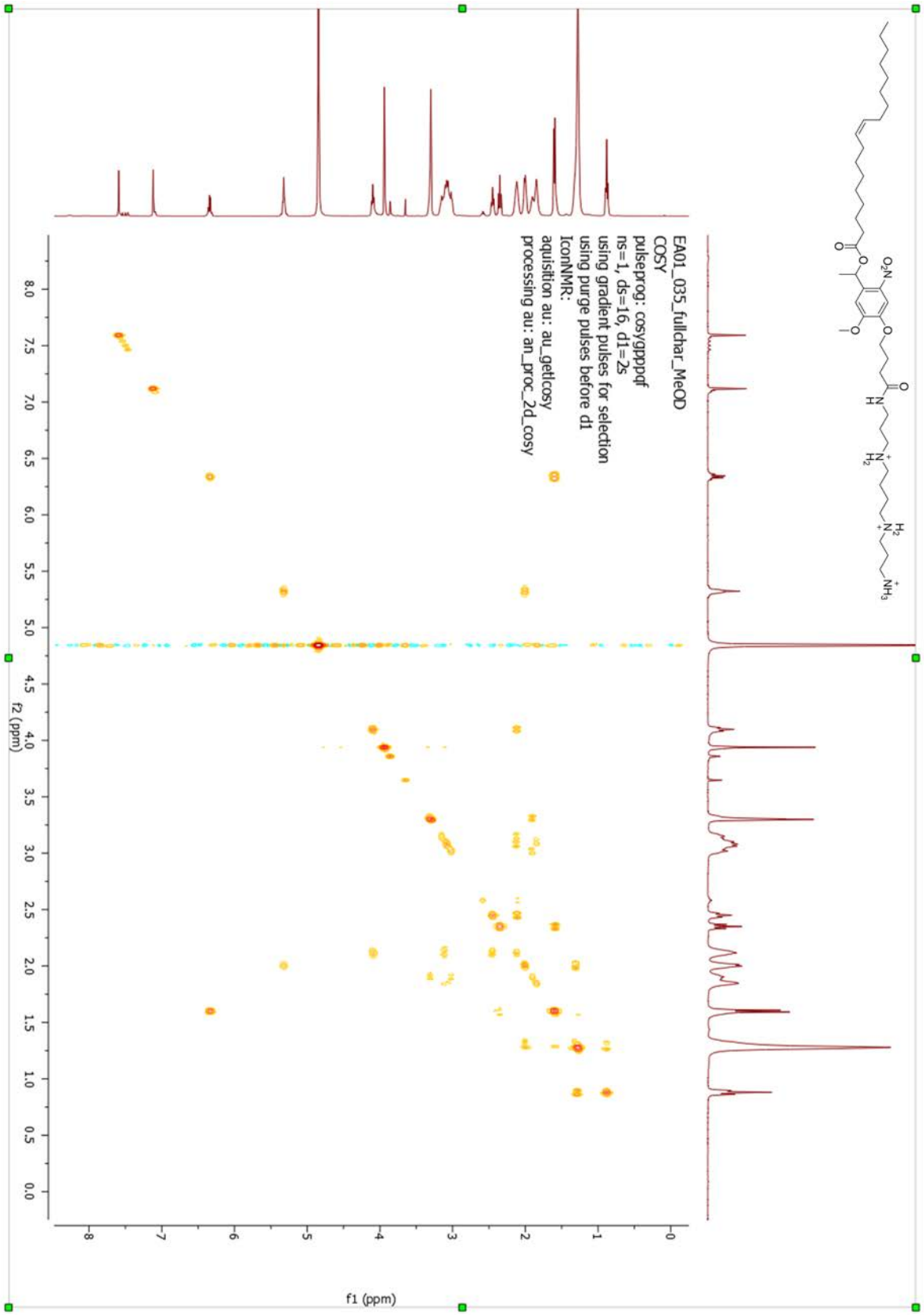


Figure S30. COSY NMR spectra (MeOD-d4, 400 MHz) of **SpIIO**.

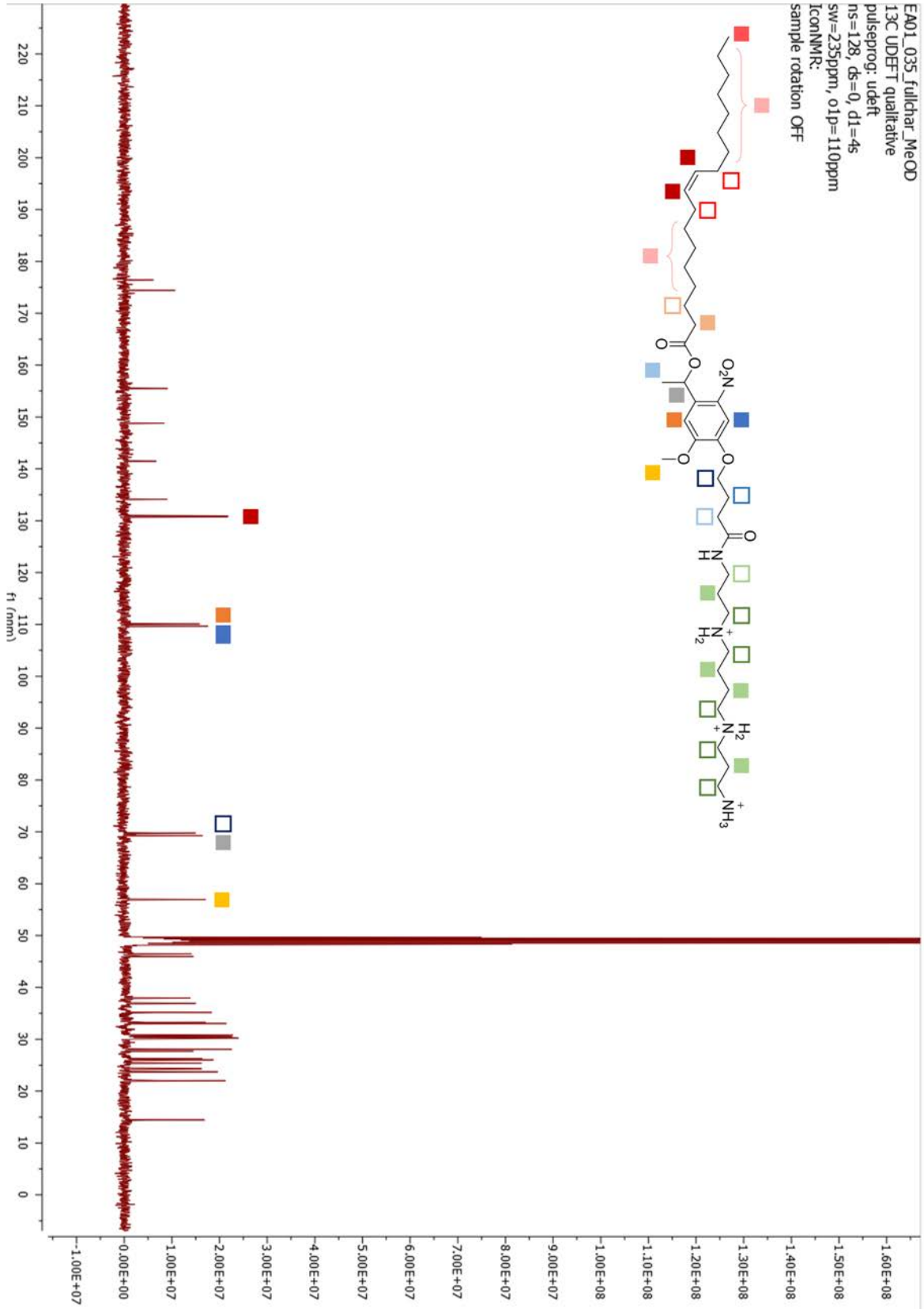


Figure S31. ^{13}C-NMR spectra (MeOD-d4, 100 MHz) of **SpIIO**. The inset shows chemical structure and peak assignment.

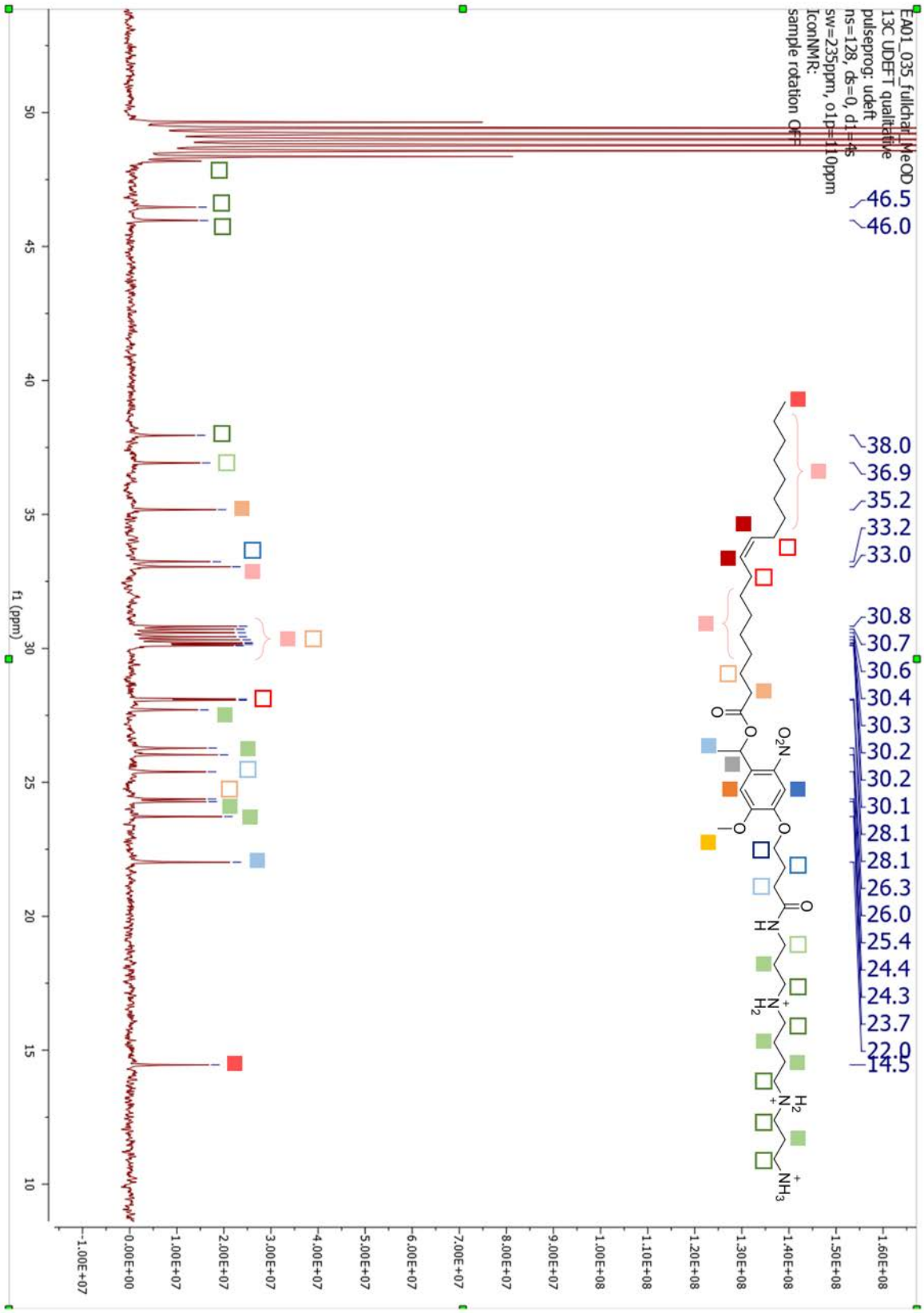


Figure S32. Detailed ^{13}C-NMR spectra (MeOD-d4, 100 MHz) of **SpIIO**. The inset shows chemical structure and peak assignment.

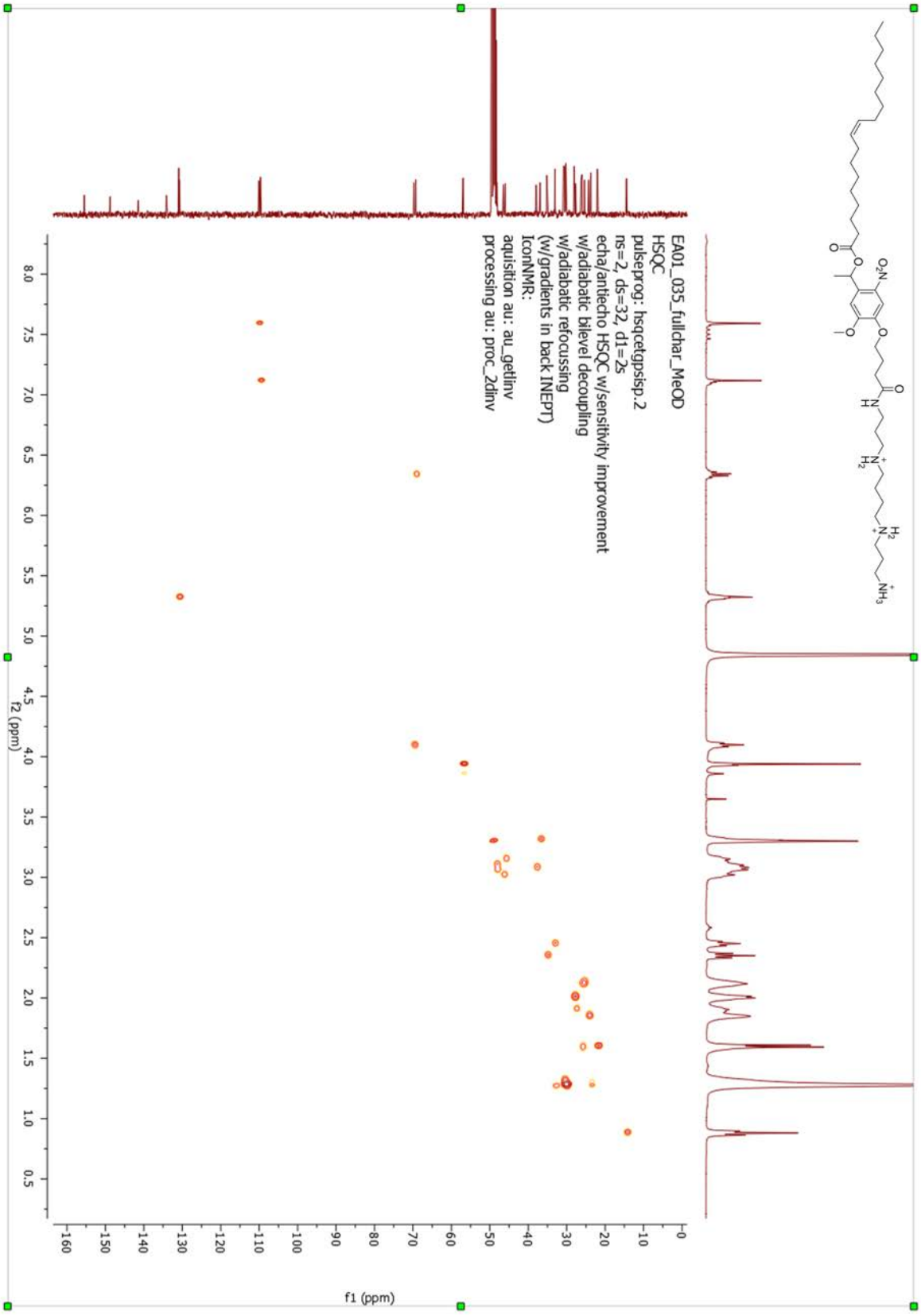


Figure S33. HSQC spectra (MeOD-d) of **SpIIO**.

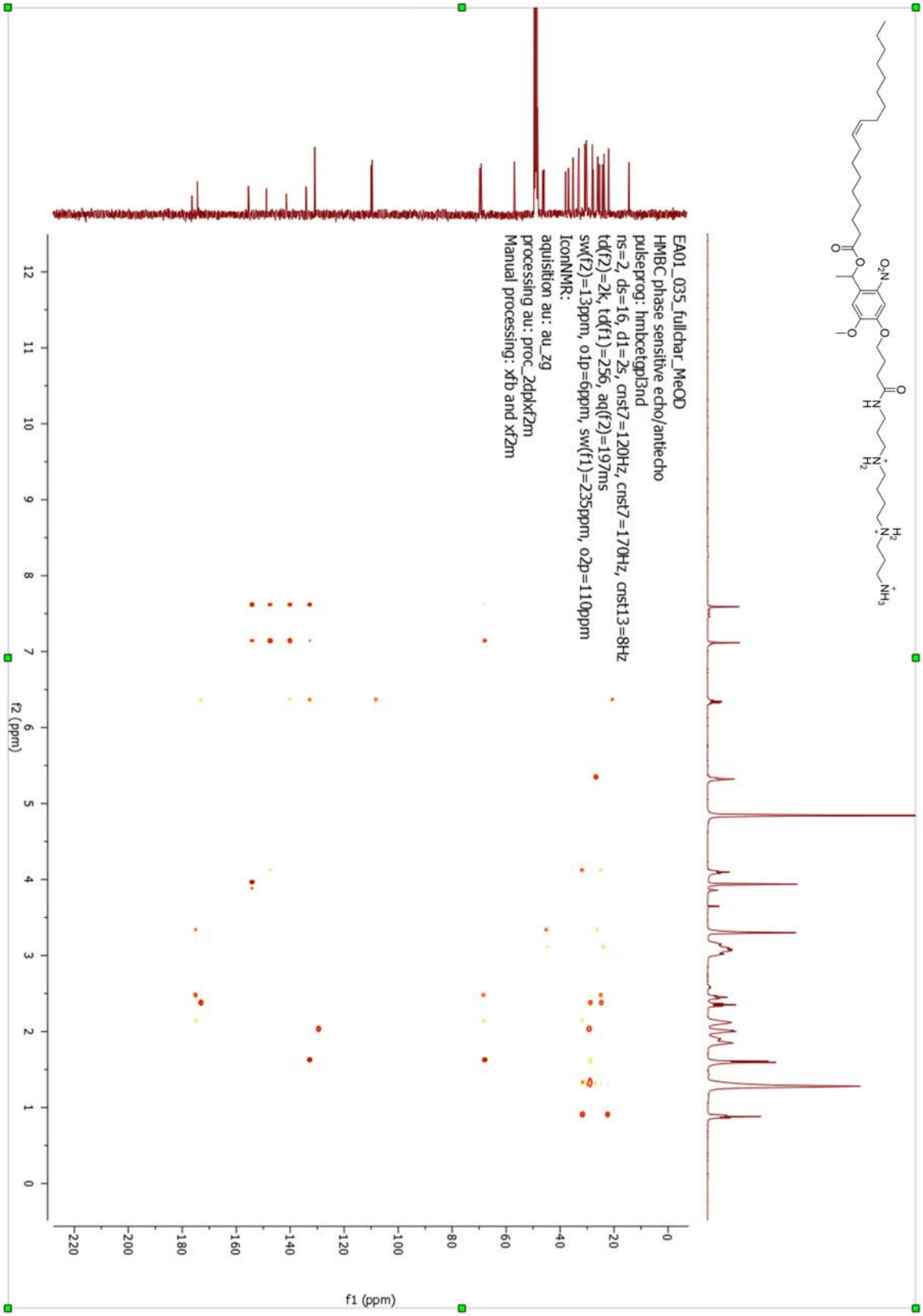


Figure S34. HMBC spectra (MeOD-d) of **SpIIO**.

9. Electrospray ionization mass spectrometry (ESI-MS)

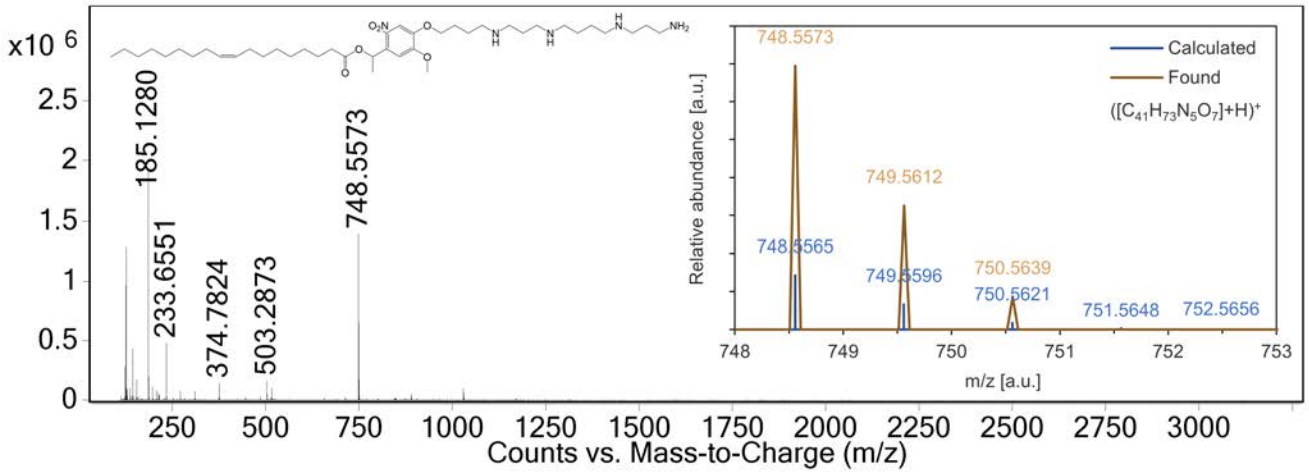


Figure S35. High resolution electrospray ionization mass spectrometry (HR-ESI-MS) of **SpIIO**. The inset shows calculated and found mass of the $M+H^+$ species.

10. Full raw data of agarose gels

Figure 2a, top (0 mM NaCl)

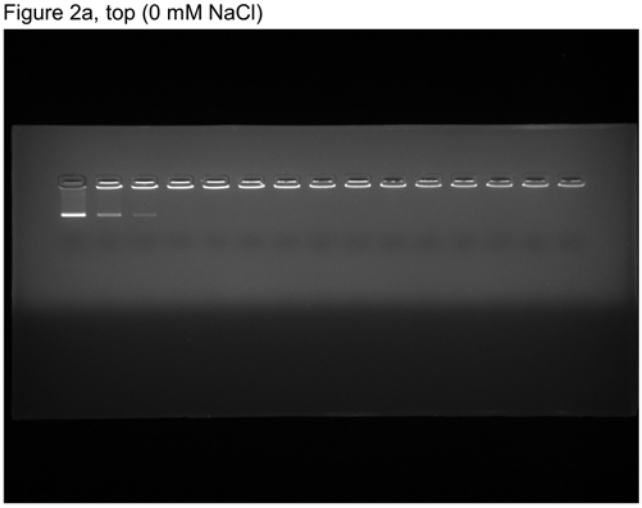


Figure 2a, middle (200 mM NaCl)

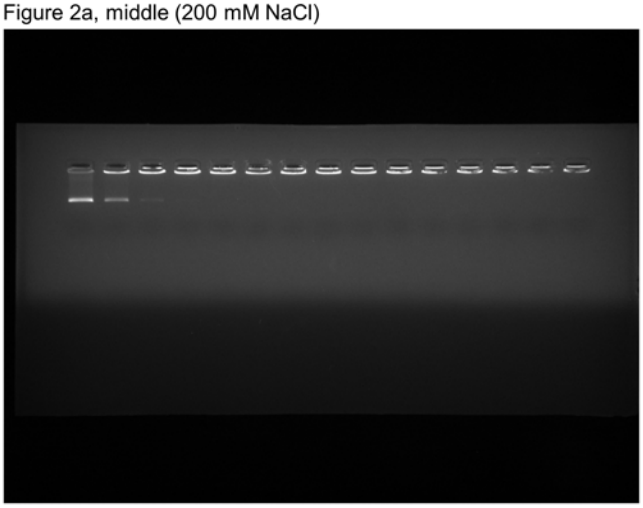


Figure 2a, bottom (500 mM NaCl)

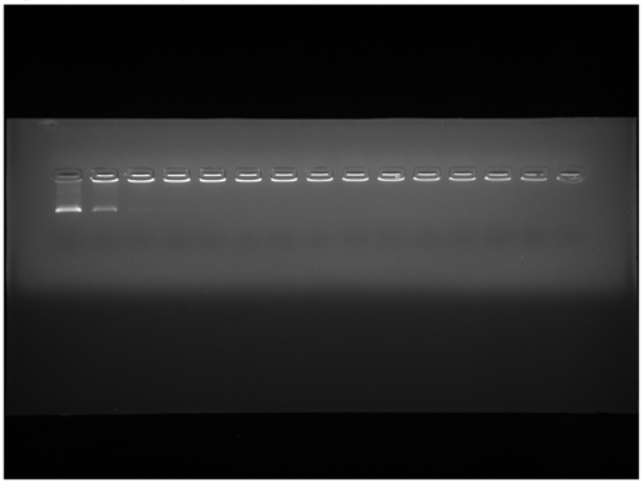


Figure 3a + Figure S3

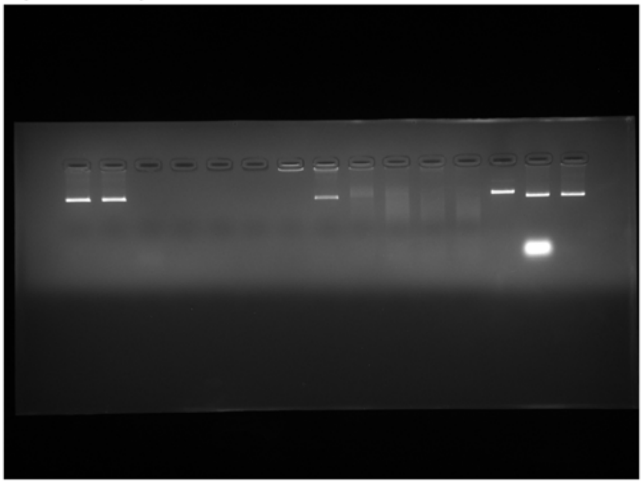


Figure 3d + Figure S22

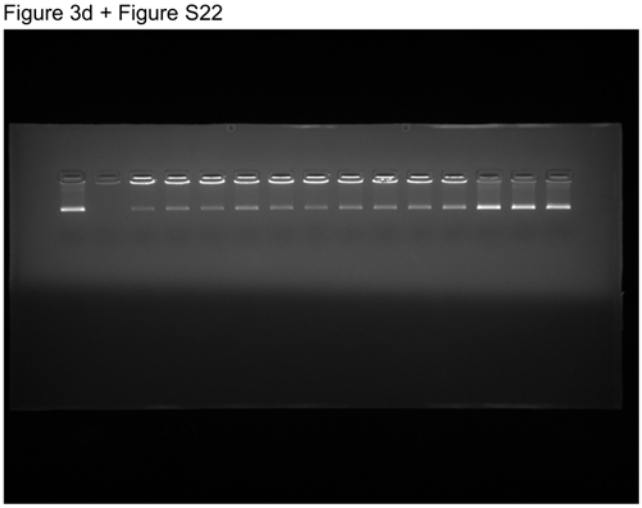


Figure S7

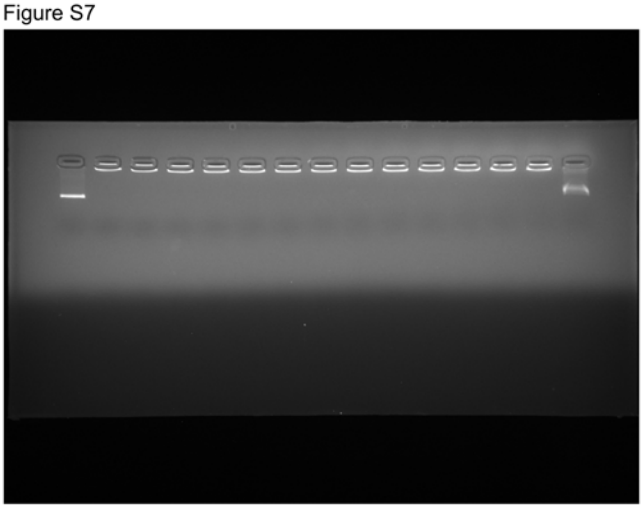
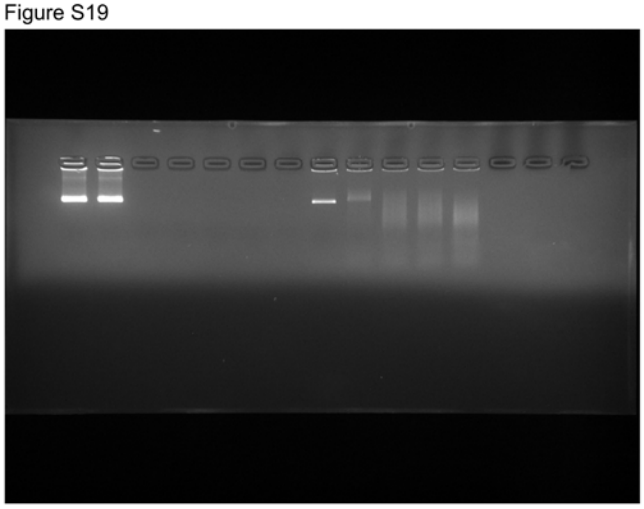


Figure S19



11. References

1. I. S. Blagbrough and A. J. Geall, *Tetrahedron Lett.*, 1998, **39**, 439–442.
2. M. A. Kostiainen, D. K. Smith and O. Ikkala, *Angew. Chem., Int. Ed.*, 2007, **46**, 7600–7604.
3. H. Bui, C. Onodera, C. Kidwell, Y. Tan, E. Graugnard, W. Kuang, J. Lee, W. B. Knowlton, B. Yurke and W. L. Hughes, *Nano Lett.*, 2010, **10**, 3367–3372.
4. E. Stahl, T. G. Martin, F. Praetorius and H. Dietz, *Angew. Chem., Int. Ed.*, 2014, **53**, 12735–12740.
5. A. M. Hung, C. M. Micheel, L. D. Bozano, L. W. Osterbur, G. M. Wallraff and J. N. Cha, *Nat. Nanotechnol.*, 2010, **5**, 121–126.
6. C. E. Castro, F. Kilchherr, D.-N. Kim, E. L. Shiao, T. Wauer, P. Wortmann, M. Bathe and H. Dietz, *Nat. Methods*, 2011, **8**, 221–229.