# Glycosyl-nucleoside-lipid-based supramolecular assembly as a nanostructured hydrogel material with Nucleic acid delivery capabilities

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## Materials and methods

**Materials. Instruments.** Unless noted otherwise, all starting materials were obtained from commercial suppliers and were used without further purification. The solvents were commercially dried quality. All compounds were characterized using standard analytical and spectroscopic data such as $^{1}H$ and $^{13}C$ spectroscopy (apparatus BRUKER Avance DPX-300, $^{1}H$ at 300.13 MHz, $^{13}C$ at 75.46 MHz) and mass spectrometry (Instrument JEOL SX 102, NBA matrix). The NMR chemical shifts are reported in ppm relative to tetramethylsilane using the residual proton of the solvent for $^{1}H$ NMR spectra and the carbon atom of the deuterated solvent for $^{13}C$ NMR spectra ($CDCl_3$ or DMSO-d6). The $^{1}H$ NMR coupling constant *J* are reported in Hz. Merck RP-18 F254S plates were used for analytical thin layer chromatography. Silica gel 60 (particle size: 40 – 60 μm) was employed for flash chromatography. The UV-absorbance experiments at 260 nm were performed on a NanoDrop® ND-1000 spectrophotometer.

Abbreviation: CAC, critical aggregation concentration; DCM, methylene chloride; DMEM, Dulbecco's modified Eagle medium; DMF, Dimethylformamide; THF, Tetrahydrofurane; DNA, Deoxyribonucleic acid; PBS, Phosphate buffer saline; GNL, Glycosyl-nucleoside-lipid; ON, Oligonucleotide.

**Hydrogel formation.** The gels are prepared by dissolving a small amount (typically 10 mg of GNL in 400 μL of water, 2.5 % w/w) of compound **5** in hot pure water (80 °C). After cooling at room temperature, the complete volume of water is immobilized and can support its own weight. The hydrogel formation is confirmed by turning the test tube upside

down at room temperature; a gel is obtained when the mixture does not run down. The gels obtained are stable for at least one week at room temperature.

**Fluorescence and optical microscopy.** Samples containing organisation of **5** were obtained from the mixture of 1mg/mL in water and from the mixture of 1mg/mL of **5** with 10 μM ON. Before observation, the mixture was incubated for 24h hours at room temperature. The observations were carried out with an Axiovert 200 fluorescent microscope (Zeiss).

**Transmission electronic microscopy.** Samples containing organisation of **5** were obtained from the mixture of 1mg/mL in pure water, from the mixture of 1mg/mL water (15 mM cacodylate buffer, pH 6.0, 8 mM $MgCl_2$, 3 mM NaCl) and from the mixture of 1mg/mL of **5** with 10 μM ON (15 mM cacodylate buffer, pH 6.0, 8 mM $MgCl_2$, 3 mM NaCl). Before TEM imaging, the mixture was incubated for 18h hours at room temperature. TEM microscopy experiments were performed on a HITACHI H 7650 (negative staining with ammonium molybdate 1% in water, Ni carbon coated grids).

**Cell culture.** Huh-7 (human hepatoma) cells were grown in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine and 1% non-essential amino acids, at 37 °C in a 5% $CO_2$ atmosphere. All culture reagents were purchased from Invitrogen.

**Cell survival.** $2.10^3$ Huh-7 cells per well were seeded into a 96-well plate and incubated the following day with increasing concentrations of GNL (from 5 to 100 μM) in complete growth medium. After 5 days in the continuous presence of the GNL, the living cells were quantified by the colorimetric CellTiter Aqueous One Solution Cell Proliferation Assay (Promega), as recommended.

**Fluorescence microscopy on cells.** $8.10^4$ cells per well were seeded into a 24-well plate containing glass coverslips. The following day, they were incubated for 24h with 0.5 μM of fluorescein-labeled ON, that has been complexed 6h before by increasing concentrations (from 1 to 10 equivalent of **5** per ON phosphate) of GNL in complete growth medium. The cell layers were then rinsed twice with PBS, fixed in 3.7% formaldehyde and mounted on glass slides using Vectashield (Vector Laboratories) mounting solution. The slides were observed with an Axiovert 200 fluorescent microscope (Zeiss).

**Oligonucleotides.** All the ONs were 2'OMe ON. The ONs were stock solutions of 3'-CGGCUCAUCACAACCCA-5'for TEM experiments and 3'-Fluorescein-CGGCUCAUCACAACCCA-5' for fluorescence microscopy experiments (Godeau, G.; Staedel, C.; Barthélémy, P. *J. Med. Chem.* **2008,** *51*, 4374–4376).

**Small-Angle X-ray Scattering.** A Rigaku Nanoviewer (Microsource generator, Micromax 007, 800 W rotating anode coupled with Confocal Maxflux Mirror) was used. The 20 % w/w hydrogel was sealed into a 1.5 mm diameter glass capillary (Glaskapillaren GLAS, Germany). Integration of the spectrum was performed with the program *R-axis Display* software.

## Synthesis.

### N-propargyloleamide. (1)

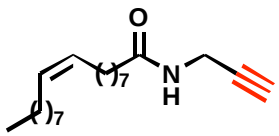
Anhydrous DCM (10 mL) was added under nitrogen to oleoyl chloride (1.08g, 3.6 mmol, 1.2eq). The DCC (928 mg, 4.5 mmol, 1.5 eq) and DMAP (550 mg, 4.5 mmol, 1.5 eq) were added. Propargyl amine (165 mg, 3 mmol, 1 eq) was then added at room temperature. The reaction was stirred over the night. 50 mL of DCM were added and the mixture was

washed successively twice with saturated $NaHCO_3$ aqueous solution (20 mL) and once with brine (20 mL). The organic phase was dried on $Na_2SO_4$ and evaporated under reduced pressure. 750 mg of product were isolated after chromatography (hexane/ethyl acetate, 9/1), (Yield: 78 %). $R_f$: 0.41 (hexane/ethyl acetate, 7/3).

NMR $^1$H (300.13 MHz, DMSO $d_6$): 0.85 (t, *J* = 6 Hz, 3H), 1.24 (s, 10H), 1.28 (s, 10H), 1.57-1.63 (m, 2H), 1.97-2.03 (m, 4H), 2.17-2.21 (m, 3H), 4.00-4.02 (m, 2H), 5,28-5.32 (m, 2H), 6.46 (t, *J* = 6 Hz, 1H).

NMR $^{13}$C (75.46 MHz, $CDCl_3$): 14.1, 22.7, 25.6, 27.2, 29.3, 29.5, 29.7, 29.8, 31.9, 36.3, 71.2, 79.8, 129.7, 130.0, 173.1.

HRMS (ESI): $[M+H]^+$ calcd 320.2953, found 320.2962



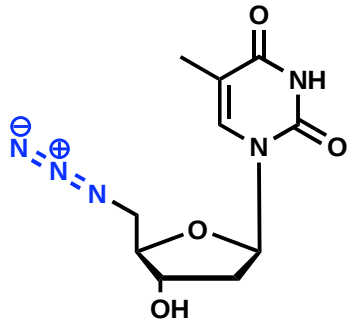

**(1)**

**5'-azido-5'-deoxythymidine. (2)**

Dried pyridine (20 mL) was added to thymidine (500 mg, 2.06 mmol). The mixture was cooled to 0 °C and methanesulfonyl chloride (284 mg, 1.05 eq, 2.07 mmol) was added drop wise. The mixture was stirred at room temperature for 4 hours. The solvent was evaporated under reduced pressure and the residual compound was used directly without further purification in the following step.

DMF (40 mL) and sodium azide (669 mg, 10.3 mmol, 5 eq) were added. The reaction mixture was maintained at 80 °C under stirring for 4 hours. The DMF was removed under reduced pressure. The residual solid was dissolved in 50 mL of ethyl acetate and was washed successively twice with $NaHCO_3$ 5% in water (20 mL) and once with brine (20 mL). The organic phase was dried on $Na_2SO_4$ and evaporated under reduced pressure. 330 mg of product were isolated after precipitation (methanol/acetonitrile), (Yield: 60%). $R_f$: 0.47 (ethyl

acetate/methanol, 9/1). All spectroscopic data agreed with the literature values (Hiebl, J. and al, *J. Med. Chem.* **1991**, *34*,1426-1430).




**(2)**

**5'-(4-((Oleamide)methyl)-1H-1,2,3-triazol-1-yl)-thymidine. (3)**

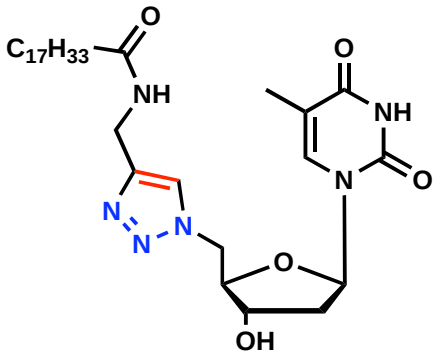
N-propargyloleamide (**1**) (118 mg, 0.37 mmol, 1 eq), sodium ascorbate (16 mg, 0.08 mmol, 0.2 eq) and copper sulfate (6 mg, 0.04 mmol, 0.1 eq) were added to 5'-azido-5'-deoxythymidine (100 mg, 0.37 mmol, 1 eq) in 5 mL of water/THF mixture (50/50). The reaction mixture was maintained at 65 °C under stirring for 10 hours. The mixture was cooled to room temperature and the solvent was evaporated. The residual solid was dissolved in DCM (100 mL) and successively washed by water (2 X 20 mL) and brine (20 mL). The organic layer was then dried on $NaSO_4$ and evaporated under reduce pressure.

150 mg of product were isolated after chromatography (ethyl acetate/methanol, 95/5), (Yield: 69 %). $R_f$: 0.4 (ethyl acetate/methanol, 8/2)

NMR $^{1}H$ (300.13 MHz, DMSO $d_6$): 0.85 (t, *J* = 6 Hz, 3H), 1.24 (s, 22H), 1.44-1.49 (m, 2H), 1.8 (s, 3H), 1.97-2.06 (m, 4H), 4.05-4.07 (m, 1H), 4.26 (d, *J* = 6 Hz, 3H), 4.59-4.67 (m, 2H), 5.32 (t, *J* = 6 Hz, 2H), 5.51 (s, 1H), 6.17 (t, *J* = 6 Hz, 1H), 7.38 (s, 1H), 7.87 (s, 1H), 8.27 (t, *J* = 6 Hz, 1H), 11.33 (s, 1H).

NMR $^{13}C$ (75.46 MHz, DMSO $d_6$): 12.5, 14.4, 22.6, 25.7, 27.0, 27.1, 29.0, 29.1, 29.2, 29.3, 29.6, 31.2 , 31.8, 34.5, 35.6, 38.3, 51.7, 71.2, 84.5, 110.3, 123.9, 130.1, 136.5, 145.6, 150.9, 164.1, 172.5.

HRMS (ESI): $[M+H]^+$ calcd 587.3921, found 587.3923




**(3)**

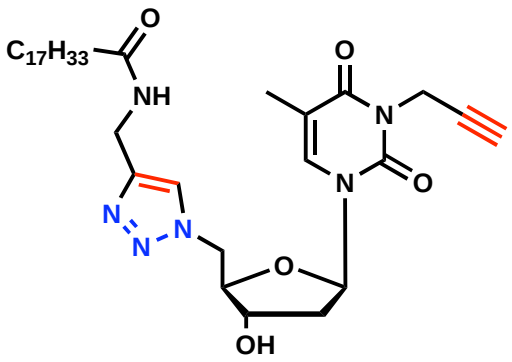
**5'-(4-((Oleamide)methyl)-1H-1,2,3-triazol-1-yl)-N3-propargylthymidine. (4)**

Anhydrous DMF (25 mL) was added under nitrogen to compound **3** (100 mg, 0.17 mmol, 1eq). $K_2CO_3$ (36 mg, 0.26 mmol, 1.5 eq) was added. Propargyl bromide (20 mg, 0.17 mmol, 1 eq) was then added at room temperature. The reaction mixture was stirred over night. The DMF was removed under reduced pressure. The residual solid was dissolved in 50 mL of DCM and was washed successively twice with water (20 mL) and once with brine (20 mL). The organic phase was dried on $Na_2SO_4$ and evaporated under reduced pressure. 80 mg of product were obtained (Yield: 65 %). $R_f$: 0.4 (hexane/ethyl acetate, 85/15). The compound **4** can be used for the next step with no further purification.

NMR $^1$H (300.13 MHz, $CDCl_3$): 0.89 (t, *J* = 6 Hz, 3H), 1.28 (s, 20H), 1.57-1.63 (m, 2H), 1.84 (s, 3H), 2.00-2.02 (m, 4H), 2.19 (t, *J* = 6Hz, 2H), 2.25 (s, 1H), 2.35-2.41 (m, 2H), 4.24-4.28 (m, 1H), 4.47-4.51 (m, 3H), 4.65-4.74 (m, 4H), 5.35 (t, *J* = 6 Hz, 2H), 6.16 (t, *J* = 6 Hz, 1H), 6.52 (t, *J* = 6 Hz, 1H), 7.78 (s, 1H).

NMR $^{13}$C (75.46 MHz, $CDCl_3$): 2, 13.2, 14.2, 22.7, 25.6, 27.2, 29.2, 29.3, 29.5, 29.7, 29.8, 30.5, 31.9, 34.7, 36.5, 38.7, 51.4, 70.9, 71.4, 77.3, 78.1, 84.1, 87.3, 110.7, 124.3, 129.7, 130.0, 134.9, 144.9, 150.0, 162.3, 173.7.

HRMS (ESI): $[M+Na]^+$ calcd 647.3897, found 647.3923




**(4)**

**5'-(4-((Oleamide)methyl)-1H-1,2,3-triazol-1-yl)-N3-(1-((β-D-glucopyranoside)-1H-1,2,3-triazol-4-yl)methyl)thymidine. (5)**

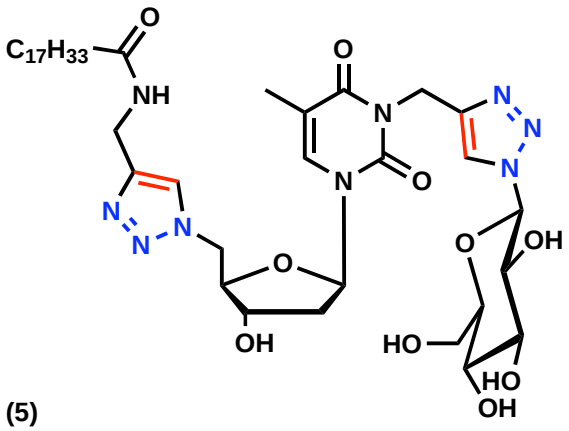
The compound **4** (80 mg, 0.13 mmol, 1 eq), sodium ascorbate (6 mg, 0.03mmol, 0.2 eq) and copper sulfate (2 mg, 0.01mmol, 0.1 eq) were added to 1-azido-β-(D)-glucopyranoside (27 mg, 0.13 mmol, 1 eq) in 5 mL of water/THF mixture (50/50). The reaction mixture was maintained at 65 °C under stirring for 10 hours. The mixture was cooled to room temperature and the solvent was evaporated.

100 mg of product were isolated after chromatography (ethyl acetate/methanol, increasing from 95/5 to 80/20), (Yield: 93 %). $R_f$: 0.5 (ethyl acetate/methanol, 6/4)

NMR $^{1}$H (300.13 MHz, DMSO $d_6$): 0.85 (t, *J* = 6 Hz, 3H), 1.24 (s, 20H), 1.45-1.49 (m, 2H), 1.88 (s, 3H), 1.96-2.0 (m, 4H), 2.03-2.09 (m, 3H), 2.15-2.20 (m, 2H), 3.18-3.24 (m, 1H), 3.66-3.75 (m, 2H), 4.08-4.12 (m, 1H), 4.27 (d , *J* = 6 Hz), 4.64-4.67 (m, 3H), 5.05 (s, 2H), 5.18 (d, *J* = 6 Hz, 1H), 5.32-5.36 (m, 4H), 5.48 (d, *J* = 9 Hz, 1H), 5.57 (s, 1H), 6.23 (t, *J* = 6 Hz, 1H), 7.50 (s, 1H), 7.90 (s, 1H), 8.11 (s, 1H), 8.29 (t, *J* = 6 Hz, 1H).

NMR $^{13}$C (75.46 MHz, DMSO $d_6$): 13.2, 14.4, 22.6, 25.7, 27.0, 27.1, 29.0, 29.1, 29.2, 29.3, 29.5, 29.6, 31.8, 34.5, 35.6, 36.6, 38.5, 51.6, 61.1, 70.0, 71.2, 72.3, 77.4, 80.4, 84.7, 85.6, 87.9, 109.6, 122.9, 123.9, 130.1, 135.6, 143.1, 145.6, 150.7, 162.8, 172.6.

HRMS (ESI): $[M+Na]^+$ calcd 852.4596, found 852.4604




(5)

**1-(4-((Oleamid)methyl)-1H-1,2,3-triazol-1-yl)-β-D-glucopyranoside. (6)**

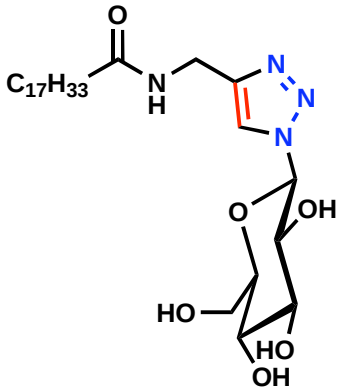
The compound **1** (200 mg, 0.63 mmol, 1 eq), sodium ascorbate (6 mg, 0.03mmol, 0.2 eq) and copper sulfate (2 mg, 0.01mmol, 0.1 eq) was added to 1-azido-β-(D)-glucopyranoside (128 mg, 0.63 mmol, 1 eq) in 30 mL of water/THF mixture (50/50). The reaction mixture was maintained at 65 °C under stirring for 10 hours. The mixture was cooled to room temperature and the solvent was evaporated.

141 mg of product were isolated after chromatography (ethyl acetate/methanol, 95/5), (Yield: 43 %). $R_f$: 0.4 (ethyl acetate/methanol, 85/15).

NMR $^{1}$H (300.13 MHz, DMSO $d_6$): 0.85 (t, *J* = 6 Hz, 3H), 1.24 (s, 20H), 1.46-1.49 (m, 2H), 1.97 (q, *J* = 6 Hz, 4H), 2.09 (t, *J* = 6 Hz, 2H), 2.51 (s, 4H), 3.17-3.25 (m, 1H), 3.42-3.46 (m, 1H), 3.66-3.74 (m, 2H), 4.29 (d, *J* = 6 Hz, 2H), 4.66 (t, *J* = 6 Hz, 1H), 5.18 (d, *J* = 6 Hz, 1H), 5.33-5.39 (m, 4H), 5.49 (d, *J* = 9 Hz, 1H), 8.05 (s, 1H), 8.30 (t, *J* = 6 Hz, 1H).

NMR $^{13}$C (75.46 MHz, DMSO $d_6$): 14.4, 22.6, 25.6, 27.0, 27.1, 29.0, 29.1, 29.2, 29.3, 29.6, 31.8, 34.5, 35.7, 61.2, 72.5, 77.5, 80.4, 87.8, 122.3, 130.1, 145.5, 172.6.

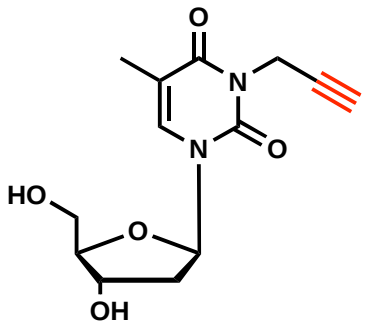
HRMS (ESI): $[M+Na]^+$ calcd 547.3472, found 547.3448




**(6)**

**N3-propargylthymine. (7)**

Anhydrous DMF (50 mL) was added under nitrogen to thymidine (1 g, 4.1 7 mmol, 1eq). $K_2CO_3$ (849.9 mg, 6.15 mmol, 1.5 eq) was added. Propargyl bromide (491.2 mg, 4.1 mmol, 1 eq) was then added at room temperature. The reaction mixture was stirred over night. The DMF was removed under reduced pressure. The residual solid was dissolved in 100 mL of ethyl acetate and was washed successively twice with water (10 mL) and once with brine (10 mL). The organic phase was dryed on $Na_2SO_4$ and evaporated under reduced pressure. 692 mg of product were obtained (Yield: 60 %). $R_f$: 0.48 (hexane/ethyl acetate, 8/2). All spectroscopic data agreed with the literature values (Nakane, M.; Ichikawa, S.; Matsuda, A. *J. Org. Chem.* **2008**, *73*, 1842-1851).




**(7)**

**Physicochemical Studies.** The critical aggregation concentration (CAC) of **5** was determined using a tensiometer DCAT11 from Dataphysics. To determine the CAC value of our GNL, a freshly prepared solution of **5** was added drop wise into distillated water. All additions and measurements were automated and done at room temperature. The CAC value

for amphiphile **5** is 3.91 ± 0.61 µmol.L$^{-1}$. Note, that this value is similar in magnitude to CACs reported in the literature for mono-oleyl polyols or cationic nucleolipid derivatives. These results suggest that the nucleoside moiety doesn't significantly affect the CAC.

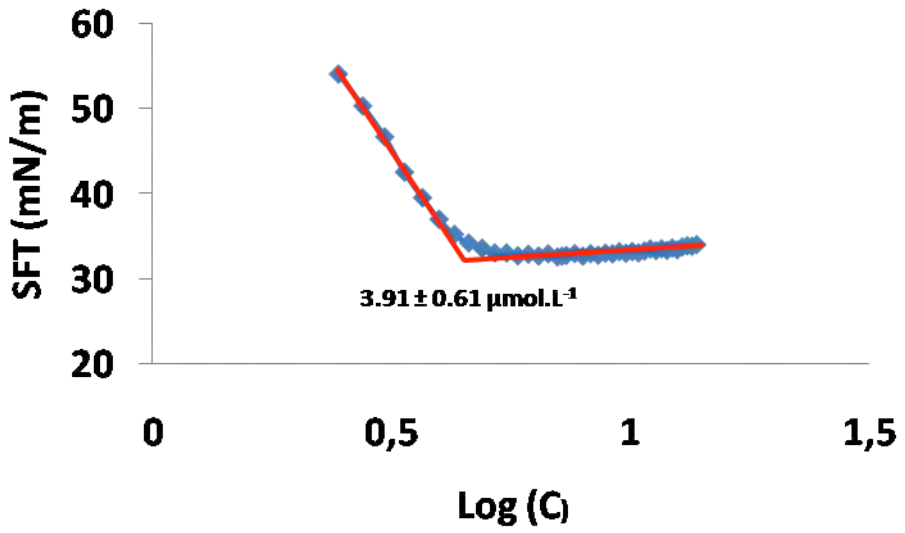



**Figure SI1:** Example of CAC determination's graphique (C = concentartion in µM).

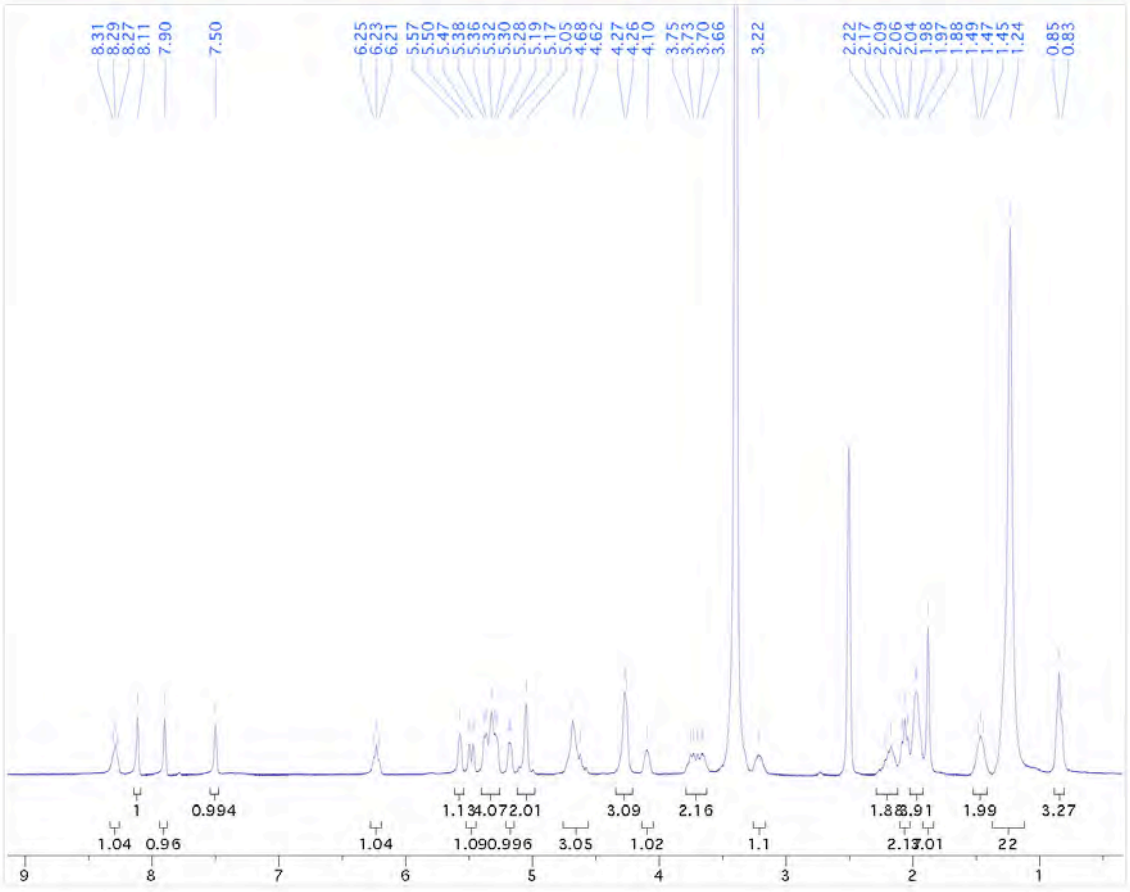



**Figure SI2:** Example of $^{1}$H NMR spectrum (Compound **5**).

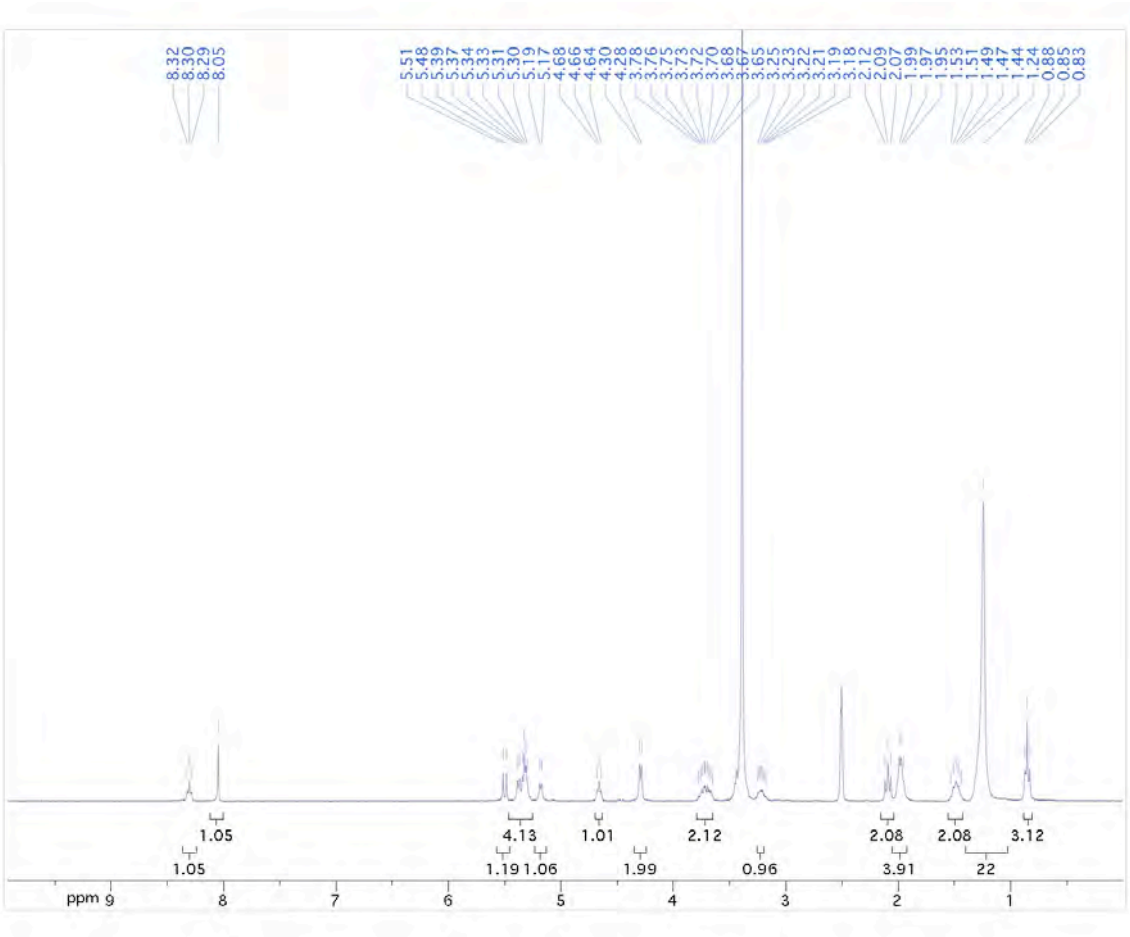



**Figure SI3:** Example of $^{1}$H NMR spectrum (Compound **6**).

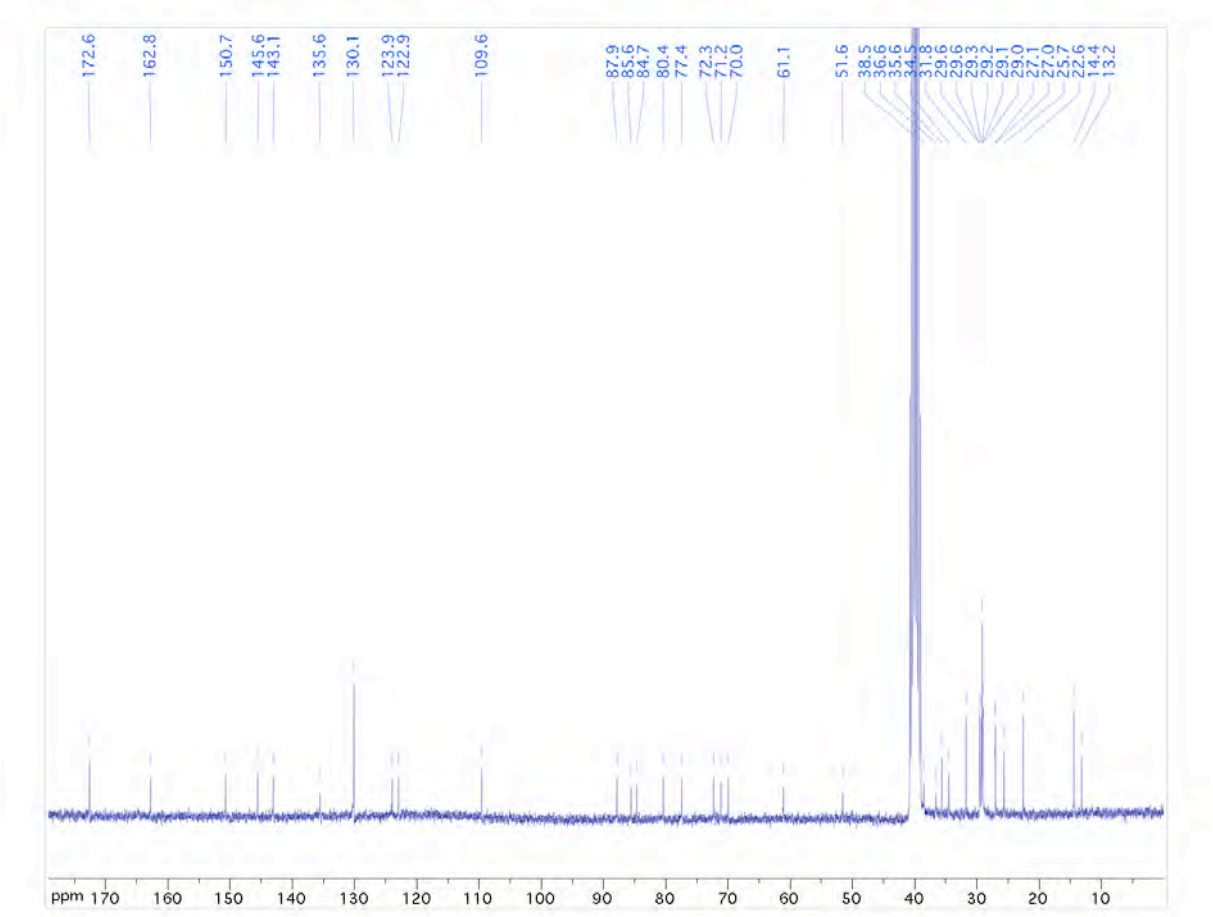



**Figure SI4:** Example of $^{13}C$ NMR spectrum (Compound **5**).

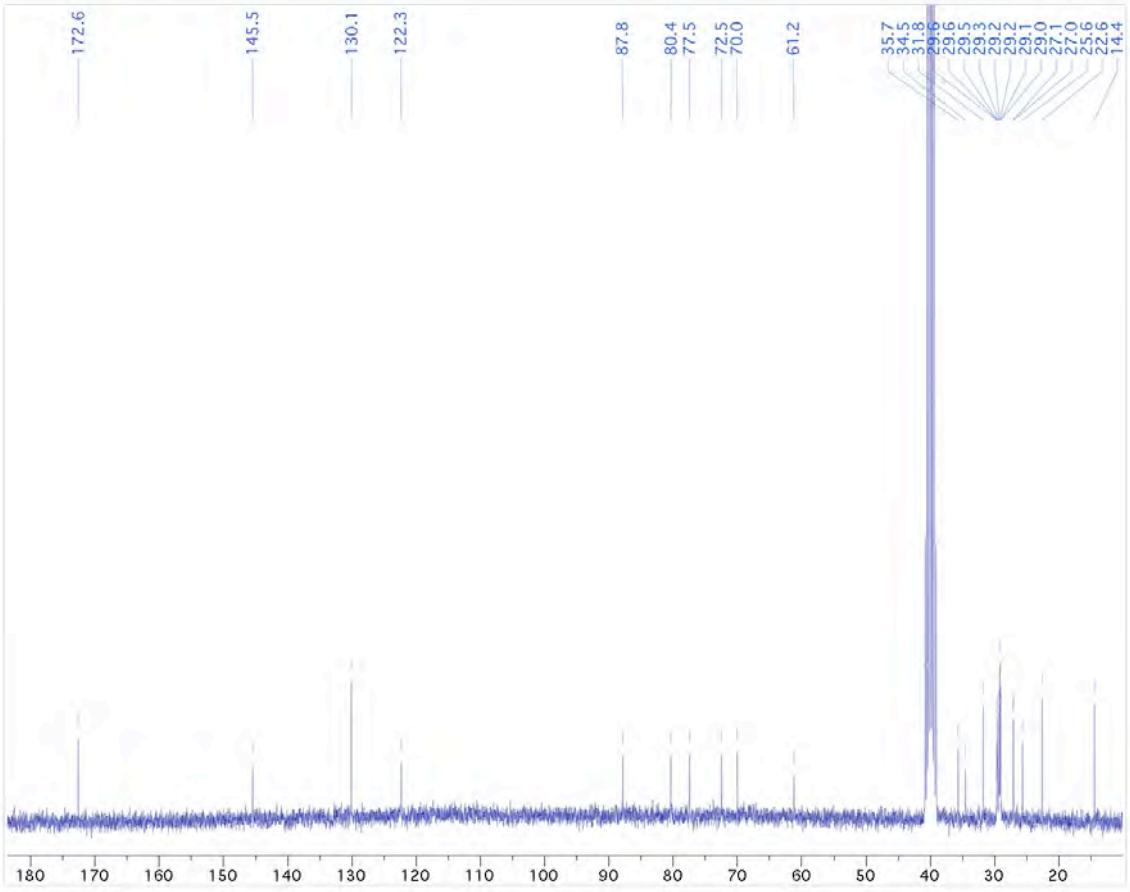



**Figure SI5:** Example of $^{13}C$ NMR spectrum (Compound **6**).

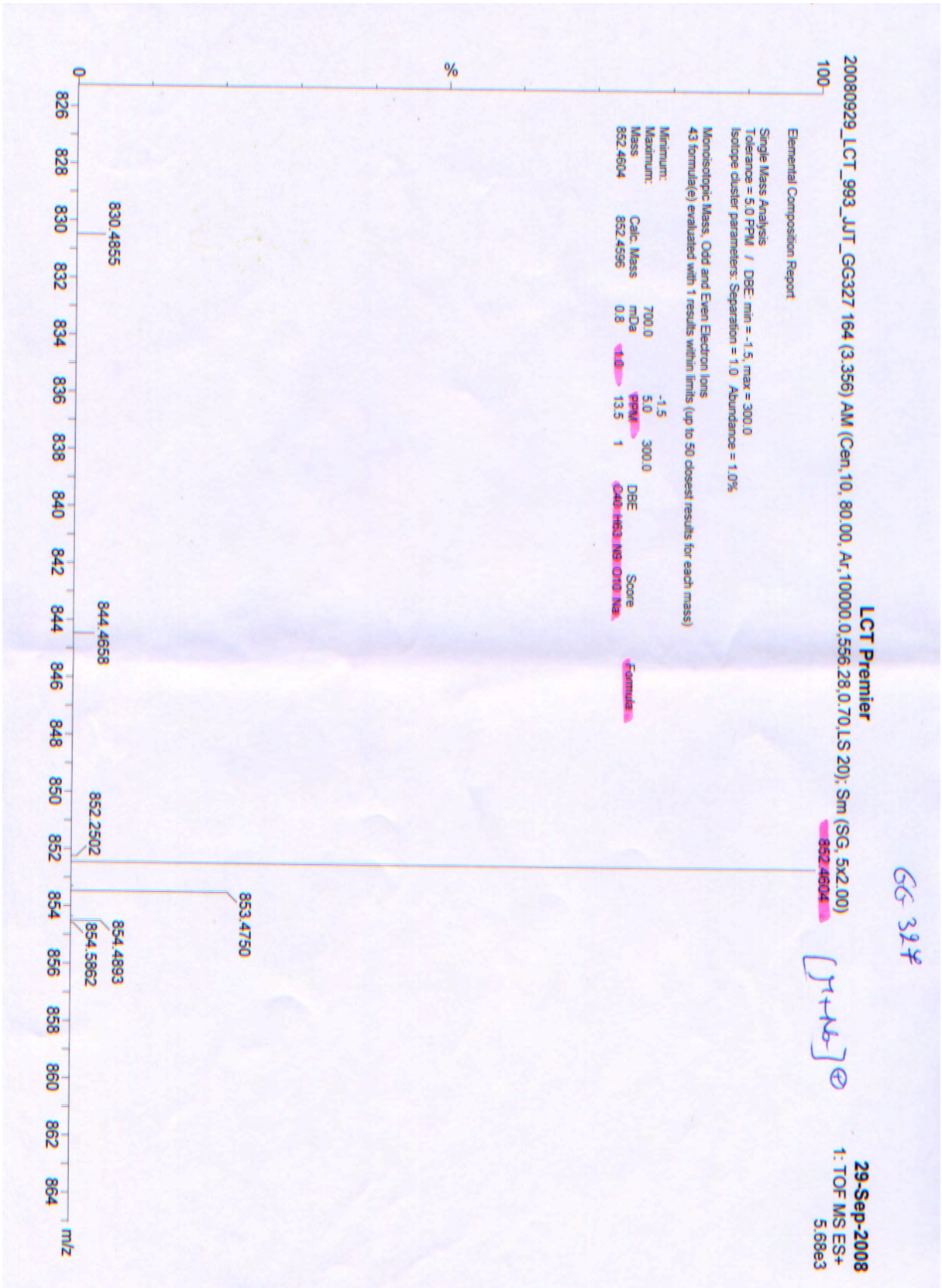



**Figure SI6:** Example of HRMS spectrum for compound **5**.

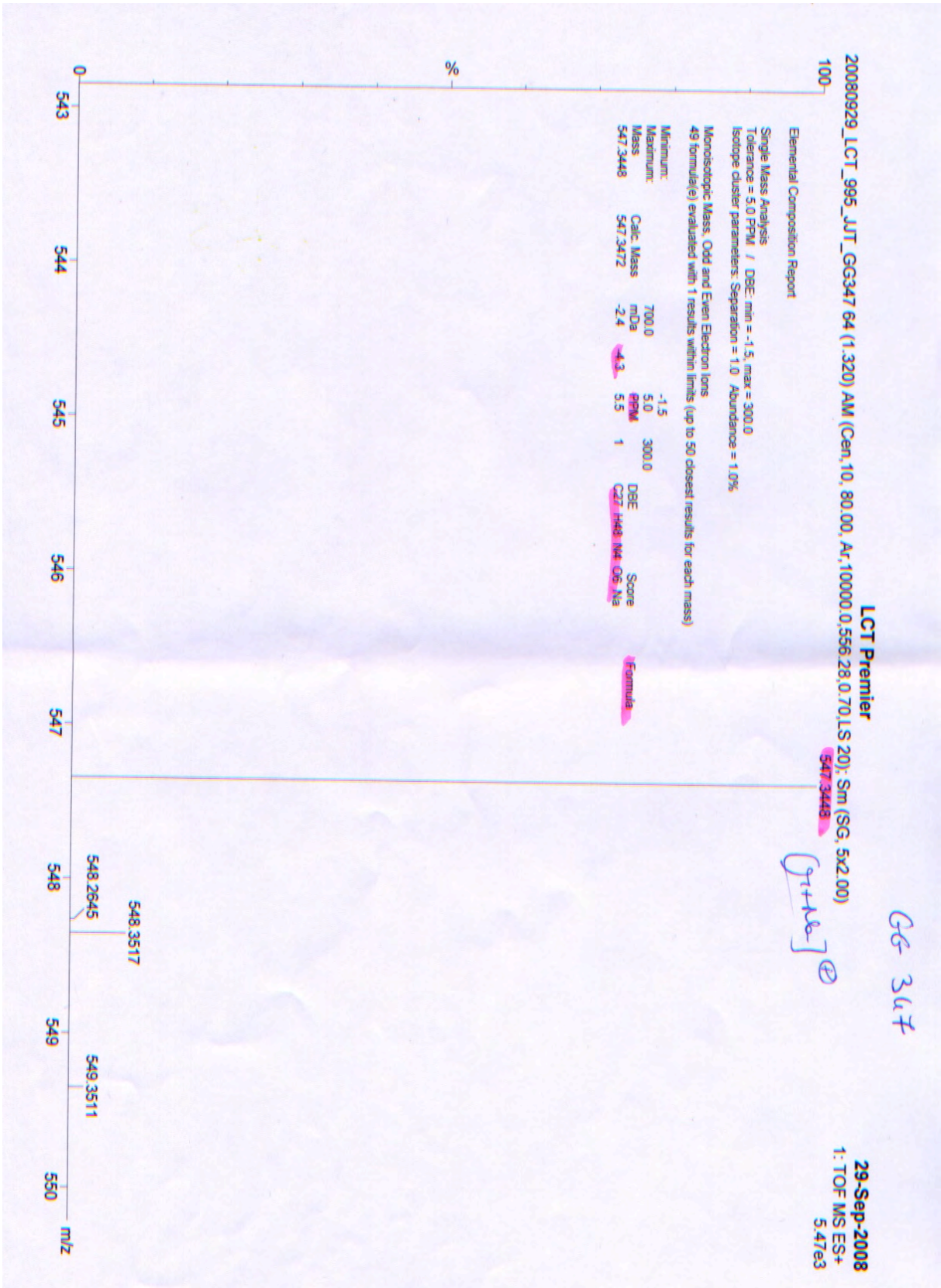



**Figure SI7:** Example of HRMS spectrum for compound **6**.

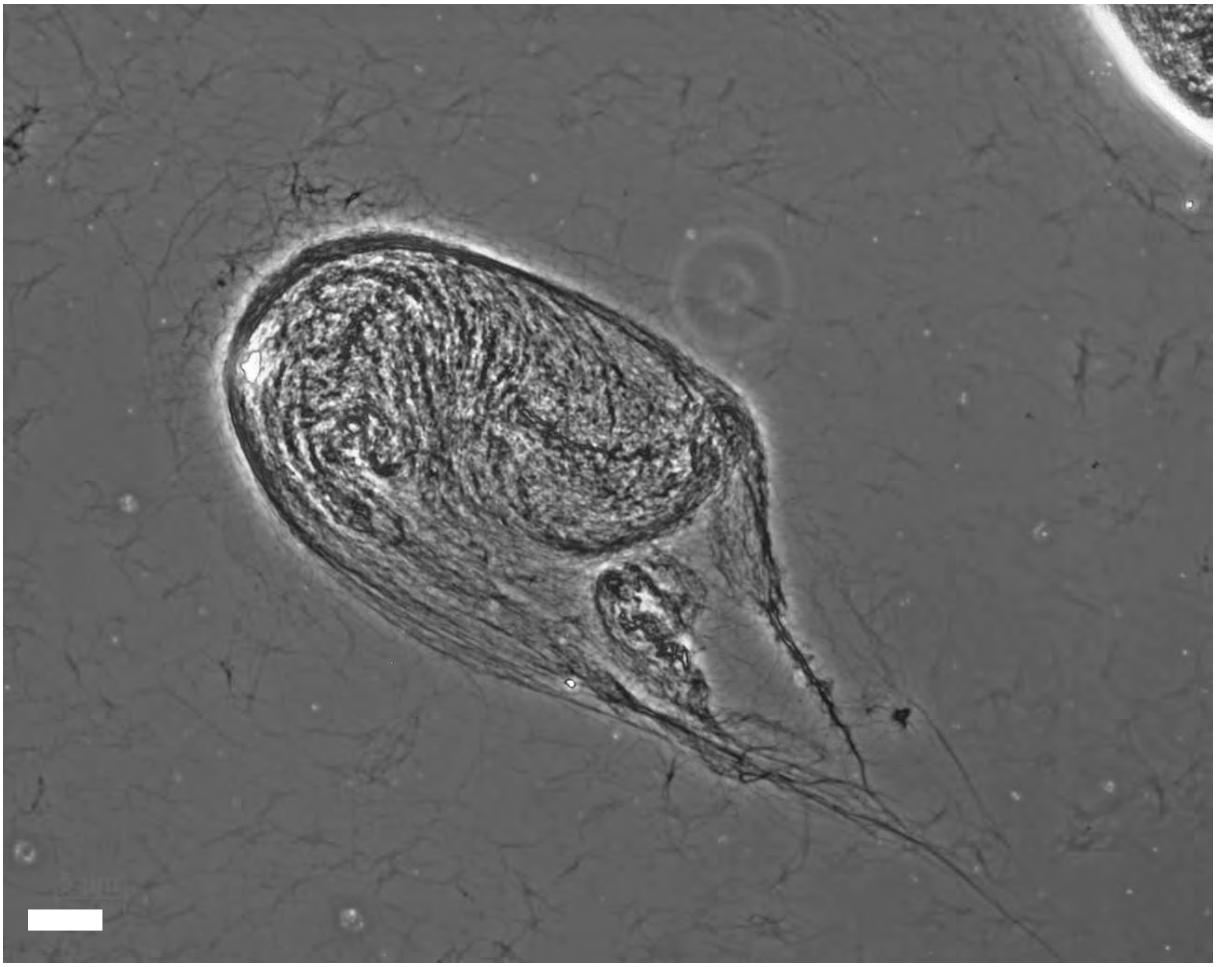


**Figure SI8:** Example of optical microscopy on a 1 mg/mL water solution of Compound **5** (Scale = 5μm).

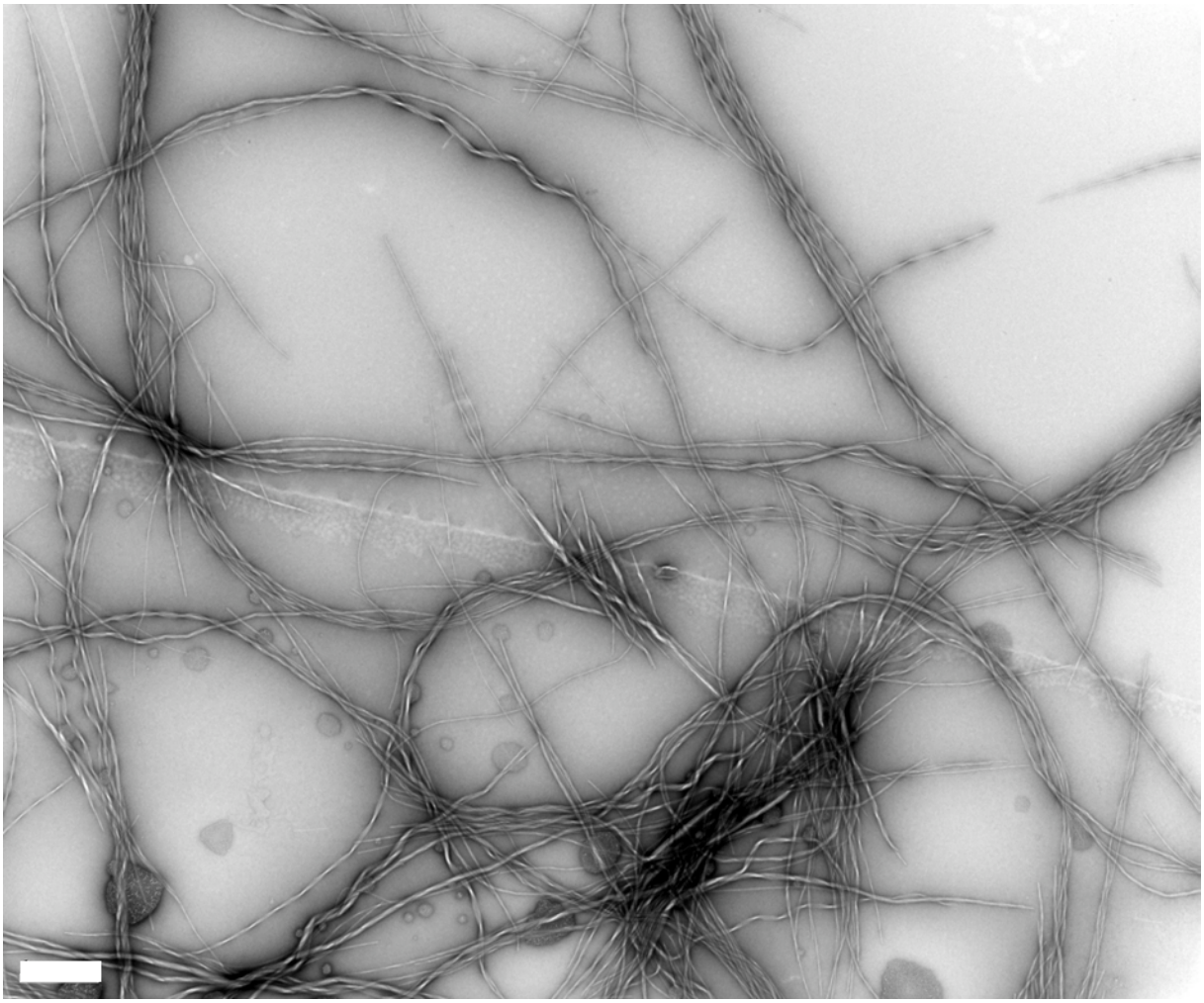


**Figure SI9:** Example of transmission electronic microscopy image of a 1 mg/mL water solution of Compound **5** (scale = 1µm).

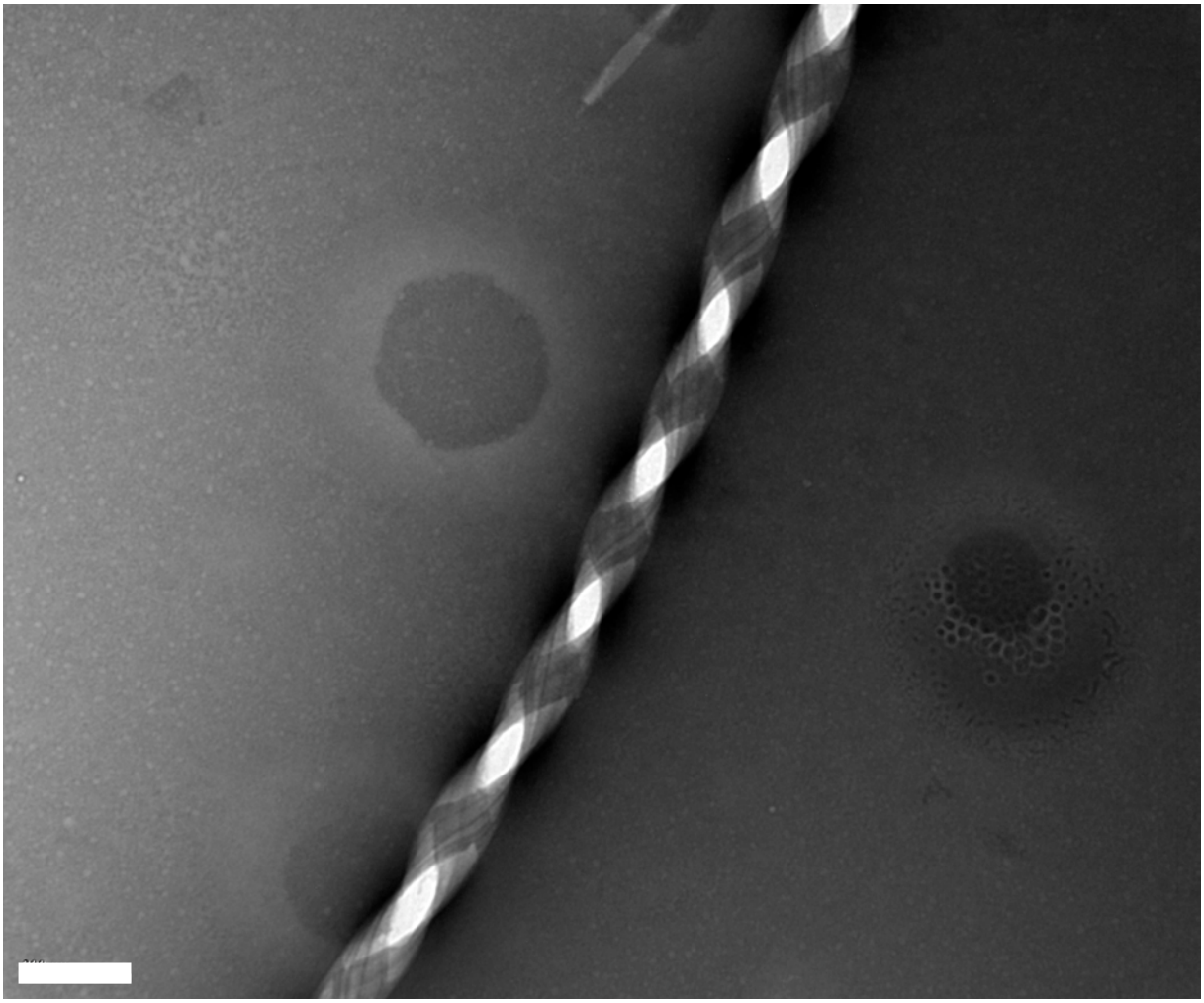


**Figure SI10:** Example of self assembling helical fibres of compound **5** observed by TEM (scale = 200 nm).

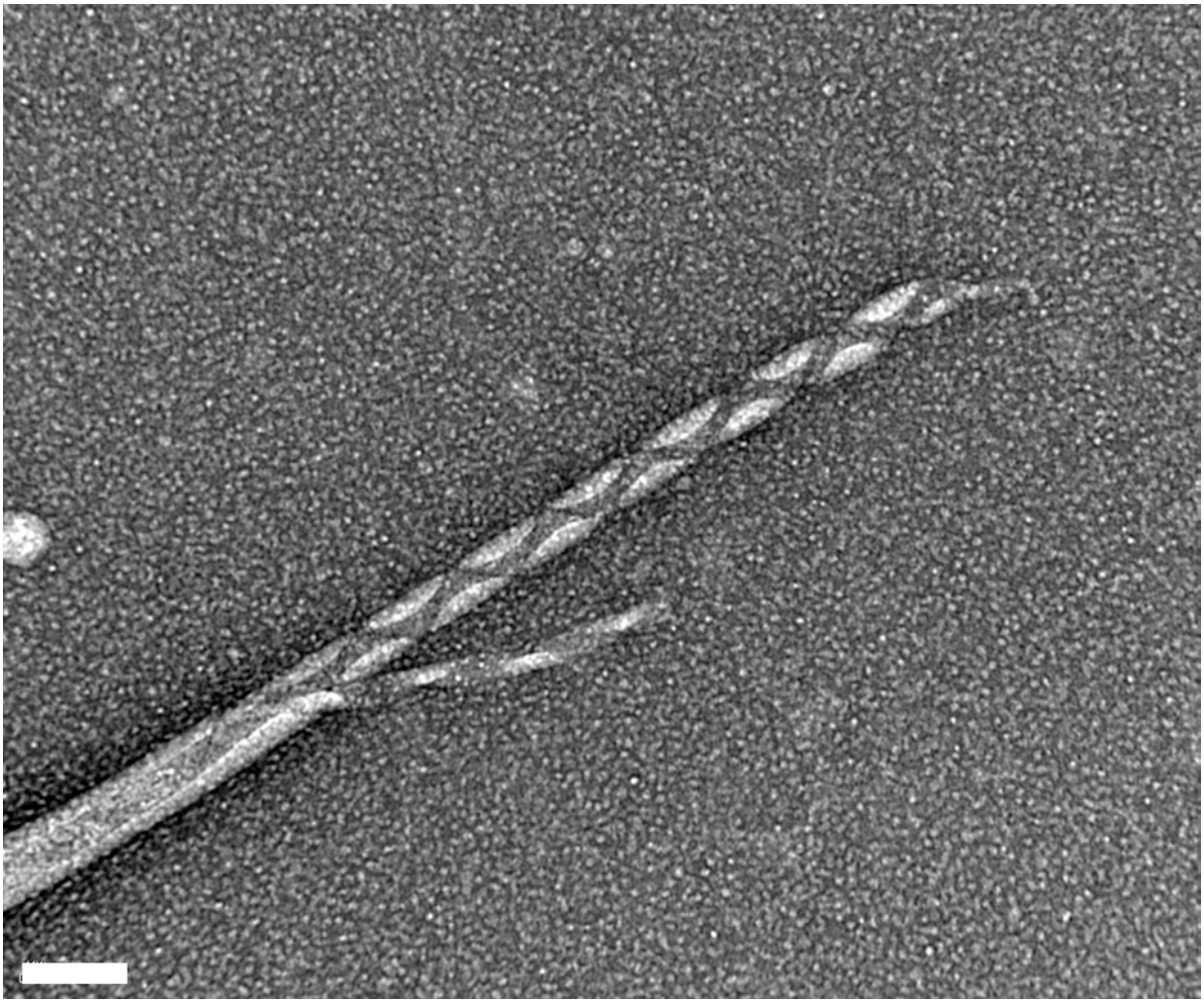


**Figure SI11:** Example of self-assembling helical fibres of compound **5** forming nano-tubular structures (scale = 100 nm).

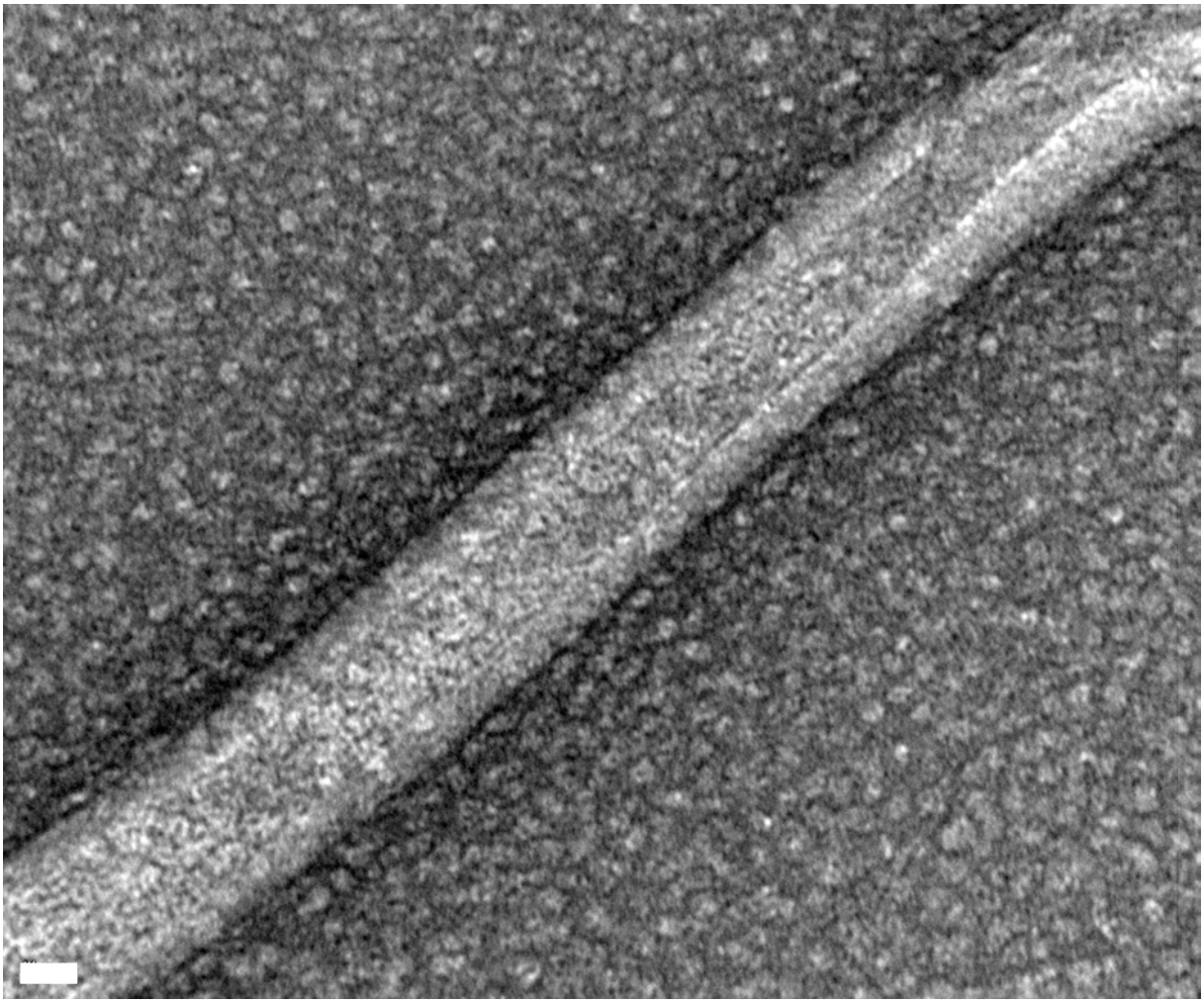


**Figure SI12:** Example of nanotube of compound **5** observed by TEM (scale = 20 nm).

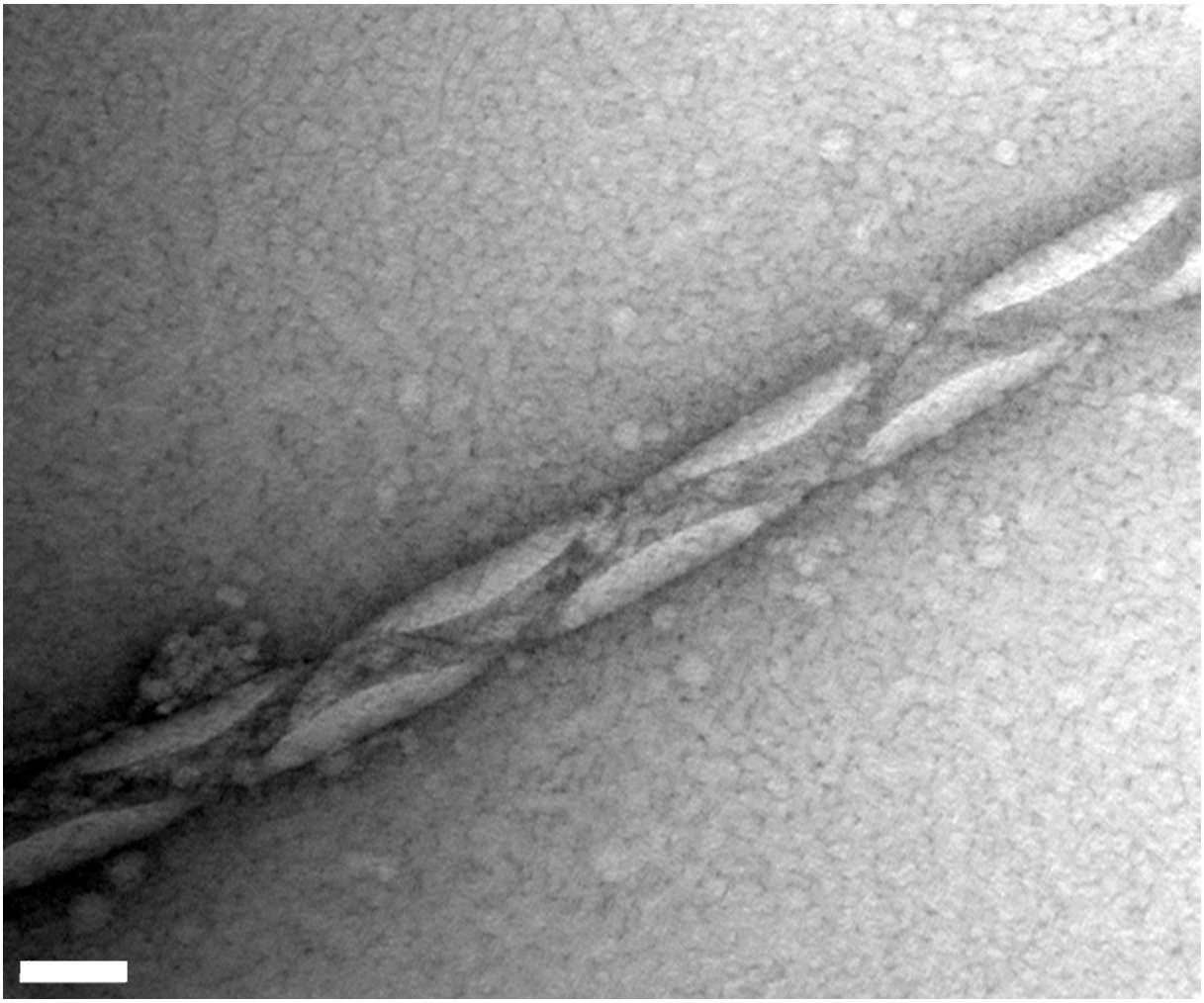


**Figure SI13:** Example of helical fibre of compound **5** observed by TEM (scale = 50 nm).

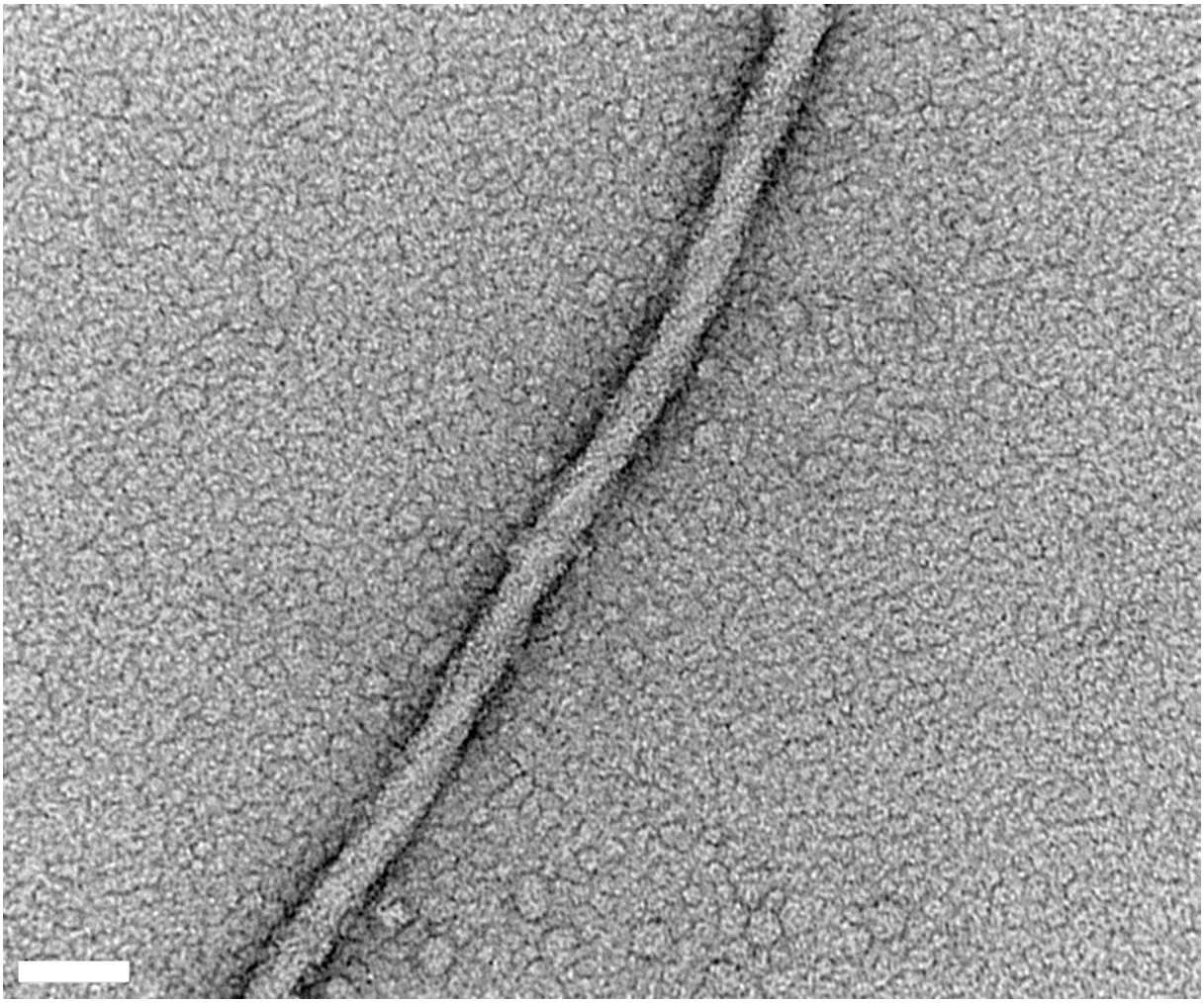


**Figure SI14:** Example of fibre formed by one helical fibre of compound **5** observed by TEM (scale = 50 nm).

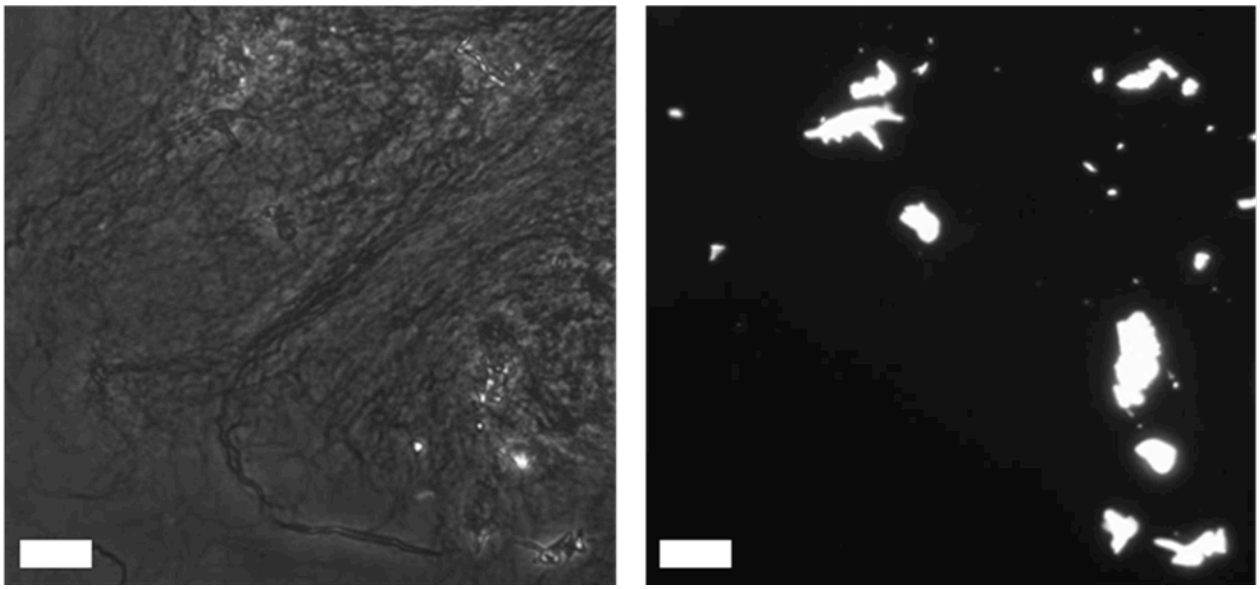


**Figure SI15:** Phase contrast and fluorescence microscopy on mixture of **5** with 3'-labelled ON (scales = 5μm).

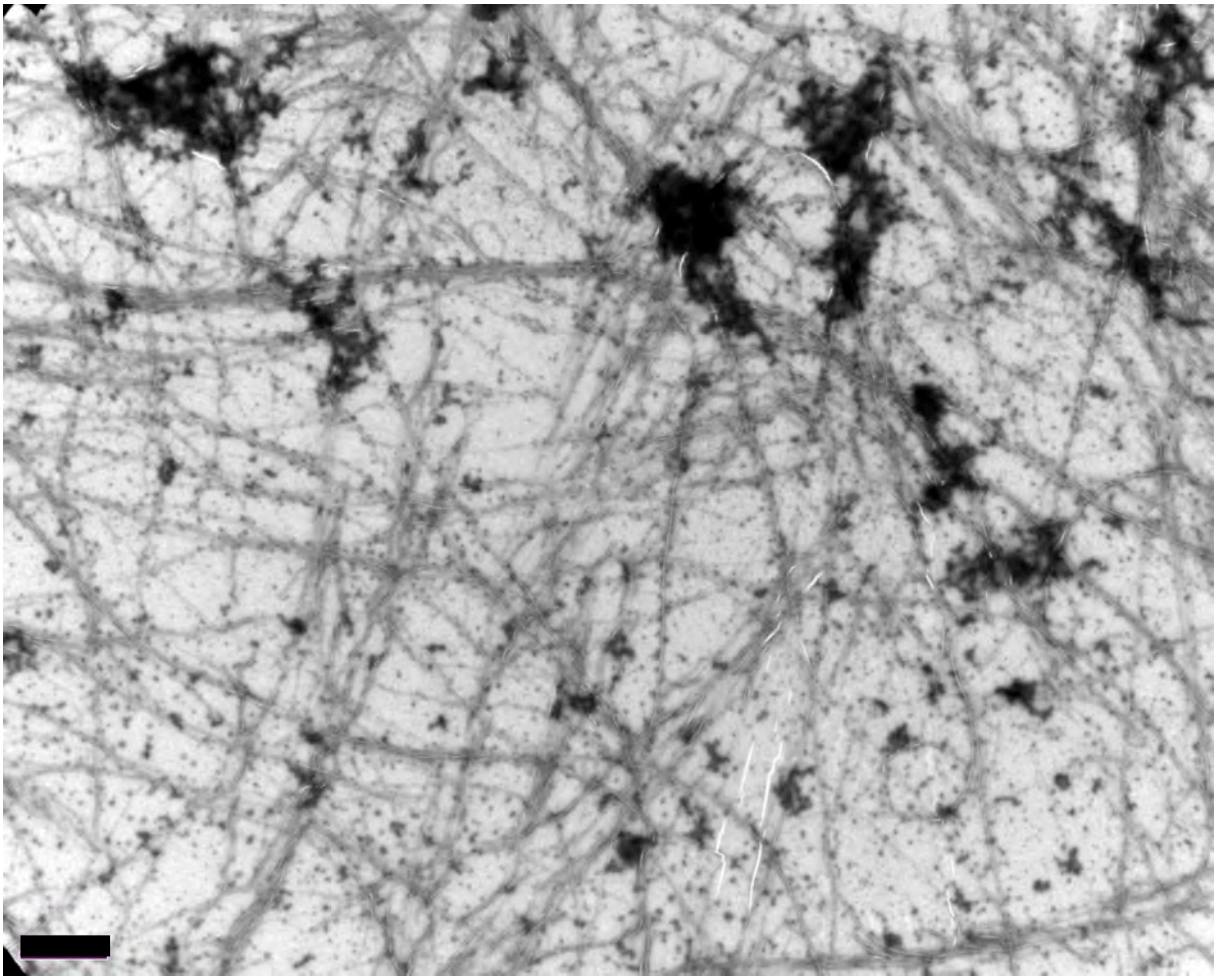


**Figure SI16:** Example of TEM microscopy on mixture of **5** with ON (Scale = 2 μm).

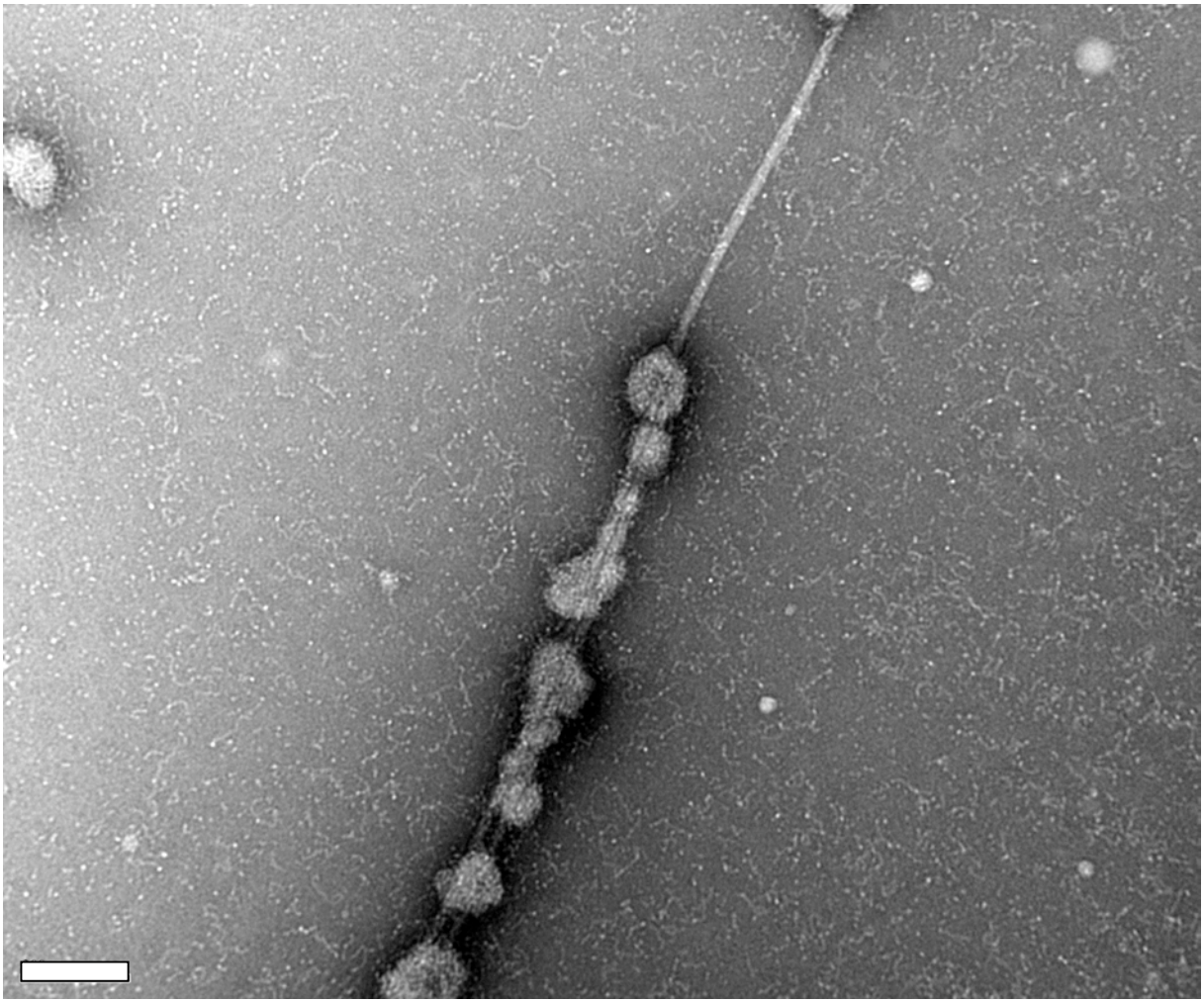


**Figure SI17:** Example of TEM microscopy: ON complexed on fibre of **5** (Scale = 200 nm).

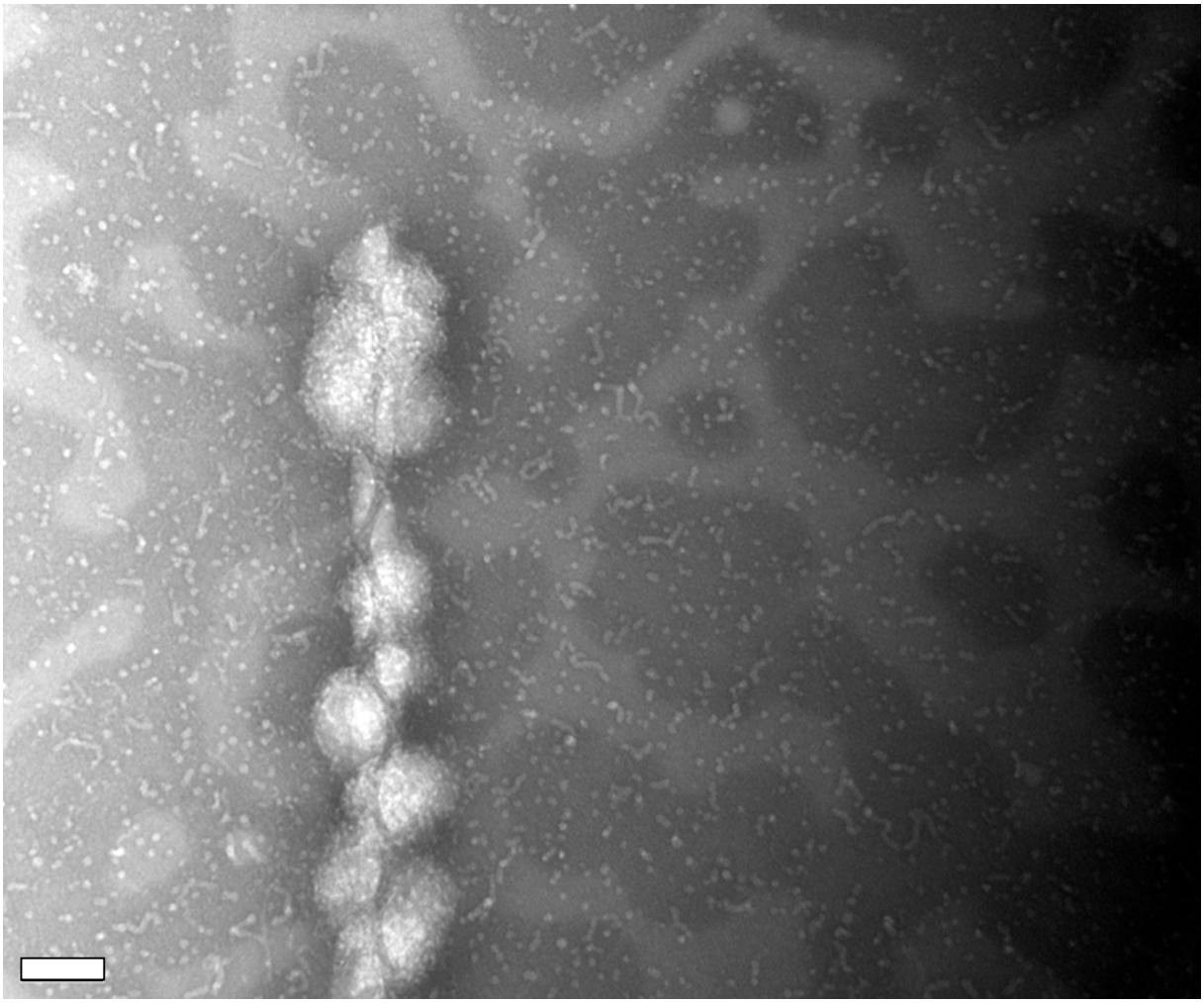


**Figure SI18:** Example of TEM microscopy: ON complexed on helical fibre (Scale = 100 nm).

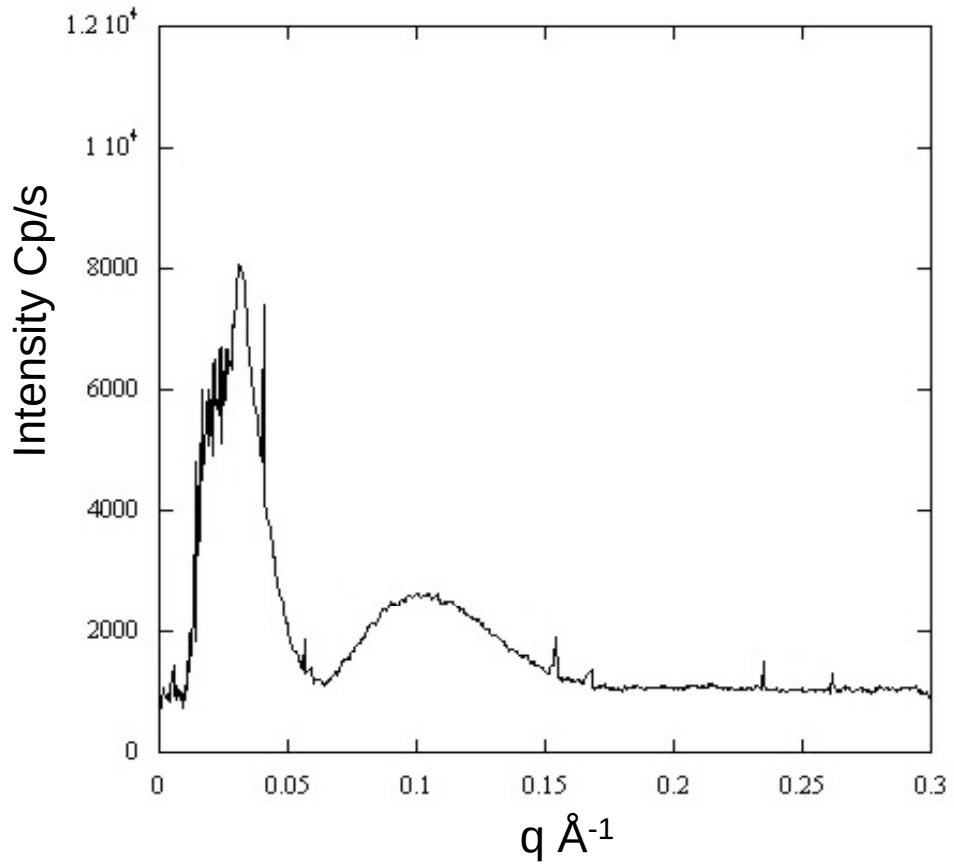



**Figure SI19:** SAXS diffraction patern of compound 5 (20 % w/w hydrogel)