

## *Supplementary information*

### **Double conjugated nanogels for selective intracellular drug delivery**

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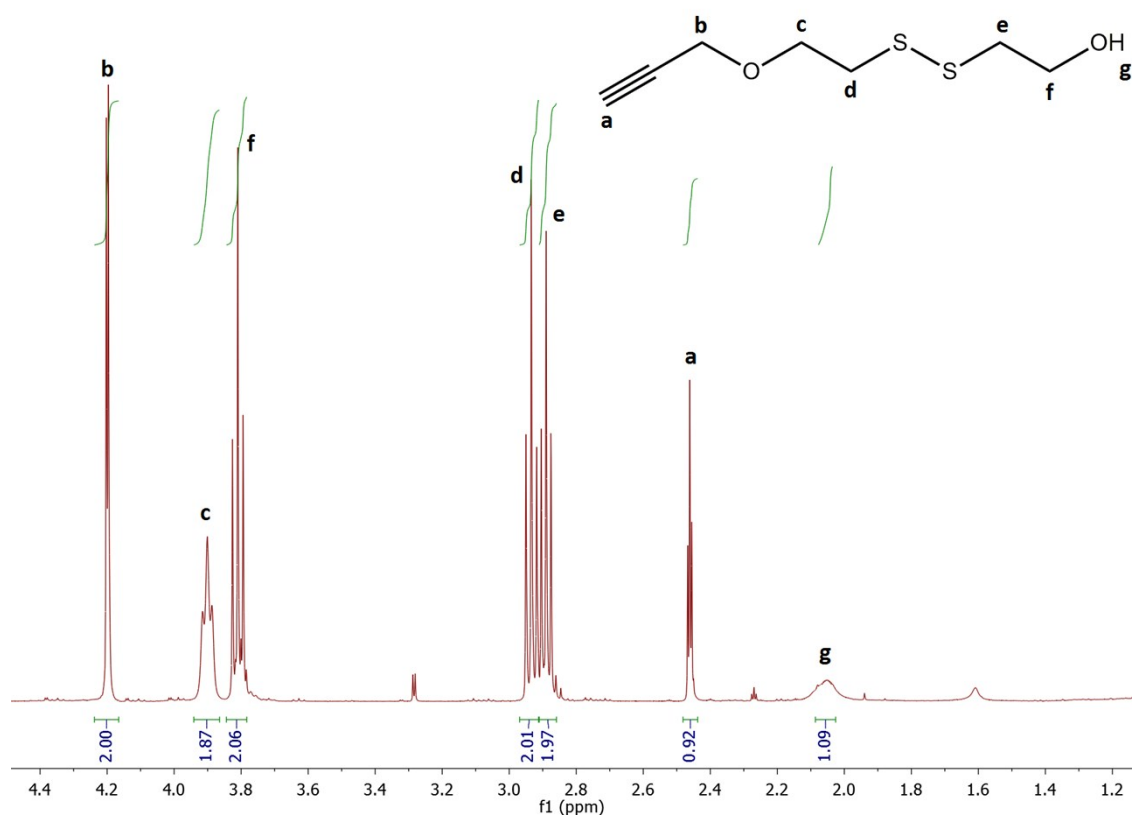
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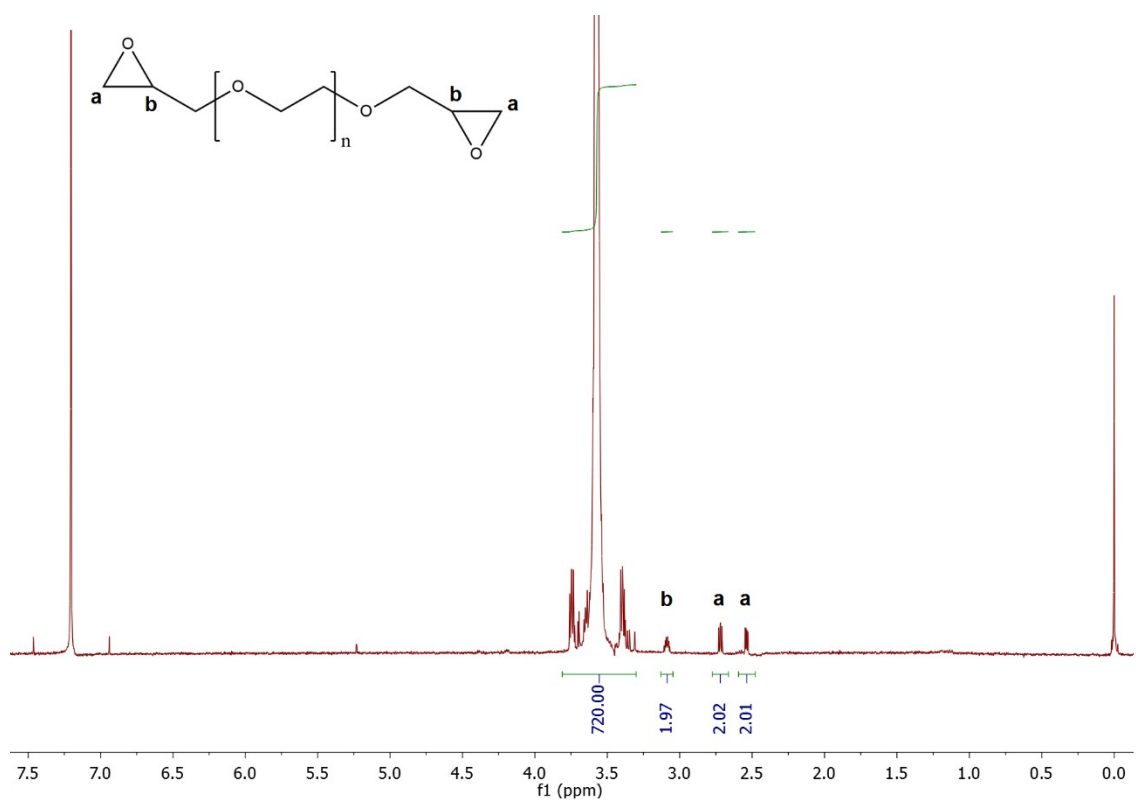
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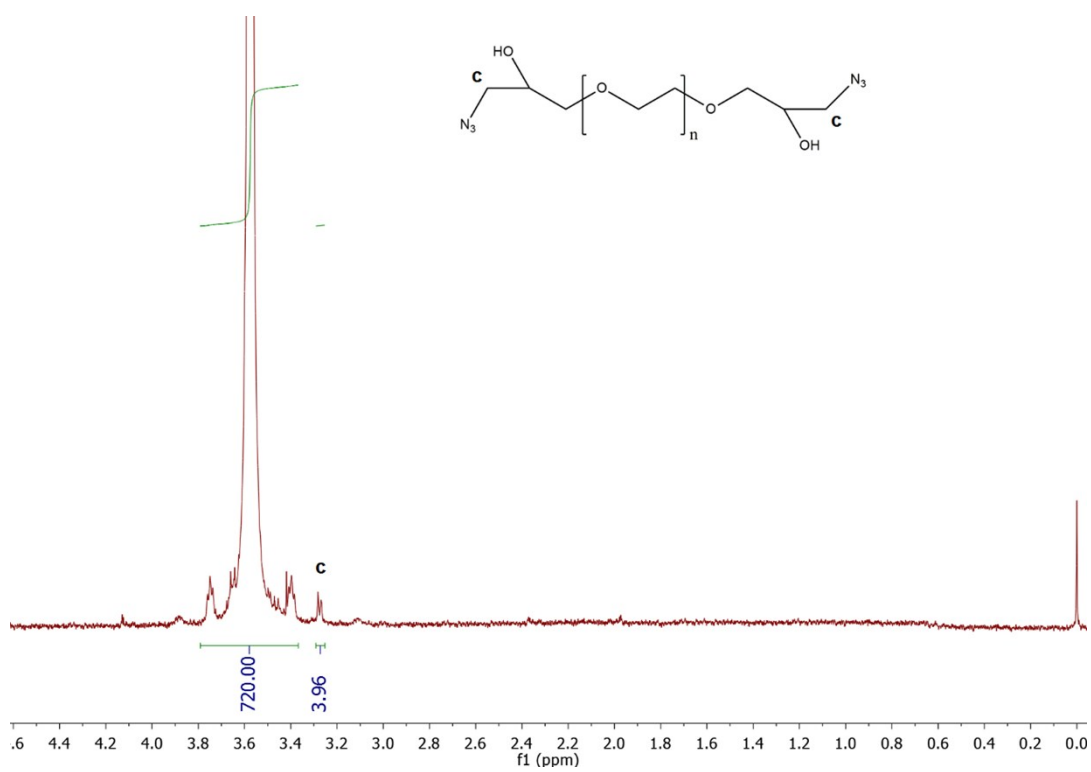
## 1. <sup>1</sup>H-NMR Spectra



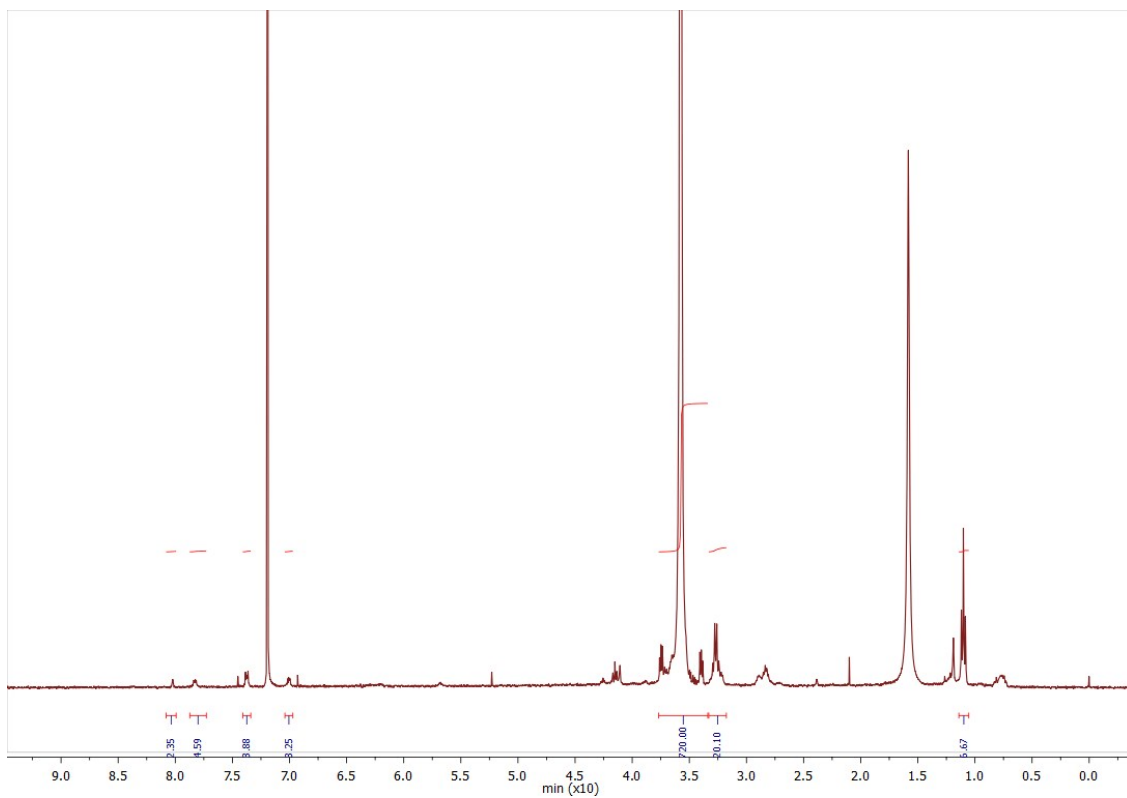
**Figure S1.** <sup>1</sup>H-NMR spectrum of 2-propynoxyethyl disulfanyl ethanol **2** in CDCl<sub>3</sub>: the integral values confirms the alkyne mono-functionalization of the product.



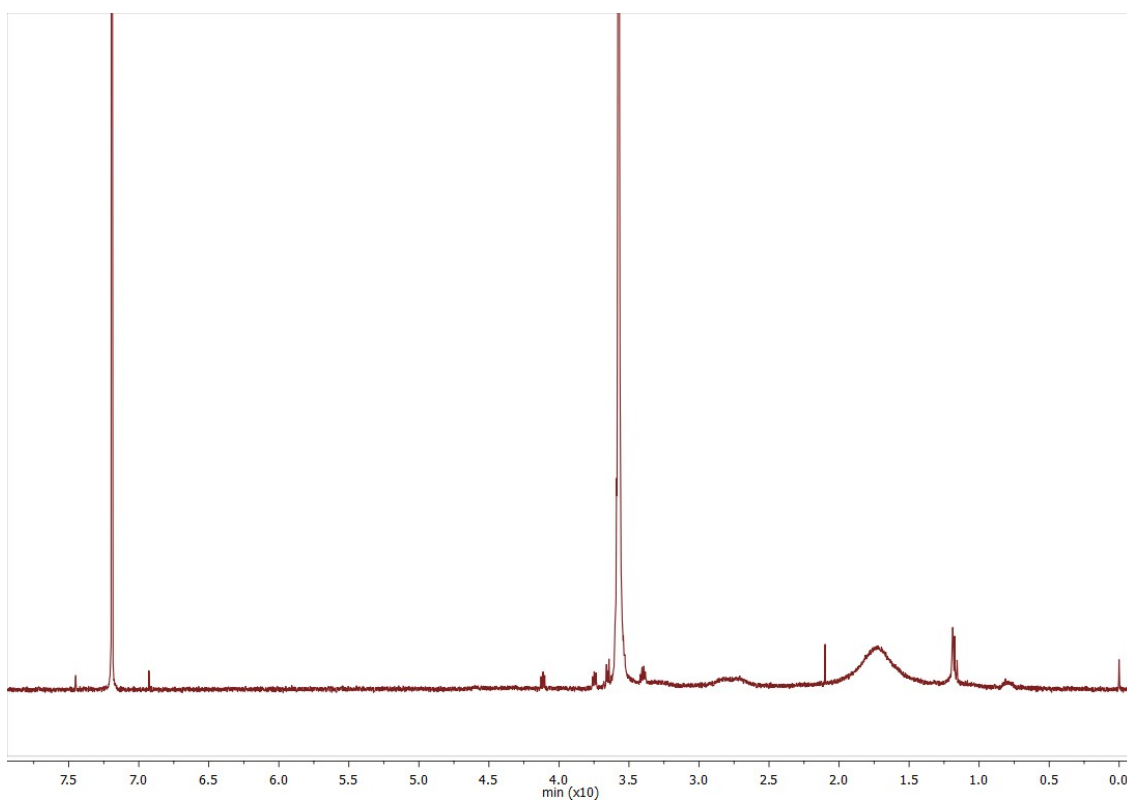
**Figure S2.** <sup>1</sup>H-NMR spectrum of diepoxy-PEG 4 in CDCl<sub>3</sub>.



**Figure S3.** <sup>1</sup>H-NMR spectrum of PEG with azide groups 5 in CDCl<sub>3</sub>.



**Figure S4.**  $^1\text{H-NMR}$  spectrum of PEG-rhodamine conjugate **5-R** in  $\text{CDCl}_3$ .



**Figure S5.**  $^1\text{H-NMR}$  spectrum of nanogel in  $\text{CDCl}_3$ .

**2. Amine modified rhodamine 1 calibration curve.** Modified rhodamine 1 (2 mg, 4.11  $\mu\text{mol}$ ) was dissolved in 2 mL of ethanol. From the resulting 2.05 mM stock solution, the calibration solutions with concentration of 1.027 mM, 513.5  $\mu\text{M}$ , 256.75  $\mu\text{M}$ , 128.38  $\mu\text{M}$ , 64.19  $\mu\text{M}$ , 32.10  $\mu\text{M}$ , 16.05  $\mu\text{M}$ , 8.03  $\mu\text{M}$  and 4.02  $\mu\text{M}$  were prepared by diluting the stock solution with ethanol. UV-vis measurements were recorded for the calibration solutions. Its related calibration curve was obtained plotting absorbance at 570 nm versus concentration.

**3. Polymer characterization: gel permeation chromatography.** The effective PEG functionalization was characterized using size exclusion chromatography (SEC) analysis with THF as eluent and a 0.5 mL/min flow rate. The instrument (Agilent, 1100 series, Germany) was equipped with differential refractive index (RI) three PL gel columns (Polymer laboratories Ltd., UK; two columns had pore sizes of the MXC type and one was an oligopore; 300 mm length and 7.5 mm ID) and a precolumn. A universal calibration was applied based on polystyrene (PS) standards from 580 Da to 3,250,000 Da (Polymer Laboratories). In Figure S6 GPC chromatograms of PEG and compound 5-R are presented. Peak (\*) corresponds to 8000 in term of molecular weight, while (\*\*) 9800 g/mol; so peak (\*) represents PEG 8000 while peak (\*\*) compound 5-R. The presence of PEG 8000 in compound 5-R is due to the cleavability of the S-S bond in GPC technique.

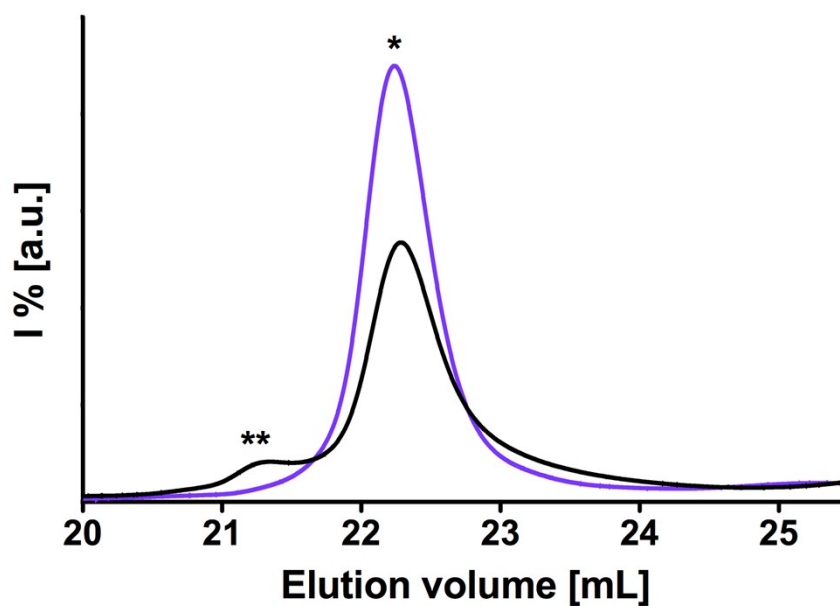


Figure S6. GPC analysis of PEG 8000 (blue line) and compound 5-R (black line).

#### 4. Nanogel cytocompatibility

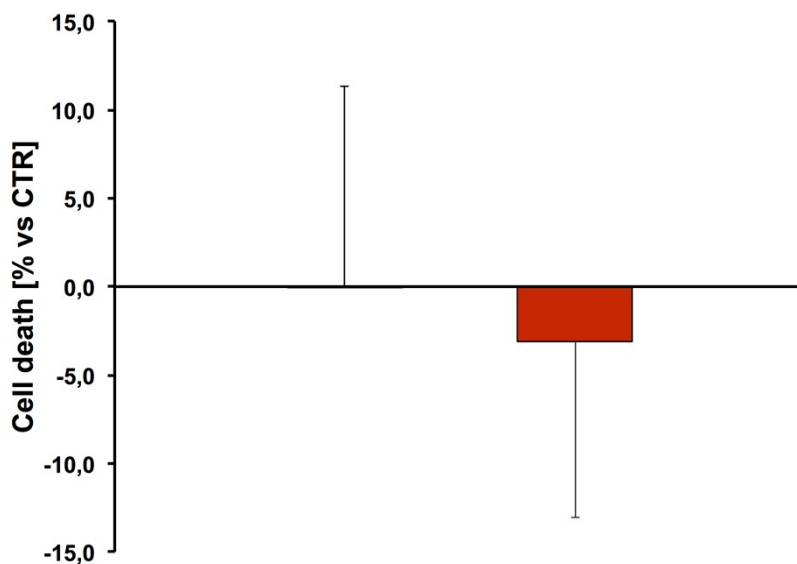


Figure S7. LDH assay reveals that viability of microglial cells after 5 days of treatment with NGs (red) is not affected. Data are presented as percentage  $\pm$  SD of viable cells normalized to treatment condition (not exposed to NGs, blue).