

Supporting Information

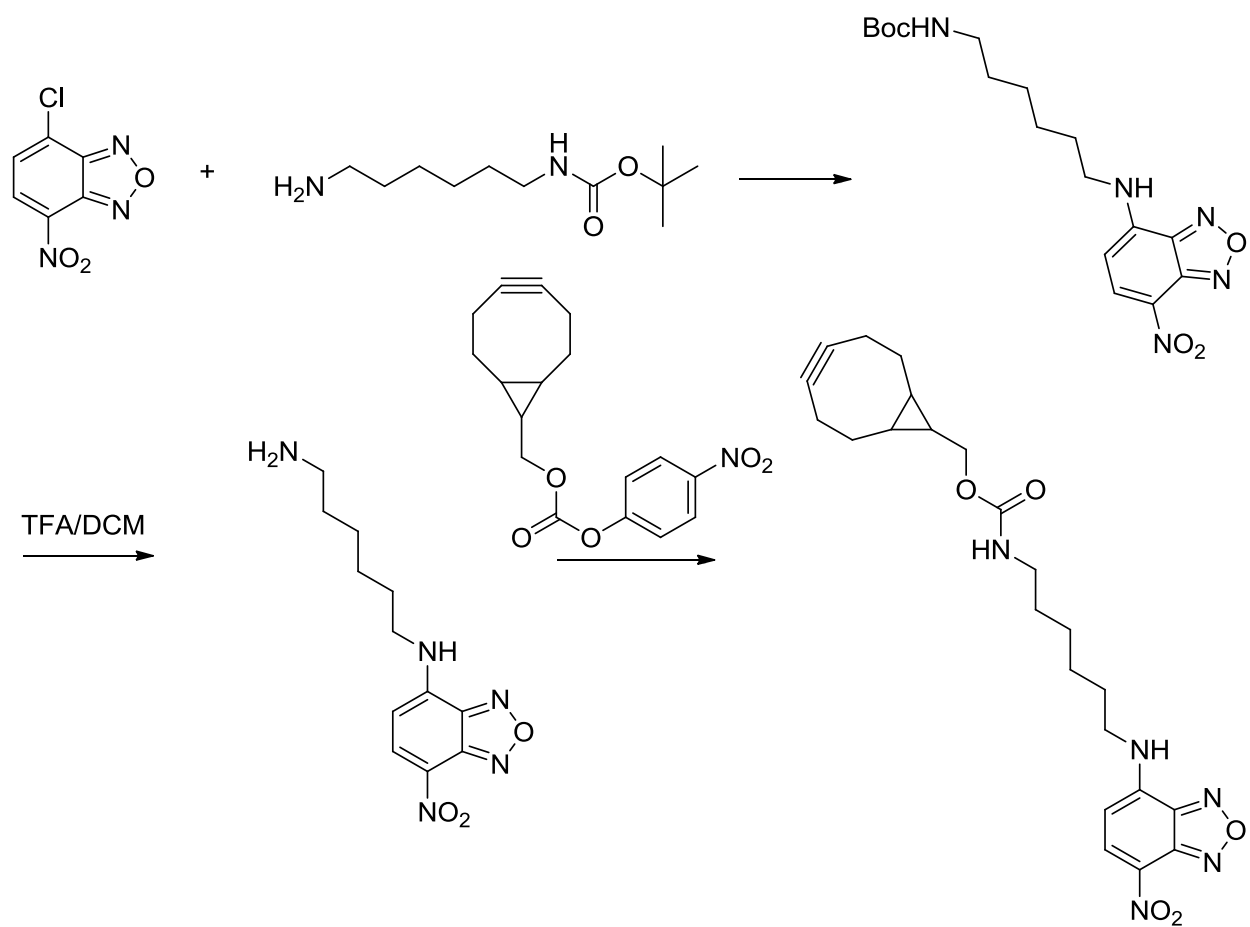
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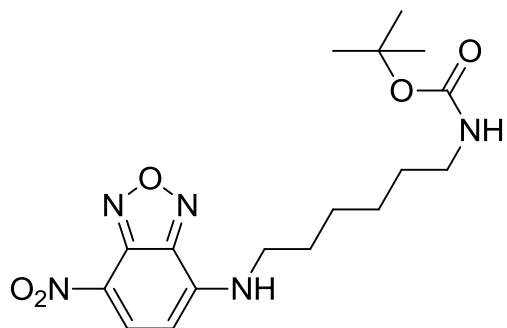
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Synthetic scheme to afford NBD dye



Scheme S1: Synthesis of NBD dye **5**

Spectral characterization of *tert*-Butyl (6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexyl)carbamate (NBoc-hexylenediamine-NBD)



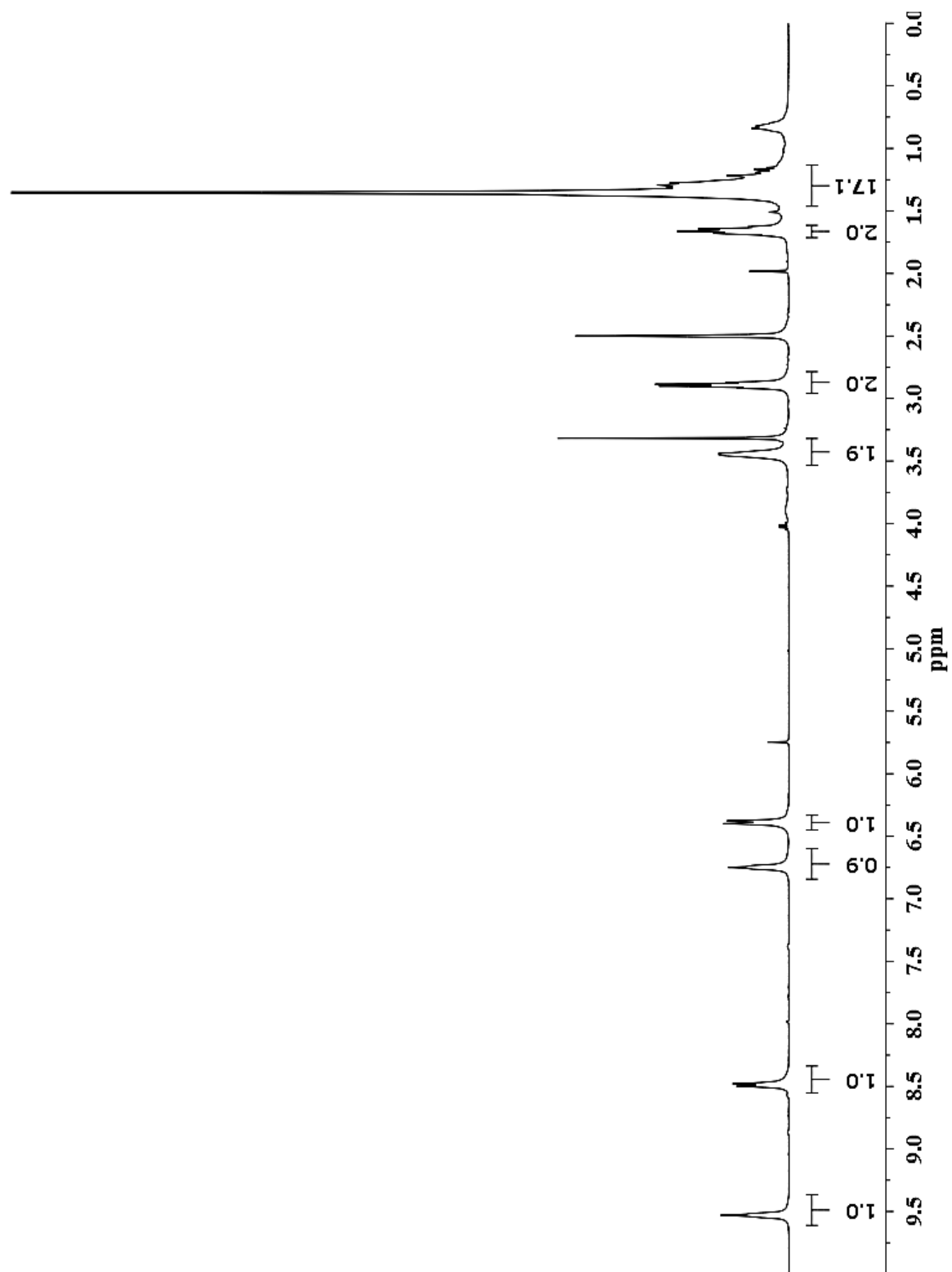


Figure S1: ^1H NMR spectrum of tert-Butyl (6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexyl)carbamate (NBoc-hexylenediamine-NBD) (400 MHz, DMSO-d_6)

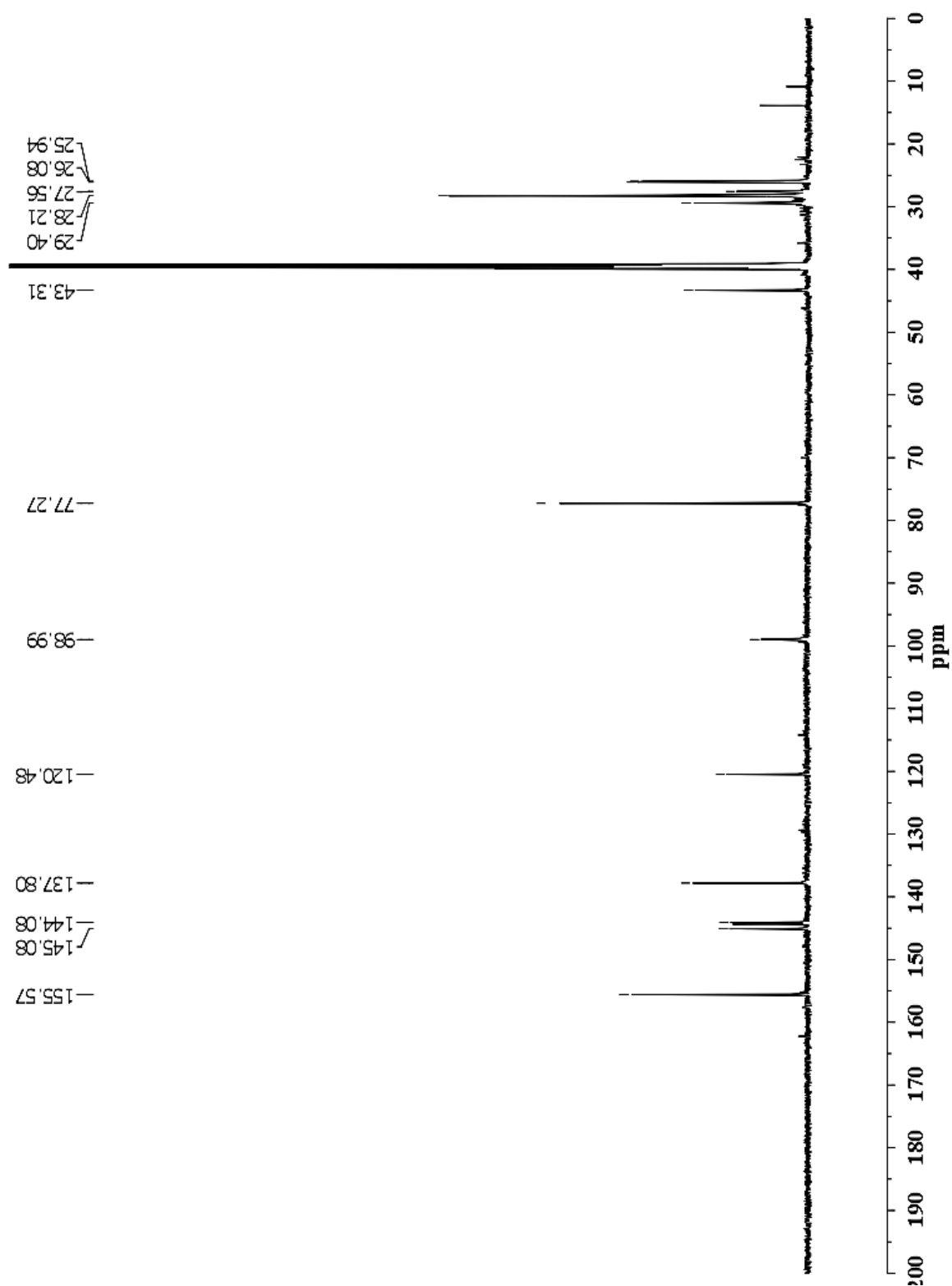
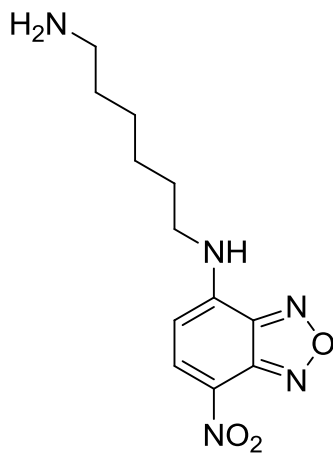


Figure S2: ¹³C NMR spectrum of tert-Butyl (6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexyl)carbamate (NBoc-hexylenediamine-NBD) (150 MHz, DMSO-d₆)

**Spectral characterization of N¹-(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)hexane-1,6-diamine
(hexylenediamine-NBD)**



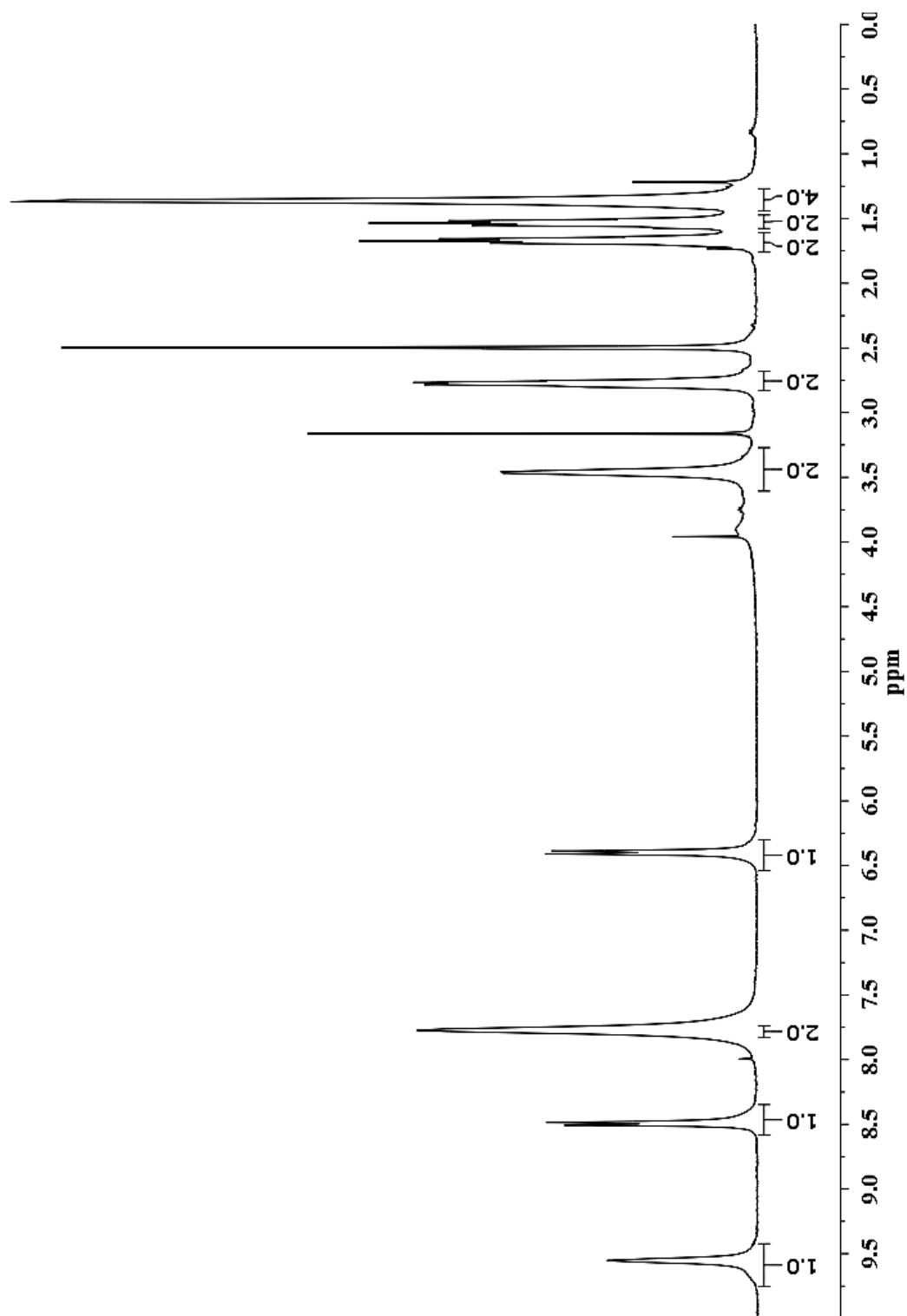


Figure S3: ^1H NMR spectrum of N1-(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)hexane-1,6-diamine (hexylenediamine-NBD) (400 MHz, DMSO-d_6)

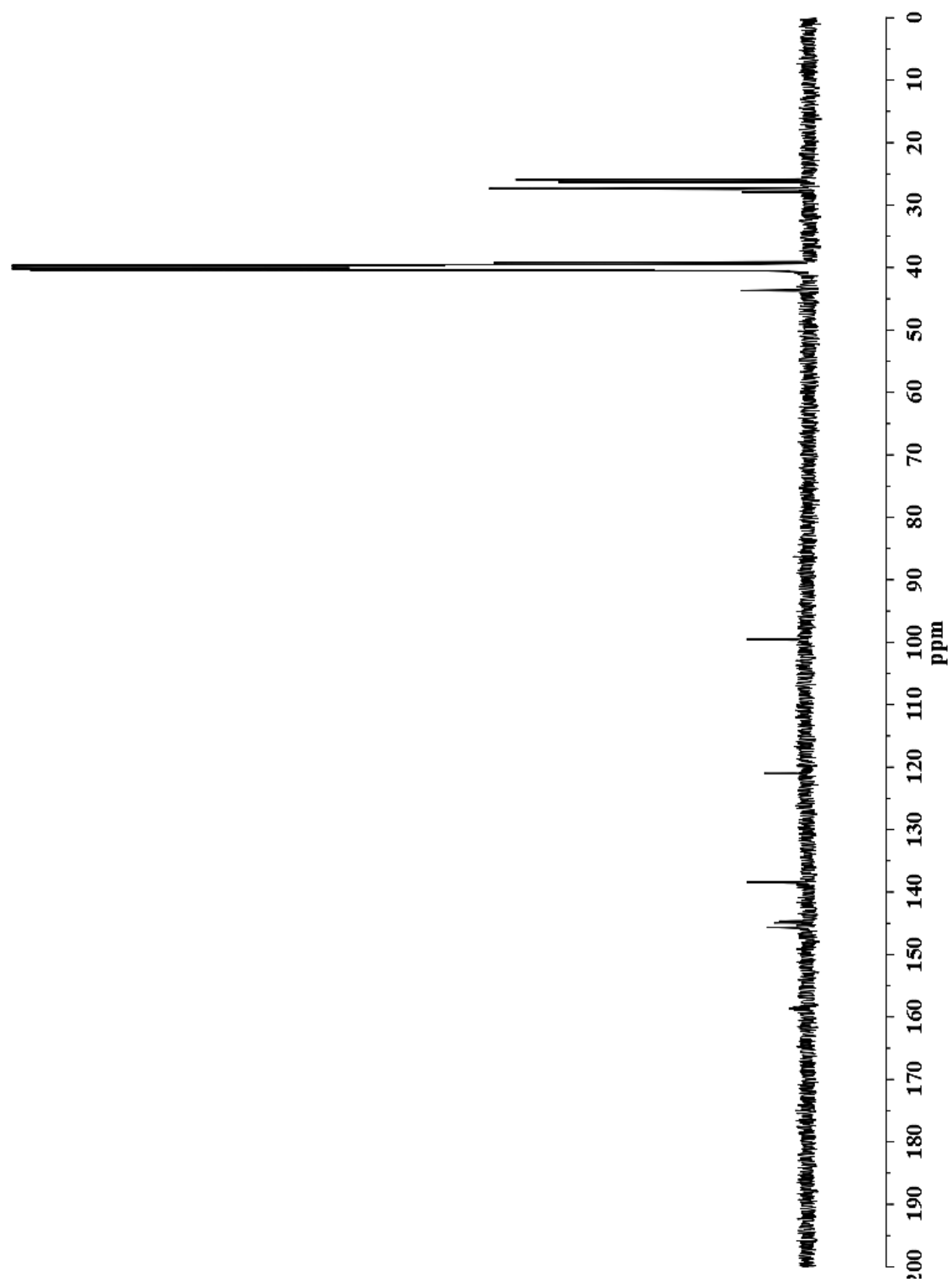
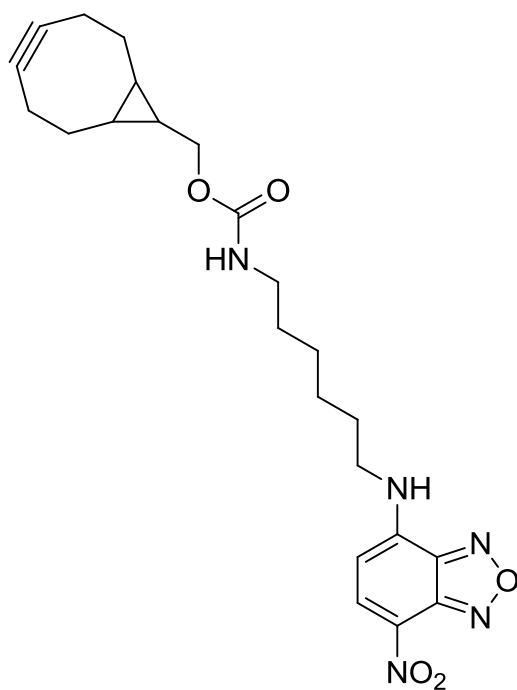


Figure S4: ^{13}C NMR spectrum of N1-(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)hexane-1,6-diamine (hexylenediamine-NBD) (150 MHz, DMSO-d_6)

Spectral characterization of BCN-hexylenediamine-NBD 5



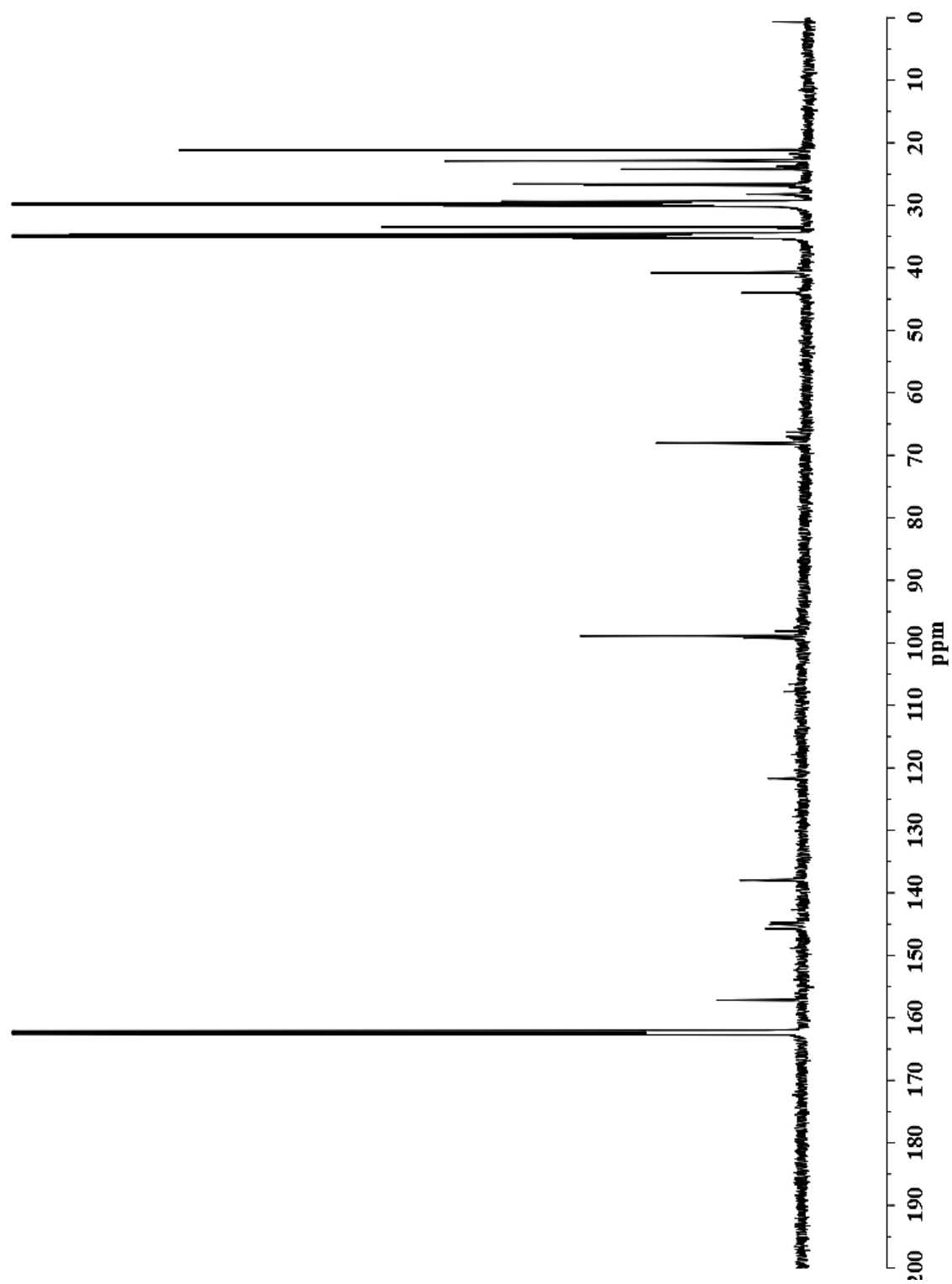
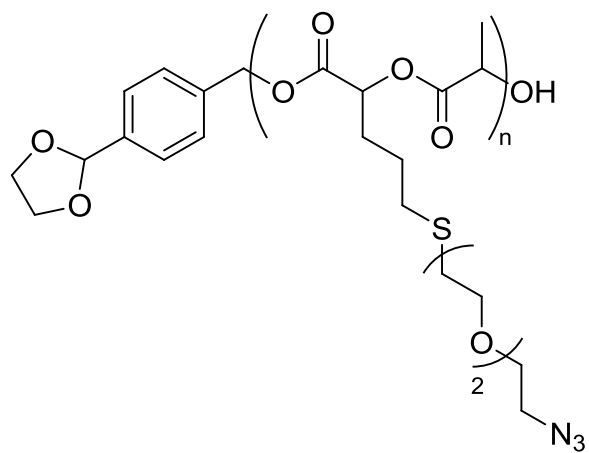


Figure S6: ^{13}C NMR spectrum of BCN-hexylenediamine-NBD **5** (150 MHz, MeCN-d_3)

Polymer characterization – dioxolane-PLA-g-PEG 3



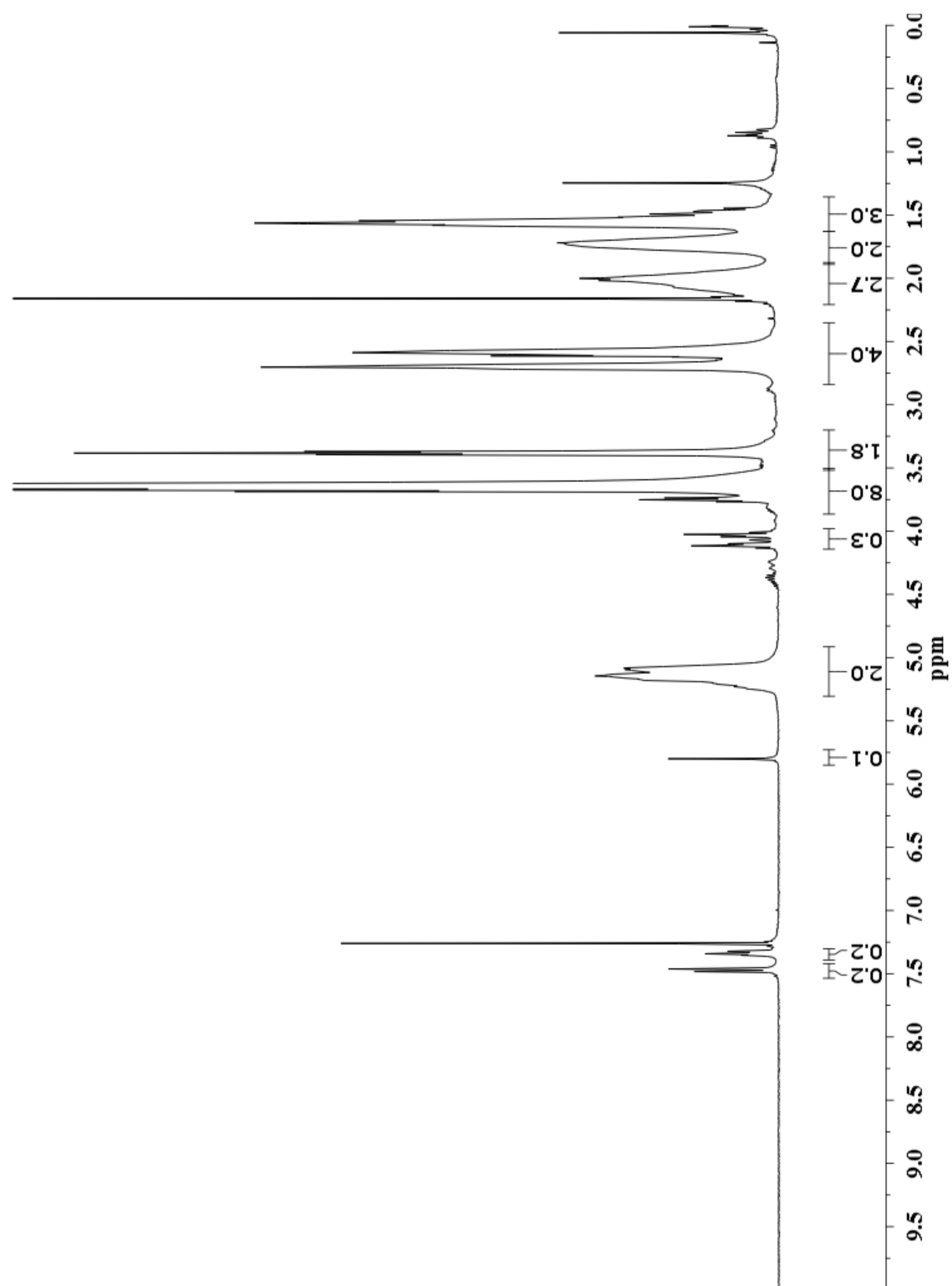


Figure S7: ^1H NMR spectrum of dioxolane-PLA-g-PEG **3** (400 MHz, CDCl_3)

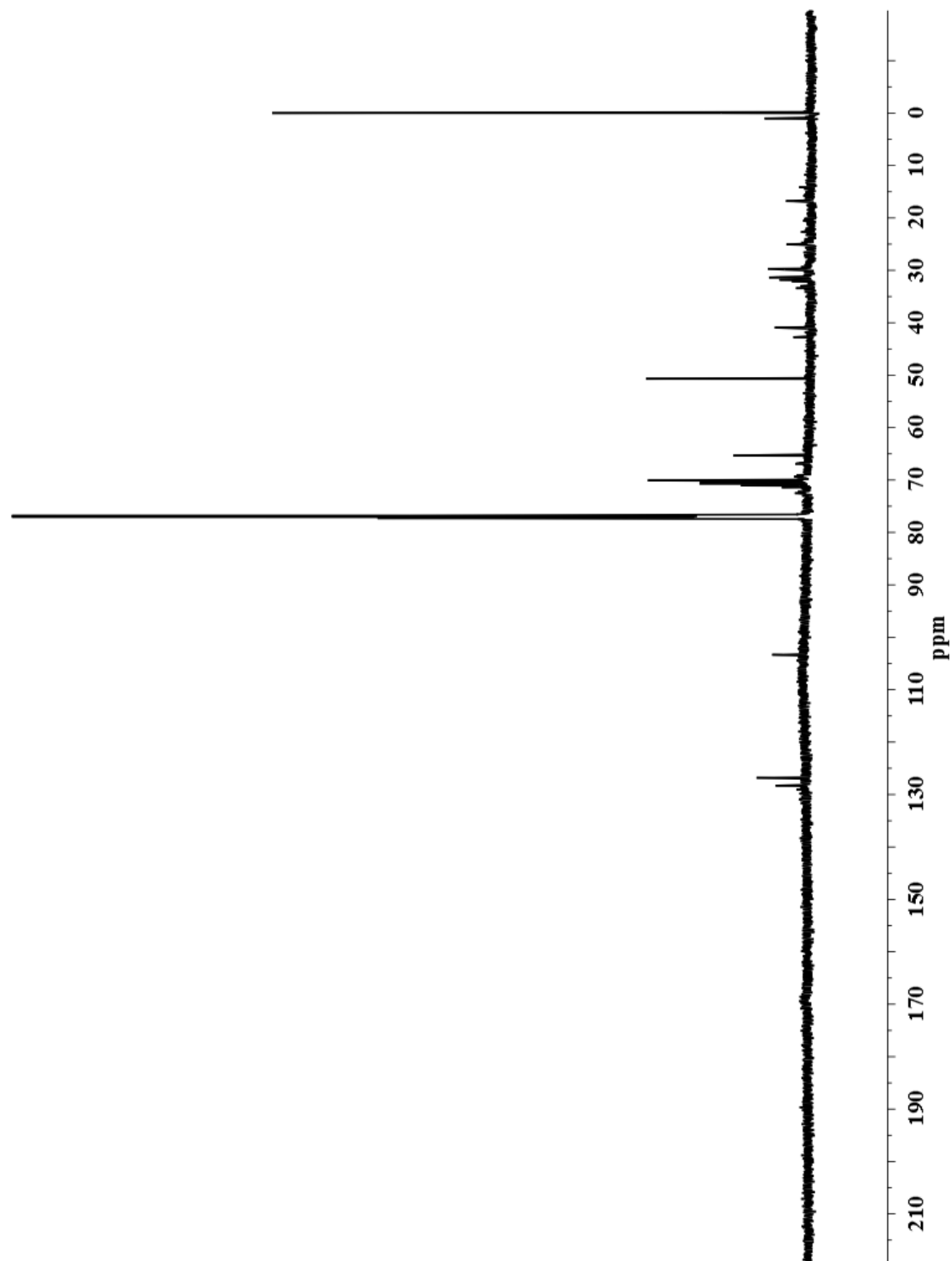
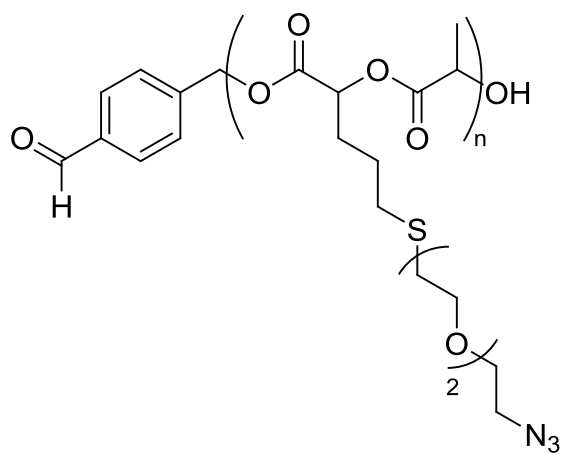


Figure S8: ^{13}C NMR spectrum of dioxolane-PLA-*g*-PEG **3** (150 MHz, CDCl_3)

Polymer characterization – aldehyde-PLA-g-PEG 4



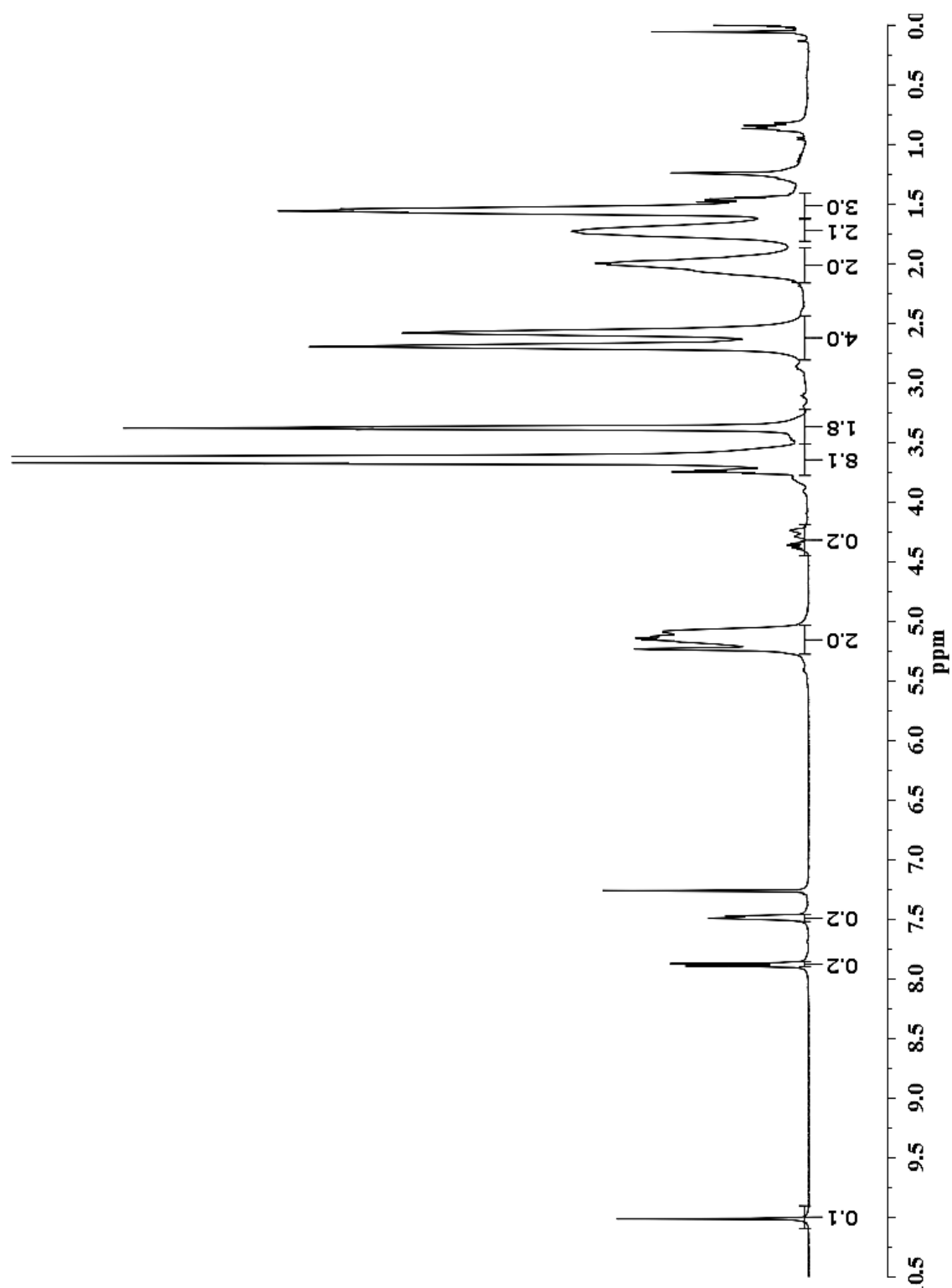


Figure S9: ^1H NMR spectrum of aldehyde-PLA-g-PEG 4 (600 MHz, CDCl_3)

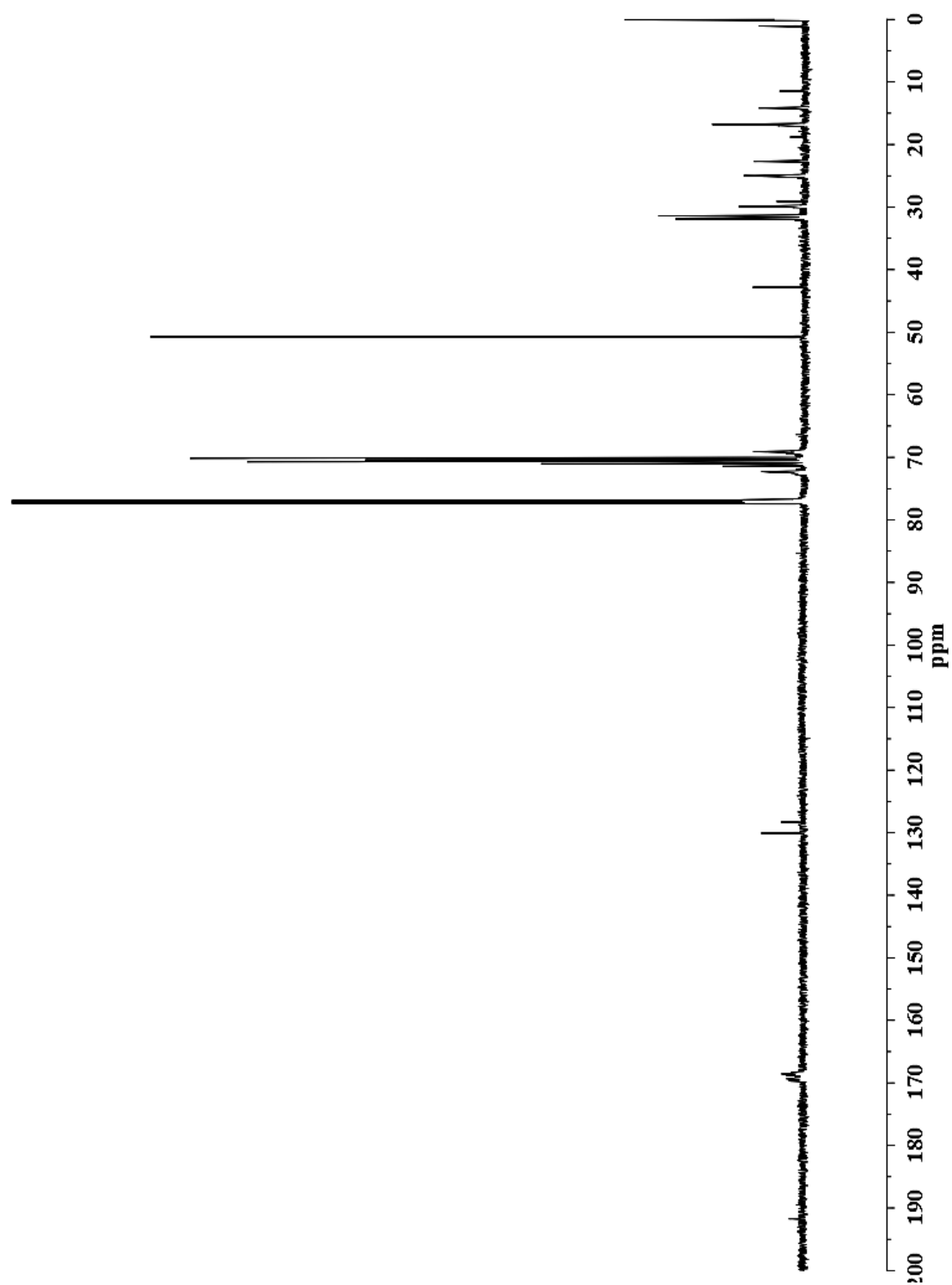
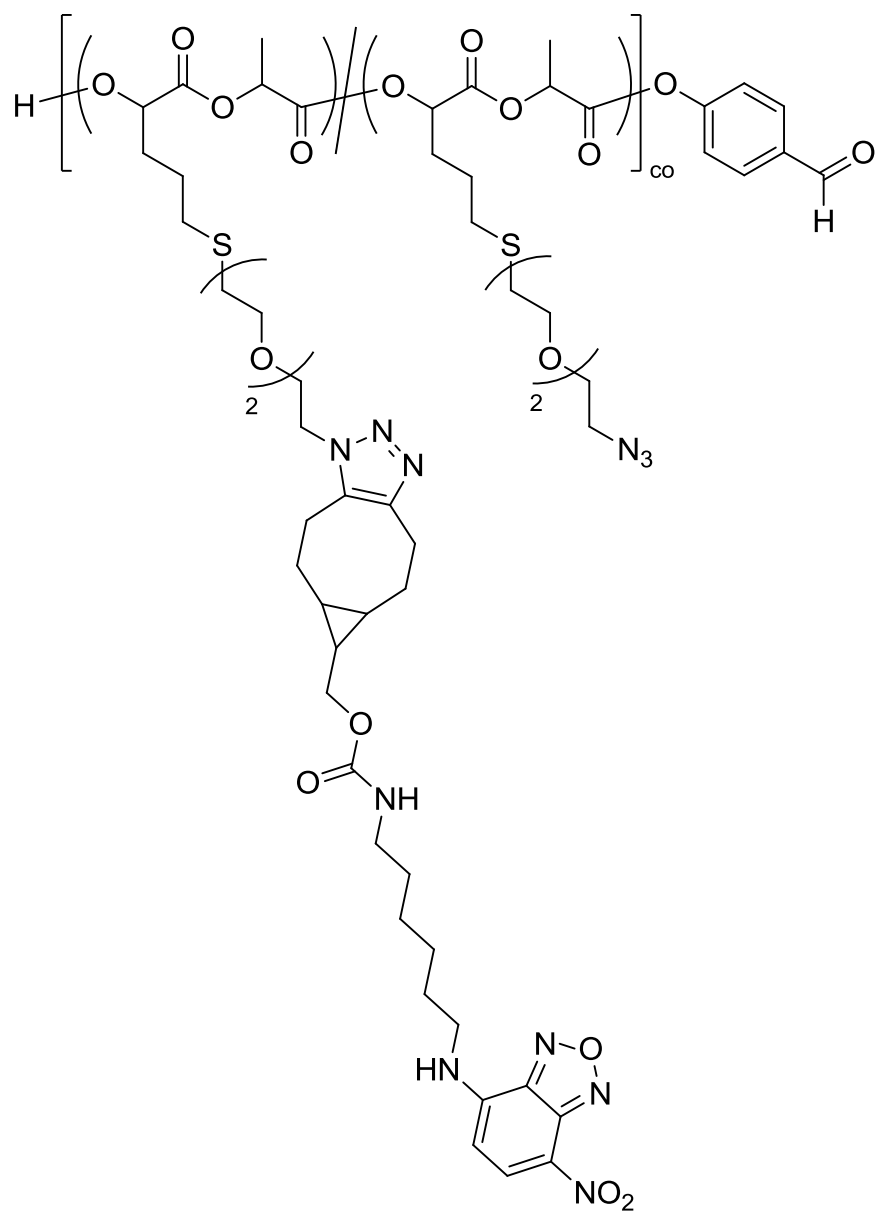
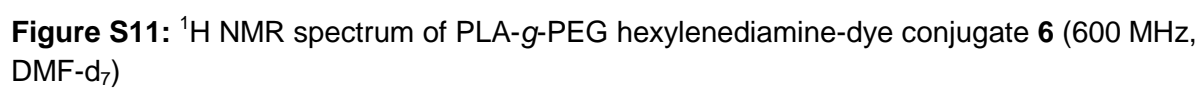


Figure S10: ^{13}C NMR spectrum of aldehyde-PLA-*g*-PEG **4** (150 MHz, CDCl_3)

Polymer characterization - PLA-g-PEG hexylenediamine-dye conjugate 6





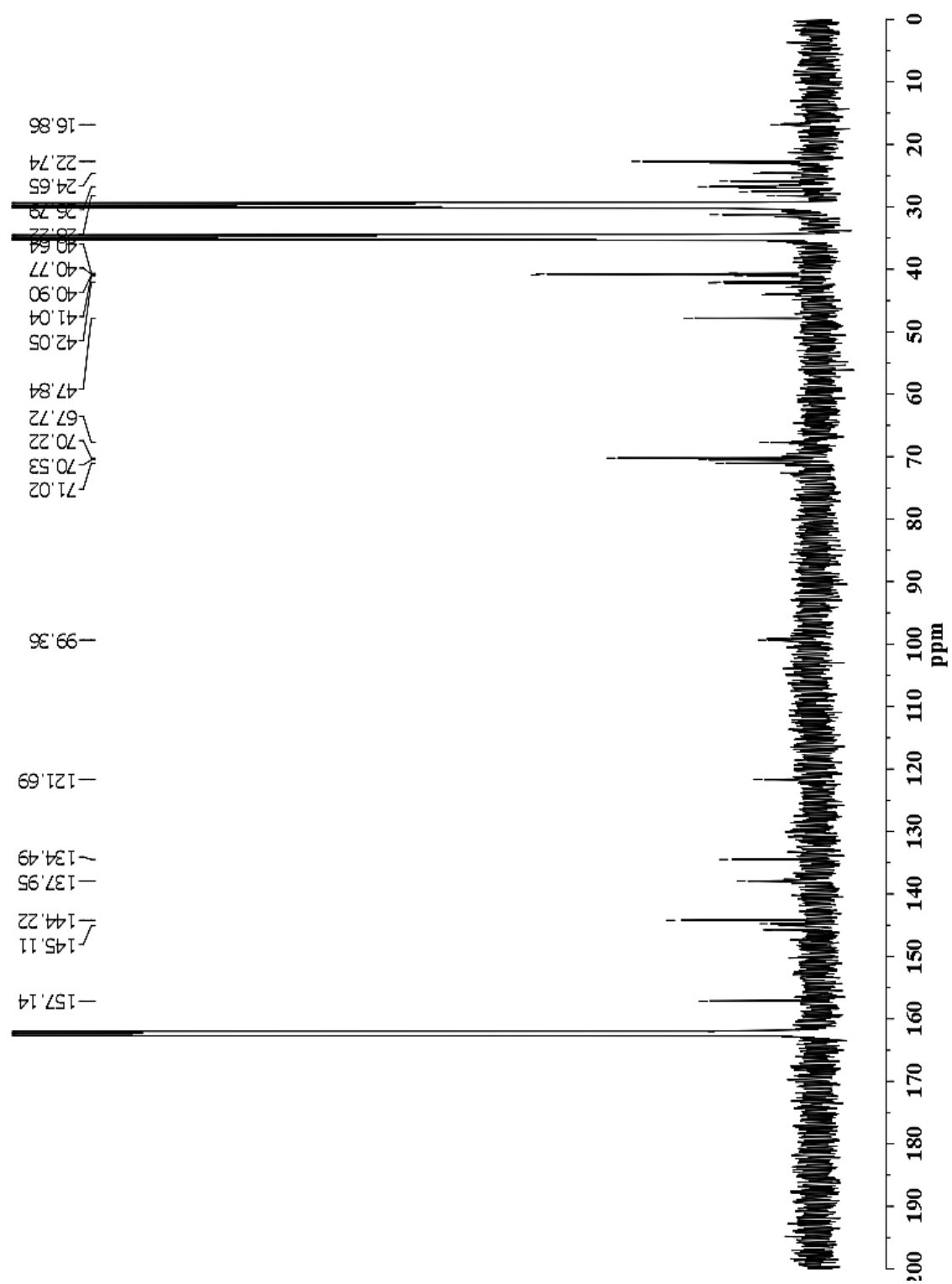


Figure S12: ^{13}C NMR spectrum of PLA-g-PEG hexylenediamine-dye conjugate **6** (150 MHz, DMF-d_7)

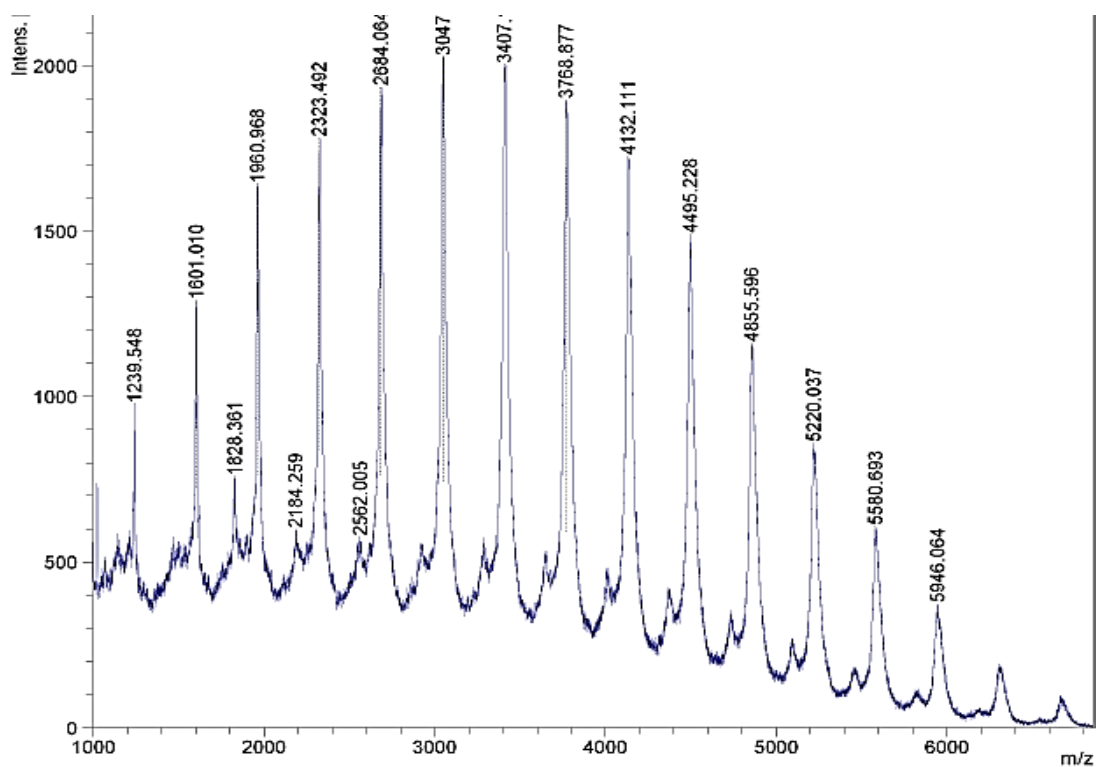


Figure S13: MALDI-ToF analysis of aldehyde-PLA-g-PEG 4

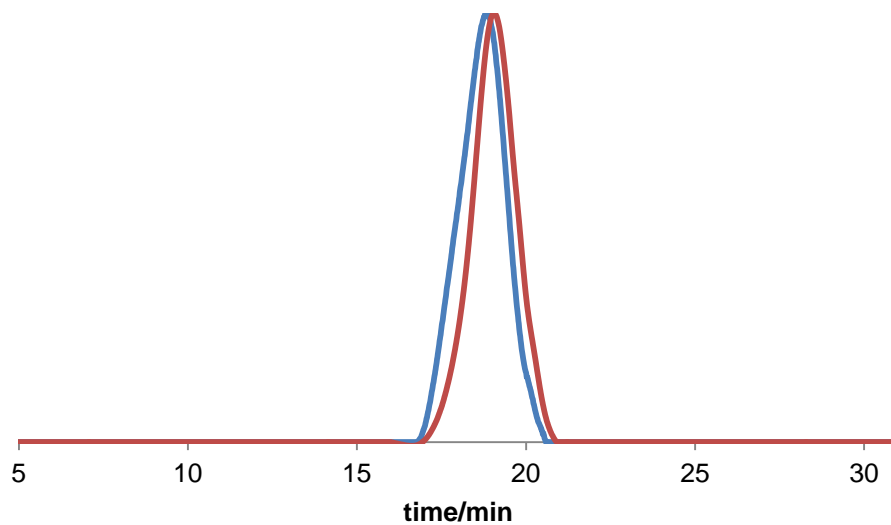


Figure S14: Enlarged SEC-trace of dioxolane-PLA-g-PEG 3 (blue) versus aldehyde-PLA-g-PEG 4 (red)

Synthetic procedure for K-modified gH625

The peptide was synthesized by standard solid phase synthesis using the Fmoc/^tBu protecting group methodology on a Syro I Multisynthes GmbH (Witten, Germany) automatic synthesizer. The Rink amide resin (substitution of 0.76 mmol/g) was used as the solid phase support, and syntheses were performed on a scale of 50 µmol. 4 equivalents (relative to resin loading) of Fmoc-amino acid were coupled according to the *N*-hydroxybenzotriazole/2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/diisopropylethylamine method [1 eq of Fmoc-protected amino acid, 1 eq of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, 1 eq of *N*-hydroxybenzotriazole (0.5 mM *N*-hydroxybenzotriazole in *N*-methyl-2-pyrrolidone), and 2 eq of diisopropylethylamine (1 mM diisopropylethylamine in *N*-methyl-2-pyrrolidone)]. The Fmoc protecting group was removed with 30% (v/v) piperidine in *N*-methyl-2-pyrrolidone. Acetylation with acetic anhydride was performed on the final histidine to avoid ambiguity during the reductive amination with the lysine later on. The peptide was fully deprotected and cleaved from the resin with trifluoroacetic acid containing 5% thioanisole, 3% ethanedithiol, and 2% anisole as scavengers and 1% water. The crude peptide was precipitated with ice cold diethyl ether, filtered, dissolved in water, lyophilized, and purified by preparative reverse-phase HPLC (LC8 Shimadzu (Kyoto, Japan) system equipped with a UV Lambda Max Model 481 detector). The sample was injected onto a Jupiter Proteo column (21.20 × 250 mm, 10 µm) eluted with a solvent mixture of H₂O and 0.1% trifluoroacetic acid (solvent A) and CH₃CN and 0.1% trifluoroacetic acid (solvent B). A linear gradient of 20–80% solvent B over 20 min at a flow rate of 20 mL/min was employed.

The pure peptide was lyophilized and analyzed by a Thermo Electron (San Jose, CA, USA) Finnigan Surveyor MSQ single quadrupole ESI LC-MS LC-MS using a Proteo analytical column (4.6 × 150 mm, 4 µm). A linear gradient of 20–80% solvent B over 10 min at a flow rate of 0.8 mL/min was used. (Ac-HGLASTLTRWAHYNALIRAFK-CONH₂ molecular weight = 2467.81 [M + 2H]⁺/2 = 1234.90 amu)

^1H NMR spectrum for gH625-polymer conjugate 7

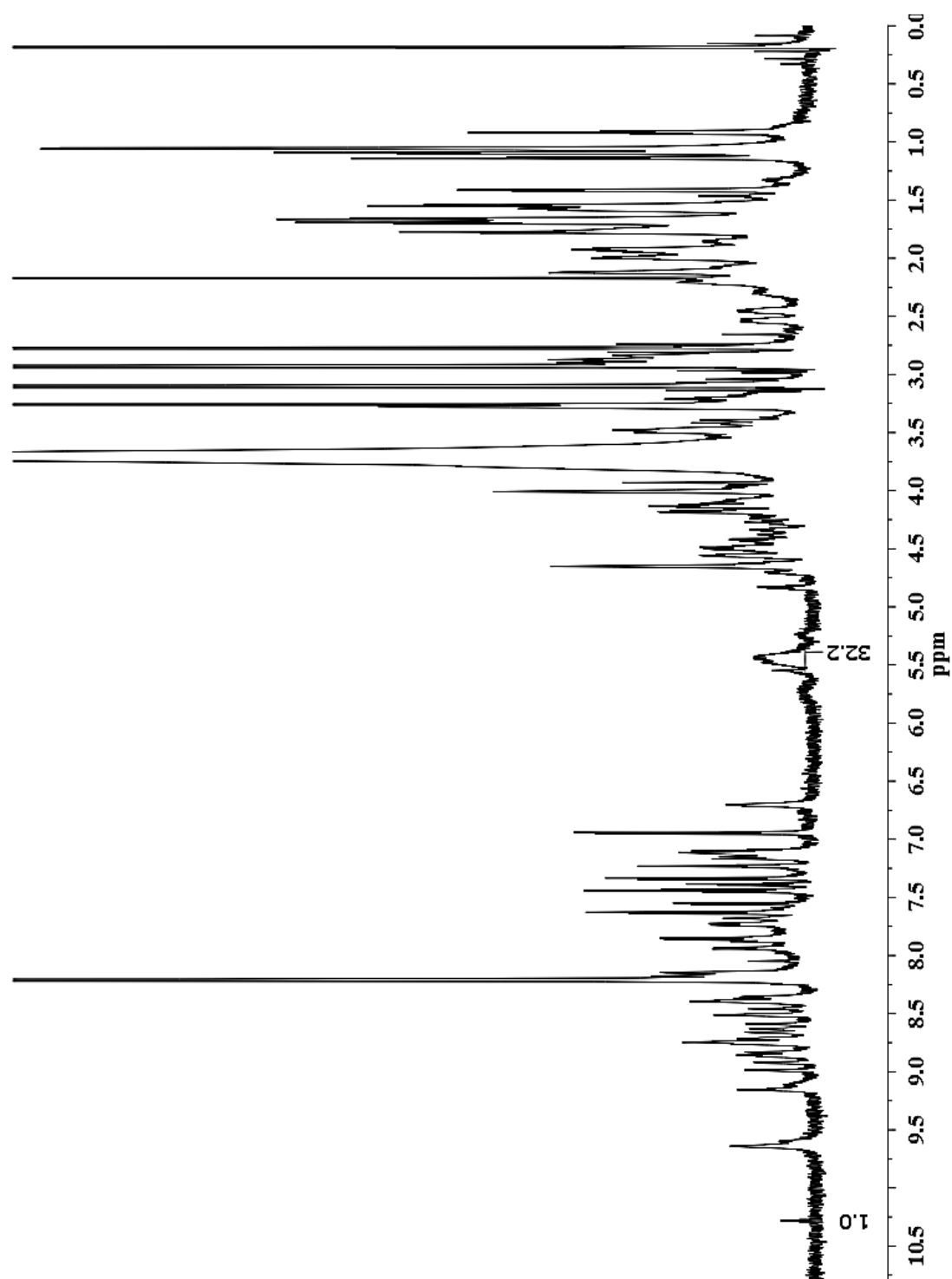


Figure S15: ^1H NMR spectrum of gH625-polymer conjugate **7** (600 MHz, DMF-d_7)

UV-Vis calibration curves for nitrobenzofurazan (NBD) determination of functionalized PLA polymer 6

A linear UV-Vis calibration curves was developed by measuring the absorbance of the NBD-dye **5** at a wavelength of 330 nm for solutions at different concentrations in *N,N*-dimethylformamide (DMF) (Figure S). The absorption spectra were recorded at room temperature on an Agilent 8453 UV-visible single beam spectrophotometer with 10 mm path length quartz cuvettes.

The calibration curves were used to evaluate the functionalization degree of NBD-dye on the dye-polymer conjugates.

The degree of functionalization was found to be 83.3% for hexylamine-NBD dye, which is in good agreement with the results obtained by ^1H NMR spectroscopy

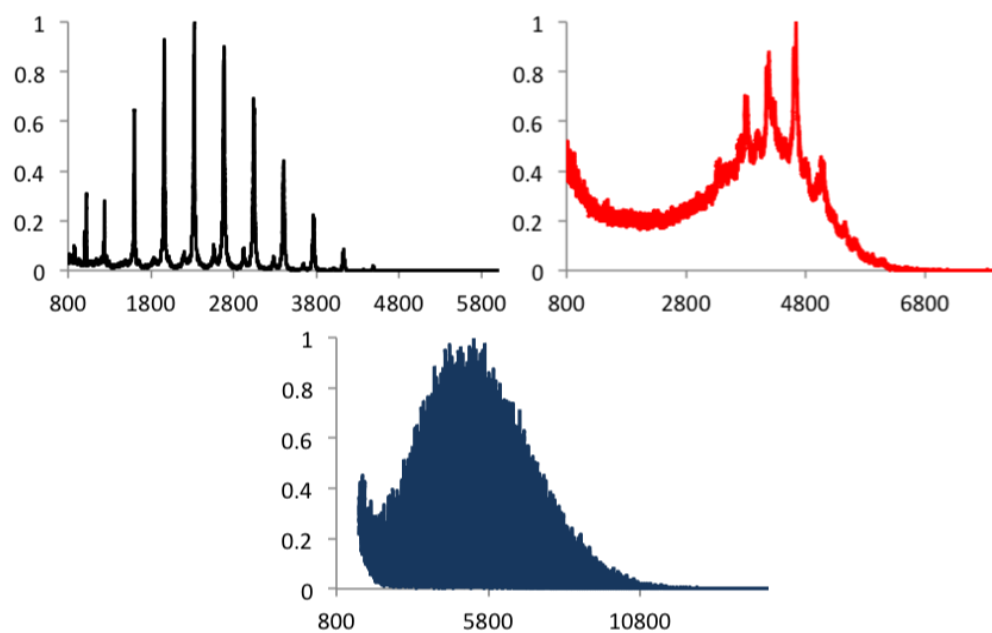


Figure S16: MALDI-ToF comparison of 4, (black), 6 (red) and 7 (blue). Y = relative intensity, X = MW in g/mol

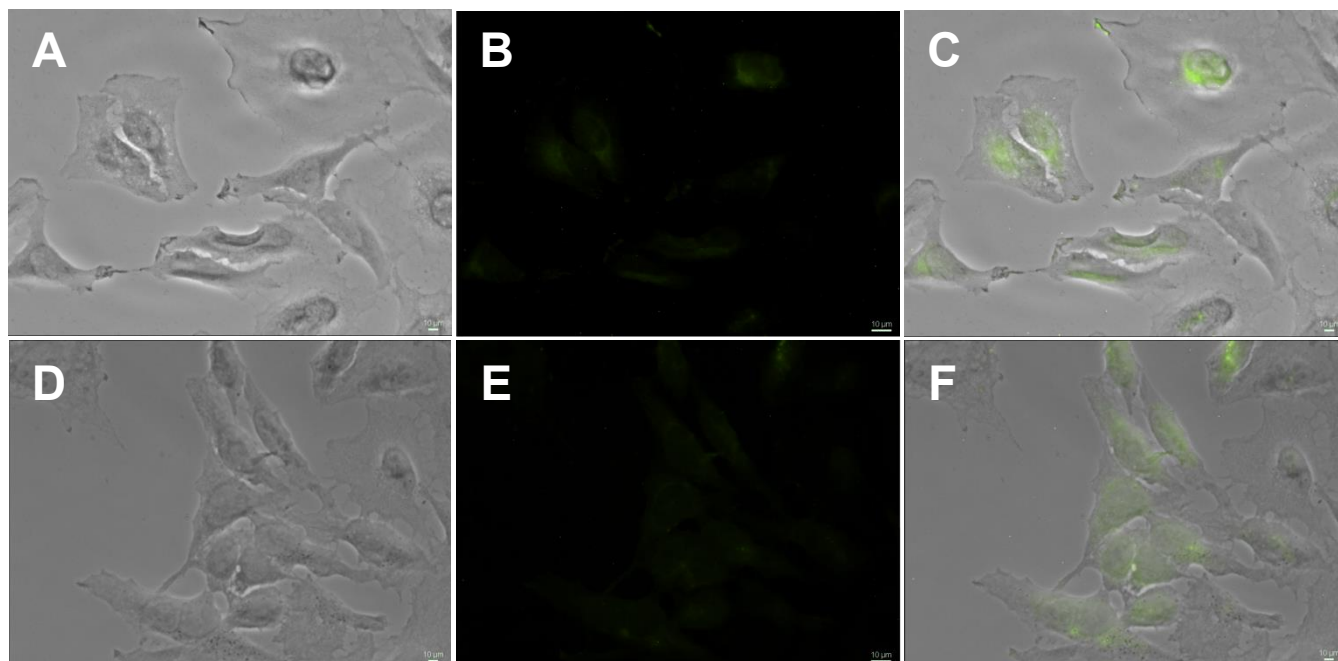


Figure S17: Cell images for NBD-PLA-*g*-PEG (A-C) and gH625-NBD-PLA-*g*-PEG at 15 μ M for 4 h, ***after preincubation with NaN₃ for 30 minutes*** (inhibition experiment); A, D = transmission; B,E = fluorescence; C,F = overlay.

