Red Light Activation of Tetrazine-Norbornene Conjugation for Bioorthogonal Polymer Crosslinking across Tissue

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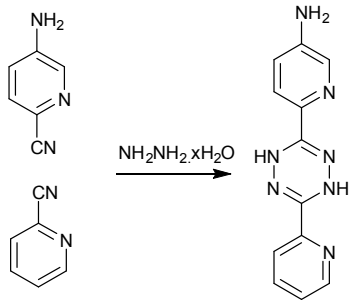
Supporting Information

*Synthesis of 6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (**S1**).*



This compound was synthesized following previously reported procedures with modification.[1] In a typical procedure, 2-cyanopyridine (3 g, 28.8 mmol) and 5-amino-2-cyanopyridine (1.71 g, 14.4 mmol) were suspended in hydrazine monohydrate (5 mL, 5.7 mmol) and the mixture was heated at 90 °C under refluxing condition for 3 h. Ice cold water was added to the mixture and the solid was filtered. The crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (10/0.5 to 10/0.7 by volume) to give product as a yellow solid (2.45 g, 68%) ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 8.7 (s, 1H), 8.65 (s, 1H), 8.62 (1H, dt, $^2J_{HH}$ = 5.4 Hz, $^3J_{HH}$ = 1 Hz), 7.98-7.9 (m, 3H), 7.65 (1H, d, $^2J_{HH}$ = 5.4), 7.23 (1H, ddt, $^2J_{HH}$ = 7.1 Hz, $^2J_{HH}$ = 4.8, $^3J_{HH}$= 1.4), 7 (1H, dd, $^2J_{HH}$ = 2.7, $^2J_{HH}$ = 8.5), 5.89 (2H, s). ^{13}C NMR (DMSO-d_6, 100 MHz) δ ppm: 148.4, 147.4 , 146.6 , 146.5, 146.5, 136, 134.1, 133.3, 125.1, 121.7, 120.7.

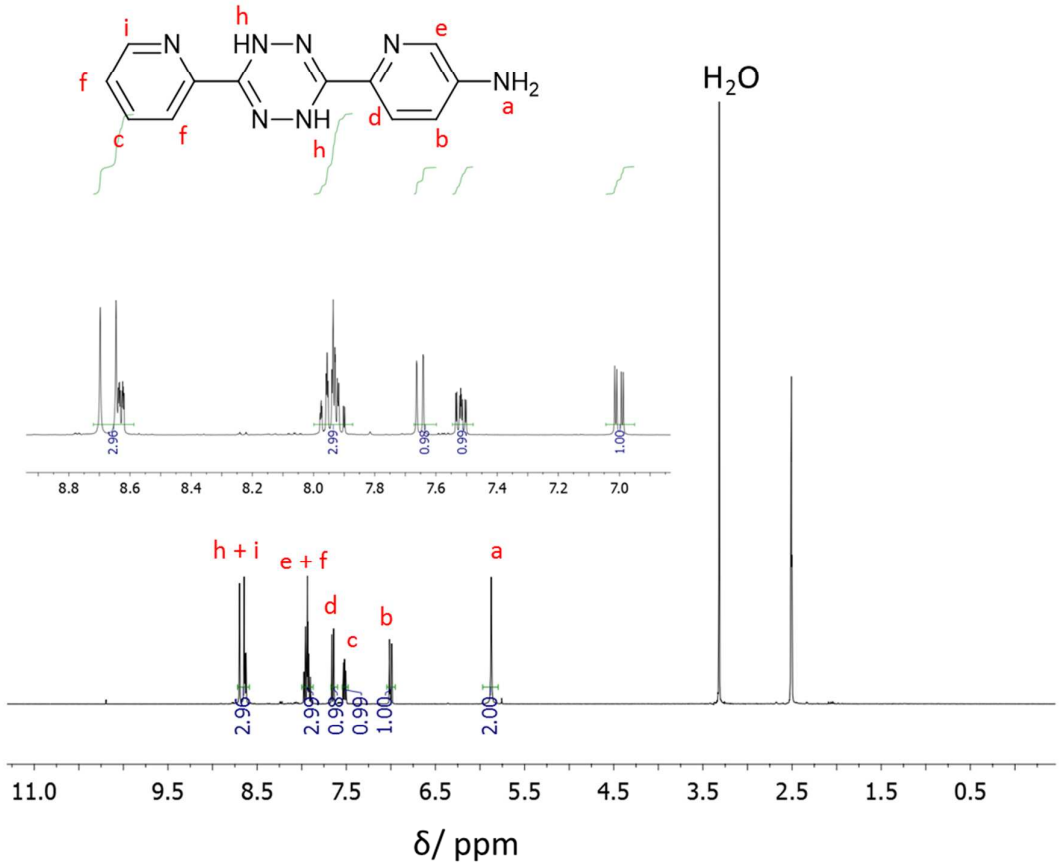


Figure S1. ^{1}H NMR spectrum of compound S1 (DMSO-d_6, 400 MHz)

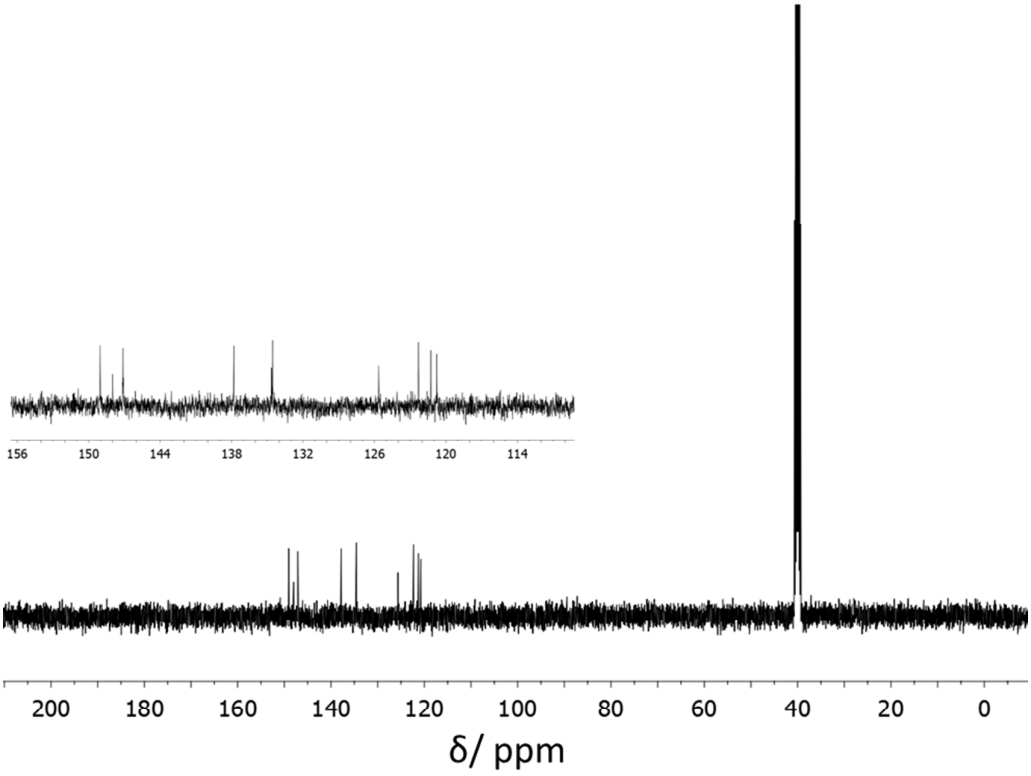
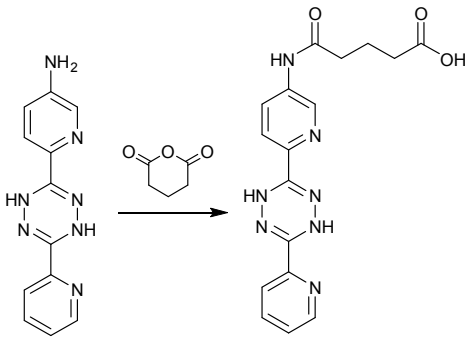


Figure S2. ^{13}C NMR spectrum of compound S1 (DMSO-d_6, 100 MHz)

*Synthesis of 5-oxo-5-((6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)amino)pentanoic acid (**S2**).*



Compound **S1** (2.1 g, 8.3 mmol) was dissolved in THF (50 mL) and glutaric anhydride (1 g, 8.9 mmol) was added. The solution was heated at 65 °C under refluxing conditions for 20 h. The solution was cooled to room temperature and kept in a freezer at -20 °C overnight. The formed precipitate was filtered, washed with diethyl ether (50 mL*2) and dried to give product as orange crystal (2.3 g, yield: 76%).^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.21 (s, 1H), 10.40 (s, 1H), 8.95 (s, 1H), 8.90 (s, 1H), 8.8 (d, $^2J_{HH}$ = 2.4 Hz, 1H), 8.67 –8.58 (m, 1H), 8.15 (dd, $^2J_{HH}$ = 8.8, 2.5 Hz, 1H), 8.01 –7.84 (m, 3H), 7.52 (ddd, $^2J_{HH}$ = 6.9, 4.8, 1.6 Hz, 1H), 2.42 (t, $^3J_{HH}$ = 7.4 Hz, 2H), 2.29 (t, $^3J_{HH}$ = 7.3 Hz, 2H), 1.89 –1.76 (m, 2H).^{13}C NMR (DMSO-d_6, 100 MHz) δ: 174.25, 171.68, 148.66, 147.35, 146.42, 146.15, 141.45, 138.91, 137.47, 137.31, 126.69, 125.37, 121.45, 121.02, 35.36, 32.96, 20.24.

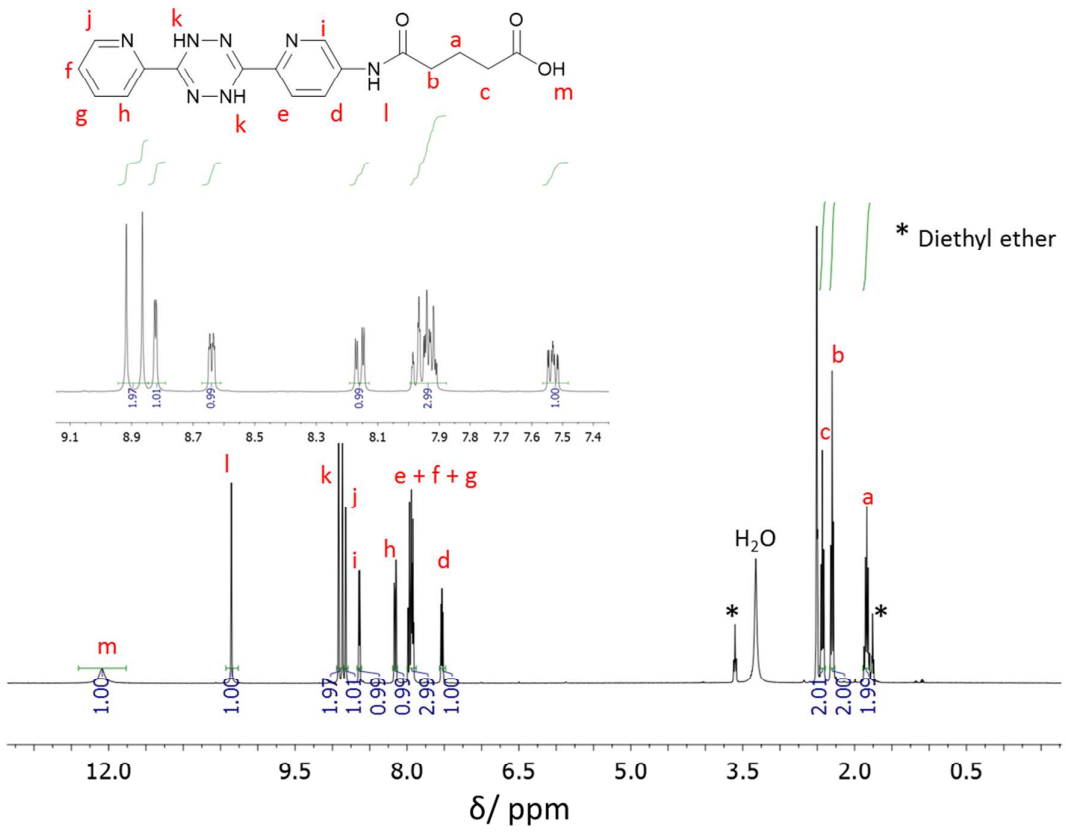


Figure S3. ^{1}H NMR spectrum of compound S2 (DMSO-d_6, 400 MHz)

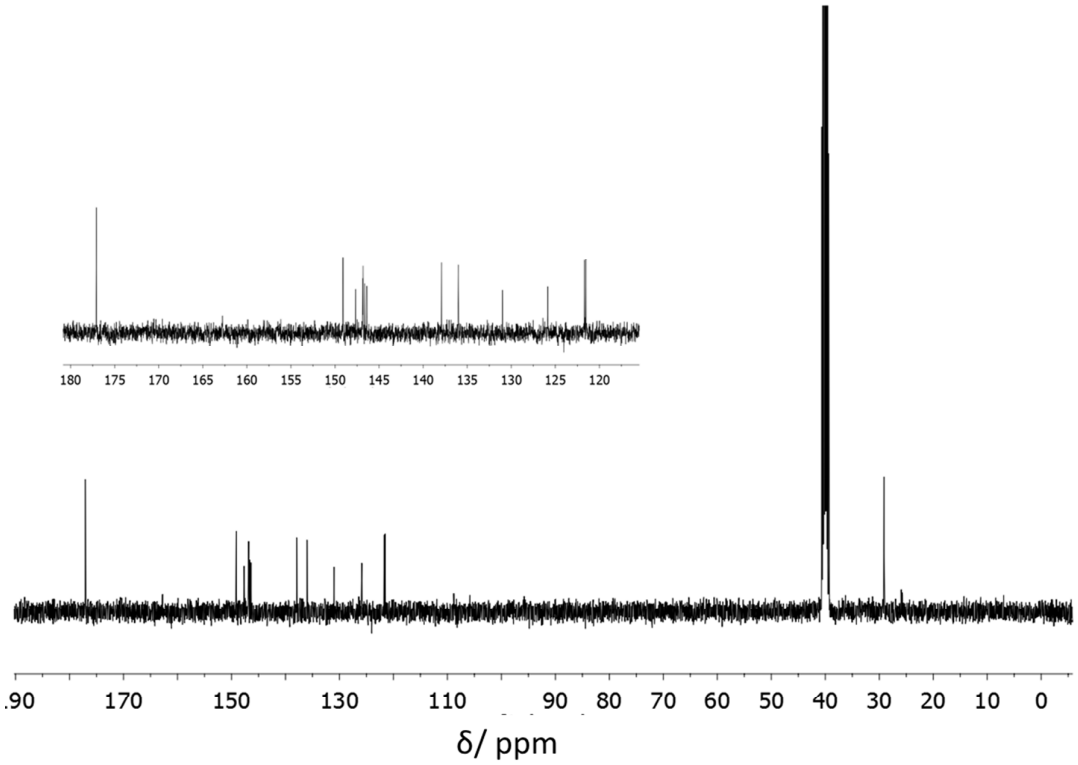
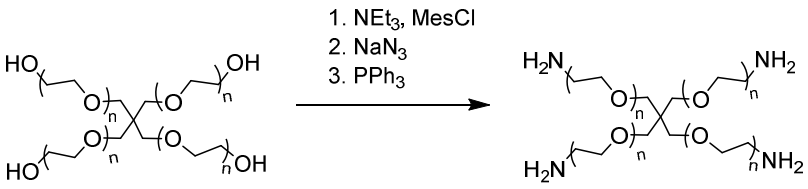


Figure S4. ^{13}C NMR spectrum of compound S1 (DMSO-d_6, 100 MHz)

Synthesis of 4-arm PEG10k-amine.



This polymer was prepared following a three-step procedure and in each step integration of the methylene protons from the pentearythritol core was used to make sure the conversion of the endgroup is over 99% by ^{1}H NMR integration. In a typical procedure, 4-arm PEG-OH (5 g, 0.5 mmol) was dissolved in CH_2Cl_2 (20 mL) followed by addition of NEt_3 (0.3 g, 3 mmol). The solution was cooled on an ice bath and methanesulfonylchloride (3 mmol) was added dropwise over 15 min. The solution was allowed to stir at room temperature for 20 h and concentrated to ca. 2 mL, precipitated in Et_2O to collect product as white powder. ^{1}H NMR (CDCl3, 400 MHz) δ: 4.36 (t, $^{3}J_{HH}$ =4.13) 3.60 (m, CH_2O of PEG), 3.38 (s CH_2O from pentearythritol core), 3.08 (s).

The above product was dissolved in DMF (10 mL) and sodium azide (0.2 g, 3 mmol) was added. The solution was heated at 80 °C for 16 h and DFM was evaporated in vacuo. The solid residue was dissolved in CH_2Cl_2 (10 mL), filtered and washed with H_2O (20 mL), brine (10 mL), dried with $MgSO_4$ and concentrated to ca. 2 mL. The product was collected as off-white powder after precipitation in Et_2O.

The above product was dissolved in MeOH (20 mL) and triphenylphosphine was added. The solution was stirred at ambient temperature for 12 h and concentrated in vacuo. The solid residue was dissolved in water (20 mL), washed with Et_2O (30 mL * 2), and extracted with CH_2Cl_2 (20 mL). The organic phase was dried ($MgSO_4$) and concentrated to ca. 2 mL. The product was collected as off-white powder after precipitation in Et_2O. (3.4 g, total yield: 68%). ^{1}H NMR (CDCl3, 400 MHz) δ: 3.60 (m, CH_2O of PEG), 3.38 (s CH_2O from pentearythritol core), 1.97 (t, $^{3}J_{HH}$ = 5.2 Hz).

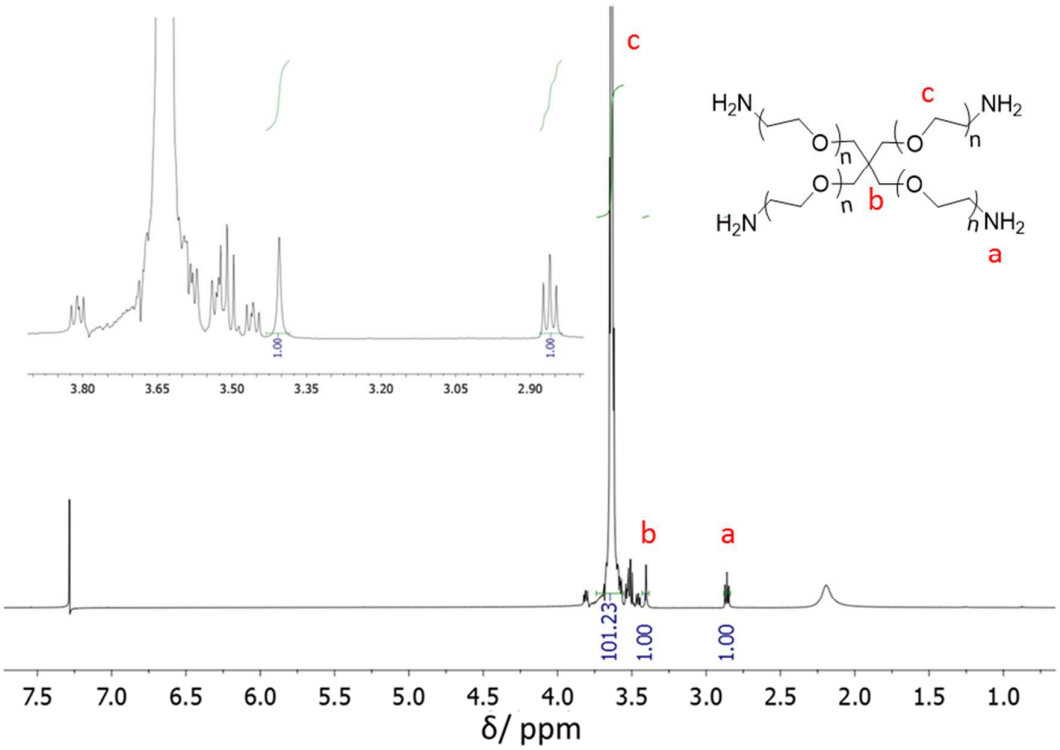


Figure S5. ^{1}H NMR spectrum of 4arm PEG-NH_2 ($CDCl_3$, 400 MHz)

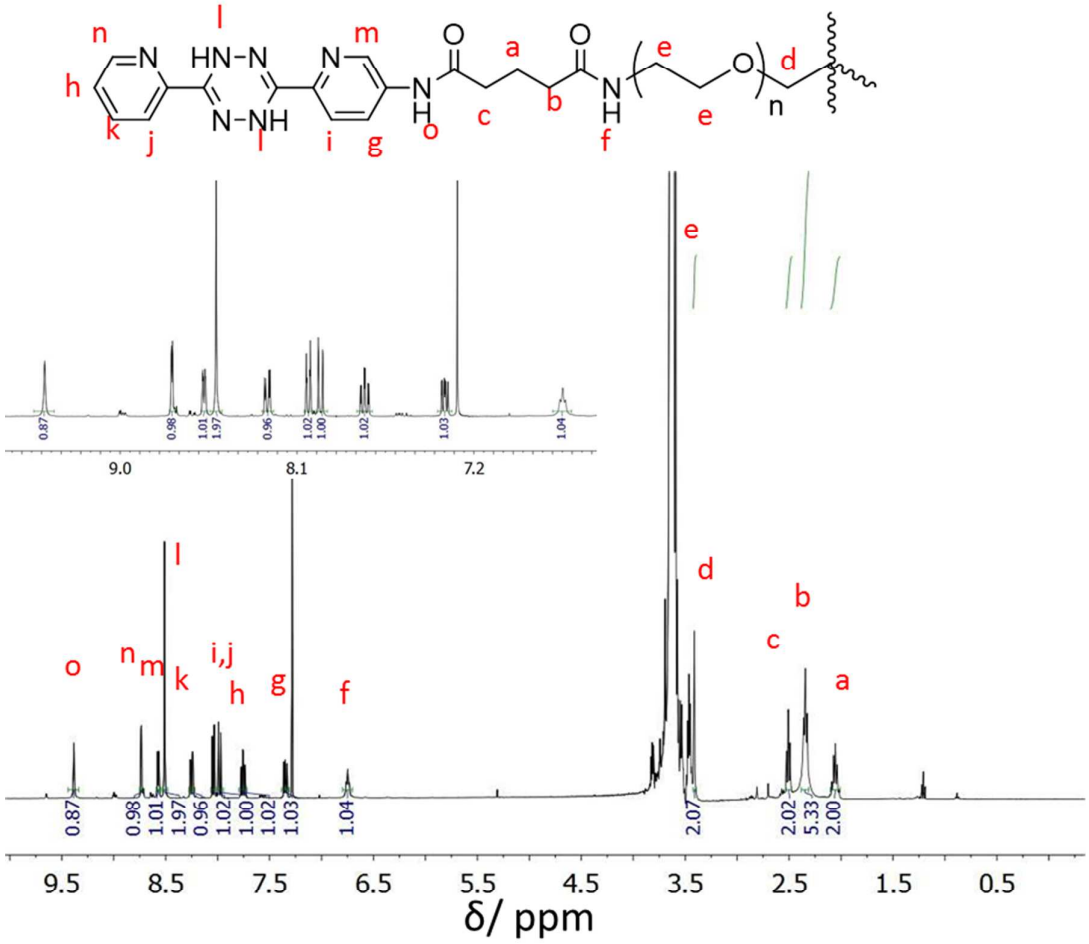


Figure S6. ^{1}H NMR spectrum of 4arm PEG-dHTz ($CDCl_3$, 400 MHz)

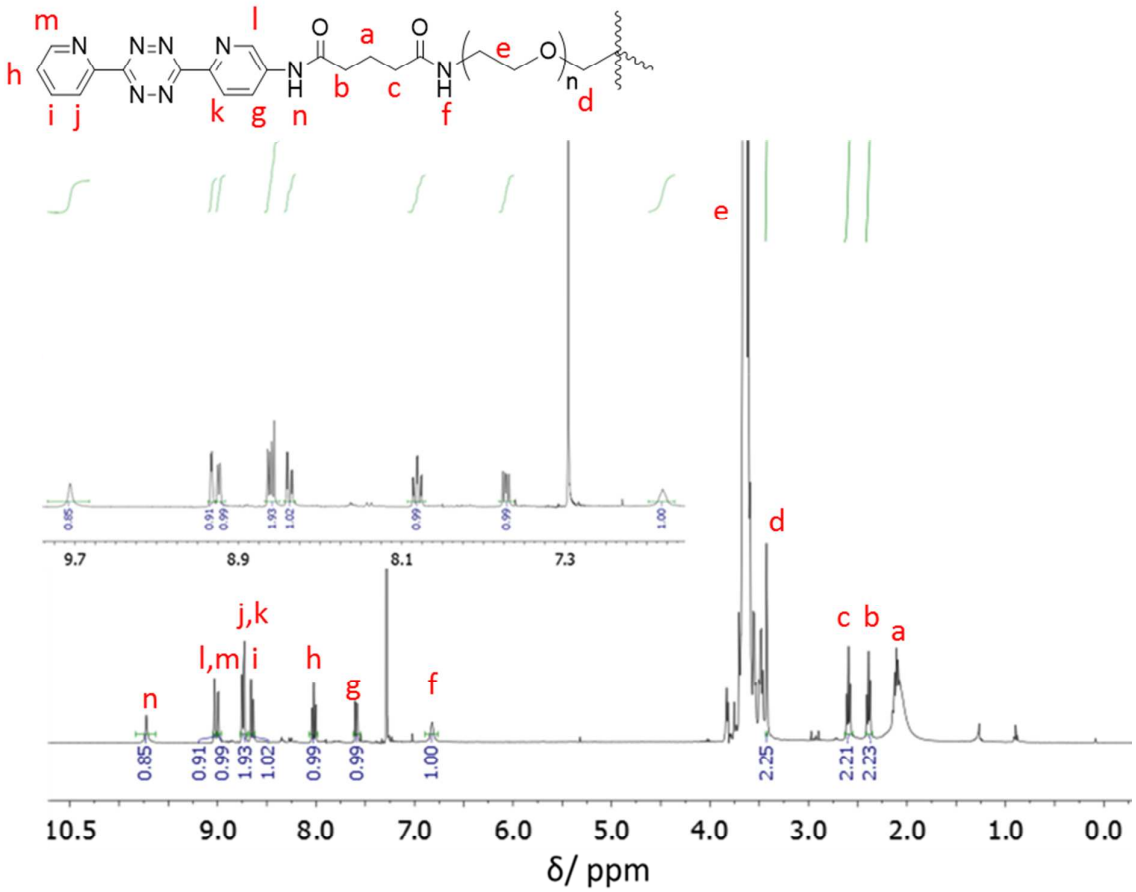
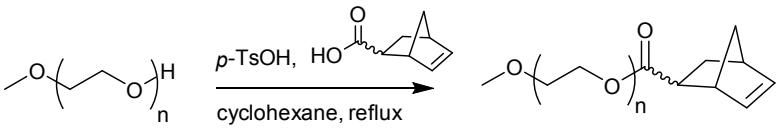


Figure S7. ^{1}H NMR of 4arm PEG-Tz ($CDCl_3$, 400 MHz)

Synthesis of MeO-PEG-Nb



MeO-PEG-OH (3 g, 4 mmol), norbornene carboxylic acid (Nb-COOH, 2.2 g, 14 mmol), and para-toluene sulfonic acid (0.1 g, catalytic amount) were suspended in cyclohexane (100 mL) and the solution was heated to refluxing (90 °C) under Dean-Stark conditions for 20 h. The solution was allowed to cool to room temperature and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (50 mL) washed with $NaHCO_3$ saturated solution (50 mL*2), brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo to give product as white amorphous solid. ^{1}H NMR (400 MHz) δ 5.98-6.24 (m, CH=CH of norbornene, endo protons at 5.98 and 6.24, exo protons at 6.13-6.15), 4.21 (m) 3.60 (m, CH_2O of PEG), 3.31 (s, OCH_3), 3.22 (s, CHCO of norbornene), 2.87 (m, CH2 bridge of norbornene) 1.99 (m, CH of norbornene) 1.33 and 1.46 (m, CH2 from norbornene).

4arm PEG-Nb was synthesized using a similar procedure as above. The product was purified by precipitation in Et_2O.

MeO-PEG-SH was synthesized from MeO-PEG-OH and mercaptopropionic acid using a similar procedure as above. The product was purified by concentration in vacuo to give product as colourless oil.

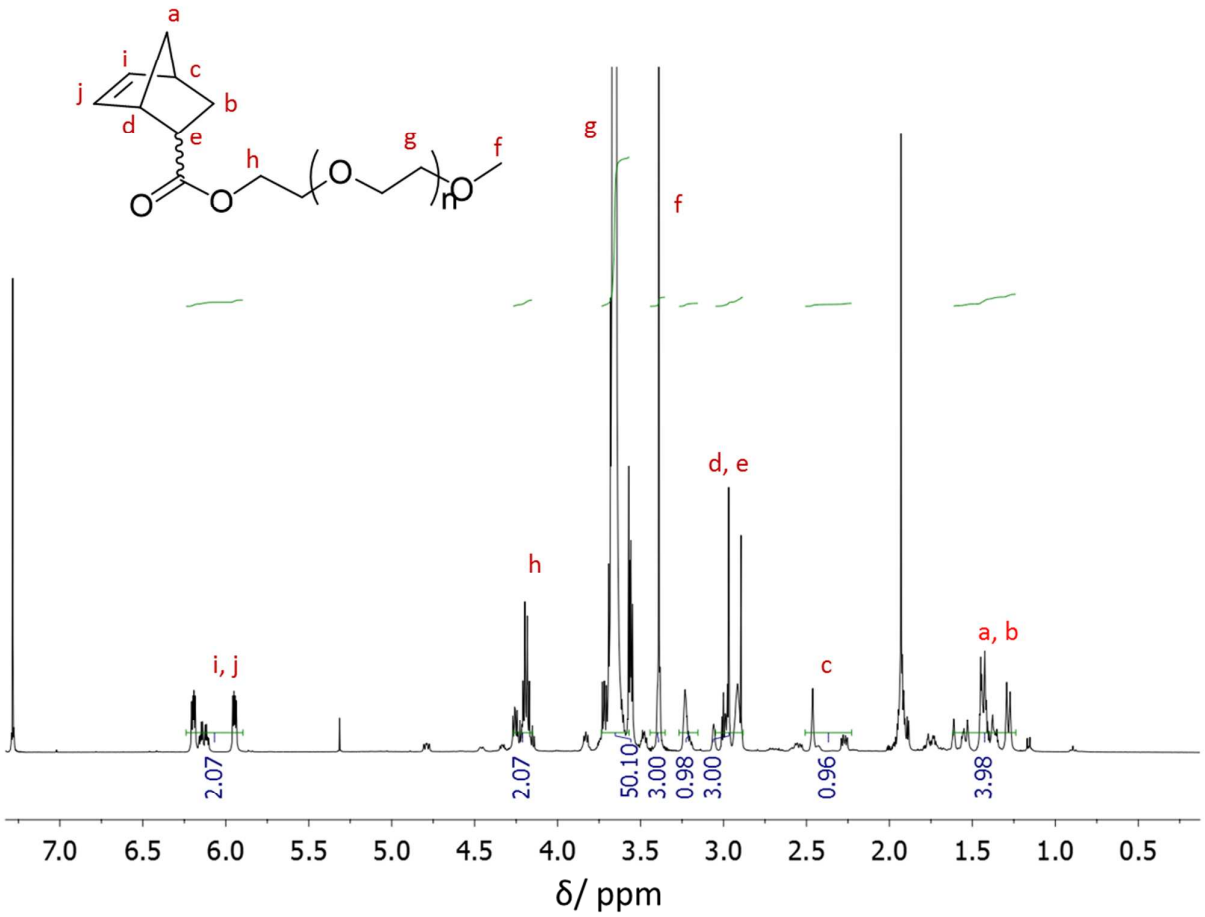


Figure S8. ^{1}H NMR of MeO-PEG-Nb ($CDCl_3$, 400 MHz)

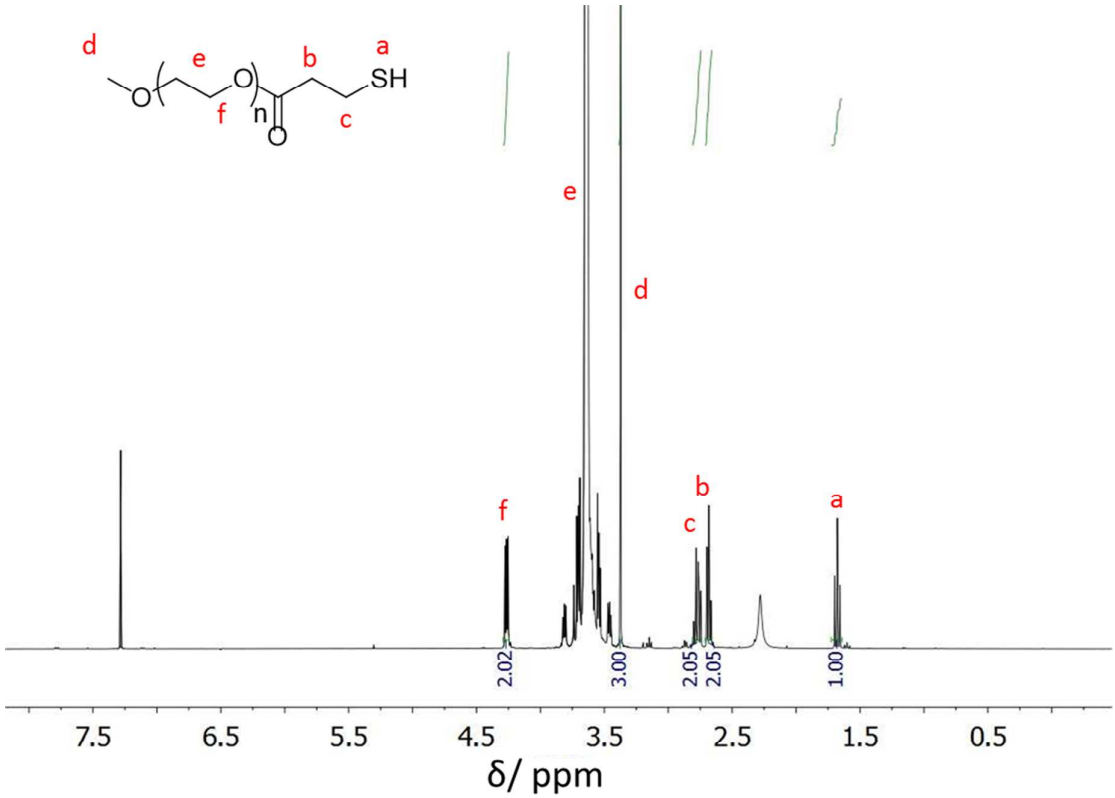


Figure S9. ^{1}H NMR of MeO-PEG-SH ($CDCl_3$, 400 MHz).

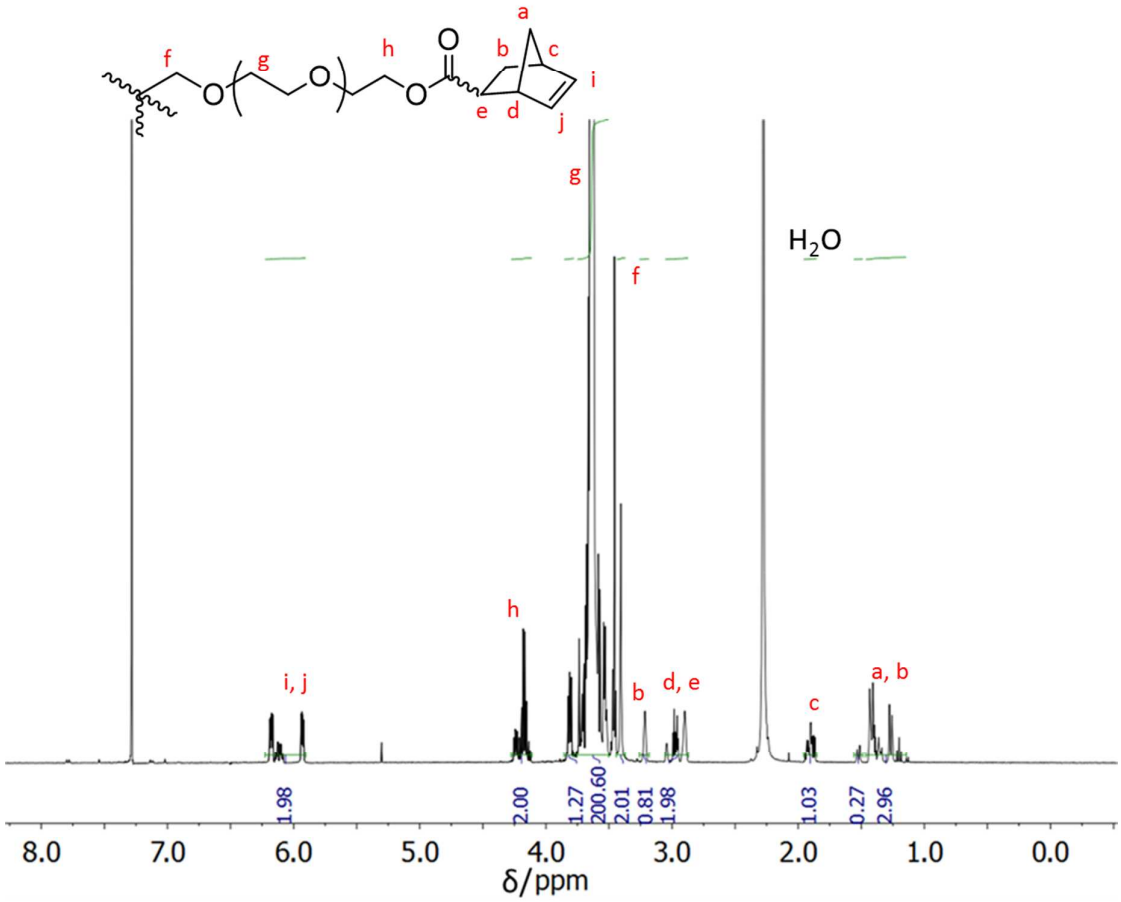


Figure S10. ^{1}H NMR of 4arm PEG-Nb ($CDCl_3$, 400 MHz)

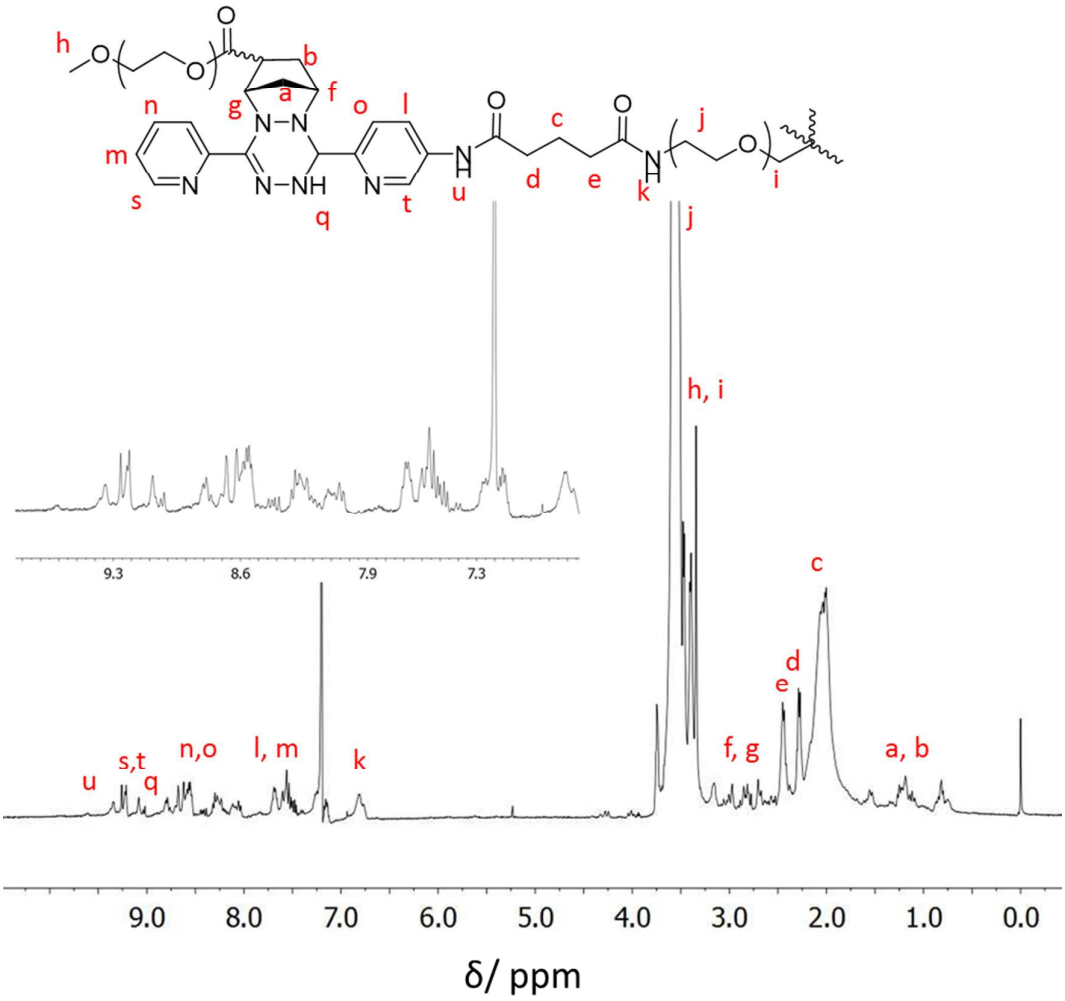


Figure S11. ^{1}H NMR of 4arm PEG-Nb ($CDCl_3$, 400 MHz). Due to the Nb starting group containing two isomers (endo/exo) the product also contains two isomers causing significant overlapping of the signal and difficulty in integrating the chemical shifts.

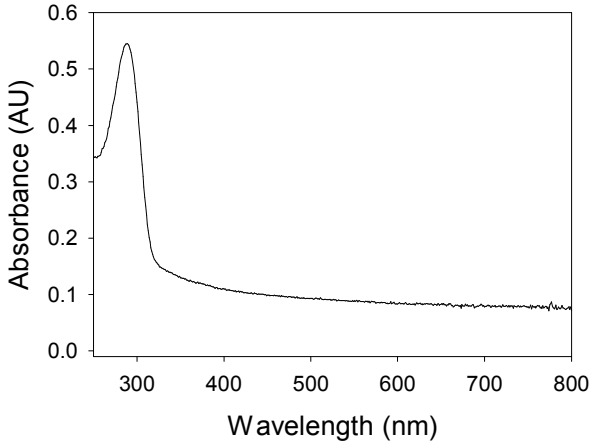


Figure S12. UV-vis spectrum of MeO-PEG-Nb.

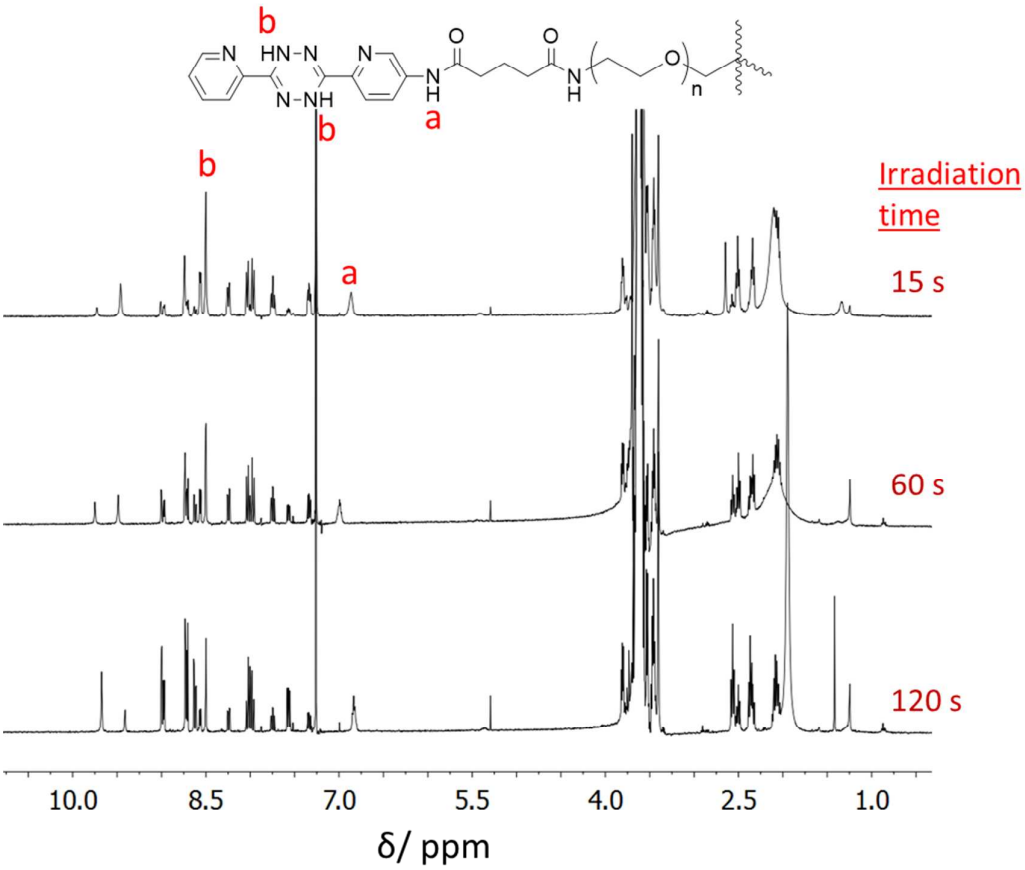


Figure S13. Representative ^{1}H NMR spectra of the 4arm-PEG-dHT (400 MHz, $CDCl_3$) after being lyophilized from PBS solution at different times of red light irradiation (625 nm, 10 mW cm^{-1}). The integration of the assigned chemical shifts is used for calculation of the reaction rate.

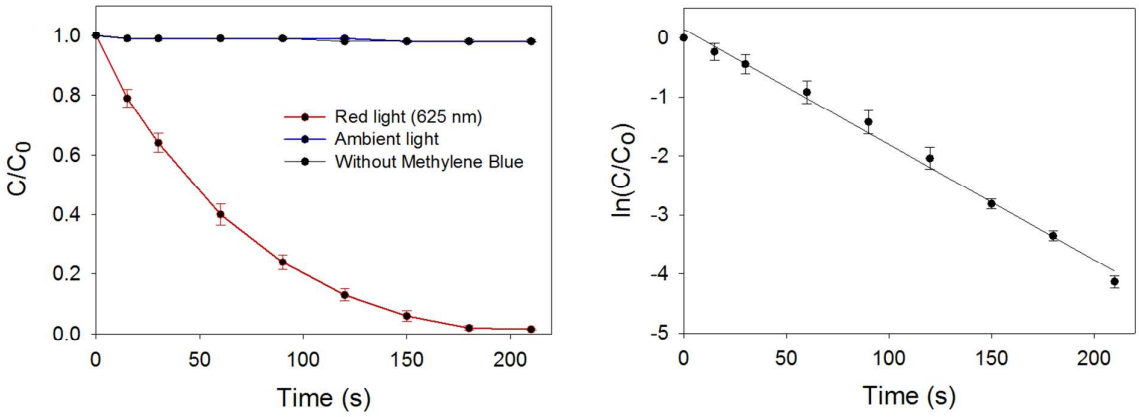


Figure S14. Kinetics of reaction between polymer **1** and MeO-PEG-Nb in PBS pH 7.4 in the presence of methylene blue and red light irradiation. Note that the reaction was carried out without stirring (the solutions were stirred using a magnetic stirrer bar in UV-Vis analysis) to simulate gelation condition.

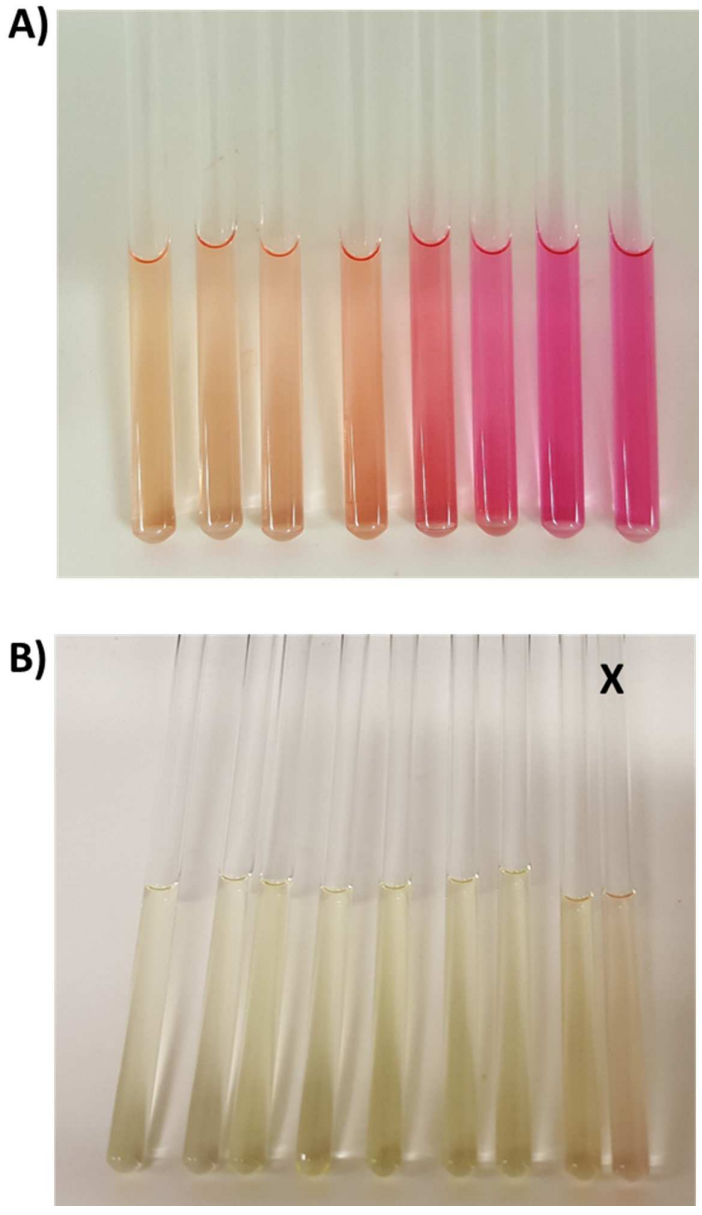


Figure S15. Photographs of 4arm PEG-dHTz in $CDCl_3$ at different time points (total time of 210 s) of photocatalytic oxidation in PBS solution containing methylene blue (5 µM) and (**A**) under irradiation of red light (625 nm, 10 mW cm^{-1}); and (**B**) under fume cupboard light, sample marked with **X** was placed under ambient light for 1 h. The polymer solution was freeze-dried before redissolving in $CDCl_3$.

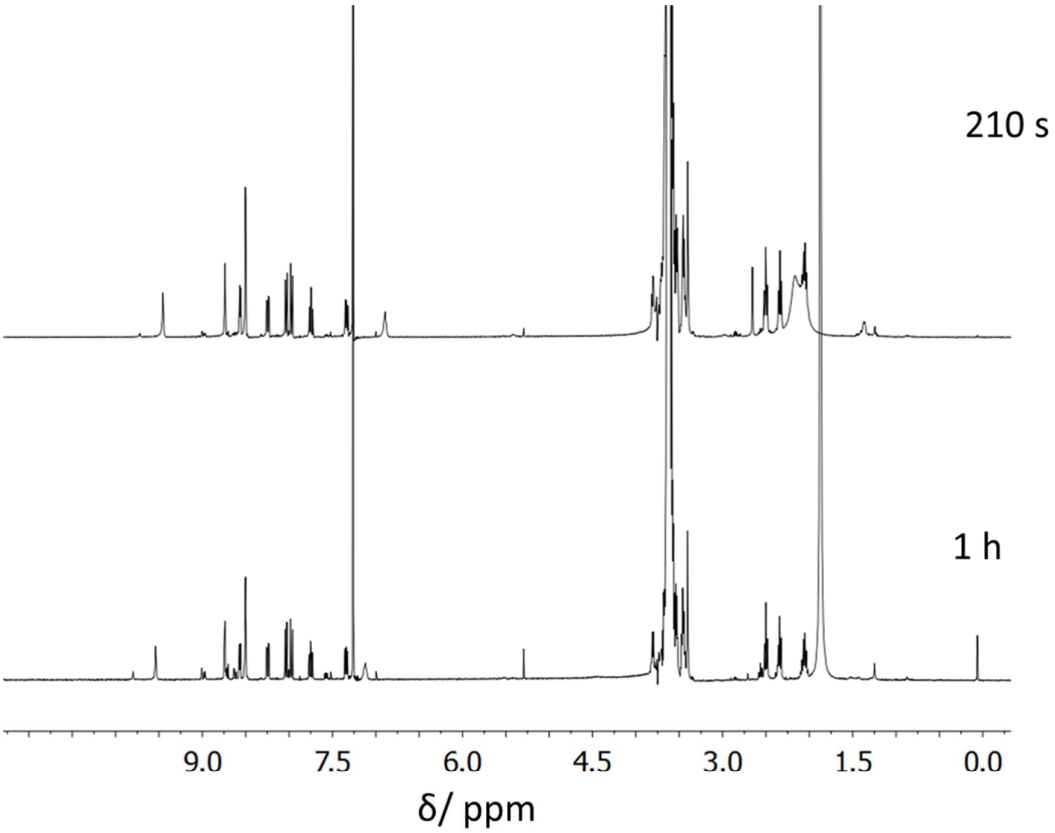


Figure S16. ^{1}H NMR spectra of the 4arm-PEG-dHT (400 MHz, $CDCl_3$) after being lyophilized from PBS solution containing methylene blue and left under ambient light at different time.

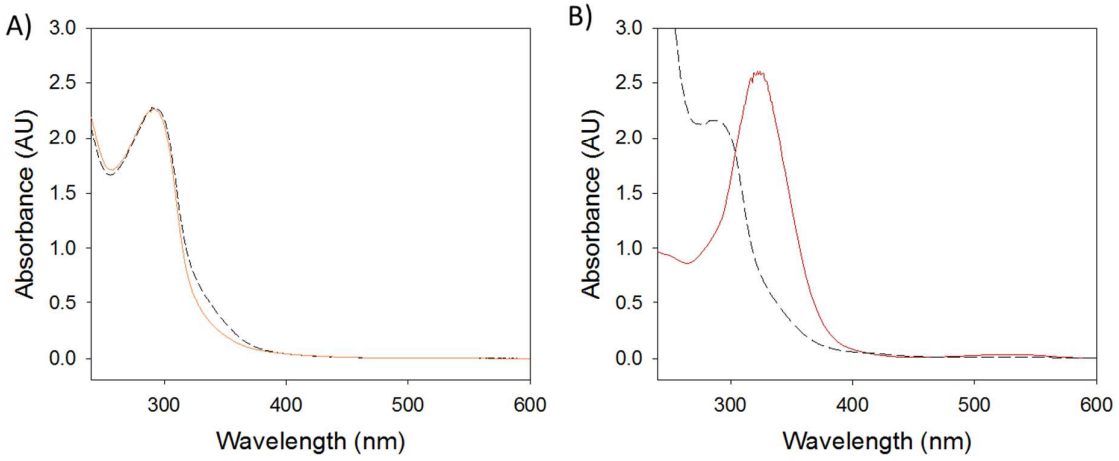


Figure S17. UV-Vis spectra of (**A**) polymer **1** and polymer **2** (**B**) in PBS solution containing cysteine (10 μM) before (solid line) and after incubation at 37 °C for 1 h (dashed line).

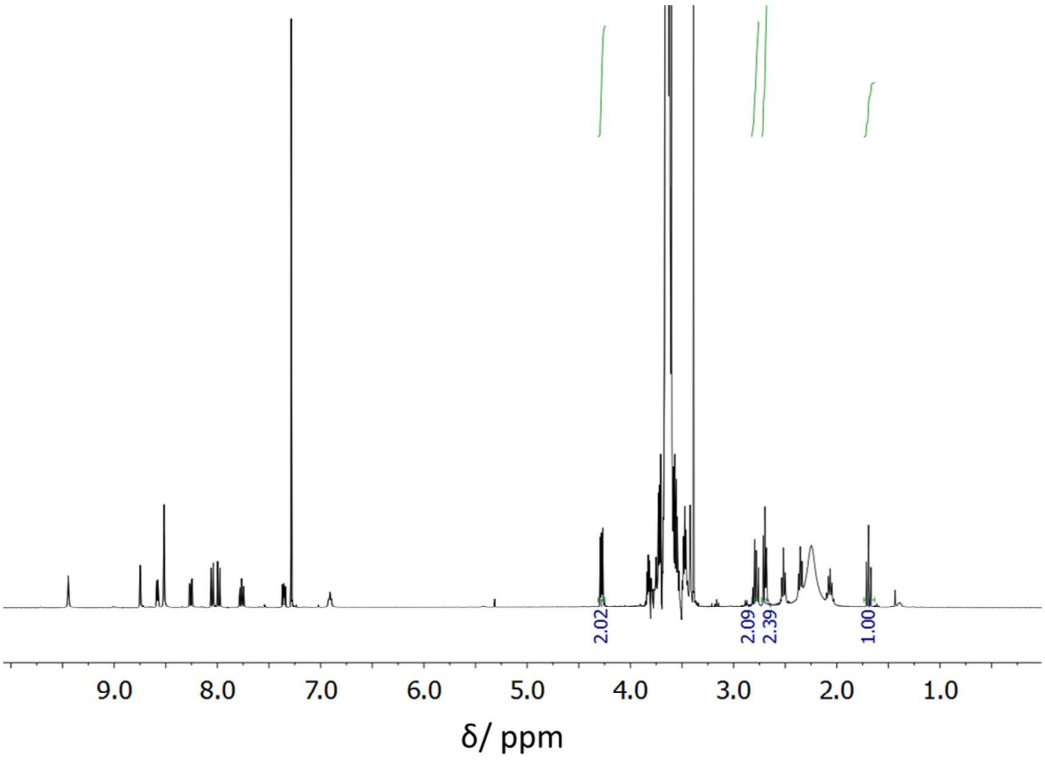


Figure S18. ^{1}H NMR spectrum of PEG-dHTz (polymer **1**) and MeO-PEG-SH after being in PBS solution for 1 h ($CDCl_3$, 400 MHz), showing no change in the chemical shifts for the dHTz and thiol groups. The solution was freeze-dried and the solid was redissolved in $CDCl_3$ for NMR analysis.

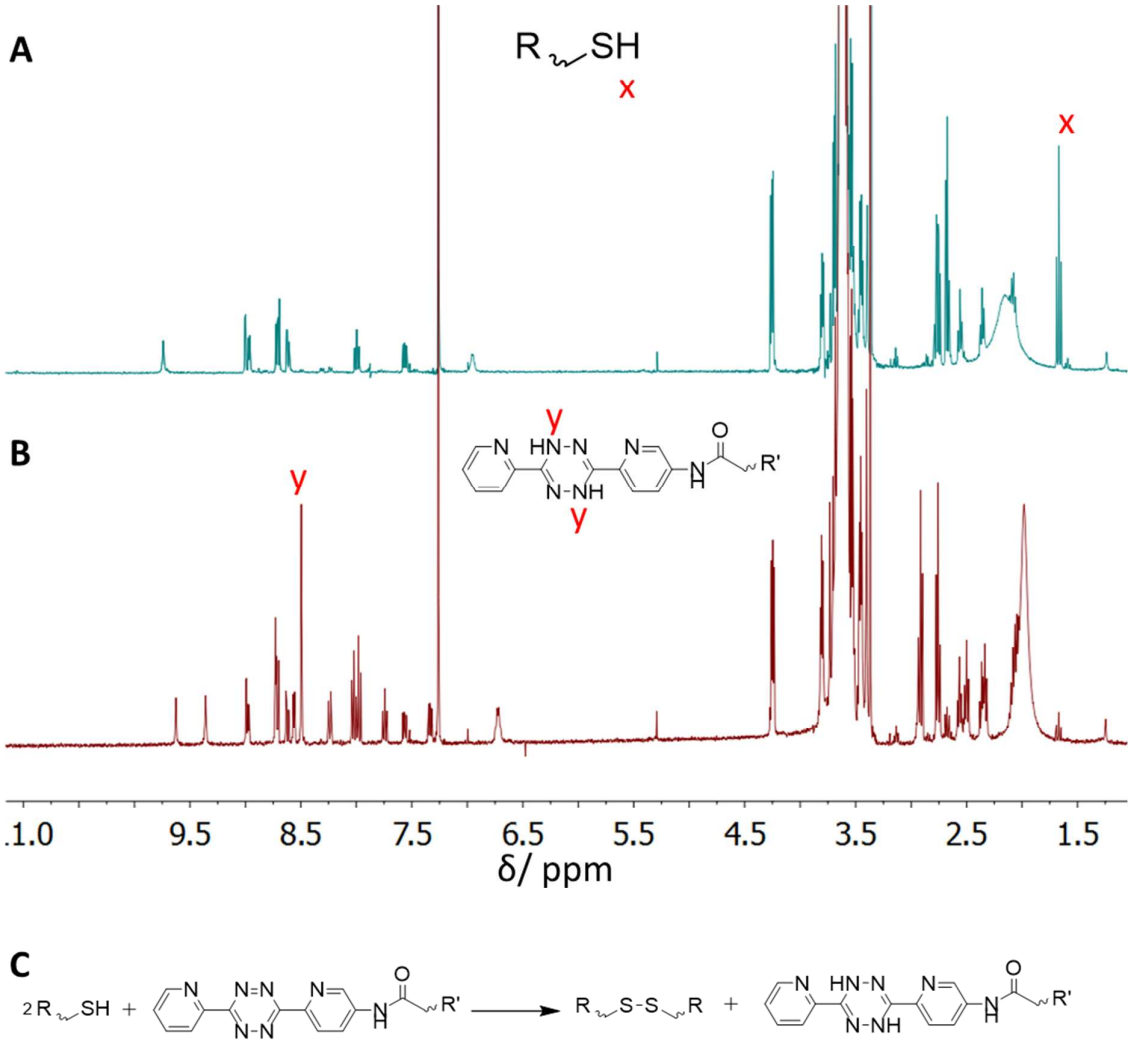


Figure S19. ^{1}H NMR spectra of 4arm PEG-Tz and MeO-PEG-SH before (**A**) and (**B**) after mixing in PBS solution for 1 h ($CDCl_3$, 400 MHz), the solution was freeze-dried and redissolved in CDCl3 for NMR analysis. The disappearance of the thiol group and the presence of the proton from dHTz could be observed after 1 h, indicating reduction of the dHTz as shown in scheme **C**.

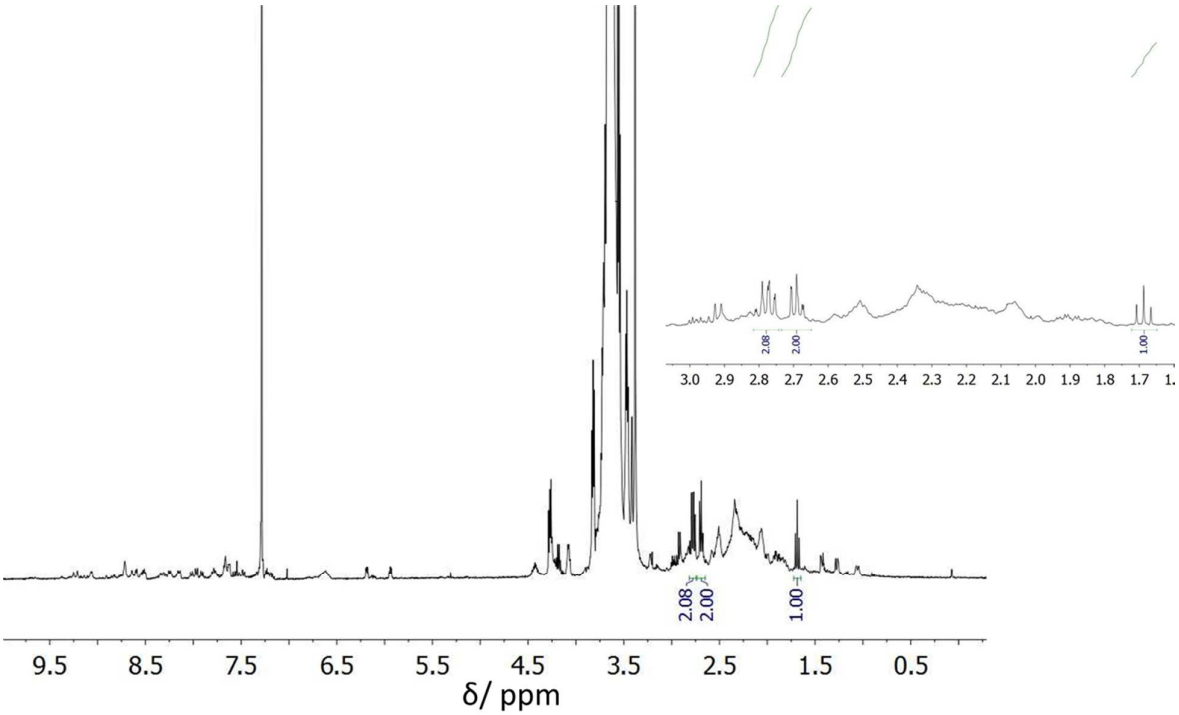


Figure S20. ^{1}H NMR spectrum of 4arm PEG-dHTz, MeO-PEG-Nb and MeO-PEG-SH after photocatalytic oxidation ($CDCl_3$, 400 MHz). The PBS solution was freeze-dried and the solid was redissolved in $CDCl_3$ for NMR analysis. The thiol group was observed to retain while the dHTz group and Nb group were consumed

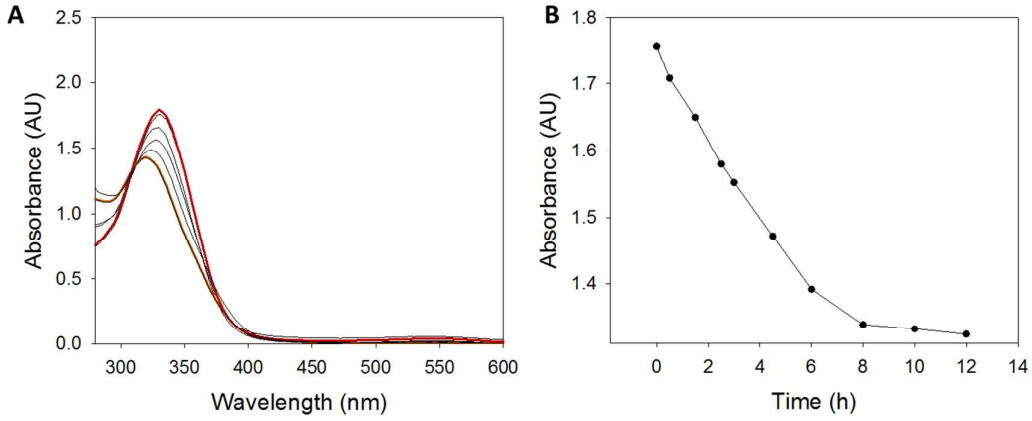


Figure S21. (A) UV-Vis spectra of polymer **2** and **3** in chloroform and (**B**) decrease of the absorbance value at 330 nm over time.

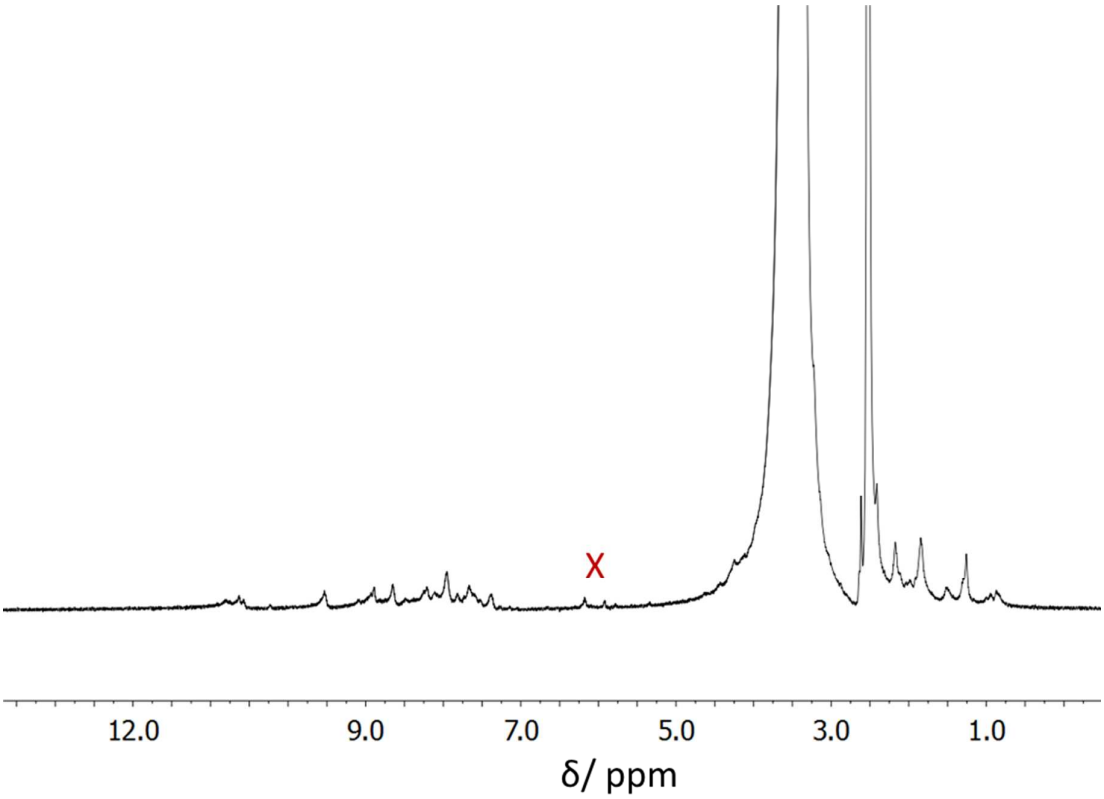


Figure S22. ^{1}H NMR spectrum of Tz-Nb gel formed by photocatalytic activation crosslinking (600 MHz, DMSO-d_6). Hydrogel was fractured and freeze-dried before redissolving in DMSO-d_6 for NMR analysis The x mark indicates possible unreacted Nb groups.

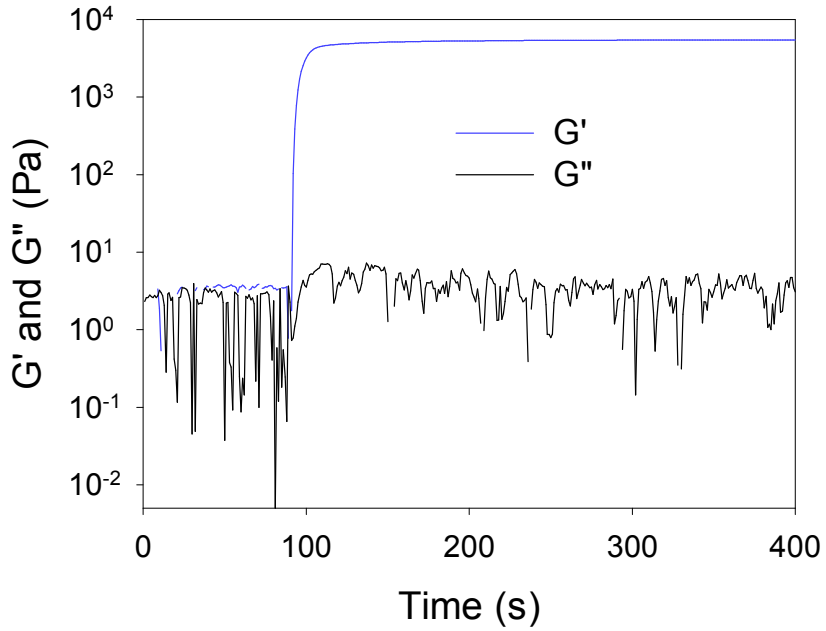


Figure S23. Gelation kinetics of crosslinking in the presence of rose Bengal (5 µM) and activated by green light (530 nm) at the intensity of 10 mW cm^{-1}.

Figure S24. Photo of the rheometer with Intralipid containing agarose gel attached underneath the quartz plate. Red light (625 nm, 10 mW cm^{-1}) can be seen passing through the phantom tissue.

Reference:

1. Hansell, C. F.; Espeel, P.; Stamenović, M. M.; Barker, I. A.; Dove, A. P.; Du Prez, F. E.; O'Reilly, R. K., Additive-Free Clicking for Polymer Functionalization and Coupling by Tetrazine–Norbornene Chemistry. *J. Am. Chem. Soc.* **2011**, 133, 13828-13831.