Supporting information

Therapeutic applications of responsive organic photocatalytic polymers, enabling *in situ* drug activation

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1. General information for materials and characterization techniques

Materials

All chemicals and solvents were purchased from chemical suppliers and used without further purification, unless specifically noted. 2-(azepan-1-yl)ethan-1-ol (95%) was obtained from Thermo Fisher Scientific. Poly(ethylene glycol) methyl ether (4-cyano-4-pentanoate dodecyl trithiocarbonate) ($mPEG_{113}$-CPDTC), methacryloyl chloride (97%), 4,4'-azobis(4-cyanovaleric acid) (ACVA, 98%), eosin Y (EY, 75%), titanium(VI) chloride ($TiCl_4$, 99.9%), anhydrous acetone, 4,4,5,5-tetramethyl-2-phenyl-1,3,2-dioxaborolane (97%), 2-hydroxyethyl methacrylate (HEMA, 99%), 1-hydroxybenzotriazole hydrate (HOBt, 97%), DMSO-d_6 (99.9 atom % D), and $CDCl_3$ (99.8 atom % D) were purchased from Sigma Aldrich. Triethylamine (TEA, 99%) was acquired from VWR. 3-Mercaptopropanoic acid (98%), (S)-(+)-camptothecin (CPT, 97%), ethyl propiolate (98%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochlorides (EDC·HCl, 98%), piperidine (99%) were purchased from CDI.

Characterization

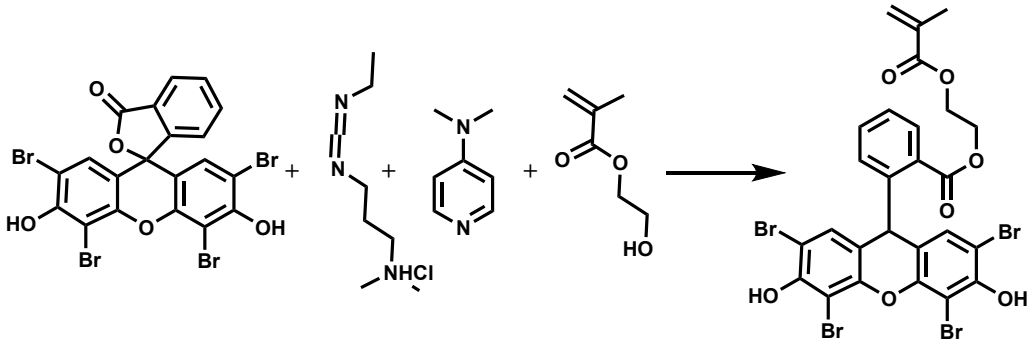
^1H NMR and ^{13}C NMR spectra were measured by Bruker Avance 400. UV-Vis transmittance spectra were taken from a Cary 60 UV/Vis spectrometer. UV/Vis absorption and emission were monitored by plate reader. Hydrodynamic diameter of the photocatalytic nanoparticles was determined by Zetasizer Nano ZS. Gas chromatograms and corresponding mass spectra were taken from GCMS-QP-2010 Ultra. FTIR spectra were measured using a Bruker Tensor II FTIR spectrometer. TEM was conducted using a JEM-1400 Transmission Electron Microscope. Samples were prepared by dispersing the particles in PBS 0.1 mM pH 6.4 buffer solution (0.1 wt%) and applying them to carbon-coated copper grids. The particles were stained using uranyl acetate stain allowing us to better visualize the particles. Zeta potential was examined applying Zetasizer Malvern, Nano Z.

2. Experimental Section

2.1 Synthesis of photocatalytic methacrylamide monomer 2-(2-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3H-xanthan-9-yl)benzamido)ethyl methacrylate (EYHEMA)

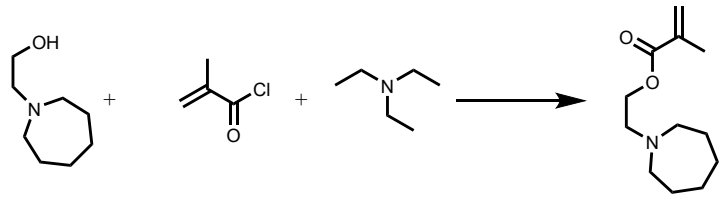
EY (2 g, 3.09 mmol), hydroxylethylmethacylate (401.7 mg, 3.09 mmol), and DMAP (377 mg, 3.1 mmol) were combined in a flame-dried round bottom Schlenk flask under Ar and dissolved in 30 mL of anhydrous DCM. The reaction mixture was cooled in an ice bath at 0 °C. After 10 min, EDC·HCl (887.7 mg, 4.6 mmol) in 10 mL of DCM was added to the reaction mixture above at 0 °C dropwise. The mixture was stirred overnight and warmed up to room temperature under an Ar atmosphere. The product was diluted with DCM and washed with HCl (1 M),

$NaHCO_3$ (saturated), water and brine. The extracted organic phase was dried over $MgSO_4$ and the solvent was evaporated using a rotary evaporator.



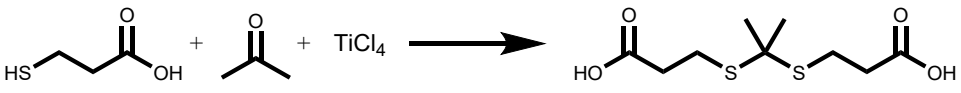
2.2 Synthesis of pH-responsive monomer 2-(azepan-1-yl)ethyl methacrylate (AEMA)

2-(azepan-1-yl)ethan-1-ol (1 g, 6.98 mmol) was added to a flame-dried Schlenk tube (100 mL) with 30 mL of anhydrous DCM under Ar, followed by the addition of TEA (1.27 mL, 9.08 mmol). The reaction mixture was cooled in an ice bath. After 15 min, methacryloyl chloride (818.5 µL, 8.38 mmol) was added dropwise. The reaction mixture was stirred overnight and warmed up to room temperature. The solvent and unreacted starting materials were evaporated.



2.3 Synthesis of thiol ketal (TK)

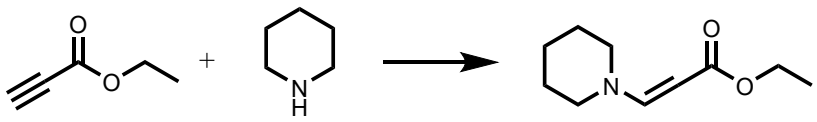
Mercaptopropionic acid (2 g, 18.84 mmol) and anhydrous acetone (1.4 mL, 18.84 mmol) were combined in a flame-dried Schlenk flask before the addition of anhydrous DCM (40 mL). The reaction mixture was cooled to -10 °C for 15 min before the addition of $TiCl_4$ (1.07 g, 5.65 mmol) dropwise. The colour of the solution turned reddish while adding $TiCl_4$ and some white precipitates were formed around the flask wall as the reaction proceeded. The mixture was allowed to warm up to room temperature and stirred overnight. The crude product was washed with aqueous HCl solution (0.2 N), DIW and brine. The organic phase was dried over $MgSO_4$ and the solvent was evaporated by a rotary evaporator. The final product was obtained as a white powder after recrystallisation in ethyl acetate/hexane.



2.4 Synthesis of ethyl 3-(piperidine-1-yl)acrylate

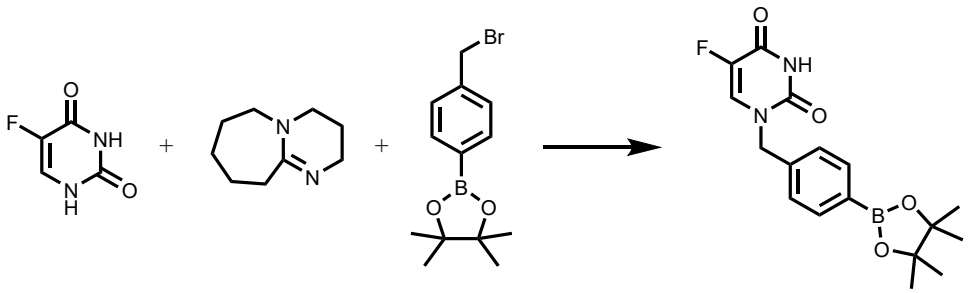
Ethyl propiolate (200 mg, 2.04 mmol) and piperidine (173.6 mg, 2.04 mmol) were dissolved in 25 mL of anhydrous THF in a flame-dried Schlenk tube. The solution was stirred at room temperature for 15

min. The solvent was removed under reduced pressure. The crude product was further purified by column chromatography (ethyl acetate/hexane 7/3), giving the final product a yellowish liquid.

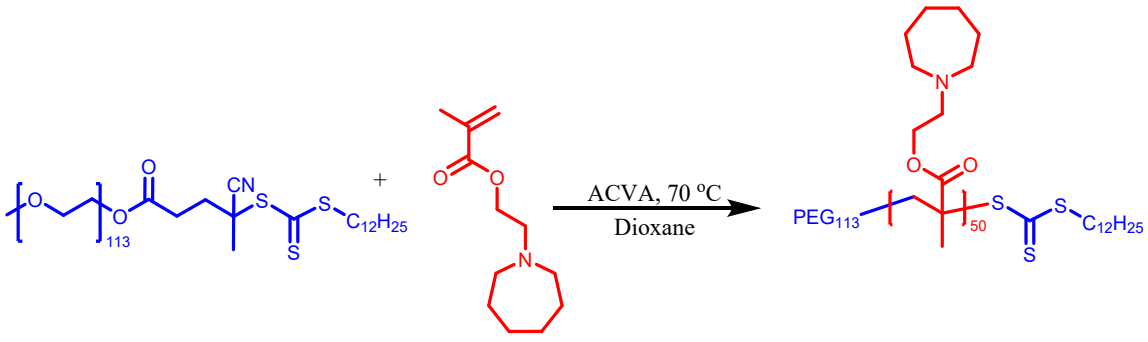


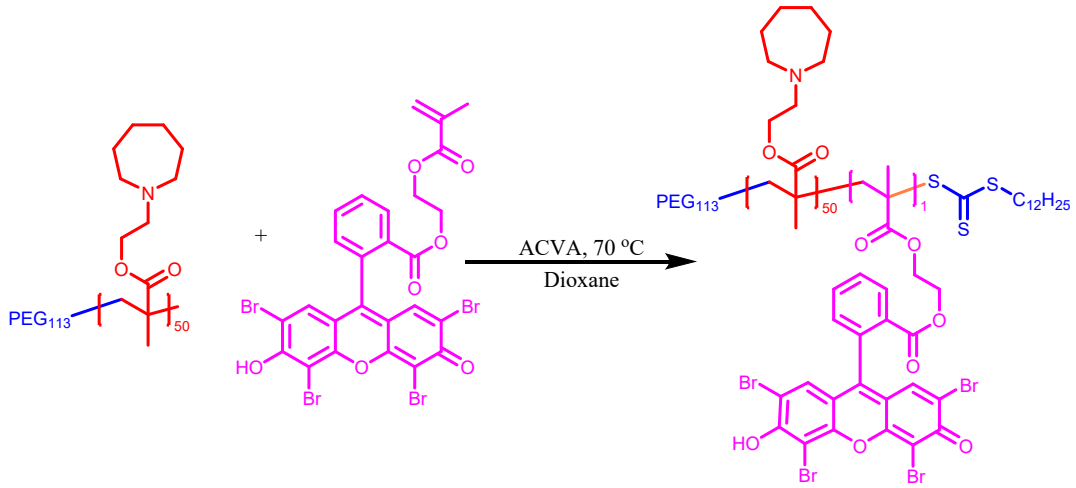
2.5 Synthesis of 5-Fluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrimidine-2,4(1H,3H)-dione

Fluorouracil (FU, 500 mg, 3.84 mmol) and diazabicycloundecene (DBU 585 mg, 3.84 mmol) were added to a flame-dried Schlenk flask and dissolved in anhydrous DMF (20 mL). The mixture was cooled in an ice bath. After 15 min, 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.14 g, 3.84 mmol) in DMF (10 mL) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stirred overnight. The solvent was removed under reduced pressure and the final product was afforded through a silica column (EtAc/Hexane 1/1).



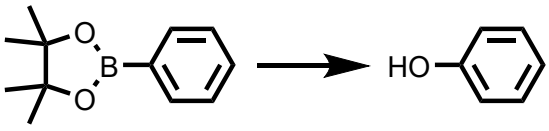
2.6 Preparation of pH-responsive polymer PEG-*b*-PAEMA and dual-responsive polymer PEG-*b*-PAEMA-*b*-EYHEMA





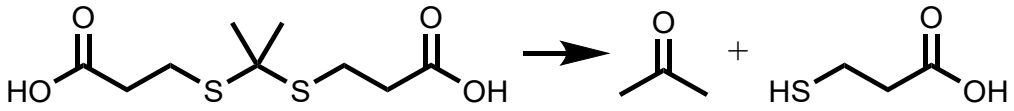
PEG-*b*-PAEMA and PEG-*b*-PAEMA-EYHEMA were synthesized by RAFT polymerization. Briefly, $mPEG_{113}$-CPDTC (0.564 g, 0.105 mmol), AEMA (3 g, 12.5 mmol), and ACVA (5.9 mg, 21 µmol) were dissolved in anhydrous dioxane (5 mL). After degassing, the mixture was stirred at 70 °C for 1 day followed by precipitating in cold hexane. The precipitate was collected by filtration and dried in a vacuum overnight. The final product was obtained as a yellow solid. The degree of polymerization of the PAEMA block was determined as 52 according to ^{1}H NMR spectrum. The dispersity was determined by GPC. The as obtained PEG-*b*-PAEMA was further used as macroCTA for the chain extension of photocatalytic moiety EYHEMA. More in detail, PEG-*b*-PAEMA (608.5 mg, 37 µmol), EYHAMA (56.4 mg, 74 µmol), and ACVA (2.1 mg, 7.4 µmol) were dissolved in anhydrous DMF (2 mL) and degassed with Ar. The reaction mixture was heated up to 70 °C overnight followed by dialysis in acetone/DIW (1/1) for 6 days and DIW for 1 day. The final product was dried by lyophilization, obtaining a fluorescent red powder. Regarding pH-responsive photocatalytic nanoparticle preparation, PEG-*b*-PAEMA-EYHEMA polymers were dissolved in DCM before the addition of PBS buffer solution (pH 7.4, 0.1 mM), respectively. The mixture was allowed to stir overnight to evaporate the DCM organic solvent, leading to the self-assembly of the amphiphilic photocatalytic polymers, generating NP-AEMA-EYAMA and NP-AEMA-EYHEMA dispersions at a concentration of 10 mg/mL.

2.7 Photocatalytic activation of prodrug model compounds using EY disodium salt

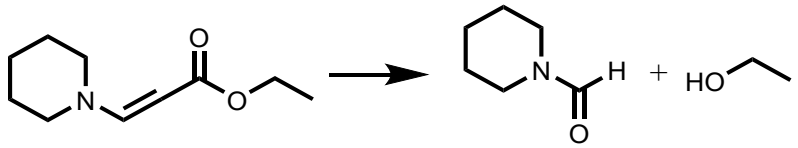


EY disodium salt (5 mol%), 4,4,5,5-tetramethyl-2-phenyl-1,3,2-dioxaborolane (30 mg, 50 mM), sodium ascorbate (150 mg, 5 eq.) was combined in an open-top screw cap vial with 3 mL PBS buffer solution (pH 7.4, 0.1 mM). The reaction mixture was supplied with oxygen through an oxygen balloon connected to the vial. The reaction mixture was irradiated with blue LED light (power: 17 mW/cm^2,

λ = 460 nm) for 2 h, with aliquots taken to monitor conversion over time. The sample aliquots (0.4 mL) were mixed with DCM (2.5 mL) to extract reagents and products. The DCM solutions were dried over $MgSO_4$ before submitting to GCMS measurements. The yield and product were obtained by GCMS measurements.

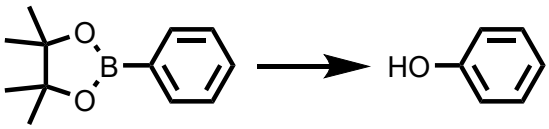


EY disodium salt (5 mol%) and 3,3'-(propane-2,2-diylbis(sulfanediyl))dipropionic acid (37.5 mg, 50 mM) were combined in an open-top screw cap vial with 3 mL PBS buffer solution (pH 7.4, 0.1 mM). The reaction mixture was supplied with oxygen through an oxygen balloon connected to the vial. The reaction mixture was then irradiated with blue LED light (power: 17 mW/cm^2, λ=460 nm) for 2.5 h, with aliquots taken to monitor conversion over time. The sample aliquots (0.4 mL) were mixed with $CDCl_3$ (2 mL) to extract reagents and products for NMR measurements. The yield was calculated based on NMR measurements.



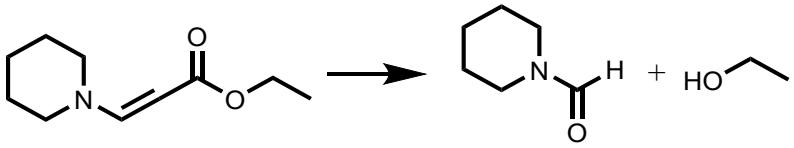
EY disodium salt (5 mol%) and ethyl 3-(piperidine-1-yl)acrylate (30 mg, 50 mM) were combined in an open-top screw cap vial with 3 mL PBS buffer solution (pH 7.4, 0.1 mM). The reaction mixture was supplied with oxygen through an oxygen balloon connected to the vial. The reaction mixture was irradiated with blue LED light (power: 17 mW/cm^2, λ = 460 nm) for 4 h, with aliquots taken to monitor conversion over time. The sample aliquots (0.4 mL) were mixed with DCM (2.5 mL) to extract reagents and products. The DCM solutions were dried over $MgSO_4$ before submitting to GCMS measurements. The yield and product were obtained by GCMS measurements.

2.8 Photocatalytic activation of prodrug model compounds using polymeric photocatalyst

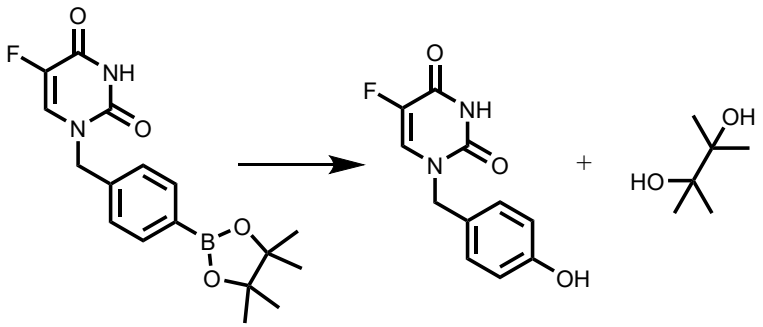


PEG-*b*-PAEMA-*b*-EYHEMA (2.5 mol%), 4,4,5,5-tetramethyl-2-phenyl-1,3,2-dioxaborolane (6 mg, 15 mM), sodium ascorbate (30 mg, 5 eq.) was combined in an open-top screw cap vial with 2 mL phosphate buffer solution (pH 6.5, 0.1 mM). The reaction mixture was supplied with oxygen through an oxygen balloon connected to the vial. The reaction mixture was irradiated with blue LED light (power: 11.9 mW/cm^2, λ = 460 nm) for 1.5 h, with aliquots taken to monitor conversion over time. The sample aliquots (0.4 mL) were mixed with DCM (2.5 mL) to extract reagents and products. The DCM solutions

were dried over $MgSO_4$ before submitting to GCMS measurements. The yield and product were obtained, in triplicate, by GCMS measurements.



PEG-*b*-PAEMA-*b*-EYHEMA (2.5 mol% photocatalytic unit) and ethyl 3-(piperidine-1-yl)acrylate (20 mg, 50 mM) were combined in an open-top screw cap vial with 2 mL phosphate buffer solution (pH 6.5, 0.1 mM). NP-EY-PAEMA (2.5 mol% photocatalytic units) and ethyl 3-(piperidine-1-yl)acrylate (20 mg, 50 mM) were combined in an open-top screw cap vial with 2 mL PBS buffer solution (pH 7.4, 0.1 mM). The reaction mixtures were supplied with oxygen through an oxygen balloon connected to the vial. The reaction mixtures were irradiated with blue LED light (power: 11.9 mW/cm^2, λ = 460 nm) for 1.5 h, with aliquots taken to monitor conversion over time. The sample aliquots (0.4 mL) were mixed with DCM (2.5 mL) to extract reagents and products. The DCM solutions were dried over $MgSO_4$ before submitting to GCMS measurements. The yield and product were obtained, in triplicate, by GCMS measurements.



PEG-*b*-PAEMA-*b*-EYHEMA (2.5 mol% photocatalytic unit), 5-fluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrimidine-2,4(1H,3H)-dione (20 mg, 39 mM), and sodium ascorbate (45 mg, 5 eq.) were combined in an open-top screw cap vial with 1.5 mL phosphate buffer solution (pH 6.5, 0.1 mM). The reaction mixtures were supplied with oxygen through an oxygen balloon connected to the vial. The reaction mixtures were irradiated with blue LED light (power: 11.9 mW/cm^2, λ = 460 nm) for 0.5 h. The sample aliquots (0.3 mL) were mixed with DMSO-d6 (0.3 mL) for the ^{19}F-NMR measurements.

2.9 Cytotoxicity Assay

The standard sample preparation for the cytotoxicity assay: PEG-*b*-PAEMA-EYHEMA (2.5 mol% photocatalytic unit), 5-fluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrimidine-2,4(1H,3H)-dione (3.5 mg, 10 mM), and sodium ascorbate (10 mg, 5 eq.) were combined in an open-top screw cap vial with 1 mL phosphate buffer solution (pH 6.5, 0.1 mM). This mixture was supplied

with oxygen through an oxygen balloon connected to the vial and irradiated with blue LED light (power: 11.9 mW/cm^2, λ = 460 nm) for 2 h.

Control conditions: (1) PEG-*b*-PAEMA-EYHEMA (2.5 mol% photocatalytic unit), 5-fluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrimidine-2,4(1H,3H)-dione (3.5 mg, 10 mM), and sodium ascorbate (10 mg, 5 eq.) were combined in an open-top screw cap vial with 1 mL phosphate buffer solution (pH 6.5, 0.1 mM). This mixture was supplied with oxygen through an oxygen balloon connected to the vial and kept in the dark for 2 h. (2) PEG-*b*-PAEMA-EYHEMA (2.5 mol% photocatalytic unit) was dissolved in 1 mL phosphate buffer solution (pH 6.5, 0.1 mM). (3) 5-fluoro-1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)pyrimidine-2,4(1H,3H)-dione (3.5 mg, 10 mM) was well dispersed in 1 mL phosphate buffer solution (pH 6.5, 0.1 mM). Each of the prepared samples were then incubated with cells.

Regarding the cell cytotoxicity, briefly, cells were seeded at a density of 10 000 cells per well in white bottom 96 well plates and incubated overnight at 37 °C and 5% CO_2. On the next day, the cells were respectively treated with PEG-*b*-PAEMA-EYHEMA, prodrug, the combination of prodrug and PEG-*b*-PAEMA-EYHEMA before and after blue light LED irradiation in various concentrations ranging from 6.25 to 100 mM. After 72 h of incubation, a volume of CellTiter-Glo® Reagent (Promega, Germany) equal to the volume of cell culture media present was added to the cells as recommended by the manufacturer. The luminescent signal was recorded with a Tecan Infinite M100 plate reader. The viability was calculated based on the average luminescent signal intensity (n = 5) for the non-treated cells.

3. Supplementary Figures

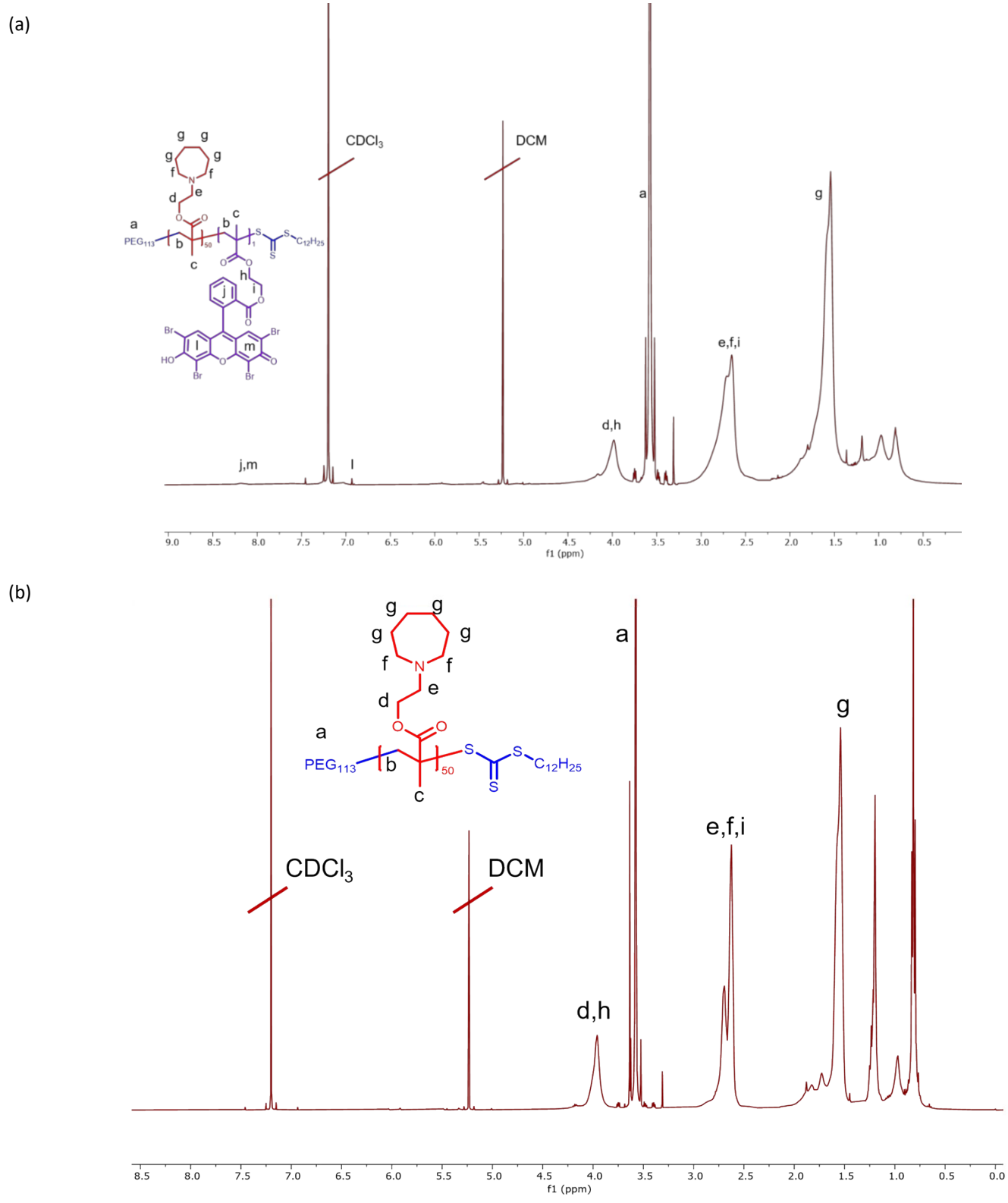


Figure S1. (a) ^{1}H NMR spectrum of PEG-*b*-PAEMA-PEYAEMA in $CDCl_3$ (400 MHz, at 298 K). (b) ^{1}H NMR spectrum of PEG-*b*-PAEMA in $CDCl_3$ (400 MHz, at 298 K).

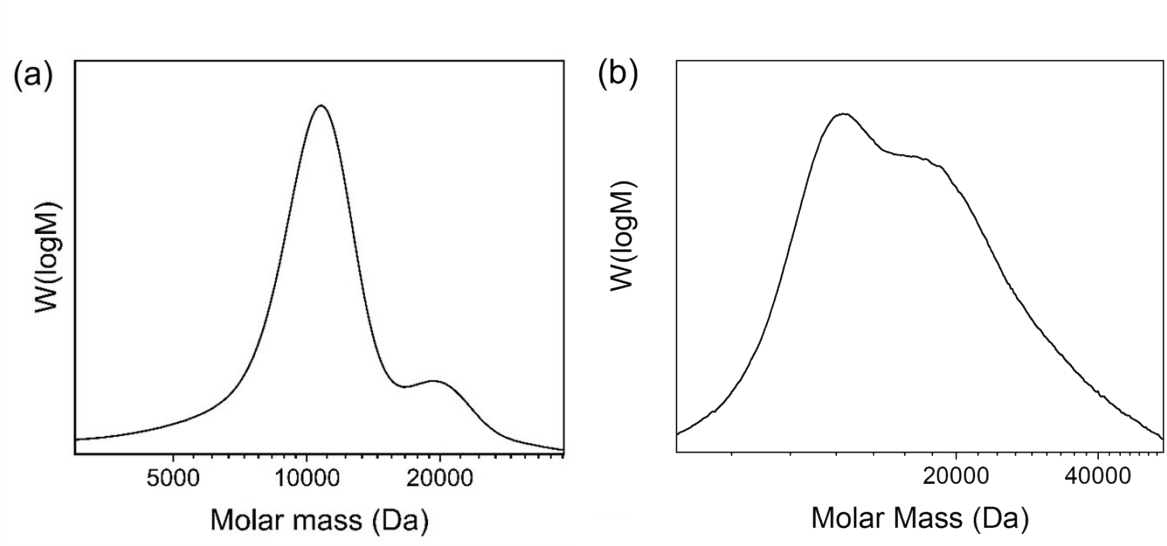


Figure S2. (a) Molecular weight distribution of PEG macroCTA (UV signal, Mn = 9.8 kDa, *Đ* = 1.20, standard: polymethylmethacrylate). (b) Molecular weight distribution of PEG-*b*-PAEMA-EYHEMA copolymer (UV signal, Mn = 13.9 kDa, *Đ* = 1.24, standard: polymethylmethacrylate).

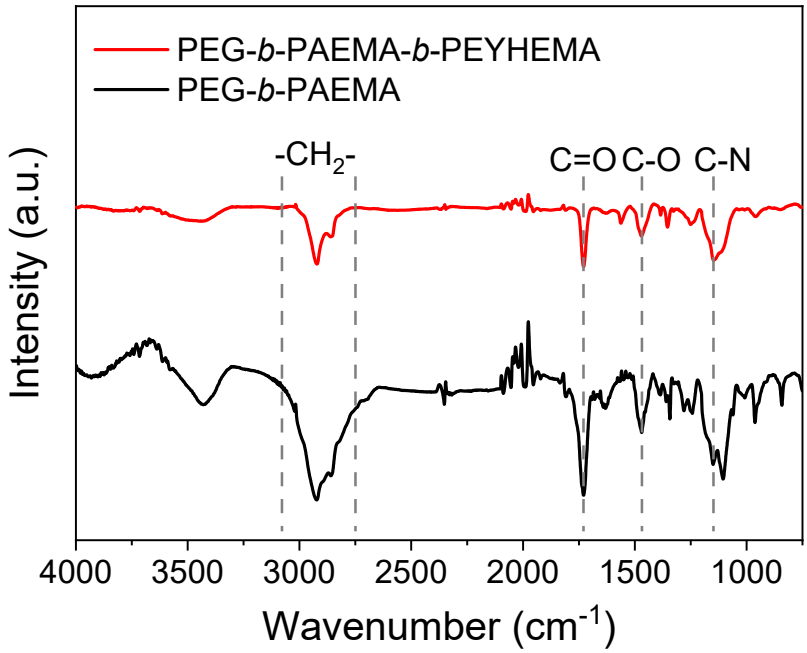


Figure S3. FTIR spectra of pH-responsive polymers without and with photocatalyst units: PEG-*b*-PAEMA (black) and PEG-*b*-PAEMA-EYHEMA (red).

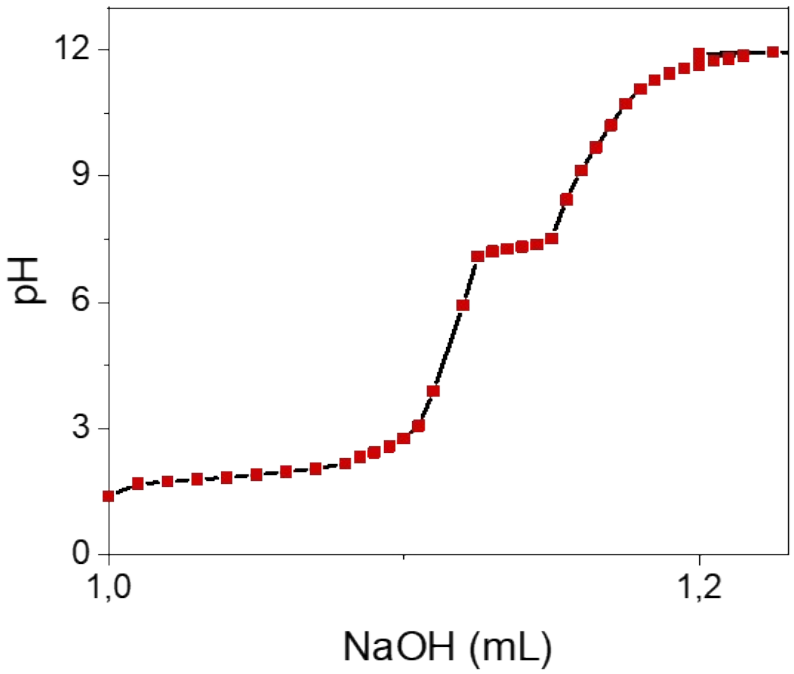


Figure S4. Titration curve of PEG-*b*-PAEMA polymer chains.

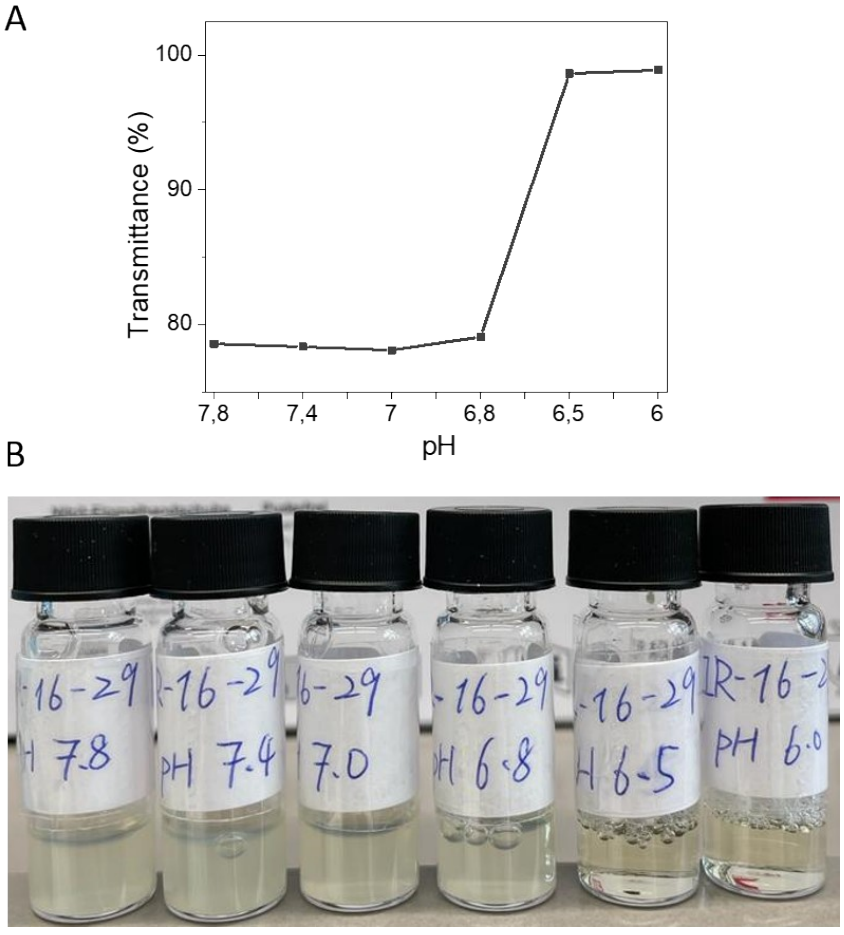


Figure S5. (A) UV/Vis transmittance of PEG-*b*-PAEMA dispersions as a function of pH value from pH 6.0 to pH 7.8 at 700 nm. (B) Photographs of the corresponding PEG-*b*-PAEMA dispersions in phosphate buffer solutions with pH values varying from pH 6.0 to pH 7.8.

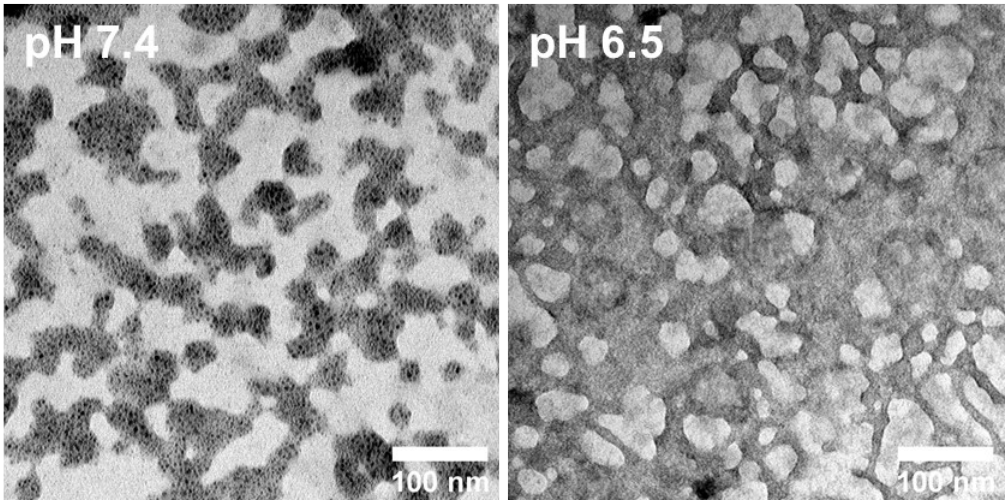


Figure S6. TEM images of PEG-*b*-PAEMA-PEYHEMA dispersion in phosphate buffer (0.1 mM) at pH 7.4 and pH 6.5.

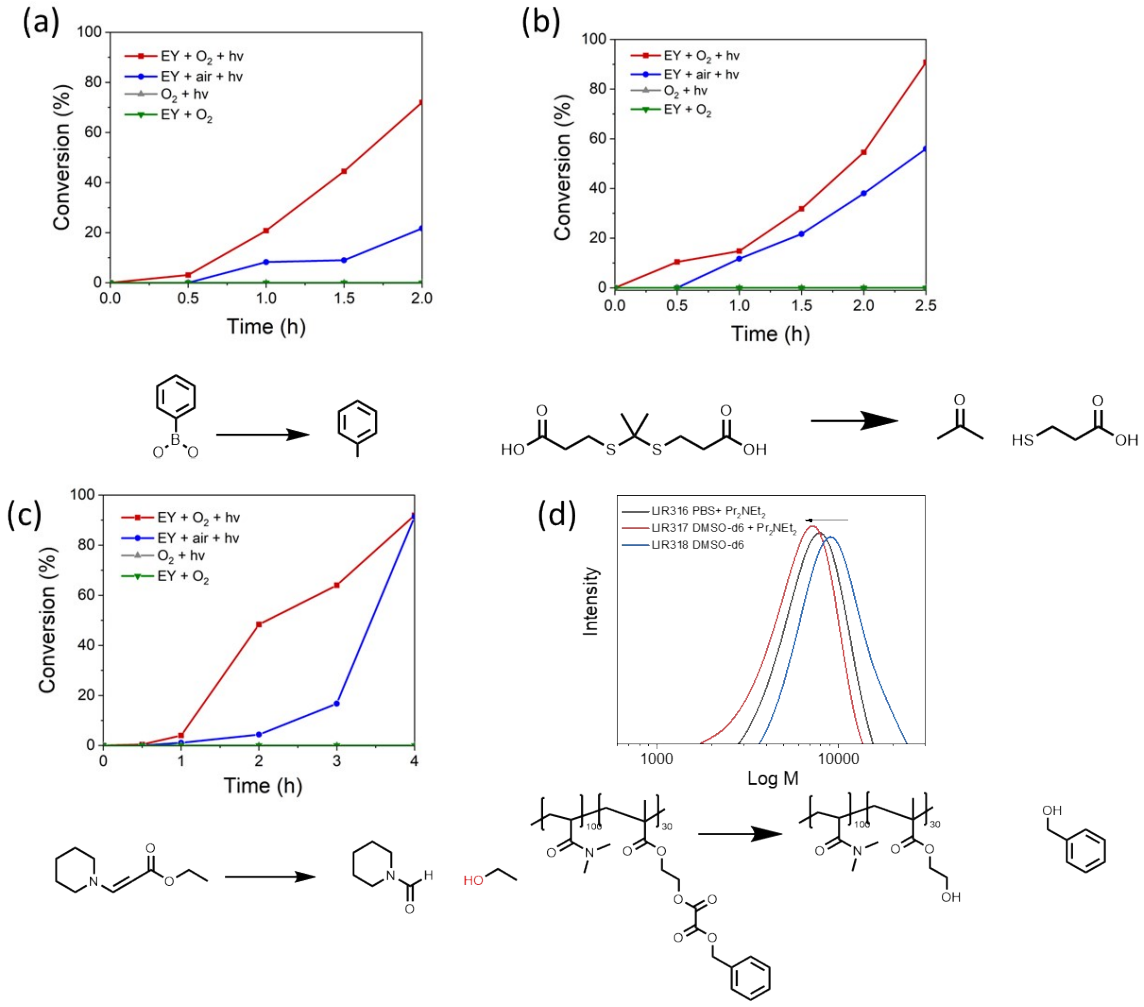
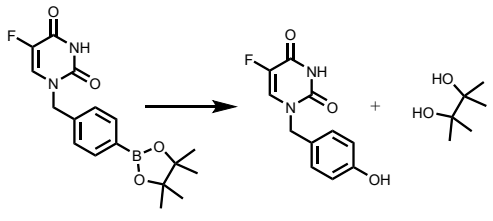


Figure S7. Prodrug model compounds activation using eosin Y disodium salt (5 mol%) loading for all reactions. (a) Kinetic profile of boronic acid pinacol ester activation, using sodium ascorbate as sacrificing agent. Conversion determined by GCMS. (b) Kinetic profile of thiol ketal activation without additive. Conversion determined by ^{1}H-NMR. (c) Kinetic profile of aminoacrylate activation without additive. Conversion determined by GCMS. (d) GPC monitored the degradation of oxalate-based model polymer using either PBS or DMSO-d_6 as solvent, as well as Pr_2NEt_2 as sacrificing agent.



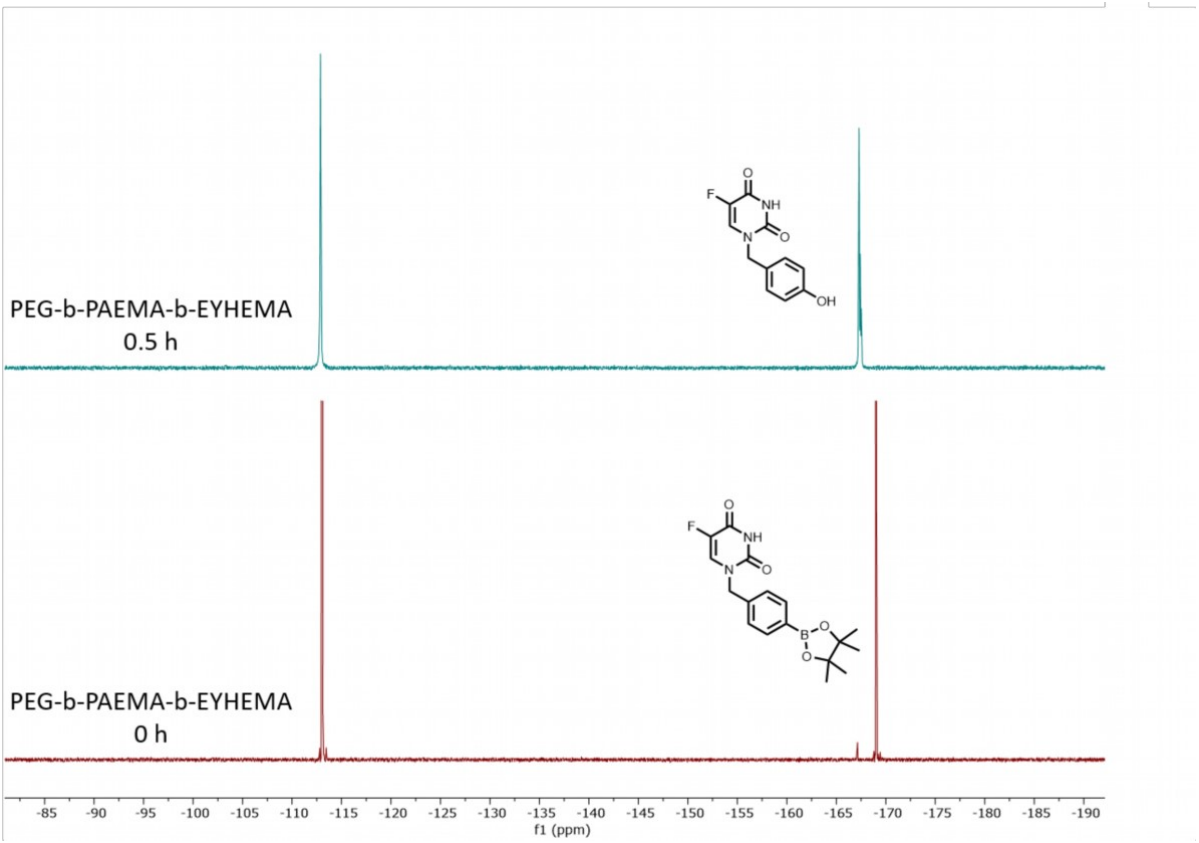
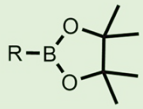
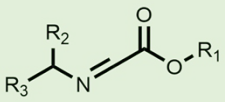
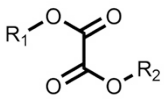


Figure S8. 19F-NMR spectrum of the activation of 5FU prodrug (40 mM) using PEG-*b*-PAEMA-EYHEMA polymeric photocatalyst (2.5 mol%) solution in phosphate buffer solution at pH 6.5 (the reference peak at -113 ppm was from fluorobenzene).

4. Supplementary table

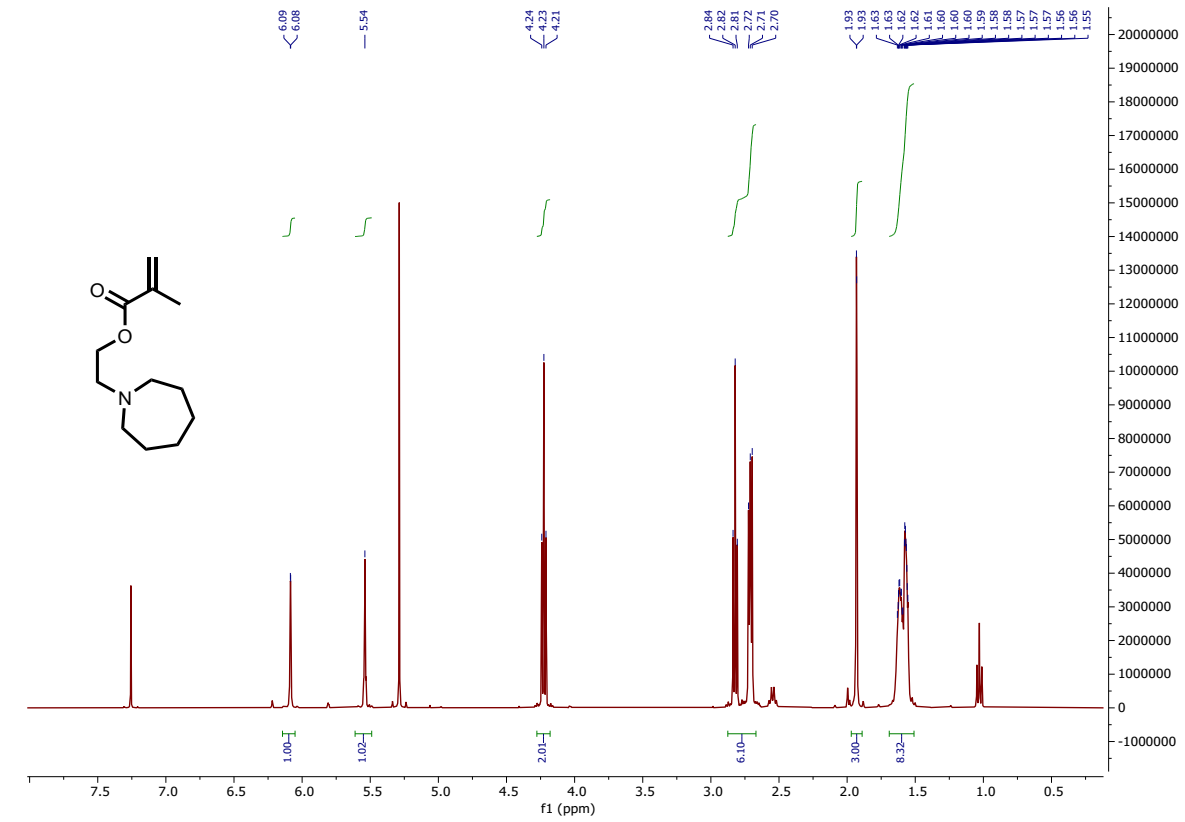
Table S1. Summary of prodrug model compounds activated by molecular eosin Y photocatalyst.

Entry	Linkage	Oxygen	Time/h	Yield/%
1		+	2	72[a]
2		-	2	22[a]
3		+	2.5	91[a]
4		-	2.5	56[a]
5		+	4	92[a]
6		-	4	91[a]
7		+	2.5	94[b]
8		-	2.5	>99[c]

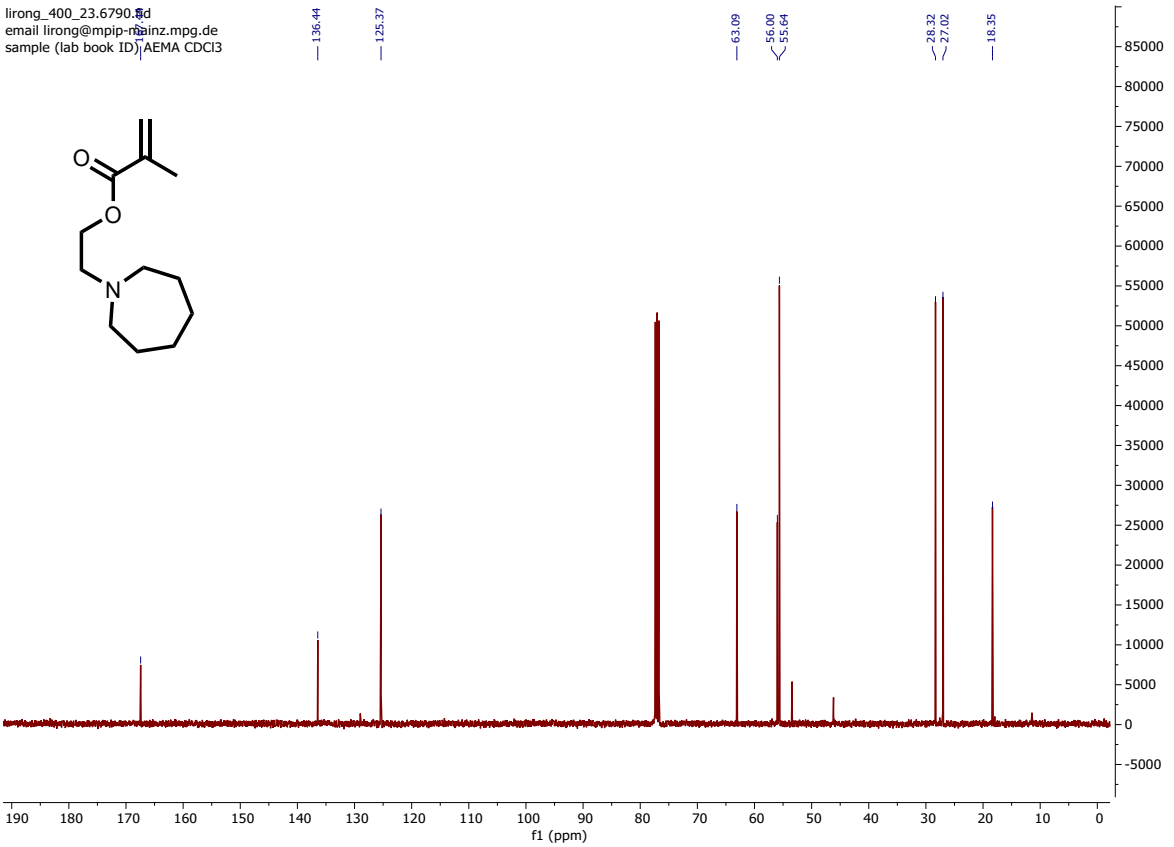
[a]Standard conditions: prodrug model molecules (50 mM), eosin Y disodium salt (5 mol%), PBS buffer (3 mL), under either oxygen or ambient environment. [b]Condition: prodrug model polymer (50 mM of the ROS sensitive linkage), eosin Y disodium salt (5 mol%), PBS buffer (1 mL), with oxygen. [c]Condition: prodrug model polymer (50 mM of the ROS sensitive linkage), eosin Y disodium salt (5 mol%), DMSO-d_6 (1 mL), with oxygen. Blue LED (λ_{max} = 460 nm) at the power of 17 mW/cm^2, temperature = 18 °C. Yield of Entry 1,2,5, and 6 were determined by GCMS. Yield of Entry 3 and 4 was measured by 1H NMR. Yield of Entry 7 and 8 was monitored by GPC.

Appendix: ^{1}H and ^{13}C NMR spectra

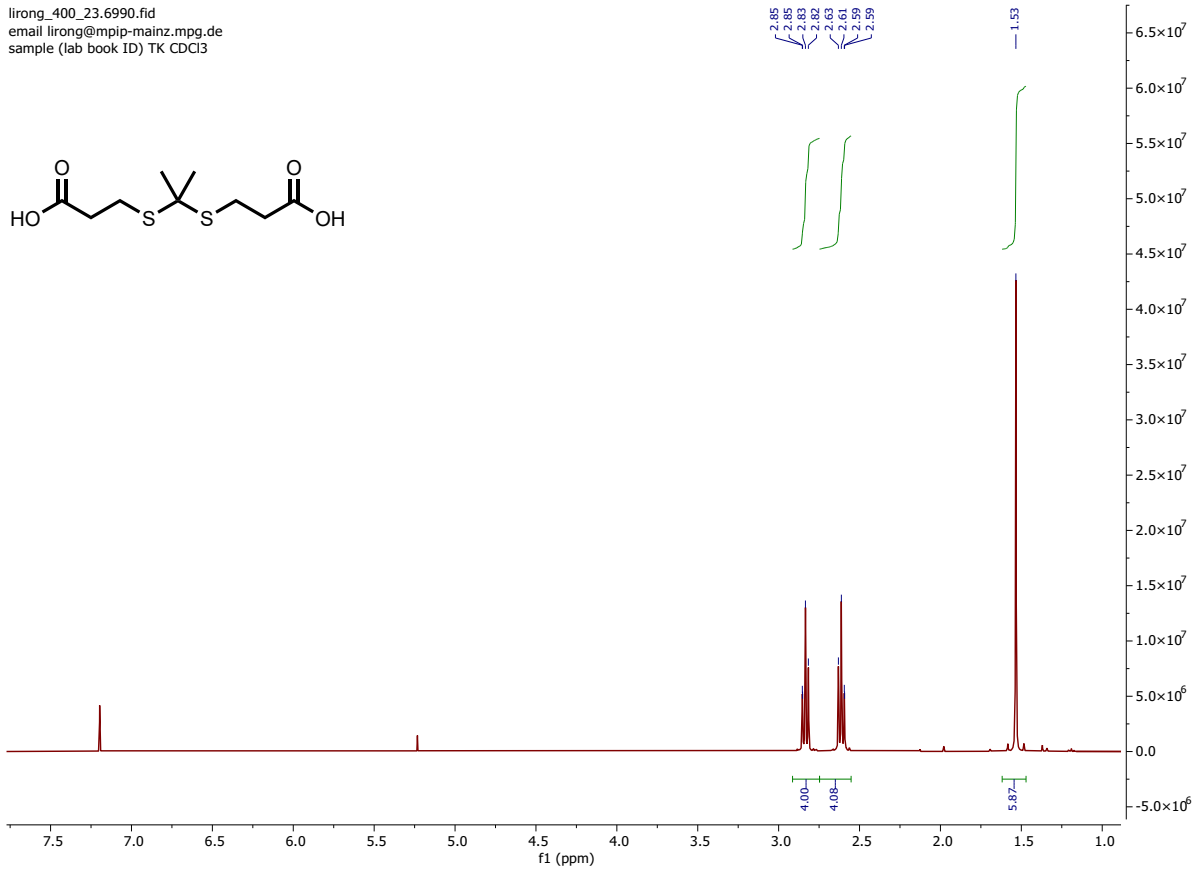
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 2-(azepan-1-yl)ethyl methacrylate



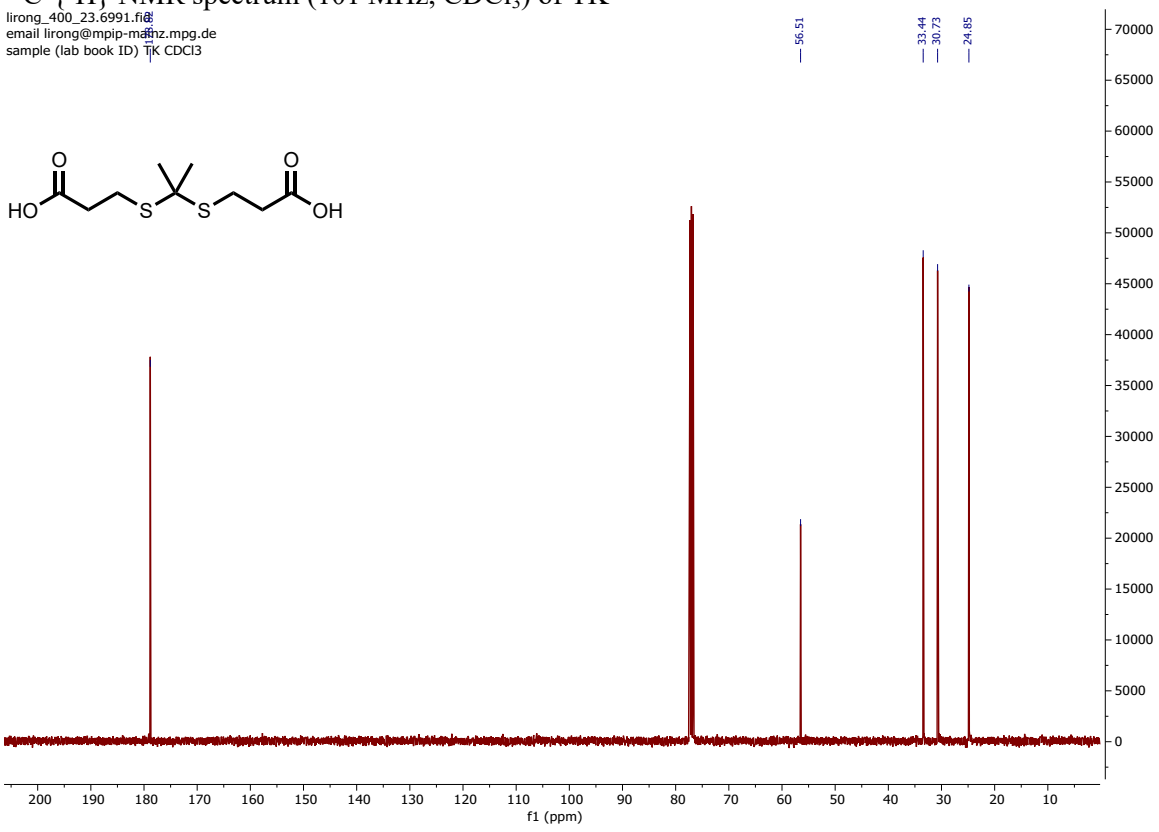
^{13}C-{^{1}H} NMR spectrum (101 MHz, $CDCl_3$) of 2-(azepan-1-yl)ethyl methacrylate



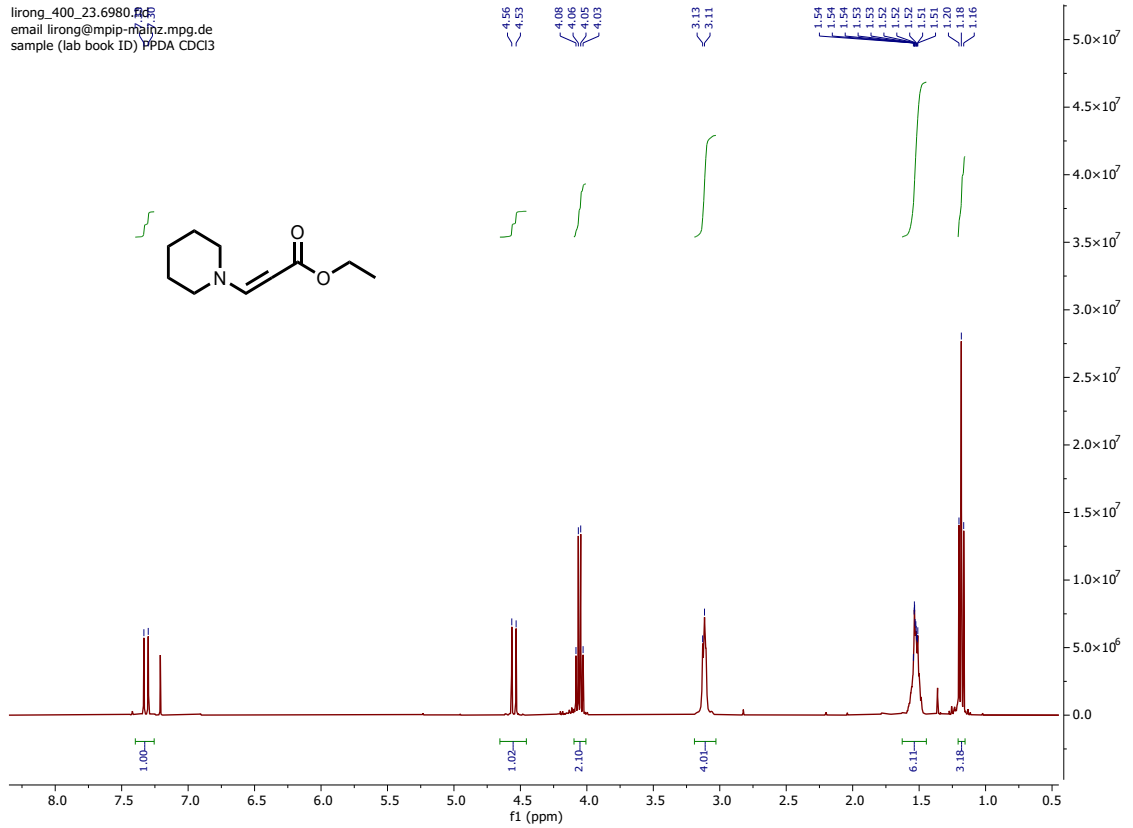
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of TK



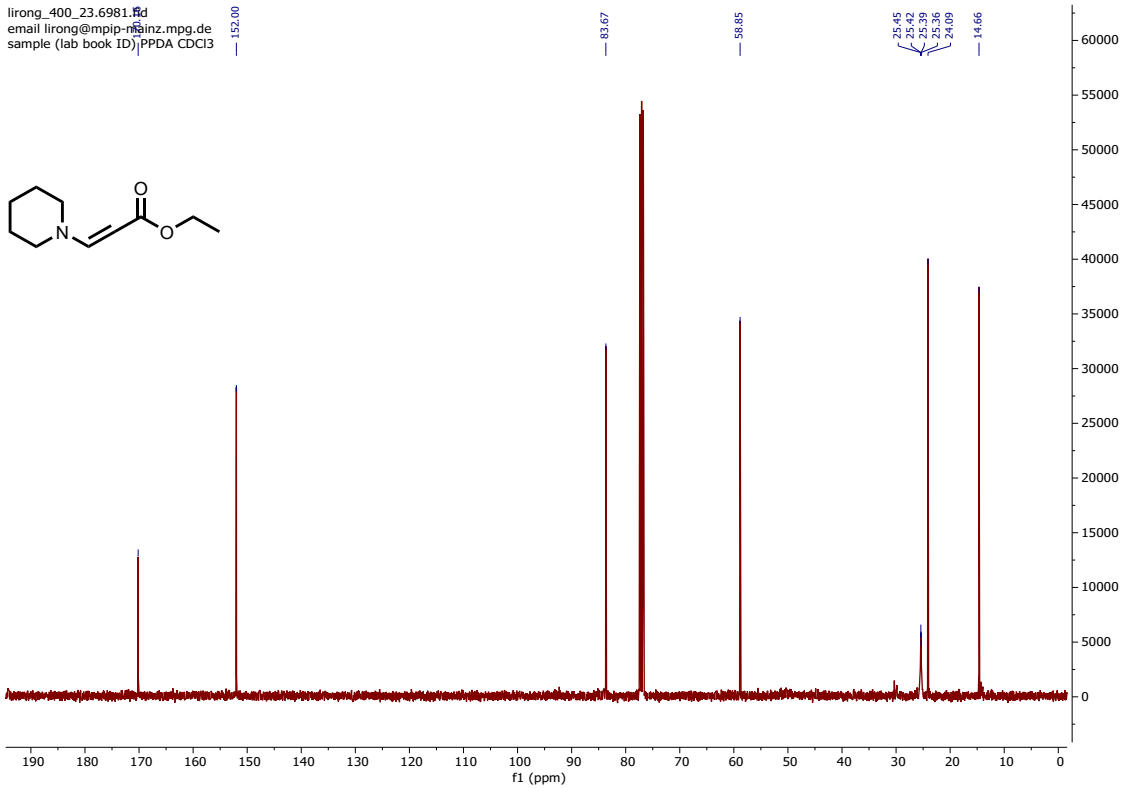
^{13}C-{^{1}H} NMR spectrum (101 MHz, $CDCl_3$) of TK



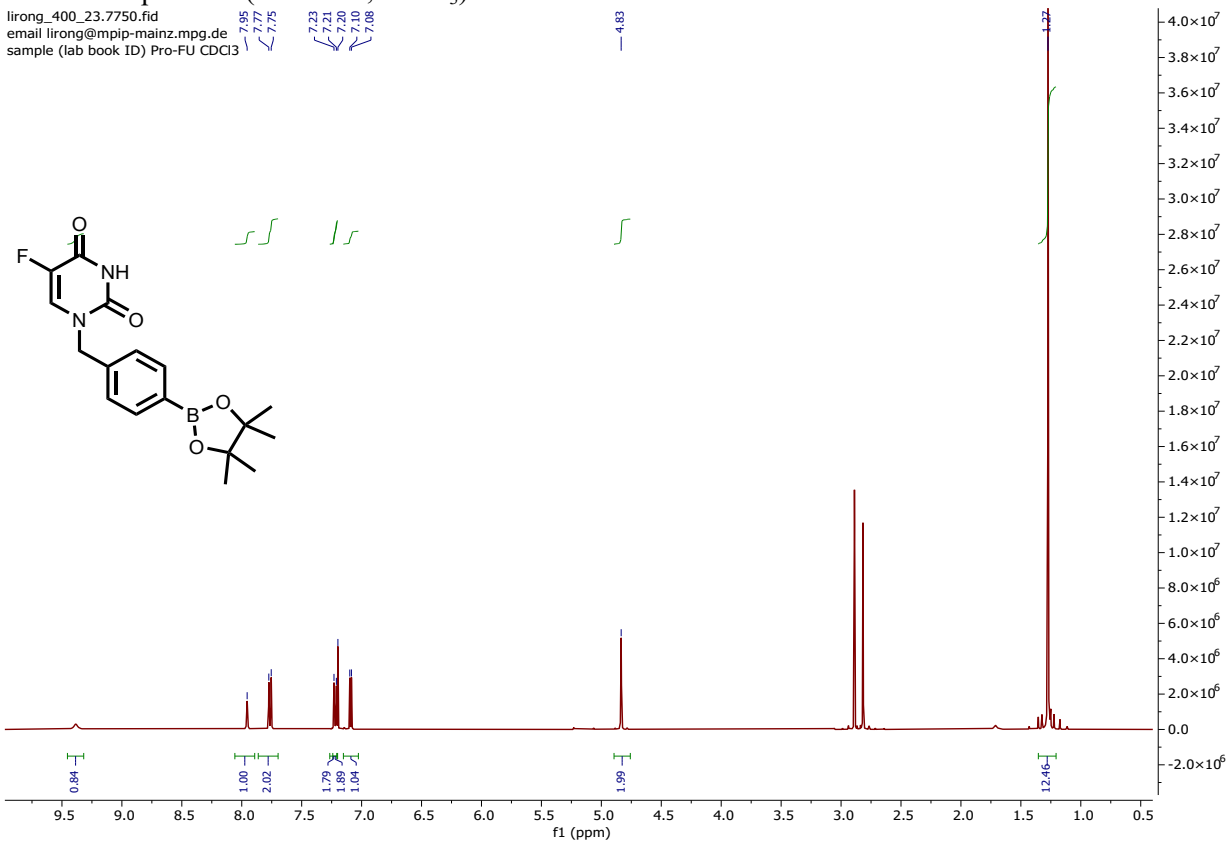
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of ethyl 3-(piperidine-1-yl)acrylate



^{13}C-{^{1}H} NMR spectrum (101 MHz, $CDCl_3$) of ethyl 3-(piperidine-1-yl)acrylate



^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 5FU



^{13}C-{^{1}H} NMR spectrum (101 MHz, $CDCl_3$) of 5FU