Electronic supplementary information for:

Covalent cross-linking approaches for all-trans retinoic acid-loaded thermo-responsive hydrogels

Xueli Mei[1], Robert C. Stewart[2], Xiao Zhen Zhou[2,3,4], Kun Ping Lu[2,3,4], Elizabeth R. Gillies[1,5]*

[1]Department of Chemistry, The University of Western Ontario, 1151 Richmond St., London, Ontario, N6A 5B7

[2]Department of Biochemistry, The University of Western Ontario, 1151 Richmond St., London, Ontario, N6G 2V4

[3]Department of Oncology, The University of Western Ontario, 1151 Richmond St., London, Ontario, N6G 2V4

[4]Robarts Research Institute, The University of Western Ontario, 1151 Richmond St., London, Ontario, N6G 2V4

[5]Department of Chemical and Biochemical Engineering, The University of Western Ontario, 1151 Richmond St., London, Ontario, N6A 5B9

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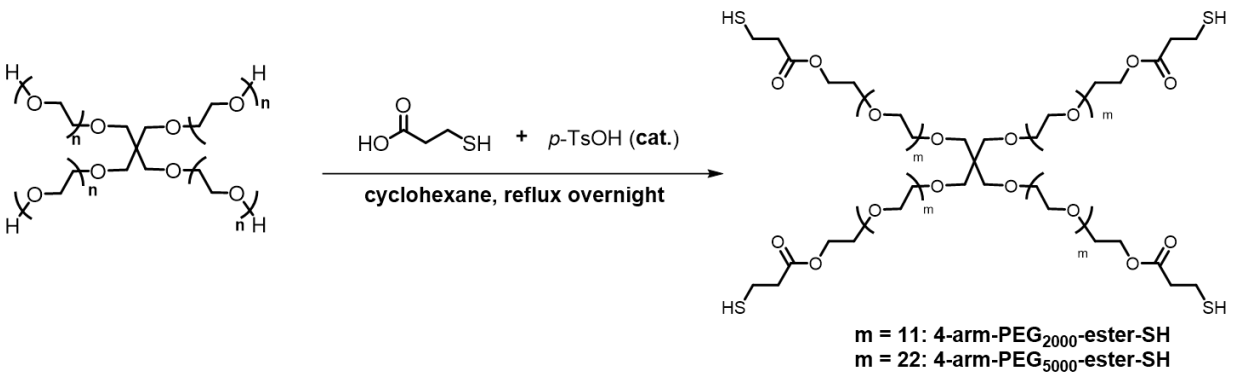
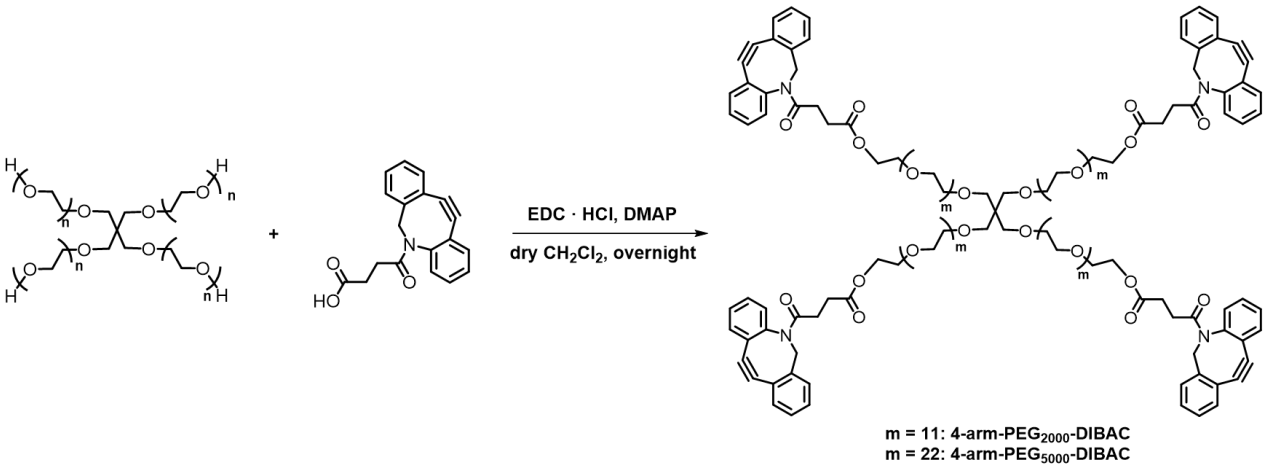


Scheme S1. Synthesis of 4-arm-PEG-ester-SH, m = n-1.



Scheme S2. Synthesis of 4-arm-PEG-DIBAC. m = n-1.

Synthesis of 4-arm-PEG$_{2000}$-ester-SH

4-arm-PEG-ester-SH was synthesized using a modification of a previously reported procedure.[1] 1.0 g (0.50 mmol, 1.0 equiv.) of 4-arm-PEG$_{2000}$ was dried azeotropically with dry toluene, then 0.65 mL (0.80 g, 7.5 mmol, 15 equiv.) of 3-mercaptopropionic acid, 0.10 g (0.60 mmol, 1 equiv.) of *p*-toluenesulfonic acid, and 30 mL of cyclohexane were added, The reaction was heated at reflux and stirred under N_2 with Dean-Stark trap overnight. Then, cyclohexane was decanted, and the residue was diluted with 50 mL of CH_2Cl_2. It was then washed with saturated $NaHCO_3$ solution (3 × 50 mL), dried over $MgSO_4$, concentrated to ~5 mL, and precipitated in cold diethyl ether. The product was a colorless oil. Yield = 0.51 g, 42 %. The product was used quickly or stored under N_2 in the freezer to avoid cross-linking by disulfide bond formation. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.26 (t, J = 4.1 Hz, 8H), 3.73 – 3.47 (m, 176H), 3.39 (s, 8H), 2.80 – 2.71 (m, 8H), 2.69 – 2.60 (m, 8H) 1.65 (t, J = 8 Hz, 4H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 171.6, 70.9– 69.6, 63.5, 38.1, 19.4. IR: 1945, 2865, 1730 cm^{-1}. DMF SEC: M_n = 1431 g mol^{-1}, M_w = 1620 g mol^{-1}, $Đ$ = 1.13.

Synthesis of 4-arm-PEG$_{5000}$-ester-SH

4-arm-PEG$_{5000}$-ester-SH was synthesized using the same procedure as described above for 4-arm-PEG-ester-SH but from 4-arm-PEG$_{5000}$. Yield = 0.52 g, 48 %. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.26 (t, J = 4.1 Hz, 8H), 3.73 – 3.47 (m, 454H), 3.39 (s, 8H), 2.80 – 2.71 (m, 8H), 2.69 – 2.60 (m, 8H) 1.65 (t, J = 8 Hz, 4H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 171.6, 71.4 – 69.6, 63.8, 61.7, 38.5, 19.8. IR: 2930, 2880, 1730 cm^{-1}. DMF SEC: M_n = 3510 g mol^{-1}, M_w = 3869 g mol^{-1}, $Đ$ = 1.10.

Synthesis of 4-arm-PEG$_{2000}$-DIBAC

To a flame-dried, three neck 100 mL round bottom flask, 0.50 g (0.10 mmol, 1.0 equiv.) of 4-arm-PEG_{2000} was added under N_2 followed by 0.12 g (0.60 mmol, 6.0 equiv.) of EDC·HCl and 0.020 g (0.15 mmol, 1.5 equiv.) of DMAP. Then, 10 mL of dry CH_2Cl_2 was added to the flask. After all the reagents were completely dissolved, 0.20 g (0.25 mmol, 6.0 equiv.) of DIBAC-CO_2H was added to the system. The reaction was stirred overnight at room temperature under N_2. The reaction mixture was then concentrated under reduced pressure, and then purified by silica gel chromatography using a gradient from 2% to 5% EtOH in CH_2Cl_2 over 8 column volumes. Yield = 0.35 g, 44 %. The product was used rapidly or stored in the freezer under N_2. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67 (d, J = 8 Hz, 4 H), 7.54 – 7.46 (m, 4 H), 7.44 – 7.27 (m, 22 H), 5.15 (d, J = 12 Hz, 4 H), 4.26 – 4.02 (m, 10 H), 3.82 – 3.45 (m, 176 H), 2.79– 2.58 (m, 8 H), 2.40– 2.28 (m, 4 H), 1.99 – 1.89 (m, 4 H). IR: 3065, 2860, 1730, 1660 cm^{-1}. DMF SEC: M_n = 1514 g mol^{-1}, M_w = 1721 g mol^{-1}, $Đ$ = 1.14.

Synthesis of 4-arm-PEG_{5000}-DIBAC

The 4-arm-PEG_{5000}-DIBAC was prepared in the same manner as 4-arm-PEG_{2000}-DIBAC with same equivalents of reagents but using 4-arm-PEG_{5000} as the starting material. Yield = 0.36 g, 58 %. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.67 (d, J = 8 Hz, 4 H), 7.54 – 7.46 (m, 4 H), 7.44 – 7.27 (m, 22 H), 5.15 (d, J = 12 Hz, 4 H), 4.26 – 4.02 (m, 10 H), 3.82 – 3.45 (m, 454 H), 2.79– 2.58 (m, 8 H), 2.40– 2.28 (m, 4 H), 1.99 – 1.89 (m, 4 H). IR: 2865, 1730, 1660 cm^{-1}. DMF SEC: M_n = 3013 g mol^{-1}, M_w = 3453 g mol^{-1}, $Đ$ = 1.31.

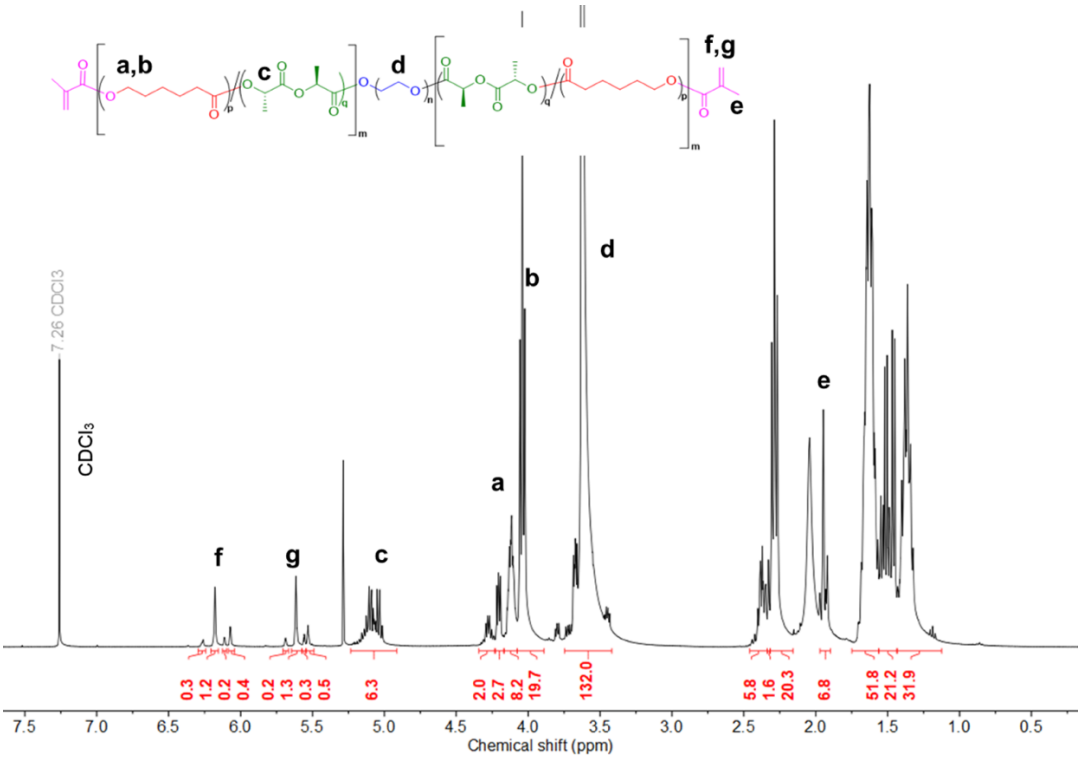


Figure S1. ^{1}H NMR spectrum of M-PCLA-PEG-PCLA-M (400 MHz, $CDCl_3$). The integration of the PEG peak was set to 132 and then 6.3 units of LA per 1500 g/mol PEG was determined based on the integration of the peak "c" while 13.0 CL units was calculated from the sum of the integrals of peaks "a" and "b" divided by 2 protons per repeat unit. The presence of the methacrylate end groups is confirmed by peaks at 6.4 – 5.4 and 1.9 ppm. Multiple peaks are observed because methacrylate groups can react with terminal caprolactone, lactide, or PEG units.

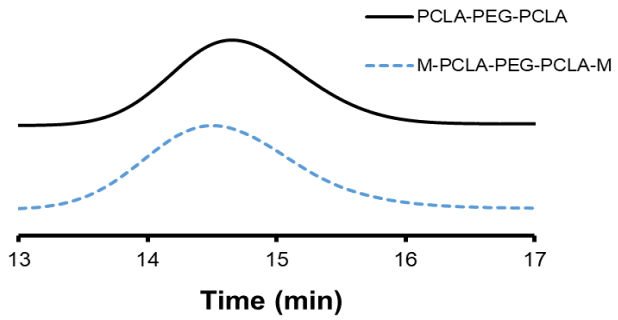


Figure S2. SEC trace of M-PCLA-PEG-PCLA-M, compared to the PCLA-PEG-PCLA starting material, run in DMF.

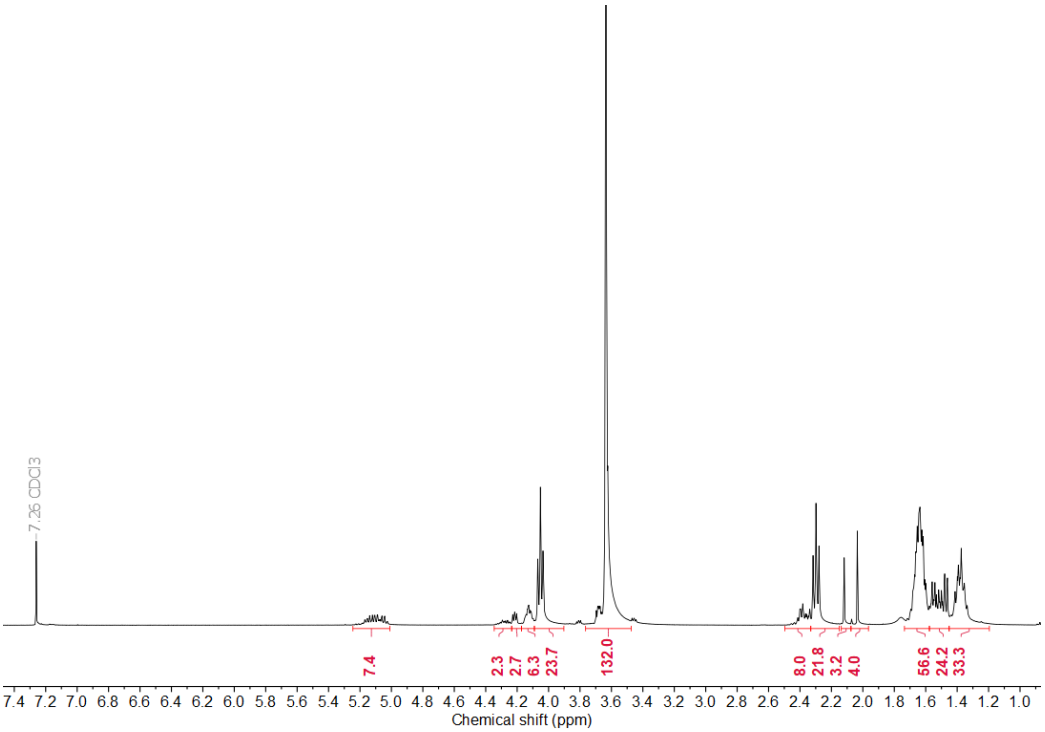


Figure S3. ^{1}H NMR spectrum of Ac-PCLA-PEG-PCLA-Ac (400 MHz, CDCl3). The presence of the acyl end groups is confirmed by the presence of peaks at 2.2 – 2.0 ppm.

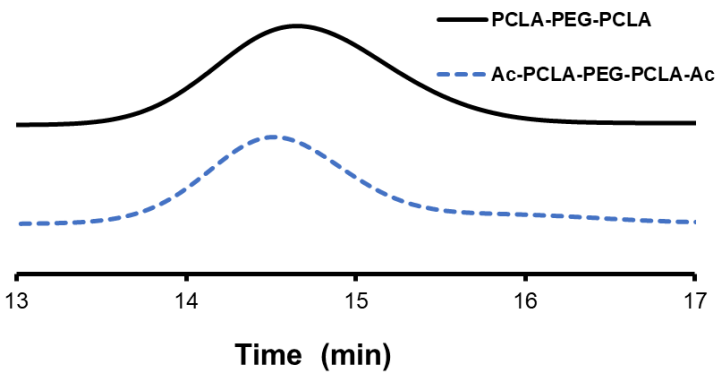


Figure S4. SEC trace of Ac-PCLA-PEG-PCLA-Ac, compared to the PCLA-PEG-PCLA starting material, run in DMF.

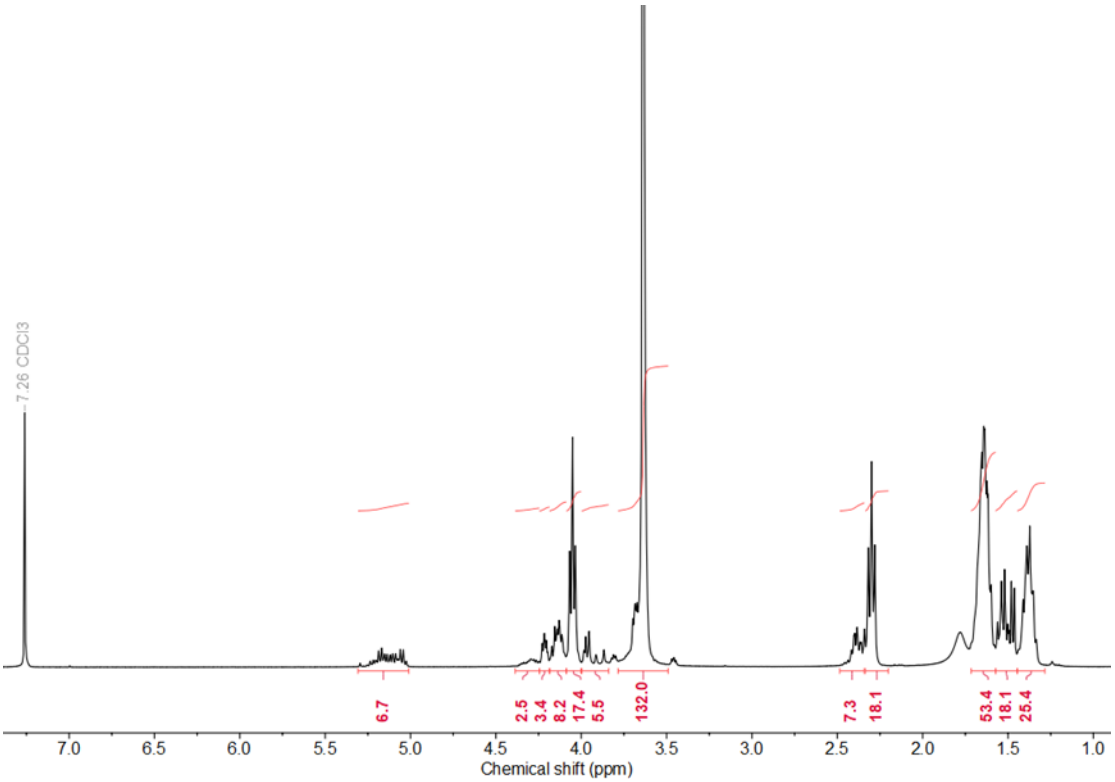


Figure S5. ^{1}H NMR spectrum of Az-PCLA-PEG-PCLA-Az (400 MHz, CDCl3). The presence of the azidoacetyl end groups is confirmed by the presence of the peaks at 4.0 – 3.8 ppm corresponding to the end group's methylene protons.

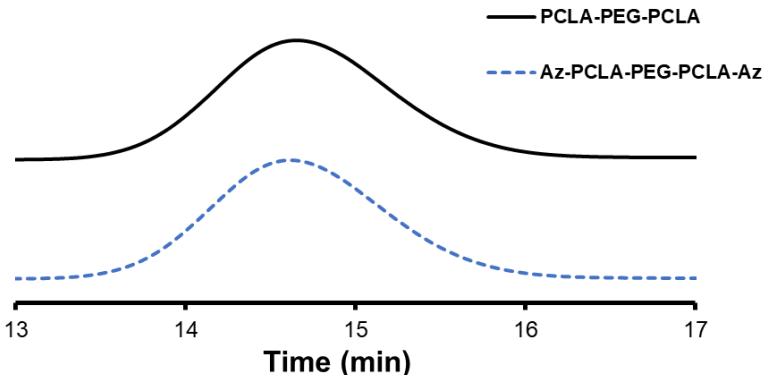


Figure S6. SEC trace of Az-PCLA-PEG-PCLA-Az, compared to the PCLA-PEG-PCLA starting material, run in DMF.

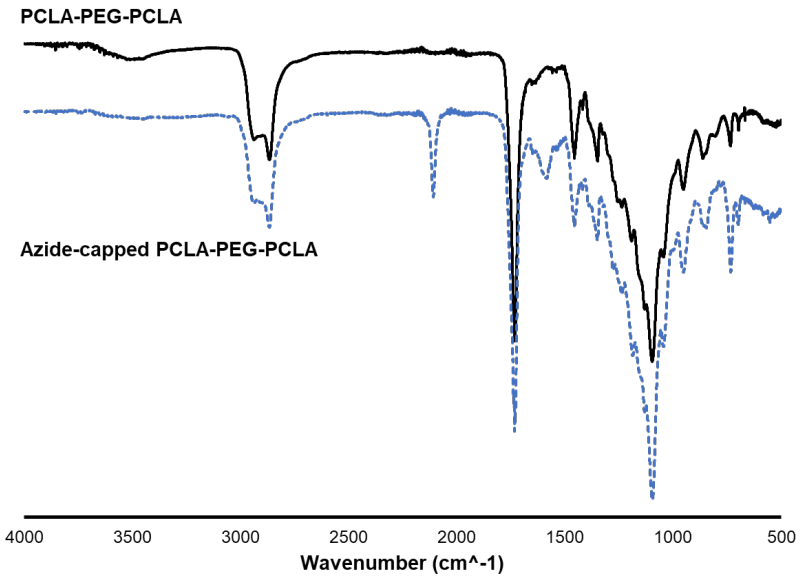


Figure S7. FT-IR trace for Az-PCLA-PEG-PCLA-Az synthesized comparing to the starting polymer PCLA-PEG-PCLA. The new peak at 2110 cm^{-1} corresponds to the azide group.

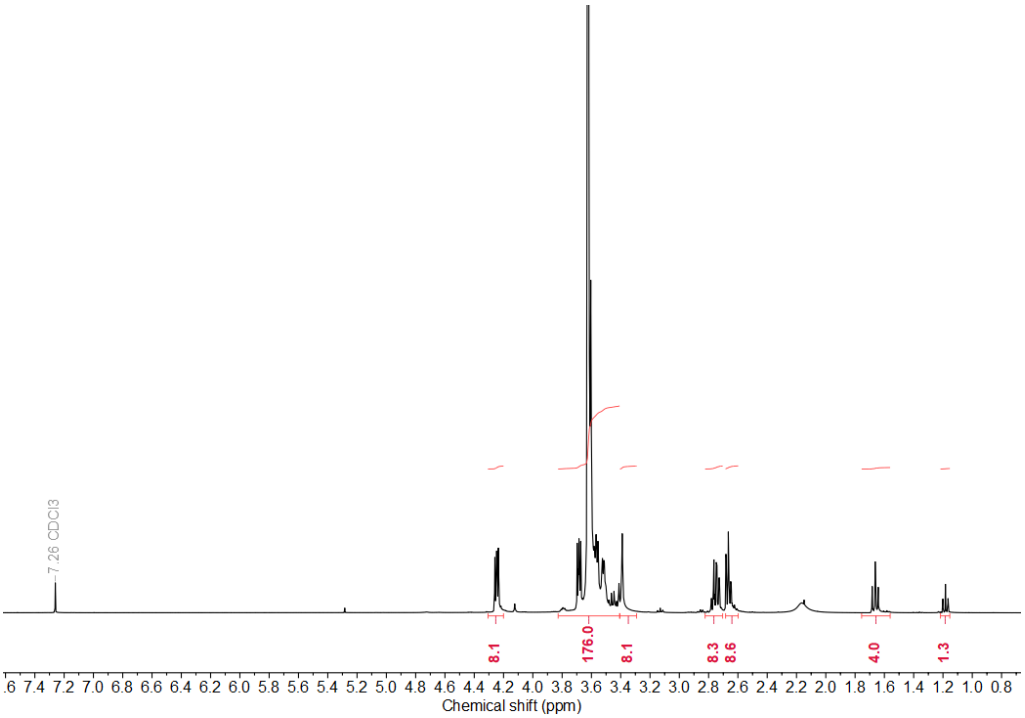


Figure S8. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 4-arm-PEG_{2000}-ester-SH. Successful derivatization with the thiols was confirmed based the peak at 4.26 ppm corresponding to the PEG methylene protons adjacent to the newly formed ester and peaks at 2.80 – 2.71 ppm, 2.69 – 2.60 ppm and 1.65 ppm corresponding to the two methylene groups and the -SH group, respectively from the 3-mercaptopropionic ester.

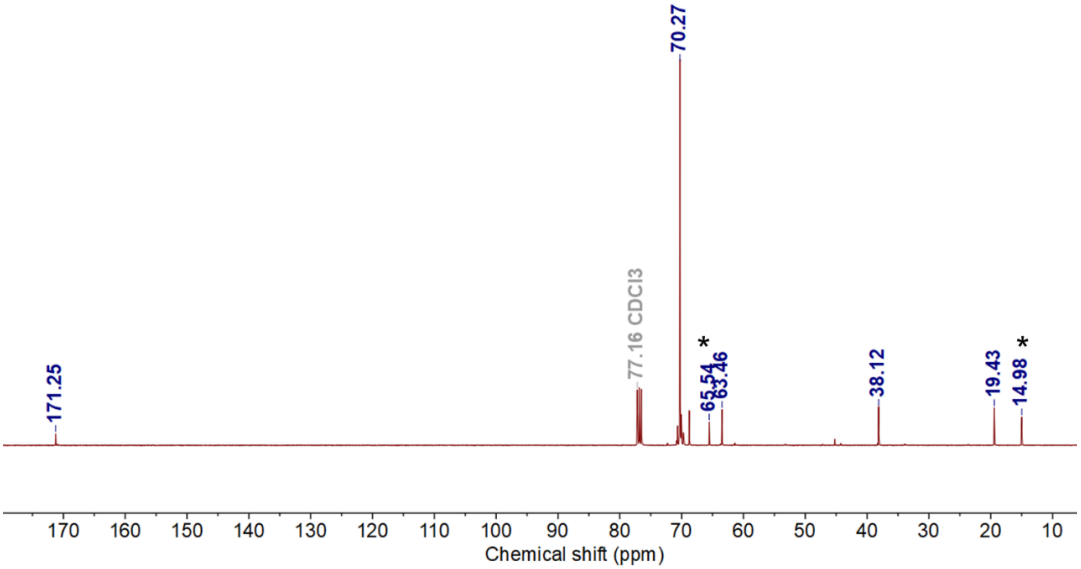


Figure S9. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of 4-arm-PEG_{2000}-ester-SH. * indicates residual diethyl ether. The newly formed ester carbonyl proton appears at 171 ppm.

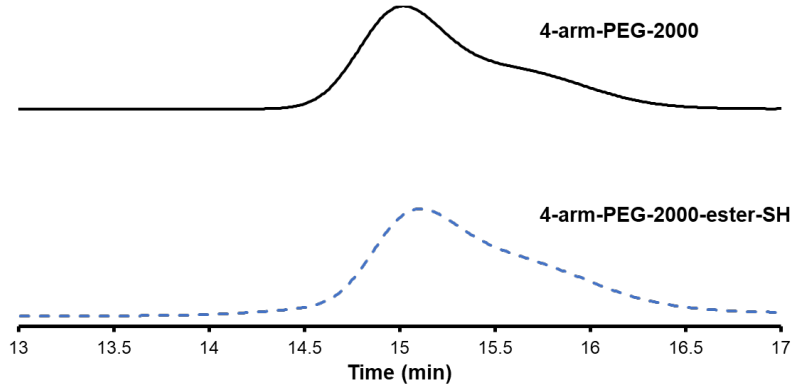


Figure S10. SEC trace for 4-arm-PEG$_{2000}$-ester-SH, compared to the 4-arm-PEG$_{2000}$ starting material, run in DMF.

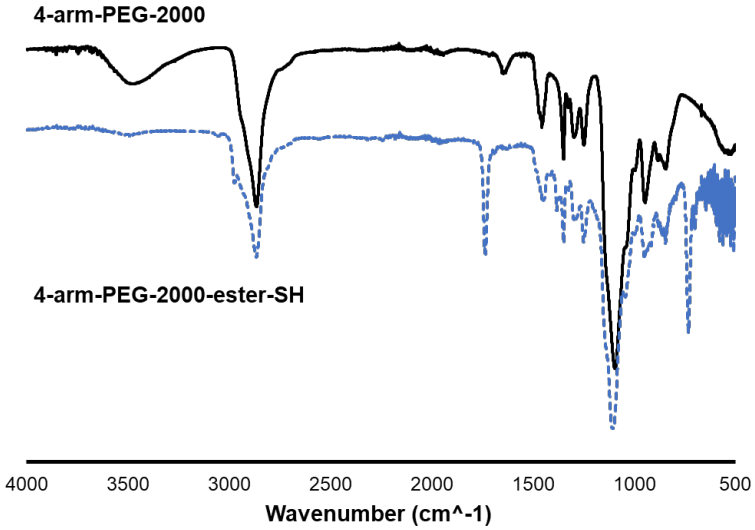


Figure S11. FT-IR trace for 4-arm-PEG$_{2000}$-ester-SH synthesized compared with the starting polymer 4-arm-PEG$_{2000}$. The new peak at ~1730 cm^{-1} corresponds to the carbonyl groups of the newly formed ester bond.

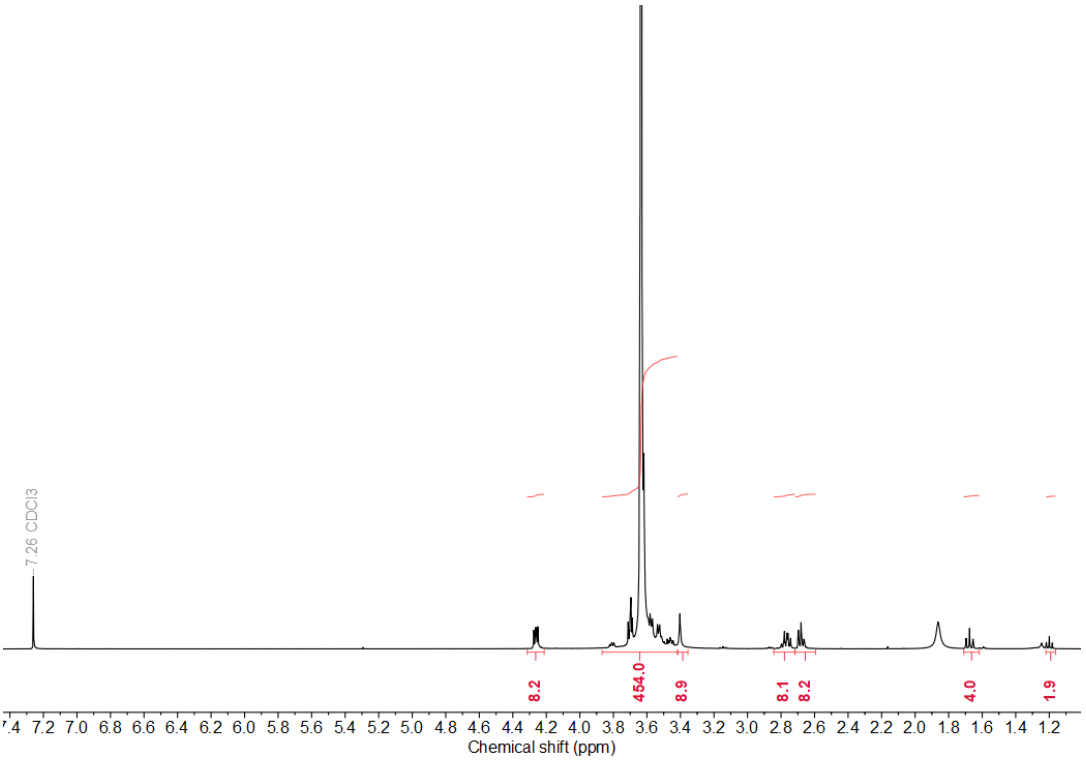


Figure S12. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 4-arm-PEG_{5000}-ester-SH. Successful derivatization with the thiols was confirmed based the peak at 4.26 ppm corresponding to the PEG methylene protons adjacent to the newly formed ester and peaks at 2.80 – 2.71 ppm, 2.69 – 2.60 ppm and 1.65 ppm corresponding to the two methylene groups and the -SH group, respectively from the 3-mercaptopropionic ester.

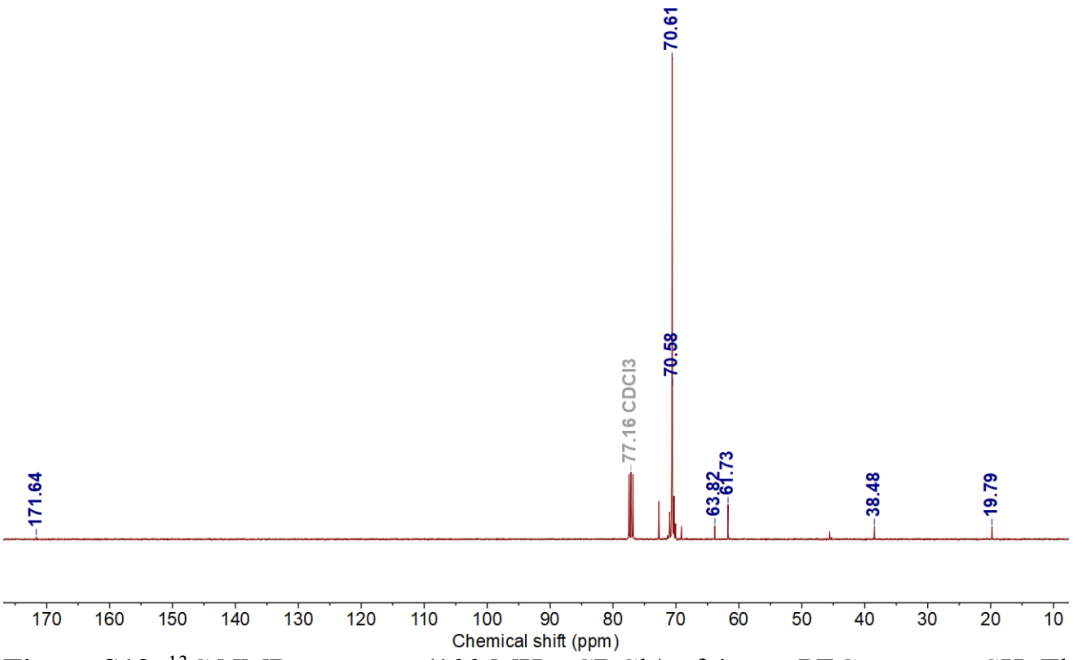


Figure S13. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of 4-arm-PEG_{5000}-ester-SH. The newly formed ester carbonyl proton appears at 171 ppm.

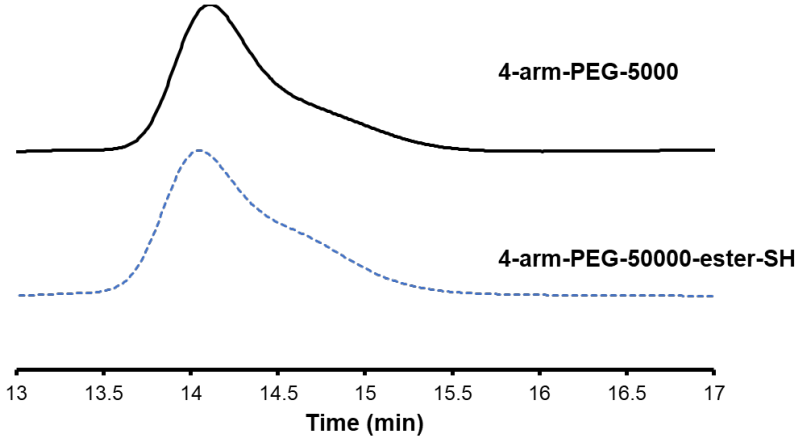


Figure S14. SEC trace for 4-arm-PEG$_{5000}$-ester-SH, compared to the 4-arm-PEG$_{5000}$ starting material, run in DMF.

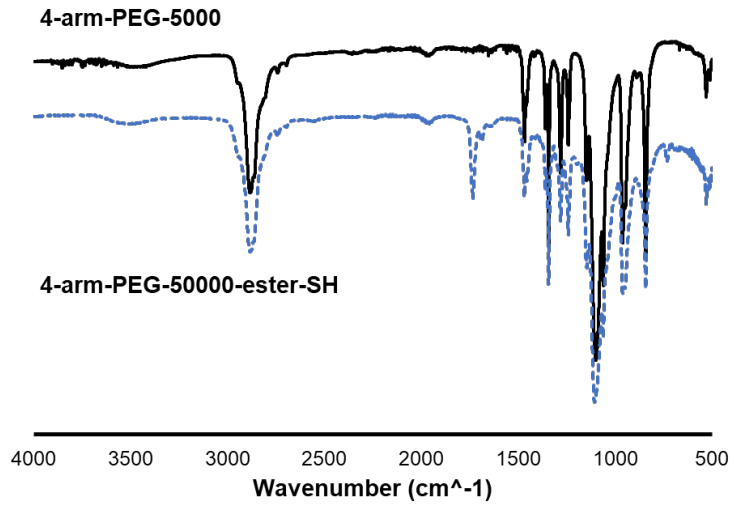


Figure S15. FT-IR spectra of 4-arm-PEG$_{5000}$-ester-SH synthesized compared to the starting polymer 4-arm-PEG$_{5000}$. The new peak at ~1730 cm^{-1} corresponds to the carbonyl groups of the newly formed ester bond.

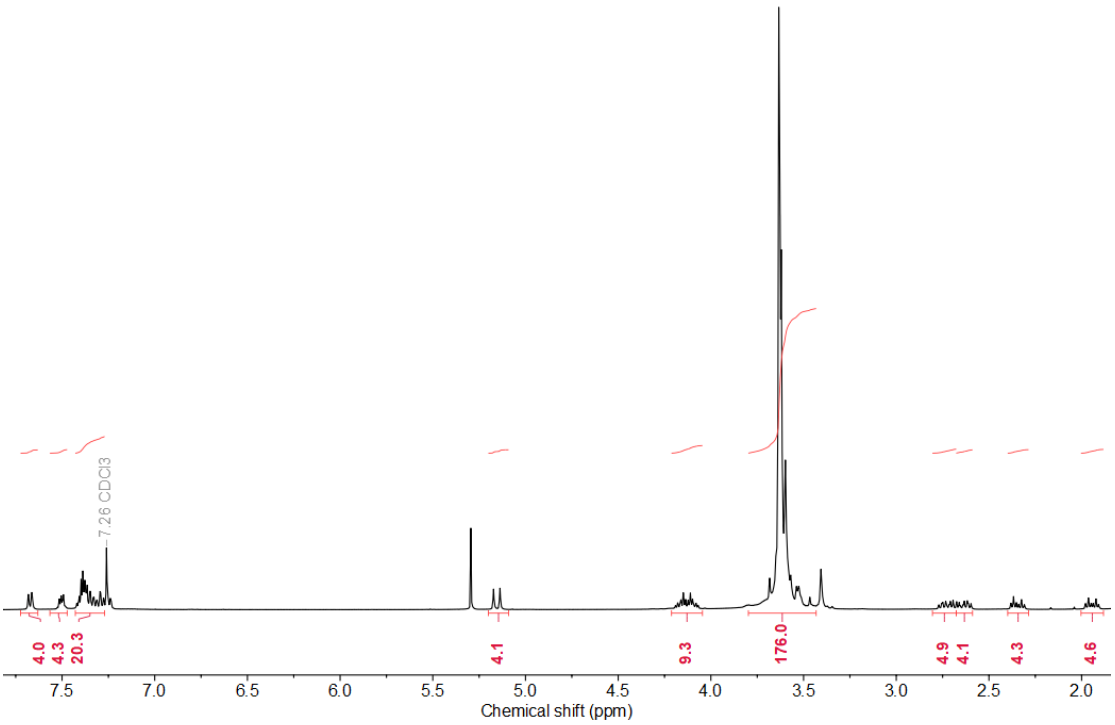


Figure S16. NMR spectrum (400 MHz, $CDCl_3$) of 4-arm-PEG_{2000}-DIBAC. Successful functionalization was confirmed based on the new peaks at 7.67 – 7.27 ppm corresponding to the aromatic protons on DIBAC, peaks at 3.82 – 3.45, 2.79– 2.58, 2.40– 2.28, 1.99 – 1.89 ppm corresponding to methylene protons of DIBAC and the linker, and the peak at 4.26 – 4.02 ppm corresponding to the methylene protons adjacent to the newly formed ester.

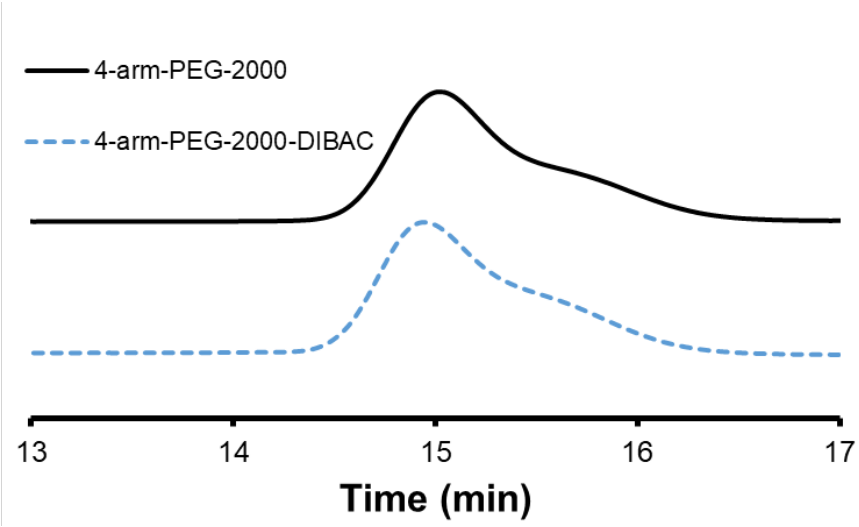


Figure S17. SEC trace for 4-arm-PEG_{2000}-DIBAC, compared to the 4-arm-PEG_{2000} starting material, run in DMF.

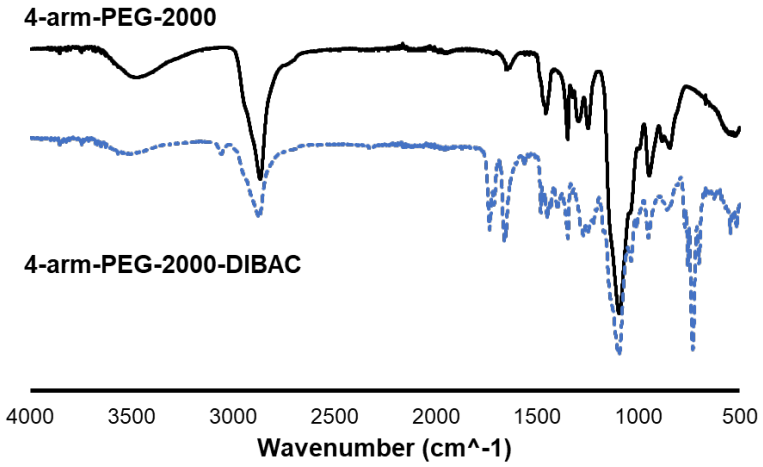


Figure S18. FT-IR spectra of 4-arm-PEG_{2000}-DIBAC. The new peak at 1733 cm^{-1} and 1662 cm^{-1} correspond to the carbonyl group of the newly formed ester bond and the carbonyl group of the amide on the DIBAC group, respectively.

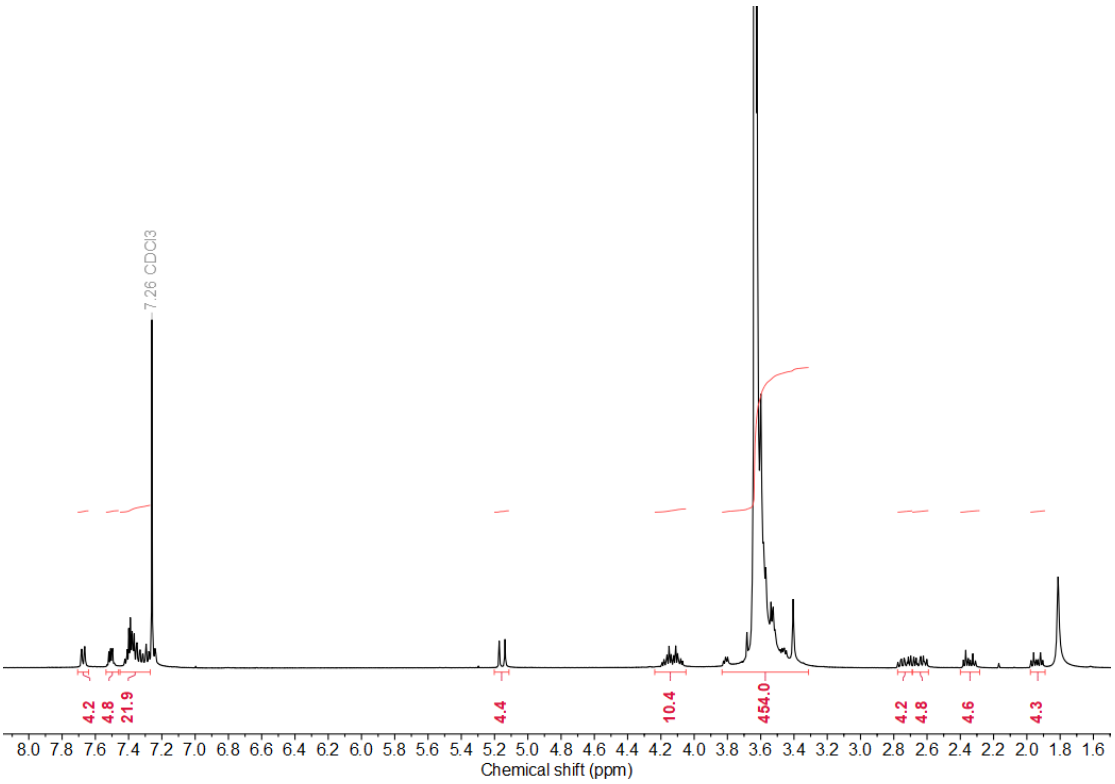


Figure S19. NMR spectrum (400 MHz, $CDCl_3$) of 4-arm-PEG_{5000}-DIBAC synthesized. Successful functionalization was confirmed based on the new peaks at 7.67 – 7.27 ppm corresponding to the aromatic protons on DIBAC, peaks at 3.82 – 3.45, 2.79– 2.58, 2.40– 2.28, 1.99 – 1.89 ppm corresponding to methylene protons of DIBAC and the linker, and the peak at 4.26 – 4.02 ppm corresponding to the methylene protons adjacent to the newly formed ester.

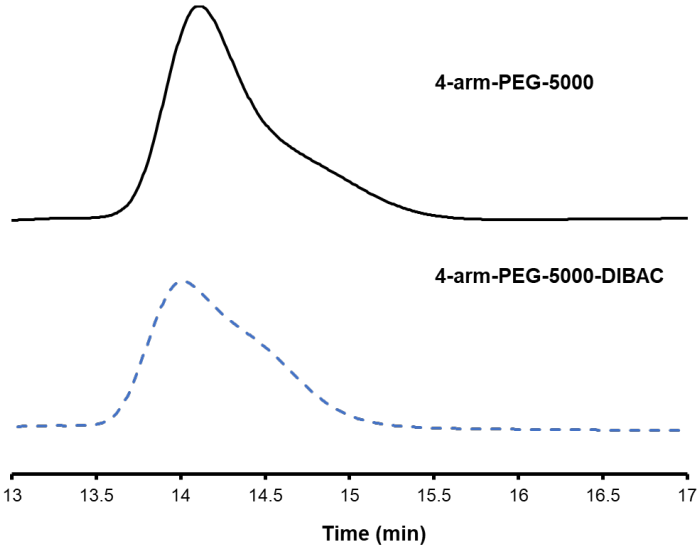


Figure S20. SEC trace for 4-arm-PEG_{5000}-DIBAC, compared to the 4-arm-PEG_{5000} starting material, run in DMF.

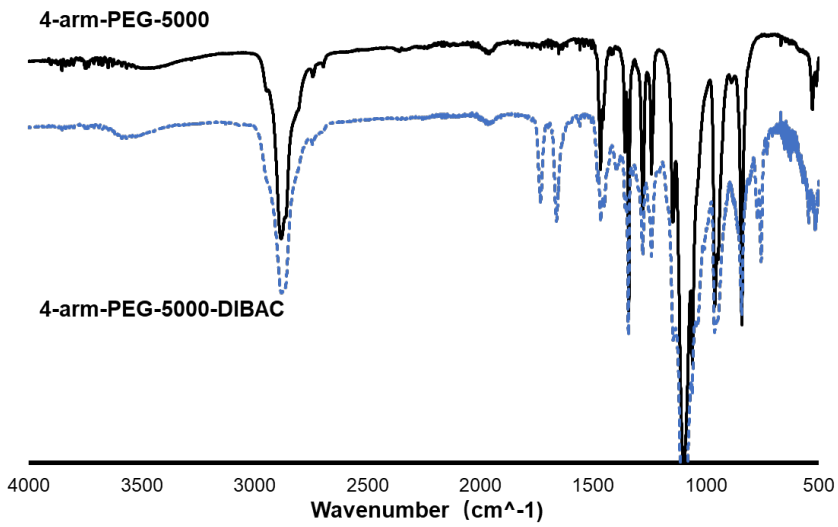


Figure S21. FT-IR spectra of 4-arm-PEG$_{5000}$-DIBAC synthesized compared with the starting polymer 4-arm-PEG$_{5000}$. The new peaks at 1733 cm^{-1} and 1662 cm^{-1} correspond to the carbonyl group of the newly formed ester bond and the carbonyl group of the amide on the DIBAC group, respectively.

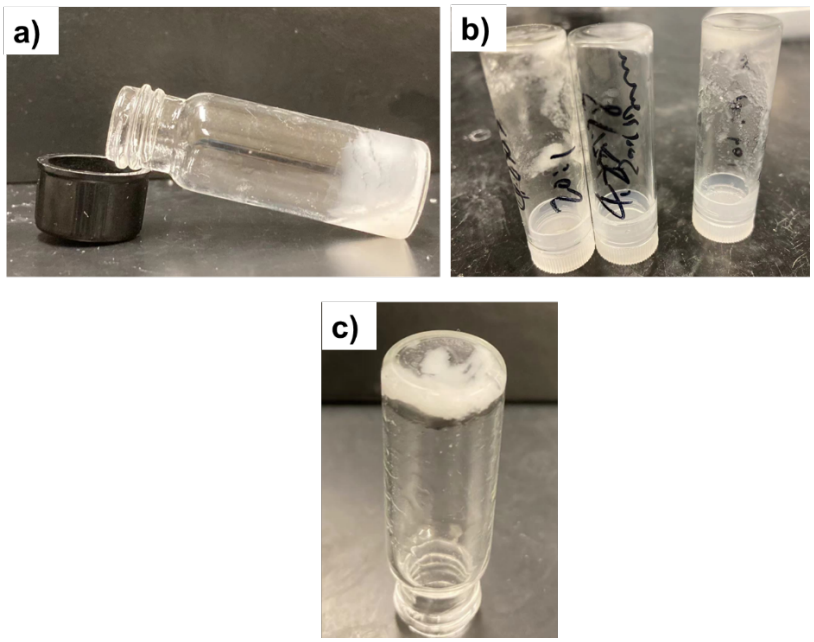


Figure S22. Pictures of the thiol-Michael addition method products: **a)** and **b)** using 4-arm-PEG-5000-ester-SH as the cross-linker with M-PCLA-PEG-PCLA-M; **c)** using 4-arm-PEG-2000-ester-SH as the cross-linker with M-PCLA-PEG-PCLA-M.

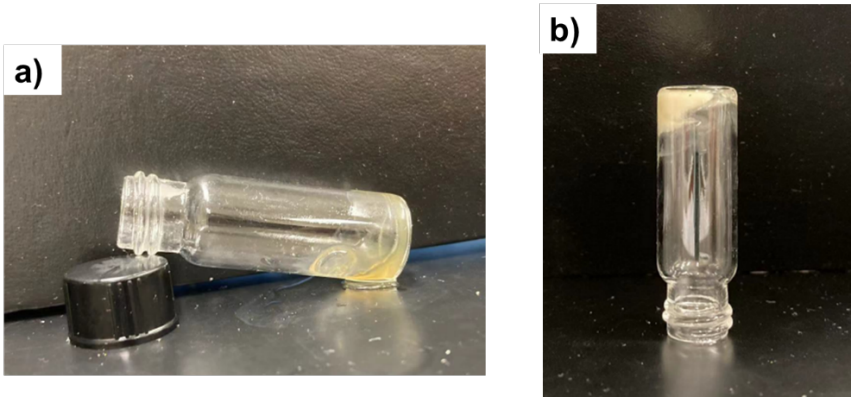


Figure S23. Pictures of the hydrogel prepared by SPAAC reaction of Az-PCLA-PEG-PCLA-Az and 4-arm-PEG_{5000}-DIBAC, **a)** before and **b)** after gelation.

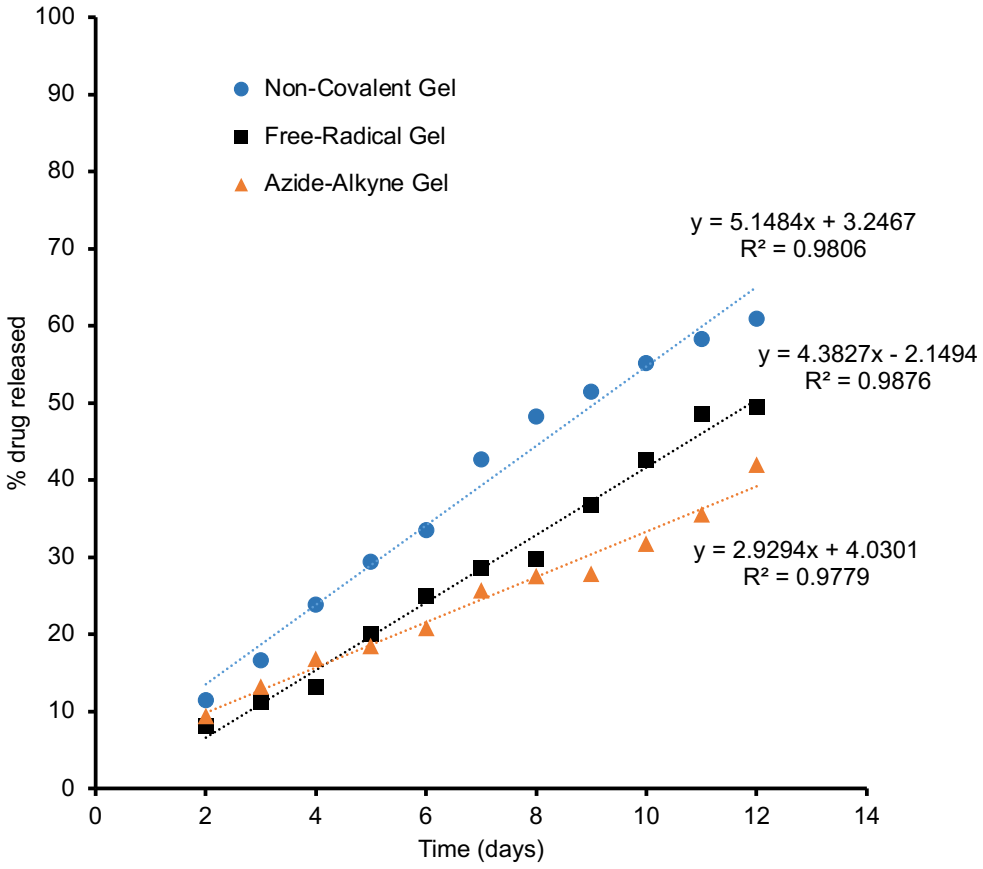


Figure S24. Linear regressions on the ATRA release data from 2 – 12 days.

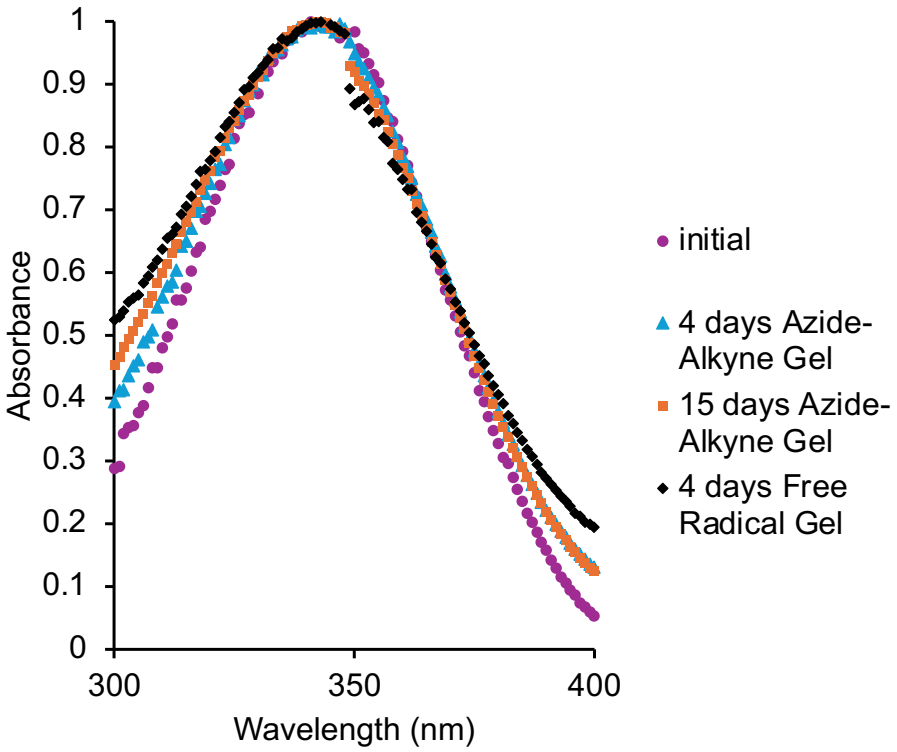


Figure S25. Comparison of normalized UV-visible spectra of initial ATRA from the calibration (not encapsulated and released), released from the Azide-Alkyne Gel at 4 and 15 days, and release from the Free Radical Gel at 4 days. The data shows minimal change in the spectra, indicating that ATRA was chemically intact after release, although the broadening of the absorption peak was largest for ATRA released from the Free Radical Gel, indicating possible ATRA degradation products.

References

1. V. X. Truong, I. Donderwinkel and J. E. Frith, *J. Polym. Sci., Part A: Polym. Chem.*, 2019, **57**, 1872-1876.