Electronic Supporting Information

for

Fluorogenic Atom Transfer Radical Polymerization in Aqueous Media as a Strategy for Detection

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General Methods

All air- and moisture-sensitive reactions were carried out in glassware that was oven-dried (>130°C) and cooled under nitrogen (N_2) gas. Reaction vessels were sealed with rubber septa and maintained in an inert environment under a positive pressure of anhydrous N_2. Stirring was accomplished via magnetic, Teflon-coated stir bars. Solid reagents were measured on a Mettler Toledo MS204TS balance. Air- and moisture-sensitive liquids were transferred via syringe under an atmosphere of N_2. Reaction temperatures refer to the bath temperature in which the reaction vessel was partially immersed. Room temperature indicates an external temperature of 20-25°C. Elevated temperatures were achieved by the use of a mineral oil bath heated by a VWR 620-HPS hot plate/stirrer. Temperatures of 0°C were maintained with ice/water mixtures. The term *in vacuo* refers to the use of a rotary evaporator with an attached vacuum membrane pump. Residual solvents were removed using a Welch vacuum pump held at <1.0 Torr. Analytical thin layer chromatography (TLC) was performed using 0.20 mm glass-backed silica gel 60F254-coated plates from Sigma Aldrich and monitored at 254 nm.

Materials

Unless otherwise noted, all commercial solvents and reagents were purchased from Sigma-Aldrich USA and used as received. Methacryloyl chloride was freshly distilled prior to use. Oligo(ethylene oxide) methyl ether methacrylate (**5**, 99%, average molecular weight 500) was passed over a column of basic alumina (Sigma-Aldrich USA) prior to use. Water, *N,N*-dimethylformamide (DMF, anhydrous), methylene chloride (CH_2Cl_2, anhydrous, 99.8%) and tetrahydrofuran (THF, anhydrous, 99.8%) were used as received. Ethyl ether, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), methanol (MeOH), toluene, hexanes, various acids and bases, and sodium sulfate were purchased from Sigma-Aldrich USA and used as received. Silica gel was purchased from Silicycle, Inc. Deuterated solvents were purchased from Sigma-Aldrich, Inc. ($CDCl_3$ and DMSO-d6 with 0.03% v/v TMS, also Methanol-d4) or from Cambridge Isotopes (D_2O). Sera-Mag Magnetic Streptavidin

Microparticles (Streptavidin-coated beads) were ordered from Thermo Scientific and washed with H_2O prior to use.

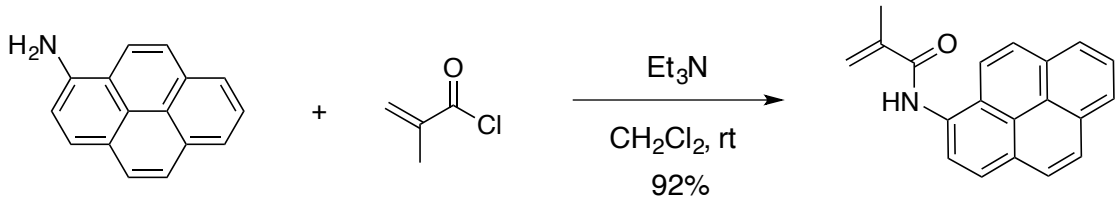
Physical and Spectroscopic Measurements

Nuclear magnetic resonance (NMR) spectra were collected on a Varian Inova 500 (^{1}H at 500 MHz, ^{13}C at 125 MHz) magnetic resonance spectrometer. Data for ^{1}H NMR spectra are reported as follows: chemical shift, multiplicity (bs = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet and m = multiplet), coupling constant (Hz) and integration. Chemical shifts are reported in ppm (δ). NMR spectra were referenced to residual solvent peaks or trimethylsilane (TMS) additives.[1]

Fluorescence spectra and intensities were determined with a Photon Technology International Quanta-Master model QM-1 fluorimeter with 2 mm slit width. Samples were run in quartz cuvettes (4 clear walls, 10 mm with screw cap and septum) purchased from Science Outlet. Infrared spectra were measured on a Thermo Nicolet Nexus 470 Fourier transform spectrometer (FTIR) equipped with an attenuated total reflection (ATR) unit. All IR peaks are reported in wavenumbers (cm^{-1}). Gel permeation chromatography (GPC) was performed in chloroform at a flow rate of 0.5 mL/min on a Waters chromatograph equipped with a Waters 515 pump and one Waters Styragel HR4 THF column connected in series. A Wyatt DAWN Heleos-II 18 angle light scattering detector was used to measure light scattering and a Wayatt Optilab T-Rex was used to measure the change in refractive index. High resolution mass spectrometry (HRMS) was performed at the University of Texas San Antonio mass spectrometry core with a Bruker microTOFMS or at Trinity University with an Agilent accurate mass TOF LC/MS. Elemental analysis was performed by Midwest Microlabs. Pictures of polymerization reactions were taken with iPhone cameras and illuminated by long-wave (365 nm) ultraviolet (UV) light using a hand-held UV lamp (UVP compact).

Fluorogenic Monomer and Biotinylated Initiator Synthesis and Characterization

Synthesis of N-(1-pyrene)methacrylamide monomer ***Py 1***



1-aminopyrene | methacryloyl chloride | **Py 1**

A solution of freshly distilled methacryloyl chloride (0.60 mL, 5.5 mmol) and triethylamine (0.42 mL, 5.5 mmol) in 5 mL of CH_2Cl_2 was added dropwise to a second solution of 1-aminopyrene (1.00 g, 4.60 mmol) in 20 mL of CH_2Cl_2. Yellow triethylammonium chloride precipitated from the solution as it was stirred for one hour at ambient temperature. Hydrochloric acid (10%, 50 mL) was added to the reaction mixture followed by water (100 mL) and CH_2Cl_2 (50 mL). The white precipitate dissolved into the organic layer after the mixture was shaken. The organic layer was removed and extracted with water (3 x 50 mL). Each aqueous layer was washed with CH_2Cl_2 (50 mL) two times and the organic layers were combined and dried with sodium sulfate. The solution was filtered and the solvent removed *in vacuo* to yield 1.28 g of a pale yellow crude solid. The crude solid was purified by column chromatography with silica gel (CH_2Cl_2:hexanes) and product containing fractions were combined and concentrated *in vacuo* to yield N-(1-pyrene)methacrylamide monomer **Py 1** (1.19 g, 92%) as a gold colored solid. The compound was chromatographically homogeneous (one spot) by TLC.

Data for Py 1:

mp = 171.9-175.3°C

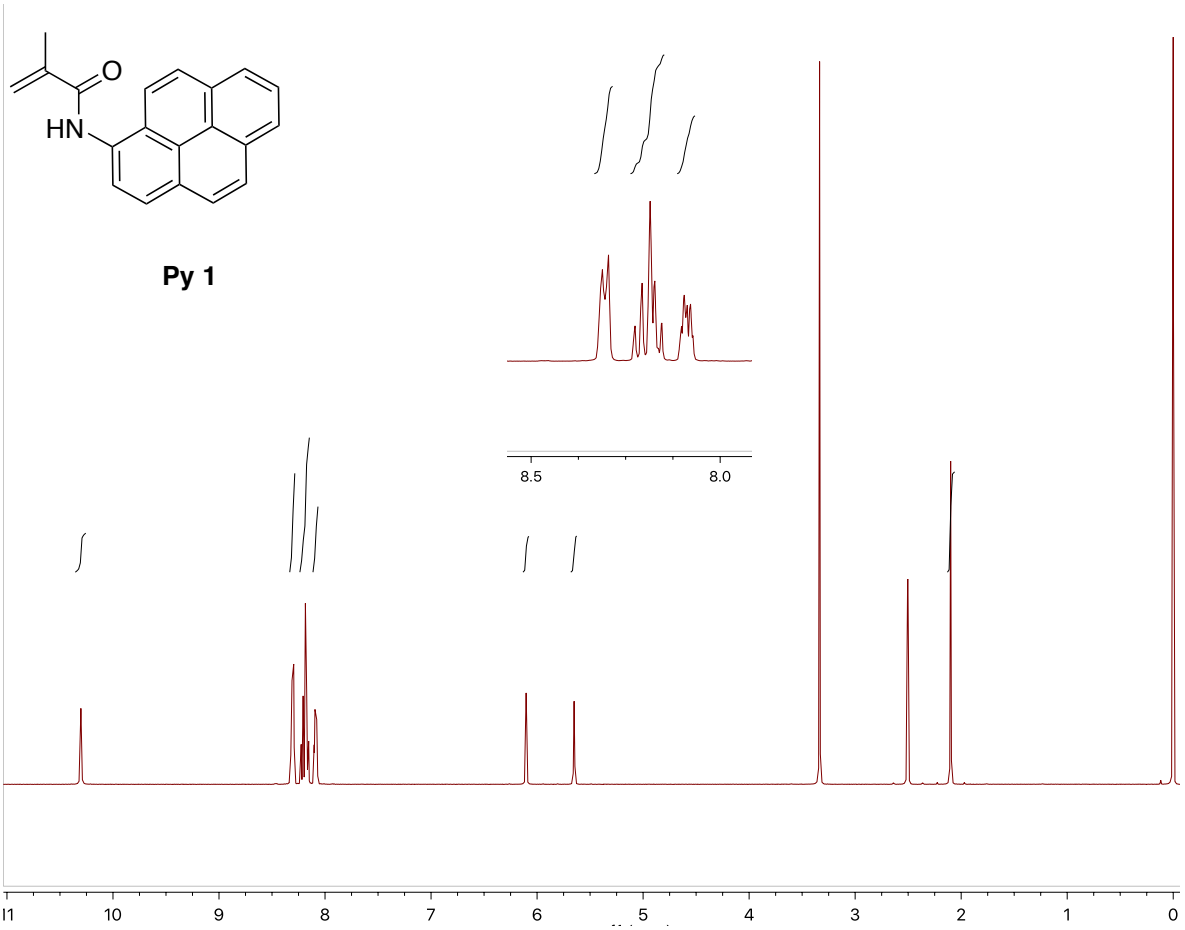
TLC: R_f = 0.28 (9:1 CH_2Cl_2:hexanes), one spot by UV.

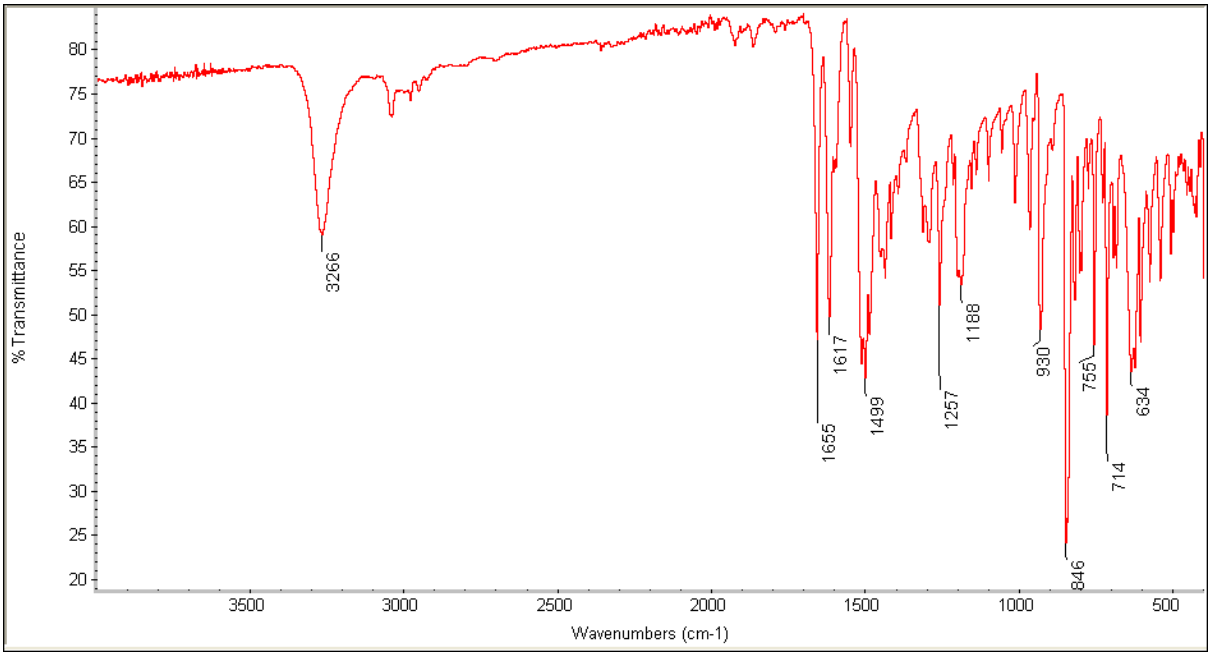
^{1}H NMR (500 MHz, C_2D_6OS): δ = 10.30 (s, 1 H), 8.30 (d, *J* = 8.50 Hz, 3 H), 8.18 (m, 4 H), 8.09 (m, 2 H) 6.10 (s, 1 H), 5.65 (s, 1 H), 2.10 (s, 3 H) ppm.

^{13}C NMR (125 MHz, C_2D_6OS): δ = 167.5, 140.0, 131.8, 130.8, 130.5, 128.8, 127.2, 127.1, 126.9, 126.4, 125.5, 125.3, 125.0, 124.9, 124.3, 123.8, 122.9, 120.6, 18.9 ppm.

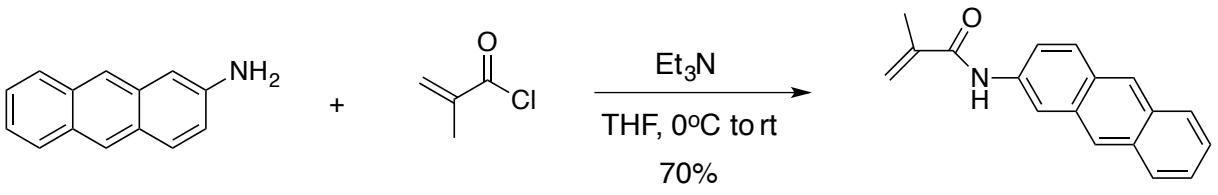
FT-IR: ν = 3266, 1655, 1617, 1499, 1257, 1188, 930, 846, 755, 714, 634 cm^{-1}.

HRMS (*m/z*): [M + H^+] calculated for $C_{20}H_{15}NO$, 286.1226; found, 286.1226; mass accuracy, 0.00 ppm.





Synthesis of N-(2-anthracene)methacrylamide ***An 2***



2-aminoanthracene | methacryloyl chloride | **An 2**

Triethylamine (Et_3N, 0.72 mL, 9.4 mmol) and 2-aminoanthracene (1.0 g, 5.17 mmol) were dissolved in 10 mL of THF and purged with N_2. A solution of freshly distilled methacryloyl chloride (0.60 mL, 5.5 mmol) dissolved in 5 mL THF was added dropwise at 0°C. After one hour the reaction was allowed to warm to room temperature and stirred for 24 hours while yellow triethylammonium chloride precipitated from the solution. The solvent was evaporated under reduced pressure. The crude solid was purified by column chromatography with silica gel (CH_2Cl_2:hexanes) and product containing fractions were combined and concentrated *in vacuo* to yield N-(2-anthracene)methacrylamide monomer **An 2** (0.95 g, 70%) as a golden brown solid. The compound was chromatographically homogeneous (one spot) by TLC.

Data for An 2:

mp = 241.2-244.3°C

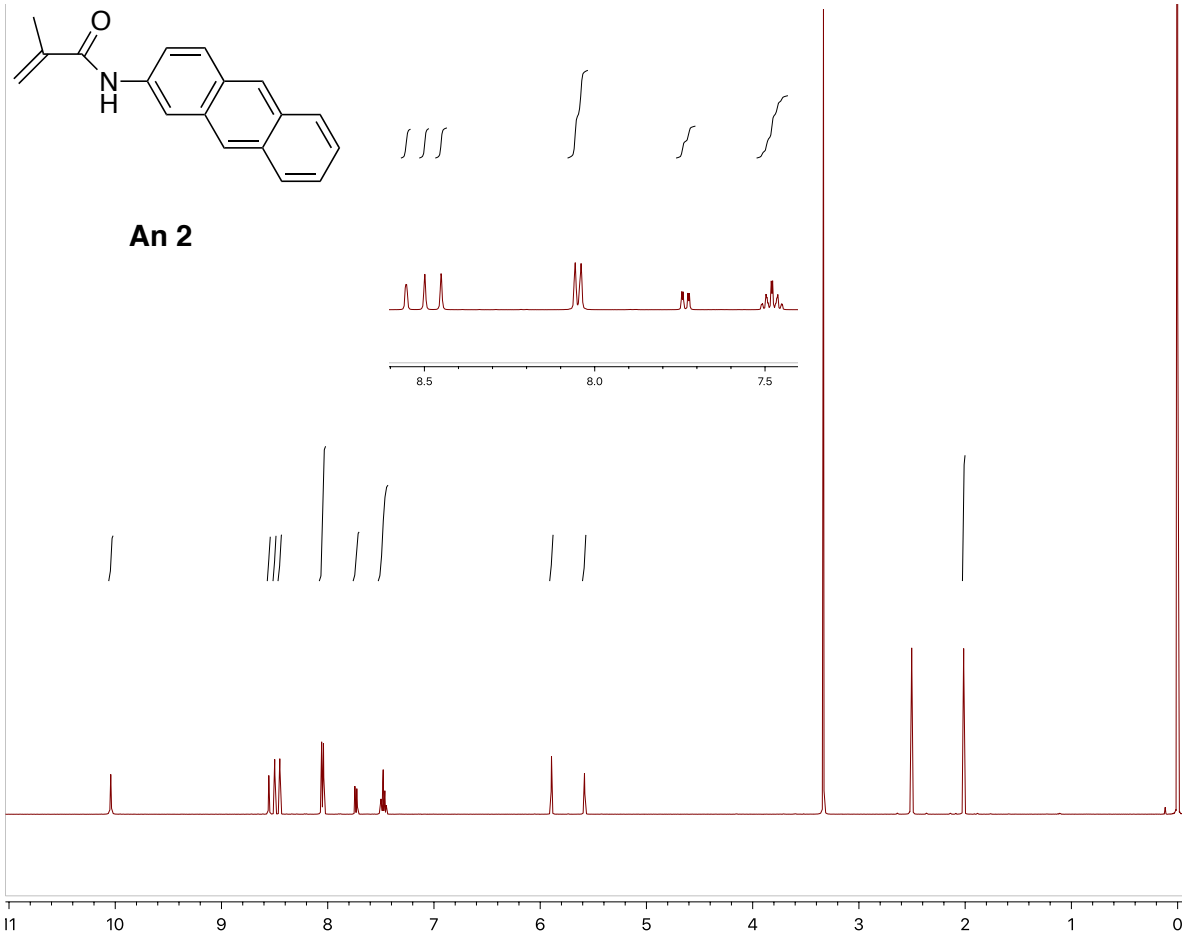
TLC: R_f = 0.40 (9:1 CH_2Cl_2:hexanes), one spot by UV.

^{1}H NMR (500 MHz, C_2D_6OS): δ = 10.02 (s, 1 H), 8.54 (s, 1 H), 8.49 (s, 1 H), 8.44 (s, 1 H), 8.04 (d, *J* = 8.65 Hz, 3 H), 7.73 (dd, *J* = 2.9 Hz, 1 H), 7.47 (m, 2 H), 5.89 (s, 1 H), 5.58 (s, 1 H), 2.01 (s, 3 H) ppm.

^{13}C NMR (500 MHz, C_2D_6OS): δ = 140.4, 136.1, 131.7, 131.5, 130.5, 128.6, 128.5, 128.1, 128.1, 127.7, 125.8, 125.1, 125.0, 121.8, 120.2, 115.0, 18.8 ppm.

FT-IR: ν = 3254, 1654, 1619, 1561, 1543, 1428, 1296, 1260, 1220, 1183, 977, 955, 910, 891, 872, 811, 735, 615, 470 cm^{-1}.

HRMS (*m/z*): [M + H^+] calculated for $C_{18}H_{15}NO$, 262.1226; found, 262.1226; mass accuracy, 0.00 ppm.


O
N
H
An 2
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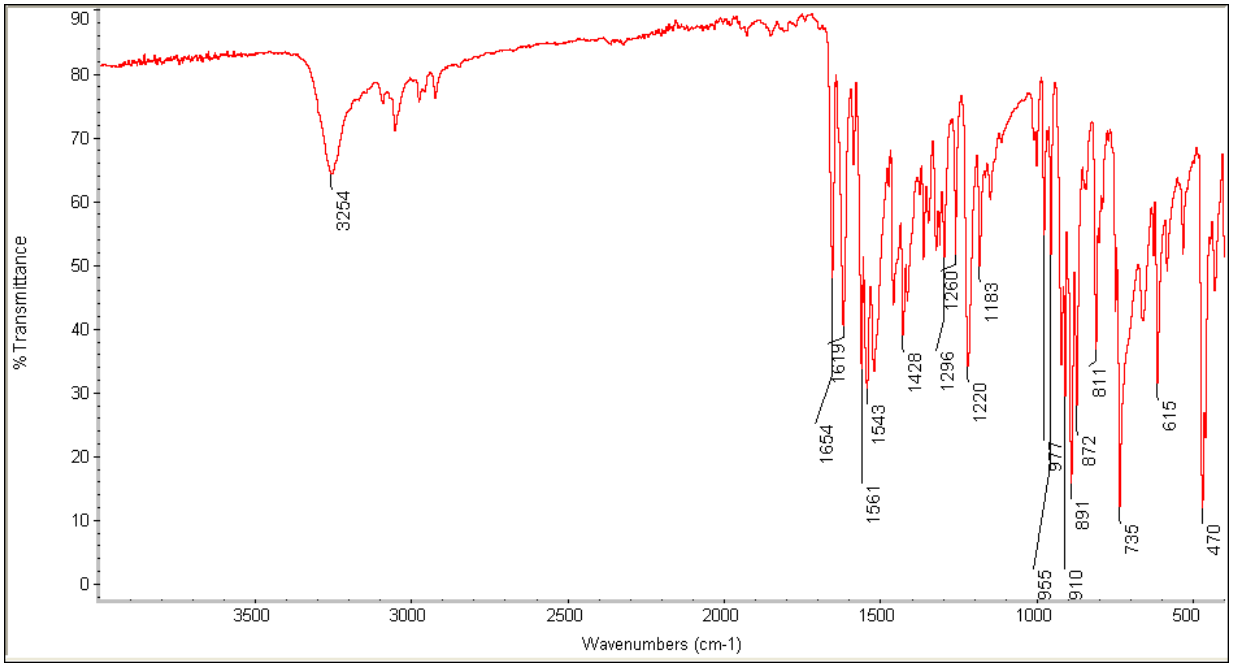
8.0
7.5
11
10
9
8
7
6
5
4
3
2
1
0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1619
1561
1543
1428
1296
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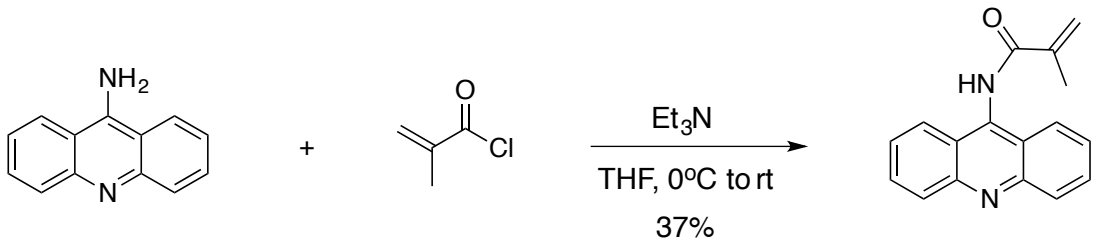
891
872
811
735
615
470

Synthesis of N-(9-acridine)methacrylamide ***Ac 3***



9-aminoacridine | methacryloyl chloride | **Ac 3**

Triethylamine (Et_3N, 0.42 mL, 5.5 mmol) and 9-aminoacridine (1.0 g, 5.17 mmol) were dissolved in 10 mL of THF and purged with N_2. A solution of freshly distilled methacryloyl chloride (0.60 mL, 5.5 mmol) dissolved in 5 mL THF was added dropwise at 0°C. After one hour the reaction was allowed to warm to room temperature and stirred for 24 hours while yellow triethylammonium chloride precipitated from the solution. The solvent was evaporated under reduced pressure. The crude solid was purified by column chromatography with silica gel (3:1 CH_2Cl_2:EtOAc, 1% Et_3N) and product containing fractions were combined and concentrated *in vacuo* to yield N-(9-acridine)methacrylamide monomer **Ac 3** (0.50 g, 37%) as a bright yellow solid. The compound was chromatographically homogeneous (one spot) by TLC.

Data for Ac 3:

mp = 171.8-175.6°C

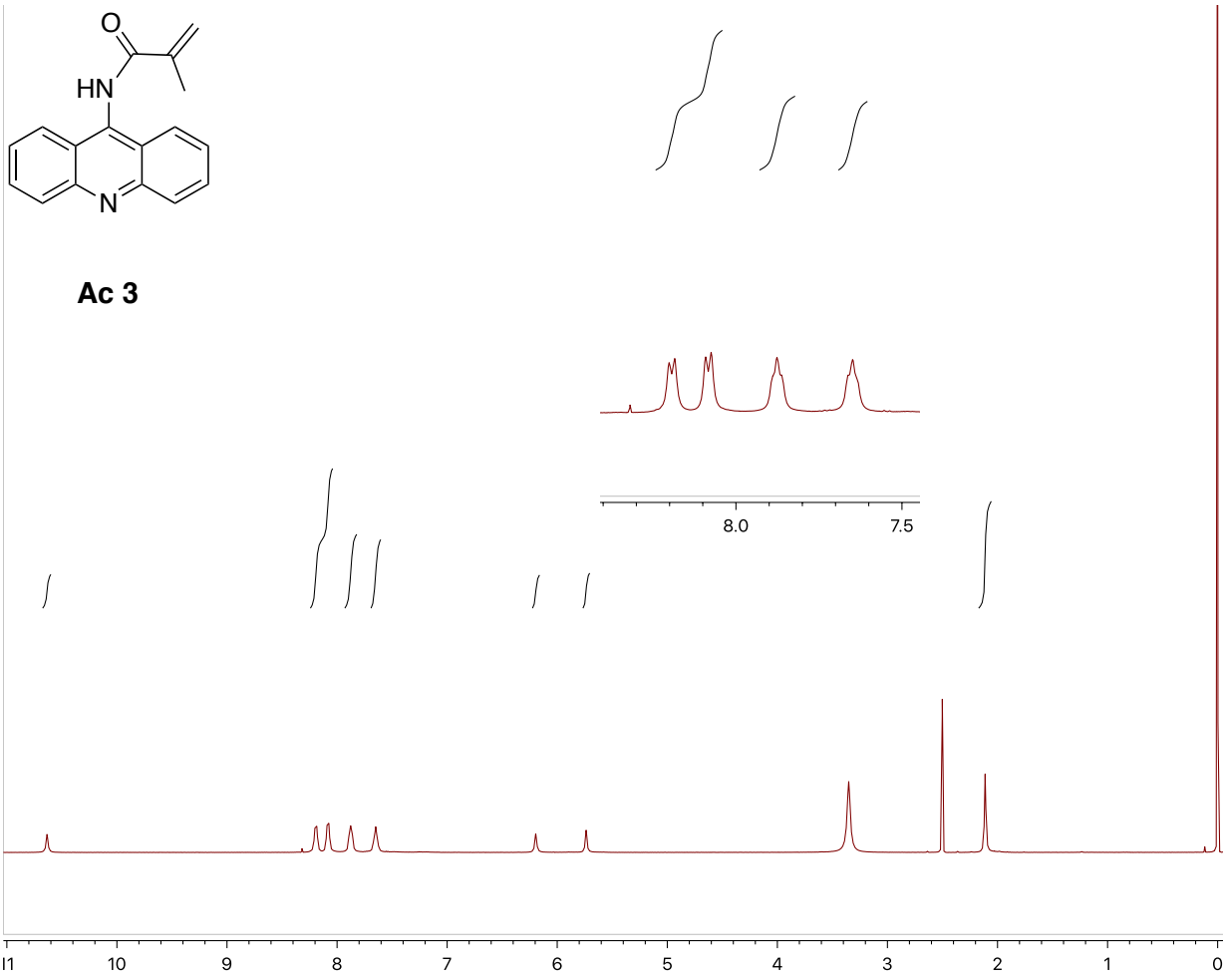
TLC: R_f = 0.42, (3:1 CH_2Cl_2:EtOAc, 1% Et_3N) one spot by UV.

^{1}H NMR (500 MHz, C_2D_6OS): δ = 10.63 (s, 1 H), 8.19 (d, *J* = 8.0, 2 H), 8.08 (d, *J* = 8.0, 2 H), 7.88 (t, *J* = 7.0 Hz, 2 H), 7.65 (t, *J* = 6.5 Hz, 2 H), 6.20 (s, 1 H), 5.74 (s, 1 H), 2.11 (s, 3 H) ppm.

^{13}C NMR (500 MHz, C_2D_6OS): δ = 167.8, 148.8, 140.6, 139.4, 130.5, 129.2, 126.1, 124.4, 123.0, 121.4, 18.8 ppm.

FT-IR: ν = 3213, 1655, 1622, 1567, 1484, 1416, 1315, 1178, 1143, 944, 902, 801, 749, 635, 595, 575, 553, 459 cm^{-1}.

HRMS (*m/z*): [M + H^+] calculated for $C_{17}H_{14}N_2O$, 263.1179; found, 263.1179; mass accuracy, 0.00 ppm.


O
HN
N
Ac 3
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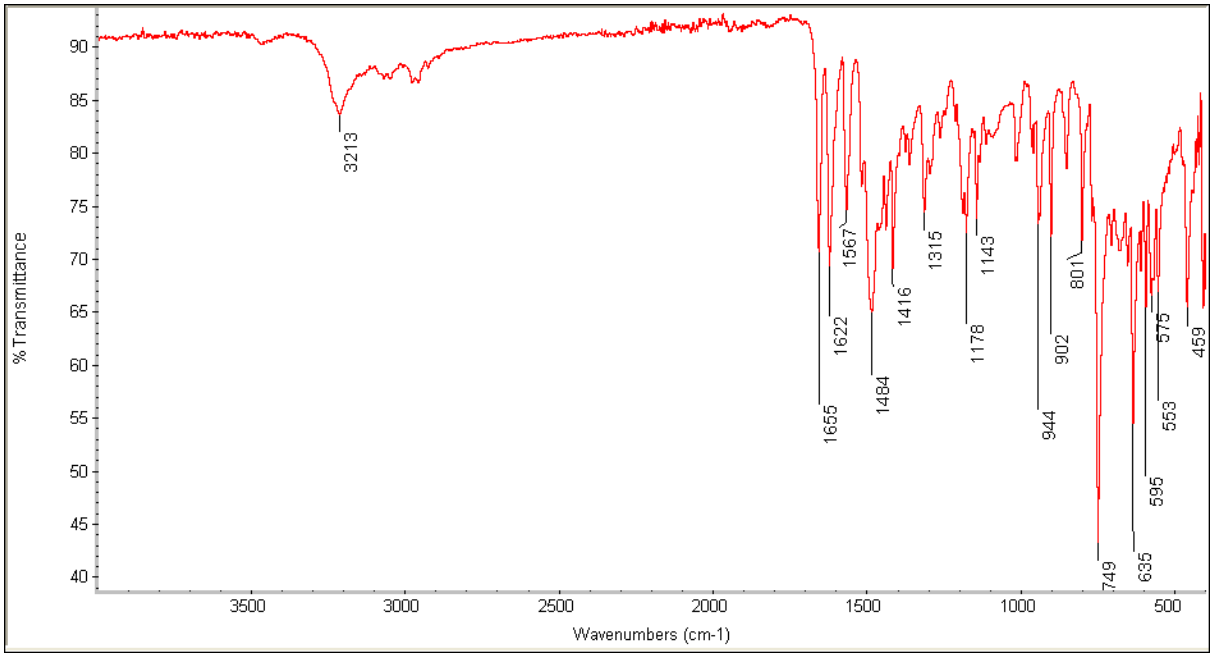
7.5
11
10
9
8
7
6
5
4
3
2
1
0


% Transmittance
Wavenumbers (cm-1)
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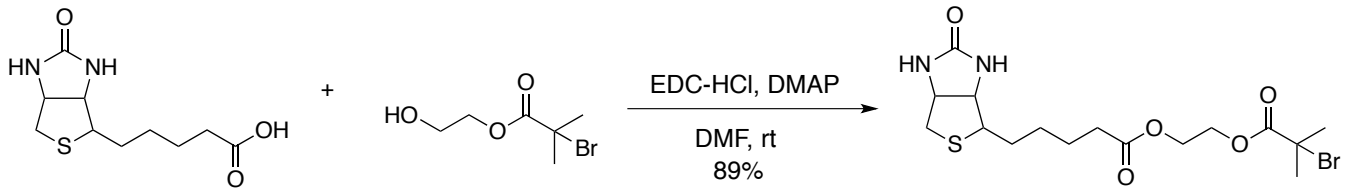
1655
1622
1567
1484
1416
1315
1178
1143
944
902
801
749
635
595
553
575
459

*Synthesis of Biotinylated Initiator **7***



Biotin **4** **7**

To an oven-dried flask equipped with a stir bar and purged under N_2 was added biotin (700 mg, 2.87 mmol), 2-hydroxyethyl-2-bromoisobutyrate **4** (456.5 μL, 3.15 mmol), *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC-HCl, 700 mg, 3.65 mmol), and 4-(Dimethylamino)pyridine (DMAP, 51.5 mg, 0.4 mmol). The reaction mixture was dissolved in 50 mL of DMF and stirred overnight at room temperature. After 12 h, the solvent was removed *in vacuo*. The residue was purified by column chromatography with silica gel (9:1 CH_2Cl_2:MeOH) and product containing fractions were combined and concentrated *in vacuo* to yield biotinylated initiator ***7*** (1.11 g, 89%) as a pale yellow oil. The compound was chromatographically homogeneous (one spot) by TLC.

Data for 7:

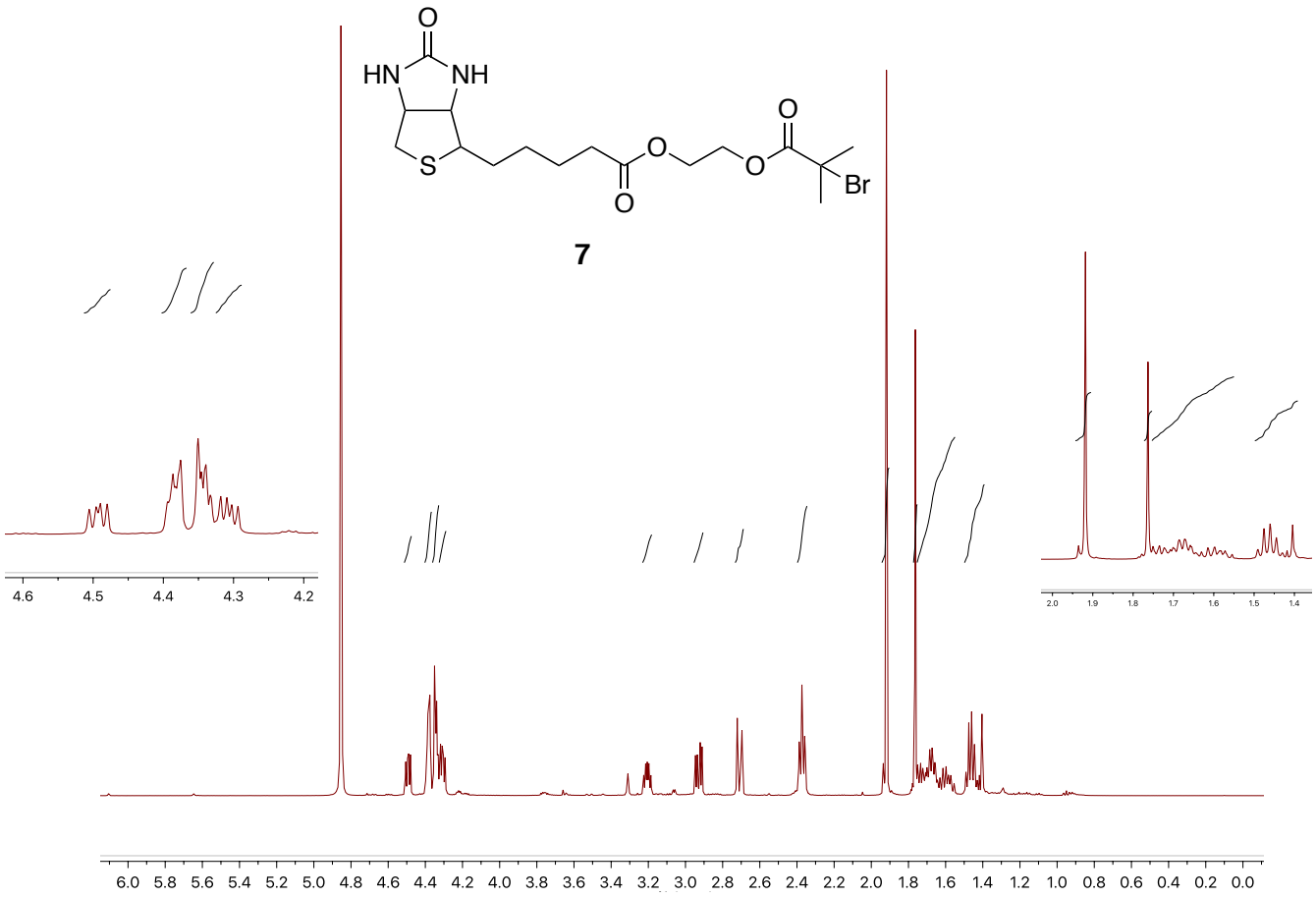
TLC: R_f = 0.32 (9:1 CH_2Cl_2:MeOH), one spot by potassium permanganate stain.

^{1}H NMR (500 MHz, CD_3OD): δ = 4.51-4.48 (m, 1 H), 4.40-4.38 (m, 2 H), 4.39-4.34 (m, 2 H), 4.31 (q, *J* = 3.5, 1 H), 3.21 (quin, 1 H), 2.93 (q, *J* = 8, 1 H), 2.71 (d, *J* = 12.5, 1 H), 2.38 (t, *J* = 7.5, 2 H), 1.94 (s, 1 H), 1.93 (s, 4 H), 1.77 (s, 1 H), 1.76-1.56 (m, 4 H), 1.47 (quin, 2 H) ppm.

^{13}C NMR (500 MHz, CD_3OD): δ = 174.9, 172.8, 166.1, 64.9, 63.4, 63.0, 61.6, 57.0, 41.0, 34.7, 31.1, 30.0, 29.7, 29.5, 25.9 ppm.

FT-IR: ν = 3251, 2931, 1732, 1687, 1459, 1371, 1333, 1270, 1158, 1111, 1077, 952, 869, 761, 685, 535, 430 cm^{-1}.

HRMS (*m/z*): [M + H^+] calculated for $C_{16}H_{25}BrN_2O_5S$, 437.0740; found, 437.0735; mass accuracy, 1.14 ppm.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O
O
O
O
Br
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4.5
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4.3
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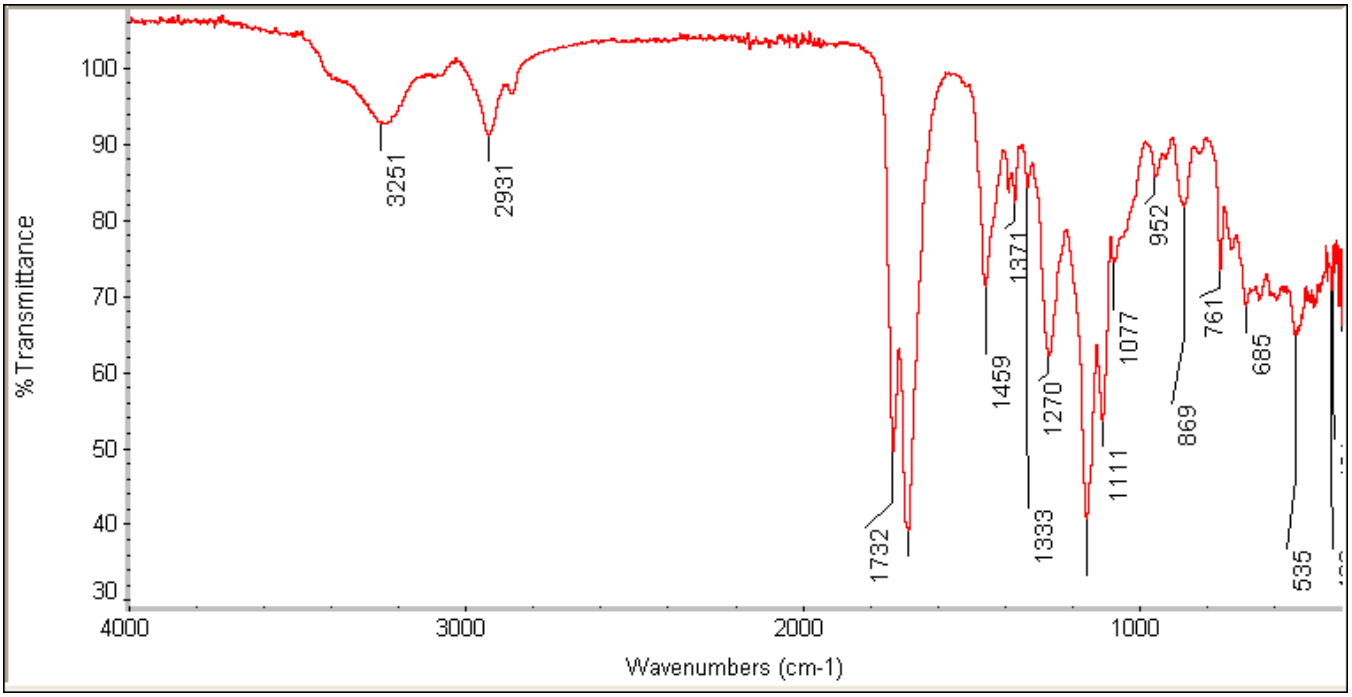
1.8
1.7
1.6
1.5
1.4
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0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1077
952
869
761
685
535
4000
3000
2000
1000
Wavenumbers (cm-1)

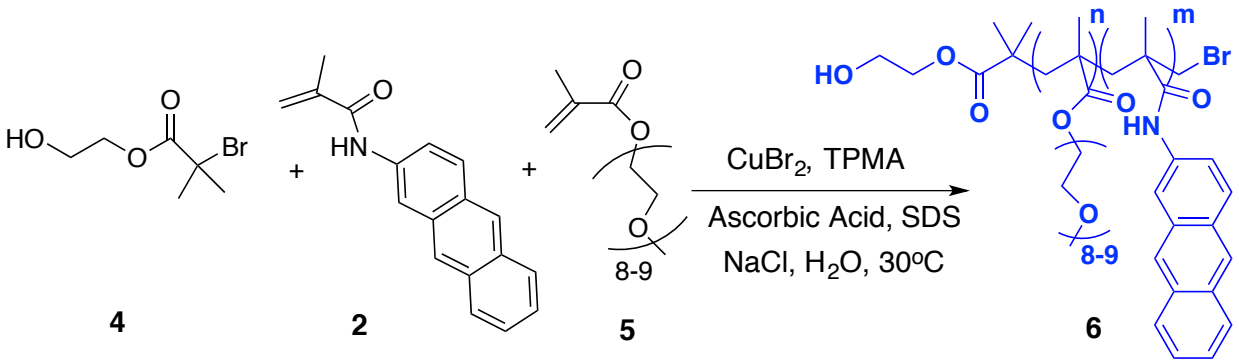
Polymerization Procedures and Characterization

General Fluorogenic Co-Polymerization ATRP Procedure

PEG methacrylate **5** (0.7043 g, 1.5 mmol), NaCl (17.4 mg, 0.3 mmol), 100 mM stock solution of 2-hydroxyethylbromoisobutyrate initiator **4** in water (0.060 mL, 0.006 mmol), stock solution of 25 mM $CuBr_2$ and 200 mM TPMA in water (6 μL, 0.15 μmol of $CuBr_2$ and 1.2 μmol of TPMA) and 5 mM stock solution of fluorogenic methacrylamide monomer (**Py 1**, **An 2** or **Ac 3**) in water with 366 mM SDS (0.300 mL, 0.150 mmol monomer, 0.102 mmol SDS) were dissolved in H_2O (2.03 mL) in a round-bottom flask. DMF was added (0.03 mL) as an internal standard for monitoring the reaction by ^1H NMR. The flask was sealed and purged with nitrogen bubbling through the solution for 30-60 min, and then the solution was transferred to a sealed quartz cuvette containing a stir bar and placed in a 30°C oil bath under nitrogen. A 16 mM ascorbic acid solution in water was separately purged with nitrogen for 30-60 min, then 0.090 mL (1.4 μmol) was slowly added to the cuvette to start the reaction. At various times, the cuvettes were removed from the oil bath and examined for fluorescence by fluorimeter emission scans and/or photographs after irradiation by long-wave UV light. After 1 h, an additional 0.090 mL (1.4 μmol) of degassed ascorbic acid solution was added and the reaction continued to be monitored for 24 h (or longer in some cases). Modifications to the described procedure were made to increase the scale up to 5x, in which case reactions were run in round-bottom flasks and aliquots periodically removed for fluorescence analysis. Other deviations from the original procedure included varying the amount of fluorogenic probe added (the described procedure is for optimized 1:930 fluorogenic monomer **1-3**:PEG **5** molar ratio), or the amount of SDS or initiator in solution.

General Polymer Isolation Procedure

When desired, the polymerization reaction was quenched by exposure to air and dilution with water (3 mL) and then the polymers were isolated by the following protocol. Approximately 500 mg of KCl was added to precipitate the SDS and the reaction was extracted with CH_2Cl_2 (4 x 15 mL). The combined organic layers were dried with sodium sulfate then filtered and concentrated *in vacuo*. The residue was dissolved in THF and precipitated into diethyl ether, and centrifuged at 4000 rpm for 5 min. The supernatant was decanted and the precipitation procedure repeated two more times. The fluorescent polymer was dried under vacuum overnight and analyzed by ^{1}H NMR, GPC, and elemental analysis.



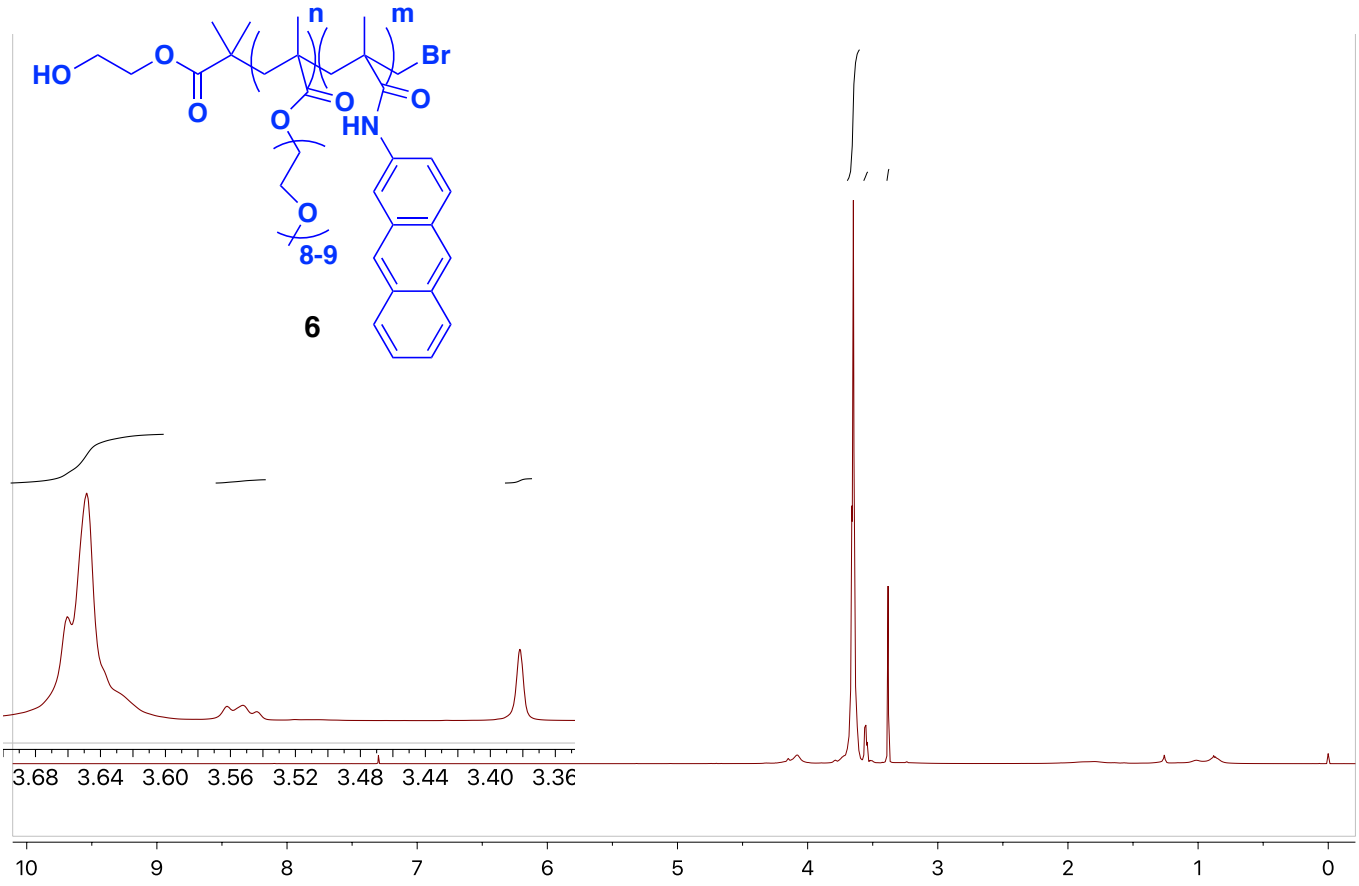
Data for Anthracene:PEG Co-Polymer 6:

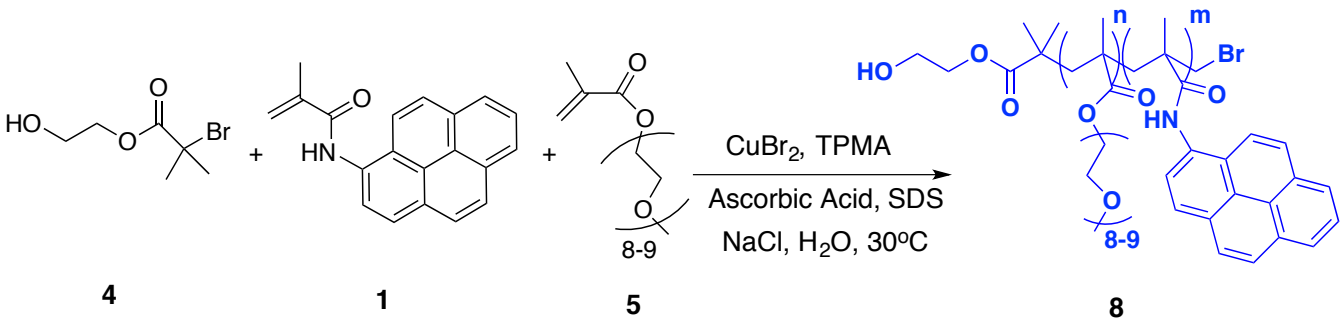
Percent Recovery: 99% (5 days of reaction time, monitored by ^{1}H NMR for full monomer conversion).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.38 (s, 3 H), 3.55 (t, *J* = 4.6, 2 H), 3.61-3.68 (m, 32 H) ppm. (Only PEG peaks visible due to relatively small amount of fluorogenic monomer in the reaction (1:930 **An 2:5** molar ratio).

GPC: M_w = 186,800, M_n = 156,000, PDI (M_w/M_n) = 1.197.

Elemental Analysis: C 51.31%, H 8.04%, N 0.10%, O 36.4% (Detectable nitrogen indicates trace incorporation of fluorogenic monomer **An 2**).





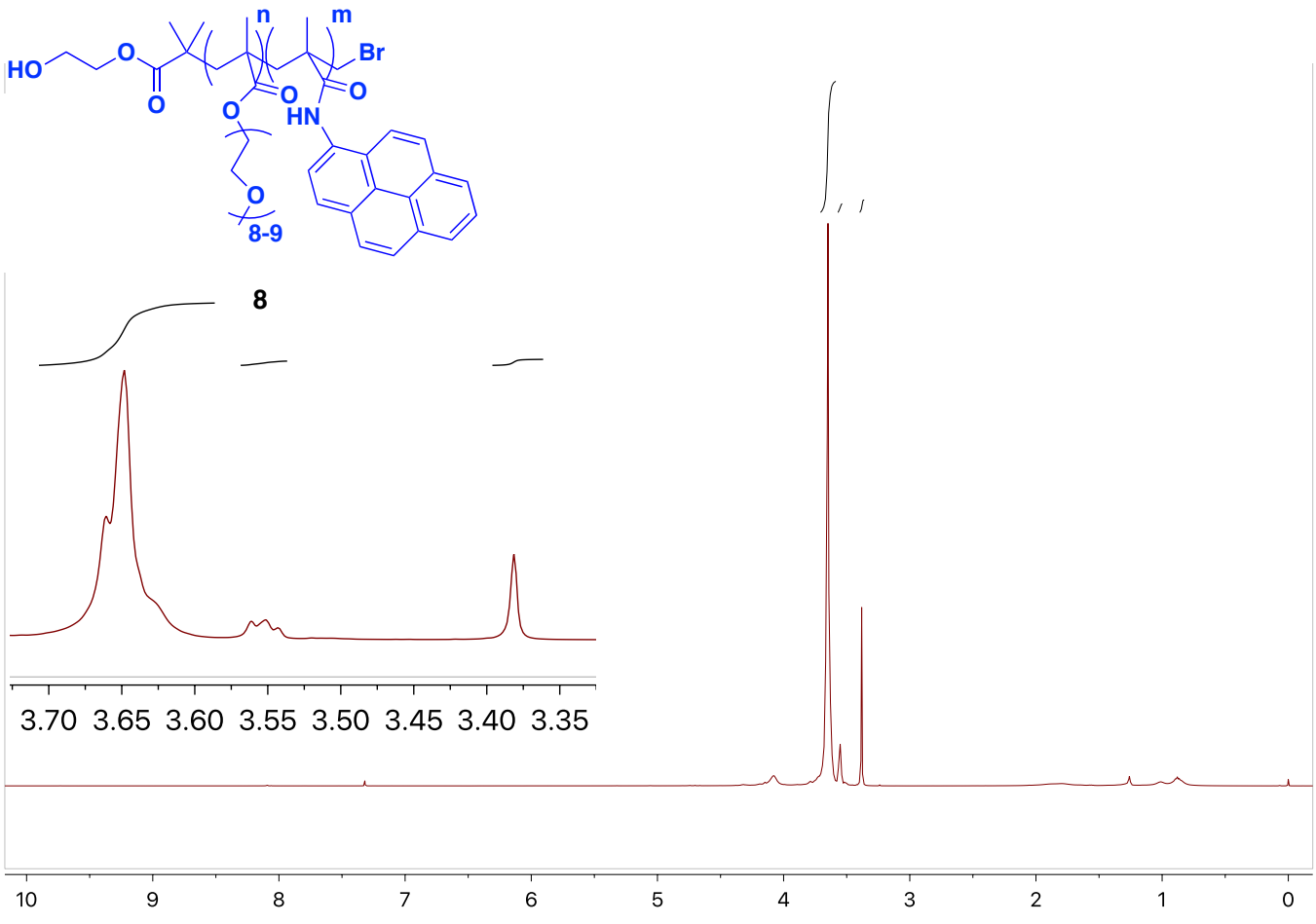
4 **1** **5** **8**

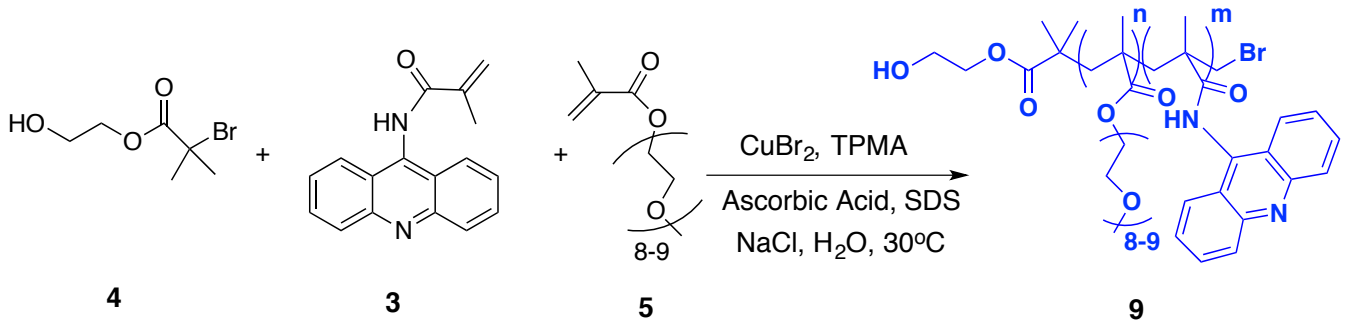
Data for Pyrene:PEG Co-Polymer 8:

Percent Recovery: 83% (5 days of reaction time, monitored by ^{1}H NMR for full monomer conversion).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.38 (s, 3 H), 3.55 (t, *J* = 4.55, 2 H), 3.60-3.69 (m, 32 H) ppm. (Only PEG peaks visible due to relatively small amount of fluorogenic monomer in the reaction (1:930 **Py 1:5** molar ratio).

Elemental Analysis: C 51.47%, H 7.99%, N 0.11%, O 36.42% (Detectable nitrogen indicates trace incorporation of fluorogenic monomer **Py 1**).





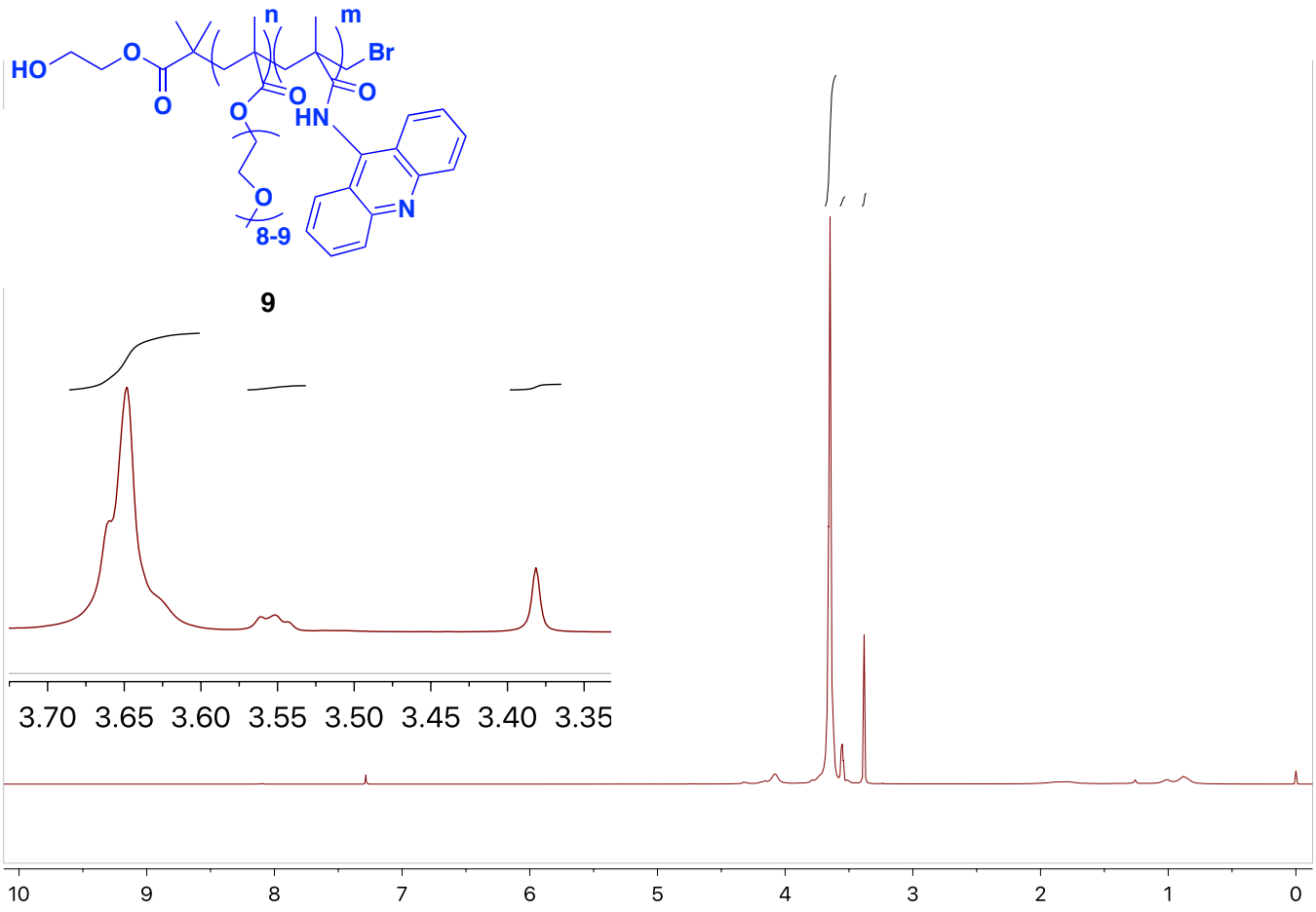
4 **3** **5** **9**

Data for Acridine:PEG Co-Polymer 9:

Percent Recovery: 92% (5 days of reaction time, monitored by ^{1}H NMR for full monomer conversion).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.38 (s, 3 H), 3.55 (t, *J* = 4.38, 2 H), 3.59-3.69 (m, 30 H) ppm. (Only PEG peaks visible due to relatively small amount of fluorogenic monomer in the reaction (1:930 **Ac 3:5** molar ratio).

Elemental Analysis: C 50.72%, H 7.70%, N 0.11%, O 38.61% (Detectable nitrogen indicates trace incorporation of fluorogenic monomer **Ac 3**)



Streptavidin Detection Assay

A stock solution of Sera-Mag magnetic Streptavidin-coated particles (100 µL, 10 mg/mL) was added to a 1.5 mL Eppendorf tube, placed on a DynaMag-2 magnet for 2 minutes, and the resulting supernatant discarded. The pellet was suspended in water (300 µL), vortexed, placed on the magnet for 2 minutes, and the resulting supernatant discarded. This step was repeated. The pellet was suspended in a NaCl solution (2 M), vortexed, placed on the magnet for 2 minutes, and the resulting supernatant discarded. A stock solution of biotinylated ATRP initiator **7** (400 µL, 60 µM, 10% DMF, 2 M NaCl) was incubated with the streptavidin-coated beads for 15 minutes with vortexing at 0, 5, 10, and 15 minutes. The solution was placed on the magnet for 2 min, and the resulting supernatant was discarded. The pellet was suspended in a stock solution of 10% DMF in water (300 µL), vortexed, placed on the magnet for 2 min, and the resulting supernatant discarded. This step was repeated. The pellet was suspended in water (300 µL), vortexed, placed on the magnet for 2 min, and the resulting supernatant discarded. This step was repeated. The pellet was suspended in water (300 µL), vortexed, and incubated to 75 °C.[2] The solution was placed on the magnet for 2 min, the supernatant was removed and added into a polymerization reaction.

Supplemental Figures

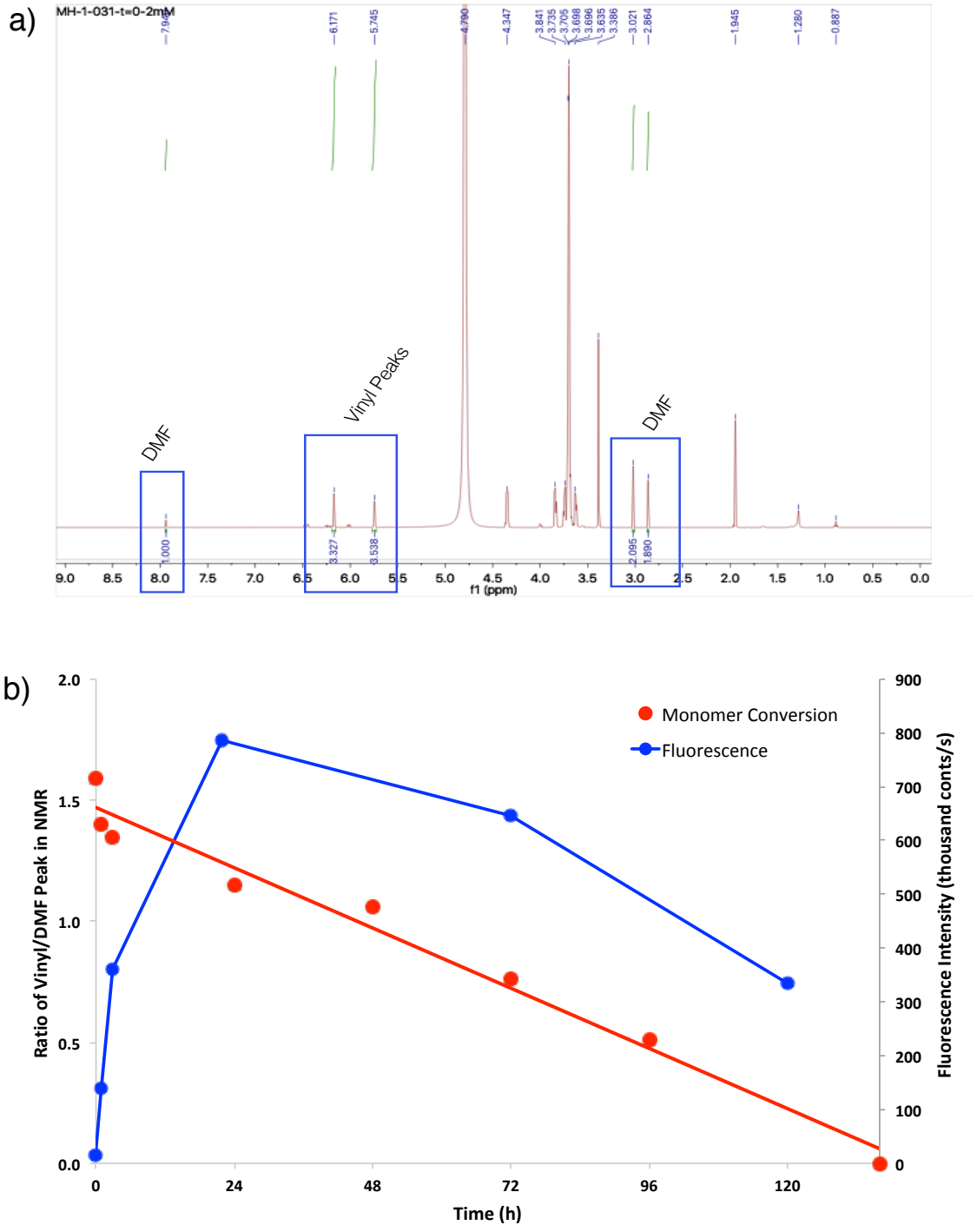


Figure S1. Monomer Conversion to Polymer is Observable with Proton NMR Analysis and Fluorescence Tracks with Early Monomer Conversion. a) ^{1}H NMR spectrum of fluorogenic ATRP polymerization reaction at t=0. b) Graph of vinyl peak/DMF peak ratio of areas measured by ^{1}H NMR and anthracene fluorescence at 426 nm following 371 nm excitation at indicated time points.

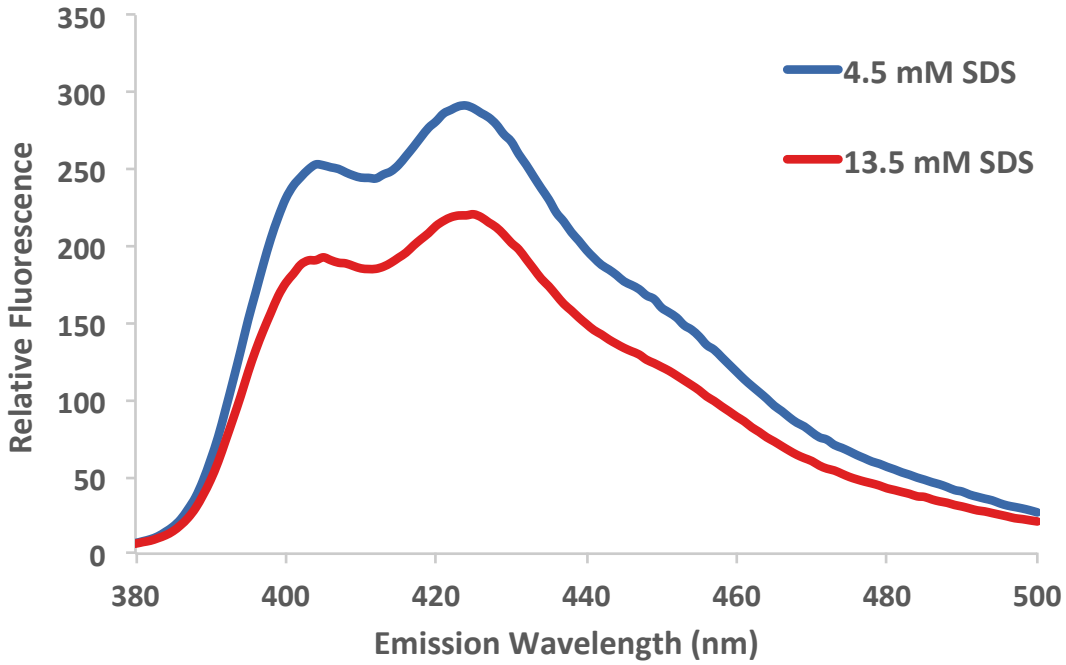


Figure S2. Reduction in SDS Concentration During Polymerization Allows for Increased Signal in Anthracene ATRP Polymerization. Emission spectra at t = 22 h of various SDS concentrations following 337 nm excitation.

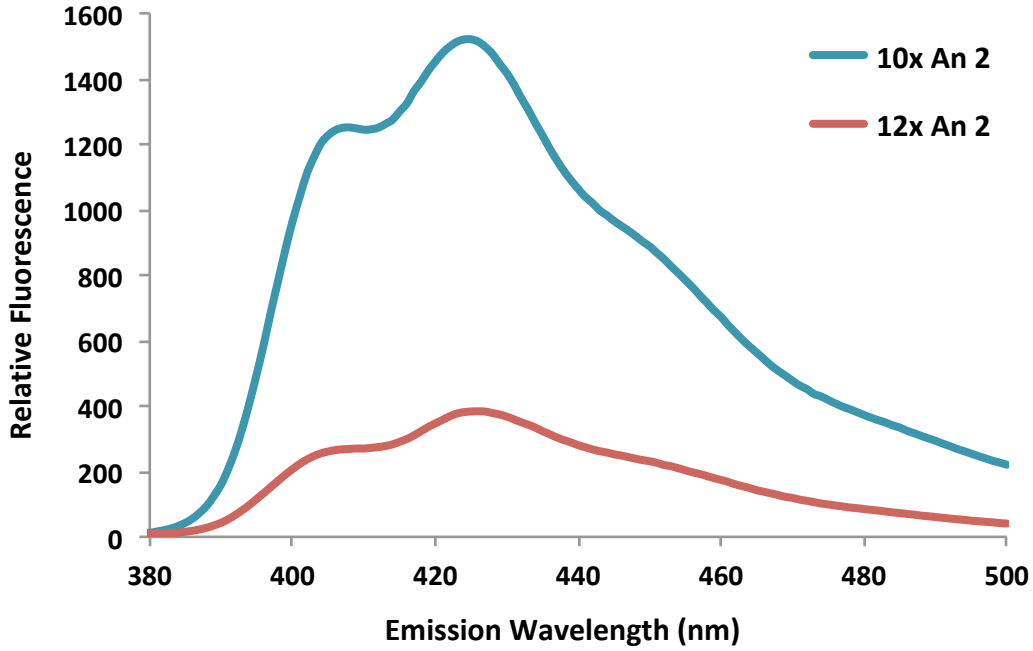


Figure S3. 10-Fold Anthracene Incorporation Over Initial Conditions are Optimal for Fluorescence Output. Emission spectra at t = 22 h of various fold increases in anthracene monomer (**An 2**) incorporation in ATRP co-polymerization following 337 nm excitation.

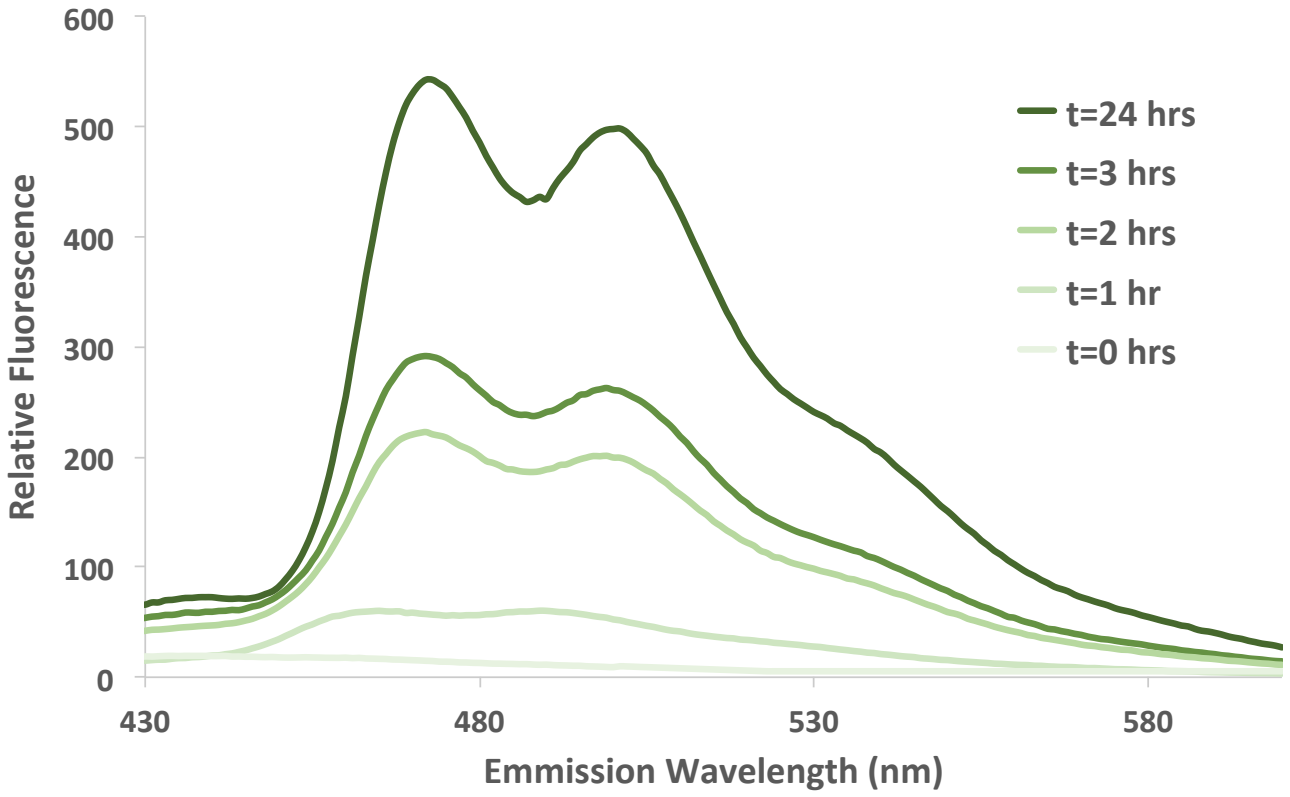


Figure S4. Acridine monomer **3** is fluorogenic upon aqueous ATRP polymerization. Emission spectra of Acridine:PEG co-polymerization at indicated times during polymerization following 421 nm excitation.

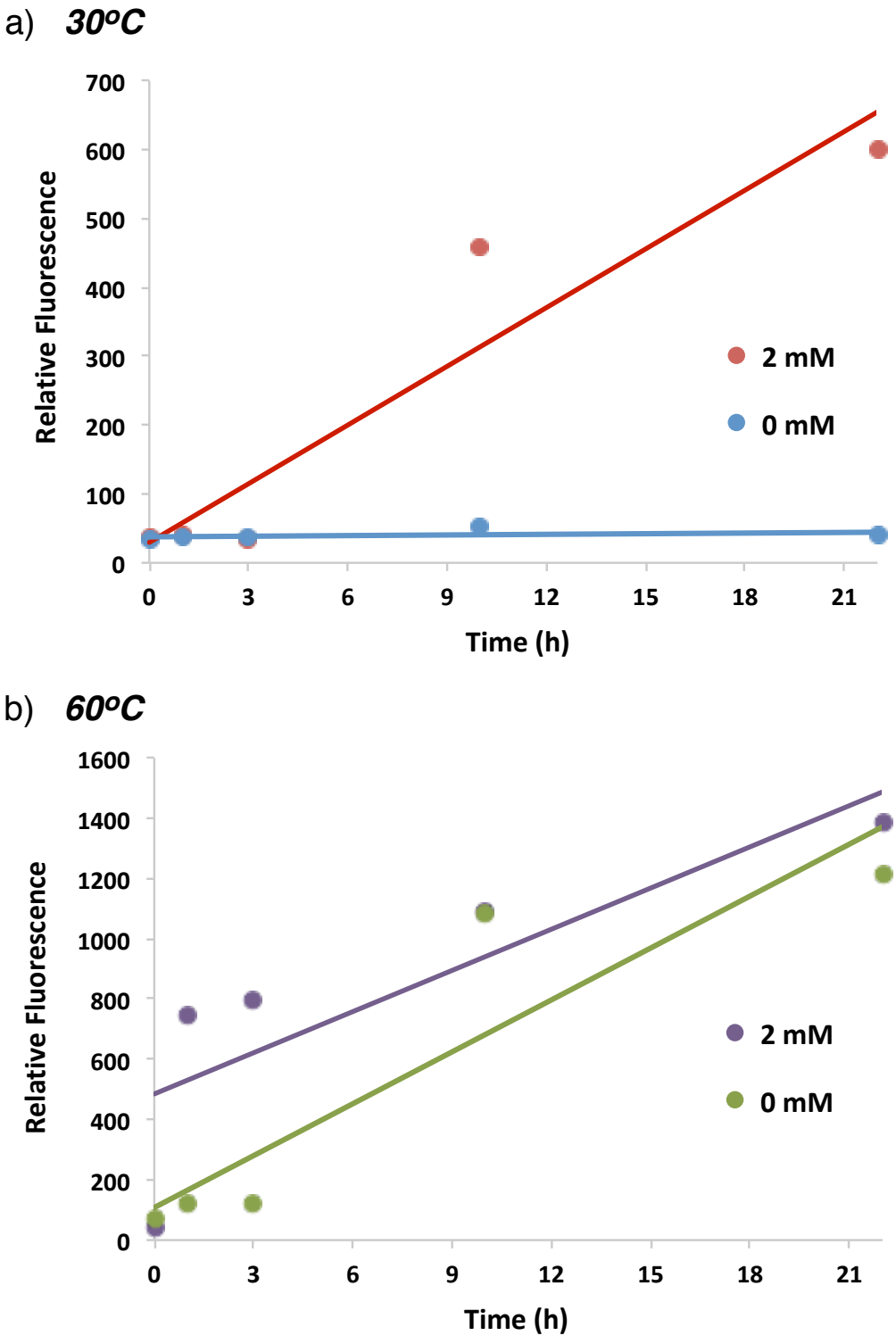


Figure S5. Kinetics of Polymerization and Background Polymerization is Temperature Dependent. Plots of fluorescence emission at 426 nm with indicated concentrations of initiator **4** versus time of anthracene:PEG co-polymerization run at 30°C (a) or 60°C (b) following 371 nm excitation.

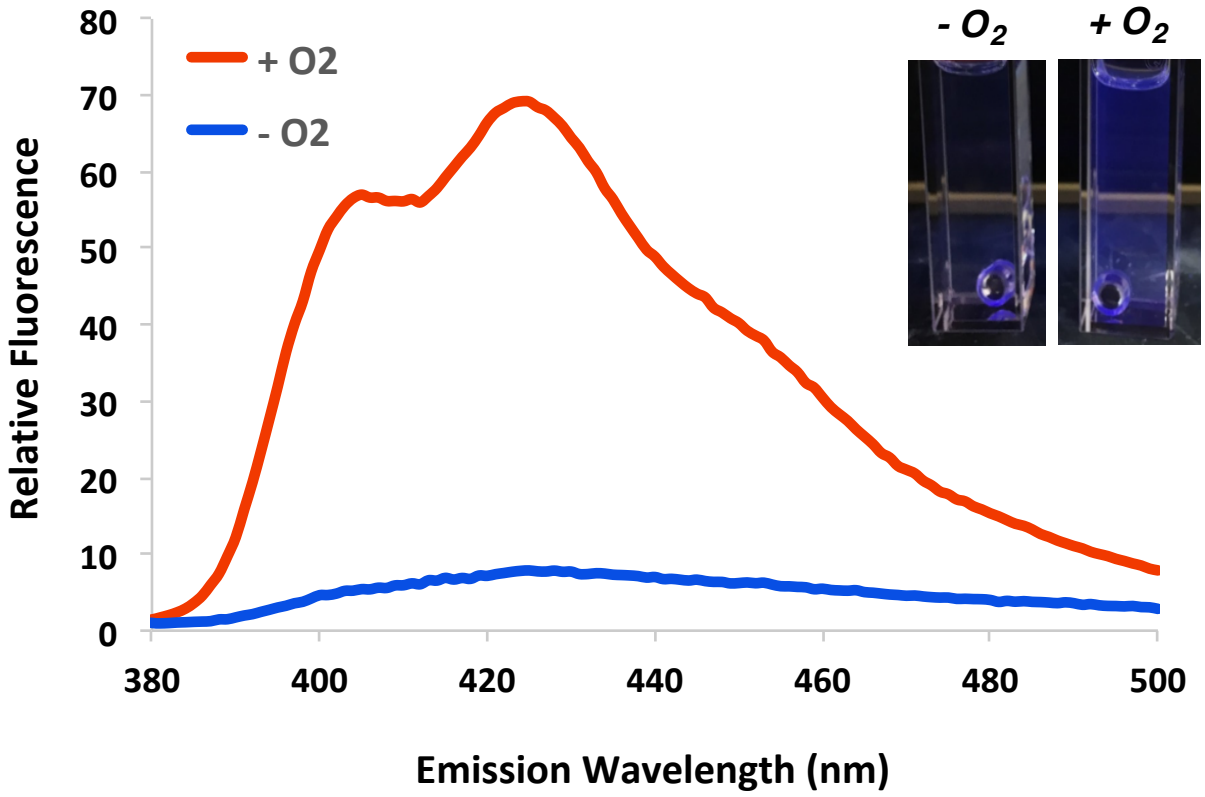


Figure S6. Trace Oxygen Initiates ATRP Polymerization in the Absence of Initiator. Plot of fluorescence emission and pictures of reactions run with anthracene:PEG co-polymerization at t = 24 h with no initiator **4** in the presence (no degassing, + O_2) and absence (with 1 h degassing under N_2, - O_2) of oxygen following 371 nm excitation.

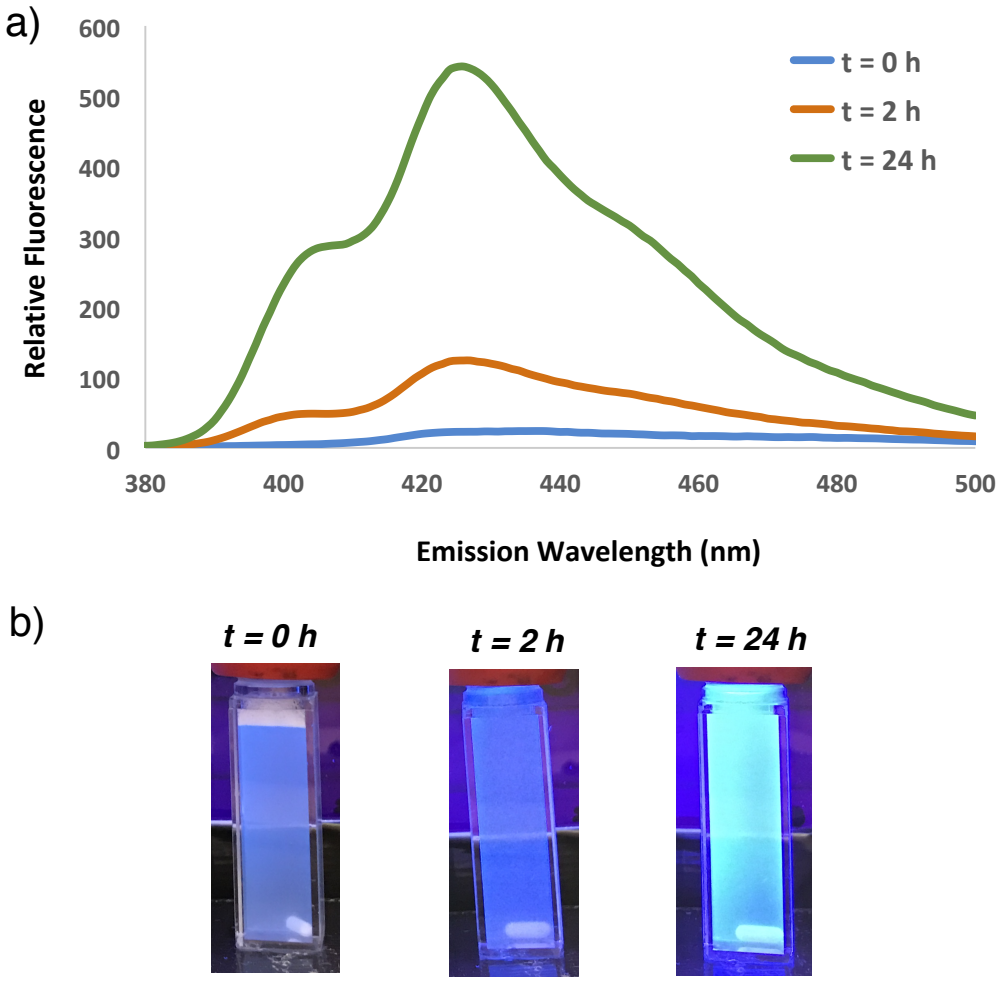


Figure S7. Biotinylated Initiator **7** Initiates Fluorogenic ATRP Polymerization. Emission spectra (a) and photos (b) of Anthracene:PEG Co-Polymerization at indicated times following excitation at 371 nm (a) or by hand-held UV lamp (b).

References

(1) Fulmer, G. R.; Miller, A. J. M.; Sherden, N. H.; Gottlieb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I. *Organometallics* **2010**, *29* (9), 2176-2179.

(2) Holmberg, A.; Blomstergren, A.; Nord, O.; Lukacs, M.; Lundeberg, J.; Uhlén, M. *Electrophoresis* **2005**, *26* (3), 501-510.