Electronic Supplementary Information

Tandem catalysis using an enzyme and a polymeric ruthenium-based artificial metalloenzyme

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1. Experimental

1.1. Instrumentation

NMR spectra were recorded using a Varian U500, VXR500, Bruker CB500, or Bruker B600 spectrometer in the NMR Lab in Roger Adams Laboratory, School of Chemical Sciences, University of Illinois. Coupling constants (*J*) are reported in Hertz. The spectra were processed using MestReNova v14.1.0-24037. The NMR spectra were plotted in OriginPro 2020 v9.7.0.188 and saved as a JPG file. Mass spectral analysis were provided by the Mass Spectrometry Lab, School of Chemical Sciences, University of Illinois, using ESI on a Waters Q-TOF Ultima ESI spectrometer and MALDI on Bruker Daltonics UltrafleXtreme MALDI TOFTOF spectrometer. Fluorescence experiments were performed on a Horiba FluoroMax-4 fluorometer with FluorEssence (v3.5) software. The UV-Vis experiments were performed in a Shimadzu UV-2501PC UV-Vis spectrometer using a quartz cuvette. Dynamic light scattering (DLS) characterization was performed using a Malvern Panalytical Ltd Zetasizer Nano ZS instrument (software v7.11). The raw data from the fluorescence, UV-Vis, and DLS experiments were extracted and processed using OriginPro 2020 v9.7.0.188. Molecular weight (M_w) and dispersity ($Đ = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC). GPC using THF as the mobile phase and flow rate of 1 mL min^{-1} was performed using a Tosoh Ecosec HLC-8320GPC at 40 ºC. This SEC is equipped with both a refractive index and UV detector. The SEC is fitted with a guard column (6.0 mm ID x 4.0 cm x 5 μm), and two analytical columns (7.8 mm ID x 30 cm x 5 μm; TSKgel GMH_{HR}-H). Polystyrene standards (16 points ranging from M_w = 200 to M_w = 2.1 million were used as the general calibration. GPC using DMF containing 0.1 M LiBr as the mobile phase and a flow rate of 1 mL min^{-1} was performed on a system equipped with

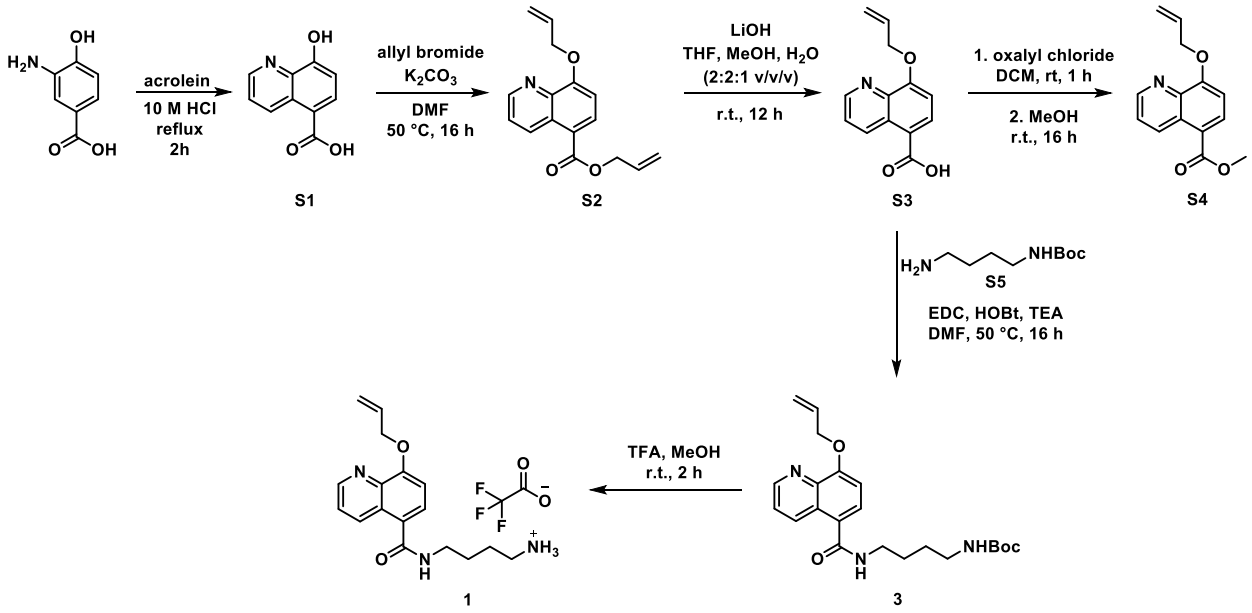
a Model1200 isocratic pump (Agilent Technology) in series with a 717 Autosampler (Waters) and size exclusion columns S3 (102 Å, 103 Å, 104 Å, 105 Å, 106 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex) which were maintained at a temperature of 60 °C. A DAWN HELEOS (Wyatt Technology) multiangle laser light scattering (MALLS) operating at a wavelength of 658 nm and an Optilab rEX refractive index detector (Wyatt Technology) operating at a wavelength of 658 nm were used as detectors. Samples were filtered through a 0.45 μm PTFE filter before analysis. Absolute molecular weights of polymers were determined using ASTRA 6.1.1.17 software (Wyatt Technology) and calculated from dn/dc values assuming 100% mass recovery.

1.2. Materials and methods

All reagents were purchased from Sigma-Aldrich, Fisher Scientific, Acros Organics, AK Scientific, or TCI America and used without further purification. The tris(acetonitrile)cyclopentadienylruthenium(II) hexafluorophosphate was purchased from STREM Chemicals, Inc. and used without further purification. Dulbecco's Modified Eagle Medium (DMEM) was purchased from the Cell Media Facility, School of Chemical Sciences, University of Illinois. For the synthetic procedures, dry DCM and dry THF were obtained from a MBRAUN solvent purification system. Anhydrous DMF, 1,4-dioxane, TEA, and DIPEA were stored over 3 Å molecular sieves. MeCN and acetone used in the Ru coordination procedures were degassed and stored over 3 Å molecular sieves. Ru coordination procedures were performed under nitrogen using a modified procedure reported by Meggers and coworkers.[1] Silica gel (SiO_2) chromatography was performed on 40-63 μm silica gel. Kinetic studies were performed in triplicate and the average rates with standard errors are reported.

2. Synthetic procedures

2.1. Synthesis of quinoline ligands



8-Hydroxyquinoline-5-carboxylic acid (S1). A modification of a reported procedure was followed for synthesis of **S1**.[2] To a 250-mL round bottom flask was added 3-amino-4-hydroxybenzoic acid (5.5 g, 33 mmol) and HCl (100 mL, 6 M aq. soln.). The suspension was magnetically stirred at 40 °C in an oil bath. To the suspension was added acrolein (3.3 mL, 49 mmol) dropwise via an addition funnel over 30 min. The mixture was refluxed at 105 °C for 2 h. The dark brown mixture was filtered via vacuum filtration. The pH of the dark brown filtrate was adjusted to pH 9 with NH_4OH (28% aq. soln.). The basic mixture was filtered by vacuum filtration. The pH of the dark brown filtrate was adjusted to pH 7 using HCl (10 M aq. soln.) and filtered by vacuum filtration. The pH of the filtrate was adjusted to pH 6 with HCl (10 M aq. soln.) and filtered by vacuum filtration. The pH of the filtrate was adjusted by the dropwise addition of HCl (10 M aq. soln.) until solid precipitated out and the heterogeneous mixture was filtered. The process of

adjusting the pH of the filtrate with HCl (10 M aq. soln.) and filtering was repeated until an orange solid was obtained (usually pH 4-5). The pH 5 filtrate was extracted with ethyl acetate and more yellow solid precipitated out. The orange solids were combined, washed twice with DCM, and dried under vacuum to afford 1.02 g (17%) of the product as a bright orange powder. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.47 (dd, J = 8.8, 1.6, 1H), δ 8.90 (dd, J = 4.0, 1.6, 1H), δ 8.24 (d, J = 8.2, 1H), δ 7.69 (dd, J = 8.8, 4.1, 1H), δ 7.12 (d, J = 8.2, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.63, 157.75, 148.11, 138.21, 134.51, 133.39, 128.05, 123.21, 116.68, 110.11. High resolution ESI-MS: m/z calculated for $C_{10}H_8NO_3^+$ ($[M+H]^+$): 190.0504; found 190.0495.

Allyl 8-(allyloxy)quinoline-5-carboxylate (S2). In a 50-mL round bottom flask, 8-hydroxyquinoline-5-carboxylic acid **S1** (1.0 g, 5.3 mmol) and K_2CO_3 (4.4 g, 32 mmol) were dissolved in DMF (15 mL). The mixture was magnetically stirred and allyl bromide (1.8 mL, 21 mmol) was added dropwise. The mixture was stirred at 50 °C for 16 h. To the reaction mixture was added water (20 mL) and ethyl acetate (20 mL). The layers were separated and the aqueous layer was extracted twice with ethyl acetate (20 mL). The combined organic layers were washed 4 times with water (20 mL) and once with sat. aq. NaCl (20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude was purified by silica gel column chromatography eluting with 30% (v/v) ethyl acetate in hexane to afford a pale yellow solid. The solid was washed with a minimal amount of diethyl ether to remove the yellow impurity and dried under vacuum to afford 550 mg (39%) of a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.47 (dd, J = 8.8, 1.7, 1H), 8.98 (dd, J = 4.1, 1.7, 1H), 8.35 (d, J = 8.4, 1H), 7.55 (dd, J = 8.8, 4.1, 1H), 7.06 (d, J = 8.4, 1H), 6.20 (ddt, J = 17.3, 10.7, 5.5, 1H), 6.09 (ddt, J = 17.3, 10.5, 5.7, 1H), 5.51-5.42 (m, 2H), 5.39-5.30 (m, J = 2H), 4.94 (dt, J = 5.6, 1.5, 2H), 4.88 (dt, J = 5.6, 1.5, 2H). ^{13}C NMR

(126 MHz, $CDCl_3$): δ 165.91, 158.30, 149.43, 140.17, 134.53, 132.57, 132.33, 128.78, 123.05, 118.97, 118.38, 118.05, 107.51, 77.22, 70.07, 65.49. High resolution ESI-MS: *m*/*z* calculated for $C_{16}H_{16}NO_3^+$ ($[M+H]^+$): 270.1130; found 270.1118.

8-(Allyloxy)-7,8-dihydroquinoline-5-carboxylic acid (S3). In a 20-mL glass vial, compound **S2** (460 mg, 1.71 mmol) and $LiOH \cdot H_2O$ (717 mg, 17.1 mmol) were dissolved in THF (2 mL), MeOH (2 mL), and water (1 mL). The mixture was stirred at room temperature for 12 h. Volatiles were removed under vacuum and the solid was resuspended in water (5 mL). The pH of the slurry was adjusted to pH 3 with HCl (1 M aq. soln.) and filtered via vacuum filtration to afford 374 mg (95%) of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.95 (s, 1H), 9.41 (dd, J = 8.8, 1.7, 1H), 8.91 (dd, J = 4.0, 1.7, 1H), 8.27 (d, J = 8.4, 1H), 7.67 (dd, J = 8.7, 4.0, 1H), 7.27 (d, J = 8.4, 1H), 6.18 (ddt, J = 17.1, 10.7, 5.4, 1H), 5.53 (dq, J = 17.2, 1.6, 1H), 5.35 (dq, J = 10.5, 1.5, 1H), 4.85 (dt, J = 5.4, 1.5, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.56, 157.68, 149.02, 139.55, 133.89, 133.10, 132.54, 127.98, 123.06, 118.30, 118.28, 108.13, 69.17. High resolution ESI-MS: *m*/*z* calculated for $C_{13}H_{12}NO_3^+$ ($[M+H]^+$): 230.0817; found 230.0808.

Methyl 8-(allyloxy)quinoline-5-carboxylate (S4). In a 25-mL round bottom flask, compound **S3** (300 mg, 1.31 mmol) was dissolved in dry DCM (7.5 mL), and DMF (11.5 µL) and oxalyl chloride (285 µL, 3.27 mmol) were added. The mixture was stirred at room temperature for 1 h. Volatiles were removed under rotary evaporation and the resulting solid was washed with dry THF. The solid was dissolved in MeOH (10 mL) and the mixture was stirred at room temperature for 16 h. Volatiles were removed under rotary evaporation and the resulting solid was dissolved in ethyl acetate (10 mL) and washed with sat. aq. $NaHCO_3$ soln. (10 mL). The solution was dried over

Na_2SO_4, filtered, and concentrated by rotary evaporation. The crude was purified by silica gel column chromatography eluting with 50% (v/v) ethyl acetate in hexane to afford a pale yellow oil. The oil was washed with hexane to afford 164 mg (52%) of a white crystalline solid. ^{1}H NMR (500 MHz, Acetone-d_6): δ 9.43 (dd, J = 8.8, 1.7, 1H), 8.92 (dd, J = 4.0, 1.7, 1H), 8.32 (d, J = 8.4, 1H), 7.65 (dd, J = 8.8, 4.0, 1H), 7.26 (d, J = 8.4, 1H), 6.23 (ddt, J = 17.3, 10.5, 5.2, 1H), 5.59 (dq, J = 17.2, 1.7, 1H), 5.34 (dq, J = 10.6, 1.5, 1H), 4.90 (dt, J = 5.1, 1.6, 2H), 3.94 (s, 3H). ^{13}C NMR (126 MHz, acetone-d_6): δ 166.28, 158.66, 149.00, 140.33, 133.71, 133.12, 132.47, 128.40, 122.95, 117.91, 117.28, 107.83, 69.41, 51.30. High resolution ESI-MS: m/z calculated for $C_{14}H_{14}NO_3^+$ ($[M+H]^+$): 244.0974; found 244.0962.

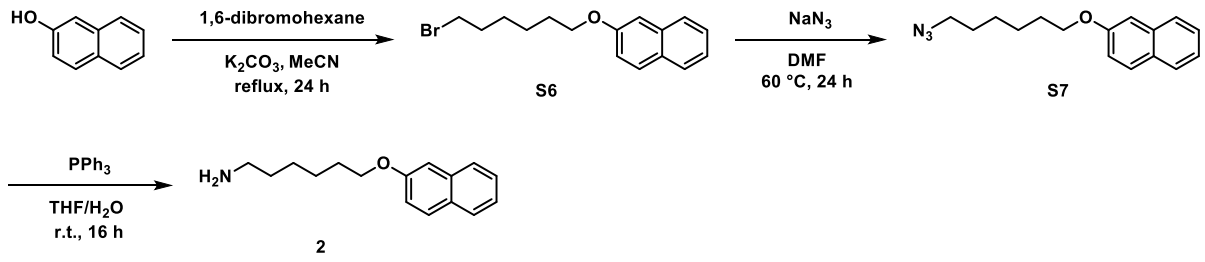
***N*-Boc-1,4-diaminobutane (S5).** This compound was prepared following reported procedure.[3]

***tert*-Butyl (4-(8-(allyloxy)quinoline-5-carboxamido)butyl)carbamate (3).** In a 25-mL round bottom flask, compound **S3** (800 mg, 3.49 mmol), EDC (803 mg, 4.19 mmol), HOBt (641 mg, 4.19 mmol), and *N*-boc-1,4-butanediamine (788 mg, 4.19 mmol) were dissolved in DMF (15 mL). Triethylamine (1.7 mL, 12 mmol) was added and the mixture was stirred at room temperature for 16 h. The mixture was diluted with water (20 mL) and extracted three times with ethyl acetate (20 mL). The organic layers were washed four times with water (20 mL) and once with sat. aq. NaCl (20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The crude was dissolved in minimal volume of DCM and the product was obtained by precipitation in diethyl ether (25 mL). The solid was dried under vacuum to afford 659 mg (47%) of the product as a beige solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.97 (dd, J = 4.1, 1.7, 1H), 8.85 (dd, J = 8.6, 1.8, 1H), 7.63 (d, J = 8.1, 1H), 7.50 (dd, J = 8.6, 4.1, 1H), 6.99 (d, J = 8.2, 1H),

6.27 (s, 1H), 6.19 (ddt, *J* = 17.4, 10.7, 5.4, 1H), 5.47 (dq, *J* = 17.2, 1.5, 1H), 5.36 (dq, *J* = 10.5, 1.4, 1H), 4.90 (dt, *J* = 5.5, 1.5, 2H), 4.63 (s, 1H), 3.54 (q, *J* = 6.6, 2H), 3.19 (q, *J* = 6.6, 2H), 1.73 – 1.66 (m, 2H), 1.63 (q, *J* = 7.2, 2H), 1.42 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.55, 156.31, 156.28, 149.91, 140.46, 134.50, 132.75, 127.47, 126.31, 126.21, 122.61, 118.86, 107.63, 79.45, 70.09, 40.19, 39.80, 28.55, 27.93, 26.93. High resolution ESI-MS: *m*/*z* calculated for $C_{22}H_{30}N_3O_4^+$ ($[M+H]^+$): 400.2236; found 400.2220.

4-(8-(Allyloxy)quinoline-5-carboxamido)butan-1-aminium 2,2,2-trifluoroacetate (1). In a 20-mL glass vial, compound **3** (74 mg, 0.19 mmol) was dissolved in DCM (2 mL) and TFA (200 µL) was added. The mixture was stirred at room temperature for 2 h. The reaction mixture was precipitated in diethyl ether (40 mL) to yield 70 mg (91%) of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.90 (dd, *J* = 4.1, 1.8, 1H), 8.75 (dd, *J* = 8.6, 1.7, 1H), 8.54 (t, *J* = 5.8, 1H), 7.69 (d, *J* = 8.1, 1H), 7.66 (s, 3H), 7.60 (dd, *J* = 8.7, 4.1, 1H), 7.22 (d, *J* = 8.1, 1H), 6.17 (ddt, *J* = 17.4, 10.5, 5.3, 1H), 5.51 (dq, *J* = 17.3, 1.8, 1H), 5.37 – 5.30 (m, 1H), 4.83 (dt, *J* = 5.4, 1.6, 2H), 2.85 (d, *J* = 6.4, 2H), 1.62 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.53, 155.33, 149.16, 139.50, 133.91, 133.36, 126.84, 126.66, 125.96, 122.31, 118.02, 108.16, 69.07, 38.71, 38.41, 26.17, 24.65. High resolution ESI-MS: *m*/*z* calculated for $C_{17}H_{22}N_3O_2^+$ ($[M]^+$): 300.1712; found 300.1707.

2.2. Synthesis of naphthalene side chain



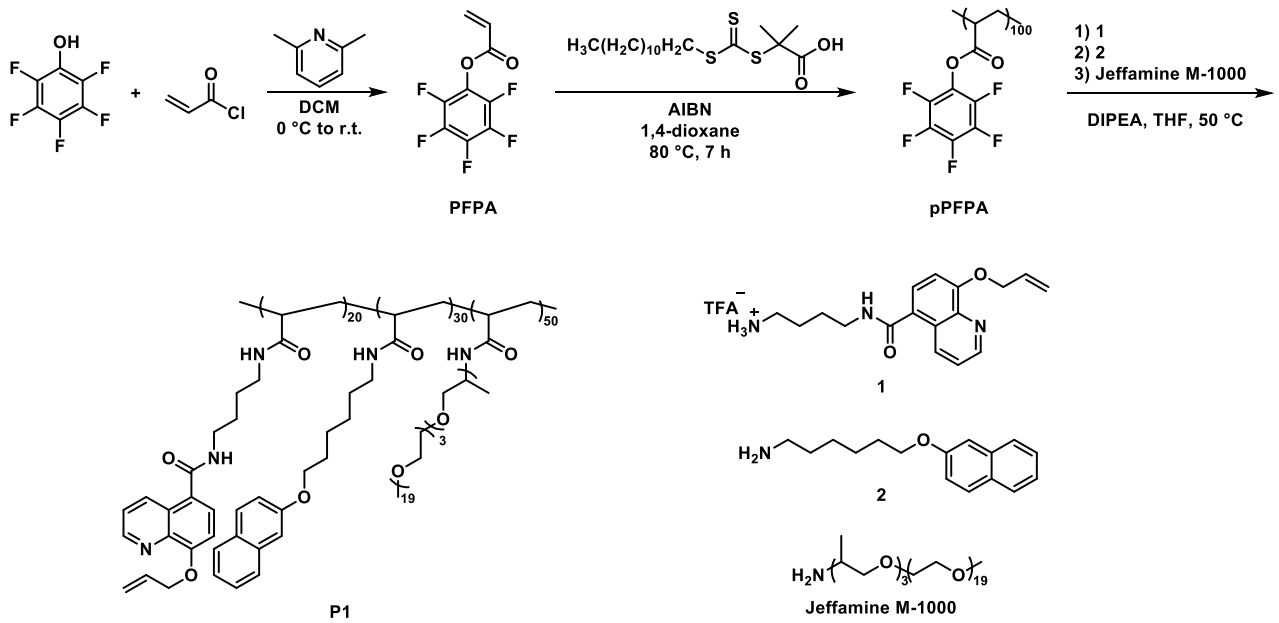
2-((6-Bromohexyl)oxy)naphthalene (S6). To a 3-neck 250-mL round bottom flask was added 2-naphthol (5.0 g, 35 mmol), K_2CO_3 (7.19 g, 52.0 mmol), and MeCN (50 mL). The flask was transferred to an oil bath preheated to 90 °C and stirred for 30 min. To the flask was added a solution of 1,6-dibromohexane (26.7 mL, 173 mmol) in MeCN (50 mL), and the mixture was stirred at reflux under N_2 atm for 24 h. The mixture was diluted with $CHCl_3$ (100 mL) and washed once with water (100 mL) and once with sat. aq. NaCl (100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The crude was purified twice by silica gel column chromatography using a gradient elution of 100% hexane to 10% (v/v) ethyl acetate in hexane to afford 8.08 g (76 %) of a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.68 (m, 3H), 7.43 (m, 1H), 7.36 – 7.29 (m, 1H), 7.17 – 7.08 (m, 2H), 4.09 (t, J = 6.4, 2H), 3.44 (t, J = 6.8, 2H), 1.90 (m, 4H), 1.55 (p, J = 3.8, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.02, 134.60, 129.36, 128.91, 127.66, 126.70, 126.33, 123.53, 118.99, 106.55, 67.74, 33.86, 32.72, 29.10, 27.98, 25.40. High resolution ESI-MS: m/z calculated for $C_{16}H_{19}BrO^+$ ($[M+H]^+$): 307.0619; found 307.0689.

2-((6-Azidohexyl)oxy)naphthalene (S7). To a 250-mL round bottom flask equipped with a magnetic stirbar was added compound **S6** (5.00 g, 19.4 mmol), sodium azide (1.59 g, 24.5 mmol), and DMF (75 mL). The mixture was transferred to an oil bath preheated to 60 °C and stirred for

24 h. The mixture was diluted with ethyl ether (100 mL) and washed five times with water (50 mL) and once with sat. aq. NaCl (50 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated by rotary evaporation to afford 3.87 g (88%) of a yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.67 (m, 3H), 7.43 (ddd, *J* = 8.2, 6.8, 1.3, 1H), 7.33 (ddd, *J* = 8.1, 6.9, 1.3, 1H), 7.15 (m, 2H), 4.09 (t, *J* = 6.4, 2H), 3.31 (t, *J* = 6.9, 6.3, 2H), 1.87 (p, *J* = 8.1, 6.5, 2H), 1.67 (p, *J* = 12.3, 7.6, 4.4, 2H), 1.62 – 1.41 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.02, 134.61, 129.37, 128.92, 127.66, 126.71, 126.34, 123.53, 118.99, 106.55, 67.73, 51.42, 29.15, 28.85, 26.57, 25.79. High resolution ESI-MS: *m*/*z* calculated for $C_{16}H_{20}N_3O^+$ ($[M+H]^+$): 270.1528; found 270.1563.

6-(Naphthalen-2-yloxy)hexan-1-amine (2). To a 300-mL round bottom flask equipped with a magnetic stir bar was added compound **S7** (1.53 g, 6.98 mmol), PPh_3 (1.79 g, 6.82 mmol), and 4:1 THF/H_2O (25 mL). The mixture was stirred at room temperature for 16 h. The mixture was concentrated by rotary evaporation, diluted with DCM (20 mL) and HCl (20 mL, 1 M aq. soln.) and allowed to sit overnight. The mixture was filtered by vacuum filtration to afford 1.37 g (86%) of a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 – 7.68 (m, 3H), 7.42 (t, *J* = 7.5, 1H), 7.32 (t, *J* = 7.5, 1H), 7.17 – 7.10 (m, 2H), 4.08 (t, *J* = 6.5, 2H), 2.73 (t, *J* = 7.0, 2H), 1.86 (dt, *J* = 14.5, 6.6, 2H), 1.52 (tt, *J* = 14.0, 7.2, 5H), 1.47 – 1.38 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.07, 134.62, 129.33, 128.89, 127.65, 126.70, 126.31, 123.49, 119.02, 106.54, 77.25, 77.03, 76.82, 67.88, 42.12, 33.57, 29.25, 26.70, 26.03. High resolution ESI-MS: *m*/*z* calculated for $C_{16}H_{21}NO^+$ ($[M+H]^+$): 244.1623; found 244.1694.

2.3. Synthesis of P1



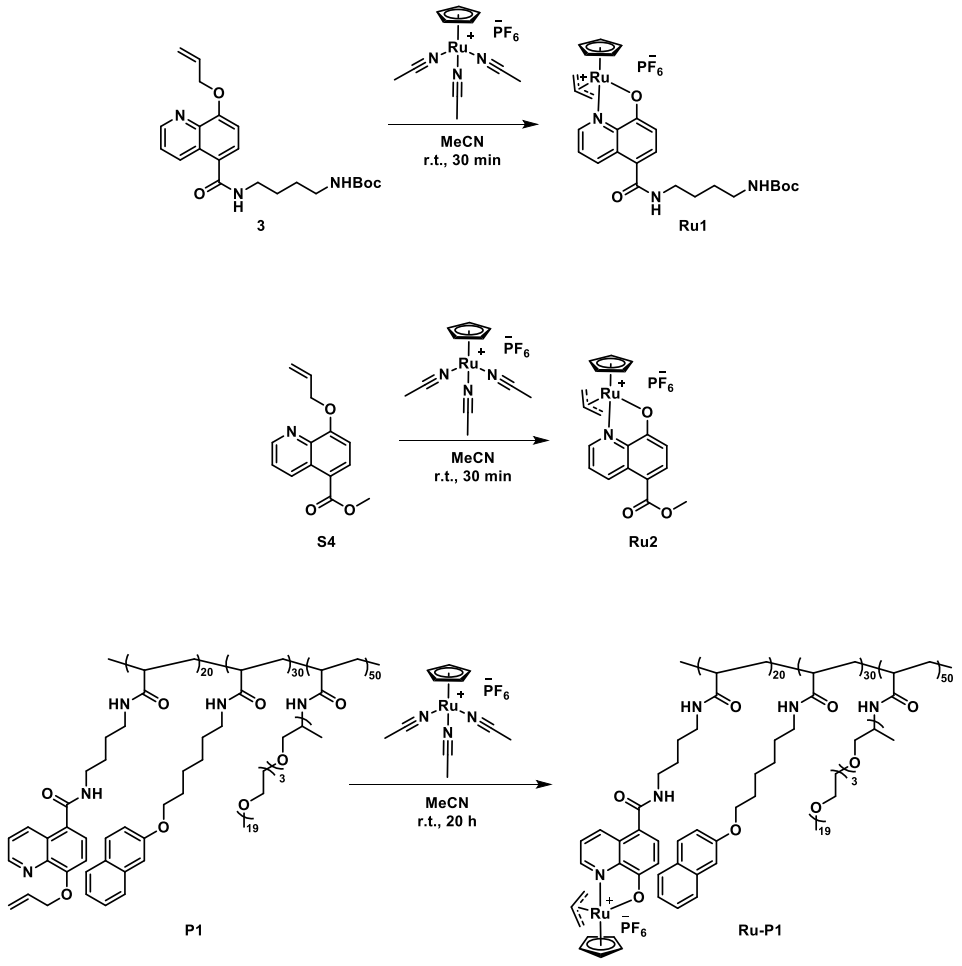
Pentafluorophenyl acrylate (PFPA). This compound was prepared following reported procedure.[4]

Poly (pentafluorophenyl acrylate) (pPFPA). This compound was prepared following reported procedure.[4]

P1. This procedure was adapted from that reported by Palmans, Meijer and coworkers.[5] In a 20-mL glass vial, pPFPA (200 mg, 8.4 μmol) and compound **1** (69.4 mg, 168 μmol) were dissolved in dry THF (1 mL) and DMF (1 mL), and DIPEA (50 μL) was added. The mixture was stirred at 50 °C for 4 h. The progress of the reaction was checked by ^{19}F NMR in $CDCl_3$ to confirm intended degree of functionalization. Compound **2** (48.6 mg, 200 μmol) and DIPEA (50 μL) were added to the mixture and stirred at 50 °C for 4 h. The progress of the reaction was checked by ^{19}F NMR. An excess of Jeffamine M-1000 (200 μL of 750 mg/mL solution in THF) was added to the vial

and stirred at 50 °C for 16 h. The polymer was dialyzed against methanol in 1 kDa cutoff dialysis tubing for 2 d followed by dialysis in 5 kDa cutoff dialysis tubing in water for 2 d. The polymer solution was dried in a lyophilizer to yield 265 mg of a light brown gel. GPC (0.1 M LiBr in DMF): M_n = 54.8 kDa, M_w = 67.9 kDa, $Đ$ = 1.2.

2.4. Synthesis of Ru catalysts



Ru1. In a 4-mL glass vial, compound **3** (20.3 mg, 50.9 μmol) was dissolved in degassed MeCN (0.5 mL). In a separate 4-mL glass vial, tris(acetonitrile)cyclopentadienylruthenium(II)

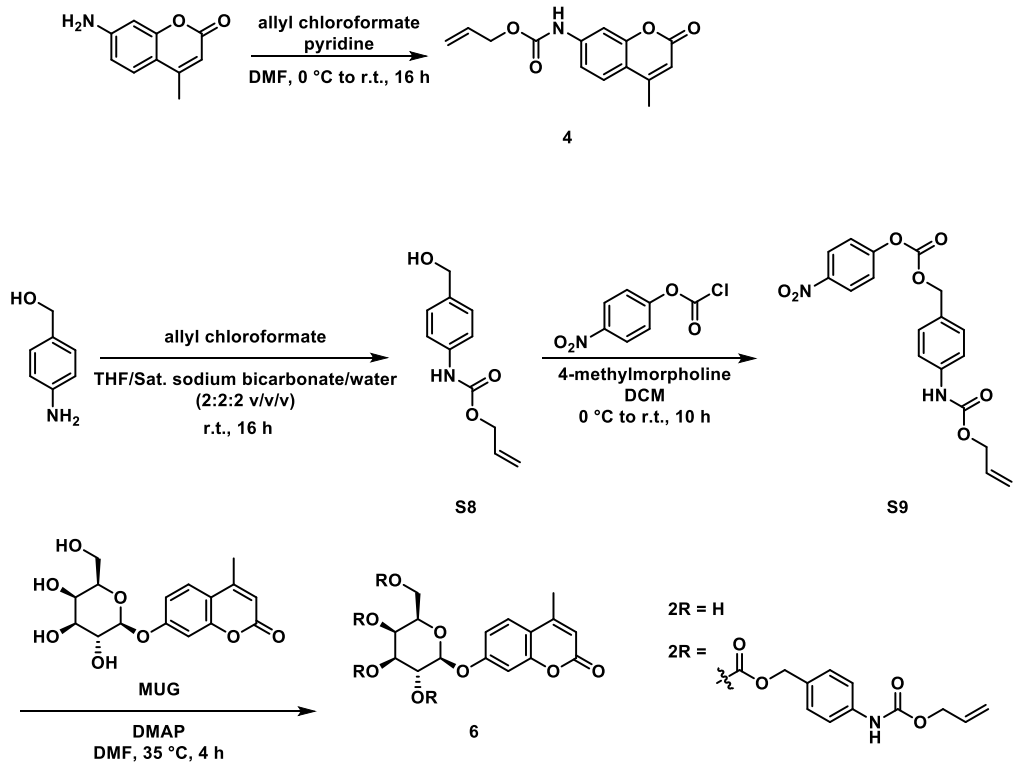
hexafluorophosphate (22.1 mg, 50.9 μmol) was dissolved in degassed MeCN (0.5 mL). The solution of compound **3** was added to the Ru solution. The mixture was stirred at room temperature for 1 h. The reaction mixture was added dropwise to diethyl ether (45 mL) in a 50-mL centrifuge tube, and a yellow solid was collected by centrifugation. The pellet was washed twice with diethyl ether (35 mL). The product was collected by centrifugation and dried under vacuum to afford 13.1 mg (36%) of a yellow solid. ^{1}H NMR (500 MHz, CD_3CN): δ 9.22 (dd, *J* = 8.7, 1.2, 1H), 8.65 (dd, *J* = 5.2, 1.2, 1H), 7.65 (d, *J* = 8.3, 1H), 7.57 (dd, *J* = 8.7, 5.1, 1H), 6.94 (s, 1H), 6.86 (d, *J* = 8.3, 1H), 5.95 (s, 5H), 5.31 (s, 1H), 4.51 (tt, *J* = 10.8, 6.2, 1H), 4.41 (d, *J* = 10.9, 1H), 4.14 (d, J = 11.0, 1H), 4.12 (dd, J = 6.1, 2.9, 1H), 4.09 (dd, *J* = 6.3, 2.8, 1H), 3.38 – 3.32 (m, 2H), 3.05 (q, *J* = 6.5, 2H), 1.57 (q, *J* = 7.1, 6.5, 2H), 1.54 – 1.46 (m, 2H), 1.39 (s, 9H). ^{13}C NMR (126 MHz, CD_3CN): δ 172.49, 168.01, 156.67, 146.78, 139.81, 131.45, 130.42, 125.22, 119.08, 114.95, 99.66, 96.78, 69.62, 66.28, 63.91, 40.74, 40.01, 28.63, 28.25, 27.54, 15.63. MALDI-TOF: *m*/*z* calculated for $C_{27}H_{34}N_3O_4Ru^+$ ($[M]^+$): 565.7; found 565.3.

Synthesis of Ru2. In a 4-mL glass vial, compound **S4** (27.6 mg, 114 μmol) was dissolved in degassed MeCN (0.5 mL). In a separate 4-mL glass vial, tris(acetonitrile)cyclopentadienylruthenium(II) hexafluorophosphate (49.3 mg, 114 μmol) was dissolved in degassed MeCN (0.5 mL). The solution of compound **S4** was added to the Ru solution. The mixture was stirred at room temperature for 1 h. The reaction mixture was added dropwise to diethyl ether (45 mL) in a 50-mL centrifuge tube, and a yellow solid was collected by centrifugation. The pellet was washed twice with diethyl ether (35 mL). The product was collected by centrifugation and dried under vacuum to afford 49.7 mg (79%) of a yellow solid. ^{1}H NMR (500 MHz, CD_3CN): δ 9.62 (dd, *J* = 8.8, 1.2, 1H), 8.68 (dd, *J* = 5.1, 1.2, 1H), 8.21 (d, *J* = 8.6, 1H),

7.65 (dd, J = 8.8, 5.1, 1H), 6.91 (d, J = 8.6, 1H), 5.98 (s, 5H), 4.54 (tt, J = 10.7, 6.2, 1H), 4.44 (d, J = 11.0, 1H), 4.18 (d, J = 10.8, 1H), 4.17 – 4.11 (m, 2H), 3.88 (s, 3H). ^{13}C NMR (126 MHz, CD_3CN): 175.10, 167.04, 164.62, 156.66, 139.61, 136.72, 131.81, 126.14, 115.63, 111.62, 99.98, 96.90, 69.69, 64.43, 52.41. MALDI-TOF: m/z calculated for $C_{19}H_{18}NO_3Ru^+$ ($[M]^+$): 409.4; found 409.2.

General synthesis of Ru-P1. In a 1.5-mL glass vial, **P1** (1.95 mg) was dissolved in degassed MeCN (0.2 mL). In a separate 1.5-mL glass vial, the tris(acetonitrile)cyclo-pentadienylruthenium(II) hexafluorophosphate was dissolved in degassed MeCN (0.2 mL). To the polymer solution was added the Ru solution (60 µL, 0.8 µmol). The 1:1 mixture of Ru complex to ligand was stirred at room temperature for 20 h. The amount of Ru coordinated to the polymer was determined via UV-vis using a calibration curve from the Ru complex **Ru1** in MeCN (see Fig. S3). This **Ru-P1** solution was used directly without further purification to prepare aqueous solutions for catalysis.

2.5. Synthesis of fluorogenic substrates



Allyl carbamate protected coumarin (4). In a 4-mL glass vial, 7-amino-4-methylcoumarin (101 mg, 577 µmol) was dissolved in DMF (2.0 mL). Pyridine (93 µL, 1.15 mmol) was added and the mixture was stirred in an ice bath. To the ice-cold mixture, allyl chloroformate (72.8 µL, 685 µmol) was added dropwise and the mixture was allowed to stir at 0 °C for 4 h and at room temperature for 12 h. HCl (4 mL, 5% (v/v) aq. soln.) and ethyl acetate (10 mL) were added to the mixture. The aqueous layer was extracted twice with ethyl acetate (5 mL) and the combined organic layers were washed twice with sat. aq. $NaHCO_3$ soln. (5 mL). Volatiles were removed under rotary evaporation and the crude was purified by silica gel column chromatography using a gradient elution of 100 %

hexane to 20% (v/v) ethyl acetate in hexane to afford 28 mg (19%) of a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 (d, *J* = 8.6, 1H), 7.43 (d, *J* = 2.1, 1H), 7.37 (dd, *J* = 8.6, 2.2, 1H), 6.85 (s, 1H), 6.19 (s, 1H), 6.03 – 5.92 (m, 1H), 5.39 (dq, *J* = 17.1, 1.7, 1H), 5.32 – 5.27 (m, 1H), 4.71 (dd, *J* = 5.8, 1.6, 2H), 2.41 (t, *J* = 0.9, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 161.11, 154.68, 152.77, 152.23, 141.43, 132.13, 125.53, 118.94, 115.78, 114.50, 113.45, 106.14, 66.49, 18.70. High resolution ESI-MS: *m*/*z* calculated for $C_{14}H_{14}NO_4^+$ ($[M+H]^+$): 260.0923; found 260.0915.

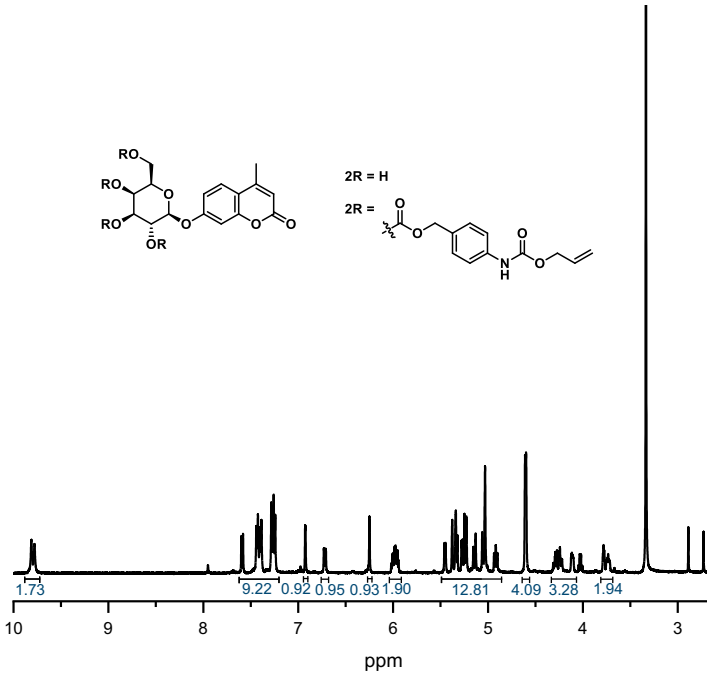
Allyl (4-(hydroxymethyl)phenyl)carbamate (S8). In a 100-mL round bottom flask, 4-aminobenzyl alcohol (1.41 g, 11.4 mmol) was dissolved in a 2:2:1 mixture of THF/sat. aq. $NaHCO_3$ soln./water and allyl chloroformate (1.34 mL, 12.6 mmol) was added dropwise over 5 min at room temperature. The mixture was stirred at room temperature for 16 h. To the reaction mixture was added ethyl acetate (20 mL) and the mixture was washed twice with sat. aq. NH_4Cl soln. (10 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The crude was purified by silica gel column chromatography using a gradient elution of 20% (v/v) ethyl acetate in hexane to 40% (v/v) of ethyl acetate in hexane to afford 1.2 g (51%) of a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (d, *J* = 8.2, 2H), 7.31 (d, *J* = 8.6, 2H), 6.66 (s, 1H), 5.97 (ddt, *J* = 17.2, 10.4, 5.7, 1H), 5.37 (dq, *J* = 17.2, 1.5, 1H), 5.27 (dq, *J* = 10.3, 1.3, 1H), 4.67 (dt, *J* = 5.8, 1.4, 2H), 4.64 (d, *J* = 5.9, 2H), 1.63 (t, *J* = 5.9, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 153.32, 137.43, 136.18, 132.53, 128.12, 118.91, 118.47, 66.05, 65.11. High resolution ESI-MS: *m*/*z* calculated for $C_{11}H_{14}NO_3^+$ ($[M+H]^+$): 208.0974; found 208.0964.

Allyl (4-((((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamate (S9). In a 100-mL round bottom flask, compound **S8** (1.17 g, 5.65 mmol) and DMAP (88.0 mg, 0.72 mmol) were dissolved in dry DCM. The flask was placed in an ice bath for 10 min and the 4-nitrophenyl chloroformate

(2.28 g, 11.3 mmol) was added while stirring followed by the dropwise addition of *N*-methyl morpholine (0.74 mL, 6.78 mmol). The mixture was stirred in the ice bath until it equilibrated to room temperature and stirred for 16 h total. Ethyl acetate (40 mL) was added and the mixture was washed twice with sat. aq. $NaHCO_3$ soln. (15 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude was purified by silica gel column chromatography eluting with 15% (v/v) ethyl acetate in hexane to afford a beige solid. The beige solid was crystalized from hot DCM to afford 1.01 g (48%) of white crystals. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29 – 8.24 (m, 2H), 7.48 – 7.35 (m, 6H), 6.70 (s, 1H), 5.97 (ddt, *J* = 16.9, 10.3, 5.7, 1H), 5.37 (dq, *J* = 17.2, 1.5, 1H), 5.31 – 5.26 (m, 1H), 5.25 (s, 2H), 4.68 (dt, *J* = 5.8, 1.4, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 155.67, 153.16, 152.59, 145.54, 138.79, 132.39, 130.12, 129.26, 125.44, 121.93, 118.83, 118.63, 70.82, 66.17. High resolution ESI-MS: *m*/*z* calculated for $C_{18}H_{17}N_2O_7^+$ ($[M+H]^+$): 373.1036; found 373.1040.

Allyl carbamate protected MUG (6). In a 50-mL round bottom flask, the 4-methylumbelliferyl β-D-galactopyranoside MUG (140 mg, 414 μmol) and DMAP (101 mg, 828 μmol) were dissolved in dry DMF (7 mL). The suspension was placed in an oil bath preheated to 45 °C and stirred until homogenous. The temperature of the oil bath was decreased to 35 °C and allowed to equilibrate to 35 °C. To the mixture was added compound **S9** (308 mg, 828 μmol) was added and the progress of the reaction was monitored by ^{1}H NMR in DMSO-d_6. The reaction mixture was diluted with water (50 mL) and placed in an ice bath for 30 min. The solution was filtered by vacuum filtration using a Buchner funnel to collect a yellow crude. The crude was purified by silica gel flash column chromatography using a gradient elution of 100 % DCM to 50% (v/v) ethyl acetate in DCM to

afford 18 mg (5%) of a white solid. High resolution ESI-MS: *m*/*z* calculated for $C_{40}H_{41}N_2O_{16}$ ($[M+H]^+$): 805.2456; found 805.2458.



^{1}H NMR of **6** in DMSO-*d*$_6$.

3. General procedure for critical micelle concentration study

To sixteen 7-mL glass vials was added 100 µL of a Nile Red solution (5 µM) in DCM. The vials were left uncapped and the DCM was evaporated. In a separate 7-mL glass vial, a 2 mg/mL solution of polymer (**P1** or polymeric catalyst **Ru-P1)** was prepared in 10 mM PBS pH 7.4. To each vial was added aliquots of a polymer solution and the corresponding volume of PBS to yield 0.5 mL of varying concentrations of the polymer. The vials were capped and stirred at room

temperature for 20 h to equilibrate. The fluorescence spectra were measured for each polymer solution with λ_{ex} = 553 nm. A plot of the relative fluorescence intensity at 619 nm versus the log of polymer concentrations in mg/mL produced a non-linear curve. A linear curve was fitted to the static data points belonging to the low polymer concentrations and a second linear curve was fitted to the data points showing steady increase of fluorescence intensity. The critical micelle concentration was calculated as the point where the two linear curves intersect.

4. General procedures for fluorescence studies

All catalysts, small molecules and polymers appeared to be fully soluble under the concentrations used with no precipitation observed after 24 h.

Ru-catalyzed cleavage of allylcarbamate groups under biologically relevant conditions. Stock solutions of glutathione (GSH) (20 mM in water), pro-fluorophore **4** (1 mM in DMSO), **Ru-P1** (500 μM in Ru in MeCN), and **Ru2** (500 μM in MeCN) were prepared. All reactions were conducted in a final volume of 500 μL of 10 mM PBS pH 7.4. To each reaction mixture was added 3, 5, or 10 μL of **Ru-P1** or **Ru2** (3, 5, and 10 μM final concentration), 7.5 μL GSH (300 μM final concentration), and 10 μL of **4** (20 μM final concentration). The cleavage reaction was also conducted under more biologically relevant conditions with 5 μM of **Ru-P1** and **Ru2**. These reactions were carried out in PBS + 5 mM GSH, DMEM, DMEM + 5 mM GSH, and cell lysate. For the reactions requiring 5 mM of GSH, 125 μM of the 20 mM stock solution was added to each reaction mixture. The increase in fluorescence was measured using a fluorometer with λ_{ex} = 355 nm and λ_{em} = 450 nm. The fluorescence was recorded every 10 sec for 20 min. The percent conversions were determined by using a linear regression curve from the linear correlation between fluorescence intensity and yield of coumarin **5**. Solutions of 20 μM of **5**:**4** mixtures in 500 μL of

PBS, DMEM, or cell lysate were prepared. Each of these solutions correlates to a percent yield. For example, 0% yield = 20 μM **4**, 20% yield = 16 μM **4** + 4 μM **5**, 100% yield = 20 μM **5**. The fluorescence of each solution was recorded with λ_{ex} = 355 nm and λ_{em} = 450 nm. The initial rates were calculated from the first derivative of the slope tangent of each timepoint between 1 and 2 min of reaction.

General procedure for tandem catalysis. Stock solutions of pro-fluorophore **6** (1 mM in DMSO), **Ru-P1** (500 μM in Ru in MeCN), and **Ru2** (500 μM in MeCN) were prepared. The reactions were conducted in a final volume of 500 μL of 10 mM PBS pH 7.4 or DMEM. To each reaction mixture was added 5 μL of **Ru-P1** or **Ru2** (5 μM final concentration), 1 μL of βGal (8.6 μM for 17.2 nM final), and 10 μL of **6** (20 μM final concentration). The increase in fluorescence was measured using a fluorometer with λ_{ex} = 340 nm and λ_{em} = 445 nm. The fluorescence was recorded every 10 sec for 20 min. The percent conversions were determined by using a linear regression curve from the linear correlation between fluorescence intensity and yield of coumarin **7**. Solutions of 20 μM of **7**:**6** mixtures in 500 μL of PBS or DMEM were prepared. Each of these solutions correlates to a percent yield. For example, 0% yield = 20 μM **6**, 20% yield = 16 μM **6** + 4 μM **7**, 100% yield = 20 μM **7**. The fluorescence of each solution was recorded with λ_{ex} = 340 nm and λ_{em} = 445 nm. There is a lag phase at the beginning of the reaction, and the initial rates were calculated from the first derivative of the slope tangent of each timepoint after the lag phase.

5. Additional figures

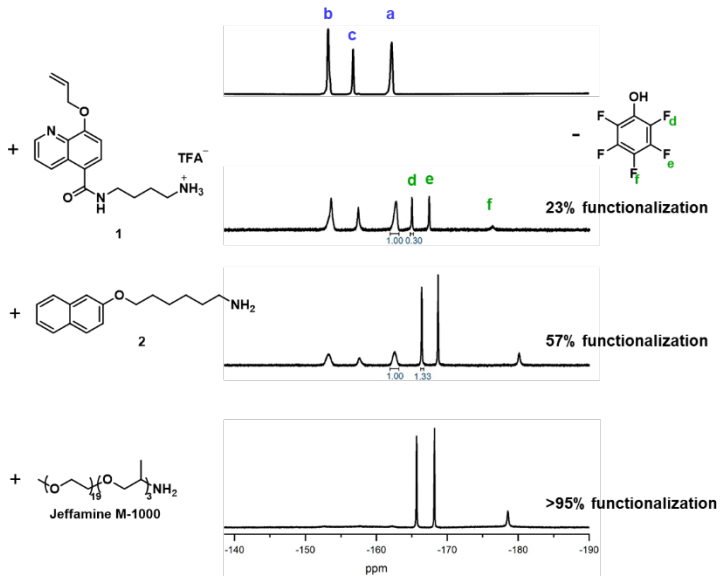


Figure S1. ^{19}F NMR in $CDCl_3$ of aliquots of post-polymerization functionalization of pPFPA with amines **1**, **2**, and Jeffamine M-1000 to obtain **P1**.

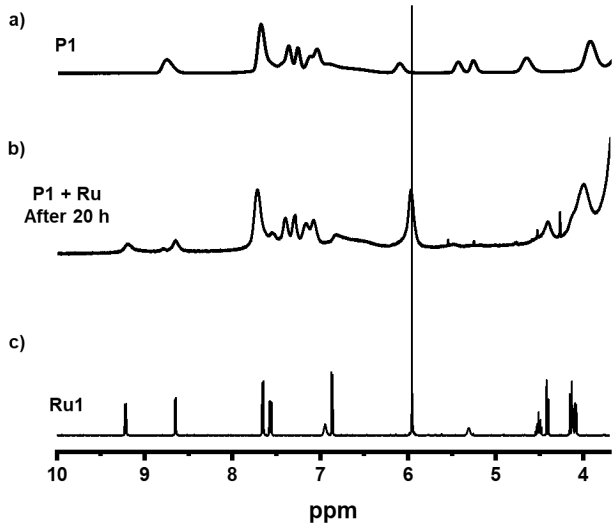


Figure S2. ^{1}H NMR spectra in CD_3CN of a) **P1**, b) after stirring **P1** with $[CpRu(MeCN)_3]PF_6$ for 20 h, and c) **Ru1**.

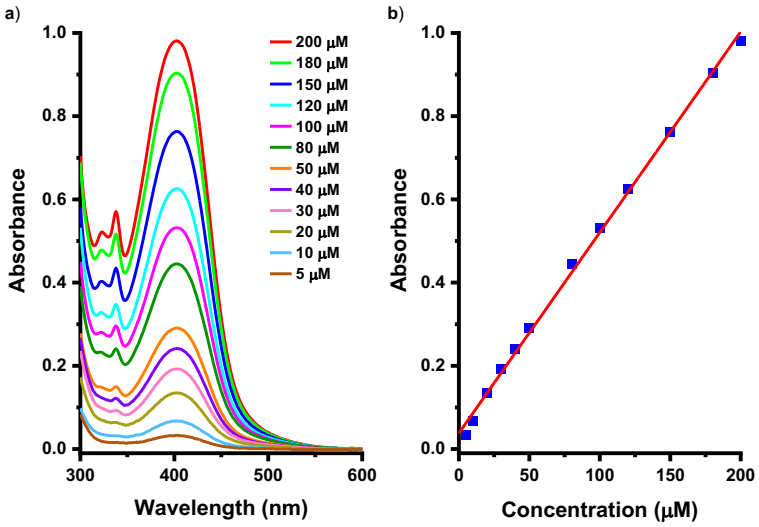


Figure S3. a) UV-vis spectra in MeCN of **Ru1** at concentrations ranging from 5 μM to 200 μM and b) calibration curve obtained from the plot of absorbance at 401 nm versus concentration.

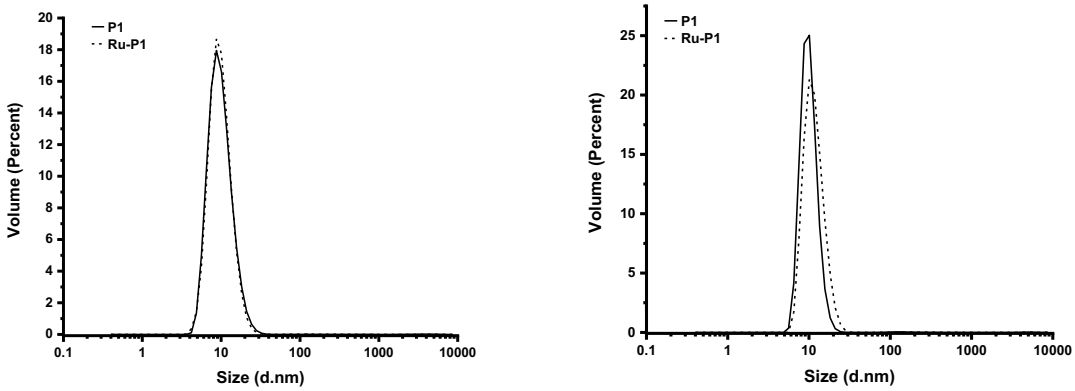


Figure S4. DLS curves of polymers **Ru-P1** and **P1** at 0.1 mg/mL (left) and 0.02 mg/mL (right) in PBS.

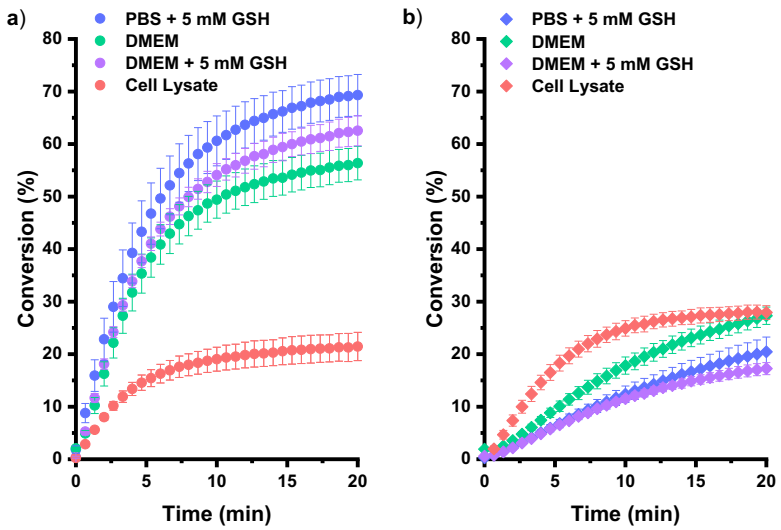


Figure S5. Fluorescence studies of Ru-catalyzed cleavage of allylcarbamates using a) **Ru-P1** and b) **Ru2** with or without 5 mM GSH. [**4**] = 20 μM, [**Ru-P1**] or [**Ru2**] = 5 μM in Ru, in PBS 1x or DMEM or HeLa cell lysate, room temperature, λ_{ex} = 375 nm, λ_{ex} = 440 nm. This is a remake of Figure 6 that includes error bars.

6. References

(1) T. Völker and E. Meggers, *ChemBioChem*, 2017, **18**, 1083–1086.

(2) R. Schiller, G. Scozzafava, A. Tumber, J. R. Wickens, J. T. Bush, G. Rai, C. Lejeune, H. Choi, T. L. Yeh, M. C. Chan, B. T. Mott, J. S. O. McCullagh, D. J. Maloney, C. J. Schofield and A. Kawamura, *ChemMedChem*, 2014, **9**, 566–571.

(3) T. J. M. Beenakker, D. P. A. Wander, W. A. Offen, M. Artola, L. Raich, M. J. Ferraz, K. Y. Li, J. H. P. M. Houben, E. R. Van Rijssel, T. Hansen, G. A. Van Der Marel, J. D. C. Codée, J. M. F. G. Aerts, C. Rovira, G. J. Davies and H. S. Overkleeft, *J. Am. Chem. Soc.*,

2017, **139**, 6534–6537.

(4) A. Das and P. Theato, *Macromolecules*, 2015, **48**, 8695–8707.

(5) Y. Liu, T. Pauloehrl, S. I. Presolski, L. Albertazzi, A. R. A. Palmans and E. W. Meijer, *J. Am. Chem. Soc.*, 2015, **137**, 13096–13106.