

Synthesis of rhodamine 6G-based compounds for the

ATRP synthesis of fluorescently-labeled biocompatible polymers

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Materials

Rhodamine 6G (99 %), *N*-(2-hydroxyethyl)piperazine (98.50 %) and lithium bromide (LiBr, 99 +%) were obtained from Acros Organics (Geel, Belgium) and were used as received. 3-Aminopropan-1-ol (99 %), 2-(methylamino)ethanol (99 %), sodium hydrogen carbonate (99.7 +%, A.C.S. grade), 2-bromoisobutyryl bromide (98 %), anhydrous methanol (MeOH, 99.8 %), CuBr (99.999 %), 2,2'-bipyridine (bpy, 99 %), morpholine (> 99%, ReagentPlus®), methacrylic acid (99 %), methacrylic anhydride (94 %), 2,6-di-*tert*-butyl-4-methylphenol (BHT, \geq 99 %), ethyl 2-bromoisobutyrate (EtOBr, 98 %), deuterated methanol (CD₃OD, 99.96 atom %), trifluoroacetic acid (TFA, 99+ %), triethylamine (Et₃N, \geq 99 %), and 2-(butylamino)ethanol (98+ %), 4-(2-Hydroxyethyl)morpholine (99 %) were all purchased from Sigma Aldrich UK (Dorset, UK) and were used as received. The silica gel 60 (0.063 – 0.200 μ m) used to remove the spent ATRP catalyst was purchased from E. Merck (Darmstadt, Germany) and was used as received. 2-Bromoisobutyric acid (>98 %) was obtained from Fluka (Dorset, UK) and was used as received. HPLC grade acetonitrile, diethyl ether, dichloromethane, methanol, tetrahydrofuran, isopropanol and n-heptane were obtained from Fisher Scientific (Loughborough, UK) and were used as received. Magnesium sulfate (MgSO₄), sodium chloride (NaCl), triethylamine (Et₃N) and sodium sulfate (Na₂SO₄) were laboratory reagent grade from Fisher Scientific (Loughborough, UK) and were used as received. Sodium bromide (NaBr, 99 + %) and hydrochloric acid (HCl, 32 %, general purpose grade) were purchased from Fisher Scientific (Loughborough, UK) and were used as received.

2-(Methacryloyloxy)ethyl phosphorylcholine monomer (MPC, 99.9 % purity) was donated by Biocompatibles UK Ltd. (Farnham, UK) and was used as received. Phosphate-buffered saline (PBS) was prepared from tablets obtained from Oxoid (Basingstoke, UK). Regenerated cellulose

dialysis membrane (1,000 MWCO) was purchased from Spectra/Por. Disposable UV-grade cuvettes were obtained from Fisher Scientific (Loughborough, UK).

The 4-(2-bromoisobutyryl ethyl)morpholine initiator (MEBr) was prepared according to a previously published procedure.¹

Preparation of 2-bromoisobutyric anhydride

2-Bromoisobutyric anhydride was prepared according to a previously published procedure.² 2-Bromoisobutyric acid (10.0133 g, 60.0 mmol) was dissolved in dichloromethane (75 mL) and *N,N'*-dicyclohexylcarbodiimide (6.8106 g, 33.0 mmol) was added to this solution. The resulting opaque mixture was stirred at 25°C overnight. The precipitate was filtered off, and the filtrate was concentrated through rotary evaporation and precipitated into cold, dry *n*-heptane. The residue was filtered, washed with cold *n*-heptane, and dried under reduced pressure to give 4.18 g (44 %) of a white solid.

¹H NMR (CDCl₃): δ 1.99 (s, 12H, CH₃) ppm.

¹³C NMR (CDCl₃): δ 165.71, 54.90, 30.12 ppm.

Reaction between rhodamine 6G and 3-aminopropan-1-ol to give rhodamine 6G N-(3-hydroxypropyl)amide, *1*

This reaction was conducted according to a previously published procedure, except that DMF was substituted for acetonitrile.^{3,4} Rhodamine 6G (10.011 g, 20.7 mmol) was dissolved in 200 mL acetonitrile. To this deep red solution was added 3-aminopropan-1-ol (4.8 mL, 63 mmol). The reaction mixture became gradually heterogeneous and lost color. After 20 h, most of the solvent was evaporated on a rotary evaporator under reduced pressure to obtain a concentrated solution (approximately a third of its original volume) and the mixture was filtered. The solid was washed thoroughly with water and dried under vacuum till constant weight to give 8.71 g (89 %) of an off-white product, **1**.

¹H NMR (400 MHz, 3:1 CDCl₃: CD₃OD) δ 8.09 (m, 1H), 7.71 (m, 2H), 7.27 (m, 1H), 6.55 (s, 1H), 6.33 (s, 1H), 3.52 (t, 2H, J = 5.5 Hz), 3.41 (m, 6H), 2.09 (s, 6H), 1.52 (t, 6H, J = 7.09 Hz), 1.34 (m, 2H) ppm

^{13}C NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 172.02, 156.26, 154.54, 150.44, 135.65, 133.26, 131.07, 130.67, 126.63, 125.24, 120.99, 107.75, 99.23, 68.75, 61.39, 40.86, 38.92, 33.19, 19.07, 16.87 ppm

ESI-MS, m/z ($\text{M}+\text{H}$) $^+$ 472

Esterification of 1 with 2-bromoisobutyryl bromide to give rhodamine 6G N-(3-(2-bromoisobutyryl)propyl)amide, 2

To **1** (3.0 g, 6.3 mmol) was added acetonitrile (200 mL) and 32 % hydrochloric acid (1.0 mL, 10 mmol). On refluxing this mixture, a dark red solution was formed within 45 minutes. Then 2-bromoisobutyryl bromide (1.0 mL, 7.9 mmol) was added to the refluxing solution. After a further 2.5 h, an additional charge of 2-bromoisobutyryl bromide was added (0.5 mL, 4.0 mmol). After a total reaction time of 5 h, the reaction mixture was evaporated to afford a viscous oil. Addition of ether (100 mL) led to precipitation overnight at $-25\text{ }^\circ\text{C}$. The precipitate was dispersed in water and an excess of sodium hydrogen carbonate was added. After stirring for 3 h, the aqueous dispersion was extracted with dichloromethane (three 50 mL portions).

The combined organic extracts were dried over dry magnesium sulfate and filtered, washing the filtrate with dichloromethane. Evaporation and drying in vacuum afforded the desired ATRP initiator **2** (3.70 g, 94 %) in its neutral form. Although more than 90 % pure by ^1H NMR, further purification could be achieved by recrystallization from methanol with a few drops of water added to initiate precipitation.

^1H NMR (400 MHz, CDCl_3) δ 7.91 (m, 1H), 7.46 (m, 2H), 7.04 (m, 1H), 6.33 (s, 2H), 6.21 (s, 2H), 3.94 (t, 2H, $J = 6.0\text{ Hz}$), 3.21 (m, 6H), 1.90 (s, 6H), 1.87 (s, 6H), 1.51 (m, 2H), 1.32 (t, 6H, $J = 7.21\text{ Hz}$) ppm

^{13}C NMR (400 MHz, CDCl_3) δ 171.37, 168.11, 153.51, 151.73, 147.41, 132.42, 131.33, 128.49, 128.04, 123.80, 122.74, 117.97, 106.07, 96.54, 65.00, 63.79, 56.01, 38.38, 37.10, 30.70, 27.27, 16.70, 14.74 ppm

ESI-MS, m/z ($\text{M}+\text{H}$) $^+$ 622

General reaction between rhodamine 6G and a secondary amine

In a round-bottomed flask, rhodamine 6G (10.0 g, 0.021 mol) was dissolved in the secondary amine (10.0 g). The flask was fitted with a reflux condenser, placed under nitrogen and heated to 90 °C for approximately 24 h. After cooling, the solution was dissolved in the minimum amount of methanol and poured into 500 mL water. After filtering, the aqueous solution was saturated with sodium chloride and extracted with 50 mL aliquots of a 2:1 isopropanol:dichloromethane mixture until only a faint color remained in the aqueous phase. The combined organic phases were dried over anhydrous sodium sulfate, filtered and evaporated. The resulting solid was recrystallized from methanol. The hydrochloride salt or hydrobromide salt were prepared by dissolving this solid in water, adding 1.1 molar equivalents of the corresponding concentrated acid and freeze-drying the aqueous solution overnight.

Reaction between rhodamine 6G and 2-(methyamino)ethanol to give rhodamine 6G N-(2-hydroxyethyl)-N-methyl amide, 3

Yield: 52 % (as HBr salt)

¹H NMR (400 MHz, 3:1 CDCl₃: CD₃OD) δ 7.45 (m, 3H), 7.13 (m, 1H), 6.73 (m, 2H), 6.50 (m, 2H), 3.23 (q, 4H, J = 7.21 Hz), 3.03 (m, 4H), 2.71 (s, 1.7H), 2.44 (s, 1.3H), 1.91 (s, 6H), 1.13 (t, 6H, J = 7.21 Hz) ppm

¹³C NMR (400 MHz, 3:1 CDCl₃: CD₃OD) δ 173.22, 161.38, 160.14, 158.89, 140.02, 134.76, 134.15, 133.93, 133.67, 133.38, 131.53, 129.17, 117.53, 97.75, 63.11, 53.63, 42.33, 36.07, 21.02, 17.31 ppm

ESI-MS, m/z (M+H)⁺ 472

Accurate Mass (Calculated), (M): 471.251960 (471.252192) corresponding to an elemental composition of C₂₉H₃₃N₃O₃ (C₂₉H₃₃N₃O₃)

Reaction between rhodamine 6G and diethanolamine to give rhodamine 6G N-(bis(2-hydroxyethyl))amide, 4

Yield: 75 % (as HCl salt)

^1H NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 7.66 (m, 1H), 7.26 (m, 2H), 7.19 (m, 1H), 6.80 (s, 2H), 6.56 (s, 2H), 3.47 (t, 2H, $J = 5.50$ Hz), 3.33 (q, 4H, $J = 7.21$ Hz), 3.15 (m, 8H), 1.99 (s, 6H), 1.20 (m, 6H, $J = 7.21$ Hz) ppm

^{13}C NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 173.24, 160.42, 159.16, 158.90, 139.07, 133.84, 132.90, 132.56, 131.48, 130.33, 128.15, 116.71, 96.81, 62.26, 61.84, 55.46, 50.36, 41.43, 20.01, 16.44

ESI-MS, m/z ($\text{M}+\text{H}$) $^+$ 502

Accurate Mass (Calculated), ($\text{M}+\text{H}$) $^+$: 502.2685 (502.2706) corresponding to an elemental composition of $\text{C}_{30}\text{H}_{36}\text{N}_3\text{O}_4$ ($\text{C}_{30}\text{H}_{36}\text{N}_3\text{O}_4$)

Reaction between rhodamine 6G and N-(2-hydroxyethyl)piperazine to give rhodamine 6G N-(4-(2-hydroxyethyl)piperazine) amide, 5

Yield: 65 % (in neutral form)

^1H NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 7.52 (m, 2H), 7.37 (m, 1H), 7.16 (m, 1H), 6.73 (s, 2H), 6.55 (s, 2H), 3.46 (t, 1H, $J = 5.50$ Hz), 3.40 (t, 2H, $J = 5.62$ Hz), 3.29 (q, 7.27 Hz), 3.16 (br m, 2H + MeOH), 2.94 (br t, 2H, $J \sim 5.1$ Hz), 2.54 (br t, 2H, $J \sim 5.0$ Hz), 2.40 (t, 1H, $J = 5.38$ Hz), 2.24 (t, 2H, $J = 5.62$ Hz), 1.97 (s, 6H), 1.17 (t, 6H, $J = 7.21$ Hz) ppm

^{13}C NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 170.42, 160.21, 159.08, 157.63, 138.00, 136.75, 133.94, 133.05, 132.82, 132.27, 130.42, 128.20, 116.52, 96.66, 61.20, 55.50, 52.98, 46.41, 41.25, 20.00, 16.23 ppm

ESI-MS, m/z ($\text{M}+\text{H}$) $^+$ 527

Accurate Mass (Calculated), ($\text{M}+\text{H}$) $^+$: 527.3026 (527.3022) corresponding to an elemental composition of $\text{C}_{32}\text{H}_{39}\text{N}_4\text{O}_3$ ($\text{C}_{32}\text{H}_{39}\text{N}_4\text{O}_3$)

Reaction between rhodamine 6G and 2-(butylamino)ethanol to give rhodamine 6G N-(4-hydroxy butyl)-N-methyl amide, 6

Yield: 56 % (in HCl salt form)

Recrystallized from chloroform

^1H NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 7.50 (m, 3H), 7.19 (m, 1H), 6.83 (ss, 2H), 6.58 (ss, 2H), 3.41 (br t, 1H, $J = 5.75$ Hz), 3.33 (q, 4H, $J = 7.21$ Hz), 3.08 (br t, 1H, $J = 5.75$ Hz), 2.97 (br m, 2H), 2.90 (br t, 1H, $J = 5.38$ Hz), 2.81 (m, 1H), 2.00 (ss, 6H), 1.20 (t, 6H, $J = 7.21$ Hz), 0.95 (br q, 1H, $J = 7.46$ Hz), 0.61 (br m, 4H), 0.46 (br t, 2H, $J = 6.11$ Hz) ppm

^{13}C NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 168.9, 159.0, 157.3, 156.1, 136.7, 131.0, 129.8, 129.2, 128.0, 126.9, 125.6, 123.5, 114.0, 94.0, 60.4, 57.8, 51.1, 48.3, 38.6, 30.6, 27.8, 19.9, 18.1, 13.9 ppm

ESI-MS, m/z ($\text{M}+\text{H}$) $^+$ 514

Accurate Mass (Calculated), ($\text{M}+\text{H}$) $^+$: 514.3055 (514.3070) corresponding to an elemental composition of $\text{C}_{32}\text{H}_{40}\text{N}_3\text{O}_3$ ($\text{C}_{32}\text{H}_{40}\text{N}_3\text{O}_3$)

Reaction between rhodamine 6G and morpholine to give rhodamine 6G N-morpholinamide, 11

Yield: 10 % (in neutral form)

Recrystallized from dichloromethane

^1H NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 7.54 (m, 2H), 7.39 (m, 1H), 7.18 (m, 1H), 6.73 (s, 2H), 6.57 (s, 2H), 3.30 (q, 4H, $J = 7.21$ Hz), 3.27-3.10 (m, br, 8H), 1.98 (s, 6H), 1.19 (t, 6H, $J = 7.21$ Hz) ppm

^{13}C NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 171.70, 161.31, 160.19, 158.640, 138.72, 135.07, 134.22, 134.07, 134.04, 133.32, 131.57, 129.30, 117.64, 97.86, 70.36, 46.11, 42.42, 21.04, 17.37 ppm

ESI-MS, m/z ($\text{M}+\text{H}$) $^+$ 484

Reaction between hydroxy-functional rhodamine derivatives and 2-bromoisobutyric anhydride to give a monofunctional ATRP initiator using 2-bromoisobutyric acid as solvent.

In a round-bottomed flask was placed hydroxy-functional rhodamine derivative (3 or 5) (neutral form, 0.50 mg, ~ 1 mmol) and 5.0 g 2-bromoisobutyric acid (30 mmol, 30 equivalent). The mixture was placed under nitrogen and heated to the stated temperature (see below). Once a

homogeneous solution had formed, 2-bromoisobutyric anhydride (635 mg, 2.0 mmol, 2 equivalent) was added. After 24 to 48 h, no further reaction occurred and the reaction mixture was cooled to room temperature and diethyl ether (100 mL) was added. After filtration and washing with diethyl ether, the solid residue was partitioned between dichloromethane (100 mL) and water (50 mL). Sodium hydrogen carbonate was added until gas evolution ceased and the aqueous phase was washed with aliquots of dichloromethane (3 x 50 mL). The combined organics were washed with water (five 50 mL portions) and finally with a saturated sodium bromide solution (50 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated. The crude product was recrystallized from THF.

Reaction between 3 and 2-bromoisobutyric anhydride to give a monofunctional initiator, rhodamine 6G N-(2-(2-bromoisobutyryl)- ethyl)-N-methyl amide, 7

Temperature: 70 °C. Yield 0.327 g (53 %) of neutral product after recrystallization.

¹H NMR (400 MHz, 3:1 CDCl₃: CD₃OD) δ 7.74 (m, 2H), 7.64 (m, 1H), 7.42 (m, 1H), 7.00 (m, 2H), 6.80 (m, 2H), 3.90 (t, 2H, J = 5.26 Hz), 3.49 (m, 5 H), 2.98 (s, 3H), 2.16 (s, 6H), 1.81 (s, 6H), 1.36 (t, 6H, J = 7.34) ppm

¹³C NMR (400 MHz, 3:1 CDCl₃: CD₃OD) δ 171.36, 168.93, 157.22, 156.03, 154.58, 135.64, 130.84, 130.23, 129.97, 129.83, 129.30, 127.57, 125.42, 113.57, 93.90, 67.89, 63.41, 49.84, 46.529, 38.42, 30.50, 17.64, 13.64 ppm

ESI-MS, m/z (M+H)⁺ 620

Accurate Mass (Calculated), (M+H)⁺: 620.2103 (620.2124) corresponding to an elemental composition of C₃₃H₃₉BrN₃O₄ (C₃₃H₃₉BrN₃O₄)

Reaction between 5 and 2-bromoisobutyric anhydride to give a monofunctional initiator, rhodamine 6G N-(4-(2-(2-bromoisobutyryloxy)ethyl))piperazine amide, 8

Temperature: 50 °C. Yield: 0.420 g (66 %) of neutral product after recrystallization.

¹H NMR (400 MHz, 3:1 CDCl₃: CD₃OD) δ 7.98 (m, 2H), 7.84 (m, 1H), 7.63 (m, 1H), 7.18 (s, 2H), 6.99 (s, 2H), 4.48 (t, 2H, J = 5.50 Hz), 3.75 (q, 4H, J = 7.21 Hz), 3.62 (br m, 4H), 2.87 (t, 2H, J = 5.50 Hz), 2.59 (br m, 4H), 2.43 (s, 6H), 2.13 (s, 6H), 1.60 (t, 6H, J = 7.21 Hz) ppm

^{13}C NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 173.05, 169.14, 158.81, 157.68, 156.17, 136.58, 132.40, 131.67, 131.47, 131.41, 130.88, 129.02, 126.73, 115.06, 95.27, 69.26, 64.38, 57.09, 43.15, 39.84, 31.79, 26.80, 18.51, 14.78 ppm

ESI-MS, m/z ($\text{M}+\text{H}$) $^+$ 675

Accurate Mass (Calculated), ($\text{M}+\text{H}$) $^+$: 675.2534 (675.2546) corresponding to an elemental composition of $\text{C}_{36}\text{H}_{44}\text{BrN}_4\text{O}_4$ ($\text{C}_{36}\text{H}_{44}\text{BrN}_4\text{O}_4$)

Reaction between **5 and methacrylic anhydride to give a monofunctional monomer, rhodamine 6G N-(4-(2-(methacryloyloxy)ethyl))piperazine amide, **9****

In a round-bottomed flask was placed **5** (neutral form, 1.0051 g, 1.9 mmol) and methacrylic acid (20.0 mL, 20.3 g, 0.236 mol). To this mixture was added 50 mL chloroform and 12.6 mg BHT. Once a homogeneous solution had formed, methacrylic anhydride (2 mL, 2.07 g, 13.4 mmol) was added. After 40 h, 2 mL methanol was added to quench residual methacrylic anhydride and the reaction mixture was left for further 2 h. Chloroform was evaporated at 30 °C under reduced pressure and the residue was poured into 200 mL diethyl ether. After filtration and washing with diethyl ether, the solid residue was partitioned between dichloromethane (100 mL) and water (50 mL). Sodium hydrogen carbonate was added until gas evolution ceased and the aqueous phase was washed with aliquots of dichloromethane (3 x 50 mL). The combined organics were washed with water (five 50 mL portions) and finally with a saturated sodium bromide solution (50 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated. This product was found to be $\geq 95\%$ pure by ^1H NMR. If necessary, the product could be recrystallized from THF.

Yield: 0.859 g (76 %) of neutral product after precipitation from diethyl ether.

^1H NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 7.78 (m, 2H), 7.65 (m, 1H), 7.46 (m, 1H), 7.00 (s, 2H), 6.85 (s, 2H), 6.09 (s, 1H), 5.63 (s, 1H), 4.23 (t, 2H, $J = 5.62$ Hz), 3.55 (q, 4H, $J = 7.15$ Hz), 3.41 (br m, 4H), 2.65 (t, 2H, $J = 5.75$ Hz), 2.37 (br m, 2H), 2.32 (br m, 2H), 2.21 (s, 6H), 1.93 (br s, 3H), 1.40 (t, 6H, $J = 7.21$ Hz) ppm

^{13}C NMR (400 MHz, 3:1 CDCl_3 : CD_3OD) δ 173.16, 167.20, 156.97, 156.08, 153.43, 136.05, 131.51, 130.28, 129.69, 128.97, 127.51, 126.05, 119.66, 113.49, 93.64, 61.84, 56.32, 53.47, 52.69, 47.53, 41.79, 38.44, 19.80, 18.52, 13.76 ppm

ESI-MS, m/z (M+H)⁺ 595

Accurate Mass (Calculated), (M+H)⁺: 595.3275 (595.3284) corresponding to an elemental composition of C₃₆H₄₃N₄O₄ (C₃₆H₄₃N₄O₄)

Polymerisation of MPC using a rhodamine-based initiator

In a typical experiment, **7** (0.17 mmol, 1.0 equivalent) and MPC (1.00 g, 3.38 mmol, 20 equivalents) was dissolved in 1.5 mL anhydrous methanol. After purging the solution for 20 minutes, CuBr (24.3 mg, 0.17 mmol, 1.0 equivalent) and bpy (52.9 mg, 0.34 mmol, 2.0 equivalents) were added. After 1.5 h, methacrylic protons were no longer detected by ¹H NMR and the reaction mixture was diluted with methanol and exposed to air. The homogeneous solution was then diluted with methanol and passed through a silica column to remove the spent copper catalyst. The dark red solution was evaporated and washed thoroughly with THF to remove residual bpy followed by acetonitrile to remove any unreacted initiator. Finally the polymer was redissolved in water and freeze-dried overnight, followed by drying in a vacuum oven at 80 °C for two days. Yield: **~75 %**.

These polymers were also efficiently purified by dialysis against methanol, typically using dialysis membranes with a MWCO of 1,000 Da.

Preparation of PMPC₂₅-PDPA₉₀ pH-responsive diblock copolymer using ATRP initiator **7 (Table 2, entry 9)**

MPC (1.00 g, 3.34 mmol, 25 equivalents) under nitrogen was dissolved in anhydrous methanol (1.5 mL) containing **7** (83.7 mg, 0.135 mmol, 1 equivalent) and purged with nitrogen for 20 minutes. Then bpy (42.7 mg, 0.273 mmol, 2 equivalents) and CuBr (19.7 mg, 0.137 mmol, 1 equivalent) were mixed as solids and added. After 37 minutes a nitrogen-purged solution of DPA (2.60 g, 12.2 mmol, 90 eq.) in anhydrous methanol (4 mL) was added to the polymerizing solution via cannula. After 63 h, the reaction mixture was exposed to air and diluted with isopropanol. The homogeneous solution was then passed through a silica column using isopropanol as eluent to remove the spent catalyst and evaporated at 60 °C under reduced pressure. The dark red residue was washed thoroughly with acetonitrile. Then the solid was dispersed in water followed by careful evaporation of the water under reduced pressure at 50 °C. This procedure was repeated twice. Finally the solid was dispersed in water and freeze-dried overnight, followed by drying in a vacuum oven at 80 °C for two days.

Preparation of PMPC₂₅-(PDPA₆₆-9**) pH-responsive diblock copolymer using monomer **9** (Table 2, entry 10)**

MPC (1.00 g, 3.34 mmol, 25 equivalents) under nitrogen was dissolved in anhydrous ethanol (1.5 mL) containing MEBR (37.9 mg, 0.135 mmol, 1 equivalent) and purged with nitrogen for 20 minutes. Then bpy (42.7 mg, 0.273 mmol, 2 equivalents) and CuBr (19.7 mg, 0.137 mmol, 1 equivalent) were mixed as solids and added. After 60 minutes a nitrogen-purged solution of DPA (2.02 g, 9.48 mmol, 70 eq.) and **9** (80.4 mg, 0.135 mmol, 1 equivalent) in anhydrous ethanol (3 mL) was added to the polymerizing solution via cannula. After 48 h, the reaction mixture was exposed to air and diluted with isopropanol. The homogeneous solution was then passed through a silica column using ethanol as eluent to remove the spent catalyst and concentrated at 60 °C under reduced pressure. The solution was dialysed against ethanol, methanol and water, followed by freeze-drying overnight and drying in a vacuum oven at 80 °C for two days.

Gel permeation chromatography

Chromatograms were assessed using two different protocols: 1: A Polymer Laboratories PL-GPC50 integrated GPC system with a PL Aquagel-OH 40 and a PL Aquagel-OH 30 7.5x300 mm columns in series with a guard column. The eluent was a 0.2 M NaNO₃, 0.05 M TRISMA buffer at pH 7.0. A series of near-monodisperse PEO standards were used to create a calibration curve using the refractive index detector.

2: Hewlett Packard HP1090 Liquid Chromatograph and two Polymer Laboratories PL Gel 5 μ m Mixed-C 7.5 x 300 mm columns in series with a guard column at 40°C connected to a Gilson Model 131 refractive index detector. The eluent was a 3:1 v/v % chloroform/methanol mixture containing 2 mM LiBr at a flow rate of 1.0 ml min⁻¹. A series of near-monodisperse PMMA samples were used as calibration standards. Toluene (2 μ l) was added to all samples as a flow rate marker. Data analysis was carried out using CirrusTM GPC Software supplied by Polymer Laboratories.

Molar absorption coefficient determination

Solutions for measuring the molar absorption coefficient of the rhodamine derivatives were prepared by weighing approximately 20.0 mg of dye in a 25 mL or a 100 mL volumetric flask using a microbalance and filling to the mark with either 0.1 M aqueous HCl or methanol or methanol containing 0.1 % v/v trifluoroacetic acid. Serial dilution of these stock solutions using pipettes and volumetric flasks gave solutions with absorbances ranging between 1.0 and 1.5. Further sequential dilutions allowed evaluation of the molar absorption coefficient, which is expressed as an average of either two or three dilutions. Stock solutions for determining the polymer molecular weight were obtained in a similar fashion; polymers were weighed into a 100 mL volumetric flask and serially diluted to give solutions with absorbances ranging between 1.0 and 1.5. This 'maximum absorbance' solution was then further diluted and the Beer-Lambert law ($A = \epsilon \cdot c \cdot l$) was used to calculate the apparent initiator concentration for each solution, using the molar absorption coefficient, ϵ , at maximum wavelength ($\lambda = 538$ nm to 541 nm depending on the polymer and initiator as given in Table 1). The stated value of ϵ is an average of three measurements. A PC-controlled Perkin-Elmer Lambda 25 uv/visible absorption spectrophotometer was used to record spectra from 300 nm to 700 nm at a scan rate of 240 nm

min⁻¹ with a slit width of 1 nm. All measurements were performed using disposable UV-grade cuvettes.

pH-dependent absorption and emission of 1 and 3

1 and **3** (2-3 mg, 50 μ mol) in a 25 mL measuring flask was dissolved in 0.1 M HCl (25 mL). This solution was further diluted with 0.1 M HCl to give a final solution with an absorbance between 0.1 and 2.0 (approximately 10^{-4} to 10^{-5} M). A 2 mL aliquot was removed after measuring the solution pH using a calibrated pH meter (Hanna Instruments). The pH was then slowly increased using NaOH concentrations of 0.50 M, 0.05 M and 0.001 M. A 2 mL aliquot was removed at approximately every pH unit. Each aliquot was analyzed by uv/visible absorption spectroscopy, fluorescence spectroscopy and dynamic light scattering.

A PC-controlled Perkin-Elmer Lambda 25 uv/visible absorption spectrophotometer was used for recording spectra from 300 nm to 700 nm at a scan rate of 240 nm min⁻¹ with a slit width of 1 nm. A PC-controlled Fluoromax-3 fluorimeter was used for obtaining fluorescence spectra under the following conditions: excitation wavelength = 530 nm, emission scans from 540-700 nm at 240 nm min⁻¹, an excitation slit width of 5 nm and an emission slit width of 2.5 nm.

Determination of relative quantum yield

Relative quantum yields were determined using the procedure of Fery-Forgues and Lavabre.^{5,6} Around 10 mg sample was weighed on a microbalance (three significant digits) and dissolved in 25 mL PBS. The solution was diluted 10-fold with PBS to give a solution with absorption around 0.5. Concentrations determined by absorption spectroscopy using the data in table 1 corresponded well to the calculated concentrations. The solution was diluted ten times with PBS and the emission spectrum was obtained at 515 nm. Spectra were integrated in Microsoft excel. Rhodamine 6G in water was used as a standard ($\Phi=0.95$ in water).⁶

A PC-controlled Perkin-Elmer Lambda 25 uv/visible absorption spectrophotometer was used for recording spectra from 300 nm to 700 nm at a scan rate of 240 nm min⁻¹ with a slit width of 1 nm. A PC-controlled Fluoromax-3 fluorimeter was used for obtaining fluorescence spectra under the following conditions: excitation wavelength = 515 nm, emission scans from 530-800 nm at 120 nm min⁻¹, an excitation slit width of 2.5 nm and an emission slit width of 5 nm.

Reverse-phase high performance liquid chromatography

HPLC chromatograms were acquired using a Varian ProStar HPLC system consisting of an autosampler (Varian Model 410), a solvent delivery module (Varian Module 230) and a UV-detector (Varian Model 310). The column was either a 150 x 4.6 mm ProGemini 5 μ C18 110 Å or a 100 x 4.6 mm Thermo Hypersil Keystone 3 μ Betabasic-18. Chromatographic conditions: 95 % 0.1 % aqueous TFA:acetonitrile to 100 % acetonitrile in 20 minutes followed by equilibration for 10 minutes at the original conditions prior to injection of a new sample. Sample: Approximately 0.5 % solution in methanol, 5 μ l injected. Detection: UV at 254 nm. Data were collected with Star Chromatography Workstation system control version 6.20.

Analysis of initiator degradation under ATRP conditions

8 (approximately 10^{-4} mol determined to three significant figures) was dissolved in 2.0 mL anhydrous methanol or deuterated methanol. The reaction mixture was purged with nitrogen for 5 minutes. Then CuBr (1 equivalent) and bpy (2 equivalent) was mixed and added. Aliquots were taken out, diluted with aerated CD₃OD and analyzed by ¹H NMR and HPLC at regular intervals. In addition, selected samples were analyzed by electrospray mass spectroscopy (ESI-MS).

A similar procedure was followed for the control experiments; CuBr was either replaced with one equivalent of CuBr₂, or omitted altogether, whereas two equivalents of bpy ligand were used in all cases.

pH-dependent dynamic light-scattering, fluorescence and absorption of PMPC-PDPA diblock copolymers

In a typical procedure, diblock copolymer **7**-PMPC₂₅-PDPA₉₀ (50.0 mg) was dissolved in 0.1 M HCl (25.0 mL). A 2 mL aliquot was removed after measuring the pH using a calibrated pH meter (Hanna Instruments). The pH was then slowly increased using NaOH concentrations of 0.50 M, 0.05 M and 0.001 M. A 2 mL aliquot was removed at approximately every pH unit. Each aliquot was analyzed by uv/visible absorption spectroscopy, fluorescence spectroscopy and dynamic light scattering. A PC-controlled Perkin-Elmer Lambda 25 spectrophotometer was used for

recording spectra from 300 nm to 700 nm at a scan rate of 240 nm min⁻¹ with a slit width of 1 nm. A PC-controlled Fluoromax-3 fluorimeter was used for obtaining fluorescence spectra under the following conditions: excitation wavelength = 530 nm, emission scans from 540 to 700 nm at 240 nm/min, an excitation slit width of 5 nm and an emission slit width of 2.5 nm (Unless otherwise stated). Dynamic light scattering experiments were performed with a Zetasizer Nano-ZS (Malvern Instruments, UK) at a scattering angle of 173°. Dispersion Technology Software version 4.20 from Malvern Instruments was used for the data analyses.

Compound	λ_{\max} nm (methanol)	λ_{\max} nm (0.10 M HCl)	ϵ_{\max} $\text{cm}^{-1}\cdot\text{M}^{-1}$ (methanol)	ϵ_{\max} $\text{cm}^{-1}\cdot\text{M}^{-1}$ (0.10 M HCl)	ϵ_{\max} $\text{cm}^{-1}\cdot\text{M}^{-1}$ (PBS, pH 7.2)	ϕ (PBS, pH 7.2)
R6G	529	527	111,000 ± 900	-	86,700 ^{a)}	0.95 ^{a)}
1	529 ^{b)}	530 ^{c)}	109,000 $\pm 1,000$ ^{b)}	52,000 ± 800 ^{c)}	n/m ^{d)}	n/m ^{d)}
2	529 ^{b)}	530 ^{c)}	88,000 $\pm 3,000$ ^{b)}	34,000 $\pm 2,000$ ^{c)}	n/m ^{d)}	n/m ^{d)}
3	533	532	114,000 ± 600	87,000 $\pm 2,500$	93,000 $\pm 2,700$	0.93
4	533	533	116,000 ± 250	87,000 $\pm 3,900$	95,000 $\pm 3,400$	0.97
5	533	534	100,000 $\pm 2,300$	84,000 $\pm 2,500$	95,000 $\pm 2,700$	0.80
6	534	533	100,000 ± 200	85,000 $\pm 5,600$	67,000 $\pm 2,000$	0.91
7	534	534	84,000 $\pm 1,900$	67,000 $\pm 1,200$	65,000 $\pm 2,400$	0.27
8	534	535	87,000 ± 850	89,000 $\pm 2,100$	87,000 $\pm 4,900$	0.55
9	534	534	93,000 $\pm 1,100$	91,000 $\pm 2,900$	73,000 $\pm 1,200$	0.43
10	533	532	87,000 $\pm 9,000$	90,000 ± 500	100,000 $\pm 2,200$	0.84

Table S1. Maximum wavelength and corresponding absorption coefficients recorded in methanol and in 0.10 M HCl for various rhodamine 6G derivatives. (a) This value was obtained from reference 6 and is obtained in pure water. (b) These measurements were performed in methanol containing 0.1 % v/v trifluoroacetic acid. (c) The dye was dissolved in 25.0 mL methanol containing 0.10 % v/v trifluoroacetic acid and diluted with 0.10 M aqueous HCl. The error is the standard error for three measurements. (d) These compounds were not soluble in PBS at pH 7.2.

Entry	Target Composition	M _n Target	¹ H NMR Composition ^{a)}	M _n ¹ H NMR ^{a)}	M _n GPC ^{b)}	M _w /M _n GPC ^{b)}	A _{max} Composition ^{c)}	M _n A _{max} ^{c)}	λ _{max} / nm ^{d)}
1	7-PMPC ₂₀	6,500	7-PMPC ₂₅	8,000	15,100	1.28	7-PMPC ₅₄	16,000	539
2	7-PMPC ₅₀	15,400	7-PMPC ₆₇	20,300	29,900	1.38	7-PMPC ₁₈₂	54,000	539
3	7-PMPC ₁₀₀	30,200	N/A ^{e)}	N/A	41,000	1.32	7-PMPC ₂₂₄	66,000	539
4	7-PMPC ₂₀₀	59,700	N/A ^{e)}	N/A	77,800	1.74	7-PMPC ₃₃₂	98,000	540
5	8-PMPC ₂₀	6,600	8-PMPC ₂₆	8,300	13,900	1.23	8-PMPC ₃₇	11,000	542
6	8-PMPC ₅₀	15,500	8-PMPC ₈₁	24,400	28,800	1.48	8-PMPC ₁₀₈	32,000	541
7	8-PMPC ₁₀₀	30,200	N/A ^{e)}	N/A	44,500	1.60	8-PMPC ₁₈₆	55,000	541
8	8-PMPC ₂₀₀	59,700	N/A ^{e)}	N/A	104,400	2.25	8-PMPC ₃₉₃	116,000	541
9	7-PMPC ₂₅ -PDPA ₉₀	27,200	7-PMPC ₂₅ -PDPA ₉₀	27,200	34,000 ^{f)}	1.22 ^{f)}	7-PMPC ₃₂ -PDPA ₁₁₅	34,000 ^{g)}	539
10	PMPC ₂₅ -P(DPA ₇₀ - 9 ₁) ^{h)}	22,800	N/A ^{e)}	22,000	20,100 ^{f)}	1.26 ^{f)}	PMPC ₂₅ -P(DPA ₆₆ - 9 ₁)	21,600 ^{g)}	546

Table S2. Summary of ¹H NMR, GPC and absorption data for various polymers prepared using the two rhodamine-based ATRP initiators, **7** and **8**. (a) ¹H NMR spectra recorded in d₄-methanol. (b) GPC data obtained in an aqueous Trisma buffer at pH 7. (c) M_n calculated from the ε_{max} value of the rhodamine compound recorded in 0.1 M HCl or PBS (see Table 1). (d) Wavelength for the absorption maximum recorded in water. (e) The signals assigned to rhodamine could not be integrated with sufficient precision to give the rhodamine content of this copolymer due to its relatively poor signal-to-noise ratio. (f) GPC data obtained in a 3:1 chloroform/methanol mixed eluent using poly(methyl methacrylate) calibration standards. (g) This value was obtained in 0.1 M HCl using the absorption coefficient of the initiator/monomer in this solvent. (h) This is a statistical copolymer where one equivalent of **9** was mixed with the DPA monomer prior to polymerization of the second block.

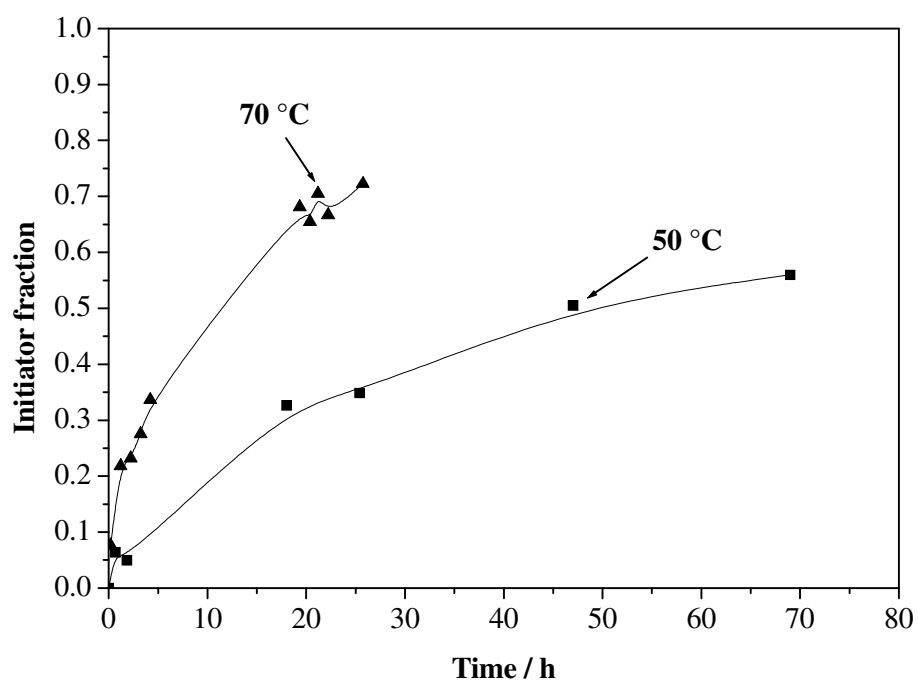


Figure S1: Kinetics of formation of rhodamine 6G-based initiator 7 versus time as determined by reverse phase HPLC.

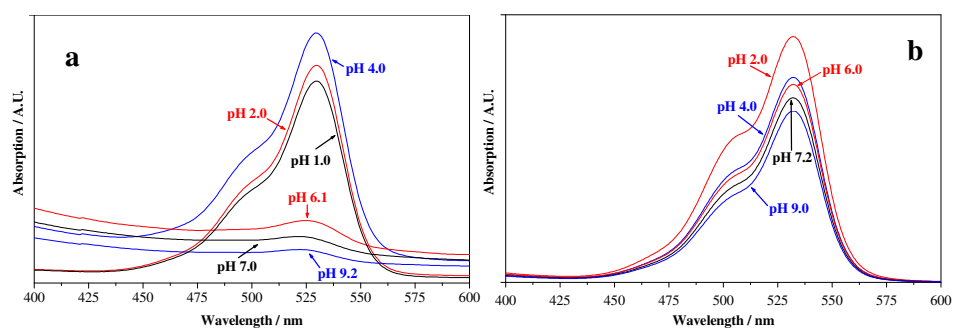


Figure S2: a) Absorption spectra of 1 versus pH. b) Absorption spectra of 3 versus pH

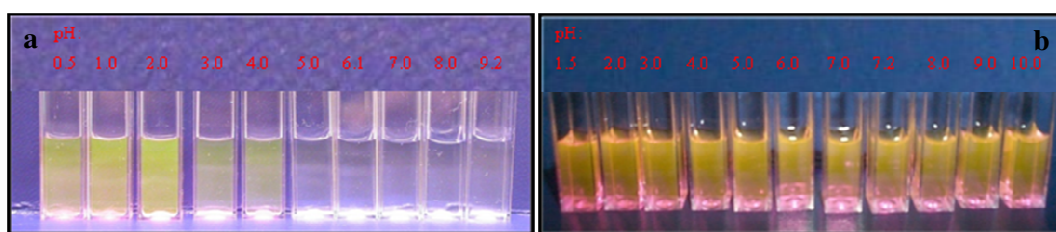


Figure S3: a) $5 \cdot 10^{-5}$ M 1 at different pH b) $1 \cdot 10^{-5}$ M 3 at different pH

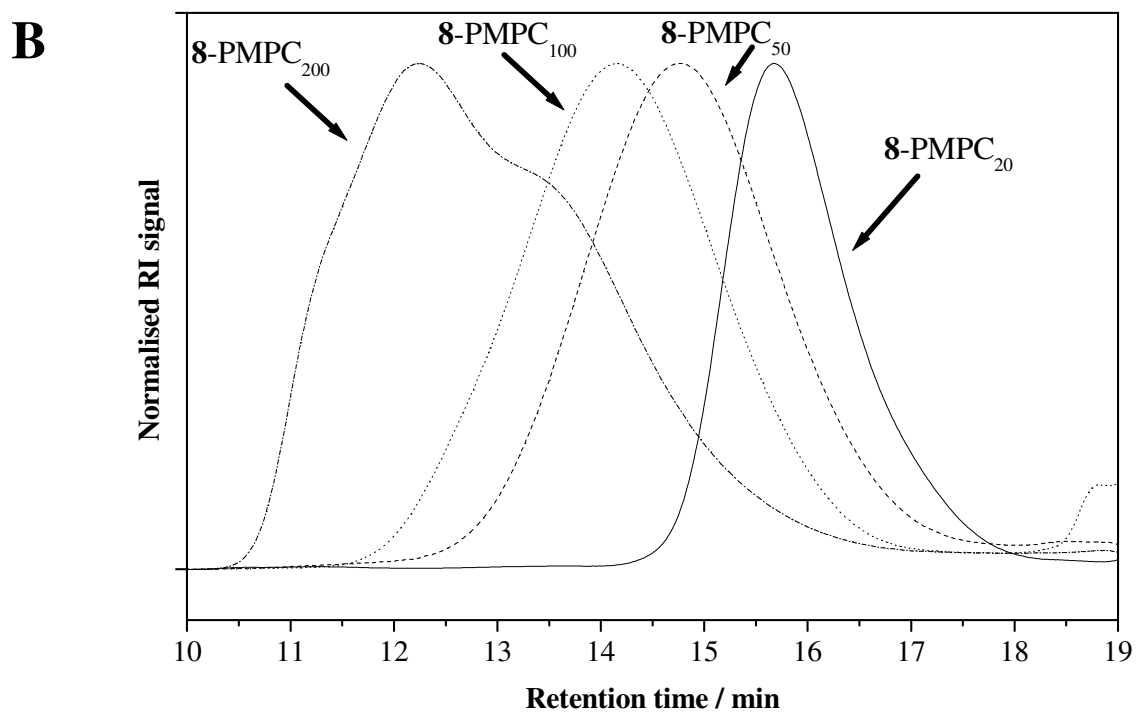
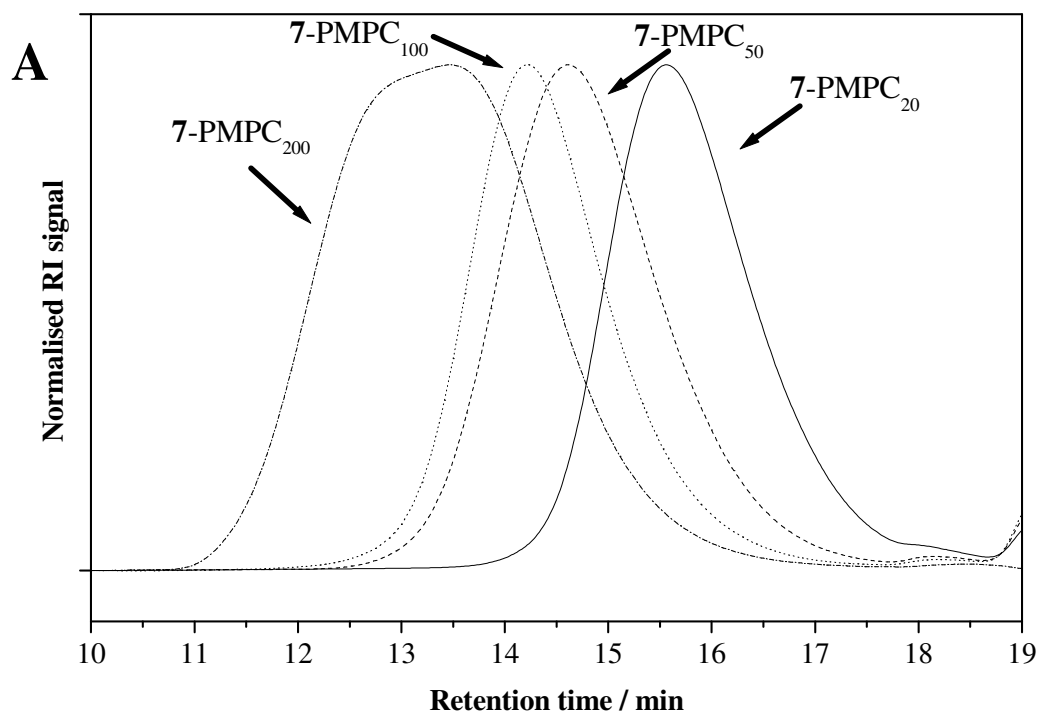


Figure S4: Aqueous gel permeation chromatograms of PMPC homopolymers based on A) Initiator 7 and B) initiator 8.

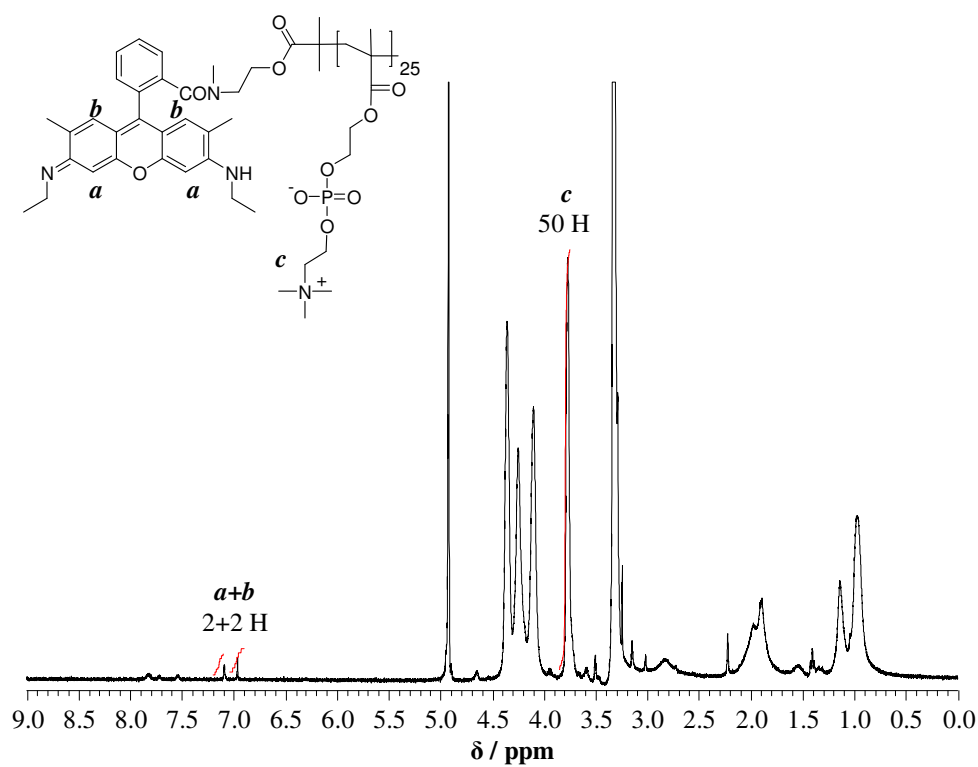


Figure S5: 400 MHz ¹H NMR spectrum in CD₃OD illustrating the peaks used for calculating the degree of polymerisation of 7-PMPC₂₅. These peaks were used for determining the degree of polymerisation when possible.

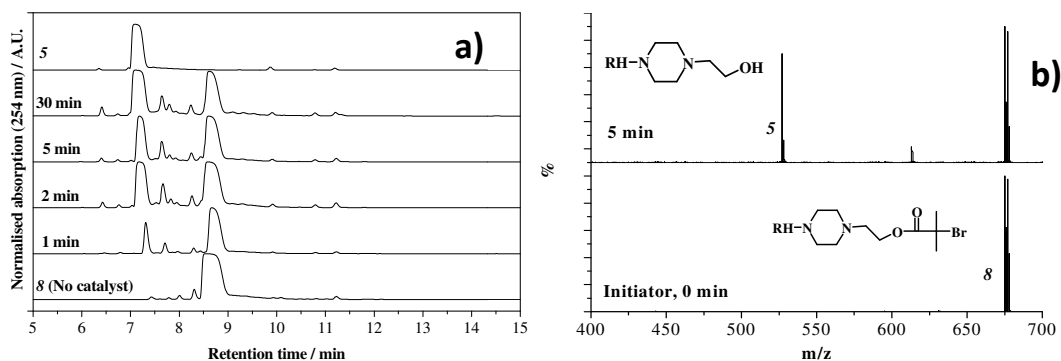


Figure S6: Analysis of the transesterification of 8 under ATRP conditions. a) HPLC chromatograms obtained for compound 5, kinetic samples of 8 with CuBr and bpy (8: CuBr: bpy = 1:1:2) and compound 8. b) ESI-MS of selected kinetic samples of 8 with CuBr and bpy (8: CuBr: bpy = 1:1:2) and initiator 8.

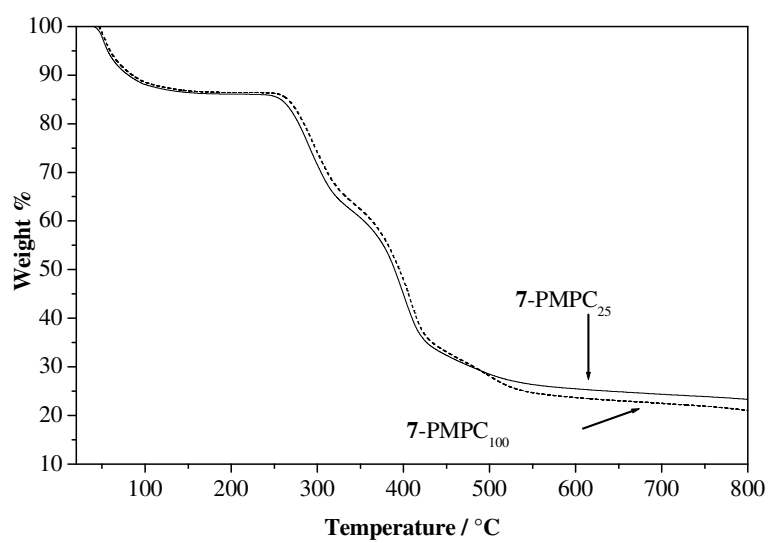


Figure S7: Weight loss as a function of heating in air of 7-PMPC₂₀ and 7-PMPC₁₀₀.

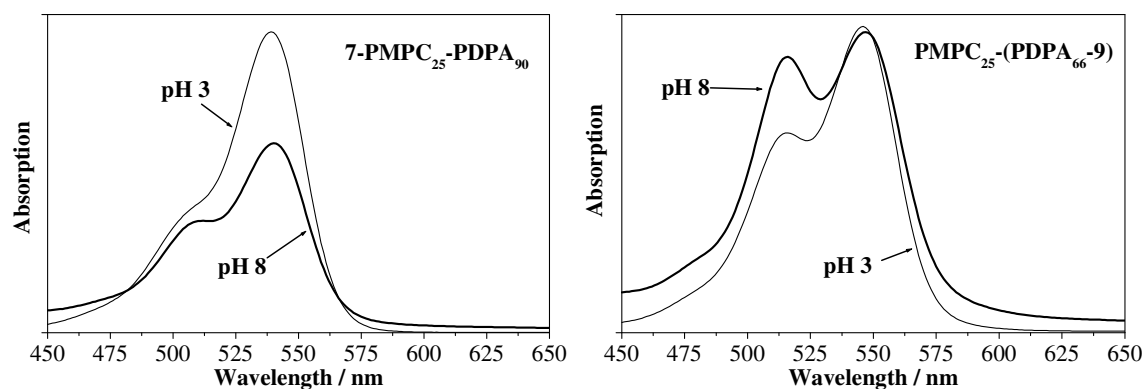


Figure S8: Absorption spectra of 7-PMPC₂₅-PDPA₉₀ and PMPC₂₅-(PDPA₆₆-9) at pH 3 and pH 8.

¹ Weaver, J. V. M., Bannister, I., Robinson, K. L., Bories-Azeau, X., Armes, S. P., Smallridge, M. , McKenna, P. *Macromolecules* **2004**, *37*, 2395-2403

² Östmark, E., Harrison, S., Wooley, K. L., Malmström, E. E. *Biomacromol.* **2007**, *8*, 1138-1148

³ Adamczyk, M., Grote, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1539-1541

⁴ Adamczyk, M., Grote, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2327-2330

⁵ Fery-Forgues, S., Lavabre, D. *Journal of Chemical Education* **1999**, *76*, 1260-1264

⁶ Nguyen, T., Francis, M.B., *Organic Letters*, **2003**, *5*, 3245-3248