Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Clickable Poly(ionic liquids): A Materials Platform for Transfection

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Supporting Information

Table of Contents

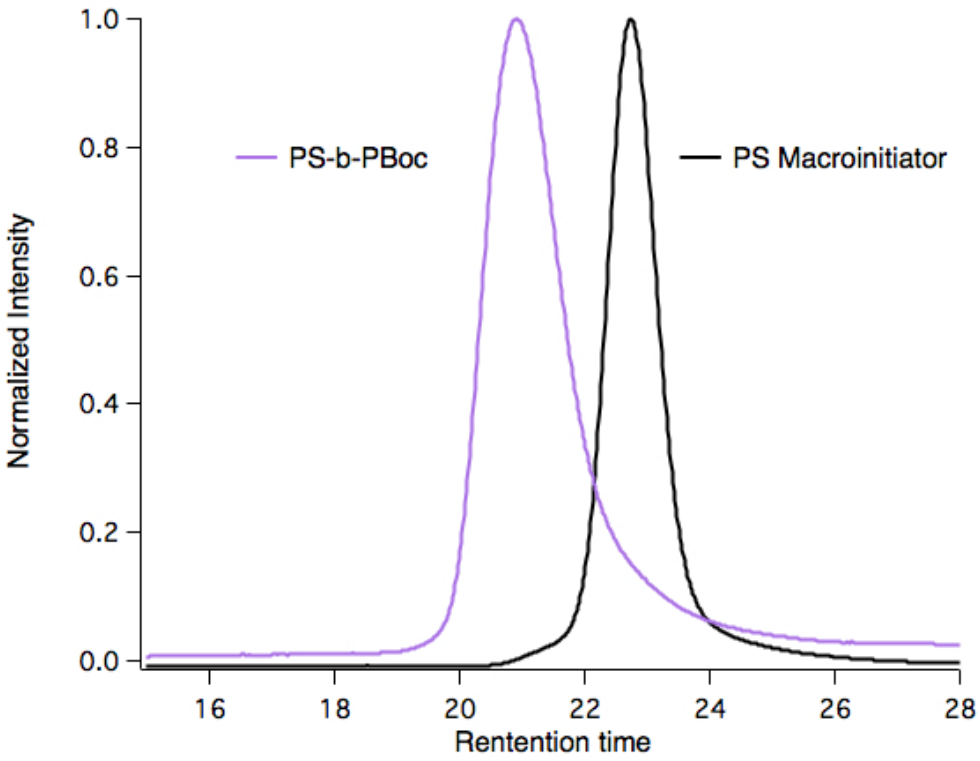
I. General Information: All materials were purchased from Sigma Aldrich and were used without further purification except as noted below. Methylene chloride (CH_2Cl_2) and *N*,*N*-dimethylformamide (DMF) were dried using a J.C. Meyer solvent purification system. Deuterated solvents used for NMR spectroscopy were purchased from Cambridge Isotope Laboratories, Inc. Eluents for column chromatography were HPLC grade and purchased from Fisher Scientific. Organic solutions were concentrated by use of a Buchi rotary evaporator. All polymerizations were carried out with temperature control via an oil bath under an argon atmosphere in Schlenk flasks.

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ (except where noted in Experimental Methods) on a Bruker AMX-300, AMX-400, or AMX-500 spectrometer. Data for ^{1}H NMR are reported as follows: chemical shift in reference to residual $CHCl_3$ at 7.26 ppm (δ ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, m = multiplet), coupling constant (Hz), and integration. Data for ^{13}C NMR are reported in terms of chemical shift in reference to the $CDCl_3$ solvent signal (77.16 ppm).

High-resolution mass spectra were obtained from the Columbia University Mass Spectrometry Facility on a JOEL JMSHX110 HF mass spectrometer using FAB+ ionization mode. Thin layer chromatography (TLC) was performed using Teledyne Silica gel 60 F254 plates and viewed under UV light. Flash column chromatography was performed using Teledyne Ultra Pure Silica Gel (230 – 400 mesh) on a Teledyne Isco Combiflash Rf.

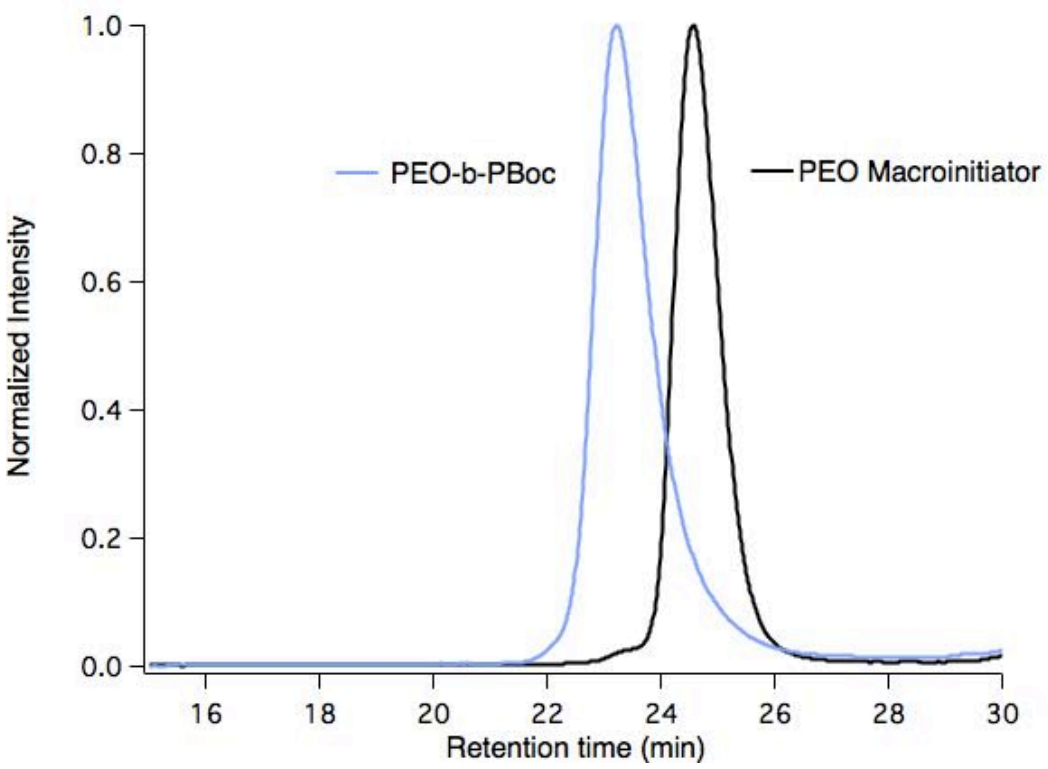
Polyplex size and zeta potential were measured on a Malvern Zetasizer Nano ZS (Malvern, United Kingdom). For all measurements, polyplexes were diluted 1:100 in Milli-Q water at neutral pH. The reported diameters are the average of three measurements, where each measurement comprises at least 10 acquisitions, and the zeta potential was calculated according to the Smoluchowski approximation.

II. Supplementary Figures



	MM (kD)	Dispersity *Đ*
PS Macroinitiator	19	1.05
PS-*b*-PBoc	42	1.14

Figure S1. GPC traces of polystyrene macroinitiator and PS-*b*-PBoc reveal narrow dispersity is maintained after copolymerization.



	MM (kD)	Dispersity (*Đ*)
PEO Macro-initiator	5 (purchased)	1.02
PEO-*b*-PBoc	13.8	1.05

Figure S2. GPC traces of poly(ethylene oxide) macroinitiator and PEO-*b*-PBoc reveal narrow dispersity is maintained after copolymerization.

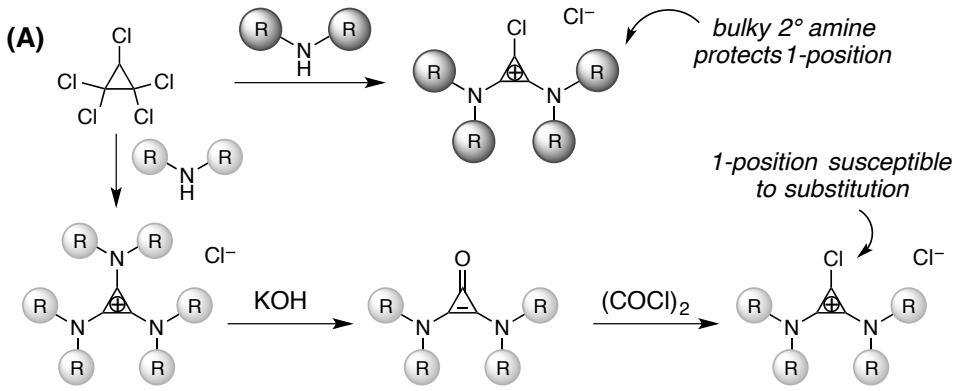


Figure S3. Synthetic path to obtain BACCl ClickabIL building blocks

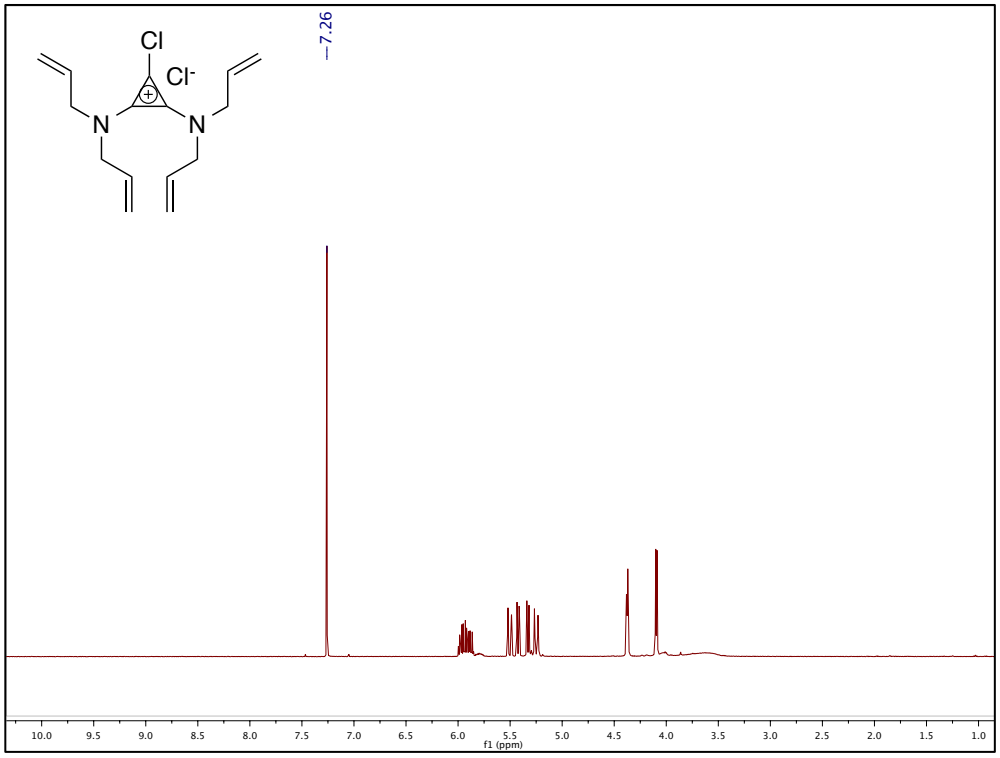


Figure S4. ^{1}H NMR spectrum of **bis-1,2-(diallylamino)-3-chlorocyclopropenium chloride**.

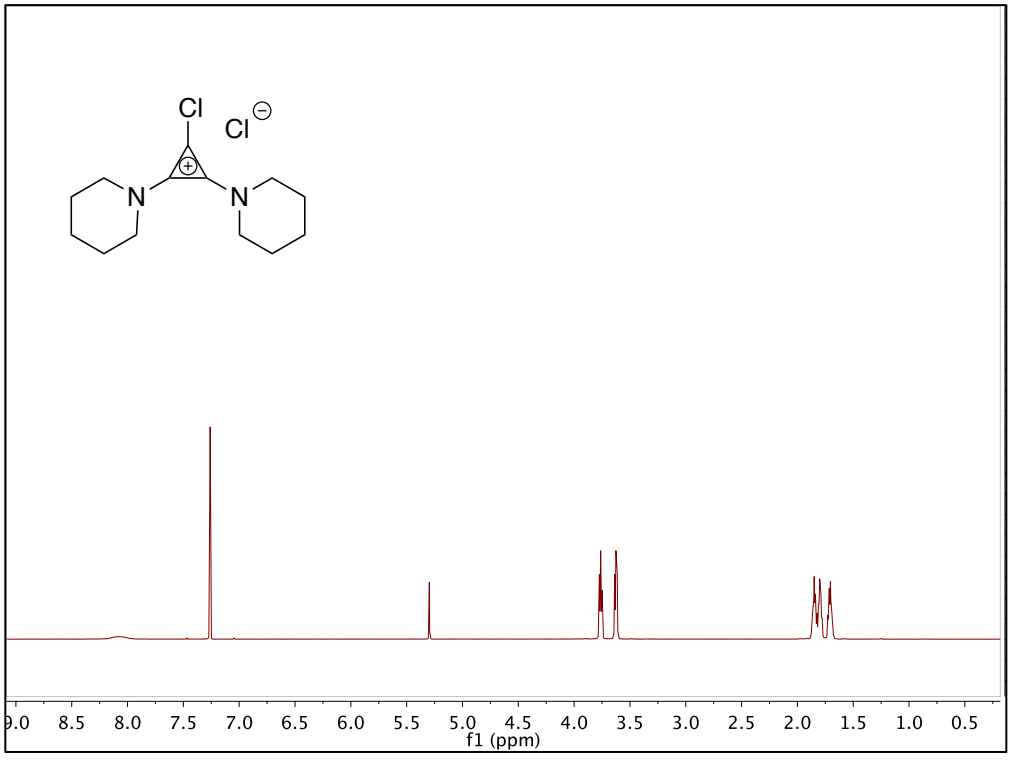


Figure S5. ^{1}H NMR spectrum of **bis-1,2-(piperidino)-3-chlorocyclopropenium chloride**

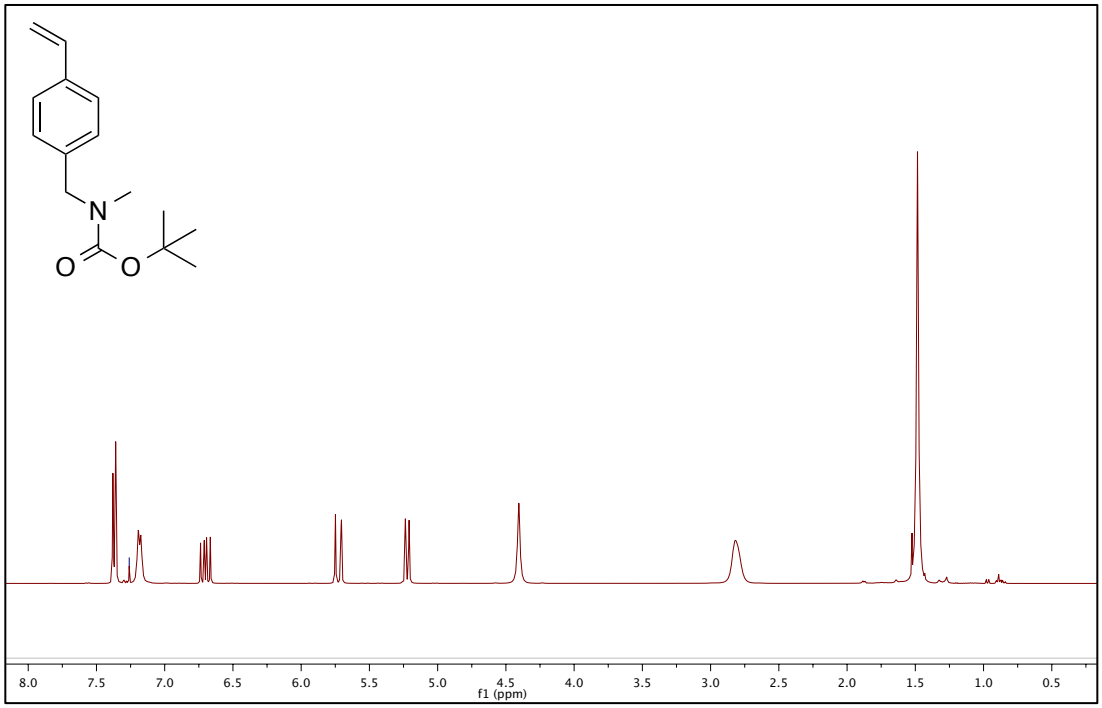


Figure S6. ^{1}H NMR spectrum of ***tert*-butyl methyl(4-vinylbenzyl)carbamate**.

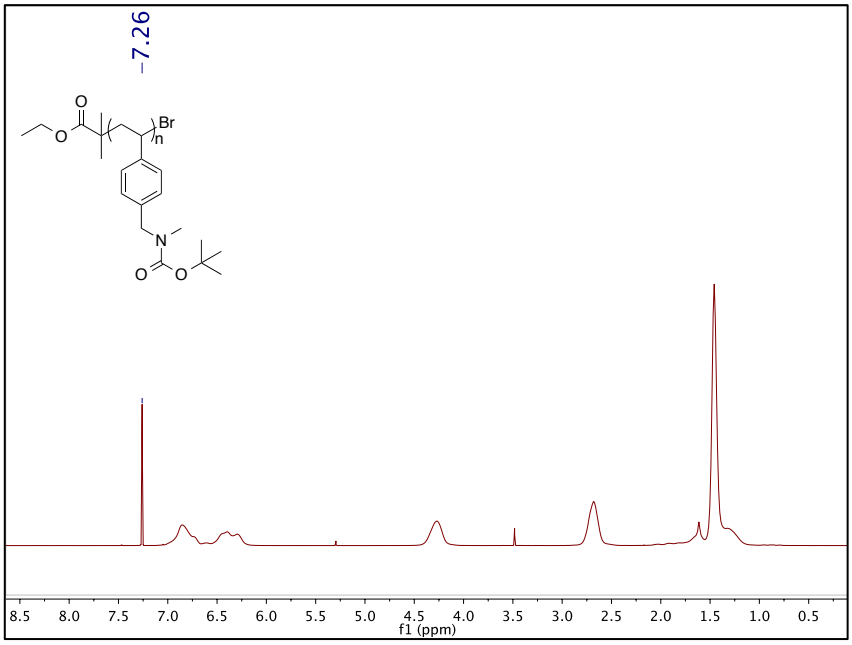


Figure S7. ^{1}H NMR spectrum of **PBoc**.

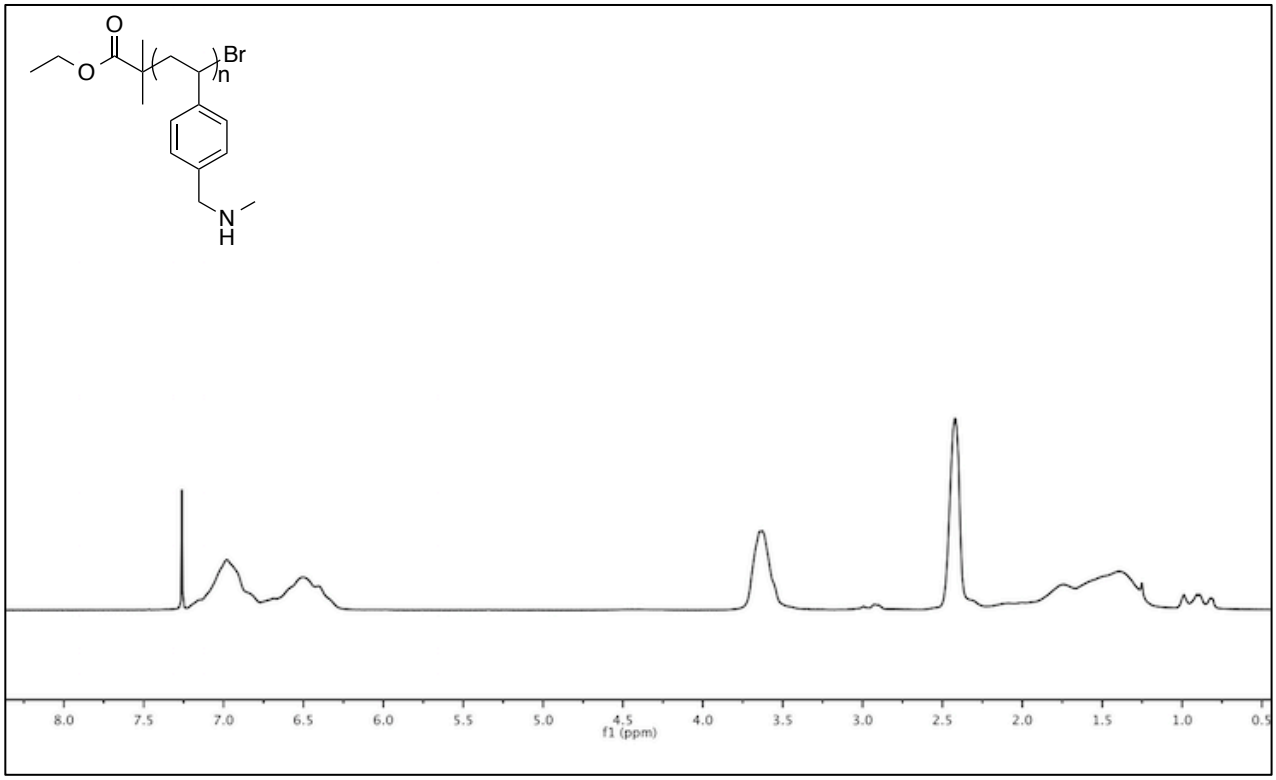


Figure S8. ^{1}H NMR spectrum of **PMAS**.

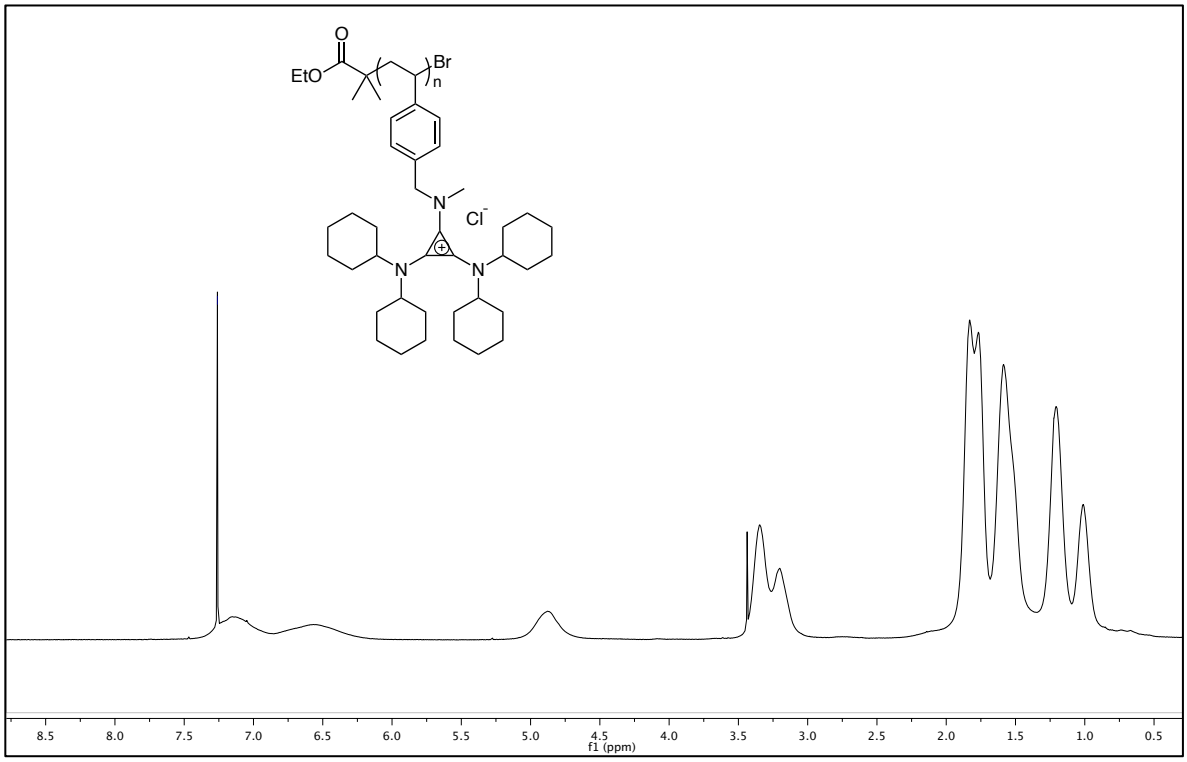


Figure S9. ^{1}H NMR spectrum of **PMAS(Cy)**.

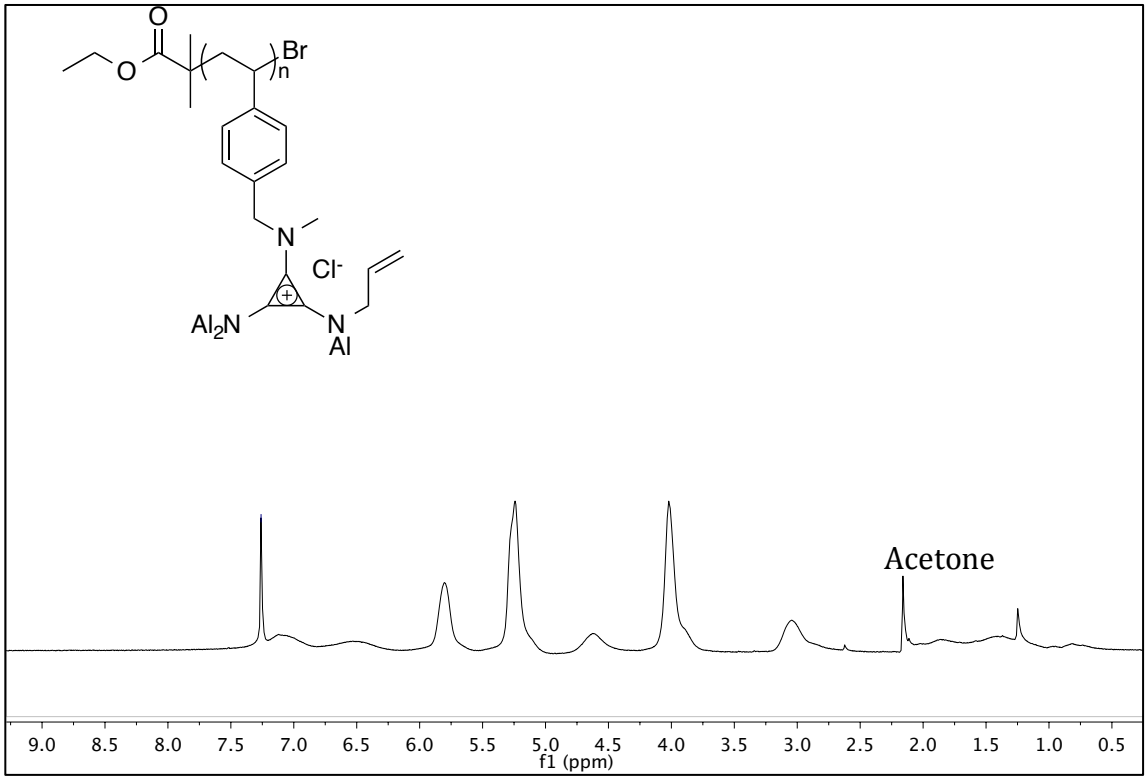


Figure S10. ^{1}H NMR spectrum of **PMAS(Al)**.

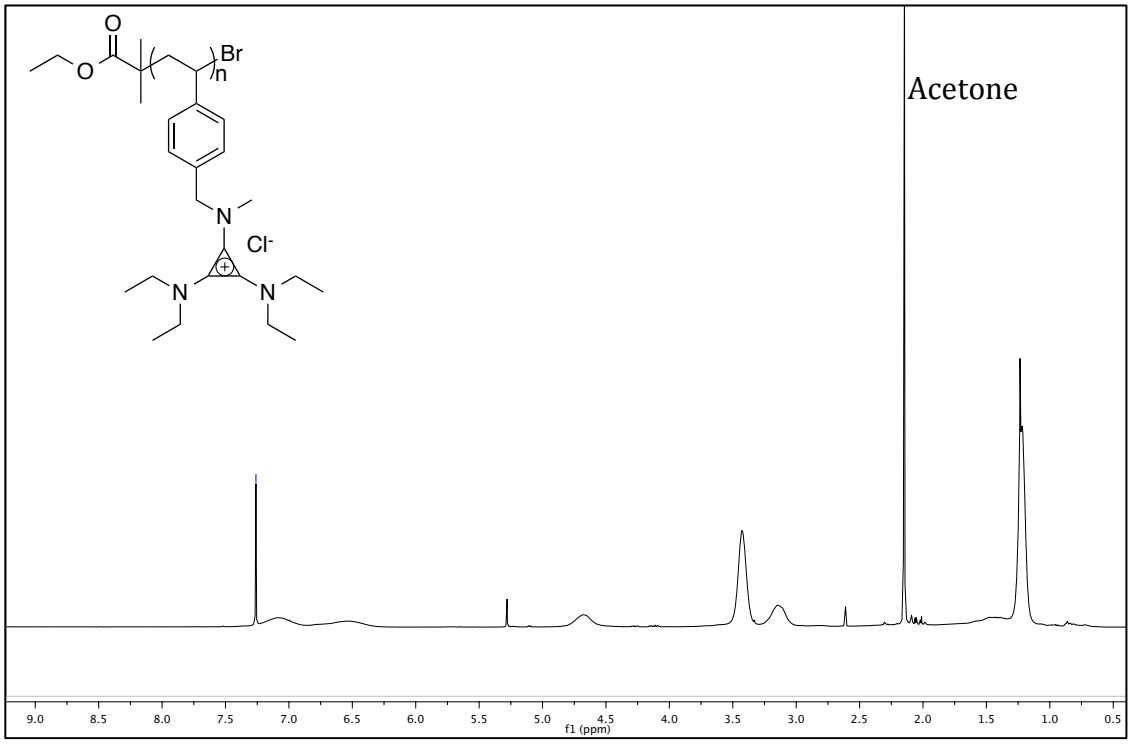


Figure S11. ^{1}H NMR spectrum of **PMAS(Et)**.

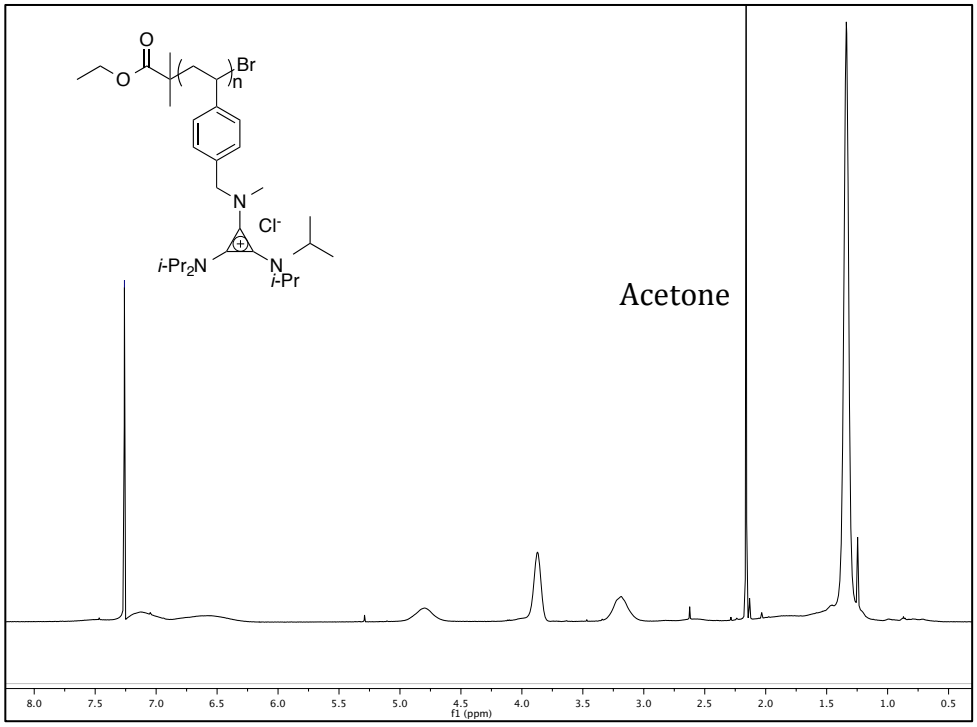


Figure S12. ^{1}H NMR spectrum of **PMAS(iP).**

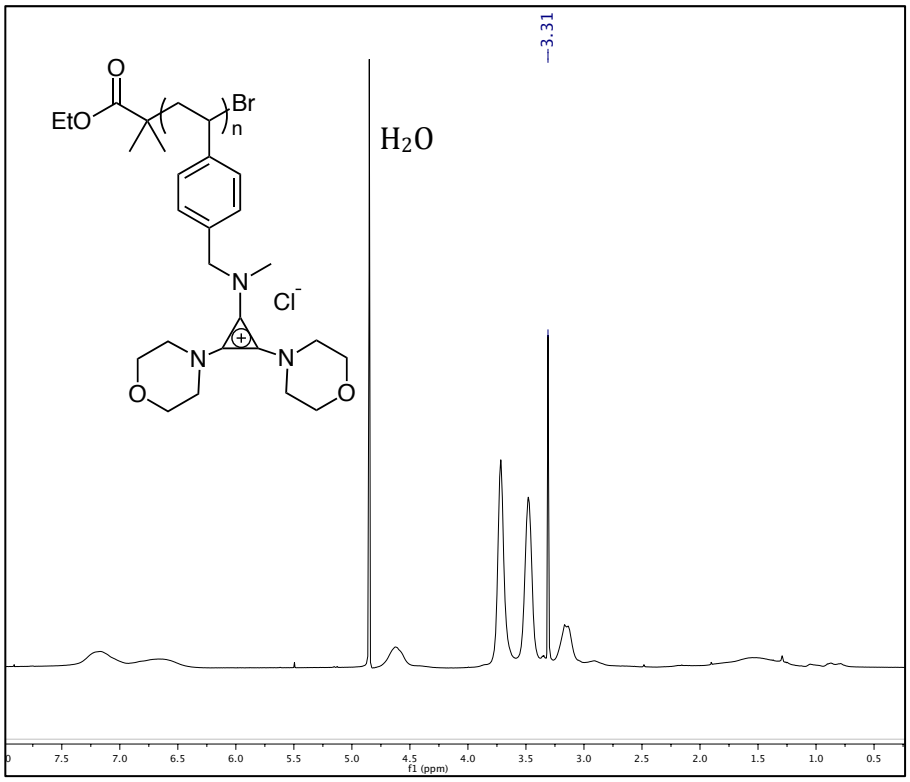


Figure S13. ^{1}H NMR spectrum of **PMAS(Mo)**

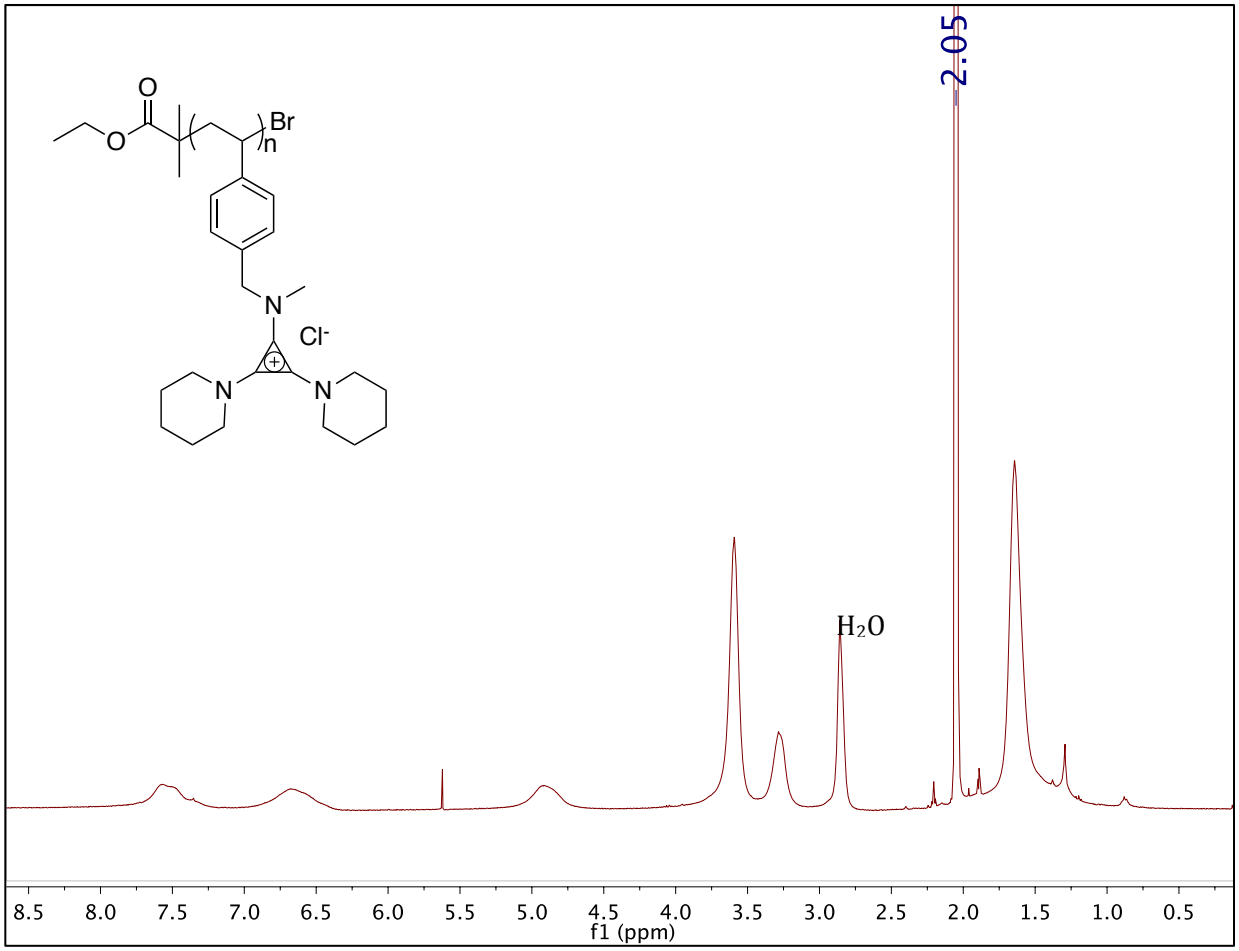


Figure S14. [1]H NMR spectrum of **PMAS(Pep)**

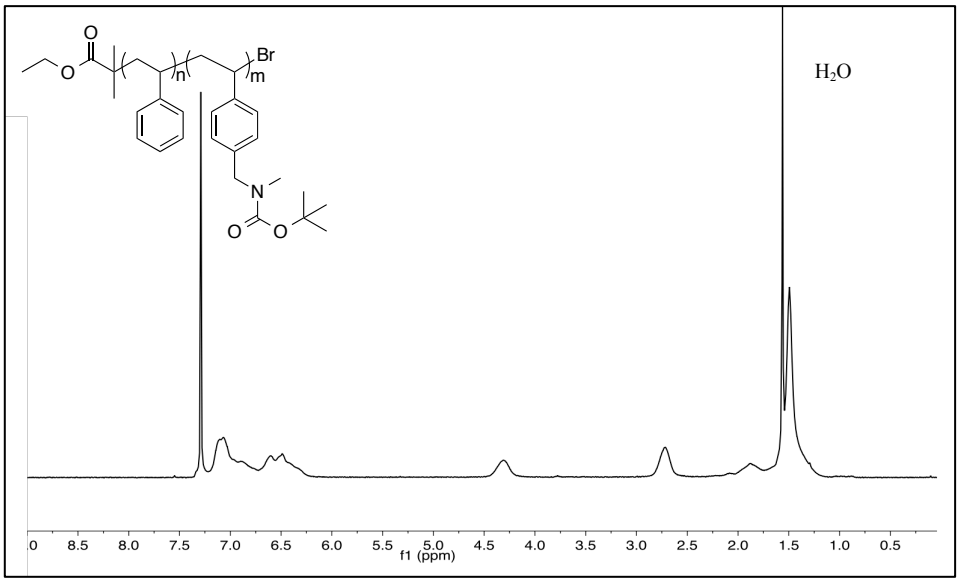


Figure S15. [1]H NMR spectrum of **PS-*b*-PBoc**.

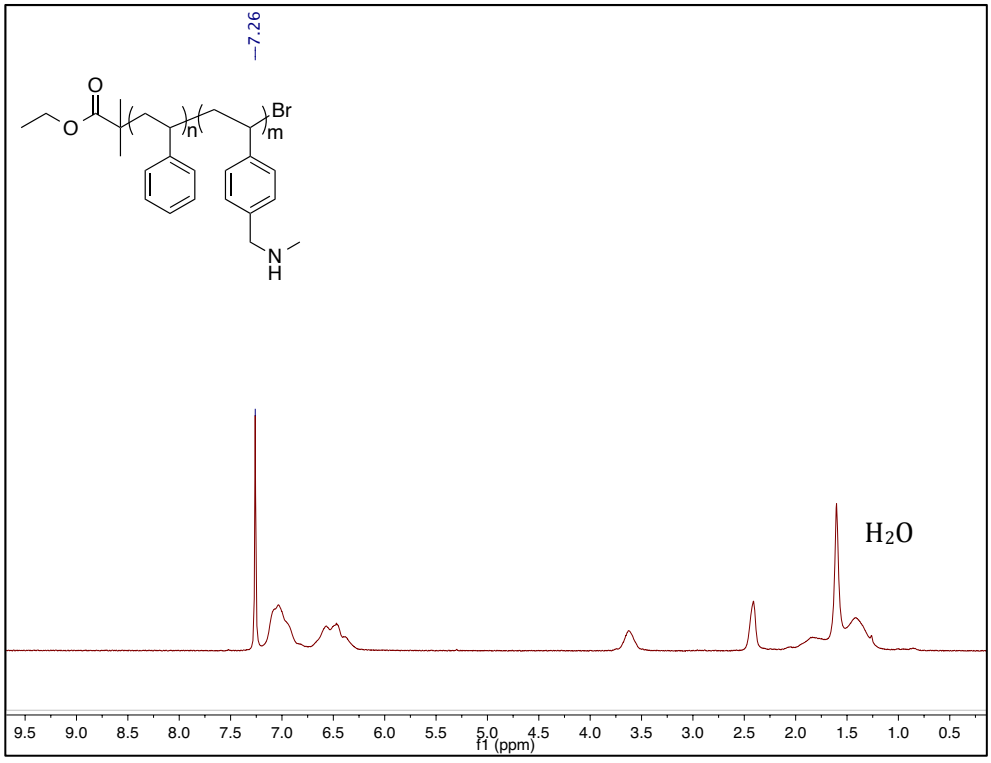


Figure S16. ^{1}H NMR spectrum of **PS-*b*-PMAS**.

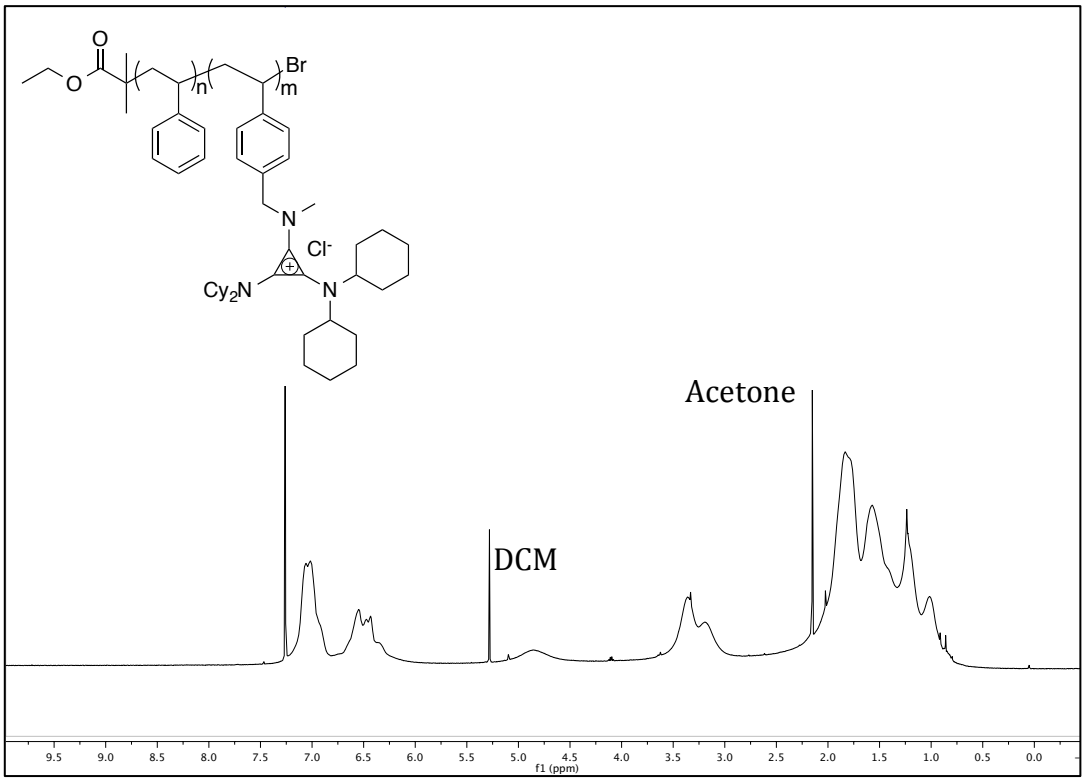


Figure S17. ^{1}H NMR spectrum of **PS-*b*-PMAS(Cy)**.

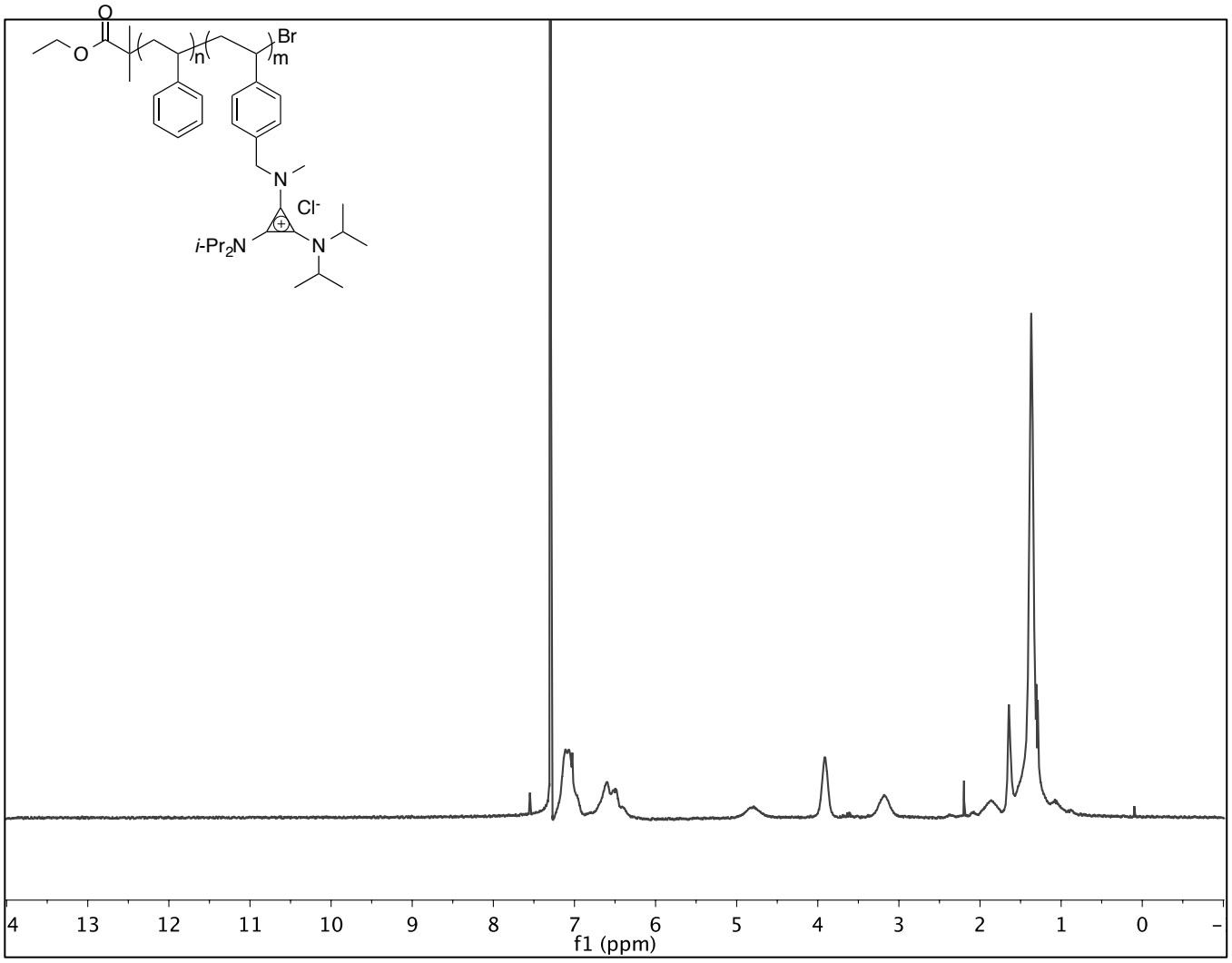


Figure S18. ^{1}H NMR spectrum of **PS-*b*-PMAS(iP)**.

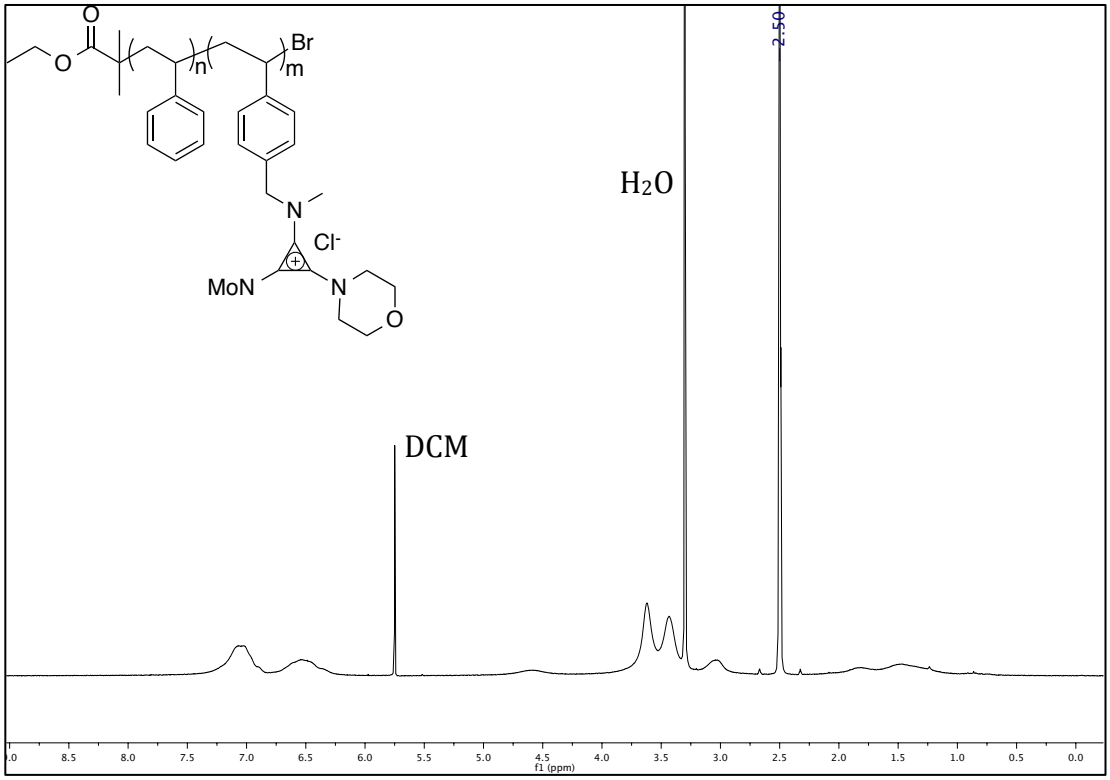


Figure S19. ^{1}H NMR spectrum of **PS-*b*-PMAS(Mo)**.

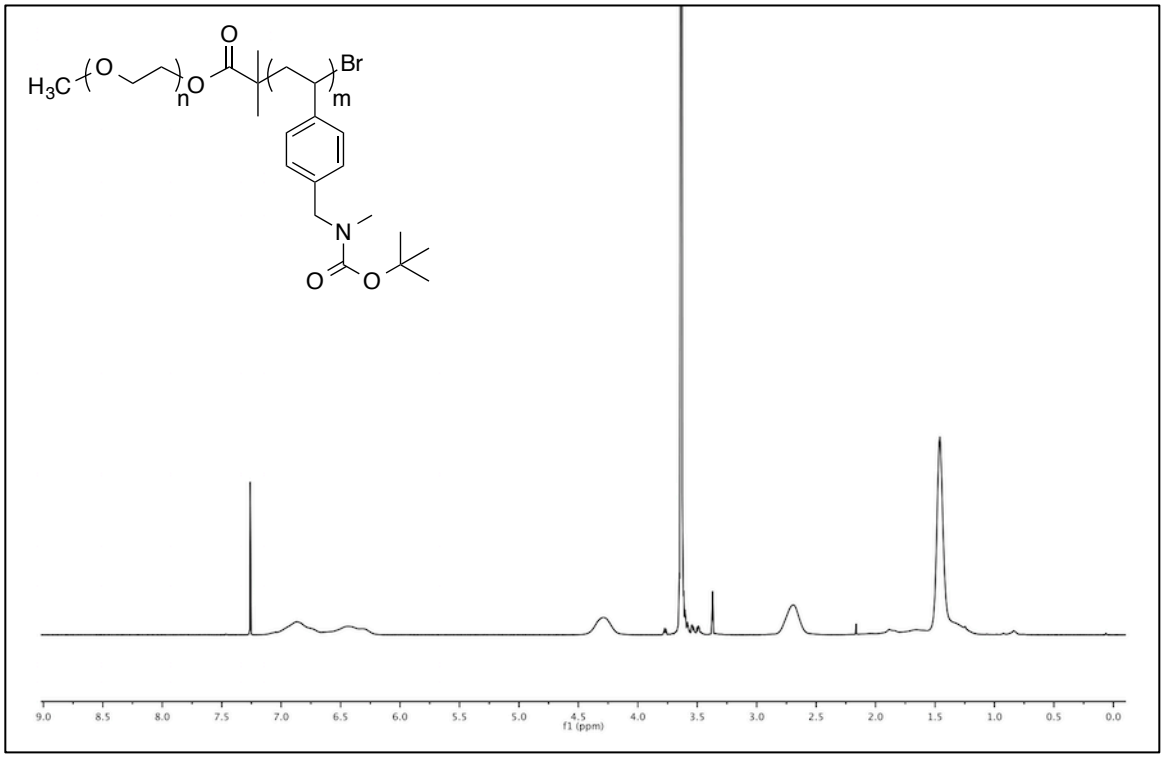


Figure S20. ^{1}H NMR spectrum of **PEO-*b*-PBoc**.

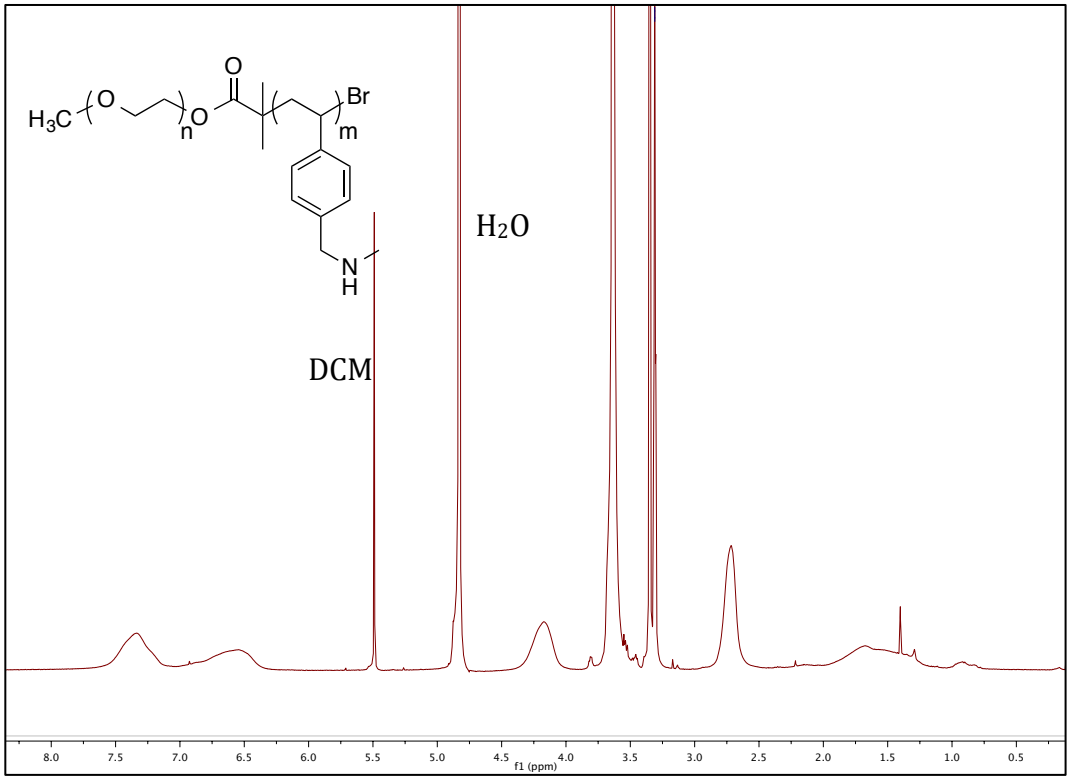


Figure S21. ^{1}H NMR spectrum of **PEO-*b*-PMAS**.

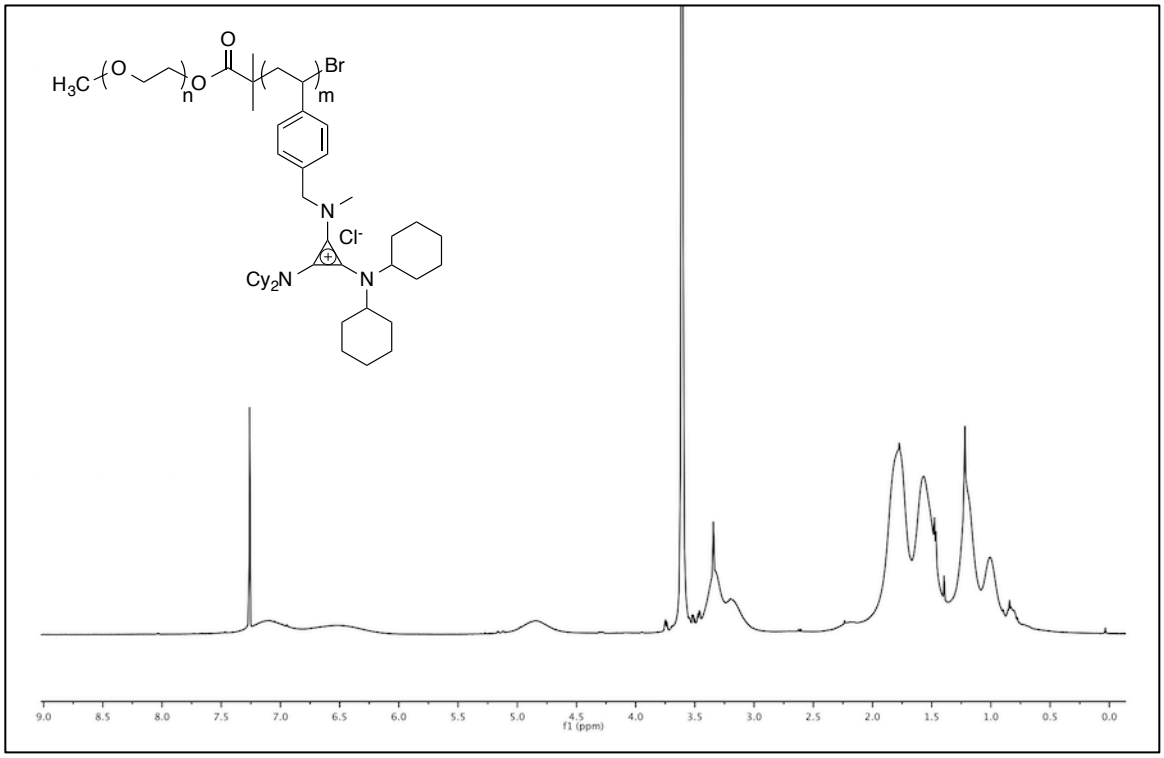


Figure S22. ^{1}H NMR spectrum of **PEO-*b*-PMAS(Cy)**.

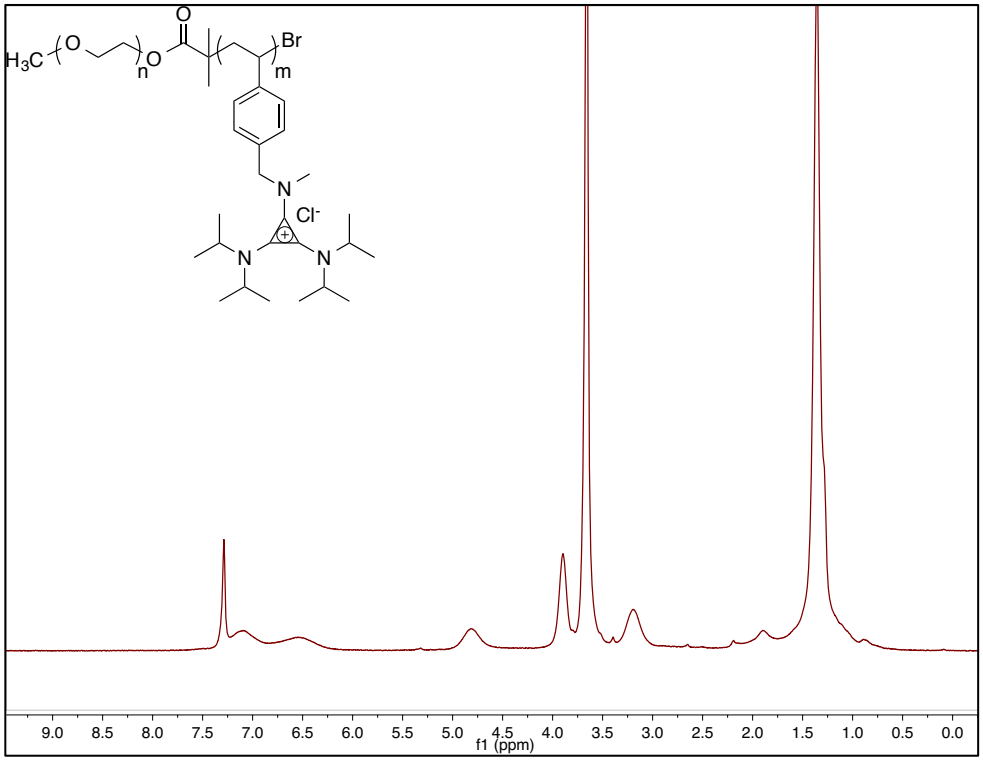


Figure S23. ^{1}H NMR spectrum of **PEO-*b*-PMAS(iP)**.

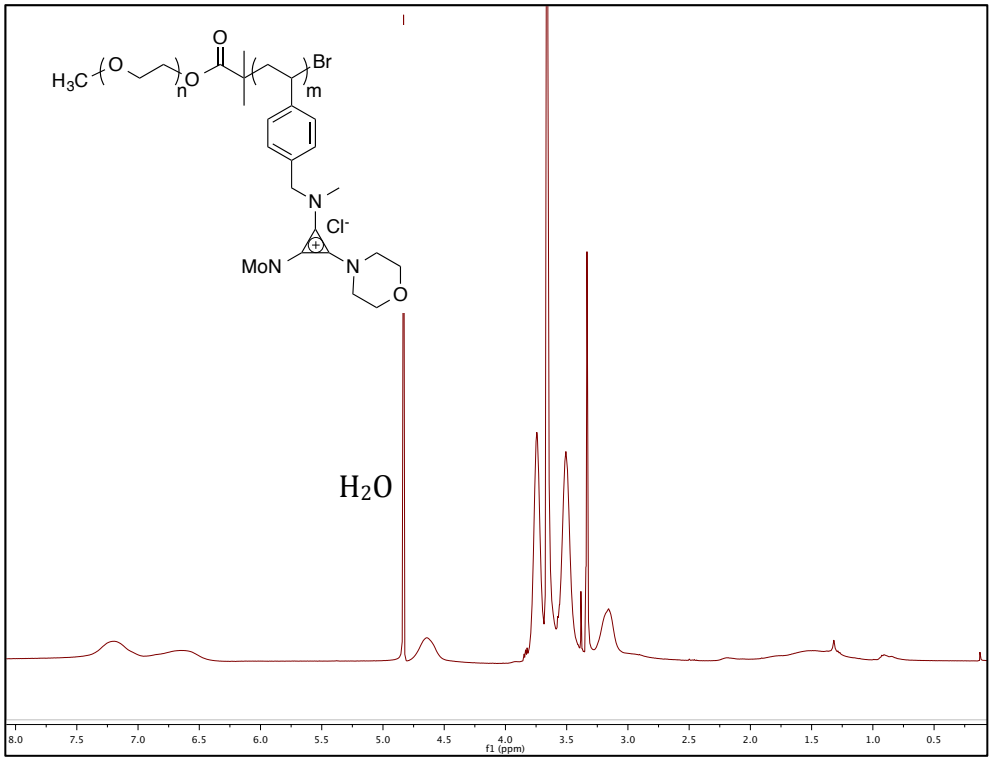


Figure S24. ^{1}H NMR spectrum of **PEO-*b*-PMAS(Mo)**.

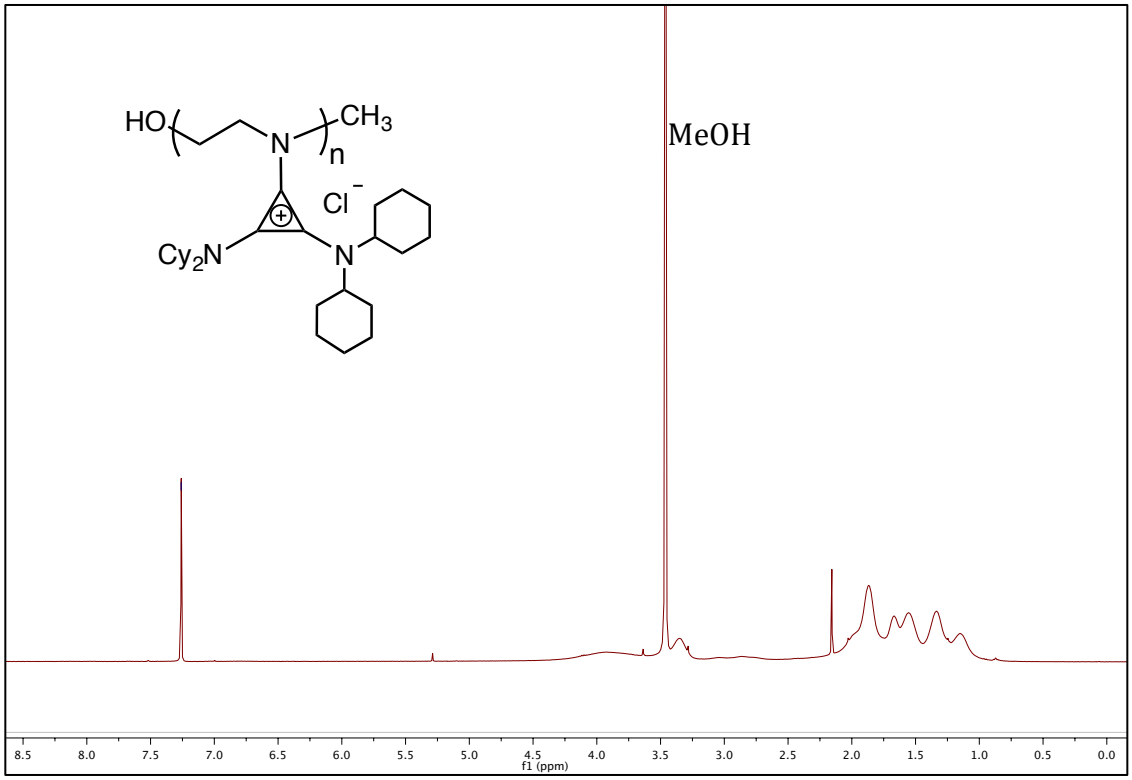


Figure S25. ^{1}H NMR spectrum of **PEI(Cy)**.

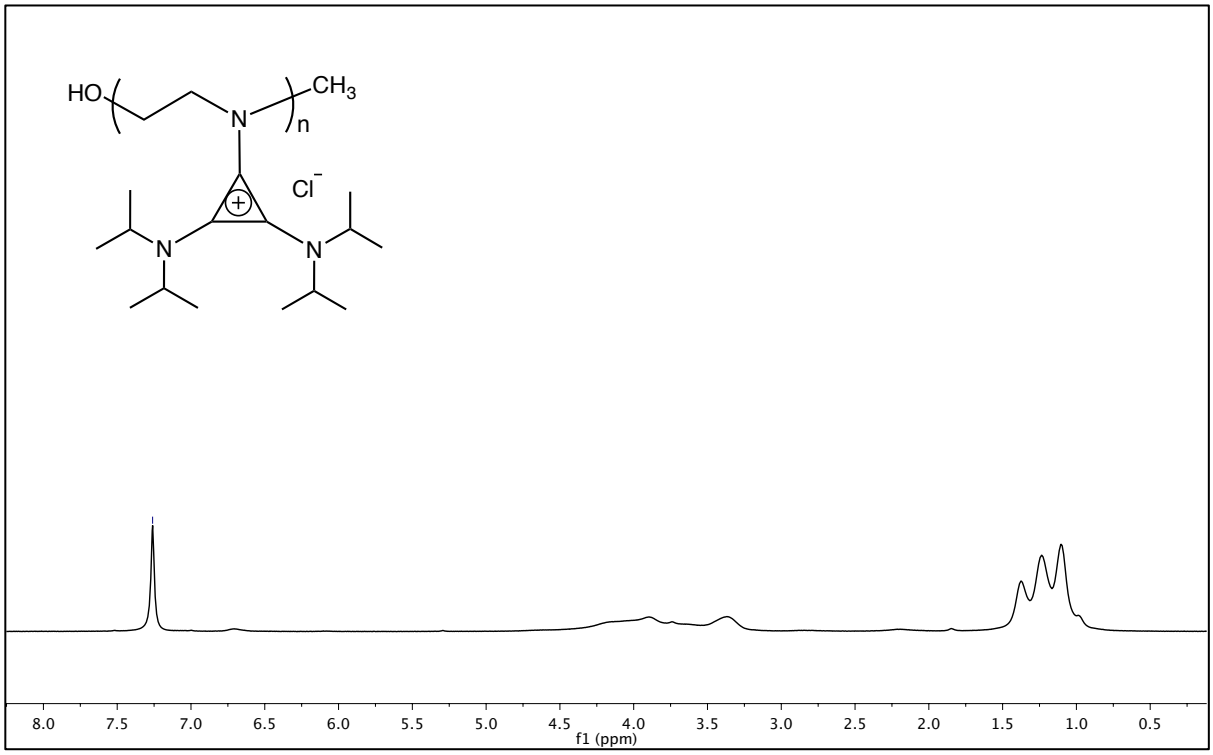


Figure S26. ^{1}H NMR spectrum of **PEI(iP)**.

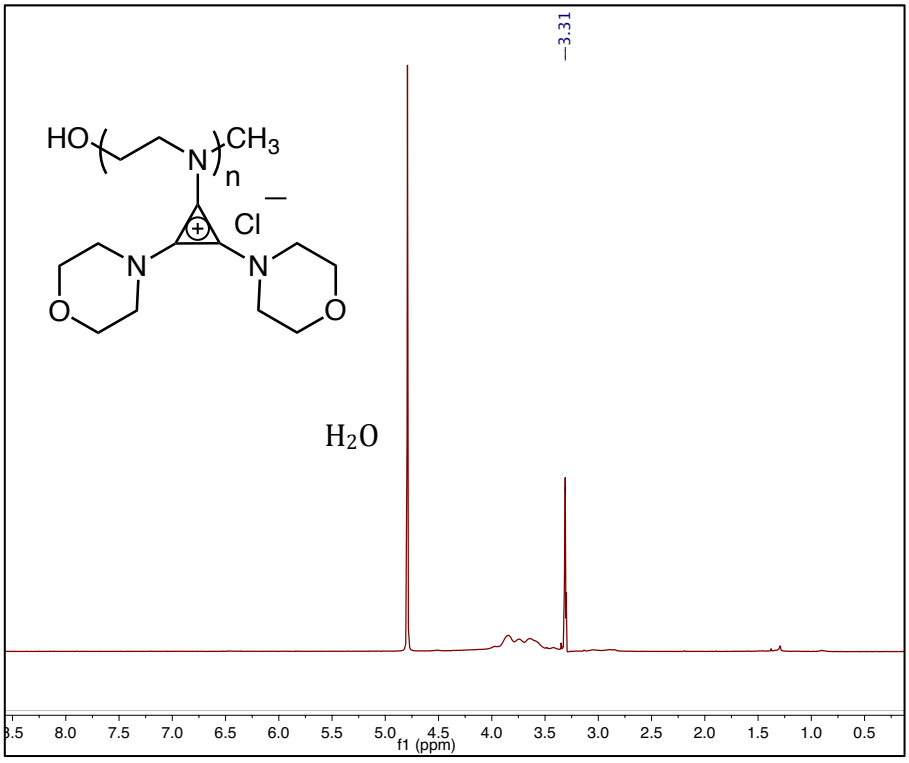


Figure S27. ^{1}H NMR spectrum of **PEI(Mo)**.

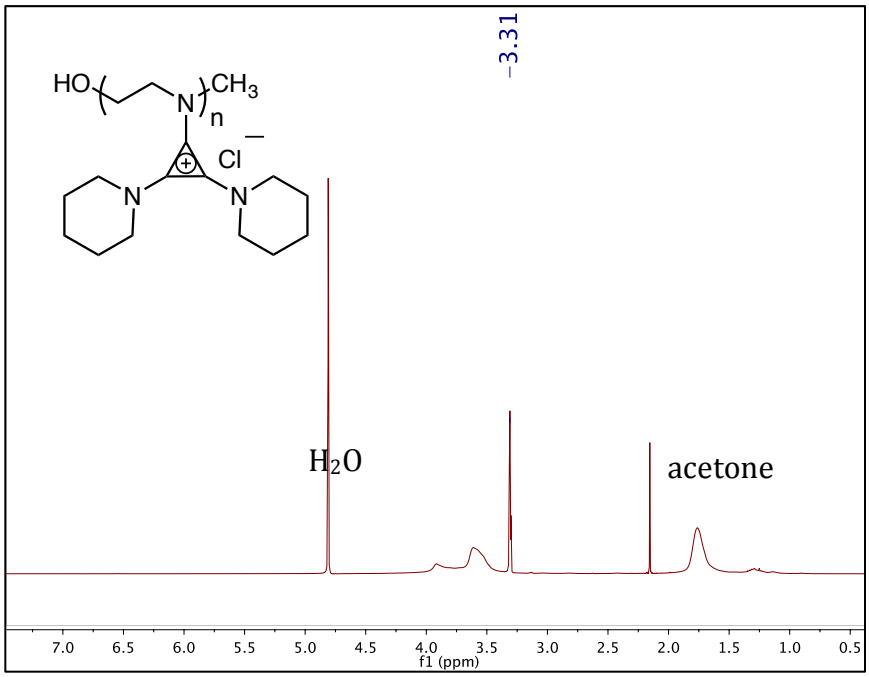


Figure S28. ^{1}H NMR spectrum of **PEI(Pep)**.

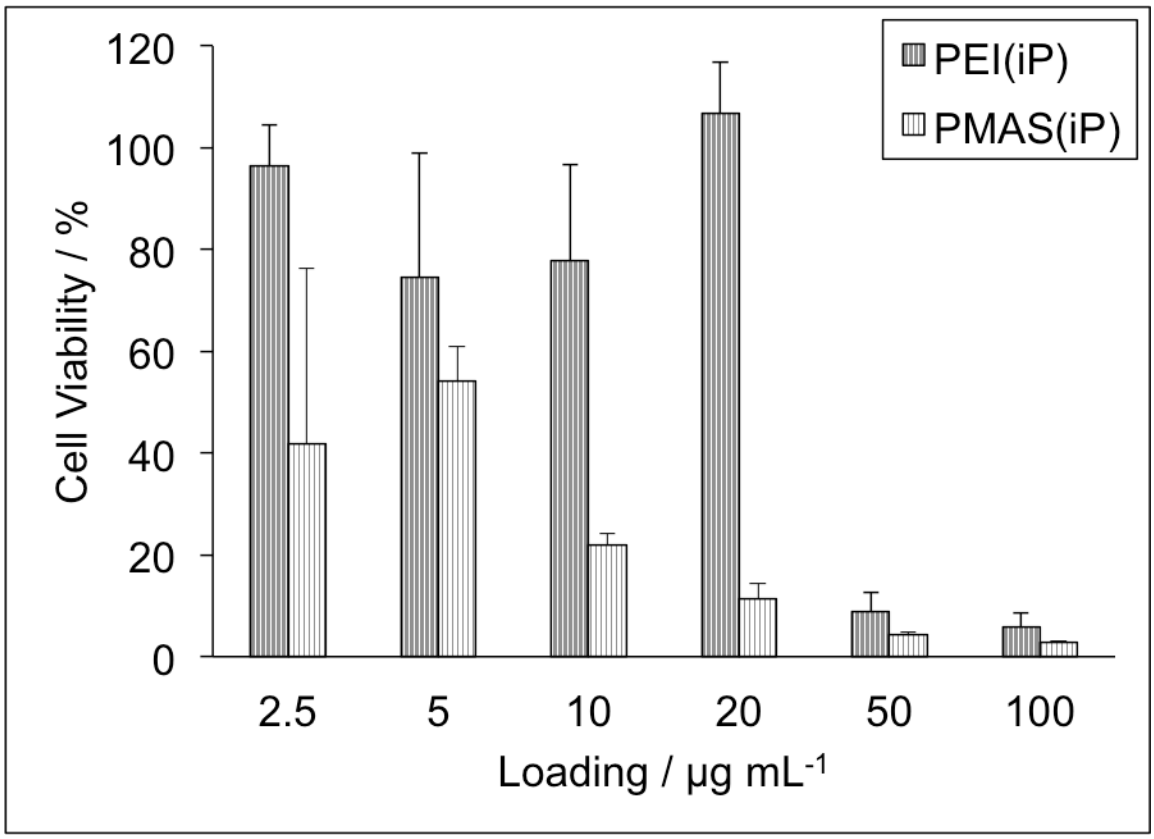


Figure S29. Cell viability of transfected 293T cells as a function of polymer loading for polymers functionalized with BACiP groups. Error bars represent the standard deviation of three measurements.

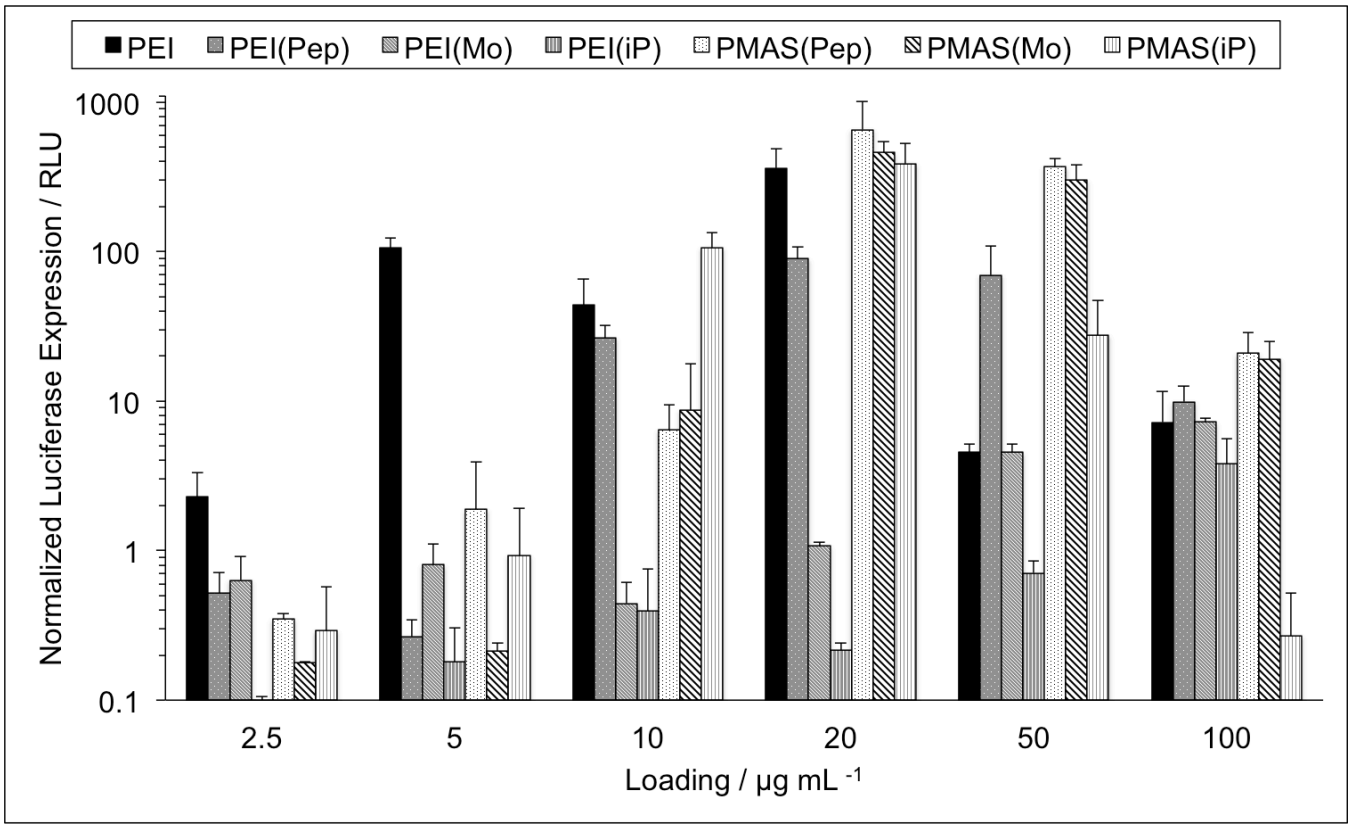
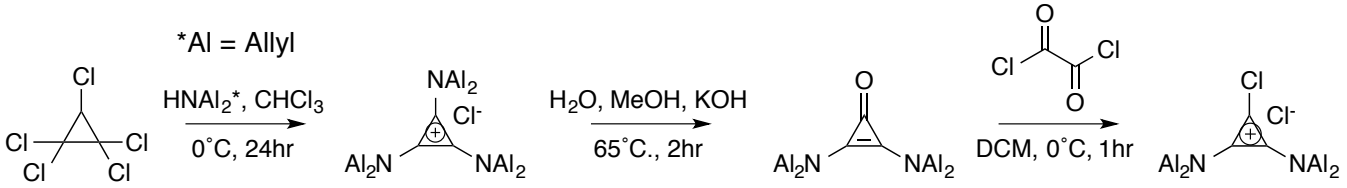


Figure S30. Luciferase expression of transfected 293T cells with all tested polymers across loading series. Error bars represent standard deviation of four measurements.

Experimental Methods

Procedures for the synthesis of bis-1,2-(diallylamino)-3-chlorocyclopropenium chloride

Preparations of BACCl derivatives have been reported,[1] but briefly, their synthesis involves in situ dehydrochlorination of pentachlorocyclopropane followed by nucleophilic substitution of the resulting tetrachlorocyclopropene with a secondary amine. If the secondary amine is sterically hindered (e.g. Cy and iP), selective double addition yields the desired BACCl in a single step. However, less sterically demanding amines (e.g. Et, Al, and Mo) lead to the tris(dialkylamino)cyclopropenium products, which require hydrolysis with base to furnish the corresponding cyclopropenone, followed by chlorination with oxalyl chloride (Figure S3).



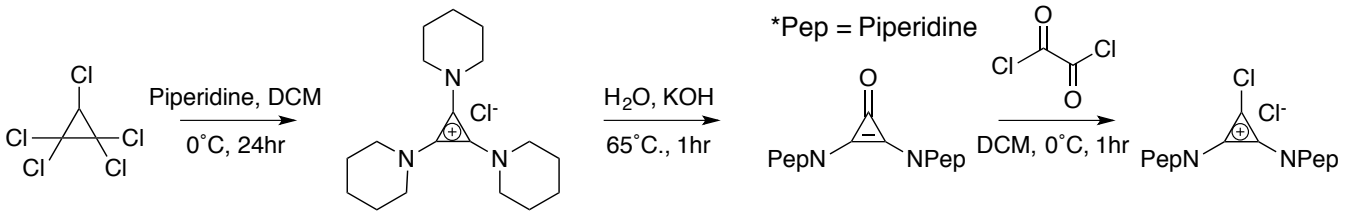
Synthesis of bis-2,3-(diallylamino)-1-cyclopropenone

This procedure was performed at ambient conditions, without deoxygenation or rigorous efforts to remove water/moisture. Diallylamine (33.0 g, 340 mmol, 7.2 equiv) was slowly added to a solution of pentachlorocyclopropane (10.0 g, 47.2 mmol, 1.0 equiv) in $CHCl_3$ (400 mL) in a 1L round bottom flask. The solution turned orange and, after stirring overnight at room temperature, was concentrated *in vacuo* to yield a crude solid of the same color. A room-temperature solution of water (125 mL), methanol (125 mL), and potassium hydroxide (45 g, 802 mmol) was used to dissolve this solid. The solution was heated to 65 ˚C and stirred for two hours. Water was removed by rotary evaporation. The resulting solid was dissolved in CH_2Cl_2 and filtered to remove salt. The organic solution was dried with anhydrous sodium sulfate, concentrated *in vacuo* yielding a crude orange solid. The crude material was purified by silica gel chromatography (20% MeOH in EtOAc) to yield the title product as an orange solid (5.26 g, 21.5 mmol, 46% two-step yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (m, 4H, $NCH_2C\mathbf{H}=CH_2$), 5.20 (dd, 8H, $NCH_2CH=C\mathbf{H_2}$), 3.78 (d, 8H, $NC\mathbf{H_2}CH=CH_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.07, 132.85, 119.83, 118.26, 118.07, 117.85, 53.46. HRMS (FAB+) m/z = 245.1654 calcd for $C_{15}H_{20}N_2Cl$ $[M+H]^+$ 245.16.

Synthesis of bis-1,2-(diallylamino)-3-chlorocyclopropenium chloride (BACAl)

Oxalyl chloride (6.86 mL, 79.4 mmol, 2.0 equiv) was slowly added to a solution of bis-2,3-(diallylamino)-1-cyclopropenone (8.9 g, 39.7 mmol, 1.0 equiv) in dry CH_2Cl_2 (250 mL) at 0 ˚C under argon. The solution was warmed to room temperature and left to react for one hour. The solution was concentrated *in vacuo* to yield the title product as a dark brown liquid in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (m, 1H, $NCH_2C\mathbf{H}=CH_2$), 5.46 (dd, 4H, $NCH_2CH=C\mathbf{H_2}$), 5.30 (dd, 4H, $NCH_2CH=C\mathbf{H_2}$), 4.35 (d, 4H, $NC\mathbf{H_2}CH=CH_2$), 4.10 (d, 4H, $NC\mathbf{H_2}CH=CH_2$).

Procedures for the synthesis of bis-1,2-(piperidino)-3-chlorocyclopropenium chloride



Synthesis of bis-2,3-(piperidino)-1-cyclopropenone

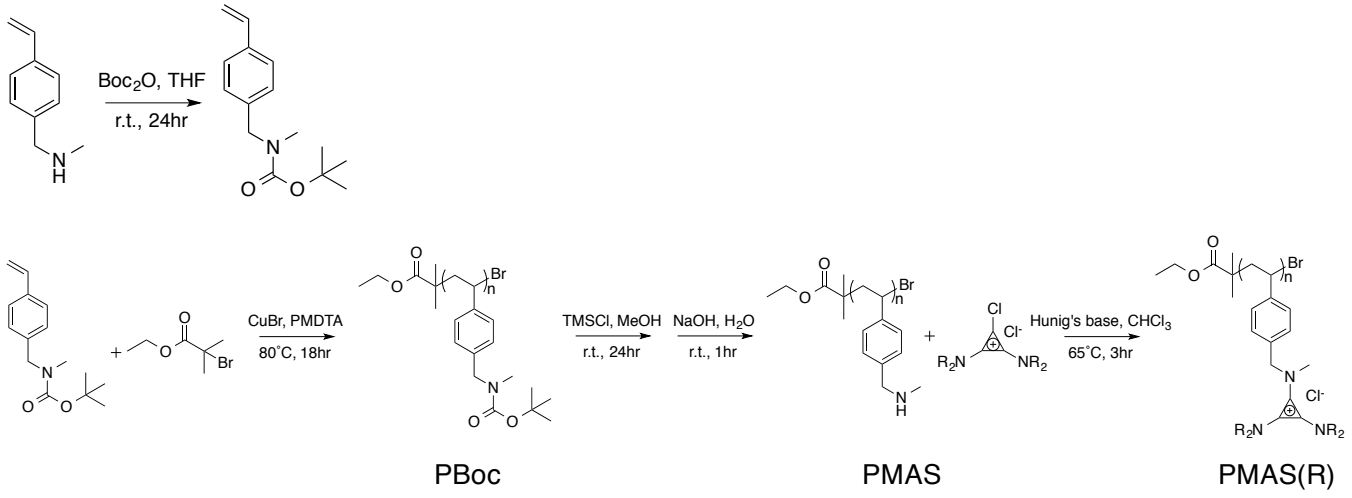
This procedure was performed at ambient conditions, without deoxygenation or rigorous efforts to remove water/moisture. Piperidine (15.6 g, 0.183 mol, 8 equiv) was slowly added to a solution of pentachlorocyclopropane (5.0 g, 22.8 mmol, 1.0 equiv) in CH_2Cl_2 (230 mL) in a 5000 mL round bottom flask. The solution turned orange and was allowed to stir overnight at room temperature. The reaction mixture was washed with 1M HCl (3x 100 mL), DI water (1 x 100 mL), and saturated NaCl solution (1 x 100 mL), dried over magnesium sulfate, and concentrated *in vacuo* to yield a crude orange/ brown solid. The crude product was dissolved in room temperature DI water (50 mL), and a solution of 10 g

potassium hydroxide in 15 mL DI water was added to this mixture. The solution was heated to 65 ˚C and left to react for one hour. Water was removed by rotary evaporation. The resulting solid was dissolved in CH_2Cl_2 and filtered to remove salt. The organic solution was dried with anhydrous sodium sulfate, concentrated *in vacuo* yielding a crude orange solid. The crude material was purified by silica gel chromatography (100% EtOAc; 5% MeOH in DCM) to yield the title product as an orange solid (1.465 g, 21.5 mmol, 30% two-step yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.28 (s, 8H, $C_3(N(C\mathbf{H_2})_2(CH_2)_3)_2$), 1.58 (s, 12H, $OC_3(N(CH_2)_2(C\mathbf{H_2})_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.85, 120.40, 50.63, 25.42, 23.61. HRMS (FAB+) m/z = 221.1647 calcd for $C_{13}H_{20}N_2O$ 220.16 $[M+H]^+$ 221.16.

Synthesis of bis-1,2-(piperidino)-3-chlorocyclopropenium chloride (BACPep)

Oxalyl chloride (0.41 mL, 4.72 mmol, 2.0 equiv) was slowly added to a solution of bis-2,3-(piperidino)-1-cyclopropenone (0.520 g, 2.36 mmol, 1.0 equiv) in dry CH_2Cl_2 (24 mL) at 0 ˚C under argon. The solution was warmed to room temperature and left to react for one hour. The solution was concentrated *in vacuo* to yield the title product as a dark brown liquid in quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.76 (t, 4H, $ClC_3(N(C\mathbf{H_2})(CH_2)(CH_2)_3)_2$), 3.62 (t, 4H, $ClC_3(N(CH_2)(C\mathbf{H_2})(CH_2)_3)_2$), 1.88-1.68 (m, 12H, $ClC_3(N(CH_2)(CH_2)(C\mathbf{H_2})_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.64, 52.52, 51.32, 24.99, 22.51. HRMS (FAB+) m/z = 275.1082 calcd for $C_{15}H_{20}N_2Cl$ $[M+H]^+$ 275.11.

Procedures for the synthesis of poly methylaminostyrene (PMAS)



Synthesis of *tert*-butyl methyl(4-vinylbenzyl)carbamate

N-methyl-4-v*i*nylbenzylamine (10.07 g, 68.4 mmol, 1 equiv) and THF (300mL) were added to a 1L round bottom flask (RBF) and the flask was sealed with a septum secured with copper wire under argon with a gas outlet. Triethylamine (10.4 mL, 74.8 mmol, 1.1 equiv) was added to the RBF, the system was cooled to 0 ºC, and di-*tert*-butyl dicarbonate (16.42 g, 74.8 mmol, 1.1 equiv) was slowly injected. The RBF was warmed to room temperature and allowed to stir overnight. The solution was concentrated under vacuum, and the translucent, crude product was dissolved in 300 mL of CH_2Cl_2 and transferred to a 1L separatory funnel. The solution was washed with 1M HCl (3 x 100mL) followed by a single brine

wash. The solution was then dried with magnesium sulfate, filtered, and concentrated under vacuum. The crude material was finally purified by silica gel chromatography (100% hexanes then 95% CH_2Cl_2/5% hexanes) to yield the title product as an translucent, colorless liquid (7.22 g, 29.2 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, 2H, Ar**H**), 7.18 (d, 2H, Ar**H**), 6.71 (dd, 1H, H_2C=C**H**Ar), 5.73 (dd, 1H, **H**$_2$C=CHAr), 5.23 (dd, 1H, **H$_2$**C=CHAr), 4.41 (s, 2H, ArCH_2N), 2.81 (s, 3H, NCH_3), 1.48 (s, 9H, NC=OtBu**H**). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.7, 136.6, 136.4, 128.0, 127.4, 126.4, 113.7, 79.7, 52.4, 51.7, 33.9, 28.5. HRMS (FAB+) m/z = 270.1470 calcd for $C_{15}H_{21}N_1O_2$ $[M+Na]^+$ 270.15.

Synthesis of PBoc

Copper (I) bromide (10 mg, 7.0E-2 mmol, 0.5 equiv) was added to a dry Schlenk flask and the material was deoxygenated via five vacuum-argon cycles. Degassed *N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDTA) (12.1 mg, 7.0e-2 mmol, 0.5 equiv) was added to the flask and allowed to stir for ten minutes to form Cu complex, a light green mixture. Degassed *tert*-butyl methyl(4-vinylbenzyl)carbamate (4.5 g, 18.2 mmol, 130 equiv) was then added to the mixture and three freeze-pump-thaw cycles were conducted. The Schlenk flask was closed under argon and degassed ethyl α-bromoisobutyrate (27.3 mg, 0.14 mmol, 1 equiv) was injected, and the reaction mixture was heated to 85 ˚C and allowed to react for 24 hours. The resulting solution was diluted with methanol and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against methanol. The resulting solution was concentrated under vacuum to yield a fine, white powder (803.3 mg, 17.9% recovered yield). From SEC: M_n = 6700 g mol^{-1}, degree of polymerization ~ 65, $Đ$ = 1.08. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15-6.20 (b, 254H, Ar**H**), 4.50-4.21 (b, 130H, ArC**H$_2$**N), 2.95-2.56 (b, 198H, NC**H$_3$**), 2.01-1.19 (b, 782H, NC=OtBu**H**, ArC**H**C**H$_2$**).

Synthesis of PMAS

The PBoc (803.3 mg, 3.25 mmol, 1 eq) was dissolved in methanol (10 mL) in a dry round bottom flask under argon. The flask was cooled to 0 ˚C and trimethylsilyl chloride (2.47 g, 22.7 mmol, 7 eq) was added. The reaction solution was allowed to stir at room temperature overnight and concentrated under vacuum to yield a white powder. The powder was then re-dissolved in a 1M solution of KOH in methanol and allowed to stir for one hour. The solution was concentrated so that a minimal amount of methanol remained. To this thickened liquid, water was added until white flecks of polymer began to precipitate out. The solution was filtered and the solid white flecks were redissolved in methanol and the previous step was repeated. The resulting polymer was dried under vacuum, yielding a fluffy, white powder (440 mg, 93.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-6.25 (b, 268H, Ar**H**), 3.77-3.43 (b, 130H, ArC**H$_2$**N), 2.57-2.23 (b, 197H, NC**H$_3$**), 1.70-1.19 (b, 205H, ArC**H**C**H$_2$**).

Procedures for the synthesis of PMAS(R) homopolymers (PMAS parent-polymer)

Synthesis of PMAS(Al)

This procedure was performed open to the atmosphere. PMAS (58.2 mg, 0.40 mmol, 1 equiv) was dissolved in chloroform (4 mL) in a scintillation vial. To polymer solution was added *N,N*-diisopropylethylamine (153 mg, 1.19 mmol, 3 equiv) and allowed to stir for 10 minutes. The BACAl (169 mg, 0.59 mmol, 1.5 equiv) was dissolved in 3 mL of chloroform and added to the reaction vial. The mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo*, diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag, and dialyzed against water and concentrated by rotary evaporation. The polymer was then dissolved in a minimal amount of acetone and precipitated one time into ethyl acetate at -78 °C and again concentrated under vacuum to yield a brown powder (107 mg, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-6.27 (b, 263H, Ar**H**), 5.96-5.60 (b, 260H, $NCH_2C\mathbf{H}{=}CH_2$), 5.43-5.04 (b, 560H, $NCH_2CH{=}C\mathbf{H_2}$), 4.84-4.42 (b, 130H, $ArC\mathbf{H_2}N$), 4.15-3.80 (b, 513H, $NC\mathbf{H_2}CH{=}CH_2$), 3.25-2.75 (b, 193H, $NC\mathbf{H_3}$), 2.27-1.03 (b, 195H, $ArC\mathbf{H}C\mathbf{H_2}$).

Synthesis of PMAS(Mo)

This procedure was performed open to the atmosphere. PMAS (51.8 mg, 0.39 mmol, 1 equiv) was dissolved in a DMF (3 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (130 mg, 1.02 mmol, 3 equiv), followed by a solution of DCM (3 mL) and BACMo (147 mg, 0.51 mmol, 1.5 equiv). ***Note:*** chloroform is not used here, as the resulting PIL is not soluble. DMF can be used as a co-solvent to soluble resultings PILs. The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water. The resulting solution was concentrated under vacuum to yield a brown powder (126 mg, 91.3% yield). ^{1}H NMR chemical shifts and integrations have been previously reported for this materials.[2]

Synthesis of PMAS(Cy)

This procedure was performed open to the atmosphere. PMAS (51.3 mg, 0.345 mmol, 1 equiv) was dissolved in chloroform (4 mL) in a scintillation vial. To the vial *N,N*-diisopropylethylamine (135 mg, 1.04 mmol, 3 equiv) was added, followed by a solution of BACCy (180 mg, 0.38 mmol, 1.1 equiv) in 3 mL chloroform. The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo* and precipitated twice from DCM into dioxane. The resulting powder was dissolved in methanol and transferred to a 3.5k MWCO Spectrum Labs dialysis bag. Dialysis was conducted against a 1:1 H_2O: methanol solution. The resulting solution was concentrated under vacuum to yield a light-brown powder (161 mg, 80% yield). This material is not water soluble. ^{1}H NMR chemical shifts and integrations have been previously reported for this materials.[2]

Synthesis of PMAS(iP)

This procedure was performed open to the atmosphere. PMAS (54 mg, 0.37 mmol, 1 equiv) was dissolved in chloroform (3 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine

(141 mg, 1.1 mmol, 3 equiv), and a solution of BACiP (202 mg, 0.40 mmol, 1.1 equiv) in 4 mL chloroform was added. The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo* and precipitated twice from acetone into ethyl acetate at -78 ˚C (if precipitated polymer does not filter nicely, make the polymer/acetone solution more concentrated). The resulting powder was dissolved in water and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water followed by concentration under vacuum to yield a light-brown powder (110 mg, 65% yield). ^{1}H NMR chemical shifts and integrations have been previously reported for this materials.[2]

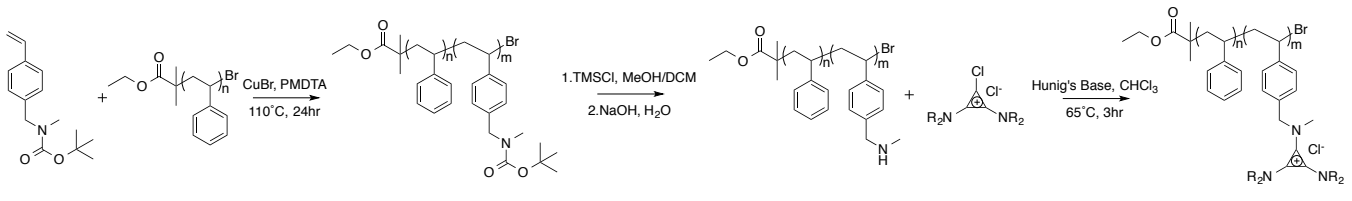
Synthesis of PMAS(Et)

This procedure was performed open to the atmosphere. PMAS (54 mg, 0.368 mmol, 1 equiv) was dissolved in chloroform (3 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (142 mg, 1.1 mmol, 3 equiv) followed by a solution of BACEt (140 mg, 0.55 mmol, 1.1 equiv) in 4 mL chloroform. The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo*, diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water, and subsequently concentrated under vacuum. The polymer was then dissolved in a minimal amount of acetone and precipitated one time into ethyl acetate at -78 ˚C to yield a brown powder (112 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-6.30 (b, 262H, Ar**H**), 4.83-4.54 (b, 130H, ArC**H$_2$**N), 3.53-3.31 (b, 502H, NC**H$_2$**CH_3), 3.26-3.02 (b, 193H, NC**H$_3$**), 1.58-1.08 (b, 1009H, NCH_2C**H$_3$**, ArC**H**C**H$_2$**).

Synthesis of PMAS(Pep)

This procedure was performed open to the atmosphere. PMAS (50 mg, 0.339 mmol, 1 equiv) was dissolved in chloroform (2 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (0.18 mL, 1.02 mmol, 3 equiv) followed by a solution of BACPep (141 mg, 0.509 mmol, 1.5 equiv) in 6 mL chloroform. The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo*, diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water, and subsequently concentrated under vacuum. The polymer was then dissolved in a minimal amount of acetone and precipitated one time into ethyl acetate at -78 ˚C to yield a brown powder (129.7 mg, 97% yield). ^{1}H NMR (400 MHz, acetone-d6) δ 7.90-6.30 (b, 609H, Ar**H**), 5.20- 4.60 (b, 274H, ArC**H$_2$**N), 3.85-3.45 (b, 1176H, C_3(N(C**H**$_2$)$_2$$(CH_2)_2CH_2)_2$)), 3.40-3.13 (b, 382H, NC**H$_3$**), 1.90-1.37 (b, 1895H,) C_3(N$(CH_2)_2$(C**H**$_2$)$_2$C**H**$_2$)$_2$), ArC**H**C**H$_2$**).

Procedures for the synthesis of PS-b-PMAS(R) block copolymers



Synthesis of PS-*b*-PBoc

Copper (I) bromide (5.5 mg, 3.87e-2 mmol, 0.5 equiv) was added to a dry Schlenk flask and the material was deoxygenated via five vacuum-argon cycles. Degassed *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA) (9.4 mg, 5.4e-2 mmol, 0.7 equiv) was added to the flask and the mixture was stirred for ten minutes, forming a light green mixture. Degassed *tert*-butyl methyl(4-vinylbenzyl)carbamate (2.6 g, 12 mmol, 150 equiv) was then added to the mixture, and three freeze-pump-thaw cycles were conducted. Lastly, a deoxygenated solution of PS (7k) (535 mg, 0.77 mmol, 1 equiv) in DMF (1 ml) was injected into the Schlenk flask and the mixture was heated to 85 ˚C and left to react for 24 hours. The resulting solution was concentrated, dissolved in THF, and then precipitated twice into a 3:1 mixture of methanol-water. The resulting powder was further dried under vacuum, yielding the title product (610 mg, 81% recovered yield). SEC and ^{1}H NMR reveal block copolymer contains ~190 units of styrene and ~127 units Boc-protected monomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15-6.20 (b, 1494H, Ar**H**), 4.50-4.21 (b, 254H, ArC**H$_2$**N), 2.95-2.56 (b, 370H, NC**H$_3$**), 2.01-1.19 (b, 782H, NC=OtBu**H**, ArC**H**C**H$_2$**).

Synthesis of PS-*b*-PMAS

The PS-*b*-PBoc (500 mg, 1.27 mmol amine-containing monomer, 1 eq amine monomer) was dissolved in a 50/50 DCM:methanol solution (15 mL) in a round bottom flask under argon. The flask was cooled to 0˚C and trimethylsilyl chloride (2.47 g, 22.7 mmol, 7 eq) was added. The reaction was allowed to stir at room temperature overnight and concentrated under vacuum to yield a white powder. The powder was then re-dissolved in DMSO and 1M NaOH was added dropwise, with stirring, until the polymer precipitated from solution. The resulting slurry was centrifuged, and the supernatant decanted. The polymer was washed two more times with DI water, and collected by centrifugation. The resulting polymer was dried under vacuum, yielding a fluffy, white powder (235.5 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23-6.25 (b, 268H, Ar**H**), 3.77-3.43 (b, 130H, ArC**H$_2$**N), 2.57-2.23 (b, 197H, NC**H$_3$**), 1.70-1.19 (b, 205H, ArC**H**C**H$_2$**).

Synthesis of PS-*b*-PMAS(Cy)

This procedure was performed open to the atmosphere. PS-*b*-PMAS (56.8 mg polymer, 0.19 mmol amine unit, 1 equiv amine unit) was dissolved in chloroform (6 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (74 mg, 0.57 mmol, 3 equiv) and the BACCy (98.4 mg, 0.21 mmol, 1.1 equiv). The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo*, diluted with methanol, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against methanol. The resulting colloidal solution was concentrated under vacuum to yield a brown powder (130 mg, 91.8% yield). ^{1}H NMR chemical shifts and integrations have been previously reported for this materials.[2]

Synthesis of PS-*b*-PMAS(iP)

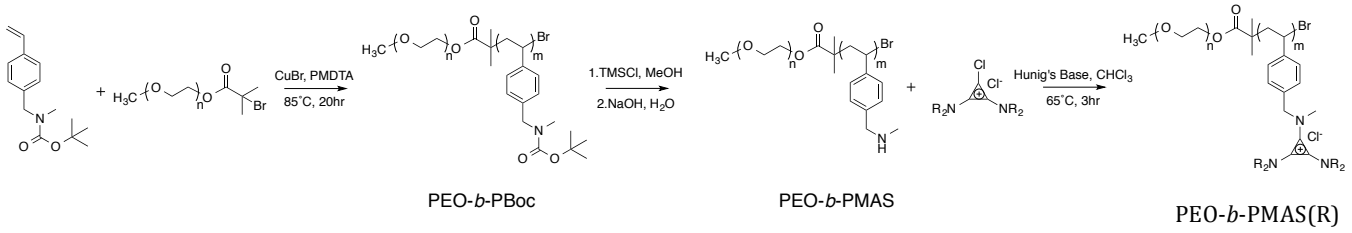
This procedure was performed open to the atmosphere. PS-*b*-PMAS (88 mg polymer, 0.30 mmol amine unit, 1 equiv amine unit) was dissolved in chloroform (7 mL) in a scintillation vial. To the vial was

added *N,N*-diisopropylethylamine (114 mg, 0.89 mmol, 3 equiv) and the BACiP (163 mg, 0.33 mmol, 1.1 equiv). The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo*, dissolved in water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against a 50/50 mixture of water and methanol. The resulting colloidal solution was concentrated under vacuum to yield a light-brown powder (134 mg, 78.8% yield). ^{1}H NMR chemical shifts and integrations have been previously reported for this materials.[2]

Synthesis of PS-*b*-PMAS(Mo)

This procedure was performed open to the atmosphere. PS-*b*-PMAS (65.7 mg polymer, 0.33 mmol amine unit, 1 equiv amine unit) was dissolved in a DMF (3 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (130 mg, 1.0 mmol, 3 equiv) and a solution of DCM (3 mL) and BACMo (140 mg, 0.50 mmol, 1.5 equiv). The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against a 50/50 mixture of water and methanol. The resulting colloidal solution was concentrated under vacuum to yield a brown powder (157 mg, 93.5% yield). ^{1}H NMR chemical shifts and integrations have been previously reported for this materials.[2]

Procedures for the synthesis of PEO-b-PMAS(R) block copolymers



Synthesis of PEO-*b*-PBoc

Copper (I) bromide (4.7 mg, 3.28e-2 mmol, 0.5 equiv) was added to a dry schlenk flask and the material was deoxygenated via five vacuum-argon cycles. Sparged *N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDTA) (5.7 mg, 3.82e-2 mmol, 0.5) was added to the flask and let stir for ten minutes, forming a light green mixture. Degassed *tert*-butyl methyl(4-vinylbenzyl)carbamate (2.23 g, 9 mmol, 150 equiv) was then added to the mixture and three freeze-pump-thaw cycles were conducted. Lastly, a solution of poly(ethylene glycol) methyl ether 2-bromoisobutyrate (300 mg, 6e-2 mmol, 1 equiv) in DMF (1 mL) was injected into the Schlenk flask and the mixture was heated to 85 ˚C and stirred for 20 hours. The resulting solution was diluted with water and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and left to dialyze against a solution of 1:1 water-methanol. The resulting solution was concentrated under vacuum to yield a fine, white powder (350 mg, 20% conversion (30 units Boc monomer per chain), 78.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15-6.20 (b, 117H, Ar**H**), 4.50-4.21 (b, 59H, ArC$\mathbf{H_2}$N), 3.72-3.58 (b, 455H, ($C\mathbf{H_2}C\mathbf{H_2}O)_{114}$), 2.95-2.56 (b, 89H, NC$\mathbf{H_3}$), 2.01-1.19 (b, 343H, NC=OtBu**H**, ArC**H**C$\mathbf{H_2}$).

Synthesis of PEO-*b*-PMAS

The PEO-*b*-PBoc (350 mg polymer, 0.85 mmol Boc unit, 1 equiv Boc unit) was dissolved in methanol (10 mL) in a dry round bottom flask under argon. The flask was cooled to 0 ˚C and trimethylsilyl chloride (1.25 g, 12.5 mmol, 14.7 equiv) was added. The reaction was allowed to stir at room temperature overnight and then a 0.5M NaOH solution (5 mL) was added to the system. This mixture was stirred for 1 hour and then transferred to a 3.5k MWCO Spectrum Labs dialysis bag and left to dialyze against water. Finally, the solution was concentrated under vacuum yielding a white powder (191.6 mg, 72% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.55-6.35 (b, 128H, Ar**H**), 4.35-4.04 (b, 59H, ArC**H$_2$**N), 3.72-3.58 (b, 455H, (C**H$_2$**C**H$_2$**O)$_{114}$), 2.95-2.56 (b, 91H, NC**H$_3$**), 2.01-1.19 (b, 93H, ArC**H**C**H$_2$**).

Synthesis of PEO-*b*-PMAS(Cy)[i]

This procedure was performed open to the atmosphere. PEO-*b*-PMAS (75.9 mg polymer, 0.24 mmol amine unit, 1 equiv amine unit) was dissolved in chloroform (4 mL) in a scintillation vial. *N,N*-diisopropylethylamine (150 mg, 1.16 mmol, 4.8 equiv) and a solution of BACCy (199 mg, 0.42 mmol, 1.75 equiv) in 3mL of chloroform were added to the vial. The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo* and dissolved in methanol before being transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against a 50/50 mixture of water and methanol. The resulting solution was concentrated under vacuum to yield a light-brown powder (118 mg, 64.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-6.15 (b, 116H, Ar**H**), 5.10-4.60 (b, 54H, ArC**H$_2$**N), 3.72-3.58 (b, 455H, (C**H$_2$**C**H$_2$**O)$_{114}$), 3.50-2.56 (b, 188H, NCy**H**, NC**H$_3$**), 1.75- (b, 102H, ArC**H**C**H$_2$**).

Synthesis of PEO-*b*-PMAS(iP)[i]

PEO-*b*-PMAS (50 mg polymer, 0.16 mmol amine unit, 1 equiv amine unit) was dissolved in chloroform (4 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (132 mg, 1.0 mmol, 6 equiv) and the BACiP (**117** mg, 0.50 mmol, 3 equiv). The reaction mixture was allowed to stir at 65˚C for three hours. The resulting solution was diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water. The resulting solution was concentrated under vacuum to yield a brown powder (71.8 mg, 77% yield). ^{1}H NMR (500 MHz**, C$\mathbf{DCl_3}$**) δ 7.40-6.20 (b, 133H, Ar**H**), 5.05-4.50 (b, 60H, ArC**H$_2$**N, NC**H**$(CH_3)_2$**),** 4.00-2.90 **(b,** 630H**,** NC**H**$(CH_3)_2$**,** (C**H$_2$**C**H$_2$**O)$_{114}$**,** NC**H$_3$**)**,** 2.00-0.80 **(b,** 805H**,** ArC**H**C**H$_2$**, NCH(C**H$_3$**)$_2$).

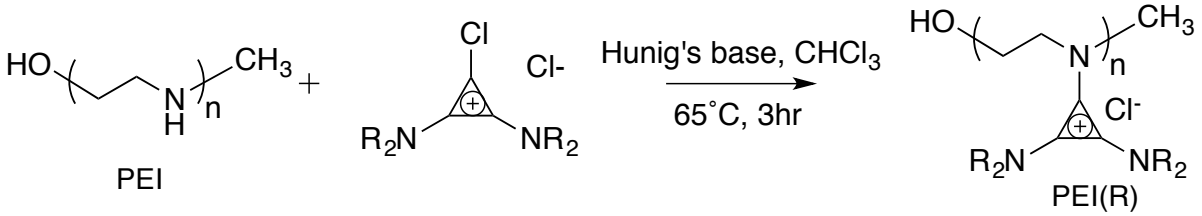
Synthesis of PEO-*b*-PMAS(Mo)[i]

This procedure was performed open to the atmosphere. PEO-*b*-PMAS (65.7 mg polymer, 0.21 mmol amine unit, 1 equiv amine unit) was dissolved in DMF (6 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (130 mg, 1.0 mmol, 5 equiv) and the BACMo (140 mg, 0.50 mmol, 2.4 equiv). The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water. The resulting solution was concentrated under vacuum to yield a brown powder (106 mg, 90.4%

yield). ^{1}H NMR (400 MHz, MeOD) δ 7.40-6.25 (b, 142H, Ar**H**), 4.85-4.40 (b, 60H, ArC**H$_2$**N), 3.85-3.35 (b, 805H, N(CH2C**H**2)$_2$O, (C**H$_2$**C**H$_2$**O)$_{114}$, N(C**H$_2$**CH$_2$)$_2$O), 3.25-3.00 (b, 90H, NC**H$_3$**), 1.70-1.10 (b, 102H, ArC**H**C**H$_2$**).

[i]For this reaction, excess of *N,N*-diisopropylethylamine and the BACCl ClickabIL was used, although stoichiometric quantities as used in other functionalization reactions would also work.

Procedures for the synthesis of PEI(R) polymers (PEI parent polymer)



Synthesis of PEI(Cy)

This procedure was performed open to the atmosphere. Linear polyethyleneimine (10k) (80 mg, 1.86 mmol, 1 equiv) was dissolved in chloroform (7 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (720 mg, 5.57 mmol, 3 equiv) and the BACCy (1.92 g, 4.1 mmol, 2 equiv). The reaction mixture was allowed to stir at 65 ˚C for 27.5 hours. ***Note***: these reaction conditions require longer time and more equivalents of BACCy than other ClickabIL conditions, potentially because of the steric hindrance of cyclohexyl substituents. The resulting solution was concentrated *in vacuo* and precipitated twice into dioxane. The resulting powder was dissolved in methanol and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against methanol. The resulting solution was concentrated under vacuum to yield a yellow-brown powder (220 mg, 25% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.45-3.15 (b, 1810, (C**H$_2$**C**H$_2$**N)$_{233}$, NCy**H**), 1.90-0.65 (10165H, NCy**H**).

Synthesis of PEI(iP)

This procedure was performed open to the atmosphere. Linear polyethyleneimine (10k; 25k was used for transfection experiments) (91 mg, 23 mmol, 1 equiv) was dissolved in chloroform (10 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (901 mg, 79 mmol, 3 equiv) and the BACiP (1.16 g, 26 mmol, 1.1 equiv). The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was concentrated *in vacuo* and then dissolved in water and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water. The resulting solution was concentrated under vacuum to yield a yellow-brown powder (394 mg, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.45-3.15 (b, 1860, (C**H$_2$**C**H$_2$**N)$_{233}$, NC**H**$(CH_3)_2$), 1.90-0.65 (5265H, NCH(C**H$_3$**)$_2$).

Synthesis of PEI(Mo)

This procedure was performed open to the atmosphere. Linear polyethyleneimine (10k; 25k was used for transfection experiments) (26.8 mg, 0.623 mmol, 1 equiv) was dissolved in a DMF (3 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (130 mg, 1.02 mmol, 3 equiv) and a

solution of DCM (3 mL) and BACMo (251 mg, 0.93 mmol, 1.5 equiv). The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water. The polymer-water solution was then washed with room temperature chloroform (3 x 50 mL). The polymer was recovered from rotary evaporation of the aqueous layer to yield a yellow-brown powder (155 mg, 87% yield). ^{1}H NMR (400 MHz, MeOD) δ 3.95-3.35 (b, 5581H, (C**H**$_2$C**H**$_2$N-C$_3$(N(C**H**$_2$)$_2$(C**H**$_2$)$_2$O)$_2$) .

Synthesis of PEI(Pep)

This procedure was performed open to the atmosphere. Linear polyethyleneimine (25k) (50 mg, 1.16 mmol, 1 equiv) was dissolved in chloroform (4 mL) in a scintillation vial. To the vial was added *N,N*-diisopropylethylamine (0.61 mL, 3.49 mmol, 3 equiv) and a solution of BACPep (480 mg, 1.74 mmol, 1.5 equiv) in 4 mL chloroform. The reaction mixture was allowed to stir at 65 ˚C for three hours. The resulting solution was diluted with water, and transferred to a 3.5k MWCO Spectrum Labs dialysis bag and dialyzed against water. The resulting solution was concentrated under vacuum, and precipitated from acetone into ethyl acetate (-78 °C) and recovered by centrifugation to yield a brown powder (68 mg, 20% recovered yield). ^{1}H NMR (400 MHz, MeOD) δ 3.95-3.40 (b, 6,972H, ((C**H**$_2$C**H**$_2$N)-C$_3$(N(C**H**$_2$)$_2$(CH$_2$)$_2$CH$_2$)$_{581}$) δ 1.90-1.60 (b, 6,972H, ((CH$_2$CH$_2$N)-C$_3$(N(CH$_2$)$_2$(C**H**$_2$)$_2$C**H**$_2$)$_2$)$_{581}$).

Information for biological experiments

Cell Culture: HEK 293T cells (American Type Culture Collection) were grown in Dulbecco's Modified Eagle Medium with L-glutamine (Gibco) supplemented with 10% FBS (Atlanta Biologicals) and 1% penicillin/streptomycin (Gibco). Cultures were incubated in humidified tissue incubators (Thermo Scientific) at 37°C and 5% CO_2.

Cell Viability Measurements: Trypan blue dye exclusion counting was performed in triplicate with an automated cell counter (ViCell, Beckman-Coulter). Cell viability under experimental conditions is reported as a percentage relative to untreated cells.

Polymer-DNA Complexation: Solutions of polymer in RNase-free water were added to 3 μg of pDNA (gWiz-Luciferase, Aldevron, Fargo, ND) at specified loadings. The solutions were then vortexed at 1500 rpm for 3 min at room temperature.

Cell Transfection and Luciferase Expression: 293T cells were seeded on 12-well plates at a density of 50,000 cells per well 24 hours prior to transfection. The media was then evacuated, replaced with fresh media, and supplemented with the polymer-pDNA complex. After 48 hours of incubation, cell viability was measured, and cells were re-plated on 96-well plates and analyzed for luciferase activity according to manufacturer's protocol. Briefly, cells were rinsed with PBS and lysed with 20 μL/well 1X Cell Lysis Buffer (Promega, Madison, WI). To the cell lysates was added 100 μL/well of Luciferase Assay Reagent (Promega) and the light produced was measured on a plate reader (PerkinElmer, Waltham, MA). Results were expressed as relative light units (RLU) normalized to cell counts, with error bars representing the standard deviation from the triplicate measurement.

Charge Ratio Calculation:

$$\frac{(3\ \mu g\ pDNA)\times(MW\ per\ polymer\ repeat\ unit)}{330\ g\ per\ pDNA\ nucleotide}$$

$$= Mass\ of\ polymer\ required\ for\ 1{:}1\ charge\ ratio\ with\ pDNA$$

Supplementary References

(1) See references discussed in: Bandar, J. S.; Lambert, T. H. *Synthesis* **2013**, *45*, 2485.

(2) Jiang, Y.; Freyer, J. L.; Cotanda, P.; Brucks, S. D.; Killops, K. L.; Bandar, J. S.; Torsitano, C.; Balsara, N. P.; Lambert, T. H.; Campos, L. M. *Nat. Commun.* **2015**, *6*.