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Chemically Modifiable *N*-Heterocycle-functionalized Polycarbonates as a Platform for Diverse Smart Biomimetic Nanomaterials

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Supporting Information

Experimental Section

Materials. MTC-OC_6F_5 was obtained from Central Glass and purified by recrystallizing twice from a mixture of ethyl acetate and hexanes. Anhydrous solvents were dried using activated alumina columns and stored over 3 Å molecular sieves. All other materials were purchased from Sigma-Aldrich and used as received.

Methods. ^{1}H NMR spectra were acquired on a Bruker Avance 400 instrument at 400 MHz. Gel permeation chromatography (GPC) was performed in tetrahydrofuran (THF) using a Waters system equipped with four 5-μm Waters columns (300 mm × 7.7 mm) connected in series with increasing pore size (100, 1000, 105, and 106 Å), a Waters 410 differential refractometer, and a 996 photodiode array detector. The system was calibrated using polystyrene standards. GPC analysis was also performed in *N*,*N*-dimethylformamide (DMF) spiked with 0.01 M LiBr using a Waters system equipped with two Agilent PolyPore columns (300 mm × 7.5 mm) connected in series, and a Waters 410 differential refractometer. The system was calibrated with poly(methyl methacrylate) standards.

Poly(MTC-OC_6F_5) (DP = 100).[1] The precursor was prepared according to our previously published protocol (ref. 1): In a nitrogen-purged glovebox, a glass vial was charged with 3-butyn-1-ol (3 mg, 0.01 mmol), MTC-OC_6F_5 (0.357 g, 1.09 mmol), and 1.45 g of dichloromethane (1.0 M with respect to initial concentration of MTC-OC_6F_5). Then, trifluoromethanesulfonic acid (triflic acid) (0.008 g, 0.05 mmol) was added to the stirred solution. As the reaction proceeded, the undissolved MTC-OC_6F_5 slowly went into solution. The reaction was monitored by ^{1}H NMR. Once the reaction was complete (~12 h at this catalyst loading), the polymer was precipitated into hexanes, isolated, and dried to obtain a white solid (yield: 0.356 g, 99 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 4.48 (s, CH_2, 4H), 1.51 (s, CH_3, 3H). GPC (RI): M_n (PDI) = 20.5 kDa (1.15).

Poly(MTC-OC_6F_5) (DP = 50). Synthesized as above, starting with benzyl alcohol (2.2 mg, 0.020 mmol), MTC-OC_6F_5 (0.357 g, 1.09 mmol), 1.45 g of dichloromethane, and triflic acid (8 mg, 0.05 mmol).

Poly(MTC-OC_6F_5) (DP = 32). Synthesized as above, starting with 3-butyn-1-ol (9.9 mg, 0.033 mmol), MTC-OC_6F_5 (0.357 g, 1.09 mmol), 1.45 g of dichloromethane, and triflic acid (8 mg, 0.05 mmol).

Polymer 1a (DP = 100). A 20-mL glass vial containing a magnetic stir-bar was charged with poly(MTC-OC_6F_5) of DP 100 (0.800 g, 2.46 mmol repeat units), anhydrous THF (5.0 mL) and triethylamine (0.273 g, 2.70 mmol), and the solution was cooled to 0 ºC in an ice-water bath. Next, a solution of 1-(3-aminopropyl)imidazole (0.307 g, 2.46 mmol) in THF (1 mL) was added dropwise with vigorous stirring. Turbidity was observed within minutes, followed by the gradual formation of an off-white precipitate. The ice bath was removed and the mixture was allowed to stir for an additional 30 minutes, after which

excess diethyl ether (15 mL) was added. The mixture was briefly sonicated and then centrifuged. The mother liquor was decanted and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded a white solid which was then dried under high vacuum for 24 h. Yield: 0.57 g (87 %). ^{1}H NMR (MeOD, 400 MHz): δ (ppm) = 7.68 (s, 1H), 7.14 (s, 1H), 6.97 (s, 1H), 4.25 (s, 4H, carbonate C$\underline{H}_2$), 4.00 (m, 2H), 3.15 (m, 2H), 1.95 (m, 2H), 1.20 (s, 3H, C$\underline{H}_3$).

Polymer 1b (DP = 50). A 20-mL glass vial containing a magnetic stir-bar was charged with poly(MTC-OC_6F_5) of DP 50 (0.200 g, 0.613 mmol repeat units), anhydrous THF (1.0 mL) and triethylamine (0.0680 g, 0.674 mmol), and the solution was cooled to 0 ºC in an ice-water bath. Next, a solution of 1-(3-aminopropyl)imidazole (0.0730 g, 0.583 mmol) in THF (0.5 mL) was added dropwise with vigorous stirring. Turbidity was observed within minutes, followed by the gradual formation of an off-white precipitate. The ice bath was removed and the mixture was allowed to stir for an additional 30 minutes, after which excess diethyl ether (15 mL) was added. The mixture was briefly sonicated and then centrifuged. The mother liquor was decanted and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded a white solid which was then dried under high vacuum for 24 h. Yield: 0.164 g (~100 %). ^{1}H NMR (MeOD, 400 MHz): δ (ppm) = 7.68 (s, 1H), 7.14 (s, 1H), 6.97 (s, 1H), 4.25 (s, 4H, carbonate C$\underline{H}_2$), 4.00 (m, 2H), 3.15 (m, 2H), 1.95 (m, 2H), 1.20 (s, 3H, C$\underline{H}_3$).

Polymer 1c (DP = 32). A 20-mL glass vial containing a magnetic stir-bar was charged with poly(MTC-OC_6F_5) of DP 32 (0.910 g, 2.79 mmol repeat units), anhydrous THF (5.0 mL) and triethylamine (0.31 g, 3.07 mmol), and the solution was cooled to 0 ºC in an ice-water bath. Next, a solution of 1-(3-aminopropyl)imidazole (0.332 g, 2.65 mmol) in THF (1 mL) was added dropwise with vigorous stirring. Turbidity was observed within minutes, followed by the gradual formation of an off-white precipitate. The ice bath was removed and the mixture was allowed to stir for an additional 30 minutes, after which excess diethyl ether (15 mL) was added. The mixture was briefly sonicated and then centrifuged. The mother liquor was decanted and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded a white solid which was then dried under high vacuum for 24 h. Yield: 0.746 g (> 99 %). ^{1}H NMR (MeOD, 400 MHz): δ (ppm) = 7.68 (s, 1H), 7.14 (s, 1H), 6.97 (s, 1H), 4.25 (s, 4H, carbonate C$\underline{H}_2$), 4.00 (m, 2H), 3.15 (m, 2H), 1.95 (m, 2H), 1.20 (s, 3H, C$\underline{H}_3$).

Polymer 2. A 20-mL glass vial containing a magnetic stir-bar was charged with poly(MTC-OC_6F_5) of DP 32 (0.910 g, 2.79 mmol repeat units), anhydrous THF (5.0 mL) and triethylamine (0.311 g, 3.07 mmol), and the solution was cooled to 0 ºC in an ice-water bath. Next, a solution of 2-(2'-aminoethyl)pyridine (0.341 g, 2.79 mmol) in THF (1.0 mL) was added dropwise with vigorous stirring. The ice bath was removed and the mixture was allowed to stir for an additional 30 minutes, after which the solution was pipetted into excess diethyl ether (15 mL) to precipitate the polymer. The flocculent mixture was briefly sonicated and then centrifuged. The mother liquor was decanted and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded the polymer as a white solid which was subsequently dried under high vacuum for 24 h. Yield: 0.729 g (~99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 8.45

(pyridine CH, 1H), 7.60 (pyridine CH, 1H), 7.43 (broad s, NH, 1H), 7.14 (pyridine CH, 1H), 7.12 (pyridine CH, 1H), 4.20 (s, carbonate CH_2, 4H), 3.58 (CH_2, 2H), 2.92 (CH_2, 2H), 1.17 (s, CH_3, 3H).

Polymer 3a. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **1a** (0.650 g, 2.43 mmol repeat units), 1,3-propanesultone (0.446 g, 3.65 mmol, 1.5 equivalents), and anhydrous DMF (2.0 mL). The reaction mixture was stirred at 18 ºC for 24 h, during which gelation occurred. The gel was dissolved by addition of a minimal volume of water (~0.5 mL), and the resulting solution was precipitated into stirred diethyl ether. The suspension was then centrifuged and the solvent decanted to give polymer **3a** as a white solid. The poly(sulfobetaine) was washed with additional portions of ether, dried under high vacuum for 24 h. Yield: 0.862 g (91 %). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 8.80 (s, 1H, imid), 7.55 (s, 1H, imid), 7.50 (s, 1H, imid), 4.34 (t, 2H, CONH-C$\underline{H}_2$), 4.24 (broad s, 4H, carbonate), 4.17 (t, 2H), 3.18 (m, 2H), 2.86 (t, 2H), 2.26 (m, 2H), 2.06 (m, 2H), 1.19 (s, 3H, C$\underline{H}_3$).

Polymer 3b. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **1b** (0.164 g, 0.613 mmol repeat units), 1,3-propanesultone (0.112 g, 0.920 mmol, 1.5 equivalents), and anhydrous DMF (1.0 mL). The reaction mixture was stirred at 18 ºC for 24 h, during which gelation occurred. The gel was dissolved by addition of a minimal volume of water (~0.5 mL), and the resulting solution was precipitated into stirred diethyl ether. The suspension was then centrifuged and the solvent decanted to give the target polymer as a white solid. The poly(sulfobetaine) was washed with additional portions of ether, dried under high vacuum for 24 h. Yield: 0.223 g (93 %). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 8.80 (s, 1H, N=C$\underline{H}$-N), 7.55 (s, 1H, imid), 7.50 (s, 1H, imid), 4.34 (t, 2H, CONH-C$\underline{H}_2$), 4.24 (broad s, 4H, carbonate), 4.17 (t, 2H), 3.18 (m, 2H), 2.86 (t, 2H), 2.26 (m, 2H), 2.06 (m, 2H), 1.19 (s, 3H, C$\underline{H}_3$).

Polymer 4. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **1c** (0.746 g, 2.79 mmol repeat units), 1-bromobutane (0.573 g, 4.18 mmol, 1.5 equivalents), and anhydrous DMF/acetonitrile (2:1 v/v, 6.0 mL). The reaction mixture was heated overnight at 60 ºC in the sealed vial, after which it was concentrated under reduced pressure and then precipitated into diethyl ether. The suspension was centrifuged and the mother liquor was decanted off, leaving a white solid which was freeze-dried under high vacuum over 3 days. Yield: 1.01 g (94 %). ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 8.75 (s, 1H), 7.49 (s, 1H), 7.47 (s, 1H), 4.10-4.30 (br, 8H), 3.17 (m, 2H), 2.05 (m, 2H), 1.79 (m, 2H), 1.25 (m, 2H), 1.19 (s, 3H, CH_3), 0.85 (s, 3H, CH_3).

Polymer 5. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **4** (0.404 g, 1.51 mmol repeat units), 1-bromobutane (0.145 g, 1.06 mmol, 0.7 equivalents), and anhydrous DMF/acetonitrile (2:1 v/v, 4.0 mL). The reaction mixture was heated overnight at 60 ºC in the sealed vial, after which it was concentrated under reduced pressure and then precipitated into diethyl ether. The suspension was centrifuged and the mother liquor was decanted off, leaving a white solid which was freeze-dried under high vacuum for 3 days. Yield: 0.483 g of 66 % quaternized polymer. ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 8.72 (s, 1H), 7.65 (s, 1H), 7.45 (br, 2H), 7.09 (s, 1H), 6.95 (s,

1H), 4.21 (br), 4.14 (br), 3.96 (br), 3.0-3.2 (br), 2.02 (br, 2H), 1.92 (br, 2H), 1.78 (br, 2H), 1.1-1.3 (m, 3H), 0.84 (s, 3H, CH_3).

Polymer 6 (mPEG-*b*-poly(MTC-OC_6F_5) diblock copolymer). In a nitrogen-purged glovebox, a small vial was charged with 5 kDa mPEG−OH (4.0 g, 0.80 mmol), MTC-OC_6F_5 (3.39 g, 10.4 mmol), and 10.4 mL of dichloromethane (1 M with respect to MTC-OC_6F_5). The solution was stirred until the 5 kDa mPEG-OH was fully dissolved. The MTC-OC_6F_5 is only partially soluble at this concentration. Finally, triflic acid (0.12 g, 0.80 mmol) was added to the stirred solution. As the reaction proceeded, the remaining MTC-OC_6F_5 slowly dissolved, giving a homogeneous solution. The reaction was monitored by ^{1}H NMR. At the end of the reaction, the polymer was precipitated into cold diethyl ether, isolated, and dried overnight under high vacuum to obtain a white solid (yield: 0.616 g, 62 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 4.46 (s, 4H, carbonate C$\underline{H}_2$), 3.64 (s, 4H, OC$\underline{H}_2$C$\underline{H}_2$), 1.51 (s, 3H, C$\underline{H}_3$). GPC (RI): M_n (PDI) = 11.26 kDa (1.10). DP of MTC-OC_6F_5 block (by ^{1}H NMR analysis) = 11.

Polymer 7. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **6** (0.60 g, 0.78 mmol of MTC-OC_6F_5 repeat units), anhydrous DMF (2.0 mL) and triethylamine (0.079 g, 0.78 mmol). Next, a solution of 1-(3-aminopropyl)imidazole (0.10 g, 0.78 mmol) in DMF (0.5 mL) was added dropwise with stirring. The mixture was allowed to stir for 30 min at room temperature, after which it was pipetted into excess diethyl ether (15 mL) to precipitate the polymer as a white solid. The mixture was briefly sonicated and then centrifuged. The mother liquor was decanted off and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded a white solid which was then dried under high vacuum for 24 h. ^{1}H NMR (MeOD, 400 MHz): δ (ppm) = 7.69 (s, 1H), 7.14 (s, 1H), 6.97 (s, 1H), 4.25 (s, 4H, carbonate C$\underline{H}_2$), 4.01 (m, 2H), 3.64 (s, 4H, OC$\underline{H}_2$C$\underline{H}_2$), 3.14 (m, 2H), 1.96 (m, 2H), 1.20 (s, 3H, C$\underline{H}_3$).

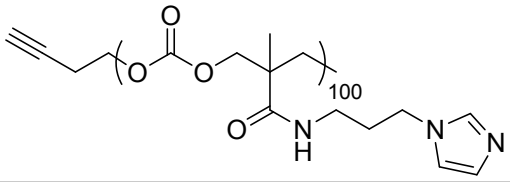
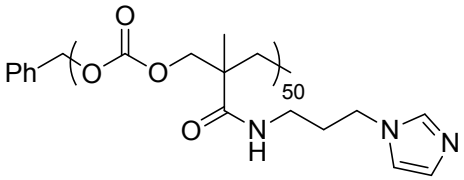
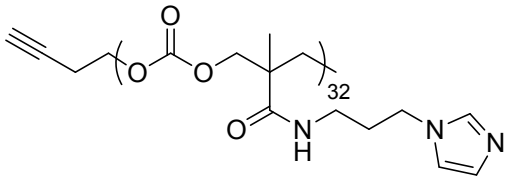
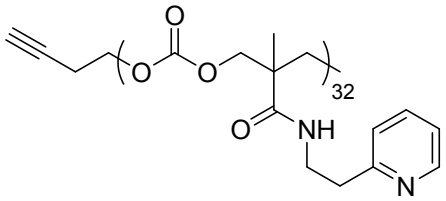
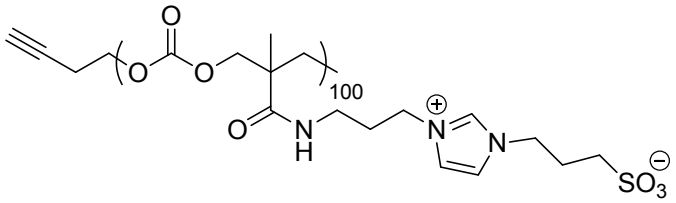
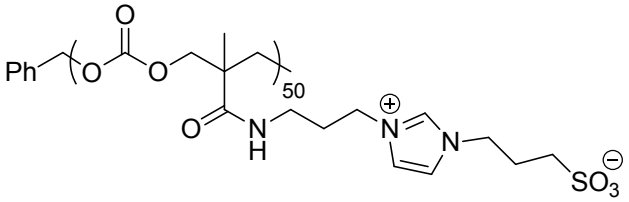
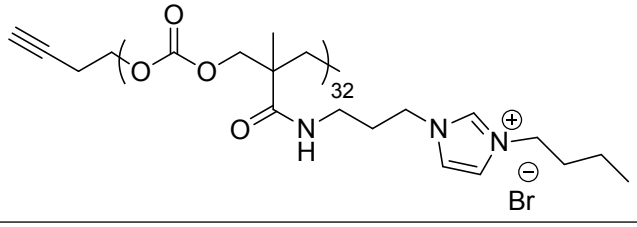
Polymer 8. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **6** (0.30 g, 0.39 mmol repeat units), anhydrous DMF (2.0 mL) and triethylamine (0.039 g, 0.39 mmol). Next, a solution of 2-(2-aminoethyl)pyridine (0.047 g, 0.39 mmol) in DMF (0.5 mL) was added dropwise with stirring. The mixture was allowed to stir for 30 min at room temperature, after which it was pipetted into excess diethyl ether (15 mL) to precipitate the polymer. The mixture was briefly sonicated and then centrifuged. The mother liquor was decanted away and additional diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded the polymer as a white solid which was then dried under high vacuum for 24 h. ^{1}H NMR (MeOD, 400 MHz): δ (ppm) = 8.44 (br, 1H), 7.74 (m, 1H), 7.28 (br, 2H), 4.24 (s, 4H, carbonate C$\underline{H}_2$), 3.65 (s, 4H, OC$\underline{H}_2$C$\underline{H}_2$), 3.50 (m, 2H), 2.95 (m, 2H), 1.19 (s, 3H, C$\underline{H}_3$).

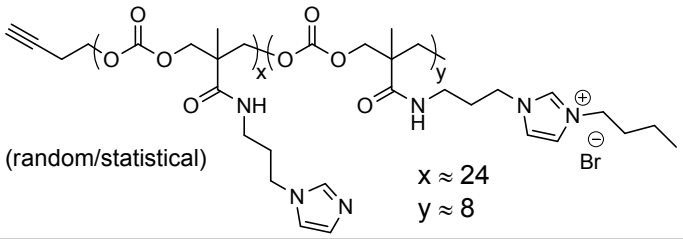
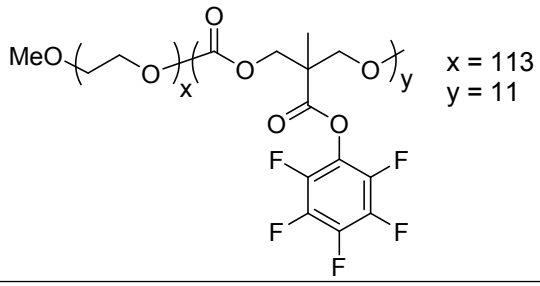
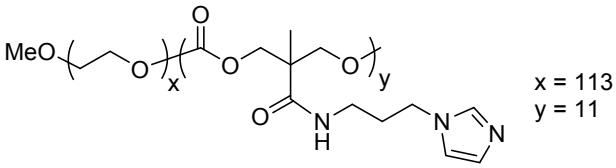
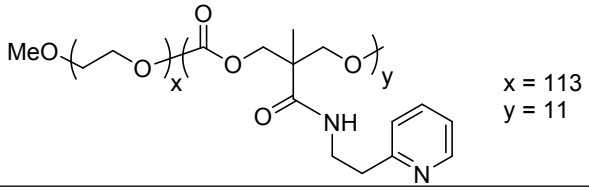
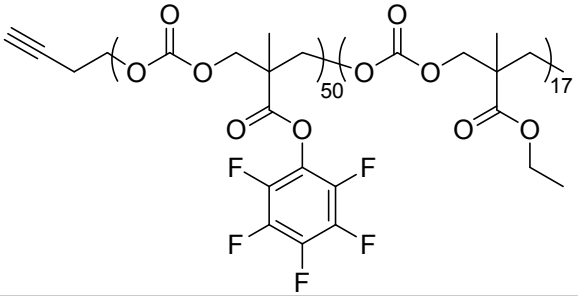
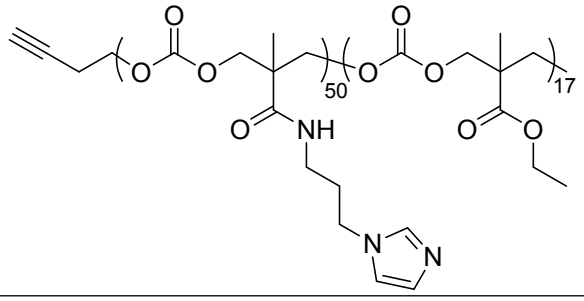
Polymer 9. Polymer **9** was synthesized according to our previously published procedure (ref. 1), starting from 3-butyn-1-ol (0.006 g, 0.02 mmol), MTC-OC_6F_5 (0.357 g, 1.09 mmol), and 1.45 g of dichloromethane (1.0 M with respect to MTC-OC_6F_5).

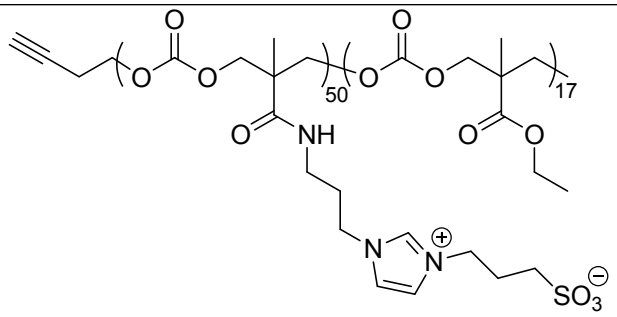
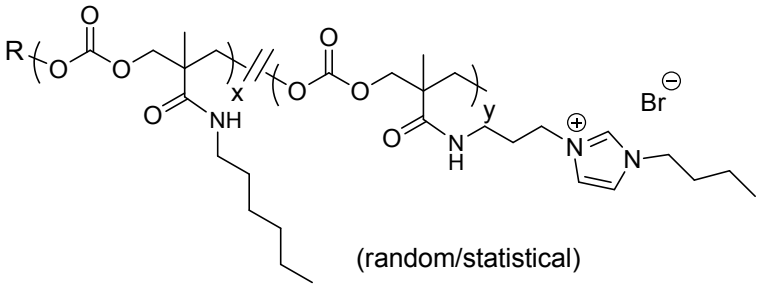
Polymer 10. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **9** (0.700 g, 1.79 mmol of MTC-OC_6F_5 repeat units), anhydrous THF (5.0 mL) and triethylamine (0.200 g, 1.97 mmol), and the solution was cooled to 0 ºC in an ice-water bath. Next, a solution of 1-(3-aminopropyl)imidazole (0.225 g, 1.79 mmol) in THF (1.0 mL) was added dropwise with stirring. The ice-bath was removed and the mixture was allowed to stir for 45 min, after which it was pipetted into excess diethyl ether (15 mL) to precipitate the polymer. The mixture was briefly sonicated and then centrifuged. The mother liquor was decanted and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded a white solid which was then dried under high vacuum for 24 h. ^{1}H NMR (MeOD, 400 MHz): δ (ppm) = 7.68 (s, 1H), 7.14 (s, 1H), 6.97 (s, 1H), 4.25 (s, 4H, carbonate C$\underline{H}_2$), 4.17 (br, 2H), 4.00 (m, 2H), 3.14 (m, 2H), 1.94 (m, 2H), 1.20 (s, 3H, C$\underline{H}_3$).

Polymer 11. A 20-mL glass vial containing a magnetic stir-bar was charged with polymer **10** (0.530 g, 1.60 mmol of imidazole-bearing repeat units), 1,3-propanesultone (0.293 g, 2.40 mmol, 1.5 equivalents), and anhydrous DMF (1.5 mL). The reaction mixture was stirred at room temperature (18 ºC) for 24 h before it was precipitated into stirred diethyl ether. The suspension was then centrifuged and the solvent was decanted to afford the target polymer as a white solid. The polymer was washed with additional portions of ether, dried under high vacuum for 24 h. ^{1}H NMR (D_2O, 400 MHz): δ (ppm) = 8.80 (s, 1H, N=C$\underline{H}$-N), 7.55 (s, 1H, imid), 7.50 (s, 1H, imid), 4.34 (t, 2H, CONH-C$\underline{H}_2$), 4.24 (m, 4H, carbonate), 4.16 (m, 2H), 3.18 (m, 2H), 2.86 (m, 2H), 2.27 (m, 2H), 2.06 (m, 2H), 1.19 (s, 3H, C$\underline{H}_3$).

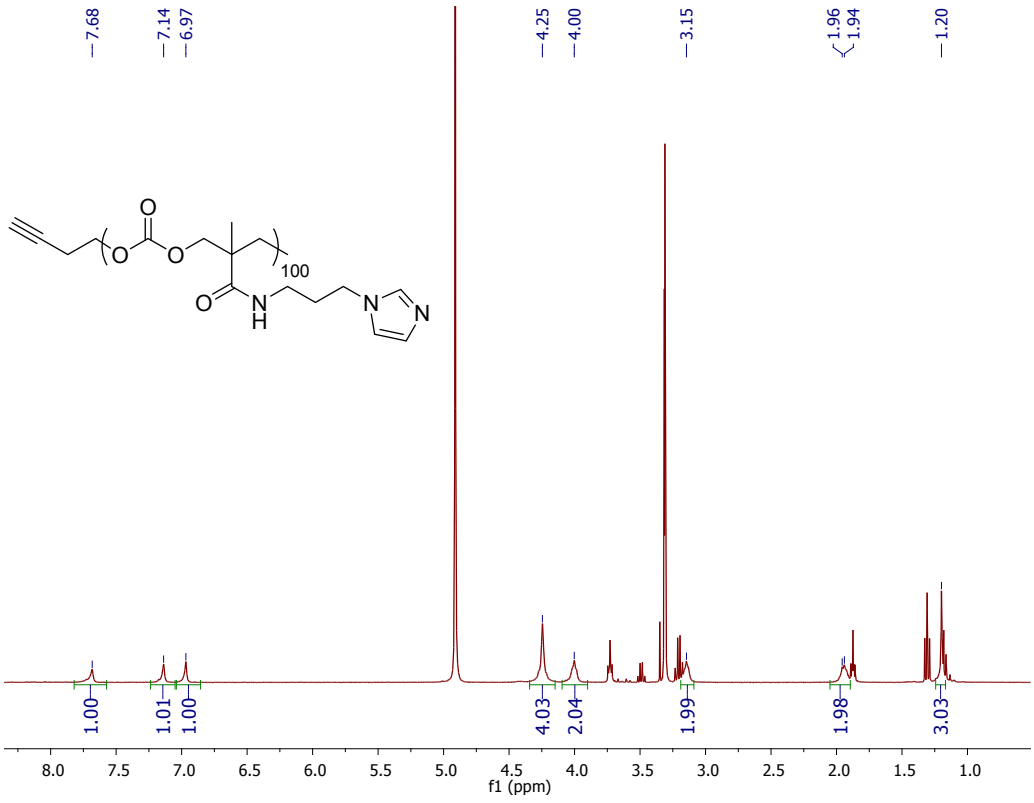
Polymer 12. A 20-mL glass vial containing a magnetic stir-bar was charged with poly(MTC-OC_6F_5) (0.150 g, 0.46 mmol repeat units), anhydrous THF (2.0 mL) and triethylamine (46.5 mg, 0.0460 mmol), and the solution was cooled to 0 ºC in an ice-water bath. Next, 1-(3-aminopropyl)imidazole (0.0460 g, 0.368 mmol) in THF (1.0 mL) and *n*-hexylamine (9.3 mg, 0.092 mmol) in THF (1.0 mL) was added sequentially with vigorous stirring. The ice bath was removed and the mixture was allowed to stir for an additional 30 minutes, after which the solution was pipetted into excess diethyl ether (15 mL) to precipitate the polymer. The flocculent mixture was briefly sonicated and then centrifuged. The mother liquor was decanted and more diethyl ether (20 mL) was added. A second round of sonication, centrifuging, and decanting afforded the functionalized polymer as a white solid which was subsequently dried under high vacuum for 24 h. This precursor/intermediate polymer was then dissolved in DMF (2.0 mL) and 1-bromobutane (79.0 mg, 0.575 mmol) was added to the solution, followed by heating overnight in a sealed vial at 60 ºC, after which it was concentrated under reduced pressure and then precipitated into diethyl ether. The suspension was centrifuged and the mother liquor was decanted off, leaving a white solid which was freeze-dried under high vacuum for 3 days. Yield: 0.g (% over 2 steps). ^{1}H NMR (H_2O, 400 MHz): δ (ppm) = 8.75 (s, 1H), 7.49 (s, 1H), 7.47 (s, 1H), 4.25 (br, 4H), 4.16 (br, 4H), 3.15 (br, 2H), 2.05 (br, 2H), 1.79 (br, 2H), 1.0-1.3 (m), 0.86 (s, 3H, C$\underline{H}_3$).

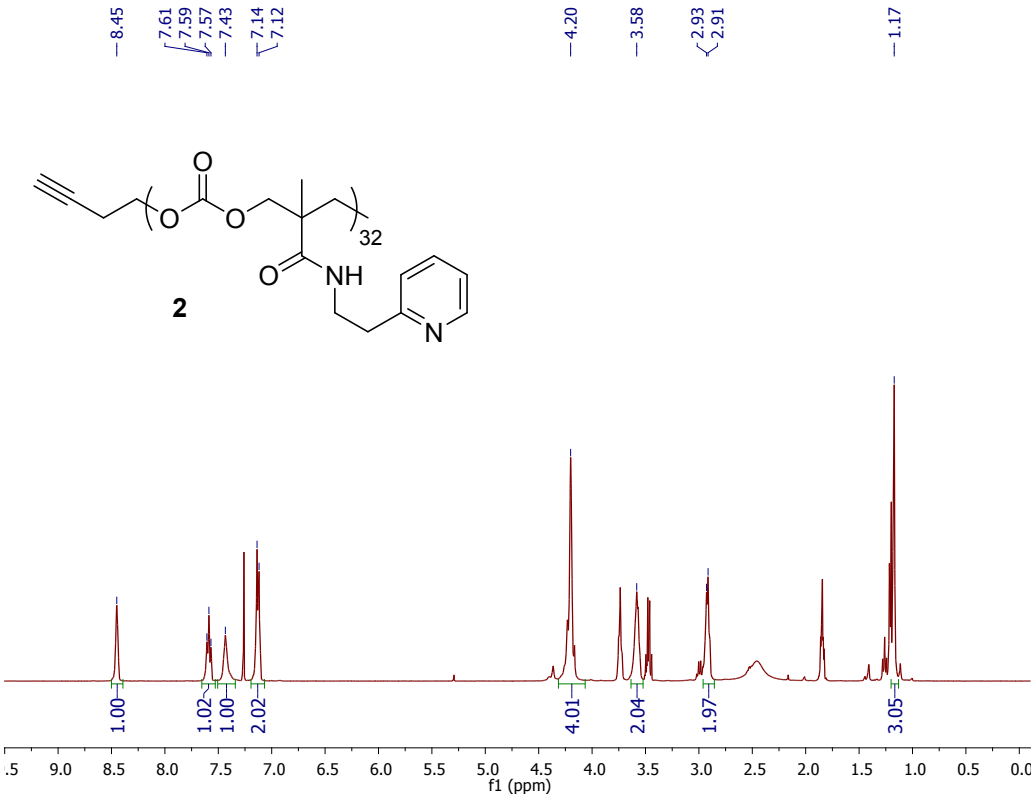
Polymer	Structure	DP	M_n (kDa)
1a		100	26.7
1b		50	13.4
1c		32	8.55
2		32	8.50
3a		100	3.90
3b		50	19.5
4		32	40.5

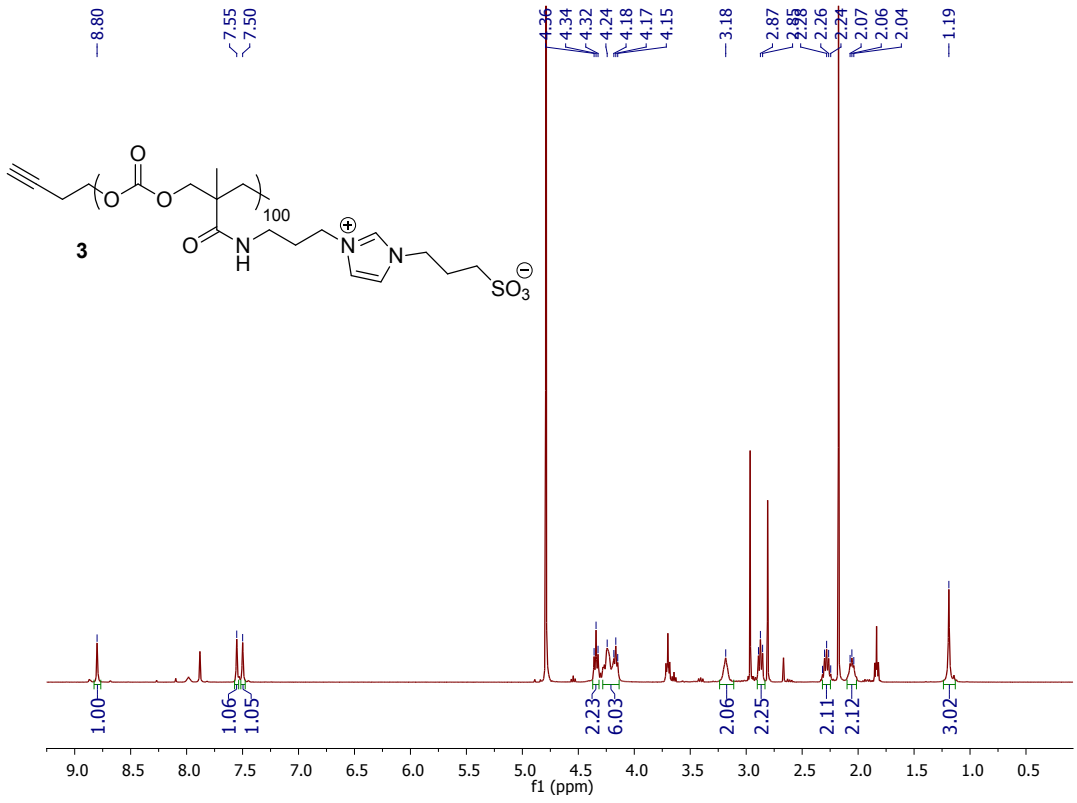
5	 (random/statistical) x ≈ 24 y ≈ 8	32	12.9
6	 x = 113 y = 11	113, 11	8.60
7	 x = 113 y = 11	113, 11	7.94
8	 x = 113 y = 11	113, 11	7.90
9		50, 17	19.5
10		50, 17	16.6

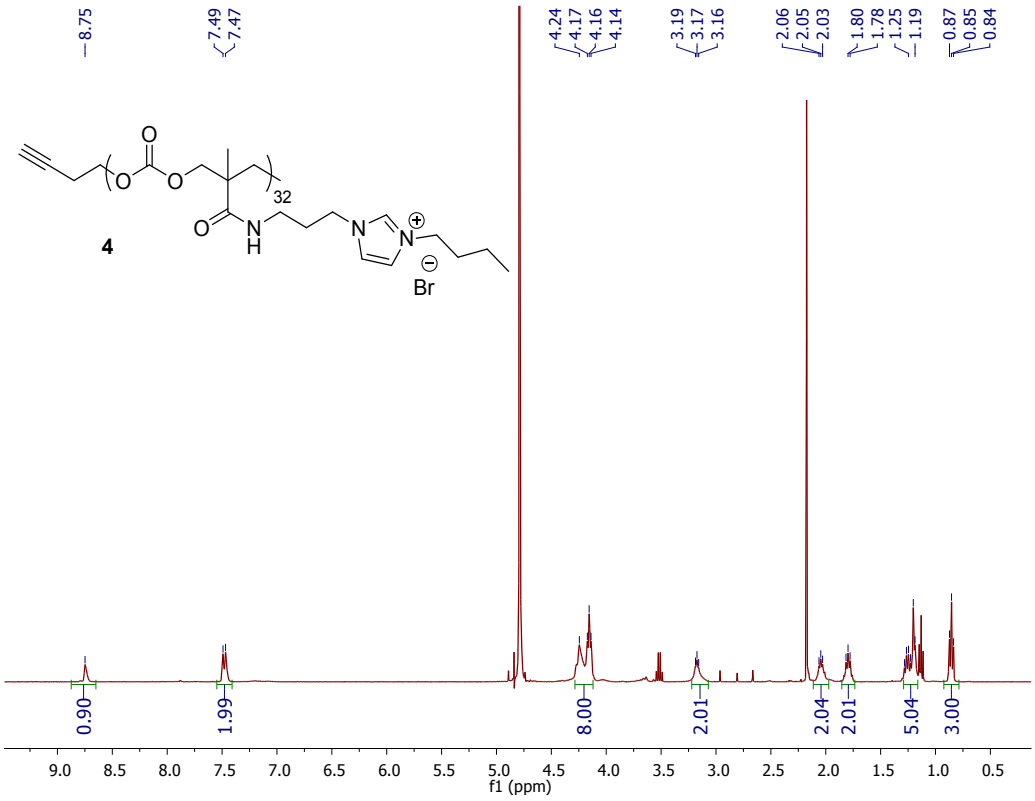
11		50, 17	22.7
12	 (random/statistical)	x=46 y=12	16.1

Polymer **1**:

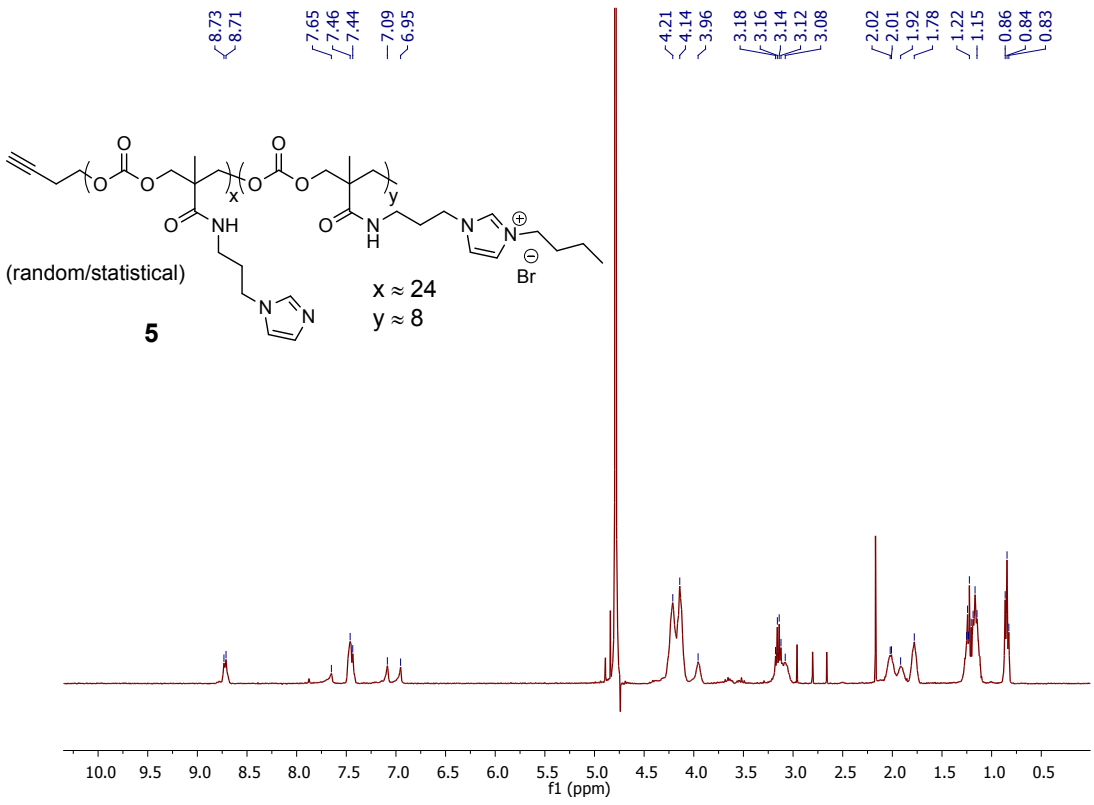



8.45
7.61
7.59
7.57
7.43
7.14
7.12
4.20
3.58
2.93
2.91
1.17
O
O
O
32
O
NH
N
2
1.00
1.02
1.00
2.02
4.01
2.04
1.97
3.05
f1 (ppm)

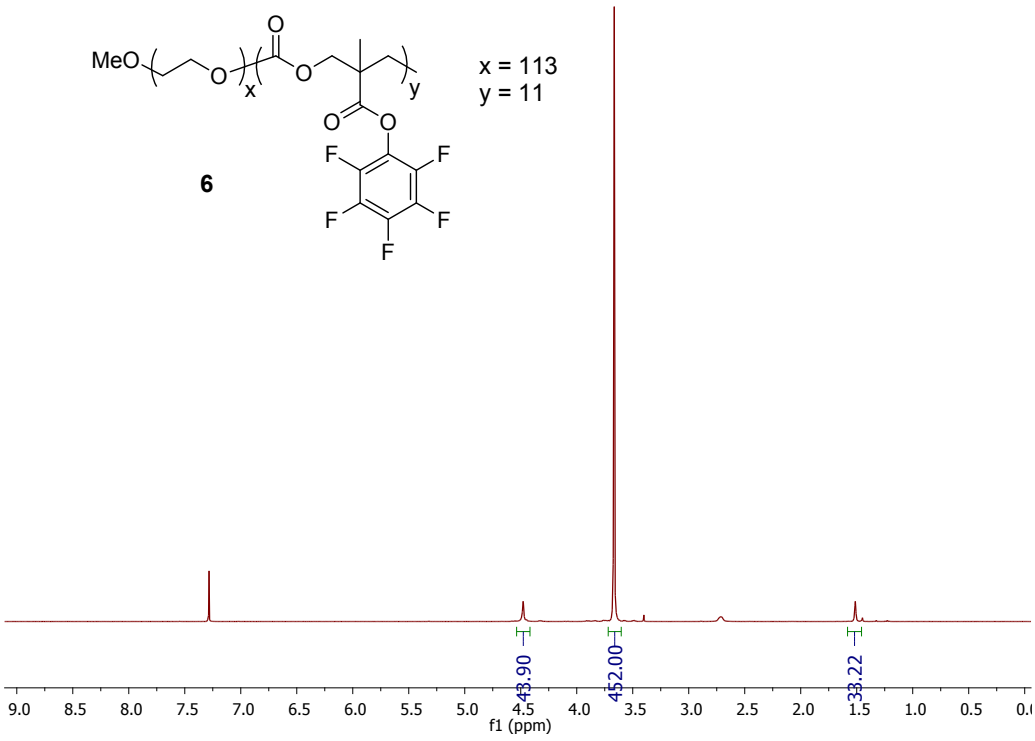

8.80
7.55
7.50
4.36
4.34
4.32
4.24
4.18
4.17
4.15
3.18
2.87
2.85
2.26
2.24
2.07
2.06
2.04
1.19
O
O
O
100
O
N
H
N
N
SO3
3
1.00
1.06
1.05
2.23
6.03
2.06
2.25
2.11
2.12
3.02
f1 (ppm)

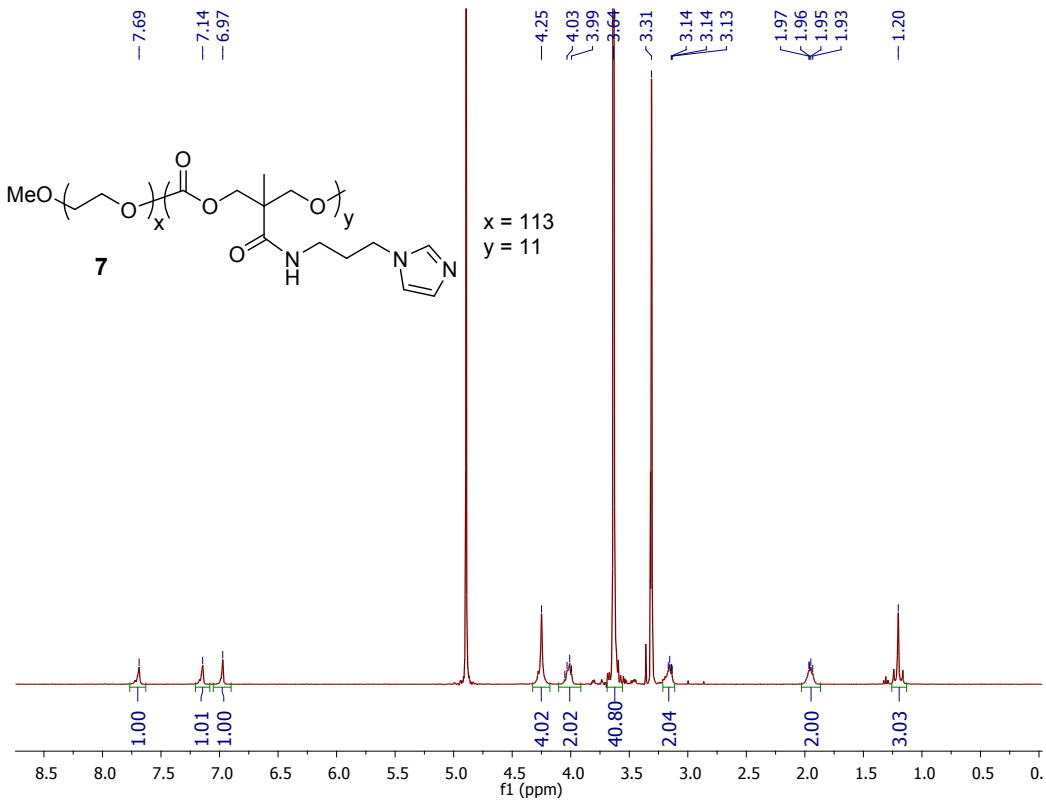

8.75
7.49
7.47
4.24
4.17
4.16
4.14
3.19
3.17
3.16
2.06
2.05
2.03
1.80
1.78
1.25
1.19
0.87
0.85
0.84
32
Br
0.90
1.99
8.00
2.01
2.04
2.01
5.04
3.00
f1 (ppm)

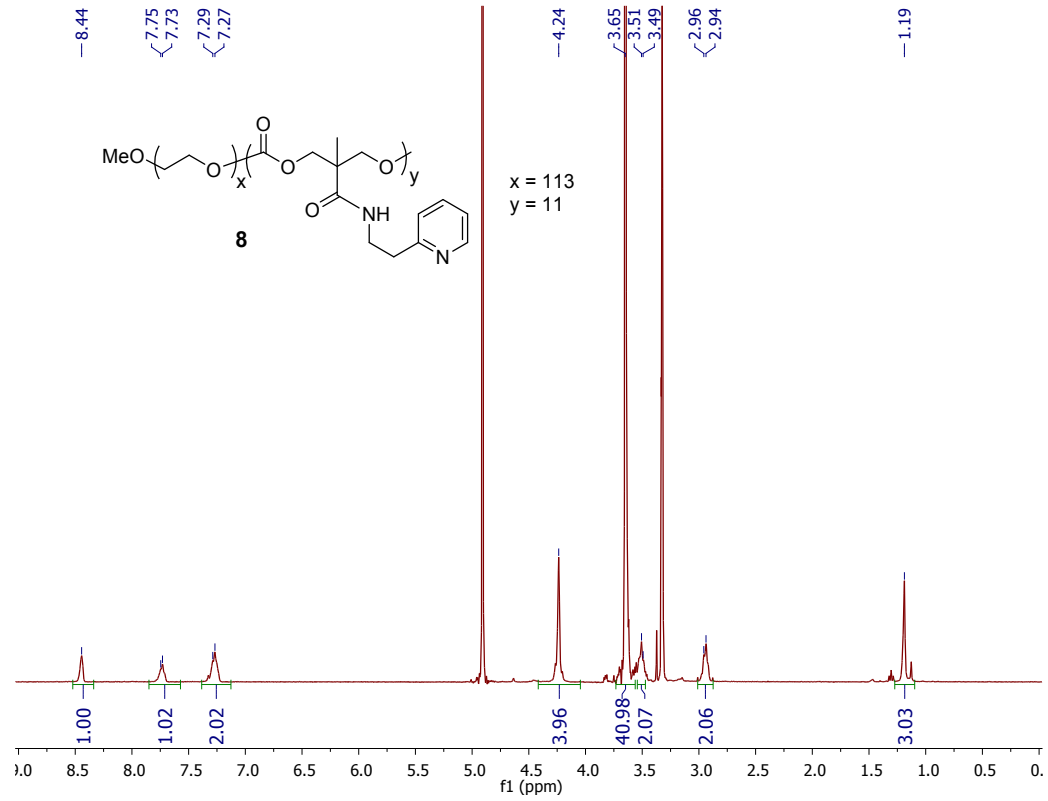
4

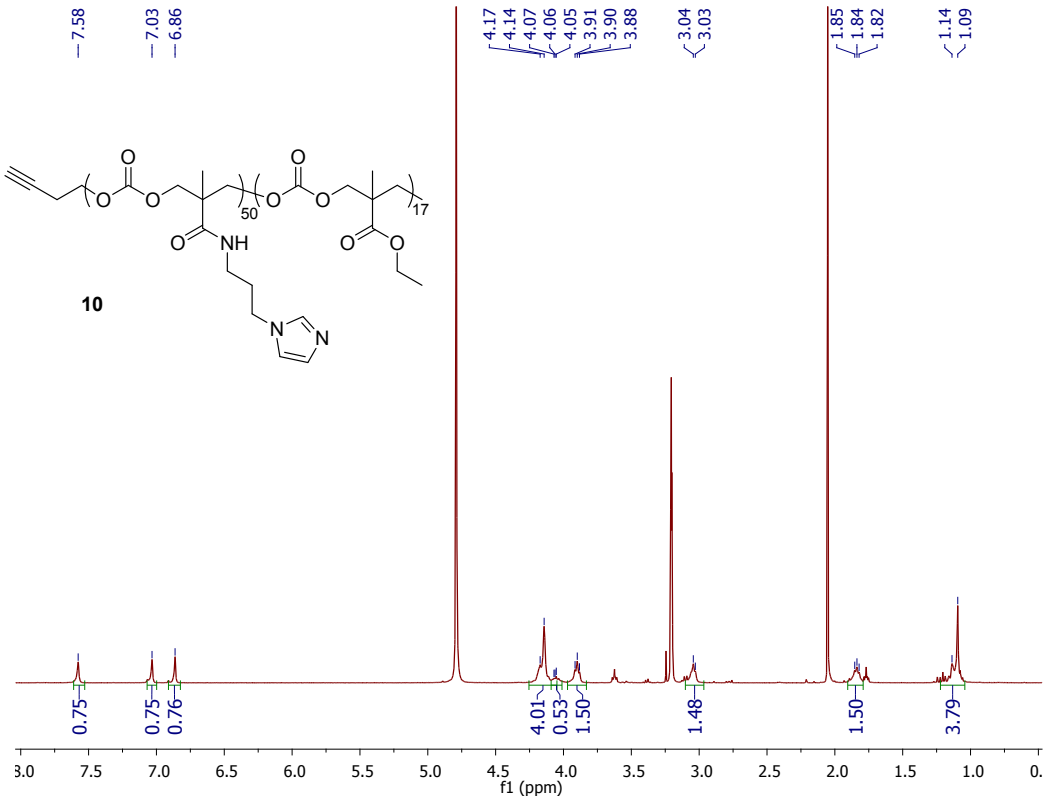

8.73
8.71
7.65
7.46
7.44
7.09
6.95
4.21
4.14
3.96
3.18
3.16
3.14
3.12
3.08
2.02
2.01
1.92
1.78
1.22
1.15
0.86
0.84
0.83
x
y
NH
Br
(random/statistical)
x ≈ 24
y ≈ 8
f1 (ppm)

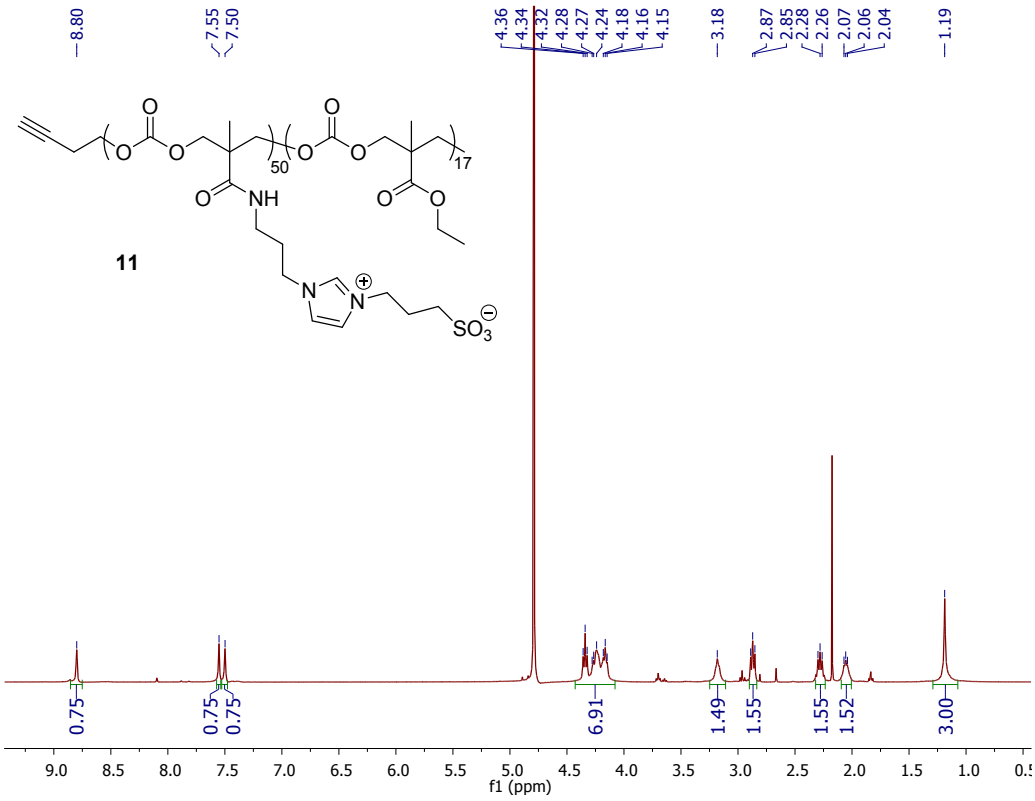
5

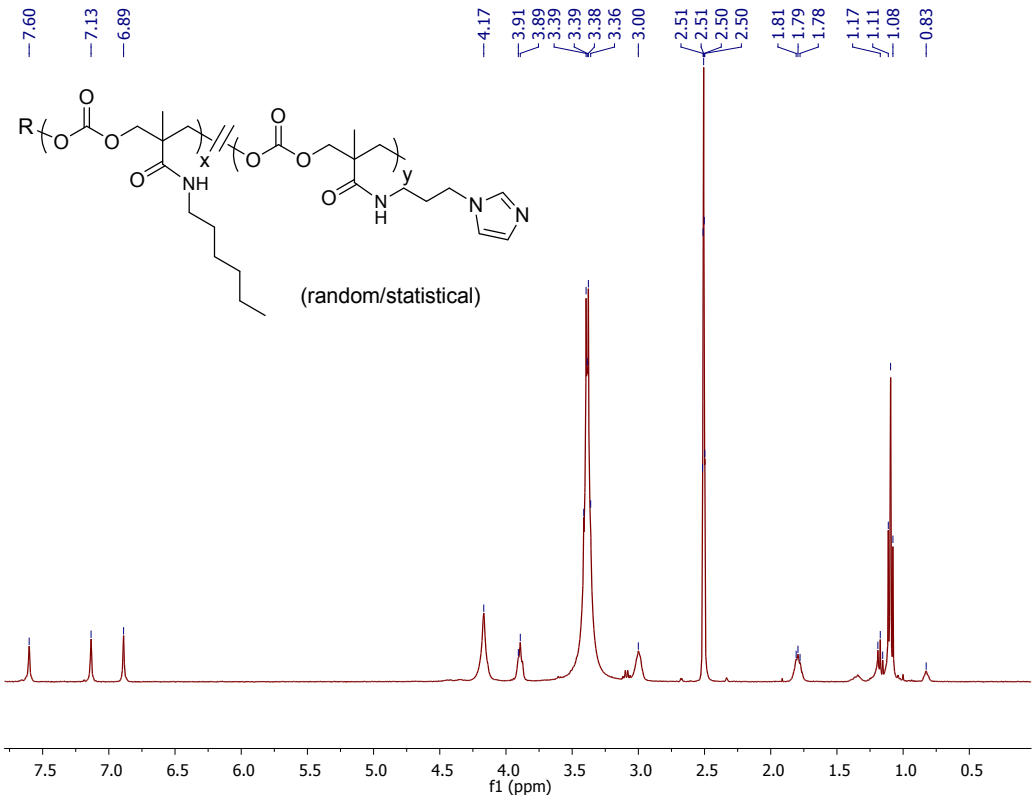

MeO
O
x
O
O
y
O
O
F
F
F
F
F
x = 113
y = 11
6
43.90
52.00
33.22
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

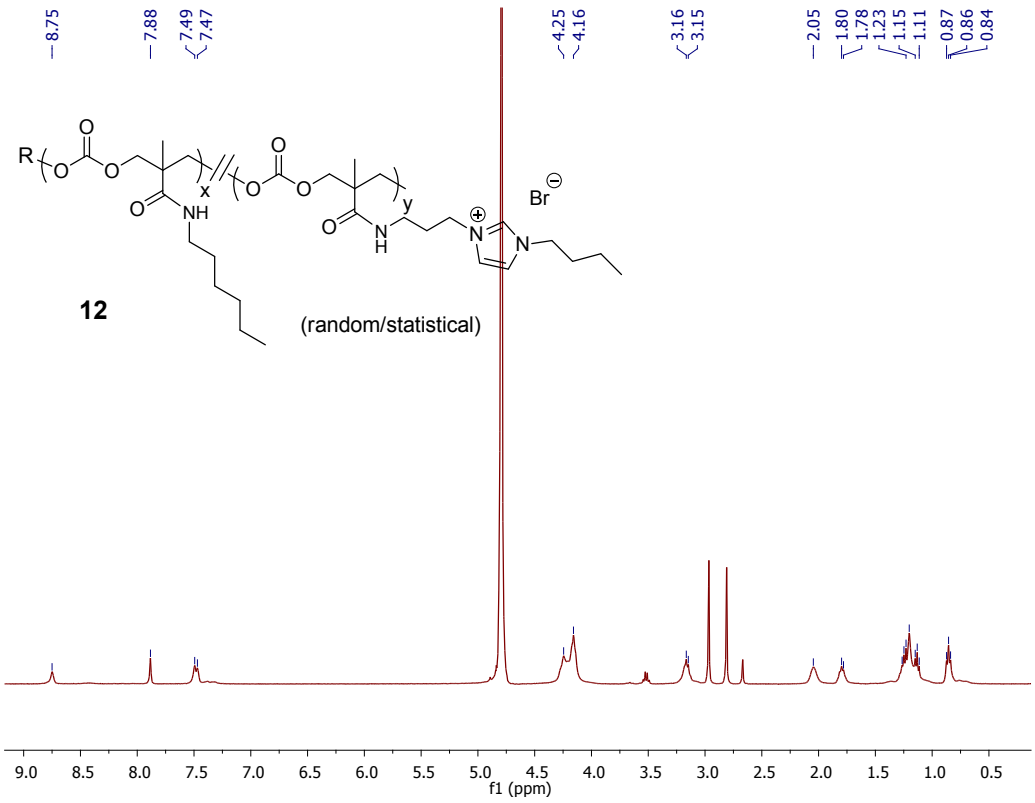

7.69
7.14
6.97
4.25
4.03
3.99
3.64
3.31
3.14
3.14
3.13
1.97
1.96
1.95
1.93
1.20
MeO
O
x
O
O
O
y
O
N
H
N
N
x = 113
y = 11
7
1.00
1.01
1.00
4.02
2.02
40.80
2.04
2.00
3.03
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


8.44
7.75
7.73
7.29
7.27
4.24
3.65
3.51
3.49
2.96
2.94
1.19
MeO
O
x
O
O
O
y
O
NH
N
x = 113
y = 11
8
1.00
1.02
2.02
3.96
40.98
2.07
2.06
3.03
f1 (ppm)


7.58
7.03
6.86
4.17
4.14
4.07
4.06
4.05
3.91
3.90
3.88
3.04
3.03
1.85
1.84
1.82
1.14
1.09
O
O
O
O
O
50
O
O
17
O
NH
O
O
N
N
10
0.75
0.75
0.76
4.01
0.53
1.50
1.48
1.50
3.79
f1 (ppm)


8.80
7.55
7.50
4.36
4.34
4.32
4.28
4.27
4.24
4.18
4.16
4.15
3.18
2.87
2.85
2.28
2.26
2.07
2.06
2.04
1.19
11
50
17
NH
SO3
0.75
0.75
0.75
6.91
1.49
1.55
1.55
1.52
3.00
f1 (ppm)


7.60
7.13
6.89
4.17
3.91
3.89
3.39
3.39
3.38
3.36
3.00
2.51
2.51
2.50
2.50
1.81
1.79
1.78
1.17
1.11
1.08
0.83
R
x
y
NH
N
H
(random/statistical)
f1 (ppm)



Biological studies

Cell viability studies

Two zwitterionic homopolymers (polymers **3a** and **3b**) and one zwitterionic amphiphilic diblock copolymer (polymer **11**) were chosen as representative polymers for the toxicity tests involving human embryonic kidney (HEK293) cell lines.

HEK293 cells were cultured in RPMI-1640 supplied with 10 % fetal bovine serum (FBS) and 1 % penicillin-streptomycin. HEK293 cells were seeded onto 96-well plates at a density of 10,000 cells/well. The cells were incubated at 37 °C, 5 % CO_2. After 24 h, the medium was replaced with fresh medium containing a polymer at various concentrations. After being incubated for 48 h, 100 μL of fresh medium and 20 μL of 5 mg/mL (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (i.e. MTT) solution was used to replace the sample medium. After 4 h of incubation, the medium was removed, and DMSO (150 μL) was added to each well to dissolve the formazan crystals. The

absorbance of each well was measured as the absorbance value at 550 nm deducted by the value at 690 nm with a microplate reader (Power-Wave X, Bio-tek Instruments, U.S.A.). The results were presented as a percentage of absorbance of the blank control.

Aggregation studies

The stability and stealth properties of the three polyzwitterions were investigated by measuring particle size change of polymers in a serum-containing medium. Polymers were dissolved in phosphate-buffered saline (PBS) containing 10 % FBS. The particle sizes of polymer solutions were analyzed using 90Plus/BI-MAS (Brookhaven Instruments Corporation, Holtsville, NY) equipped with a He-Ne laser beam at 658 nm (scattering angle: 90°) over 24 h or 48 h. The concentration of the polymers was 500 mg/L. Each sample was measured 3 times and an average particle size was obtained.

Evaluation of polymer cytotoxicity

Polymer cytotoxicity was evaluated by analyzing the viability of HEK293 cells (as a model cell line) after incubation with polymers for 48 h. PEG polymers (5 kDa and 10 kDa) were used as controls. Similar to both PEG polymers, all the three polycarbonates did not show significant cytotoxicity (Figure A).

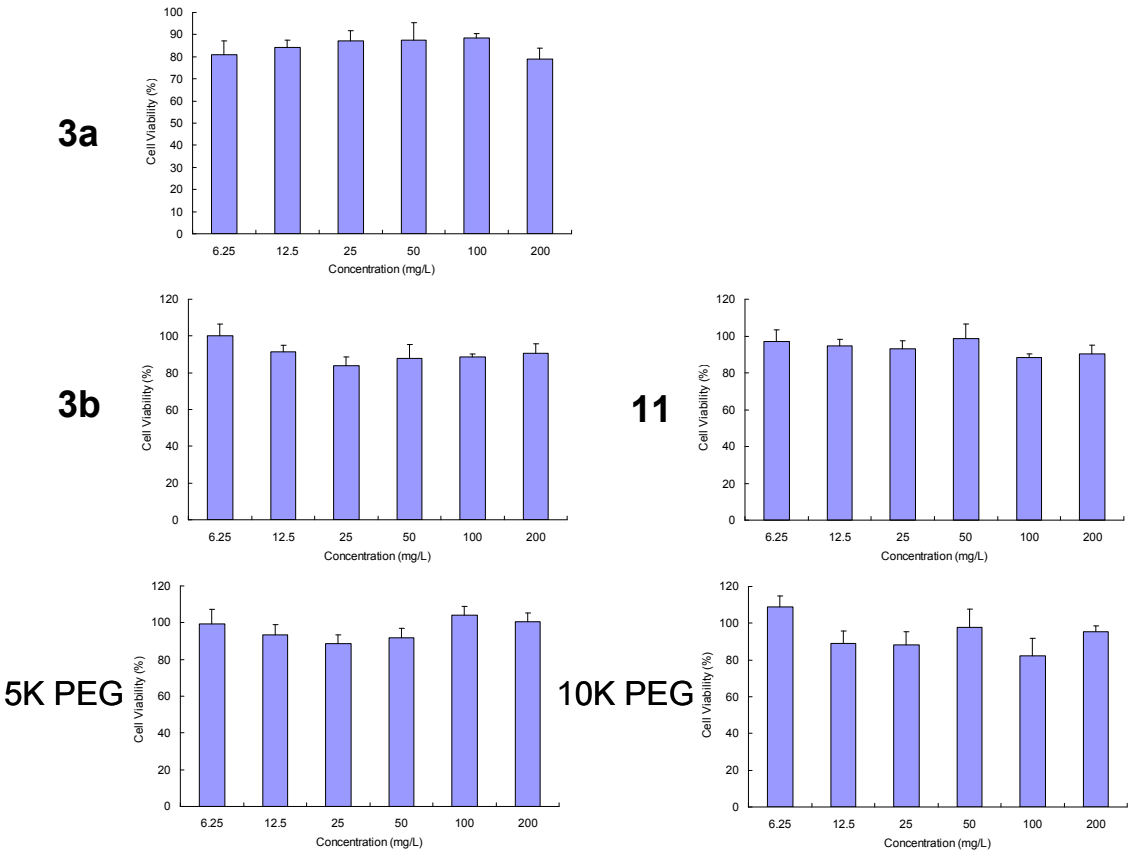


Figure A. Viability of HEK293 cells after incubation with polyzwitterions and poly(ethylene glycol) (PEG) for 48 h.

Stability and stealth properties

To investigate if these polymers had stealth characteristics, the polymers were dissolved in serum-containing phosphate-buffered saline (PBS, pH 7.4) and particle sizes of the polymer solutions were then monitored to see if there is any aggregation caused by polymer-protein interactions. Similar to serum in PBS, the particle size of polyzwitterionic homopolymers (**3a** and **3b**) in PBS containing 10 % serum was stable over a period of 48 h (Figure B), indicating that there was no aggregation caused by the polymers. However, the amphiphilic copolymer (polymer **11**) caused aggregation after 2 h of incubation. This is likely due to the presence of the hydrophobic MTC-OEt block.

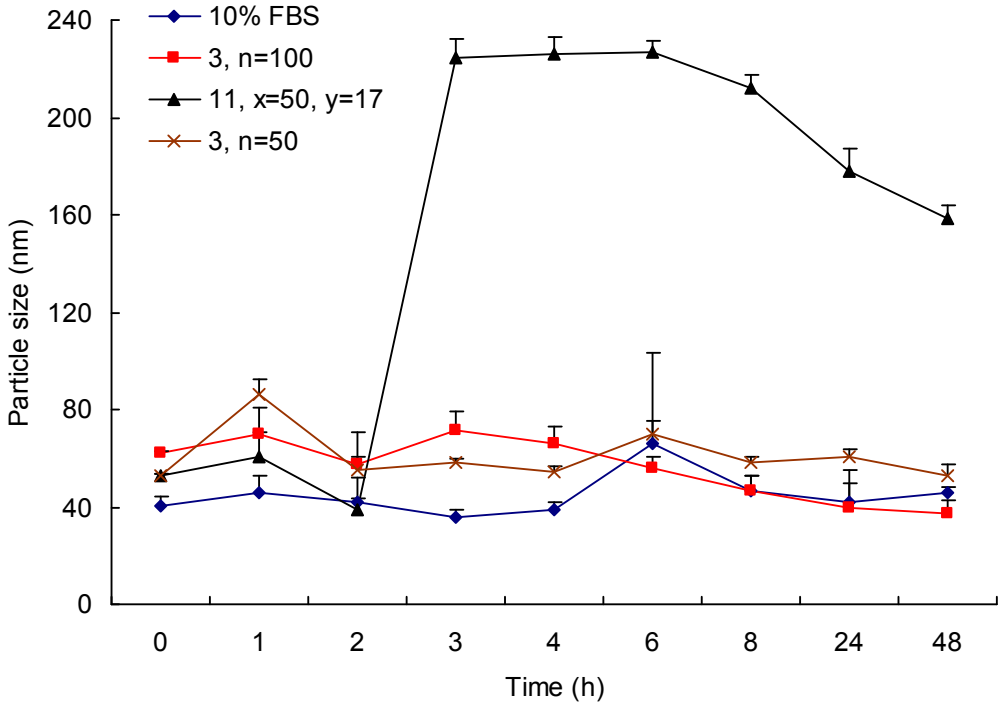


Figure B. Particle size changes of the polyzwitterions **3a** (DP 100), **3b** (DP 50), and **11** (diblock copolymer) in PBS containing 10 % fetal bovine serum (FBS) over time.

References

[1] A. C. Engler, J. M. W. Chan, D. J. Coady, J. M. O'Brien, H. Sardon, A. Nelson, D. P. Sanders, Y. Y. Yang, J. L. Hedrick, *Macromolecules* **2013**, *46*, 1283-1290.