Supporting information for:

Influence of Charge Density on Host-Guest Interactions Within Amphiphilic Polymer Assemblies in Apolar Media

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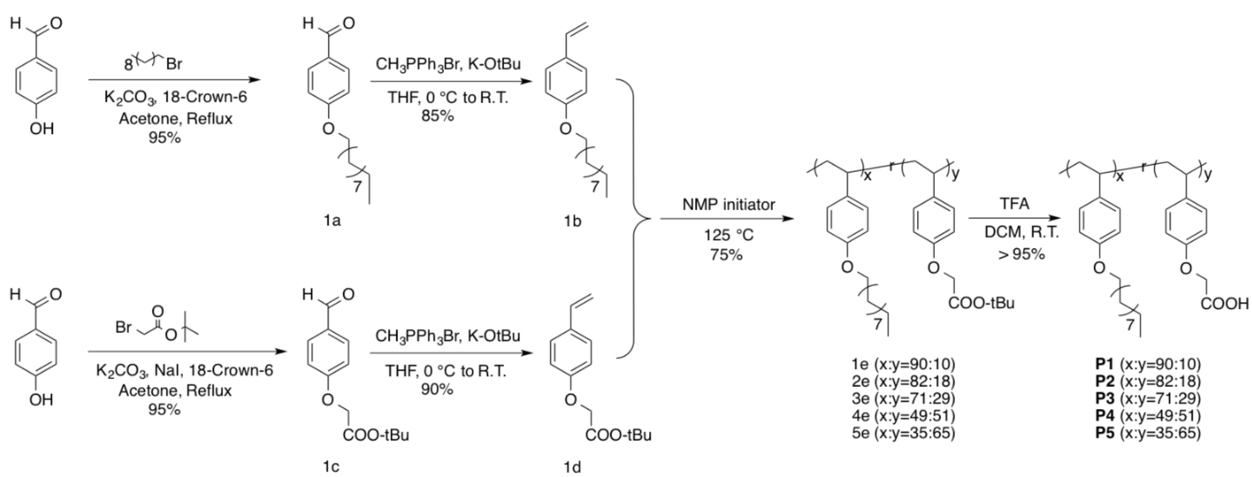
Polymer synthesis and characterization

General methods: All reagents were commercially available and used as received unless stated otherwise. ^{1}H-NMR spectra were recorded on a 400 MHz and a 500 MHz NMR spectrometer using residual proton resonance of the solvents as internal standard. Chemical shifts are reported in parts per million (ppm). Mass spectra were obtained by a Bruker AmaZon quadrupole ion trap mass spectrometer coupled with electrospray ionization source. Gel permeation chromatography (GPC) was used to estimate the molecular weight of polymers using THF as eluent and 1 μL of toluene was added as the internal reference. Polystyrene standards were used for calibration and data analysis. Dynamic light scattering (DLS) was measured by a Malvern Zetasizer. Transmission electron microscopy (TEM) images were taken from JEOL JEM-2000FX.

Synthesis of random co-polymer P1-P5



<u>Synthesis of compound **1a**:</u>

To a solution of acetone mixed with K_2CO_3 (11.84 g, 85.65 mmol) and 18-crown-6 (1.13 g, 4.28 mmol), 4-Hydroxybenzaldehyde (5.23 g, 42.83 mmol) was added and stirred for 5 min. To this mixture, 1-Bromodecane (14.21 g, 64.24 mmol) was added and stirred while refluxing for 20 h. The reaction mixture was then cooled to room temperature and filtered to afford the crude product in acetone solution. The solvent was evaporated to dryness and purified by silica gel column chromatography (8-10% ethyl acetate in hexanes) to obtain 10.5 g (95% yield) of **1a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.86 (s, 1H), δ 7.80-7.82 (d, 2H), δ 6.96-6.99 (d, 2H), δ 4.00-4.04 (t, 2H), δ 1.76-1.83 (quint, 2H), δ 1.47-1.26 (m, 14H), δ 0.85-0.89 (t, 3H).

Synthesis of compound **1b**:

Methyltriphenylphosphonium bromide (6.58 g, 25.11 mmol) and Potassium tert-butoxide (3.94 g, 35.15 mmol) were mixed in a round bottom flask, and dry THF (20 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 15 min to yield the bright yellow solution. **1a** (6.58 g, 25.11 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline 3 times. The organic layer was evaporated to dryness and purified by silica gel column chromatography (3-5% ethyl acetate in hexanes) to afford 5.5 g (85% yield) of **1b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.31-7.33 (d, 2H), δ 6.83-6.85 (d, 2H), δ 6.61-6.68 (q, 1H), δ 5.57-5.61 (d, 1H), δ 5.09-5.12 (d, 1H), δ 3.93-3.96 (t, 3H), δ 1.73-1.80 (quint, 2H), δ 1.27-1.46 (m, 14H), δ 0.86-0.89 (t, 3H).

Synthesis of compound **1c**:

To a solution of acetone mixed with K_2CO_3 (6.79 g, 49.13 mmol), NaI (7.36 g, 49.13 mmol) and 18-crown-6 (0.65 g, 2.46 mmol), 4-Hydroxybenzaldehyde (3.00 g, 24.57 mmol) was added and stirred for 5 min. To this mixture, tert-Butyl bromoacetate (9.58 g, 49.13 mmol) was added and stirred while refluxing for 20 h. The reaction mixture was then cooled to room temperature and filtered to afford the crude product in acetone solution. The solvent was evaporated to dryness and purified by silica gel column chromatography (10-13% ethyl acetate in hexanes) to obtain 5.5 g (95% yield) of **1c**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.88 (s, 1H), δ 7.82-7.84 (d, 2H), δ 6.97-6.99 (d, 2H), δ 4.59 (s, 2H), δ 1.47 (s, 9H); ESI-MS (expected: $[m+H]^+$= 237.1, obtained: $[m+Na]^+$= 259.1)

Synthesis of compound **1d**:

Methyltriphenylphosphonium bromide (7.94 g, 22.24 mmol) and Potassium tert-butoxide (2.50 g, 22.24 mmol) were mixed in a round bottom flask, and dry THF (15 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 15 min to yield the bright yellow solution. **1c** (3.5 g, 14.83 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline 3 times. The organic layer was evaporated to dryness and purified by silica gel column chromatography (3-5% ethyl acetate in hexanes) to afford 3.1 g (90% yield) of **1d**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.35 (d, 2H), δ 6.84-6.87 (d, 2H), δ 6.63-6.68 (q, 1H), δ 5.60-5.64 (q, 1H), δ 5.13-5.15 (q, 1H), δ 4.51 (s, 2H), δ 1.49 (s, 9H); ESI-MS (expected: $[m+H]^+$= 235.1, obtained: $[m+Na]^+$= 257.1)

Synthesis of random co-polymer **1e-5e**:

A mixture of the compound **1b** (108 mg, 0.41 mmol), **1d** (11 mg, 0.046 mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 3 mg, 0.009 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 ^{o}C under argon for 12 h. After the reaction cooled down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in MeOH. The precipitate was collected and dried under vacuum to yield 90 mg (75% yield) of **1e**. GPC (PMMA/THF): M_n= 12K Da, Đ= 1.14; Same method was used for **2e-5e** except for feeding ratios.

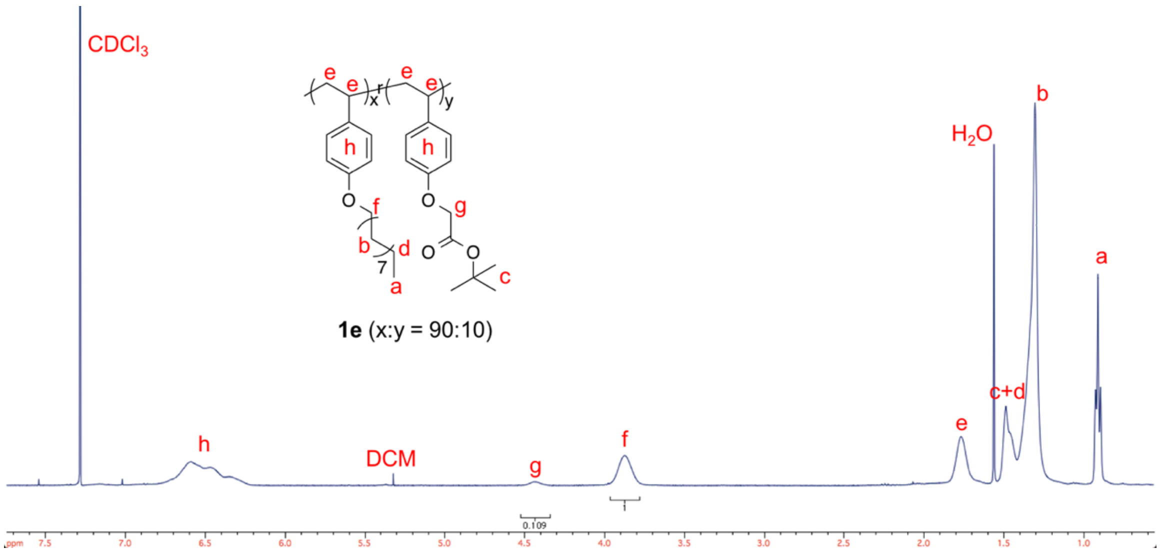
1b (160 mg, 0.61 mmol), **1d** (36 mg, 0.15 mmol) and NMP (5 mg, 0.015 mmol) were polymerized to get **2e** (GPC (PMMA/THF): M_n= 15K Da, Đ= 1.08).

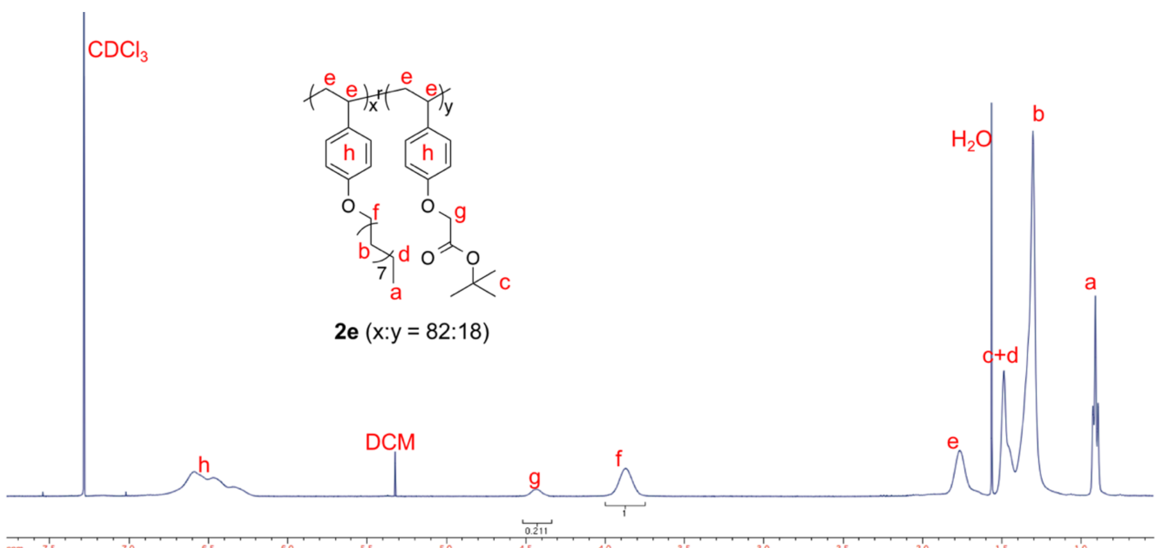
1b (84 mg, 0.32 mmol), **1d** (32 mg, 0.14 mmol) and NMP (3 mg, 0.009 mmol) were polymerized to get **3e** (GPC (PMMA/THF): M_n= 12K Da, Đ= 1.12).

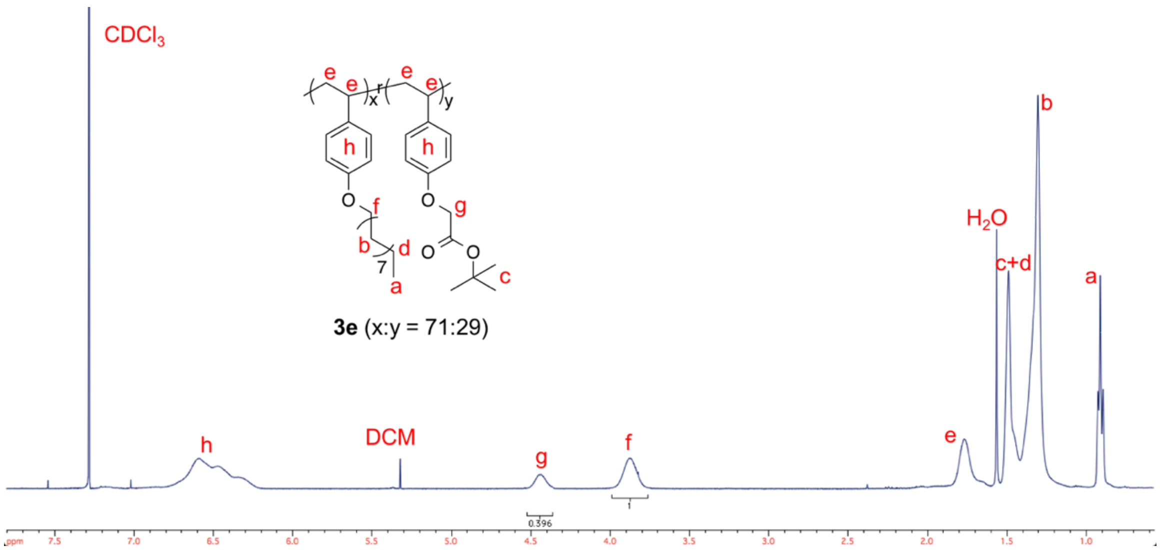
1b (300 mg, 1.15 mmol), **1d** (269 mg, 1.15 mmol) and NMP (15 mg, 0.046 mmol) were polymerized to get **4e** (GPC (PMMA/THF): M_n= 10K Da, Đ= 1.08).

1b (36 mg, 0.14 mmol), **1d** (76 mg, 0.32 mmol) and NMP (3 mg, 0.009 mmol) were polymerized to get **5e** (GPC (PMMA/THF): M_n= 11K Da, Đ= 1.16).

^{1}H NMR (400MHz, $CDCl_3$) are shown below.



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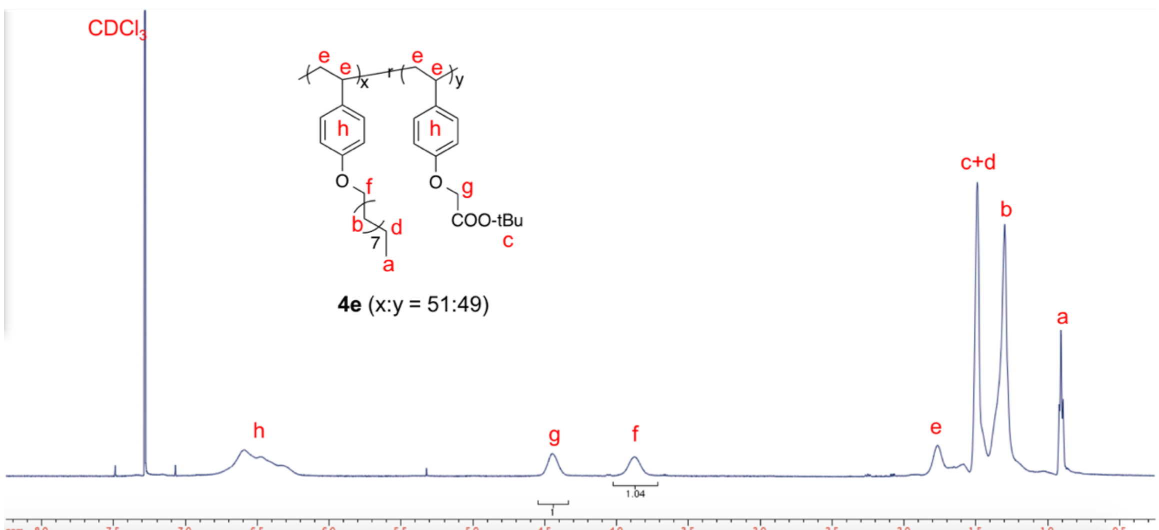
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c+d
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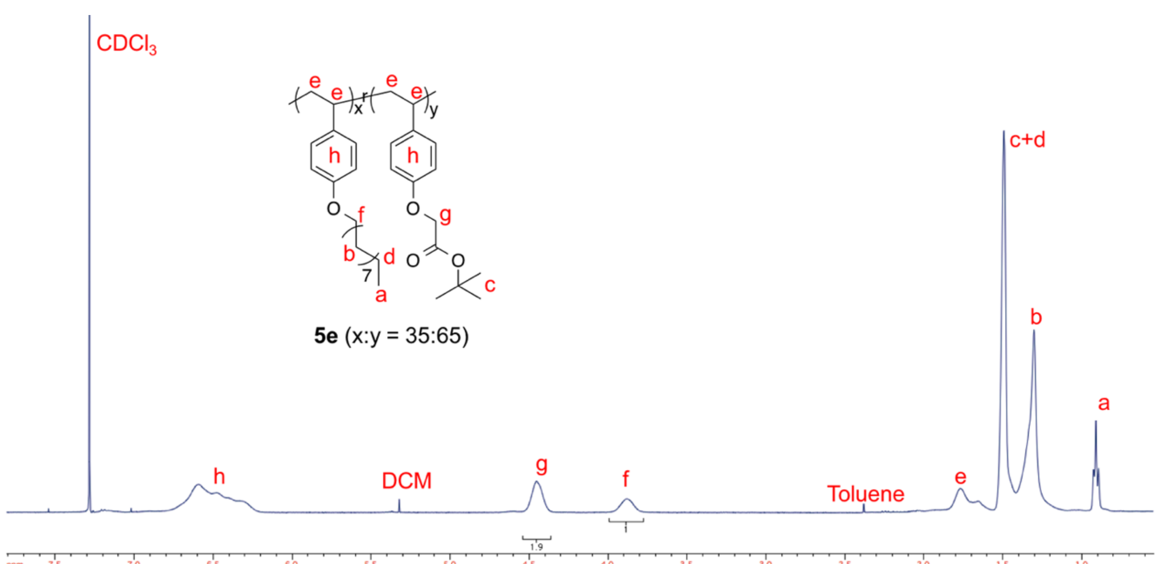
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DCM
g
f
Toluene
e
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b
a
1.9
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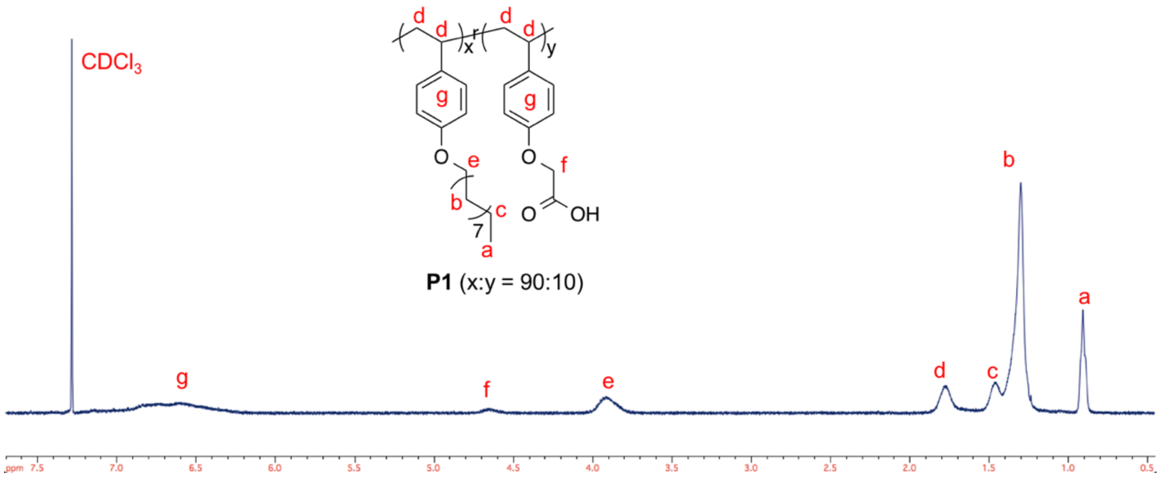
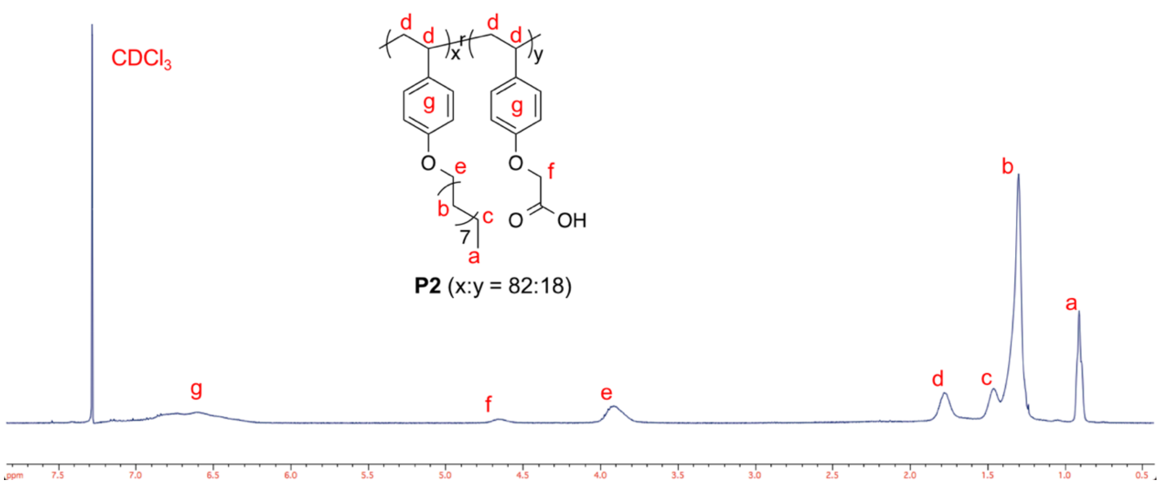
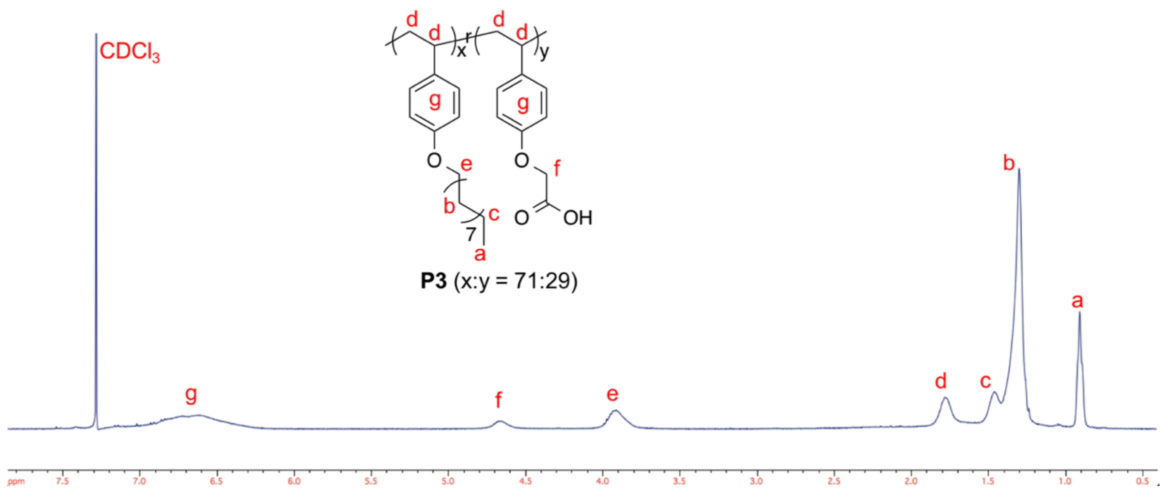
ppm

Synthesis of random co-polymer **P1-P5**:

DCM (2 mL) was added to dissolve the dried random co-polymer **1e-5e**. Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum to obtain the final product **P1-P5**. ^{1}H NMR (400MHz, $CDCl_3$) are shown below.



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r
d
d
y
g
g
O
e
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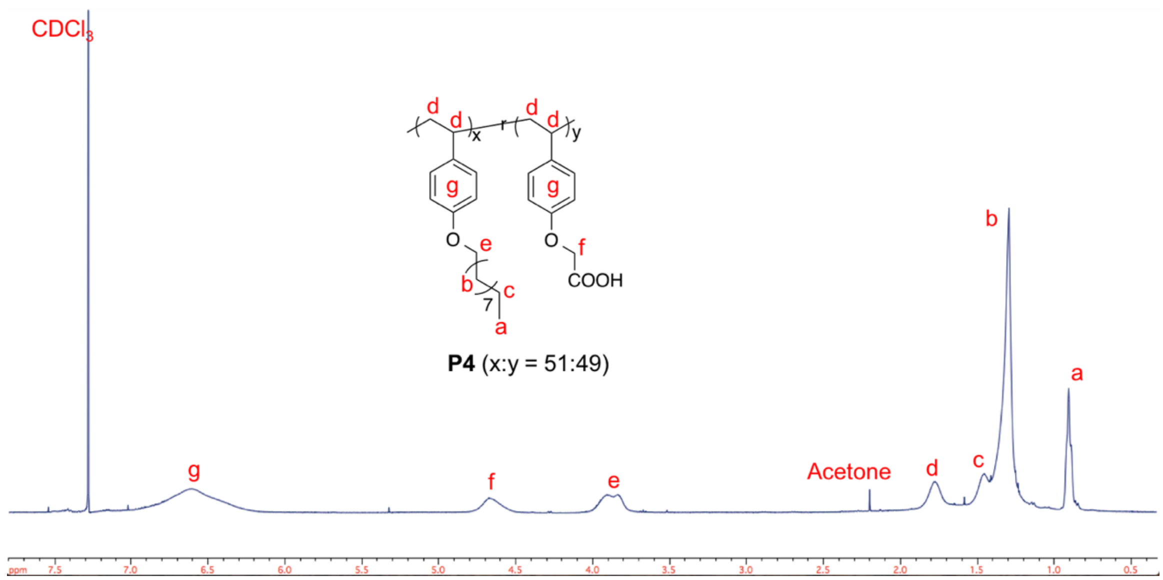
a
g
f
e
d
c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d
x
r
d
d
y
g
g
O
e
O
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COOH
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c
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P4 (x:y = 51:49)
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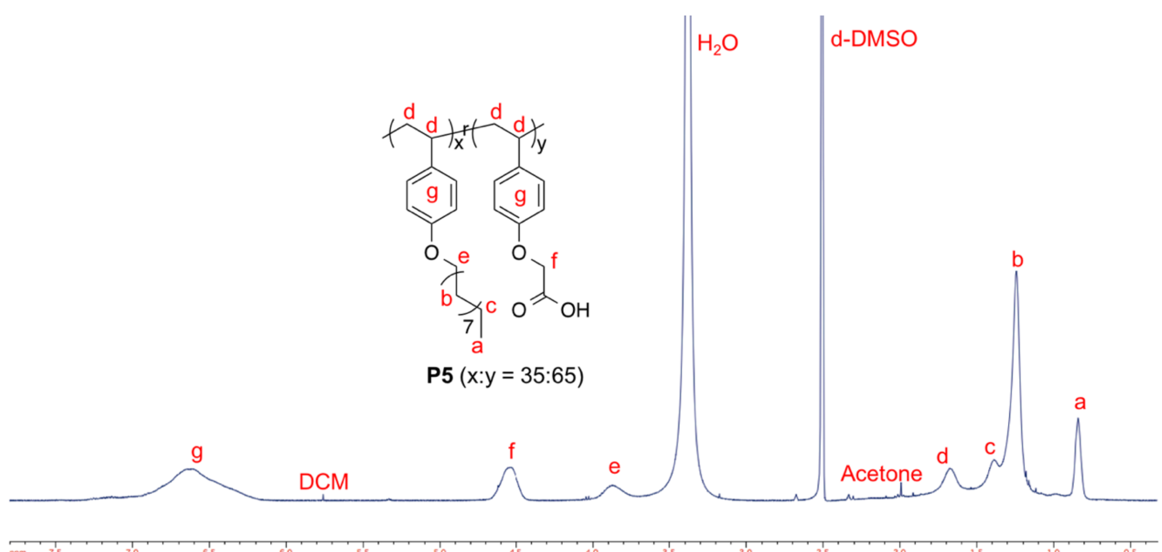
a
g
f
e
Acetone
d
c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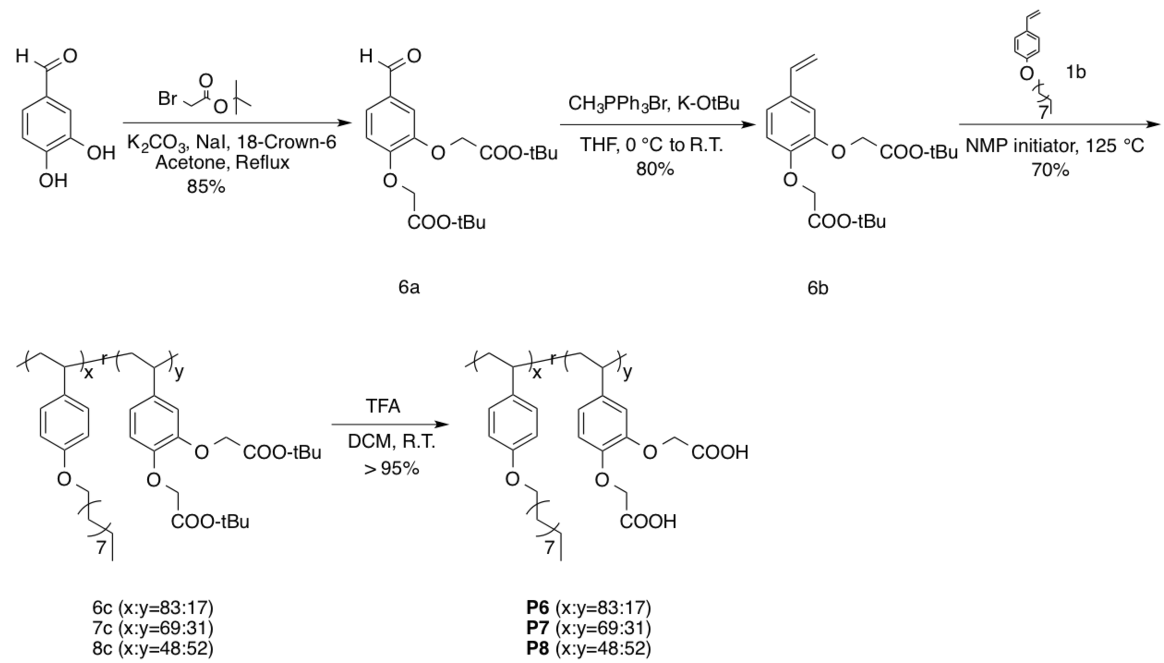
d
x
r
d
d
y
g
g
O
e
O
f
b
c
7
a
O
OH
P5 (x:y = 35:65)
b
a
g
DCM
f
e
Acetone
d
c

Synthesis of random co-polymer P6-P8



Synthesis of compound **6a**:

To a solution of acetone mixed with K_2CO_3 (6.00 g, 43.44 mmol), NaI (3.91 g, 26.06 mmol) and 18-crown-6 (0.57 g, 2.17 mmol), 3,4-Dihydroxybenzaldehyde (1.50 g, 10.86 mmol) was added and stirred for 5 min. To this mixture, tert-Butyl bromoacetate (5.08 g, 26.06 mmol) was added and stirred while refluxing for 20 h. The reaction mixture was then cooled to room temperature and filtered to afford the crude product in acetone solution. The solvent was evaporated to dryness and purified by silica gel column chromatography (10-13% ethyl acetate in hexanes) to obtain 3.4 g (85% yield) of **3a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.83 (s, 1H), δ 7.45-7.47 (q, 1H), δ 7.35-7.36 (d, 1H), δ 6.88-6.91 (d, 1H), δ 4.66-4.69 (d, 4H), δ 1.47-1.48 (d, 18H); ESI-MS (expected: $[m+H]^+$= 367.2, obtained: $[m+Na]^+$= 389.2)

Synthesis of compound **6b**:

Methyltriphenylphosphonium bromide (4.24 g, 11.88 mmol) and Potassium tert-butoxide (1.33 g, 11.88 mmol) were mixed in a round bottom flask, and dry THF (20 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 15 min to yield the bright yellow solution. **3a** (2.9 g, 7.92 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline 3 times. The organic layer was evaporated to dryness and purified by silica gel column chromatography (3-5% ethyl acetate in hexanes) to afford 2.3 g (80% yield) of **3b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 6.94-6.96 (t, 2H), δ 6.78-6.80 (d, 1H), δ 6.57-6.64 (q, 1H), δ 5.55-5.60 (q, 1H), δ 5.13-5.16 (q, 1H), δ 4.59-4.61 (d, 4H), δ 1.47-1.48 (d, 18H); ESI-MS (expected: $[m+H]^+$= 365.2, obtained: $[m+Na]^+$= 387.2)

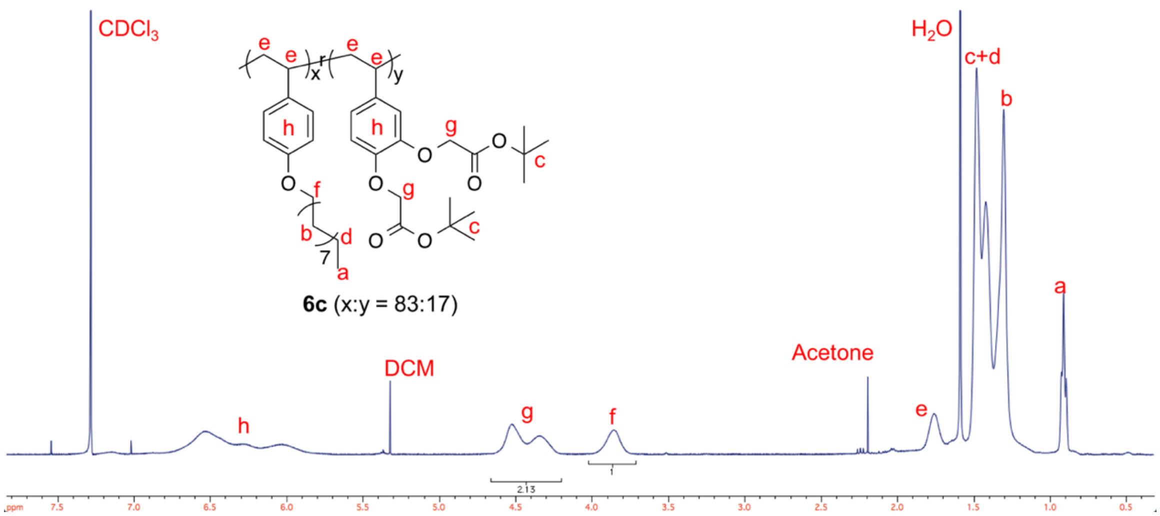
Synthesis of random co-polymer **6c-8c**:

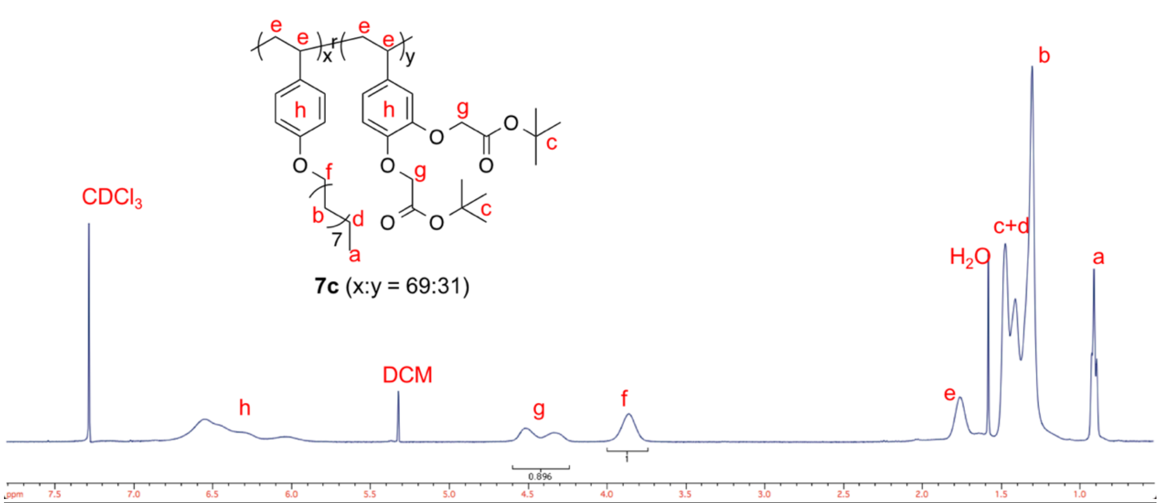
A mixture of the compound **1b** (200 mg, 0.77 mmol), **3b** (49 mg, 0.14 mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 6 mg, 0.018 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 °C under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in the MeOH. The precipitate was collected and dried under vacuum to yield 175 mg (70% yield) of **6c**. GPC (PMMA/THF): M_n= 13K Da, Đ= 1.11; Same method was used for **7c-8c** except for feeding ratios.

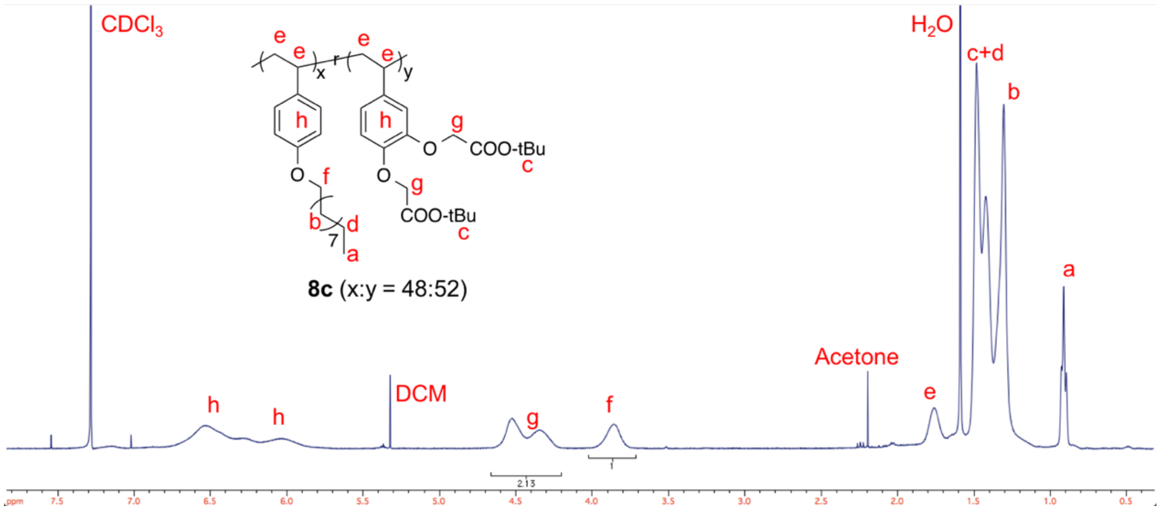
1b (280 mg, 1.08 mmol), **6b** (168 mg, 0.46 mmol) and NMP (10 mg, 0.031 mmol) were polymerized to get **7c** (GPC (PMMA/THF): M_n= 18K Da, Đ= 1.17).

1b (200 mg, 0.77 mmol), **6b** (280 mg, 0.77 mmol) and NMP (10 mg, 0.031 mmol) were polymerized to get **8c** (GPC (PMMA/THF): M_n= 14K Da, Đ= 1.07).

^{1}H NMR (400MHz, $CDCl_3$) are shown below.

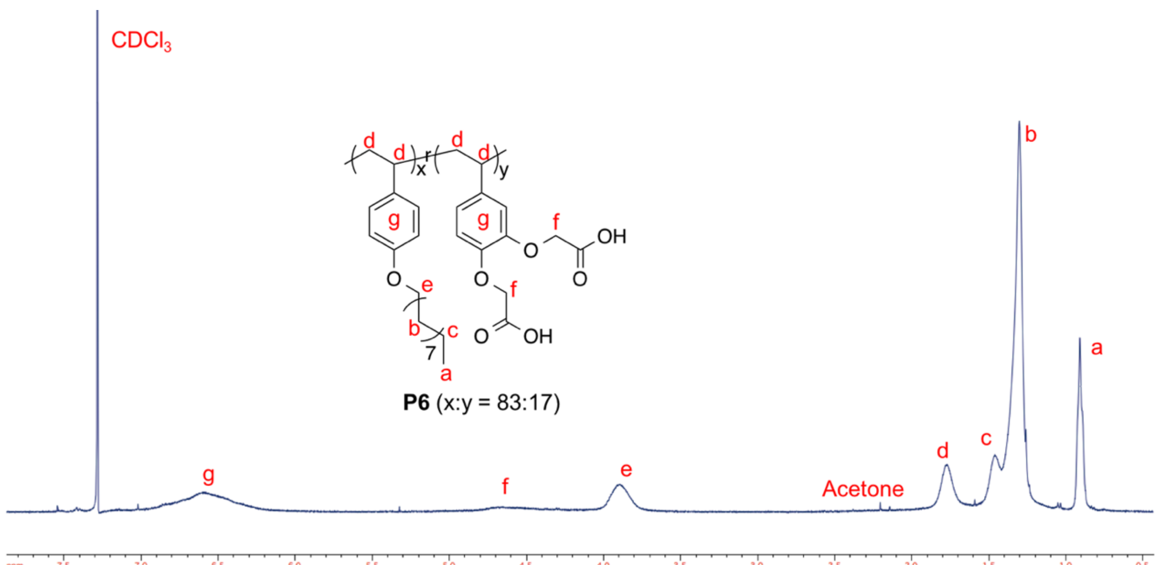


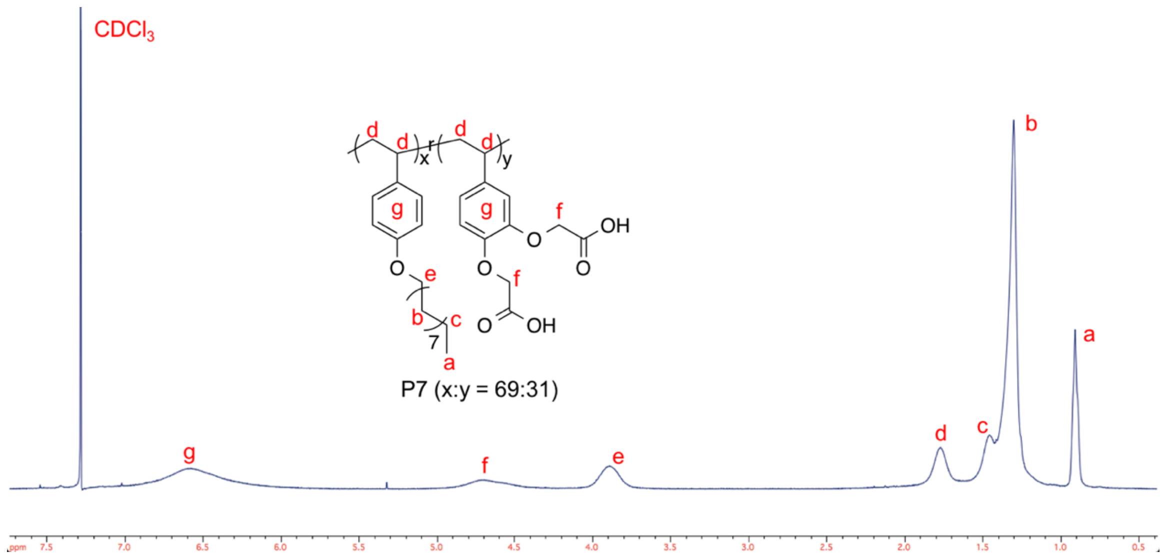


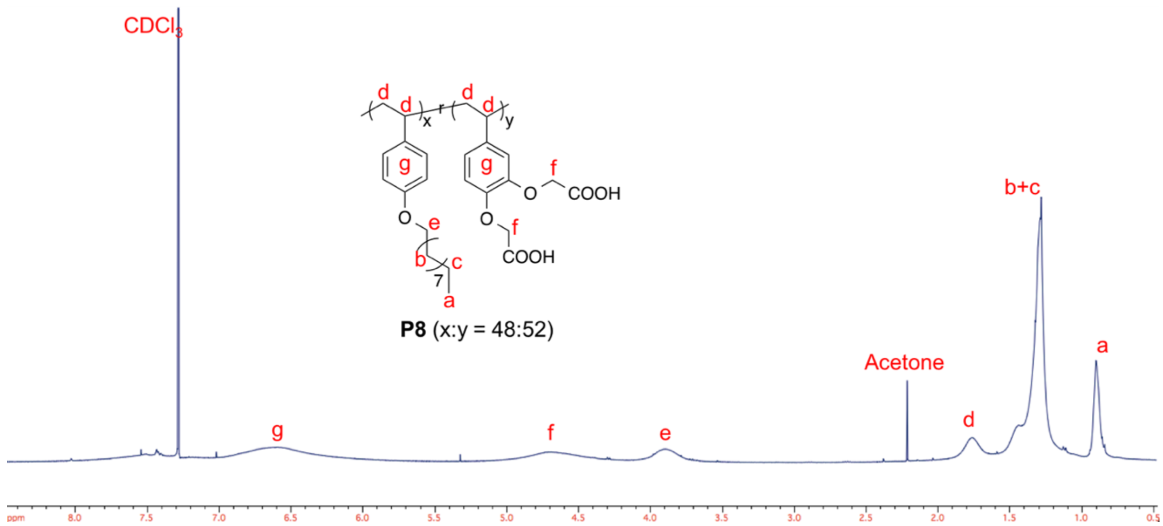


Synthesis of random co-polymer **P6-P8**:

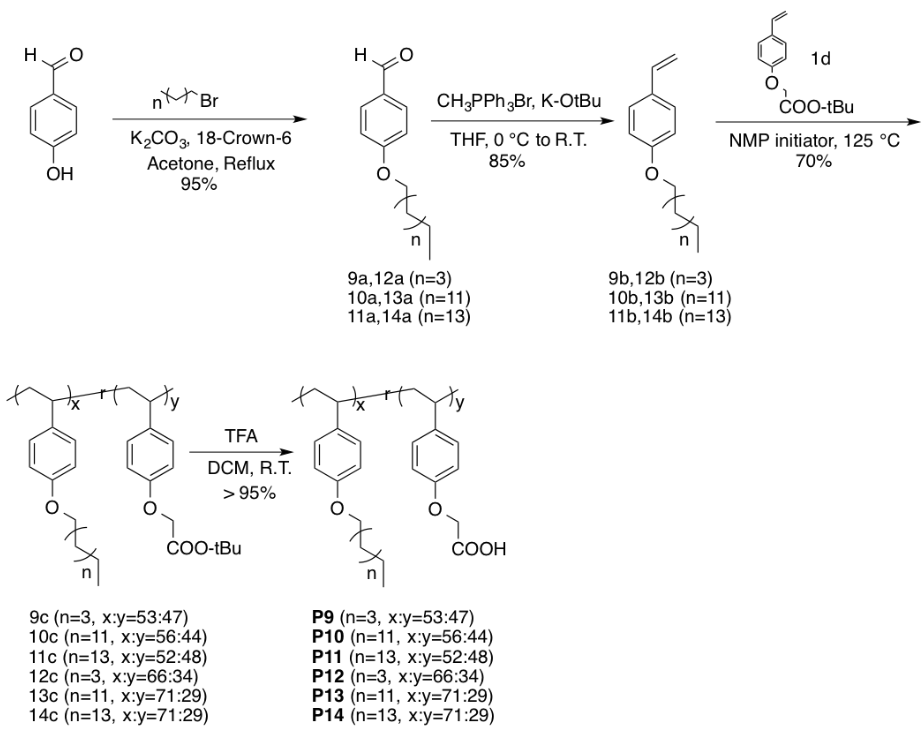
DCM (2 mL) was added to dissolve the dried random co-polymer **6c-8c**. Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum to obtain the final product **P6-P8**. ^{1}H NMR (400MHz, $CDCl_3$) are shown below.







Synthesis of random co-polymer P9-P14



Synthesis of compound **9a-14a**:

The method of synthesizing **9a-14a** is as same as **1a** above, except that the reagents were 1-Bromohexane (for **9a** and **12a**), 1-Bromotetradecane (for **10a** and **13a**) and 1-Bromohexadecane (for **11a** and **14a**). ^{1}H NMR (400MHz, $CDCl_3$) of **9a**, δ 9.87 (s, 1H), δ 7.81-7.83 (d, 2H), δ 6.97-6.99 (d, 2H), δ 4.01-4.05 (t, 2H), δ 1.77-1.82 (quint, 2H), δ 1.34-1.46 (m, 6H), δ 0.90 (s, 3H); ^{1}H NMR (400MHz, $CDCl_3$) of **10a**, δ 9.88 (s, 1H), δ 7.81-7.83 (d, 2H), δ 6.98-7.00 (d, 2H), δ 4.02-4.05 (t, 2H), δ 1.77-1.84 (quint, 2H), δ 1.27-1.50 (m, 24H), δ 0.87-0.90 (t, 3H); ^{1}H NMR (400MHz, $CDCl_3$) of **11a**, δ 9.88 (s, 1H), δ 7.81-7.84 (q, 2H), δ 6.98-7.00 (q, 2H), δ 4.02-4.05 (t, 2H), δ 1.79-1.83 (quint, 2H), δ 1.26-1.55 (m, 26H), δ 0.88-0.90 (t, 3H);

Synthesis of compound **9b-14b**:

The method of synthesizing **9b-14b** is as same as **1b** above. ^{1}H NMR (400MHz, $CDCl_3$) of **9b**, δ 7.33-7.35 (d, 2H), δ 6.84-6.86 (d, 2H), δ 6.63-6.70 (q, 1H), δ 5.58-5.63 (d, 1H), δ 5.10-5.13 (d, 1H), δ 3.94-3.98 (t, 2H), δ 1.43-1.50 (quint, 2H), δ 1.32-1.36 (m, 4H), δ 0.92-0.94 (t, 3H); ^{1}H NMR (400MHz, $CDCl_3$) of **10b**, δ 7.32-7.34 (d, 2H), δ 6.84-6.86 (d, 2H), δ 6.62-6.69 (q, 1H), δ 5.58-5.62 (d, 1H), δ 5.10-5.13 (d, 1H), δ 3.94-3.97 (t, 2H), δ 1.74-1.81 (quint, 2H), δ 1.27-1.49 (m, 22H), δ 0.87-0.90 (t, 3H); ^{1}H NMR (400MHz, $CDCl_3$) of **11b**, δ 7.32-7.34 (q, 2H), δ 6.84-6.86 (q, 2H), δ 6.62-6.69 (q, 1H), δ 5.58-5.62 (q, 1H), δ 5.10-5.13 (q, 1H), δ 3.94-3.97 (t, 2H), δ 1.76-1.79 (quint, 2H), δ 1.26-1.54 (m, 26H), δ 0.87-0.90 (t, 3H);

Synthesis of random co-polymer **9c-14c**:

The method of synthesizing **9c-14c** is as same as **1e** above with corresponding monomer **9b-14b** and **1d**. **9b** (157 mg, 0.77 mmol), **1d** (180 mg, 0.77 mmol) and NMP (10 mg, 0.031 mmol) were polymerized to get **9c** (GPC (PMMA/THF): M_n= 8K Da, Đ= 1.20).

10b (243 mg, 0.77 mmol), **1d** (180 mg, 0.77 mmol) and NMP (10 mg, 0.031 mmol) were polymerized to get **10c** (GPC (PMMA/THF): M_n= 12K Da, Đ= 1.11).

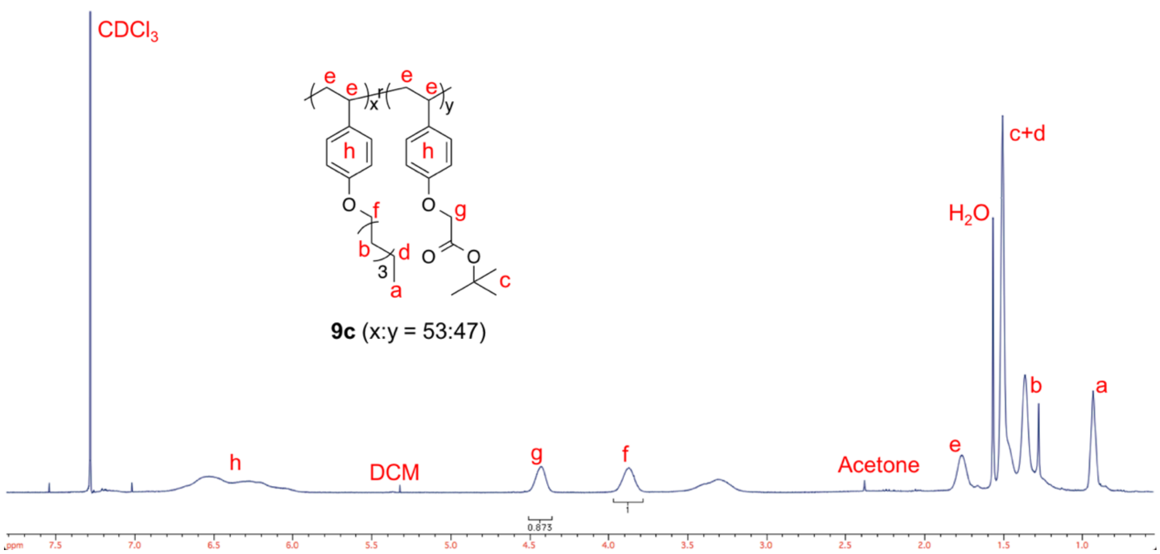
11b (133 mg, 0.39 mmol), **1d** (90 mg, 0.39 mmol) and NMP (5 mg, 0.015 mmol) were polymerized to get **11c** (GPC (PMMA/THF): M_n= 17K Da, Đ= 1.31).

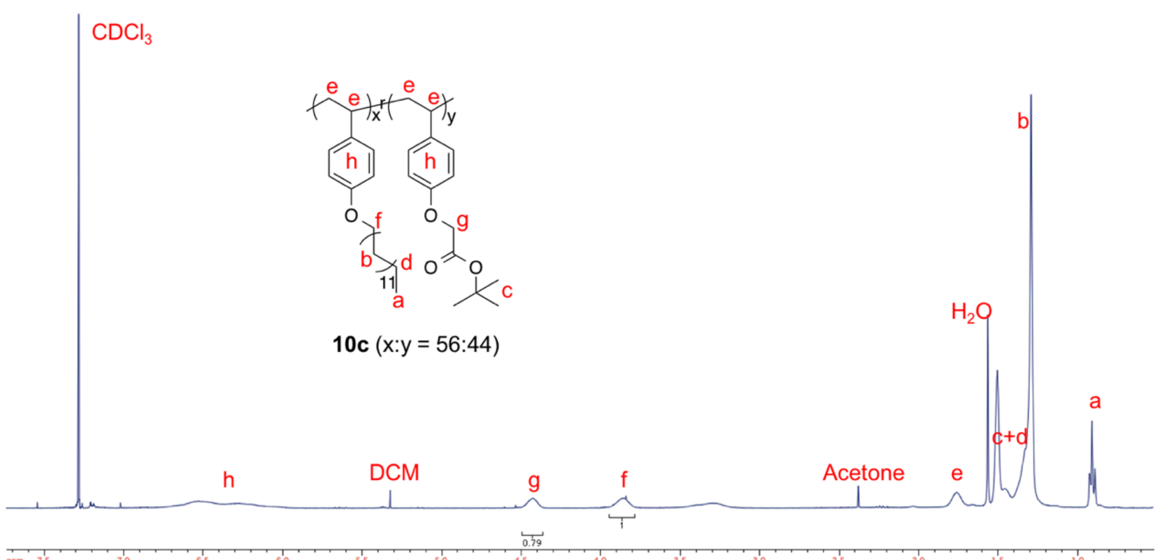
12b (110 mg, 0.54 mmol), **1d** (54 mg, 0.23 mmol) and NMP (5 mg, 0.015 mmol) were polymerized to get **12c** (GPC (PMMA/THF): M_n= 12K Da, Đ= 1.64).

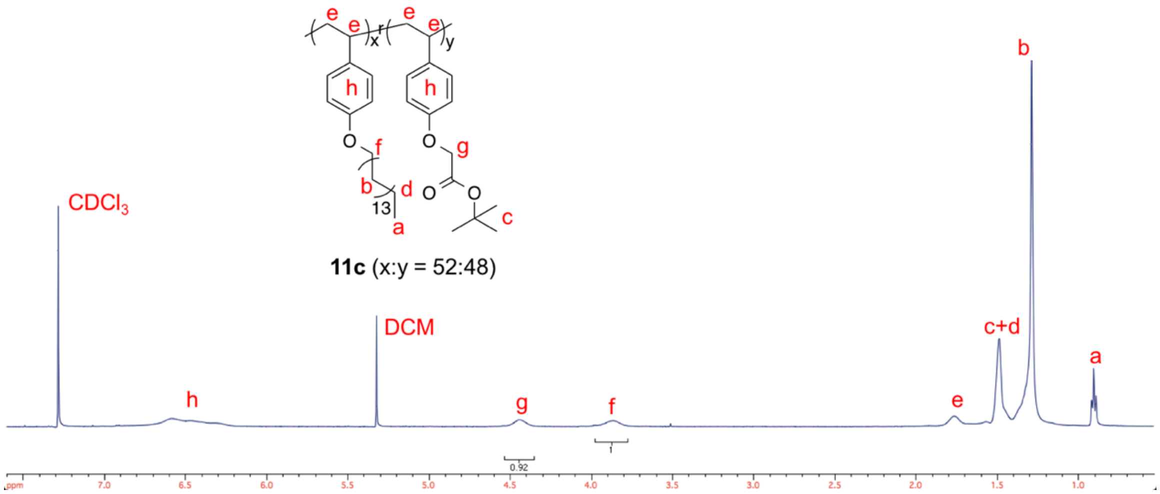
13b (340 mg, 1.08 mmol), **1d** (108 mg, 0.46 mmol) and NMP (10 mg, 0.031 mmol) were polymerized to get **13c** (GPC (PMMA/THF): M_n= 12K Da, Đ= 1.16).

14b (185 mg, 0.54 mmol), **1d** (54 mg, 0.23 mmol) and NMP (5 mg, 0.015 mmol) were polymerized to get **14c** (GPC (PMMA/THF): M_n= 10K Da, Đ= 1.45).

^{1}H NMR (400MHz, $CDCl_3$) are shown below.







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13
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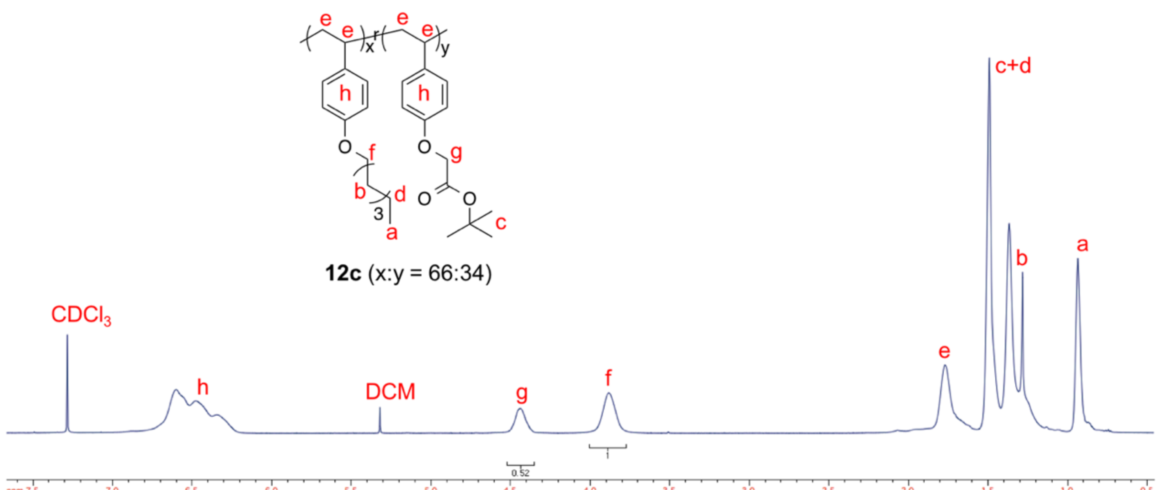

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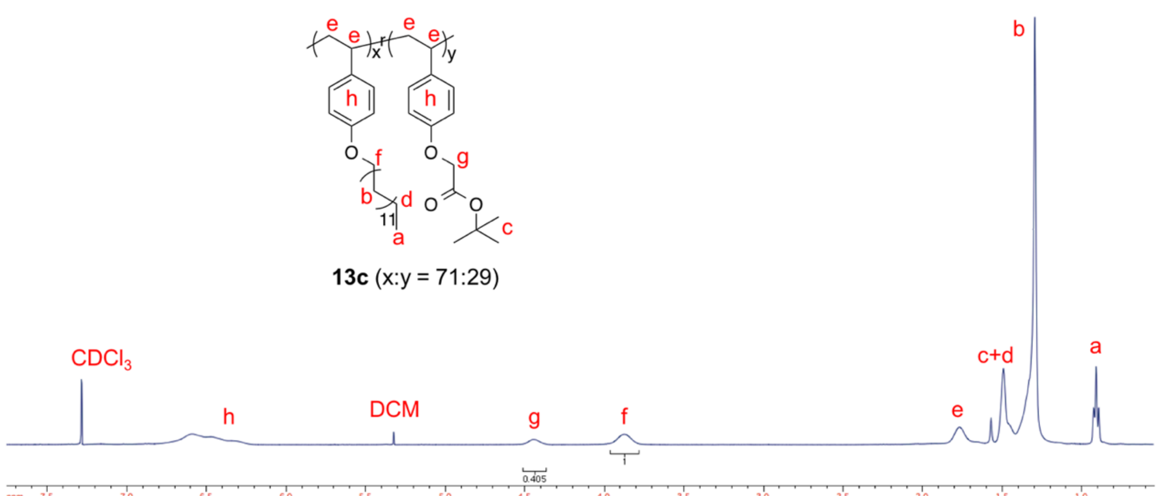

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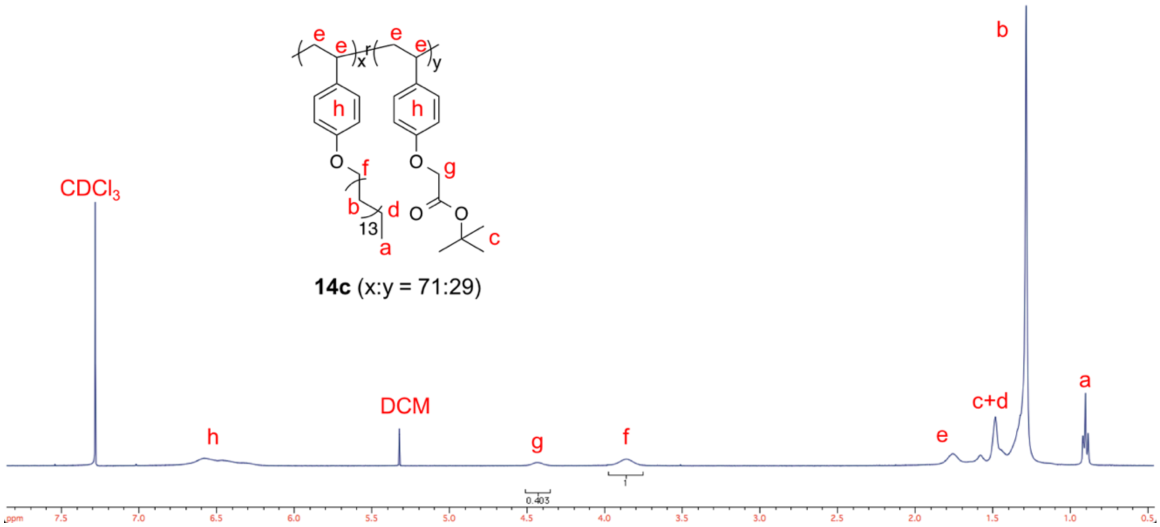
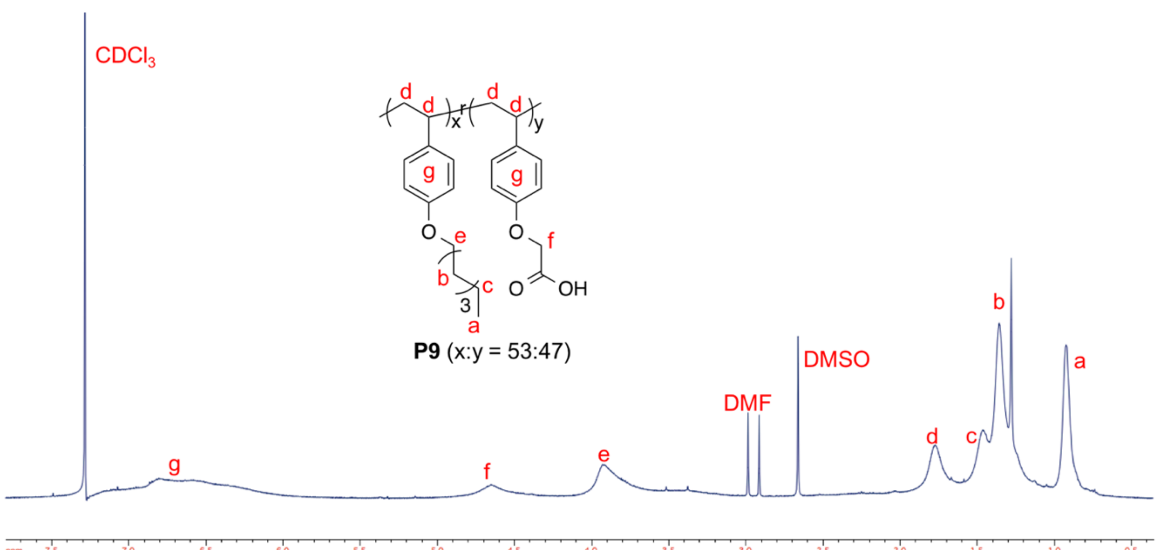
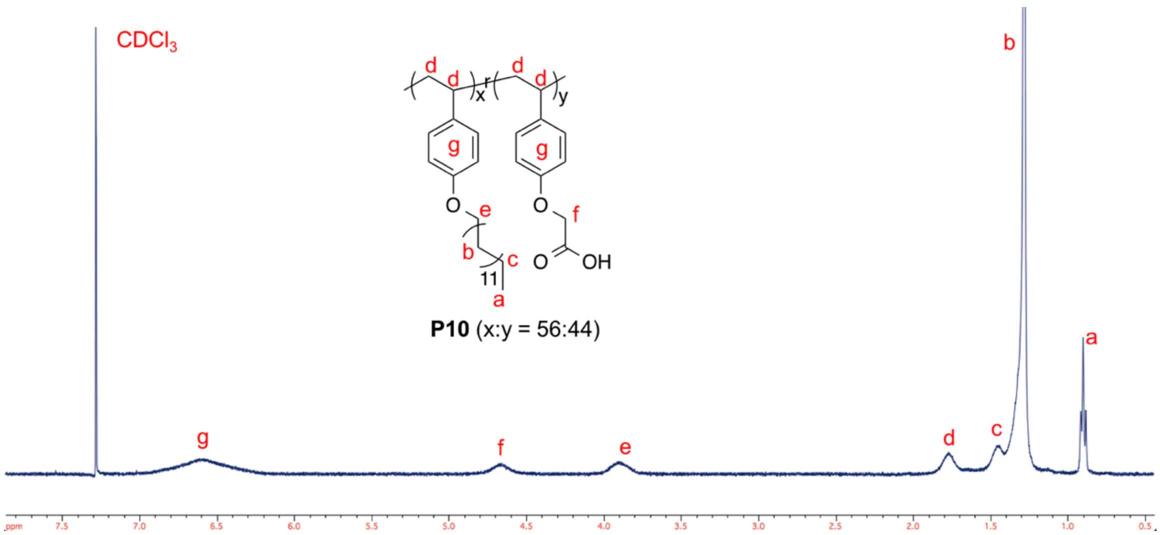
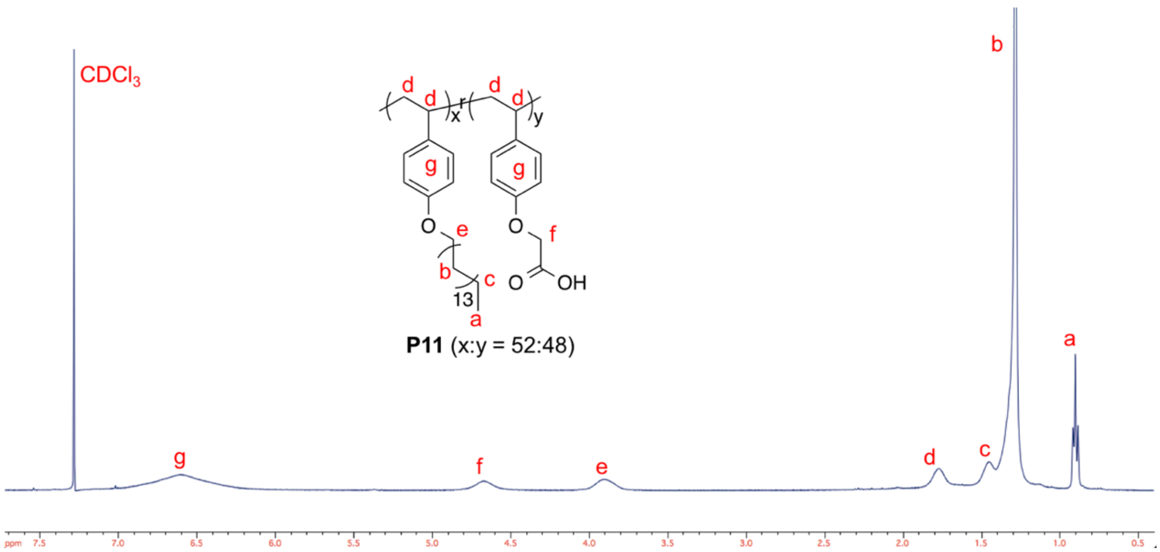


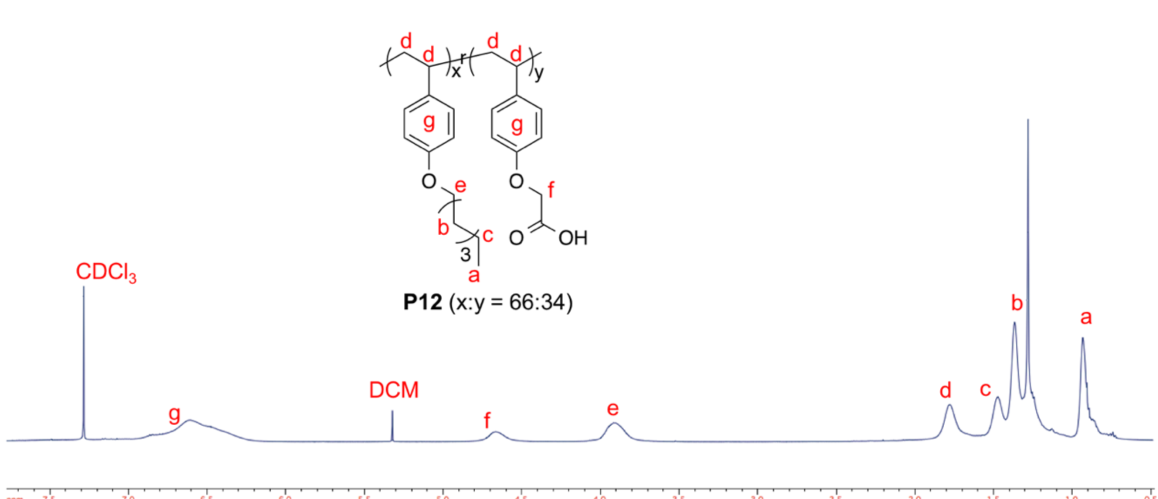
Synthesis of random co-polymer **P9-P14**:

DCM (2 mL) was added to dissolve the dried random co-polymer **9c-14c**. Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum to obtain the final product **P9-P14**. ^{1}H NMR (400MHz, $CDCl_3$) are shown below.




CDCl3
d d x r d d y
g g
O e
O f
b c O OH
11
a
P10 (x:y = 56:44)
b
a
g
f
e
d
c
ppm


CDCl3
d d x r d d y
g g
O e
O f
b c O OH
13
a
P11 (x:y = 52:48)
b
a
g
f
e
d
c
ppm


d d x r d d y
g g
O e
O f
b c O OH
3
a
CDCl3
P12 (x:y = 66:34)
b
a
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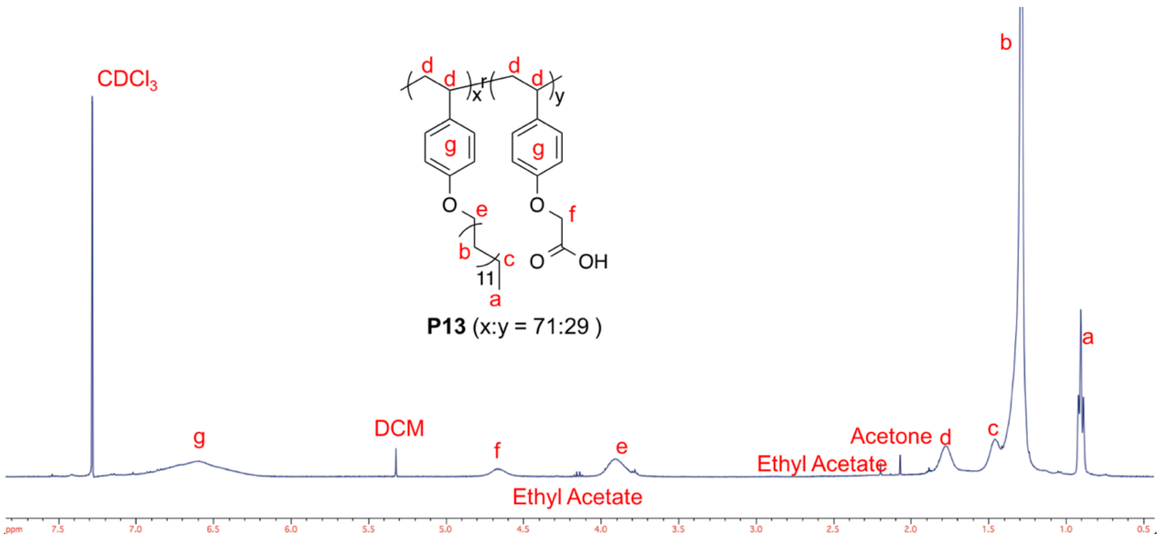
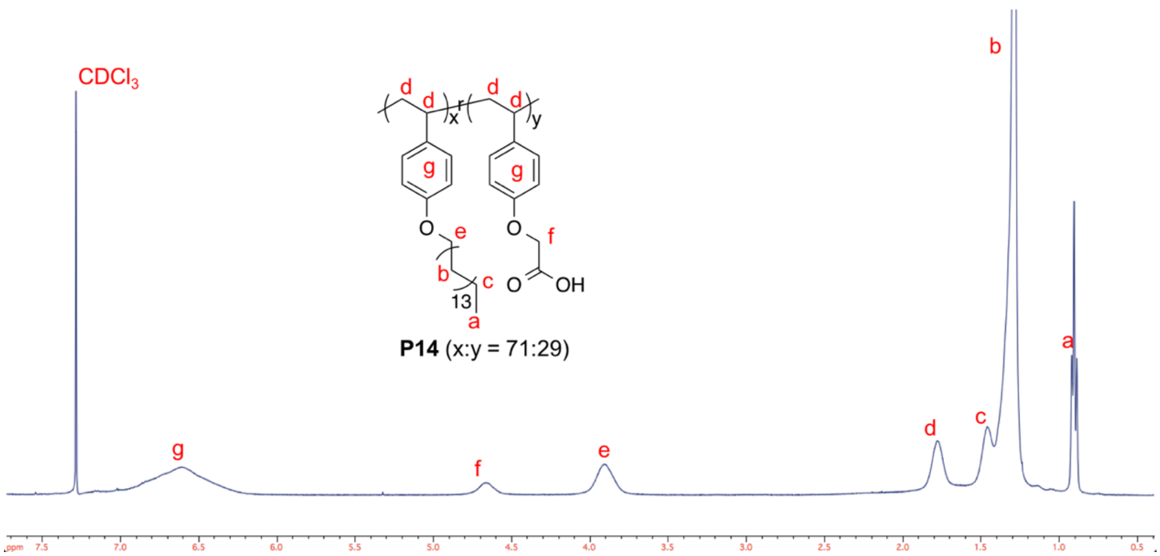
f
e
d
c
ppm





Dynamic light scattering (DLS) of P1-P5

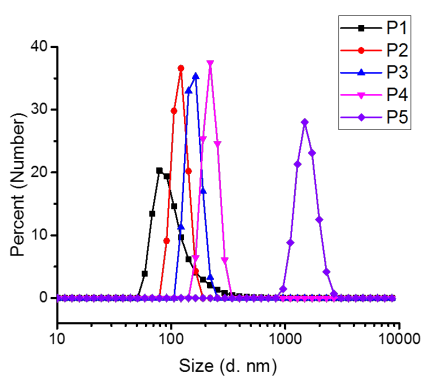


Figure S1. Size of reverse micelle solutions of **P1-P5.**

Transmission electron microscopy (TEM)

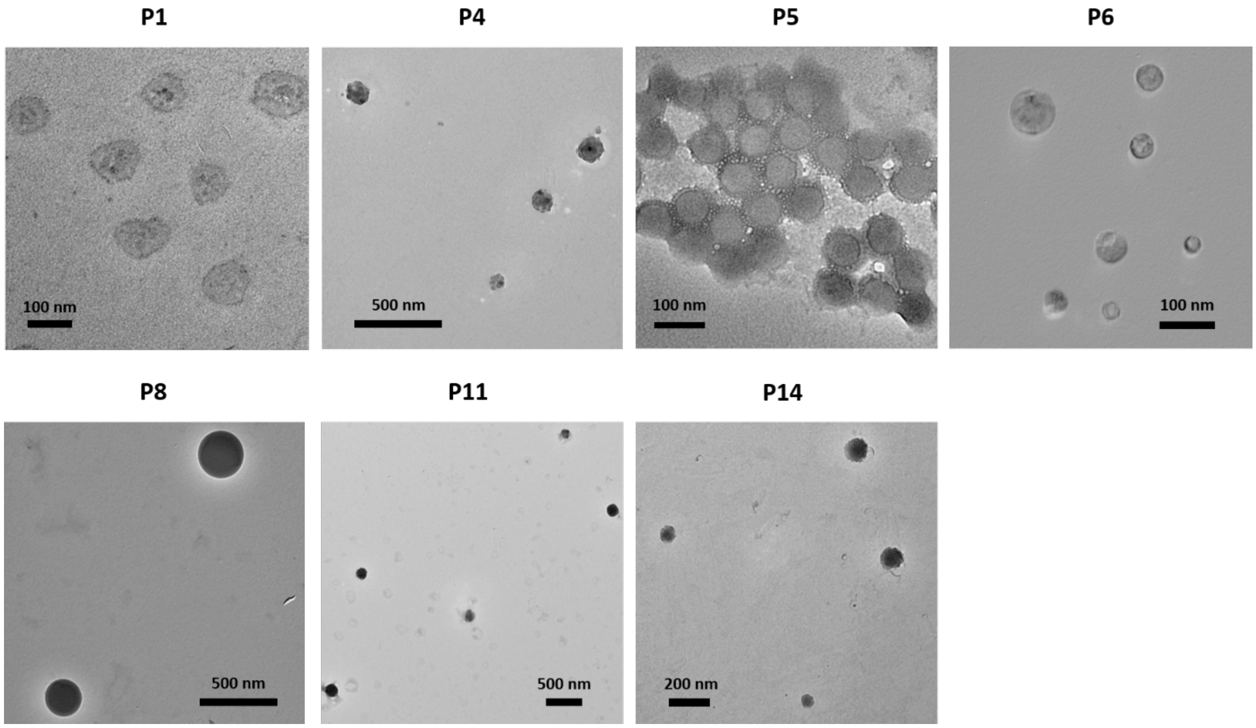


Figure S2. Exemplified TEM images of selected reverse micelles

Stability of reverse micelles of P2-P4

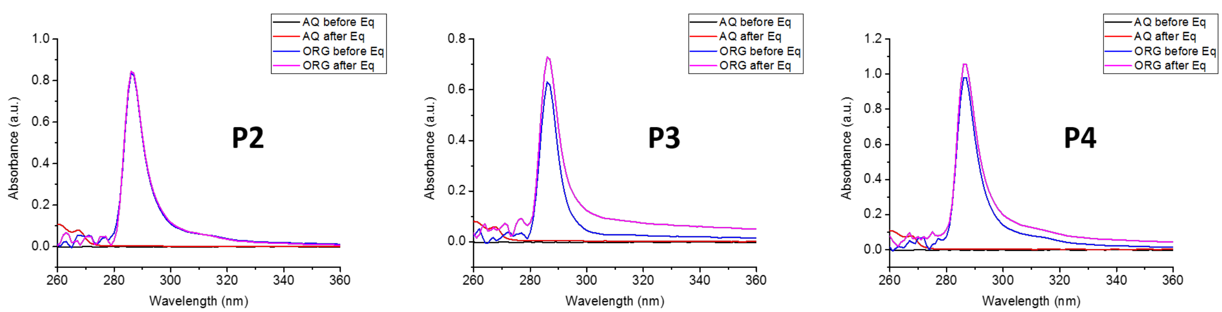


Figure S3. UV-Vis measurements with reverse micelles of **P2-P4** starting in toluene (ORG), before and after equilibration (Eq) with aqueous phase (AQ).

Reverse micelles with size variations

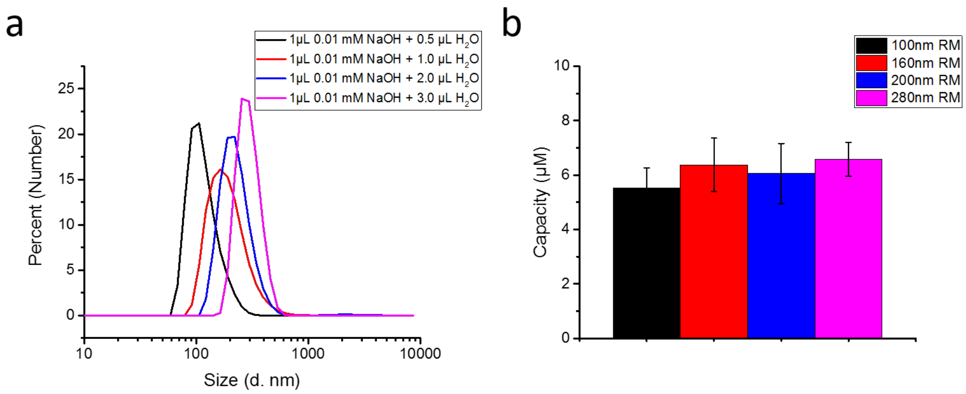


Figure S4. (a) Size of reverse micelle solution of **P4** with different amount of water addition. (b) Capacities of reverse micelle solutions of **P4** with different sizes at pH 7.6.

Calculated percent occupancy of carboxylates

The total amount (in mol unit) of positively charged peptides were obtained from Figure 2a. Since bradykinin (RPPGFSPFR) peptide has a net charge of 2 at pH 7.6, the total amount of postive charge provided by bradykinin is twice of the amount of extracted peptide. The amount of negatively charged carboxylate is calculated from Figure 2g using the concentration of carboxylate multipled by the 100 μL volume. Assuming one bradykinin can bind to two carboxylates in reverse micelle, the ratio of the occupied carboxylate is calculated using (amount of positive charge provided by peptide)/ (amount of carboxylate in reverse micelles).

	Amount of negatively charged carboxylate in reverse micelles (mol)	Amount of extracted bradykinin (mol)	Amount of positive charge provided by peptide (mol)	Ratio of the occupied carboxylate
P1	4.6 E-8	7.0 E-10	1.41 E-9	3.0 %
P2	1.2 E-8	3.9 E-9	7.76 E-9	6.6 %
P3	1.4 E-7	8.8 E-9	1.76 E-8	12.4 %
P4	2.0 E-7	5.7 E-9	1.15 E-8	5.7 %
P5	2.9 E-7	2.9 E-10	5.86 E-10	0.2 %

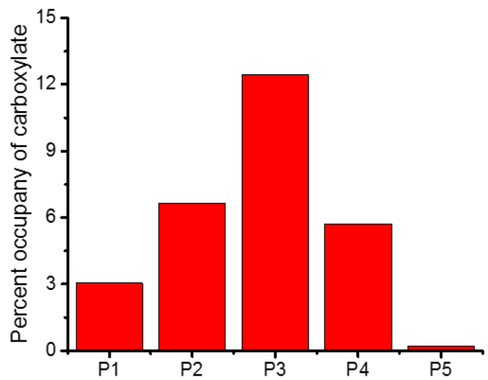


Figure S5. Percent occupancy of carboxylates in the reverse micelles by bradykinin peptides (net charge is 2 at pH 7.6) using extraction capacities in Figure 2a.

Optimal charge density on different peptides

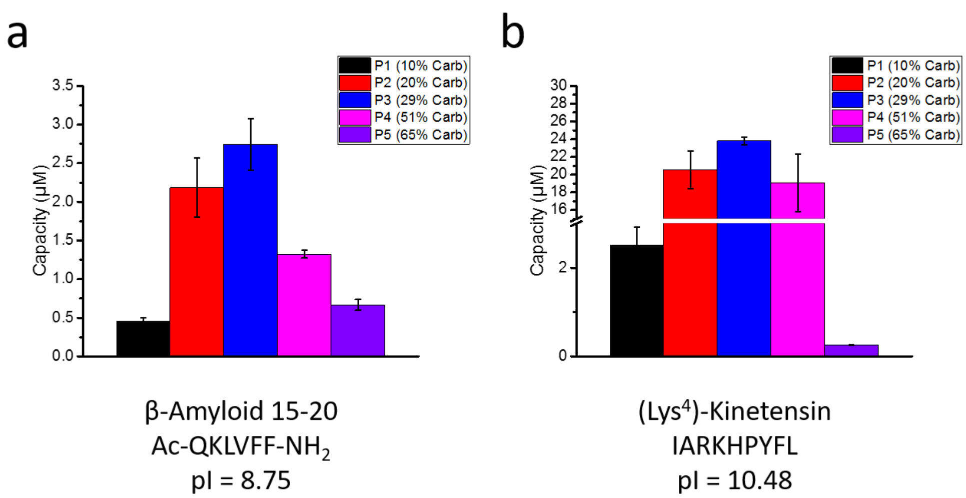


Figure S6. (a) Beta-amyloid 15-20 peptide capacities of **P1-P5** at pH 7.6. (b) (Lys[4])-kinetensin peptide capacities of **P1-P5** at pH 7.6.

Stability of reverse micelles of P8

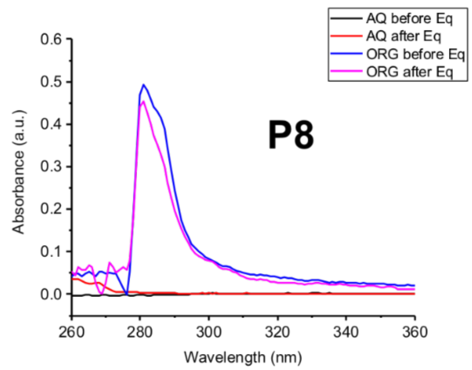


Figure S7. UV-Vis measurements with reverse micelles of **P8** starting in toluene (ORG), before and after equilibration (Eq) with aqueous phase (AQ).

Dynamic light scattering (DLS) of P6-P14

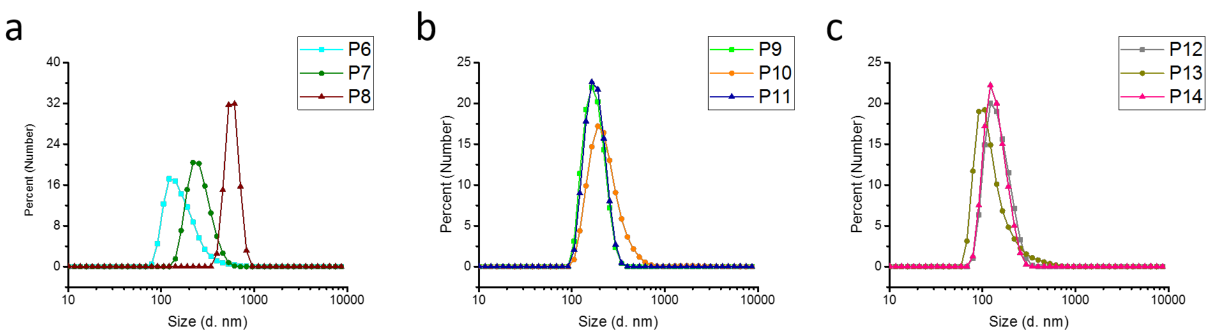


Figure S8. DLS of reverse micelles of **P6-P14**