Supplementary information

Fluorene-functionalized aliphatic polycarbonates: Design, syntheses and aqueous self-assembly of amphiphilic block copolymers

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Materials and methods

Materials

Unless, specifically mentioned, all materials were purchased from Sigma-Aldrich or TCI and all other solvents were of analytical grade, purchased from Fisher Scientific or J. T. Baker and used as received. Macroinitiators, mPEG-OH were purchased from Polymer Source Inc., Canada. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was distilled from CaH2 under dry N₂ and transferred to a glove box. Before transferring into the glove box, monomers and other reagents (like mPEG-OH) were dried extensively by freeze-drying under high vacuum.
Methods

Nuclear magnetic resonance (NMR) spectroscopy
The $^1$H- and $^{13}$C-NMR spectra of monomers and polymers were recorded using a Bruker Avance 400 spectrometer, and operated at 400 and 100 MHz respectively, with the solvent proton signal as the internal reference standard.

Molecular weight determination by size exclusion chromatography (SEC)
SEC was conducted using THF as the eluent for monitoring the polymer conversion and also for the determination of polystyrene equivalent molecular weights of the macro-transfer agents. THF-SEC was recorded on a Waters 2695D (Waters Corporation, USA) Separation Module equipped with an Optilab rEX differential refractometer (Wyatt Technology Corporation, U.S.A.) and Waters HR-4E as well as HR-1 columns (Waters Corporation, USA). The system was equilibrated at 30 °C in THF, which served as the polymer solvent and eluent with a flow rate of 1.0 mL/min. Polymer solutions were prepared at a known concentration (ca. 3 mg/mL) and an injection volume of 100 μL was used. Data collection and analysis were performed using the Astra software (Wyatt Technology Corporation, USA; version 5.3.4.14). The columns were calibrated with series of polystyrene standards ranging from $M_p = 360$ Da to $M_p = 778$ kDa (Polymer Standard Service, USA).

Transmission electron microscopy (TEM)
The morphologies of self-assembled nanostructures of the polymers were observed under a FEI Tecnai G2 F20 electron microscope using an acceleration voltage of 200 keV.
TEM samples were prepared by first placing a drop of self-assembled polymer solution (4.0 microliters, prepared as described above) onto a formvar coated 200 mesh copper grid (Ted Pella Inc., USA). After 1.0 min, the excess solution was wicked off by using filter paper. Then the staining agent phosphotungstic acid (2 % w/v; 4.0 µL) was placed on the grid and after 1.0 min, the excess solution was wicked off and the grid was left to dry under the ambient conditions.

**UV-Vis spectroscopy:**

UV-Vis spectra were recorded on Agilent 8543 spectrophotometer by using quartz cuvette (Hellma Analytics) of path length 10 mm, at 25 °C.

**Synthesis of spiro[fluorene-9,5'-[1,3]dioxan]-2'-one (F-TMC):**

In a 500 mL round bottom flask, 9H-fluorene-9,9-dimethanol (5.0 g, 22 mmol, 1.0 equiv.) and ethyl chloroformate (8.4 mL, 9.6 g, 88 mmol, 4.0 equiv.) were allowed to stir in THF (250 mL) for about 30 minutes in ice-cold conditions, followed drop-wise addition of triethylamine (12.3 mL, 9.0 g, 88 mol, 4.0 equiv.) over 30 minutes. The reaction was allowed to proceed at ice-cold conditions for about 1 hour and later at room temperature for about 15 hours. The precipitated triethylamine.HCl salt was removed by filtration, followed by evaporation of volatiles under vacuum to yield the crude product, which was further purified by flash column chromatography using DCM and gradient of ethylacetate (0 – 20 %), to yield the purified product as half-white powder. (3.7g, 70.6%)
$^1$H NMR (400 MHz, CDCl$_3$, $\delta$, ppm): 7.2 – 7.85 (m, 8H, Fluorene), 4.6 (s, 4H, CH$_2$OCOO); $^{13}$C NMR (100 MHz, CDCl$_3$, $\delta$, ppm): 148.2, 142.4, 140.6, 129.4, 128.1, 124.0, 120.7, 74.9 and 46.7.

**Procedures for block copolymer (2a-f) synthesis:**

**Synthesis of mPEG$_{36}$-b-P(F-TMC)$_{20.0}$ (2a):** In a 7 mL vial containing a magnetic stir bar, in glove box, mPEG-OH (1.6 kDa, $DP_n = 36$, 126 mg, 78.8 $\mu$mol, 1.0 equiv.) and F-TMC (406 mg, 1610 $\mu$mol, 20.4 equiv.) were dissolved in DCM (2.0 mL). To this solution, DBU (11.7 $\mu$L, 11.9 mg, 78.4 $\mu$mol, 1.0 equiv.) was added to initiate polymerization. The reaction mixture was allowed to stir at room temperature for 15 minutes. The reaction was quenched by the addition of about 20 mg of benzoic acid and the reaction mixture was precipitated in ice-cold diethyl ether (2 × 50 mL). Polymer 2a was dried in a tared vial for about 1 to 2 days until a constant sample mass was obtained as white powder (0.389 mg).

**Synthesis of mPEG$_{54}$-b-P(F-TMC)$_{19.8}$ (2b):** In a 7 mL vial containing a magnetic stir bar, in glove box, mPEG-OH (2.4 kDa, $DP_n = 54$, 176 mg, 73.3 $\mu$mol, 1.0 equiv.) and F-TMC (385 mg, 1530 $\mu$mol, 20.8 equiv.) were dissolved in DCM (2.0 mL). To this solution, DBU (10.9 $\mu$L, 11.1 mg, 78.4 $\mu$mol, 1.0 equiv.) was added to initiate polymerization. The reaction mixture was allowed to stir at room temperature for 15 minutes. The reaction was quenched by the addition of about 20 mg of benzoic acid and the reaction mixture was precipitated in ice-cold diethyl ether (2 × 50 mL).
Polymer 2b was dried in a tared vial for about 1 to 2 days until a constant sample mass was obtained as white powder (431 mg).

**Synthesis of mPEG\textsubscript{88}-b-P(F-TMC)\textsubscript{19,0} (2c):** In a 7 mL vial containing a magnetic stir bar, in glove box, mPEG-OH (3.9 kDa, $DP_n = 88$, 250 mg, 64.1 µmol, 1.0 equiv.) and F-TMC (338 mg, 1340 µmol, 20.9 equiv.) were dissolved in DCM (2.0 mL). To this solution, DBU (9.6 µL, 9.8 mg, 64.3 µmol, 1.0 equiv.) was added to initiate polymerization. The reaction mixture was allowed to stir at room temperature for 15 minutes. The reaction was quenched by the addition of about 20 mg of benzoic acid and the reaction mixture was precipitated in ice-cold diethyl ether (2 × 50 mL). Polymer 2c was dried in a tared vial for about 1 to 2 days until a constant sample mass was obtained as white powder (493 mg).

**Synthesis of mPEG\textsubscript{113}-b-P(F-TMC)\textsubscript{18,4} (2d):** In a 7 mL vial containing a magnetic stir bar, in glove box, mPEG-OH (5.0 kDa, $DP_n = 113$, 250 mg, 50 µmol, 1.0 equiv.) and F-TMC (263 mg, 1040 µmol, 20.9 equiv.) were dissolved in DCM (2.0 mL). To this solution, DBU (7.6 µL, 7.7 mg, 50.9 µmol, 1.0 equiv.) was added to initiate polymerization. The reaction mixture was allowed to stir at room temperature for 15 minutes. The reaction was quenched by the addition of about 20 mg of benzoic acid and the reaction mixture was precipitated in ice-cold diethyl ether (2 × 50 mL). Polymer 2d was dried in a tared vial for about 1 to 2 days until a constant sample mass was obtained as white powder (430 mg).
Synthesis of mPEG$_{158}$-b-P(F-TMC)$_{18.5}$ ($2e$): In a 7 mL vial containing a magnetic stir bar, in glove box, mPEG-OH (7.0 kDa, $DP_n = 158$, 304 mg, 43.4 µmol, 1.0 equiv.) and F-TMC (224 mg, 888 µmol, 20.4 equiv.) were dissolved in DCM (4.5 mL). To this solution, DBU (6.4 µL, 6.5 mg, 42.9 µmol, 1.0 equiv.) was added to initiate polymerization. The reaction mixture was allowed to stir at room temperature for 15 minutes. The reaction was quenched by the addition of about 20 mg of benzoic acid and the reaction mixture was precipitated in ice-cold diethyl ether (2 × 50 mL). Polymer $2e$ was dried in a tared vial for about 1 to 2 days until a constant sample mass was obtained as white powder (448 mg).

Synthesis of mPEG$_{226}$-b-P(F-TMC)$_{19.2}$ ($2f$): In a 7 mL vial containing a magnetic stir bar, in glove box, mPEG-OH (10.0 kDa, $DP_n = 226$, 351 mg, 35.1 µmol, 1.0 equiv.) and F-TMC (198 mg, 785 µmol, 22.4 equiv.) were dissolved in DCM (4.5 mL). To this solution, DBU (5.2 µL, 5.3 mg, 34.8 µmol, 1.0 equiv.) was added to initiate polymerization. The reaction mixture was allowed to stir at room temperature for 15 minutes. The reaction was quenched by the addition of about 20 mg of benzoic acid and the reaction mixture was precipitated in ice-cold diethyl ether (2 × 50 mL). Polymer $2f$ was dried in a tared vial for about 1 to 2 days until a constant sample mass was obtained as white powder (451 mg).

Aqueous self-assembly of block copolymers

Block copolymer (5.0 mg) was taken in a scintillation vial (20 mL) with a magnetic stir bar and dissolved with THF (5.0 mL) by stirring for about 1-2 hours. The polymer
solution in THF was then transferred to a pre-washed dialysis membrane having a molecular weight cut-off (MWCO) of 1000 Da (Spectra/Por) and was dialyzed at room temperature against DI water (2 L) with magnetic stirring (~ 500 rpm), The water in the beaker was changed at 3, 6 and 18 hours, respectively. Typically the final concentration of the polymer solution after dialysis was about 0.35 - 0.45 mg/mL.

**Hydrolytic degradation experiment**

In a 20 mL scintillation vial, polymer 2a (10.6 mg) was dissolved in THF (0.5 mL) and the solvent was removed with first by a gentle stream of N₂ gas, followed by high vacuum (~ 2 h), to leave behind a thin polymer film. NaOD solution in D₂O (1 N, 1 mL) was added to the polymer and the mixture was allowed to stir by using a mini magnetic stirrer at RT (500 rpm) for 18 h. For ¹H NMR spectroscopy, 200 µL of the crude mixture was diluted with 400 µL of DMSO-d₆.
SI. Figure 1. Normalized UV-vis absorption spectra of monomer 1 (50 ppm) and polymers 2a-f (100 ppm) in CHCl₃.
**SI. Figure 2.** $^1$H NMR spectra of (A) degraded 2a in DMSO-$d_6$ : D$_2$O = 2 : 1** and (B) 9H-Fluorene-9,9-dimethanol in DMSO-$d_6$ : D$_2$O = 2 : 1.

**NaOD used for the degradation experiment was not removed.**