

Modulating the Depolymerization of Self-immolative Brush Polymers with Poly(benzyl ether) Backbones

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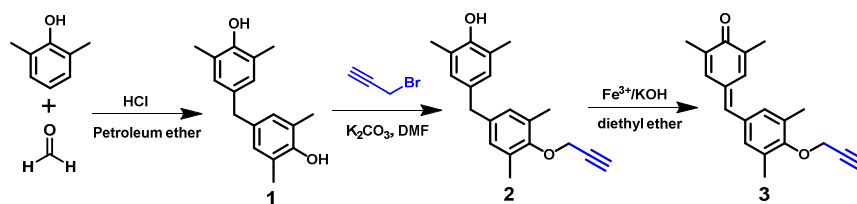
1. Materials and Methods

All reactions were performed in flame-dried glassware under a positive pressure of nitrogen unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe. Organic solvents used in column chromatography, including ethyl acetate (EA), dichloromethane (DCM), tetrahydrofuran (THF), methanol (MeOH), were purchased from Greagent and used directly without any purification. Super-dry solvents (DCM, THF, pyridine) were purchased from J&K Scientific Ltd. The HPLC-grade solvents, N,N-dimethylformamide (DMF) and THF, were

purchased from Fisher Scientific and Adamas Inc. Styrene (99%, Aldrich) was purified by passing through a column filled with basic alumina twice to remove the inhibitor prior to polymerization. Azido-terminated poly(ethylene glycol) (N₃-PEG) methyl ether (M_n=2 kDa, PDI=1.05) was purchased from Shanghai Ponsure Biotech Inc. Azido-terminated polystyrene (N₃-PS) was synthesized according to the literature.¹ All other reagents were purchased from J&K Scientific Ltd and used as received unless otherwise noted.

¹H and ¹³C NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. Chemical shifts (δ) were reported in ppm. Fourier transform infrared (FT-IR) spectra were recorded on a Varian 2000 spectrometer in the range from 3800-400 cm⁻¹, and the samples were prepared by mixing with KBr and compacted to form thin pellets. DMF GPC was carried out on a Waters Breeze 2 GPC system equipped with one or two PLgel 5 μm MIXED-C, 300×7.5mm columns and RI/2998 PDA detectors. HPLC-grade DMF with 1% LiBr was used as the mobile phase, and samples were run at a flow rate of 1 mL/min and 60 °C. The GPC was calibrated using PS standards (Easivial PS-M 2 mL) or PEG standards kit (EasiVial PEG/PEO 2 mL) from Agilent Technologies. THF GPC was performed on a Waters Breeze 2 GPC system equipped with two PLgel 5 μm MIXED-C, 300×7.5mm column and a 2998 PDA detector. HPLC-grade THF was used as the eluent running at a flow rate of 1 mL/min.

2. Synthesis of monomer 3



Compound 1. In a 250 mL round bottom flask, concentrated hydrochloric acid (36% w/v, 15 mL) was added dropwise to a biphasic mixture of 2,6-dimethylphenol (10.7 g, 87.6 mmol) in petroleum ether (PE) (80 mL) and formaldehyde in water (37% w/v, 16 mL, 198 mmol) over 10 min at room temperature with vigorous stirring. The reaction mixture was stirred for another 3 h, and a white precipitation appeared during the reaction. Then the mixture was poured into 400 mL of water, mixed well, and filtered through a Buchner funnel to collect a white solid, which was washed with water (300 mL), and dried at 70 °C under vacuum (~1 mmHg) overnight to afford compound **1** without additional purification (10 g, 39 mmol, 89%). ^1H NMR (400MHz, CDCl_3): δ 6.79 (s, 4H), 4.46 (s, 2H), 3.70 (s, 2H), 2.20 (s, 12H); ^{13}C NMR (400MHz, CDCl_3): δ 150.34, 133.41, 128.87, 122.86, 40.29, 15.91. The NMR data matches the known spectrum for this compound.²

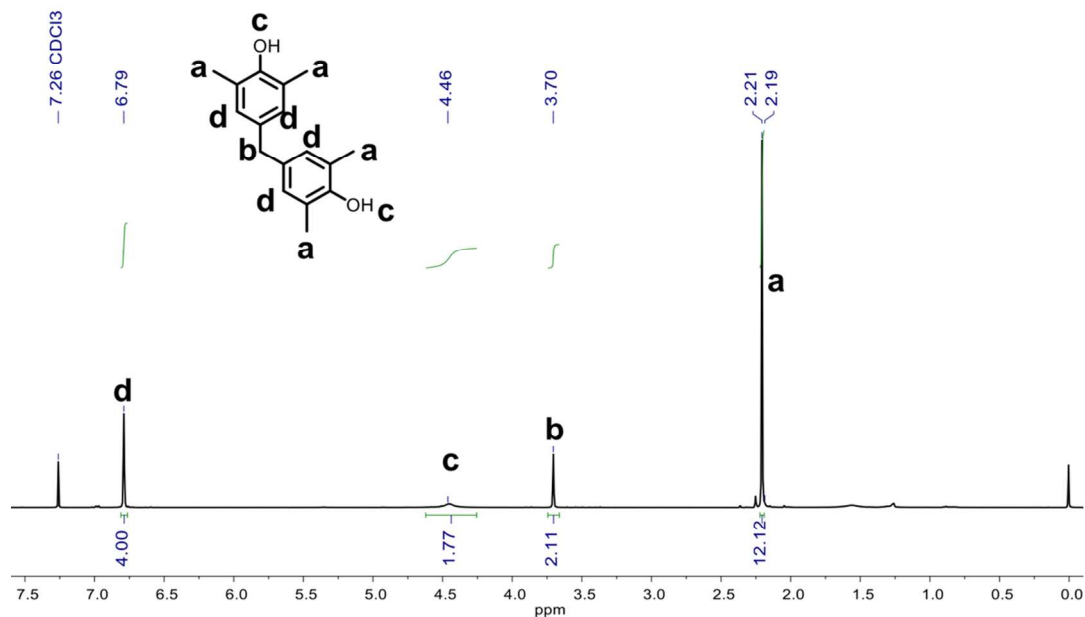


Figure S1. ^1H NMR spectrum of compound **1** in CDCl_3 .

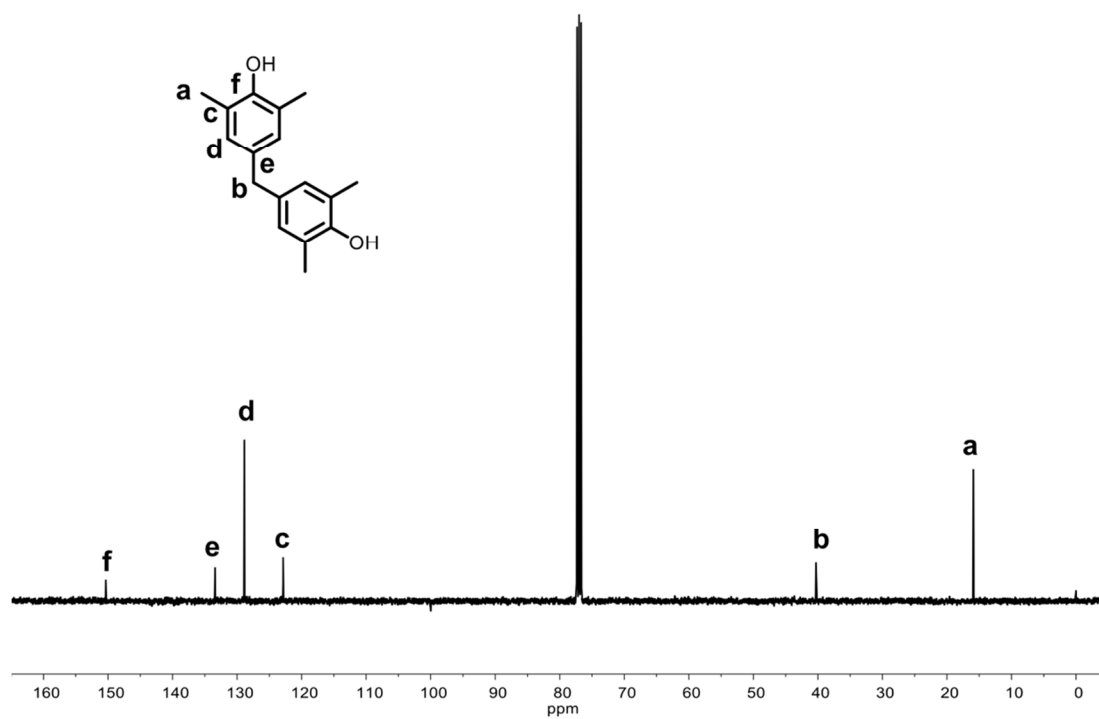


Figure S2. ^{13}C NMR spectrum of compound **1** in CDCl_3 .

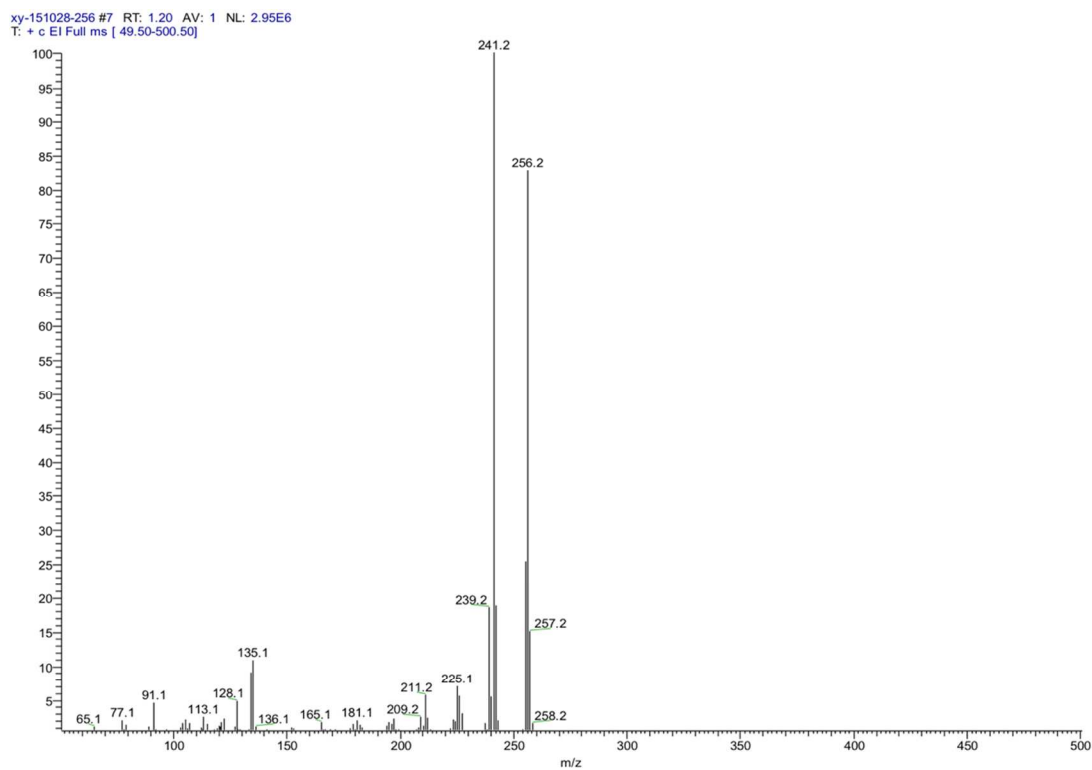


Figure S3. EI-MS of compound **1**. Calculated: 256.35, found: 256.2.

Compound 2. Propargyl bromide (1.70 mL, 19.5 mmol) in 15 mL dry DMF was added dropwise to a dry DMF solution (20 mL) of compound **1** (5.0 g, 19.5 mmol) and potassium carbonate (2.98 g, 21.5 mmol). The reaction mixture was stirred at room temperature for 24 h. The solids were removed by filtration and the solution was concentrated via rotary evaporation at 60 °C to remove the most of the DMF solvent. The crude product was then diluted by 100 mL of EA and then washed with saturated ammonium chloride solution (100 mL, 1×), water (100 mL, 2×), brine (100 mL, 1×). The combined organic extracts were dried over anhydrous sodium sulfate and the solvent was removed by rotary evaporation. The remaining yellow residue was purified by silica gel column chromatography (gradient elution with 5 – 10% EA in PE), affording compound **2** as a white solid. (1.9 g, 6.45 mmol, 32%). ^1H NMR (400MHz, CDCl_3): δ 6.86 (s, 2H), 6.84 (s, 2H), 4.63 (s, 1H), 4.50 (s, 2H), 3.76 (s, 2H), 2.54 (s, 1H), 2.32 (s, 6H), 2.25 (s, 6H); ^{13}C NMR (400MHz, CDCl_3): δ 148.65, 145.63, 132.90, 128.03, 126.06, 124.23, 124.11, 118.19, 74.71, 70.06, 54.96, 35.66, 11.70, 11.11.

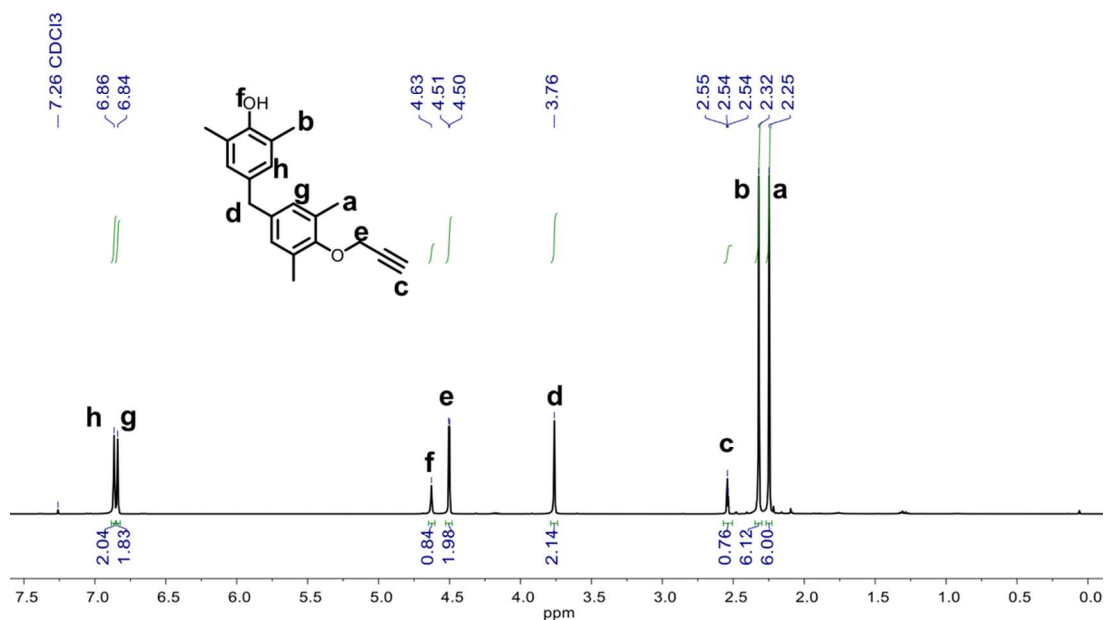


Figure S4. ^1H NMR spectrum of compound **2** in CDCl_3 .

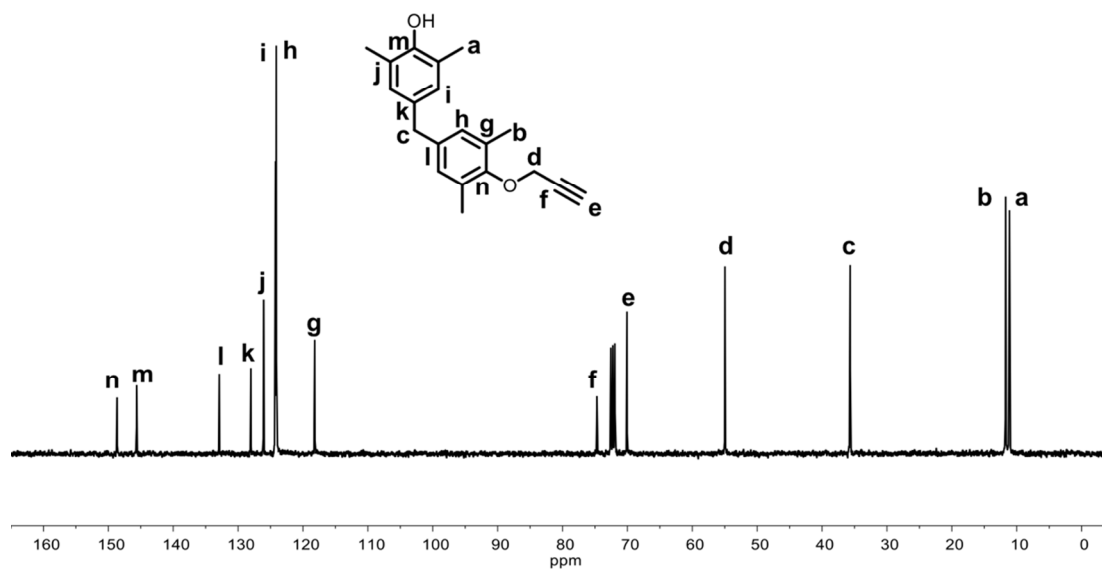


Figure S5. ^{13}C NMR spectrum of compound **2** in CDCl_3 .

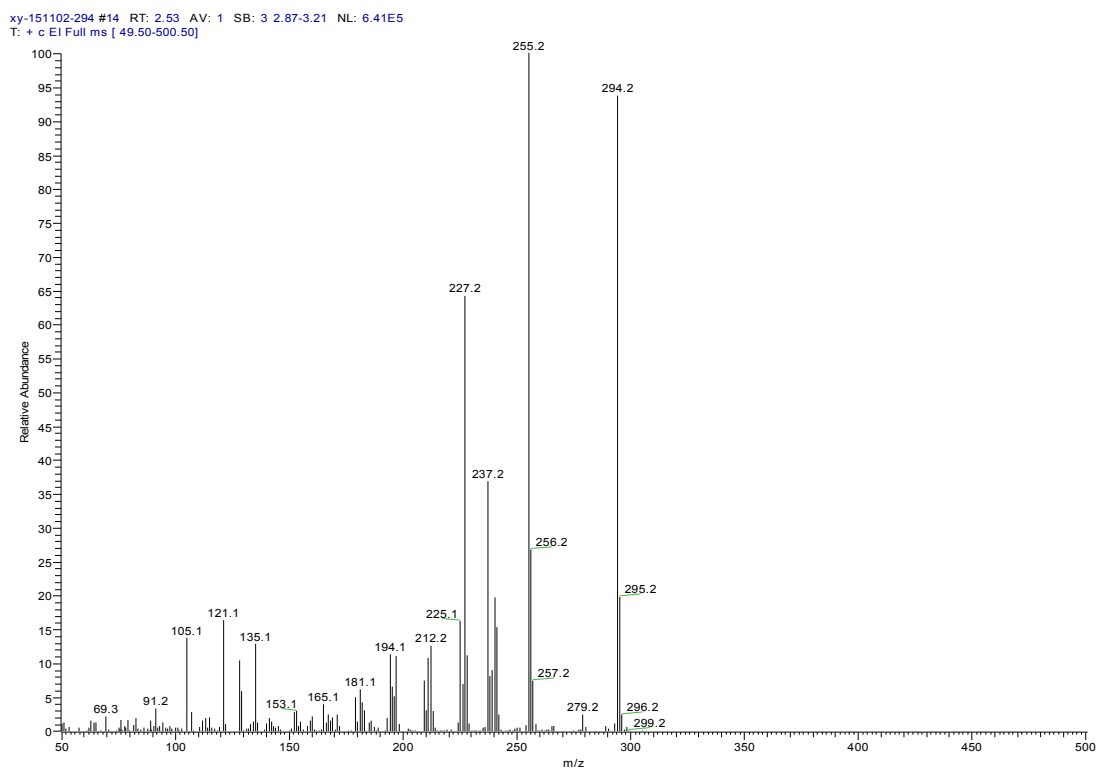


Figure S6. EI-MS of compound **2**. Calculated: 294.39, found: 294.2.

Compound 3. Potassium ferricyanide (4.48 g, 13.61 mmol, 4 equiv) was added to a solution of compound **2** (1.0 g, 3.40 mmol) in diethyl ether (90 mL) under N₂ atmosphere. Potassium hydroxide (0.8 g, 14.27 mmol) in deionized water (20 mL) was added into the mixture. The reaction mixture was stirred vigorously for 2 h at room temperature and monitored by TLC. The mixture was then extracted with diethyl ether (100 mL, 3×) and the combined organic layers were washed with brine (100 mL, 1×) and dried over sodium sulfate. The solids were removed by filtration and the solution was concentrated by rotary evaporation to give pure oxidized product as yellow needle-like solid without column purification (0.9 g, 3.08 mmol, 90%). Compound **3** was dried under vacuum for several days and stored in a desiccated atmosphere. ¹H NMR (400MHz, CDCl₃): δ 7.52 (s, 1H), 7.13 (s, 2H), 7.00-7.06 (d, 2H), 4.56 (s, 2H), 2.54 (s, 1H), 2.36 (s, 6H), 2.04-2.07 (d, 6H); ¹³C NMR (400MHz, CDCl₃): δ 187.31, 156.45, 142.66, 139.04, 137.42, 135.46, 131.94, 131.20, 78.93, 75.47, 59.93, 16.98, 16.26.

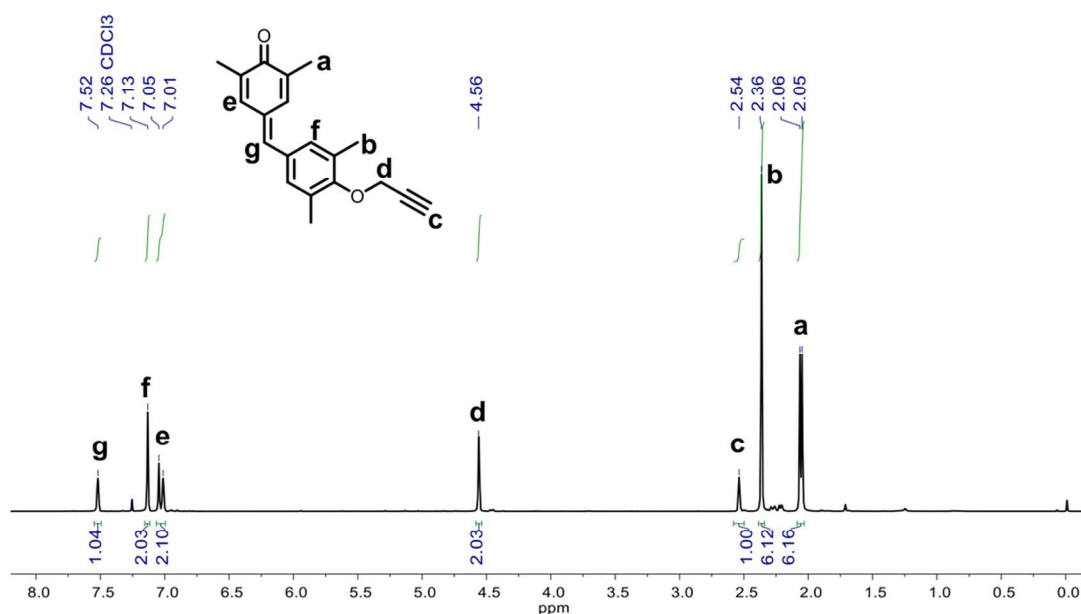


Figure S7. ¹H NMR spectrum of compound **3** in CDCl₃.

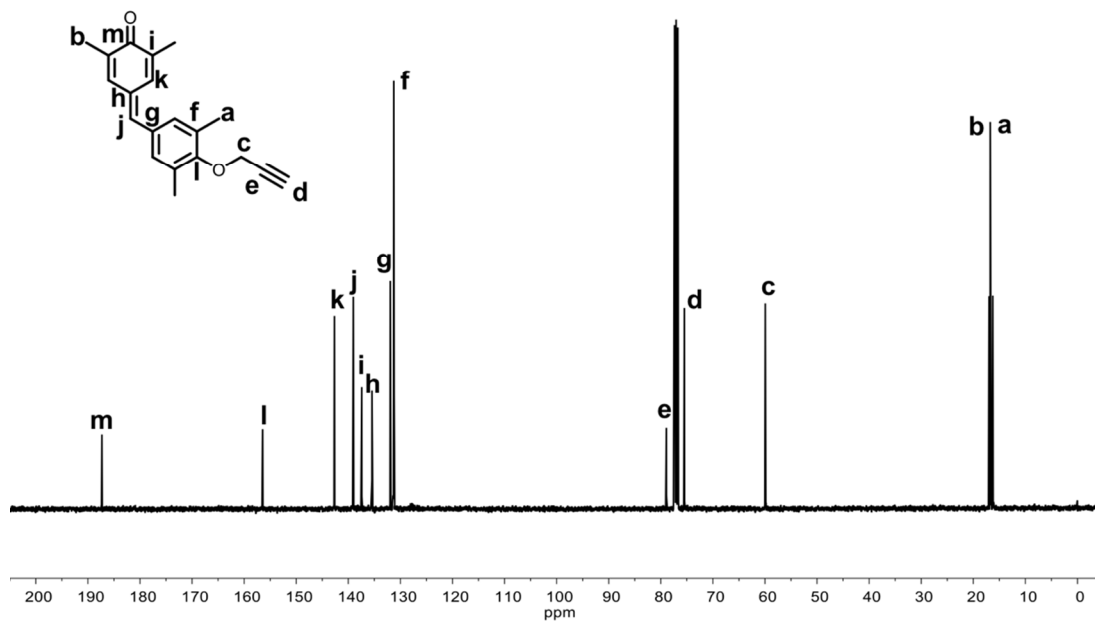


Figure S8. ^{13}C NMR spectrum of compound **3** in CDCl_3 .

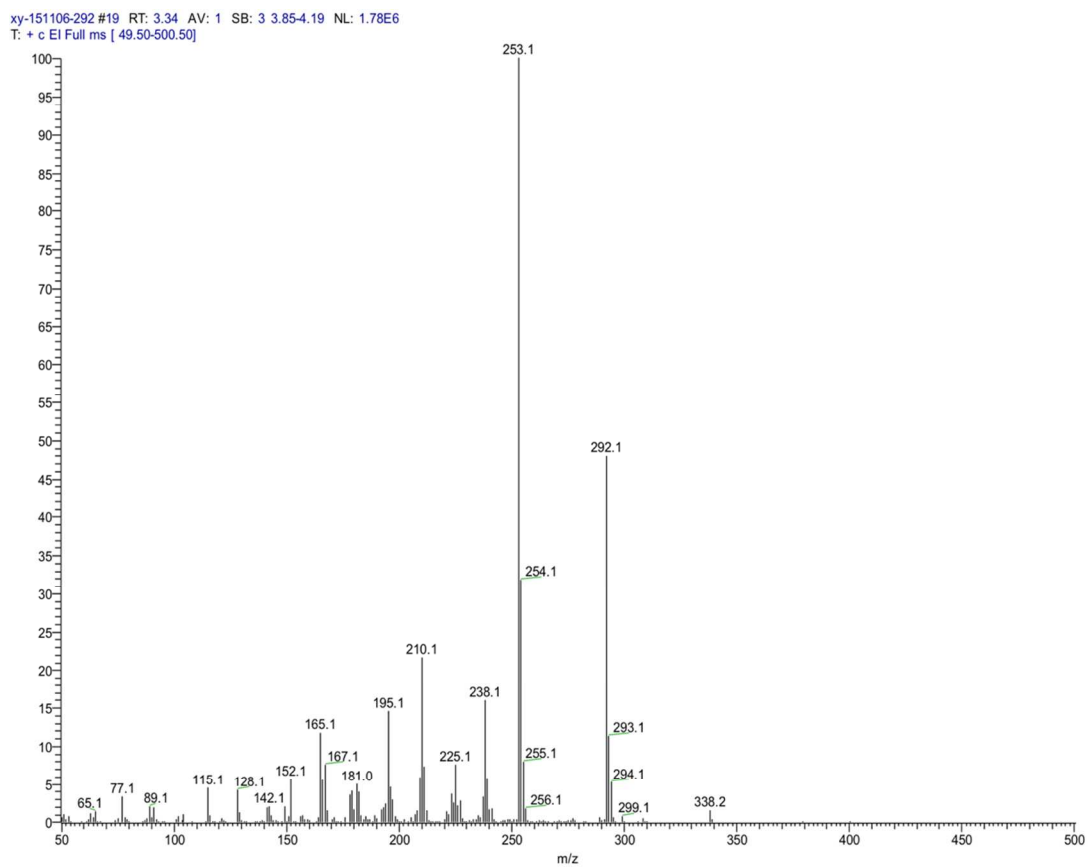


Figure S9. EI-MS of compound **3**. Calculated: 292.38, found: 292.1.

3. Synthesis of self-immolative PBE

General polymerization procedure. A 10 mL Schlenk flask with a stir bar was flame-dried under vacuum and purged with N₂. Compound **3** (0.25 mg, 0.86 mmol, 1 equiv) was added to the flask, which was backfilled with N₂ and then super-dry THF (1 mL, 0.86 M) with a syringe. The solution was degassed by 3× freeze-pump-thaw cycles, and the flask was backfilled with N₂ on the final cycle. The solution was allowed to equilibrate to 0 °C. Then, 20 µL of a stock solution of super-dry MeOH (69 µL) in super-dry THF (2 mL) (prepared by degassed via freeze-pump-thaw 3×) was added dropwise to the reaction mixture (0.0171 mmol MeOH, 0.02 equiv to monomer **3**), immediately followed by the addition of P₂-*t*-Bu phosphazene base solution in THF (21 µL of a 2 M stock solution; 0.0427 mmol, 0.05 equiv to monomer **3**). The reaction mixture was left to stir for 1 h, before the capping agent (1.2 equiv, *t*-butyldimethylsilyl chloride (TBSCl) or allyl chloroformate (ACF)) and base (1.2 equiv, imidazole or 4-(dimethylamino)-pyridine (DMAP)) were added to the solution. The solution was stirred at room temperature for 15 h. The polymer was precipitated 3× by addition to cold MeOH (60 mL) at -20 °C. Imidazole was used with the TBSCl capping agent while DMAP was used with the ACF capping agent. TBS- and ACF-capped PBEs were obtained as white solids in 80% and 72% yields, respectively.

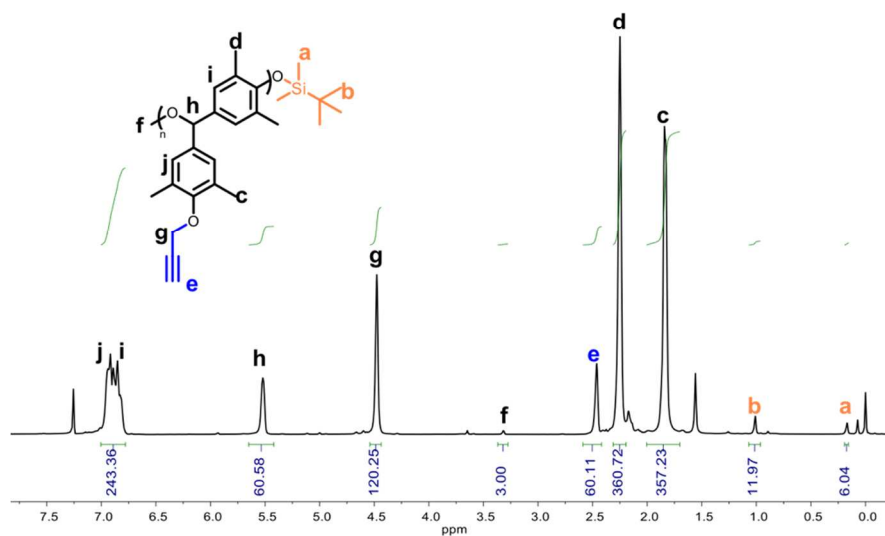


Figure S10. ^1H NMR spectrum of TBS-capped PBE in CDCl_3 .

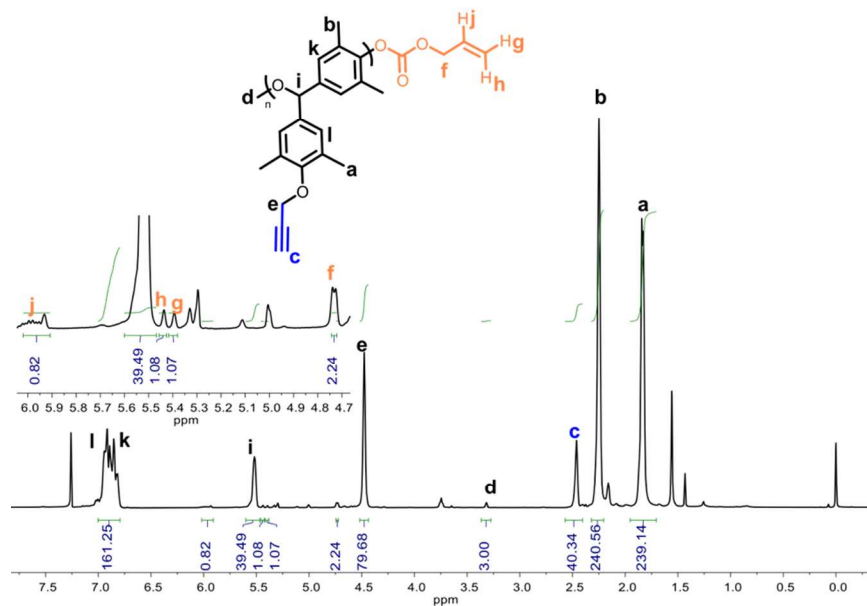


Figure S11. ^1H NMR spectrum of ACF-capped PBE in CDCl_3 .

Two-step polymerization for improved PDI. The polymerization using a separate initiation step was conducted according to literature.³ Briefly, two Schlenk flasks (10 mL) equipped with stir bars were flame dried under vacuum. One of the flasks was then loaded with compound **3** (100 mg, 0.342 mmol) and by super-dry THF (0.30 mL), and was degassed by 3× freeze-pump-thaw cycles. Next, super-dry MeOH (6.9 μ L, 0.171 mmol) and 2.0 M P₂-t-Bu phosphazene base solution in THF (80 μ L, 0.171 mmol) was sequentially added to this flask at 20 °C. The solution was stirred for 45 min. Then, a 20 μ L aliquot of this initiator solution was added to a degassed solution of compound **3** (0.25 g, 0.86 mmol) in super-dry THF (1 mL) pre-chilled to -20 °C in ice-salt bath. After 1 h, the reaction mixture was quenched with TBSCl (0.16 g, 1.03 mmol) and imidazole (0.07 g, 1.2 mmol) and stirred at room temperature for 15 h. The polymer was precipitated 3× by addition to cold MeOH (60 mL) at -20 °C to give TBS-capped PBE as a white solid (0.16 g, 64%). $M_{n, GPC} = 36.2$ kDa, $M_{w, GPC} = 40.2$ kDa, PDI = 1.1.

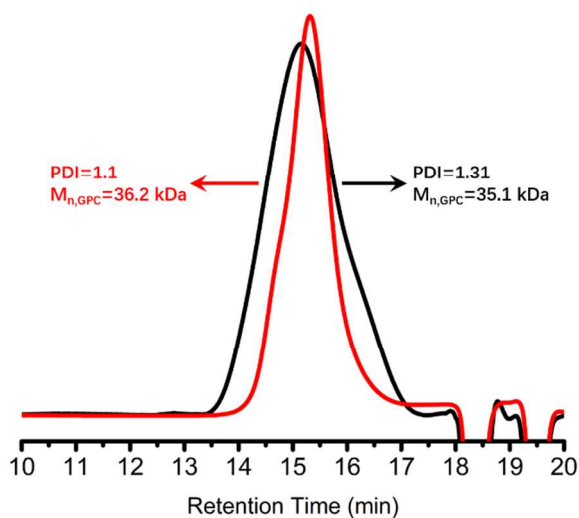


Figure S12. DMF GPC chromatograms for TBS-capped PBE prepared using single-step (black) or two-step (red) polymerization procedures.

4. Synthesis of self-immolative brush polymers

Synthesis of azide-terminated PS side chains. The N₃-PS was prepared by ATRP followed by azide displacement of the chain-end bromide according to a previous report.¹ Briefly, Br-PS was dissolved in DMF containing NaN₃ (2 equiv to bromide). The mixture was stirred at 25 °C overnight, and the product was purified by precipitation 3× into cold methanol/water mixture (1:1 v:v).

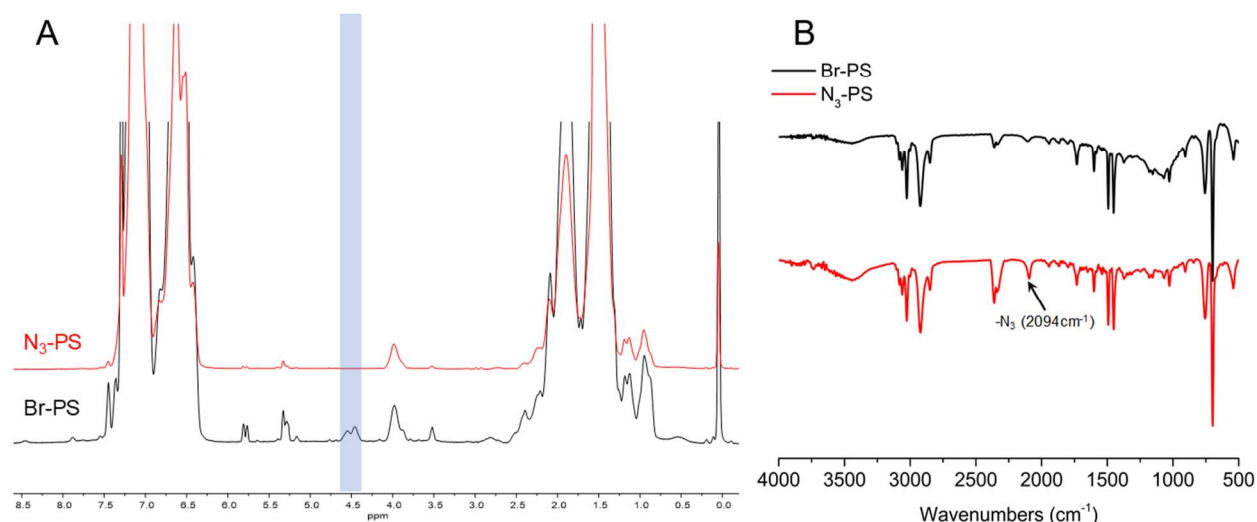


Figure S13. (A) ¹H NMR spectra of Br-PS and N₃-PS in CDCl₃. The highlighted region indicates the loss of the chain-end bromides. (B) FT-IR spectra of Br-PS and N₃-PS, showing the appearance of the azide vibration at 2094 cm⁻¹.

General procedure for the graft-onto synthesis of brush polymers. The following ratio of reagents was used for all coupling reactions: [azide]/[alkyne]/[CuBr]/[PMDETA] = 1.50/1.0/0.3/0.3. The click coupling reactions between side chains (0.045 mmol azide) and PBE (0.03 mmol alkyne) were conducted in a 10 mL Schlenk flask with 2 mL CH₂Cl₂ as the solvent and CuBr/PMDETA as the catalyst system. The reagents were mixed and stirred at room temperature for 3 h. Thereafter, the reaction mixture was exposed to air, diluted with THF, and passed through

neutral alumina to remove the copper catalyst. The solution was then concentrated via rotary evaporation and precipitated in cold diethyl ether. The crude product was obtained after centrifugation, and dried under vacuum. To remove excess side chains, the polymer mixtures were dissolved in THF (for PS conjugation) or DMF (for PEG conjugation), and injected into semi-prep DMF or THF GPC. The fraction for the conjugates were collected, combined, and dried in vacuo. The coupling kinetics and efficiency were determined via DMF GPC measurement of the samples withdrawn periodically from the reaction mixture. The grafting density of the PS side chain coupling was also calculated by monitoring the alkyne C-H vibration in the IR using the C-O vibration as an internal reference (for the PEG side chain there is substantial peak overlapping). Calculations were made using the following equation:

$$\text{grafting density}_{\text{PS brushes, IR}} = 1 - \frac{A_{\nu_{\text{C-H}}(\text{PBE-g-PS})} : A_{\nu_{\text{C-O}}(\text{PBE-g-PS})}}{A_{\nu_{\text{C-H}}(\text{PBE})} : A_{\nu_{\text{C-O}}(\text{PBE})}}$$

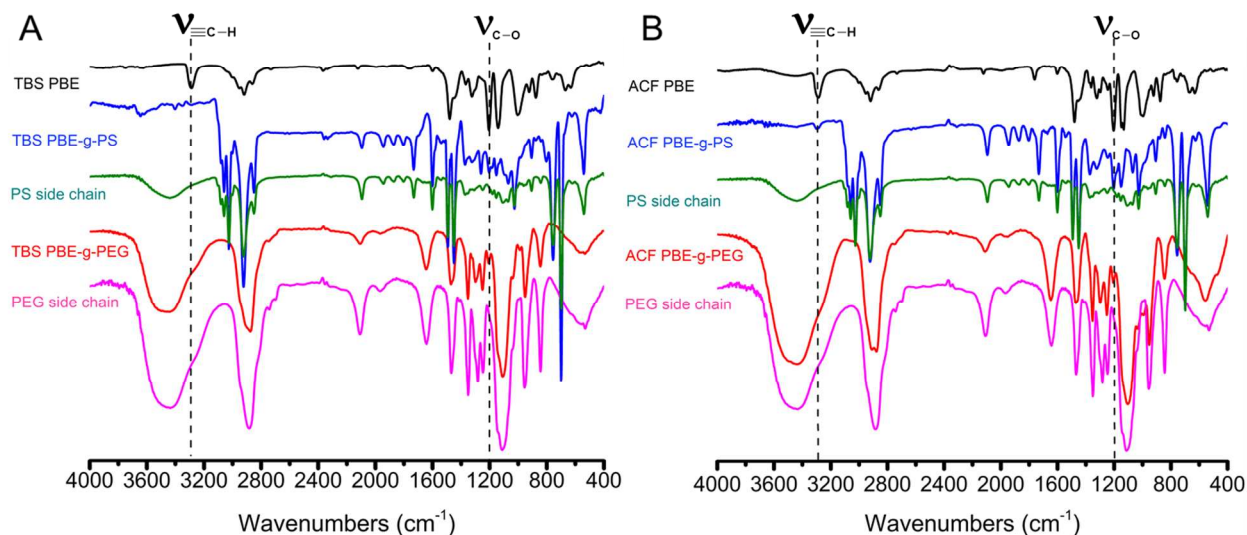


Figure S14. FT-IR spectra of TBS- and ACF-capped PBE and molecular brushes. The grafting densities of ACF-capped PBE-g-PS based on IR peak integration is 64%, while that for the TBS-capped PBE-g-PS is 92%, agreeing well with the GPC values (69% and 89%, respectively).

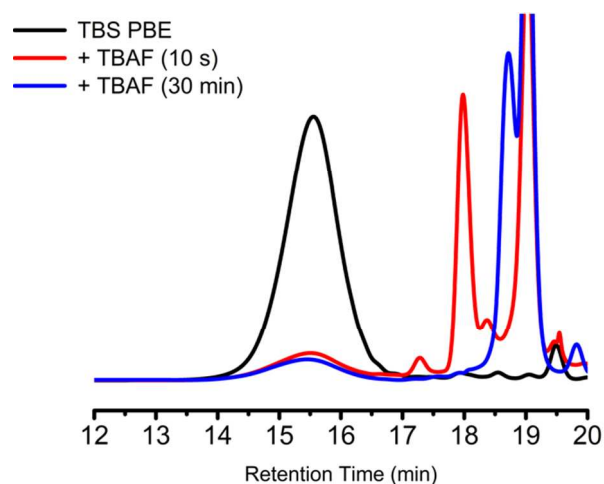


Figure S15. GPC profiles for the depolymerization of TBS PBE synthesized from super-dry CH_2Cl_2 , showing that the polymer cannot be fully degraded (with ~5-10% remaining).

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