Tailored Silyl Ether Monomers Enable Backbone-Degradable Polynorbornene-Based Linear, Bottlebrush, and Star Copolymers through ROMP

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Supplementary Information

Table of Contents

Materials and Methods.................................................................................................................................. 2
Monomer Synthesis ........................................................................................................................................ 2
Polymer Synthesis ........................................................................................................................................ 8
Polymer Degradation and Characterization of Degradation Products.................................................... 9
Reaction Optimization and Characterization .................................................................................................12
Computational Chemistry...............................................................................................................................12
In Vitro Experiments....................................................................................................................................13
In Vivo Experiments ....................................................................................................................................14
Statistics and Reproducibility .......................................................................................................................15
Scheme 1.....................................................................................................................................................17
Table 1.........................................................................................................................................................18
Figures 1-40................................................................................................................................................19
References .................................................................................................................................................... 60
Materials and Methods

All reagents were purchased from commercial suppliers and used without further purification unless noted otherwise. Grubbs 3\textsuperscript{rd} generation catalyst, norbornene monomers NB1,\textsuperscript{1} NB2,\textsuperscript{2} NB3,\textsuperscript{3} NB4,\textsuperscript{4} PEG-MM,\textsuperscript{5} PS-MM,\textsuperscript{6} PLA-MM,\textsuperscript{7} Cy5.5-MM,\textsuperscript{8} Tel-MM,\textsuperscript{9} and crosslinker AcXL\textsuperscript{10} were prepared following reported procedures. PS-MM and PLA-MM were further purified by preparative gel permeation chromatography before use. Dry tetrahydrofuran (THF) and dichloromethane (DCM) were passed through an activated alumina column prior to use. Thin-layer chromatography was performed using Baker-flex pre-coated TLC plates containing F254 indicator.

\(^1\)H nuclear magnetic resonance (\(^1\)H-NMR) and \(^{13}\)C nuclear magnetic resonance (\(^{13}\)C-NMR) spectra were acquired at the MIT Department of Chemistry Instrumentation Facility on a Varian Mercury 300, Bruker AVANCE III DRX 400, or Varian Inova 500. Chemical shifts are reported in ppm relative to signals from the NMR solvent: for CDCl\textsubscript{3}, this corresponds to 7.26 for \(^1\)H and 77.0 for \(^{13}\)C spectra. High-resolution mass spectrometry (HRMS) measurements were obtained on a JEOL AccuTOF system at the MIT Department of Chemistry Instrumentation Facility. MALDI-TOF analyses were performed on a Bruker microflex LRF using a 0.1% alpha-cyano-4-hydroxycinnamic acid matrix.

Gel permeation chromatography (GPC) analyses of polyethylene glycol (PEG)-containing polymers were performed on an Agilent 1260 Infinity system with dual Agilent PL1110-6500 columns and a 0.025 M LiBr in DMF mobile phase at 60 °C. The differential refractive index (dRI) of each compound was monitored using a Wyatt Optilab T-rEX detector. For polymers containing primarily polystyrene (PS) or polyactic acid (PLA), GPC analysis was performed on a Tosoh EcoSEC HLC-8320 with dual TSKgel SuperH3000 columns and a chloroform mobile phase.

Dynamic light scattering (DLS) measurements were performed using a Wyatt Technology Mobius DLS instrument. Samples were prepared at 1.0 mg/mL in the requisite buffer. The solutions were filtered through a 0.2 \(\mu\)m nylon filter into disposable polystyrene cuvettes, which were pre-cleaned with compressed air. The solutions were immediately capped after addition of the solution to the cuvette. Measurements were made in sets of 10 acquisitions; the average hydrodynamic diameters were calculated using the DLS-correlation function via a regularization fitting method (Dynamics 7.4.0.72 software package from Wyatt).

Monomer Synthesis

\[
\begin{align*}
\text{OH} & \quad \text{TBSCI, imidazole} \\
\text{THF} & \quad \text{OTBS} \\
\text{S1}
\end{align*}
\]

The synthesis of TBS-protected alkyne S1 was adapted from a literature protocol (Al-Shuhaid, Z. et al, \textit{Tet. Lett.} 2013, 54, 6716-6718). 20.0 g (285 mmol) of 3-butynol, a clear liquid, was dissolved in 200 mL of dry THF in an oven-dried 1 L flask charged with stir bar. Next, 43.0 g (285 mmol, 1 eq.) of tert-butylimidethylsilyl chloride and 29.1 g (428 mmol, 1.5 eq.) of imidazole were added. The reaction was stirred for two hours at room temperature, during which time a significant amount of white precipitate formed. The mixture was filtered to remove imidazolium salt and was concentrated by rotary evaporation. The remaining residue was diluted with 200 mL of diethyl
ether, washed with 200 mL of saturated ammonium chloride solution and 200 mL of saturated sodium bicarbonate solution, dried over sodium sulfate, and concentrated to yield 51.3 g (98%) of S1, which was utilized in the next step without further purification. NMR spectra matched those reported in the literature.

The synthesis of TBS-protected diol S2 was adapted from a literature protocol (Trost, B. M.; Kainmals, C. A. Org. Lett. 2017, 19, 2346-2349). 48.5 g (263 mmol) of TBS butynol was dissolved in 400 mL of dry THF in an oven-dried 1 L flask charged with stir bar. The solution was placed under a dry nitrogen atmosphere and cooled to -78 °C using an acetone/dry ice bath. To the mixture was carefully added 110 mL of 2.5 M n-butyllithium in hexanes (276 mmol, 1.05 equiv.) over the course of 15 minutes. The now yellow reaction mixture was stirred for 1 h at -78 °C, and then was allowed to warm to 0 °C. Next, 9.5 g (316 mmol, 1.2 eq.) of paraformaldehyde was added in one portion and the reaction was allowed to stir at room temperature overnight. The solution was then quenched with the addition of 300 mL of saturated ammonium chloride and 300 mL of diethyl ether was added. The organic layer was collected and the aqueous layer was extracted with another 300 mL of diethyl ether. The organic layers were then dried over sodium sulfate and concentrated to yield a pale yellow oil, which was purified by silica gel chromatography with 6:1 hexanes/ethyl acetate to yield 38.7 g (68%) of S2 as a clear oil. NMR spectra matched those reported in the literature.

The reduction of TBS-protected alkynol was performed via an adaptation of literature procedure (Al-Shuhaib, Z. et al, Tet. Lett. 2013, 54, 6716-6718). To a 1 L flask charged with stir bar was added 210 mL of absolute ethanol and 3.57 (14.4 mmol, 0.13 equiv.) of nickel acetate tetrahydrate. The solution was placed under a hydrogen atmosphere using hydrogen-filled balloons. To the mixture was carefully added a suspension of 735 mg (19.5 mmol, 0.178 equiv.) of sodium borohydride in 45 mL of ethanol, turning the light teal solution a dark black color. After stirring for 30 min, 3 mL (3.9 g, 64.5 mmol, 0.586 equiv.) of ethylene diamine were added followed by a solution of 23.7 g (110 mmol) of S2 in 75 mL of ethanol. The reaction was stirred at room temperature and monitored via NMR. After 5 h, 1H NMR spectroscopy showed full conversion of starting material to product. The flask was then evacuated of hydrogen gas. The mixture was diluted with 500 mL of ethyl acetate and poured through a pad of silica to remove Ni salts. The solution was then concentrated under vacuum and purified with a short plug of silica using 4:1 hexanes/ethyl acetate to yield 12.0 g (50%) of S3 as a clear oil. 1H NMR (400 MHz, Chloroform-d) δ 5.87 – 5.76 (m, 1H), 5.63 – 5.52 (m, 1H), δ 4.13 (t, J = 5.6 Hz, 2H), 3.64 (t, J = 6.1 Hz, 2H), 2.34 (q, J = 6.6 Hz, 2H), 2.16 (s, 1H), 0.89 (s, 3H), 0.05 (s, 6H). 13C NMR (101 MHz, CDCl3) δ 130.84, 129.36, 77.32, 77.00, 76.68, 62.21, 57.92, 30.83, 25.89, 18.36, -5.46. HRMS (DART+): Calculated for C11H25O2Si (M+H)+: 217.1623, found 217.1796. Rf = 0.40 (4:1 hexanes/ethyl acetate, KMnO4)
12.0 g (55.4 mmol) of **S3** was dissolved in 200 mL of methanol containing 1% concentrated hydrochloric acid. The reaction was stirred at room temperature while being closely monitored by TLC. After 15 min, TLC showed conversion to product. The reaction was neutralized through the addition of 30% aqueous sodium hydroxide, concentrated, and then purified via silica gel chromatography with 2:1 hexanes/ethyl acetate to ethyl acetate to yield 4.44 g (44 mmol, 80%) of **S4** as a clear oil. **1H NMR** (300 MHz, Chloroform-\(d\)) \(\delta\) 5.93 – 5.78 (m, 1H), 5.68 – 5.52 (m, 1H), 4.14 (d, \(J = 7.1\) Hz, 2H), 3.66 (t, \(J = 5.9\) Hz, 2H), 2.82 (s, 1H), 2.69 (s, 1H), 2.36 (dd, \(J = 7.5, 5.9, 1.4\) Hz, 2H). **13C NMR** (101 MHz, CDCl\(3\)) \(\delta\) 130.68, 129.03, 60.86, 57.24, 30.32. \(R_f = 0.50\) (ethyl acetate, KMnO\(_4\)).

1.02 g (10 mmol) of **S4** and 1.36 g of imidazole (20 mmol, 2 equiv.) were dissolved in 500 mL of dry DCM in an oven-dried flask. Next, 1.22 mL of dichlorodimethylsilane (1.29 g, 10 mmol, 1 equiv.) were added dropwise over 5 min, during which time the solution turned cloudy. The mixture was stirred for 1 h, filtered, and concentrated. The yellow residue was then transferred into a small flask and distilled under vacuum (140 °C, 10 mtorr) to yield a clear oil. The oil was further purified via silica gel chromatography with 20:1 hexanes/ethyl acetate to yield 0.837 g (53%) of **MeSi** as a somewhat volatile clear oil that was kept at -20 °C. **1H NMR** (500 MHz, Chloroform-\(d\)) \(\delta\) 5.88 (dt, \(J = 11.4, 5.7\) Hz, 1H), 5.74 (app. dtt, \(J = 11.1, 8.5, 1.2\) Hz, 1H), 4.31 (dd, \(J = 5.8, 1.2\) Hz, 2H), 3.88 – 3.81 (m, 2H), 2.49 (dt, \(J = 8.6, 5.3\) Hz, 2H), 0.14 (s, 6H). **13C NMR** (101 MHz, CDCl\(3\)) \(\delta\) 130.87, 129.38, 60.65, 57.13, 30.15, 0.66. \(R_f = 0.60\) (20:1 hexanes/ethyl acetate, KMnO\(_4\)).

1.02 g (10 mmol) of **S4** and 1.36 g of imidazole (20 mmol, 2 equiv.) were dissolved in 500 mL of dry DCM in an oven-dried flask. Next, 1.50 mL of dichlorodiethylsilane (1.57 g, 10 mmol, 1 equiv.) were added dropwise over 5 min, during which time the solution turned cloudy. The mixture was stirred for 1 h, filtered, and concentrated. The yellow residue was then transferred into a small flask and distilled under vacuum (140 °C, 10 mtorr) to yield a clear oil. The oil was further purified via column chromatography with 20:1 hexanes/ethyl acetate to yield 1.15 g (62%) of **EtSi** as a clear oil that was kept at -20 °C. **1H NMR** (300 MHz, Chloroform-d) \(\delta\) 5.97 – 5.83 (m, 1H), 5.74 (app. dtt, \(J = 10.9, 8.5, 1.1\) Hz, 1H), 4.35 – 4.26 (m, 2H), 3.90 – 3.81 (m, 2H), 2.55 – 2.42 (m, 2H), 0.97 (app. td, \(J = 8.0, 0.6\) Hz, 6H), 0.62 (app. qd, \(J = 7.9, 0.9\) Hz, 4H). **13C NMR** (101 MHz, CDCl\(3\))
δ 131.64, 129.15, 62.91, 59.96, 30.44, 6.27, 4.30. HRMS (DART+): Calculated for C₉H₁₉O₂Si (M+H)⁺: 187.1154, found 187.1318. Rᵣ = 0.65 (20:1 hexanes/ethyl acetate, KMnO₄)

1.02 g (10 mmol) of S₄ and 1.36 g of imidazole (20 mmol, 2 equiv.) were dissolved in 500 mL of dry DCM in an oven-dried flask. Next, 1.50 mL of dichlorodiisopropylsilane (1.57 g, 10 mmol, 1 equiv.) were added dropwise over 5 min, during which time the solution turned cloudy. The mixture was stirred for 3 h, filtered, and concentrated. The yellow residue was then transferred into a small flask and distilled under vacuum (140 °C, 10 mtorr) to yield a clear oil. The oil was further purified via column chromatography with 20:1 hexanes/ethyl acetate to yield 1.05 g (49%) of iPrSi as a clear oil that was kept at -20 °C. ¹H NMR (400 MHz, Chloroform-d) δ 5.92 (dt, J = 11.5, 6.0 Hz, 1H), 5.73 (dt, J = 10.9, 8.5 Hz, 1H), 4.40 – 4.25 (m, 2H), 4.05 – 3.90 (m, 2H), 2.44 (dt, J = 8.6, 5.2 Hz, 2H), 1.00 (s, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 131.85, 129.38, 63.54, 60.34, 30.75, 17.58, 17.43, 17.33, 12.47. HRMS (DART+): Calculated for C₁₁H₂₃O₂Si (M+H)⁺: 215.1467, found 215.1436. Rᵣ = 0.70 (20:1 hexanes/ethyl acetate, KMnO₄)

0.300 g (3 mmol) of S₄ and 0.408 g (6 mmol, 2 equiv.) of imidazole were dissolved in 150 mL dry DCM. Next, dichlorodiphenylsilane (1.52 g, 6 mmol, 2 equiv.) was added dropwise over 5 min, during which time the solution turned cloudy. The mixture was stirred for 3 h, filtered, and concentrated. The mixture was purified using a short column of hexanes to 10:1 hexanes/ethyl acetate. The resulting oil, which was a mixture of cyclic monomer and higher order cyclic oligomers, was purified by preparative GPC (with chloroform eluent) to yield 93 mg (11%) of pure PhSi as a clear oil that was kept at -20 °C. ¹H NMR (400 MHz, Chloroform-d) δ 7.71 – 7.63 (m, 4H), 7.45 – 7.32 (m, 4H), 5.90 (dt, J = 11.0, 5.4 Hz, 1H), 5.78 – 5.66 (m, 1H), 4.49 (dd, J = 5.4, 1.3 Hz, 2H), 4.04 – 3.97 (m, 2H), 2.54 (dt, J = 8.6, 5.3 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 134.58, 133.40, 131.80, 130.10, 128.81, 127.82, 77.32, 77.00, 76.68, 67.05, 63.42, 61.06, 30.67. HRMS (DART+): Calculated for C₁₇H₁₉O₂Si (M+H)⁺: 283.1154, found 283.1138. Rᵣ = 0.80 (20:1 hexanes/ethyl acetate, UV, KMnO₄)
0.264 g (3 mmol) of cis-1,4-butenediol and 0.408 g (6 mmol, 2 equiv.) of imidazole were dissolved in 60 mL of dry DMF. Next, dichlorodisopropylsilane (0.555 g, 3 mmol) was added dropwise over 5 min. The solution was stirred overnight, quenched with 150 mL H₂O, extracted with 2 x 200 mL hexanes, dried over sodium sulfate, and concentrated. The product was purified with 10:1 hexanes/ethyl acetate to yield 0.750 g (quant.) of the desired product (7-iPrSi) as a clear oil. ¹H NMR (400 MHz, Chloroform-d) δ 5.67 (t, J = 1.9 Hz, 2H), 4.50 (d, J = 1.8 Hz, 4H), 1.08 (d, J = 1.7 Hz, 14H). ¹³C NMR (101 MHz, CDCl₃) δ 129.83, 62.54, 17.30, 12.03. HRMS (DART+): Calculated for C₁₀H₂₁O₂Si (M+H)⁺: 201.1310, found 201.1065. Rf = 0.70 (20:1 hexanes/ethyl acetate, UV, KMnO₄).

S₅ was prepared according to reported procedures (O’Rourke, N. F. et al. Org. Lett. 2016, 18, 1250-1253). To a 300 mL flask charged with stir bar was added 40 mL of absolute ethanol and 0.714 g (2.87 mmol, 0.13 equiv.) of nickel acetate tetrahydrate. The solution was placed under a hydrogen atmosphere using a hydrogen-filled balloon. To the mixture was carefully added a suspension of 147 mg (3.89 mmol, 0.178 equiv.) of sodium borohydride in 9 mL of ethanol, turning the light teal solution a dark black color. After stirring for 30 min, 0.6 mL (0.54 g, 8.98 mmol, 0.586 equiv.) of ethylene diamine were added followed by a solution of S₅ (6.20 g, 22 mmol) in 15 mL of ethanol. The reaction was stirred at room temperature and monitored by ¹H NMR spectroscopy. After 5 h, NMR showed full conversion of starting material to product. The flask was then evacuated of hydrogen gas and placed under ambient atmosphere. The mixture was diluted with 500 mL of ethyl acetate and poured through a pad of silica to remove Ni salts. The solution was then concentrated under vacuum and purified with a short plug of silica using 4:1 hexanes/ethyl acetate to yield 3.58 g (57%) of S₆ as a clear oil. ¹H NMR (400 MHz, Chloroform-d) δ 5.51 (t, J = 4.9 Hz, 2H), 4.59 (t, J = 3.5 Hz, 2H), 3.87 (ddd, J = 11.1, 7.6, 3.3 Hz, 2H), 3.74 (dt, J = 9.3, 7.0 Hz, 2H), 3.49 (dt, J = 10.7, 4.8 Hz, 2H), 3.41 (dt, J = 9.5, 7.0 Hz, 2H), 2.38 (q, J = 6.5 Hz, 4H), 1.83 (qd, J = 7.6, 7.1, 3.4 Hz, 2H), 1.71 (ddd, J = 12.3, 7.9, 4.4 Hz, 2H), 1.64 – 1.41 (m, 8H). ¹³C NMR (101 MHz, CDCl₃) δ 127.61, 98.63, 66.86, 62.15, 30.61, 27.97, 25.40, 19.49. HRMS (DART+): Calculated for C₁₆H₂₉O₄ (M+H)⁺: 285.2065, found 285.1765. Rf = 0.50 (10:1 hexanes/ethyl acetate, KMnO₄).
In a round bottom flask, 0.5 mL of acetyl chloride were added to 50 mL of methanol. The solution was stirred for 5 min, then 3.0 g (10.6 mmol) of S6 were added. The mixture was stirred for 15 min, after which time TLC showed full conversion to product. The mixture was then neutralized with 30% sodium hydroxide and concentrated under vacuum. The remaining residue was passed through a short silica column, starting with 1:1 hexanes/ethyl acetate and eluting the product with ethyl acetate. The solution was concentrated to yield 0.694 g (56%) of S7 as a clear oil.

\[ \text{S6} \xrightarrow{\text{AcCl, MeOH}} \text{S7} \]

To 300 mL of dry DCM was added 0.408 g (6 mmol, 2 equiv.) of imidazole. Next, solutions of 0.348 g (3 mmol) of S7 and dichlorodiisopropylsilane (0.555 g, 3 mmol, 1 equiv.), each in 5 mL of dry DCM, were added dropwise over 5 min, during which time the solution turned cloudy. The mixture was stirred for 3 h, filtered, and concentrated. The mixture was purified using a short column of hexanes to 10:1 hexanes/ethyl acetate. The resulting oil, which was a mixture of cyclic monomer and higher order cyclic oligomers, was purified by preparative GPC (using chlorofor eluent) to yield 0.244 g (36%) of 9-iPrSi as a clear oil.

\[ \text{S7} \xrightarrow{iPr_2SiCl_2, \text{imidazole}} \text{9-iPrSi} \]

Synthesis of Cy3-MM

Cy3-MM was prepared in an analogous fashion to that reported for Cy5.5-MM. Briefly, 13.4 mg of Cy3-Azide (Lumiprobe) was added to a 2-dram vial. Next, 60 mg of an alkyne-containing macromonomer were added. The combined solids were transferred into a nitrogen-filled glovebox and dissolved in 1 mL of DCM. Next, a spatula tip of copper (I) acetate was added and the solution stirred for 1 h, after which time LC-MS analysis indicated full conversion of Cy3-Azide. The mixture was then concentrated and purified by preparative HPLC using an acetonitrile-0.1% AcOH in water gradient. The desired fractions were partially concentrated by rotary evaporation until ~25% of the residual solvent remained, then 250 mL of DCM were added and the entire mixture was dried with sodium sulfate. The desired product was collected by removal of the sodium sulfate and concentration to yield Cy3-MM as a magenta solid. Calculated mass (M)\(^+\): 3698.84 found 3696.08 (see Figure S23).
Polymer Synthesis

Linear Copolymer Synthesis

NB1-NB4 and the requisite silyl ether monomers were transferred into a water- and air-free glovebox under N₂ atmosphere and dissolved in dioxane to form a 0.5 M solution. A pre-measured amount of Grubbs’ 3rd generation bispyridyl catalyst in a vial was diluted with dioxane to form a freshly prepared 0.01 M stock solution.

Inside the glovebox, to a 0.5-dram vial containing a stir bar were added 105 µL of dioxane, 15 µL of 0.5 M norbornene stock, and 15 µL of 0.5 M silyl ether stock (or dioxane for polynorbornene homopolymers). Finally, 15 µL of the catalyst solution were added to generate polymers with a target DP of 50 for each monomer. The mixture was stirred for 30 min, removed from the glovebox, quenched with a drop of ethyl vinyl ether (EVE), and analyzed by DMF GPC.

Bottlebrush Copolymer Synthesis

Bottlebrush polymers were synthesized using 200 mg of PEG-MM in 800 µL of dioxane. 200 µL of solution were added into each of four one-dram vials, followed by 30 µL of 0.5 M silyl ether in dioxane or 30 µL of dioxane. Finally, 75 µL of 0.02 M Grubbs’ 3rd generation catalyst in dioxane were added to target a DP of 30 for each monomer. The mixture was stirred for 30 min, quenched with a drop of EVE, and analyzed by GPC. The reaction mixtures were used directly for the degradation experiments described in the following section.

Drug-conjugated, PLA, and PS bottlebrush copolymers were synthesized in an analogous fashion.

Brush-Arm Star Polymer Synthesis

50 mg of PEG-MM were dissolved in 199 µL of dioxane in a glovebox. Next, 40 µL of PEG-MM solution were added to each of four one-dram vials loaded with stir bars. Next, 6.2 µL of 0.5 M silyl ether or dioxane were added. 22.1 µL of 0.02 M Grubbs’ 3rd generation catalyst in dioxane were added and the solution was stirred for 15 min. Next, 88.4 µL total of 0.1 M AcXL in dioxane were added in four portions over 15 min. The solutions were allowed to crosslink for 90 min, removed from the glovebox, and quenched with EVE and analyzed by GPC. The solutions were concentrated under vacuum before dissolution in 100 µL dioxane for degradation experiments.

Linear Block Copolymer Synthesis

In a N₂ glovebox, 80 µL of dioxane were added to a 0.5-dram vial with a stir bar. 10 µL of a 1M solution of NB4 in dioxane were added, followed by 10 µL of a freshly prepared solution of 0.02 M Grubbs’ 3rd generation catalyst in dioxane for a target DP of 50. The solution was stirred for 30 min. Next, 17.5 µL of a 4:3 mixture of 1 M NB4 and 1 M iPrSi were added to introduce another 50 units of each monomer onto the polymer. The solution was stirred for another 2 h, taken out of the glovebox, quenched with a drop of EVE, concentrated under vacuum, and redissolved in 100 µL dioxane before degradation experiments. A sample was taken for analysis by DMF GPC.

Bottlebrush Block Copolymer Synthesis

In a N₂ glovebox, 100 mg of PEG-MM and 100 mg of PS-MM were each dissolved into 400 µL of dioxane. 106 µL of PEG-MM solution were added to a 0.5-dram vial. To the solution were added 12.8 µL of 0.02 M Grubbs’ 3rd generation catalyst in dioxane. The solution was stirred for 30 min
for a target DP of 30 for the PEG block. Next, a mixture of 106 µL of PS-MM stock and 6 µL of 1M iPrSi stock in dioxane was added in one portion for a target DP of 30 for the PS block. The solution was stirred for another 2 h, removed from the glovebox, and quenched with a drop of EVE. The solution was then concentrated under vacuum and diluted in 200 µL of dioxane before use in degradation experiments. A sample was taken for analysis by GPC.

**Block Copolymer Synthesis with Degradable Linkers**

In a glovebox, 100 mg of PEG-MM were dissolved in 400 µL of dioxane. 106 µL of solution were added to each of two vials. Next, 12.5 µL of freshly prepared 0.02 M Grubbs’ 3rd generation catalyst in dioxane were added. The mixture was stirred for 1 h. Next, a combination of 20 µL of 1M NB3 and 10 µL of iPrSi were combined. 7.5 µL of this solution were added to one of the vials, while 5 µL of NB3 were added to the other. The solution was stirred for 30 min. Finally, 300 mg of PS-MM in 300 µL of dioxane were added. The solution was stirred for 6 h, quenched with EVE, and characterized by GPC. The solution was then concentrated under vacuum before use in degradation experiments.

**Polymer Degradation and Characterization of Degradation Products**

**Acidic Hydrolysis of Linear Copolymers**

To force degradation of the material, the polymerization solution was concentrated in a vacuum chamber at room temperature to remove residual EVE and diluted in 100 µL of dioxane. To the solution were added 10 µL of 2M HCl. The mixture was stirred for 30 minutes. Excess sodium sulfate was added and the mixture was allowed to sit for 5 min. Finally, the mixture was extracted with DCM, filtered with a 0.2 µm nylon filter, concentrated, and analyzed by GPC.

Gel permeation chromatography (GPC) analyses were performed on an Agilent 1260 Infinity system with a 0.025 M LiBr in DMF mobile phase at 60 °C. The differential refractive index (dRI) of each compound was monitored using a Wyatt Optilab T-rEX detector.

**Acidic Hydrolysis of Bottlebrush Polymers and Star Polymers**

30 µL of the polymer mixture were concentrated under vacuum to remove EVE and taken up in 100 µL of dioxane. 10 µL of 2M HCl were added and the mixture was stirred for 30 min. Excess sodium sulfate was added and the reaction mixture was allowed to sit for 5 min. Finally, the mixture was suspended in DCM, filtered with a 0.2 µm Nylon filter, concentrated under vacuum, and analyzed by GPC.

**Degradation of Bottlebrush Polymers under Buffered Conditions**

200 µL of each crude reaction mixture, prepared as described above, were placed in a vial containing 10 mL of the requisite buffer. The solution was incubated at 37 °C for the indicated times. The pH 5.5, 6.5, and 7.4 solutions were prepared using mixtures of 0.2 M Na₂HPO₄ and 0.1 M citric acid.

At set timepoints, 1 mL of the material was flash frozen into liquid N₂ and lyophilized; the resulting material was extracted with DCM, filtered, and concentrated under vacuum. The residue was dissolved in DMF with 0.025 M LiBr and analyzed by gel permeation chromatography. A similar approach was used for the brush-arm star polymer samples as well, where samples from the
dynamic light scattering experiments were flash frozen, lyophilized, and extracted before characterization by DMF GPC.

To assess the size of the degradation fragments, **PEG-MM** was polymerized at target DP values of 2, 3, or 4 and analyzed by DMF GPC.

**Characterization of Bottlebrush Polymer Degradation Fragments**

To further confirm the nature of the degradation fragments, we independently synthesized short polymer fragments by combining **PEG-MM** and G3 catalyst in a 2:1 molar ratio. This reaction generated an oligomer mixture with an average DP of 2 that contained macromonomer as well as oligomers of DP = 3 and larger.

**Quantification of Bottlebrush Polymer Degradation**

The area under the curve of each GPC trace after a specific retention time (13.75 minutes, $A$) was determined for all samples. This value was divided by the total integrated value under each peak ($A_o$) to yield a ratio $R$. The cutoff retention time was chosen to minimize $R$ in the intact bottlebrush polymer while maximizing this value for the fully-degraded sample.

To convert the $R$ value into a percent degradation, we normalized this value using the $A/A_o$ derived from the fully-intact (0 min, $R_{initial}$) and fully degraded (after HCl treatment, $R_{final}$) bottlebrush polymers, which were set at 0 and 100% degradation, respectively on a linear scale. In this case, degradation refers to the fraction of silyl ether linkages that are degraded within the polymer. This normalization was performed separately for each type of bottlebrush polymer. $R$ was then calculated from each timepoint using the corresponding GPC traces and converted to a percent degradation using our normalization procedure.

Specifically, percentage degradation for each timepoint is calculated by:

$$\text{Percent degradation} = \frac{(R-R_{initial})}{(R_{final}-R_{initial})} \times 100\%$$

An example image of the fully intact, fully degraded, and an intermediate timepoint with the cutoff retention time are shown below.
Degradation of Non-PEG Containing Bottlebrush Polymers

For the PS and PLA-based bottlebrush polymers, 15 µL of the crude ROMP reaction mixture were diluted with 15 µL of dioxane. Next, 7 µL of 1M tetrabutylammonium fluoride in THF were added. The solution was incubated for 15 min before being diluted with 1 mL of chloroform, filtered through a 0.2 µm Teflon filter (A ChemTek), and analyzed by chloroform GPC.

GPC analysis was performed in a Tosoh EcoSEC HLC-8320 with dual TSKgel SuperH3000 columns and a chloroform mobile phase.

Characterization of Brush-Arm Star Polymer Degradation

Solutions of brush-arm star polymers in dioxane were diluted to 1.0 mg/mL in the requisite buffer. Dynamic light scattering (DLS) measurements were performed using a Wyatt Technology Mobius DLS instrument. The solutions were filtered through a 0.2 µm Nylon filter (A ChemTek) into disposable polystyrene cuvettes, which were pre-cleaned with compressed air. The solutions were immediately capped after addition of the solution to the cuvette. Measurements were made in sets of 10 acquisitions; the average hydrodynamic diameters were calculated using the DLS-correlation function via a regularization fitting method (Dynamics 7.4.0.72 software package from Wyatt). The cuvettes were sealed with parafilm and stored at room temperature between measurements.

For GPC analysis, a sample was flash frozen and lyophilized. Afterwards the polymer was extracted with DCM, filtered with a 0.2 µm Nylon filter, concentrated under gentle vacuum, and analyzed by GPC.

Degradation of Block Copolymers

To 30 µL of the polymer mixture were added 10 µL of 2 M HCl. The mixture was stirred for 30 min. Excess sodium sulfate was added and the reaction mixtures allowed to sit for five minutes. Finally, the mixture was suspended in DCM, filtered with a 0.2 µm nylon filter, concentrated, and analyzed by GPC.

Linker Degradation of Bottlebrush Copolymers

The concentrated polymer reaction mixture was redissolved in 200 µL of dioxane. 20 µL of this solution were diluted with 80 µL of dioxane. 10 µL of 2 M HCl were added and the solution was allowed to sit for 30 min. Excess sodium sulfate was added and, after 5 min, the mixture was extracted with DCM, filtered with a 0.2 µm nylon filter, concentrated, and analyzed by GPC to assess degradation.

To study the potential impact of polymer degradation toward generating responsive materials, 20 µL of sample were diluted in 1 mL of dioxane. A solution in 10 mL of MeOH was prepared. 1 mL of each solution was transferred to a vial and 10 µL of 2 M HCl were added and the solutions were monitored over time visually. Pictures were acquired with a smartphone camera.
Reaction Optimization and Characterization

Silyl Ether Screening for Bottlebrush Copolymer Synthesis

To assess the role of silyl ether ring size on bottlebrush copolymer synthesis, solutions of 0.5 M silyl ether (7-iPrSi, iPrSi, and 9-iPrSi) were added to PEG-MM before polymerization at a 1:1 molar ratio. Bottlebrush polymers with a target DP of 30 for each monomer were synthesized and degraded in an analogous manner to that described above.

To assess how excess of silyl ether impacts polymerization efficiency, different volumes of iPrSi stock were added in order to generate mixtures of 1:3 or 1:5 molar ratios of PEG-MM/iPrSi before the addition of G3 catalyst.

Probing Backbone Accessibility to Chain Transfer Reactions

To assess chain-transfer upon prolonged reaction times, the reaction mixtures were quenched at the designated timepoints (beyond 30 min) by removal from the glovebox and the addition of a drop of EVE. Copolymer characterization by GPC before and after degradation were performed following the protocol described above.

To further study the susceptibility of the polymer backbone to chain-transfer, samples of polymer were removed from a glovebox and quenched with EVE. The solutions were then concentrated under vacuum to remove residual EVE. The mixtures were then taken up in 100 µL of dioxane.

To the mixtures were added 10 µL of cis-octene and 10 µL of 0.02 M Grubbs’ 2nd generation catalyst. After incubation for 1 h, the solutions were quenched with EVE and characterized by GPC.

Computational Chemistry

Calculations were performed using a combination of Avogadro 1.2.0 and ORCA 4.1. Structures were optimized by first using molecular mechanics (UFF) and a systematic rotor search followed by further geometry optimization with B3LYP/6-31G(d). Strain energies were calculated by comparing the heats of formation of the cyclic monomer and ethylene and the corresponding ring-opened product derived from a homodesmotic ring-opening metathesis reaction.
In Vitro Experiments

Dye-Labeled Bottlebrush Polymer Synthesis for In Vitro Experiments

Bottlebrush polymer was synthesized using 400 mg of PEG-MM and 4 mg of Cy3-MM in 1600 µL of dioxane. 425 µL of the resulting solution were added into each of four vials, followed by 60 µL of 0.5 M silyl ether in dioxane or 60 µL of dioxane. Finally, 50 µL of 0.02 M G3 in dioxane were added to yield a target DP of 30. The mixture was stirred for 30 min and quenched with a drop of EVE. Polymers were concentrated under vacuum at room temperature, then diluted to a concentration of 10 mg/mL immediately before uptake and toxicity experiments.

In Vitro Polymer Uptake Assayed by Flow Cytometry

OVCAR8 cells were plated at 100,000 cells/well overnight in 100 µL DMEM + 10% FBS in a 96 well plate. Next, 7.5 µL of 10 mg/mL of bottlebrush were added and the cells were incubated for 1, 3, or 12 h. The cells were washed with 2 x 150 µL PBS and suspended by treatment with 100 µL of trypsin at 37 °C. After 15 min, the cells were transferred into a Thermo-Fisher 97-well cell trainer and transferred into a V-bottom plate by centrifugation at 1300 RPM for 5 min. The supernatant was removed and the cells were resuspended in 150 µL of PBS and analyzed by flow cytometry.

Jurkat cells were grown to confluence and then diluted to 500,000 cells/mL in RPMI with 10% FBS. 150 µL of cells were added to each of 6 x 9 wells in a 150 µL V-bottom plate. To the solutions were added 7.5 µL of bottlebrush polymer solution in PBS or nothing to cells. Incubation was performed for 1, 4, or 12 h at 37 °C. The cells were then washed by centrifugation at 1300 rpm for 5 min, removal of the supernatant, and resuspension in 150 µL PBS. This wash step was repeated once more before analysis of the cells by flow cytometry. An analogous approach was taken with OVCAR8 cells. Flow cytometry experiments were performed at the Flow Cytometry Core as a part of the Koch institute for Integrative Cancer Research at MIT on a BD LSR II and analyzed with FlowJo 10.4.2. Statistical significance was assessed through two-sample Student’s t tests.

In Vitro Polymer Toxicity Assayed by Flow Cytometry

OVCAR8 cells were plated at 10,000 cells per well in 100 µL of DMEM in a 96-well tissue-culture treated polystyrene plate (VWR) and allowed to adhere overnight. The media was then replaced with media containing solutions of bottlebrush polymer at a concentration of 1 mg/mL and the cells were incubated for another 36 h. The cells were then washed with PBS and assayed for viability using DAPI staining for 30 min, washing with PBS, and characterization via flow cytometry. As a positive control, cells were treated with 100 µL of EtOH immediately before washing and DAPI staining. An analogous approach was used to assay Jurkat cell viability. An example of the gating used is shown below:
In Vitro Polymer Uptake Assayed by Confocal Microscopy

OVCAR8 cells were plated at 100,000 cells/well overnight in 200 µL DMEM + 10% FBS in a Nunc Lab-Tek Chambered Coverglass. Next, 7.5 µL of 10 mg/mL of bottlebrush polymer were added and the cells were incubated for 12 h. Finally, the cells were stained with LysoTracker Far Red at 50 nM for 30 min and Hoescht 33342 at 5 µg/mL for 10 min before washing with PBS. Cells were imaged on a Nikon A1R confocal microscope at the Koch Institute Microscopy Core.

In Vivo Experiments

Animal Usage

All experiments involving animals were reviewed and approved by the MIT Committee for Animal Care (CAC). BALB/c mice (female, 8-12 weeks old, Taconic) were used for in vivo toxicity, pharmacokinetic, and biodistribution studies (n = 3-4). All animals received an alfalfa-free diet (TestDiet) for two weeks prior to the start of these studies to minimize residual auto-fluorescence.

Dye-Labeled Bottlebrush Synthesis for In Vivo Experiments

Bottlebrush polymer was synthesized in an analogous fashion to polymers used in in vitro experiments, except Cy5.5-MM was used in place of Cy3-MM. Polymers were dialyzed for five h before lyophilization and storage at 4 °C. Lyophilized polymer was diluted in 5% glucose solution to a final concentration of 25 mg/mL and filtered through a sterile 0.2 µm nylon filter immediately before use.

Pharmacokinetic Studies

Bottlebrush polymers were dissolved in 5% glucose solution and filtered through a sterile 0.2 µm nylon filter. Solutions were prepared fresh before each set of injections. 200 µL of solution were administered via tail vein injection at different timepoints (5 mg per animal). Animals were euthanized and blood was collected via cardiac puncture into a heparin-coated tube (Sarstedt). Blood samples were stored at 4 °C immediately following collection. 100 µL of sample from each mouse was added to a 96-well plate and characterized by fluorescence imaging (IVIS, Cy 5.5, λex/λem = 675/720 nm, Xenogen). Statistical significance was assessed through two-sample Student’s t tests.
Biodistribution Studies

For biodistribution studies, organs were harvested from each mouse following blood collection and imaged by fluorescence imaging for whole organ images (IVIS, Cy 5.5, $\lambda_{ex}/\lambda_{em} = 675/720$ nm, Xenogen).

To quantify the amount of polymer in each sample, the samples were then homogenized by diluting each sample 5x w/v with PBS buffer (VWR) in a 5 mL Eppendorf tube. This dilution step was chosen to standardize the amount of tissue present in each homogenate. Two 3.5 mm UFO stainless steel beads were added to each tube and the sample was homogenized twice for two minutes at “Speed 16” on a Next Advance Bullet Blender Gold homogenizer at 4 °C. The homogenates were then transferred into a smaller Eppendorf tube and stored at 4 °C until analysis. 100 µL of each homogenate solution was added to each well of a black 96-well polystyrene plate and the mixtures were characterized by fluorescence imaging (IVIS, Cy 5.5, $\lambda_{ex}/\lambda_{em} = 675/720$ nm, Xenogen). A vehicle only control was used for background correction and fluorescence was normalized by the weight of tissue being measured. Statistical significance was assessed through two-sample Student’s $t$ tests.

Blood Chemistry Analysis

Serum was isolated from freshly acquired blood using a VACUETTE serum clot activator tube (Greiner) and a full blood chemistry panel analysis performed by the Charles River Laboratories. Blood samples were collected 10 w after treatment by cardiac puncture before harvesting the organs of the mice for biodistribution studies.

Histology and Pathology

Organs were fixed in 30% formalin overnight and stored in ethanol. Samples were processed for histology at the Hope Tang Histology Facility at the Koch institute for Integrative Cancer Research at MIT. Organ samples were collected from mice at the 6 w timepoint before tissue homogenization for biodistribution studies.

Statistics and Reproducibility

For Figure 5b: For the 3 w timepoint, the $p$-value between the PEG$_{30}$ and iPrSi$_{30}$PEG$_{30}$ samples is 0.0024 and the $p$-value between the PEG$_{30}$ and PhSi$_{30}$PEG$_{30}$ samples is 0.0036. For the 6 w timepoint, the $p$-value between the PEG$_{30}$ and iPrSi$_{30}$PEG$_{30}$ samples is 0.0024 and the $p$-value between the PEG$_{30}$ and PhSi$_{30}$PEG$_{30}$ samples is 0.0052.

For Figure 5c: For the 3 w Liver timepoint, the $p$-value between the PEG$_{30}$ and iPrSi$_{30}$PEG$_{30}$ samples is 0.0021 and the $p$-value between the PEG$_{30}$ and PhSi$_{30}$PEG$_{30}$ samples is 0.0052. For the 10 w Liver timepoint, the $p$-value between the PEG$_{30}$ and iPrSi$_{30}$PEG$_{30}$ samples is 0.0118 and the $p$-value between the PEG$_{30}$ and PhSi$_{30}$PEG$_{30}$ samples is 0.0424. For the 72 h Spleen timepoint, the $p$-value between the PEG$_{30}$ and iPrSi$_{30}$PEG$_{30}$ samples is 0.0009 and the $p$-value between the PEG$_{30}$ and PhSi$_{30}$PEG$_{30}$ samples is 0.0022. For the 3 w Spleen timepoint, the $p$-value between the PEG$_{30}$ and iPrSi$_{30}$PEG$_{30}$ samples is 0.0001 and the $p$-value between the PEG$_{30}$ and PhSi$_{30}$PEG$_{30}$ samples is <0.0001. For the 10 w Spleen timepoint, the $p$-value between the PEG$_{30}$ and iPrSi$_{30}$PEG$_{30}$ samples is <0.0001 and the $p$-value between the PEG$_{30}$ and PhSi$_{30}$PEG$_{30}$ samples is <0.0001.
Supplementary Figure 23: For OVCAR cells treated with PhSi$_{30}$PEG$_{30}$, the amount of DAPI positive cells were significantly higher than cells treated with other polymer samples. The $p$-value for the PhSi$_{30}$PEG$_{30}$ and PEG$_{30}$, MeSi$_{30}$PEG$_{30}$, EtSi$_{30}$PEG$_{30}$, and iPrSi$_{30}$PEG$_{30}$ samples are 0.0020, 0.0028, 0.0099, and 0.0006, respectively.

Supplementary Figure 24: The $p$-value between the iPrSi$_{30}$PEG$_{30}$ or PhSi$_{30}$PEG$_{30}$ and the PEG$_{30}$, MeSi$_{30}$PEG$_{30}$, and EtSi$_{30}$PEG$_{30}$ samples is <0.0001 for all pairs.
Scheme 1: Synthesis of silyl ether monomers MeSi, EtSi, iPrSi, and PhSi
| Sample Name       | Target DP                                      | Theoretical $M_n$ | Observed $M_n$ | $M_w/M_n$ |
|-------------------|-----------------------------------------------|------------------|----------------|----------|
| PEG$_{100}$       | 100 PEG-MM                                    | $3.23 \times 10^5$ | $3.69 \times 10^5$ | 1.05     |
| iPrSi$_{100}$PEG$_{100}$ | 100 PEG-MM/100 iPrSi                        | $3.41 \times 10^5$ | $3.25 \times 10^5$ | 1.14     |
| NB$_{150}$        | 50 NB1                                        | $7.60 \times 10^3$ | $8.28 \times 10^5$ | 1.02     |
| iPrSi$_{50}$NB$_{150}$ | 50 NB1/50 iPrSi                              | $1.85 \times 10^4$ | $8.93 \times 10^5$ | 1.14     |
| NB$_{250}$        | 50 NB2                                        | $8.30 \times 10^3$ | $9.53 \times 10^3$ | 1.02     |
| iPrSi$_{50}$NB$_{250}$ | 50 NB2/50 iPrSi                             | $1.90 \times 10^4$ | $9.97 \times 10^3$ | 1.13     |
| NB$_{350}$        | 50 NB3                                        | $8.85 \times 10^3$ | $1.07 \times 10^4$ | 1.01     |
| iPrSi$_{50}$NB$_{350}$ | 50 NB3/50 iPrSi                             | $1.96 \times 10^4$ | $1.12 \times 10^4$ | 1.11     |
| NB$_{450}$        | 50 NB4                                        | $8.85 \times 10^3$ | $7.64 \times 10^3$ | 1.02     |
| iPrSi$_{50}$NB$_{450}$ | 50 NB4/50 iPrSi                             | $1.96 \times 10^4$ | $1.08 \times 10^4$ | 1.09     |
| PEG$_{30}$        | 30 PEG-MM                                     | $9.69 \times 10^4$ | $1.02 \times 10^5$ | 1.02     |
| MeSi$_{30}$PEG$_{30}$ | 30 PEG-MM/30 MeSi                          | $1.02 \times 10^5$ | $1.22 \times 10^5$ | 1.24     |
| EtSi$_{30}$PEG$_{30}$ | 30 PEG-MM/30 EtSi                          | $1.03 \times 10^5$ | $1.30 \times 10^5$ | 1.14     |
| PhSi$_{30}$PEG$_{30}$ | 30 PEG-MM/30 PhSi                          | $1.05 \times 10^5$ | $1.28 \times 10^5$ | 1.17     |
| iPrSi$_{30}$PEG$_{30}$ | 30 PEG-MM/30 iPrSi                        | $1.03 \times 10^5$ | $1.27 \times 10^5$ | 1.15     |
| BASP              | 7 PEG-MM, 20 AcXL                              | --               | $7.87 \times 10^5$ | 1.30     |
| MeSi-BASP        | 7 PEG-MM, 7 MeSi, 20 AcXL                      | --               | $1.28 \times 10^6$ | 1.40     |
| EtSi-BASP        | 7 PEG-MM, 7 EtSi, 20 AcXL                      | --               | $9.58 \times 10^5$ | 1.31     |
| iPrSi-BASP       | 7 PEG-MM, 7 PhSi, 20 AcXL                      | --               | $9.54 \times 10^5$ | 1.32     |

Table 1: Reference table with polymer samples presented in the main text and their target DPs.
Figure 1: $^1$H NMR spectra of crude reaction mixtures of the iPrSi/PEG-MM copolymerizations (target DP = 30/30 or 100/100) as well as spectra for polymer obtained after GPC purification. For comparison, the spectra for a PEG-MM homopolymer, iPrSi, and PEG-MM are provided.
Figure 2: GPC trace of crude polymerization reaction generated from a 2:1 mixture of PEG-MM and G3 catalyst, resulting in peaks corresponding to trimers, dimers, and unimers/macromonomer (starred).
Figure 3: GPC traces of PEG-MM/iPrSi bottlebrush copolymers of different lengths (10, 30, or 100 target PEG-MM units, 1:1 PEG-MM/iPrSi) before and after degradation. The resulting fragments after acidic hydrolysis are independent of polymer length. The star corresponds to unreacted monomer in the GPC traces prior to degradation.
Figure 4: GPC traces of PS or PLA bottlebrush polymers (target DP = 30 macromonomer units) prepared in the presence of iPrSi and treated with TBAF.
Figure 5: GPC traces of copolymers (target DP = 10 macromonomer units) using a branched telmisartan-conjugated macromonomer and their subsequent degradation under acidic conditions. The star corresponds to unreacted monomer in the solid GPC traces (prior to degradation).
Figure 6: GPC traces for PEG-MM-derived bottlebrush polymers (target DP = 30 PEG-MM units) copolymerized with different equivalents of iPrSi (1-3 equivalents relative to PEG-MM), demonstrating that excess iPrSi negatively impacts the resulting molar mass dispersity. The star indicates unreacted macromonomer.
Figure 7: Comparison of copolymerization reactions performed with seven- versus eight- versus nine-membered silyl ether monomers for synthesizing bottlebrush polymers (target DP = 30 PEG-MM units). The star indicates unreacted macromonomer in the solid GPC traces.
Figure 8: (A) DFT calculations of reaction enthalpy of hypothetical ring opening metathesis reactions for norbornene and silyl ether derivatives of different ring sizes. (B) 3D models of iPrSi, showing the potential role that silyl ether substituents may play in influencing the accessibility of backbone olefins.
Figure 9: GPC traces of linear and bottlebrush copolymers prepared by combining (A) NB4 or (B) PEG-MM with iPrSi or cyclooctene (COE) at a 1:1 molar ratio (target DP = 50/50 for the linear polymer and DP = 30/30 for the bottlebrush polymer). Dramatically higher dispersity and the formation of smaller fragments is observed in both cases, indicative of substantial chain transfer reactions. The star corresponds to unreacted monomer in the solid GPC traces.
Figure 10: $^1$H NMR spectra of $iPrSi$ and crude polymerization mixtures between $iPrSi$ and norbornene monomers (target DP = 50/50). Red arrows indicate peaks in the olefin region that may be indicative of copolymer formation (connection between an $iPrSi$ monomer and an $NBx$ monomer).
Figure 11: Mayo-Lewis plot of the copolymerization of NB4 and iPrSi, indicating statistical copolymerization of the two monomers (n = 3 experiments, mean/S.D.).
Figure 12: $^1$H NMR spectra of crude polymerization mixtures between silyl ether monomers and PEG-MM (target DP = 30/30), highlighting additional peaks unique to the family of silyl ether monomers within the copolymer.
Figure 13: NMR spectra of crude polymerization reaction mixtures between all reported silyl ether monomers and PEG-MM (target DP = 30/30), showing high conversion of monomer under these conditions. Spectra from PEG-MM and each unreacted silyl ether monomers are provided for comparison.
Figure 13 (continued).
**Figure 14:** GPC traces for silyl ether containing bottlebrush copolymers (target DP = 30/30) after storage at different pH values (5.5, 6.5, 7.4) over time at 37 °C.
Figure 15: GPC traces of bottlebrush copolymers (target DP = 30/30) after storage for two months in solution at RT.
Figure 16: GPC traces of NB3/silyl ether copolymers (target DP = 50/50) before and after subjecting to forced chain-transfer reactions using chain-transfer agent cis-4-octene (CT) and additional Grubbs II catalyst, showing minimal conversion into smaller fragments. Overall, the amount of conversion appears to rank from Ph < iPr < Et < Me, which is consistent with the steric bulk of these substituents.
Figure 17: GPC traces of linear NB4 polymer containing degradable and non-degradable blocks, before and after acidic hydrolysis. After acidic hydrolysis, a primary peak with similar molecular weight to that of an NB4 homopolymer (target DP = 50), along with additional smaller fragments, was observed. The slightly earlier retention time of the high molecular weight peak after degradation suggests the incorporation of additional NB4 units to the end of the initial polynorbornene block, which are not cleaved by acidic hydrolysis. The $M_n$ of the copolymer is $2.23 \times 10^4$ ($M_w/M_n = 1.04$), which is then degraded to two sets of peaks: one with an $M_n$ of $8.50 \times 10^3$ ($M_w/M_n = 1.02$) corresponding to the NB4 homopolymer and a broader set of fragments with $M_n$ of $7.07 \times 10^2$ ($M_w/M_n = 1.33$)
Figure 18: GPC traces of either (A) PEG-PS or (B) PS-PEG bottlebrush polymers (target DP = 30/30/30 PEG-MM/PS-MM/iPrSi), demonstrating that either the PS or PEG blocks can be selectively degraded depending on the location of the silyl ether monomer. Differences in refractive index traces between the two samples correspond to the different refractive indices of PS and PEG.
Figure 19: (A) GPC traces of PEG-PS copolymers before and after treatment with HCl, demonstrating degradation only with polymers containing a silyl ether-containing block. (B) Images of vials containing PS-PEG block brush polymers with or without a degradable silyl ether segment. Only the polymer containing silyl ether precipitates in the presence of acid. Solutions contained 3 mg/mL of polymer in methanol. The solution was acidified through the addition of 10 µL of 2M HCl.
Figure 20: Representative DLS histograms of BASPs over time in various pH media. Samples bearing more labile silyl ethers show more rapid aggregation. The silyl-ether containing BASPs aggregate more quickly at pH 5.0 than at pH 7.4.
Figure 21: GPC trace of BASP after aggregation as observed by DLS, showing the presence of large quantities of residual crosslinked polymer. These results support the hypothesis that the BASP cores remain crosslinked over the course of these experiments, degrading via an ‘outside-in’ mechanism as the PEG fragments are shed the hydrophobic core of the particle is revealed, which eventually leads to aggregation as observed by DLS. We note that this type of degradation mechanism stands in contrast to previous observations on the degradation of the non-silyl ether BASPs with degradable cores, which undergo ‘inside-out’ degradation upon cleavage of the crosslinker to its component bottlebrushes.
Figure 22: GPC traces of Cy3 (A) and Cy5.5 (B) labeled bottlebrush polymers used for *in vitro* and *in vivo* experiments. The star corresponds to unreacted monomer.
Figure 23: Viability of (A) Jurkat and (B) OVCAR8 cells treated with bottlebrush polymers (0.75 mg/mL, 36 h, Target DP = 30/30) as assessed via flow cytometry ($n = 3$ replicates, mean/S.D.). Excitation was performed with a 405 nm laser and emission was measured after passing through a 440/40 nm bandpass filter. V = vehicle. The $p$-values were determined by a two-sided Student’s t-test.
Figure 24: Quantification of cell uptake of bottlebrush polymers in (A) Jurkat and (B) OVCAR8 cells by flow cytometry. Cells were incubated with bottlebrush polymers (0.75 mg/mL, 36 h, Target DP = 30/30) for the indicated time points and analyzed by flow cytometry ($n = 3$ replicates, mean/S.D.). Excitation was performed with a 561 nm laser and emission was measured after passing through a 582/15 nm bandpass filter. $V$ = vehicle. The $p$-values were determined by a two-sided Student’s t-test.
**Figure 25:** Observation of cell uptake of bottlebrush polymers in OVCAR8 cells by confocal microscopy. Cells were incubated with bottlebrush polymers, then stained with LysoTracker Deep Red and Hoescht 33342. The punctate fluorescence signal from the polymer, which colocalizes with the LysoTracker stain, suggests localization of all three polymers within acidic compartments after uptake.
Figure 26: Additional data on the concentrations of bottlebrush polymers with or without silyl ether co-monomers in the brain and muscle. Minimal fluorescence signal is observed in all samples, suggesting little polymer accumulation at these sites (n = 3 mice for 72 h, 4 mice for 3, 6, and 10 w, mean/S.D.).
Figure 27: Representative image of organs harvested from mice 10 w after injection with PEG$_{30}$, iPrSi$_{30}$PEG$_{30}$, or PhSi$_{30}$PEG$_{30}$ bottlebrush polymers.
**Figure 28**: Blood chemistry results from mice treated with PEG\textsubscript{30}, iPrSi\textsubscript{30}PEG\textsubscript{30}, or PhSi\textsubscript{30}PEG\textsubscript{30} bottlebrush polymers as compared to untreated controls. Blood samples were collected 10 weeks after treatment. Note: a mild elevation in blood glucose levels was observed for all three bottlebrush samples.

| Assay | Units | Control Mouse | 10 WK PEG\textsubscript{30} Brush | 10 WK iPrSi\textsubscript{30}PEG\textsubscript{30} Brush | 10 WK PhSi\textsubscript{30}PEG\textsubscript{30} Brush |
|-------|-------|---------------|---------------------------------|---------------------------------|---------------------------------|
| CHOL  | mg/dL | 99            | 126                             | 124                             | 107                             |
| TRIG  | mg/dL | 86            | 262                             | 195                             | 194                             |
| ALT   | U/L   | 26            | 20                              | 22                              | 25                              |
| AST   | U/L   | 162           | 82                              | 108                             | 121                             |
| ALP   | U/L   | 81            | 76                              | 82                              | 69                              |
| GLU   | mg/dL | 103           | 293                             | 224                             | 290                             |
| PHOS  | mg/dL | 9.2           | 7.7                             | 8.4                             | 11.3                            |
| Ca    | mg/dL | 9.1           | 10.8                            | 10.3                            | 10.9                            |
| TP    | g/dL  | 5.1           | 5.7                             | 5.6                             | 5.5                             |
| ALB   | g/dL  | 3.0           | 3.2                             | 3.2                             | 3.2                             |
| GLOB  | g/dL  | 2.1           | 2.5                             | 2.4                             | 2.3                             |
| A/G   | -     | 1.4           | 1.3                             | 1.3                             | 1.4                             |
| BUN   | mg/dL | 14            | 15                              | 13                              | 15                              |
| CREAT | mg/dL | 0.2           | 0.1                             | 0.1                             | 0.2                             |
| TBIL  | mg/dL | 0.30          | 0.33                            | 0.35                            | 0.32                            |
| Na    | mEq/L | 151           | --                              | 150                             | 150                             |
| K     | mEq/L | 9.4           | --                              | 9.0                             | 10.0                            |
| Cl    | mEq/L | 113           | --                              | 112                             | 113                             |
| Na/K  | -     | 16.06         | --                              | 16.67                           | 15.00                           |
Figure 29: Representative images of formalin fixed tissue slices from the liver, kidney, and spleens of mice treated with PEG$_{30}$, iPrSi$_{30}$PEG$_{30}$, or PhSi$_{30}$PEG$_{30}$ bottlebrush polymers as compared to untreated controls.
Figure 30: MALDI-TOF spectra for Cy3-MM.
Figure 31: $^1$H (top) and $^{13}$C (bottom) NMR spectra for S3.
Figure 32: $^1$H (top) and $^{13}$C (bottom) NMR spectra for S4.
Figure 33: $^1$H (top) and $^{13}$C (bottom) NMR spectra for MeSi.
**Figure 34:** $^1$H (top) and $^{13}$C (bottom) NMR spectra for EtSi.
Figure 35: $^1$H (top) and $^{13}$C (bottom) NMR spectra for iPrSi.
Figure 36: $^1$H (top) and $^{13}$C (bottom) NMR spectra for PhSi.
Figure 37: $^1$H (top) and $^{13}$C (bottom) NMR spectra for 7-iPrSi.
Figure 38: $^1$H (top) and $^{13}$C (bottom) NMR spectra of S6.
Figure 39: $^1$H (top) and $^{13}$C (bottom) NMR spectra of S7.
Figure 40: NMR spectra of iPrSi-9
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