Supplementary information for

Orthogonally functionalizable polyacetals: A versatile platform for the design of acid sensitive amphiphilic copolymers

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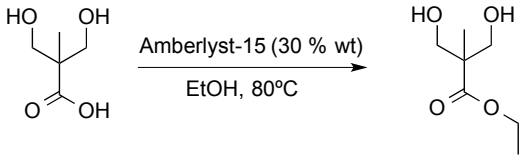
SI-1. Synthesis of ethyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (M1)

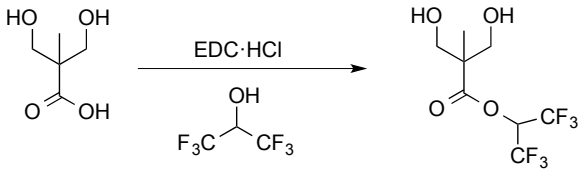


In a 100 mL round-bottom flask, bis-MPA (5 g, 37 mmol) and 1.5 g of Amberlyst-15 (30 %wt) were charged. 35 mL of absolute ethanol was added and the reaction mixture was stirred and heated at reflux temperature for 6 hours. The crude reaction was filtered from a short basic alumina column and evacuated. Finally, the crude product was purified by column chromatography using hexane: ethyl acetate (10:1) as eluent, to afford the pure compound as colorless viscous oil (90% yield).

^{1}H NMR [$CDCl_3$, TMS, δ (ppm)] (Figure S1): 4.24 (q, $COOC\underline{H}_2$-CH_3, 2H), 3.90 (dd, C-$C\underline{H}_2$-OH, 2H), 3.73 (dd, C-$C\underline{H}_2$-OH, 2H), 3.01 (s, OH, 2H), 1.30 (t, $COOCH_2$-$C\underline{H}_3$, 3H),, 1.06 (s, C-$C\underline{H}_3$, 3H).

^{13}C NMR [$CDCl_3$, TMS, δ (ppm)] (Figure S2): 176.0, 68.4, 68.1, 48.9, 17.1, 14.1.

SI-2. Synthesis of 1,1,1,3,3,3-hexafluoropropan-2-yl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (M2)



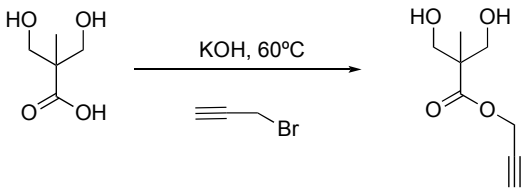
In a 25 mL round-bottom flask, bis-MPA (1.5 g, 11.8 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) (2.35 g, 12.2 mmol) were charged. The reagents were deoxygenated by six argon-vacuum cycles (20 seconds each cycle) and 18 mL (166 mmol) of HFIP was added under argon atmosphere. The reaction mixture was stirred at room temperature. Initially the reaction mixture was heterogeneous, however after 30 min it becomes a homogeneous solution that was allowed to stir for 24 hours. The crude reaction was distilled to recover the excess of non-reacted HFP (*bp*=60 ºC) and the resulting solution was diluted with DCM and filtered to discriminate the starting material. The filtered solution was washed with water, dried over $MgSO_4$ and concentrated. Finally, the crude product was purified by column chromatography using hexane: ethyl acetate (8:2) as eluent, to afford the pure compound as white powder (75% yield).

^{1}H NMR [$CDCl_3$, TMS, δ (ppm)] (Figure S4): 5.82 (sept, C<u>H</u>-$(CF_3)_2$, 1H), 3.96 (dd, C-C<u>H</u>$_2$-OH, 2H), 3.82 (dd, C-C<u>H</u>$_2$-OH, 2H), 2.62 (s, OH, 2H), 1.17 (s, C-C<u>H</u>$_3$, 3H).

^{13}C NMR [$CDCl_3$, TMS, δ (ppm)] (Figure S5): 172.4, 121.7, 118.9, 67.3, 66.5 (sept, <u>C</u>H-$(CF_3)_2$), 49.9, 16.7.

^{19}F NMR [$CDCl_3$, TMS, δ (ppm)] (Figure S6): -73.3.

SI-3. Synthesis of prop-2-yn-1-yl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (M3)

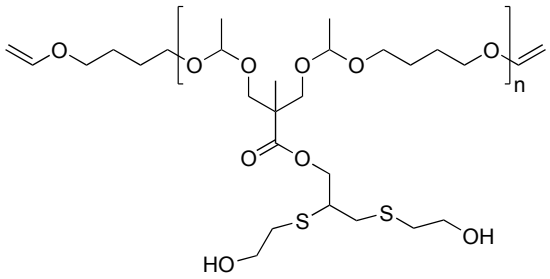


To a 100 mL round-bottom flask bis-MPA (10g, 74.6 mmol) and 4.49 g of potassium hydroxide (79.9 mmol) were charged, followed by the addition of 50 mL of dimethylformamide. The reaction mixture was stirred at 100 ºC for 1 hour, when the initial heterogeneous solution become a homogeneous solution. After that, the reaction temperature was decreased to 60 ºC and 8.2 mL of propargyl bromide was added. The reaction mixture was stirred for 24 hours at 60 ºC and the solvent was eliminated under reduced pressure. The obtained residue was dissolved in 200 mL of DCM, washed with water, brine, dried with $MgSO_4$ and concentrated. Finally, the crude product was purified by column chromatography using hexane: ethyl acetate (7:3) as eluent, to afford the pure compound as white powder (86% yield).

^{1}H NMR [$CDCl_3$, TMS, δ (ppm)] (Figure S8): 4.76 (s, COO-C$\underline{H}_2$-C≡CH, 2H), 3.91 (dd, C-C$\underline{H}_2$-OH, 2H), 3.74 (dd, C-C$\underline{H}_2$-OH, 2H), 2.89 (s, OH, 2H), 2.50 (C≡C$\underline{H}$, 1H), 1.09 (s, C-C$\underline{H}_3$, 3H).

^{13}C NMR [$CDCl_3$, TMS, δ (ppm)] (Figure S9): 175.1, 77.3, 75.3, 68.0, 52.5, 49.3, 17.0.

SI-4. Thiol-yne click modification of PA3 with mercaptoethanol. Synthesis of PA3-100, PA3-63, PA3-36, PA3-14

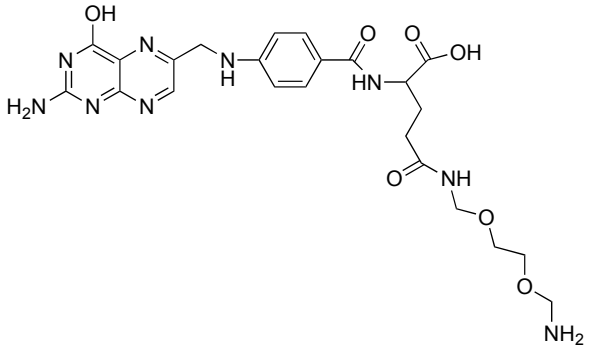


To a 5 mL round-bottom flask, **PA3** (0.5 g, 0.98 mmol) was added under argon flow and dissolved in 1 mL of anydrous tetrahydrofurane containing DMPA (0.05 mmol). After that, mercaptoethanol (0.6 mL, 5.88 mmol) was added by a syringe. The reaction mixture was irradiated using a UV nail-lamp (365 nm x4 lamps) at room temperature for 5 minutes. The crude reaction mixture was added to cold hexane and the obtained oil was treated several times with ethyl acetate. Finally, the obtained polymer was dried under vaccum until constant weight. (96%, yield, 100% modification degree **PA3-100**). By using 2.10, 1.05 and 0.65 mmol of mercaptoethanol, 63, 36 and 14% modification degrees were obtained, respectively (**PA3-63**, **PA3-36** and **PA3-14**).

PA3-100: ^{1}H NMR [$CDCl_3$, TMS, δ (ppm)] (Figure 3b): 4.69 (m, O-C<u>H</u>-O, 2H), 4.32 (dd, COOC<u>H$_2$</u>-CH, 1H), 4.16 (dd, COOC<u>H$_2$</u>-CH, 1H), 3.71 (m, S-CH$_2$-<u>CH$_2$</u>-OH, 4H) 3.66 (m, C-C<u>H$_2$</u>-O, 2H), 3.53 (m, O-C<u>H$_2$</u>-CH$_2$, 2H), 3.44 (m, C-C<u>H$_2$</u>-O, 2H), 3.38 (dd, COOC<u>H$_2$</u>-CH, 1H), 3.35 (m, O-C<u>H$_2$</u>-CH$_2$, 2H), 3.03 (m, COOCH$_2$-C<u>H</u>, 1H), 2.82 (m, CH-C<u>H$_2$</u>-S-CH$_2$-CH$_2$-OH, 2H), 2.74-2.67 (m, S-C<u>H$_2$</u>-CH$_2$-OH, 4H), 1.79 (t, S-CH$_2$-CH$_2$-O<u>H</u>, 2H), 1.55 (m, O-CH$_2$-C<u>H$_2$</u>, 4H), 1.21 (s, O-CH-C<u>H$_3$</u>, 6H), 1.17 (s, C-C<u>H$_3$</u>, 3H).

PA3-100: ^{13}C NMR [$CDCl_3$, TMS, δ (ppm)]: 171.1, 100.1, 67.9, 67.8, 66.5, 66.3, 66.0, 65.9, 65.6, 48.5, 44.9, 34.9, 27.8, 26.6, 25.5, 17.9, 14.7.

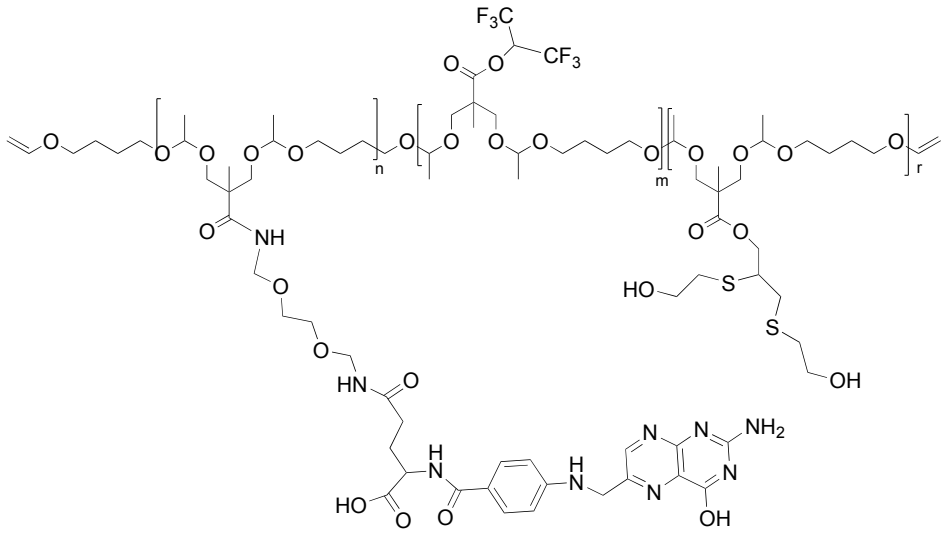
SI-5. Synthesis of 2,2-(ethylendioxy)bis(ethylamine) folate (Folate spacer, FS)



0.45 g of FA (1 mmol) was dissolved in 30 mL of dry dimethylsulfoxide and transferred to a 25 mL Schlenk tube, followed by the addition of 0.25 g of (DCC; 1.2 mmol) and 0.11 g (1 mmol) of *N*-hydroxysuccinimide (NHS) to the initial solution. The reaction mixture was stirred for 6 h at 40 ºC. The resulting activated folate-NHS was filtered and added dropwise to a solution of 1.5 g of 2,2-(ethylendioxy)bis(ethylamine) (10 mmol) and 0.1 g of triethylamine (1 mmol) in 20 mL of dimethylsulfoxide. The reaction was allowed to proceed overnight at 30 ºC. The reaction mixture was filtered and the filtrate was precipitated in cold diethyl ether (1L). Finally, the crude product was collected by filtration, washed with diethyl ether to remove the dimethylsulfoxide traces and lyophilized to afford the pure FS compound as intense orange powder (75% yield).

^{1}H NMR [$CDCl_3$, TMS, δ (ppm)]: 8.79 (s, C-C<u>H</u>=N, 1H), 7.65 (d, Ar-C<u>H</u>, 2H), 6.62 (d, Ar-C<u>H</u>, 2H), 4.51 (s, NH_2-C<u>H_2</u>-O, 2H), 4.40 (dd, NH-C<u>H</u>-COOH, 1H), 4.35 (s, NH-C<u>H_2</u>-Ar, 2H), 3.52 (dt, O-C<u>H_2</u>-C<u>H_2</u>-O, 4H), 2.10 (m, COOH-C<u>H_2</u>-C<u>H_2</u>-COONH, 4H). ESI-MS: calcd, 571.2; found, 572.7 (M+H).

SI-6. Amidation of PA2,3-3 with FS. Synthesis of PA2,3-4



To a 5 mL round-bottom vial, **PA2,3-3** (50 mg, 0.30 mmol) and triethylamine (41 μL, 0.30 mmol) were added under argon flow and dissolved with 1 mL of absolute dimethylsulfoxide. After that, a solution containing 50 mg, 0.15 mmol of FS in dimethylsulfoxide was added. The reaction was stirred at 45 ºC until complete conversion was confirmed by ^{19}F NMR (7 h). The resulting reaction mixture was concentrated and precipitated in cold hexane. Finally, the polymer was purified through dialysis against acetone to afford the pure folate conjugated polyacetal (83% of yield).

^{1}H NMR [$CDCl_3$, TMS, δ (ppm)]: 8.79 (s, C-C<u>H</u>=N, 1H), 7,65 (d, Ar-C<u>H</u>, 2H), 7.10 (d, Ar-C<u>H</u>, 2H), 5.78 (sept, C<u>H</u>-$(CF_3)_2$, 1H), 4.66 (m, O-C<u>H</u>-O, 4H), 4.51 (s, NH_2-C<u>H_2</u>-O, 2H), 4.40 (dd, NH-C<u>H</u>-COOH, 1H), 4.35 (s, NH-C<u>H_2</u>-Ar, 2H), 4.32 (dd, COOC<u>H_2</u>-CH, 1H), 4.22 (dd, COOC<u>H_2</u>-CH, 1H), 3.76 (m, S-CH_2-<u>CH_2</u>-OH, 4H) 3.67 (m, C-C<u>H_2</u>-O, 2H), 3.57 (m, O-C<u>H_2</u>-CH_2, 2H), 3.34 (m, C-C<u>H_2</u>-O, 2H), 3.31 (dd, COOC<u>H_2</u>-CH, 1H), 3.15 (m, O-C<u>H_2</u>-CH_2, 2H), 3.01 (m, $COOCH_2$-C<u>H</u>, 1H),

2.81 (m, CH-C<u>H</u>$_2$-S-CH$_2$-CH$_2$-OH, 2H), 2.74-2.67 (m, S-C<u>H</u>$_2$-CH$_2$-OH, 4H), 1.55 (m, O-CH$_2$-C<u>H</u>$_2$, 4H), 1.21 (s, O-CH-C<u>H</u>$_3$, 6H), 1.17 (s, C-C<u>H</u>$_3$, 3H).

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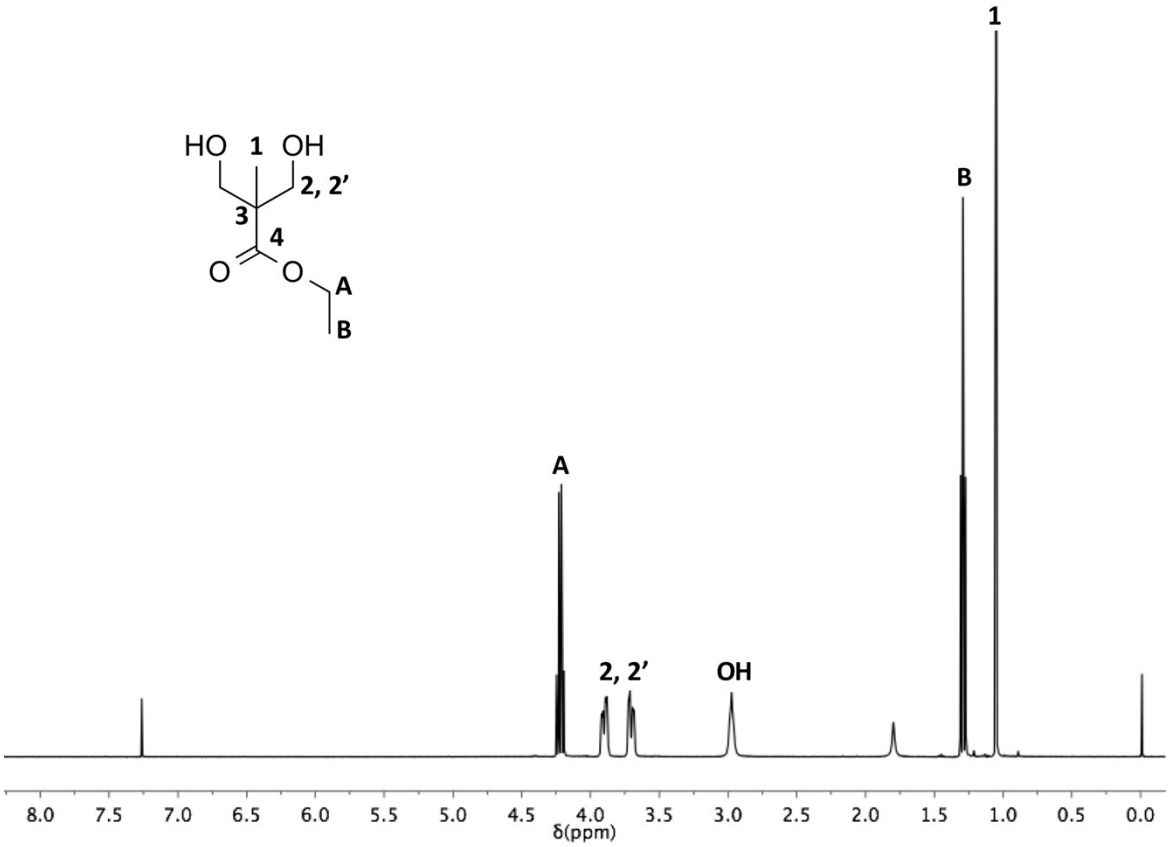


Figure S1. ^{1}H NMR spectrum of **M1** (δ (ppm), $CDCl_3$)

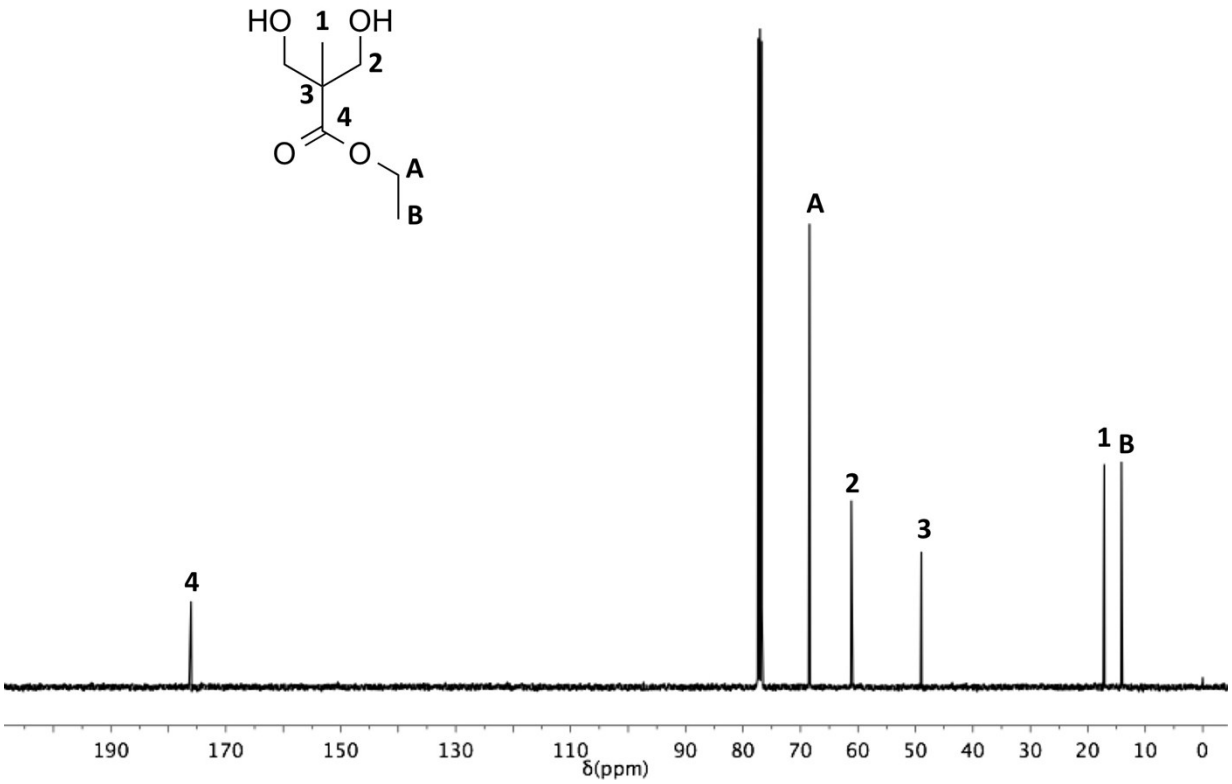


Figure S2 ^{13}C NMR spectrum of **M1** (δ (ppm), $CDCl_3$)

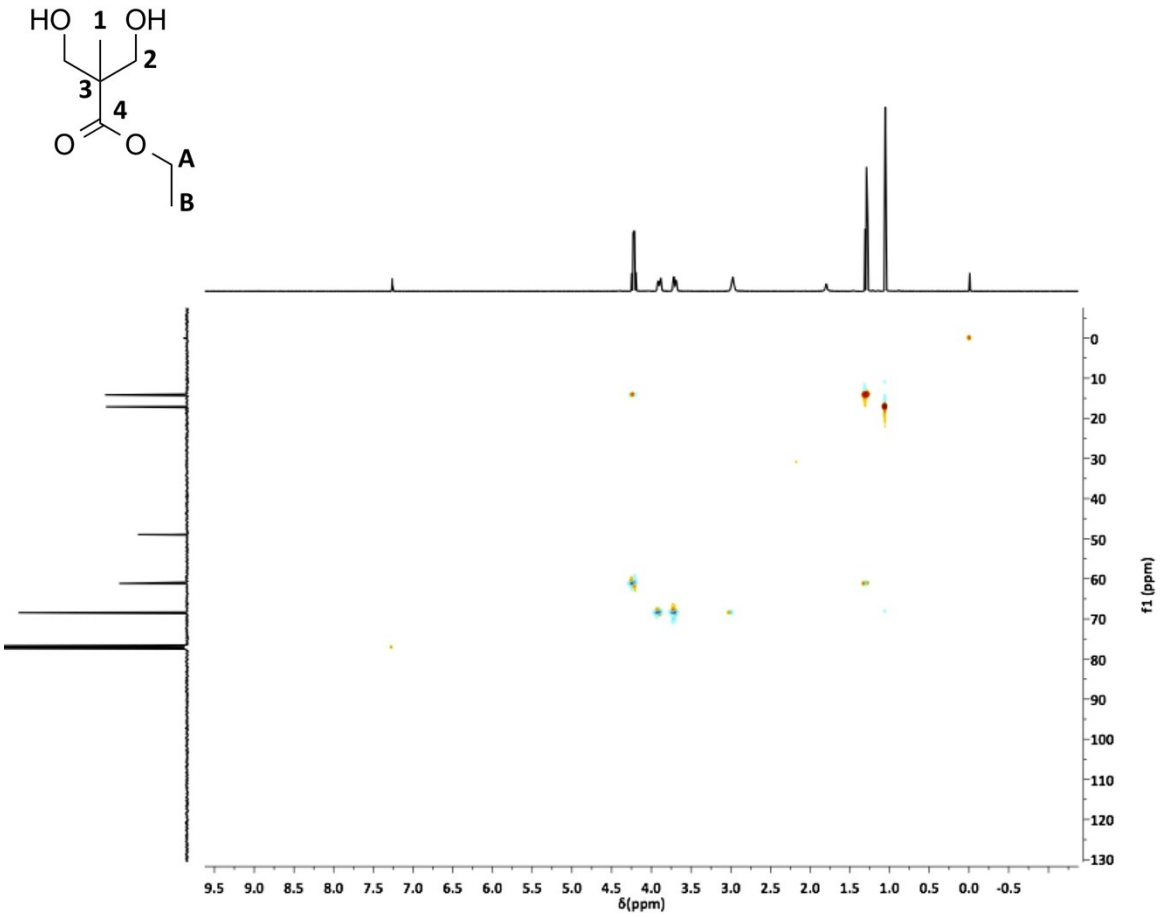


Figure S3 2D ^{1}H-^{13}C HSQC NMR spectrum of **M1** (δ (ppm), $CDCl_3$)

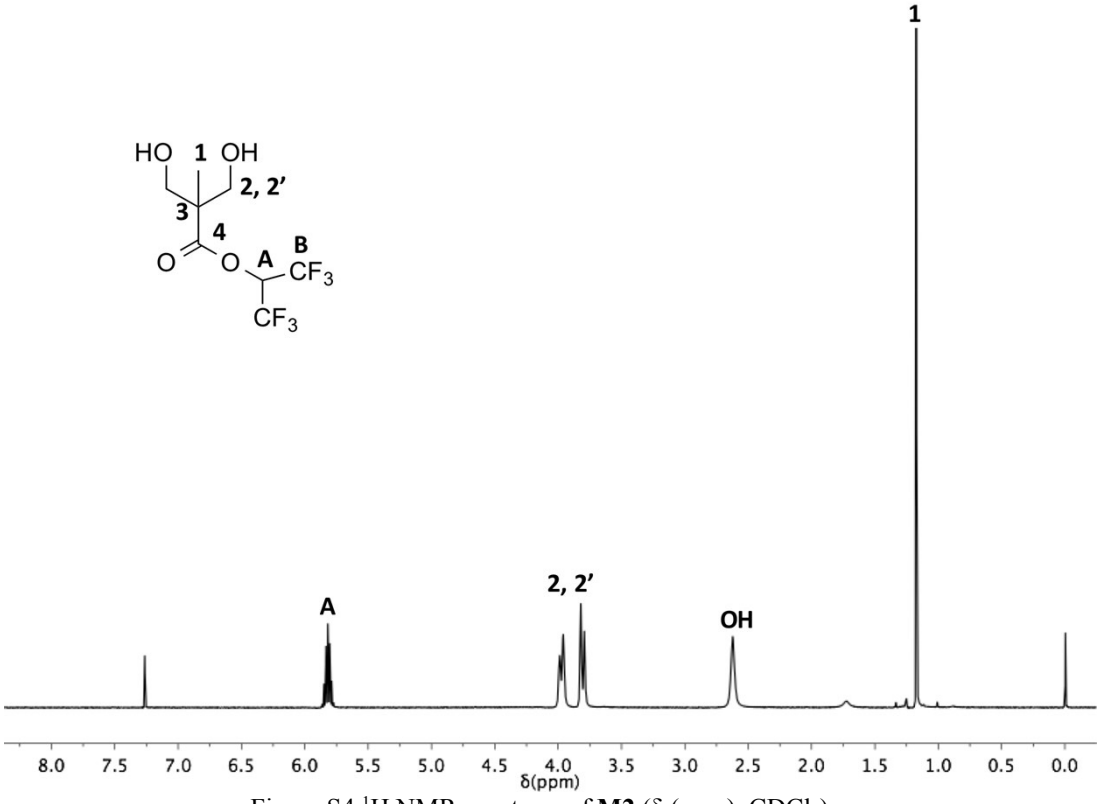


Figure S4 ^{1}H NMR spectrum of **M2** (δ (ppm), $CDCl_3$)

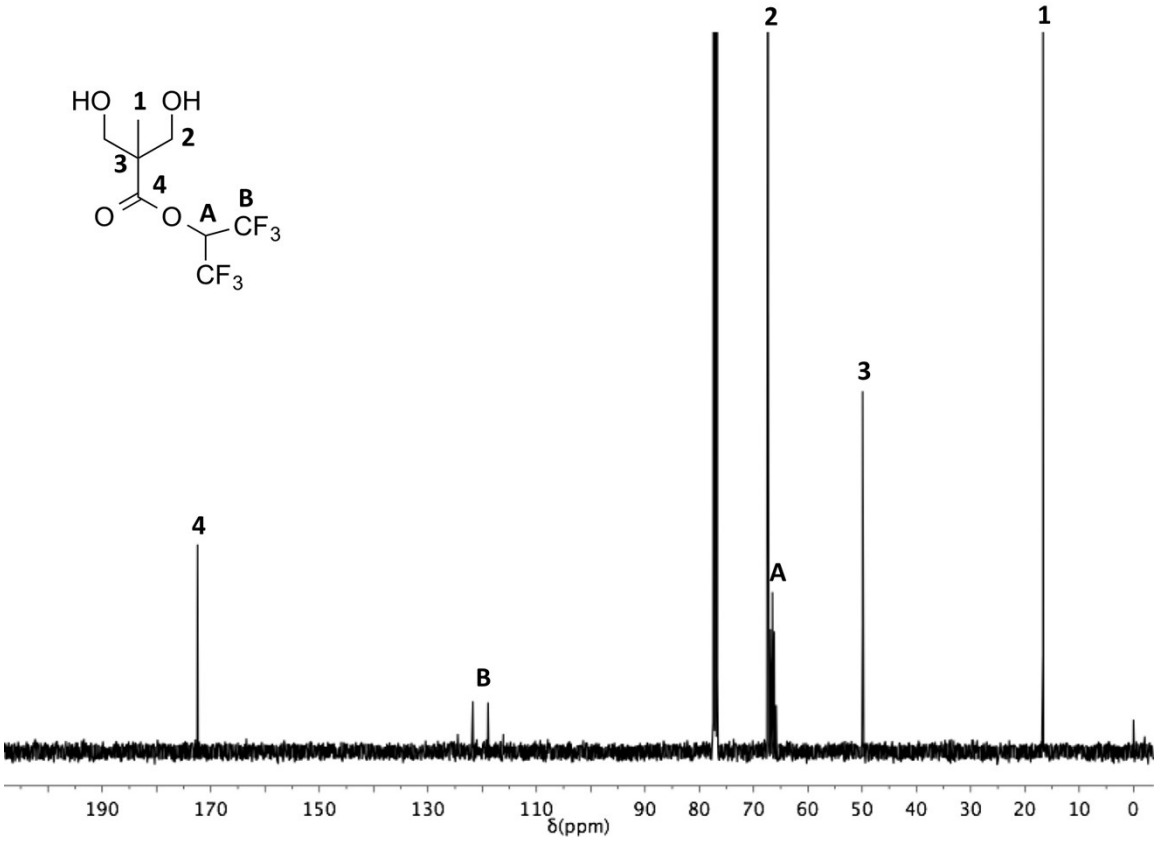


Figure S5 ^{13}C NMR spectrum of **M2** (δ (ppm), $CDCl_3$)

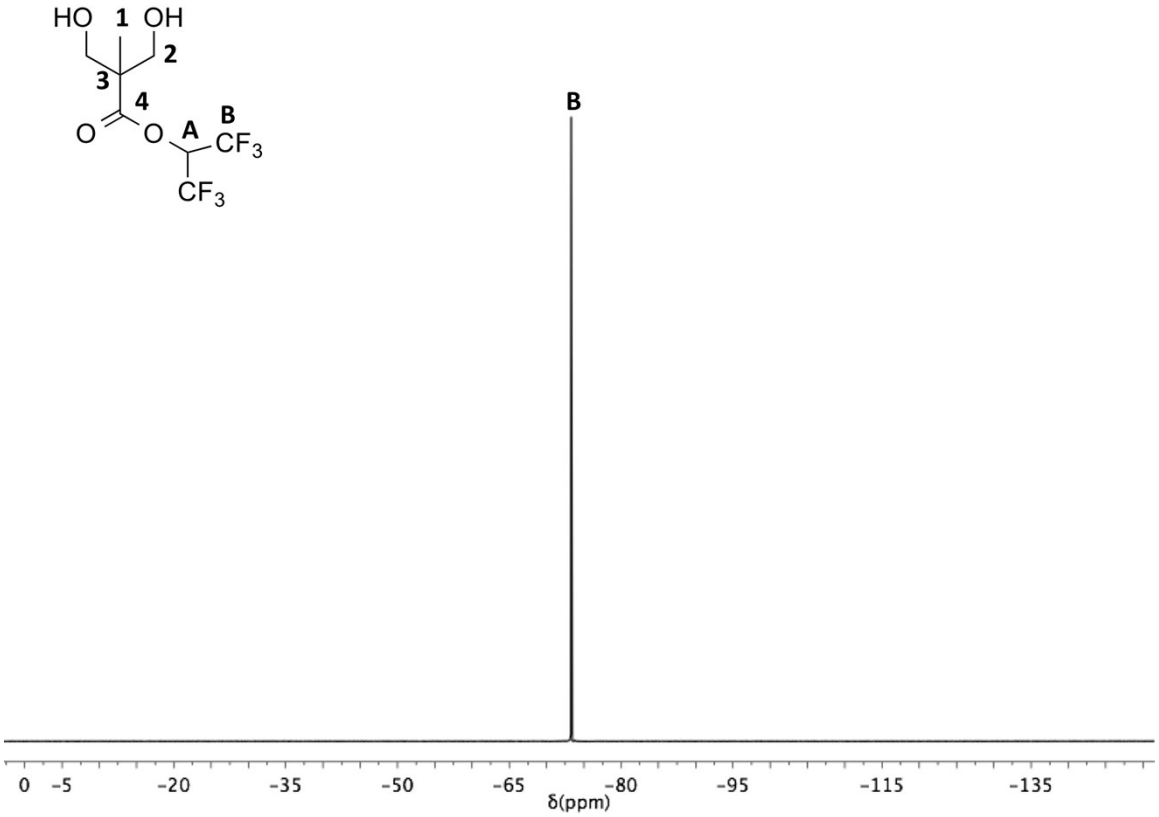


Figure S6 ^{19}F NMR spectrum of **M2** (δ (ppm), $CDCl_3$)

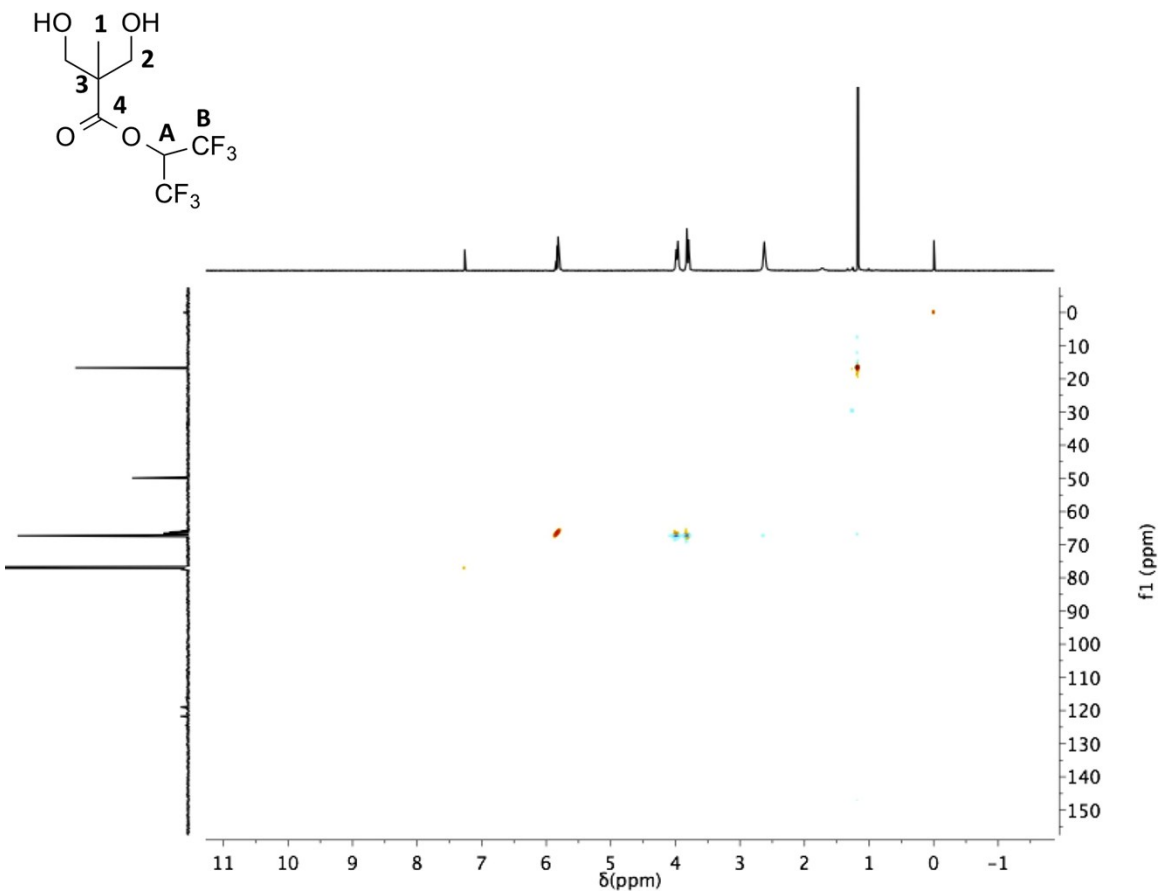


Figure S7 2D ^{1}H-^{13}C HSQC NMR spectrum of **M2** (δ (ppm), $CDCl_3$)

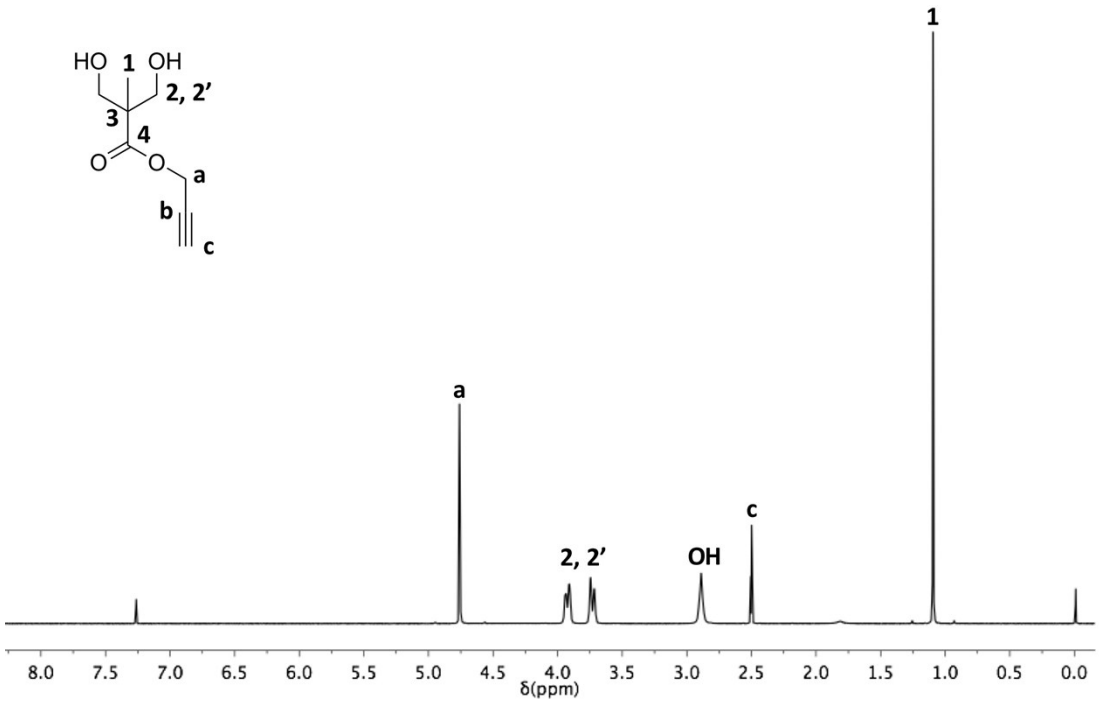


Figure S8 ^{1}H NMR spectrum of **M3** (δ (ppm), $CDCl_3$)

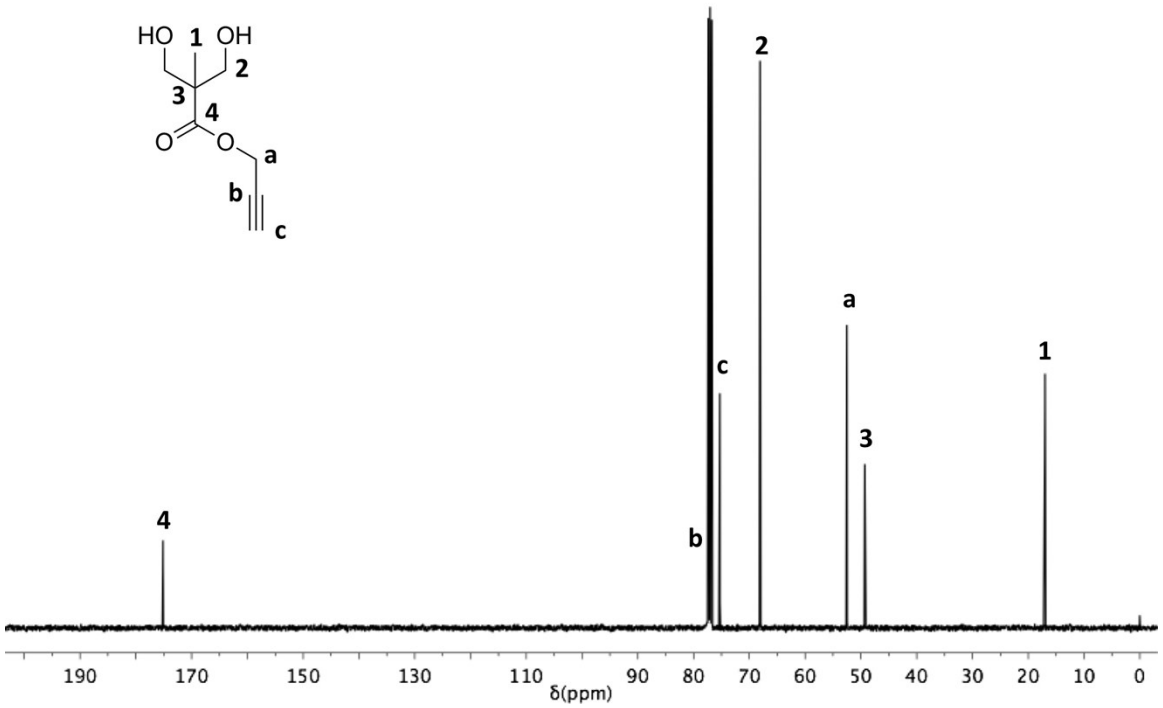


Figure S9 ^{13}C NMR spectrum of **M3** (δ (ppm), $CDCl_3$)

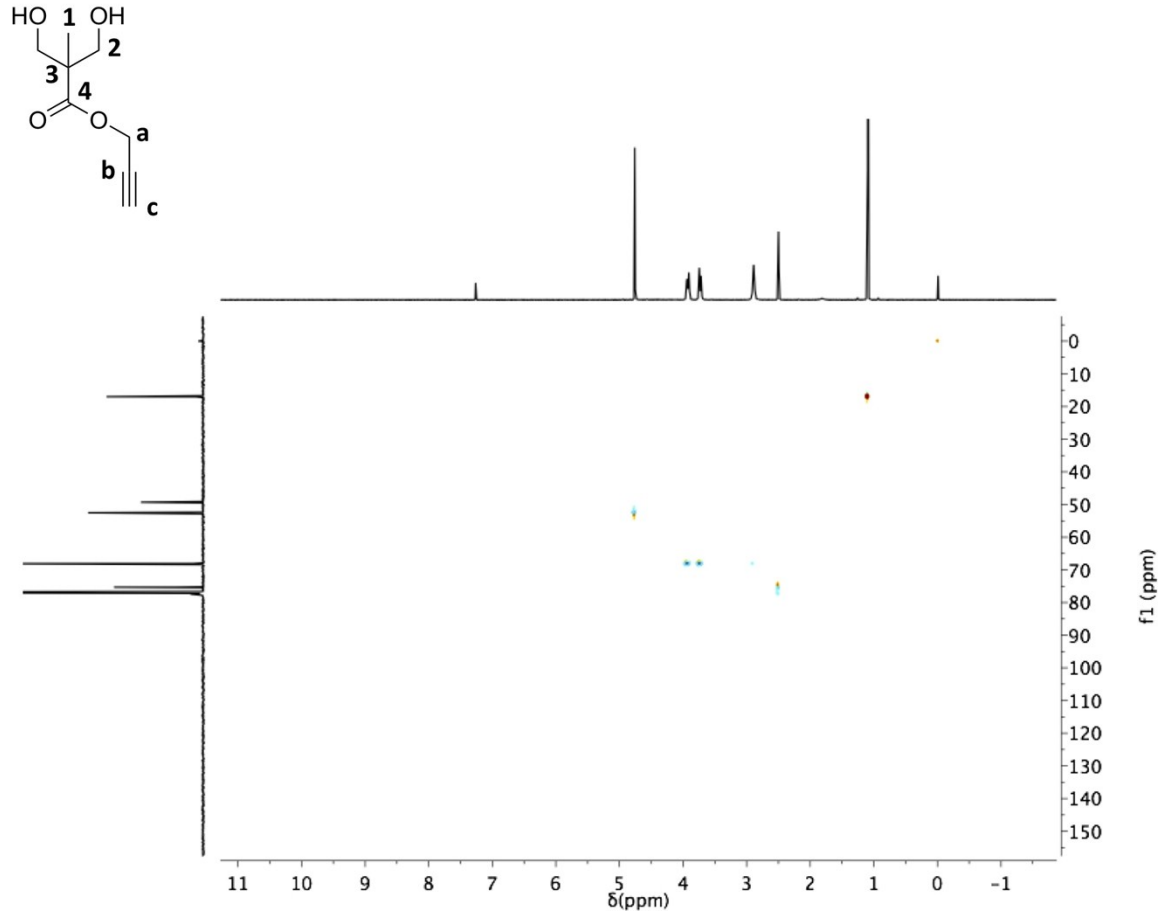


Figure S10 2D ^{1}H-^{13}C HSQC NMR spectrum of **M3** (δ (ppm), $CDCl_3$)

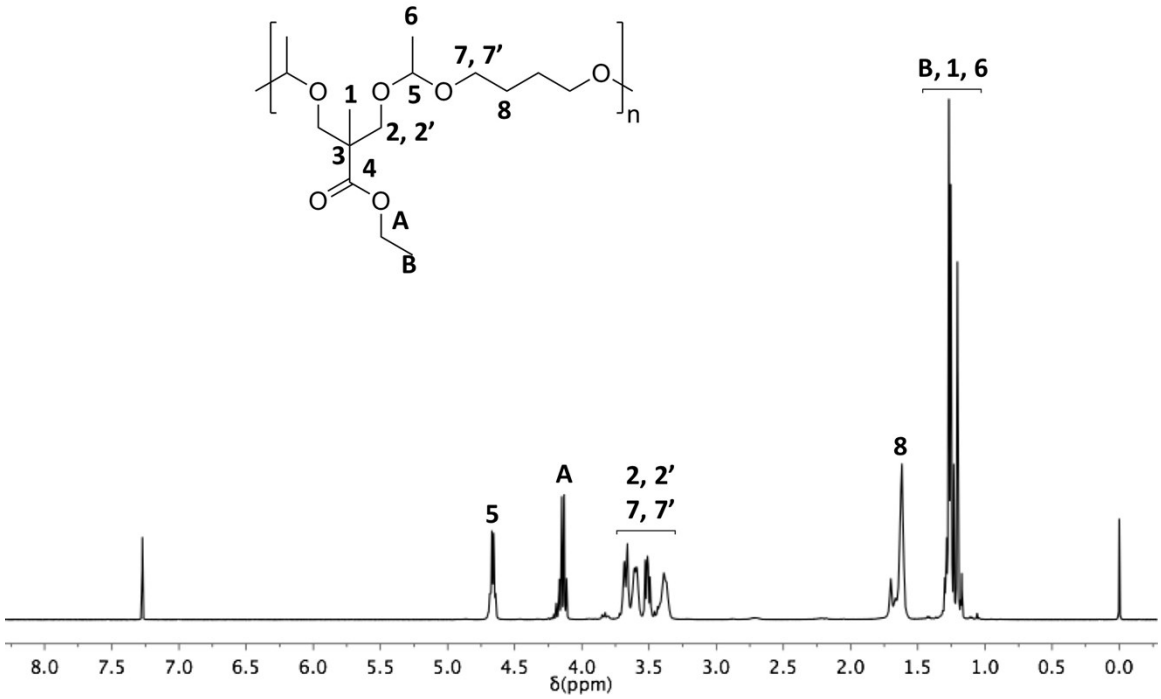


Figure S11 ^{1}H NMR spectrum of **PA1** (δ (ppm), $CDCl_3$)

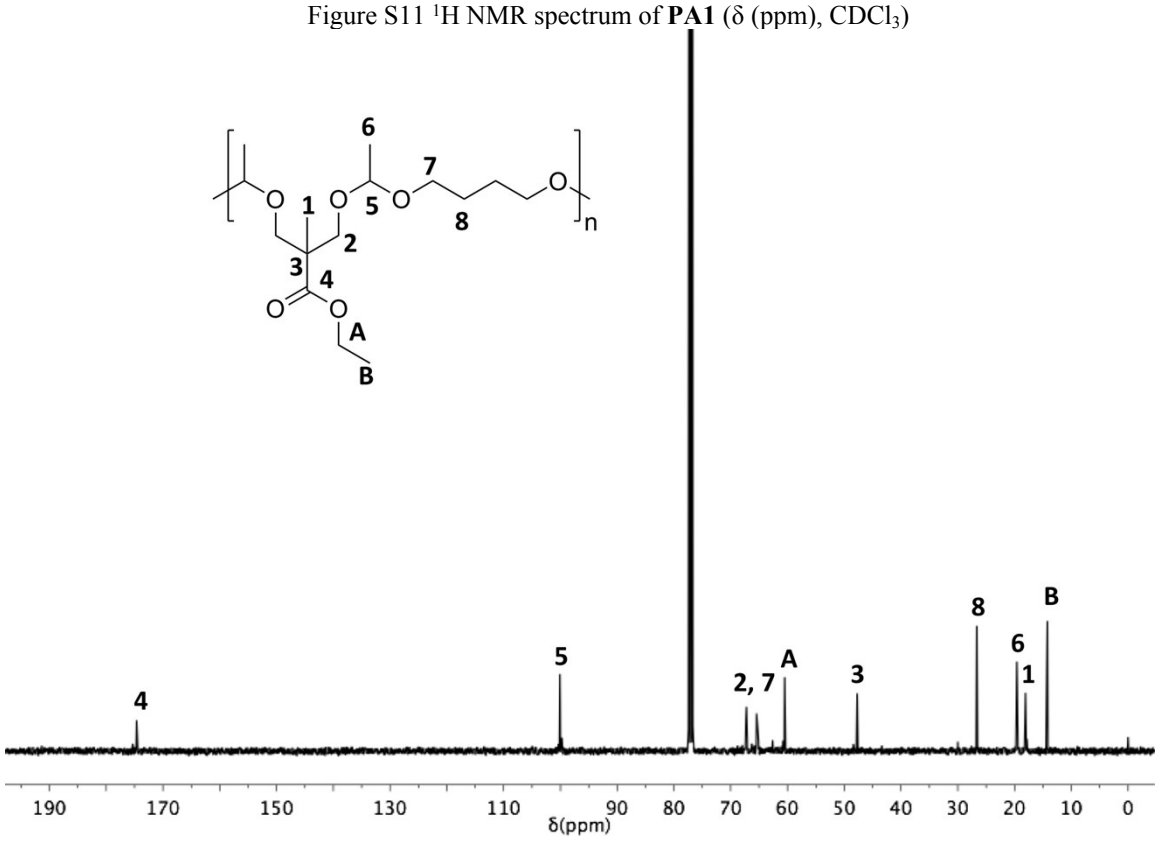


Figure S12 ^{13}C NMR spectrum of **PA1** (δ (ppm), $CDCl_3$)

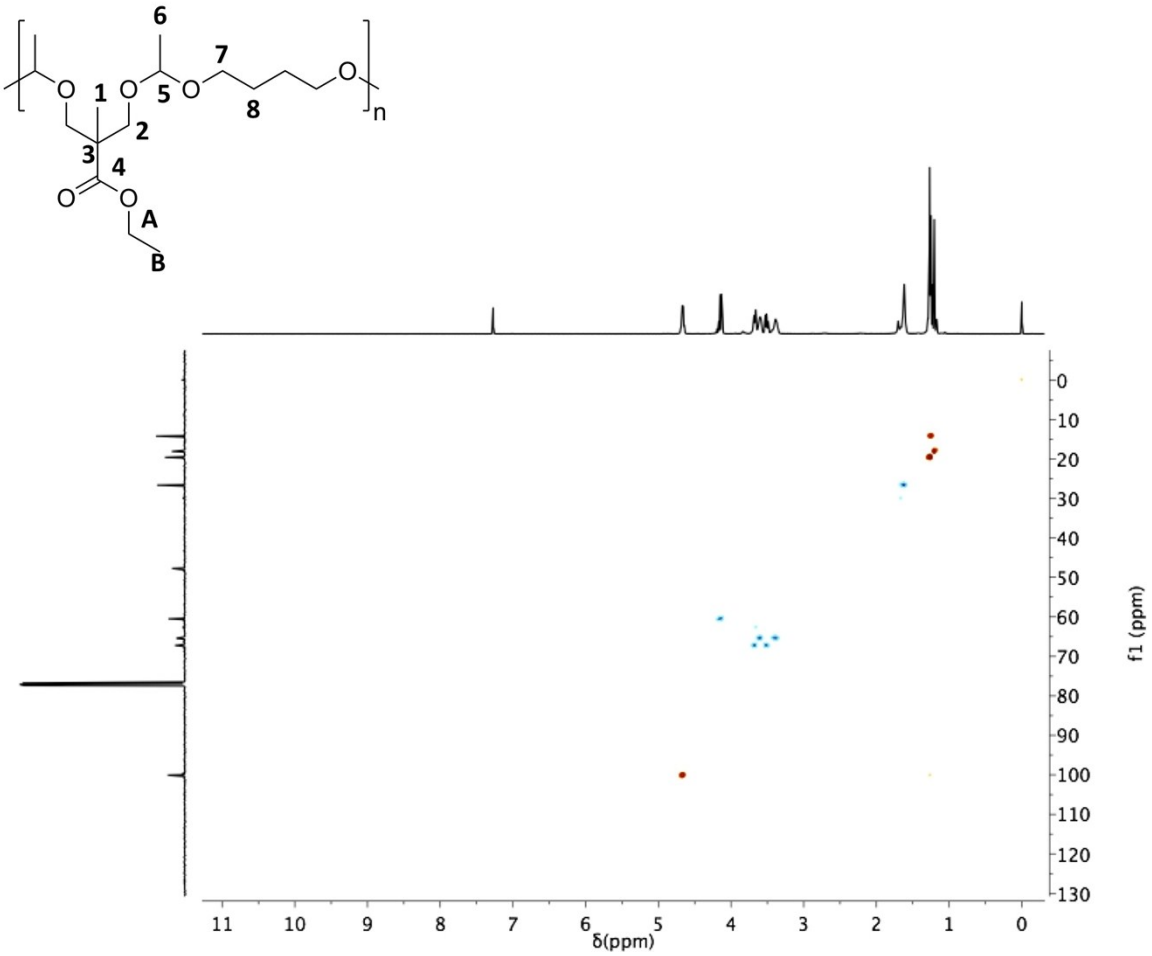


Figure S13 2D ^{1}H-^{13}C HSQC NMR spectrum of **PA1** (δ (ppm), $CDCl_3$)

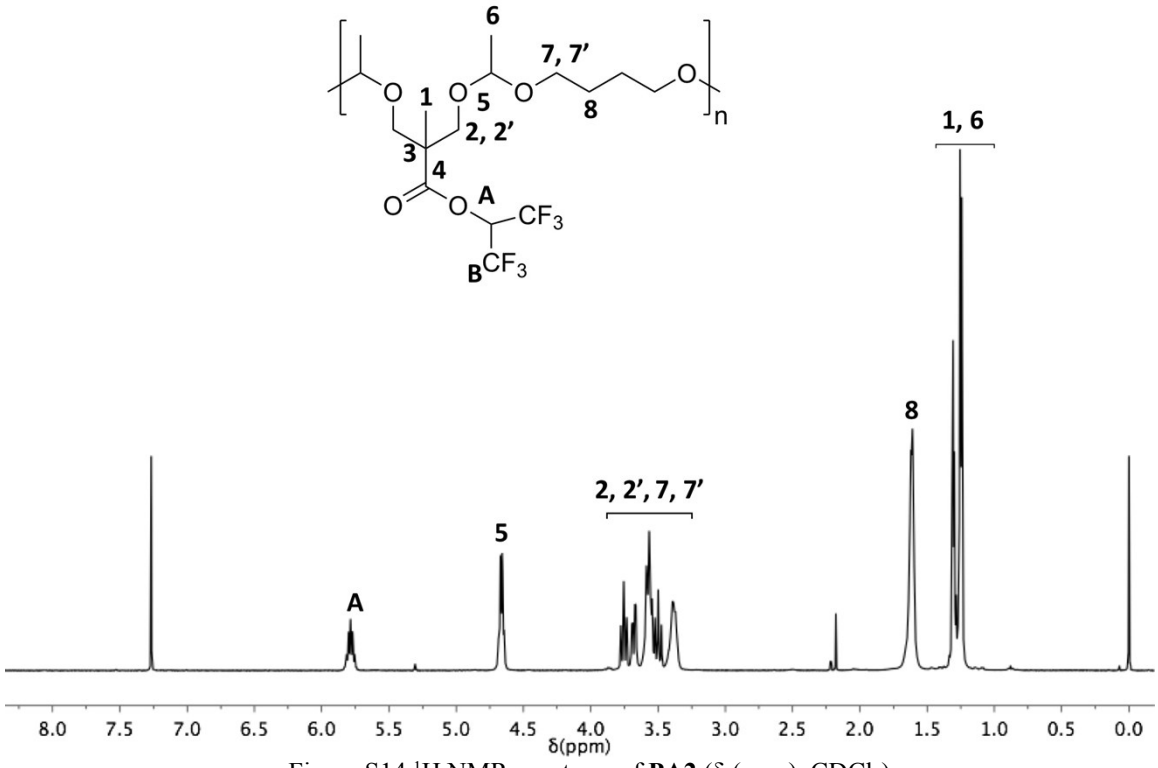


Figure S14 ^{1}H NMR spectrum of **PA2** (δ (ppm), $CDCl_3$)

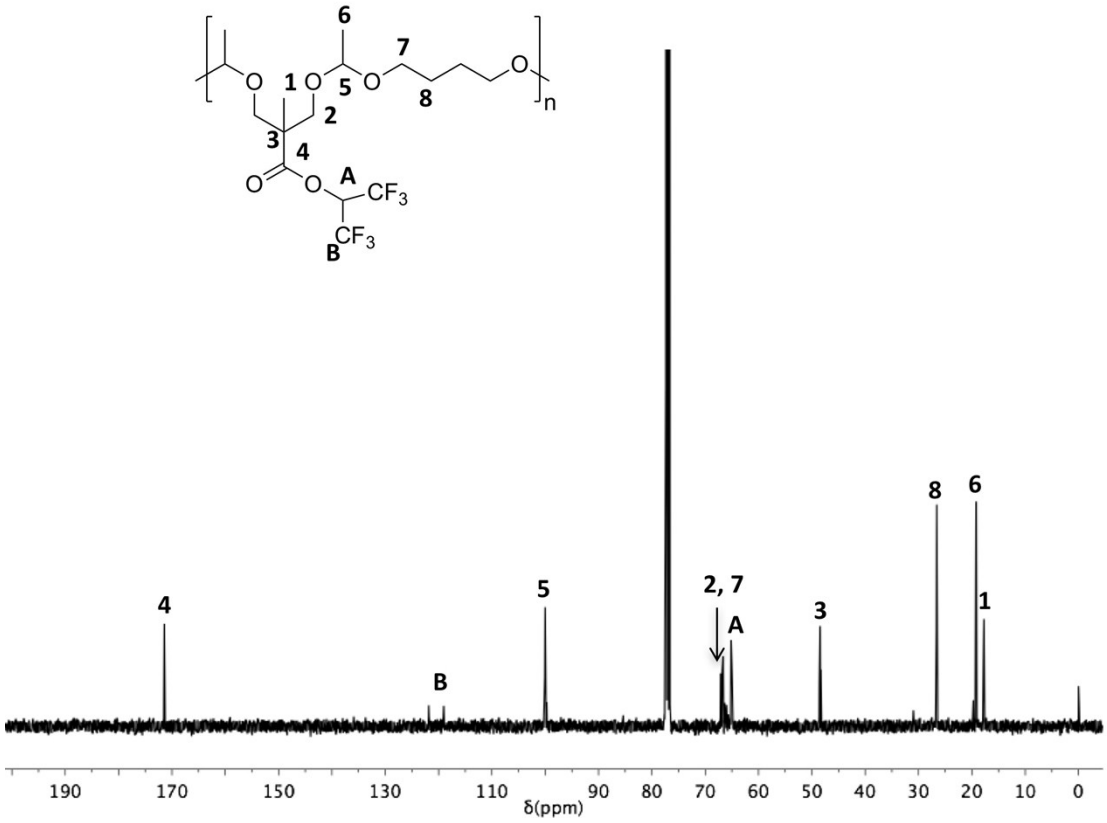


Figure S15 ^{13}C NMR spectrum of **PA2** (δ (ppm), $CDCl_3$)

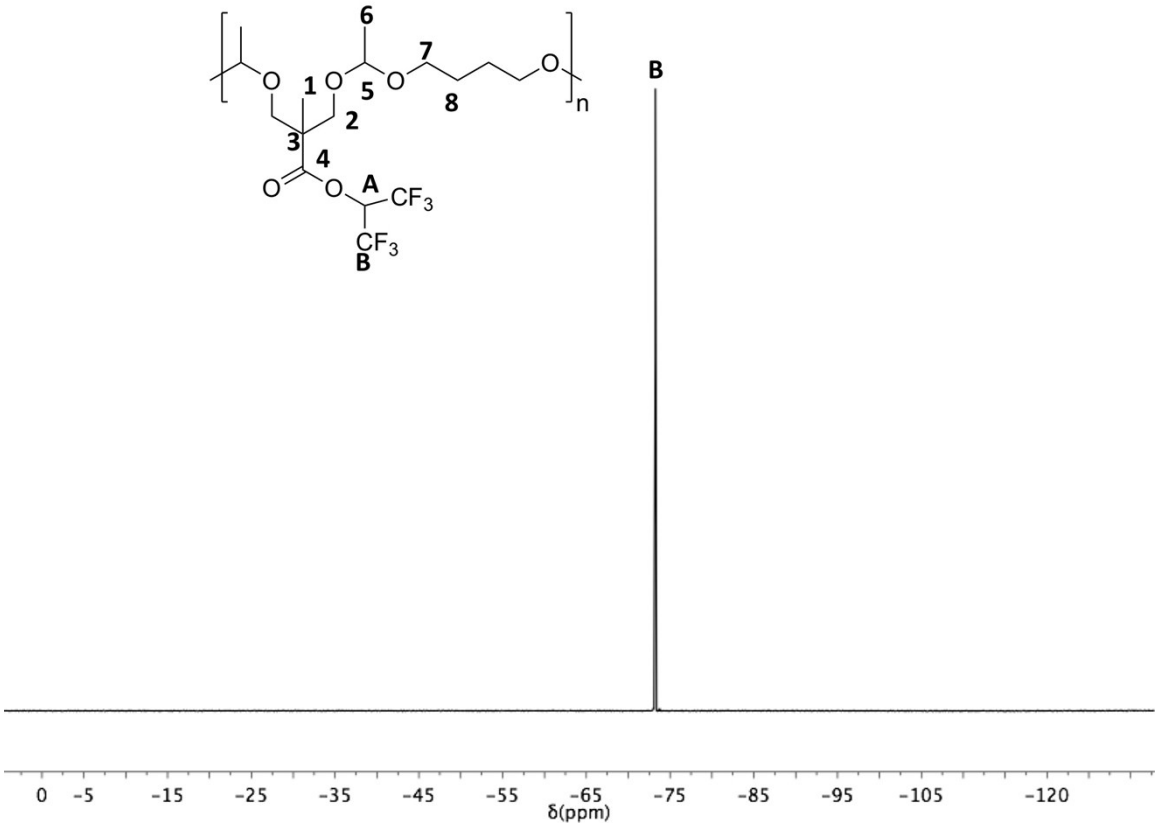


Figure S16 ^{19}F NMR spectrum of **PA2** (δ (ppm), $CDCl_3$)

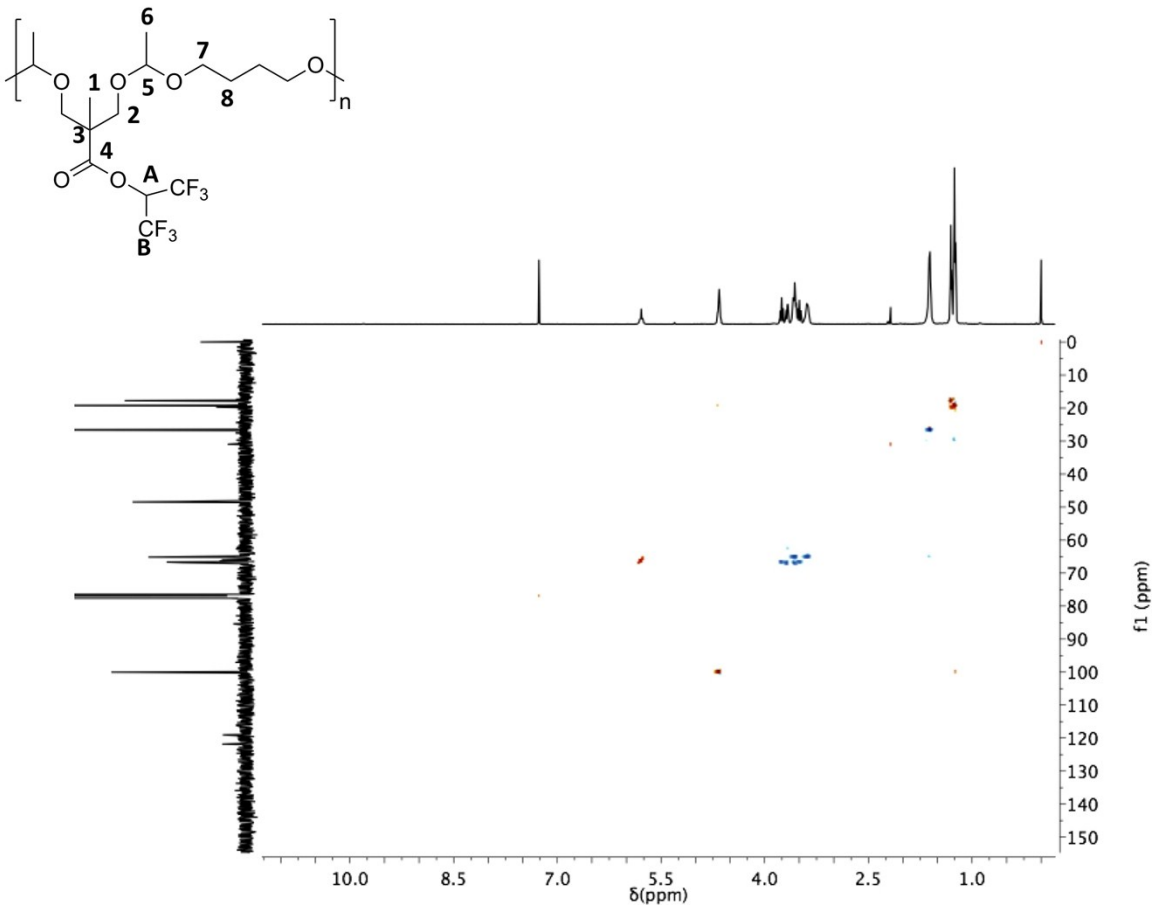


Figure S17 2D ^{1}H-^{13}C HSQC NMR spectrum of **PA2** (δ (ppm), $CDCl_3$)

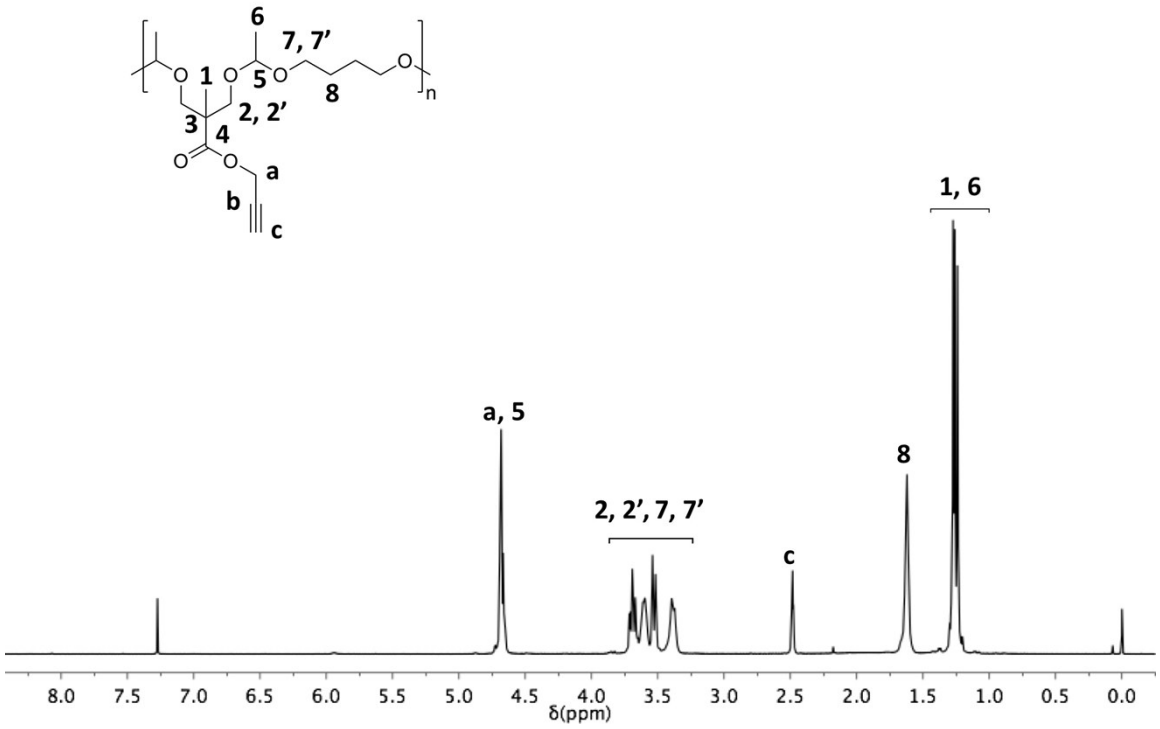


Figure S18 ^{1}H NMR spectrum of **PA3** (δ (ppm), $CDCl_3$)

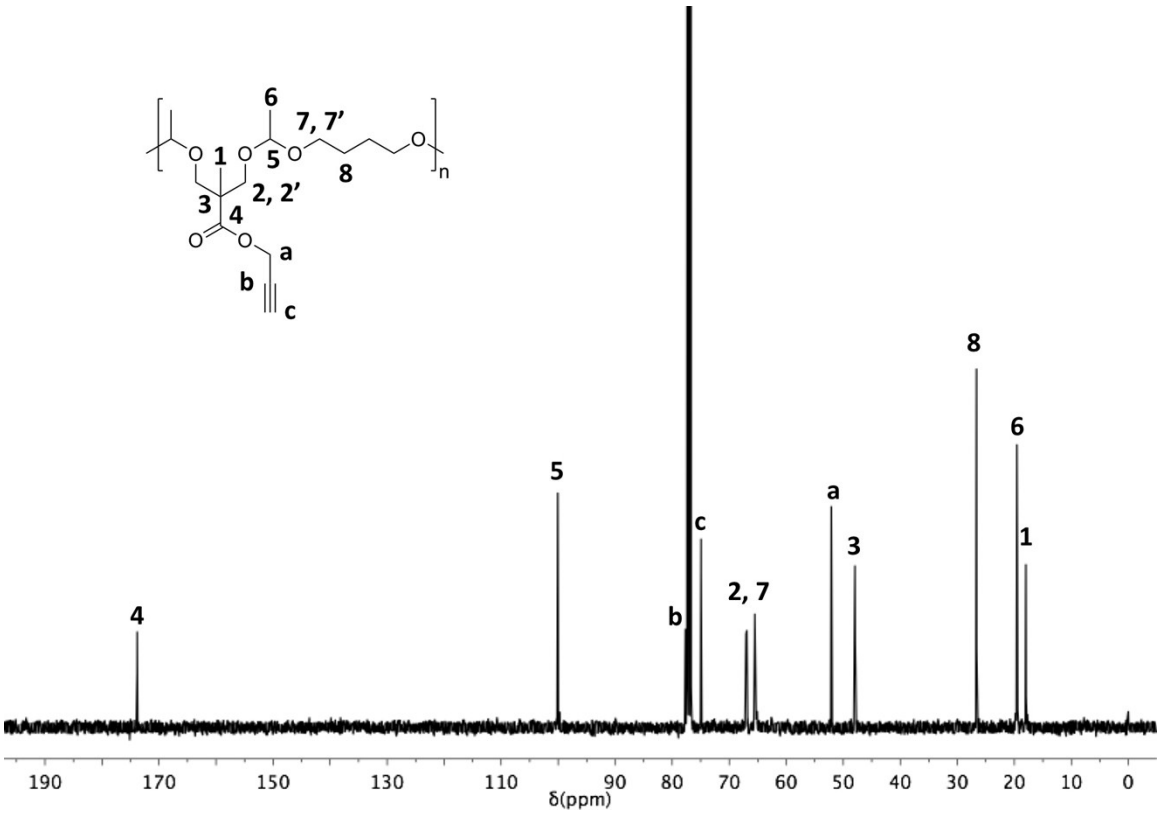


Figure S19 ^{13}C NMR spectrum of **PA3** (δ (ppm), $CDCl_3$)

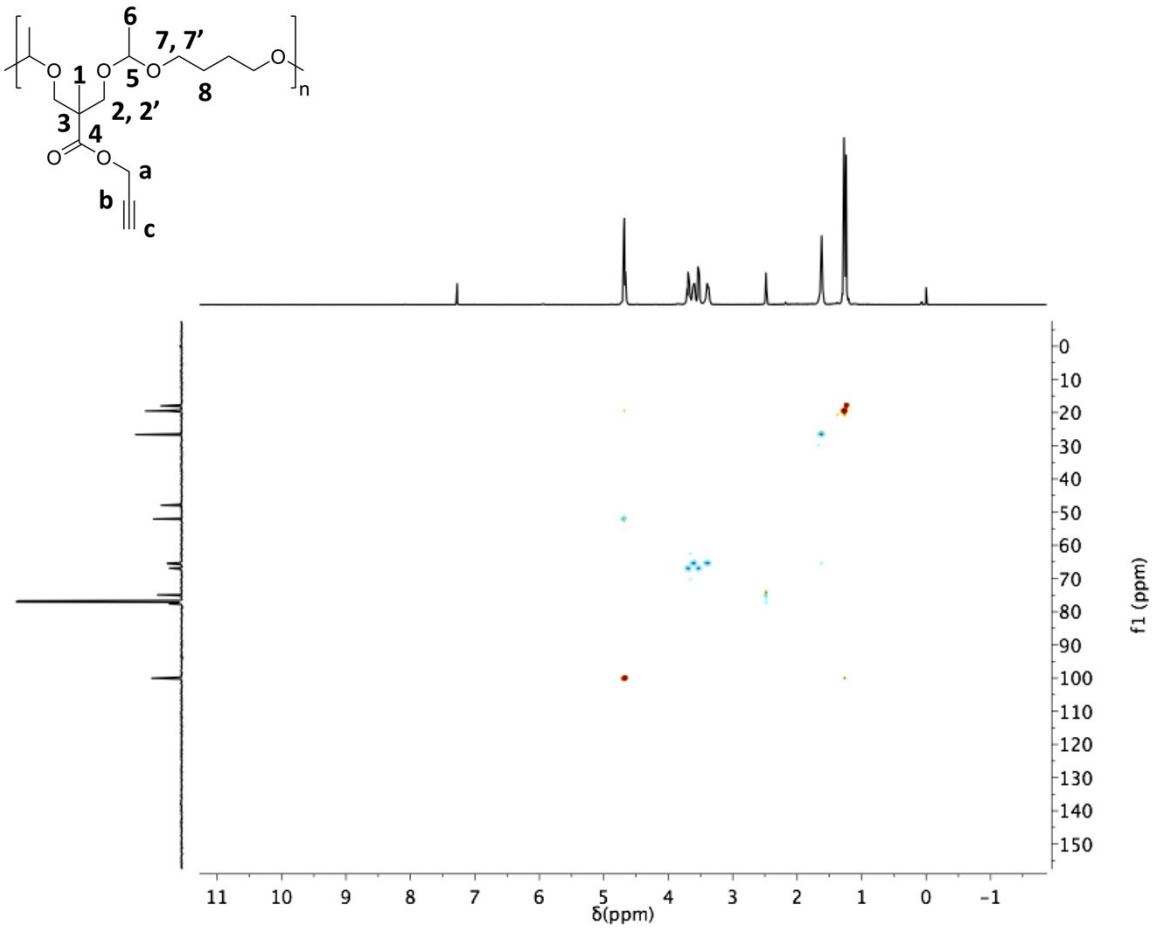


Figure S20 2D ^{1}H-^{13}C HSQC NMR spectrum of **PA3** (δ (ppm), $CDCl_3$)

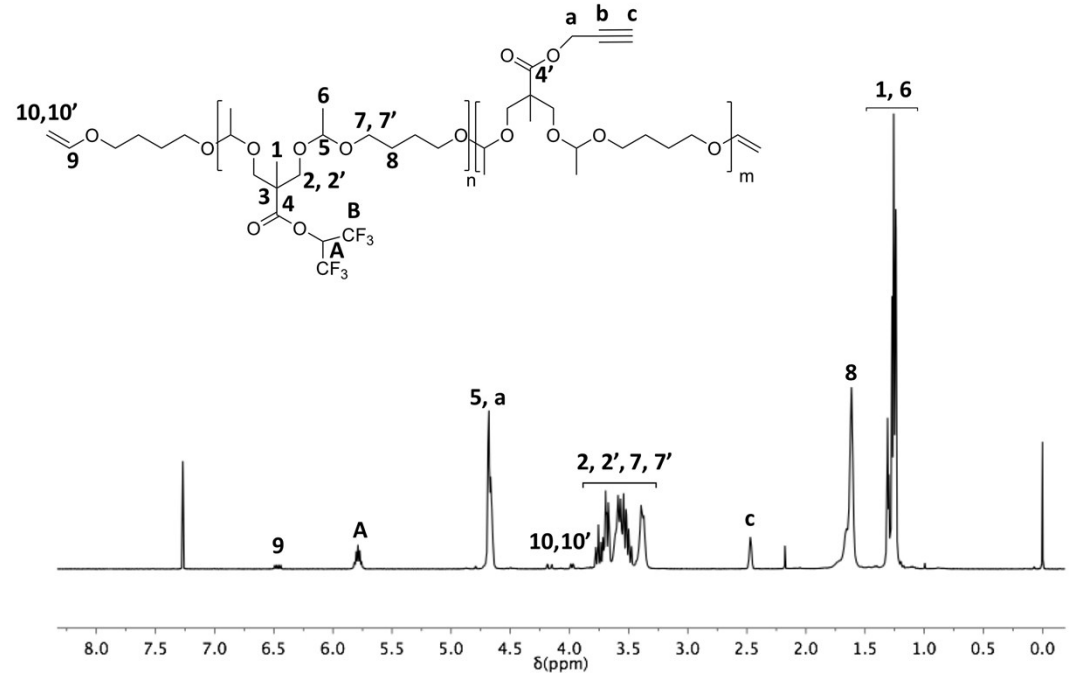


Figure S21 ^{1}H NMR spectrum of **PA2,3** (δ (ppm), $CDCl_3$)

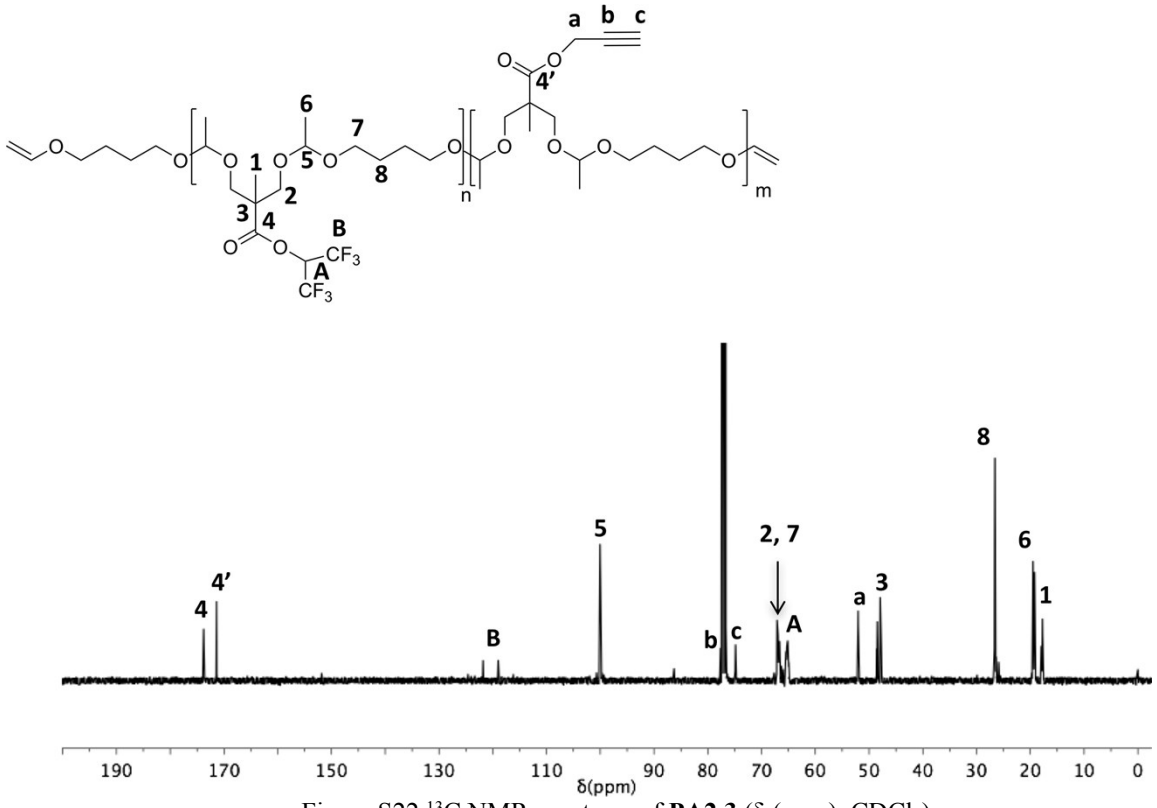


Figure S22 ^{13}C NMR spectrum of **PA2,3** (δ (ppm), $CDCl_3$)

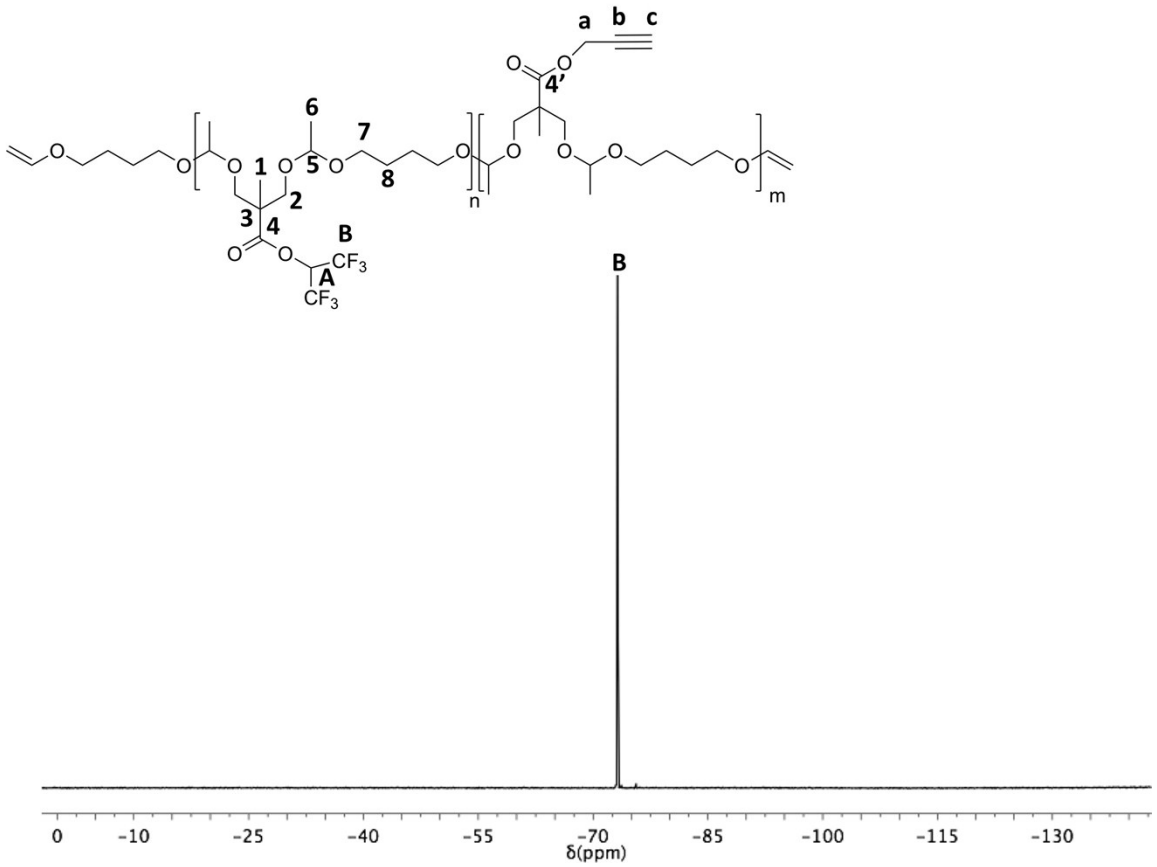


Figure S23 ^{19}F NMR spectrum of **PA2,3** (δ (ppm), $CDCl_3$)

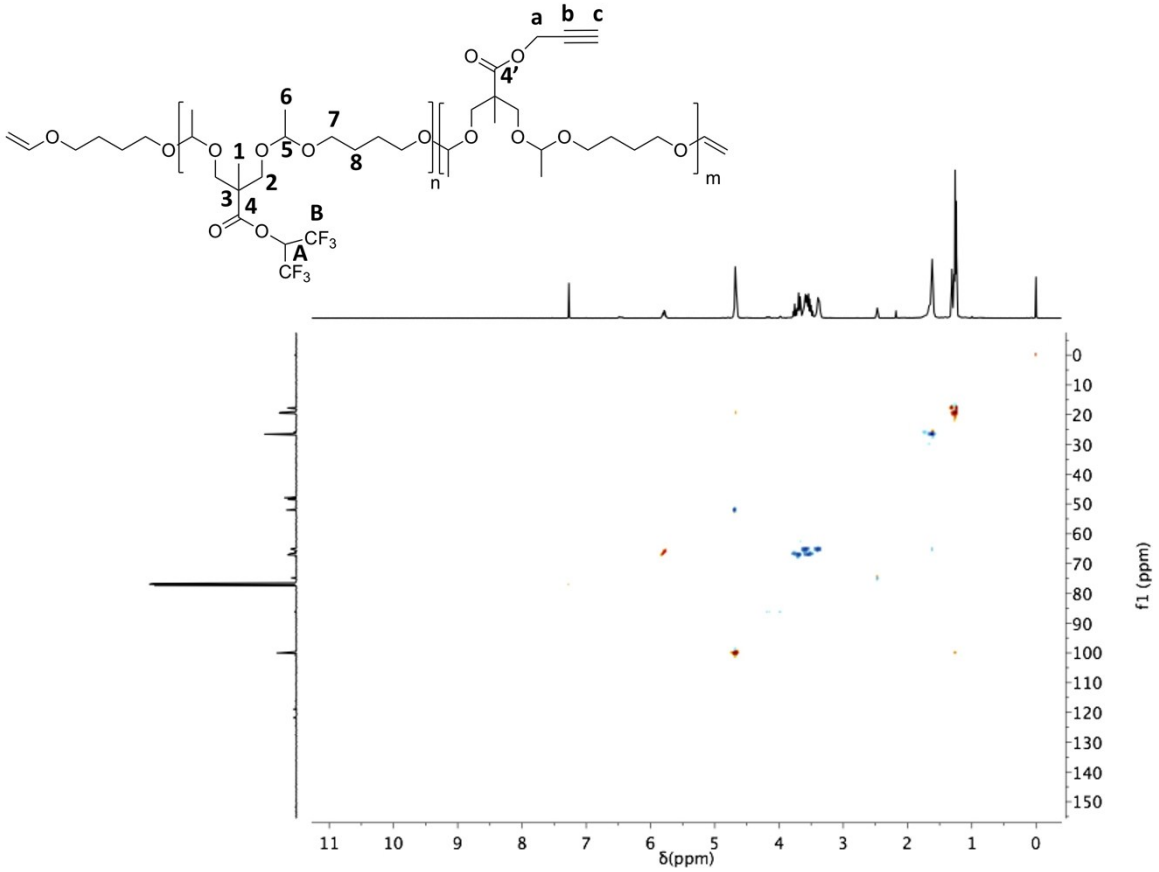


Figure S24 2D ^{1}H-^{13}C HSQC NMR spectrum of **PA2,3** (δ (ppm), $CDCl_3$)

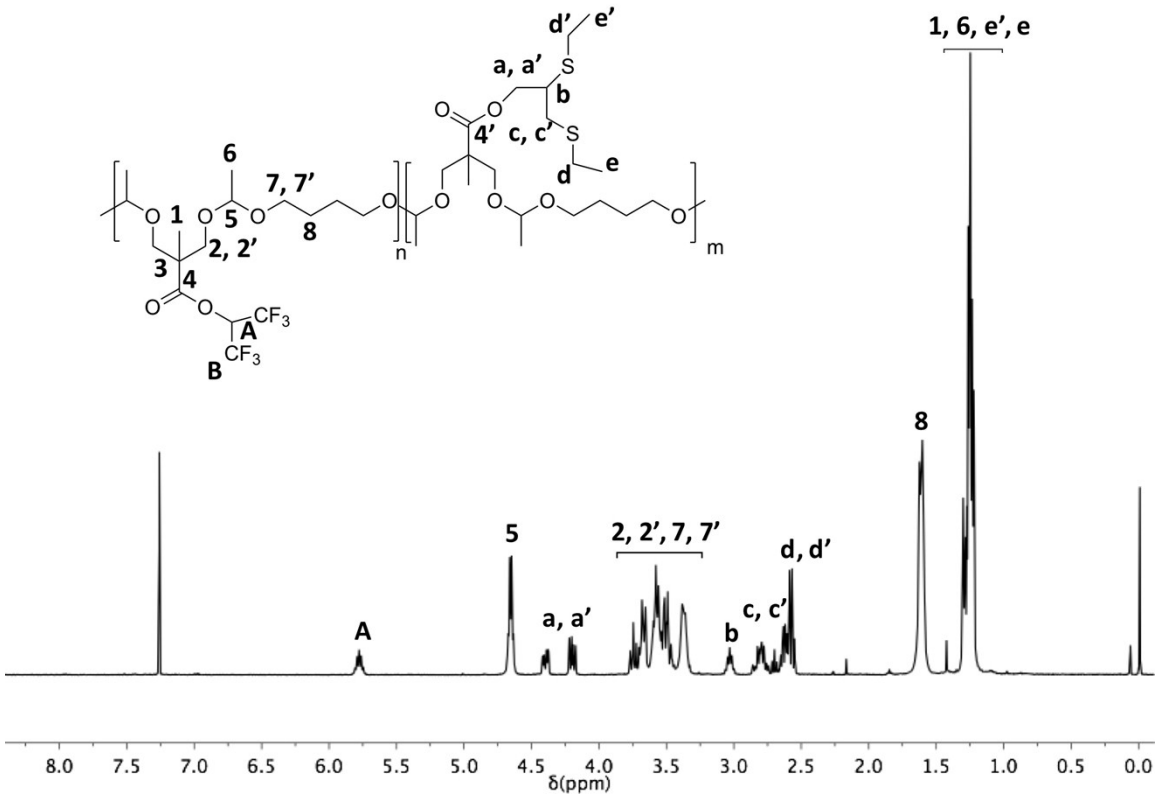


Figure S25 ^{1}H NMR spectrum of **PA2,3-1** (δ (ppm), $CDCl_3$)

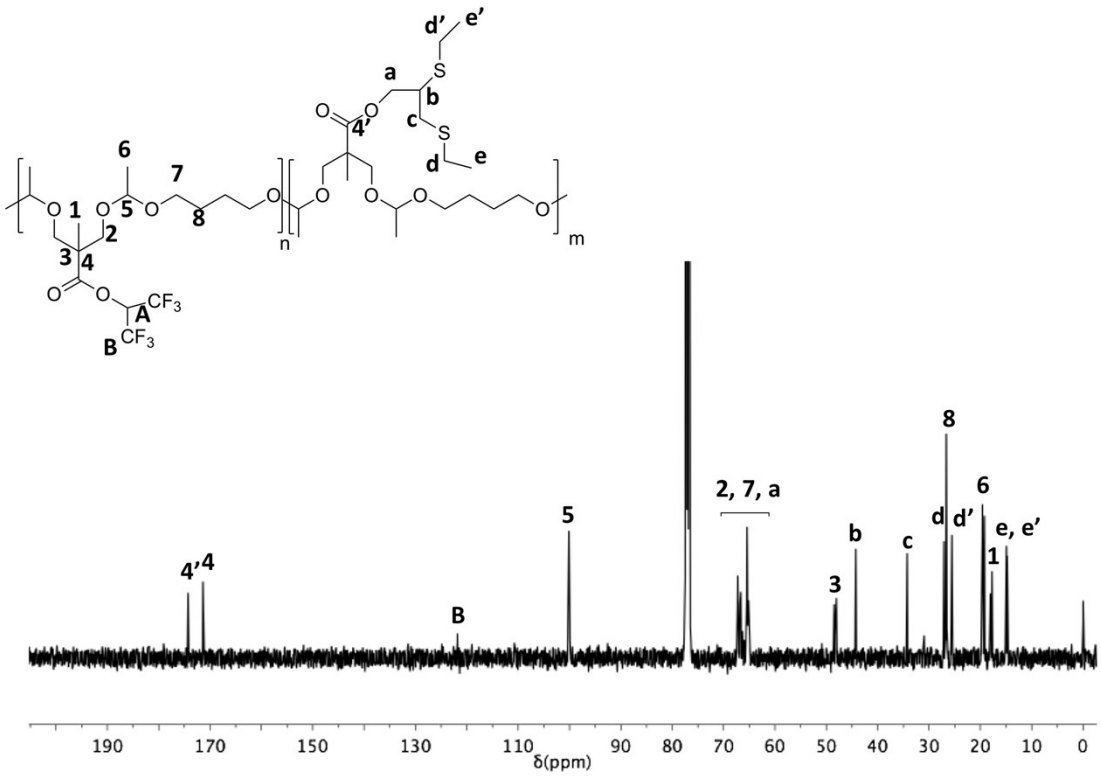


Figure S26 ^{13}C NMR spectrum of **PA2,3-1** (δ (ppm), $CDCl_3$)

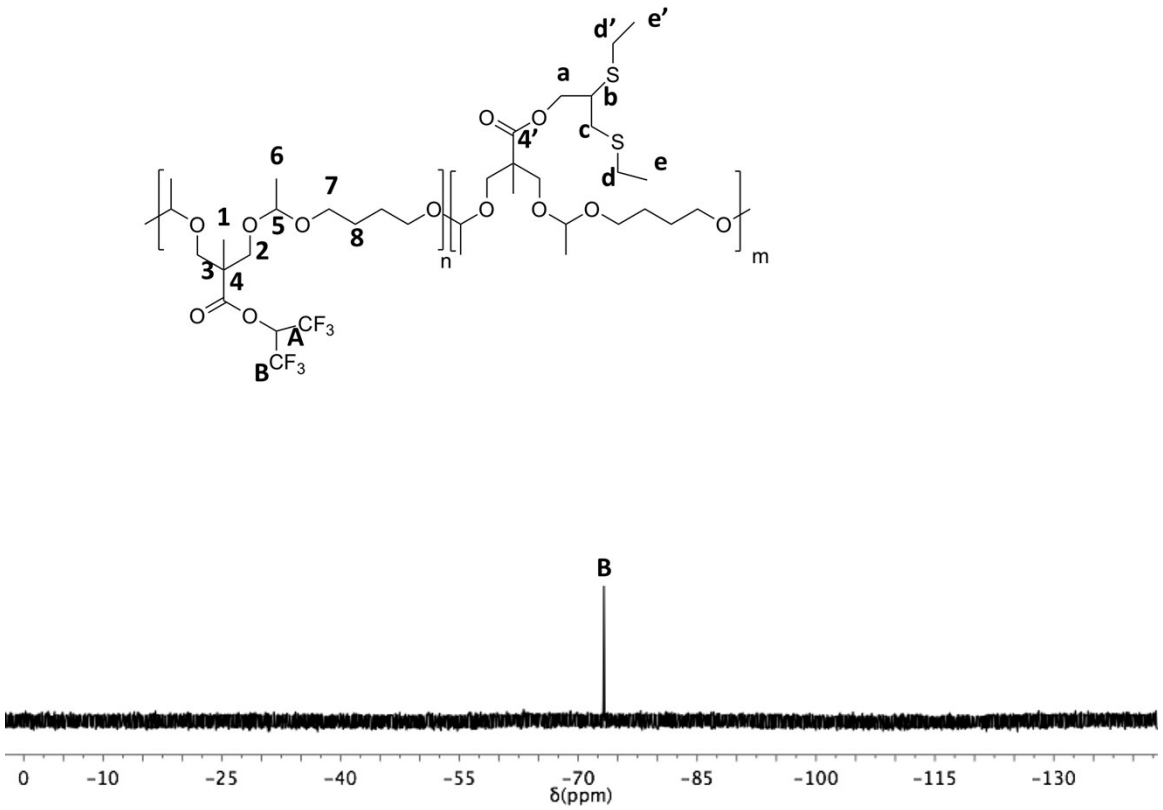


Figure S27 ^{19}F NMR spectrum of **PA2,3-1** (δ (ppm), $CDCl_3$)

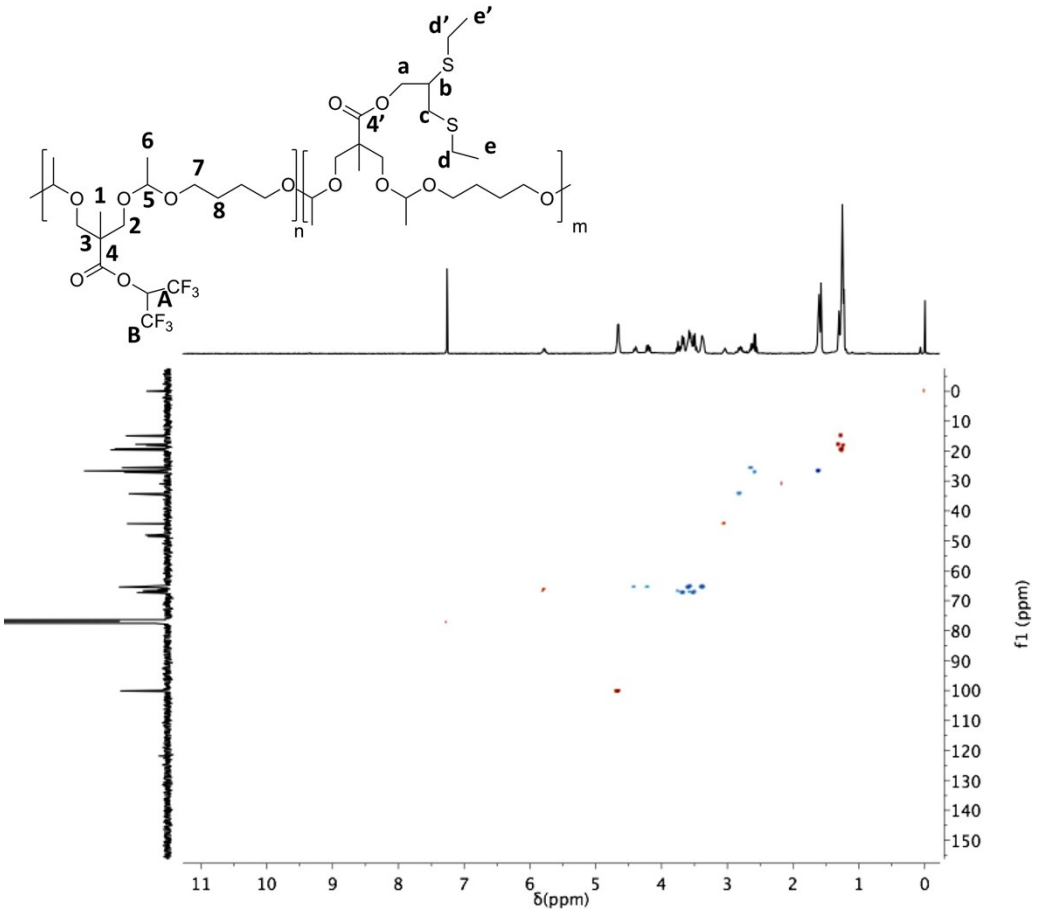


Figure S28 2D ^{1}H-^{13}C HSQC NMR spectrum of **PA2,3-1** (δ (ppm), $CDCl_3$)

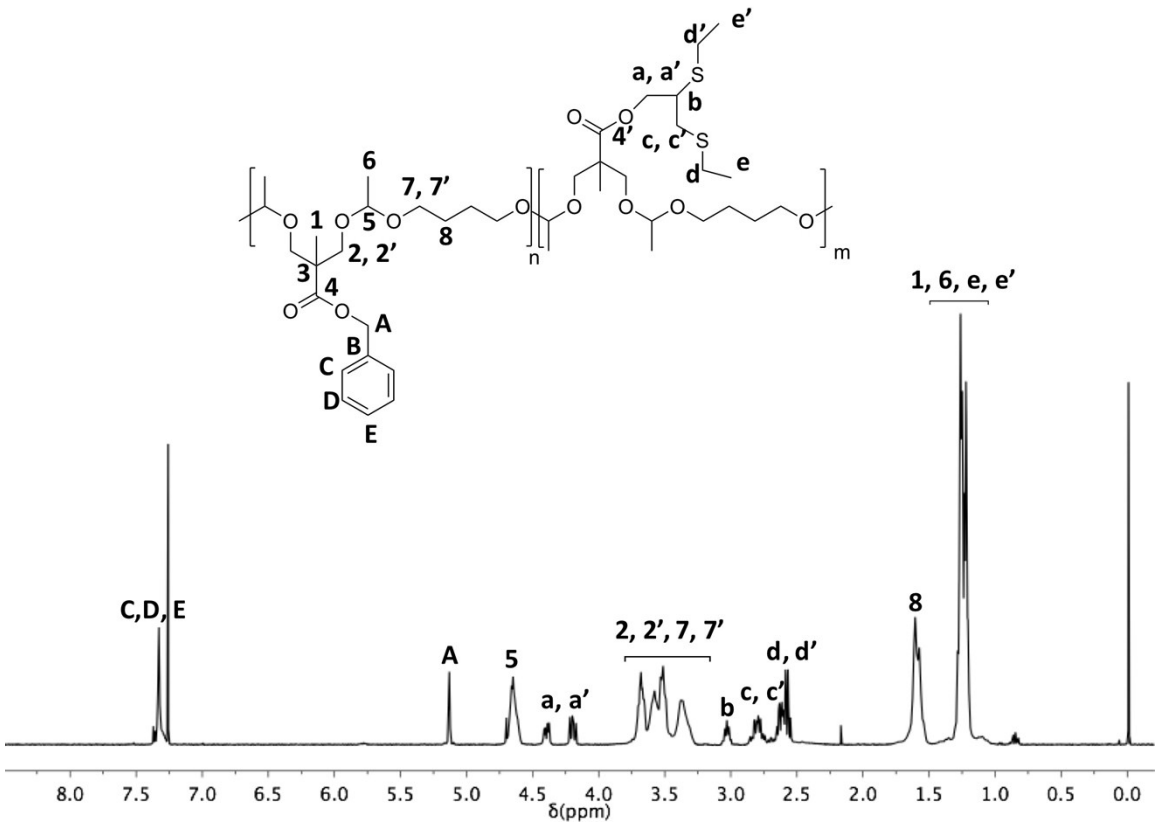


Figure S29 ^{1}H NMR spectrum of **PA2,3-2** (δ (ppm), $CDCl_3$)

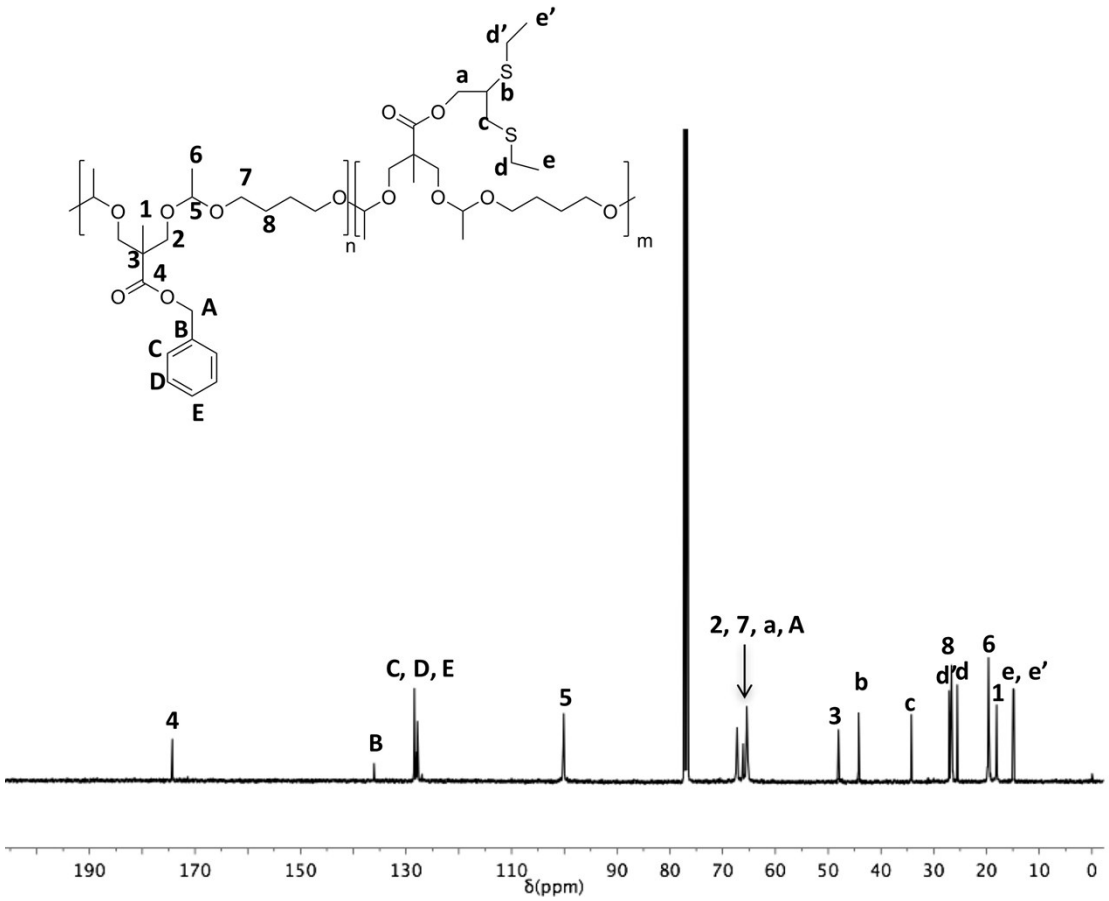
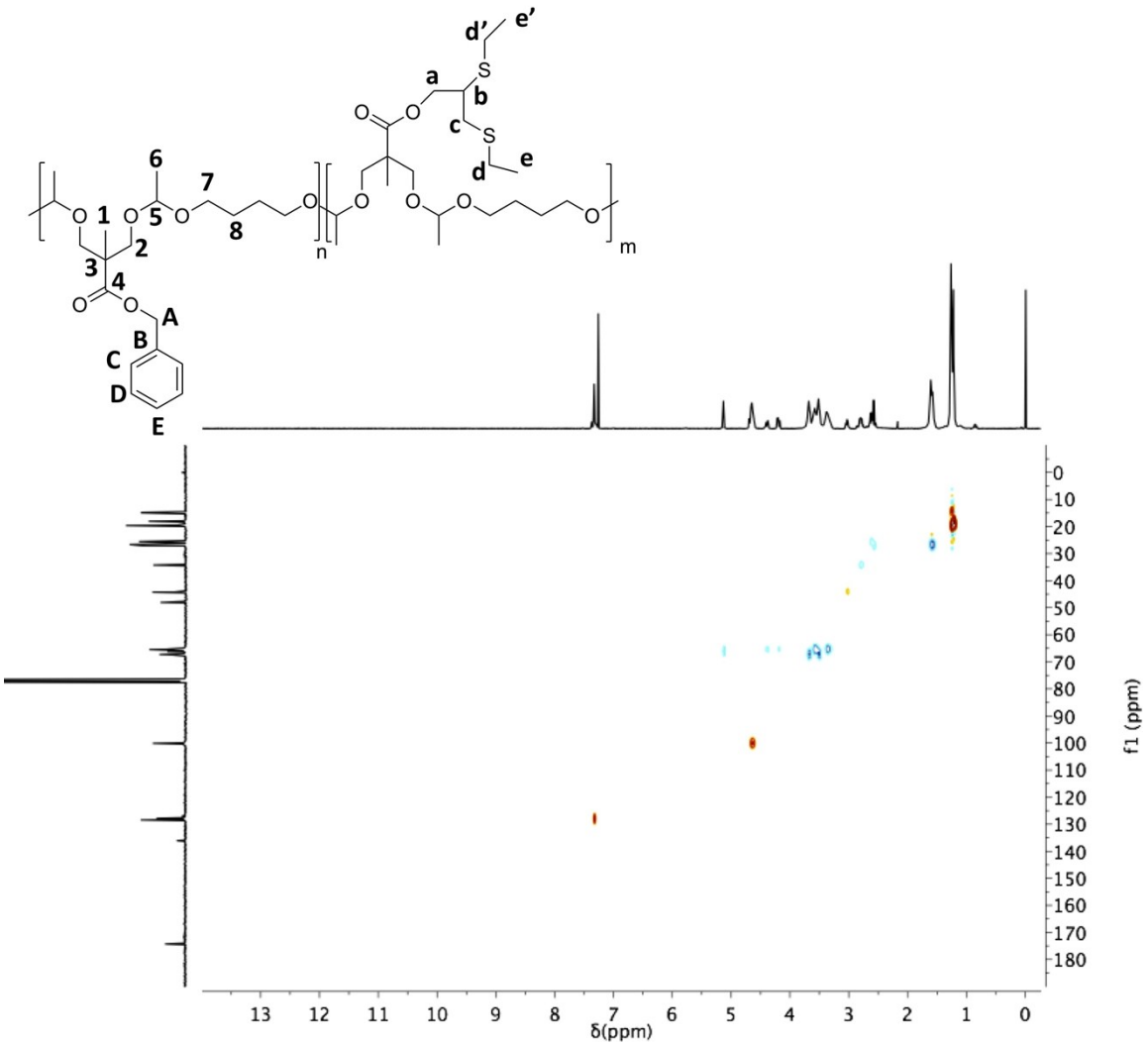


Figure S30 ^{13}C NMR spectrum of **PA2,3-2** (δ (ppm), $CDCl_3$)



.. Figure S31 2D ^{1}H-^{13}C HSQC NMR spectrum of **PA2,3-2** (δ (ppm), $CDCl_3$)

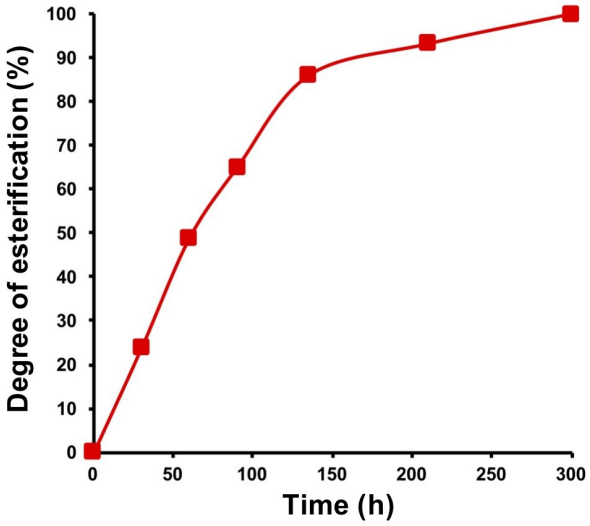


Figure S32 Kinetics plot, degree of esterification vs. time for **PA2,3-1** with BnOH

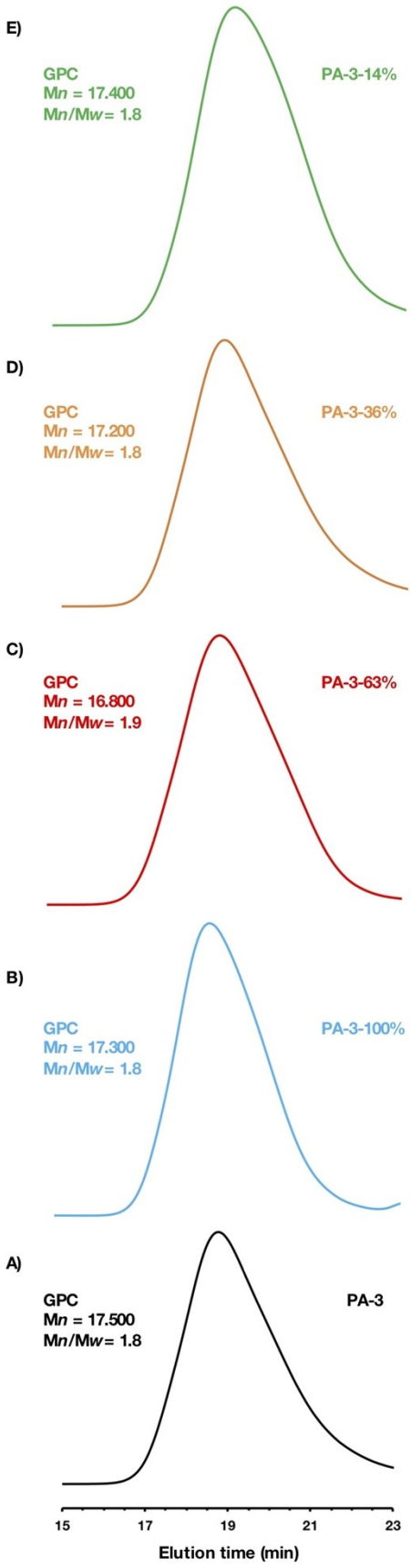


Figure S33. SEC traces for **PA3** (A), **PA3-100** (B), **PA3-63** (C), **PA3-36** (D) and **PA3-14** (E).

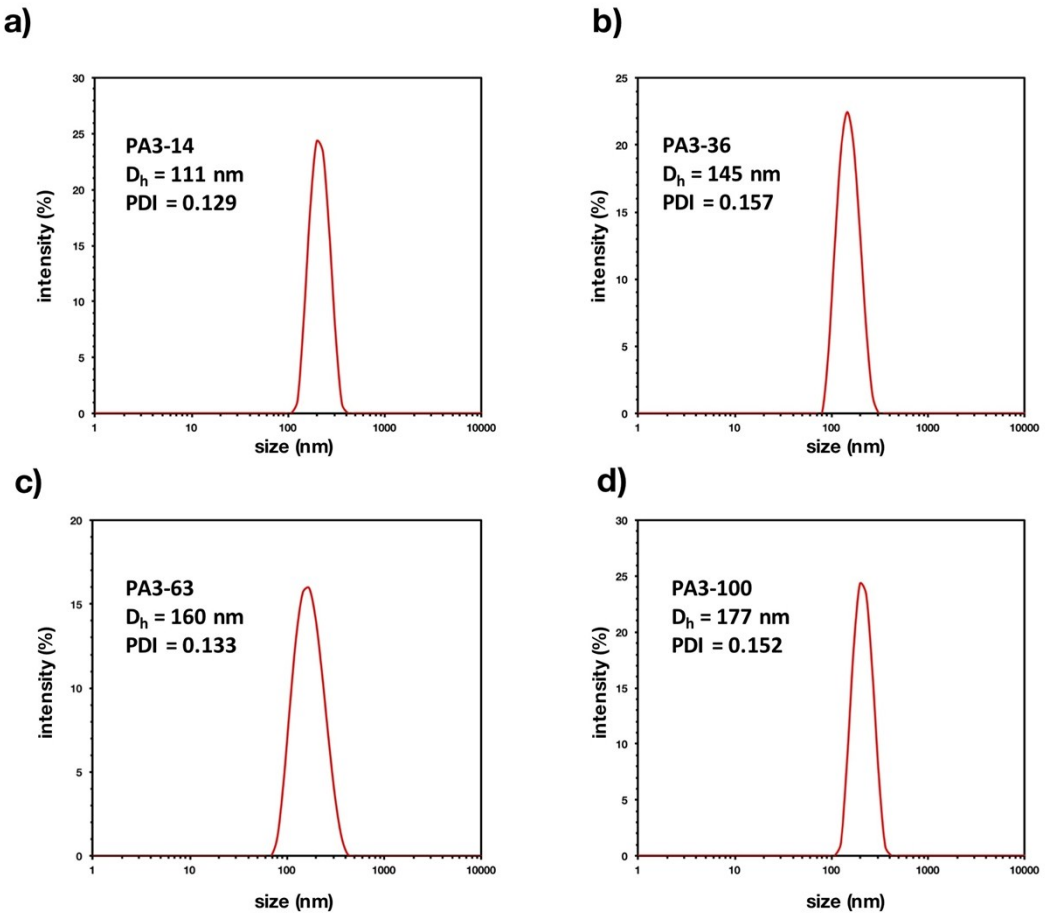


Figure S34. DLS size distribution for (a) **PA3-14**, (b) **PA3-36**, (c) **PA3-63** and (d) **PA3-100**

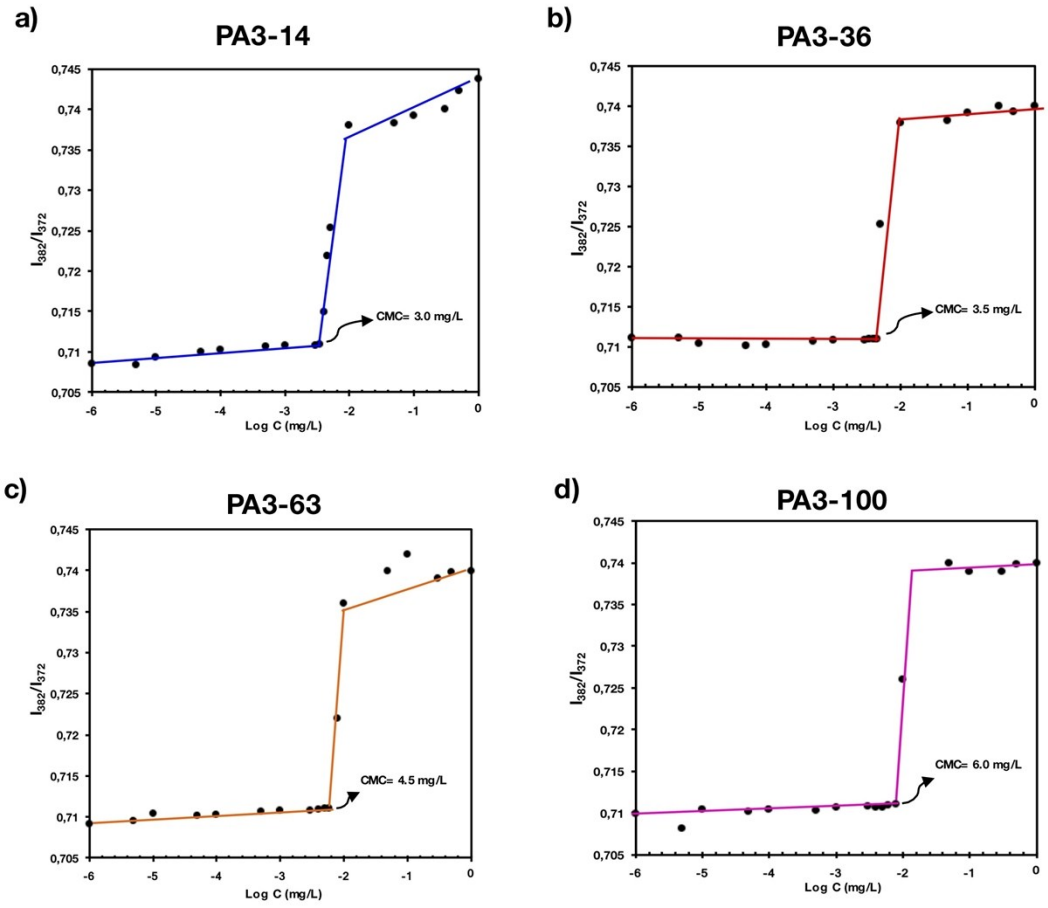


Figure S35 Plot of the fluorescence intensity ratio (I_{382}/I_{372}) for pyrene vs. the log of micelle concentration for: **PA3-100**, **PA3-63**, **PA3-36** and **PA3-14**

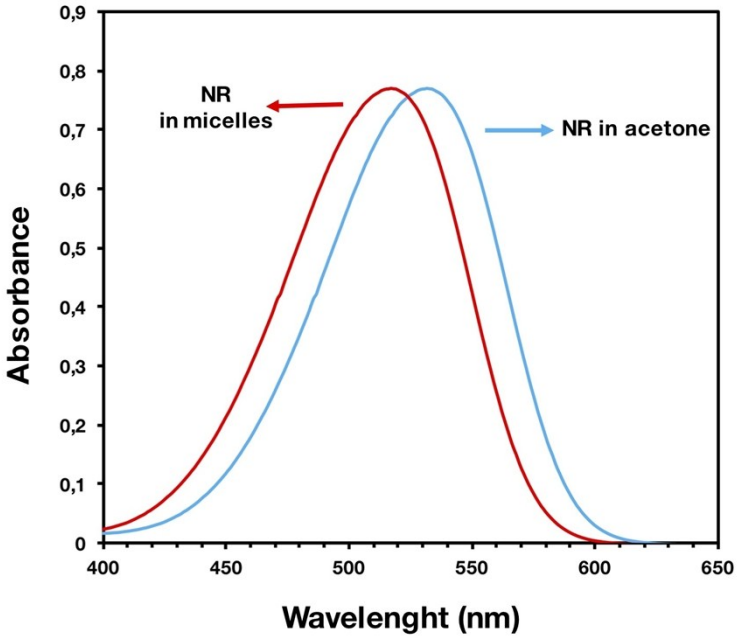


Figure S36 UV spectra of NR in acetone (blue) and NR encapsulated in **PA2,3-3** in water (red)