Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

A Sequential Native Chemical Ligation−Thiol-Michael Addition Strategy for Polymer-Polymer Ligation

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Experimental details and characterisation

2,2-Dimethylthiazolidine-4-carboxylic acid (Tz4CA)

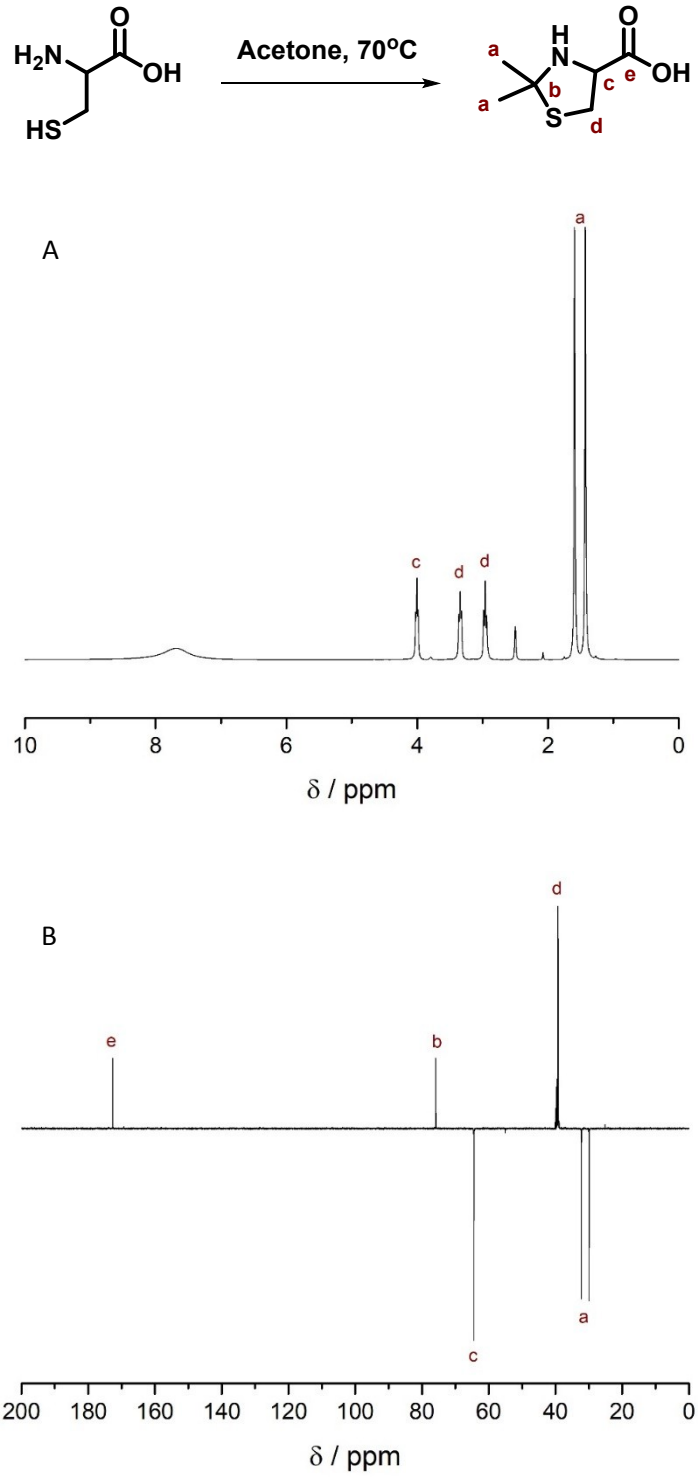
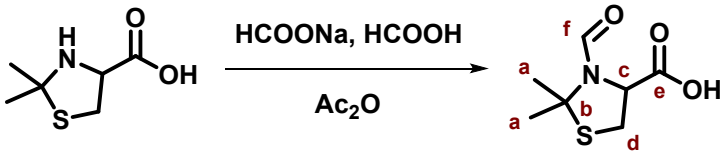


Fig. S1. `A) ^{1}H NMR (top) and B) ^{13}C NMR in d_6-DMSO of Tz4CA.

2,2-Dimethylthiazolidin-3-(N-formyl)-4-carboxylic acid (FTz4CA)



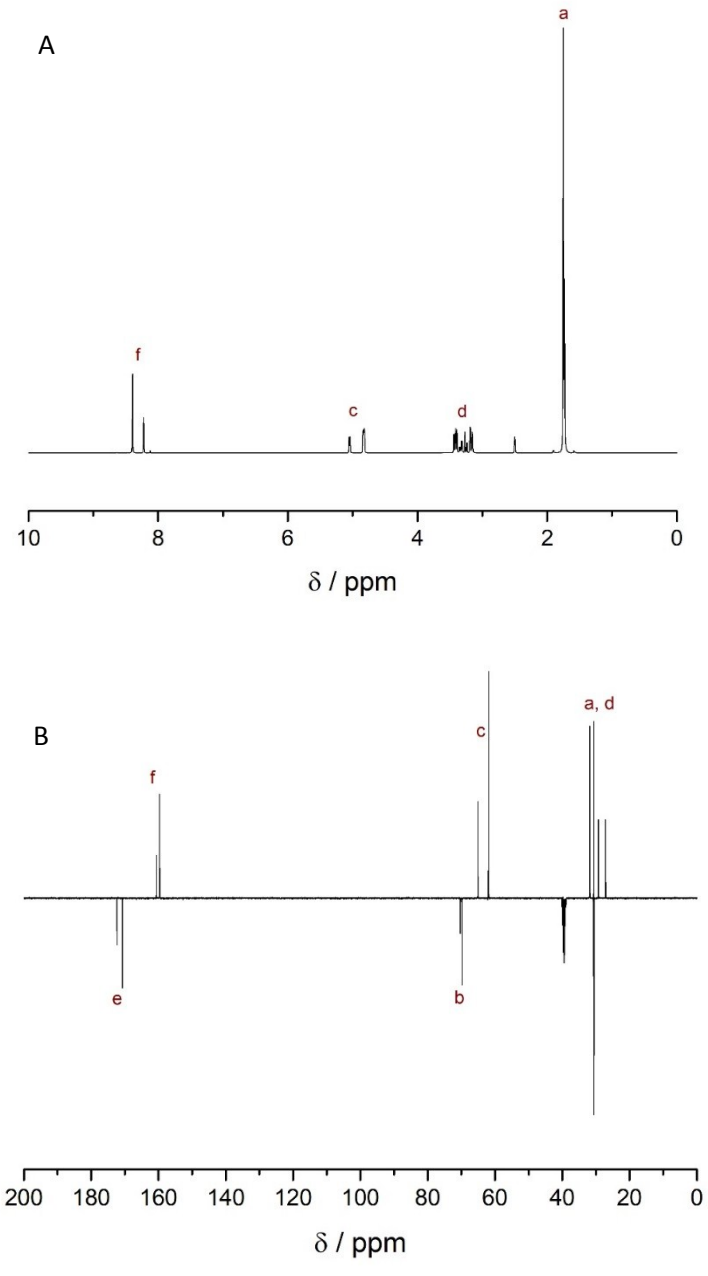
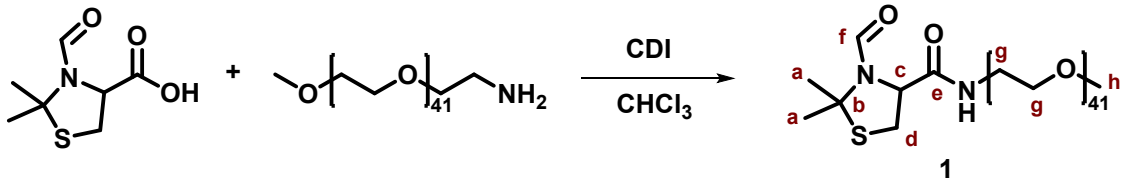
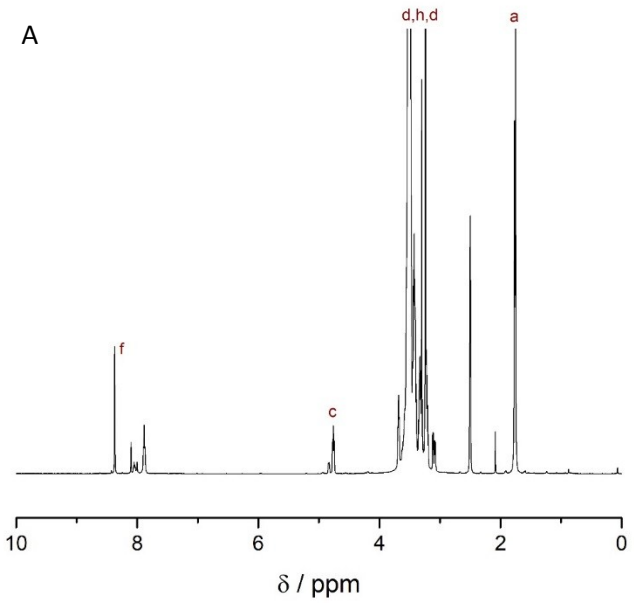
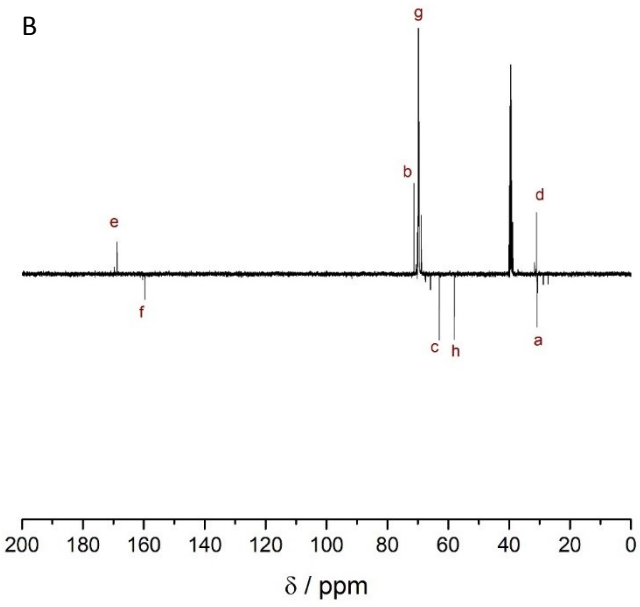


Fig. S2. A) ^{1}H NMR and B) ^{13}C NMR in d_6-DMSO of FTz4CA.

PEG-FTz4CA (1)


CDI
$CHCl_3$
1


A
d,h,d
a
f
c
10
8
6
4
2
0
δ / ppm


B
g
b
d
e
f
c
h
a
200
180
160
140
120
100
80
60
40
20
0
δ / ppm

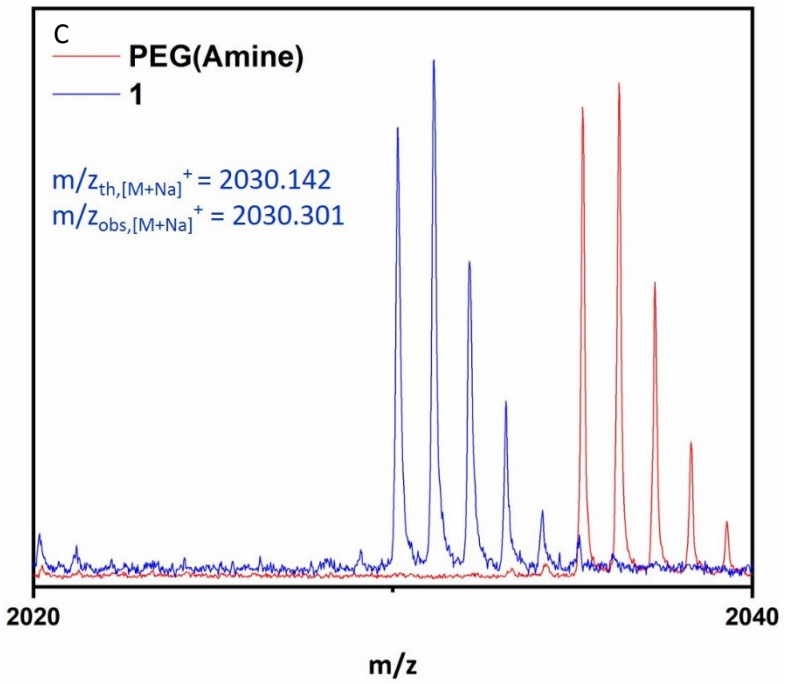


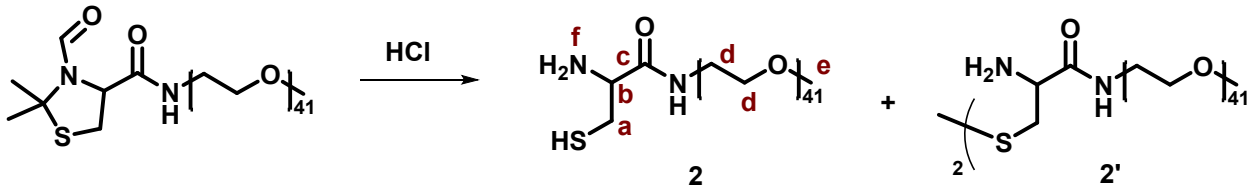
Fig. S3. A) ^{1}H NMR and B) ^{13}C NMR of PEG-FTz4CA (**1**), in d_6-DMSO; C) Enlarged MALDI-ToF-MS from Fig 1. of **1** with DP_n = 41 corresponding to $C_{90}H_{178}N_2NaO_{43}S$ (Table S1).

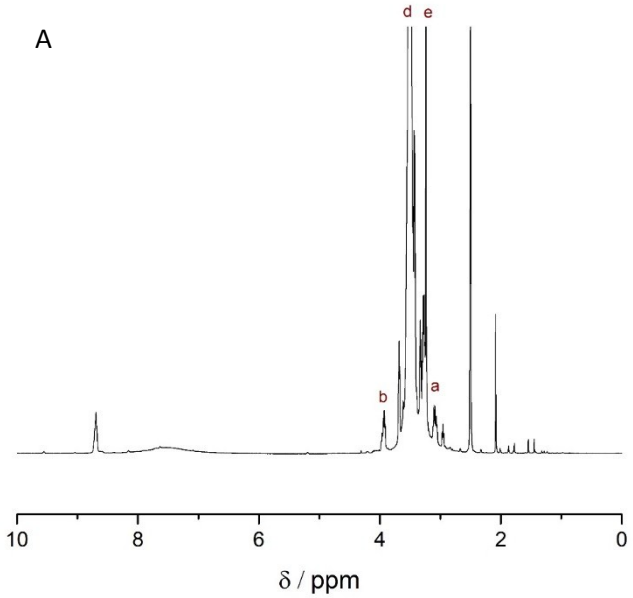
Table S1. Molecular weight data obtained from MALDI-ToF-MS for α-methoxy-ω-functional-PEG polymers **1 – 4**.

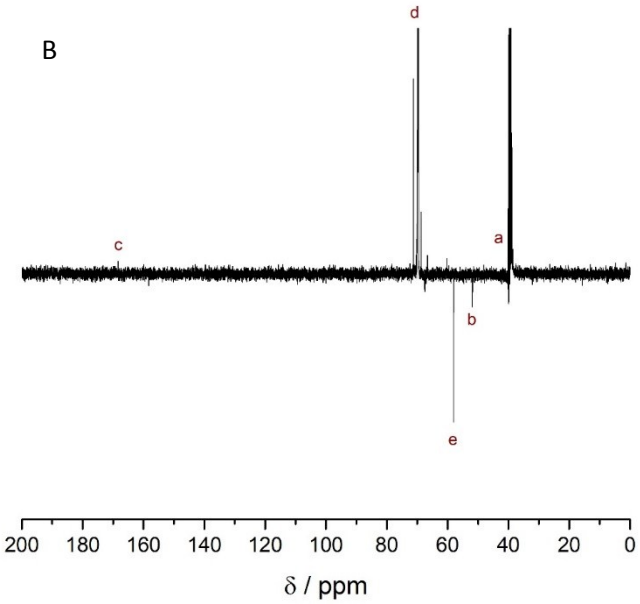
Sample	n[a]	Empirical Formula	Adduct[b]	m/z_{th}[b]	m/z_{obs}[c]
PEG(Amine)	41	$C_{83}H_{169}NO_{41}$	M+Na	1859.106	1859.166
1	41	$C_{90}H_{178}N_2O_{43}S$	M+Na	2030.142	2030.301
2	41	$C_{86}H_{174}N_2O_{42}S$	M+Na	1962.115	1962.119
3	41	$C_{100}H_{191}N_3O_{45}S$	M+Na	2209.236	2209.188
4	41	$C_{105}H_{196}F_3N_3O_{47}S$	M+Na	2363.260	2363.406

[a]Number of repeating units of PEG in the observed species composition; [b]Determined by Bruker Isotope Pattern software; [c]Determined by MALDI spectrum.

PEG-cysteine (2)







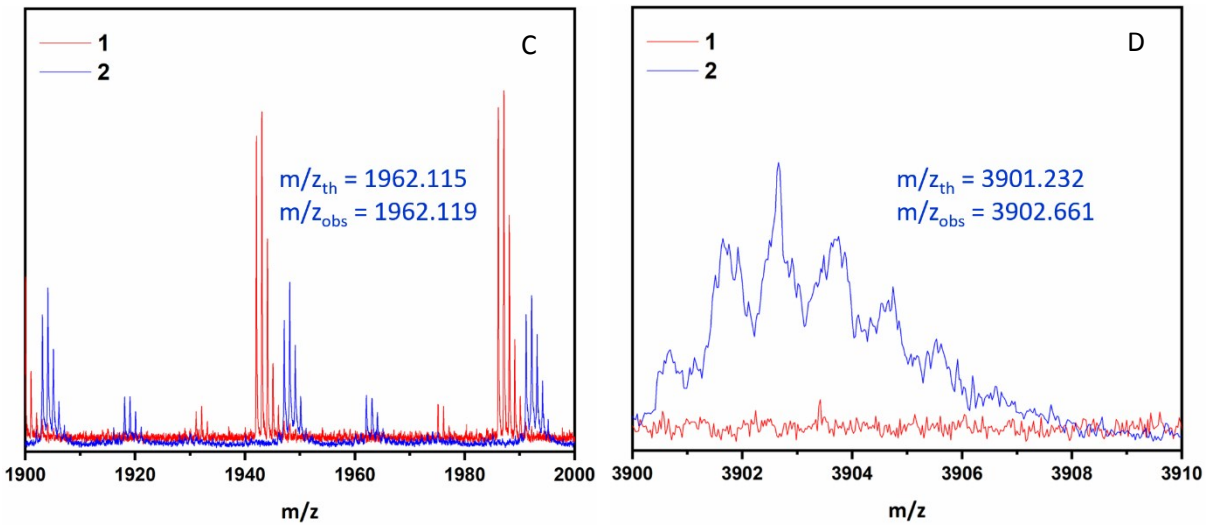
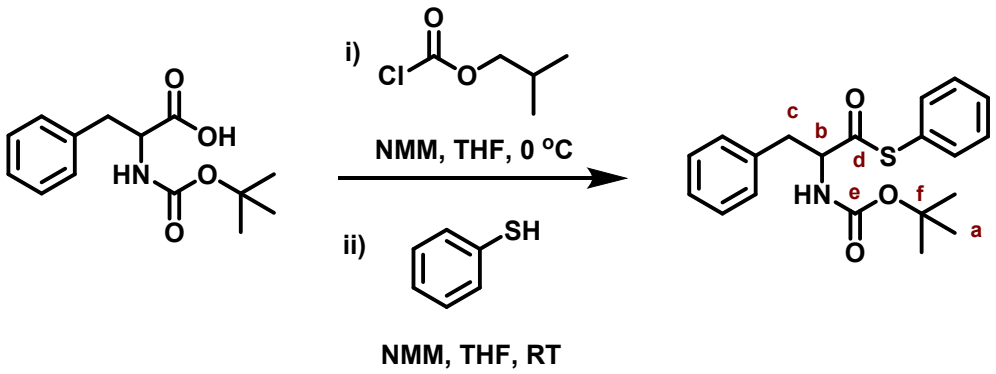


Fig. S4. A) ^{1}H NMR and B) ^{13}C NMR of Cys_PEG (**2**), in d_6-DMSO; C) Enlarged MALDI-ToF-MS of **2** with DP$_n$ = 41 corresponding to $C_{86}H_{174}N_2NaO_{42}S$ (Table S1); D) Enlarged MALDI-ToF-MS of **2'**.

Phenyl 2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanethioate (BocPheSPh)



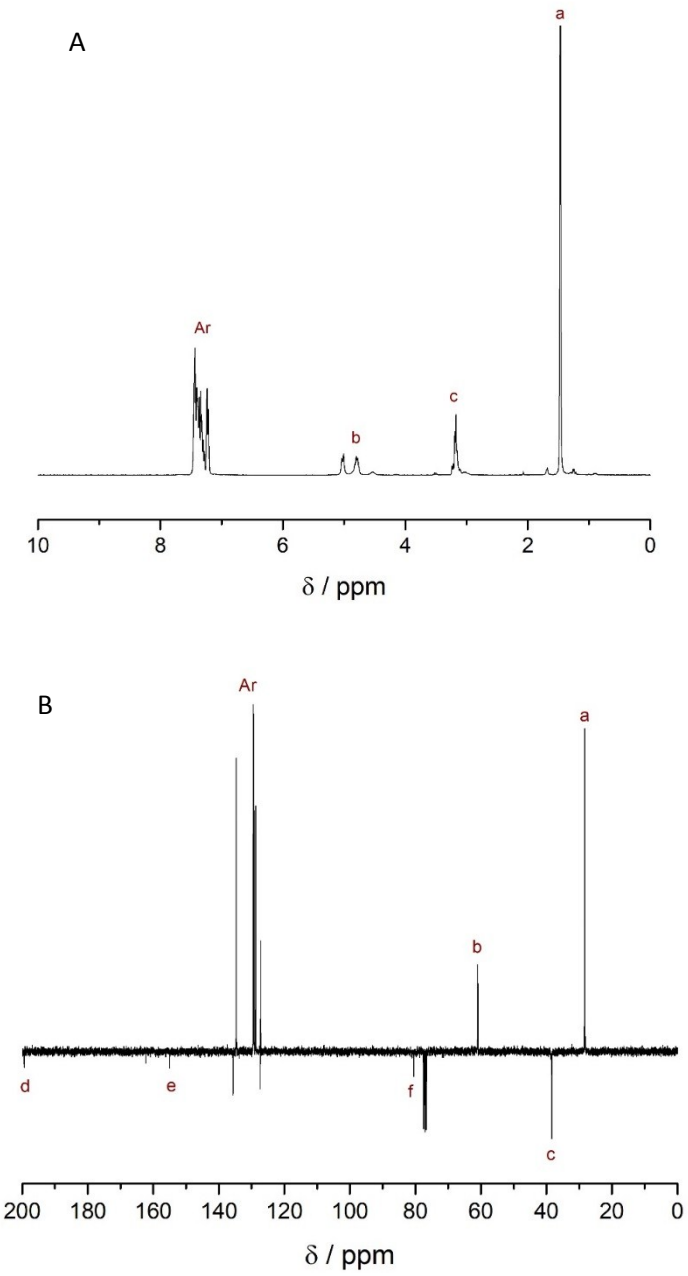
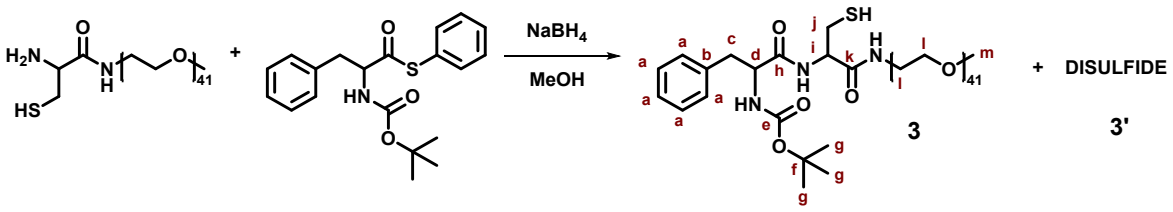


Fig. S5. A) ^{1}H NMR and B) ^{13}C NMR of BocPheSPh in $CDCl_3$.

Native chemical ligation using BocPheSPh



The general procedure for native chemical ligation was followed using BocPheSPh as the thioester to yield **3** as a white solid.

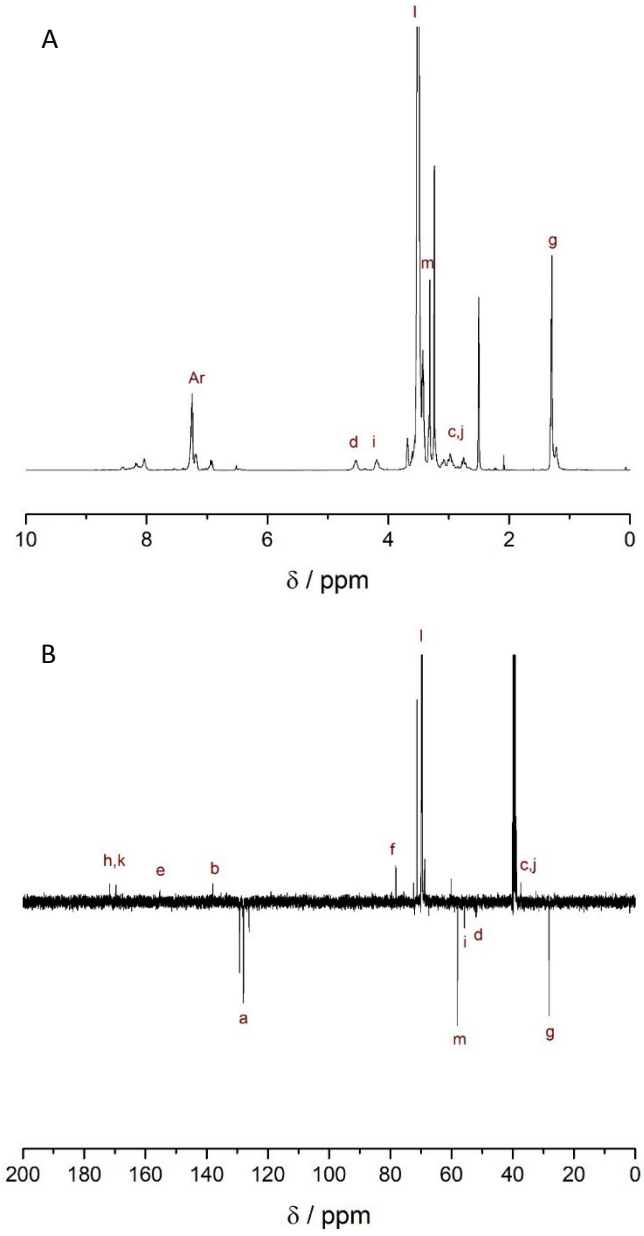


Fig. S6. A) ^{1}H NMR and B) ^{13}C NMR of **3/3'** in d_6-DMSO.

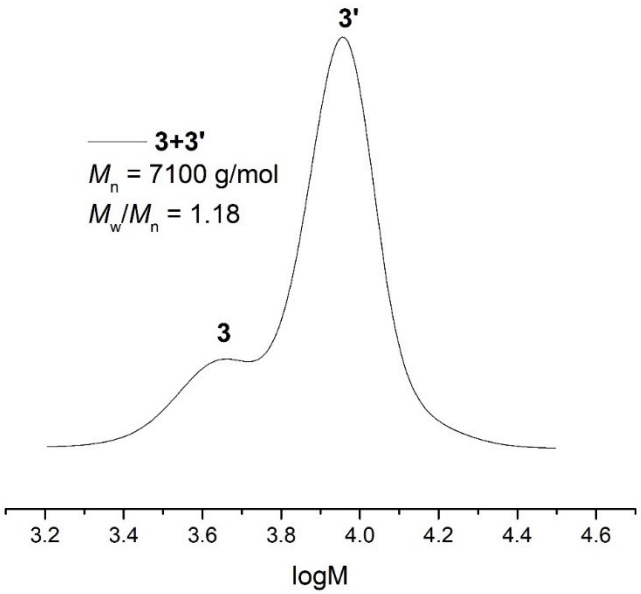


Fig. S7. SEC trace (THF) of **3/3'**.

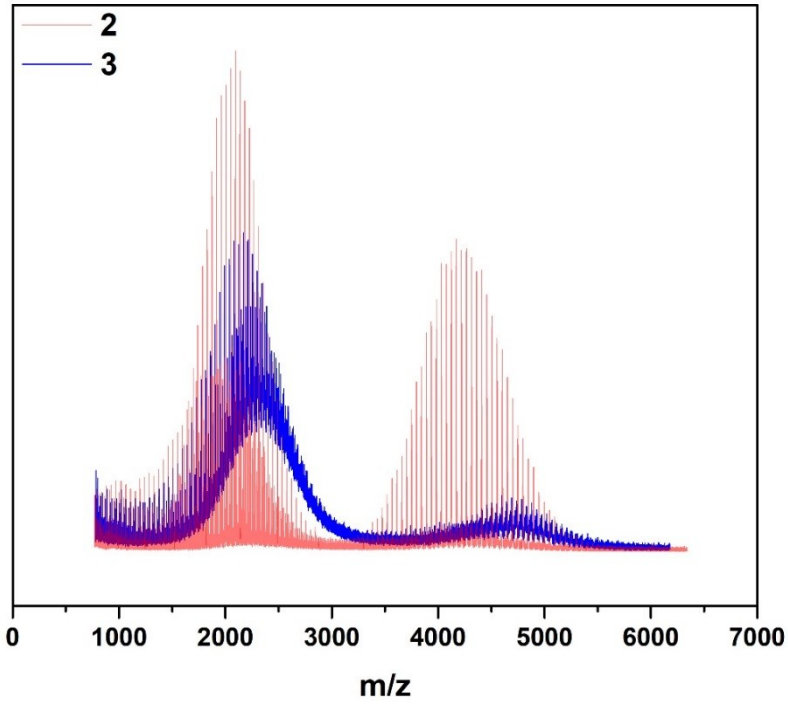


Fig. S8. MALDI-ToF-MS overlay showing the shift in distribution following the NCL reaction of **2/2'** (red) with BocPheSPh to form **3/3'** (blue). The 44 Da PEG repeating unit was retained (Table S1).

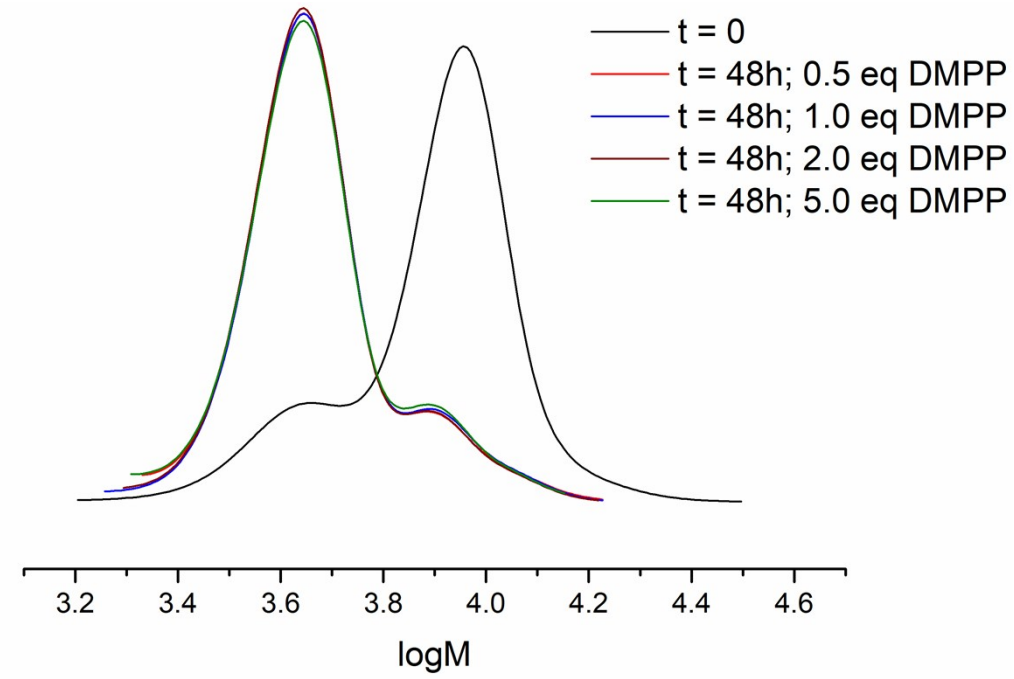
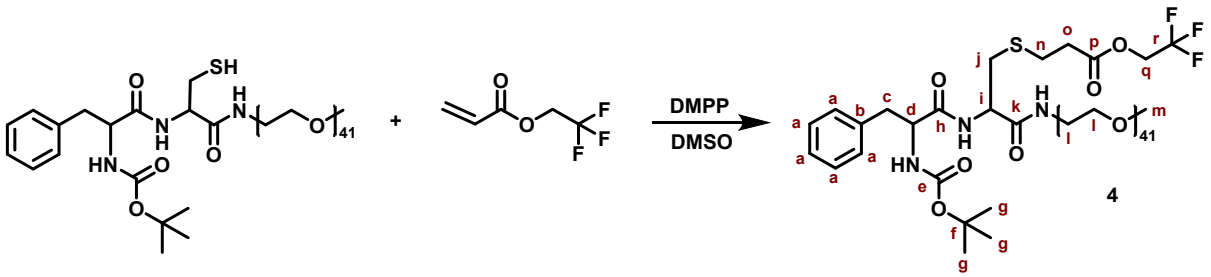


Fig. S9. SEC (THF) chromatograms at t = 48h, demonstrating the in-situ reduction of **3**' to **3** in the presence of increasing amounts of DMPP.

Thiol-Michael addition using 2,2,2-trifluoroethyl acrylate (TFEA)



The general procedure for thiol-Michael addition was followed using 2,2,2-trifluoroethyl acrylate. The pure product **4** was isolated as a white solid.

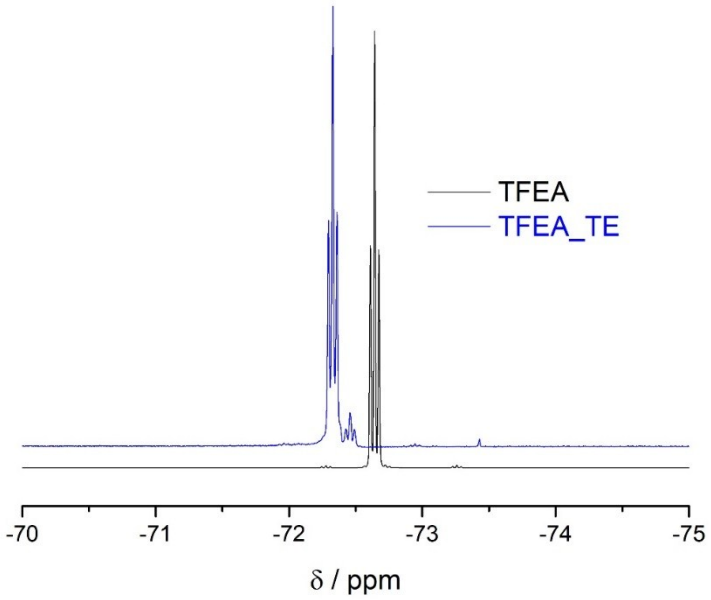


Fig. S10. ^{19}F NMR of TFEA (black) and thiol-ene product **4** (blue).

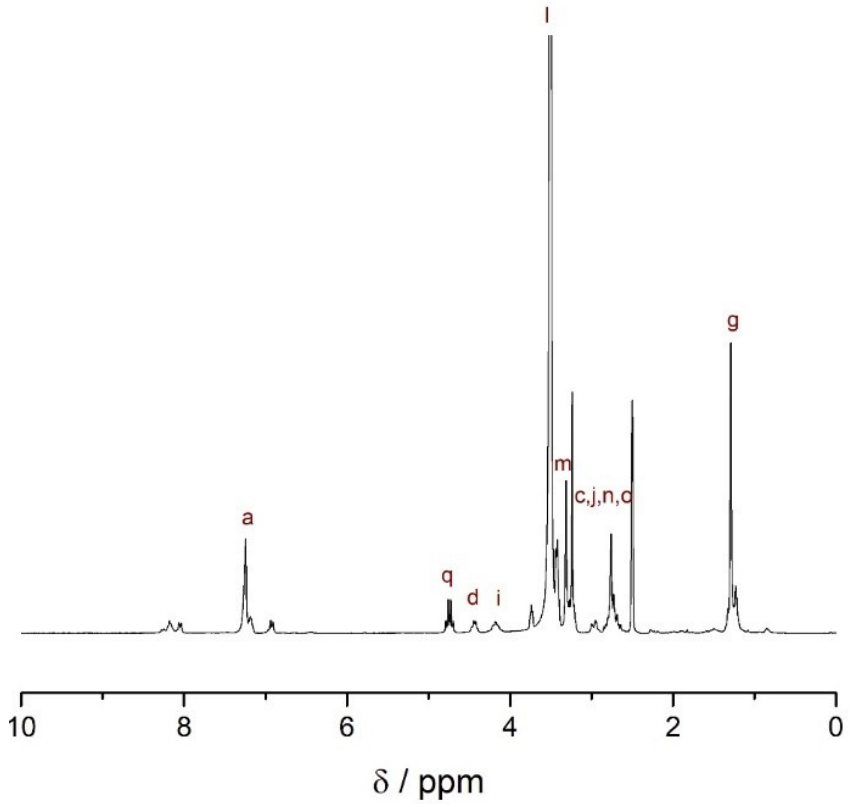


Fig. S11. ^{1}H NMR of modified polymer **4** upon *in-situ* reduction (DMPP) and thiol-Michael addition using polymer **3** and TFEA as a model acrylate.

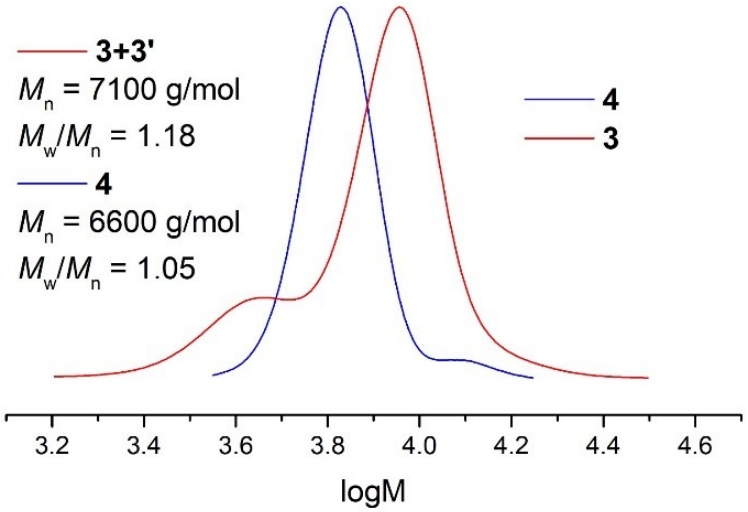
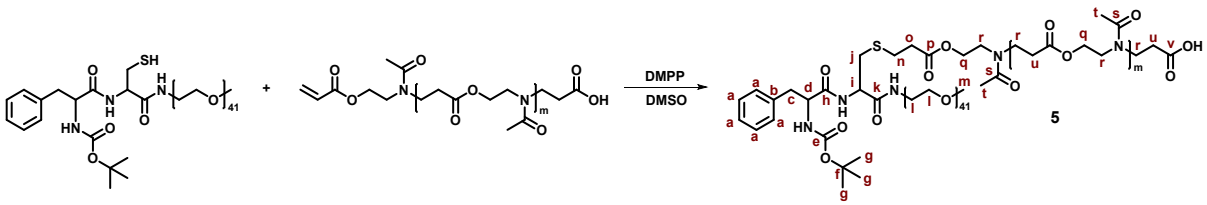


Fig. S12 SEC (DMF) thiol-Michael addition product **4** of the reaction between polymer **3** and TFEA.

Thiol-Michael addition using oligo(MeOx-alt-AA)$_n$A macromonomer



The general procedure for thiol-Michael addition was followed using oligo(MeOx-alt-AA)$_n$A . The pure product **5** was isolated as a white solid.

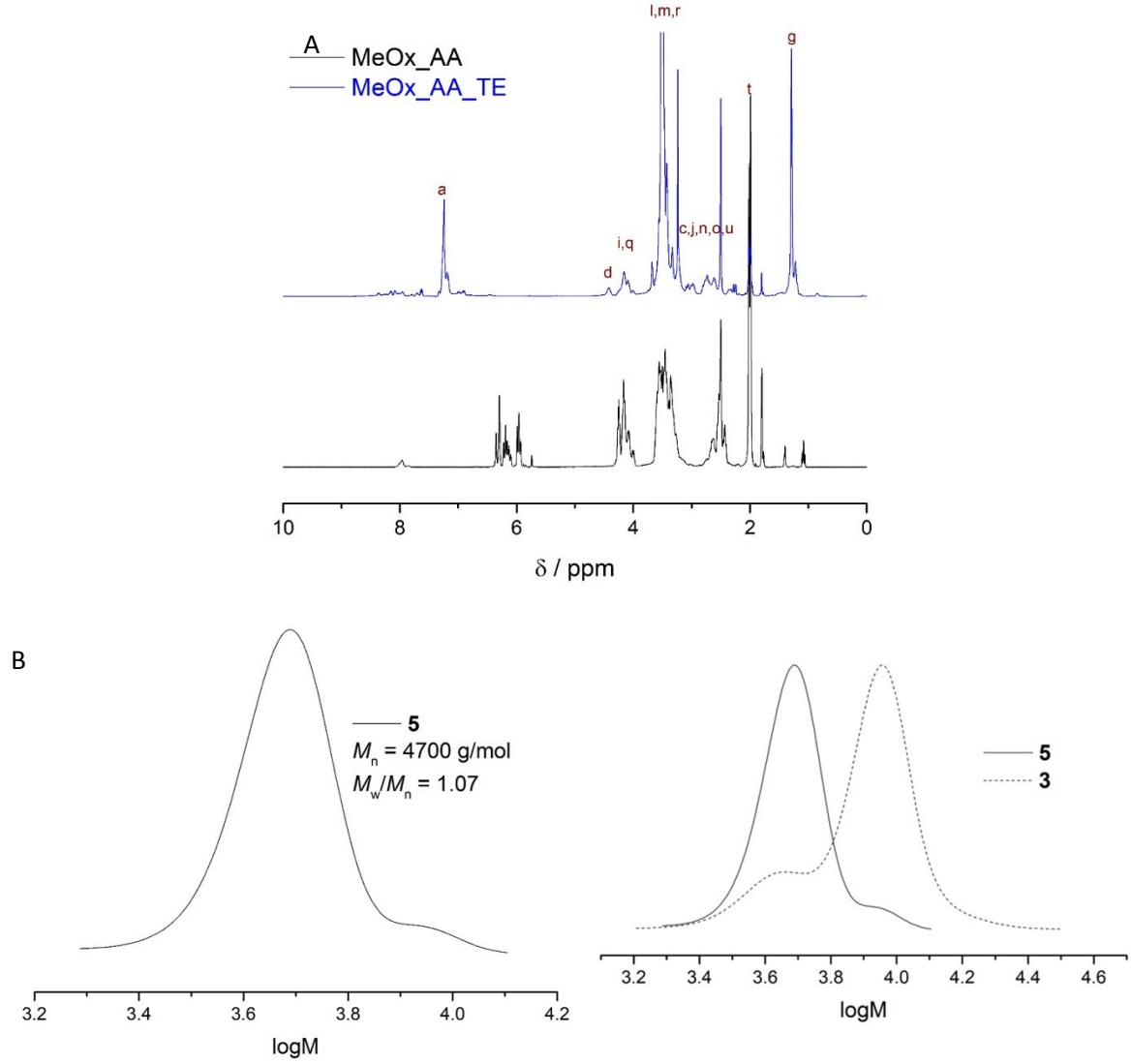
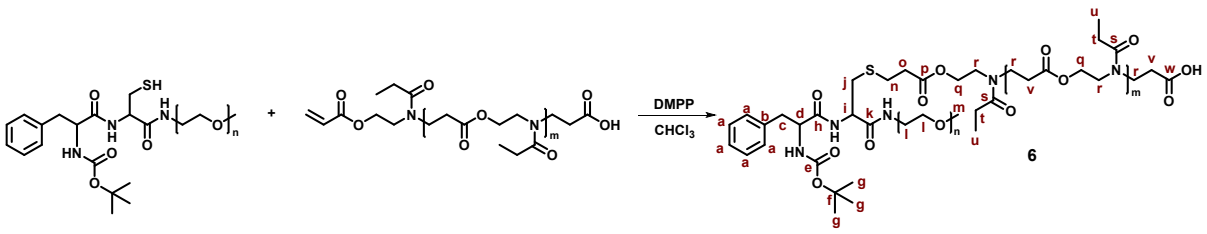


Fig. S13. (A) ^{1}H NMR (d_6-DMSO) overlay of oligo(MeOx-alt-AA)$_n$A (MeOx_AA) before (black) and after (**5**, blue) thiol-ene reaction with NCL product **3**; (B) SEC (THF) showing molecular weight data for **5** and the change in molecular weight distribution relative to **3**.

Thiol-Michael addition using oligo(EtOx-alt-AA)$_n$A macromonomer



The general procedure for thiol-Michael addition was followed using oligo(EtOx-alt-AA)$_n$A. The pure product **6** was isolated as a white solid.

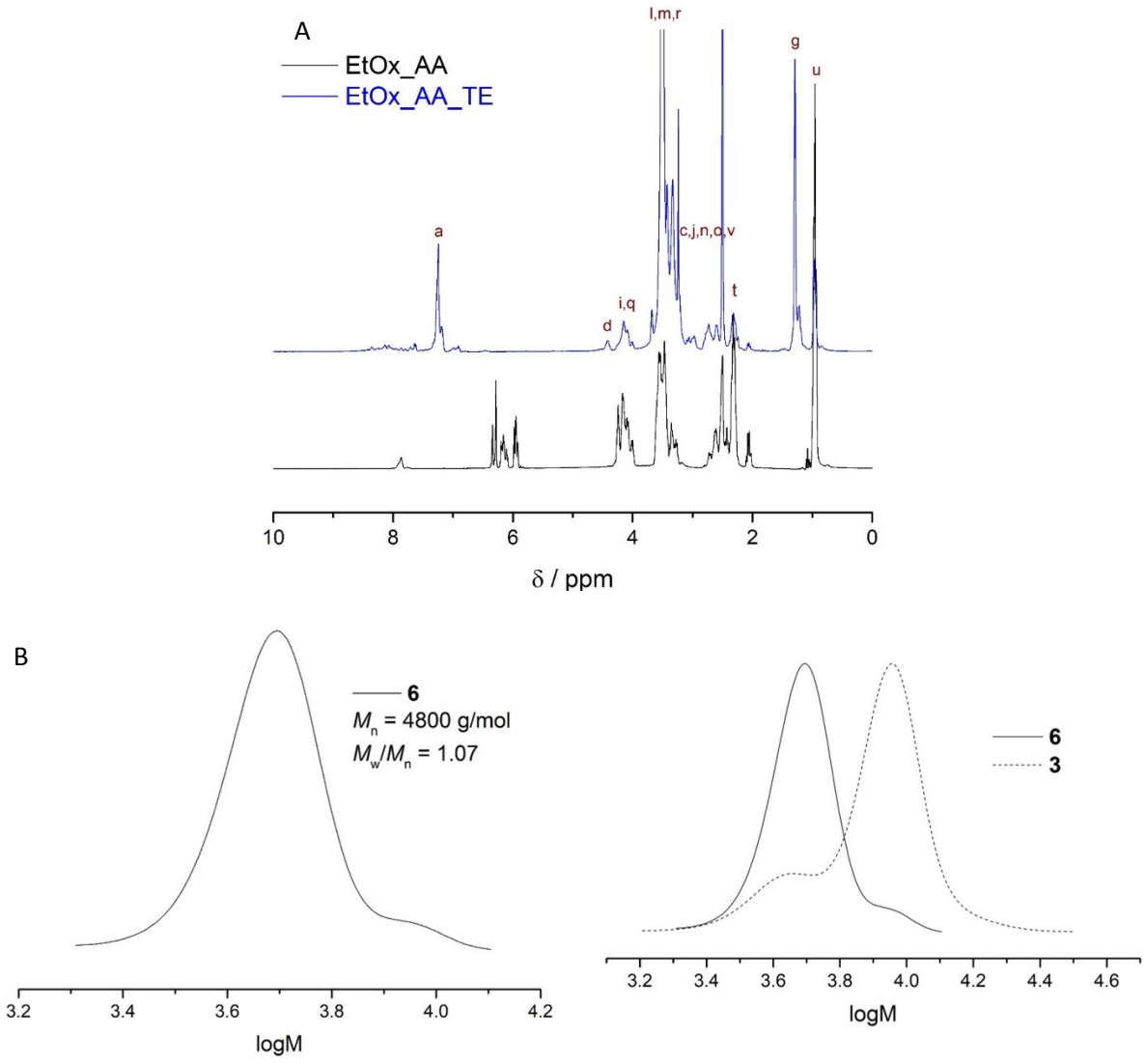
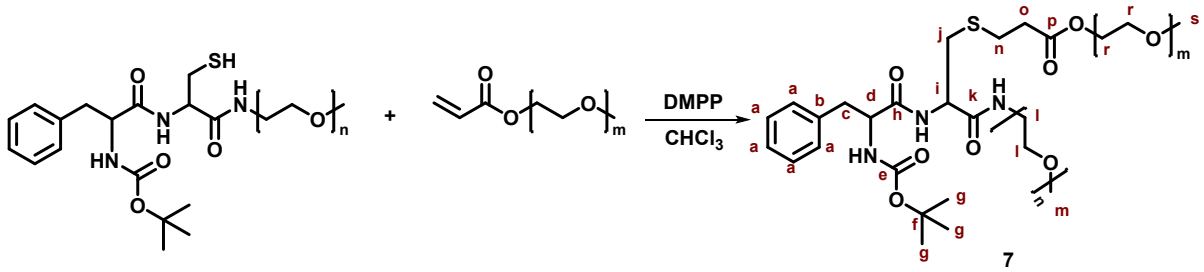


Fig. S14. (A) ^{1}H NMR (d_6-DMSO) overlay of oligo(MeOx-alt-AA)$_n$A (EtOx_AA) before (black) and after (**6**, blue) thiol-ene reaction with NCL product **3**; (B) SEC (THF) showing molecular weight data for **6** and the change in molecular weight distribution relative to **3**.

Thiol-Michael addition using $PEGA_{480}$



The general procedure for thiol-Michael addition was followed using $PEGA_{480}$. The pure product **7** was isolated as a white solid.

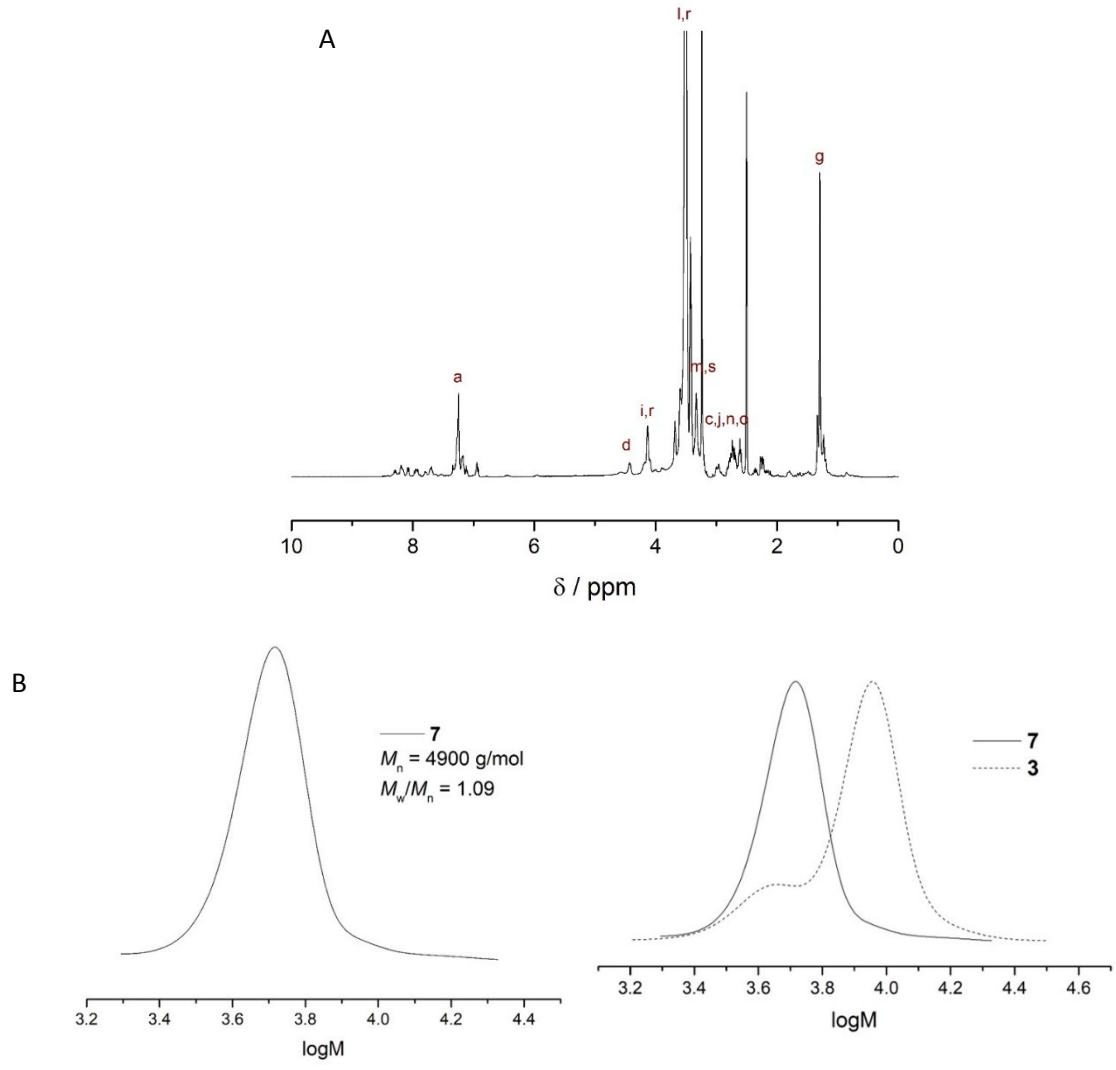
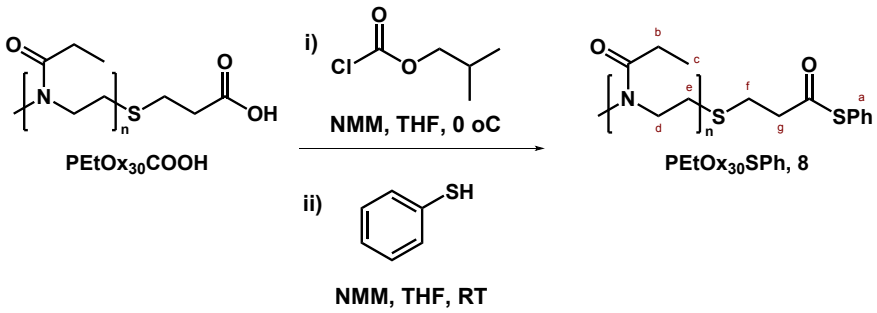


Fig. S15. (A) ^{1}H NMR (d_6-DMSO) overlay of $PEGA_{480}$ before (black) and after (**7**, blue) thiol-ene reaction with NCL product **3**; (B) SEC (THF) showing molecular weight data for **7** and the change in molecular weight distribution relative to **3**.

Synthesis of $PEtOx_{30}$-COSPh, 8



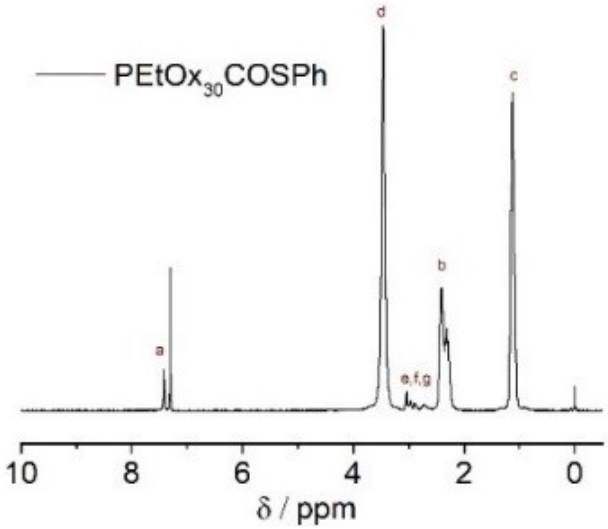


Fig. S16. ^{1}H NMR ($CDCl_3$) of $PEtOx_{30}COSPh$, **8**

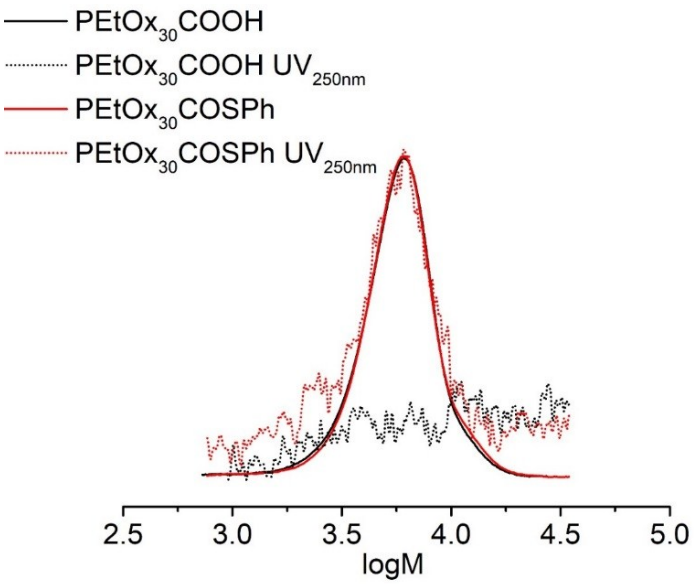
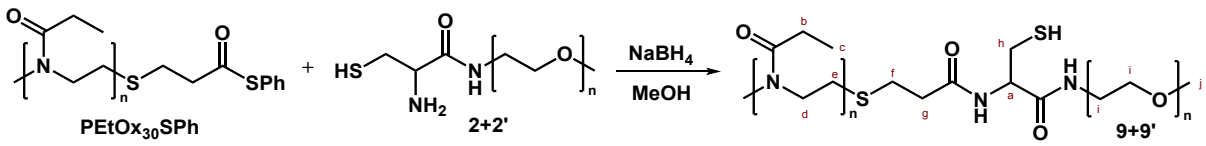


Fig. S17. SEC (DMF) of $PEtOx_{30}COOH$ (black) and $PEtOx_{30}COSPh$ (red) showing traces collected from DRI (solid) and UV (λ = 250 nm, dashed) detectors.

Native chemical ligation using $PEtOx_{30}$-COSPh



The general procedure for native chemical ligation was followed using $PEtOx_{30}$-COSPh as the thioester to yield **9** as a white solid.

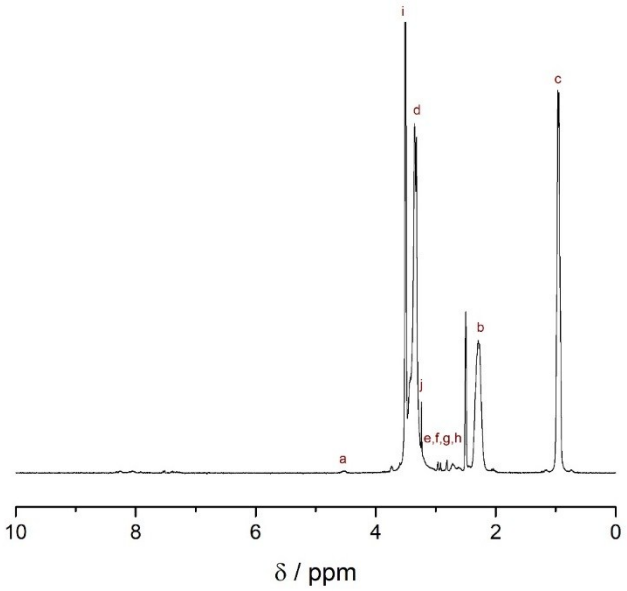


Fig. S18. ^{1}H NMR (d_6-DMSO) of the **9**.

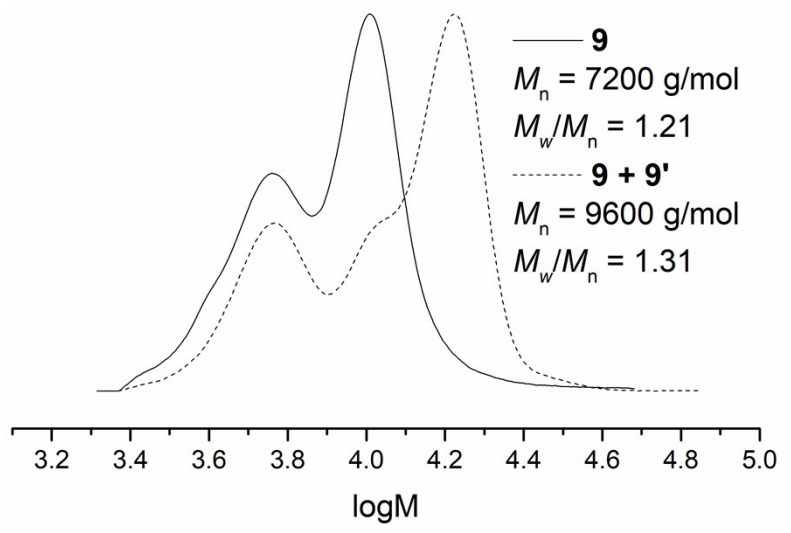
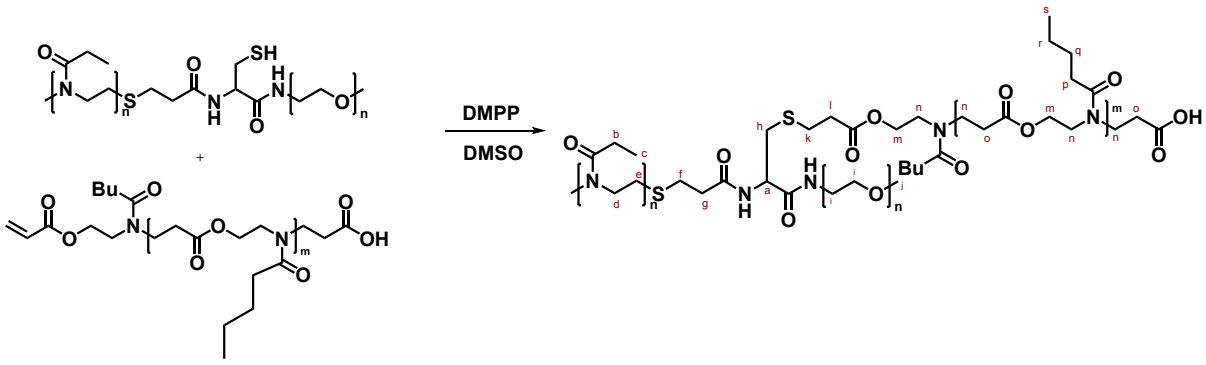


Fig. S19. SEC (DMF) of **9+9'** before (dash) and after reduction to yield **9** (solid). Low molecular weight shoulder corresponds to unreacted $PEtOx_{30}COSPh$ (**8**) which was not removed during dialysis against water (nMWCO = 3500 g/mol).

Thiol-Michael addition of 9 to macromonomer oligo(ButOx-alt-AA)$_n$A



The general procedure for thiol-Michael addition was followed using oligo(ButOx-alt-AA)$_n$A. The crude product was initially purified against water using a regenerated cellulose membrane (nMWCO = 3500 g/mol) which furnished a bimodal distribution (Fig. 3A, blue dash). Further purification by centrifugal filtration (nMWCO = 10000 g/mol) removed the low

molecular weight impurity (PEtOx derived from **8**, $M_n \approx 3100$ g/mol) to furnish pure miktoarm star polymer **10** as a white solid (Fig. 3A, blue solid; Fig 3B).

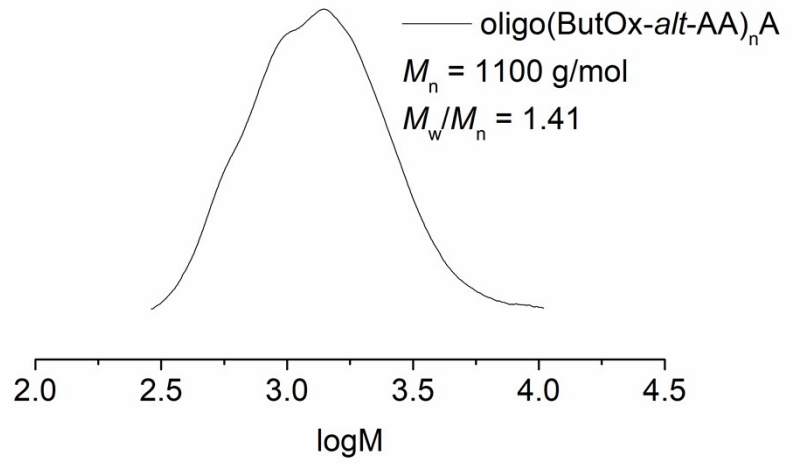


Fig. S20. SEC (DMF) of oligo(ButOx-alt-AA)nA .

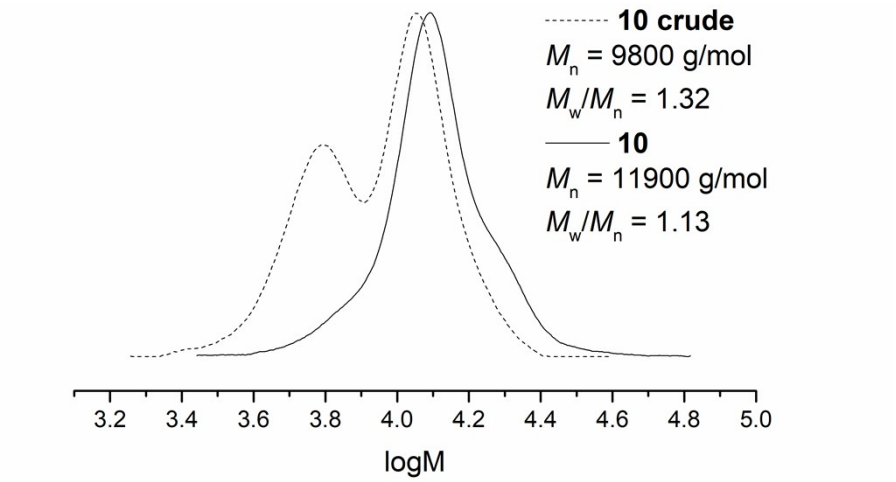


Fig. S21. SEC (DMF) of **10** before (dash) and after purification (solid). Low molecular weight shoulder corresponds to unreacted $PEtOx_{30}COSPh$ (**8**) which was removed via centrifugal filtration (nMWCO = 10000 g/mol).