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)

![Chemical structure of Tz4CA]

Acetone, 70°C

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)

\[
\begin{align*}
\text{HCOONa, HCOOH} & \quad \text{Ac}_2O \\
\end{align*}
\]

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

\[
\text{N}-\text{O} \quad \text{O} \\
\text{S} \quad \text{N} \\
\text{O} \quad \text{H} \\
\text{O} \quad \text{O} \\
\text{O} \quad \text{O} \\
\text{N} \quad \text{H} \\
\text{N} \quad \text{H} \\
\text{O} \\
\text{O} \\
\text{CDI} \\
\text{CHCl}_3
\]

A

\[\delta / \text{ppm}\]

B

\[\delta / \text{ppm}\]
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 $D_P = 41$ corresponding to $C_{90}H_{178}N_2O_{43}S$ (Table S1).

Table S1. Molecular weight data obtained from MALDI-ToF-MS for $\alpha$-methoxy-$\omega$-functional-PEG polymers 1 – 4.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Empirical Formula</th>
<th>Adduct</th>
<th>$m/z_{th}$</th>
<th>$m/z_{obs}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG(Amine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>$C_{84}H_{169}NO_{41}$</td>
<td>M+Na</td>
<td>1859.106</td>
<td>1859.166</td>
</tr>
<tr>
<td>1</td>
<td>$C_{90}H_{178}N_2O_{43}S$</td>
<td>M+Na</td>
<td>2030.142</td>
<td>2030.301</td>
</tr>
<tr>
<td>2</td>
<td>$C_{88}H_{174}N_2O_{42}S$</td>
<td>M+Na</td>
<td>1962.115</td>
<td>1962.119</td>
</tr>
<tr>
<td>3</td>
<td>$C_{100}H_{191}N_3O_{45}S$</td>
<td>M+Na</td>
<td>2209.236</td>
<td>2209.188</td>
</tr>
<tr>
<td>4</td>
<td>$C_{105-}H_{196}F_3N_3O_{47}S$</td>
<td>M+Na</td>
<td>2363.260</td>
<td>2363.406</td>
</tr>
</tbody>
</table>

$^a$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)

\[
\begin{align*}
\text{PEG-cysteine} & \xrightarrow{\text{HCl}} \text{HSNH}_2C\text{O}_2\text{H} + \text{HSNH}_2C\text{O}\text{H}_2
\end{align*}
\]
Fig. S4. A) $^1H$ 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)

![Chemical structure of BocPheSPh](image)

**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 $3$. 

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Thiol-Michael addition using PEGA

The general procedure for thiol-Michael addition was followed using PEGA\textsubscript{480}. The pure product 7 was isolated as a white solid.

**Fig. S15.** (A) \textsuperscript{1}H NMR (\textit{d}_{6}-DMSO) overlay of PEGA\textsubscript{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 PEtO$_{30}$-COSPh, 8

Fig. S16. $^1$H NMR (CDCl$_3$) of PEtO$_{30}$COSPh, 8

Fig. S17. SEC (DMF) of PEtO$_{30}$COOH (black) and PEtO$_{30}$COSPh (red) showing traces collected from DRI (solid) and UV ($\lambda = 250$ nm, dashed) detectors.
Native chemical ligation using PEtOx\textsubscript{30}-COSPh

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

Fig. S18. \textsuperscript{1}H NMR (\textit{d\textsubscript{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 PEtO$_3$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)$_n$A.

![SEC (DMF) of oligo(ButOx-alt-AA)$_n$A](image)

10 crude
$M_n = 9800$ g/mol
$M_w/M_n = 1.32$

10
$M_n = 11900$ g/mol
$M_w/M_n = 1.13$

**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).