Electronic Supplementary Material (ESI) for Polymer Chemistry.

Enhancing Conjugation Yield of Brush Polymer-Protein Conjugates by Increasing Linker Length at the Polymer End-Group

Peter C. Nauka, Juneyoung Lee, Heather D. Maynard*

Department of Chemistry and Biochemistry and California NanoSystems Institute, University of California, Los Angeles, 607 Charles E. Young Drive South, Los Angeles, California 90095-1569, United States

*Email: maynard@chem.ucla.edu Phone: +1 (310) 267-5162

Supporting Information

Synthesis of CTA1

Due to the availability of 2-mercaptoethanol, **CTA1** was synthesized using a shortened scheme. **3** and **CTA1** were synthesized according to previous reports.[1] **3** (489.2 mg, 2.61 mmol) and 2-(ethylsulfanylthiocarbonyl sulfanyl)-propionic acid (493.0 mg, 2.34 mmol) were dissolved in 50 mL of dry DCM. The reaction was stirred at 0 °C for 20 minutes, after which, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1367 mg, 7.13 mmol) and 4-(dimethylamino) pyridine (DMAP, 58.1 mg, 0.48 mmol) were added. The reaction changed color from yellow to red. The ice bath was removed after 5 hours the solution became yellow, and the reaction was stopped after 24 hours. The resulting solution was washed with 30 mL of H_2O three times and the organic layer was dried with $MgSO_4$. Silica gel column chromatography (100% EtOAc) was performed to elute a yellow oil, which was then further purified by HPLC (10:90 H_2O:MeOH) to yield 338 mg product (34% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.48-8.46(m, 1H), 7.70-7.63 (m, 2H), 7.12-7.09 (m, 1H), 4.81 (q, J = 7.46, 1H), 4.43-4.36 (m, 2H), 3.36 (q, J = 7.4 Hz, 2H), 3.04 (t, J = 6.43 Hz, 2H), 1.60 (d, J = 7.55 Hz, 3H), 1.35 (t, J = 7.43, 3H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 221.77, 170.94, 159.67, 149.76, 137.15, 120.91, 119.80, 63.35, 47.74, 37.16, 31.58, 16.75, 12.99. MS (ESI-MS) calc. for $C_{13}H_{17}NO_2S_5H$: 379.99 observed: 379.99

Synthesis CTA2

CTA2 was synthesized according to previous reports.[1] **2a** (726.0 mg, 2.64 mmol) and 2-(ethylsulfanylthiocarbonyl sulfanyl)-propionic acid (504.0 mg, 2.40 mmol) were dissolved in 24 mL of dry DCM. The reaction was stirred at 0 °C for 20 minutes, after which, EDC (1103 mg, 5.76 mmol) and DMAP (58.55 mg, 0.48 mmol) were added. The ice bath was removed after 5 hours, and the reaction was stopped after 24 hours. The solution was washed with 30 mL of H_2O three times and the organic layer was dried with $MgSO_4$. Silica gel column chromatography (100% EtOAc) was performed to elute a yellow oil, which was then further purified by HPLC (10:90 H_2O:MeOH) to yield 456 mg of product (41% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.46-8.45 (m, 1H), 7.79-7.77 (m, 1H), 7.68-7.64 (m, 1H), 7.10-7.08 (m, 1H), 4.83 (q, J = 7.31 Hz, 1H), 4.33-4.26 (m, 2H), 3.74-3.67 (m, 4H), 3.64-3.61 (m, 2H), 3.59-3.56 (m, 2H), 3.00 (t, J = 6.3 Hz, 2H), 1.60 (d, J = 7.45 Hz, 3H), 1.34 (t, J = 7.43 Hz, 3H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 221.82, 171.15, 160.46, 149.58, 137.13, 120.67, 119.63, 70.596, 70.46, 69.03, 68.99, 64.92, 47.90, 38.44, 31.55, 16.90, 12.99. MS (ESI-MS) calc. for $C_{17}H_{25}NO_4S_5H$: 468.05 observed 468.05.

Synthesis of 1b

A previous literature procedure was adapted for this reaction.[2] Tetraethylene glycol (5 g, 25.74 mmol) and *p*-tosyl chloride (2.45 g, 12.85 mmol) were cooled to 0 °C in a reaction flask. Triethylamine (3.23 mL, 23.12 mmol) was added dropwise into the reaction flask and the reaction stirred for 1 hour at 0 °C. The reaction was then allowed to warm up to 25 °C for 16 hours. Triethylamine was quenched with concentrated HCl and the solution was washed with 50 mL of H_2O and DCM three times. The organic layer was removed and dried with $MgSO_4$. Silica gel column chromatography (100% EtOAc) produced 2.35 g product (**1b**, 43% yield) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.80 (d, J = 8.25 Hz, 2H), 7.33 (d, J = 8 Hz, 2H), 4.16 (t, J = 4.8 Hz, 2H), 3.72-3.59 (m, 16H), 2.45 (s, 3H), 1.66 (broad s, 2H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 144.71, 132.89, 129.71, 127.88, 72.35, 70.64, 70.55, 70.36, 70.23, 69.12, 68.61, 61.65, 21.53. MS (ESI-MS) calc. for $C_{15}H_{24}O_7SNa^+$: 371.1140 observed: 371.1550. UV/Vis (DMF) λ=263, ε=665.59 $cm^{-1}M^{-1}$.

Synthesis of 2b

1b (1.94 g, 5.6 mmol) and thiourea (0.425 g, 5.6 mmol) were dissolved in 18 mL of absolute EtOH.[2] The reaction solution was refluxed at 90 °C for 24 hours. Sodium hydroxide (0.57 g, 14.39 mmol) was dissolved in 2 mL H_2O and 18 mL EtOH and added to the reaction flask. The reaction was refluxed at 90 °C for 2.5 additional hours prior to being cooled to 25 °C. Concentrated HCl was added until pH 2 was reached. The resultant solution was filtered with EtOAc/EtOH 5:1 and solvent evaporated to yield yellow oil. In order to prevent disulfide formation, the unpurified product was immediately used in the next reaction.

Synthesis of 3b

Aldrithiol (3.69 g, 16.74 mmol) was dissolved in 23mL MeOH and 1.85 mL glacial acetic acid in a reaction flask. **2b** (1.17 mg, 5.58 mmol) was dissolved in 20 mL MeOH and slowly added to the reaction flask. The solution color changed from clear to yellow-green. The reaction was stirred rapidly for 24 hours. Afterwards, the solvent was evaporated and the resultant solid washed with 30 mL of H_2O three times. The organic layer was removed and dried with $MgSO_4$. Silica gel column chromatography (100% EtOAc) produced 244.9 mg of clear oil (**3b**, 12.2% yield, two steps). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.45-8.44 (m, 1H), 7.77-7.76 (m, 1H), 7.67-7.63 (m, 1H), 7.09-7.06 (m, 1H), 3.73-3.70 (m, 4H), 3.68-3.61 (m, 6H), 3.61-3.57 (m, 4H), 2.99 (t, J = 6.4 Hz, 2H), 2.61 (broad s, 1H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 160.30, 149.39, 137.01, 120.52, 119.54, 72.38, 70.55, 70.37, 70.26, 70.24, 68.89, 61.62, 38.24. MS (ESI-MS) calc. for $C_{13}H_{21}NO_4S_2H$: 320.0990 observed: 320.0994. UV/Vis (DMF) λ=282, ε=6268 $cm^{-1}M^{-1}$.

Synthesis of CTA3

This synthesis was followed according to literature procedure.[1] **3b** (647.1 mg, 2.03 mmol) and 2-(ethylsulfanylthiocarbonyl sulfanyl)-propionic acid (406.00 mg, 1.93 mmol) were dissolved in 22 mL of dried DCM. The reaction was stirred at 0 °C for 20 minutes, after which, EDC (889 mg, 4.63 mmol) and DMAP (47.4 mg, 0.39 mmol,) were added. After 5 hours, the ice bath was removed and the reaction was stopped after 24 hours. The resultant solution was washed with 30 mL of H_2O three times and the organic layer was dried with $MgSO_4$. Silica gel column chromatography (100% EtOAc) was performed to elute a yellow oil, which was then further purified by HPLC (10:90 H_2O:MeOH) to yield 552.3 mg product (51% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.45-8.44 (m, 1H), 7.78-7.76 (m, 1H), 7.68-7.64 (m, 1H), 7.10 (m, 1H), 4.82 (q, J = 7.4 Hz, 1H), 4.32-4.26 (m, 2H), 3.74-3.67 (m, 4H), 3.66-3.62 (m, 6H), 3.59-3.57 (m, 2H), 3.40-3.30 (m, 2H), 2.99 (t, J = 6.5 Hz, 2H), 1.60 (t, J = 3.7 Hz, 3H), 1.34 (t, J = 7.45, 3H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 221.81, 171.16, 160.43, 149.56, 137.15, 120.64, 119.64, 70.70, 70.59, 40.45, 68.99, 68.89, 64.93, 47.92, 38.43, 31.54, 19.92, 13.00. MS (ESI-MS) calc. for $C_{19}H_{29}NO_5S_5H$: 512.0729 observed: 512.0745. UV/Vis (DMF) λ=304, ε=11844 $cm^{-1}M^{-1}$.

Synthesis of 1c

A previous literature procedure was adapted for this reaction.[2] Hexaethylene glycol (2.61 g, 9.25 mmol) and tosyl chloride (0.88 g, 4.62 mmol) were cooled to 0 °C in a reaction flask. Triethylamine (0.84 g, 8.31 mmol) was added dropwise into the reaction flask and the reaction was stirred for 1 h at 0 °C. The reaction was then allowed to warm up to 25 °C for 16 h. Triethylamine was quenched with concentrated HCl and the solution was washed with 50 mL of H_2O and DCM three times. The organic layer was removed and dried with $MgSO_4$. Silica gel

column chromatography (100% EtOAc) produced 1.01 g product (**1c**, 52% yield) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.79 (d, J = 8.25 Hz, 2H), 7.33 (d, J = 8 Hz, 2H), 4.15 (t, J = 4.85 Hz, 2H), 3.71-3.57 (m, 24H), 2.44 (s, 3H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 144.81, 132.98, 129.84, 128.00, 72.61, 70.70, 70.59, 70.53, 70.51, 70.49, 70.25, 69.30, 68.68, 61.70, 21.66. MS (ESI-MS) calc. for $C_{19}H_{32}O_9SNa^+$: 459.1665 observed: 459.1655. UV/Vis (DMF) λ=263, ε=672 $cm^{-1}M^{-1}$.

Synthesis of 2c

1c (1.01 g, 2.39 mmol) and thiourea (0.182 g, 2.39 mmol) were dissolved in 7.8 mL of absolute EtOH.[2] The reaction solution was refluxed at 90 °C for 24 hours. Sodium hydroxide (0.246 g, 6.15 mmol) was dissolved in 0.75 mL H_2O and 7.8 mL EtOH and added to the reaction flask. The reaction was refluxed at 90 °C for 2.5 additional hours prior to being cooled to 25 °C. Concentrated HCl was added until pH 2. The resultant solution was filtered with EtOAc/EtOH 5:1 and solvent evaporated to yield yellow oil. In order to prevent degradation, the unpurified product was immediately used in the next reaction.

Synthesis of 3c

Aldrithiol (1.581 g, 7.18 mmol) was dissolved in 29 mL MeOH and 1.85 mL glacial acetic acid in a reaction flask. **2c** (713.9 mg, 2.39 mmol) was dissolved in 13 mL MeOH and slowly added to the reaction flask, changing solution color from clear to yellow-green. The reaction was stirred rapidly for 24 hours. Afterwards, the solvent was evaporated and resultant solid washed with 30 mL of H_2O three times. The organic layer was removed and dried with $MgSO_4$. Silica gel column chromatography (100% EtOAc) produced 116.8 mg of clear oil (**3c**, 60% yield. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.45-8.44 (m, 1H), 7.78-7.76 (m, 1H), 7.67 (m, 1H), 7.67-7.64 (m, 1H), 7.09-7.07 (m, 1H), 3.97-3.34 (m, 24H), 2.99 (d, J = 6.38 Hz, 2H), 2.0 (broad s, 1H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ: 160.47, 149.52, 137.15, 120.62, 119.63, 72.63, 70.65, 70.60, 70.54, 70.51, 70.49, 70.38, 70.26, 68.95, 61.71, 38.42. MS (ESI-MS) calc. for $C_{17}H_{29}NO_6S_2$: 408.1515 observed: 408.1508. UV/Vis (DMF) λ=283, ε=4241.6 $cm^{-1}M^{-1}$.

Synthesis of CTA4

A previous literature procedure was adapted for this reaction.[1] **3c** (576.8 mg, 1.42 mmol) and 2-(ethylsulfanylthiocarbonyl sulfanyl)-propionic acid (270.6 mg, 1.29 mmol) were dissolved in 23 mL of dry DCM. The reaction was stirred at 0 °C for 20 minutes before EDC, 591 mg, 3.08 mmol, 2.4 eq) and DMAP (31 mg, 0.25 mmol) were added. After 5h stirring under an ice bath, the reaction was moved to room temperature and stopped after 24 h. The resultant solution was washed with 30 mL of H_2O three times and the organic layer was dried with $MgSO_4$. Silica gel column chromatography (100% EtOAc) was performed to elute a yellow oil, which was then further purified by HPLC (10:90 H_2O:MeOH) to yield 535.5 mg product (66% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.45-8.44 (m, 1H), 7.78-7.77 (m, 1H), 7.68-7.64 (m, 1H), 7.09-7.07 (m, 1H), 4.83 (q, J = 5.49 Hz, 1H), 4.29 (dd, J = 0.9 Hz, J = 9.7, 2H), 3.72-3.68 (m, 6H), 3.65-3.61 (m, 15H), 3.58-3.56 (m, 3H), 3.35 (sextet, J = 6.92 Hz, 2.99 (t, J = 6.38, 2H), 1.60 (d, J = 7.4 Hz, 3H), 1.34 (t, J = 7.45 Hz, 3H). MS (ESI-MS) calc. for $C_{23}H_{37}NO_7S_5H$: 600.1252 observed: 600.1227. UV/Vis (DMF) λ=302, ε=10251 $cm^{-1}M^{-1}$.

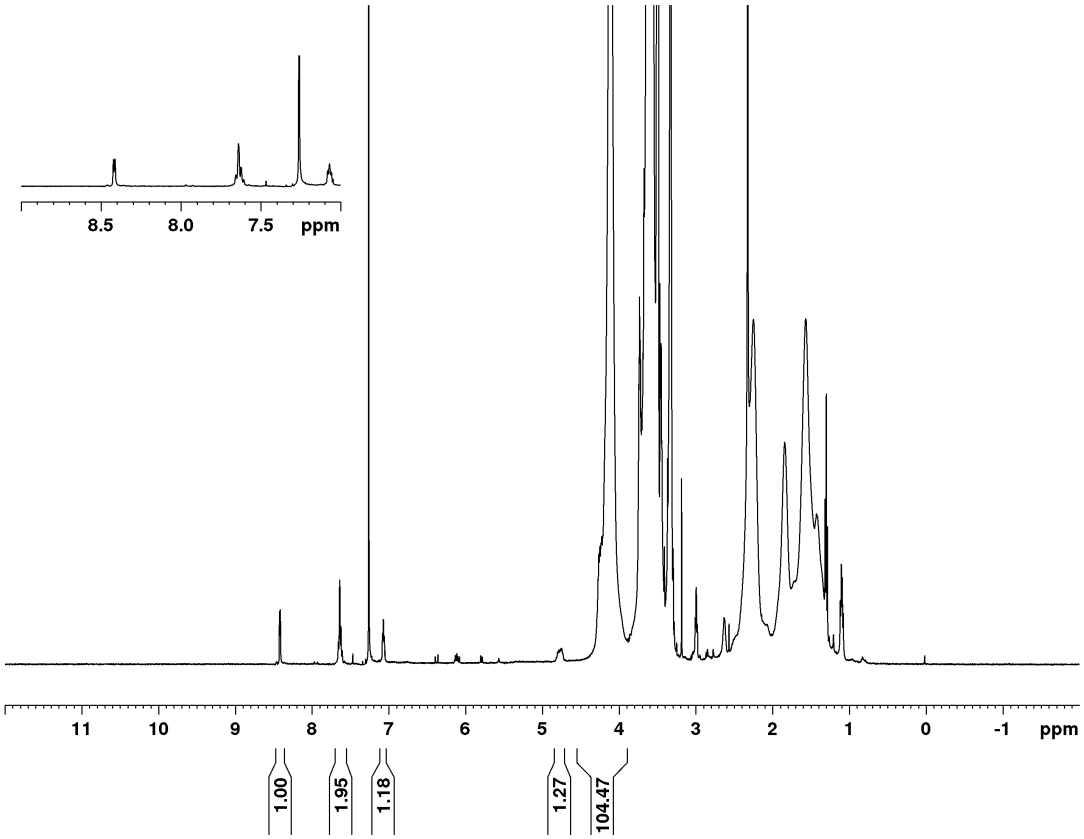

Figure S1. ^{1}H-NMR spectrum of **pPEGA1**.

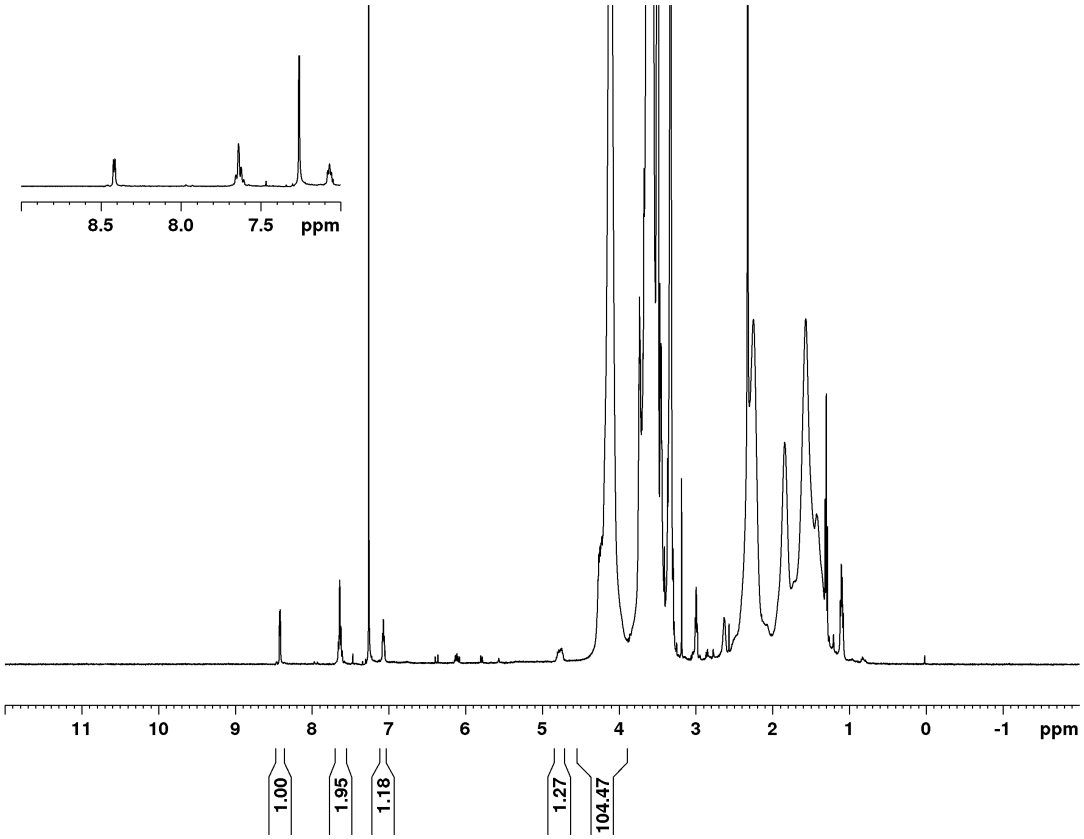

Figure S2. ^{1}H-NMR spectrum of **pPEGA2**.

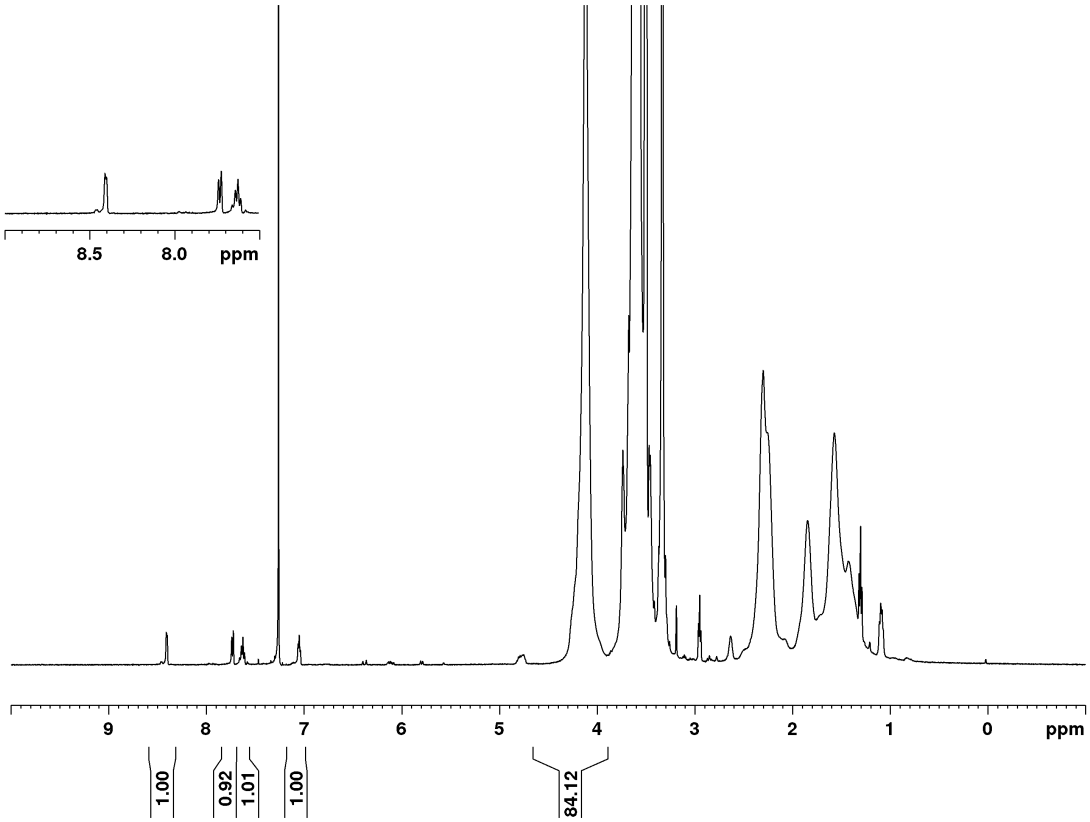

Figure S3. ^{1}H-NMR spectrum of **pPEGA3**.

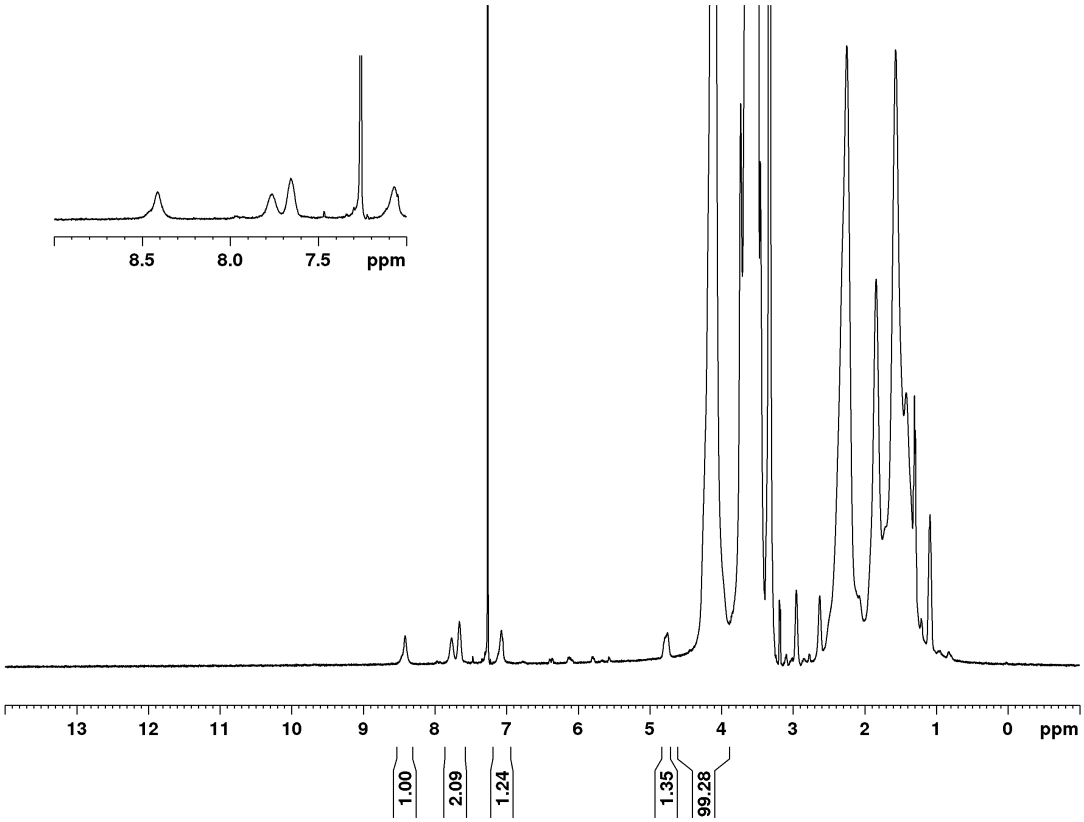

Figure S4. ^{1}H-NMR spectrum of **pPEGA4**.

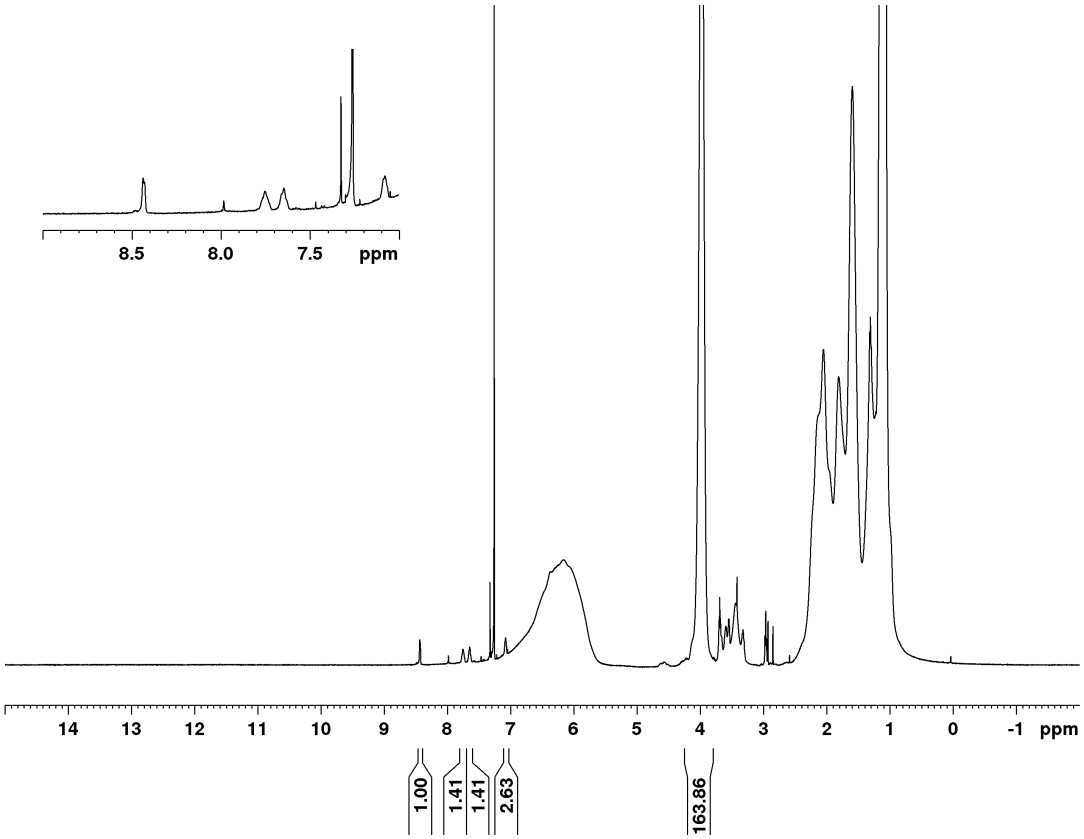

Figure S5. ^{1}H-NMR spectrum of **pNIPAAm**.

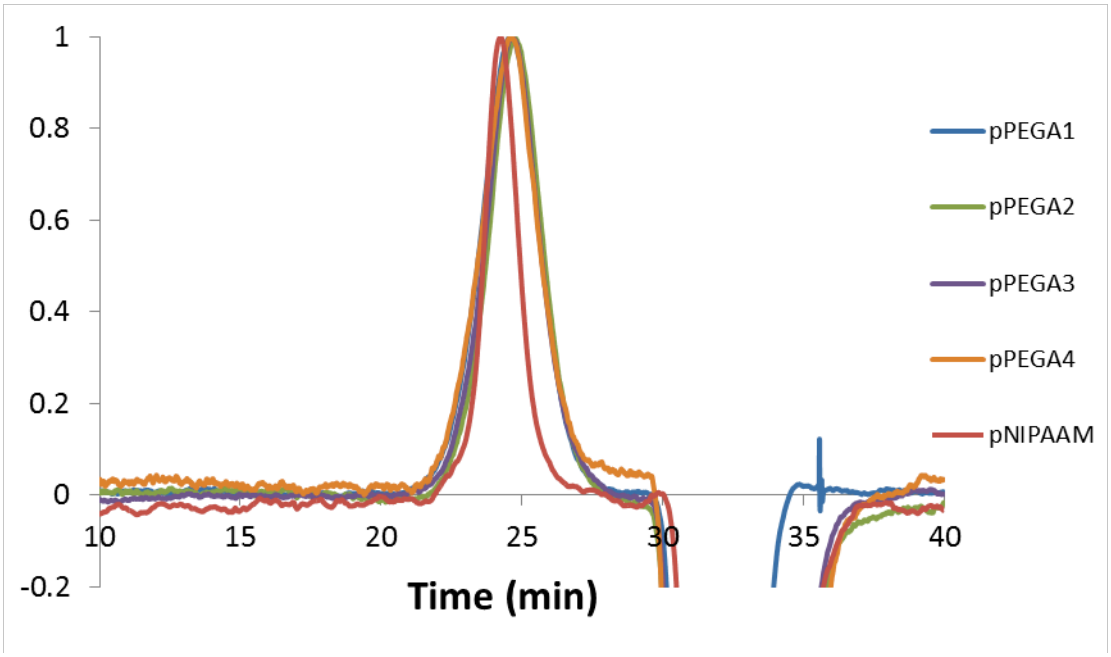

Figure S6. GPC Traces of **pPEGA1-pPEGA4, pNIPAAm**.

REFERENCES

1. R. J. Mancini, J. Lee and H. D. Maynard, *J. Am. Chem. Soc.*, 2012, **134**, 8474-8479.
2. C. Steinem, A. Janshoff, K. von dem Bruch, K. Reihs, J. Goossens and H. J. Galla, *Bioelectroch. Bioener.*, 1998, **45**, 17-26.