

## Supporting information

### **Tailored Polymeric Membranes for Mycobacterium Smegmatis Porin A (MspA) Based Biosensors**

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### Protein Insertion Study Protocols:

Experiments were carried out using a ~50 micron Teflon aperture (Eastern Scientific LLC). Lipid painting solutions of diphytanol phosphatidylcholine DPhPC (Avanti Polar Lipids, AL) were made by dissolving 1.5 mg of lipid in 75  $\mu$ L *n*-decane and 25  $\mu$ L heptanol. ABA copolymer prepaint solutions were made by dissolving 1.5mg of ABA in 100  $\mu$ L of chloroform ( $\text{CHCl}_3$ ). The ABA membrane forming solutions were made by dissolving 1.5mg of ABA in 30  $\mu$ L *n*-decane and 70  $\mu$ L of chloroform ( $\text{CHCl}_3$ ). A 1 M KCl, 10 mM HEPES/KOH solution buffered at pH ~7 was added to the cis and trans chamber of the setup. An additional 1.5  $\mu$ L of ABA in 30/70 *n*-decane/ $\text{CHCl}_3$  was added to the cis chamber and planar bilayers were formed using air bubbles from a micropipette.

Once the thinned portion of the membrane was visible, 4  $\mu$ L of 1  $\mu$ g/mL M2-MspA in 0.1% OPOE was added to the 1mL chamber and stirred gently (1  $\mu$ L for lipid to reduce insertion rate). MspA mutant porin M2-MspA was selectively prepared and extracted from *M. smegmatis*.

Conductance measurements were taken using an Eastern Scientific Picoamp-300B with picoamp resolution modified to apply voltages up to 2V. Data was acquired using a NI PCI-6024E Card with NI LabView Software at a rate of 10 kHz with a 2nd-order Bessel filter applied from the Picoamp-300B at 1 kHz. Subsequent data processing was performed in Mathworks MATLAB software. Current traces were filtered again with a 10-pole butterworth filter at 100Hz.

The average noise values for the polymers were calculated to be  $4.06 \pm 1.94 \text{ pA}_{\text{rms}}$  (ABA935),  $1.80 \pm 0.70 \text{ pA}_{\text{rms}}$  (ABA1135), and  $7.67 \pm 7.47 \text{ pA}_{\text{rms}}$  (ABA1125). Calculations were obtained using 10 second samples from sections of traces with no gating. Average  $1/f^\alpha$  slopes for the spectral density were calculated to be (ABA1135)  $\alpha = 0.60 \pm 0.24$ , (ABA935)  $\alpha = 0.80 \pm 0.15$ , (ABA1125)  $\alpha = 0.82 \pm 0.52$ .

Current versus voltage (IV) curves are measured to determine an appropriate applied voltage below the gating threshold of the protein, as shown in Figure S1. The IV curve was taken 5 times and linearly fit between -100 mV and 100 mV. From this test, 60mV applied voltage was chosen to determine the conductance of the traces shown in the paper.

For accurate comparison, each protein spectra was taken at the same applied voltage with similar current due to the noise dependence on bias as shown in Figure S2. As is evident from this figure, changing the magnitude of the applied voltage or flipping the polarity has a significant effect on both the slope and the amplitude of the noise. As a result, all protein spectra were taken at +60mV.

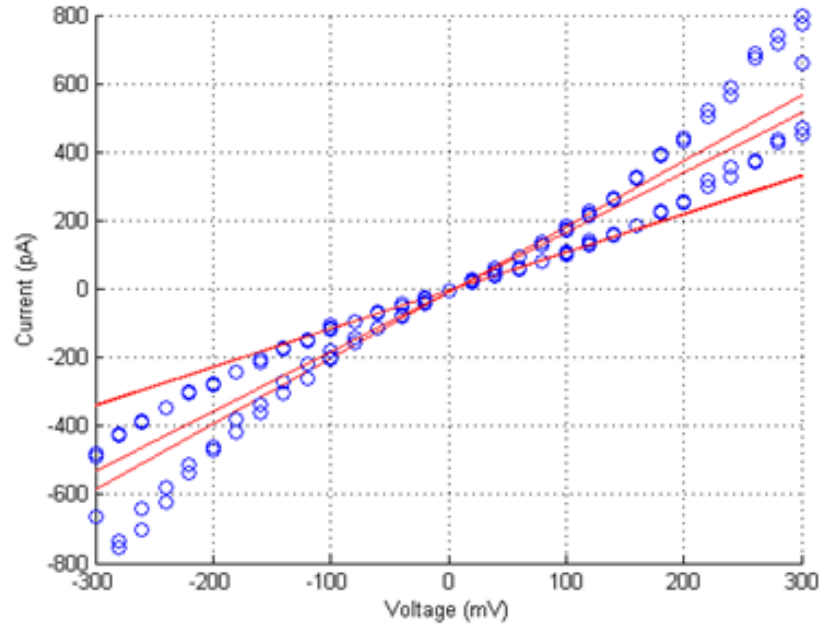


Figure S1: Example I-V curves of a single MspA protein insertion into ABA935 bilayer membrane used to determine an applied voltage below the gating threshold of MspA. I-V curves were recorded using a 1M KCl HEPES buffer at 25°C and pH ~7.

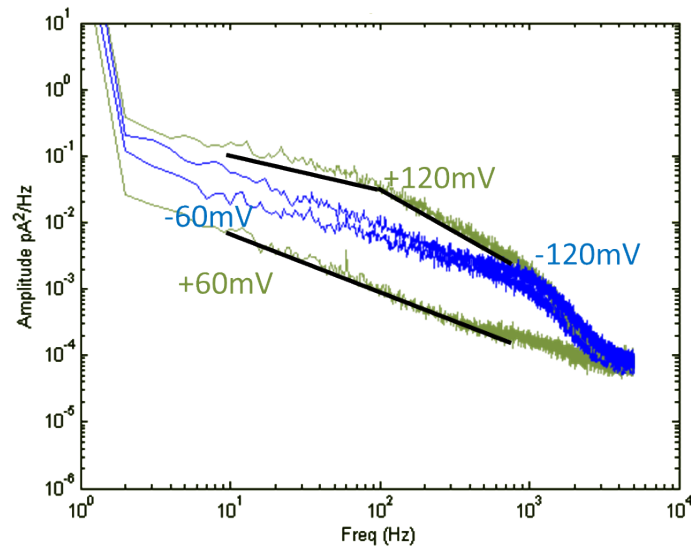


Figure S2: Spectra of a single protein insertion observed at various bias conditions for 5 minutes each to demonstrate the noise dependence on bias conditions. Measurements were recorded using a 1M KCl HEPES buffer at 25°C and pH ~7.

Polymer membrane stability was tested using a linear rising signal method as previously established for lipid bilayer membranes.<sup>2</sup> Minimum breakdown voltage ( $V_b$ ) was obtained using nonlinear regression.

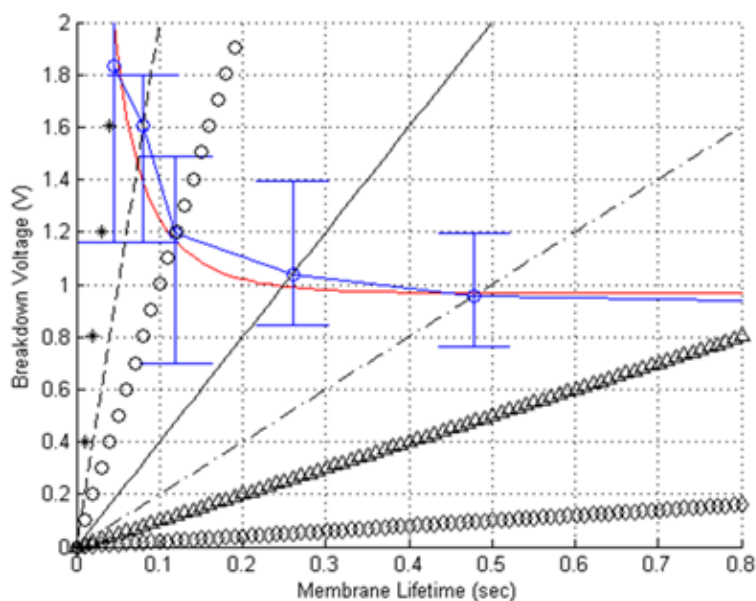


Figure S3: Breakdown (ABA1125) ( $V_b=968$  mV)

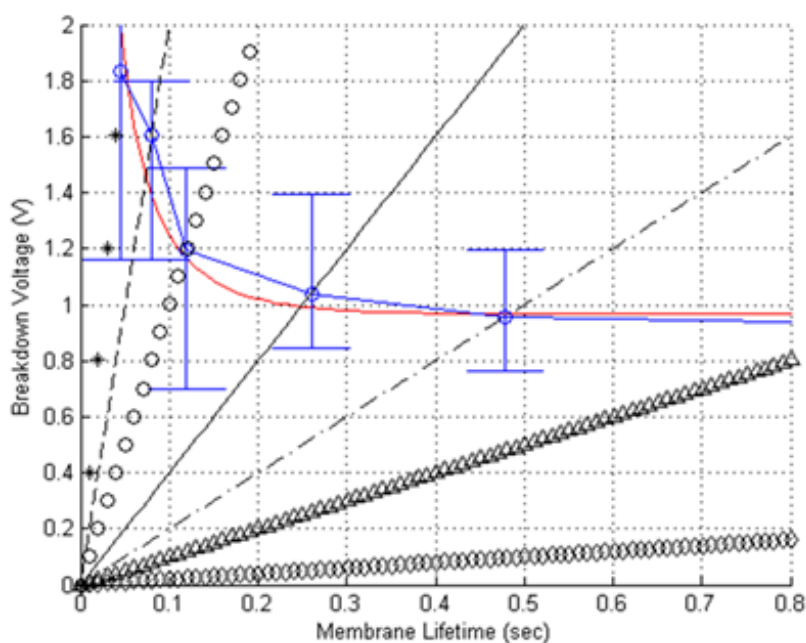


Figure S4: Breakdown (ABA1135) ( $V_b=891$  mV)

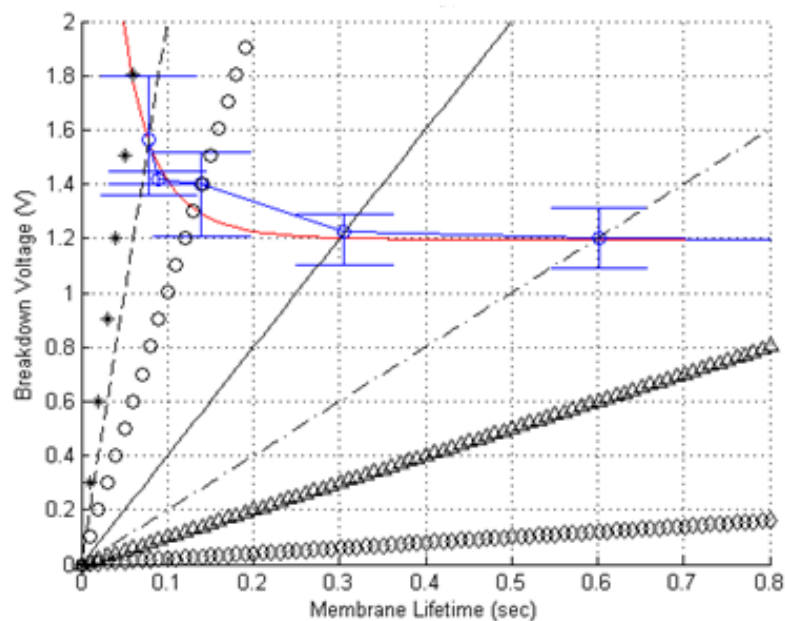


Figure S5: Breakdown (ABA935) ( $V_b=1.20$  V)

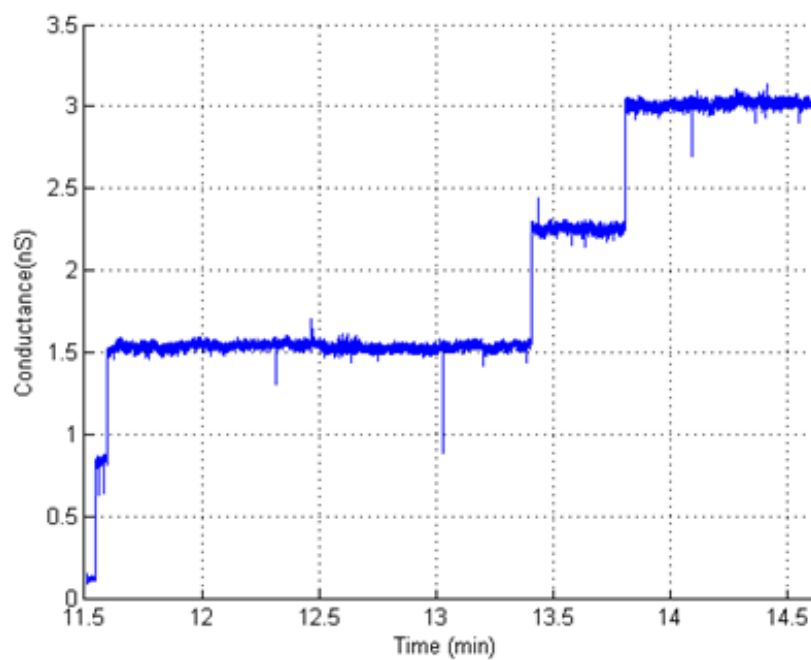


Figure S6:  $\alpha$ -Hemolysin insertion in ABA 1100-2500-1100 performed to corroborate the results obtained with previously published data<sup>3</sup> (Filtered at 1kHz)

## Additional current traces

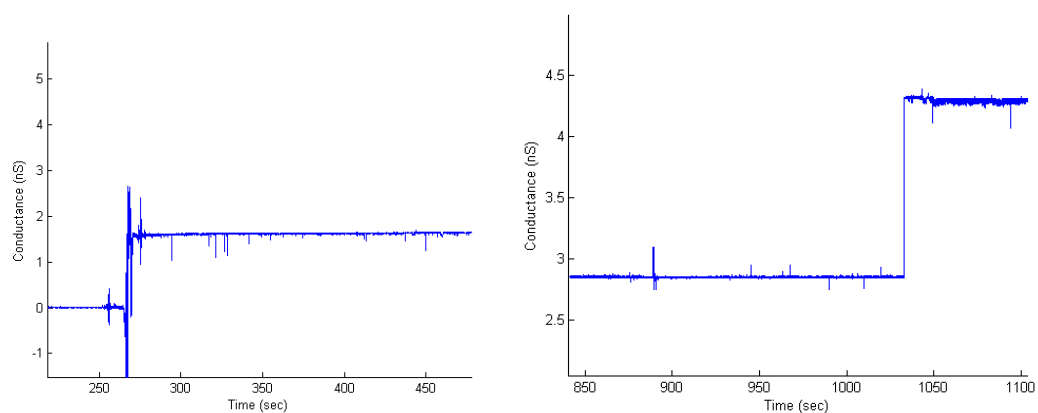


Figure S7: Additional conductance traces showing protein insertions into ABA1135. A single insertion of MspA (left) indicated by a sudden increase of conductance to  $\sim 1.6$  nS. Polymers have shown capability of multiple insertions as well (right) (Filtered at 1 kHz).

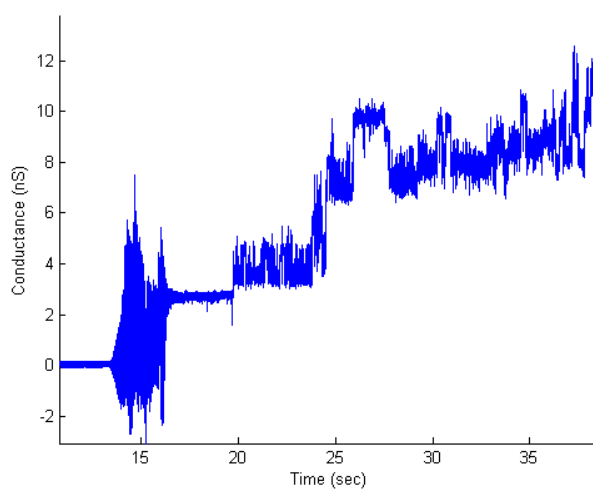


Figure S8: Conductance trace of multiple MspA insertions under 180mV into ABA935 polymer membrane (Filtered at 1kHz).

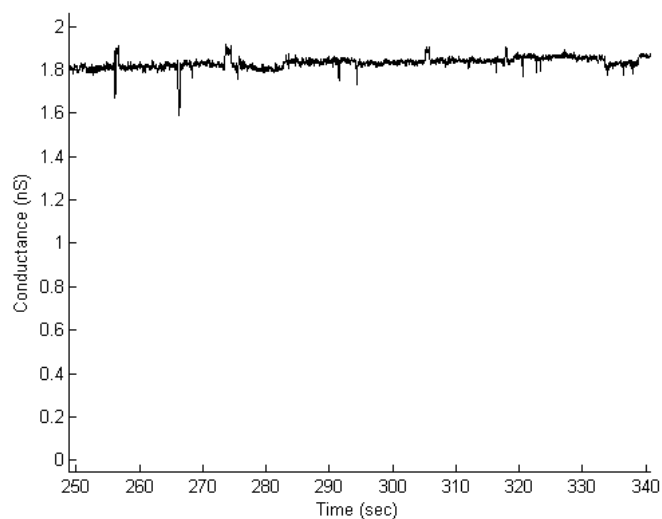


Figure S9: MspA insertion into commercially available 550-2600-550 (PMOXA-PDMS-PMOXA) ABA copolymer (purchased from Polymer Source Inc). Measurements were recorded using a 1M KCl HEPES buffer at 25°C and pH ~7 at 60 mV. Associated noise spectral density (below) shows similar noise properties to ABA1125 and ABA935 indicating noise likely arises from ABA component lengths.

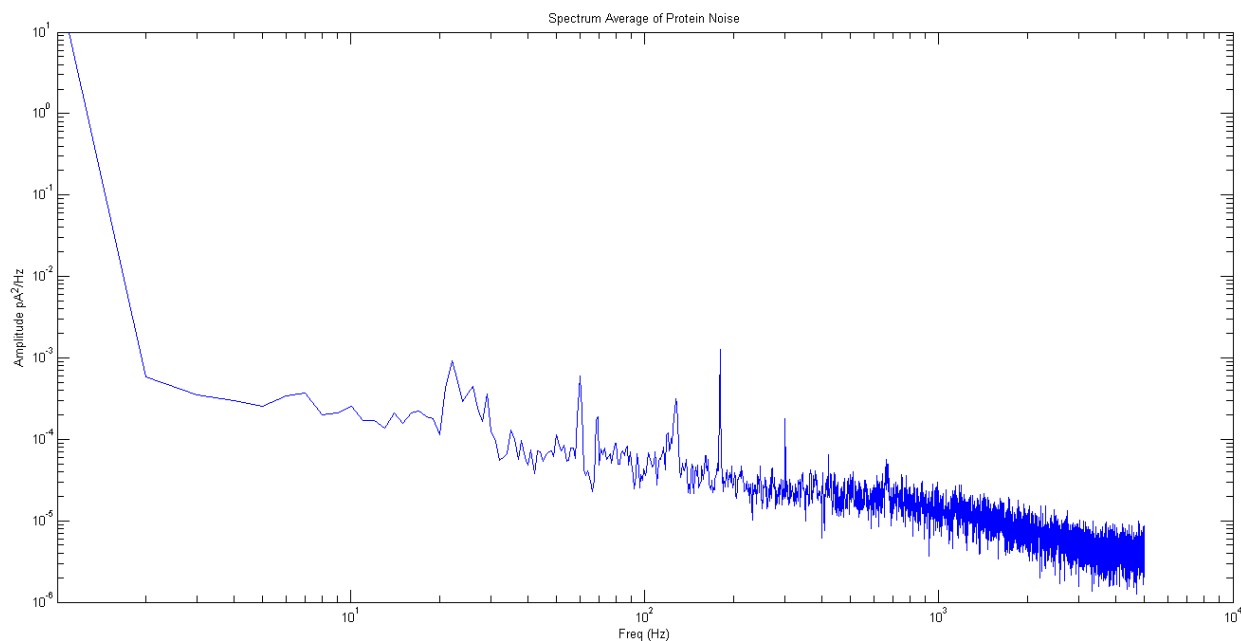


Figure S10: Noise power spectral density of MspA into 550-2600-550 ABA polymer membrane purchased from polymer source.

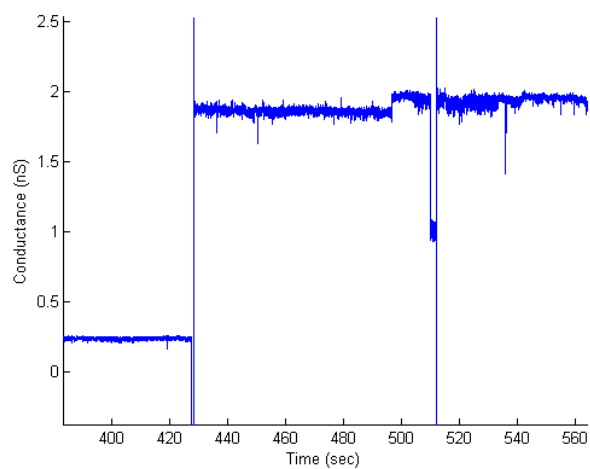
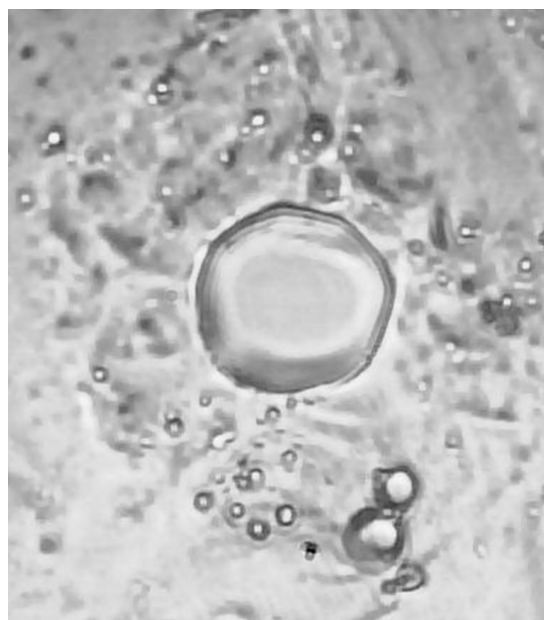


Figure S11: Conductance trace of single MspA insertion into ABA1125 polymer membrane (Filtered at 1kHz).



S12: Example webcam image of 1100-3500-1100 polymer membrane formed in 50 micron aperture. 4X magnification applied from microscope. The lighter region corresponds to the membrane.

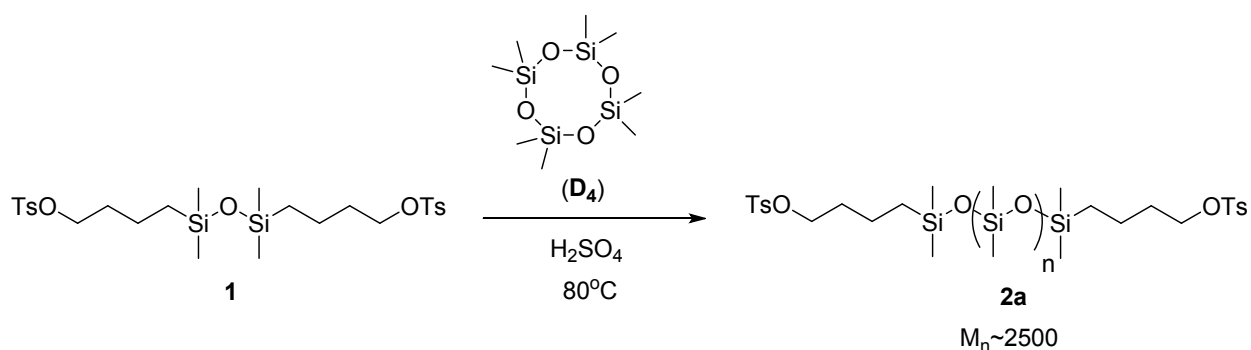


### ABA Triblock Copolymer Synthesis:

ABA triblock copolymers were prepared in a manner previously described by the group.<sup>1</sup> 2-methyl-2-oxazoline was purified by stirring over CaH<sub>2</sub> overnight and followed by distillation. Octamethylcyclotetrasiloxane (D<sub>4</sub>) was distilled under reduced pressure. Copper nanopowder (CuNPs) was purchased from Aldrich (<50 nm particle size (TEM), 99.5%). All other materials were used as received. <sup>1</sup>H-NMR (NMR) spectra were recorded on a Bruker Avance DMX500MHz SB NMR Spectrometer by dissolving polymers in deuterated chloroform (CDCl<sub>3</sub>). Gel permeation chromatography (GPC) studies were performed using a Waters 2695 Separation Module equipped with a 2414 Refractive Index Detector and 2996 Photodiode Array Detector. When forming solutions of polymer in n-decane/CHCl<sub>3</sub> mixtures the chloroform was always added first to completely dissolve polymer.

### B block synthesis

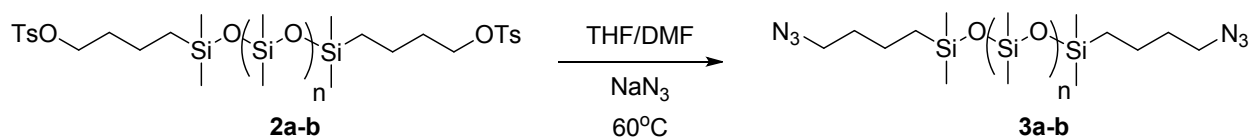
Synthesis of 2500 TsO-PDMS-OTs (**2**):



A round bottom flask was charged with bis-tosylate<sup>1</sup> (**1**) (400 mg, 0.68 mmol) was dried under reduced pressure at 60°C for three hours before freshly distilled D<sub>4</sub> was added (1.7 mL, 5.5 mmol). The mixture was stirred at room temperature for 15 minutes before a drop of 98% H<sub>2</sub>SO<sub>4</sub> was added to the reaction to initiate polymerization. The reaction mixture was then fitted with a reflux condenser and heated to 80°C for 17 hours. The reaction mixture was then allowed to cool to room temperature before removing any excess D<sub>4</sub> *in vacuo*. The crude was partitioned between hexanes (25 mL) and water (15 mL) before washing with MeOH, and brine. The organic phases were combined and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed to give 1.6 g (93% yield) of a clear oil (**2a**).  $M_{n,1\text{H NMR}} \sim 2500$ .

$^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.82 (d, 4H, arom. H), 7.29 (d, 4H, arom. H), 4.06 (t, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OTs}$ ), 2.47 (s, 6H,  $\text{CH}_3\text{-Ph}$ ), 1.71 (m, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OTs}$ ), 1.35 (m, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OTs}$ ), 0.48 (t, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OTs}$ ), 0.07 (br. s, 209H,  $-\text{Si}(\text{CH}_3)_2\text{-O-}$ ).

#### Synthesis of 2500 $\text{N}_3$ -PDMS- $\text{N}_3$ (**3a**)



TsO-PDMS-OTs (**2a**) was dissolved in a solvent mixture of THF (10 mL) and DMF (5 mL).  $\text{NaN}_3$  was added to the mixture and the reaction was heated to  $60^\circ\text{C}$  and allowed to stir for 22 hours before cooling to room temperature. The reaction was filtered through a fritted funnel to remove white precipitate and THF was removed under reduced pressure. The crude was then diluted with hexanes (20 mL) to remove remaining DMF. The hexanes layer was washed with water and brine before drying over  $\text{Na}_2\text{SO}_4$ . The solvent was removed to give 1.3 g (81% yield) of a clear oil.

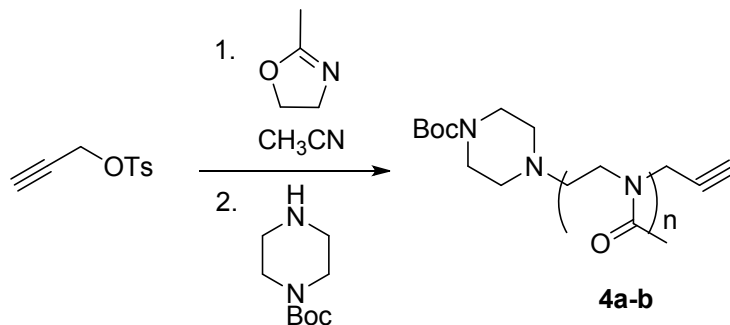
$^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 3.30 (t, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 1.65 (m, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 1.47 (m, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 0.57 (t, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 0.08 (br. s, 207H,  $-\text{Si}(\text{CH}_3)_2\text{-O-}$ ).

#### Synthesis of 3500 $\text{N}_3$ -PDMS- $\text{N}_3$ (**3b**)

The same substitution procedure of above was applied to 3500 OTs-PDMS-OTs (**2b**) to give 4.11 g (76% yield) of a clear oil.

$^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 3.30 (t, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 1.67 (m, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 1.46 (m, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 0.58 (t, 4H,  $\text{Si}(\text{CH}_3)_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ ), 0.11 (br. s, 292H,  $-\text{Si}(\text{CH}_3)_2\text{-O-}$ ).

Synthesis of 900 boc-PMOXA (**4a**):



Propargyl tosylate (50 mg, 0.24 mmol) was added to an oven dried round bottom flask and placed under reduced pressure for 18 hours to remove trace water. CH<sub>3</sub>CN (5 mL) was added to the flask followed by the addition of distilled 2-methyl-2-oxazoline (174 mg, 2.0 mmol). The flask was fitted with a reflux condenser and the reaction was heated to 80°C and stirred for 20 hours. The mixture was allowed to cool to room temperature before adding 1-Boc-piperazine (134 mg, 0.72 mmol) in portions. The reaction was allowed to stir for three hours before removing the solvent and washing the crude with Et<sub>2</sub>O to remove excess 1-Boc-piperazine. The crude was then redissolved in CH<sub>3</sub>CN (5 mL) and treated with K<sub>2</sub>CO<sub>3</sub> (2.4 mmol) and allowed to stir at room temperature for 18 hours. The solvent was removed and the crude was redissolved in DCM, filtered, and concentrated again to yield 210 mg of **4a** (97% yield).  $M_{n,1H\text{NMR}} \sim 870$ , PDI 1.50 (PS standards, Eluent: CHCl<sub>3</sub> + 0.25% triethylamine)

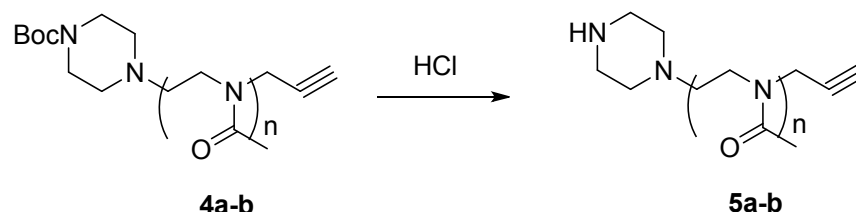
<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ = 4.25-4.05 (br. m, 2H, alkyne-CH<sub>2</sub>-N(C=O)), 3.50 (br. m, 40H, N(C=O)-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.92 (br. m, 6H, pip (CH<sub>2</sub>-CH<sub>2</sub>-NBoc-CH<sub>2</sub>-CH<sub>2</sub> and pip-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O))), 2.41 (br. m, 2H, pip-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.17 (br. m, 27H, N(C=O)CH<sub>3</sub>), 1.46 (s, 9H, Boc (C(CH<sub>3</sub>)<sub>3</sub>)).

Synthesis of 1100 boc-PMOXA (**4b**)

The same polymerization procedure was used as listed above except 268 mg (3.12 mmol, 13 eq.) of 2-methyl-2-oxazoline was used to obtain 248 mg (94% yield) of **4b**.  $M_{n,1H\text{NMR}} \sim 1120$ , PDI 1.63 (PS standards, Eluent: CHCl<sub>3</sub> + 0.25% triethylamine)

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ = 4.25-4.05 (br. m, 2H, alkyne-CH<sub>2</sub>-N(C=O)), 3.48 (br. m, 52H, N(C=O)-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.83 (br. m, 5H, pip (CH<sub>2</sub>-CH<sub>2</sub>-NBoc-CH<sub>2</sub>-CH<sub>2</sub> and pip-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O))), 2.51 (br. s, 1H, pip-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.43 (br. m, 2H, pip-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.18 (br. m, 35H, N(C=O)CH<sub>3</sub>), 1.48 (s, 9H, Boc (C(CH<sub>3</sub>)<sub>3</sub>)).

Synthesis of 900 PMOXA (**5a**):



Polymer (**4a**) (210 mg, 0.23 mmol) was added to an oven dried round bottom flask and treated with 2 M HCl (5 mL) and allowed to stir at room temperature overnight. The reaction was basified using  $\text{K}_2\text{CO}_3$  (317 mg, 2.3 mmol) and the reaction was stirred for one hour at room temperature before the solvent was removed. The crude was redissolved in  $\text{CH}_2\text{Cl}_2$  and filtered before removing solvent. The product **5a** was obtained as a white powder (158 mg, 75% yield).

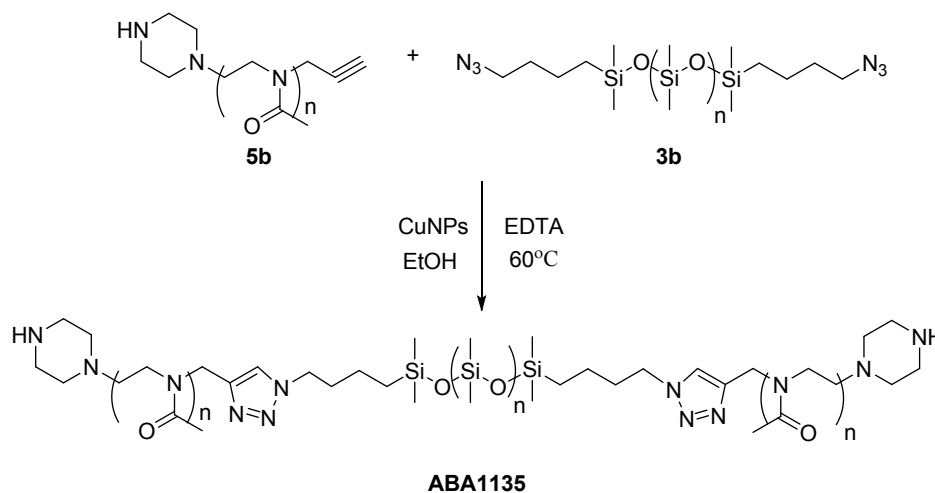
$^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 4.25-4.05 (br. m, 2H, alkyne- $\text{CH}_2$ -N(C=O)), 3.50 (br. m, 40H, N(C=O)- $\text{CH}_2$ - $\text{CH}_2$ -N(C=O)), 2.82 (br. m, 4H, pip ( $\text{CH}_2$ - $\text{CH}_2$ -NH- $\text{CH}_2$ - $\text{CH}_2$ ), 2.41 (br. m, 2H, pip- $\text{CH}_2$ - $\text{CH}_2$ -N(C=O)), 2.17 (br. m, 31H, N(C=O) $\text{CH}_3$  and pip( $\text{CH}_2$ - $\text{CH}_2$ -NH- $\text{CH}_2$ - $\text{CH}_2$ )).

Synthesis of 1100 PMOXA (**5b**):

The same deprotection procedure was used as above on compound **4b** to obtain 179 mg (72% yield) of **5b**.

$^1\text{H}$ -NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 4.29-4.10 (br. m, 2H, alkyne- $\text{CH}_2$ -N(C=O)), 3.50 (br. m, 49H, N(C=O)- $\text{CH}_2$ - $\text{CH}_2$ -N(C=O)), 2.82 (br. m, 2H, pip ( $\text{CH}_2$ - $\text{CH}_2$ -NH- $\text{CH}_2$ - $\text{CH}_2$  and pip- $\text{CH}_2$ - $\text{CH}_2$ -N(C=O)), 2.52-2.43 (br. m, 5H, pip- $\text{CH}_2$ - $\text{CH}_2$ -N(C=O) pip ( $\text{CH}_2$ - $\text{CH}_2$ -NH- $\text{CH}_2$ - $\text{CH}_2$ ), 2.18 (br. m, 35H, N(C=O) $\text{CH}_3$ ).

Synthesis of **ABA1135**:



To an oven-dried round bottom flask was added 100 mg (0.06 mmol) of **5b** and 66 mg (0.03 mmol) of **3b**. EtOH (5 mL) and 4 mg (0.01 mmol) of EDTA were added to mixture before degassing with bubbling argon. CuNPs (5 mg) was added and the flask was heated to 60°C under argon for two hours. The mixture was cooled to room temperature and filtered through a pad of celite before removing the solvent. The crude was redissolved in 1 mL of CH<sub>2</sub>Cl<sub>2</sub> and treated with 1 M Na<sub>2</sub>S (190 µL) and allowed to stir for one hour before filtering through a pad of celite. The solvent was removed and the crude was then taken up in i-PrOH (5 mL) and treated with 500 mg of MgSO<sub>4</sub> overnight at room temperature. The mixture was filtered and the solvent was removed before being taken up in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and filtered through a 0.2 µL PTFE filter. The solvent was removed to obtain 120 mg of **ABA1135** (75% yield).

<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ= 7.55 (br. m, 2H, triazole-H), 4.58 (br. m, 4H, triazole-CH<sub>2</sub>-N(C=O)), 4.38-4.32 (br. m, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-triazole), 3.51 (br. m, 106H, -N(C=O)-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.59-2.53 (br. m, 8H, pip(CH<sub>2</sub>-N-CH<sub>2</sub>)), 2.17 (br. m, 80H, N(C=O)CH<sub>3</sub>), 1.96 (br. m, 6H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-pip + pip-NH), 1.42 (br. m, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-triazole), 0.58 (br. t, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-pip), 0.08 (br. s, 286H, Si(CH<sub>3</sub>)<sub>2</sub>-O-)

Synthesis of **ABA1125**:

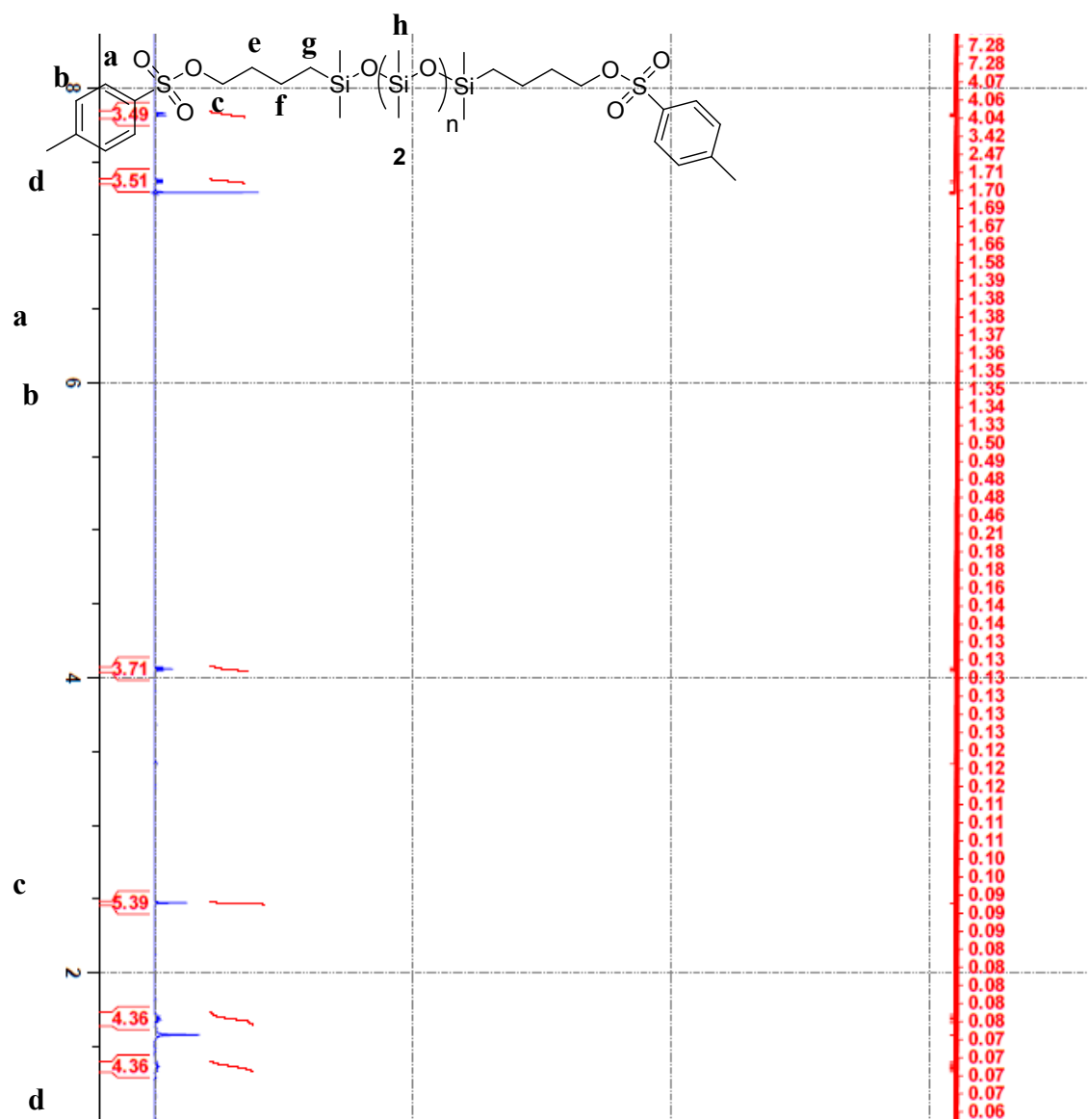
The same procedure was used as above except using **3a** in place of **3b**. After solvent was removed 157 mg (84% yield) of **ABA1125** was obtained.

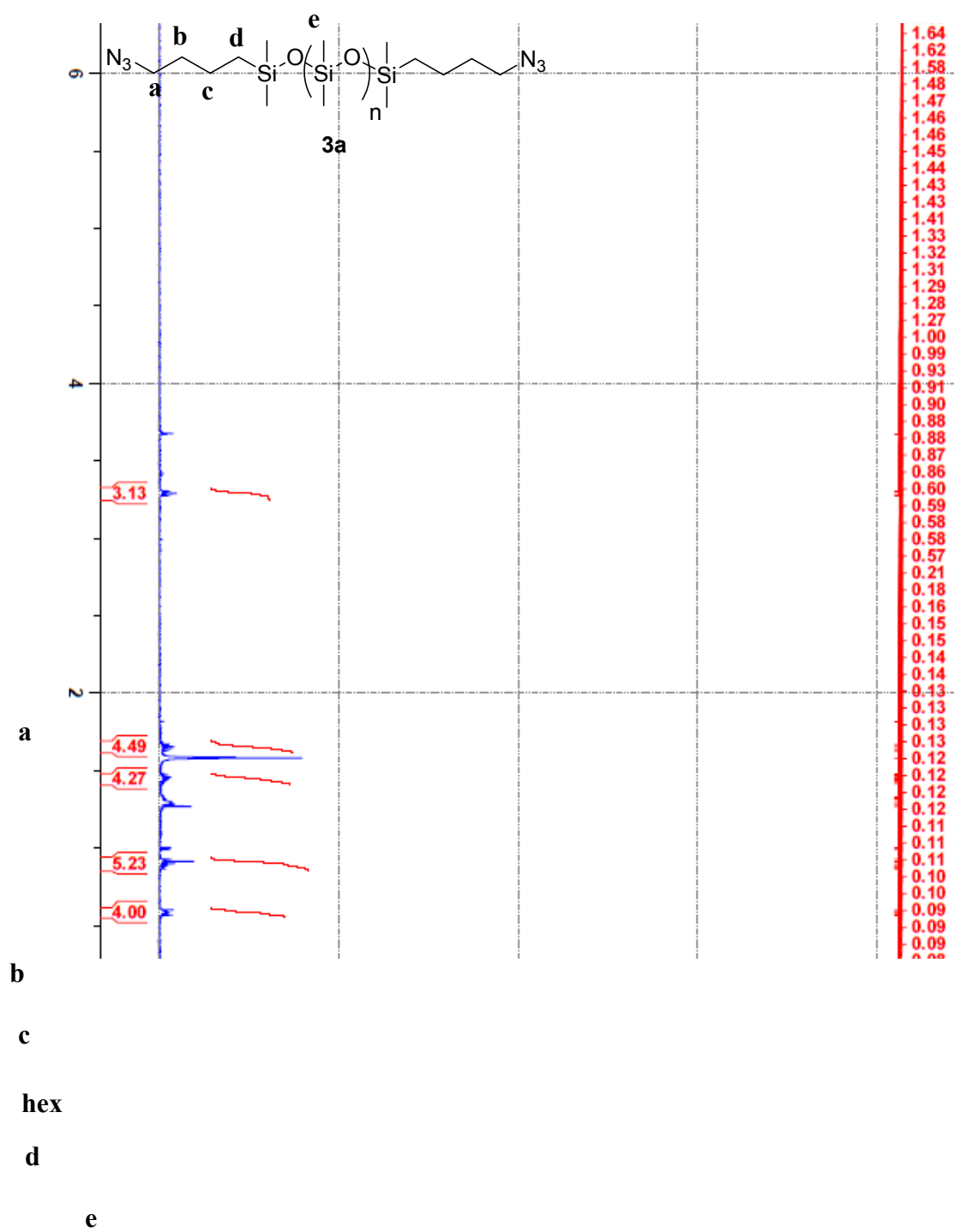
<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ= 7.49 (br. m, 2H, triazole-H), 4.60 (br. m, 4H, triazole-CH<sub>2</sub>-N(C=O)), 4.40-4.32 (br. m, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-triazole), 3.51 (br. m, 118H, -N(C=O)-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.61-2.53 (br. m, 8H, N(C=O)-CH<sub>2</sub>-CH<sub>2</sub>-pip + pip(CH<sub>2</sub>-N-CH<sub>2</sub>)), 2.17 (br. m, 87H, N(C=O)CH<sub>3</sub>), 1.96 (br. m, 6H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-pip + pip-NH), 1.45 (br. m, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-triazole), 0.58 (br. t, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-pip), 0.09 (br. s, 206H, Si(CH<sub>3</sub>)<sub>2</sub>-O-)

#### Synthesis of **ABA935**:

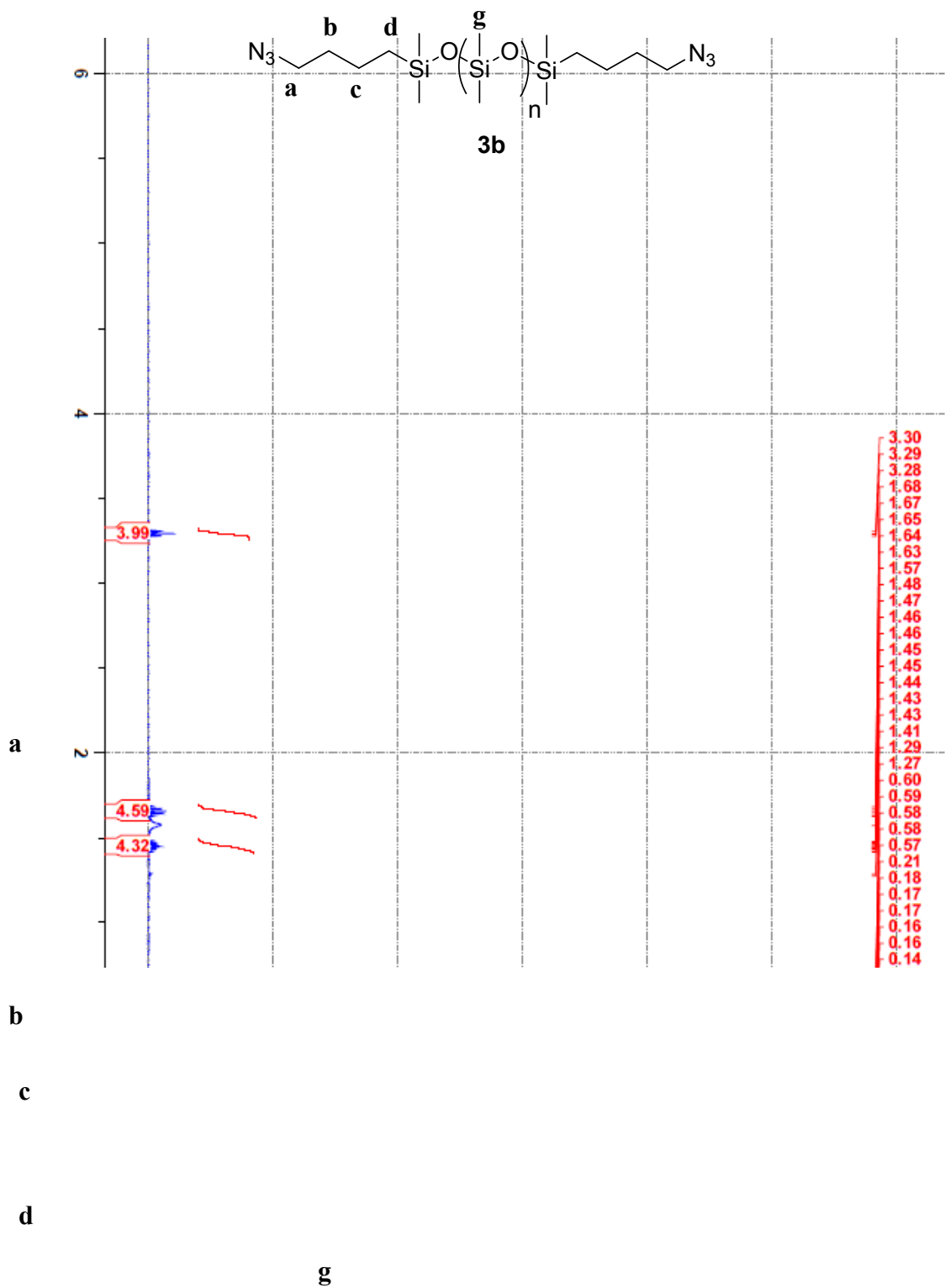
The same procedure was used as above except using **5a** in place of **5b**. After solvent was removed 108 mg (71% yield) of **ABA935** was obtained.

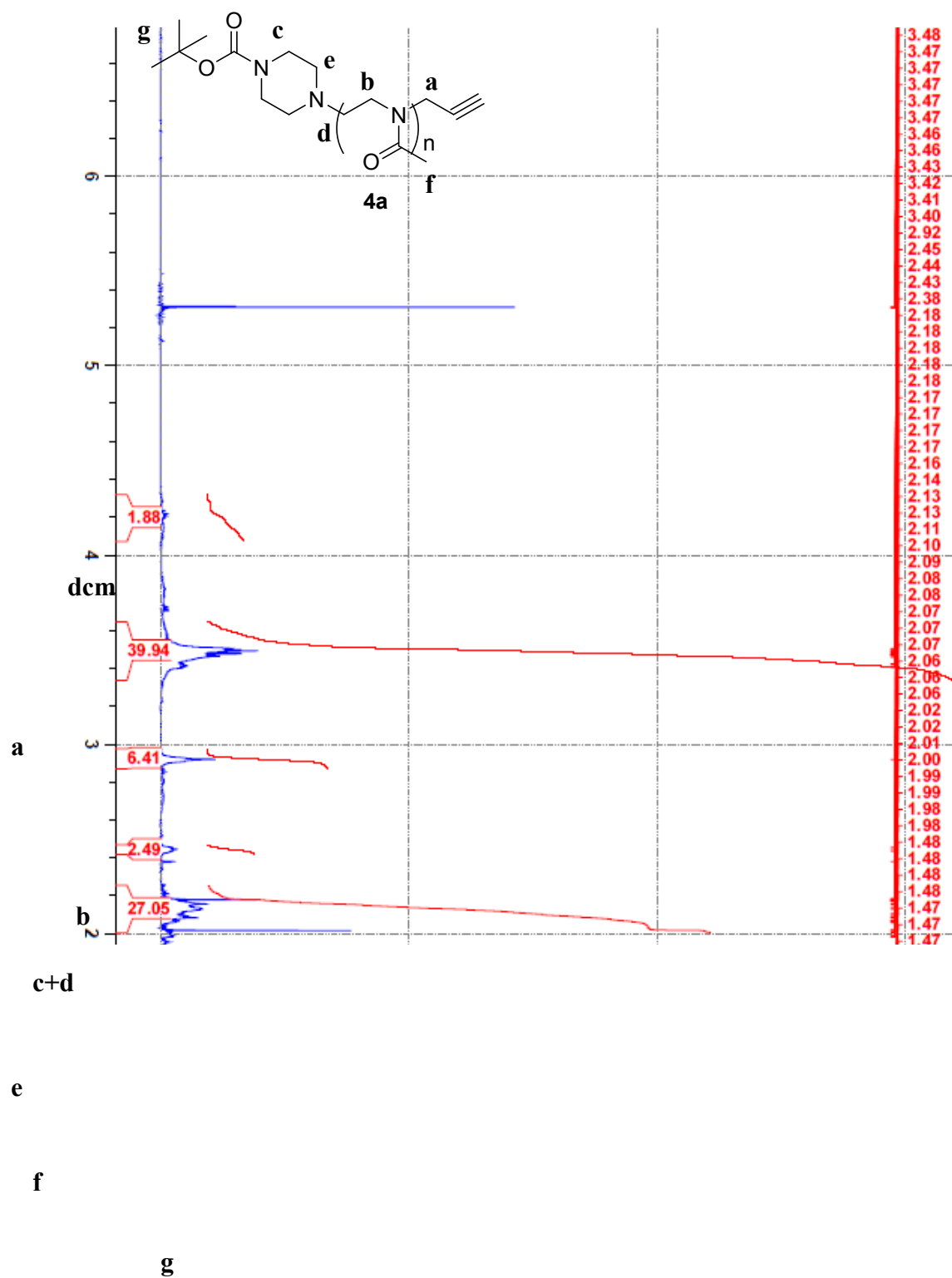
<sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): δ= 7.54 (br. m, 2H, triazole-H), 4.57 (br. m, 4H, triazole-CH<sub>2</sub>-N(C=O)), 4.43-4.34 (br. m, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-triazole), 3.51 (br. m, 86H, -N(C=O)-CH<sub>2</sub>-CH<sub>2</sub>-N(C=O)), 2.70-2.53 (br. m, 8H, N(C=O)-CH<sub>2</sub>-CH<sub>2</sub>-pip + pip(CH<sub>2</sub>-N-CH<sub>2</sub>)), 2.17 (br. m, 60H, N(C=O)CH<sub>3</sub>), 1.96 (br. m, 6H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-pip + pip-NH), 1.45 (br. m, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-triazole), 0.58 (br. t, 4H, Si(CH<sub>3</sub>)<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-pip), 0.09 (br. s, 291H, Si(CH<sub>3</sub>)<sub>2</sub>-O-).

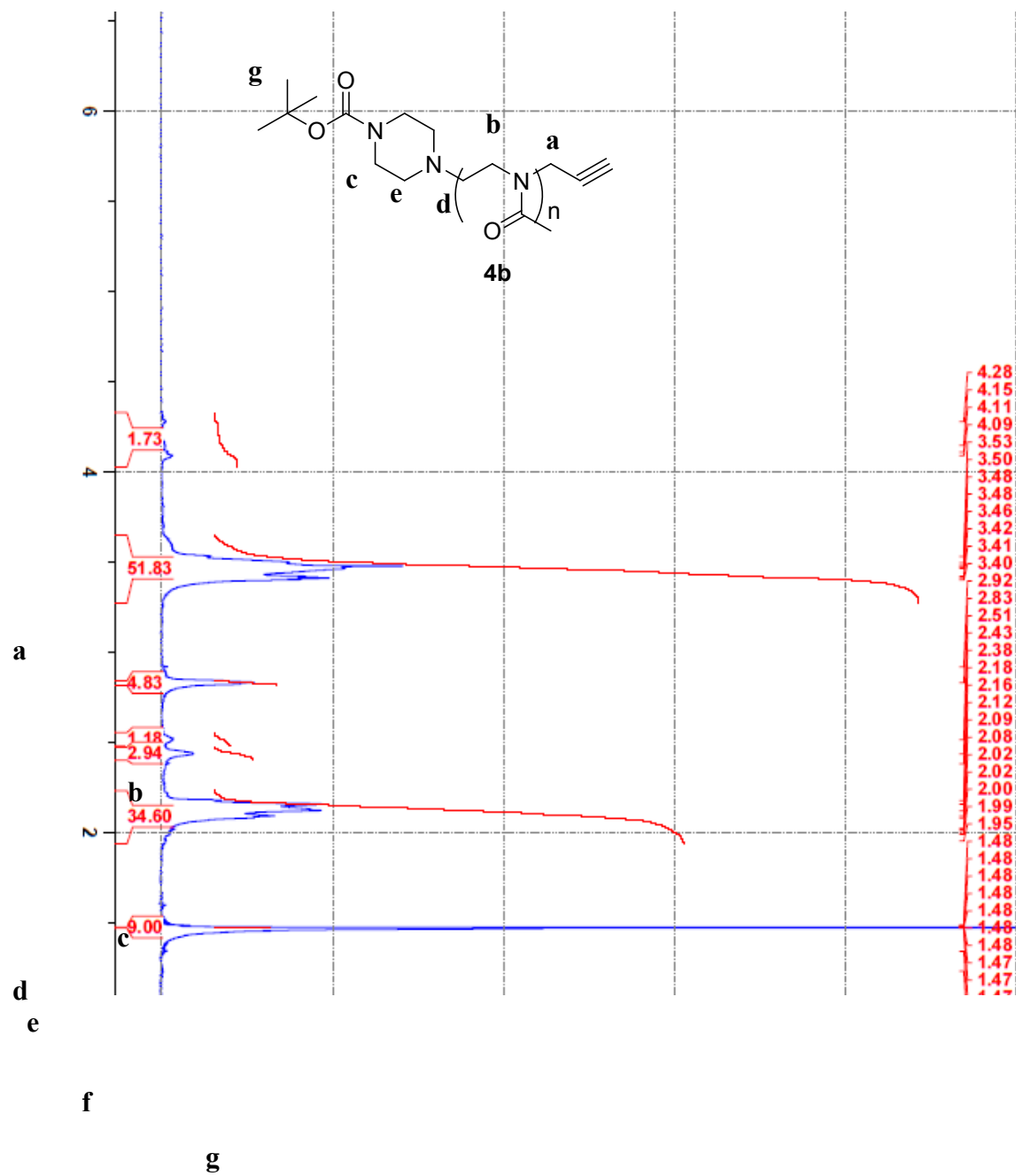


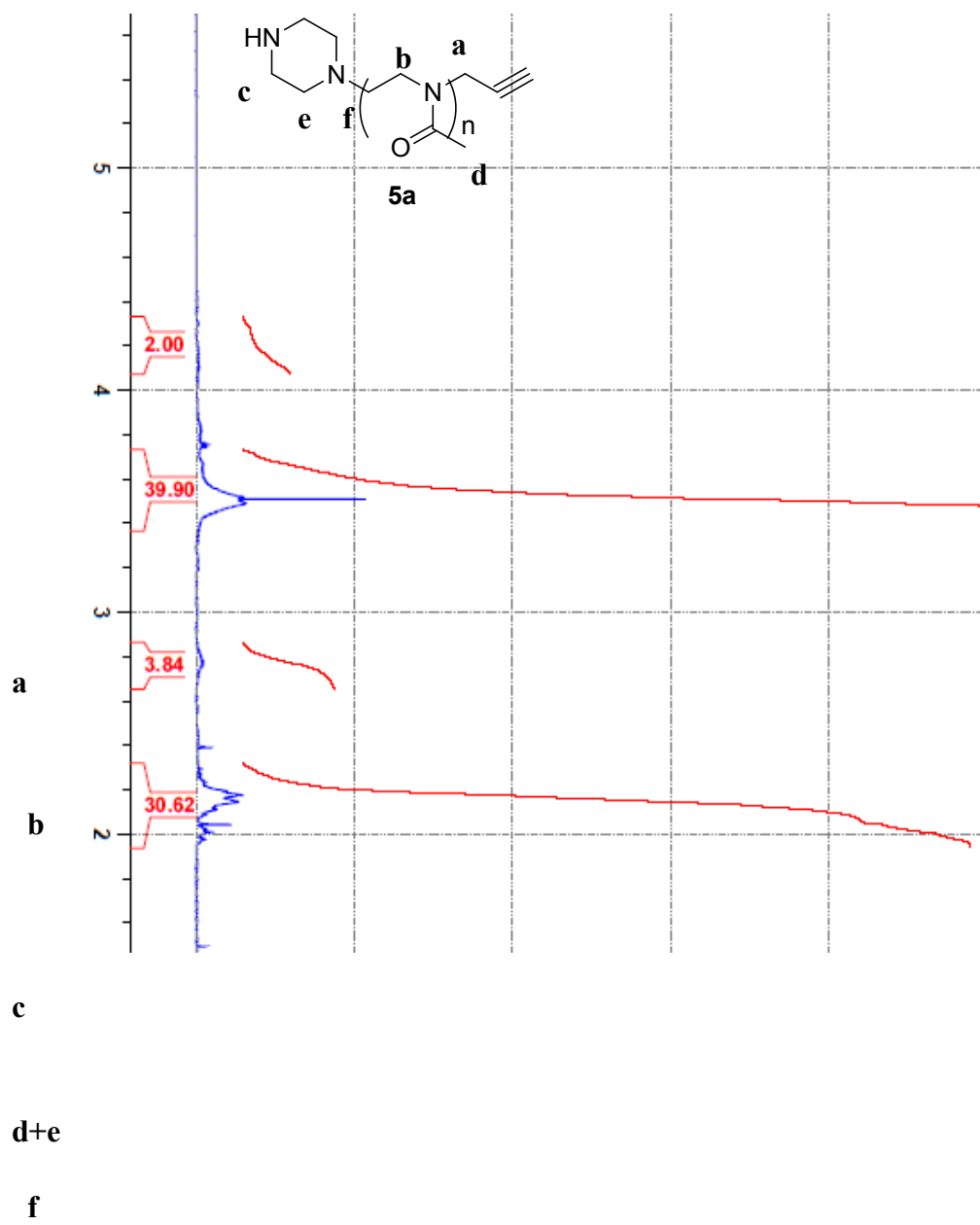


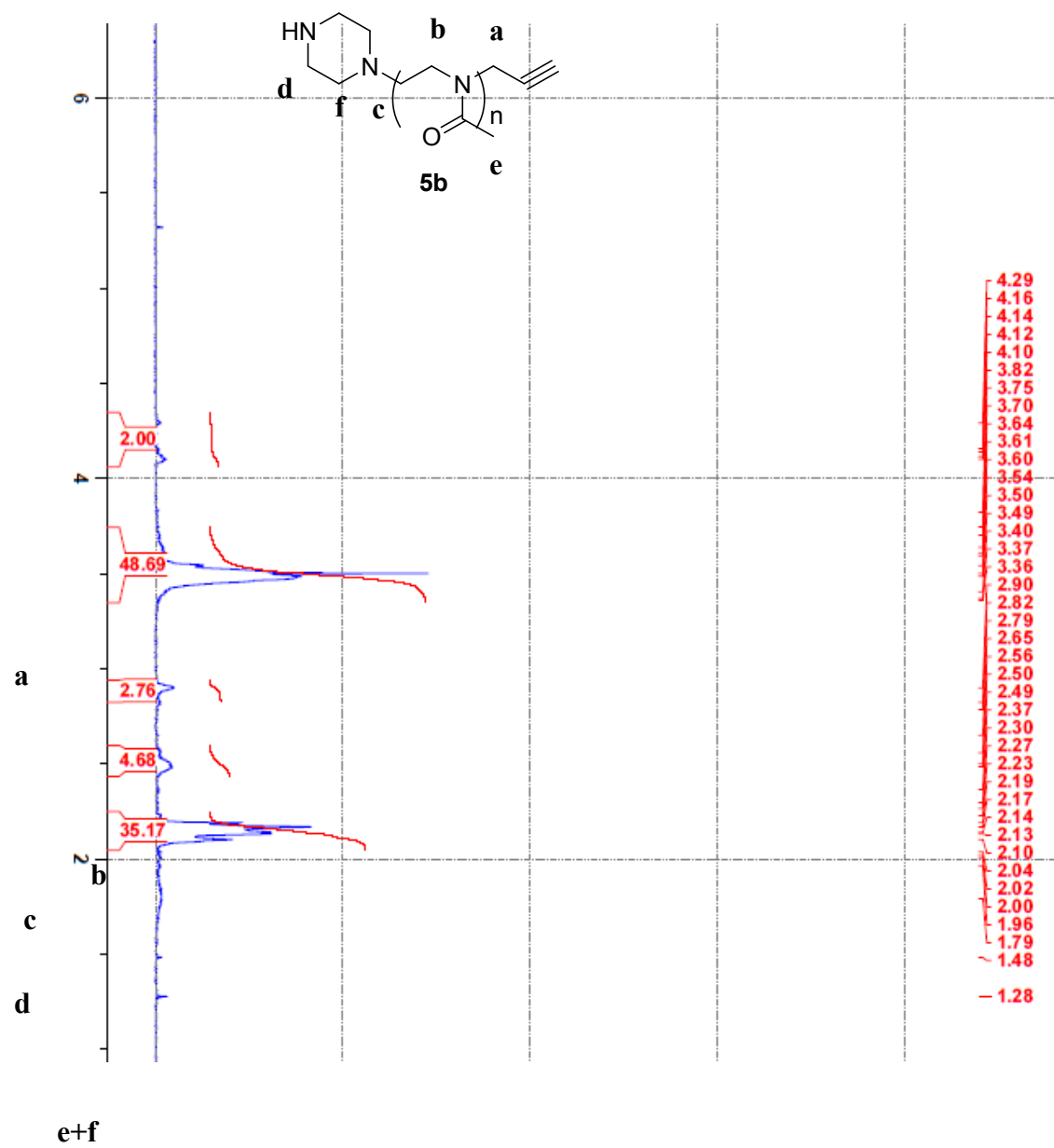


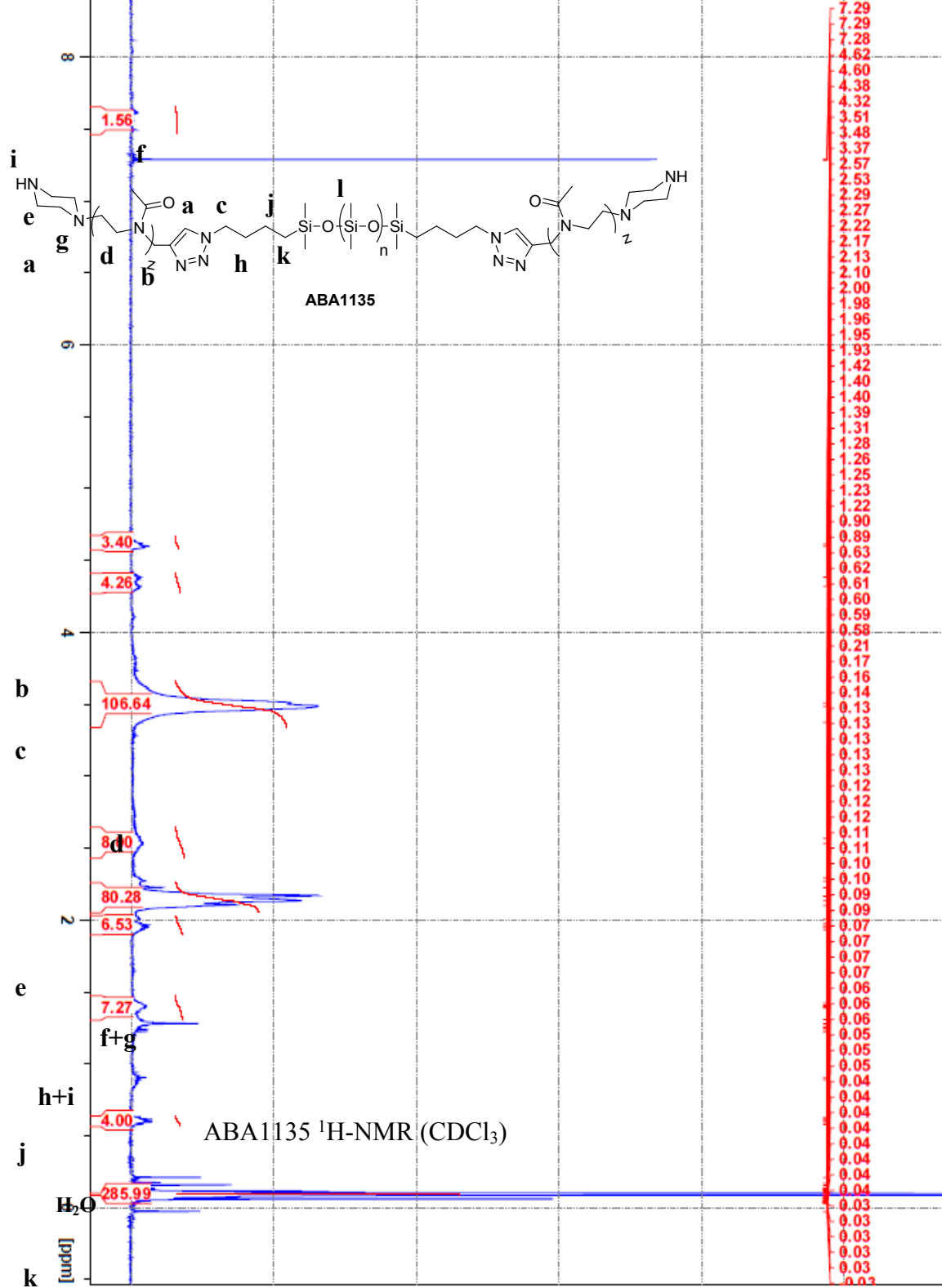




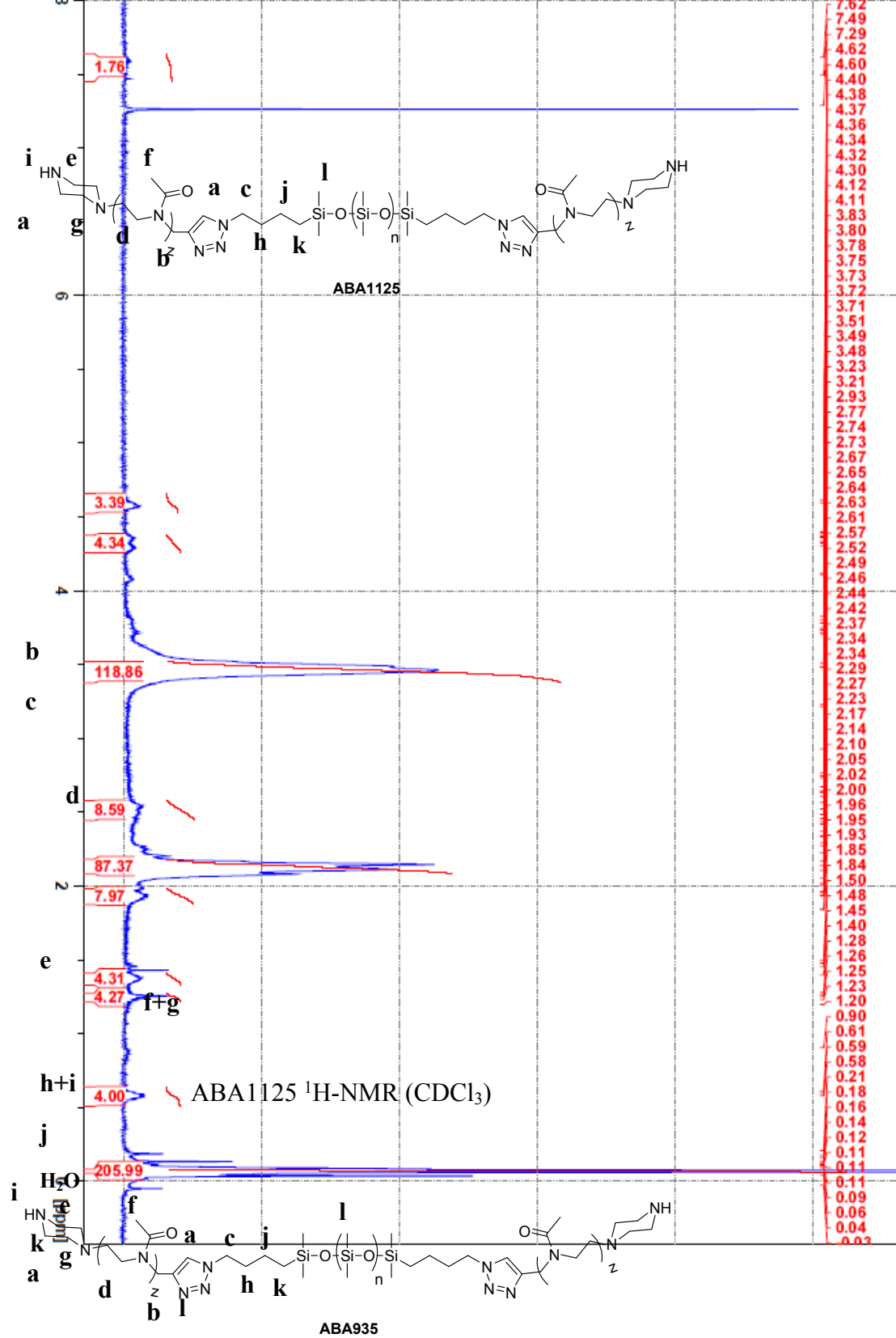






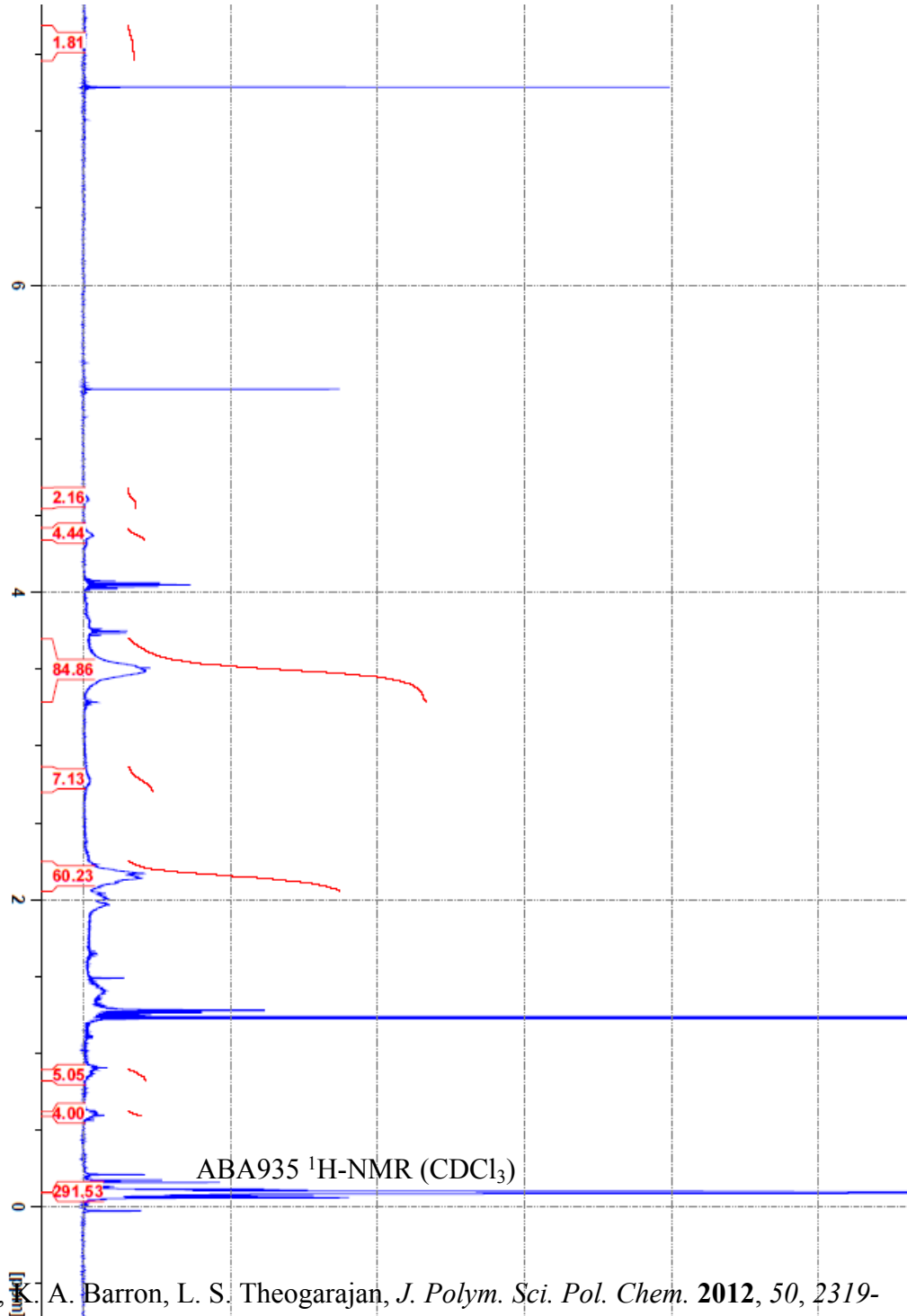


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b



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