Supporting information

Minimization of synthetic polymer ligands for specific recognition and neutralization of a toxic peptide

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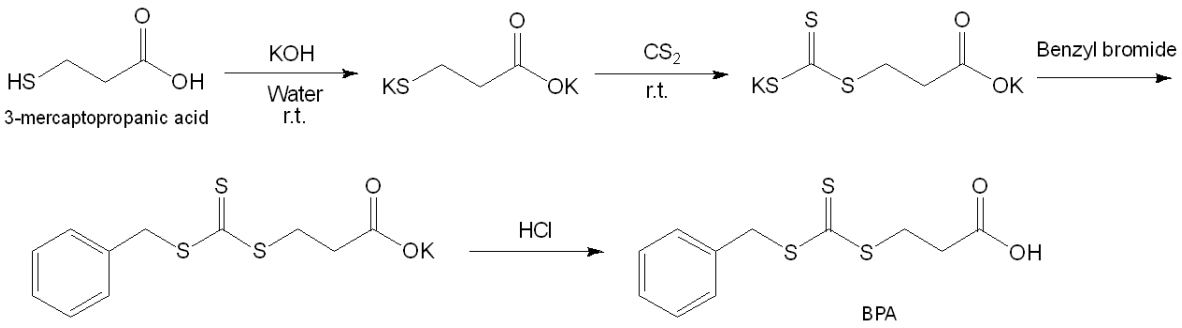
S1. Preparation of minimized synthetic polymer ligands

1. Preparation of BPA



Scheme S1-1.

BPA was prepared according to Davis and his coworkers[1]. 3-Mercaptopropionic acid (10 mL, 115 mmol) was added to potassium hydroxide (12.9 g in 125 mL H_2O, 230 mmol). Subsequently, 15.3 mL CS_2 was added dropwise. The resulting orange-colored solution was stirred for 5 h at room temperature, after which 13.65 mL benzyl bromide (55.4 mmol) was added. Benzyl bromide turned the color of the solution to opaque yellow. The mixture was heated to 80 ºC for 12 h and cooled. Chloroform (100 mL) was added next, and 100 mL HCl (1 M in H_2O) was slowly added until the organic layer turned yellow. The aqueous layer was extracted with chloroform (3 x 100 mL), while the organic layer was washed with distilled water (3 x 100 mL). Finally, the organic layer was dried over $MgSO_4$. BPA was recrystallized in hexane. Yield: 65 %.

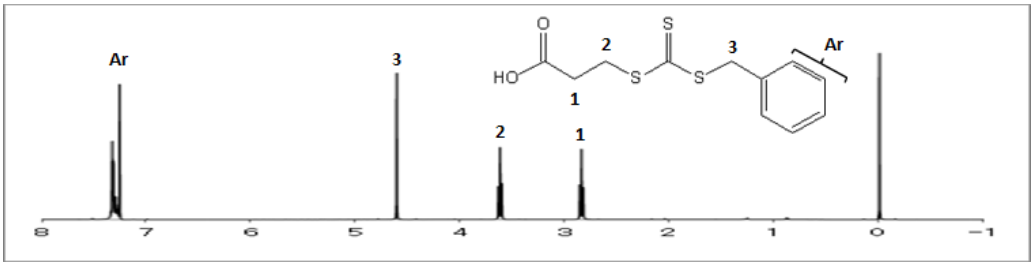
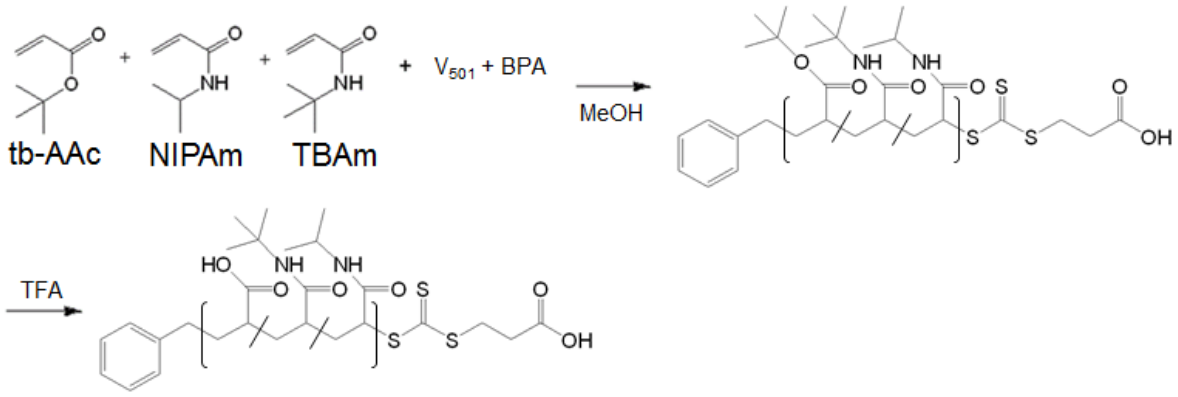


Figure S1-1. ^{1}H NMR of BPA in $CDCl_3$

2. Preparation of 300-mer, 30-mer, 15-mer and 9-mer polymer ligands



Scheme S1-2.

Reversible addition-fragmentation chain-transfer (RAFT) was used to obtain 300-mer, 30-mer, 15-mer and 9-mer polymer ligands with narrow molecular weight distribution. BPA was used as chain transfer agent. *Tert*-butyl acrylate (*tb*-AAc), *N*-isopropyl acrylamide (NIPAm), *N-tert*-butylacrylamide (TBAm), BPA (20.4 or 40.8 mg) and V_{501} (6.3 mg) were dissolved in methanol (~1mL) according to the feed ratio listed in Table S1-1. Solutions were degassed in three cycles of freeze-evacuate-thaw, sealed in an ampoule, and heated at 70 °C for 3 h in an oil bath. Products were precipitated with brine and distilled water, and conversion rates were calculated from ^{1}H NMR in MeOD. After the composition and molecular weight were determined by ^{1}H NMR and GPC, polymer ligands were deprotected with TFA.

Table S1-1. Feed ratio and characteristics of resulting polymers

	TBAm (x)	*tb*-AAc (y)	NIPAm (100-x-y)	Conversion rate	GPC		
					M_n	M_w	PDI
30-mer (T0%A5%)	0	5 mol% 14.1 mg, 0.11 mmol	95 mol% 244.2 mg, 2.15 mmol	97	3400	4600	1.4
30-mer (T0%A10%)	0	10 mol% 28.2 mg, 0.22 mmol	90 mol% 231.3 mg, 2.04 mmol	98	2800	3700	1.3
30-mer (T0%A20%)	0	20 mol% 56.4 mg, 0.44 mmol	80 mol% 205.6 mg, 1.82 mmol	98	2600	3400	1.3
30-mer (T10%A0%)	10 mol% 28.6 mg, 0.23 mmol	0	90 mol% 231.3 mg, 2.04 mmol	97	2800	3800	1.4
30-mer (T10%A10%)	10 mol% 28.6 mg, 0.23 mmol	10 mol% 28.2 mg, 0.22 mmol	80 mol% 205.6 mg, 1.82 mmol	94	2700	3600	1.3
30-mer (T20%A0%)	20 mol% 57.2 mg, 0.45 mmol	0	80 mol% 205.6 mg, 1.82 mmol	93	3100	4100	1.4
30-mer (T20%A5%)	20 mol% 57.2 mg, 0.45 mmol	5 mol% 14.1 mg, 0.11 mmol	75 mol% 192.8 mg, 1.7 mmol	98	2500	3300	1.3
30-mer (T20%A10%)	20 mol% 57.2 mg, 0.45 mmol	10 mol% 28.2 mg, 0.22 mmol	70 mol% 179.9 mg, 1.59 mmol	96	2800	3600	1.3
30-mer (T20%A20%)	20 mol% 57.2 mg, 0.45 mmol	20 mol% 56.4 mg, 0.44 mmol	60 mol% 154.2 mg, 1.36 mmol	96	2600	3400	1.3
30-mer (T40%A0%)	40 mol% 114.4 mg, 0.9 mmol	0	60 mol% 154.2 mg, 1.36 mmol	99	2700	3600	1.3
30-mer (T40%A5%)	40 mol% 114.4 mg, 0.9 mmol	5 mol% 14.1 mg, 0.11 mmol	55 mol% 141.35 mg, 1.25 mmol	98	2500	3400	1.4
30-mer (T40%10%)	40 mol% 114.4 mg, 0.9 mmol	10 mol% 28.2 mg, 0.22 mmol	50 mol% 129.0 mg, 1.14 mmol	95	2600	3500	1.3
30-mer (T40%20%)	40 mol% 114.4 mg, 0.9 mmol	20 mol% 56.4 mg, 0.44 mmol	40 mol% 102.8 mg, 0.91 mmol	97	2500	3200	1.3
15-mer (T20%A0%)	20 mol% 57.2 mg, 0.45 mmol	0	80 mol% 205.6 mg, 1.82 mmol	93	1500	1700	1.2
15-mer (T20%A5%)	20 mol% 57.2 mg, 0.45 mmol	5 mol% 14.1 mg, 0.11 mmol	75 mol% 192.8 mg, 1.7 mmol	96	1400	1600	1.1
15-mer (T20%A10%)	20 mol% 57.2 mg, 0.45 mmol	10 mol% 28.2 mg, 0.22 mmol	70 mol% 179.9 mg, 1.59 mmol	98	1400	1600	1.1
15-mer (T20%A20%)	20 mol% 57.2 mg,	20 mol% 56.4 mg,	60 mol% 154.2 mg,	99	1600	2000	1.3

	0.45 mmol	0.44 mmol	1.36 mmol				
15-mer	40 mol%	0	60 mol%	96	1500	1700	1.1
(T40%A0%)	114.4 mg,		154.2 mg,				
	0.9 mmol		1.36 mmol				
15-mer	40 mol%	5 mol%	55 mol%	95	1400	1500	1.1
(T40%A5%)	114.4 mg,	14.1 mg,	141.35 mg,				
	0.9 mmol	0.11 mmol	1.25 mmol				
15-mer	40 mol%	10 mol%	50 mol%	98	1600	2100	1.3
(T40%A10%)	114.4 mg,	28.2 mg,	129.0 mg,				
	0.9 mmol	0.22 mmol	1.14 mmol				
15-mer	40 mol%	20 mol%	40 mol%	98	1500	1800	1.2
(T40%A20%)	114.4 mg,	56.4 mg,	102.8 mg,				
	0.9 mmol	0.44 mmol	0.91 mmol				

3. ^{1}H NMR of polymer ligands in MeOD

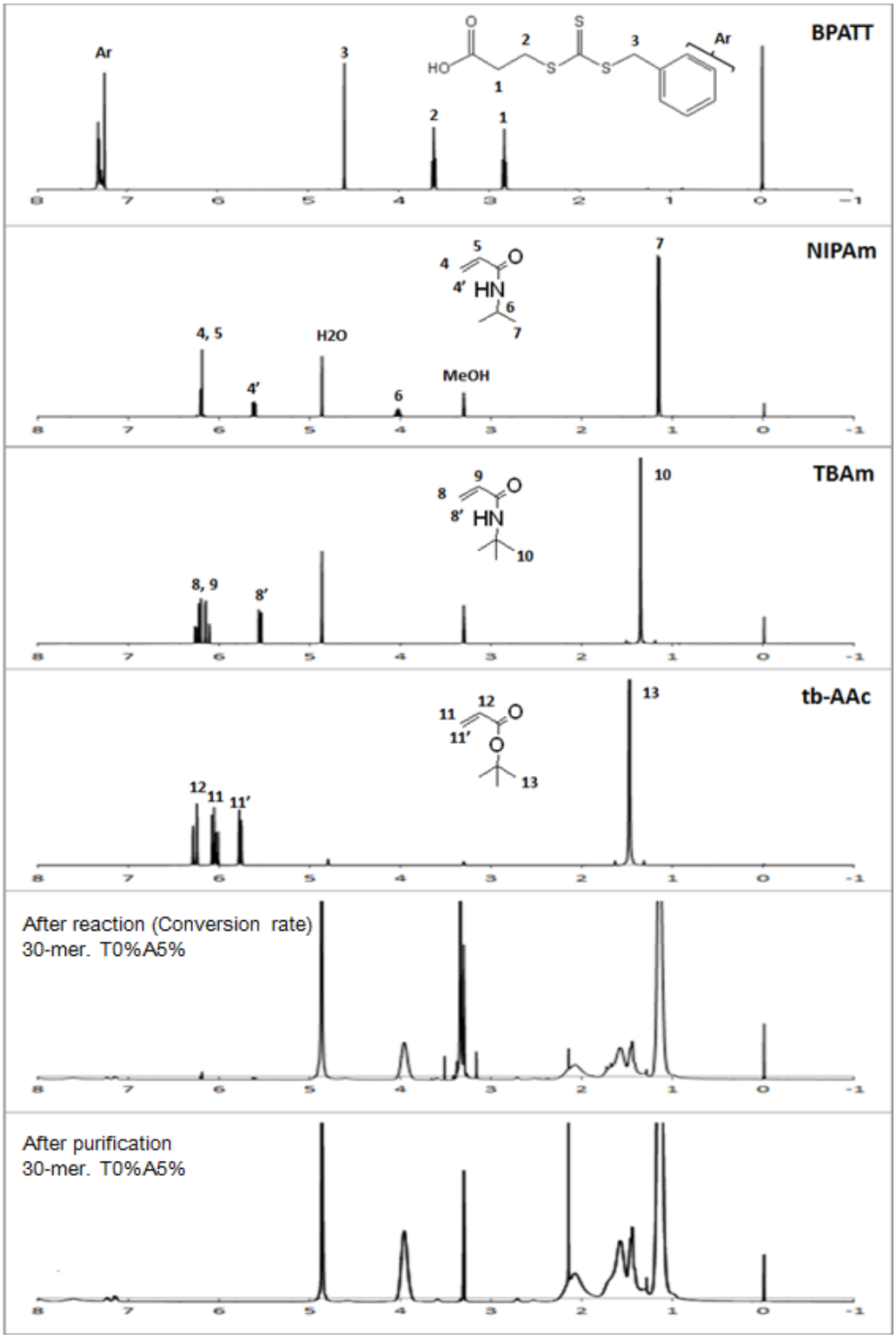


Figure S1-2. ^{1}H NMR of 30-mer PL, containing 0 % TBAm and 5 % *tb*-AAc

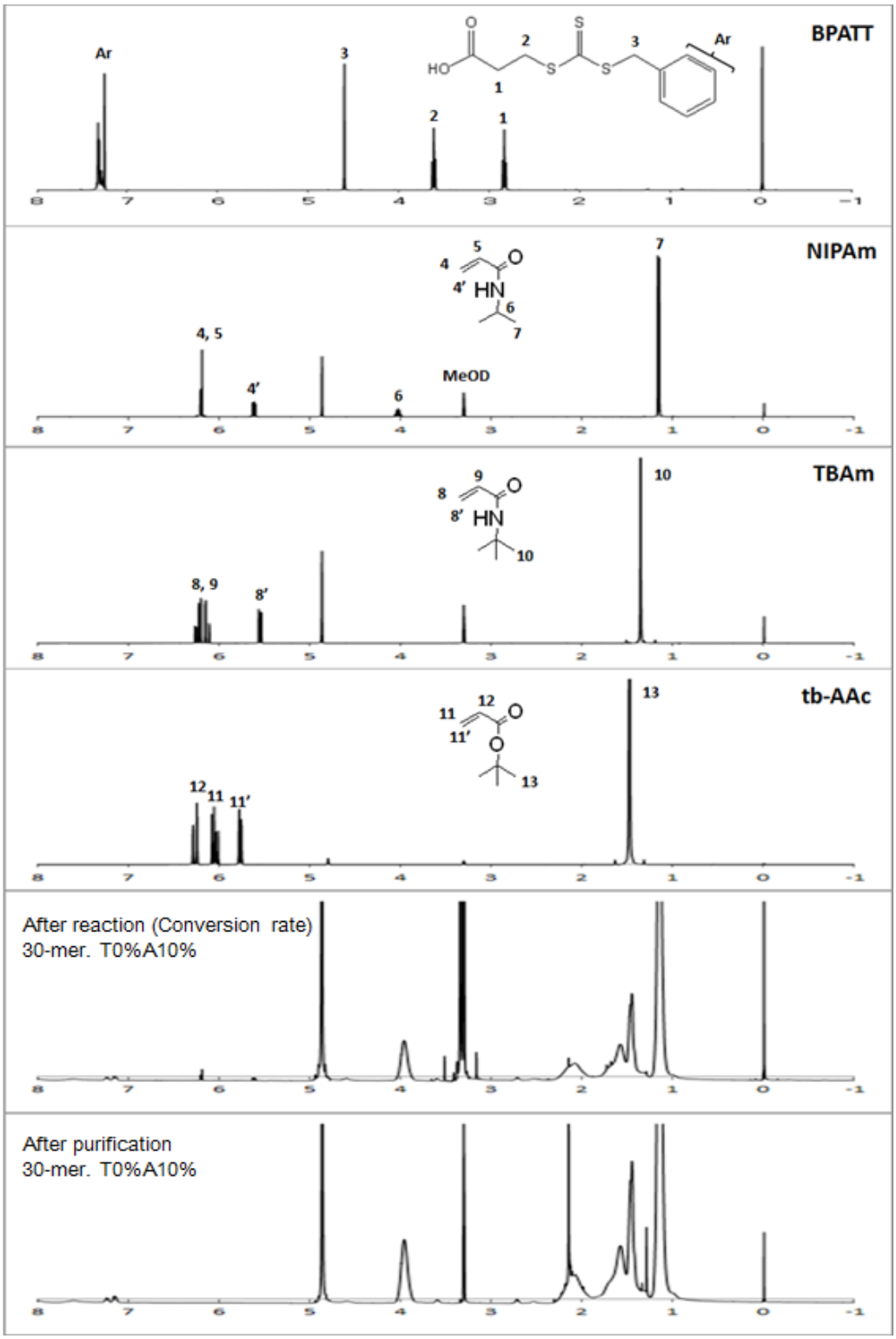


Figure S1-3. ^{1}H NMR of 30-mer PL, containing 0 % TBAm and 10 % *tb*-AAc

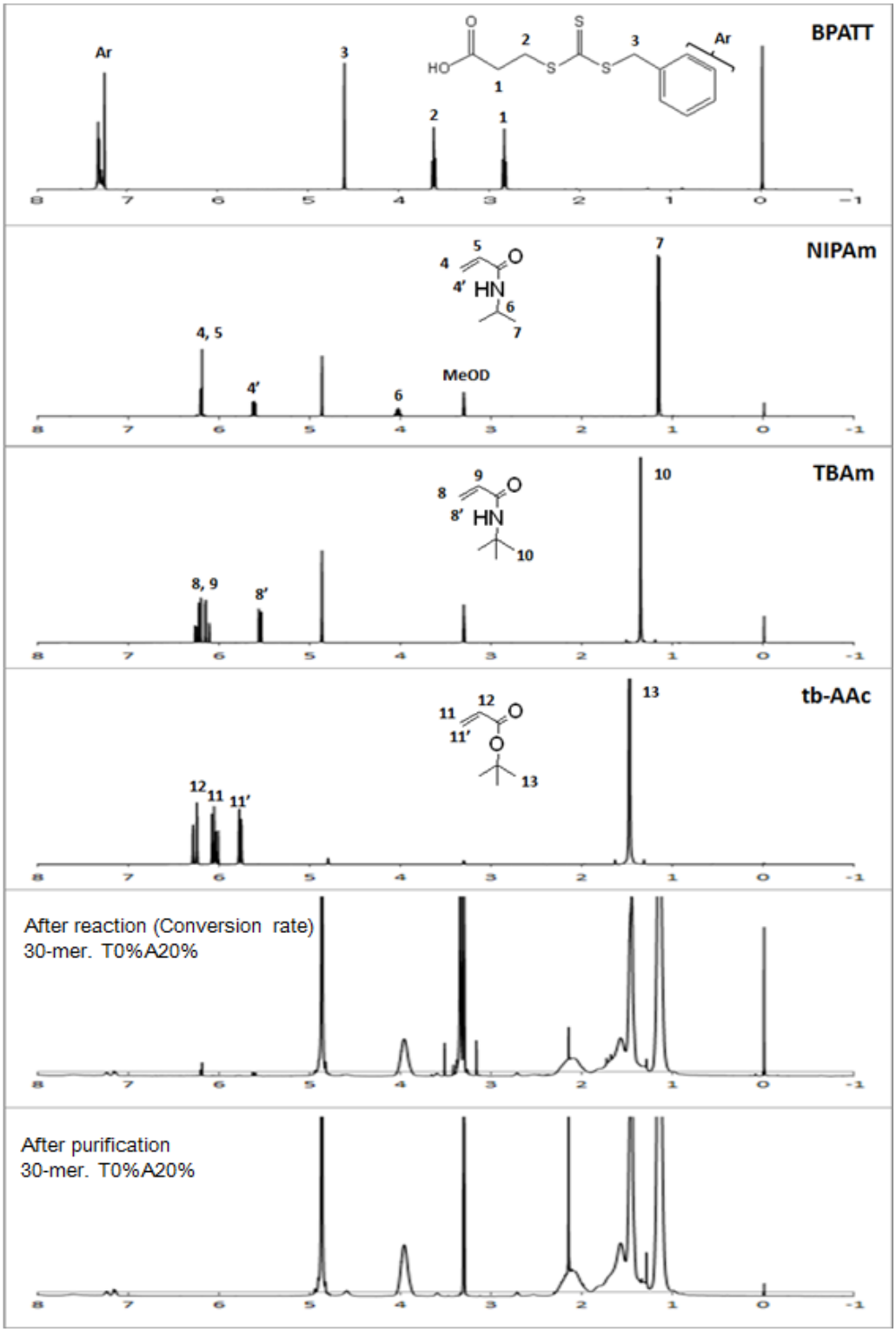


Figure S1-4. ^{1}H NMR of 30-mer PL, containing 0 % TBAm and 20 % *tb*-AAc

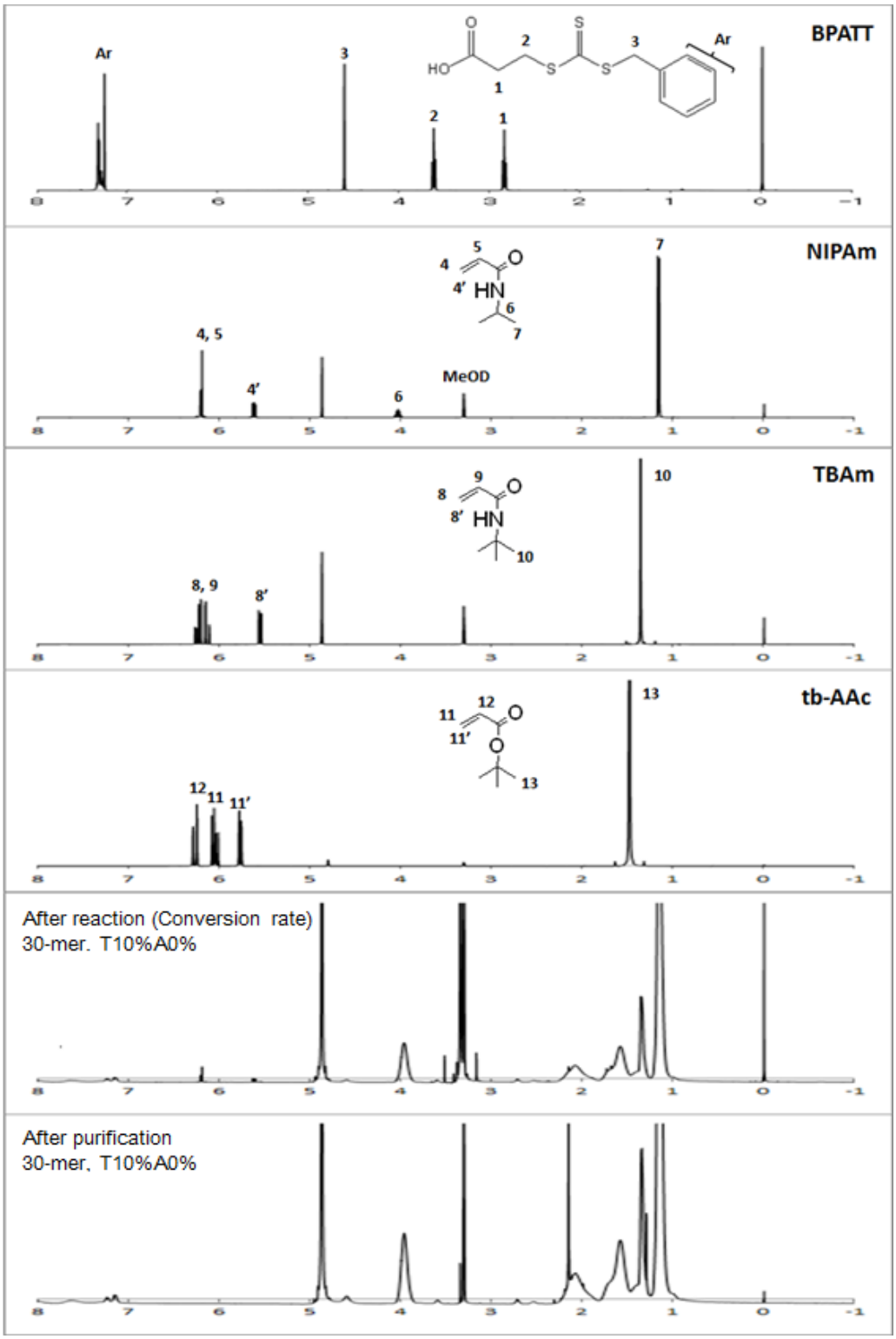


Figure S1-5. ^{1}H NMR of 30-mer PL, containing 10 % TBAm and 0 % *tb*-AAc

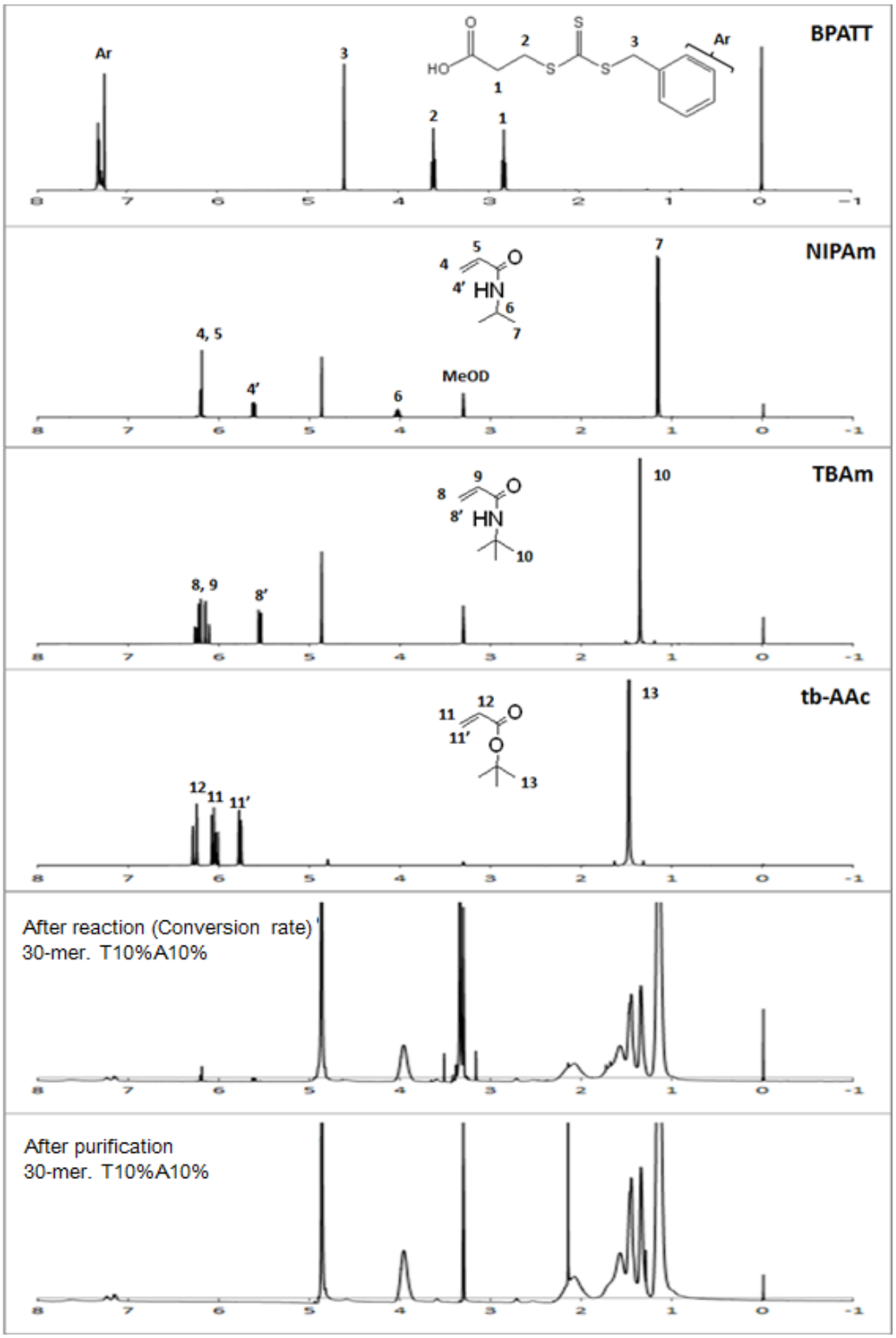


Figure S1-6. ^{1}H NMR of 30-mer PL, containing 10 % TBAm and 10 % *tb*-AAc

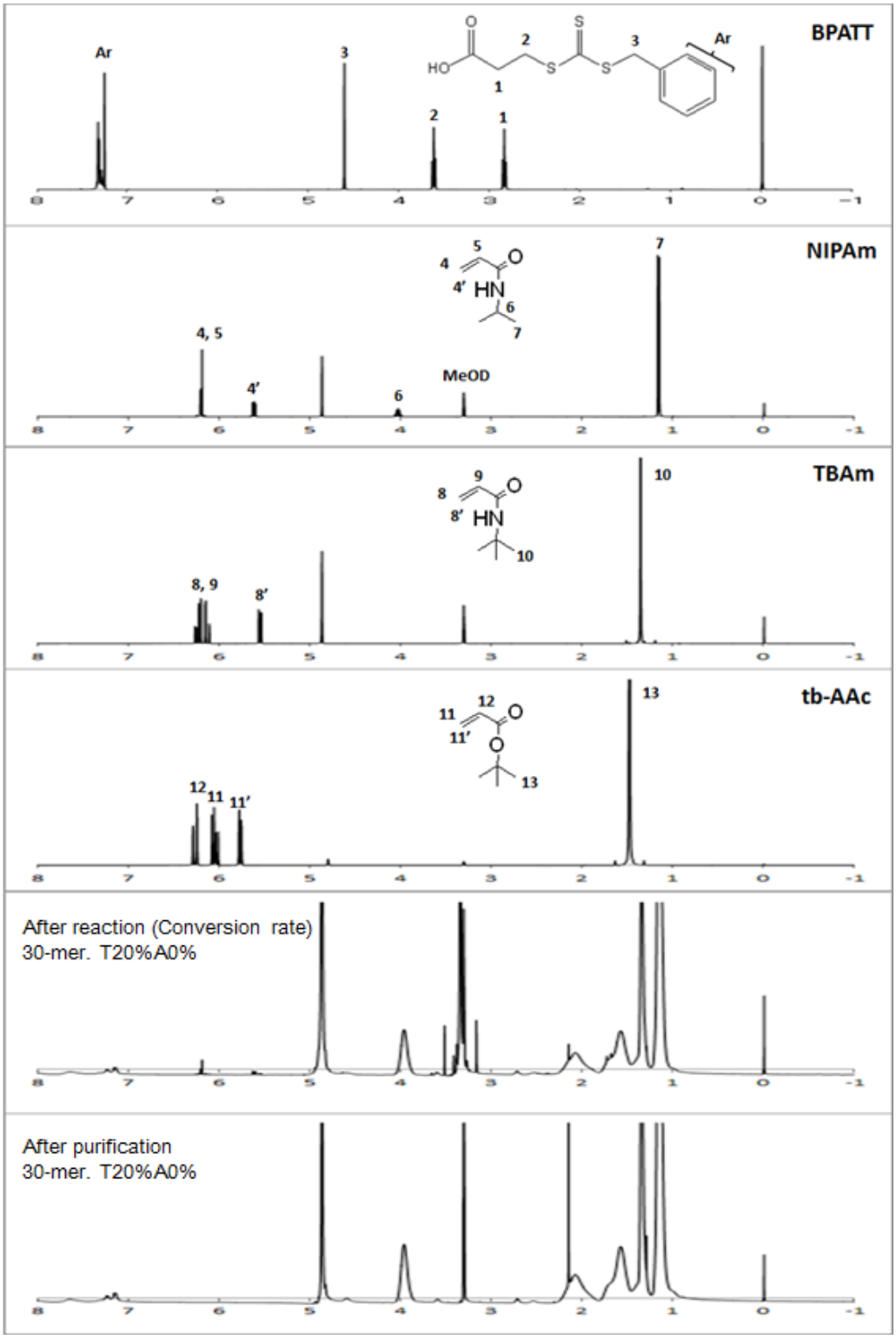


Figure S1-7. ^{1}H NMR of 30-mer PL, containing 20 % TBAm and 0 % *tb*-AAc

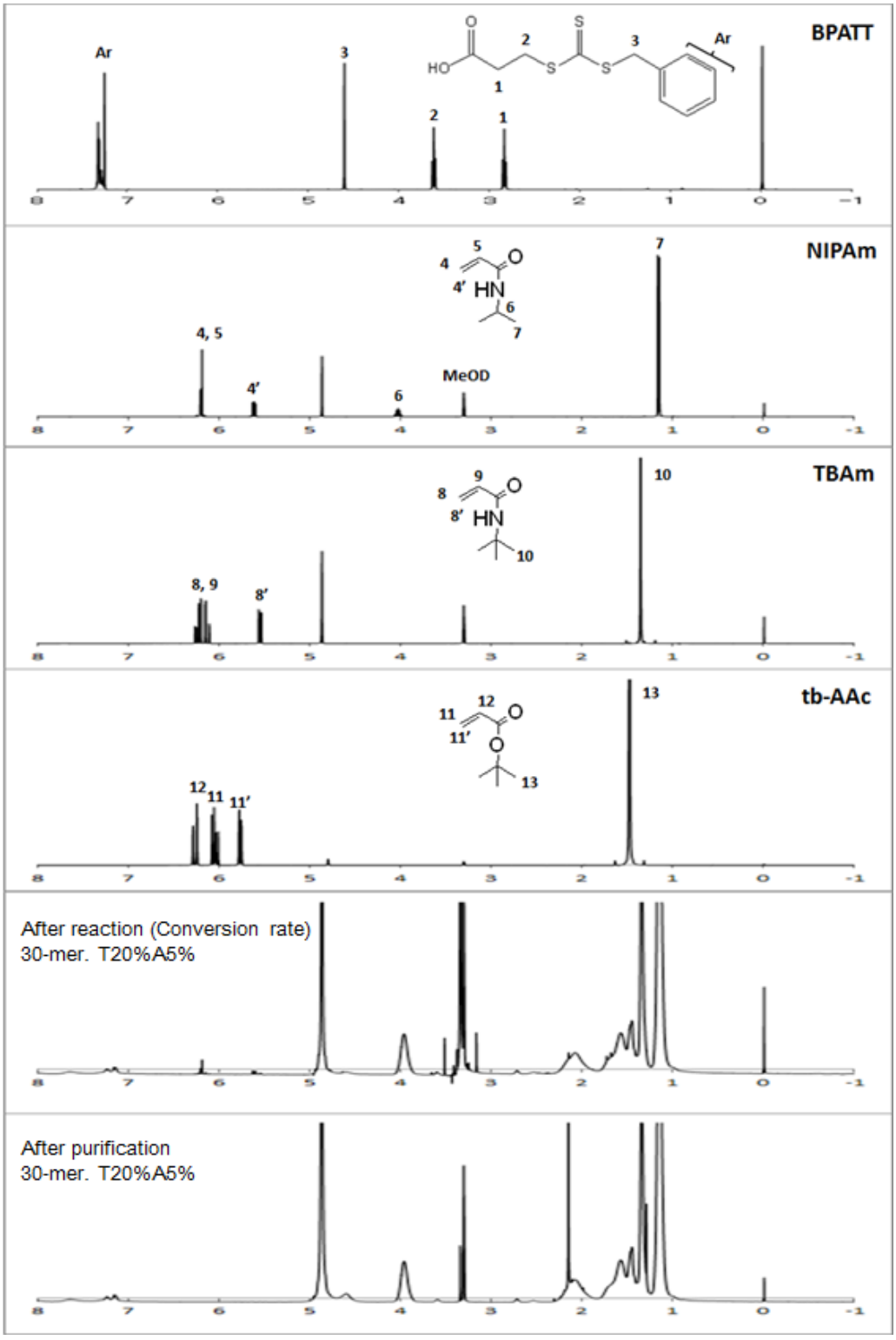


Figure S1-8. ^{1}H NMR of 30-mer PL, containing 20 % TBAm and 5 % *tb*-AAc

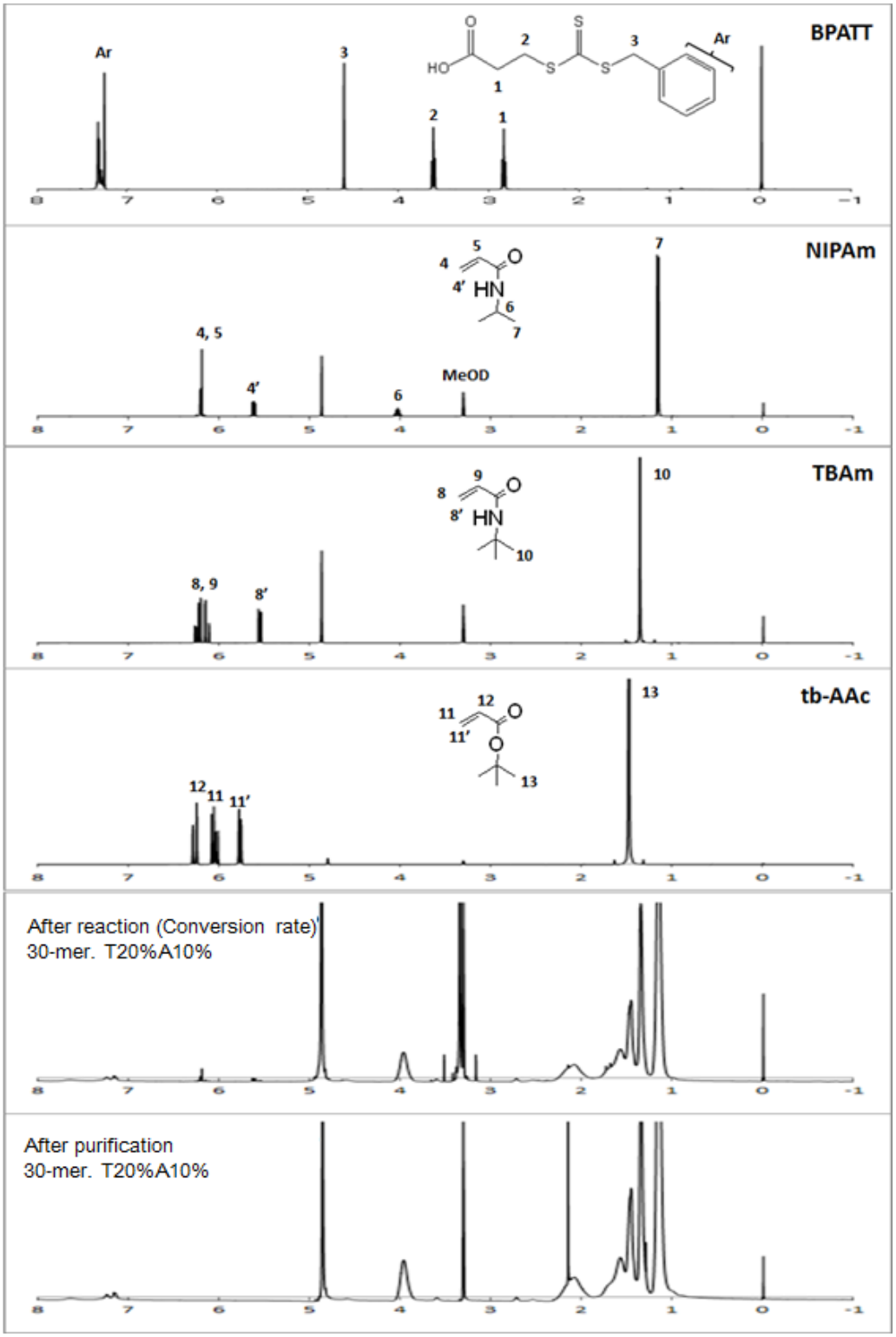


Figure S1-9. ^{1}H NMR of 30-mer PL, containing 20 % TBAm and 10 % *tb*-AAc

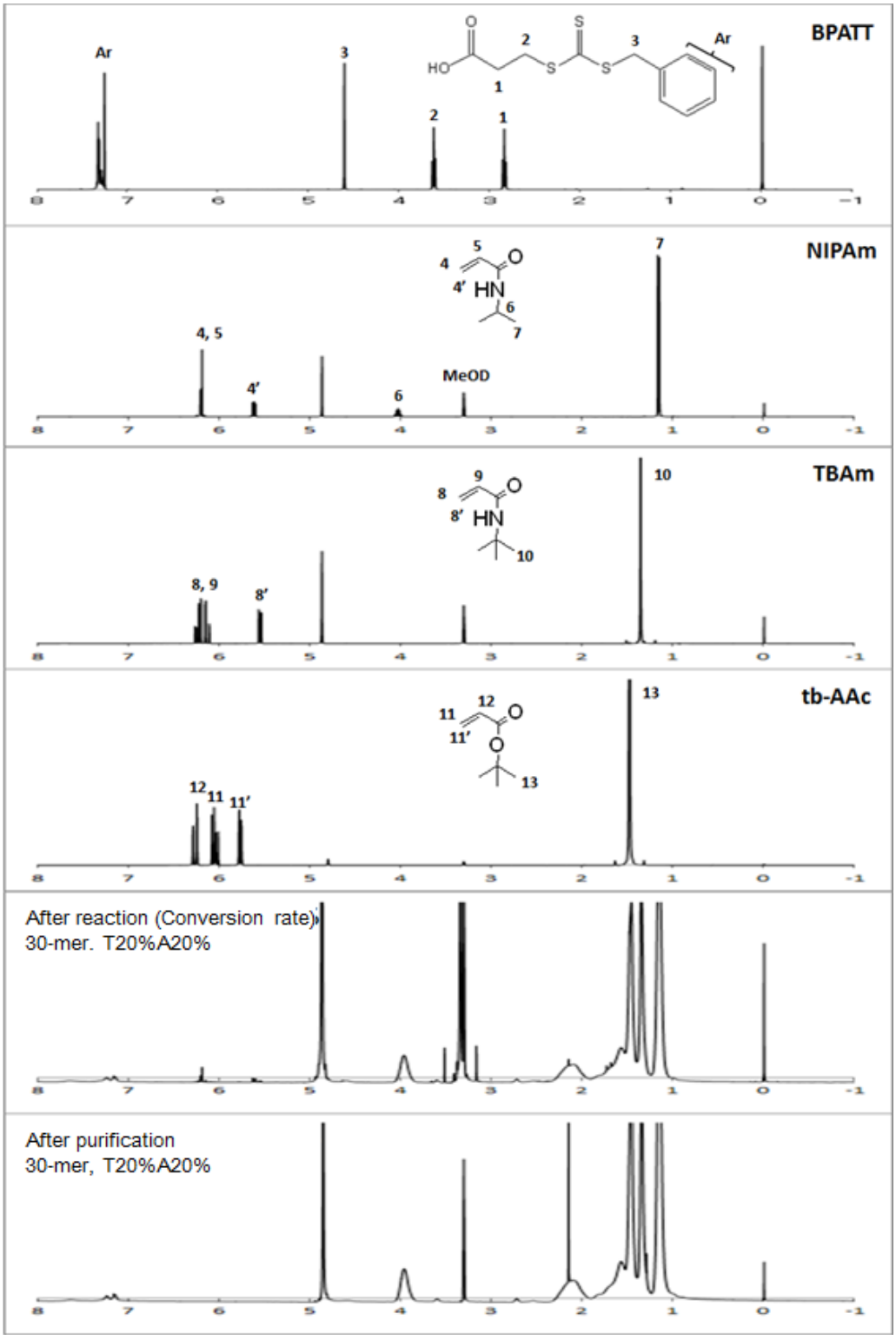


Figure S1-10. ^{1}H NMR of 30-mer PL, containing 20 % TBAm and 20 % *tb*-AAc

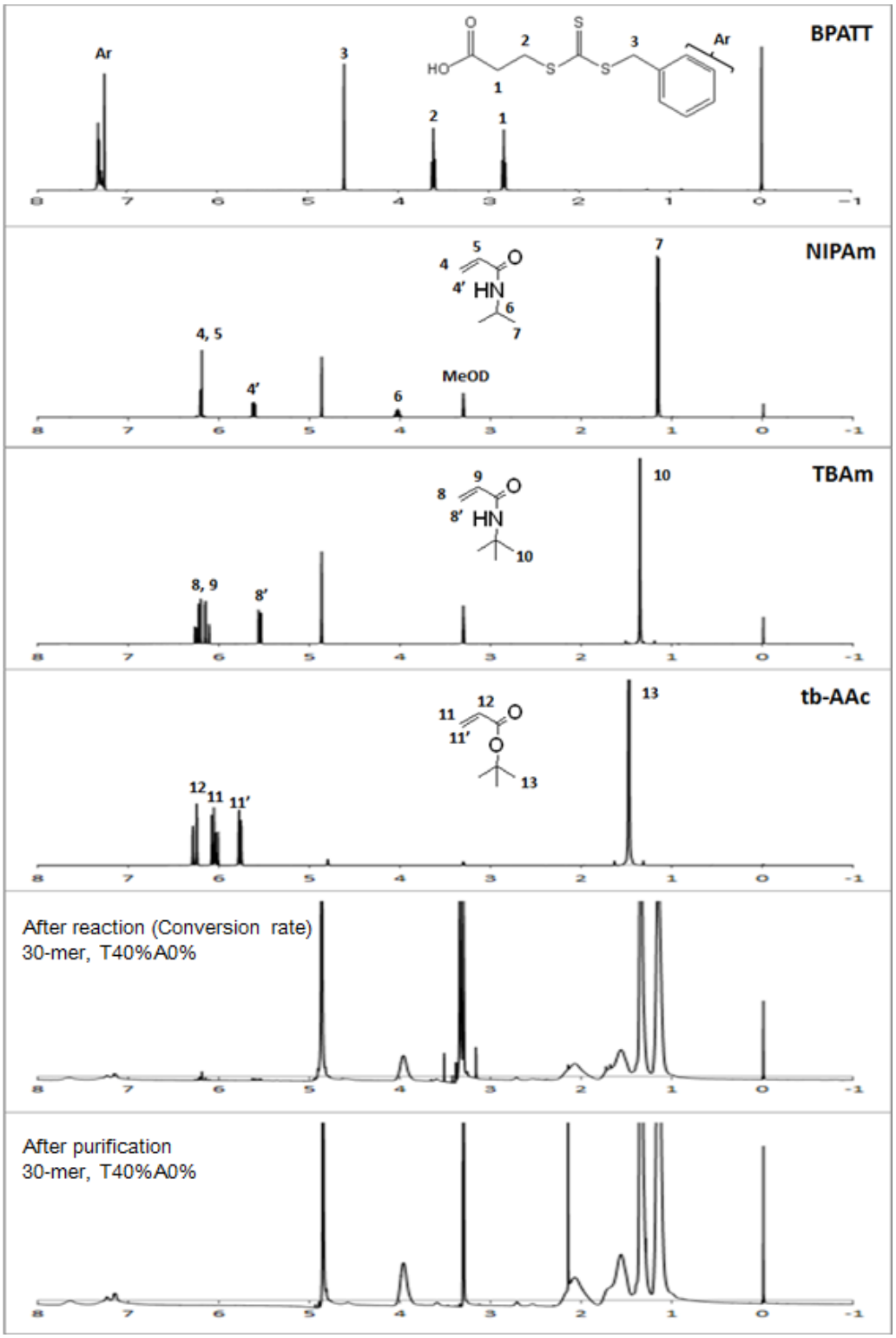


Figure S1-11. ^{1}H NMR of 30-mer PL, containing 40 % TBAm and 0 % *tb*-AAc

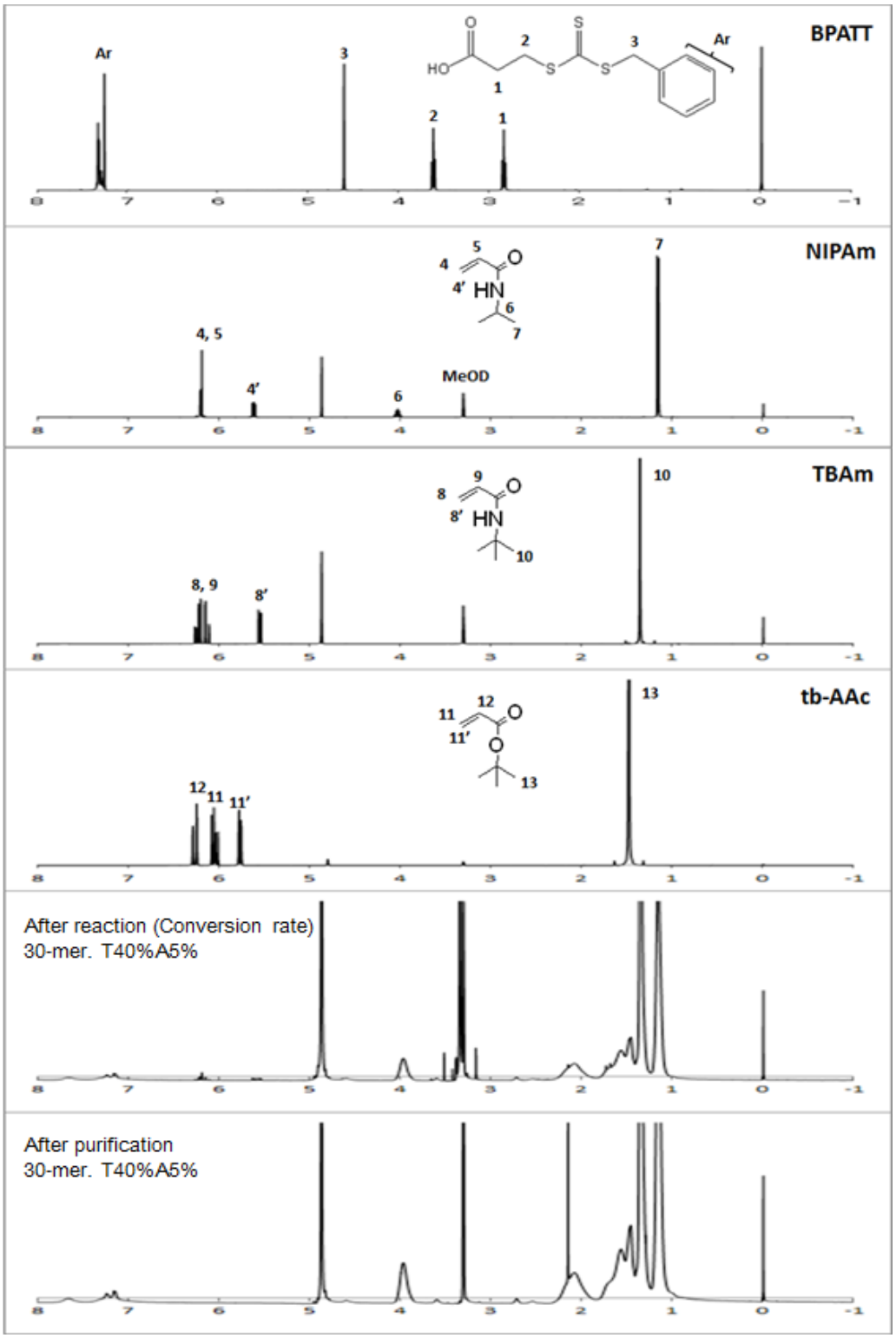


Figure S1-12. ^{1}H NMR of 30-mer PL, containing 40 % TBAm and 5 % *tb*-AAc

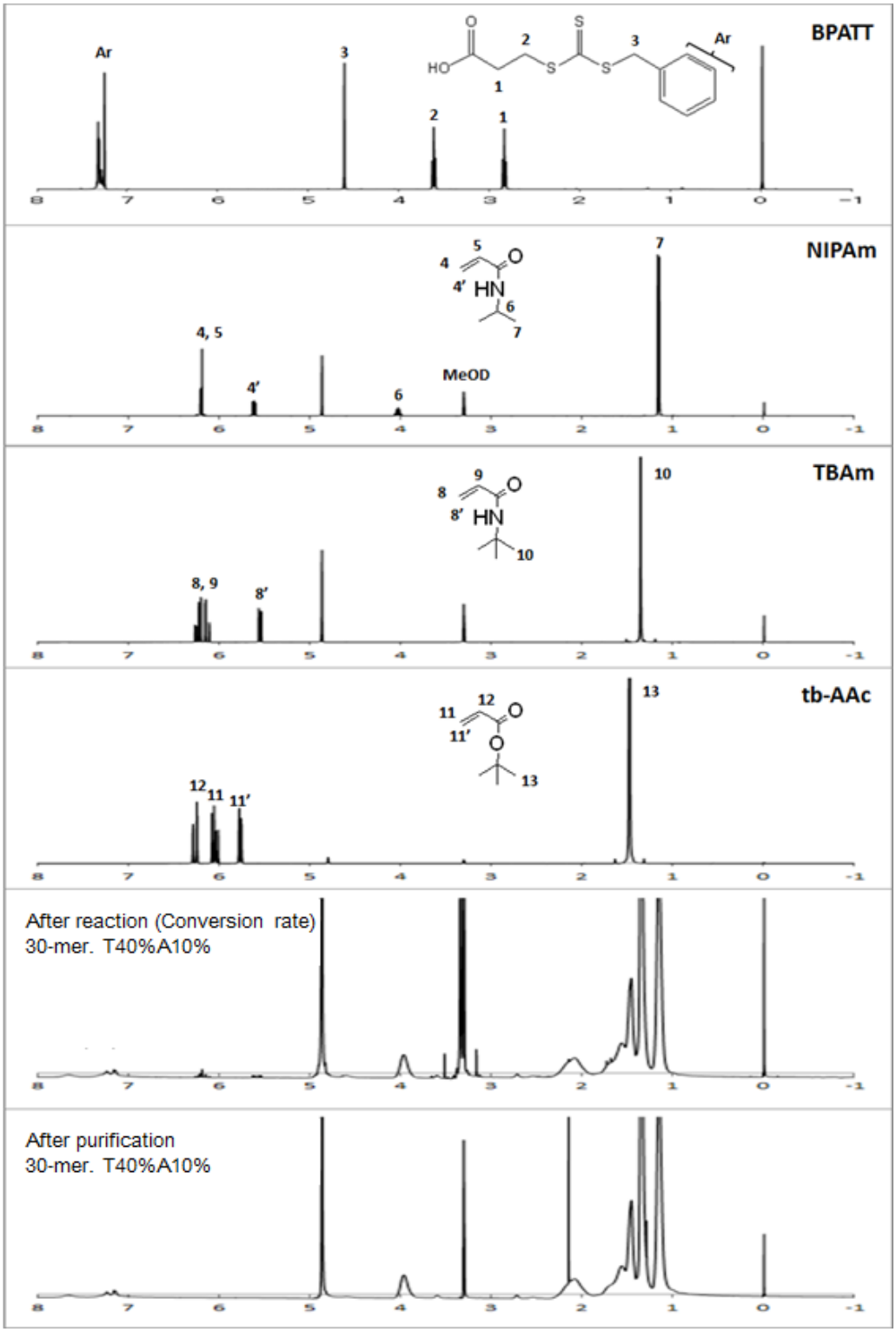


Figure S1-13. ^{1}H NMR of 30-mer PL, containing 40 % TBAm and 10 % *tb*-AAc

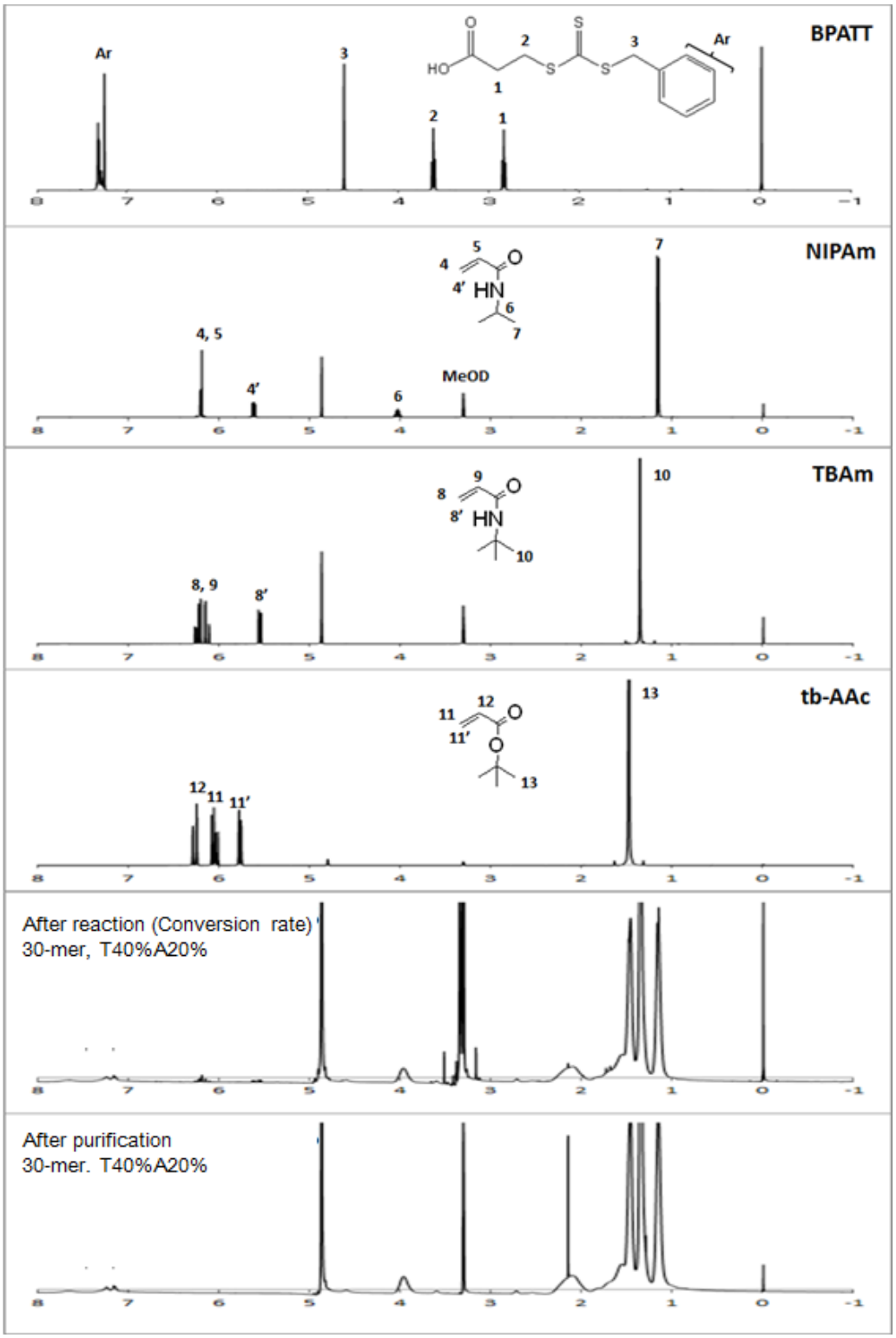


Figure S1-14. ^{1}H NMR of 30-mer PL, containing 40 % TBAm and 20 % *tb*-AAc

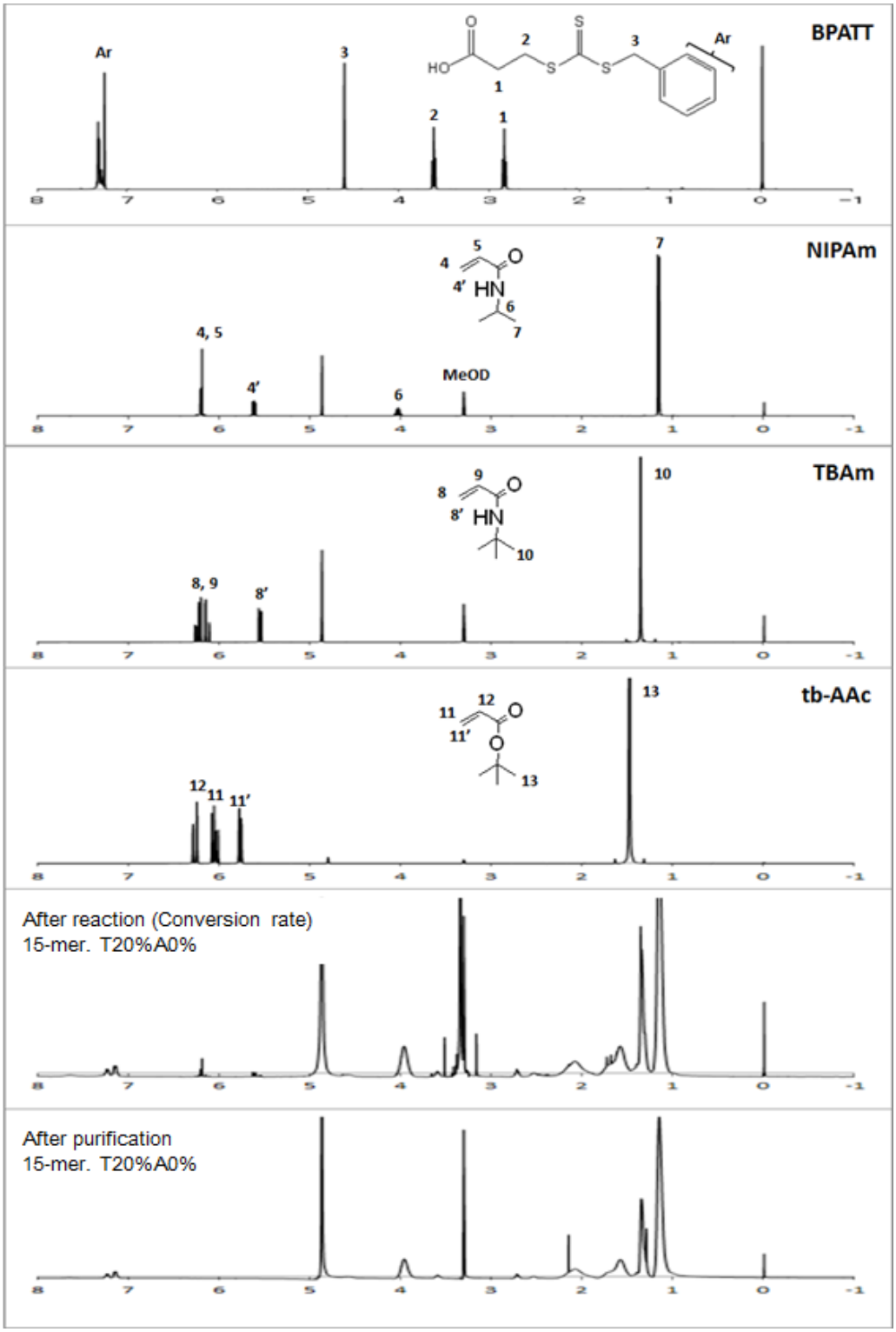


Figure S1-15. ^{1}H NMR of 15-mer PL, containing 20 % TBAm and 0 % *tb*-AAc

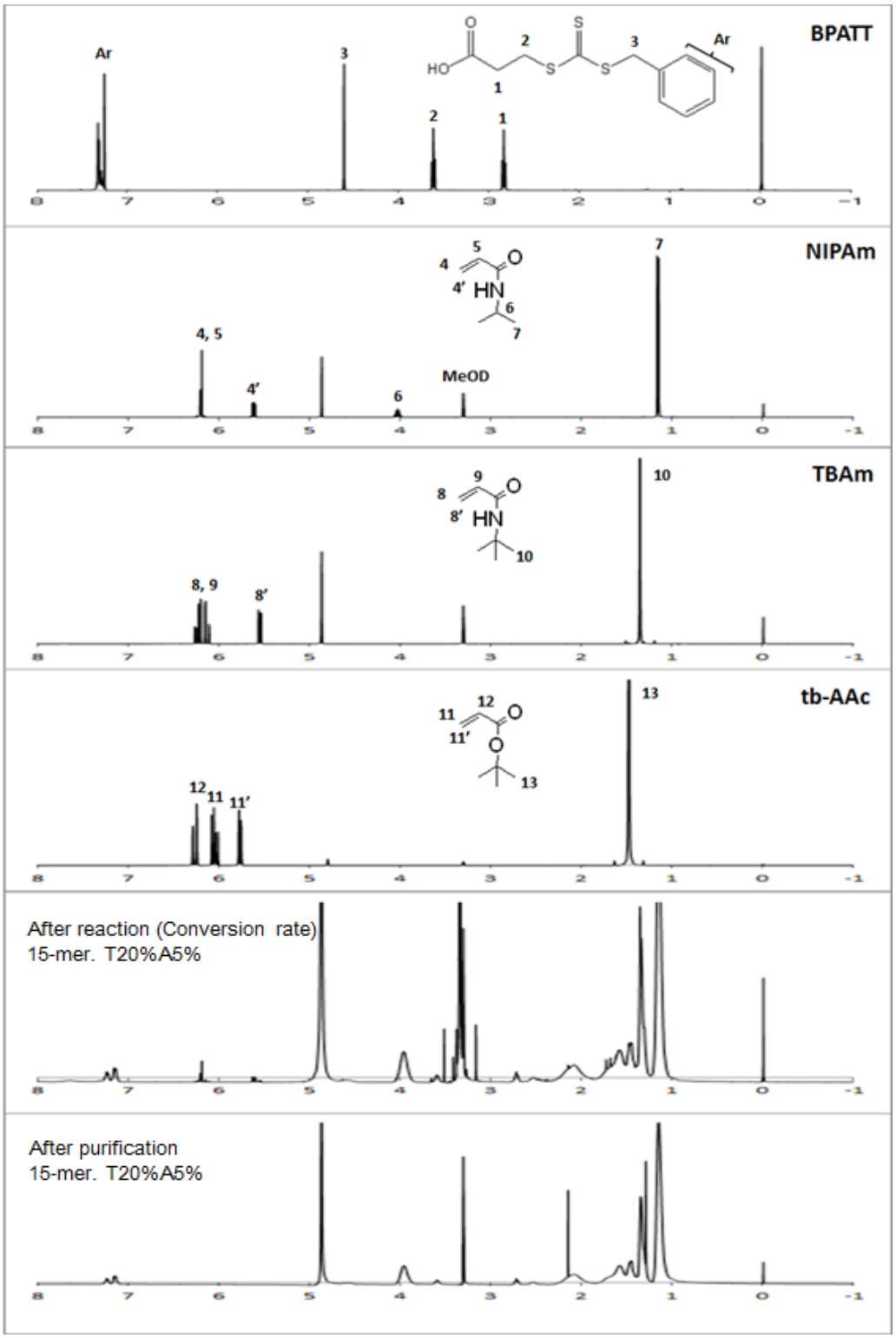


Figure S1-16. ^{1}H NMR of 15-mer PL, containing 20 % TBAm and 5 % *tb*-AAc

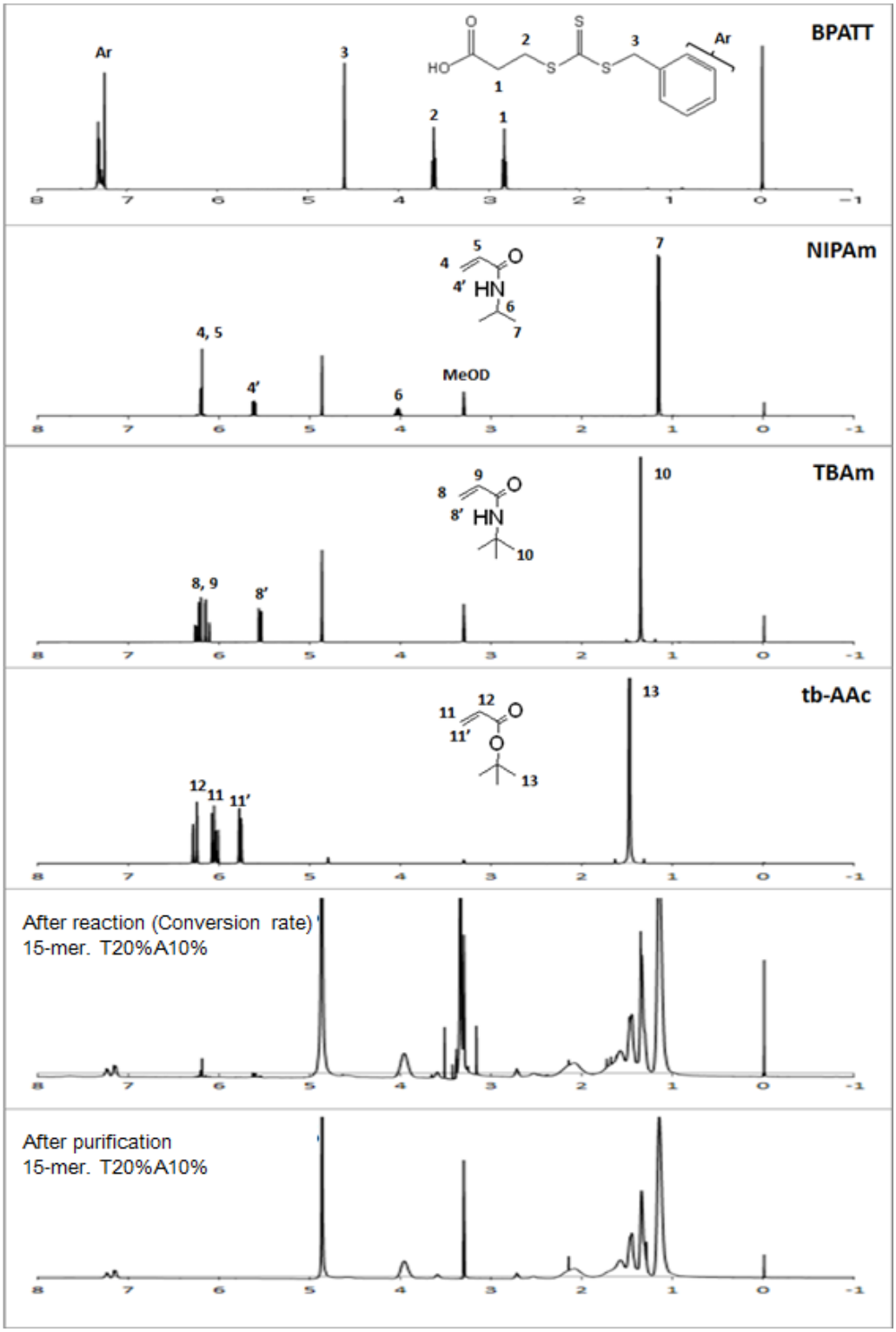


Figure S1-17. ^{1}H NMR of 15-mer PL, containing 20 % TBAm and 10 % *tb*-AAc

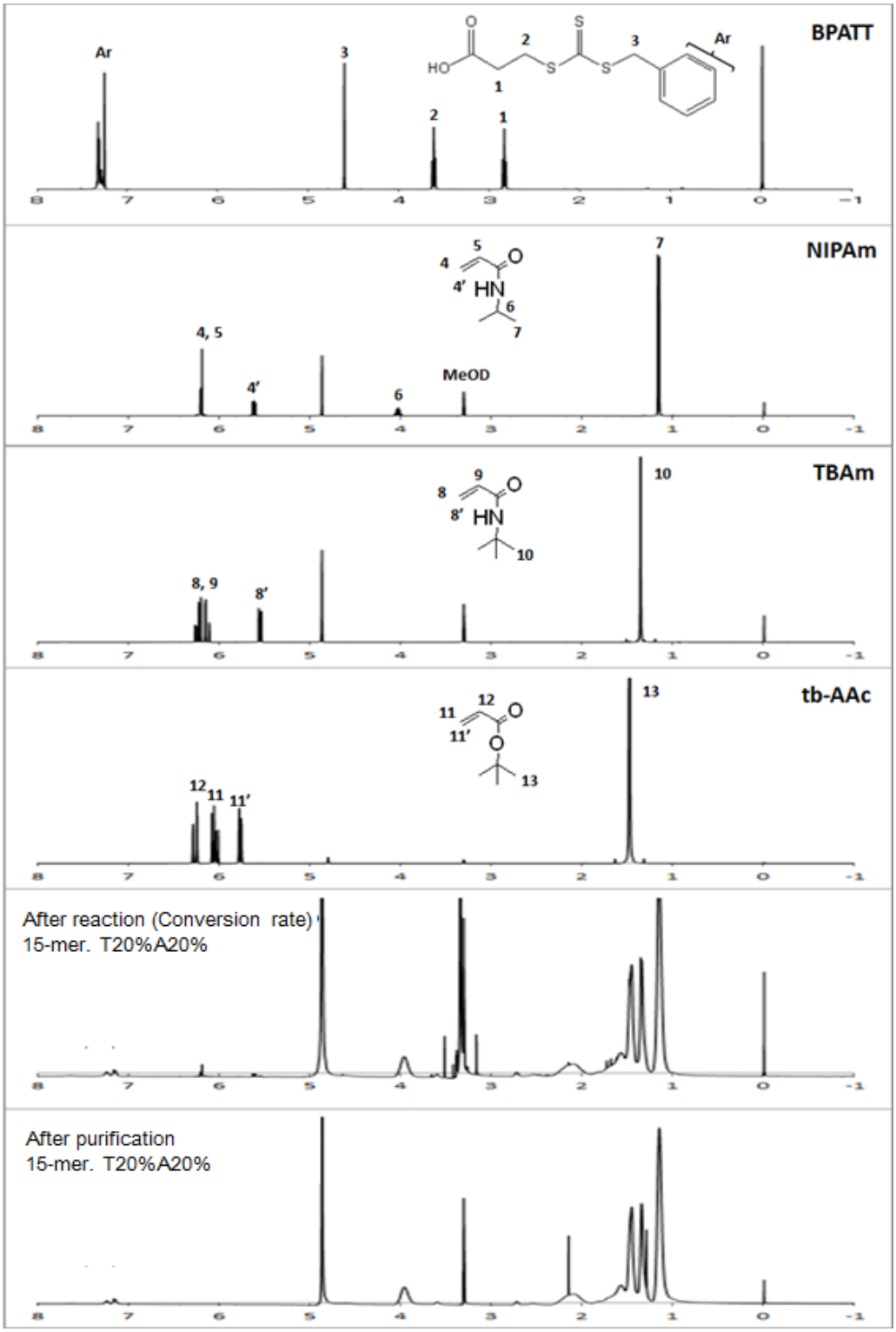


Figure S1-18. ^{1}H NMR of 15-mer PL, containing 20 % TBAm and 20 % *tb*-AAc

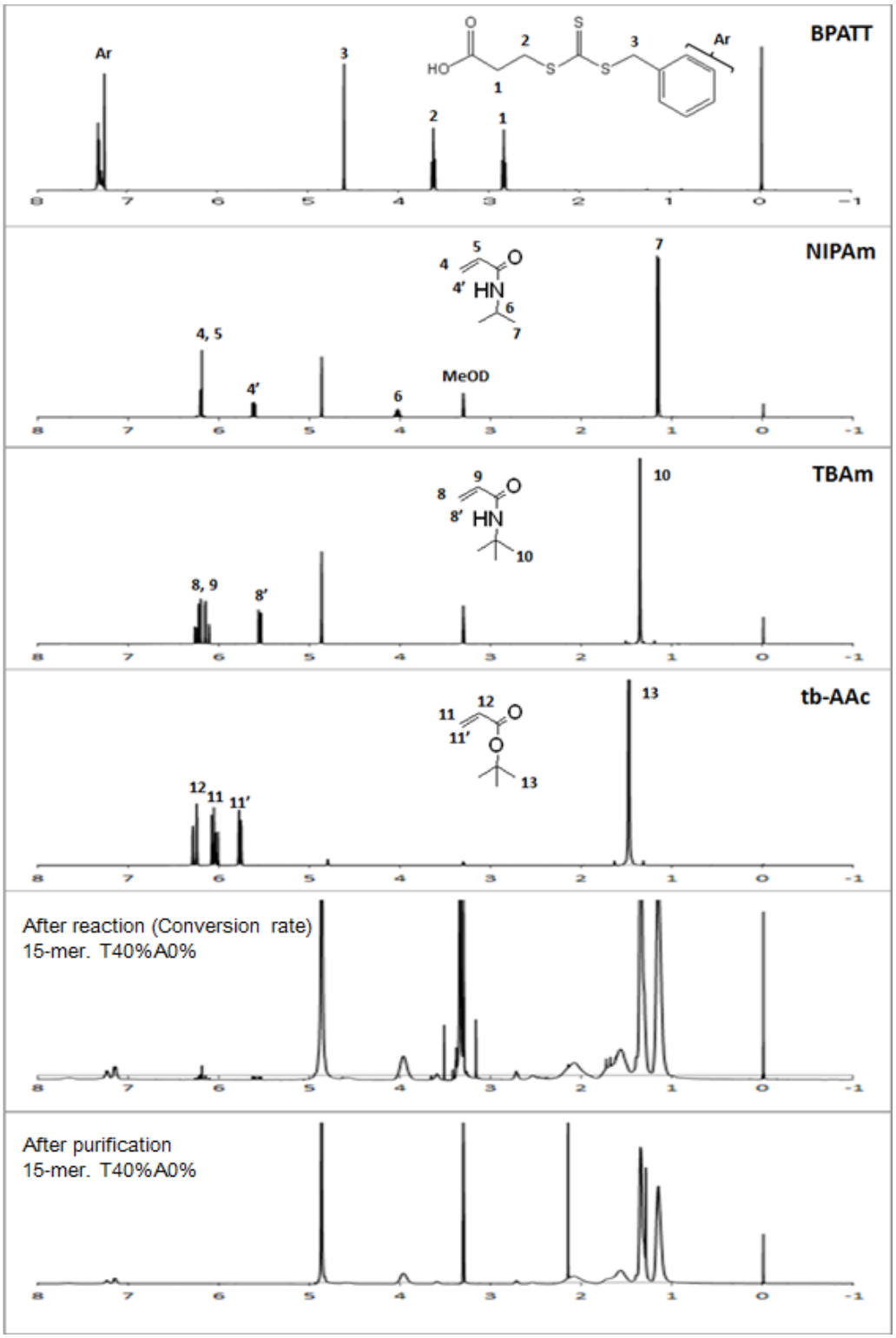


Figure S1-19. ^{1}H NMR of 15-mer PL, containing 40 % TBAm and 0 % *tb*-AAc

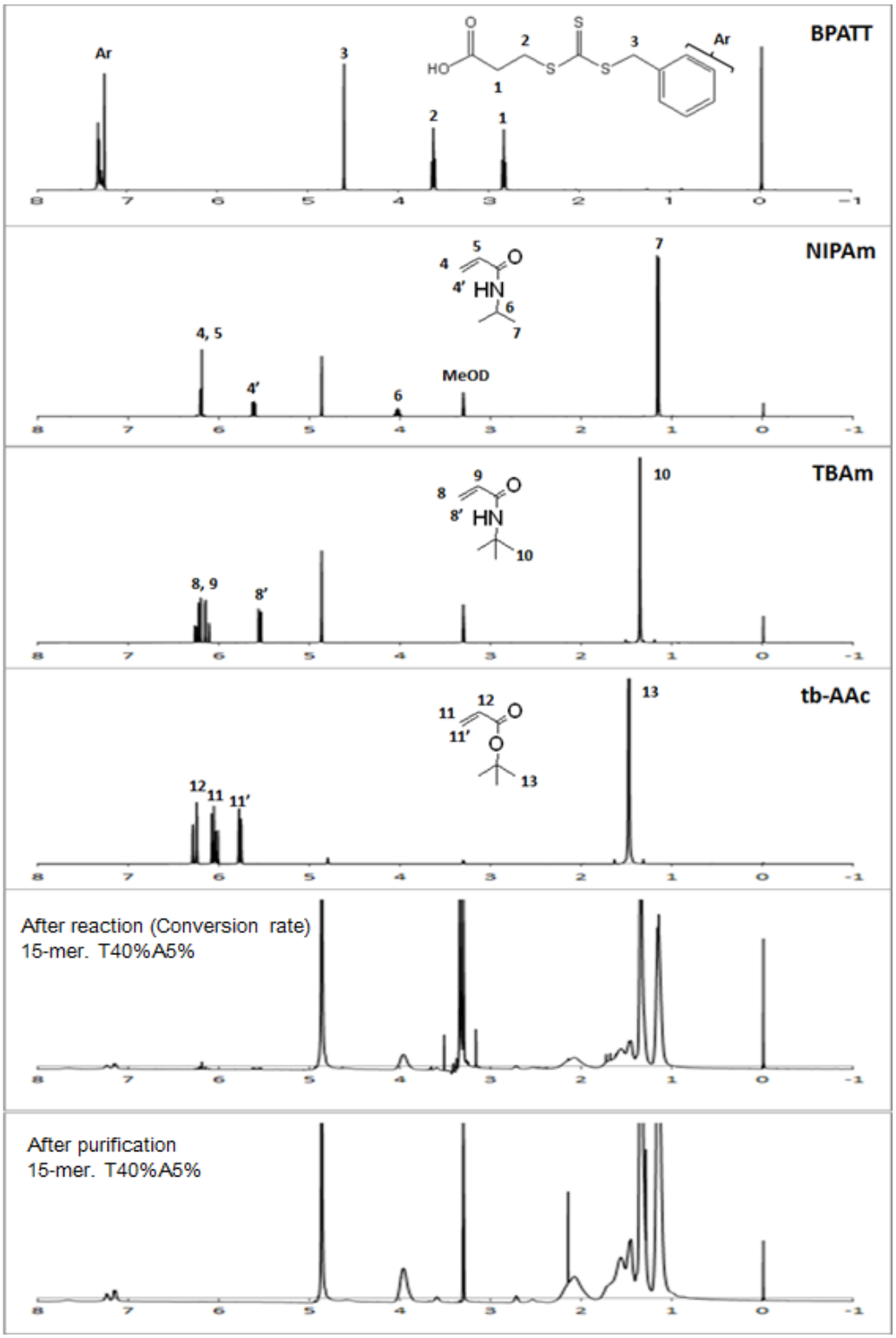


Figure S1-20 ^{1}H NMR of 15-mer PL, containing 40 % TBAm and 5 % *tb*-AAc

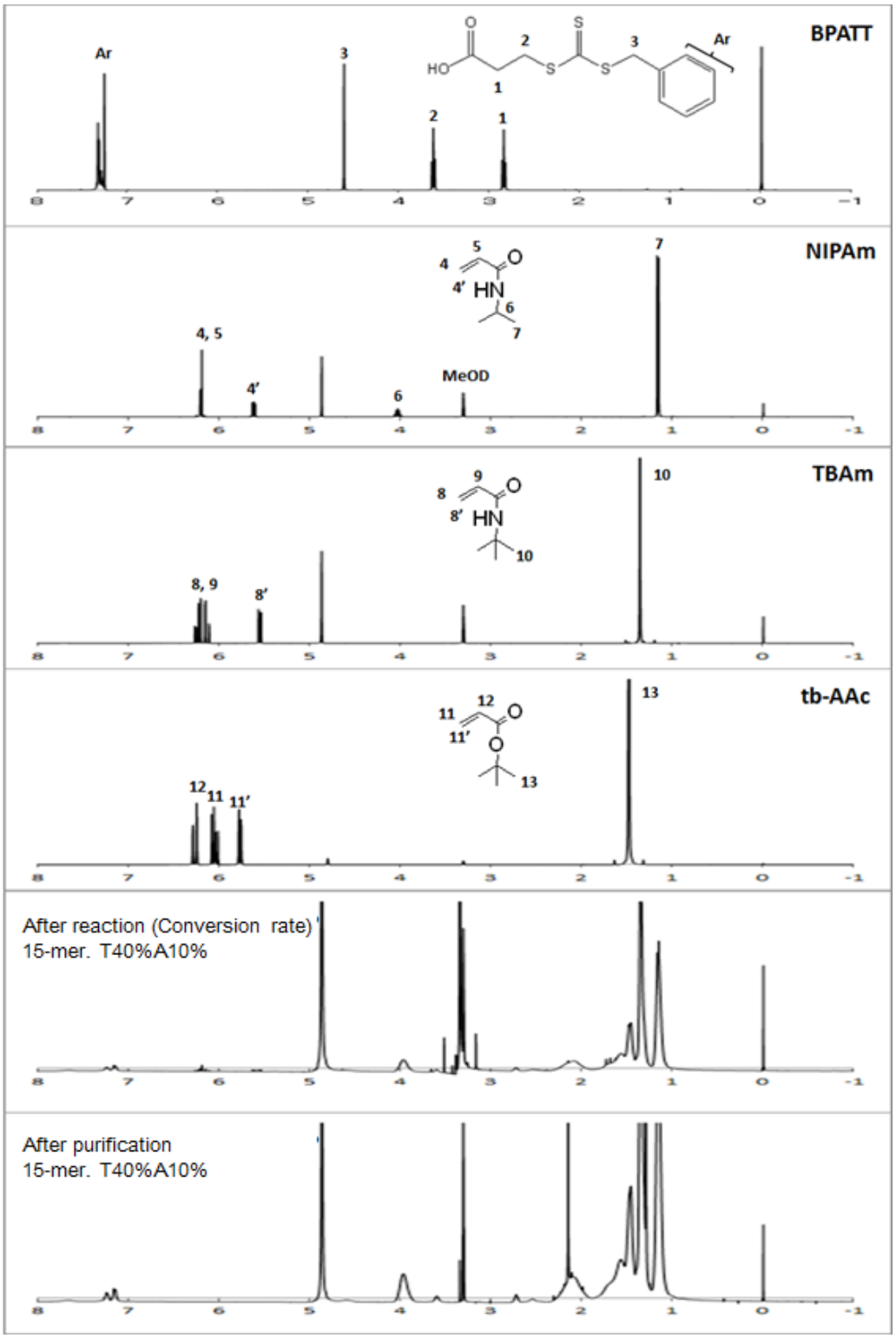


Figure S1-21. ^{1}H NMR of 15-mer PL, containing 40 % TBAm and 10 % *tb*-AAc

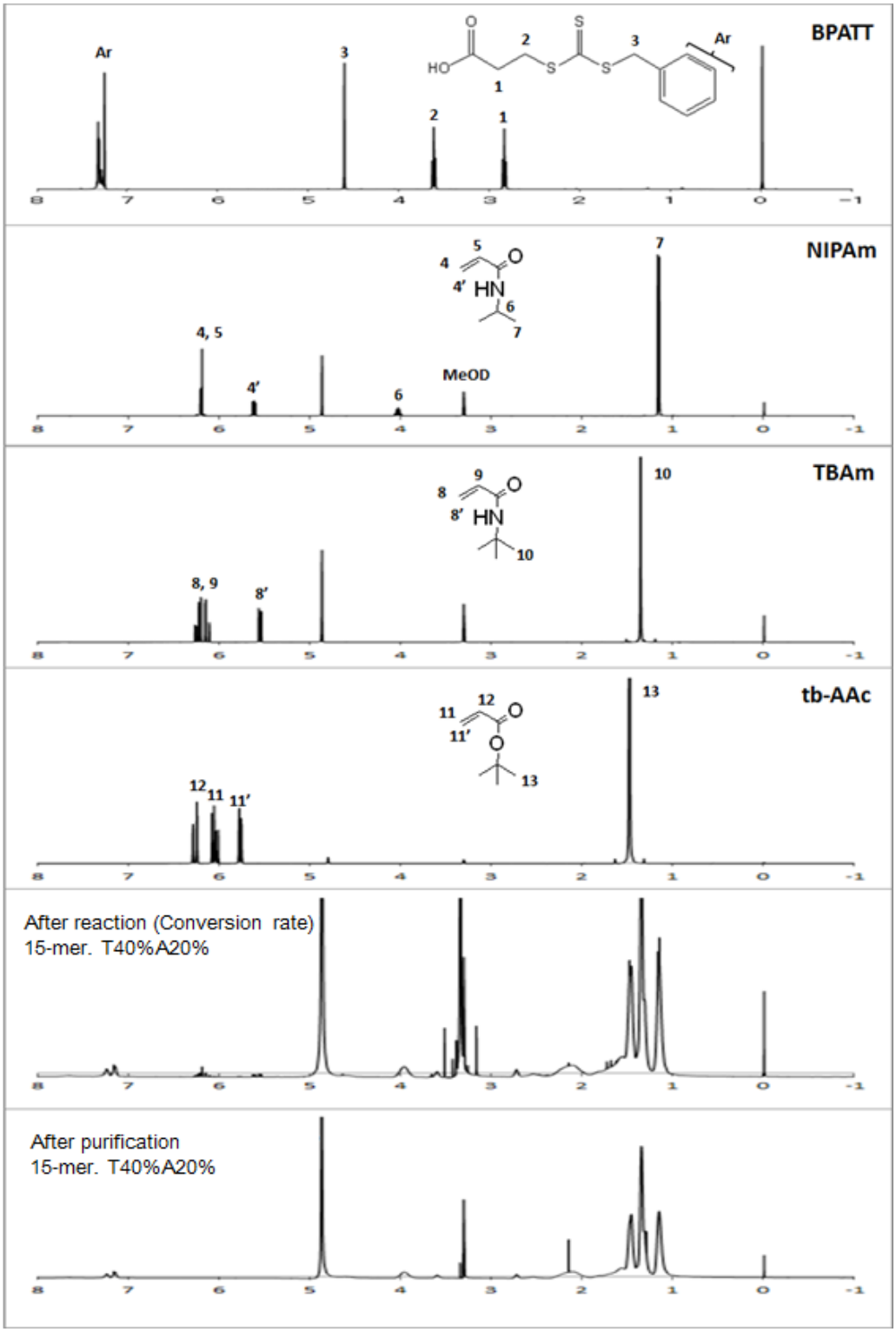


Figure S1-22. ^{1}H NMR of 15-mer PL, containing 40 % TBAm and 20 % *tb*-AAc

S2. HPLC analysis of peptides unbound by synthetic PL

All peptides and polymers were dissolved in 35 mM PBS (0.15 M NaCl, pH 7.3) and stored at 4°C. It was confirmed that all PLs were soluble in the buffer and the solutions were transparent even after incubation at 37 °C for 0.5 hr (Figure S2-0 a-c). 1.9 mg/mL of NP, 300-, and 30-mer PLs were added into the peptide mixed solutions (melittin, ponericin and magainin1, 0.1 mM each) in 35 mM PBS (0.15 M NaCl, pH 7.3) and incubated at 37°C for 0.5 hr. Then, the incubated solutions were filtered with centrifugal filter system for 0.5 hr (Milipore Co., Amicon Ultra-0.5, NMWL 10K, 8000 G at 37°C). We confirmed that PLs were separated from the solution by this filtration process (Figure S2-0 d). After centrifugation, 7 μL of the filtrate were injected on a CAPCELL PAK C18 column for separation by high performance liquid chromatography (HPLC). The mobile phase consisted of 0.1 % TFA dissolved in acetonitrile/water. The linear elution gradient is illustrated in **Figure S2-1**. Flow rate of the mobile phase was 1 mL/min. The column was kept at 37 °C, and elution was monitored at 220 nm.

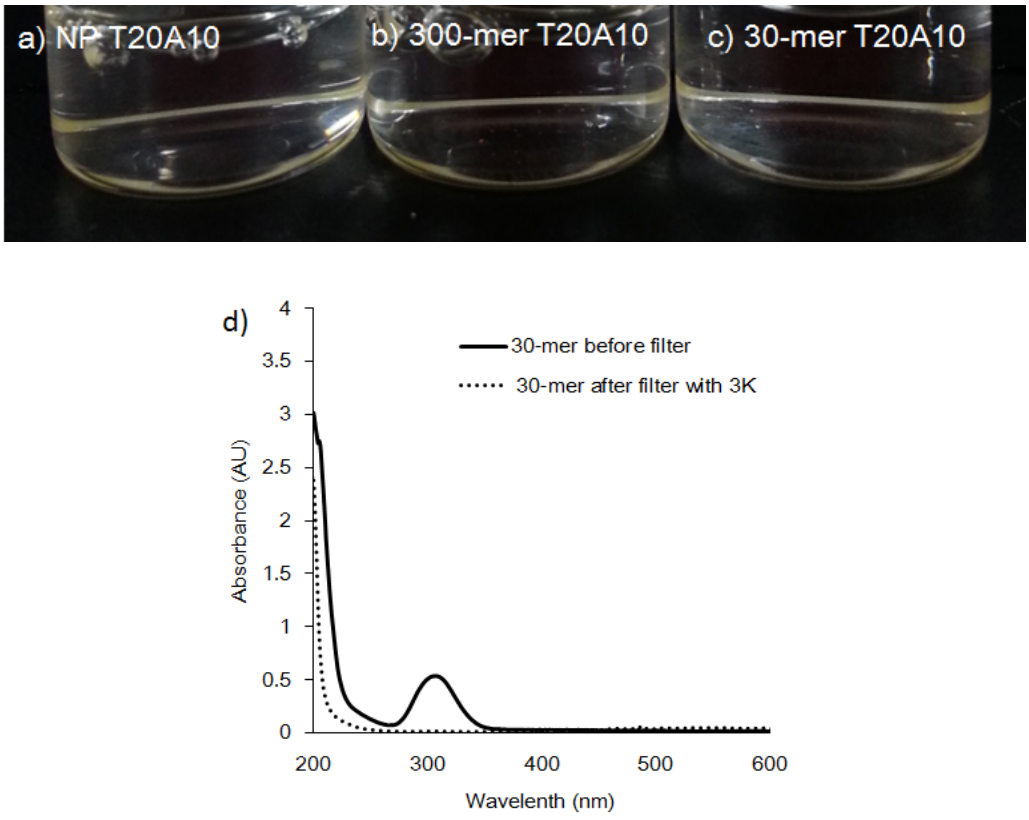


Figure S2-0. a-c Photographs of polymer solutions (0.1 mM) after incubation at 37°C for 0.5 hr. **d** UV-vis spectra of buffer solution of 30-mer PLs before and after filtration.

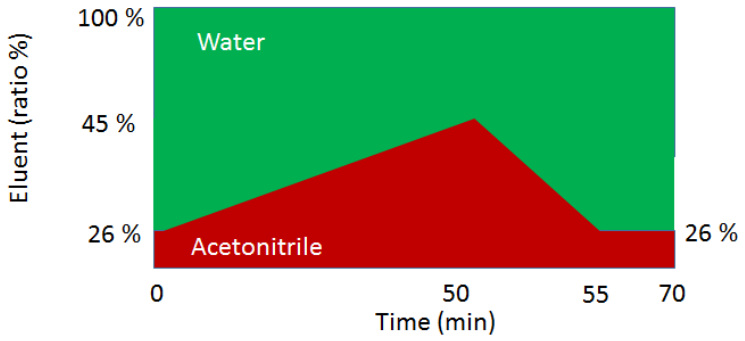


Figure S2-1. HPLC scheme

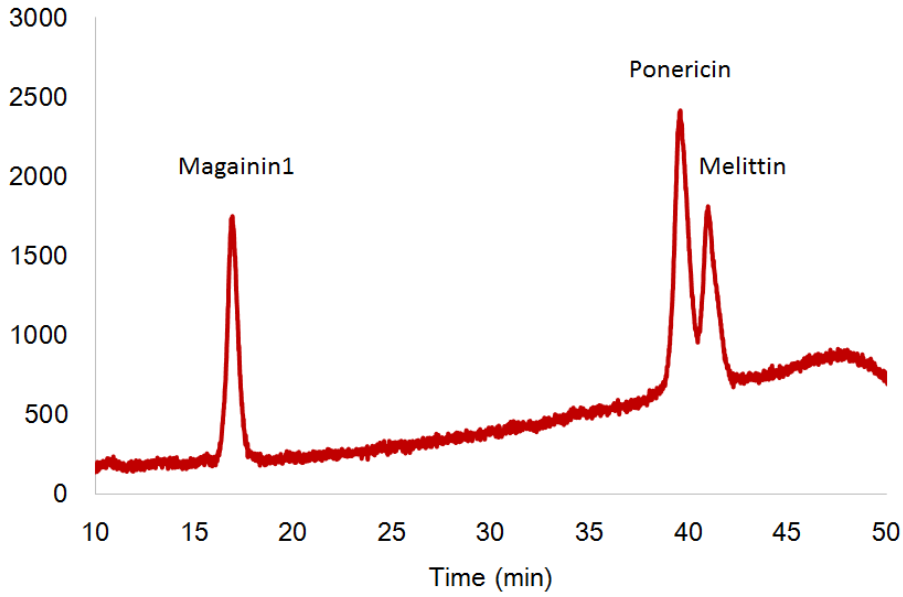


Figure S2-2. A mixture of free lytic peptides, consisting of magainin 1, ponericin, and melittin (concentration of peptides: 0.1 mM each).

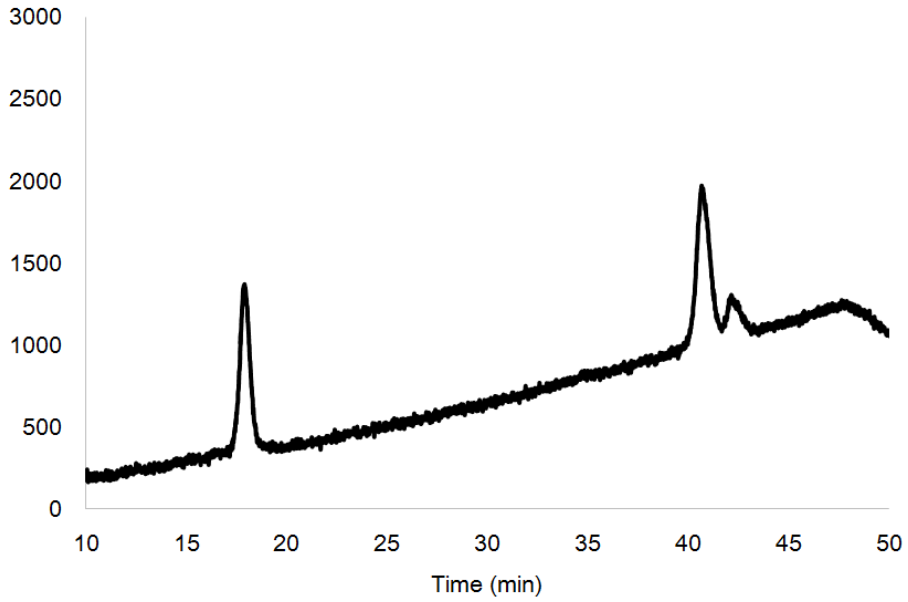


Figure S2-3. Peptides unbound by NP (T20%A10%)
(concentration of peptides: 0.1 mM each, concentration of NP: 1.9 mg/mL)

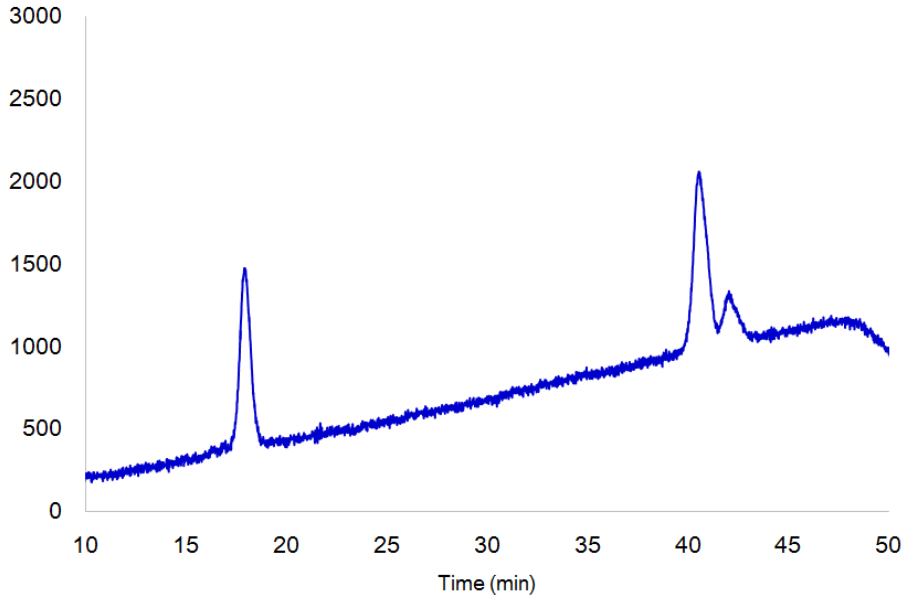


Figure S2-4. Peptides not bound by 300-mer PL (T20%A10%)
(concentration of peptides: 0.1 mM each, concentration of 300-mer PL: 1.9 mg/mL)

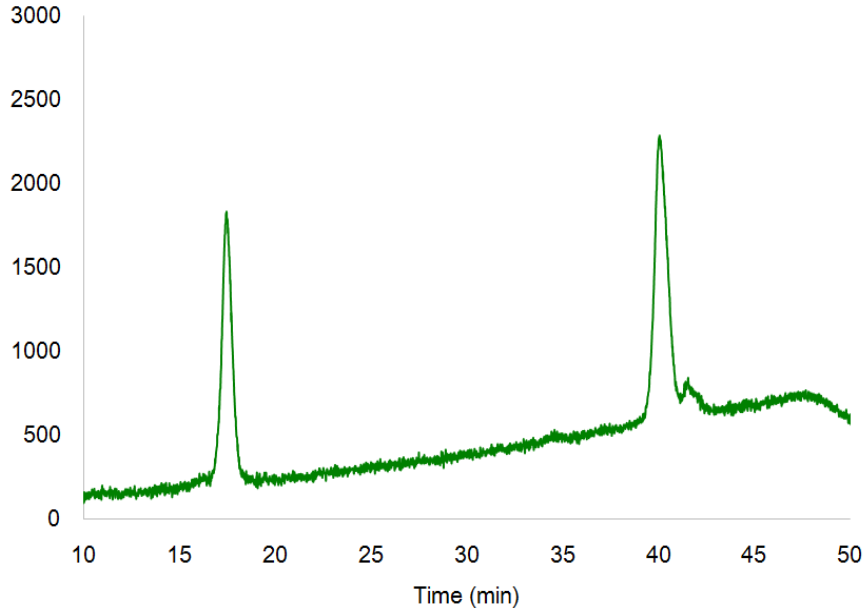


Figure S2-5. Peptides not bound by 30-mer PL (T20%A10%)
(concentration of peptides: 0.1 mM each, concentration of 30-mer PL: 1.9 mg/mL)

S3. ITC titration of magainin 1 into synthetic PL

[General ITC titration procedure]

All peptides and polymers were dissolved in 35 mM PBS (0.15 M NaCl, pH 7.3) and stored at 4°C. After degassing the polymer ligands solution (0.38 mg/mL), it was loaded into a ITC reaction cell and the reference cell was loaded with degassed MilliQ water. The instrument was equilibrated at 37°C until the baseline was flat and stable. Peptide solution (0.5 mM) was degassed and loaded in syringe. Each titration was programmed for 25 injections with 10 μL. The spacing between injections was 150 s. The reference power was 10 μcal/s. Titrations were conducted at 37°C with a stirring speed of 310 rpm. The raw data was analyzed by ORIGIN software.

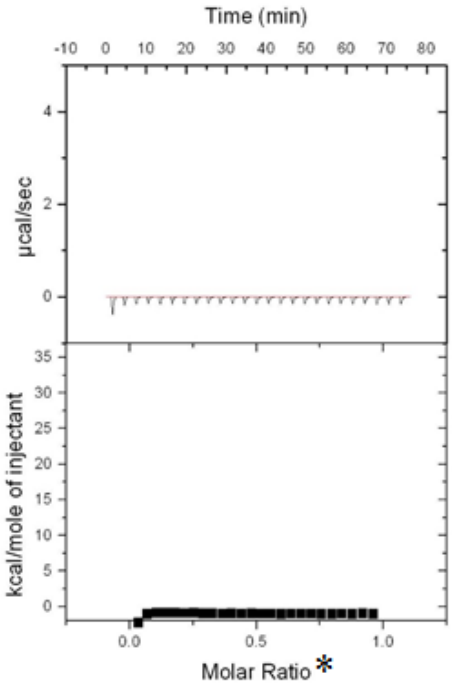


Figure S3-1. ITC titration of magainin 1 into NP (T20%A10%)

Cell	0.38 mg/mL NP with 20 %TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM magainin 1 in 35 mM PBS (pH 7.3, 0.15M NaCl)

* Apparent molar ratio

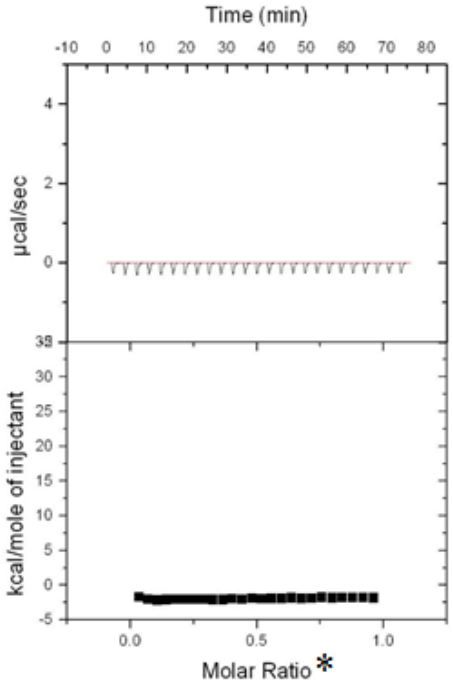


Figure S3-2. ITC titration of magainin 1 into 300-mer PL (T20%A10%)

Cell	0.38 mg/mL 300-mer PL with 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM magainin 1 in 35 mM PBS (pH 7.3, 0.15M NaCl)

* Apparent molar ratio

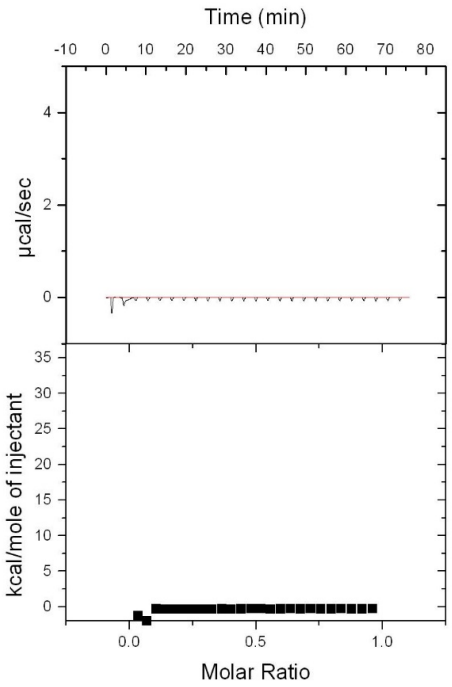


Figure S3-3. ITC titration of magainin 1 into 30-mer PL (T20%A10%)

Cell	0.38 mg/mL 30-mer PL with 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM magainin 1 in 35 mM PBS (pH 7.3, 0.15M NaCl)

S4. ITC titration of melittin or ponericin into synthetic PL

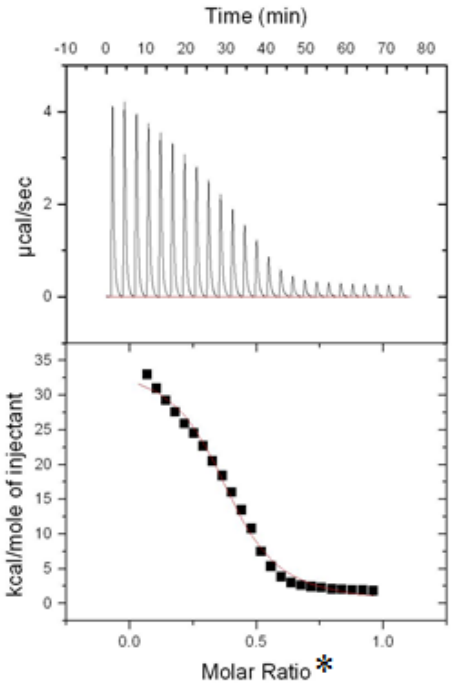


Figure S4-1. ITC titration of melittin into NP (T20%A10%)

Cell	0.38 mg/mL NP that contains 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM melittin in 35 mM PBS (pH 7.3, 0.15M NaCl)

* Apparent molar ratio

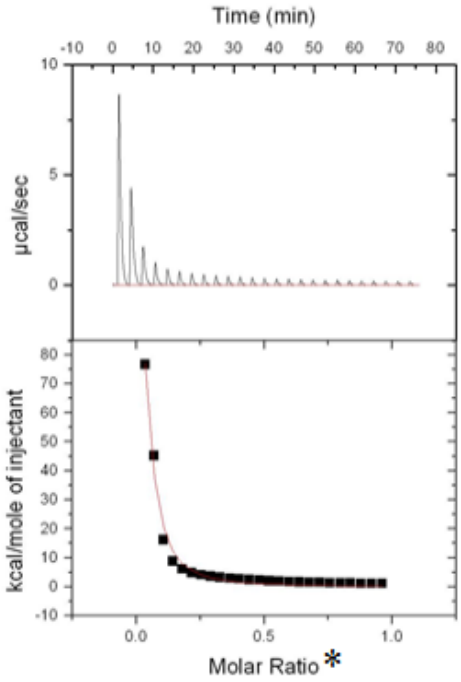


Figure S4-2. ITC titration of melittin into 300-mer PL (T20%A10%)

Cell	0.38 mg/mL 300-mer with 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM melittin in 35 mM PBS (pH 7.3, 0.15M NaCl)

* Apparent molar ratio

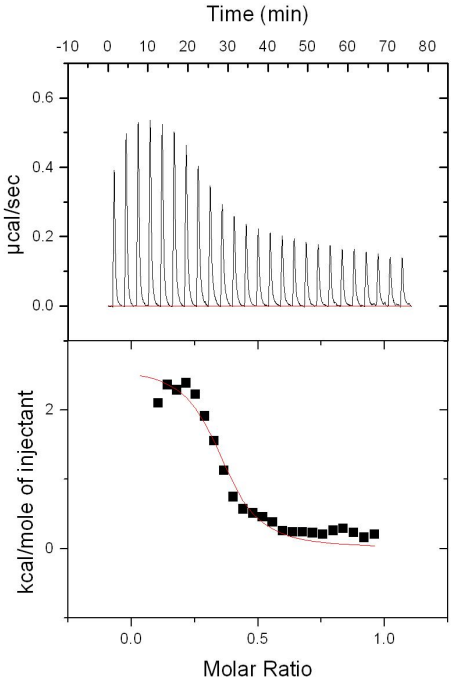


Figure S4-3. ITC titration of melittin into 30-mer PL (T20%A10%)

Cell	0.38 mg/mL 30-mer PL with 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM melittin in 35 mM PBS (pH 7.3, 0.15M NaCl)

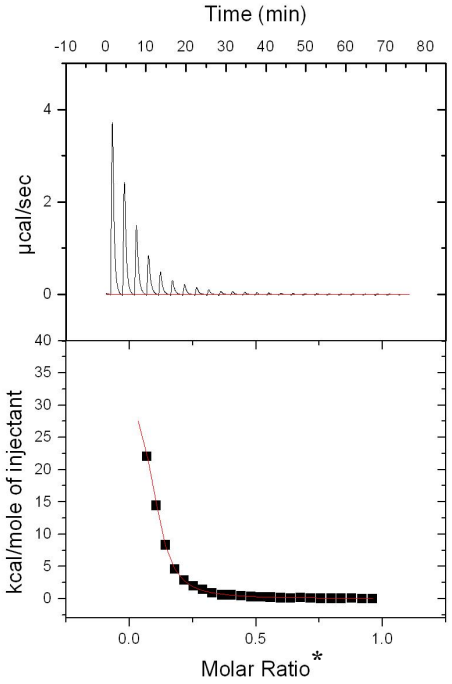


Figure S4-4. ITC titration of ponericin into NP (T20%A10%)

Cell	0.38 mg/mL NP functionalized with 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM ponericin in 35 mM PBS (pH 7.3, 0.15M NaCl)

* Apparent molar ratio

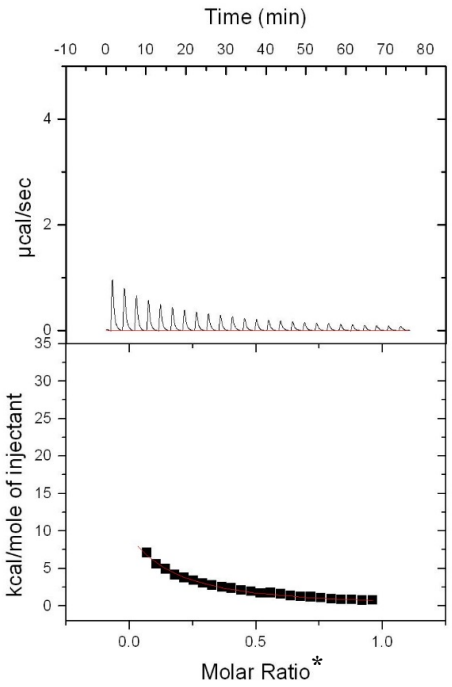


Figure S4-5. ITC titration of ponericin into 300-mer PL (T20%A10%)

Cell	0.38 mg/mL 300-mer PL with 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM ponericin in 35 mM PBS (pH 7.3, 0.15M NaCl)

* Apparent molar ratio

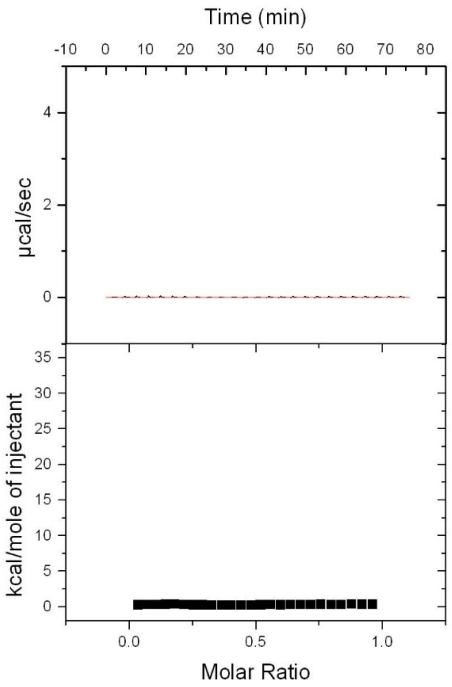


Figure S4-6. ITC titration of ponericin into 30-mer PL (T20%A10%)

Cell	0.38 mg/mL 30-mer PL with 20 % TBAm and 10 % AAc in 35 mM PBS (pH 7.3, 0.15M NaCl)
syringe	0.5 mM ponericin in 35 mM PBS (pH 7.3, 0.15M NaCl)

S5. Hemolysis neutralization assay

Red blood cells (RBC) were washed with PBS, collected by centrifugation for 10 min at 800× *g*, and then resuspended in PBS. The RBC was then incubated with PLs for 30 min at 37 °C in PBS. A mixture of melittin and PL was then added to 100 mL cells to a final volume of 200 mL. The resulting suspension was incubated at 37 °C for 30 min. Samples were then centrifuged at 800× *g* for 10 min. Released hemoglobin was measured by measuring the absorbance of the supernatant at 415 nm ($Abs_{polymer\ ligand}$). Control values for 0% neutralization and 100% neutralization were obtained, respectively, from cells incubated with 1.8 mM melittin only ($Abs_{0\%}$) and RBC only ($Abs_{100\%}$). Percent neutralization was calculated according to S6.

S6. Formula for % neutralization (E1)

$$\text{Neutralization } \% = \frac{Abs_{\ 100\%} - Abs_{\ \text{Polymer ligand}}}{Abs_{\ 100\%} - Abs_{\ 0\%}} \times 100$$

S7. HPLC analysis of lytic peptides unbound by low-MW PL

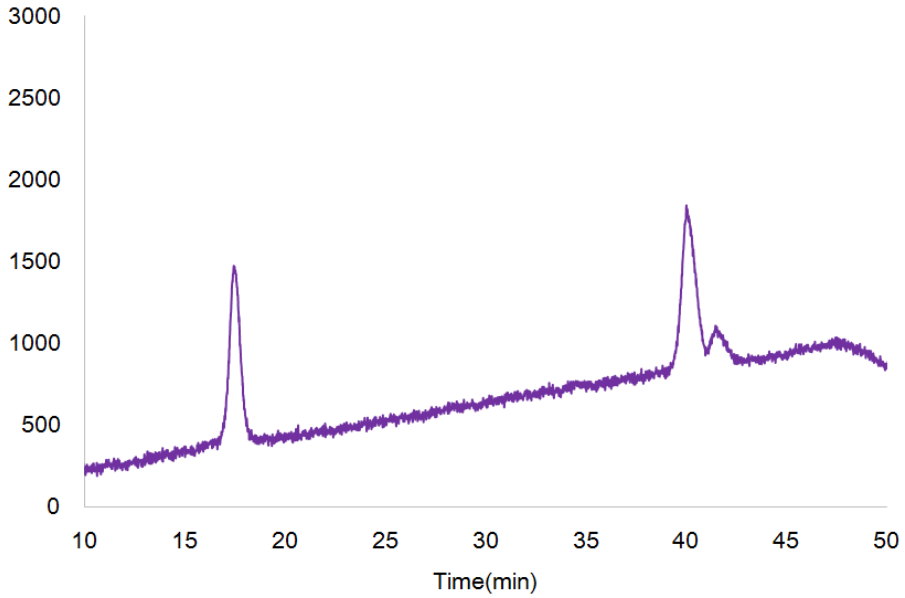


Figure S7-1. Peptides unbound by 30-mer PL (T20%A20%)
(concentration of peptides: 0.1 mM each, concentration of 30-mer PL: 1.9 mg/mL)

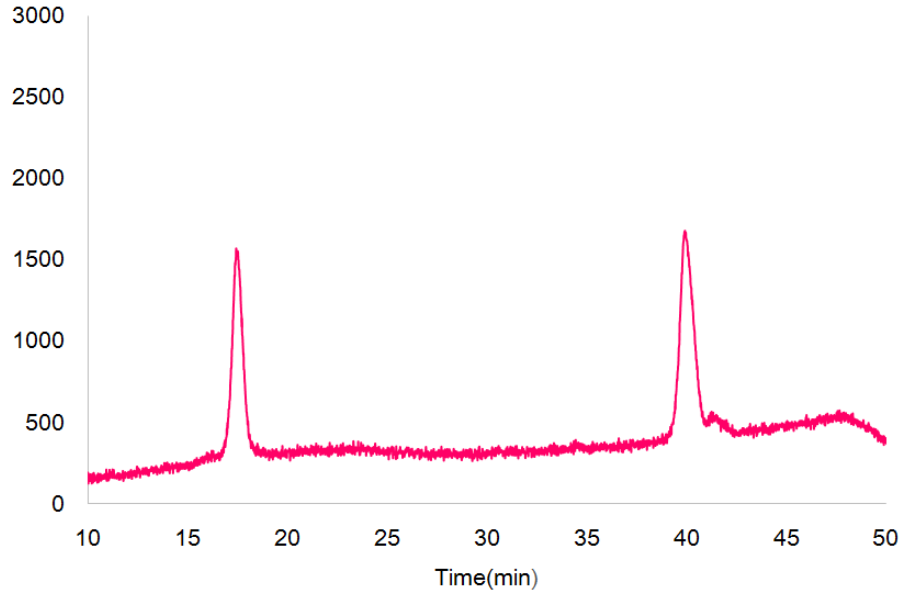


Figure S7-2. Peptides unbound by 30-mer PL (T40%A10%)
(concentration of peptides: 0.1 mM each, concentration of 30-mer PL: 1.9 mg/mL)

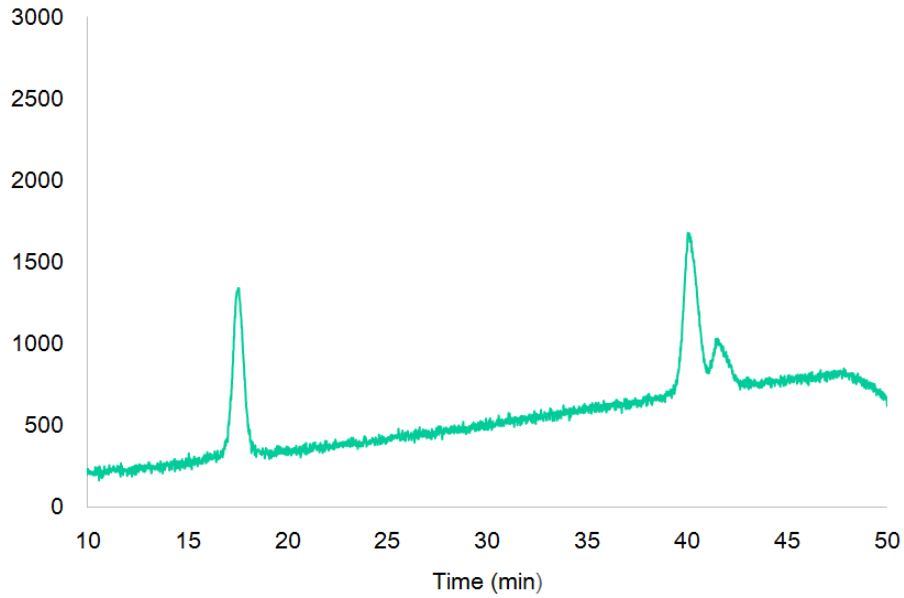


Figure S7-3. Peptides unbound by 15-mer PL (T40%A20%) (concentration of peptides: 0.1 mM each, concentration of 15-mer PL: 1.9 mg/mL)

Reference

1. Stenzel, M.H.; Davis, T. P. *J. Polym. Sci. A.*, **2002**, *40*, 4498-4512