Electronic Supplementary Material (ESI) for Polymer Chemistry.


# SUPPORTING INFORMATION

## Sequence-defined nucleobase containing oligomers via reversible addition-fragmentation chain transfer single monomer addition

Lowie Maes,[a] Daniel Massana Roqeuro,[a] Louis M. Pitet,[c] Peter Adriaensens[d,e] and Tanja Junkers [a,b]

[a] Polymer Reaction Design group, Hasselt University – Institute for Materials Research, Wetenschapspark 1, B-3590 Diepenbeek, Belgium.

[b] School of Chemistry, Monash University, 19 Rainforest Walk, Clayton VIC 3800, Australia.

[c] Advanced Polymer Functionalization group, Hasselt University – Institute for Materials Research, Wetenschapspark 1, B-3590 Diepenbeek, Belgium

[d] Nuclear Magnetic Resonance Spectroscopy Group, Institute for Materials Research (IMO-IMOMEC), Universiteit Hasselt, Agoralaan Building D, B-3590 Diepenbeek, Belgium

[e] IMEC vzw–Division IMOMEC, Wetenschapspark 1, B-3590 Diepenbeek, Belgium

# Table of contents:

# S1. Materials

Adenine (A, TCI, >99%), Thymine (T, TCI, >98%), Cytosine (C, TCI, >98%), Uracil (U, abcr, 99%), 2-amino-6-chloropurine (abcr, 98%), 2,6-Di-tert-butyl-4-methylphenol (BHT, Alfa Aesar, 99%), Potassium carbonate ($K_2CO_3$, Acros Organics, 99+%), Triethylamine (TEA, Acros Organics, 99%), Potassium tert-butoxide (KtBuO, Acros Organics, 98+ %), 1,4-butanediol diacrylate (BDDA, Sigma Aldrich, 90%), Formic acid (HCOOH, abcr, 97%), 1-dodecanethiol (Acros Organics, 98%), Carbon disulphide ($CS_2$ , Acros organics, 99.9%), Potassium phosphate tribasic ($K_3PO_4$, Sigma Aldrich, > 98%) and (1-bromoethyl) benzene (Sigma Aldrich, 97%) were used as received. 2,2'-azobis(2-methylpropionitrile) (AIBN, Sigma-Aldrich, 98%) was recrystallized twice from methanol prior to use. All solvents used are obtained from either Sigma Aldrich or Fisher Scientific and used as received.

# S2. Characterization

Proton Nuclear Magnetic Resonance ($^1$H NMR) spectra of solutions in DMSO-$d_6$ were recorded at room temperature on a Agilent/Varian 400 MHz Inova spectrometer using a 5 mm four-nucleus PFG probe. The chemical shift scale (δ) in ppm was calibrated relative to TMS (0 ppm). Free induction decays were collected with a 90° pulse of 5.0 µs, a spectral width of 6 kHz, an acquisition time of 4 s, a preparation delay of 12 s and 64 accumulations. A line-broadening factor of 0.2 Hz was applied before Fourier transformation to the frequency domain. Spectra were analyzed in Mestrenova software.

Electron spray ionisation mass spectrometry (ESI-MS) was performed using an LTQ orbitrap velos pro mass spectrometer (ThermoFisher Scientific) equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electro spray mode. The instrument was calibrated in the m/z range 220-2000 using a standard solution containing caffeine, MRFA and Ultramark 1621. A constant spray voltage of 5 kV was used and nitrogen at a dimensionless sheath gas flow-rate of 7 was applied. Capillary temperature was set to 275°C. A mixture of THF and methanol (THF:MeOH = 3:2), all HPLC grade, were used as solvent. Spectra were analyzed in Thermo Xcalibur Qual Browser software.

MALDI-TOF spectra were recorded on a Bruker Daltonics Ultraflex II Tof/Tof. 1 µL of the matrix solution (4 mg mL-1 DTCB (trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile) in $CHCl_3$) was spotted onto an MTP Anchorchip 600/384 MALDI plate. The spot was allowed to dry and 1 µL of the analyte solution (0.5 mg mL-1 in CHCl3) was spotted on top of the matrix. Spectra were analyzed in MestReNova software.

# S3. Synthetic procedures

## *Adenine acrylate monomer (AAM)*

Adenine acrylate monomer was synthesized according to literature.[1] Adenine (10.00 g, 1 eq.), $K_2CO_3$ (0.46 g, 0.04 eq.) and BHT (0.69 g, 0.04 eq.) were suspended in 200 mL DMSO. The mixture was heated to 50°C and stirred for 1 h. BDDA (28.00 mL, 2 eq.) was added to the reaction mixture. After 5 h, the mixture was diluted with water (1500 mL) and washed with hexane (350 mL) to remove excess of BDDA followed by extraction with DCM (3 x 200 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (90/10 vol%). After removing of the solvent and drying under vacuum 14.80 g of the pure product was obtained (60% yield).

## *Thymine acrylate monomer (TAM)*

Thymine acrylate monomer was synthesized according to literature.[1] Thymine (1.00 g, 1 eq.), TEA (0.22 mL, 0.20 eq.) and BHT (0.06 g, 0.04 eq.) were suspended in 20 mL DMSO. The mixture was stirred for 1 h at room temperature. BDDA (3.00 mL, 2 eq.) was added and the reaction mixture was stirred for 24 h. Then, the mixture was diluted with water (150 mL) and washed with hexane (35 mL) to remove excess of BDDA followed by extraction with DCM (3 x 20 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (95/5 vol%). After removing of the solvent and drying under vacuum 1.75 g of the pure product was obtained (68% yield).

## *Uracil acrylate monomer (UAM)*

Uracil acrylate monomer was synthesized according to an adapted literature procedure.[1] Uracil (6.00 g, 1 eq.), TEA (1.50 mL, 0.20 eq.) and BHT (0.40 g, 0.03 eq.) were suspended in 120 mL DMSO. The mixture was stirred for 1 h at room temperature. BDDA (20.00 mL, 2 eq.) was added and the reaction mixture was stirred for 24 h. Then, the mixture was diluted with water (900 mL) and washed with hexane (150 mL) to remove excess of BDDA followed by extraction with DCM (4 x 100 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (97/3 vol%). After removing of the solvent and drying under vacuum 9.63 g of the pure product was obtained (58% yield).

## *Cytosine acrylate monomer (CAM)*

Cytosine acrylate monomer was synthesized according to an adapted literature procedure.[1] Cytosine (4.00 g, 1 eq.), KtBuO (0.16 g, 0.04 eq.) and BHT (0.32 g, 0.04 eq.) were suspended in 80 mL DMSO. The mixture was stirred for 1h at room temperature. BDDA (14.00 mL, 2 eq.) was added and the reaction mixture was stirred for 24 h. Then, the mixture was diluted with water (600 mL) and washed with hexane (100 mL) to remove excess of BDDA followed by extraction with DCM (3 x 80 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The residual mixture was purified by column chromatography using a $CHCl_3$/MeOH mixture (93/7 vol%). After removing of the solvent and drying under vacuum 5.09 g of the pure product was obtained (45% yield).

## *Guanine acrylate monomer (GAM)*

Guanine acrylate monomer was synthesized according to an adapted combination of literature procedures.[1-3] 2-amino-6-chloropurine (1.00 g, 1 eq.), KtBuO (0.03 g, 0.04 eq.), BHT (0.06 g, 0.04 eq.) were suspended in 20 mL DMSO. The mixture was stirred for 1 h at 50 °C. BDDA (2.39 mL, 2 eq.) was added and the reaction mixture was stirred for 5 h at 50 °C. Then, the mixture was diluted with water (600ml) and washed with hexane (75 mL) to remove excess of BDDA followed by extraction with DCM (4 x 75 mL). The organic phase was dried with $MgSO_4$, filtered and concentrated under reduced pressure resulting in 1.29 g of the crude intermediate mixture.

In a second step 1.29 g of the crude intermediate mixture was dissolved in 15 mL HCOOH/$H_2O$ mixture (80/20 vol%) and stirred for 2 h at 75 °C. The mixture was concentrated under reduced pressure and purified by column chromatography using a $CHCl_3$/MeOH mixture (93/7 vol%). After removing of the solvent and drying under vacuum 0.68 g of the pure product was obtained (33% yield).

## *2-dodecyl-1-phenylethyl trithiocarbonate (DPE-TTC)*

DPE-TTC was synthesized according to literature.[4] $K_3PO_4$ (10.5 g, 1 eq.) and 1-dodecanethiol (10.0 g, 1 eq.) were suspended in 280 mL of acetone and stirred for 10 minutes at room temperature. $CS_2$ (11.3 g, 3 eq.) was added to the suspension and stirred continued for 1 hour. Subsequently, 1-bromoethyl benzene (9.1 g, 1 eq.) was added. After 5 hours of stirring the solvent was evaporated under reduced pressure and the crude mixture was purified using column chromatography (100% petroleum ether). After evaporation of the solvent and drying under vacuum 16.8 g of the pure product was obtained (89% yield). ESI-MS: 405.1746 [$M+Na^+$]

## *MUMI U*

UAM (4.05 g, 5 eq.), DPE-TTC (1.00 g, 1 eq.) and AIBN (0.02 g, 0.05 eq.) were suspended in 15 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 2% MeOH and ending at 10%.

## *SUMI A*

AAM (12.63 g, 3 eq.), DPE-TTC (4.80 g, 1 eq.) and AIBN (0.10 g, 0.05 eq.) were suspended in 60 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated *via* gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 2% MeOH. After eluding unreacted RAFT agent and SUMI A the percentage MeOH was increased to 5% to elude the unreacted monomer faster. After evaporation of the solvent and drying under vacuum 5.45 g of pure SUMI A was obtained (60% yield).

## *SUMI AA*

AAM (0.910 g, 1 eq.), SUMI A (1.950 g, 1 eq.) and AIBN (0.02 g, 0.05 eq.) were suspended in 6 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 2% MeOH and ending at 5%. After evaporation of the solvent and drying under vacuum 0.44 g of pure SUMI AA was obtained (15% yield).

## *SUMI AAA*

AAM (0.27 g, 1 eq.), SUMI AA (0.44 g, 1 eq.) and AIBN (3.40 mg, 0.05 eq.) were suspended in 2 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 2% MeOH and ending at 10%. After evaporation of the solvent and drying under vacuum 45.20 mg of pure SUMI AAA was obtained (8% yield).

## *SUMI AAAA*

AAM (10.7 mg, 1 eq.), SUMI AAA (45.2 mg, 1 eq.) and AIBN (0.3 mg, 0.05 eq.) were suspended in 0.3 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 7.5% MeOH and ending at 15%. After evaporation of the solvent and drying under vacuum 12.7 mg of pure SUMI AAA was obtained (23% yield).

## *SUMI AC*

CAM (1.55 g, 1 eq.), SUMI A (3.60 g, 1 eq.) and AIBN (0.04 g, 0.05 eq.) were suspended in 15 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 2% MeOH and ending at 7.5%. After evaporation of the solvent and drying under vacuum 0.61 g of pure SUMI AA was obtained (12% yield).

## *SUMI ACA*

AAM (0.19 g, 1 eq.), SUMI AC (0.60 g, 1 eq.) and AIBN (4.80 mg, 0.05 eq.) were suspended in 1 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 5% MeOH and ending at 10%. After evaporation of the solvent and drying under vacuum 138.90 mg of pure SUMI ACA was obtained (17% yield).

## *SUMI ACAC*

CAM (31.6 mg, 1 eq.), SUMI ACA (138.9 mg, 1 eq.) and AIBN (0.8 mg, 0.05 eq.) were suspended in 0.25 ml dioxane and purged for 10 minutes with N2. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 5% MeOH and ending at 15%. After evaporation of the solvent and drying under vacuum 44.3 mg of pure SUMI ACAC was obtained (26% yield).

## *SUMI ACT*

TAM (0.16 g, 1 eq.), SUMI AC (0.50 g, 1 eq.) and AIBN (4.00 mg, 0.05 eq.) were suspended in 1 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 5% MeOH and ending at 8%. Fractions were collected and individually analysed *via* MALDI-TOF. Fractions containing pure ACT were combined, solvent was evaporated and 31.80 mg of the trimer was obtained (5% yield). Impure fractions containing ACT were combined and a second flash column with adjusted conditions (liquid loading, slower flowrate and gradient) was performed. Pure fractions were combined after MALDI-TOF analysis, solvent was evaporated and 50.90 mg of pure product was obtained (7% yield) resulting and an overall amount of 82.70 mg of ACT (12% overall yield).

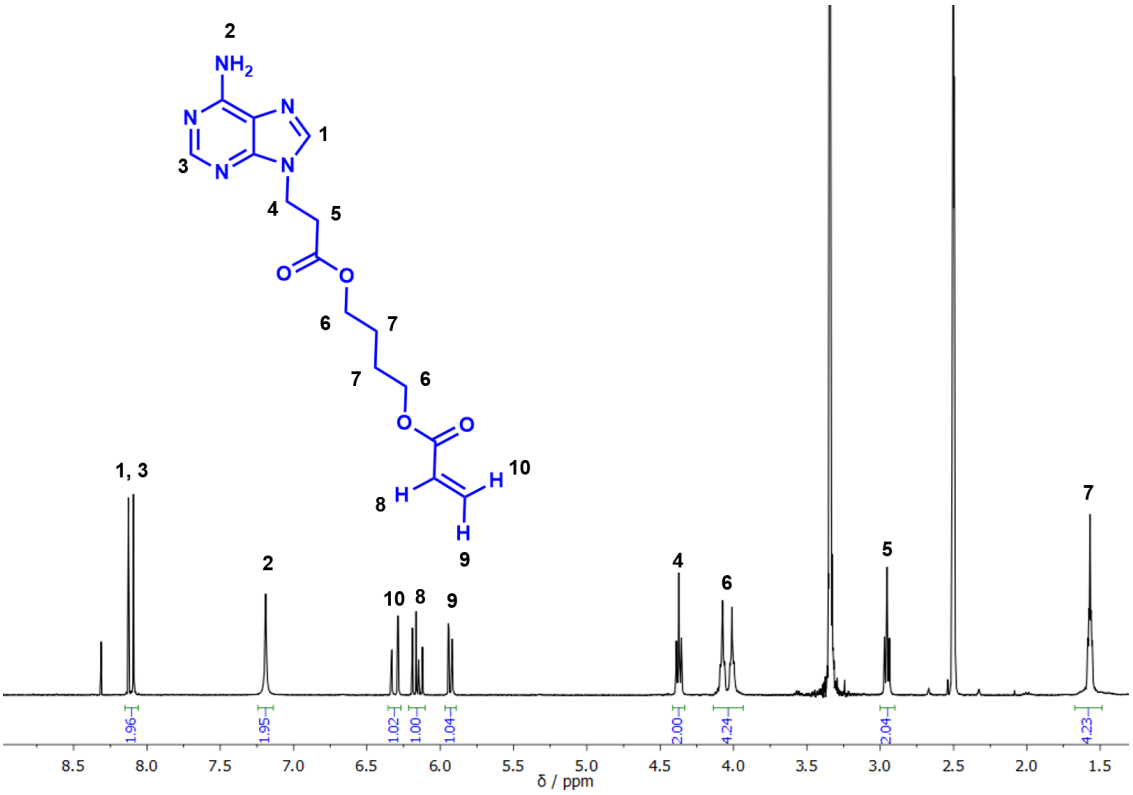
## *SUMI ACTG*

GAM (21.4 mg, 1 eq.), SUMI ACT (82.7 mg, 1 eq.) and AIBN (0.5 mg, 0.05 eq.) were suspended in 0.15 mL dioxane and purged for 10 minutes with $N_2$. The mixture was heated at 100 °C for 45 minutes. After evaporation of the solvent the crude mixture was separated via gradient flash column chromatography using a $CHCl_3$/MeOH mixture starting from 8% MeOH and ending at 15%. After evaporation of the solvent and drying under vacuum 18.4 mg of pure SUMI ACTG was obtained (18% yield).

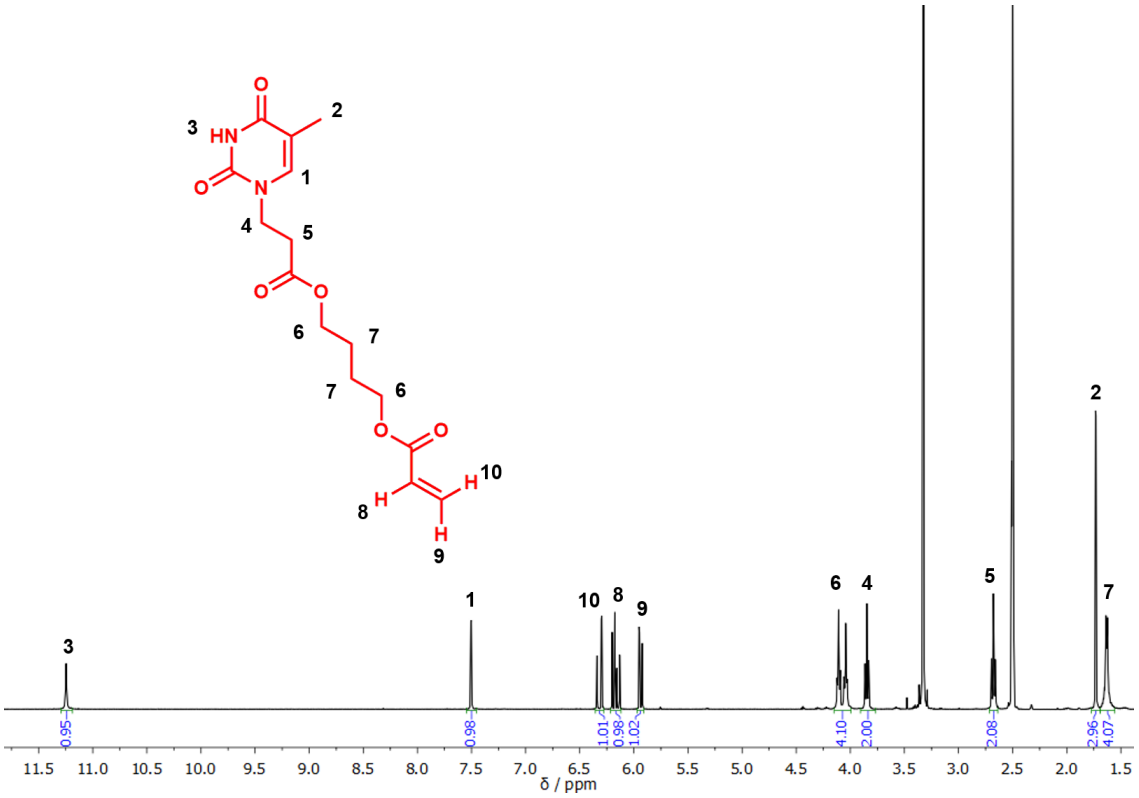
# S4. $^1$H NMR

Chemical shifts of solvents visible in $(CD_3)_2SO$: $CHCl_3$ (8.32 ppm), DCM (5.76 ppm), 1,4-dioxane (3.57 ppm), $H_2O$ (3.33 ppm), MeOH (4.01 ppm, 3.16 ppm) and residual signal of $(CD_3)_2SO$ (2.50 ppm).

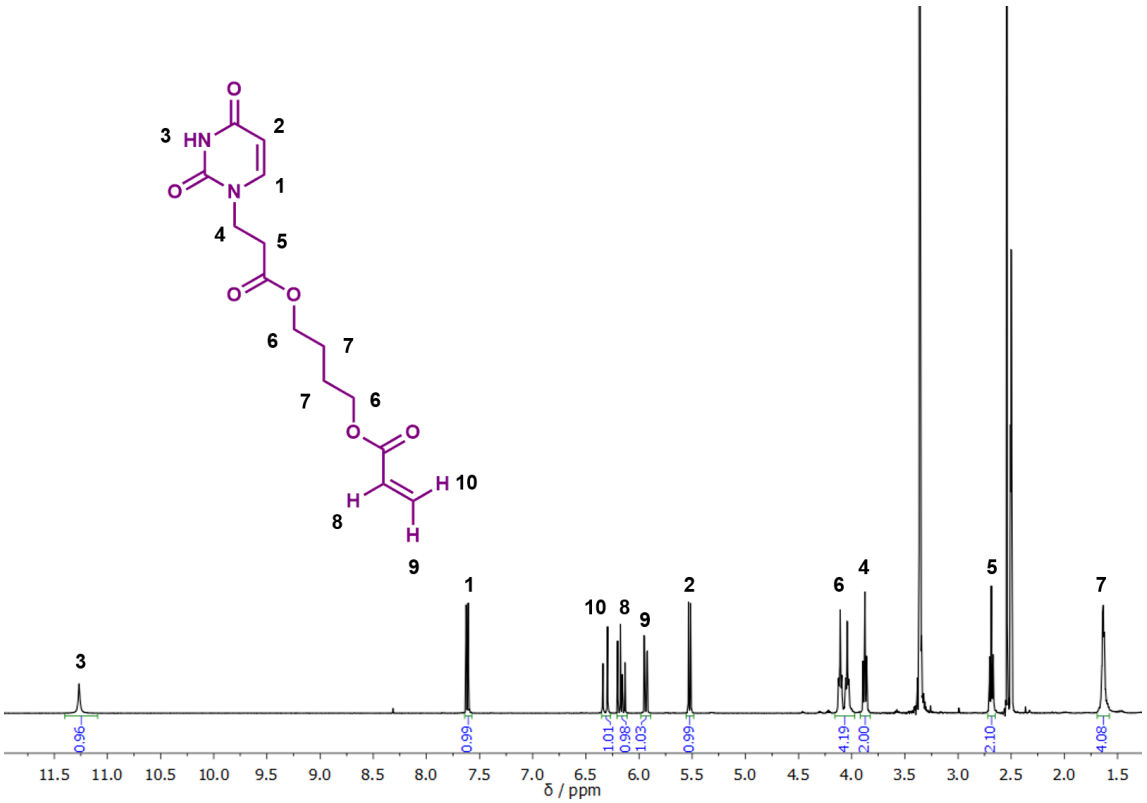
*AAM*



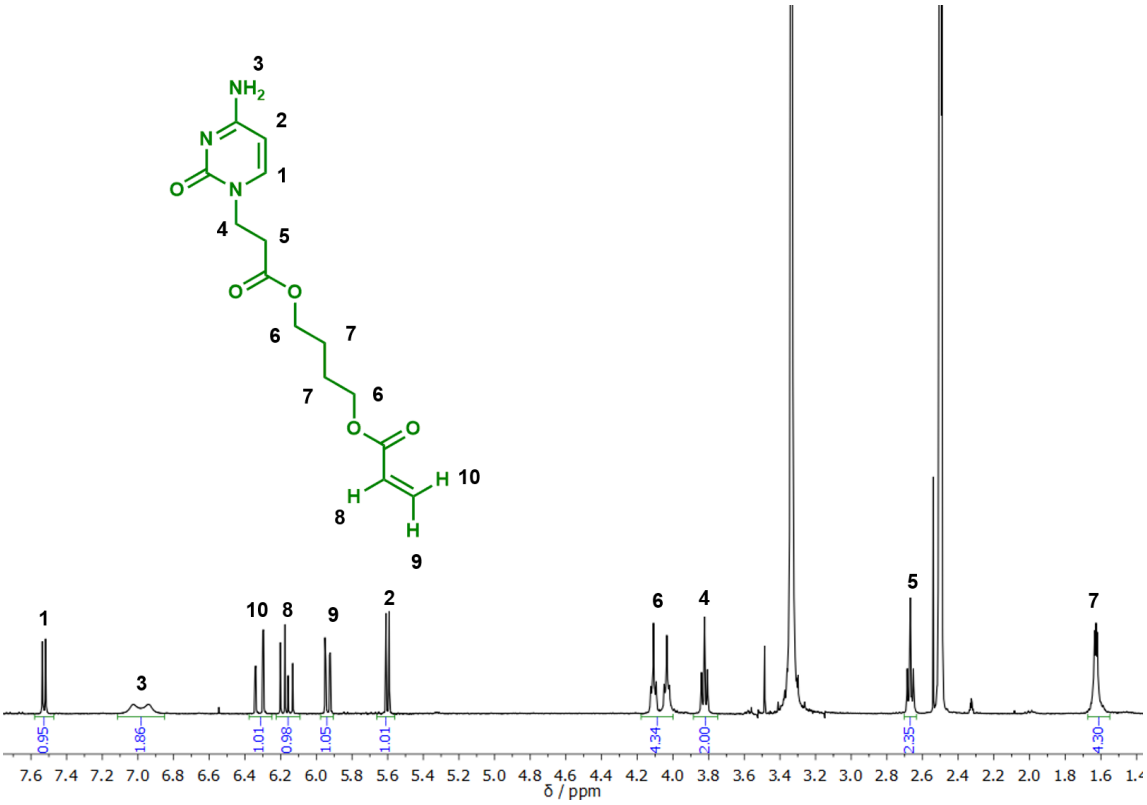
## *TAM*


3
HN
O
2
1
N
4
5
O
O
6
7
7
6
O
O
H
10
8
H
H
9
1
10
8
9
6
4
5
2
7
3
0.95
0.98
1.01
0.98
1.02
4.10
2.00
2.08
2.96
4.07
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
δ / ppm

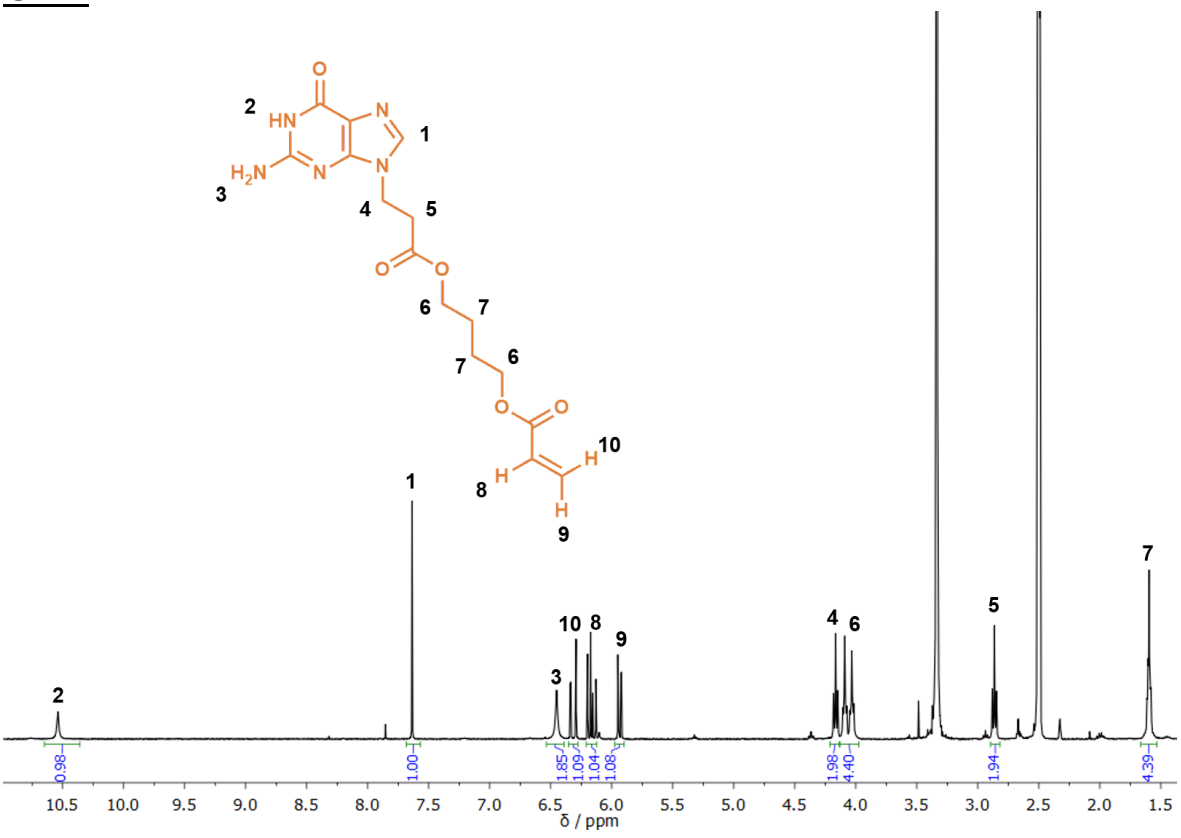
## *UAM*


3
HN
O
2
1
N
4
5
O
O
6
7
7
6
O
O
H
10
8
H
H
9
1
10
8
9
2
6
4
5
7
3
0.96
0.99
1.01
0.98
1.03
0.99
4.19
2.00
2.10
4.08
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5
δ / ppm

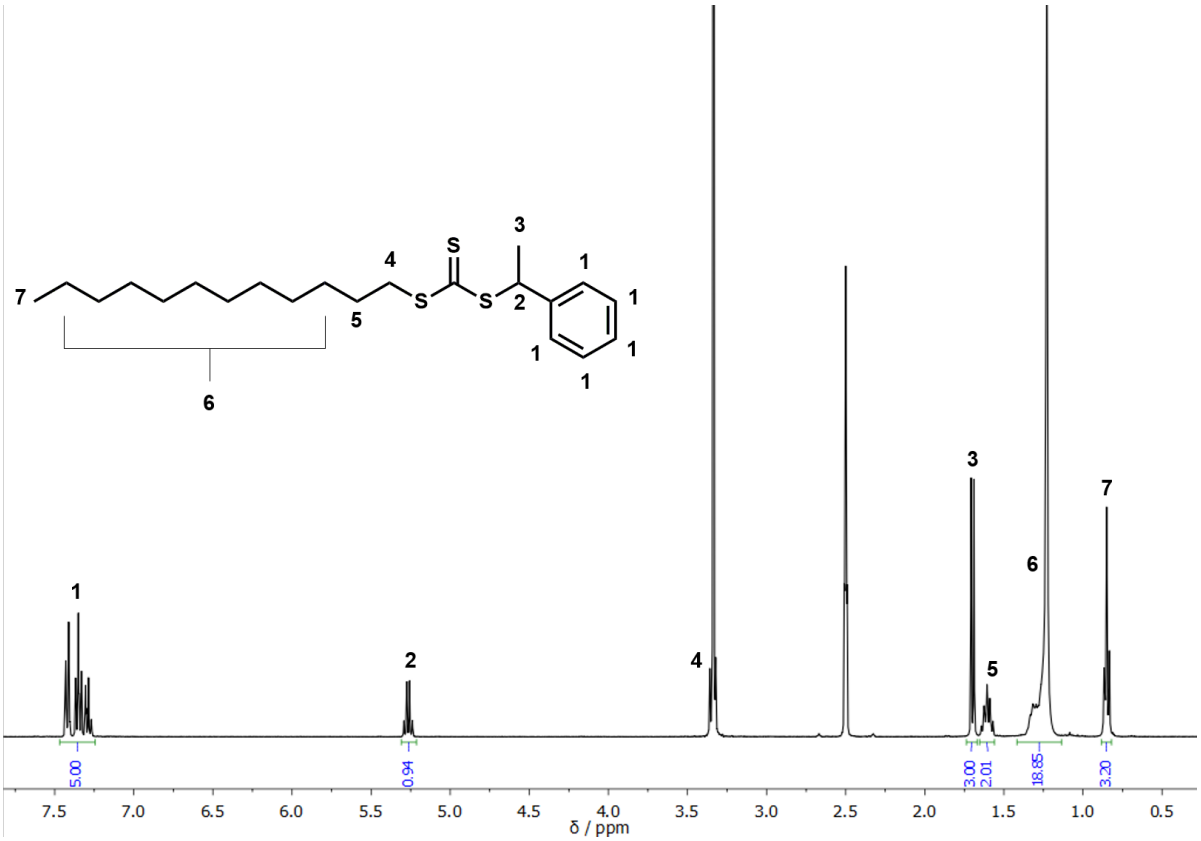
## *CAM*



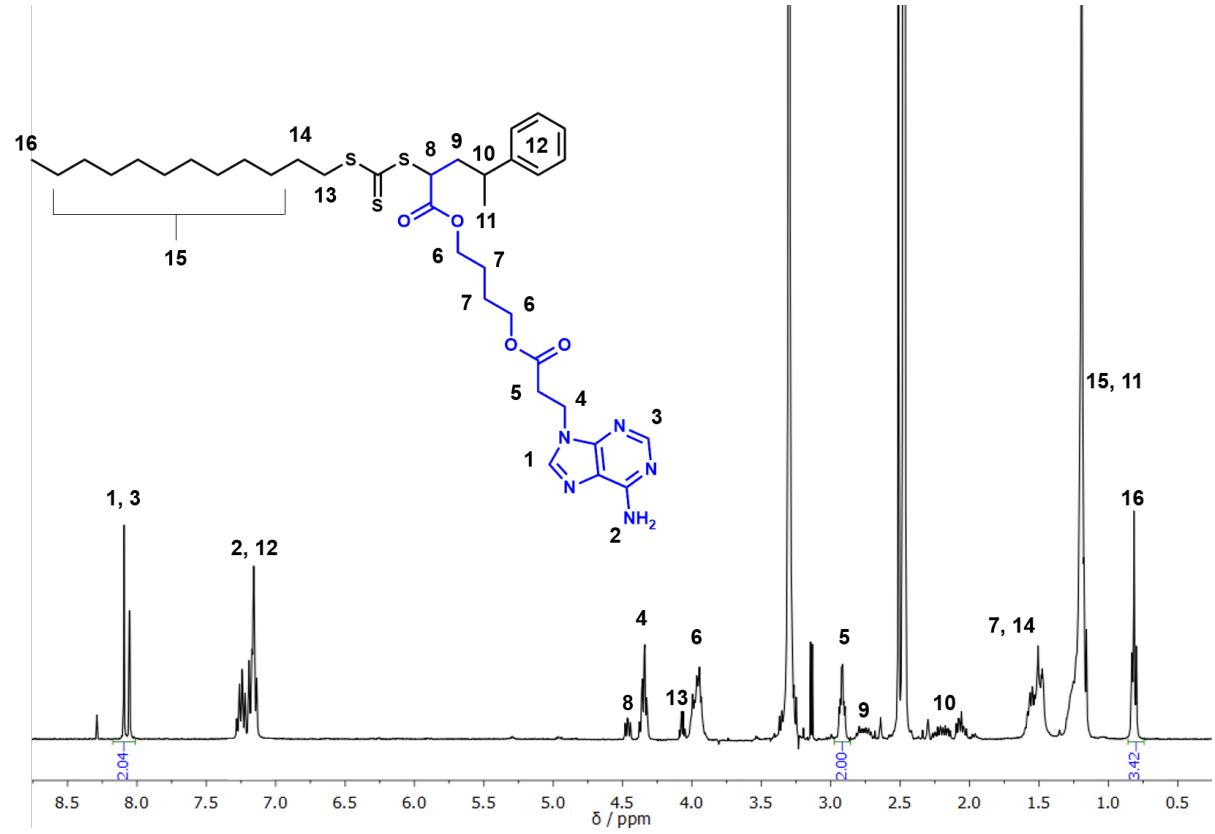

## *GAM*



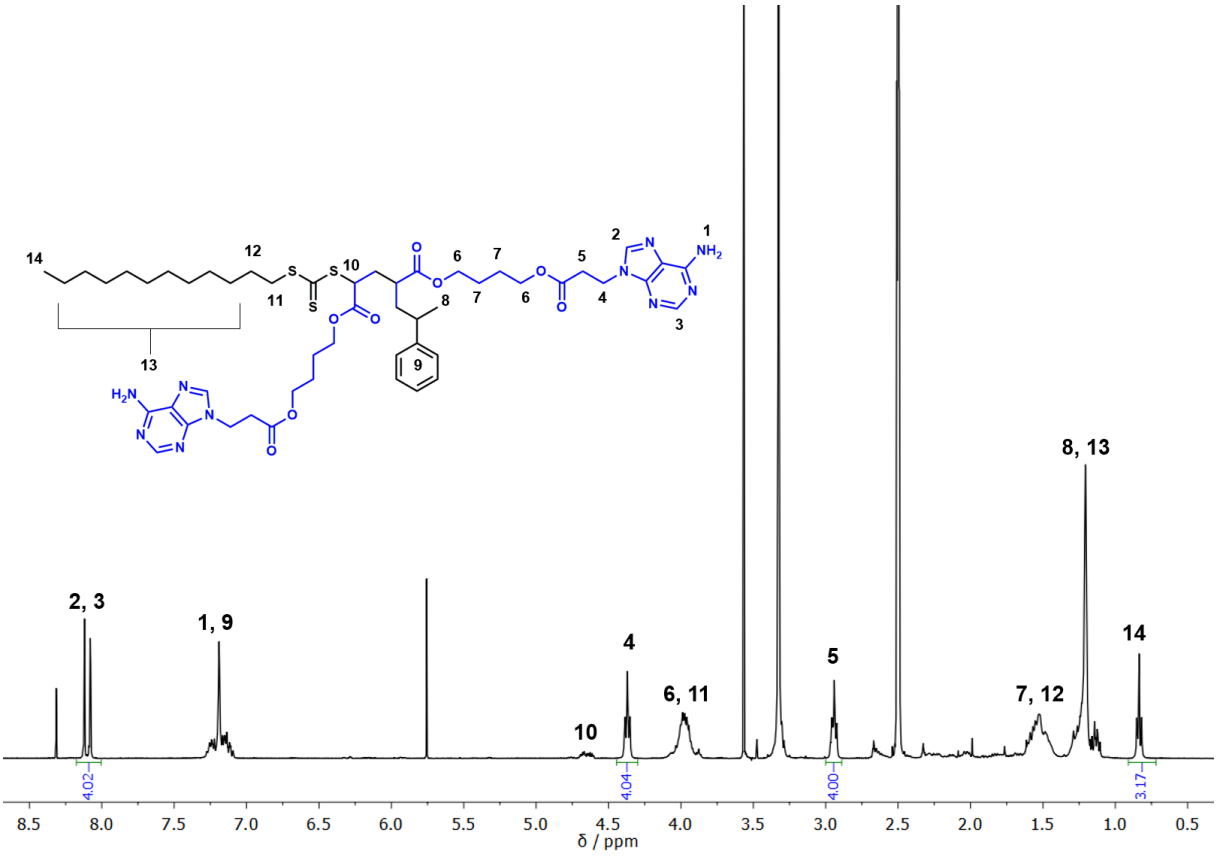
## *DPE-TTC*



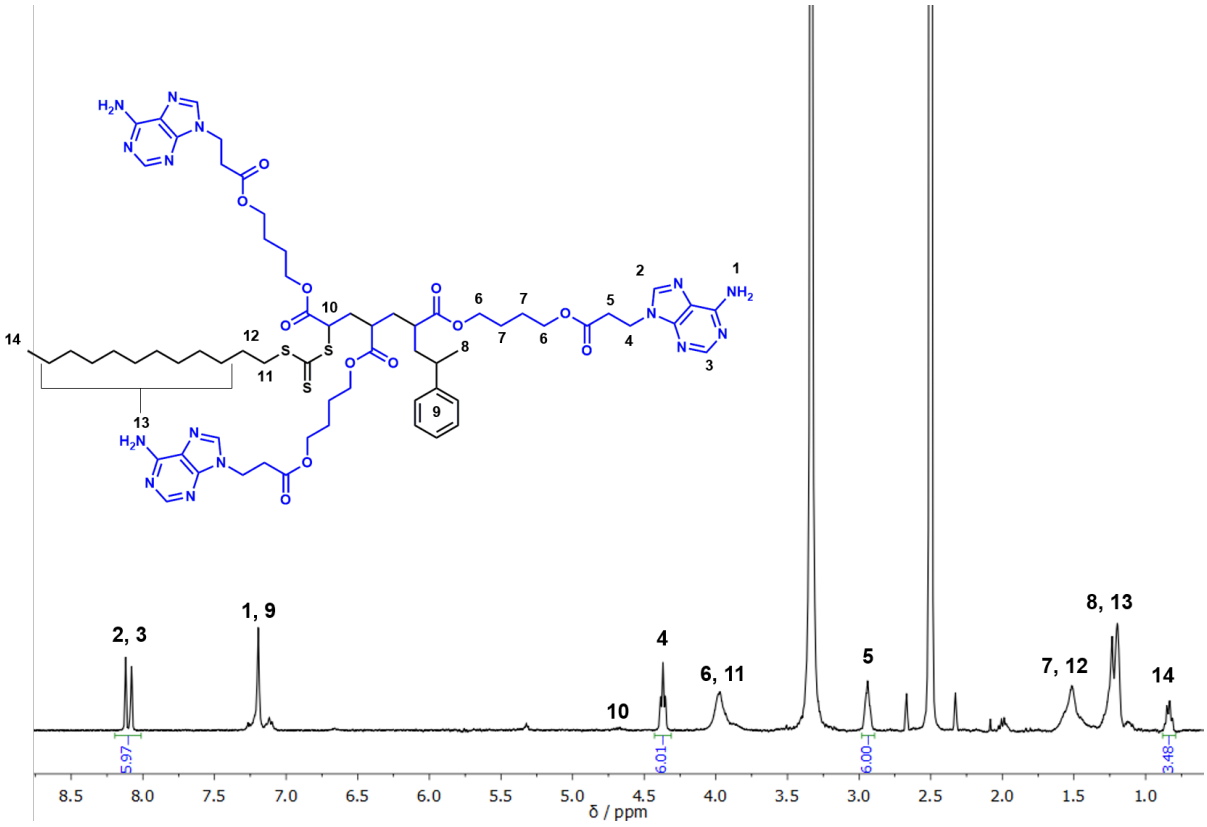

## *SUMI A*



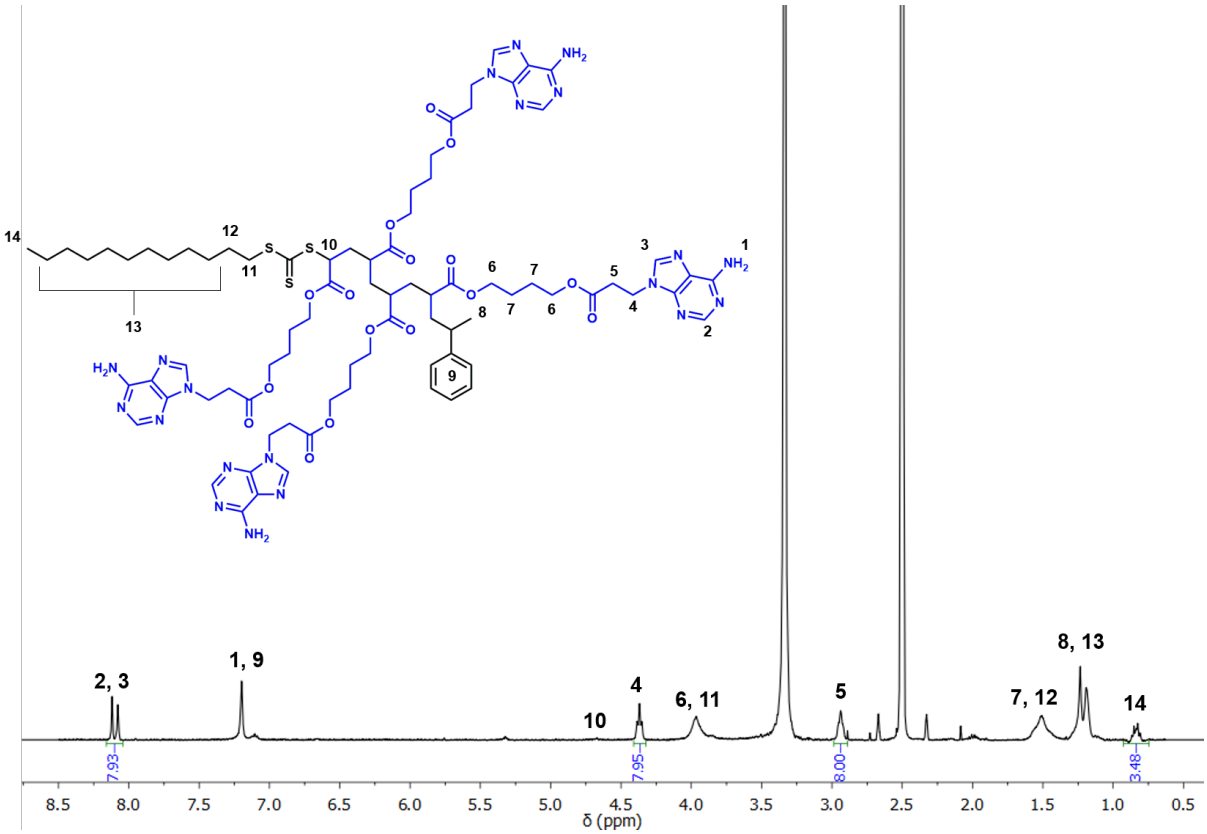
## *SUMI AA*



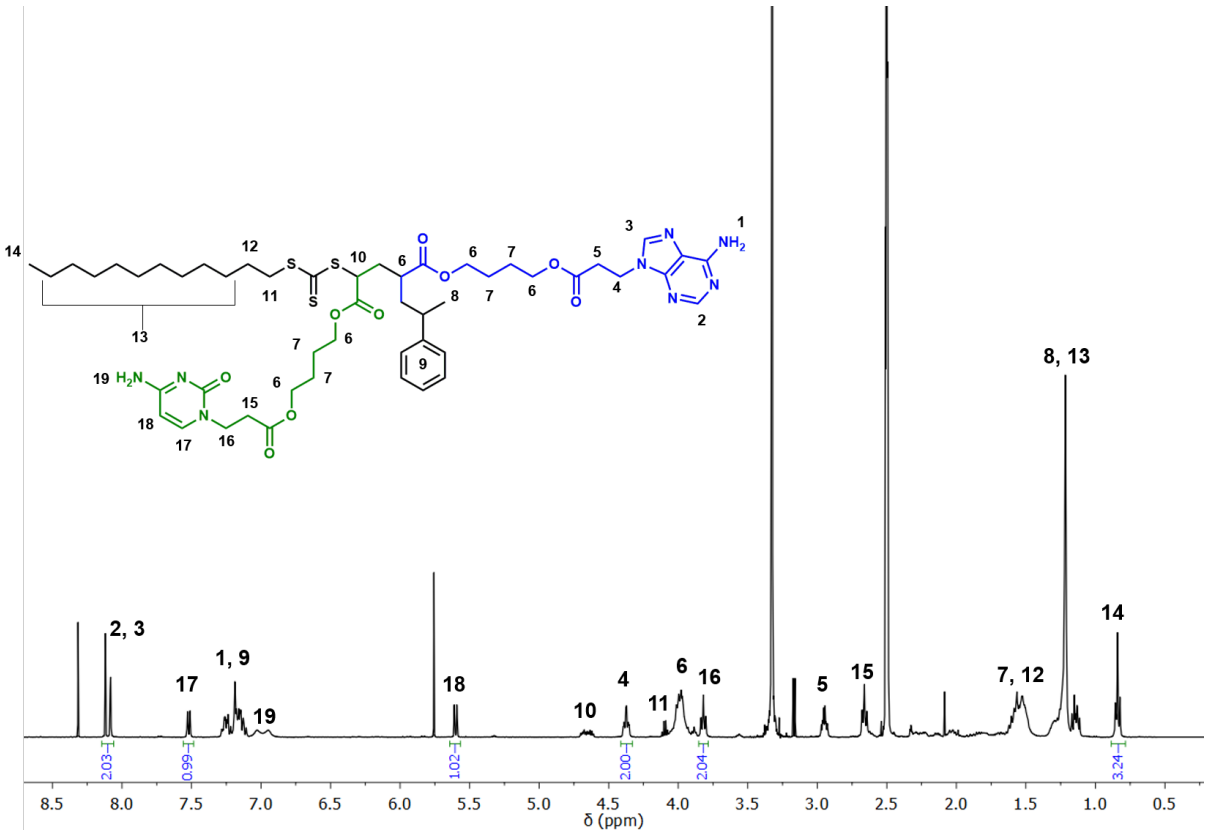

## *SUMI AAA*



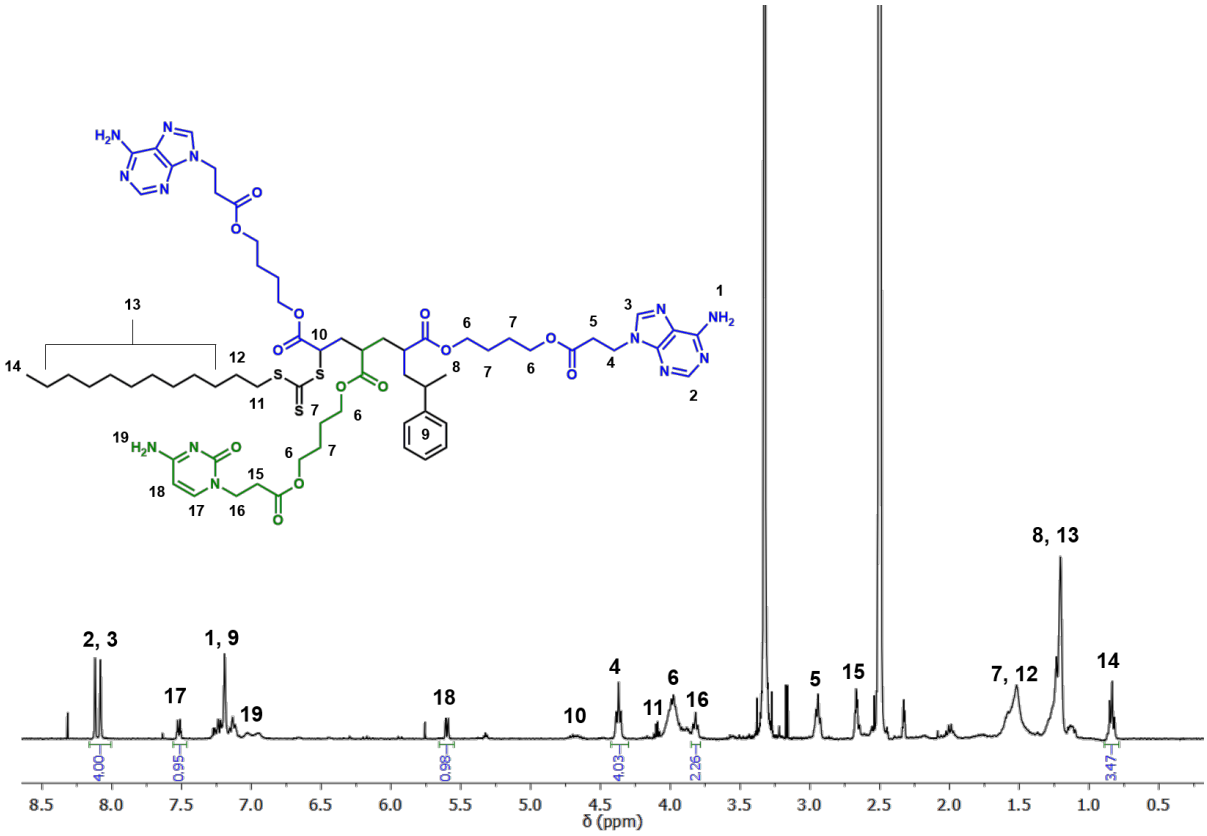
## *SUMI AAAA*



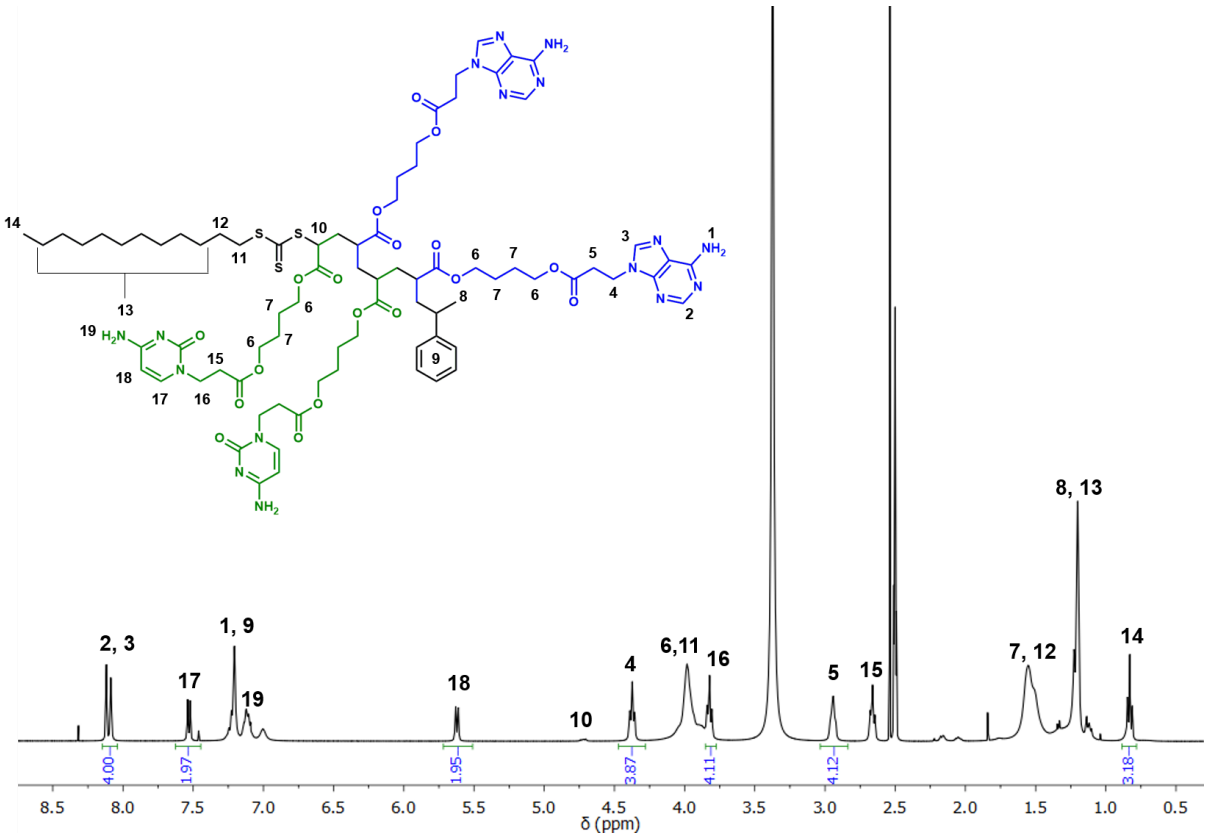

## *SUMI AC*



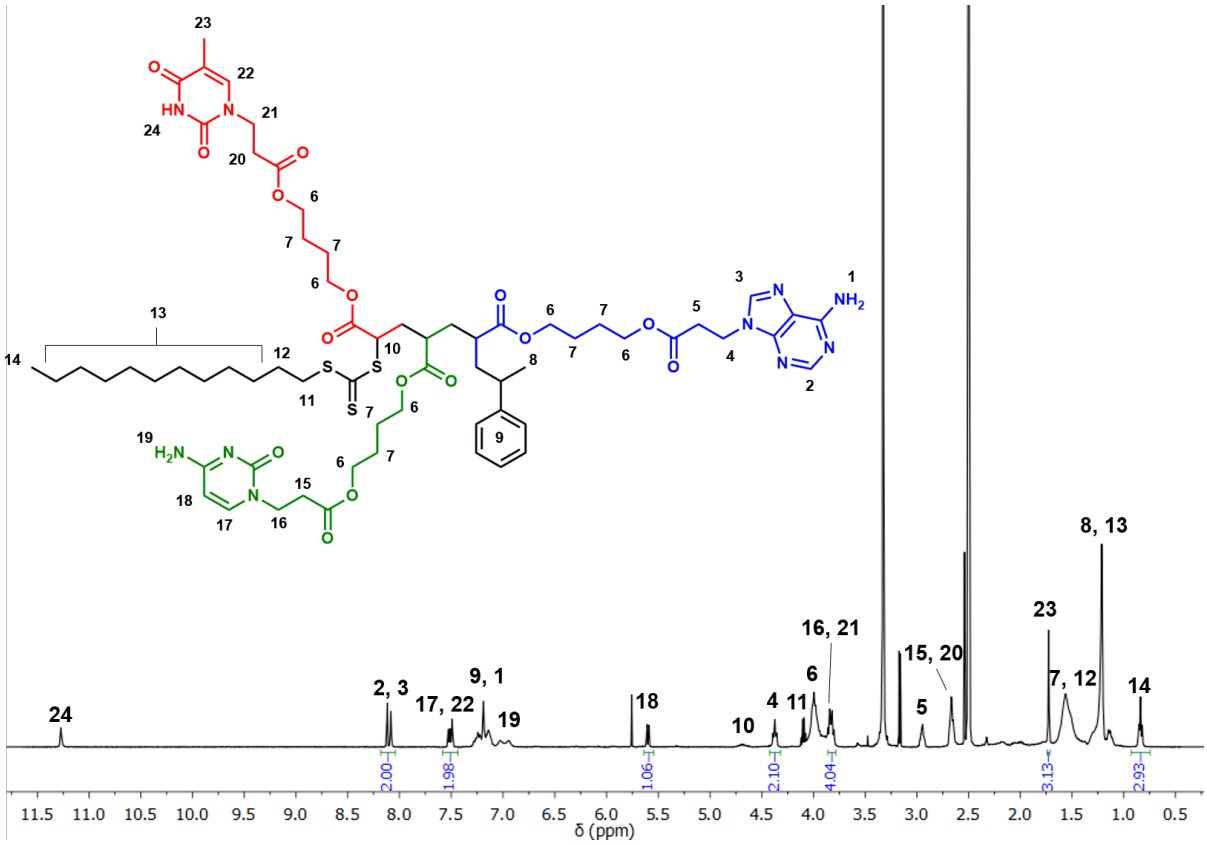
## *SUMI ACA*



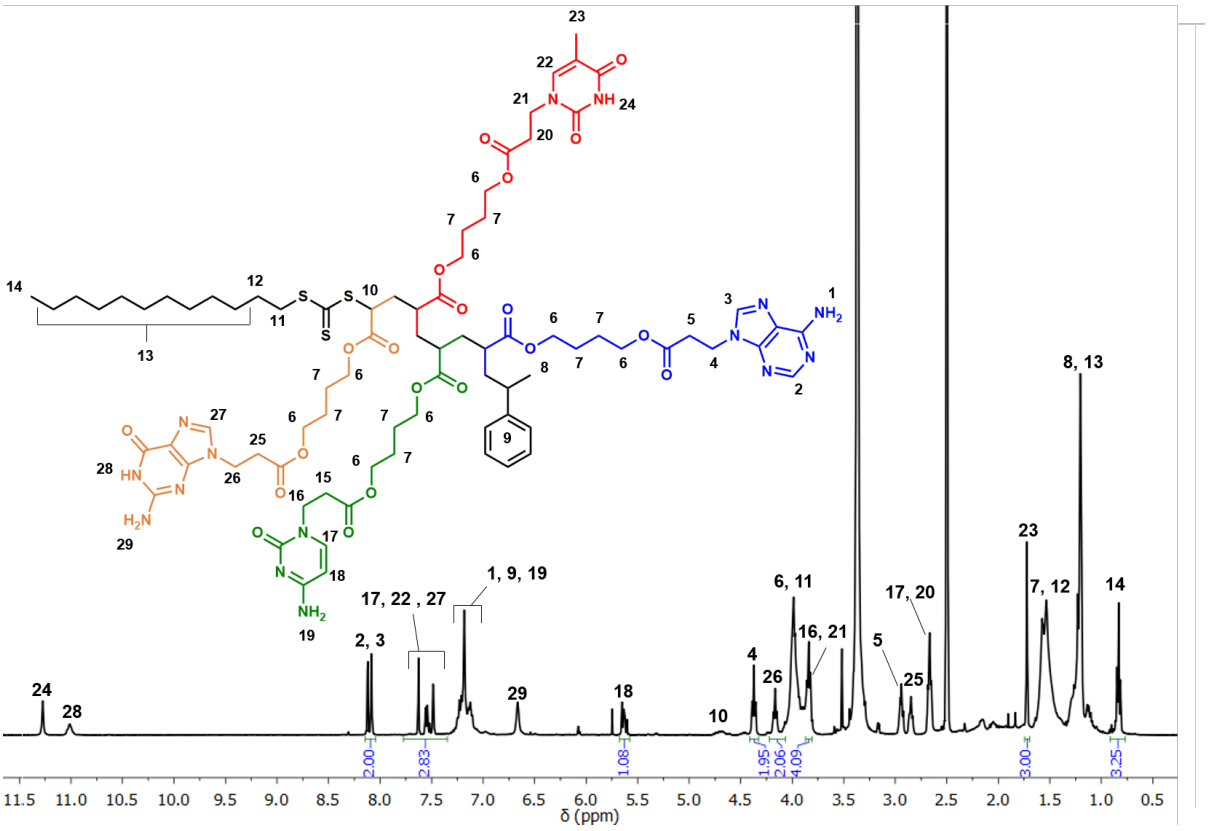

## *SUMI ACAC*



## *SUMI ACT*

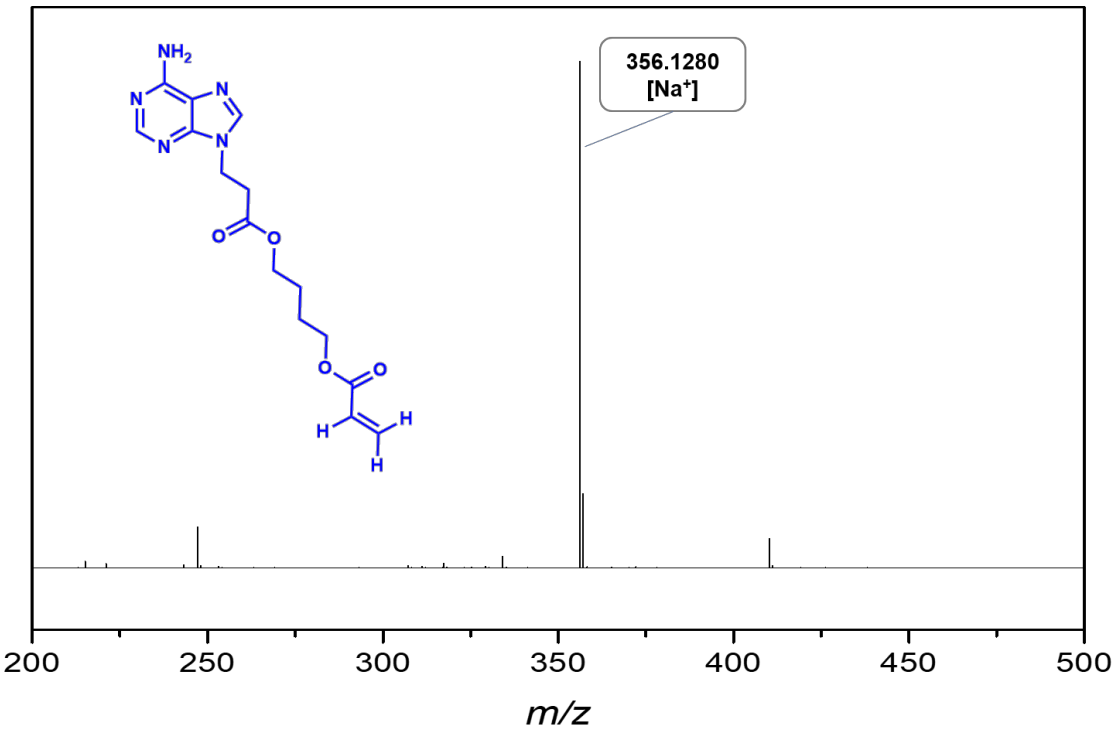



## *SUMI ACTG*

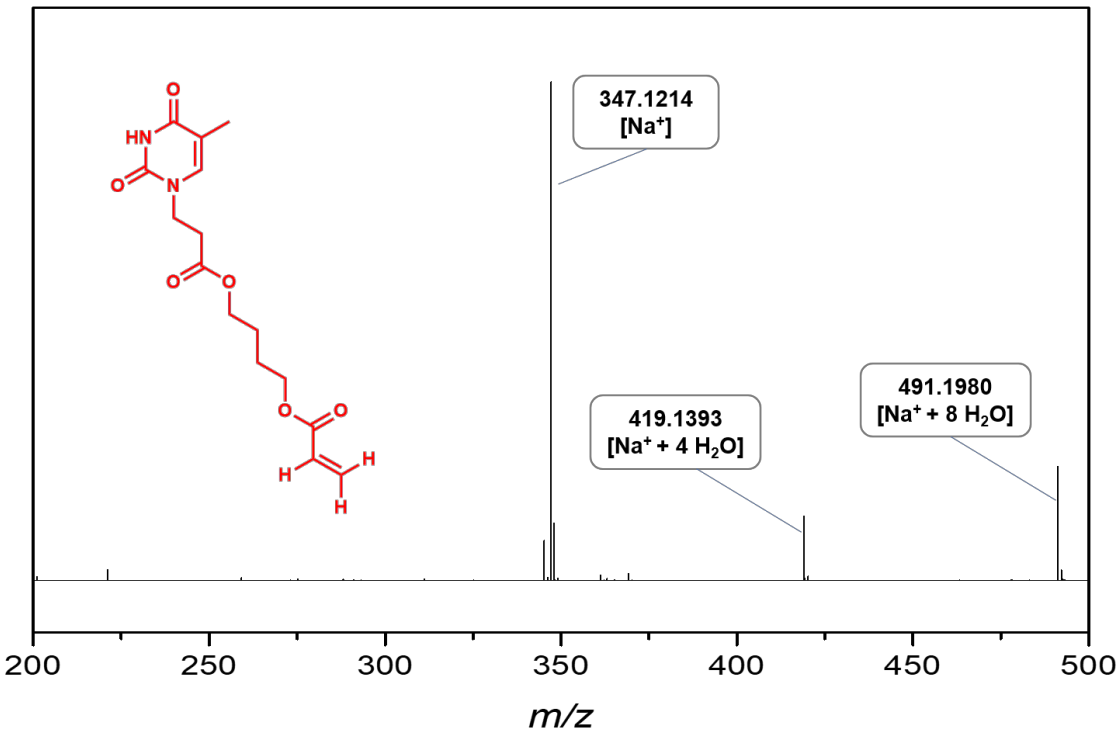


## S5. ESI-MS

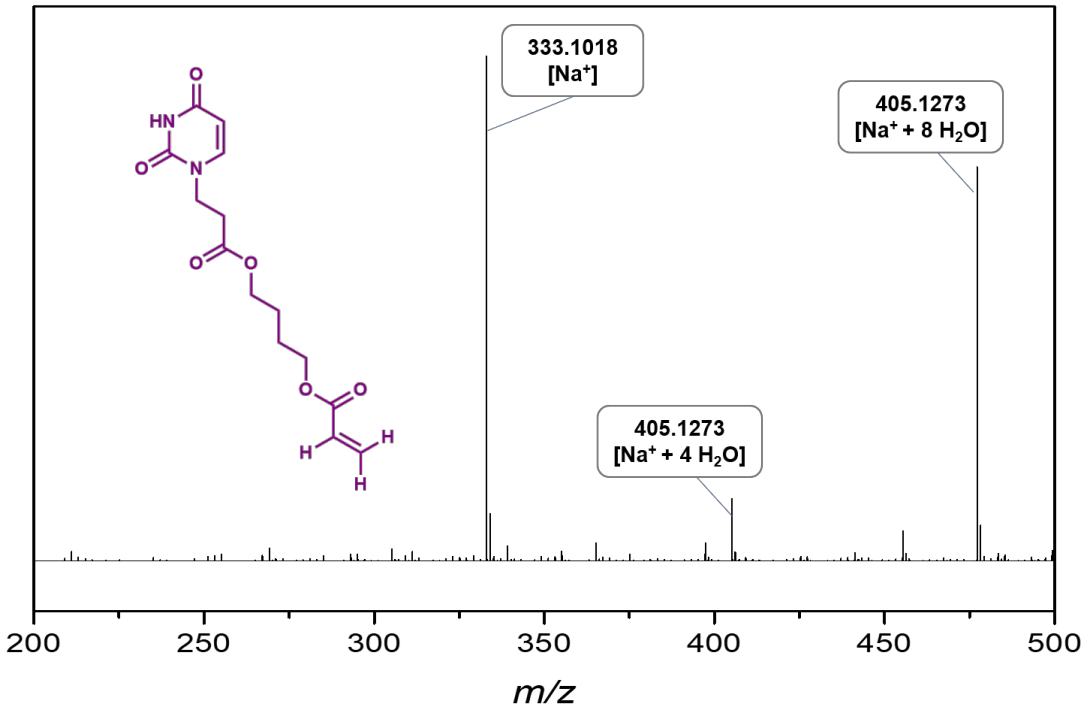
*AAM*



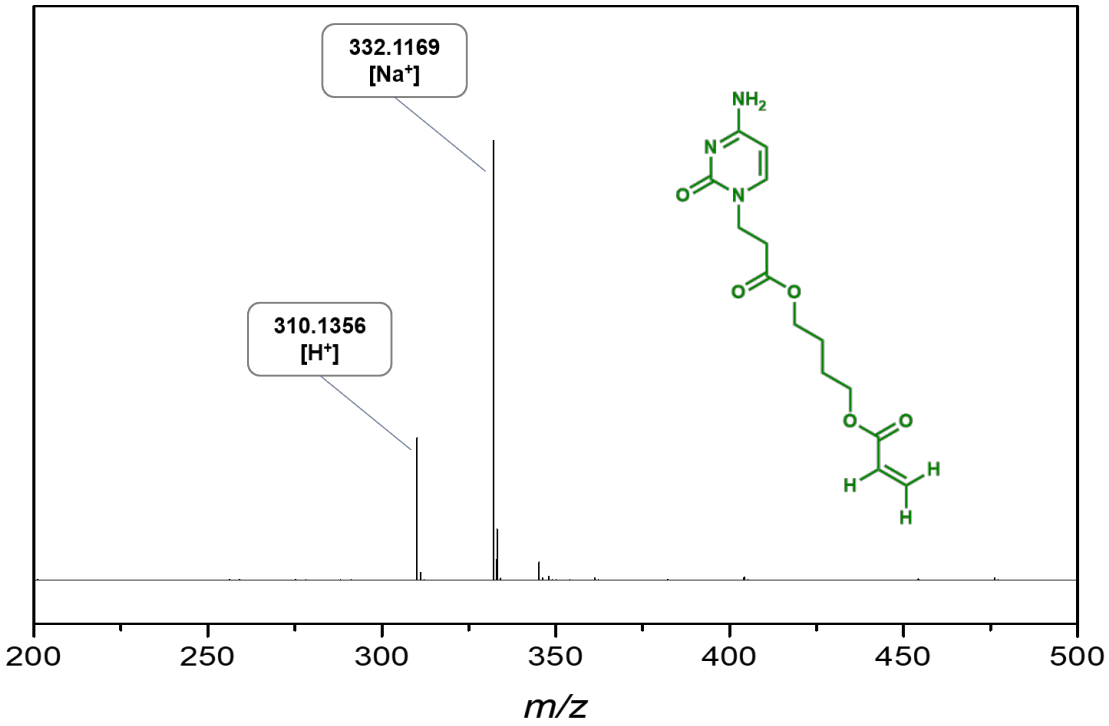

*TAM*



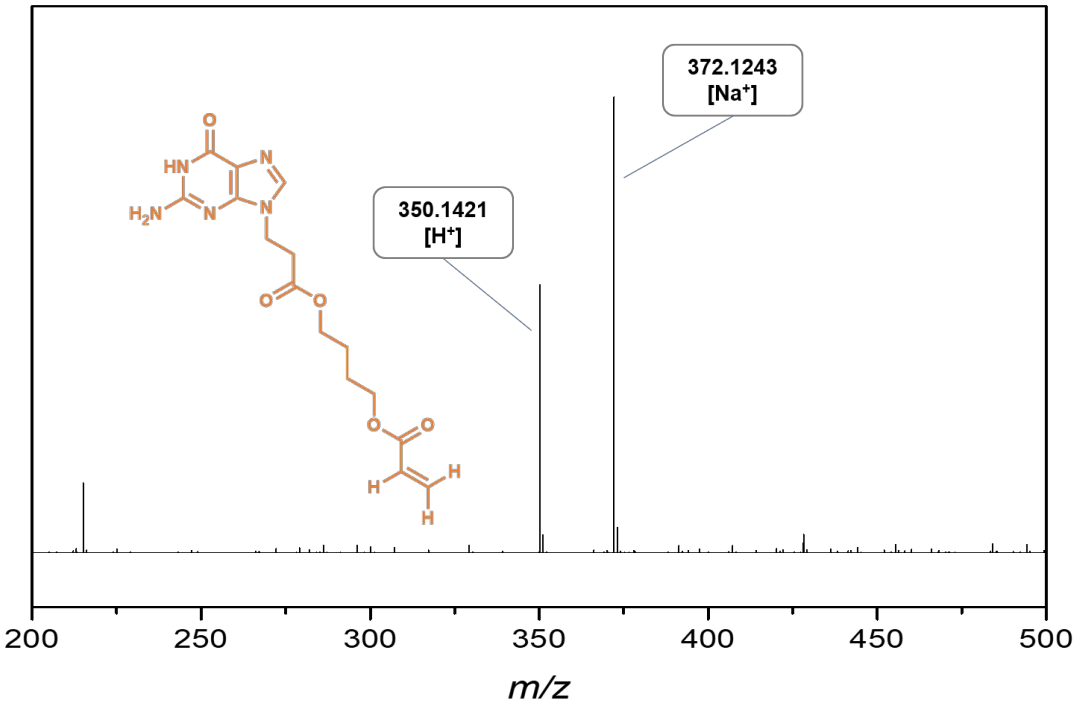
*UAM*


333.1018
[Na+]
405.1273
[Na+ + 8 H2O]
405.1273
[Na+ + 4 H2O]
200
250
300
350
400
450
500
m/z

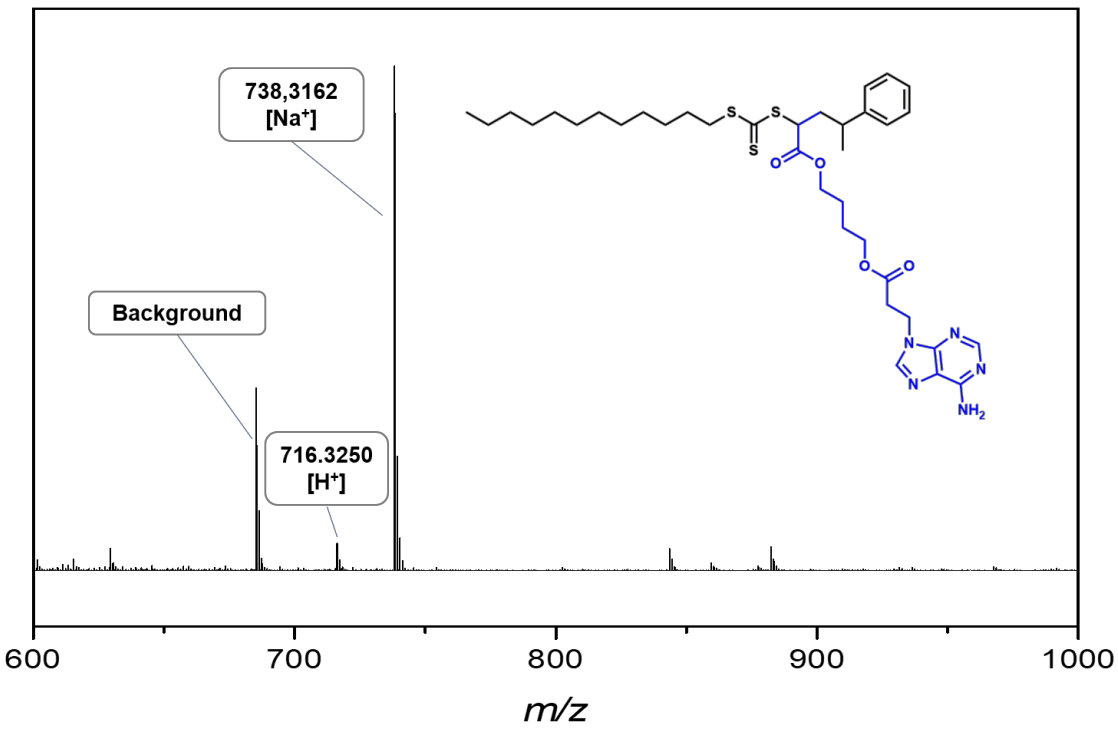
*CAM*


332.1169
[Na+]
310.1356
[H+]
200
250
300
350
400
450
500
m/z

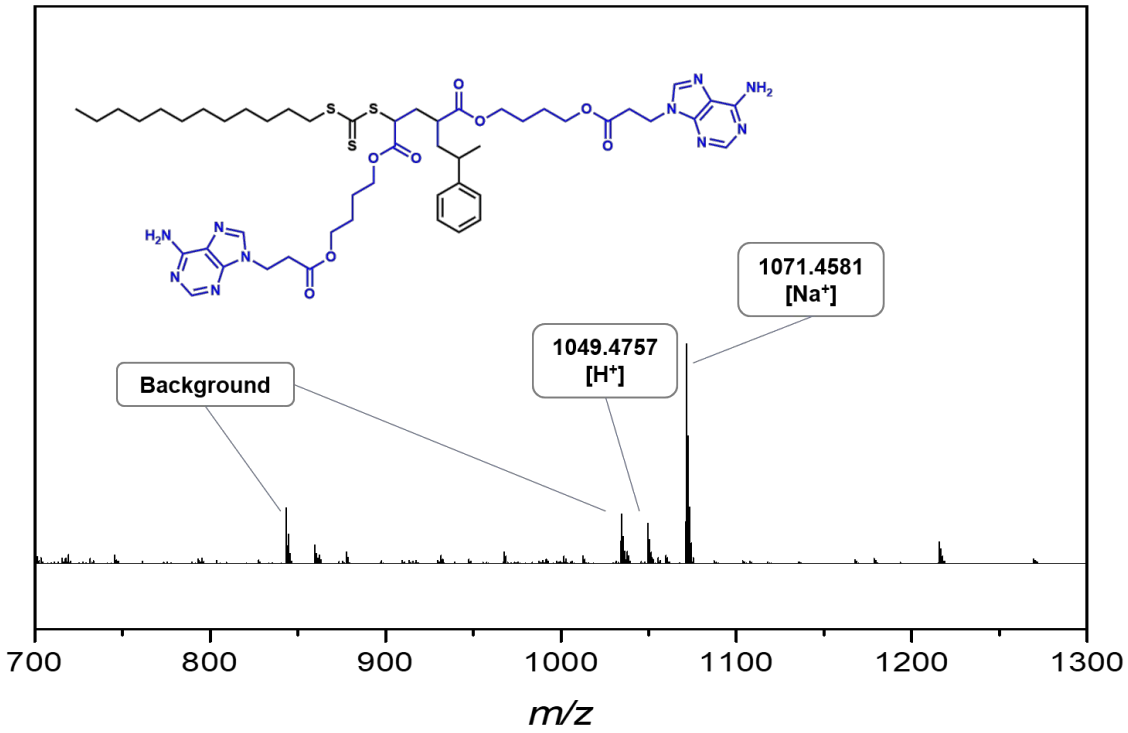
*GAM*


372.1243
[Na+]
350.1421
[H+]
200
250
300
350
400
450
500
m/z

*SUMI A*


738,3162
[Na+]
Background
716.3250
[H+]
600
700
800
900
1000
m/z

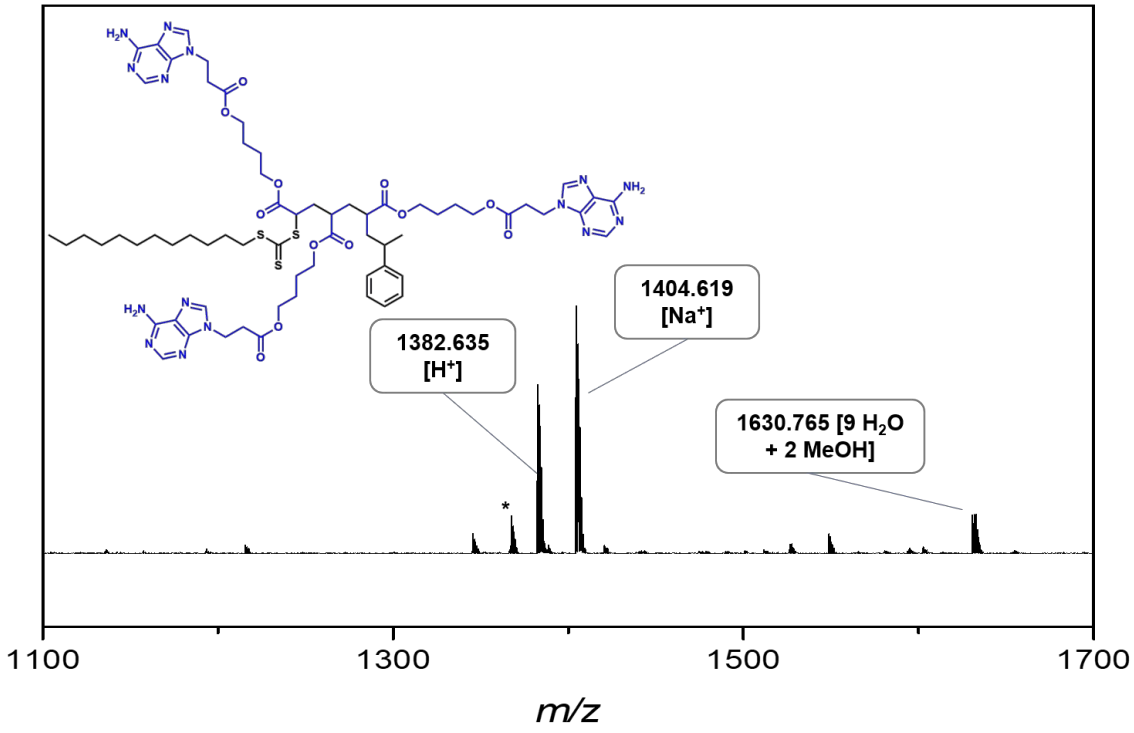
*SUMI AA*


1071.4581
[Na+]
1049.4757
[H+]
Background
700
800
900
1000
1100
1200
1300
m/z

# S6. MALDI-TOF

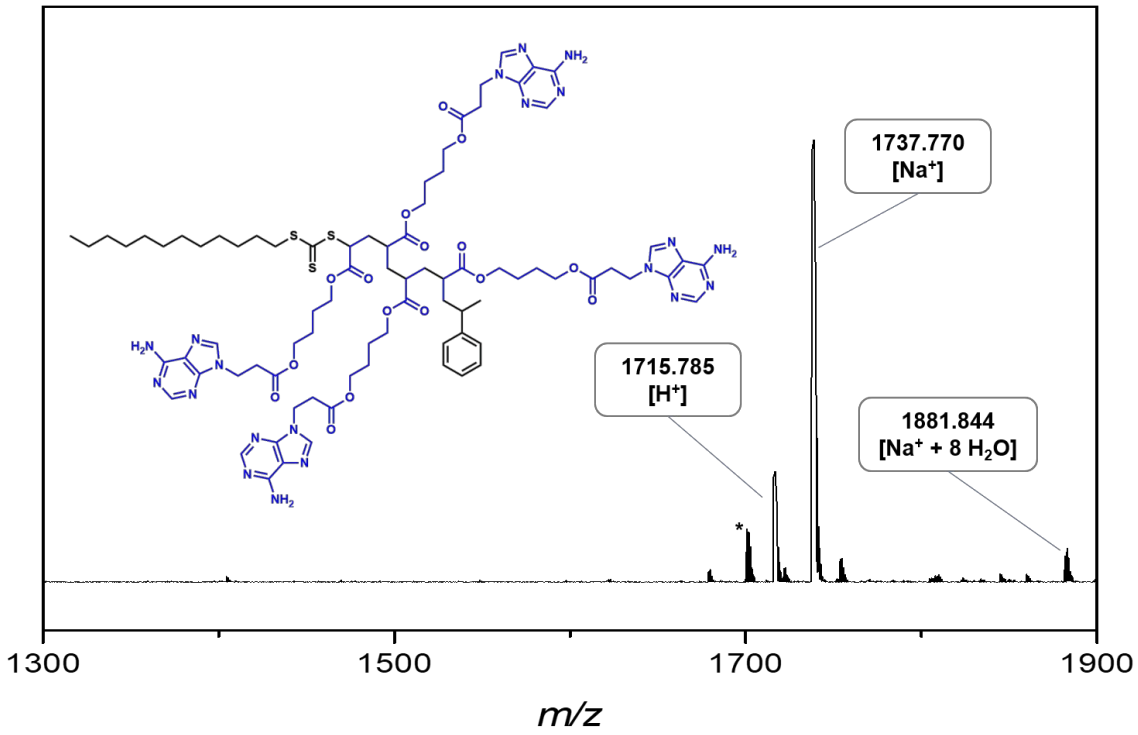
## *SUMI AAA*

*: 1-penylethyl group is exchanged by an isobutyronitrile end-group

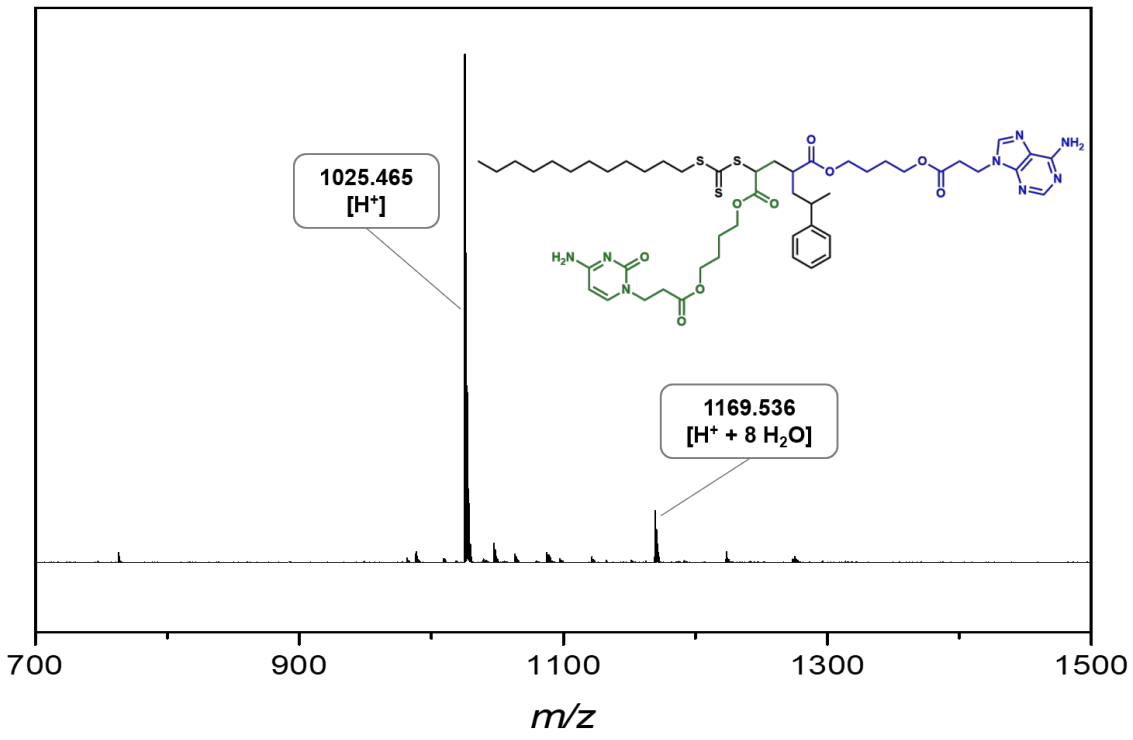


## *SUMI AAAA*

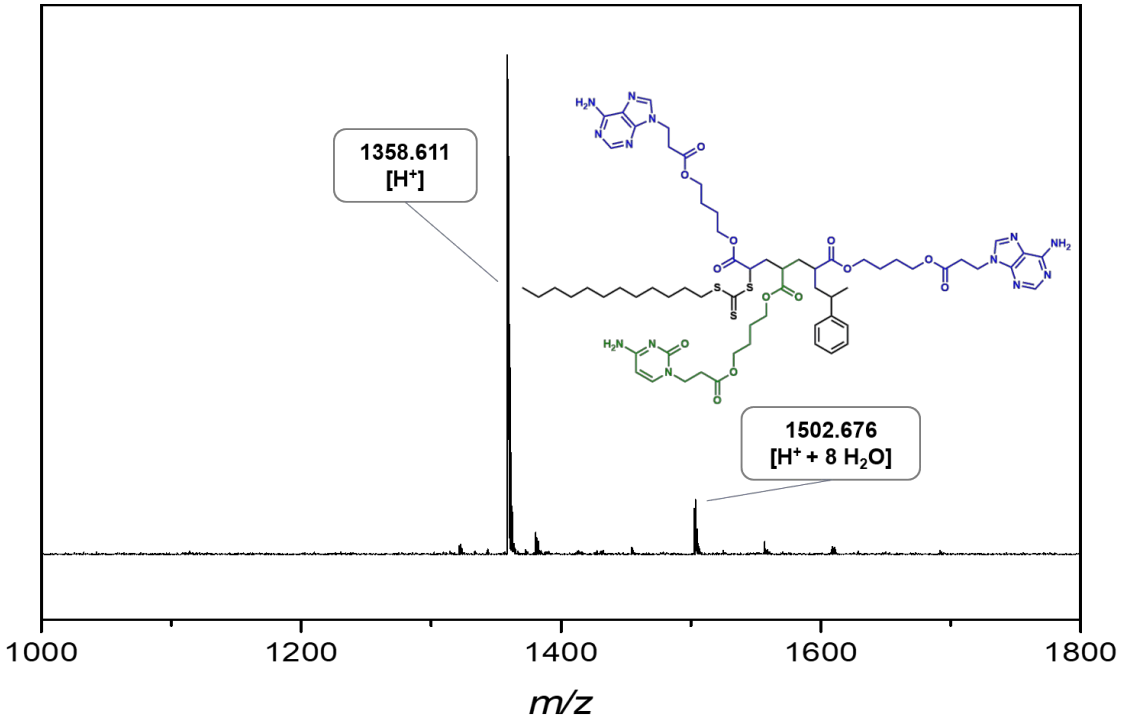
*: 1-penylethyl group is exchanged by an isobutyronitrile end-group



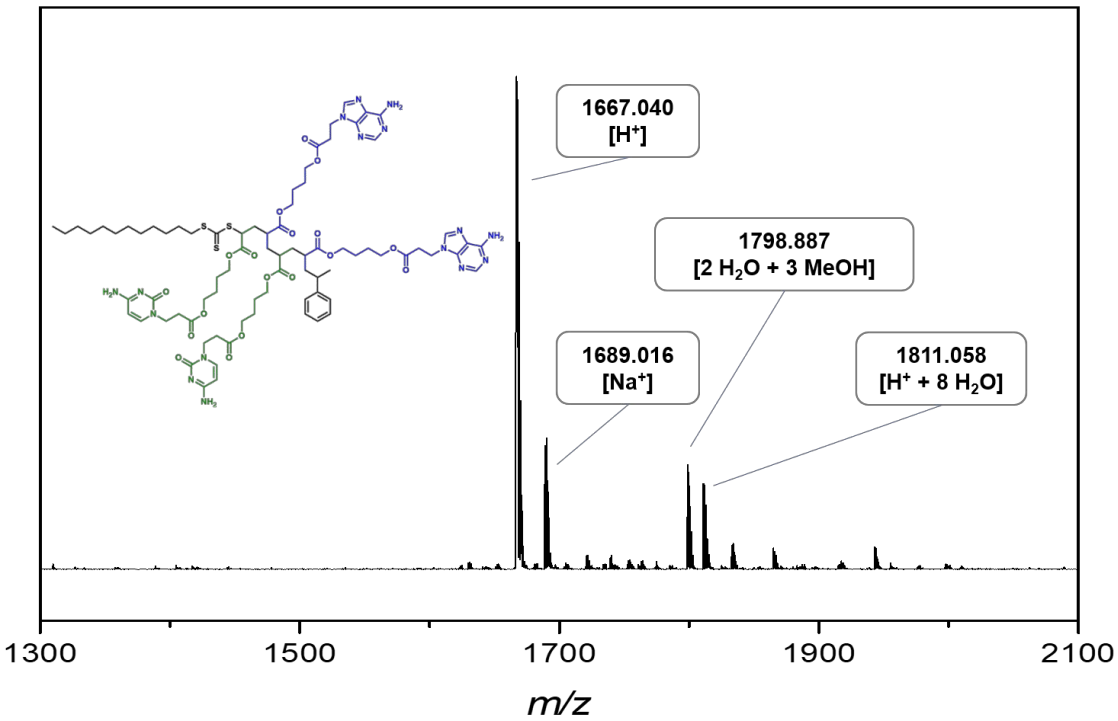

## *SUMI AC*



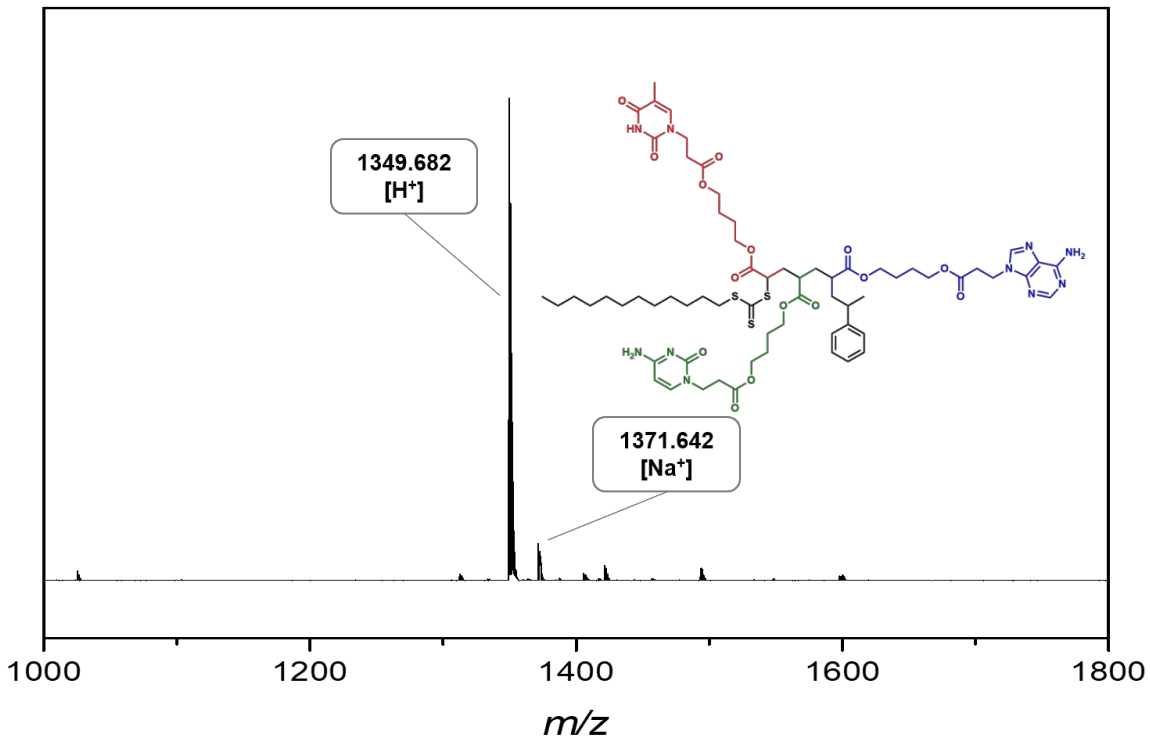
## *SUMI ACA*




## *SUMI ACAC*

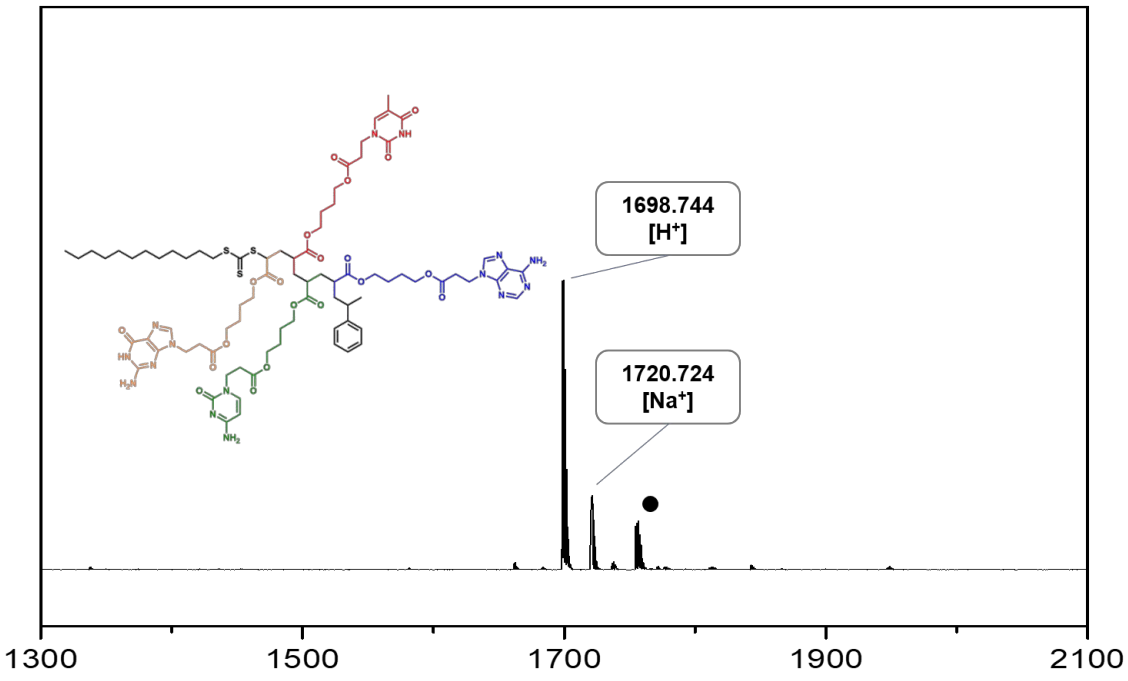


## *SUMI ACT*




## *SUMI ACTG*

● :Guanine oxidation in MALDI-TOF instrument (ACTG chain with 8-oxoguanine)



1. S. Cheng, M. Zhang, N. Dixit, R. B. Moore and T. E. Long, *Macromolecules*, 2012, **45**, 805-812.
2. M. Sedlák, P. Šimůnek and M. Antonietti, *Journal of Heterocyclic Chemistry*, 2003, **40**, 671-675.
3. K. Zhang, G. B. Fahs, E. Margaretta, A. G. Hudson, R. B. Moore and T. E. Long, *The Journal of Adhesion*, 2019, **95**, 146-167.
4. A. Lu, T. P. Smart, T. H. Epps, D. A. Longbottom and R. K. O'Reilly, *Macromolecules*, 2011, **44**, 7233-7241.