Supporting Information

Cationic mucic acid polymer-based siRNA delivery systems

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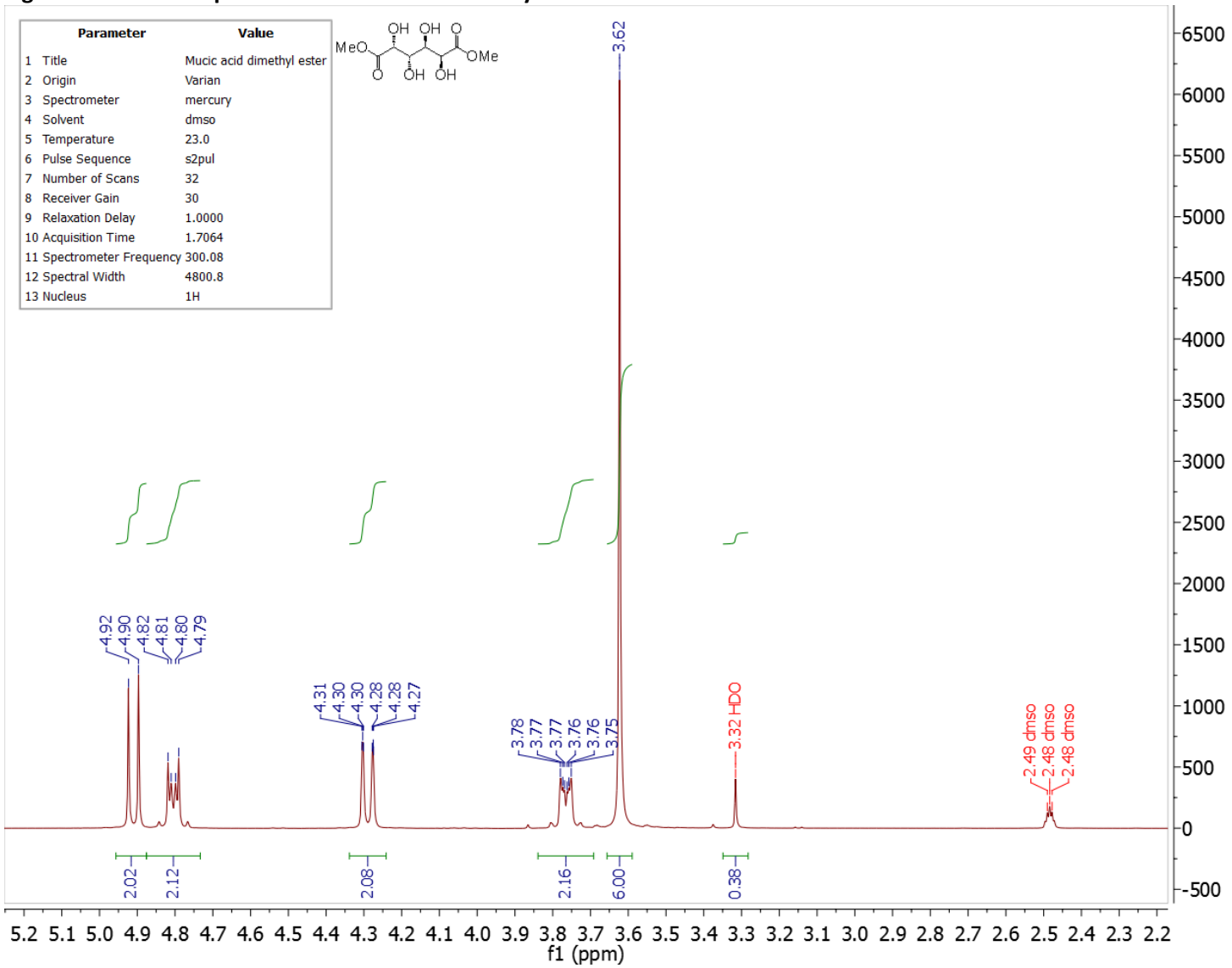
1. cMAP precursors: NMR and mass spectra

Mucic acid dimethyl ester.

Figure S1: 1H NMR Spectrum of mucic acid dimethyl ester.



N-boc protected mucic acid ethylenediamine.

Figure S2: 1H NMR Spectrum of N-boc protected mucic acid ethylenediamine.

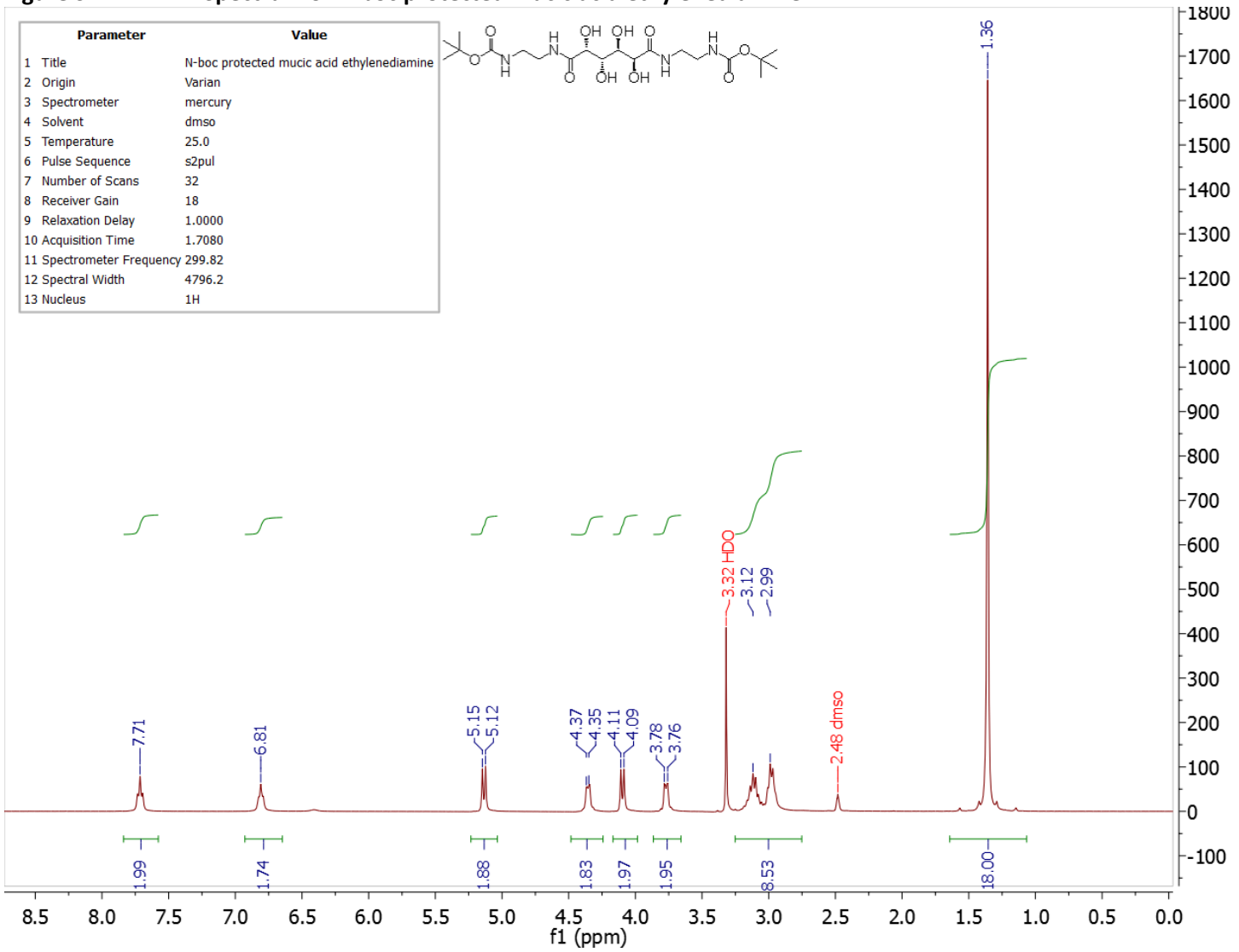
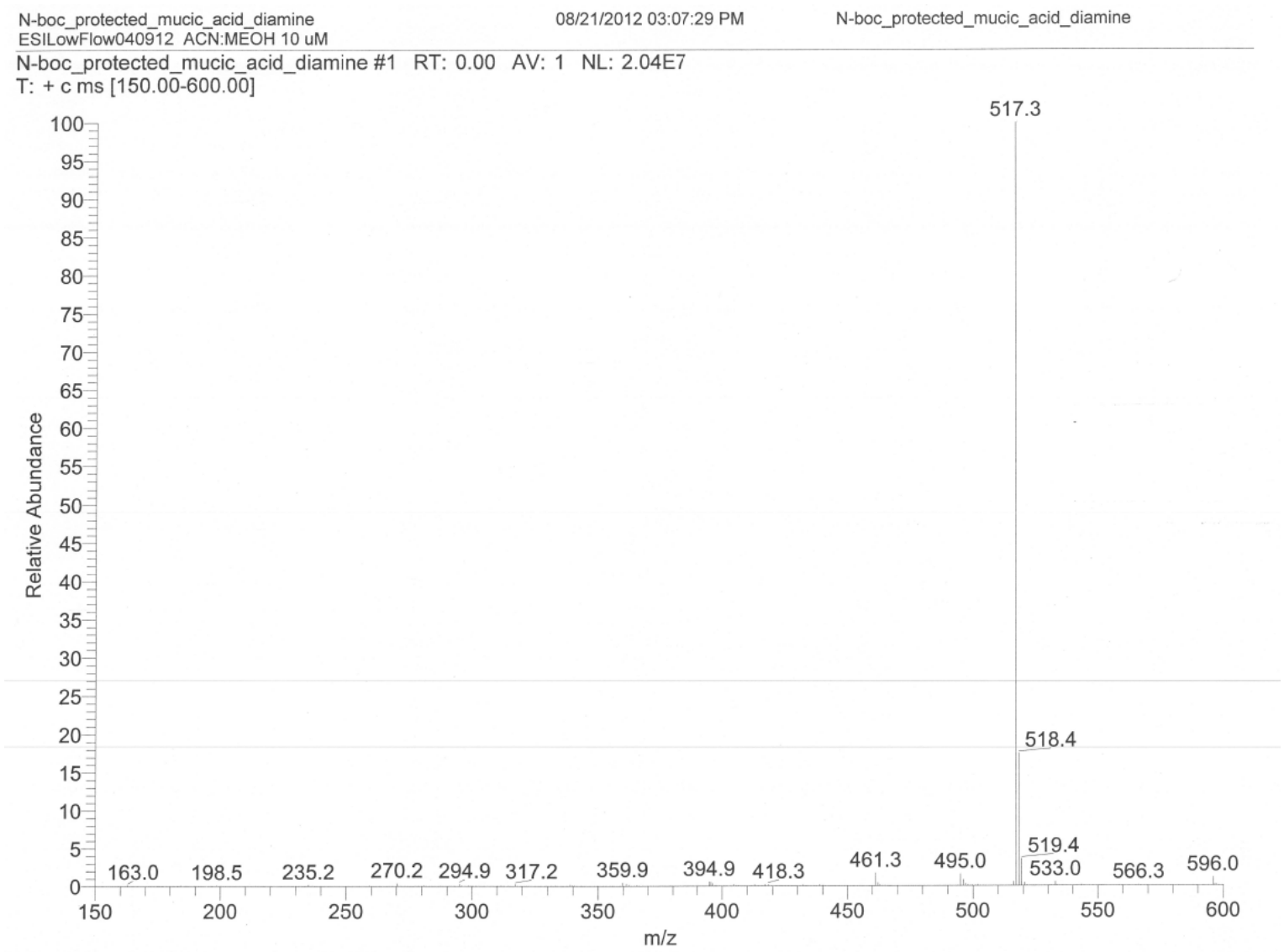
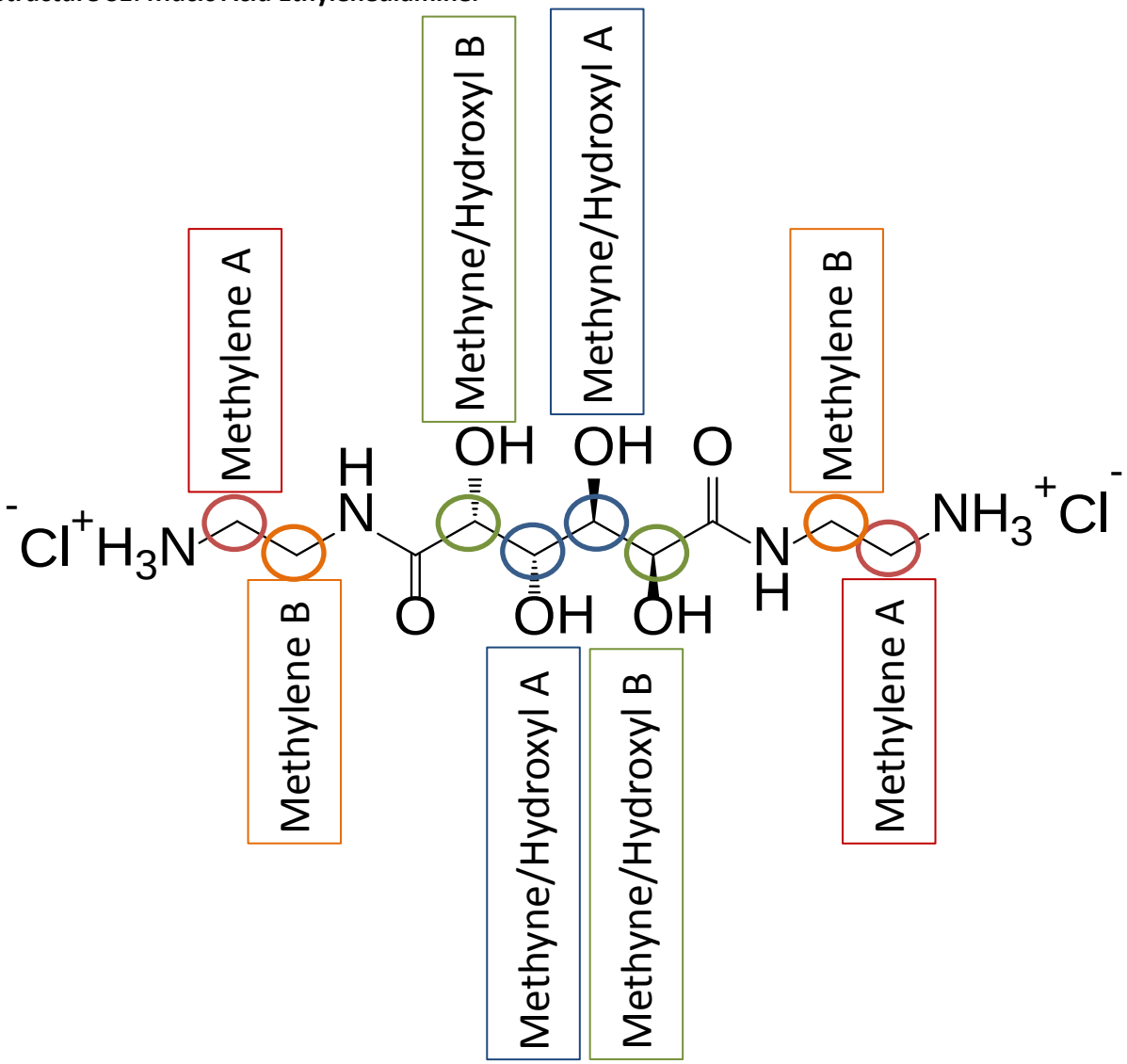


Figure S3: Electrospray Ionization (ESI) Mass Spectrum of N-boc protected mucic acid ethylenediamine.



Mucic acid ethylenediamine.

Structure S1: Mucic Acid Ethylenediamine.



Mucic acid ethylenediamine

Figure S4: 1H NMR Spectrum of Mucic Acid Ethylenediamine in D_2O.

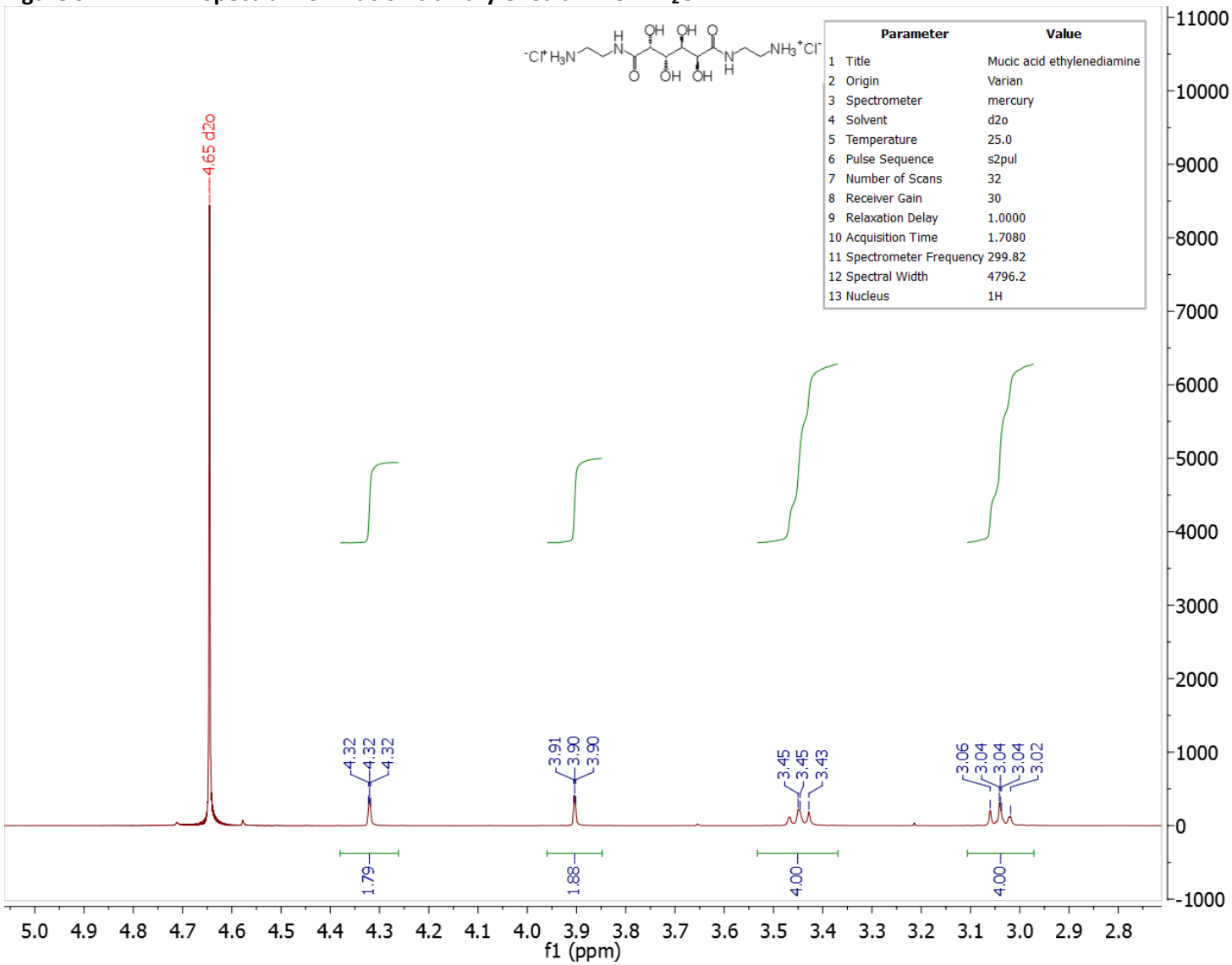


Figure S5: 1H NMR Spectrum of Mucic Acid Ethylenediamine in DMSO.

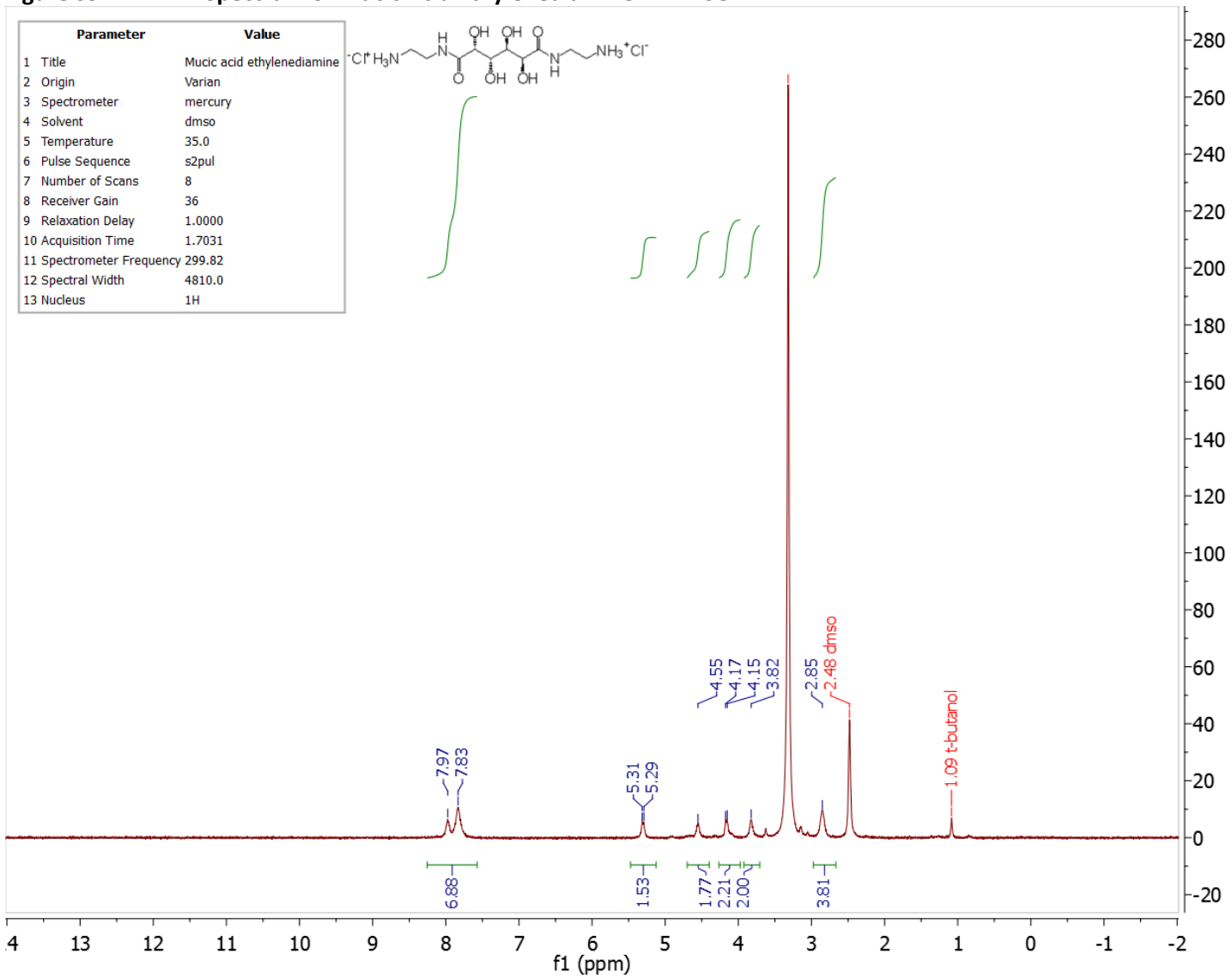


Figure S6: 13C NMR Spectrum of Mucic Acid Ethylenediamine in DMSO.

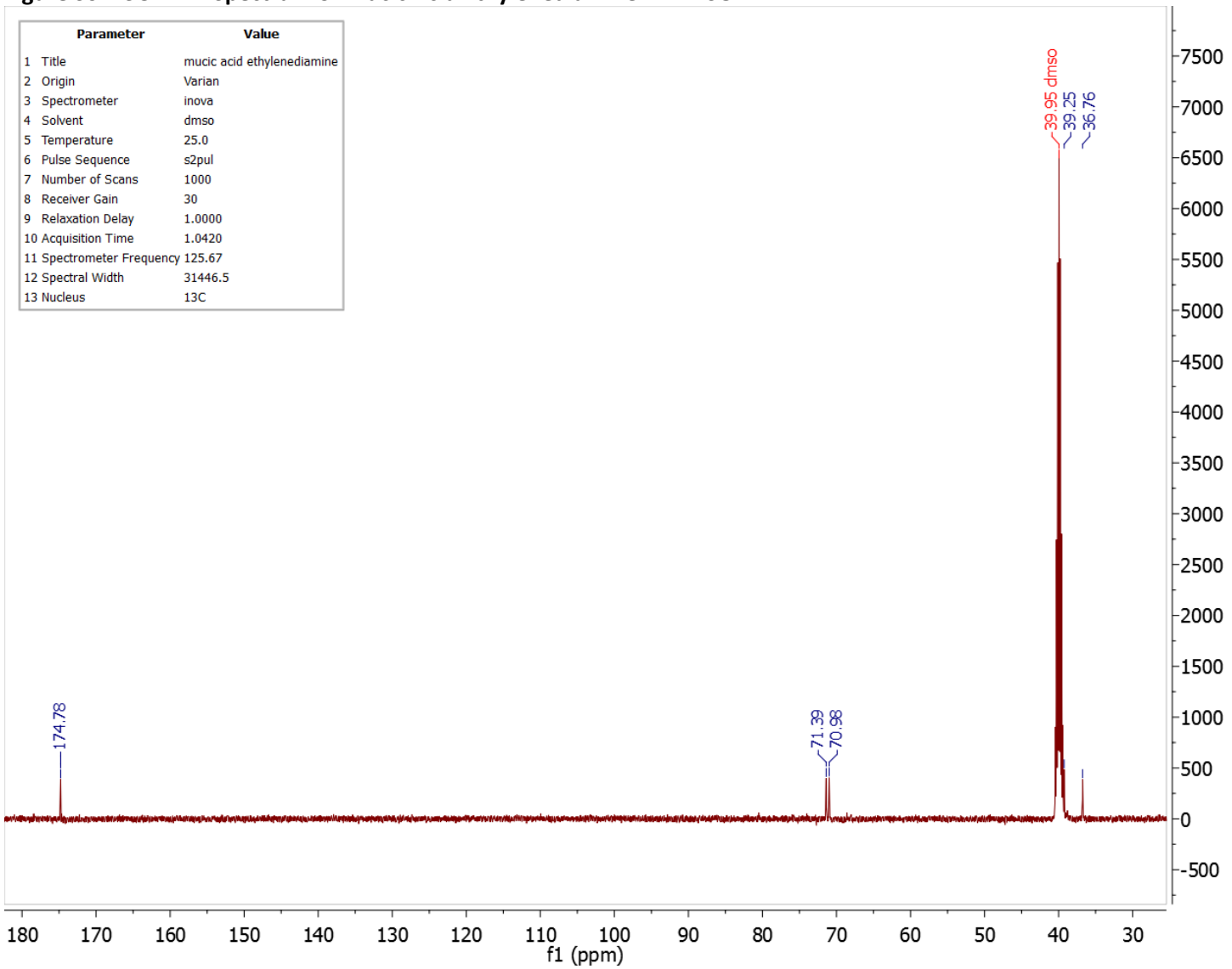
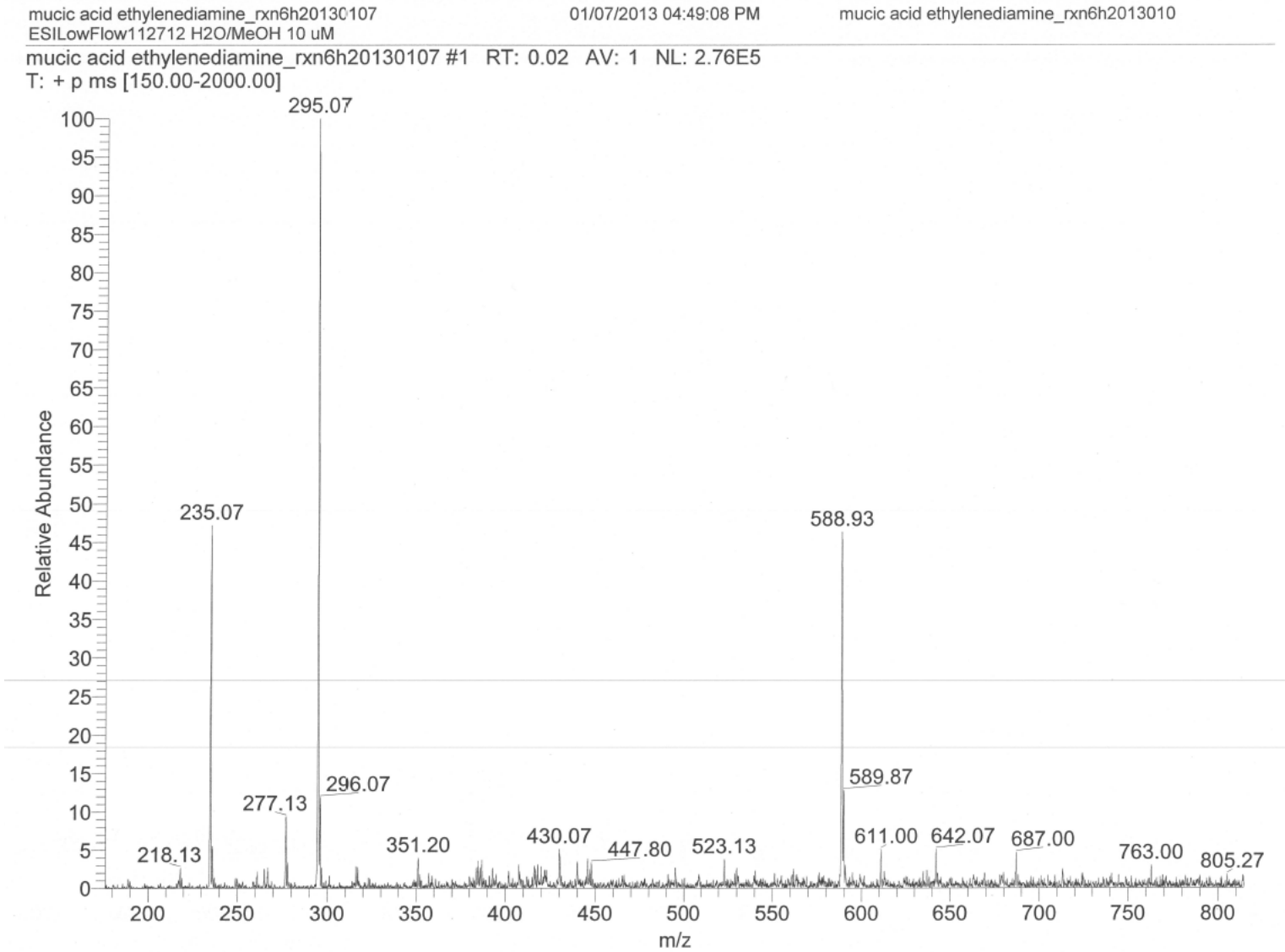


Table S1: 1H and 13C NMR peak assignments for Mucic Acid Ethylenediamine.

	1H in D_2O Chemical Shift (ppm)	1H in DMSO Chemical Shift (ppm)	13C in DMSO Chemical Shift (ppm)
Methylene A	3.04	2.85	36.76
Methylene B	3.45	Covered by H2O	39.25
Methyne A	3.90	3.82	70.98
Methyne B	4.32	4.15	71.39
Hydroxyl A	Not observed	4.55	N/A
Hydroxyl B	Not observed	5.30	N/A
Amide and amines	Not observed	7.83, 7.97	N/A
Amide Carbonyl carbon	N/A	N/A	174.78

Figure S7: ESI Mass Spectrum of Mucic Acid Ethylenediamine.



2. Dimethyl Suberimidate (DMS) and DMS hydrolysis: NMR and mass spectra

Dimethyl Suberimidate (DMS).

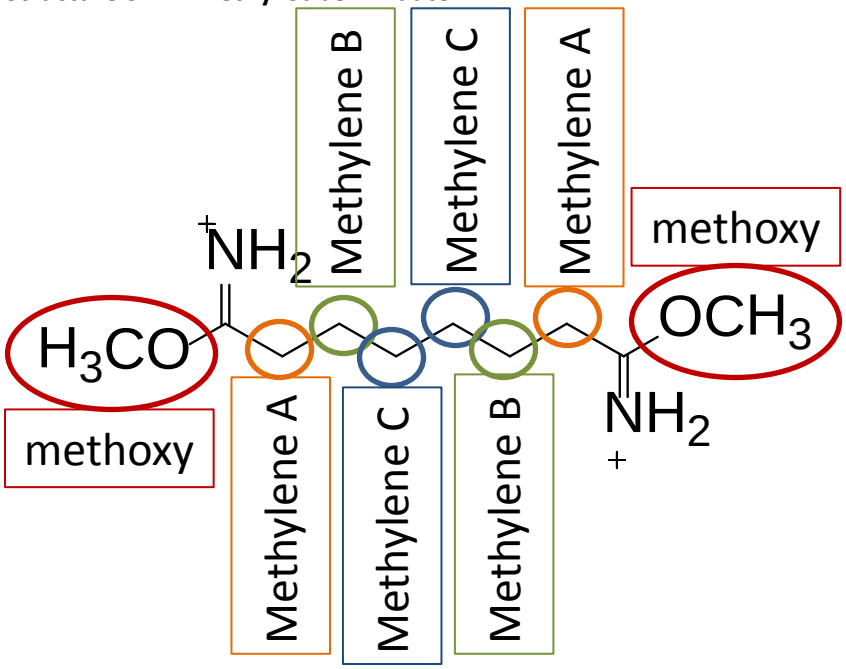
Dimethyl suberimidate, the charged monomer with which mucic acid ethylenediamine was polymerized, was used as purchased from Thermo Scientific or Sigma-Aldrich. In order to assign peaks in the proton and carbon spectra of cMAP, NMR spectra of dimethyl suberimidate were acquired. Both proton and carbon NMR spectra of DMS were more complex than expected, suggesting that some hydrolysis was present in a freshly opened bottle. For example, the three protons of the methoxy peak for DMS have a chemical shift of 4.08 ppm, which completely shift to 3.55 ppm in a sample hydrolyzed in D_2O. Similarly, the methylene peak adjacent to the methoxy (methylene A) has a chemical shift of 2.64 ppm in DMS, which when hydrolyzed shifts more upfield to 2.25 ppm.

Additionally there was the presence of dimethyl suberimidate containing one methoxy group which was completely hydrolyzed to a carboxylate group, as determined by both the ESI mass spectrum peak at *m/z* of 187.9 and the ^{1}H-^{13}C HMBC NMR spectrum. The methylene A peak in this case was present on ^{1}H NMR with a chemical shift of 2.01 ppm.

Table S2: 1H NMR peak assignments for Dimethyl Suberimidate with varying degrees of hydrolysis.

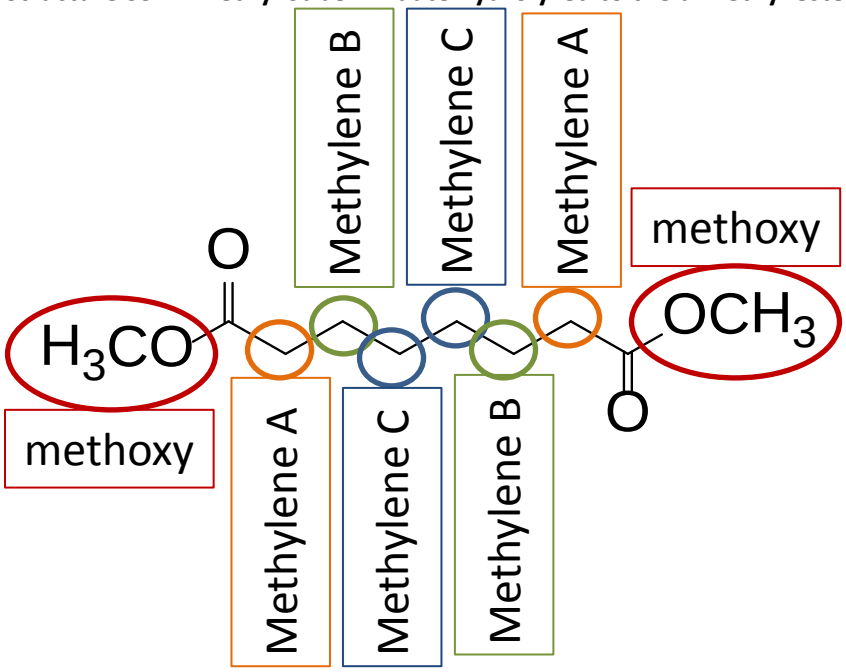
	Methoxy	Methylene A	Methylene B	Methylene C
Dimethyl suberimidate	4.08	2.64	1.60	1.30
Dimethyl suberimidate hydrolyzed to the dimethyl ester	3.55	2.25	1.45	1.20
Dimethyl suberimidate hydrolyzed to carboxylate	None	2.01	1.46	1.20

Structure S2: Dimethyl Suberimidate.



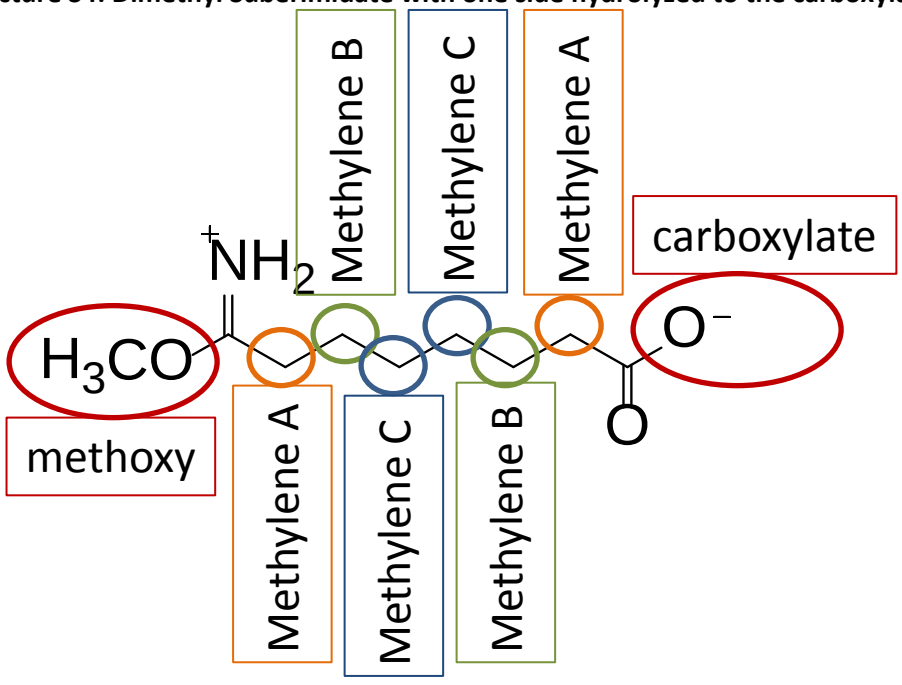
Dimethyl Suberimidate

Structure S3: Dimethyl Suberimidate hydrolyzed to the dimethyl ester.



Dimethyl Suberimidate - hydrolyzed

Structure S4: Dimethyl Suberimidate with one side hydrolyzed to the carboxylate.



Dimethyl Suberimidate – hydrolyzed to carboxylate

Figure S8: 1H NMR Spectrum of Dimethyl Suberimidate.

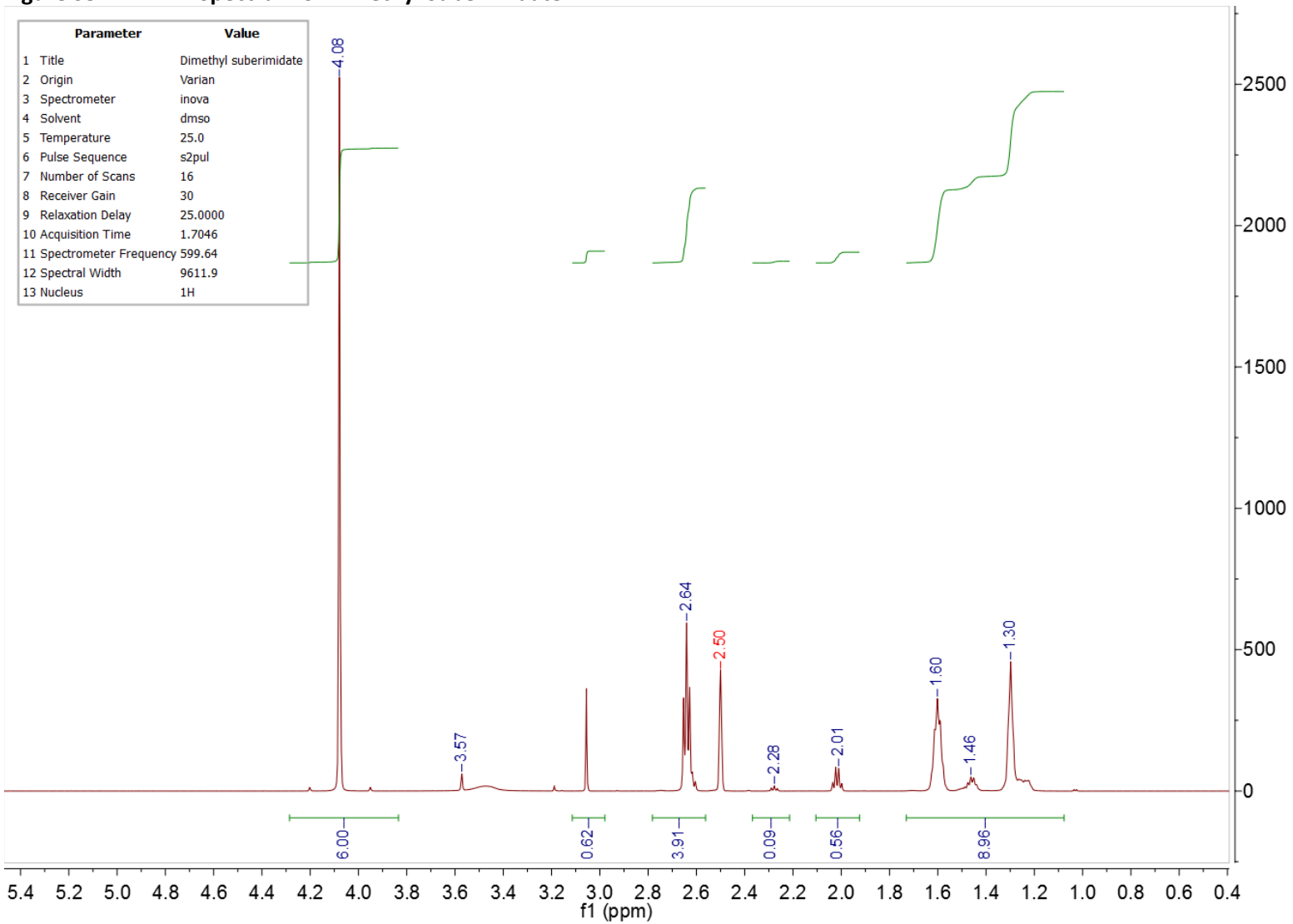


Figure S9: 13C NMR Spectrum of Dimethyl Suberimidate.

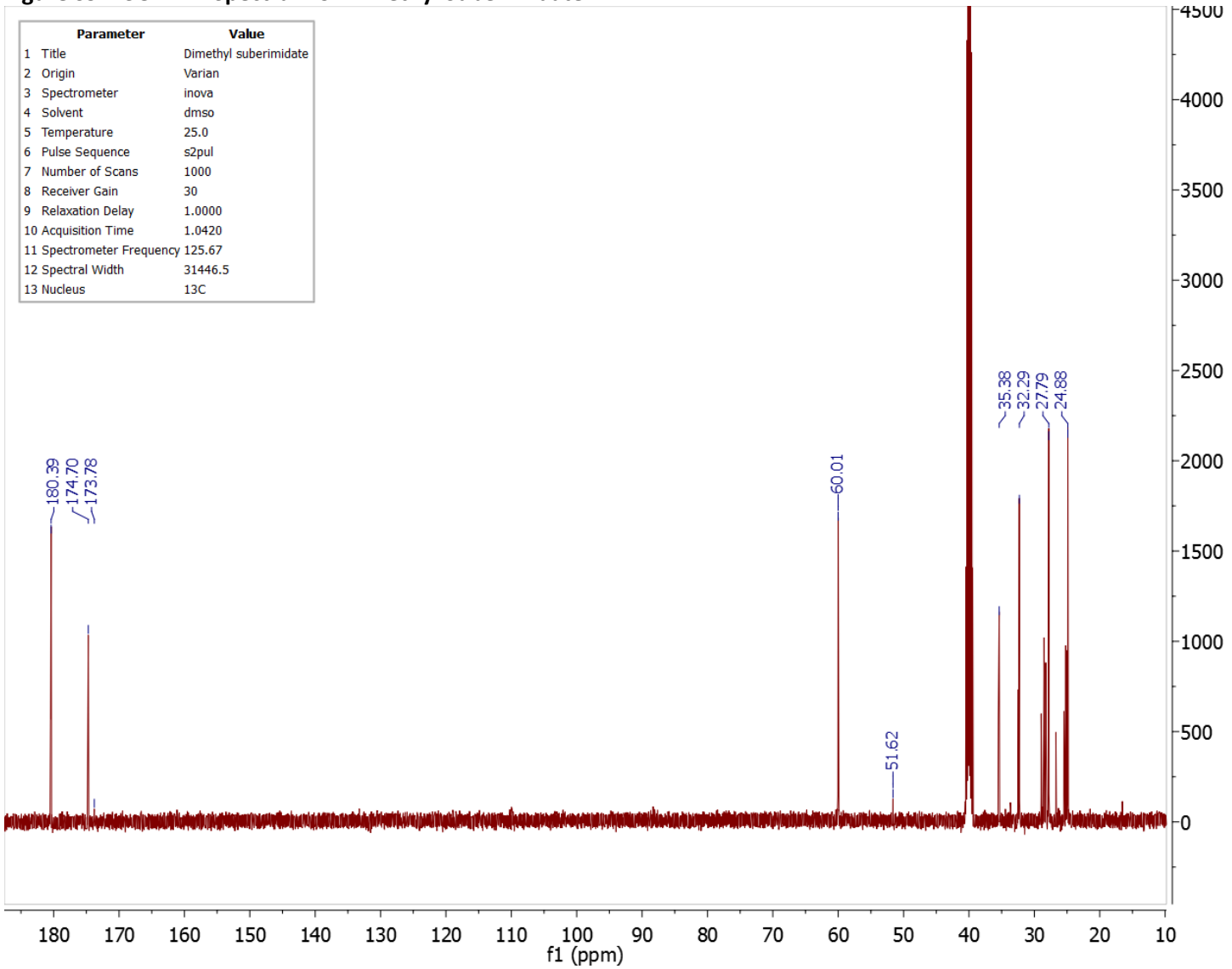


Figure S10: 1H-13C HMBC Spectrum of Dimethyl Suberimidate.

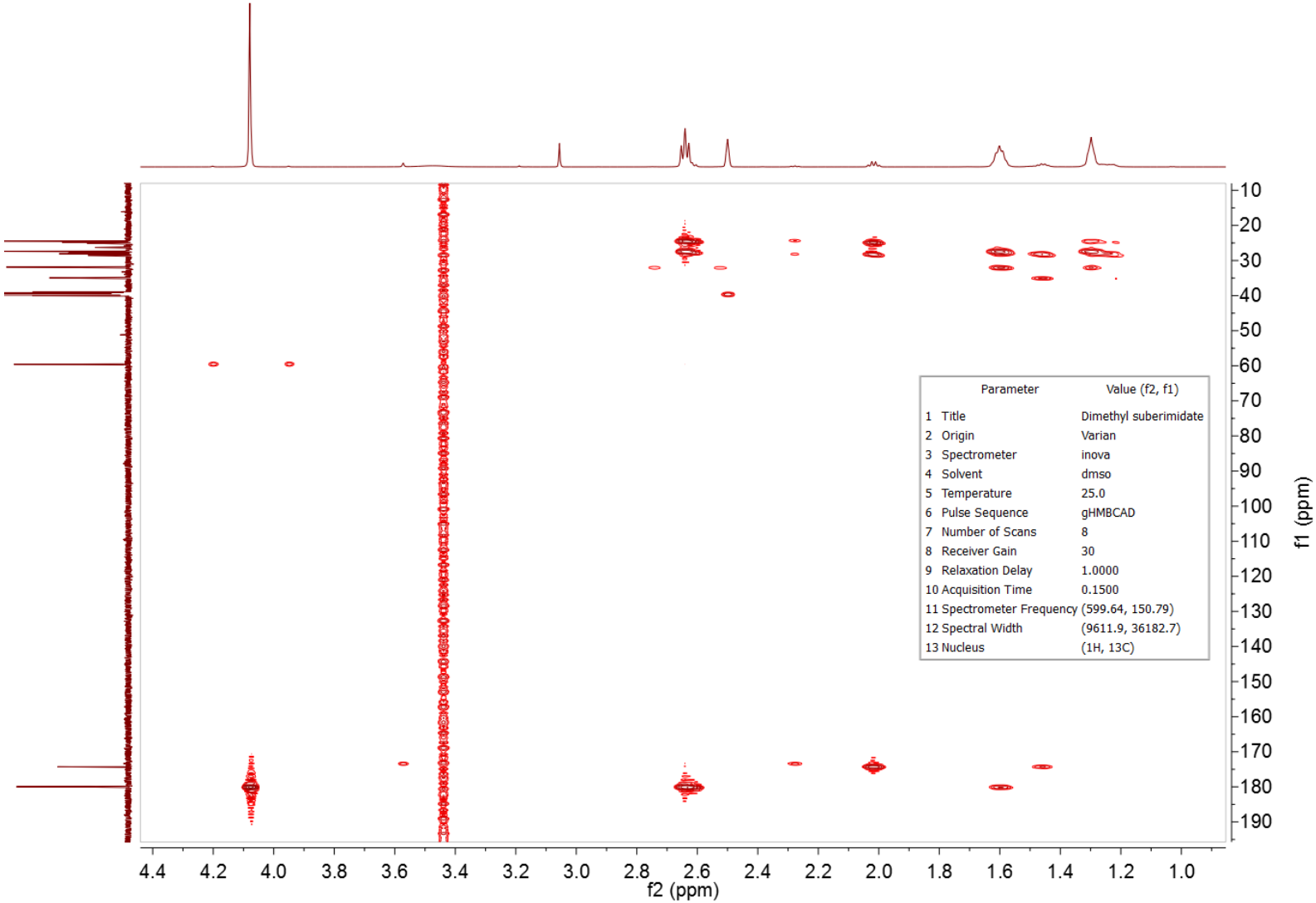
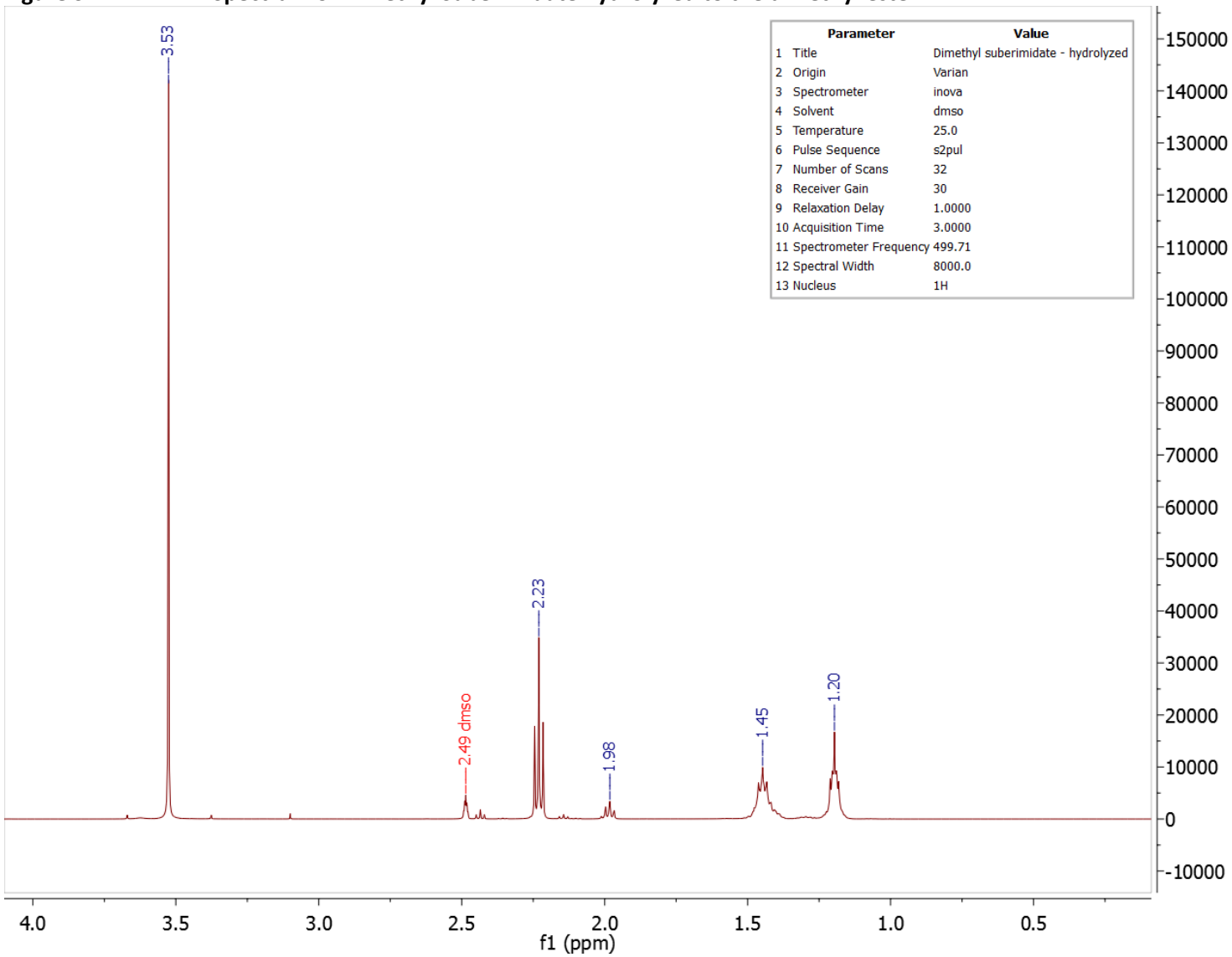


Figure S11: 1H NMR Spectrum of Dimethyl Suberimidate hydrolyzed to the dimethyl ester.



	Parameter	Value
1	Title	Dimethyl suberimidate - hydrolyzed
2	Origin	Varian
3	Spectrometer	inova
4	Solvent	dmso
5	Temperature	25.0
6	Pulse Sequence	s2pul
7	Number of Scans	32
8	Receiver Gain	30
9	Relaxation Delay	1.0000
10	Acquisition Time	3.0000
11	Spectrometer Frequency	499.71
12	Spectral Width	8000.0
13	Nucleus	1H

Figure S12: 13C NMR Spectrum of Dimethyl Suberimidate hydrolyzed to the dimethyl ester.

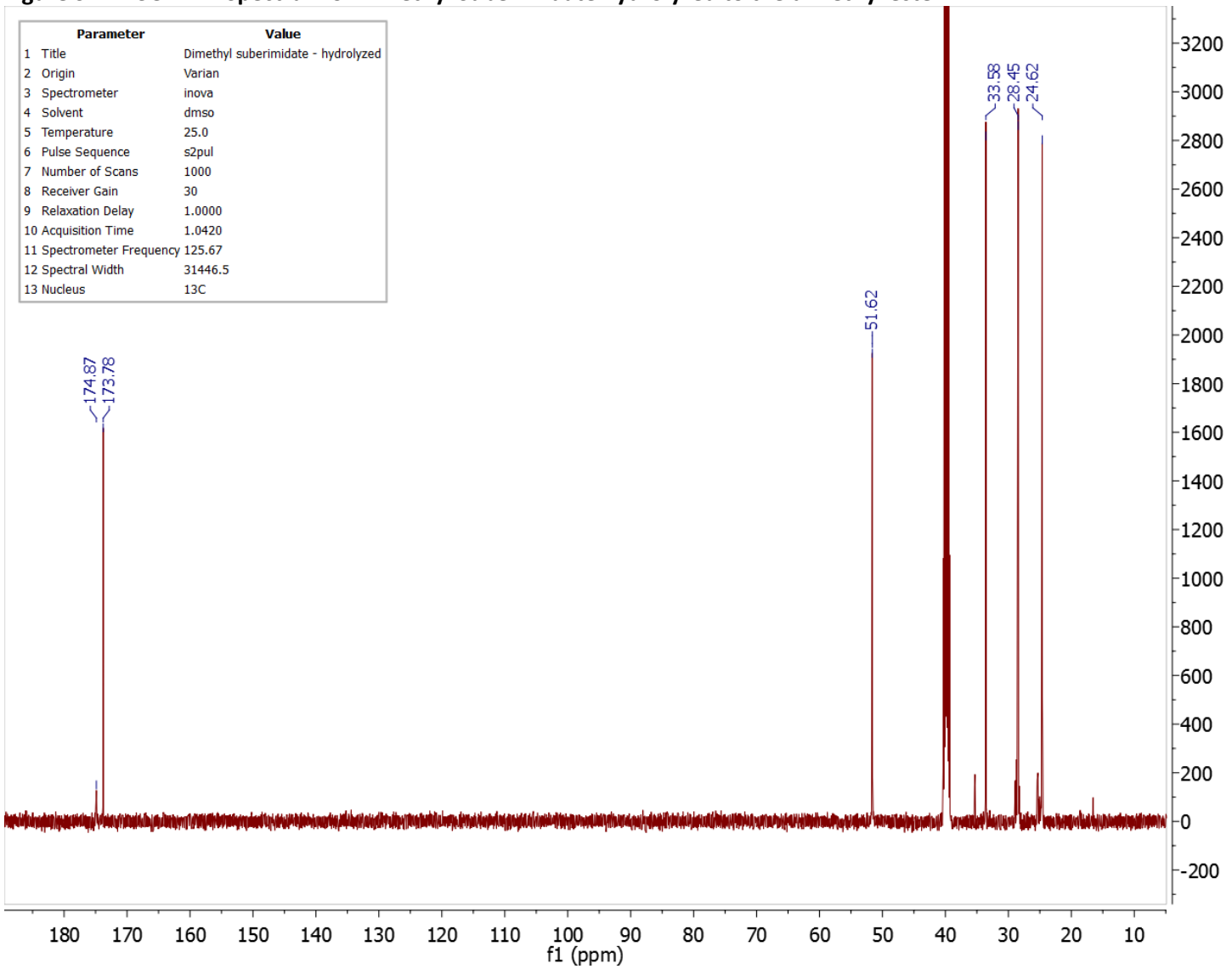
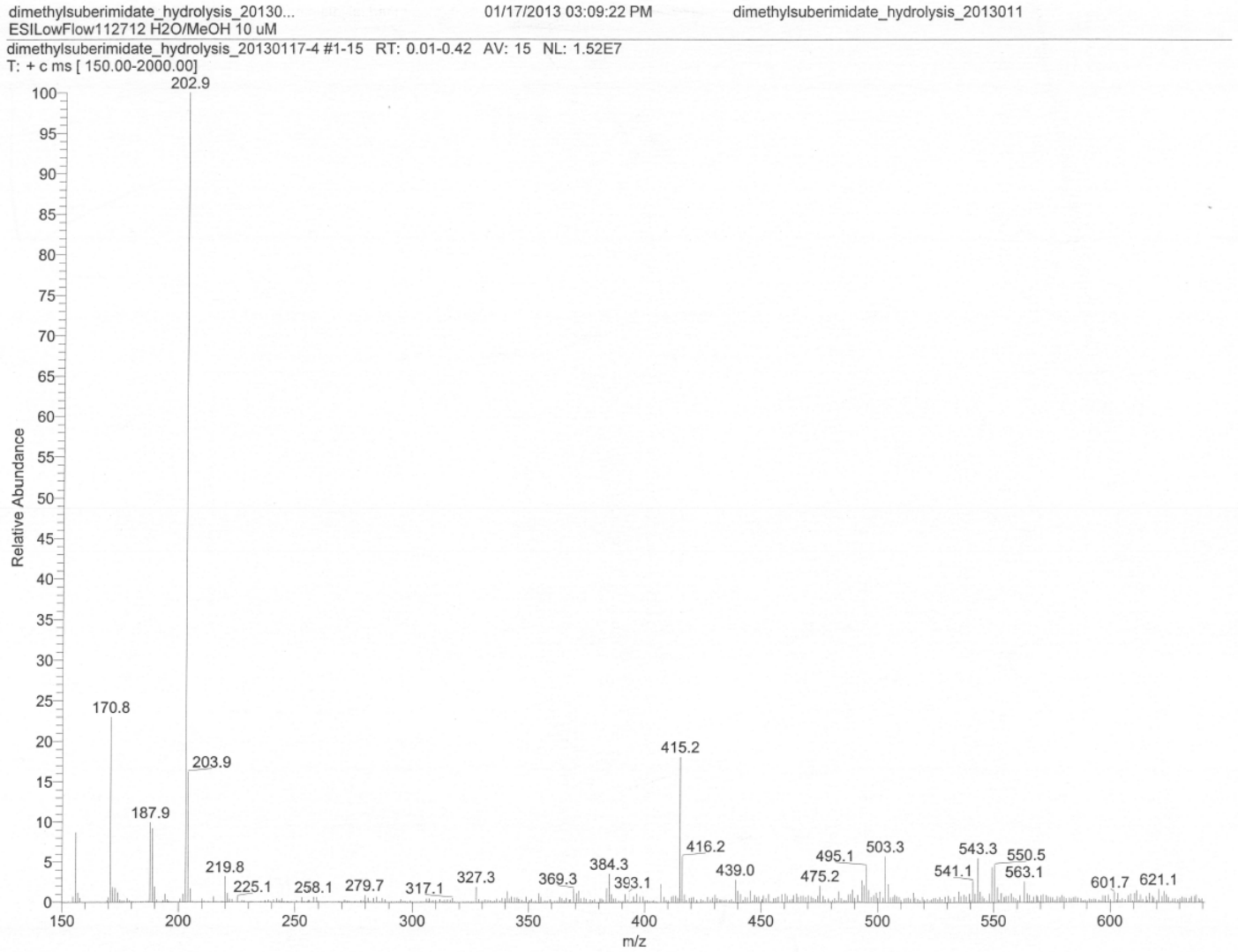


Figure S13: ESI Mass Spectrum of Dimethyl Suberimidate hydrolyzed to the dimethyl ester (*m/z* 202.9). Dimethyl suberimidate with one end hydrolyzed to the carboxylate is the peak with *m/z* 187.9. Dimethyl suberimidate with both ends hydrolyzed to the carboxylate is the peak with *m/z* 170.8.



3. cMAP: NMR spectra

cationic Mucic Acid Polymer (cMAP).

Structure S5: cMAP.

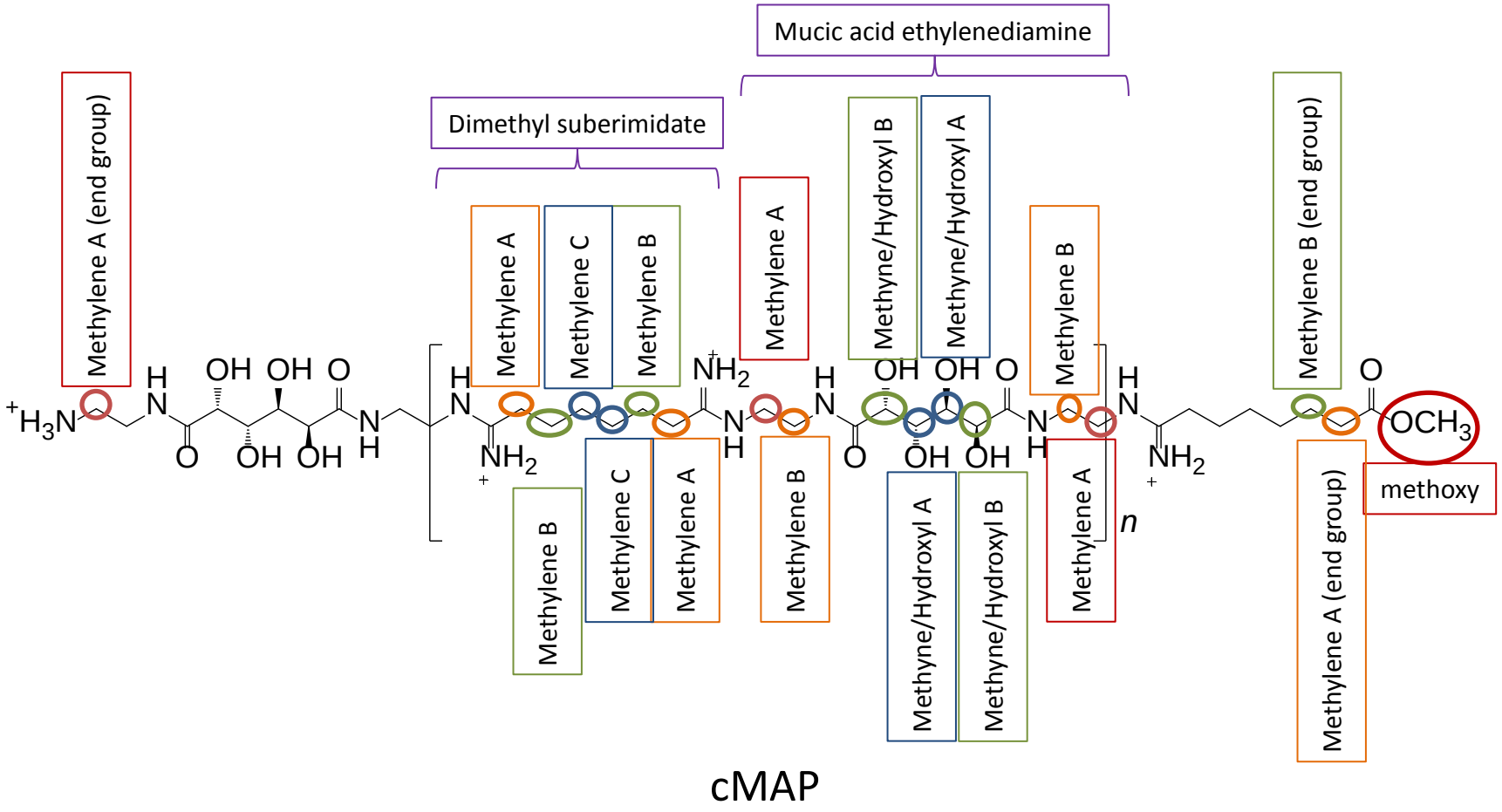


Figure S14: 1H NMR Spectrum of cMAP.

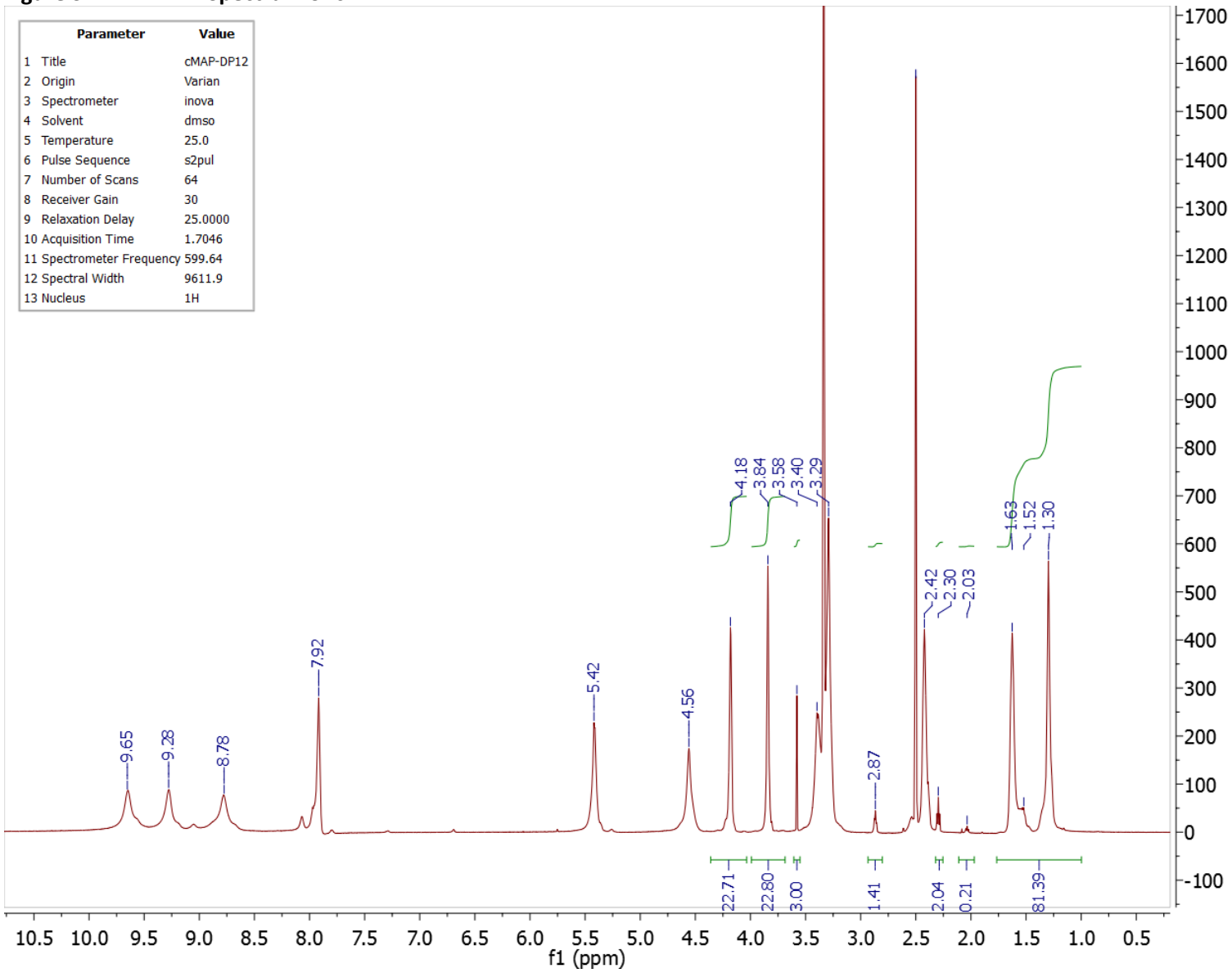


Table S3: 1H NMR peak assignments for cMAP.

	Originating Monomer	1H in DMSO Chemical Shift (ppm)
Amidine	Mucic acid and dimethyl suberimidate	9.65
Amidine	Mucic acid and dimethyl suberimidate	9.28
Amidine	Mucic acid and dimethyl suberimidate	8.78
Amide	Mucic acid and dimethyl suberimidate	7.92
Hydroxyl B	Mucic acid	5.42
Hydroxyl A	Mucic acid	4.56
Methyne B	Mucic acid	4.18
Methyne A	Mucic acid	3.84
Methoxy (end group)	Dimethyl suberimidate	3.58
Methylene B	Mucic acid	3.40
Methylene A	Mucic acid	3.29
Methylene A (end group)	Mucic acid	2.87
Methylene A	Dimethyl suberimidate	2.42
Methylene A (end group)	Dimethyl suberimidate	2.30
Methylene A (end group)	Dimethyl suberimidate – hydrolyzed to carboxylate	2.03
Methylene B	Dimethyl suberimidate	1.63
Methylene B (end group)	Dimethyl suberimidate	1.52
Methylene C	Dimethyl suberimidate	1.30

Figure S15: 13C NMR Spectrum of cMAP.

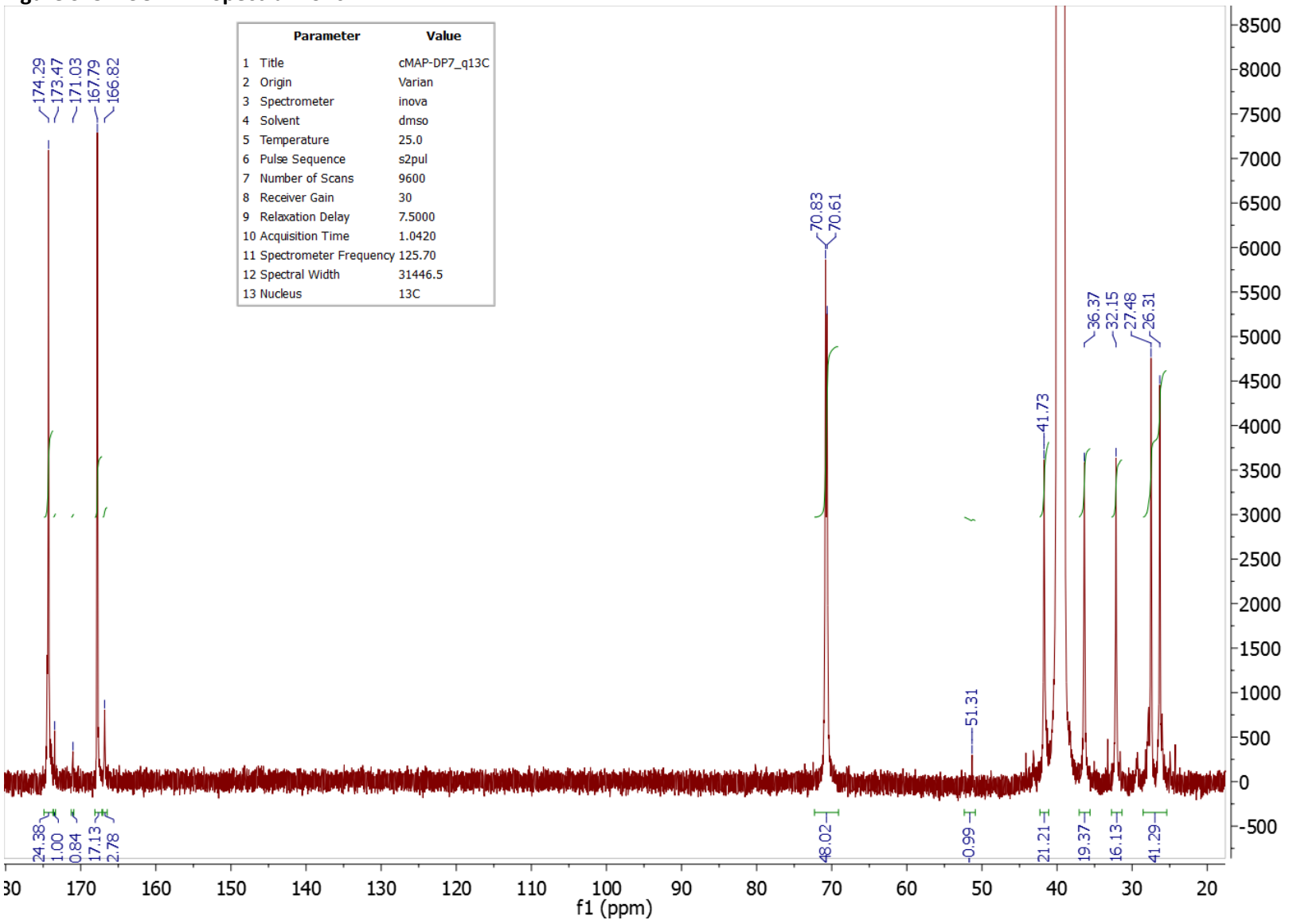


Table S4: 13C NMR peak assignments for cMAP.

	Originating Monomer	13C in DMSO Chemical Shift (ppm)
Amide Carbonyl	Mucic acid	174.29
Methoxy Carbonyl (end group)	Dimethyl suberimidate	173.47
Amide Carbonyl (end group)	Mucic acid	171.03
Amidine Carbonyl	Dimethyl suberimidate	167.79
		166.82
Methyne B	Mucic acid	70.83
Methyne A	Mucic acid	70.61
Methoxy (end group)	Dimethyl suberimidate	51.31
Methylene A	Mucic acid	41.73
Methylene B	Mucic acid	36.37
Methylene A	Dimethyl suberimidate	32.15
Methylene C	Dimethyl suberimidate	27.48
Methylene B	Dimethyl suberimidate	26.31
Methylene A (end group)	Dimethyl suberimidate – hydrolyzed to carboxylate	35.06
Methylene A (end group)	Dimethyl suberimidate	33.22
Methylene B (end group)	Dimethyl suberimidate	24.23

Figure S16: 1H-13C HSQC Spectrum of cMAP used to correlate proton and carbon chemical shifts.

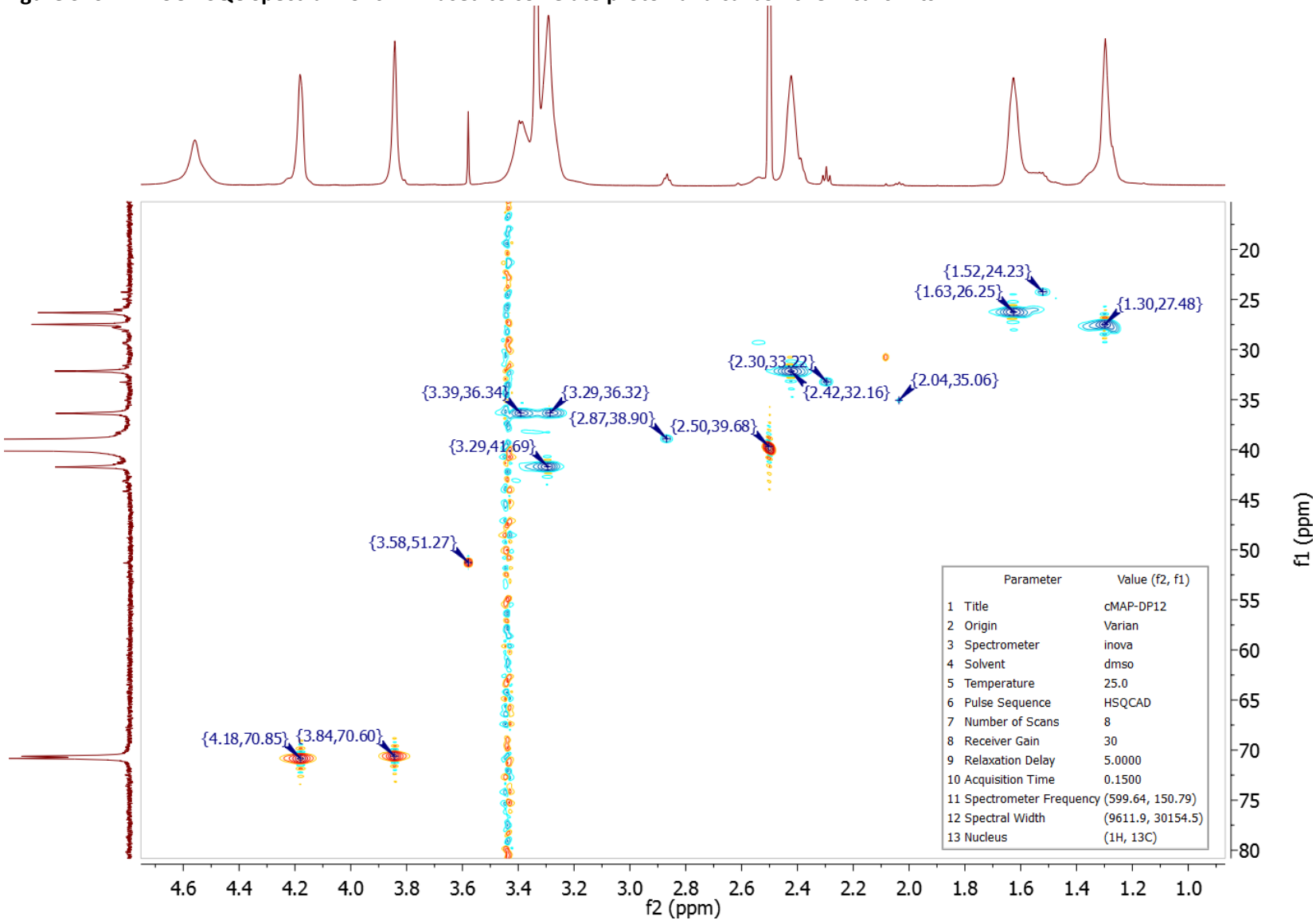


Table S5: 1H-13C HSQC NMR peak assignments for cMAP.

	Originating Monomer	1H Chemical Shift (ppm)	13C Chemical Shift (ppm)
Methyne B	Mucic acid	4.18	70.85
Methyne A	Mucic acid	3.84	70.60
Methoxy (end group)	Dimethyl suberimidate	3.58	51.27
Methylene A	Mucic acid	3.29	41.69
DMSO	(Solvent)	2.50	39.68
Methylene A (end group)	Mucic acid	2.87	38.90
Methylene B	Mucic acid	3.39	36.34
Methylene A	Mucic acid	3.29	36.32
Methylene A (end group)	Dimethyl suberimidate (hydrolyzed to carboxylate)	2.04	35.06
Methylene A (end group)	Dimethyl suberimidate	2.30	33.22
Methylene A	Dimethyl suberimidate	2.42	32.16
Methylene C	Dimethyl suberimidate	1.30	27.48
Methylene B	Dimethyl suberimidate	1.63	26.25
Methylene B (end group)	Dimethyl suberimidate	1.52	24.23

Figure S17: 1H-1H COSY Spectrum of cMAP used to correlate protons to nearby protons more than one bond away.

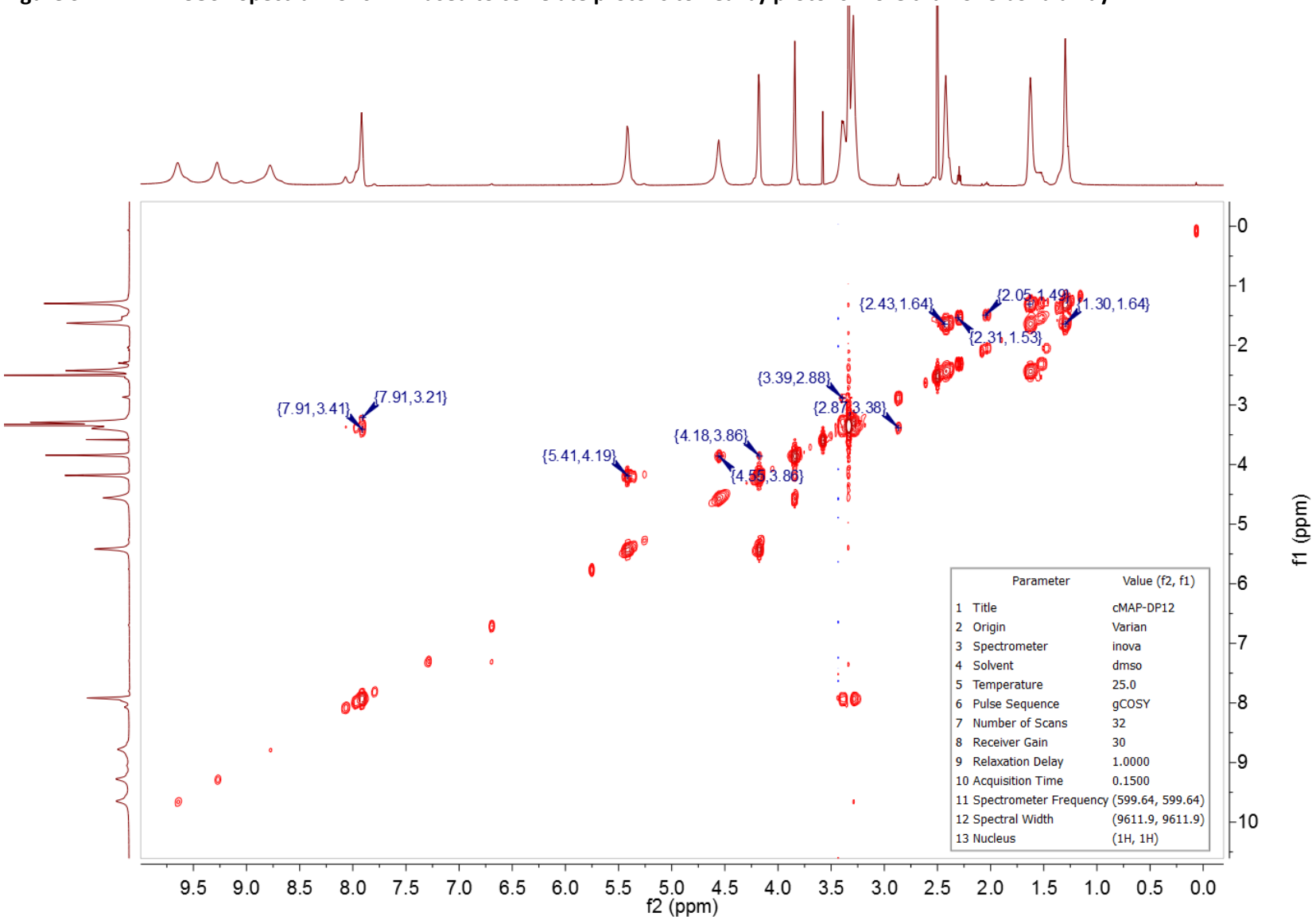


Table S6: 1H-1H COSY NMR peak assignments for cMAP.

Correlation		1H Chemical Shift (ppm)	1H Chemical Shift (ppm)
Methyne B	Hydroxyl B	4.19	5.41
Methyne B	Methyne A	3.86	4.18
Methyne A	Hydroxyl A	3.86	4.55
Methylene B (mucic acid)	Amide	3.41	7.91
Methylene B (mucic acid)	Methylene A (end group, mucic acid)	3.38	2.87
Methylene A (mucic acid)	Amide	3.21	7.91
Methylene A (end group, mucic acid)	Methylene B (mucic acid)	2.88	3.39
Methylene B (DMS)	Methylene A (DMS)	1.64	2.43
Methylene B (DMS)	Methylene C (DMS)	1.64	1.30
Methylene B (end group, DMS)	Methylene A (end group, DMS)	1.53	2.31
Methylene B (end group, DMS)	Methylene A (end group, DMS hydrolyzed to carboxylate)	1.49	2.05
Methylene C (DMS)	Methylene B (DMS)	1.31	1.63

Figure S18: 1H-13C HMBC Spectrum of cMAP used to correlate protons to carbons more than one bond away.

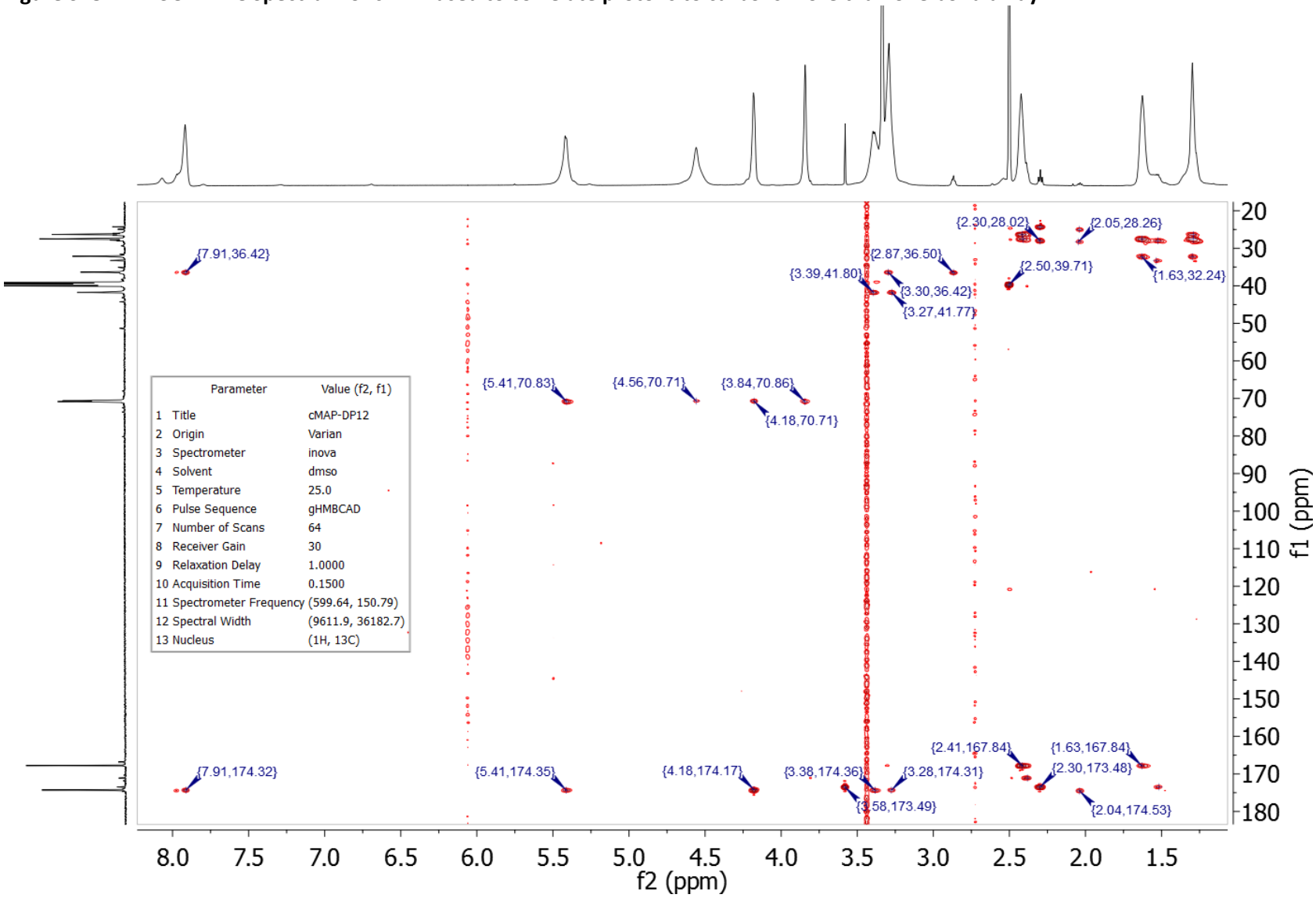
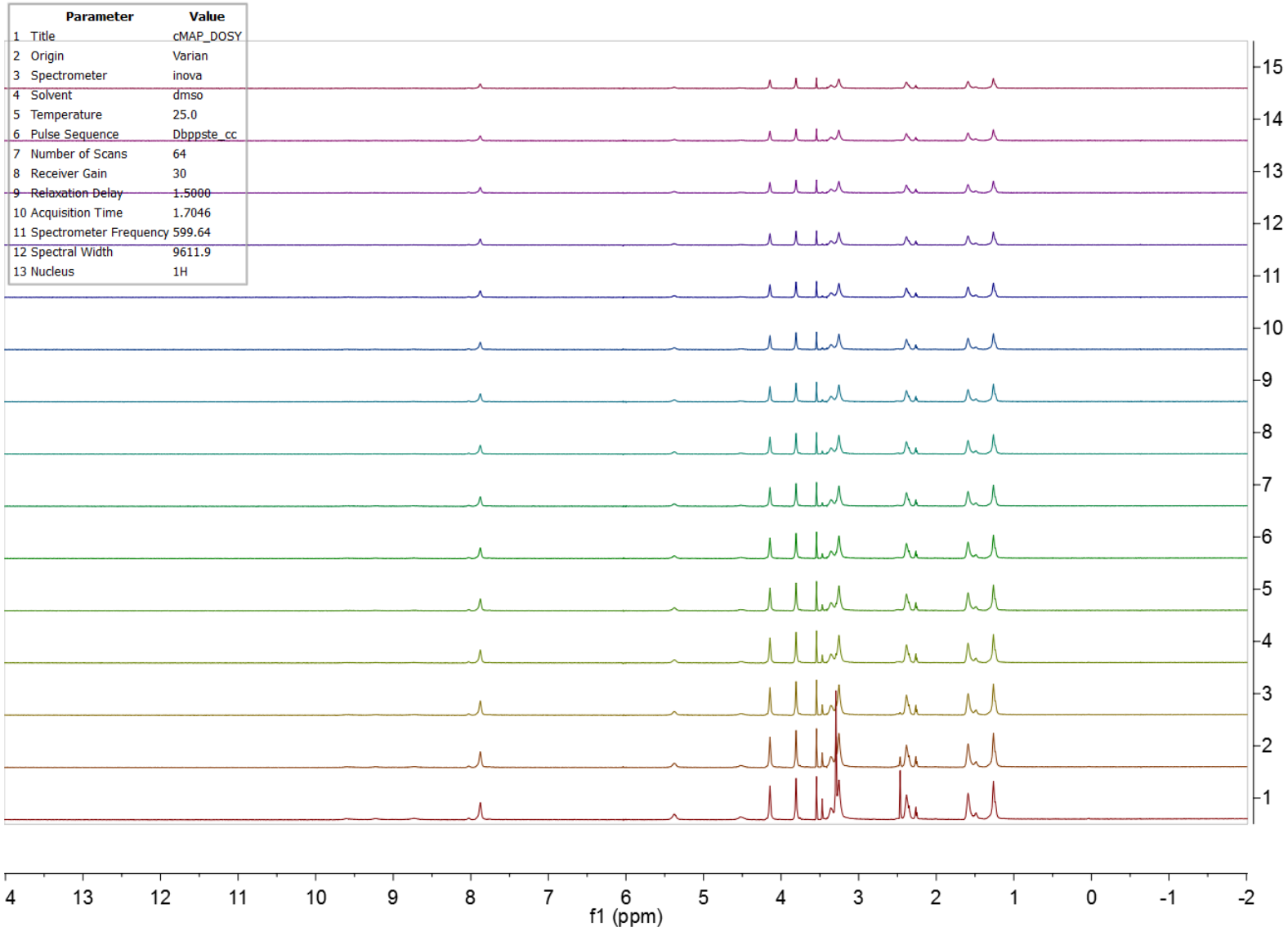


Table S7: 1H-13C HMBC NMR peak assignments for cMAP.

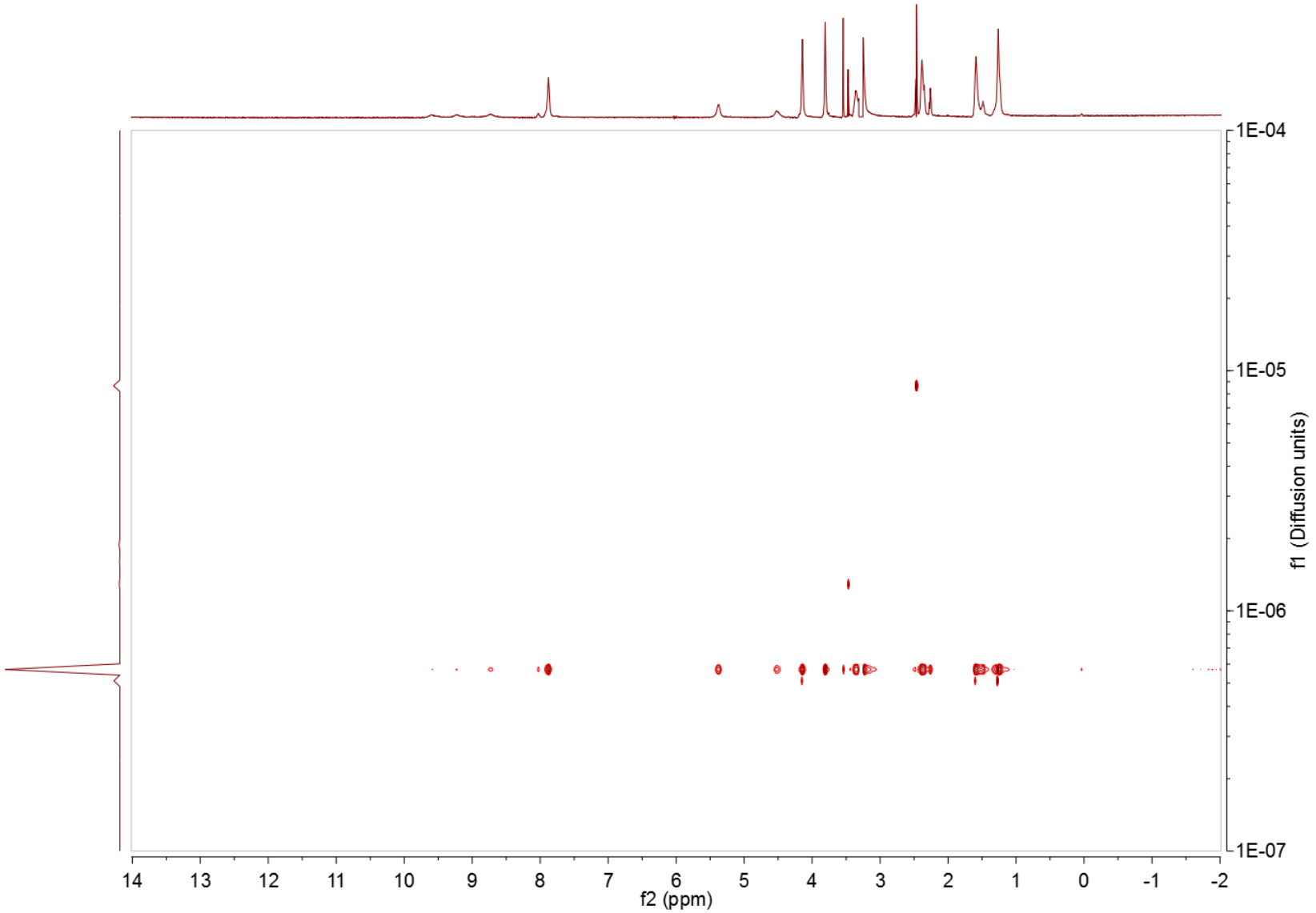
Correlation		13C Chemical Shift (ppm)	1H Chemical Shift (ppm)
Carbonyl Carbon (DMS hydrolyzed to carboxylate)	Methylene A (end group, DMS hydrolyzed to carboxylate)	174.53	2.04
Amide Carbonyl (mucic acid)	Methylene B (mucic acid)	174.36	3.38
Amide Carbonyl (mucic acid)	Hydroxyl B (mucic acid)	174.35	5.41
Amide Carbonyl (mucic acid)	Amide proton (mucic acid)	174.32	7.91
Amide Carbonyl (mucic acid)	Methylene A (mucic acid)	174.31	3.28
Amide Carbonyl (mucic acid)	Methyne B (mucic acid)	174.17	4.18
Methyl ester carbonyl (DMS)	Methoxy (DMS)	173.49	3.58
Methyl ester carbonyl (DMS)	Methylene A (end group, DMS)	173.48	2.30
Methyl ester carbonyl (DMS)	Methylene B (end group, DMS)	173.44	1.52
Amide Carbonyl (end group)	Methylene B (end group, mucic acid)	171.09	2.38
Amidine Carbonyl	Methylene A (DMS)	167.84	2.41
Amidine Carbonyl	Methylene B (DMS)	167.84	1.63
Methyne B (mucic acid)	Methyne A (mucic acid)	70.86	3.84
Methyne B (mucic acid)	Hydroxyl B (mucic acid)	70.83	5.41
Methyne A (mucic acid)	Methyne B (mucic acid)	70.71	4.18
Methyne A (mucic acid)	Hydroxyl A (mucic acid)	70.71	4.56
Methylene A (mucic acid)	Methylene B (mucic acid)	41.80	3.39
Methylene A (mucic acid)	Methylene A (mucic acid)	41.77	3.27
DMSO	DMSO	39.71	2.50
Methylene B (mucic acid)	Methylene A (end group, mucic acid)	36.50	2.87
Methylene B (mucic acid)	Amide Proton (mucic acid)	36.42	7.91
Methylene B (mucic acid)	Methylene A (mucic acid)	36.42	3.30
Methylene A (end group, DMS)	Methylene B (end group, DMS)	33.33	1.53
Methylene A (DMS)	Methylene B (DMS)	32.24	1.63
Methylene A (DMS)	Methylene C (DMS)	32.24	1.30
Methylene B (end group, DMS)	Methylene A (end group, DMS hydrolyzed to carboxylate)	28.26	2.05
Methylene B (end group, DMS)	Methylene A (end group, DMS)	28.02	2.30
Methylene B (end group, DMS)	Methylene B (end group, DMS)	28.00	1.52
Methylene C (DMS)	Methylene C (DMS)	27.61	1.29
Methylene C (DMS)	Methylene A (DMS)	27.61	2.41
Methylene C (DMS)	Methylene B (DMS)	27.57	1.63
Methylene B (DMS)	Methylene C (DMS)	26.41	1.29
Methylene B (DMS)	Methylene A (DMS)	26.34	2.42
Methylene C (end group, DMS hydrolyzed to carboxylate)	Methylene A (end group, DMS hydrolyzed to carboxylate)	25.00	2.04
Methylene B (end group, DMS)	Methylene A (end group, DMS)	24.33	2.30

Figure S19: 1H stacked DOSY Spectrum of cMAP showing that all the peaks on the cMAP polymer, including the small end group peaks, diffuse at the same rate. H_2O and DMSO diffuse much faster as shown by their quick disappearance after the first spectrum (bottom).



Figure

Figure S20: 1H transformed DOSY Spectrum of cMAP showing that all the peaks on the cMAP polymer, including the small end group peaks, diffuse at the same rate. H_2O and DMSO diffuse much faster.



4. cMAP: End group ratios

Table S8: Ratios of amine: methoxy: carboxylate end groups in 8 batches of cMAP by comparing NMR integrations.

Batch	% amine	% methoxy	% carboxylate
11	52.52	33.96	13.52
12	38.45	54.15	7.40
13	48.58	45.89	5.53
14	47.07	45.71	7.22
15	47.75	51.17	1.08
16	55.58	37.14	7.28
17	63.22	29.92	6.86
18	38.76	39.50	21.75
Average	48.99 ± 2.93	42.18 ± 2.99	6.22 ± 2.20

5. cMAP-PEG copolymer: NMR spectra

Figure S21: 1H NMR of cMAP-PEG5k copolymer. The large resonance at 3.5 ppm corresponds to the PEG block.

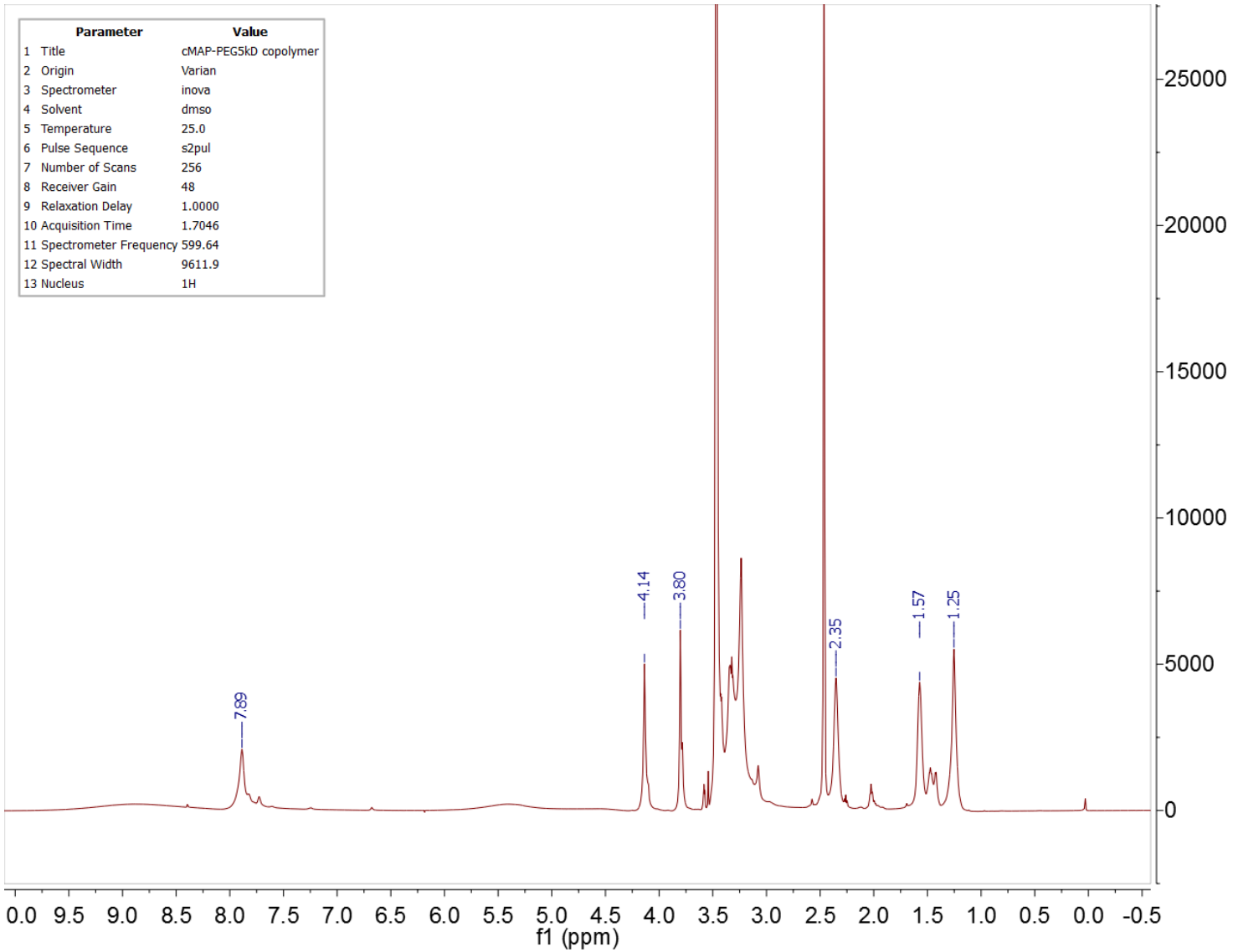


Figure S22: 1H-13C HSQC spectrum of cMAP-PEG5k copolymer.

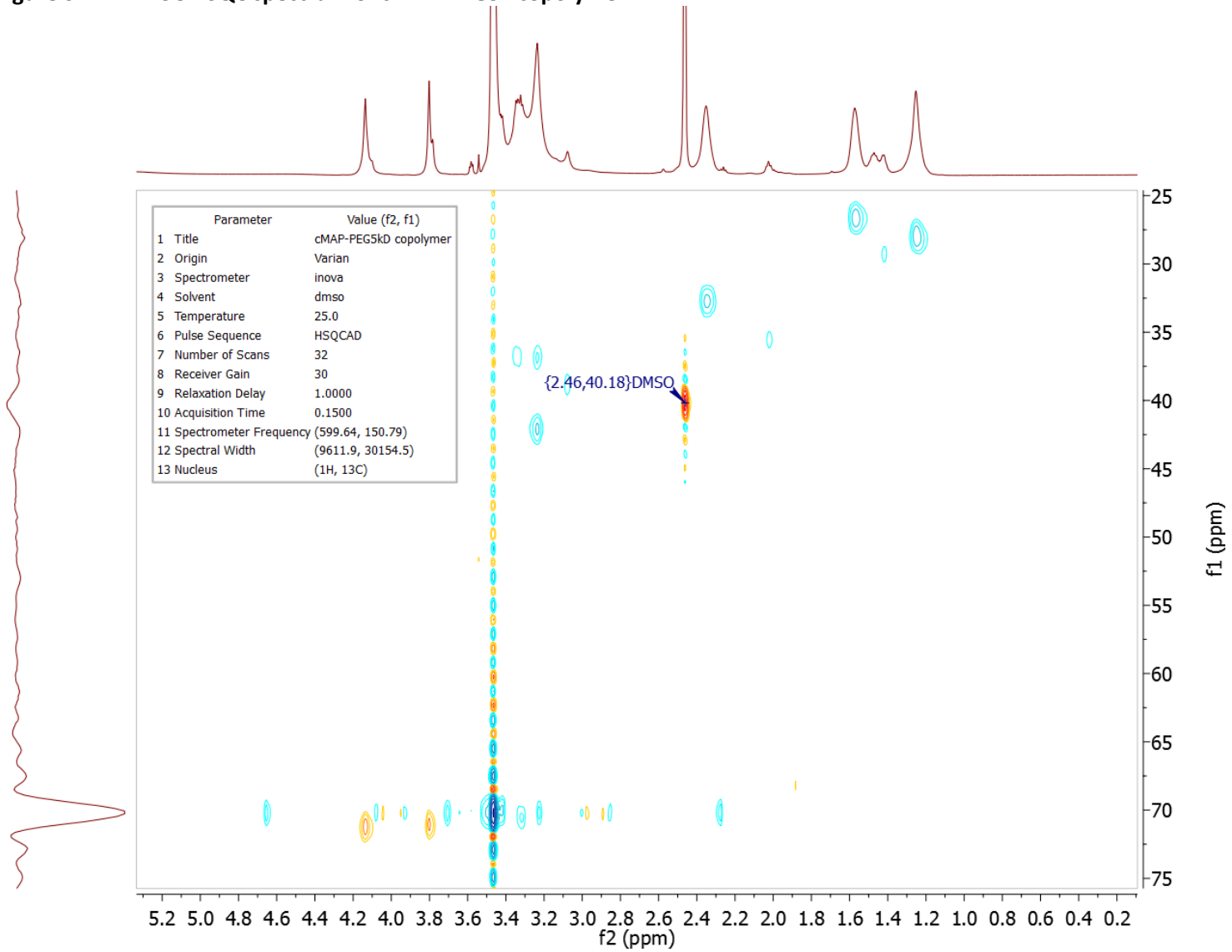


Figure S23: 1H DOSY transformed spectrum of cMAP-PEG5k copolymer.

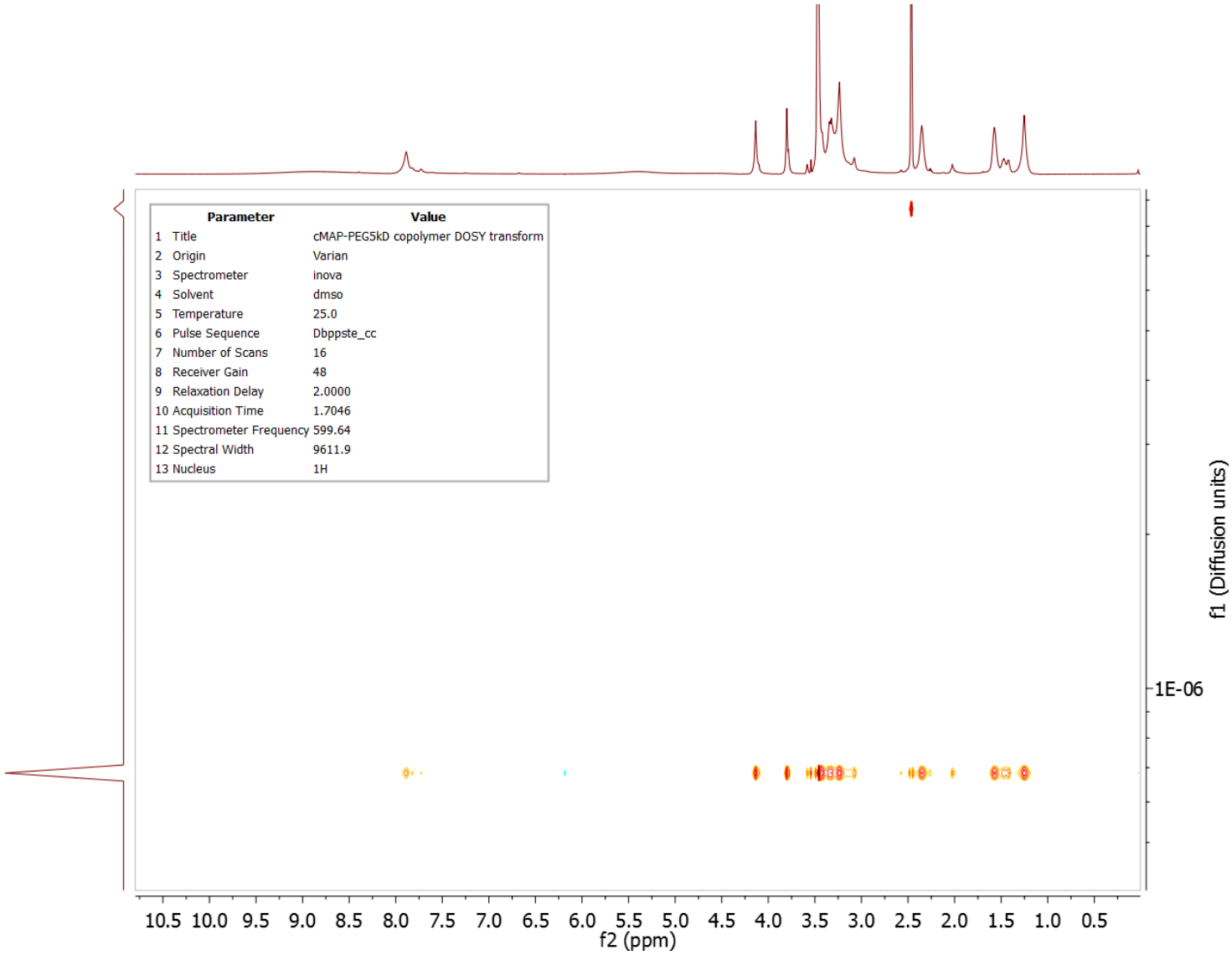


Figure S24: 1H NMR of cMAP-PEG3.4k copolymer.

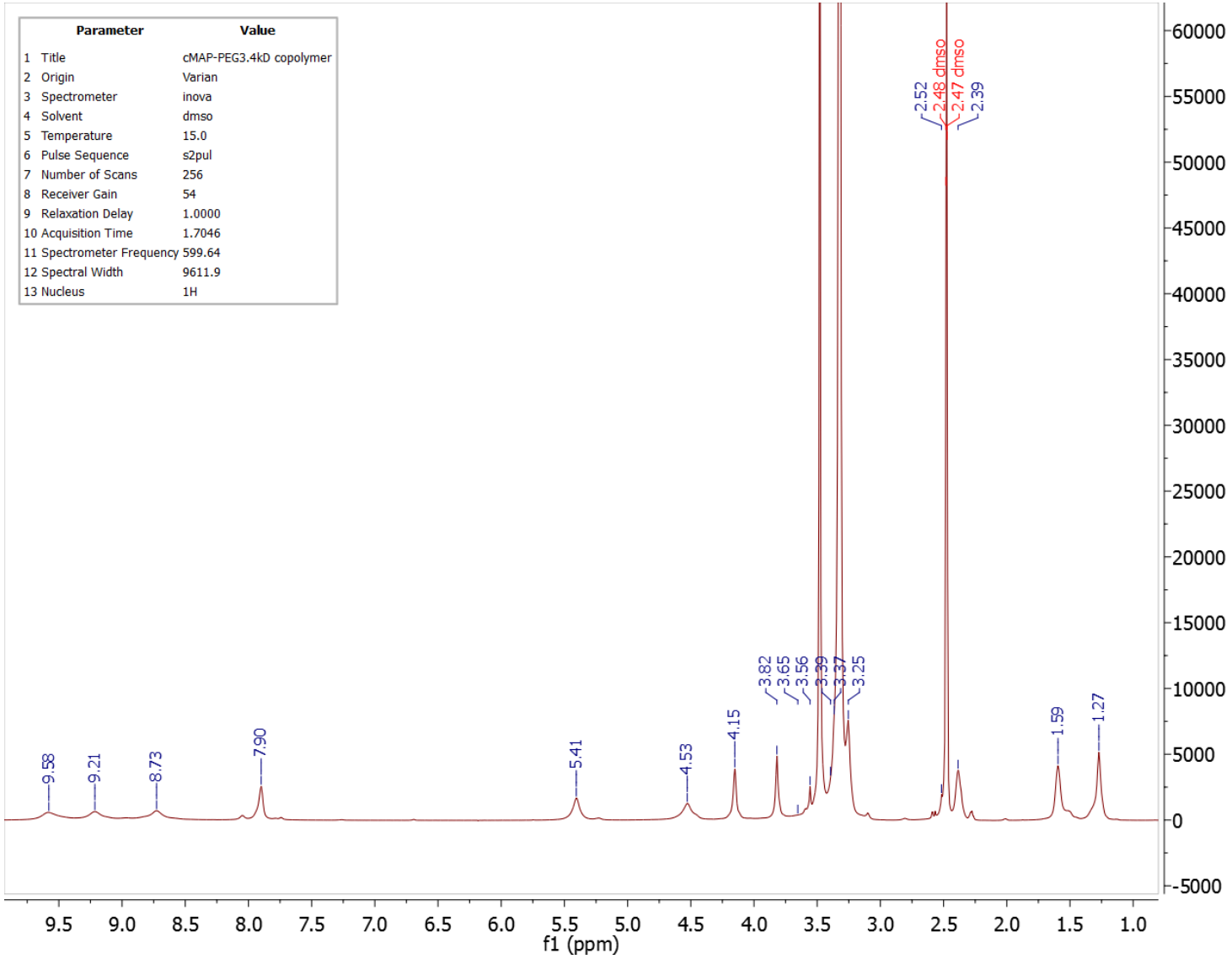


Figure S25: 13C NMR of cMAP-PEG3.4k copolymer.

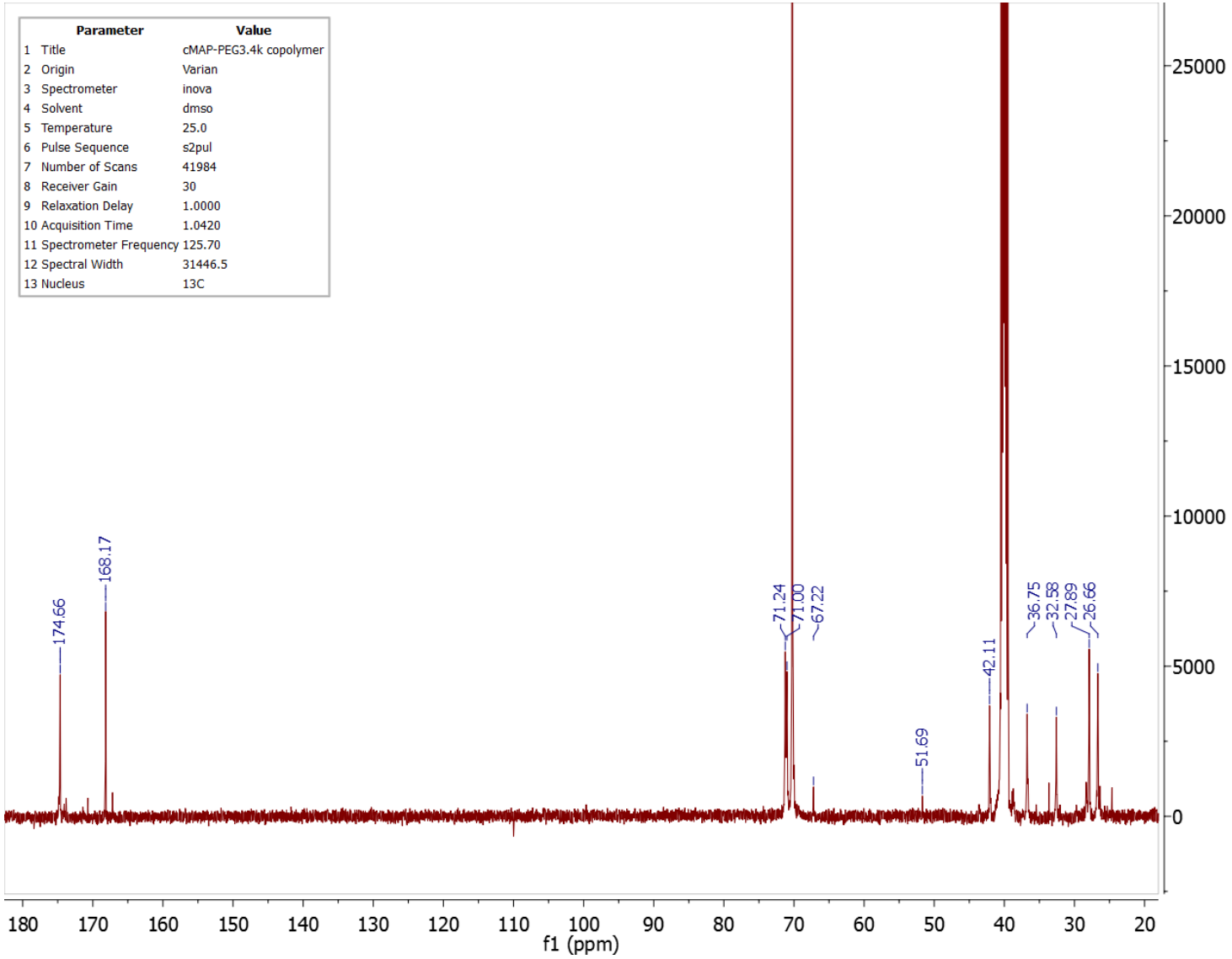


Figure S26: 1H-13C HSQC spectrum of cMAP-PEG3.4k copolymer.

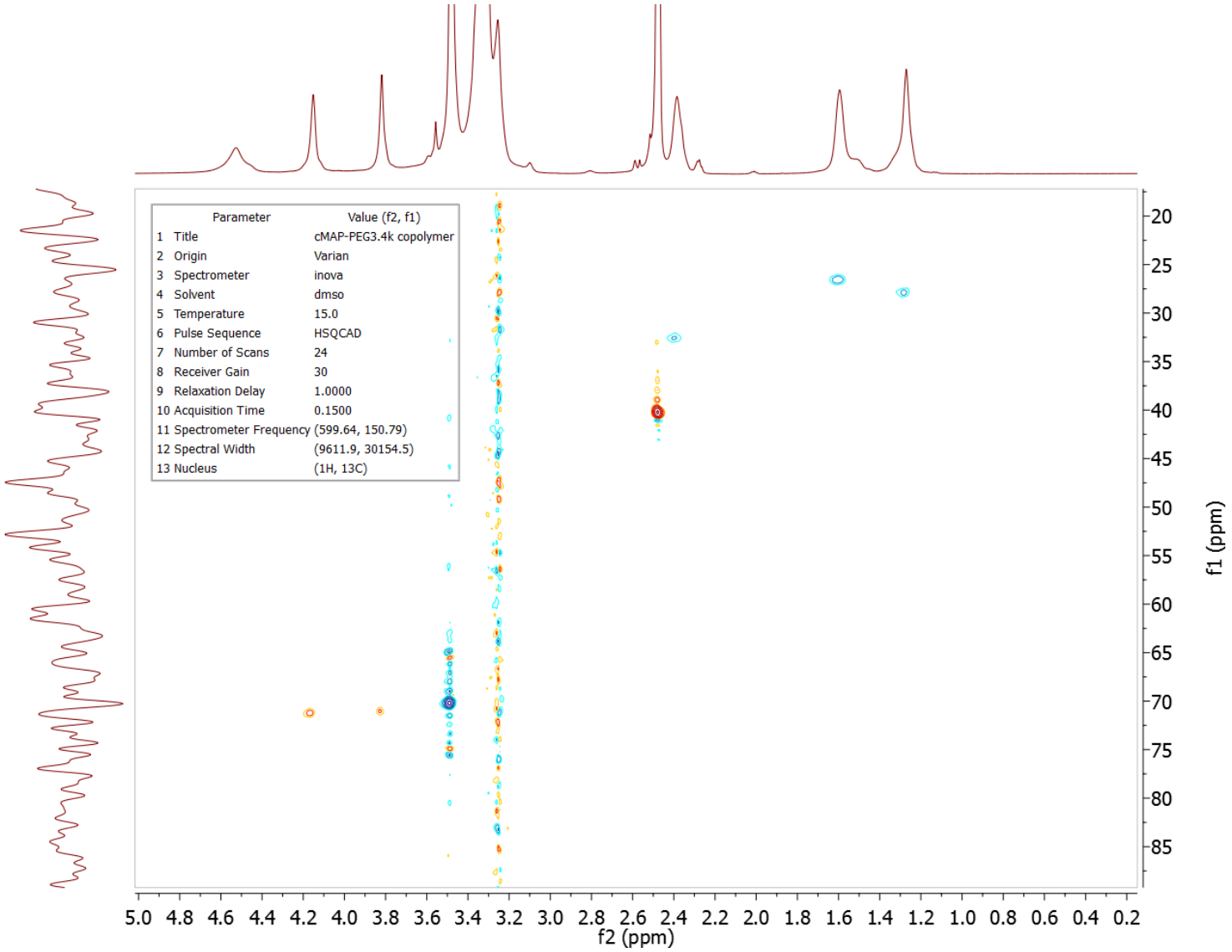
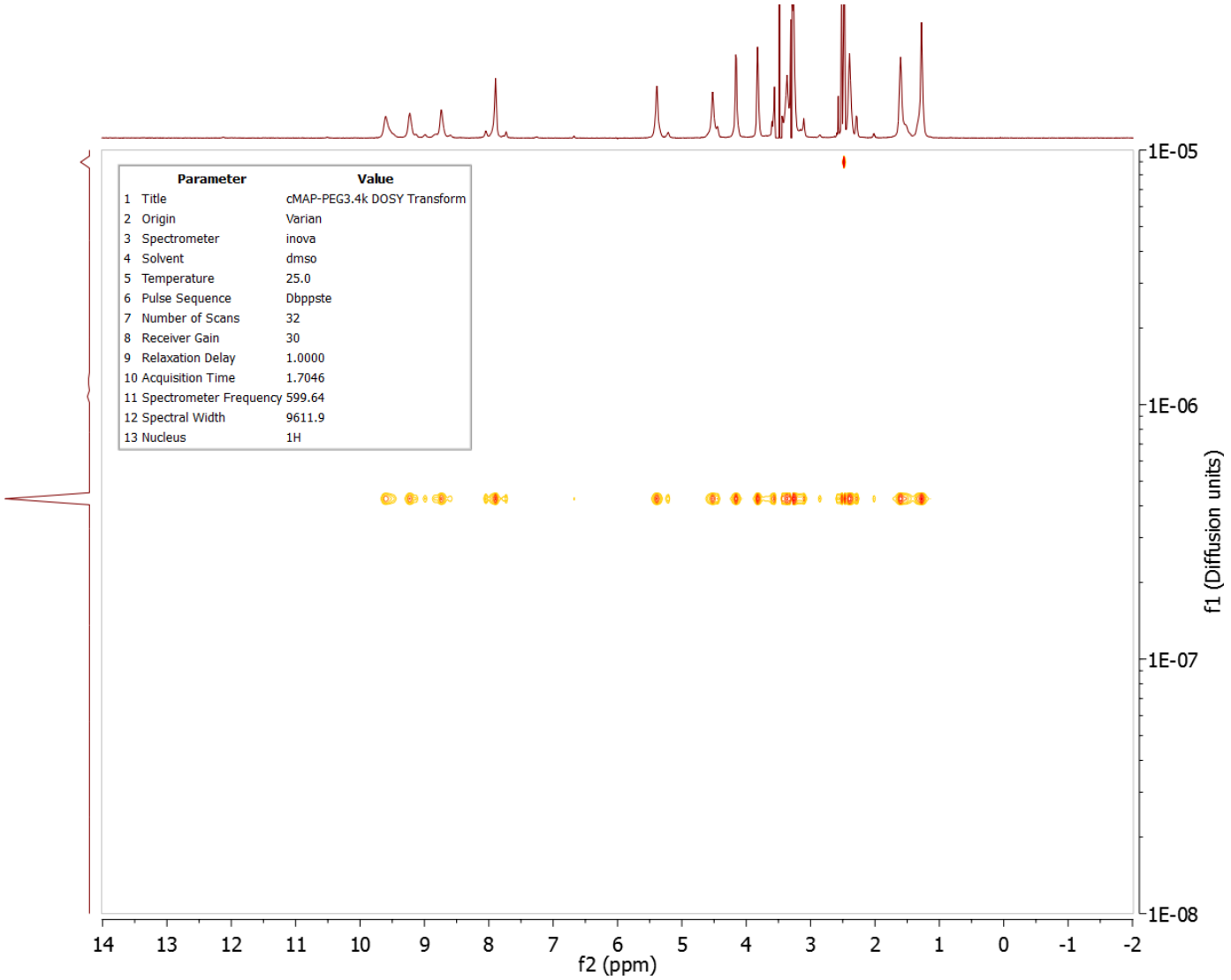


Figure S27: 1H DOSY transformed spectrum of cMAP-PEG3.4k copolymer.



5. cMAP-PEG copolymer fractionation yields

A reaction was started with 50 mg of cMAP and 16.5 mg di-SPA-PEG3.4k. After stirring for 24 hours, the reaction was diluted in water and filtered through sequentially smaller molecular weight cutoff (MWCO) Amicon centrifugal spin filters. Some material loss does occur onto the filter membrane and during transfer steps, but the retained material on each of these filters after lyophilization to dryness is shown in the table below. A significant amount of high molecular weight cMAP-PEG3.4k copolymer is formed in the synthesis due to the presence of diamine end groups on cMAP.

Table S9: Retained mass on each MWCO filter after fractionating crude cMAP-PEG3.4k copolymer.

MWCO (kD)	Mass (mg)	Comment
100	9.5	High molecular weight cMAP-PEG copolymer
50	3.4	High molecular weight cMAP-PEG copolymer
30	5	High molecular weight cMAP-PEG copolymer
20	12.3	
10	10.6	**Pure cMAP-PEG-cMAP triblock**
3	13.2	Unreacted cMAP, excess PEG
Total	54	

Similarly, a reaction was started with 50 mg of cMAP and 22.3 mg di-SVA-PEG5k. After stirring for 24 hours, the reaction was diluted in water and filtered through sequentially smaller molecular weight cutoff (MWCO) Amicon centrifugal spin filters. Some material loss does occur onto the filter membrane and during transfer steps, but the retained material on each of these filters after lyophilization to dryness is shown in the table below. A significant amount of higher molecular weight cMAP-PEG5k copolymer is formed in this synthesis similar to the analogous reaction above.

Table S10: Retained mass on each MWCO filter after fractionating crude cMAP-PEG5k copolymer.

MWCO (kD)	Mass (mg)	Comment
30	18.3	High molecular weight cMAP-PEG copolymer
20	20.8	
10	12	**Pure cMAP-PEG-cMAP triblock** + cMAP-PEG diblock
Total	51.1	

6. mPEG-cMAP-PEGm NMR spectra

Figure S28: 1H NMR of mPEG5k-cMAP-PEG5km. The resonance at 3.5 ppm corresponds to the PEG blocks, and the resonance at 3.2 ppm is that of the methoxy end group on PEGm.

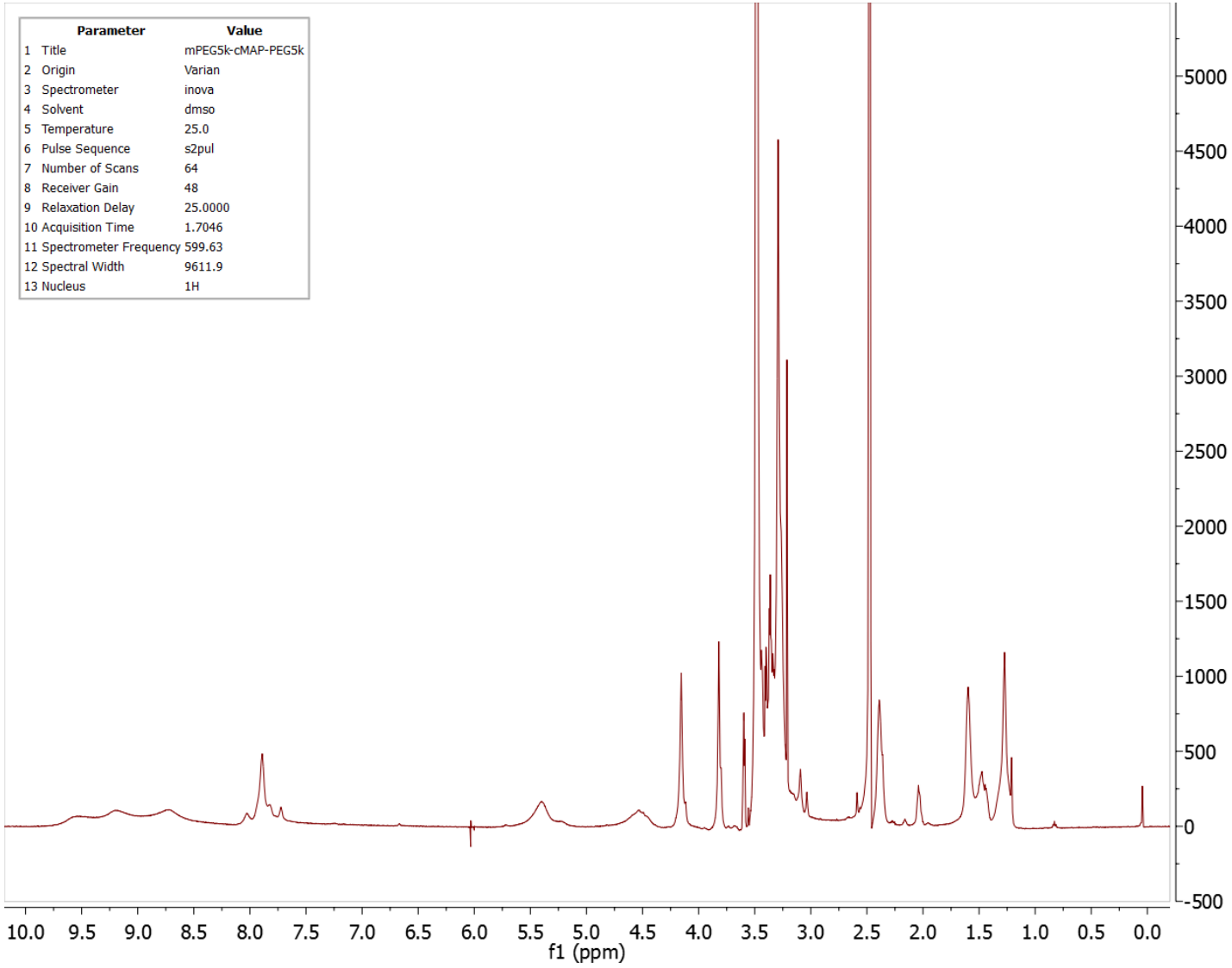


Figure S29: 1H-13C HSQC of mPEG5k-cMAP-PEG5km.

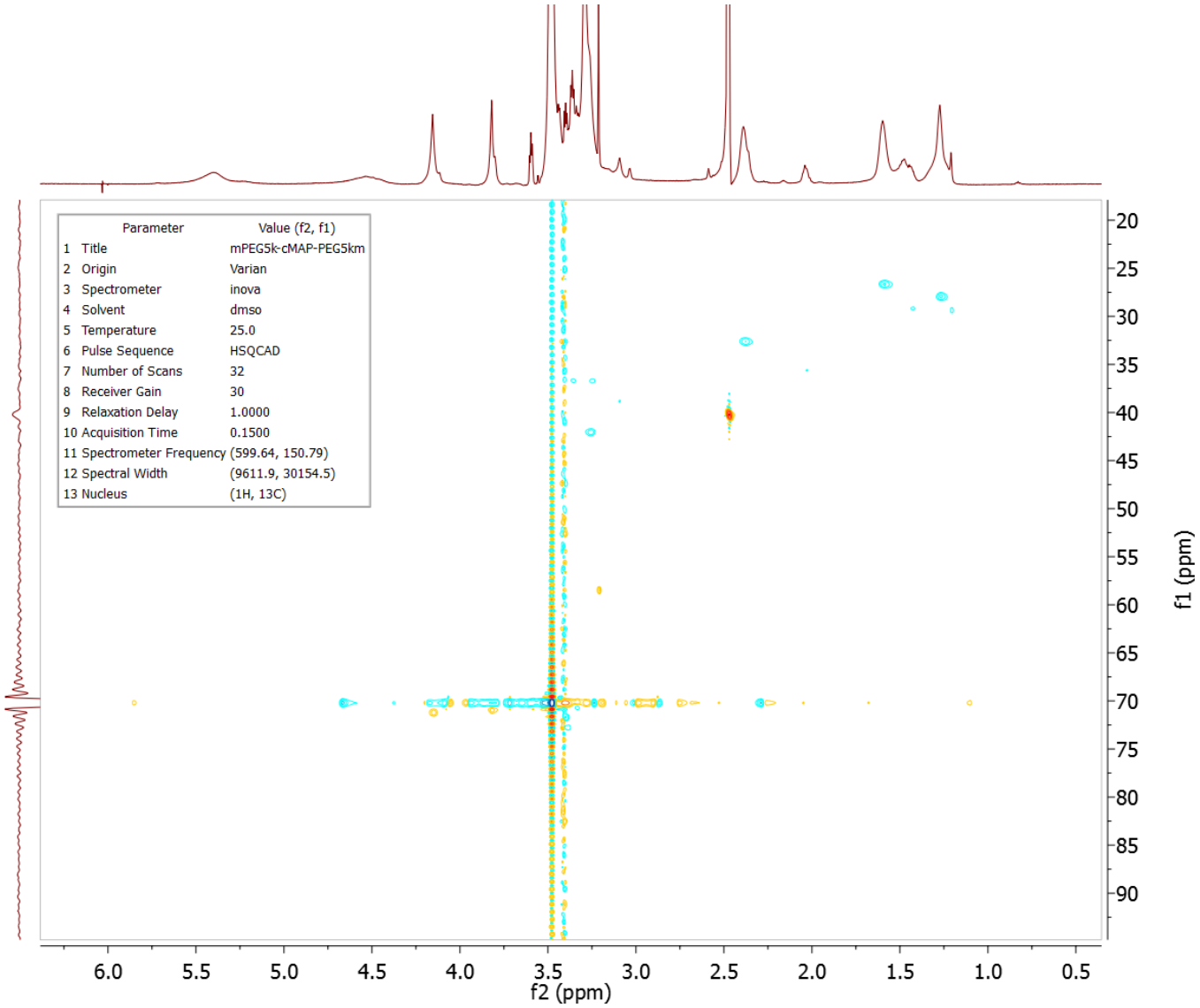


Figure S30: 1H DOSY transform of mPEG5k-cMAP-PEG5km.

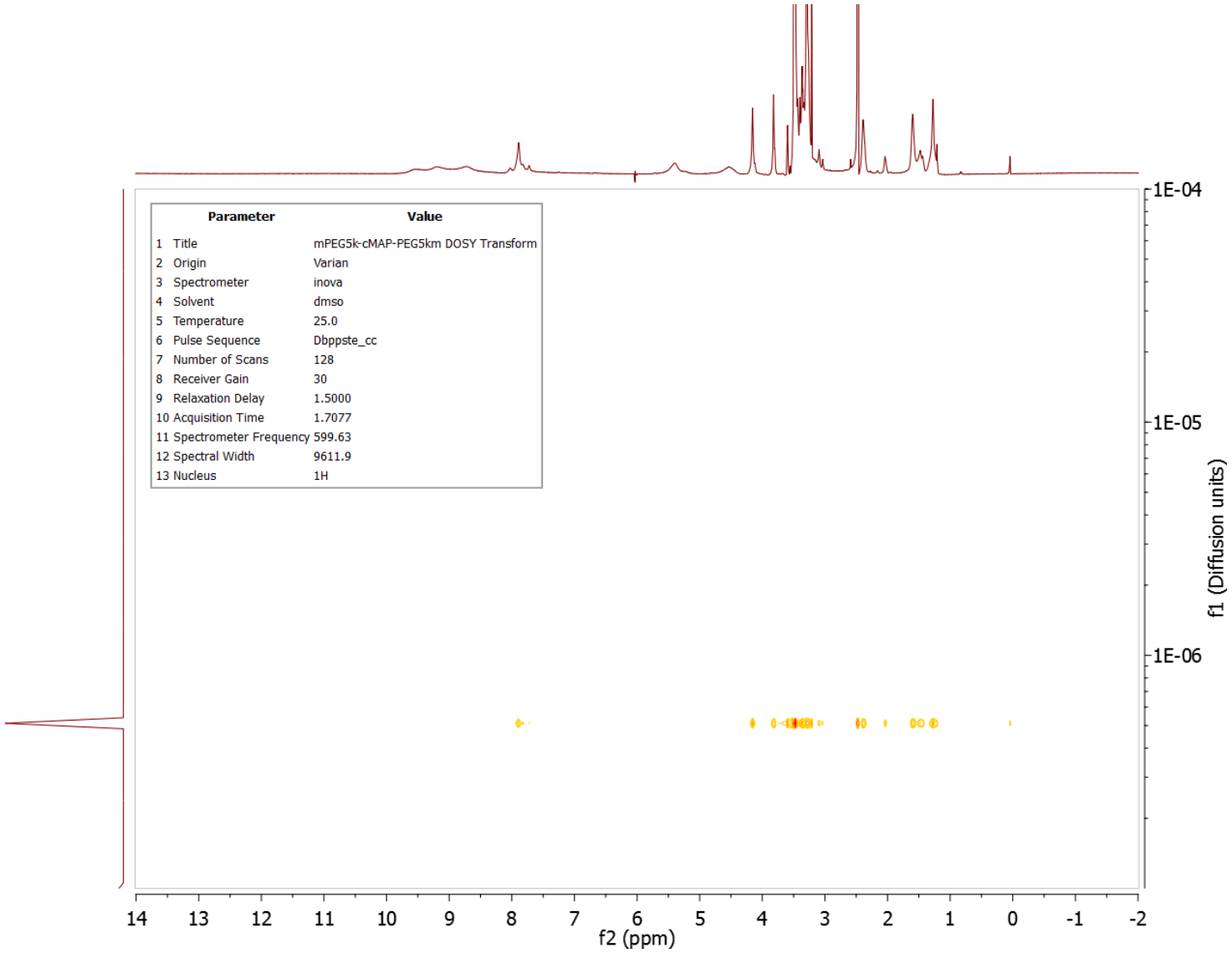


Figure S31: 1H NMR of mPEG2k-cMAP-PEG2km.

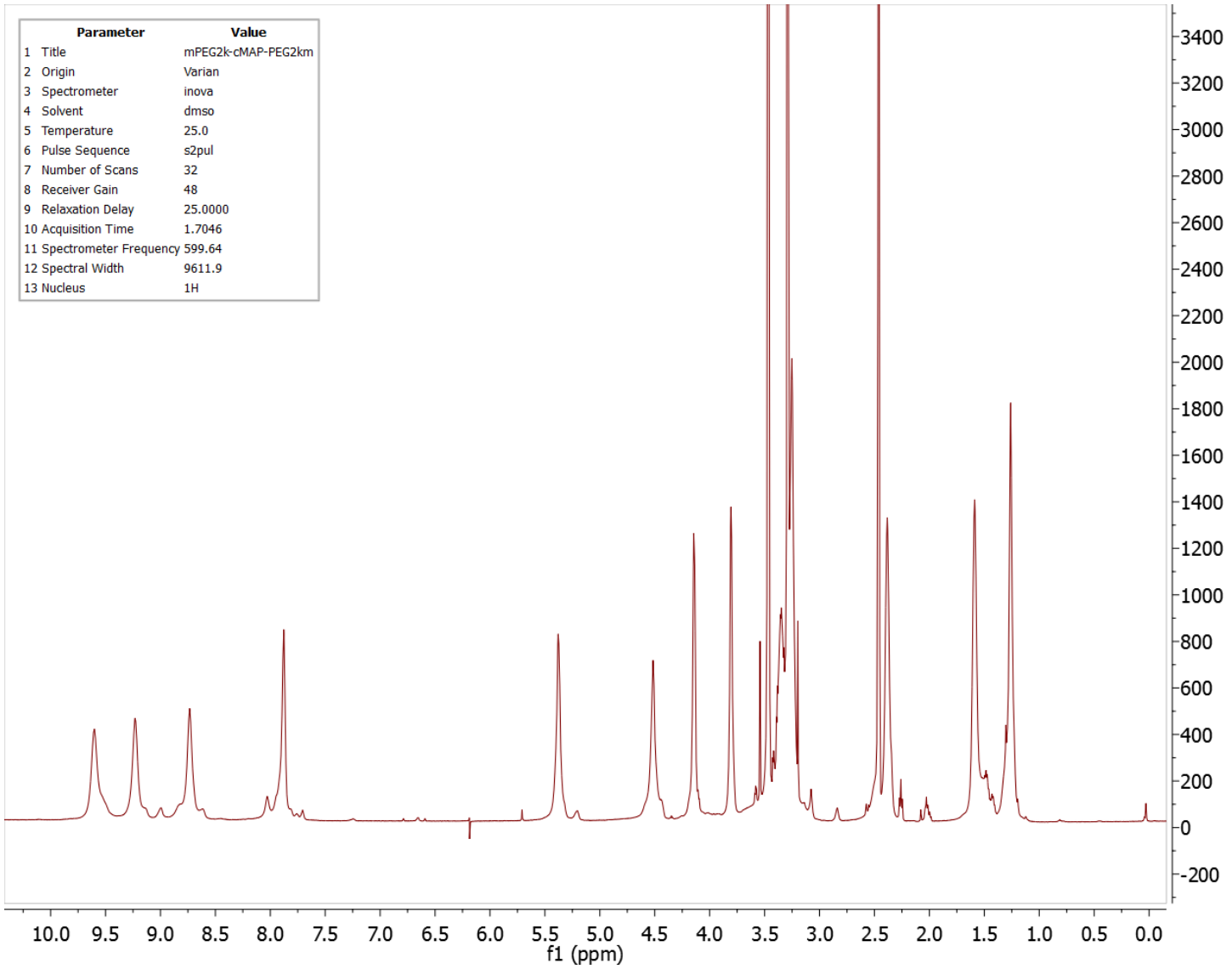


Figure S32: 1H-13C HSQC of mPEG2k-cMAP-PEG2km.

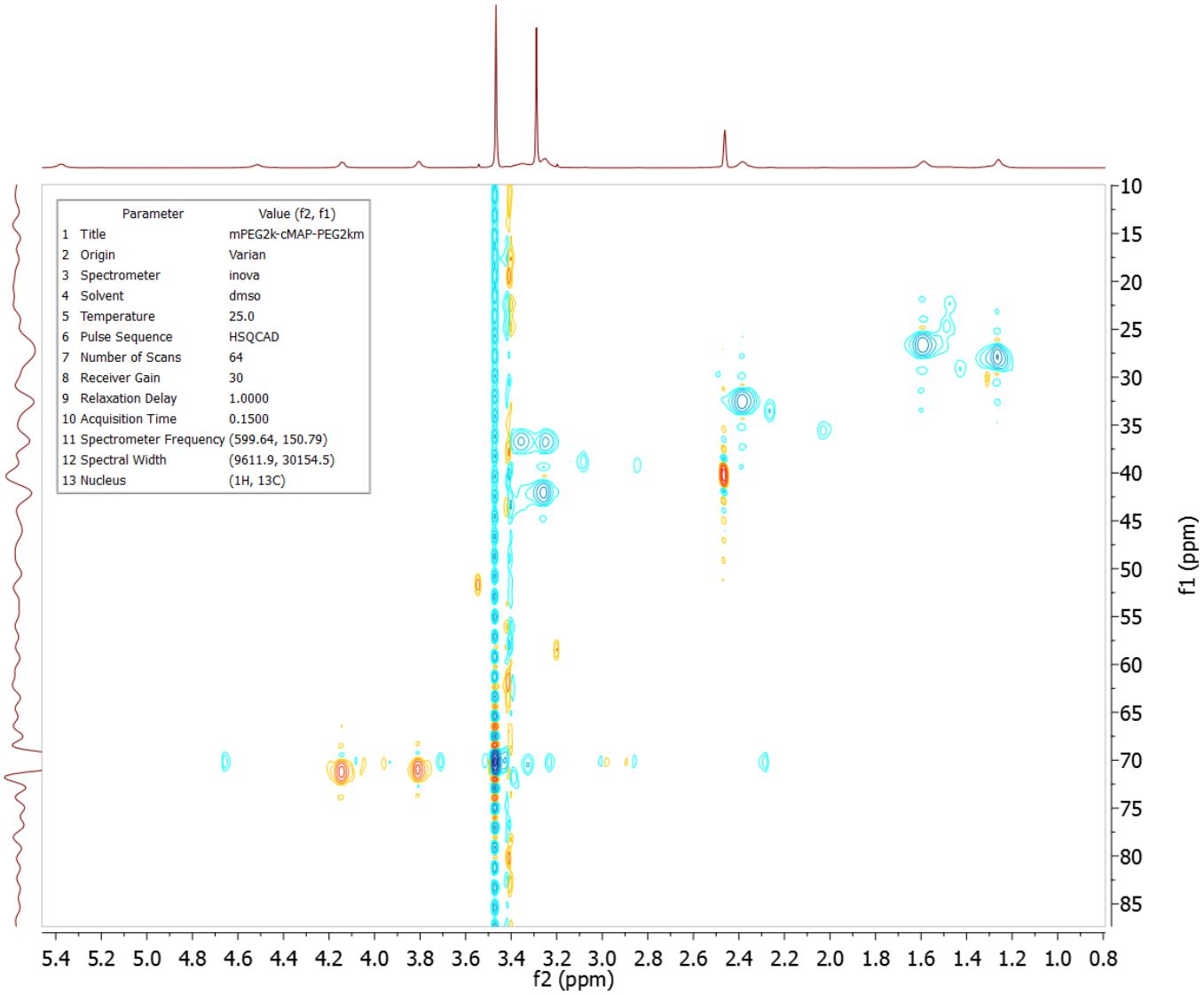
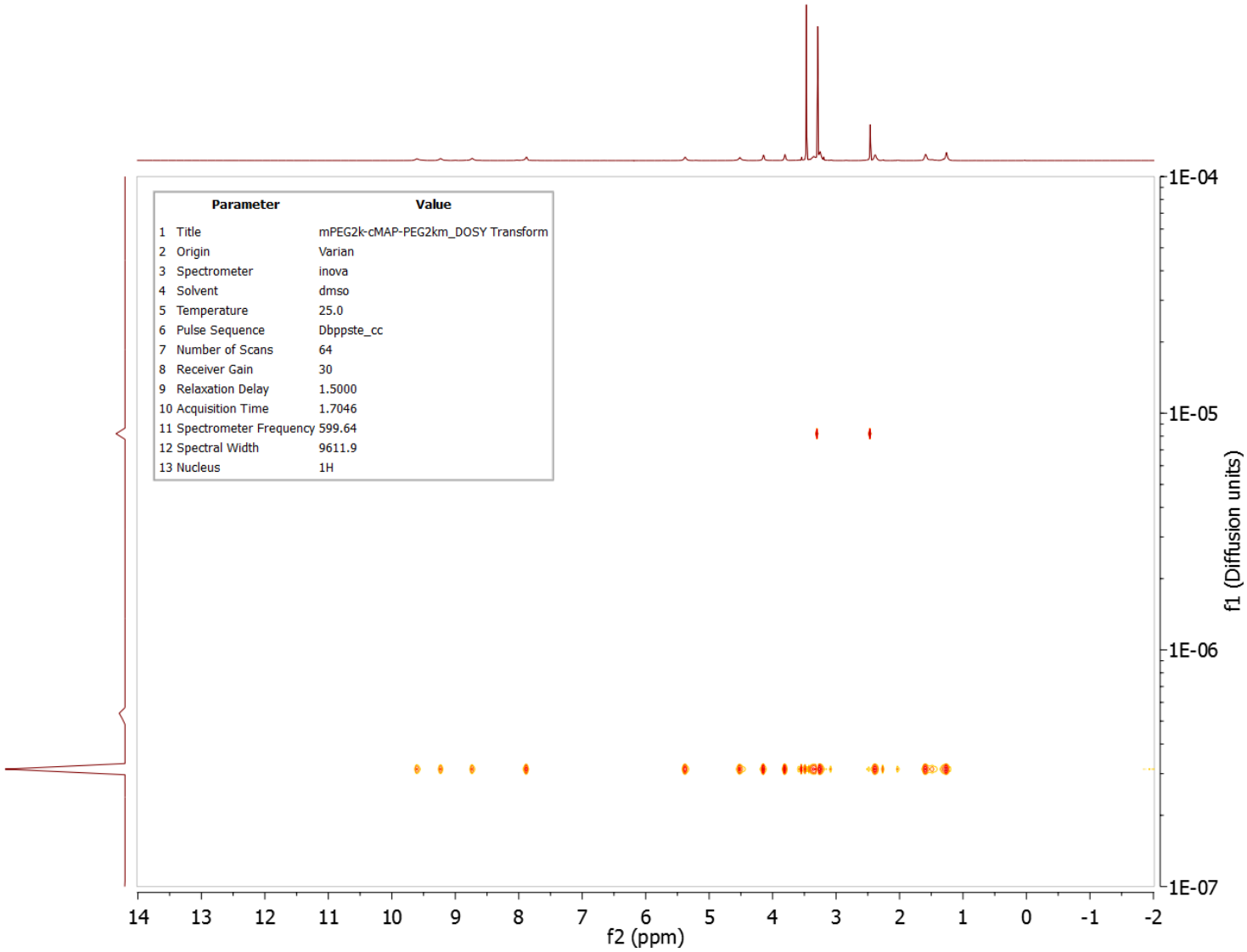


Figure S33: 1H DOSY transform of mPEG2k-cMAP-PEG2km.



7. 5-nitrophenylboronic acid-PEGm NMR and MALDI spectra

Figure S34: 1H NMR of 5-nPBA-PEGm.

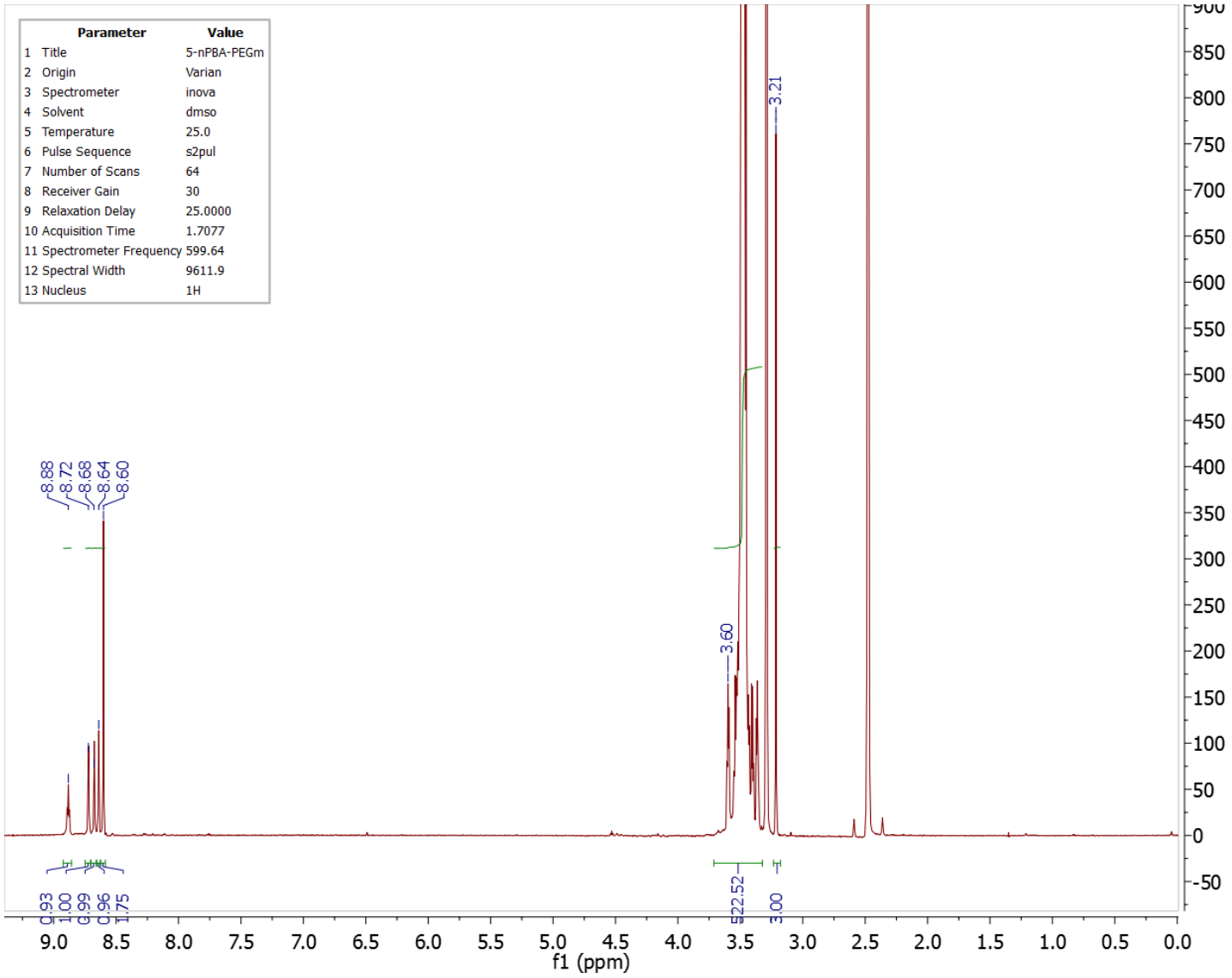


Figure S35: 1H DOSY of 5-nPBA-PEGm showing that the 5-nitrophenylboronic acid group is attached to PEGm by the downfield protons' persistence with PEG throughout the gradient sequence (compared to DMSO and H_2O, which disappear after the first gradients applied).

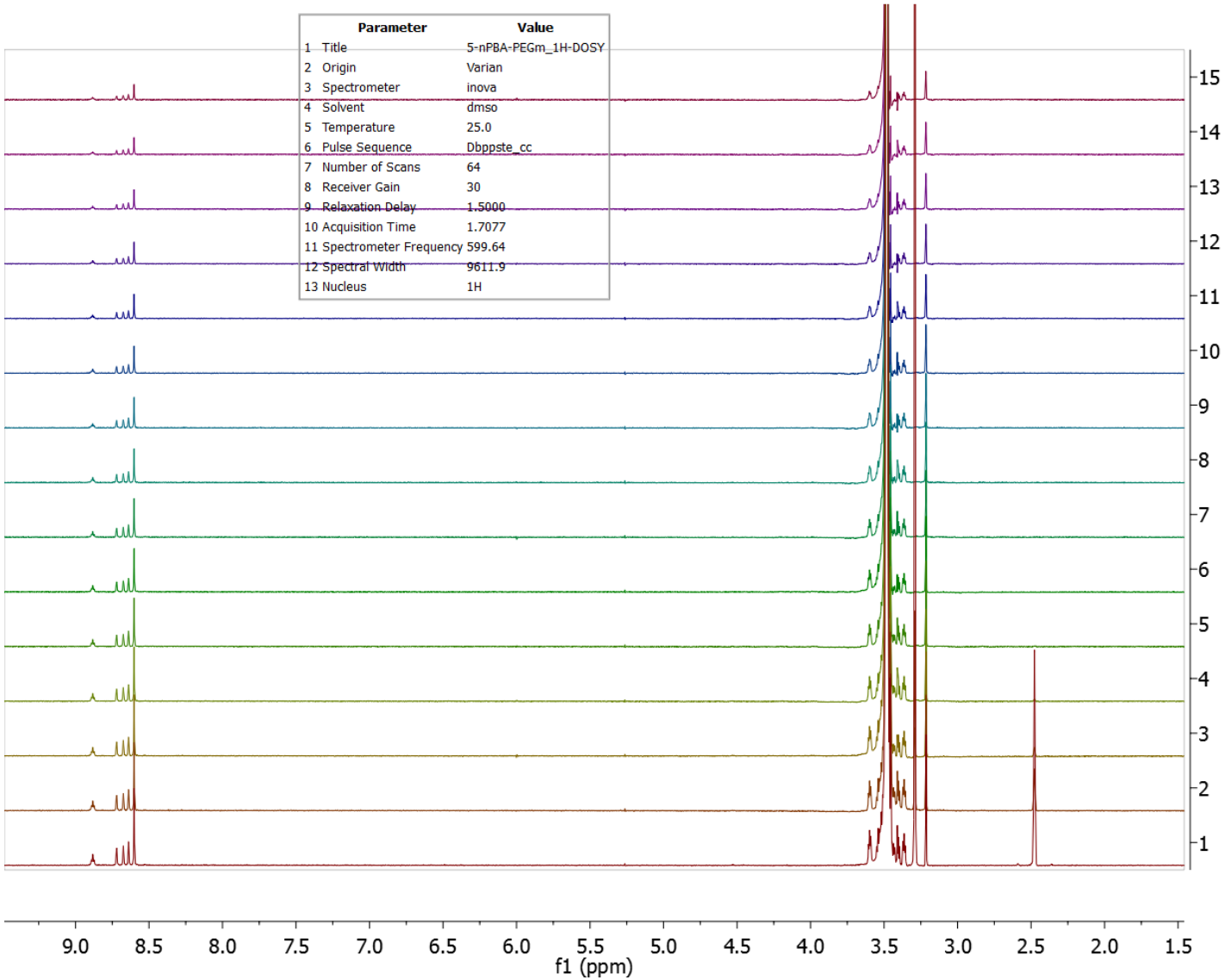


Figure S36: 1H DOSY transform of 5-nPBA-PEGm showing that the downfield 5-nitrophenylboronic acid peaks diffuse at the same rate as the PEG peaks.

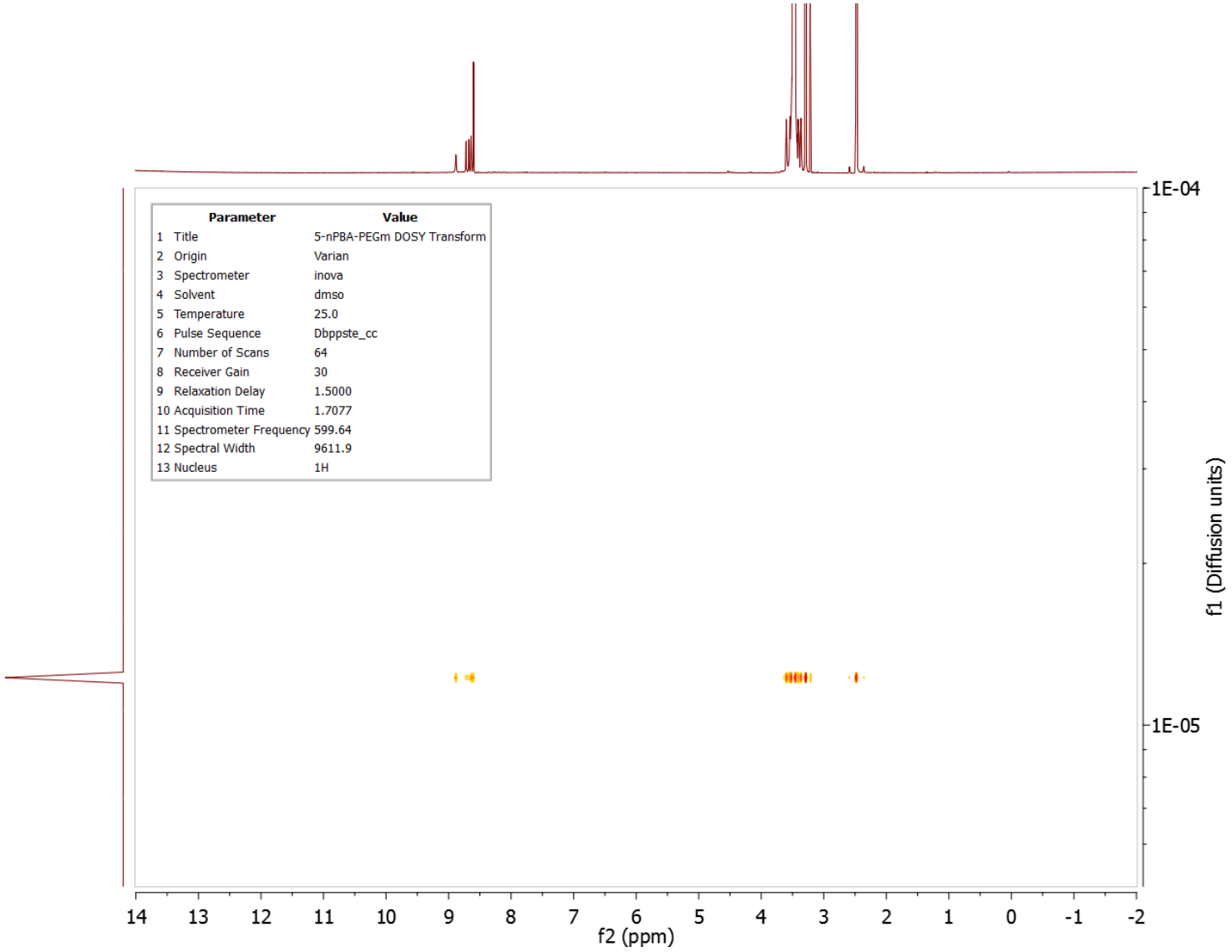


Figure S37: 11B NMR of 5-nPBA-PEGm. 11.26 ppm is the boronic acid peak. 19.54 ppm is boric acid (impurity).

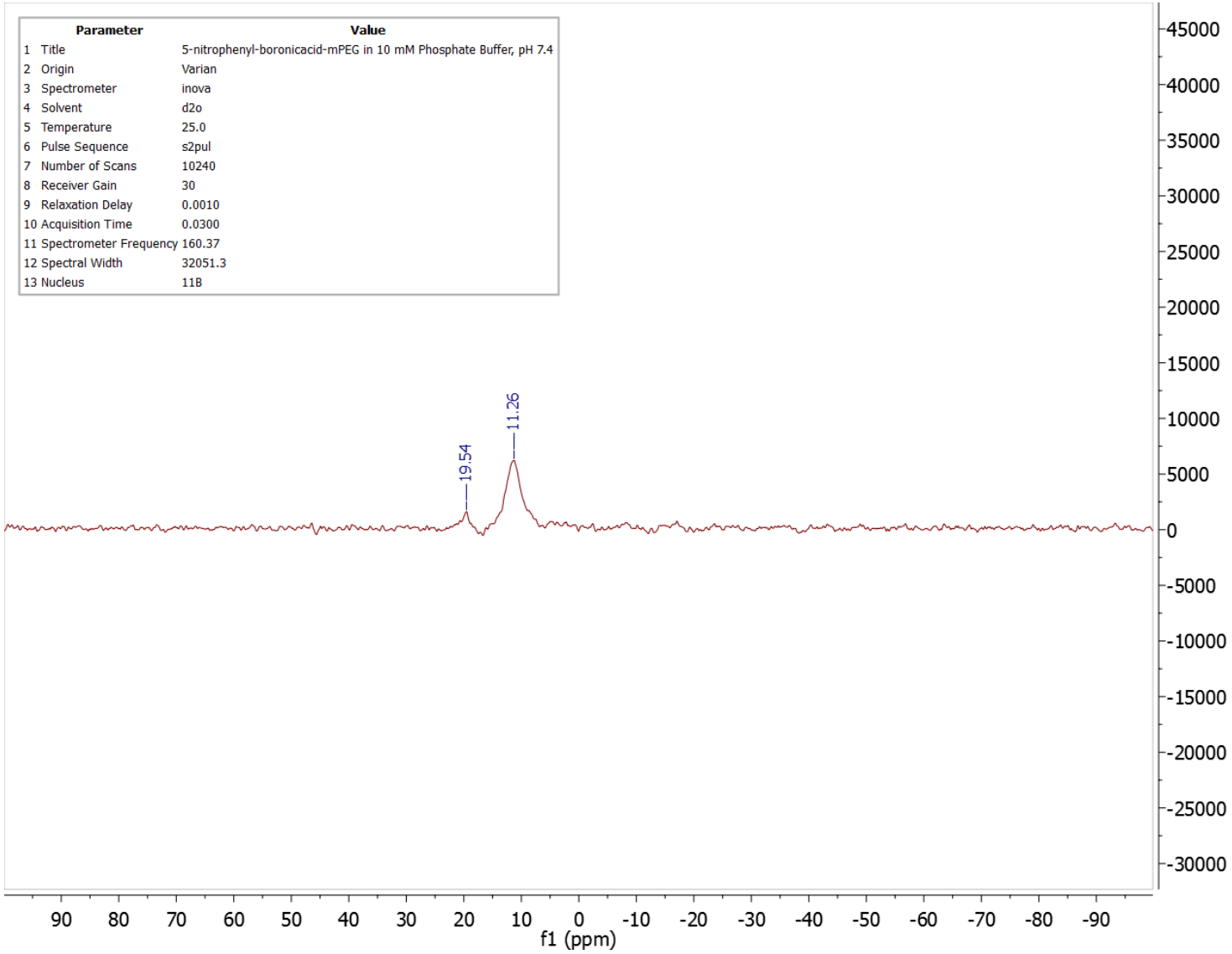
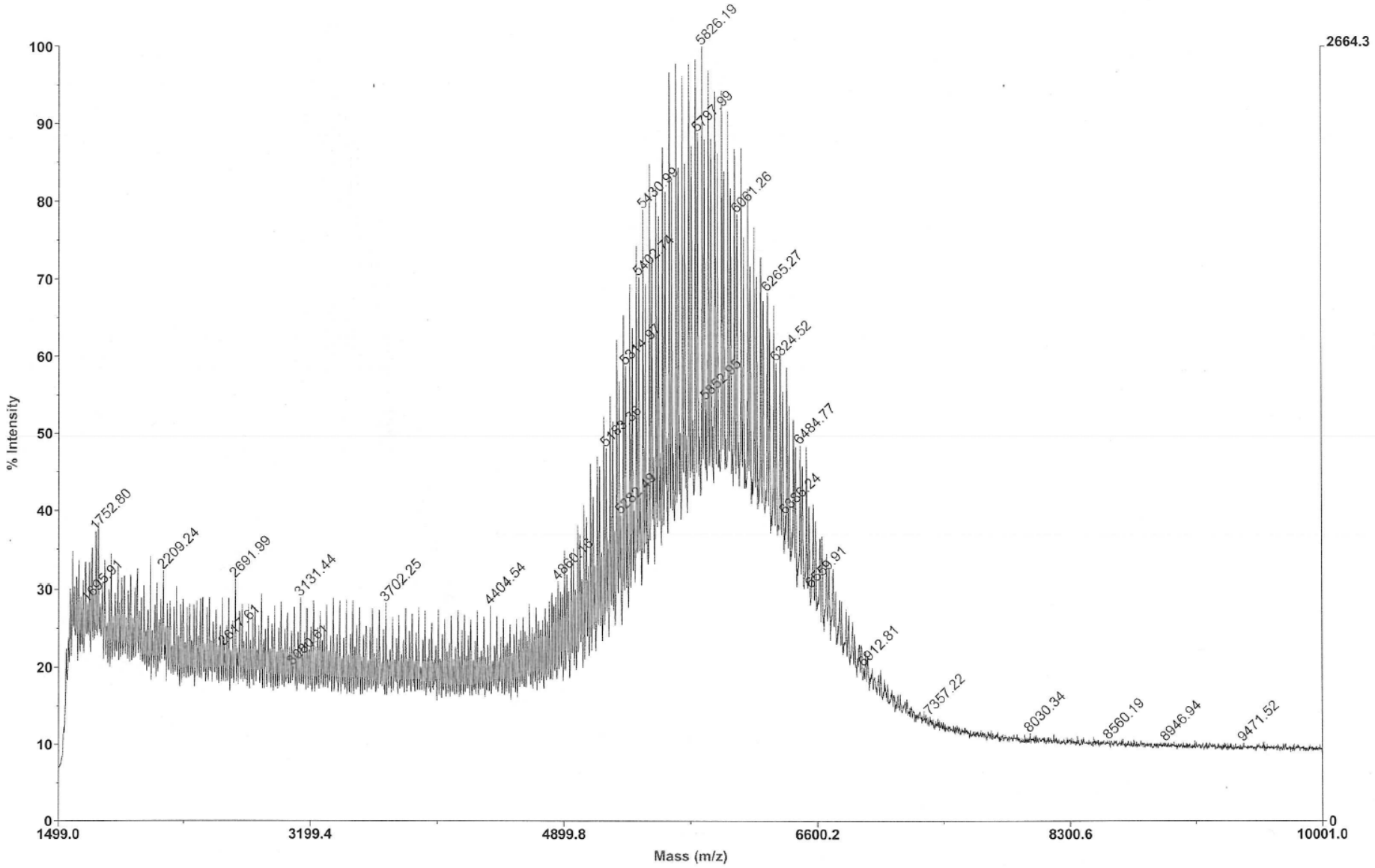


Figure S38: MALDI mass spectrum of 5-nPBA-PEGm.



8. GPC: cMAP individual batch MW.

Table S11: Gel Permeation Chromatography Analysis of cMAP Batches.

Sample	Mn	Mw	Mw/Mn (PDI)
cMAP-DP11	5323	6068	1.14
cMAP-DP12	5213	5839	1.12
cMAP-DP13	5936	6365	1.07
cMAP-DP14	5747	5940	1.03
cMAP-DP15	5050	5568	1.10
cMAP-DP16	6102	6357	1.04
cMAP-DP17	7712	8235	1.07
cMAP-DP18	7313	7524	1.03
cMAP-DP19	8353	8984	1.08

Values are reported as the average of 3 runs.

9. GPC: other PEG length copolymer and triblock MW.

Table S12: GPC Analysis of cMAP copolymers and triblocks (other than 5 kD PEG reported in main article text).

Polymer	dn/dc (mL/g)	Mn (kD)	Mw (kD)	PDI (Mw/Mn)
cMAP-PEG3.4k Copolymer	0.1660	128.30	289.25	2.27
cMAP-PEG3.4k -cMAP	0.1660 used (not for pure triblock)	12.89	14.20	1.10
cMAP-PEG5k-cMAP	0.1660 used (not for pure triblock)	24.17	26.84	1.11
mPEG2k-cMAP-PEG2km	0.1654	9.75	9.81	1.01

10. siRNA encapsulation: Gel Retardation images

Figure S39. cMAP gel retardation showing complete siRNA encapsulation at a charge ratio of 1+/-.

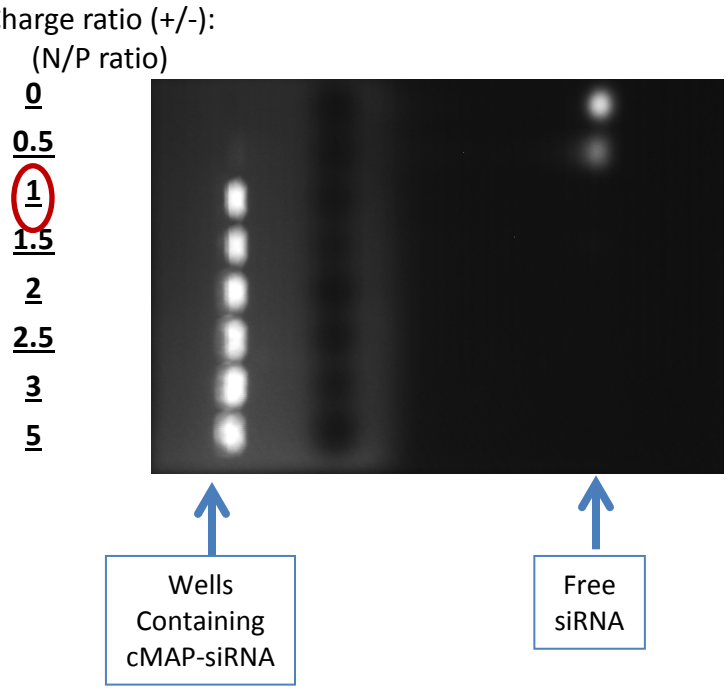


Figure S40. cMAP-PEG3.4k copolymer gel retardation assay showing siRNA encapsulation at a charge ratio of 1+/- .

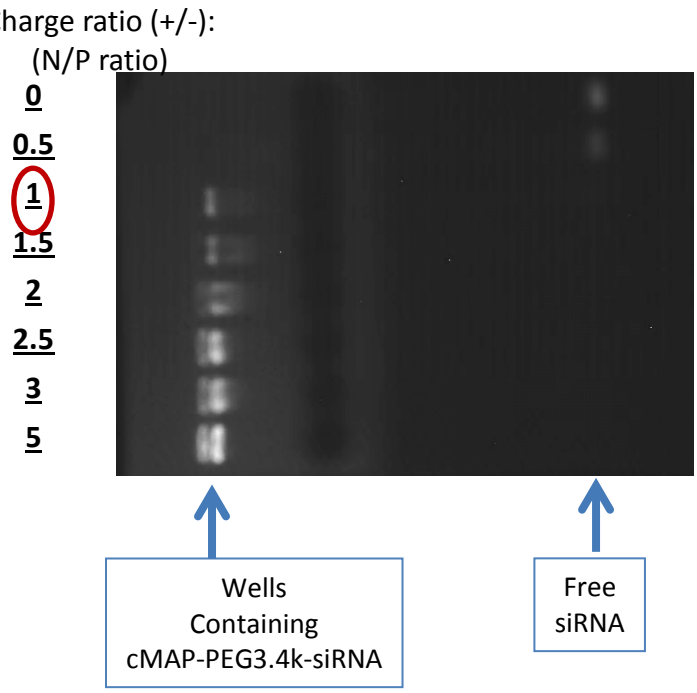
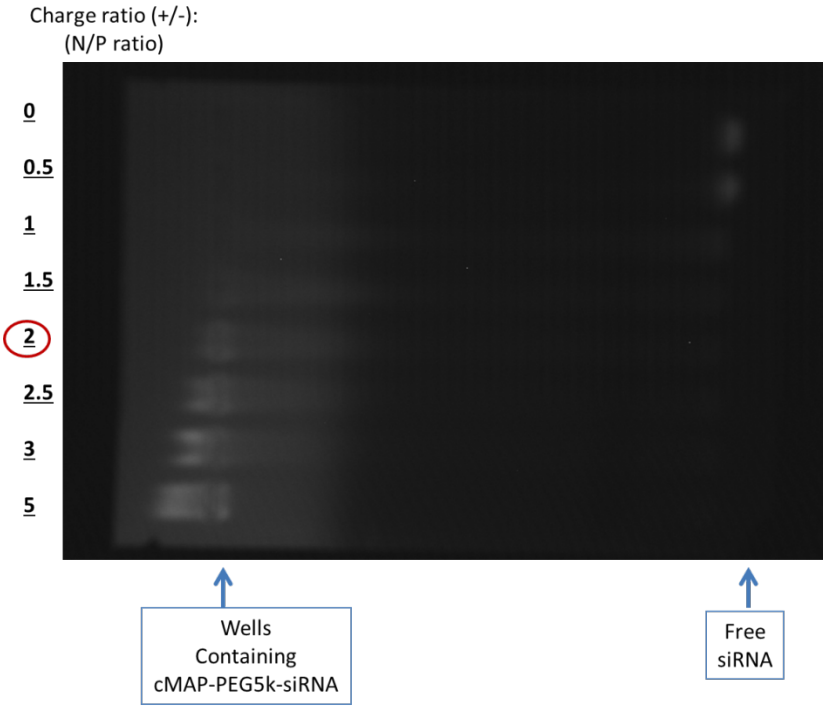


Figure S41. cMAP-PEG5k copolymer gel retardation assay showing siRNA encapsulation at a charge ratio of 2+/- .



11. siRNA encapsulation: RiboGreen comparing PEG lengths for copolymer and triblock

Figure S42. cMAP-PEG copolymer RiboGreen assay showing siRNA encapsulation by a charge ratio of 3+/- for 3.4k and 5k PEG blocks.

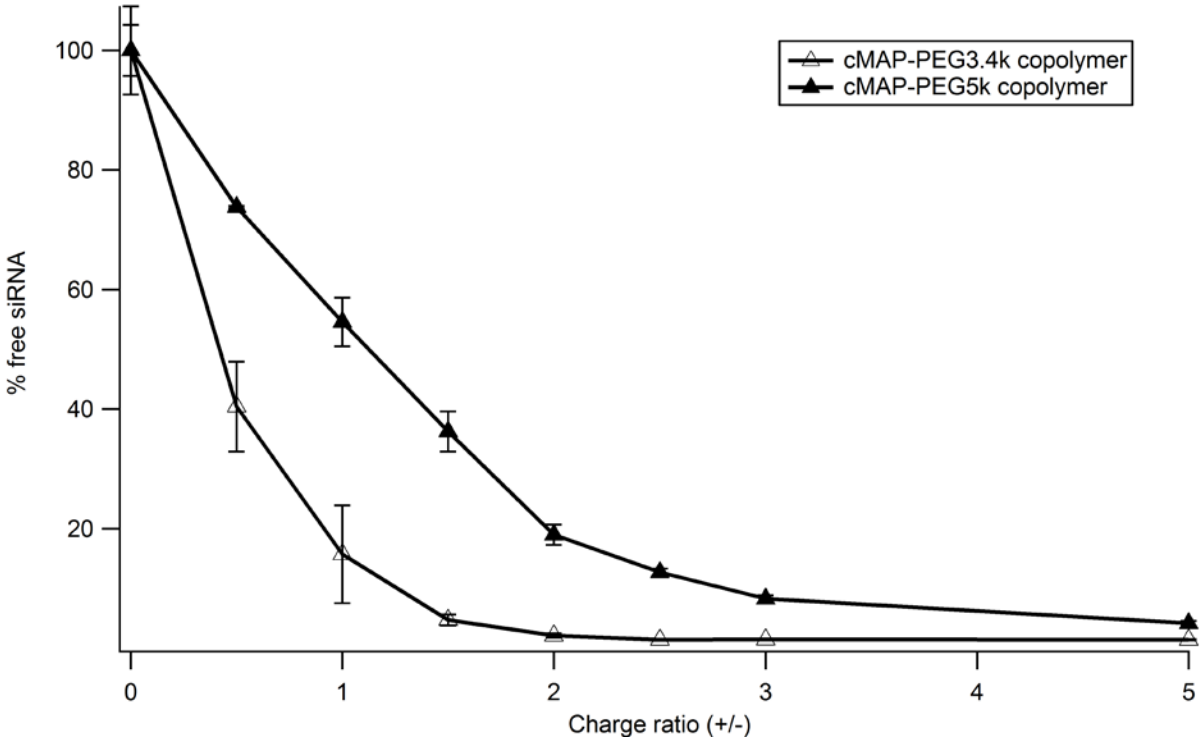
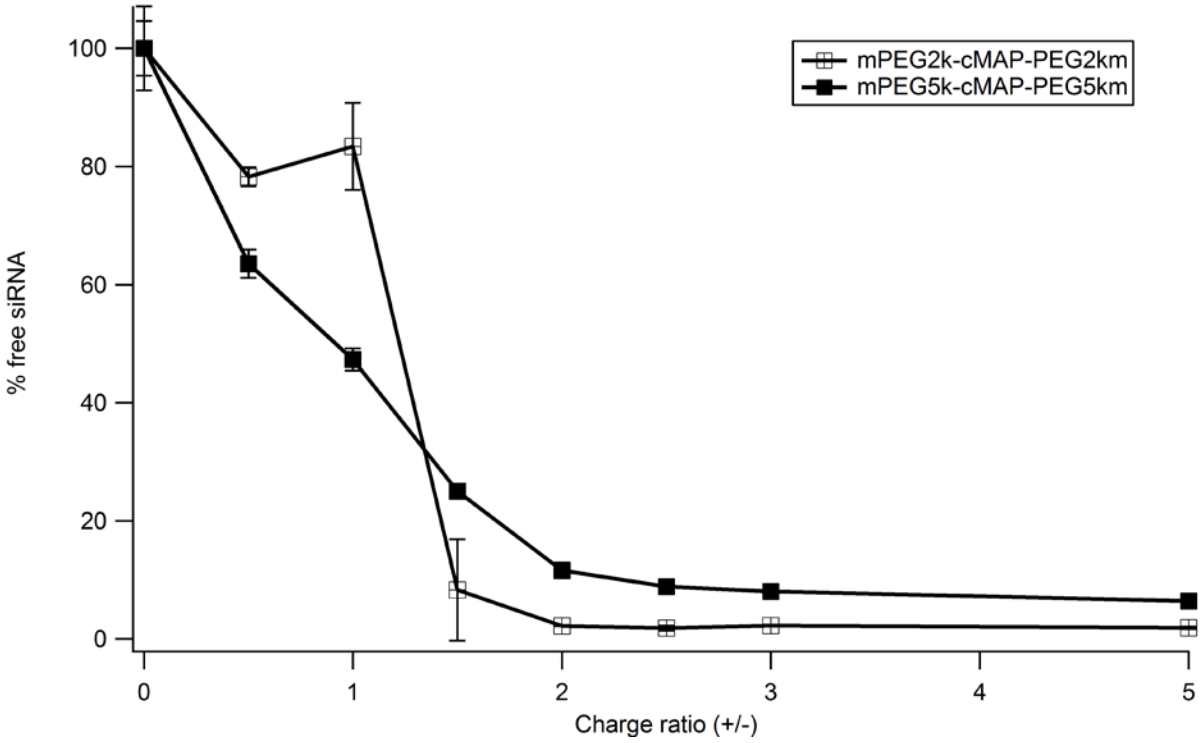
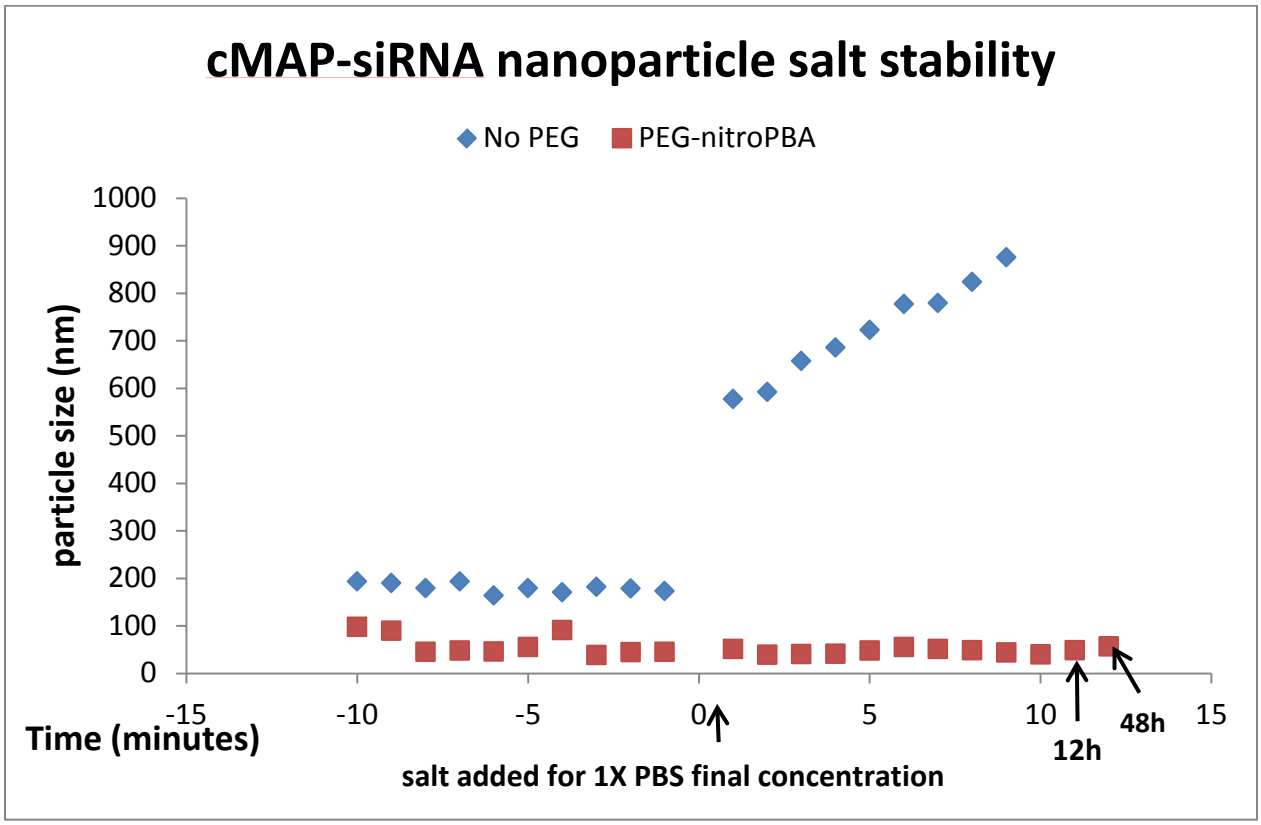


Figure S43. mPEG-cMAP-PEGm triblock RiboGreen assay showing siRNA encapsulation by a charge ratio of 3+/- for 2k and 5k PEG blocks.



12. cMAP siRNA NP Salt Stability

Figure S44. Without PEG, the cMAP-siRNA NP is unstable once in 1X PBS, but is stable for 2 days when 5-nPBA-PEGm is used to stabilize the NP.



13. cMAP-PEG-cMAP pure triblock siRNA NP Salt Stability

Table S13: NPs formed without extra 5-nPBA-PEGm using cMAP-PEG-cMAP triblock isolated from cMAP-PEG copolymer aggregates in 1X PBS, but is stable when at least one PEG per 2 diol groups is added to the formulation. The average sizes of the data presented in the following Figures are shown.

Formulation	10 mM phosphate buffer	PBS
	Avg. size (nm)	Avg. size (nm)
3.4k triblock, 1:1, 0 PEG	176.6 ± 1.0	aggregates
3.4k triblock, 1:1, 0.5 PEG	100.1 ± 0.8	57.9 ± 0.9
3.4k triblock, 1:1, 1 PEG	149.5 ± 1.6	56.5 ± 1.5
3.4k triblock, 1:1, 2 PEG	85.9 ± 2.0	74.5 ± 3.4
3.4k triblock, 3:1, 0 PEG	392.3 ± 5.0	aggregates
3.4k triblock, 3:1, 0.5 PEG	42.8 ± 2.0	47.3 ± 2.0
3.4k triblock, 3:1, 1 PEG	61.3 ± 2.3	55.7 ± 1.2
3.4k triblock, 3:1, 2 PEG	115.9 ± 11.4	96.6 ± 1.6
5k triblock, 1:1, 0 PEG	165.2 ± 4.7	aggregates
5k triblock, 1:1, 0.5 PEG	141.3 ± 5.2	99.8 ± 3.8
5k triblock, 1:1, 1 PEG	109.2 ± 3.6	97.5 ± 4.2
5k triblock, 1:1, 2 PEG	179.1 ± 19.9	143.5 ± 5.4

Figure S45. Without added 5-nPBA-PEGm, the cMAP-PEG3.4k-cMAP siRNA NP formulated at a 1+/- charge ratio aggregates once in 1X PBS, but is stable when at least one 5-nPBA-PEGm per two diol groups (0.5 PEG) on cMAP is added to the formulation.

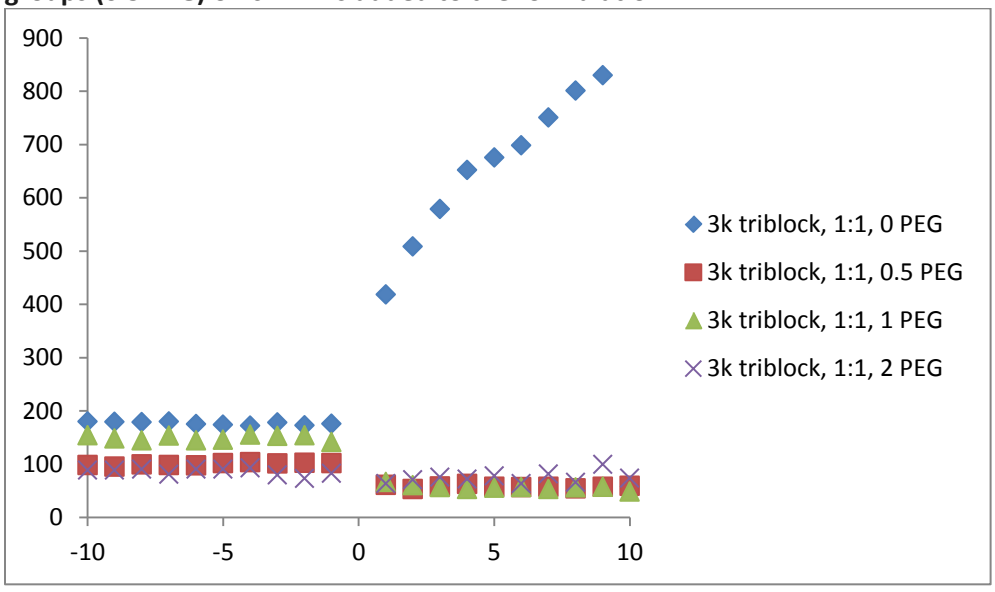


Figure S46. Without added 5-nPBA-PEGm, the cMAP-PEG3.4k-cMAP siRNA NP formulated at a 3+/- charge ratio aggregates once in 1X PBS, but is stable when at least one 5-nPBA-PEGm per two diol groups (0.5 PEG) on cMAP is added to the formulation.

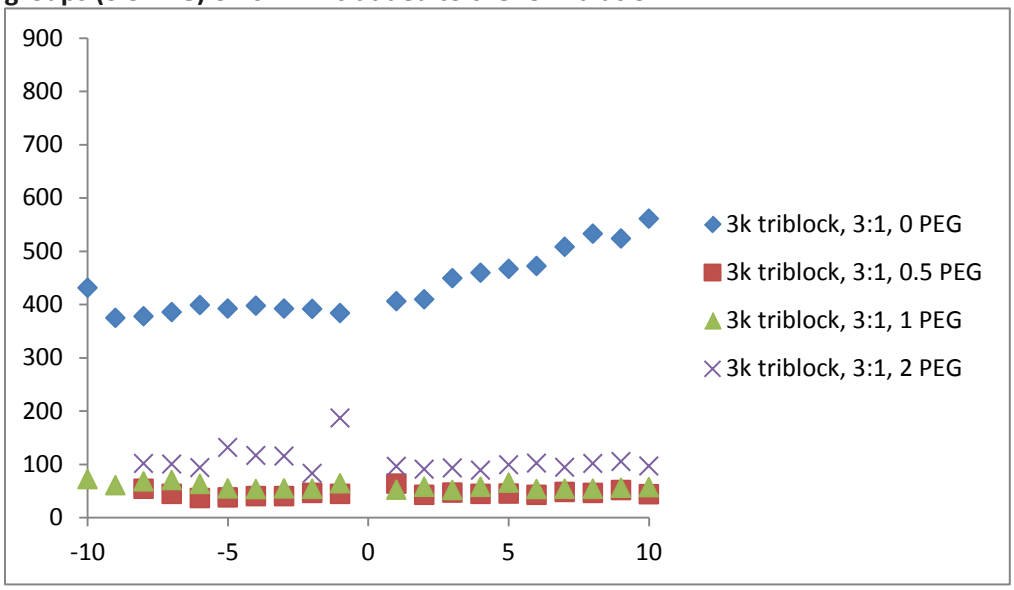
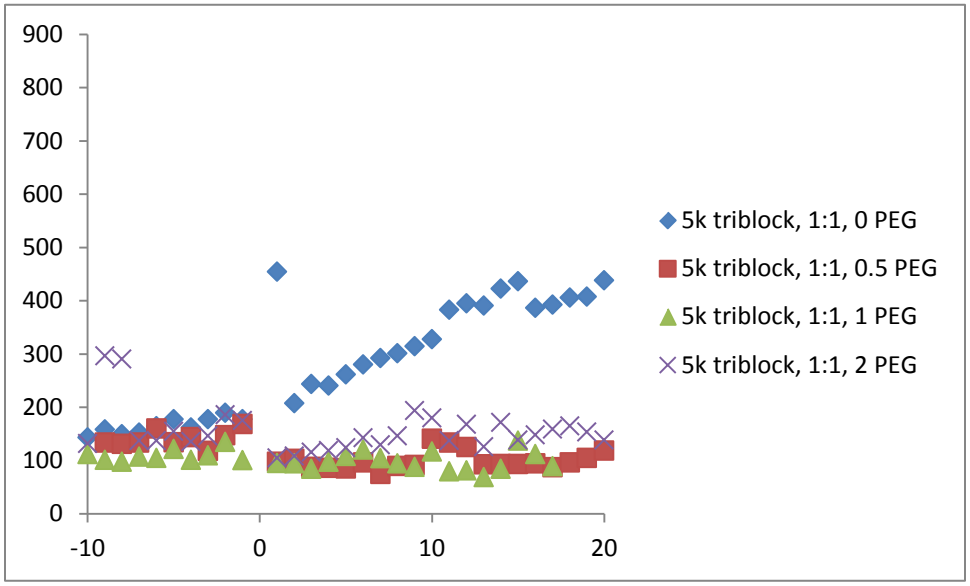


Figure S47. Without added 5-nPBA-PEGm, the cMAP-PEG5k-cMAP siRNA NP formulated at a 1+/- charge ratio aggregates once in 1X PBS, but is stable when at least one 5-nPBA-PEGm per two diol groups (0.5 PEG) on cMAP is added to the formulation.



14. DLS Nanoparticle Size Distributions

Figure S48: Lognormal size distribution by DLS for the cMAP + 5-nPBA-PEGm NP.

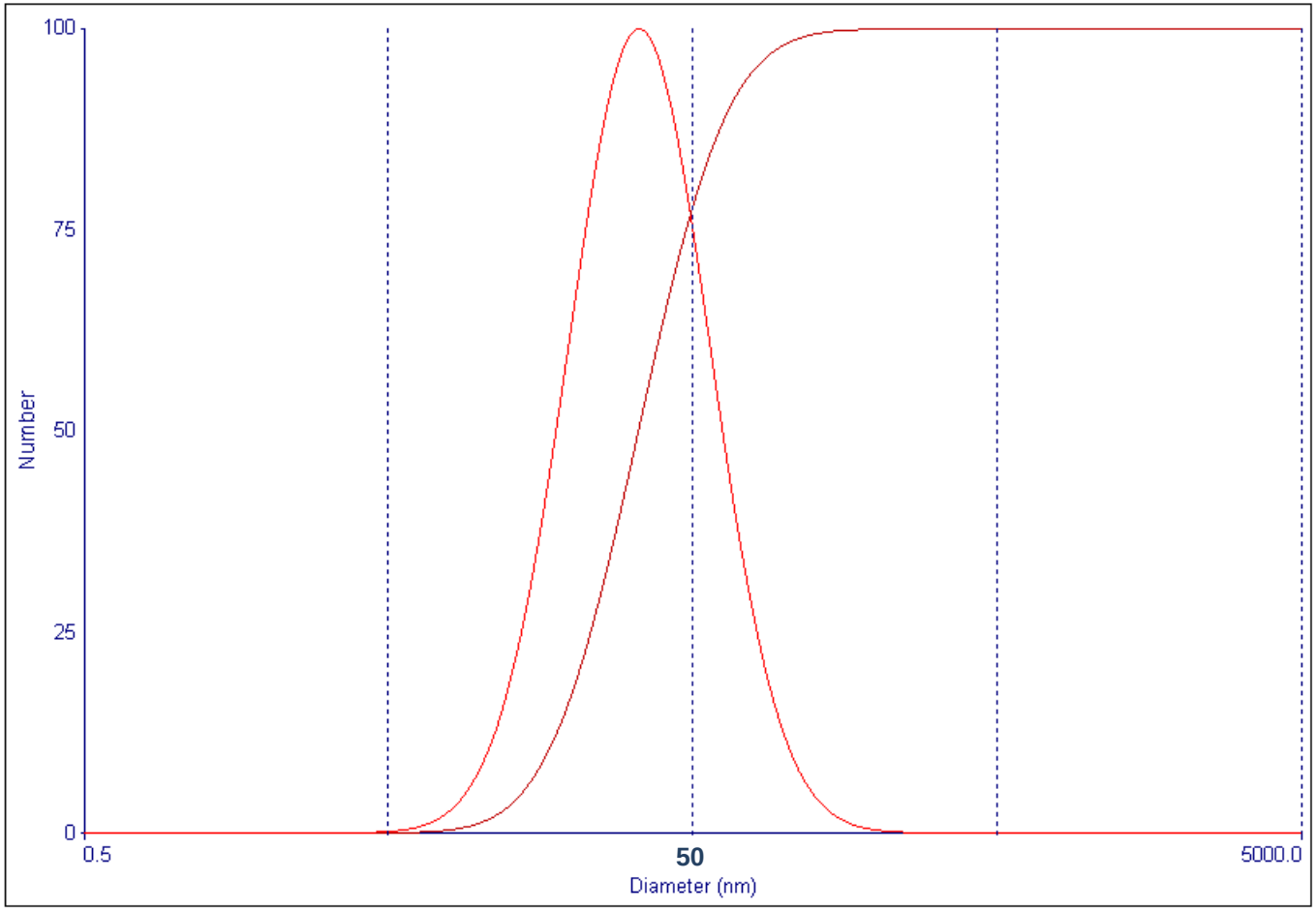


Figure S49: Lognormal size distribution by DLS for the CMAP-PEG copolymer NP.

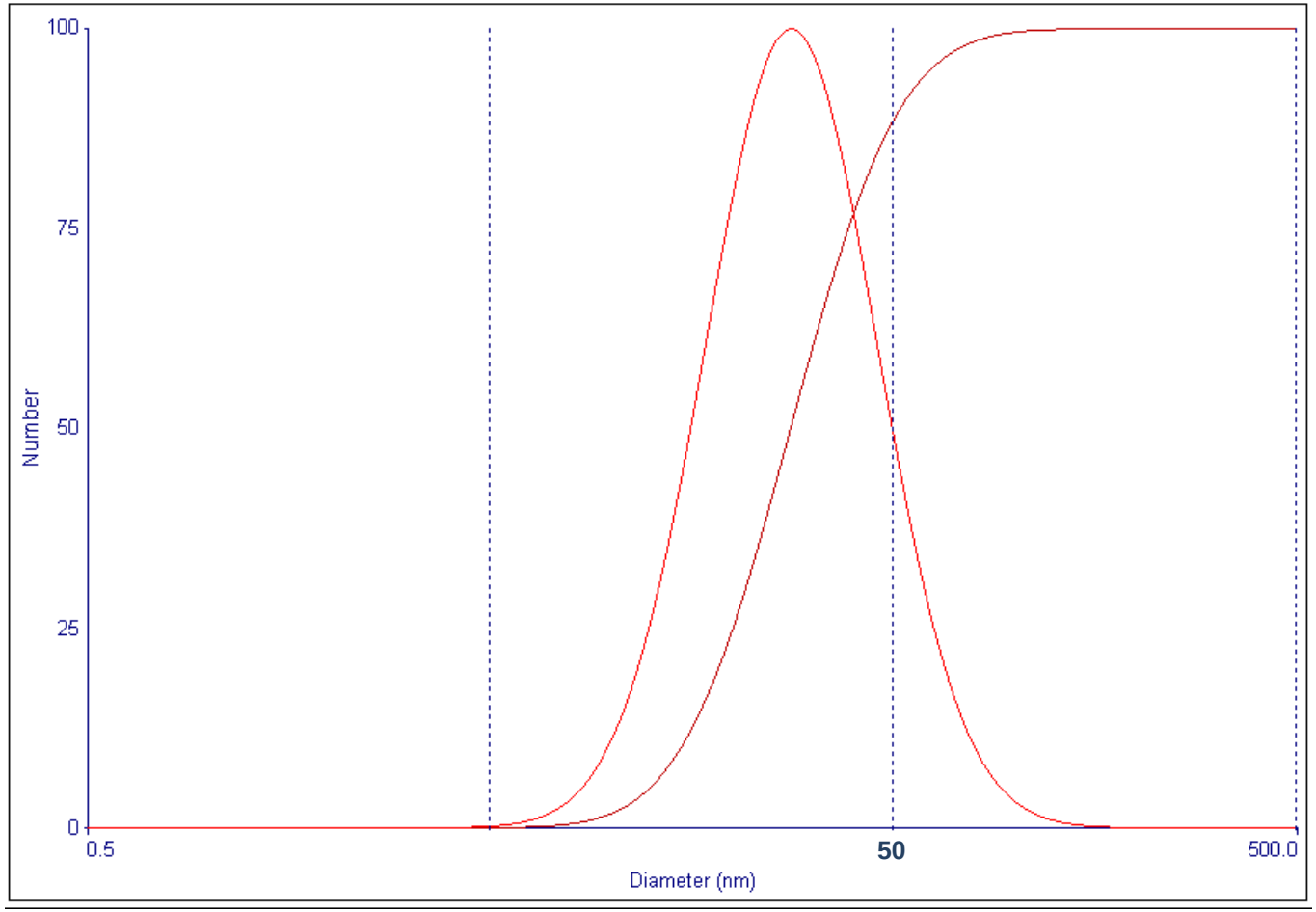


Figure S50: Lognormal size distribution by DLS for the cMAP-PEG copolymer + 5-nPBA-PEGm NP.

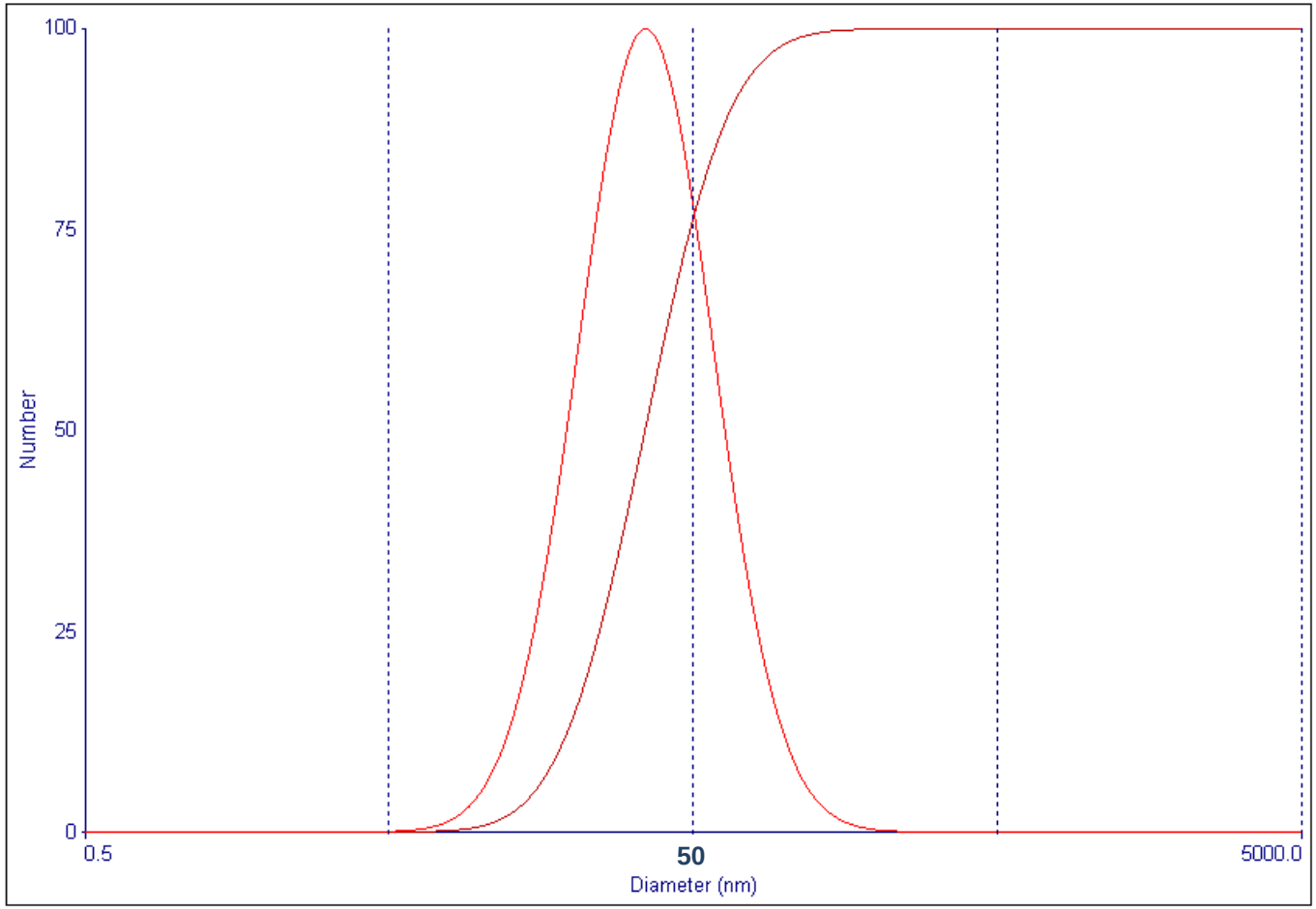


Figure S51: Lognormal size distribution by DLS for the mPEG-cMAP-PEGm NP.

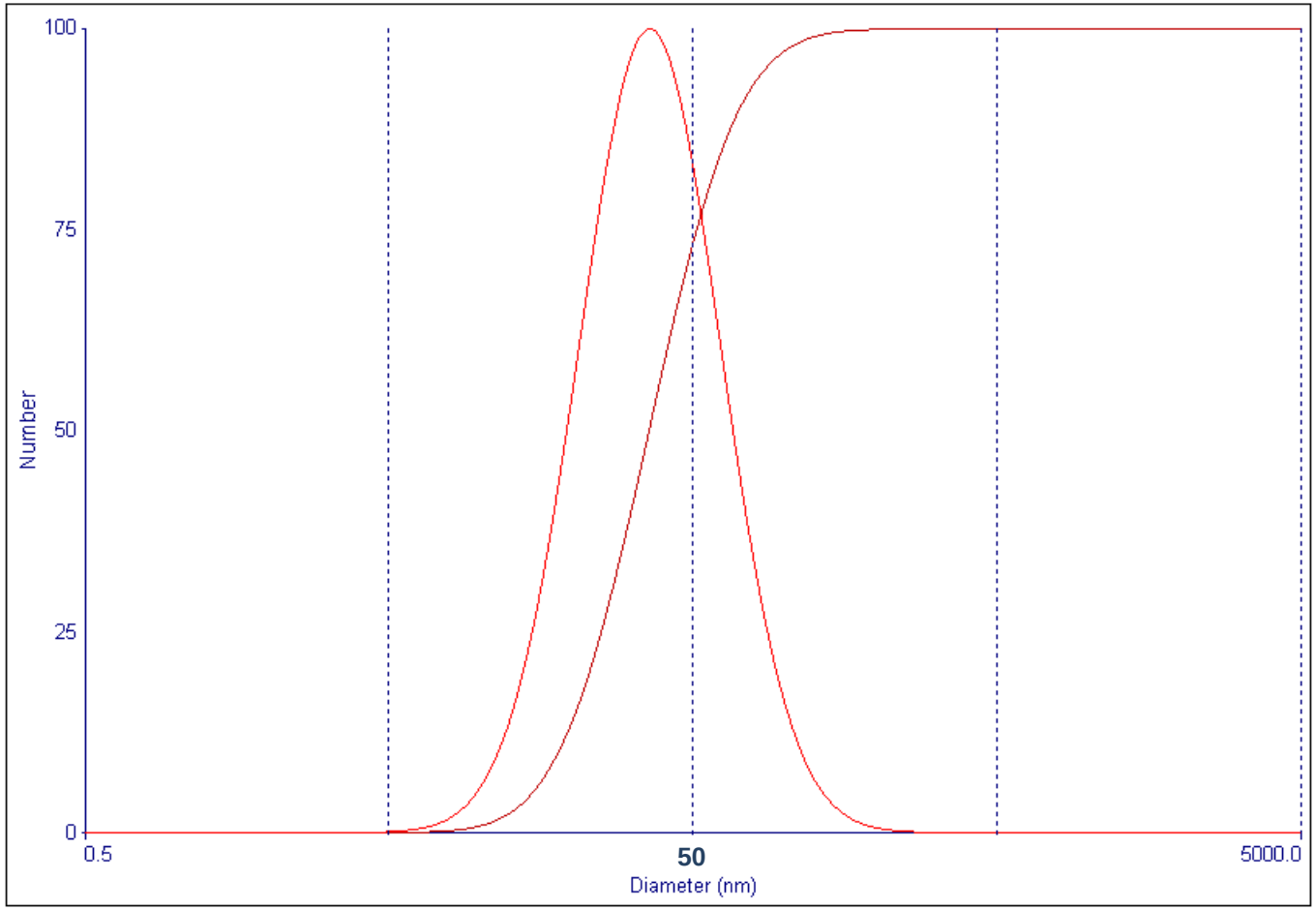
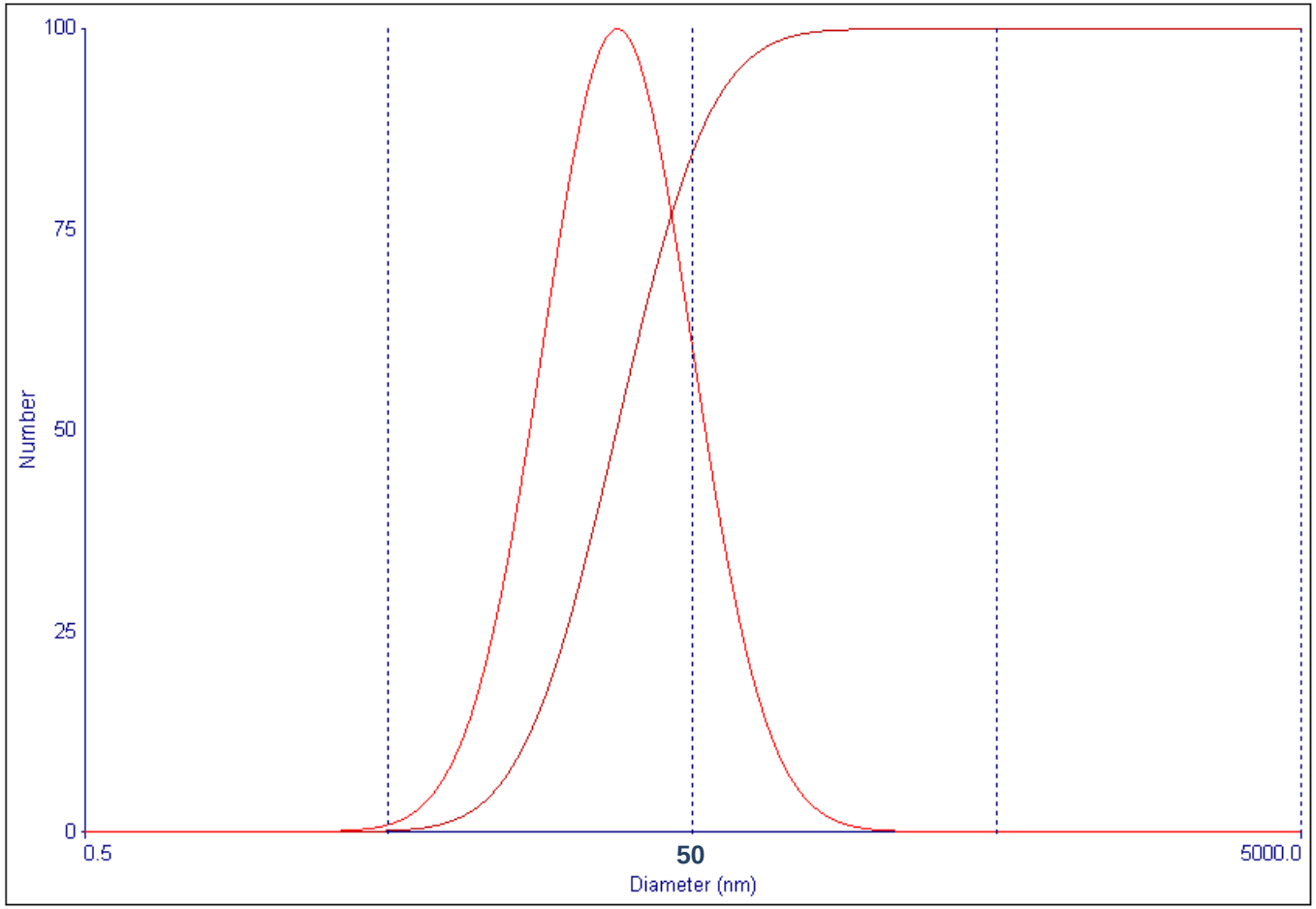


Figure S52: Lognormal size distribution by DLS for the mPEG-cMAP-PEGm + 5-nPBA-PEGm NP.



15. CryoTEM Nanoparticle Size Distributions and additional images

Figure S53: Size distribution by CryoTEM for the cMAP + 5-nPBA-PEGm NP.

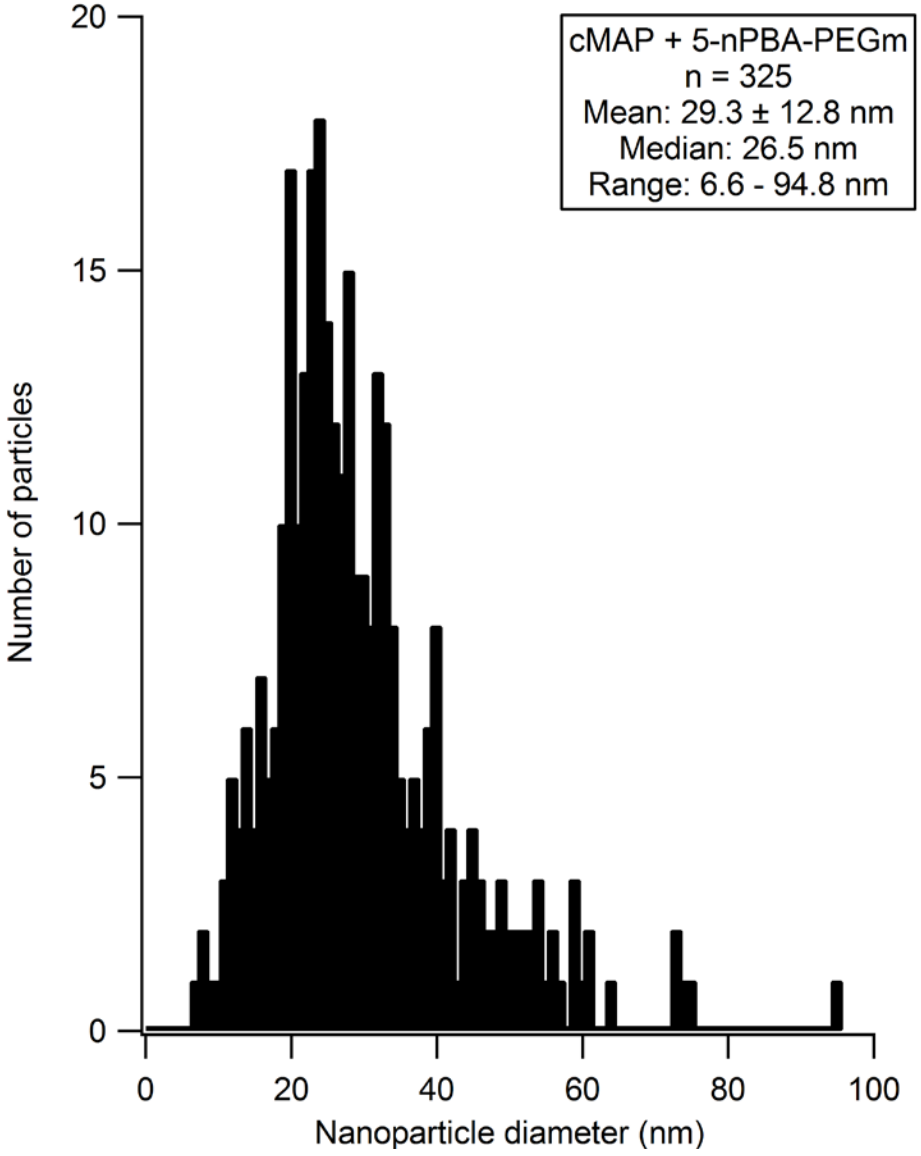


Figure S54: Additional CryoTEM images for the cMAP + 5-nPBA-PEGm NP.

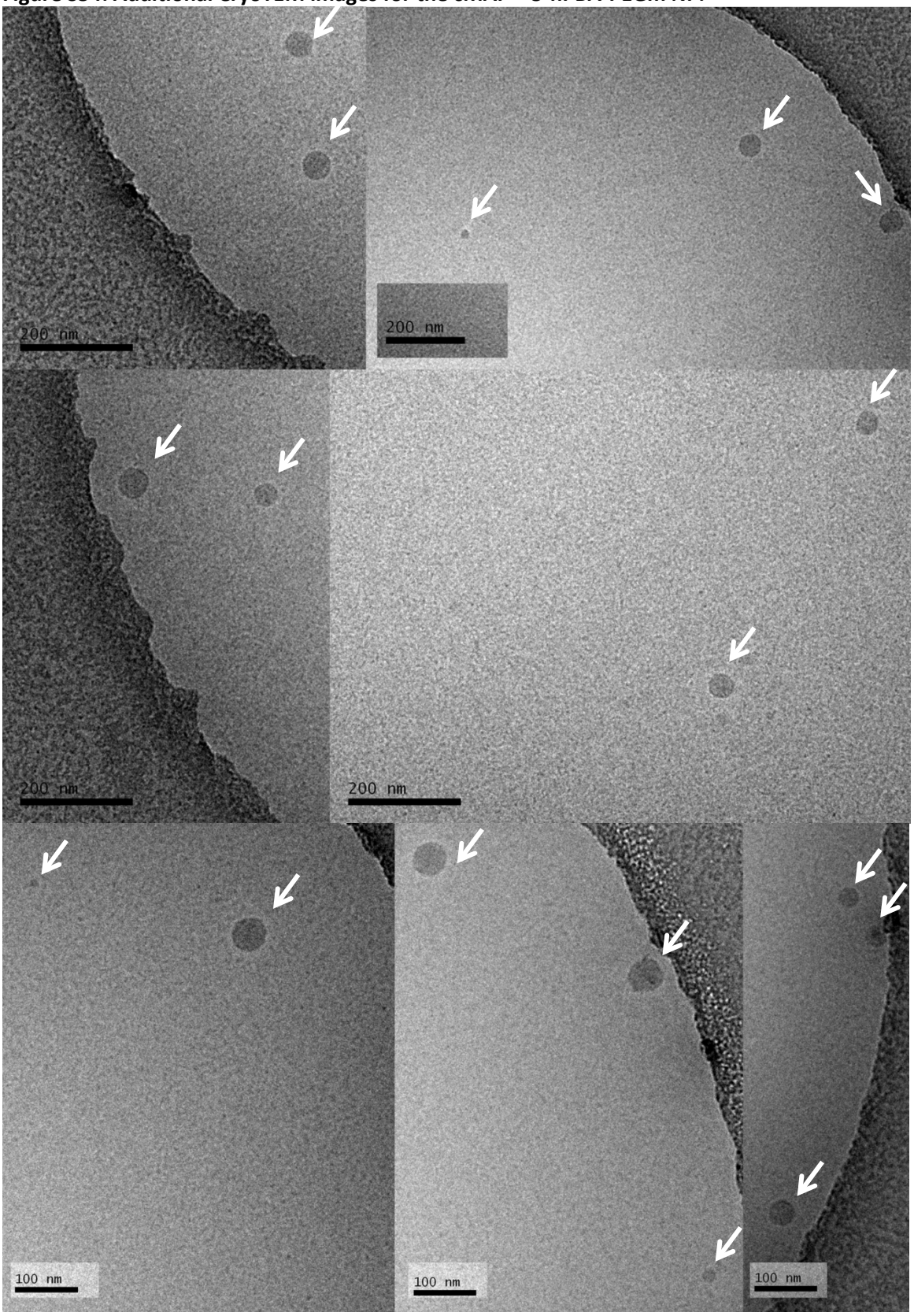


Figure S55: Size distribution by CryoTEM for the cMAP-PEG copolymer NP.

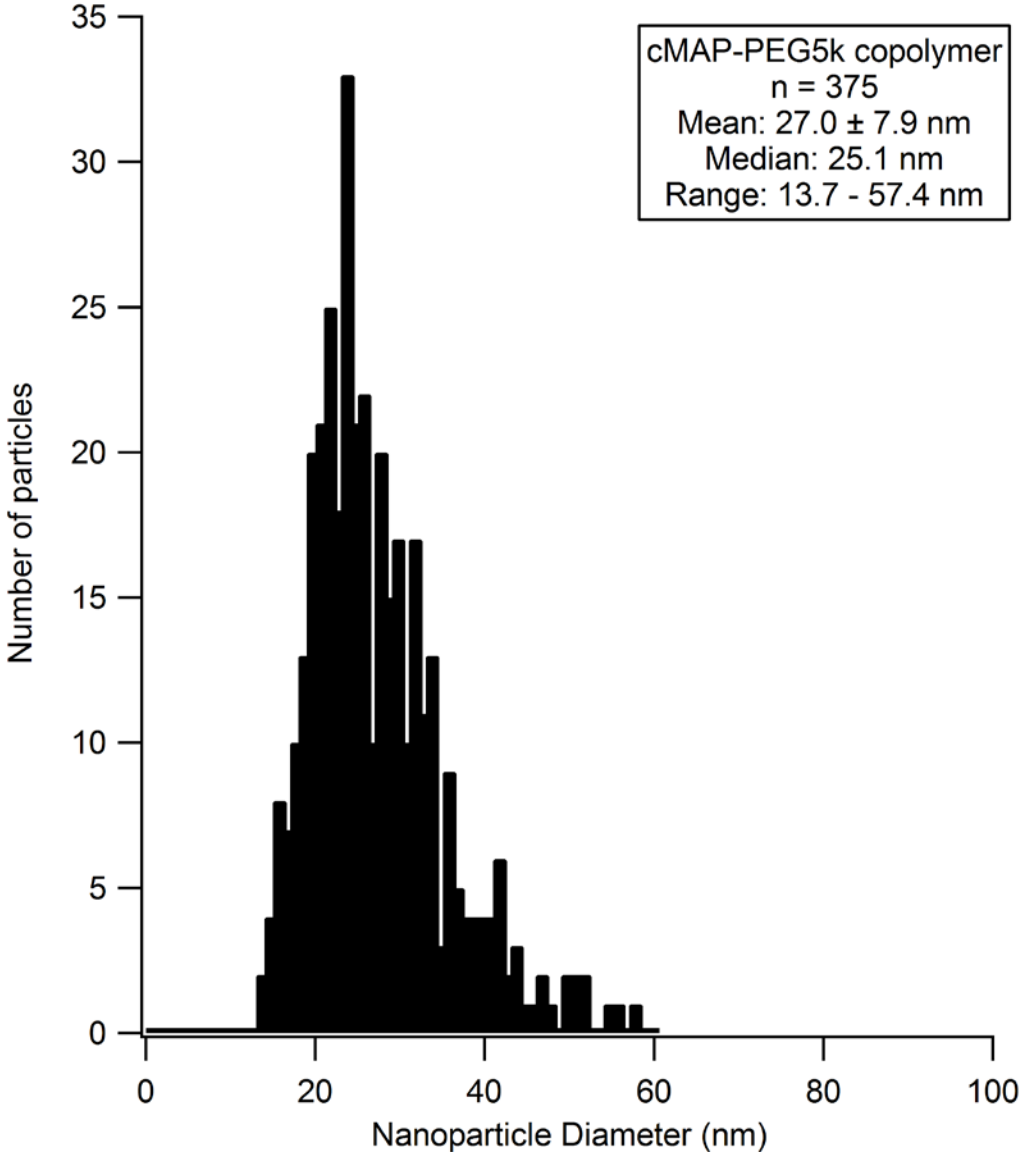
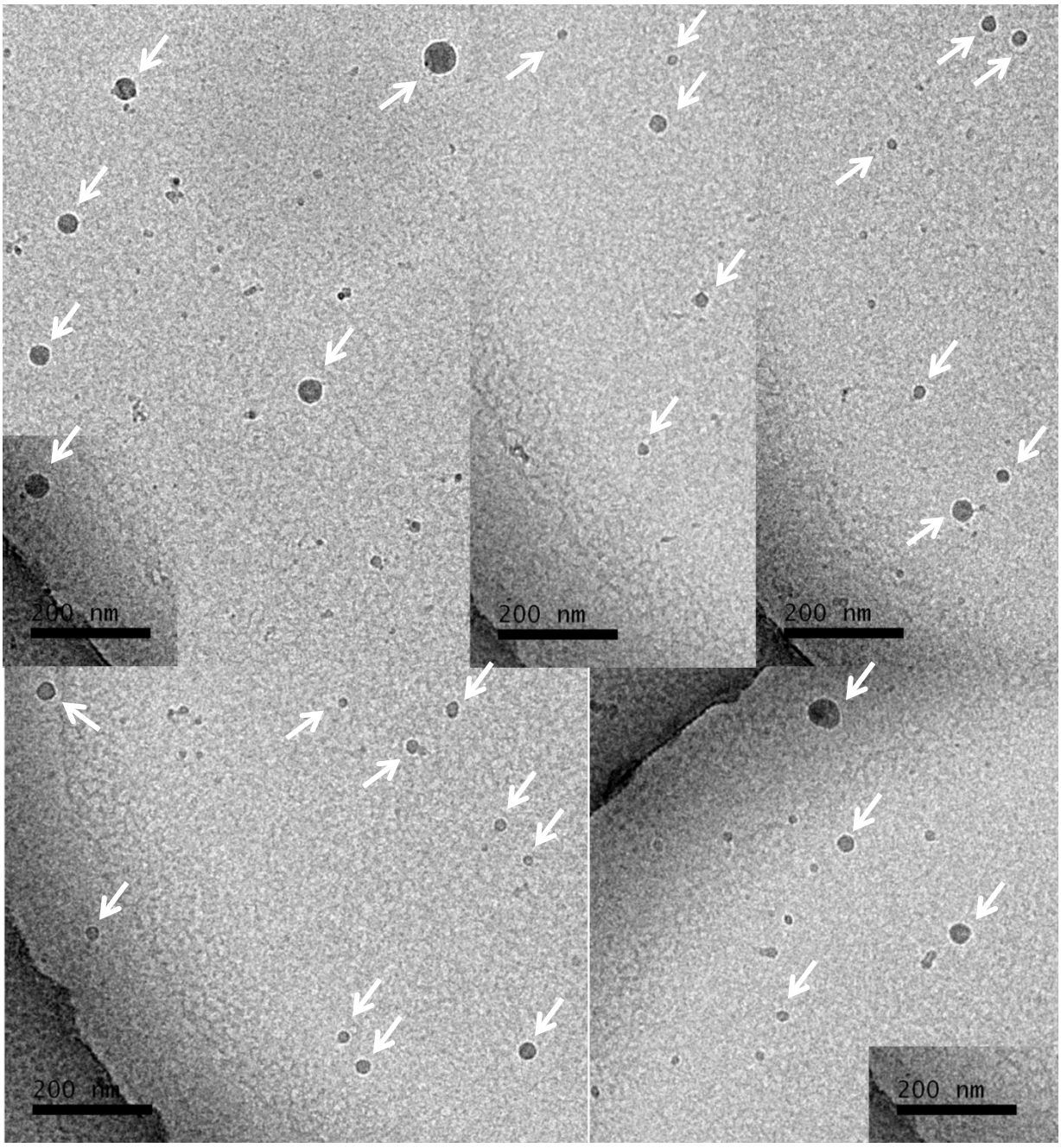


Figure S56: Additional CryoTEM images for the cMAP-PEG copolymer NP.



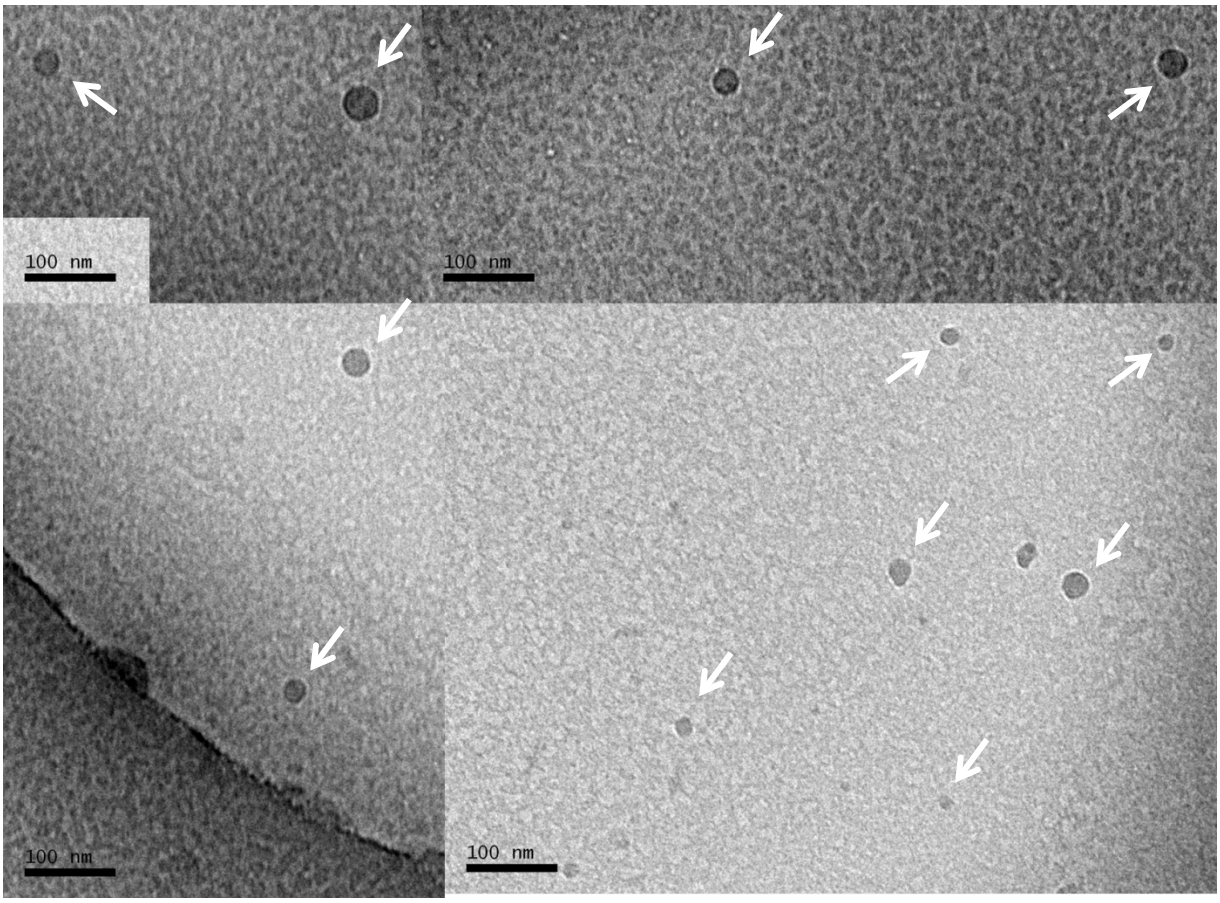

100 nm
100 nm
100 nm
100 nm

Figure S57: Size distribution by CryoTEM for the cMAP-PEG copolymer + 5-nPBA-PEGm NP.

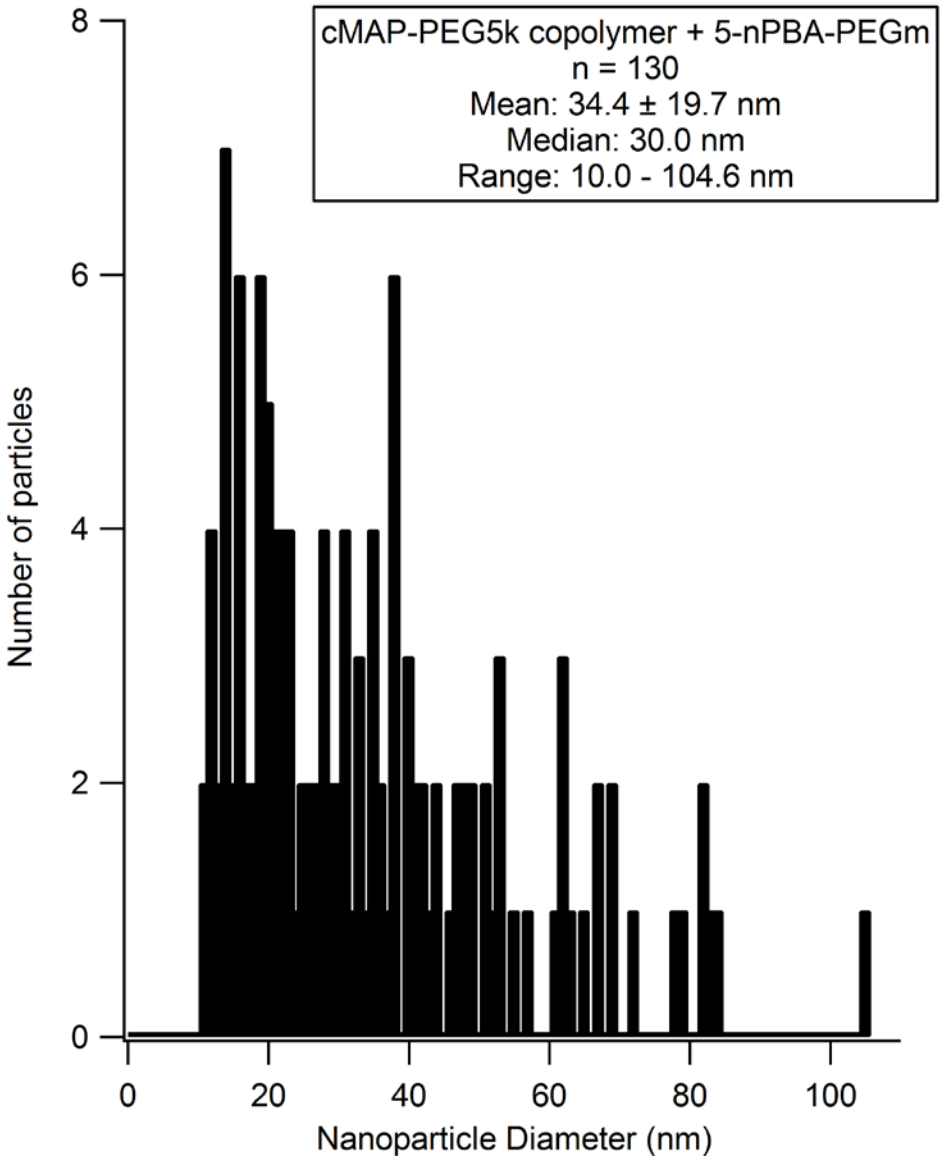


Figure S58: Additional CryoTEM images for the cMAP-PEG copolymer + 5-nPBA-PEGm NP.

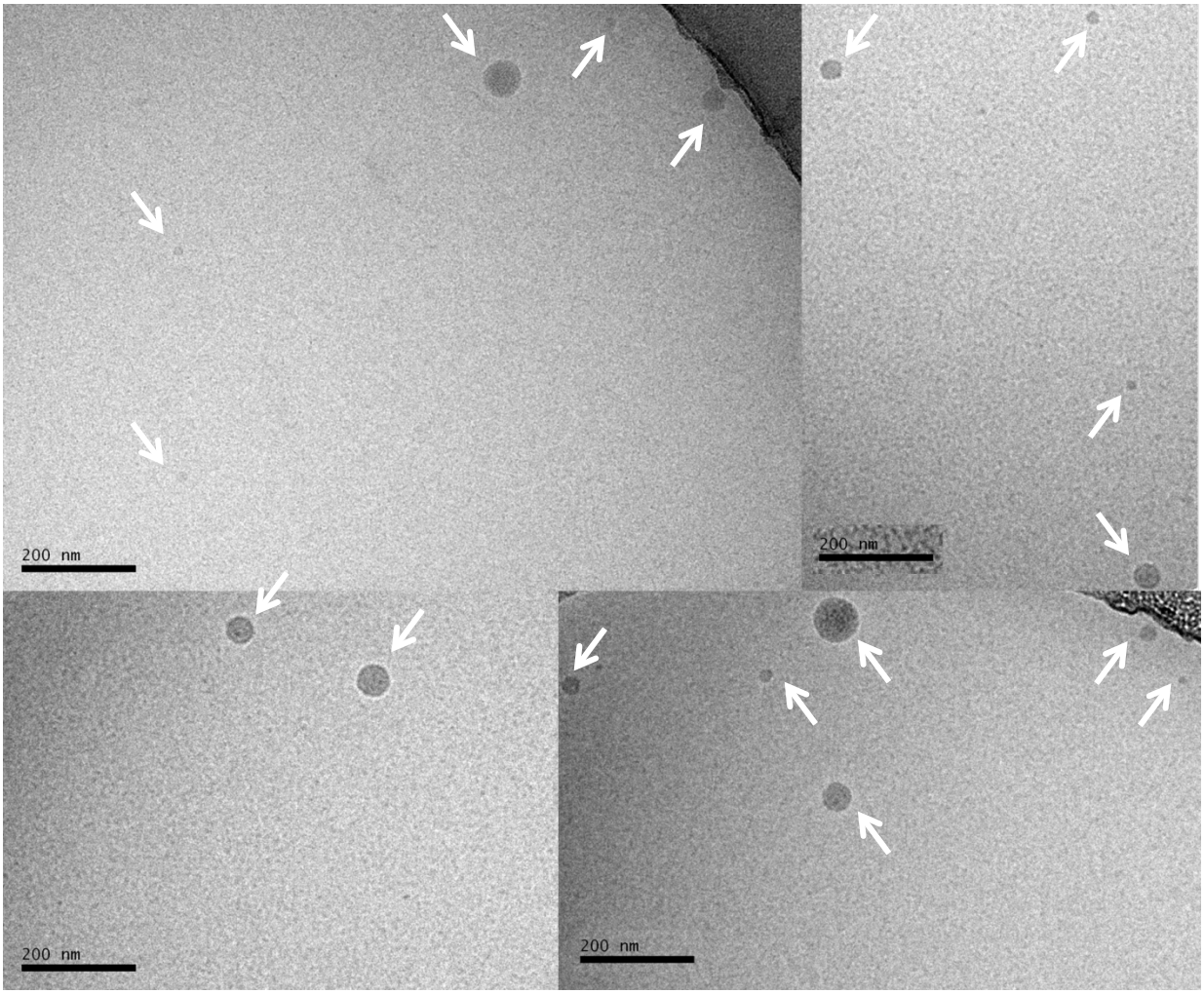


Figure S59: Size distribution by CryoTEM for the mPEG-cMAP-PEGm NP.

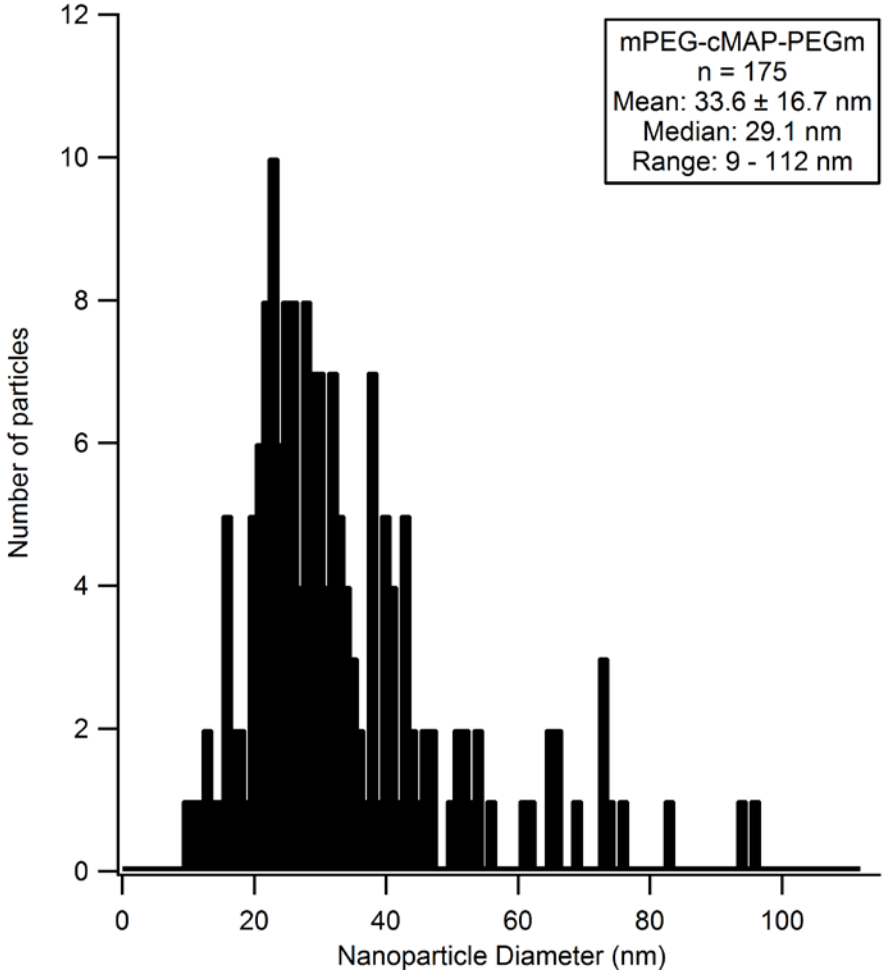
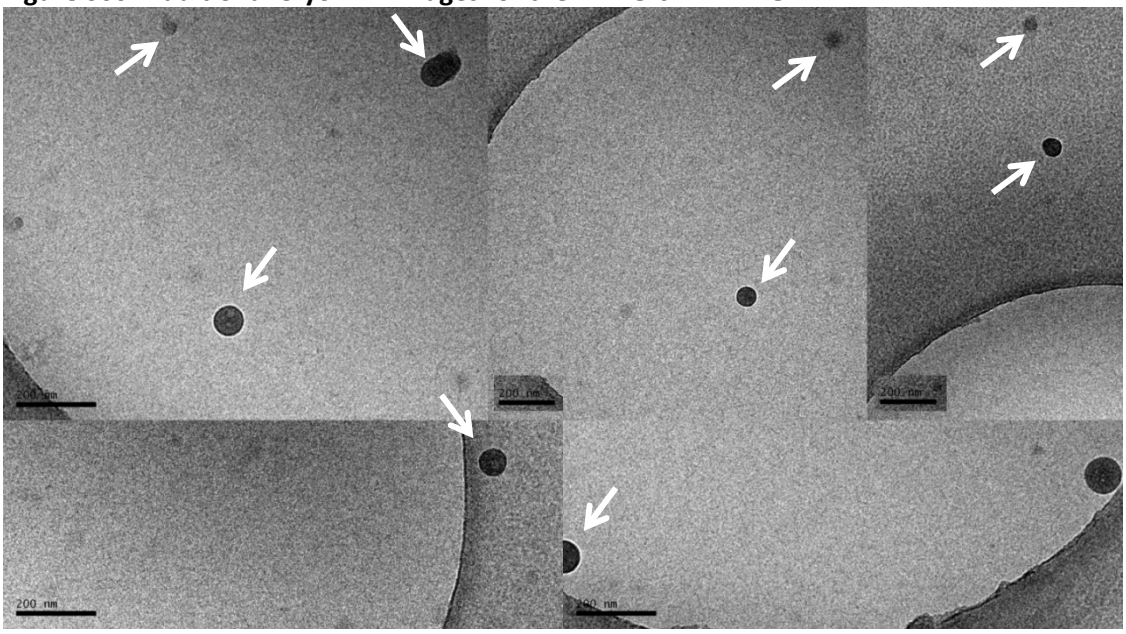


Figure S60: Additional CryoTEM images for the mPEG-cMAP-PEGm NP.



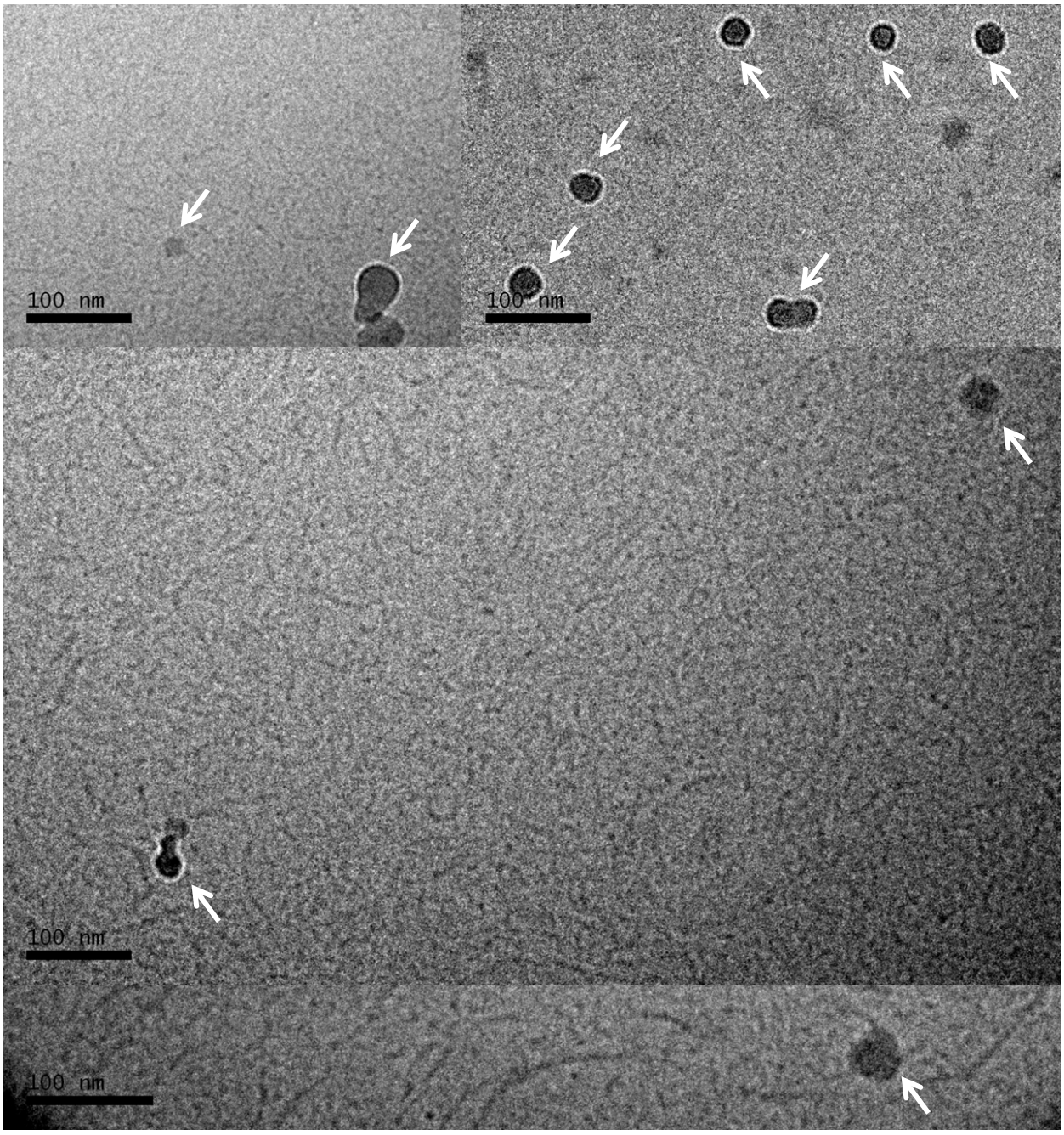

100 nm
100 nm
100 nm
100 nm

Figure S61: Size distribution by CryoTEM for the mPEG-cMAP-PEGm + 5-nPBA-PEGm NP.

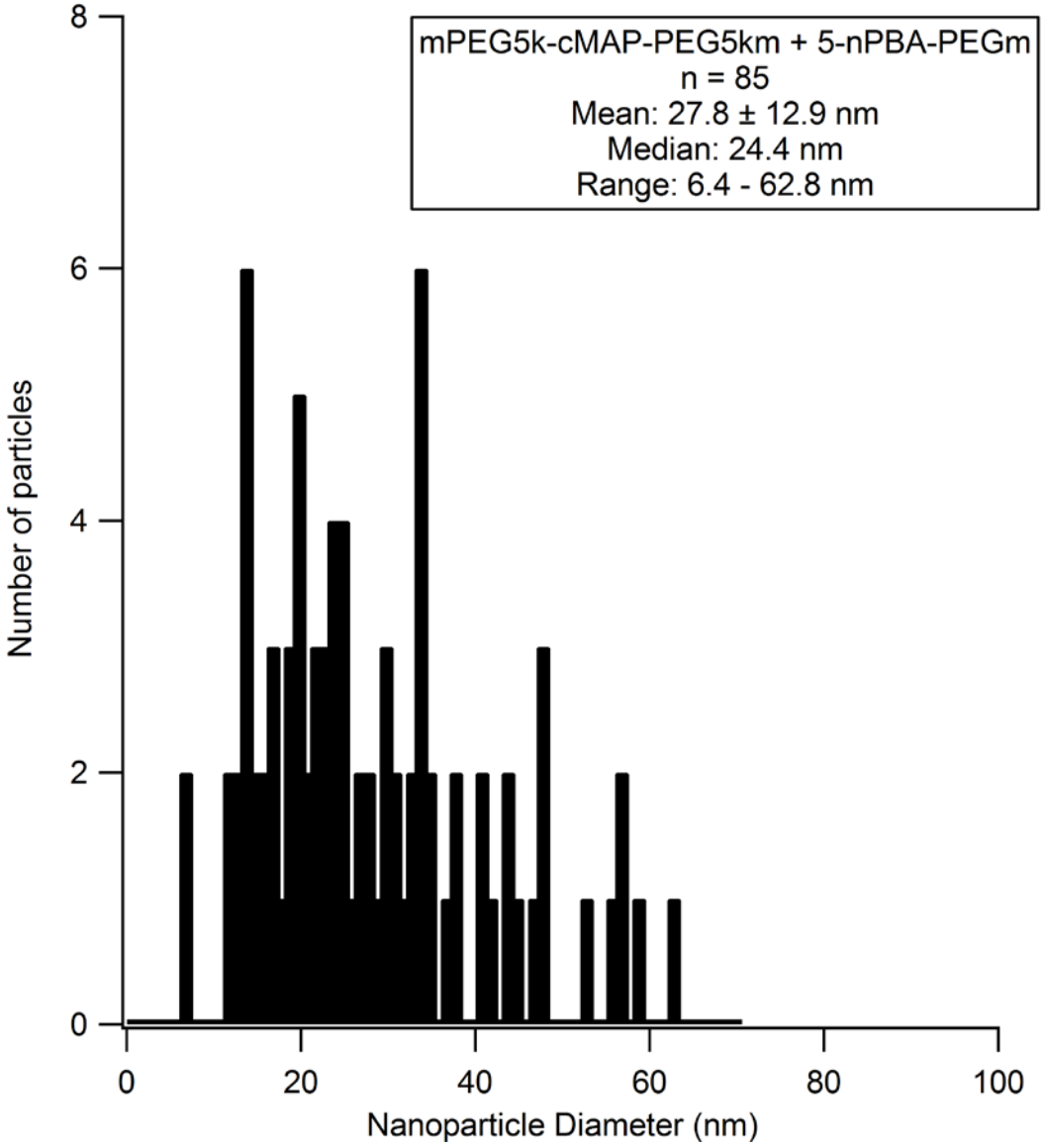
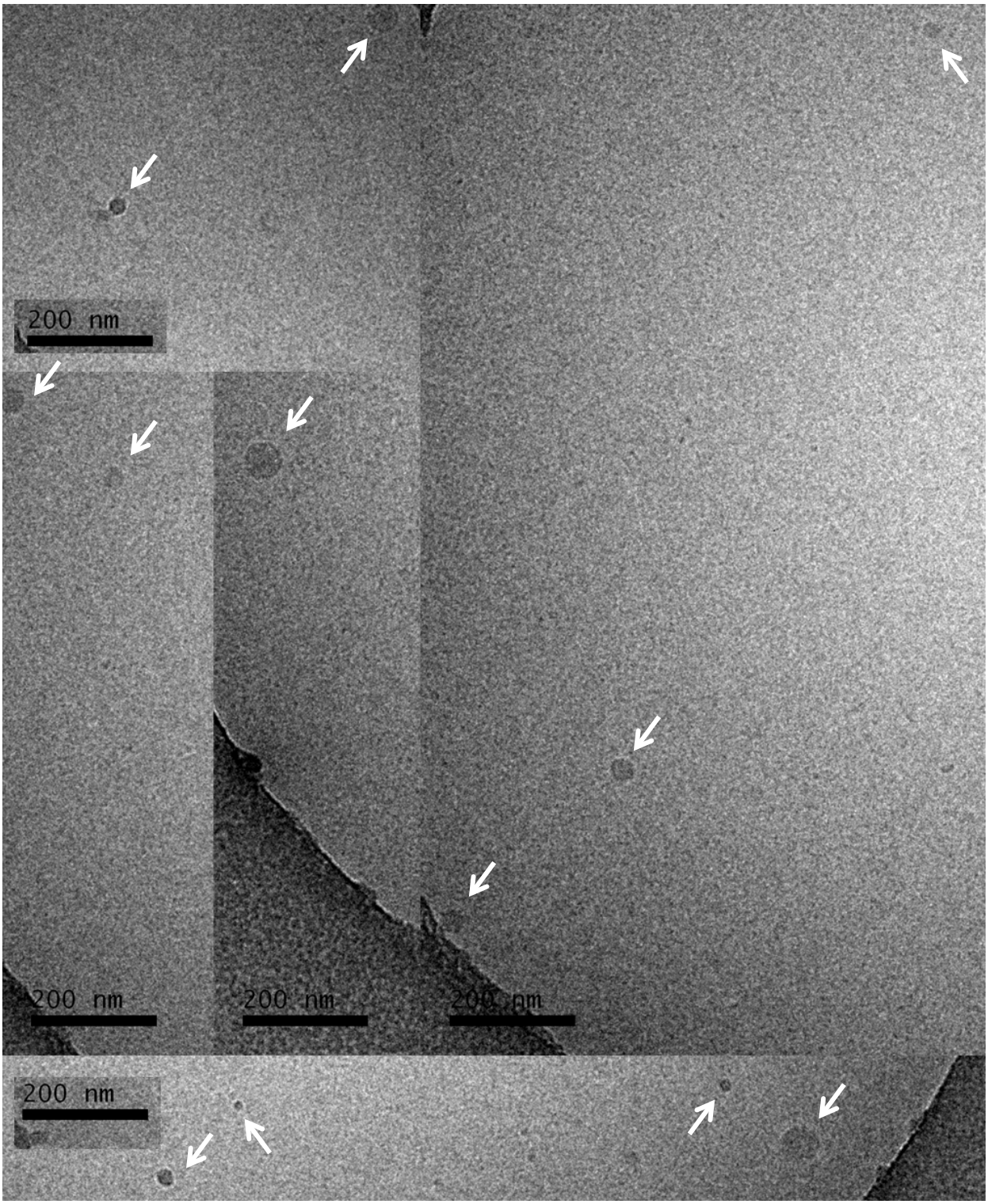
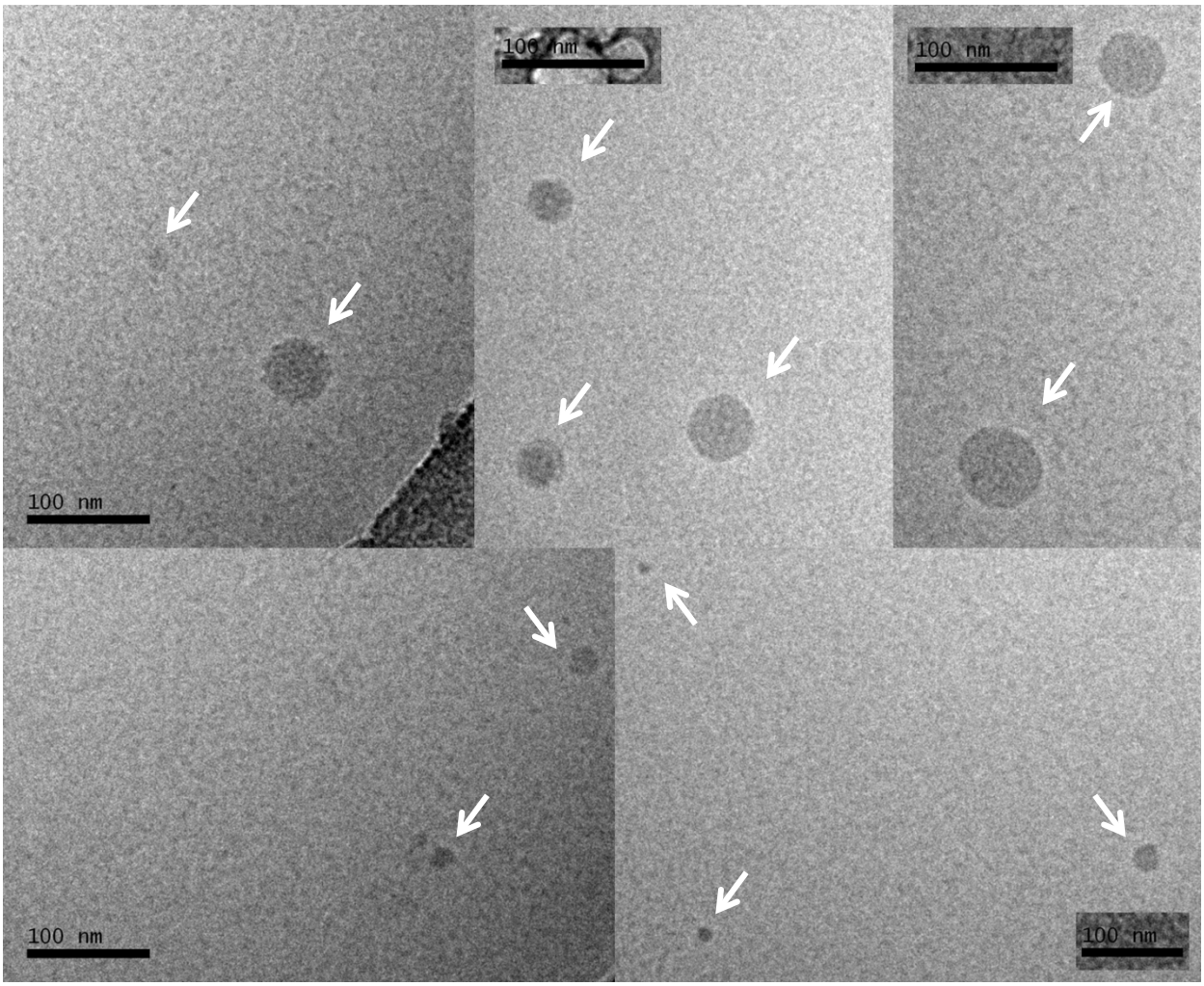


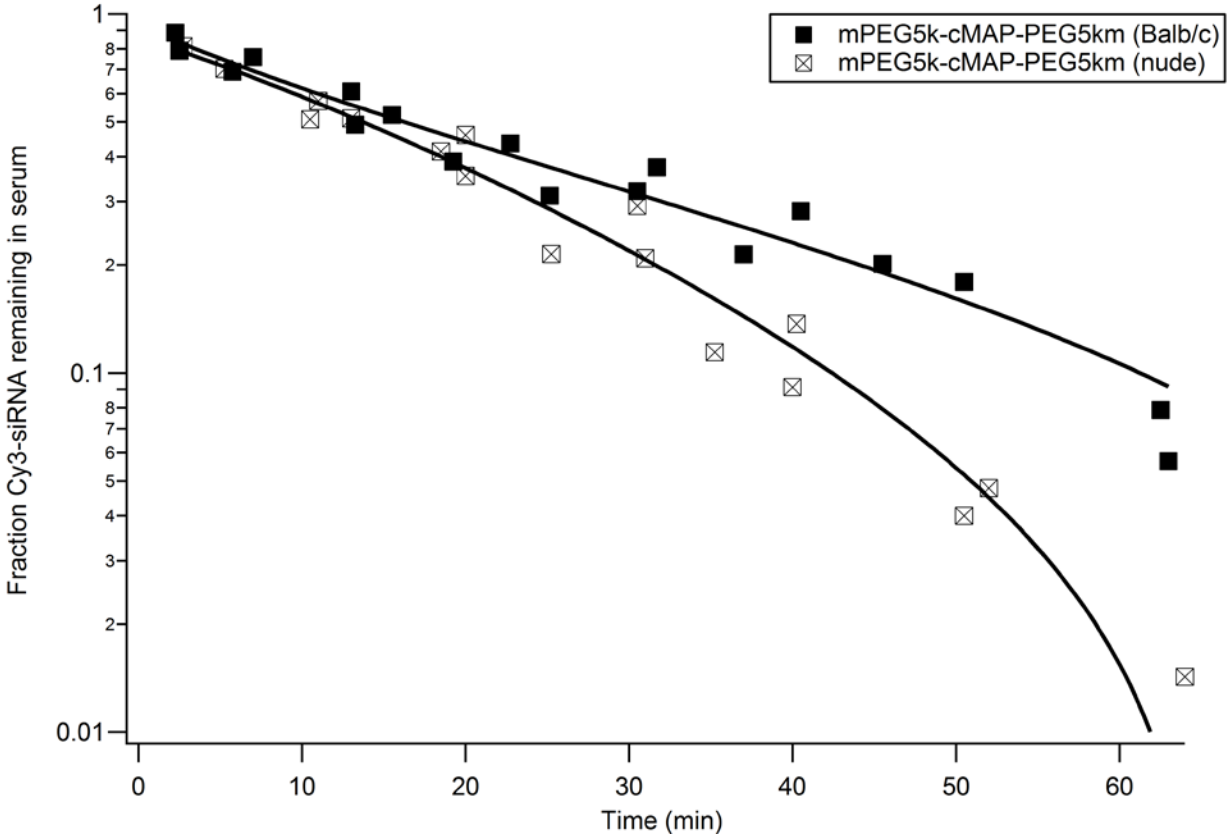
Figure S62: Additional CryoTEM images for the mPEG-cMAP-PEGm + 5-nPBA-PEGm NP.




100 nm
100 nm
100 nm
100 nm
100 nm

16. Phamacokinetics of mPEG-cMAP-PEGm NP in Balb/c vs. nude mice

Figure S63: The circulation time of the mPEG-cMAP-PEGm siRNA NP is similar in Balb/c and nude mice. n=3 mice.



17. Gel images of mPEG-cMAP-PEGm NP in mouse serum

Figure S64: This gel shows mPEG-cMAP-PEGm NPs with and without 5-nPBA-PEGm in mouse serum. The samples are run on a 0.5% agarose gel in Tris-Borate-EDTA buffer (pH 8) at 90V, and are detected by the Cy3 fluorophore labeled siRNA on a Typhoon gel scanner with laser excitation 532 nm, emission 580 nm. Lanes are labeled as follows. This shows that the NPs shift further down the gel due to protein adsorption but are intact in mouse serum. SDS dissociates the nanoparticle allowing the siRNA to run down the gel.

1. mPEG-cMAP-PEGm NP
2. mPEG-cMAP-PEGm NP in mouse serum
3. mPEG-cMAP-PEGm NP in mouse serum + SDS
4. mPEG-cMAP-PEGm + 5-nPBA-PEGm NP
5. mPEG-cMAP-PEGm + 5-nPBA-PEGm NP in mouse serum
6. mPEG-cMAP-PEGm + 5-nPBA-PEGm NP in mouse serum + SDS
7. siRNA in mouse serum
8. siRNA in mouse serum + SDS

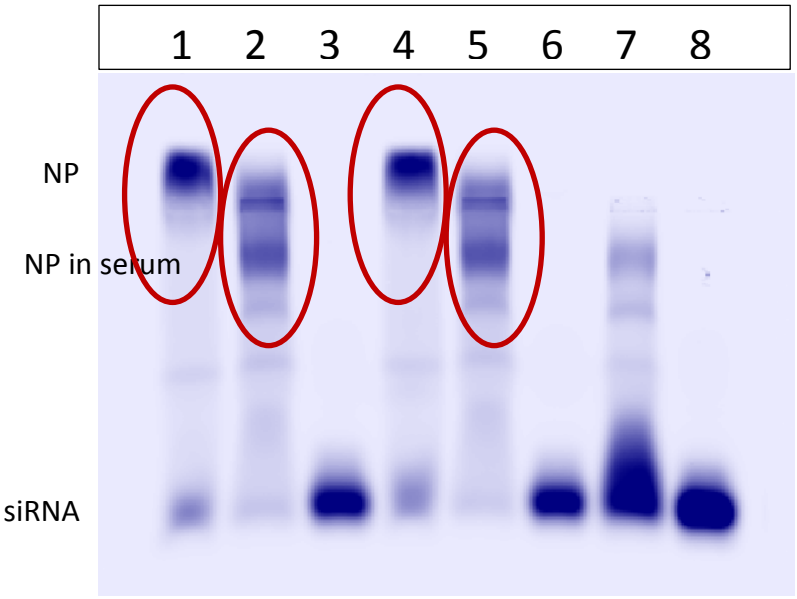


Figure S65: A 0.5% agarose gel (made as described in Figure S64) is detected by ethidium bromide intercalating siRNA on a Biodocit gel imager. Lanes are labeled as follows. SDS dissociates the NP so the siRNA runs down the gel. This shows that the mPEG-cMAP-PEGm NP is intact in mouse serum, including in serum after 20 minutes of circulation post-injection.

1. Mouse Serum
2. Mouse Serum + SDS
3. siRNA
4. siRNA + SDS
5. mPEG-cMAP-PEGm NP
6. mPEG-cMAP-PEGm NP + SDS
7. Serum collected at 20 min after mPEG-cMAP-PEGm NP injection
8. Serum collected at 20 min after mPEG-cMAP-PEGm NP injection + SDS

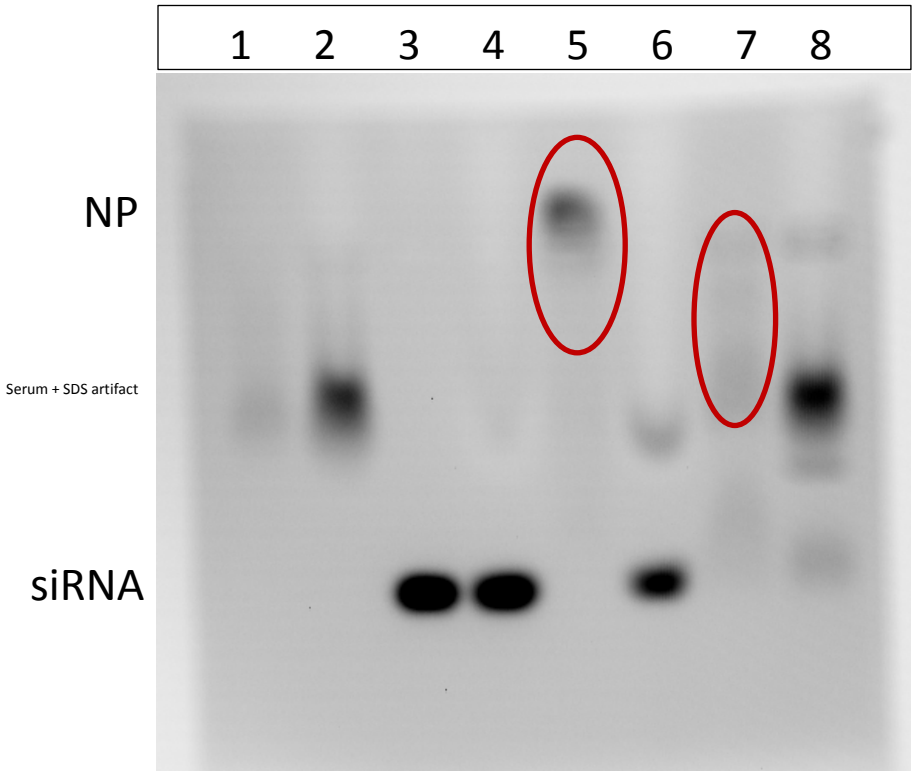


Figure S66: This is the same gel as in Figure S60, but detected by the Cy3 fluorophore labeled siRNA on a Typhoon gel scanner with laser excitation 532 nm, emission 580 nm. Lanes are labeled as follows. SDS dissociates the NP. The bands detected by fluorescence line up well with the bands detected by ethidium bromide staining. This shows that the mPEG-cMAP-PEGm NP is intact in mouse serum, including in serum after 20 minutes of circulation after injection.

1. Mouse Serum
2. Mouse Serum + SDS
3. siRNA
4. siRNA + SDS
5. mPEG-cMAP-PEGm NP
6. mPEG-cMAP-PEGm NP + SDS
7. Serum collected at 20 min after mPEG-cMAP-PEGm NP injection
8. Serum collected at 20 min after mPEG-cMAP-PEGm NP injection + SDS

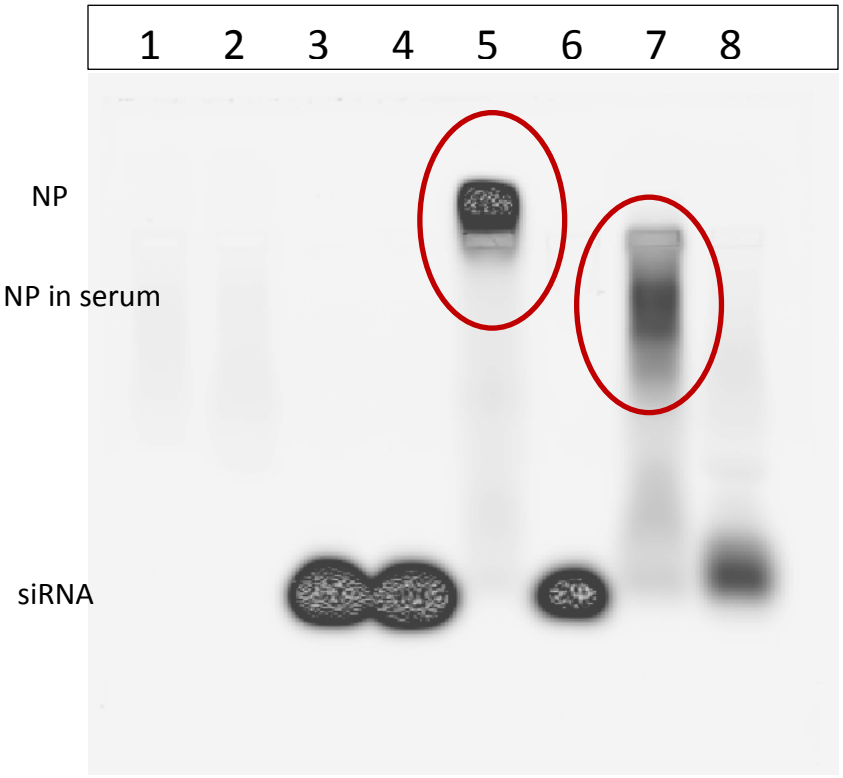


Figure S67: This gel is detected by the Cy3 fluorophore labeled siRNA on a Typhoon gel scanner with laser excitation 532 nm, emission 580 nm. Lanes are labeled as follows. SDS dissociates the NP. This shows that the mPEG-cMAP-PEGm NP is intact in mouse serum, including in serum after 20 minutes of circulation after injection.

1. Mouse Serum
2. mPEG-cMAP-PEGm NP
3. mPEG-cMAP-PEGm NP in mouse serum
4. mPEG-cMAP-PEGm NP in mouse serum + SDS
5. Serum collected at 20 min after mPEG-cMAP-PEGm NP injection
6. siRNA
7. siRNA in mouse serum
8. siRNA in mouse serum + SDS