Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary data

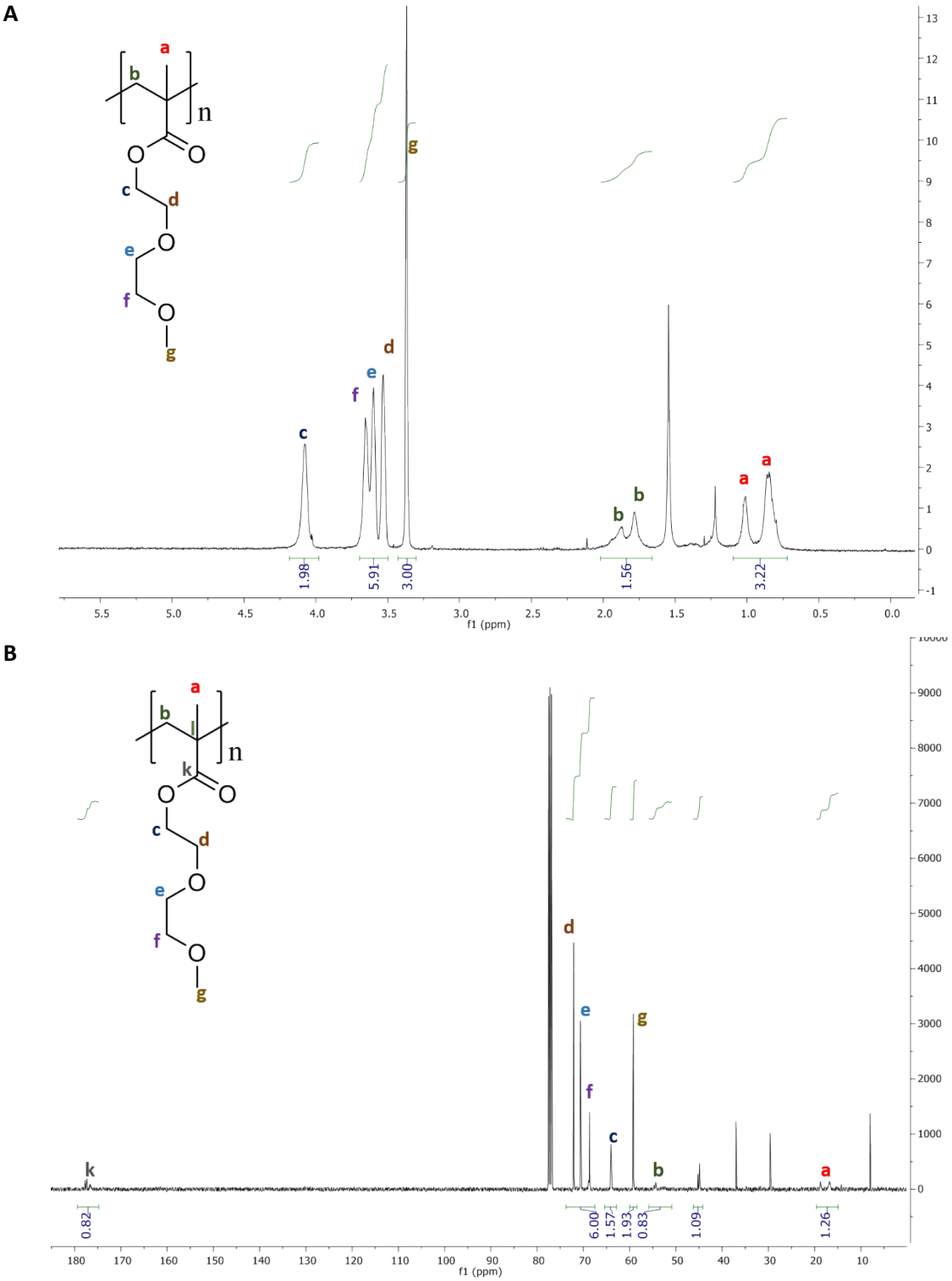

Figure S1. A) H^1-NMR of PDEGMA and B) C^{13}-NMR of PDEGMA.

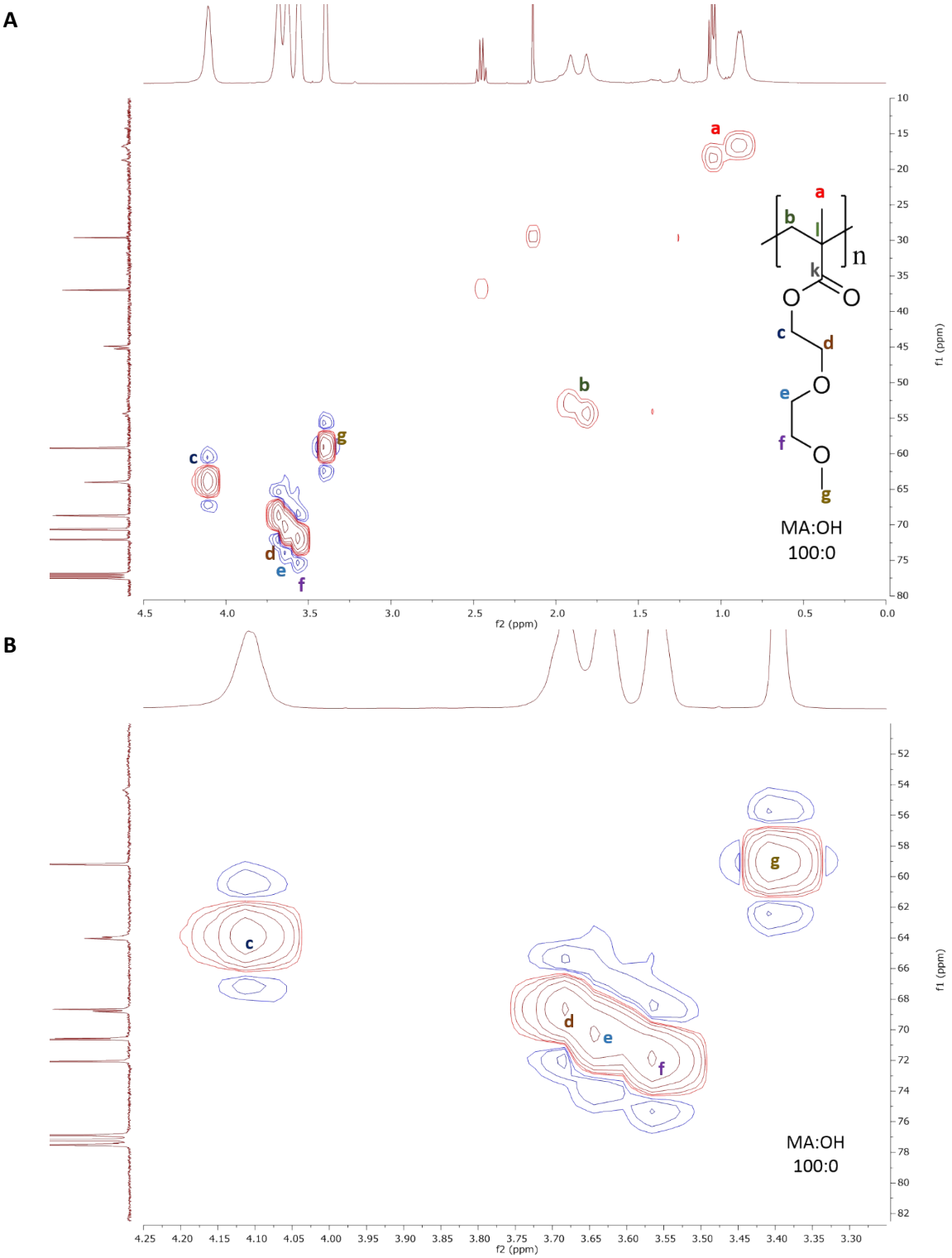

Figure S2. 2D NMR of A) PDEGMA and b) zoomed in 2D NMR of PDEGMA.

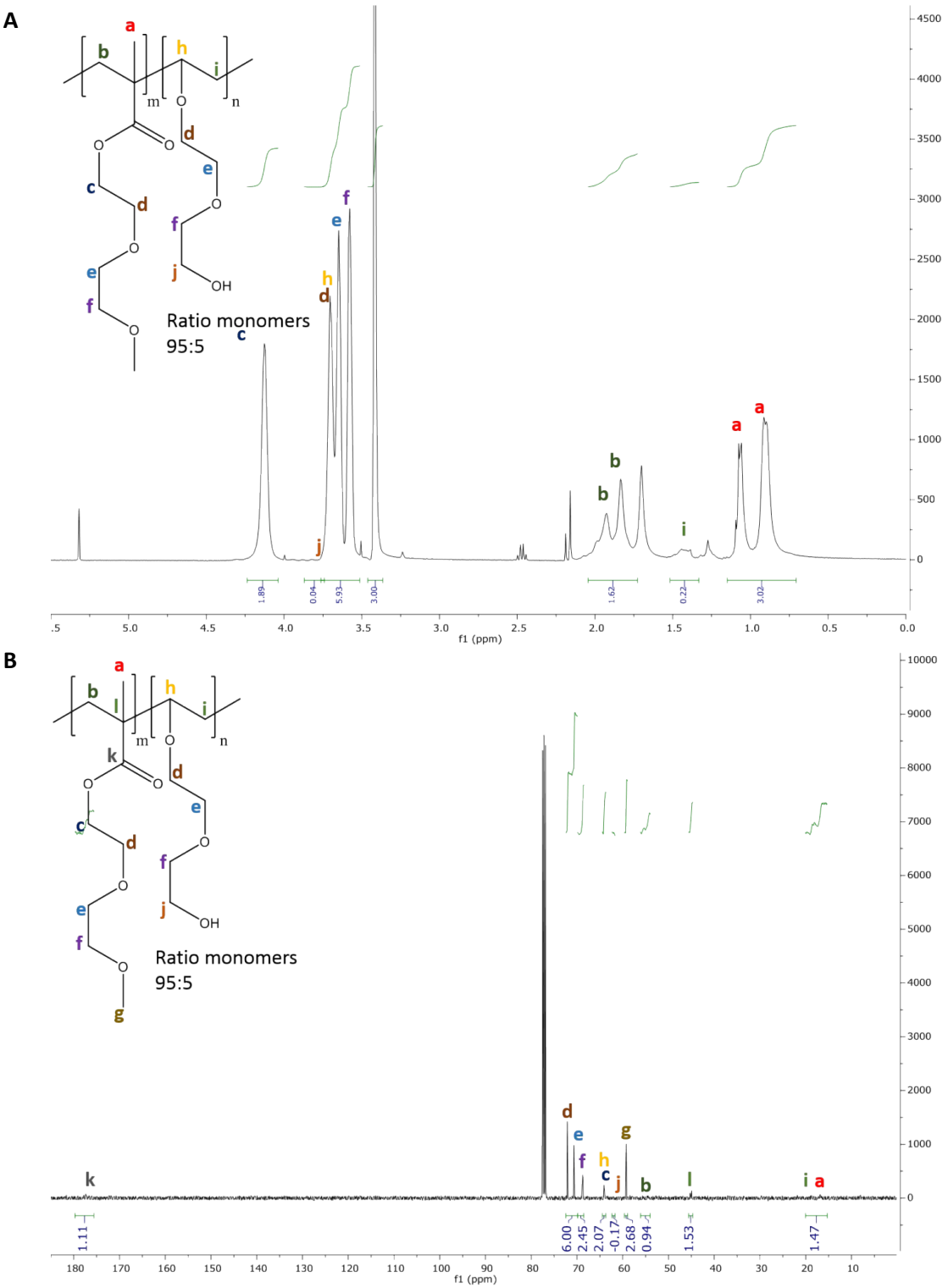

Figure S3. A) H^1-NMR of PDEGMA/PDEGOH (98:2) and B) C^{13} -NMR of PDEGMA/PDEGOH (98:2). Ratios of monomer used was 95:5, DEGMA:PDEGOH respectively.

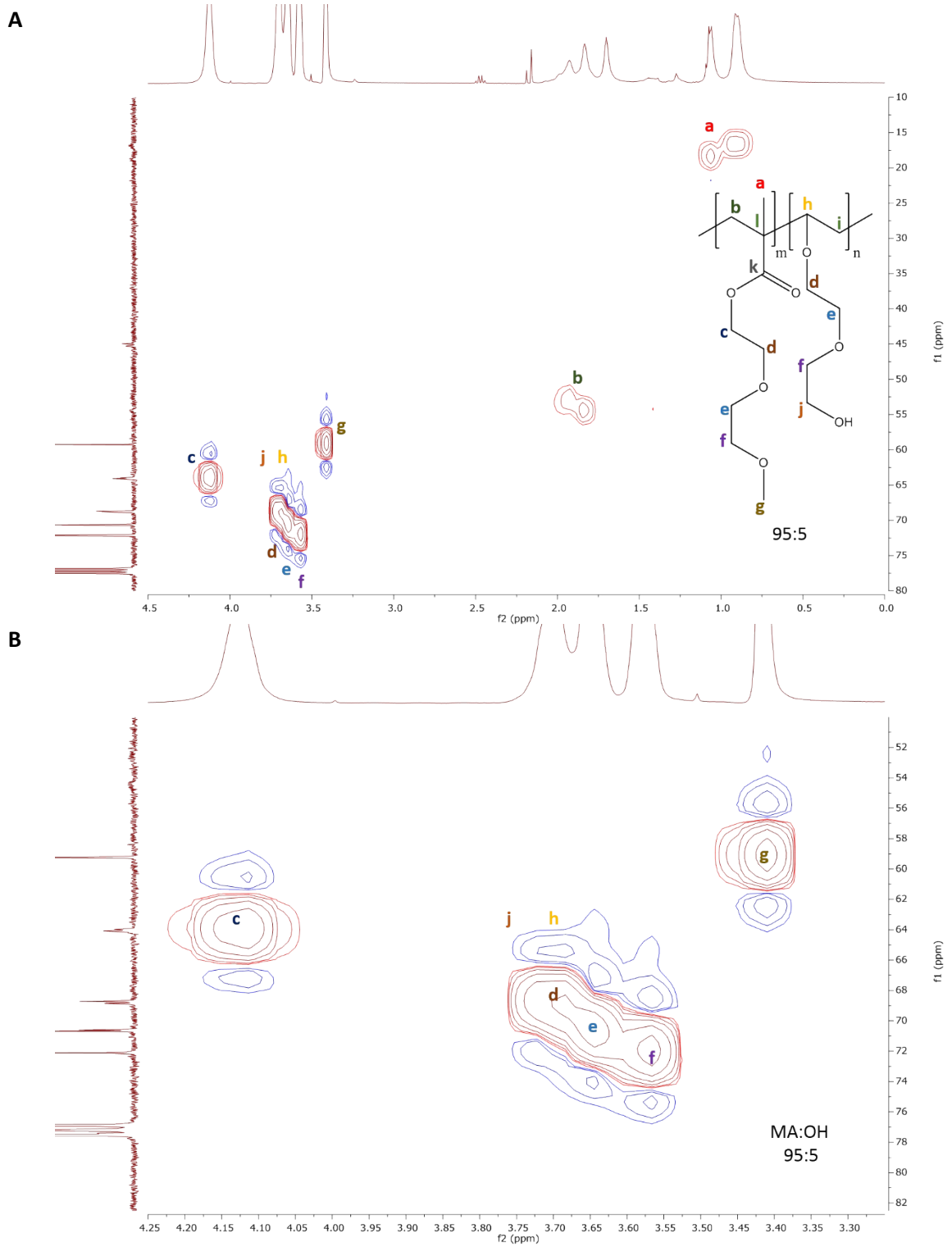

Figure S4. 2D NMR of A) PDEGMA/PDEGOH (98:2) and B) zoomed in 2D NMR of PDEGMA/PDEGOH (98:2). Ratios of monomer used was 95:5, DEGMA:DEGOH respectively.

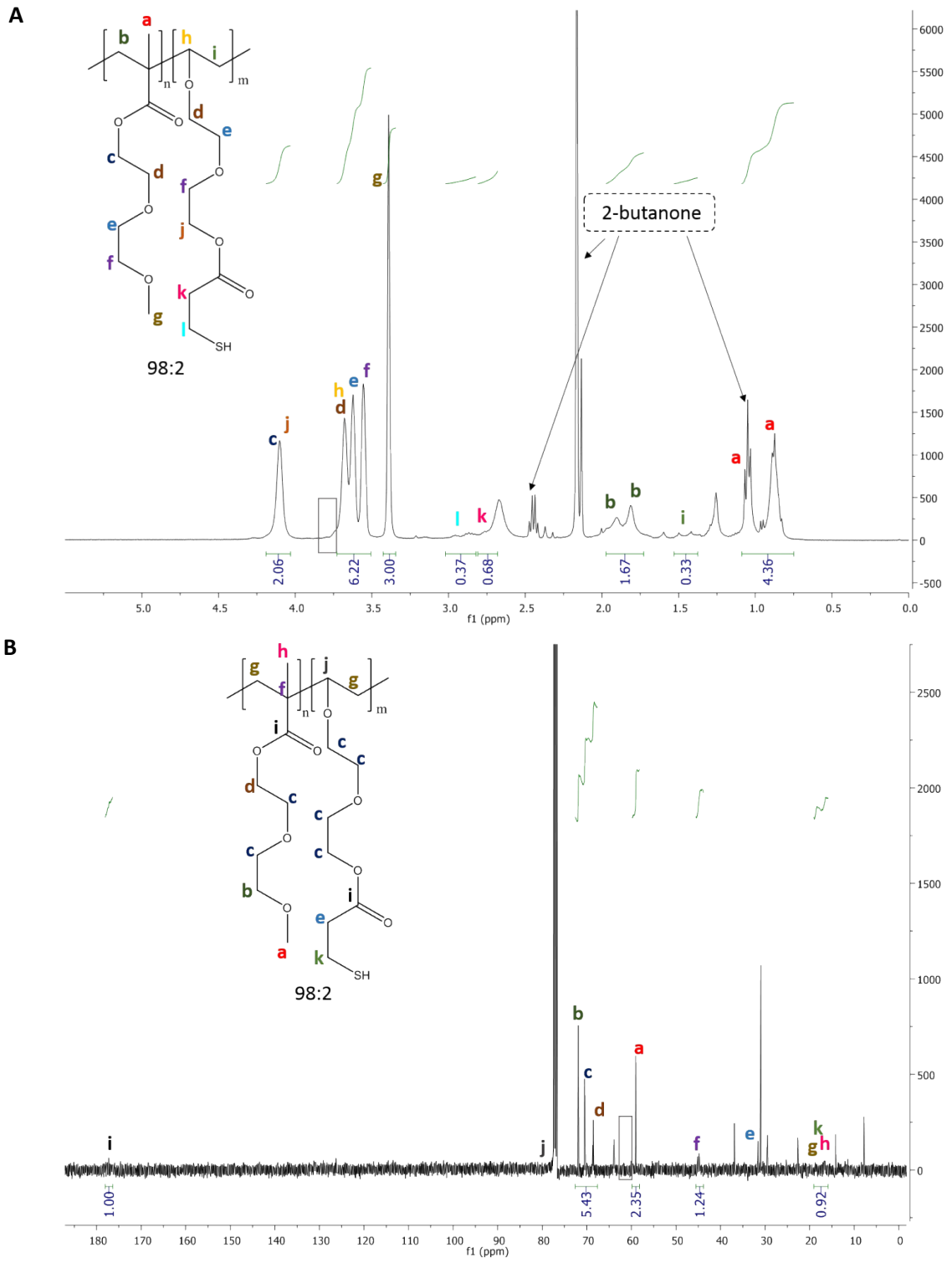

Figure S5. A) H^1-NMR of PDEGMA/PDEGSH (98:2) and B) C^{13} -NMR of PDEGMA/PDEGSH (98:2), synthesised from the PDEGMA/PDEGOH (95:5) polymer starting product.

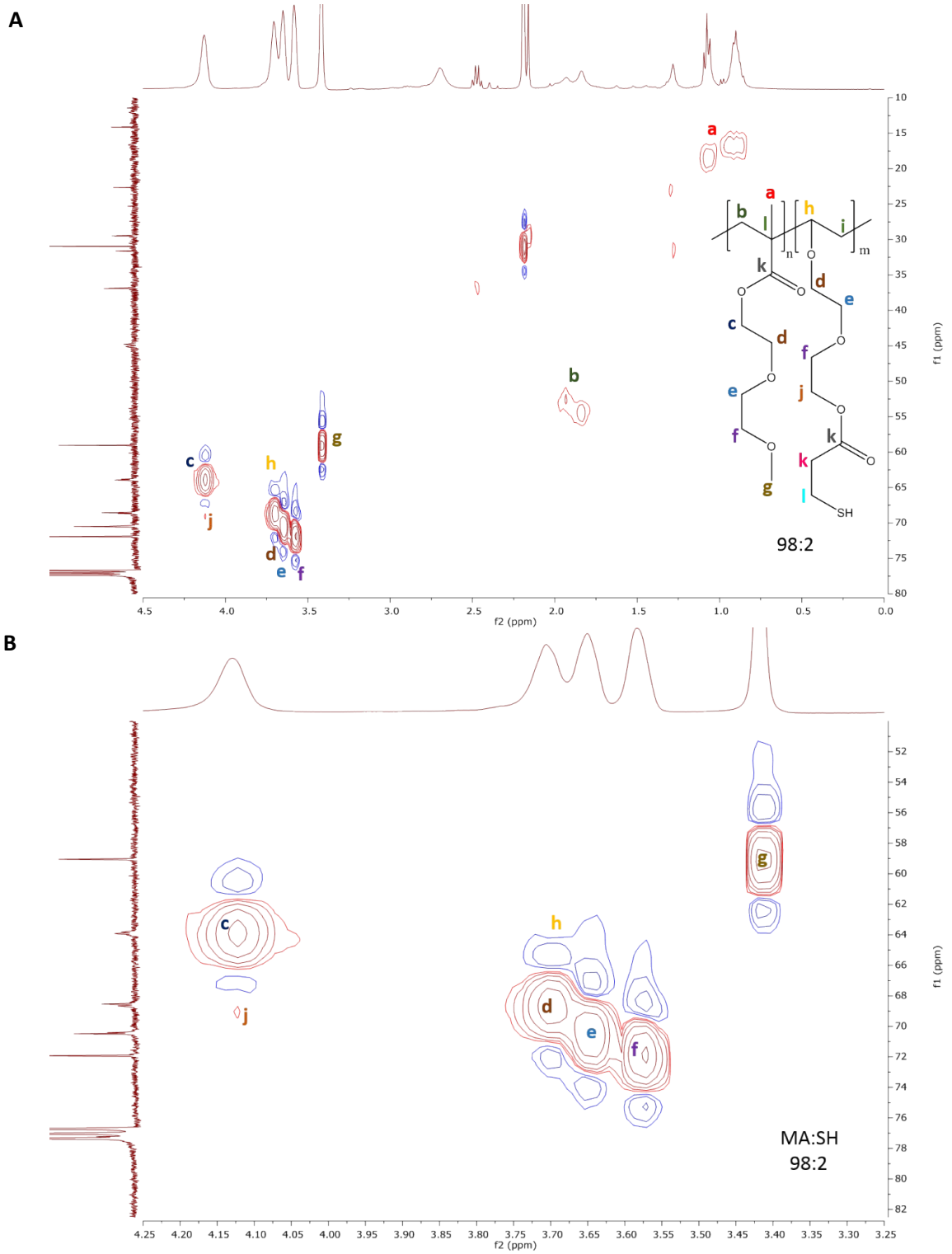

Figure S6. 2D NMR of A) PDEGMA/PDEGSH (98:2) and B) zoomed in 2D NMR of PDEGMA/PDEGSH (98:2), synthesised from the PDEGMA/PDEGOH (95:5) polymer starting product.

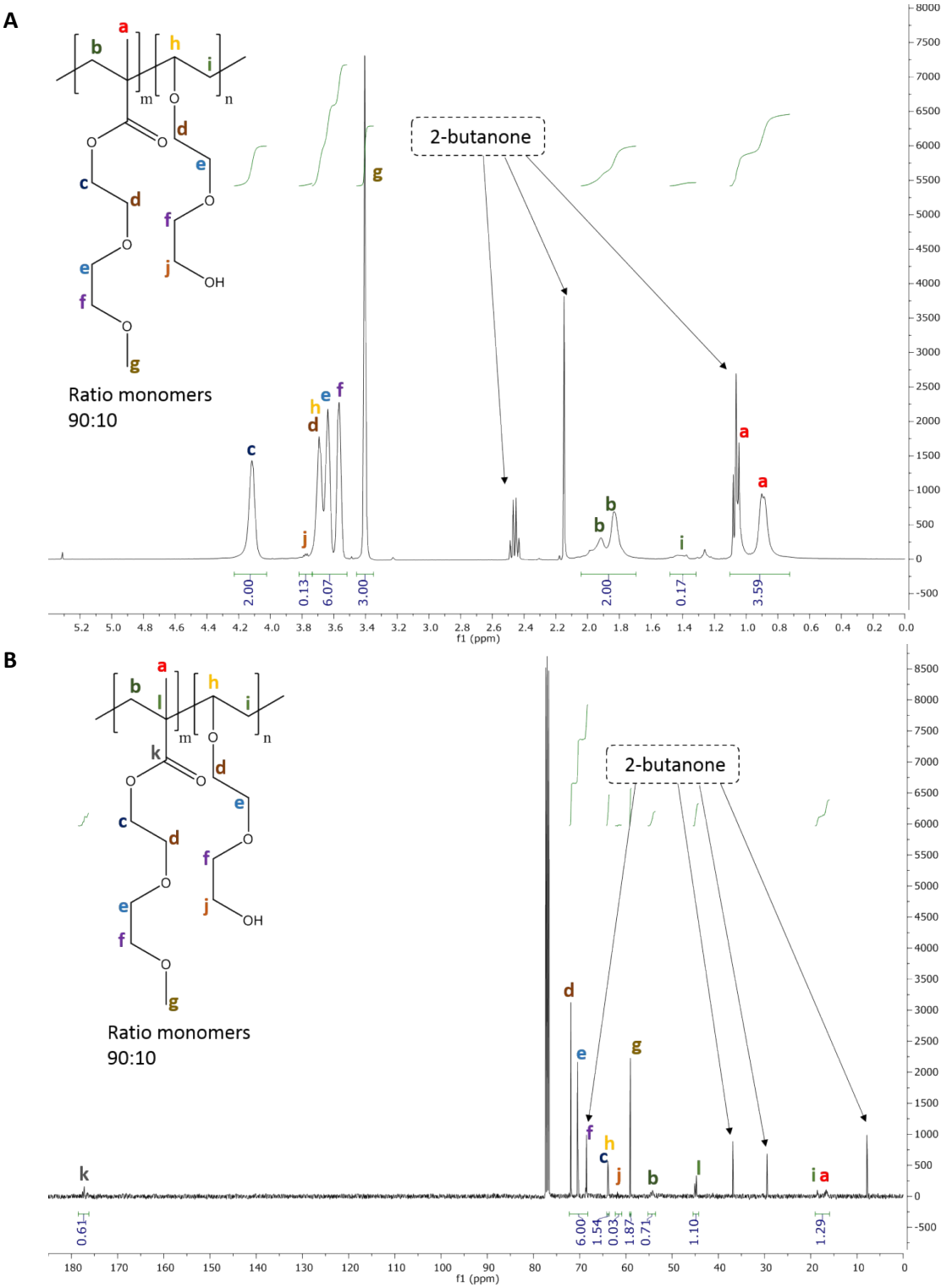

Figure S7. A) H^1-NMR of PDEGMA/PDEGOH (97:3) and B) C^{13} -NMR of PDEGMA/PDEGOH (97:3). Ratios of monomer used was 90:10, DEGMA:PDEGOH respectively.

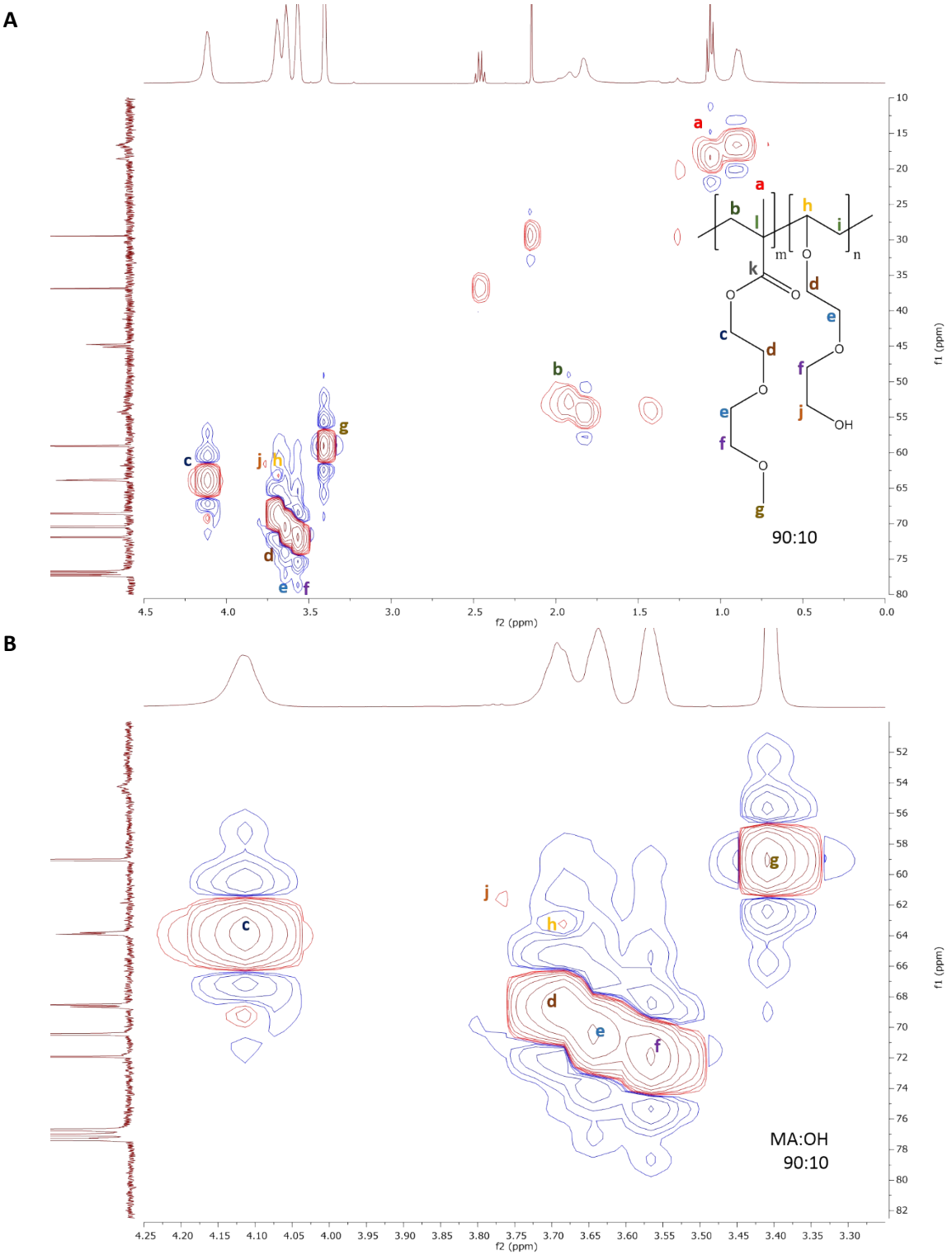

Figure S8. 2D NMR of A) PDEGMA/PDEGOH (97:3) and B) zoomed in 2D NMR of PDEGMA/PDEGOH (97:3). Ratios of monomer used was 90:10, DEGMA:PDEGOH respectively.

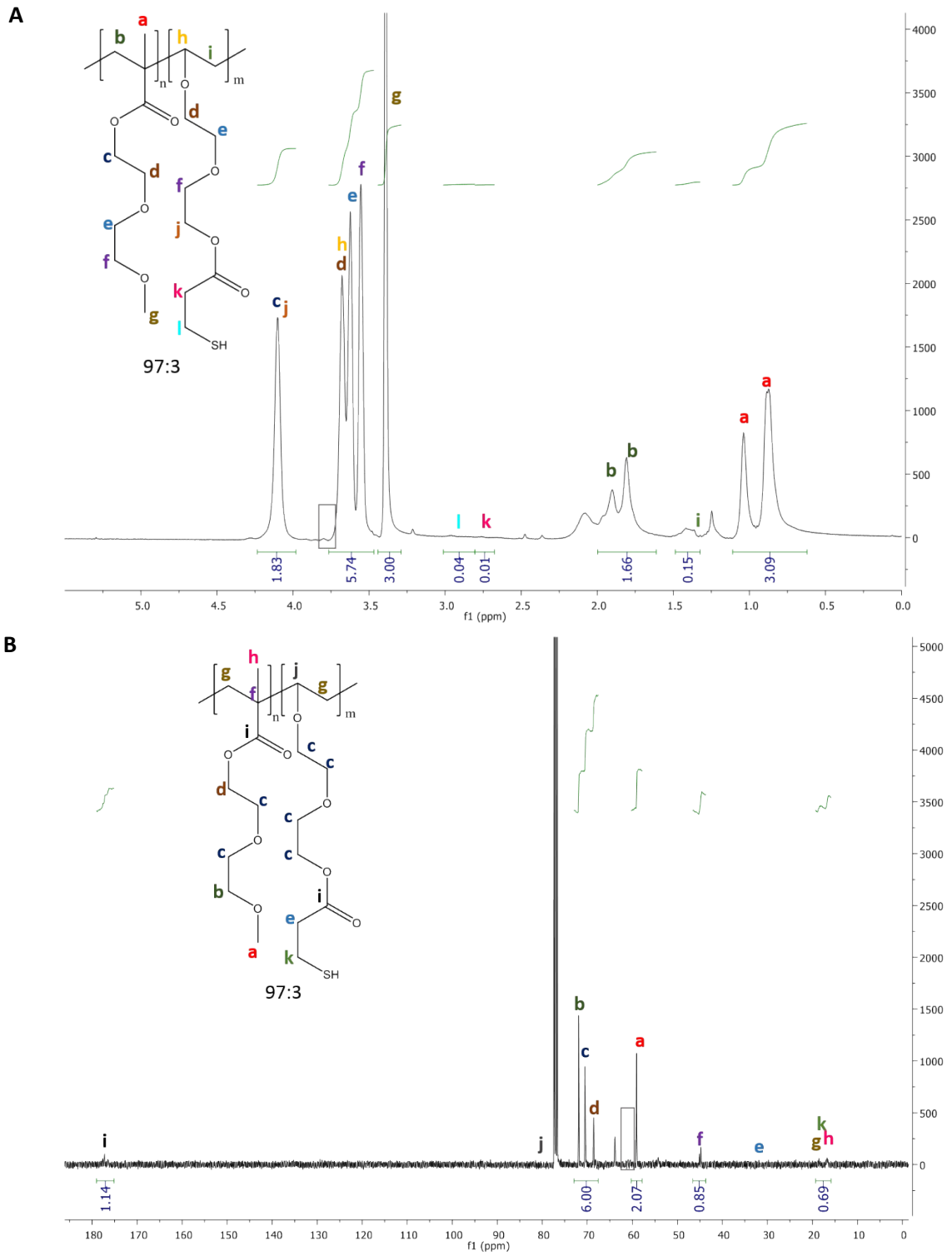

Figure S9. A) H^1-NMR of PDEGMA/PDEGSH (97:3) and B) C^{13} -NMR of PDEGMA/PDEGSH (97:3), synthesised from the PDEGMA/PDEGOH (90:10) polymer starting product.

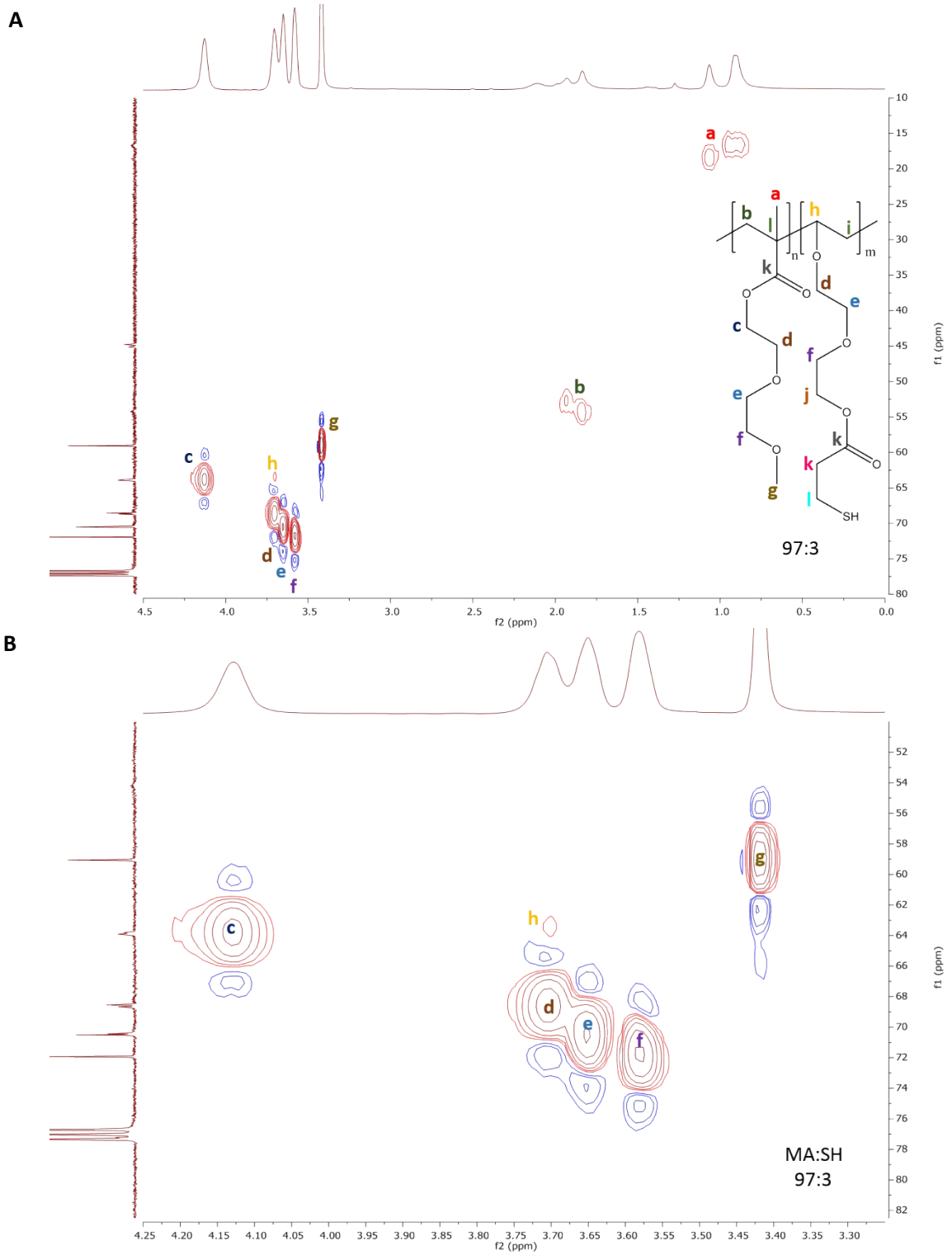

Figure S10. 2D NMR of A) PDEGMA/PDEGSH (97:3) and B) zoomed in 2D NMR of PDEGMA/PDEGSH (97:3), synthesised from the PDEGMA/PDEGOH (90:10) polymer starting product.

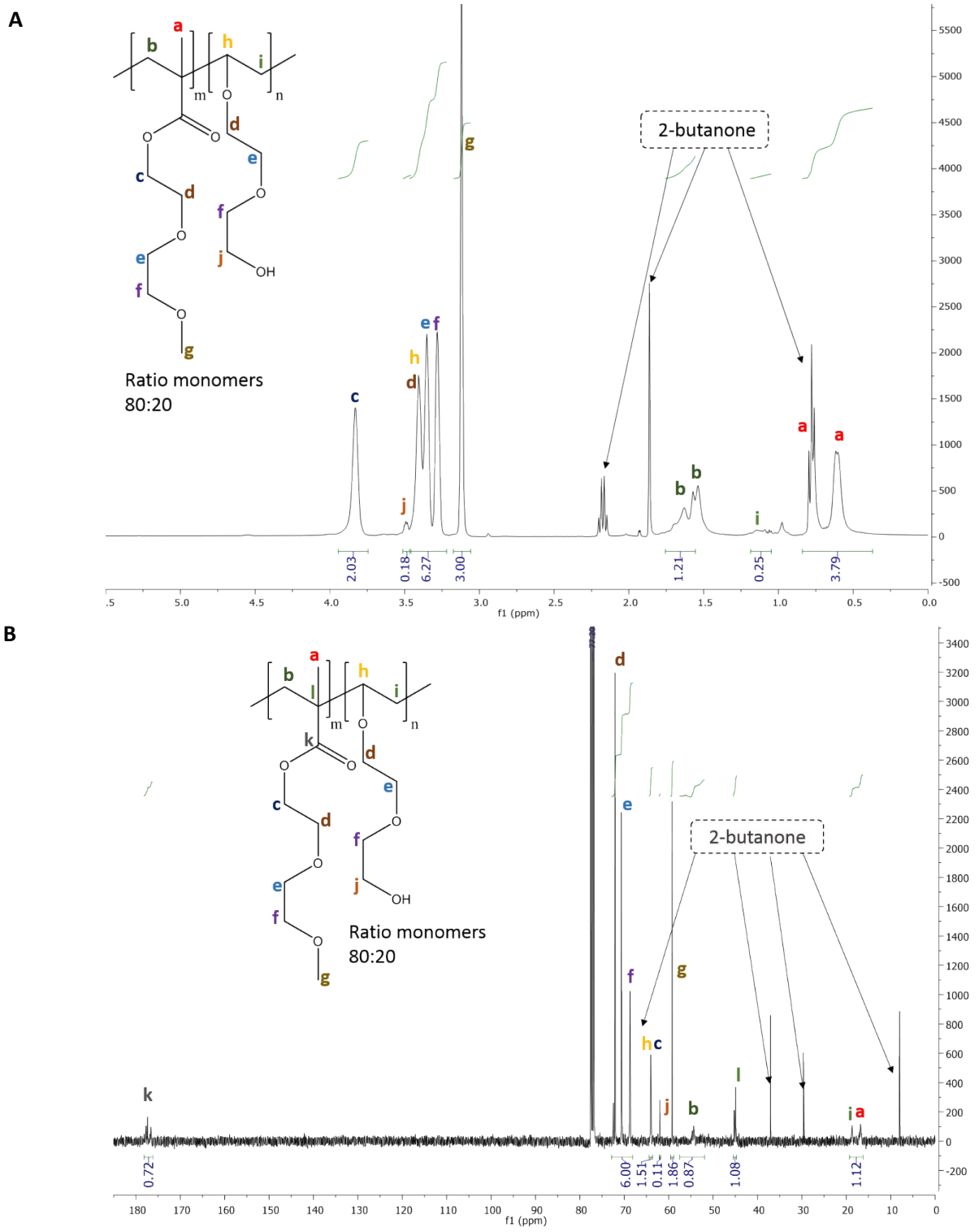

Figure S11. A) H^1-NMR of PDEGMA/PDEGOH (96:4) and B) C^{13} -NMR of PDEGMA/PDEGOH (96:4). Ratios of monomer used was 80:20, DEGMA:PDEGOH respectively.

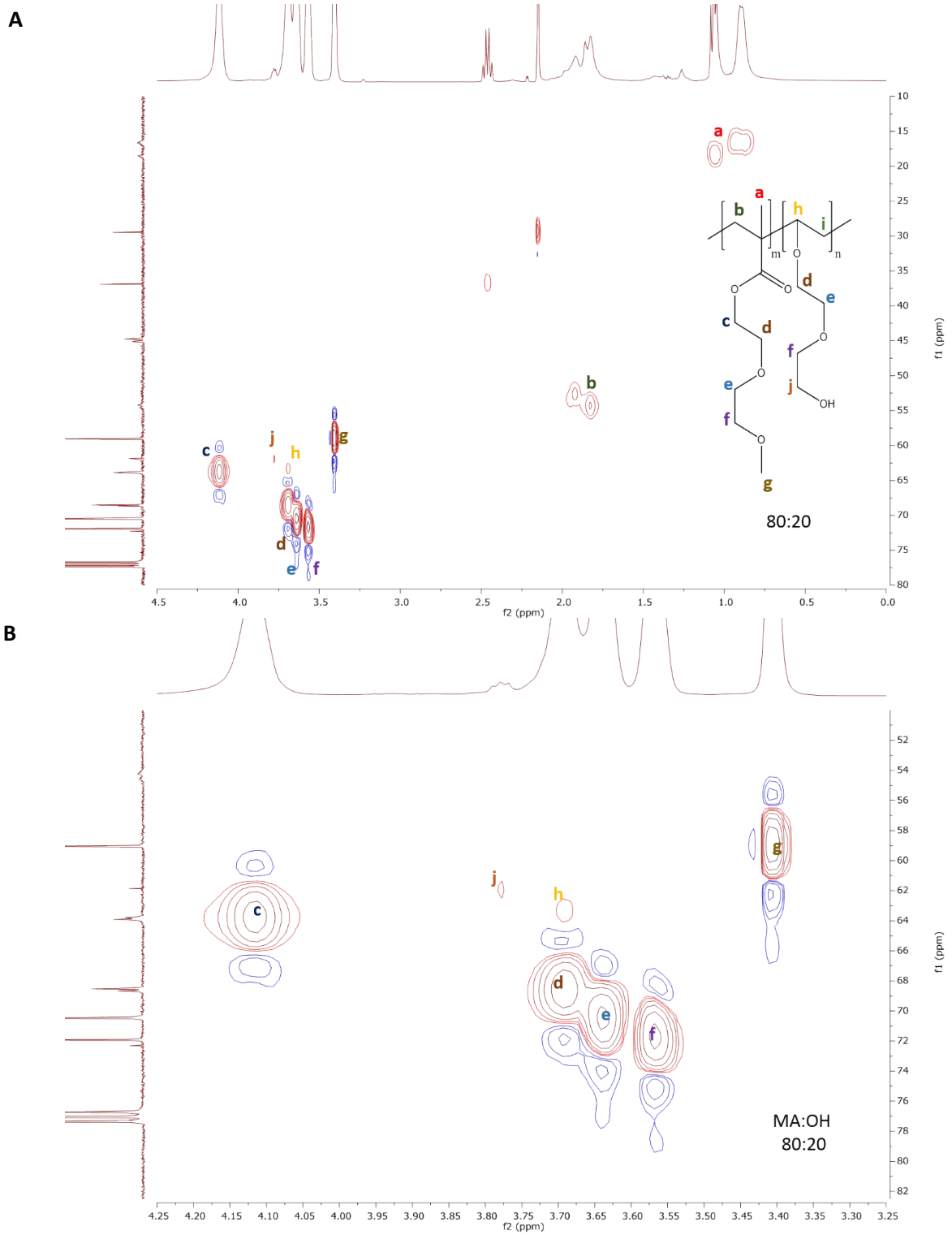

Figure S12. 2D NMR of A) PDEGMA/PDEGOH (96:4) and B) zoomed in 2D NMR of PDEGMA/PDEGOH (96:4). Ratios of monomer used was 80:20, DEGMA:PDEGOH respectively.

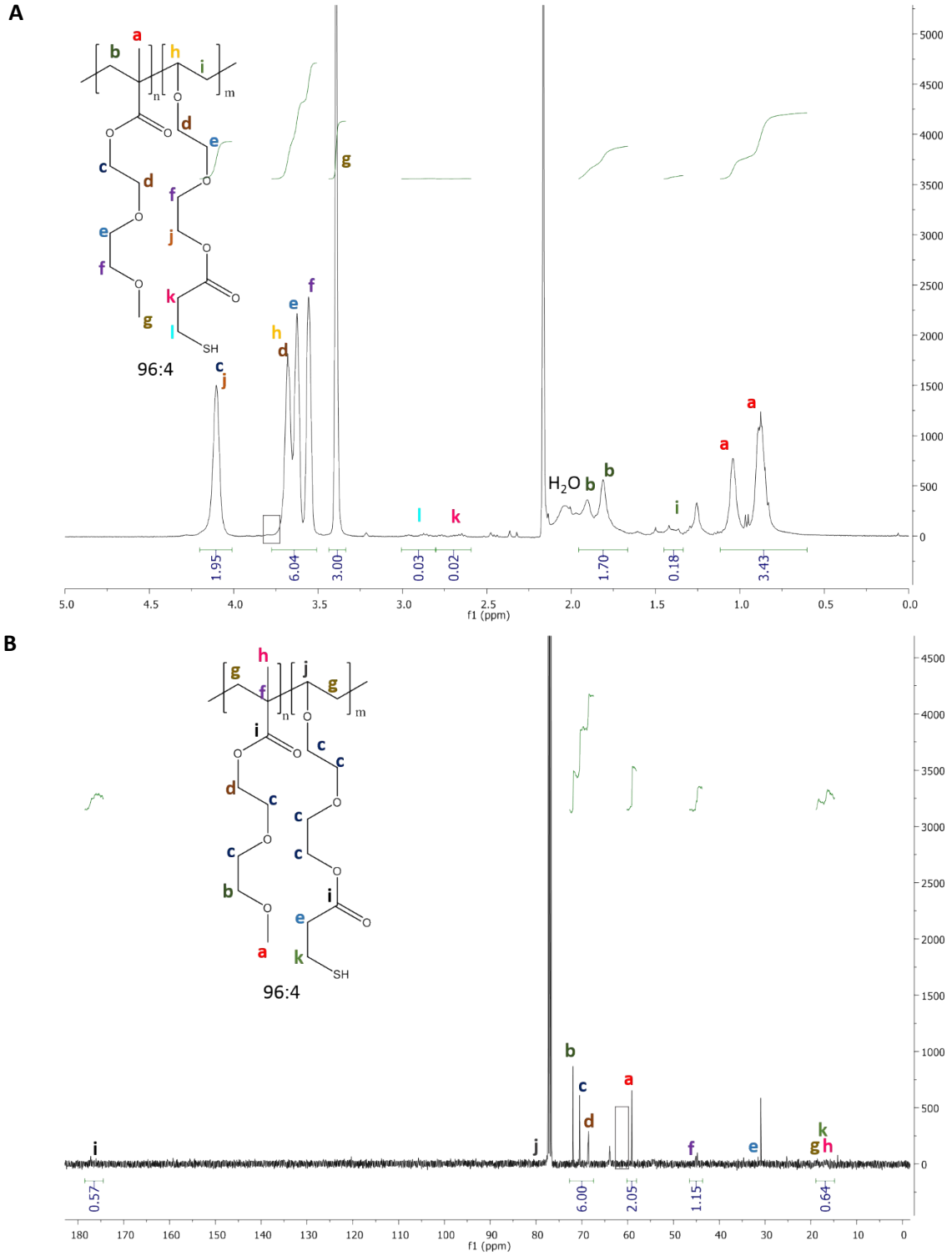

Figure S13. A) H^1-NMR of PDEGMA/PDEGSH (96:4) and B) C^{13} -NMR of PDEGMA/PDEGSH (96:4), synthesised from the PDEGMA/PDEGOH (80:20) polymer starting product.

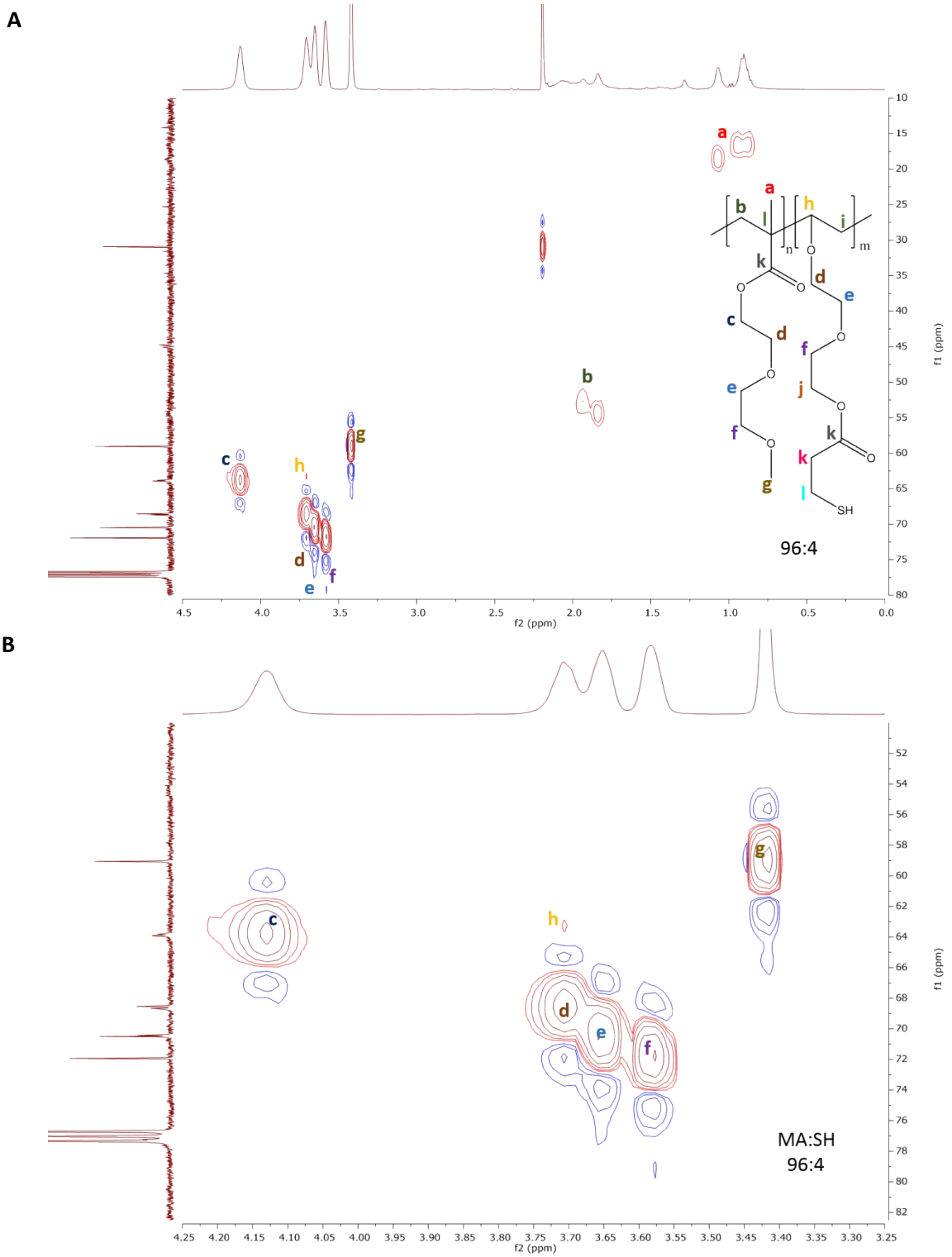

Figure S14. 2D NMR of A) PDEGMA/PDEGSH (96:4) and B) zoomed in 2D NMR of PDEGMA/PDEGSH (96:4), synthesised from the PDEGMA/PDEGOH (80:20) polymer starting product.

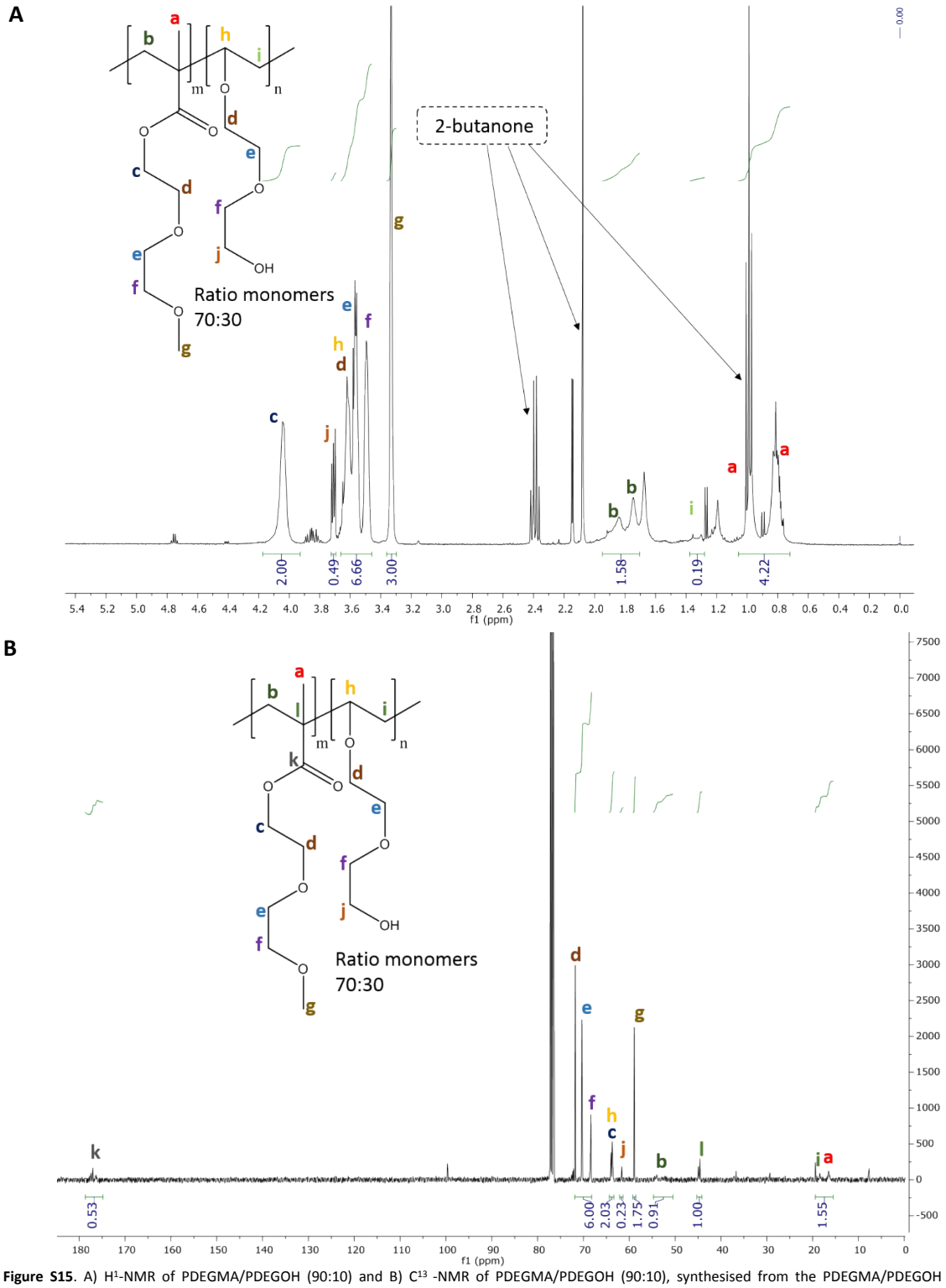

Figure S15. A) H[1]-NMR of PDEGMA/PDEGOH (90:10) and B) C[13] -NMR of PDEGMA/PDEGOH (90:10), synthesised from the PDEGMA/PDEGOH (70:30) polymer starting product.

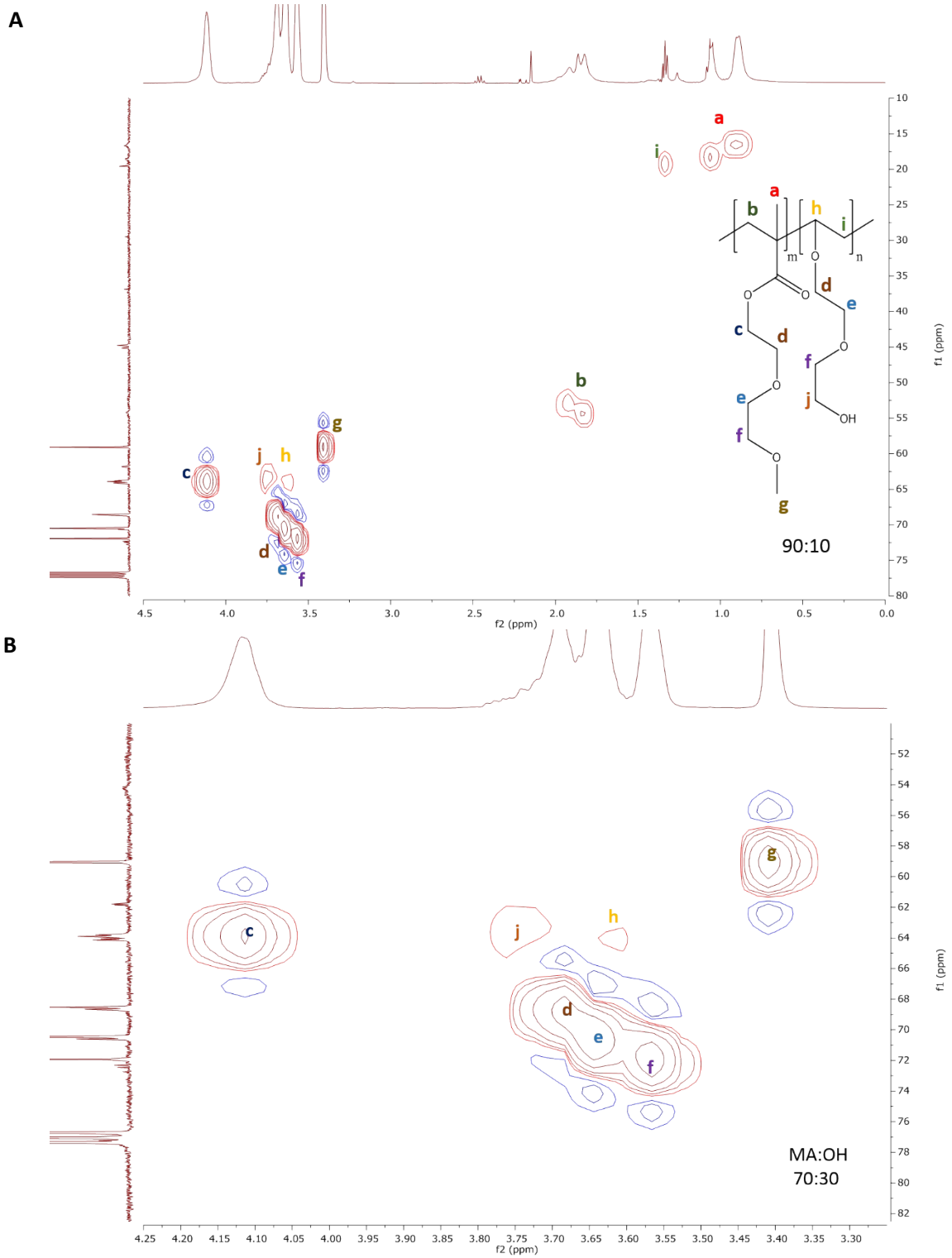

Figure S16. 2D NMR of A) PDEGMA/PDEGOH (90:10) and B) zoomed in 2D NMR of PDEGMA/PDEGOH (90:10), synthesised from the PDEGMA/PDEGOH (70:30) polymer starting product.

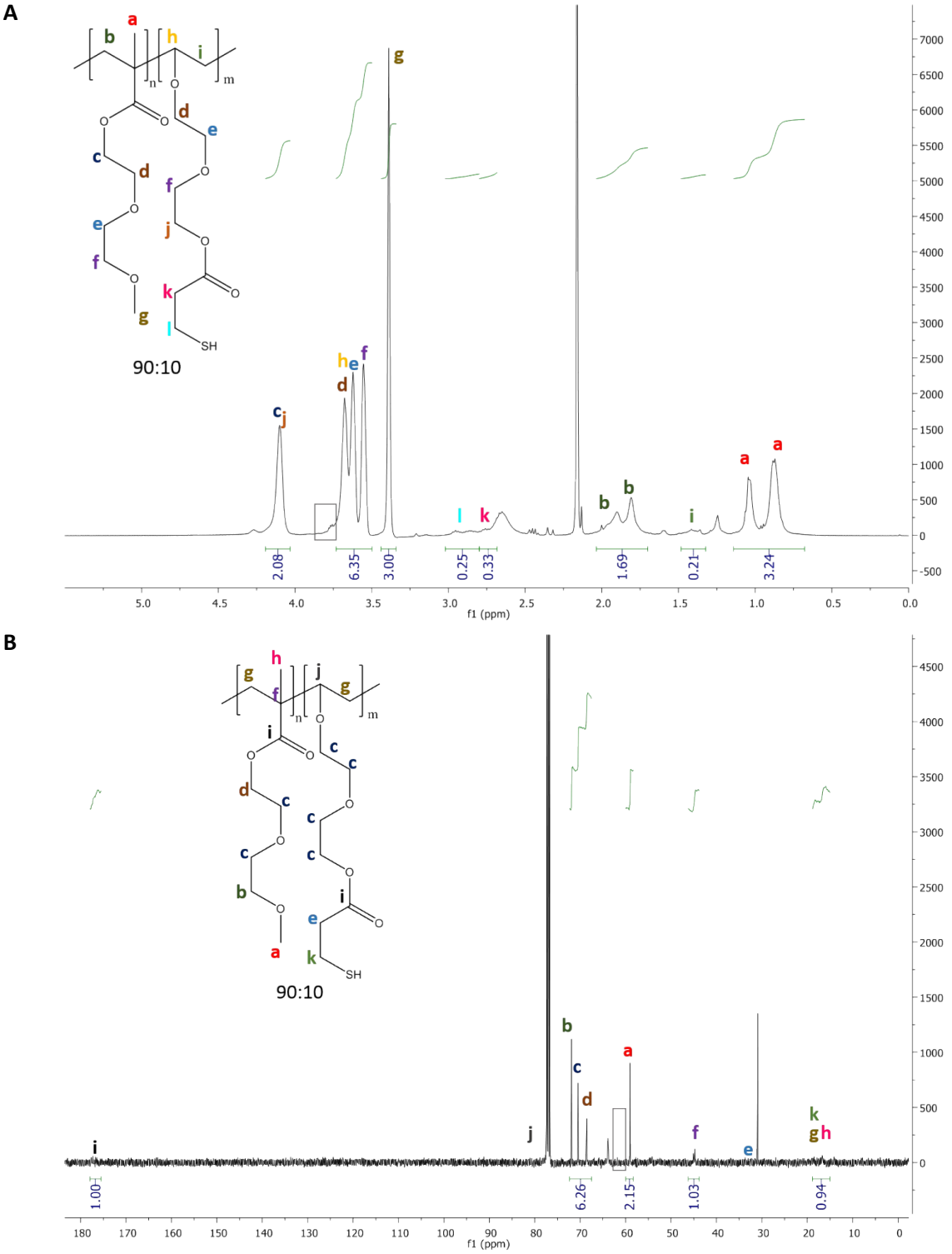

Figure S17. A) H^1-NMR of PDEGMA/PDEGSH (90:10) and B) C^{13}-NMR of PDEGMA/PDEGSH (90:10), synthesised from the PDEGMA/PDEGOH (70:30) polymer starting product.

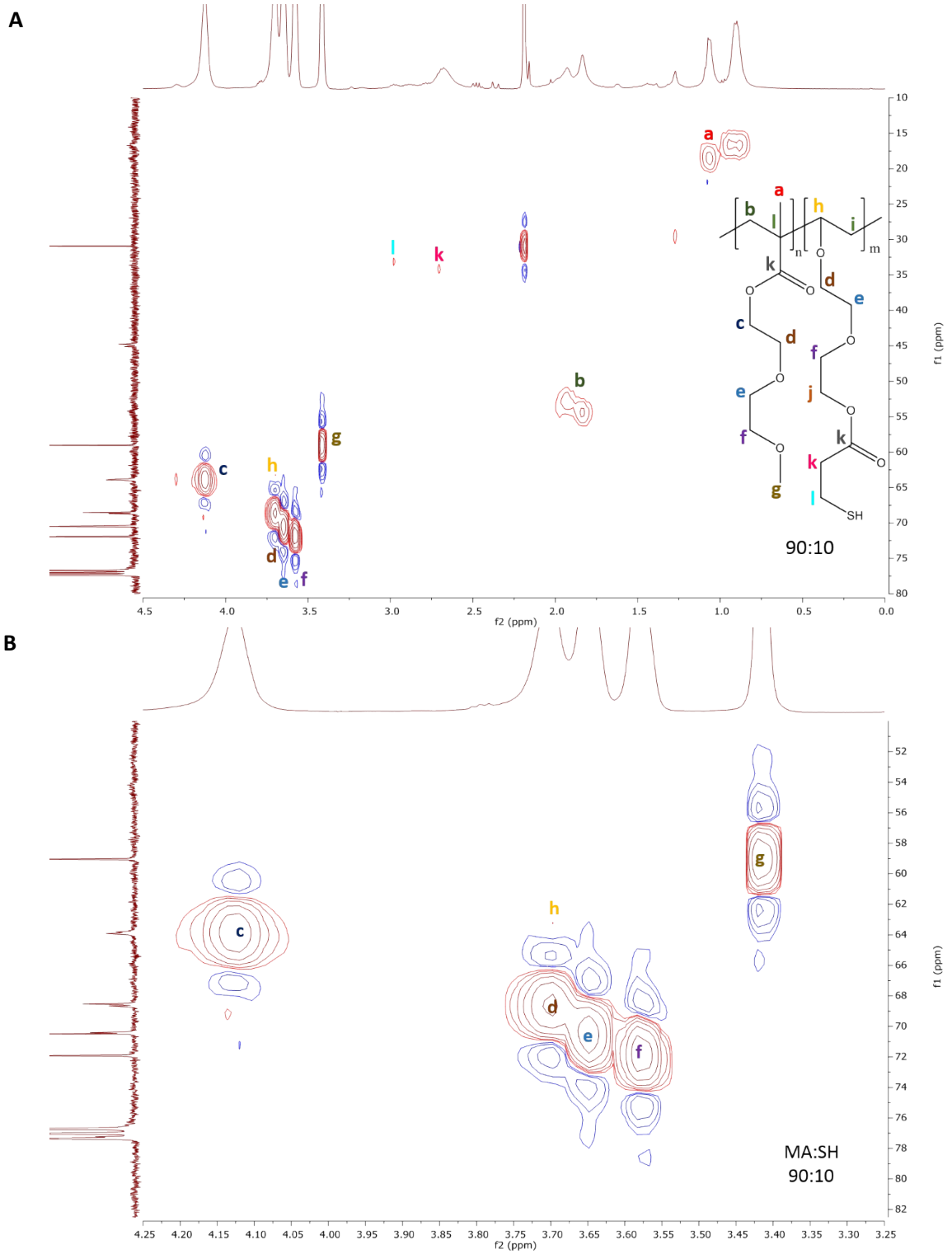

Figure S18. 2D NMR of A) PDEGMA/PDEGSH (90:10) and B) zoomed in 2D NMR of PDEGMA/PDEGSH (90:10), synthesised from the PDEGMA/PDEGOH (70:30) polymer starting product.

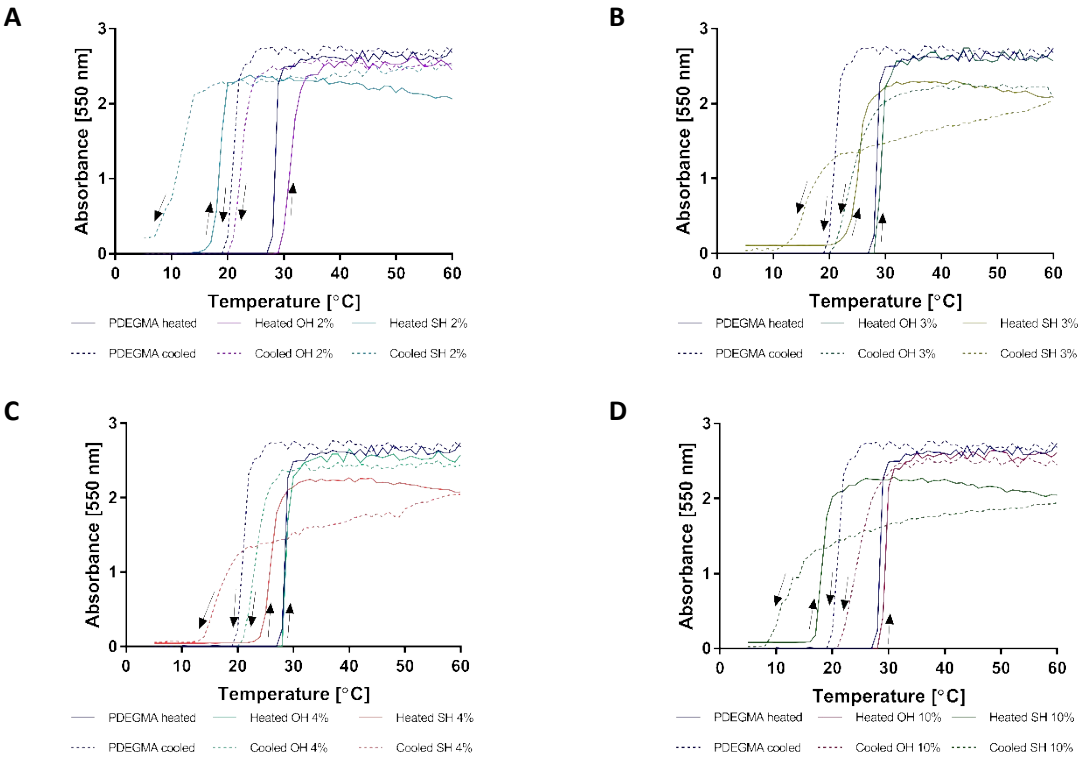

Figure S19. A) H^1-NMR of PDEGMA/PDEGOH (90:10), B) C^{13} -NMR of PDEGMA/PDEGOH (90:10), C) 2D NMR of PDEGMA/PDEGOH (90:10), D) zoomed in 2D NMR of PDEGMA/PDEGOH (90:10), E) H^1-NMR of PDEGMA/PDEGSH (90:10), F) C^{13} -NMR of PDEGMA/PDEGSH (90:10), G) 2D NMR of PDEGMA/PDEGSH (90:10) and H) zoomed in 2D NMR of PDEGMA/PDEGSH (90:10).

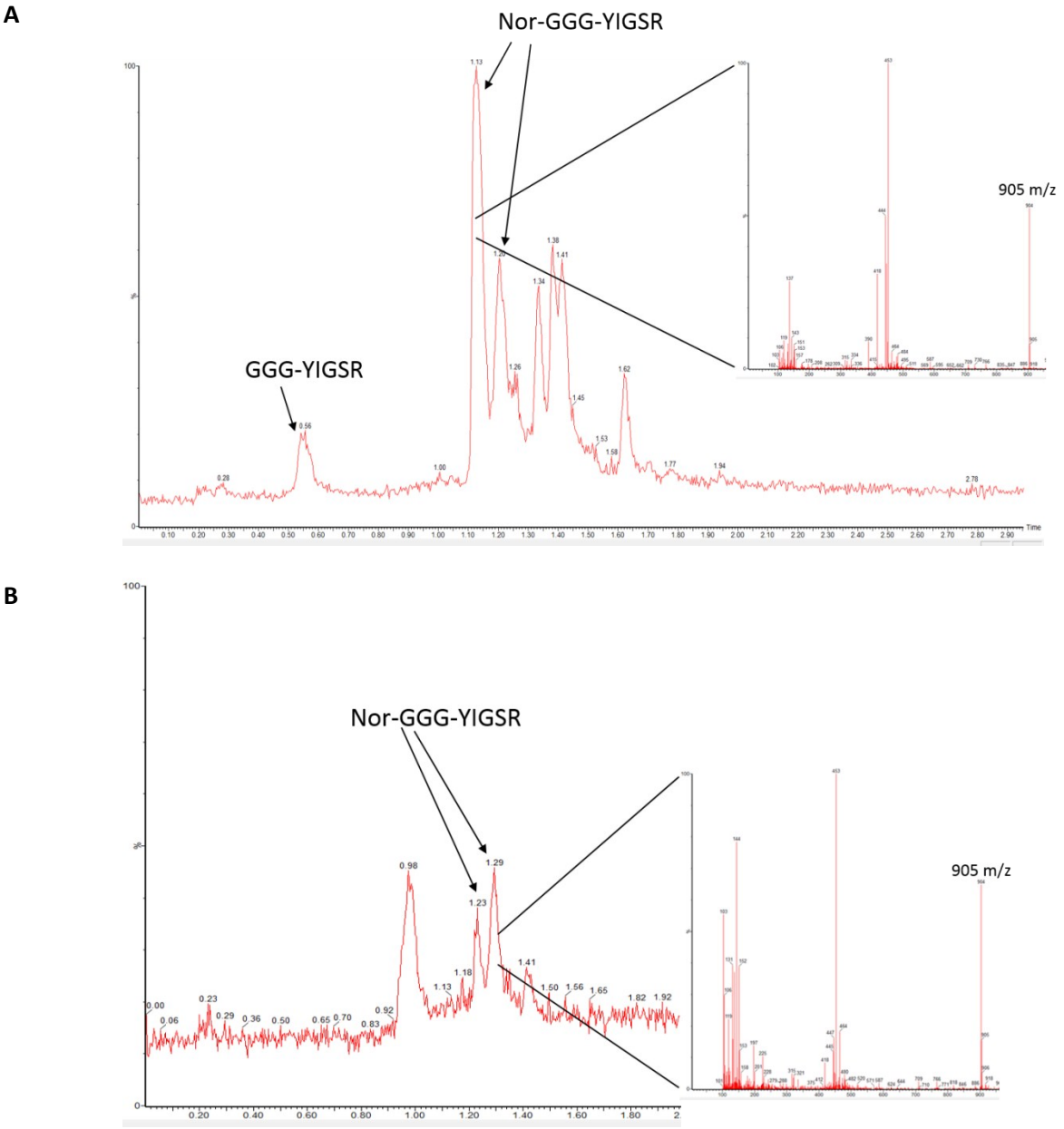

Figure S20. **A)** HPLC-MS spectra before purification Nor-GGG-YIGSR and **B)** HPLC-MS spectra after purification Nor-GGG-YIGSR.

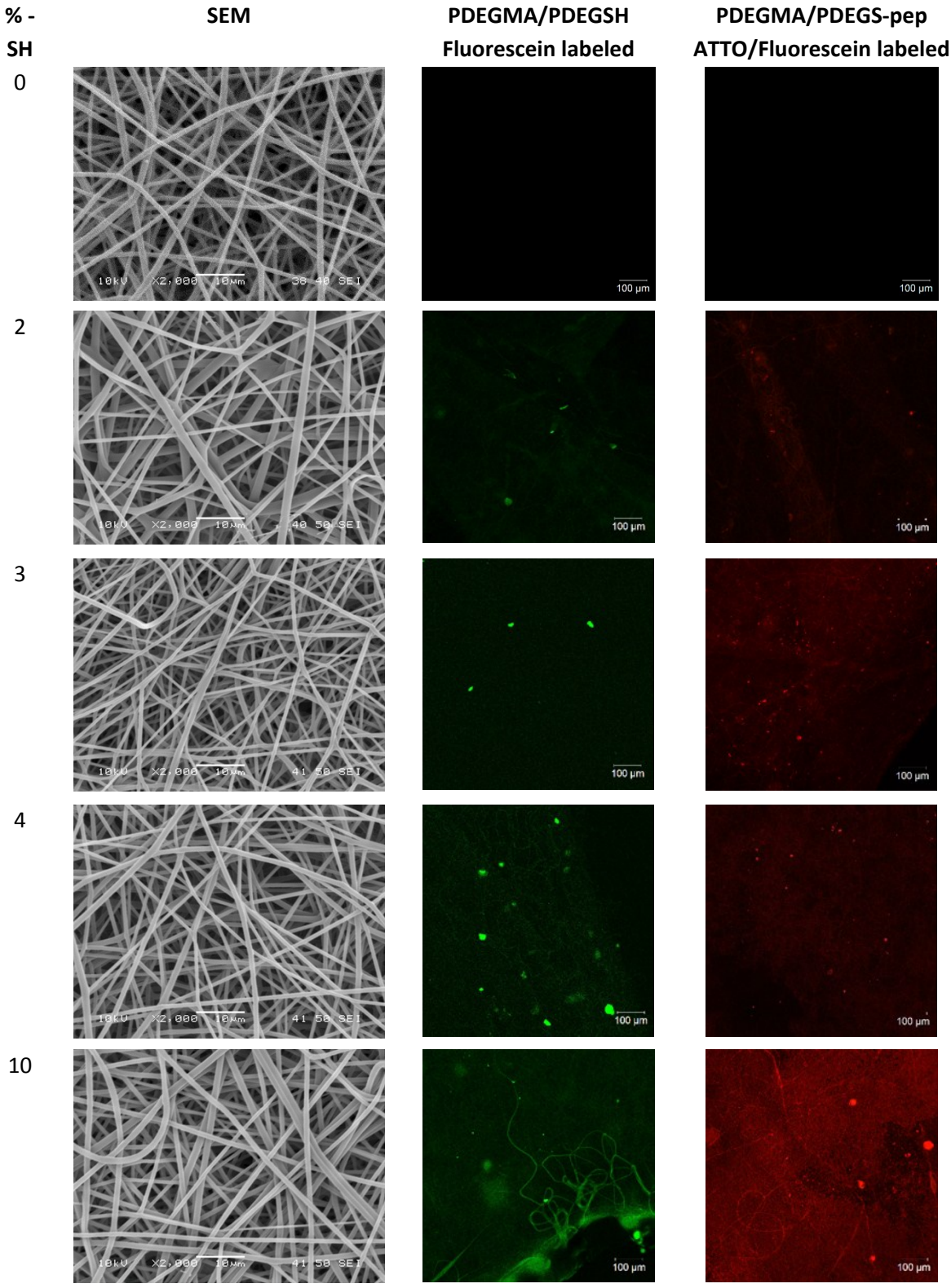

Figure S21. SEM images and average fibre diameters of co-electrospun scaffolds of PLLA with 10 w/v% PDEGMA/PDEGSH at different MA/SH ratios 100:0, 98:2, 97:3, 96:4 and 90:10 PDEGMA/PDEGSH ratios. Scale bar= 10 µm. Fluorescein and ATTO-stained PDEGMA/PDEGSH and derivatised PDEGMA/PDEGS-Nor-GGG-YIGSR co-electrospun scaffolds confocal images. Thiol presence was observed by Fluorescein staining on the PDEGMA/PDEGSH scaffolds and peptide presence after the thiol-ene reaction was observed by ATTO staining with subsequent Fluorescein staining to confirm full conversion. Scale bar = 100 µm.

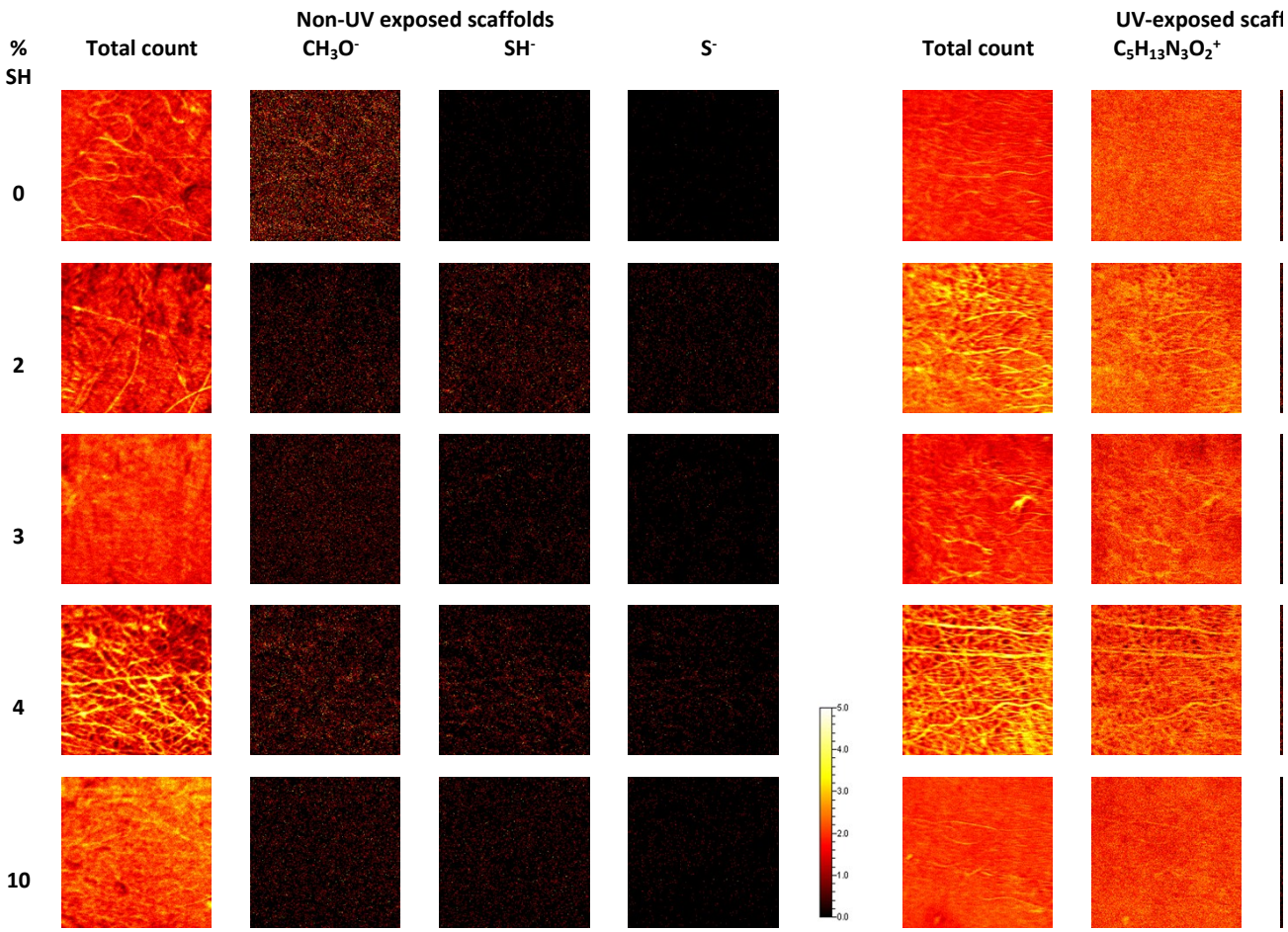

Figure S22. ToF-SIMS measurement of the free thiol containing scaffolds. **A)** Total count images**, B)** CH_3O^- group of the PDEGMA thermo-responsive part of the co-polymer PDEGMA/PDEGSH, **C)** SH^- group of the PDEGSH and **D)** S^- group of the PDEGSH (image surface area is 500 x 500 nm, normalized to MC of 200 for total count and MC of 3 for all other signals). Tof-SIMS measurement of the derivatised scaffolds. **E)** Total count images, **F)** $C_5H_{13}N_3O_2^+$ lysine (R) part of the Nor-GGG-YIGSR sequence, **G**) $C_{17}H_{27}N_7O_6^+$ IGSR part of the Nor-GGG-YIGSR sequence and **H)** $C_{26}H_{37}N_8O_9^+$ GGG-YIGSR part of the Nor-GGG-YIGSR sequence (image surface area is 500 x 500 µm, normalised to MC of 200 for total count and MC of 3 for all other signals).

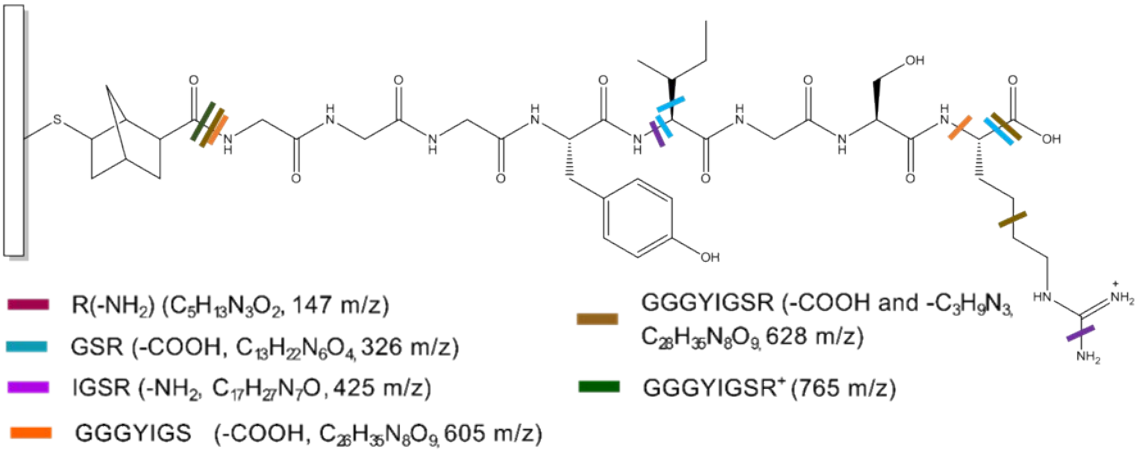

Figure S23. Schematic representation of peptide fragments observed during ToF-SIMS analysis.

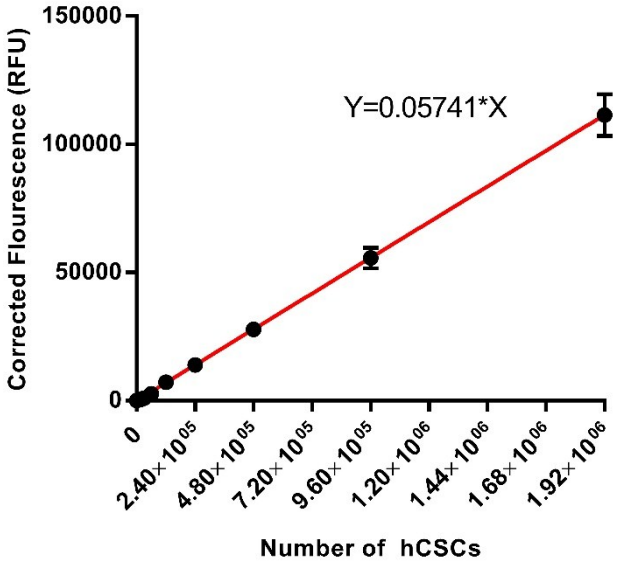

Figure S24. Standard curve for Alamar blue cell viability assays.

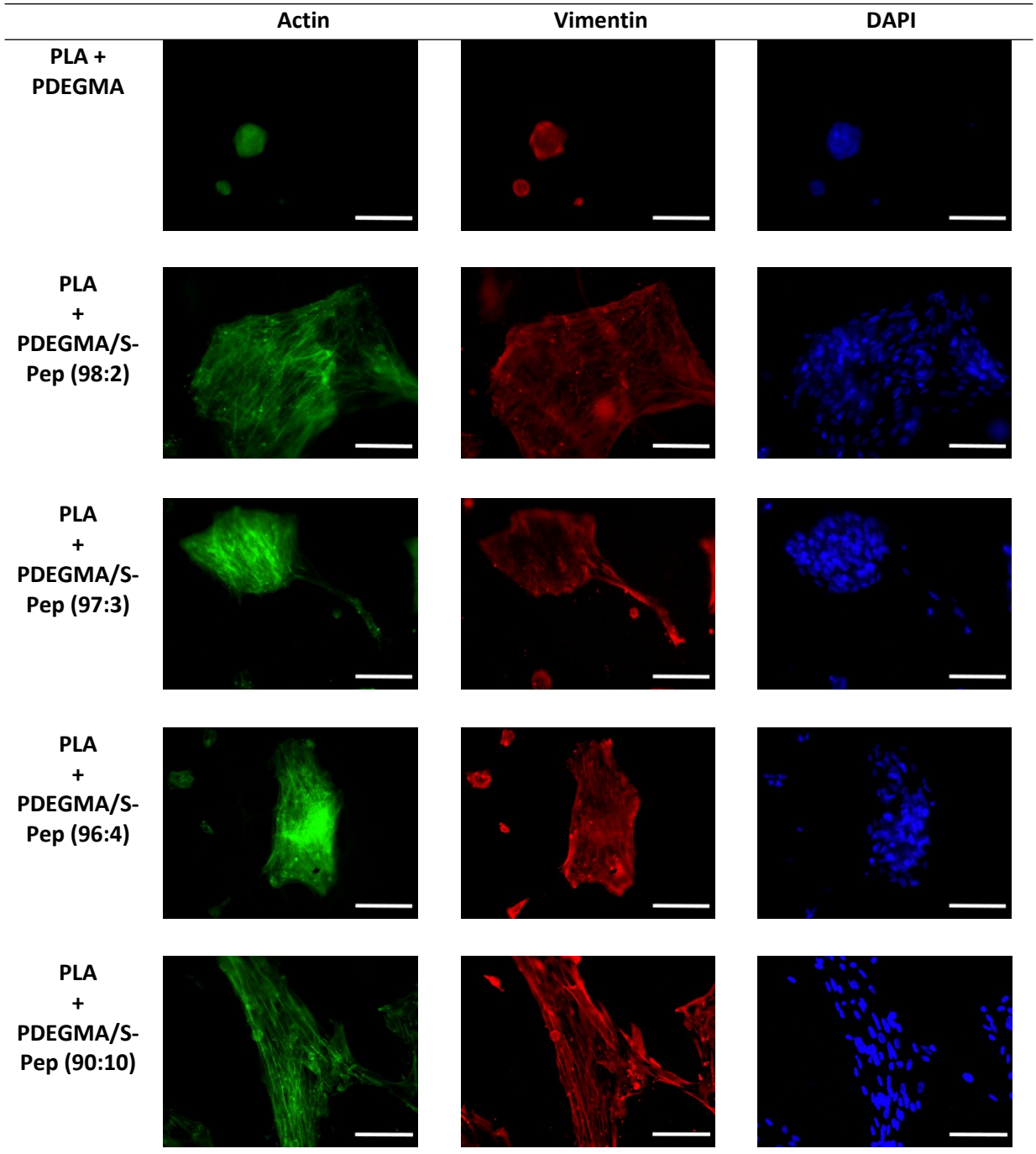

Figure S25. Representative confolcel images of hCSCs cultures on PLA fibrous scaffodls with different surface chemistry and their correspoding morphology. Cells immuno-stained with Actin, Vimentin and the nuclei with DAPI. Scale bar = 100 µm.

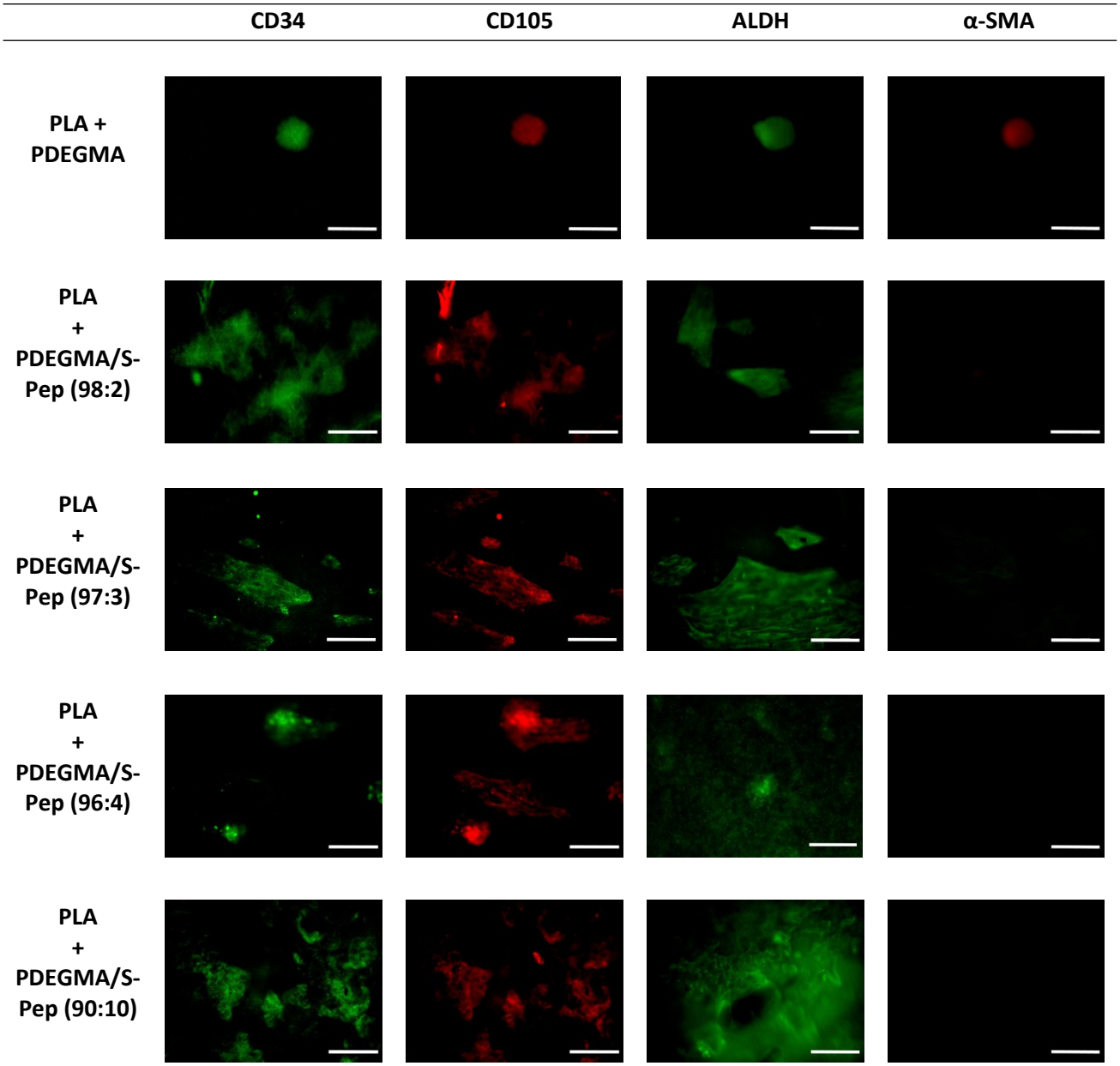

Figure S26. Representative confolcel images of hCSCs cultures on PLA fibrous scaffodls with different surface chemistry and their correspoding morphology. Cells immuno-stained with Actin, Vimentin and the nuclei with DAPI. Scale bar = 100 µm. 0SH= PLA scaffold with PDEGMA. 10SH= PLA scaffold with PDEGMA/PDEGSH and no peptide and 10S-P= PLA scaffolds with PDEGMA/PDEGS-NOR-GGG-YIGSR peptide attached.

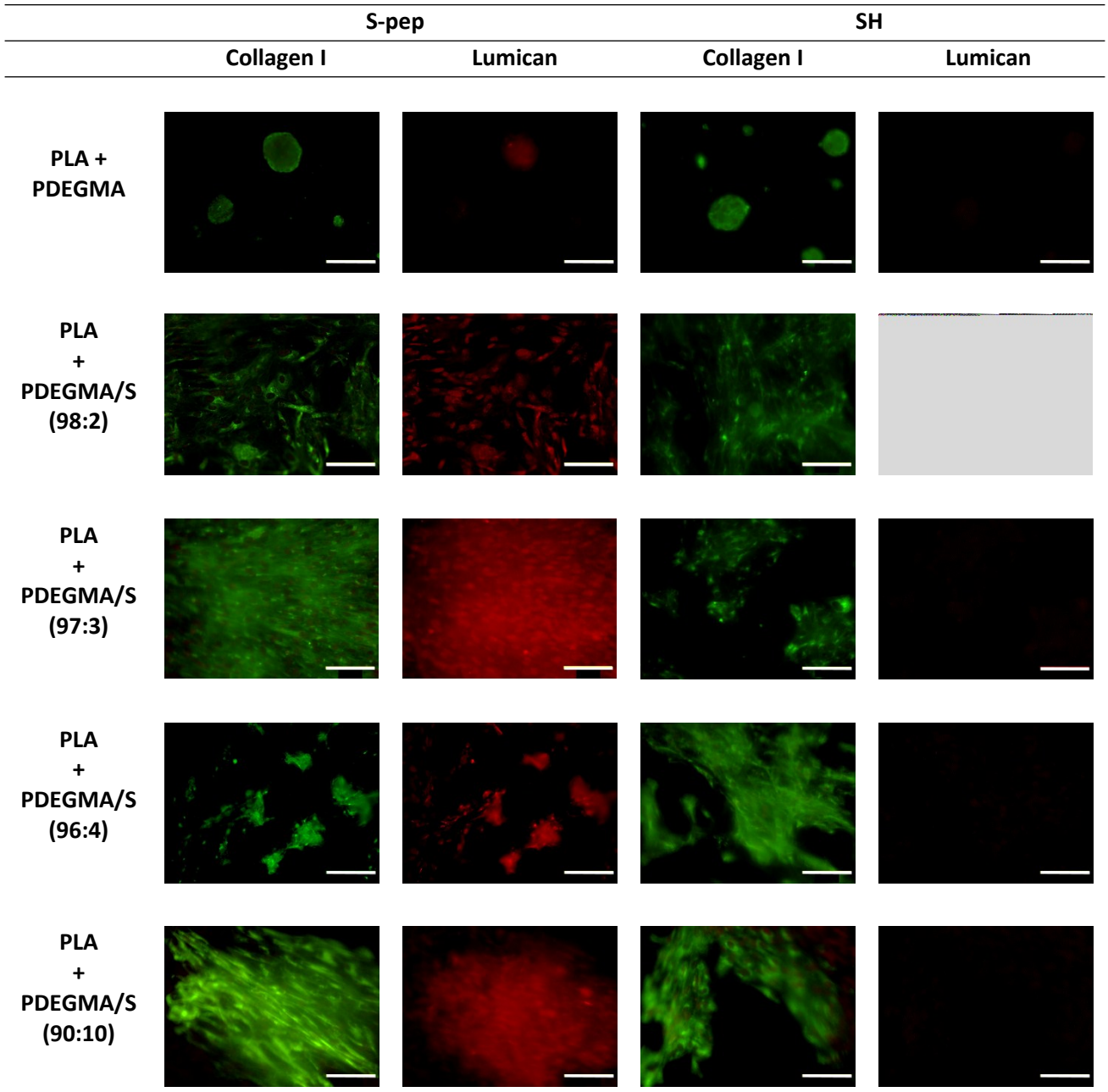

Figure S27. Representative confolcel images of hCSCs cultures on PLA fibrous scaffodls with different surface chemistry and their correspoding morphology. Cells immuno-stained with Actin, Vimentin and the nuclei with DAPI. Scale bar = 100 µm. 0SH= PLA scaffold with PDEGMA. 10SH= PLA scaffold with PDEGMA/PDEGSH and no peptide and 10S-P= PLA scaffolds with PDEGMA/PDEGS-NOR-GGG-YIGSR peptide attached.

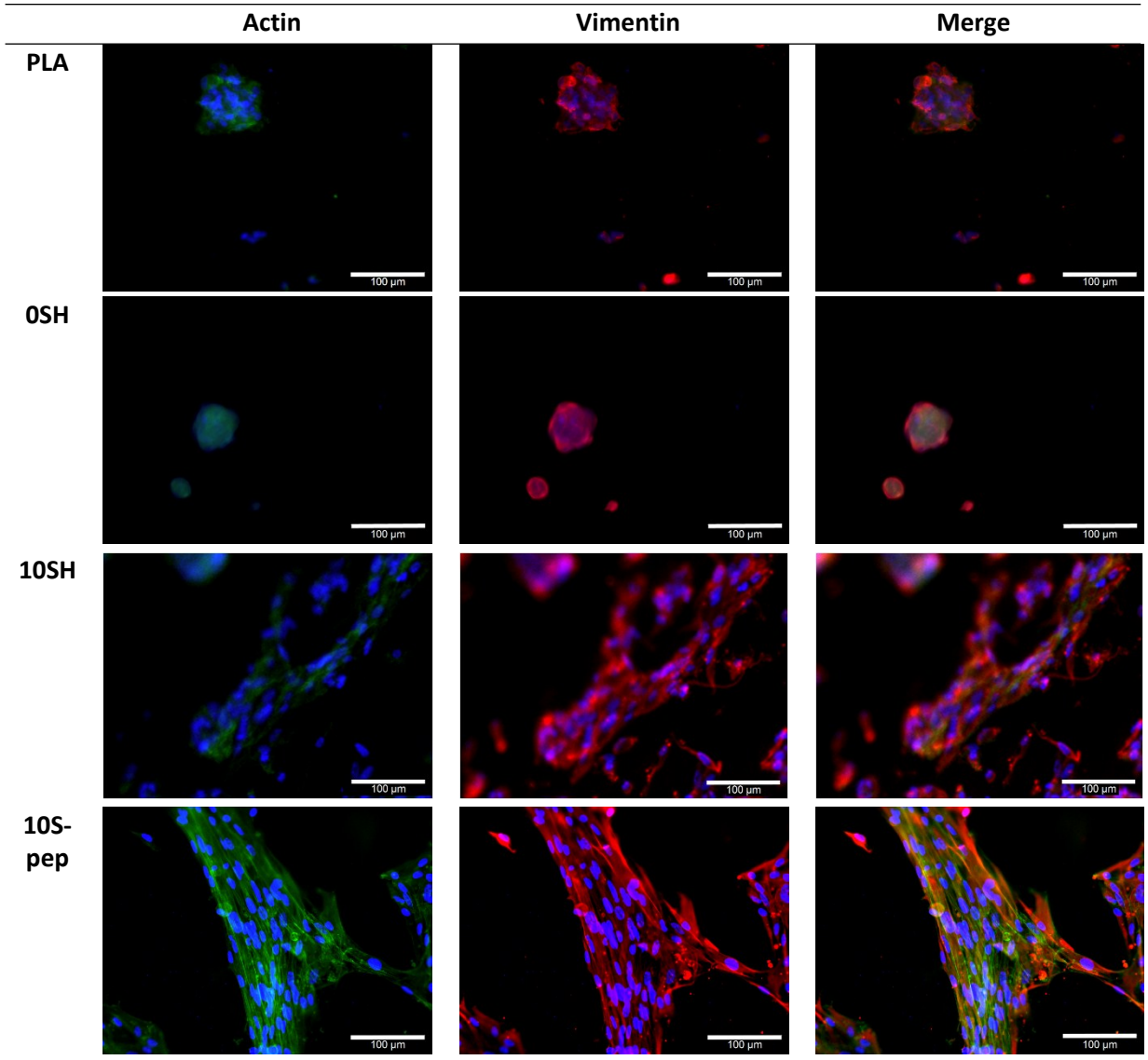

Figure S28. Representative confolcel images of hCSCs cultures on PLA fibrous scaffodls with different surface chemistry and their correspoding morphology. Cells immuno-stained with Actin, Vimentin and the nuclei with DAPI. Scale bar = 100 µm. 0SH= PLA scaffold with PDEGMA. 10SH= PLA scaffold with PDEGMA/PDEGSH and no peptide and 10S-P= PLA scaffolds with PDEGMA/PDEGS-NOR-GGG-YIGSR peptide attached.

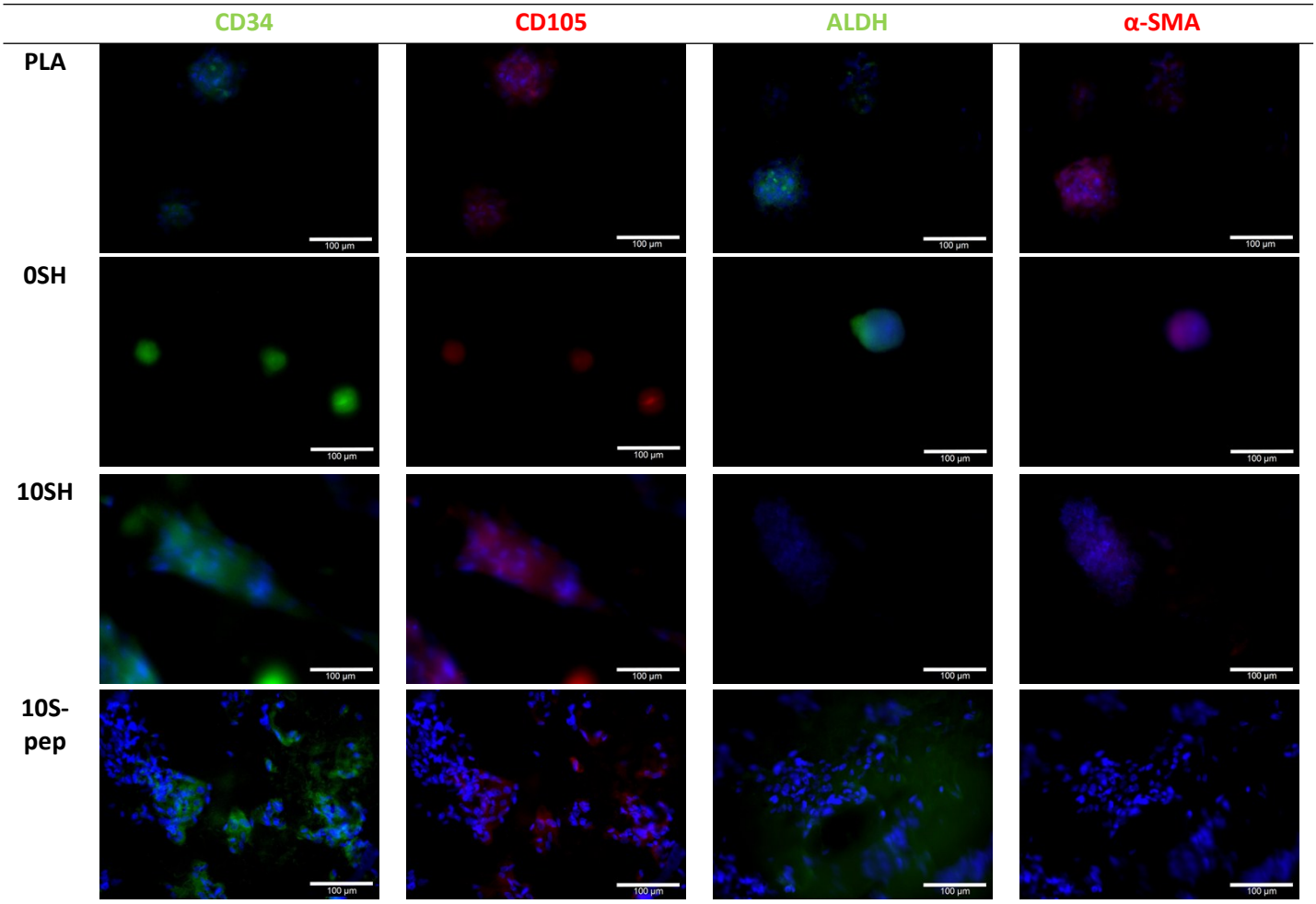

Figure S29. Representative confolcel omages of hCSCs cultures on PLA fibrous scaffodls with different surface chemistry and their correspoding phenotypic expression. Cells immuno-stained with the keatocyte markets CD34 and ALDH, MSC marker CD105 and the fibroblast marker α-SMA. The nucleis were stained with DAPI. Scale bar = 100 µm. 0SH= PLA scaffold with PDEGMA. 10SH= PLA scaffold with PDEGMA/PDEGSH and no peptide and 10S-P= PLA scaffolds with PDEGMA/PDEGS-NOR-GGG-YIGSR peptide attached.

Table S1. Details of primary, secondary antibodies and chemical staining used in immunostainings.

Antigen	Clone	Source (Catalog No.)	Host	Conjugates
		Primary antibodies		
Vimentin	V9	Vector Laboratories (VPV684)	Mouse	-
CD34	QBEND10	Sigma-Aldrich (SAB4700736)	Mouse	-
CD105	Polyclonal	R&D Systems (AF1097)	Goat	-
ALDH3A1	Polyclonal	Abcam (Ab76976)	Rabbit	-
α-SMA	1A4	LSBio (LS-C210475)	Mouse	-
Lumican	Polyclonal	R&D Systems (AF2846)	Goat	-
Collagen I	Polyclonal	Abcam (Ab34710)	Rabbit	-
		Secondary antibodies		
Mouse IgG[a]	Polyclonal	Life Technologies (A-21202)	Donkey	AF[b]-488
Goat IgG[a]	Polyclonal	Life Technologies (A-11056)	Donkey	AF[b]-546
Rabbit IgG[a]	Polyclonal	Life Technologies (A-10040)	Donkey	AF[b]-546
		Chemical Staining		
Phalloidin	-	Thermo-Fisher (A12379)	-	AF[b]-488
DAPI	-	Thermo-Fisher (D1306)	-	-

[a] IgG, immunoglobine; [b] AF, Alexa FLour

Table S2. List of primary and secondary antibodies and their counter combinations for A) and B) phenotype marker expression, C) morphology staining and D) ECM expression of fixed hCSCs cells to confirm the phenotype of the cells in interaction with the electrospun scaffolds. Antibody dilution is presented in the brackets. m= mouse, g= goat, r= rabbit.

Double staining sample code	Primary antibody	Secondary antibody	Primary antibody	Secondary antibody	Cells permeabilised (Yes/No)
A	CD34-m (1:200)	AF488-m (1:300)	CD105-g (1:200)	AF546-g (1:300)	No
B	ALDH3A1-r (1:100)	AF488-r (1:300)	α-SMA-m (1:200)	AF594-m (1:300)	Yes
C	-	Phalloidin AF488 (1:60)	Vimentin-m (1:100)	AF594-m (1:300)	Yes
D	Collagen I-r	AF488-r (1:300)	Lumican-g (1:100)	AF546-g (1:300)	Yes

Table S3. GPC data of PDEGMA/PDEGOH polymers in THF

% of -OH	Mn [kDa]	Mp [kDa]	Mw [kDa]	PD [Đ]
0	31	43	59	2.1
2	35	56	79	2.2
3	34	59	86	2.5
4	30	52	75	2.5
10	16	33	43	2.7

Table S4. Transition temperature (T_t) estimated from cloud-point measurements of all different PDEGMA, PDEGMA/PDEGOH and PDEGMA/PDEGSH polymer ratios when heated and cooled.

	Ratio % -OH or -SH	Heated	Cooled
PDEGMA	0	28	22
PDEGMA/PDEGOH	2	27	23
	3	25	24
	4	27	26
	10	28	25
PDEGMA/PDEGSH	2	18	11
	3	22	18
	4	25	19
	10	18	13
PDEGMA/PDEGS-Nor-GGG-YIGSR	4	25	19

Table S5. Representative range, average and standard deviation (SD) of the different scaffolds fibre diameters.

% -SH	Fibre diameter range in nm	Average fibre diameter in nm	±SD
0	450-1000	789	219
2	550-2700	1150	492
3	470-1660	887	220
4	570-2230	994	267
10	650-3100	1187	325

Table S6. XPS data from the four different electrospun scaffolds. Pure PLA, blend PLA and PDEGMA, blend PLA and PDEGMA/PDEGSH and the conjugated blend PLA and PDEGMA/PDEGS-peptide (n=3 scaffold areas per scaffold).

	O 1s (532.4 eV)	N 1s (399.8 eV)	C 1 s (284.9 eV)	S 2p (167.9 eV)
PLA	33.5	-	66.5	-
PLA + PDEGMA	31.3	-	68.7	-
PLA + PDEGMA/PDEGSH	31.2	-	68.7	0.1
PLA + PDEGMA/PDEGS-peptide	31.1	0.2	68.7	-

Table S7. Negative and positive secondary ions reported by ToF-SIMS from the co-electrospun scaffolds of the different chemical compositions

			Assignment deviation [ppm]									
	m/z	**Structure**	**PLA**	**0SH**	**2SH**	**3SH**	**4SH**	**10SH**	**0S-P**	**2S-P**	**3S-P**	**4S-P**	**10S-P**
PLA	55.022	$C_3H_3O^-$	-15.4	25	-15.8	-39.3	5.8	-16.6	-22.3	-11.6	-59.3	-6.3	-9.7
	56.028	$C_3H_4O^-$	28.3	97.1	56.9	33.3	86.5	43	112.9	100.3	84.7	118.7	84.4
PDEGMA	31.026	CH_3O^+	-	-49.1	-55.6	31	36.4	-127.6	34.6	11.9	48.5	20.5	-5.2
	44.050	$C_2H_4O^+$	-	4	-7.5	-34.1	9.2	-6.1	-22.3	-25.1	-60.1	-6.3	-20.7
PDEGSH	31.969	S^-	-	-30.4	-16.3	-101.2	-88.9	-65.9	-206.9	-144.6	-129.5	140.2	-125
	32.984	SH^-	-	311.9	122.6	112.2	104.5	152	330.1	161.1	173.3	242	196.6
Peptide	32.984	CNO^-	-	-	-	-	-	-	141.3	130.7	102.9	136.6	132.7
	147.091	$C_5H_{13}N_3O_2^+$	-	-	-	-	-	-	-65.0	-58.6	-59.2	-63.7	-76.3
	425.258	$C_{17}H_{27}N_7O_2^+$	-	-	-	-	-	-	128.5	138.7	167.3	167.1	166.9
	605.236	$C_{26}H_{35}N_8O_9^+$	-	-	-	-	-	-	-59.4	-64.5	-75.2	-68.9	-77.5
	627.207	$C_{28}H_{35}N_8O_9^+$	-	-	-	-	-	-	-72.7	-86.9	-88.9	-93.3	-93.4
	765.366	$C_{32}H_{51}N_{11}O_{11}^+$	-	-	-	-	-	-	-36.7	20.9	6	0.3	13.2

Table S8. Flow cytometry % of cell population of keratocyte phenotype expression and combined total expression % of cell population. Two group of combination staining were performed (CD34/CD105 and ALDH/a-SMA). -/- is population of cells with no staining observed. -/+ is population of cells with the undesired activated phenotype expression. +/- is population of cells with the desired quiescent phenotype expression and +/+ is population of cells with the both desired and undesired activated phenotype expression (n=3, scaffold per staining group).

Population [%]

	CD34 / CD105				ALDH / α-SMA			
	-/-	**-/+**	**+/-**	**+/+**	**-/-**	**-/+**	**+/-**	**+/+**
0S-P	68.11	9.3	5.76	16.83	22.11	10.89	34.49	32.51
2S-P	37.38	31.07	7.22	24.33	46.30	10.49	29.63	13.58
3S-P	31.45	23.98	15.96	28.62	38.91	3.04	32.83	25.23
4S-P	45.40	16.25	3.58	34.78	37.71	4.97	44.24	13.61
10S-P	28.81	21.15	3.52	46.51	38.94	16.13	28.80	16.13
2D-Gel	9.07	21.44	4.48	65.01	0.75	0.13	6.54	92.58

Total % of cell population

	CD34	**CD105**	**ALDH**	**α-SMA**
0S-P	22.59	26.13	67	43.4
2S-P	31.55	55.37	43.21	24.07
3S-P	44.58	52.38	58.06	28.27
4S-P	38.36	51.02	57.58	18.58
10S-P	50.03	67	44.93	32.26
2D-Gel	69.49	86.45	92.96	99.12

Table S9. Flow cytometry Y and X- mean of cell population of keratocyte phenotype expression of the two group of combination staining (CD34/CD105 and ALDH/a-SMA). -/- is population of cells with no staining observed. -/+ is population of cells with the undesired activated phenotype expression. +/- is population of cells with the desired quiescent phenotype expression and +/+ is population of cells with the both desired and undesired activated phenotype expression (n=3, scaffold per staining group).

X-axis median

	CD34 / CD105				ALDH / α-SMA			
	-/-	**-/+**	**+/-**	**+/+**	**-/-**	**-/+**	**+/-**	**+/+**
0S-P	$4.84E^{+05}$	$1.34E^{+06}$	$5.08E^{+06}$	$6.25E^{+06}$	$8.36E^{+05}$	$1.52E^{+06}$	$6.48E^{+06}$	$7.12E^{+06}$
2S-P	$6.65E^{+05}$	$1.94E^{+06}$	$3.58E^{+06}$	$4.02E^{+06}$	$7.34E^{+05}$	$7.51E^{+05}$	$1.95E^{+07}$	$3.61E^{+07}$
3S-P	$7.61E^{+05}$	$1.96E^{+06}$	$3.08E^{+06}$	$4.56E^{+06}$	$9.67E^{+05}$	$1.55E^{+05}$	$6.76E^{+06}$	$9.31E^{+07}$
4S-P	$6.09E^{+05}$	$1.69E^{+06}$	$3.88E^{+06}$	$4.98E^{+06}$	$8.36E^{+05}$	$1.23E^{+05}$	$1.35E^{+07}$	$9.18E^{+07}$
10S-P	$6.79E^{+05}$	$1.82E^{+06}$	$3.56E^{+06}$	$4.90E^{+06}$	$7.26E^{+05}$	$7.22E^{+05}$	$8.61E^{+06}$	$4.00E^{+07}$
2D-Gel	$1.39E^{+06}$	$1.72E^{+06}$	$3.88E^{+06}$	$5.60E^{+06}$	$1.16E^{+06}$	$1.04E^{+06}$	$7.67E^{+07}$	$1.30E^{+08}$

Y-axis median

	CD34 / CD105				ALDH / α-SMA			
	-/-	**-/+**	**+/-**	**+/+**	**-/-**	**-/+**	**+/-**	**+/+**
0S-P	$5.19E^{+05}$	$2.44E^{+07}$	$1.52E^{+06}$	$7.54E^{+07}$	$4.61E^{+05}$	$5.59E^{+06}$	$3.76E^{+05}$	$8.04E^{+06}$
2S-P	$5.67E^{+05}$	$3.47E^{+07}$	$7.94E^{+05}$	$6.00E^{+07}$	$3.66E^{+05}$	$1.56E^{+07}$	$5.21E^{+05}$	$5.84E^{+06}$
3S-P	$7.13E^{+05}$	$2.25E^{+07}$	$5.71E^{+05}$	$5.92E^{+07}$	$3.83E^{+05}$	$6.84E^{+06}$	$7.38E^{+05}$	$6.56E^{+06}$
4S-P	$6.08E^{+05}$	$1.96E^{+07}$	$1.88E^{+06}$	$6.88E^{+07}$	$4.27E^{+05}$	$5.25E^{+06}$	$6.34E^{+05}$	$6.54E^{+06}$
10S-P	$6.38E^{+05}$	$1.49E^{+07}$	$1.82E^{+06}$	$2.80E^{+07}$	$5.18E^{+05}$	$1.15E^{+07}$	$5.80E^{+05}$	$6.05E^{+06}$
2D-Gel	$1.22E^{+06}$	$1.14E^{+07}$	$8.97E^{+04}$	$1.67E^{+07}$	$2.59E^{+05}$	$8.61E^{+06}$	$2.20E^{+06}$	$4.22E^{+06}$

Table S10. ANOVA value of Y-mean and X-mean of FACS. Two-way ANOVA analysis by Turkey test of the X-axis medians for $CD34^+/CD105^+$ and Y-medians of $ALDH^+/\alpha\text{-}SMA^+$. (*=<0.05, **=<0.01 and ***=<0.001)

				X-axis medians $ALDH^+/\alpha\text{-}SMA^+$		
	0S-P	**2S-P**	**3S-P**	**4S-P**	**10S-P**	**2D-gelatin**
0S-P	-	ns	*	*	ns	***
2S-P	ns	-	ns	ns	ns	**
3S-P	*	ns	-	ns	ns	ns
4S-P	*	ns	ns	-	ns	ns
10S-P	ns	ns	ns	ns	-	*
2D-gelatin	***	**	ns	ns	*	-
				Y-axis medians $CD34^+/CD105^+$		
	0S-P	**2S-P**	**3S-P**	**4S-P**	**10S-P**	**2D-gelatin**
0S-P	-	ns	ns	ns	**	***
2S-P	ns	-	ns	ns	ns	*
3S-P	ns	ns	-	ns	ns	*
4S-P	ns	ns	ns	-	ns	**
10S-P	**	ns	ns	ns	-	ns
2D-gelatin	***	*	*	**	ns	-

Table S11. Two-way ANOVA analysis by Turkey test of the total % of cell population expressing CD34, CD105, ALDH or α-SMA (*=<0.05, **=<0.01 and ***=<0.001)

				% of cell population expressing CD34		
	0S-P	**2S-P**	**3S-P**	**4S-P**	**10S-P**	**2D-gelatin**
0S-P	-	ns	ns	ns	ns	ns
2S-P	ns	-	ns	ns	ns	ns
3S-P	ns	ns	-	ns	ns	ns
4S-P	ns	ns	ns	-	ns	ns
10S-P	ns	ns	ns	ns	-	ns
2D-gelatin	ns	ns	ns	ns	ns	-
				% of cell population expressing CD105		
	0S-P	**2S-P**	**3S-P**	**4S-P**	**10S-P**	**2D-gelatin**
0S-P	-	ns	ns	ns	ns	*
2S-P	ns	-	ns	ns	ns	ns
3S-P	ns	ns	-	ns	ns	ns
4S-P	ns	ns	ns	-	ns	ns
10S-P	ns	ns	ns	ns	-	ns
2D-gelatin	*	ns	ns	ns	ns	-
				% of cell population expressing ALDH		
	0S-P	**2S-P**	**3S-P**	**4S-P**	**10S-P**	**2D-gelatin**
0S-P	-	ns	ns	ns	ns	ns
2S-P	ns	-	ns	ns	ns	ns
3S-P	ns	ns	-	ns	ns	ns
4S-P	ns	ns	ns	-	ns	ns
10S-P	ns	ns	ns	ns	-	ns
2D-gelatin	ns	ns	ns	ns	ns	-
				% of cell population expressing α-SMA		
	0S-P	**2S-P**	**3S-P**	**4S-P**	**10S-P**	**2D-gelatin**
0S-P	-	ns	*	*	ns	*
2S-P	ns	-	ns	ns	ns	*
3S-P	ns	ns	-	ns	ns	**
4S-P	ns	ns	ns	-	ns	*
10S-P	ns	ns	ns	ns	-	*
2D-gelatin	*	*	**	*	*	-