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Electronic Supplementary Information

Soft hydrogels from tetra-functional PEGs using UV-induced thiol-ene coupling chemistry: A structure-to-property study

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Synthesis of PEG-G1-allyl

Synthesis of PEG-2k-G1-allyl

PEG-OH ($\bar{M}_n$ = 2 kDa) (19.05 g, 9.525 mmol), DMAP (465.0 mg, 3.806 mmol), and pyridine (4.600 mL, 57.10 mmol) were dissolved in 12.00 mL DCM. Using an ice-bath, BAPA anhydride **1** (12.51 g, 30.47 mmol) dissolved in 3.000 mL DCM was added drop wise and the solution was allowed to react over-night. An additional amount of DMAP (230.0 mg, 1.883 mmol) was added and the solution was slightly concentrated after which the reaction was allowed to run for 24 more hours to ensure complete substitution. Full conversion of OH-groups was confirmed using ^{13}C NMR analysis. Unreacted anhydride was quenched with 15.00 mL H_2O for 24 hours. 600.0 mL DCM was added to the solution before PEG-2k-G1-allyl was purified through extraction with $NaHSO_4$ (10%) four times followed by extraction with $NaHCO_3$ (10%) two times, after which the organic phase was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. (19.24 g, 8.043 mmol, 84.45% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.17 (s, -CH_3), 3.52 (d, ┼C-C*H*$_2$-O-), 3.60 (s, -C*H*$_2$- (PEG)), 3.94 (dd, -O-C*H*$_2$-CH=), 4.21 (t, PEG-C*H*$_2$-OOC-G1), 5.11 (dd, -CH=C*H*$_2$ (cis)), 5.21 (dd, -CH=C*H*$_2$ (trans)), 5.82 (m, -C*H*=CH_2).
^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.25 (-*C*H_3), 48.46 (┼*C*-CH_3), 63.74 (-PEG-*C*H_2-OOC-G1), 69.18 (-O-*C*H_2-CH_2-COO-G1), 70.62 (-*C*H_2- (PEG)), 72.12 (-O-*C*H_2-CH=), 72.31 (┼C-*C*H_2-O-), 116.55 (=*C*H_2), 134.91 (-*C*H=CH_2), 174.61 (PEG-OO*C*-G1).

SEC: $\bar{M}_n$ = 2147 g mol^{-1}, Đ = 1.03.

Synthesis of PEG-10k-G1-allyl

PEG-OH ($\bar{M}_n$ = 10 kDa) (20.00 g, 2.000 mmol), DMAP (195.5 mg, 1.600 mmol), and pyridine (967.0 µL, 1.200 mmol) were dissolved in 20.00 mL DCM. Using an ice-bath, BAPA anhydride **1** (2.627 g, 6.400 mmol) dissolved in 2.000 mL DCM was added drop wise and the solution was allowed to react for 72 hours. Full conversion of OH-groups was confirmed using ^{13}C NMR analysis. Purification was achieved through precipitation from DCM in diethyl ether (3 times). (18.61 g, 1.791 mmol, 89.54% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.11 (s, -CH_3), 3.45 (d, ┼C-C*H*$_2$-O-), 3.53 (s, -C*H*$_2$- (PEG)), 3.86 (dd, -O-C*H*$_2$-CH=), 4.14 (t, PEG-C*H*$_2$-OOC-G1), 5.03 (dd, -CH=C*H*$_2$ (cis)), 5.13 (dd, -CH=C*H*$_2$ (trans)), 5.75 (m, -C*H*=CH_2).
^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.64 (-*C*H_3), 48.05 (┼*C*-CH_3), 63.34 (-PEG-*C*H_2-OOC-G1), 68.77 (-O-*C*H_2-CH_2-COO-G1), 70.24 (-*C*H_2- (PEG)), 71.74 (-O-*C*H_2-CH=), 71.88 (┼C-*C*H_2-O-), 116.13 (=*C*H_2), 134.54 (-*C*H=CH_2), 174.11 (PEG-OO*C*-G1).

SEC: $\bar{M}_n$ = 11571 g mol^{-1}, Đ = 1.03.

Synthesis of PEG-20k-G1-allyl

PEG-OH ($\bar{M}_n$ = 20 kDa) (9.960 g, 0.4980 mmol), DMAP (40.00 mg, 0.3274 mmol), and pyridine (240.0 µL, 2.979 mmol) were dissolved in 12.00 mL DCM. Using an ice-bath, BAPA anhydride **1** (572.0 mg, 1.393 mmol) dissolved in 1.000 mL DCM was added drop wise and the solution was allowed to react over-night. An additional amount of DMAP (48.00 mg, 0.3929 mmol) was added and the reaction was allowed to continue for 24 additional hours to ensure complete reaction. Full conversion of OH-groups was confirmed using ^{13}C NMR analysis. Purification was achieved through precipitation from DCM in diethyl ether (2 times). (8.342 g, 0.4091 mmol, 82.14% yield).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.21 (s, -CH_3), 3.46 (d, ┼C-C*H*$_2$-O-), 3.64 (s, -C*H*$_2$- (PEG)), 3.96 (dd, -O-C*H*$_2$-CH=), 4.25 (t, PEG-C*H*$_2$-OOC-G1), 5.13 (dd, -CH=C*H*$_2$ (cis)), 5.23 (dd, -CH=C*H*$_2$ (trans)), 5.85 (m, -C*H*=CH_2).
^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.61 (-*C*H_3), 48.02 (┼*C*-CH_3), 63.30 (-PEG-*C*H_2-OOC-G1), 68.74 (-O-*C*H_2-CH_2-COO-G1), 70.21 (-*C*H_2- (PEG)), 71.70 (-O-*C*H_2-CH=), 71.84 (┼C-*C*H_2-O-), 116.09 (=*C*H_2), 134.51 (-*C*H=CH_2), 174.06 (PEG-OO*C*-G1).

SEC: $\bar{M}_n$ = 21915 g mol^{-1}, Đ = 1.06.

General procedures for hydrogel formation

Hydrogels were produced by crosslinking PEG-G1-allyl 2 with TMP-$(SH)_3$ using an [allyl]:[thiol] ratio of 1:1 and a solid content of 50 w% in EtOH (96%). Hydrogels for swelling tests and the degradation study were cured in a poly(tetrafluoroethylene) (PTFE) mold with dimensions 58*8*1 mm covered with a glass slide to prevent evaporation of the solvent during curing. Hydrogel samples for rheology measurements were cured between two glass slides separated by a distance of 1 mm. Circular samples with a diameter of 25 mm were made from fully swollen hydrogels using a punch.

PEG-2k-G1-allyl based hydrogels

PEG-2k-G1-allyl (2.000 g, 0.8359 mmol) and TMP-$(SH)_3$ (367.0 µL, 1.114 mmol) were dissolved in EtOH (96%, 3.113 mL) along with initiator I2959 (12.2 mg, 0.50 wt% of total solid content). Hydrogels were cured by irradiation with a Blak-Ray® XX-15BLB UV Bench Lamp (UVP, Cambridge, UK, 365 nm, 28.5 mW cm^{-2}) for 20 min. After curing, hydrogels were washed in DI water (overnight + 2h + 2h) and ethanol (96%, 2h + 2h) to remove residual reactants and then dried in air overnight followed by 12 h in a vacuum oven at 50°C. GF = 79 ± 9 %

PEG-10k-G1-allyl based hydrogels

PEG-10k-G1-allyl (2.000 g, 0.1924 mmol) and TMP-$(SH)_3$ (84.50 µL, 0.2565 mmol) were dissolved in EtOH (96%, 2.678 mL) along with initiator I2959 (10.6 mg, 0.50 wt% of total solid content). Hydrogels were cured by irradiation with a Blak-Ray® XX-15BLB UV Bench Lamp (UVP, Cambridge, UK, 365 nm, 28.5 mW cm^{-2}) for 20 min. After curing, hydrogels were washed in DI water (overnight + 2h + 2h) and ethanol (96%, 2h + 2h) to remove residual reactants and then dried in air overnight followed by 12 h in a vacuum oven at 50°C. GF = 91 ± 1 %

PEG-20k-G1-allyl based hydrogels

PEG-20k-G1-allyl (2.000 g, 0.09808 mmol) and TMP-$(SH)_3$ (43.10 µL, 0.1308 mmol) were dissolved in EtOH (96%, 2.613 mL) along with initiator I2959 (10.3 mg, 0.50 wt% of total solid content). Hydrogels were cured by irradiation with a Blak-Ray® XX-15BLB UV Bench Lamp (UVP, Cambridge, UK, 365 nm, 28.5 mW cm^{-2}) for 20 min. After curing, hydrogels were washed in DI water (overnight + 2h + 2h) and ethanol (96%, 2h + 2h) to remove residual reactants and then dried in air overnight followed by 12 h in a vacuum oven at 50°C. GF = 90 ± 1 %

Calculation of $\overline{M}_c$ from swelling data

Molecular weights between crosslinks ($\bar{M}_c$) for the synthesized hydrogels have been calculated from swelling data using the Peppas-Merrill model[1] derived from the equation originally proposed by Flory[2].

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\frac{\bar{v}}{V_1}\left[ln(1-\nu_{2,s}) + \nu_{2,s} + \chi(\nu_{2,s})^2\right]}{(\nu_{2,r})\left[\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3} - \frac{1}{2}\frac{\nu_{2,s}}{\nu_{2,r}}\right]}$$

The factor $\left(1-\frac{2\bar{M}_c}{\bar{M}_n}\right)$ is a correction factor for network imperfections such as dangling chain ends. For perfect infinite networks, this factor reduces to 1, and since the specific volume of the polymer, $\bar{v}$, is the inverse of the polymer density, ρ_p, the equation can be rearranged to[3]:

$$\bar{M}_c = -\frac{\rho V_1(\nu_{2,r})\left[\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3} - \frac{1}{2}\frac{\nu_{2,s}}{\nu_{2,r}}\right]}{\left[ln(1-\nu_{2,s}) + \nu_{2,s} + \chi(\nu_{2,s})^2\right]}$$

ρ_p	polymer density (1.125 g cm^{-3} for PEG)[4]
V_1	molar volume of swelling medium (18 cm^3 mol^{-1} for water)
$\nu_{2,r} = \left[1 + \frac{(q_F-1)\rho_p}{\rho_{solvent}}\right]^{-1}$	volume fraction of polymer in the relaxed gel during curing
q_F	weight ratio after curing (weight of cured gel / weight of dry gel)
$\rho_{solvent}$	density of solvent used during gel formation (0.789 g cm^{-3} for ethanol)
$\nu_{2,s} = \left[1 + \frac{(q_w-1)\rho_p}{\rho_{sm}}\right]^{-1}$	volume fraction of polymer in swollen gel
χ	Flory's polymer-solvent interaction parameter (0.426 for PEG-water)[5]
q_w	weight swelling ratio at equilibrium (weight of swollen gel / weight of dry gel)
ρ_{sm}	density of the swelling medium (1.00 g/cm^3 for water)

Table S1. Values of $\bar{M}_c$ calculated from swelling data, q_w.

Hydrogel system	q_w	$\bar{M}_c$
PEG-2k-G1-allyl	3.412	333
PEG-6k-G1-allyl	7.491	2520
PEG-10k-G1-allyl	8.816	3640
PEG-20k-G1-allyl	14.763	10830

[1] N. A. Peppas and E. W. Merrill, *J Appl Polym Sci*, 1977, **21**, 1763-1770.
[2] P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, NY, USA, 1953.
[3] N. A. Peppas, *Hydrogels in medicine and pharmacy*, CRC Press, Boca Raton, FL, USA, 1987.
[4] D. W. van Krevelen and K. te Nijenhuis, *Properties of Polymers: Their Correlation with Chemical Structure; their Numerical Estimation and Prediction from Additive Group Contributions*, Third Edition edn., Elsevier Science Publishers B.V., Amsterdam, Netherlands, 1990.
[5] E. W. Merrill, K. A. Dennison and C. Sung, *Biomaterials*, 1993, **14**, 1117-1126.

Results from tensile tests

Tensile test were conducted on an Instron 5944 tensile testing machine equipped with a standard video extensometer. Measurements were done on dog-bone-shaped hydrogels swollen to equilibrium in DI water at 23 °C using a cross-head speed of 5 mm min-1 and a 500 N load cell. Hydrogels based on PEG-20k-G1-allyl could not be tested, since the samples broke during clamping under their own weight.

Table S2. Results from tesnsile tests.

Hydrogel system	Strain at break (%)	Stress at break (kPa)	Tensile toughness (kJ m^{-3})	Young's modulus (kPa)
PEG-2k-G1-allyl	30 ± 6	85 ± 23	12.4 ± 5.0	314 ± 26
PEG-6k-G1-allyl	23 ± 4	81 ± 6	10.9 ± 3.8	333 ± 42
PEG-10k-G1-allyl	26 ± 7	26 ± 5	2.0 ± 0.8	153 ± 34

Due to large standard deviations, it was difficult to draw any significant conclusions from the tensile tests. It can however be seen in Table S2 that the 10k-hydrogel system exhibited a lower stress at break, a lower tensile toughness, and a lower Young's modulus than the 2k- and 6k-hydrogel systems. It can also be noted that the Young's moduli acquired through tensile tests were higher than the values estimated from the shear modui obtained with rheology.

Degradation study

Freeze-dried samples from hydrogels degraded in pH 10 buffer solution were analysed using ^{1}H NMR and ^{13}C NMR spectroscopy in D_2O. The spectra for the degraded samples are shown in Fig. S1-S4. NMR spectra for undegraded PEG-6k-G1-allyl (Fig. S5), TMP-$(SH)_3$ (Fig. S6), TMP (Fig. S7), and Na_2CO_3 (Fig. S8), as well as estimated NMR spectra for the supposed degradation product (compound 4 in the main article) (Fig. S9), have also been included.

Comparison of the spectra show that apart from PEG, there are peaks corresponding to TMP (Fig S7) in the degraded hydrogel samples (Fig. S1-S4). There are also peaks at 2.45 – 2.74 ppm that indicate that the proposed degradation product, compound **4**, is present in the degraded samples.

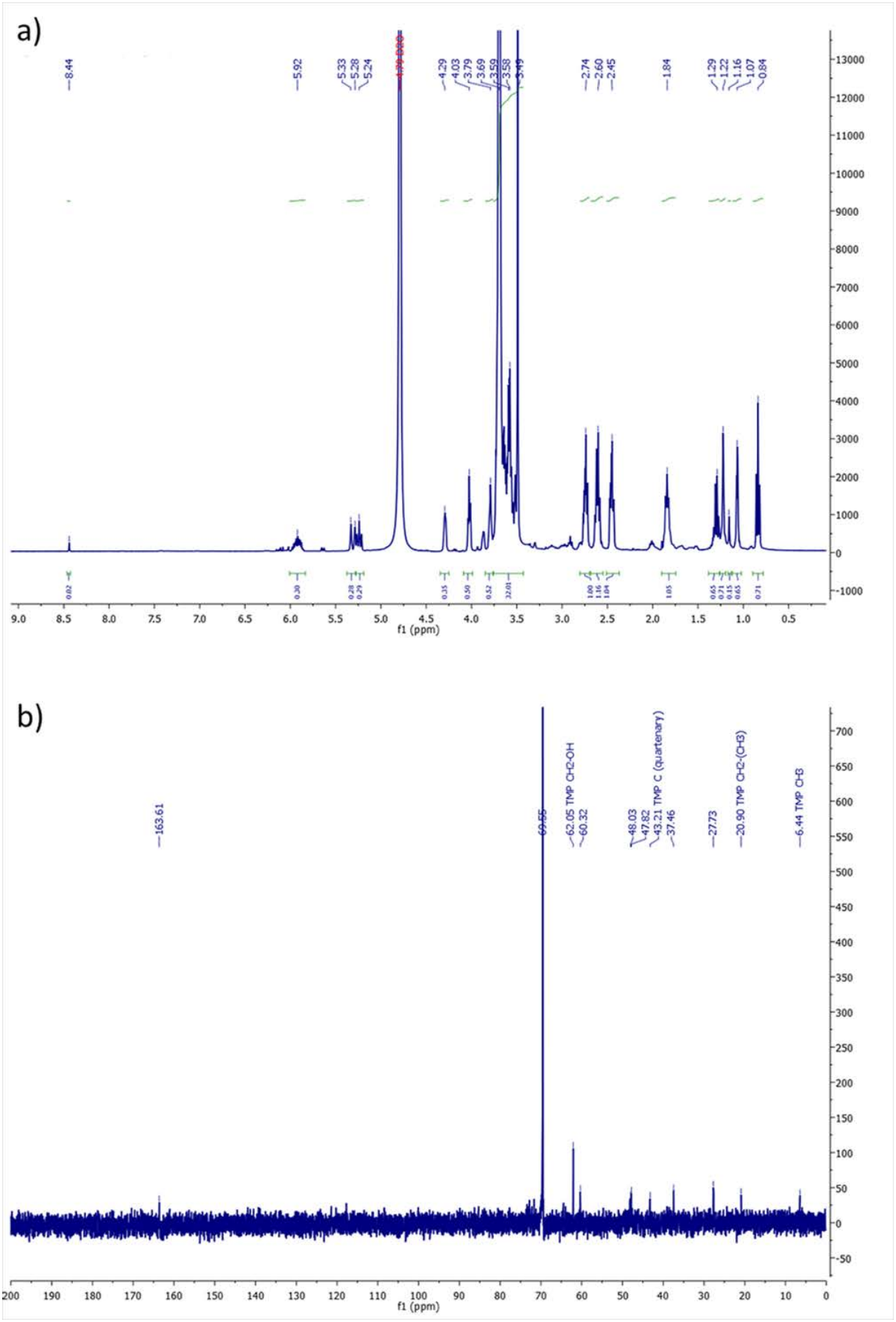

Fig. S1 a) ^{1}H NMR and b) ^{13}C NMR spectra for a 2k-hydrogel degraded in pH 10 buffer solution for 3 months.

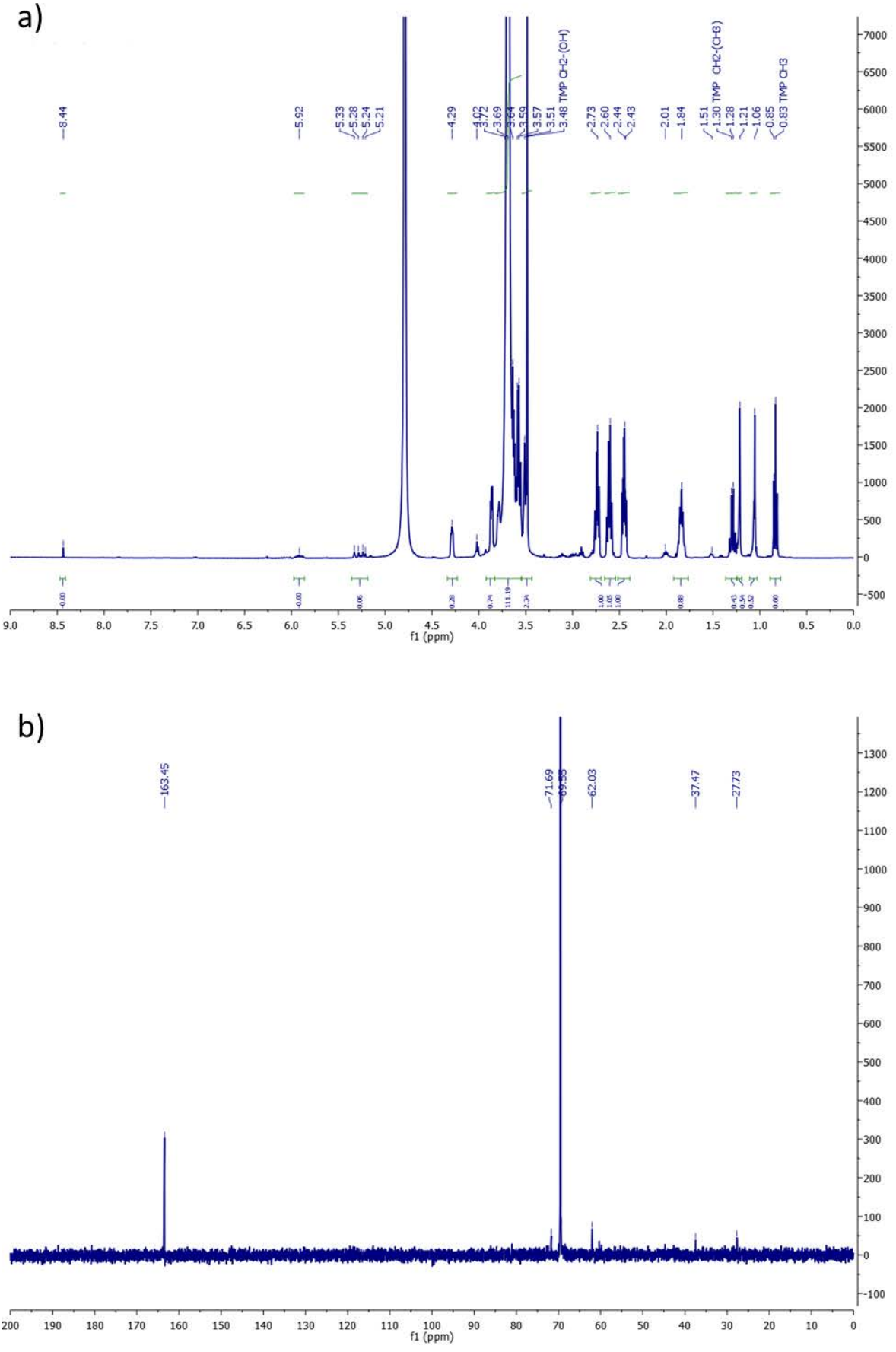

Fig. S2 a) ^{1}H NMR and b) ^{13}C NMR spectra for a 6k-hydrogel degraded in pH 10 buffer solution for 3 months.

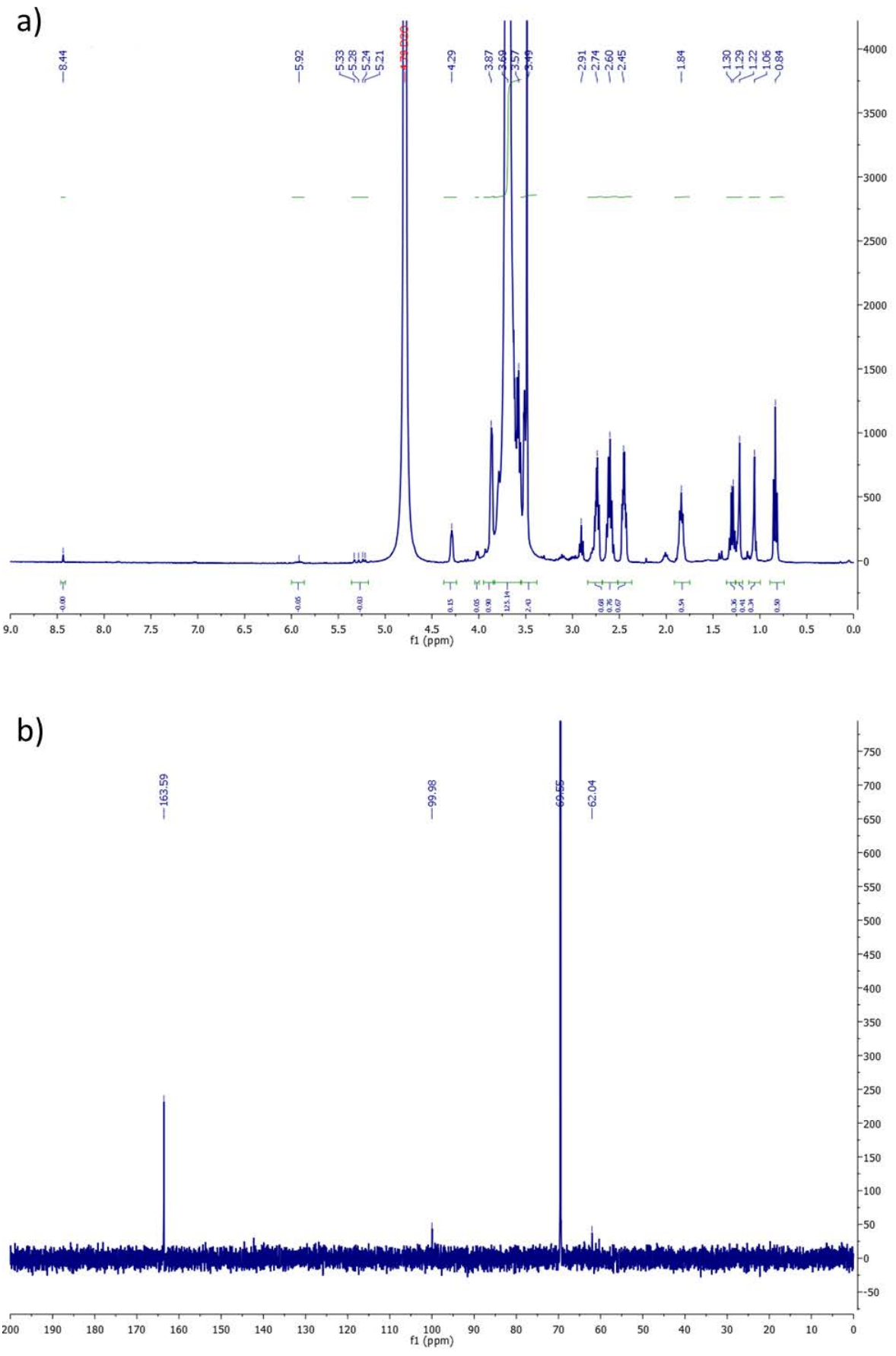

Fig. S3 a) ^{1}H NMR and b) ^{13}C NMR spectra for a 10k-hydrogel degraded in pH 10 buffer solution for 3 months.

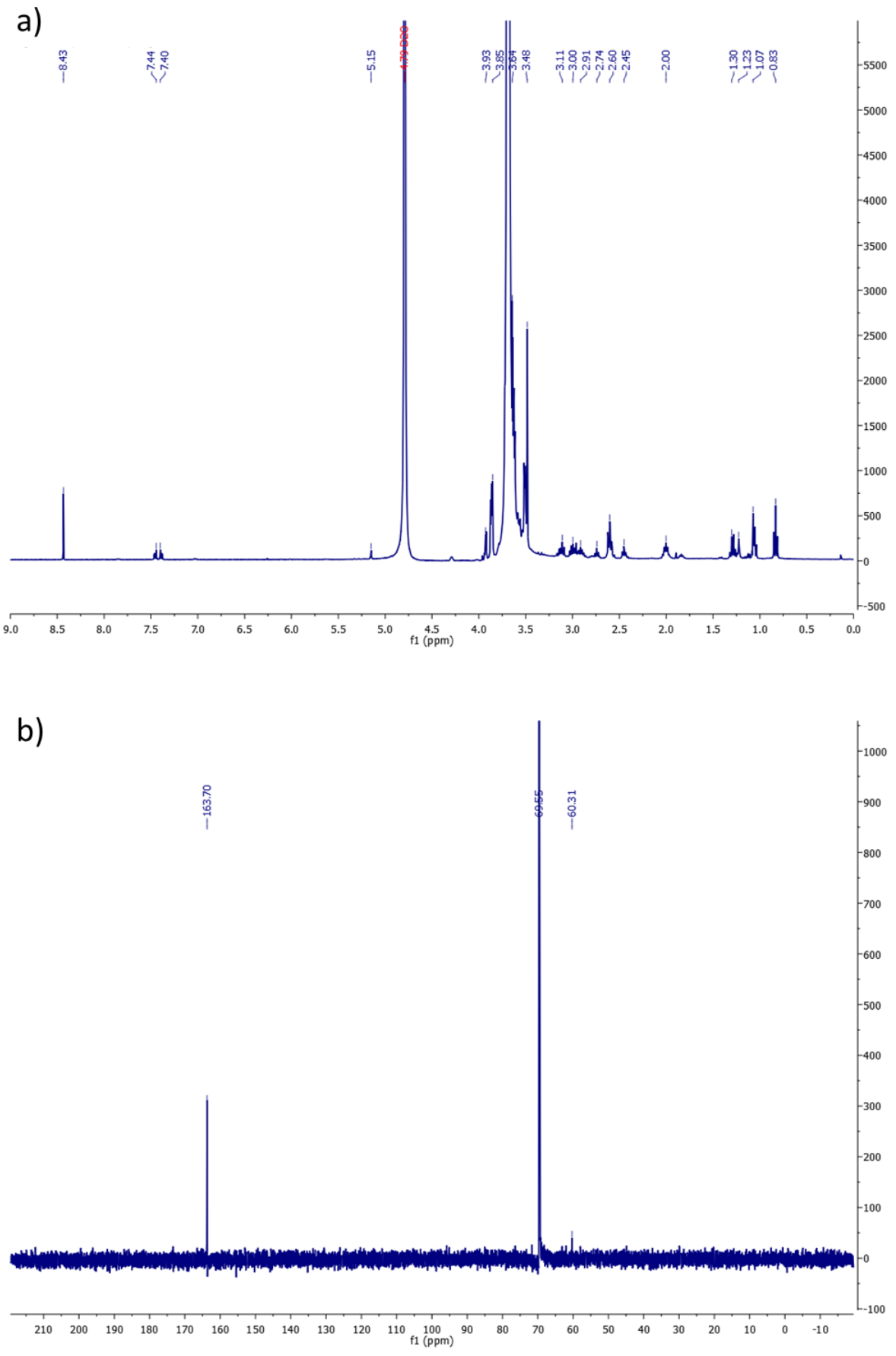

Fig. S4 a) ^{1}H NMR and b) ^{13}C NMR spectra for a 20k-hydrogel degraded in pH 10 buffer solution for 3 months.

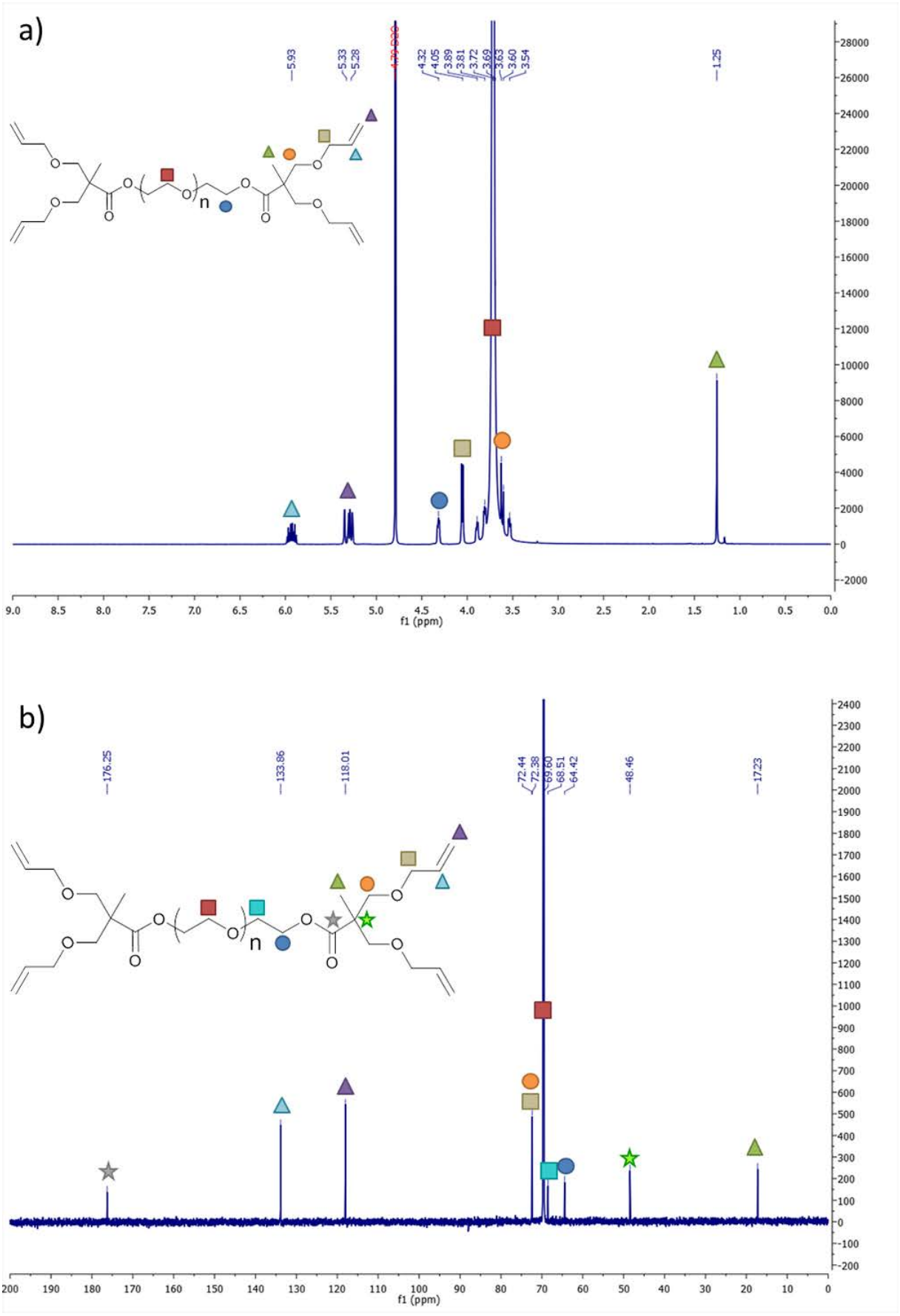

Fig. S5 a) ^{1}H NMR and b) ^{13}C NMR spectra for undegraded PEG-6k-G1-allyl in D_2O.

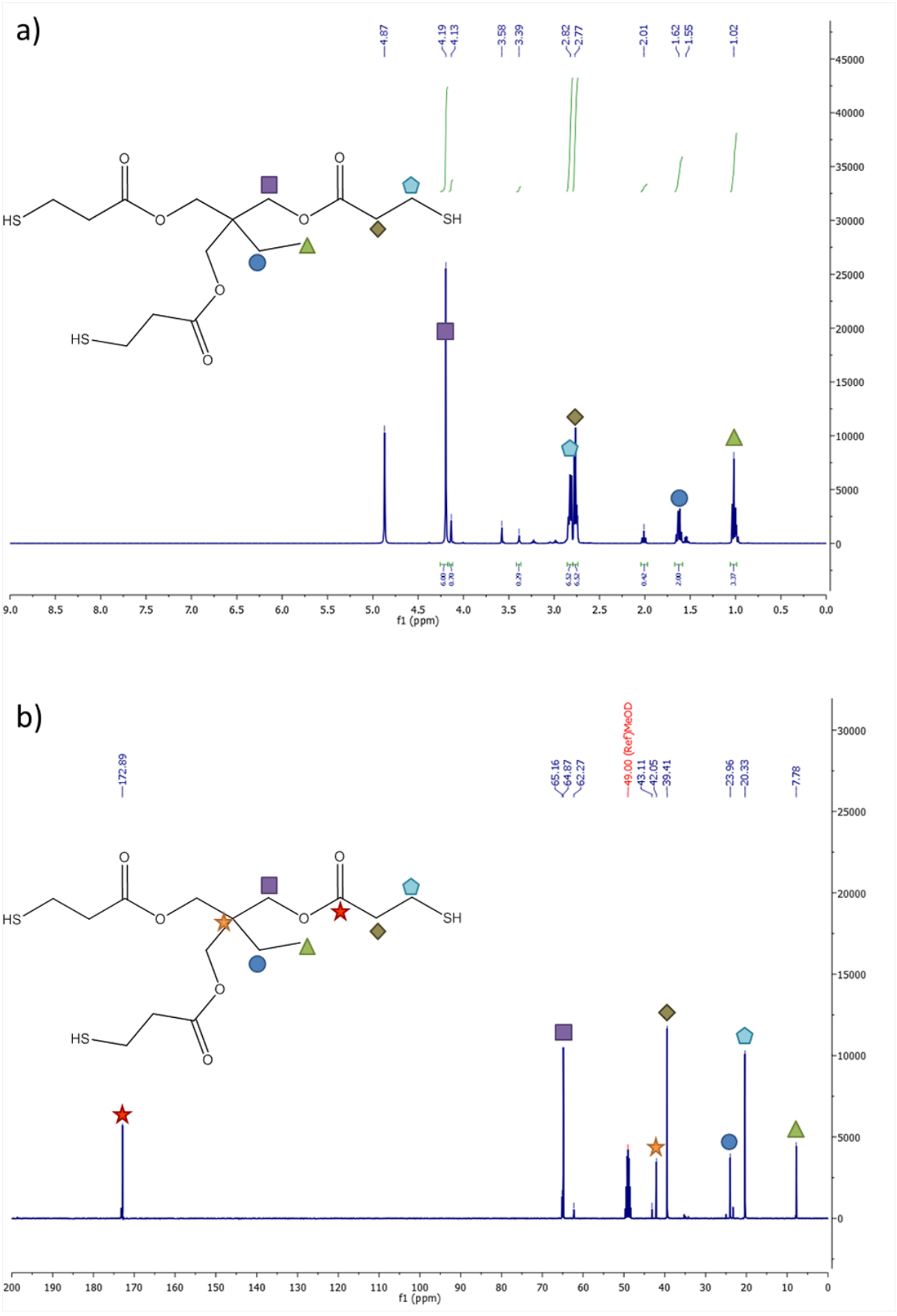

Fig. S6 a) ^{1}H NMR and b) ^{13}C NMR spectra for TMP-$(SH)_3$ in MeOD.

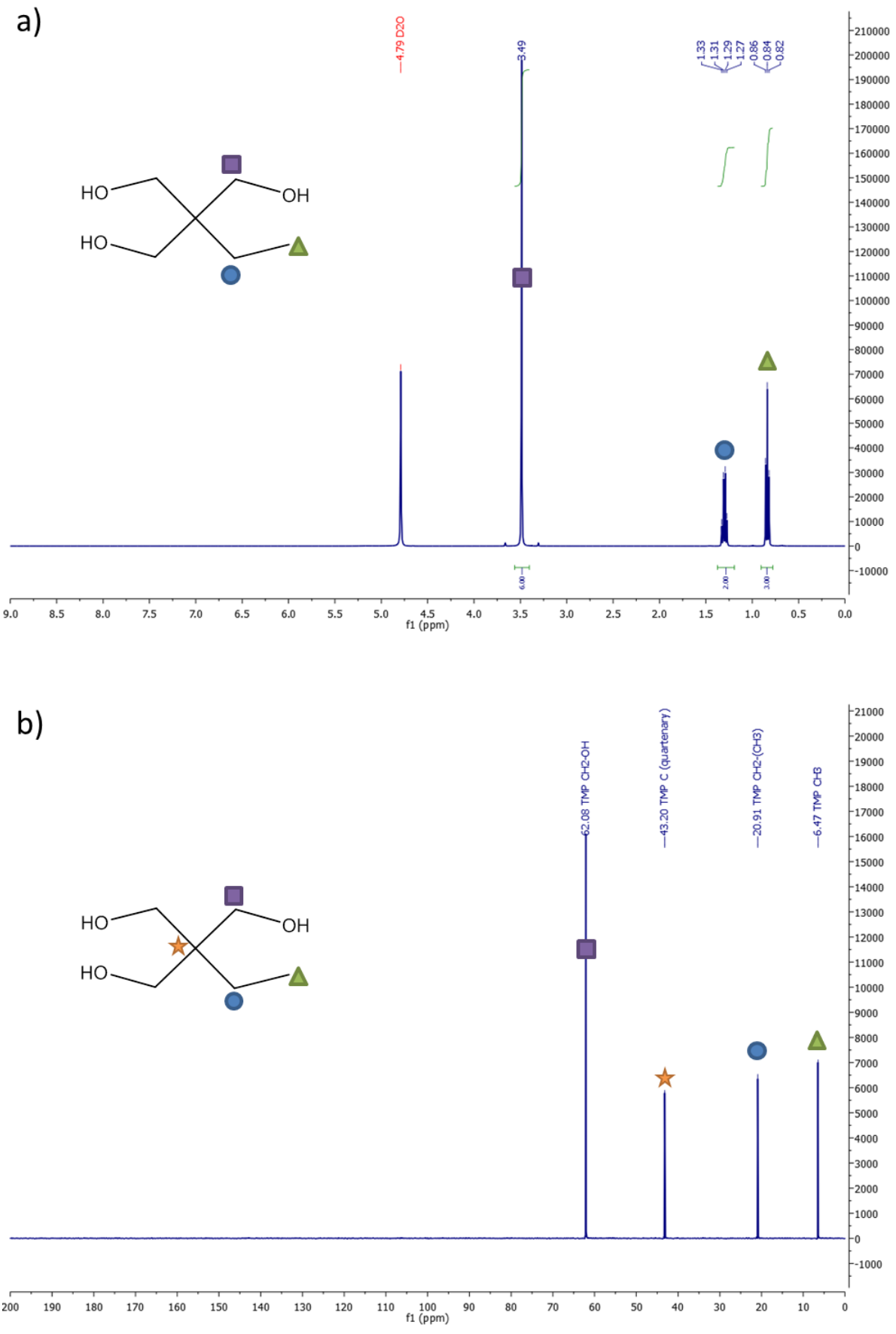

Fig. S7 a) ^{1}H NMR and b) ^{13}C NMR spectra of TMP in D_2O.

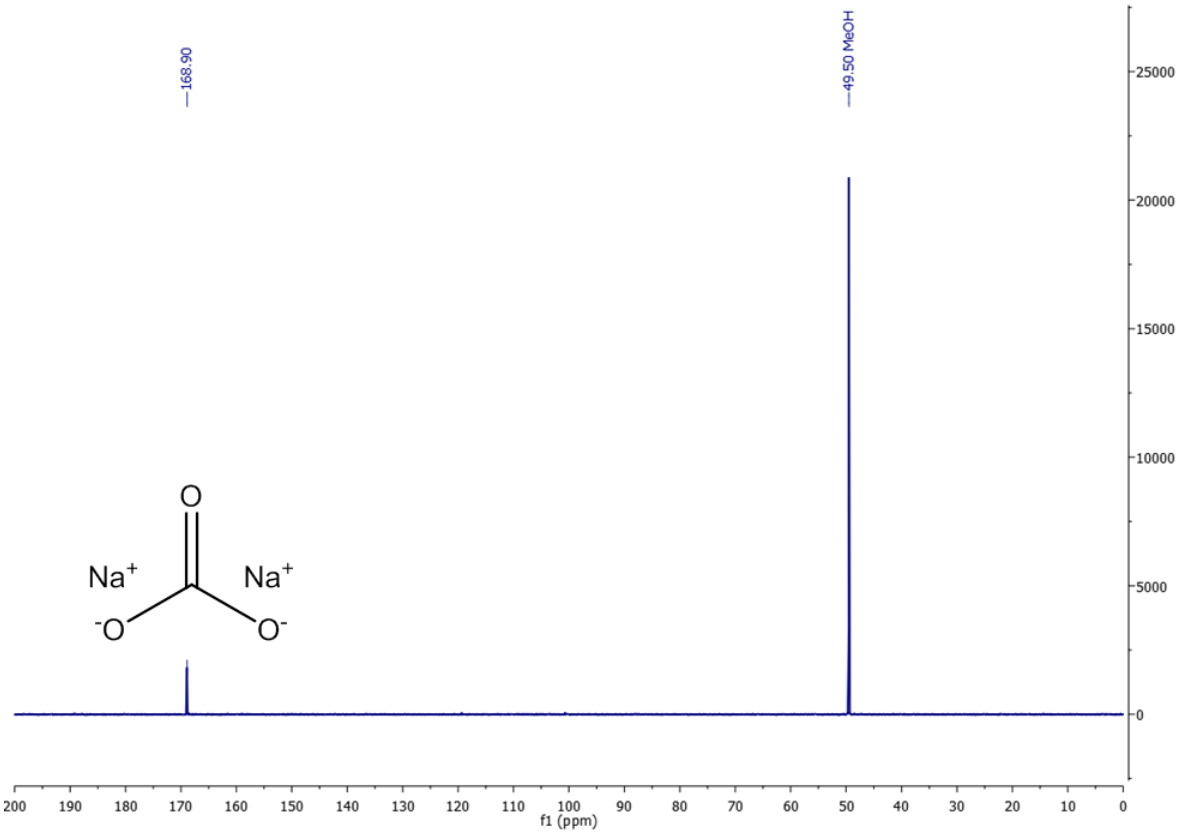

Fig. S8 ^{13}C NMR spectrum of Na_2CO_3 (present in the pH 10 buffer solution) in D_2O. MeOH was used as internal reference.

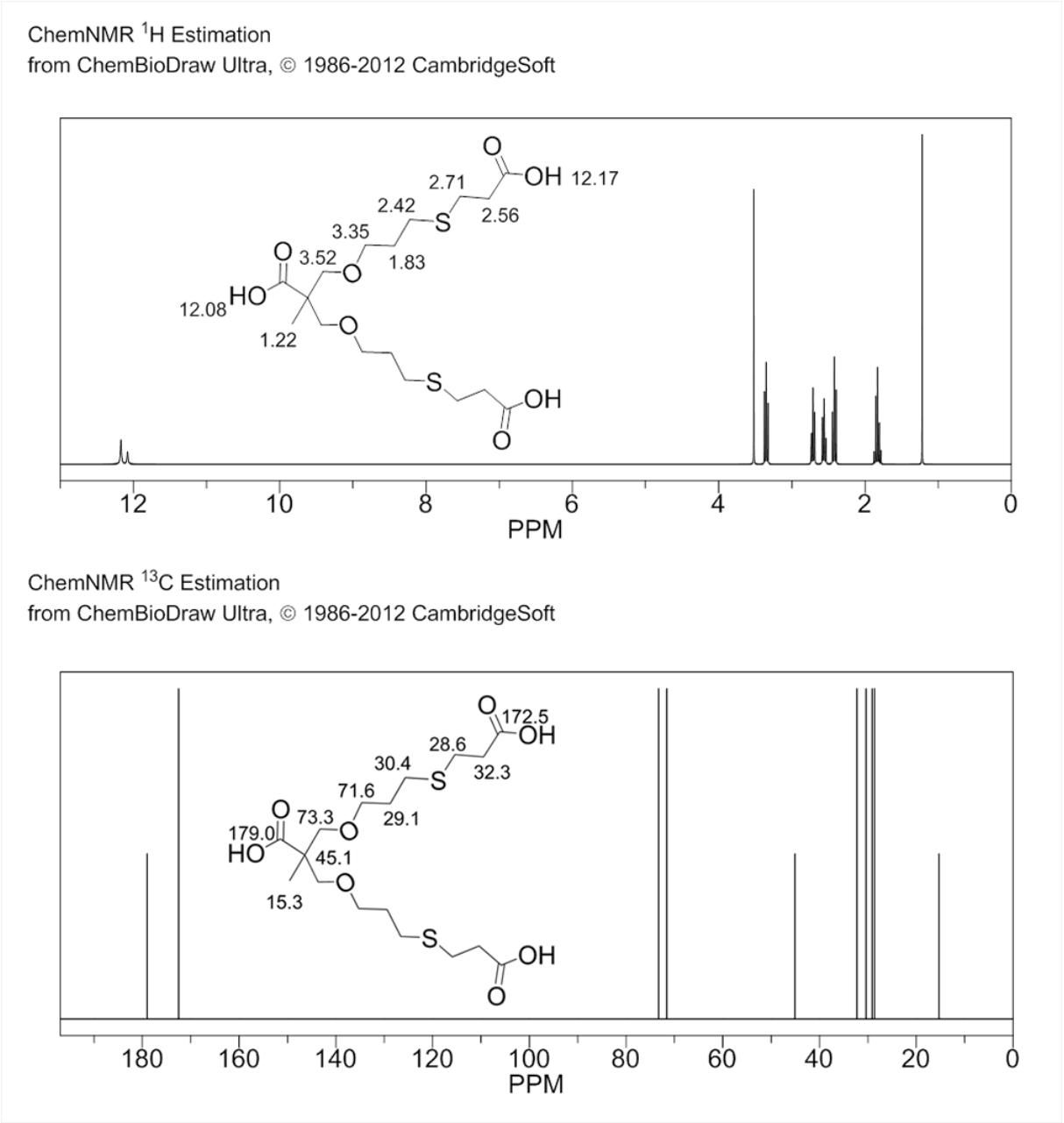

Fig. S9 ^{1}H and ^{13}C estimation of NMR spectra for the proposed degradation product, compound **4**.