Supporting Information for

# Mechanochemical and Aging-Based Reductive Amination with Chitosan and Aldehydes Affords High Degree of Substitution Functional Biopolymers.

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Number of schemes: 2

Number of figures: 28

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## 1. Method

### Data statistic methods

### a. Determination of Chs DA and DDA with $^{13}C$ ssNMR

The degree of acetylation (DA) and DDA of chitosan was calculated by $^{13}C$ ssNMR. The DA value was determined by taking the ratio of the average of the area of the acetylglucosamine C=O (δ=170.06) and $CH_3$ (δ=20.68) to that of $C_1$ (δ=101.65) of the polysaccharide chain. The DDA value was determined by subtracting the DA value from 100%.

$$DA = \frac{1}{2}\left(\frac{\int Chs\ acetyl\ amide\ C=O}{\int Chs\ C_1} \times 100\% + \frac{\int Chs\ acetyl\ amide\ CH_3}{\int Chs\ C_1} \times 100\%\right)$$

$$DDA = 100\% - DA$$

**Example calculation:**

$$DA = \frac{1}{2}\left(\frac{0.62}{2.66} \times 100\% + \frac{0.60}{2.66} \times 100\%\right) = 23\%$$

$$DDA = 100\% - 23\% = 77\%$$

### b. Determination of Chs DS with $^{1}H$ NMR

The Chs DS was calculated by $^{1}H$ NMR. Since $^{1}H$ NMR affords better sensitivity and higher resolution than $^{13}C$ ssNMR, the values reported in the main text were calculated by $^{1}H$ NMR for all alkylated Chs samples that were soluble in 0.1% HCl acidified $D_2O$. The parameter was defined as the percentage of modified glucosamine monomers on Chs to the total glucosamine units available for functionalization. The number of monomers was calculated by dividing the intensity of the characteristic peak of grafting by the number of nuclides of respective peaks. The acetylglucosamine $CH_3$ peak (δ=1.97) was taken as an internal standard for the calculation as its

intensity is intrinsic to the starting material. Then, the ratio of modified glucosamine monomers to the acetylglucosamine monomers was multiplied with Chs DA to calculate the percentage of modified monomers to the total monomers of the polysaccharide chain, followed by dividing by Chs DDA to yield Chs DS.

$$DS = \frac{\int characteristic\ peak\ of\ grafting}{nH_{characteristic\ peak\ of\ gradting}} \div \frac{\int Chs\ acetyl\ CH_3}{3} \times \frac{ChsDA}{ChsDDA} \times 100\%$$

**Example calculation:**

$$DS_3 = \frac{1.04}{1} \div \frac{0.94}{3} \times \frac{23\%}{77\%} \times 100\% = 99.14\%$$

**c. Determination of aldehyde total %conversion with $^1$H NMR**

The aldehyde total % conversion was calculated by $^1$H NMR. The parameter was defined as the ratio of the total amount of aldehyde derivatives (grafted on Chs and free aldehyde from the hydrolysis of Chs SB). Since 1 equivalent of aldehyde was added in each trial of reactions, the aldehyde total % conversion can also be seen as the sum of the percentage of aldehyde derivatives to the total glucosamine units available for functionalization. Similar to the Chs DS calculation, The acetylglucosamine $CH_3$ peak (δ=1.97) was taken as an internal standard.

$$tot\%conversion = \sum \left( \frac{\int characteristic\ peak\ of\ grafting}{nH_{characteristic\ peak\ of\ gradting}} \div \frac{\int Chs\ acetyl\ CH_3}{3} \times \frac{ChsDA}{ChsDDA} \times 100\% \right)$$

**Example calculation:**

$$tot\%conversion_3 = \frac{1.04}{1} \div \frac{0.94}{3} \times \frac{23\%}{77\%} \times 100\% + \frac{0.03}{1} \div \frac{0.94}{3} \times \frac{23\%}{77\%} \times 100\% = 99.14\% + 2.86\% = 102.00\%$$

**d. Determination of Chs DS with $^{13}C$ ssNMR**

The Chs DS could also be calculated by $^{13}C$ ssNMR as an alternative. However, since $^{1}H$ NMR affords better sensitivity and higher resolution than $^{13}C$ ssNMR, the DS values calculated by $^{13}C$ ssNMR were only reported in the main text when the alkylated Chs samples were insoluble in 0.1% HCl acidified $D_2O$ (samples **4h**, **4i** and **4j**). With $^{13}C$ ssNMR the number of monomers was calculated by dividing the intensity of the characteristic peak of grafting by the number of carbon of respective peaks. The acetylglucosamine $CH_3$ peak (δ=20.68) was taken as an internal standard for the calculation as its intensity is intrinsic to the starting material. Then, the ratio of modified glucosamine monomers to the acetylglucosamine monomers was multiplied with Chs DA to calculate the percentage of modified monomers to the total monomers of the polysaccharide chain, followed by dividing by Chs DDA to yield Chs DS.

$$DS = \frac{\int characteristic\ peak\ of\ grafting}{} \div \frac{\int Chs\ acetyl\ CH_3}{1} \times \frac{ChsDA}{ChsDDA} \times 100\%$$

**Example calculation:**

$$DS_{4h} = \frac{3.05}{1} \div \frac{1}{1} \times \frac{23\%}{77\%} \times 100\% = 91.1\%$$

**e. Determination of %isolated yield of the mechanochemical and aging-based alkylated chitosan with selected scope of aldehyde**

The %isolated yield of the samples followed the mechanochemical and aging-based chitosan reductive alkylation with the scope of aldehydes is defined as the percentage of recovered sample mass to the theoretical mass of the samples with calculated DS. The theoretical mass of the sample was calculated as the starting mass of Chs in addition to the mole number of the selected

aldehyde added to the reaction multiplied by the Chs DS, and multiplied by the molar mass of the selected aldehyde minus 16 g/mol.

$$\%\ isolated\ yield = \frac{m_{recovered}}{m_{Chs} + n_{aldehyde} \times (Mw_{aldehyde} - 16) \times DS} \times 100\%$$

**Example calculation:**

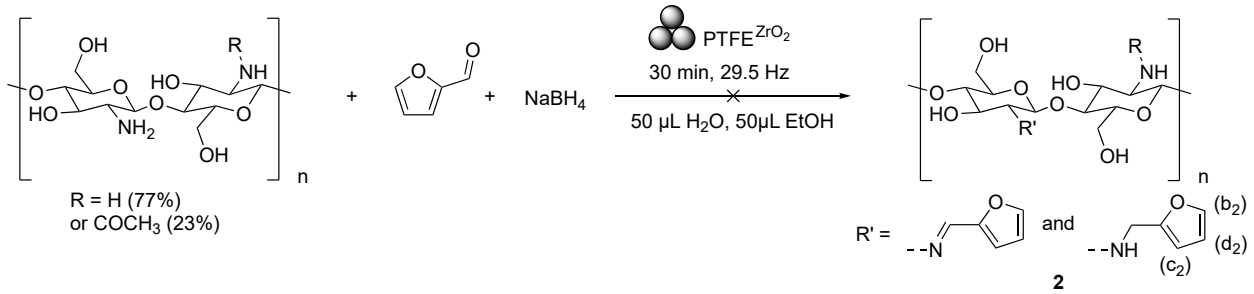
$$\%\ isolated\ yield_3 = \frac{254.4mg}{250mg + 0.99mmol \times \frac{1mol}{1000mmol}\left(96\frac{g}{mol} - 16\frac{g}{mol}\right) \times \frac{1000mg}{1g} \times 99.14\%} \times 100\% = 77.2\%$$

**Sample work-up procedure**

After set milling time, the reaction mixture was dispersed and washed in ethanol (EtOH), filtered, and washed repeatedly with deionized (DI) water to remove all excess reagents, reaction by-products, and EtOH bound on functionalized chitosan (Chs) samples during the first washing step. Finally, Chs Schiff base (SB) samples were collected and dried in a vacuum oven at 50 ˚C overnight for storage and characterization.

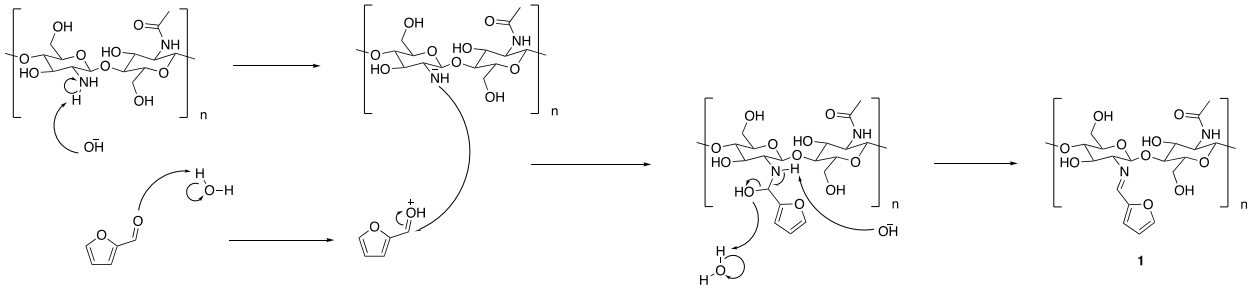
**SB condensation and reduction in one-pot experiment**

The addition of reducing agent $NaBH_4$ inhibited the Chs furfural SB (**1**) formation by reducing furfural to furfuryl alcohol, which did not react with Chs. This trial indicated that furfural to furfuryl alcohol reduction by $NaBH_4$ is kinetically favoured over the formation of **1**. In our system, the Chs reductive alkylation requires two individual steps of imine pre-formation and its consecutive reduction.




**Scheme S1.** Mechanochemical one-pot synthesis and reduction of **1**. Typical experimental conditions: Chs (250 mg), 1 eq. of furfural (95.0 mg), and 1 eq of $NaBH_4$ (37.4 mg) were loaded into a PTFE jar with a 7 mm $ZrO_2$ ball and milled for 30 min at 29.5 Hz in LAG condition with $H_2O$ (50 µL) and EtOH (50 µL).

**The role of water in the formation of 1**




**Scheme S2.** Water as a Lewis acid in the formation of **1**.

**Mechanochemical and aging-based reductive alkylation of chitosan with furfural and $NaBH_4$ $^1H$ NMR interpretation**

For the $^1H$ NMR spectrum of **1** (Figure 1), there were two groups of peaks (δ9.47, 7.91, 7.57, 6.75, and δ8.80, 8.25, 7.98, 6.99) outside of intrinsic chemical shifts of Chs. Four peaks were sharp at 9.49, 7.91, 7.57, and 6.75 ppm that of similar intensity. The chemical shift of 9.49 suggested to be a dissolvent aldehyde of furfural. The chemical shift was nearly identical to the $^1H$ spectrum of furfural starting material, consistently shifted 0.05 ppm from a furfural $^1H$ spectrum acquired in the same acidified $D_2O$ solvent.

For the $^1H$ NMR spectrum of **2** (Figure 2), along with the Chs peaks in the aliphatic region, there are two groups of additional peaks. To begin with we can still see the four sharp peaks (a, b, c, d) of free furfural. As demonstrated by the one-pot trial experiment, the free furfural reduction to furfuryl alcohol reaction is kinetically favoured over **1** formation. The amount of furfural used in the experiments would be completely reduced to its alcohol derivative and washed away from the sample during work-up, leaving an imperceptible amount of furfural residue to be seen on the spectra. Thus, we can definitively affirm all the free furfural detected on the spectra was from the hydrolysis of **1**. With the average integral of free furfural peaks and the Chs acetyl peak, we calculated that after incomplete reduction by $NaBH_4$, there was still a significant amount of unreacted imine that contributed to 62.83% conversion from the original furfural starting material. Other than the free furfural peaks, there were three broad peaks in the aromatic region at 7.64 ($b_2$), 6.68 ($d_2$), and 6.53 ppm ($c_2$) respectively. Such observation was in line with the hypothesis that once the imine of **1** was reduced to a secondary amine, the imine proton RHC=N peak previously at 8.80 ppm will become a more shielded secondary amine α proton in $RCH_2$-N.

At the same time, the protons on the furan ring grafted on Chs by a C-N bond will also be upfield because of more shielding from the chemical environment compared to those grafted by a C=N bond. Therefore, the three broad peaks belong to Chs SB that was reduced by milling with $NaBH_4$, which contributed to 40.82% conversion from the original furfural starting material. With the % conversion of furfural to imine and secondary amine, we can calculate the % conversion in the first step of the mechanochemical synthesis of **1** at 103.65%. The two numbers also allowed us to calculate the yield of an imine reduction of 39.38%.

The spectrum of aging-based reduced **1** (Figure 1) did not show any additional peaks compared to those quenched immediately after milling with $NaBH_4$. However, the proportions between the two furfural derivatives observed on the spectra, namely the free furfural coming from the hydrolysis of **1**, and the furan grafted onto Chs by C-N bonds reduced from **1** have drastically changed. The total % conversion of free furfural for the samples aged 0, 1, 2, and 3 d are 91.78%, 87.94%, 87.69%, and 101.69%, respectively. Since the samples all underwent the same amount of milling during the initial synthesis of **1**, the fluctuation was more likely from the non-perfectly homogeneous nature of the starting Chs that had a slight variation in its degree of deacetylation (DDA). The quantification assumed that Chs is homogenous with the same experimental DDA of 77%, or degree of acetylation (DA) of 23%. The acetyl $CH_3$ peaks of Chs were used as internal standards, and the fluctuation in Chs DA/DDA between batches would thus be amplified and reflected on calculated % conversion.

**4i, 4h, 4j hydrolysis and dissolution attempts for $^1$H NMR**

First, we tried the acid hydrolysis of chitosan to oligomers using hydrochloric acid reported by Beg and coworkers (Aljbour *et al.*, 2019). The HCl/$D_2O$ NMR solvent was further acidified to 2.0 M (pH -0.3) for the hydrolysis and NMR characterization of the samples. However, the NMR spectra did not suggest any improved dissolution. We hypothesized this is due to the reliance on the dissolution of chitosan in the acid solution for hydrolysis. As our samples were not soluble in acidic aqueous conditions, the hydrolysis did not occur.

We then switched to the controlled chitosan molecular weight reduction by mechanochemical and aging-based phosphoric acid hydrolysis developed by our research group (Yang *et al.*, 2023). The hydrolyzed sample of **4j** was still nearly insoluble in the NMR solvent. While the hydrolyzed samples **4h** and **4i** exhibited significantly improved solubility in the NMR solvent of choice. The $^1$H NMR spectra of **4h** and **4i** also have a good signal intensity with the dissolved fraction of hydrolyzed samples. Below we attach the $^1$H NMR of **4h** hydrolyzed in 2.0 M HCl/$D_2O$ and **4h** hydrolyzed by 85% $H_3PO_4$ by mechanochemistry and aging as an example. As the spectra showed (Figure S4.h.2) the signal intensity of **4h** hydrolyzed by 85% $H_3PO_4$ by mechanochemistry and aging was strong enough for DS calculation

$$DS_{4h} = \frac{3.01}{3} \div \frac{1.00}{3} \times \frac{23\%}{77\%} \times 100\% = 89.9\%$$

The $H_3PO_4$ hydrolyzed **4h** DS was comparable to the value calculated with $^{13}$C ssNMR (91.1%). This is also the case with **4i** as the DS of hydrolyzed sample was calculated at 25.7%, which was comparable to the result calculated by $^{13}$C ssNMR (22.4%). However, since the $H_3PO_4$ hydrolyzed **4j** was not still not soluble in the NMR solvent, the solid-state hydrolysis cannot be used as a universal method to access aqueous insoluble alkylated Chs DS values. Given the reliability of $^{13}$C ssNMR demonstrated with $H_3PO_4$ hydrolyzed **4h** and **4i**, we decided

to not expand the scope of this study to avoid introducing bias with a destructive method to the sample DS calculations.

**Supplementary Tables and Figures**

**1. Mechanochemical and aging-based reductive alkylation of chitosan with furfural and $NaBH_4$**

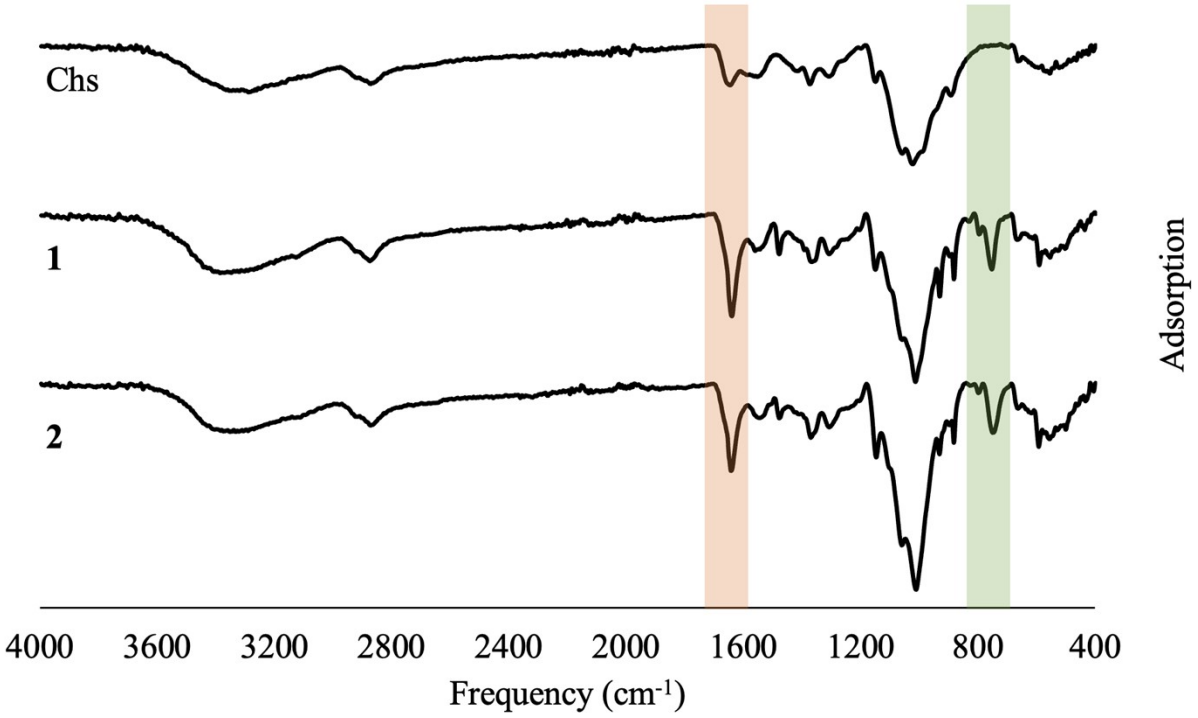



**Figure S1.** FT-IR spectra of Chs, **1**, and **2**. The peak characteristic of the bound furan alkene is highlighted in green and the peak characteristic of the imine is highlighted in orange.

A weak peak at 1170 $cm^{-1}$ and another medium peak at 800 $cm^{-1}$ was observed on the sample spectra, which aligned with the adsorption of the C-O stretching of grafted furfural vinyl ether, and that of the C=C bending of the same grafted units.

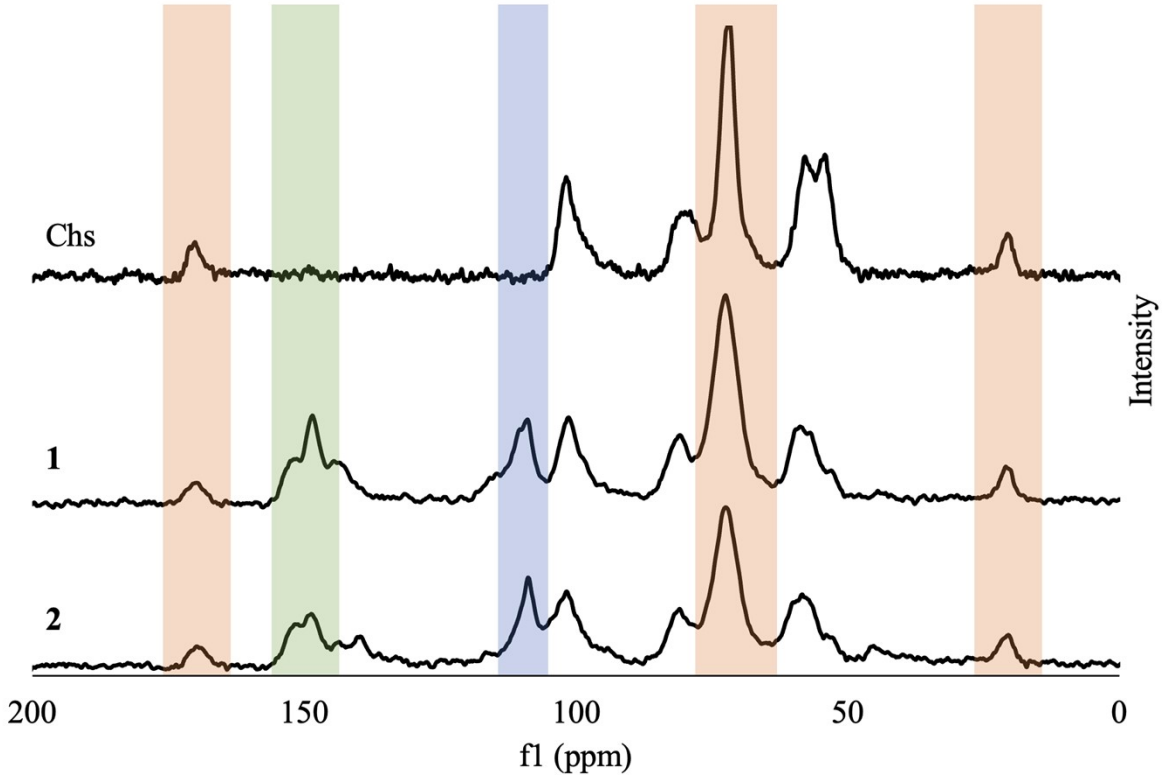



**Figure S2.** $^{13}C$ Solid-state NMR spectra of Chs, **1**, and **2.** The peak characteristic of the bound furan alkene is highlighted in blue, the peak characteristic of the imine is highlighted in green; and the peaks characteristic if the chitosan backbone are highlighted in orange.

$^{13}C$ ssNMR was used to probe structural medication from the introduction of the furfural ring structure to Chs. In Figure S2, the starting Chs bears an acetamide carbonyl peak at 170.18 ppm and an acetamide $CH_3$ peak at 20.64 ppm, with the rest of the carbon on the six-membered polysaccharide backbone and the primary alcohol $\alpha$ carbon all having chemical shift falling into the range between 101.65 and 54.26 ppm. With **1**, on the other hand, we observed carbon peaks of the imine at 148.16 ppm, furfural vinyl ether at 151.40 ppm, and the alkene at 109.54 ppm. This observation provided NMR spectroscopic evidence that agrees with the FT-IR analysis.

## 2. One pot mechanochemical reduction of 1 with $NaBH_4$

Solid samples recovered from the experiment were dissolved in 0.1% HCl $D_2O$ for $^1H$ NMR spectrum acquisition. The EtOH wash was collected and evaporated under reduced pressure. After evaporation, the thick pale-yellow oil was collected and dissolved in $CDCl_3$ for $^1H$ NMR spectrum acquisition.

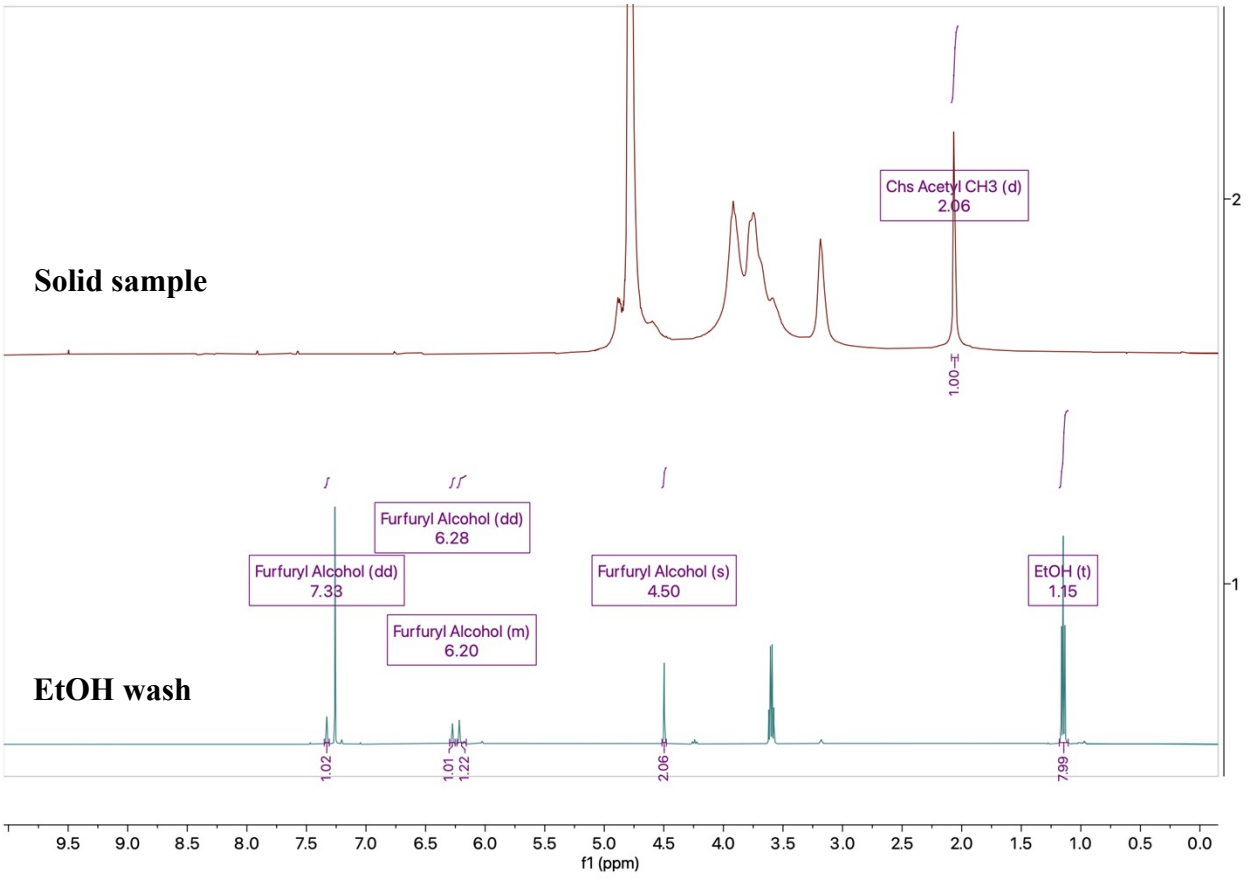



**Figure S3.** $^1H$ NMR spectra of one pot mechanochemical reduction of **1** with $NaBH_4$. (Maroon: recovered solid sample in 0.1% HCl $D_2O$, Teal: solvent-removed EtOH wash in $CDCl_3$.)

## 4. The aldehyde scope of the mechanochemical and aging-based chitosan reductive alkylation

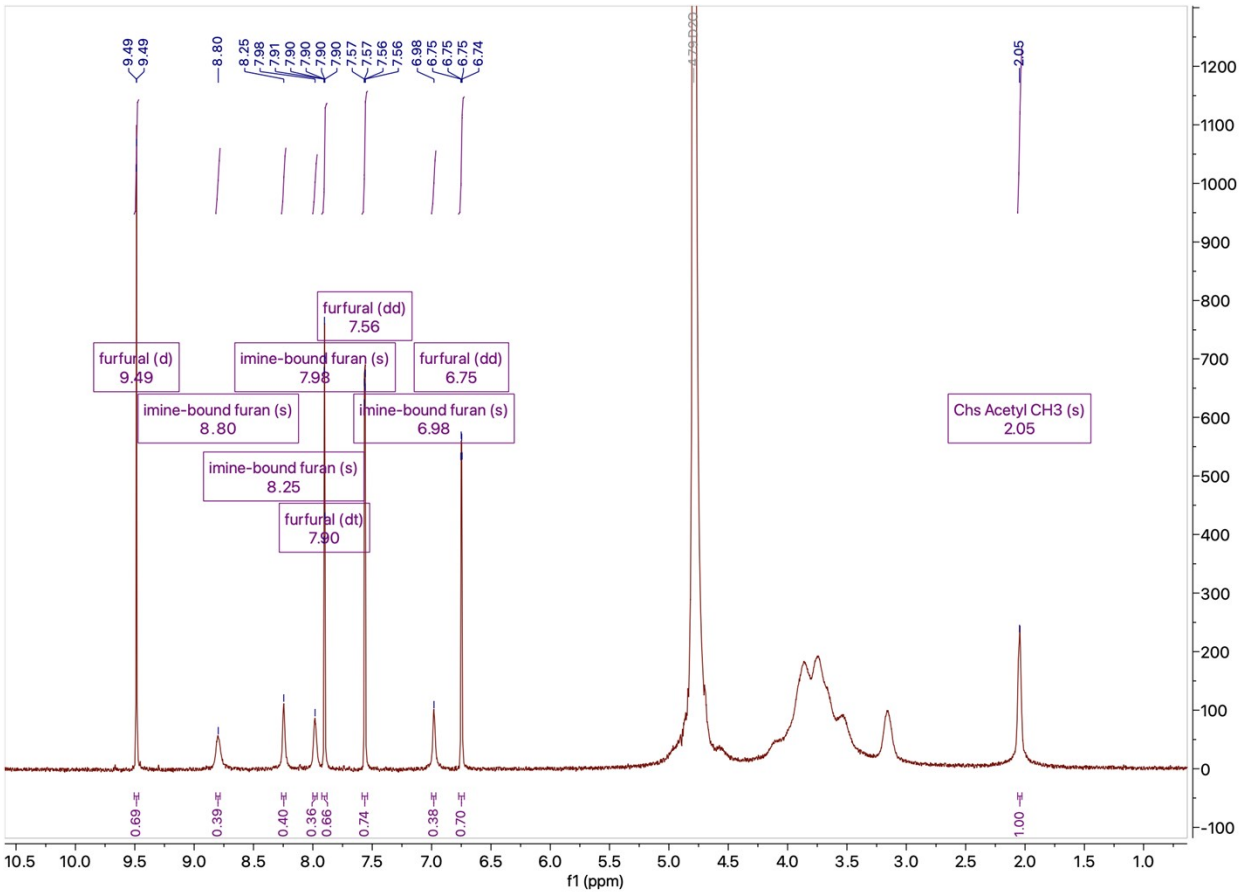



**Figure S4.1.** $^{1}$H spectrum of **1**.

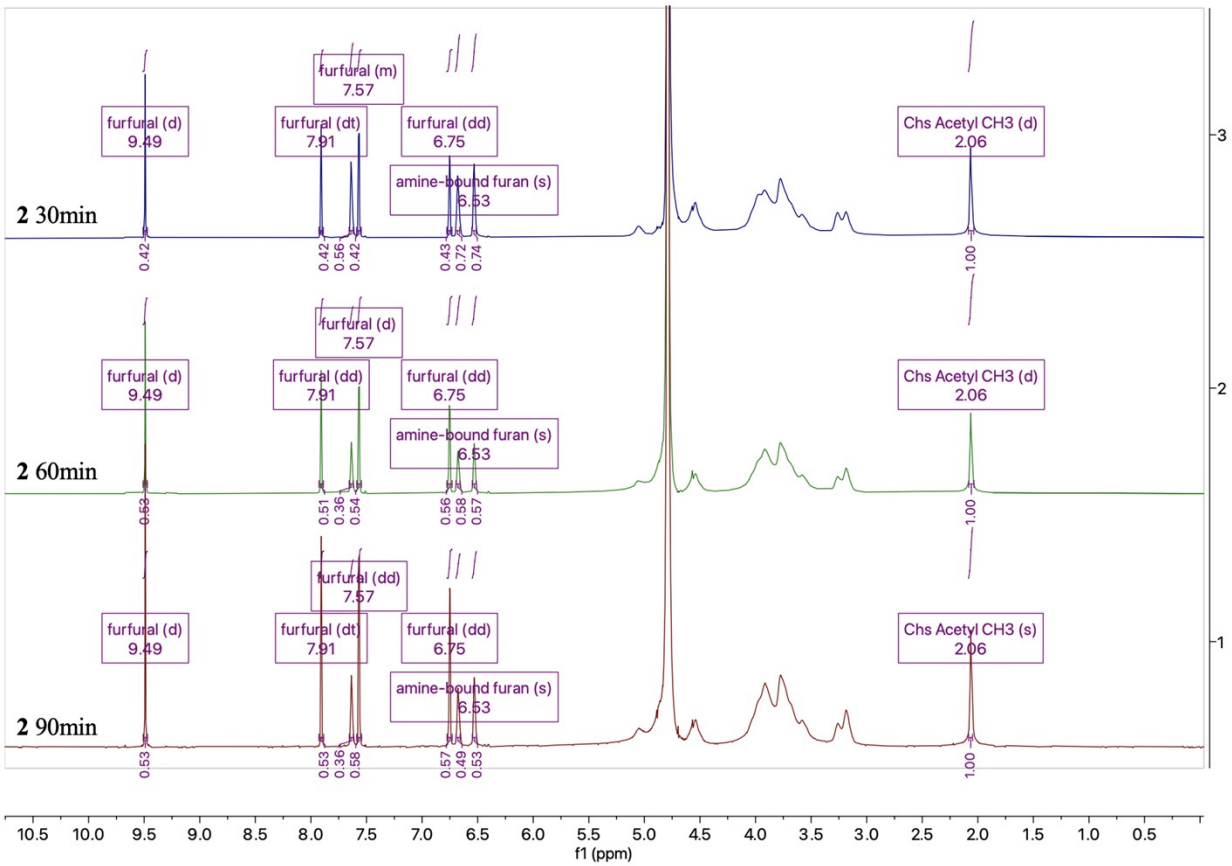



**Figure S4.2.** $^{1}$H spectrum of **2**.

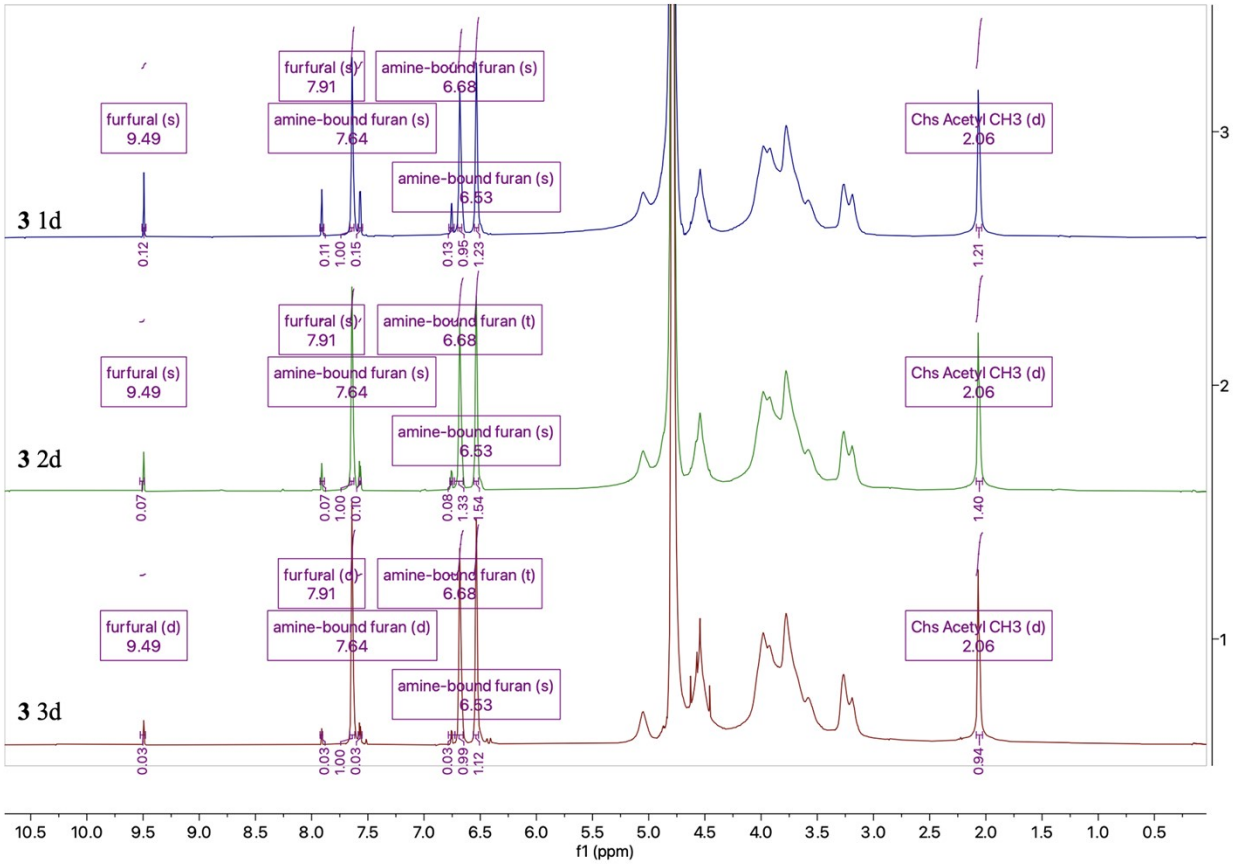



**Figure S4.3.** $^{1}$H spectrum of **3**.

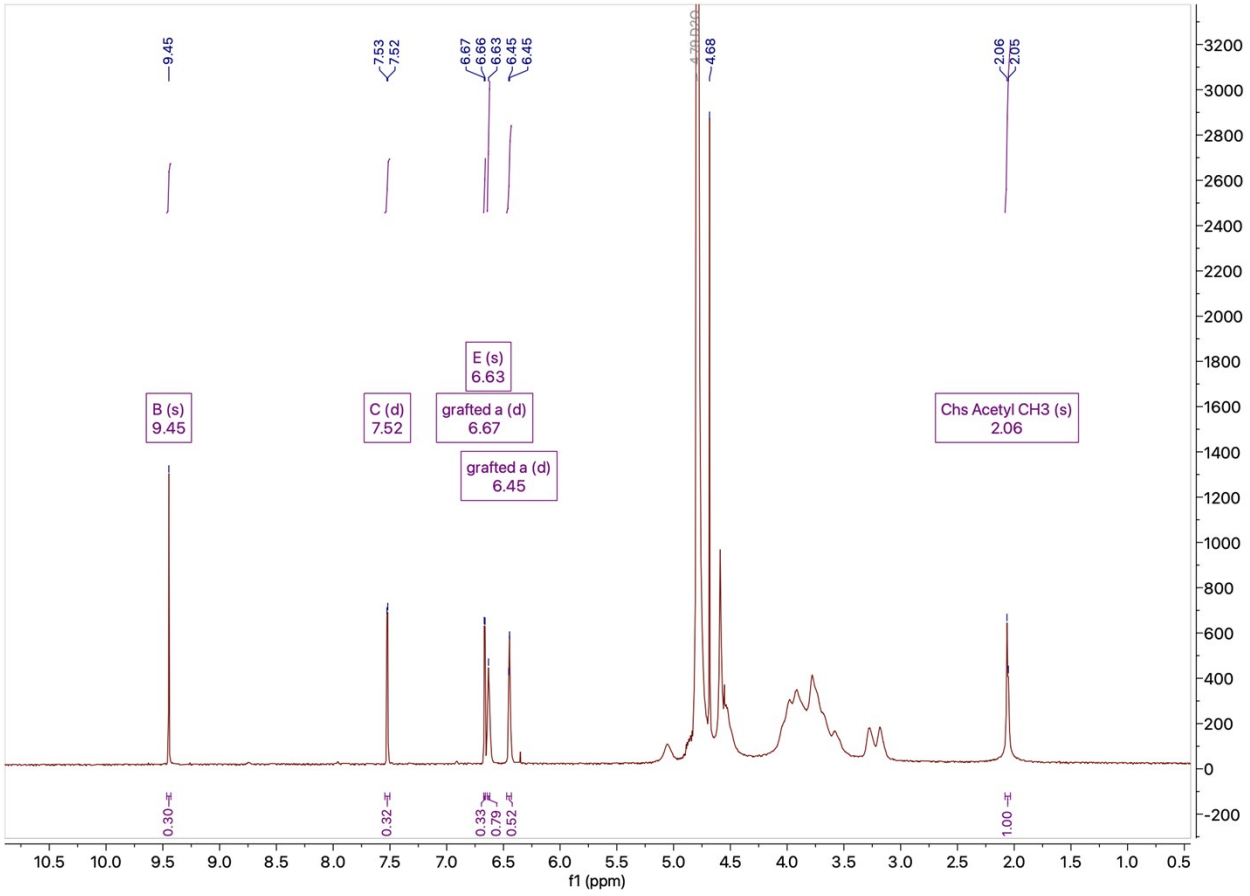



**Figure S4.a.** $^{1}$H spectrum of **4a**.

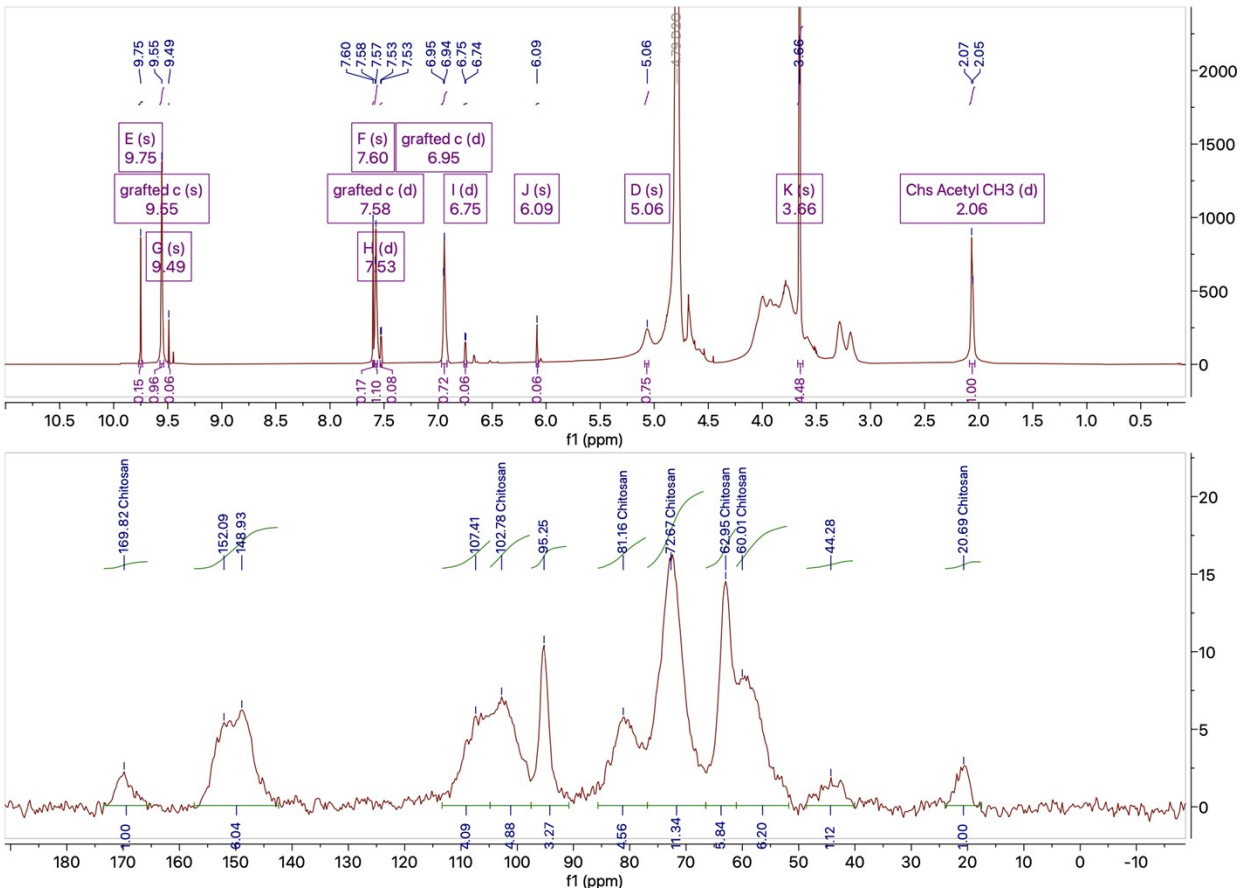



**Figure S4.b.** $^{1}H$ and $^{13}C$ spectra of **4b**.

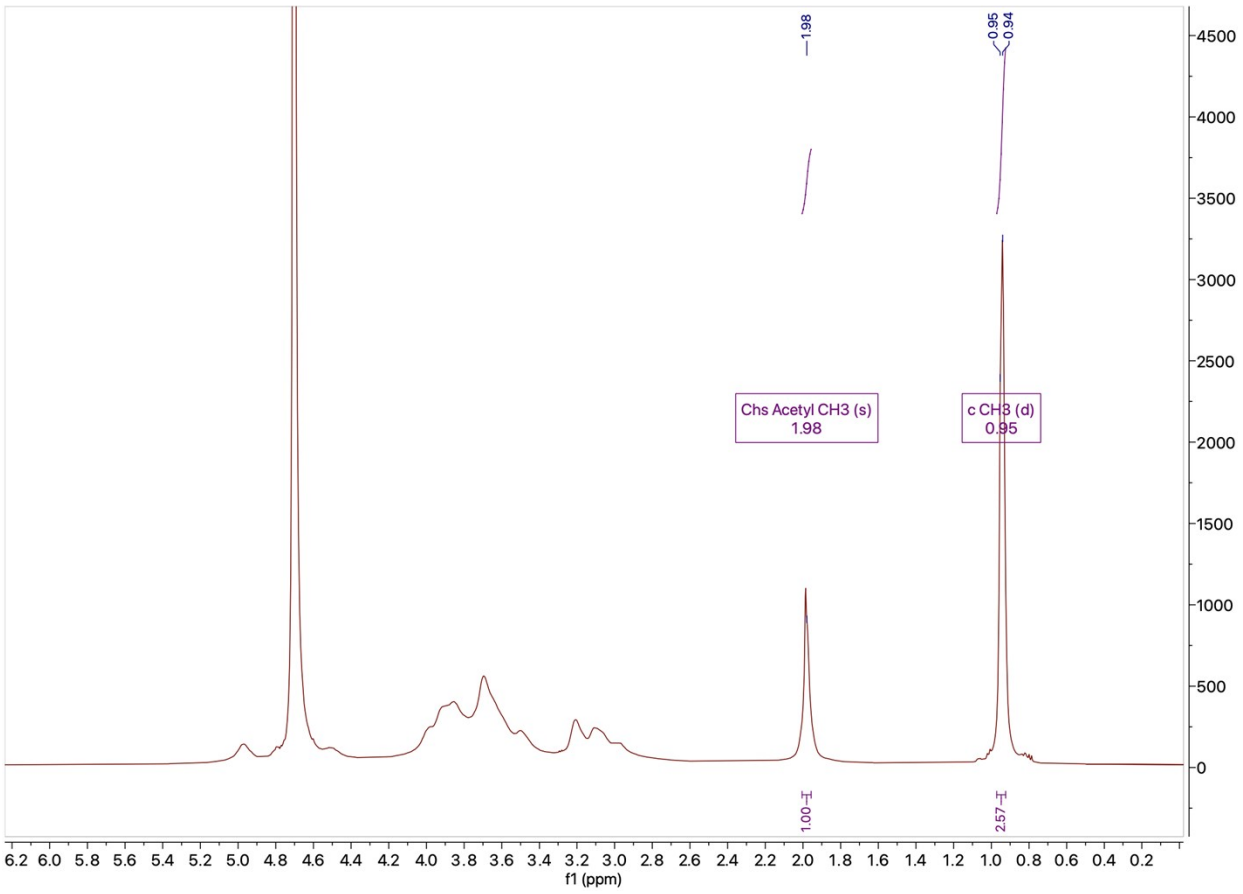



**Figure S4.c.** $^{1}$H spectrum of **4c**.

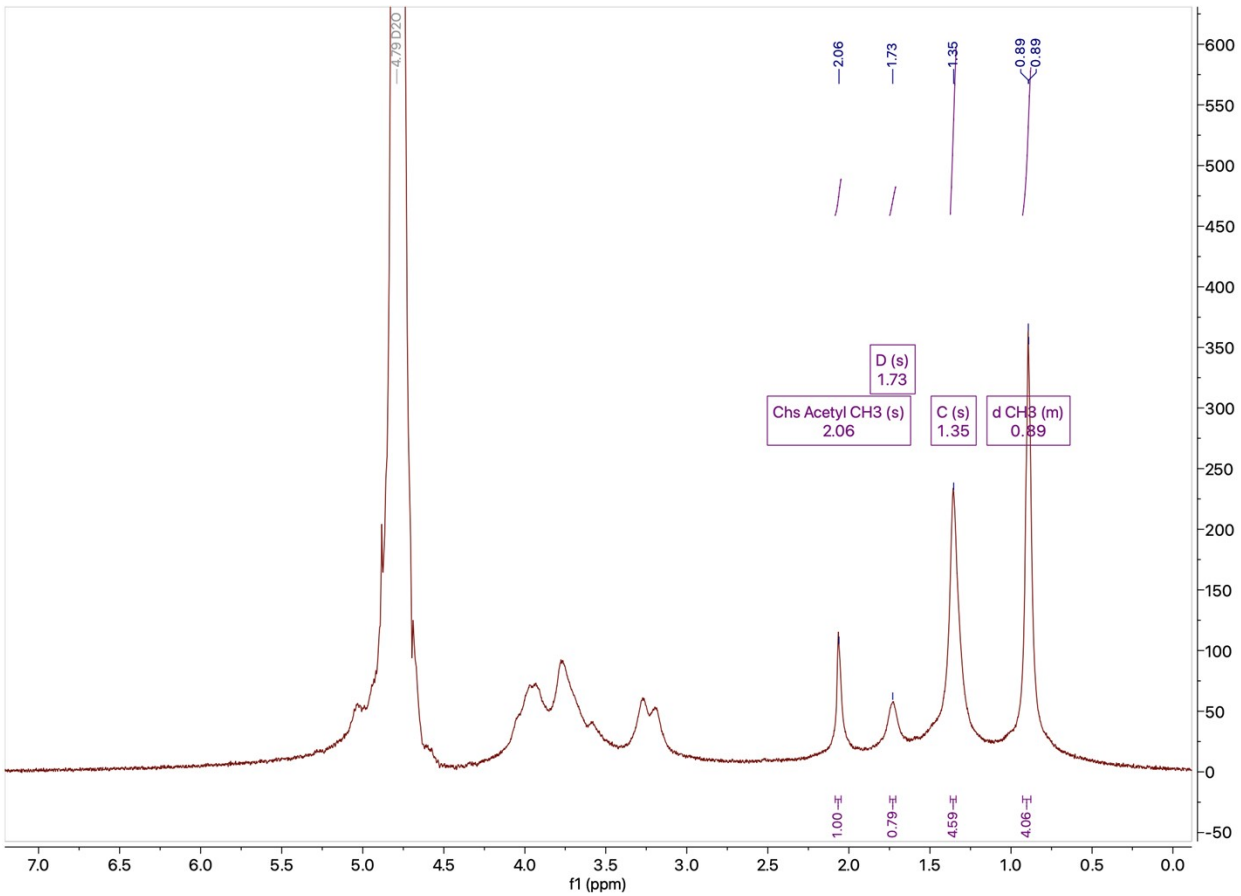



**Figure S4.d.** $^{1}$H spectrum of **4d**.

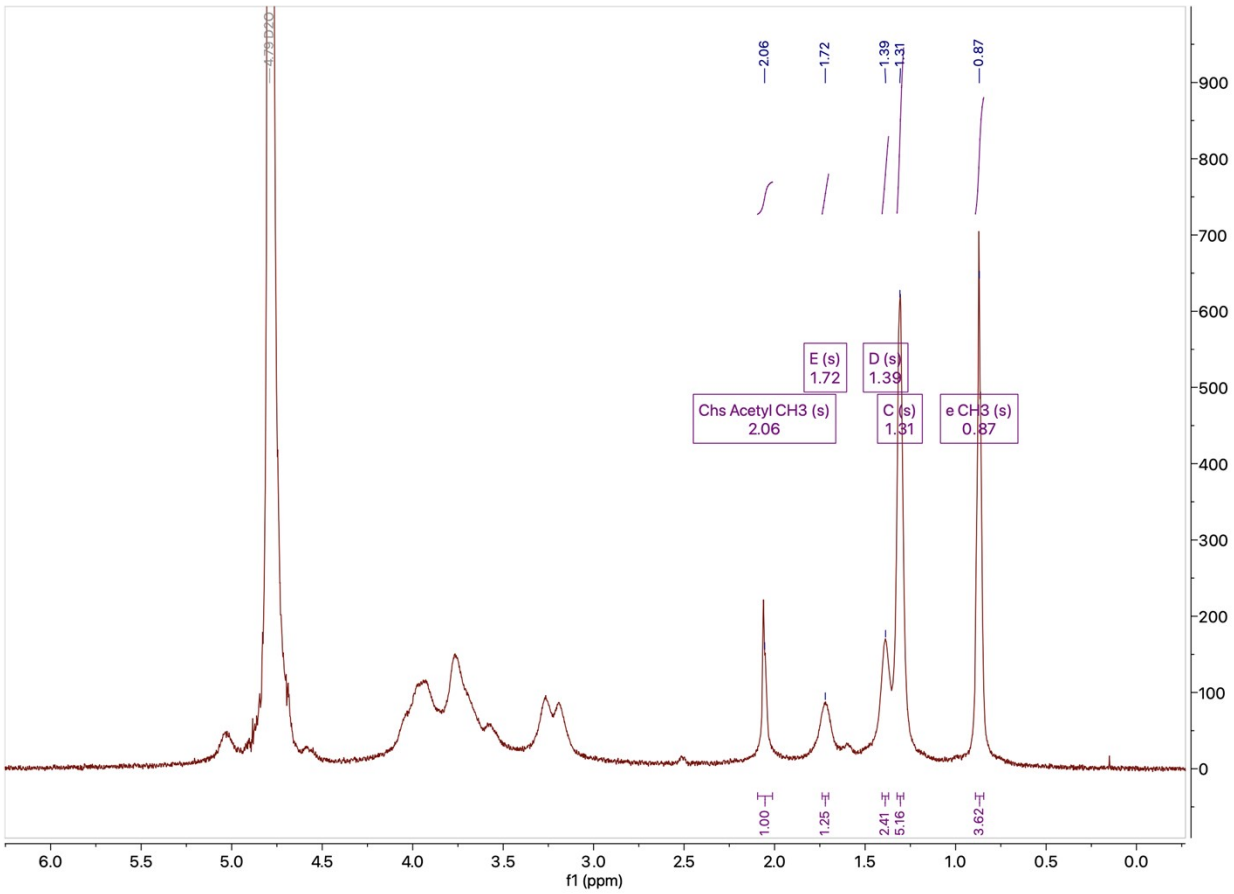



**Figure S4.e.** $^{1}$H spectrum of **4e**.

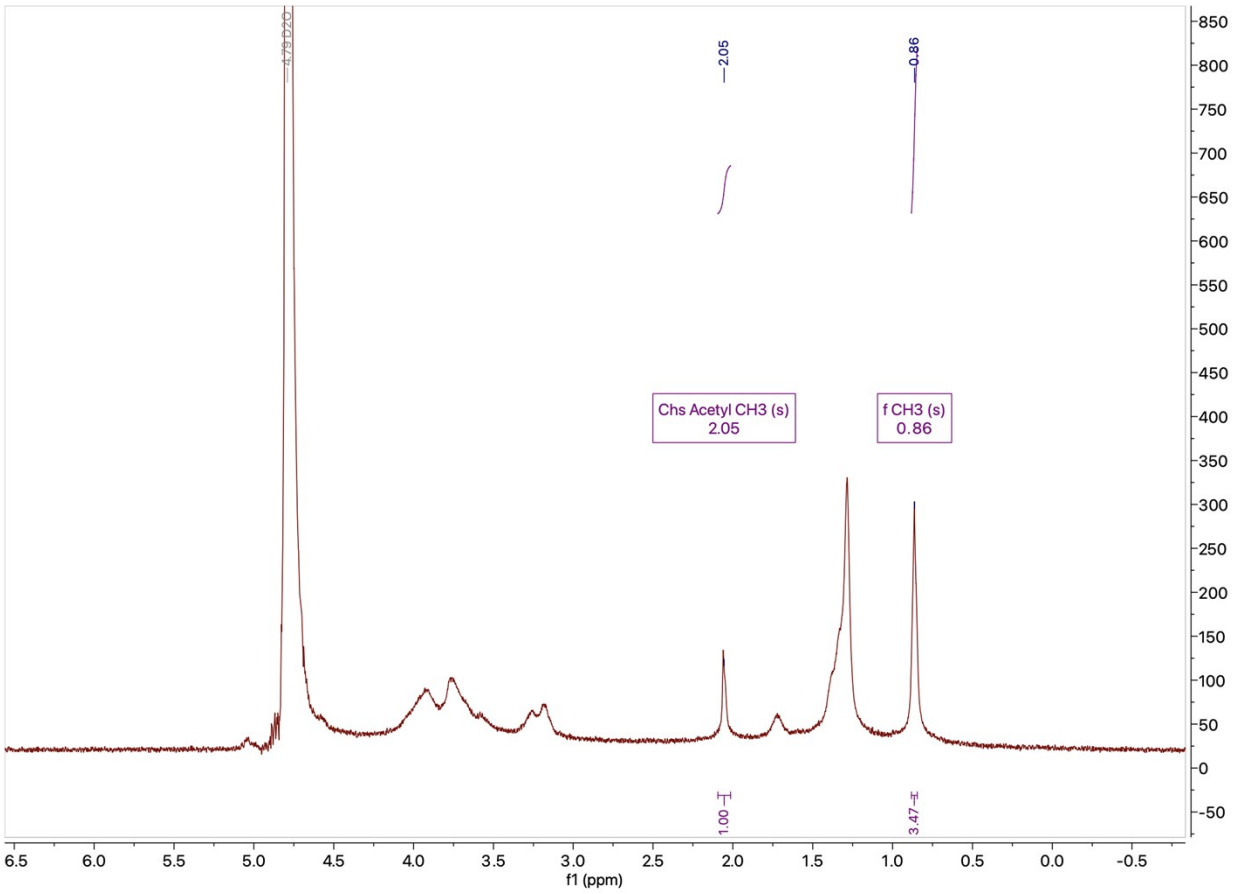



**Figure S4.f.** $^{1}$H spectrum of **4f**.

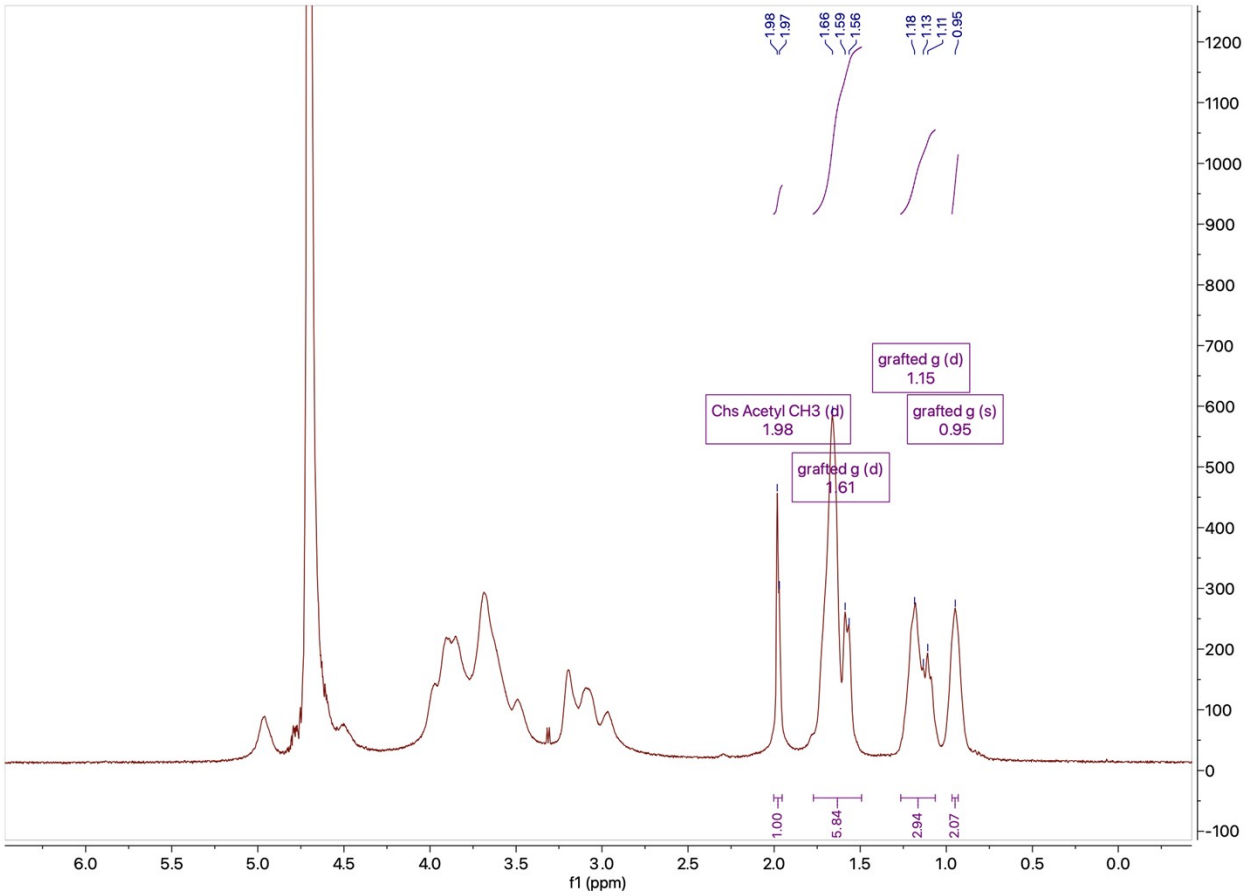



**Figure S4.g.** $^{1}$H spectrum of **4g**.

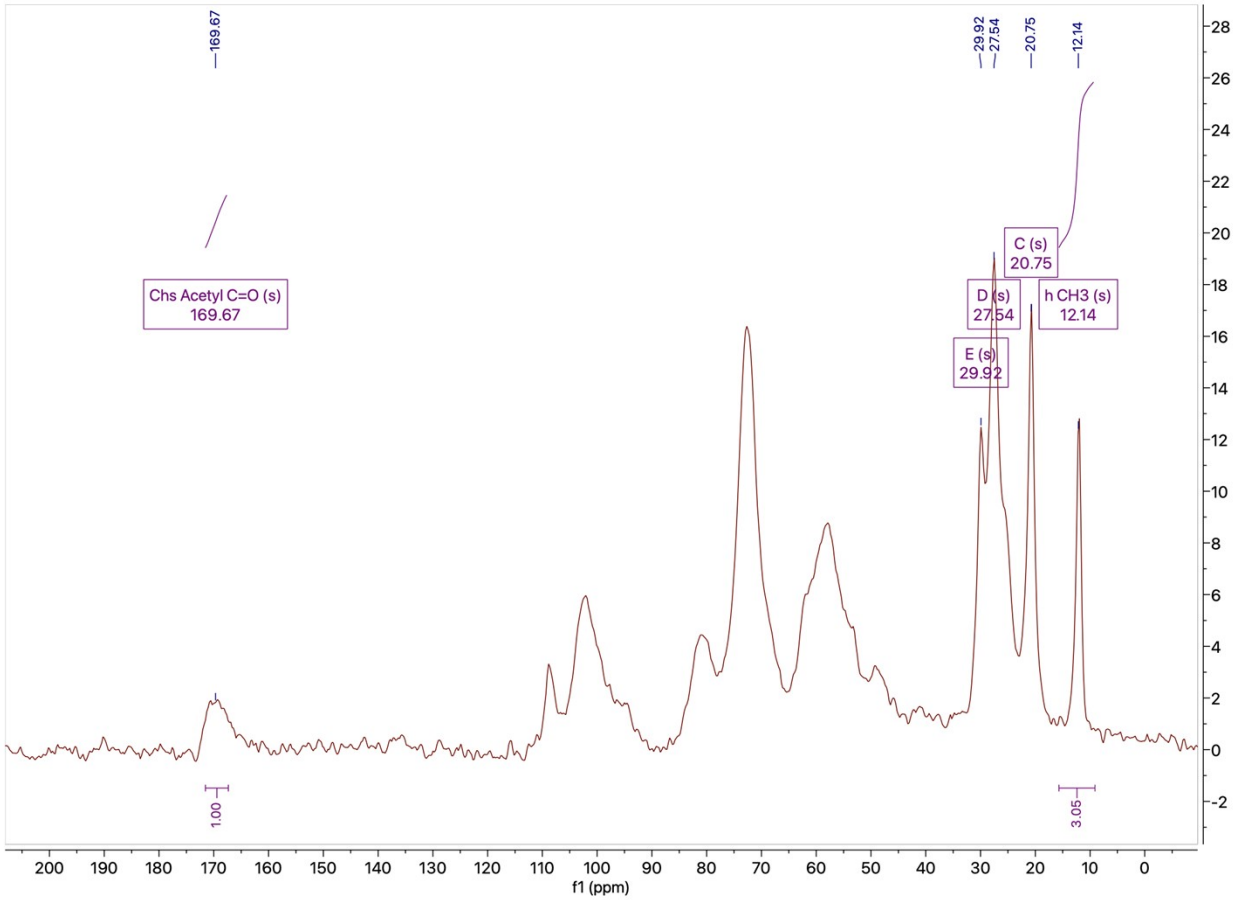



**Figure S4.h.** $^{13}C$ spectrum of **4h**.

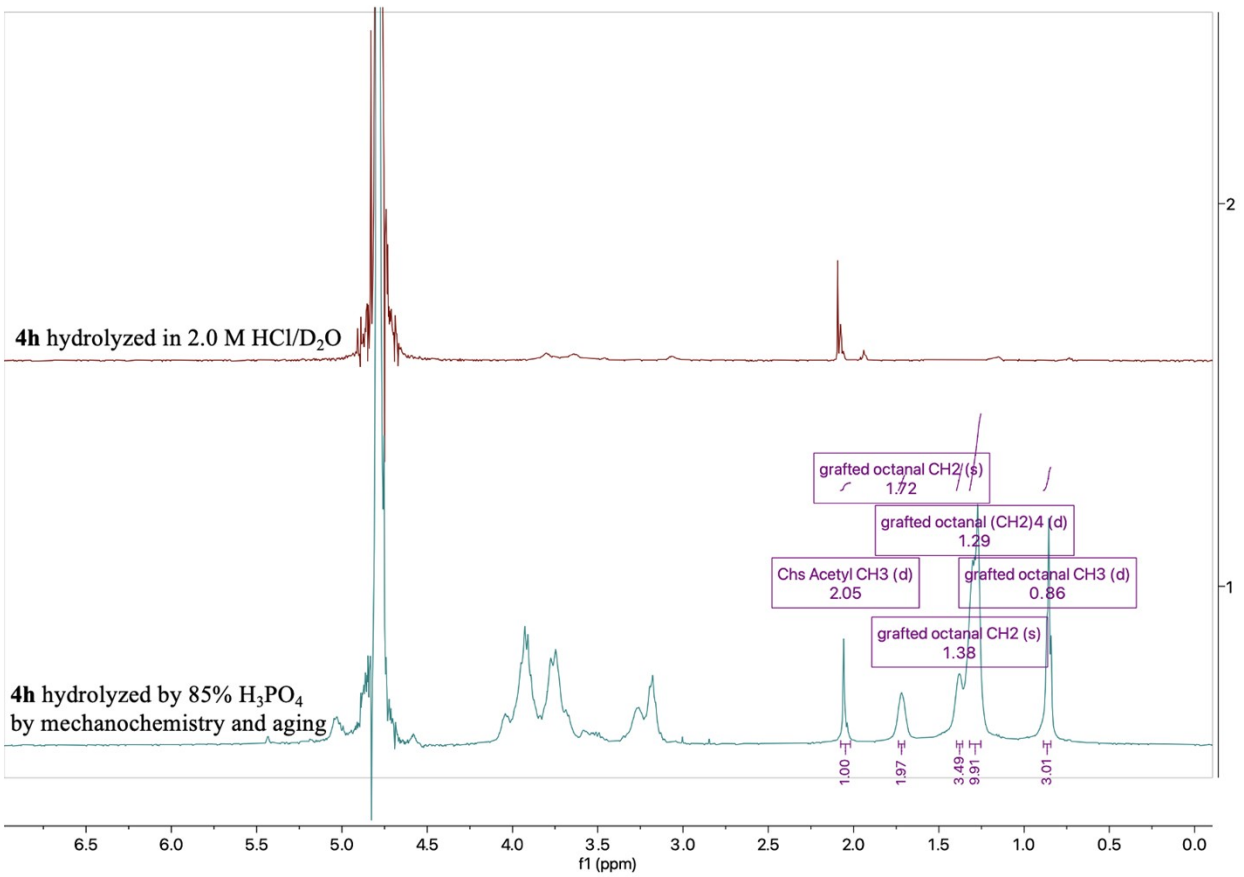



**Figure S4.h.2** $^{1}$H spectra of acid hydrolyzed **4h**.

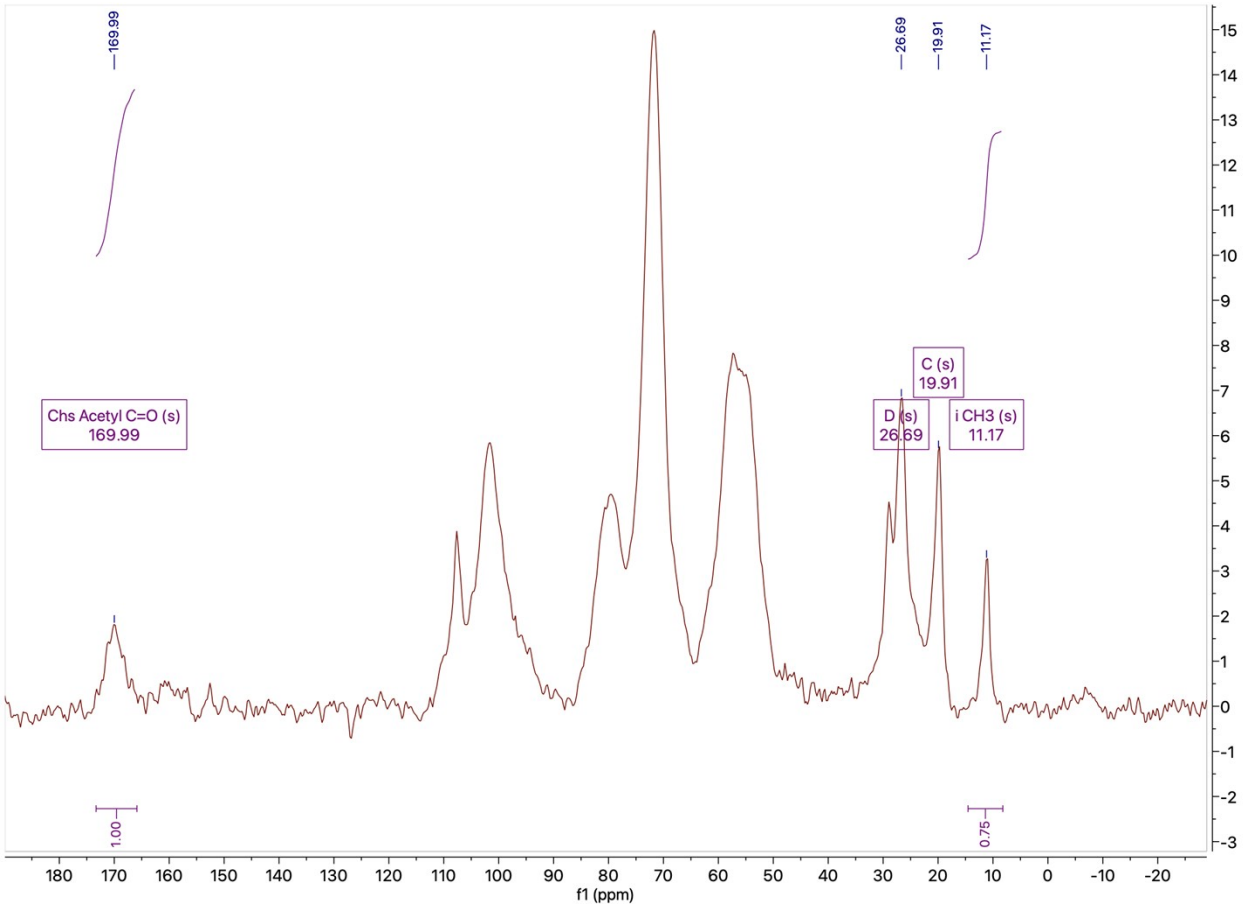



**Figure S4.i.** $^{13}C$ spectrum of **4i**.

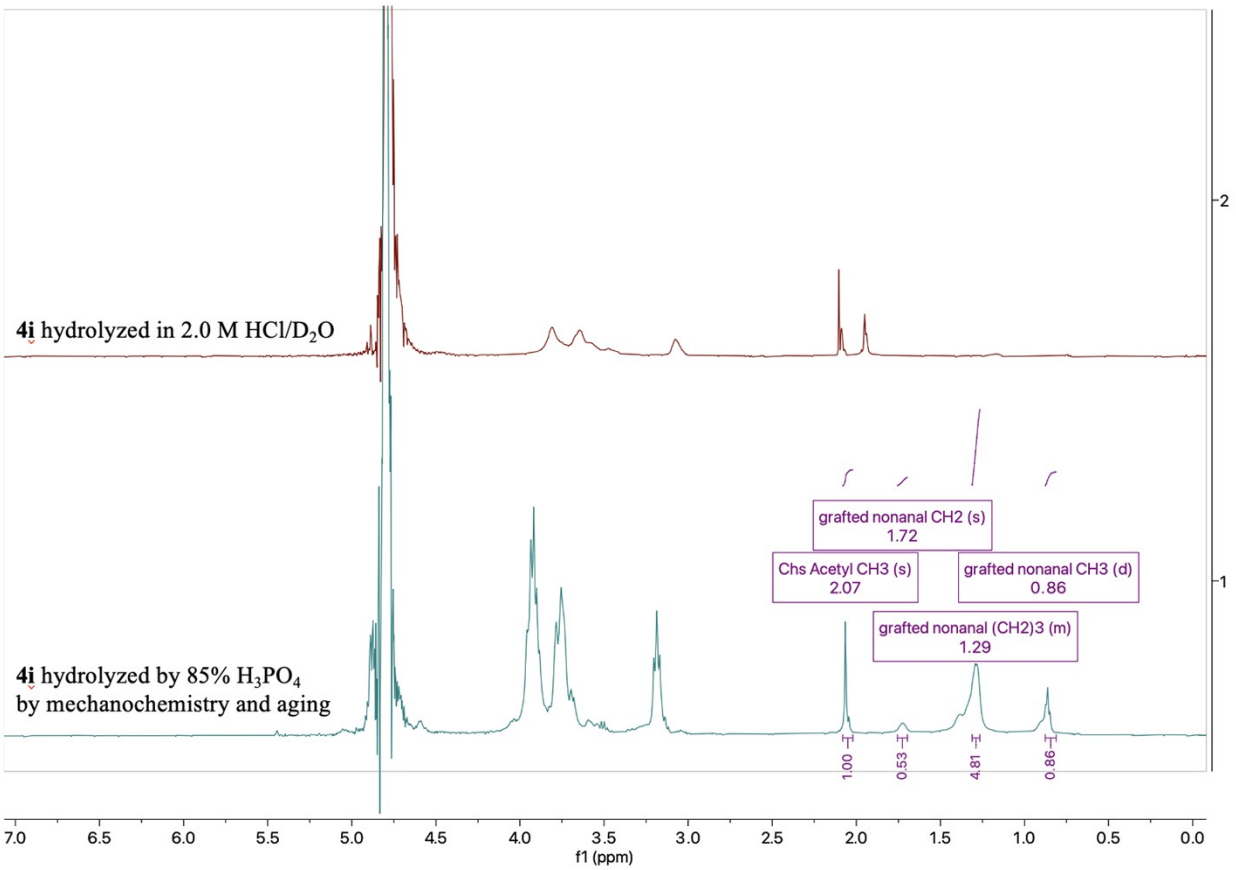



**Figure S4.i.2** $^{1}$H spectra of acid hydrolyzed **4i**.

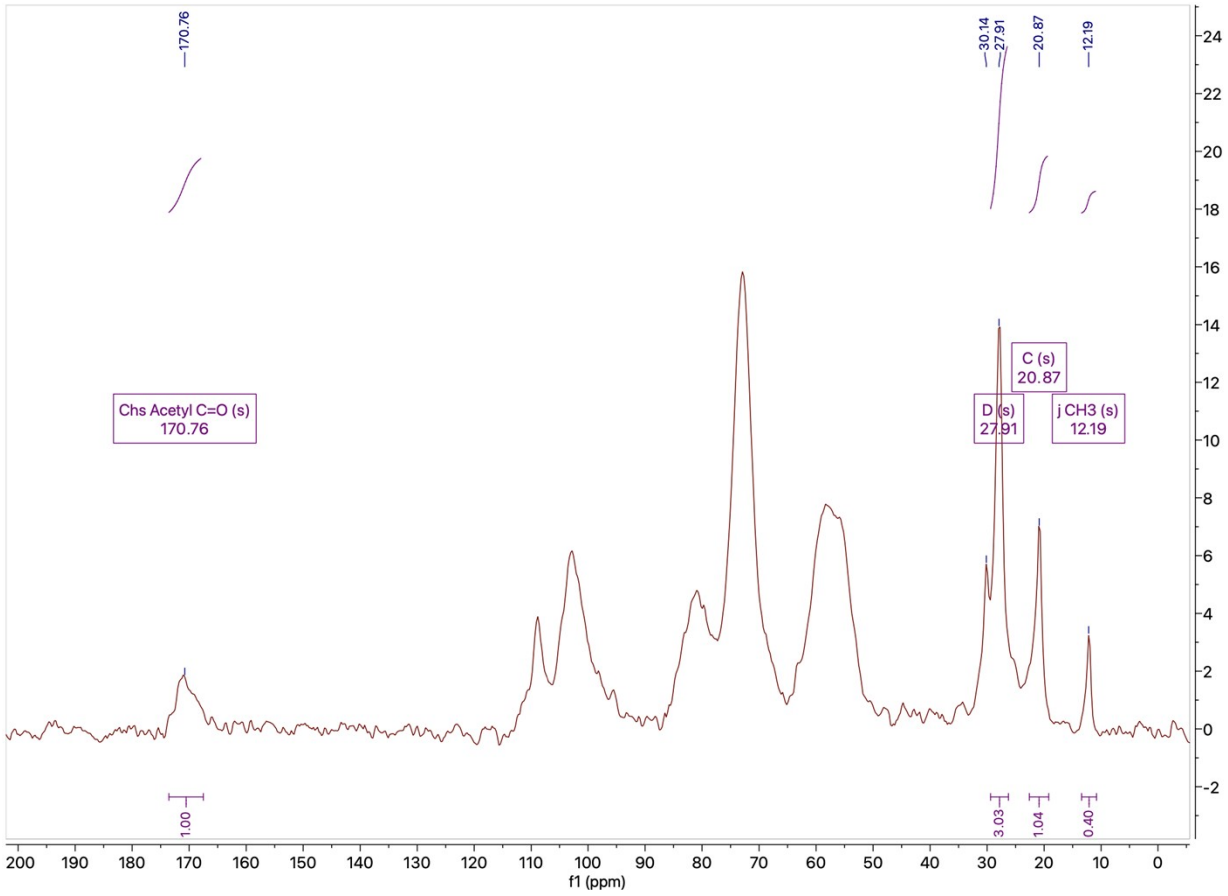



**Figure S4.j.** $^{13}C$ spectrum of **4j**.

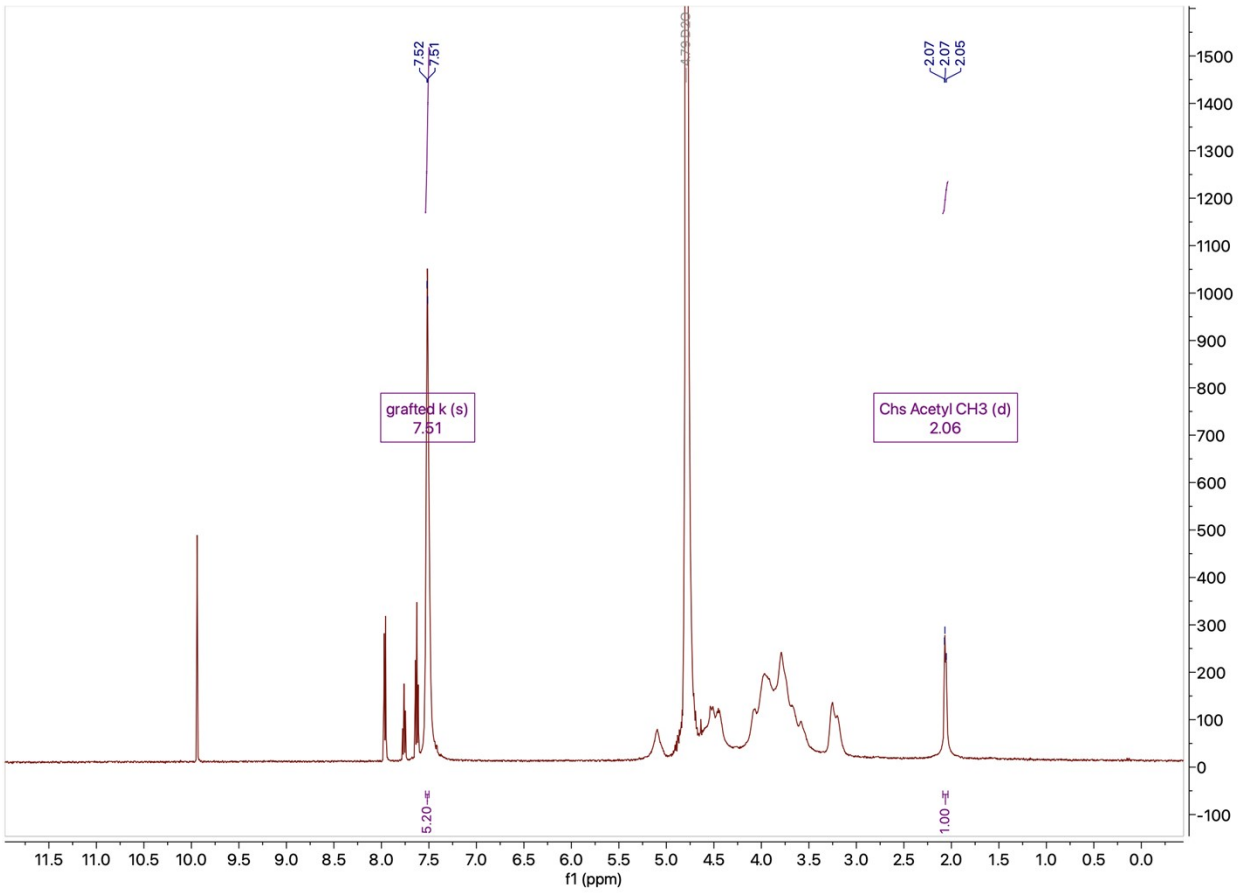



**Figure S4.k.** $^{1}$H spectrum of **4k**.

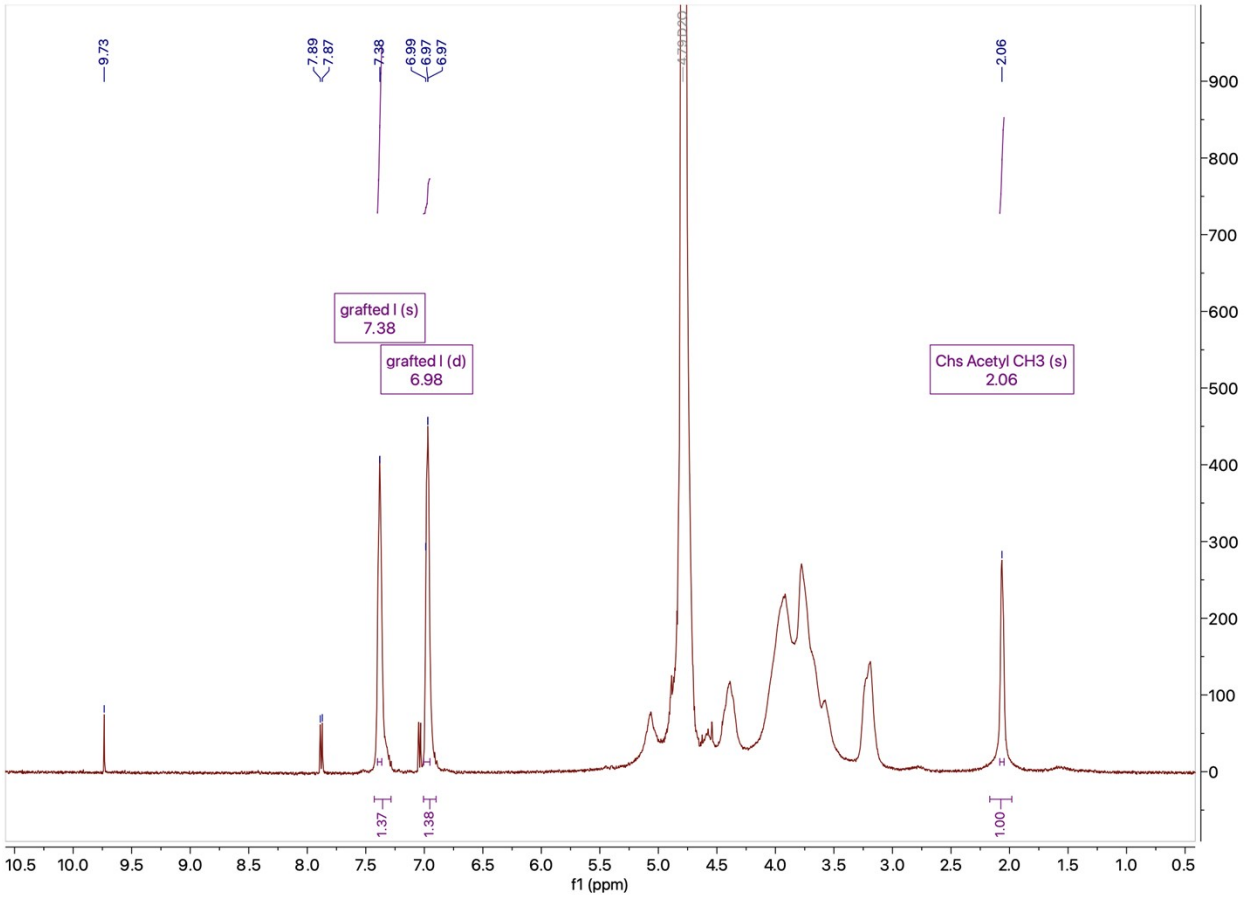



**Figure S4.l.** $^{1}$H spectrum of **4l**.

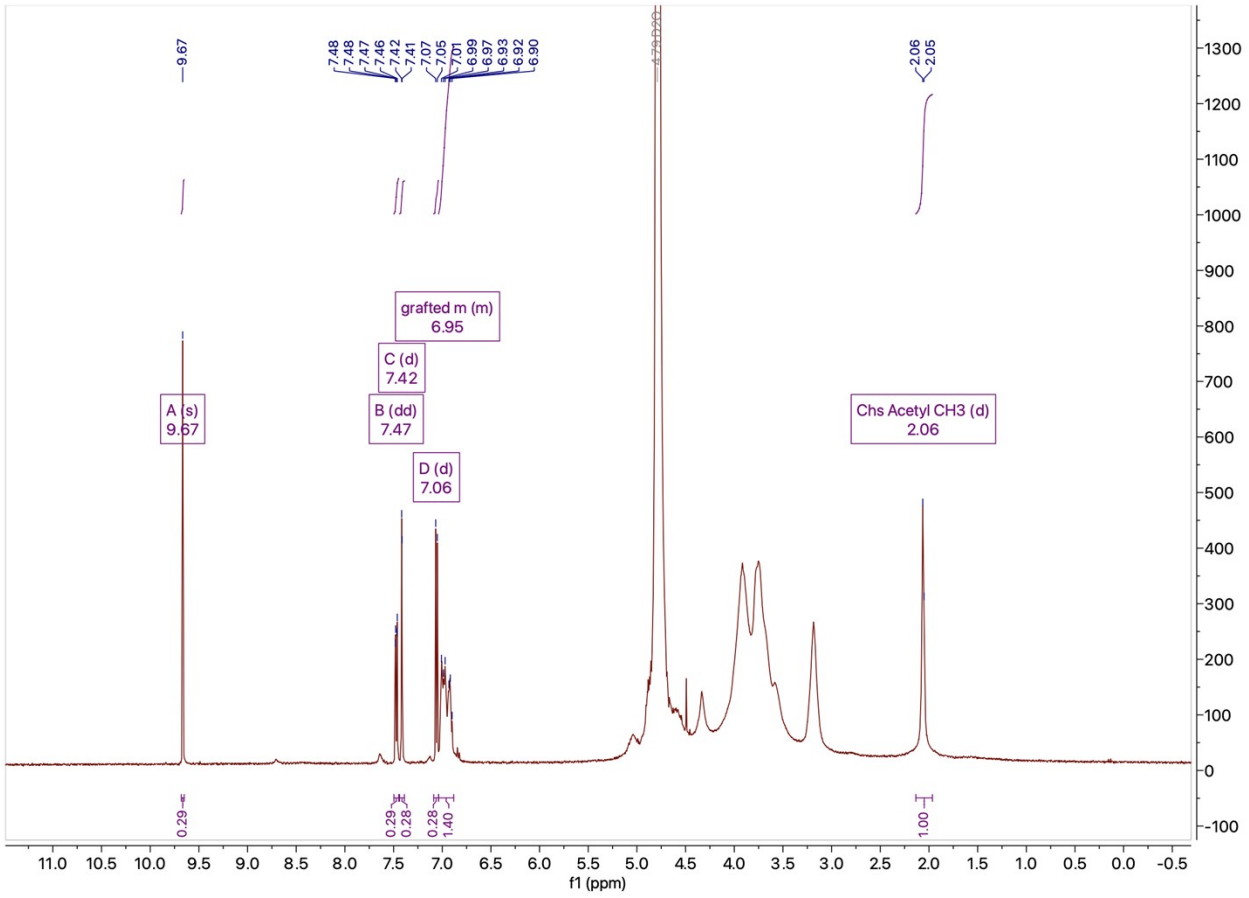



**Figure S4.m.** $^{1}$H spectrum of **4m**.

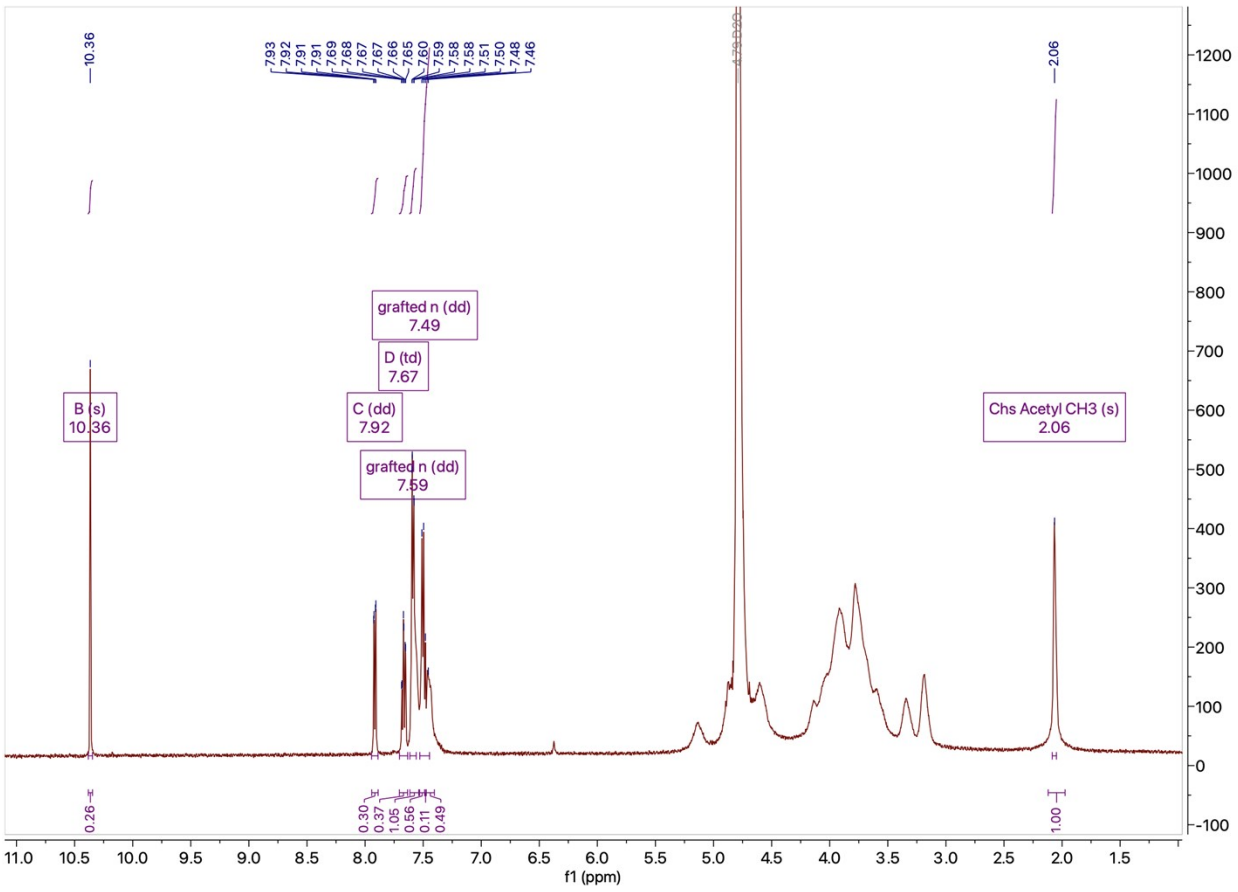



**Figure S4.n.** $^{1}$H spectrum of **4n**.

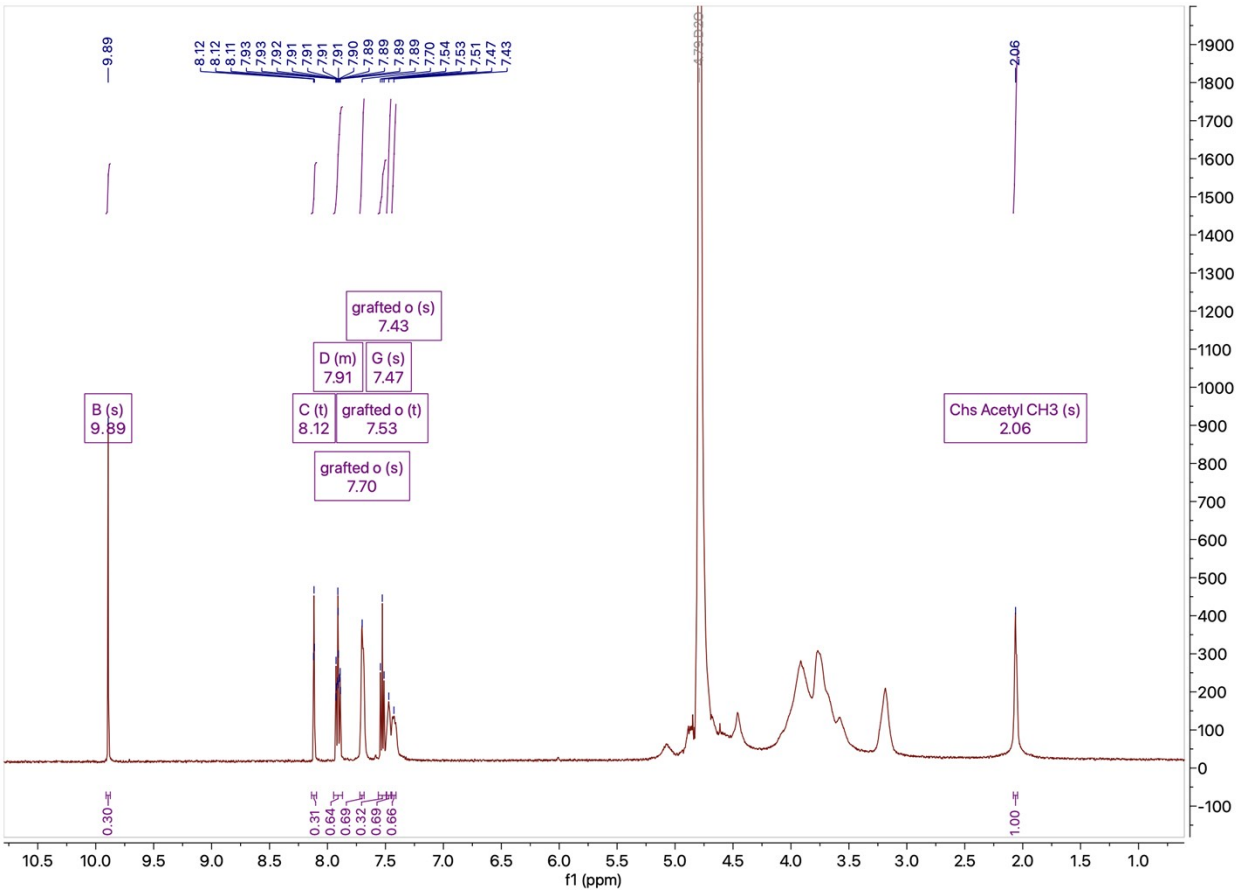



**Figure S4.o.** $^{1}$H spectrum of **4o**.

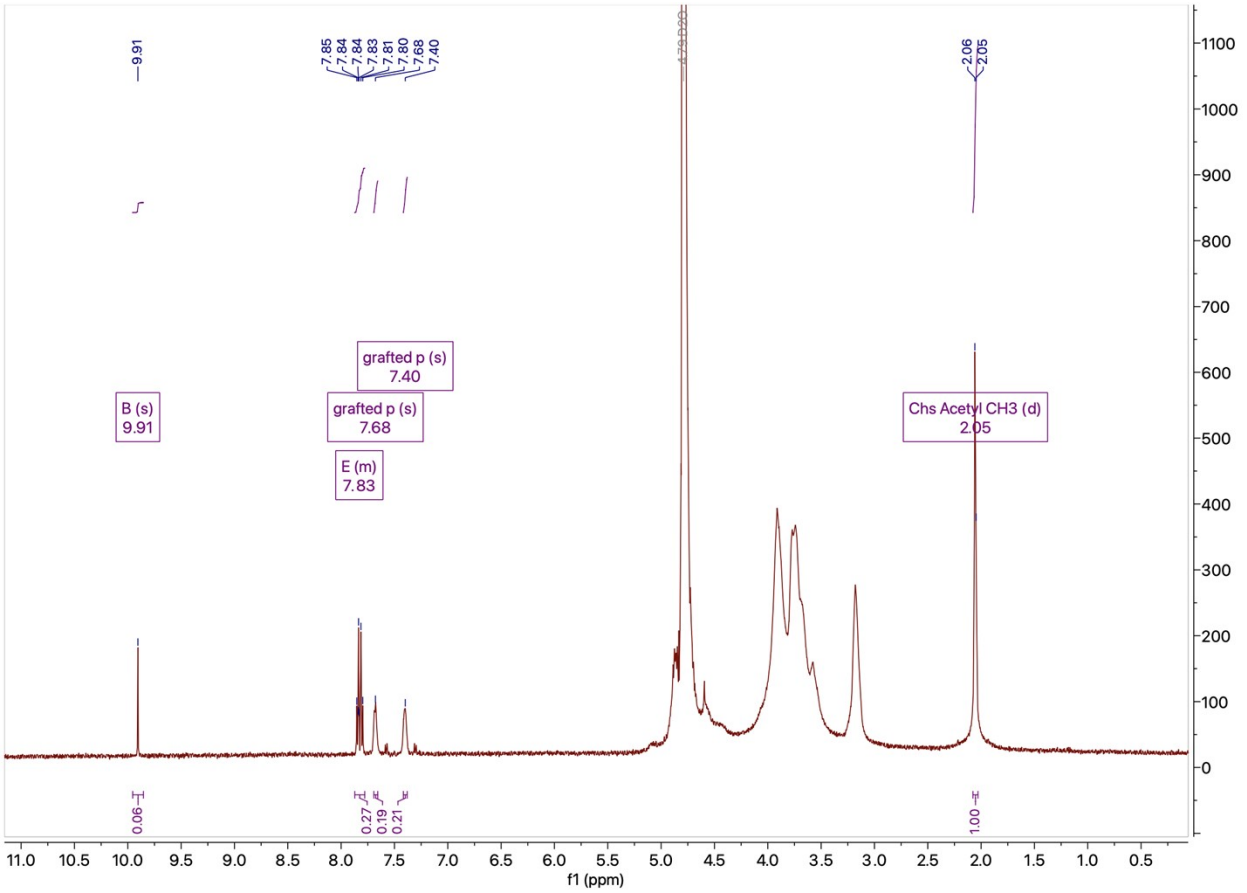



**Figure S4.p.** $^{1}$H spectrum of **4p**.

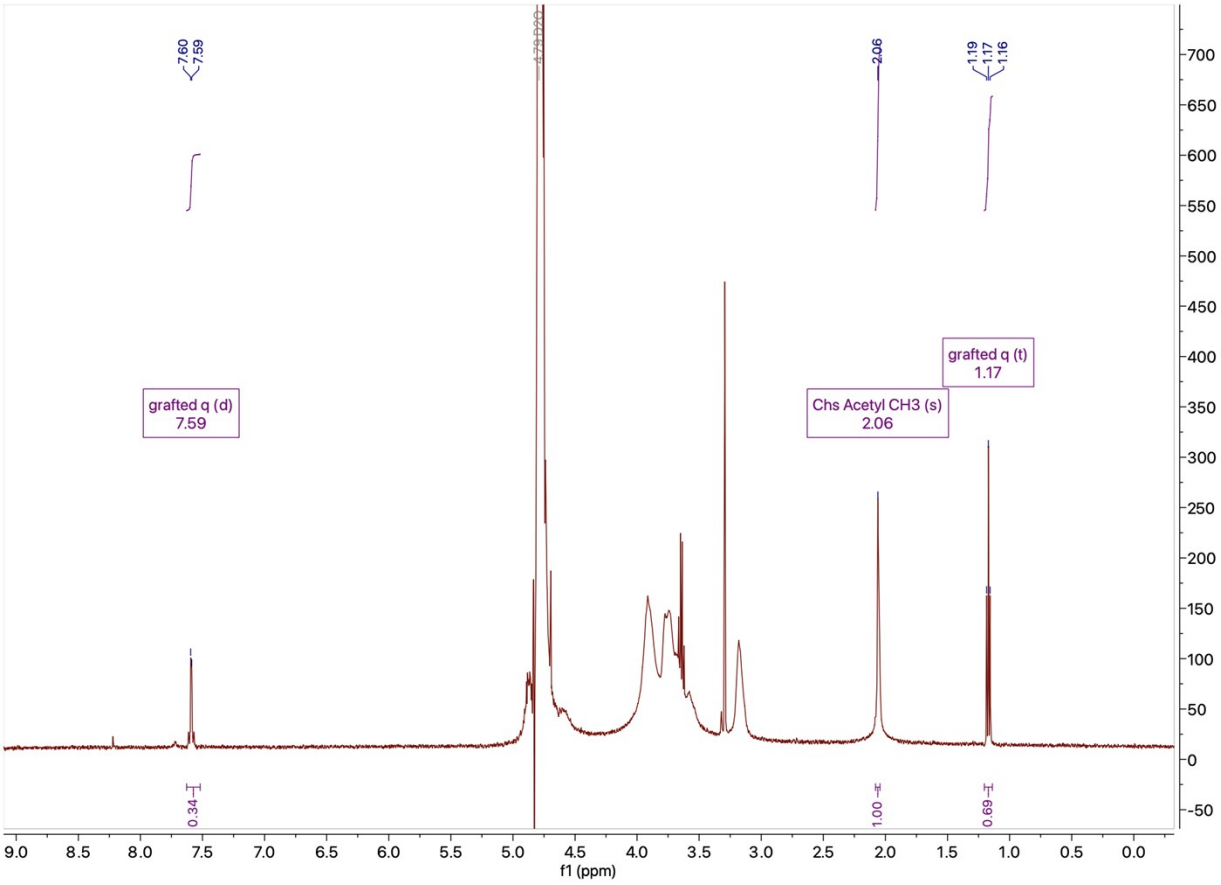



**Figure S4.q.** $^{1}$H spectrum of **4q**.

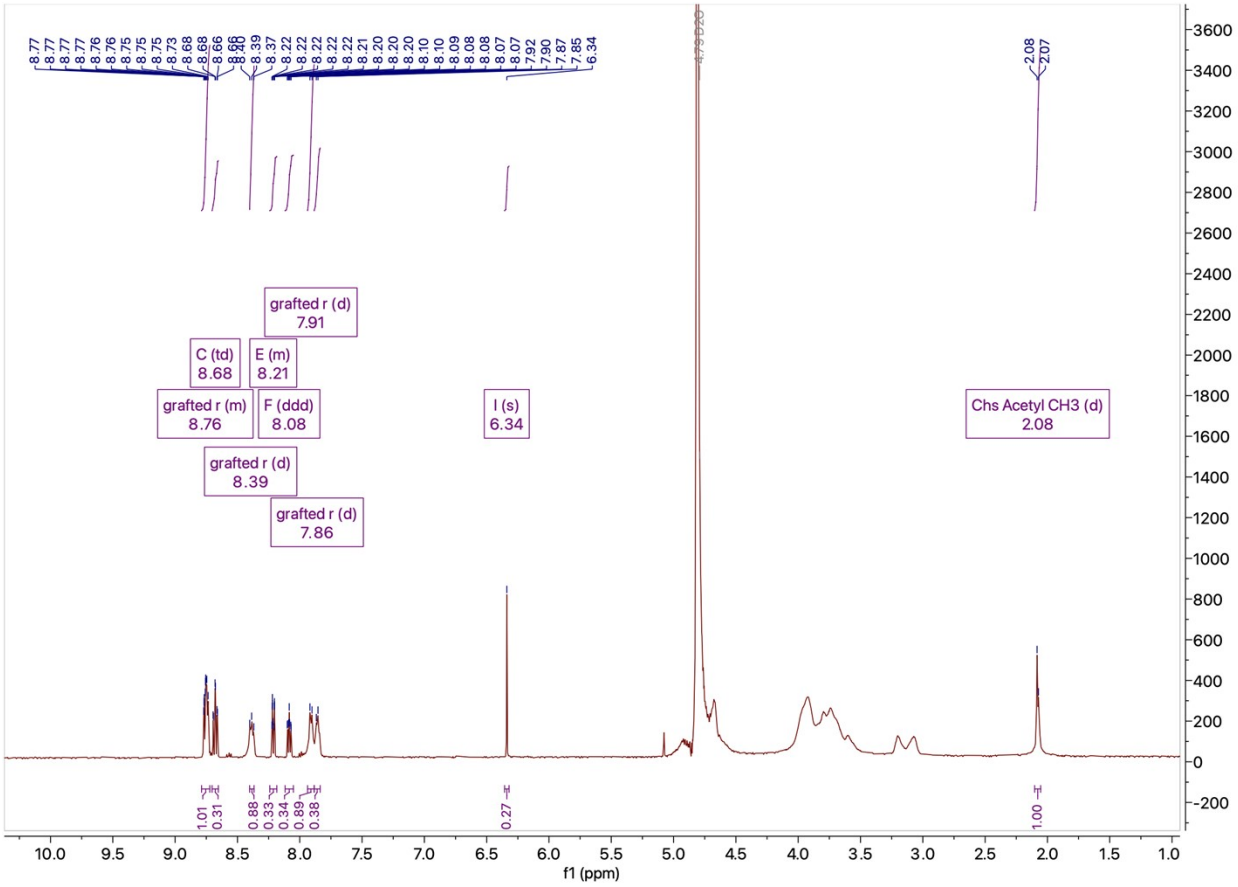



**Figure S4.r.** $^{1}$H spectrum of **4r**.

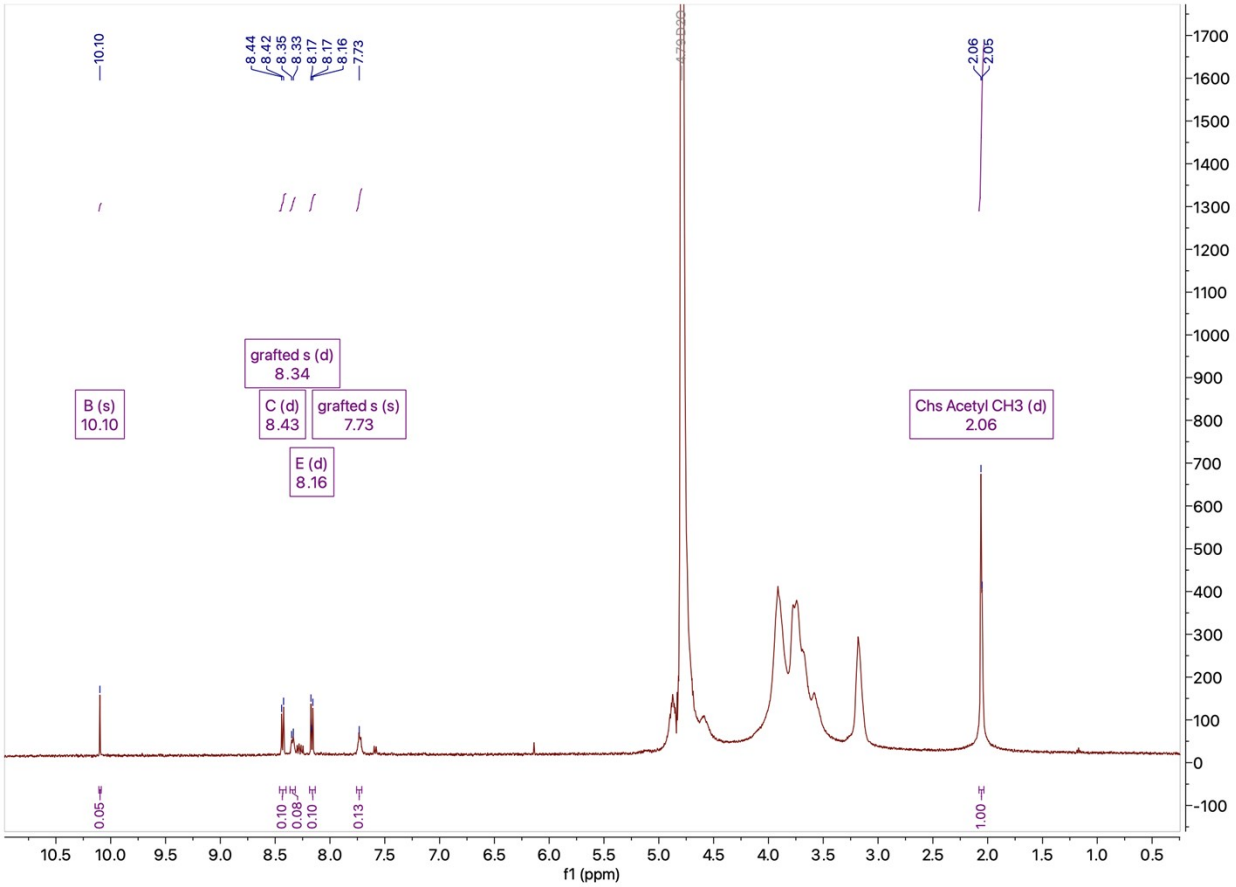



**Figure S4.s.** $^{1}$H spectrum of **4s**.

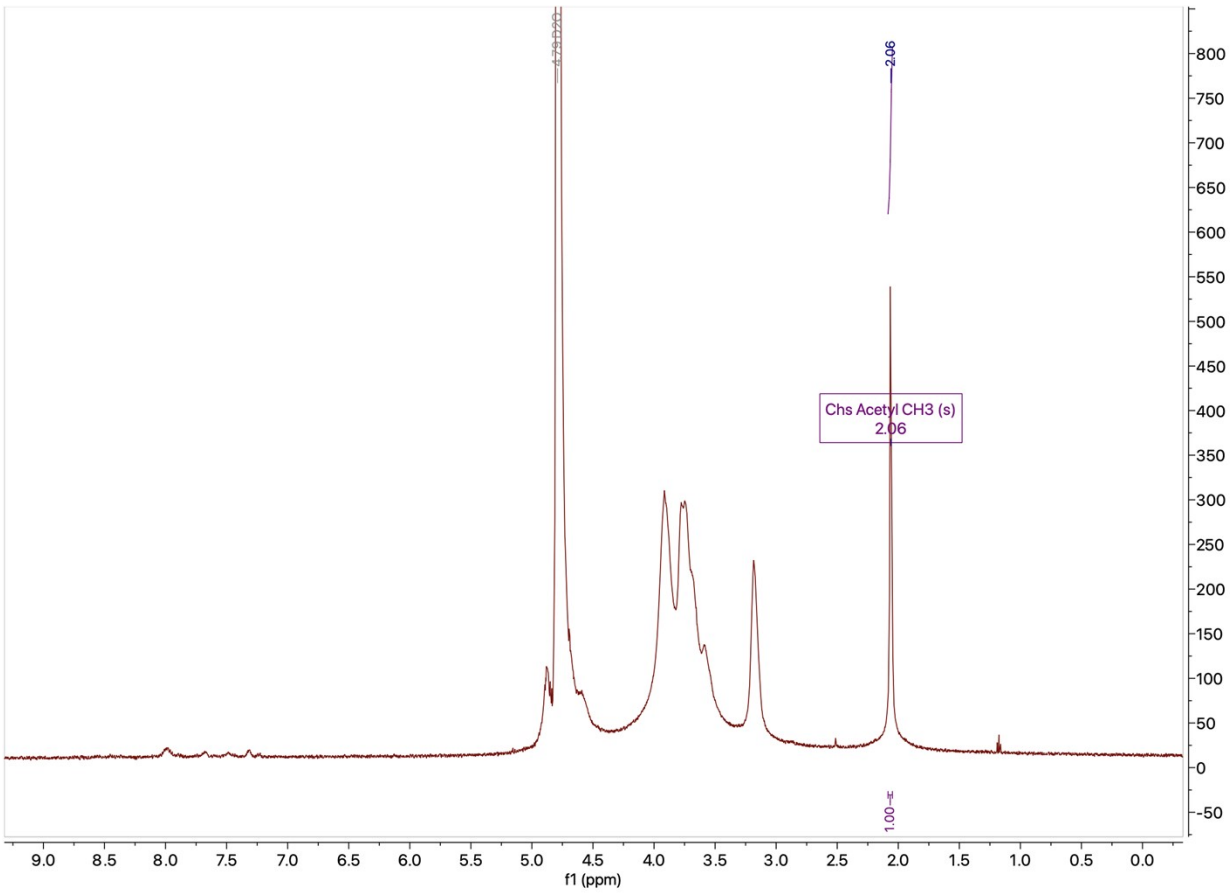



**Figure S4.t.** $^{1}$H spectrum of **4t**.