

Electronic Supporting Information

Protein Aggregation Nucleated by Functionalized Dendritic Polyglycerols

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Mass Determination by SEC-HPLC

Glycopolymer products were evaluated by gel filtration chromatography using Phenomenex GFC 4000 guard cartridge system followed by Waters Ultrahydrogel 500 and 250 columns and monitored at 235 nm with a Shimadzu SPD-M20A photodiode array. Back-pressure remained constant and smooth (461-488 psi) throughout the calibration and analysis; deviation from this metric was used to diagnose the need for guard cartridge replacement. Eluent was prepared from a stock 250 mM KBr aqueous solution (HPLC grade water, chromatography grade KBr; Sigma Aldrich). It was diluted to 100 mM, and filtered three times (Millipore, Nylon, HN, 0.45 μm). Solutions for sample injections (2.5 mg/mL, 100 μL) were prepared in eluent and allowed to equilibrate overnight. Solution was run through a syringe filter (0.45 μm , Nylon) prior to injection. Calibration samples were run in triplicate, but due to extremely low UV-VIS detection, stock dPG were prepared at 10 mg/mL and only injected once.

Table S.1: Description of dPG products **11** - **17**. Here, n corresponds to the number of lactose terminal endgroups and y corresponds to the number of PEG terminal endgroups. Endgroup values are based on final product NMR data. Percent functionalization was based on the propargyl functionalized intermediate **8** - **10** NMR spectral data. Mass characteristics were determined by SEC-HPLC. Polymer radii were determined by DLS, in PBS at 20 °C.

Label	Scaffold	Functionalization (%func./ n/y)	M_W (kDa)	M_N (kDa)	PDI	ΔFWHM (%)
11	2.5 kDa dPG	48% / 10 / 0	14.7	7.2	2.0	18.2
12	5 kDa dPG	48% / 21 / 0	16.9	8.5	2.0	-15.6
13	10 kDa dPG	44% / 33 / 0	35.7	17.3	2.1	2.3
14	10 kDa dPG	44% / 27 / 6	46.8	20.6	2.3	-1.8
15	10 kDa dPG	44% / 20 / 13	62.0	25.7	2.4	0.0
16	10 kDa dPG	44% / 4 / 29	33.5	13.3	2.5	18.6
17	10 kDa dPG	44% / 1 / 32	36.7	13.7	2.7	15.6

The calibration standards used were lactose functionalized PAMAM dendrimers, which were previously characterized by MALDI-TOF MS (Table S.2).

Table S.2: Lactose functionalized PAMAM dendrimers characterized by MALDI-TOF. Stock dPG purchased from Nanopartica GmbH and characterized by Haag research group GPC-HPLC.

Compound	M_p
Lactose func. G2 PAMAM	6985 amu
Lactose func. G4 PAMAM	30341 amu
Lactose func. G6 PAMAM	90317 amu
Alcohol terminal dPG	8271 amu

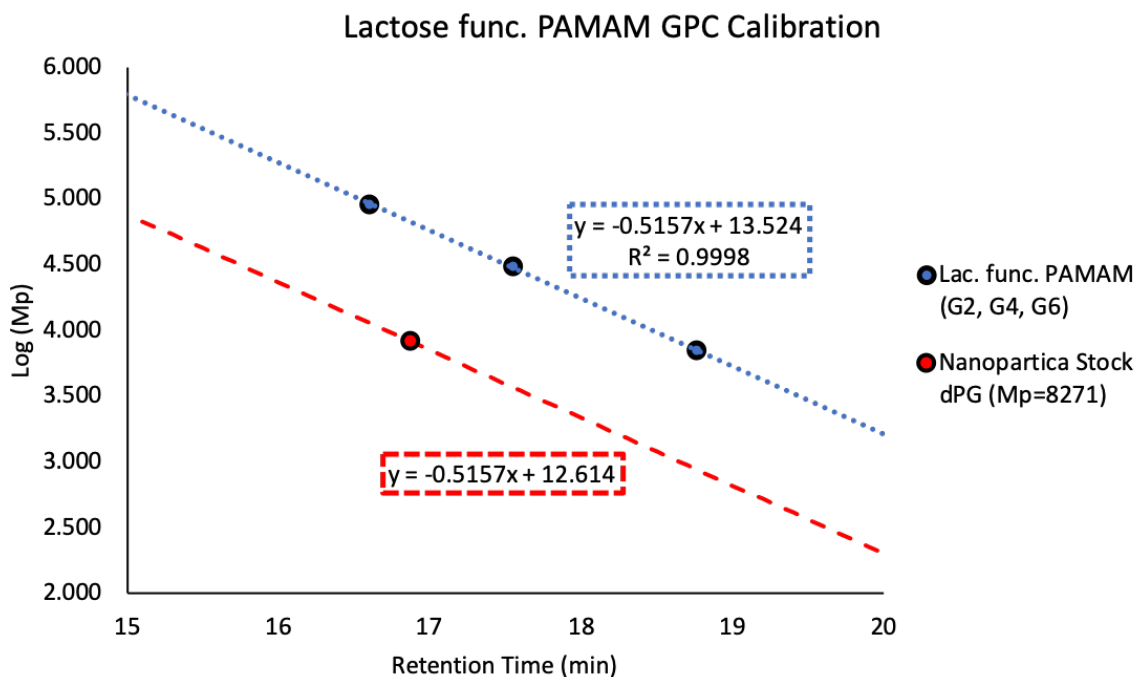


Figure S.1: Linear calibration of the SEC-HPLC with lactose functionalized PAMAM dendrimers (blue, Equation S.1) and a translational shift to accommodate observed stock dPG data (red, Equation S.2). This calibration was applied to chromatograms for lactose functionalized dPG.

For the calibration, m/z at maximum intensity (M_p) was compared to characteristic retention times based on maximum mAU (254 nm). A linear trend-line was applied to a plot of $\log(M_p)$ vs. retention time to obtain;

$$Y = -0.5157X + 13.524 \quad (\text{S.1})$$

as a calibration curve. A translational shift was applied to the calibration curve so that it passed through the data point obtained from the stock 10 kDa dPG chromatogram (235 nm; $M_p = 8271$ Da, 16.864 min), without altering the slope;

$$Y = -0.5157X + 12.614 \quad (\text{S.2})$$

Chromatograph data (Intensity (mAU) vs. time) was truncated to best represent the characteristic polymer peak, excluding disruptions caused by sample/eluent salt concentration differences and small molecule impurities ($M_w < 250$ amu). This is accomplished by selecting the local minima in the trough on the tail end of the major peak and then the equivalent intensity on the front of the peak. A mass profile was generated from the truncated curve, based on calibration information (Equation S.2), and then used

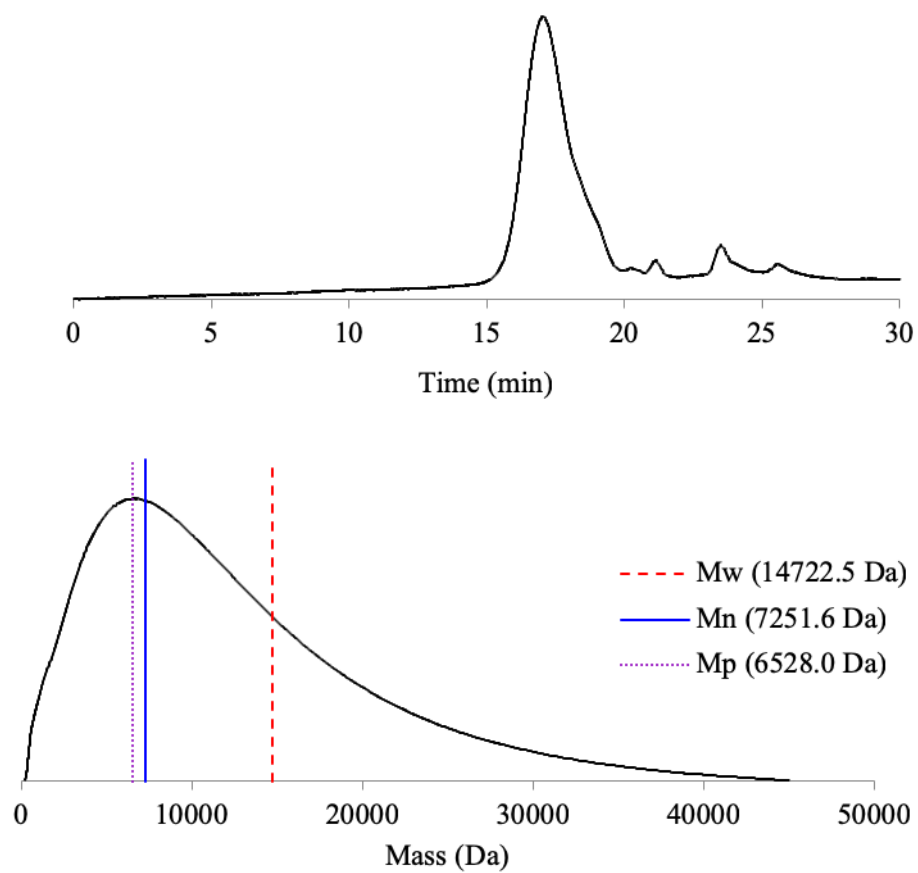
to calculate important molar mass distributions (MMD; M_w , M_n , M_p) and the polydispersity index (PDI).

$$M_w = \left(\frac{\sum M_i^2 N_i}{\sum M_i N_i} \right) \quad (\text{S.3})$$

$$M_n = \left(\frac{\sum M_i N_i}{\sum N_i} \right) \quad (\text{S.4})$$

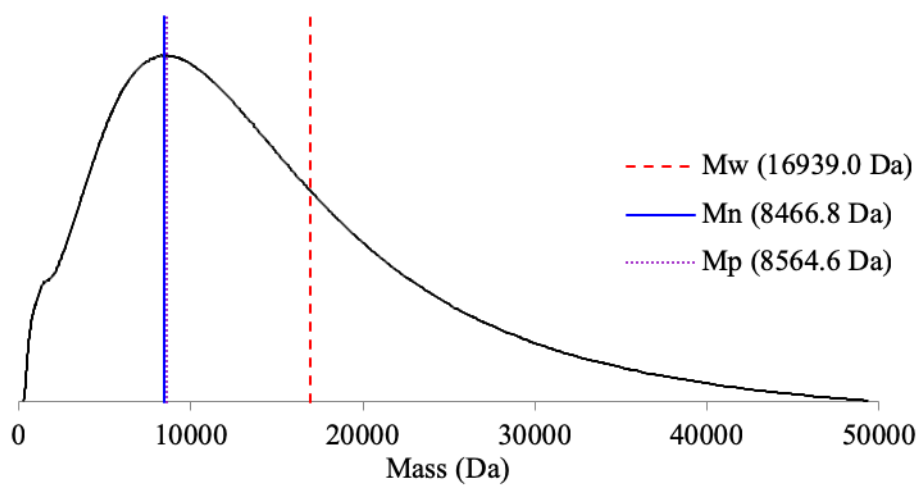
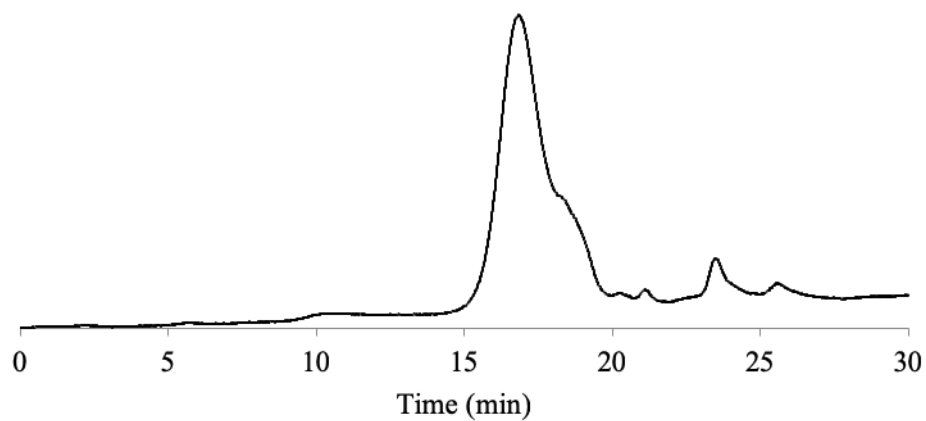
$$PDI = \left(\frac{M_w}{M_n} \right) \quad (\text{S.5})$$

To evaluate the increase in polydispersity of the compound introduced by functionalization, the full width at half max was taken for the stock dPG and compared to final polymers. Stock polymers were not dialyzed prior to evaluation, potentially contributing to inflated FWHM values. Lactose functionalized dPG demonstrates a decrease in FWHM after functionalization, suggesting a significant contribution of species removed by dialysis.



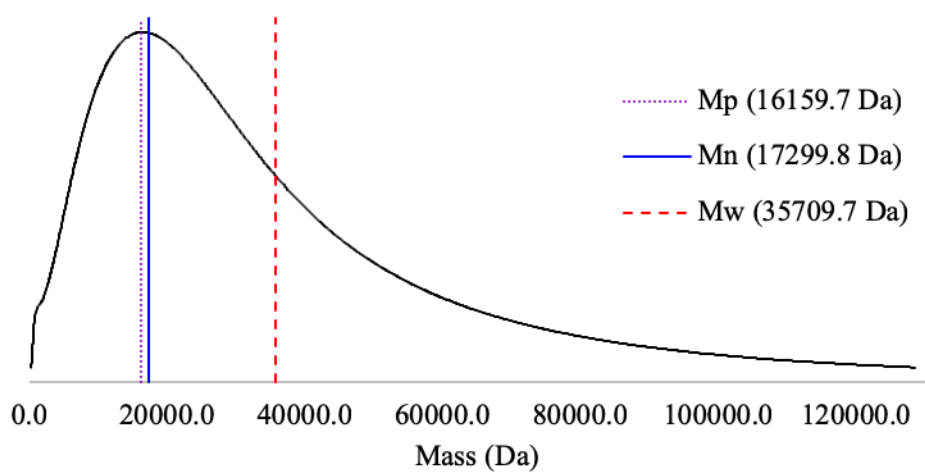
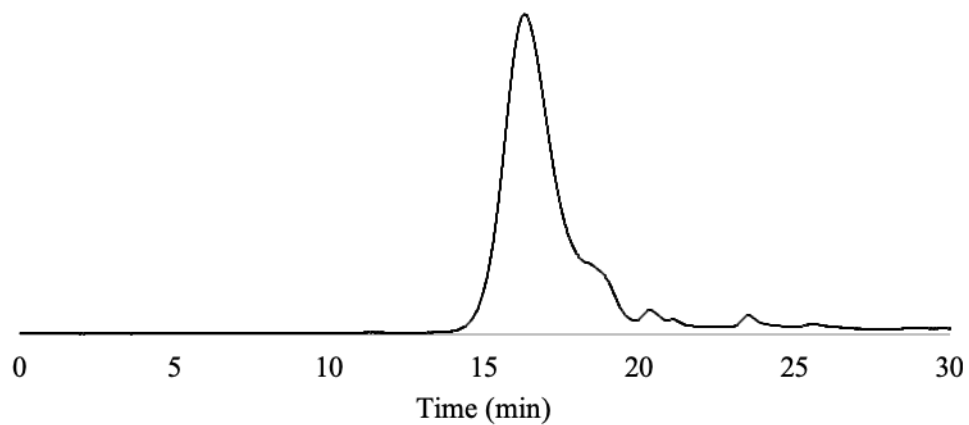
M_w	14.7 kDa
M_n	7.3 kDa
M_p	6.5 kDa
PDI	2.01

Figure S.2: SEC chromatogram and mass profile for product **11**.



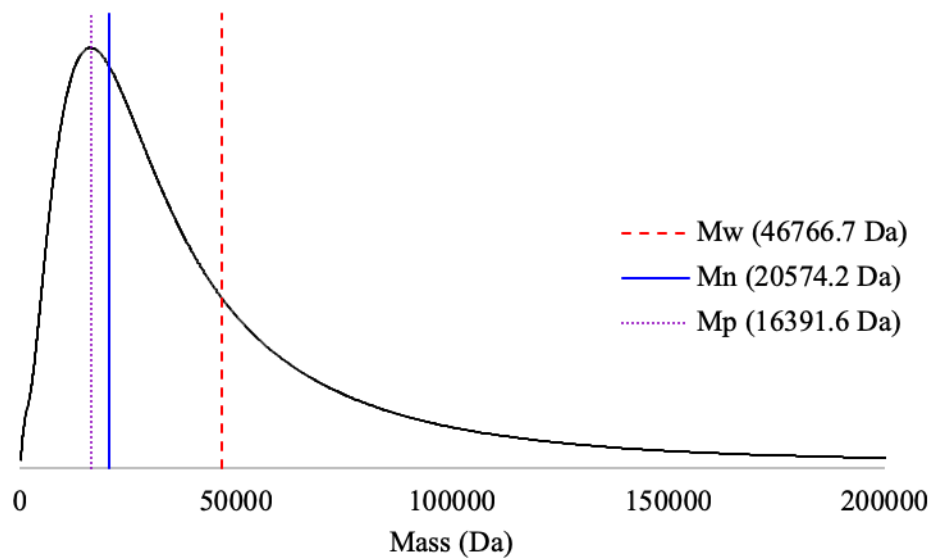
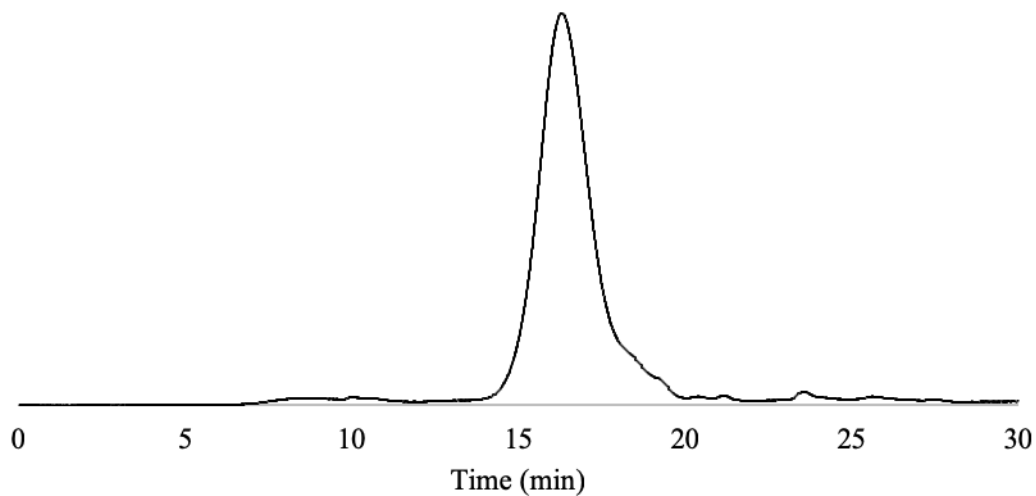
M_w	16.9 kDa
M_n	8.5 kDa
M_p	8.6 kDa
PDI	1.99

Figure S.3: SEC chromatogram and mass profile for product **12**.



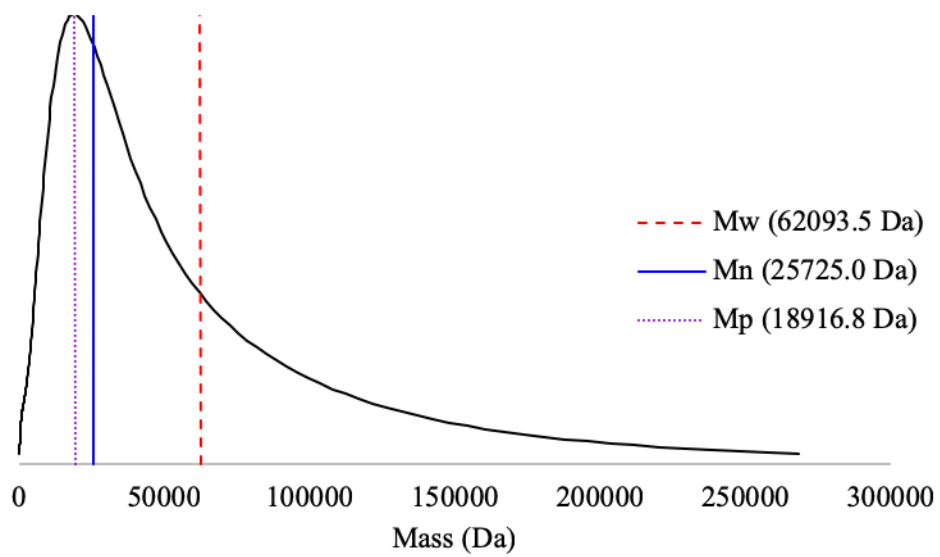
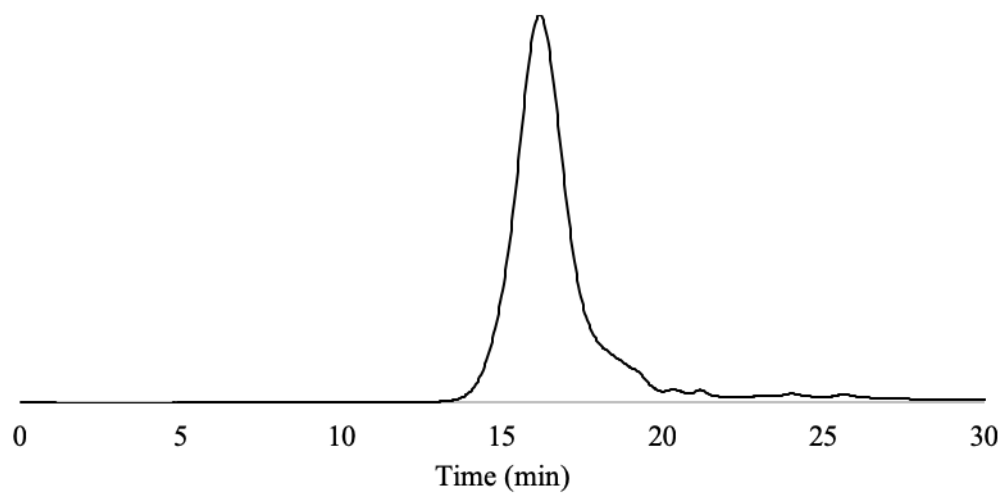
M_w	35.7 kDa
M_n	17.3 kDa
M_p	16.2 kDa
PDI	2.06

Figure S.4: SEC chromatogram and mass profile for product **13**.



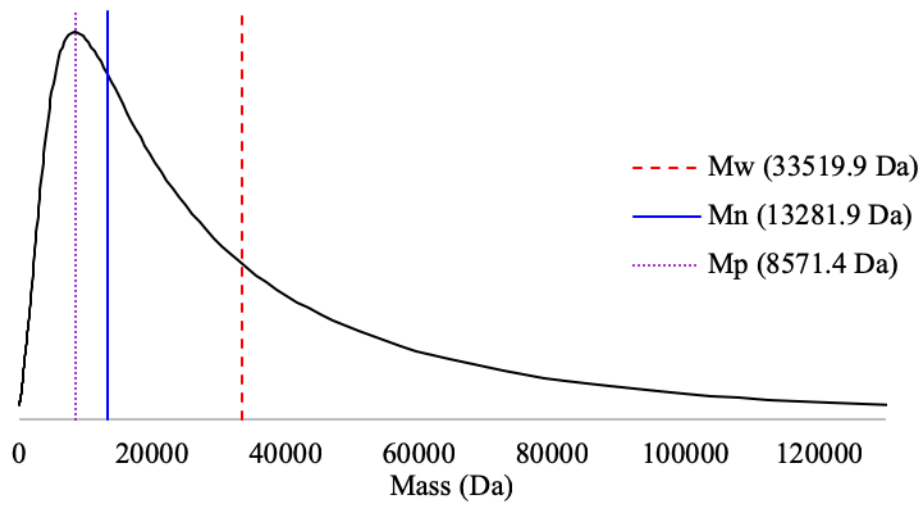
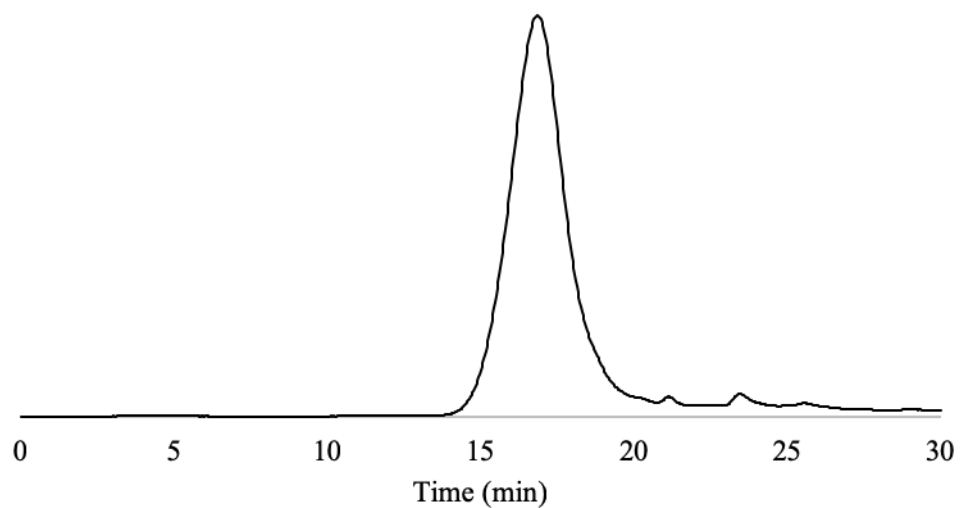
M_w	46.8 kDa
M_n	20.5 kDa
M_p	16.4 kDa
PDI	2.27

Figure S.5: SEC chromatogram and mass profile for product **14**.



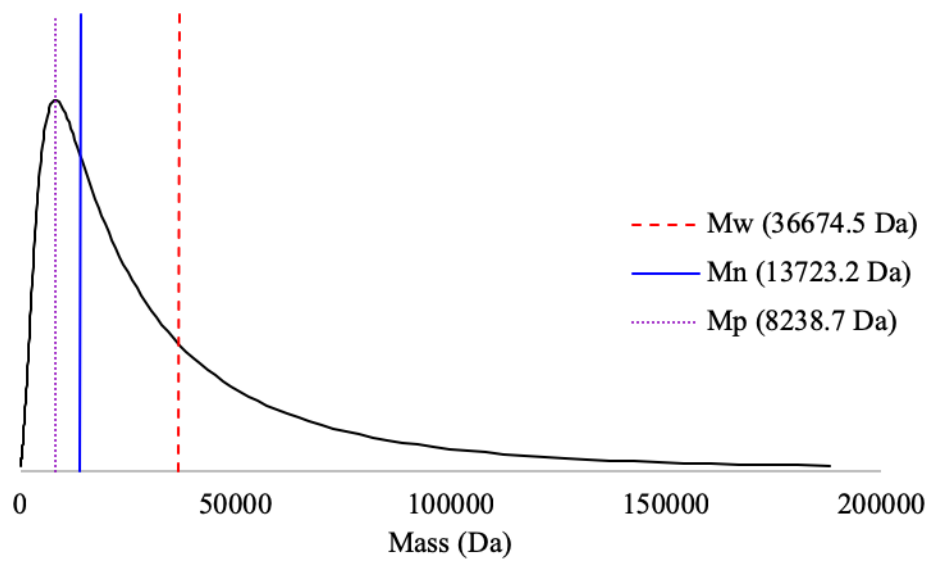
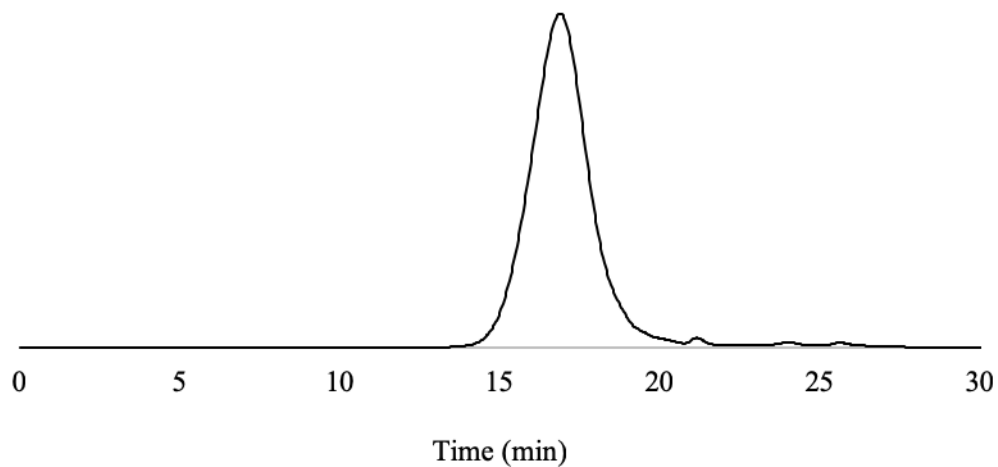
M_w	62.0 kDa
M_n	25.7 kDa
M_p	18.9 kDa
PDI	2.41

Figure S.6: SEC chromatogram and mass profile for product **15**.



M_w	33.5 kDa
M_n	13.3 kDa
M_p	8.6 kDa
PDI	2.52

Figure S.7: SEC chromatogram and mass profile for product **16**.



M_w	36.7 kDa
M_n	13.7 kDa
M_p	8.2 kDa
PDI	2.67

Figure S.8: SEC chromatogram and mass profile for product **17**.

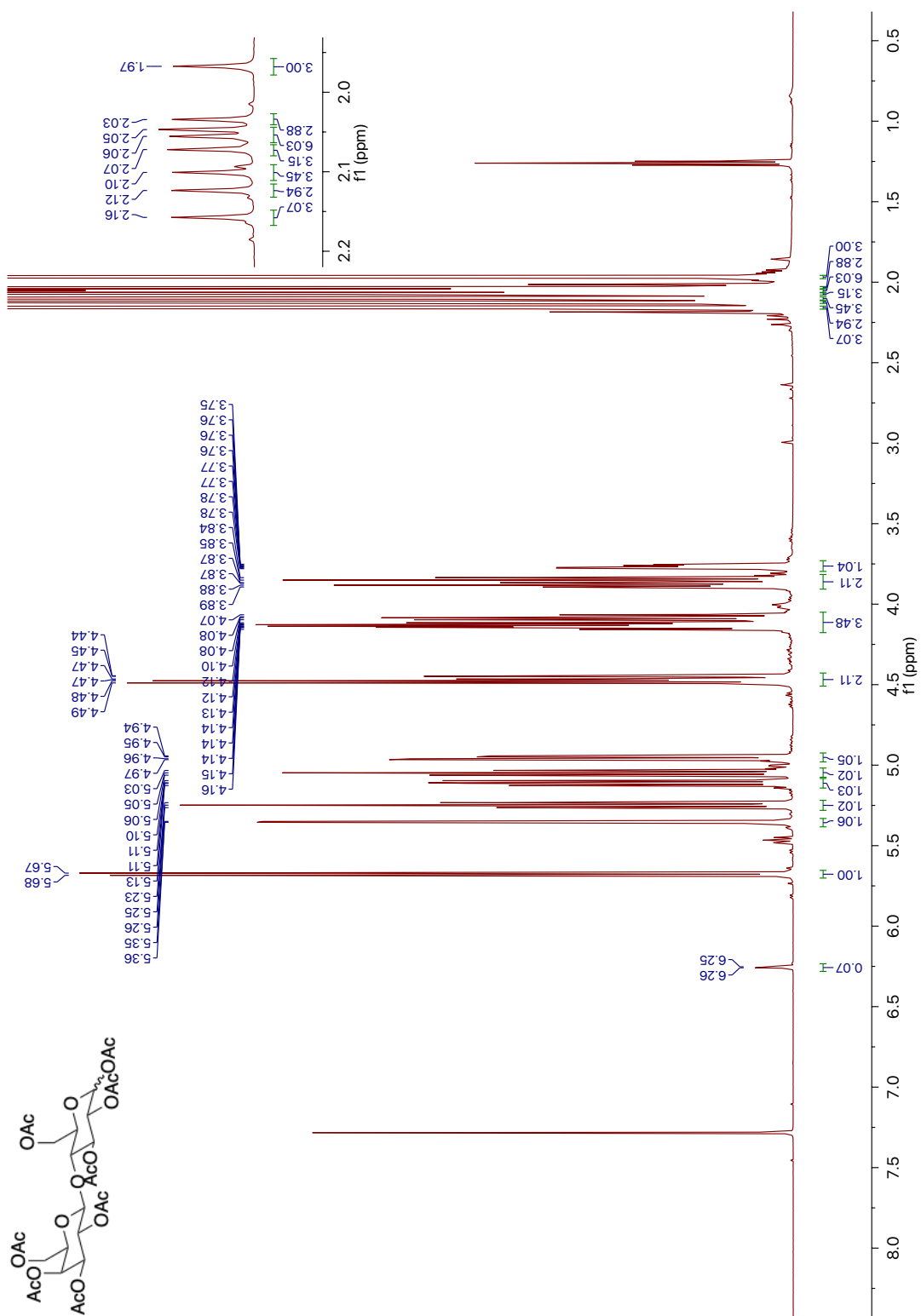


Figure S.9: ¹H NMR of peracetylated lactose.

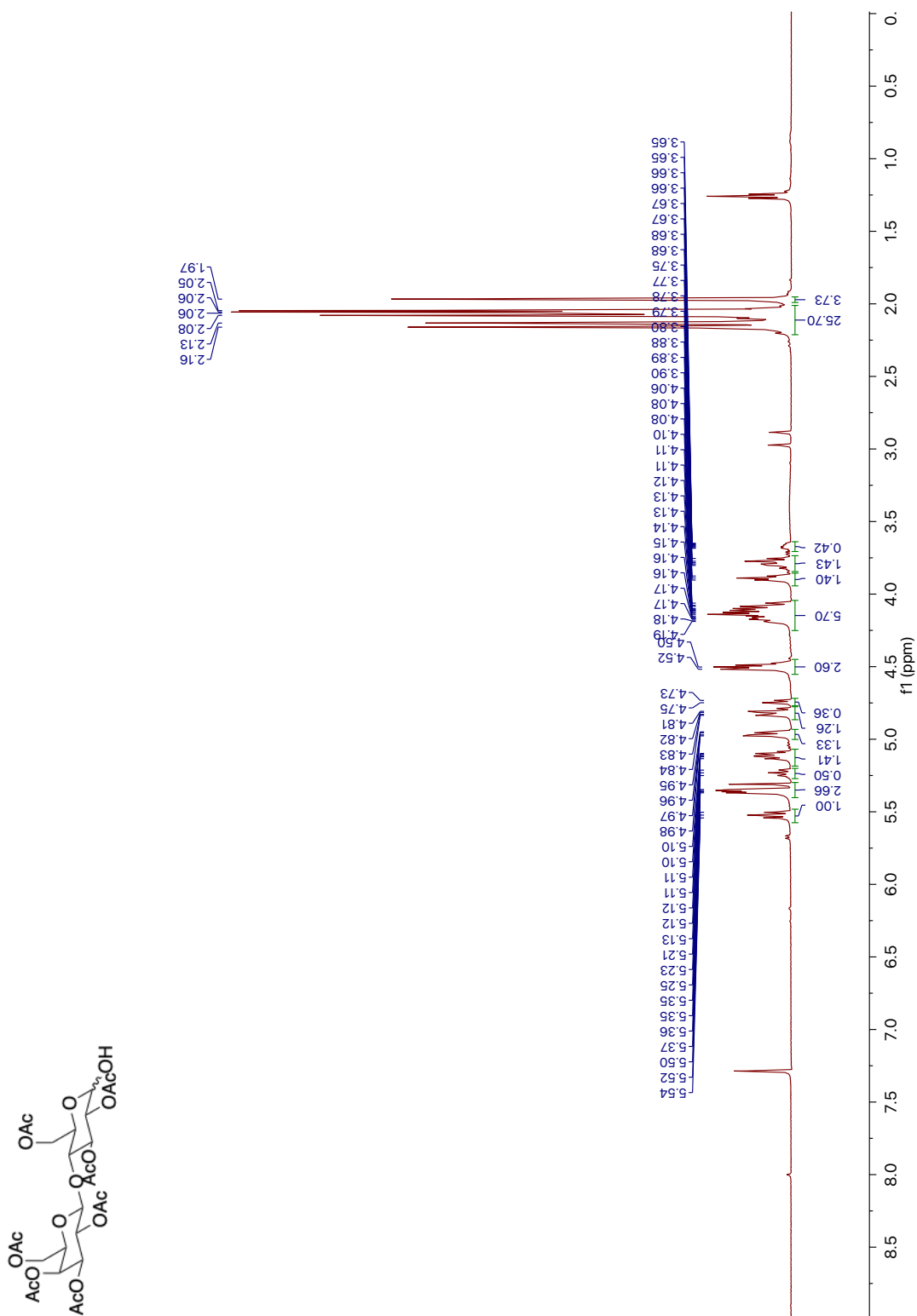


Figure S.10: ¹H NMR of product 1.

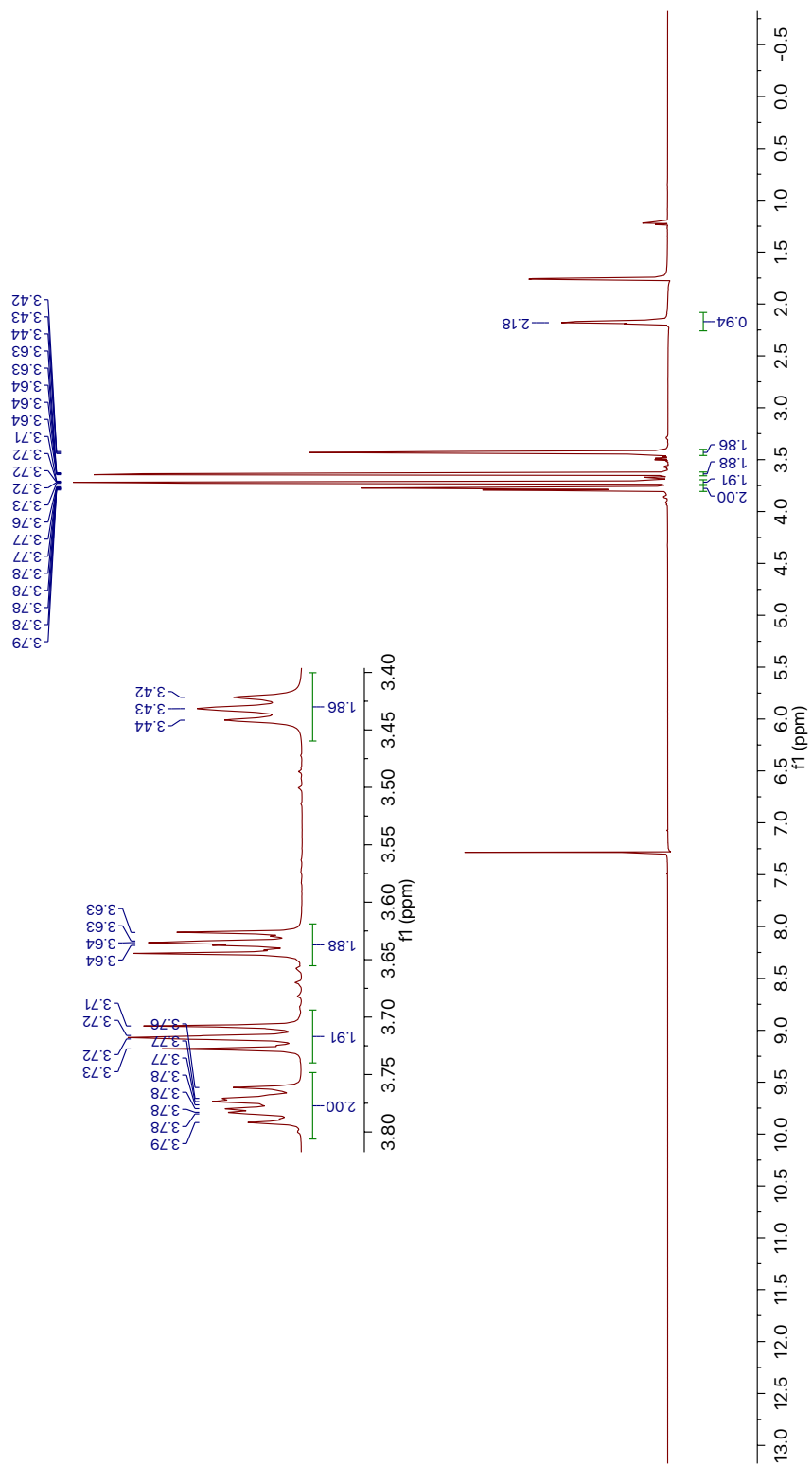
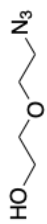


Figure S.12: ^1H NMR of 2-(2-azidoethoxy)ethanol **3**.

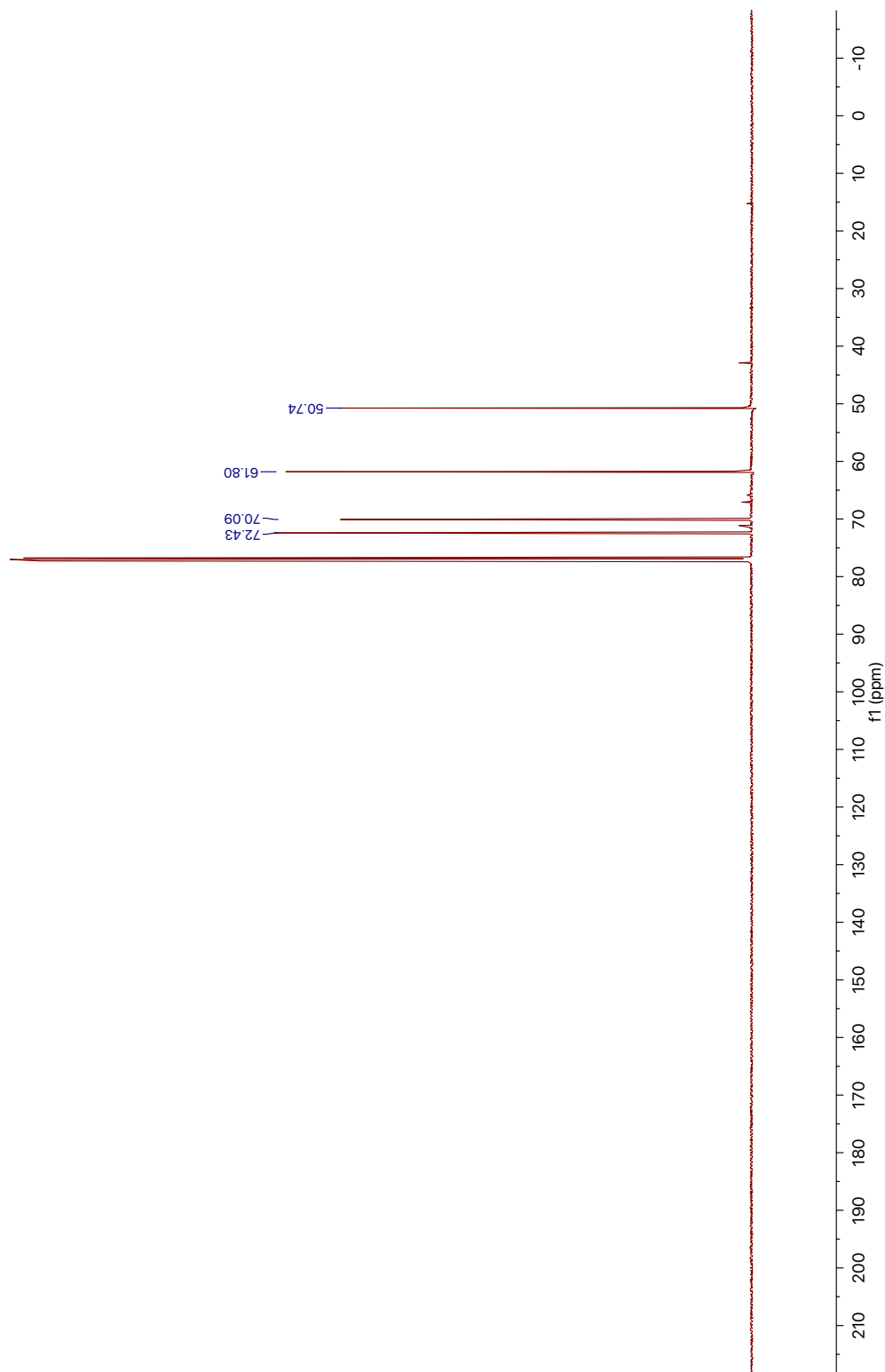
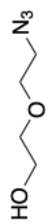


Figure S.13: ^{13}C NMR of product 2-(2-azidoethoxy)ethanol **3**.

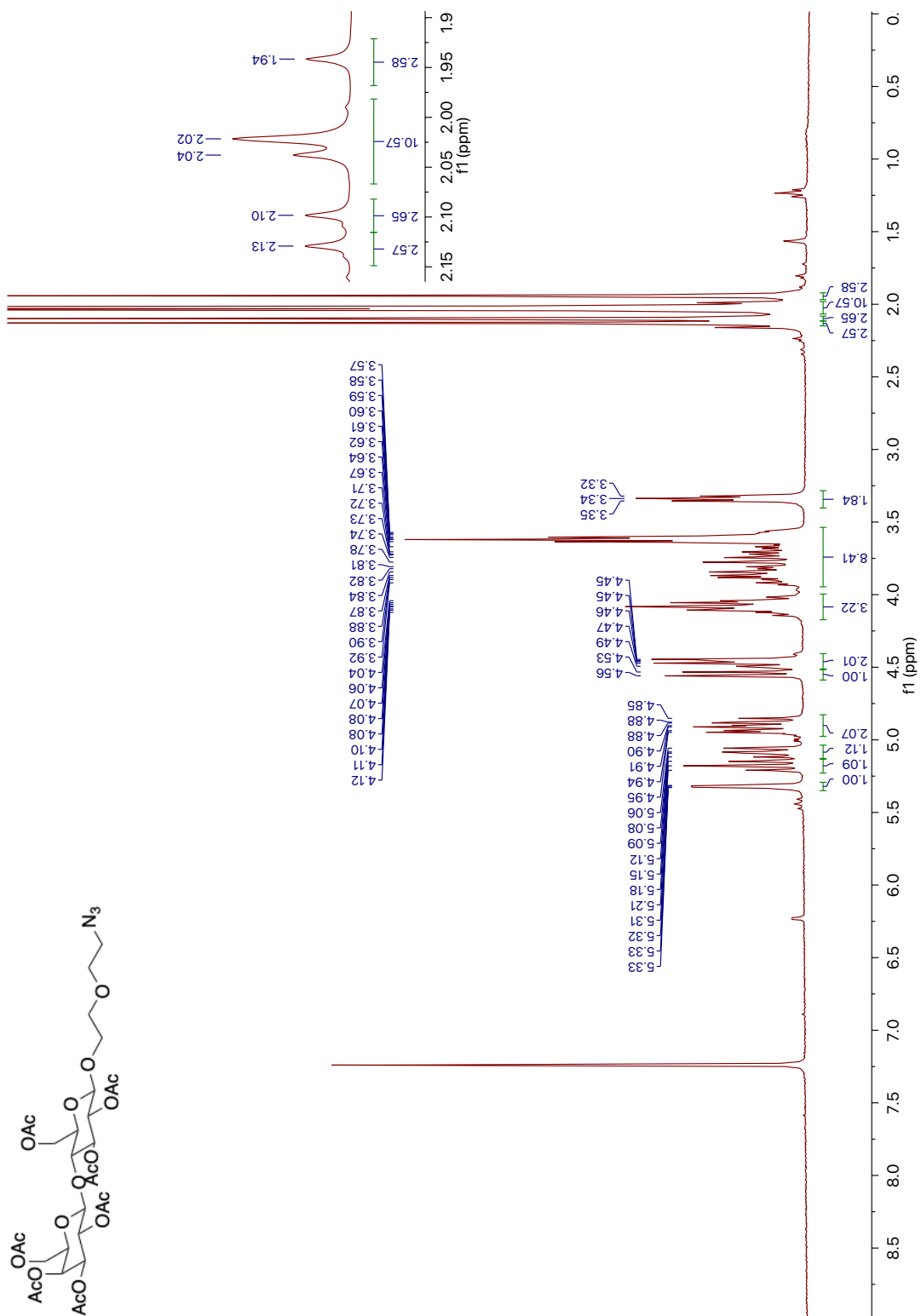


Figure S.14: ¹H NMR of product 4.

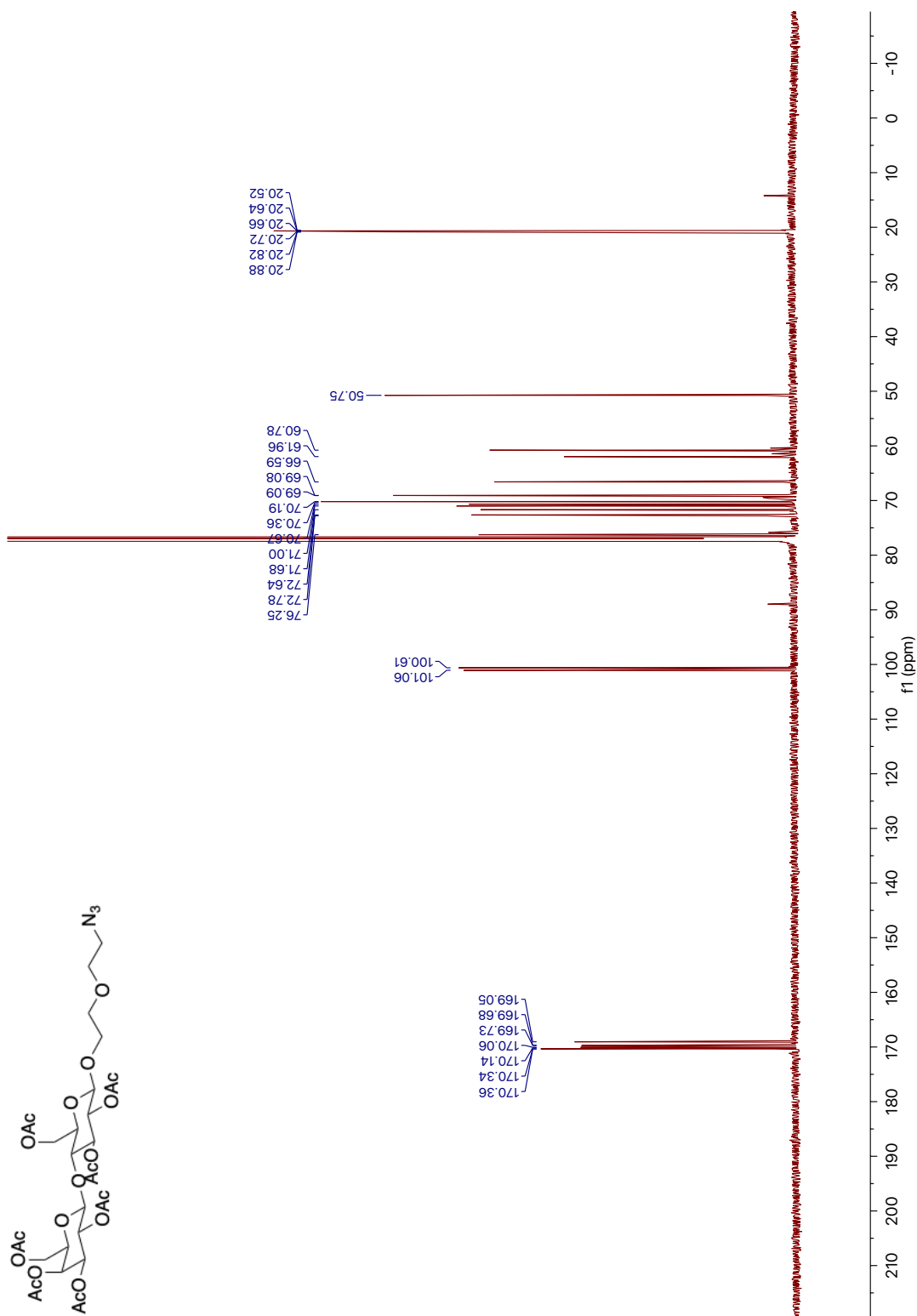


Figure S.15: ^{13}C NMR of product 4.

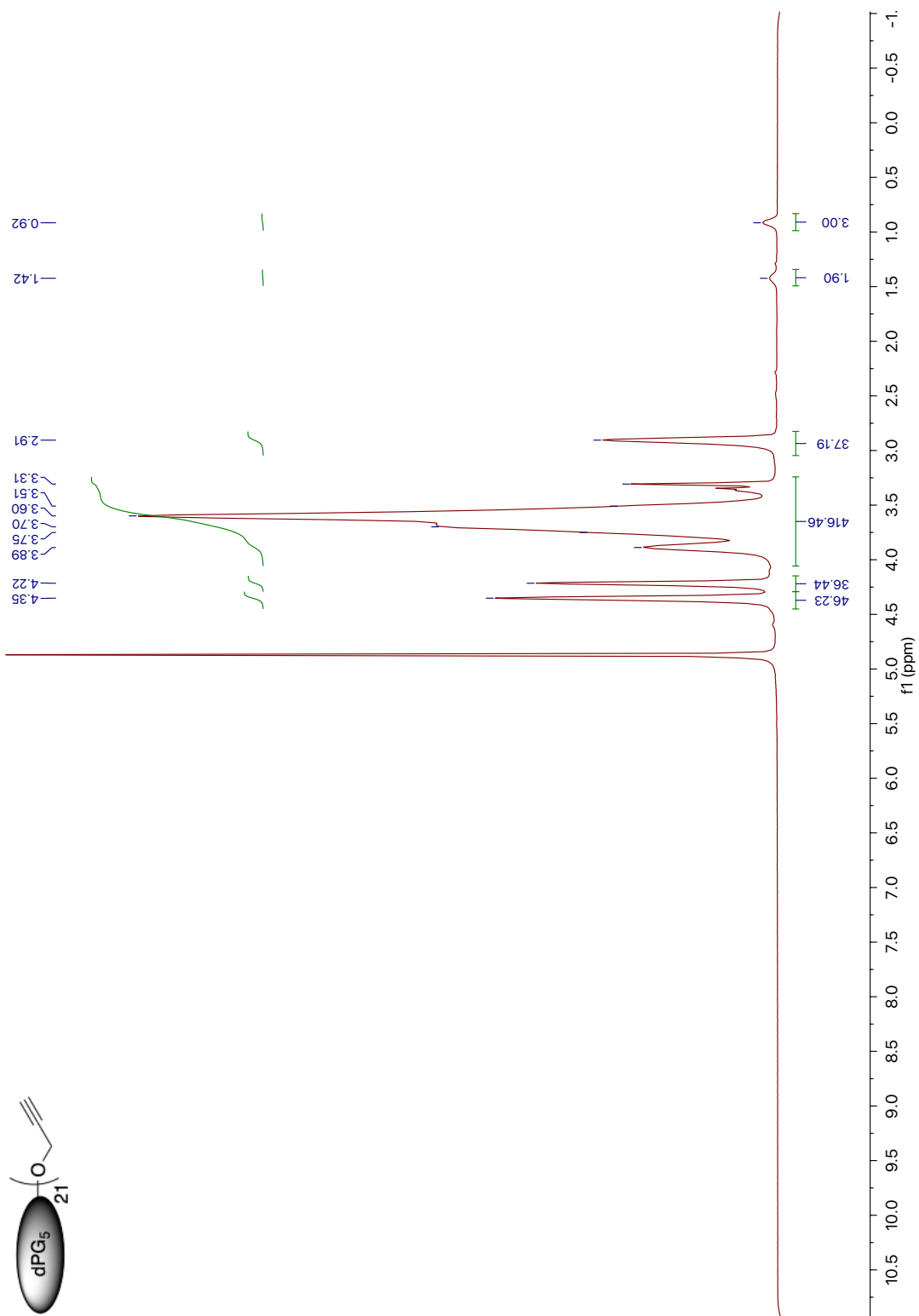


Figure S.17: ^1H NMR of product **6**.

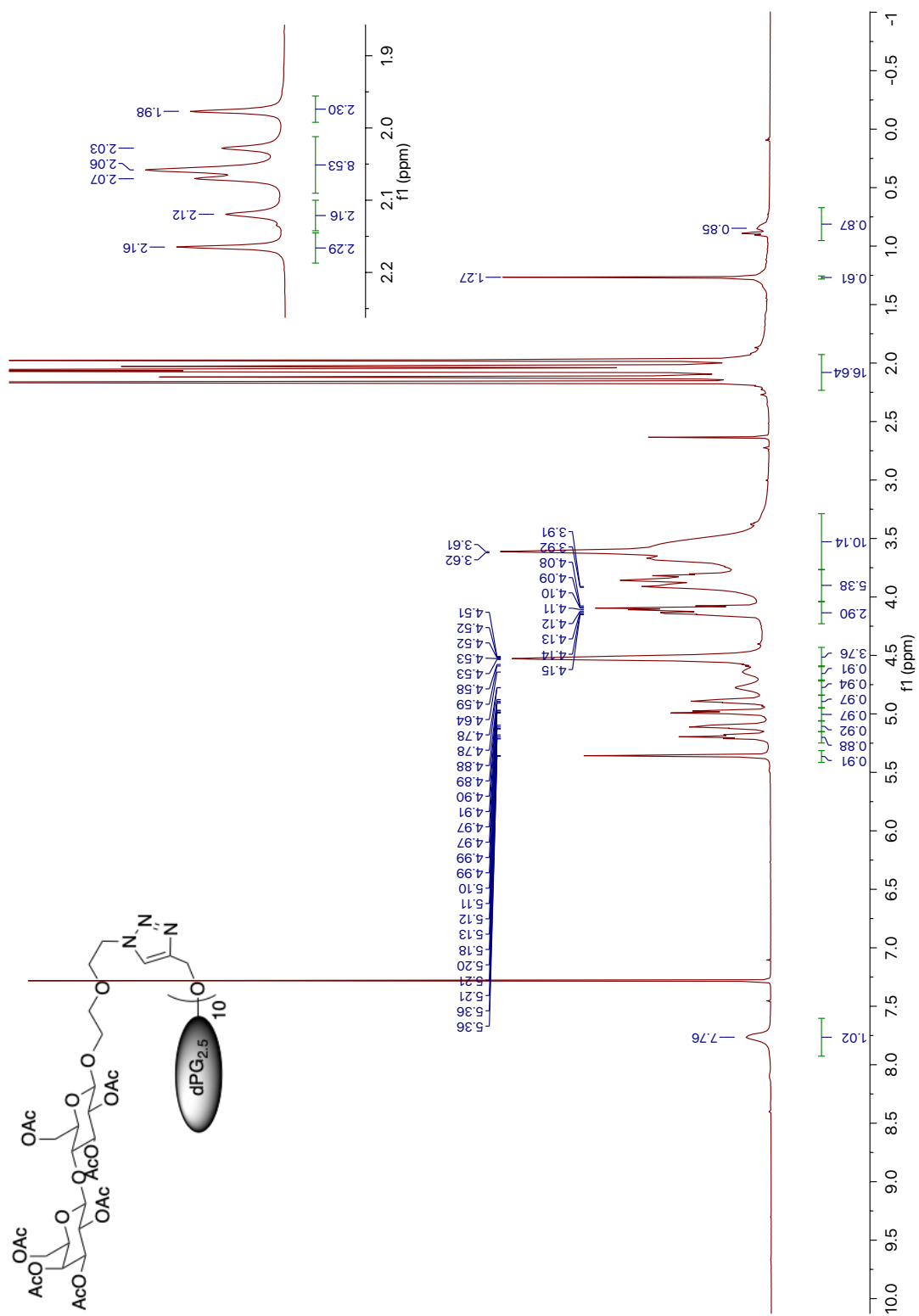


Figure S.19: ¹H NMR of product **8**.

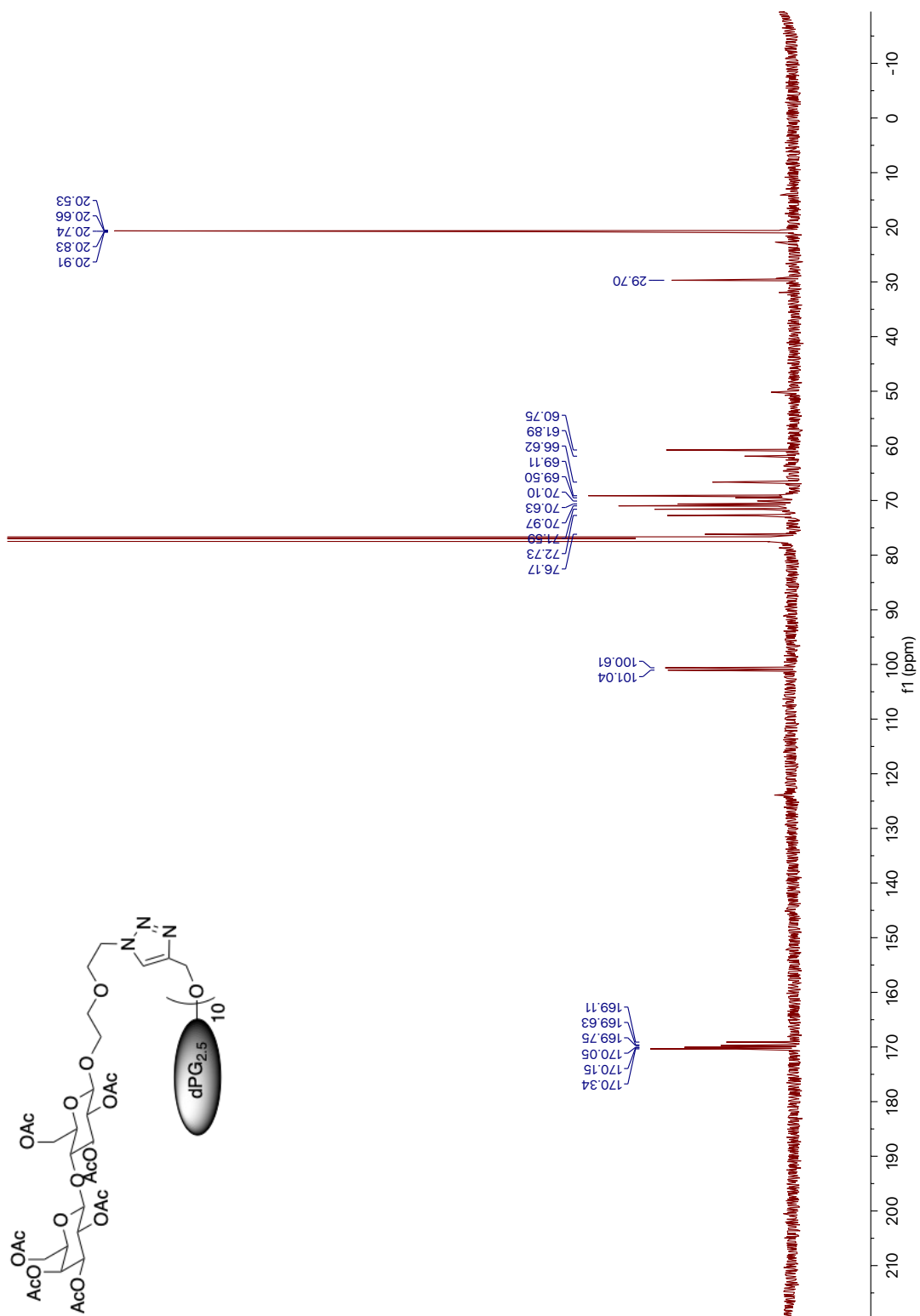


Figure S.20: ^{13}C NMR of product 8.

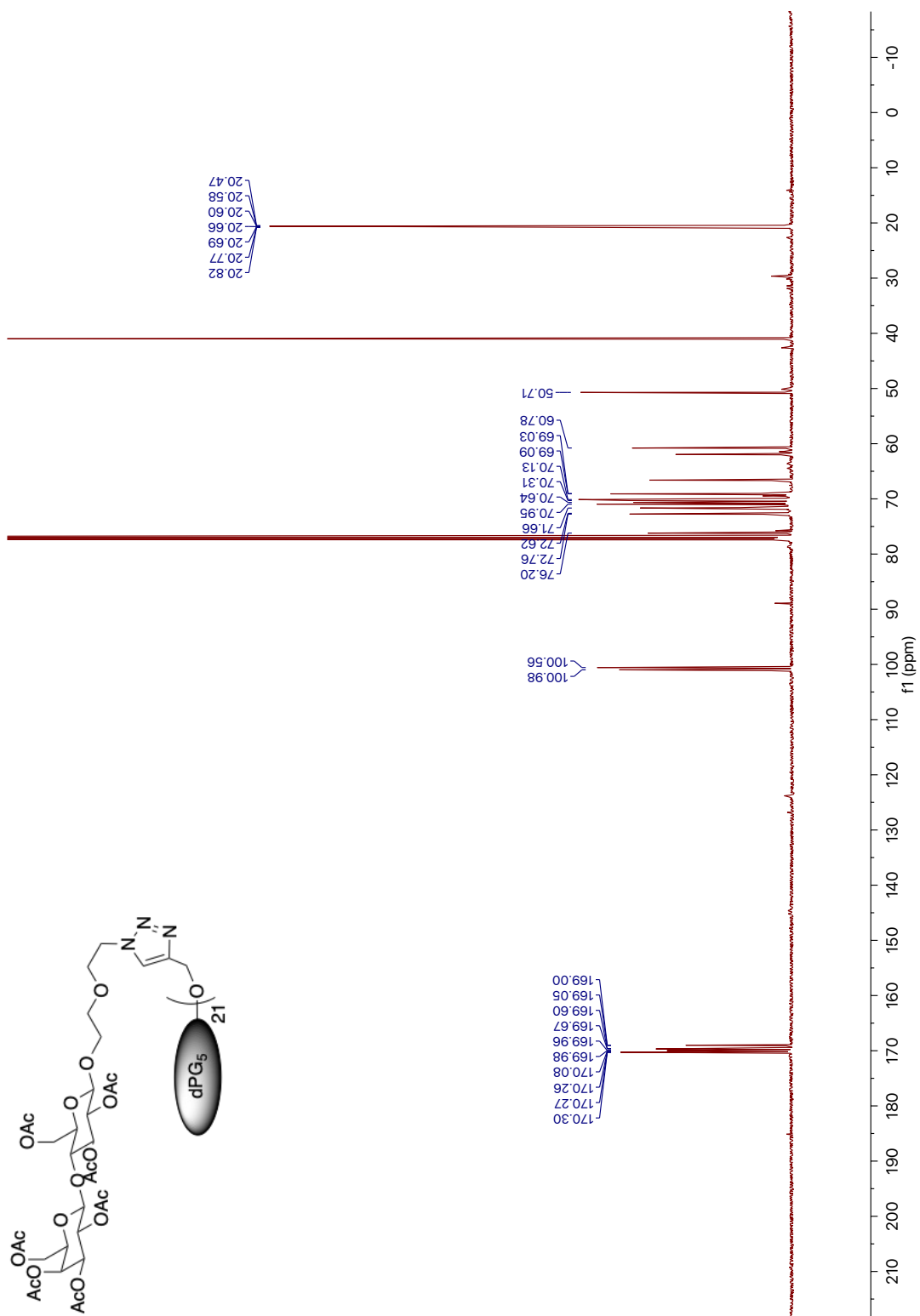


Figure S.22: ¹³C NMR of product **9**.

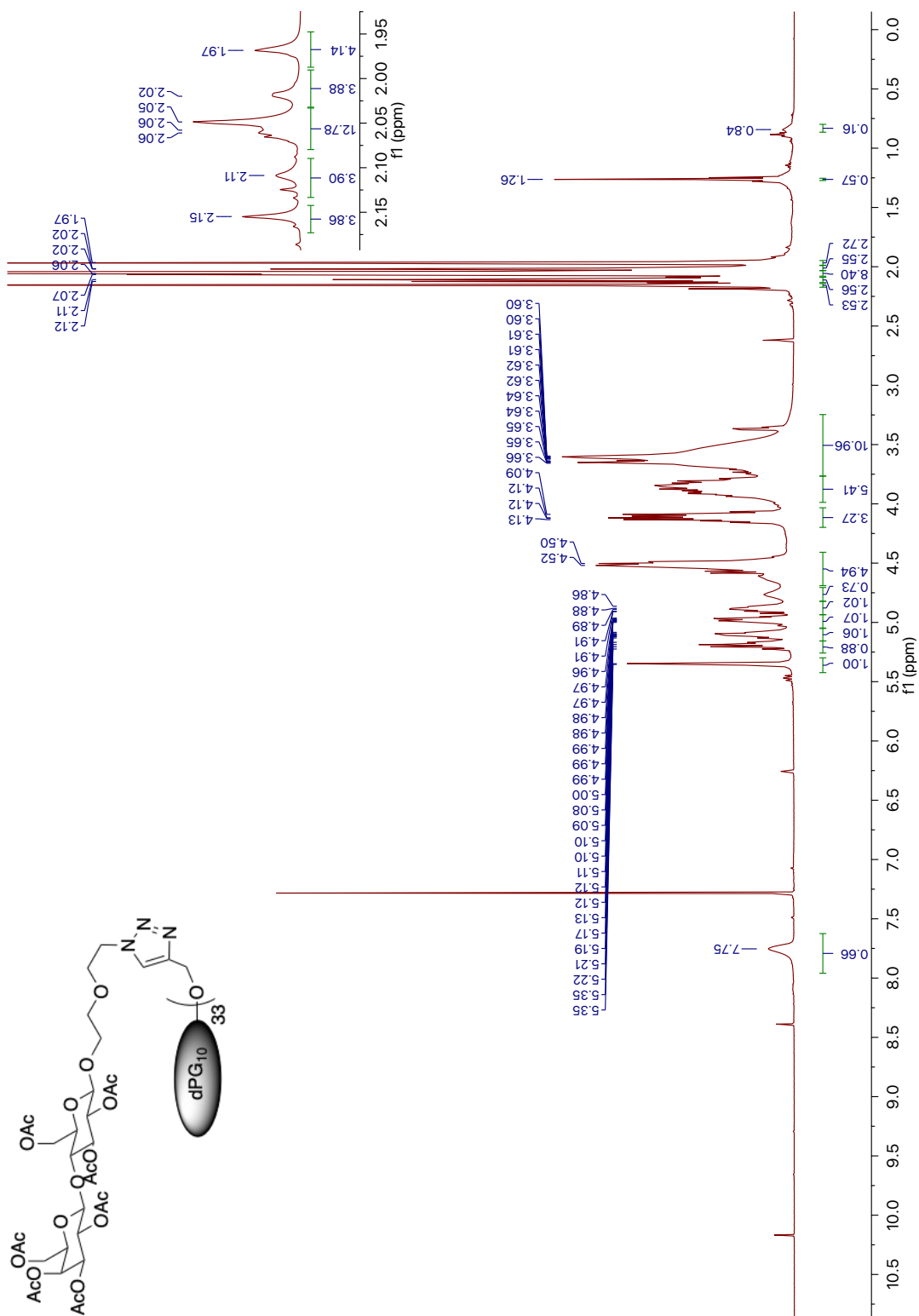


Figure S.23: ¹H NMR of product 10.

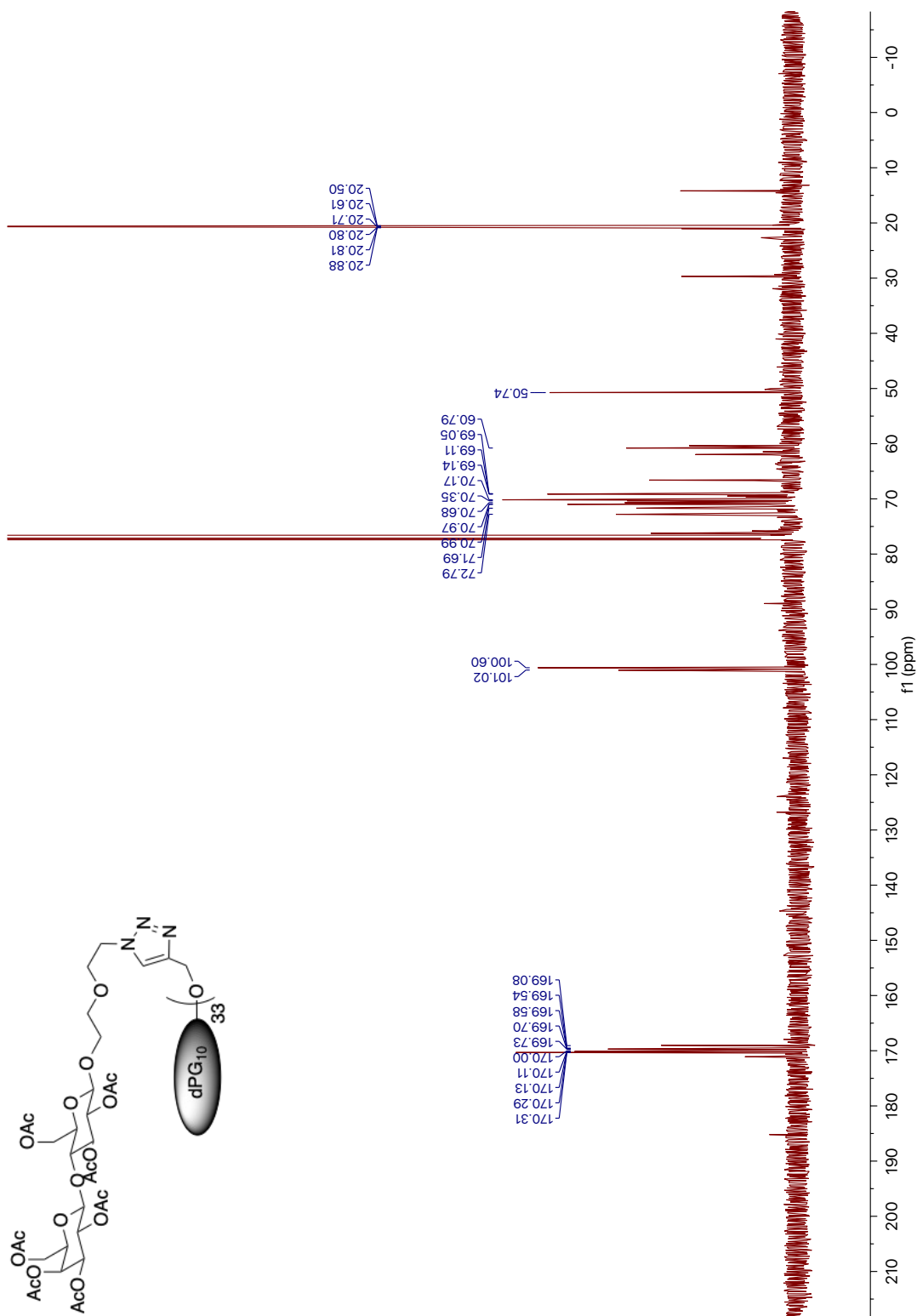


Figure S.24: ¹³C NMR of product 10.

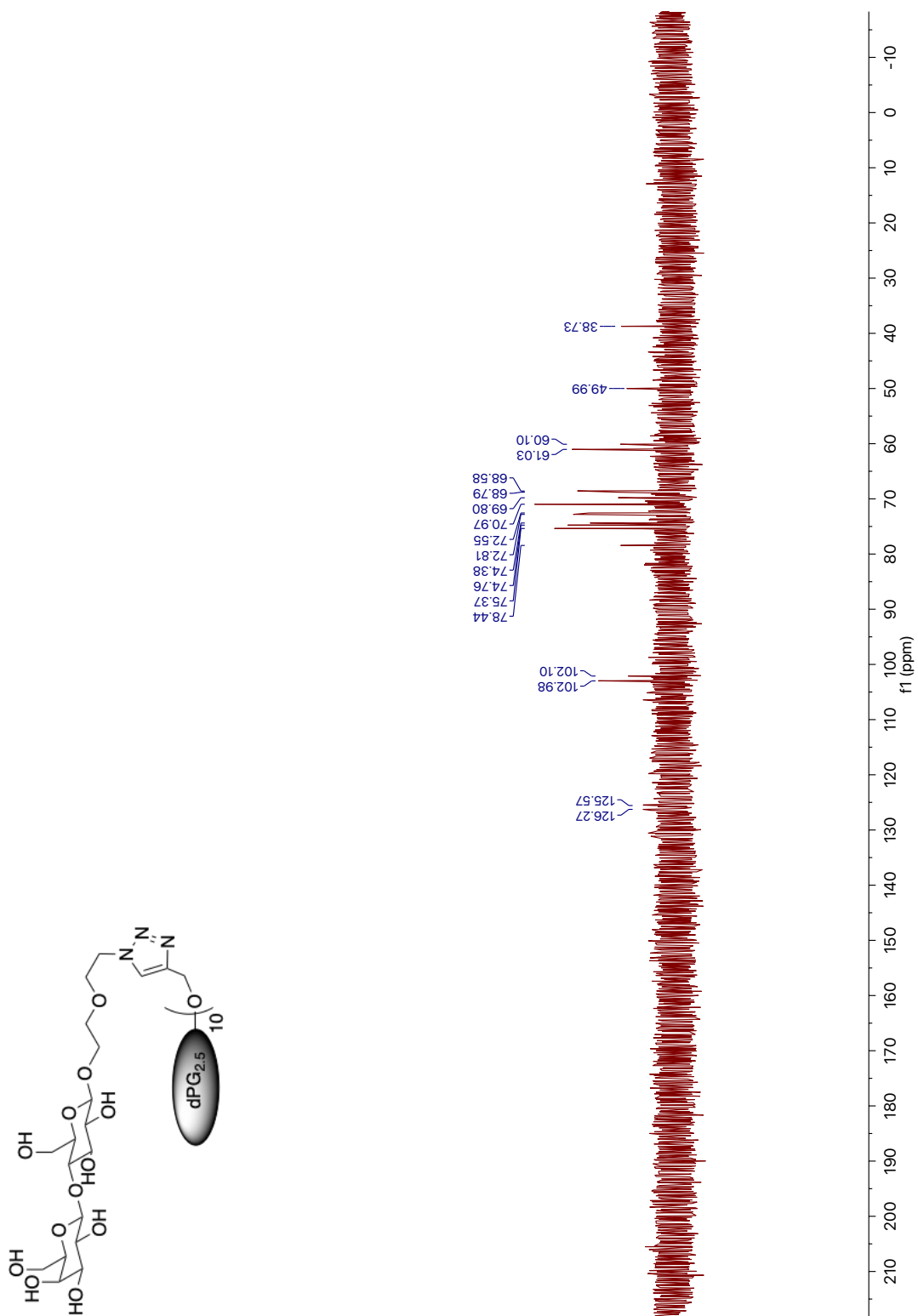


Figure S.26: ¹³C NMR of product **11**.

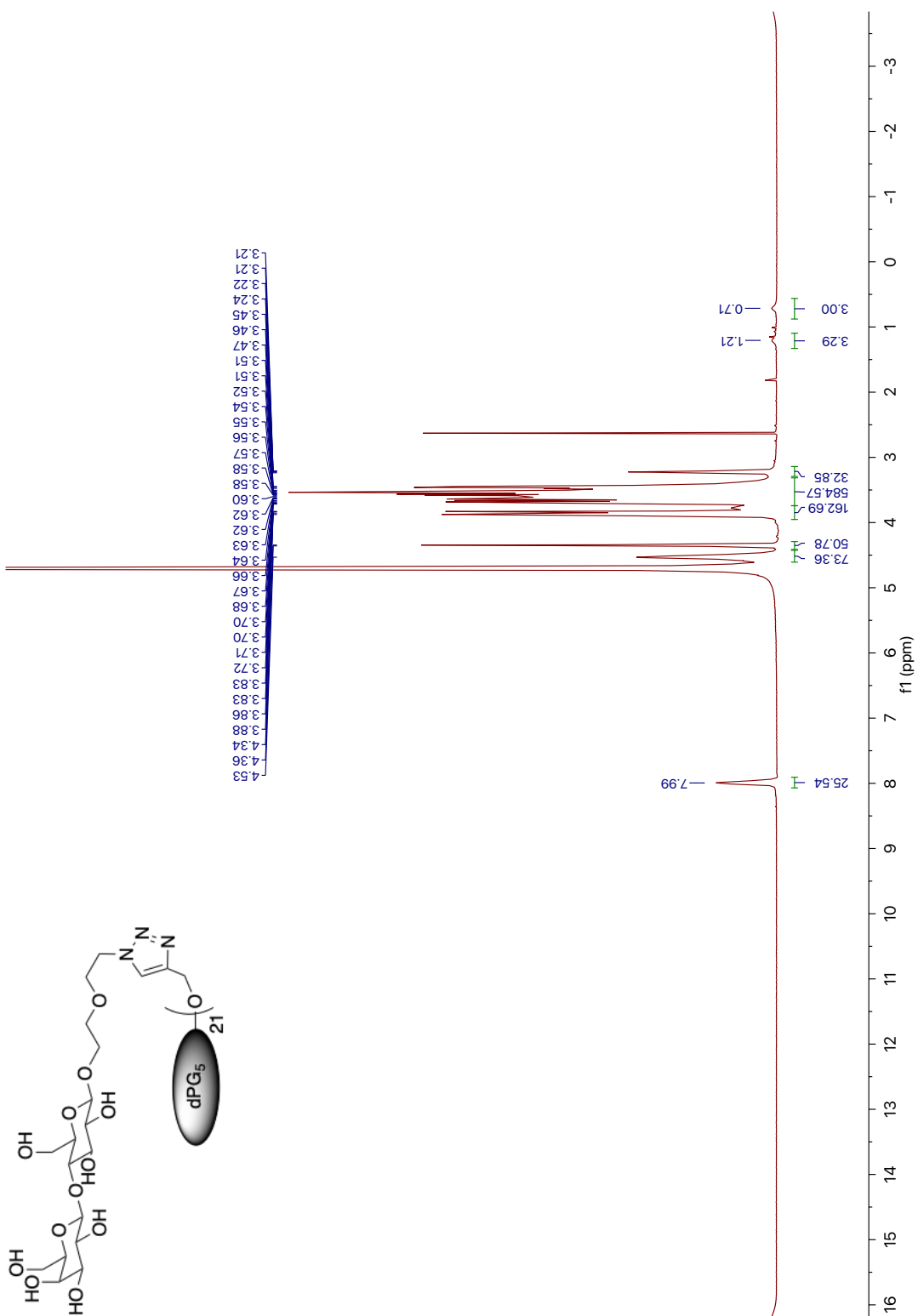


Figure S.27: ¹H NMR of product **12**.

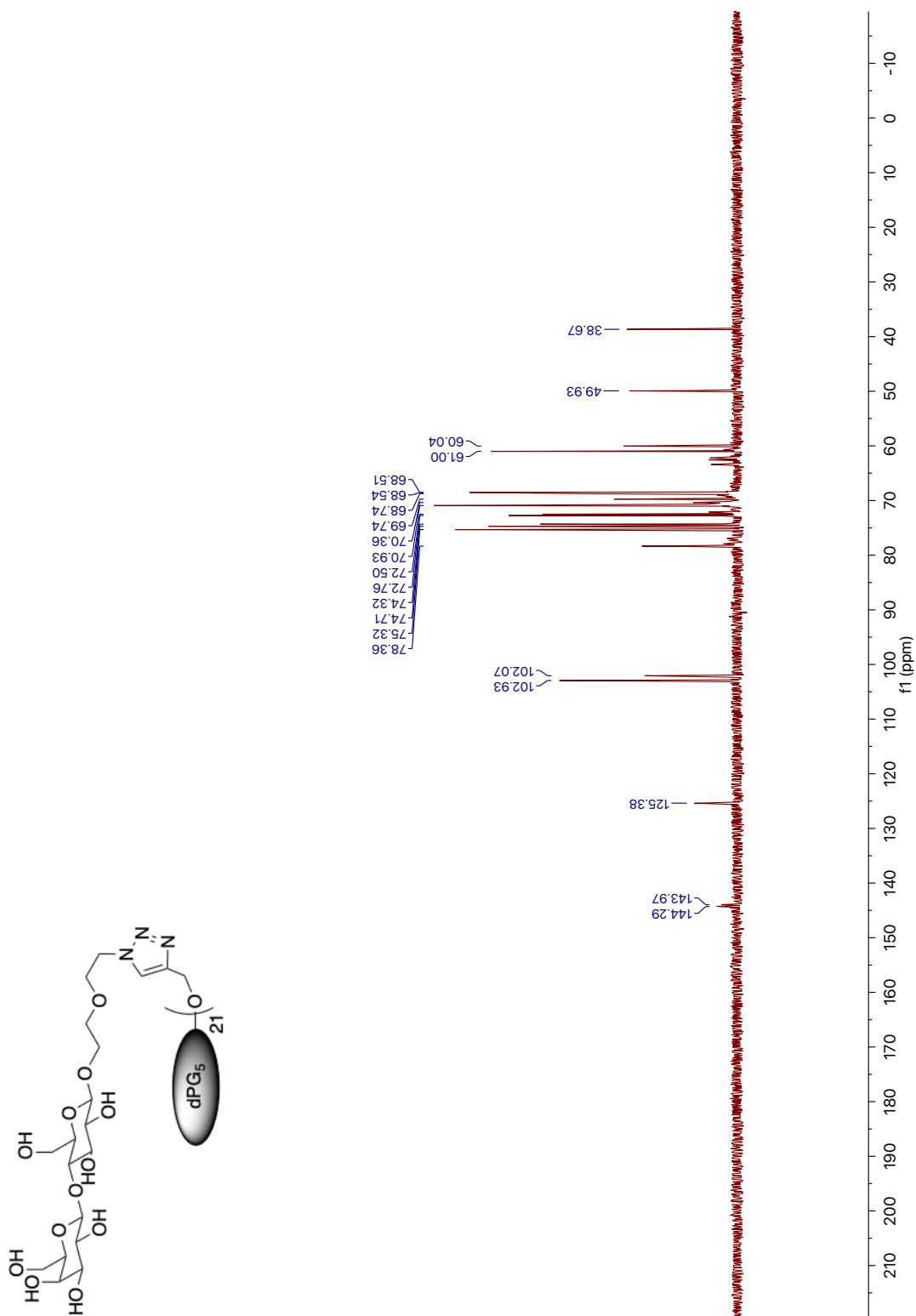


Figure S.28: ¹³C NMR of product **12**.

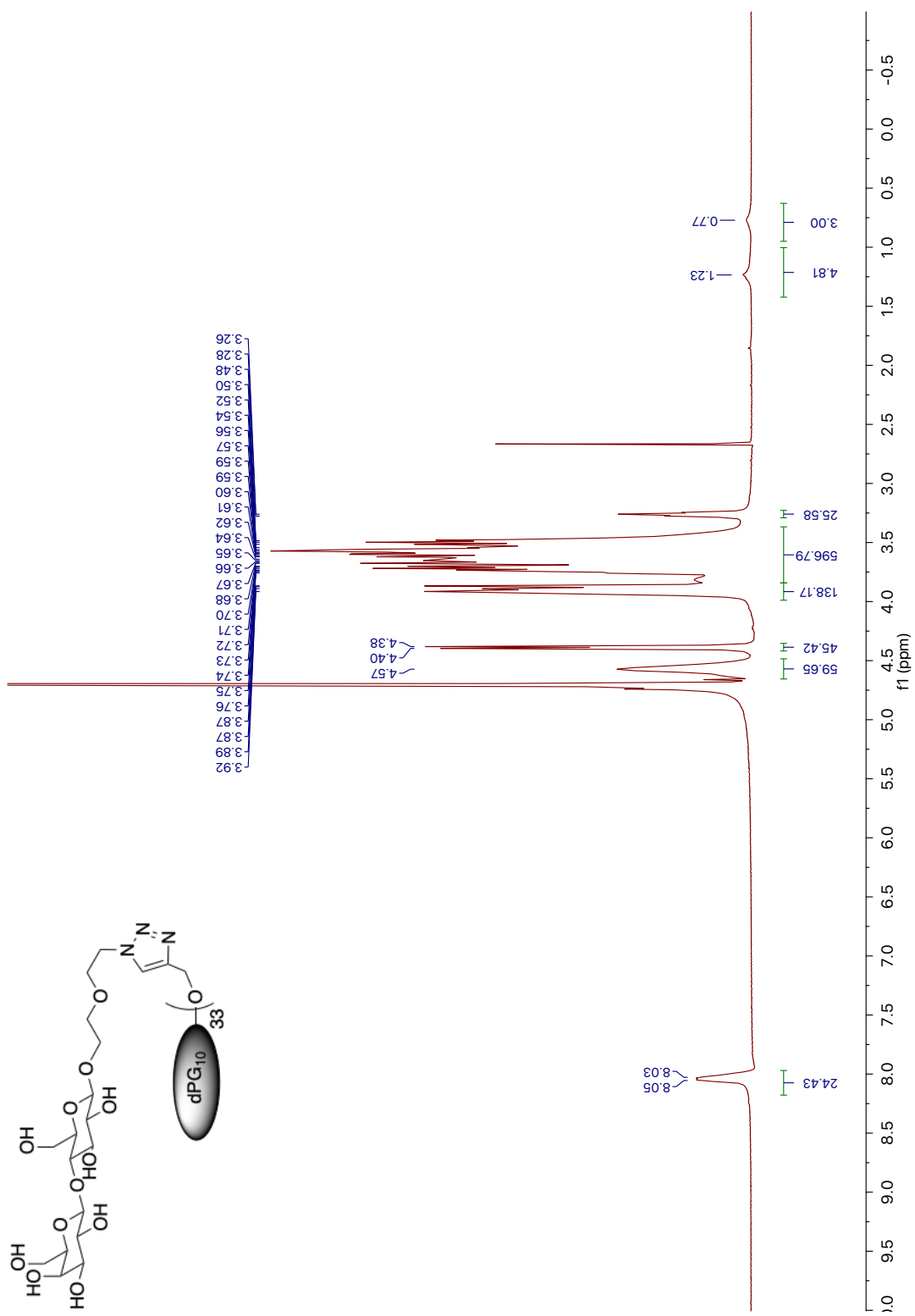


Figure S.29: ¹H NMR of product **13**.

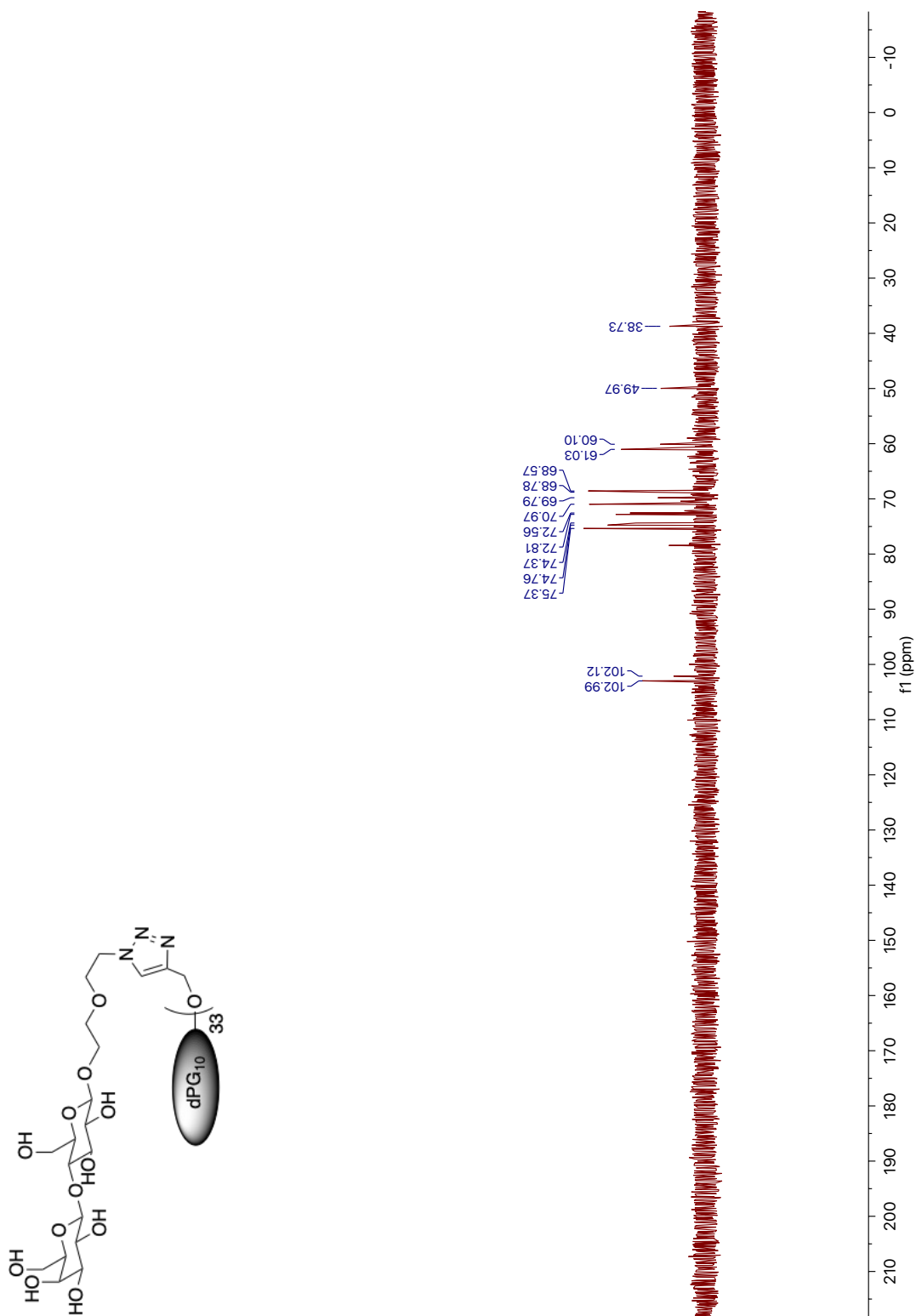


Figure S.30: ¹³C NMR of product **13**.

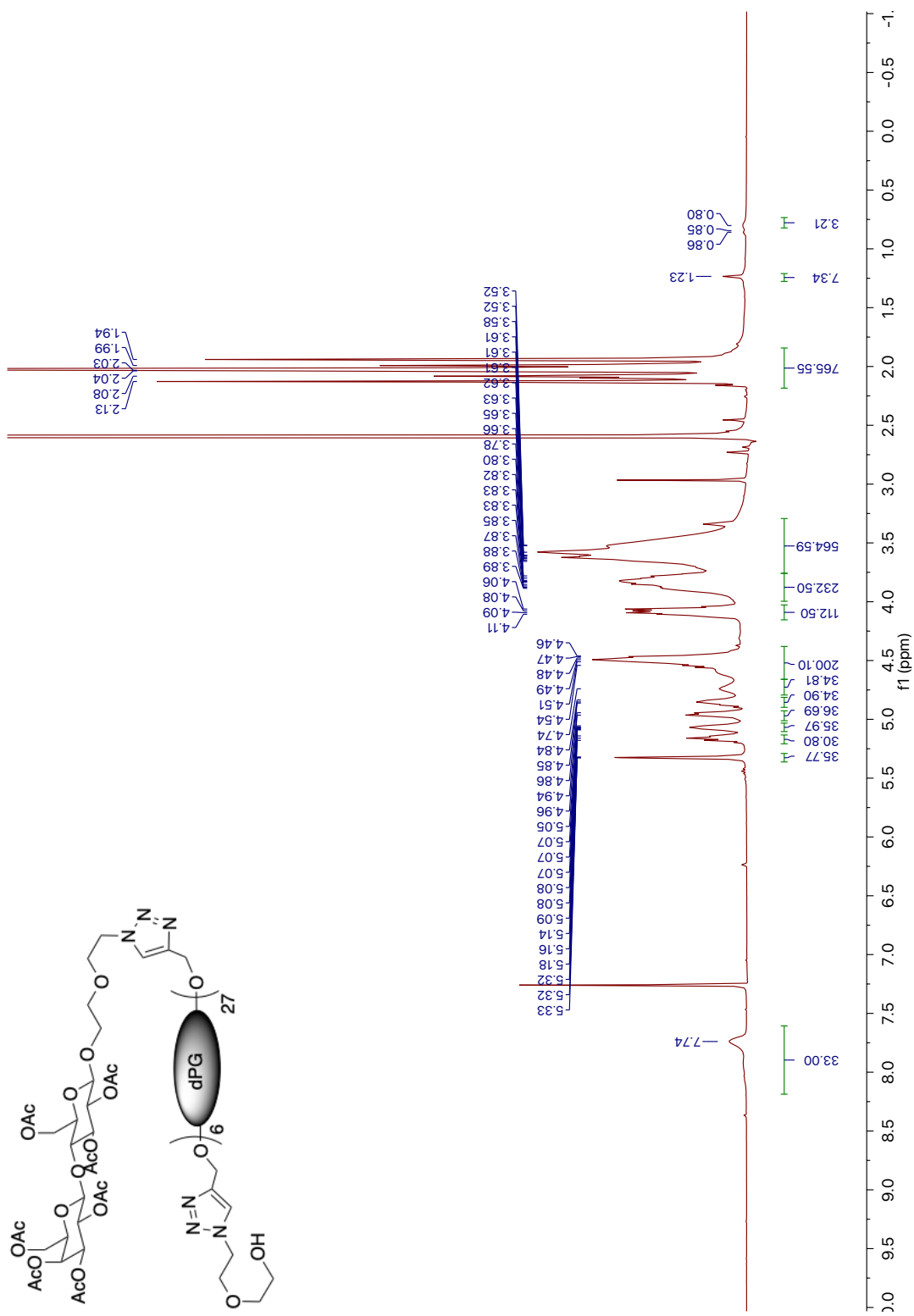


Figure S.31: ^1H NMR of product **14a**.

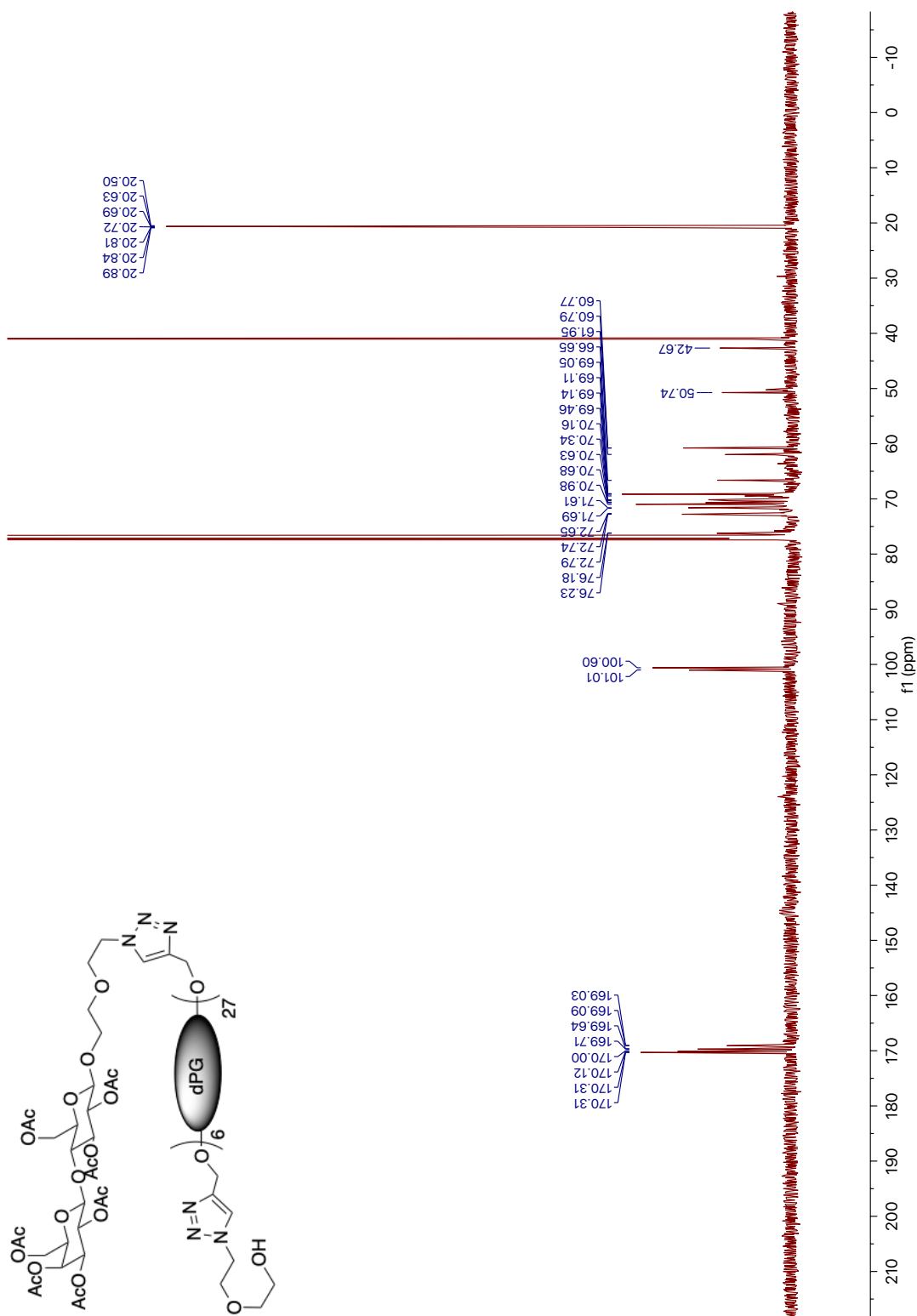


Figure S.32: ^{13}C NMR of product 14a.

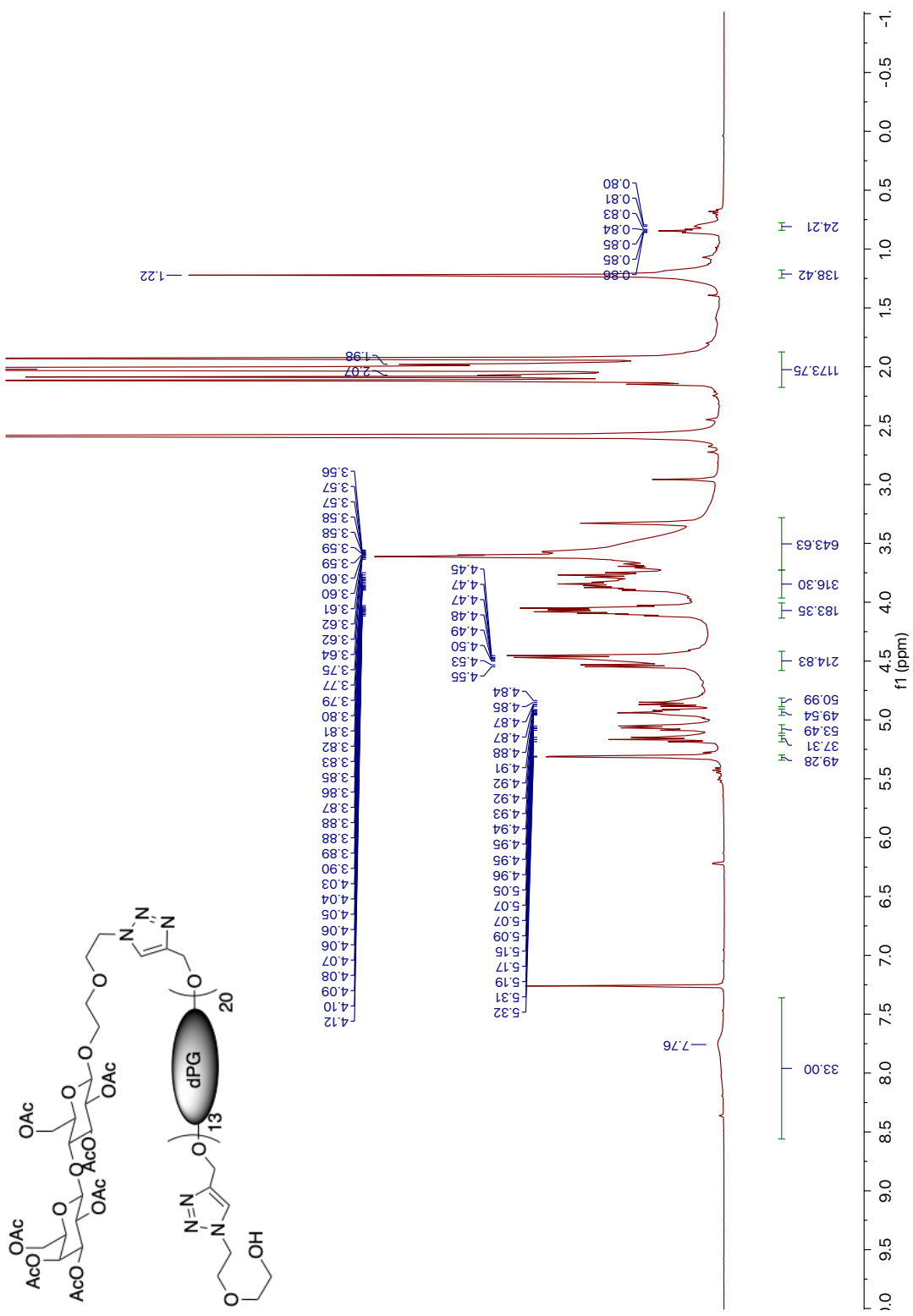


Figure S.33: ¹H NMR of product **15a**.

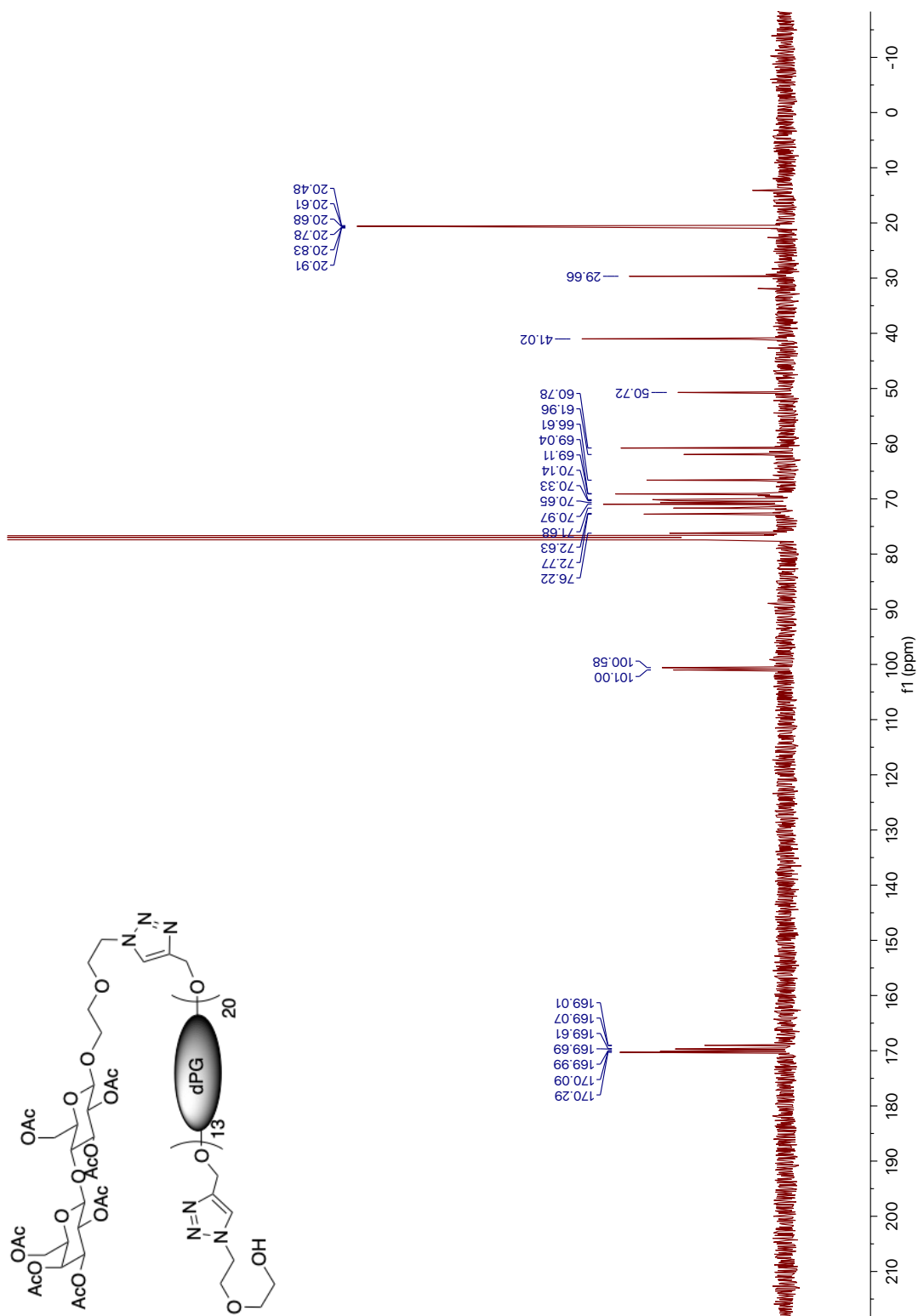


Figure S.34: ^{13}C NMR of product **15a**.

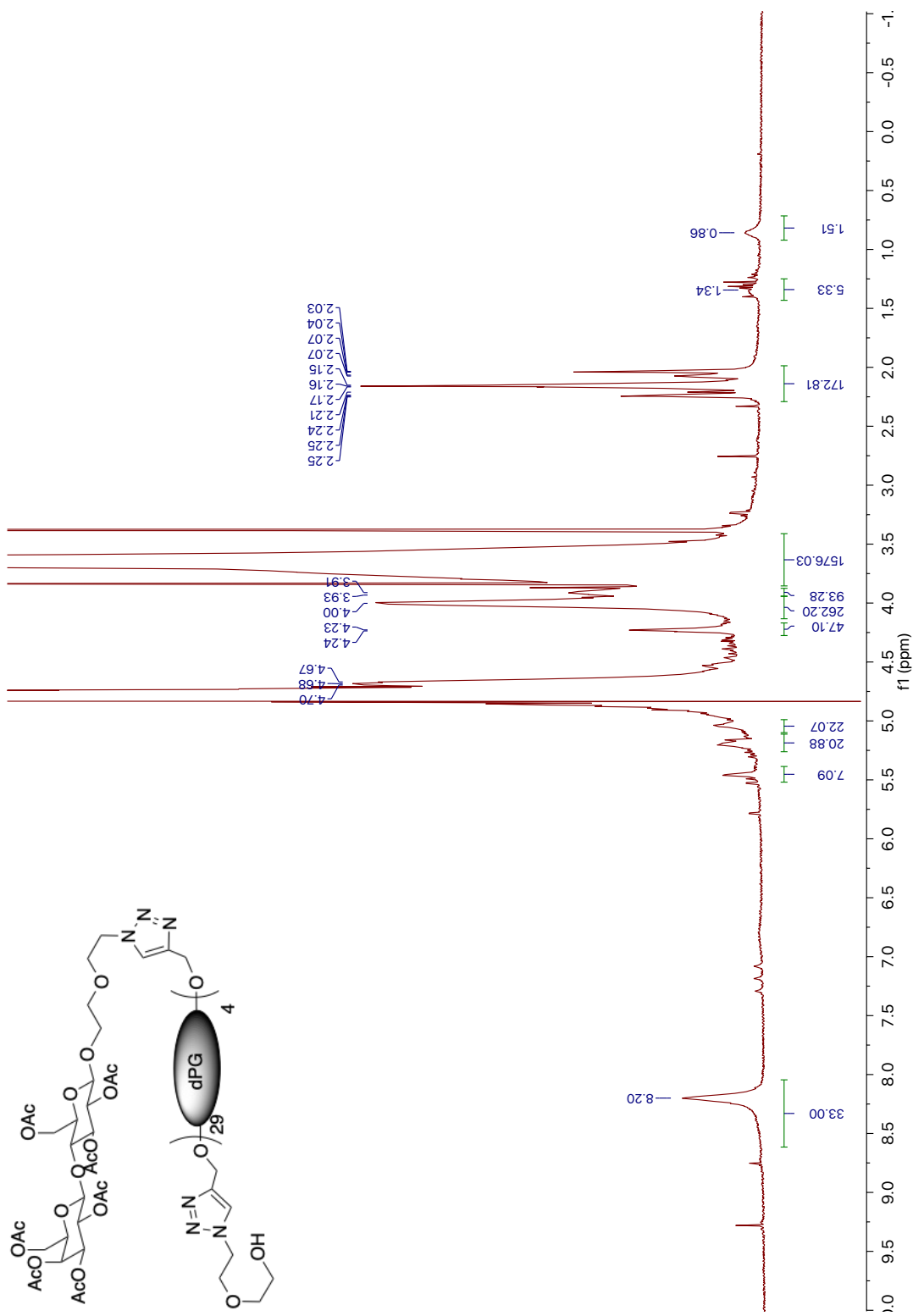


Figure S.35: ^1H NMR of product **16a**.

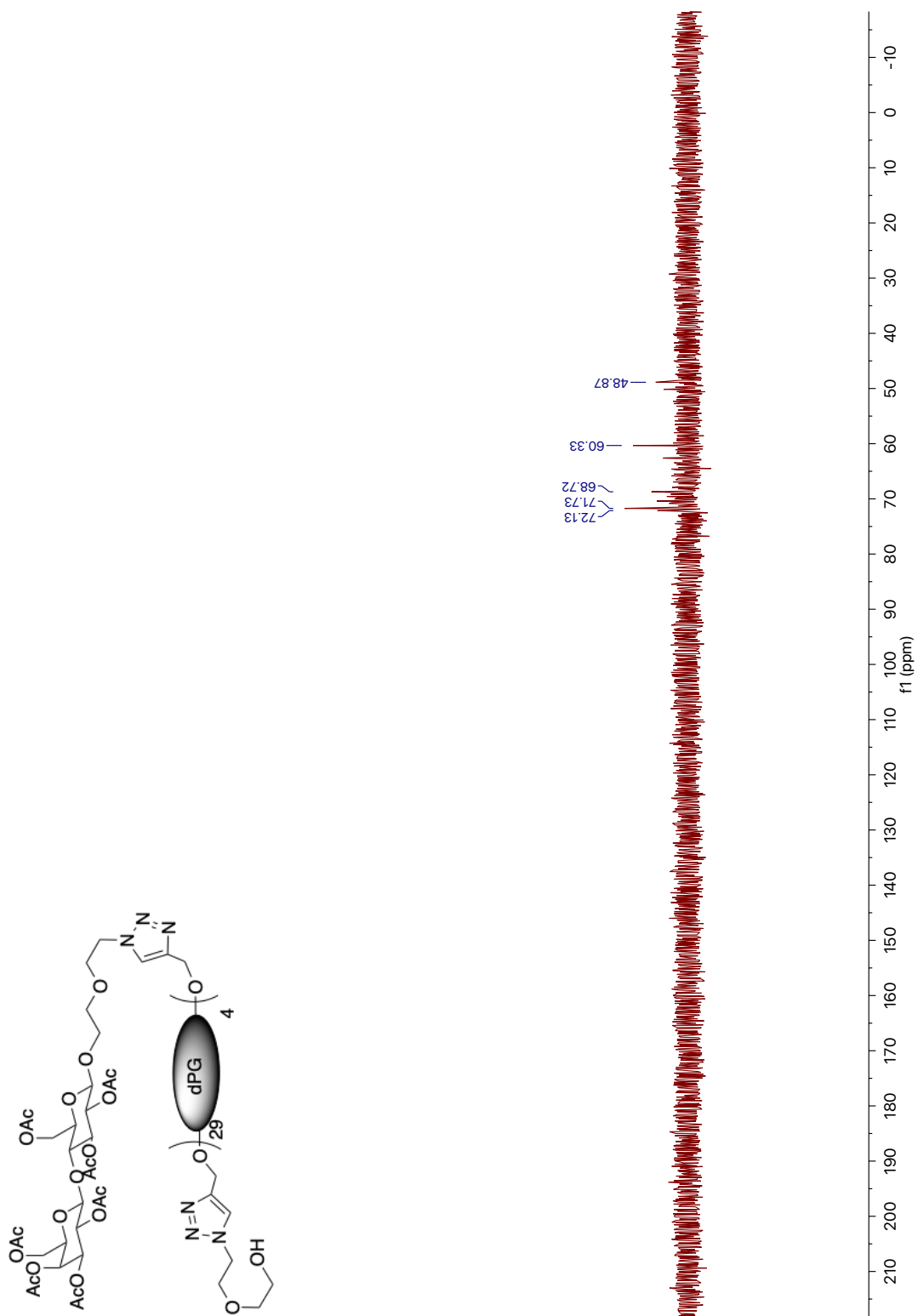


Figure S.36: ^{13}C NMR of product **16a**.

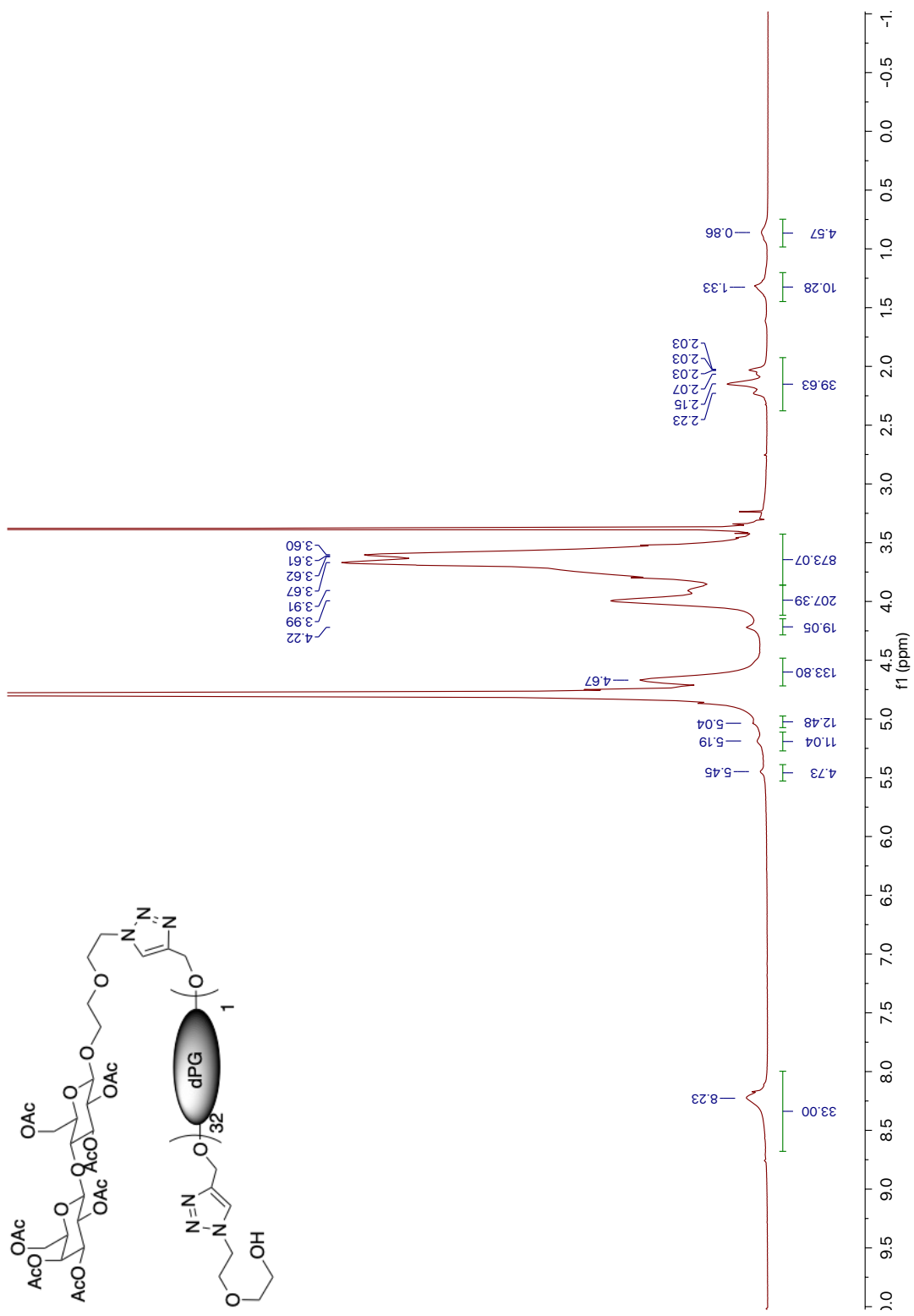


Figure S.37: ^1H NMR of product **17a**.

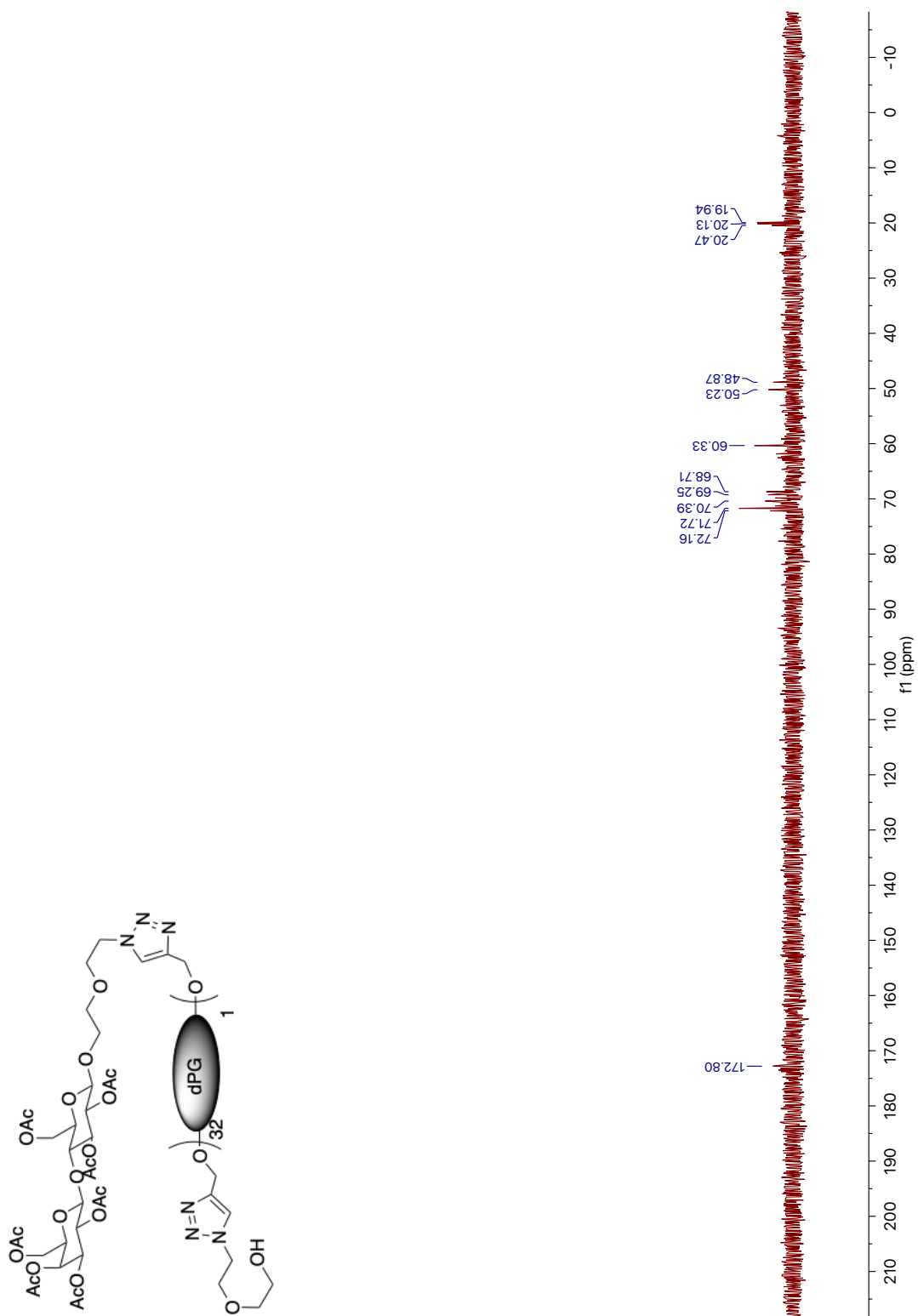


Figure S.38: ^{13}C NMR of product **17a**.

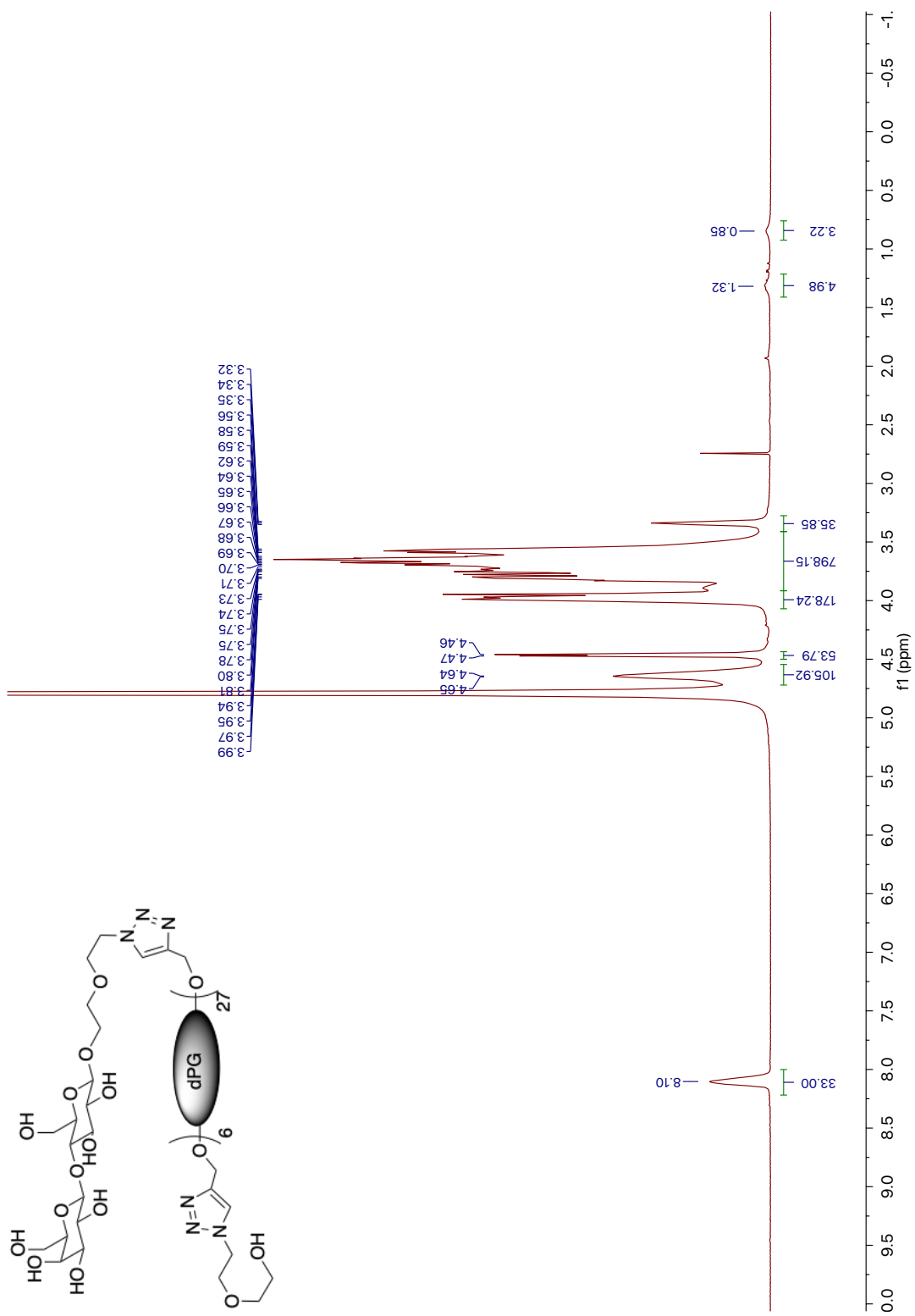


Figure S.39: ¹H NMR of product 14.

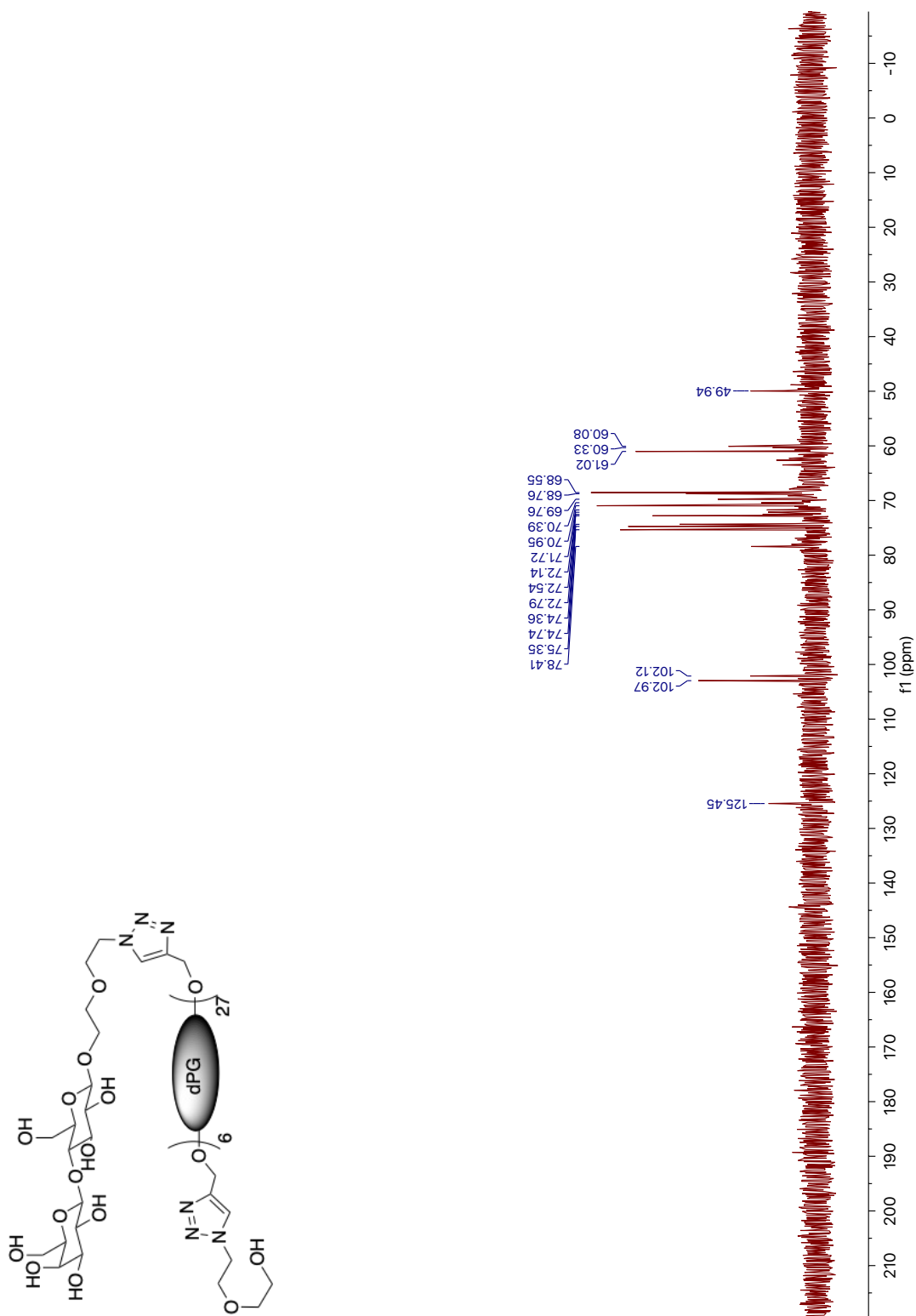


Figure S.40: ^{13}C NMR of product 14.

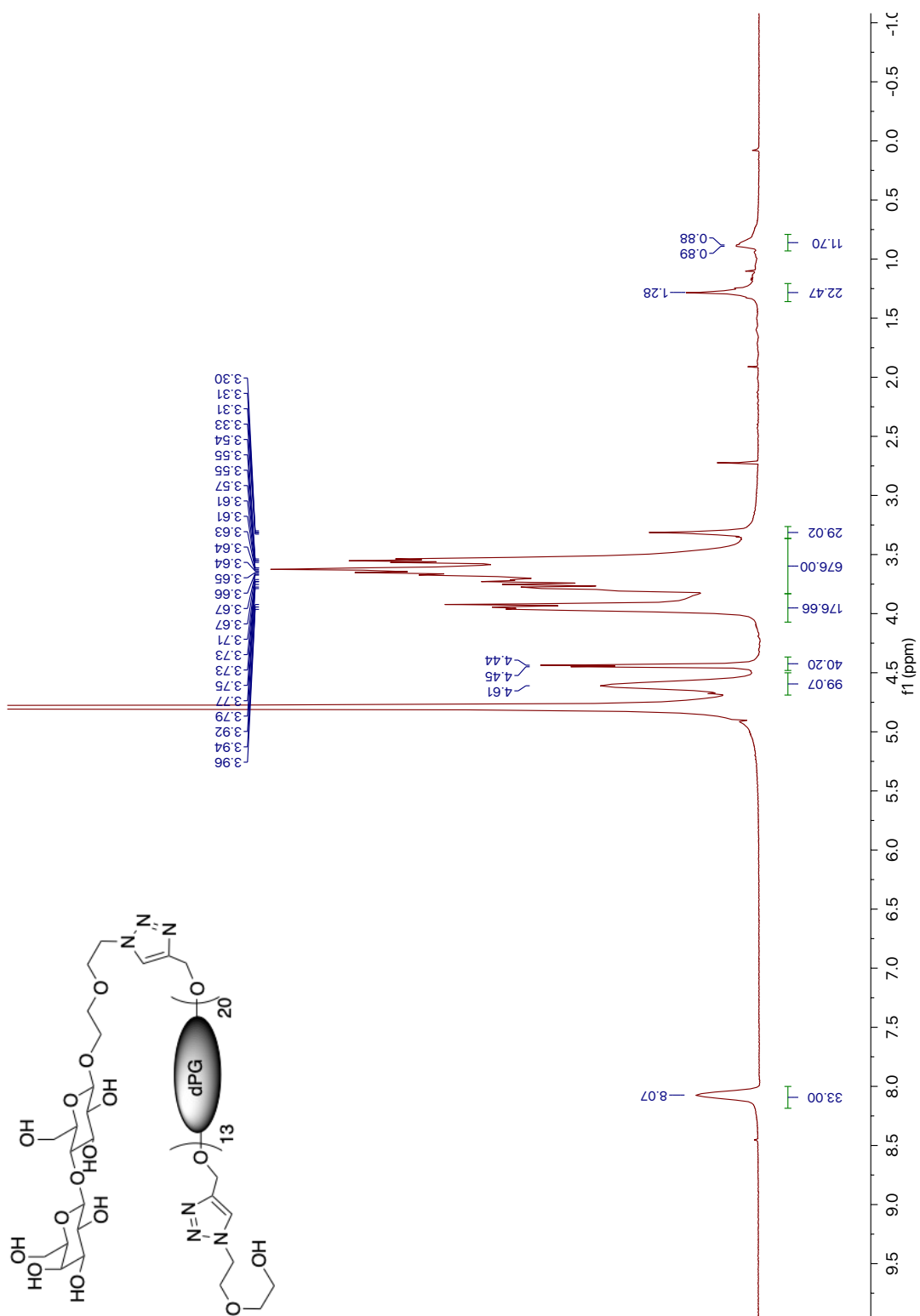


Figure S.41: ^1H NMR of product **15**.

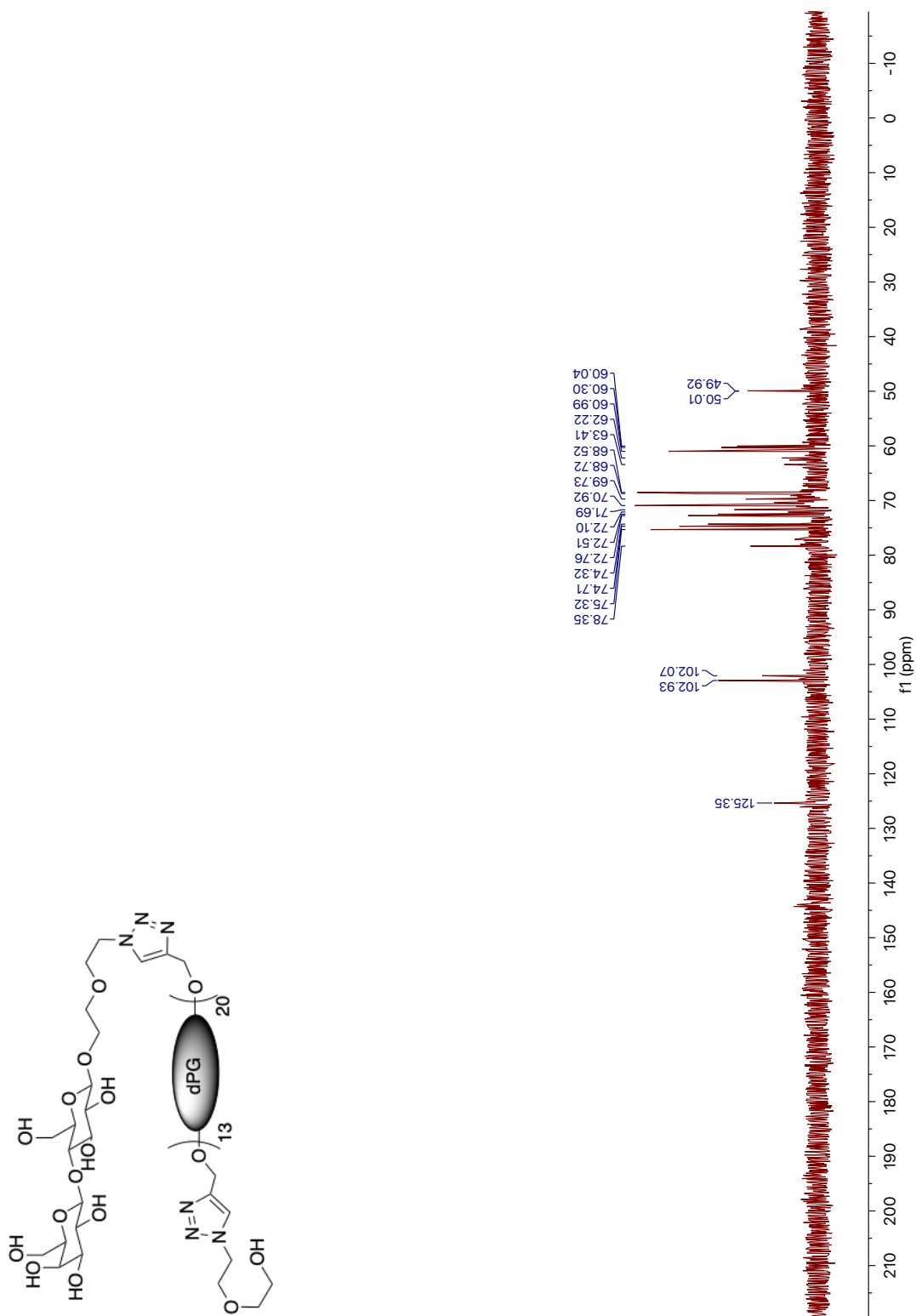


Figure S.42: ^{13}C NMR of product **15**.

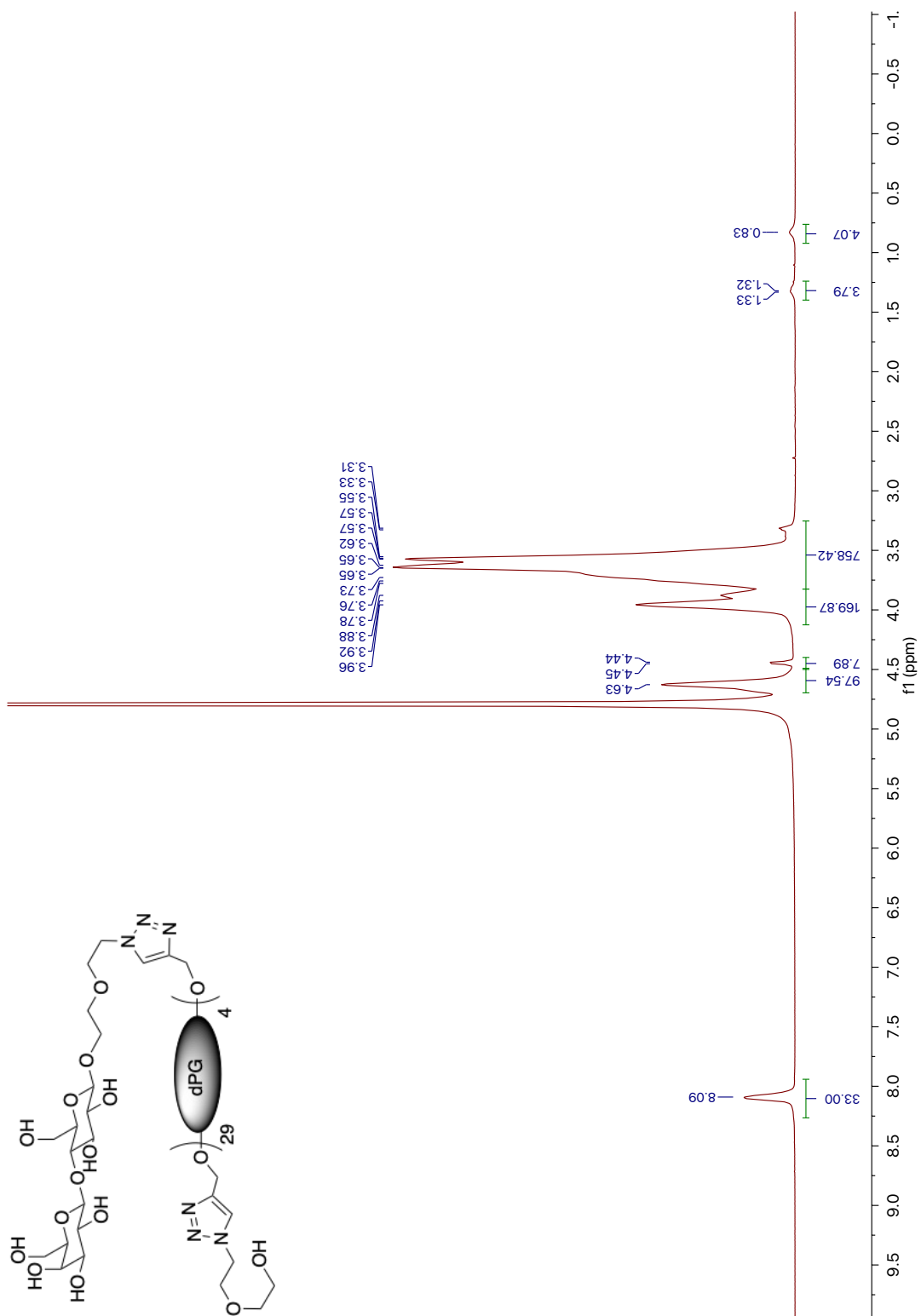


Figure S.43: ^1H NMR of product 16.

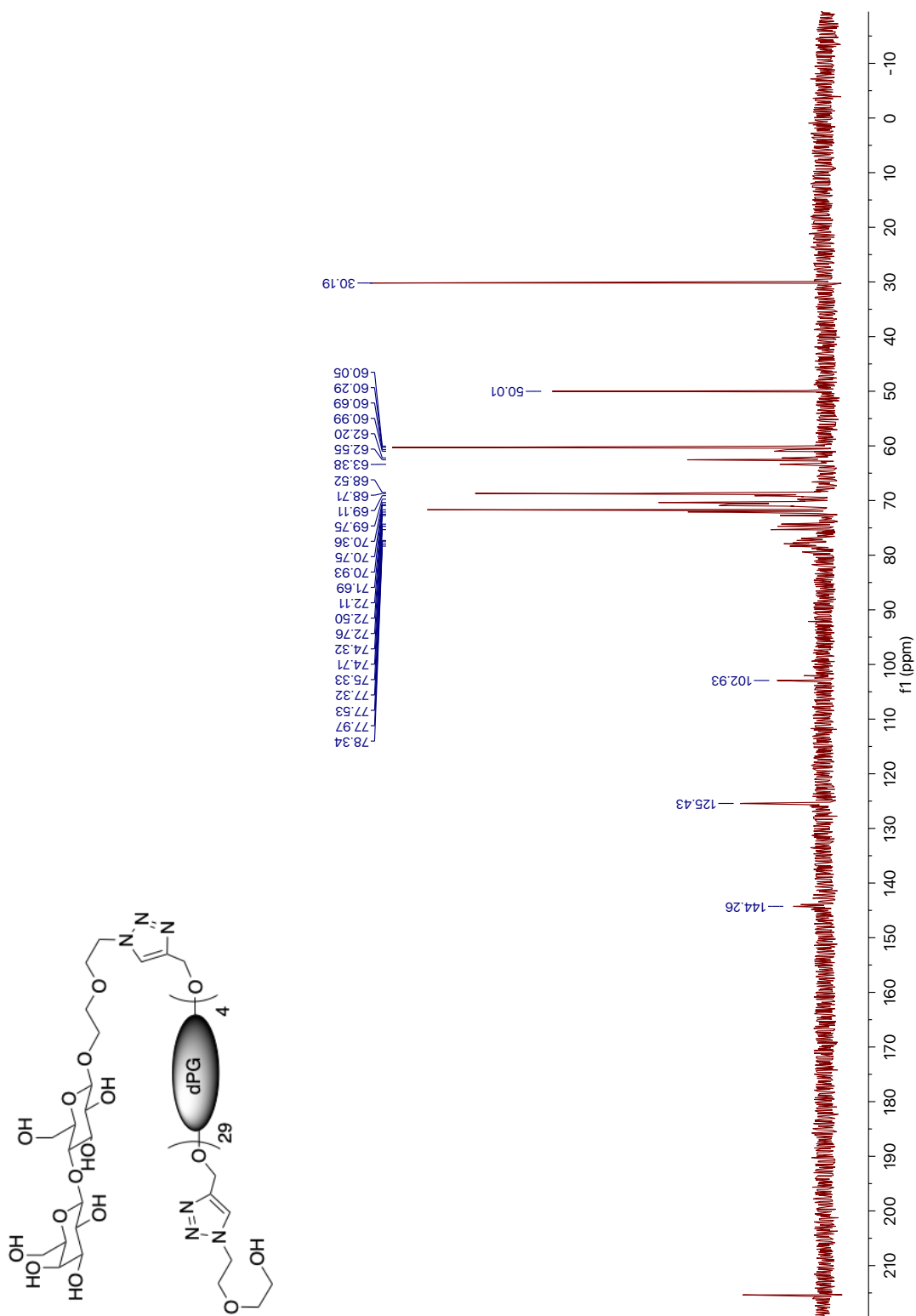


Figure S.44: ^{13}C NMR of product **16**.

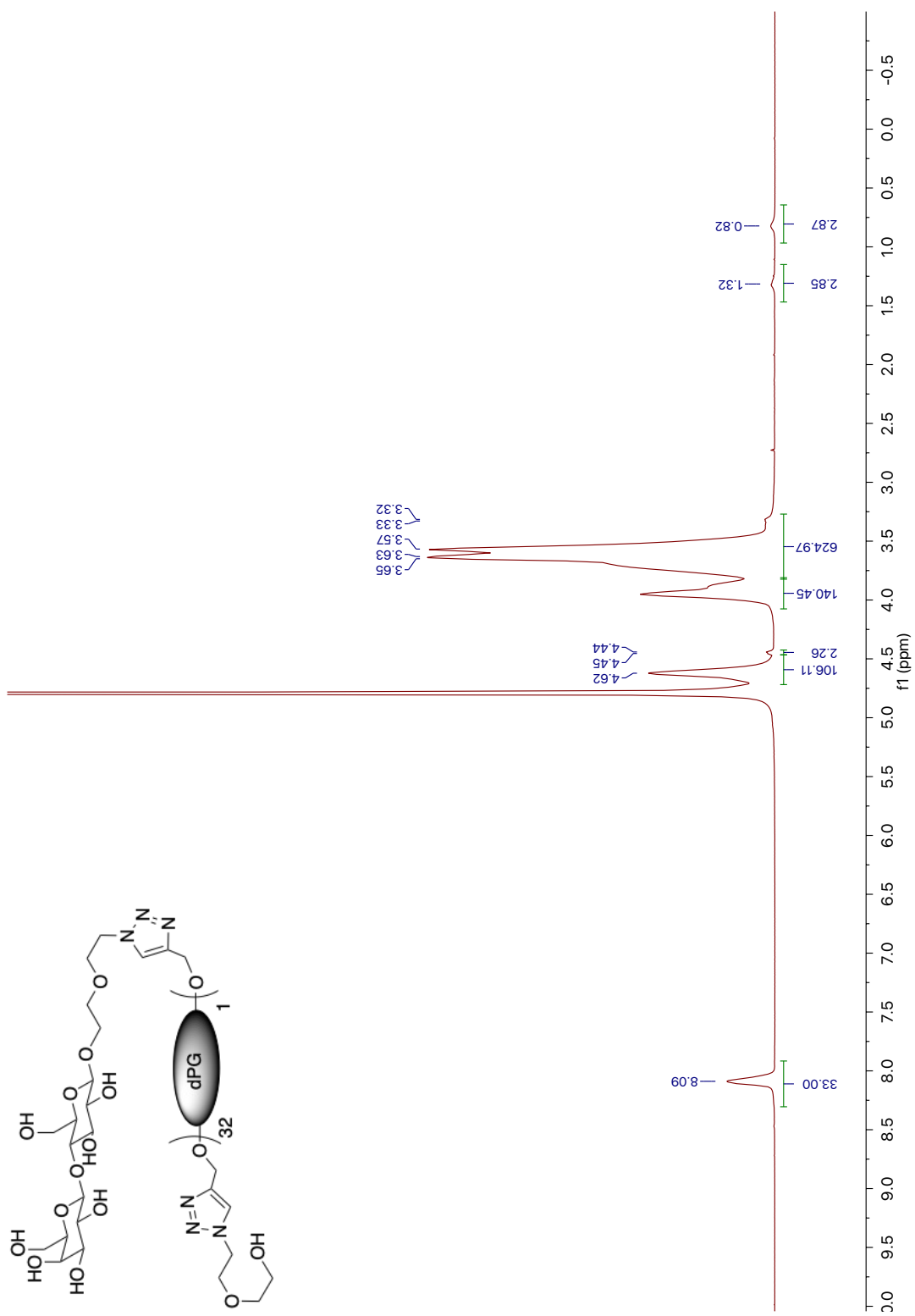


Figure S.45: ^1H NMR of product **17**.

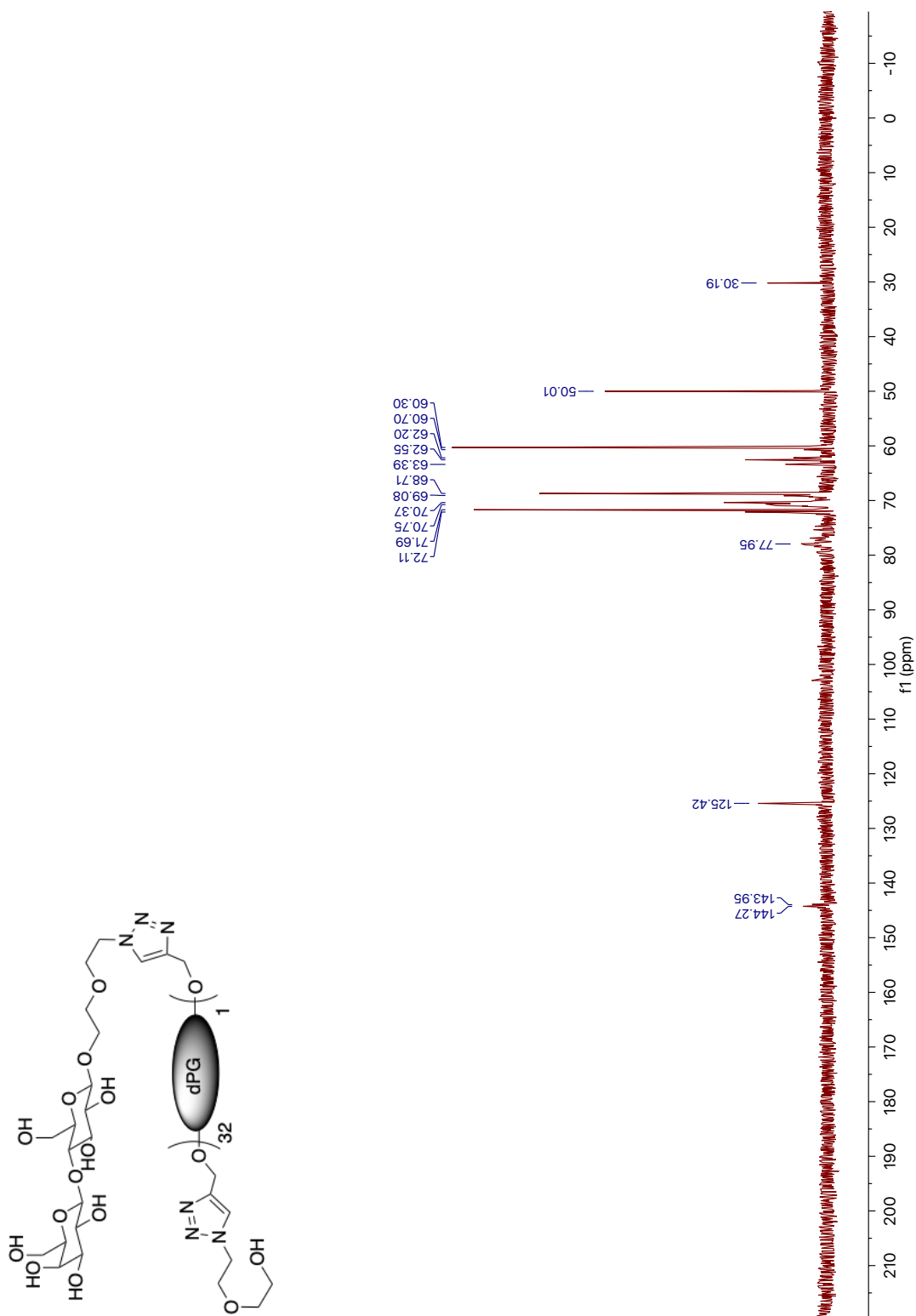


Figure S.46: ^{13}C NMR of product **17**.

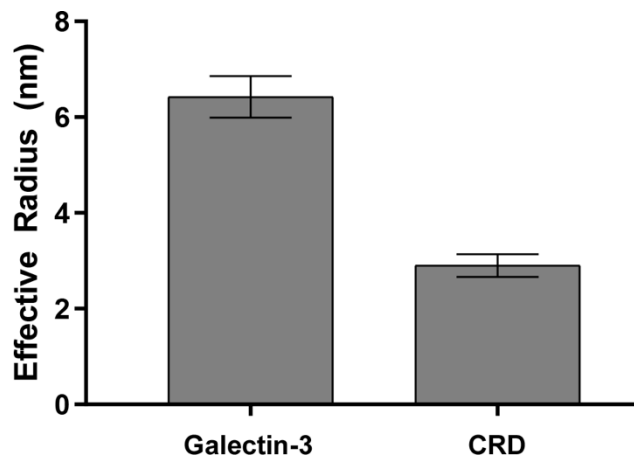


Fig. S47 DLS measurements indicating the long term stability of galectin-3 and CRD.