Supporting Information for publication

Synthesis of choline sulfonates buffers and their effect on cytochrome *c* dissolution and oxidation state

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General

All reagents and solvents were obtained commercially, unless otherwise noted, and appropriately purified, if necessary. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker AVANCE II+ 400 MHz NMR spectrometer, using D_2O as solvent and the residual solvent peak as reference.

Elemental analyses and DSC measurements were conducted by REQUIMTE-DQ-FCT laboratory (Portugal) and carried out with a Thermo Finnigan Flash EA 111 and Setaram DSC 131 calorimeter at a scanning rate of 10 ºC min^{-1} respectively.

Horse heart cytochrome c was obtained from CalbioChem. The Good's buffers were obtained from Sigma. Sodium dithionite was obtained from Fisher Scientific, Potassium ferricyanide was obtained from Biochemica-Applichem. Double-distilled water was used in all experiments. The concentration of cytochrome c was determined by using ε550 nm=27.6 mM−1·cm−1.1

Titration curve

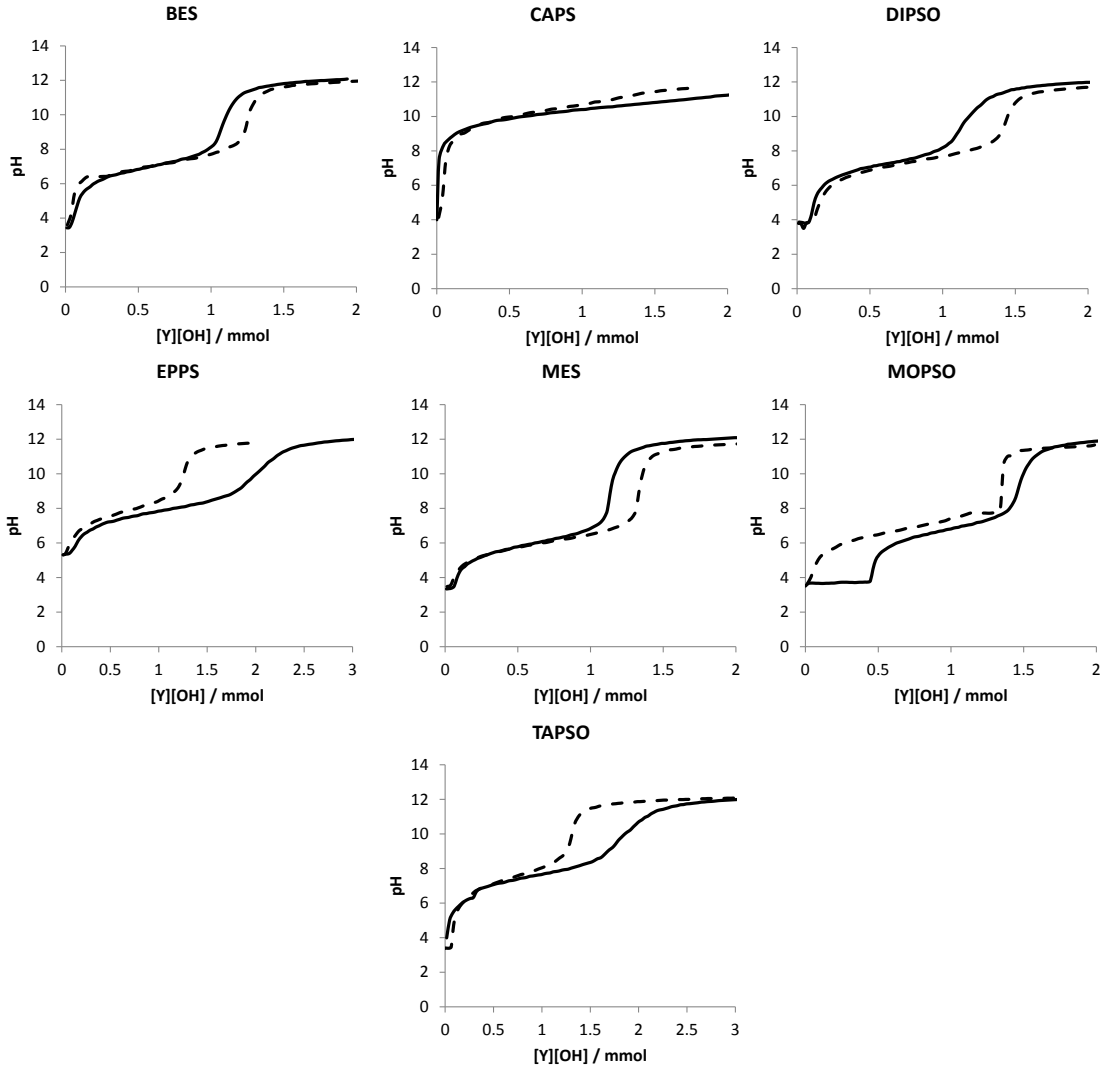

Figure 1- Titration curve for 10 mL of 0.1 M of different acids *versus* [Choline][OH] 0.1 M (full line) or [Na][OH] 0.1 M (dash line). The electrode is standardized with two aqueous primary standard buffer solutions. The electrode pH limit is 12.

Choline sulfonic buffers	Buffer Range
[Ch][BES]	6.46 – 7.66
[Ch][CAPS]	9.77 – 11.82[a]
[Ch][DIPSO]	6.81 – 7.64
[Ch][EPPS]	7.23 – 8.73
[Ch][MES]	5.33 – 6.69
[Ch][MOPSO]	5.65 – 7.33
[Ch][TAPSO]	6.87 – 8.23

Table 1- pH range of choline sulfonate buffers.
[a]The electrode pH limit is 12.

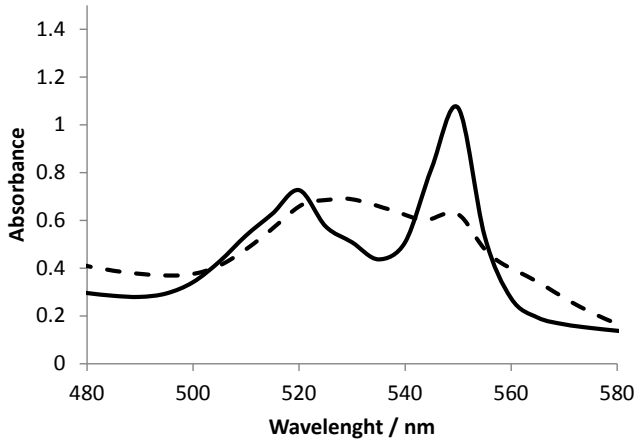

Figure 2- Q-bands of cyt *c* in [Ch][TAPSO] (full line) and 0.1M TAPSO buffer (dash line).

Cyclic Redox Assay

The assay was performed using 0.3mg/ml of cytochrome c in phosphate buffer and choline sulfonates buffers by measuring the optical density at 550 nm. Cytochrome c was reduced with a few grains of sodium dithionite and the effectiveness of the reduction was checked by calculating the ratio of the absorbance at 550 nm versus 565 nm, greater than 6. After adjusting the ratio, the experiment was followed for some minutes by adding alternately a few grains of $K_3[Fe(CN)_6]$ or sodium dithionite.

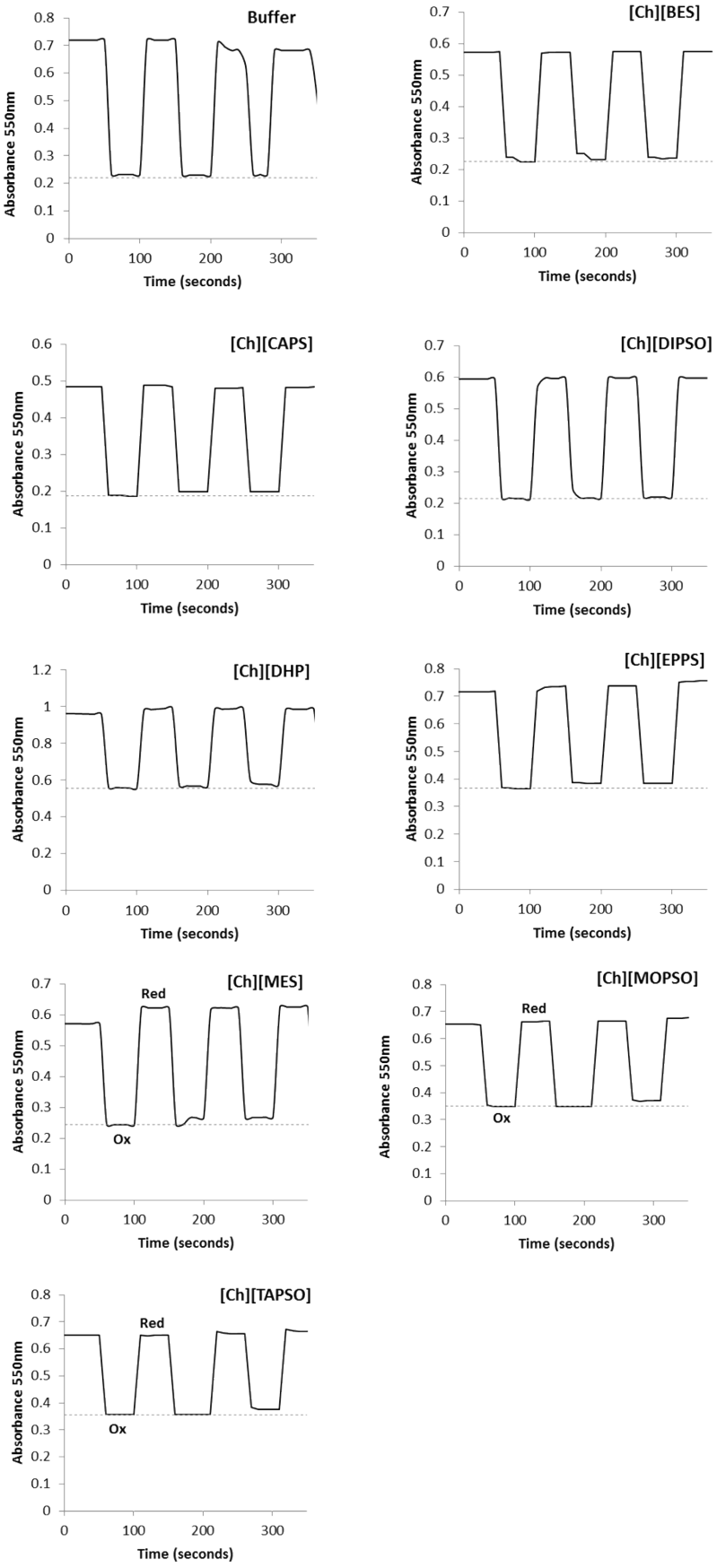

Figure 3- Cyclic redox assay of 0.3mg/ml cytochrome c solution in choline buffers at 25ºC.

Choline sulfonates buffers' regeneration

Choline sulfonate buffers were hydrated with 30% (w/w) of Milliq water. After hydration, water was removed under continuous vacuum (0.02 mbar) for 2 days at room temperature. In order to access their regeneration profile 1H and 13C NMR spectra were performed. According to the weight variation and NMR spectra it is possible to confirm that most of choline buffers can be regenerated after hydration. The exception was the Choline EPPS that gave a different cation:anion ratio after their dissolution in water and evaporation.

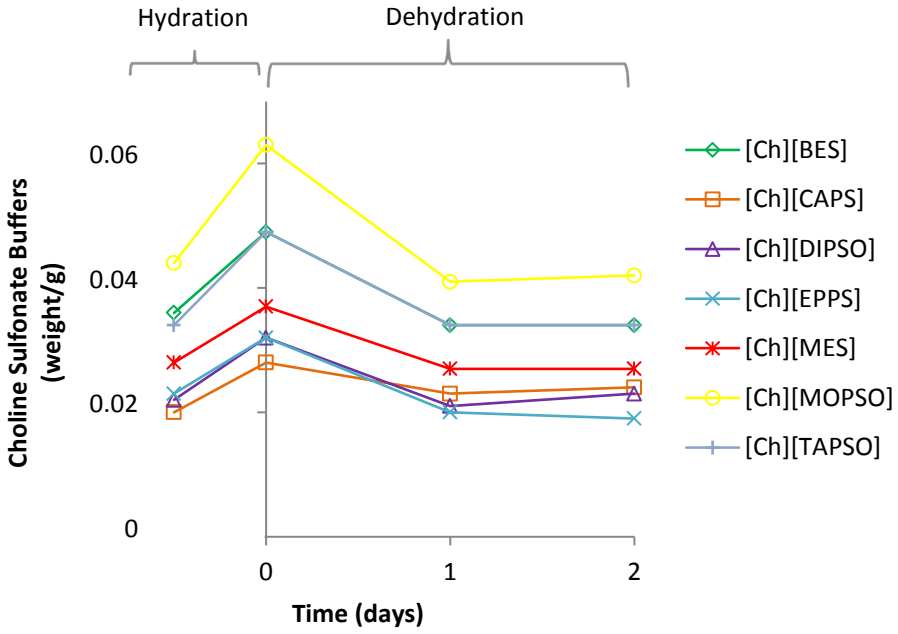

Figure 4- Weight variation of 30% hydrated Choline Buffers under 0.02 mbar and room temperature.

Choline Buffer	Sample	T_m (ºC)	T_c (ºC)	T_g (ºC)	T_d (ºC)
[Ch][BES]	0	-	-	-83.39	>200
	I	-	-	-84.74	n.d
	II	-	-	-77.3	n.d
[Ch][DIPSO]	0	-	-	-57.63	>210
	I	-	-	-59.64	n.d
	II	-	-	-44.33	n.d
[Ch][EPPS]	0	-	-	-62.28	>200
	I	-	-	-64.11	n.d
	II	-	-	-58.55	n.d
[Ch][MOPSO]	0	-	-	-66.49	>210
	I	-	-	-68.41	n.d
	II	-	-	- 65.41	n.d
[Ch][TAPSO]	0	-	-	-46.34	>225
	I	-	-	-42.18	n.d
	II	-	-	-42.39	n.d
[Ch][CAPS]	0	100.4	89.04	-	>200
	I	101.4	n.d	-	>200
	II	99.89	n.d	-	>200
[Ch][DHP]	0	75.59; 112.33	-	-	>175
	I	88.34; 122.7	-	-	>175
	II	41.8; 54.98	-	-	>120
[Ch][MES]	0	89.18; 112.39	72.94	-	>200
	I	72.72; 107.44	n.d	-	>200
	II	85,59; 111.07	n.d	-	>200

Table 2 – Differential scanning calorimetry results of Choline Buffers under different conditions.
$\mathbf{T_m}$ melting temperature, $\mathbf{T_c}$ cristalization temperature, $\mathbf{T_g}$ glass transition temperature, T_d degradation temperature. 0 –dry choline buffer stored at room temperature; I – dry choline buffer stored at 40ºC for 1 week; II- 30% hydrated choline buffer stored at 40ºC for 1 week. (Results from 1st heating).
n.d -not determined

NMR Spectra

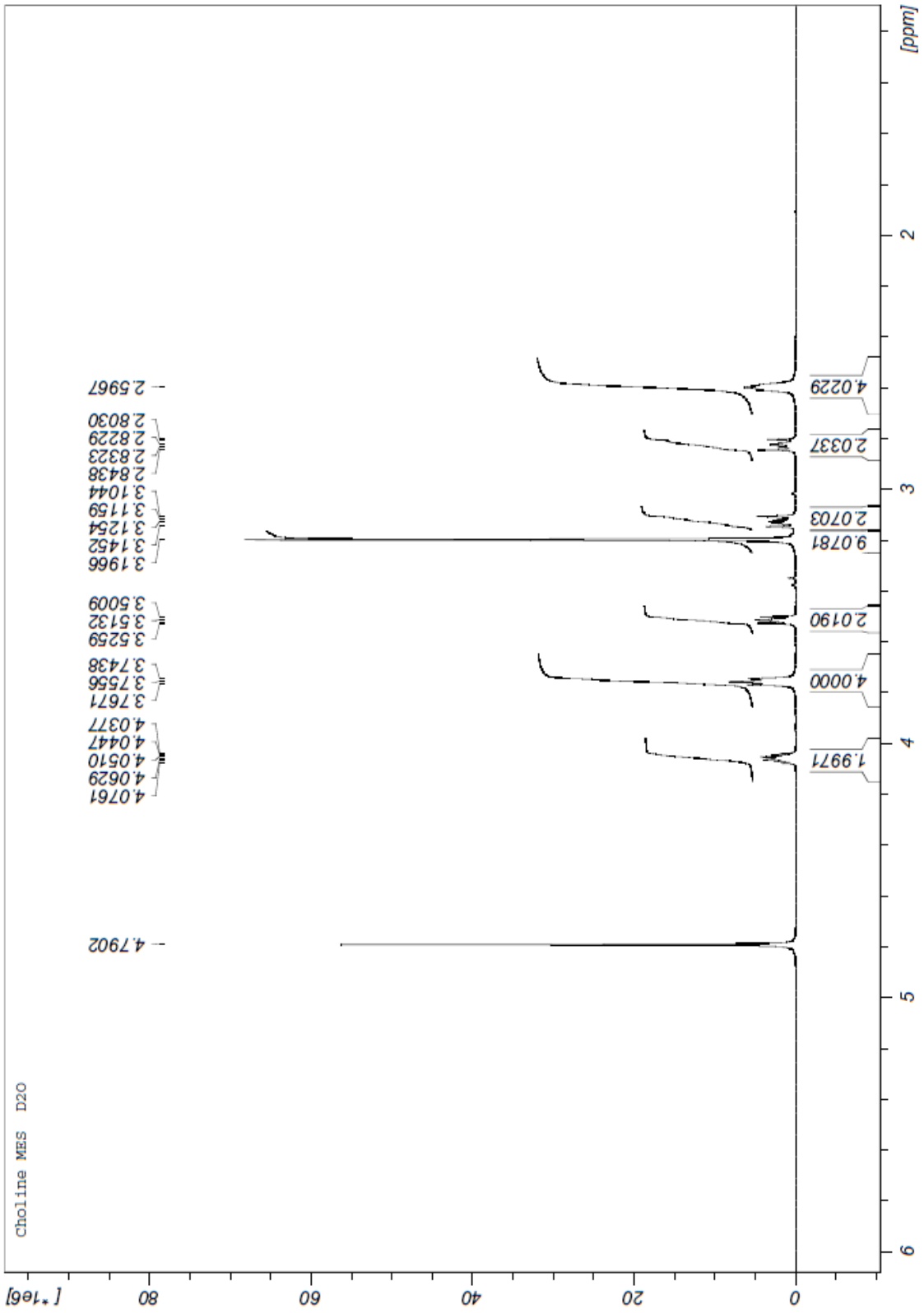

Figure 5 – ^{1}H NMR spectrum of choline 2-(N-morpholino)ethanesulfonate **[Ch][MES]** in D_2O at room temperature.

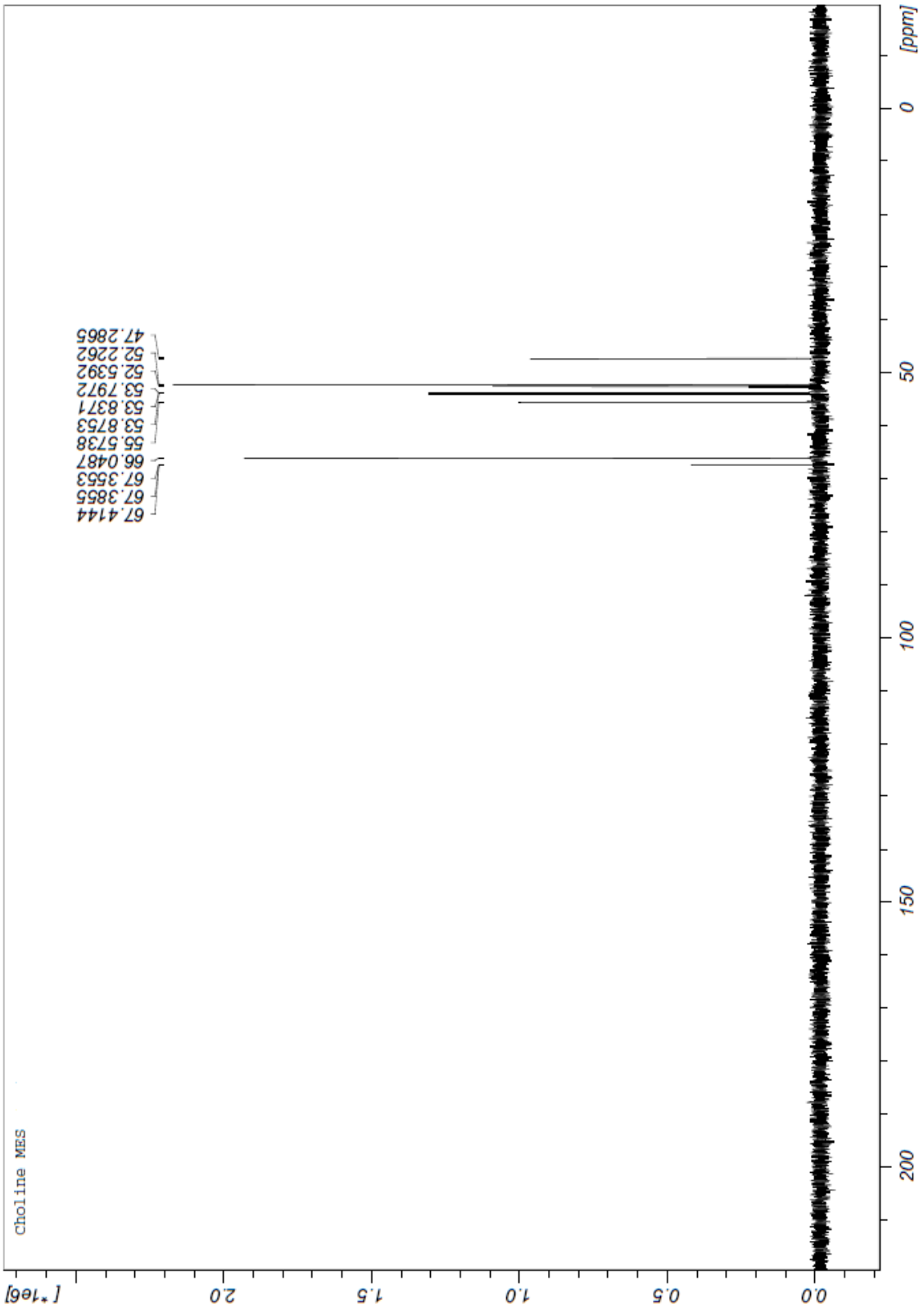

Figure 6 – ^{13}C NMR spectrum of choline 2-(N-morpholino)ethanesulfonate **[Ch][MES]** in D_2O at room temperature.

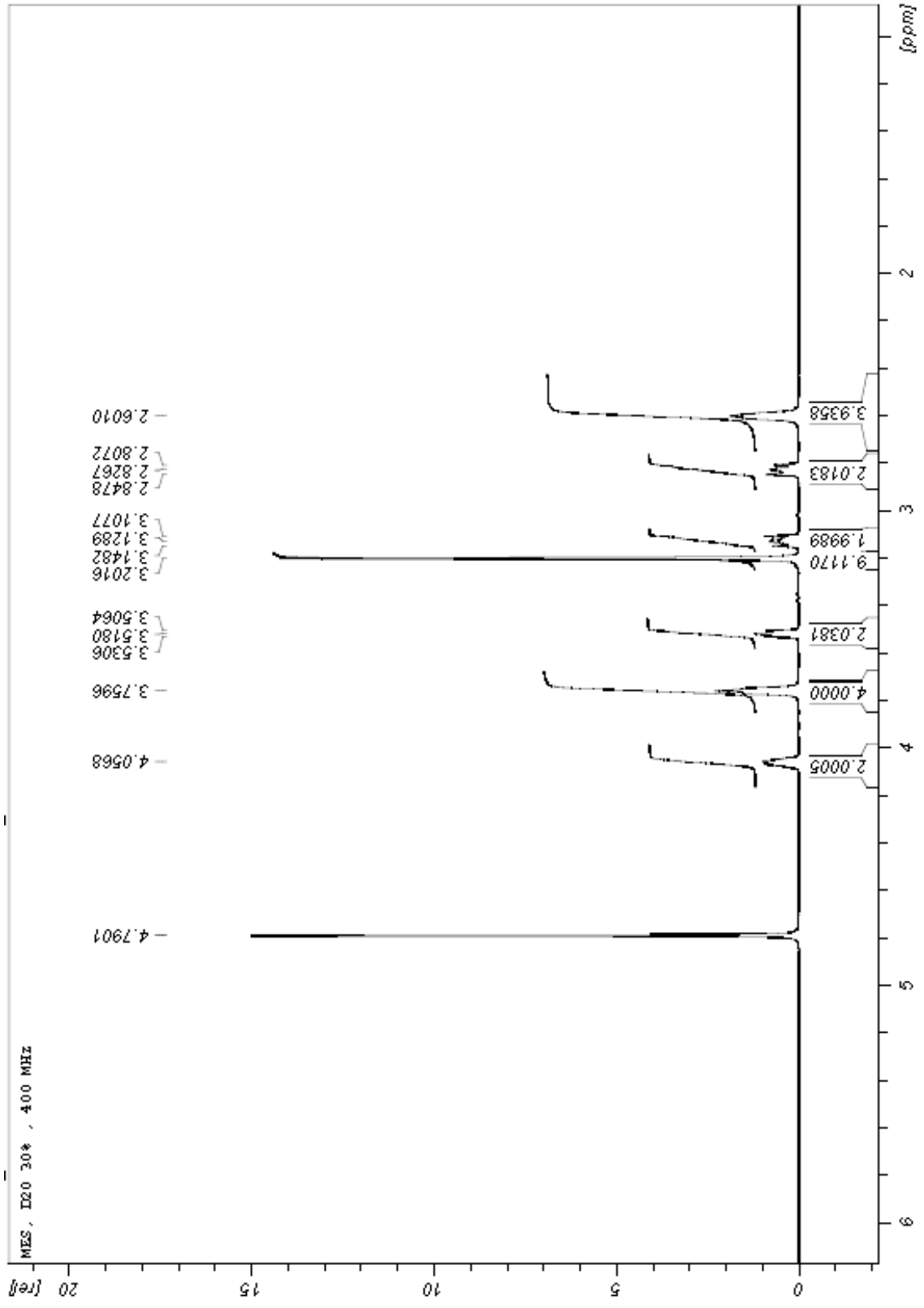

Figure 7 – ^{1}H NMR spectrum of 30% hydrated choline 2-(N-morpholino)ethanesulfonate **[Ch][MES]** in D_2O at room temperature.

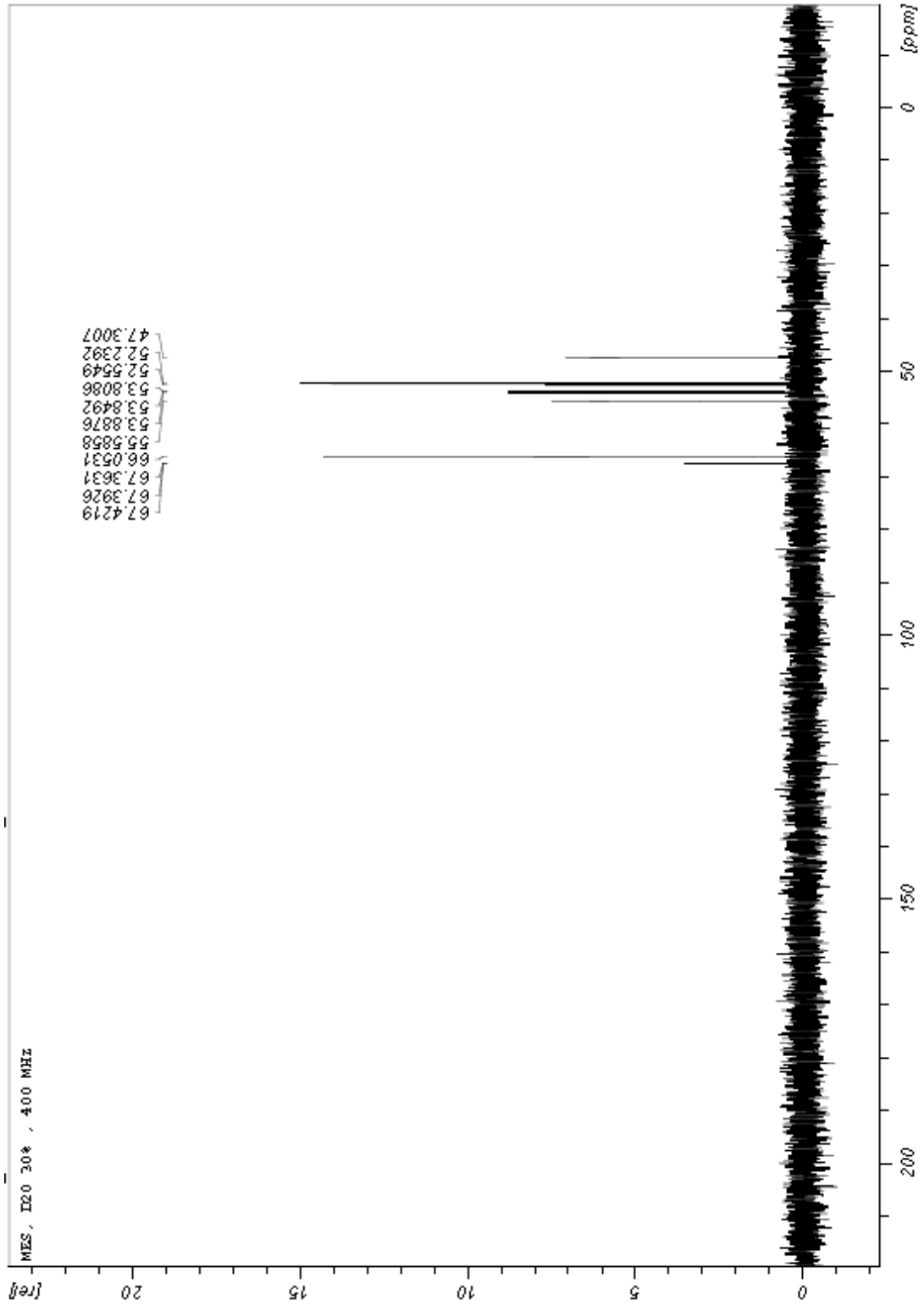

Figure 8 – ^{13}C NMR spectrum of 30% hydrated choline 2-(N-morpholino)ethanesulfonate **[Ch][MES]** in D_2O at room temperature.

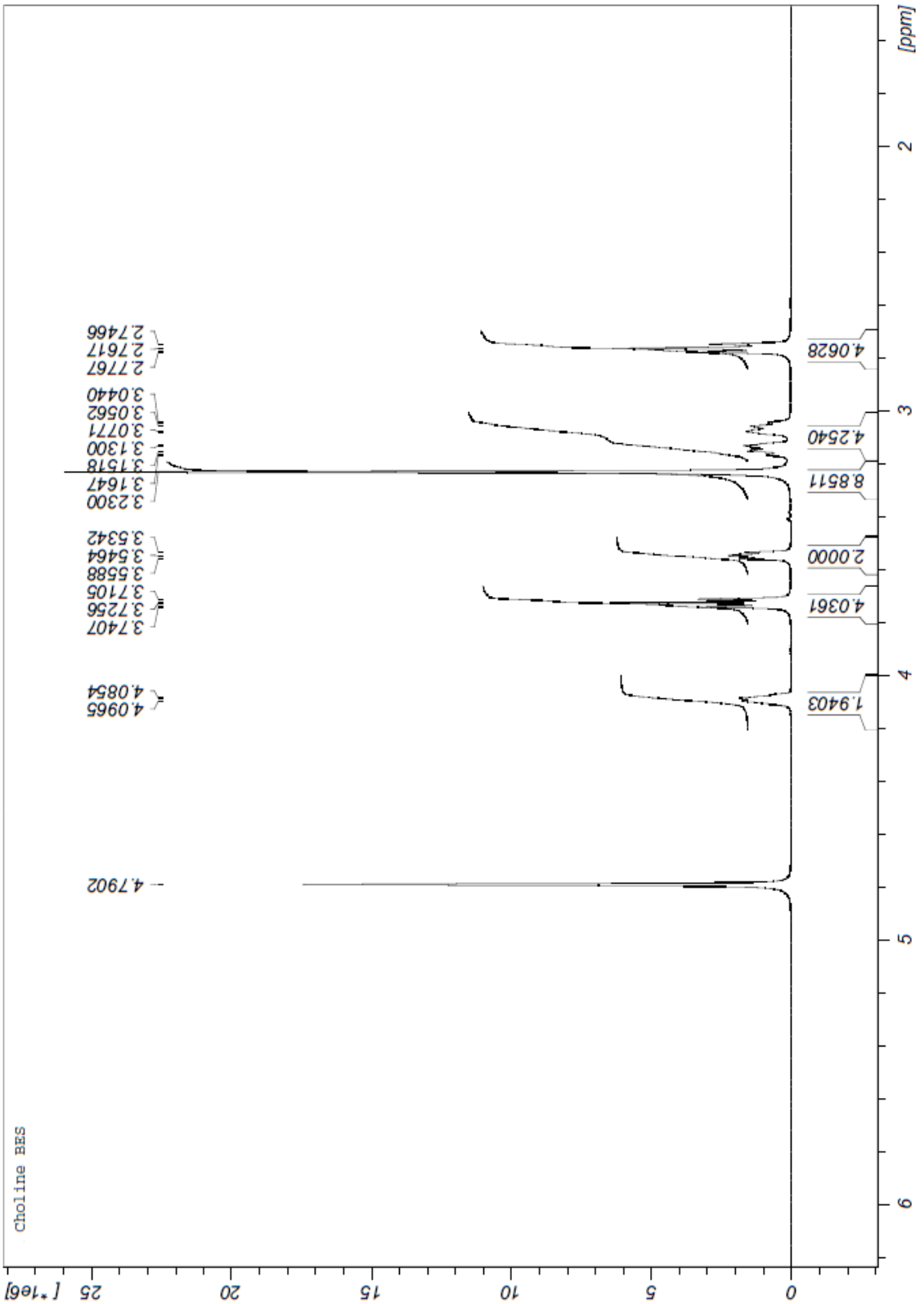

Figure 9 – ^{1}H NMR spectrum of choline 2-[bis(2-hydroxyethyl)amino]ethanesulfonate **[Ch][BES]** in D_2O at room temperature.

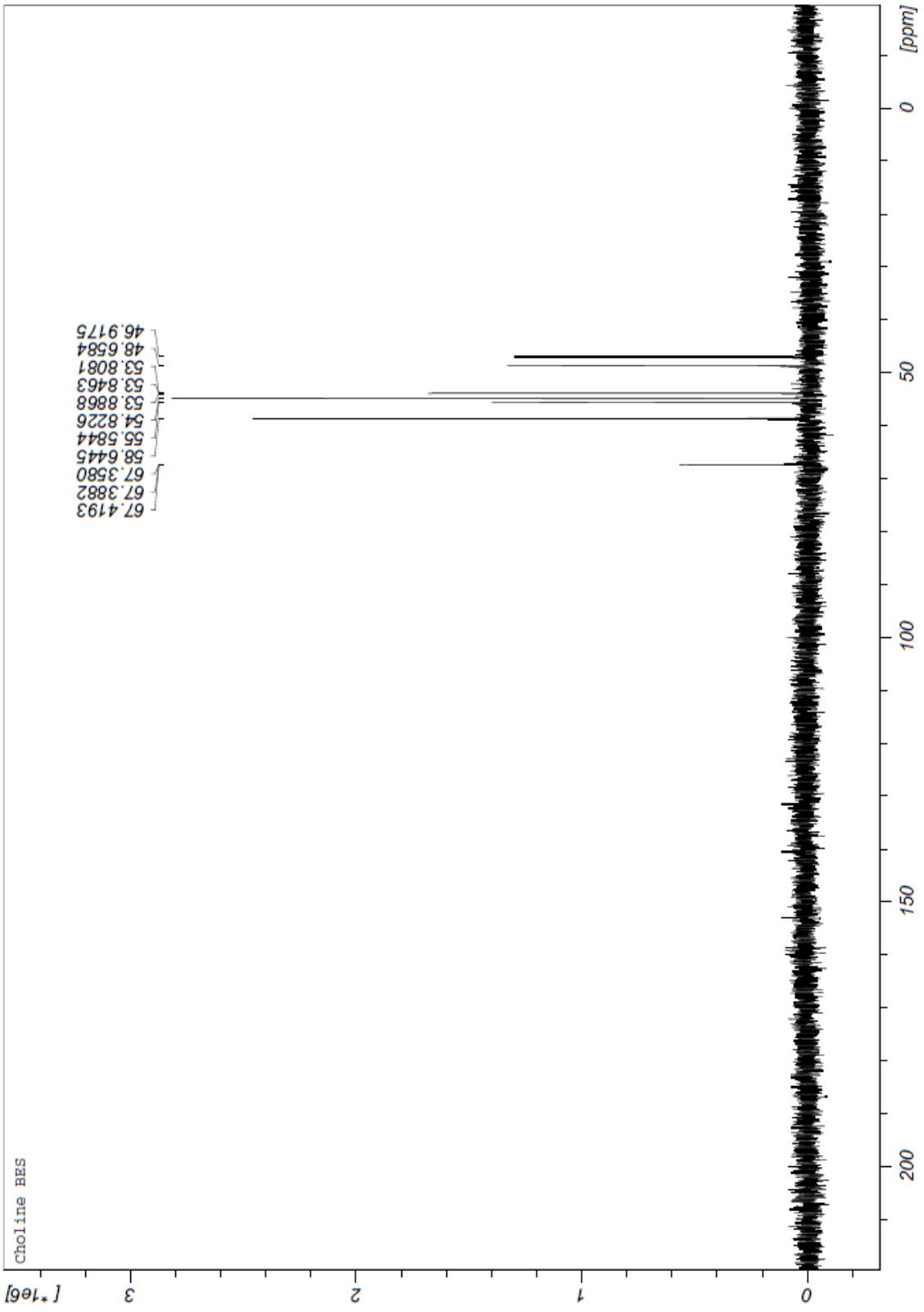

Figure 10 – ^{13}C NMR spectrum of choline 2-[bis(2-hydroxyethyl)amino]ethanesulfonate **[Ch][BES]** in D_2O at room temperature.

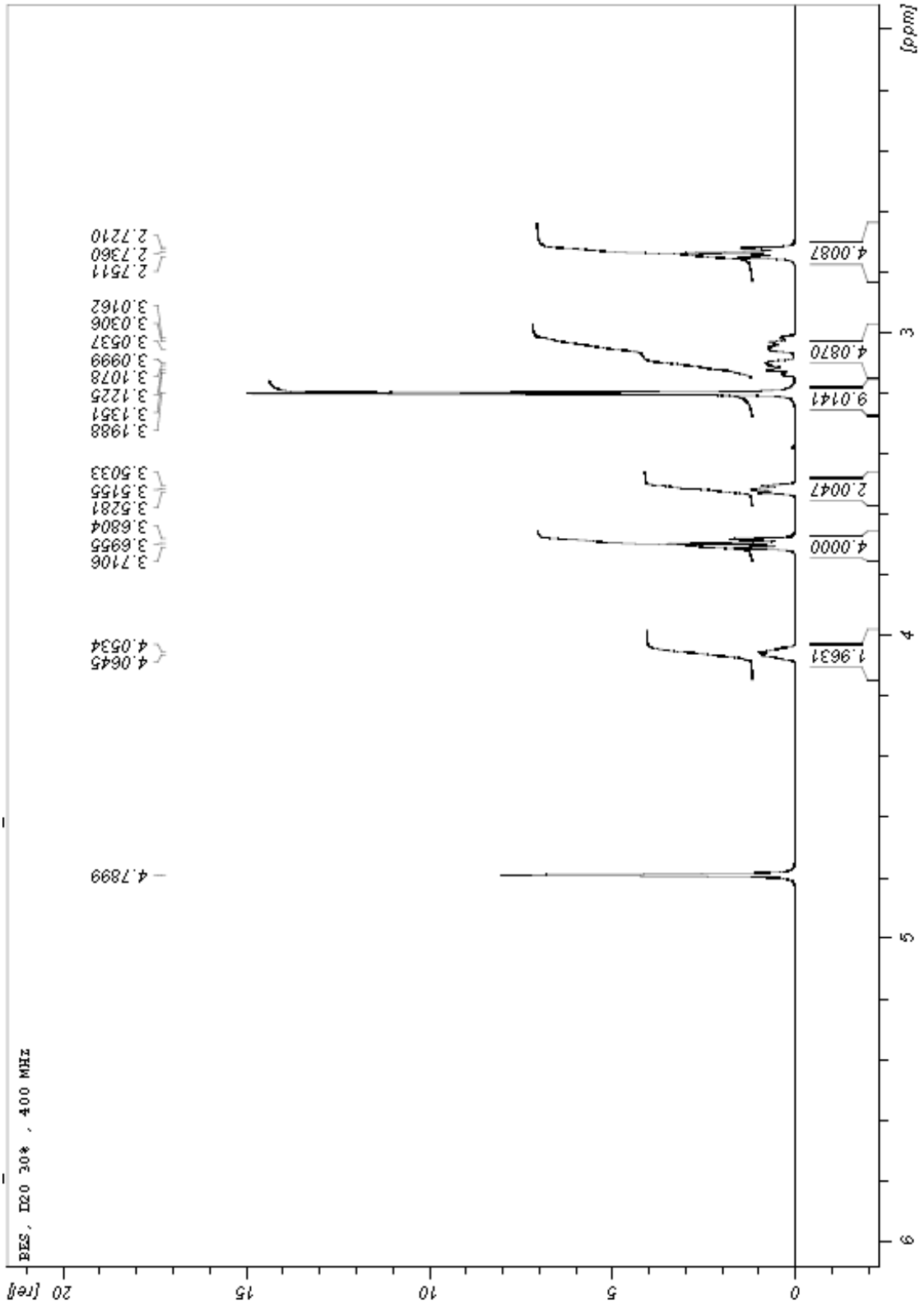

Figure 11 – ^{1}H NMR spectrum of 30% hydrated choline 2-[bis(2-hydroxyethyl)amino]ethanesulfonate **[Ch][BES]** in D_2O at room temperature.

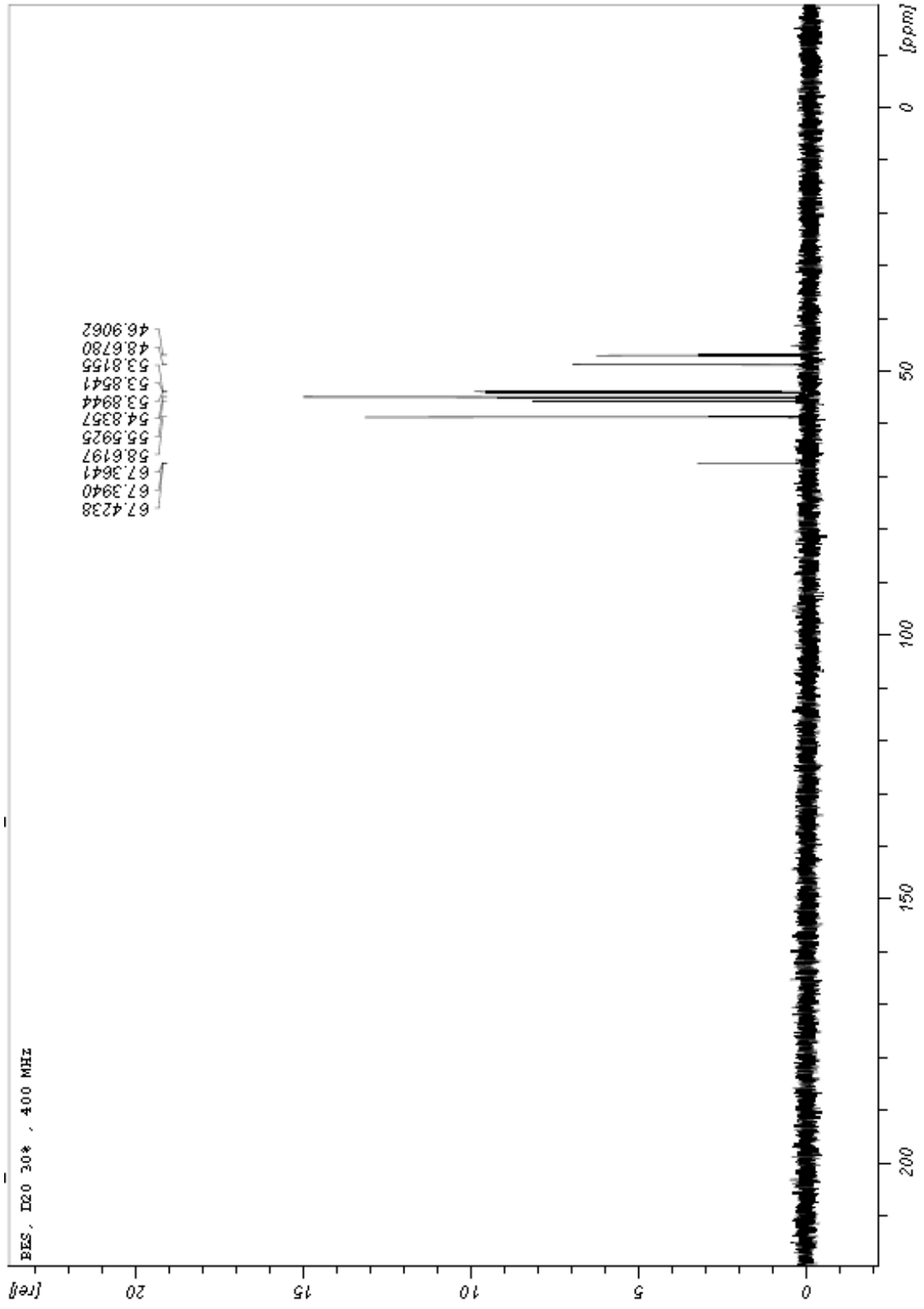

Figure 12 – ^{13}C NMR spectrum of 30% hydrated choline 2-[bis(2-hydroxyethyl)amino]ethanesulfonate **[Ch][BES]** in D_2O at room temperature.

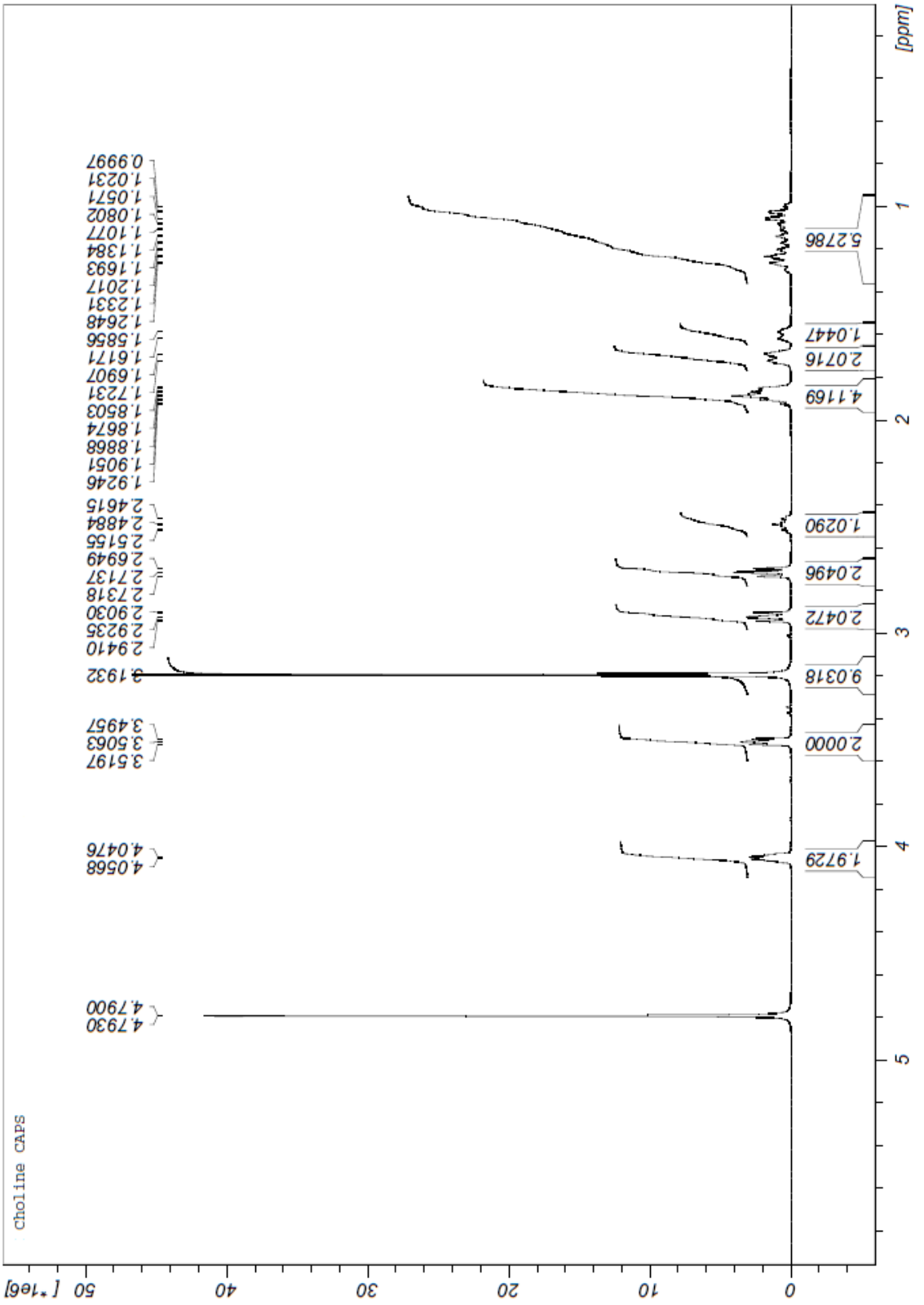

Figure 13 – ^{1}H NMR spectrum of choline 3-(cyclohexylamino)propanesulfonate **[Ch][CAPS]** in D_2O at room temperature.

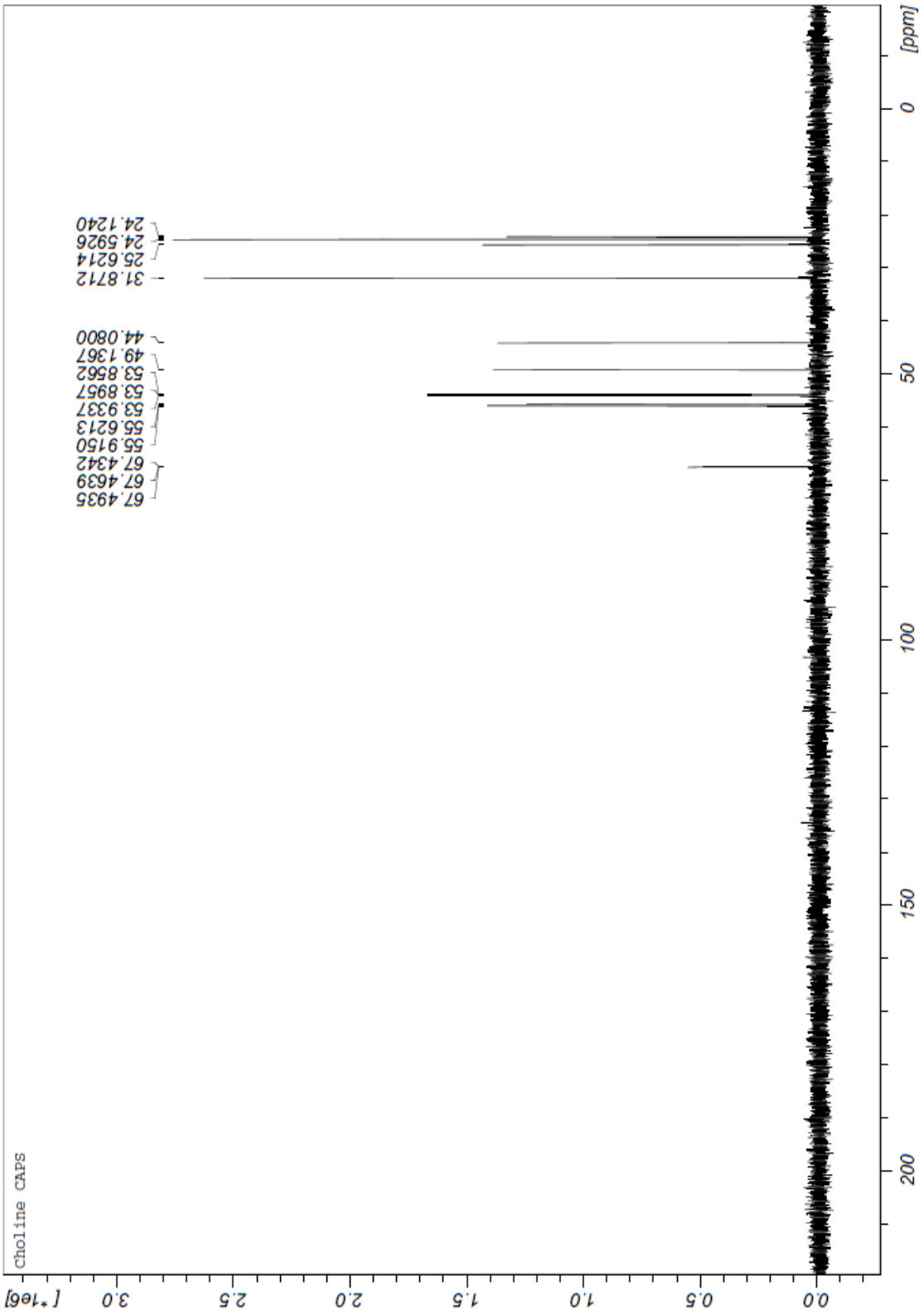

Figure 14 – ^{13}C NMR spectrum of choline 3-(cyclohexylamino)propanesulfonate **[Ch][CAPS]** in D_2O at room temperature.

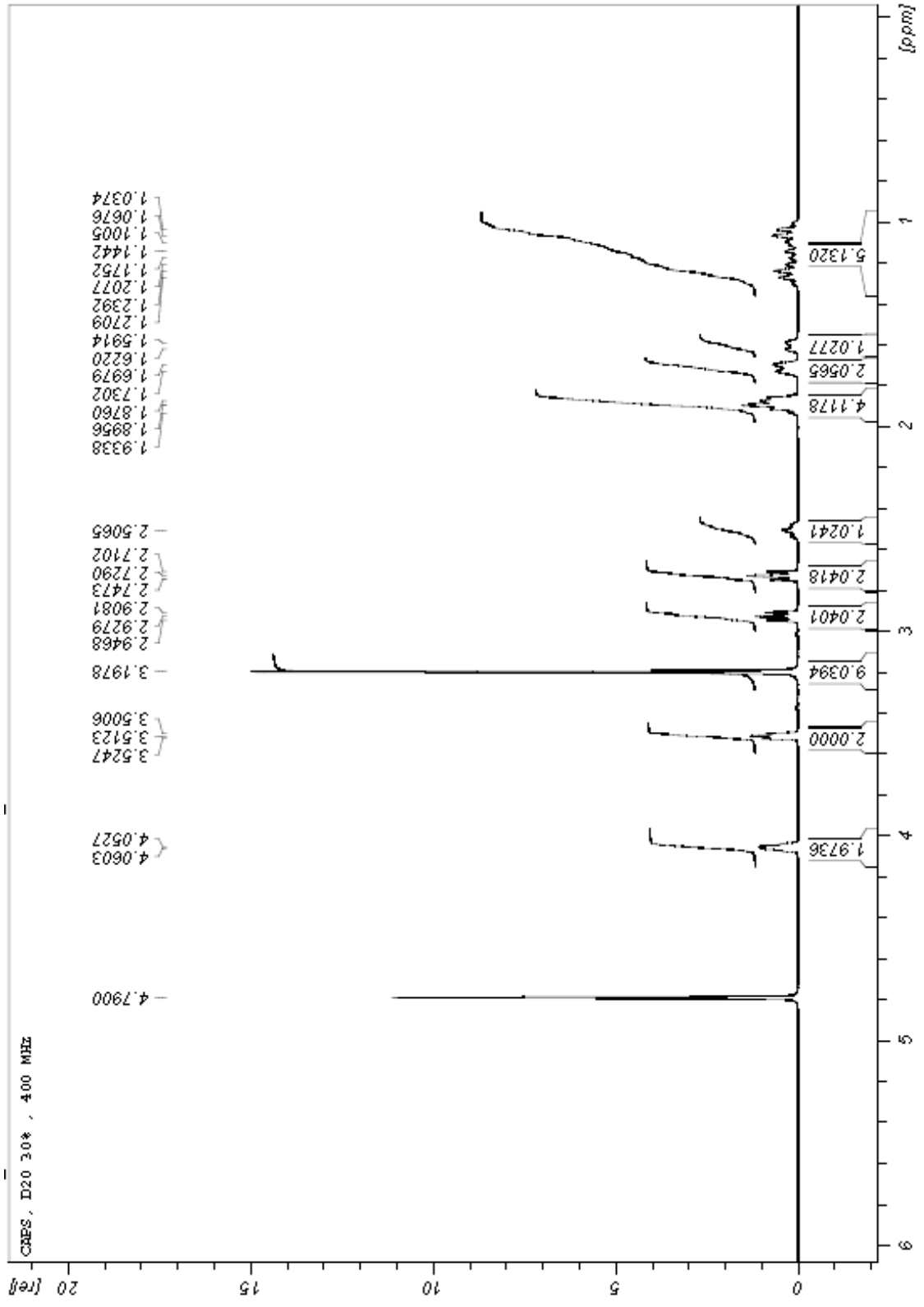

Figure 15 – ^{1}H NMR spectrum of 30% hydrated choline 3-(cyclohexylamino)propanesulfonate **[Ch][CAPS]** in D_2O at room temperature.

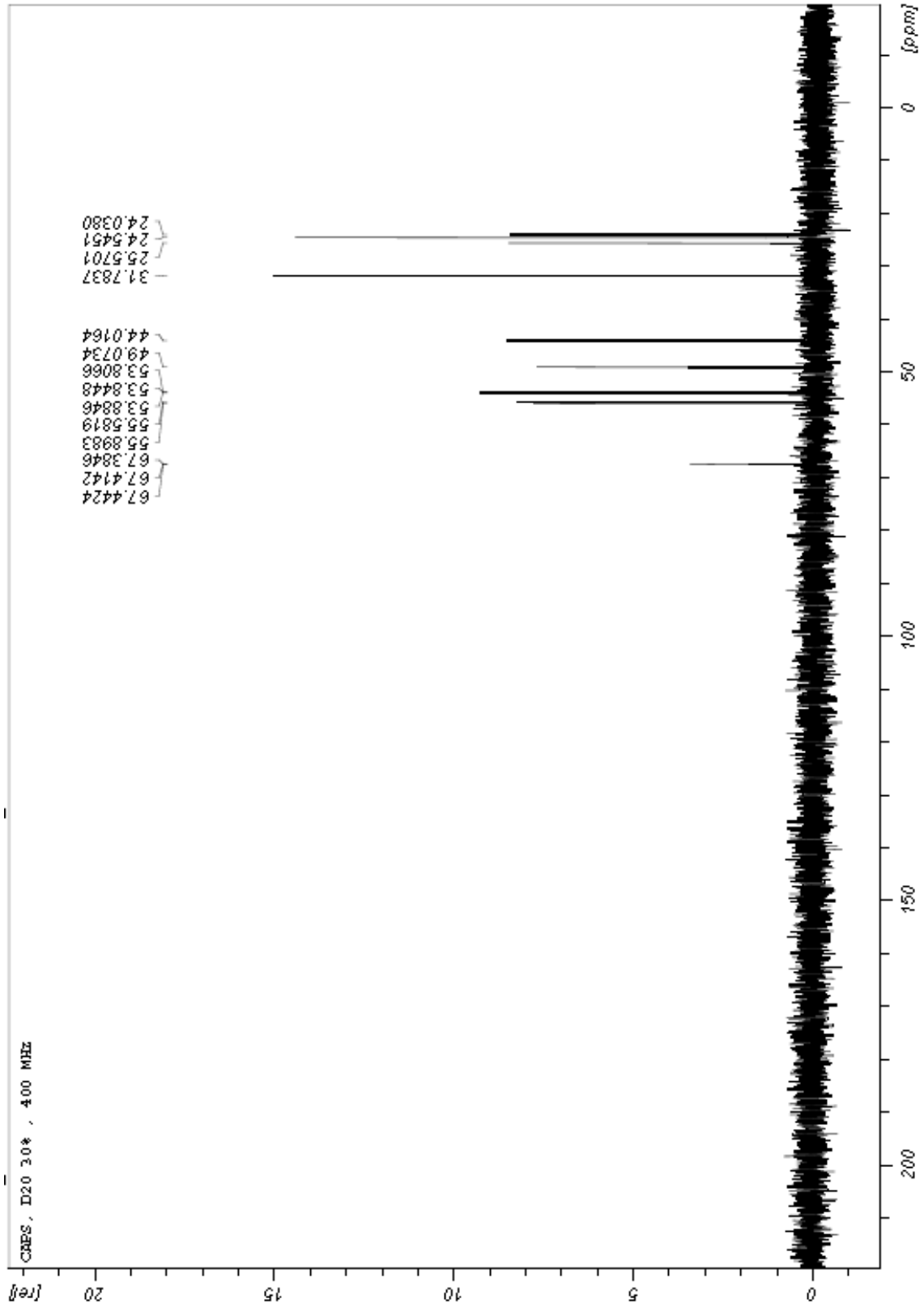

Figure 16 – ^{13}C NMR spectrum of 30% hydrated choline 3-(cyclohexylamino)propanesulfonate **[Ch][CAPS]** in D_2O at room temperature.

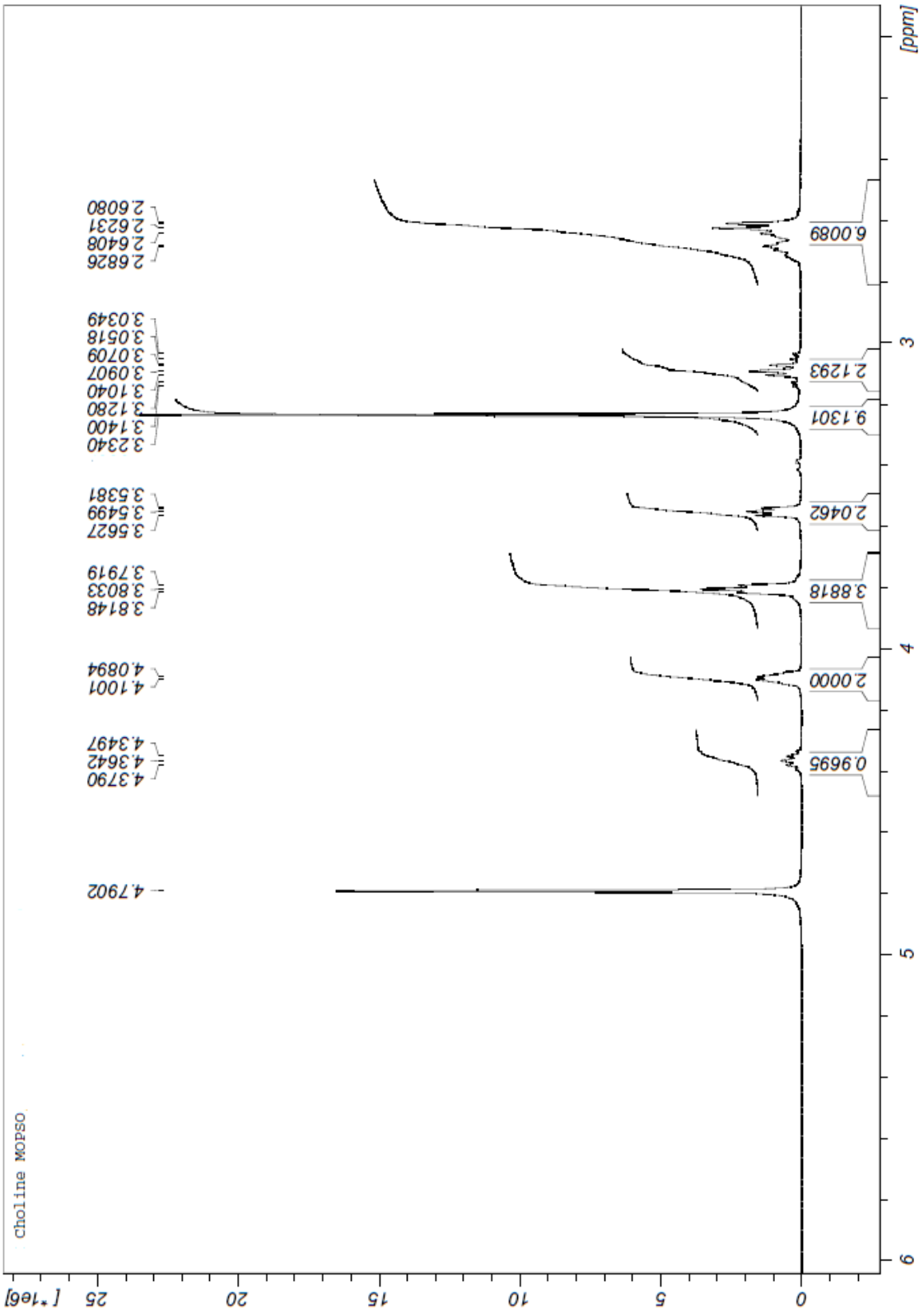

Figure 17 – ^{1}H NMR spectrum of choline 3-(N-morpholino)-2-hydroxypropanesulfonate **[Ch][MOPSO]** in D_2O at room temperature.

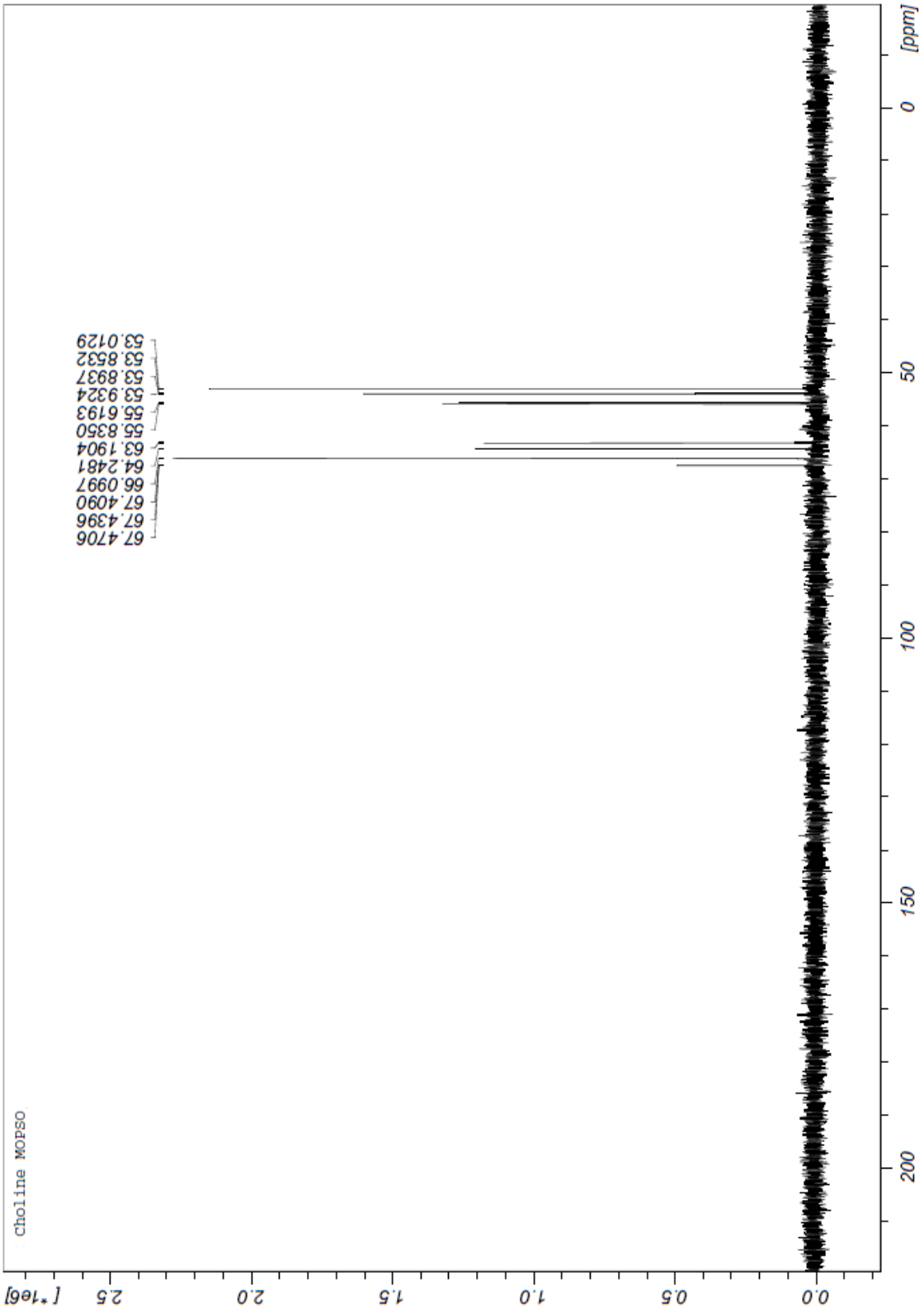

Figure 18– ^{13}C NMR spectrum of choline 3-(N-morpholino)-2-hydroxypropanesulfonate **[Ch][MOPSO]** in D_2O at room temperature.

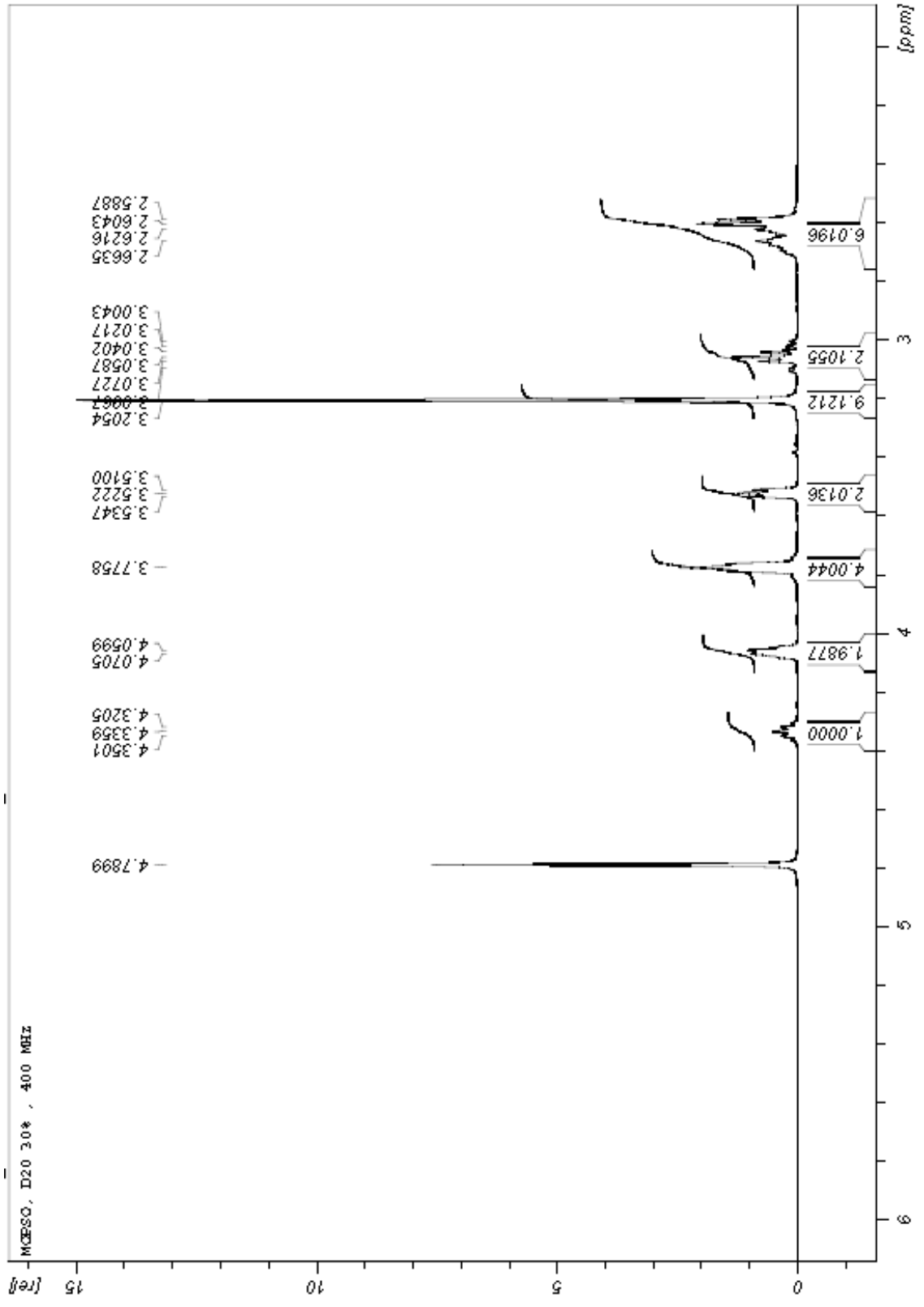

Figure 19 – ^{1}H NMR spectrum of 30% hydrated choline 3-(N-morpholino)-2-hydroxypropanesulfonate **[Ch][MOPSO]** in D_2O at room temperature.

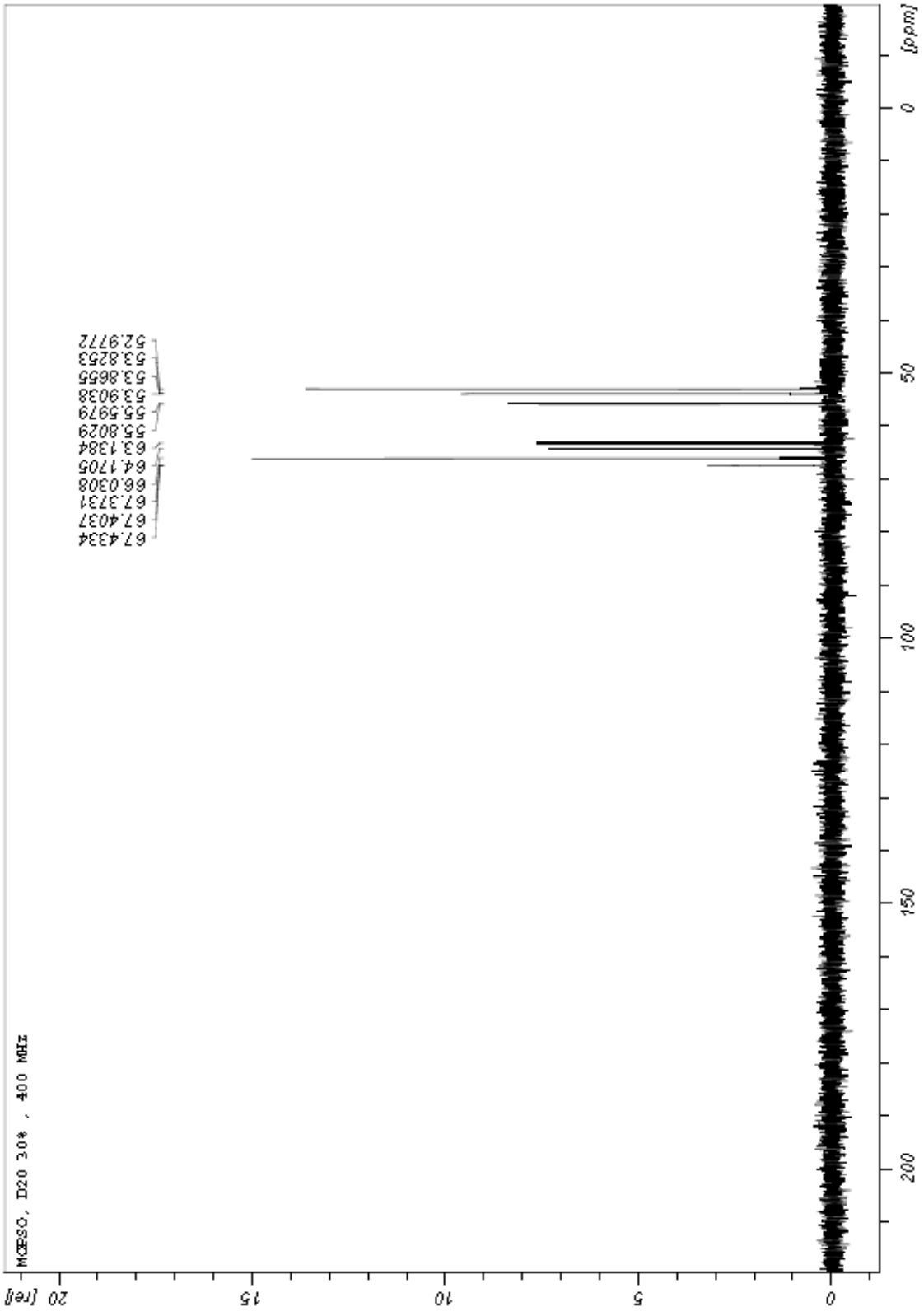

Figure 20– ^{13}C NMR spectrum of 30% hydrated choline 3-(N-morpholino)-2-hydroxypropanesulfonate **[Ch][MOPSO]** in D_2O at room temperature.

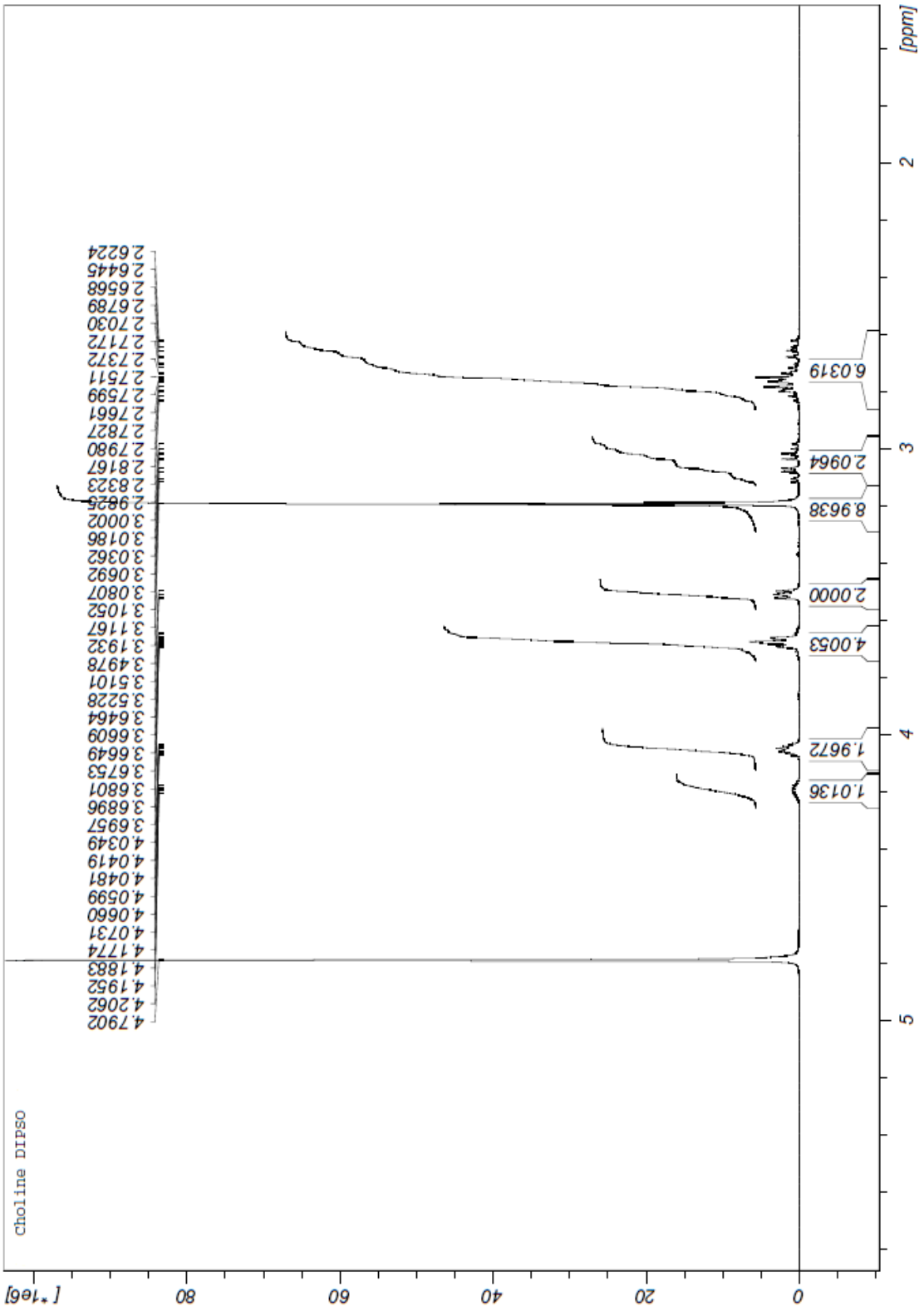

Figure 21 – ^{1}H NMR spectrum of choline 3-[bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonate **[Ch][DIPSO]** in D_2O at room temperature.

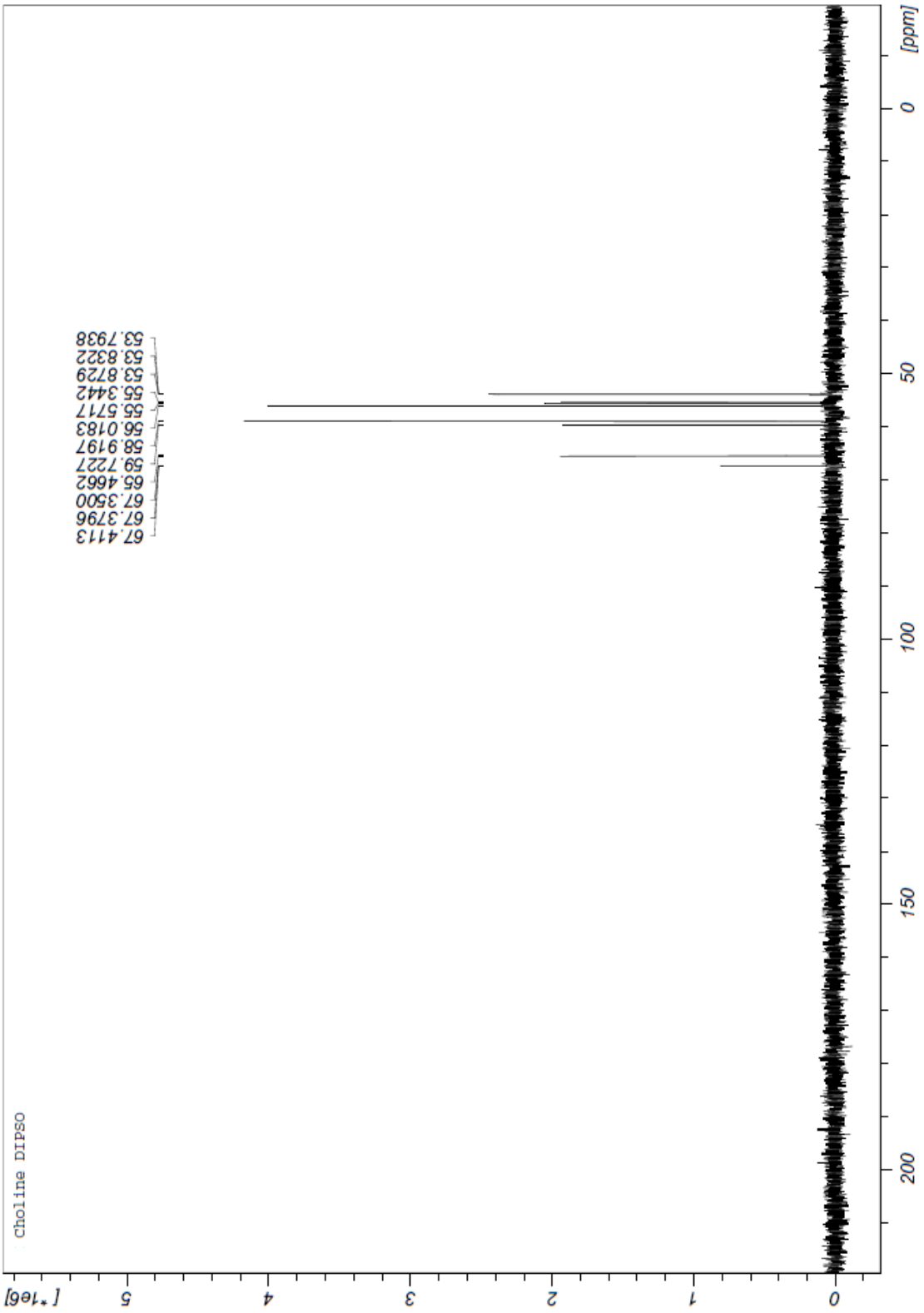

Figure 22 – ^{13}C NMR spectrum of choline 3-[bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonate **[Ch][DIPSO]** in D_2O at room temperature.

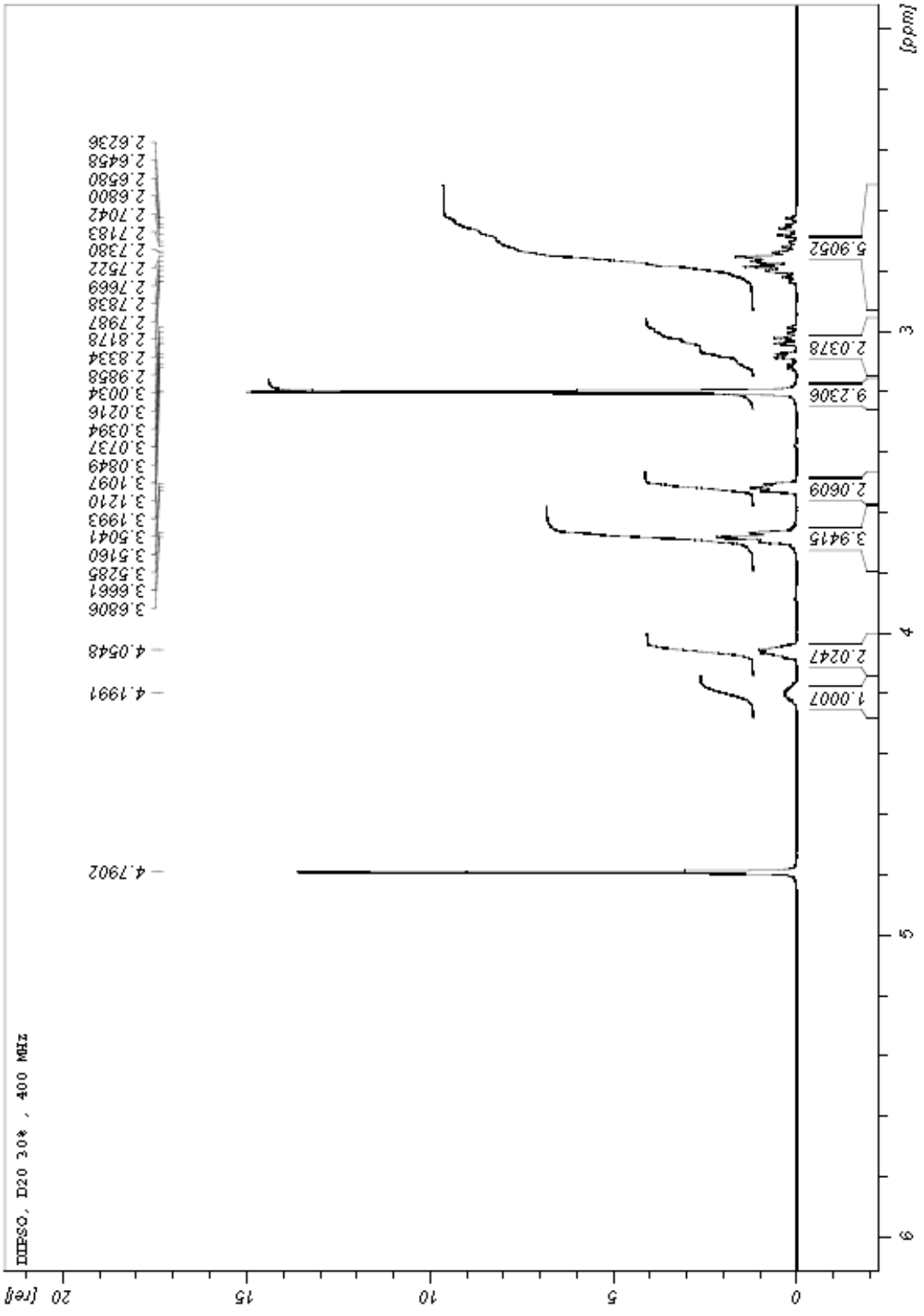

Figure 23 – ^{1}H NMR spectrum of 30% hydrated choline 3-[bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonate **[Ch][DIPSO]** in D_2O at room temperature.

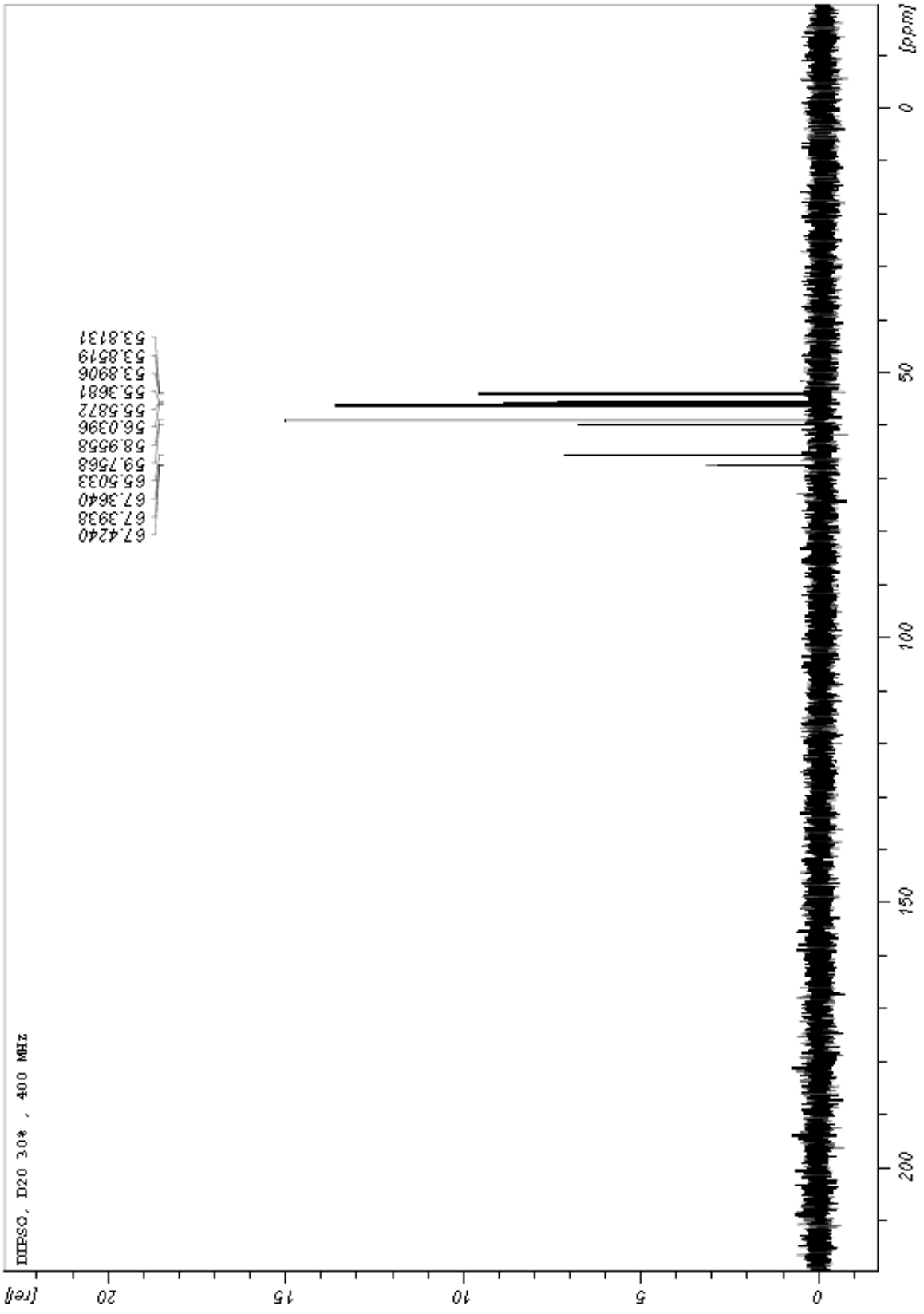

Figure 24 – ^{13}C NMR spectrum of 30% hydrated choline 3-[bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonate **[Ch][DIPSO]** in D_2O at room temperature.

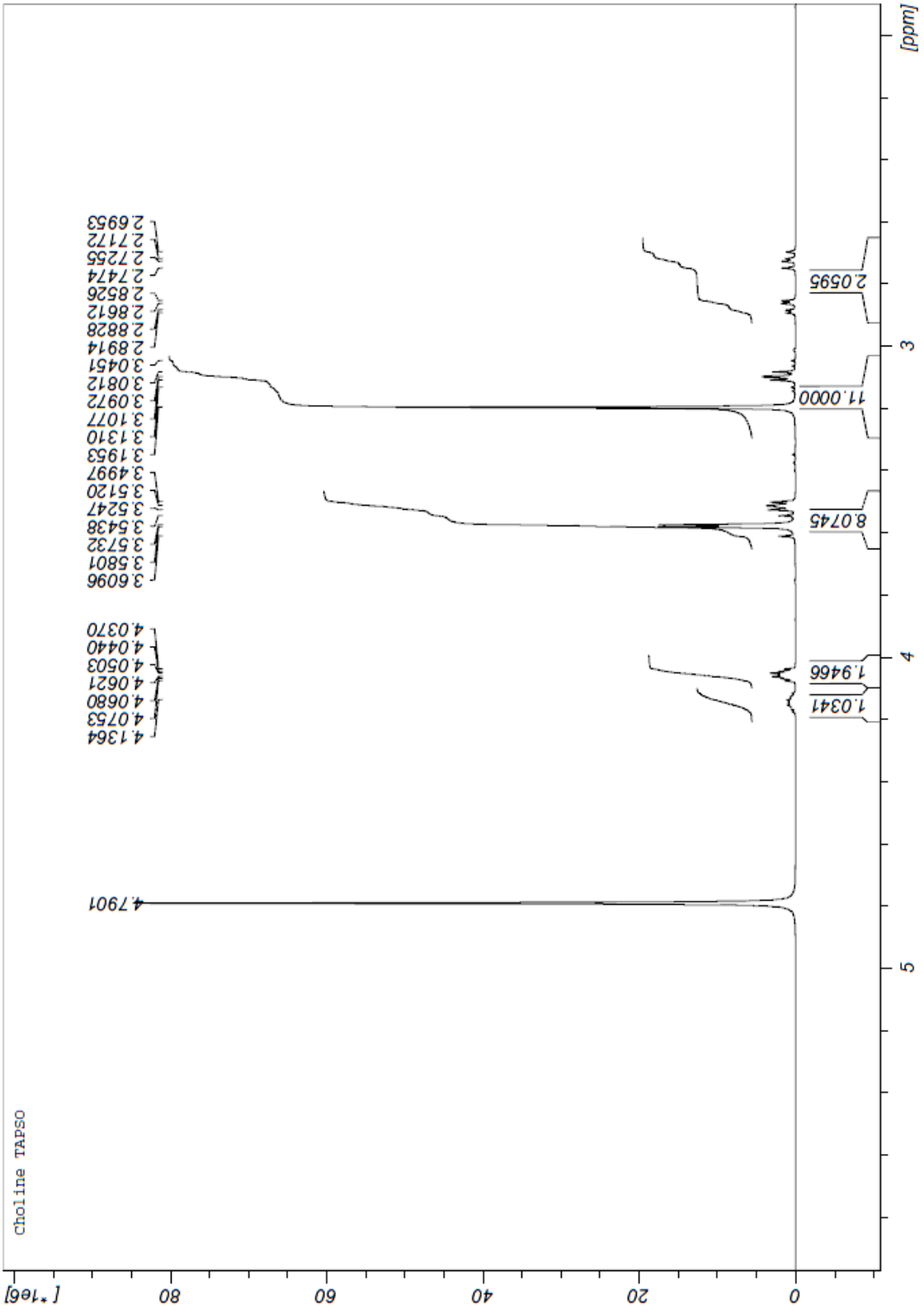

Figure 25– ^{1}H NMR spectrum of 3-[tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate **[Ch][TAPSO]** in D_2O at room temperature.

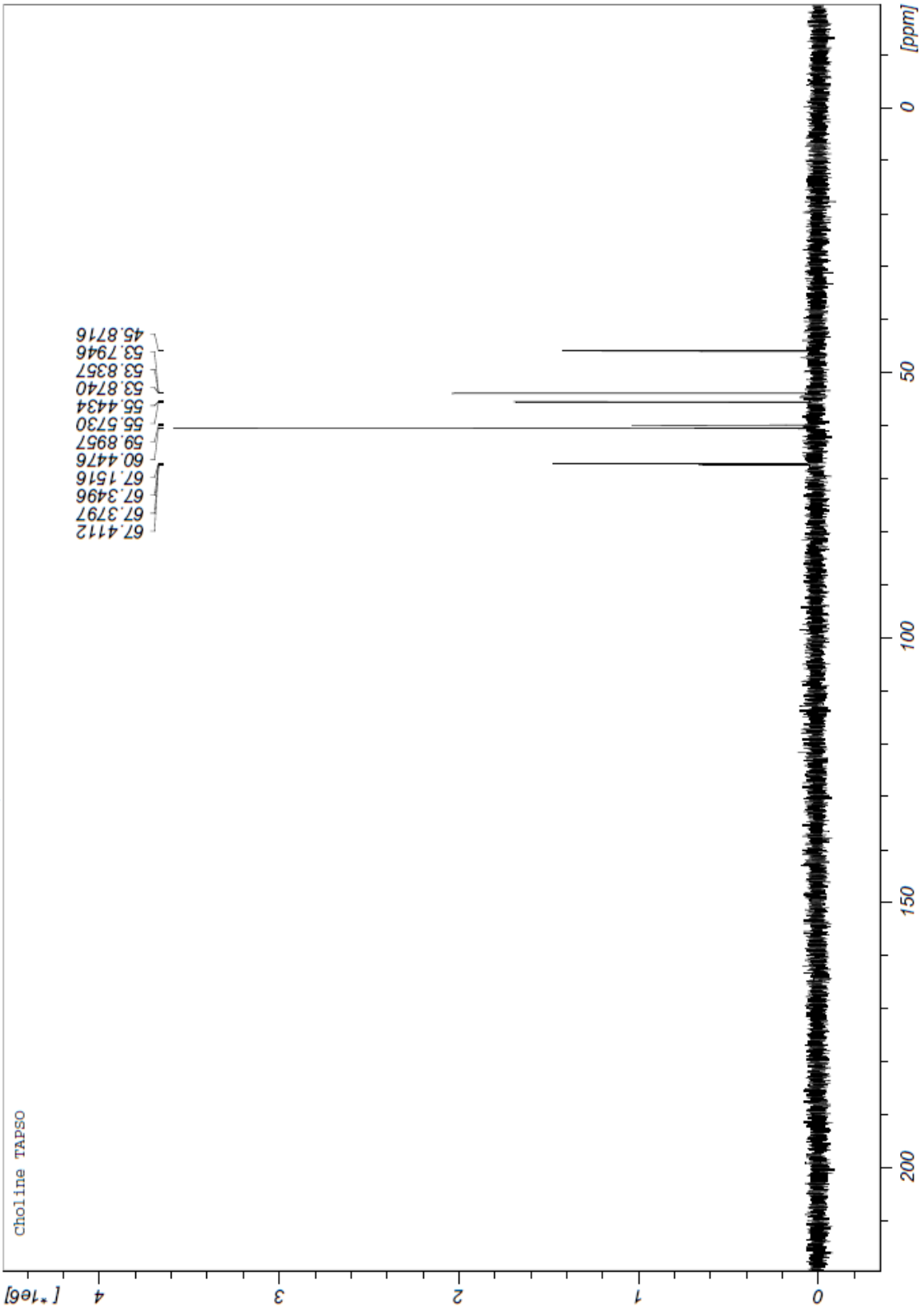

Figure 26– ^{13}C NMR spectrum of 3-[tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate **[Ch][TAPSO]** in D_2O at room temperature.

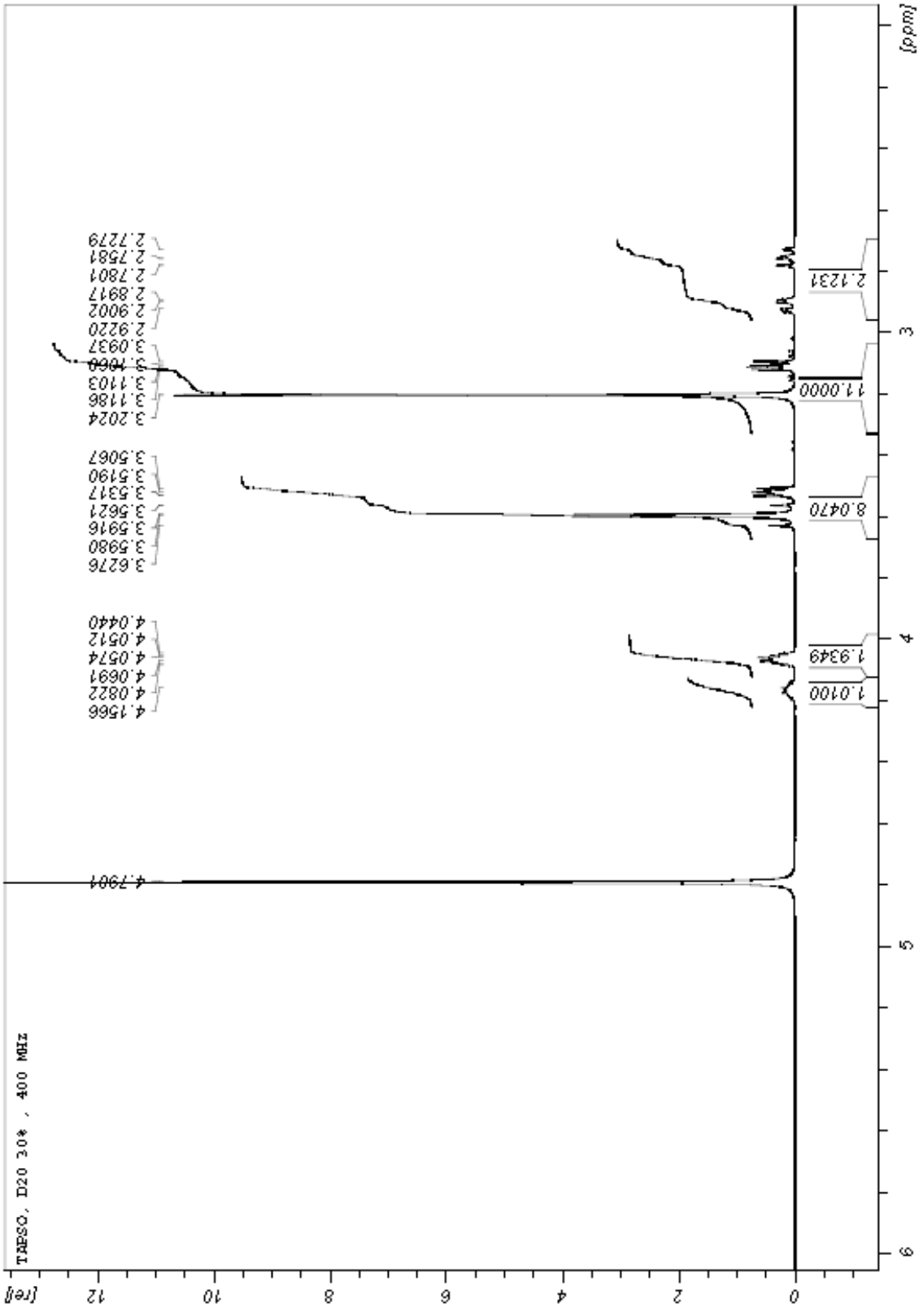

Figure 27– ^{1}H NMR spectrum of 30% hydrated choline 3-[tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate **[Ch][TAPSO]** in D_2O at room temperature.

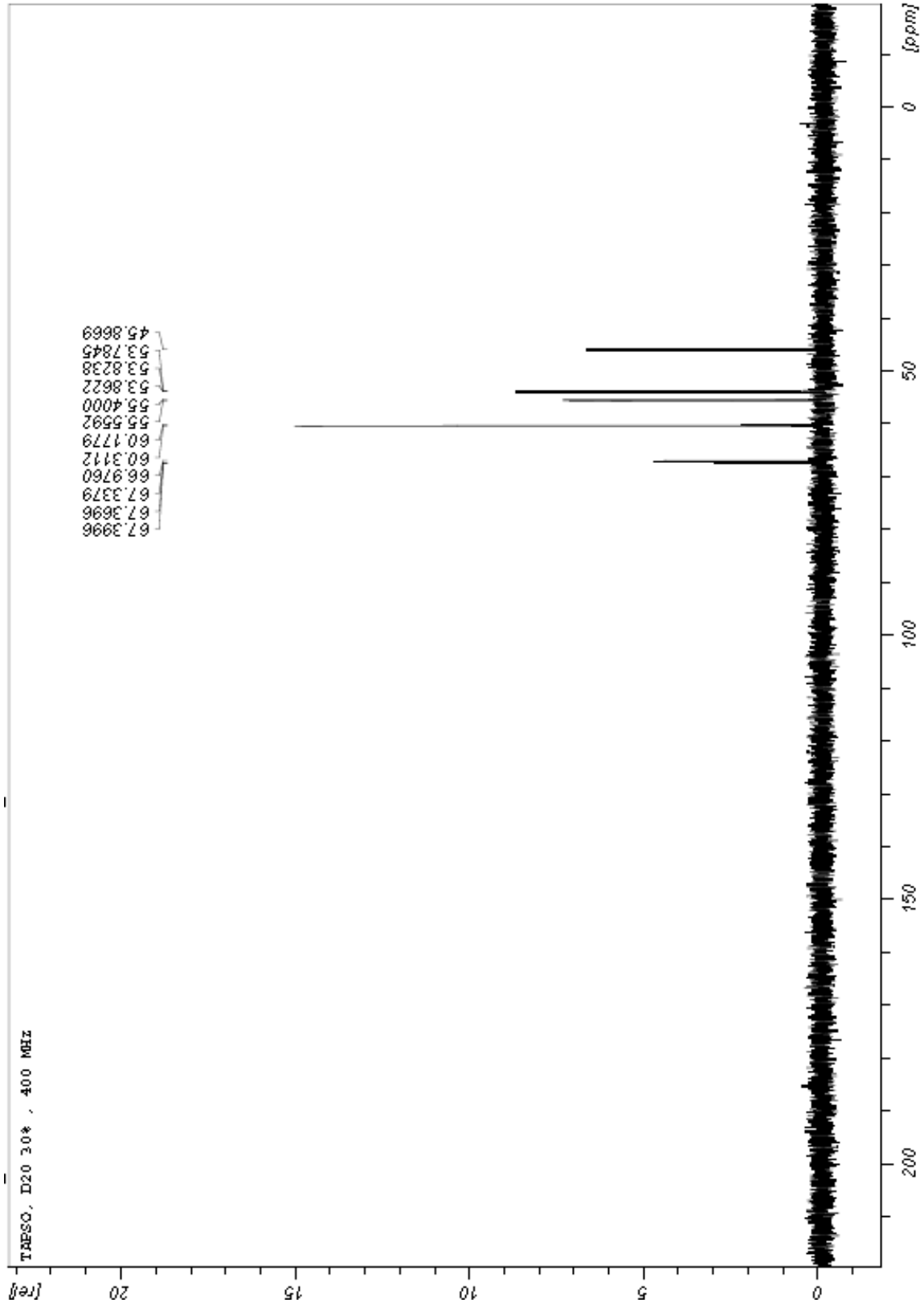

Figure 28– ^{13}C NMR spectrum of 30% hydrated 3-[tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonate **[Ch][TAPSO]** in D_2O at room temperature.

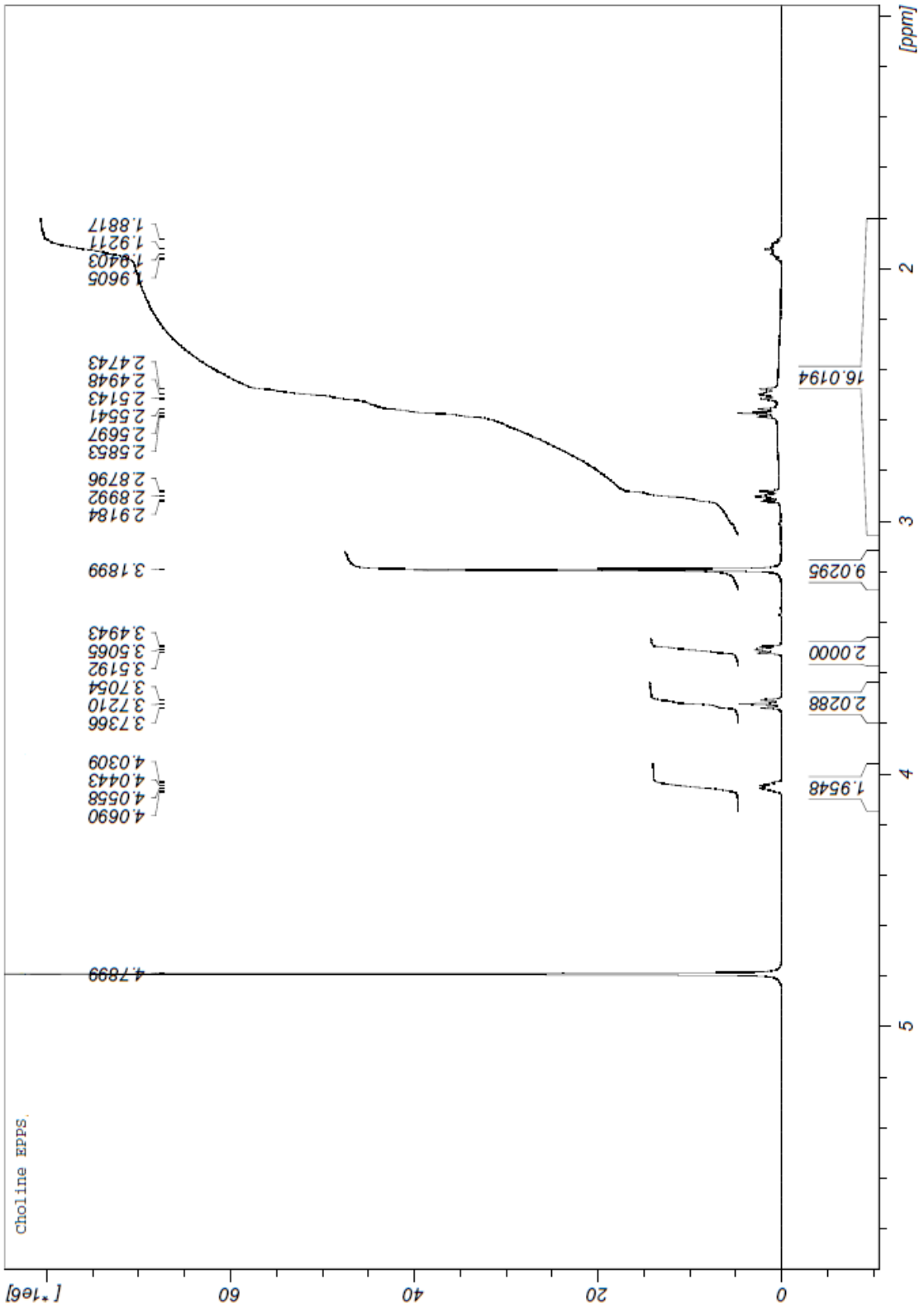

Figure 29– ^{1}H NMR spectrum of choline 3-[4-(2-hydroxyethyl)-1-piperazine]propanesulfonate **[Ch][EPPS]** in D_2O at room temperature.

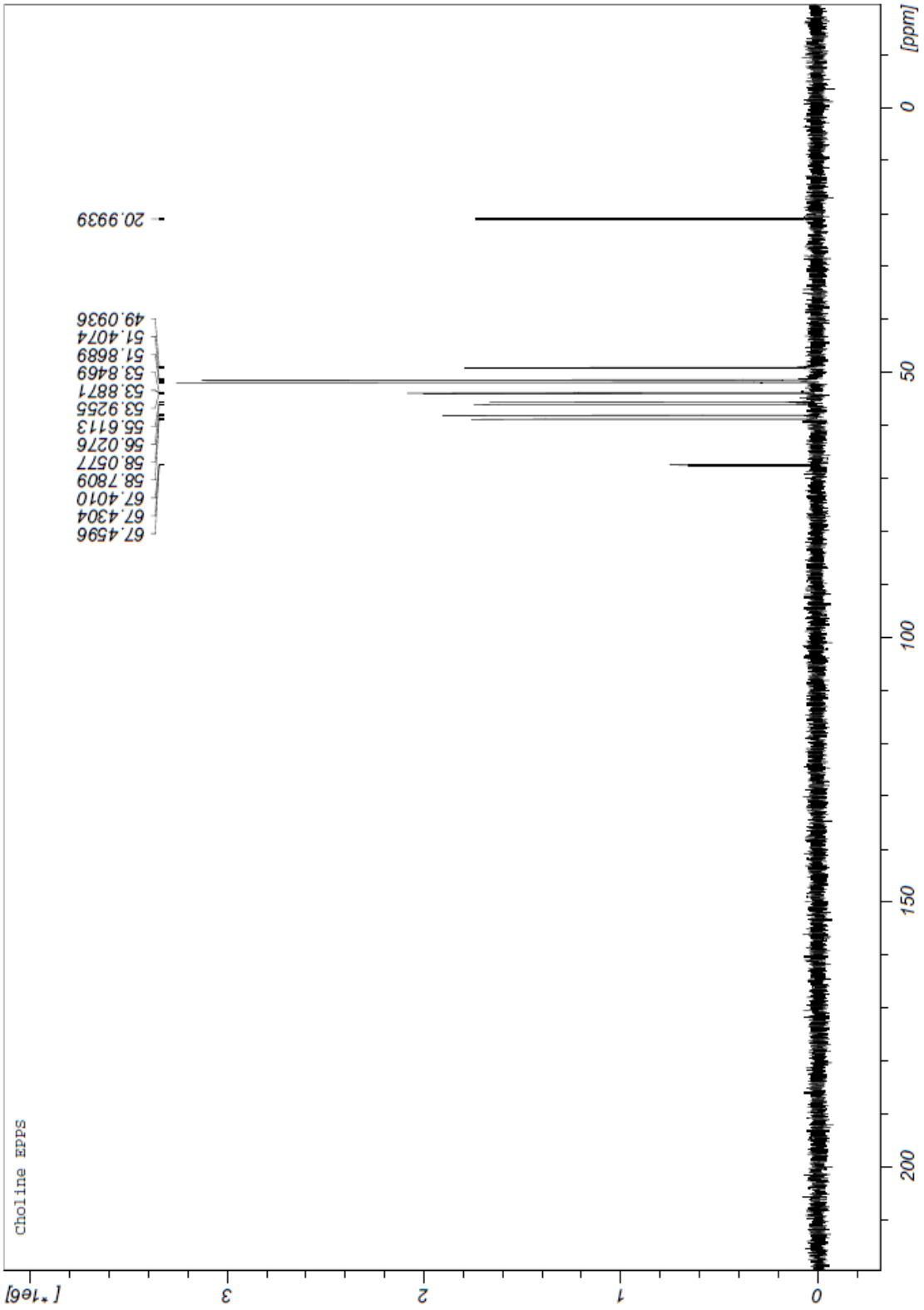

Figure 30– ^{13}C NMR spectrum of choline 3-[4-(2-hydroxyethyl)-1-piperazine]propanesulfonate **[Ch][EPPS]** in D_2O at room temperature.

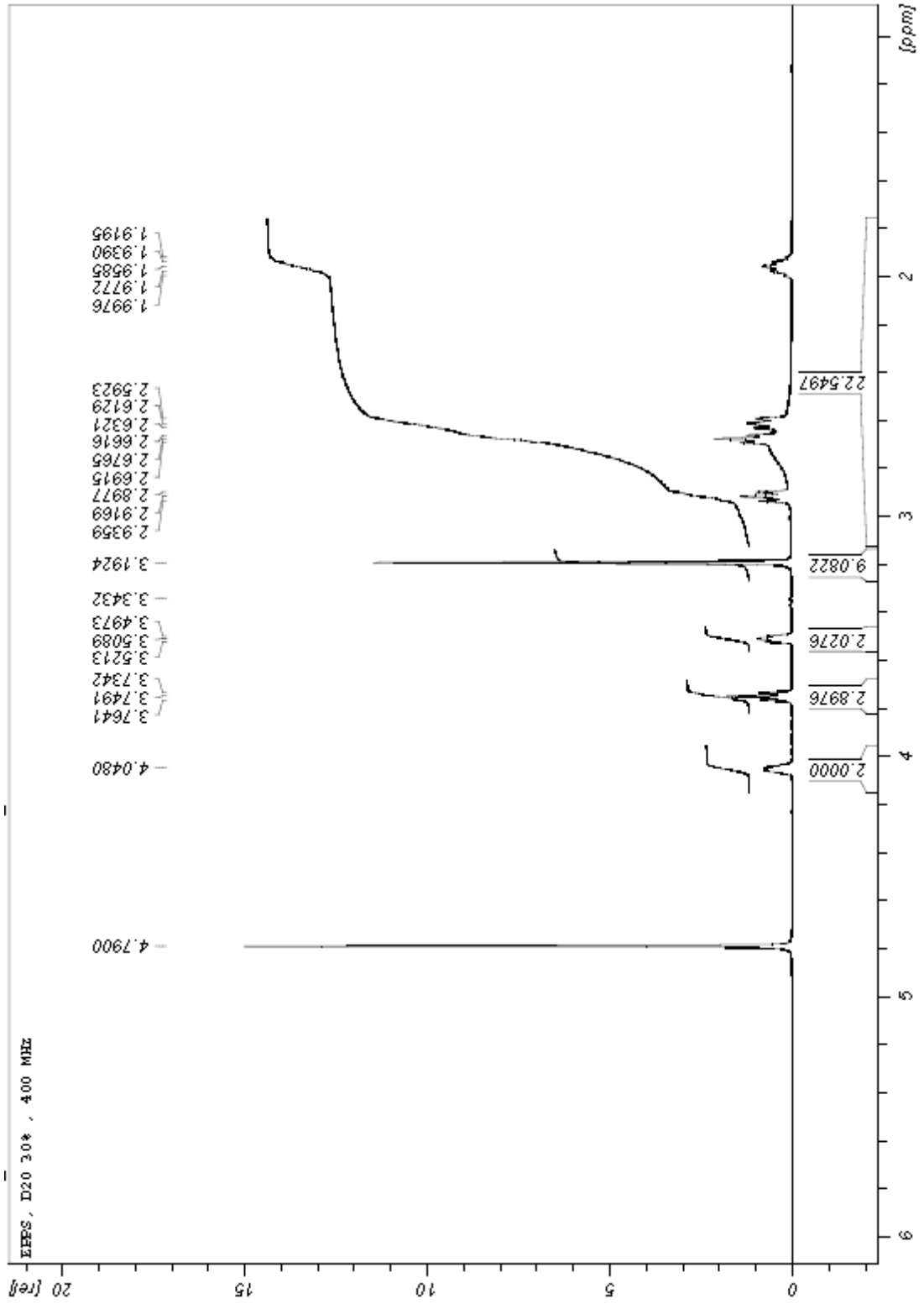

Figure 31– ^{1}H NMR spectrum of 30% hydrated choline 3-[4-(2-hydroxyethyl)-1-piperazine]propanesulfonate **[Ch][EPPS]** in D_2O at room temperature.

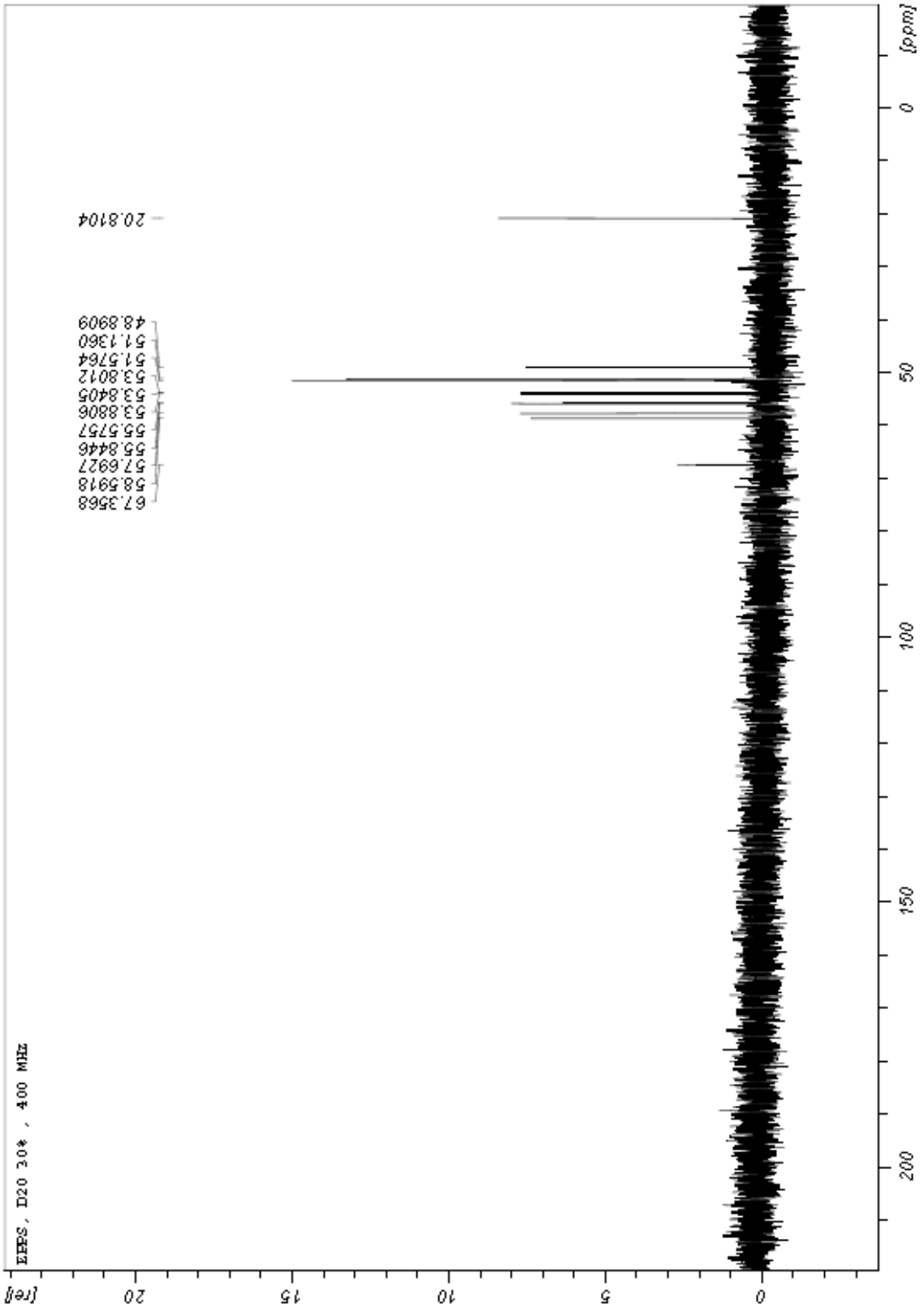

Figure 32– ^{13}C NMR spectrum of 30% hydrated choline 3-[4-(2-hydroxyethyl)-1-piperazine]propanesulfonate **[Ch][EPPS]** in D_2O at room temperature.

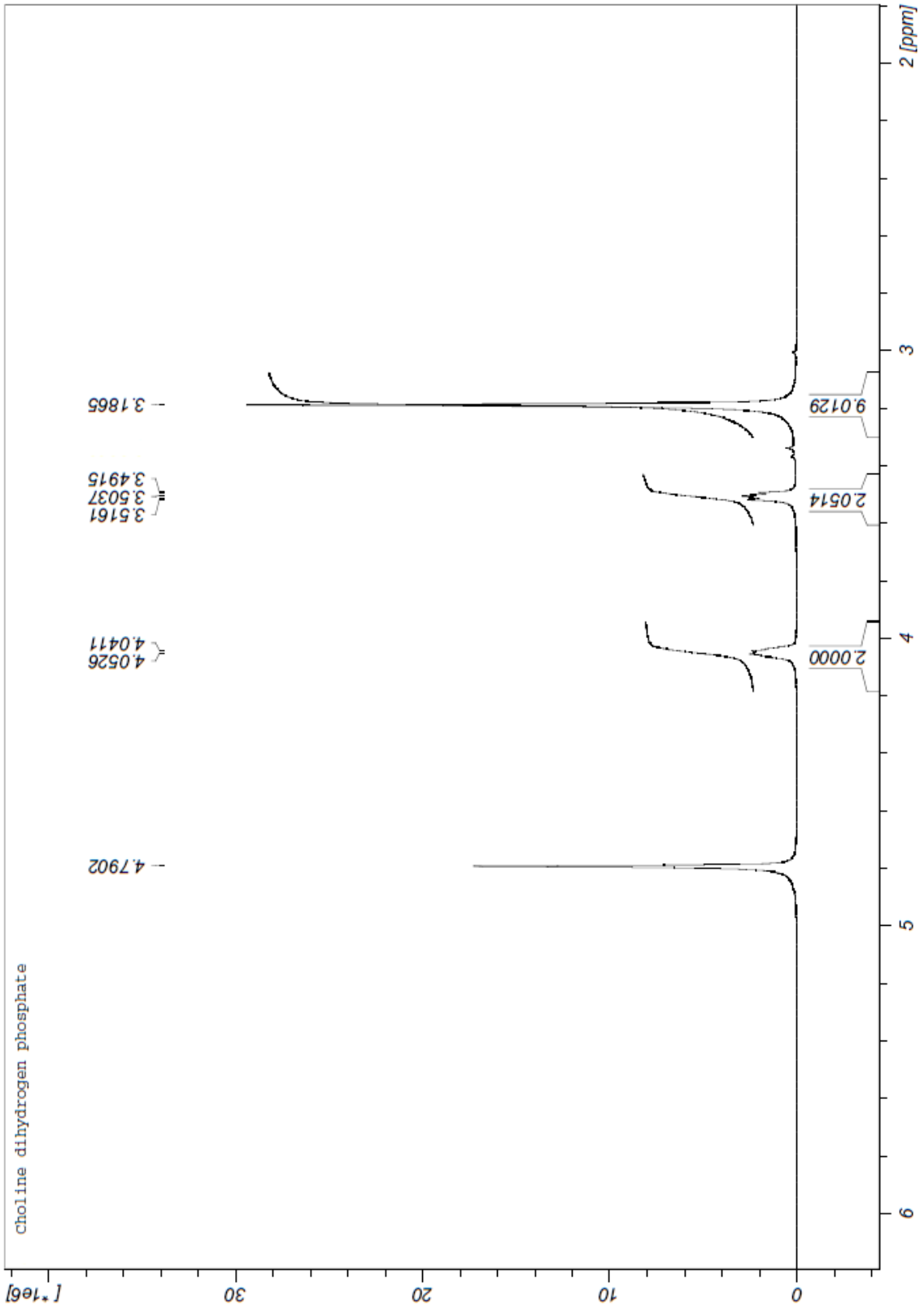

Figure 33– ^{1}H NMR spectrum of choline dihydrogen phosphate **[Ch][DHP]** in D_2O at room temperature.

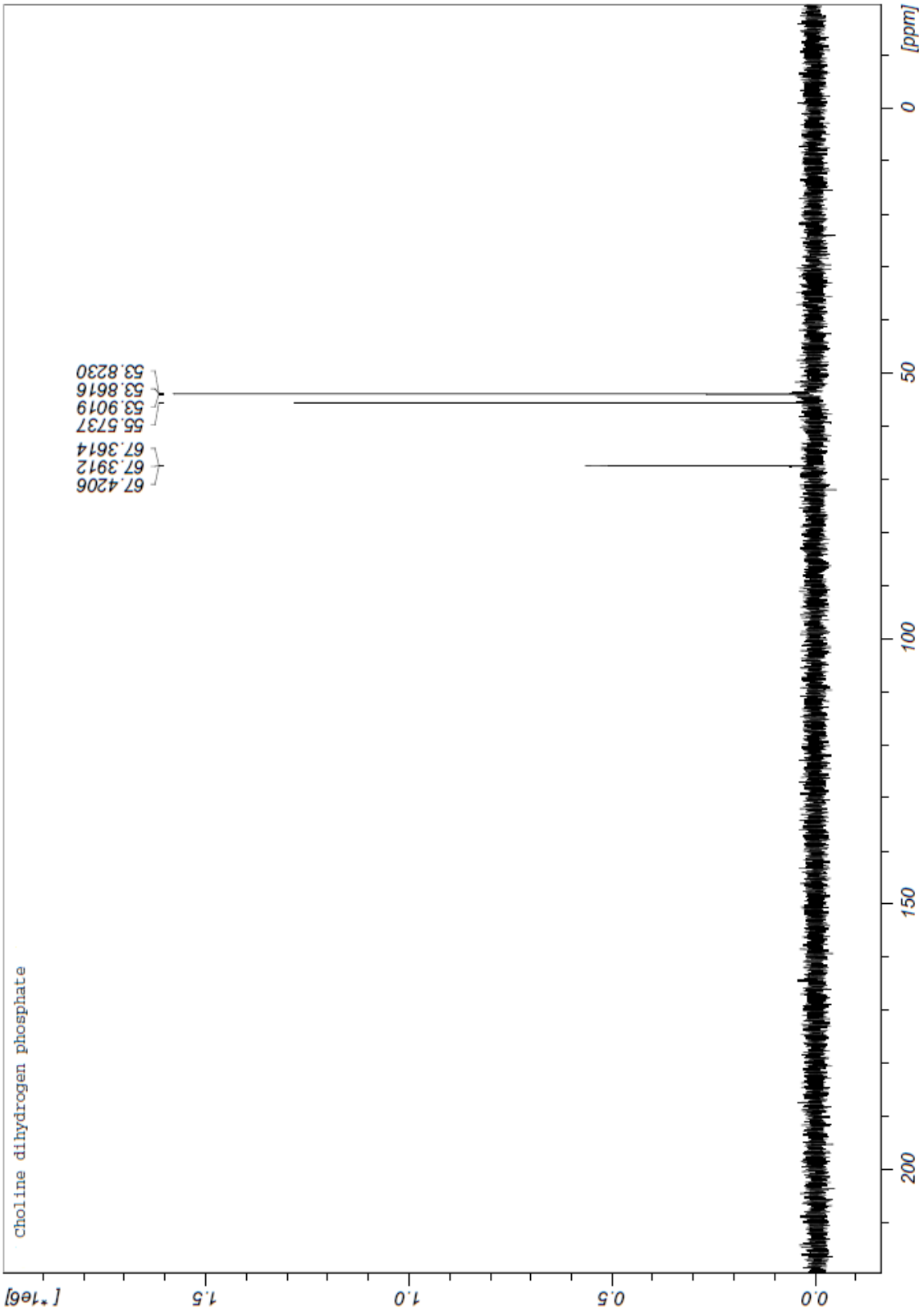

Figure 34 – ^{13}C NMR spectrum of choline dihydrogen phosphate **[Ch][DHP]** in D_2O at room temperature.

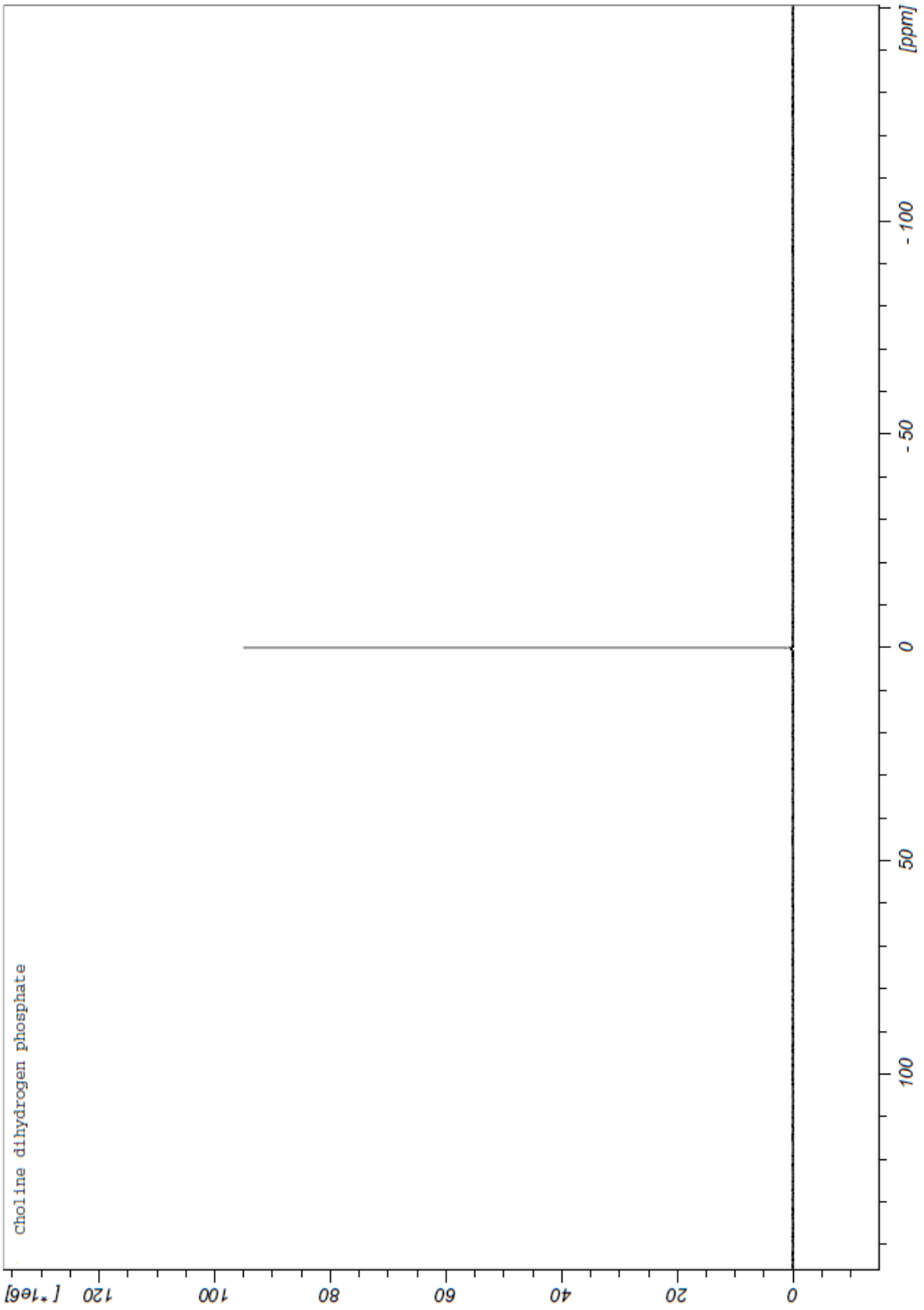

Figure 35– ^{31}P NMR spectrum of choline dihydrogen phosphate **[Ch][DHP]** in D_2O at room temperature.

1. X. Zhang, *Investigating Quinone Function in Bacterial Photosynthetic Reaction Centers*, City University of New York, 2006