On the hunt for truly biocompatible ionic liquids for lipase-catalyzed reactions

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Electronic Supplementary Information

Reagents used

The substrate for lipolytic activity determination, *p*-nitrophenyl laurate, the compounds for buffer preparation in lipolytic activity assay, $CaCl_2 \cdot 2H_2O$ and Trizma base, Cholinium chloride (> 98%), the fluorescence probe Sypro Orange and amino acids L-Alanine (>99.5%), L-Glycine (>99%) and L-Lysine (98%) were purchased from Sigma Aldrich. The cholinium-based ionic liquids used in this work were synthesized on the basis of the route proposed by Tao et al (2013),[S1] and optimized as follows: ChOH aqueous solution was obtained after passing ChCl through an Amberlite IRN-78 anion exchange resin packed column. ChOH was then neutralized with an equimolar aqueous solution of amino acid by stirring at room temperature for 12 h. After neutralization, water was evaporated under vacuum at 323.15 K. The excess of amino acid was precipitated by adding methanol. The structures of ChAA ILs were confirmed by ^{1}H NMR, ^{13}C NMR and FT-IR spectra, and the main data are shown below. The purity of the synthesized ionic liquids was always higher than 95%, as checked by NMR spectra. Coulometric Karl-Fischer titrations yielded final water contents below 1500 ppm. The concentration of Cl^- in each ChAA was measured with a chloride specific ion electrode, and the related impurity was lower than 0.05 wt %.The pure ionic liquids were stored in amber glass vials with screw caps provided with a septum to ensure a secure seal and to prevent humidity. *Thermomyces lanuginosus* lipase was also purchased from Sigma Aldrich and kept at 4 ºC until use.

a) Data for ChGly
^{1}H NMR δ/ppm (400 MHz, D_2O): 3.18 (9H, s, $(\mathbf{CH_3})_3N$), 3.19 (2H, s, $\mathbf{CH_2}NH_2$), 3.50 (2H, m, $\mathbf{CH_2}OH$), 4.03 (2H, m, $\mathbf{CH_2}CH_2N$).
^{13}C NMR δ/ppm (100 MHz, D_2O): 44.75 (CH_2NH_2), 3 x 54.2 ((CH_3)$_3$N), 55.93 (CH_2OH), 67.78 (CH_2CH_2N), 180.94(C=O).
IR: ν = 3347, 2943, 1568, 1479, 1398, 1085, 954 cm^{-1}.
Tg: -80.44 ºC

b) Data for ChAla
^{1}H NMR δ/ppm (400 MHz, D_2O): 1.22 (3H, d, $\mathbf{CH_3}CH$), 3.20 (9H, s, $(\mathbf{CH_3})_3N$), 3.35 (1H, q, $\mathbf{CH}NH_2$), 3.52 (2H, m, $\mathbf{CH_2}OH$), 4.05 (2H, m, $CH_2\mathbf{CH_2}N$).
^{13}C NMR δ/ppm (100 MHz, D_2O): 19.95 (CH_3), 51.31 ($CHNH_2$), 3 x 53.8 ((CH_3)$_3$N), 55.54 (CH_2OH), 67.39 (CH_2CH_2N), 183.63 (C=O).
IR: ν = 3356, 2255, 1563, 1478,1406, 1362, 1135, 1083, 954, 856 cm^{-1}
Tg: -76.48 ºC

c) Data for ChLys
^{1}H NMR δ/ppm (400 MHz, D_2O): 1.36 (2H, m, ***CH***2), 1.56 (2H, m, ***CH***2), 2.79 (2H, t, $\mathbf{CH_2}NH_2$), 3.20 (9H, s, $(\mathbf{CH_3})_3N$), 3 26 (1H, t, $\mathbf{CH}NH_2$), 3.52 (2H, m, $\mathbf{CH_2}OH$), 4.06 (2H, m, $CH_2\mathbf{CH_2}N$).
^{13}C NMR δ/ppm (100 MHz, D_2O): 22.02 ($\mathbf{C}H_2$), 28.38 ($\mathbf{C}H_2$), 33.78 ($\mathbf{C}H_2CHNH_2$), 39.61 ($\mathbf{C}H_2NH_2$), 3 x 53.8 (($\mathbf{C}H_3$)$_3$N), 55.55 ($\mathbf{C}HNH_2$), 57.5 (CH_2OH), 67.36 (CH_2CH_2N), 182.77 (C=O).
IR: v= 2923, 2852, 1569, 1480, 1386, 1089, 954, 865 cm^{-1}.
Tg: -64.55 ºC

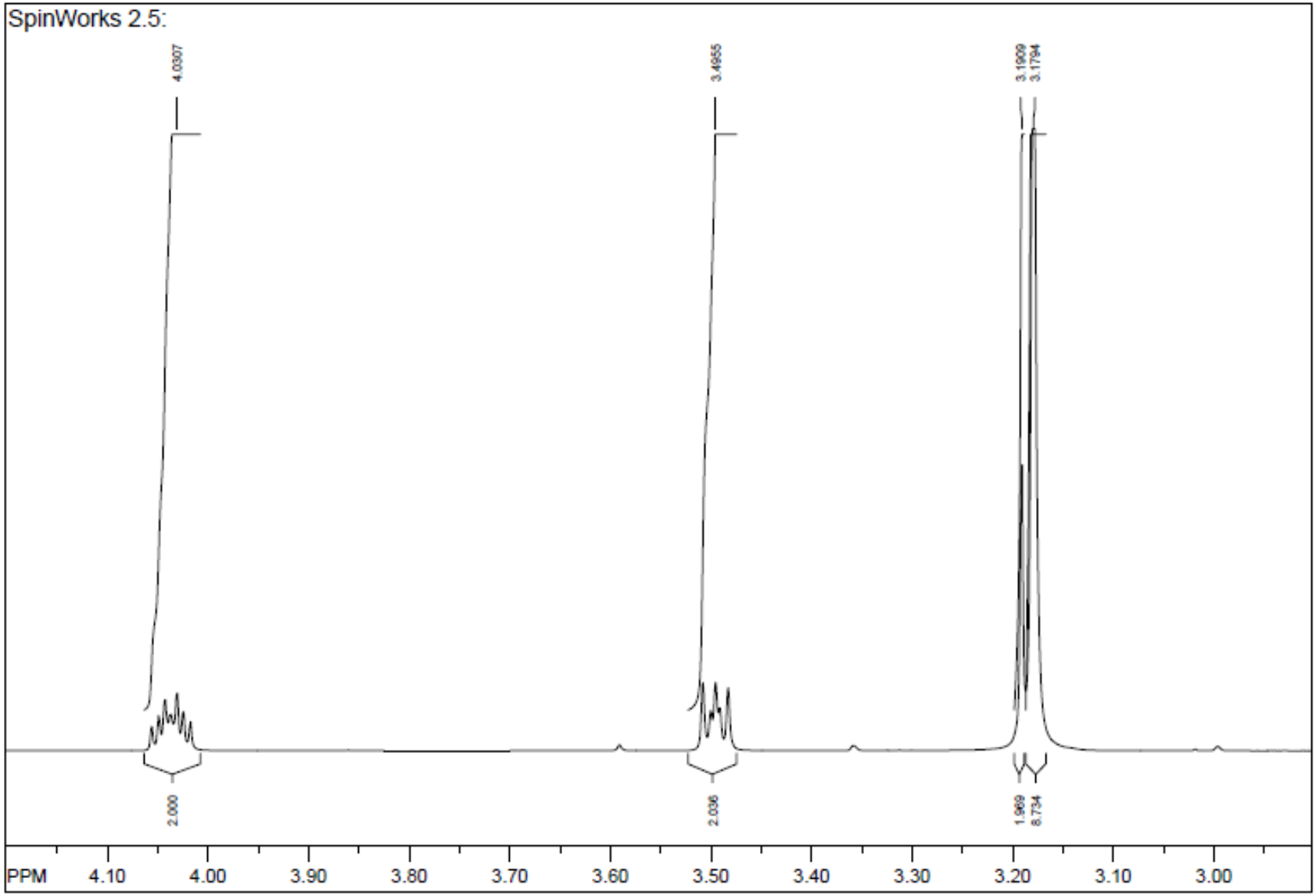

Fig. S1 ^{1}H NMR Spectrum of ChGly

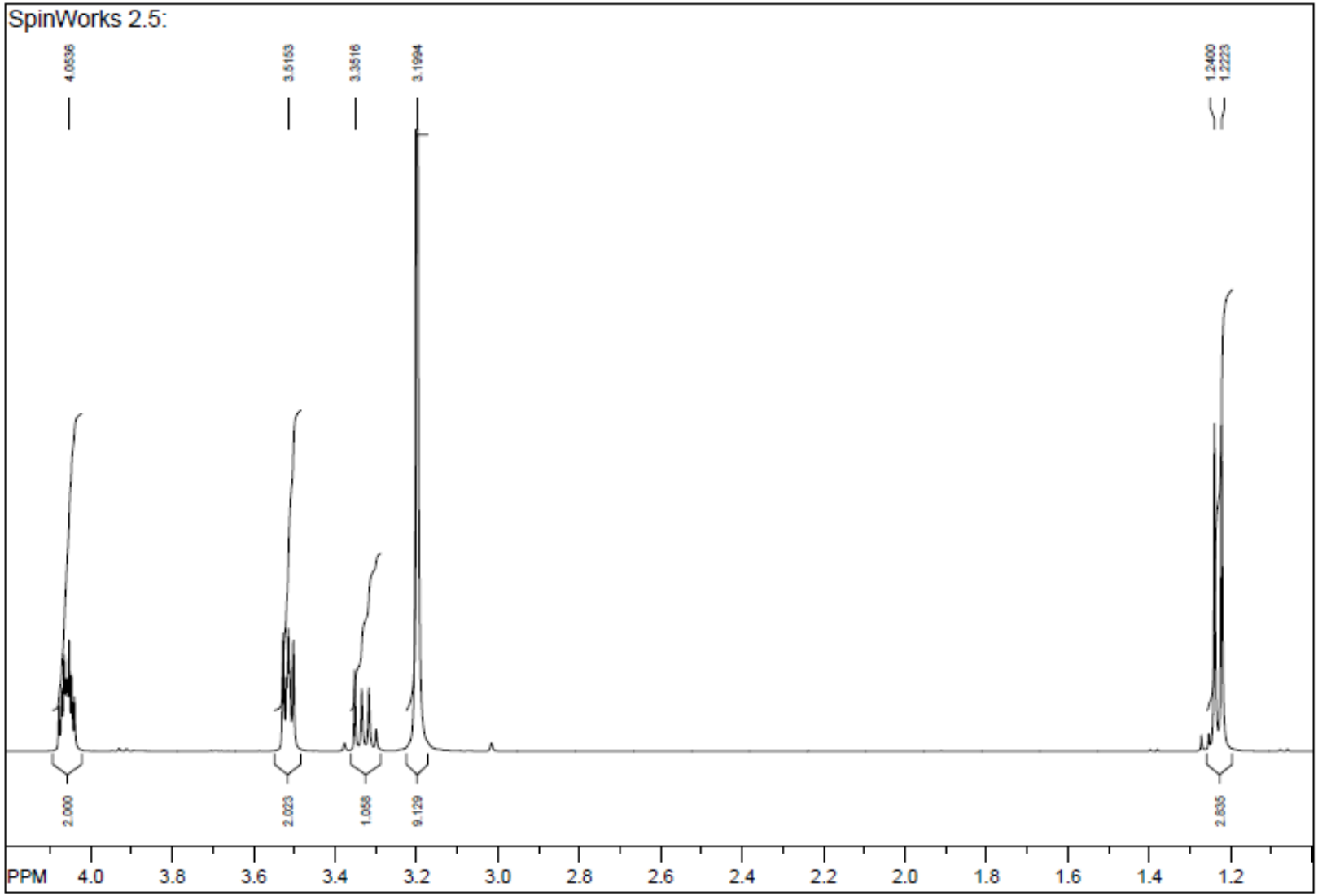

Fig. S2 ^{1}H NMR spectrum of ChAla

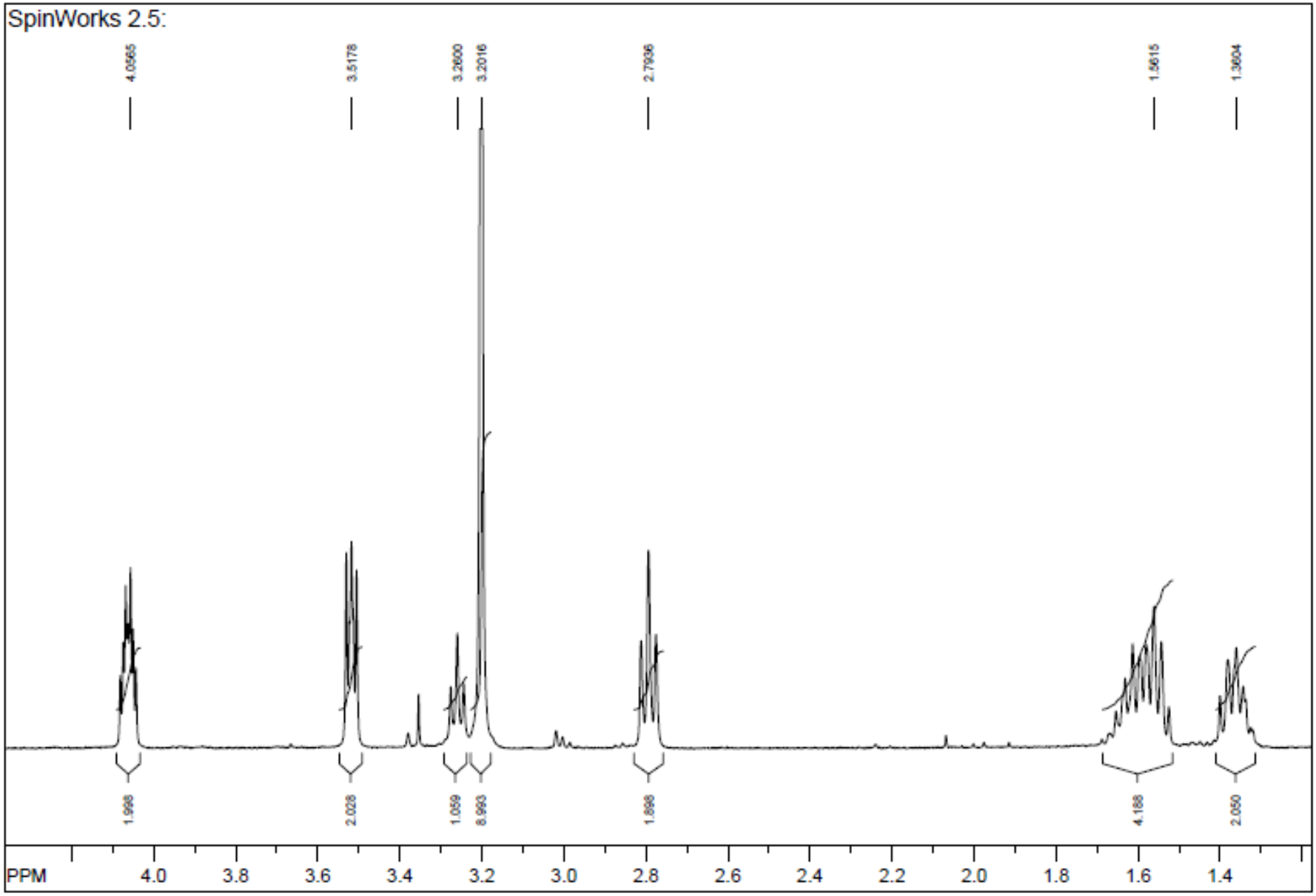

Fig. S3 ^{1}H NMR spectrum of ChLys

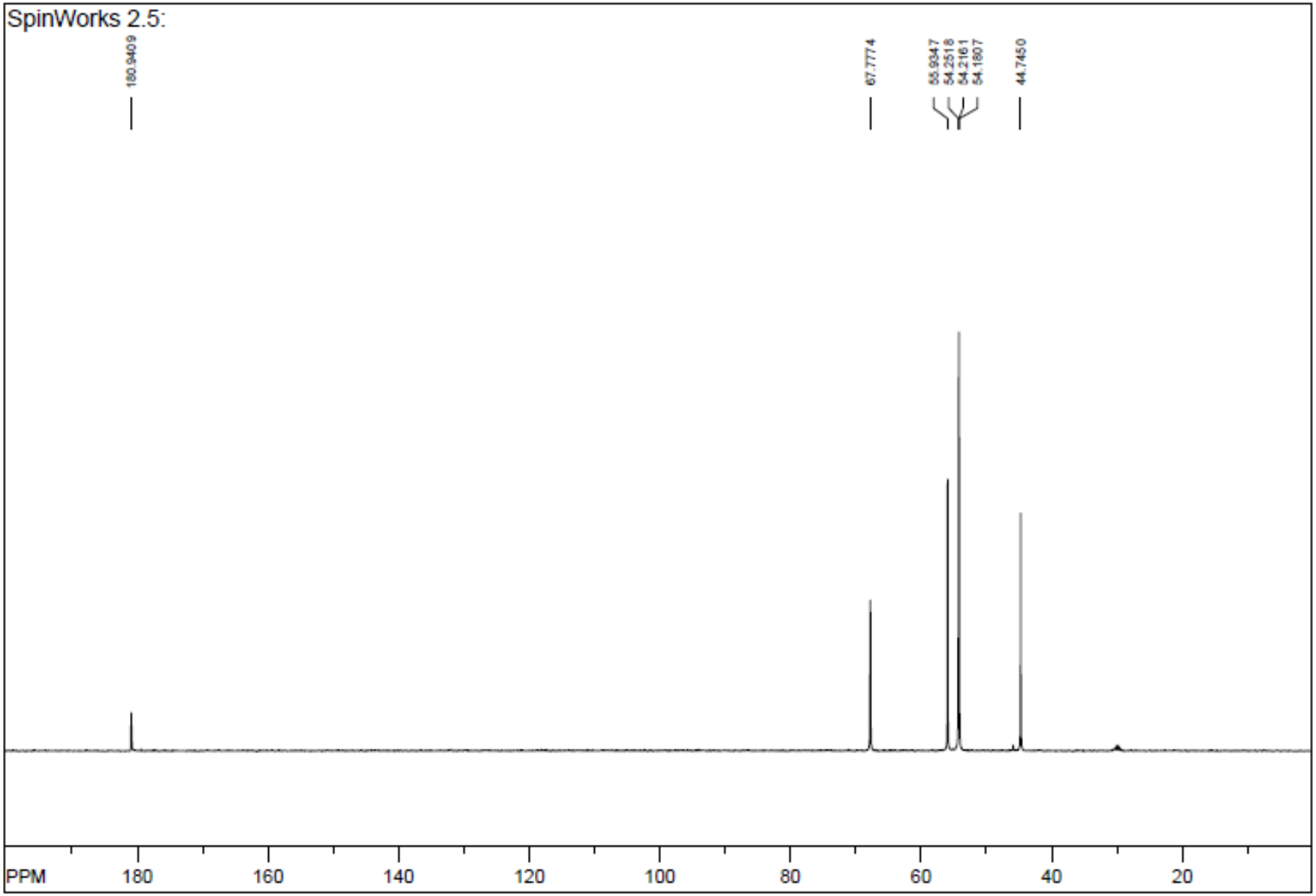

Fig. S4 ^{13}C NMR Spectrum of ChGly

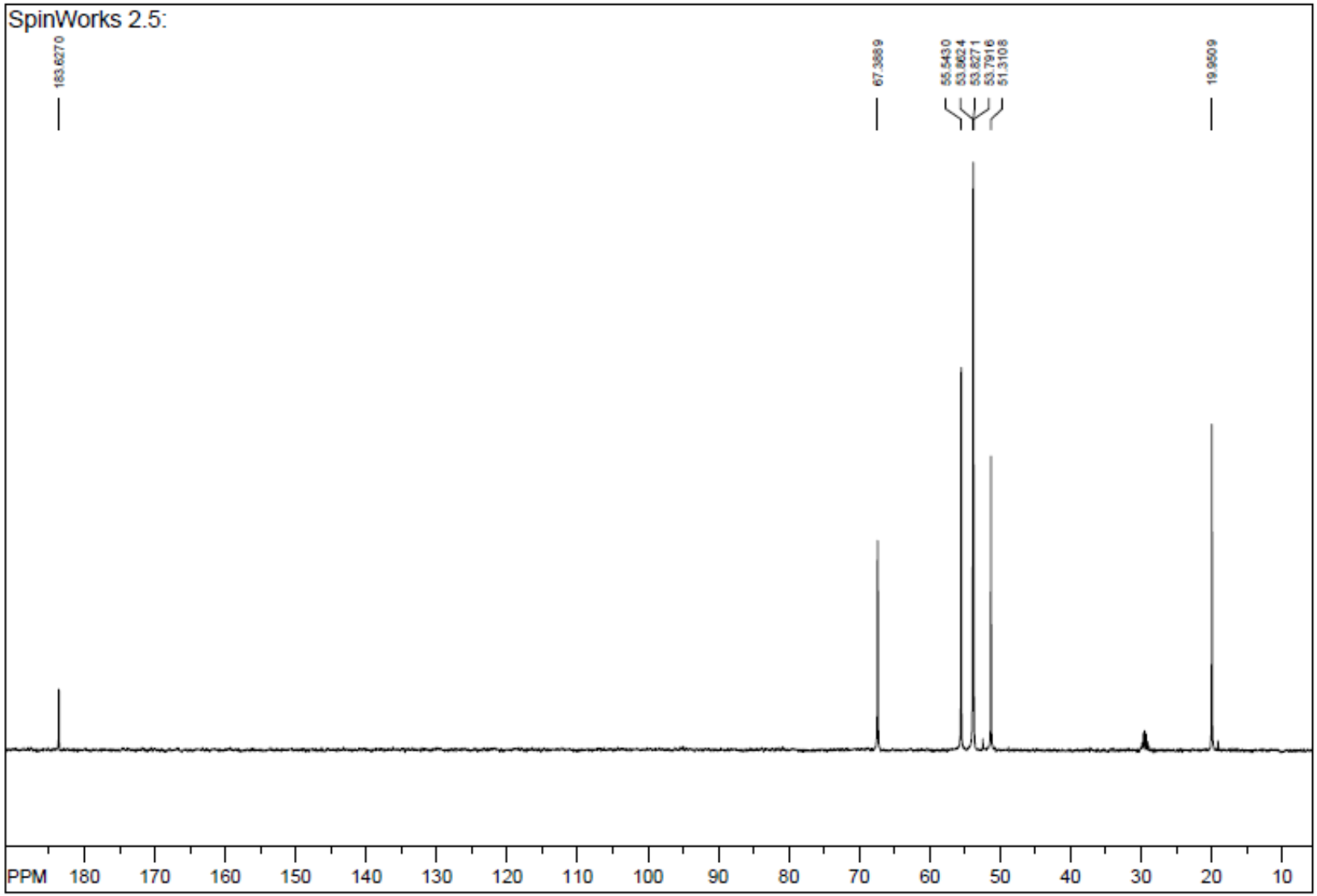

Fig. S5 ^{13}C NMR spectrum of ChAla

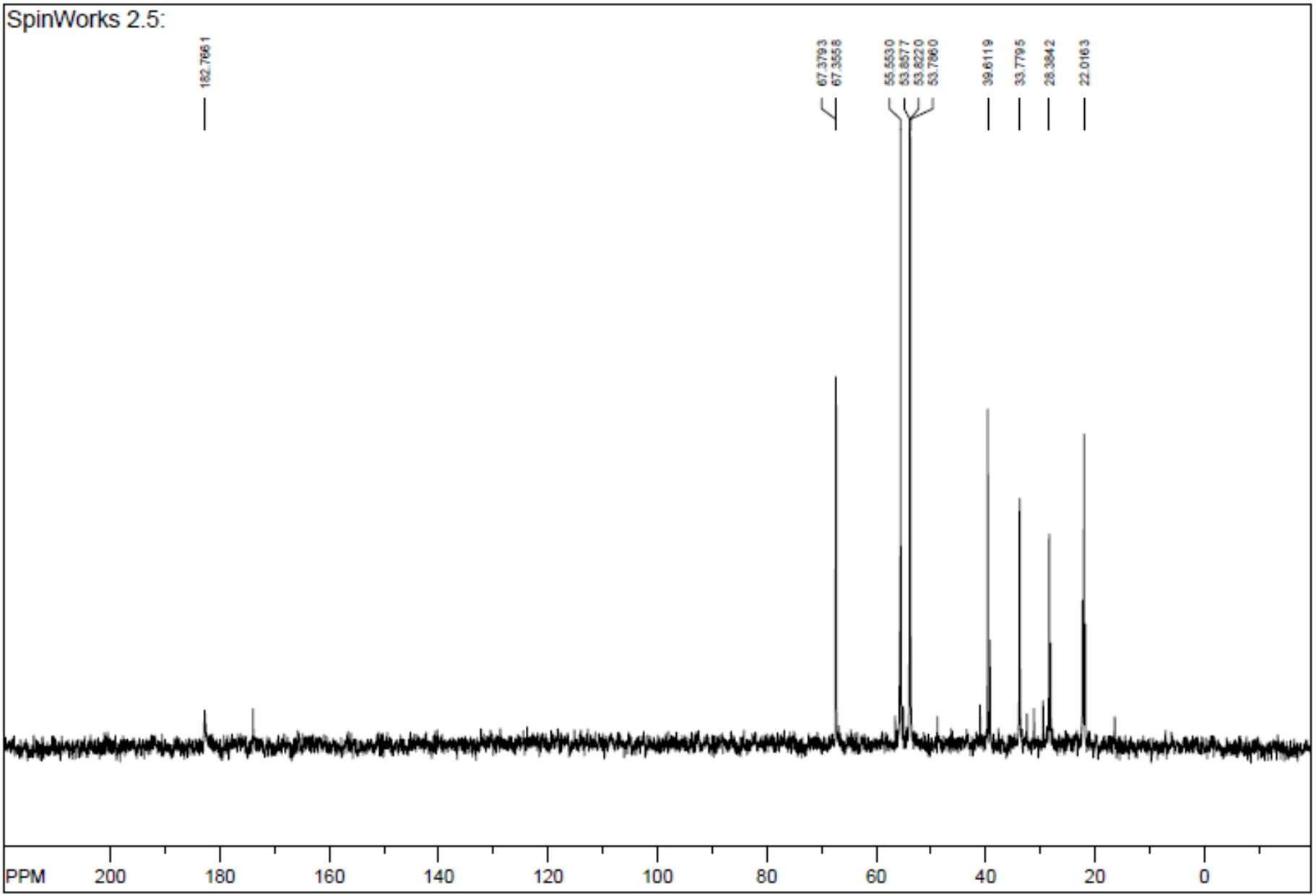

Fig. S6 ^{13}C NMR spectrum of ChLys

Methods

Lipolytic activity was determined spectrophotometrically using an aqueous solution of 2.5 mM of p-nitrophenyl laurate as substrate at pH 7.0, 25ºC and 20 min reaction time (Sigugirladotir et al, 1993)[18] One of the products of the hydrolysis reaction, p-nitrophenol, was monitored by the increase in the absorbance band at 400 nm. One activity unit is defined as the amount of enzyme that produced 1 μmol of p-nitrophenol per minute under standard assay conditions. The activities were expressed in (U/L) and all the results shown in the graphs are mean values of triplicates and the error bars are the standard deviation calculated from three independent measurements.

Differential Scanning Calorimetry (DSC) measurements were carried out in a MicroCal VP-DSC MicroCalorimeter controlled by the VP-viewer program and equipped with 0.51 mL cells. Studies were made using 0.473 mM of enzyme in the selected solvent (water or ionic liquid). Heating rates of 1 ºC min-1 were used from 40 to 90 ºC. For each rate at least five blank measurements were performed with the respective reference solvent in both compartments. These runs were used as the baseline for the run with Tl lipolytic enzyme in the sample compartment and the corresponding solvent in the reference compartment. In all experiments solutions were degassed for 10 min under vacuum. An overpressure of about 30 psi was applied to the calorimeter cells. Calorimetric data were converted to heat capacity by subtracting the solvent baseline and dividing by the scan rate and protein concentration.

Differential scanning fluorimetry (DSF) data were obtained monitoring the fluorescence of the exogenous probe Sypro Orange, emission of which increases upon interaction with the hydrophobic moieties that become exposed upon protein unfolding induced by temperature rise. Using a real-time PCR instrument, it was possible to test a number of conditions in a 96-well plate in a single experiment.

Table S1 T_m from DSC and DSF techniques at different concentrations (in molarity) of the selected cholinium-based ionic liquids. T_m1 and T_m2 are the first and the second structural transitions

Concentration (M)	Solvent	DSC			DSF					
		T_m1 (ºC)	T_m2 (ºC)	ΔT_m	T_m (ºC)			T_m average (ºC)	Std Dev	ΔT_m
	Water	69.14	74.06		74.00	74.00	75.00	74.33	0.58	
0.5 M	ChAla		62.24	11.82	63.00	62.00	62.00	62.33	0.58	12.00
	ChGly		62.72	11.34	63.00	63.00	63.00	63.00	0.00	11.33
	ChLys		65.09	8.97	65.00	65.00	64.00	64.67	0.58	9.67
	ChCl	68.73	73.00	1.06	72.00	72.00	70.00	71.33	1.15	3.00
1 M	ChAla		61.48	12.58	62.00	62.00	62.00	62.00	0.00	12.33
	ChGly		61.54	12.52	63.00	62.00	62.00	62.33	0.58	12.00
	ChLys	63.47	65.73	8.33	64.00	64.00	64.00	64.00	0.00	10.33
	ChCl	67.97	71.85	2.21	70.00	69.00	69.00	69.33	0.58	5.00
2 M	ChAla		60.86	13.20	61.00	61.00	60.00	60.67	0.58	13.67
	ChGly		61.83	12.23	63.00	61.00	61.00	61.67	1.15	12.67
	ChLys		65.35	8.71	64.00	64.00	63.00	63.67	0.58	10.67
	ChCl	66.47	70.37	3.69	69.00	69.00	69.00	69.00	0.00	5.33

Table S2 Water content, hydrophobicity, pH and activity of the ionic liquid-water mixtures under study

Ionic liquid	Concentration (M)	% (v/v)	% water	logP (cation)	logP (anion)	logP	pH	% Activity
ChAla	0.5	9.613	90.387	-1.574	-2.84	-4.414	10.9	138.63
ChGly		8.912	91.088	-1.574	-3.41	-4.984	10.9	173.13
ChLys		12.468	87.532	-1.574	-3.21	-4.784	9.8	129.21
ChCl		6.981	93.019	-1.574	0.61	-0.964	5.3	90.41
ChAla	1	19.226	80.774	-1.574	-2.84	-4.414	11	142.22
ChGly		17.824	82.176	-1.574	-3.41	-4.984	11	159.42
ChLys		24.936	75.064	-1.574	-3.21	-4.784	9.8	122.99
ChCl		13.962	86.038	-1.574	0.61	-0.964	5.5	97.25
ChAla	2	38.452	61.548	-1.574	-2.84	-4.414	11.3	139.51
ChGly		35.648	64.352	-1.574	-3.41	-4.984	11.1	138.18
ChLys		49.872	50.128	-1.574	-3.21	-4.784	10.0	128.68
ChCl		27.924	72.076	-1.574	0.61	-0.964	4.7	56.72

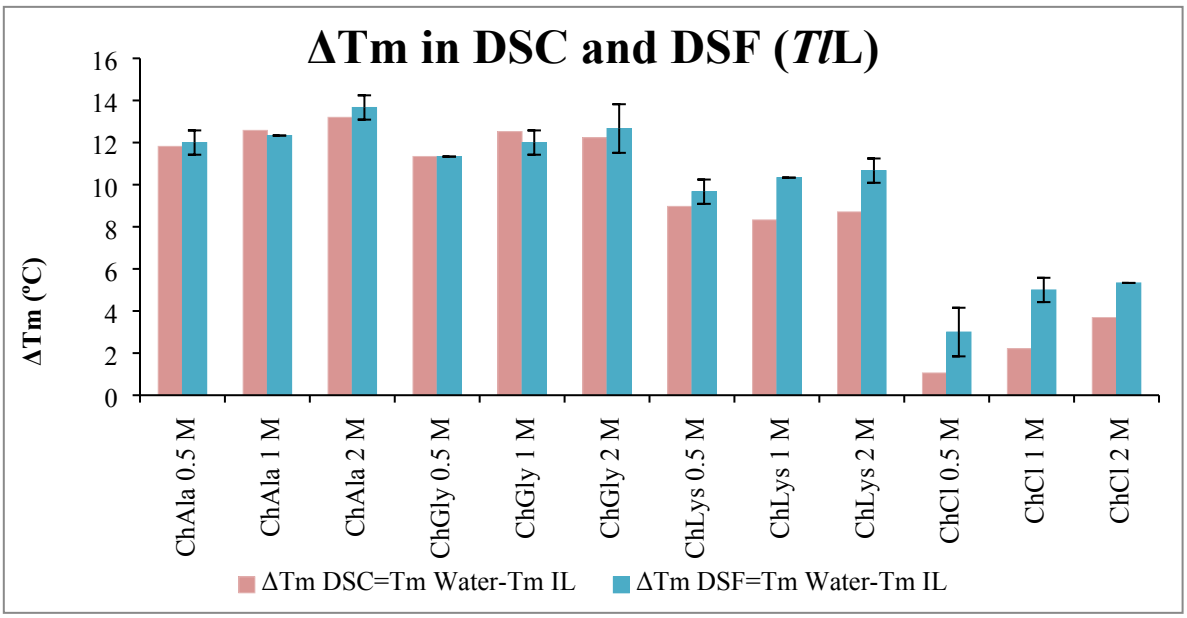

Fig. S7 Effect of the selected cholinium amino acids and cholinium chloride on the structure of *Tl*L

References

[S1] D. J. Tao, Z. Cheng, F. F. Chen, Z. M. Li, N. Hu, X. S. Chen, J. Chem. Eng. Data 2013, 58, 1542-1548.

[S2] S. Sigurgísladóttir, M. Konráðsdóttir, A. Jónsson, J. K. Kristjánsson, E. Matthiasson, Biotechnol. Lett. 1993, 15, 361.