Structural-functional evaluation of ionic liquid libraries for the design of co-solvents in lipase-catalysed reactions

ELECTRONIC SUPPLEMENTARY INFORMATION

Materials and methods

Lipolytic activity assay method - 2,3-dimercapto-1-propanol tributyrate (Sigma Aldrich), DMPTB, was dissolved in Triton X-100 (Sigma Aldrich) and in 50 mM trizma base buffer (Sigma Aldrich), pH 7.2. The final stock solution is 10 mM DMPTB and 2% Triton X-100 and was stored at -20 ºC. A 40 mM solution of 5,5'-dithiobis(2-nitro benzoic acid) (Sigma Aldrich), DTNB, in isobutanol was prepared daily. Thermomyces lanuginosus lipase (Sigma Aldrich, L0777), TlL, was dissolved in 50 mM trizma base buffer pH 7.2 with 0.0025% of triton X-100. The standard reaction mixture contained 4.44 mM DMPTB, 0.88 mM DTNB, 0.0013% Triton X-100, and 26.70 mM Trizma base, pH 7.2. The concentration of enzyme used was 70 nM. Each well of the 96-well microplate was filled with 100 µl of a 50% IL (prepared in water), 50 µl of the standard reaction mixture and 50 µl of the enzyme sample. Controls without the enzyme and/or the IL were also performed.

The time-dependent absorbance change was monitored at 405 nm for 10 min of reaction in a Biotek Synergy 2 multi-mode microplate reader. The lipolytic activity was calculated based on the initial velocity of the reaction (Abs/s). Lipolytic activity is calculated as a percentage of the activity of TlL in buffer in the presence of the selected ionic liquids and represents the average of at least 2 replicates.

Differential Scanning Fluorimetry (DSF)- Differential scanning fluorimetry, runs were performed by monitoring the fluorescence of the exogenous probe Sypro Orange (Life Technologies, 5000x concentrate in DMSO) in a Bio Rad IQ5 Multicolor Real-Time PCR detection system equipped with a charge-coupled device (CCD) camera and a Cy3 filter with excitation and emission wavelengths of 490 and 575 nm, respectively. 75 µL of 6.67 µM enzyme solution in 20x DMSO concentrated sypro orange probe was left in contact with 25 µL of 25% (w/w) IL solution for 15 minutes. Each well of the 96-Well PCR plate was filled with 20 µL of this mixture. The assays were performed in a Bio Rad IQ5 Multicolor Real-Time PCR detection system with heating rates of 1.0 ºC min-1 from 20 to 90 ºC. Controls without the enzyme and/or the IL were also performed.

Table S1 – List of ionic liquids used in this work and respective activity and stability data for *Tl*L

Compound	Activity (%)	Normalized intensity	Apparent T_m by DSF (°C)	ΔT_m (ºC)	Transition steepness (%)	logP (Cation)	logP (Anion)	LogP (IL)
Control (Water)	100	0	70	0	100	-	-	-
$(CH_3)_2N_2CNH_2$ $N(CN)_2$	19	#N/D	#N/D	#N/D	#N/D	-2.043	0.282	-1.761
$N_{1\,1\,1\,(2OH)}$ Bicarbonate	10	3	59	-11	68.3	-1.574	-0.516	-2.09
$N_{1\,1\,1\,(2OH)}$ NO_3	12	8	54	-16	55.1	-1.574	1.31	-0.264
$N_{1\,1\,1\,(2OH)}$ C_3COO	39	5	64	-6	67.2	-1.574	0.215	-1.359
$N_{1\,1\,1\,(2OH)}$ $(C_1)_2PO_4$	42	6	69	-1	93.6	-1.574	-1.094	-2.668
$N_{1\,1\,1\,(2OH)}$ C_2COO	44	6	67	-3	80.2	-1.574	-0.241	-1.815
$N_{1\,1\,1\,(2OH)}$ C_4COO	46	12	60	-10	36.5	-1.574	0.672	-0.902
$N_{1\,1\,1\,(2OH)}$ C_2SO_3	54	12	63	-7	74.0	-1.574	-0.768	-2.342
$N_{1\,1\,1\,(2OH)}$ Pivalate	54	19	60	-10	38.9	-1.574	0.639	-0.935
$N_{1\,1\,1\,(2OH)}$ Salicylate	57	22	35	-35	38.6	-1.574	0.489	-1.085
$N_{1\,1\,1\,(2OH)}$ Cl	61	7	40	-30	63.1	-1.574	0.77	-0.804
$N_{1\,1\,1\,(2OH)}$ OTf	71	9	55	-15	59.9	-1.574	1.019	-0.555
$N_{1\,1\,1\,(2OH)}$ C_1COO	87	4	70	0	86.1	-1.574	-0.908	-2.482
$N_{1\,1\,1\,(2OH)}$ $(C_1)_2CCOO$	88	7	60	-10	59.9	-1.574	0.222	-1.352
$N_{1\,1\,1\,(2OH)}$ C_1SO_3	100	9	66	-4	89.7	-1.574	-1.117	-2.691
$N_{1\,1\,1\,(2OH)}$ C_5COO	#N/D	92	54	-16	8.0	-1.574	1.128	-0.446
$N_{1\,1\,1\,(2OH)}$ H_2PO_4	#N/D	2	37	-33	27.0	-1.574	-1.911	-3.485
$N_{1\,1\,2\,(2OH)}$ Br	33	29	63	-7	60.1	-1.225	0.89	-0.335
$N_{1\,1\,1\,(2O1)}$ Cl	70	6	57	-13	65.0	-1.189	0.77	-0.419
$N_{1\,1\,3\,(2OH)}$ Br	23	35	50	-20	53.8	-0.702	0.89	0.188
$N_{1\,1\,4\,(2O1)}$ Cl	97	15	60	-10	71.2	-0.316	0.77	0.454
$N_{1\,1\,4\,(2OH)}$ Br	6	30	59	-11	16.2	-0.072	0.89	0.818
C_1C_1im C_1SO_3	46	42	40	-30	40.7	0.025	-1.117	-1.092
$N_{1\,1\,5\,(2OH)}$ Br	7	75	53	-17	2.8	0.211	0.89	1.101
C_2C_1im $N(CN)_2$	0	85	No transition	No transition	No transition	0.374	0.282	0.656
C_2C_1im HCOO	11	42	59	-11	25.7	0.374	-0.516	-0.142
C_2C_1im C_2SO_3	12	21	65	-5	19.1	0.374	-0.768	-0.394
C_2C_1im Cl	17	38	57	-13	10.5	0.374	0.77	1.144
C_2C_1im Pivalate	19	29	55	-15	21.2	0.374	0.639	1.013
C_2C_1im C_2COO	19	12	62	-8	29.4	0.374	-0.241	0.133
C_2C_1im C_1COO	24	35	64	-6	40.6	0.374	-0.908	-0.534
C_2C_1im C_1SO_3	24	88	67	-3	35.8	0.374	-1.117	-0.743
C_2C_1imC_6SO_3	27	76	No transition	No transition	No transition	0.374	1.124	1.498
C_2C_1im C_4SO_3	35	57	No transition	No transition	No transition	0.374	0.212	0.586
C_2C_1im Gala	71	1	66	-4	41.5	0.374	-3.153	-2.779
C_2C_1im C_4SO_4	#N/D	50	No transition	No transition	No transition	0.374	0.058	0.432
C_2C_1im C_8SO_4	#N/D	2	No transition	No transition	No transition	0.374	0.97	1.344
$N_{1\,1\,Bz\,(2OH)}$ Cl	1	60	38	-32	2.9	0.469	0.77	1.239
C_4C_1pyrr Lactate	19	31	58	-12	24.6	0.755	-0.989	-0.234
C_4C_1pyrr Cl	#N/D	23	49	-21	12.4	0.755	0.77	1.525
C_3C_1im C_3SO_3	12	44	54	-16	27.8	0.898	-0.245	0.653
C_3C_1im C_2SO_3	13	36	61	-9	15.0	0.898	-0.768	0.13
C_3C_1im Cl	20	38	43	-27	12.0	0.898	0.77	1.668
C_3C_1im C4SO3	35	55	45	-25	3.5	0.898	0.212	1.11
C_4C_1pip Cl	36	45	No transition	No transition	No transition	1.211	0.77	1.981
C_4C_1im $N(CN)_2$	2	78	50	-20	1.0	1.354	0.069	1.423
C_4C_1im C_2SO_3	6	44	53	-17	3.4	1.354	-0.768	0.586
C_4C_1im Cl	10	42	No transition	No transition	No transition	1.354	0.77	2.124
C_4C_1im C_1COO	18	29	58	-12	16.6	1.354	-0.908	0.446
C_6C_1pyrr Cl	#N/D	34	No transition	No transition	No transition	1.667	0.77	2.437
C_5C_1im Cl	20	78	No transition	No transition	No transition	1.81	0.77	2.58
C_6C_1im C_1SO_3	2	69	No transition	No transition	No transition	2.267	-1.117	1.15
C_6C_1im Cl	6	96	No transition	No transition	No transition	2.267	0.77	3.037
C_6C_1im C_2SO_3	19	59	No transition	No transition	No transition	2.267	-0.768	1.499
C_8C_1im Cl	2	96	45	-25	5.9	3.179	0.77	3.949
$N_{1\,1\,4\,8}$ Cl	3	66	No transition	No transition	No transition	3.448	0.77	4.218
$N_{8\,8\,8\,H}$ C_7COO	12	78	48	-22	13.7	8.22	2.04	10.26
$N_{8\,8\,8\,H}$ C_1COO	56	15	36	-34	70.4	8.22	-0.908	7.312
$N_{8\,8\,2\,1}$ C_2SO_4	3	100	41	-29	4.8	5.272	-0.922	4.35
$N_{8\,8\,8\,H}$ $(2\text{-}C_4)C_7COO$	39	28	72	2	55.7	8.22	3.871	12.091
C_4C_1im OTf	2	85	No transition	No transition	No transition	1.354	1.019	2.373

#N/D = not determined

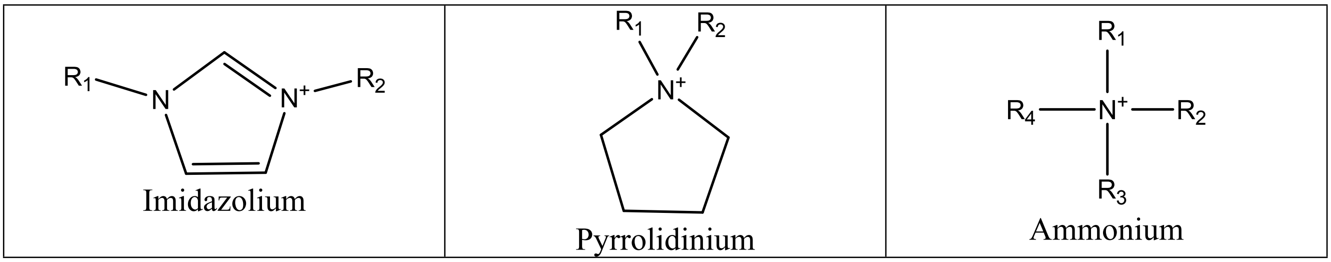

Figure S1 –Main cation structure of ILs

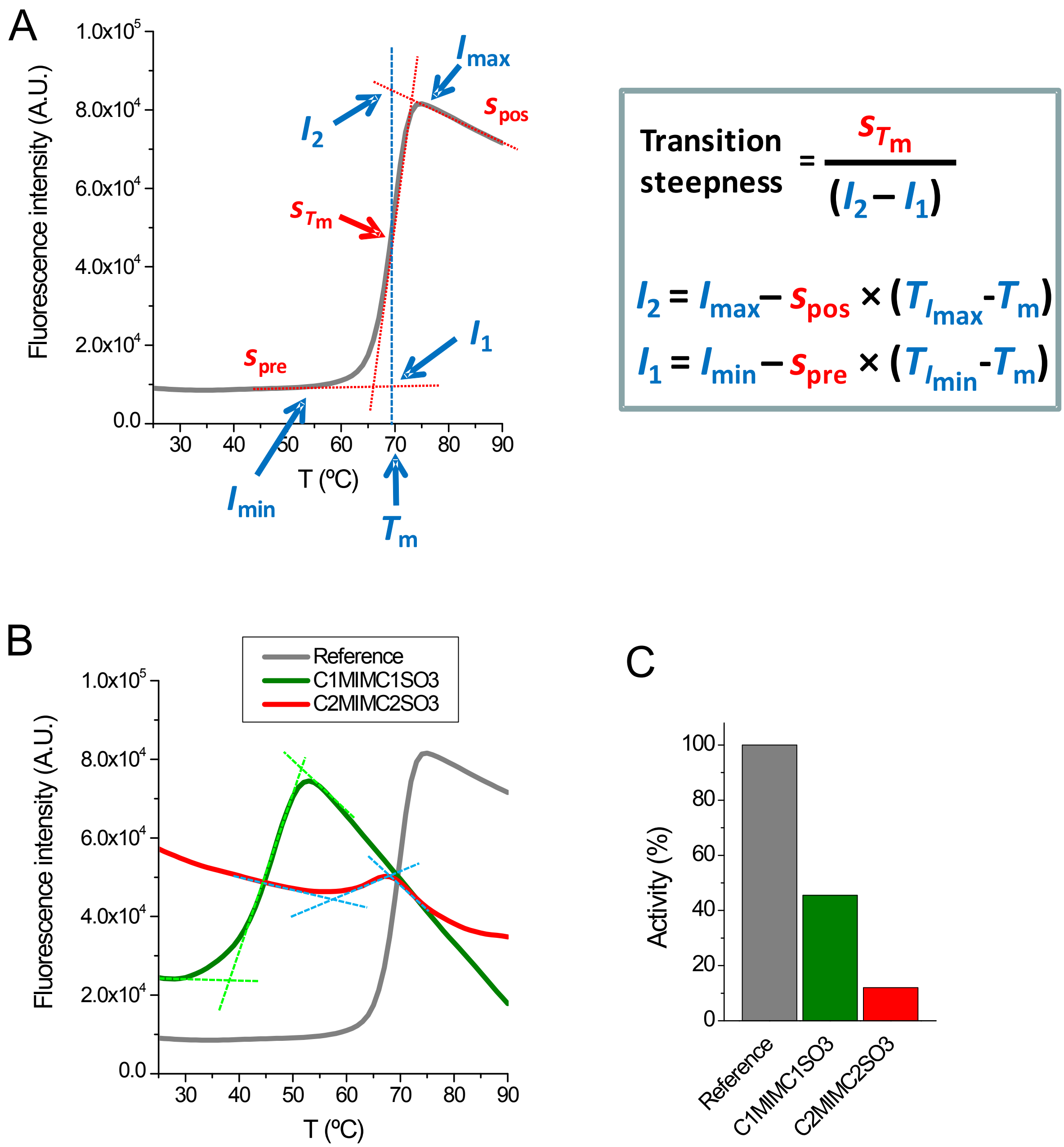

Figure S2 –Examples of DSF curves and parameter determination. A) The melting temperature (T_m) is the temperature at midpoint transition, and the transition steepness is the slope of the intensity change at T_m, normalized by the amplitude of the transition, which is calculated by the difference in maximum and minimum intensities extrapolated through T_m using slopes for the post- and pre-transitions, respectively. B) Different types of curves obtained by DSF using distinct solvents and C) activity data for the corresponding examples.

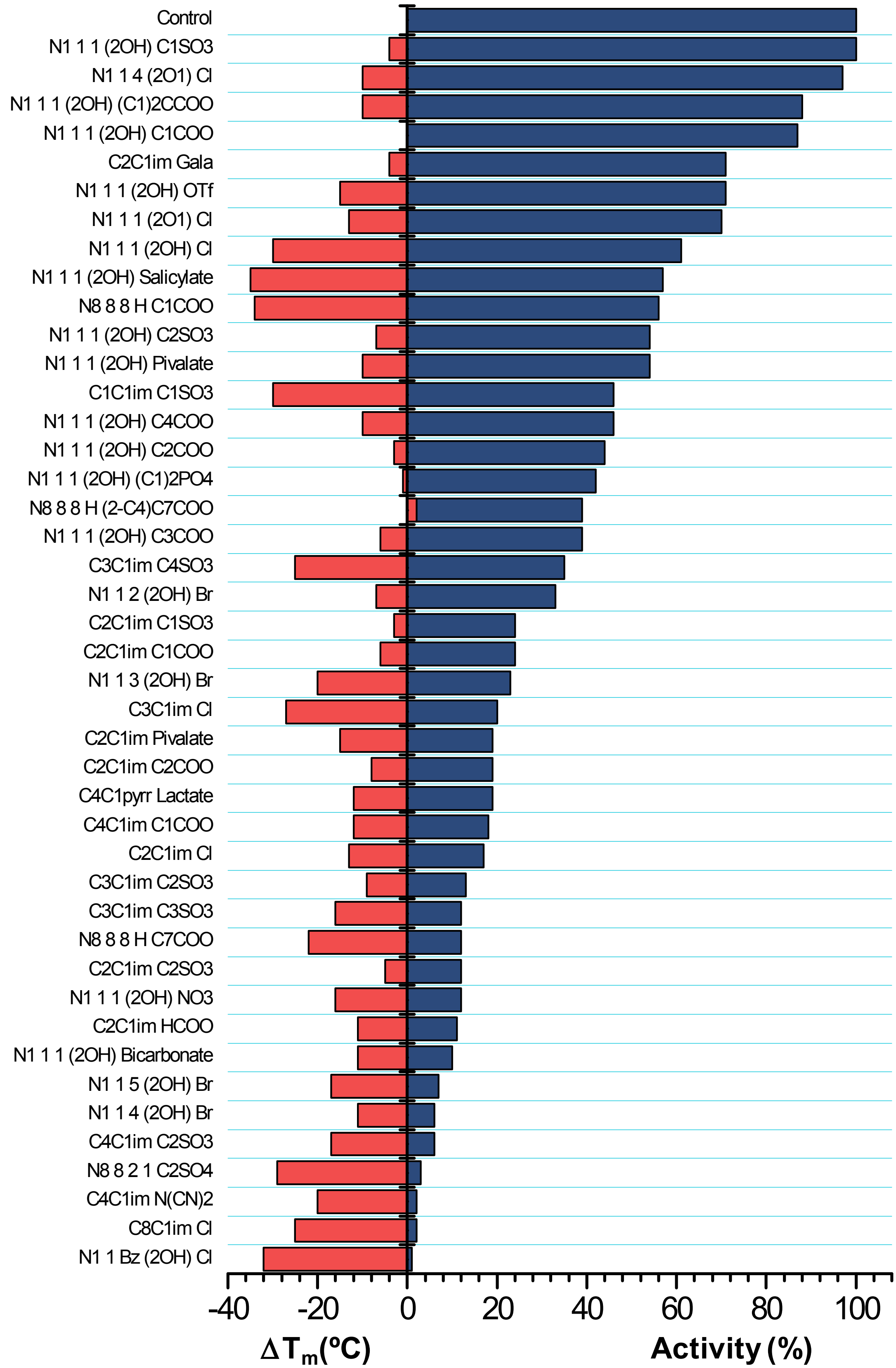

Figure S3 –Comparison between the activity of *Tl*L and the T_m determined by DSF

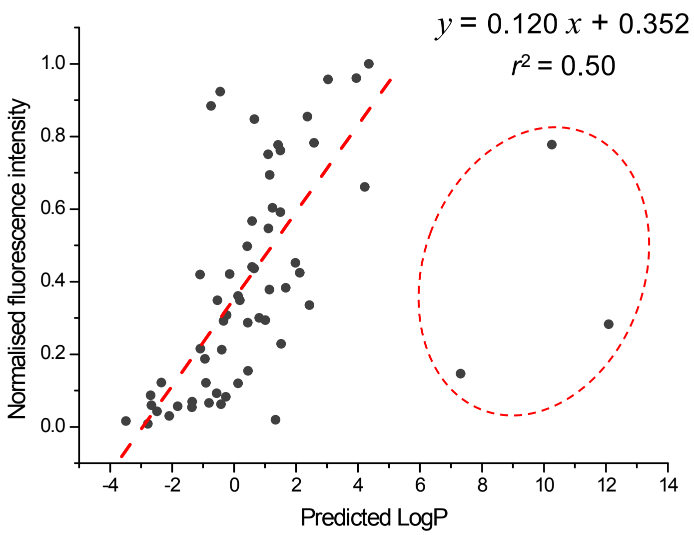

Figure S4 – Effect of the hydrophobic character of IL on the fluorescence intensity of Sypro Orange in the corresponding solvent condition.

Table S2 – List of ionic liquids used in this work and source

Ionic liquids	Source
$(CH_3)_2N_2CNH_2$ $N(CN)_2$	GVSM Carrera, RFM Frade, J Aires de Sousa, CAM Afonso, LC Branco, *Tetrahedron* 2010, **12**, 643
$N_{1\,1\,1\,(2OH)}$ Bitartrate	Sigma Aldrich
$N_{1\,1\,1\,(2OH)}$ H_2Citrate	Sigma Aldrich
$N_{1\,1\,1\,(2OH)}$ Bicarbonate	Sigma Aldrich
$N_{1\,1\,1\,(2OH)}$ NO_3	Iolitec
$N_{1\,1\,1\,(2OH)}$ C_3COO	M Petkovic, JL Ferguson, HQ Nimal Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo, C Silva Pereira, *Green Chem* 2010, **12**, 643
$N_{1\,1\,1\,(2OH)}$ $(C_1)_2PO_4$	Iolitec
$N_{1\,1\,1\,(2OH)}$ C_2COO	M Petkovic, JL Ferguson, HQ Nimal Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo, C Silva Pereira, *Green Chem* 2010, **12**, 643
$N_{1\,1\,1\,(2OH)}$ C_4COO	M Petkovic, JL Ferguson, HQ Nimal Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo, C Silva Pereira, *Green Chem* 2010, **12**, 643
$N_{1\,1\,1\,(2OH)}$ C_2SO_3	A.J.L. Costa, M.R.C. Soromenho, K. Shimizu, J.M.S.S. Esperança, J.N. Canongia Lopes, L.P.N. Rebelo, *EUCHEM* 2012, Wales, UK, August 5-10, 2012
$N_{1\,1\,1\,(2OH)}$ Pivalate	S Shahriari, LC Tomé, JMM Araújo, LPN Rebelo, JAP Coutinho, IM Marrucho, MG Freire, *RSC Adv* 2013, **3**, 183
$N_{1\,1\,1\,(2OH)}$ Salicylate	Sigma Aldrich
$N_{1\,1\,1\,(2OH)}$ Cl	Sigma Aldrich
$N_{1\,1\,1\,(2OH)}$ OTf	Iolitec
$N_{1\,1\,1\,(2OH)}$ C_1COO	M Petkovic, JL Ferguson, HQ Nimal Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo, C Silva Pereira, *Green Chem* 2010, **12**, 643
$N_{1\,1\,1\,(2OH)}$ $(C_1)_2CCOO$	M Petkovic, JL Ferguson, HQ Nimal Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo, C Silva Pereira, *Green Chem* 2010, **12**, 643
$N_{1\,1\,1\,(2OH)}$ C_1SO_3	A.J.L. Costa, M.R.C. Soromenho, K. Shimizu, J.M.S.S. Esperança, J.N. Canongia Lopes, L.P.N. Rebelo, *EUCHEM* 2012, Wales, UK, August 5-10, 2012
$N_{1\,1\,1\,(2OH)}$ C_5COO	M Petkovic, JL Ferguson, HQ Nimal Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo, C Silva Pereira, *Green Chem* 2010, **12**, 643
$N_{1\,1\,1\,(2OH)}$ H_2PO_4	Iolitec
$N_{1\,1\,2\,(2OH)}$ Br	Sigma Aldrich
$N_{1\,1\,1\,(2O1)}$ Cl	AJL Costa, P Papis, PM Reis, K Shimizu, J Szydłowski, JN Canongia Lopes, JMSS Esperança, LPN Rebelo, *COIL-5*, Vilamoura, Portugal, April 21-25, 2013
$N_{1\,1\,3\,(2OH)}$ Br	AJL Costa, MRC Soromenho, K Shimizu, IM Marrucho, JMSS Esperança, JN Canongia Lopes, LPN Rebelo, *ChemPhysChem* 2012, **13**, 1902
$C_{2OH}C_1$im Cl	Iolitec
$N_{1\,1\,3\,(2O1)}$ Cl	AJL Costa, P Papis, PM Reis, K Shimizu, J Szydłowski, JN Canongia Lopes, JMSS Esperança, LPN Rebelo, COIL-5, Vilamoura, Portugal, April 21-25, 2013
$N_{1\,1\,4\,(2OH)}$ Br	AJL Costa, MRC Soromenho, K Shimizu, IM Marrucho, JMSS Esperança, JN Canongia Lopes, LPN Rebelo, ChemPhysChem 2012, 13, 1902
C_1C_1im C_1SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
$N_{1\,1\,5\,(2OH)}$ Br	AJL Costa, MRC Soromenho, K Shimizu, IM Marrucho, JMSS Esperança, JN Canongia Lopes, LPN Rebelo, ChemPhysChem 2012, 13, 1902
C_2C_1im $N(CN)_2$	Iolitec
C_2C_1im OTf	Iolitec
C_2C_1im SCN	Iolitec
C_2C_1im HCOO	M Petkovic, JL Ferguson, HQ Nimal Gunaratne, R Ferreira, MC Leitão, KR Seddon, LPN Rebelo, C Silva Pereira, *Green Chem* 2010, **12**, 643
C_2C_1im Br	Iolitec
C_2C_1im C_2SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_2C_1im Cl	Iolitec
C_2C_1im Pivalate	It was prepared by several of the authors in accordance with the protocol cited in J Blath, N Deubler, T Hirth, T Schiestel, *Chem Eng J* 2012, **181**, 152
C_2C_1im C_2COO	B Zhao, L Greiner, W Leitne, *RSC advances* 2012, **2**, 2476
C_2C_1im C_1COO	Sigma Aldrich
C_2C_1im C_1SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_2C_1imC_6SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_2C_1im C_4SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_2C_1im Galactate	They were prepared by some of the authors in QUILL centre (Queen's University Ionic Liquids Laboratory, Belfast, UK).
C_2C_1im C_2SO_4	Merck
C_2C_1im C_4SO_4	Merck
C_2C_1im C_8SO_4	Merck
$N_{1\,1\,Bz\,(2OH)}$ Cl	Sigma Aldrich
C_4C_1pyrr Lactate	They were prepared by some of the authors in QUILL centre (Queen's University Ionic

	Liquids Laboratory, Belfast, UK).
C_4C_1pyrr OTf	Iolitec
C_4C_1pyrr Cl	Iolitec
C_3C_1im C_3SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_3C_1im C_1SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_3C_1im C_2SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_3C_1im Cl	Iolitec
C_3C_1im C_4SO3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_2py Cl	Iolitec
C_4C_1pip Cl	Iolitec
C_4C_1im C_1SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_4C_1im $N(CN)_2$	Iolitec
C_4C_1im C_2SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_4C_1im BF_4	Iolitec
C_4C_1im Cl	Iolitec
C_4C_1im Alalinate	Ohno H, Fukumoto K, *Acc Chem Res* 2007, **40**, 1122
C_4C_1im C_1COO	Sigma Aldrich
C_6C_1pyrr Cl	Iolitec
C_5C_1im C_1SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_5C_1im Cl	They were prepared by some of the authors in QUILL centre (Queen's University Ionic Liquids Laboratory, Belfast, UK).
C_4py Cl	Iolitec
C_6C_1im C_1SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_6C_1im Cl	Iolitec
C_6C_1im C_2SO_3	Blesic et al. *Phys Chem Chem Phys* 2009, **11**, 8939
C_8C_1pyrr Cl	They were prepared by some of the authors in QUILL centre (Queen's University Ionic Liquids Laboratory, Belfast, UK).
C_4C_1py Cl	Iolitec
C_8C_1im Cl	They were prepared by some of the authors in QUILL centre (Queen's University Ionic Liquids Laboratory, Belfast, UK).
$N_{1\,1\,4\,8}$ Cl	Cheng Chen, Guang Xian Zhang, Feng Xiu Zhang, Hui Zheng, *Adv Materials Res* 2012, **549**, 278
C_6C_1py Cl	Iolitec
C_6C_1py C_1SO_3	AB Pereiro, A Rodríguez, M Blesic, K Shimizu, JNC Lopes, LPN Rebelo, *J Chem Eng Data* 2011, **56**, 4356
C_8C_1py Cl	AB Pereiro, A Rodríguez, M Blesic, K Shimizu, JNC Lopes, LPN Rebelo, *J Chem Eng Data* 2011, **56**, 4356
$N_{8\,8\,8\,H}$ C_7COO	Bioniqs
$N_{8\,8\,8\,H}$ C_1COO	Bioniqs
$P_{4\,4\,4\,2}$ $(C2)_2PO_4$	Cytec
$N_{8\,8\,2\,1}$ C_2SO_4	Bioniqs
$N_{8\,8\,8\,H}$ (2-C_4)C_7COO	Bioniqs
C_4C_1im OTf	Iolitec