

Biodegradable, non-bactericidal oxygen-functionalised imidazolium esters: A step towards 'greener' ionic liquids†

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A series of imidazolium ionic liquids was prepared and screened against 7 bacterial strains. The incorporation of ether groups into the ester side-chain significantly reduced the toxicity compared with alkyl ester derivatives. Biodegradation data are also presented for 15 of the ionic liquids—including 6 examples which can be classed as readily biodegradable.

Introduction

Ionic liquids (ILs) have been under study as a replacement for volatile organic compounds (VOCs) and are now used as media for a variety of organic and inorganic reactions, ranging from catalytic hydrogenation^{1,2} and hydroformylation³ to the Friedel–Crafts⁴ and Diels–Alder⁵ reactions. ILs have also proved useful in the physical sciences, with applications emerging in analytical separations,⁶ near-infrared luminescence⁷ and photo-magnetism.⁸

A significant research effort ensued when ILs emerged as a possible 'green' alternative to common organic solvents.⁹ Although their negligible vapour pressures¹⁰ render ILs attractive alternatives to VOCs, other release routes aside from evaporation to the environment must be addressed before ILs can officially be categorised as 'green'.

In recent years investigations have commenced into the biological impact of ILs on the environment. With the field of ILs continuing to shift from the research laboratory to an industrial setting,^{11–13} toxicity and biodegradability have become additional factors to be considered before ILs gain acceptance as viable alternatives to VOCs.

Toxicological and ecotoxicological data for ILs have been collected using test systems based on different biological complexity levels. Toxicity and antimicrobial studies have been performed on a range of bacteria and fungi,^{14–17} acute toxicity studies have been carried out on fish,¹⁸ growth inhibition studies have been carried out on algae,^{19,20,21} and also on terrestrial plants.^{22,23} The variety of organisms studied now extends to higher classes such as the soil nematode²⁴ and the freshwater snail.²⁵

Most of the ionic liquids studied have been of the imidazolium and pyridinium classes, with alkyl or alkyloxy side-chains,

containing the anions, bromide (Br[−]), chloride (Cl[−]), hexafluorophosphate (PF₆[−]), and tetrafluoroborate (BF₄[−]). Preliminary experiments on the effects of ILs on rat cell lines and also human Caco-2 cell lines have been conducted,^{26,27,28} as well as enzymatic studies.²⁹

Results from these investigations prove that the toxicity of ILs increases with increasing alkyl chain length. This could be explained by the increased lipophilic character of the IL with increasing alkyl chain length, which may lead to IL incorporation into biological membranes and disruption of membrane proteins (polar narcosis).³⁰ The resulting increase in cell membrane permeability may also adversely affect the ability of cells to resist or repair membrane disruption.²¹

The contribution of ILs to anthropogenic waste is a major factor hindering their valid classification as 'green solvents'. To date, limited research has been carried out to determine the biodegradability of ILs.^{31–39}

Although an extensive range of ILs have been synthesized and tested for environmental toxicity, little research has been conducted in which the design of the ILs is the chief factor in reducing their environmental impact.⁴⁰

The work of Gathergood and Scammells in the field of biodegradable ILs began in 2002³¹ when they applied the same principles that are used in the synthesis of biodegradable surfactants to the design of ILs. A modified Sturm test (ISO9439) was used to evaluate the biodegradability of imidazolium ILs that have ester-linked side-chains. In these studies the most readily degraded IL gave a biodegradation of 59%, close to the pass level for this test (60%).

Gathergood *et al.*³² proceeded to test ILs for biodegradability, using the 'Closed Bottle Test' (OECD 301D) together with a modified Sturm test (OECD 301B). The ILs that incorporated an ester linkage in the side-chain showed a significant increase in biodegradation (close to 40%) when compared with their commercially available counterparts, [bmim][BF₄] and [bmim][PF₆] that show negligible biodegradation.

After studies by Garcia *et al.*³³ confirmed that the presence of an ester linkage promoted biodegradation, a range of ILs both with and without ester groups, were examined. Garcia determined that in both classes of IL, the octylsulfate counter ion showed the highest biodegradation. However, none of the ILs synthesized could yet be described as readily biodegradable.

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Ultimately, by combining the features of a propyl or pentyl ester side-chain with the octylsulfate counterion, Gathergood *et al.*³⁴ were able to synthesize the first 'readily biodegradable ILs'. Two complementary test methods were chosen to investigate the biodegradability of Scammells and Gathergood's ILs. Firstly, using the 'Closed Bottle Test' (OECD 301D), reference test compounds [bmim][Br] and [bmim][BF₄] showed the lowest biodegradation level (0–1%). Imidazolium bromide salts containing ester moieties showed improved biodegradation (23–33%), while the octylsulfate ILs containing esters gave a further improvement in biodegradation levels (49–56%). These results were corroborated using the CO₂ Headspace test (ISO 14593). It was with ISO 14593 that the most impressive biodegradation levels so far were obtained (60–66%), with ILs containing an ester moiety and also the octylsulfate counter anion. Monitoring the disappearance of substrates during biodegradation tests by a battery of analytical tests, including NMR and HPLC is highly desirable, but if methods that take account of respirometric parameters, such as O₂ depletion (OECD 301D) and CO₂ formation (*e.g.* ISO9439, OECD 301B, ISO 14593) are neglected then the analysis cannot be relied upon to reflect biodegradation of a test sample.⁴¹

Herein we disclose the synthesis of a library of imidazolium-based ILs that enables us to compare the toxicity and biodegradability of the reported butyl (**1a–f**) and pentyl (**2a–f**) imidazolium esters³⁴ with decyl imidazolium ester **12a**⁴² as well as novel esters derived from ethylene glycol, diethylene glycol and triethylene glycol, with oxygenated side-chains ranging from 4 to 10 atoms in length. (Fig. 1).

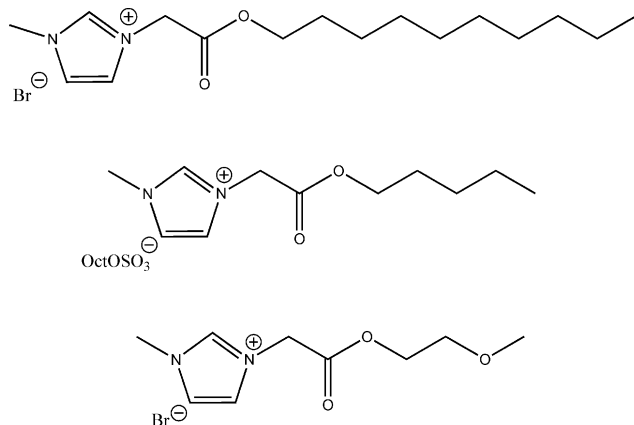


Fig. 1 Oxygen functionalized ILs.

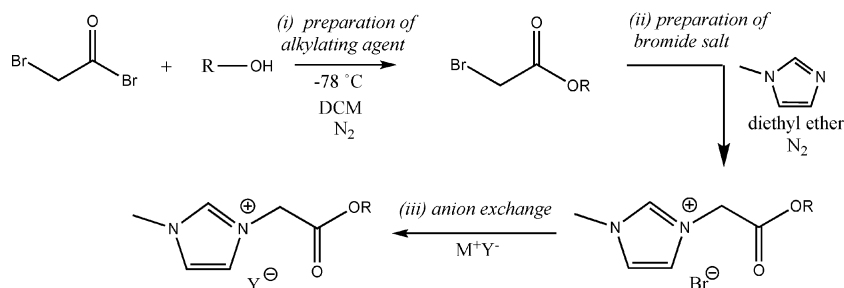


Fig. 2 2-Step synthesis of IL followed by anion exchange.

Results and discussion

Synthesis

Synthesis of the ILs described herein is carried out in two steps followed by anion exchange (Fig. 2).

The commercially available alcohols were reacted with bromoacetyl bromide to form the desired alkylating agents in good yields (62–88%). Pure 2-bromoesters were then obtained by distillation of the crude product under high vacuum to give colourless to pale yellow oils. This reaction was performed at scales up to 500 mmol with no compromise in yield or purity.

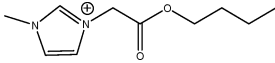
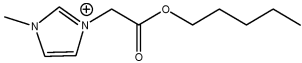
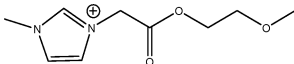
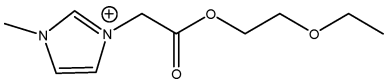
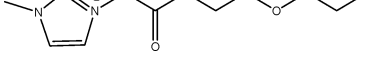
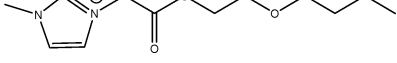
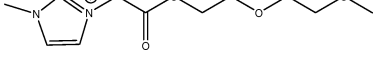
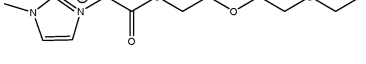
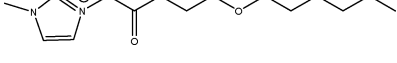
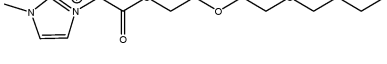
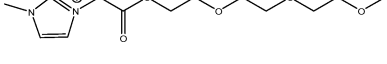
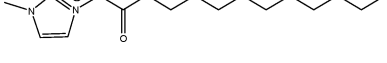
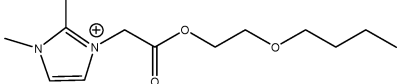
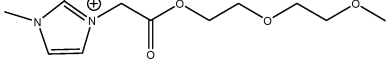
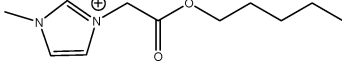
Subsequent alkylation of either 1-methylimidazole or 1,2-dimethylimidazole by the 2-bromoester at –15 °C for 3 hours, then at 20 °C for 18 hours resulted in precipitation of the desired bromide salt in good yields (82–98%). However, only moderate yields (55% and 51%, respectively) were recorded for two of the long-chain esters, **11a** and **12a**.

Although four of the bromide salts were viscous liquids (**1a**, **2a**, **13a**, **15a**) in most cases the bromides were solids at 20 °C. However, with only five exceptions (solids, **3d**, **10e**, **13f**, **15d** and **15f**) amongst the other counter-ions the remaining 66 esters were liquid at 20 °C, and even considering the solid examples, in no case did the melting point of the ester-functionalised ILs exceed 75 °C (significantly below the 100 °C limit for definition as ILs, Table 1).

The final counter-ion metathesis was carried out using potassium hexafluorophosphate, sodium tetrafluoroborate, sodium dicyanoamide, lithium trifluoromethanesulfonimide or sodium octylsulfate. This exchange is important as it results in changes to the bulk solvent properties of the corresponding IL. In most cases, the anion exchange reaction leads to ILs with decreased melting points, compared with their bromide salt analogues. This alteration in physical properties is especially marked in the case of the trifluoromethanesulfonimide (NTf₂[–]) salts, which were all liquids at 20 °C and appeared to be significantly less viscous than the other salts. The NTf₂[–] salt was readily prepared by a simple reaction of the bromide IL with lithium trifluoromethanesulfonimide in water over 4–18 hours, which gave the desired IL as a separate phase, beneath the aqueous layer. The ion-exchange reactions proceeded in good yields (69–96%) and simply washing the hydrophobic IL with water proved to be an effective means of removing trace impurities.

In common with the trifluoromethanesulfonimide ILs, the PF₆[–] salts formed a biphasic mixture with water. In most cases the hexafluorophosphate ILs exhibit slightly increased melting points, when compared with the NTf₂[–] ILs (Table 1). A different

Table 1 IL data

IL Cation						
% yield						
Melting point	Br ⁻	NTf ₂ ⁻	BF ₄ ⁻	PF ₆ ⁻	N(CN) ₂ ⁻	OctOSO ₃ ⁻
	1a 82 <i>liquid</i>	1b 86 <i>liquid</i>	1c 97 <i>liquid</i>	1d 93 <i>liquid</i>	1e 87 <i>liquid</i>	1f 61 <i>liquid</i>
	2a 97 <i>liquid</i>	2b 93 <i>liquid</i>	2c 95 <i>liquid</i>	2d 98 <i>liquid</i>	2e 98 <i>liquid</i>	2f 96 <i>liquid</i>
	3a 89 53–55 °C	3b 91 <i>liquid</i>	3c 95 <i>liquid</i>	3d 96 58–60 °C	3e 80 <i>liquid</i>	3f 95 <i>liquid</i>
	4a 93 24–26 °C	4b 90 <i>liquid</i>	4c 96 <i>liquid</i>	4d 98 <i>liquid</i>	4e 99 <i>liquid</i>	4f 96 <i>liquid</i>
	5a 88 25–27 °C	5b 68 <i>liquid</i>	5c 97 <i>liquid</i>	5d 97 <i>liquid</i>	5e 91 <i>liquid</i>	5f 85 <i>liquid</i>
	6a 89 28–30 °C	6b 84 <i>liquid</i>	6c 96 <i>liquid</i>	6d 95 <i>liquid</i>	6e 51 <i>liquid</i>	6f 93 <i>liquid</i>
	7a 97 52–54 °C	7b 91 <i>liquid</i>	7c 94 <i>liquid</i>	7d 91 <i>liquid</i>	7e 94 <i>liquid</i>	7f 82 <i>liquid</i>
	8a 92 28–30 °C	8b 87 <i>liquid</i>	8c 96 <i>liquid</i>	8d 96 <i>liquid</i>	8e 99 <i>liquid</i>	8f 93 <i>liquid</i>
	9a 98 32–34 °C	9b 82 <i>liquid</i>	9c 93 <i>liquid</i>	9d 91 <i>liquid</i>	9e 85 <i>liquid</i>	9f 98 <i>liquid</i>
	10a 94 48–50 °C	10b 86 <i>liquid</i>	10c 92 <i>liquid</i>	10d 79 <i>liquid</i>	10e 98 34–36 °C	10f 92 <i>liquid</i>
	11a 55 59–61 °C	11b 93 <i>liquid</i>	11c 94 <i>liquid</i>	11d 57 <i>liquid</i>	11e 75 <i>liquid</i>	11f 84 <i>liquid</i>
	12a 51 49–51 °C	12b 95 <i>liquid</i>	n/a	n/a	n/a	12f 79 <i>liquid</i>
	13a 92 <i>liquid</i>	13b 83 <i>liquid</i>	13c 95 <i>liquid</i>	13d 97 <i>liquid</i>	13e 78 <i>liquid</i>	13f 84 50–52 °C
	14a 88 74–75 °C	14b 96 <i>liquid</i>	14c 94 <i>liquid</i>	14d 95 <i>liquid</i>	14e 99 <i>liquid</i>	14f 94 <i>liquid</i>
	15a 81 <i>liquid</i>	15b 95 <i>liquid</i>	15c 93 <i>liquid</i>	15d 97 40–42 °C	15e 85 <i>liquid</i>	15f 86 63–65 °C

method of anion exchange was used to prepare the hexafluorophosphates, in which the 3-substituted 1-methylimidazolium bromide salt was refluxed in acetone for four days in the presence of potassium hexafluorophosphate to give the PF_6^- ILs in high yields (> 90%). The BF_4^- ILs were synthesized analogously to the PF_6^- salts using sodium tetrafluoroborate, giving ILs in equally good yields (> 90%). The corresponding $\text{N}(\text{CN})_2^-$ salts were again formed by reaction of the bromide IL with the sodium salt for four days, however the solvent was changed from acetone to acetonitrile and refluxing was unnecessary, with good yields (51–99%) of exchanged dicyanoamide IL obtained even at 20 °C. To obtain the IL octylsulfate, the bromide salt of the IL and sodium octylsulfate were stirred in water for 2 hours at 60 °C, according to the literature method.⁴³ The water was then slowly removed under vacuum. The resulting precipitate was subsequently dissolved in dichloromethane and washed with a small amount of distilled water. After evaporation of the solvent, the exchanged octylsulfate ILs were obtained in good yields, usually between 80 and 98%. Although two of the octylsulfate ILs (**13f** and **15f**) are solids at 20 °C, they still exhibit melting points significantly below 100 °C (Table 1). A study of hydrogenation vs. hydrogenolysis of benzyl cinnamate in ILs (**2b**, **3b**, **4b**, **5b**, **6b**, **9b**, **11b** and **15b**; **2f** and **5f**) prepared above has recently been reported by Gathergood *et al.*⁴⁴

Preparation of amide ILs

The method of preparation of the amide ILs is analogous to that for the ester derivatives. The only notable difference was that in the case of **19a** recrystallisation from diethyl ether was used to purify the intermediate bromoacetamide, rather than vacuum distillation. Good yields were obtained for the majority of the bromide and octylsulfate amide derivatives, with elevated melting points again recorded for the bromide salts (Table 2).

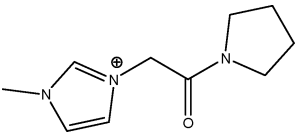
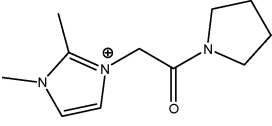
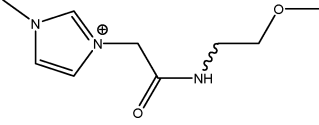
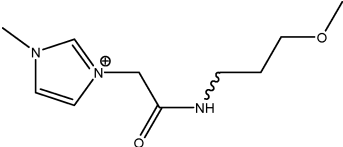
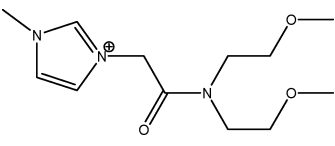
Although the 3-methoxypropylamide and bis(2-methoxyethyl)amide 3-methylimidazolium bromide salts (**19a** and **20a**, respectively) were solids at 20 °C, they both had melting points under 100 °C, technically qualifying them as ionic liquids. However, the pyrrolidine amide 3-methylimidazolium **16a** and 2,3-dimethylimidazolium bromide **17a** salts had melting points above 100 °C and hence were not classified as ionic liquids. In contrast, 2-methoxyethyl 3-methylimidazolium bromide was a liquid at 20 °C, as were octylsulfates **16f** and **20f**, derived from **16a** and **20a** by the previous anion-exchange process.

Toxicity

Seven strains of bacteria were used to assess the antimicrobial activity of the ILs: 4 Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella sp.*, *Salmonella sp.*) and three Gram positive (*Staphylococcus aureus*, *Enterococcus sp.*, *Bacillus subtilis*) organisms.

The minimum inhibitory concentrations were measured for those ILs that showed activity against any of the seven strains. A wide concentration range was tested (0–20 mg/mL). The MIC values for typical cationic antiseptic/antibacterial agents generally lie in the range of 8 µg/mL to 500 µg/mL.⁴⁵ The commercial disinfectants, BAC (benzalkonium chloride, a mixture of quaternary ammonium salts with hydrocarbon chains of 8, 10, 12, 14, 16 and 18 atoms length) and CPC

Table 2 Amide IL data

Amide (cation)	% yield	
Melting point	[Amide cat.]-[Br ⁻]	[Amide cat.]-[OctOSO ₃ ⁻]
	16a 56 109–111 °C	16f 97 liquid
	17a 82 142–144 °C	17f 98 liquid
	18a 97 liquid	n/a
	19a 91 75–77 °C	n/a
	20a 91 68–70 °C	20f 92 liquid

(cetylpyridinium chloride, a pyridinium salt with a 16-carbon aliphatic chain) both have reported MIC values of 8 µg/mL for *S. aureus* 209, *S. aureus* R209 and *B. subtilis*. In the case of CPC, which is a discrete entity, having a molecular mass of 340 Da, this represents a MIC of 23.5 µM. Ionic liquids with long hydrocarbon chains also exhibit toxic properties, with the C-12-substituted 1-methyl-3-dodecylimidazolium bromide (MIC from 8 µg/mL to 32 µg/mL,⁴⁵ *i.e.* 24.2 to 96.7 µM) and especially C-14/C-16 showing pronounced biocidal properties (1-methyl-3-tetradecylimidazolium chloride/1-methyl-3-hexadecylimidazolium bromide – MICs from 4 µg/mL to 8 µg/mL,⁴⁵ (12.7 to 25.4 µM, C-14/10.3 to 20.7 µM, C-16) over a range of 10 bacteria and 2 fungi). The C-10 chain ionic liquid, 1-methyl-3-decylimidazolium chloride is also a good antiseptic, with MIC values of 8 µg/mL (30.9 µM) for *E. coli*, 16 µg/mL (61.8 µM) for *S. aureus* 209, and 32 µg/mL (123.6 µM) for *S. aureus* R209. Higher MICs were recorded for *S. typhimurium* and *B. subtilis* (both 125 µg/mL, *i.e.* 482.9 µM) and fungal strains *C. albicans* and *C. regularis* (both 250 µg/mL, 965.9 µM).⁴⁵ In fact imidazolium ionic liquids in which an ester linkage is used to attach a hydrocarbon chain (C-1 to 18) to the cationic core have recently been the subject of a patent application for anti-microbial compositions in the preservation of personal care products and cosmetics.⁴² In contrast, a number of our ILs containing ethereal side-chains linked *via* an ester to

the imidazole core exhibited no toxicity even at concentrations above 20 mg/mL, against any of 7 bacterial strains screened. Non-toxicity across a broad range of microorganisms has also been reported by Pernak, who synthesized 1-alkylimidazolium DL-lactates (5 examples, alkyl varying from methyl to pentyl) with MIC values >5.814 mM across a range of 5 Gram-negative rods, 5 Gram-positive cocci and 2 fungal strains.¹⁶ For our non-toxic ILs, MIC values greater than 20 mg/mL correspond to a lack of toxicity at concentrations from >27 mM to >75 mM, depending on the molecular mass of the IL. In most cases, all 7 bacterial strains showed no sensitivity towards each IL (MIC is quoted as $>$ test concentration when no toxicity was shown at the concentration tested). These data are summarised in Table 3.

A toxicity study with the ester, 1-methyl-3-(decyloxy-carbonyl)methylimidazolium bromide (**12a**) which is known to exhibit toxicity due to its long alkyl chain, was completed as a reference. This experiment compares, for the same side chain length, the impact of the presence of the ether oxygens (in **11a**) on toxicity. As expected **12a** was toxic to all the different bacteria screened (Table 4), and in some cases even at low concentrations (Table 4 *E. coli*, *Enterococcus sp.* and *S. aureus*). A comparison of the results in Table 4 with those from Table 3, (**11a**), indicates that the presence of oxygen in the side chain is crucial to suppress the toxicity.

From the data collected it is apparent that all the bromide salts with an oxygenated side chain are non-toxic. At the forefront of

our results is the fact that IL series **1** and **2** (which lack ethereal side-chains) are the most toxic tested in this study (each showing toxicity with 3 different anions). These ILs, which have highly lipophilic cations, are the only ones to display toxicity with three different anions. The lipophilic octylsulfate salts (**1f**, **8f**, **10f**, **13f**, **14f**) together with the NTf_2^- (**1b**, **2b**, **3b**, **5b**, **7b**) salts were among the most toxic imidazolium salts studied, showing the highest number of ILs with MIC values in the range 2.5–10 mg/mL.

Results from the BF_4^- series provided further evidence for the toxicity of an alkyl chain, with the only toxic examples out of all the BF_4^- salts occurring in cases **2c** and **15c** (both containing pentyl ester side-chains without ether linkages). A similar trend was observed for the $\text{N}(\text{CN})_2^-$ salts in which only butyl (**1e**) and pentyl (**2e**) esters exhibited toxicity. However, a notable exception was observed when incorporation of a methyl group at C-2 of the imidazole ring in pentyl ester **15e** abolished the toxicity previously recorded for C-2 unsubstituted pentyl ester **2e**.

As previously mentioned, toxicity is frequently encountered with ionic liquids containing an extended hydrocarbon chain. Notably, Bodor *et al.*^{46–50} had shown that a long chain ester derivative of methyl imidazole (compound **21** in Fig. 3) shows effective antimicrobial activity at ppm concentrations.

The results in Table 3 show that all the ionic liquids prepared show significantly lower toxicity than derivatives without ester and ether or poly ether functional groups. ILs **17a**, **19a**, **20a** and **20f** demonstrate that the presence of oxygen atoms in the

Table 3 MIC^a values (low toxicities [high MIC] were recorded for all bacterial strains)

Strain	Counter Anion																			
(I–VII)	Bromide																			
	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	13a	14a	15a	16a	17a	18a	19a	20a	
	>72	>69	>72	>68	>65	>62	>62	>59	>60	>55	>54	>60	>59	>66	>73	>69	>72	>68	>60	
(I–VII)	Trifluoromethanesulfonimide																			
	1b	2b	3b	4b	5b	6b	7b	8b	9b	10b	11b	13b	14b	15b	16b					
	21	20	21	>41	19	>38	19	>37	>36	>35	>35	>37	>37	>40	n/a					
(I–VII)	Tetrafluoroborate																			
	1c	2c	3c	4c	5c	6c	7c	8c	9c	10c	11c	13c	14c	15c	16c					
	>70	34	>70	>67	>64	>61	>61	>58	>56	>54	>53	>27	>27	7.2	n/a					
(I–VII)	Hexafluorophosphate																			
	1d	2d	3d	4d	5d	6d	7d	8d	9d	10d	11d	13d	14d	15d	16d					
	>58	>56	>58	>56	>54	>52	6.4	>50	>48	>47	>46	>50	>50	>54	n/a					
(I–VII)	Dicyanoamide																			
	1e	2e	3e	4e	5e	6e	7e	8e	9e	10e	11e	13e	14e	15e	16e					
	19	36	>75	>72	>68	>65	>65	>62	>59	>57	>57	>28	>28	>30	n/a					
(I–VII)	Octylsulfates																			
	1f	2f	3f	4f	5f	6f	7f	8f	9f	10f	11f	13f	14f	15f	16f	17f	18f	19f	20f	
	12	>48	>49	>47	>46	>44	>44	21	>42	20	>40	22	>21	>46	>50	>48	n/a	n/a	>42	

^a In mM, the number of microorganisms in 1 mL range from 10^4 to 10^5 n/a indicates compound was not synthesized.

Table 4 Percentage kill for seven strains of bacteria at various concentrations of **12a**

Strain (I–VII)	12a Concentration (μg/mL)								
	1000	500	250	125	63	31.5	15.75	7.88	3.94
	2.8 mM	1.4 mM	692.6 μM	346.3 μM	173.2 μM	86.6 μM	43.3 μM	21.6 μM	10.8 μM
(I) <i>E. coli</i>	91	82	92	92	37	28	17	9	10
(II) <i>Enterococcus sp.</i>	96	84	83	50	49	19	23	6	7
(III) <i>P. aeruginosa</i>	100	82	82	56	0	6	0	0	0
(IV) <i>Salmonella sp.</i>	86	88	74	58	10	0	0	0	0
(V) <i>Klebsiella sp.</i>	92	68	64	21	7	0	0	0	0
(VI) <i>S. aureus</i>	—	96	94	81	90	86	88	90	66
(VII) <i>B. subtilis</i>	100	83	82	80	0	0	0	0	0

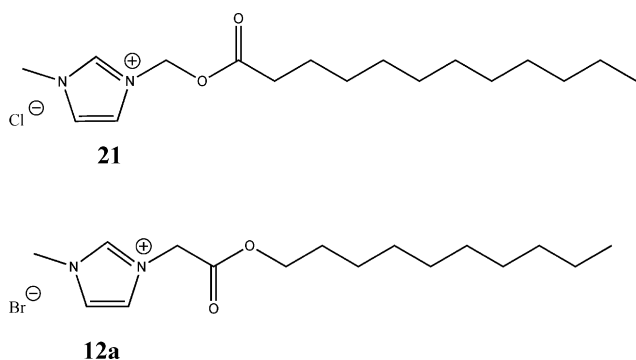


Fig. 3 Comparison of structures of **21** 1-[(*n*-undecylcarbonyloxy)-methyl]-3-methylimidazolium chloride and **12a** (3-methyl-1-(*n*-decyloxy-carbonylmethyl) imidazolium bromide. Both are potent antibacterials.

side chain of amide derivatives also leads to low toxicity ILs, when compared with ILs containing hydrocarbon side-chains of similar length. (e.g. If **20a** is compared with dodecyl substituted imidazolium salts *vide supra*). These results have significant implications for the usefulness of the ILs, as the toxicity is exceptionally low.

Biodegradability

CO₂ Headspace test. To evaluate the biodegradability of the test ionic liquids, the “CO₂ Headspace” test (ISO 14593)³¹ was implemented. This method allows the evaluation of the ultimate aerobic biodegradability of an organic compound in an aqueous medium at a given concentration of microorganisms by analysis of inorganic carbon. The test ionic liquid, as the sole source of carbon and energy, was added at a concentration of 40 mg L⁻¹ to a mineral salt medium. These solutions were inoculated with activated sludge collected from an activated sludge treatment plant, washed and aerated prior to use and incubated in sealed vessels with a headspace of air. Biodegradation (mineralization to carbon dioxide) was determined by measuring the net increase in total organic carbon (TOC) levels over time.

Biodegradation data. Results for biodegradation of the octylsulfates at weekly intervals over 28 days are tabulated in the ESI† and represented graphically in Figs. 4–6.

ILs, **2f**, **5f**, **6f**, **9f**, **10f** and **13f** passed the CO₂ Headspace test (at least 60% over 28 days duration) and clearly are “readily biodegradable” according to this test (see Fig. 4 and Fig. 5). ILs **1f**, **3f**, **4f**, **7f**, and **8f** all show significant biodegradation (between 55–59% in CO₂ Headspace test) and represent a marked improvement over the negligible biodegradation result obtained for the imidazolium based ILs, [bmim][BF₄] and [bmim][PF₆].^{31,33,34} A decrease in biodegradation potential can be seen for the amide ILs, with none surpassing a threshold of 40% in the CO₂ Headspace test after 28 days (Fig. 6). This fact emphasises the importance of an ester linkage for successful biodegradation of these imidazolium ILs.

Conclusions

Several factors that are important in the development of green solvents for industry are melting point, toxicity and biodegradation. We have demonstrated that for a series of ionic liquids containing a wide range of ether and poly ether esters

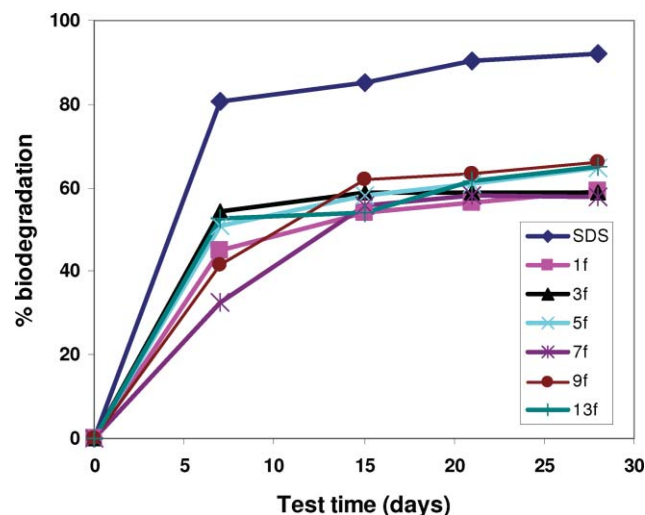


Fig. 4 Biodegradation of selected ILs.

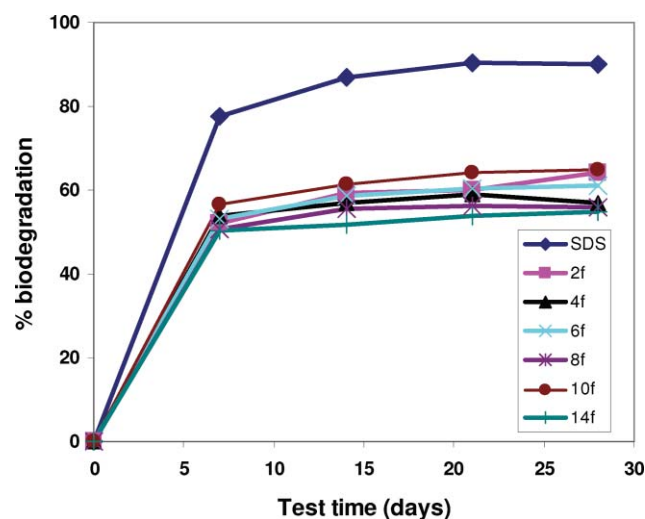


Fig. 5 Biodegradation of selected ILs.

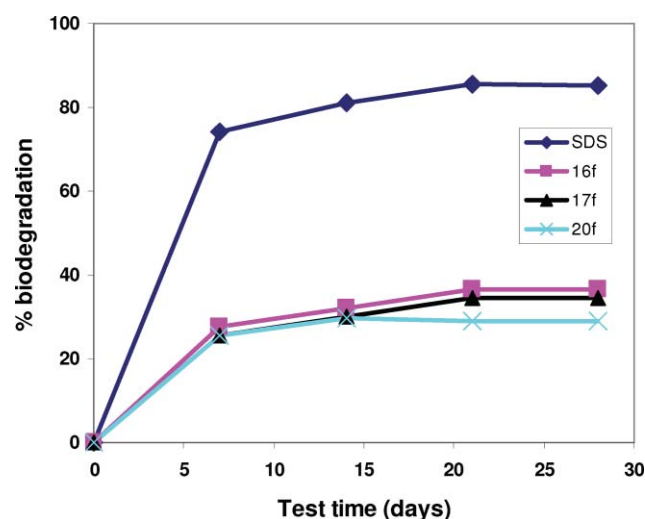


Fig. 6 Biodegradation of selected ILs.

many are liquid at room temperature and all have melting points below 100 °C. Examples containing extended poly ether side chains, *e.g.* **9b-f**, are liquid at room temperature. Toxicity studies show a clear trend that for all the ionic liquids prepared containing ether or poly ether side-chains a significant reduction in toxicity is observed, compared with the long chain alkyl substituted derivatives. **9a-f** and **10a-f** contain extended side-chains but nevertheless show no antimicrobial toxicity even at concentrations above 20 mg/mL (36–59 and 20–57 mM respectively, depending on molecular mass). Biodegradation results support previous studies demonstrating that an ester group is preferred over an amide. Whilst ILs with a butoxy- or propoxy- terminus are readily biodegradable, those with methoxy- or ethoxy- terminal substitution remained slightly below the 60% threshold for biodegradability.

Experimental

All chemicals were purchased from Aldrich, with the exception of lithium trifluoromethanesulfonimide, which was purchased from Solvionic. 1-Methylimidazole and bromoacetyl bromide were used without further purification. 1-Butanol, 1-pentanol, 2-(2-methoxyethoxy) ethanol, 2-(2-ethoxyethoxy) ethanol, 2-(2-propoxyethoxy) ethanol, 2-(2-butoxyethoxy) ethanol, 2-methoxyethanol, 2-ethoxyethanol, 2-propoxyethanol, 2-butoxyethanol, and 1-decanol were dried over 4 Å molecular sieves and used without further purification. 1,2-Dimethylimidazole was distilled before use. All organic solvents were dried and distilled before use. All NMR spectra of ILs were recorded in deuterated chloroform, acetonitrile or acetone on a Bruker 400 MHz spectrometer.

A representative synthetic method is given for each group of compounds prepared. All other information may be found in the ESI.†

CO₂ Headspace test

To evaluate the biodegradability of the test ionic liquids, the “CO₂ Headspace” test (ISO 14593) was also applied.⁵² This method allows the evaluation of the ultimate aerobic biodegradability of an organic compound in aqueous medium at a given concentration of microorganisms by analysis of inorganic carbon. The test ionic liquid, as the sole source of carbon and energy, was added at a concentration of 40 mg L⁻¹ to a mineral salt medium. These solutions were inoculated with activated sludge collected from an activated sludge treatment plant, washed and aerated prior to use and incubated in sealed vessels with a headspace of air. Biodegradation (mineralization to carbon dioxide) was determined by measuring the net increase in total organic carbon (TOC) levels over time compared with blanks. Sodium n-dodecyl sulfate (SDS) was used as a reference substance. The tests ran for 28 days and the extent of biodegradation was expressed as a percentage of the theoretical amount of inorganic carbon, based on the amount of test compound added at the start. CO₂ was estimated by GC according to the equation:

$$\% \text{CO}_2 (\text{headspace}) = \frac{(\text{GC peak height}_{\text{sample}} - \text{GC peak height}_{\text{air}})}{\text{GC peak height}_{\text{standard}}} \times \% \text{CO}_2 (\text{standard})$$

Toxicity studies

Minimum inhibitory concentrations (MICs) for the compounds were determined by serial two-fold dilutions in Mueller-Hinton broth using the broth microdilution method described by Amsterdam.⁵²

Strains were grown in Mueller-Hinton broth overnight. The compound solution and 96-well plates were ready before the cultures reached the desired growth phase. The compound to be tested was dissolved in sterile water and diluted in the test medium to twice the maximum concentration desired in the test, *i.e.*, the highest desired concentration was 20 mg/mL, so the ionic liquid was diluted to 40 mg/mL.

μL of Mueller-Hinton broth was dispensed into all wells of a microtitre plate. 100 μL of the 2× compound solution was pipetted into the wells in column 1 (far left of plate). The compound was mixed into the wells in column 1 by pipetting up and down 6–8 times. 100 μL was withdrawn from column 1 and added to column 2. This made column 2 a two-fold dilution of column 1. This was mixed up and down 6–8 times. 100 μL was transferred to column 3. This procedure was repeated down to column 10 only. 100 μL was discarded from column 10 rather than putting it in column 11. 5 μL of (2 × 10⁴ CFU/mL) the strain to be tested was dispensed into wells in the columns 11 to 1 in that order. Column 12 was used as a sterility control. The plates were incubated at 37 °C. Growth on the plates was noted and recorded after 18–36 hours. The MIC was the lowest concentration of the compound that completely inhibited growth of the organism in the microdilution wells as detected by the unaided eye.

Compounds that were water-insoluble were diluted in methanol. The MICs for these compounds were obtained using the same procedure described above. However, instead of diluting the compound directly in Mueller-Hinton broth, 20 μL of the solution (to give a final concentration of 20 mg/mL) was added to the first well in the row. The methanol was allowed to evaporate and then 200 μL of Mueller-Hinton broth was added, mixed and diluted using serial two-fold dilutions as described above.

Preparation of 2-propoxyethyl 2-bromoacetate

To a stirred solution of dichloromethane (350 mL), propoxyethanol (47.84 mL, 460 mmol), and triethylamine (69.3 mL, 500 mmol), under a nitrogen atmosphere at –78 °C was added dropwise bromoacetyl bromide (92.92 g, 460 mmol). After stirring at –78 °C for 3 h, the reaction mixture was allowed to warm up to –20 °C and quenched by addition of water (50 mL). The organic phase was washed with distilled water (3 × 25 mL), saturated ammonium chloride (3 × 25 mL), saturated sodium bicarbonate (3 × 25 mL) and brine (2 × 25 mL). The organic phase was then dried over magnesium sulfate, filtered and solvents removed *via* rotary evaporation. The crude product was distilled (bp 100–102 °C) to give a pale yellow liquid in 83% yield (85.91 g, 382 mmol).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 4.34 (t, *J* = 4.6 Hz, 2H), 3.88 (s, 2H), 3.67 (t, *J* = 4.6 Hz, 2H), 3.47 (t, *J* = 6.9 Hz, 2H), 1.66 (tq, *J* = 6.9, 7.3 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ (ppm) 167.31, 73.07, 68.10, 65.42, 25.90, 22.81, 10.52.

Preparation of 3-methyl-1-(2-(2-*n*-propoxyethoxy)-ethoxycarbonylmethyl) imidazolium bromide (9a)

To a stirred solution of 1-methylimidazole (50 mmol, 4.10 g) in diethyl ether (100 mL) at -15°C under a nitrogen atmosphere was added dropwise 2-(2-propoxyethoxy)ethyl-2-bromoacetate (60 mmol, 16.14 g). The reaction mixture was stirred vigorously at -15°C for 3 h, then at 20°C for 16 h. The diethyl ether phase was decanted and the IL washed with diethyl ether (2×30 mL), then residual solvent removed on the rotary evaporator. The product was dried under high vacuum for 8 h yielding a white solid in 98% yield (17.16 g, 48.89 mmol).

^1H NMR (400 MHz, CDCl_3) δ (ppm) 10.13 (s, 1H), 7.54 (t, $J = 1.8$ Hz, 1H), 7.34 (t, $J = 1.8$ Hz, 1H), 5.45 (s, 2H), 4.31 (t, $J = 4.6$ Hz, 2H), 4.02 (s, 3H), 3.68 (t, $J = 4.6$ Hz, 2H), 3.59–3.57 (m, 2H), 3.53–3.50 (m, 2H), 3.35 (t, $J = 6.9$ Hz, 2H), 1.53 (tq, $J = 6.9$, 7.3 Hz, 2H), 0.84 (t, $J = 7.3$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 166.21, 138.54, 123.85, 122.84, 73.10, 70.62, 69.92, 68.55, 65.69, 50.29, 36.93, 22.76, 10.52.

MP ($^{\circ}\text{C}$) 32–34 $^{\circ}\text{C}$.

IR (KBr disc) (cm^{-1}) 2959, 2926, 2859, 1751, 1558, 1639, 1495, 1452.

MS m/z , Found 271.1648 $[\text{M}-\text{Br}]^+$, Calcd. $\text{C}_{13}\text{H}_{23}\text{N}_2\text{O}_4$ 271.1658.

MS m/z , 271.2 $[\text{M}-\text{Br}]^+$; MS: m/z , 79 and 81 $[\text{Br}]^-$.

Preparation of 3-methyl-1-(2-(ethoxy)ethoxycarbonylmethyl) imidazolium NTf_2^- (4b)

A flask was charged with 3-methyl-1-(2-(ethoxy)ethoxycarbonylmethyl) imidazolium bromide (2.93 g, 10.0 mmol) and distilled water (10 mL). LiNTf_2 (4.59 g, 16.0 mmol) in distilled water (3 mL) was added in one portion and the suspension was stirred vigorously for 4 h at 20°C . The top aqueous layer was removed and the IL was washed with distilled water (3×10 mL). The solvent was then removed on the rotary evaporator and under high vacuum for 8 h to give a liquid at 20°C in 90% yield (4.42 g, 8.97 mmol).

^1H NMR (400 MHz, CDCl_3) δ (ppm) 8.82 (s, 1H), 7.39 (t, $J = 1.8$ Hz, 1H), 7.34 (t, $J = 1.8$ Hz, 1H), 5.06 (s, 2H), 4.38 (t, $J = 4.6$ Hz, 2H), 3.97 (s, 3H), 3.68 (t, $J = 4.6$ Hz, 2H), 3.56 (q, $J = 7.1$ Hz, 2H), 1.22 (t, $J = 7.1$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 165.76, 137.63, 123.80, 123.25, 119.70 (q, $J = 319$ Hz, 2CF_3 's), 67.62, 66.67, 65.97, 49.92, 36.56, 15.01.

IR (thin film on salt plate) (cm^{-1}) 3169, 3116, 2967, 2927, 2859, 1751, 1581, 1569, 1558, 1495, 1452, 1352, 1196, 1135.

MS m/z , 213.1 $[\text{M}-\text{NTf}_2]^-$; MS: m/z , 280.0 $[\text{NTf}_2]^-$.

Preparation of 3-methyl-1-(2-(2-methoxyethoxy)-ethoxycarbonylmethyl) imidazolium PF_6^- (7d)

A flask was charged with 3-methyl-1-(2-(2-methoxyethoxy)-ethoxycarbonylmethyl) imidazolium bromide (3.55 g, 11.0 mmol) and acetone (10 mL). KPF_6 (3.31 g, 18.0 mmol) in acetone (5 mL) was added in one portion and the suspension was stirred vigorously for 4 days under reflux. The fine white precipitate was then filtered and washed with acetone (2×5 mL). The solvent was removed from the product on the rotary evaporator. The product was then dried under high vacuum

for 4 h to give viscous liquid at 20°C in 91% yield (3.87 g, 9.97 mmol).

^1H NMR (400 MHz, CDCl_3) δ (ppm) 8.60 (s, 1H), 7.53 (tt, $J = 1.8$, 1.8 Hz, 2H), 5.59 (s, 2H), 4.45 (t, $J = 4.6$ Hz, 2H), 4.00 (s, 3H), 3.82 (t, $J = 4.6$ Hz, 2H), 3.72 (t, $J = 4.6$ Hz, 2H), 3.62 (t, $J = 4.6$ Hz, 2H), 3.44 (s, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 165.98, 136.76, 123.33, 123.17, 71.16, 69.69, 67.85, 65.23, 57.54, 49.44, 35.84.

IR (thin film on salt plate) (cm^{-1}) 3172, 3124, 2926, 1751, 1580, 1569, 1559, 1495, 1457, 1218, 1181, 1106.

MS m/z , 243.2 $[\text{M}-\text{PF}_6]^-$; MS: m/z , 145.0 $[\text{PF}_6]^-$.

Preparation of 3-methyl-1-(2-(2-*n*-propoxyethoxy)-ethoxycarbonylmethyl) imidazolium BF_4^- (9c)

A dry flask was charged with 3-methyl-1-(2-(2-*n*-propoxyethoxy)ethoxycarbonylmethyl) imidazolium bromide (2.94 g, 8.38 mmol) and acetone (10 mL) under a nitrogen atmosphere. NaBF_4 (1.11 g, 10.1 mmol) was added in one portion and the suspension was stirred vigorously for 4 days under reflux. The fine white precipitate was filtered quickly in air and washed with dry acetone (2×3 mL). The filtrate and washings were combined, solvent removed by rotary evaporation and then under high vacuum to give a slight viscous oil at 20°C in 93% yield (2.79 g, 7.79 mmol).

^1H NMR (400 MHz, CDCl_3) δ (ppm) 8.95 (s, 1H), 7.45 (t, $J = 1.8$ Hz, 1H), 7.37 (t, $J = 1.8$ Hz, 1H), 5.12 (s, 2H), 4.38 (t, $J = 4.7$ Hz, 2H), 3.97 (s, 3H), 3.75 (t, $J = 4.7$ Hz, 2H), 3.67 (t, $J = 3.2$ Hz, 2H), 3.60 (t, $J = 3.2$ Hz, 2H), 3.44 (t, $J = 6.8$ Hz, 2H), 1.64 (tq, $J = 6.8$, 7.6 Hz, 2H), 0.94 (t, $J = 7.6$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 166.23, 137.96, 123.79, 123.13, 73.06, 70.54, 69.89, 68.54, 65.66, 49.85, 36.52, 22.75, 10.49.

IR (thin film on salt plate) (cm^{-1}) 3166, 3121, 2964, 2927, 2866, 1750, 1581, 1574, 1569, 1558, 1495, 1452, 1220, 1181.

MS m/z , 271.3 $[\text{M}-\text{BF}_4]^-$; MS: m/z , 87.0 $[\text{BF}_4]^-$.

Preparation of 3-methyl-1-(*n*-butoxycarbonylmethyl) imidazolium $\text{N}(\text{CN})_2^-$ (1e)

A dry flask was charged with 3-methyl-1-(*n*-butoxycarbonylmethyl) imidazolium bromide (3.05 g, 11.0 mmol) and acetonitrile (10 mL) under a nitrogen atmosphere. $\text{NaN}(\text{CN})_2$ (1.42 g, 16.0 mmol) was added in one portion and the suspension was stirred vigorously for 4 days at 20°C . The fine white precipitate was filtered quickly in air and washed with dry acetonitrile (2×1 mL). The filtrate and washings were combined, solvent removed by rotary evaporation and then under high vacuum to give a light yellow oil at 20°C in 87% yield (2.51 g, 9.54 mmol).

^1H NMR (400 MHz, CDCl_3) δ (ppm) 9.82 (s, 1H), 7.56 (t, $J = 1.8$ Hz, 1H), 7.46 (t, $J = 1.8$ Hz, 1H), 5.32 (s, 2H), 4.15 (t, $J = 6.8$ Hz, 2H), 4.02 (s, 3H), 1.61 (tt, $J = 6.8$, 7.2 Hz, 2H), 1.33 (tq, $J = 7.2$, 7.5 Hz, 2H), 0.87 (t, $J = 7.5$ Hz, 3H).

^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 164.10, 136.12, 121.89, 121.18, 64.85, 48.22, 34.87, 28.31, 16.96, 11.67 Note: C's from anion are not visible in ^{13}C NMR.

IR (thin film on salt plate) (cm^{-1}) 2962, 2931, 2861, 2241, 2139, 1750, 1569, 1558, 1539, 1495, 1452, 1217, 1177.

MS m/z , 197.1 $[\text{M}-\text{N}(\text{CN})_2]^-$; MS: m/z , 66.0 $[\text{N}(\text{CN})_2]^-$.

Preparation of 3-methyl-1-(2-(*n*-propoxy)ethoxycarbonylmethyl)imidazolium octylsulfate (5f)

To a solution of 3-methyl-1-(2-(*n*-propoxy)ethoxycarbonylmethyl)imidazolium bromide (3.68 g, 12.0 mmol) in distilled water (20 mL) was added in one portion sodium octylsulfate (2.09 g, 9.00 mmol). The reaction was stirred at 60 °C for 2 h and then water was slowly removed under vacuum. The precipitate was dissolved in DCM (10 mL) and washed with distilled water (2 × 5 mL). The product remaining was dried on the rotary evaporator and then under high vacuum for 8 h to yield a yellow grease in 85% yield (3.33 g, 7.62 mmol).

¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.45 (s, 1H), 7.48 (t, *J* = 1.6 Hz, 1H), 7.41 (t, *J* = 1.6 Hz, 1H), 5.25 (s, 2H), 4.36 (t, *J* = 4.7 Hz, 2H), 4.01 (m, 5H), 3.67 (t, *J* = 4.7 Hz, 2H), 3.43 (t, *J* = 6.8 Hz, 2H), 1.63–1.58 (m, 4H), 1.56–1.29 (m, 10H), 0.92–0.86 (m, 6H).

¹³C NMR (100 MHz, CDCl₃) δ (ppm) 166.45, 138.89, 123.71, 123.06, 73.04, 67.92, 67.89, 65.67, 49.91, 36.58, 31.83, 29.50, 29.36, 29.26, 25.87, 22.73, 22.66, 14.13, 10.47.

IR (thin film on salt plate) (cm⁻¹) 3118, 2958, 2927, 2855, 1750, 1569, 1558, 1539, 1495, 1455, 1217, 1178, 1108.

MS *m/z*, 227.1 [M-OctOSO₃]⁺; MS: *m/z*, 209.0 [OctOSO₃]⁻.

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