Further studies on the biodegradation of ionic liquids†

Leigh Ford,[a] Jitendra R. Harjani,[a] Farzad Atefi,[a] M. Teresa Garcia,[b] Robert D. Singer[c] and Peter J. Scammells*[a]

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A range of ionic liquids (ILs) containing a pyridinium cation were synthesised and their biodegradability was evaluated using the CO_2 headspace test (ISO 14593). ILs bearing a 1-(2-hydroxyethyl) side chain were prepared from either pyridine or nicotinic acid derivatives. These ILs showed high levels of biodegradation under aerobic conditions and can be classified as 'readily biodegradable'. In contrast, pyridinium ILs with methyl or ethyl ether side chains showed significantly lower levels of biodegradability in the same test. Biodegradation studies on a range of novel ILs with acetal and carbamate functionalities, as well as thiazolium-based salts, also showed low levels of mineralization.

Introduction

The study of ionic liquids (ILs) has increased exponentially during the last decade.[1] ILs no longer simply represent an alternative reaction or extraction media, but are utilised in such diverse applications as waste recycling, gas treatment, hydrogen storage and energy production.[2] Both the industrial and academic sectors have realised their potential, resulting in the constant development of novel applications.

Several advantages exist in the use of ILs over more conventional solvents, including reusability, thermal and chemical stability, high solvating capacity and a wide electrochemical window. ILs are often touted as being "green" solvents due to their low vapour pressure, flammability and toxicity, though the latter is not always the case.[3] Despite the "green" aspects of ILs, it is irresponsible to ignore the ultimate fate of any chemical released into the environment; bioaccumulation may result in chronic toxicity issues, and thus for any IL to be considered "green" with respect to conventional reaction media, its long term impact must be evaluated, particularly as the use of ILs increases.

Previous studies within our group[4] and by others,[5] have focused on synthesising non-toxic ILs that undergo aerobic biodegradation by microorganisms, leading to innocuous by-products (water, CO_2 and/or biomass), as a pathway that represents a minimal environmental impact and a means of generating truly green solvents. The biodegradability and toxicity of ILs has been the subject of a number of reviews.[6]

A common motif of many biodegradable ILs identified to date is a pyridinium core, a design instigated by several studies showing an enhanced susceptibility of the pyridinium ring towards microbial degradation.[4e,4f,7] Our studies highlighted the excellent biodegradation of pyridinium ILs bearing ester groups in the 1- and 3-positions, whereas those bearing linear alkyl chains showed poor degradation. The latter result may be due to acute biotoxicity of such compounds to microbes.[4c,8] Despite the encouraging biodegradation data, chemical limitations exist when using ester-containing solvents, and therefore it was desirable to explore other biodegradable ILs with more robust functional groups.

To design a species that is susceptible to effective microbial attack in every given environment is a considerable task, in fact such a universally biodegradable moiety is unlikely due to the diversity of microbial strains in any particular environment, each with their own physiological and catabolic idiosyncrasies.[9] Years of biodegradation studies have revealed several "rules of thumb" that can be utilised to increase the propensity for aerobic decay, indicating various structural features which, when incorporated into an IL, should improve the efficiency of biodegradation by microbes. It was these "rules" that led the synthetic design of our new ILs.

As with previous studies, in order to evaluate the biodegradability of the test ILs, the "CO_2 headspace" test (ISO 14593) was applied. 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 the analysis of CO_2 generated.

Results and discussion

Synthesis

The majority of the ILs were prepared using a standard methodology. Alkylation of the appropriate heterocycle with an alkyl halide afforded the corresponding quaternized halide salt (Scheme 1). Anion exchange, where relevant, occurred by a metathesis reaction with potassium hexafluorophosphate,

[a]*Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, 3052, Victoria, Australia. E-mail: peter.scammells@monash.edu; Fax: +61 3 99039582; Tel: +61 3 9903 9542*
[b]*Department of Surfactant Technology, IQAC-CSIC, Jordi Girona 18-26, 08034, Spain. E-mail: mtgbet@iiqab.csic.es; Fax: +34 93 204 5904; Tel: +34 93 400 6100*
[c]*Department of Chemistry, Saint Mary's University, Halifax, Nova Scotia, Canada B3H 3C3. E-mail: robert.singer@smu.ca; Fax: +1 902 496 8104; Tel: +1 902 496 8189*

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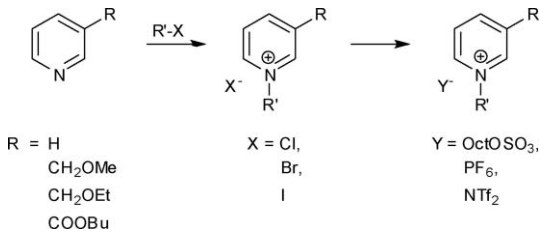


Scheme 1 Synthesis of pyridinium ILs.

lithium bis(trifluormethanesulfonimide) or ammonium octyl sulfate under aqueous conditions.

Carbamate derivative **10** was synthesized by refluxing 1-(2-hydroxyethyl)pyridinium bis(trifluoromethylsulfonyl)amide (**2b**) with propyl isocyanate in CH_2Cl_2 in the presence of a catalytic amount of pyridine for 48 h.

Thiazolium ILs were synthesized by the treatment of thiazole with a corresponding alkyl halide. In the synthesis of **12**, the reaction of thiazole with 2-chloroethanol was particularly sluggish and gave poor yields. However, switching from conventional heating to microwave irradiation, following the procedure of Aupoix and Vo-Thanh,[10] gave excellent conversion after 10 min.

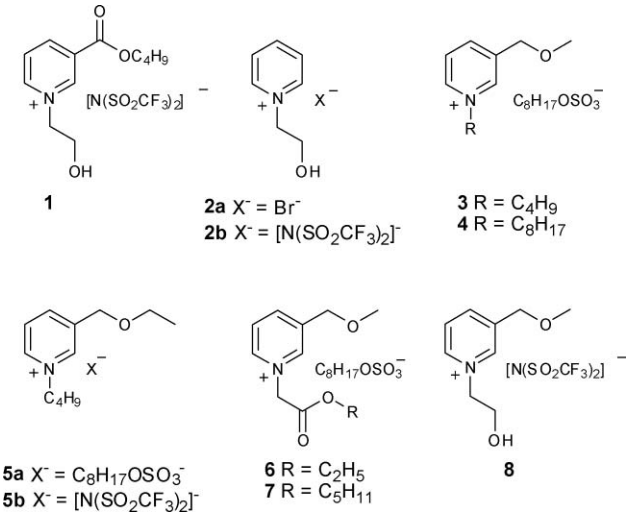
Biodegradation studies

Given our previous success with pyridinium ILs containing ester side chains, we sought to improve mineralization (*i.e.* complete degradation to carbon dioxide) through manipulation of the nitrogen substituent. Hence IL **1** was synthesised, containing a 1-(2-hydroxyethyl) side chain. The hydroxy group has previously been shown to aid biodegradability.[11] Indeed IL **1** showed high levels of mineralization, reaching the threshold of 60% degradation after only 14 d (see Table 1). Furthermore the parent 1-(2-hydroxyethyl)pyridinium salts **2a** and **2b** showed biodegradation levels of 65 and 62%, respectively, after 28 d. However, the initial rate of biodegradation was significantly higher for IL **1** than for pyridinium salts **2a** and **2b**, which showed a lag time. The presence of an easily hydrolizable ester group in the side chain of IL **1** clearly enhances the biodegradation.

In our previous studies, we have demonstrated that many commonly used anions, such as halide and bis(trifluoromethanesulfonyl)imide, have a negligible influence on biodegradation. One notable exception is the octyl sulfate anion, which makes a significant contribution to biodegradation and obviously needs to be considered in any discussion or comparison of the overall biodegradability. Accordingly, the high biodegradability of **1**, **2a** and **2b** can be attributed to the mineralisation of the 1-(2-hydroxyethyl) substituted pyridinium cation.

In order to build on these encouraging results, several ILs containing ether side chains were synthesized in the hope of developing ILs that tolerate a wider range of conditions (*e.g.* organometallic reagents and other basic species). It is known that the ether functionality does not aid biodegradation. However, it was hoped that given the high degree to which previous pyridinium ILs were biodegraded, manipulation of the other substituents could be accommodated. Stasiewicz and co-workers showed that 1-undecyloxymethyl-3-hydroxypyridinium saccharinate undergoes 72% biodegradation in a Closed Bottle test (OECD 1992); however, other similar derivatives in that study were only moderately mineralized.[12]

Table 1 Percentage of biodegradation of pyridinium ILs after 7, 14, 21 and 28 d, as determined by the CO_2 headspace test



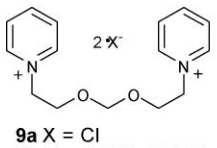
Compound	% Biodegradation[a] 7 d	14 d	21 d	**28 d**	95% CI[b]
1	47	64	73	**71**	68–74
2a	1	6	25	**65**	62–68
2b	2	14	55	**62**	58–66
3	27	28	30	**32**	39–35
4	23	31	32	**31**	30–32
5a	32	35	39	**36**	34–38
5b	0	1	0	**1**	0–2
6	38	46	47	**47**	43–51
7	42	50	48	**51**	49–53
8	0	1	8	**6**	5–7
SDS	*75*	*79*	*82*	***81***	*77–85*

[a] IL initial concentration = 20 mg C/L. [b] Confidence limits were calculated from 5 replicates.

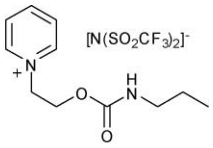
Unfortunately, ILs possessing an ether side chain showed diminished biodegradability. ILs **3**, **4** and **5a**, containing methyl ethers in the 3-position and linear alkyl substituents in the 1-position, showed only moderate (32, 31 and 36%, respectively) mineralization. The mineralization observed may reflect solely the degradation of the octyl sulfate anion, as replacement with the bis(trifluoromethanesulfonyl)imide anion (IL **5b**) resulted in a complete loss of biodegradation. Ether-substituted ILs **6** and **7**, containing 1-alkyl ester side chains complemented by the octyl sulfate anion, showed some improvement, yet ultimately failed to reach the 60% degradation threshold. Disappointingly, IL **8**, bearing the 1-(2-hydroxyethyl) substituent, showed extremely poor degradation.

Given the low biodegradation of the ether-containing ILs, we shifted focus to bis(pyridinium) salts (**9a–c**), bridged through an acetal linkage, in the hope that a balance between chemical resistance and biodegradation could be found. Hydrolysis of the acetal to the free alcohol (*cf.* ILs **2a** and **2b**) should result in further biodegradation. Dicationic ILs have previously been reported to possess superior thermal stability and a lack of volatility relative to traditional ILs, and have potential

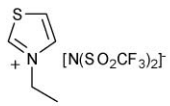
Table 2 Percentage of biodegradation of pyridinium ILs after 7, 14, 21 and 28 d, as determined by the CO_2 headspace test



9a X = Cl
9b X = $[N(SO_2CF_3)_2]$
9c X = PF_6



10



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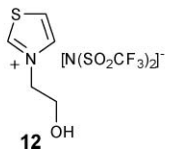

12

	% Biodegradation[a]				
Compound	7 d	14 d	21 d	**28 d**	95% CI[b]
9a	2	1	4	**5**	4–6
9b	1	3	3	**4**	3–5
9c	0	3	4	**3**	2–4
10	1	1	4	**3**	2–4
11	4	2	1	**3**	2–4
12	2	4	5	**7**	6–8
SDS	*53*	*82*	*92*	***95***	*91–99*

[a] IL initial concentration = 20 mg C/L. [b] Confidence limits were calculated from 5 replicates.

applications as media for high temperature reactions[13] and as high temperature lubricants.[14] Bis-pyridinium surfactants and ILs, in which the cations are joined by an alkyl linker, have been prepared previously.[15,16] "Bis-detergents" that contain pyridinium cations joined by an acetal linkage have also been synthesised and evaluated as agents for the cytosolic delivery of macromolecules.[16] However, little is known about the biodegradability of these species.

Bis(pyridinium) ILs **9a–c**, containing an acetal linkage with Cl^-, NTf_2^- and PF_6^- anions, were synthesized and their biodegradation tested (see Table 2). Structurally-related bis-pyridinium detergents were reported to be susceptible to hydrolysis under mildly acidic conditions (pH 5.0) with an approximate half-life of 3 h at 37 °C.[17] In this case, hydrolysis of the acetal would release the readily biodegradable 1-(2-hydroxyethyl)pyridinium cation and formaldehyde. Unfortunately, all of the bis(pyridinium) based ILs tested showed extremely poor mineralization (≤5%) after 28 d. This suggests that the acetal functionality is not hydrolysed under the assay conditions. Alternatively, since formaldehyde is a known biocide,[18] its formation upon cleavage of bis(pyridinium) ILs themselves may be toxic to aerobic microorganisms, thereby limiting biodegradation. To evaluate the toxicity of the bis(pyridinium) ILs and formaldehyde to microbes, solutions containing a mixture of formaldehyde or **9** and the reference substance (SDS) were evaluated using the CO_2 headspace test (data in the ESI†). No toxic effects were found due to the addition of formaldehyde to the standard (SDS) at relatively high formaldehyde concentrations (from 2–10 times higher than those theoretically released by acetal cleavage). Furthermore, the formaldehyde appears to be completely mineralised at these concentrations.

Biodegradation percentages for the mixtures of SDS and **9** were calculated based on the theoretical inorganic carbon yield anticipated from only the reference substance, SDS. For compounds **9a** and **9c**, a low level of SDS biodegradation inhibition was observed. The inhibition percentages were much lower than the limits commonly accepted for inhibitory effects (< 25%).

These results support the conclusion that bis(pyridinium) ILs are only slightly toxic to the microorganisms responsible for biodegradation under the assay conditions. The toxicity of these ILs to aerobic microorganisms appears to play a minor role in their low biodegradation; moreover, the failure of the acetal linkage to be cleaved under the test conditions[19] is the likely impediment of substantial biodegradation.

A carbamate derivative of the biodegradable IL **2b** was also prepared (compound **10**). Carbamates are typically more stable than esters to basic and nucleophilic conditions, which would be advantageous for numerous applications. Additionally, it is known that certain waste water microorganisms are able to metabolise carbamate-containing compounds, such as the insecticides Aldicarb and Carbaryl.[20] However, IL **10** was subsequently found to be very poorly degraded (3%) after 28 d, highlighting the importance of the free alcohol for biodegradability.

Thiazolium-based ILs have been reported to be excellent solvents for several transformations,[21] though, to the best of our knowledge, no aerobic degradation studies have been undertaken. Given the structural similarities to imidazolium ILs, we were interested to determine if they showed similar degradation characteristics. Both thiazolium salts tested showed minimal degradation (<10%) after a 28 d period. Surprisingly, the incorporation of the hydroxyethyl group in **12** failed to significantly improve the mineralization, as compared with the linear alkyl chain present in **11**. There is some indication that thiazolium salts possess antimicrobial activity,[22] which could account for the poor degradation of these compounds.

Conclusions

It can be concluded that incorporation of the 1-(2-hydroxyethyl) group into pyridinium salts generally results in ILs possessing an improved biodegradation profile. Interestingly, the presence of a 1-(2-hydroxyethyl) group has previously been shown to confer high levels of biodegradability in the tetraalkylammonium (*i.e.* cholinium) class of IL,[23] but this was not the case for dialkylimidazolium ILs.[4g] The biodegradable pyridinium ILs that we had previously identified all possess an ester moiety, which limits their range of potential applications. The discovery of 1-(2-hydroxyethyl)pyridinium ILs that are readily biodegradable expands this range of possibilities, just as alcohols such as methanol and ethanol have proven to be versatile solvents for a range of different reactions. In contrast, ILs containing a methyl or ethyl ether side chain generally showed poor mineralization, except in those cases where an ester side chain was also present. These ILs showed moderate biodegradation, but ultimately failed to reach the desired 60% threshold. Bis(pyridinium) ILs with an acetal linkage (**9a–c**) showed very poor mineralization. However, it should be noted that bis(pyridinium) ILs of this type could be chemically hydrolysed to the constituent

biodegradable 1-(2-hydroxyethyl)pyridinium salts under acidic conditions. Pyridinium carbamate **10**, which was predicted to be prone to enzymatic hydrolysis, showed unexpectedly little degradation after 28 d. In contrast to the pyridinium series, thiazolium ILs bearing a 1-(2-hydroxyethyl) substituent showed very poor levels of biodegradation.

Experimental

Synthesis

Lithium bis(trifluoromethanesulfonyl)imide (Aldrich, 97%), potassium hexafluorophosphate (Fluka, 98%), sodium octyl sulfate (Sigma, ~95%), methyl iodide (Sigma-Aldrich, 99%), butyl iodide (Fluka ≥ 98%) ethyl bromoacetate (Merck, > 98%), toluene (Merck, GR, ≤ 0.03% H_2O), acetonitrile (Merck, Chromatography grade, ≤ 0.02% H_2O) and diethyl ether (Merck, GR, ≤ 0.03% H_2O) were procured from commercial suppliers and used without any pre-treatment. All ^{1}H NMR spectra were recorded on a Bruker Avance DPX 300 spectrometer at 300.13 MHz. ^{13}C NMR spectra were recorded on a Varian Unity Inova 600 spectrometer at 150.8 MHz, or on a Bruker Avance DPX 300 spectrometer at 75.4 MHz. Unless stated otherwise, DMSO-d_6 (Cambridge Isotope Laboratories Inc., 99.9% D) was used as the solvent for NMR samples. High resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE (TOF) spectrometer fitted with an electrospray ion source. Where applicable, all ILs were subjected to an $AgNO_3$ precipitate test, washing with distilled water until negative, to ensure no halide impurities were present. All ILs were dried to uniform weight under high vacuum.

3-(Methoxymethyl)pyridine. A solution of picolyl chloride hydrochloride (52.74 mmol, 8.65 g) in dimethyl sulfoxide (80 mL) was gradually added to a suspension of sodium methoxide (158.22 mmol, 8.55 g) in dimethyl sulfoxide (80 mL) at room temperature. The reaction mixture was allowed to stir for 24 h. The crude ether was separated from dimethyl sulfoxide by diluting it with water (200 mL) and extracting its aqueous solution with ethyl acetate (3 × 100 mL). The ethyl acetate extracts were dried and evaporated to yield a bright orange liquid. The crude product was purified by column chromatography using neat ethyl acetate as an eluent. Yield 64%, light yellow oil, ^{1}H NMR ($CDCl_3$): δ 3.31 (s, 3H), 4.37 (s, 2H), 7.17–7.21 (m, 1H), 7.57–7.60 (m, 1H) 8.43–8.48 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 58.3, 72.0, 123.4, 133.6, 135.4, 149.0, 149.0.

3-(Ethoxymethyl)pyridine. Absolute ethanol (25 mL) was added dropwise to freshly cut and dried Na chunks (4.2 g, 0.18 mol) under an N_2 atmosphere. The mixture was subsequently refluxed for 90 min until no solids could be observed. Excess ethanol was removed under reduced pressure and the sludge was dried over high vacuum at 100 °C for 60 min. The resulting fine powder was suspended in DMSO (100 mL) and a mixture of picolyl chloride hydrochloride (10 g, 60.96 mol) in DMSO (100 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature and subsequently quenched with H_2O (250 mL). The product was extracted into ethyl acetate (3 × 120 mL), the combined organic layers were dried over $MgSO_4$, filtered and the solvents removed under reduced pressure. Compound **9** (8.34 g, 98%) was obtained as red oil after column chromatography (silica gel, ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 8.57 (s, 1H), 8.52 (d, J = 4.8 Hz, 1H), 7.68 (d, J = 7.7 Hz, 1H), 7.26 (dd, J = 7.7, 4.4 Hz, 1H), 4.48 (s, 1H), 3.55 (q, J = 7.0 Hz, 2H), 1.24 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CHCl_3$, 150.8 MHz): δ 15.0, 65.9, 69.9, 123.2, 133.8, 135.3, 148.7, 148.8. ESI-MS calc. for $C_8H_{11}NO + H^+$, 138.1; found, 138.2.

Synthesis of bis(trifluoromethanesulfonyl)imides 1, 2b, 5b and 8. The heterocyclic amine (4.07 mmol), 2-bromoethanol (4.88 mmol, 0.61 g, 0.35 mL) and sodium iodide (4.07 mmol, 0.61 g) were stirred at 110 °C for 24 h. The reaction was set up in a moisture guarded assembly and reflux condenser was used while it was been heated. The quaternary salt was extracted into water (50 mL) and its aqueous solution was purified by extractions with diethyl ether (4 × 20 mL). The aqueous solution of quaternary halide was treated with the solution of bis(trifluormethanesulfonimide) lithium salt (4.47 mmol, 1.28 g) in water (10 mL) and the resultant solution was stirred for 10 min. The product was isolated by solvent extraction using dichloromethane (2 × 20 mL). The extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to yield the required product. In the synthesis of **2b**, ethyl acetate was used instead of dichloromethane. In the synthesis of **8**, dichloromethane was used for purification of the aqueous quaternary salt solution. The bis(trifluoromethanesulfonyl)imide **8** being water soluble was isolated by evaporating the aqueous reaction mixture of bis(trifluoromethanesulfonyl)imide and quaternary halide(s) to complete dryness and subsequently extracting the IL with dichloromethane.

3-(Butoxycarbonyl)-1-(2-hydroxyethyl)pyridinium bis(trifluoromethylsulfonyl)amide (1). Yield 70%, light yellow liquid. ^{1}H NMR: δ 0.92–0.97 (t, J = 7.5 Hz, 3H), 1.39–1.52 (m, 2H), 1.70–1.79 (m, 2H), 3.88 (bs, 2H), 4.39–4.44 (t, J = 6.5 Hz, 2H), 4.77–4.80 (t, J = 4.8 Hz, 2H), 5.25 (bs, 1H), 8.27–8.32 (m, 1H), 9.00–9.04 (m, 1H), 9.19–9.21 (m, 1H), 9.53 (s, 1H). ^{13}C NMR: δ 13.5, 18.6, 30.1, 60.0, 63.5, 66.3, 119.5 (–CF_3), 128.1, 129.7, 145.3, 146.2, 148.6, 161.8. HRMS (ESI, +ve) calc. for $C_{12}H_{18}NO_3$ 224.1281, found 224.1292, HRMS (ESI, –ve) calc. for $N(SO_2CF_3)_2$ 279.9178, found 279.9188.

1-(2-Hydroxyethyl)pyridinium bis(trifluoromethylsulfonyl)-amide (2b). Yield 97%, pale yellow liquid. ^{1}H NMR: δ 3.84–3.88 (t, J = 5.0 Hz, 2H), 4.64–4.67 (t, J = 5.0 Hz, 2H), 5.24 (bs, 1H), 8.14–8.18 (m, 2H), 8.59–8.64 (m, 1H), 8.99–9.01 (m, 2H). ^{13}C NMR: δ 60.1, 63.3, 119.6 (–CF_3), 127.8, 145.2, 145.6 ppm. HRMS (ESI, +ve) calc. for $C_7H_{10}NO$ 124.0757, found 124.0751, HRMS (ESI, –ve) calc. for $N(SO_2CF_3)_2$ 279.9178, found 279.9180

***N*-Butyl-3-(ethoxymethyl)pyridinium bis(trifluoromethanesulfonyl)imide (5b).** Yield 71%, light yellow liquid. ^{1}H NMR: δ 9.05 (s, 1H), 9.01 (d, J = 6.0 Hz, 1H), 8.52 (d, J = 8.0 Hz, 1H), 8.13 (dd, J = 8.0, 6.1 Hz, 1H), 4.70 (s, 2H), 4.63 (t, J = 7.5 Hz, 2H), 3.61 (q, J = 7.0 Hz, 2H), 1.97–1.87 (m, 2H), 1.38–1.26 (m, 2H), 1.21 (t, J = 7.0 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (150.8 MHz): δ 13.0, 14.7, 18.6, 32.6, 60.6, 65.8, 67.5, 119.4 (q, J = 322 Hz, C–F), 127.5, 139.8, 142.8, 143.5, 143.6. HRMS (ESI, +ve) calc. for $C_{12}H_{20}NO$ 194.1539, found,

191.1545, HRMS (ESI, –ve) calc. for $(CH_3SO_2)_2N$ 279.9178, found 279.9174.

1-(2-Hydroxyethyl)-3-(methoxymethyl)pyridinium bis(trifluoromethylsulfonyl)amide (8). Quantitative, light yellow liquid. ^{1}H NMR: δ 3.41 (s, 3H), 3.84–3.89 (m, 2H), 4.66–4.68 (m, 4H), 5.23–5.26 (t, J = 5.3 Hz, 1H), 8.12–8.16 (m, 1H), 8.52–8.55 (m, 1H), 8.92–8.98 (m, 2H) ppm. ^{13}C NMR: δ 58.3, 60.1, 63.4, 69.6, 119.6 (CF_3), 127.4, 139.1, 143.4, 143.9, 144.3 ppm. HRMS (ESI, +ve) calc. for $C_9H_{14}NO_2$ 168.1019, found 168.1018, HRMS (ESI, –ve) calc. for $N(SO_2CF_3)_2$ 279.9178, found 279.9192.

Synthesis of octyl sulfates 3, 4, 5a, 6 and 7. The heterocyclic amine (4.07 mmol), alkyl bromide (4.88 mmol) and sodium iodide (0.61 g, 4.07 mmol) were stirred at 110 °C for 24 h. The reaction apparatus was moisture guarded and a reflux condenser was used while the reaction mixture was heated. The quaternary salt was extracted into water (50 mL) and its aqueous solution was purified by extraction with diethyl ether (2 × 20 mL). The aqueous solution of quaternary halide was treated with aqueous ammonium octyl sulfate (0.92 g, 4.07 mmol in 20 mL of water) and the resultant was stirred for 10 min. The product was isolated by solvent extraction with dichloromethane (3 × 20 mL). The extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to yield a pure product.

1-Butyl-3-(methoxymethyl)pyridinium octyl sulfate (3). Yield 92%, bright yellow liquid (solidifies at 4 °C). ^{1}H NMR: δ 0.83–0.93 (m, 6H), 1.24–1.33 (m, 12H), 1.45–1.47 (m, 2H), 1.88–1.95 (m, 2H), 3.40 (s, 3H) 3.65–3.70 (t, J = 6.6 Hz, 2H), 4.60–4.65 (m, 4H), 8.13–8.18 (m, 1H), 8.52–8.55 (m, 1H) 9.04–9.09 (m, 2H). ^{13}C NMR: δ 13.3, 14.0, 18.8, 22.1, 25.5, 28.7, 28.8, 29.1, 31.3, 32.8, 58.3, 60.6, 65.5, 69.5, 127.7, 139.4, 143.1, 143.8 ppm. HRMS (ESI, +ve) calc. for $C_{11}H_{18}NO$ 180.1383, found 180.1385, HRMS (ESI, –ve) calc. for $C_8H_{17}OSO_3$ 209.0853, found 209.0843.

1-Octyl-3-(Methoxymethyl)pyridinium octyl sulfate (4). Yield 97%, yellow waxy solid (tends to solidify at room temperature). ^{1}H NMR: δ 0.82–0.87 (m, 6H), 1.23–1.27 (m, 20H), 1.44–1.49 (m, 2H), 1.91–1.93 (m, 2H), 3.39 (s, 3H) 3.65–3.69 (t, J = 6.6 Hz, 2H), 4.58–4.65 (m, 4H), 8.12–8.17 (m, 1H), 8.52–8.54 (m, 1H) 9.03–9.08 (m, 2H). ^{13}C NMR: δ 13.9, 13.9, 22.1, 22.1, 25.4, 25.6, 28.4, 28.5, 28.7, 28.8, 29.1, 30.8, 31.2, 31.3, 58.3, 60.8, 65.5, 69.5, 127.7, 139.4, 143.1, 143.8 ppm. HRMS (ESI, +ve) calc. for $C_{15}H_{26}NO$ 236.2009, found 236.2005, HRMS (ESI, –ve) calc. for $C_8H_{17}OSO_3$ 209.0853, found 209.0835.

1-Butyl-3-(ethoxymethyl)pyridinium octyl sulfate (5a). Yield 67%, light yellow liquid. ^{1}H NMR: δ 9.10 (s, 1H), 9.07 (d, J = 6.1 Hz, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.15 (dd, J = 7.9, 6.1 Hz, 1H), 4.70 (s, 2H), 4.65 (t, J = 7.4 Hz, 2H), 3.70 (t, J = 6.7 Hz, 2H), 3.59 (q, J = 7.0 Hz, 2H), 1.96–1.86 (m, 2H), 1.49–1.43 (m, 2H), 1.37–1.17 (m, 15H), 0.91 (t, J = 7.4 Hz, 3H), 0.84 (t, J = 6.7 Hz, 3H). ^{13}C NMR (150.8 MHz): δ 13.1, 13.7, 14.7, 18.6, 21.9, 25.4, 28.5, 28.6, 28.9, 31.1, 32.6, 60.4, 65.4, 65.7, 67.4, 127.6, 139.6, 142.9, 143.5, 143.6. HRMS (ESI, +ve) calc. for $C_{12}H_{20}NO$ 194.1539, found 194.1535. HRMS (ESI, –ve) calc. for $C_8H_{17}OSO_3$ 209.0853, found 209.0848.

1-(2-Ethoxy-2-oxoethyl)-3-(methoxymethyl)pyridinium octyl sulfate (6). Yield 77%, brown liquid. ^{1}H NMR: δ 0.84–0.87 (m, 3H), 1.21–1.29 (m, 13H), 1.45–1.47 (m, 2H), 3.42 (s, 3H), 3.65–3.70 (t, J = 6.6 Hz, 2H), 4.23–4.28 (q, J = 4.8 Hz, 2H), 4.69 (s, 2H), 5.68 (s, 2H) 8.21–8.26 (m, 1H), 8.62–8.65 (m, 1H), 8.98–9.00 (m, 1H), 9.06 (s, 1H). ^{13}C NMR: δ 14.0, 15.0, 22.1, 25.6, 28.7, 28.8, 29.1, 31.3, 58.3, 60.4, 62.3, 65.6, 69.5, 127.5, 139.2, 144.4, 145.1, 145.4, 166.5 ppm. HRMS (ESI, +ve) calc. for $C_{11}H_{16}NO_3$ 210.1125, found 210.1119, HRMS (ESI, –ve) calc. for $C_8H_{17}OSO_3$ 209.0853, found 209.0851.

3-(Methoxymethyl)-1-(2-oxo-2-(pentyloxy)ethyl)pyridinium octyl sulfate (7). Yield 87%, light brown liquid. ^{1}H NMR: δ 0.84–0.91 (m, 6H), 1.26–1.34 (m, 14H), 1.46–1.50 (m, 2H), 1.61–1.66 (m, 2H), 3.44 (s, 3H), 3.66–3.70 (t, J = 6.6 Hz, 2H), 4.16–4.21 (t, J = 6.6 Hz, 2H), 4.70 (s, 2H), 5.69 (s, 2H) 8.22–8.27 (m, 1H), 8.63–8.66 (m, 1H), 8.99–9.01(m, 1H), 9.08 (s, 1H). ^{13}C NMR: δ 13.8, 14.0, 21.7, 22.1, 25.6, 27.4, 27.6, 28.7, 28.8, 29.1, 31.3, 58.3, 60.4, 65.5, 66.2, 69.5, 127.5, 139.2, 144.4, 145.1, 145.3, 166.4 ppm. HRMS (ESI, +ve) calc. for $C_{14}H_{22}NO_3$ 252.1594, found 252.1603, HRMS (ESI, –ve) calc. for $C_8H_{17}OSO_3$ 209.0853, found 209.0843.

1,1′-(2,2′-Methylenebis(oxy)bis(ethane-2,1-diyl))dipyridinium dichloride (9a). Pyridine (1.9 mL, 23.49 mmol) was added to bis(2-chloroethoxy)methane[24] (1.7 g, 9.82 mmol) in one portion and the resulting solution was stirred at room temperature for 1 h before being heated to 110 °C for 24 h. The solution was cooled and the volatiles removed *in vacuo* to give the desired product as a light brown solid. ^{1}H NMR: δ 3.83 (t, J = 5.1 Hz, 4H), 4.57 (s, 2H), 4.83 (t, J = 5.1 Hz, 2H), 8.19 (dt, J = 6.9, 0.9 Hz, 4H), 8.65 (tt, J = 7.8, 1.5 Hz, 2H), 9.12 (m, 4H). ^{13}C NMR: δ 60.2, 65.7, 94.1, 127.8, 145.3, 145.9. HRMS (ESI, +ve) $[M^{2+} - Cl^-]^+$ calc. for $C_{15}H_{20}ClN_2O_2$ 295.1208, found 295.1210.

1,1′-(2,2′-Methylenebis(oxy)bis(ethane-2,1-diyl))dipyridinium bis(trifluoromethylsulfonyl)amide (9b). To **9a** (0.61 g, 1.83 mmol) in 10 mL distilled water was added lithium bistrifluoromethanesulfonimidate (1.15 g, 4.00 mmol), the resulting solution was stirred at room temperature for 30 min. EtOAc (20 mL) was added to the reaction mixture and stirring was continued for 10 min. The aqueous phase was washed with a further 2 × 20 mL EtOAc, combined organic phases were washed with distilled water until $AgNO_3$ test gave a negative result, then dried ($MgSO_4$), concentrated *in vacuo* then placed under high vacuum at 40 °C to give a clear oil (1.44 g, 97%). ^{1}H NMR: δ 3.81 (t, J = 4.8, 4H), 4.56 (s, 2H), 4.76 (t, J = 4.8 Hz, 4H), 8.17 (m, 4H), 8.64 (tt, J = 7.8, 1.2 Hz, 2H), 8.99 (m, 4H). ^{13}C NMR: δ 60.4, 65.7, 94.2, 119.5 (q, J_{C-F} = 320.1 Hz), 127.9, 145.2, 146.0. HRMS (ESI, +ve) $[M^{2+} - NTf_2^-]^+$ calc. for $C_{17}H_{20}F_6N_3O_6S_2$ 540.0692 found 540.0672, HRMS (ESI, –ve) calc. for $N(SO_2CF_3)_2$ 279.9178, found 279.9178.

1,1′-(2,2′-Methylenebis(oxy)bis(ethane-2,1-diyl))dipyridinium hexafluorophosphate (9c). To dichloride salt **9a** (0.53 g, 1.59 mmol) in 2 mL distilled water (sonication required) was added KPF_6 (0.64 g, 3.49 mmol) in 1.5 mL distilled water, washing with 1 mL distilled water. The solution became viscous and was sonicated for 15 min then stirred at room temperature for 4 h. Solution filtered and residue washed with cold distilled

water then placed under vacuum (0.5 mmHg for 72 h) to give the desired product (0.81 g, 93%) as a white solid. ^{1}H NMR: δ 3.85 (t, J = 4.2 Hz, 4H), 4.59 (s, 2H), 4.80 (t, J = 4.2 Hz, 4H), 8.21 (m, 4H), 8.68 (m, 2H), 9.03 (m, 4H). ^{13}C NMR: δ 60.4, 65.6, 94.2, 127.9, 145.1, 146.0. HRMS (ESI, +ve) $[M^{2+} - PF6^-]^+$ calc. for $C_{15}H_{20}F_6N_2O_2P$ 405.1161, found 405.1160. HRMS (ESI, −ve) calc. for PF_6 144.9647, found 144. 9641.

1-(2-(Propylcarbamoyloxy)ethyl)pyridinium bis(trifluoromethylsulfonyl)amide (10). To **2b** (0.5 g, 1.24 mmol), dried overnight (0.1 mm Hg) in anhydrous CH_2Cl_2 (5 mL) was added PrNCO (275 μL, 2.93 mmol) dropwise. To this mixture was added 2 drops of pyridine and the resulting solution was refluxed for 48 h. The solvent and volatiles were removed *in vacuo* to give IL **10** (0.6 g, 99%) as a yellow oil. ^{1}H NMR: δ 0.78 (t, J = 7.2 Hz, 3H), 1.33 (sext., J = 7.2 Hz, 2H), 2.85 (q, J = 6.6 Hz, 2H), 4.46 (t, J = 5.1 Hz, 2H), 4.85 (t, J = 5.1 Hz, 2H), 7.24 (t, J = 5.7 Hz, 1H), 8.18 (m, 2H), 8.64 (m, 1H), 9.04 (m, 2H). ^{13}C NMR: δ 11.2, 22.5, 42.0, 60.3, 62.0, 119.5 (q, J_{C-F} = 319.9 Hz), 127.9, 145.4, 146.0, 155.3. HRMS (ESI, +ve) calc. for $C_{11}H_{17}N_2O_2$ 209.1285, found 209.1294, HRMS (ESI, −ve) calc for $N(SO_2CF_3)_2$ 279.9178, found 279.9170.

3-Ethylthiazolium bis(trifluoromethylsulfonyl)amide (11). To thiazole (0.5 mL, 7.05 mmol) at room temperature was added ethyl iodide (0.51 mL, 6.40 mmol) the resulting solution was stirred for 1 h at room temperature and then heated to 50 °C for 48 h in the dark. The solution was cooled to room temperature diluted with 10 mL distilled water and washed with diethyl ether (3 × 35 mL). The aqueous layer was then treated with lithium bistrifluoromethanesulfonimidate (2.00 g, 6.97 mmol) in 10 mL distilled water at room temperature for 30 min. The aqueous solution was extracted with CH_2Cl_2, dried with $MgSO_4$ and concentrated *in vacuo* to give the desired product as a clear oil (1.7 g, 68%). ^{1}H NMR: δ 1.49 (t, J = 7.5 Hz, 3H), 4.56 (q, J = 7.5 Hz, 2H), 8.34 (m, 1H), 8.59 (m, 1H), 10.18 (s, 1H). ^{13}C NMR (d^6-DMSO) δ 15.3, 50.2, 119.5 (q, J_{C-F} = 325.8 Hz), 126.7, 136.9, 158.9. HRMS (ESI, +ve) calc. for C_5H_8NS 114.0372, found 114.0372, HRMS (ESI, −ve) calc. for $N(SO_2CF_3)_2$ 279.9178, found 279.9184.

3-(2-Hydroxyethyl)thiazolium bis(trifluoromethylsulfonyl)-amide (12). Thiazole (0.25 mL, 3.5 mmol) and 2-chloroethanol (0.22 mL, 3.3 mmol) were heated in a microwave reactor at 150 °C for 10 min. The solution was allowed to cool to room temperature before being diluted with distilled water (10 mL). The aqueous solution was washed with diethyl ether (3 × 10 mL) then treated with lithium bistrifluoromethanesulfonimidate (1 g, 3.5 mmol) in 10 mL distilled water. The mixture was stirred at room temperature for 40 min then extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed (3 × 10 mL distilled water), dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil (1.12 g, 83%). ^{1}H NMR: δ 3.81 (q, J = 5.1 Hz, 2H), 4.60 (t, J = 5.1 Hz, 2H), 5.24 (t, J = 5.1 Hz, 1H), 8.32 (m, 1H), 8.51 (m, 1H), 10.11 (m, 1H). ^{13}C NMR: δ 57.2, 59.3, 119.5 (q, J_{C-F} = 319.7 Hz), 126.3, 137.4, 159.7. HRMS (ESI, +ve) calc. for C_5H_8NOS 130.0321, found 130.0321, HRMS (ESI, −ve) calc. for $N(SO_2CF_3)_2$ 279.9178, found 279.9176.

ISO 14593: CO_2 headspace test

To evaluate the biodegradability of the test ILs, the "CO_2 headspace" test (ISO 14593, OECD 310) was applied. 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 (IC). The test IL, as the sole source of carbon and energy, was added at a concentration of 20 mg C/L to a mineral salts buffer 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 (mineralisation to carbon dioxide) was determined by measuring the IC produced in the test bottles in excess of that produced in blank vessels containing inoculated medium only. Sodium *n*-dodecyl sulfate (SDS) was used as the reference substance; the test ran for 28 d. The extent of biodegradation was expressed as a percentage of the theoretical amount of inorganic carbon (ThIC) based on the amount of test compound added initially.

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Notes and references

1 *Ionic Liquids in Synthesis*, ed. P. Wasserscheid and T. Welton, Wiley-VCH, Weinheim, 2nd edn, 2008.
2 N. V. Plechkova and K. R. Seddon, *Chem. Soc. Rev.*, 2008, **37**, 123–150.
3 (*a*) D. Zhao, Y. Lioa and Z. Zhang, *Clean*, 2007, **35**, 42–48; (*b*) M. J. Rosen, F. Li, S. W. Morrall and D. J. Versteg, *Environ. Sci. Technol.*, 2001, **35**, 954; (*c*) A. S. Wells and V. T. Coombe, *Org. Process Res. Dev.*, 2006, **10**, 794; (*d*) T. D. Landry, K. Brooks, D. Poche and M. Woolhiser, *Bull. Environ. Contam. Toxicol.*, 2005, **74**, 559–565; (*e*) T. P. Thuy Pham, C.-W. Cho and Y.-S. Yu, *Water Res.*, 2010, **44**, 352–72.
4 (*a*) N. Gathergood and P. J. Scammells, *Aust. J. Chem.*, 2002, **55**, 557–560; (*b*) N. Gathergood, M. T. Garcia and P. J. Scammells, *Green Chem.*, 2004, **6**, 166–175; (*c*) M. T. Garcia, N. Gathergood and P. J. Scammells, *Green Chem.*, 2005, **7**, 9–14; (*d*) N. Gathergood, P. J. Scammells and M. T. Garcia, *Green Chem.*, 2006, **8**, 156–160; (*e*) J. R. Harjani, R. D. Singer, M. T. Garcia and P. J. Scammells, *Green Chem.*, 2008, **10**, 436–438; (*f*) J. R. Harjani, R. D. Singer, M. T. Garcia and P. J. Scammells, *Green Chem.*, 2009, **11**, 83–90; (*g*) J. R. Harjani, J. Farrell, M. T. Garcia, R. D. Singer and P. J. Scammells, *Green Chem.*, 2009, **11**, 821–829; (*h*) F. Atefi, M. T. Garcia, R. D. Singer and P. J. Scammells, *Green Chem.*, 2009, **11**, 1595–1604.
5 (*a*) A. Romero, A. Santos, J. Tojo and A. Rodriguez, *J. Hazard. Mater.*, 2008, **151**, 268–273; (*b*) S. Stolte, S. Abdulkarim, J. Arning, A. K. Blomeyer-Nienstedt, U. Bottin-Weber, M. Matzke, J. Ranke, B. Jastorff and J. Thoming, *Green Chem.*, 2008, **10**, 214–224; (*c*) K. M. Docherty, J. K. Dixon and C. F. Kulpa, *Biodegradation*, 2007, **18**, 481–493; (*d*) P. Wasserscheid, R. van Hal and A. Bosmann;, *Green Chem.*, 2002, **4**, 400–404; (*e*) S. Morrissey, B. Pegot, D. Coleman, M. T. Garcia, D. Ferguson, B. Quilty and N. Gathergood, *Green Chem.*, 2009, **11**, 475–483; (*f*) Y. Fukaya, Y. Iizuka, K. Sekikawa and H. Ohno, *Green Chem.*, 2007, **9**, 1155–1157.
6 (*a*) P. J. Scammells, J. L. Scott and R. D. Singer, *Aust. J. Chem.*, 2005, **58**, 155–169; (*b*) S. Zhu, R. Chen, Y. Wu, Q. Chen, X. Zhang and Z. Yub, *Chem. Biochem. Eng. Q.*, 2009, **23**, 207–211; (*c*) D. Coleman and N. Gathergood, *Chem. Soc. Rev.*, 2010, **39**, 600–637; (*d*) T. P. Thuy Pham, C.-W. Cho and Y.-S. Yun, *Water Res.*, 2010, **44**,

352–372; (*e*) R. F. M. Frade and C. A. M. Afonso, *Hum. Exp. Toxicol.*, 2010, DOI: 10.1177/0960327110371259.
7 (*a*) E. Grabinska-Sota and J. Kalka, *Environ. Int.*, 2003, **28**, 687–690; (*b*) D. Pijper, E. Bulten, J. Smisterova, A. Wagenaar, D. Hoekstra, J. B. F. N. Engberts and R. Hulst, *Eur. J. Org. Chem.*, 2003, 4406–4412; (*c*) T. P. Thuy Pham, C.-W. Cho, C.-O. Jeon, Y.-J. Chung, M.-W. Lee and Y.-S. Yun, *Environ. Sci. Technol.*, 2009, **43**, 516–521; (*d*) K. M. Docherty, M. V. Joyce, K. J. Kulacki and C. F. Kulpa, *Green Chem.*, 2010, **12**, 701–712; (*e*) C. Zhang, H. Wang, S. V. Malhotra, C. J. Dodge and A. J. Francis, *Green Chem.*, 2010, **12**, 851–858.
8 (*a*) R. S. Boethling, *ACS Symp. Ser.*, 1996, **640**, 156; (*b*) P. H. Howard, R. S. Boethling, W. Stiteler, W. Meylan and J. Beauman, *Sci. Total Environ.*, 1991, **109–110**, 635; (*c*) R. S. Boethling, *Cationic Surfactants, Surfactant Science Series Vol. 53*, Marcel Dekker, New York, 1994, pp. 95–135.
9 M. Alexander, *Biodegradation and Bioremediation*, Academic Press, New York, 2nd edn, 1999.
10 A. Aupoix and G. Vo-Thanh, *Synlett*, 2009, **12**, 1915–1921.
11 R. S. Boethling, Elizabeth Sommer and D. DiFiore, *Chem. Rev.*, 2007, **107**, 2207–2227.
12 M. Stasiewicz, E. Mulkiewicz, R. Tomczak-Wandzel, J. Kumirska, E. M. Siedlecka, M. Golebiowski, J. Gajdus, M. Czerwicka and P. Stepnowski, *Ecotoxicol. Environ. Saf.*, 2008, **71**, 157–165.
13 (*a*) J. L. Anderson, R. Ding, A. Ellern and D. W. Armstrong, *J. Am. Chem. Soc.*, 2005, **127**, 593–604; (*b*) X. Han and D. W. Armstrong, *Org. Lett.*, 2005, **7**, 4205–4208.
14 G. Yu, S. Yan, F. Zhou, X. Liu, W. Liu and Y. Liang, *Tribol. Lett.*, 2007, **25**, 197–205.
15 L. Zhou, X. Jiang, Y. Li, Z. Chen and X. Hu, *Langmuir*, 2007, **23**, 11404–11408.
16 X.-Z. Yang, J. Wang, Z.-Z. Zhang and G.-S. Li, *J. Chem. Eng. Data*, 2009, **54**, 1385–1388.
17 A. Asokan and M. J. Cho, *Bioconjugate Chem.*, 2004, **15**, 1166–1173.
18 Formaldehyde; MSDS no. F5522; Mallinckrodt Baker, Phillipsburg, NJ, September 8, 2009 (http://www.jtbaker.com/msds/englishhtml/f5522.htm (accessed 23/02/10)).
19 5 mg of IL **9c** was dissolved in a 5 : 1 mixture of d^4-MeOH and D_2O in an NMR tube. The stability of the IL was monitored by ^{1}H NMR spectroscopy over 1 month at 25 and 45 °C. No appreciable acetal cleavage was observed under these conditions.
20 G. H. Chaudhry and W. B. Wheeler, *Water Sci. Technol.*, 1988, **20**, 89–94.
21 (*a*) J. H. Davis and K. J. Forrester, *Tetrahedron Lett.*, 1999, **40**, 1621–1622; (*b*) T. Takeuchi, M. Suzuki and N. Kitagashi, *Jpn. Kokai Tokkyo Koho*, 2009 JP 2009084193.
22 J. L. Selph, J. J. Partridge and J. F. Reinhard, Thiazolium Compounds And Uses Thereof, *WO Pat.*, 20080009416, United States Mycosol, Inc., 2008.
23 Y. Yu, X. Lu and Q. Zhou, *Chem.–Eur. J.*, 2008, **14**, 11174–11182.
24 R. M. Anker and A. H. Cook, *J. Chem. Soc.*, 1948, 806–810.