Synthesis, Antibiotic Structure-Activity-Relationships, and Cellulose Dissolution Studies of New Room-Temperature Ionic Liquids Derived from Lignin

Shihong Liu  
Queens University of Charlotte

Michael Gonzalez  
Queens University of Charlotte

Celine Kong  
Queens University of Charlotte

Scott Weir  
Queens University of Charlotte

Aaron Socha (sochaa@queens.edu)  
Queens University of Charlotte  https://orcid.org/0000-0001-7963-1638

Research

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Abstract

Background: Ionic liquids (ILs) are promising pretreatment solvents for lignocellulosic biomass, but are largely prepared from petroleum precursors. Benzaldehydes from depolymerized lignin, such as vanillin, syringaldehyde and 4-methoxy benzaldehyde, represent renewable feedstocks for the synthesis of ionic liquids. We herein report syntheses of novel lignin-derived ionic liquids, with extended N-alkyl chains, and examine their melting points, cellulose dissolution capacities, and toxicity profiles against *Daphnia magna* and *E. coli* strain 1A1. The latter organism has been engineered to produce isoprenol, a drop-in biofuel and precursor for commodity chemicals.

Results: The new N,N-diethyl and N,N-dipropyl methyl benzylammonium ILs were liquids at room temperature, showing 75-100°C decreased melting points as compared to their N,N,N-trimethyl benzyl ammonium analog. Extension of N-alkyl chains also increased antibacterial activity 3-fold, while ionic liquids prepared from vanillin showed 2- to 4-fold lower toxicity as compared to those prepared from syringaldehyde and 4-methoxybenzaldehyde. The trend of antibacterial activity for anions of lignin-derived ILs was found to be methanesulfonate < acetate < hydroxide. Microcrystalline cellulose dissolution, from 2-4 wt% after 20 min at 100°C, was observed in all new ILs using light microscopy and IR spectroscopy.

Conclusions: Ionic liquids prepared from S, G and H-lignin oxidation products provided differential cytotoxic activity against *E.coli* and *D. magna*, suggesting these compounds could be tailored for application specificity within a biorefinery.

Background

Ionic liquids (ILs) are salts with melting points below 100°C, super-solvents comprised entirely of paired ions. The physical properties of ILs, *e.g.* viscosity, conductivity, vapor pressure and thermal stability, are defined by structural features [1], and have been adopted in over 50 commercial applications [2]. Chemical “tuning” of ionic liquids has been described [3], exploited to solvate a wide range of natural products [4], and extended to protein stabilization [5] and enzyme catalysis [6]. Depending on their degree of Lewis basicity, ILs can dissolve cellulose [7], hemicellulose [8] and/or lignin [9], effectively pretreating plant biomass for enzymatic hydrolysis to monomeric sugars [10, 11]. When compared to other biomass pretreatment methods, ILs typically provide higher space-time yields of glucose and xylose [12, 13], and fewer inhibitors of downstream fermentation [14]. Bokinski *et. al.* [15] demonstrated microbial production of butanol, fatty acid ester, and terpene biofuels from switchgrass pretreated with 1-ethyl-3-methyl imidazolium acetate (1). Integrated pretreatment and fermentation unit operations, using bio-based ILs, *e.g.* from amino acids [16, 17], have been demonstrated, as well as aqueous IL pretreatment systems using methanesulfonate anions [18]. Applications of ILs for the production of consumer products, such as textiles [19], and foods [20] have also garnered attention for bio-based ILs [21].
In many biochemical processes, toxicity is tantamount to efficacy. Mechanistic studies have shown IL toxicity generally increases with cation alkyl chain length as a result of cell membrane destabilization [22, 23]. N-alkylated pyridinium, imidazolium and quaternary ammonium cations showed increased toxicity across trophic levels, including studies with *C. elegans*, *D. magna*, and mammalian cell cultures [24–30]. Degree of functionalization of cation side chains [31], localization of heteroatoms on the cation and anion [28], anion chain length [32], and/or ion coordination [33] can all influence bioactivity. Introduction of polar functional groups into shorter alkyl chains reduced the aquatic toxicity of a broad range of cation classes [34].

Quaternary ammonium ILs have diverse engineering applications [35], and have been listed as suitable COVID-19 disinfectants for hard, non-porous surfaces [36]. Our research focuses on biomass applications using benzylammonium ionic liquids and deep eutectic solvents derived from lignin [37–39], and we have recently demonstrated isolation techniques for aminophenol IL precursors from oxidized kraft lignin [40].

Diez et al. prepared a series of trimethyl benzylammonium acetate ILs (6–8) from 4-methoxy benzaldehyde, syringaldehyde, and vanillin, respectively representing major oxidative depolymerization products from H-, S- and G-type lignin [38]. Though these compounds remained solid at room temperature, cellulose dissolution, switchgrass pretreatment, mass balances, and saccharification yields for 6–8 were comparable to 1. In the present study, modification of N-alkyl chains using diethyl and dipropyl amines provided room-temperature ILs 9–10. Cellulose solubility of the new compounds was examined using light microscopy and confirmed with IR spectroscopy. In efforts to inform *in planta* lignin engineering of biorefinery feedstocks [41], the toxicity of the lignin-derived ILs were determined using an *E. coli* strain recently optimized for the production of isoprenol (3-methyl-3-buten-1-ol) [42]. Several compounds were then evaluated with *Daphnia magna*, a model organism used to determine environmental toxicity. Of the lignin-derived ILs tested, those with elongated N-alkyl chains showed greater antibacterial activity, and those prepared from vanillin displayed the lowest toxicity against both *E. coli* and *D. magna*.

**Results And Discussion**

**Synthesis of Ionic Liquids**

Compound 1 was obtained from BASF, and compounds 3 and 5 were purchased from Sigma Aldrich. Compounds 2 and 4 were prepared from compounds 3 and 5, respectively, using ion exchange. Compounds 6–8 were prepared using CBILS™ methyl carbonate chemistry as reported elsewhere [38]. Compounds 11 and 12 were synthesized from their corresponding methyl carbonates by ion exchange with methanesulfonic acid.

Synthesis of 9 was accomplished using the vanillin-derived benzylamine, 4-((diethylamino)methyl)-2-methoxyphenol [39]. Bismethylation with methyl iodide provided the isolable intermediate, N-(3,4-dimethoxybenzyl)-N-ethyl-N-methylethanaminium iodide. Lastly, 9 was achieved by ion exchange with silver acetate in 85% yield over 2 steps (Scheme 1a). Similarly, 10 was prepared from N-(3,4-
dimethoxybenzyl)-N-propylpropan-1-amine using methyl iodide followed by silver acetate (77% yield over 2 steps) as shown in Scheme 1b. In contrast to 8, which formed a solid at room temperature, 9 and 10 remained liquid after extensive vacuum drying (Fig. 2). This result corresponds to those observed for alkyl and hydroxyalkyl ammonium ILs, whereby increasing N-alkyl chain length decreases melting point [43].

**Cellulose dissolution experiments**

Cellulose solubility in 8–10 was determined gravimetrically, and light microscopy of ILs containing different concentrations of cellulose is provided in Fig. 3. For compound 8, cellulose precipitation was observed at 4 wt% at 120 °C, which is in good agreement with previous studies compound 8 at 100 °C [38]. Cellulose dissolution was readily observed in compounds 9 and 10 at 2 wt% at 100 °C. To further examine cellulose solubility, washed samples of cellulose precipitated from ILs 8–10 (2 wt%) were centrifuged and analyzed by FTIR. For spectral comparison, untreated microcrystalline cellulose, and cellulose dissolved in 1 (10 wt%) were also analyzed, and are shown in Fig. 4.

The FTIR spectra show subtle, yet distinct differences between untreated cellulose and IL-treated cellulose. The broad resonance from 3600–3100 cm⁻¹, can be assigned to the OH-stretching vibration of hydrogen bonds [44], and signal shift from 3333 cm⁻¹ to 3407 cm⁻¹ can be observed for cellulose recovered from ILs 1, 8, 10. These results agree with work performed by Ciolacu et al. [45], who reported similar wavenumber increases corresponding to disruption of intra- and intermolecular H-bonds. The peak at 897 cm⁻¹ represents C-O-C stretching at β-(1–4)-glycosidic linkages, an indicator for increased amorphous regions in the cellulose [44, 45]. As shown in Fig. 4, the peak at 897 cm⁻¹ displayed stronger intensity in cellulose dissolved in 1, 8, 10, and weaker intensity in untreated cellulose, and cellulose suspended in 9.

The peaks at 1105 cm⁻¹ and 1054 cm⁻¹ are respectively assigned to CH stretching vibrations and C-O-C skeletal vibration of cyclic polysaccharides [44, 46]. It can be seen from Fig. 4 that the intensities of these signals are also related to decreased cellulose crystallinity. The peak intensity at 1105 cm⁻¹ is decreased in all dissolved cellulose samples. Additionally, decreased signal intensity at 1054 cm⁻¹ was clearly observed in all IL dissolved celluloses, except for compound 9. Lastly, the signal at 1031 cm⁻¹, which is assigned to C-O-C stretching vibration for untreated cellulose [46], shifted to a lower wavenumber (1017 cm⁻¹) in cellulose recovered from ILs 1, 8 and 10, yet this result is not observed in cellulose treated with 9.

Based on the results from Fig. 3 and Fig. 4, it can be concluded that lignin-derived ILs 8 and 10 had the greatest ability to dissolve and decrease the crystallinity of cellulose, under the temperature and time conditions tested. Among the selected lignin-derived ILs, 9 demonstrated slightly less effective cellulose dissolution and decrystallization, which may are arisen from minor, yet persistent impurities as observed in the ¹H NMR spectrum (Figure S5).

**Antibiotic properties of selected ILs**
The results of broth dilution assays with *E. coli* are shown in Fig. 5 as inhibitory concentration of IL required to decrease bacterial growth by 50% (IC$_{50}$). The results indicate that IL toxicity is a function of both cation and anion structure. As expected, increasing cation side chain length increases toxicity approximately 2.5-fold as observed when substituting N,N-dimethyl (8) with N,N-diethyl (9) and N,N-dipropyl (10). Compound 10 (IC$_{50}$ = 17.7 ± 0.9 mM) gave a slightly greater toxicity than 9 (IC$_{50}$ = 19.6 ± 0.4 mM). This result can be explained by the increase of lipophilicity with the increase of the alkyl side chain length, which facilitates disruption of bacterial cell membranes. Among the tetrabutylammonium ILs evaluated in this study, the acetate (2, IC$_{50}$ = 13.2 ± 2.9 mM) showed slightly less toxicity when compared to the bromide (3, IC$_{50}$ = 10.0 ± 1.3 mM, $p = 0.055$), and the toxicity of tetrabutylammonium ILs were generally greater than that of the benzylammonium ILs (4, 6–10). Benzylammonium hydroxide (5) showed IC$_{50}$ value of 10.4 ± 0.3 mM, which could be due to its pH influence on the broth dilution assay.

*denotes statistically significant difference ($p = 0.002$, $t = 4.00$) between 9 ($n = 7$) and 10 ($n = 5$).

Interestingly, when holding constant the acetate anion, and the N,N,N-trimethyl side chains of the cation, the toxicity of benzyl trimethylammonium ILs (4, 6–8) was modulated by the naturally-occurring frequency and position of methoxy substitutions on the aromatic ring. A single methoxy substitution at the *para* position increased toxicity 4-fold, as can be seen by comparing antibacterial values of compound 4 (IC$_{50}$ = 47.8 ± 0.6 mM) to 6 (IC$_{50}$ = 11.6 ± 1.7 mM). In compound 7, derived from syringaldehyde, where methoxy groups are found in *para* and both *meta* positions, the IC$_{50}$ value is 22.2 ± 0.8 mM. In compound 8, derived from vanillin, a methoxy group occupies the *para* and a single *meta* position, and the IC$_{50}$ is 48.2 ± 3.3 mM.

Anion type also affected the antibacterial activity of the lignin-derived ILs tested. Trimethyl benzylammonium acetate (4) was found to be 4-fold less toxic than its corresponding hydroxide (5). Holding the N,N,N-trimethyl vanillin-derived cation constant, introduction of a methanesulfonate anion (11, IC$_{50}$ = 78.7 ± 2.5 mM) reduced toxicity 1.6-fold as compared to the acetate anion (8, IC$_{50}$ = 48.2 ± 3.3 mM). Therefore, it can be concluded that the toxicity of anions showed a trend of methanesulfonate < acetate < hydroxide for N,N,N-trimethyl benzylammonium ILs.

Analogous structure-activity-relationships were observed from lignin-derived cations in acute toxicity assays with *D. magna*. The least antibacterial anion, methanesulfonate, was selected for evaluation, and paired with cations derived from G- and S-type lignin (11 and 12, respectively). Similar to the antibacterial trend, syringaldehyde-derived IL 12 (IC$_{50}$ = 0.123 ± 0.012 mM) was 2.6-fold more toxic to *D. magna* than vanillin-derived IL 11 (0.319 ± 0.066 mM). It should be noted that the IC$_{50}$ of 11 (0.319 mM) against *D. magna* is approximately 250-fold lower than its IC$_{50}$ against *E. coli* (78.7 mM) indicating that *D. magna* is far more sensitive to N,N,N-trimethyl benzylammonium ILs.

**Conclusion**
New synthetic approaches to lignin-derived quaternary ammonium compounds provided room-temperature ILs 9 and 10 that demonstrated rapid cellulose dissolution capacity at 100 °C. FTIR analysis confirmed that cellulose dissolution also reduced cellulose crystallinity. The IC\textsubscript{50} data of ILs assayed on engineered *E. coli* suggest that toxicity was affected by both the structure of the cations and the type of anions. Anion toxicity followed the trend of methanesulfonate < acetate < hydroxide. As compared to ILs derived from syringaldehyde and 4-methoxybenzaldehyde, asymmetric methoxy substitution on the benzyl ring of the cation may have caused the observed decrease in toxicity for vanillin-derived ILs. The antibacterial mechanism of action for compounds 9 and 10 is likely due to IL disruption of the lipopolysaccharide cell membrane, as evidenced by the trend of increasing toxicity with increasing cation N-alkyl chain length. The lower toxicity of ILs derived from vanillin (8, 11) against both *E.coli* and *D. magna* suggests that ILs derived from softwood lignin (G-type) could be favorable pretreatment solvents for biofuel and bioproduct applications with integrated fermentation, and/or involving consumer products. These results warrant additional consideration for *in planta* feedstock engineering within a lignocellulose biorefinery.

**Methods**

NMR spectra were acquired by a Bruker Advance III HD spectrometer at the frequency of 400 MHz for \textsuperscript{1}H (100 MHz for \textsuperscript{13}C). High-resolution mass spectrometry (HR-MS) was performed on an Agilent accurate-mass 6520B Q-TOF mass spectrometer. A Perkin Elmer Spectrum Two FTIR spectrometer equipped with a universal diamond ATR unit was used for the analysis of dissolved cellulose. Spectra of samples were recorded between range of 450 cm\textsuperscript{-1} and 4500 cm\textsuperscript{-1}, at a resolution of 4 cm\textsuperscript{-1}. The spectra shown in Fig. 4 represent the accumulation of 4 scans/sample with baseline correction applied. All purchased chemicals were used without purification.

**Procedure for the synthesis of ILs 2, 4, 9 and 10.**

Tetrabutylammonium acetate (2) was prepared by dissolving tetrabutylammonium bromide (3) (322 mg, 1 mmol, 1 eq) of in 20 mL of acetonitrile. Silver acetate (167 mg, 1 mmol, 1 eq) was then added to the mixture. The reaction was allowed to stir at RT for 1 hr. After the reaction, the solution was centrifuged at 4000 x G for 10 min, and the supernatant was collected and dried under vacuum (0.3 Torr, 1 hr) at 50 °C. The product was obtained as a light yellow oil in 89% yield. \textsuperscript{1}H NMR (acetone -d\textsubscript{6}): 0.97 (12H, t), 1.41 (8H, m), 1.68 (3H, s), 1.78 (8H, m), 3.48 (8H, t). \textsuperscript{13}C NMR: 13.93, 20.34, 24.50, 25.81, 59.14, 59.16, 174.47. HR-MS: [C\textsubscript{16}H\textsubscript{36}N]\textsuperscript{+}, found 242.2845, calcd. 242.2842 (1.2 ppm). The \textsuperscript{1}H and \textsuperscript{13}C NMR of compound 2 are shown in Figure S1-S2.

Benzytrimethylammonium acetate (4) was prepared by mixing 418 mg (1 mmol, 1 eq) of benzytrimethylammonium hydroxide (5, 40 wt% aqueous solution) with 60 mg glacial acetic acid (1 mmol, 1 eq). The reaction was allowed to stir at RT for 1 hr. After the reaction, the solution was dried under vacuum vacuum (0.3 Torr, 1hr) at 50 °C. The product was obtained as a clear liquid in quantitative
yield. $^1$H NMR ($D_2$O): 1.86 (3H, s), 3.06 (9H, s), 4.44 (2H, s), 7.52 (5H, m). $^{13}$C NMR: 23.42, 52.36, 52.40, 52.44, 69.63, 127.43, 129.25, 130.90, 132.86, 181.29. HR-MS: [C$_{10}$H$_{16}$N]$^+$, found 150.1278, calcd. 150.1277 (0.7 ppm). The $^1$H and $^{13}$C NMR of compound 4 are shown in Figure S3-S4.

$N$-(3,4-dimethoxybenzyl)-$N$-ethyl-$N$-methylethanaminium acetate (9) was prepared by dissolving 209 mg (1 mmol, 1 eq) of 4-((diethylamino)methyl)-2-methoxyphenol in 2 mL of acetone. Dry $K_2$CO$_3$ (138 mg, 1 mmol, 1 eq) was added, followed by methyl iodide (568 mg, 4 mmol, 4 eq). The reaction was kept at 70 °C for 24 hr. After the reaction, the solution was filtered and then dried under vacuum (0.3 Torr, 1 hr) at 50°C. The product was then re-dissolved in 20 mL of acetonitrile and mixed with silver acetate (167 mg, 1 mmol, 1 eq). After 1 hr of reaction at RT, the solution was centrifuged at 4000 x G for 10 min, and the supernatant was collected and dried under vacuum (0.3 Torr, 1 hr) at 50°C. The final product was obtained as a dark brown oil in 85% overall yield. $^1$H NMR (DMSO-$d_6$ with 1 µL acetone as calibration standard): 1.19 (6H, t), 1.73 (3H, s), 2.96 (6H, s), 3.29 (4H, q), 3.71 (3H, m), 4.91 (2H, m), 6.86 (3H, m). $^{13}$C NMR: 7.42, 24.29, 48.52, 55.17, 56.66, 57.54, 73.71, 112.17, 115.04, 120.40, 127.22, 147.41, 147.53, 173.10. HR-MS: [C$_{14}$H$_{24}$NO$_2$]$^+$, found 238.1803, calcd. 238.1802 (0.4 ppm). The $^1$H and $^{13}$C NMR of compound 9 are shown in Figure S5-S6.

$N$-(3,4-dimethoxybenzyl)-$N$-propylpropan-1-amine was prepared by dissolving 3,4-dimethoxybenzaldehyde (10.0 g, 60 mmol, 1 eq) into 200 mL dry acetonitrile. While stirring, dipropylamine (7.92 g, 78 mmol, 1.3 eq) was added followed by sodium triacetoxyborobydride (17.85 g, 84.2 mmol, 1.4 eq). The reaction was allowed to stir at RT overnight and worked up as reported elsewhere [37]. After vacuum drying (0.3 Torr, 1 hr) at 50°C, the product (14.1 g, 55.8 mmol) was obtained as a pale yellow oil in 93% yield. $^1$H NMR (CDCl$_3$): 0.86 (6H, t), 1.49 (4H, m), 2.40 (4H, t), 3.53 (2H, s), 3.86 (3H, s), 3.88 (3H, s), 6.79 (2H, m), 6.99 (1H, s). $^{13}$C NMR: 11.99, 19.97, 55.61, 55.94, 55.98, 58.40, 110.76, 112.09, 120.92, 148.01, 148.95. HRMS: [C$_{15}$H$_{26}$NO$_2$]$^+$, found 252.1955, calcd. for 252.1958 (1.2 ppm). The $^1$H and $^{13}$C NMR of N-(3,4-dimethoxybenzyl)-N-propylpropan-1-amine are shown in Figure S7-S8.

$N$-(3,4-dimethoxybenzyl)-$N$-methyl-$N$-propylpropan-1-aminium acetate (10) was prepared by dissolving N-(3,4-dimethoxybenzyl)-N-propylpropan-1-amine (325 mg, 1 mmol) in 2 mL of acetone. Methyl iodide (284 mg, 2 mmol, 2 eq) was then added to the mixture. The reaction was kept at 70 °C for 24 hr. After the reaction, the solution was filtered and dried under vacuum (0.3 Torr, 1 hr) at 50°C, and the product was then re-dissolved in 20 mL of acetonitrile and mixed with silver acetate (167 mg, 1 mmol, 1 eq). After 1 hr of reaction at RT, the solution was centrifuged at 4000 x G for 10 min, and the supernatant was collected and dried under vacuum (0.3 Torr, 1 hr) at 50°C. The product was obtained as a dark brown oil in 77% overall yield. $^1$H NMR: 0.89 (6H, t), 1.60 (3H, s), 1.75 (4H, m), 2.92 (3H, s), 3.14 (4H, m), 3.78 (6H, s), 4.52(2H, s), 7.09 (3H, m). $^{13}$C NMR (DMSO-$d_6$): 10.58, 15.28, 25.58, 46.82, 48.43, 55.51, 55.67, 61.31, 64.44, 111.54, 116.34, 120.18, 125.90, 148.54, 150.12, 173.18. HR-MS: [C$_{16}$H$_{26}$NO$_2$]$^+$, found 266.2118, calcd. 266.2115 (1.1 ppm). The $^1$H and $^{13}$C NMR of compound 10 are shown in Figure S9-S10.
Cellulose dissolution

Avicel PH 101 microcrystalline cellulose obtained from Sigma Aldrich was used in the dissolution experiments. To test cellulose solubility, 500 mg of selected ILs were collected in a 7 mL transparent glass tube and heated in a sand bath at 100 °C for 1 hr (compound 8 was heated to 120 °C for 1 hr for due to its higher melting point). Cellulose (10 mg, 2 wt%) was added slowly into the tube, and the solution was stirred with a spatula. After 20 min of incubation, a drop of the solution was taken and observed under a light microscope (10x magnification). The procedure was repeated with 20 mg (4%) of total cellulose addition.

FTIR spectroscopy of dissolved cellulose

The IL solutions with 2 wt% cellulose concentration were used for FTIR analysis. Methanol (5 mL) was added into the tube to dissolve the IL and precipitate the dissolved cellulose. The solution was then transferred to a 50 mL centrifuge tube and centrifuged at 4000 x G for 10 min. After removal of the supernatant, the cellulose at bottom was dried in an oven at 70 °C for 1 h before FTIR analysis.

Antibiotic assays

*E. coli* strain 1A1 [40] was obtained from the microbial strain repository at the Joint Bioenergy Institute at Lawrence Berkeley National Laboratory. A 10 mL culture in Luria Broth (LB) media was grown for 18 hr at 37 °C with shaking at 200 rpm. The culture was then diluted into LB media to an absorbance of 0.05 at 600 nm, and added into the wells of a 96-well plate (first row = 190 µL, all other wells = 100 µL/well). Approximately 700 mg of IL was dissolved into 1 mL of methanol, and 10 µL of each IL solution was added to first row wells of the plate. A 2-fold serial dilution was performed down the plate by mixing and transferring 100 µL using a multi-channel pipette. To maintain isochoric conditions, 100 µL was discarded from bottommost wells. The plate was then incubated at 37° C with shaking at 150 rpm. After 24 hr incubation, *E. coli* growth was quantified at 600 nm using an Epoch Microplate Spectrophotometer (Gen5 software). Cells grown with 10 µL of methanol were used as negative controls, and resulted in zero growth inhibition. All experiments were repeated 3–7 times and error bars in Fig. 5 represent standard deviation. Statistical F-test was performed to determine the appropriate t-test (i.e., equal or unequal variance) to analyze significant difference between IC$_{50}$ data.

Daphnia magna cultivation

Procedures for cultivating and performing acute toxicity assays with *D. magna* generally followed the US EPA protocol [47]. *Daphnia magna* was cultured from stocks supplied by Aquatic Bio Systems Inc. (Fort Collins, CO). The organisms were cultured in 900 mL jars filled with moderately hard water from the recipe: 0.473 g CaSO$_4$, 0.959 g NaHCO$_3$, 1.223 g MgSO$_4$ • 7H$_2$O, and 0.039 g KCl per 10L of deionized water. 2 mL of algae (*Selenastrum capricornutum*) containing $3.0 \times 10^7$ cells/mL were fed to *D. magna* on both Tuesdays and Thursdays with water changes as well as feedings on Saturdays. All glass jars were placed in a Thermo Scientific Precision incubator (Fischer Scientific, Hampton, NH) at a holding
temperature of 20°C and on a 12 hr: 12 hr light: dark cycle. This procedure was conducted approximately a month prior to the beginning of the project. To prepare *D. magna* for use in acute experiments, three hundred adult daphnids were separated into five 1L beakers with 60 daphnids each. These daphnids were used a week later in the experiment. All daphnid cultures followed the same protocol for feeding and water changes as mentioned previously.

**Acute Toxicity Study of ILs on D. magna**

A 48-hour acute toxicity study was carried out to examine the toxic effects of 11 and 12 on *D. magna*, Glass jars were filled with moderately hard water and spiked with the appropriate concentration of IL from stock solution to a total volume of 50 mL. For each IL, 5 different concentrations were studied. Control jars were set up with only 50 mL of moderately hard water. Four replicates were used for each concentration and each replicate had four daphnids. The daphnids were placed into glass jars for each concentration of ILs. All glass jars were placed in a Thermo Scientific Precision incubator (Fischer Scientific, Hampton, NH) at a holding temperature of 20°C and on a 12 hr: 12 hr light: dark cycle. Mortality in each jar was recorded every 24-hour.

**Declarations**

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government, and the publisher, by accepting the article for publication, acknowledge that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work or allow others to do so, for U.S. Government purposes.

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**Authors’ contributions:** AS and SL conceived of the study, designed the experiments, examined all spectroscopic data and wrote the manuscript. SL performed all synthesis, cellulose dissolution experiments, microscopy and spectroscopy. SL and MG performed all antibacterial assays and performed statistical analysis of the data. SW and CK designed and performed all assays with *D. magna* and SW assisted with statistical analysis of antibacterial data.

**Availability of data and material:** Data and materials used in this study are available upon request.

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