

Contents lists available at ScienceDirect

### Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

# Amino acid-based dicationic ionic liquids as complex crop protection agents



Damian Krystian Kaczmarek <sup>a,\*</sup>, Daniela Gwiazdowska <sup>b</sup>, Katarzyna Marchwińska <sup>b</sup>, Tomasz Klejdysz <sup>c</sup>, Marta Wojcieszak <sup>a</sup>, Katarzyna Materna <sup>a</sup>, Juliusz Pernak <sup>a</sup>

<sup>a</sup> Department of Chemical Technology, Poznan University of Technology, ul. Berdychowo 4, Poznan 60-965, Poland

<sup>b</sup> Department of Natural Science and Quality Assurance, Institute of Quality Science, Poznań University of Economics and Business, al. Niepodległości 10, Poznan 61-875, Poland <sup>c</sup> Institute of Plant Protection – National Research Institute, ul. W. Węgorka 20, Poznan 60-318, Poland

#### ARTICLE INFO

Article history: Received 4 March 2022 Revised 28 April 2022 Accepted 7 May 2022 Available online 13 May 2022

Keywords: Amino acid Dicationic ionic liquids Phytotoxicity Feeding deterrents Antimicrobial activity Synthesis

#### ABSTRACT

The utilization of efficient synthesis methods and natural raw materials to obtain novel compounds with biological activity is crucial due to the requirement to reduce the negative effect on the environment. In this study, we responded to recent trends and developed a procedure to synthesize a new group of ionic liquids containing L-histidinate or L-prolinate anions and bis-ammonium or bis-phosphonium cations with an alkyl linker or with a linker containing two ester bonds. The structures of the obtained compounds were confirmed by performing spectral analyses (FT-IR, <sup>1</sup>H and <sup>13</sup>C NMR). Based on analyses of the effect of the obtained compounds on the deterrent activity against storage insects, the obtained ionic liquids exhibited at least weak deterrent activity and the length of the alkyl linker and the presence of an ester bond in the cation were essential determinants of biological activity. Furthermore, in the case of several salts, we observed significant positive effects on root system development and limited inhibition of shoot growth, indicating reduced phytotoxicity. Analyses of relevant physicochemical properties (differential scanning calorimetry, thermogravimetric analysis, surface activity, and solubility analysis) were performed to determine the effect of structure on the measured parameters. Furthermore, antimicrobial activity assays were performed to analyze the environmental effects of the products. Our results indicate a significant future potential use of these novel ionic liquids as agrochemicals.

© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http:// creativecommons.org/licenses/by/4.0/).

#### 1. Introduction

The increased demand for cereal products results in the necessity to secure cereal silos against of negative effects of all types of harmful organisms. The most notable threats that may occur in granaries include the growth of bacteria and fungi or the feeding of storage pests on grains [1,2]. Bacteria and/or fungi deteriorate grain in two ways, mainly by accelerating grain decomposition and by producing toxic substances that are absorbed by the grain, and can then affect living organisms [3,4]. A completely different mechanism is observed in the case of insects. Storage pests treat grain as a source of food, which in turn reduces its mass and decreases its aesthetic value. In addition, insects contaminate the warehouses with their excrements, excretions and dead bodies. Therefore, various methods should be used to protect the harvest and obtain high-quality grains stored in cereal silos [5].

\* Corresponding author. *E-mail address:* damian.kaczmarek@put.poznan.pl (D.K. Kaczmarek).

The preparation of chemical agents with all the aforementioned properties is possible by designing new compounds belonging to the group of ionic liquids (ILs), which consist of an organic cation and an organic or inorganic anion [6,7]. In addition, the designability of ILs makes them an attractive group of compounds with uses in a wide variety of scientific and industrial fields (replacements for traditional solvents, media for chemical or enzymatic reactions, surfactants, disinfectants, antistatic or softening substances, extractants, and wood preservatives) [8]. Their low volatility is undoubtedly an additional advantage because it notably reduces their negative effects on people directly exposed through contact. Notably, a slight modification of the structures of the active substance enables the formation of multifunctional ILs with specific physicochemical and biological properties (third-generation ionic liquid) [9–14]. The structure of ILs is also a decisive element determining the environmental impact, since inappropriate selection of ions and their modification may contribute to changes in toxicity. Extensive research regarding this issue indicates a significant effect of the cation structure on toxicity toward wheat, barley, and garden cress [15,16]. Moreover, cation and anion structural elements



contribute to a change in toxicity toward bacteria and fungi, and influence the rate and level of biodegradation [17–19].

Dicationic ionic liquids (DILs) are an interesting group of ILs composed of a cation that contains two centers with a positive charge in its structure. Commonly used cations in DILs are bisammonium or bis-phosphonium cations [20]. Moreover, the original chemical structure and properties of DILs have resulted in many important scientific studies in which gemini compounds were utilized as novel surfactants or antibacterial and antifungal compounds [21-23]. Recent reports describe the use of gemini compounds with a bis-ammonium cation as herbicidal ionic liquids or antifeedant ionic liquids [24-26]. These reports indicate bis-ammonium appropriately designed and bisthat phosphonium cations achieve the desired physicochemical and biological properties.

The selection of the appropriate counterion is also very important during the synthesis of ILs. In recent years, the use of amino acids as anions has been one of the main interests. Indeed, this concept represents a crucial aspect of the synthesis of nature-inspired compounds. Based on published data, these compounds are nontoxic and biodegradable, are commonly found in living organisms, and have numerous useful applications, e.g., as medicines or nutrients and as dietary supplements [27–29].

The purpose of the study was to synthesize novel dicationic ionic liquids containing L-histidine or L-proline as an anion. The developed DILs contained two different linkers in the cations (a straight alkyl chain or an alkyl chain containing additional ester bonds). As a result, novel bioinspired compounds with biological activity and reduced toxicity were obtained. Since these DILs have not been described previously, the development of an efficient synthesis method and confirming the designed structures were crucial parts of the study. The research also includes an extensive assessment of the potency of the obtained compounds to protect cereal products. Therefore, we conducted a thorough analysis of the effect of the cation structure on the bactericidal, fungicidal, and antifeedant activities of the synthesized DILs. Furthermore, their positive or negative effects on plant development were determined.

#### 2. Experimental

#### 2.1. Materials

Tributylamine (99.5%, CAS Number 102-82-9), tributylphosphine (97%, CAS Number 998-40-3), 1,4-dibromobutane (99%, CAS Number 110-52-1), 1.8-dibromooctane (98%, CAS Number 4549-32-0), 1,12-dibromododecane (98%, CAS Number 3344-70-5), L-histidine (>99%, CAS Number 71-00-1), and L-proline (99%, CAS Number 147-85-3) were purchased from Sigma-Aldrich. Potassium hydroxide (85%, CAS Number 1310-58-3) was purchased from Alfa Aesar. Potassium chloroacetate (CAS Number 7748-25-6) was obtained at the Department of Chemical Technology of Poznan University of Technology. All solvents (methanol, dimethylsulfoxide (DMSO), acetonitrile, 2-propanol, ethyl acetate, chloroform, and hexane) were also provided by Sigma-Aldrich. Water for the solubility and surface activity measurements was deionized, with a conductivity below 0.1  $\mu$ S cm<sup>-1</sup>, using the HLP Smart 1000 demineralizer (Hydrolab). Microbiological media were purchased from Oxoid (United Kingdom).

#### 2.2. Synthesis

A. Synthesis of alkane-1,ω-bis(tributylammonium) dibromide with an alkyl linker.

Alkane-1, $\omega$ -bis(tributylammonium) dibromides were obtained by performing a quaternization reaction between the appropriate dibromoalkane (0.1 M) and tributylamine (0.2 M). Reactions were conducted in a 50 cm<sup>3</sup> mixture of acetonitrile:methanol (10:1 v:v) at 60 °C for 24 h. Next, the solvents were removed by a vacuum evaporator, and the product of the reaction was mixed with ethyl acetate. After the solvent was added, the dibromide bisammonium precipitated as a white solid and was isolated by filtration. The final product was dried under reduced pressure at 70 °C for 24 h.

B. Synthesis of alkane-1, $\omega$ -bis(tributylphosphonium) dibromide with an alkyl linker.

Alkane-1, $\omega$ -bis(tributylphosphonium) dibromides were obtained through a quaternization reaction between the appropriate dibromoalkane (0.1 M) and tributylphosphine (0.2 M). The reactions were conducted in 50 cm<sup>3</sup> of chloroform at 40 °C for 72 h under nitrogen. Next, the solvents were removed by a vacuum evaporator, and the product of the reaction was mixed with acetonitrile. The mixture was then transferred to a 250 cm<sup>3</sup> separation funnel. The remaining substrates were extracted from acetonitrile by washing them three times with 50 cm<sup>3</sup> of hexane. Next, acetonitrile was removed using a rotary evaporator and the product was dried under reduced pressure at 70 °C for 24 h.

#### C. Synthesis of tributylammonioacetate.

Tributylamine (0.05 M) was dissolved in 50 cm<sup>3</sup> of methanol and inserted into a reactor with a magnetic stirrer and a heating bowl. Next, 0.05 M potassium chloroacetate was added, and the reaction was carried out at 60 °C for 72 h. After the reaction, the mixture was cooled to 5 °C, which led to the precipitation of potassium chloride. The precipitated by-product was filtered off, and than methanol was evaporated. The obtained product was further dried in a vacuum dryer at 60 °C for 48 h. The product was stored in a vacuum desiccator on molecular sieves to maintain the low water content of the sample. The product was obtained at an 92% yield.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ [ppm] = 0.98 (9H, t); 1.39 (6H, m); 1.75 (6H, m); 3.02 (6H, m); 4.06 (2H, s).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ [ppm] = 13.2, 19.7, 24.7, 51.6, 60.8, 171.1.

#### D. Synthesis of tributylphosphonioacetate.

Tributylphosphine (0.05 M) was dissolved in 50 cm<sup>3</sup> of methanol and inserted into a reactor with a magnetic stirrer and a heating bowl. Next, 0.05 M potassium chloroacetate was added, and the reaction was carried out at 60 °C for 96 h. Nitrogen gas was used to remove oxygen from the system and to prevent the formation of the by-product. Following the reaction, the mixture was cooled to 5 °C, which led to precipitation of potassium chloride and by-product was filtered off. After the filtration, methanol was evaporated. Multiple acetonitrile:hexane extractions were used to purify the main product from unreacted substrates. After purification, acetonitrile was further dried in a vacuum dryer at 60 °C for 48 h. The product was stored in a vacuum desiccator over molecular sieves to maintain the low water content of the sample. The product was obtained with a 70% yield.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ [ppm] = 0.97 (9H, t); 1.53 (12H, m); 2.04 (2H, d); 2.40 (6H, m).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ [ppm] = 12.9, 19.0/19.5, 23.1/23.3, 26.7/27.3, 159.8.

E. Synthesis of alkane-1,ω-bis(carboxymethyltributylammo nium) or alkane-1,ω-bis(carboxymethyltributylphospho nium) dibromide with two ester bond linker.

Tributylammonio acetate or tributylphosphonio acetate (0.05 M) was dissolved in 50 cm<sup>3</sup> of methanol or acetone. Then 1,4-dibromobutane, 1,8-dibromooctane or 1,12-dibromododecane (0.025 M) was added and the reaction was carried out at 60 °C for 120 h. Following the reaction, the solvent was evaporated from the product. In the next step, hexane was added to the obtained DILs to precipitate the reaction product. This step was repeated 5 times to remove impurities. The obtained product was then dried in a vacuum dryer at 70 °C for 72 h. The synthesized products were stored in a vacuum desiccator on molecular sieves to maintain the low water content of the product.

#### F. Exchange reaction.

Potassium salts of L-histidine or L-proline (0.05 M), which were obtained in a previous reaction of an amino acid with potassium hydroxide, were placed in a reaction vessel and dissolved in 30 cm<sup>3</sup> of methanol. Bis-ammonium or bis-phosphonium dibromide was then added in a stoichiometric ratio (0.025 M). The reaction mixture was stirred at 25 °C until the inorganic salts (KBr) precipitated. The mixture was then cooled to 5 °C and the inorganic salt was removed by filtration. In the next step, methanol was evaporated from the filtrate. The product was dissolved in acetonitrile and the mixture was cooled to 5 °C to remove the inorganic salt residues. The precipitated residue was filtered, and the solvent was evaporated. Finally, the product was dried in a laboratory dryer under reduced pressure at 60 °C for 24 h. All synthesized salts were stored under vacuum at 5 °C on P<sub>4</sub>O<sub>10</sub>.

#### 2.3. General

The methods were performed as previously described. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were collected using a Varian VNMR-S 400 spectrometer operating at 400/300 and 100/75 MHz, respectively. Tetramethylsilane (TMS) was used as an internal standard, and deuterated methanol and water (CD<sub>3</sub>OD:D<sub>2</sub>O at a 1:4 ratio) were used as solvents in the analysis. FTIR spectra were recorded using an IFS 66v/S spectrometer (Bruker Optics, Ettlinger, Germany). Data were sampled from 4000 to 400 cm<sup>-1</sup> and visualized using Spectragryph 1.2.13<sup>S1</sup> (IFS 66v/S). The samples for measurement were prepared as KBr pellets (solids) or as thin films between KBr plates (liquids). The water content in all obtained products was measured with a TitroLine 7500 KF trace apparatus (SI Analytics, Germany) using the Karl Fischer titration method. First, each compound was dissolved in anhydrous methanol. After the concentration of water in the pure methanol and in the obtained methanolic solutions was determined, the water content in the products was calculated.

#### 2.4. Thermal properties

Thermal analysis was performed based on the method described in the literature [6]. Thermal gravimetric analysis (TGA) was performed using a Mettler Toledo Stare TGA/DSC1 unit (Leicester, UK) under nitrogen. Samples (2–10 mg) were placed in aluminium pans and heated from 30 to 450 °C at a heating rate of 10 °C min<sup>-1</sup>. Phase transition temperatures were determined by differential scanning calorimetry (DSC), with a Mettler Toledo Stare DSC1 (Leicester, UK) unit under nitrogen. The samples (5–15 mg) were placed in aluminium pans and heated from 25 to 100 °C at a heating rate of 10 °C min<sup>-1</sup> and cooled with an intracooler at a cooling rate of 10 °C min<sup>-1</sup> to - 100 °C and then heated again to 100 °C.

#### 2.5. Solubility

The solubility of the obtained DILs was determined following the methodology described in Vogel's Textbook of Practical Organic Chemistry [30]. Six solvents (water, methanol, DMSO, acetonitrile, 2-propanol and hexane) were selected for the solubility test on the basis of their usefulness in synthesis and agrochemistry. According to the methodology, a sample of the compound  $(0.1 \pm 0.0001 \text{ g})$ was introduced into three volumes of each solvent and the behavior of the sample was recorded. The term 'good solubility' refers to compounds that dissolved in 1 cm<sup>3</sup> of the solvent; 'limited solubility' applies to ILs that dissolved in 3 cm<sup>3</sup> of the solvent; and 'poor solubility' refers to compounds that were insoluble in 3 cm<sup>3</sup> of the solvent. All analyses were performed at 25 °C.

#### 2.6. Phytotoxicity

Effect on the germination and early development of plants. The experiment involved the assessment of the effect of synthesized salts on the germination and early development of plants of white mustard (*Sinapis alba*) using the phytotoxicity test based on the international standard ISO-11269–2:2003. The germination test was conducted in vertical plastic containers (Phytotoxkit, Tigret, Belgium). The containers were filled with 100 g of soil, and then the analyzed substance was dissolved in water (25 cm<sup>3</sup>) and added to the soil to achieve effective concentrations of 10 g/ kg of soil dry weight. In the next step, 10 white mustard seeds were placed separately on the soil layer in a single row. Then the Phytotoxkit plastic containers were closed, placed in the dark, and maintained at a temperature of  $25 \pm 1$  °C for 7 days. At the end of the experiment, the number of germinated seeds was counted, and the lengths of the roots and shoots were measured.

Soil with the following elemental composition was used for the experiment: 67 mg P kg<sup>-1</sup>, 55 mg K kg<sup>-1</sup>, 54 mg Mg kg<sup>-1</sup>, 100 mg Fe kg<sup>-1</sup>, pH of 5.75 (in CaCl<sub>2</sub>), and an organic C content of 1.90% (19.00 g kg<sup>-1</sup>). Based on the obtained data, the germination capacity was calculated using the following equation:

$$G = (\frac{G_S}{G_E}) \times 100\%$$

where  $G_s$  is the number of germinated seeds and  $G_E$  is the total number of examined seeds.

All experiments were conducted in triplicate, and all error bars presented in the figures represent SEM values.

#### 2.7. Feeding deterrent experiment

The deterrent activity of the obtained salts toward two species of essential storage grain pests, the granary weevil (Sitophilus granarius (Linnaeus, 1758)) and the khapra beetle (Trogoderma granarium Everts, 1898), was evaluated. These insects were reared in the laboratory in an incubator at  $26 \pm 10$  °C and a relative humidity of 60 ± 5% on uncrushed wheat grain (granary weevil) and crushed wheat grain products: flour, bran, and others (khapra beetle). Azadirachtin was used as a reference substance in the experiment. Choice and no-choice tests were conducted using a method described in the literature [31]. Wafer disks that were 1 cm in diameter and 1 mm thick (average weight of approximately 15 mg) were composed of wheat flour. The prepared wafers were saturated by dipping them in methanol (control) or a 1% solution of the obtained salt in methanol and then air-dried for 30 min. After the evaporation of the solvent, the wafers were weighed and provided to insects in plastic boxes as the sole food source for five days. The wafers were placed in the Petri dishes according to the type of test:

- two disks treated with solvent (control for an experiment in a test variant without choice),
- one disk previously dipped in solvent and one disk dipped in a 1% solution of the test compound (choice test),
- two disks previously dipped in a 1% solution of the test compound (no-choice test).

Both variants of the experiment and control were performed in 5 replicates, where 3 adults of the granary weevil or 10 larvae of the khapra beetle were placed in each dish. The number of insects used in the experiments depended on the feeding intensity. After the 5th day, the wafers were weighed again and the average weight of the food consumed was calculated. The values of coefficients A (absolute coefficient of deterrence) and R (relative coefficient of deterrence) were calculated as follows:

$$R = \frac{(C - E)}{(C + E)} \times 100$$
(choice test)

$$A = \frac{(CC - EE)}{(CC + EE)} \times 100(no - choice test)$$

where C and CC correspond to the amount of food consumed from the control disks, and E and EE correspond to the amount of food treated with the tested compound that was consumed.

The total coefficient of deterrence (T) was the sum of the relative and absolute coefficients.

Deterrent activity was defined according to a predetermined scale, where the total coefficient of deterrence was used. Biological activities with T values ranging from 151 to 200 indicate very good deterrents, those with values ranging from 101 to 150 indicate good deterrents, those with values ranging from 51 to 100 indicate moderate deterrents, and those with values < 50 indicate weak deterrents. The deterrence coefficients of the group of compounds were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test with homogenous subsets.

#### 2.8. Microbial toxicity assay

#### 2.8.1. Microorganisms

The following microorganisms used in the research - (i) Grampositive bacteria: Staphylococcus aureus ATCC 33862, Staphylococcus epidermidis ATCC 12228, Enterococcus faecalis ATCC 19433, Bacillus subtilis ATCC 11774, Micrococcus luteus ATCC 4698, Clostridium perfringens ATCC 13124, Lactiplantibacillus plantarum DKK 003 (Department of Natural Science and Quality Assurance, PUEB collection), (ii) Gram-negative bacteria: Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Serratia marcescens ATCC 8100, Proteus vulgaris ATCC 49132, Moraxella catarrhalis ATCC 25238, Salmonella enteritidis ATCC 13076, and (iii) fungi: Candida albicans ATCC 10231, Rhodotorula mucilaginosa DKK 040 (Department of Natural Science and Quality Assurance, PUEB collection), Botrytis cinerea BPR 187 and Fusarium graminearum KZF 1 (Research Centre for Registration of Agrochemicals, Institute of Plant Protection-National Research Institute collection). Before the experiments, the microorganisms were cultivated on appropriate media for 24 h for bacteria and yeast, and 5-7 days for filamentous fungi.

### 2.8.2. Determination of MIC and MBC/MFC values by two-fold dilution in microplates

The minimal inhibitory concentration (MIC) and minimal bactericidal/fungal concentration (MBC/MFC) of 24 DILs were determined using the broth microdilution method and spot inoculation method, respectively. Before the experiments, the ionic liquids were dissolved in water or water with DMSO (1:1) (compounds: 7, 8, 9, 10, 19, 20, and 21), resulting in a starting concentration of 2000  $\mu$ g cm<sup>-3</sup>. Didecyldimethylammonium chloride [DDA][Cl] and benzalkonium chloride [BA][Cl] were tested as substances to compare the antimicrobial activity.

#### a. Bacteria and yeast

The microbial suspensions prepared in Mueller-Hinton broth for bacteria and in Sabouraud broth for yeasts from 24-hour cultures were standardized to obtain MacFarland's optical density of 0.5 with a final cell concentration of 10<sup>6</sup> cfu cm<sup>-3</sup>. Two-fold series dilutions of the tested ionic liquids were prepared in 96-well microtiter plates at concentrations ranging from 0.5 to 1000  $\mu$ g cm<sup>-3</sup>. For this purpose, 100  $\mu$ l of Mueller-Hinton or Sabouraud broth were introduced into the wells of sterile microplates, except for the first row that contained ionic liquid solutions at a concentration of 2000  $\mu$ g cm<sup>-3</sup>. Subsequently, dilutions of the ionic liquids were prepared, and then 100 µl of the bacterial or veast suspensions were added to all wells, obtaining a final inoculum density of  $5 \times 10^5$  cfu cm<sup>-3</sup>. Medium containing the ionic liquid was used as a negative control, while the positive control was the culture of microorganisms without the addition of the inhibitory agent. The plates were incubated at a temperature of 30 or 37 °C, as appropriate for the growth of each of the tested microorganisms, for 24 h. The optical density of microorganism growth was determined by measuring the absorbance at 600 nm using a BioTek Epoch 2 microplate reader.

#### b. Filamentous fungi

Fungal suspensions were prepared by harvesting the hyphae and conidia from fresh cultures on PDA agar (Potato Dextrose Agar) and mixing them with 10 cm<sup>3</sup> of 0.85% sterile saline. The cell concentration was adjusted to  $10^6$  cells/cm<sup>3</sup> by enumeration using a hemocytometer chamber. Serial two-fold dilutions of the tested ionic liquids were prepared as described above. Then, 100 µl of a suspension of hyphae and fungal spores were introduced into all wells. The medium containing ionic liquids was used as a negative control, while the fungal culture lacking the inhibitory agent was used as a positive control. The plates were incubated at 30 °C under aerobic conditions for 5–7 days. The interaction of the ionic liquids was assessed visually.

The results are reported as the averages of three parallel replicates. The concentration of the ionic liquid that inhibited the growth of the microorganism by at least 90% was defined as the MIC value. The MBC/MFC value was determined by plating 10  $\mu$ l of culture along with the ionic liquids at a concentration equal to or greater than the determined MIC value on plates with an appropriate agar medium, and spectrophotometric measurements or a visual assessment in the case of filamentous fungi revealed 100% inhibition of microbial growth.

#### 2.9. Surface activity

Measurements such as contact angle and surface tension were performed using a DSA 100E analyzer (Krüss, Germany with accuracy  $\pm$  0.01 mN m^{-1}) at a controlled temperature of 25 °C. The particular steps of the method are described in our previous studies. The surface tension was determined using the drop shape method, while the contact angle was determined based on the sessile drop method. As mentioned above, the surface tension ( $\gamma$  in mN m^{-1}) was measured to investigate the surface activity of the analyzed compounds by performing an automatic examination of the drop profile using the Laplace equation. The wettability of the synthesized compounds was tested using paraffin as the wetting surface.

#### 3. Results and discussion

#### 3.1. Synthesis

#### Synthesis of the precursors

Syntheses of bis-ammonium and bis-phosphonium salts with an alkyl linker (Compounds 1–3 and 7–9).

Bis-ammonium dibromides and bis-phosphonium dibromides with an alkyl linker were synthesized using methods described in the literature with minor modifications. During the synthesis of salts 1-3, a mixture of acetonitrile:methanol (10:1 v:v) was used instead of acetonitrile to increase the solubility of tributylamine. As a result, the reaction yield was increased compared to the reaction that proceeded in acetonitrile or methanol only [24]. During the synthesis of salts 7–9, the solvent was changed from acetone to chloroform. Furthermore, the reaction was conducted under a nitrogen atmosphere to prevent the oxidation of tributylphosphine. In addition, the hazardous ethers used to precipitate the main product after the reaction were changed to extraction with hexane and acetonitrile to purify the compound [32,33]. The obtained salts were stored in a vacuum desiccator over a silicon deposit. Products 1-3 and 7-9 were obtained with yields exceeding 75% and are listed in Table 1.

Synthesis of bis-ammonium and bis-phosphonium salts with a linker containing two ester bonds (Compounds **4–6** and **10–12**).

Alkane-1, $\omega$ -bis(carboxymethyltributylammonium) dibromides were synthesized using different methods from those presented in the literature [34-36]. Moreover, to the authors' knowledge, the methods used to synthesize alkane-1,  $\omega$ -bis(carboxymethyltri butylphosphonium) dibromides were first reported in this publication. In the first stage, tributylammonio acetate and tributylphosphonio acetate were obtained in a novel manner, as described in detail in the experimental part in subsections C and D. Furthermore, the synthesized products were O-alkylated to obtain bisammonium and bis-phosphonium dibromides with ester bonds in the linker. This reaction was conducted in methanol or acetone at 60 °C for 120 h. A shorter reaction time led to a significant decrease in the reaction yield. Following the reaction, the solvent was evaporated and the product was purified by leaching the impurities with hexane. The precipitated product was separated from the solvent and dried in a vacuum dryer for 72 h at 70 °C. The compounds were stored in a vacuum desiccator on a silicon deposit to prevent the absorption of water. The experimental results of the synthesis for salts **4–6** and **10–12** are presented in Table 1.

## Synthesis of DILs with bis-ammonium and bis-phosphonium cations (**1a–12a** and **1b–12b**)

The final stage of the synthesis was the metathesis reaction between the bis-ammonium and bis-phosphonium dibromides obtained above and the potassium salts of L-histidine or Lproline. The reaction was performed using the methods described in the literature, where methanol was used as a solvent [24,39,40]. Following the reaction, the mixture was cooled to 5 °C and the inorganic salt was removed by filtration. The solvent was then evaporated on a rotary vacuum evaporator. Subsequently, in contrast to the methods described above, acetonitrile was added to precipitate the inorganic salts (the use of acetone turned some solutions yellow, which indicated an undesirable side reaction). The prepared mixture was cooled to 5 °C and then the byproduct was filtered off. For each salt obtained, the potassium bromide level was determined by performing a test using AgNO3 and was < 1000 ppm. The outcome of the synthesis reactions was the synthesis of 24 new amino acid ionic liquids with bisammonium or bis-phosphonium cations with yields exceeding 93% (Table 2). The water content of the compounds tested ranged from 1.9 to 2.5%. The structure of the synthesized DILs is presented in Fig. 1.

#### 3.2. Spectral analysis of synthesized DILs

The structures of the synthesized products were confirmed by FT-IR, and <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. All spectra and descriptions for DILs **1a–12a** and **1b–12b** are presented in Figures S1-S72 in the Supplementary Material.

An analysis of the FT-IR spectra of the obtained DILs confirmed the presence of bands at 2800–3100 cm<sup>-1</sup>, and their intensity increased with an increasing number of carbon atoms in the linker. The bands in this region were assigned to the stretching vibrations of alkyl C–H bonds. Additionally, all products exhibited a characteristic band at 3800–3100 cm<sup>-1</sup> associated with stretching vibrations of the N-H ( $\nu \sim N-H$ ) and O-H ( $\nu \sim O-H$ ) groups in the anions that originated from L-proline (**a**) and L-histidine (**b**). Furthermore, in the FT-IR spectra of the DILs, intense peaks originating from amino acid anions were identified, as bands from the carboxyl group ( $\nu \sim C=O$ ) at approximately 1670 cm<sup>-1</sup>. In addition, a band was observed at approx. 1740 cm<sup>-1</sup> originating from C=O stretching vibrations ( $\nu \sim C=O$ ) for cations containing a linker with two ester bonds. Several other characteristic bands were also noted at

Table 1

ŝ	vnthesized	salts	com	orising	bis	-ammoniu	n oi	bis-	phos	phonium	as a	cation	and	bromide	as ar	n anion	(1 - 12)	).
	J																(	

5		· · · ·			
No.	Dication	-R-	Anions	Yield [%]	<b>T</b> <sub>m</sub> [℃]
1	C <sub>4</sub> H <sub>9</sub> C <sub>4</sub> H <sub>9</sub>	-C <sub>4</sub> H <sub>8</sub> -	$2Br^{-}$	80	157–158 <sup>a</sup>
2		-C <sub>8</sub> H <sub>16</sub> -		82	121–124 <sup>a</sup>
3		-C <sub>12</sub> H <sub>24</sub> -		85	81-85
4	C4H9	-C4H8-		95	_b
5	$\mathbf{R}$	-C <sub>8</sub> H <sub>16</sub> -		97	_b
6	C4H9       C4H9 C1H0 O O C4H9	-C <sub>12</sub> H <sub>24</sub> -		89	221-225
7	C <sub>4</sub> H <sub>9</sub> C <sub>4</sub> H <sub>9</sub>	-C4H8-		75	_b
8	CAHO.	-C <sub>8</sub> H <sub>16</sub> -		80	_c
9	$C_{A}H_{0}$ $+$ $R$ $+$ $C_{A}H_{0}$	-C <sub>12</sub> H <sub>24</sub> -		81	_c
10	$C_4H_9$ , $C_4H_9$ , $C_4H_9$	-C4H8-		80	_b
11		-C <sub>8</sub> H <sub>16</sub> -		78	_b
12	$\begin{array}{c c} C_4H_9 & \  & \  &   \\ C_4H_9 & O & O & C_4H_9 \end{array}$	-C <sub>12</sub> H <sub>24</sub> -		75	_b

<sup>a</sup> solid [37];

<sup>b</sup> wax form;

<sup>c</sup> amorphous solids[38].

### Table 2

Synthesized DILs comprising bis-ammonium or bis-phosphonium as a cation and L-prolinate (1a-12a) or L-histidinate (1b-12b) as an anion.

No.	Cation	-R-	Anion	Yield [%]
1a	C <sub>4</sub> H <sub>9</sub> C <sub>4</sub> H <sub>9</sub>	-C <sub>4</sub> H <sub>8</sub> -	0	98
2a		-C <sub>8</sub> H <sub>16</sub> -		99
3a		-C <sub>12</sub> H <sub>24</sub> -	$\sim$ $\checkmark$ -	97
	$C_4H_9$ + $R$ + $C_4H_9$		~ `o	
4a	C <sub>4</sub> H <sub>9</sub> , , , , , , , , , , , , , , , , , , ,	-C4H8-	ŃH	95
5a	$\mathbf{R}$	-C <sub>8</sub> H <sub>16</sub> -		97
6a		-C <sub>12</sub> H <sub>24</sub> -		99
7a		-C₄H₀-		93
8a	с.н. С.н.	-C <sub>8</sub> H <sub>16</sub> -		95
9a		$-C_{12}H_{24}$ -		96
10a	$C_4H_9$ + $C_4H_9$	-C <sub>4</sub> H <sub>8</sub> -		98
11a		-C <sub>8</sub> H <sub>16</sub> -		97
12a		-C <sub>12</sub> H <sub>24</sub> -		93
1b	$C_4H_9$ $C_4H_9$	-C₄H <sub>8</sub> -	N	94
2b		-C <sub>8</sub> H <sub>16</sub> -	ji > o	98
3b		$-C_{12}H_{24}$ -		99
	C <sub>4</sub> H <sub>9</sub> + R + C <sub>4</sub> H <sub>9</sub>		H H N O	
4b	$C_4H_9$ + $O_1 O_1$ + $C_4H_9$	-C <sub>4</sub> H <sub>8</sub> -		95
5b	$\mathbf{N}$ $\mathbf{N}$ $\mathbf{R}$ $\mathbf{N}$	-C <sub>8</sub> H <sub>16</sub> -		96
6b	C <sub>4</sub> H <sub>9</sub> C <sub>4</sub> H <sub>9</sub>	-C <sub>12</sub> H <sub>24</sub> -		93
	Ċ₄H <sub>9</sub> Ö Ö Ċ₄H <sub>9</sub>			
7b	C <sub>4</sub> H <sub>9</sub> C <sub>4</sub> H <sub>9</sub>	-C <sub>4</sub> H <sub>8</sub> -		95
8b	C <sub>4</sub> H <sub>9</sub> C <sub>4</sub> H <sub>9</sub>	$-C_8H_{16}-$		97
9b	P P	$-C_{12}H_{24}$ -		98
	$C_4H_9$ + $C_4H_9$			
10b		-C <sub>4</sub> H <sub>8</sub> -		99
11b	$\mathbf{P}$ $\mathbf{R}$ $\mathbf{P}$	-C <sub>8</sub> H <sub>16</sub> -		96
12b	$\begin{array}{c c} C_4H_9 &   &    &   & C_4H_9 \\ \hline C_4H_0 & O & O & C_4H_0 \end{array}$	$-C_{12}H_{24}-$		94

**Cations:** 









**R**: -C<sub>4</sub>H<sub>8</sub>-; -C<sub>8</sub>H<sub>16</sub>-; -C<sub>12</sub>H<sub>24</sub>-

Anions:



Fig. 1. Structure of DILs 1a-12a and 1b-12b.

1470–1200 cm<sup>-1</sup>, originating from stretching vibrations between 3.3. Solubility C–N ( $\nu \sim$  C–N) or C–P ( $\nu \sim$  C–P). Furthermore, all products showed multiple significantly strong bands in the range of 930–1280 cm<sup>-1</sup>,

that were assigned to stretching vibrations from the C–O ( $v \sim$  C–O) and C–P ( $v \sim$  C–P) groups, as well as out-of-plane bending vibrations from the O–H ( $\delta \sim$  O–H) group [41–44]. We were able to distinguish characteristic signals at approx. 3 ppm (protons from the methyl group of the L-histidinate anion), 3.2–3.8 ppm (protons located in the surroundings of the amino group) and approx. 7.0 and 7.6 ppm (protons originating from hydrogen atoms in the imidazolium ring) in the <sup>1</sup>H NMR spectra. The lack of a signal from the proton of the amino group and the N-H bond in the imidazolium ring was attributed to exchange with the deuterated solvent used (CD<sub>3</sub>OD:D<sub>2</sub>O). As shown in Fig. 2, the cation signals occurred in a range of 0.9 to 5.0 ppm. Signals at 0.9 ppm (triplet of the methyl group) were not shifted by changing the type of alkyl linker and the guaternary nitrogen or phosphorus atom. Considering the other signals from hydrogen atoms in the cation structures presented in Fig. 2, we observed signals at different chemical shift positions. The signal at approximately 3.0 to 3.5 ppm (protons in the methylene group on the alkyl chains of bis-ammonium cations) changed to a value of approximately 2.0 ppm by switching from a nitrogen atom to a phosphorus atom. Notable observations included the introduction of an ester bond into the linker for significant changes in value. The signals from the methylene groups (at the quaternary atom)

shifted by 0.9 (bis-ammonium cation) or 0.5 ppm (bisphosphonium cation) with the substitution of an alkyl linker for a linker with two ester bonds. Furthermore, an additional signal of approximately 1.8 or 4.0 ppm was observed to originate from the CH<sub>2</sub> group attached to oxygen.

The solubility characteristics of the obtained DILs 1a-12a and 1b-12b were essential aspects of the study due to the determination of solvents necessary for chemical reactions, the purification procedure, and the application. The compounds reported in this study will be applied in the preservation of granaries, therefore, an appropriate solvent is crucial for the development of suitable spray solutions. Consequently, we determined the solubility in 6 representative solvents for the prepared DILs **1a–12a** and **1b–12b** at room temperature using the procedure described by Vogel [30]. The results of the solubility test are shown in Table S1. An analysis of the results suggests that the solubility in water, DMSO, acetonitrile. 2-propanol, and hexane was not altered by the cation and anion structure. None of the DILs dissolved in DMSO. 2propanol, or hexane, while they dissolved in water and acetonitrile. Indeed, the main difference in the solubility attributed to the structure of the obtained compounds was that DILs 1a-12a dissolved very well in methanol, while DILs 1b-12b exhibited reduced solubility in that solvent.

Regarding the application of the obtained DILs 1a-12a and 1b-12b as feeding deterrents, solubility in water and methanol was a positive outcome, as spray applications using these solvents are possible to prepare. The use of other solvents is not recommended for the preparation of application formulations because of insolubility or solubility limitations.

#### 3.4. Thermal properties



Table 3 summarizes the glass transition temperature  $(T_{\sigma})$  and decomposition temperature  $(T_{0.05})$  for the synthesized DILs, the

Fig. 2. FT-IR spectra of DILs 1a (blue), 4a (green), 7a (orange) and 10a (red), and <sup>1</sup>H NMR spectra of DILs 1b (blue), 4b (green), 7b (orange) and 10b (red).

#### Table 3

Thermal analysis (DSC and TGA) of the synthesized DILs.

No.	T <sub>g</sub> [°C]	T <sub>0.05</sub> [°C]	No.	Т <sub>g</sub> [°С]	T <sub>0.05</sub> [°C]
1a	_34	189	1b	-13	199
2a	-26	188	2b	-25	195
3a	-13	181	3b	-8	191
4a	-12	201	4b	-15	232
5a	-12	197	5b	5	222
6a <sup>a</sup>	-	193	6b	24	205
7a	-30	239	7b	-9	300
8a	-42	239	8b	-21	294
9a	-25	223	9b	-17	277
10a	-45	225	10b	-51	272
11a	-45	216	11b	-49	240
12a	-41	213	12b	-48	227

<sup>a</sup> T<sub>c</sub> = 11.00 °C; T<sub>m</sub> = 22.43 °C (T<sub>peak</sub> values); T<sub>g</sub> – glass transition temperature; T<sub>c</sub> – temperature of crystallization; T<sub>m</sub> – melting point; T<sub>0.05</sub> – decomposition temperature of 5%.

latter corresponding to a 5% mass loss. All compounds were waxes at 25 °C, exhibiting glass transition temperatures between - 51 and 24 °C (except DIL **6a** –  $T_c$  = 11.00 °C;  $T_m$  = 22.43 °C). The phase transitions determined for the analyzed DILs gualified all synthesized salts as ILs according to the definition [6]. Additionally, thermogravimetric analysis revealed that the obtained DILs exhibited simple thermal decomposition behavior with a single decomposition step. The T<sub>0.05</sub> values of all the obtained DILs ranged from 181 to 339 °C, whereas the thermal stabilities of L-proline and L-histidine were 233 °C [45] and 380 °C [46], respectively. Although the  $T_{0.05}$  values of the synthesized DILs (higher than 181 °C) were lower than those of the amino acids used, they were still sufficiently high to enable their application. The most stable DILs were salts 7a and 8a with L-prolinate anions and salts 7b and 8b with L-histidinate anions, whereas DILs 3a and 3b were the least thermally stable. In general, a slight decrease in the thermal stability of all ILs was observed with the increase in the linker length in the cation [47]. Moreover, DILs composed of a phosphonium cation (7a–12a and 7b–12b) were more thermally stable than DILs with an ammonium cation (1a-6a and 1b-6b). These results are consistent with those previously reported, where ILs based on a quaternary phosphorus atom exhibited higher T<sub>0.05</sub> values than ILs containing a quaternary nitrogen atom [48]. Carvalho et al. suggested that this difference is related to the occurrence of weaker interion interactions due to the increased ionic volume [49]. Interesting differences were observed for DILs containing an ester bond in the linker compared with those containing a simple alkyl linker. The presence of an ester bond in the surroundings of a quaternary nitrogen atom increases the stability of ILs compared to DILs with alkyl linkers. The opposite effect was observed for the synthesized salts with a bis-phosphonium cation. Further analyses are required to explain this phenomenon. Nonetheless, the analysis of phase transitions and thermal stability indicated that the synthesized DILs may be applied at temperatures < 181 °C and, due to their presence in liquid form at ambient temperature, their use should not be problematic.

#### 3.5. Feeding deterrent experiment

Due to the enormous risks associated with the damage to grains in warehouses by insects, continuous searches for novel solutions to prevent these effects are important. Therefore, for several years, a search for ionic liquids that would be more affordable and efficient formulations than the currently used commercial formulations has been ongoing. An analysis of deterrent activity was performed using the standard method described in the literature to determine whether the synthesized DILs **1a–12a** and **1b–12b** are feeding deterrents [24]. The control substance was azadirachtin, a chemical compound of natural origin with very good biological activity. Studies were conducted using beetles and larvae of common storage pests: beetles of granary weevil (*S. granarius*) and larvae of the khapra beetle (*T. granarium*). The antifeedant activity results are presented in Fig. 3A and B. Detailed values with statistical analyses are available in Table S2 (Supplementary Material).

The obtained results should be considered in two categories. The first is the effect of anions, in which the obtained DILs exhibited at least weak deterrent activity. According to the literature, amino acids affect taste organs, resulting in their repellent effect [50]. The obtained data indicated that DILs with the L-histidinate anion (**1b–12b**) were more effective in repelling storage insects than DILs with the L-prolinate anion (**1a–12a**). However, further studies of the effects of amino acids and their ionic forms on deterrent activity are needed to explain this phenomenon.

The effect of the cation in the synthesized DILs on the deterrent activity should be considered separately. The first intriguing result observed was that the presence of an ester bond in the cation (DILs 4a-6a, 10a-12a, 4b-6b, and 10b-12b) reduced insect feeding deterrence and therefore rendered these compounds relatively weak feeding deterrents. This effect might be explained by the increased hydrophilicity of compounds containing an ester bond [51,52]. Furthermore, Niemczak et al. verified that as hydrophobicity increases, biological activity against storage pests also increases [39]. Additionally, by analyzing the effect of the length of the alkyl linker on the cation, two correlations were observed. In compounds with a simple alkyl linker (DILs 1a-3a, 1b-3b, 7a-9a, and 7b-9b), biological activity increased with the elongation of the linker. This effect is well known and described in the literature [24]. However, in salts with an ester bond, the opposite result was observed; as the distance between the ester bonds in the linker (DILs 4a-6a, 4b-6b, 10a-12a, and 10b-12b) increased, the deterrent activity decreased. Therefore, the presence of an ester bond must affect insect taste organs differently, and the distance of ester bonds is very important. However, a larger group of insects must be studied and the interaction of these compounds with their receptors must be studied to confirm this hypothesis. We tentatively concluded that the salts obtained in the present study prevented the feeding of pests present in the granaries. More importantly, salts 8a, 8b, 9a, and 9b may be alternatives to the popularly used and expensive compound azadirachtin.

#### 3.6. Determination of antimicrobial activity

The antimicrobial properties of the tested DILs against a wide spectrum of microorganisms, including gram-positive and gramnegative bacteria, as well as fungi, are presented in Table 4 and



Fig. 3. Deterrent activity of DILs (1a-12a and 1b-12b) toward adult granary weevil and larvae of the khapra beetle compared to that of azadirachtin (Ref.); A – DILs 1a-12a (L-prolinate anion), B – DILs 1b-12b (L-histidinate anion).

#### Table 4

MIC (µg cm <sup><math>-3</math></sup> ) and MBC or MFC (µg cm <sup><math>-1</math></sup> )	<sup>3</sup> ) determined for the obtained DILs <b>1a-12a</b>	(L-prolinate anion) and <b>1b-12b</b>	(L-histidinate anion)
--	---	---------------------------------------	-----------------------

No.	Gram-positive bacteria				Gram-negative bacteria				Fungi			
	M. luteus	is C. perfring		gens	E. coli		M. catarrhalis		C. albicans		F. graminearum	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
1a	1000	1000	1000	1000	>1000	>1000	1000	1000	>1000	>1000	>1000	>1000
2a	125	125	>1000	>1000	>1000	>1000	31	31	>1000	>1000	>1000	>1000
3a	< 0.5	<0.5	125	125	125	125	< 0.5	< 0.5	31	31	500	500
4a	125	250	>1000	>1000	>1000	>1000	1000	1000	>1000	>1000	>1000	>1000
5a	1000	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
6a	4	8	125	125	500	>1000	4	8	31	31	500	500
7a	250	500	>1000	>1000	>1000	>1000	250	1000	>1000	>1000	>1000	>1000
8a	< 0.5	< 0.5	31	31	62	62	500	1000	16	16	250	250
9a	< 0.5	< 0.5	16	16	62	62	31	62	8	8	125	125
10a	500	>1000	>1000	>1000	1000	>1000	1000	>1000	>1000	>1000	>1000	>1000
11a	500	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	>1000	>1000
12a	250	250	500	>1000	>1000	>1000	500	>1000	1000	1000	>1000	>1000
1b	250	250	1000	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
2b	125	125	1000	1000	>1000	>1000	>1000	>1000	1000	1000	1000	1000
3b	125	125	1000	1000	1000	1000	>1000	>1000	1000	1000	1000	1000
4b	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	500	500
5b	250	250	>1000	>1000	>1000	>1000	>1000	>1000	1000	1000	500	500
6b	31	62	250	250	500	>1000	>1000	>1000	125	125	>1000	>1000
7b	31	31	500	500	1000	1000	>1000	>1000	1000	1000	>1000	>1000
8b	31	31	500	500	1000	1000	>1000	>1000	1000	1000	>1000	>1000
9b	< 0.5	< 0.5	16	16	31	31	1000	1000	8	8	1000	1000
10b	500	1000	>1000	>1000	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
11b	250	250	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
12b	250	250	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000

Tables S3 and S4 (Supplementary Material). Most of the tested compounds (19 of 24) exhibited no activity or slight inhibition of some tested microorganisms. The strongest antagonistic effect was observed for DILs **3a**, **6a**, **8a**, **9a** and **9b**, and the detected activity against selected microorganisms was at a level similar to the compounds used for comparative purposes: [DDA][Cl] and [BA]

[Cl]. These results were observed mainly for gram-positive bacteria, while the effect was weaker on gram-negative bacteria and fungi. The MIC ranged from < 0.5 to 125  $\mu$ g cm<sup>-3</sup> for gram-positive bacteria, from < 0.5 to > 1000  $\mu$ g cm<sup>-3</sup> for gram-negative bacteria, and from 8 to 1000  $\mu$ g cm<sup>-3</sup> for the tested fungi. Compounds **6b** and **8b** showed activity against the majority of

gram-positive bacteria and selected gram-negative bacteria and yeasts, but the antibacterial effect was much weaker than that of DILs **6a** and **8a**. The MIC values for DILs **8a**, **9a** and **9b** against *S. aureus* and *M. luteus* were less than or equal to the values obtained for the reference compounds. The lowest concentration of the reference compounds and DIL **6a** inhibiting the growth of *M. luteus* was the same at < 0.5  $\mu$ g cm<sup>-3</sup>. The MIC results for DILs **6a** and **3a** showed significant inhibition of the growth of the gramnegative bacterium *M. catarrhalis* at the same and lower concentrations, as the compounds [DDA][CI] and [BA][CI], respectively.

The remaining DILs showed activity toward only a few of the tested microorganisms; however, the MIC was > 125  $\mu$ g cm<sup>-3</sup>. In some samples, lower MIC values were noted for *S. aureus* (**8b**), *M. luteus* (**6–9b**), *S. marcescens* (**8b**), *M. catarrhalis* (**2b**, **3b**, and **6a**) and the yeast *C. albicans* (**8b**) and *R. mucilaginosa* (**6b**).

The effect of linker structure in the cation of the analyzed DILs on antimicrobial activity was observed. Salts containing a linker with 12 carbon atoms in its structure were characterized as displaying the highest antimicrobial activity, and the exceptions were DILs 12a and 12b (additionally containing two ester bonds in the linker). The lack of ester bonds resulted in good antimicrobial properties, especially against gram-positive bacteria, with lower activity against gram-negative bacteria. Interestingly, DILs with quaternary nitrogen atoms (1a-3a and 1b-3b) were less effective at inhibiting microbial growth than DILs with quaternary phosphorus atoms (7a-9a and 7b-9b). Similar observations were reported previously, in which the type of quaternary atom and the presence of the ester bond exerted a significant effect on the toxicity of the ILs tested [53–56]. In the present study, DILs containing two ester bonds in the cation at the linker of quaternary nitrogen atoms were some of the least effective compounds, except for IL 6a and 6b (the length of the alkyl chain between the two ester bonds was 12 carbon atoms).

After comparing the effect of the studied DILs on the growth of individual groups of microorganisms, we concluded that grampositive bacteria and fungi showed greater sensitivity to the tested compounds than gram-negative bacteria. Among gram-negative bacteria, the most sensitive bacteria were *M. catarrhalis* and *S. marcescens*.

#### 3.7. Phytotoxicity

Phytotoxicity is a crucial criterion for evaluating the suitability of a new compound for agricultural purposes. In particular, these parameters indicate the safety of substances released to the environment during controlled spraying or accidental leakage toward plants. A phytotoxicity analysis was performed to determine the effect of the obtained DILs **1a–12a** and **1b–12b** at a 1% concentration on the germination rate, and the stimulation of root and shoot growth of white mustard. The results are presented in Fig. 4 A and B and Table S5 (Supplementary Material).

#### **Germination**

The white mustard germination test indicated a significant tendency of the formulated aqueous solutions of the examined DILs to stimulate seed germination. Thus, we confirm that these compounds permeate through the endosperm of seeds and encourage their development (Table S5 in the Supplementary Material). The effect of the obtained DILs on germination was more favorable than that of the control sample containing demineralized water. The only notable exceptions were the aqueous solutions of DILs **6a**, **9a** and **12a** and **6b** and **9b**, which did not contribute to acceler-



Fig. 4. Stimulation or inhibition of root (A) and shoot (B) length of white mustard seedlings by DILs 1a-12a and 1b-12b.

Ta	ble	5

Surface activity parameters of the studied ILs.

No.	γ [mN m <sup>-1</sup> ]	CA [°]	No.	γ [mN m <sup>-1</sup> ]	CA [°]
1a	49.7	88	1b	31.7	90
2a	49.5	85	2b	35.4	88
3a	48.1	80	3b	40.1	79
4a	65.1	109	4b	67.7	106
5a	52.6	98	5b	67.5	104
6a	46.7	96	6b	46.9	96
7a	49.9	92	7b	36.4	86
8a	44.3	80	8b	34.8	79
9a	47.8	78	9b	35.2	77
10a	49.5	98	10b	52.7	98
11a	44.9	93	11b	43.2	82
12a	39.2	71	12b	39.1	73

 $\gamma$  – surface tension (±) 0.3 [mN m<sup>-1</sup>] and **CA** – contact angle (±) 2.0 [°] of 1% aqueous solutions of DILs.

ated germination. No significant correlation was observed between structure and this aberration - the lack of stimulation of germination by the abovementioned compounds was noted for some compounds with the longest linker in the bis-ammonium or bisphosphonium cation.

#### The length of the shoot and the root

Studies aiming to determine the effect of the obtained DILs on rooting and shoot length revealed their statistically significant effects on plant growth. Root growth was stimulated by DILs 1a-5a and 7a-9a, and DILs 1b-4b and 7b-9b (Fig. 4A). DILs that contain the bis-ammonium cation and bis-phosphonium cation in their structure with an alkyl linker (1a-3a, 7a-9a, 1b-3b and 7b-9b) promoted faster root system growth than the control experiment. Moreover, DILs 7-9 (bis-phosphonium cation with alkyl linker) stimulated growth by 40-70% more than DILs 1-3 (bis-ammonium cation with alkyl linker), regardless of the anion used. The ester bond in the linkers of bis-ammonium (4-6) and bis-phosphonium (10-12) cations increased the phytotoxicity to white mustard and mainly inhibited root system growth compared to the control experiment. The potential explanation for this phenomenon is the increased transport of DILs containing ester bonds through cell membranes and the better penetration of epicuticular waxes. Consequently, the concentration of the obtained DILs was sufficiently high to permeate the roots of germinated plants, resulting in inhibition of root system growth [57,58]. Nevertheless, in monocationic ionic liquids, the occurrence of an ester bond decreased phytotoxicity [59].

An examination of the effects of synthesized DILs on shoot growth revealed an effect of all salts on the growth of white mustard (Fig. 4B). All tested compounds reduced the growth of the aboveground part of the plant, regardless of the structure. Nevertheless, a similar trend was observed in this experiment, where DILs with ester bonds in the linker (4a–6a, 10a–12a, 4b–6b, and 10b–12b) resulted in increased inhibition of shoot growth compared to their analogs without ester bonds (1a–3a, 7a–9a, 1b–3b, and 7b–9b). We did not observe a correlation between the anion structure and shoot growth inhibition.

#### 3.8. Surface activity and its correlation with biological activity

In general, the surface properties of ILs are valuable for an effective interpretation of their biological activity [60]. Therefore, aqueous solutions of DILs with the same concentration as those used in biological studies were used to analyze surface activity. The surface activity of DILs with anions that originated from L-proline and Lhistidine was determined by measuring parameters such as surface tension and contact angle (Table 5).

All the tested DILs decreased the surface tension of water, in the range of 39.2 to 65.1 mN m<sup>-1</sup> for compounds with L-prolinate anions (1a-12a) and 31.7 to 67.5 mN  $m^{-1}$  for DILs with Lhistidinate anions (1b-12b). The interfacial activity evidently depended on the structure of the cation linker in the DILs investigated. A trend was observed toward a decreased surface tension with the elongation of the alkyl chain in the linker of the cation. The exceptions are DILs **7a–9a** and **1b–3b**, in which the tendency was not noticed. Moreover, the  $\gamma$  values of phosphonium and ammonium DILs suggested that changes in the central atom of the cation influenced their behavior in aqueous solution. This conclusion is consistent with data presented by Sintra et al., 2019, who examined the effect of changing the structure of ILs (phosphonium and ammonium) on their interfacial activities [61]. For the analysis of surface properties, the presence of an ester bond in the cation did not exert a significant effect (exceptions include DILs 1b-6b), in contrast to the previously mentioned biological activity of the studied DILs.

A major goal was to determine the wettability of aqueous solutions on a hydrophobic paraffin surface. The CA values for DILs with L-prolinate and L-histidinate anions ranged from 71 to 106°, respectively. The improved wettability of the paraffin surface may be an advantage in future industrial applications of these DILs.

Nevertheless, no linear relationship was observed between surface activity and biological activity. The most significant findings were that the DILs (**3a**, **8a**, **9a**, and **9b**) with excellent wettability properties induced the greatest inhibition of microbial growth. Regarding the deterrent activity of DILs with an alkyl linker (**1–3** and **7–9**), with the elongation of the linker, the values of the contact angle increased, and the deterrent activity subsequently improved. Interestingly, the deterrent activity of DILs with two ester bonds in the linker (**4–6** and **10–12**) decreased as the wettability of paraffin improved. This trend requires further analysis.

#### 4. Conclusions

In this study, a series of novel bioinspired ionic liquids with Lhistidinate or L-prolinate anions and bis-ammonium or bisphosphonium cations with different linkers were described and characterized. The synthesis methodology developed here allowed the precursors and DILs to be obtained in high yields (exceeding 75% for precursors and 93% for DILs) and purity, as confirmed by spectral analyses (FT-IR and <sup>1</sup>H and <sup>13</sup>C NMR). The experiments revealed that the chemical structure of the cation negligibly altered the solubility in most of the organic solvents used here, and that only the anion contributed to the change in solubility in methanol. Furthermore, the synthesized ionic forms of amino acids exhibited biological activity, enabling them to be included in thirdgeneration ionic liquids. Ionic liquids with bis-ammonium or bisphosphonium cations containing an alkyl bond showed moderate antibacterial or antifungal activity, and good repellent properties against common pests feeding on stored products. However, they were not phytotoxic to white mustard. In contrast, DILs with bisammonium or bis-phosphonium cations containing two ester bonds in the linker exhibited weak fungicidal and bactericidal activity, while they exhibited insect deterrent activity and were phytotoxic. To the best of our knowledge, this report is the first in which the biological activity of a dicationic ionic liquid was correlated with the occurrence of an ester bond in the linker.

The obtained results confirm that multifunctional ionic liquids with desired antibacterial, antifungal, antifeedant and phytotoxic activities can be obtained by modifying the linker in the bisammonium or bis-phosphonium cation. These studies also indicate that dicationic ionic liquids prepared with amino acids may be an economically justified alternative to chemicals commonly used for the protection of cereal seeds. Therefore, the use of DILs to replace commercial formulations may become a promising solution based on the concept of using products obtained from compounds of natural origin.

#### CRediT authorship contribution statement

**Damian Krystian Kaczmarek:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Daniela Gwiazdowska:** Methodology, Writing – review & editing, Investigation, Resources. **Katarzyna Marchwińska:** Methodology, Writing – review & editing, Investigation, Resources. **Tomasz Klejdysz:** Methodology, Writing – review & editing, Formal analysis, Investigation, Resources. **Marta Wojcieszak:** Methodology, Writing – review & editing, Investigation, Resources. **Katarzyna Materna:** Methodology, Writing – review & editing, Investigation, Resources. **Katarzyna Materna:** Methodology, Writing – review & editing, Investigation, Resources, Supervision, Project administration.

#### **Declaration of Competing Interest**

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Damian Krystian Kaczmarek has patent P.437804 pending to Poznan University of Technology. Damian Krystian Kaczmarek has patent P.437807 pending to Poznan University of Technology. Damian Krystian Kaczmarek has patent P.437806 pending to Poznan University of Technology. Damian Krystian Kaczmarek has patent P.437805 pending to Poznan University of Technology.

#### Acknowledgements

This work was supported by the National Science Centre, Poland (PRELUDIUM 17:2019/33/N/ST4/02292). We thank Dr. inż. F. Walkiewicz for performing the thermal stability and phase transition analysis.

#### Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2022.119357.

#### References

- N.K. Dubey (Ed.), Natural products in plant pest management, CABI International, Wallingford, 2011.
- [2] N. Manu, G.P. Opit, E.A. Osekre, F.H. Arthur, G. Mbata, P. Armstrong, J.K. Danso, S.G. McNeill, J.F. Campbell, Moisture content, insect pest infestation and

mycotoxin levels of maize in markets in the northern region of Ghana, J. Stored Prod. Res. 80 (2019) 10–20, https://doi.org/10.1016/j.jspr.2018.10.007.

- [3] M.R. Khan, F.M. Doohan, Bacterium-mediated control of Fusarium head blight disease of wheat and barley and associated mycotoxin contamination of grain, Biol. Control. 48 (2009) 42–47, https://doi.org/10.1016/j.biocontrol.2008.08.015.
- [4] Ž. Savković, M. Stupar, N. Unković, A. Knežević, J. Vukojević, M.L. Grbić, Fungal deterioration of cultural heritage objects, in: K.F. Mendes, R.N. de Sousa, K.C. Mielke (Eds.), Biodegrad. Technol. Org. Inorg. Pollut., IntechOpen, 2021. https://doi.org/10.5772/intechopen.98620.
- [5] A.J. Mobolade, N. Bunindro, D. Sahoo, Y. Rajashekar, Traditional methods of food grains preservation and storage in Nigeria and India, Ann. Agric. Sci. 64 (2019) 196–205, https://doi.org/10.1016/j.aoas.2019.12.003.
- [6] T. Welton, Ionic liquids: a brief history, Biophys. Rev. 10 (2018) 691–706, https://doi.org/10.1007/s12551-018-0419-2.
- [7] R.D. Rogers, K.R. Seddon, Ionic liquids-solvents of the future?, Science 302 (2003) 792-793, https://doiorg/10.1126/science.1090313.
- [8] N.V. Plechkova, K.R. Seddon, Applications of ionic liquids in the chemical industry, Chem. Soc. Rev. 37 (2008) 123–150, https://doi.org/10.1039/ B006677J.
- [9] W.L. Hough, M. Smiglak, H. Rodríguez, R.P. Swatloski, S.K. Spear, D.T. Daly, J. Pernak, J.E. Grisel, R.D. Carliss, M.D. Soutullo, J.H. Davis Jr., R.D. Rogers, The third evolution of ionic liquids: active pharmaceutical ingredients, New J. Chem. 31 (2007) 1429–1436, https://doi.org/10.1039/B706677P.
- [10] D.K. Kaczmarek, T. Rzemieniecki, D. Gwiazdowska, T. Kleiber, T. Praczyk, J. Pernak, Choline-based ionic liquids as adjuvants in pesticide formulation, J. Mol. Liq. 327 (2021), https://doi.org/10.1016/j.molliq.2020.114792 114792.
- [11] S. Dewilde, W. Dehaen, K. Binnemans, Ionic liquids as solvents for PPTA oligomers, Green Chem. 18 (2016) 1639–1652, https://doi.org/10.1039/ C5GC02185E.
- [12] J. Feder-Kubis, P. Czerwoniec, P. Lewandowski, H. Pospieszny, M. Smiglak, Ionic liquids with natural origin component: A path to new plant protection products, ACS Sustain. Chem. Eng. 8 (2020) 842–852, https://doi.org/10.1021/ acssuschemeng.9b04859.
- [13] J. Pernak, M. Niemczak, Ł. Chrzanowski, Ł. Ławniczak, P. Fochtman, K. Marcinkowska, T. Praczyk, Betaine and carnitine derivatives as herbicidal ionic liquids, Chem. A Eur. J. 22 (2016) 12012–12021, https://doi.org/10.1002/chem.201601952.
- [14] Ł. Ławniczak, A. Syguda, A. Borkowski, P. Cyplik, K. Marcinkowska, Ł. Wolko, T. Praczyk, Ł. Chrzanowski, J. Pernak, Influence of oligomeric herbicidal ionic liquids with MCPA and Dicamba anions on the community structure of autochthonic bacteria present in agricultural soil, Sci. Total Environ. 563–564 (2016) 247–255, https://doi.org/10.1016/j.scitotenv.2016.04.109.
- [15] I. Čobanov, A. Tot, S. Gadžurić, I. Maksimović, M. Putnik-Delić, M. Daničić, M. Bešter-Rogač, M. Vraneš, Influence of structural changes of cation and anion on phytotoxicity of selected surface active ionic liquids, J. Mol. Liq. 342 (2021), https://doi.org/10.1016/j.molliq.2021.117458 117458.
- [16] C. Zhang, F. Cui, G. Zeng, M. Jiang, Z. Yang, Z. Yu, M. Zhu, L. Shen, Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment, Sci. Total Environ. 518–519 (2015) 352–362, https://doi.org/ 10.1016/j.scitotenv.2015.03.007.
- [17] D. Coleman, N. Gathergood, Biodegradation studies of ionic liquids, Chem. Soc. Rev. 39 (2010) 600–637, https://doi.org/10.1039/B817717C.
- [18] A.G. Santos, B.D. Ribeiro, D.S. Alviano, M.A.Z. Coelho, Toxicity of ionic liquids toward microorganisms interesting to the food industry, RSC Adv. 4 (2014) 37157–37163, https://doi.org/10.1039/C4RA05295A.
- [19] C.W. Cho, T.P.T. Pham, Y. Zhao, S. Stolte, Y.S. Yun, Review of the toxic effects of ionic liquids, Sci. Total Environ. 786 (2021) 147309, https://doi.org/10.1016/j. scitotenv.2021.147309.
- [20] J. Niu, Z. Zhang, J. Tang, G. Tang, J. Yang, W. Wang, H. Huo, N. Jiang, J. Li, Y. Cao, Dicationic ionic liquids of herbicide 2,4-dichlorophenoxyacetic acid with reduced negative effects on environment, J. Agric. Food Chem. 66 (2018) 10362–10368, https://doi.org/10.1021/acs.jafc.8b02584.
- [21] J.F. Vélez, M.B. Vazquez-Santos, J.M. Amarilla, P. Tartaj, B. Herradón, E. Mann, C. del Río, E. Morales, Asymmetrical imidazolium-trialkylammonium room temperature dicationic ionic liquid electrolytes for Li-ion batteries, Electrochim. Acta. 280 (2018) 171–180, https://doi.org/10.1016/j.electacta.2018.05.103.
- [22] F. Brunel, C. Lautard, F. Garzino, J.-M. Raimundo, J.-M. Bolla, M. Camplo, Phosphonium-ammonium-based di-cationic ionic liquids as antibacterial over the ESKAPE group, Bioorg. Med. Chem. Lett. 30 (2020) 127389. https://doi.org/ 10.1016/j.bmcl.2020.127389.
- [23] F.M. Menger, J.S. Keiper, Gemini Surfactants, Angew. Chemie Int. Ed. 39 (2000) 1906–1920, https://doi.org/10.1002/1521-3773(20000602)39:11<1906::AID-ANIE1906>3.0.CO;2-Q.
- [24] D.K. Kaczmarek, K. Czerniak, T. Klejdysz, Dicationic ionic liquids as new feeding deterrents, Chem. Pap. 72 (2018) 2457–2466, https://doi.org/10.1007/ s11696-018-0495-6.
- [25] A. Zajac, R. Kukawka, A. Pawlowska-Zygarowicz, O. Stolarska, M. Smiglak, Ionic liquids as bioactive chemical tools for use in agriculture and the preservation of agricultural products, Green Chem. 20 (2018) 4764–4789, https://doi.org/ 10.1039/C8GC01424H.
- [26] M. Niemczak, T. Rzemieniecki, Ł. Sobiech, G. Skrzypczak, T. Praczyk, J. Pernak, Influence of the alkyl chain length on the physicochemical properties and biological activity in a homologous series of dichlorprop-based herbicidal ionic liquids, J. Mol. Liq. 276 (2019) 431-440, https://doi.org/10.1016/ j.molliq.2018.12.013.

Damian Krystian Kaczmarek, D. Gwiazdowska, K. Marchwińska et al.

- [27] W. Leuchtenberger, K. Huthmacher, K. Drauz, Biotechnological production of amino acids and derivatives: current status and prospects, Appl. Microbiol. Biotechnol. 69 (2005) 1–8, https://doi.org/10.1007/s00253-005-0155-y.
- [28] H. Ohno, K. Fukumoto, Amino acid ionic liquids, Acc. Chem. Res. 40 (2007) 1122-1129, https://doi.org/10.1021/ar700053z.
- [29] K. Fukumoto, M. Yoshizawa, H. Ohno, Room temperature ionic liquids from 20 natural amino acids, J. Am. Chem. Soc. 127 (2005) 2398–2399, https://doi.org/ 10.1021/ja043451i.
- [30] A.I. Vogel, Textbook of practical organic chemistry, 5th ed., Wiley, New York, 1989.
- [31] D.K. Kaczmarek, D. Gwiazdowska, K. Juś, T. Klejdysz, M. Wojcieszak, K. Materna, J. Pernak, Glycine betaine-based ionic liquids and their influence on bacteria, fungi, insects and plants, New J. Chem. 45 (2021) 6344–6355, https:// doi.org/10.1039/d1nj00498k.
- [32] P.I. Liu, D.C. Wang, C.H. Ho, Y.C. Chen, L.C. Chung, T.M. Liang, M.C. Chang, R.Y. Horng, Exploring the performance-affecting factors of monocationic and dicationic phosphonium-based thermoresponsive ionic liquid draw solutes in forward osmosis, Desalin. Water Treat. 200 (2020) 1–7, https://doi.org/ 10.5004/dwt.2020.25987.
- [33] A.W. Widmer, K.A. Jolliffe, L.C. Wright, T.C. Sorrell, Bis-cationic compounds and use thereof, WO2005047230A1, 2005.
- [34] S. Guo, X. Sun, Q. Zou, J. Zhang, H. Ni, Antibacterial activities of five cationic gemini surfactants with ethylene glycol bisacetyl spacers, J. Surfactants Deterg. 17 (2014) 1089–1097, https://doi.org/10.1007/s11743-014-1620-3.
- [35] Z. Gao, S. Tai, Q. Zhang, Y. Zhao, B. Lü, Y. Ge, L. Huang, X. Tang, Synthesis and surface activity of biquaternary ammonium salt gemini surfactants with ester bond, Wuhan Univ, J Nat. Sci. 13 (2008) 227–231, https://doi.org/10.1007/ s11859-008-0219-9.
- [36] B.E. Brycki, I.H. Kowalczyk, A. Szulc, O. Kaczerewska, M. Pakiet, Multifunctional gemini surfactants: Structure, synthesis, properties and applications, in: R. Najjar (Ed.), Appl. Charact. Surfactants, IntechOpen, Rijeka, 2017. https://doi. org/10.5772/intechopen.68755.
- [37] H. Nakayama, Hydrates of organic compounds. IV. Clathrate hydrates of various bolaform salts, Bull. Chem. Soc. Jpn. 52 (1979) 52–56, https://doi.org/ 10.1246/bcsj.52.52.
- [38] A. Ohki, M. Yamura, M. Takagi, S. Maeda, Dionium cations as novel sensing and separation reagents for phthalates in ion-selective electrode method and in ion-pair chromatography, Anal. Sci. 6 (1990) 585–588, https://doi.org/ 10.2116/analsci.6.585.
- [39] M. Niemczak, D.K. Kaczmarek, T. Klejdysz, D. Gwiazdowska, K. Marchwińska, J. Pernak, Ionic liquids derived from Vitamin C as multifunctional active ingredients for sustainable stored-product management, ACS Sustain. Chem. Eng. 7 (2019) 1072–1084, https://doi.org/10.1021/acssuschemeng.8b04696.
- [40] T. Rzemieniecki, D. Gwiazdowska, K. Rybak, K. Materna, K. Juś, J. Pernak, Synthesis, properties, and antimicrobial activity of 1-alkyl-4-hydroxy-1methylpiperidinium ionic liquids with mandelate anion, ACS Sustain. Chem. Eng. 7 (2019) 15053–15063, https://doi.org/10.1021/acssuschemeng.9b03690.
- Eng. 7 (2019) 15053–15063, https://doi.org/10.1021/acssuschemeng.9b03690.
  B.T. Gowda, K. Jyothi, J.D. D'Souza, Infrared and NMR Spectra of Arylsulphonamides, 4-X-C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>NH<sub>2</sub> and i-X, j-YC<sub>6</sub>H<sub>3</sub>SO<sub>2</sub>NH<sub>2</sub> (X=H; CH<sub>3</sub>; C<sub>2</sub>H<sub>5</sub>; F; Cl; Br; I or NO<sub>2</sub> and i-X, j-Y=2,3-(CH<sub>3</sub>)<sub>2</sub>; 2,4-(CH<sub>3</sub>)<sub>2</sub>; 2,5-(CH<sub>3</sub>)<sub>2</sub>; 2,2-CH<sub>3</sub>, 4-Cl; 2-CH<sub>3</sub>, 5-Cl; 3-CH<sub>3</sub>, 4-Cl; 2,4-Cl<sub>2</sub> or 3,4-Cl<sub>2</sub>), Zeitschrift Für Naturforsch. A. 57a (2002) 967–973. https://doi.org/10.1515/zna-2002-1210.
- [42] L.D.S. Yadav, Infrared (IR) Spectroscopy, in: L.D.S. Yadav (Ed.), Org. Spectrosc., Springer, Dordrecht, 2005: pp. 52–106. https://doi.org/10.1007/978-1-4020-2575-4\_3.
- [43] A.M. Turner, M.J. Abplanalp, T.J. Blair, R. Dayuha, R.I. Kaiser, An infrared spectroscopic study toward the formation of alkylphosphonic acids and their precursors in extraterrestrial environments, Astrophys. J. Suppl. Ser. 234 (2018) 6, https://doi.org/10.3847/1538-4365/aa9183.
- [44] A. Mackowiak, P. Galek, K. Fic, Deep eutectic solvents for high-temperature electrochemical capacitors, ChemElectroChem. 8 (2021) 4028–4037, https:// doi.org/10.1002/celc.202100711.
- [45] V. Pokorný, V. Štejfa, J. Havlín, K. Růžička, M. Fulem, Heat capacities of Lhistidine, L-phenylalanine, L-proline, L-tryptophan and L-tyrosine, Molecules 26 (2021) 4298, https://doi.org/10.3390/molecules26144298.

- [46] P. Kumaresan, S. Moorthy Babu, P.M. Anbarasan, Thermal, dielectric studies on pure and amino acid (L-glutamic acid, L-histidine, L-valine) doped KDP single crystals, Opt. Mater. 30 (2008) 1361–1368, https://doi.org/10.1016/j. optmat.2007.07.002.
- [47] R.A. Patil, M. Talebi, C. Xu, S.S. Bhawal, D.W. Armstrong, Synthesis of thermally stable geminal dicationic ionic liquids and related ionic compounds: An examination of physicochemical properties by structural modification, Chem. Mater. 28 (2016) 4315–4323, https://doi.org/10.1021/acs. chemmater.6b01247.
- [48] C. Maton, N. De Vos, C.V. Stevens, Ionic liquid thermal stabilities: decomposition mechanisms and analysis tools, Chem. Soc. Rev. 42 (2013) 5963–5977, https://doi.org/10.1039/C3CS60071H.
- [49] I.S. Cardoso, E.L.P. de Faria, A.J.D. Silvestre, M.G. Freire, A. Mohamadou, Synthesis and characterization of analogues of glycine-betaine surface-active ionic liquids, J. Mol. Liq. 342 (2021), https://doi.org/10.1016/ j.mollig.2021.117440 117440.
- [50] L.M. Schoonhoven, L. Lin-er, Multiple mode of action of the feeding deterrent, toosendanin, on the sense of taste in *Pieris brassicae* larvae, J. Comp. Physiol. A. 175 (1994) 519–524, https://doi.org/10.1007/BF00199258.
- [51] A.R. Tehrani-Bagha, R.G. Singh, K. Holmberg, Solubilization of two organic dyes by cationic ester-containing gemini surfactants, J. Colloid Interface Sci. 376 (2012) 112–118, https://doi.org/10.1016/j.jcis.2012.02.016.
- [52] J. Pernak, K. Wasiński, T. Praczyk, J. Nawrot, A. Cieniecka-Rosłonkiewicz, F. Walkiewicz, K. Materna, Sweet ionic liquids-cyclamates: Synthesis, properties, and application as feeding deterrents, Sci. China Chem. 55 (2012) 1532–1541, https://doi.org/10.1007/s11426-012-4631-9.
- [53] S.P.M. Ventura, C.S. Marques, A.A. Rosatella, C.A.M. Afonso, F. Gonçalves, J.A.P. Coutinho, Toxicity assessment of various ionic liquid families towards Vibrio fischeri marine bacteria, Ecotoxicol. Environ. Saf. 76 (2012) 162–168, https:// doi.org/10.1016/j.ecoenv.2011.10.006.
- [54] M. Sivapragasam, J.R. Jaganathan, J.M. Levêque, M. Moniruzzaman, M.I. Abdul Mutalib, Microbial biocompatibility of phosphonium- and ammonium-based ionic liquids, J. Mol. Liq. 273 (2019) 107–115, https://doi.org/10.1016/ j.molliq.2018.10.022.
- [55] P. Oulego, D. Blanco, D. Ramos, J.L. Viesca, M. Díaz, A. Hernández Battez, Environmental properties of phosphonium, imidazolium and ammonium cation-based ionic liquids as potential lubricant additives, J. Mol. Liq. 272 (2018) 937–947, https://doi.org/10.1016/j.molliq.2018.10.106.
- [56] E.V. Nikitina, M.I. Zeldi, M.V. Pugachev, S.V. Sapozhnikov, N.V. Shtyrlin, S.V. Kuznetsova, V.E. Evtygin, M.I. Bogachev, A.R. Kayumov, Y.G. Shtyrlin, Antibacterial effects of quaternary bis-phosphonium and ammonium salts of pyridoxine on *Staphylococcus aureus* cells: A single base hitting two distinct targets?, World J Microbiol. Biotechnol. 32 (2016) 5, https://doi.org/10.1007/s11274-015-1969-0.
- [57] C.E. Price, N.H. Anderson, Uptake of chemicals from foliar deposits: Effects of plant species and molecular structure, Pestic. Sci. 16 (1985) 369–377, https:// doi.org/10.1002/ps.2780160411.
- [58] P.S. Gerogianni, M.V. Chatziathanasiadou, D.A. Diamantis, A.G. Tzakos, D. Galaris, Lipophilic ester and amide derivatives of rosmarinic acid protect cells against H<sub>2</sub>O<sub>2</sub>-induced DNA damage and apoptosis: The potential role of intracellular accumulation and labile iron chelation, Redox Biol. 15 (2018) 548–556, https://doi.org/10.1016/j.redox.2018.01.014.
- [59] B. Pawłowska, A. Telesiński, R. Biczak, Phytotoxicity of ionic liquids, Chemosphere 237 (2019), https://doi.org/10.1016/j. chemosphere.2019.124436 124436.
- [60] D.K. Kaczmarek, T. Rzemieniecki, K. Marcinkowska, J. Pernak, Synthesis, properties and adjuvant activity of docusate-based ionic liquids in pesticide formulations, J. Ind. Eng. Chem. 78 (2019) 440–447, https://doi.org/10.1016/j. jiec.2019.05.023.
- [61] T.E. Sintra, M. Vilas, M. Martins, S.P.M. Ventura, A.I.M.C. Lobo Ferreira, L.M.N.B. F. Santos, F.J.M. Gonçalves, E. Tojo, J.A.P. Coutinho, Synthesis and characterization of surface-active ionic liquids used in the disruption of *Escherichia Coli* cells, ChemPhysChem 20 (2019) 727–735, https://doi.org/ 10.1002/cphc.201801127.