Journal of Molecular Liquids 374 (2023) 121285

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Design of sustainable ionic liquids based on L-phenylalanine and L-alanine dipeptides: Synthesis, toxicity and biodegradation studies

Illia V. Kapitanov [a,1,2], Grete Raba [a,1], Marcel Špulák [b], Raivo Vilu [c], Yevgen Karpichev [a,*], Nicholas Gathergood [a,d,*]

[a] Department of Chemistry and Biotechnology, Faculty of Science, Tallinn University of Technology (TalTech), Akadeemia tee 15, 12618 Tallinn, Estonia
[b] Department of Organic and Bioorganic Chemistry, Faculty of Pharmacy, Charles University, Heyrovskeho 1203, Hradec Kralove 500 03, Czech Republic
[c] Center of Food and Fermentation Technologies (TFTAK), Akadeemia tee 15a, 12618 Tallinn, Estonia
[d] School of Chemistry, College of Science, University of Lincoln, Brayford Pool, Lincoln, LN6 7TS, United Kingdom

ARTICLE INFO

Article history:
Received 10 November 2022
Revised 6 January 2023
Accepted 17 January 2023
Available online 20 January 2023

Keywords:
Amino acid ionic liquids
Dipeptide ionic liquids
Sustainable chemicals
Biodegradability
Transformation products
Closed bottle test

ABSTRACT

A series of dipeptide ionic liquids (ILs) with L-phenylalanine and L-alanine fragments in structure were synthesized and their possible degradation pathways were analyzed. Based on this analysis, potential transformation products (PTPs) were proposed and synthesized. All of these compounds (25 in total) went through microbial toxicity screening and aerobic biodegradation testing. Obtained results demonstrated that by investigating ILs and PTPs with a dipeptide fragment (in tandem with single amino acid analogues), the design of ILs with high biodegradation values in closed bottle test can be accomplished. One finding was that within the scope of the compounds studied, L-phenylalanine containing compounds were more biodegradable than L-alanine derivatives. In addition to the choice of amino acid residue, its position in the dipeptide IL structure also had a significant effect on biodegradability. $PyCH_2CO$-Phe-Ala-OEt IL, where L-phenylalanine was in close proximity to the positively charged pyridinium sub-unit, gave higher biodegradation percentages compared to $PyCH_2CO$-Ala-Phe-OEt IL, where alanine was closer to pyridinium than the phenylalanine residue. Analysis of PTPs data showed that the presence of an alanine residue resulted in undesirable (less green) PTPs more often compared to PTPs containing phenylalanine, especially when alanine was in close proximity to the pyridinium headgroup. Based on both toxicity and biodegradation testing results preferable and less preferable subunits can be chosen for the design of new sustainable chemicals based on amino acids. Results from this study demonstrate a potential of designing new sustainable chemicals using amino acid moieties as part of their structure.

1. Introduction

The history of ionic liquids (ILs) is usually considered to have begun in 1914 when Paul Walden reported the synthesis of ethylammonium nitrate $[C_2H_5NH_3]^+[NO_3]^-$ – a salt which is liquid at room temperature [1]. Over the next hundred years ILs have been utilized in diverse applications as alternatives to traditional volatile organic solvents [2]. More than 115,000 research papers on „ionic liquids“ were published by 2022 (SciFinder ®) principally due to their desirable properties, such as thermal and chemical stability, low vapour pressure and modularity [3]. In addition to research, ILs are used in applied industrial processes [4,5]. For example, the BASIL™ process used for the production of photoinitiator precursor alkoxyphenylphosphines and the Dimersol process for dimerisation of alkenes and butenes to more valuable branched hexenes and octenes [5–10].

However, even though ILs with their low vapor pressure have been proposed as *green* solvents, often a dearth of their toxicity and biodegradability assessment is observed [11,12]. These are key elements in the evaluation of the environmental effect of chemicals [13–17]. Ideally, from an environmental point of view, a *green* solvent should be non-toxic, biodegradable, use bioresources as starting materials and synthesized using effective environmentally friendly synthesis procedures and be recyclable [18–20]. Most ILs are designed to be robust and stable under the various chemical and physical conditions of their target application

* Corresponding authors at: Department of Chemistry and Biotechnology, Faculty of Science, Tallinn University of Technology (TalTech), Akadeemia tee 15, 12618 Tallinn, Estonia (N. Gathergood).
E-mail addresses: yevgen.karpichev@taltech.ee (Y. Karpichev), ngathergood@lincoln.ac.uk (N. Gathergood).
[1] Contributed equally to the publication.
[2] Current address: Gemini PharmChem Mannheim GmbH, 68305 Mannheim, Germany.

https://doi.org/10.1016/j.molliq.2023.121285
0167-7322/

[21]. Concerns have been raised that the design of inert ILs can lead to examples which tend to show poor degradability in environmental conditions [22–25]. Although progress has been made in IL recyclability, reuse is limited to a certain number of cycles before inevitable contamination and the need for ILs to be discarded at the end of their use [18,26]. For that reason, it is extremely important that ILs used in industrial applications result in a minimal impact to the environment [11,27]. This would be best achieved by using ILs which are mineralizable under wastewater treatment plant conditions and in soil and surface water. If microbial communities could metabolize ILs into inorganic CO_2, O_2, water and other innocuous substances, IL persistency would be avoided, and toxicity concerns mitigated. As there are claimed at least 10^{18} accessible combinations of cations and anions to form various ILs, a systematic approach is required in order to find mineralizable building blocks for ILs and to propose fragments for a benign-by-design synthesis [18,28–32].

The idea of finding mineralizable IL cations made from renewable sources was the basis of our previously published work, in collaboration with the Kümmerer's group, of a fully mineralizable IL **1** (see Fig. 1) with pyridinium headgroup and L-phenylalanine moiety [33–35].

We concluded, surprisingly, that amide bond cleavage was taking place before esterolysis, resulting in biodegradable and mineralizable transformation products [33]. This was unexpected and thus further studies to analyze the effect of amide bond nature in such type of structures were essential. In essence, to establish the specific influence of amino acid hydrophobicity on biodegradability, and if the presence of the sterically bulky CH_2Ph (c.f. Me; Ala) hydrophobic unit in IL **1** was critically required for effective binding to the active sites of biodegrading enzymes.

To answer these questions L-alanine ionic liquid **2** and a series of L-phenylalanine and L-alanine based dipeptide ILs **3**–**6** (see Fig. 1) were synthesized, and biodegradability of compounds (**1**–**6**) was investigated. The overall strategy of IL structure modifications was consistent with our design of imidazolium salts with high antimicrobial activity, but that time our focus was on identifying biodegradable ILs with low toxicity [36].

These novel dipeptides **3**–**6** have an additional amide bond (compared to amino acid ILs **1** and **2**) and the structural variations enable us to ascertain how the position of amide bond and amino acid residue affects biodegradation. It is widely reported that pyridinium ILs containing an amide not derived from an amino acid generally have poor biodegradability [22,23,37,38]. By extending the amino acid substructure from one residue (**1** and **2**) to two residues (**3**–**6**), we can determine the effect of an increase of peptide sequence on biodegradation. To investigate the effect of the sterics, π-π stacking and hydrophobicity of the -CH_2Ph of mineralizable Phe IL **1**, the Ala derivative was studied. This CH_3 analogue was selected due to the reduced steric hindrance, absence of π-π interactions and lower hydrophobicity.

Pyridinium ILs toxicity was reported to increase in antimicrobial activity as the substituting alkyl chain lengthened [39–43]. As the focus of this study was to determine the effect of the amino acid residues, the ethyl ester group (e.g. **1**) was selected to minimize potential toxicity complications due to the alkyl ester tail.

We submit that the amino acid side chains of ILs **1**–**6** are consistent with the concept of cation surfactants ('Charged head group substituted with hydrophobic tail') and thus the results have broad applicability to the synthesis of biodegradable cationic surfactants.

In addition, for each parent compound **1**–**6** possible breakdown sites and degradation pathways were proposed and analyzed. Based on this analysis potential transformation products (PTPs) were suggested, then synthesized and studied for their biodegradability. Studying PTPs together with parent compounds is of critical importance when complete mineralization of chemical compounds (including ILs) is the desired outcome. IL parent compounds **1**–**6** and their PTPs represent the series of compounds selected to enable biodegradation and toxicity SAR studies underpinning the benign-by-design approach.

2. Materials and methods

Biodegradation of two L-amino acid based ILs, four L-phenylalanine and L-alanine based dipeptide ILs with pyridinium sub-unit and their proposed transformation products were studied. 25 compounds (**1**–**25**) in total were synthesized and underwent antimicrobial activity screening and biodegradation testing under aerobic conditions.

2.1. Selection of target PTPs

Selection of target PTPs for synthesis and study (Fig. 1) was performed from analysis of possible biodegradation pathways (see Scheme 1; **S1**). The proposed first step of biodegradation for studied molecules is hydrolysis to lower molecular mass fragments which will then be ultimately transformed to CO_2, H_2O and N_2 [33,34].

Amino acid ILs **1** and **2** contain two hydrolysable bonds (one ester and one amide bond), either of which could be hydrolyzed first, and can therefore initiate two different biodegradation pathways (Schemes S1-1 and S1-2). Dipeptide ILs **3** – **6** contain three hydrolysable bonds (one ester and two amide bonds) and, correspondingly, can initiate three distinct biodegradation pathways with different transformation products being formed in the breakdown process (**Schemes S1-3, S1-4, S1-5, and S1-6**). For all pathways the potential ultimate result is the same (full hydrolysis of all ester/amide bonds), although some metabolites will be different due to a change in the order of the bonds hydrolysis. Analysis of each pathway shows that all PTPs for amino acid ILs **1** and **2** can be found also in PTPs for dipeptide ILs **3** – **6** (see Scheme 1 and S1-7). Significant overlap also occurs when comparing the structures of PTPs for dipeptide ILs **3** – **6** which can be formed through different pathways, and this considerably decreased the number of target molecules. This reduces the resources and time required for the synthesis and testing for their aerobic biodegradability, resulting in an efficient strategy for this type of study. The structures of these target compounds are presented in Fig. 1.

2.2. Chemicals

Reagents and solvents were purchased from Sigma-Aldrich, Acros Organics, Alfa Aesar, Honeywell and used without further purification. Dipeptides (*N*-L-Phe-L-Phe-OH; *N*-L-Phe-L-Ala-OH; *N*-L-Ala-L-Phe-OH; *N*-L-Ala-L-Ala-OH) were provided by Fluorochem ltd. All other chemicals (amino acid ILs, dipeptide ILs and their PTPs) were synthesized by methods similar to procedures from [34,36,44,45] and are described in **S2** (Appendix A1). Characterization by NMR, HRMS spectra and melting points of studied compounds is also collected in supporting information (see **S2**).

The general synthetic routes to prepare compounds **1**–**10**, and **12**–**21** are shown in Scheme 2: corresponding L-amino acid (**12** and **13**) was transformed into ethyl ester hydrochloride (**27, 28)** with $SOCl_2$ in ethanol. The obtained product was then neutralized with sodium carbonate and gave an ester (**9** and **10**) in free base form. Next, the L-amino acid ethyl esters (**9**, **10**) was used for the synthesis of *N*-bromoacetyl derivatives (**29** and **30**) via acylation with bromoacetyl bromide in dichloromethane in the presence of sodium carbonate. The Boc-protected dipeptide ethyl esters (**31**–**34**) were synthesized by coupling with corresponding Boc-

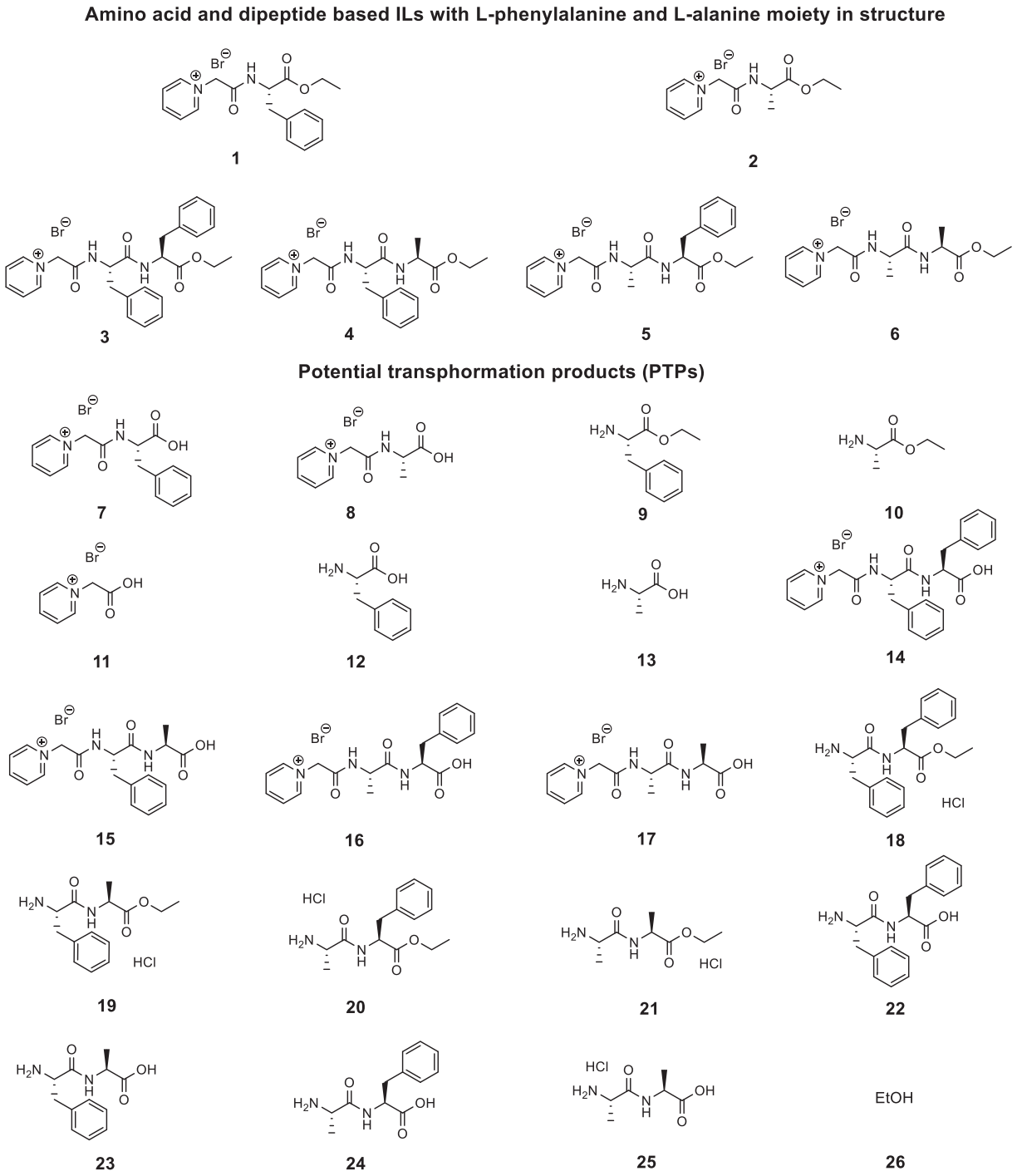
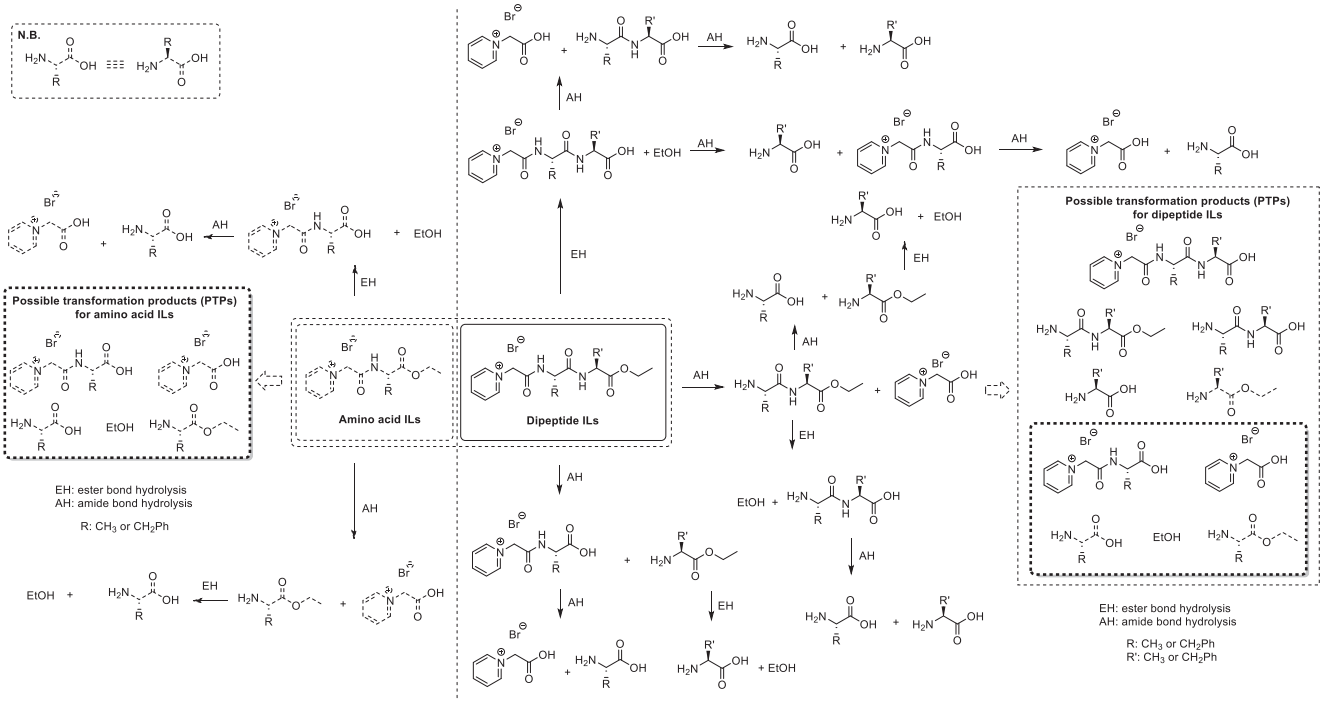


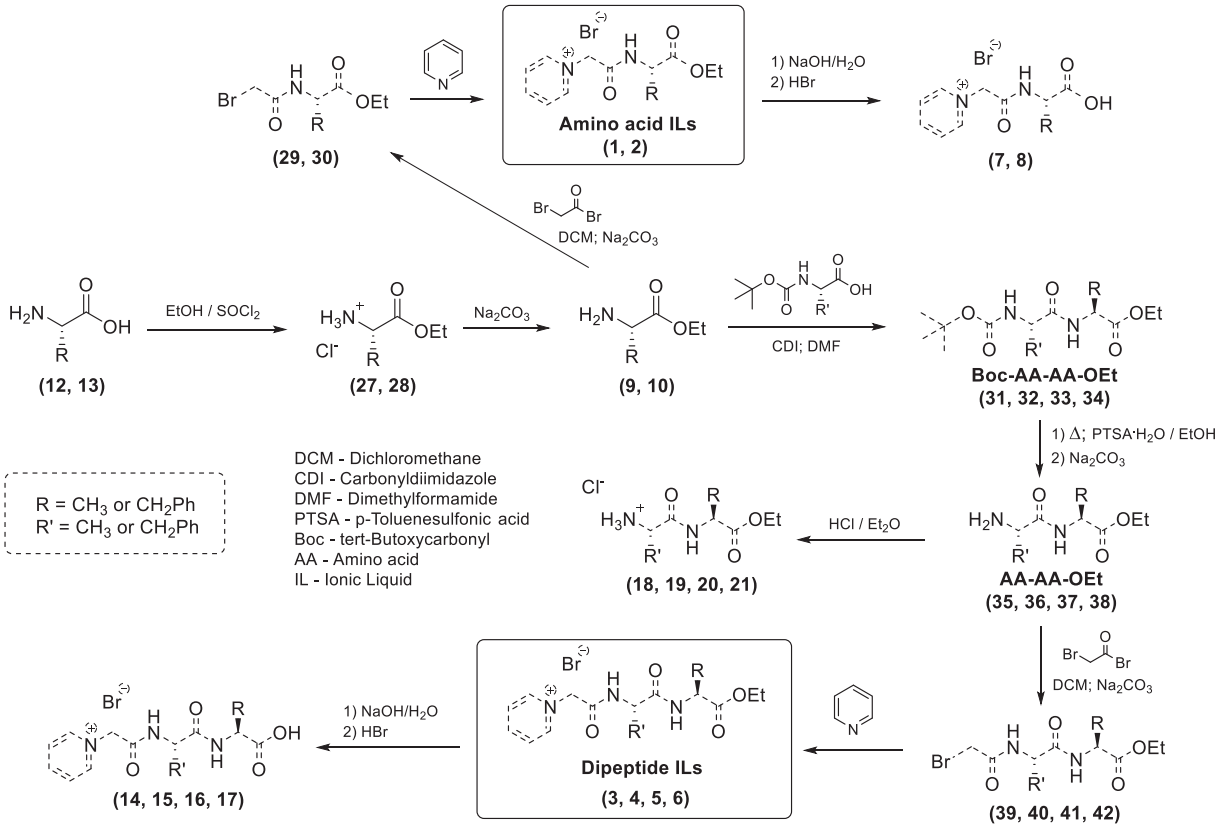
Fig. 1. Amino acid ILs (**1**, **2**) and dipeptide ILs (**3**–**6**) with L-phenylalanine and L-alanine moiety in structure and target PTPs (**7**–**26**) selected for synthesis and study.

protected L-amino acid). The alkylation of pyridine with *N*-bromoacetyl derivative of L -amino acid ethyl ester (**29** and **30**) resulted in target amino acid ILs **1** and **2**, which could be hydrolyzed to corresponding acids **7** and **8** in mild conditions. The Boc-protected dipeptide ethyl esters (**31**–**34**) were deprotected by reflux with PTSA in ethanol and then transformed into free base (**35**–**38**) with sodium carbonate. The dipeptide ethyl ester in free base form (**35**–**38**) were used for synthesis of hydrochloride salts **18**–**21** (via neutralization of free base in diethyl ether with solution of hydrochloric acid in diethyl ether). The *N*-bromoacetyl derivatives (**39**–**42**) were also prepared from (**35**–**38**) by acylation with bromoacetyl bromide in dichloromethane in presence of sodium carbonate. The products of alkylation of pyridine with *N*-bromoacetyl derivative of dipeptide ethyl ester (**39**–**42**) were target dipeptide ILs **3**–**6**, which could also be hydrolyzed to corresponding acids in mild conditions (**14**–**17**). Compound **11** was prepared according to our previous paper [34].

Resource efficient synthesis of chemical compounds is an integral part of our strategy to design greener chemicals. Analysis of the synthetic procedures according to Green Chemistry principles were performed with J. Clark's Green Chemistry metrics toolkit [46]. The key parameters of reactions are collected in Table S3. All reactions quantitatively analyzed by yield, conversion and selectivity can be marked with green flag (>90 %), except one case



Scheme 1. Biodegradation pathway analysis and selection of possible transformation products – a synergistic approach.



Scheme 2. Synthesis of amino acid ILs, dipeptide ILs and their PTPs. General strategy.

(AlaAlaOEt synthesis; yield and selectivity only 87 %; amber flag). Atom economy (AE) for most reactions (32/35) is >70 %. For ILs formation step (i.e. pyridine alkylation) the AE is 100 %. Reaction mass efficiency (RME) parameter for reactions, which were analyzed was in the range 40.8–99.4 %, however the majority of reactions have RME > 70 %. Optimum efficiency (OE) was also high with > 80 % determined in 31/35 cases. Analysis of process mass intensity (PMI) parameters shows that PMI workup solvents parameter is

the major contributor to the PMI total and reduced solvent usage in the workup step is recommended in future studies to make the synthesis greener. Several solvents which were used in the synthetic procedures and workup are rated green (water, ethanol, ethyl acetate, acetone), but in some steps problematic solvents (e.g., DMF, DCM, diethyl ether and petroleum ether were used [46]. To reduce the negative impact on the environment all problematic solvents were specifically targeted to be recovered, with recovery yields > 80 % achieved). Evaluation of critical elements used in the synthesis has been marked with green (>500 years supply remaining) or amber flag (50–500 years supply remaining). Based on evaluation of energy parameters (see **Table S3**) none of the reactions could be marked with red flag (reactions did not run below –20 or above 140 °C). All reaction were done in batch (amber flag), but potentially the hydrolysis step (synthesis of compounds **7**, **8**, **14**, **15**, **16**, **17**) can be performed under flow conditions in the presence of immobilized enzymes (green flag) [46]. The health & safety parameters (by H-code) for used synthetic procedures were also quantitatively assessed (see Green Chemistry Metrics files in Appendix A2).

2.3. Toxicity screening

Toxicity screening was performed with a similar method used in the previous papers [34,36], however the panel of bacterial and fungal strains involved more ATCC species: bacterial strains SA, *Staphylococcus aureus* (ATCC 29213); MRSA, *Staphylococcus aureus* (ATCC 43300); SE, *Staphylococcus epidermis* (clinical isolate); EF, *Enterococcus faecalis* (ATCC 29212); EC, *Escherichia coli* (ATCC 25922); KP, *Klebsiella pneumoniae* (clinical isolate); SEMA, *Serratia marcescens* (clinical isolate); PA, *Pseudomonas aeruginosa* (ATCC 27853); yeasts CA1, *Candida albicans* (ATCC 24433); CA2, *Candida albicans* (ATCC 90028); CK, *Candida krusei* (ATCC 6258); CG, *Candida glabrata* (ATCC 90030); CP, *Candida parapsilosis* (ATCC 22019); CT, *Candida tropicalis* (ATCC 750); PQ, *Pichia quilliermondii* (ATCC 90877); SC, *Saccharomyces cerevisiae* (ATCC 9763); fungal strains AF, *Aspergillus fumigatus* (ATCC 204305); AFla, *Aspergillus flavus* (CCM 8363); AC, *Absidia corymbifera* (CCM 8077); MC, *Microsporum canis* (CCM8353); TI, *Trichophyton interdigitale* (ATCC 9533). Minimum inhibitory concentration (MIC) values for evaluating antibacterial activity were obtained with microdilution broth method based on EUCAST (The European Committee on Antimicrobial Susceptibility Testing) instructions [47–49]. For experimental method and results see Appendix A1 (**S4,** Table S4-1, S4-2).

2.4. Inoculum

Effluent from wastewater treatment plant was collected from a municipal wastewater treatment plant in Tallinn, Estonia (Paljassaare wastewater treatment plant, 59°27′55.5″N 24°42′08.8″E). WWTP effluent was filtered through a cellulose filter (membrane ø 240 mm) before being used as inoculum for aerobic biodegradation testing.

2.5. Aerobic biodegradation according to modified OECD 301D

Aerobic biodegradation testing was performed using modified closed bottle test (CBT) based on OECD 301D guidelines [50,51]. CBT setup with modification where biological oxygen consumption is measured with an optode oxygen sensor system using PTFE-lined PSt3 oxygen sensor spots (Fibox 3 PreSens, Regensburg, Germany) allows measuring BOD without opening the flasks and thereby reducing the number of parallels needed for each compound and increasing test throughput. It has also shown to improve reproducibility compared to the original OECD 301D guideline [50]. Compared to other standard aerobic biodegradation tests, CBT is better suited for testing compounds with various physico-chemical properties. It is also one of the strictest tests as the amount of inoculum added is very low and thereby compounds passing CBT should show good biodegradation not only under artificial wastewater treatment conditions but also in soil and groundwater systems. Description of experimental setup and detailed results can be found in **S5**, Appendix A1 (Figures S5-1 _ S5-25).

3. Results and discussion

Six IL parent compounds (**1**–**6**) and their PTPs (**7**–**25**) were synthesized, screened for antimicrobial activity and their biodegradability under aerobic conditions was determined using modified CBT. Standard aerobic biodegradation tests enable us to have a standardized test where the results can be used to intelligently design chemicals with high biodegradability. However, they only approximate to the conditions found in wastewater treatment plants and/or surface water, soil. They suffer from two drawbacks. Firstly, they do not consider the variability of physico-chemical conditions and microbial communities which can be present in different parts of sewage lines before reaching treatment plants. Secondly, the CBT protocols do not require an evaluation and identification of TPs formed during the test. The common unknown in both these scenarios is the identification of the TPs, the additional chemical compounds present during the timeframe of the test. When considering which are the most labile (or reactive) groups in our ILs in an aqueous environment, the ester and amide functional groups were proposed. It is assumed that the compounds resulting from hydrolysis of the peptide ILs would also be generated in the environment (after accidental release) or during a CBT. For that reason, we included all possible hydrolysis degradation pathways and all resulting PTPs in our study. This strategy gave us more comprehensive information about the possible benign effect on the environment (or harm) of our designed compounds. In addition to the dipeptide ILs, identifying biodegradable PTPs with low toxicity is also a worthwhile endeavor, as they are good candidates to support future green chemical design.

3.1. Microbial toxicity screening

All six IL parent compounds and their PTPs were screened for antimicrobial activity against 4 Gram-positive bacteria strains, four Gram-negative bacteria strains, 8 yeast strains and 4 fungal strains. This was performed to ascertain their possible toxicity and to assist our biodegradation testing strategy. In particular, if any of the compounds showed high antimicrobial activity, it would be taken into consideration that the compound may also inhibit biotransformations in CBT. Results from microbial toxicity screening are summarised in Tables S4-1 and S4-2. Compounds **12** (L-phenylalanine) and **13** (L-alanine) were insoluble in DMSO and could not be screened for antimicrobial activity by the method used but are known to be common metabolites in microbes and other living organisms. Although PTP **26** (ethanol) is a widely used antiseptic it is expected to show no toxic effects at the concentrations used in CBT (<5 mg/L). This screening serves as a starting point for a more thorough investigation of IL toxicity.

The main goal of the microbial toxicity screen was identifying high-toxicity compounds which would be given a low priority in the subsequent time-consuming biodegradability testing. High antimicrobial activity is an undesirable property when designing sustainable ILs with broad applicability and could also negatively affect the microbes used as inoculum in CBT. From the results of this preliminary screening (see Tables S4-1 and S4-2) it was decided that all compounds would be studied in CBT as none

showed high activity (MIC < 10 μM in 24 h) against the strains of bacteria, yeast and fungi they were screened against.

Out of all studied compounds only **6** showed minor antibacterial activity against Gram-positive SA (MIC 125 μM in 24 h), MRSA (MIC 500 μM in 24 h), SE (MIC 1000 μM in 24 h), EF (MIC 2000 μM in 24 h) and Gram-negative EC (MIC 1000 μM in 24 h) and KP (MIC 2000 μM in 24 h), see Table S4-1. The **6** did not show any activity against yeast and fungi at the maximum concentration screened. None of the other ILs and PTPs (compounds **1**–**5**, **7**–**25**) exhibited antibacterial properties in the maximum concentrations used against the strains chosen for screening. Screening results with yeast and fungi showed minor antimicrobial activity for **9, 10** and **18**, but no antibacterial properties were demonstrated. Overall, only minor antimicrobial activity was found with compounds **1**–**25**. Concentrations used in this screening (up to 2000 μM) exceeded the concentrations which were later used for CBT (5.2–52.1 μM) and resulted in the decision that all 25 compounds were the same priority for aerobic biodegradability testing. The toxicity data was in accordance with previously published results where pyridinium ILs showed low microbial toxicity [34]. Due to the similarity of obtained MIC values no clear SAR comparison can be made.

In addition to antimicrobial activity screening, toxicity was also evaluated during CBT using „toxicity series" data recorded concurrently. If any of the bottles in this series showed<25 % biodegradation by day 14, this compound would be classified as toxic [50]. None of the studied 25 compounds were toxic to microbes in wastewater treatment plant effluent used as inoculum. This is consistent with our toxicity screening results which showed low antimicrobial activity of studied compounds (Tables S4-1 and S4-2).

Toxicity screening results complement our previously reported findings of a mineralizable pyridinium substituted phenylalanine derived IL (**1**) with low toxicity [33,34]. However, when Hou and co-workers studied cholinium ILs with amino acids as anions, they reported that the phenylalanine analogue had one of the highest antibacterial activities (including SA (MIC 62.5 mM) and EC (MIC 31.3 mM)) among 20 standard amino acids [52]. Similar work with cholinium-amino acid ILs was carried out by Yazdani who reported EC_{50} values 251 – 1120 mg/L (0.93–5.80 mM) for alanine and phenylalanine anions against two Gram-positive and two Gram-negative bacteria [53]. As the method used in our study was not suitable for screening pure alanine and phenylalanine samples, data cannot be compared directly. However, compounds in our study containing these two amino acid residues did not show high antimicrobial activity. Our aim was not to determine MIC values but to check for high toxicity which could have negatively affected the following biodegradability studies. The MIC values reported previously for phenylalanine and alanine were higher than the concentrations used in CBT (>2 mM) and based on this we concluded that **12** and **13** should not show any inhibition in aerobic biodegradation tests.

These examples demonstrate that the overall structure and charge of a compound can play a big role in toxicity mechanism. Minor changes in the chemical structure of a compound can still have a significant effect when determining its '*greenness*'.

3.2. Aerobic biodegradation testing

Microbial toxicity screening results indicated that none of the tested compounds would be expected to show inhibition towards microbes in inoculum during CBT. Thus, based on results from antimicrobial activity testing all six IL parent compounds (**1**–**6**) and their PTPs (**7**–**25**) were given equal priorities for further studies and were tested for their aerobic biodegradability. Biodegradation testing was performed using modified CBT method and each test ran for up to 33–42 days. Most of the tested compounds contained a pyridinium sub-unit which had previously been reported to have a long induction period after which fast biodegradation occurs [33]. To better observe the effects of this phenomenon we prolonged CBT from standard 28 days (suggested by OECD 301 D protocol) to 33–42 days [51]. A general scheme for the proposed breakdown pathways and resulting PTPs are shown in Scheme 1 and Fig. 1, respectively. For specific proposed breakdown pathways for **1**–**6**, see ESI-1. Biodegradation graphs for amino acid ILs (**1** and **2)** and their PTPs (**7**–**13**) and dipeptide ILs (**3**–**6)** and their PTPs (**7**–**25**) are shown on Figs. 2 and 3. The results are summarized in Table 1. Individual biodegradation curves for each compound together with quality and toxicity controls are presented in **S5** (Figures S5-1 _ S5-25). The minor antimicrobial activity demonstrated by IL **6** and PTPs **9**, **10** and **18** did not affect their biodegradability as all of them passed the 60 % threshold in extended CBT with 42 days.

Phenylalanine IL **1** passed the 60 % biodegradation threshold on day 25 and reached 66 % after the extended CBT of 42 days (Fig. 3). These are similar results compared to biodegradation data of **1** previously obtained in our study with the Kümmerer group [33]. All of the evaluated PTPs of **1** (i.e., **7**, **9**, **11** and **12**) also showed high final biodegradation values (61–93 %) (Table 1). Switching amino acid moiety from phenylalanine to alanine, however, significantly decreased biodegradability (Fig. 2) and **2** reached only 30 % in 42 days (25 % in 28 days which is the standard length of CBT) (Table 1). We propose that hydrophobic phenylalanine residue in close proximity to the positively charged pyridinium headgroup makes the cation a more reactive substrate for enzymes responsible for observed biodegradation. A similar trend was shown by PTPs **7** and **8,** esterolysis products of phenylalanine and alanine ILs (**1** and **2**, respectively). **7** passed the 60 % threshold in the CBT standard 28 days (66 % in 42 days) while **8** reached only 43 % in 42 days (40 % in 28 days) (Table 1). Amidolysis products of ILs **1** and **2** are ethyl esters **9** and **10.** Both of these amino esters can be classified as biodegradable (after 28 days, **9** 60 % and **10** 73 %, Table 1), suggesting that amino acid containing TPs resulting from amidolysis of ILs **1** and **2** are more biodegradable compared to amino acid containing TP formed via the alternative initial esterolysis pathway. Amino esters **9** and **10** can also hydrolyse to the corresponding amino acids. These PTPs **12** and **13** were screened, and the results showed a similar trend as seen with the carboxylic acids **7** and **8**, esterolysis products of **1** and **2**, respectively. L-phenylalanine **12** was biodegradable in 28 days (65 %) while L-alanine **13** biodegraded only 48 % in standard 28 days (Table 1). However, while L-alanine pyridinium salt **8** did not further biodegrade on extending the test from 28 to 42 days, L-alanine **13** increased from 48 % to 62 % biodegradation due to the extra 14 days. The pyridinium product of amidolysis of both **1** and **2**, PTP **11**, had a long induction period before starting rapid biodegradation reaching 93 % biodegradation after 42 days. This is in agreement with our previous study of biodegradation of phenylalanine IL **1** [33,34].

In case of monoamino acid ILs, **1** with phenylalanine residue showed significantly improved biodegradability compared to less hydrophobic salt **2**. The Fig. 2 overall shows that high levels of biodegradation for IL **1** and PTPs can be achieved. Although PTP **11** had a long induction period, high biodegradation was observed with 42 days. By plotting the biodegradation data for the IL and PTPs on the same graph, a more thorough analysis of whether the IL has favourable biodegradable properties can be made. The Fig. 2 illustrates a complimentary dataset where again additional analysis of the biodegradation data is possible compared to a study only reporting the CBT results for IL **2**. Although **2** and ester hydrolysis product **8** do not show high biodegradation (and thus applications using **2**, or ILs which degrade to form **8**, are not preferred), ILs

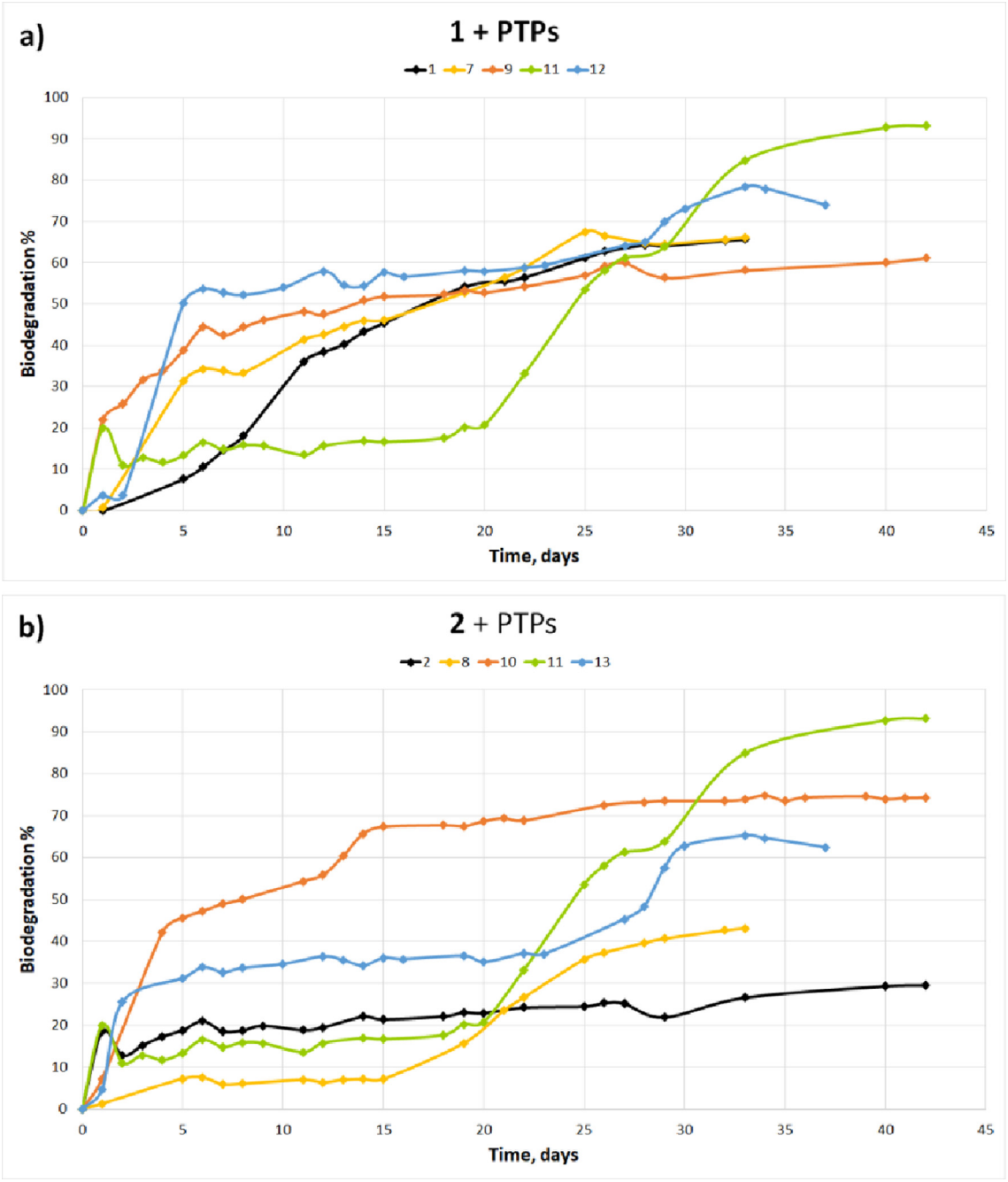


Fig. 2. Aerobic biodegradation curves of a) **1** and PTPs **7**, **9**, **11** and **12**; b) **2** and PTPs **8**, **10**, **11** and **13** using modified CBT. More detailed biodegradation graphs for each compound are presented in **Appendix A1** (Figures S5-1 (**1**), **S5-2** (**2**), **S5-7** (**7**), **S5-8** (**8**), **S5-9** (**9**), **S5-10** (**10**), **S5-11** (**11**), **S5-12** (**12**) and **S5-13** (**13**)).

which degrade to yield L-alanine ethyl ester **10** and L-alanine **13** are preferred. Results for **2** and its PTPs also indicate that just combining biodegradable „building blocks" does not always guarantee good biodegradability of the product: biodegradation values for all PTPs of **2** showed higher D% compared to **2**. This shows the importance of a systematic study of breakdown pathways of parent compounds and their transformation products if mineralizable ILs are to be developed. While this is undoubtedly the ideal approach, the resources required to complete this for a series of compounds is considerable. We propose that the targeted approach we describe herein, enables researchers to select the preferred compounds for further study.

Biodegradation was evaluated using the monoamino acid ILs (**1** and **2**) as well as dipeptide ILs (**3**–**6**) and their PTPs (**7**–**25**). In regular CBT conditions with 28 days, **3** and **4** passed the 60 % threshold and could be classified as biodegradable (see Fig. 3). After extending the test to 42 days dipeptide ILs **5** and **6** also reached biodegradation values > 60 % (see Fig. 3). Out of the four dipeptide IL parent compounds **4** ($PyCH_2CO$-Phe-Ala-OEt IL) had highest biodegradability: 91 % after 42 days. **3** ($PyCH_2CO$-Phe-Phe-OEt IL) (84 %) and **5** ($PyCH_2CO$-Ala-Phe-OEt IL) (73 %) followed and **6** ($PyCH_2CO$-Ala-Ala-OEt IL) had the lowest biodegradability with 61 % after 42 days, as shown in Table 1. Lower biodegradability of **6** is in accordance with results from toxicity screening where **6** showed some antibacterial activity against tested bacterial

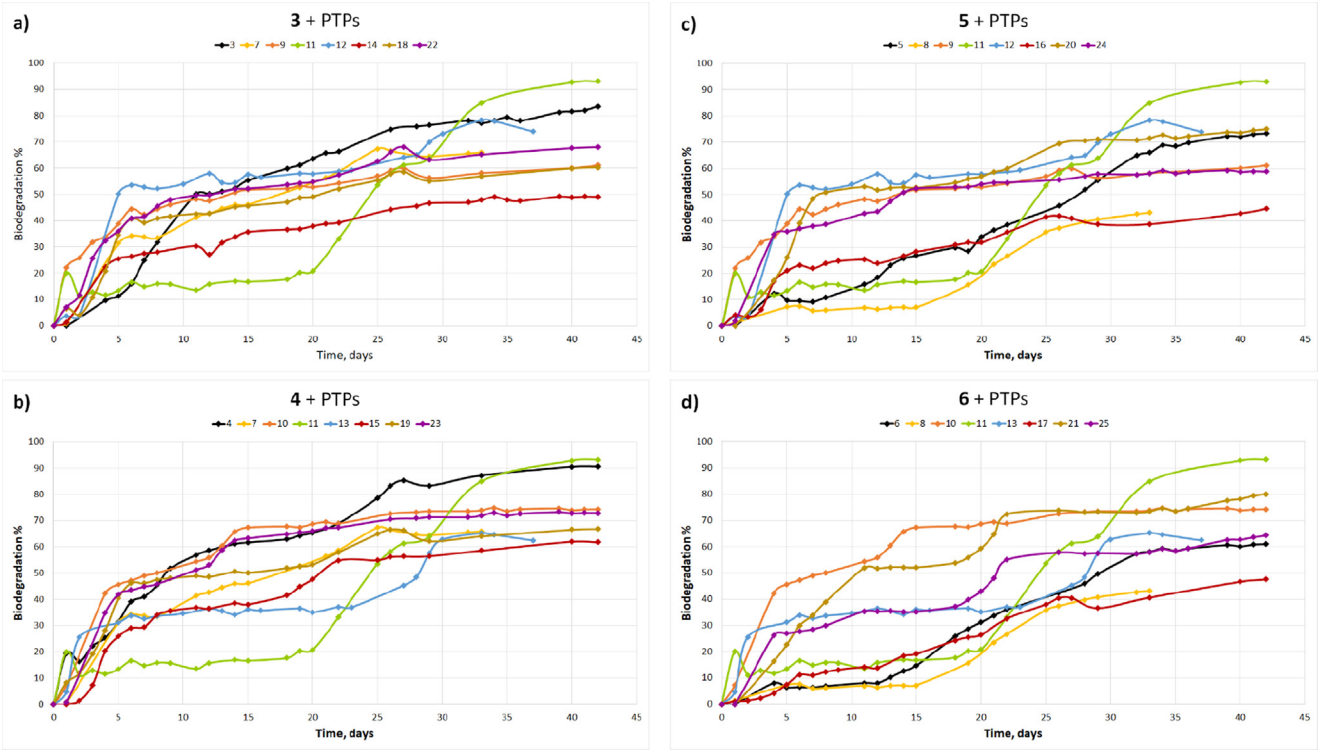


Fig. 3. Aerobic biodegradation curves of a) **3** and PTPs **7**, **9**, **11**, **12**, **14**, **18** and **22;** b) **4** and PTPs **7**, **10**, **11**, **13**, **15**, **19** and **23**; c) **5** and PTPs **8**, **9**, **11**, **12**, **16**, **20** and **24**; d) **6** and PTPs **8**, **10**, **11**, **13**, **17**, **21** and **25** using modified CBT. More detailed **21**), biodegradation graphs for each compound are found in Appendix A1 (Figures S5-3 (**3**), **S5-4** (**4**), **S5-5** (**5**), **S5-6** (**6**), **S5-7** (**7**), **S5-8** (**8**), **S5-9** (**9**), **S5-10** (**10**), **SI5-11** (**11**), **S5-12** (**12**), **S5-13** (**13**), **S5-14** (**14**), **S5-15** (**15**), **S5-16** (**16**), **S5-17** (**17**), **S5-18** (**18**), **S5-19** (**19**), **S5-20** (**20**), **S5-21** (**21**), **S5-22** (**22**), **S5-23** (**23**), **S5-24** (**24**), and **S5-25** (**25**).

strains, most notably SA, and therefore could exhibit similar inhibition to some of the strains in CBT inoculum. These results further prove that inclusion of the phenylalanine moiety into pyridinium ILs leads to dipeptide compounds with high biodegradability under our test conditions. The common structural unit of the three amino acid derived ILs (**1**, **3** and **4**) with the recorded highest values of biodegradation is $PyCH_2COPhe$-. Interestingly, it seems that the position of the phenylalanine moiety in the structure of IL cation is important. Significantly lower biodegradation was observed for **5** the $PyCH_2CO$-Ala-Phe-OEt IL compared to $PyCH_2CO$-Phe-Ala-OEt IL **4**. The lowest biodegradation (albeit a good result after 42 days, 61 %) was found when only alanine residues were present in IL **6**. This shows that both the amino acid moiety and its position in IL structure are important factors when biodegradability is considered.

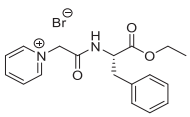
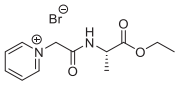
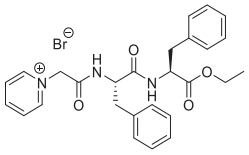
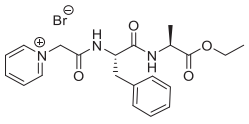
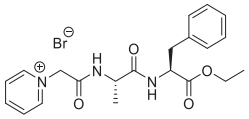
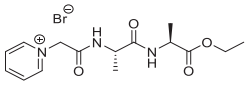
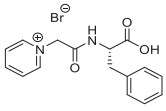
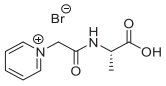
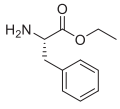
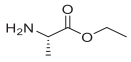
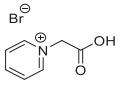
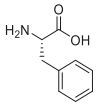
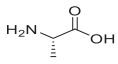
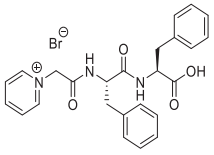
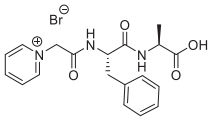
There are three sites susceptible to hydrolysis in ILs **3**–**6**, two amide bonds and an ethyl ester. A reasonable assumption is that the ethyl ester is more labile and accessible than the two amide bonds and would be cleaved first. After hydrolysis of the ester bond, PTPs **14**–**17** would be formed. However, all 4 dipeptide ILs **3**, **4**, **5** and **6** esterolysis products (compounds **14**, **15**, **16** and **17,** respectively) showed significantly lower biodegradation values – 49 % vs 84 %; 62 % vs 91 %; 45 % vs 73 %; and 48 % vs 61 %) (see Table 1). **15**, PTP of **4**, showed the highest biodegradability out of the four hydrolysis PTPs, reaching 56 % in 28 days and 62 % in 42 days. None of the other hydrolysis PTPs passed the 60 % threshold in 42 days. This indicates that the hydrophilic carboxylic acid residue, which is formed after esterolysis of **3**–**6**, inhibits biodegradation.

This effect is not apparent with monoamino acid ILs. Ethyl ester **1** and the carboxylic acid derivative **7** CBT results were almost identical, ca. 65 % biodegradation after 28 and 42 days. After 42 days there was no significant difference in biodegradation values (30 % and 43 %) for the ethyl ester and carboxylic acid alanine ILs, **2** and **8** respectively (see Table 1).

Considering ILs **3**–**6**, if the amide bond connecting the two amino acids hydrolyses first, then the PTPs are the carboxylic acids **7** and **8**, and the amino ethyl esters **9** and **10**. Biodegradation values for **7**, **9** and **10** are all above 60 % threshold after 28 days. However, **8** is only 43 %, even after 42 days. Therefore, hydrolysis between the two amino acids of $PyCH_2COPhe$- ILs **3** and **4**, would yield PTPs with biodegradation values>60 % (after 28 days). This result is consistent with the high biodegradation values obtained for **3** and **4**. However, comparing the biodegradation data for $PyCH_2COAla$- IL **5** and hydrolysed product **8**, a different trend is observed. Although **5** attained 73 % degradation after 42 days, a significantly lower value was recorded for its PTP **8** (43 %). One possibility is that the degradation of **5** does not have a dominant breakdown pathway via **8**. To further support this claim, we also considered the alternative scenario where the amide bond closest to the pyridinium head group hydrolysed first. IL **5** would be converted into PTPs **11** and **20**. High biodegradation values 93 % and 75 % (after 42 days) were recorded for these PTPs and are consistent with the high biodegradation observed for IL **5** (73 %, 42 days).

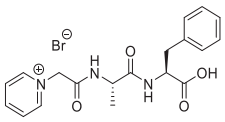
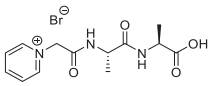
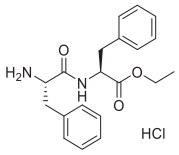
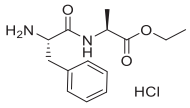
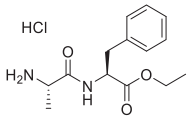
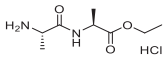
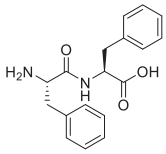
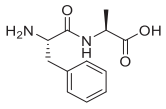
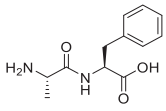
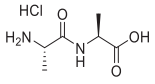
If, instead of esterolysis, breaking of the amide bond near the pyridinium headgroup would be the first transformation during biodegradation, PTPs **18**–**21** would be formed. These PTPs all showed good biodegradability: **19**–**21** passed the 60 % threshold in 28 days and **18** reached 59 %. Especially high values were obtained with extended CBT: 60 % for **18**; 67 % for **19**; 75 % for **20** and 80 % for **21**. If amidolysis and loss of pyridinium headgroup is the first step in biodegradation of **3**–**6** good biodegradation is still achieved. Interestingly, losing pyridinium headgroup would also increase biodegradability of esterolysis products: PTPs **22**–**25** showed higher biodegradation values compared to PTPs **14**–**17** which had pyridinium headgroup attached to the molecule. **22**;

Table 1
Biodegradation results for the periods of 14, 28, and 42 days.

#	Structure	Conc. in CBT, μM	10-day-window analysis				D% 14 days	D% 28 days	D% 42 days
			Start day	Start D%	End D%	ΔD%			
1		7.8	6	11 ± 2	45 ± 4	34	43 ± 6	64 ± 3	66[a] ± 3
2		12.0	2	13 ± 1	20 ± 1	7	22 ± 1	25 ± 2	30 ± 1
3		5.2	5	11 ± 2	55 ± 6	44	52 ± 4	76 ± 1	84 ± 1
4		6.8	2	16 ± 1	59 ± 14	38	61 ± 14	83 ± 8	91 ± 5
5		6.8	4	12 ± 4	26 ± 16	14	26 ± 16	52 ± 4	73 ± 4
6		9.8	13	10 ± 5	36 ± 11	26	13 ± 6	46 ± 7	61 ± 5
7		9.2	5	31 ± 1	46 ± 2	15	46 ± 2	65 ± 6	66[a] ± 6
8		15.6	19	16 ± 7	41 ± 5	25	7 ± 3	40 ± 6	43[a] ± 4
9		12.0	2	26 ± 2	48 ± 3	22	51 ± 3	60 ± 5	61 ± 3
10		26.0	4	42 ± 1	66 ± 1	24	66 ± 1	73 ± 1	74 ± 1
11		22.3	2	11 ± 1	16 ± 2	5	17 ± 2	61 ± 16	93 ± 3
12		15.6	5	50 ± 4	58 ± 1	8	54 ± 1	65 ± 2	74[b] ± 4
13		52.1	2	26 ± 9	36 ± 1	10	34 ± 1	48 ± 1	62[b] ± 3
14		5.8	4	22 ± 2	34 ± 1	12	34 ± 1	46 ± 2	49 ± 2
15		7.8	4	20 ± 1	39 ± 11	19	39 ± 11	56 ± 2	62 ± 3

(*continued on next page*)

Table 1 (*continued*)

#	Structure	Conc. in CBT, μM	10-day-window analysis				D% 14 days	D% 28 days	D% 42 days
			Start day	Start D%	End D%	ΔD%			
16		7.8	4	17 ± 10	27 ± 11	10	27 ± 11	41 ± 9	45 ± 10
17		12.0	6	11 ± 1	19 ± 4	8	18 ± 4	40 ± 1	48 ± 1
18		6.8	3	11 ± 1	43 ± 1	32	45 ± 1	59 ± 4	60 ± 3
19		9.8	2	12 ± 4	49 ± 1	37	51 ± 1	66 ± 4	67 ± 7
20		9.8	4	17 ± 1	53 ± 1	36	53 ± 2	71 ± 2	75 ± 1
21		17.4	4	16 ± 1	52 ± 1	36	52 ± 1	73 ± 2	80 ± 1
22		7.8	2	12 ± 1	50 ± 1	38	52 ± 1	68 ± 1	68 ± 1
23		12.0	4	35 ± 1	62 ± 1	27	62 ± 1	71 ± 1	73 ± 1
24		12.0	4	35 ± 1	51 ± 2	16	51 ± 2	57 ± 2	59 ± 2
25		26.0	4	26 ± 1	35 ± 1	9	35 ± 1	57 ± 1	64 ± 1

[a] 33 days.
[b] 37 days.

23 and **24** reached biodegradation plateaus in 28 days: 68 %, 71 % and 57 % respectively. **22** and **23** could be classified as biodegradable. **25** reached 57 % in 28 days but after extended CBT passed the 60 % threshold and reached 64 % in 42 days. Overall, analysis of PTPs of **3**–**6** shows that, in general, hydrophobic phenylalanine moiety improves biodegradability compared to alanine residue. All PTPs of Phe-Ala dipeptide IL **4** passed the 60 % threshold in extended CBT with 42 days.

All four studied dipeptide ILs contained a pyridinium headgroup. Pyridinium cations with short alkyl chains have previously shown poor biodegradability [54,55]. Docherty and Neumann also reported, however, very substantial improvement in biodegradability when pyridinium headgroup was substituted with a long alkyl chain or if the alkyl chain contained hydrolysable groups. Our results support these and other previously reported findings about substituted pyridinium headgroup's good biodegradability and ability to be mineralizable [33,34,56–58]. However, we also identified a long induction period in pyridinium sub-unit's biodegradation. This induction period, lasting up to ~ 20 days is illustrated well in biodegradation curve of **11** (Figs. 2, 3, and S5-11). Similar pyridinium-induced induction period was reported by Zhang who studied effect of [EtPy][BF_4] on *P. fluorescens* [59]. This long induction period was also the reason why CBT duration was extended from standard 28 days to 33–42 days to allow better observation of biodegradation and to gain more information which could help with future studies of IL biodegradability.

Alanine and phenylalanine biodegradability have been studied using amino acids as anions to cholinium cation [52,53]. In both studies alanine and phenylalanine ILs passed biodegradation test and results are similar to the ones reported in this paper: alanine biodegradation was 80 % (Yazdani. [53]); 64 % (Hou. [52]) and 62 % (current study) in these three studies while phenylalanine biodegradation was 71 % (Yazdani. [53]); 74 % (Hou. [52]) and 74 % (current study). This is a promising result as amino acids, with their possibility of sourcing from renewable materials, are good candidates to prepare environmentally friendly and *green* chemicals.

Standard biodegradation tests are usually considered passed when a threshold representing degradation of 60 % is achieved. In case of ready biodegradability this threshold has to be reached fast, usually in a 10-day-window after degradation process has begun [51]. This rapid degradation is good as it means less time for the compound to have any effect on the environment, but it also means that a compound passing standard biodegradation test can leave up to 40 % persistent organic pollutants. Furthermore, this type of evaluation gives little indication to what transformations take place with the compound. It is possible that the transformation product itself is recalcitrant or even toxic. For this reason, we argue that it can also be beneficial to study compounds which might go through slower biodegradation but eventually are completely mineralized: transformed into inorganic carbon dioxide, water etc.

OECD 301D guidelines state that for a compound to be classified as „readily biodegradable" it must achieve at least 60 % biodegradation in the following 10 days after reaching 10 % degradation [51]. This is not achieved by phenylalanine IL **1** (Table 1; Figure S5-1) which has been previously studied and is known to be completely mineralizable in 42 days, but was biodegraded 45 % at the end of its 10-day-window. Even lower biodegradation was shown by alanine IL **2** which passed 10 % already at second day of the test but was only degraded by 20 % 10 days later (Table 1; Figure S5-2). Interestingly, PTP **11**, which is also previously known to be completely mineralizable, showed a long induction period for the first 20 days, after which it started to rapidly biodegrade. By OECD guidelines, PTP **11** could not be classified as readily biodegradable, but after 42 days it showed 93 % biodegradation, giving strong indication that PTP **11** is mineralizable in used test conditions. PTP **11** was a key transformation product forming from all our IL parent compounds through various possible degradation pathways and this observed long induction period (and following mineralization) was one of the reasons for prolonging CBT tests to 33–42 days to allow seeing if other compounds behaved similarly. Another compound with long ca 20-day induction period was PTP **8**, the esterolysis product of IL **2**. Unlike **11**, **8** never passed the 60 % threshold during 42 days of CBT.

None of the dipeptide IL parent compounds were shown to be readily biodegradable, however **3** and **4** were very close and reached 55 % and 59 % respectively in the 10-day-window. In addition to giving the highest biodegradability, **3** and **4** were also degraded rapidly, further confirming that changing the amino acid residue to hydrophobic phenylalanine near the positively charged pyridinium sub-unit helped improve the cation's biodegradability. Dipeptide ILs **5** and **6** showed much slower biodegradation and **6** had a long induction period for the first 13 days. Out of all tested 25 compounds only PTPs **10** and **23** can be classified as readily biodegradable having met the criteria of being biodegraded > 60 % in the first 10 days after they had been degraded by 10 %. This shows the importance of studying both the parent compounds and their PTPs, as it gives more information about how the compounds could behave when disposed. Extending the test for another two weeks (42 days in total) is another good practice if mechanisms of biodegradation are studied as even more information is gathered and biodegradability of test compounds can be evaluated more objectively.

Based on results from toxicity screening and biodegradation studies we propose preferable ILs and PTPs to be used in design of benign and sustainable chemicals: phenylalanine IL **1**, Phe-Phe and Phe-Ala ILs **3** and **4**, esterolysis product **7**, amidolysis products **9**, **10**, **11**, **19**, **20**, **21**, transformation products **22**, **23** and amino acid phenylalanine (**12**) which all can be classified as biodegradable. A little more problematic were Ala-Phe and Ala-Ala ILs **5** and **6**, esterolysis product **15**, amidolysis products **18**, PTP **25** and amino acid alanine (**13**) which showed slower biodegradation but passed the 60 % threshold in prolonged 42 days of CBT. Caution is advised when integrating alanine IL **2**, its esterolysis product **8** or dipeptide esterolysis products **14**, **16**, **17** PTP **24** into the design of new chemicals as these compounds were shown to be poorly biodegradable.

4. Conclusions

To gain an improved understanding of pyridinium ILs biodegradation a series of L-phenylalanine and L-alanine ILs were evaluated. Their possible degradation pathways and resulting TPs were proposed and all required ILs and TPs synthesized. These compounds (25 in total) underwent microbial toxicity screening, aerobic biodegradation testing and resulted in a valuable dataset which enabled concurrent analysis of ILs and their PTPs.

One of the key findings was that addition of a second amino acid resulted in dipeptide ILs with improved biodegradability. Specifically, the addition of a second alanine or phenylalanine residue improved biodegradation percentages for all studied ILs. In addition to the amino acid residue present, its position in the dipeptide IL structure also significantly affected the biodegradability of the compound. For example, Phe-Ala IL (**4**), where phenylalanine is in close proximity to the positively charged pyridinium sub-unit, gave higher biodegradation percentages compared to Ala-Phe IL (**5**). Overall, the best biodegradability was observed with Phe-Ala IL (**4**) which was biodegraded 83 % in 28 days and reached up to 91 % in a prolonged CBT of 42 days.

Analysis of PTPs also showed that presence of an alanine residue gave problematic (i.e. more persistent) PTPs compared to most PTPs containing phenylalanine. This was especially the case when alanine was in close proximity to the pyridinium headgroup. Based on both toxicity and biodegradation testing results preferable and less preferable fragments can be chosen for design of new sustainable chemicals based on amino acids.

The modified CBT allows for a measurement of biodegradability with many datapoints (potentially daily) which together with extension of the test period can provide valuable information for future studies of biodegradability and TP investigations. Where biodegradable TPs are found they could be incorporated into the structure of ILs and released via known breakdown pathways.

Results from this study demonstrate a potential of designing new sustainable ILs using amino acid moieties as part of their structure. Combining these functional groups together can create biodegradable ILs with low toxicity which can be used as model compounds when studying biodegradation mechanisms.

CRediT authorship contribution statement

Illia V. Kapitanov: Methodology, Data curation, Writing – original draft, Conceptualization, Writing – review & editing. **Grete Raba:** Methodology, Data curation, Writing – original draft, Conceptualization. **Marcel Špulák:** Methodology, Data curation, Funding acquisition. **Raivo Vilu:** Conceptualization, Writing – review & editing. **Yevgen Karpichev:** Conceptualization, Methodology, Funding acquisition, Data curation, Writing – original draft, Writ-

ing – review & editing. **Nicholas Gathergood:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors acknowledge funding from European Union's Seventh Framework Programme for Research, Technological Development, and Demonstration under Grant Agreement no. 621364 (TUTIC-Green), and Estonian Research Council grants PUT1656 (IK, GR, YK, and NG) and COVSG5 (YK). The project was partially funded by the Czech Science Foundation (project No. 22-19209S). The authors thank Jelena Valtin and AS Tallinna Vesi for providing wastewater treatment plant effluent necessary for performing aerobic biodegradation tests in this study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2023.121285.

References

[1] P. Walden, Molecular weights and electrical conductivity of several fused salts, Bul. Imp. Acad. Sci. 1800 (1914) 405–422.
[2] M. Koel, Ionic liquids in chemical analysis, Crit. Rev. Anal. Chem. 35 (3) (2005) 177–192, https://doi.org/10.1080/10408340500304016.
[3] J.S. Wilkes, A short history of ionic liquids - from molten salts to neoteric solvents, Green Chem. 4 (2) (2002) 73–80, https://doi.org/10.1039/b110838g.
[4] N.V. Plechkova, K.R. Seddon, Applications of Ionic Liquids in the Chemical Industry, Chem. Soc. Rev. 37 (1) (2008) 123–150, https://doi.org/10.1039/b006677j.
[5] A.J. Greer, J. Jacquemin, C. Hardacre, Industrial applications of ionic liquids, Molecules 25 (21) (2020) 5207, https://doi.org/10.3390/molecules25215207.
[6] M. Freemantle, BASF's smart ionic liquid, Chem. Eng. News (2003) 9, https://doi.org/10.1021/cen-v081n013.p009.
[7] M. Maase, K. Massonne, K. Halbritter, Method for the separation of acids from chemical reaction mixtures by means of ionic fluids. US7351339B, 2008.
[8] G.W. Phillips, S.N. Falling, S.A. Godleski, J.R. Monnier, Continuous Process for the Manufacture of 2,5-Dihydrofurans from γ, δ-Epoxybutenes. US5315019A. US5315019A, 1994.
[9] P.J. Piedade, E. Kochańska, R.M. Lukasik, Biodegradable ionic liquids in service of biomass upgrade, Curr. Opin. Green Sustain. Chem. 35 (2022), https://doi.org/10.1016/j.cogsc.2022.100609.
[10] P. Pillai, M. Maiti, A. Mandal, Mini-review on recent advances in the application of surface-active ionic liquids: petroleum industry perspective, Energy Fuel 36 (15) (2022) 7925–7939, https://doi.org/10.1021/acs.energyfuels.2c00964.
[11] S.S. de Jesus, R. Maciel Filho, Are ionic liquids eco-friendly?, Renew Sustain. Energy Rev. 157 (2022), https://doi.org/10.1016/j.rser.2021.112039.
[12] A.R.P. Gonçalves, X. Paredes, A.F. Cristino, F.J.V. Santos, C.S.G.P. Queirós, Ionic Liquids—A review of their toxicity to living organisms, Int. J. Mol. Sci. 22 (11) (2021) 5612, https://doi.org/10.3390/ijms22115612.
[13] P. Anastas, N. Eghbali, Green chemistry: principles and practice, Chem. Soc. Rev. 39 (1) (2010) 301–312, https://doi.org/10.1039/b918763b.
[14] D. Kowalska, J. Maculewicz, P. Stepnowski, J. Dołżonek, Ionic liquids as environmental hazards – crucial data in view of future PBT and PMT assessment, J. Hazard. Mater. 403 (2021), https://doi.org/10.1016/j.jhazmat.2020.123896.
[15] F. Billeci, F. D'anna, M. Feroci, P. Cancemi, S. Feo, A. Forlino, F. Tonnelli, K.R. Seddon, H.Q.N. Gunaratne, N.V. Plechkova, When functionalization becomes useful: ionic liquids with a "Sweet" appended moiety demonstrate drastically reduced toxicological effects, ACS Sustain. Chem. Eng. 8 (2) (2020) 926–938, https://doi.org/10.1021/acssuschemeng.9b05507.
[16] A.-K. Amsel, O. Olsson, K. Kümmerer, Inventory of biodegradation data of ionic liquids, Chemosphere 299 (2022) 134385, https://doi.org/10.1016/j.chemosphere.2022.134385.
[17] A.A. Quintana, A.M. Sztapka, V. de C. Santos Ebinuma, C. Agatemor, Enabling sustainable chemistry with ionic liquids and deep eutectic solvents: A fad or the future?, Angew Chem. Int. Ed. 61 (37) (2022), https://doi.org/10.1002/anie.202205609.
[18] M. Cvjetko Bubalo, K. Radošević, I. Radojčić Redovniković, J. Halambek, V. Gaurina Srček, A brief overview of the potential environmental hazards of ionic liquids, Ecotoxicol. Environ. Saf. 99 (2014) 1–12, https://doi.org/10.1016/j.ecoenv.2013.10.019.
[19] A. Brzęczek-Szafran, P. Więcek, M. Guzik, A. Chrobok, Combining amino acids and carbohydrates into readily biodegradable, task specific ionic liquids, RSC Adv. 10 (31) (2020) 18355–18359.
[20] J.B. Zimmerman, P.T. Anastas, H.C. Erythropel, W. Leitner, Designing for a green chemistry future, Science, 367 (6476) (2020) 397–400, https://doi.org/10.1126/science.aay3060.
[21] E.M. Siedlecka, M. Czerwicka, J. Neumann, P. Stepnowski, J.F. Fernández, J. Thöming, Ionic liquids: methods of degradation and recovery, in: A. Kokorin (Ed.), Ionic Liquids: Theory, Properties, New Approaches, InTech, 2011, https://doi.org/10.1016/j.colsurfa.2011.12.014.
[22] A. Jordan, N. Gathergood, Biodegradation of ionic liquids - a critical review, Chem. Soc. Rev. 44 (22) (2015) 8200–8237, https://doi.org/10.1039/C5CS00444F.
[23] D. Coleman, N. Gathergood, Biodegradation studies of ionic liquids, Chem. Soc. Rev. 39 (2) (2010) 600–637, https://doi.org/10.1039/B817717C.
[24] J. Ranke, S. Stolte, R. Störmann, J. Arning, B. Jastorff, Design of sustainable chemical products - the example of ionic liquids, Chem. Rev. 107 (6) (2007) 2183–2206.
[25] L. Ferrazzano, M. Catani, A. Cavazzini, G. Martelli, D. Corbisiero, P. Cantelmi, T. Fantoni, A. Mattellone, C. de Luca, S. Felletti, W. Cabri, A. Tolomelli, Sustainability in peptide chemistry: current synthesis and purification technologies and future challenges, Green Chem. 24 (3) (2022) 975–1020, https://doi.org/10.1039/D1GC04387K.
[26] S.I. Abu-Eishah, Ionic liquids recycling for reuse, in: S. Handy (Ed.), Ionic Liquids - Classes and Properties, InTech, 2011, https://doi.org/10.1016/j.colsurfa.2011.12.014.
[27] W. Wilms, M. Woźniak-Karczewska, A. Syguda, M. Niemczak, Ł. Ławniczak, J. Pernak, R.D. Rogers, Ł. Chrzanowski, Herbicidal ionic liquids: A promising future for old herbicides? Review on synthesis, toxicity, biodegradation, and efficacy studies, J. Agric. Food Chem. 68 (39) (2020) 10456–10488.
[28] M.J. Earle, K.R. Seddon, Ionic liquids: green solvents for the future, ACS Symp. Ser. 819 (7) (2002) 10–25, https://doi.org/10.1021/bk-2002-0819.ch002.
[29] X. Xu, F. He, W. Yang, J. Yao, Effect of homochirality of dipeptide to polymers' degradation, Polymers (Basel) 12 (9) (2020) 2164, https://doi.org/10.3390/polym12092164.
[30] L. Morandeira, A. Martínez-Baltasar, M.Á. Sanromán, A. Rodríguez, F.J. Deive, Designing novel biocompatible oligopeptide-based ionic liquids for greener downstream processes, J. Clean. Prod. 279 (2021), https://doi.org/10.1016/j.jclepro.2020.123356.
[31] D. Skarpalezos, A. Tzani, E. Avraam, C. Politidis, A. Kyritsis, A. Detsi, Synthesis, structure-properties relationship and biodegradability assessment of novel protic ionic liquids, J. Mol. Liq. 344 (2021), https://doi.org/10.1016/j.molliq.2021.117754.
[32] S.J. Pandya, I.V. Kapitanov, Z. Usmani, R. Sahu, D. Sinha, N. Gathergood, K.K. Ghosh, Y. Karpichev, An example of green surfactant systems based on inherently biodegradable IL-derived amphiphilic oximes, J. Mol. Liq. 305 (2020) 112857.
[33] A. Haiß, A. Jordan, J. Westphal, E. Logunova, N. Gathergood, K. Kümmerer, On the way to greener ionic liquids: identification of a fully mineralizable phenylalanine-based ionic liquid, Green Chem. 16 (2016) 4361–4373, https://doi.org/10.1039/C6GC00417B.
[34] A. Jordan, A. Haiß, M. Špulak, Y. Karpichev, K. Kümmerer, N. Gathergood, Synthesis of a series of amino acid derived ionic liquids and tertiary amines: green chemistry metrics including microbial toxicity and preliminary biodegradation data analysis, Green Chem. 16 (2016) 4374–4392, https://doi.org/10.1039/C6GC00415F.
[35] M. Suk, A. Haiß, J. Westphal, A. Jordan, A. Kellett, I.V. Kapitanov, Y. Karpichev, N. Gathergood, K. Kümmerer, Design rules for environmental biodegradability of phenylalanine alkyl ester linked ionic liquids, Green Chem. 22 (14) (2020) 4498–4508.
[36] D. Coleman, M. Špulak, M.T. Garcia, N. Gathergood, Antimicrobial toxicity studies of ionic liquids leading to a "hit" MRSA selective antibacterial imidazolium salt, Green Chem. 14 (5) (2012) 1350–1356, https://doi.org/10.1039/C2GC16090K.
[37] I.F. Mena, E. Diaz, J. Palomar, J.J. Rodriguez, A.F. Mohedano, Cation and anion effect on the biodegradability and toxicity of imidazolium- and choline-based ionic liquids, Chemosphere (2020) 240, https://doi.org/10.1016/j.chemosphere.2019.124947.
[38] N. Delgado-Mellado, M. Ayuso, M.M. Villar-Chavero, J. García, F. Rodríguez, Ecotoxicity evaluation towards vibrio fischeri of imidazolium- and pyridinium-based ionic liquids for their use in separation processes, SN Appl. Sci. (2019) 896, https://doi.org/10.1007/s42452-019-0916-3.
[39] I.V. Kapitanov, A. Jordan, Y. Karpichev, M. Špulak, L. Perez, A. Kellett, K. Kümmerer, N. Gathergood, Synthesis, self-assembly, bacterial and fungal toxicity, and preliminary biodegradation studies of a series of L-

phenylalanine-derived surface-active ionic liquids, Green Chem. 21 (7) (2019) 1777–1794, https://doi.org/10.1039/C9GC00030E.
[40] D.K.A. Kusumahastuti, M. Sihtmäe, I.V. Kapitanov, Y. Karpichev, N. Gathergood, A. Kahru, Toxicity profiling of 24 L-phenylalanine derived ionic liquids based on pyridinium, imidazolium and cholinium cations and varying alkyl chains using rapid screening vibrio Fischeri assay, Ecotoxicol. Environ. Saf. 172 (2019) 556–565, https://doi.org/10.1016/j.ecoenv.2018.12.076.
[41] M. Trush, L. Metelytsia, I. Semenyuta, L. Kalashnikova, O. Papeykin, I. Venger, O. Tarasyuk, L. Bodachivska, V. Lagodatnyi, S. Rogalsky, Reduced ecotoxicity and improved biodegradability of cationic biocides based on ester-functionalized pyridinium ionic liquids, Environ. Sci. Pollut. Res. 26 (2019) 4878–4889, https://doi.org/10.1007/s11356-018-3924-8.
[42] K.M. Docherty, C.F. Kulpa, Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids, Green Chem. 7 (4) (2005) 185–189, https://doi.org/10.1039/b419172b.
[43] D.K.A. Kusumahastuti, M. Sihtmäe, V. Aruoja, N. Gathergood, A. Kahru, Ecotoxicity profiling of a library of 24 L-phenylalanine derived surface-active ionic liquids (SAILs), Sustain. Chem. Pharm. 19 (2021), https://doi.org/10.1016/j.scp.2020.100369.
[44] G. Kaur, L.A. Abramovich, E. Gazit, S. Verma, Ultrastructure of metallopeptide-based soft spherical morphologies, RSC Adv. 4 (110) (2014) 64457–64465, https://doi.org/10.1039/C4RA10532J.
[45] E. Schröder, K. Lübke, The Peptides Volume 1: Methods of Peptide Synthesis, Academic Press, New York and London, 1965.
[46] C.R. McElroy, A. Constantinou, L.C. Jones, L. Summerton, J.H. Clark, Towards a holistic approach to metrics for the 21st century pharmaceutical industry, Green Chem. 17 (5) (2015) 3111–3121, https://doi.org/10.1039/C5GC00340G.
[47] ESCMID. (ESCMID) European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases. Eucast Discussion Document E. Dis 5.1. Determination of Minimum Inhibitory Concentrations (MICs) of An. Clinical Microbiology and Infection, 2003.
[48] EUCAST. Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Conidia Forming Moulds - EUCAST Definitive Document EDEF 9.3.1.; 2015.
[49] EUCAST. Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Yeasts - EUCAST Definitive Document EDEF 7.3.1; 2017.
[50] J. Friedrich, A. Längin, K. Kümmerer, Comparison of an electrochemical and luminescence-based oxygen measuring system for use in the biodegradability testing according to closed bottle Test (OECD 301D), Clean (Weinh) 41 (3) (2013) 251–257, https://doi.org/10.1002/clen.201100558.
[51] OECD. OECD Guideline for Testing of Chemicals - Ready Biodegradability. 1992.
[52] X.-D. Hou, Q.-P. Liu, T.J. Smith, N. Li, M.-H. Zong, V. Bansal, Evaluation of toxicity and biodegradability of cholinium amino acids ionic liquids, PLoS One 8 (3) (2013) e59145.
[53] A. Yazdani, M. Sivapragasam, J.M. Leveque, M. Moniruzzaman, Microbial biocompatibility and biodegradability of choline-amino acid based ionic liquids, J. Microb. Biochem. Technol. 08 (05) (2016), https://doi.org/10.4172/1948-5948.1000318.
[54] J. Neumann, S. Steudte, C.-W. Cho, J. Thöming, S. Stolte, Biodegradability of 27 pyrrolidinium, morpholinium, piperidinium, imidazolium and pyridinium ionic liquid cations under aerobic conditions, Green Chem. 4 (2014) 2174–2184, https://doi.org/10.1039/C3GC41997E.
[55] K.M. Docherty, J.K. Dixon, C.F. Kulpa Jr, Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community, Biodegradation 18 (2007) 481–493, https://doi.org/10.1007/s10532-006-9081-7.
[56] Y. Deng, I. Beadham, M. Ghavre, M.F. Gomes, N. Gathergood, P. Husson, B. Légeret, B. Quilty, M. Sancelme, P. Besse-Hoggan, When can ionic liquids be considered readily biodegradable? Biodegradation pathways of pyridinium, pyrrolidinium and ammonium-based ionic liqudis, Green Chem. 3 (2015) 1479–1491, https://doi.org/10.1039/C4GC01904K.
[57] K.M. Docherty, M.V. Joyce, K.J. Kulacki, C.F. Kulpa, Microbial biodegradation and metabolite toxicity of three pyridinium-based cation ionic liquids, Green Chem. 12 (4) (2010) 701.
[58] T.P.T. Pham, C.-W. Cho, C.-O. Jeon, Y.-J. Chung, M.-W. Lee, Y.-S. Yun, Identification of metabolites involved in the biodegradation of the ionic liquid 1-butyl-3-methypyridinium bromide by activated sludge microorganisms, Environ. Sci. Tech. (2009) 516–521, https://doi.org/10.1021/es703004h.
[59] C. Zhang, S.V. Malhotra, A.J. Francis, Toxicity of imidazolium- and pyridinium-based ionic liquids and the co-metabolic degradation of N-ethylpyridinium tetrafluoroborate, Chemosphere 82 (11) (2011) 1690–1695.