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8 ABSTRACT. In this manuscript we present the synthesis and characterization of herbicidal v Article Online DOI: 10.1039/C7NJ01474K (HILs) based on betaine derivatives (N-dodecylbetaine 9 ionic liquids 10 cocoamidopropyl)betaine) playing role of cation and four firmly established synthetic auxin 11 herbicides (2,4-D, MCPA, MCPP or dicamba) applied as anions. The syntheses were conducted 12 in two steps: protonation of alkylbetaines with hydrochloric acid followed by the ion exchange 13 reaction between formed intermediates and potassium salts of herbicides. All the synthesized 14 salts were classified as ionic liquids (ILs) owing to their melting points below 100 °C. The products exhibited high thermal stability with decomposition temperatures (Tonset) varied from 15 16 240 to 273 °C. The herbicidal efficacy experiments performed under greenhouse and field 17 conditions confirmed their biological activity. The enhancement of their herbicidal activity 18 results from the potent surface-active properties generated by alkylbetaine cations. The spray 19 solutions of investigated HILs display low contact angle (approx. 50-62°) and surface tension 20 (approx. 30-33 mN·m⁻¹) contributing to the increase of contact surface between leaves and 21 active ingredient and thereby the increase of absorption of herbicide into the plant. The highest 22 susceptibility to biodegradation (approx. 60-75% efficiency) was proven for salts containing 23 phenoxyacetates (2,4-D and MCPA) anions.

KEYWORDS. Herbicidal ionic liquids, 2,4-D, MCPA, MCPP, dicamba, weed control,
 dodecylbetaine, cocoamidopropylbetaine.

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26 INTRODUCTION

One of the newest, promising possibilities of using ionic liquids (ILs) is to incorporate a herbicidal 27 28 anion into their structure. This action leads to the formation of a new group of compounds defined as herbicidal ionic liquids (HILs).^{1,2} Due to their activity against unwanted vegetation, they may 29 30 be applied as anti-weed agents in crop protection causing the improvement of both quality and quantity of crops.³ HILs belong to the third generation of ILs; they simultaneously exhibit targeted 31 biological activity and selected physical and chemical properties.⁴ The anions used for synthesis 32 of HILs are popular and commercially available herbicides such as phenoxyacids derivatives: 33 2,4-D,^{2,3} MCPA,^{1,4-6} MCPP^{1,4,6} and MCPB⁷. Other utilized herbicides include derivatives of 34 benzoic acid, e.g. dicamba,⁸ nicotinic acid, e.g. clopyralid,⁹ sulfonylureas, e.g. metsulfuron-methyl 35 (MSM),¹⁰ bentazone,¹¹ fomesafen¹² or one of the most popular non-selective herbicides – 36 glyphosate¹³. The greatest advantage of HILs in comparison to commercial herbicidal formulations 37 is their low volatility.⁸ Hence, the negative influence of toxic vapors is reduced due to the ionic 38 39 form of the pesticide which does not spread to the neighboring fields minimizing the risk of 40 environmental pollution. HILs also possess unique physicochemical properties, including high thermal stability and reduced water solubility which minimizes the risk of unintended drift.^{6-8,13,14} 41 42 The first reports on HILs focused on economically reasoned cations such as cationic surfactants, 43 quaternary ammonium or phosphonium halides. Their high surface activity resulted in enhanced 44 herbicidal efficiency and allowed to reduce significantly the required dose of active ingredient per 45 hectare. This strategy eliminates the necessity of using additional adjuvants often recommended for commercial crop protection agents, which are often characterized by an unspecified toxicity 46 and negative environmental impact.¹⁻³ Recently, the second generation of herbicidal ionic liquids 47 48 has been described and characterized. The firmly established herbicidal anions was successfully

49 combined with a 2-chloroethyltrimethylammonium cation likewise exhibiting biological activity 50 as a plant growth regulator^{15,16} or fungicide, such as tebuconazole or propiconazole¹⁷. Moreover, 51 the chemical structure of the cation may be modified to regulate the toxicity and biodegradability of HILs.^{1,14,18-20} Therefore, the reports describing HILs derived from renewable sources such as 52 betaine and carnitine²¹ or D-glucose²² started to appear. Recently, ionic liquids have been proved 53 54 to be effective plant resistance inducers; they are able to stimulate the resistance system of the 55 plant to act against pathogenic microorganisms, especially viruses - due to the fact there are no plant protection agents acting directly on them.^{23,24} 56

N-Dodecylbetaine and N-(3-cocoamidopropyl)betaine are two well-known representatives of
amphoteric surfactants, which structures are similar to amino acid in proteins.^{25,26} As zwitterionics,
they exist in aqueous solution in the form of inner salt possessing both the carboxylate anion as
well as a quaternary ammonium cation.²⁷ (Fig. 1).



Figure 1. Structures of N-dodecylbetaine (A) and N-(3-cocoamidopropyl)betaine (B), where *coco*is a mixture of alkyl substituents C₈-C₁₈.

Alkylbetaines are commonly used as foam boosters in shampoos, shower gels, liquid soaps, hand cleaners or as antistatic agents mostly due to their very low irritation levels and excellent capacity to promote mildness in cosmetic formulations.²⁷⁻³⁰ Comparing to cationic surfactants, alkylbetaines also have the advantages of good water-solubility, high biocompatibility and biodegradability as well as fine stability in a wide pH range.^{25,26} The unique, multifunctional properties allowed to successfully apply them as solubilizers of phosphatidylcholine liposomes,³⁰ components enhancing the resonance light scattering (RLS) signals in the quantitative analysis of

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nucleic acids,²⁵ additives to anionic surfactants promoting the surface elasticity^{31,32} or adjuvants

facilitating the mixing, application or effectiveness of agrochemicals (e.g., phenoxyacids,

74 The idea of incorporating alkylbetaines into HILs structure appeared as the consequence of 75 substantial benefits obtained from utilizing the cationic surface active compounds as the source of the cation.^{1-3,8,10-13,36} Considering several reports describing excellent biodegradability of 76 amphoteric surfactants,^{37,38} we expected that alkylbetaine-based HILs will preserve this feature. 77 78 Here we present a convenient synthesis methodology of biologically active ILs consisting of cation 79 derived from cost-effective and easily available commercial amphoteric surfactants. This study 80 involves also an evaluation of the influence of the structure of both cation and anion on the HILs 81 physicochemical properties (*i.e.*, solubility, thermal stability, surface activity), their herbicidal 82 efficiency (determined in greenhouse and field experiments) as well as susceptibility to

glyphosate)32-35.

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85 MATERIALS AND METHODS

biodegradation.

86 Materials

N-Dodecylbetaine ([$C_{12}Bet$], CAS Registry No. 66455-29-6, 35% aqueous solution, EMPIGEN® BB detergent) and N-(3-cocoamidopropyl)betaine ([CAPBet], CAS Registry No. 61789-40-0, 30% aqueous solution, Dehyton[®] PK 45) were purchased from Sigma Aldrich and BASF, respectively. After dehydration of both solutions, betaines were dissolved in methanol. Then, inorganic contaminants were separated *via* vacuum filtration and the filtrate was thoroughly evaporated. Purified betaines were dried in vacuum for 24 h. Hydrochloric acid, potassium hydroxide and solvents (methanol, DMSO, acetonitrile, acetone, isopropanol, ethyl acetate, chloroform, toluene, hexane), were purchased from Sigma Aldrich and used without further
purification. All herbicidal acids (2,4-D, MCPA, MCPP and dicamba) were supplied by OrganikaSarzyna S.A. Poland and PESTINOVA Poland.

97

98 Preparation of HILs

99 Protonation of N-dodecylbetainium chloride [C₁₂Bet][Cl]

N-dodecylbetaine (0.20 mol) and 200 cm³ of acetone were placed in a 500 cm³ Erlenmeyer flask equipped with a mechanical stirrer and the obtained mixture was vigorously stirred. Then, stoichiometric amount (0.20 mol) of hydrochloric acid (36%) was added and the reaction was conducted at 60 °C for 1 h. As a result of betaine protonation , a white solid of N-dodecylbetainium chloride precipitated, which was filtered off and washed with small amounts of acetone. Afterwards, the product was dried under reduced pressure at 50 °C for 24 h. The melting point of N-dodecylbetainium chloride was in the range 158 – 160 °C.

107 Protonation of N-(3-cocoamidopropyl)betainium chloride [CAPBet][Cl]

N-(3-cocoamidopropyl)betaine (0.20 mol) and 200 cm³ of acetone were placed in a 500 cm³ Erlenmeyer flask equipped with a mechanical stirrer and the obtained mixture was vigorously stirred. Then, stoichiometric amount (0.20 mol) hydrochloric acid (36%) was added and the reaction was conducted at 60 °C for 3 h. Then, acetone and water were removed using a rotary evaporator. In order to evaporate traces of water and hydrochloric acid the product was dried under reduced pressure at 60 °C for 120 h. The obtained N-(3-cocoamidopropyl)betainium chloride was a yellowish grease at room temperature.

115 Anion exchange reactions

116 All reactions were conducted in methanol using an EasyMaxTM reactor. The appropriate amount 117 (0.05 mol) of N-dodecylbetainium chloride or N-(3-cocoamidopropyl)betainium chloride was dissolved in 25 cm³ of methanol. Afterwards, 25 cm³ of the methanolic suspension containing the 118 119 potassium salt of 2,4-D (0.05 mol) or the methanolic solution of MCPA, MCPP or dicamba 120 potassium salts (0.05 mol) were slowly added. The reaction was conducted under constant stirring 121 at 60 °C for 3 h. As a result of the anion exchange reaction, the by-product (potassium chloride) 122 precipitated from the reaction solution. After cooling the post-reaction mixture to 0 °C, the 123 inorganic salt was separated by filtration and methanol was evaporated from the filtrate. Then, the raw product was dried and dissolved in 50 cm³ of anhydrous acetone. The obtained precipitate was 124 125 separated via vacuum filtration and the solvent was evaporated. Finally, the product was dried 126 under reduced pressure at 50 °C for 24 h.

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129 General

130 ¹H NMR spectra were recorded on Mercury Gemini 300 and Varian VNMR-S 400 MHz spectrometers operating at 300 MHz and 400 MHz with TMS as the internal standard. ¹³C NMR 131 132 spectra were obtained with the same instruments at 75 and 100 MHz, respectively. Elemental 133 analyses (CHN) were performed at the Adam Mickiewicz University, Poznan (Poland). NMR 134 spectra descriptions as well as elemental analysis results are provided in the Supporting 135 Information (Table S1 and Fig. S1-S20). The water content was determined using an Aquastar 136 volumetric Karl Fischer titration with Composite 5 solution as the titrant and anhydrous methanol 137 as a solvent. The water content in synthesized products was determined by Karl-Fischer 138 measurements and was found to be between 1000 - 1500 ppm.

140 *Density measurements*

Density was determined using an automatic Density Meter DDM2911 with a mechanical oscillator method. The density of the samples (approx. 2.0 cm³) was measured with respect to temperature controlled conditions *via* Peltier, from 20 to 80 °C. The used apparatus was calibrated using deionized water as the reference substance.

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146 Viscosity measurements

147 Viscosity was determined using a rheometer (Rheotec RC30-CPS) with cone-shaped geometry 148 (C50-2). The viscosity of the samples (approx. 1.5 cm^3) was measured with respect to temperature, 149 from 20 to 80 °C. The uncertainty of the viscosity measurement was estimated to be less than 10^{-1} 150 ⁴ Pa·s.

151

152 *Refractive index measurements*

Refractive index was determined using Automatic Refractometer J357 with electronic temperature control in range from 20 to 80 °C. The accuracy of the temperature stabilization was 0.02 °C and uncertainty of the refractive index measurement was less than 0.00005.

156

157 Thermal analysis

Differential scanning calorimetry (DSC) and thermogravimetric (TG) analyses were performed on
 Mettler Toledo Star^e TGA/DSC1 unit (Leicester, UK) under nitrogen at a heating/cooling rate of
 10 °C·min⁻¹. During DSC analyses samples between 5 and 15 mg were placed in aluminum pans

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and were heated from 25 to 160 °C and cooled to -100 °C. During TG experiments samples
between 2 and 10 mg were placed in aluminum pans and were heated from 30 to 500 °C.

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164 Solubilities

165 The solubilities of the obtained products in ten representative solvents were determined according to the protocols in Vogel's Textbook of Practical Organic Chemistry.³⁹ The solvents chosen for 166 167 study were selected in order of decreasing polarity expressed as the Snyder polarity index: water, 168 9.0; methanol, 6.6; DMSO, 6.5; acetonitrile, 6.2; acetone, 5.1; isopropanol, 4.3; ethyl acetate, 4.3; 169 chloroform, 4.1; toluene, 2.3; hexane, 0.0. A 0.1 g sample of each salt was added to a certain 170 volume of solvent and the samples were thermostated in water bath MEMMERT model WNB 7 171 at 25 °C. On the basis of the volume of solvent used, three types of behaviors were recorded: 'soluble' applies to compounds which dissolved in 1.0 cm^3 of solvent, 'limited solubility' – applies 172 to compounds that dissolved in 3.0 cm^3 of solvent, and 'not soluble' – applies to the compounds 173 which did not dissolve in 3.0 cm³ of solvent. 174

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176 Greenhouse Experiments

177 Common lambsquarters (*Chenopodium album* L.), cornflower (*Centaurea cyanus* L.) and rapeseed 178 (*Brassica napus* L.) were used as test plants. The plants were grown in 0.5 dm³ plastic pots 179 containing commercial peat-based potting material under controlled environmental conditions: 180 temperature of $20(\pm 2)$ °C, humidity of 60%, and a photoperiod of 16/8 h day/night. After 181 emergence, the plants were thinned to 5 plants in each pot. Treatments were applied at the 4–6 182 leaves growth stage. Commercial products: Aminopielik Standard 600 SC (600 g dimethylamine 183 salt of 2,4-D in 1 dm³), Chwastox Extra 300 SL (300 g of sodium and potassium salts of MCPA

184 in 1 dm³) and Dikamba 700 SC (700 g sodium salt of dicamba in 1 kg) were used as reference 185 compounds. The synthesized salts were applied at a dose corresponding to 400 g of active 186 ingredient per 1 hectare for compounds with 2,4-D, MCPA, and MCPP or 200 g of active 187 ingredient per 1 hectare for compounds with dicamba. Reference herbicides were used at the same 188 doses. All tested salts were dissolved in a mixture of water and ethanol (1:1 v/v), whereas reference 189 compounds were dissolved in water. The applications were conducted using a moving sprayer 190 (APORO, Poznan, Poland) with a TeeJet[®] VP 110/02 (TeeJet Technologies, Wheaton, IL, USA) flat-fan nozzle capable of delivering 200 dm³ \cdot ha⁻¹ of spray solution at 0.2 MPa operating pressure. 191 192 The sprayer was moved above the plants (40 cm distance) at a constant speed of $3.1 \text{ m} \cdot \text{s}^{-1}$. After 193 treatment, the plants were again placed in a greenhouse under the environmental conditions 194 mentioned above. A weed control was evaluated visually two weeks after treatment (2 WAT) using 195 a scale of 0 (no effect) to 100% (complete weed destruction). The study was carried out in four 196 independent experiments in a randomized setup. Tukey's test was used for the comparison of 197 averages and lowest significant difference (LSD) was determined at the level of 5%. All 198 calculations were performed using Agriculture Research Manager (ARM) software. To compare 199 the studied treatments, a multiple Tukey's post hoc test (α =0.05) was applied.

201 Field Experiments

The field trials were carried out in spring barley in 2014 and 2015 at the Experimental Station of Institute of Plant Protection in Winna Gora (E: $17^{\circ}26'$, N: $52^{\circ}12'$). Cereals were cultivated according to the local agricultural practice. The plot size was equal to 16.5 m^2 . The experimental design was based on a randomized block with four replications. The plots were infested by cornflower (*Centaurea cyanus* L.) in 2014 and oilseed rape volunteers (*Brasica napus* L.) as well as common lambsquarters (*Chenopodium album* L.) in 2015. The treatments were applied at the

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stage of the end of crop tillering (BBCH 29) using a knapsack sprayer (APORO, Poznan, Poland) 208 209 equipped with TeeJet® AXR 110/03 flat-fan nozzles (TeeJet Technologies, Wheaton, IL, USA) delivering 200 dm³·ha⁻¹ of spray solution at 0.3 MPa of operating pressure. A weed control was 210 211 evaluated visually five weeks after a herbicide application (5 WAT) using a scale of 0 (no effect) 212 to 100% (complete weed destruction). Commercial products: Aminopielik Standard 600 SC (600 213 g dimethylamine salt of 2.4-D in 1 dm³), Chwastox Extra 300 SL (300 g of sodium and potassium 214 salts of MCPA in 1 dm³) and Dikamba 700 SC (700 g sodium salt of dicamba in 1 kg) were used 215 as reference compounds. Each error margin range represents standard errors of the mean (SEM). 216 The SEM values were calculated according to equation 1:

217 Equation 1. Calculation of SEM values.

$$SEM = \frac{S}{n^{0.5}}$$

where SEM – standard error of the mean, s – sample standard deviation, n – number of samples. 220

221 Surface Activity

222 Surface tension and contact angle measurements were carried out using a DSA 100 analyzer (Krüss, Germany, accuracy $\pm 0.01 \text{ mN} \cdot \text{m}^{-1}$), at 25 °C. The surface tension was determined using 223 224 the shape drop method. Basically, the principle of this method is to form an axisymmetric drop at 225 the tip of a needle of a syringe. The image of the drop (3 cm^3) is taken with a CCD camera and digitized. The surface tension (γ in mN·m⁻¹) was calculated by analyzing the profile of the drop 226 227 according to the Laplace equation. Temperature was controlled using a Fisherbrand FBH604 228 thermostatic bath (Fisher, Germany, accuracy +0.1 °C). The determination of the contact angle 229 was based on the sessile drop method, *i.e.* drops of liquid are deposited on a solid surface (paraffin).

230



Wistar rats (symbol lmp: WIST, outbred) used in the studies originated from a culture in the 232 233 Medical Institute of Work in Lodz/Poland and were kept in cages of the conventional type. Before 234 the study, the animals were quarantined for a minimum 5 days and observed daily during this 235 period. The animals were marked individually. During guarantine and the experiments, the animals 236 were kept in a conditioned room of the following parameters: temperature 21-22 °C, relative air 237 humidity 40-75%, and artificial illumination 12 h light/12 h darkness. Rats were kept in cages with 238 plastic bottom and wired superstructure, with the dimensions of 58 x 37 x 21 cm (length x width 239 x height). The animals were kept in cages individually (in the observation study the rats received a dosage of 2000 or 300 mg \cdot kg⁻¹ b.w.) or 4 rats per cage (in the main study the dose of 300 mg \cdot kg⁻¹ 240 241 ¹ b.w.). UV-sterilized wooden shavings were used as litter. Each cage was equipped with a label 242 containing the following information: name of test material, study code, used dose, start date and 243 planned ending date of the experiment, sex, and animal numbers. The rats were given standard 244 granulated GLM fodder and tap water ad libitum.

245

246 Ready Biodegradability According To The OECD 301 F Test – Manometric Respirometry

247 The test was performed according to OECD guideline for 301 F test. The biological oxygen 248 demand (BOD) was determined every 24 h for 28 days using the OxiTop system (WTWGmbH 249 Weilheim Germany) in a thermostated incubator (IKA Germany) covered with aluminium foil. 250 The activated sludge was collected from a local municipal wastewater treatment plant 251 (Kozieglowy, Poznan, Poland). Prior to use, the activated sludge was aerated for 7 days in mineral 252 medium which was also used for subsequent tests. The mineral medium consisted of KH₂PO₄ - 85 $mg \cdot dm^{-3}$, $K_2HPO_4 - 220 mg \cdot dm^{-3}$, $Na_2HPO_4 \cdot 2H_2O - 220 mg \cdot dm^{-3}$, $NH_4Cl - 17 mg \cdot dm^{-3}$, 253 $CaCl_2 \cdot 2H_2O - 37 \text{ mg} \cdot dm^{-3}$, $MgSO_4 \cdot 2H_2O - 23 \text{ mg} \cdot dm^{-3}$, $FeCl_3 - 0.25 \text{ mg} \cdot dm^{-3}$. The measured pH 254 255 was at 7.2. The test was performed in brown glass bottles containing mineral medium, inoculum

- Hach, USA) and tested ILs at a concentration of approx. 10-30 mg·dm⁻³, which was equal to 100
- 258 mg·dm⁻³ of Theoretical Oxygen Demand (ThOD, calculated based on equation 1).
- 259 Equation 1. Theoretical Oxygen Demand for a chemical formula given as C_cH_hN_nO_oP_p:

ThOD =
$$\frac{16[2c + \frac{1}{2}(h - 3n) + \frac{5}{2}p - o]\frac{mg}{mg}}{\text{molecular mass of the test substance }\frac{mmol}{mg}}$$

Allylthiourea (1.16 mg \cdot dm⁻³) was added to inhibit nitrification. All samples were analyzed in triplicates together with controls (sodium benzoate without inoculum, tested substances without inoculum) and blanks (medium and inoculum without tested substances). Gas tight flasks were equipped with a CO₂ trap (solid NaOH) and incubated in the dark at 20 °C for 28 days. The biodegradation efficiency was calculated based on the oxygen uptake in each bottle (measured automatically by the electronic OxiTop head) and corrected for the oxygen demand of the blank, with the respect to the Theoretical Oxygen Demand and the amount of ILs tested.

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269 RESULTS AND DISCUSSION

270 Synthesized ILs

The substrates used for the syntheses were commercially available products containing common 271 272 amphoteric surfactants: N-dodecylbetaine $[C_{12}Bet]$ (CAS: 66455-29-6) and 273 N-(3-cocoamidopropyl)betaine [CAPBet] (CAS: 61789-40-0), where coco refers to the mixture of 274 saturated linear alkyl substituents C₈-5%, C₁₀-6%, C₁₂-50%, C₁₄-19%, C₁₆-14%, C₁₈-10%. In 275 the first stage, both dehydrated substances have been protonated with hydrochloric acid. As a result 276 of the protonation reaction N-dodecylbetainium chloride - [C₁₂Bet][Cl] and N-(3-277 cocoamidopropyl)betainium chloride - [CAPBet][Cl] were obtained and then separated from the

278 reaction mixture, in accordance with scheme 1. Reaction yields for both precursors were 90 and

279 92%, respectively.

1 step - alkylbetaine protonation



2 step - anion exchange reaction



Scheme 1. Synthesis of alkylbetainium HILs *via* protonation followed by an anion exchange
reaction, where R is dodecyl or 3-cocoamidopropyl and A is 2,4-D, MCPA, MCPP or dicamba.

In the next step, the chloride anion was exchanged for herbicidally active anions (2,4dichlorophenoxyacetate - 2,4-D, 4-chloro-2-methylphenoxyacetate – MCPA, 2-(4-chloro-2methylphenoxy)propionate – MCPP and 3,6-dichloro-2-methoxybenzoate - dicamba). Structures of used herbicides are presented in Fig. 2. All salts were obtained in high yields exceeding 90% (Table 1).



Figure 2. Structures of used herbicidal acids.

291

Salt	R	Anion	Abbreviation	Yield	State	Melting point ^b
					at 25°C	(°C)
1	$C_{12}H_{25}$	2,4-D	[C ₁₂ Bet][2,4-D]	93	Solid	64-66
2	$C_{12}H_{25}$	MCPA	[C ₁₂ Bet][MCPA]	94	Solid	37-40
3	$C_{12}H_{25}$	MCPP	[C ₁₂ Bet][MCPP]	96	Liquid	
4	$C_{12}H_{25}$	dicamba	[C ₁₂ Bet][Dicamba]	95	Solid	28-30
5	CAP ^a	2,4-D	[CAPBet][2,4-D]	92	Wax	
6	CAP	MCPA	[CAPBet][MCPA]	91	Liquid	
7	CAP	MCPP	[CAPBet][MCPP]	93	Liquid	
8	CAP	dicamba	[CAPBet][Dicamba]	92	Liquid	
^a 3-cocoamidopropyl; ^b METTLER TOLEDO MP 90 melting point system						

290 **Table 1**. Synthesized alkylbetainium salts **1-8**.

One of the obtained compounds (5) was a wax and four (3, 6-8) were liquids at room temperature. 292 293 Any of the synthesized salts did not undergo phase transfer to the solid state at 100 °C, therefore 294 they could be classified as ionic liquids (ILs). Salts with N-dodecylbetainium cation and anions 295 2.4-D (1), MCPA (2) and dicamba (4) were solids at room temperature with melting points at 296 approx. 65, 39 and 29 °C, respectively. Moreover, owing to their low melting points 3 and 5-8, are 297 classified as room temperature ionic liquids (RTILs). Therefore, the 3-cocoamidopropyl 298 substituent was more suitable for synthesis of RTILs compared to dodecyl chain. This 299 phenomenon is mainly associated with the presence of an amide group affecting the geometric 300 arrangement of the whole molecule and resulting in a decrease of its lattice energy. Furthermore, 301 we discovered that alkyl chain elongation in betaine type cation may result in a decrease of HILs 302 melting point, which is also consistent with data collected for betaine (N.N.N-trimethylglycine)based HILs recently described by our research group.²¹ This characteristic behaviour of ILs has 303 been described repeatedly in the literature^{40,41} and explains the differences between betainium and 304 305 N-dodecylbetainium cations. Further reduction of the melting point between 1-4 and 5-8 is due to

308 On the basis of ¹H NMR spectra it was established that the structure of the anion generally had a 309 marginal influence on the chemical shift values originating from protons in the cation (Table S2, 310 Supporting Information). However, the main differences between precursors and synthesized ILs have been noted for protons from the methylene group (N-CH₂-COOH), as both chlorides were 311 312 characterized by approx. 0.4-0.7 ppm higher values compared to 1-8. The observed shifts on NMR 313 spectra may become an evidence of efficient anion exchange as well as the absence of trace substrates in the obtained ILs. A similar analysis was conducted for ¹³C NMR spectra (Table S3). 314 315 We found that the structure of the anion has a noticeable influence on the values of chemical shifts 316 only between precursors and the appropriate ILs, which were obtained from them. The signals 317 from the methylene group (N-CH₂-COOH) for 1-8 occurred at approx. 63 ppm, whereas the same 318 signal from precursors appeared at lower values of approx. 61 ppm.

319

320 Physicochemical properties

Physicochemical properties, such as viscosity, density and the refractive index, were determined for the ILs in the range from 20 to 80 °C (Figs. 3-5; collected physicochemical data and calculated linear regression coefficients were presented in the Supporting Information in Tables S4-S8). The greasy state of N-(3-cocoamidopropyl)betainium 2,4-dichlorophenoxyacetate (**5**) did not allow to determine its viscosity and density. Moreover, measurements for ILs **1**, **2** and **4** were performed at temperatures above their melting points.

According to the obtained viscosity results, it was found that all analysed ILs are Newtonian fluids
and their sheer stress values increases linearly with the increase of shear rate – the correlation

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329 coefficients (\mathbb{R}^2) exceeded 0.99 (see Fig. S21, ESI). The influence of temperature on the viscosity 330 values for [$C_{12}Bet$][2,4-D] (1), [$C_{12}Bet$][MCPA] (2), [$C_{12}Bet$][MCPP] (3), [$C_{12}Bet$][Dicamba] (4),

331 [CAPBet][MCPA] (6), [CAPBet][MCPP] (7) and [CAPBet][Dicamba] (8) is presented on Fig. 3.



Figure 3. Influence of temperature on the viscosity values for ILs (1-4, 6-8).

334 At 20 °C values of viscosity for the synthesized RTILs differed from 64.7 Pass for 3 to 3461.8 Pass 335 for 8 (Table S4, ESI). However, the measured values for both RTILs comprising MCPP anion (3 336 and 7) were significantly lower compared to RTILs with MCPA (5) as well as dicamba anion (8). 337 We also noted that the viscosity of the obtained RTILs was notably higher compared to the previously described HILs.^{5,7,14} vet, they were similar to results obtained for betaine and carnitine-338 based HILs.²¹ We explained this phenomenon as the effect of the considerable amount of hydrogen 339 340 bonds between the ions caused by the presence of carboxyl group in the ILs cation. Moreover, the 341 collected values for betaine 2-(4-chloro-2-methylphenoxy)propionate amounted to approx. 60 and 10 Pa·s at temperature values of 20 and 30 °C,²¹ accordingly, and were lower in comparison to **3** 342 343 comprising a long dodecyl substituent instead of a methyl group. Hence, we may assume that the 344 straight alkyl chain elongation in ILs with betaine type cation resulted in an increase of viscosity parameter, which complies with other reports about ILs.^{7,42} It is worth noting that ILs with the 345 346 dicamba anion (4 and 8) possessed relatively higher viscosities than ILs comprising phenoxyacids.

As for other viscous ILs, the viscosity of the obtained ILs decreased nonlinearly with increasing temperature. With regard to literature, further extension of the alkyl chain as well as the presence of the amide group (capable of forming subsequent intermolecular hydrogen bonds) should cause a substantial rise of viscosity. Except for ILs with dicamba (**4** and **8**), the obtained results are compatible with the foregoing assumption. Finally, on the basis of the obtained results, it is possible to classify all tested ILs as *high viscosity ionic liquids*.

The density of prepared RTILs **3** and **5-8** at 20 °C was higher compared to the density of pure water (Fig. 4). The obtained values varied from 1.068 g·cm⁻³ for **3** to 1.156 g·cm⁻³ for **8** (Table S5, ESI) and were similar to other described HILs.^{5,7,14}



Figure 4. Influence of temperature on the density values for ILs (1-4, 6-8).

This shows that the density of alkylbetaine ILs is decreasing with elongation of the alkyl chain from methyl to dodecyl or 3-cocoamidopropyl, which is consistent with the findings on ILs containing ammonium cation.⁴³ The density values at room temperature lead to the conclusion that the presence of MCPP anion in the IL results in lower density values in comparison to ILs containing MCPA or dicamba. A similar tendency was observed for ILs based on esterquats.¹⁴ As expected, the increase of temperature led to a linear decrease of density and the line describing this relationship was expressed as the equation $y = a \cdot x + b$ (Table S6, ESI), where *a* was equal to

378

approx. -0.0007 while *b* ranged from 1.08 g·cm⁻³ for HIL with MCPP anion (**3**) to 1.17 g·cm⁻³ for both ILs comprising dicamba (**4** and **8**). As an effect of increasing the temperature from 20 to 80 °C, the analysed parameter was reduced by approx. 0.04 g·cm⁻³ for all RTILs, similarly to our previous reports.^{7,21} The presented results confirm the previous observation that dicamba-based ammonium ILs possess higher density values in comparison to phenoxyacids.¹⁴

370 The refractive index, as a value characterizing the deviation of a light beam during propagation 371 from one medium to another, is an important property not only as the characteristic value used for 372 verification the identity or purity of each IL, but also as the measure of ILs electric polarizability.^{44,45} The measured indices of ILs often vary in the range from 1.40 to 2.20 depending 373 on the structure of both the cation as well as the anion.^{7,46} Refractive indices of the synthesized 374 375 RTILs (3, 5-8) at 20 °C ranged from 1.499 for 6 to 1.515 for 8 (Table S7, ESI). Collected data presented in Fig. 5 coincide with the previous reports, ^{5,7,14,21} in which the determined values for 376 377 different ammonium HILs varied from approx. 1.50 to 1.54.





Heating all samples caused, similarly as in case of density measurements, a linear and slight decrease of analysed parameter. The linear regression allowed to describe this relationship using the equation $y = a \cdot x + b$ (Table S8, ESI), where *a* varied from -0.00035 for **8** and **7** to -0.00030

for 5 and 7. The b parameter ranged from 1.5048 for 3 to 1.5219 for 4 and 8. Eventually, at 80 °C, 383 384 the refractive indices were lower by approx. 0.020 and reached values below 1.500 for all ILs. 385 In general, the refractive index of ILs increases with the increase of their density. The refractive 386 index of a substance is higher when its molecules are more tightly packed, *i.e.*, when the compound possesses higher density.⁴⁴ Taking into account this hypothesis, we observed that the refractive 387 388 indices presented in this work are partly correlated with the values of ILs density described above. 389 The highest densities for dicamba-based ILs (4, 8) correspond with their values of refractive index. 390 Therefore, IL 3 was characterized by the lowest values of refractive index as well as density. The 391 relationship 'density-refractive index' additionally explains why the alkyl substituent elongation 392 in ILs comprising ammonium as well as betaine type cations caused a decrease of their refractive 393 indices.

394 Thermal Properties of synthesized ILs

395 Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) data for the396 synthesized ILs are presented in Table 2.

397	Table 2. Thermal	properties of t	the obtained ILs.
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п	Tg ^a	Tcryst ^b	Tmc	Tonset5% ^d	Tonset ^e
117	(°C)	(°C)	(°C)	(°C)	(°C)
1	-14	70	64	228	250
2	-20		37	230	272
3	-17			219	260
4	-6	33	27	200	240
5	-6			171	273
6	-13			159	265
7	-11			185	263
8	-9			184	246

398 $\overline{{}^{a}T_{g}}$ - glass transition temperature; ${}^{b}T_{cryst}$ - temperature of crystallization; ${}^{c}T_{m}$ - melting point;

 $^{d}T_{onset5\%}$ – decomposition temperature of 5% sample; $^{e}T_{onset}$ – decomposition temperature

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Among all the prepared ILs, five (**3**, **5-8**) were liquids below 20 °C. For each of them neither melting nor crystallization were observed in the analysed temperature range. The lowest melting point was detected for **4** ($T_m = 27$ °C), which additionally possessed a crystallization event on cooling at 33 °C. Other ILs with N-dodecylbetainium cation (**1** and **2**) melted at higher temperatures amounting to 37 °C for **2** and 64 °C for **1**, respectively. None of the obtained compounds exceeded the threshold T_m value of 100 °C, therefore all may be classified as ILs. Moreover, the crystallization temperature (T_{cryst}) for **1** was equal to 70 °C, however, this parameter could not be determined for the obtained IL with the MCPA anion (**2**). The supercooling behaviour of **2**, for which a melting transition was observed on first heating but could not be reproduced within several heating/cooling cycles, was also noted for other HILs with betainium and carnitinium cations²¹ as well as HILs comprising cationic surfactants as cations^{1,2,8,10,13}.

The glass transition temperatures (Tg) determined for each of the obtained ILs ranged from -20 to 412 -6 °C. Among ILs derived from phenoxyacids (2,4-D, MCPA and MCPP), ILs with the N-(3-413 cocoamidopropyl)betainium cation (5-8) exhibited higher T_g values, ranging from -13 to -6 °C, 414 whereas in case of ILs with the N-dodecylbetainium cation (1-3) the values were slightly lower 415 (from -20 to -14 °C). Likewise, the structure of the anion influenced the glass transition 416 temperature values for both cations, which can be displayed by the following order: 2,4-D > 2417 MCPP> MCPA. However, due to the lack of the long alkyl substituent in HILs derived from 418 betaine, their T_g values were notably higher in comparison to HILs presented in this manuscript 419 and exceeded 0 °C. Thus, when analysing the influence of the cation structure on glass transition, 420 we may arrange them in the order of decreasing T_g values: betainium > N-(3-421 cocoamidopropyl)betainium > N-dodecylbetainium.

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422 Analysis of structure vs glass transition confirmed that alkyl chain elongation in the cation of ILs 423 results in lowering their glass transition temperatures. In literature this is mainly caused by 424 disruption of the crystal packing and reduction of the lattice energy due to the extension of substituent attached to the nitrogen atom.⁴⁷ However, it should be mentioned that the further 425 426 increase of alkyl chain length may enhance the attractive van der Waals interactions and then led to increased Tg values.⁴⁸ These findings explain why ILs with N-dodecylbetainium cation are 427 428 characterized N-(3by lower glass transition temperatures compared to 429 cocoamidopropyl)betainium-based ILs.

430 The thermogravimetric analysis results confirmed the high stability of the obtained ILs (Table 2). 431 The measured T_{onset5%} values were in a range from 159 for 6 to 230 °C for 2. The ILs comprising 432 the N-dodecylbetainium cation were characterized by a higher thermal stability, since their Tonset5% 433 values were higher by 14 to even 60 °C compared to ILs based on the N-(3-434 cocoamidopropyl)betainium cation. The reason for such behaviour is the presence of an amide 435 group in the cation which is more susceptible to thermal degradation in comparison to straight 436 alkyl chain. Among ILs with the N-dodecylbetainium cation (1-4), the phanoxyacetates (1 and 2) 437 exhibited slightly higher T_{onset5%} values compared to IL with MCPP (3).

Generally, the temperature values of 50% decomposition of the synthesized ILs ranged from 240 to 273 °C. It was observed that the presence of phenoxyacids caused an increase of thermal stability of 1-3 and 5-7 compared to ILs comprising the dicamba anion (4 and 8). This observation is consistent with the previous papers regarding the lower thermal stability of HILs with the dicamba anion compared to ILs comprising phenoxycarboxylates as anions.^{6,14,21} On the other hand, the highest values of T_{onset} which exceeded 270 °C were observed for 2 and 5 - based on phenoxyacetates. The results obtained for 5-8 were only slightly higher compared to 1-4 which

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445 suggest that in this case the influence of the cation structure on the T_{onset} parameter is 446 inconsiderable.

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448 Solubilities

449 The solubilities of the prepared ILs were tested in ten representative solvents, varying from high

450 polarity to low polarity. The obtained results are presented in Table 3.

451	Table 3. Solubility of prepared salts at 25 °C.

IL	Water	Methanol	DMSO	Acetonitrile	Acetone	Isopropanol	Ethyl acetate	Chloroform	Toluene	Hexane
	9.0 ^a	6.6	6.5	6.2	5.1	4.3	4.3	4.1	2.3	0.0
1	_	+	+	+	+	+	+	+	+	-
2	_	+	+	+	+	+	+	+	±	_
3	±	+	+	+	+	+	+	+	+	+
4	_	+	+	+	+	+	+	+	+	±
5	_	+	±	_	+	+	_	±	+	_
6	_	+	+	_	+	+	_	+	+	_
7	_	+	+	_	+	+	_	+	+	±
8	_	+	+	±	+	+	_	+	+	_

^a Snyder polarity index + complete solubility; ± limited solubility; – insoluble

It was observed that all tested ILs are well soluble in organic solvents with high polarity, such as methanol and DMSO, which corresponds well with our previous reports describing ILs based on phenoxyacids or dicamba anions and long alkyl ammonium ester cations,¹⁴ bisammonium cations⁶ and betaine cation²¹. However, alkylbetaine-based ILs exhibited low solubility in water. The high hydrophobicity of the obtained ILs may be explained by the presence of a long alkyl chain (reported for other ILs)^{7,10,14} as well as a carboxylic group (reported for ILs with betainium 459 cation)²¹. Interestingly, both betaines in zwitterionic form as commercial surfactants are 460 characterized by excellent water solubility. Low water solubility may be a particularly useful 461 property in terms of controlling soil and groundwater mobility of a new herbicide or good wetting 462 of plants leading to a better substance absorption. Slightly less polar acetone and isopropanol also 463 dissolved all ILs regardless of both the cation and anion structure. However, N-(3-464 cocamidopropyl) betainium cation caused a deterioration of ILs (5-8) solubility in other less polar 465 solvents, such as acetonitrile or ethyl acetate. Furthermore, despite similar Snyder polarity index 466 values (4.3) of isopropanol and ethyl acetate, the obtained results were significantly different. 467 Owing to the ionic bond in the structure of ILs, isopropanol as the semipolar protic solvent was a 468 better diluent than the aprotic semipolar ester - ethyl acetate. Due to presence of a long alkyl 469 substituent, the synthesized ILs were also soluble in nonpolar solvents, such as chloroform and 470 toluene. In addition, both ILs with MCPP anion (3 and 7) as well as N-dodecylbetainium 471 3,6-dichloro-2-methoxybenzoate (4) also dissolved in the most non-polar solvent - hexane, which 472 was previously reported only for esterguats HILs comprising a highly hydrophobic 473 di(tallowoyloxyethyl)dimethylammonium cation.¹⁴

474

475 Herbicidal Activity

The herbicidal activity of the obtained ILs was determined both in greenhouse and field tests. Their efficiency was compared to the reference herbicides comprising dimethylammonium salt of 2,4-D (1, 5), sodium-potassium salt of MCPA (2, 3, 6, 7) and sodium salt of dicamba (4, 8). The herbicidal efficacy of the synthesized alkylbetainium ILs in the greenhouse experiments was examined using common lambsquarters (*Chenopodium album* L.), rapeseed (*Brassica napus* L.) and cornflower (*Centaurea cyanus* L.) as test plants. The results are shown in Fig. 6. No significant

487

differences were observed in the case of results obtained for cornflower, whereas the results of experiments on common lambsquarters and rapeseed differed notably depending on the salt used for treatment. The main symptoms of plant damage such as deformation of shoots, leaves and disruption of plant growth caused by auxin-like herbicides (*i.e.* phenoxyacids or dicamba) were observed for the examined ILs during the experiment (see Fig. S22, ESI).



Figure 6. Herbicidal efficacy of tested ILs against common lambsquarters, cornflower andrapeseed under greenhouse conditions.

490 Generally, all of the tested ILs demonstrated high efficacy (exceeding 90%) which underlines the 491 beneficial role of both utilized alkylbetainium cations. For example, ILs 1, 4, 5 and 8 were 492 characterized by higher herbicidal activity towards rapeseed compared to the applied standards. 493 According to the results of greenhouse experiments, none of the studied ILs, excluding salts with 494 MCPP anion (3, 7) toward common lambsquarters, exhibited lower efficacy in comparison to 495 reference herbicides. Moreover, it was noted that ILs with dicamba anion (4, 8) were characterized 496 by multiple times higher herbicidal activity compared to the reference herbicide towards rapeseed 497 at the applied dose of the active ingredient. Among all synthesized ILs, 6 turned out to be the most active – its efficiency was higher than 95% against all three plants. The presented studies confirm
that biological activity of the synthesized ILs has been preserved – they may be classified as new
herbicidal ionic liquids (HILs).

501 Biological activity of $[C_{12}Bet][MCPA]$ (2) has been tested in a preliminary field experiment in 502 spring barley in 2014. It was noted that this IL is characterized by better or comparable 503 effectiveness compared to the reference herbicide containing MCPA. This HIL reached an average 504 effectiveness equal to 100% towards common lambsquarters and 90% towards cornflower, while 505 the reference herbicide efficiency was lower and amounted to 90 and 70% for both weeds, respectively. The next field experiment performed in 2015 in spring barley confirmed the 506 507 substantial enhancement of biological activity of the tested HILs caused by the presence of 508 alkylbetaine cation (Fig. 7).



509

Figure 7. Weed control in spring barley in 2015 against two weed species evaluated 5 weeks after
treatment (5 WAT).

512 All HILs demonstrated similar or significantly higher efficiency towards common lambsquarters 513 and rapeseed as compared to the applied reference herbicide. In case of 2,4-D-based HILs (1, 5)

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the control of common lambsquarters was five-times higher compared to 2,4-D reference herbicide. The studied HILs in the field tests exhibited very high efficacy (> 90%) which indicates that they could be used for successful weed control in spring cereal crops. The enhancement of herbicidal efficiency caused by the incorporation of amphoteric surfactant (alkylbetaine) into HIL is also consistent with literature, as a similar phenomenon has been noted for many HILs based on cationic surfactants and phenoxyacids^{1-3,14,36} as well as well as dicamba^{8,14,36}.

521 Surface properties

The main surface activity parameters, such as contact angle (CA) and surface tension (γ) of spray solutions utilized for evaluation of herbicidal activity of the synthesized HILs, were determined (collected values are provided in ESI – Table S9) and presented in Fig. 8.



525

Figure 8. Surface tension (*y*) and contact angle (CA) of spray solutions containing HILs (1-8) and
reference herbicides.

528 As the derivatives of amphoteric surfactants – all the obtained HILs were characterized by good 529 surface-active and wetting properties. Their values of surface tension between 30.4 for **8** and 32.6

 $mN \cdot m^{-1}$ for 4 are comparable to the results obtained by using the most effective adjuvants.^{49,50} In 530 general, surface tension of HILs comprising phenoxyacids (1-3, 5-7) was over 40 mN \cdot m⁻¹ lower 531 532 compared to reference herbicides, whereas the difference in γ values between dicamba-based HILs Published on 27 June 2017. Downloaded by University of California - San Diego on 27/06/2017 11:39:14. 533 (4, 8) and reference herbicide was approx. 34 mN \cdot m⁻¹. 534 Low values of contact angle and surface tension should enhance the wetting of plants, spray 535

retention, absorption, cuticle penetration and translocation of the active ingredient. This 536 assumption has been confirmed in previous reports regarding HILs comprising cationic surfactants 537 and once again underline the role of cation selection for synthesis of biologically active ILs.^{2,3} 538 According to Fig. 8, the synthesized HILs were characterized by low values of contact angle 539 ranging from approx. 50° to 62°. The structure of the herbicidal anion had a minor influence on 540 the measured values, which differed by a few degrees. Furthermore, CA of solutions containing 541 the synthesized salts were substantially lower than values observed for standards which, due to 542 their lack of surface active properties, exceeded 100°. The differences in CA by 40-55° between 543 HILs and standards manifest in different shape of droplets of spray solution on the leaf of the weed 544 - droplets with HILs possess a greater contact area (see Fig. S23, ESI), which facilitates the 545 penetration of the active substance into the interior of the plant and also prevents droplets from falling from the leaf. 546

547

548 Toxicity of selected HILs

549 Two HILs with the MCPA anion (2, 6) were selected for determination of LD₅₀ value towards rats. 550 The presence of the alkylbetainium cation did not alter the toxicity level of the new herbicides in 551 comparison to MCPA in acidic form (Table 4).

552



Table 4. Acute oral LD₅₀ for rats of the synthesized alkylbetaine derivatives.

Compound	Category GHS ^a	LD50 (mg·kg ⁻¹)
[C ₁₂ Bet][MCPA] (2)	4	300-2000
[CAPBet][MCPA] (6)	4	300-2000
MCPA free acid	4	962 ⁵¹
N-dodecylbetaine	3	71 ^{37,52}
N-(3-cocoamidopropyl)betaine	e 5	$\geq 4900^{53}$

^a Globally Harmonized System of Classification and Labelling of Chemicals

Both tested betaine derivatives could be included into category 4. In case of **2** we found that the combination of N-dodecylbetainium cation with MCPA anion resulted in the reduction of toxicity in comparison to data reported for pure N-dodecylbetaine.^{37,52} Moreover, regarding our previous results for HILs with the MCPA anion and betaine or carnitine cation²¹, it was found that the influence of the presence of long alkyl chain (dodecyl as well as 3-cocoamidopropyl) in cation of betaine has no considerable impact on the acute toxicity towards rats. Hence, all tested betainiumbased HILs were classified into 4 category thus far.

564

565 Susceptibility to biodegradation

566 Generally, betaine derivatives are considered as readily biodegradable compounds, which 567 distinguishes this group of surfactants. The primary biodegradation of N-dodecylbetaine and N-568 (3-cocoamidopropyl)betaine was almost complete – results for both exceed 90% in the same 569 OECD test.³⁷ This fact additionally showed us the significance and potential of utilization of 570 alkylbetainium-based HILs in plant protection research. The comparison of HILs with the same anions and different cations (Table 5) leads to a general conclusion, that the use of N-(3cocoamidopropyl)betainium cation contributed to a slightly higher biodegradation efficiency (from 47% for **8** to 73% for **5**), whereas HILs based on the N-dodecylbetainium cation were characterized by lower biodegradability (from 42% for **4** to 72% for **1**).

575 **Table 5.** Biodegradation results of the obtained HILs (OECD 301 F tests).

IL	Biodegradation efficiency after	Error margin	Time window between 10 and	Class
	28 days [%]	[%]	60% [days]	
1	72	2	9	А
2	59	4	Х	В
3	51	2	Х	С
4	42	2	х	С
5	73	0	9	А
6	62	1	12	А
7	55	0	Х	С
8	47	1	Х	С

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576 A - readily biodegradable; B - inherently biodegradable; C- not readily biodegradable

577 The obtained results revealed that both HILs comprising the 2,4-D anion (1 and 5) exhibited high 578 biodegradation efficiency, which exceeded the 60% threshold within the 9 days time window, 579 therefore they could be classified as readily biodegradable. In accordance to calculated error 580 margin, there were no significant differences in biodegradation efficiency between both HILs. On 581 the other hand, the most notable differences in the obtained results are associated with the presence 582 of specific herbicidal anions in the synthesized HILs. Hence, the used herbicidal anions could be 583 descending order biodegradation which their arranged in of rate. refers to 584 susceptibility to biodegradation: 2,4-D > MCPA > MCPP > dicamba.

585 In case of HILs comprising the MCPA anion, **6** fulfilled the requirements (biodegradation rate 586 \geq 60% within the 12 days time window), which allow to classify this HIL as readily biodegradable.

587 Salt 2 exhibited a biodegradation rate slightly lower than the test threshold (59%), however it may 588 be classified as inherently biodegradable. None of the HILs comprising the MCPP (3 and 7) or 589 dicamba (4 and 8) anion reached the test threshold and could not be classified as readily 590 biodegradable. Moreover, we found that alkyl chain elongation from methyl (HILs with betaine 591 cation)²¹ to dodecyl or 3-cocoamidopropyl did not cause a rapid decrease of biodegradability of 1-8, which has been already observed for other ILs.⁵⁴ Furthermore, some of the obtained HILs (1, 2, 3)592 593 5, 6) proved to be comparably or even more susceptible to biodegradation than recently described 594 by us esterquat - di(tallowoyloxyethyl)dimethylammonium 4-chloro-2-methylphenoxyacetate, 595 that reached the value of $63\% (\pm 5)$.¹⁴

597 CONSLUSIONS

598 In this study, a group of new herbicidal ionic liquids comprising popular and commercially 599 available analogues of betaine (N-dodecylbetaine and N-(3-cocoamidopropyl)betaine) as cations 600 were synthesized by using a two-step approach. The protonation reaction as well as the subsequent 601 ion exchange for anions derived from popular herbicides such as 2,4-dichlorophenoxyacetic acid 602 (2,4-D), 4-chloro-2-methylphenoxyacetic acid (MCPA), 2-(4-chloro-2-methyl-phenoxy)propionic 603 acid (MCPP) and 3,6-dichloro-2-methoxybenzoic (dicamba) were characterized by high yields 604 which exceeded 90%. To the best of our knowledge, this is the first successful attempt to 605 incorporate an amphoteric surfactant and a pesticide into the structure of an ionic liquid. The TGA 606 and DSC analyses confirmed that all the prepared salts were characterized by good thermal stability ($T_{onset} \ge 240$ °C) and exhibited melting points below 100 °C. Therefore all may be 607 608 classified as ionic liquids, including five room temperature ionic liquids (RTILs). Due to the 609 presence of carboxyl group capable of forming intermolecular hydrogen bonds, the obtained

RTILs were highly viscous liquids at 20 °C. It was observed that the most significant differences 610 611 in physicochemical properties of the obtained ionic liquids could be attributed to the presence of 612 specific herbicidal anions. On the other hand, the most significant differences in solubility were 613 affected by the structure of the cation, therefore the presence of N-(3-cocoamidopropyl)betainium 614 cation decreased the solubility in acetonitrile and ethyl acetate. Furthermore, the herbicidal activity 615 of the ionic liquids towards common lambsquarters, cornflower and rapeseed was similar or better 616 compared with the commercial reference herbicides (herbicidal efficacy in field trials > 90%) – 617 therefore, they may be classified as herbicidal ionic liquids. The obtained excellent results of 618 biological activity are consistent with data collected in surface activity experiments. The 619 synthesized herbicidal ionic liquids display potent surface-active properties, which contributed to 620 the increase of contact surface between leaves and active ingredient and thereby the enhancement 621 of adsorption of the herbicide into the plant. The effect of the anion was also notable in 622 biodegradation tests. Ionic liquids containing 2,4-D were readily biodegradable (biodegradation 623 efficiency > 70%), followed by those with MCPA (approx. 60%), whereas ionic liquids which 624 contained MCPP and dicamba were inherently biodegradable. Utilisation of both alkylbetainium 625 cations resulted in low toxicity of the synthesized herbicidal ionic liquids (category 4, LD_{50}) towards rats in the range of $300 - 2000 \text{ mg} \cdot \text{kg}^{-1}$). 626

627

628 ASSOCIATED CONTENT

629 Supporting information. ¹H and ¹³C NMR spectra, elemental analysis results, values of density, 630 viscosity and refractive index for synthesized ILs as well as values of surface tension and contact 631 angle and shape of drop of their spray solutions are provided in Electronic Supporting Information. 632

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Graphic for Table of Content



TEXT:

Herbicidal ionic liquids derived from alkylbetaines proved to be biodegradable, thermally stable and highly effective anti-weed agents.