Green Chemistry

Cite this: *Green Chem.*, 2011, **13**, 2901

www.rsc.org/greenchem

PAPER

Synthesis, toxicity, biodegradability and physicochemical properties of 4-benzyl-4-methylmorpholinium-based ionic liquids†

Juliusz Pernak,*[a] Nina Borucka,[a] Filip Walkiewicz,[a] Bartosz Markiewicz,[a] Przemysław Fochtman,[b] Stefan Stolte,[c] Stephanie Steudte[c] and Piotr Stepnowski[c]

Received 27th April 2011, Accepted 22nd June 2011
DOI: 10.1039/c1gc15468k

A series of 4-benzyl-4-methylmorpholinium salts have been synthesized producing morpholinium ionic liquids with inorganic (chloride, nitrate, tetrafluoroborate, hydrogen sulphate) and organic (formate, acetate, methoxyacetate, 2-(2-methoxyethoxy)acetate, hexanoate, octanoate, dodecyl sulphate, 2-ethylbutyrate, lactate, crotonate, maleate, salicylate, saccharinate) anions. Their physicochemical properties, cytotoxicity to the promyelocytic leukaemia rat cell line IPC-81, oral toxicity and biodegradability were determined. The anion significantly determined the state of aggregation and cytotoxicity. The results enabled 4-benzyl-4-methylmorpholinium ionic liquids to be classified as being of moderate or low toxicity (EC_{50} between 0.15 and 14.13 mM). The acute toxicity for 4-benzyl-4-ethylmorpholinium acetate is between 300–2000 mg kg^{-1} b.w. in female rats. The activity of these morpholinium salts against bacteria and fungi was very low. It was found that new 4-benzyl-4-methylmorpholinium-based ionic liquids with anions commonly used for dissolving cellulose, such as formate or acetate, can also be used as new biomass solvents.

Introduction

Ionic liquids (ILs) with nitrogen, sulphur, or phosphorus as the central atom of the cations have been extensively investigated. These include imidazolium, pyrrolidinium, tetraalkylammonium, pyridinium, piperidinium, sulphonium and phosphonium-based ILs.[1,2]

ILs are called 'green' usually because of their negligible vapour pressure. Low volatility, however, does not completely eliminate potential environmental hazards and can pose serious threats to aquatic and terrestrial ecosystems.[3] There are many other factors that determine whether ILs are or are not green. Today, it is very important that not only ILs themselves should be green (low toxicity or biodegradability), but also their synthesis.[4] In this respect, however, extensive studies on the toxicity and biodegradability of ILs are necessary. The vast majority of toxicological studies on ILs available to date have focused on imidazolium ILs.[5] Their acute ecotoxicity has been studied intensively using several different test systems and species.[6–10] For IL cations a general trend was found, namely that acute (eco)toxic effects can be related to a compound's hydrophobicity. Substances with greater hydrophilicity exhibit higher (eco)toxicities. Thus, compounds with long alkyl substituents have greater influence on toxicity than cations with shorter alkyl residues or side chains containing polar functional groups. Besides the cationic head group of ILs, the anion plays a central role in defining physicochemical as well as toxicological properties. Several anions, especially highly fluorinated and hydrophobic ones, show higher activity compared to, for example, halides in different biological test systems.[11]

Recently, interest in morpholinium-based ILs has increased because of their properties. In fact, they are less toxic than the commonly used imidazolium, pyridinium or tetraalkylammonium-based ILs.[12] Morpholinium ILs are applied as electrolytes[13,14] and gel electrolytes,[15] ionic liquid crystals (the class of liquid-crystalline compounds),[16,17] reaction media[18] or corrosion inhibitors.[19] In particular, ILs based on the morpholinium cation are preferred because of their electrochemical stability.[20] However, little data on the (eco)toxicity of morpholinium-based ILs are available. The functionalized morpholinium compounds tested so far have shown low acute cytotoxic effects up to concentrations of 20 mM in tests with the promyelocytic leukaemia rat cell line IPC-81.[21]

Apart from a low (eco)toxicity, current environmental legislation makes insistent demands for non-persistent chemicals.

[a]*Department of Chemical Technology, Poznan University of Technology, pl. M. Sklodowskiej-Curie 2, 60-965, Poznan, Poland. E-mail: juliusz.pernak@put.poznan.pl; Tel: +48 61 665 36 82*
[b]*Institute of Industrial Organic Chemistry, Pszczyna Branch, ul. Doświadczalna 27, 43-200, Pszczyna, Poland. E-mail: fochtman@ipo-pszczyna.pl; Fax: +48 32 210 30 81 ext. 120; Tel: +48 32 210 30 81 ext. 124*
[c]*Department of Environmental Analysis, Faculty of Chemistry, University of Gdańsk, ul. Sobieskiego 18, 80-952, Gdańsk, Poland. E-mail: sox@chem.univ.gda.pl; Fax: +48 58 523 55 72; Tel: +48 58 523 54 48*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1gc15468k

For chemicals to be described as 'sustainable' or 'green', complete and rapid biotic degradation is a crucial requirement. The biodegradability of IL cations is strongly dependent on structure. Pyridinium compounds with ethyl and octyl side chains are completely mineralized within 28 days,[22,23] whereas imidazolium-based cations are only partially degradable, with the core structure itself remaining resistant to biodegradation.[24] However, investigations examining the biodegradability of different head groups such as morpholinium are still required.

This study reports on the synthesis of novel 4-benzyl-4-methylmorpholinium and 4-benzyl-4-ethylmorpholinium cations combined with 16 different anions: inorganic (chloride, nitrate, tetrafluoroborate or hydrogen sulphate), organic aromatic (salicylate or saccharinate) and aliphatic (formate, acetate, hexanoate, octanoate, maleate, crotonate or lactate) acids and dodecyl sulphate. Besides typical physicochemical properties such as melting points, viscosities, and thermal stabilities, data describing their toxicological properties (acute toxicity towards bacteria, fungi, *in vitro* cell line and rat) and environmental properties (ready biodegradability) are also presented.

Experimental

Materials

4-Methylmorpholine, 4-ethylmorpholine, benzyl chloride, potassium nitrate, sodium tetrafluoroborate, sulphuric acid, formic acid, acetic acid, methoxyacetic acid, 2-(2-methoxyethoxy)acetic acid, hexanoic acid, octanoic acid, sodium dodecyl sulphate, lactic acid, salicylic acid, sodium saccharin, dimethylsulphoxide, trifluoroacetic acid, potassium phosphate monobasic, potassium phosphate dibasic, sodium phosphate dibasic dihydrate, calcium chloride dihydrate, magnesium sulphate heptahydrate, iron(III) chloride and microcrystalline cellulose were obtained from Sigma Aldrich and used without further purification. Crotonic acid, maleic acid, 2-ethylbutyric acid, acetonitrile, ammonium chloride, potassium hydroxide and mercury(II) chloride were purchased from Fluka. All solvents were used as obtained without further purification. Cell culture media, sera and phosphate buffer were purchased from Invitrogen Life Technologies (Frankfurt, Germany), WST-1 reagent was obtained from Roche Diagnostics GmbH (Mannheim, Germany).

General

^{1}H NMR spectra were recorded on a Mercury Gemini 300 spectrometer operating at 300 MHz with TMS as the internal standard. ^{13}C NMR spectra were obtained with the same instrument at 75 MHz. CHN elemental analyses were performed at the Adam Mickiewicz University, Poznan (Poland). The water content was determined using an Aquastar volumetric Karl-Fischer titration with Composite 5 solution as titrant and anhydrous methanol as solvent. Melting points were determined by visual observation using a hot-plate apparatus. Viscosity was determined using a rheometer (Rheotec RC30-CPS) with cone-shaped geometry (C50-2). The viscosity of the samples, about 1.5 mL, was measured with respect to temperature, from 20 to 55 °C. The pH values of aqueous solutions of IL, 0.1 M, were determined with a pH-meter (inoLab pH720) using a combined electrode (ERH-11S).

Synthesis of 4-benzyl-4-methylmorpholinium salts

4-Benzyl-4-methylmorpholinium and 4-benzyl-4-ethylmorpholinium chlorides have recently been described.[25,26]

Method I: Morpholinium salts were synthesized by treating equimolar quantities of sodium or potassium salts with 4-benzyl-4-methylmorpholinium chloride in methanol. The solutions were stirred at room temperature for 1 h, after which the precipitated sodium or potassium chloride was removed by filtration. After evaporation of the methanol, the product was dissolved in acetone and the remaining inorganic salt removed. The product (**1**, **2**, **10** and **16**) was dried under vacuum at 45 °C for 10 h.

Method II: Two salts (**3** and **14**) were synthesized by the dropwise addition of sulphuric acid or maleic acid (0.02 mol) dissolved in dichloromethane to 4-benzyl-4-methylmorpholinium chloride (0.02 mol) in dichloromethane, with initial cooling in ice, followed by warming to room temperature and overnight stirring. Dry air was passed through the salts obtained to remove hydrochloric acid. The products were then separated from dichloromethane and dried under vacuum at 55 °C for 8 h.

Method III: The remaining morpholinium salts were synthesized in two stages. In the first stage, a stoichiometric quantity of potassium hydroxide was added to a portion of 4-benzyl-4-methylmorpholinium chloride (0.015 mol) or 4-benzyl-4-ethylmorpholinium chloride (0.015 mol), dissolved in methanol. The solutions were stirred at room temperature for 0.5 h, after which the partially precipitated potassium chloride was removed by filtration. In the second stage, a stoichiometric amount of an appropriate carboxylic acid was added to the filtrate. The solutions were stirred again at room temperature for 0.5 h and, then, after evaporation of the methanol, the product was dissolved in acetone and the remaining inorganic salt removed. The products (**4–9**, **11–13**, **15** and **17**) were dried under vacuum at 50 °C for 9 h.

4-Benzyl-4-methylmorpholinium nitrate (1). ^{1}H NMR (DMSO-d_6) δ (ppm) 3.11 (s, 3H); 3.38 (t, J = 4.29 Hz, 2H); 3.58 (qw, J = 2.61 Hz, 2H); 3.99 (t, J = 4.31 Hz, 4H); 4.78 (s, 2H); 7.54 (m, 3H); 7.60 (m, 2H); ^{13}C NMR (DMSO-d_6) δ (ppm) 45.0; 58.5; 59.9; 67.6; 127.2; 128.9; 130.4; 133.3. Elemental analysis calc. (%) for $C_{12}H_{18}N_2O_4$ (254.28): C 56.68, H 7.13, N 11.02. Found: C 57.03, H 6.85, N 11.46.

4-Benzyl-4-methylmorpholinium tetrafluoroborate (2). solid. ^{1}H NMR (DMSO-d_6) δ (ppm) 3.06 (s, 3H); 3.32 (t, J = 4.25 Hz, 2H); 3.53 (qw, J = 5.21 Hz, 2H); 3.98 (t, J = 3.29 Hz, 4H); 4.68 (s, 2H); 7.54 (m, 5H); ^{13}C NMR (DMSO-d_6) δ (ppm) 44.9; 58.6; 59.8; 67.7; 127.1; 129.0; 130.4; 133.2. Elemental analysis calc. (%) for $C_{12}H_{18}NOBF_4$ (279.08): C 51.64, H 6.50, N 5.02. Found: C 51.93, H 6.12, N 5.49.

4-Benzyl-4-methylmorpholinium hydrogen sulfate (3). ^{1}H NMR (DMSO-d_6) δ (ppm) 3.10 (s, 3H); 3.17 (s,1H); 3.37 (t, J = 4.29 Hz, 2H); 3.56 (qw, J = 5.24 Hz, 2H); 3.98 (t, J = 1.71 Hz, 4H); 4.77 (s, 2H); 7.54 (m, 3H); 7.60 (m, 2H); ^{13}C NMR (DMSO-d_6) δ (ppm) 45.0; 58.5; 59.9; 67.6; 127.3; 129.0; 130.4;

133.4. Elemental analysis calc. (%) for $C_{12}H_{19}NO_5S$ (289.35): C 49.81, H 6.62, N 4.84. Found: C 49.37, H 7.03, N 4.42.

4-Benzyl-4-methylmorpholinium formate (4). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 3.13 (s, 3H); 3.41 (t, J = 4.27 Hz, 2H); 3.60 (qw, J = 5.13 Hz, 2H); 3.99 (t, J = 6.35 Hz, 4H); 4.84 (s, 2H); 7.53 (m, 3H); 7.62 (m, 2H); 8.62 (s, 1H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 44.8; 58.4; 59.9; 67.4; 127.4; 128.9; 130.3; 133.4; 165.7. Elemental analysis calc. (%) for $C_{13}H_{19}NO_3$ (237.29): C 65.80, H 8.07, N 5.90. Found: C 65.21, H 8.84, N 5.39.

4-Benzyl-4-methylmorpholinium acetate (5). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 1.61 (s, 3H); 3.15 (s, 3H); 3.43 (t, J = 4.27 Hz, 2H); 3.60 (qw, J = 5.24 Hz, 2H); 3.98 (t, J = 5.86 Hz, 4H); 4.88 (s, 2H); 7.52 (m, 3H); 7.64 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 26.1; 44.7; 58.3; 59.9; 67.2; 127.6; 128.8; 130.2; 133.4; 173.2. Elemental analysis calc. (%) for $C_{14}H_{21}NO_3$ (251.32): C 66.91, H 8.42, N 5.57. Found: C 67.47, H 8.93, N 4.96.

4-Benzyl-4-methylmorpholinium methoxyacetate (6). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 3.12 (s, 3H); 3.21 (s, 3H); 3.40 (t, J = 4.27 Hz, 2H); 3.59 (qw, J = 4.06 Hz, 2H); 3.98 (t, J = 2.50 Hz, 4H); 4.11 (s, 2H); 4.82 (s, 2H); 7.53 (m, 3H); 7.62 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 44.8; 57.5; 58.4; 59.9; 67.4; 72.0; 127.4; 128.9; 130.3; 133.3; 172.1. Elemental analysis calc. (%) for $C_{15}H_{23}NO_4$ (281.35): C 64.03, H 8.24, N 4.98. Found: C 64.48, H 7.84, N 4.47.

4-Benzyl-4-methylmorpholinium 2-(2-methoxyethoxy)acetate (7). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 3.11 (s, 3H); 3.23 (s, 3H); 3.41 (t, J = 1.83 Hz, 2H); 3.49 (qw, J = 1.98 Hz, 2H); 3.57 (t, J = 3.66 Hz, 4H); 3.76 (s, 2H); 3.98 (t, J = 6.23 Hz, 4H); 4.81 (s, 2H); 7.53 (m, 3H); 7.61 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 44.8; 58.0; 58.4; 59.9; 67.5; 68.7; 71.0; 71.4; 127.4; 128.9; 130.3; 133.4; 172.2. Elemental analysis calc. (%) for $C_{17}H_{27}NO_5$ (325.40): C 62.75, H 8.36, N 4.30. Found: C 63.18, H 7.84, N 4.84.

4-Benzyl-4-methylmorpholinium hexanoate (8). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 0.83 (t, J = 4.66 Hz, 3H); 1.21 (m, 4H); 1.42 (qw, J = 5.92 Hz, 2H); 1.89 (t, J = 4.93 Hz, 2H); 3.15 (s, 3H); 3.43 (t, J = 4.26 Hz, 2H); 3.62 (qw, J = 5.26 Hz, 2H); 3.98 (t, J = 2.36 Hz, 4H); 4.88 (s, 2H); 7.51 (m, 3H); 7.64 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 14.0; 22.2; 26.1; 31.7; 38.3; 44.6; 58.3; 59.9; 67.2; 127.5; 128.7; 130.1; 133.4; 175.6. Elemental analysis calc. (%) for $C_{18}H_{23}NO_3$ (307.43): C 70.32, H 9.51, N 4.56. Found: C 69.84, H 9.02, N 4.97.

4-Benzyl-4-methylmorpholinium octanoate (9). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 0.84 (t, J = 4.52 Hz, 3H); 1.21 (qw, J = 8.42 Hz, 8H); 1.40 (qw, J = 5.64 Hz, 2H); 1.86 (t, J = 5.01 Hz, 2H); 3.12 (s, 3H); 3.41 (t, J = 4.27 Hz, 2H); 3.59 (qw, J = 5.20 Hz, 2H); 3.95 (t, J = 2.20 Hz, 4H); 4.83 (s, 2H); 7.52 (m, 3H); 7.62 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 14.0; 22.1; 26.6; 28.8; 29.5; 31.4; 38.6; 44.7; 58.3; 60.0; 67.4; 127.4; 128.8; 130.2; 133.3; 175.6. Elemental analysis calc. (%) for $C_{20}H_{33}NO_3$ (335.48): C 71.60, H 9.91, N 4.17. Found: C 71.04, H 10.37, N 4.59.

4-Benzyl-4-methylmorpholinium dodecyl sulfate (10). solid. ^{1}H NMR (DMSO-d$_6$) δ (ppm) 0.85 (t, J = 4.56 Hz, 3H); 1.23 (qw, J = 4.32 Hz, 18H); 1.47 (qw, J = 4.00 Hz, 2H); 3.10 (s, 3H); 3.36 (t, J = 4.33 Hz, 2H); 3.57 (qw, J = 4.22 Hz, 2H); 3.69 (t, J = 4.76 Hz, 2H); 3.98 (t, J = 2.90 Hz, 4H); 4.78 (s, 2H); 7.53 (m, 3H); 7.60 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 13.9; 22.1; 25.5; 28.71; 28.77; 29.0; 31.3; 44.8; 58.4; 59.8; 65.5; 67.4; 127.2; 128.8; 130.3; 133.2. Elemental analysis calc. (%) for $C_{24}H_{43}NO_5S$ (457.66): C 62.98, H 9.47, N 3.06. Found: C 62.46, H 9.93, N 2.67.

4-Benzyl-4-methylmorpholinium 2-ethylbutyrate (11). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 0.78 (t, J = 4.90 Hz, 6H); 1.23 (qw, J = 4.04 Hz, 2H); 1.40 (qw, J = 4.28 Hz, 2H); 1.73 (qw, J = 3.38 Hz, 1H); 3.14 (s, 3H); 3.43 (t, J = 4.30 Hz, 2H); 3.61 (qw, J = 5.19 Hz, 2H); 3.98 (t, J = 2.42 Hz, 4H); 4.85 (s, 2H); 7.52 (m, 3H); 7.63 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 12.6; 25.7; 44.8; 52.1; 58.3; 59.9; 67.3; 127.5; 128.8; 130.2; 133.4; 178.1. Elemental analysis calc. (%) for $C_{18}H_{29}NO_3$ (307.43): C 70.32, H 9.51, N 4.56. Found: C 70.02, H 10.11, N 3.95.

4-Benzyl-4-methylmorpholinium lactate (12). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 1.32 (d, J = 3.36 Hz, 3H); 3.29 (s, 3H); 3.56 (t, J = 4.26 Hz, 2H); 3.68 (qw, J = 5.27 Hz, 2H); 3.92 (d, J = 3.36 Hz, 1H); 3.97 (t, J = 3.89 Hz, 4H); 4.46 (m, 1H); 4.94 (s, 2H); 7.43 (m, 3H); 7.56 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 21.3; 45.1; 58.2; 60.3; 68.0; 68.9; 126.2; 129.0; 130.5; 133.1; 179.6. Elemental analysis calc. (%) for $C_{15}H_{23}NO_4$ (281.35): C 64.03, H 8.24, N 4.98. Found: C 63.55, H 8.79, N 4.31.

4-Benzyl-4-methylmorpholinium crotonate (13). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 1.68 (d, J = 4.20 Hz, 3H); 3.26 (s, 3H); 3.52 (t, J = 4.20 Hz, 2H); 3.59 (qw, J = 5.28 Hz, 2H); 3.93 (t, J = 5.93 Hz, 4H); 4.83 (s, 2H); 5.88 (d, J = 7.70 Hz, 1H); 6.53 (qw, J = 4.44 Hz, 1H); 7.37 (m, 3H); 7.54 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 17.2; 45.4; 58.0; 60.4; 69.1; 126.6; 128.9; 130.2; 130.4; 133.4; 136.8; 172.3. Elemental analysis calc. (%) for $C_{16}H_{23}NO_3$ (277.36): C 69.29, H 8.36, N 5.05. Found: C 69.86, H 8.78, N 4.64.

4-Benzyl-4-methylmorpholinium maleate (14). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 3.11 (s, 3H); 3.38 (t, J = 4.10 Hz, 2H); 3.58 (qw, 2H); 3.98 (t, 4H); 4.82 (s, 2H); 6.28 (d, 2H); 7.53 (m, 3H); 7.61 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 44.9; 58.5; 59.9; 67.4; 127.3; 128.9; 130.3; 130.4; 133.3; 166.7. Elemental analysis calc. (%) for $C_{16}H_{21}NO_5$ (307.34): C 62.53, H 6.89, N 4.56. Found: C 62.98, H 7.47, N 3.94.

4-Benzyl-4-methylmorpholinium salicylate (15). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 3.08 (s, 3H); 3.35 (t, J = 4.21 Hz, 2H); 3.56 (qw, J = 5.22 Hz, 2H); 3.98 (t, J = 2.70 Hz, 4H); 4.74 (s, 2H); 6.66 (m, 2H); 7.18 (t, J = 5.72 Hz, 1H); 7.53 (m, 3H); 7.57 (m, 2H); 7.69 (d, J = 4.67 Hz, 1H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 44.9; 58.5; 59.8; 67.6; 116.0; 116.3; 119.6; 127.2; 128.9; 130.0; 130.3; 131.9; 133.2; 162.7; 171.5. Elemental analysis calc. (%) for $C_{19}H_{23}NO_4$ (329.39): C 69.28, H 7.04, N 4.25. Found: C 68.74, H 7.55, N 4.82.

4-Benzyl-4-methylmorpholinium saccharinate (16). ^{1}H NMR (DMSO-d$_6$) δ (ppm) 3.28 (s, 3H); 3.55 (t, J = 4.26 Hz, 2H); 3.65 (qw, J = 5.30 Hz, 2H); 3.92 (t, J = 6.13 Hz, 4H); 4.91 (s, 2H); 7.35 (m, 3H); 7.56 (m, 4H); 7.75 (m, 2H); ^{13}C NMR (DMSO-d$_6$) δ (ppm) 45.3; 58.2; 60.4; 69.3; 119.5; 123.2; 126.1; 129.1; 130.6; 131.4; 132.0; 133.4; 134.6; 144.4; 170.1. Elemental analysis calc. (%) for $C_{19}H_{22}N_2O_4S$ (374.45): C 60.94, H 5.92, N 7.48. Found: C 60.86, H 5.84, N 7.59.

4-Benzyl-4-ethylmorpholinium acetate (17). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.36 (t, J = 4.78 Hz, 3H); 1.64 (s, 3H); 3.44 (m, 6H); 3.97 (t, J = 2.83 Hz, 4H); 4.74 (s, 2H); 7.53 (m, 3H); 7.56 (m, 2H); ^{13}C NMR (DMSO-d_6) δ (ppm) 7.2; 25.1; 51.2; 55.9; 59.7; 62.3; 127.3; 129.0; 130.3; 133.1; 173.5. Elemental analysis calc. (%) for $C_{15}H_{23}NO_3$ (265.35): C 67.90, H 8.74, N 5.28. Found: C 67.42, H 9.25, N 4.76.

Thermal analysis

Thermal transition temperatures of the morpholinium salts were determined by DSC, with a Mettler Toledo Stare DSC1 (Leicester, UK) unit, under nitrogen. Samples between 5 and 15 mg were placed in aluminium pans and heated from 25 to 120 °C at a heating rate of 10 °C min^{-1} and cooled with an intracooler at a cooling rate of 10 °C min^{-1} to −100 °C and then heated again to 120 °C. Thermogravimetric analysis was performed using a Mettler Toledo Stare TGA/DSC1 unit (Leicester, UK) under nitrogen. Samples between 2 and 10 mg were placed in aluminium pans and heated from 30 to 450 °C at a heating rate of 10 °C min^{-1}.

Antimicrobial activity

The antimicrobial activity was determined by the tube dilution method and described earlier.[27] The lowest concentration of compound at which there was no visible growth (turbidity) was taken as representing the MIC (minimum inhibitory concentration). The lowest concentration of compound supporting no colony formation was defined as the MBC for bacteria and MFC for fungi. The following microorganisms were used: *Micrococcus luteus* NCTC 7743, *Staphylococcus aureus* NCTC 4163, *Staphylococcus epidermidis* ATCC 49134, *Enterococcus faecium* ATCC 49474, *Moraxella catarrhalis* ATCC 25238, *Escherichia coli* ATCC 25922, *Serratia marcescens* ATCC 8100, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* NCTC 6749, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, and *Rhodothorula rubra* (Demml 1889, Lodder 1934). Standard strains were supplied by the National Collection of Type Cultures (NCTC) London and the American Type Culture Collection (ATCC). *Rhodothorula rubra* was obtained from the Department of Pharmaceutical Bacteriology, University of Medical Sciences, Poznan.

Cell viability assay

The cytotoxicity of the morpholinium salts was determined for the promyelocytic leukaemia rat cell line IPC-81.[28] Cultures of IPC-81 were grown in RPMI medium (with L-glutamine, without $NaHCO_3$, supplemented with 1% penicillin–streptomycin and 1% glutamine, pH 7) with 10% horse serum at 37 °C (5% CO_2).

The cytotoxicity assay was carried out according to Ranke *et al.*[29] Cell viability was measured using a colorimetric assay for 96 well plates with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2*H*-tetrazolium monosodium salt (WST-1) reagent. Each plate contained blanks (no cells), controls (no toxicants), and substance in 1 : 1 dilution series. Stocks of ILs were prepared in a culture medium with 0.5% dimethylsulphoxide (DMSO) to improve the solubility of the substances. This DMSO concentration has been proven not to be cytotoxic. For the test, IPC-81 cells in a concentration of 15×10^5 cells mL^{-1} (in RPMI with 8% foetal calf serum) were incubated for 44 h in 96-well plates in the presence of a morpholinium salt and for an additional 4 h in the presence of WST-1 reagent. Cell viability as the ability to reduce WST-1 was observed photometrically at 450 nm in a microplate reader (MRX, Dynatech Laboratories, Chantilly, USA). The cytotoxicity of the compounds was expressed as the percentage of cell viability measured as WST-1 reduction compared to the controls. Each dose–response curve was recorded for at least 9 parallel dilution series on three different 96 well plates. Dose–response curve parameters and plots were obtained using the drift package (version 0.05–63) for R.[30] Positive controls with carbendazim were checked at regular intervals.

Biodegradation tests

The primary biodegradation test was carried out using a modified version of OECD guideline 301 D.[31] The primary biodegradation of the compound was monitored *via* HPLC-UV for 28 days. The inoculum was obtained from the wastewater treatment plant at Gdańsk (Poland) and aerated for 48 h. A mineral medium containing final concentrations of 8.5 g L^{-1} KH_2PO_4, 21.75 mg L^{-1} K_2HPO_4, 22.13 mg L^{-1} $Na_2HPO_4{\cdot}2H_2O$, 1.7 mg L^{-1} NH_4Cl, 36.4 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 22.5 mg L^{-1} $MgSO_4{\cdot}7H_2O$ and 0.25 mg L^{-1} $FeCl_3$ (pH 7.2) was added to the suspension. The dry weight of the sludge was determined gravimetrically (10 g L^{-1}). Samples containing 200 µM (*ca.* 45 mg L^{-1}) 4-benzyl-4-methylmorpholinium chloride were prepared, as well as blank samples (inoculated media without test substance) and abiotic control samples (inoculated media containing 200 µM 4-benzyl-4-methylmorpholinium chloride poisoned with 50 mg L^{-1} $HgCl_2$), each in replicates. All samples were aerated at 20 °C in the dark during the test. Losses due to evaporation were checked regularly by weighing and made good by the addition of water. The oxygen content was checked as well. For analysis of the biodegradation 1000 µL of all samples were taken at regular intervals and centrifuged (5000 rpm, 15 min, MiniSpin, Eppendorf, Hamburg, Germany). 500 µL of the supernatant were analysed *via* HPLC-UV (212 nm) on a C6-Phenyl column (Gemini 5u C6-Phenyl 110 Å 150 × 4.60 mm). An isocratic method using 7% acetonitrile and 93% 0.1% trifluoroacetic acid and a flow rate of 0.7 mL min^{-1} was applied (retention time for 4-benzyl-4-methylmorpholinium 8.05 min, limit of detection 1 µM, limit of quantification 3 µM). The percentage of biodegradation was determined by integrating the peak area in comparison to the initial concentration (day 0).

The manometric respirometry test was performed according to OECD guideline 301 F.[32] The biological oxygen demand of the substance was determined for 28 days using BOD TRAK (Hach Lange GmbH). The inoculum used was obtained from the wastewater treatment plant at Delmenhorst (Germany). The inoculum was filtered and aerated for 24 h before use. A mineral medium containing final concentrations of 85 mg L^{-1} KH_2PO_4, 217.5 mg L^{-1} K_2HPO_4, 221.3 mg L^{-1} $Na_2HPO_4{\cdot}2H_2O$, 17 mg L^{-1} NH_4Cl, 36.4 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 22.5 mg L^{-1}, $MgSO_4{\cdot}7H_2O$ and 0.25 mg L^{-1} $FeCl_3$ (pH 7.2) was added to the filtrate. Samples containing 20 mg L^{-1} and 100 mg

L^{-1} 4-benzyl-4-methylmorpholinium chloride respectively were prepared, as well as blank samples (inoculated media without test substance). The bottles containing vessels with potassium hydroxide to ensure absorption of the carbon dioxide produced were hermetically sealed and kept in the dark at 20 °C. The oxygen consumption was determined manometrically. The biodegradation of the test substance was calculated by the oxygen uptake for the test substance (corrected by the oxygen demand of blank samples) related to the theoretical oxygen demand (ThOD) of the substance and the amount of substance present in the sample:

$$B = \frac{O_{2(s)} - O_{2(z)}}{C_{(s)} \times ThOD_{(s)}} \times 100\%$$

where:

B is percentage of biodegradation of the test substance, %;

$O_{2(s)}$ is uptake of oxygen in the sample, $mg_{(O_2)}\ L^{-1}$;

$O_{2(z)}$ is the oxygen demand of blank samples, $mg_{(O_2)}\ L^{-1}$;

$C_{(s)}$ is the concentration of substance present in the sample, $mg_{(s)}\ L^{-1}$;

$ThOD_{(s)}$ is the theoretical oxygen demand (ThOD) of the substance, $mg_{(O_2)}\ mg_{(s)}^{-1}$.

Acute oral toxicity test

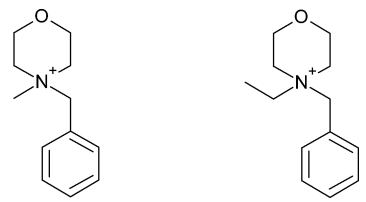
The studies were conducted at the Institute of Industrial Organic Chemistry, Pszczyna Branch, Poland. The toxicity was tested according to the acute toxic class method (OECD No. 420: Method UE B.1.BIS). The studies were started with a preliminary experiment in which **17** was administered once to a female Wistar rat, 171 g body weight (b.w.), at a dose of 2000 mg kg^{-1} b.w. and to another female rat, b.w. of 167 g, at a dose of 300 mg kg^{-1} b.w. **17** was first suspended in distilled water and then administered intragastrically at doses of 300 and 2000 mg kg^{-1} b.w. In the experiment proper, four 9-week-old rats were used (mean body weight 172 g). After the dose was administered, the rats were observed for 14 days.

Dissolution of cellulose in the morpholinium ILs

Microcrystalline cellulose (MCC) was added to a 20 mL vial containing 1.0 g of the dried IL. The vial, sealed with parafilm, was then immersed in an oil bath; the instability of the bath temperature was estimated to be ±1 °C. The mixture was heated at a given temperature (95 °C) and stirred. Additional cellulose was added until the solution became optically clear under a microscope (Bresser Biolux LCD Microscope). When the cellulose became saturated, this was judged to be the point where cellulose could not be dissolved further within 1 h.

Results and discussion

Seventeen new 4-benzyl-4-methylmorpholinium salts (structure of cations in Scheme 1 and of organic anions in Scheme 2) were synthesized *via* anion-exchange reaction but in three different ways (Scheme 3) with high yields (Table 1, only two salts **7** and **9** gave a yield of 70%). Although it was possible to carry out the reactions in aqueous solution, better yields were obtained in an organic solvent. The yield of anion-exchange reaction and total yield for the reaction of quaternization and anion-exchange are showed in Table 1. Potassium or sodium chloride were a by-product of the exchange reaction according to methods I and III, and were removed from anhydrous acetone solution. In method II hydrochloric acid was the by-product; it was removed by passing dry air, which was then



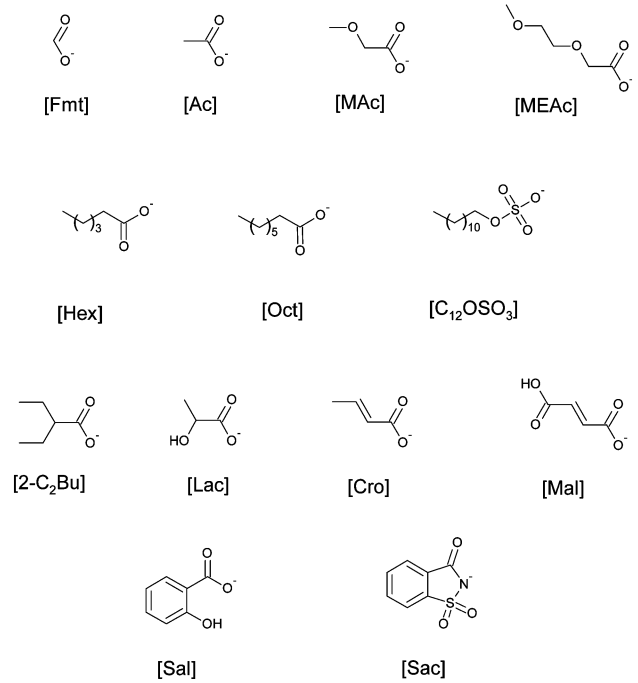
[BMmorf] [BEmorf]

Scheme 1 Structure of cations.

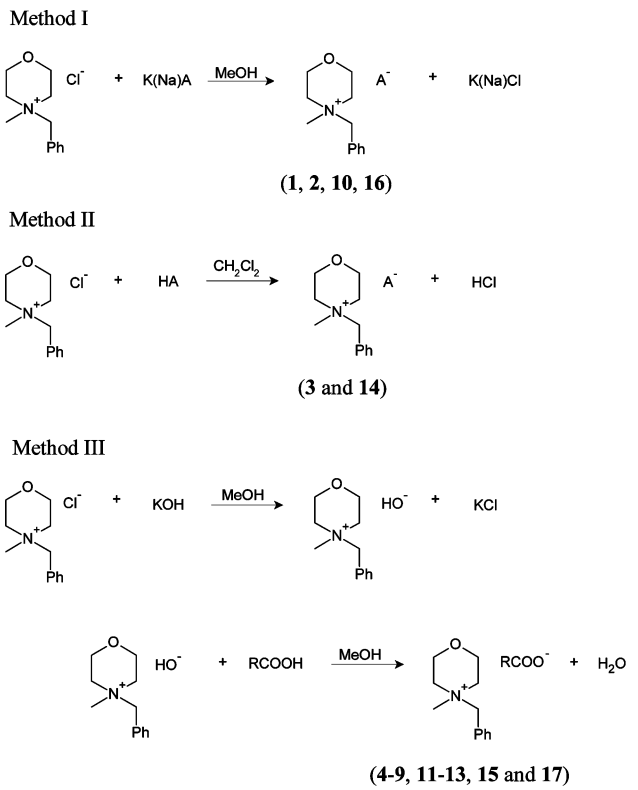
Table 1 4-Benzyl-4-methylmorpholinium [BMmorf] (**1–16**) and 4-benzyl-4-ethylmorpholinium [BEmorf] (**17**) salts

		Yield/%				
Salt	Anion	Metathesis	Total	mp[a]/°C	Viscosity[c]/Pa s	pH[d]
1	Nitrate, [NO_3]	88.0	83.0	Liquid	143.64	7.18
2	Tetrafluoroborate, [BF_4]	83.0	78.5	88–92[b]	—	2.81
3	Hydrogen sulfate, [HSO_4]	94.0	89.0	Liquid	8.31	0.87
4	Formate, [Fmt]	83.0	78.5	Wax	—	6.84
5	Acetate, [Ac]	90.5	85.5	Liquid	5.81	8.33
6	Methoxyacetate, [MAc]	85.5	81.0	Wax	—	3.98
7	2-(2-Methoxyethoxy)acetate, [MEAc]	70.0	66.0	Wax	—	4.43
8	Hexanoate, [Hex]	86.0	81.5	Liquid	5.41	5.48
9	Octanoate, [Oct]	70.5	66.5	Liquid	0.49	6.28
10	Dodecyl sulfate, [$C_{12}OSO_3$]	98.5	93.0	65–68	—	7.62
11	2-Ethylbutyrate, [2-C_2Bu]	83.0	78.5	Liquid	6.12	5.29
12	Lactate, [Lac]	89.0	84.0	Liquid	4.16	7.33
13	Crotonate, [Cro]	98.0	92.5	Wax	—	5.22
14	Maleate, [Mal]	93.0	88.0	Liquid	11.54	2.48
15	Salicylate, [Sal]	88.0	83.0	Liquid	80.51	3.15
16	Saccharinate, [Sac]	88.0	83.0	Wax	—	4.48
17	Acetate, [Ac]	84.5	80.0	Liquid	0.33	5.25

[a] Melting point determined by hot-plate apparatus. [b] Crystallized from water. [c] Measured at 25 °C. [d] For concentration 100 mM.



Scheme 2 Structure of organic anions.



Scheme 3 Scheme of the synthesis methods.

reacted with potassium hydroxide in a trap until the colour of phenolphthalein disappeared. This method was used for acids, whose pK_a did not exceed 2 (sulphuric acid pK_a = 1.99, maleic acid pK_{a1} = 1.93[33,34]).

Table 2 Chemical shifts

	^{1}H NMR		^{13}C NMR
IL	$^+$N-C$\underline{H}_3$	$^+$N-C$\underline{H}_2$-Ph	$^+$N-$\underline{C}H_2$-Ph
[BMmorf][Cl]	3.10	4.81	67.3
2	3.06	4.68	67.7
3	3.10	4.77	67.6
5	3.15	4.88	67.2
8	3.15	4.88	67.2
11	3.14	4.85	67.3
12	3.29	4.94	68.9
13	3.26	4.83	69.1
16	3.28	4.91	69.3

Of the seventeen synthesized salts, ten were liquids, two were crystalline and the other five were a wax. The liquid products were dried under vacuum and stored over P_4O_{10}. The synthesized salts were air and moisture stable and soluble in water, methanol, dichloromethane (except **3** and **14**) and acetone. They were immiscible with hexane and toluene. The water content, determined by Karl-Fischer measurements, was found to be less than 500 ppm. All the morpholinium salts obtained were ILs with melting points below 100 °C and were characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental CHN analysis. In the ^{1}H NMR spectra, proton chemical shifts as large as 0.25 ppm were observed for the protons located around the quaternary nitrogen atom (see Table 2). On the other hand, the ^{13}C NMR spectra indicated weak variation in the carbon signal shifts at the level of 2 ppm (Table 2).

The anions significantly determined the state of aggregation on morpholinium ILs. By selecting an appropriate anion, it was possible to obtain an IL resembling water (low viscosity), a viscous liquid, wax or a solid substance with a high melting point. Correspondingly, the viscosity of the liquid ILs at room temperature (Table 1) varied widely over a wide range from 0.33 for **17** to 143.64 Pa s for **1**. The value was strongly temperature-dependent, as evident from Fig. 1. The results show how strongly the viscosity, measured at room temperature, depended on the type of anion. With increasing temperature the effect of the anion on viscosities was overridden and similar for each counterion (Fig. 1). The dependence of shear stress *versus* shear rate at 25 °C was rectilinear, so morpholinium ILs exhibited the character of a Newtonian liquid.

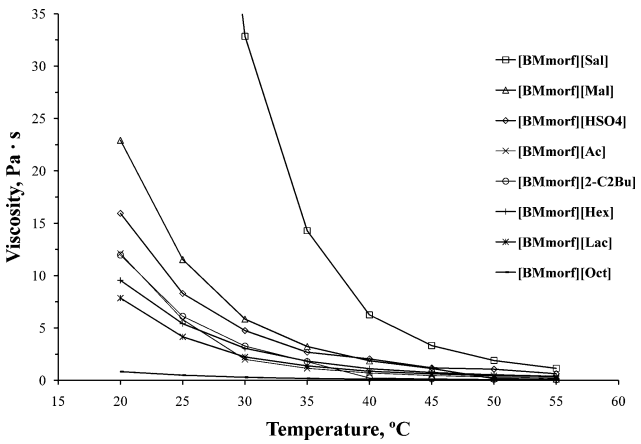


Fig. 1 Viscosity of the liquid compounds.

Table 3 Thermal stability and phase transition of the ILs

IL	T_g[a]/°C	T_m[b]/°C	$T_{onset(5\%)}$[c]/°C	$T_{onset(50\%)}$[d]/°C	T_1[e]/°C
1	−19.4	—	196	250	—
2	−1.3	88–92	240	312	—
4	−52.4	—	114	178	—
5	−56.7	—	107	175	—
6	−57.6	—	95	189	—
7	−57.3	—	116	247	247 (50.0)
8	−55.7	—	100	180	189 (47.3)
9	−56.2	—	112	210	191 (55.8)
10	−42.0	74–77	226	276	—
11	−44.8	—	120	193	185 (45.5)
12	−34.8	—	146	198	—
13	−29.8	—	113	184	—
14	−43.4	—	115	236	—
15	−12.4	—	145	223	220 (49.8)
16	8.6	—	182	280	255 (57.5)
17	−56.6	—	120	172	—

[a] Glass transition temp. determined by DSC. [b] Melting point determined by DSC. [c] Decomposition temp. determined from onset to 5 wt% mass loss. [d] Decomposition temp. determined from onset to 50% mass loss. [e] Second step decomposition (percentage decomposition in parentheses).

Since the ILs synthesized were soluble in water, it was decided to measure the pH of their 100 mM solutions in water. The results are listed in Table 1. Following dissolution in water, morpholinium ILs with the anions presented in Scheme 2 yielded solutions with pH values ranging from 8.33 to 0.87. The pH values for 4-benzyl-4-methylmorpholinium salts changed in the following sequence:

$$[Ac] > [C_{12}OSO_3] > [Lac] > [NO_3] > [Fmt] > [Oct] > [Cl]\ (pH = 5.76) > [Hex] > [2\text{-}C_2Bu] > [Cro] > [Sac] > [MEAc] > [MAc] > [Sal] > [BF_4] > [Mal] > [HSO_4].$$

To a large extent the pH of the aqueous solution was determined by the anion.

As shown in Table 3, the thermal stability of morpholinium ILs also depended on the anion. The most stable ILs were those with $[BF_4]$ (**2**) and $[C_{12}OSO_3]$ (**10**) anions, where decomposition started above 200 °C. In six morpholinium ILs, a second decomposition step was observed – in **7** at 247 °C (50% mass loss), in **8** at 189 °C (47% mass loss), in **9** at 191 °C (56% mass loss), in **11** at 185 °C (46% mass loss), in **15** at 220 °C (50% mass loss), and in **16** at 255 °C (58% mass loss). A glass transition was observed with almost every salt. The melting point was observed in two morpholinium ILs.

For a first toxicity evaluation we investigated the antimicrobial and *in vitro* toxicity with IPC-81 cells isolated from rats. These test systems provide reproducible results for measuring the acute toxicity of ILs, and many data are available for comparison.[35–37]

For four ILs (**6**, **10**, **12**, **17**) and 4-benzyl-4-methylmorpholinium chloride the determined values of MIC, MBC and MFC are listed in Table 4. The IL with the dodecyl sulphate anion (**10**) was slightly more effective than IL with lactate (**12**) because of its long alkyl chain. Summing up, the activity of morpholinium salts against bacteria and fungi was very low. This was confirmed by tests on the antimicrobial activity of 4-carbalkoxymethyl-4-methylmorpholinium chlorides.[38]

The cytotoxicity of the compounds synthesized to the IPC 81 rat leukaemia cell line is summarized in Table 5. We used the concept of "anion effect ratio" to classify relative toxicities of anions in ILs.[11,39] It is defined the ratio (AR) of the EC_{50} value of the chloride-containing reference ionic liquid (here 4-benzyl-4-methylmorpholinium chloride) and the EC_{50} value measured for the same cation combined with a different anion. Anions in ionic liquids with AR values smaller than 5 are considered as non-cytotoxic or only marginal cytotoxic. In contrast, anions exhibiting AR values greater than 5 are considered as significantly influencing the cytotoxicity of the corresponding IL. In general, all the compounds showed a cytotoxicity level from 0.25 to 14.13 mM and AR vaules from 1 to 43. Comparison of 4-benzyl-4-methylmorpholinium chloride (EC_{50} 11 mM) with 1-methyl-3-octylimidazolium chloride (EC_{50} 0.10 mM[28]) shows that the cytotoxicity of the morpholinium cation itself is low.

The cytotoxicity effect is dramatically changed by the anion to lower EC_{50} and higher AR values. When combining the 4-benzyl-4-methylmorpholinium cation with nitrate $[NO_3]$, hydrogen sulphate $[HSO_4]$, formate [Fmt], acetate [Ac], methoxyacetate [Mac], 2-(2-methoxyethoxy)acetate [MEAc], hexanoate [Hex], 2-ethylbutyrate [2-C_2Bu], lactate [Lac], crotonate [Cro] or saccharinate [Sac]] anion, the cytotoxicity of the IL is not considerably increased (AR values < 5).

Whereas the combination with, tetrafluoroborate $[BF_4]$, octanoate [Oct], dodecyl sulphate $[C_{12}OSO_3]$, maleate [Mal], and salicylate [Sal], gives a lower EC_{50} values and AR values ranging from 5 to 43. The low EC_{50} value of the dodecyl sulphate anion can be related to the long alkyl side chain, which is responsible for the high lipophilicity. The cytotoxicity of sodium dodecyl sulphate was already known to be 0.5 μM;[24] the results obtained in this study verified this value. Surprisingly, the IL containing $[BF_4]$ anion also had a low EC_{50} value (AR 26). Toxicity studies of this anion combined with an inorganic cation have indicated its much lower toxicity.[11]

The acute toxicity values listed in Fig. 2 indicate that substitution of chloride by an organic anion containing two oxygen atoms results in decreased toxicity, whereas three or more oxygen atoms in the anion or the presence of a linear alkyl group containing five or more carbon atoms, or the presence of an aromatic ring is associated with an increase in toxicity.

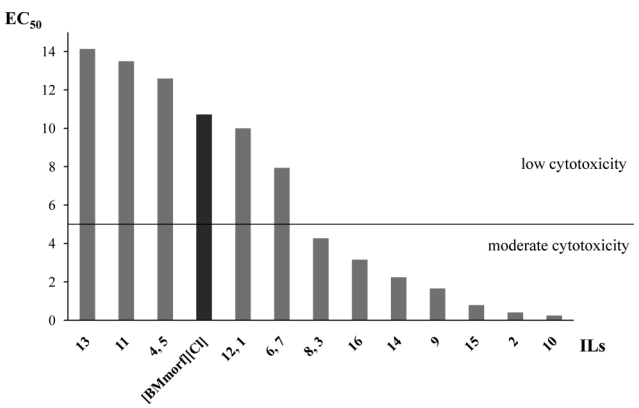


Fig. 2 Acute *in vitro* cytotoxicity of the ILs and 4-benzyl-4-methylmorpholinium chloride (EC_{50} values in mM).

The acute oral toxicities of 4-benzyl-4-ethylmorpholinium acetate (**17**) was determined in six female Wistar rats, which

Table 4 MIC[a], MBC[b] and MFC[c] values[d] for the ILs and [BMmorf][Cl][e]

Strain		ILs				
		6	**10**	**12**	**17**	[BMmorf][Cl][e]
M. luteus	MIC	>1.8	0.5	>1.8	>1.9	>2.2
	MBC	>1.8	0.5	>1.8	>1.9	>2.2
S. aureus	MIC	>1.8	0.3	0.9	>1.9	>2.2
	MBC	>1.8	0.3	>1.8	>1.9	>2.2
S. epidermidis	MIC	>1.8	0.3	0.9	>1.9	>2.2
	MBC	>1.8	>1.1	>1.8	>1.9	>2.2
E. faecium	MIC	0.9	0.5	0.9	>1.9	>2.2
	MBC	>1.8	>1.1	0.9	>1.9	>2.2
M. catarrhalis	MIC	0.9	0.1	0.9	0.9	1.1
	MBC	0.9	0.3	0.9	0.9	1.1
E. coli	MIC	>1.8	0.3	0.9	0.9	1.1
	MBC	>1.8	0.3	>1.8	>1.9	>2.2
S. marcescens	MIC	>1.8	>1.1	>1.8	>1.9	>2.2
	MBC	>1.8	>1.1	>1.8	>1.9	>2.2
P. vulgaris	MIC	>1.8	>1.1	0.9	>1.9	>2.2
	MBC	>1.8	>1.1	>1.8	>1.9	>2.2
P. aeruginosa	MIC	>1.8	>1.1	>1.8	>1.9	>2.2
	MBC	>1.8	>1.1	>1.8	>1.9	>2.2
B. subtilis	MIC	0.9	0.3	0.9	0.9	1.1
	MBC	0.9	0.3	0.9	0.9	1.1
C. albicans	MIC	>1.8	>1.1	>1.8	>1.9	>2.2
	MFC	>1.8	>1.1	>1.8	>1.9	>2.2
R. rubra	MIC	>1.8	0.5	>1.8	>1.9	>2.2
	MFC	>1.8	>1.1	>1.8	>1.9	>2.2

[a] minimum inhibitory concentration; [b] minimum bactericidal concentration; [c] minimum fungicidal concentration; [d] in mM; [e] 4-benzyl-4-methylmorpholinium chloride.

Table 5 EC_{50} values for the acute toxicity of IL towards IPC-81 cells

IL	anion	EC_{50}/mM	Anion effect ratio (AR)
	[Cl]	10.72	1
1	[NO_3]	10.00	1
2	[BF_4]	0.41	26
3	[HSO_4]	4.37	3
4	[Fmt]	12.59	1
5	[Ac]	12.59	1
6	[MAc]	7.94	1
7	[MEAc]	7.76	1
8	[Hex]	4.27	3
9	[Oct]	1.66	7
10	[$C_{12}OSO_3$]	0.25	43
11	[2-C_2Bu]	13.49	1
12	[Lac]	>10.00	<1
13	[Cro]	14.13	1
14	[Mal]	2.24	5
15	[Sal]	0.79	14
16	[Sac]	3.16	3

received a dose of 300 mg kg^{-1} b.w. (mg of substance per kg of body weight) and 2000 mg kg^{-1} b.w. of **17**. In the preliminary experiment, a single administration of **17** to a rat at a dose of 2000 mg kg^{-1} b. w. was followed within 30 min by development of clinical signs, which persisted until the rat died two hours later. In the experiment proper, following a single administration of **17** at a dose of 300 mg kg^{-1} b.w. to four other rats no signs of toxicity were observed. In the second week of the experiment two of the animals demonstrated a slight decrease in body weight, while the remaining animals gained weight. The above results indicate that the acute toxicity range for **17** is between 300–2000 mg kg^{-1} b.w. in female rats (Imp: WIST – outbred strain). This IL would thus be classified as a category 4 (R22 Harmful if swallowed) toxin, according to standard GHS grading.

Compared to the imidazolium and pyridinium ILs[5] described, the low biological activity of morpholinium ILs and the moderate oral acute toxicity towards rats deserves attention: this trait significantly influences the application of morpholinium-based ILs.

The biodegradability of 4-benzyl-4-methylmorpholinium cation combined with chloride has been tested in primary biodegradation and oxygen consumption tests. As neither test showed any biodegradation of the cation, the cation cannot be classified as readily biodegradable. It does not necessarily follow that it will not degrade in the environment, but further testing (*e.g.* for inherent biodegradation) will be necessary to clear this initial suspicion of persistence. Also, the introduction of functionalized side chains instead of the phenyl group could help to improve biodegradability.

Dissolution of cellulose was tested for six morpholinium ILs with carboxylate anions – **4**, **5**, **7**, **8**, **9** and **17**. Because of the low stability of ILs, the dissolution was carried out at 95 °C. All compounds showed the ability to dissolve microcrystalline cellulose but needed different times to dissolve 1% of cellulose: for example, **7** mixed with cellulose for 3 h showed a significant fragmentation of cellulose pulp and partial dissolution of cellulose. Morpholinium acetate dissolved cellulose best, and elongation of the alkyl chain in the cation shortened the dissolution time. As shown in Fig. 3, 1% cellulose dissolved completely after just 0.5 h of heating the cellulose with 4-benzyl-4-ethylmorpholinium acetate, **17**. This means that the morpholinium ILs have the ability to dissolve cellulose, thus forming a new class of compounds able to dissolve cellulose.[40]

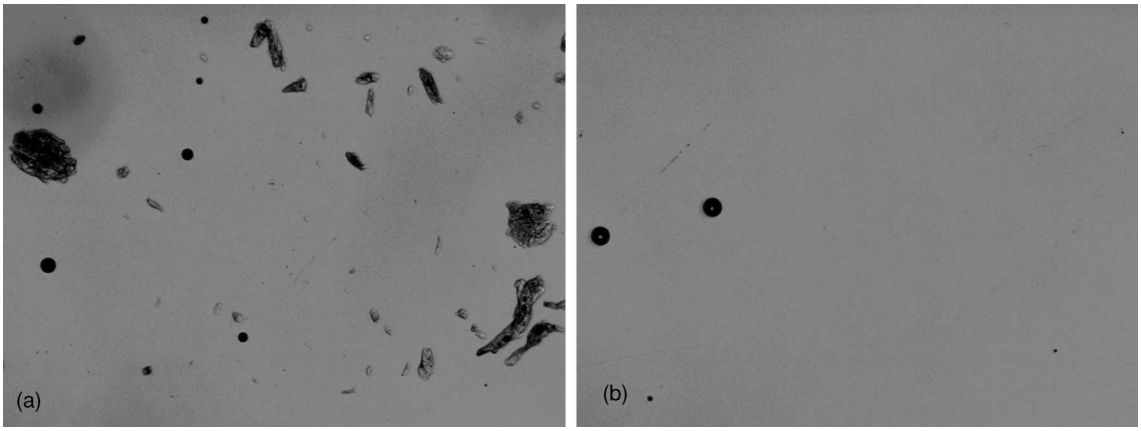


Fig. 3 Micrographs of 1% of microcrystalline cellulose dissolved in 4-benzyl-4-ethylmorpholinium acetate - **17** (a) after adding cellulose, (b) after 0.5 h of stirring (temperature: 95 °C; fourfold enlargement).

Conclusions

By using an anion-exchange reaction, a number of new morpholinium ILs were synthesized. Optimal methods of synthesizing various salts were developed to obtain the highest possible yield. All the salts obtained are stable in air, water and commonly used organic solvents. The anions determined the state of aggregation on morpholinium ILs to a significant extent. By selecting an appropriate anion it was possible to obtain an IL resembling water, a viscous liquid, wax or a solid salt, and also to regulate thermal stability.

The activity of 4-benzyl-4-methylmorpholinium ILs against bacteria and fungi was very low. The cytotoxicity of these compounds can be classified as moderate or low, but may vary dramatically – from 0.25 to 14.13 mM – depending on the anion. The acute toxicity of 4-benzyl-4-ethylmorpholinium acetate is between 300–2000 mg kg^{-1} b.w. in female rats. This IL would thus be classified as a category 4 (R22 Harmful if swallowed) toxin, according to standard GHS grading. The biodegradation tests cannot classify the 4-benzyl-4-methylmorpholinium cation as readily biodegradable. However, this does not mean that these compounds will not decompose in the environment, only that to determine their behaviour in the environment other, more comprehensive tests should be carried out.

In addition, 4-benzyl-4-ethylmorpholinium acetate, because it has the lowest viscosity, can be effectively used as a solvent for biomass.

Acknowledgements

This work was supported by the Foundation for Polish Science, academic grants for professors, MISTRZ Programme, 2009 edition. This research was also supported by the Polish Ministry of Research and Higher Education under grants DS 8200-4-0085-11 and SPB/260/7PR/2010.

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