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Supplementary Information

Controlled Fragrance Delivery in Functionalised Ionic Liquid-Enzyme Systems

Marijana Blesic,* H. Q. Nimal Gunaratne, Peter Nockemann, Philip McCarron, and Kenneth R. Seddon

mblesic@chem.polimi.it

The QUILL Centre, School of Chemistry and Chemical Engineering, The Queen's University of Belfast, Stranmillis Road, Belfast BT9 5AG, United Kingdom.

Experimental

Materials: 1-Butylimidazole (99%), imidazole (99%), dichloromethane, N,N-dimethylformamide, chloroacetyl chloride, racemic menthol (≥99%), 1-chlorooctane (99%), 1-chlorotetradecane (99%), N,N-dimethyloctylamine, (≥99%), N,N-dimethylaniline (≥99.5%) and pyrene were purchased from Aldrich and used as received. Lipase B from *Candida Antarctica* was kindly provided by Almac, Belfast. Double distilled deionised water was obtained from a Bernstead Nanopure water purification system.

Synthesis: The four ionic liquids used in this study were synthesised following the general procedure described below.

(±) Menthyl chloroacetate: (±) Menthol (0.2 mol) and N,N-dimethylaniline (0.2 mol) were dissolved in dichloromethane (100 cm^3), placed in a round-bottomed flask, and stirred vigorously. The mixture was cooled in an ice-bath, and chloroacethyl chloride (0.2 mol) dissolved in dichloromethane (50 cm^3) was added dropwise to maintain the reaction temperature close 0^oC. The reaction mixture was then stirred for 2h at room temperature. Dichloromethane was removed using a rotary evaporator to yield the crude liquid product. To the crude product was partitioned with cyclohexane (60 ml) and water (40 ml). After separation, organic layer was washed firstly with 10% H_2SO_4 (50 cm^3), and afterwards with saturated $NaHCO_3$ aqueous solution (30 cm^3) and then dried over anhydrous $MgSO_4$. Cyclohexane was removed using a rotary evaporator, and product was purified by fractional vacuum distillation to yield a colourless liquid. Yield 62%.

1-Alkylimidazole: Imidazole (1 mol eq), 1-chloroalkane (1 mol eq.) and K_2CO_3 (2 mol eq.) were dissolved in dry N,N-dimethylformamide and stirred vigorously in round-bottomed flask at 80 ^oC for two days. Solid precipitate obtained after cooling the reaction mixture was removed by filtration, and N,N-dimethylformamide removed on a rotary evaporator. A small amount of hexane was added (20ml) and the mixture was heated and then left to cool down. Solid precipitate was filtered off, hexane removed on rotary evaporator, and the product dried under vacuum at 60 ^oC. Yield 72%.

$[C_4menim]Cl$, $[C_8menim]Cl$, $[C_{14}menim]Cl$: Menthol ester (1 mol eq) and 1-alkylimidazole (1 mol eq) were placed in a round-bottom flask and dissolved in acetonitrile (150 cm^3). The reaction mixture was stirred under reflux for 24 hours. Upon completion of the reaction, acetonitrile was removed and diethylether was added. White

solid precipitate was formed, filtered off, and dried under vacuum.

[$N_{1\ 1\ 8}$men]Cl: Menthol ester (15mmol) and N,N-dimethyloctylamine (15mmol) were placed in a screw-cap tube and dissolved in acetonitrile ($3cm^3$). The reaction mixture was stirred under reflux for 18 hours at 60°C. Upon completion of the reaction, acetonitrile was removed and diethylether was added. White solid precipitate was formed, filtered off, and dried under vacuum. Yield 81%.

^1H and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance spectrometer DPX 300, using $CDCl_3$ as solvent.

[C_4menim]Cl

^1H δ ($CDCl_3$, 400 MHz):10.58 (1H, s, NC**H**N), 7.48, 7.41 (2H, s, s, NC**H**C**H**N), 5.44 (2H, q, NC**H_2**CH_2), 4.78 (1H, m, OC**H**), 4.33 (2H, t, OCC**H_2**N), 2.03 (1H, m, C**H**$(CH_3)_2$), 1.92 (2H, m (NCH_2C**H_2**), 1.85 (1H, m, CH_2C**H(**$CH)_2$), 1.69 (2H, d, (CHC**H_2**CH), 1.41 (4H, m, (CH(C**H_2**$)_2$CH,), 1-0.89 (12H, m, (C**H(**$CH_2)_2CH_3$,**CH_2CH_3,** CH(C**H_3**$)_2$) 0.76 (3H, d, (C**H_3**CH)).

^{13}C δ ($CDCl_3$, 101 MHz): (166.2, 139.2, 123.8, 121.75, 50.9, 50.6, 50.5, 47.2, 50.0, 34.3, 32.41, 31.8, 26.5, 23.6, 22.3, 221.8, 19.9, 16.6, 13.8.).

For $C_{19}H_{33}ClN_2O_2 \cdot H_2O$ C 60.86, H 9.41, N 7.47; Found C 60.91, H 8.97, N 7.38.

Melting point 52°C.

[C_8menim]Cl

^1H δ ($CDCl_3$, 400 MHz):10.91 (1H, s, NC**H**N), 7.39, 7.25 (2H, s, s, **NCHCHN**), 5.45 (2H, q, NC**H_2**), 4.78 (1H, m, OC**H**), 4.30 (2H, t, OCC**H_2N**), 2.02 (1H, m, C**H**$(CH_3)_2$), 1.92 (2H, m (NCH_2C**H_2**), 1.79 (1H, m, CH_2C**H(**$CH)_2$), 1.69 (2H, d, (CHC**H_2**CH), 1.47-1.26 (11H, brd, (CH(C**H_2**$)_2$CH, C**H(**$CH_2)_2CH_3$, $N(CH_2)_2$(C**H_2**$)_3$). 1.2 (2H, m, (C**H_2**CH_2CH_3) 0.93-0.85 (11H, brd, (C**H_2**C**H_3**, CH(C**H_3**$)_2$) 0.75 (3H, d, (C**H_3**CH)).

^{13}C δ ($CDCl_3$, 101 MHz): (165.9, 138.9, 123.5, 121.3, 50.4, 50.3, 46.8, 40.7, 34.0, 31.7, 31.5, 30.2, 29.1, 28.9, 29.0, 26.3, 26.2, 23.3, 22.6, 22.0, 20.1, 16.3, 14.11).

For $C_{23}H_{41}ClN_2O_2 \cdot H_2O$ C 64.09, H 10.05, N 6.50; Found C 64.55, H 10.05, N 6.49.

Melting point 76°C.

[C_{14}menim]Cl

^{1}H δ (CDCl$_3$, 400 MHz):10.53 (1H, s, NC**H**N), 7.54, 7.45 (2H, s, s, **NCHCHN**), 5.47 (2H, q, NC**H$_2$**), 4.78 (1H, m, OC**H**), 4.31 (2H, t, OCC**H$_2$N**), 2.05 (1H, m, C**H**(CH$_3$)$_2$), 1.95 (2H, m (NCH$_2$C**H$_2$**), 1.81 (1H, m, CH$_2$C**H**CH), 1.69 (2H, d, (CHC**H$_2$**CH), 1.46-1.25 (23H, brd, (CH(C**H$_2$**)$_2$CH, C**H(**CH$_2$)$_2$CH$_3$, N(CH$_2$)$_2$(C**H$_2$**)$_9$). 1.21 (2H, m, C**H$_2$**CH$_2$CH$_3$) 0.92-0.85 (11H, brd, (C**H$_2$**C**H$_3$**, CH(C**H$_3$**)$_2$) 0.76 (3H, d, (C**H$_3$**CH)).

^{13}C δ (CDCl$_3$, 101 MHz): (165.8, 138.6, 123.5, 121.4, 50.3, 50.2, 46.7, 40.6, 33.9, 31.9, 31.4, 30.2, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.0, 26.2, 26.1, 23.2, 22.6, 21.9, 20.7, 16.2, 14.1).

For $C_{29}H_{53}ClN_2O_2 \cdot H_2O$ C 67.61, H 10.76, N 5.44. Found C 68.35, H 10.36, N 5.16.

Melting point 93°C.

[$N_{1\,1\,8}$men]Cl

^{1}H δ (CDCl$_3$, 400 MHz): 4.92(1H, d, CO**CH$_2$**N), 4.80(1H, m, OC**H**), 4.58(1H, d, CO**CH$_2$**N), 3.78 (2H, m, -COCH$_2$N**CH$_2$**), 3.68, 3.66 (6H, s,s, 2x**CH$_3$**N), 1.97(1H, brd, C**H**-ring), 1.79(2H,m, NCH$_2$**CH$_2$**), 1,71-1.26(14H, brm, ring & chain), 1.05(2H, q, **CH$_2$**-chain), 0.90(11H, m, **CH$_3$**,**CH$_2$**,(**CH$_3$**)$_2$,0.75(3H, d, **CH$_3$**CH)

^{13}C δ (CDCl$_3$, 101 MHz): 164.5, 64.7, 61.1, 52.4, 52.3, 46.8, 40.7, 33.9, 31.7, 31.6, 29.2, 29.1, 26.4, 26.3, 23.3, 23.1, 22.7, 21.9, 20.9, 16.2, 14.2

For $C_{22}H_{44}ClNO_2 \cdot 0.5H_2O$ C 66.21, H 11.37, N 3.51; Found C 66.03, H 10.82 N 3.43.

Melting point 75°C.

Fluorescence Spectroscopy. For the fluorescence measurements, aqueous stock solutions of four ionic liquids were prepared using water containing 8.7×10^{-8} M pyrene, and all studied solutions were prepared from the stock solutions, by diluting with the same pyrene aqueous solution. Pyrene was used as a fluorescent probe to ascertain the onset of the aggregation of the ionic liquids in water. Steady-state fluorescence spectra of the pyrene containing solutions in 1 cm quartz cuvettes were recorded at room temperature with a Perkin–Elmer LS55 luminescence spectrometer equipped with a R928 photomultiplier. Excitation was set to a wavelength of 337 nm. Intensities of first ($I1$) and third ($I3$) vibronic bands in the pyrene emission spectra located around 373 and 384 nm, respectively, were measured and used to determine the ratio $I3/I1$.

Interfacial Tension (IFT). Interfacial tensions were measured using a Drop Shape Analysis Tensiometer (Contact Angle System OCA, Carl Stuart Ltd) working in the pendant drop mode at a constant temperature of 23±2 ºC. IFT is derived from the fit of the pendant drop profile. The drops were left to equilibrate close to the rupture point and at least three consistent measurements per solution were recorded.

Enzyme activity. Ester hydrolysis rate was determined by measuring the concentration of the released menthol. A typical procedure was as following: samples of certain amount of ionic liquid were dissolved in 100ml phosphate buffer at pH 7.5. Then, 150mg of the liquid enzyme was incubated, quickly mixed and the prepared solution was distributed in 10ml vials. These vials were left under a gentle agitation at room temperature, and periodically withdrawn for analysis. 2ml of cyclohexane was added to the solution to be analysed, and mixed well. After centrifugation clear cyclohexane solution with menthol was analysed by GC chromatography. The menthol concentration was determined by comparing the sample's peak area with a calibrated curve.

GC Method

Analysis was performed on Agilent Technologies 6890N GC system with FID and autosampler. A cyclodextrin column, as supplied by BGB Analytik AG (p/n: 27430-025, 30 m x 0.25 mm, 0.25 µm film) was used for chiral separation. Method test parameters include inlet temperature of 280 ^{o}C, makeup flow of 1 ml min^{-1}, and initial oven temperature of 100 ^{o}C, ramped at 2 ^{o}C min^{-1}.

Crystallography

Single crystals of $C_{20}H_{44}N_2O_2Cl$ were obtained after slow evaporation of the compound from an aqueous solution. A suitable crystal was selected and measured on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 120 K during data collection. Using Olex2[1], the structure was solved with the ShelXS[2] structure solution program using Direct Methods and refined with the ShelXL[3] refinement package using Least Squares minimisation.[4]

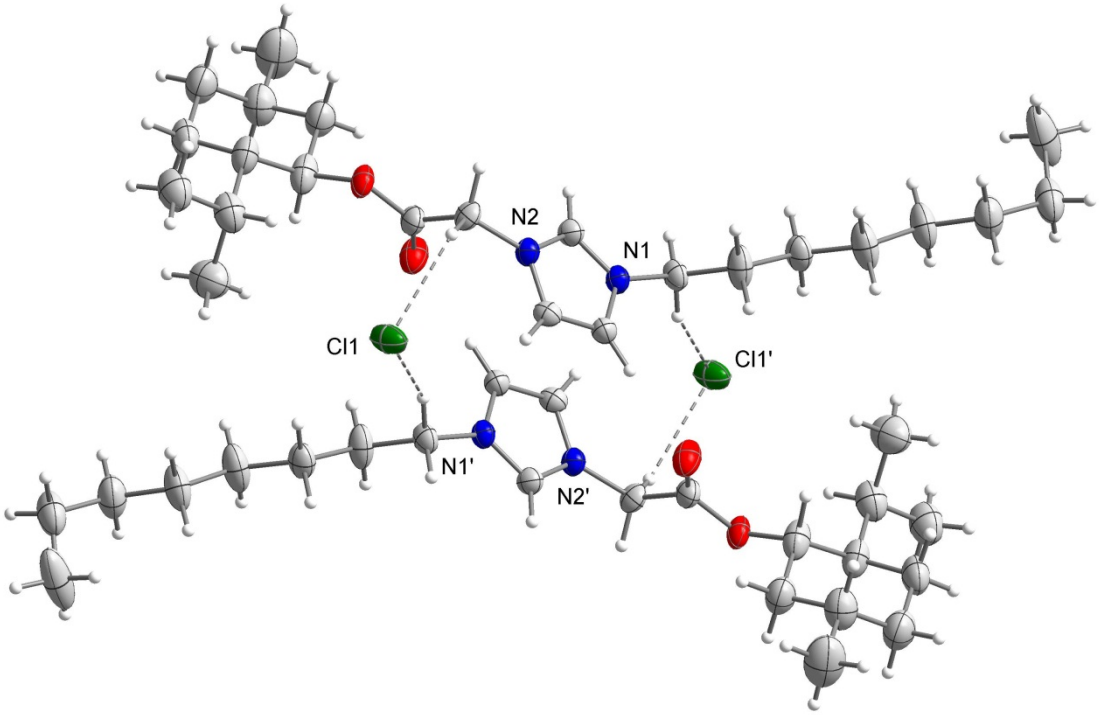

Figure S1: Hydrogen bonding interactions in the crystal structure of [C_8menim]Cl.

Crystal Data. $C_{23}H_{41}ClN_2O_3$, M =429.03, triclinic, a = 8.663(3) Å, b = 10.555(4) Å, c = 15.180(6) Å, α = 72.18(2)°, β = 80.99(3)°, γ = 74.98(3)°, V = 1271.8(8) Å^3, T = 120, space group P-1 (no. 2), Z = 2, μ(Molybdenum) = 0.174, 12742 reflections measured, 5796 unique (R_{int} = 0.0405) which were used in all calculations. The final wR_2 was 0.2694 (all data) and R_1 was 0.0927 (>2sigma(I)).

1. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program. *J. Appl. Cryst.* (2009). 42, 339-341.
2. SHELXS-97 (Sheldrick, 1990).
3. SHELXL, G.M. Sheldrick, *Acta Cryst.* (2008). A64, 112-122.
4. S. J. Coles, P. A. Gale, *Chem. Sci.,* 2012, **3** (3), 683-689.