Electronic Supplementary Information

Experimental procedures

General
Reagents and enzymes were purchased from Sigma-Aldrich, except where described below. Ionic liquids were purchased from Solvent Innovation (Köln, Germany). SABP2 was expressed and purified according to Kumar, et al.1. PFE was expressed and purified according to Cheeseman, et al.2. In all cases, blank reactions were run alongside the enzymatic reactions and gave negligible conversion, with the exception of ChCl:MA, as shown in Table 1 of the text.

Gas chromatography
The detector and injector temperatures were 275 ºC and 250 ºC, respectively. Analysis of transesterification and aminolysis products was performed using a 30 m HP-5 column (J&W Scientific, Folsom, CA) with 0.32 mm inner diameter and 0.25 μm film size. The initial column temperature of 60 ºC was held for 6 min, then increased to 165 ºC at 15 ºC min⁻¹, then further increased to 200 ºC at 25 ºC min⁻¹ and held at 200 ºC for 5 min. Products of styrene oxide hydrolysis were analyzed on a 25 m CP 7502 column (Varian) with 0.25 mm inner diameter and 0.25 μm film size. The initial column temperature of 50 ºC was held for 5 min, then increased to 200 ºC at 10 ºC min⁻¹ held at 200 ºC for 5 min.

Transesterification reaction
Enzyme (2.0 mg solid; note that iCALB contains only ~10 wt% protein) was suspended in solvent (0.2 ml) in a glass vial. Ethyl valerate (40 mM) and butanol (400 mM) were added to the suspension and the resulting mixture was stirred at 60 ºC for up to 24 h. The reaction products were extracted with toluene (1.0 ml) and analyzed by GC.

Aminolysis reaction
Enzyme (2.0 mg solid; note that iCALB contains only ~10 wt% protein) was suspended in solvent (0.2 ml) in a glass vial. Ethyl valerate (100 mM) and butylamine (110 mM) were added to the suspension and the resulting mixture was stirred at 60 ºC for up to 24 h. The reaction products were extracted with toluene (1.0 ml) and analyzed by GC.

Hydrolysis of p-nitrophenyl acetate
A 0.5 mg protein ml⁻¹ solution in 5 mM BES at pH 7.2 (10 μl) was added to a p-nitrophenyl acetate solution (30 mM, 90 μl), acetonitrile (7.4 % v/v), and a mixture of 5 mM BES at pH 7.2 and ChCl:Gly. The final DES volume fraction was 0, 0.1, 0.25, 0.5, 0.75, or 0.9. The absorbance was monitored at 404 nm at 6-second intervals with a microplate reader (SpectraMax 384 Plus, Molecular Devices) at room temperature for up to 40 minutes.

Hydrolysis of styrene oxide
A mixture (0.2 ml) of styrene oxide (100 mM), 5 mM BES at pH 7.2, and sufficient ChCl:Gly to give DES volume fractions of 0, 0.1, 0.25, 0.5, 0.75, and 0.9 was prepared. A 10 mg ml⁻¹ protein solution in 5 mM BES at pH 7.2 (2 μl) was added to the suspension to start the reaction. The reaction mixture was incubated at 37 ºC for 2 h, after which the mixture was extracted with ethyl acetate (1.0 ml) and analyzed by GC.

Polarity estimation
Reichardt’s dye (2,6-diphenyl-4-(2,4,6-triphenylpyridinio)phenolate, 0.4 mg) was dissolved in DES (0.5 ml). An aliquot was transferred to a 96-well microplate. The wavelength of the absorption maximum of the long-wavelength transition (λmax) was measured at room temperature using a Spectra Max 384 Plus. Normalized polarity values (EtN) were calculated from the equation:

\[
EtN = \frac{E_t(\text{solvent}) - E_t(\text{TMS})}{E_t(\text{water}) - E_t(\text{TMS})} = \frac{E_t(\text{solvent}) - 30.7}{32.4}
\]

where \(E_t(\text{solvent})\) is the energy (kcal mol⁻¹) of the maximum of the long wavelength transition and is given by

\[
E_t(\text{solvent})(\text{kcal mol}^{-1}) = \frac{28591}{\lambda_{\text{max}}(\text{nm})}
\]
Supporting data

Stability of CALB in ChCl:U

We preincubated CALB (2 mg ml\(^{-1}\)) in either 10 M urea or 5 M choline chloride in deionized water (0.3 ml) for 30, 60, and 90 min at 60°C. After the allotted time, we examined the hydrolysis activity of \(p\)-nitrophenyl acetate and compared it a mixture with no preincubation. After 90 min in 10 M urea, CALB lost 70% of its initial activity, and the first-order rate constant for the degradation had a value of 7 \(\times\) 10\(^{-3}\) min\(^{-1}\). For 5 M choline chloride, the value of the degradation rate constant was 4 \(\times\) 10\(^{-3}\) min\(^{-1}\), corresponding to an activity loss of 25% after 90 min. We then preincubated CALB (0.2 mg ml\(^{-1}\)) in ChCl:U (0.3 ml) for up to 90 min. We then examined the transesterification activity of the enzyme, and found a change of < 1% in activity over the course of the experiment, corresponding to a rate constant of less than 2 \(\times\) 10\(^{-4}\) min\(^{-1}\). Based on these results, CALB is at least 20- and 35-fold more stable in ChCl:U than in 5 M choline chloride or 10 M urea, respectively.

Transesterification in glycerol-containing DES: ChCl:Gly

An iCALB-catalyzed transesterification of pure glycerol with ethyl valerate in tert-butanol (glycerol and its esters of valeric acid are poorly soluble in toluene.) showed a peak in the GC trace consistent with glyceryl monoester of ethyl valerate (9.9 min) in toluene. C, D: the same transesterification as B done in ChCl:Gly; the glyceryl ester peak accounts for <0.5% of conversion (peak shown in D). No other products were observed at longer times in any of the reactions. The broadness and differences in the glycerol peak were typical - depending on the glycerol concentration, the broad peak would begin around 8.2 min and continue up to 10.5 min.

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valeric acid, panel A in Fig. S1. A similar reaction with 1-butanol as the nucleophile and toluene as the solvent showed the expected 1-butyl valerate, panel B in Fig. S1. The same reaction in ChCl:Gly after extraction with tert-butanol (1.0 ml) instead of toluene, Panels C and D, showed 95% conversion to 1-butyl valerate and <0.5% conversion to the glyceryl monoester of valeric acid.

**Fig. S2** Hydrolytic activity of \(p\)-nitrophenyl acetate (330 \(\mu\)M) by lipases and esterases (0.05 mg ml\(^{-1}\)) at room temperature at different concentrations of ChCl:Gly in 5 mM BES buffer at pH 7.2. Activity is relative to the initial rate in the buffer only. Points are averages of quadruplicate runs and represent activity above the background reaction. Error was typically less than five percent.

**Hydrolysis of \(p\)-nitrophenyl acetate by lipases and esterases**

We ran hydrolysis assays in 0% to 90% ChCl:Gly with three lipases and four esterases, Fig. S2. Four enzymes showed higher activity in 10% DES as compared to buffer only: CALB (125%), PFE (125%), PLE (284%), and ROE (328%). At 25% DES, three enzymes still had higher activity as compared to buffer only: CALB (130%), PLE (215%), and ROE (299%). At 50%, only CALB retained higher activity as compared to buffer only: 112%. At 25% to 90% DES, all enzymes showed lower activity as compared to buffer only. At 90% DES PLE and PCL showed the lowest activity as compared to buffer only: 1% and 6%, respectively.

**References**