Improved Preparation and Use of Room-Temperature Ionic Liquids in Lipase-Catalyzed Enantio- and Regioselective Acylations

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Polar organic solvents such as methanol or *N*-methylformamide inactivate lipases. Although ionic liquids such as 3-alkyl-1-methylimidazolium tetrafluoroborates have polarities similar to these polar organic solvents, they do not inactivate lipases. To get reliable lipase-catalyzed reactions in ionic liquids, we modified their preparation by adding a wash with aqueous sodium carbonate. Lipase-catalyzed reactions that previously did not occur in untreated ionic liquids now occur at rates comparable to those in nonpolar organic solvents such as toluene. Acetylation of 1-phenylethanol catalyzed by lipase from *Pseudomonas cepacia* (PCL) was as fast and as enantioselective in ionic liquids as in toluene. Ionic liquids permit reactions in a more polar solvent than previously possible. Acetylation of glucose catalyzed by lipase B from *Candida antarctica* (CAL-B) was more regioselective in ionic liquids because glucose is up to one hundred times more soluble in ionic liquids. Acetylation of insoluble glucose in organic solvents yielded the more soluble 6-*O*-acetyl glucose, which underwent further acetylation to give 3,6-*O*-diacetyl glucose (2−3:1 mixture). However, acetylation of glucose in ionic liquids yielded only 6-*O*-acetyl glucose (>13:1 and up to >50:1).

Introduction

Although enzymes are environmentally friendly reagents, some enzyme-catalyzed reactions require environmentally harmful organic solvents. One potential solution is to replace organic solvents with room-temperature ionic liquids. Room-temperature ionic liquids are organic salts whose ions do not pack well and remain liquid at room temperature. Ionic liquids are completely nonvolatile and can usually be recycled and reused.[1]

Several groups recently reported enzyme-catalyzed reactions in ionic liquids and identified some potential advantages besides environmental ones. Thermolysin for peptide synthesis was more stable in ionic liquids as compared to ethyl acetate, but the reaction rates were lower.[2] On the other hand, reaction rates of lipase-catalyzed alcoholysis, ammoniolysis and perhydrolysis were comparable or slightly better in ionic liquids as compared to organic solvents.[3] Similarly, Schöfer et al. reported faster reactions in some ionic liquids, but no reaction at all in others, even when the structures were very similar. In addition, they as well as Kim et al. found that the enantioselectivity of lipase-catalyzed acetylation of secondary alcohols was higher in some ionic liquids.[4] Although one can finely tune the properties of an ionic liquid by varying its structure, one must first identify which solvent properties are important. When enzyme-catalyzed reactions work in one ionic liquid, but not in a similar one, it is impossible to identify the most important solvent properties for enzyme-catalyzed reactions.

In this paper, we report an improved preparation of ionic liquids that yields ionic liquids that work reliably in enzyme-catalyzed reactions. Minor changes in structure of the ionic liquids no longer cause dramatic changes in reaction rate. We also measured the polarity of common ionic liquids and show that they are comparable to methanol and *N*-methylformamide. As an example of a lipase-catalyzed transformation of a polar substrate, we report the lipase-catalyzed acetylation of glucose in ionic liquids.

Results

Synthesis of Ionic Liquids. Ten ionic liquids were prepared either by literature procedures or by straightforward modification of literature procedures, Scheme 1.[5] For example, alkylation of *N*-methylimidazole with an alkyl halide yielded 3-alkyl-1-methylimidazolium halides as white solids. Metathesis with sodium tetrafluoroborate yielded the desired tetrafluoroborate salts as viscous oils. Unfortunately, lipase-catalyzed reactions in these un-

(1) Review: Seddon, K. R. *J. Chem. Technol. Biotechnol.* **1997**, *68*, 351−356. Welton, T. *Chem. Rev.* **1999**, *99*, 2071−2083. Wasserscheid, P.; Keim, W. *Angew. Chem., Intl. Ed.* **2000**, *39*, 3772−3789.

(2) Erbeldinger, M.; Mesiano, A. J.; Russell, A. J. *Biotechnol. Prog.* **2000**, *16*, 1131−1133. Alston, W. C., II; Ng, K. *Book of Abstracts*; 217th ACS National Meeting, Anaheim, CA, March 21−25 1999; American Chemical Society: Washington, DC, 1999; BIOT-131.

(3) Lau, R. M.; Rantwijk, F. van; Seddon, K. R.; Sheldon, R. A. *Org. Lett.* **2000**, *2*, 4189−4191.

(4) Itoh, T.; Akasaki, E.; Kudo, K.; Shirakami, S. *Chem. Lett.* **2001**, 262−263. Schöfer, S. H.; Kaftzik, N.; Wasserscheid, P.; Kragl, U. *Chem. Commun.* **2001**, 425−426. Kim, K.-W.; Song, B.; Choi, M.-Y.; Kim, M.-J. *Org. Lett.* **2001**, *3*, 1507−1509.

(5) BMIM·BF_4 or BMIM·PF_6 via metathesis of the halide with $NaBF_4$, $NaPF_6$, or HPF_6: (a) Huddleston, J. G.; Willauer, H. D.; Swatloski, R. P.; Visser, A. E.; Rogers, R. D. *J. Chem. Soc., Chem. Commun.* **1998**, 1765−1766. (b) Suarez, P. A. Z.; Dullius, J. E. L.; Einloft, S.; De Souza, R. F.; Dupont, J. *Polyhedron* **1996**, *15*, 1217−1219. EMIM·BF_4 or PMIM·BF_4 via $AgBF_4$: (c) Holbrey, J. D.; Seddon, K. R. *J. Chem. Soc., Dalton Trans.* **1999**, 2133-2139.

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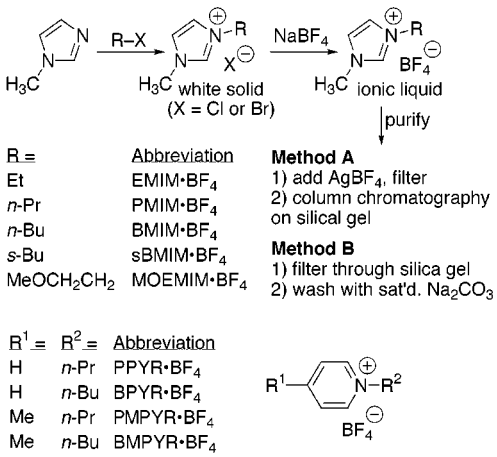
Scheme 1. Ten Ionic Organic Liquids Investigated in This Paper[a]



[a] The cations are either 3-alkyl-1-methylimidazolium or *N*-alkylpyridinium. The anions were tetrafluoroborate in all nine salts shown, but the tenth was a hexafluorophosphate salt: 3-butyl-1-methylimidazolium hexafluorophosphate, BMIM·PF_6.

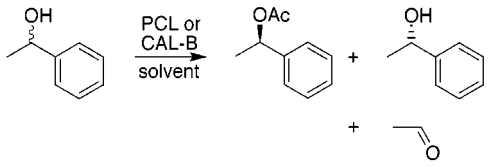
purified ionic liquids were either slow or did not occur; see below. We suspected that an impurity in these ionic liquids might inhibit the lipase-catalyzed reactions, so we tested several purification methods.

One known impurity in these crude ionic liquids is 3-alkyl-1-methylimidazolium halide, which remains from an incomplete metathesis reaction.[5] We confirmed that crude ionic liquids contain halide because a precipitate formed upon addition of aqueous silver nitrate. Previous researchers removed the contaminating halide either by precipitation with silver tetrafluoroborate or by carrying out the metathesis in acetone from which the tetrafluoroborate salts separated as viscous oils. The acetone method does not completely remove the halide, so we used the silver tetrafluoroborate method. Precipitation of the halide using silver tetrafluoroborate followed by chromatography on silica gel, which we call purification method A, removed the halide as shown by no precipitate upon addition of silver nitrate solution. Although this purification method improved some ionic liquids, others still gave slow reactions or, in some cases, no reaction; see below.

We developed an alternative purification that avoids the use of silver ion, which we call purification method B. The ionic liquid was diluted with methylene chloride, filtered through silica gel to remove the 3-alkyl-1-methylimidazolium halide, and washed with saturated aqueous sodium carbonate.[6] Finally, we dried the ionic liquid with anhydrous magnesium sulfate followed evaporation of the methylene chloride under vacuum. This purification method B yielded ionic liquids that worked reliably in all lipase-catalyzed reactions tested in this paper.

These procedures yielded five 3-alkyl-1-methylimidazolium tetrafluoroborate ionic liquids. One additional ionic liquid, 3-butyl-1-methylimidazolium hexafluorophosphate, was prepared from the halide by metathesis with hexafluorophosphoric acid followed by purification using method B. Similar reactions and purification methods yielded four more ionic liquids based pyridinium and 4-methylpyridinium cations for a total of 10 different ionic liquids, Scheme 1.

Lipase-Catalyzed Enantioselective Acetylation Reactions in Ionic Liquids. As a model reaction, we used the acetylation of 1-phenylethanol with vinyl acetate catalyzed by lipase from *Pseudomonas cepacia*, PCL, eq 1. This is a highly enantioselective reaction so the



maximum conversion was 50%. We compared the rates of reaction and enantioselectivities in ionic solvents to those in normal organic solvents such as toluene and acetone, Table 1. Lipases did not dissolve in ionic liquids, but remained suspended as powders as they do in organic solvents. The enantioselectivity of the acetylation remained high, $E > 200$, in all ionic liquids. However, the reaction rates, as measured by the degree of conversion after 24 h, varied dramatically. Reaction rates in unpurified ionic liquids (Table 1, entries 9−12) were at least two to five times slower than in toluene, THF, or acetone (Table 1, entries 1−4). In many ionic liquids, of which one example is shown, PMIM·BF_4 (Table 1, entry 12), no reaction occurred. Upon purification of the ionic liquids using method A (Table 1, entries 13−16), the reaction rate in BMIM·BF_4 (compare entries 9 and 14 of Table 1) doubled, while that in PMIM·BF_4 increased from no reaction to a rate similar to that in toluene or acetone (compare entries 12 and 15 of Table 1). Nevertheless, a number of structurally similar ionic liquids still gave no reaction after purification by method A (Table 1, entries 13 and 16).

On the other hand, ionic liquids purified by method B showed consistent behavior (Table 1, entries 21−30). Reaction rates varied moderately with moderate changes in structure of the ionic liquid, and the fastest rates were the same as in toluene or acetone.

To identify the impurities that cause of the lack of reaction in some ionic solvents, we measured the effect of additives on the reaction, Table 1, entries 31−43. For ionic solvents purified by method B, addition of silica gel, bromide salt of the ionic liquid, or sodium carbonate had no detectable effect on either the rate or enantioselectivity of the reaction. However, addition of silver tetrafluoroborate completely stopped the reaction,while addition of acetic acid slowed the reaction by approximately a factor of 2. Thus, silver ion stops the PCL-catalyzed reaction, while acetic acid slows it down. Another lipase, lipase B from *Candida antarctica*, CAL-B, showed a similar inactivation upon addition of silver tetrafluoroborate.[7] (Data not shown.) Thus, the most likely causes of

(6) Although BMIM·PF_6 is not miscible with water, the tetrafluoroborate ionic liquids dissolve in water. The ionic liquids were diluted with methylene chloride to permit washing with an aqueous solution.

(7) The inactivation of these two lipases by traces of silver ion is not surprising. PCL contains two cysteine residues that form a disulfide link on the surface of the protein (C190 and C270). Similarly, CAL-B contains six cysteine residues that form three disulfide links on the lipase surface (C22 and C64, C216 and C258, C293 and C311). Silver presumably disrupts these links and inactivates the lipase. Unlike the other ionic liquids, lipases PCL and CAL-B remained active in sBMIM·BF_4 even after the addition of silver ion, Table 1, entry 38. (Data for CAL-B not shown.) We do not understand the reason for this phenomenon.

Table 1. Rate and Enantioselectivity of the Acetylation of 1-Phenylethanol with Vinyl Acetate by Lipase from *Pseudomonas cepacia* in Organic Solvents and in Room Temperature Ionic Liquids[a]

entry	solvent	purification method[b]	polarity[c]	additive	ee_s, %	ee_p, %	*c*, %	E
1	toluene	none	0.10	none	78	99	44	>200
2	toluene[e]	none	0.10	none	99	99	49	>200
3	THF	none	0.21	none	47	99	32	>200
4	acetone	none	0.36	none	52	99	34	>200
5	DMF	none	0.37	none	3.0	99	3.0	>200
6	DMSO	none	0.44	none	nr	nr	0	nr
7	acetonitrile	none	0.46	none	32	99	25	>200
8	*N*-methylformamide	none	0.72	none	nr	nr	0	nr
9	BMIM·BF_4	none	nd	none	8.4	99	7.8[d]	>200
10	BMIM·PF_6	none	nd	none	18	99	15[d]	>200
11	BMIM·PF_6	none	nd	none	94	99	48[e]	>200
12	PMIM·BF_4	none	nd	none	nr	nr	0[d]	nr
13	EMIM·BF_4	A	nd	none	nr	nr	0	nr
14	BMIM·BF_4	A	nd	none	15	99	13	>200
15	PMIM·BF_4	A	nd	none	63	99	39	>200
16	MOEMIM·BF_4	A	nd	none	nr	nr	0	nr
17	BPyr·BF_4	A	nd	$NaHCO_3$	nr	nr	0	nr
18	BPyr·BF_4	A	nd	Na_2CO_3	47	99	32	>200
19	EMIM·BF_4	A	nd	Na_2CO_3	87	99	46	>200
20	MOEMIM·BF_4	A	nd	Na_2CO_3	71	99	42	>200
21	EMIM·BF4	B	0.71	none	85	99	46	>200
22	MOEMIM·BF4	B	0.70	none	73	99	42	>200
23	PMIM·BF_4	B	0.69	none	62	99	38	>200
24	BMIM·BF4	B	0.68	none	55	99	36	>200
25	sBMIM·BF4	B	0.68	none	54	99	35	>200
26	BMIM·PF_6	B	0.68	none	41	99	29	>200
27	BMPyr·BF_4	B	0.63	none	34	99	25	>200
28	PMPyr·BF_4	B	0.67	none	47	99	33	>200
29	BPyr·BF_4	B	0.64	none	62	99	38	>200
30	PPyr·BF_4	B	0.66	none	59	99	37	>200
31	EMIM·BF_4	B	nd	silica gel	81	99	45	>200
32	MOEMIM·BF_4	B	nd	silica gel	77	99	43	>200
33	EMIM·BF_4	B	nd	EMIM·Br	75	99	43	>200
34	MOEMIM·BF_4	B	nd	MOEMIM·Cl	63	99	39	>200
35	EMIM·BF_4	B	nd	$AgBF_4$	nr	nr	0	nr
36	PMIM·BF_4	B	nd	$AgBF_4$	nr	nr	0	nr
37	BMIM·BF_4	B	nd	$AgBF_4$	nr	nr	0	nr
38	sBMIM·BF_4	B	nd	$AgBF_4$	37	99	27	>200
39	MOEMIM·BF_4	B	nd	$AgBF_4$	nr	nr	0	nr
40	EMIM·BF_4	B	nd	Na_2CO_3	77	99	44	>200
41	MOEMIM·BF_4	B	nd	Na_2CO_3	71	99	42	>200
42	EMIM·BF_4	B	nd	acetic acid[f]	28	99	22	>200
43	MOEMIM·BF_4	B	nd	acetic acid[f]	25	99	20	>200

[a] Conditions: 1 mmol of vinyl acetate, 1 mmol of *sec*-phenethyl alcohol, 1 mL of solvent, 20 mg of PCL or 5 mg of CAL-B, 10 mg of additive, 24 h, room temperature, stirred with magnetic stirring bar. PCL was the lipase unless otherwise noted. Some reactions were run at twice this scale. nr = no reaction, nd = not determined, *E* = enantiomeric ratio as defined by Chen, C. S.; Fujimoto, Y.; Girdaukas, G.: Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299. These reactions are highly enantioselective, so the maximum conversion is 50%. The values of conversion, *c*, were calculated using the measured enantiomeric excess of the starting material (ee_s) and product (ee_p). The values in the box are our recommended reaction conditions for lipase-catalyzed reactions in ionic solvents. These data in the box as well as the data for normal organic solvents are also plotted in Figure 1. [b] Purification method A: add silver tetrafluoroborate, remove silver halide precipitate by filtration, followed by chromatography on silica gel. Purification method B: filtration through silica gel plug, wash with saturated aqueous sodium carbonate. [c] Solvent polarity according to Reichardt's normalized polarity scale, E_T^N. On this scale, tetramethylsilane has a polarity of 0 and water has a polarity of 1. The values for the organic solvents were taken from a recent review (Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319−2358.). The values for the ionic liquids were calculated using the measured absorbance maximum of the long-wavelength transition of 2,6-diphenyl-4-(2,4,6-triphenylpyridinio)phenolate as described in the Experimental Section. [d] 108 h reaction time, but using only 5 mg of PCL. [e] CAL-B used in place of PCL. [f] 20 μL added.

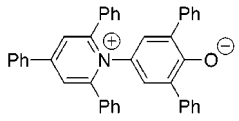
slow reaction or no reaction in ionic solvents purified by method A are traces of remaining silver ion or acidic impurities.

Consistent with this explanation, the addition of solid sodium carbonate to ionic liquid purified by method A dramatically increased the rate of reaction to the same level as that for ionic liquid purified by method B (Table 1, entries 18−20). For example, without sodium carbonate, no reaction was observed in MOEMIM·BF_4, (Table 1, entry 16), but with sodium carbonate (Table 1, entry 20), the yield was the same as that for MOEMIM·BF_4 purified by method B (Table 1, entry 22). The reason for this increase may be due to removal of traces of silver ion by precipitation as the carbonate and/or neutralization of acidic impurities in the ionic liquid. Addition of sodium bicarbonate instead of carbonate did not increase the reaction rates. (Compare entries 17 and 18 of Table 1.) Thus, ionic liquids purified by method A can be made suitable for lipase-catalyzed reactions by addition of solid sodium carbonate. We recommend purification method B because is simpler and avoids the use of expensive silver salts.[8]

Polarity of Ionic Liquids As Compared to Organic Solvents. The color of Reichardt's dye (a substituted *N*-(4-oxidophenyl)pyridinium, Chart 1) varies

(8) Most researchers use organic solvents "as is" and do not control the amount of trace water. Similarly, we did not do anything special to the ionic liquids to control the amount of trace water, but we expect that they contained little water. We dried them with anhydrous magnesium sulfate and then removed organic solvent under vacuum.

Chart 1. Reichardt's Dye



strongly with the polarity of the solvent—from $\lambda_{max} = 453$ nm in water to $\lambda_{max} = 810$ nm in diphenyl ether.[9] The ground state of this dye is highly polarized, while the first excited state is less polarized due to charge transfer. Polar solvents, especially those that form a hydrogen bond to the phenoxide oxygen, stabilize the ground state, thereby increasing the difference between the ground and excited states and increasing the energy of the absorption. In nonpolar solvents, the energy difference between ground and excited state is much smaller and the absorption is at lower energy.

We measured the polarity of the different ionic solvents by measuring the color of Reichardt's dye dissolved in the different ionic solvents. We used Reichardt's normalized scale where the tetramethylsilane has a value of zero and water has a value of one. The polarity values for the 10 ionic liquids in Scheme 1 ranged from 0.63 to 0.71 with the most polar being EMIM·BF_4 and the least polar being BMPyr·BF_4, Table 1. Muldoon et al.[10] recently measured the polarity of several ionic liquids using this dye. Carmichael and Seddon recently measured the polarity of several ionic liquids using another solvatochromic dye, Nile Red,[11] and Aki et al. used fluorescent probes to measure the polarity of ionic solvents.[12] Although only a few ionic liquids are the same as the ones we measured, our values are similar and the relative ranking of the polarities is the same.

Ionic liquids permit researchers to run lipase-catalyzed reactions in a solvent polarity range that was previously inaccessible. Organic solvents with polarities similar to the ionic liquids include the following: methanol, 2-chloroethanol, *N*-methylformamide, diethylene glycol, or 1,2 propanediol. Most of these are hydroxylic solvents, which are not suitable for acylation reactions since the solvent would compete with the substrate alcohol for the acyl donor. The one potentially suitable organic solvent, *N*-methylformamide, showed no reaction presumably because it denatured the lipase, Table 1.

With normal organic solvents, the trend is toward higher reaction rates in less polar solvents. However, for the PCL-catalyzed acetylation in ionic liquids the trend was in the opposite direction—toward higher reaction rates in the more polar ionic liquids, Figure 1. However, for the acetylation of glucose below, the reaction rate showed no correlation with the polarity of the ionic solvent. In this case, the solubility of the substrate glucose and acetylated products likely influences reaction rates.

Regioselective 6-O-Acetylation of β-D-Glucose in Ionic Liquids. Since ionic liquids are polar solvents that do not denature lipases, they may be ideal for lipase-catalyzed transformations of polar substrates. To test this idea, we examined the lipase-catalyzed acylation of glucose, eq 2, Table 2.

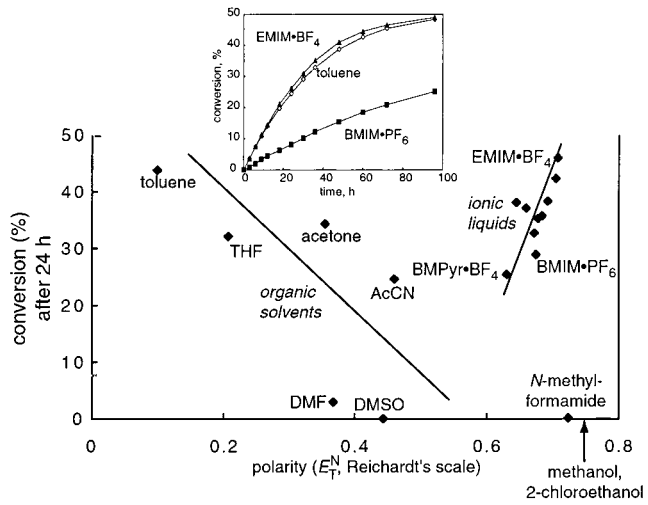
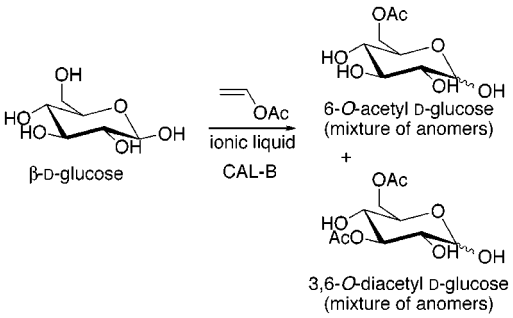


Figure 1. Rates of lipase-catalyzed reactions as a function of solvent polarity for normal organic solvents and for ionic liquids. The model reaction is a PCL-catalyzed acetylation of racemic 1-phenylethanol with vinyl acetate, which is highly enantioselective, so the maximum conversion is 50%. Inset: A comparison of the time course of the acetylation reaction in several solvents. The reaction rate in both toluene and the polar ionic liquid, EMIM·BF_4, are similar, but the initial reaction rate in a less polar ionic liquid, BMIM·PF_6, was approximately three times slower. (Reaction conditions: 4 mL of solvent, 30 mg of PCL, 4 mmol of vinyl acetate, 4 mmol of 1-phenylethanol, 96 h, room temperature.) Main graph: Correlation between degree of conversion in a lipase-catalyzed acylation and solvent polarity (Reichardt's normalized polarity scale) for organic solvents and ionic liquids. For normal organic solvents, the acetylation reaction proceeds well in nonpolar solvent, but not in polar solvents. The reaction is nearly completed in toluene and partially completed in tetrahydrofuran (THF), acetone, or acetonitrile (AcCN), but proceeds very slowly or not at all in the more polar *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), or *N*-methylformamide. Although the ionic liquids are highly polar solvents (similar to *N*-methylformamide), the acetylation reaction proceeds well in all ionic liquids tested. Alcoholic solvents such as methanol or 2-chloroethanol have polarities similar to the ionic liquids, but are not suitable for this acylation reaction because they would react with the acyl donor. The trend for ionic liquids is for higher degrees of conversion as the polarity of the ionic liquid increases, while the trend for organic solvents is the opposite—lower degrees of conversion as the polarity increases. The trend lines are not a fit to any theory, but are included only to guide the eye. Using ionic liquids as solvents permits lipase-catalyzed reactions to be run in a previously inaccessible polarity region. (Reactions are similar to those in Table 1: 1 mL of solvent, 20 mg of PCL, 1 mmol of vinyl acetate, 1 mmol of 1-phenylethanol, 24 h, room temperature).



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(10) Muldoon, M. J.; Gordon, C. M.; Dunkin, I. R. *J. Chem. Soc., Perkin Trans. 2*, **2001**, 433−434.
(11) Carmichael, A. J.; Seddon, K. R. *J. Phys. Org. Chem.* **2000**, *13*, 591−595.
(12) Aki, S. N. V. K.; Brennecke, J. F.; Samanta, A. *Chem. Commun.* **2001**, 413−414.

Table 2. Regioselective CAL-B Catalyzed Acylation of β-D Glucose in Ionic Liquids and in Organic Solvents[a]

solvent	conversion,[b] %	monoacylation, %	D-glucose, % (α/β)	6-*O*-acyl-D-glucose, % (α/β)	3,6-*O*-diacyl-D-glucose,[c] % (α/β)
EMIM·BF_4	50	99	9.7/40	19/31	0.0 /0.0
MOEMIM·BF_4	99	93	0.0/0.0	39/54	6.6/0.3
PMIM·BF_4	28	99	12/61	12/16	0.0/0.0
BMIM·BF_4	78	89	4.4/18	31/38	4.9/3.8
sBMIM·BF_4	90	88	4.1/5.7	35/44	6.8/4.0
BMIM·PF_6	29	39	7.5/63	4.4/6.9	9.1/9.0
BPyr·BF_4	42	89	8.1/50	15/22	2.1/2.6
PPyr·BF_4	44	88	8.1/48	15/24	2.2/2.8
acetone	72	76	5.5/22	29/26	11/6.7
acetone	42[d]	82	3.6/54	19/15	4.9/2.7
THF	99	53	0.0/0.0	31/22	32/15
THF	50[d]	85	2.1/48	25/18	4.9/2.3

[a] Conditions: 0.5 mmol of β-D-glucose, 1 mmol of vinyl acetate, 1 mL of solvent, 30 mg of Novozyme SP435, 36 h, 55 °C. [b] Conversion and product distribution was measured by gas chromatography after derivatization with chlorotrimethylsilane and 1,1,1,3,3,3-hexamethyldisilazane according to: Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497−2507. [c] The acylation position was determined from 2D ^{1}H NMR COSY spectra. [d] A reaction using one-third of the amount of lipase to show the initial regioselectivity.

Although this 6-O-acetylation reaction proceeds in organic solvents such as acetone and THF, further acetylation of the 3-O-position also occurs. In acetone, acetylated products formed in 72% yield, of which 76% was the desired 6-*O*-acetyl compound (∼3:1 selectivity). In THF, the products formed in 99% yield, but only 53% was the desired 6-*O*-acetyl compound (∼2:1 selectivity). Even at a lower extent of conversion, the regioselectivity remained low: In acetone at 42% conversion, 82% was the 6-*O*-acetyl compound (∼5:1 selectivity), while in THF at 50% conversion, 85% was the 6-*O*-acetyl compound (∼6:1 selectivity). The low selectivity is likely related to the poor solubility of glucose in these organic solvents (0.02−0.04 mg/mL at 60 °C[19]). Glucose remains a suspended solid and the initial 6-O-acetylation yields a more soluble compound, which then undergoes further acetylation to the 3,6-*O*-diacetyl derivative.

No reaction occurred in unpurified ionic liquids. However, acylation of glucose proceeded smoothly in all ionic liquids after purification by method B. In addition, the selectivity for monoacetylation is much higher in ionic liquids than in organic solvents. The 6-O-acetylation proceeds with 88−99% selectivity in the seven ionic liquids containing a tetrafluoroborate anion. The degree of conversion varied from 42 to 99%. The best ionic liquid for this reaction was MOEMIM·BF_4, where the acetylated products formed in 99% yield, of which 93% was the desired 6-*O*-acetyl compound. The one ionic liquid with a hexafluorophosphate anion, BMIM·PF_6, showed both slow reaction (29% conversion) and low selectivity (39% monoacetyl).

Approximately 100 times more glucose dissolves in the best ionic liquid, MOEMIM·BF_4 ∼5 mg/mL at 55 °C, than in acetone or THF. On the other hand, glucose is not very soluble in the worst ionic liquid, BMIM·PF_6, <1 mg/mL at 55 °C. For the acetylation of glucose in ionic liquids, the ability of the ionic liquid to dissolve glucose is an important factor.

Although we started with the β-anomer of glucose, we observed anomerization of both the starting materials and the products in both ionic liquids and in organic solvents. The higher temperature of the reaction (55 °C, 36 h) and traces of acetic acid formed by lipase-catalyzed hydrolysis of vinyl acetate most likely caused this anomerization. Hydrolysis of vinyl acetate is a significant side reaction even in "dry" organic solvents.[13]

Initial experiments show that CAL-B also catalyzes the regioselective acylation of maltose monohydrate, a disaccharide that is even more polar than glucose. Using the reaction conditions as in Table 2, but only half the amount of maltose (0.25 mmol instead of 0.5 mmol) and MOEMIM·BF_4 as the solvent, yielded 50% of acetylated products.

Discussion

Besides potential environmental benefits, ionic liquids permit enzyme-catalyzed reactions in a solvent polarity range that was previously inaccessible. Although there was no reaction in a polar organic solvent like *N*-methylformamide, ionic liquids with similar polarities on Reichardt's polarity scale gave excellent reactions. These more polar solvents offer major advantages with polar substrates such as glucose and maltose. Reactions with these polar substrates either do not proceed at all in organic solvent or proceed with low regioselectivity due to further acylation of the more soluble products. The higher solubility of glucose and maltose in ionic liquids facilitates their reaction. Previous enzyme catalyzed acylations of maltose required refluxing *tert*-butyl alcohol (82 °C) as the solvent.[14]

Although the catalyst usually controls the regioselectivity of a reaction, with poorly soluble substrates and

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products such as glucose and its derivatives, the relative solubility also contributes to the regioselectivity. For example, most researchers chose to acylate not glucose, but the more organic-solvent soluble alkyl glucosides (e.g., 1-*O*-ethyl glucoside).[15] These acylations show high regioselectivity for the primary alcohol because the CAL-B favors the primary alcohol position, and both the starting alkyl glucosides and the products 6-*O*-acyl-1-*O*-alkyl glucosides have similar solubilities in the reaction media. Similarly, acylation of other organic solvent soluble derivatives of glucose such as borate complexes[16] or isopropylidene ether derivatives[17] also shows high regioselectivity for the primary alcohol. In these cases, the relative solubilities of the starting glucose derivative and the product are similar and do not significantly affect the regioselectivity.

On the other hand, acylation of unmodified glucose varies with length of the acyl group because the solubility of the product 6-*O*-acyl derivative varies with the length of the acyl group. Acylation with shorter chain acyl groups (e.g., C2 to C6) gives a mixture of regioisomers because the initially formed 6-*O*-acyl derivative is soluble and undergoes further acylation. Pig pancreatic lipase catalyzed acylation of glucose in pyridine with activated acetyl esters gave a 5.6: 1 mixture of regioisomers.[18] A CAL-B catalyzed acylation of glucose with shorter chain acids such as caproic acid gave a "small" amount of the diester.[19]

However, acylation with longer chain acyl groups (≥C12) showed high regioselectivity for monacylation at the 6-position because the product 6-*O*-acyl glucose is also poorly soluble. Pig pancreatic lipase catalyzed acylation in pyridine with activated lauryl esters (C12) gave a 20:1 mixture of regioisomers instead of the 5.6:1 mixture with the acetyl ester.[18] A CAL-B-catalyzed acylation of glucose with longer chain acids such as palmitic (C16) gave only monacylation at the 6-position.[19] Similarly, Tsitsimpokou et al. acylated glucose absorbed on silica gel with lauric acid using CAL-B and observed high regioselectivity for the 6-position.[20]

To increase the regioselectivity for acylation of unmodified glucose with short acyl chains, one needs a solvent where either the glucose is more soluble or the 6-*O*-acyl derivative is less soluble. Degn et al. found that *tert*-butyl alcohol dissolves glucose to 2.4 mg/mL at 45 °C and CAL-B was highly regioselective for the primary alcohol position in this solvent.[21] However, these reaction conditions required dilute solutions − acylation of 100 mg of glucose would require 40 mL of solvent. Ionic liquids also increase the solubility of glucose in the reaction medium and thereby increase in regioselectivity. Our reaction conditions were about 40 times more concentrated than those of Degn et al. Acylation of 100 mg of glucose would require only 1 mL of ionic liquid. There is no need to propose a special interaction of the ionic liquid and lipase to explain the increased regioselectivity.

When the acyl chains are long, 6-*O*-acyl glucose and similar derivatives are surfactants,[22] but the surface-active properties make it an inconvenient synthetic intermediate. Derivatives with a short acyl chain are more useful as synthetic intermediates. Although researchers have also developed chemical methods for selective 6-O-acylation of unprotected glucose, these methods are less selective and lower yielding than enzymatic methods.[23] The ionic solvent method here may be the best way to protect the 6-position of glucose and quite likely other sugars also.

As with organic solvents, one ionic liquid is not best for all reactions. By varying the structure of the ionic liquid, one can optimize both the rates and the selectivities for each reaction. For 1-phenylethanol, acetylation was fastest in EMIM·BF_4 and slowest, by about a factor of 2, in BMIM·PF_6. The enantioselectivity remained high in all ionic liquids. For glucose, acetylation was fastest in MOEMIM·BF_4 and slowest, by about a factor of 3, in either PMIM·BF_4 or BMIM·PF_6. The regioselectivity was high in all ionic liquids except for one, BMIM·PF_6.

The key structural features that control enzyme-catalyzed reactions in ionic liquids remain unclear at this time. For the enantioselective acetylation of 1-phenylethanol, we found a correlation between the polarity of the ionic solvent and the reaction rate. A possible explanation relates to the relative solvation of the substrate in the solvent vs, enzyme. A more polar ionic liquid does not solvate a nonpolar substrate well, so the substrate binds to the enzyme and reacts. However, for the acetylation of glucose, we did not see a correlation between reaction rate and solvent polarity. For this poorly soluble substrate, reaction was fastest in the solvent most able to dissolve the substrate glucose. The nature of the anion had no effect on the enantioselectivity of the acetylation of 1-phenylethanol, but the hexafluorophosphate anion caused lower regioselectivity in the acylation of glucose.

The wash with aqueous sodium carbonate yields ionic solvents that are suitable for reactions in ionic liquids. For example, in the acetylation of 1-phenylethanol Schöfer et al. reported no reaction for CAL-B-catalyzed reaction in BMIM·BF_4 or BMIM·PF_6, no reaction for the PCL-catalyzed reaction in BMIM·PF_6 and slow reaction in BMIM·BF_4. Upon preparation of ionic liquids using the purification method B involving the wash with aqueous sodium carbonate, all these reactions proceeded at rates comparable to those in nonpolar organic solvents. We do not know why the sodium carbonate wash improves the reaction rates, but speculate that it may add small amounts of a buffer or water to the ionic liquid.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded in acetone-d_6 at 400 MHz. Lipase from *Pseudomonas cepacia* (commercial name PS30) was purchased from Amano USA (Lombard, IL). An immobilized form of lipase B from *Candida antarctica* (SP435) was used for acylation of glucose and maltose, while a soluble form was used to test the acetylation of 1-phenylethanol. Other chemicals were purchased from Sigma-Aldrich.

Synthesis of Ionic Liquids. BMIM·PF_6 was prepared according to a literature procedure using hexafluorophosphoric acid. The tetrafluoroborate salts of the ionic liquids were made according to literature procedures.[6] A mixture of alkyl halide (0.40 mol) and 1-methylimidazole (0.40 mol, 31.9 mL) was

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stirred at 70−80 °C (50 °C for EMIM·BF_4) for 1 or 2 days under nitrogen. The mixture was cooled to room temperature, and ethyl acetate (70 mL) was added causing precipitation of 1-alkyl-3-methyl imidazolium halide as a white solid. This solid was recovered by filtration and washed with ethyl acetate followed by ethyl ether: the crude yield was 90−100%.

To prepare the tetrafluoroborate salts, the 1-alkyl-3-methylimidazolium halide salt (0.40 mol) was added to a suspension of $NaBF_4$ (1.2 equiv, 52.7 g, 0.48 mol) in acetone (150 mL). After the mixture was stirred for 48 h at room temperature, the sodium halide precipitate was removed by filtration and the filtrate concentrated to an oil (∼100 mL) by rotary evaporation. This oil still contained some 1-alkyl-3-methyl imidazolium halide because it gave a precipitate when mixed with aqueous silver nitrate.

Purification of Tetrafluoroborate Salts: Method A.[7b,c] The oil (∼100 mL) was dissolved in methyl alcohol (100 mL), and an aqueous solution of $AgBF_4$ (generated from Ag_2O and HBF_4) was added dropwise until no more precipitate was formed. The mixture was filtered through Celite (no. 545), concentrated by rotary evaporation, dissolved in dichloromethane (100 mL), and filtered again to remove insoluble material. The product was purified by column chromatography in three portions on silica gel (∼400 g) eluted with methylene chloride/methanol (9:1). The solvent removed under vacuum yielded pale yellow oil, 60−80% yield.

Purification of Tetrafluoroborate Salts: Method B. The crude ionic liquid was diluted with methylene chloride (200 mL) and filtered through silica gel (∼100 g). This step removed the 1-alkyl-3-methylimidazolium halide since the filtrate no longer gave a precipitate mixed with aqueous silver nitrate. The solution was washed twice with saturated sodium carbonate aqueous solution (40 mL) and dried over anhydrous magnesium sulfate. Removal of solvent under vacuum yielded a pale yellow oil, 50−70% yield. Washing a solution of EMIM·BF_4 in methylene chloride with aqueous sodium carbonate yielded three layers: water on the top, EMIM·BF_4 in the middle, and methylene chloride at the bottom. The two bottom layers were separated, evaporated to remove water dissolved in the EMIM·BF_4 layer, diluted with methylene chloride (200 mL), dried over anhydrous magnesium sulfate, and concentrated to an oil.

EMIM·BF_4. ^{1}H NMR: δ 8.98 (1H, s); 7.75 (1H, dd); 7.68 (1H, dd); 4.37 (2H, q); 4.03 (3H, s); 1.54 (3H, t).

MOEMIM·BF_4. ^{1}H NMR: δ 8.95 (1H, s); 7.71 (1H, dd); 7.68 (1H, dd); 4.51 (2H, t); 4.05 (3H, s); 3.80 (2H, t); 3.34 (3H, s).

PMIM·BF_4. ^{1}H NMR: δ 8.99 (1H, s); 7.75 (1H, dd); 7.71 (1H, d); 4.31 (2H, t); 4.04 (3H, s); 1.95 (2H, m); 0.95 (3H, t).

BMIM·BF_4. ^{1}H NMR: δ 8.99 (1H, s); 7.75 (1H, d); 7.70 (1H, d); 4.35 (2H, t); 4.04 (3H, s); 1.91 (2H, m); 1.37 (2H, m); 0.94 (3H, t).

***s*BMIM·BF_4.** ^{1}H NMR: δ 9.05 (1H, s); 7.82 (1H, dd); 7.73 (1H, dd); 4.57 (1H, m); 4.04 (3H, s); 1.94 (2H, m); 1.60 (3H, d); 0.87 (3H, t).

BMIM·PF_6. ^{1}H NMR: δ 8.99 (1H, s); 7.76 (1H, dd); 7.71 (1H, dd); 4.36 (2H, t); 4.06 (3H, s); 1.93 (2H, m); 1.38 (2H, m); 0.94 (3H, t).

PMPyr·BF_4. ^{1}H NMR: δ 8.95 (2H, d); 8.06 (2H, d); 4.70 (2H, t); 2.73 (3H, s); 2.09 (2H, q); 0.99 (3H, t).

BMPyr·BF_4. ^{1}H NMR: δ 8.96 (2H, d); 8.05 (2H, d); 4.73 (2H, t); 2.72 (3H, s); 2.06 (2H, m); 1.42 (2H, m); 0.96 (3H, t).

PPyr·BF_4. ^{1}H NMR: δ 9.13 (2H, d); 8.72 (1H, t); 8.26 (2H, t); 4.78 (2H, t); 2.13 (2H, m); 1.00 (3H, t).

BPyr·BF_4. ^{1}H NMR: δ 9.16 (2H, d); 8.73 (1H, t); 8.27 (2H, t); 4.83 (2H, t); 2.10 (2H, m); 1.45 (2H, m); 0.97 (3H, t).

Transesterification of 1-Phenylethanol. Vinyl acetate (92 μL, 1.0 mmol) and 1-phenylethanol (120 μL, 1.0 mmol) were added to a suspension of PCL (20 mg) in solvent (1.0 mL of either organic solvents or ionic liquids) and stirred at 25 °C. The reactions were monitored by TLC (ethyl acetate/hexane, 1:3). After 24 h, the reaction mixture was extracted with hexane (3 mL), and the hexane extract was analyzed by GC with a chiral capillary column (Chromopak Chiralsil-Dex CB column (25 m × 0.25 mm, Raritan, NJ)): initial column temperature 125 °C for 2 min, then ramp to 150 °C over 10 min: 1-phenylethanol (α = 1.06, k_R = 3.32, k_S = 3.35); 1-methylbenzyl acetate (α = 1.12, k_R = 2.53, k_S = 2.26) The conversion, *c*, was calculated from the enantiomeric excess of the product, ee_p, and of the starting material, ee_s, using the equation below.[24]

$$c = \frac{ee_s}{ee_s + ee_p}$$

Transesterification of β-D-Glucose. Vinyl acetate (92 μL, 1.0 mmol), β-D-glucose (90 mg, 0.5 mmol), and Novozyme SP435 (30 mg) were mixed with solvent (1.0 mL of either organic solvents or ionic liquids) and stirred at 55 °C. After 36 h, pyridine (2 mL), 1,1,1,3,3,3-hexamethyldisilazane (1 mL), and chlorotrimethylsilane (1 mL) were added to the reaction mixture, and the mixture was extracted with hexane (5 mL). The hexane extract was analyzed by gas chromatography on the column noted above. The derivatives of glucose and acetyl glucose were separated using the following temperature program: initially 2 min at 180 °C, then gradient to 190 °C at 1 °C/min, then held at 190 °C for 28 min. In a separate experiment, we confirmed that this derivatization method does not cause anomerization of glucose.

Polarity Determination Using Reichardt's Polarity Scale. Reichardt's dye (2,6-diphenyl-4-(2,4,6-triphenylpyridinio)phenolate, 0.4 mg) was dissolved in ionic liquid (0.5 mL), and an aliquot was transferred to a 96-well microplate. The wavelength of the absorption maximum of the long-wavelength transition was measured at 25 °C using a microplate reader (Spectra Max 340, Molecular Devices Co., Sunnyvale, CA). Normalized polarity values (E^N_T), which range from 0.0 for tetramethylsilane to 1.0 for water, were calculated using the equation

$$E^N_T = \frac{E_T(\text{solvent}) - E_T(\text{TMS})}{E_T(\text{water}) - E_T(\text{TMS})} = \frac{E_T(\text{solvent}) - 30.7}{32.4}$$

where E_T(solvent) is the energy, in kilocalories per mole, of the maximum of the long wavelength transition and is given by

$$E_T(\text{solvent})(\text{kcal/mol}) = \frac{28591}{\lambda_{max}(\text{nm})}$$

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