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Enzymatic reactions in ionic liquids: lipase-catalysed kinetic resolution of racemic, *P*-chiral hydroxymethanephosphinates and hydroxymethylphosphine oxides

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Abstract—Lipase-mediated acetylation of racemic *P*-chiral hydroxymethanephosphinates and hydroxymethylphosphine oxides was performed in ionic liquids under kinetic resolution conditions. Lipase AK (Amano) and lipase from *Pseudomonas fluorescens* (Fluka) were up to six times more enantioselective in BMIM·PF_6 solutions than in common organic solvents. On the contrary, the analogous reactions performed in BMIM·BF_4 were practically non-stereoselective. © 2002 Published by Elsevier Science Ltd.

1. Introduction

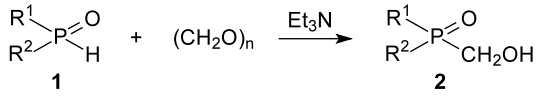
Ionic liquids are becoming more and more popular as effective solvents for a great variety of organic reactions.[1] Very recently some reports appeared on their application as reaction media for enzyme-promoted transformations, in which several advantages of this approach were presented. The first example concerned a thermolysin-catalysed synthesis of *Z*-aspartame, performed in BMIM·PF_6 containing 5% water, which proved to enhance the stability of the enzyme and the reaction rate.[2] In turn, the first example of the use of an enzyme in water-free ionic liquids was a series of lipase-catalysed transformations of carboxylic acid derivatives.[3] Those two papers were quickly followed by two reports on the successful use of ionic liquids to enhance the enantioselectivity of the lipase-promoted kinetic resolution of secondary alcohols.[4,5] Finally, the most recent paper by Park and Kazlauskas described modifications in the preparation of ionic liquids and their influence on the reactivity of lipases and stereoselectivity of the enzymatic reactions.[6]

In the course of our investigations on the enzyme-promoted syntheses of chiral heteroatom compounds,[7] we have recently applied a lipase-catalysed acetylation under kinetic resolution conditions for the synthesis of optically active *P*-chiral hydroxymethanephosphinates and phosphonates.[8] The latter are gaining increasing attention due to their applicability as substrates for the synthesis of biologically active compounds,[9] among them herbicides.[10] As the enantiomeric excess values of the products obtained in the above reaction were moderate (only in one case reached 90%), we have decided to check whether the use of ionic liquids will improve stereoselectivity of the reaction. This paper describes a successful lipase-mediated kinetic resolution of racemic *P*-chiral hydroxymethanephosphinates and a hydroxymethylphosphine oxide achieved in ionic liquids as solvents. It is noteworthy that this is the first example of the application of ionic liquids in an enzyme-catalysed resolution of primary alcohols bearing a remote stereogenic heteroatom centre.

2. Results and discussion

2.1. Synthesis of racemic hydroxymethanephosphinates

The required racemic hydroxymethanephosphinates **2** were synthesised in the reaction of the corresponding *H*-phosphinates **1** with paraformaldehyde in the presence of triethylamine (Eq. (1)). The compounds **2d**, **3d** and the hydroxymethylphosphine oxide **2e** were obtained previously.[10,11]



a: R^1 = Ph, R^2 = MeO
b: R^1 = Ph, R^2 = EtO
c: R^1 = Ph, R^2 = i-PrO
d: R^1 = Et, R^2 = i-PrO
e: R^1 = Ph, R^2 = t-Bu

(1)

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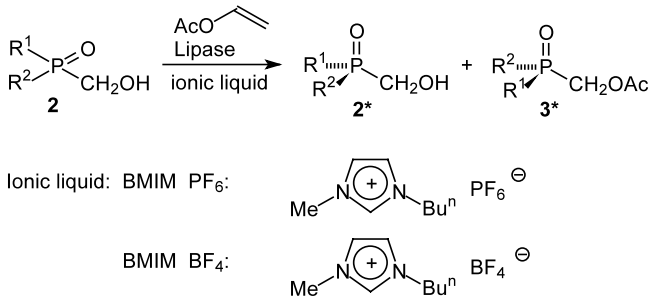
2.2. Kinetic resolution of racemic substrates 2

To perform the title reaction we chose 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM·PF_6) and 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM·BF_4) as our model ionic solvents. The former one is stable and immiscible with water and selected organic solvents, whereas the latter can be mixed with water in various proportions. The racemic hydroxymethanephosphinates were dissolved in an ionic liquid and acetylated using vinyl acetate in the presence of selected lipases, which were added directly to the reaction mixture (Eq. (2)). The reaction was carried out under kinetic control conditions, i.e. it was stopped at ca. 50% conversion, which was determined by ^{31}P NMR of the aliquots taken directly from the reaction mixture. It should be added that in the case of BMIM·PF_6 the ^{31}P NMR signal of the PF_6 anion of the solvent appears at $\delta = -145$ ppm and does not affect the observation of the signals of **2** and **3**, which appear in the region of 30–40 ppm. The product and the unconsumed substrate were then extracted from the ionic liquid solution (see Section 4) and separated by column chromatography or TLC. The enantiomeric excess (ee) values were determined both by ^1H NMR, using (−)-(*S*) or (+)-(*R*)-*tert*-butylphenylphosphinothioic acid as a chiral solvating agent[12] and by HPLC using a chiral column. The results are summarised in Table 1.[13]

Of the two ionic liquids applied, only BMIM·PF_6 proved to meet the expectations for enhancing the stereoselectivity of the enzymatic kinetic resolution. In



(2)

no case was the *E* value lower than that seen in the analogous reactions performed in common organic solvents. The improvement in the *E* values was particularly marked (three to six times) for substrates having larger organic substituents. In contrast to BMIM·PF_6, its tetrafluoroborate analogue, BMIM·BF_4, surprisingly showed an opposite effect. In spite of the fact that the enzymatic resolution of secondary alcohols was found to proceed in BMIM·BF_4 with high stereoselectivity for the lipases from *Pseudomonas cepacia* and *Candida antarctica*,[4–6] in our experiments practically no enantioselectivity was observed, although the reactions proceeded with rates comparable to those seen for reactions in BMIM·PF_6. At the moment no satisfactory explanation for this phenomenon can be provided due to the lack of systematic studies on the influence of ionic liquids on enzymes. However, it would be reasonable to assume that the lack of stereoselectivity might be attributed to the miscibility of BMIM·BF_4 with water. It is known that relatively hydrophilic solvents are capable of stripping off the essential water from the

Table 1. Kinetic resolution of **2** in ionic liquids

Entry	Substr.	Lipase	Ionic liquid	Recovered alcohol **2***				Acetate **3***				E[14]	E[b]
				Yield[a] (%)	$[\alpha]_D$ ($CHCl_3$)	E.e. (%)	Abs. conf.	Yield[a] (%)	$[\alpha]_D$ ($CHCl_3$)	E.e. (%)	Abs. conf.		
1	**2a**	AK	PF	33.3	−21.5	89	*R*	37.7	+49.8	89	*S*	51	45[c]
2	**2a**	PFL	PF	40.0	−20.0	75	*R*	42.0	+41.0	78	*S*	18	
3	**2a**	AK	BF	44.0	+0.4	1.2	*S*	10.0	−1.2	4.8	*S*	1.1	
4	**2a**	PFL	BF	14.0	−0.83	1.4	*R*	37.0	+0.5	0.3	*S*	1.0	
5	**2b**	AK	PF	36.0	−12.1	79	*R*	36.5	+39.6	83	*S*	26	5[c]
6	**2b**	PFL	PF	44.0	−9.5	63	*R*	30.0	+37.3	76	*S*	14	
7	**2b**	AK	BF	24.0	−1.1	0.4	*R*	33.0	+0.2	1	*S*	1.0	
8	**2c**	AK	PF	36.0	−21.3	95	*R*	48.5	+31.0	80	*S*	32	5[c]
9	**2c**	PFL	PF	39.0	−16.1	86	*R*	49.0	+26.0	68	*S*	15	
10	**2c**	AK	BF	56.0	+0.4	4.0	*S*	38.0	−0.3	4.5	*R*	1.3	
11	**2d**	PFL	PF	32.0	−12.1	95	N.d.	55.0	+10.4	50	N.d.	12	12[d]
12	**2e**	AK	PF	33.0	−18.7[f]	43	*S*	42.0	+7.0[f]	53	*R*	5	4[e]
13	**2e**	AK	BF	43.0	+0.1[f]	1	*R*	42.0	−1.4[f]	2	*S*	1.1	

Lipases: AK: Lipase AK (AMANO); PFL: Lipase from *Pseudomonas fluorescens* (FLUKA).
Ionic liquids: PF: BMIM·PF_6; BF: BMIM·BF_4.
N.d. = not determined.
[a] Yields after separation.
[b] The highest *E* values calculated for the same reactions performed in diisopropyl ether with the same enzymes and under comparable conditions, taken from:
[c] Ref. 8.
[d] Ref. 10.
[e] Ref. 15.
[f] In C_6H_6.

enzyme surface, leading to insufficient hydration of the enzyme which may in some cases exert a strong influence on the enzyme and decrease its activity.[16] BMIM·BF_4, which can mix with water in any proportions, may thus be considered responsible for such an interaction with certain kinds of enzymes.

3. Conclusion

The results described above clearly demonstrate that ionic liquids may be a promising medium for enzymatic transformations involving chiral heteroatom substrates. However, a lot of detailed investigations will be necessary to gain sufficient knowledge about the influence of ionic liquids on enzymes in general, and in particular, the interactions of various types of ionic liquids with different kinds of enzymes.

4. Experimental

4.1. General

BMIM·PF_6 was prepared according to the procedure described by Rogers et al.[17] BMIM·BF_4 was prepared by a straightforward adaptation of the procedure described by Carlin et al[18] for EMIM·BF_4 and purified according to the procedures described by Park and Kazlauskas.[6]

The enzymes were purchased from AMANO or FLUKA. NMR spectra were recorded on Bruker instruments at 200 MHz for ^{1}H and 81 MHz for ^{31}P, with C_6D_6 or $CDCl_3$ as solvents. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. Column chromatography was carried out using Merck 60 silica gel. TLC was performed on Merck 60 F_{254} silica gel plates. The HPLC analyses were performed on an LKB instrument, using CHIRALCEL OD column; flow 0.25 mL/min; hexane:*i*-PrOH 98:2; $\lambda = 254$ nm.

4.2. Synthesis of racemic 2—general procedure

Equimolar amounts of **1** and paraformaldehyde and a few drops of triethylamine were heated under argon at 80°C until the precipitate dissolved. The crude reaction mixture was then evacuated under vacuum at ca. 60°C to remove triethylamine and volatile by-products and finally purified either by column chromatography using chloroform–methanol (in gradient 100:1 to 15:1) as eluent, or by bulb-to-bulb distillation, or crystallisation.

4.3. Methyl hydroxymethanephenylphosphinate 2a

After purification by column chromatography yield 60%. ^{31}P NMR (C_6D_6): $\delta = 41.9$. ^{1}H NMR (C_6D_6): $\delta = 3.31$ (d, $J = 10.5$, 3H), 4.0–4.28 (m, 2H), 6.0 (br. s, 1H), 6.9–7.15 and 7.7–7.9 (m, 5H). Anal. calcd for $C_8H_{11}O_3P$: C, 51.62; H, 5.96; P, 16.64. Found: C, 51.40; H, 5.88; P, 16.30%.

4.4. Ethyl hydroxymethanephenylphosphinate 2b

Purified by a bulb-to-bulb distillation (ca. 140°C/0.2 mmHg), yield 80%. ^{31}P NMR (C_6D_6): $\delta = 39.4$. ^{1}H NMR (C_6D_6): $\delta = 0.96$ (t, $J = 7.05$, 3H), 3.6–4.0 (m, 2H), 4.05–4.30 (m, 2H), 6.4 (br. s, 1H), 6.9–7.1 and 7.7–7.9 (m, 5H). Anal. calcd for $C_9H_{13}O_3P$: C, 54.00; H, 6.55; P, 15.47. Found: C, 53.60; H, 6.57; P, 15.29%.

4.5. *i*-Propyl hydroxymethanephenylphosphinate 2c

Purified by column chromatography, followed by crystallisation from benzene; yield 60%, mp 70–72°C. ^{31}P NMR (C_6D_6): $\delta = 38.1$. ^{1}H NMR (C_6D_6): $\delta = 0.94$ (d, $J = 6.2$, 3H), 1.22 (d, $J = 6.2$, 3H), 4.0–4.35 (m, 2H), 4.5–4.7 (m, 1H), 6.1 (br. s, 1H), 6.95–7.1 and 7.75–7.9 (m, 5H). Anal. calcd for $C_{10}H_{15}O_3P$: C, 56.05; H, 7.05; P, 14.45. Found: C, 56.16; H, 6.99; P, 14.25%.

4.6. Kinetic resolution of 2—general procedure

A mixture of a racemic **2** (1 mmol), the enzyme (10–20 mg) and vinyl acetate (1 mL) was stirred in the ionic liquid (3 mL) at room temperature. The reaction was monitored by ^{31}P NMR and stopped at ca. 50% conversion. The unconsumed substrate and the acetate formed **3** were extracted from the ionic liquid solution with ether. To ensure full extraction of the products, the ionic liquid layer was treated with water. In the case of BMIM·PF_6, which is immiscible with water, the layers were separated and the aqueous layer was extracted with chloroform. In the case of BMIM·BF_4, the homogeneous solution formed after water addition was also extracted with chloroform. In both cases the organic layers were combined, dried over $MgSO_4$ and the solvents evaporated. The products were separated by column chromatography using chloroform–methanol (in gradient 100:1 to 15:1) as solvent or by preparative TLC (chloroform–methanol 20:1).

4.7. Methyl acetoxymethanephenylphosphinate 3a

^{31}P NMR (C_6D_6): $\delta = 34.1$. ^{1}H NMR (C_6D_6): $\delta = 1.49$ (s, 3H), 3.30 (d, $J = 10.9$, 3H), 4.27–4.56 (2×AB, 2H), 6.9–7.1 and 7.7–7.9 (m, 5H).

4.8. Ethyl acetoxymethanephenylphosphinate 3b

^{31}P NMR (C_6D_6): $\delta = 32.4$. ^{1}H NMR (C_6D_6): $\delta = 0.97$ (t, $J = 7.05$, 3H), 1.50 (s, 3H), 3.65–4.0 (m, 2H), 4.25–4.60 (2×AB, 2H), 7.0–7.1 and 7.8–7.95 (m, 5H).

4.9. Isopropyl acetoxymethanephenylphosphinate 3c

^{31}P NMR (C_6D_6): $\delta = 31.2$. ^{1}H NMR (C_6D_6): $\delta = 0.98$ (d, $J = 6.2$, 3H), 1.17 (d, $J = 6.2$, 3H), 1.49 (s, 3H), 4.2–4.65 (2×m, 4H), 7.0–7.1 and 7.85–7.95 (m, 5H).

4.10. Acetoxymethyl-*tert*-butylphenylphosphine oxide 3e

^{31}P NMR (C_6D_6): $\delta = 40.4$. ^{1}H NMR (C_6D_6): $\delta = 0.94$ (d, $J = 14.5$, 9H), 1.52 (s, 3H), 4.63 and 4.65 (2×AB, 2H), 7.0–7.15 and 7.6–7.75 (2×m, 5H).

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