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Supercritical carbon dioxide as a reaction medium for enzymatic kinetic resolution of P-chiral hydroxymethanephosphinates

Małgorzata Albrycht,[a] Piotr Kiełbasiński,[a,*] Józef Drabowicz,[a] Marian Mikołajczyk,[a] Tomoko Matsuda,[b,*] Tadao Harada[c] and Kaoru Nakamura[d]

[a]*Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Department of Heteroorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland*
[b]*Department of Bioengineering, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan*
[c]*Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan*
[d]*Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*

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Abstract—Kinetic resolution of racemic P-chiral hydroxymethanephosphinates via their lipase-promoted acetylation in supercritical carbon dioxide as the reaction medium was for the first time investigated under various conditions. The reactivity and selectivity could be controlled by changing the pressure.

1. Introduction

Supercritical carbon dioxide, $scCO_2$, has become a versatile solvent, which is more and more widely used in organic synthesis, extraction and chromatography.[1] Due to the high compressibility of $scCO_2$, it is possible to easily change its density, viscosity and other properties, which in turn allows the reaction conditions to be tuned so that the outcome of a given transformation can be maximised. Recently, $scCO_2$ has been proven to be a suitable medium for biocatalytic reactions.[2] We have applied it as a solvent for enzyme-promoted transformations—both using isolated lipases[3] and immobilised resting cells.[4] To date, very simple compounds, such as α-phenylethanol derivatives, have been kinetically resolved by lipases and the advantages of using $scCO_2$ for biocatalysis demonstrated: improvement in the reaction efficiency and environmental friendliness. However, there are currently no examples of the application of $scCO_2$ in more difficult enzymatic transformations, for example, involving more demanding substrates, such as organosulfur or organophosphorus compounds, especially those with a stereogenic centre located on the heteroatom.

Over the course of our investigations on the enzyme-promoted syntheses of non-racemic chiral heteroorganic compounds,[5] we found out that a lipase-catalysed acetylation of racemic P-chiral hydroxymethanephosphinates *rac*-**1** under kinetic resolution conditions proceeded stereoselectively to give both unreacted substrates **1** and products, *O*-acetoxymethanephosphinates **2**, with ees over 90%.[6,7] The reaction was performed in both organic solvents[6] and in ionic liquids;[7] the latter proved to increase the stereoselectivity of the transformation. Since the hydroxyalkanephosphorus derivatives have gained increasing attention due to their applicability as substrates for the synthesis of biologically active compounds,[8] among them herbicides,[9] while the synthesis of optically active phosphorus compounds still remains a real challenge, we decided to study whether the use of $scCO_2$ as a reaction medium for their enzymatic kinetic resolution will enhance its chemical outcome and stereoselectivity. Thus, we have investigated in detail the reaction shown in Scheme 1, using racemic P-chiral hydroxymethanephosphinates **1a**–**d** and several enzymes. It is noteworthy that this is the first example of the use of $scCO_2$ in an enzymatic transformation of

* Corresponding authors. Fax: +48 426847126 (P.K.), +81 459245757 (T.M.); e-mail addresses: piokiel@bilbo.cbmm.lodz.pl; tmatsuda@bio.titech.ac.jp

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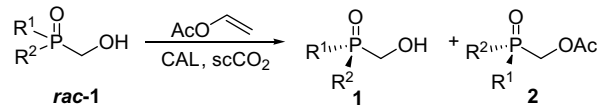

1a: R^1 = Ph, R^2 = *i*-PrO
1b: R^1 = Ph, R^2 = EtO
1c: R^1 = Ph, R^2 = MeO
1d: R^1 = Et, R^2 = *i*-PrO

Scheme 1.

heteroorganic substrates and particularly of primary alcohols bearing a remote stereogenic heteroatom centre.

2. Results and discussion

2.1. Screening of enzymes

In preliminary experiments, we screened several lipases for the enantioselective acetylation of **1a**. Table 1 clearly shows that some lipases proved to be totally unreactive. The best results were obtained with the lipase from *Candida antarctica* (CAL), which was then used in all ensuing experiments.

2.2. Effect of pressure

The effect of pressure on the enzymatic acetylation of *rac*-**1a** using vinyl acetate in the presence of CAL in $scCO_2$ was investigated at 40 °C, while the reaction time was 2 h. The data collected in Table 2 show that at pressures up to 8 MPa, that is, below the critical density, the reaction does not proceed at all. However, the reaction conversion could be largely improved by changing the pressure. It was found that it was important to tune the pressure so that the density of CO_2 exceeds the critical density. The reaction was fastest when pressure was closer to the critical pressure: at 11 MPa the reaction rate reached its maximum and then gradually decreased as the pressure was increased to 15 MPa. The optimal conditions were obtained at 13 MPa, at which point the reaction stopped at ca. 50% conversion and the enantioselectivity was highest. Thus, by modifying the pressure, both the reactivity and stereoselectivity were controlled. The control of the reaction outcome by changing the solvent properties is a very special characteristic of supercritical fluids.

2.3. Effect of reaction time

Examination of the data included in Table 3 reveals that the CAL-mediated acetylation of **1a** reaches its ca. 50% conversion approximately after 2 h, which means that this is the optimum reaction time to achieve kinetic resolution. As a result, the yields of both **1a** and **2a** are close to 50% and exhibit the same enantiomeric excess.

2.4. Substrate specificity

Finally, we used a series of hydroxymethanephosphinates **1a–d**, which were substrates in our previous investigations,[6,7] to check the scope of the reaction carried out in $scCO_2$. Since the conversion could not be monitored, all the experiments were carried out under the same conditions and stopped after the same reaction time. The results collected in Table 4 indicate that the reaction rates differ substantially in each case, which is shown by the totally different conversion degrees. The large differences seem to be a result of a competitive influence of two factors: the bulkiness of the substituents at phosphorus and the solubility of the substrates in $scCO_2$. As $scCO_2$ is a non-polar solvent, the solubility

Table 1. Screening of lipases for enantioselective acetylation of **1a**

Enzyme	Conversion (%) (^{31}P NMR)	Recovered substrate **1a**			Product **2a**			*E*
		Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	
AK	0	95	—	—	—	—	—	—
LPL	0	100	—	—	—	—	—	—
Lipozyme	0	99	—	—	—	—	—	—
PS-C	9	93	−1.8	8	6	+14.1	36	3
CAL (Novozyme)	37	56	−4.1	18	25	+12.6	32	3

Conditions: T = 40 °C, P = 13 MPa, t = 2 h.
Substrate **1a**: 0.23–0.30 mmol, vinyl acetate: 0.4 mL.
Lipase: 5 mg.

Table 2. Effect of pressure on the CAL-mediated acetylation of *rac*-**1a**

Pressure (MPa)	Conversion (%) (^{31}P NMR)	Recovered substrate **1a**			Product **2a**			*E*
		Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	
8	0	95	—	—	—	—	—	—
11	88	21	−16.3	73	88	+1.7	4	2
13	46	52	−6.2	28	46	+10.4	27	3
15	12	83	−2.7	12	12	+12.5	33	2.5

Conditions: T = 40 °C, t = 2 h.
Substrate **1a**: 0.23–0.30 mmol, vinyl acetate: 0.4 mL.
Lipase: 5 mg.

Table 3. Effect of time on the stereoselectivity of CAL-mediated acetylation of *rac*-**1a**

Time (h)	Conversion (%) (^{31}P NMR)	Recovered substrate **1a**			Product **2a**			*E*
		Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	
0.2	8	85	−0.9	4	7	+18.0	45	3
1	18	78	−2.9	13	16	+16.9	42	3
2	46	52	−6.2	28	46	+10.4	27	3

Conditions: $T = 40$ °C, $P = 13$ MPa.
Substrate **1a**: 0.23–0.30 mmol, vinyl acetate: 0.4 mL.
Lipase: 5 mg.

Table 4. Substrate specificity for CAL-mediated enantioselective acetylation in $scCO_2$

Substrate	Conversion (%) (^{31}P NMR)	Recovered substrate **1**			Product **2**			*E*
		Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	Yield (%)	$[\alpha]_D$ ($CHCl_3$)	ee (%)	
rac-**1a**	46	52	−6.2	28	46	+10.4	27	3
rac-**1b**	93	6	−13.4	88	82	+2.9	6	3
rac-**1c**	8	81	−1.1	4	5	+3.4	7	1.5
rac-**1d**	100	—	—	—	90	—	—	—

Conditions: $T = 40$ °C, $P = 13$ MPa, $t = 2$ h.
Substrates **1a–d**: 0.23–0.30 mmol, vinyl acetate: 0.4 mL.
Lipase: 5 mg.

of the substrates, which contain a hydroxy moiety, should generally be low. The solubility should be, however, enhanced by the presence of hydrophobic substituents. Therefore, the difference in reactivity between **1b** (the ethoxy group) and **1c** (the methoxy group) seems understandable. On the other hand, the lower reactivity of **1a** (the isopropoxy group), whose solubility should be the highest, can be explained in terms of a larger steric hindrance exerted by the isopropyl group. In turn, **1d** seems to combine both features (higher solubility and lower steric hindrance), which makes it very reactive and thus excludes the possibility of achieving any stereoselectivity in this set time. On the contrary, the remaining three racemic substrates were successfully resolved under these conditions to give non-racemic products in up to 88% ee. A search for the methods, which would improve the stereoselectivity of this reaction, is currently in progress in our laboratories.

3. Conclusions

Supercritical carbon dioxide, $scCO_2$, was for the first time used as a reaction medium for an enzyme-promoted kinetic resolution of racemic heteroorganic compounds with a stereogenic centre located on the heteroatom. Moreover, the substrates, P-chiral hydroxymethanephosphinates, were the first primary alcohols bearing a remote stereogenic centre that were ever resolved under the conditions described. The procedure applying $scCO_2$ allowed us to obtain optically active products, whose yield and enantiomeric purity could be controlled by the pressure applied.

4. Experimental

4.1. General

Racemic hydroxymethanephosphinates were prepared according to the procedure described.[6] Lipase LPL was kindly supplied by Toyobo, the lipases AK and PS-C were kindly supplied by Amano Enzymes Inc., while the lipases Lipozyme and CAL (*Novozyme*) were kindly supplied by Novozymes. Chemicals were purchased from Nacalai Tesque, Inc., Wako Pure Industries, Ltd., and Aldrich Chemical Co and used without further purification unless otherwise indicated. Vinyl acetate was distilled and dried over MS-4A before use. NMR spectra were recorded on Bruker instruments at 300 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 HORIBA High Sensitive Polarimeter SEPA-200. Column chromatography was carried out using Merck 60 silica gel.

The enantiomeric excess (ee) values were determined both by ^{1}H NMR, using either (−)-(*S*)- or (+)-(*R*)-*tert*-butylphenylphosphinothioic acid as a chiral solvating agent[10] or by comparing the $[\alpha]_D$ values with the corresponding data given in Refs. 6 and 7.

4.2. Experimental apparatus for $scCO_2$ reactions

The apparatus consisted of a CO_2 gas cylinder, cooler (−10 °C), pump (Jasco PU-1580 pump), manometer (Taiatsu Techno, Co., Osaka, 25 MPa), stainless steel pressure-resistant vessel (Taiatsu Techno, Co., Osaka, TVS-N2 type, 10 mL), stop valve (Swagelok, SS3NBS4), oven and magnetic stirrer (Koike, HE-16GA).

4.3. Enantioselective acetylation of *rac*-1 in $scCO_2$

The enzyme (5 mg), racemic alcohol **1** (0.23–0.30 mmol), vinyl acetate (0.4 mL) and a magnetic stirrer bar were placed in the vessel (the chemicals were placed in a glass tube to prevent them from contacting the biocatalyst before supercritical conditions were achieved). The vessel was then warmed to 40 °C, and CO_2, preheated to 40 °C, introduced at 13 MPa or at adequate pressure indicated in Table 2. The mixture was then stirred at this

temperature for 2 h or for adequate time indicated in Table 3. Next, the $scCO_2$ was liquefied at −10 °C and the gas pressure released. The resulting residue was dissolved in chloroform, and the mixture put on Extrelut and washed with chloroform. The solvent was then evaporated. The products were separated by column chromatography using chloroform–methanol (in gradient 100:1 to 15:1) as solvent.

4.4. *i*-Propyl hydroxymethanephenylphosphinate 1a

Purified by column chromatography using chloroform–methanol (in gradient 100:1 to 15:1) as eluent, 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.

4.5. Ethyl hydroxymethanephenylphosphinate 1b

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.

4.6. Methyl hydroxymethanephenylphosphinate 1c

After purification by column chromatography using chloroform–methanol (in gradient 100:1 to 15:1) as eluent 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.

4.7. Isopropyl acetoxymethanephenylphosphinate 2a

^{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).

4.8. Ethyl acetoxymethanephenylphosphinate 2b

^{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. Methyl acetoxymethanephenylphosphinate 2c

^{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).

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