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Enzyme-mediated enantioselective hydrolysis of poly(ethylene glycol)-supported carbonates

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Abstract—Kinetic resolution of poly(ethylene glycol)(PEG)-supported carbonates by enzymatic hydrolysis is discussed. Water-soluble carbonates are prepared by immobilization of racemic secondary alcohols onto low-molecular weight monomethoxy PEG (MPEG) through a carbonate linker. Porcine pancreas lipase (PPL) enantioselectively catalyzes the hydrolysis of the substrates to give optically active compounds. In this system, the separation of the resulting alcohols and the remaining substrates is achieved by an extraction process without laborious column chromatography. The carbonates are easily hydrolyzed with K_2CO_3 to afford the corresponding alcohols.

Enzyme-mediated kinetic resolution of racemic alcohols and esters is one of the practical methods for the preparation of optically active compounds, and a great number of examples have been published.[1] Although, in the reaction process, the products and the substrates could be separated mainly by column chromatography, the most tedious and wasteful purification step is the bottleneck to a sustainable and large scale production. In order to resolve this irritating problem, several examples of an easy separation have been published,[2–8] but facile and efficient methods are still desired. On the other hand, organic synthesis based on polymer supports has made rapid progress. Although enzymatic transformation on a polymer support is also of contemporary interest and can be potentially useful for the easy isolation of the products, there have been relatively few reports on polymer-supported reactions by enzymes so far.[7–11] Recently, poly(ethylene glycol) (PEG) has been recognized as an inexpensive and convenient water-soluble polymer.[12,13] We have noted that a PEG-supported strategy could be suitable for enzymatic transformation because the broad solubility of PEG facilitates the analysis of the PEG-supported substrates and could greatly enhance the reactivity under homogeneous conditions.[14–16] In this paper, we report the first example of a hydrolase-mediated kinetic resolution of PEG-supported substrates with a carbonate linker to afford the corresponding optically active compounds, and the method enables us to achieve the easy separation of the resulting alcohols and the remaining substrates by an extraction process without laborious column chromatography.

We used low-molecular weight monomethoxy PEG (MPEG, av MW 750 or 550), which had the desired solubility profile, as the matrix because the loading capacity (1.3 or 1.8 mmol/g, respectively) is higher than that of $MPEG_{5000}$ (av MW 5000), which has been used in many previous reports. Also, the terminal methyl group becomes a reference for the determination of the loading ratio in the reaction steps.[17]

For a screening test of enzymes, we chose the substrate *dl*-**1a** ($MPEG_{750}$), which was afforded by a coupling of racemic 4-benzyloxy-2-butanol (*dl*-**2**) with $MPEG_{750}$–OH through a carbonate linker. The carbonate is a typical linker for organic synthesis on a polymer support and can be easily constructed.[18,19] The substrate *dl*-**1** was readily prepared as shown in Scheme 1. The reaction of *dl*-**2** with *N*,*N*′-carbonyldiimidazole in CH_2Cl_2 at rt proceeded to give the corresponding *dl*-**3**. Immobilization of *dl*-**3** onto MPEG–OH was carried out with DMAP in DMF at 130 °C to afford nearly pure

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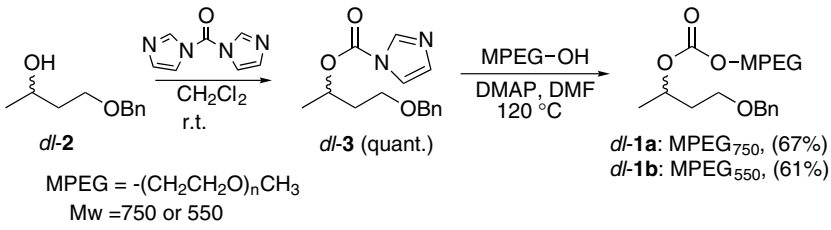


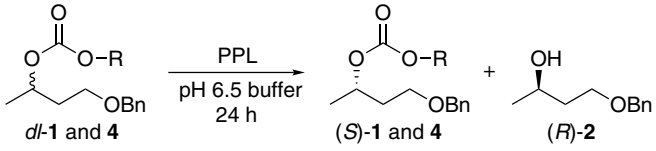
Scheme 1.

$MPEG_{750}$-supported *dl*-**1a**, which was identified by NMR analysis, in 67% yield.[20,21] In the same way, $MPEG_{550}$-supported *dl*-**1b** was also prepared.

In the first screening test using 12 commercially available enzymes,[22] the selection of the enzyme was performed on the basis of hydrolytic activity without taking enantioselectivity into account by examining the production of **2** using thin layer chromatography. In the second screening, we then focused on the enantioselectivity of the hydrolysis. Finally, porcine pancreas lipase (PPL, EC 3.1.1.3, Type II from Sigma) was found to be the best enzyme. In a typical experiment, 200 mg of *dl*-**1a** (sub. concn 5 mM) and 50 mg of PPL were added to 40 mL of 0.1 M sodium phosphate buffer (pH 6.5) and incubated at 30 °C for 24 h. In this case, not only the product **2** but also the remaining **1a** were extracted with AcOEt.[23] After evaporation, the mixture was passed through a pad of silica gel (ca. 1–2 g) with hexane/AcOEt (3/1) as the first eluent to give the alcohol **2**.[24] The MPEG-supported **1a** was eluted with AcOEt/MeOH (3/1). The ee of **2** was determined by HPLC analysis (Chiralcel OD-H, Daicel Chemical Industries, Ltd), and a similar analysis of **2** derived from **1a** with K_2CO_3 was also performed (Table 1). Under the reaction conditions, the reaction of *dl*-**1a** proceeded with high enantioselectivity (conv = 0.29, E value = 23)[25] to afford optically active (*S*)-**1a** (50%, 36% ee) and (*R*)-**2** {28%, 89% ee; $[\alpha]_D^{26}$ − 12.2 (*c* 0.24, MeOH)}. The absolute configurations of the products were determined by comparing the optical rotation of **2** with that of an authentic sample {$[\alpha]_D^{27}$ + 19.0 (*c* 0.95, MeOH)} derived from ethyl (*S*)-3-hydroxybutanoate. As expected, the reaction of lower molecular weight $MPEG_{550}$-supported **1b** also proceeded with high enantioselectivity (conv = 0.35, E value = 28). In the reaction of *dl*-**1b** at 10 °C, the E value was up to 32 and (*R*)-**2** with 93% ee was obtained, although the conversion was apparently decreased. Interestingly, the substrate *dl*-**4** (R = Me), which was not supported on MPEG, was also hydrolyzed with PPL, but the enantioselectivity was very low (E value = 1.4). These results indicate that the hydrophilic MPEG matrix could change the physical property of the alcohol **2** and that the substrate would favorably fit the enzyme active site.

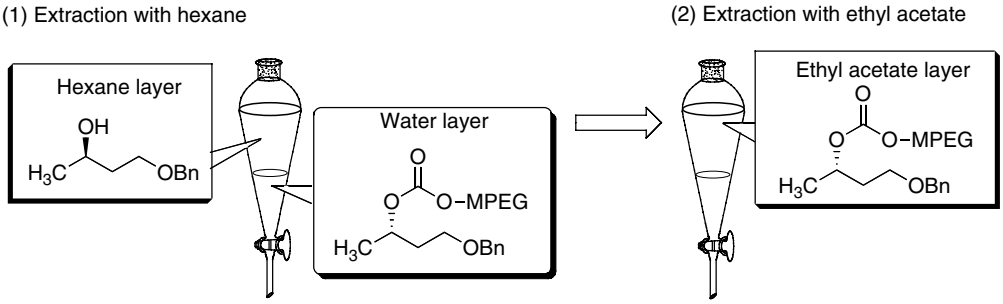
The suitable water solubility of the MPEG-supported substrate enables us to establish a more facile separation of the resulting alcohol **2** and the remaining substrate **1**; this procedure is illustrated in Scheme 2. After the enzymatic reaction, the first extraction process was performed with hexane. In this step, only the alcohol **2** was selectively extracted into the hexane layer. In the following second extraction with AcOEt, the substrate **1** was then successfully obtained in the organic layer while MPEG–OH, which was removed from **1**, remained in the aqueous layer. The yields and the ees of the compounds were comparable to those obtained from the separation method using a pad of silica gel mentioned above.

Table 1. Enantioselective hydrolysis of carbonates **1** and **4** with PPL[a]



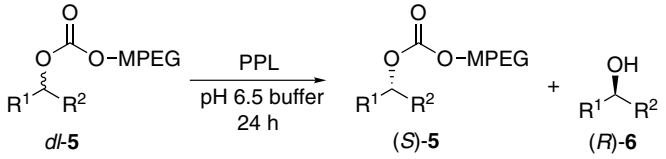
Substrate	R	Temp (°C)	Carbonate		Alcohol		Conv[e]	E[f]
			Yield[b] (%)	ee (%)[c]	Yield (%)	ee (%)[d]		
1a	$MPEG_{750}$	30	50	36	28	89	0.29	23
1b	$MPEG_{550}$	30	54	47	30	89	0.35	28
1b	$MPEG_{550}$	10	76	10	21	93	0.10	32
4	Me	30	65	5	28	12	0.27	1.4

[a] The reaction was performed using 5 mM of the substrate with PPL in 0.1 M phosphate buffer (pH 6.5) for 24 h.
[b] Determined by its weight on the basis of the weight of the racemic substrate.
[c] Determined by HPLC analysis after the hydrolysis of the carbonate.
[d] Determined by HPLC analysis.
[e] Calculated by ee(carbonate)/[ee(carbonate) + ee(alcohol)].
[f] Calculated by ln[(1−conv)(1−ee(carbonate))]/ln[(1−conv)(1 + ee(carbonate))].



Scheme 2.

Table 2. Enantioselective hydrolysis of carbonates **5** with PPL[a]



Substrate	R^1	R^2	Carbonate **5**		Alcohol **6**		Conv[e]	E[f]
			Yield (%)[b]	ee (%)[c]	Yield (%)	ee (%)[d]		
5a	CH_3	Ph	36	95	31	77	0.55	29
5b	CH_3	CH_2CH_2Ph	37	23	47	27	0.46	2
5c	Vinyl	CH_2CH_2OBn	47	56	41	80	0.41	16

[a] The reaction was performed using 5 mM of *dl*-**5** with PPL in 0.1 M phosphate buffer (pH 6.5) for 24 h.
[b] Determined by its weight on the basis of the weight of the racemic substrate.
[c] Determined by HPLC analysis after the hydrolysis of the carbonate.
[d] Determined by HPLC analysis.
[e] Calculated by ee(**5**)/[ee(**5**) + ee(**6**)].
[f] Calculated by ln[(1−conv)(1−ee(**5**))]/ln[(1−conv)(1 + ee(**5**)].

We applied this procedure to several substrates supported on $MPEG_{550}$ under the same conditions, and the results are shown in Table 2. In all cases, the alcohols **6** and the substrates **5** were successfully obtained by the two-step extraction procedure as expected. While the reaction of *dl*-**5b** (R^1 = Me, R^2 = CH_2CH_2Ph) showed a low enantioselectivity (E value = 2), the other substrates were enantioselectively hydrolyzed. In particular, the hydrolysis of 1-phenylethanol derivative **5a** (R^1 = Me, R^2 = Ph) proceeded with higher enantioselectivity (conv = 0.55, E value = 29) to afford the corresponding alcohol (*R*)-**6a** (77% ee) and (*S*)-**5a** (95% ee) in 31% and 36% yields, respectively.

In conclusion, we have demonstrated for the first time the hydrolase-mediated kinetic resolution of racemic carbonates on a low-molecular weight MPEG support to afford the optically active compounds. In our method, separation of the resulting alcohols from the remaining substrates was achieved by an extraction process without time- and solvent-consuming column chromatography. Further investigations are now in progress.

Acknowledgements

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20. After the reaction of *dl*-**3** with MPEG–OH, the mixture was washed with 2 M HCl in order to remove DMAP, the resulting imidazole and the remaining MPEG–OH. The product **1a** was purified by column chromatography on silica gel (eluent, AcOEt → AcOEt/MeOH = 3/1). Compound **1a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, J = 6.5 Hz, 3H), 1.77–2.03 (m, 2H), 3.38 (s, 3H, CH_3 O–PEG), 3.50–3.57 (m, 2H, PEG), 3.55–3.70 (m, ca. 46H, PEG), 3.70 (t, J = 4.5 Hz, 2H, PEG), 4.17–4.33 (m, 2H), 4.49 (s, 2H), 4.89–5.01 (m, 1H), 7.26–7.37 (m, 5H); IR (neat) 2870, 2359, 1742, 1454, 1350, 1263, 1105, 949, 847 cm^{-1}.
21. The yields of MPEG-supported compounds were determined by the weights with the assumption that the MW was 750 or 550 for MPEG–OH.
22. In the screening test, we used the following enzymes: PPL (Sigma), Lipase AK, Lipase PS, Lipase D, Lipase AP, Lipase AY, Newlase F, PLE (Amano Enzyme, Inc.), Lipase OF (Meito Sangyo Co., Ltd), Esterase SNSM-87 (Nagase & Co., Ltd), Tripsin, α-Chymotrypsin (E. Merck). The hydrolysis of **1a** with PPL or Esterase SNSM-87 proceeded with low enantioselectivity. The other enzymes did not catalyze the reaction.
23. In the process, the resulting MPEG–OH could not be extracted with an organic solvent.
24. Compound **2**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, J = 6.0 Hz, 3H), 1.66–1.85 (m, 2H), 2.82 (br s, 1H), 3.60–3.75 (m, 2H), 3.95–4.07 (m, 1H) 4.53 (s, 2H), 7.25–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 38.1, 67.4, 69.0, 73.2, 127.6, 127.7, 128.4, 137.9; IR (neat) 3416, 2965, 2864, 1494, 1454, 1368, 1206, 1099, 1028, 737, 698 cm^{-1}. The spectral data were in full agreement with those reported.[26] HPLC conditions: column, Chiralcel OD-H (Daicel Chemical

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