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Easy separation of optically active products by enzymatic hydrolysis of soluble polymer-supported substrates

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Abstract—The easy separation of optically active compounds from enzymatic kinetic resolution products by simple precipitation using poly(ethylene glycol)(PEG)-supported carbonates is disclosed. The water-soluble substrate was prepared by the immobilization of (±)-1-phenylethanol onto a middle-molecular weight (av M_w 5000) monomethoxy PEG (MPEG) through a carbonate linker. The enantioselective hydrolysis using Lipase from porcine pancreas (PPL; Type II, Sigma) in a mixed solvent (hexane/buffer = 9:1) proceeded to afford the corresponding optically active compounds. In this system, the separation of the products was achieved by a simple procedure without laborious column chromatography. A hydrophobic spacer between the MPEG moiety and the carbonate linker affected both the reactivity and enantioselectivity, and the substrate with a phenylethyl spacer was hydrolyzed with the highest enantioselectivity (E value = 270).

The kinetic resolution of racemic alcohols and esters using hydrolytic enzymes is one of the practical methods for the preparation of optically active alcohols, and a significant number of examples have been published.[1] During the reaction process, the enantiomers, the remaining substrate and the resulting product, could be mainly separated by column chromatography. However, the tedious and wasteful purification step is a bottleneck to an easy operation and a sustainable synthesis. Although several studies of easy separation processes have been published,[2–5] facile and efficient procedures are still desired. Recently, poly(ethylene glycol) (PEG) has been recognized as an inexpensive and convenient soluble polymer,[6–9] and we noticed that a PEG-supported strategy could be suitable for an enzymatic transformation and potentially useful for the easy isolation of the products. We have already succeeded in the kinetic resolution of a low-molecular weight monomethoxy PEG (MPEG, av M_w 550 or 750)-supported substrate with a carbonate linker using a hydrolytic enzyme (porcine pancreas lipase (PPL), lipase Type II from Sigma).[10] The broad solubility of PEG facilitated the

analysis of the PEG-supported substrates, and the easy separation of the products by an extraction procedure was achieved. However, for the isolation of the MPEG-supported compounds through all processes of the substrate syntheses, the column chromatography steps were still needed because the compounds were liquids. In this Letter, we report the enzyme-mediated kinetic resolution of higher-molecular weight (av M_w 5000) MPEG-supported carbonates, which are solids and easier to handle. Furthermore, the introduction of an appropriate hydrophobic spacer between the MPEG moiety and the carbonate linker affects both the reactivity and enantioselectivity.

We chose the carbonate (±)-**1** and **2** as the substrates with and without a spacer, respectively. The carbonate is a typical linker used in organic synthesis on a polymer support, and (±)-**1** was easily prepared by the coupling of the racemic 1-phenylethanol ((±)-**3**) with MPEG$_{5000}$–OH (Scheme 1).[11,12] We are also interested in the affect of a hydrophobic spacer between the MPEG moiety and the carbonate linker. The substrates (±)-**2** were also synthesized in 4 steps from MPEG$_{5000}$–OH.[12] By making use of the MPEG$_{5000}$, which has been utilized in many studies, it allows us to isolate and purify the substrates (±)-**1** and **2** by a simple precipitation procedure from diethyl ether.[7]
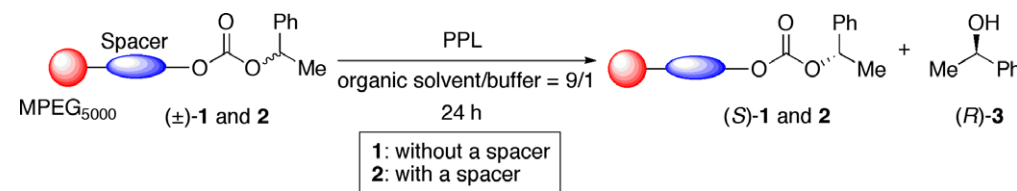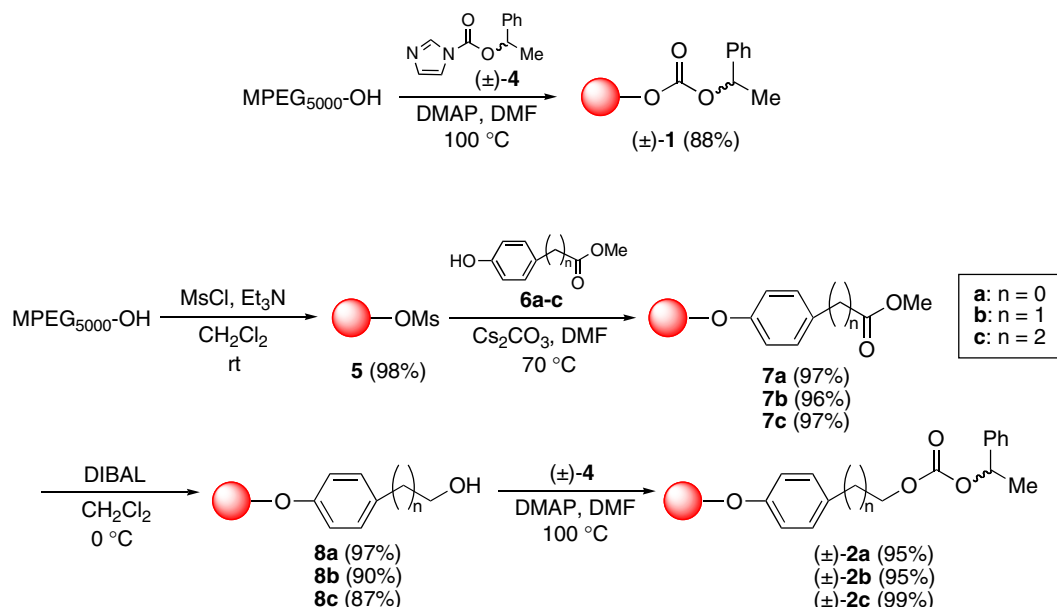
Scheme 1.

At first, we examined the PPL-catalyzed reaction of (±)-**1** under the same reaction conditions as those for the MPEG$_{550}$-supported substrates (0.1 M phosphate buffer (pH 6.5) medium; 24 h; 30 °C).[10] As expected, it was found that the hydrolysis of (±)-**1** proceeded to afford the optically active (S)-**1** (56% ee) and (R)-**3** (82% ee) (conv. = 0.41, E value = 18),[13,14] although the reactivity and enantioselectivity were lower than those of the corresponding substrate with MPEG$_{550}$ (conv. = 0.55, E value = 29).[10] In this case, the extraction process was also necessary for recovering the unreacted **1**, and there were not many advantages for the easy preparation versus using MPEG$_{550}$. We then tried to examine the reaction in a mixed solvent (organic solvent/buffer = 9:1). In a typical experiment, 125 mg of (±)-**1** (sub. concn 5 mM) and 10 mg of PPL were added to a hexane–buffer (4.5 mL:0.5 mL), and stirred at 30 °C (Table 1, entry 1). Fortunately, the enantioselective hydrolysis smoothly proceeded even in this case. While the use of the organic solvent did not improve the enantioselectivity, the conversion was up to 0.58 (conv. = 0.58, E value = 13). Changing the organic solvent to toluene and i-Pr$_2$O (entries 2 and 3) decreased both the reactivity and enantioselectivity. For the kinetic controlled reaction, the temperature could be one of the important factors in many cases. We then investigated the temperature effect of the reaction (entries 4–6). As expected, lowering the temperature apparently improved the enantioselectivity. In particular, for the reaction at 0 °C (entry 6), the E value was up to 58 and (R)-**3** with 96% ee was obtained, although the conversion apparently decreased to 0.16.[15] We considered that a suitable spacer could increase the affinity to the active site of the enzyme, which had many hydrophobic amino residues inside the binding pocket. Beyond our expectation, the introduction of the spacer drastically increased not only the conversion, but also the enantioselectivity. For the cases of the phenylmethyl and phenylpropyl spacers (entries 7 and 9), the conversions

were up to 0.44 and 0.30, respectively, besides the carbonates (±)-**2a** (n = 0) and **2c** (n = 2) were hydrolyzed with very high enantioselectivities and E values were also up to 151 and 74, respectively. These results indicate that the substrates would more favorably fit to the enzyme active site. On the other hand, the phenylethyl spacer in the substrate **2b** (n = 1) gave a different effect (entry 8). The hydrolysis of (±)-**2b** proceeded with the highest enantioselectivity (E value = 270) to afford the almost optically pure (R)-**3**, although the conversion was slightly improved against that of the original substrate (±)-**1**.[16] The result suggests that the introduction of the phenylethyl spacer could drastically lower the affinity of the slow reactive enantiomer of **2b** for the enzyme.

The use of the MPEG$_{5000}$ and the two-phase system also enabled us to achieve an easier work-up procedure (Scheme 2). Only the resulting alcohol (R)-**3** was extracted in the hexane layer, and was isolated after evaporation. On the other hand, the aqueous layer was diluted with CH$_2$Cl$_2$, and the dehydration was performed with anhydrous Na$_2$SO$_4$. After evaporation, the residue was poured into Et$_2$O to precipitate the mixture of the MPEG$_{5000}$-supported carbonate and MPEG$_{5000}$–OH as a white solid, which was collected by simple filtration. The chemical hydrolysis with NaOH in MeOH–H$_2$O gave the optically active (S)-**3**.

The concept of this reaction was applicable to the preparation of not only the optically active **3**, but also other optically active compounds. For example, the reaction of substrate (±)-**9**, which was constructed from MPEG$_{5000}$ and (±)-4-benzyloxy-2-butanol (**10**), also proceeded with enantioselectivity to afford the corresponding optically active compounds, although the reaction conditions were not necessarily optimized (Scheme 3).[15] The work-up was performed as the same procedure as that mentioned above.

Table 1. Enantioselective hydrolysis of MPEG$_{5000}$-supported carbonates (±)-**1** and **2**[a]

| Entry	Substrate	Organic solvent	Temp (°C)	ee of carbonate[b] (%)	ee of alcohol[c] (%)	Conv.[d]	E value[e]
1	**1**	Hexane	30	88	65	0.58	13
2	**1**	Toluene	30	15	78	0.17	9
3	**1**	*i*-Pr$_2$O	30	18	78	0.19	10
4	**1**	Hexane	20	50	88	0.36	26
5	**1**	Hexane	10	41	93	0.31	39
6	**1**	Hexane	0	18	96	0.16	58
7	**2a**	Hexane	0	76	97	0.44	151
8	**2b**	Hexane	0	31	99	0.24	270
9	**2c**	Hexane	0	42	96	0.30	74

[a] The reaction was performed using 5 mM of the substrates with PPL in a mixed medium (organic solvent/0.1 M phosphate buffer (pH 6.5) = 9:1) for 24 h.
[b] Determined by GLC analysis after the hydrolysis of the unreacted substrates.
[c] Determined by GLC analysis.
[d] Calculated by ee(carbonate)/[ee(carbonate) + ee(**3**)].
[e] Calculated by $\ln[(1 - \text{conv.})(1 - \text{ee(carbonate)})]/\ln[(1 - \text{conv.})(1 + \text{ee(carbonate)})]$.
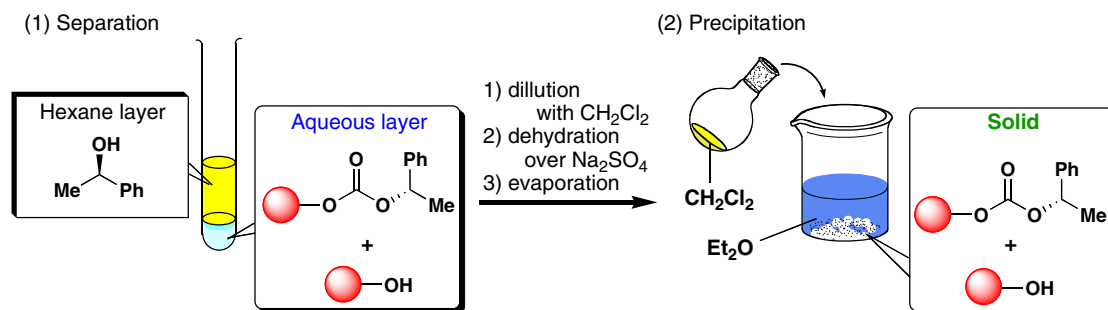
Scheme 2.
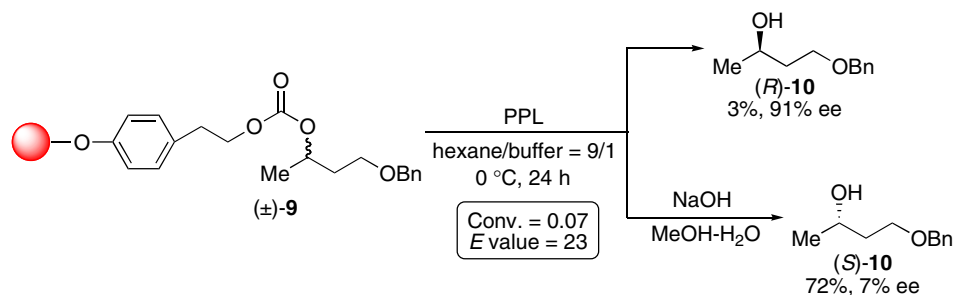
Scheme 3.

In conclusion, we have demonstrated the enzyme-mediated kinetic resolution of soluble polymer (MPEG$_{5000}$)-supported carbonates to afford optically active secondary alcohols (**3** and **10**). In our method, the separation and isolation of the reaction products were achieved by a simple precipitation technique without the time- and solvent-consuming column chromatography. Furthermore, we have disclosed that the reactivity and enantioselectivity can be controlled using a suitable hydrophobic spacer between the MPEG moiety and the carbonate linker. We anticipate that the use of a soluble polymer as the matrix of the substrates will provide an operationally simple and eco-friendly protocol.

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11. The synthetic method of the imidazolide (±)-**4** has already been reported.[10] Compound **1**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.59 (d, $J = 6.5$ Hz, 3H), 3.38 (s, 3H, CH_3O–PEG), 3.50 (t, $J = 5.0$ Hz, 2H), 3.55 (t, $J = 4.5$ Hz, 2H), 3.57–3.68 (m, PEG–methylenes), 3.70 (t, $J = 4.5$ Hz, 2H, PEG), 3.78 (t, $J = 5.0$ Hz, 2H), 4.23 (td, $J_1 = 4.5$ Hz, $J_2 = 12.0$ Hz, 1H), 4.28 (td, $J_1 = 5.0$ Hz, $J_2 = 12.0$ Hz, 1H), 5.72 (q, $J = 6.5$ Hz, 1H), 7.23–7.39 (m, 5H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 22.2, 58.8, 66.7, 68.7, 70.37, 70.40, 70.6, 71.7, 76.3, 125.9, 127.9, 128.3, 140.8, 154.3; IR (KBr) 2887, 1744, 1636, 1466, 1342, 1281, 1113, 964, 843 cm^{-1}.

12. The yields of the MPEG-supported compounds were based on the weights of the starting materials. The purities were determined by a ^{1}H NMR analysis, and the terminal methyl group and/or the PEG–methylenes were used as the reference. The purities of the substrates **1**, **2a**, **2b**, and **2c** were ca. 91%, 94%, 89%, and 93%, respectively. The detail of the synthetic procedure of the substrates will be reported separately.

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14. The ee of **3** was determined by GLC analysis, and a similar analysis of **3** derived from **1** was also performed. GLC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm × 50 m; injection, 160 °C; detection, 160 °C; oven, 140 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 8.9 (*R*) and 9.2 (*S*) min.

15. For the reaction of a methyl carbonate of (±)-**3** under the same conditions, the reaction scarcely proceeded. On the other hand, an acetate of (±)-**3** was slowly hydrolyzed (conv. = 0.11) without any enantioselectivity. The hydrolysis of a methyl carbonate of (±)-**10** was also proceeded with very low enantioselectivity (*E* value = 1.4).[10]

16. The reaction was also performed using 3.6 g of the substrate (±)-**2b**. In this case, we finally obtained (*R*)-**3** (98% ee, $[\alpha]_D^{18}$ +32.8 (*c* 0.96, MeOH)) in 12% and (*S*)-**3** (58% ee, $[\alpha]_D^{25}$ −21.0 (*c* 1.06, MeOH)) in 46% isolated yields (conv. = 0.37, *E* value = 179).[17]

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