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Enzyme-mediated enantioselective hydrolysis of soluble polymer-supported dendritic carbonates

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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 dendritic carbonates is disclosed. The water-soluble polymer-supported substrates were prepared by immobilization of (±)-1-phenylethanol onto a monomethoxy PEG (MPEG; av MW 5000) bearing a dendritic spacer through a carbonate linker. The enantioselective hydrolysis of the dendritic substrates of the 1st and 2nd generations using lipase from porcine pancreas (PPL; Type II, Sigma) smoothly proceeded, and a multimolecule of the corresponding (*R*)-alcohols was released from one molecule of the racemic substrates. The *E* values of the reactions at 0 °C in a mixed solvent (hexane/buffer = 9/1) were up to >200.

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, should be separated, and the tedious and wasteful purification step by column chromatography is still a big problem for an easy operation. We noticed that poly(ethylene glycol) (PEG) was an inexpensive and convenient soluble polymer,[2–5] and 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 low- and middle-molecular weight monomethoxy PEG (MPEG, av MW 550, 750, and 5000)-supported substrates with a carbonate linker using a hydrolytic enzyme (porcine pancreas lipase (PPL), lipase Type II from Sigma),[6,7] and the easy separation of the products has been achieved. The reaction of $MPEG_{5000}$-supported substrates, which were soluble solids and easier to handle, was preferable in terms of both their reactivity and their enantioselectivity. However, the number of alcohols immobilized per gram of the substrate (the loading capacity) is very low (ca. 0.2 mmol/g), and this drawback is a bottleneck to an effective synthesis. In order to overcome this problem, we decided to incorporate the basic principle of dendrimer chemistry[8] into our strategy.[9] In this Letter, we disclose the first example of the hydrolase-mediated kinetic resolution of PEG-supported dendritic substrates with a carbonate linker. The substrates have a higher loading capacity (ca. 0.4–0.8 mmol/g), and a multimolecule of the corresponding optically active alcohols can be released from one molecule of the substrate.

We first examined the PPL-catalyzed hydrolysis of the MPEG (av MW 5000)-supported carbonates (±)-**1a** (R = Ph) and **1b** (R = $(CH_2)_2OBn$) bearing two functional moieties (Scheme 1).[10–12] The dendritic substrates of the 1st generation were easily synthesized by coupling of the racemic imidazolides (±)-**7a** and **7b** derived from 1-phenylethanol (**2a**) and 4-benzyloxy-2-butanol (**2b**), respectively, with the diol **6**, which was prepared by a modification procedure of a reference (Scheme 1).[9] Both substrates, as white solids, are soluble in water, but not in hexane.

The reaction was carried out under the same reaction conditions as those already reported.[7] In a typical experiment, 125 mg of the substrate (sub. concd 5 mM) and 10 mg of PPL were added to a mixed solvent (hexane, 4.5 mL; 0.1 M phosphate buffer (pH 6.5), 0.5 mL) in a test tube, and stirred at 30 °C (Table 1). Fortunately, the enantioselective hydrolysis of **1a** smoothly proceeded even in this case, and the corresponding optically active (*R*)-**2a** was obtained (entries 1–4).[13] After the reaction, the separation of products was already finished, because only the resulting alcohol (*R*)-**2a** was extracted in the hexane layer. The aqueous layer was diluted with CH_2Cl_2, and the dehydration was performed with anhydrous Na_2SO_4. After evaporation, the remaining **1a** was easily precipitated by pouring the residue into Et_2O. As a matter of course, the chemical hydrolysis of **1a** gave (*S*)-**2a**. To the best of our knowledge, this is a first example of the enzyme-mediated

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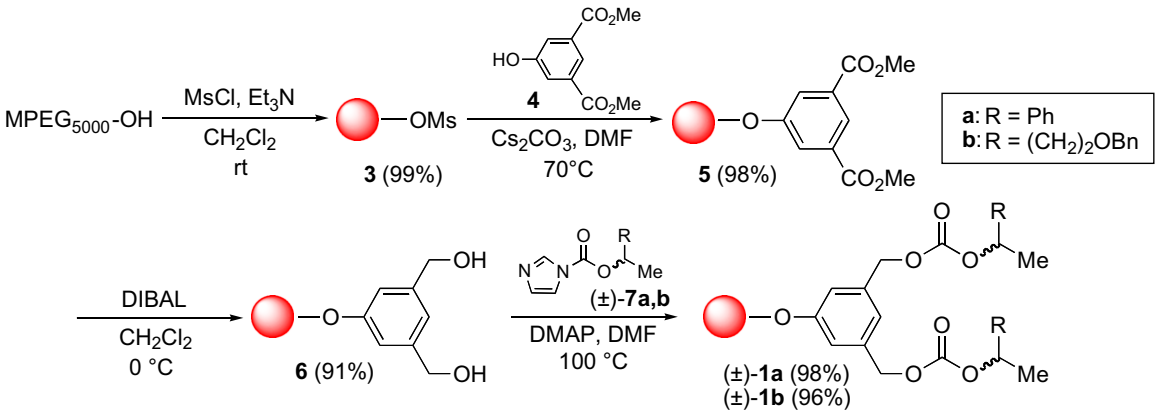
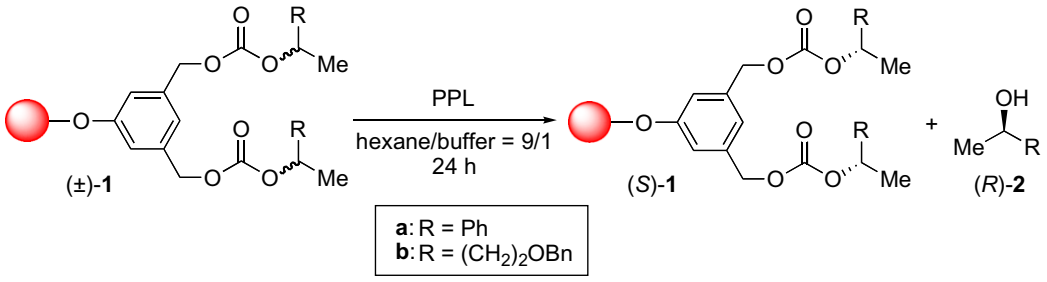


Scheme 1.

Table 1
Enantioselective hydrolysis of $MPEG_{5000}$-supported dendritic carbonates (±)-**1**[a]



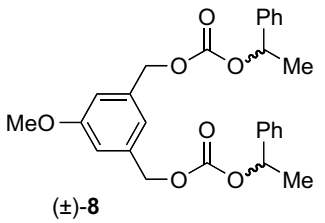
Entry	Substrate	Temperature (°C)	ee of carbonate **1**[b] (%)	ee of alcohol **2**[c] (%)	Conv.	*E* value
1	**1a**	30	82	89	0.48	44
2	**1a**	20	81	92	0.47	60
3	**1a**	10	72	97	0.43	142
4	**1a**	0	40	99	0.29	>200
5[d]	**1a**	0	73[e,f]	98[f,g]	0.43	>200
6[d]	**1b**	0	13[f,h]	97[f,i]	0.12	75

[a] The reaction was performed using 125 mg of the substrate in a test tube.
[b] Determined by GLC analysis after the chemical hydrolysis of **1**.
[c] Determined by GLC analysis.
[d] The reaction was performed on a gram scale of the substrate in a recovery flask.
[e] Isolated in 52% yield as (*S*)-**2a**; $[\alpha]_D^{29}$ −29.5 (*c* 1.03, MeOH).
[f] The isolated yields of **2** were calculated on the basis of the amount of **1**, and the ideal combined yields of both enantiomers of **2** were 200%. We assumed that the MW of MPEG-OH was 5000.
[g] Isolated in 46% yield; $[\alpha]_D^{29}$ +40.1 (*c* 0.38, MeOH).
[h] Isolated in 154% yield as (*S*)-**2b**; $[\alpha]_D^{29}$ +2.1 (*c* 1.05, MeOH).
[i] Isolated in 22% yield; $[\alpha]_D^{29}$ −14.9 (*c* 1.08, MeOH).

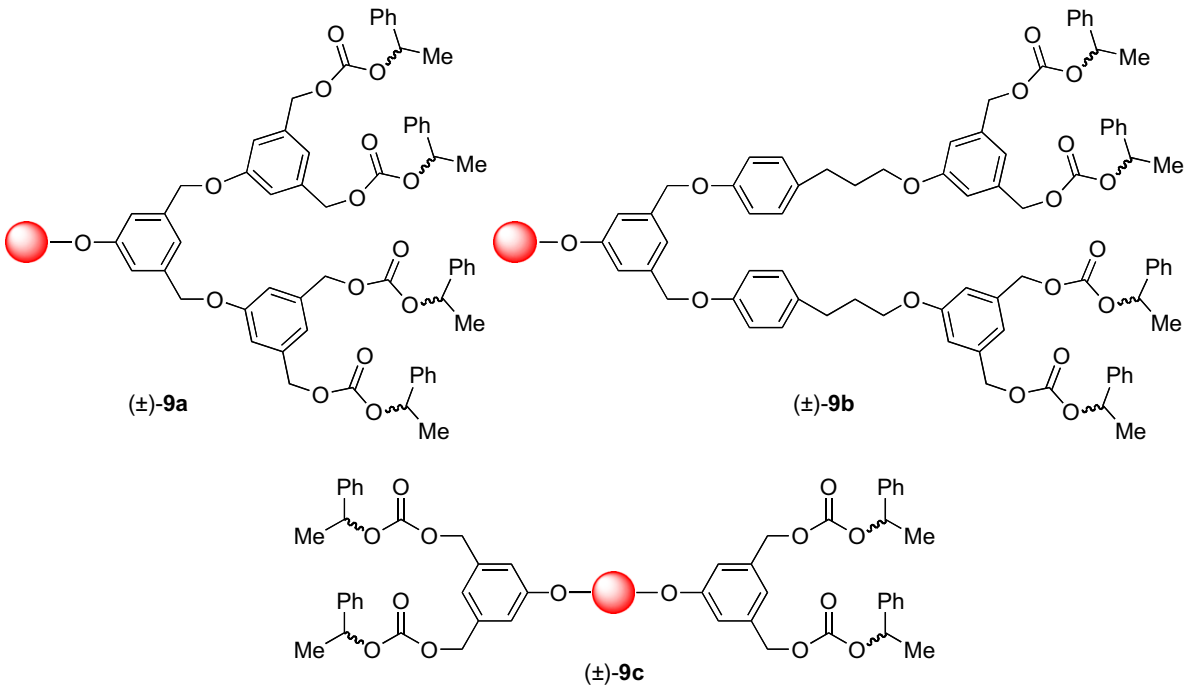
enantioselective hydrolysis of a dendritic substrate. As expected, lowering the temperature apparently improved the enantioselectivity, while the conversion gradually decreased. For the reaction at 0 °C, the *E* value[14] was up to >200 and (*R*)-**2a** with a 99% ee was obtained.[15] On the other hand, we also tried to examine the enzymatic hydrolysis of the dendritic substrate **8**, which was not supported by MPEG, under the same reaction conditions (Scheme 2). The reaction, however, did not proceed at all. This indicates that the solubility of MPEG plays an important role in the enzymatic hydrolysis.

This reaction was also useful in a preparative-scale operation (>1 g of (±)-**1a**) using a recovery flask, and a comparable enantioselectivity was obtained with a higher conversion (entry 5).[16,17] The substrate (±)-**1b** was also hydrolyzed with a high enantioselectivity (*E* value = 75) to afford the optically active (*R*)-**2b** with 97% ee, although the conversion was apparently lower than that of **1a** (entry 6).[18,19]

Next, we tried to examine the enzymatic reactions of several substrates bearing a higher loading capacity as shown in Scheme 3,[20] the results of which are summarized in Table 2. The compound (±)-**9a** is an $MPEG_{5000}$-supported dendritic carbonate of 2nd generation, and has four functional moieties (entry 1). The enzyme also



Scheme 2.



Scheme 3.

catalyzed the enantioselective hydrolysis of **9a** for 48 h to give (*R*)-**2a** (*E* value = 36), but the reactivity was quite low (conv. = 0.03). We assumed that the steric hindrance of **9a** might inhibit the reaction, and the elongation of the spacer could cancel the repulsion between the reactive parts. As expected, introducing a longer spacer ((±)-**9b**) apparently improved the reactivity, and the conversion was up to 0.17 (entry 2). Interestingly, the *E* value also increased to 120. On the other hand, the enantioselective hydrolysis of the substrate (±)-**9c**, which was constructed on PEG (av MW 4600) as the matrix and had twice the functional groups of MPEG, proceeded even in this case (entry 3), and the *E* value was up to >200.

In conclusion, we succeeded in producing the first example of the enzyme-mediated kinetic resolution of soluble polymer-supported dendritic carbonates to afford optically active alcohols (**2a** and **2b**). In our method, a multimolecule of optically active alcohols could be released from one molecule of the racemic substrates, and the separation and isolation of the reaction products were achieved by a simple precipitation technique. We anticipate that the concept of the coupling of dendrimer chemistry with PEG chemistry can provide a useful and eco-friendly protocol in not only organic chemistry, but also medicinal chemistry for the development of PEG-supported prodrugs, which gradually release native drugs by enzymatic hydrolysis.

Table 2
Enantioselective hydrolysis of $MPEG_{5000}$- and PEG_{4600}-supported dendritic carbonates[a]

Entry	Substrate	ee of (*S*)-**9**[b] (%)	ee of (*R*)-**2a**[c] (%)	Conv.	*E* value
1	**9a**	3	96	0.03	50
2	**9b**	20	98	0.17	120
3[d]	**9c**	20	99	0.17	>200

[a] Unless otherwise noted, the reaction was performed using 125 mg of the substrate in a test tube for 48 h at 0 °C.
[b] Determined by GLC analysis after the chemical hydrolysis of **9**.
[c] Determined by GLC analysis.
[d] The reaction was performed for 24 h.

Acknowledgment

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10. 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.
11. Compound (±)-**1a** (purity, ca. 90%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.59 (d, J = 6.5 Hz, 6H), 3.38 (s, 3H), 3.44–3.80 (m, PEG–methylenes), 3.83 (t, J = 5.0 Hz, 2H), 4.08 (t, J = 5.0 Hz, 2H), 5.02 (d, J = 12.5 Hz, 2H), 5.08 (d, J = 12.0 Hz, 2H), 5.73 (q, J = 6.5 Hz, 2H), 6.86 (s, 2H), 6.91 (s, 1H), 7.27–7.39 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.2, 58.9, 67.3, 68.8, 69.4, 70.4 (PEG), 70.5, 70.6, 71.7, 76.5, 114.1, 119.9, 125.8, 128.0, 128.4, 136.8, 140.8, 154.2, 158.9; IR (KBr) 2886, 1742, 1645, 1466, 1344, 1281, 1252, 1113, 953, 843 cm^{-1}; ESI-TOF MS m/z 2634.30 Da (most abundant ion; 2632.50 Da calcd for $[MeO(CH_2CH_2O)_{108}C_{26}H_{25}O_6{\cdot}2Na]^{2+}$), $[MeO(CH_2CH_2O)_{81-134}C_{26}H_{25}O_6{\cdot}2Na]^{2+}$ (m/z range 2040.40–3206.49 Da; z = 2).
12. Compound (±)-**1b** (purity, ca. 94%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (d, J = 6.0 Hz, 6H), 1.79–1.89 (m, 2H), 1.91–2.01 (m, 2H), 3.37 (s, 3H), 3.46–3.80 (m, PEG–methylenes), 3.82 (t, J = 5.0 Hz, 2H), 4.10 (t, J = 5.0 Hz, 2H), 4.46 (s, 4H), 4.97 (tq, J_1 = 1.0 Hz, J_2 = 6.5 Hz, 2H), 5.04 (d, J = 12.5 Hz, 2H), 5.08 (d, J = 12.0 Hz, 2H), 6.878 (s, 1H), 6.880 (s, 1H), 6.94 (s, 1H), 7.18–7.37 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.0, 35.8, 58.9, 66.2, 67.4, 68.7, 69.5, 70.4 (PEG), 70.6, 70.7, 71.8, 72.9, 73.0, 114.1, 120.0, 127.4, 127.5, 128.2, 137.0, 138.1, 154.4, 159.0; IR (KBr) 2886, 1742, 1645, 1468, 1342, 1281, 1242, 1115, 964, 843 cm^{-1}; ESI-TOF MS m/z 2692.58 Da (most abundant ion; 2690.54 Da calcd for $[MeO(CH_2CH_2O)_{108}C_{32}H_{37}O_8{\cdot}2Na]^{2+}$), $[MeO(CH_2CH_2O)_{84\text{-}135}C_{32}H_{37}O_8{\cdot}2Na]^{2+}$ (m/z range 2163.64–3287.75 Da; z = 2).
13. GC analysis of **2a**: 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.
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15. The conversion was calculated by ee(**1**)/[ee(**1**) + ee(**2**)], and the E value was calculated by ln[(1 − conv.)(1 − ee(**1**))]/ln[(1 − conv.)(1 + ee(**1**))].
16. Although the total isolated yield (98%) of (*R*)- and (*S*)-**2a** was low due to the high volatility, the number was almost twice for of that a non-dendritic substrate.[7]
17. The absolute configurations of the products were determined by comparing the optical rotation of **2a** with the reported one; lit.[21] $[\alpha]_D^{20}$ +45 (*c* 5.15, MeOH) for the (*R*)-enantiomer.
18. The absolute configurations of the products were determined by comparing the optical rotation of **2b** with the reported one; lit.[6b] $[\alpha]_D^{27}$ +19.0 (*c* 0.95, MeOH) for the (*S*)-enantiomer.
19. HPLC analysis of **2b**: column, CHIRALCEL OD-H (Daicel Chemical Industries, Ltd); eluent, hexane/2-propanol = 90/10; flow rate, 0.5 mL/min; 254 nm; temperature, 25 °C; retention time, 13 (*S*) and 14 (*R*) min.
20. The substrates were synthesized by the sequential coupling of $MPEG_{5000}$-OH or PEG_{4600}-OH with their respective units.
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