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Tetrahedron 62 (2006) 7300–7306
Tetrahedron

Kinetic resolution of poly(ethylene glycol)-supported carbonates by enzymatic hydrolysis

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Received 7 April 2006; revised 9 May 2006; accepted 11 May 2006
Available online 5 June 2006

Abstract—The enzyme-mediated enantioselective hydrolysis of poly(ethylene glycol) (PEG)-supported carbonates is disclosed. The water-soluble carbonates were prepared by immobilization of a racemic secondary alcohol (4-benzyloxy-2-butanol) onto low-molecular weight (av MW 550 and 750) monomethoxy PEG through a carbonate linker. For the screening of the hydrolytic enzymes, the substrate was enantioselectively hydrolyzed by commercially available lipase from porcine pancreas (PPL; Type II, Sigma) to afford the optically active compounds. In this system, the separation of the remaining (*S*)-substrate and the resulting (*R*)-alcohol was achieved by an extraction process without a laborious column chromatography. The (*S*)-carbonate was easily hydrolyzed with K_2CO_3 to afford the corresponding (*S*)-alcohol. Other MPEG-supported substrates were also hydrolyzed to afford the corresponding optically active alcohols.

1. Introduction

Optically active secondary alcohols are versatile intermediates in organic syntheses. The use of enzymes in the preparation of such compounds is especially attractive due to its benign effect on the environment. In particular, the kinetic resolution of racemic alcohols and esters using hydrolytic enzymes is one of the practical methods for the preparation of the optically active compounds, and a significant number of examples have been reported.[1] In the reaction process, the enantiomers, the remaining substrate and the resulting product, could be separated mainly by column chromatography. However, the tedious and wasteful separation step is the bottleneck to an easy operation and a sustainable product. Although, in order to resolve this irritating problem, several studies of an easy separation have been published,[2–9] facile and efficient procedures are still desired.

On the other hand, organic synthesis based on polymer supports has made rapid progress, especially in the field of combinatorial chemistry. Because insoluble polymers (polystyrene, silica gel, and so on) are usually used, it is called 'solid-phase' chemistry. While the methodology provides us an easy separation of the products, the heterogeneous reaction causes a low reactivity and a difficult analysis of the polymer-supported intermediate. 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.[6–12] Recently, poly(ethylene glycol) (PEG) has been recognized as an inexpensive and convenient soluble polymer.[13,14] The synthetic approach using a soluble polymer is termed as 'liquid-phase' chemistry and couples the advantages of homogeneous solution chemistry with those of solid-phase chemistry. 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 significantly enhance the reactivity under homogeneous conditions. Actually, PEG-supported esters and amides have been studied as prodrugs, which are hydrolyzed in vitro or in vivo to gradually release native drugs.[15] In this report, we disclose the first example of the kinetic resolution of PEG-supported substrates with a carbonate linker by a hydrolytic enzyme to afford the corresponding optically active compounds, and the method enables us to achieve the easy separation of the remaining substrates and the resulting alcohols by an extraction process without laborious column chromatography.[16]

In general, previously reported PEG use during enzymatic synthesis has been restricted as the reagent for the modification of enzymes[17] and the additive for improving the enzyme

Keywords: Carbonates; Enantioselective hydrolysis; Enzymes; Hydrolase; Poly(ethylene glycol)-supported substrate.
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doi:10.1016/j.tet.2006.05.020

activities.[18] To the best of our knowledge, there has been only one report on the PEG-supported substrate used for enzymatic transformation.[19] However, the substrate only worked as a nucleophile to an acyl–enzyme intermediate, and the example did not make the most of the advantage of PEG. We now present a new aspect of PEG use.

2. Results and discussion

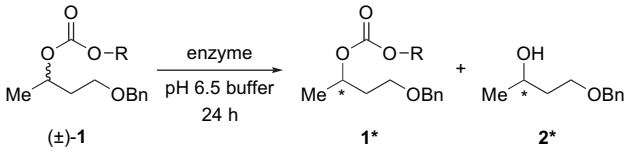
2.1. Screening test of enzymes

We used low-molecular weight monomethoxy PEG (MPEG, av MW 750 and 550) as the matrix.[20] It has the desired solubility profile and the higher loading capacity ($MPEG_{750}$, 1.3 mmol/g; $MPEG_{550}$, 1.8 mmol/g), while that of $MPEG_{5000}$ (av MW 5000), which has been used in many previous reports, is only 0.2 mmol/g. In addition, the terminal methyl group becomes a reference for the determination of the loading ratio in the reaction steps.

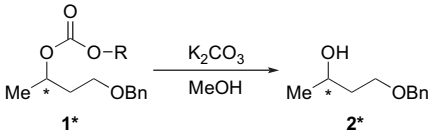
For the screening test of enzymes, we selected the carbonate (±)-**1a** ($MPEG_{750}$), which was afforded by the coupling of racemic 4-benzyloxy-2-butanol ((±)-**2**) with $MPEG_{750}$–OH through a carbonate linker. The carbonate is a typical linker for organic synthesis on a polymer support and can be easily constructed. In general, carbonate is recognized as a poor substrate for hydrolase. However, we have already succeeded in the development of the enzymatic hydrolysis of cyclic carbonates,[21] and realize that carbonate is merely a kind of ester, which can be hydrolyzed by enzymes.

The substrate (±)-**1a** was readily synthesized as shown in Scheme 1. The reaction of (±)-**2** with *N*,*N*′-carbonyldiimidazole in CH_2Cl_2 proceeded to afford the corresponding (±)-**3**. The compound (±)-**3** was immobilized on MPEG–OH with DMAP in DMF at 120 °C to give nearly the pure $MPEG_{750}$-supported (±)-**1a** in 67% yield. In the same way, the $MPEG_{550}$-supported (±)-**1b** and other substrates were also prepared. The yields of substrates were determined by the weights with the assumption that the MW was 750 or 550 for MPEG–OH.

In the first screening test, 12 hydrolytic enzymes were used. The selection of the enzyme was carried out on the basis of hydrolytic activity without paying attention to the enantioselectivity. The assay was performed by checking the production of **2** using TLC, and we selected three enzymes. In the second screening, the enantiomeric excesses (ees) of the products were determined after purification (Scheme 2). 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 (Scheme 3). These results are shown in Table 1. Although pig liver esterase (PLE; Amano Enzyme, Inc.) smoothly catalyzed the hydrolysis of **1a** to afford (*R*)-**2**, the enantioselectivity was quite low (*E* value=3).[22] Interestingly, the esterase SNSM-87 (Nagase & Co., Ltd) preferentially hydrolyzed the opposite enantiomer with moderate enantioselectivity to give the alcohol (*S*)-**2**, but the conversion was very low (conv.=0.08, *E* value=11). Finally, lipase from porcine pancreas (PPL; Type II, Sigma) was found to be the best enzyme. Under the given reaction conditions, the reaction of (±)-**1a** with PPL proceeded with a higher enantioselectivity (conv.=0.29, *E* value=23) to afford the 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 (Scheme 4). Changing the MPEG part to a lower molecular weight $MPEG_{550}$ did not negatively affect the reactivity. The reaction of the $MPEG_{550}$-supported **1b** also proceeded with a high enantioselectivity (conv.=0.35, *E* value=28). In the reaction of (±)-**1b** at 10 °C, the *E* value was up to 32 and (*R*)-**2** with 93% ee was obtained, although the conversion apparently decreased. On the other hand, the methyl carbonate (±)-**1c** (R=Me) was also hydrolyzed, but the enantioselectivity was very low (*E* value=1.4). The substrate (±)-**1c** is not supported on MPEG and basically insoluble in water. These facts indicate that the hydrophilic MPEG matrix could change the physical property of the alcohol **2** and that the substrate would favorably fit into the enzyme active site.



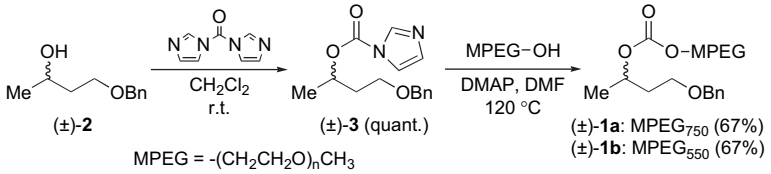
Scheme 2.



Scheme 3.

2.2. Separation of the products

During the reaction process, we succeeded in the establishment of a more facile separation of the remaining substrate (*S*)-**1a**,**b** and the resulting alcohol (*R*)-**2** due to the suitable water-solubility of the MPEG-supported substrates. Scheme 5 illustrates this extraction procedure. First, the extraction

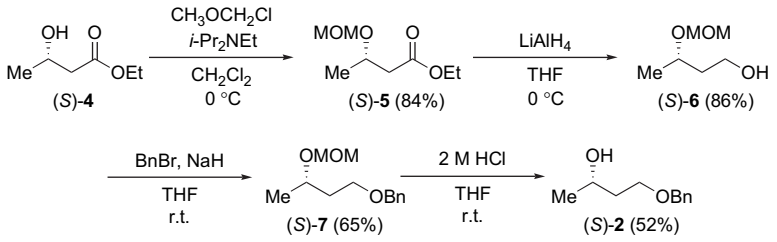


Scheme 1.

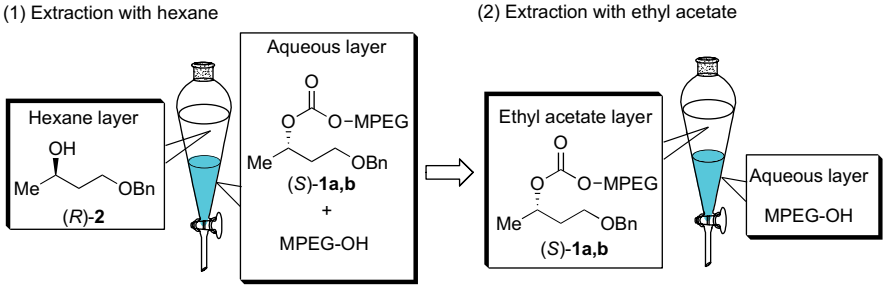
Table 1. Enantioselective hydrolysis of carbonates (±)-**1**[a]

	Substrate	R	Temp (°C)	Carbonate **1**		Alcohol **2**		Conv.[e]	E[f]
				Yield [b](%)	ee (%)[c]	Yield (%)	ee (%)[d]		
PLE	**1a**	$MPEG_{750}$	30	4	96(*S*)	76	7(*R*)	0.93	3
Esterase SNSM-87	**1a**	$MPEG_{750}$	30	56	7(*R*)	11	82(*S*)	0.08	11
PPL	**1a**	$MPEG_{750}$	30	50	36(*S*)	28	89(*R*)	0.29	23
PPL	**1b**	$MPEG_{550}$	30	54	47(*S*)	30	89(*R*)	0.35	28
PPL	**1b**	$MPEG_{550}$	10	76	10(*S*)	21	93(*R*)	0.10	32
PPL	**1c**	Me	30	65	5(*S*)	28	12(*R*)	0.27	1.4

[a] The reaction was performed using 5 mM of the substrate with an enzyme 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 4.

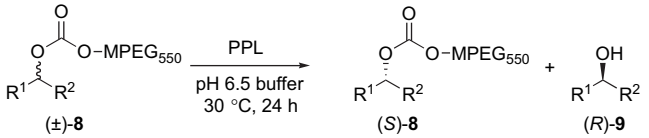


Scheme 5.

process is performed with hexane after the enzymatic reaction. In this step, only the alcohol (*R*)-**2** is selectively extracted into the hexane layer. Second, the substrate (*S*)-**1a**,**b** is successfully extracted from the aqueous layer with AcOEt. MPEG–OH, which is removed from (*R*)-**1a**,**b**, still remains in the aqueous layer. In order to purify these compounds, only a pad of silica gel is needed.

2.3. Application of the enzymatic reaction

We next examined the enzymatic reactions of several substrates supported on $MPEG_{550}$ under the same conditions (Scheme 6). These results are summarized in Table 2. As expected, the hydrolysis of 1-phenylethanol derivative (±)-**8a** (R^1=Me, R^2=Ph) enantioselectively proceeded, and the conversion was greater than those of **1** (conv.=0.55, E value=29). In this case, the corresponding optically active compounds, (*S*)-**8a** (36%, 95% ee) and (*R*)-**9a** (31%, 77% ee), were obtained. While the reaction of (±)-**8c** (R^1=vinyl, R^2=CH_2CH_2OBn) showed a good enantioselectivity (E value=16), (±)-**8b** (R^1=Me, R^2=CH_2CH_2Ph) was



Scheme 6.

Table 2. Enantioselective hydrolysis of carbonates (±)-**8** with PPL[a]

Substrate	R^1	R^2	Carbonate (*S*)-**8**		Alcohol (*R*)-**9**		Conv.	E
			Yield (%)[b]	ee (%)	Yield (%)	ee (%)		
8a	Me	Ph	36	95[c]	31	77[d]	0.55	29
8b	Me	CH_2CH_2Ph	37	23[c]	47	27[d]	0.46	2
8c	Vinyl	CH_2CH_2OBn	47	56[e]	41	80[f]	0.41	16

[a] The reaction was performed using 5 mM of (±)-**8** 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] Determined by ^{1}H NMR analysis of the corresponding MTPA ester after the hydrolysis of the carbonate.
[f] Determined by ^{1}H NMR analysis of the corresponding MTPA ester.

hydrolyzed with a very low enantioselectivity. Because the bulkiness of the substituent of **8c** is not much different from those of **1** and **8b**, the interaction between the enzyme and the oxygen atom in the benzyloxy group should be important for the enantioselectivity. In all cases, the substrates **8** and the alcohols **9** were successfully separated by the two step-extraction procedure as expected.

2.4. What is the real active enzyme?

Although commercially available PPL (Type II, Sigma) works well in this enzymatic reaction, the crude enzyme contains a number of hydrolases besides the true PPL. The existence of several active enzymes might affect the reactivity and enantioselectivity. In addition, the active-site model proposed by Jones for the PPL-catalyzed hydrolysis of a primary ester does not predict the reaction mode in this case.[23] In order to research the accurate result of the reaction for MPEG-supported substrates, we then investigated the reaction using the commercially available purified PPL (lipase Type VI-S, Sigma) and two kinds of major contaminant hydrolases, α-chymotrypsin (Type II, Sigma) and cholesterol esterase (Sigma). We selected (±)-**8a** as the substrate because it was the most reactive amongst all the examined substrates (Table 3). It is noteworthy that all enzymes show no or very low enantioselectivities. These results suggest that the activity could be due, not to the true PPL, but to another unknown enzyme. Further detailed investigations are now in progress.

Table 3. Enantioselective hydrolysis of carbonates **8a**[a]

Enzyme	Carbonate (*S*)-**8a**	Alcohol (*R*)-**9a**	Conv.	*E*
	ee (%)	ee (%)		
Purified PPL[b]	2.1	30	0.07	2
α-Chymotrypsin[c]	1.2	21	0.06	1.5
Cholesterol esterase[d]	1.2	0.4	0.77	~1

[a] The reaction was performed using 5 mM of (±)-**8a** with the enzyme in 0.1 M phosphate buffer (pH 6.5, 4 mL) for 24 h at 30 °C.
[b] Using 0.3 mg (81,000 U/mg).
[c] Using 5 mg (30,000 U/mg).
[d] Using 1 mg (54 U/mg).

3. Conclusions

In summary, we have demonstrated the first example for the hydrolase-mediated kinetic resolution of low-molecular weight MPEG-supported carbonates. We succeeded in the highly enantioselective hydrolysis of several substrates to give the substituted methyl (**2** and **9a**) and vinyl (**9c**) carbinols, which were optically active. In our method, the separation of the resulting alcohols from the remaining substrates was achieved by an extraction process without time- and solvent-consuming column chromatography. We anticipate that the use of a soluble polymer as the matrix of the substrates will provide an operationally simple and eco-friendly protocol.

4. Experimental

4.1. General

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were measured on JEOL JNM AL-300, with tetramethylsilane (TMS) as the internal standard. ^{1}H (500 MHz) NMR spectra were measured on JEOL α-500. IR spectra were recorded with a Shimadzu IR Prestige-21 spectrometer. Mass spectra were obtained with a JEOL EI/FAB mate BU25 instrument (EI method). The optical rotations were measured with a Jasco DIP-1000 polarimeter. HPLC data were obtained on Shimadzu LC-10AD$_{VP}$, SPD-10A$_{VP}$, and sic 480II data station (System Instruments Inc.). Kieselgel 60 F_{254} Art.5715 (E. Merck) was used for analytical thin-layer chromatography (TLC). Preparative TLC was performed on a Kieselgel 60 F_{254} Art.5744 (E. Merck). Flash column chromatography was performed with Silica Gel 60N (63–210 mm, Kanto Chemical Co. Inc.). MPEG$_{550}$–OH and MPEG$_{750}$–OH were purchased from Aldrich and the containing water was removed as the toluene azeotrope prior to use. Racemic secondary alcohols were prepared from the suitable starting material in the usual way. All other chemicals and enzymes were also obtained from commercial sources.

4.2. Preparation of carbonates as the substrate

4.2.1. MPEG$_{750}$-supported substrate coupled with 4-benzyloxy-2-butanol ((±)-1a). Under an argon atmosphere, to a solution of *N*,*N*′-carbonyldiimidazole (1.98 g, 12.2 mmol) in CH_2Cl_2 (10 mL) was added a solution of 4-benzyloxy-2-butanol ((±)-**2**, 2.00 g, 11.1 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred overnight at room temperature. After the mixture was diluted with CH_2Cl_2, the solution was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, 4-(benzyloxy)butan-2-yl 1*H*-imidazole-1-carboxylate ((±)-**3**) was obtained as a colorless oil (3.17 g, quant). This was used in the following reaction without further purification; IR (neat) 2862, 2359, 1759, 1472, 1393, 1319, 1290, 1242, 1180, 1096, 1003, 743 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (d, J=6.0 Hz, 3H), 1.94–2.11 (m, 2H), 3.51–3.59 (m, 2H), 4.44 (d, J=12.0 Hz, 1H), 4.50 (d, J=12.0 Hz, 1H), 5.33 (dqd, J_1=5.0 Hz, J_2=6.0 Hz, J_3=8.0 Hz, 1H), 7.04 (t, J=0.8 Hz, 1H), 7.19–7.34 (m, 5H), 7.36 (t, J=1.3 Hz, 1H), 8.07 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 35.8, 65.8, 73.1, 73.9, 117.0, 127.6, 127.7, 128.3, 130.4, 137.0, 137.9, 148.2; MS *m*/*z* (EI, rel intensities) 274 (M^+, 6.1%), 168 (100), 162 (93), 107 (42), 91 (100); HRMS *m*/*z* (EI) 274.1315 (calcd for $C_{15}H_{18}O_3N_2$: 274.1318, M^+).

Under an argon atmosphere, to a solution of *N*,*N*-dimethylaminopyridine (DMAP) (445 mg, 3.65 mmol) in DMF (10 mL) were added a solution of MPEG$_{750}$–OH (2.74 g, 3.65 mmol) in DMF (20 mL) and (±)-**3** (1.00 g, 3.65 mmol) in DMF (10 mL) at 0 °C. After the mixture was stirred overnight at 120 °C, it was washed with 2 M HCl in order to remove DMAP, the resulting imidazole, and the remaining MPEG–OH. After evaporation under reduced pressure, the residue was purified by column chromatography on silica gel (AcOEt → AcOEt/MeOH=3/1) to give the MPEG$_{750}$-supported carbonate (±)-**1a** as a colorless oil (3.50 g, 67%). The yield was determined by the weight with the assumption that the molecular weight was 750 for MPEG–OH; IR (neat) 2870, 2359, 1742, 1454, 1350, 1263, 1105, 949, 847 cm^{-1}; ^{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_3O–PEG), 3.50–3.57 (m, 2H, PEG), 3.55–3.70 (m, ca. 62H,

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).

Other substrates were synthesized by the same procedure.

4.2.2. MPEG$_{550}$-supported substrate coupled with 4-benzyloxy-2-butanol ((±)-1b). Yield 61% from 4-benzyloxy-2-butanol (*dl*-**2**); IR (neat) 2870, 2359, 1742, 1454, 1350, 1263, 1107, 949, 851 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, J=6.5 Hz, 3H), 1.77–2.04 (m, 2H), 3.38 (s, 3H, CH_3O–PEG), 3.51–3.57 (m, 2H, PEG), 3.57–3.67 (m, ca. 44H, 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).

4.2.3. MPEG$_{550}$-supported substrate coupled with 1-phenylethanol ((±)-8a). Yield 53% from 1-phenylethanol ((±)-**9a**); IR (neat) 2872, 2359, 1744, 1454, 1348, 1261, 1103, 949, 849 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (d, J=6.5 Hz, 3H), 1.77–2.03 (m, 2H), 3.38 (s, 3H, CH_3O–PEG), 3.53–3.57 (m, 2H, PEG), 3.60–3.72 (m, ca. 44H, PEG), 3.70 (t, J=5.0 Hz, 2H, PEG), 5.72 (q, J=6.5 Hz, 1H), 7.25–7.40 (m, 5H).

4.2.4. MPEG$_{550}$-supported substrate coupled with 4-phenyl-2-butanol ((±)-8b). Yield 52% from 4-phenyl-2-butanol (*dl*-**9b**); IR (neat) 2870, 2359, 1740, 1454, 1350, 1267, 1107, 949, 847 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, J=6.0 Hz, 3H), 1.77–2.03 (m, 2H), 3.38 (s, 3H, CH_3O–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), 4.73–4.84 (m, 1H), 7.14–7.31 (m, 5H).

4.2.5. MPEG$_{550}$-supported substrate coupled with 5-benzyloxy-1-hepten-3-ol ((±)-8c). Yield 60% from 5-benzyloxy-3-ol (*dl*-**9c**); IR (neat) 3113, 2870, 2359, 1744, 1454, 1350, 1261, 1105, 947, 851 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.38 (s, 3H, CH_3O–PEG), 3.50–3.60 (m, 2H, PEG), 3.60–3.72 (m, ca. 46H, PEG), 3.70 (t, J=5.0 Hz, 2H, PEG), 4.22–4.33 (m, 2H), 4.49 (s, 2H), 5.21 (td, J_1=1.5 Hz, J_2=10.5 Hz, 1H), 5.31 (td, J_1=1.5 Hz, J_2=17.0 Hz, 1H), 5.81 (ddd, J_1=7.0 Hz, J_2=10.5 Hz, J_3=17.0 Hz, 1H), 7.25–7.37 (m, 5H).

4.2.6. 4-(Benzyloxy)butan-2-yl methyl carbonate ((±)-1c). Under an argon atmosphere, to a solution of (±)-**2** (400 mg, 2.23 mmol) in CH_2Cl_2 (20 mL) were added pyridine (1.08 mL, 13.3 mmol) and methyl chlorocarbonate (0.34 mL, 4.44 mmol), and the mixture was stirred overnight at room temperature. After the addition of methyl chlorocarbonate (0.68 mL, 8.88 mmol), the mixture was stirred overnight at room temperature again. The reaction was stopped with 0.1 M phosphate buffer (pH 6.5) and the products were extracted with CH_2Cl_2 (×3). The combined organic layer was washed with 2 M HCl (×2), brine, satd $NaHCO_3$ aqueous solution and brine, and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=5/1) to give (±)-**1c** as a colorless oil (454 mg, 86%); IR (neat) 2955, 2859, 2359, 1746, 1443, 1271, 1096, 941 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, J=7.0 Hz, 3H), 3.50–3.75 (m, 8H), 4.33 (q, J=7.0 Hz, 1H), 4.47 (d, J=11.5 Hz, 1H), 4.59 (d, J=11.5 Hz, 1H), 7.26–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.8, 42.5, 45.6, 66.7, 67.0, 71.1, 75.3, 127.8, 127.9, 128.5, 137.4, 170.5; MS m/z (EI, rel intensities) 238 (M^+, 5.5%), 162 (100), 147 (6.8), 131 (30), 105 (100), 91 (100), 77 (100); HRMS m/z (EI) 238.1205 (calcd for $C_{13}H_{18}O_4$: 238.1205, M^+).

4.3. First screening of enzymes

In the screening test, we used the following enzymes: Lipase from porcine pancreas (PPL; Type II, 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), Trypsin, α-Chymotrypsin (E. Merck). The substrate (±)-**1a** (200 mg) and 50 mg of enzyme were incubated in 40 mL of 0.1 M phosphate buffer (pH 6.5) for 24 h at 30 °C. The products were extracted with AcOEt (×3) and detected by TLC (hexane/AcOEt=3/1).

4.4. Typical procedure of enantioselective hydrolysis of MPEG-supported substrates

To a 200-mL Erlenmeyer flask containing 200 mg (ca. 0.208 mmol; sub. concn, 0.5 mM) of (±)-**1a** was added 40 mL of 0.1 M phosphate buffer (pH 6.5). To the mixture was added 50 mg of Lipase from porcine pancreas (PPL; Type II, Sigma) (994 U/mg, using olive oil at pH 7.7), and the solution was incubated for 24 h at 30 °C. First, only the resulting alcohol **2** was extracted with hexane (×3), and the hexane layer was dried over Na_2SO_4. After evaporation, the residue was passed through a pad of silica gel with hexane/AcOEt (3/1) to give the alcohol (*R*)-**2** (10.1 mg, 28%, 89% ee). Second, the remaining carbonate **1a** was re-extracted with AcOEt from the water layer, and the organic layer was dried over Na_2SO_4. After evaporation, the residue was passed through a pad of silica gel (AcOEt/MeOH=3/1) to give the carbonate (*S*)-**1a** (99.7 mg, 50%, 36% ee). The yields of **1a** and **2** were determined by their weights on the basis of the weight of the substrate (±)-**1a**. The carbonate (*S*)-**1a** was easily hydrolyzed with K_2CO_3 in MeOH to afford the corresponding alcohol (*S*)-**2**.

4.5. Chemical hydrolysis of (*S*)-1a

To a solution of (*S*)-**1a** (40.3 mg, 0.053 mmol) in MeOH (6 mL) was added K_2CO_3 (36.6 mg, 0.265 mmol), and the mixture was stirred for 1 h at room temperature. After the reaction was stopped with water, MeOH was evaporated in vacuo. The products were extracted with AcOEt (×3), and the combined organic layer was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=4/1) to give (*S*)-**2** as a colorless oil (8.1 mg, 85%).

4.6. Several data of alcohols

4.6.1. 4-Benzyloxy-2-butanol (2). Compound (*R*)-**2**, $[\alpha]_D^{26}$ −12.2 (*c* 0.24, MeOH) (89% ee); IR (neat) 3416, 2965, 2864, 1494, 1454, 1368, 1206, 1099, 1028, 737, 698 cm^{-1}; ^{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; MS *m/z* (EI, rel intensities) 180 (M^+, 14%), 161 (57), 107 (100), 91 (100), 89 (42); HRMS *m/z* (EI) 180.1146 (calcd for $C_{11}H_{16}O_2$: 180.1150, M^+). The spectral data were in full agreement with those reported.[24] HPLC conditions: 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.

Enantioselective hydrolysis of the other cases was carried out by the same procedure. In the case of (±)-**1c**, the remaining **1c** and the resulting **2** were separated by flash column chromatography (hexane/AcOEt=5/1→hexane/AcOEt=3/1).

4.6.2. 1-Phenylethanol (9a). Compound (*R*)-**9a**, $[\alpha]_D^{21}$ +24.8 (*c* 0.85, MeOH) (77% ee), lit.[25] $[\alpha]_D^{20}$ +45 (*c* 5.15, MeOH) for the (*R*)-enantiomer. The spectral data were in full agreement with that of commercial source. The ee of (*R*)-**9a** was determined by GLC analysis. 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.

4.6.3. 4-Phenyl-2-butanol (9b). Compound (*R*)-**9b**, $[\alpha]_D^{27}$ −3.4 (*c* 0.69, $CHCl_3$) (27% ee), lit.[26] $[\alpha]_D^{27}$ +17.45 (*c* 2.04, $CHCl_3$) for the (*S*)-enantiomer; IR (neat) 3358, 2965, 2926, 2361, 1713, 1603, 1495, 1454, 1373, 1128, 1055, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (d, *J*=4.0 Hz, 3H, CH_3), 1.72–1.82 (m, 2H), 2.60–2.82 (m, 2H), 3.83 (tq, J_1=J_2=6.0 Hz, 1H), 7.14–7.32 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5, 32.1, 40.8, 67.5, 125.8, 128.4, 142.0; MS *m/z* (EI, rel intensities) 150 (M^+, 25%), 132 (100), 117 (100), 105 (47), 91 (100); HRMS *m/z* (EI) 150.1042 (calcd for $C_{10}H_{14}O$: 150.1045, M^+). HPLC conditions: 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, 15 (*R*) and 20 (*S*) min.

4.6.4. 5-Benzyloxy-1-hepten-3-ol (9c). Compound (*R*)-**9c**, $[\alpha]_D^{28}$ +5.5 (*c* 0.40, MeOH) (80% ee); IR (neat) 3417, 2862, 2359, 1454, 1366, 1277, 1099, 1028, 993, 922, 737 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67–1.97 (m, 2H), 2.90 (br s, 1H), 3.59–3.76 (m, 2H), 4.30–4.40 (m, 1H), 4.52 (s, 2H), 5.10 (td, J_1=1.5 Hz, J_2=10.5 Hz, 1H), 5.26 (td, J_1=1.5 Hz, J_2=17.0 Hz, 1H), 5.87 (ddd, J_1=5.5 Hz, J_2=10.5 Hz, J_3=17.0 Hz, 1H), 7.25–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 36.2, 68.1, 71.6, 73.2, 114.3, 127.6, 128.4, 137.8, 140.5; MS *m/z* (EI, rel intensities) 192 (M^+, 9.3%), 107 (93), 91 (100), 68 (100); HRMS *m/z* (EI) 192.1177 (calcd for $C_{12}H_{16}O_2$: 192.1150, M^+). The ee of **9c** was determined by ^{1}H NMR analysis of the corresponding (+)-methoxytrifluoromethylphenylacetate (MTPA) ester, which was converted from **9c**.

^{1}H NMR of the MTPA ester (500 MHz, $CDCl_3$) δ 4.40 (d, *J*=6.5 Hz, 1H, OC*H*HPh) and 4.42 (d, *J*=6.5 Hz, 1H, OCH*H*Ph)(*S*), 4.48 (s, 2H, OC*HH*Ph)(*R*). The absolute configuration was determined by comparing the NMR signal pattern of the MTPA ester with that of the authentic sample.

4.7. Preparation of authentic (*S*)-2

Under an argon atmosphere, to a solution of ethyl (*S*)-(+)-3-hydroxybutanoate (**4**, 1.00 g, 7.57 mmol) in CH_2Cl_2 (20 mL) were added diisopropylethylamine (5.27 mL, 30.3 mmol) and a solution of chloromethylmethylether (1.82 g, 22.8 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction was stopped with 0.1 M phosphate buffer (pH 6.5) and the products were extracted with AcOEt (×3). The organic layer was washed with brine (×2) and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by column chromatography (hexane/AcOEt=5/1) to give ethyl (*S*)-3-(methoxymethoxy)butanoate as a colorless oil (**5**, 1.12 g, 84%); IR (neat) 2978, 1738, 1449, 1377, 1300, 1186, 1150, 1103, 1036, 918 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (d, *J*=6.0 Hz, 3H), 1.26 (t, *J*=7.0 Hz, 3H), 2.41 (dd, J_1=5.5 Hz, J_2=15.0 Hz, 1H), 2.60 (dd, J_1=7.5 Hz, J_2=15.0 Hz, 1H), 3.36 (s, 3H), 4.08–4.22 (m, 1H), 4.15 (q, *J*=7.0 Hz, 2H), 4.66 (d, *J*=7.0 Hz, 1H), 4.67 (d, *J*=7.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 20.5, 42.4, 55.3, 60.3, 70.3, 95.3, 171.2.

Under an argon atmosphere, to a suspension of $LiAlH_4$ (200 mg, 5.26 mmol) in THF (5 mL) was added a solution of (*S*)-**5** (901 mg, 5.12 mmol) in THF (10 mL) at 0 °C. After the mixture was stirred for 1 h at room temperature, the reaction was quenched with water (200 μL), 15% NaOH aqueous solution (200 μL), and water (400 μL). After filtration thorough a Celite pad and evaporation, the residue was purified by column chromatography (hexane/AcOEt=1/1→AcOEt) to give (*S*)-3-(methoxymethoxy)-1-butanol as a colorless oil (**6**, 588 mg, 86%); IR (neat) 3428, 2963, 1449, 1411, 1377, 1261, 1103, 1036, 797 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (d, *J*=6.0 Hz, 3H), 1.75 (dt, J_1=J_2=6.0 Hz, 2H), 2.69 (br s, 1H), 3.39 (s, 3H), 3.68–3.85 (m, 2H), 3.93 (tq, J_1=J_2=6.0 Hz, 1H), 4.63 (d, *J*=7.0 Hz, 1H), 4.72 (d, *J*=7.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.2, 39.1, 55.4, 59.9, 72.0, 94.9.

Under an argon atmosphere, to a suspension of NaH (60% in oil, 337 mg, 8.43 mmol) in THF (5 mL) were added a solution of (*S*)-**6** (501 mg, 3.74 mmol) in THF (10 mL) and benzyl bromide (0.44 mL, 3.74 mmol) at 0 °C. The mixture was stirred for 4 h at room temperature and the reaction was quenched with 0.1 M phosphate buffer (pH 6.5). The products were extracted with AcOEt (×3), and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=10/1→5/1) to give (*S*)-1-benzyloxy-3-(methoxymethoxy)butane as a colorless oil (**7**, 541 mg, 65%); IR (neat) 2930, 2882, 1452, 1375, 1207, 1103, 1040, 918, 737, 698 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (d, *J*=6.0 Hz, 3H), 1.70–1.89 (m 2H), 3.35 (s, 3H), 3.50–3.64 (m, 2H), 3.87 (tq, J_1=J_2=6.5 Hz, 1H), 4.50 (s, 3H), 4.60 (d, *J*=7.0 Hz, 1H), 4.67 (d, *J*=7.0 Hz, 1H), 7.22–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6, 37.2, 55.3, 66.9, 70.6, 73.0, 95.1, 127.5, 127.7, 128.3, 138.4.

To a solution of (*S*)-**7** (400 mg, 1.79 mmol) in THF (10 mL) was added 2 M HCl (4 mL). After the mixture was stirred overnight at room temperature, the reaction mixture was diluted with water. The products were extracted with AcOEt

(×3), and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation under reduced pressure, the residue was purified by flash column chromatography (hexane/AcOEt=10/1→4/1) to give the remaining (*S*)-**7** (193 mg, 48%) and (*S*)-**2** as colorless oils (167 mg, 52%); (*S*)-**2**, $[\alpha]_D^{27}$ +19.0 (*c* 0.95, MeOH). The spectral data were in full agreement with those of the (*R*)-**2** obtained by the enzymatic reaction.

Acknowledgements

We are grateful to Amano Enzyme, Inc. and Nagase & Co., Ltd for the gift of enzymes. M.S. was supported by a grant from Research Fellowships of the Japan Society for the Promotion of Science (JSPS) for Young Scientists. We thank Material Science Research Center (Meisei University) for NMR analysis.

References and notes

1. (a) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Wiley-VCH: Weinheim, 1999; (b) Bommarius, A. S.; Riebel, B. R. *Biocatalysis*; Wiley-VCH: Weinheim, 2004; (c) Faber, K. *Biotransformations in Organic Chemistry: A Textbook*, 5th ed.; Springer: Berlin, Heidelberg, New York, 2004.
2. For a review of modern separation techniques, see: Tzschucke, C. C.; Markert, C.; Bannwarth, W.; Roller, S.; Hebel, A.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 3964–4000.
3. For enzymatic acylation with succinic acid, see: (a) Terao, Y.; Tsuji, K.; Murata, M.; Achiwa, K.; Nishio, T.; Watanabe, N.; Seto, K. *Chem. Pharm. Bull.* **1989**, *37*, 1653–1655; (b) Gutman, A. L.; Brenner, D.; Boltanski, A. *Tetrahedron: Asymmetry* **1993**, *4*, 839–844.
4. For lipase-catalyzed transformation followed by sulfation, see: Yamano, T.; Kikumoto, F.; Yamamoto, S.; Miwa, K.; Kawada, M.; Ito, T.; Ikemoto, T.; Tomimatsu, K.; Mizuno, Y. *Chem. Lett.* **2000**, 448–449.
5. For using fluorous-tagged esters with fluorous solvents, see: (a) Beier, P.; O'Hagan, D. *Chem. Commun.* **2002**, 1680–1681; (b) Luo, Z.; Swaleh, S. M.; Theil, F.; Curran, D. P. *Org. Lett.* **2002**, *4*, 2585–2587.
6. For enzymatic transesterification of esters with PEG as a nucleophile, see: (a) Wallace, J. S.; Reda, K. B.; Williams, M. E.; Morrow, C. J. *J. Org. Chem.* **1990**, *55*, 3544–3546; (b) Whalen, L. J.; Morrow, C. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1279–1288.
7. For enzyme-mediated kinetic resolution of carboxylic acids anchored on polystyrene based resin, see: Nanda, S.; Rao, B.; Yadav, J. S. *Tetrahedron Lett.* **1999**, *40*, 5905–5908.
8. For lipase-catalyzed kinetic resolution with poly(acrylamide)-ethylene glycol (PEGA)-based resin as a nucleophile, see: Ulijn, R. V.; Bisek, N.; Flitsch, S. L. *Org. Biomol. Chem.* **2003**, *1*, 621–622.
9. Córdova, A.; Tremblay, M. R.; Clapham, B.; Janda, K. D. *J. Org. Chem.* **2001**, *66*, 5645–5648.
10. Halcomb, R. L.; Huang, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 11315–11322.
11. (a) Waldmann, H.; Reidel, A. *Angew. Chem., Int. Ed.* **1997**, *36*, 647–649; (b) Sauerbrei, B.; Jungmann, V.; Waldmann, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 1143–1146.
12. (a) Ulijn, R. V.; Baragaña, B.; Halling, P. J.; Flitsch, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 10988–10989; (b) Humphrey, C. E.; Turner, N. J.; Easson, M. A. M.; Flitsch, S. L.; Ulijn, R. V. *J. Am. Chem. Soc.* **2003**, *125*, 13952–13953; (c) Basso, A.; Ulijn, R. V.; Flitsch, S. L.; Margetts, G.; Brazendale, I.; Ebert, C.; De Martin, L.; Linda, P.; Verdelli, S.; Gardossi, L. *Tetrahedron* **2004**, *60*, 589–594; (d) Basso, A.; Ebert, C.; Gardossi, L.; Linda, P.; Phuong, T. T.; Zhu, M.; Wessjohann, L. *Adv. Synth. Catal.* **2005**, *347*, 963–966.
13. (a) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489–509; (b) Wentworth, P.; Janda, K. D. *Chem. Commun.* **1999**, 1917–1924; (c) Dickerson, T. J.; Reed, N. N.; Janda, K. D. *Chem. Rev.* **2002**, *102*, 3325–3344; (d) Bergbreiter, D. E. *Chem. Rev.* **2002**, *102*, 3345–3384.
14. (a) Benaglia, M.; Celentano, G.; Cozzi, F. *Adv. Synth. Catal.* **2001**, *343*, 171–173; (b) Pozzi, G.; Cavazzini, M.; Quici, S.; Benaglia, M.; Dell'Anna, G. *Org. Lett.* **2004**, *6*, 441–443; (c) Oikawa, M.; Tanaka, T.; Kusumoto, S.; Sasaki, M. *Tetrahedron Lett.* **2004**, *45*, 787–790; (d) Reed, N. N.; Dickerson, T. J.; Boldt, G. E.; Janda, K. D. *J. Org. Chem.* **2005**, *70*, 1728–1731.
15. (a) Veronese, F. M.; Morpurgo, M. *Il Farmaco* **1999**, *54*, 497–516; (b) Greenwald, R. B. *J. Controlled Release* **2001**, *74*, 159–171.
16. Shimojo, M.; Matsumoto, K.; Nogawa, M.; Nemoto, Y.; Ohta, H. *Tetrahedron Lett.* **2004**, *45*, 6769–6773.
17. (a) Inada, Y.; Yoshimoto, T.; Matsushima, A.; Saito, Y. *Trends Biotechnol.* **1986**, *4*, 68–73; (b) Inada, Y.; Takahashi, K.; Yoshimoto, T.; Ajima, A.; Matsushima, A.; Saito, Y. *Trends Biotechnol.* **1986**, *4*, 190–194.
18. (a) Otamiri, M.; Adlerkreutz, P.; Mattiasson, B. *Biotechnol. Bioeng.* **1994**, *44*, 73–78; (b) Ruppert, S.; Gais, H. J. *Tetrahedron: Asymmetry* **1997**, *8*, 3657–3664.
19. López-Pelegrín, J. A.; Wentworth, P., Jr.; Sieber, F.; Metz, W. A.; Janda, K. D. *J. Org. Chem.* **2000**, *65*, 8527–8531.
20. Low-molecular weight PEG was used as a tag for real time reaction monitoring in the synthesis of oligosaccharides. (a) Manabe, S.; Ito, Y. *J. Am. Chem. Soc.* **2002**, *124*, 12638–12639; (b) Hanashima, S.; Manabe, S.; Ito, Y. *Synlett* **2003**, 979–982.
21. (a) Matsumoto, K.; Sato, Y.; Shimojo, M.; Hatanaka, M. *Tetrahedron: Asymmetry* **2000**, *11*, 1965–1973; (b) Matsumoto, K.; Nakamura, Y.; Shimojo, M.; Hatanaka, M. *Tetrahedron Lett.* **2002**, *43*, 6933–6936.
22. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
23. Hultin, P. G.; Jones, J. B. *Tetrahedron Lett.* **1992**, *33*, 1399.
24. (a) Casara, P.; Darwin, C.; Metcalf, B.; Jung, M. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2201–2208; (b) Cadot, C.; Dalko, P. I.; Cossy, J.; Ollivier, C.; Chuard, R.; Renaud, P. *J. Org. Chem.* **2002**, *67*, 7193–7202.
25. Laumen, K.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1988**, 598–600.
26. Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129–6139.