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Enzyme-mediated enantioselective hydrolysis of soluble polymer-supported carboxylates

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ABSTRACT

The enzyme-mediated enantioselective hydrolysis of water-soluble polymer-supported carboxylates is disclosed. The representative monomethoxy poly(ethylene glycol) (MPEG, av MW 5000)-supported substrate was synthesized by immobilization of (±)-1-phenylethanol onto the modified MPEG (MPEG/NH_2) through an carboxylate linker with a succinate spacer. For the screening of the hydrolytic enzymes, the substrate was enantioselectively hydrolyzed by lipase from *Candida antarctica* (Novozym 435) in a mixed solvent (hexane/buffer=9/1) at 30 °C to afford the remaining (*S*)-substrate and the resulting (*R*)-alcohol (*E* value>200). The products were easily separated by a simple procedure without any laborious column chromatography. The substrate was hydrolyzed with NaOH in MeOH/H_2O to afford the corresponding (*S*)-alcohol. We also found that the structure of the spacer between the MPEG moiety and the carboxylate linker strongly affected both the reactivity and enantioselectivity, and the substrate bearing a glutarate spacer gave the best result. Our procedure was applicable for the preparation of several optically active alcohols.

1. Introduction

The hydrolase-mediated kinetic resolution of racemic alcohols and esters is one of the attractive methods for the preparation of optically active compounds, and there have already been a great number of studies about enzymatic hydrolysis and esterification.[1] During the reaction, however, the remaining substrate and the resulting product must be separated, and the tedious and wasteful separation step by column chromatography is still a big problem for developing an easy operation and a sustainable product. In order to simplify the separation of products, an organic reaction on polymer supports is a practical method. On the other hand, the usual organic compounds are insoluble in water, and the solubility profile prevents the enzymatic reaction of such compounds in some cases. Recently, poly(ethylene glycol) (PEG) has been recognized as an inexpensive and convenient soluble material, and the reactions using several PEG-supported compounds in organic synthesis have also been developed under homogeneous conditions.[2–4] We postulated that a PEG-supported strategy could be preferable for an enzymatic reaction in water, and could also be useful for the easy separation of the products. In this context, we have already disclosed the enzymatic kinetic resolution of the low- and middle-molecular weight monomethoxy PEG (MPEG, av Mw 550, 750, and 5000)-supported substrate with a carbonate linker using porcine pancreas lipase (PPL, lipase Type II from Sigma), and we have succeeded in the easy separation of the products.[5,6] In particular, the $MPEG_{5000}$-bound carbonates were soluble solids and easier to handle, and the reaction of the substrates also gave better results in terms of both the reactivity and the enantioselectivity. Although a carbonate bond is one of the representative linkers for polymer-support reactions, this structure is not necessarily suitable for enzymatic hydrolysis, and there have been a relatively few examples for the lipase-catalyzed kinetic resolution of carbonates. In our previous study, many commercial enzymes scarcely hydrolyzed the MPEG-supported carbonates.[5] In addition, the coupling of alcohols with MPEG through a carbonate linker should be constructed at high temperature (>120 °C), and the purity of the resulting compounds was low in some cases. In order to extend the scope of the application of our PEG-strategy, we then focused on the enzymatic hydrolysis of the MPEG-supported carboxylates, which had the usual ester bond as the linker. In this paper, we disclose the easy preparation of new types of MPEG-supported compounds with a carboxylate linker and the hydrolase-mediated kinetic resolution of these substrates. We also report that the structure of an appropriate hydrophobic spacer between the MPEG moiety and the carboxylate linker significantly affects both the reactivity and enantioselectivity, and our procedure is applicable for the preparation of several optically active secondary alcohols.

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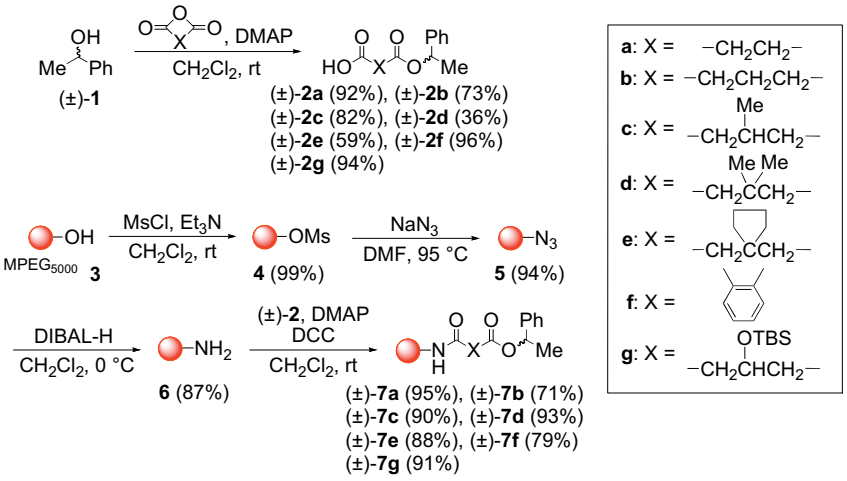
2. Results and discussion

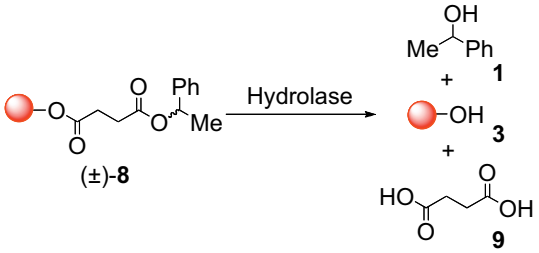
2.1. Preparation of MPEG-supported compounds

Initially, we tried to prepare new MPEG-supported carboxylates with a high purity by an easy and effective procedure. We selected 1-phenylethanol (**1**) as the representative target alcohol, and the racemate (±)-**1** was combined with succinic anhydride using DMAP in CH_2Cl_2 to give the dicarboxylic monoester (±)-**2a** in 92% yield (Scheme 1).



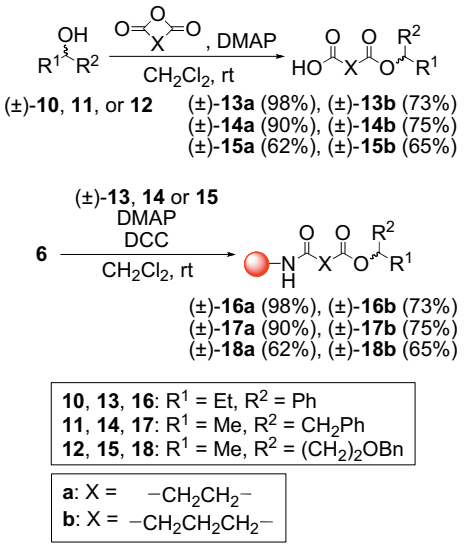
Scheme 1.

The succinate part could be utilized as the spacer between the MPEG and carboxylate linker, and the reactions using other carboxylic anhydrides gave the corresponding intermediates (±)-**2b**–**g**. When (±)-**2a** was directly coupled with $MPEG_{5000}$–OH (**3**, av Mw 5000), the diester (±)-**8** was obtained. However, both the two ester bonds of **8** was hydrolyzed by lipases and esterases in many cases, and the substrate was consequently decomposed into three components, **1**, **3**, and succinic acid (**9**) (Scheme 2).



Scheme 2.

We noticed that the coupling of the MPEG part with the spacer should be constructed by the amide bond, which could be difficult to hydrolyze with lipases. We then tried to change the hydroxyl group of MPEG to the amino group. The mesylate **4** derived from **3** was treated with sodium azide, and the resulting **5** was reduced by DIBAL-H to afford the modified MPEG–NH_2 (**6**). The coupling of **6** with compound (±)-**2a** using DMAP and DCC in CH_2Cl_2 at room temperature gave the $MPEG_{5000}$-supported carboxylic ester (±)-**7a** bearing a succinate spacer. The reactions of **6** using other dicarboxylic monoesters (±)-**2b**–**g** afforded the corresponding esters (±)-**7b**–**g** with various spacers, respectively. In a similar manner, the MPEG-supported compounds (±)-**16**, **17**, and **18** were prepared from the corresponding secondary alcohols, (±)-1-phenyl-1-propanol (**10**), 1-phenyl-2-propanol (**11**), and 4-benzyloxy-2-butanol (**12**), respectively (Scheme 3). The preparations of the MPEG-supported substrates were confirmed by ^1H and ^{13}C NMR and ESI-TOF MS analyses.



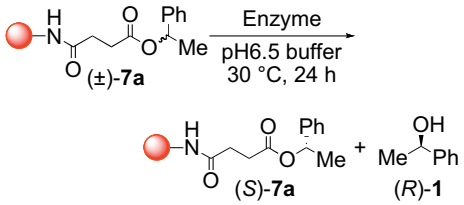
Scheme 3.

2.2. Screening test of enzymes

Next, we screened the enzymes having the enantioselective hydrolytic ability of the carboxylate (±)-**7a** as the representative substrate. The reaction of the screening test was performed in 0.1 M phosphate buffer (pH 6.5) for 24 h at 30 °C, and the selection of the enzyme was carried out on the basis of the enantioselectivity by checking the enantiomeric excess (ee) of the product **1** by GC (CP-Cyclodextrin-B-236-M19, Chrompack). In our previous study, almost every lipase, except for PPL, could not catalyze the hydrolysis of the MPEG-supported substrates with a carbonate linker.[5] Interestingly, all the enzymes hydrolyzed the MPEG-supported carboxylate (±)-**7a** to afford the corresponding alcohol (*R*)-**1**, although the ees varied with the type of enzyme (Table 1).

In the case of the reaction using PPL, which was the best enzyme for the enantioselective hydrolysis of the MPEG-supported carbonates, the ee of the resulting (*R*)-**1** was low (30% ee). These results indicated that the enzymes essentially recognize the structure

Table 1
Enantioselective hydrolysis of $MPEG_{5000}$-supported carboxylic ester with hydrolytic enzyme[a]



Enzyme	ee of **1** (%)[b]
Lipase A (Amano)	10
Lipase D (Amano)	28
Lipase D-360 (Amano)	~0
Lipase PS (Amano)	48
Newlase F (Amano)	11
PLE (Amano)	32
Lipase OF (Meito)	42
Lilipase (Nagase)	12
Esterase SNSM-87 (Nagase)	~0
PPL (Sigma)	31
Lipase Type VII (Sigma)	27
α-Chymotrypsin (E. Merck)	~0
Novozym 435 (Novozymes)	96

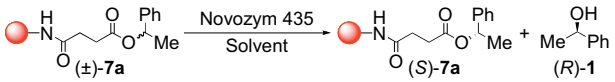
[a] The reaction was performed using 5 mM of the substrate with the hydrolytic enzyme in 0.1 M phosphate buffer (pH 6.5) for 24 h at 30 °C.
[b] Determined by GC analysis.

of the linker, and the carboxylate linker could be widely accepted by hydrolases for use of a soluble polymer as the matrix. Finally, we selected Novozym 435 from *Candida antarctica* (Novozymes) as the best enzyme, and the ee of the obtained (*R*)-**1** was 96% ee.

2.3. Enzymatic hydrolysis of MPEG-supported carboxylates with Novozym 435

We next tried to evaluate the determination of the enantioselectivity of the reaction using Novozym 435, and these results are shown in Table 2.

Table 2
Enantioselective hydrolysis of $MPEG_{5000}$-supported carboxylate (±)-**7a** with Novozym 435[a]



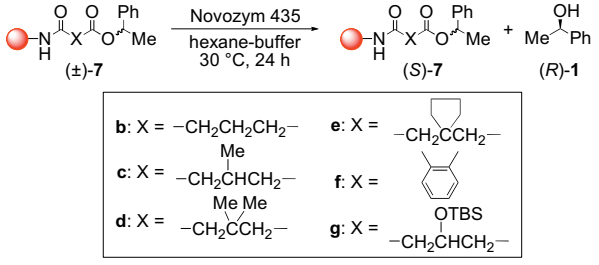
Entry	Solvent	Temp (°C)	Time (h)	ee of **7a**/%[b]	ee of **1**/%[c]	Conv.	*E* value
1	Buffer	30	24	17	96	0.15	58
2	Hexane/Buffer	30	24	55	98	0.36	172
3	Hexane/Buffer	0	24	23	>99	0.19	>249
4	Hexane/Buffer	0	72	>99	>99	0.50	>1057
5[d]	Hexane/Buffer	0	24	95	>99	0.49	>747

[a] Unless otherwise noted, the reaction was performed using 5 mM of the substrate (125 mg) with Novozym 435 (20 mg) in 0.1 M phosphate buffer (pH 6.5) or a mixed medium (hexane/0.1 M phosphate buffer (pH 6.5)=9/1).
[b] Determined by GC analysis after chemical hydrolysis of the unreacted substrate.
[c] Determined by GC analysis.
[d] Using 60 mg of Novozym 435.

In a typical experiment, 125 mg of (±)-**7a** (sub. concn, 5 mM) and 20 mg of Novozym 435 were added to a medium (5 mL) in a test tube, and the mixture was stirred for 24 h at a constant temperature. The reaction was first carried out in 0.1 M phosphate buffer (pH 6.5) at 30 °C (entry 1). Although the hydrolysis of (±)-**7a** proceeded, the reactivity was low (conv.=0.15) and the enantioselectivity was moderate (*E* value=58).[7] In this case, the resulting (*R*)-**1** should be extracted from the mixture with AcOEt, and the remaining substrate (*S*)-**7a** was obtained by extraction with CH_2Cl_2 after acidification of the aqueous layer. After the chemical hydrolysis of the unreacted substrate **7a** with NaOH aq in MeOH, the ee of the resulting (*S*)-**1** was also determined by GC analysis. In our previous study, we already found that the reaction of the MPEG-supported carbonates in a mixed solvent of buffer and hexane gave a better result than that in a homogeneous system. We then examined the reaction in the two-phase system. As expected, changing the medium improved both the reactivity and enantioselectivity, and the conversion and *E* value of the reaction in a mixed solvent containing 90% hexane were up to 0.36 and 172, respectively (entry 2). It was found that the reaction at a lower temperature proceeded with a higher enantioselectivity. For the reaction at 0 °C (entry 3), the *E* value increased to >249 and the optically pure (*R*)-**1** was obtained, although the conversion apparently decreased to 0.19. A longer reaction time improved the conversion and almost complete optical resolution of **1** was accomplished, but the time taken to thoroughly react (*R*)-**7a** needed 72 h (entry 4). As expected, increasing the amount of the enzyme (60 mg) also improved the conversion without lowering the enantioselectivity (entry 5). In this two-phase reaction system, the property of a medium-molecular weight $MPEG_{5000}$ allows us to facilitate the easy work-up and separation of the products the same as our previous studies.[6] After the enzymatic reaction, the resulting alcohol (*R*)-**1** was already extracted in the hexane layer, and then isolated after evaporation. On the other hand, CH_2Cl_2 was added to the aqueous layer, and dried over anhydrous Na_2SO_4. After evaporation, the residue was poured into Et_2O to precipitate the mixture of the (*S*)-**7a** and MPEG-supported carboxylic acid as a white solid, which was collected by simple filtration.

We speculated that a suitable spacer could increase the affinity to the active site of the enzyme. We then investigated changing the spacer of the substrate in order to improve the conversion and enantioselectivity of the reaction for 24 h at 30 °C (Table 3).

Table 3
Enantioselective hydrolysis of $MPEG_{5000}$-supported carboxylates **7b**–**g** with Novozym 435[a]



Entry	Substrate	ee of **7**/%[b]	ee of **1**/%[c]	Conv.	*E* value
1	(±)-**7b**	>99	>99	0.50	>1057
2	(±)-**7c**	93	99	0.48	684
3	(±)-**7d**	2	89	0.02	18
4	(±)-**7e**	1	57	0.02	4
5	(±)-**7f**	~0	3	0.05	1
6	(±)-**7g**	~0	84	~0	12

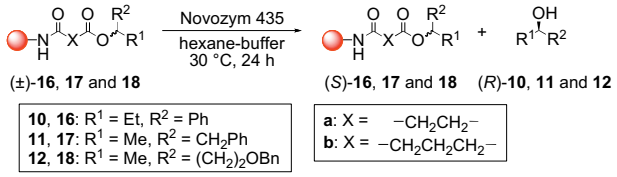
[a] The reaction was performed using 5 mM of the substrate with Novozym 435 in a mixed medium (hexane/0.1 M phosphate buffer (pH 6.5)=9/1) for 24 h at 30 °C.
[b] Determined by GC analysis after hydrolysis of the unreacted substrates.
[c] Determined by GC analysis.

Beyond our expectation, changing the spacer significantly affected not only the conversion, but also the enantioselectivity. Surprisingly, the substrate (±)-**7b** bearing the glutarate spacer was smoothly hydrolyzed, and the conversion was up to 0.50 (entry 1). In addition, the reaction also proceeded with an excellent enantioselectivity (*E* value>1057) to afford both almost optically pure enantiomers. The reaction was also performed using 3.0 g of the

substrate (±)-**7b** in a recovery flask, and we finally obtained (*R*)-**1** (>99% ee, $[\alpha]_D^{20}$ +34.5 (*c* 1.77, MeOH); lit.[8] $[\alpha]_D^{20}$ +45 (*c* 5.15, MeOH)) in 25% and (*S*)-**1** (94% ee) in 24% isolated yields (conv.=0.49, *E* value>712). Interestingly, the reaction of compound (±)-**7c** bearing the 3-methylglutarate spacer gave almost the same result as the case of **7b** (entry 2, conv.=0.48, *E* value=684). However, the introduction of a dimethyl group to the glutarate spacer drastically decreased both the reactivity and enantioselectivity, and the conversion and *E* value of the reaction using **7d** bearing the 3,3-dimethylglutarate spacer were only 0.02 and 18, respectively (entry 3). As expected, the compounds **7e**, **7f**, and **7g** bearing the bulky spacer (3,3-tetramethyleneglutarate, phthalate, and 3-(*tert*-butyldimethylsilyloxy)glutarate spacers, respectively) were scarcely hydrolyzed, and the enantioselectivities were quite low in all cases (entries 4–6, respectively).

Next, in order to apply the concept of this reaction for the kinetic resolution of the other secondary alcohols, we examined the enzymatic hydrolysis of several MPEG-supported carboxylates bearing the succinate or glutarate spacer. These results are shown in Table 4. While the enantioselective hydrolysis of the 1-phenyl-1-propanol derivative (±)-**16a** bearing the succinate spacer hardly proceeded (entry 1), changing the spacer to the glutarate ((±)-**16b**) significantly improved both the reactivity and enantioselectivity (entry 2; conv.=0.53, *E* value=51). In the case of the 1-phenyl-2-propanol derivative (±)-**17b** bearing the glutarate spacer (entry 4), the enzyme also catalyzed the hydrolysis of the compound with an excellent enantioselectivity to afford the highly optically active (*R*)-**11** (>99% ee) and (*S*)-**17b** (95% ee) (conv. 0.49, *E* value>747). On the other hand, the reaction of the carboxylate (±)-**17a**, which had the succinate spacer, slowly proceeded with a moderate enantioselectivity (entry 3; conv. 0.09, *E* value=54). These results indicate that the enzyme apparently prefers the *R*-enantiomer of the substrates having not the succinate, but the glutarate spacer in all cases. It was noteworthy that both reactions of the 4-benzyloxy-2-butanol derivatives (±)-**18a** (entry 5) and **18b** (entry 6) proceeded to afford the optically active compound (*R*)-**12** in both cases, although the conversions were low (conv.=0.16 and 0.18, respectively). The excellent enantioselectivities (*E* value>239 and >249, respectively) were substantially the same as that in the reaction of **17b**. Although the reactions of **18a** and **18b** were not carried out under the optimized conditions, we supposed that prolonging the reaction time and increasing the amount of the enzyme could improve the conversions in a manner similar to the substrate **7a**. Interestingly, the hydrolysis of the acetate of the alcohol **12** with Novozym 435 in phosphate buffer for 24 h at 30 °C smoothly proceeded (conv.=0.52), but the *E* value was only 46.

Table 4
Enantioselective hydrolysis of $MPEG_{5000}$-supported carboxylates **16**–**18** with Novozym 435[a,b]



Entry	Substrate	ee of substrate/%[c]	ee of product/%[d]	Conv.	*E* value
1	(±)-**16a**	~0	68	~0	<5
2	(±)-**16b**	97	85	0.53	51
3	(±)-**17a**	10	94	0.09	54
4	(±)-**17b**	95	>99	0.49	>747
5	(±)-**18a**	19	>99	0.16	>239
6	(±)-**18b**	23	>99	0.19	>249

[a] The reaction was performed using 5 mM of the substrate with Novozym 435 in a mixed medium (hexane/0.1 M phosphate buffer (pH 6.5)=9/1) for 24 h at 30 °C.
[b] The absolute configurations of the compounds **10**–**12** were determined by GC analysis comparing the retention times to those of the authentic samples.
[c] Determined by GC analysis after hydrolysis of the unreacted substrates.
[d] Determined by GC analysis.

3. Conclusions

In summary, we have demonstrated the enzyme-mediated kinetic resolution of soluble polymer $MPEG_{5000}$-supported carboxylates to afford optically active secondary alcohols **1**, **10**, **11**, and **12**. We also have disclosed that the reactivity and enantioselectivity can be controlled using a suitable hydrophobic spacer between the MPEG moiety and the carboxylate linker. In our method, the separation and isolation of the reaction products were achieved by a simple precipitation technique without using time- and solvent-consuming column chromatography. Because the use of the PEG matrix could change the physical properties of the insoluble organic compounds to solubilize them in an aqueous medium, the PEG-strategy could pave the way for a novel enzymatic system.

4. Experimental

4.1. General

^1H (500 MHz or 300 MHz) and ^{13}C (125 MHz or 75 MHz) NMR spectra were recorded on a JEOL α-500 with tetramethylsilane as the internal standard. IR spectra were recorded with a Shimadzu IR Prestige-21 spectrometer. ESI-TOF mass spectra were measured in MeOH/H_2O solution including AcONa with a JEOL JMS-T100. All enzymatic reactions were performed in a SANYO incubator MIR-253. Kieselgel 60 F_{254} Art.5715 (E. Merck) was used for analytical thin-layer chromatography (TLC), and preparative TLC was performed on Kieselgel 60 F_{254} Art. 5744 (E. Merck). The optical rotations were measured with a Jasco DIP-1000 polarimeter. HPLC data were obtained on a Shimadzu LC-10AD_{vp}, SPD-10A_{vp}, and sic 480II date station (System Instruments Inc.). GLC data were obtained on GL Sciences GC 353B and sic 480II. $MPEG_{5000}$–OH (**3**) was purchased from Fluka, and all other chemicals and enzymes were also obtained from commercial sources.

The yields of the MPEG-supported compounds were based on the weights of the starting materials. The purities were determined by ^1H NMR analysis, and the terminal methyl group and/or the PEG-methylenes were used as the reference.

4.2. Preparation of dicarboxylic monoesters

*4.2.1. 3-((1-Phenylethoxy)carbonyl)propanoic acid ((±)-**2a**).* Under an argon atmosphere, succinic anhydride (489.5 mg, 4.892 mmol), and DMAP (598.7 mg, 4.901 mmol) were added to a solution of 1-phenyletanol ((±)-**1**, 299.1 mg, 2.448 mmol) in CH_2Cl_2 (10 mL), and the solution was stirred for 2 h at room temperature. After the product was extracted with satd $NaHCO_3$ aq, the separated water layer was acidified by 2 M HCl. The products were extracted with AcOEt, washed with brine, and dried over Na_2SO_4. After evaporation in vacuo, the residue was purified by column chromatography on silica gel (hexane/AcOEt=3/1) to give the (±)-**2a** as a colorless oil in 97% yield (527.9 mg); IR (KBr) 2982, 2934, 1736, 1713, 1495, 1450, 1375, 1285, 1207, 1169, 1063, 959, 762, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.53 (d, *J*=6.5 Hz, 3H), 2.56–2.74 (m, 4H), 5.89 (q, *J*=6.5 Hz, 1H), 7.21–7.41 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.1, 28.9, 29.1, 72.9, 126.0, 127.9, 128.5, 141.3, 171.4, 178.2; HRMS *m*/*z* (ESI) 245.0788 (calcd for $C_{12}H_{14}O_4Na$: 245.0790, M+Na^+).

The other compounds (±)-**2**, **13**, **14**, and **15** were synthesized by the same procedure. In the cases of (±)-**2e**, **2f**, **2g**, **13a**, **13b**, **14b**, **15a**, and **15b**, the products were directly extracted with AcOEt without the extraction step using satd $NaHCO_3$.

*4.2.2. 4-((1-Phenylethoxy)carbonyl)butanoic acid ((±)-**2b**).* Yield 73% from (±)-**1** with glutaric anhydride; IR (KBr) 2980, 2936, 1732, 1709, 1495, 1452, 1375, 1287, 1246, 1207, 1155, 1063, 935, 762, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.53 (d, J=6.5 Hz, 3H), 1.95 (tt, J_1=7.5 Hz, J_2=7.5 Hz, 2H), 2.35–2.48 (m, 4H), 5.89 (q, J=6.5 Hz, 1H), 7.21–7.41 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.8, 22.2, 32.9, 33.4, 72.5, 126.0, 127.9, 128.5, 141.5, 172.1, 179.0; HRMS m/z (ESI) 259.0953 (calcd for $C_{13}H_{16}O_4Na$: 259.0946, M+Na^+).

*4.2.3. 3-Methyl-4-((1-phenylethoxy)carbonyl)butanoic acid ((±)-**2c**).* Yield 82% from (±)-**1** with 3-methylglutaric anhydride; IR (KBr) 2974, 2878, 1732, 1709, 1495, 1456, 1375, 1287, 1207, 1065, 974, 935, 762, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.02 (d, J=6.5 Hz, 3H), 1.536 (d, J=6.5 Hz) and 1.539 (d, J=6.5 Hz) (3H), 2.20–2.34 (m, 2H), 2.36–2.54 (m, 3H), 5.90 (q, J=6.5 Hz, 1H), 7.20–7.43 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.66 and 19.71, 22.18 and 22.20, 27.2, 40.42 and 40.47, 41.0, 72.4, 126.1, 127.9, 128.5, 141.5, 171.6, 178.4; HRMS m/z (ESI) 273.1096 (calcd for $C_{14}H_{18}O_4Na$: 273.1103, M+Na^+).

*4.2.4. 3,3-Dimethyl-4-((1-phenylethoxy)carbonyl)butanoic acid ((±)-**2d**).* Yield 36% from (±)-**1** with 3,3-dimethylglutaric anhydride; IR (KBr) 2963, 2876, 1734, 1707, 1495, 1450, 1371, 1287, 1234, 1152, 1063, 964, 760, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.11 (s, 3H), 1.12 (s, 3H), 1.54 (d, J=6.5 Hz, 3H), 2.42 (d, J=14.5 Hz, 1H), 2.44 (d, J=14.5 Hz, 1H), 2.46 (d, J=14.0 Hz, 1H), 2.49 (d, J=14.0 Hz, 1H), 5.90 (q, J=6.5 Hz, 1H), 7.21–7.42 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.2, 27.72, 27.74, 32.6, 45.0, 45.2, 72.4, 126.1, 127.9, 128.4, 141.4, 171.4, 177.5; HRMS m/z (ESI) 287.1257 (calcd for $C_{15}H_{20}O_4Na$: 287.1259, M+Na^+).

*4.2.5. 2-(1-(((1-Phenylethoxy)carbonyl)methyl)cyclopentyl)acetic acid ((±)-**2e**).* Yield 59% from (±)-**1** with 3,3-tetramethyleneglutaric anhydride; IR (KBr) 2955, 2872, 1732, 1705, 1495, 1454, 1371, 1285, 1209, 1161, 1062, 953, 762, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.53 (d, J=6.5 Hz, 3H), 1.55–1.69 (m, 8H), 2.54 (d, J=15.5 Hz, 1H), 2.55 (d, J=15.5 Hz, 1H), 2.57 (d, J=14.5 Hz, 1H), 2.60 (d, J=15.0 Hz, 1H), 5.89 (q, J=6.5 Hz, 1H), 7.20–7.41 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.2, 23.9, 37.98, 38.02, 42.1, 42.3, 43.0, 72.4, 126.1, 127.8, 128.4, 141.5, 171.8, 177.7; HRMS m/z (ESI) 313.1411 (calcd for $C_{17}H_{22}O_4Na$: 313.1416, M+Na^+).

*4.2.6. 2-((1-Phenylethoxy)carbonyl)benzoic acid ((±)-**2f**).* Yield 96% from (±)-**1** with phthalic anhydride; IR (KBr) 2976, 2833, 2683, 2581, 1724, 1694, 1601, 1493, 1449, 1418, 1315, 1288, 1258, 1125, 1063, 932, 795, 743, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.66 (d, J=6.5 Hz, 3H), 6.14 (q, J=6.5 Hz, 1H), 7.26 (tt, J_1=1.5 Hz, J_2=7.5 Hz, 1H), 7.34 (tt, J_1=1.5 Hz, J_2=7.5 Hz, 2H), 7.41 (td, J_1=1.5 Hz, J_2=8.0 Hz, 2H), 7.56 (td, J_1=1.5 Hz, J_2=7.5 Hz, 1H), 7.59 (td, J_1=1.5 Hz, J_2=7.5 Hz, 1H), 7.69 (dd, J_1=2.0 Hz, J_2=7.0 Hz, 1H), 7.88 (dd, J_1=2.0 Hz, J_2=7.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.7, 74.2, 126.2, 128.0, 128.5, 128.8, 129.7, 130.0, 130.8, 132.1, 133.4, 141.0, 167.2, 172.4; HRMS m/z (ESI) 293.0779 (calcd for $C_{16}H_{14}O_4Na$: 293.0790, M+Na^+).

*4.2.7. 3-(tert-Butyldimethylsilyloxy)-4-((1-phenylethoxy)carbonyl) butanoic acid ((±)-**2g**).* Yield 94% from (±)-**1** with 3-(*tert*-butyldimethylsilyloxy)glutaric anhydride; IR (KBr) 2930, 2857, 1736, 1713, 1495, 1462, 1377, 1256, 1204, 1099, 1063, 972, 837, 779, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (s), 0.059 (s) and 0.065 (s) (6H), 0.81 (s) and 0.84 (s, 9H), 1.54 (d, J=6.5 Hz, 3H), 2.50–2.66 (m, 4H), 4.50–4.58 (m, 1H), 5.883 (q, J=6.5 Hz) and 5.887 (q, J=6.5 Hz) (1H), 7.25–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.05 and −5.03, −4.92 and −4.91, 17.8 and 17.9, 22.2 and 22.3, 25.59 and 25.62, 42.0 and 42.1, 42.4 and 42.5, 65.98 and 66.03, 72.7, 126.07 and 126.12, 127.9, 128.5, 141.38 and 141.41, 170.10 and 170.15, 176.63 and 176.68; HRMS m/z (ESI) 389.1756 (calcd for $C_{19}H_{30}O_5SiNa$: 389.1760, M+Na^+).

*4.2.8. 3-((1-Phenylpropyloxy)carbonyl)propanoic acid ((±)-**13a**).* Yield 98% from (±)-**10** with succinic anhydride; IR (KBr) 2970, 2936, 2880, 1738, 1713, 1495, 1454, 1381, 1258, 1169, 1084, 964, 843, 758, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, J=7.5 Hz, 3H), 1.75–1.98 (m, 2H), 2.55–2.74 (m, 4H), 5.68 (t, J=7.0 Hz, 1H), 7.16–7.40 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.8, 28.9, 29.1, 29.2, 77.9, 126.4, 127.8, 128.3, 140.2, 171.5, 178.2; HRMS m/z (ESI) 259.0946 (calcd for $C_{13}H_{16}O_4Na$: 259.0946, M+Na^+).

*4.2.9. 4-((1-Phenylpropyloxy)carbonyl)butanoic acid ((±)-**13b**).* Yield 73% from (±)-**10** with glutaric anhydride; IR (KBr) 2970, 2938, 2878, 1734, 1709, 1495, 1452, 1383, 1246, 1204, 1084, 968, 758, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.88, (t, J=7.5 Hz, 3H), 1.76–1.86 (m, 1H), 1.87–2.00 (m, 4H), 2.34–2.49 (m, 4H), 5.67 (t, J=7.0 Hz, 1H), 7.22–7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.9, 19.8, 29.3, 32.9, 33.4, 77.5, 126.5, 127.8, 128.4, 140.4, 172.2, 179.1; HRMS m/z (ESI) 273.1104 (calcd for $C_{14}H_{18}O_4Na$: 273.1103, M+Na^+).

*4.2.10. 3-((1-Methyl-2-phenylethoxy)carbonyl)propanoic acid ((±)-**14a**).* Yield 90% from (±)-**11** with succinic anhydride; IR (KBr) 2980, 2932, 1730, 1713, 1497, 1454, 1377, 1233, 1173, 1049, 962, 845, 748, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.22 (d, J=6.5 Hz, 3H), 2.49–2.69 (m, 4H), 2.77 (dd, J_1=6.5 Hz, J_2=13.5 Hz, 1H), 2.91 (dd, J_1=6.5 Hz, J_2=13.5 Hz, 1H), 5.14 (qt, J_1=6.0 Hz, J_2=6.5 Hz, 1H), 7.15–7.34 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.3, 28.9, 29.1, 42.1, 72.0, 126.5, 128.3, 129.4, 137.4, 171.5, 178.1; HRMS m/z (ESI) 259.0947 (calcd for $C_{13}H_{16}O_4Na$: 259.0946, M+Na^+).

*4.2.11. 4-((1-Methyl-2-phenylethoxy)carbonyl)butanoic acid ((±)-**14b**).* Yield 75% from (±)-**11** with glutaric anhydride; IR (KBr) 2978, 2934, 1728, 1713, 1497, 1454, 1379, 1248, 1155, 1057, 943, 822, 746, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.23 (d, J=6.0 Hz, 3H), 1.80–1.94 (m, 4H), 2.25–2.39 (m, 2H), 2.77 (dd, J_1=6.0 Hz, J_2=13.5 Hz, 1H), 2.90 (dd, J_1=7.0 Hz, J_2=13.5 Hz, 1H), 5.14 (qt, J_1=6.0 Hz, J_2=6.0 Hz, 1H), 7.14–7.33 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.5, 19.7, 32.8, 33.4, 42.2, 71.6, 126.5, 128.3, 129.3, 137.5, 172.3, 178.9; HRMS m/z (ESI) 273.1091 (calcd for $C_{14}H_{18}O_4Na$: 273.1103, M+Na^+).

*4.2.12. 3-((1-Methyl-4-(benzyloxy)propyloxy)carbonyl)propanoic acid ((±)-**15a**).* Yield 62% from (±)-**12** with succinic anhydride; IR (KBr) 2978, 2932, 2870, 1732, 1713, 1497, 1454, 1377, 1258, 1171, 1099, 1028, 961, 739, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.24 (d, J=6.5 Hz, 3H), 1.76–1.95 (m, 2H), 2.46–2.68 (m, 4H), 3.43–3.55 (m, 2H), 4.47 (d, J=12.0 Hz, 1H), 4.48 (d, J=12.0 Hz, 1H), 5.05–5.16 (m, 1H), 7.22–7.39 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 28.9, 29.1, 35.9, 66.4, 69.1, 73.0, 127.6, 127.7, 128.3, 138.2, 171.6, 178.1; HRMS m/z (ESI) 303.1181 (calcd for $C_{14}H_{18}O_4Na$: 303.1203, M+Na^+).

*4.2.13. 4-((1-Methyl-4-(benzyloxy)propyloxy)carbonyl)butanoic acid ((±)-**15b**).* Yield 65% from (±)-**12** with glutaric anhydride; IR (KBr) 2976, 2936, 2870, 1732, 1709, 1497, 1452, 1377, 1248, 1206, 1146, 1099, 1028, 941, 739, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.24 (d, J=6.0 Hz, 3H), 1.71–2.02 (m, 4H), 2.19–2.51 (m, 4H), 3.39–3.58 (m, 2H), 4.47 (d, J=12.0 Hz, 1H), 4.48 (d, J=12.0 Hz, 1H), 5.01–5.17 (m, 1H), 7.15–7.47 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.8, 20.2, 32.9, 33.4, 35.9, 66.4, 68.7, 73.0, 127.6, 127.7, 128.3, 138.2, 172.4, 178.8; HRMS m/z (ESI) 317.1337 (calcd for $C_{14}H_{18}O_4Na$: 317.1359, M+Na^+).

4.3. Preparation of MPEG$_{5000}$-supported compounds

*4.3.1. Compound (±)-**7a**.* Under an argon atmosphere, methanesulfonyl chloride (0.35 mL, 4.51 mmol) and triethylamine (1.50 mL,

10.8 mmol) were added to a solution of **3** (3.00 g, 0.60 mmol) in CH_2Cl_2 (13 mL), and the solution was stirred overnight at room temperature. After evaporation in vacuo, the residue was poured into Et_2O to precipitate a white solid. After the solid was washed with 2-propanol, evaporation and precipitation gave the mesylate **4** as a white solid in 99% yield (3.03 g; purity, ca. >99%); IR (KBr) 2886, 1636, 1466, 1360, 1342, 1281, 1242, 1113, 962, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.09 (s, 3H) 3.38 (s, 3H), 3.46–3.82 (m, PEG-methylenes), 4.39 (t, J=4.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 37.6, 58.9, 68.9, 69.2, 70.4 (PEG), 70.5, 70.6, 71.8.

Under an argon atmosphere, sodium azide (256 mg, 3.94 mmol) was added to a solution of **4** (4.00 g, 0.788 mmol) in toluene (10.5 mL), and the solution was stirred overnight at 95 °C. The mixture was evaporated in vacuo, and the residue was filtrated through a Celite pad. After evaporation, the residue was poured into Et_2O to precipitate the compound **5** as a white solid in 95% yield (3.75 g; purity, ca. >99%); IR (KBr) 2886, 2100, 1636, 1468, 1342, 1281, 1242, 1115, 963, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.38 (s, 3H), 3.40 (t, J=5.0 Hz, 2H), 3.46–3.82 (m, PEG-methylenes); ^{13}C NMR (125 MHz, $CDCl_3$) 50.5, 58.9, 69.9, 70.4 (PEG), 70.55, 70.62, 71.8.

Under an argon atmosphere, DIBAL-H (7.1 mL, 1.0 M solution in toluene) was added to a solution of **5** (2.70 g, 0.537 mmol) in CH_2Cl_2 (22 mL) at 0 °C, and the solution was warmed to room temperature. After stirring overnight, the reaction was quenched with water, and the suspension was filtrated through a Celite pad. After evaporation, the residue was poured into Et_2O to precipitate the compound **6** as a white solid in 87% yield (2.33 g; purity, ca. 97%); IR (KBr) 3464, 2887, 1651, 1468, 1342, 1281, 1242, 1113, 964, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.88 (t, J=5.0 Hz, 2H), 3.38 (s, 3H), 3.47–3.83 (m, PEG-methylenes); ^{13}C NMR (125 MHz, $CDCl_3$) δ 41.6, 58.9, 70.1, 70.4 (PEG), 70.6, 71.8, 72.9.

Under an argon atmosphere, (±)-**2a** (269.2 mg, 1.211 mmol), DCC (261.6 mg, 1.268 mmol) and DMAP (73.2 mg, 0.599 mmol) were added to a solution of **6** (2.000 g, 0.4001 mmol) in CH_2Cl_2 (13 mL), and the solution was stirred overnight at room temperature. After evaporation, the residue was poured into Et_2O to precipitate the compound (±)-**7a** as a white solid in 95% yield (1.985 g; purity, ca. 84%); IR (KBr) 2886, 1734, 1653, 1466, 1342, 1281, 1242, 1113, 964, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.53 (d, J=6.5 Hz, 3H), 2.44 (dt, J_1=7.0 Hz, J_2=15.0 Hz, 1H), 2.49 (dt, J_1=7.0 Hz, J_2=15.0 Hz, 1H), 2.65 (td, J_1=7.0 Hz, J_2=17.0 Hz, 1H), 2.73 (td, J_1=7.5 Hz, J_2=17.0 Hz, 1H), 3.38 (s, 3H), 3.40–3.46 (m, 2H), 3.47–3.82 (m, PEG-methylenes), 5.87 (q, J=6.5 Hz, 1H), 6.50 (br s, 1H), 7.23–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.2, 29.7, 30.7, 39.1, 58.9, 69.7, 70.0, 70.4 (PEG), 70.6, 71.8, 72.4, 125.9, 127.7, 128.3, 141.5, 171.3, 172.0; ESI-TOF MS m/z 2454 Da (2452 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{12}H_{14}NO_3\cdot 2Na]^{2+}$).

Other $MPEG_{5000}$-supported compounds were synthesized by the same procedure.

*4.3.2. Compound (±)-**7b**.* Yield 71% (purity, ca. 70%) from **6** with (±)-**2b**; IR (KBr) 2886, 1732, 1651, 1468, 1342, 1281, 1242, 1113, 962, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.53 (d, J=6.5 Hz, 3H), 1.90–2.00 (m, 2H), 2.16–2.23 (m, 2H), 2.35–2.43 (m, 2H), 3.37 (s, 3H), 3.39–3.45 (m, 2H), 3.46–3.81 (m, PEG-methylenes), 5.88 (q, J=6.5 Hz, 1H), 6.12 (br s, 1H), 7.18–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.7, 22.2, 33.6, 35.2, 39.0, 58.9, 69.7, 70.0, 70.4 (PEG), 70.6, 71.8, 72.1, 125.9, 127.7, 128.4, 141.6, 172.1, 172.3; ESI-TOF MS m/z 2461 Da (2459 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{13}H_{16}NO_3\cdot 2Na]^{2+}$).

*4.3.3. Compound (±)-**7c**.* Yield 90% (purity, ca. 61%) from **6** with (±)-**2c**; IR (KBr) 2886, 1732, 1651, 1468, 1342, 1281, 1242, 1115, 964, 843; ^1H NMR (500 MHz, $CDCl_3$) δ 1.02 (d, J=6.5 Hz, 3H), 1.53 (d, J=6.5 Hz, 3H), 2.16–2.46 (m, 5H), 3.38 (s, 3H), 3.39–3.83 (m, PEG-methylenes), 5.88 (q, J=6.5 Hz, 1H), 7.09 (br s, 1H), 7.22–7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.51, 19.55, 22.12, 22.14, 27.3, 40.50, 40.55, 40.9, 58.9, 63.2, 68.9, 70.4 (PEG), 70.6, 71.8, 72.1, 125.9, 127.7, 128.3, 141.45, 141.47, 171.4, 172.1; ESI-TOF MS m/z 2468 Da (2466 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{14}H_{18}NO_3\cdot 2Na]^{2+}$).

*4.3.4. Compound (±)-**7d**.* Yield 93% (purity, ca. 98%) from **6** with (±)-**2d**; IR (KBr) 2886, 1732, 1653, 1466, 1342, 1281, 1242, 1109, 962, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.05 (s, 3H), 1.09 (s, 3H), 1.55 (d, J=6.5 Hz, 3H), 2.21 (s, 2H), 2.39 (d, J=13.5 Hz, 1H), 2.42 (d, J=13.5 Hz, 1H), 3.38 (s, 3H), 3.39–3.44 (m, 2H), 3.47–3.81 (m, PEG-methylenes), 5.89 (q, J=6.5 Hz, 1H), 6.77 (br s, 1H), 7.15–7.41 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.1, 28.26, 28.27, 33.4, 38.8, 45.0, 47.2, 58.9, 69.8, 70.0, 70.4 (PEG), 70.6, 71.8, 72.3, 126.0, 127.8, 128.4, 141.3, 171.0, 171.9; ESI-TOF MS m/z 2475 Da (2473 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{15}H_{20}NO_3\cdot 2Na]^{2+}$).

*4.3.5. Compound (±)-**7e**.* Yield 88% (purity, ca. 83%) from **6** with (±)-**2d**; IR (KBr) 2886, 1732, 1653, 1466, 1342, 1281, 1242, 1113, 964, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.40–1.73 (m, 8H), 1.55 (d, J=6.5 Hz, 3H), 2.29 (s, 2H), 2.48 (d, J=14.0 Hz, 1H), 2.49 (d, J=14.0 Hz, 1H), 3.38 (s, 3H), 3.45–3.83 (m, PEG-methylenes), 5.89 (q, J=6.5 Hz, 1H), 6.78 (br s, 1H), 7.21–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.1, 23.7, 37.92, 37.98, 38.8, 42.2, 44.1, 58.9, 69.8, 70.0, 70.4 (PEG), 70.6, 71.8, 72.4, 126.0, 127.8, 128.4, 141.4, 171.5, 172.3; ESI-TOF MS m/z 2487 Da (2486 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{17}H_{22}NO_3\cdot 2Na]^{2+}$).

*4.3.6. Compound (±)-**7f**.* Yield 79% (purity, ca. 48%) from **6** with (±)-**2f**; IR (KBr) 2886, 1715, 1653, 1466, 1342, 1281, 1242, 1113, 964, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.64 (d, J=6.5 Hz, 3H), 3.38 (s, 3H), 3.46–3.83 (m, PEG-methylenes), 6.09 (q, J=6.5 Hz, 1H), 6.64 (br s, 1H), 7.22–7.56 (m, 7H), 7.72 (dd, J_1=3.0 Hz, J_2=5.5 Hz, 1H), 7.85 (dd, J_1=3.0 Hz, J_2=5.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.1, 59.0, 64.5, 68.7, 70.4, 70.7, 71.8, 73.7, 126.1, 127.9, 128.4, 128.8, 129.0, 130.8, 131.1, 131.7, 132.3, 141.2, 166.4, 167.6; ESI-TOF MS m/z 2478 Da (2476 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{16}H_{14}NO_3\cdot 2Na]^{2+}$).

*4.3.7. Compound (±)-**7g**.* Yield 91% (purity, ca. 80%) from **7** with (±)-**2g**; IR (KBr) 2884, 1734, 1651, 1468, 1342, 1281, 1242, 1115, 962, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.03 (s), 0.041 (s), 0.047 (s), 0.057 (s) and 0.062 (s) (6H), 0.79 (s), 0.826 (s), 0.829 (s) and 0.84 (s) (9H), 1.54 (d, J=6.5 Hz, 3H), 2.31–2.65 (m, 4H), 3.38 (s, 3H), 3.43–3.85 (m, PEG-methylenes), 4.49–4.58 (m, 1H), 5.87 (q, J=6.5 Hz, 1H), 6.55 (br s, 1H), 7.21–7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.07, −5.02, −4.96, −4.94, 17.76, 17.79, 22.2, 22.3, 25.59, 25.61, 39.0, 41.9, 42.1, 43.9, 44.1, 58.9, 66.55, 66.59, 69.7, 70.1, 70.4 (PEG), 70.5, 70.6, 71.8, 72.5, 125.98, 126.04, 127.8, 128.4, 141.39, 141.43, 170.07, 170.13, 170.8, 170.9; ESI-TOF MS m/z 2525 Da (2524 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{19}H_{30}NO_4Si\cdot 2Na]^{2+}$).

*4.3.8. Compound (±)-**16a**.* Yield 95% (purity, ca. 89%) from **6** with (±)-**13a**; IR (KBr) 2886, 1732, 1651, 1468, 1342, 1281, 1242, 1111, 962, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, J=7.5 Hz, 3H), 1.75–1.97 (m, 2H), 2.45 (dt, J_1=7.0 Hz, J_2=15.5 Hz, 1H), 2.48 (dt, J_1=7.0 Hz, J_2=15.5 Hz, 1H), 2.66 (td, J_1=7.0 Hz, J_2=17.0 Hz, 1H), 2.76 (td, J_1=7.5 Hz, J_2=17.0 Hz, 1H), 3.38 (s, 3H), 3.39–3.45 (m, 2H), 3.46–3.83 (m, PEG-methylenes), 5.65 (t, J=7.0 Hz, 1H), 6.23 (br s, 1H), 7.20–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.8, 29.2, 29.7, 30.7, 39.1, 58.9, 69.7, 70.1, 70.4 (PEG), 70.6, 71.8, 77.4, 126.3, 127.6, 128.2, 140.4, 171.3, 172.1; ESI-TOF MS m/z 2461 Da (2459 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{13}H_{16}NO_3\cdot 2Na]^{2+}$).

*4.3.9. Compound (±)-**16b**.* Yield 83% (purity, ca. 76%) from **6** with (±)-**13b**; IR (KBr) 2886, 1732, 1647, 1466, 1342, 1281, 1242, 1113, 964, 843 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, J=7.5 Hz, 3H), 1.75–1.86 (m, 1H), 1.87–2.00 (m, 3H), 2.15–2.24 (m, 2H), 2.33–2.48 (m, 2H), 3.38 (s, 3H), 3.40–3.46 (m, 2H), 3.47–3.82 (m, PEG-

methylenes), 5.66 (t, J=7.0 Hz, 1H), 6.28 (br s, 1H), 7.18–7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.8, 20.8, 29.2, 33.5, 35.2, 39.0, 58.9, 69.7, 70.1, 70.4 (PEG), 70.5, 70.6, 71.8, 77.2, 126.4, 127.7, 128.3, 140.4, 172.0, 172.4; ESI-TOF MS m/z 2468 Da (2466 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{14}H_{18}NO_3\cdot 2Na]^{2+}$).

*4.3.10. Compound (±)-**17a**.* Yield 94% (purity, ca. 74%) from **6** with (±)-**14a**; IR (KBr) 2886, 1734, 1655, 1468, 1342, 1281, 1242, 1115, 962, 843 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.21 (d, J=6.0 Hz, 3H), 2.39–2.47 (m, 2H), 2.53–2.69 (m, 2H), 2.76 (dd, J_1=7.0 Hz, J_2=13.5 Hz, 1H), 2.92 (dd, J_1=6.5 Hz, J_2=13.5 Hz, 1H), 3.38 (s, 3H), 3.41–3.46 (m, 2H), 3.48–3.82 (m, PEG-methylenes), 5.11 (qt, J_1=6.0 Hz, J_2=6.5 Hz, 1H), 6.25 (br s, 1H), 7.15–7.33 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.2, 29.7, 30.7, 39.2, 42.0, 58.9, 69.7, 70.0, 70.4, 70.6, 71.6, 71.8, 126.3, 128.2, 129.3, 137.4, 171.4, 172.2; ESI-TOF MS m/z 2460 Da (2459 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{13}H_{16}NO_3\cdot 2Na]^{2+}$).

*4.3.11. Compound (±)-**17b**.* Yield 95% (purity, ca. 83%) from **6** with (±)-**14b**; IR (KBr) 2886, 1734, 1653, 1466, 1342, 1281, 1242, 1115, 964, 843 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23 (d, J=6.5 Hz, 3H), 1.83–1.94 (m, 2H), 2.10 (t, J=7.5 Hz, 2H), 2.30 (dt, J_1=3.5 Hz, J_2=7.5 Hz, 2H), 2.77 (dd, J_1=6.5 Hz, J_2=13.5 Hz, 1H), 2.89 (dd, J_1=7.0 Hz, J_2=13.5 Hz, 1H), 3.38 (s, 3H), 3.41–3.46 (m, 2H), 3.47–3.83 (m, PEG-methylenes), 5.14 (qt, J_1=6.0 Hz, J_2=6.5 Hz, 1H), 6.08 (br s, 1H), 7.15–7.34 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.5, 20.7, 33.6, 35.1, 39.0, 42.1, 58.9, 69.8, 70.1, 70.4, 70.6, 71.2, 71.8, 126.3, 128.2, 129.3, 137.5, 172.1, 172.5; ESI-TOF MS m/z 2468 Da (2466 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{14}H_{18}NO_3\cdot 2Na]^{2+}$).

*4.3.12. Compound (±)-**18a**.* Yield 80% (purity, ca. 67%) from **6** with (±)-**15a**; IR (KBr) 2884, 1732, 1676, 1468, 1342, 1281, 1242, 1113, 962, 843 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.24 (d, J=6.0 Hz, 3H), 1.76–1.95 (m, 2H), 2.41–2.49 (m, 2H), 2.55–2.63 (m, 2H), 3.38 (s, 3H), 3.40–3.82 (m, PEG-methylenes), 4.47 (d, J=12.0 Hz, 1H), 4.48 (d, J=12.0 Hz, 1H), 5.08 (q, J=6.5 Hz, 1H), 6.46 (br s, 1H), 7.22–7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 29.7, 30.7, 35.9, 39.3, 58.9, 66.5, 68.7, 69.6, 70.2, 70.5, 70.8, 71.8, 72.9, 127.5, 127.6, 128.2, 138.3, 171.6, 172.2; ESI-TOF MS m/z 2482 Da (2481 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{15}H_{20}NO_4\cdot 2Na]^{2+}$).

*4.3.13. Compound (±)-**18b**.* Yield 74% (purity, ca. 69%) from **6** with (±)-**15b**; IR (KBr) 2884, 1730, 1668, 1466, 1342, 1281, 1242, 1111, 962, 843 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23 (d, J=6.0 Hz, 3H), 1.75–2.00 (m, 4H), 2.21 (t, J=7.5 Hz, 2H), 2.30 (t, J=7.5 Hz, 2H), 3.38 (s, 3H), 3.42–3.83 (m, PEG-methylenes), 4.47 (d, J=12.0 Hz, 1H), 4.48 (d, J=12.0 Hz, 1H), 5.08 (q, J=6.5 Hz, 1H), 6.33 (br s, 1H), 7.22–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.2, 20.9, 33.6, 35.1, 35.9, 39.2, 58.9, 66.4, 68.4, 69.6, 70.1, 70.4, 70.8, 71.8, 72.9, 127.5, 127.6, 128.3, 138.2, 172.3, 172.5; ESI-TOF MS m/z 2489 Da (2488 Da calcd for $[CH_3(OCH_2CH_2)_{105}C_{16}H_{22}NO_4\cdot 2Na]^{2+}$).

4.4. Screening of enzymes

In the screening test, we used the following enzymes: Lipase immobilized from *C. antarctica* (Novozym 435, BioChemika), Lipase from porcine pancreas (PPL; Type II), Lipase Type VII from *Candida cylindracea* (Sigma), Lipase A, Lipase D, Lipase D-360, Lipase PS, Newlase F, PLE (Amano Enzyme, Inc.), Lipase OF (Meito Sangyo Co., Ltd.), Esterase SNSM-87, Lilipase (Nagase & Co., Ltd.), α-Chymotrypsin (E. Merck). To a test tube containing 62.5 mg of (±)-**9a** (sub. concn, 5 mM) was added 2.5 mL of 0.1 M phosphate buffer (pH 6.5). To the mixture was added 10 mg of Enzyme, and the solution was incubated for 24 h at 30 °C. The products were extracted with 2.5 mL of Et_2O, and detected by TLC (hexane/AcOEt=3/1). The ee of the product was determined by GC analysis.

4.5. Typical experimental procedure for enantioselective hydrolysis of MPEG-supported substrates

To a test tube containing 125 mg of (±)-**7a** (sub. concn, 5 mM) was added 4.5 mL of hexane and 0.5 mL of 0.1 M phosphate buffer (pH 6.5). To the mixture was added 20 mg of Novozym 435 (2.0 U/mg, using tributyrin at pH 8.0 and 40 °C), and the solution was stirred for 24 h at 30 °C. After the organic layer was separated and evaporated in vacuo, the ee of the resulting (*R*)-**1** (98% ee) in the residue was determined by GC analysis. On the other hand, the aqueous layer was diluted with CH_2Cl_2, and dried over Na_2SO_4. After evaporation, the residue was hydrolyzed with 2 M NaOH aq (1.0 mL) in MeOH (4.0 mL). The products were extracted with hexane (×3), and the organic layer was dried over Na_2SO_4. After evaporation, the ee of the corresponding (*S*)-**1** (55% ee) was determined by GC analysis.

Other reactions were carried out by the same procedure.

4.6. Typical preparative scale reaction of MPEG-supported substrates

To a recovery flask containing 3.0 g of (±)-**7b** (sub. concn, 5 mM) was added 108 mL of hexane and 12 mL of 0.1 M phosphate buffer. To the mixture was added 479.5 mg of Novozym 435, and the solution was stirred for 24 h at 30 °C. After the organic layer was separated and evaporated in vacuo, the residue was purified by preparative TLC (hexane/AcOEt=3/1) to give (*R*)-**1** (17.7 mg, 25%, 99% ee, $[\alpha]_D^{20}$ +34.5 (*c* 1.77, MeOH)); lit.[8] $[\alpha]_D^{20}$ +45 (*c* 5.15, MeOH). On the other hand, the aqueous layer was diluted with CH_2Cl_2, and dried over Na_2SO_4. After the solution was evaporated in vacuo, the residue was poured into Et_2O to precipitate the compound (*S*)-**7b** as a white solid. To a solution of (*S*)-**7b** in MeOH (96 mL) was added 2 M NaOH aq (24 mL), and the mixture was stirred for 1 h at room temperature. The products were extracted with ether (×3), and the organic layer was washed with brine and dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography (hexane/EtOAc=2/1) on silica gel to give the corresponding (*S*)-**1** (16.8 mg, 24%, 94% ee, $[\alpha]_D^{21}$ −21.0 (*c* 1.68, MeOH)).

4.7. Several data of alcohols

*4.7.1. 1-Phenylethanol (**1**).* The spectral data were in full agreement with that of a commercial source. GC 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.

*4.7.2. 1-Phenyl-1-propanol (**10**).* The spectral data were in full agreement with that of a commercial source. GC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm×50 m; injection, 140 °C; detection, 140 °C; oven, 120 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 22.6 (*R*) and 23.4 (*S*) min. The authentic sample (*R*)-**10**: $[\alpha]_D^{30}$ +41.4 (*c* 1.20, $CHCl_3$) (94% ee); lit.[9] (*S*)-form, $[\alpha]_D^{20}$ −47.0 (*c* 1.00, $CHCl_3$).

*4.7.3. 1-Phenyl-2-propanol (**11**).* The spectral data were in full agreement with that of a commercial source. GC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm×50 m; injection, 130 °C; detection, 130 °C; oven, 110 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 27.1 (*R*) and 27.5 (*S*) min. The authentic sample (*R*)-**11**: $[\alpha]_D^{30}$ −32.8 (*c* 0.67, $CHCl_3$) (99% ee); lit.[10] (*R*)-form, $[\alpha]_D^{25}$ −37.57 (*c* 5.00, $CHCl_3$).

*4.7.4. 4-Benzyloxy-2-butanol (**12**).* The spectral data were in full agreement with those reported.[5b] 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. The authentic sample (*R*)-**12**: $[\alpha]_D^{29}$ −14.9 (*c* 1.08, MeOH)(97% ee); lit.[5b] (*S*)-form, $[\alpha]_D^{27}$ +19.0 (*c* 0.95, MeOH).

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