Enzymatic Hydrolysis in Organic Solvents for Kinetic Resolution of Water-Insoluble α-Acyloxy Esters with Immobilized Lipases[1)]

Hiroyuki AKITA,*,[a] Isao UMEZAWA,[b,2)] and Hiroko MATSUKURA[b]

School of Pharmaceutical Science, Toho University,[a] 2–2–1, Miyama, Funabashi, Chiba 274, Japan and Riken Institute (The Institute of Physical and Chemical Research),[b] 2–1, Hirosawa, Wako-shi, Saitama 351–01, Japan.
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Enantioselective hydrolysis of water-insoluble α-acyloxy esters, (±)-5 and (±)-6, was carried out using lipases immobilized with Celite or a synthetic prepolymer (ENTP-4000 or ENT-4000) in a water-saturated organic solvent to produce chiral intermediates, (2*S*,3*S*)-5 and (2*S*,3*R*)-4, for the synthesis of diltiazem hydrochloride 1 and (−)-indolmycin 2, respectively. Furthermore, enantioselective hydrolysis of (±)-6 using lipid-lipase aggregates in water-saturated organic solvent was also found to give (2*S*,3*R*)-4.

Key words enantioselective hydrolysis; water-insoluble substrate; α-acyloxy ester; immobilized lipase; diltiazem hydrochloride; (−)-indolmycin

When substrates are highly lipophilic or essentially insoluble in water, enzymatic reactions often may not proceed at all. This difficulty may be overcome if the aqueous medium can be replaced by an organic solvent. However, in such cases, the enzymes should be protected from denaturation by the organic solvent. Immobilization would serve effectively for this purpose. Indeed, for many syntheses of chiral compounds using enzymes, the use of immobilized enzymes is advantageous.[3)] Such immobilized biocatalysts are particularly easy to handle and can be easily recovered from the reaction medium by simple filtration. Furthermore, many of the immobilized enzymes can be repeatedly used without much loss of enzymatic activity, and sometimes the stability of the proteins is enhanced. We now report the enantioselective hydrolysis of the water-insoluble α-acyloxy esters (±)-**5** and (±)-**6** with immobilized lipase in water-saturated organic solvents, giving the (2*S*,3*S*)-α-acyloxy ester **5** and (2*S*,3*R*)-indolmycenic ester **4**, which are intermediates for the synthesis of medicinally active diltiazem hydrochloride **1** and (−)-indolmycin **2**, respectively.

Enantioselective Hydrolysis of (±)-α-Acyloxy Esters 5 by Using Immobilized Lipases Diltiazem hydrochloride **1** is a representative calcium antagonist which is widely used to treat ischemic heart diseases.[4)] Its important chiral intermediate (2*S*,3*S*)-**3** has been produced industrially by optical resolution of the corresponding racemic α-hydroxy acid. In the present study, we examined the lipase-catalyzed kinetic resolution of (±)-**5a**. Initially, enantioselective hydrolysis of (±)-**5a** with several lipases was attempted in a phosphate buffer, but the reaction did not proceed at all, which may be attributable to insolubility of (±)-**5a**. Thus, (±)-**5a** was dissolved in a water-saturated mixture of isooctane and benzene (10:3) and the solution was exposed to lipases immobilized on Celite[5)] or photo-crosslinkable resin prepolymer (ENTP-4000 or ENT-4000).[6)] When the acyl group of a substrate is susceptible to hydrolysis, the use of lipases immobilized on Celite is advantageous, since immobilization enables enzymes to be used even in organic solvents with only small amounts of water.[5)] Lipases immobilized on Celite were prepared by mixing lipases (*ca.* 100 mg) with Celite 535 (Johns–Manville Co., Ltd., *ca.* 300 mg) containing water (0.2 ml). On the other hand, various types of prepolymers possessing photosensitive functional groups have been developed by Fukui and Tanaka.[6)] The structure of a typical photo-crosslinkable resin prepolymer is shown in Chart 2.

Typical immobilization procedures with photo-cross-

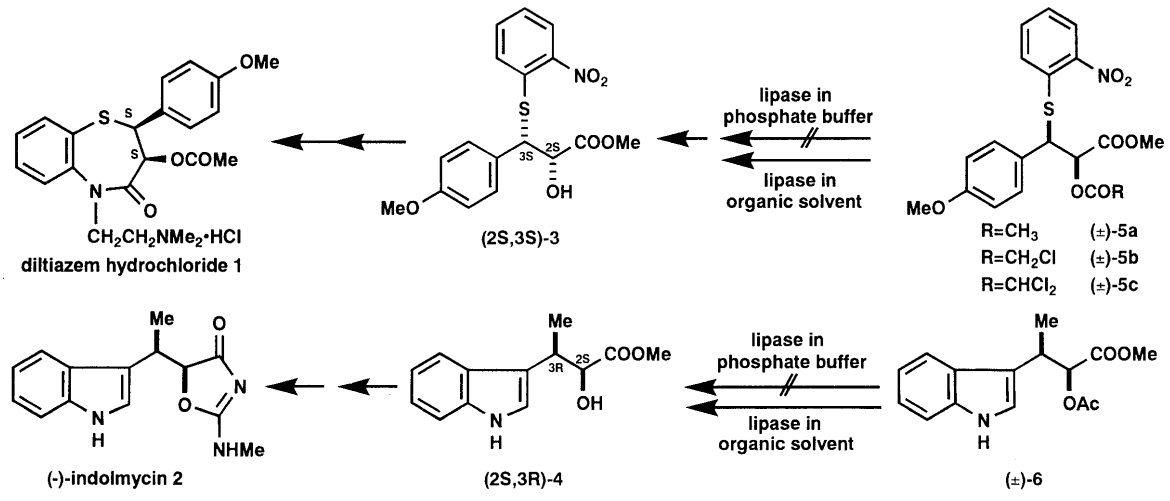


Chart 1

* To whom correspondence should be addressed.

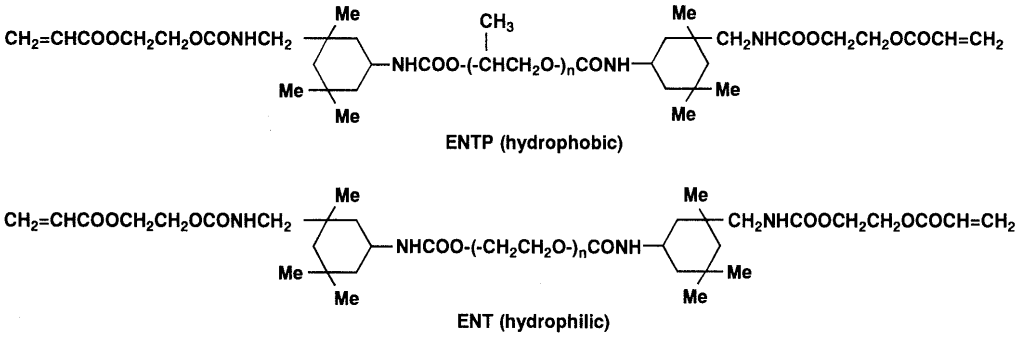


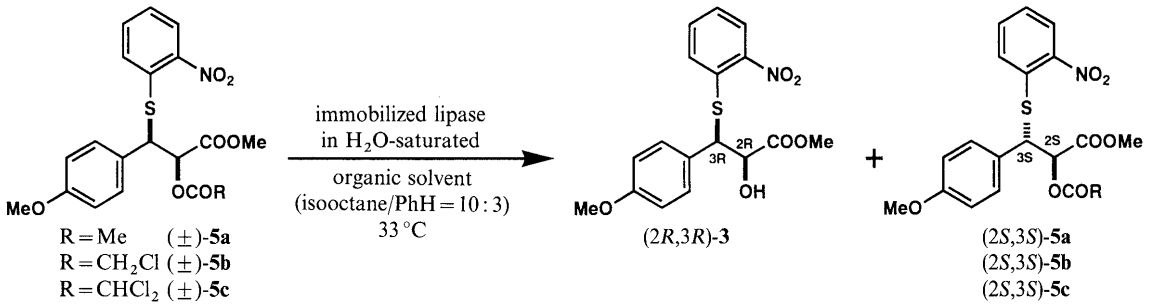
Chart 2

linkable resin prepolymer are as follows. One gram of ENTP-4000 is mixed with 10 mg of a photosensitizer, benzoin ethyl ether. The mixture is melted completely at 60 °C. The powdered lipase (100 mg) is added to the molten mixture under continuous mixing. The prepolymer-lipase mixture is layered on a sheet of transparent polyester film (thickness, *ca.* 0.5 mm). The layer is covered with transparent thin film and then illuminated with chemical lamps (wavelength range, 300—400 nm) for 3 min. The gel film thus formed is cut into small pieces (0.5 × 5 × 5 mm) and used for bioconversion reaction. In preliminary screening experiments using α-acetoxy ester (±)-**5a** as a substrate, the following eight lipases were found to be effective: "Amano A" and "Amano A-6" (from *Aspergillus niger*), "Sigma Acylase I" (from *Aspergillus melleus*), "Amano D-10" (from *Rhizopus delemar*), "Amamo F-AP-15" (from *Rhizopus javanicus*), "Amano P" (from *Pseudomonas* sp.), "Meito MY-30" (from *Candida rugosa*), "Meito PL-266" (from *Alcaligenes* sp.). The reaction did yield the desired (2*S*,3*S*)-**5a** when (±)-**5a** was exposed to the immobilized lipases in a water–saturated mixture of isooctane and benzene (10 : 3).[7] The absolute structure and optical purity of the hydrolyzed products were confirmed by an analysis of the 400 MHz NMR spectra of their (+)-α-methoxy-α-trifluoromethylacetates ((+)-MTPA esters)[8] in comparison with the spectra of authentic (2*S*,3*S*)-**3**-(+)-MTPA[9] (δ 3.71, 3H, s, COOMe) and a mixture of two (+)-MTPA esters ((2*S*,3*S*)-**3**-(+)-MTPA and (2*R*,3*R*)-**3**-(+)-MTPA (δ 3.67, 3H, s, COOMe) obtained by the reaction of (±)-**3** and (*R*)-(+)-MTPACl.[8] The absolute structure and optical purity of the unchanged acyloxy esters were also confirmed by the NMR analysis of their (+)-MTPA esters obtained by chemical hydrolysis and subsequent (+)-MTPA esterification. The results are shown in Table 1.

In every case, the hydrolyzed product was found to be (2*R*,3*R*)-**3**. Among the eight lipases used, "Amano P" gave the best results, producing the desired (2*S*,3*S*)-**5a** with moderate optical purity (48—52% ee, entries 8 and 9). When Celite was replaced by ENTP-4000 or ENT-4000, the optical yield of (2*S*,3*S*)-**5a** was increased appreciably (81—94% ee, entries 10 and 12). Then the acyloxyl group was changed from an acetoxyl group ((±)-**5a**) to a chloroacetoxyl group ((±)-**5b**) or dichloroacetoxyl group ((±)-**5c**). In the case of the enantioselective hydrolysis of the α-chloroacetoxy ester (±)-**5b** using the lipase "Amano P" immobilized on Celite, the reaction rate was found to be somewhat accelerated (38—43 h, entries 14 and 18) and the optical purity of (2*S*,3*S*)-**5b** was increased appreciably (69—72% ee, entries 14 and 18) compared with the case of entries 8 and 9. The enantioselective hydrolysis of the α-dichloroacetoxy ester (±)-**5c** using the lipase "Amano P" immobilized on Celite gave a moderate result (entry 22). From these experiments, the chloroacetoxyl group was considered to be the most suitable acyloxyl group for enzymatic hydrolysis. The desired (2*S*,3*S*)-α-chloroacetoxy ester **5b** was obtained with high optical purity when "Amano P" immobilized on ENTP-4000 or ENT-4000 was employed (90—95% ee, entries 16 and 20). When the recovered "Amano P" immobilized with ENTP-4000 or ENT-4000 was used repeatedly, the yield and optical purity of the reaction product were maintained at extremely high levels (entries 17 and 21). From these data, the lipase "Amano P" immobilized with ENTP-4000 or ENT-4000 retains its enzymatic activity well, without appreciable denaturation. "Amano P" itself is claimed to be fairly stable in various organic solvents.[10] However, the use of "Amano P" in the same organic solvent system, as noted above, gave only a poor result (entry 13). This clearly demonstrates the effectiveness of enzyme-immobilization.

Enantioselective Hydrolysis of the (±)-α-Acetoxy Ester 6 by Using Immobilized Lipases For the synthesis of (−)-indolmycin (**2**), which is an antibiotic isolated from an African strain of *Streptomyces albus*, and shows antibacterial activity against *Staphylococci*, it is advantageous to synthesize (2*S*,3*R*)-**4**[11] by the direct enantioselective hydrolysis of the (±)-α-acetoxy ester **6** obtained by the acetylation of (±)-**4**.[12] Initially, asymmetric hydrolysis of (±)-**6** with several lipases was attemped in a phosphate buffer, but the reaction did not proceed at all, which may be attributable to (±)-**6** being a water-immisible oil. Thus, (±)-**6** was dissolved in water–saturated isooctane–benzene (5 : 1)[7] and the solution was exposed to the enzymatic reaction using lipases immobilized on Celite or ENTP-4000. The following six lipases were used: "Amano A" and "Amano A-6", "Meito MY-30", "Meito OF-360", and "Sigma Lipase type VII" (from *Candida rugosa*), and "Saiken Lilipase" (from *Rhizopus javanicus*). The reaction did yield the desired (2*S*,3*R*)-**4**. The absolute structure and optical purity of the hydrolyzed products were confirmed by an analysis of the 400 MHz NMR spectra of their (+)-α-methoxy-α-trifluoromethylacetates ((+)-MTPA esters)[8] in comparison with the spectra of authentic (2*R*,3*S*)-**4**-(+)-MTPA[13] (δ 3.76, 3H, s, COOMe) and a mixture of two (+)-MTPA esters ((2*R*,3*S*)-**4**-(+)-MTPA and (2*S*,3*R*)-**4**-(+)-MTPA (δ 3.81, 3H, s, COOMe) obtained by the reaction of (±)-**4** and (*R*)-(+)-MTPACl.[8]

Table 1. The Results of Enzymatic Reaction with *ca.* 100 mg of (±)-**5**



R = Me (±)-**5a**
R = CH_2Cl (±)-**5b**
R = $CHCl_2$ (±)-**5c**

(2*R*,3*R*)-**3**

(2*S*,3*S*)-**5a**
(2*S*,3*S*)-**5b**
(2*S*,3*S*)-**5c**

Entry	Substrate	Lipase	Time	Yield, % (optical purity, % ee)	
				(2*R*,3*R*)-**3**	(2*S*,3*S*)-**5**
1	(±)-**5a**	Amano A/Celite	87 h	47 (26)	43 (6)
2	(±)-**5a**	Amano A-6/Celite	72 h	50 (39)	37 (18)
3	(±)-**5a**	Acylase/Celite	72 h	10 (93)	87 (10)
4	(±)-**5a**	Amano D-10/Celite	87 h	12 (63)	80 (19)
5	(±)-**5a**	Amano F-AP-15/Celite	16 d	20 (>99)	61 (14)
6	(±)-**5a**	MY-30/Celite	16 d	22 (75)	67 (9)
7	(±)-**5a**	PL-266/Celite	72 h	18 (97)	81 (22)
8	(±)-**5a**	Amano P/Celite	68 h	29 (>99)	67 (48)
9	(±)-**5a**	Amano P/Celite	10 d	36 (97)	59 (52)
10[a]	(±)-**5a**	Amano P/Celite, ENTP-4000	16 d	44 (98)	52 (81)
11	(±)-**5a**	Amano P/ENTP-4000	21 d	24 (>99)	75 (31)
12	(±)-**5a**	Amano P/ENT-4000	21 d	49 (97)	50 (94)
13	(±)-**5b**	Amano P	7 d	14 (70)	82 (8)
14	(±)-**5b**	Amano P/Celite	38 h	44 (91)	49 (69)
15[b]	(±)-**5b**	Amano P/Celite ENT-4000, ENTP-4000	19 d	51 (84)	48 (89)
16	(±)-**5b**	Amano P/ENTP-4000	19 d	49 (91)	50 (90)
17[c]	(±)-**5b**	Amano P/ENTP-4000	21 d	49 (95)	48 (94)
18	(±)-**5b**	Amano P/Celite	43 h	43 (93)	49 (72)
19[d]	(±)-**5b**	Amano P/Celite, ENTP-4000	19 d	44 (94)	53 (74)
20	(±)-**5b**	Amano P/ENT-4000	21 d	56 (74)	40 (95)
21[e]	(±)-**5b**	Amano P/ENT-4000	12 d	51 (88)	48 (94)
22	(±)-**5c**	Amano P/Celite	21 d	45 (71)	42 (47)

a) The present immobilized lipase was prepared by entrapping Amano P/Celite used in entry 8 with ENTP-4000. *b*) The present immobilized lipase was prepared by entrapping Amano P/Celite used in entry 14 with ENTP-4000 (0.3 g) and ENT-4000 (1 g). *c*) The same immobilized lipase as used in entry 16 was repeatedly employed. *d*) The present immobilized lipase was prepared by entrapping Amano P/Celite used in entry 18 with ENTP-4000. *e*) The same immobilized lipase as used in entry 20 was repeatedly employed.

The absolute structure and optical purity of the unchanged acetoxy esters were also confirmed by the NMR analysis of their (+)-MTPA esters obtained by chemical hydrolysis and subsequent (+)-MTPA esterification. The results are shown in Table 2.

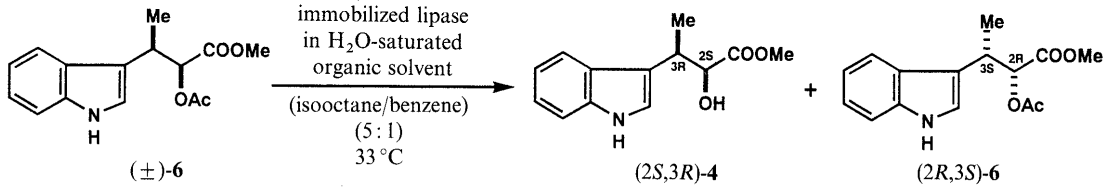
In every case, the hydrolyzed product was found to be (2*S*,3*R*)-**4**. Among the six lipases used, "OF-360" gave the best results, producing the desired (2*S*,3*R*)-**4** with reasonably high optical purity (91—93% ee, entries 6—8). The chemical and optical yields show the average values of two experiments (entry 7). From these data, lipase immobilized on ENTP-4000 was found to retain fully its activity after the reaction.

Enantioselective Hydrolysis of the α-Acetoxy Ester (±)-6 by Using Lipid-Lipase Aggregates Immobilizing enzymes is apparently a method of choice for protecting them from denaturation by organic solvents. In chiral synthesis, phospholipid-lipase aggregates in organic media are sometimes more useful than the native enzymes. We reported previously that a phospholipid-lipase aggregate with an ether linkage functions as a new type of immobilized enzyme for enantioselective hydrolysis or esterification in organic solvents.[14] In particular, when (±)-indolmycenic ester **4** was exposed to the lipase "OF-360" aggregated with phospholipid bearing an ether linkage in the presence of an acylating reagent, the (2*S*,3*R*)-2-acetoxy ester **6** (15%) with high optical yield (97% ee) and (2*R*,3*S*)-**4** (79%, 21% ee) were obtained.[15]

For comparison with the above-mentioned case, enantioselective hydrolysis of (±)-**6** using various kinds of ether-linked lipid-lipase aggregates in organic solvents was examined. The 27 kinds of phospholipid analogues with dialkyl ether-linkages used are shown in Chart 4.[14] Preparation of lipid-lipase "OF-360" aggregates bearing O-hexadecyl groups and the determination of the optical purity and chemical yield of the present enzymatic reaction products ((2*S*,3*R*)-**4** and (2*R*,3*S*)-**6**) by HPLC analysis were carried out by our previously reported method.[15] Yields of various kinds of aggregate and the results of enantioselective hydrolysis of (±)-**6** are shown in Tables 3 and 4, respectively.

In comparison with the enantioselective esterification,

Table 2. The Results of Enzymatic Reaction with *ca.* 100 mg of (±)-**6**



(±)-**6** (2*S*,3*R*)-**4** (2*R*,3*S*)-**6**

Entry	Lipase	Time (h)	Yield, % (optical purity, % ee)	
			(2*S*,3*R*)-**4**	(2*R*,3*S*)-**6**
1	Amano A-6/Celite	46	41 (45)	56 (36)
2	MY-30/Celite	75	36 (34)	54 (32)
3	Lilipase/Celite	216	10 (84)	81 (17)
4	Amano A/Celite	48	21 (35)	72 (19)
5	Sigma lipase Type VII/Celite	48	29 (38)	68 (19)
6	OF-360/Celite	24	28 (93)	61 (48)
7[a)]	OF-360/ENTP-4000	72	37 (93)	62 (54)
8	OF-360/ENTP-4000	240	40 (91)	51 (72)

a) The data show the average value after the reaction was repeated two times.

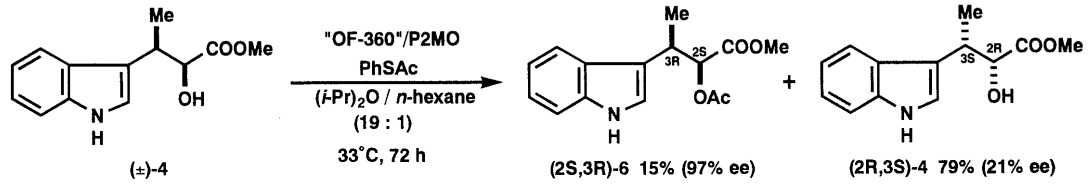


Chart 3

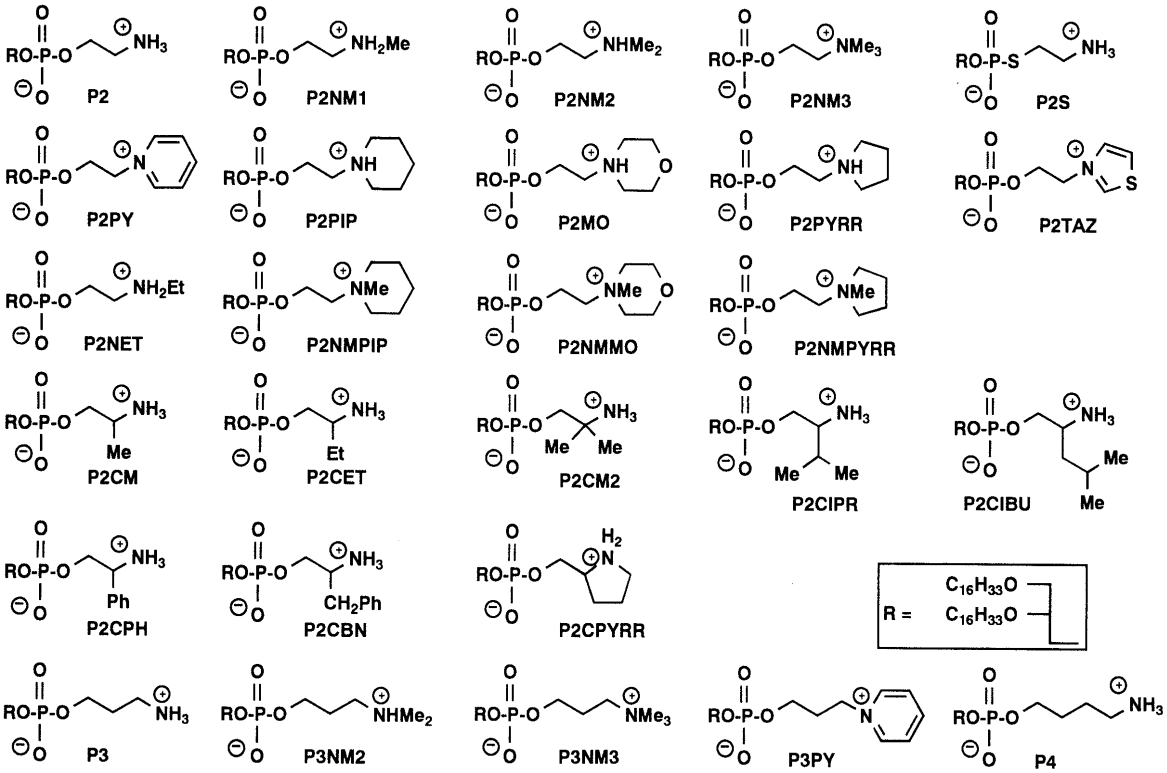


Chart 4

the rate of hydrolysis was found to be somewhat faster than that of esterification. In the case of entries 12 and 14, the chemical yield (25%) and optical purity (95% ee) of the desired (2*S*,3*R*)-**4** were high. The existence of a bulkier substituent around the quaternary ammonium cation could increase the reaction rate and enhance the enantioselectivity.

In conclusion, it became apparent that even water-insoluble compounds can be substrates for enzymatic reactions provided that the lipases are immobilized properly

and suitable organic solvents are used. The only drawback in the present method is that the reactions proceed extremely slowly. This problem may be overcome by developing new immobilization techniques.

Experimental

All melting points were measured on a Yanaco MP-S3 micro melting point apparatus and are uncorrected. NMR spectra were measured on a JEOL GX-400 spectrometer and spectra were taken as 5—10% (w/v) solutions in $CDCl_3$ with Me_4Si as an internal reference. IR spectra (CCl_4) were measured on a JASCO A-3 spectrometer. High-resolution mass spectra (HRMS) were obtained with a JEOL JMS-D 300 spectrometer. Optical rotations were measured on a Perkin-Elmer model 241 MC polarimeter. The high-performance liquid chromatography (HPLC) system was composed of two SSC instruments (ultraviolet (UV) detector 3000B and flow system 3100). All the reactions were carried out in an atmosphere of argon. All evaporations were performed under reduced pressure.

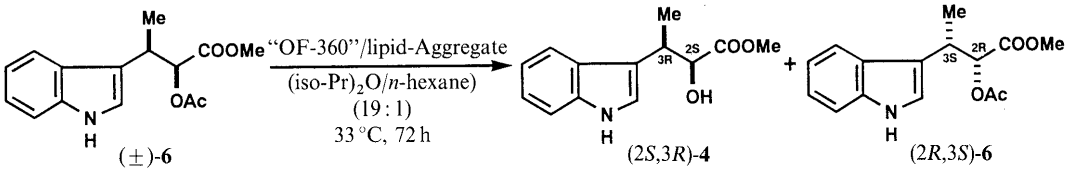
Table 3. Yields (mg) of Various Kinds of "Dry Aggregates"

1	OF-360/P2	65.5	15	OF-360/P2CM	47
2	OF-360/P1NM1	69	16	OF-360/P2CET	84
3	OF-360/P2NM2	66	17	OF-360/P2CM2	47.5
4	OF-360/P2NM3	72	18	OF-360/P2CIPR	45.5
5	OF-360/P2S	60	19	OF-360/P2CIBU	64.5
6	OF-360/P2PY	61.5	20	OF-360/P2CPH	24.5
7	OF-360/P2PIP	85.5	21	OF-360/P2CBN	37
8	OF-360/P2MO	50.5	22	OF-360/P2CPYRR	58.5
9	OF-360/P2PYRR	36	23	OF-360/P3	50.5
10	OF-360/P2TAZ	34.5	24	OF-360/P3NM2	70
11	OF-360/P2NET	31.5	25	OF-360/P3NM3	59.3
12	OF-360/P2NMPIP	48.8	26	OF-360/P3PY	46.5
13	OF-360/P2NMMO	23	27	OF-360/P4	123.7
14	OF-360/P2NMPYRR	39.2			

Methyl (2*RS*,3*RS*)-2-Acetoxy-3-(*p*-methoxyphenyl)-3-(*o*-nitrophenylthio)propanoate ((±)-5a) A mixture of methyl (2*RS*,3*RS*)-2-hydroxy-3-(*p*-methoxyphenyl)-3-(*o*-nitrophenylthio)propanoate ((±)-**3**, 5 g), Ac_2O (10 ml) and pyridine (10 ml) in the presence of 4-dimethylaminopyridine (DMAP, 200 mg) was stirred for 24 h at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, and dried over $MgSO_4$. Evaporation of organic solvent gave crude crystals, which were recrystallized from AcOEt–*n*-hexane to afford pale yellow prisms (±)-**5a** (5.48 g, 98% yield). (±)-**5a**: mp 106—107.5 °C. *Anal.* Calcd for $C_{19}H_{19}NO_7S$: C, 56.30; H,4.69; N, 3.46; S,7.90. Found: C, 56.32; H, 4.64; N, 3.41; S, 7.77. IR (CCl_4): 1755 cm^{-1}. NMR δ: 2.10 (3H, s, 2-OAc), 3.64 (3H, s, COOMe), 3.78 (3H, s, OMe), 4.89 (1H, d, J=5.4 Hz, 3-H), 5.40 (1H, d, J=5.4 Hz, 2-H).

Methyl (2*RS*,3*RS*)-2-Chloroacetoxy-3-(*p*-methoxyphenyl)-3-(*o*-nitrophenylthio)propanoate ((±)-5b) A mixture of (±)-**3** (3.63 g), monochloroacetic anhydride (3 g) and pyridine (10 ml) in the presence of DMAP (200 mg) was stirred for 2 h at 0 °C. The reaction mixture was worked up in the same manner as described for the synthesis of (±)-**5a**. Recrystallization of a crude (±)-**5b** from AcOEt–*n*-hexane afforded pale yellow prisms (±)-**5b** (3.37 g, 77% yield). (±)-**5b**: mp 80.5—81.5 °C. *Anal.* Calcd for $C_{19}H_{18}ClNO_7S$: C, 51.88; H, 4.10; Cl, 8.08; N, 3.19; S, 7.28. Found: C, 51.85; H, 4.03; Cl, 8.35; N, 3.19; S, 7.16. IR (CCl_4): 1750, 1760(sh), 1780(sh) cm^{-1}. NMR δ: 3.67 (3H, s, COOMe), 3.79 (3H, s, OMe), 4.14 (2H, q, J=15.4 Hz, 2-OCOC$\underline{H}_2$Cl), 4.91 (1H, d, J=4.9 Hz, 3-H), 5.47 (1H, d, J=4.9 Hz, 2-H).

Table 4. The Results of Enzymatic Reaction with *ca.* 10 mg of (±)-**6**



(±)-**6** (2*S*,3*R*)-**4** (2*R*,3*S*)-**6**

Entry	Lipase	Yield, % (optical purity, % ee)	
		(2*S*,3*R*)-**4**	(2*R*,3*S*)-**6**
1	OF-360/P2	17.2 (>99)	80.5 (24)
2	OF-360/P1NM1	11.0 (>99)	82.7 (15)
3	OF-360/P2NM2	12.5 (>99)	86.3 (15)
4	OF-360/P2NM3	15.7 (>99)	84.2 (19)
5	OF-360/P2S	8.8 (>99)	84.6 (10)
6	OF-360/P2PY	15.2 (>99)	75.4 (29)
7	OF-360/P2PIP	24.1 (>99)	74.4 (34)
8	OF-360/P2MO	22.9 (>99)	76.1 (31)
9	OF-360/P2PYRR	17.6 (91)	75.8 (24)
10	OF-360/P2TAZ	9.5 (88)	87.5 (8)
11	OF-360/P2NET	14.9 (95)	78.5 (17)
12	OF-360/P2NMPIP	25.0 (95)	73.6 (34)
13	OF-360/P2NMMO	21.2 (95)	77.1 (24)
14	OF-360/P2NMPYRR	25.4 (95)	73.1 (35)
15	OF-360/P2CM	13.8 (95)	76.6 (15)
16	OF-360/P2CET	20.1 (97)	79.5 (25)
17	OF-360/P2CM2	17.6 (99)	68.1 (26)
18	OF-360/P2CIPR	16.8 (96)	67.3 (22)
19	OF-360/P2CIBU	23.8 (98)	70.2 (32)
20	OF-360/P2CPH	7.6 (87)	90.3 (5)
21	OF-360/P2CBN	11.0 (95)	82.5 (14)
22	OF-360/P2CPYRR	18.3 (94)	71.4 (28)
23	OF-360/P3	7.8 (>99)	79.5 (11)
24	OF-360/P3NM2	15.5 (86)	76.6 (18)
25	OF-360/P3NM3	18.1 (94)	80.4 (23)
26	OF-360/P3PY	9.3 (>99)	89.2 (12)
27	OF-360/P4	16.6 (92)	82.2 (20)

Methyl (2*RS*,3*RS*)-2-Dichloroacetoxy-3-(*p*-methoxyphenyl)-3-(*o*-nitrophenylthio)propanoate ((±)-5c) A mixture of (±)-**3** (3.63 g), dichloroacetyl chloride (2.94 g) and pyridine (10 ml) in the presence of DMAP (200 mg) was stirred for 2 h at 0 °C. The reaction mixture was worked up in the same manner as described for the synthesis of (±)-**5a**. The residue was chromatographed on silica gel (120 g) to give crude crystals from the *n*-hexane–AcOEt (9 : 1) eluate, and these were recrystalized from AcOEt–*n*-hexane to provide pale yellow prisms (±)-**5c** (1.45 g, 31% yield). (±)-**5c**: mp 101—102 °C. *Anal.* Calcd for $C_{19}H_{17}Cl_2NO_7S$: C, 48.10; H, 3.59; Cl, 14.98; N, 2.95; S, 6.75. Found: C, 48.29; H, 3.47; Cl. 15.45; N, 2.93; S, 5.98. IR (CCl_4): 1755, 1775 cm^{-1}. NMR δ: 3.67 (3H, s, COOMe), 3.79 (3H, s, OMe), 4.95 (1H, d, J=4.8 Hz, 3-H), 5.48 (1H, d, J=4.8 Hz, 2-H). 6.03 (1H, s, 2-OCOC<u>H</u>Cl_2).

Preparation of a Mixture of (2*R*,3*R*)-3-(*R*)-(+)-MTPA Ester and (2*S*,3*S*)-3-(*R*)-(+)-MTPA Ester from (±)-3 Pyridine (0.3 ml) was added to a mixture of (±)-**3** (20 mg) and (*R*)-(+)-MTPACl (40 mg) in the presence of DMAP (5 mg), and the reaction mixture was stirred for 24 h at room temperature, then diluted with H_2O and extracted with ether. The ether extract was washed with saturated brine and dried over $MgSO_4$. Removal of the solvent gave an oily product, which was subjected to preparative thin layer chromatography (prep. TLC: Kieselgel 60 F_{254}, 200 × 200 × 0.5 mm; solvent, AcOEt–*n*-hexane (1 : 1)) to afford a mixture of (+)-MTPA esters (28.5 mg) as a homogeneous oil. NMR δ: 3.67, 3.71 (each 3H, s, COOMe), 3.75, 3.79 (each 3H, s, aromatic-OMe), 4.86 (1H, d, J=4.4 Hz, 3-H), 4.92 (1H, d, J=4.9 Hz, 3-H), 5.50 (1H, d, J=4.4 Hz, 2-H), 5.55 (1H, d, J=4.9 Hz, 2-H).

Preparation of Authentic (2*S*,3*S*)-3-(*R*)-(+)-MTPA Ester from (2*S*,3*S*)-2-Hydroxy-3-(*p*-methoxyphenyl)-3-(*o*-nitrophenylthio)propanoic Acid (2*S*,3*S*)-2-Hydroxy-3-(*p*-methoxyphenyl)-3-(*o*-nitrophenylthio)propanoic acid (56 mg) was treated with CH_2N_2 ether solution to give the corresponding methyl ester (2*S*,3*S*)-**3** (59 mg). A part (4.7 mg) of (2*S*,3*S*)-**3** was converted to the (2*S*,3*S*)-**3**-(*R*)-(+)-MTPA ester (8.5 mg) as a homogeneous oil in the same manner as described for the preparation of a mixture of (2*R*,3*R*)-**3**-(*R*)-(+)-MTPA ester and (2*S*,3*S*)-3-(*R*)-(+)-MTPA ester. NMR δ: 3.71 (3H, s, COOMe), 3.75 (3H, s, aromatic-OMe), 4.86 (1H, d, J=4.4 Hz, 3-H), 5.50 (1H, d, J=4.4 Hz, 2-H).

Immobilization of Lipase on Celite The procedure was described in the text.

Immobilization of Lipase on Photocross-Linkable Resin Prepolymer (ENTP-4000 or ENT-4000) The procedure was described in the text.

General Procedure of Enantioselective Hydrolysis of (±)-2-Acyloxy Esters 5a—c Using Immobilized Lipase A solution of each substrate (*ca.* 100 mg) in a water–saturated mixture of isooctane (10 ml) and benzene (3 ml) was incubated with immobilized lipase at 33 °C. Progress of the reaction was monitored by TLC and when the spots due to acyloxy esters and alcohols became the same in size, the hydrolysis was terminated. The immobilized lipase was filtered off and washed with AcOEt. The filtrate and washing were combined and evaporated to give a crude product, which was chromatographed on silica gel (30 g) to afford an acyloxy ester **5** from the *n*-hexane–AcOEt (9 : 1) eluate and a hydroxy ester **3** from the *n*-hexane–AcOEt (5 : 1) eluate. A part of each hydroxy ester **3** (*ca.* 15 mg) was converted to the corresponding (*R*)-(+)-MTPA ester in the same way as mentioned above, and this was analyzed by 400 MHz NMR. A part of each acyloxy ester **5** (*ca.* 20 mg) in MeOH (0.5 ml) was treated with K_2CO_3 (*ca.* 15 mg) to give a crude hydroxy ester **3** by a usual method. The obtained crude **3** was converted to the corresponding (*R*)-(+)-MTPA ester in the same way as mentioned above, and this was analyzed by 400 MHz NMR. The chemical yield and the enantiomeric excess of each reaction product are summarized in Table 1.

Methyl (2*RS*,3*SR*)-2-Acetoxy-3-(3-indolyl)butanoate ((±)-6) A mixture of methyl (2*RS*,3*SR*)-2-hydroxy-3-(3-indolyl)butanoate ((±)-**4**, 1.67 g), Ac_2O (5 ml) and pyridine (5 ml) was stirred for 24 h at room temperature. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine and dried over $MgSO_4$. Evaporation of the organic solvent gave a residue, which was chromatographed on silica gel (50 g) to afford a homogeneous oil (±)-**6** (1.473 g, 75% yield) from *n*-hexane–AcOEt (4 : 1) eluate. (±)-**6**: *Anal.* HRMS Calcd for $C_{15}H_{17}NO_4$ (M^+) *m/z*: 275.116. Found: 275.124. IR (CCl_4): 1756, 3530 cm^{-1}. NMR δ: 1.45 (3H, d, J=7.3 Hz, 3-Me), 2.10 (3H, s, 2-OAc), 3.64 (3H, s, COOMe), 3.73 (1H, dq, J=4.6, 7.3 Hz, 3-H), 5.23 (1H, d, J=4.6 Hz, 2-H).

Preparation of a Mixture of (2*R*,3*S*)-4-(*R*)-(+)-MTPA Ester and (2*S*,3*R*)-4-(*R*)-(+)-MTPA Ester from (±)-4 Pyridine (0.3 ml) was added to a mixture of (±)-**4** (30 mg) and (*R*)-(+)-MTPACl (50 mg) in the presence of DMAP (*ca.* 10 mg), and the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was diluted with H_2O and extracted with ether. The ether extract was washed with saturated brine and dried over $MgSO_4$. Removal of the solvent gave an oily product, which was subjected to preparative thin layer chromatography (prep. TLC: Kieselgel 60 F_{254}, 200 × 200 × 0.5 mm; solvent, AcOEt–*n*-hexane (1 : 1)) to afford a mixture of (+)-MTPA esters (28.5 mg) as a homogeneous oil. *Anal.* HRMS Calcd for $C_{23}H_{22}FNO_5$ (M^+) *m/z*: 449.145. Found: 449.144. NMR δ: 1.37, 1.43 (each 3H, d, J=7.1 Hz, 3-Me), 3.76, 3.81 (each 3H, s, COOMe).

Preparation of Authentic (2*R*,3*S*)-4-(*R*)-(+)-MTPA Ester from (2*R*,3*S*)-4 (2*R*,3*S*)-**4** (25 mg) was converted to the (2*R*,3*S*)-**4**-(*R*)-(+)-MTPA ester (41 mg) as a homogeneous oil in the same manner as described for the preparation of a mixture of (2*R*,3*S*)-**4**-(*R*)-(+)-MTPA ester and (2*S*,3*R*)-**4**-(*R*)-(+)-MTPA ester. NMR δ: 1.37 (3H, d, J=7.1 Hz, 3-Me), 3.76 (3H, s, COOMe).

General Procedure of Enantioselective Hydrolysis of (±)-2-Acetoxy Ester 6 Using Immobilized Lipase A solution of each substrate (*ca.* 100 mg) in a water-saturated mixture of isooctane (10 ml) and benzene (2 ml) was incubated with immobilized lipase at 33 °C. Progress of the reaction was monitored by TLC and when the spots due to acetoxy esters and alcohols became the same in size, the hydrolysis was terminated. The immobilized lipase was filtered off and washed with AcOEt. The filtrate and washing were combined and evaporated to give a crude product, which was chromatographed on silica gel (30 g) to afford an acetoxy ester **6** from the *n*-hexane–AcOEt (9 : 1) eluate and a hydroxy ester **4** from the *n*-hexane–AcOEt (5 : 1) eluate. A part of each hydroxy ester **4** (*ca.* 15 mg) was converted to the corresponding (*R*)-(+)-MTPA ester in the same way as mentioned above, and this was analyzed by 400 MHz NMR. A part of each acetoxy ester **6** (*ca.* 20 mg) in MeOH (0.5 ml) was treated with K_2CO_3 (*ca.* 15 mg) to give a crude hydroxy ester **4** by a usual method. The obtained crude **4** was converted to the corresponding (*R*)-(+)-MTPA ester in the same way as mentioned above, and this was analyzed by 400 MHz NMR. The chemical yield and the enantiomeric excess of each reaction product are summarized in Table 2.

Preparation of Lipid-Lipase Aggregates A mixture of 100 mg of lipase "OF-360" from *Candida rugosa* in water (5 ml) and 50 mg of dialkyl phospholipid analogues in benzene (40 ml) was sonicated for 30 min at 0 °C. The resulting precipitate was centrifuged at 3000 × *g* and the solvent was decanted off. The residual precipitate was dried under reduced pressure to provide a "dry aggregate" (Table 3).

HPLC Analysis of the Two Racemates ((±)-4 and (±)-6) by Using a Chiral Column A 1 : 1 mixture of the two racemates ((±)-**4** and (±)-**6**) gave four well separated peaks ((±)-**4**, 29.9 and 34.2 min; (±)-**6**, 15.3 and 16.7 min) corresponding to each enantiomer under the following analytical conditions: eluent, *n*-hexane/EtOH/iso-PrOH (94 : 4 : 2); detection, UV at 280 nm; flow rate, 1.2 ml/min. The assignment of these peaks was achieved by comparing them with those of authentic ((2*S*,3*R*)-**4**[11] and (2*R*,3*S*)-**6**). Namely, the peak with shorter retention time (t_R = 15.3 min) was found to correspond to that of the (2*R*,3*S*)-**6** enantiomer and the peak with longer retention time (t_R = 34.2 min) to that of the (2*S*,3*R*)-**4** enantiomer. (2*S*,3*R*)-**4**: *Anal.* HRMS Calcd for $C_{13}H_{15}NO_3$ (M^+) *m/z*: 233.106. Found: 233.109. $[\alpha]_D^{23}$ +4.53° (*c*=0.53, MeOH), corresponds to >99% ee. IR(CCl_4): 3500, 1736 cm^{-1}. NMR δ: 1.33 (3H, d, J=7.1 Hz, 3-Me), 2.76 (1H, d, J=5.1 Hz, 2-OH), 3.64 (1H, dq, J=3.2, 7.1 Hz, 3-H). 3.79 (3H, s, COOMe), 4.51 (1H, dd, J=3.2, 5.1 Hz, 2-H). (2*R*,3*S*)-**6**: *Anal.* HRMS Calcd for $C_{15}H_{17}NO_4$ (M^+) *m/z*: 275.116. Found: 275.124. $[\alpha]_D^{22}$ +15.4° (*c*=1.0, MeOH), corresponds to 48% ee. Spectral data (IR and NMR) were identical with those of (±)-**6**.

General Procedure of Enantioselective Hydrolysis Using Lipid-Lipase Aggregates A mixture of (±)-**6** (10 mg) and lipid-lipase aggregates (5 mg) in a mixed solvent (iso-Pr_2O (0.25 ml) and cyclohexane (4.75 ml)) was shaken at 33 °C for 3 d. The reaction mixture was dried over $MgSO_4$ and evaporated to afford a crude product, which was analyzed by HPLC. The results are shown in Table 4.

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