Enantioselective Acetylation of an α-Hydroxy Ester by Using Ether-Linked Lipid–Lipase Aggregates in Organic Solvents

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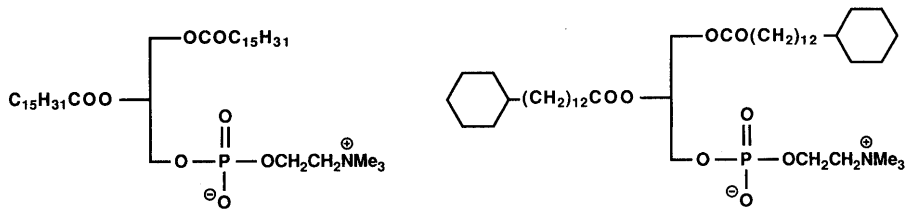
Phospholipids of a new type having ω-cyclohexyltridecyl groups were synthesized in order to investigate the influence of the structure of lipophilic part of phospholipids upon the enzymatic reaction. Enantioselective acetylation of an α-hydroxy ester was carried out in the presence of lipid–lipase aggregates bearing *O*-ω-cyclohexyltridecyl and *O*-hexadecyl groups. The chemical yield of α-acetoxy ester was improved by using lipid–lipase aggregates compared to the case of enzymatic reaction using native lipase OF-360 from *Candida cylindracea*. It was found that the influence of the ω-cyclohexyltridecyl group on the chemical and optical yields of α-acetoxy ester was essentially the same as that of the hexadecyl group.

Keywords enantioselective acetylation; α-hydroxy ester; indolmycin; lipid–lipase aggregate; ω-cyclohexyltridecyl group

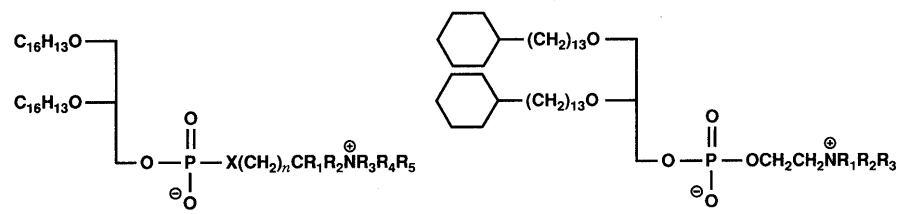
In chiral synthesis, phospholipid–lipase aggregates in organic media are sometimes more useful than the native enzymes.[3a,b)] 1,2-Di-13-cyclohexyltridecanoyl-L-α-phosphatidylcholine (13_{CY}PC) is found in acidothermophilic bacterial membranes, but is rare in nature.[4)] In contrast with a usual phosphatidylcholine, 1,2-dipalmitoyl-L-α-phosphatidylcholine (DPPC), the role of the ω-cyclohexyl-fatty acid residue is considered to make the bilayers fluid below the phase transition temperature, while making them rigid above the phase transition temperature.[4)] In order to investigate the effect of the structure of the lipophilic portion of phospholipid upon the enzymatic reaction, the ω-cyclohexyltridecyl group was selected as a suitable lipophilic moiety. We have already reported syntheses of many kinds of 1,2-di-*O*-hexadecyl ether-type phospholipid analogues A, which correspond to DPPC, as well as enantioselective hydrolysis of α-acyloxy ester by using ether-linked lipid–lipase aggregates.[3a,b)]

We now report the synthesis of 1,2-di-*O*-ω-cyclohexyltridecyl ether-type phospholipid analogues B, corresponding to 13_{CY}PC, and enantioselective acetylation of an α-hydroxy ester by using lipid–lipase aggregates bearing *O*-ω-cyclohexyltridecyl or *O*-hexadecyl groups.

Synthesis of 1,2-Di-*O*-ω-cyclohexyltridecyl Ether-Type Phospholipid Analogues. i) Synthesis of *rac*-1,2-Di-*O*-ω-cyclohexyltridecyl Glycerol 1 Direct bromination of 1,12-dodecanediol **2** with carbon tetrabromide (CBr_4) and triphenylphosphine (Ph_3P) gave a monobromo alcohol **3** (45% yield) which was treated with dihydropyran in the presence of pyridinium *p*-toluenesulfonate (PPTS) to provide the tetrahydropyranyl ether **4** in 91% yield. The monobromide **4** was reacted with 1-benzyloxy glycerol **5**[3b)] in the presence of KH in dimethylformamide (DMF) to afford the di-tetrahydropyranyl ether **6** in 63% yield. Deprotection of the tetrahydropyranyl group of **6** by using camphorsulfonic acid (CSA) afforded quantitatively a diol **7**. Swern oxidation of **7** gave a dialdehyde **8** in 96% yield. Wittig reaction of **8** and cyclohexylmethyltriphenylphosphonium salt, which was obtained by the reaction of cyclohexylmethyl bromide and Ph_3P, produced a mixture of *cis* and *trans* isomers **9** in 92% yield. Catalytic hydrogenation of **9** in the presence of 20% $Pd(OH)_2$–C gave



DPPC 13_{CY}PC



A X=O, S n=1, 2, 3 B

Chart 1

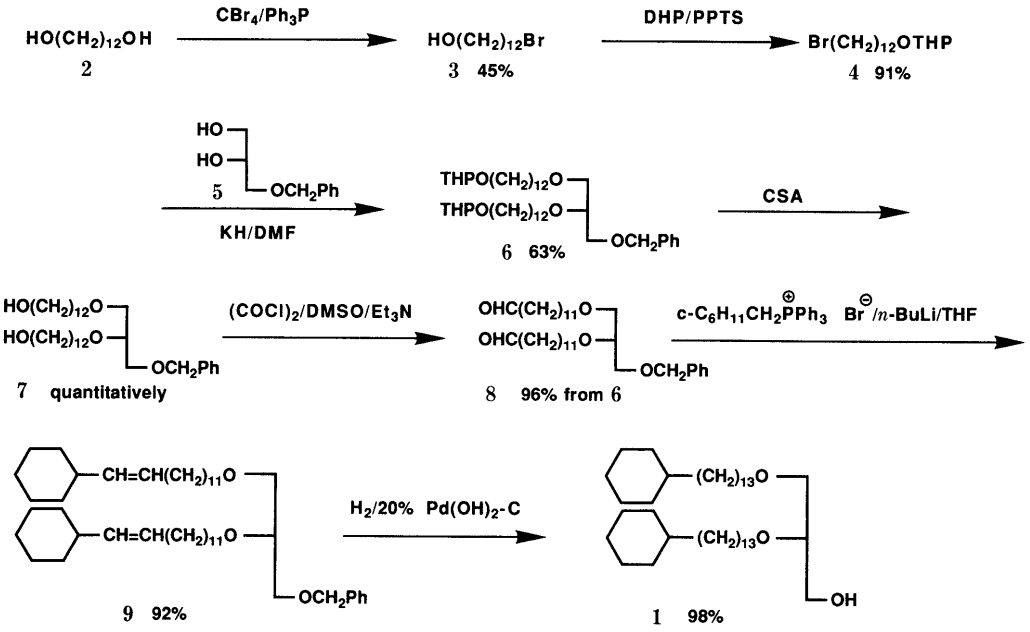


Chart 2

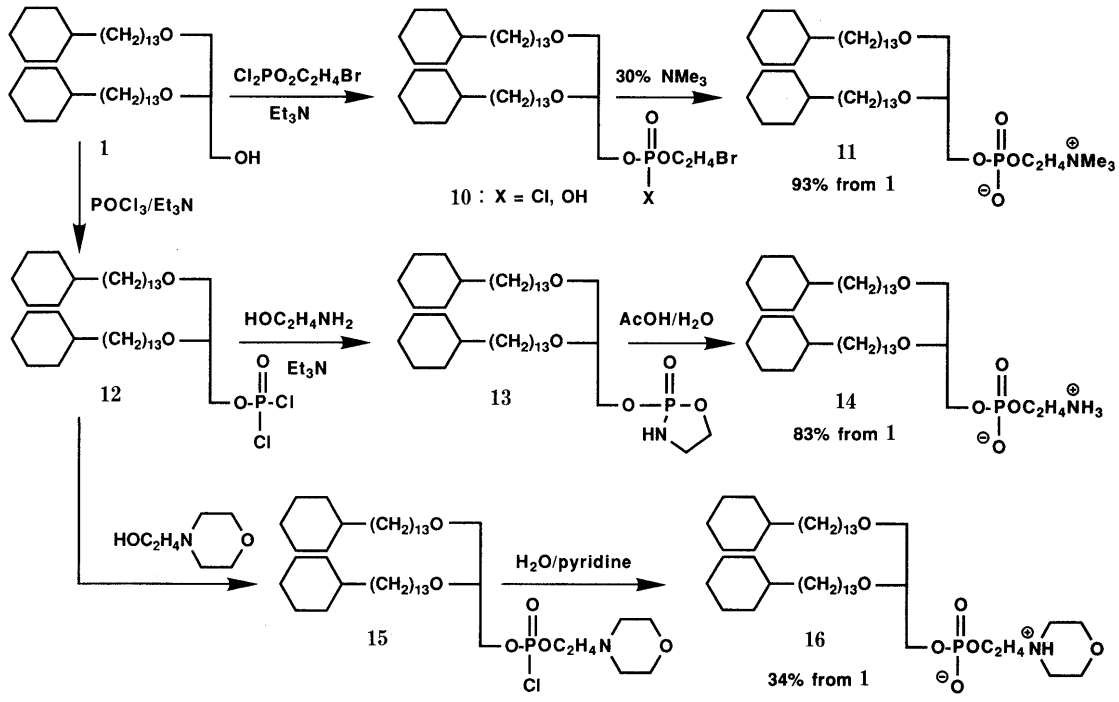


Chart 3

the desired alcohol **1** in 98% yield.

ii) Syntheses of Phospholipid Analogues A mixture of **1**, Et_3N and 2-bromoethyl phosphoryl dichloride[3b] was stirred to give an intermediate **10**, which was treated with 30% aqueous NMe_3 to provide *rac*-1,2-di-*O*-ω-cyclohexyltridecyl glycero-3-phosphonocholine ($P_{CY}2NM3$)[5] **11** in 93% overall yield from **1**. Treatment of **1** with $POCl_3$ in the presence of NEt_3 gave a crude *rac*-1,2-di-*O*-ω-cyclohexyl glycero-3-phosphoric acid dichloride **12**, which was treated with ethanolamine and NEt_3 to afford an intermediate **13**. Hydrolysis of **13** with aqueous AcOH produced *rac*-1,2-*O*-ω-cyclohexyltridecyl glycero-3-phosphonoxyethylamine (inner salt; $P_{CY}2$[5] **14**; 83% overall yield from **1**). Crude **12** was treated with *N*-(2-hydroxyethyl)morpholine to provide an intermediate **15**, which was hydrolyzed with aqueous pyridine to produce *rac*-1,2-di-*O*-ω-cyclohexyltridecyl glycero-3-phosphonoxy ethyl morpholium (inner salt; $P_{CY}2MO$[5] **16**; 34% overall yield from **1**).

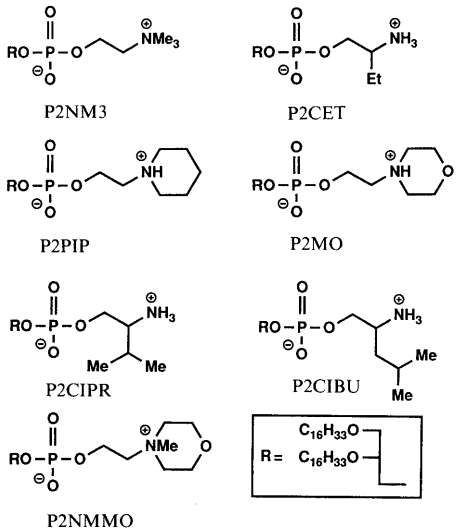
Preparation of Lipid–Lipase Aggregates Bearing *O*-ω-Cyclohexyltridecyl or *O*-Hexadecyl Groups A mixture of 100 mg of lipase OF-360 from *Candida cylindracea* in water (5 ml) and 50 mg of **11** in benzene (40 ml) was sonicated for 30 min at 0 °C. The resulting precipitate was centrifugated at 3000 × *g* and the solvent was decanted off. The residual precipitate was dried under reduced pressure to provide dry aggregate. The aggregates of OF-360 and **14** or **16** were

prepared similarly. From previously reported phospholipid analogues[3b] (*rac*-1,2-di-*O*-hexadecyl glycero-3-phosphonocholine (P2NM3), *rac*-1,2-di-*O*-hexadecyl glycero-3-phosphonoxy 2′-ethylethylamine (inner salt; P2CET), *rac*-1,2-di-*O*-hexadecyl glycero-3-phosphonoxy 2′-isopropylethylamine (inner salt; P2CIPR), *rac*-1,2-di-*O*-hexadecyl glycero-3-phosphonoxy 2′-isobutylethylamine (inner slat; P2CIBU), *rac*-1,2-di-*O*-hexadecyl glycero-3-phosphonoxy ethyl piperidium (inner salt; P2PIP), *rac*-1,2-di-*O*-hexadecyl glycero-3-phosphonoxy ethyl morpholium (inner slat; P2MO) and *rac*-1,2-di-*O*-hexadecyl glycero-3-phosphonoxy ethyl *N*-methyl morpholium (inner salt; P2NMMO), the corresponding aggregates were obtained. The results are summarized in Table I.

Enantioselective Acetylation by Using Lipid–Lipase Aggregates In order to investigate enantioselective acetylation of α-hydroxy ester by using lipid–lipase aggregates, (±)-indolmycenic ester **17** was chosen as a suitable substrate because its optically active congener, the (2*S*,3*R*)-α-acetoxy ester **18**, is an important chiral intermediate for the synthesis of the pharmacologically and optically active indolmycin **19**.[6]

Table I. Yields of Various Kinds of Dry Aggregate

OF-360/P_{CY}2NM3	(32 mg)	OF-360/P_{CY}2	(35 mg)
OF-360/P_{CY}2MO	(39 mg)	OF-360/P2NM3[3b]	(72 mg)
OF-360/P2CET[3b]	(72 mg)	OF-360/P2CIPR[3b]	(45 mg)
OF-360/P2CIBU[3b]	(64 mg)	OF-360/P2PIP[3b]	(69 mg)
OF-360/P2MO[3b]	(67 mg)	OF-360/P2NMMO[3b]	(49 mg)



In a preliminary experiment, it was found that a 1 : 1 mixture of two racemates ((±)-**17** and (±)-**18**) was well separated by high-performance liquid chromatographic (HPLC) analysis with a chiral column (Chiracel OD (4.6 × 250 mm)). By using this method, it has become feasible to determine the chemical and optical yields of each reaction product. For the purpose of finding a suitable acetylating reagent, a screening experiment using lipase OF-360 itself was carried out in a mixed solvent (iso-Pr_2O : cyclohexane = 1 : 19) and the results are given in Table II. Table II shows that isopropenyl acetate and phenyl thioacetate were effective acetyl donors.

The catalytic activity of lipid–lipase aggregates was investigated in the enantioselective acetylation of (±)-**17** using isopropenyl acetate or phenyl thioacetate in the mixed solvent mentioned above. Selected data from the enzymatic reaction are given in Table III.

The absolute structure of enzymatic reaction products was determined by comparison with authentic specimens.[6a] From the results, chemical and optical yields of the acetate, (2*S*,3*R*)-**18**, were found to be governed by the aggregates used. In the reaction of 10 mg of substrate (±)-**17** under the standard incubation conditions, the best result (chemical yield; 15.5%, optical purity; >99% ee) was obtained in entry 6. The influence of the ω-cyclohexylytridecyl group as the lipophilic portion of B on the chemical yield (%) and optical purity (% ee) was found to be essentially the same as that of the hexadecyl group as the lipophilic part of A. Chemical and optical yields were found to be governed mainly by the structure of the hydrophilic part of the lipid. Namely, when the structure of the hydrophilic part of the lipid in the aggregates was the same, similar data were obtained (entries 3 and 7, 6 and 15). In the reaction of 100 mg of substrate under stirring (Table IV), the chemical yield was increased without decrease of the optical purity, but the reason for this is not clear.

Further investigations aimed at finding simple synthetic

Table II. Screening Experiments for Finding a Suitable Acylating Reagent

Entry	Acylating reagent (mg)	Product (Yield (%))	(Optical purity (% ee))
1	$AcOCH=CH_2$ (20)	(2*S*,3*R*)-**18** (4.0) (>99)	(2*R*,3*S*)-**17** (83.0) (5)
2	$AcOC(Me)=CH_2$ (20)	(2*S*,3*R*)-**18** (8.2) (>99)	(2*R*,3*S*)-**17** (83.7) (10)
3	$AcOC_6H_4NO_2$-*p* (10)	(2*S*,3*R*)-**18** (1.8) (>99)	(2*R*,3*S*)-**17** (85.7) (2)
4	$AcOC_6H_4NO_2$-*m* (20)	(2*S*,3*R*)-**18** (1.2) (>99)	(2*R*,3*S*)-**17** (87.3) (3)
5	$AcOC_6H_4NO_2$-*o* (20)	(2*S*,3*R*)-**18** (7.6) (>99)	(2*R*,3*S*)-**17** (78.5) (9)
6	$AcOCH_2CCl_3$ (20)	(2*S*,3*R*)-**18** (2.9) (>99)	(2*R*,3*S*)-**17** (86.9) (4)
7	$AcSC_6H_5$ (20)	(2*S*,3*R*)-**18** (8.3) (>99)	(2*R*,3*S*)-**17** (82.2) (11)

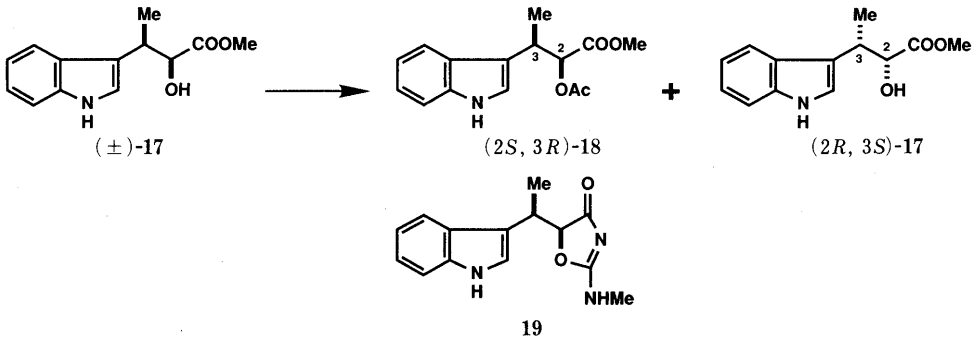


Chart 4

TABLE III. Enantioselective Acetylation Using 10 mg of (±)-**17** under Incubation

Entry	Aggregate	Acylating reagent (mg)	Product (Yield (%))	(Optical purity (% ee))
1	OF-360/$P_{CY}2$	A (30)	(2*S*,3*R*)-**18** (3.5) (>99)	(2*R*,3*S*)-**17** (95.8) (3)
2	OF-360/$P_{CY}2$	B (50)	(2*S*,3*R*)-**18** (3.4) (>99)	(2*R*,3*S*)-**17** (93.7) (4)
3	OF-360/$P_{CY}2NM3$	A (30)	(2*S*,3*R*)-**18** (4.0) (97)	(2*R*,3*S*)-**17** (94.2) (6)
4	OF-360/$P_{CY}2NM3$	B (50)	(2*S*,3*R*)-**18** (6.5) (>99)	(2*R*,3*S*)-**17** (87.1) (7)
5	OF-360/$P_{CY}2MO$	A (30)	(2*S*,3*R*)-**18** (6.0) (>99)	(2*R*,3*S*)-**17** (93.5) (6)
6	OF-360/$P_{CY}2MO$	B (50)	(2*S*,3*R*)-**18** (15.5) (>99)	(2*R*,3*S*)-**17** (83.4) (17)
7	OF-360/P2NM3	A (30)	(2*S*,3*R*)-**18** (3.7) (>99)	(2*R*,3*S*)-**17** (96.1) (4)
8	OF-360/P2NM3	B (50)	(2*S*,3*R*)-**18** (3.4) (>99)	(2*R*,3*S*)-**17** (96.1) (4)
9	OF-360/P2CET	A (30)	(2*S*,3*R*)-**18** (13.6) (>99)	(2*R*,3*S*)-**17** (86.0) (16)
10	OF-360/P2CET	B (50)	(2*S*,3*R*)-**18** (11.0) (>99)	(2*R*,3*S*)-**17** (88.7) (12)
11	OF-360/P2CIPR	A (30)	(2*S*,3*R*)-**18** (7.2) (>99)	(2*R*,3*S*)-**17** (92.5) (8)
12	OF-360/P2CIPR	B (50)	(2*S*,3*R*)-**18** (8.3) (>99)	(2*R*,3*S*)-**17** (91.6) (9)
13	OF-360/P2PIP	A (30)	(2*S*,3*R*)-**18** (12.2) (>99)	(2*R*,3*S*)-**17** (87.0) (13)
14	OF-360/P2PIP	B (50)	(2*S*,3*R*)-**18** (9.1) (>99)	(2*R*,3*S*)-**17** (89.9) (9)
15	OF-360/P2MO	B (50)	(2*S*,3*R*)-**18** (15.4) (97)	(2*R*,3*S*)-**17** (79.0) (21)
16	OF-360/P2NMMO	A (30)	(2*S*,3*R*)-**18** (9.0) (>99)	(2*R*,3*S*)-**17** (90.0) (10)
17	OF-360/P2NMMO	B (50)	(2*S*,3*R*)-**18** (7.7) (>99)	(2*R*,3*S*)-**17** (91.6) (9)

A: $AcOC(Me)=CH_2$, B: $AcSC_6H_5$.

TABLE IV. Enantioselective Acetylation Using 100 mg of (±)-**17** and Isopropenyl Acetate (100 mg) under Stirring

Entry	Aggregate (mg)	Product (Yield (%))	(Optical purity (% ee))
1	OF-360/P2CET (139)	(2*S*,3*R*)-**18** (23.9) (98)	(2*R*,3*S*)-**17** (69.9) (40)
2	OF-360/P2PIP (89)	(2*S*,3*R*)-**18** (28.3) (98)	(2*R*,3*S*)-**17** (70.9) (38)
3	OF-360/P2MO (149)	(2*S*,3*R*)-**18** (32.1) (98)	(2*R*,3*S*)-**17** (67.4) (46)

methods for amphiphiles other than phospholipids and applying artificially prepared aggregates in enzymatic reactions are in progress.

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-4000 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. FAB-MS were obtained with a JEOL JMS-HS 100 instrument. Optical rotations were measured on a Perkin-Elmer model 241 MC polarimeter. The 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.

The Synthesis of Phospholipid Analogues. (1) Synthesis of *rac*-1,2-Di-*O*-ω-cyclohexyltridecyl Glycerol 1 i) A solution of 1,12-dodecanediol **2** (20.6 g), CBr_4 (35.5 g) and Ph_3P (28 g) in dry tetrahydrofuran (THF, 200 ml) was stirred for 30 min at room temperature. The solution was concentrated to one-third of its original volume, and the residue was diluted with ether. The resulting precipitate was filtered off and the filtrate was washed with brine and dried over $MgSO_4$. Removal of the solvent gave an oily product, which was chromatographed on silica gel (250 g) to provide a homogeneous oil **3** (12.2 g; 45% yield) from the *n*-hexane–AcOEt (4 : 1, v/v) eluate. ii) A mixture of **3** (12.2 g) and dihydropyran (4.7 g) in CH_2Cl_2 (50 ml) in the presence of PPTS (1 g) was stirred for 12 h at room temperature. The reaction mixture was diluted with ether. The ether layer was washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. Removal of the solvent gave an oily product which was chromatographed on silica gel (250 g) to give a homogeneous oil **4** (14.6 g; 91% yield) from the *n*-hexane–AcOEt (10 : 1, v/v) eluate. **4**: EI-MS *m/z*: 349 ($M^+ + 1$). iii) A solution of KH (35% in mineral oil; 2.9 g) in DMF (5 ml) was added to a solution of **5**[3b] (2.09 g) in DMF (30 ml) with stirring at 0 °C. After the generation of hydrogen gas ceased, a solution of **4** (8.02 g) in DMF (15 ml) was added dropwise to the above-mentioned solution and the whole was stirred for 6 h at room temperature. The reaction mixture was diluted with water and extracted with benzene. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave an oily product, which was chromatographed on silica gel (200 g) to provide a homogeneous oil **6** (5.19 g, 63% yield) from the *n*-hexane–AcOEt (20 : 1, v/v) eluate. **6**: EI-MS *m/z*: 718 (M^+). NMR δ: 4.55 (2H, s, $\underline{OCH_2}Ph$). iv) A mixture of **6** (4.48 g) and CSA (100 mg) in a mixed solvent (MeOH (25 ml)–CH_2Cl_2 (25 ml)) was stirred for 5 h at room temperature. Then 7% aqueous $NaHCO_3$ (6 ml) was added, and the reaction mixture was stirred for 10 min then diluted with CH_2Cl_2 (100 ml). The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to give a crude mixture, which was chromatographed on silica gel (80 g) to afford a solid **7** (3.43 g; quantitative yield) from the *n*-hexane–AcOEt (3 : 1—1 : 1) eluate. **7**: EI-MS *m/z*: 551 ($M^+ + 1$). IR ($CHCl_3$): 3600 cm^{-1}. NMR δ: 4.56 (2H, s, $\underline{OCH_2}Ph$). v) Oxalyl chloride (1.36 ml) was added to a solution of dimethylsulfoxide (DMSO) (2.44 g) in CH_2Cl_2 (30 ml) under dry ice–acetone cooling (−78 °C) and the reaction mixture was stirred for 20 min at the same temperature. A solution of **7** (3.43 g) in CH_2Cl_2 (10 ml) was slowly added to the above reaction mixture and the reaction mixture was stirred for 1 h at −78 °C. Finally, Et_3N (6.96 ml) was added to the reaction mixture at the same temperature and the whole reaction mixture was stirred for 1 h. The reaction mixture was diluted with water (50 ml) and CH_2Cl_2 (100 ml). The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was chromatographed on silica gel (150 g) to provide a homogeneous oil **8** (3.28 g, 96% yield) from the *n*-hexane–AcOEt (10 : 1, v/v) eluate. **8**: NMR δ: 4.55 (2H, s, $\underline{OCH_2}Ph$), 9.76 (2H, t, $J=2$ Hz, CHO). vi) *n*-BuLi/*n*-hexane solution (1.55 M; 15 ml) was added to a solution of cyclohexylmethyl phosphonium bromide[7] (10.1 g) in dry THF (120 ml) with stirring at 0 °C. The mixture was stirred for 20 min, then a solution of **8** (5.7 g) in dry THF (10 ml) was added. The reaction mixture was stirred for 30 min at 0 °C, then diluted with water and ether. The organic layer was washed with brine and dried over $MgSO_4$. Evaporation of the solvent gave a crude product, which was chromatographed on silica gel (200 g) to provide an oily product **9** (6.78 g, 92% yield) from the *n*-hexane–AcOEt (30 : 1, v/v) eluate. **9**: EI-MS *m/z*: 706 (M^+). NMR δ: 5.16—5.34 (4H, m, olefinic-H). vii) A mixture of **9** (6.85 g) and 20% $Pd(OH)_2$–C (600 mg) in AcOEt (40 ml) was subjected to catalytic hydrogenation (H_2 atm; 4 kg/cm^2) and the reaction mixture was filtered with the aid of Celite. The filtrate was evaporated to give a crude product, which was chromatographed on silica gel (100 g) to provide a homogeneous oil **1** (5.88 g, 98% yield) from the *n*-hexane–AcOEt (19 : 1, v/v) eluate. **1**: *Anal.* Calcd for $C_{41}H_{80}O_3$: C, 79.29; H, 12.98. Found: C, 79.24; H, 13.07. IR ($CHCl_3$): 3580 cm^{-1}. NMR: No characteristic signals.

(2) Synthesis of Phospholipid Analogues i) A solution of 2-bromoethyl phosphoryl dichloride[3b] (59 mg) in toluene (5 ml) was added to a mixture of **1** (100 mg) and Et_3N (33 mg) in toluene (5 ml) with stirring at 0 °C and the reaction mixture was stirred for 3 d at room temperature, then filtered. The filtrate was concentrated to provide a residue, which was chromatographed on silica gel (50 g) to give **10** (159 mg) from the $CHCl_3$–MeOH–H_2O (45 : 15 : 1, v/v) eluate. A mixture of **10**, 30% aqueous NMe_3 (2.5 ml), $CHCl_3$ (2 ml), CH_3CN (2 ml) and iso-PrOH (2 ml) was

stirred at 70 °C for 12 h in a screw-top pressure tube. The precipitated salt was filtered off and washed with $CHCl_3$. The filtrate and washing were combined and evaporated to give a crude product, which was chromatographed on silica gel (50 g) to provide crude P_{CY}2NM3 (134 mg) from the $CHCl_3$–MeOH–H_2O (65 : 35 : 5, v/v) eluate. The crude P_{CY}2NM3 was re-chromatographed on Sephadex LH-20 (750 ml) to yield P_{CY}2NM3 (118 mg, 93% overall yield from **1**) as a colorless amorphous powder from the $CHCl_3$–MeOH (2 : 3, v/v) eluate. P_{CY}2NM3: mp 195—197 °C. *Anal.* Calcd for $C_{46}H_{92}NO_6P \cdot 3/2H_2O$: C, 67.94; H, 11.78; N, 1.71. Found: C, 67.91; H, 11.69; N, 1.78. FAB-MS *m/z*: 786 ($M^+ + 1$). NMR δ: 3.44 (9H, s, NMe_3).

ii) A mixture of **1** (110 mg) and Et_3N (26 mg) in toluene (2 ml) was added to a solution of $POCl_3$ (38 mg) in toluene (1 ml) with stirring at 0 °C. Stirring was continued for 1 h at room temperature, then the precipitated salt was filtered off and washed with toluene. The filtrate and washing were combined and evaporated. A mixture of the resulting residue **12** and Et_3N (100 mg) in toluene (3 ml) was added to a solution of ethanolamine (32 mg) in toluene (1 ml) with stirring at 0 °C. Stirring was continued for 30 min at room temperature, then the precipitated salt was filtered off and washed with toluene. The filtrate and washing were combined and evaporated to give a crude product **13**, which was stirred for 2 h at room temperature after addition of 50% aqueous AcOH (4 ml). The reaction mixture was diluted with toluene and evaporated to give a crude product, which was chromatographed on silica gel (50 g) to provide crude P_{CY}2 **14** (127 mg) from the $CHCl_3$–MeOH–H_2O (65 : 35 : 5, v/v) eluate. The crude **14** was re-chromatographed on Sephadex LH-20 (750 ml), and the $CHCl_3$–MeOH (2 : 3, v/v) eluate afforded P_{CY}2 **14** (110 mg, 83% overall yield from **1**) as a colorless amorhpous powder. P_{CY}2 **14**: mp 154.5—156 °C. *Anal.* Calcd for $C_{43}H_{86}NO_6P \cdot H_2O$: C, 67.76; H, 11.64; N, 1.84. Found: C, 67.84; H, 11.40; N, 1.85. FAB-MS *m/z*: 744 ($M^+ + 1$). NMR: No chracteristic signals.

iii) A solution of *N*-(2-hydroxyethyl)morpholine (0.3 g) in toluene (5 ml) was added to a solution of the crude **12** obtained from **1** (1 g) by the above-mentioned method ii) in toluene (10 ml) at 0 °C, and the reaction mixture was stirred for 1 h at room temperature. The resulting salt was filtered off and washed with toluene. The filtrate and washing were combined and evaporated to give a crude product **15**, which was refluxed for 1 h in H_2O (1 ml) and pyridine (3 ml). The reaction mixture was diluted with a mixed solvent (toluene : MeOH = 5 : 1, v/v) and evaporated to give a residue, which was chromatographed on silica gel (100 g). The $CHCl_3$–MeOH–H_2O (45 : 15 : 1, v/v) eluate afforded crude P_{CY}2MO **16**. The crude P_{CY}2MO **16** was re-chromatographed on Sephadex LH-20 (750 ml) and the $CHCl_3$–MeOH (2 : 3, v/v) eluate gave P_{CY}2MO **16** (450 mg, 34% overall yield from **1**) as a colorless amorphous powder. P_{CY}2MO: mp 140—142 °C. *Anal.* Calcd for $C_{47}H_{92}NO_7P \cdot 1/2H_2O$: C, 68.57; H, 11.39; N, 1.70. Found: C, 68.44; H, 11.36; N, 1.63. FAB-MS *m/z*: 814 ($M^+ + 1$). NMR: No characteristic signals.

Preparation of Lipid–Lipase Aggregate The procedure was described in the text.

HPLC Analysis of the Two Racemates ((±)-17 and (±)-18) by Using a Chiral Column A 1 : 1 mixture of the two racemates ((±)-**17** and (±)-**18**) gave four well separated peaks ((±)-**17**; 29.9, 34.2 min, (±)-**18**; 15.3, 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 an authentic sample[6a,b] ((2*S*,3*R*)-**17** and (2*R*,3*S*)-**18**). Namely, the peak with shorter retention time (t_R = 15.3 min) was found to correspond to that of the (2*R*,3*S*)-**18** enantiomer and the peak with longer retention time (t_R = 34.2 min) to that of the (2*S*,3*R*)-**17** enantiomer. (2*S*,3*R*)-**17**: *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: 3500, 1736 cm^{-1}. NMR δ: 1.326 (3H, d, *J* = 7.1 Hz, 3-Me), 2.755 (1H, d, *J* = 5.1 Hz, 2-OH), 3.636 (1H, dq, *J* = 3.2, 7.1 Hz, 3-H), 3.788 (3H, s, COOMe), 4.509 (1H, dd, *J* = 3.2, 5.1 Hz, 2-H). (2*R*,3*S*)-**18**: *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. IR: 1756, 3530 cm^{-1}. NMR δ: 1.453 (3H, d, *J* = 7.3 Hz, 3-Me), 2.097 (3H, s, 2-OAc), 3.644 (3H, s, COOMe), 3.733 (1H, dq, *J* = 4.6, 7.3 Hz, 3-H), 5.231 (1H, d, *J* = 4.6 Hz, 2-H).

Screening Experiment for Finding Suitable Acetylating Reagent A mixture of (±)-**17** (10 mg), acetylating reagent (10 or 20 mg) and lipase OF-360 from *Candida cylindracea* (10 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 provide a crude mixture, which was analyzed by HPLC. The results are given in Table II.

General Procedure of Enantioselective Acetylation i) Using 10 mg of Substrate (±)-**17**: A mixture of (±)-**17** (10 mg), acetylating reagent (see Table III) and lipid–lipase aggregate (10 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 III.

ii) Using 100 mg of Substrate (±)-**17**: A mixture of (±)-**17** (100 mg), isopropenyl acetate (300 mg), and lipid–lipase aggregate (see Table IV) in a mixed solvent (iso-Pr_2O (2.5 ml) and cyclohexane (47.5 ml)) was stirred at 33 °C for 3 d. The reaction mixture was dried over $MgSO_4$ and evaporated to afford a crude product, which was subjected to silica gel (30 g) column chromatography. The fraction with *n*-hexane–AcOEt (9 : 1, v/v) gave (2*S*,3*R*)-**18**. The second fraction eluted with *n*-hexane–AcOEt (7 : 1, v/v) provided (2*R*,3*S*)-**17**. Both fractions were analyzed by HPLC and the results are given in Table IV.

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5) Abbreviations of the synthetic phospholipid are the same as in the previous paper.[3b] The abbreviation P_{CY} indicates the presence of cyclohexyl moieties in the lipophilic part of the phospholipid, as used previously.[4]
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7) A mixture of cyclohexylmethyl bromide (17.7 g) and Ph_3P (26.3 g) in toluene (100 ml) was refluxed with stirring for 3 d. The reaction mixture was concentrated to one-third of its original volume and the resulting precipitate was filtered off and washed with hexane to provide colorless crystals (34.5 g; 79% yield).