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Asymmetric Synthesis of (+)- and (−)-Batyl Alcohol, a Key Synthetic Intermediate for Platelet-Activating Factor, by Using Biocatalysts

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Asymmetric synthesis of platelet-activating factor (PAF) and its enantiomer by using biocatalysts was studied. Microbial reduction of the pro-chiral α-ketoester (**3**) afforded (+)-**4** (>99% ee), which could be converted to (+)- and (−)-batyl alcohol (**12**), a key synthetic intermediate for PAF. Compound (−)-**4** (96% ee), with the requisite configuration for the synthesis of natural PAF, could also be obtained by enzyme-catalyzed enantioselective hydrolysis of (±)-**15**.

Keywords——platelet-activating factor; batyl alcohol; asymmetric reduction; microbial reduction; enantioselective hydrolysis; enzymic hydrolysis; kinetic resolution

Platelet-activating factor (PAF, **1**) was first discovered as a stimulator of rabbit platelets, and its structure was shown by Hanahan *et al.*[1] to be 1-*O*-hexadecyl (or octadecyl)-2-acetyl-*sn*-glycero-3-phosphorylcholine. PAF is able to provoke platelet and neutrophil activation, hypotension and broncho-constriction.[2-4]

As a part of our attempts to develop a simple synthetic route to PAF, we previously reported the asymmetric synthesis[5,6] of PAF intermediates by the (*S*)-BINAL-H[7] reduction of octadecyloxymethyl (*E*)-2-cyclohexylvinyl ketone and by enzyme-catalyzed hydrolysis of a *meso* compound, 1,3-di-*O*-acetyl-2-*O*-benzylglycerol. However, in each case, the optical purity (40—80% ee) was unsatisfactory.

Therefore, we designed a new chiral synthon ((*S*)-**4**) for natural PAF as shown in Chart 1. This sequence starts with the synthesis of methyl 2-oxo-4,4-propylenedithiopentanoate (**3**), followed by microbial reduction. Regioselective protection of the ketone function in

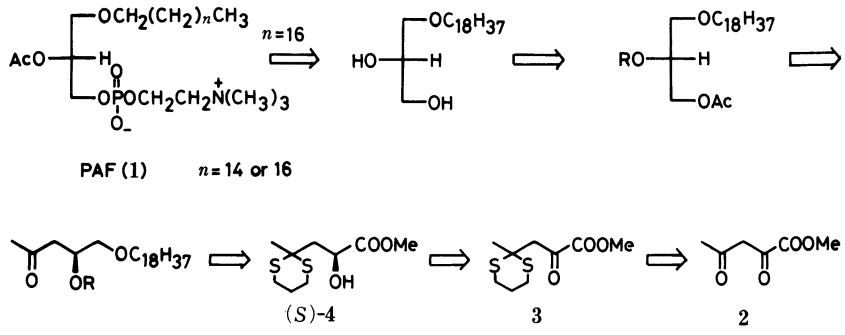
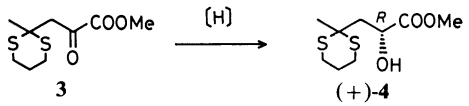


Chart 1

TABLE I. Asymmetric Reduction of 3 with Yeast



3 (+)-4

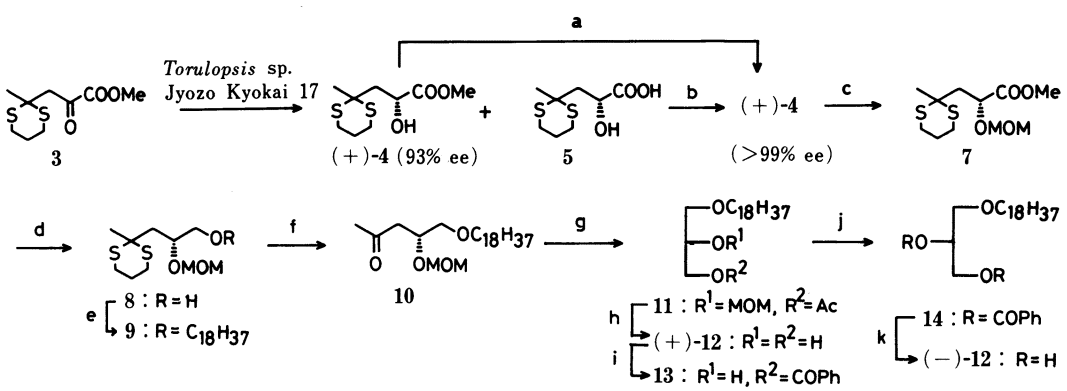
Run	Microorganism	Chemical yield as (+)-MTPA ester (%)	Optical purity (% ee)	Absolute configuration
1	*Hansenula anomala* NI-7572	15	64	*R*
2	*Pichia membranaefaciens*	34	32	*S*
3	*Saccharomyces acidifaciens*	31	50	*R*
4	*Saccharomyces delbrueckii*	33	49	*R*
5	*Saccharomyces fermentati* IFO-0422	37	88	*R*
6	*Saccharomyces tokyo* Jyozo Kyokai 2013	41	85	*R*
7	*Schizosaccharomyces octosporus*	41	23	*R*
8	*Torulopsis famata* NI-7550	19	81	*R*
9	*Torulopsis* sp. Jyozo Kyokai 17	15	>99	*R*
10	*Saccharomyces cerevisiae* Kitasato Inst.	22	89	*R*
11	*Trichosporon fermentans* IFO-1199	34	71	*R*
12	*Candida utilis* IFO-0619	25	80	*R*
13	*Saccharomyces cerevisiae*[a] (baker's yeast)	80[b]	64	*R*

a) The reaction was performed on a preparative scale (500 mg of **3**). *b*) Isolated yield of (+)-**4**.

methyl 2,4-dioxopentanoate (**2**) was performed by treatment with 1,3-propanedithiol–BF_3 etherate in CH_2Cl_2 to afford the monoacetal (**3**) in 61% yield.

Thin layer chromatographic screening of forty strains of yeast[8] for ability to reduce **3** indicated that thirteen strains afforded the hydroxy ester (**4**). Secondary screening on a larger scale (30 mg of substrate) was performed using these strains, and the results are summarized in Table I.

The enantiomeric excess (ee) of the reduction products was determined from the 400 MHz proton nuclear magnetic resonance (^{1}H-NMR) spectra after conversion to the (+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA)[9] esters by treatment with (+)-MTPA chloride. The absolute stereochemistry was finally determined by conversion into optically active batyl alcohol ((+)-**12**).



a) enhancement of optical purity b) CH_2N_2 c) MOMCl, $(iso\text{-}Pr)_2NEt$ d) $LiAlH_4$
e) $C_{18}H_{37}OMs$, KH f) $Tl(NO_3)_3$ g) CF_3CO_3H, Na_2HPO_4 h) aq. HCl, MeOH
i) PhCOCl, Py j) EtOOCN=NCOOEt, Ph_3P, PhCOOH k) NaOH, MeOH

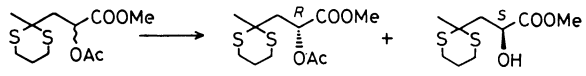
Chart 2

In most cases, (+)-**4**, which was finally found to have *R* configuration, was predominantly obtained except for run 2 (*Pichia membranaefaciens*) in Table I. This result is in harmony with that of microbial reduction of other α-ketoesters.[10] Among the tested yeasts in Table I, *Torulopsis* sp. Jyozo Kyokai 17 (run 9 in Table I) afforded optically pure (+)-**4** (>99% ee). A large-scale reaction (substrate **3**, 1.0 g × 13) using this strain of yeast afforded (+)-**4** (40% yield, 93% ee) and the hydroxyacid (**5**), which was purified after esterification with CH_2N_2 to afford (+)-**4** (33% yield from **3**, >99% ee). The optical purity of (+)-**4** (93% ee) was increased to >99% ee by a single recrystallization of the corresponding 3,5-dinitrobenzoate (**6**) from MeOH.

Optically pure (+)-**4** obtained in this manner was used as a starting material for the synthesis of PAF. The alcohol function in (+)-**4** was protected as the methoxymethyl (MOM) ether by treatment with MOM chloride–*N*,*N*-diisopropylethylamine, and subsequent reduction with $LiAlH_4$ yielded the alcohol (**8**) in 88% yield from (+)-**4**. The octadecyl ether function in **9** was introduced in 78% yield by the reaction with $C_{18}H_{37}OMs$ in the presence of KH.[11] Compound **9** has three different functional groups: dithioacetal, MOM ether and alkyl ether. The selective deprotection of the dithioacetal function in **9** was achieved by treatment with thallium(III) trinitrate to afford the ketone (**10**) in 90% yield. Subsequent Baeyer–Villiger oxidation of **10** with CF_3CO_3H–Na_2HPO_4 yielded the acetate (**11**) in 69% yield, although oxidation with *m*-chloroperbenzoic acid did not proceed at all. Concurrent hydrolysis of the MOM ether and acetate in **11** with HCl–MeOH afforded (+)-batyl alcohol ((+)-**12**) ($[\alpha]_D^{25}$ +2.41° (*c*=2.42, tetrahydrofuran (THF)); reported value $[\alpha]_D$ +2.28° (*c*=3.5, THF)).[12] Based on the sign of the specific rotation of (+)-**12**, the absolute stereochemistry of the starting material (+)-**4** was determined to be *R*.

(−)-Batyl alcohol, required for the synthesis of natural PAF, was synthesized as follows. After protection of the primary alcohol in (+)-**12** as the benzoate (**13**), inversion of the secondary alcohol was performed by the Mitsunobu method[13] to afford the dibenzoate (**14**) in 78% yield from (+)-**12**. Methanolysis of **14** gave (−)-**12**[14] in 90% yield. The optical

TABLE II. Enzyme-Catalyzed Hydrolysis of (±)-**15**



(±)-**15** (+)-**15** (−)-**4**

Run	Lipase	(+)-**15**		(−)-**4**	
		Chemical yield (%)	Optical purity (% ee)[a]	Chemical yield (%)	Optical purity (% ee)[a]
1	*Candida cylindracea* Meito "OF-360"	17	33	43	12
2	*Candida cylindracea* Meito "MY-30"	56	20	24	25
3	*Candida cylindracea* Sigma	50	18	30	22
4	*Pseudomonas fluorescens* Amano "P"	41	97	26	96
5	Porcine pancreas Amano	3	36	28	87
6	*Aspergillus niger* Amano "A"	18	14	60	0
7	*Aspergillus niger* Amano "A-6"	4	0	71	16[b]
8	*Rhizopus niveus* Amano "F"	53	58	17	92
9	*Rhizopus japonicus* Saiken "Lilipase A-10"	62	28	14	92
10	*Rhizopus japonicus* Nagase	56	19	11	61
11	*Rhizopus javanicus* Amano "F-AP-15"	42	46	18	90

a) The optical purities of (+)-**15** and (−)-**4** were determined from the 400 MHz ^{1}H-NMR spectra after conversion into the corresponding (+)-MTPA esters. *b*) Compound (+)-**4** was obtained as the hydrolyzed product.

purities of the obtained (+)- and (−)-**12** were confirmed to be >99% ee from the 400 MHz ^{1}H-NMR spectra after conversion into di-(+)-MTPA esters.[15] These findings suggest that racemization did not occur during the synthetic route from (+)-**4** to (+)-**12** and the inversion process from (+)-**12** to (−)-**12**.

In attempts to prepare (−)-**4** as a chiral synthon, inversion of (+)-**4** (>99% ee) using Ikegami's method [i) mesylation, ii) AcOCs–18-crown-6, iii) K_2CO_3][16] afforded partially racemized (−)-**4** (88% ee), and the Mitsunobu method afforded a complex mixture. To develop a synthetic route that would give optically pure (−)-**4**, the racemic acetate ((±)-**15**) derived from **3** was submitted to enzyme-catalyzed enantioselective hydrolysis. Enzymic hydrolyses of (±)-**15** were performed in 0.1 M phosphate buffer solution (pH 7.25) at 33 °C for 48 h, and the results are summarized in Table II.

Among the eleven enzymes tested, lipase "Amano-P" from *Pseudomonas fluorescens* (run 4 in Table II) afforded (+)-**15** (41% yield, 97% ee) as the recovered substrate and (−)-**4** (26% yield, 96% ee) as the hydrolyzed product. Lipase "Amano F" from *Rhizopus niveus* and lipase "Saiken-Lilipase A-10" from *Rhizopus japonicus* (runs 8 and 9 in Table II) also afforded (−)-**4** (17% yield, 92% ee and 14% yield, 92% ee, respectively). This (−)-**4**, formed in high enantiomeric excess, could be converted to (−)-**12** for the synthesis of natural PAF in a manner similar to that described for the sequence from (+)-**4** to (+)-**12**.

This is the first report of the asymmetric synthesis of enantiomerically pure batyl alcohol.

Experimental

Infrared (IR) spectra were measured with a JASCO A-202 spectrometer. ^{1}H-NMR spectra were measured on a JEOL LNP-PS-100 spectrometer unless otherwise stated. Mass spectra (MS) were taken on a JEOL JMS-D 300 spectrometer. Specific rotations were measured on a JASCO DIP-4 polarimeter. For column chromatography, silica gel (Merck, Kieselgel 60, 70—230 mesh) was used. All organic solvent extracts were washed with brine and dried on anhydrous sodium sulfate.

Methyl 2-Oxo-4,4-propylenedithiopentanoate (3)——1,3-Propanedithiol (30 ml, 0.299 mol) in CH_2Cl_2 (20 ml) was added dropwise to a stirred mixture of methyl 2,4-dioxopentanoate (**2**, 35 g, 0.243 mol) and BF_3 etherate (10 ml) in CH_2Cl_2 (60 ml) at 0 °C, and the whole was stirred at room temperature for 23 h, then poured into ice-water (100 ml), and extracted with ether. The ether extract was successively washed with 5% aq. $NaHCO_3$ and brine, then dried. Removal of the solvent *in vacuo* afforded an oily residue, which was subjected to column chromatography on silica gel (300 g). The fraction eluted with 2—5% AcOEt in hexane (v/v) afforded **3** (34.8 g, 61%) as a pale yellow solid, mp 38.9—39.5 °C. IR (Nujol): 1725, 1265, 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.63 (3H, s, CH_3), 3.52 (2H, s, CH_2CO), 3.86 (3H, s, $COOCH_3$). MS *m/z*: 234 (M^+), 147, 133.

Screening of Yeasts——i) Preliminary Screening: The microorganisms in a previous paper[8] were examined for ability to reduce **3**. Test tubes (25 × 200 mm) containing 10 ml of the culture medium (5% glucose, 0.1% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.05% urea, 0.05% $MgSO_4 \cdot 7H_2O$, 0.1% yeast extract and tap water (pH 7.0)) were inoculated with microorganisms and cultured at 30 °C for 3 d with continuous shaking. Then the substrate (*ca.* 5 mg of compound **3**) was added to the test tube, which was further incubated for 3 d under the same conditions. The mixture was extracted with AcOEt. The AcOEt extract was dried and concentrated *in vacuo*. Monitoring of the residue by thin layer chromatography (TLC) indicated that thirteen strains of yeast (listed in Table I) were effective for the reduction of **3**.

ii) Secondary Screening: Reduction with these effective microorganisms using 30—40 mg of **3** in 100 ml of culture was carried out under essentially the same conditions as noted above. In order to examine the stereochemistry and optical purity, the reduction products were converted into (+)-MTPA esters. 400 MHz ^{1}H-NMR ($CDCl_3$) δ: (+)-MTPA ester of *R*-(+)-**4**: 3.756 (3H, s, OCH_3), 3.814 (3H, s, $COOCH_3$); (+)-MTPA ester of *S*-(−)-**4**: 3.532 (3H, s, OCH_3), 3.785 (3H, s, $COOCH_3$).

Asymmetric Reduction of 3 on a Preparative Scale——The above-mentioned seed culture of *Torulopsis* sp. Jyozo Kyokai 17 (1 ml) was transferred to 800 ml of the same culture medium. After cultivation at 30 °C for 3 d, the substrate **3** (1 g) was added to this seed culture, and the cultivation was continued for a further 3 d under the same conditions. Similar reduction of **3** on a preparative scale (1 g × 13) was carried out. The reaction mixture was filtered with the aid of celite and the filtrate was extracted with AcOEt. The AcOEt extract was washed with brine and dried. Removal of the solvent *in vacuo* gave an oily residue, which was chromatographed on silica gel (150 g). The fraction eluted with 10% AcOEt in hexane (v/v) afforded (+)-**4** (5.25 g, 40%, 93% ee). The optical purity of (+)-**4** was enhanced by recrystallization from MeOH after conversion to the corresponding 3,5-dinitrobenzoate (**6**) in the usual manner, followed by methanolysis with K_2CO_3 in MeOH to afford optically pure (+)-**4** (4.2 g, 32% from **3**, >99%

ee) as a colorless oil. The fraction eluted with AcOEt afforded the crude hydroxyacid (**5**), which was purified after conversion to (+)-**4** (4.33 g, 33% from **3**, >99% ee) by treatment with CH_2N_2. (+)-**4**: $[\alpha]_D^{25}$ +8.02° (c=5.12, $CHCl_3$). IR (neat): 3460, 1740, 1375, 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.67 (3H, s, CH_3), 3.81 (3H, s, $COOCH_3$), 4.51 (1H, m, CH). MS m/z: 236 (M^+), 147, 133. **6**: Yellow needles, mp 114.0—114.5 °C, recrystallized from MeOH. $[\alpha]_D^{18}$ +34.4° (c=1.16, $CHCl_3$). IR (Nujol): 1765, 1750, 1548, 1465, 1350 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.69 (3H, s, CH_3), 3.83 (3H, s, $COOCH_3$), 5.70 (1H, dd, J=4, 8 Hz, CH), 9.22 (3H, m, Ar-H). MS m/z: 430 (M^+), 230, 216, 212. **5**: ^{1}H-NMR ($CDCl_3$) δ: 1.62 (1H, s, CH_3), 2.31 (1H, dd, J=9, 15 Hz, C_3-H), 2.66 (1H, dd, J=2, 15 Hz, C_3-H), 4.56 (1H, dd, J=2, 9 Hz, C_2-H), 5.54 (2H, br, COOH, OH).

Methyl (*R*)-2-Methoxymethoxy-4,4-propylenedithiopentanoate (7)——MOM chloride (1.92 g) was added to a stirred solution of (+)-**4** (820 mg) in *N*,*N*-diisopropylethylamine (5.3 g) at 0 °C. The whole was stirred at room temperature for 3 h, poured into 2% aq. HCl, and then extracted with ether. The ether extract was successively washed with 5% aq. $NaHCO_3$ and brine, then dried. The solvent was removed *in vacuo* to afford an oily residue, which was purified by column chromatography on silica gel (24 g). The fraction eluted with 7.5% AcOEt in hexane (v/v) gave **7** (924 mg, 95%) as a pale yellow oil. $[\alpha]_D^{24}$ −1.86° (c=6.48, $CHCl_3$). IR (neat): 1740, 1435, 1365, 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.63 (3H, s, CH_3), 3.41 (3H, s, OCH_3), 3.76 (3H, s, $COOCH_3$), 4.34 (1H, dd, J=4, 8 Hz, CH), 4.68, 4.71 (1H each, d, J=10 Hz, OCH_2O). MS m/z: 280 (M^+), 235, 147.

(*R*)-2-Methoxymethoxy-4,4-propylenedithio-1-pentanol (8)——Compound **7** (915 mg) in ether (10 ml) was added dropwise with stirring to a suspension of $LiAlH_4$ (136 mg) in ether (4 ml) at 0 °C, and the mixture was stirred for 10 min. The usual work-up afforded a crude oil, which was subjected to column chromatography on silica gel (30 g). The fraction eluted with 20—25% AcOEt in hexane (v/v) afforded **8** (767 mg, 93%) as a colorless oil. $[\alpha]_D^{24}$ −69.5° (c=0.965, $CHCl_3$). IR (neat): 3440, 1440, 1375, 1035 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.64 (3H, s, CH_3), 3.34 (1H, br, OH), 3.44 (3H, s, OCH_3), 3.58 (2H, m, CH_2OH), 3.86 (1H, m, CH), 4.72, 4.76 (1H each, d, J=11 Hz, OCH_2O). MS m/z: 252 (M^+), 220, 147.

(*R*)-2-Methoxymethoxy-1-octadecyloxy-4,4-propylenedithiopentane (9)——A dispersion of KH in mineral oil (35% w/v, 1 ml, 10 mmol) was added to a stirred solution of **8** (750 mg) in benzene (10 ml) at 0 °C under an Ar atmosphere. After 10 min, octadecyl methanesulfonate (1.61 g) in benzene (20 ml) was added dropwise, then the whole was refluxed for 15 min. The reaction mixture was diluted with hexane (10 ml), quenched with EtOH (2 ml) and H_2O (5 ml), then extracted with ether. The ether extract was successively washed with 1% aq. HCl, 5% aq. $NaHCO_3$ and brine, then dried. The solvent was removed *in vacuo* to give an oily residue, which was chromatographed on silica gel (25 g). The fraction eluted with 2% AcOEt in hexane (v/v) gave **9** (1.16 g, 78%) as a pale yellow oil. $[\alpha]_D^{23}$ −10.4° (c=4.01, $CHCl_3$). IR (neat): 1460, 1345, 1165 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7 Hz, CH_3), 1.20—1.70 (32H, m, CH_2 × 16), 1.64 (3H, s, CH_3), 3.38 (3H, s, OCH_3), 3.44—3.52 (4H, m, CH_2OCH_2), 3.94 (1H, m, CH), 4.72, 4.80 (1H each, d, J=10 Hz, OCH_2O). MS m/z: 504 (M^+), 442, 133.

(*R*)-4-Methoxymethoxy-5-octadecyloxy-2-pentanone (10)——Thallium (III) trinitrate trihydrate (2.42 g) in MeOH (2 ml) was added at 0 °C to a stirred mixture of **9** (1.15 g), MeOH (6 ml) and ether (2 ml), and the mixture was stirred for 15 min at room temperature. After removal of the resulting precipitate by filtration, the filtrate was concentrated *in vacuo*, diluted with brine, and then extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried and concentrated *in vacuo* to afford an oily residue, which was subjected to column chromatography on silica gel (30 g). The fraction eluted with 7% AcOEt in hexane (v/v) afforded **10** (852 mg, 90%) as a colorless oil. $[\alpha]_D^{24}$ +7.20° (c=4.30, $CHCl_3$). IR (neat): 1720, 1105, 1040 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7 Hz, CH_3), 2.18 (3H, s, $COCH_3$), 2.71 (2H, d, J=7 Hz, $COCH_2$), 3.34 (3H, s, OCH_3), 3.41—3.48 (4H, m, CH_2OCH_2), 4.15 (1H, m, CH), 4.68 (2H, s, OCH_2O). MS m/z: 353 ($M^+ - OCH_2OCH_3$), 310, 131. *Anal.* Calcd for $C_{25}H_{50}O_4$: C, 72.41; H, 12.15. Found: C, 72.59; H, 12.10.

1-*O*-Acetyl-2-*O*-methoxymethyl-3-*O*-octadecyl-*sn*-glycerol (11)——CF_3CO_3H [freshly prepared from $(CF_3CO)_2O$ (42 ml), 60% H_2O_2 (5 ml) and Na_2HPO_4 (74 g) in CH_2Cl_2 (100 ml)] was added dropwise to a well-stirred suspension of **10** (830 mg) and Na_2HPO_4 (12 g) in CH_2Cl_2 (24 ml) at 0 °C. The whole was stirred for 7 h at room temperature, diluted with 5% aq. $NaHCO_3$ and then extracted with CH_2Cl_2. The CH_2Cl_2 extract was successively washed with 2% aq. KI, 5% aq. $Na_2S_2O_3$, and brine, then dried. Removal of the solvent gave an oily residue, which was purified by column chromatography on silica gel (30 g). The fraction eluted with 3% AcOEt in hexane (v/v) afforded **11** (595 mg, 69%) as a colorless oil. $[\alpha]_D^{26}$ +10.61° (c=3.08, $CHCl_3$). IR (neat): 1740, 1235, 1115 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7 Hz, CH_3), 1.20—1.70 (32H, m, CH_2 × 16), 2.08 (3H, s, $OCOCH_3$), 3.39 (3H, s, OCH_3), 3.44—3.54 (4H, m, CH_2OCH_2), 3.96 (1H, m, CH), 4.20 (2H, m, CH_2OCO), 4.72 (2H, s, OCH_2). MS m/z: 431 (M^+ + 1), 399, 385, 369. *Anal.* Calcd for $C_{25}H_{50}O_5$: C, 69.72; H, 11.70. Found: C, 69.77; H, 11.83.

3-*O*-Octadecyl-*sn*-glycerol ((+)-Batyl Alcohol) ((+)-12)——Solution of **11** (313 mg) in MeOH (10 ml) was heated at 50 °C for 3 h in the presence of 35% aq. HCl (0.1 ml). After additon of 5% aq. $NaHCO_3$ (2 ml), the whole was diluted with brine, and extracted with ether. The ether extract was dried and concentrated *in vacuo* to give a colorless solid, which was purified by column chromatography on silica gel (9 g). The fraction eluted with 20% AcOEt in hexane (v/v) gave **12** (225 mg, 90%) as colorless needles, mp 70.8—71.2 °C, recrystallized from AcOEt–hexane. $[\alpha]_D^{25}$ +2.41° (c=2.42, THF). IR (Nujol): 3370, 1460, 1368, 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7 Hz, CH_3), 1.20—1.70 (32H, m, CH_2 × 16), 2.34, 2.73 (1H each, br, OH × 2), 3.39—3.92 (7H, m). MS m/z: 345 (M^+ + 1), 313, 253. High-MS

for $C_{21}H_{44}O_3$ (M^+): Calcd *m/z* 344.32893; Found 344.32841.

1-*O*-Benzoyl-3-*O*-octadecyl-*sn*-glycerol (13)——Benzoyl chloride (64 mg) in CH_2Cl_2 (1 ml) was added to a mixture of (+)-**12** (156 mg) and pyridine (0.1 ml) in CH_2Cl_2 (3 ml) at 0 °C, and the mixture was stirred for 30 min at room temperature. The whole was diluted with 5% aq. HCl and extracted with ether. The ether extract was successively washed with 5% $NaHCO_3$ and brine, then dried. Removal of the solvent *in vacuo* gave a crystalline residue, which was chromatographed on silica gel (2 g). The fraction eluted with 10% AcOEt in hexane (v/v) afforded **13** (148 mg, 73%), mp 48.5—49.5 °C, recrystallized from hexane, and the 2-*O*-benzoate of (+)-**12** (19 mg, 9%) as a colorless solid. Compound (+)-**12** (22 mg, 14%) was also recovered from the eluate with 20% AcOEt–hexane (v/v). **13**: $[\alpha]_D^{25}$ −0.54° (*c*=3.09, $CHCl_3$). IR (Nujol): 3470, 1690, 1600, 1582, 1295 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, *J*=7 Hz, CH_3), 2.60 (1H, br, OH), 3.42—3.58 (4H, m, CH_2OCH_2), 4.14 (1H, m, CH), 4.40 (2H, d, *J*=5 Hz, CH_2OCO), 7.36—7.58 (3H, m, Ar-H), 8.00—8.10 (2H, m, Ar-H). MS *m/z*: 448 (M^+), 375, 325. 2-*O*-Benzoate of (+)-**12**: IR (Nujol): 3470, 1687, 1600, 1581, 1285 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, *J*=7 Hz, CH_3), 3.38 (1H, br, OH), 3.50 (2H, t, *J*=6 Hz, $OCH_2C_{17}H_{35}$), 3.77 (2H, d, *J*=5 Hz, CH_2O-alkyl), 3.94 (2H, d, *J*=5 Hz, CH_2OH), 5.26 (1H, tt, *J*=5, 5 Hz, CH), 7.34—7.70 (3H, m, Ar-H), 8.02—8.15 (2H, m, Ar-H).

2,3-Di-*O*-benzoyl-1-*O*-octadecyl-*sn*-glycerol (14)——Diethyl azodicarboxylate (62 mg) in ether (2 ml) was added to a mixture of **13** (107 mg), triphenylphosphine (95 mg) and benzoic acid (44 mg) in ether (5 ml). The reaction mixture was stirred for 2 h at 0 °C, diluted with ether, and washed with brine, then dried. The solvent was removed *in vacuo* to afford an oily residue, which was purified by column chromatography on silica gel (4 g). The fraction eluted with 2.5% AcOEt in hexane (v/v) afforded **14** (125 mg, 95%) as a colorless oil. $[\alpha]_D^{26}$ −9.57° (*c*=2.16, $CHCl_3$). IR (neat): 1720, 1600, 1260, 1110 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, *J*=7 Hz, CH_3), 3.50 (2H, t, *J*=6 Hz, $OCH_2C_{17}H_{35}$), 3.76 (2H, d, *J*=6 Hz, CH_2O-alkyl), 4.65 (2H, d, *J*=7 Hz, CH_2OCO), 5.60 (1H, m, CH). MS *m/z*: 552 (M^+), 479, 430.

1-*O*-Octadecyl-*sn*-glycerol ((−)-Batyl Alcohol) ((−)-12)——A mixture of **14** (63 mg) and NaOH (34 mg) in MeOH (3 ml) was stirred for 1 h at room temperature. Usual work-up afforded a crystalline residue, which was chromatographed on silica gel (1.5 g). The fraction eluted with 20% AcOEt in hexane (v/v) afforded (−)-**12** (35 mg, 90%), mp 70.8—71.4 °C, recrystallized from AcOEt–hexane. $[\alpha]_D^{29}$ −2.35° (*c*=2.10, THF). High-MS for $C_{21}H_{44}O_3$ (M^+): Calcd *m/z* 344.32893; Found 344.32813.

Di-*O*-(+)-MTPA Esters of (+)- and (−)-12——Compounds (+)- and (−)-**12**[15] were converted into the corresponding di-*O*-(+)-MTPA esters in the usual manner. For determination of the enantiomeric excess, the following signals in the 400 MHz ^{1}H-NMR spectra ($CDCl_3$) were examined. 1,2-Di-*O*-(+)-MTPA ester of (+)-**12**: δ: 3.424, 3.494 (3H each, $OCH_3 \times 2$), 4.367 (1H, dd, *J*=4.88, 12.21 Hz, CH_aH_bOMTPA), 4.623 (1H, dd, *J*=3.18, 12.21 Hz, CH_aH_bOMTPA), 2,3-Di-*O*-(+)-MTPA ester of (−)-**12**: δ: 3.399, 3.482 (3H each, $OCH_3 \times 2$), 4.429 (1H, dd, *J*=6.45, 12.20 Hz, CH_aH_bOMTPA), 4.733 (1H, dd, *J*=2.93, 12.20 Hz, CH_aH_bOMTPA).

Methyl (±)-2-Hydroxy-4-oxopentanoate Propylene 1,3-Dithioacetal ((±)-4)——$NaBH_4$ (626 mg) was added portionwise to a stirred mixture of **3** (3.52 g) and $CeCl_3$ (6.17 g) in MeOH (50 ml) at −78 °C, and the whole was stirred for 1 h at −78 °C. The reaction mixture was quenched with acetone (3 ml) and diluted with brine, then extracted with AcOEt. The AcOEt extract was dried and concentrated *in vacuo* to afford an oily residue, which was subjected to column chromatography on silica gel (80 g). Compound **3** (492 mg, 14%) was recovered from the fraction eluted with 10% AcOEt in hexane (v/v). The fraction eluted with 10—20% AcOEt in hexane (v/v) afforded (±)-**4** (2.49 g, 70%) as a colorless oil.

Methyl (±)-2-Acetoxy-4-oxopentanoate Propylene 1,3-Dithioacetal ((±)-15)——Ac_2O (1.8 ml) was added to a solution of (±)-**4** (2.26 g) in pyridine (10 ml) at 0 °C. The reaction mixture was stirred for 2 h at room temperature and poured into 3% aq. HCl, then extracted with ether. The ether extract was washed successively with 5% aq. $NaHCO_3$ and brine, then dried. The solvent was removed *in vacuo* to afford an oily residue, which was purified by column chromatography on silica gel (60 g). The fraction eluted with 7.5% AcOEt in hexane (v/v) afforded (±)-**15** (2.44 g, 92%) as a colorless solid, mp 86.0—86.2 °C. IR (Nujol): 1755, 1740, 1435, 1380, 1225 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.58 (3H, s, CH_3), 2.14 (3H, s, $OCOCH_3$), 2.38 (1H, dd, *J*=8, 15 Hz, C_3-H), 2.68 (1H, dd, *J*=3, 15 Hz, C_3-H), 3.76 (3H, s, $COOCH_3$), 5.30 (1H, dd, *J*=3, 8 Hz, CH). MS *m/z*: 278 (M^+), 216, 112.

General Procedure for Enzyme-Catalyzed Hydrolysis of (±)-15——An enzyme (100 mg) was added to a stirred suspension of substrate ((±)-**15**, 200 mg) in phosphate buffer (pH 7.25, 0.1 M, 40 ml). The reaction mixture was stirred for 48 h at 33 °C, and extracted with AcOEt (100 ml × 2), and the combined extracts were dried. After removal of the solvent *in vacuo*, the crude product was purified by column chromatography on silica gel (8 g). The results are summarized in Table II. Specific rotations of (+)-**15** and (−)-**4** obtained by the use of lipase "Amano P" (run 4 in Table II) were as follows. (+)-**15**: $[\alpha]_D^{24}$ +4.44° (*c*=3.68, $CHCl_3$). (−)-**4**: $[\alpha]_D^{25}$ −8.01° (*c*=4.38, $CHCl_3$).

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References and Notes

1) C. A. Demopoulos, R. N. Pinkard, and D. J. Hanahan, *J. Biol. Chem.*, **254**, 9355 (1979).

2) B. B. Vargaftig, M. Chignard, J. Benveniste, J. Lefort, and F. Wal, *Ann. N. Y. Acad. Sci.*, **370**, 119 (1981), and references cited therein.
3) R. N. Pinkard, L. M. McManus, and D. J. Hanahan, *Adv. Inflammation Res.*, **4**, 147 (1982).
4) J. Benveniste, E. Jouvin, E. Pirotzky, B. Arnoux, J. M. Mencia-Huerta, R. Roubin, and B. B. Vargaftig, *Int. Arch. Allergy Appl. Immunol.*, **66** (suppl. 1), 121 (1981).
5) H. Suemune, A. Akashi, and K. Sakai, *Chem. Pharm. Bull.*, **33**, 1055 (1985), and references cited therein.
6) H. Suemune, Y. Mizuhara, H. Akita, and K. Sakai, *Chem. Pharm. Bull.*, **34**, 3440 (1986).
7) R. Noyori, I. Tomino, and M. Nishizawa, *J. Am. Chem. Soc.*, **101**, 5843 (1979).
8) K. Horikoshi, A. Furuichi, H. Koshiji, H. Akita, and T. Oishi, *Agric. Biol. Chem.*, **47**, 435 (1983).
9) J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
10) *a*) D. H. R. Barton, B. D. Brown, D. D. Ridley, D. A. Widdowson, A. J. Keys, and C. J. Leaver, *J. Chem. Soc., Perkin Trans. 1*, **1975**, 2069; *b*) B. S. Deol, D. D. Ridley, and G. Simpson, *Aust. J. Chem.*, **29**, 2459 (1976); *c*) H. Akita, A. Furuichi, H. Koshiji, K. Horikoshi, and T. Oishi, *Chem. Pharm. Bull.*, **32**, 1342 (1984).
11) M. Ohno, K. Fujita, H. Nakai, S. Kobayashi, K. Inoue, and S. Nojima, *Chem. Pharm. Bull.*, **33**, 572 (1985).
12) Compound (+)-**12** synthesized from D-mannitol should have *R*-configuration. G. Hirth and R. Barner, *Helv. Chim. Acta*, **65**, 1059 (1982).
13) O. Mitsunobu, *Synthesis*, **1981**, 1.
14) Compound (−)-**12** was converted to natural PAF.[12)]
15) Compounds (+)- and (−)-**12** were used without recrystallization for conversion into the corresponding di-*O*-(+)-MTPA esters.
16) Y. Torisawa, H. Okabe, and S. Ikegami, *Chem. Lett.*, **1984**, 1555.