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FACILE SYNTHESIS OF RACEMIC 2-HEXYL-3-HYDROXY-4-PENTANOLIDE(NFX-2) AND ITS OPTICAL RESOLUTION

Takuo Nishida[b], Takuya Nihira[a] and Yasuhiro Yamada[a*]
a)Department of Biotechnology, Faculty of Engineering, Osaka University,
2-1 Yamada-oka, Suita-shi, Osaka 565, Japan
b)Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., LTD.,
16-89 Kashima 3-chome, Yodogawa-ku, Osaka 532, Japan

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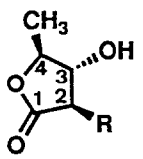
Abstract. A facile stereoselective synthesis of a constituent of antimycin A_1 and also the new virginiamycin inducing factor, NFX-2((-)-1) has been achieved. (2RS,3RS,4SR)-2-Hexyl-3-hydroxy-4-pentanolide((±)-1) was obtained by stereoselective intramolecular aldol condensation of 2-(2-bromooctanoyloxy)-1-propanal(7) as the key reaction. (±)-1 was kinetically resolved by asymmetric esterification with a lipase, affording optically pure (-)-1 and its enantiomer (+)-1.

Some of Streptomycetes have endogenous and exogenous signal molecules, which regulate the production of secondary metabolites and cyto-differentiation of these microorganisms.[1] These substances have sometimes been called autoregulators. Recently, we isolated a new virginiamycin inducing factor, NFX-2((-)-1) from the culture broth of Streptomyces antibioticus NF-18 and determined its structure.[2]



R=n-C_4H_9; **Blastmycinolactol**

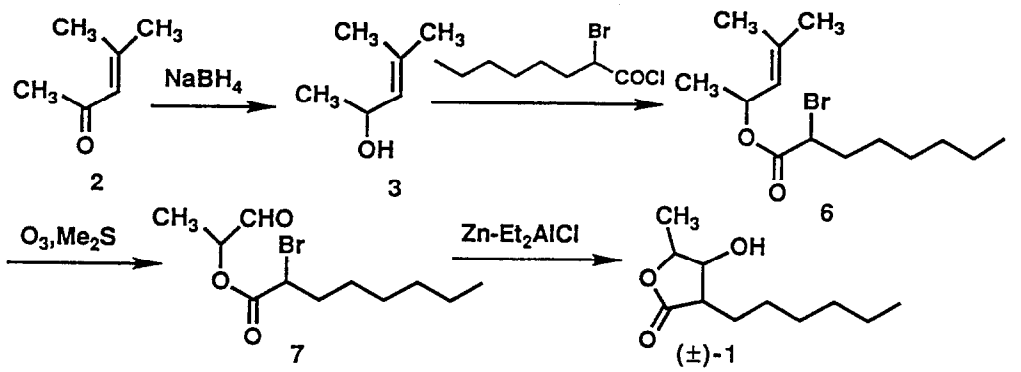
R=n-C_6H_{13}; **NFX-2 (-)-1**

On the other hand, blastmycinolactol which is the hydrolyzed product of antimycin A_3 and turned out to be analogue of NFX-2 has been an attractive target for chemical synthesis. Therefore, many groups already published syntheses of this compound in both racemic[3] and optically active form[4]. However there was no simple method to construct three contiguous chiral centers at once on this butanolide skeleton.

In the course of the studies on the structure of NFX-2, we noticed that it is one of the component of antimycin hydrolysates, namely blastmycinolactol homologue, and that it was not once clearly isolated and characterized. So, we developed a convenient method to prepare NFX-2((-)-1) to confirm the proposed structure and also to check the relation of the structure and biological activities.

In this report we describe a facile stereoselective synthesis of NFX-2 by applying intramolecular aldol condensation[5] of 2-(2-bromooctanoyloxy)-1-propanal(7) with Zn-Et_2AlCl, as the key reaction, and also its effective kinetic resolution by the transesterification and hydrolysis catalyzed with lipases.

Firstly, we attempted a stereoselective synthesis of racemic NFX-2((±)-1) from inexpensive commercially available mesityl oxide(2) via four-step procedures which is shown in Scheme 1.



Scheme 1

Crude 4-methyl-3-penten-2-ol(3) was obtained by reduction of 2 in EtOH with $NaBH_4$ in 75% yield (contains ca.10% 2-methyl-1-penten-4-ol(4) and ca.10% 4-methyl-2-pentanol(5)).[6] The esterification of crude 3 with 2-bromooctanoyl chloride yielded 4-(2-bromooctanoyloxy)-2-methyl-2-pentene(6) (54%), which contained esters of 4 and 5. Ozonolysis of 6 in MeOH, and subsequent treatment with Me_2S gave crude 2-(2-bromooctanoyloxy)-1-propanal(7). Crude 7 was purified by column chromatography. The yield of pure 7 from 6 was 67%.

Aldehyde 7, thus obtained, in dry THF was added slowly (5h) to suspension of activated zinc[7] and Et_2AlCl in THF at 55℃. Nozaki et al. obtained 13, 15 or 16-membered macrocyclic lactones from ω-hydroxy-aldehyde α-bromocarboxylic acid esters using this intramolecular condensation.[5(a),(c)] But they did not apply this reaction to small-sized

lactone ring formation. According to Baldwin rule, it seems that 5-membered ring closure of compound 7 by ordinary aldol reaction is disfavored.[8] But Zn, Et_2AlCl mediated reaction was successful, and the NMR spectrum of major product purified by chromatography was identical with that of NFX-2. Consequently, racemic NFX-2 having all trans configuration((±)-1) was prepared as the major product by this method. From this NMR spectrum, two small peaks of minor product were observed at 4.32ppm (dt, J=3Hz, J=5Hz) and 4.45ppm (dq, J=3Hz, J=6.5Hz). This stereostructure was assigned to be all cis isomer((±)-8) by comparing coupling constants of four diastereomers blastmycinolactol.[4(c)] The ratio of objective all trans isomer to all cis isomer is 10:1.

The mechanism of this reaction was assumed as shown in Fig.1.[5(a),(c)]

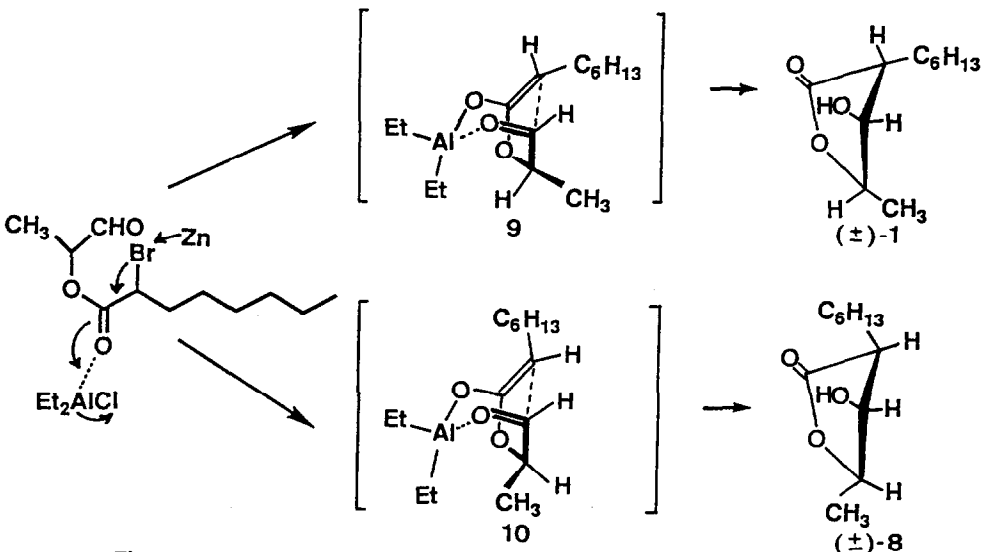


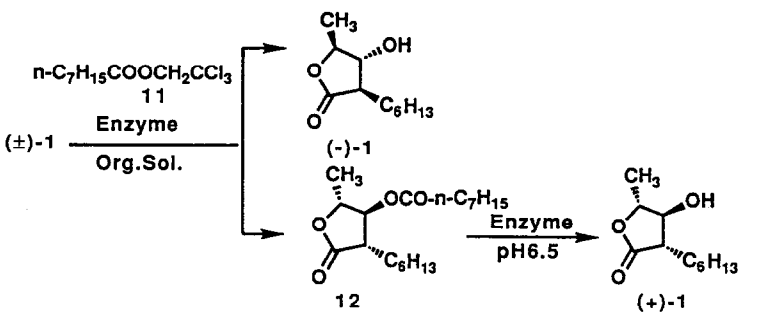
Fig.1

Aluminum enolates are generated by the coupled attack of Et_2AlCl and Zn on the α-bromoketone. (E)-enolate will be more stable than (Z)-one. (E)-enolate, thus obtained gives major product (±)-1 via a seven-membered ring system intermediate 9. Similarly, minor product (±)-8 might be obtained from (Z)-enolate through a intermediate 10. 5-Membered ring closure will be caused by intermediate 9 and 10 which possibly be stabilized under the coordination of aluminum enolate.

Next, hydrolytic enzyme-catalyzed kinetic resolution of (±)-1 was carried out using 2,2,2-trichloroethyl octanoate(11) as acyldonor in organic solvent.[9] After the screening of 31 commercially available lipases and esterases, lipase Type II from Candida cylindracea (Sigma

Chemical Company), porcine pancreas lipase (PPL, Wako Chemicals CO.,LTD.) and lipase P from Pseudomonas sp. (Nagase Biochemicals LTD.) were found to have strong activity for esterification of (±)-1. All these three enzymes under the same reaction conditions (Table I), preferentially transformed (+)-1 to give (2S,3S,4R)-2-hexyl-3-octanoyloxy-4-pentanolide(12). This ester 12 was hydrolyzed with corresponding enzyme to afford (+)-1. Although lipase P was the most effective and lipase Type II was the weakest of the three in catalytic activity, lipase P was inferior to PPL and lipase Type II in enantioselectivity. (-)-1 was obtained from remaining unesterified lactols after column chromatography. The optical purity of the product (+)-1 and (-)-1 was determined by HPLC analysis after converting them to the corresponding diastereomeric (R)-MTPA esters.[10]

Table I

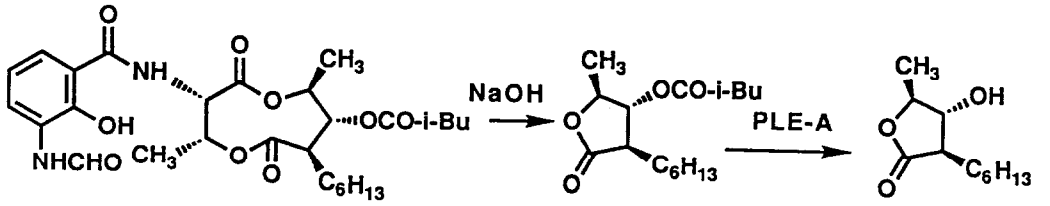


		(-)-1[f]	12[f]	(+)-1[g]
Entry	Enzyme	Yield, e.e.	Yield	Yield, e.e.
1	Lipase Type II[c]	73%, 36%ee	22%	85%, 99%ee
2	Lipase (PPL)[d]	65%, 50%ee	25%	89%,>99%ee
3	Lipase P[e]	60%, 50%ee	33%	99%, 80%ee

c:from Candida cylindracea(Sigma Chemical Company)
d:from porcine pancreas(Wako Chemicals CO.,LTD.)
e:from Pseudomonas sp.(Nagase Biochemicals LTD.)
f:the yield from (±)-1
g:the yield from 12
Reaction Conditions : see General Procedure in EXPERIMENTAL SECTION

(-)-1 prepared from antimycin A_1(13) by hydrolysis was identified in every respects with natural NFX-2 which was obtained in trace amount (610μg) from 60L of culture broth. Yonehara et al. yielded blastmycinone

by hydrolysis of antimycin A_3 with 5% NaOH, and it was further hydrolyzed with 10% NaOH to blastmycinolactol.[11] On treating antimycin A_1(13) in the similar manner, β-elimination preferentially took place to give 2-hexyl-2-penten-4-olide as by-product and the yield of (-)-1 was rather poor. Therefore we hydrolyzed (2R,3R,4S)-2-hexyl-3-isovaleryloxy-4-pentanolide(14), the mild alkaline hydrolysate of 13, with PLE-A from pig liver esterase (Amano Pharmaceutical CO.,LTD.) in the place of 10% NaOH to produce (-)-1 without β-elimination (Scheme 2).



Antimycin A_1 (13) 14 (-)-1

Scheme 2

By application of Mosher's rule[12] to (R)-MTPA ester of (-)-1 obtained from antimycin A_1(13), the absolute configuration at the C-3 carbon of (-)-1 was reaffirmed to be "R".

Next we attempt to prepare enough amount of (+)-1 and (-)-1 for biological activities tests by enzyme catalyzed optical resolution. We used PPL for kinetic resolution of (±)-1 by transesterification in benzene due to its high enantioselectivity (Table I). After transesterification was carried out with much amount of PPL and 11 or prolong reaction time until (+)-1 disappeared, unesterified optically pure (-)-1 was afforded in the 34% yield after column chromatography and recrystallization. PPL catalyzed transesterification virtually stopped below 50% conversion. The produced ester was separated and then hydrolyzed again using PPL in McIlvaine Buffer (pH6.5), and purified by column chromatography and recrystallization to afford optically pure (+)-1.

Finally, synthetic specimens (-)-1 and (+)-1 and natural (-)-1(NFX-2) were tested for their virginiamycin inducing activity (Table II). Synthetic (-)-1 and natural (-)-1 were the same in a minimum effective concentration of 1.25μg/ml. While, very interestingly (+)-1 the enantiomer of natural one was 2-times more active than natural NFX-2. But by comparing the structures of the endogeneous autoregulators virginiae butanolides(VB)(Fig.2)[1(i)(j)] from Streptomyces virginiae, (-)-1(NFX-2) and (+)-1, we found that these unexpected results were reasonable. The

synthesized unnatural (+)-1 has the same orientation on C-3 hydroxy group and on C-2 alkyl group as VB. Therefore, the configuration of two substituents at C-2 and C-3 in γ-lactone ring is found to be important for virginiamycin inducing activity in Streptomyces virginiae.

Table II Induction of Virginiamycin Production

Entry	Compounds	Minimum Effective Concentration (μg/ml)
1	NFX-2	1.25
2	(-)-1	1.25
3	(+)-1	<0.63

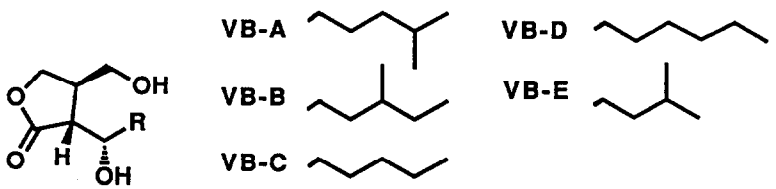


Fig.2 Structures of Virginiae Butanolides

EXPERIMENTAL SECTION

Melting points are uncorrected. ^{1}H NMR spectra were obtained with Hitachi R-24B (60MHz), JEOL JNM-GSX-400 (400MHz) and Bruker AM-600 (600MHz). IR spectra and FT-IR spectra were measured on Hitachi 215 and JEOL JIR-AQS 20M respectively. Mass spectra were obtained with JEOL JMS-DX 303. Optical rotations were measured by using JASCO DIP-181. CD spectra were obtained with JASCO J-600 spectropolarimeter. Silica gel 60 F_{254} plates (Merck) were used for analytical TLC and 70-230mesh silica gel (Merck) for column chromatography. HPLC analyses were performed on a JASCO TRI ROTAR-V with Zorbax CN column 4.6mm x 25cm (Du Pont Instruments). All reactions employing dry solvents were run under nitrogen or argon, and concentrations were carried out at reduced pressure below 40℃.

Assay of Virginiamycin Inducing Activity. Yanagimoto's method[1(b)(c)] was improved. The procedure was described in the previous paper.[1(i)]

4-Methyl-3-penten-2-ol(3). To a stirred and ice-cooled solution of 2 (98.2g, 1.0mole) in EtOH, $NaBH_4$ (10.5g, 0.28mole) was added and the mixture was stirred for 3h at r.t.. The solution was concentrated under

reduced pressure, added water (500ml) and extracted with ether (2 x 500ml). The combined extracts were dried over Na_2SO_4. The solvent was removed and residue was distilled under reduced pressure to afford crude 3 (75.0g, 75% yield) as a colorless oil (contains ca.10% 2-methyl-1-penten-4-ol and ca. 10% 4-methyl-2-pentanol). The crude product was used directly in the next reaction. : bp 75-78℃/25mmHg; IR (film) 3350, 2975, 2925, 1680, 1455, 1385cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 1.22(3H, d, J=6.4Hz, CH_3), 1.68(3H, d, J=1.5Hz, CH_3-C=), 1.70(3H, d, J=1.0Hz, CH_3-C=), 4.53(1H, dq, J=6.4Hz, J=8.3Hz, Me-C<u>H</u>-O-), 5.20(1H, d, J=8.3Hz, -CH=); EI-MS (m/e) 100(M^+) (GC-MS;OV-17).

4-(2-Bromooctanoyloxy)-2-methyl-2-pentene(6). To the solution of crude 3 (8.0g, 0.080mole), triethylamine (45ml), 4-N,N-dimethylaminopyridine (1.0g) in benzene (200ml) 2-bromooctanoyl chloride (19.5g, 0.081mole) was slowly added at less than 10℃. After the addition, the reaction mixture was kept at r.t. for 30min with stirring. The mixture was poured into ice-water (300ml) and extracted with ether (3 x 300ml). The organic layer was washed with cold 10% HCl (300ml), sat.NaCl (300ml), sat.$NaHCO_3$ and sat.NaCl (300ml), and then dried over $MgSO_4$. The solvent was removed at reduced pressure and the residue was chromatographed on a silica gel column (95g, hexane as eluent) to afford crude 6 (13.0g, 54%yield) as a light brown oil. The crude product was used directly in the next reaction. : IR (film) 2970, 2930, 2860, 1745, 1680, 1475, 1460, 1385cm^{-1}; ^1H NMR (60MHz, $CDCl_3$) δ 0.89(3H, t, J=5.0Hz, CH_3), 1.28(3H, d, J=6.0Hz, C<u>H</u>$_3$-CH-O-), 1.68-1.80(6H, $(CH_3)_2C$=), 1.05-2.50(10H, m, -$(CH_2)_5$-), 4.12(1H, t, J=7.5Hz, CHBr), 5.10(1H, d, J=8.8Hz, -CH=), 5.25-5.75(1H, m, Me-C<u>H</u>-O-). <u>CAUTION</u>:"This ester caused some injury on the skin, peeling off the outer skin of hands without any inflammation. We recommend to handle it carefully with latex gloves on."

2-(2-Bromooctanoyloxy)-1-propanal(7). Ozone was bubbled through a solution of crude 6 (10.0g, 33mmole) in MeOH (200ml) at -5℃ for 7h. Excess ozone was purged with nitrogen, and dimethyl sulfide (20ml, 0.27mole) was added at -5℃. The mixture was stirred overnight at r.t. and concentrated. The residue was chromatographed on a silica gel column twice (first; 10:1 hexane/ethyl acetate, second; methylene chloride as eluent) to give pure 7(6.1g, 67%yield) as a colorless liquid: IR (film) 3480, 2970, 2940, 2870, 1750, 1470, 1390cm^{-1}; ^1H NMR (60MHz, $CDCl_3$) δ 0.87(3H, t, J=5.0Hz, CH_3), 1.42(3H, d, J=7.3Hz, C<u>H</u>$_3$-CH-O-), 1.06-2.36(10H, m, -$(CH_2)_5$-), 4.24(1H, t, J=7.3Hz, CHBr), 4.80-5.30(1H, m, Me-C<u>H</u>-O-),

9.44(1H, s, CHO); CI-MS (m/e) 279, 281(M^+).

(2RS,3RS,4SR)-2-Hexyl-3-hydroxy-4-pentanolide((±)-1). A solution of diethyl aluminum chloride (21mmole, 25.6ml of a ca.15% solution) in hexane was added to a slurry of zinc-silver couple (34.6g, 0.53mole) in anhydrous THF (50ml) under argon for 30min at 55℃. A solution of 7 (2.96g, 10.6mmole) in THF (280ml) was added slowly over 5h. After the addition stirring was continued for 20min, and the reaction was quenched by the addition of pyridine (12.5ml). After zinc-silver couple was removed by filtration, the reaction mixture was diluted with ether (2L), washed with 2N HCl in ice water (500ml) and sat.NaCl (400ml). The organic layer was dried over $MgSO_4$ and concentrated. The residue was chromatographed on a silica gel column (50g, 5:1 hexane/ethyl acetate as eluent) to afford (±)-1 (1.16g, 55%yield) as a colorless solid (contains ca.9% (±)-8). This solid was purified by recrystallization and used next reaction. : mp 60.5-65℃; IR (Nujol) 3400cm^{-1}(OH), 1730cm^{-1}(C=O); FT-IR ($CHCl_3$) 1776cm^{-1} (C=O); ^{1}H NMR (600MHz, $CDCl_3$)δ 0.88(3H, t, J=6.9Hz, CH_3), 1.45(3H, d, J=6.3Hz, 4-CH_3), 1.25-1.65(9H, m, -$(CH_2)_5$-), 1.82-1.91(1H, m, -C<u>H</u>$_2$-C_5H_{11}), 2.02(1H, d, J=5.4Hz, OH), 2.55(1H, ddd, J=5.7Hz, J=7.7Hz, J=8.6Hz, 2-H), 3.84(1H, ddd, J=5.4Hz, J=7.0Hz, J=8.6Hz, 3-H), 4.20(1H, dq, J=6.3Hz, J=7.0Hz, 4-H); EI-MS (m/e) 201$(M+H)^+$; Anal. Calcd for $C_{11}H_{20}O_3$: C, 65.97; H, 10.07. Found: C, 65.57; H, 10.14

(2R,3R,4S)-2-Hexyl-3-hydroxy-4-pentanolide((-)-1) from Antimycin A_1(13). Antimycin A(mixture of antimycin A_1(13) and A_3, Sigma Chemical Company) (180mg) was hydrolyzed with 5% NaOHaq. (3ml) at r.t. until the crystal disappeared. To this solution was added water (30ml) and extracted with petroleum ether (3 x 30ml). The organic layer was dried over $MgSO_4$, the solvent was removed under vacuum to afford the mixture of (2R,3R,4S)-2-hexyl-3-isovaleryloxy-4-pentanolide(14) and blastmycinone(2-butyl isomer) as a colorless oil (70mg). Then, the oil (25mg), thus obtained was added to the solution of PLE-A from pig liver esterase (0.3g) in the McIlvaine Buffer (30ml, pH6.5) and stirred for 3h at 30℃. The reaction mixture was extracted with ether and dried over $MgSO_4$. The solvent was evaporated in vacuo. The residue was chromatographed on a silica gel column (3.5g, 10:1 hexane/ethyl acetate as eluent) afforded blastmycinolactol 15 (3.1mg), (-)-1 (6.0mg) and mixture of 15 and (-)-1 (3.0mg). (-)-1: a colorless solid; mp 58-59℃; FT-IR ($CHCl_3$) 1776cm^{-1} (C=O); ^{1}H NMR (600MHz, $CDCl_3$)δ 0.88(3H, t, J=7.0Hz, CH_3), 1.46(3H, d, J=6.4Hz, 4-CH_3), 1.25-1.65(9H, m, -$(CH_2)_5$-), 1.82-1.91(1H, m, -C<u>H</u>$_2$-C_5H_{11}),

1.95(1H, d, J=5.3Hz, OH), 2.55(1H, ddd, J=5.5Hz, J=7.5Hz, J=8.6Hz, 2-H), 3.84(1H, ddd, J=5.3Hz, J=7.1Hz, J=8.6Hz, 3-H), 4.20(1H, dq, J=6.4Hz, J=7.1Hz, 4-H); EI-MS (m/e) 201$(M+H)^+$; $[\alpha]_D^{20}$-11.7 (c=0.268, MeOH); $[\theta]_{217}^{MeOH}$-5700.

General Procedure(Table I).

i)Enzymatic Resolution of (2RS,3RS,4SR)-2-Hexyl-3-hydroxy-4-pentanolide ((±)-1). Enzyme (1.0g) shown in Table I was suspended in dry benzene (15ml). The reaction was started by adding (±)-1 (80mg, 0.4mmole), 2,2,2-trichloroethyl octanoate 11 (66mg, 0.24mmole) and 4Å-molecular sieves and shaken at 200r.p.m. for 48h at 30℃. The reaction was terminated by the filtration of enzyme. The solvent was evaporated in vacuo. The residue was chromatographed on a silica gel column (4g, 20:1-5:1 hexane/ethyl acetate as eluent) to afford (-)-1 as a colorless solid and (2S,3S,4R)-2-hexyl-3-octanoyloxy-4-pentanolide 12 as a colorless oil. 12: IR (film) 2930, 2860, 1790, 1750, 1475cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 0.83-0.91(6H, 2t, $2CH_3$), 1.45(3H, d, J=6.4Hz, 4-CH_3), 1.16-1.68(19H, m, -$(CH_2)_5$-Me, -CH_2-$(CH_2)_5$-Me), 1.80-1.90(1H, m, -CH_2-C_5H_{11}), 2.32(2H, t, J=7.6Hz, -CH_2-C_6H_{13}), 2.67(1H, dt, J=5.6Hz, J=8.3Hz, 2-H), 4.35(1H, dq, J=4.6Hz, J=6.4Hz, 4-H), 4.92(1H, dd, J=4.6Hz, J=5.6Hz, 3-H); EI-MS (m/e) 326(M^+). The yield and optical purity are shown in Table I.

Then, 12 (25mg, 0.077mmole) thus obtained was added to the solution of the same enzyme (0.4g) used for transesterification in the McIlvaine Buffer (30ml, pH6.5) and shaken at 200r.p.m. for 36h at 30℃. After the solution was extracted with ether, the organic layer was washed with sat.NaCl and dried over $MgSO_4$. The solvent was evaporated in vacuo. The residue was chromatographed on a silica gel column (2g, 10:1-5:1 hexane/ethyl acetate as eluent) to afford (+)-1. The yield and optical purity are shown in Table I.

ii)Determination of Optical Purity (MTPA ester). To a stirred and ice-cooled solution of (-)-1 (or (+)-1) (5mg, 0.025mmole), triethylamine (20μl), 4-N,N-dimethylaminopyridine (1mg) in benzene (2ml), (R)-MTPA-Cl (8mg, 0.032mmole) was added. The mixture was stirred for 2h at r.t. and poured into ice-water and extracted with ethyl acetate. The organic layer was washed with water, $CuSO_4$aq. and sat.NaCl and dried $MgSO_4$. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column (1g, 10:1 hexane/ethyl acetate as eluent) to afford MTPA ester of (-)-1 (or MTPA ester of (+)-1) in 82-91% yield as colorless

oil. MTPA ester of (-)-1: IR (film) 2930, 2855, 1790, 1755, 1460cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$) δ 0.87(3H, t, J=6.8Hz, CH_3), 1.51(3H, d, J=6.8Hz, 4-CH_3), 1.20-1.67(9H, m, -$(CH_2)_5$-), 1.74-1.88(1H, m, -CH_2-C_5H_{11}), 2.67(1H, dt, J=5.9Hz, J=8.3Hz, 2-H), 3.53(3H, d, J=1.0Hz, OCH_3), 4.42(1H, dq, J=4.9Hz, 6.8Hz, 4-H), 5.10(1H, dd, J=4.9Hz, J=5.9Hz, 3-H), 7.39-7.51(5H, arom.).; EI-MS (m/e) 416(M^+).
MTPA ester of (+)-1: IR (film) 2930, 2855, 1790, 1755, 1460cm^{-1}; ^{1}H NMR (400Mz, $CDCl_3$) δ 0.88(3H, t, J=6.9Hz, CH_3), 1.47(3H, d, J=6.8Hz, 4-CH_3), 1.20-1.70(9H, m, -$(CH_2)_5$-), 1.81-1.95(1H, m, -CH_2-C_5H_{11}), 2.74(1H, dt, J=5.9Hz, J=8.3Hz, 2-H), 3.51(3H, s, OCH_3), 4.32(1H, dq, J=4.9Hz, J=6.8Hz, 4-H), 5.11(1H, dd, J=4.9Hz, J=5.9Hz, 3-H), 7.39-7.51(5H, arom.).; EI-MS (m/e) 416(M^+)
The optical purity was determined by HPLC analysis. HPLC conditions: Pump flow rate 1.0ml/min; Solvent 40:1 hexane/ethyl acetate; Detection UV254nm; Retention time: MTPA ester of (+)-1: 25.4min, MTPA ester of (-)-1: 29.4min.

(2R,3R,4S)-2-Hexyl-3-hydroxy-4-pentanolide((-)-1). PPL (Wako Chemicals CO.,LTD.) (2.0g) was suspended in dry benzene (20ml). The reaction was started by adding (±)-1 (160mg, 0.80mmole), 11 (500mg, 1.8mmole) and 4Å molecular sieves and shaken at 200r.p.m. for 3 days at 30℃. The reaction was terminated by the filtration of enzyme. The solvent was evaporated in vacuo. The residue was chromatographed on a silica gel column (5g, 20:1-5:1 hexane/ethyl acetate as eluent) and recrystallized from hexane-ether to give pure (-)-1 (54mg, 34%yield) as a colorless solid:mp 57-58.5℃; $[\alpha]_D^{21}$-11.9 (c=0.986, MeOH); $[\theta]_{217}^{MeOH}$-5300.

(2S,3S,4R)-2-Hexyl-3-hydroxy-4-PENTANOLIDE((+)-1). The colorless solid (16.5mg) obtained by general procedure (Table I,entry 1+2) was recrystallized from hexane-ether to give pure (+)-1 (11mg) as a colorless solid: mp 61.5-62.5℃; $[\alpha]_D^{21}$+12.0 (c=0.553, MeOH); $[\theta]_{217}^{MeOH}$ +5700.

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