Electronic Supplementary Information

Enzymatic resolution and evaluation of enantiomers of *cis*-5'-hydroxythalidomide

Takeshi Yamamoto,[a] Norio Shibata,[a]* Masayuki Takashima,[a] Shuichi Nakamura,[a] Takeshi Toru,[a] Nozomu Matsunaga,[b] and Hideaki Hara[b]

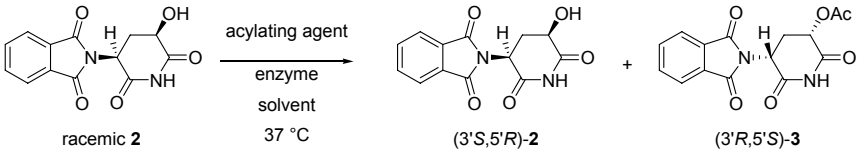
[a]*Department of Frontier Materials, Graduate School of Engineering, Nagoya Institute of Technology, Gokiso, Showa-ku, Nagoya 466-8555, Japan,* [b]*Department of Biofunctional Evaluation, Molecular Pharmacology, Gifu Pharmaceutical University, Mitahora-higashi, Gifu 502-8585, Japan*

General Methods

All reactions were performed in oven-dried glassware under a positive pressure of nitrogen. Solvents were transferred *via* syringe and were introduced into the reaction vessels though a rubber septum. All of the reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Merck silica-gel (60-F254). The TLC plates were visualized with UV light and 7% phosphomolybdic acid or *p*-anisaldehyde in ethanol/ heat. Column chromatography was carried out on a column packed with silica-gel 60N spherical neutral size 63-210 μm. The ^{1}H-NMR (200 MHz) and ^{13}C NMR (50.3 MHz) spectra for solution in d_6-DMSO were recorded on a Varian Gemini-200. Chemical shifts (δ) are expressed in ppm downfield from internal DMSO. HPLC analyses were performed on a JASCO PU-2080 Plus. Optical rotations were measured on a HORIBA SEPA-300. CD spectra were recorded on a JASCO J-820. Racemic-**2** was prepared according to ref 9a and 9b. Racemic-**1** was prepared according to Muller et. al.[1] (*S*)- and (*R*)-**1** was prepared according to ref 10b.

[1] Muller, G. W.; Konnecke, W. E.; Smith, A. M.; Khetani, V. D. *Org. Process Res. Dev.* **1999**, *3*, 139-140.

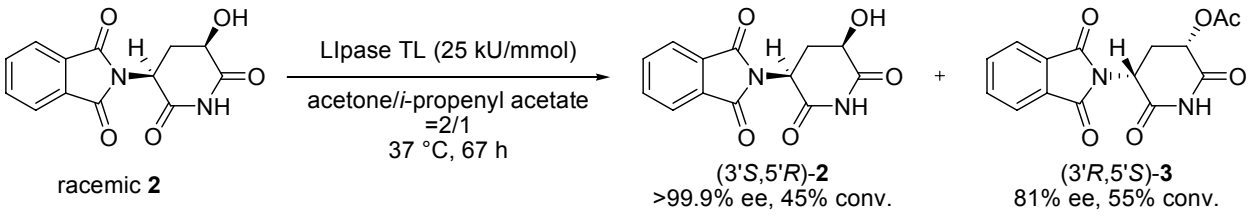
Table S-1. Optimization of enzymatic kinetic resolution[a]



Run	Enzyme	Solvent	Acylating agent	Time (h)	(3'*S*,5'*R*)-**2** Ee (%)[b]	(3'*R*,5'*S*)-**3** Ee (%)[b]	Conv.**3** (%)	E
1	Lipase PL	1,4-dioxane	vinyl acetate	24	<1	35	<3	<2
2	Lipase PS-SD	1,4-dioxane	vinyl acetate	24	<1	27	<4	<2
3	Lipase SL	1,4-dioxane	vinyl acetate	24	<1	46	<2	<3
4	Lipase OF	1,4-dioxane	vinyl acetate	72	3	5	38	1
5[c]	Lipase MY-30	1,4-dioxane	vinyl acetate	24	6	66	8	5
6[c]	Lipase AYS	1,4-dioxane	vinyl acetate	12	9	53	15	4
7	CALA	1,4-dioxane	vinyl acetate	12	0	0	0	–
8	Lipase UL	1,4-dioxane	vinyl acetate	12	3	12	20	1
9	Lipase AK	1,4-dioxane	vinyl acetate	12	3	21	13	2
10	Lipase TL	1,4-dioxane	methyl acetate	12	2	44	4	3
11	Lipase TL	1,4-dioxane	*n*-butyl acetate	12	1	65	2	5
12	Lipase TL	1,4-dioxane	phenyl acetate	12	58	79	42	15
13	Lipase TL	1,4-dioxane	acetic anhydride	12	0	0	0	–
14	Lipase TL	hexane	vinyl acetate	12	24	58	29	5
15	Lipase TL	DMSO	vinyl acetate	18	4	13	24	1
16	Lipase TL	MeOH	vinyl acetate	67	1	4	20	1
17	Lipase TL	MeCN/acetone=1/1	*i*-propenyl acetate	67	99	76	58	53
18[d]	Lipase TL	MeCN	*i*-propenyl acetate	12	<1	66	<1.5	<5
19[d]	Lipase TL	acetone	*i*-propenyl acetate	12	1	71	1.4	6
20[e]	Lipase TL	1,4-dioxane	vinyl acetate	48	41	63	39	7
21[f]	Lipase TL	1,4-dioxane	vinyl acetate	48	28	80	26	12

[a]All reactions were performed on a 7.3 μmol scale with 4 mg (25 kU) of enzyme in 0.2 mL of solvent with 0.1 mL of acylating agent. [b]Enantiomeric excess were determined by HPLC using a CHIRALCEL OD-RH with ethanol as elute. [c]Product was obtained (3'*R*,5'*S*)-**2** and (3'*S*,5'*R*)-**3**. [d]The raction was carried out at 24 ºC. [e]The reaction was carried out in the presence of 1-ethyl-3-methyl imidazolium trifluorate (50 mol%). [f]The reaction was carried out in the presence of 18-crown-6-ether (50 mol%).

Typical procedure for the enzymatic kinetic resolution of racemic-2



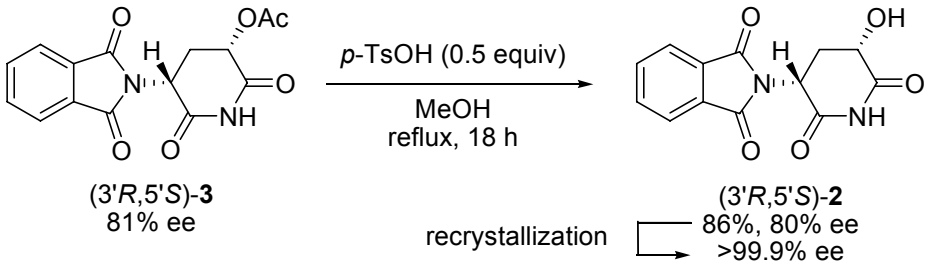
Iso-propenyl acetate (5.0 ml) was added to a mixture of racemic-**2** 100 mg (0.37 mmol), Lipase TL (50.0 mg, 25 kU) and acetone (10.0 ml). The mixture was shaken at 37 ºC. The reaction was monitored by HPLC (CHIRALCEL OD-RH 4.6 × 150 mm, EtOH 100%, 0.5 ml/min). After monitoring for 67 h ((3'*S*,5'*R*)-**2**; >99.9% ee, 45% conversion yield, (3'*R*,5'*S*)-**3**; 81% ee, 55% conversion yield), the enzyme was filtered through Celite™ and washed with acetone. The filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (hexane/ethyl acetate=50/50) and gave (3'*S*,5'*R*)-**2** as a white solid (44 mg, 44%, >99.9% ee) and (3'*R*,5'*S*)-**3** as a white solid (60 mg, 52%, 81% ee).

(3'*S*,5'*R*)-**2**: ^{1}H NMR : (d_6-DMSO) δ 2.30 (m, 1H, CH_2), 2.52 (m, 1H, CH_2), 4.52 (dd, *J*=5.2, 12.6 Hz, 1H, CH_2CHN), 5.28 (dd, *J*=5.2, 13.2 Hz, 1H, C*H*OH), 5.84 (brs, 1H, O*H*), 7.94 (m, 4H, *Ar*); 11.17 (brs, 1H, N*H*), ^{13}C NMR : (d_6-DMSO) δ 18.84,31.31, 48.43, 56.19,66.48, 123.23, 123.51, 131.08, 134.77, 166.64, 166.94, 169.48, 174.47; IR (KBr) 3410, 1715, 1392, 1228, 720; EIMS calculated for $C_{13}H_{10}N_2O_5$ ([M - H]$^-$) 273.23, found 273.00; HRMS calculated for $C_{13}H_{10}N_2O_5$: 274.0590, found 274.0587; m.p. 238.5-240.0 °C; $[\alpha]^{22}_D$ -9.0 (c 0.167, MeOH); HPLC (DAICEL CHIRALCEL OJ-RH, 4.6×250 mm, EtOH=100, flow rate 0.5 ml/min, λ=254 nm), t_R=6.24 min(major), 6.96 min(minor), >99.9% ee

(3'*R*,5'*S*)-**3**: ^{1}H NMR : (d_6-DMSO) δ 2.11 (s, 3H, CH_3), 2.25-2.80 (m, 2H, CH_2), 5.48 (dd, *J*=5.8, 12.9 Hz, 1H, CH_2CHN), 5.83 (dd, *J*=6.0, 13.1 Hz, 1H, C*H*OAc), 7.89 (m, 4H, *Ar*), 11.50 (s, 1H, N*H*); ^{13}C NMR : (d_6-DMSO) δ20.07, 27.85, 28.14, 46.03, 47.90, 66.97, 67.50, 123.38, 131.01, 134.79, 166.92, 168.38, 168.55, 168.90, 169.36.; IR (KBr) 3210, 1718, 1395, 1232, 728.; MS calculated for $C_{15}H_{12}N_2O_5$ ([M - H]$^-$) 315.27, found 315.00.; HRMS calculated for $C_{15}H_{12}N_2O_5$: 316.0695, found 316.0678; m.p. 258.0-259.0 °C; $[\alpha]^{22}_D$ +33

(c 0.125, MeOH); HPLC (DAICEL CHIRALCEL OD-RH, 4.6×150 mm, EtOH=100, flow rate 0.5 ml/min, λ=254 nm), t_R=7.93 min(major), 8.96 min(minor), 81% ee

Deacetylation of (3'*R*,5'*S*)-3



A solution of (3'*R*,5'*S*)-**3** 60.0 mg (0.18 mmol, 81% ee) and *p*-toluenesulfonic acid (18.0 mg, 0.095 mmol) was refluxed in 30 ml of methanol for 18 h. After cooling, the solvent was removed under reduced pressure. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate= 50/50) to give product in 86%, 80% ee. The optically pure (3'*R*,5'*S*)-**2** was obtained by single recrystallization from ethanol (25.5 mg, 49%, >99.9% ee); $[\alpha]^{22}_D$ +9.0 (*c* 0.184, MeOH); HPLC (DAICEL CHIRALCEL OJ-RH, 4.6×250 mm, EtOH=100, flow rate 0.5 ml/min, λ=254 nm), t_R=6.24 min(major), 6.96 min(minor), >99.9% ee. Spectral data for (3'*R*,5'*S*)-**2** (^{1}H NMR, ^{13}C NMR, IR, MS) corresponded to (3'*S*,5'*R*)-**2**.

Incubation experiments of 1 and 2

A stock solution was prepared by dissolving (*S*)-**1** or (3'*S*,5'*R*)-**2** (20.0 mg) in dimethylsulfoxide (100 ml). The 5 mL of the stock solution was diluted with 9.5 mL of buffer (phosphate buffers for pH 6 and 7, Tris-HCl buffers for pH 8 and 9); the mixture was incubated at 37 ºC. Racemization monitored by HPLC (DAICEL CHIRALCEL OJ-H, 4.6×250 mm, EtOH=100%, flow rate 0.5 ml/min, λ=254 nm) at regular intervals (0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 12, 16, 22, 28, 36, 48 h). Racemization rate was obtained to plot with incubation time as abscissa and *R*/*S* area ratio as ordinate. Hydrolysis rate was obtained to plot with incubation time as abscissa and (*R*+*S*) area/(R_0+S_0) area ratio as ordinate. (R_0 and S_0 area is peak area of incubation time 0.0 h.)

Tube Formation Assay

An angiogenesis assay kit (Kurabo) was used according to the manufacturer's instructions. Briefly, HUVECs co-cultured with fibroblasts were cultivated in the presence or absence of various concentrations of test drugs plus VEGF-A (10 ng/ml). After 11 days, cells were fixed in 70% ethanol. The cells were incubated with diluted primary antibody (mouse anti-human CD31, 1:4000) for 1 h at 37 °C, and with the secondary antibody (goat anti-mouse IgG alkaline phosphatase-conjugated antibody, 1:500) for 1 h at 37 °C, and visualization was achieved using 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT). Images were obtained from five different fields (5.5 mm^2 per field) for each well, and tube area, length, joints, and paths were quantified using Angiogenesis Image Analyzer Ver.2 (Kurabo).

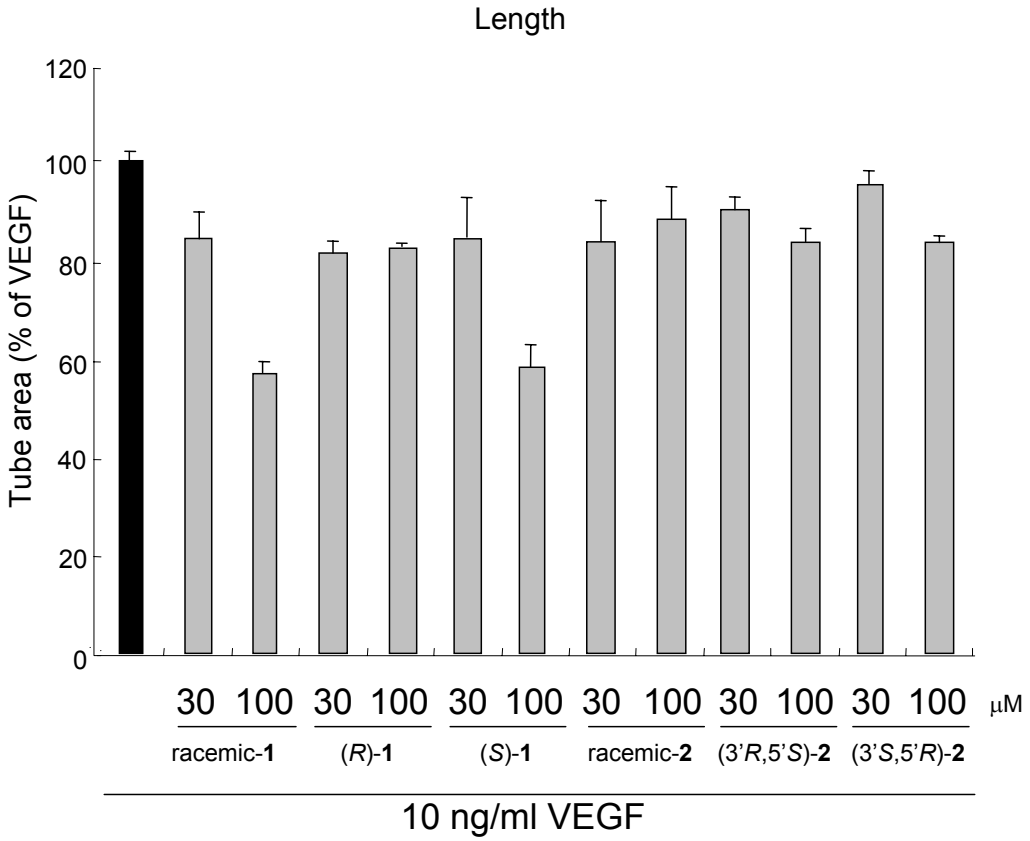


Figure S-2. Effects of thalidomide on tube length. Comparison of angiogenesis induced by VEGF: Mean ±S.E.M (VEGF:n=9, VEGF+compounds:n=3).

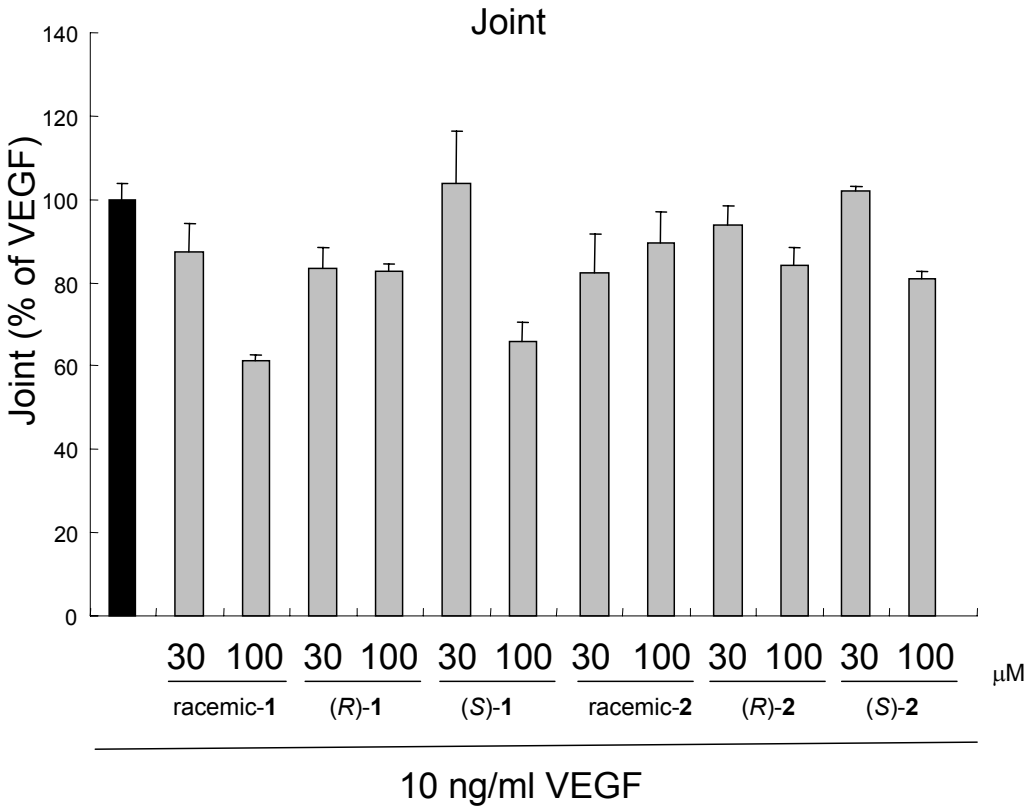


Figure S-3. Effects of thalidomide on tube joint. Comparison of angiogenesis induced by VEGF: Mean ±S.E.M (VEGF:n=9, VEGF+compounds:n=3).

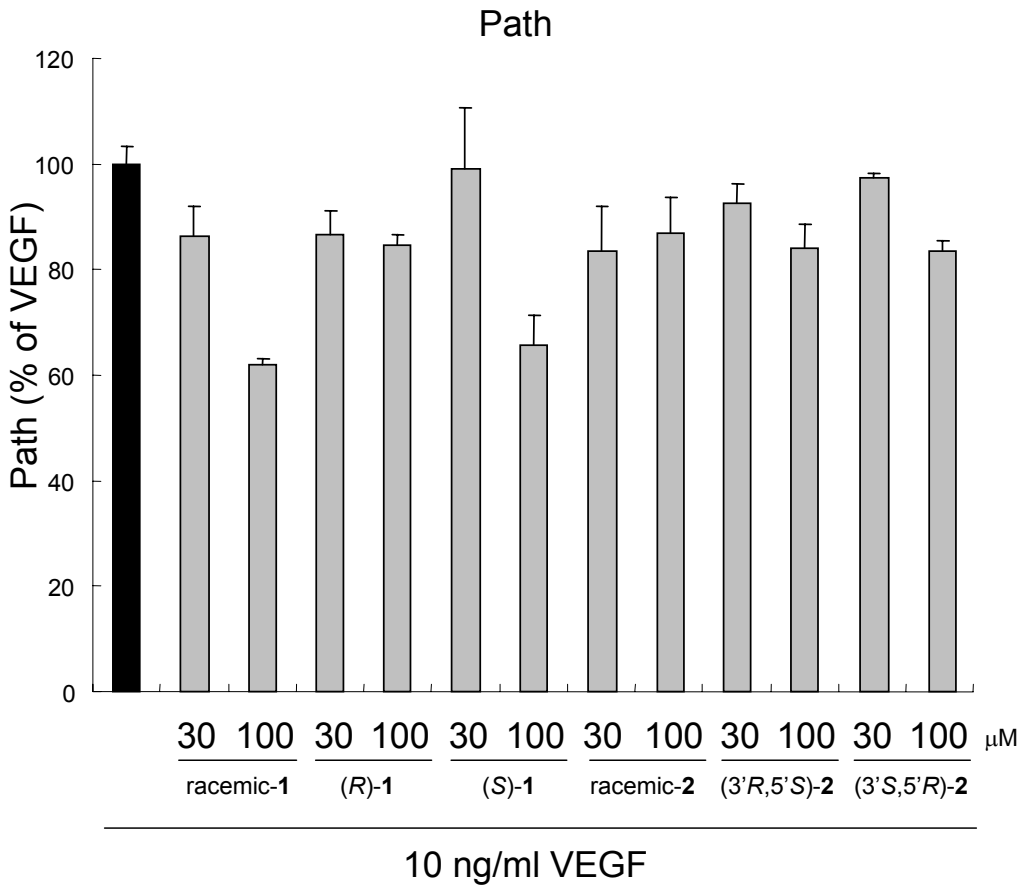
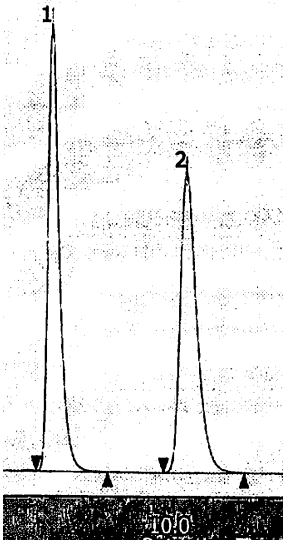
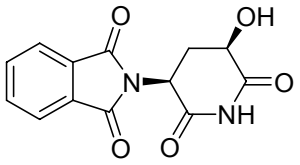


Figure S-4. Effects of thalidomide on tube path. Comparison of angiogenesis induced by VEGF: Mean ±S.E.M (VEGF:n=9, VEGF+compounds:n=3).

HPLC chromatograms of racemic-, (3'*S*,5'*R*)-, and (3'*R*,5'*S*)-2

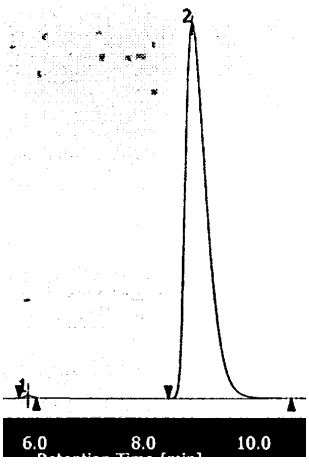
DAICEL CHIRALCEL OJ-RH, 4.6×250 mm, EtOH=100, flow rate 0.5 ml/min, λ=254 nm

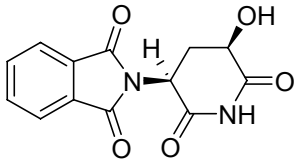




racemic **2**

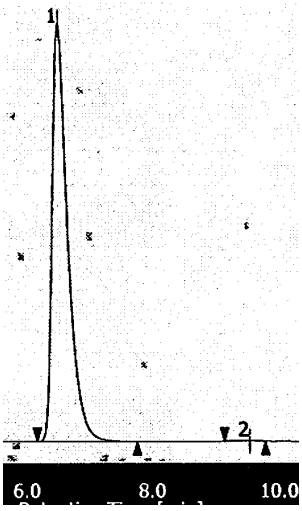
PK No.	Time	Area%	Height%
1	7.183	49.997	59.827
2	10.383	50.003	40.173

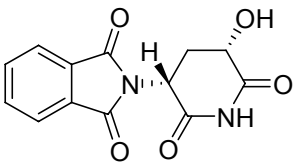




(3'*S*,5'*R*)-**2**

PK No	Time	Area%	Heiqht%
1	5.867	0.027	0.606
2	8.900	99.973	99.394





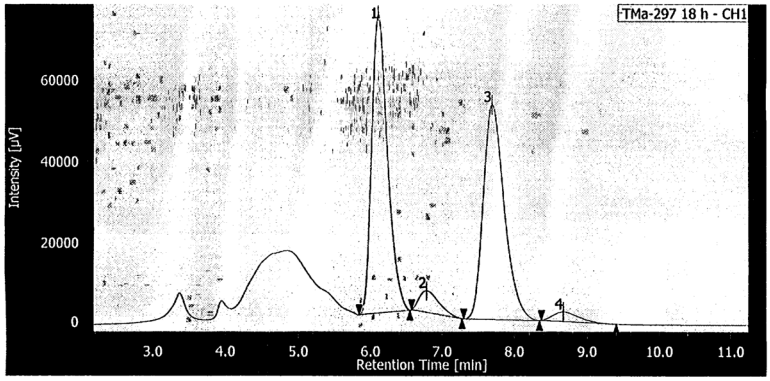
(3'*R*,5'*S*)-**2**

PK No	Time	Area%	Height%
1	6.500	99.976	99.812
2	9.533	0.024	0.188

HPLC chromatograms of enzymatic kinetic resolution

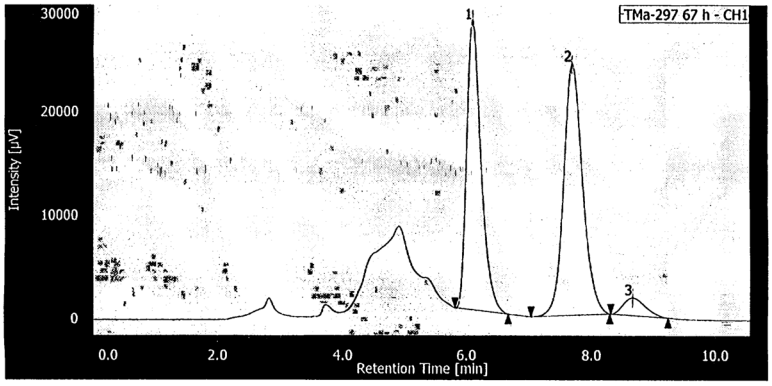
DAICEL CHIRALCEL OD-RH, 4.6×150 mm, EtOH=100, flow rate 0.5 ml/min, λ=254 nm

Enzyme: Lipase TL,acyl donor:*i*-propenyl acetate, solvent:acetone, incubation time:18 h

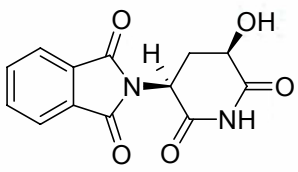


PK No	Compound	Time	Area%	Height%
1	(3'*S*,5'*R*)-**2**	6.108	47.975	54.857
2	(3'*R*,5'*S*)-**2**	6.783	4.321	3.953
3	(3'*R*,5'*S*)-**3**	7.692	45.304	39.478
4	(3'*S*,5'*R*)-**3**	8.667	2.399	1.712

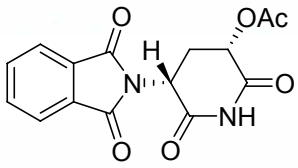
Enzyme: Lipase TL,acyl donor:*i*-propenyl acetate, solvent:acetone, incubation time:67 h



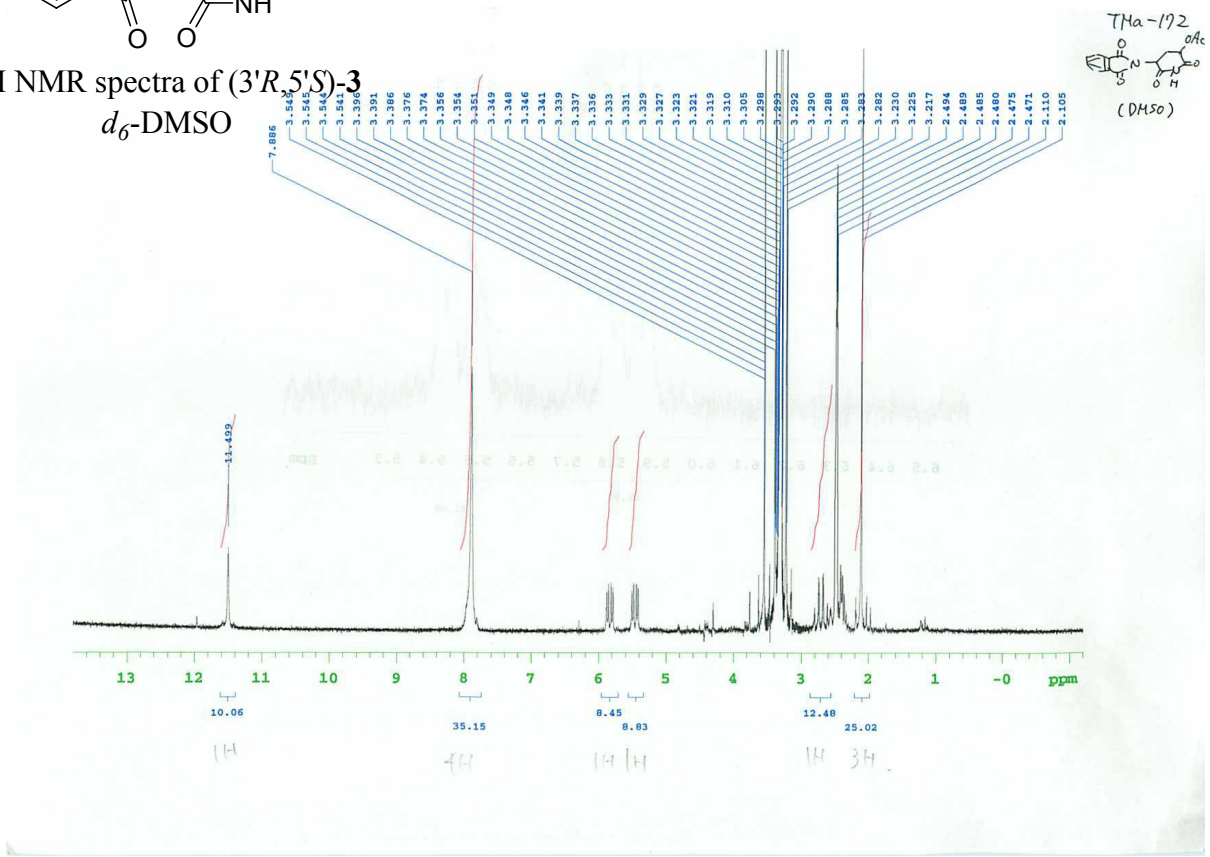
PK No	Compound	Time	Area%	Height%
1	(3'*S,5'R*)-**2**	6.100	45.20	51.994
2	(3'*R*,5'*S*)-**3**	7.683	49.707	44.408
3	(3'*S*,5'*R*)-**3**	8.650	5.274	3.598

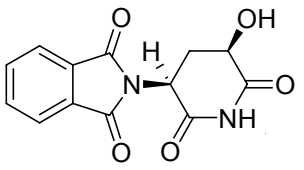


^{1}H NMR spectra of (3'*S*,5'*R*)-**2**
d_6-DMSO

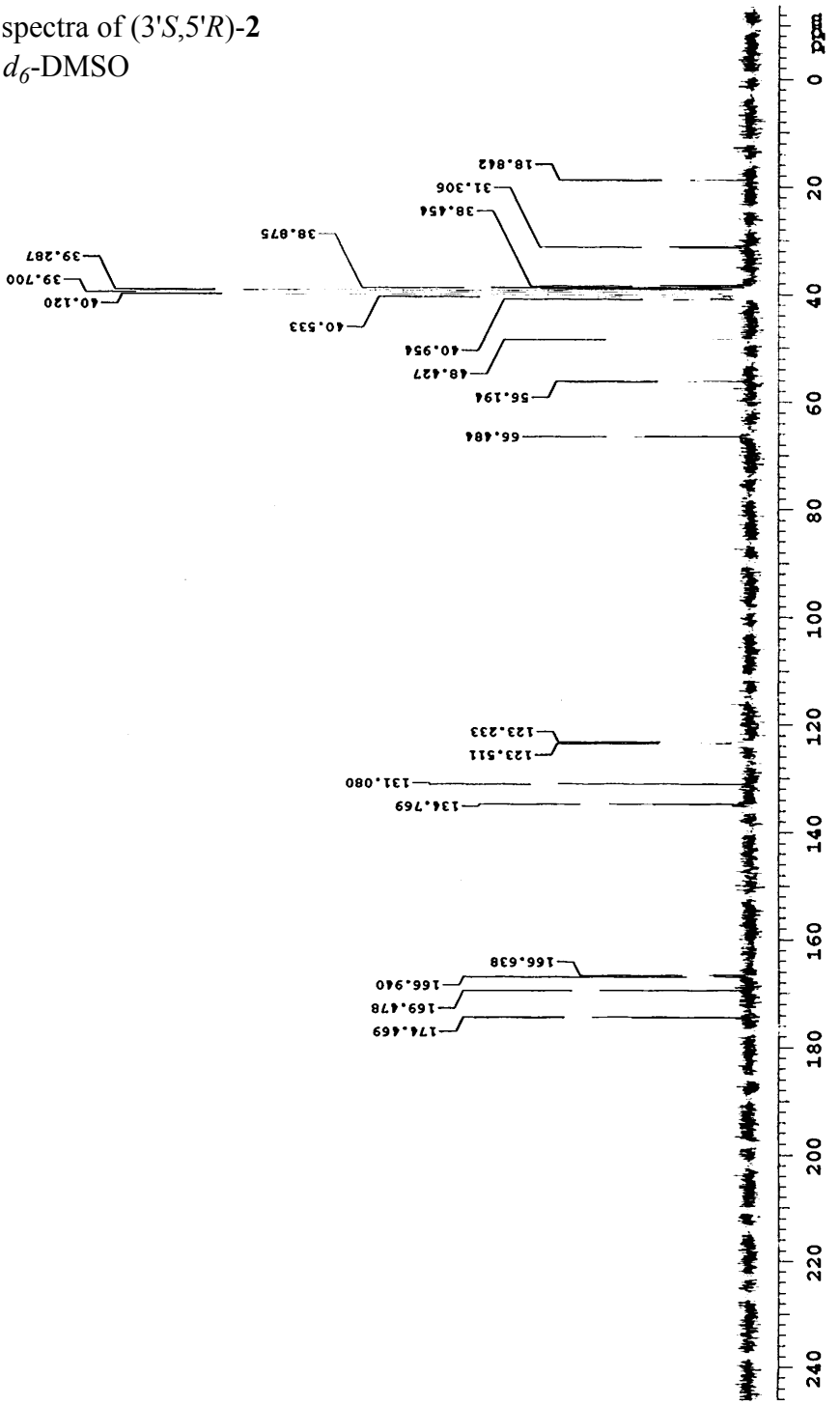


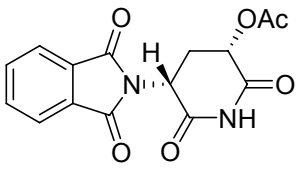
^{1}H NMR spectra of (3'*R*,5'*S*)-**3**
d_6-DMSO





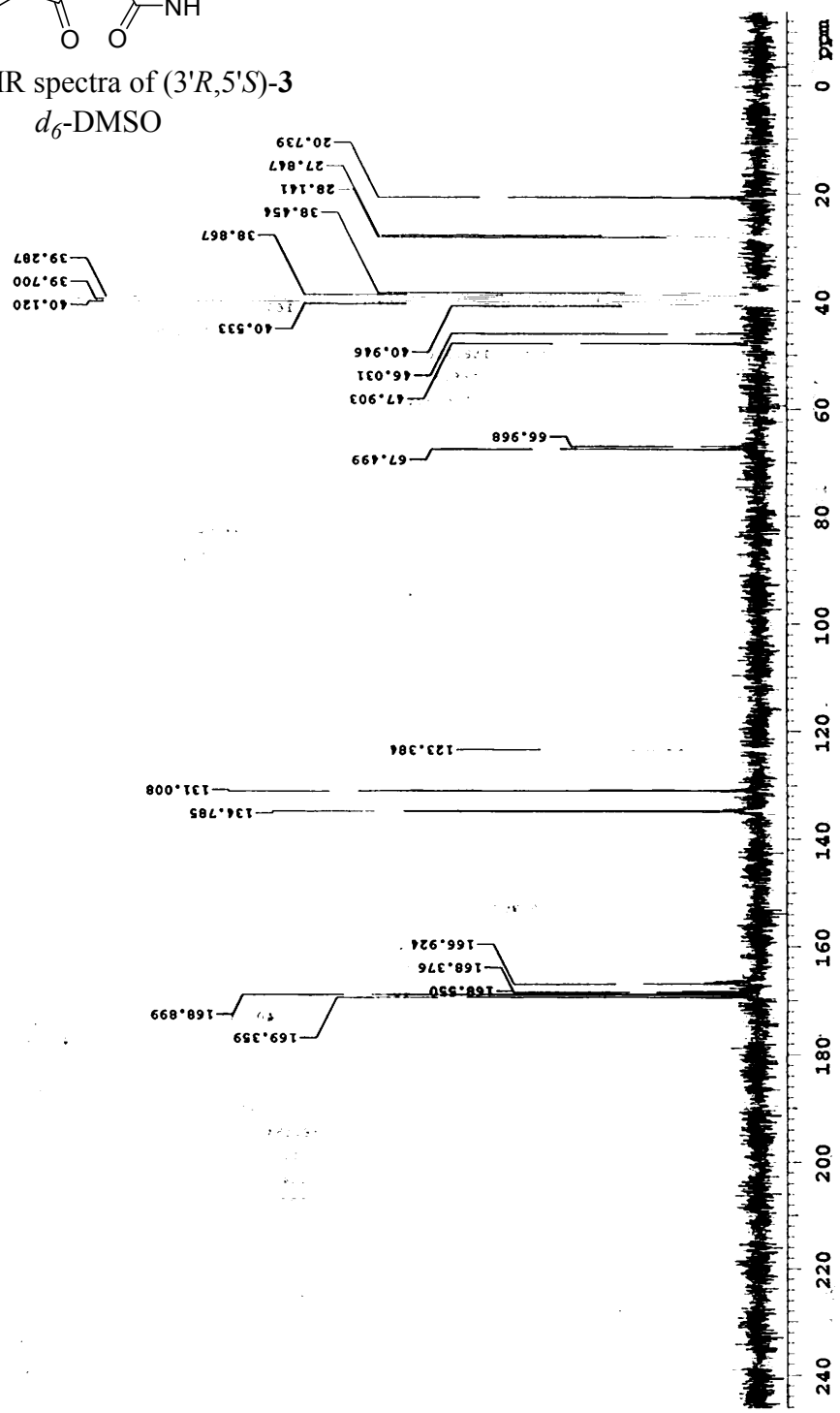
^{13}C NMR spectra of (3'*S*,5'*R*)-**2**
d_6-DMSO



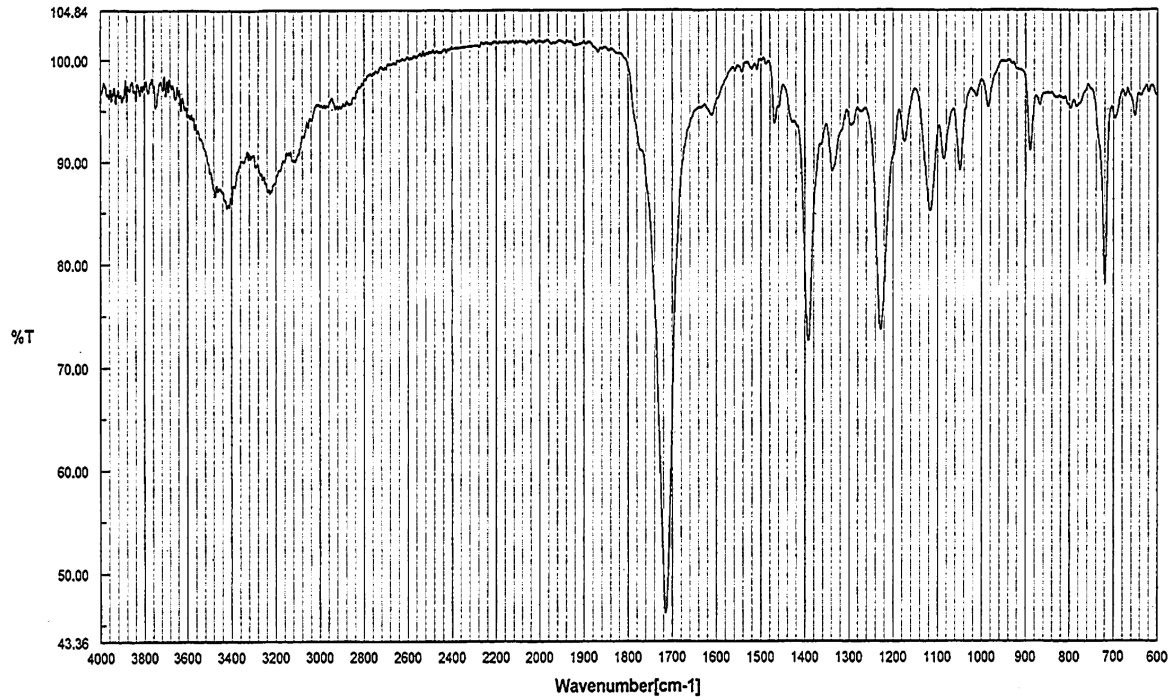


^{13}C NMR spectra of (3'*R*,5'*S*)-**3**

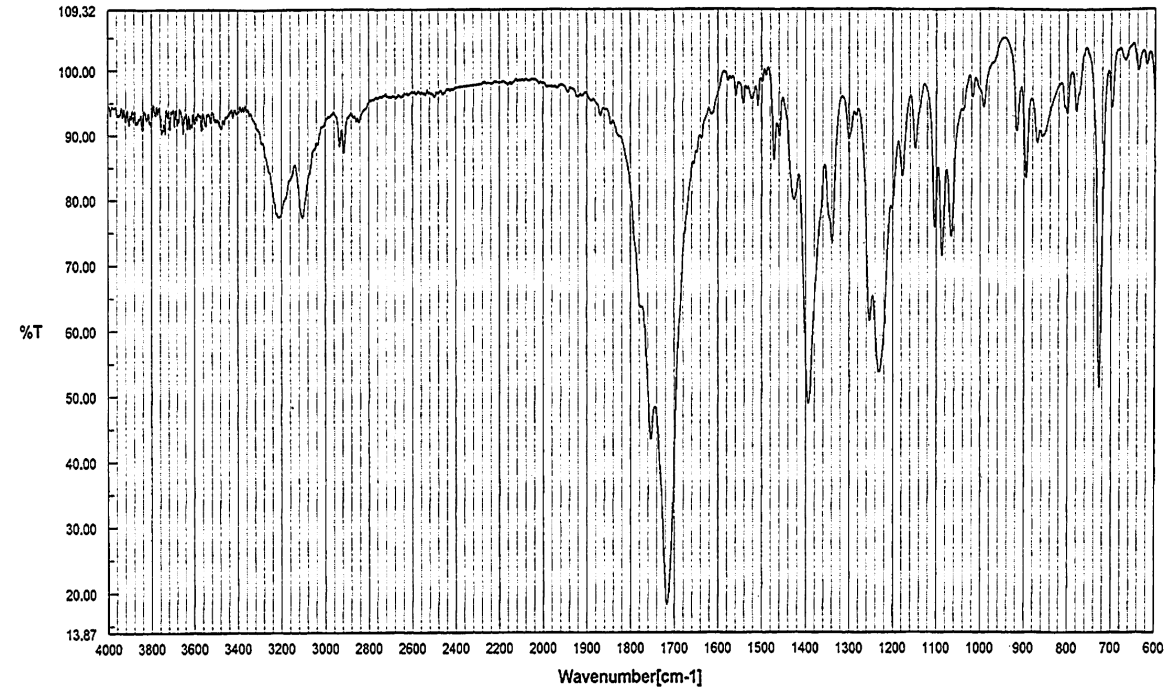
d_6-DMSO



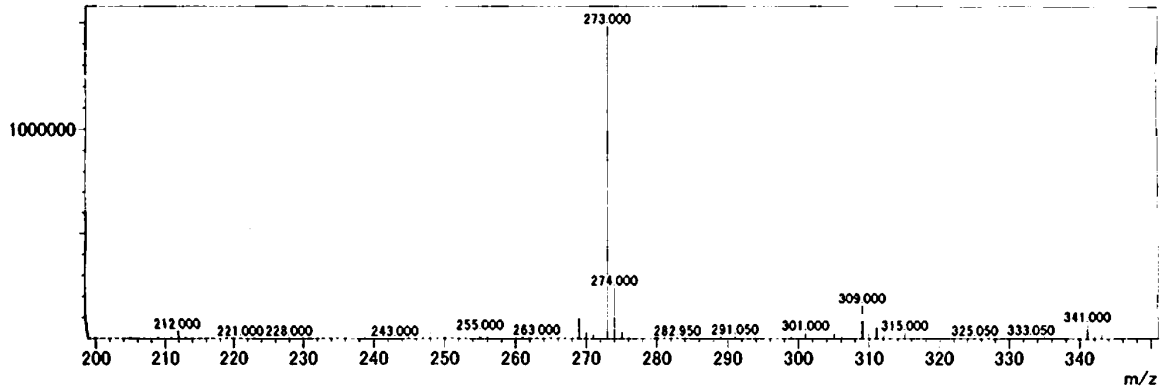
IR Spectra of (3'*S*,5'*R*)-**2**



IR Spectra of (3'*R*,5'*S*)-**3**



EIMS Spectra of (3'*S*,5'*R*)-**2**



EIMS Spectra of (3'*R*,5'*S*)-**3**