Journal of Molecular Catalysis B: Enzymatic 123 (2016) 160–166

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Preparation of (*S*)-γ-cyclogeraniol by lipase-catalyzed transesterification and synthesis of (+)-trixagol and (+)-luffarin-P

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ARTICLE INFO

Article history:
Received 24 August 2015
Received in revised form
17 November 2015
Accepted 17 November 2015
Available online 22 November 2015

Keywords:
Lipase CAL-B
γ-Cyclogeraniol
Lufferin-P
Lactone
Trixagol

ABSTRACT

Lipase-catalyzed kinetic resolution of γ-cyclogeraniol by *Candida antarctica* lipase B yielded 23% of enantiomerically pure (*S*)-γ-cyclogeraniol. (+)-trixagol and (+)-luffarin-P were synthesized from the obtained (*S*)-γ-cyclogeraniol, and the absolute configuration of natural (+)-luffarin-P was determined to have an *S* configuration by our first synthesis of (*S*)-luffarin-P for the first time.

1. Introduction

Several natural products possess γ-cyclogeranyl unit, including (+)-trixagol **1**, [1] (+)-luffarin-P **2** [2] and (*S*)-(+)-luffarin-W **3** [2] (Fig. 1). Compounds **1** and **2** are precursors of antibacterial terpenes such as agelasines, manoalide, and lufferins, which has prompted us to study their synthesis [2–4]. Enantiomatically enriched γ-cyclogeraniol (**4**) is considered to be an important chiral building block for the synthesis of these products. Thus facile preparation methods of enantiomerically active **4** are desired [5–8]. Fehr and Galindo reported two methods to prepare enantiomatically enriched **4** (Scheme 1) [5]: enantiomeric resolution by sequential recrystallization of γ-cyclogeranic acid and asymmetric protonation of α-cyclogeranic acid phenylthioester using *N*-isopropylephedrin. Previously, lipase-catalyzed enantiomeric resolution of *rac*-**4** was tested; however, the results were unsatisfactory [6]. As an alternative method, lipase-catalyzed transesterification of β-hydroxyester was conducted (Scheme 1). The enantiomatically enriched β-hydroxyester and its acetate were converted to the corresponding enantiomerically active **4** [6]. While preparation methods of enantiomerically active **4** were developed, the direct enantiomeric resolution of **4** has several advantages including avoiding racemization during the derivation of enantiomerically active **4** from the chiral compounds and reducing the conversion steps necessary to produce enantiomerically active **4**. In this study, we report the lipase-catalyzed enantiomeric resolution of *rac*-**4** and the syntheses of (*S*)-**1** and (*S*)-**2** from (*S*)-**4**.

2. Experimental

2.1. General

The NMR spectra were recorded on JEOL AL-400 spectrometer. Mass spectra were measured by JEOL JMS-AM II 50. IR spectra were carried out on JASCO FT/IR 4100. Specific rotation was measured on JASCO P-2200. HPLC analysis was performed on SSC-3210 pump equipped with chiral column, SSC-5200 UV detector and SIR Chromatocorder 21. Chemicals were purchased from Tokyo Chemical Industry Co. Ltd., Wako Pure Chemicals Industry, Ltd. or Sigma–Aldrich Inc. LLC., otherwise indicated. *rac*-γ-Cyclogeraniol (*rac*-**4**) was synthesized by previously reported procedure [6]. Geraniol, geranylacetone, lipase CAL-B (polymer supported *Candida antarctica* lipase B) and PPL (Porcine pancreas lipase) were purchased from Sigma–Aldrich Inc. LLC. Amano PS-C (*Burkholderia cepacia* lipase on ceramic) and Amano AK (*Pseudomonas fluorescens* lipase) were gifted from Amano Enzymes. Inc. Lipase QL (*Alcaligenes*

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http://dx.doi.org/10.1016/j.molcatb.2015.11.019
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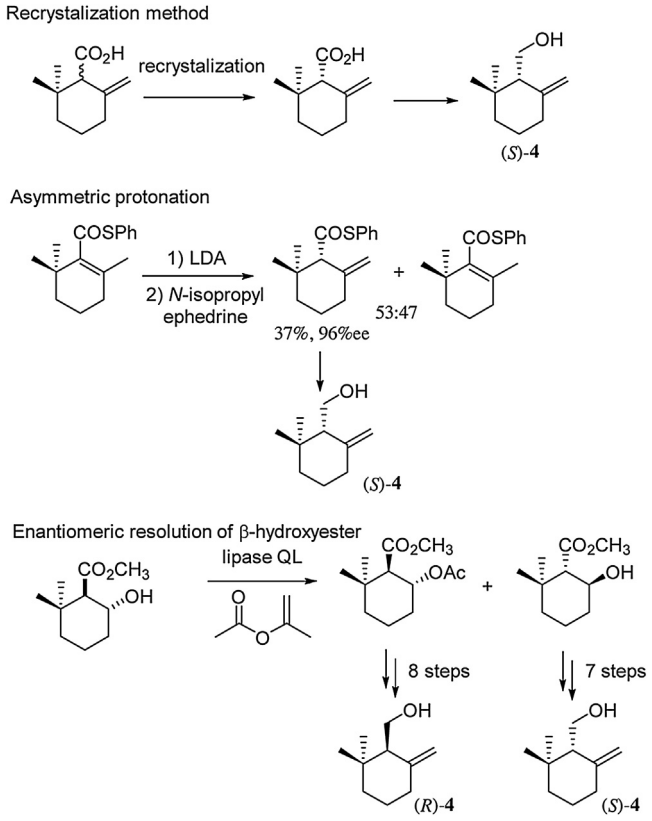


Scheme 1. Reported methods for synthesis of enantiomerically active **4**.

sp. lipase), lipase OF (*Candida rugosa* lipase) and lipase MY (*C. rugosa* lipase) were purchased from Meito Sangyo Co. Ltd.

2.2. General procedure for enzymatic kinetic resolution of γ-cyclogeraniol.

To a solution of *rac*-**4** (100 mg, 0.45 mmol) and acyl donor (10 eq.) in diisopropyl ether (1 mL) was added lipase (100 mg) and the resulting mixture was stirred at 25 °C. The conversion was determined by the measurement of ^{1}H NMR of the reaction mixture. The reaction was stopped by filtration through a pad of Celite 545. The residue was washed with ether, then the filtrate and ether solution were combined and evaporated under reduced pressure to afford the mixture of the substrate and the corresponding product. The residue was treated with benzoyl chloride (300 mg) in pyridine

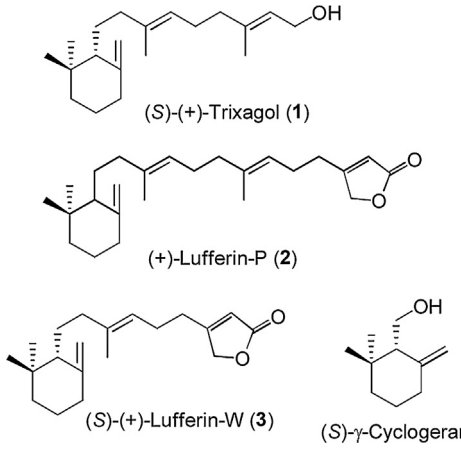
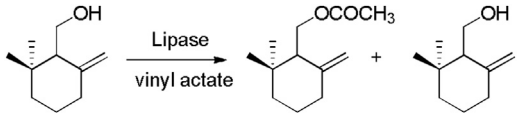


Fig. 1. Structures of natural terpenoids possessing γ-cyclogeranyl unit.

Table 1
Screening of enzymes for the enantiomeric resolution of *rac*-**4**.



Enzyme	Time (h)	Ee of **4** (%)[a]	Ee of ester (%)[b]	Conv. (%)[c]	E[d]
Amano PS–C	23	13.6 (*S*)	34.6 (*R*)	28.2	2.3
PPL	143	2.3 (*S*)	9.0 (*R*)	20.4	1.2
Amano AK	180	14.0 (*S*)	30.0 (*R*)	31.8	2.1
Lipase QL	6	15.5 (*S*)	27.0 (*R*)	36.5	2.0
Lipase OF	6	33.1 (*R*)	24.8 (*S*)	57.2	2.2
Lipase MY	180	3.0 (*R*)	31.9 (*S*)	8.6	2.0
CAL-B	48	20.2 (*S*)	70.8 (*R*)	22.2	7.1

[a] Determined by Chiral HPLC analysis (CHIRALPAK OD-H, Daicel Corp.) after benzoylation.
[b] Calculated based on the ee of 4 and the conversion [12].
[c] Determined by ^{1}H NMR spectra of the reaction mixture.
[d] Calculated based on ee of recovered 4 and the conversion [12].

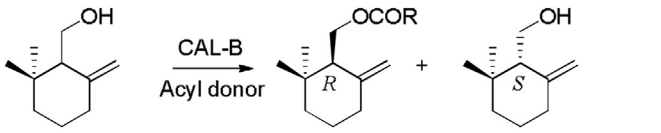
(1 mL) to convert unreacted **4** to the corresponding benzoyl ester and the ee of **4** was determined by HPLC analysis equipped with chiralpak OD-H. E value of the reaction was calculated based on the ee of recovered **4** and the conversion. HPLC analysis: *n*-hexane/i-PrOH = 100/1, Flow rate; 1.0 mL/min, Detection; 220 nm. Benzoate of (*S*)-4; t_R = 11.8 min, benzoate of (*R*)-**4**: t_R = 13.2 min. The results are summarized in Tables 1 and 2. The signals of ^{1}H NMR that were used to calculate the conversion are listed below.

4: ^{1}H NMR ($CDCl_3$) δ 3.60 (1H, dd, J = 4.8, 11.2 Hz), 3.69 (1H, dd, J = 10.6, 11.2 Hz). Acetate of **4**: ^{1}H NMR ($CDCl_3$) δ 4.20 (1H, dd, J = 5.2, 10.8 Hz), 4.24 (1H, dd, J = 9.2, 10.8 Hz). Propanoate of **4**: ^{1}H NMR ($CDCl_3$) δ 4.21 (1H, dd, J = 5.2, 10.8 Hz), 4.26 (1H, dd, J = 9.2, 10.8 Hz). Butanoate of **4**; ^{1}H NMR: δ 4.21 (1H, dd, J = 5.2, 10.8 Hz), 4.25 (1H, dd, J = 9.2, 10.8 Hz), 4.58 (1H, s), 4.80 (1H, s). EI-MS *m*/*z*: 224 (Calced for $C_{14}H_{24}O_2$: 224). Hexanoate of **4**; ^{1}H NMR: δ 4.22 (1H, dd, J = 5.2, 11.2 Hz), 4.25 (1H, dd, J = 9.2, 11.2 Hz), 4.58 (1H, s), 4.80 (1H, s). EI-MS *m*/*z*: 252 (Calced for $C_{16}H_{28}O_2$: 252). Octanoate of **4**; ^{1}H NMR: δ 4.22 (1H, dd, J = 4.8, 11.0 Hz), 4.25 (1H, dd, J = 8.8, 11.0 Hz), 4.59 (1H, s), 4.81 (1H, s). EI-MS *m*/*z*: 280 (Calced for $C_{18}H_{32}O_2$: 280). Decanoate of **4**; ^{1}H NMR: δ 4.23(1H, dd, J = 4.8, 11.0 Hz), 4.28 (1H, dd, J = 8.8, 11.0 Hz), 4.59 (1H, s), 4.81 (1H, s). EI-MS *m*/*z*: 308 (Calced for $C_{20}H_{36}O_2$: 308). Laurate of **4**; ^{1}H NMR: δ 4.22 (1H, dd, J = 4.8, 11.0 Hz), 4.25 (1H, dd, J = 8.8, 11.2 Hz), 4.58 (1H, s), 4.80 (1H, s). EI-MS *m*/*z*: 336 (Calced for $C_{22}H_{40}O_2$: 336). Myristate of **4**; ^{1}H NMR: δ 4.22 (1H, dd, J = 4.8, 11.0 Hz), 4.26 (1H, dd, J = 8.8, 11.2 Hz), 4.59 (1H, s), 4.81 (1H, s). EI-MS *m*/*z*: 364 (Calced for $C_{24}H_{44}O_2$: 364). Crotonate of **4**; ^{1}H NMR: δ 4.22 (1H, dd, J = 4.8, 11.0 Hz), 4.26 (1H, dd, J = 8.8, 11.2 Hz), 4.62 (1H, s), 4.83 (1H, s). EI-MS *m*/*z*: 222 (Calced for $C_{14}H_{22}O_2$: 222). Benzoate of **4**; ^{1}H NMR: δ 4.44 (1H, dd, J = 10.0, 11.2 Hz), 4.53 (1H, dd, J = 5.4, 11.2 Hz).

2.3. Preparation of (S)-γ-cyclogeraniol

To a solution of *rac*-**4** (3.00 g, 19.5 mmol) and vinyl propanoate (10 mL) in diisopropyl ether (100 mL) was added lipase CAL-B (1.0 g) and the resulting mixture was stirred at room temperature for 7 days. The reaction mixture was filtrated through a pad of Celite 545. The residue was washed with ether, then the filtrate and ether solution were combined, and evaporated under reduced pressure. The residue was treated with the same conditions described above {vinyl propanoate (10 mL), diisopropyl ether (100 mL) and lipase CAL-B (1.0 g)}, and then the residue was purified by gel column chromatography [silica gel (80 g), hexane/ethyl acetate (15/1)] to afford (*S*)-**4** (702 mg, 4.55 mmol, 23%, >99% ee) and the propyl ester of **4** (2.95 g, 14.0 mmol, 72%, 32% ee). After small amount of

Table 2
Screening of acyl donor on the lipase-catalyzed enantiomeric resolution of *rac*-**4**.



Acyl donor	Time (h)	Ee of **4**(%)[a]	Ee of ester(%)[b]	Conv. (%)[c]	E[d]
Vinyl acetate	48	20.2	70.8	22.2	7.1
Vinyl propanoate	50	24.3	68.4	26.2	6.7
Vinyl butanoate	24	25.7	66.4	27.9	6.3
Vinyl hexanoate	11	57.0	50.1	53.2	5.2
Vinyl octanoate	11	53.0	46.6	53.2	4.5
Vinyl decanoate	24	37.5	27.0	58.1	2.4
Vinyl laurylate	15	38.7	39.2	49.7	3.2
Vinyl myristate	15	34.0	48.7	41.1	4.0
Vinyl benzoate	157	41.5	48.8[e]	46.0	4.3[f]
Vinyl crotonate	282	1.9	4.6	29.4	1.1

[a] Determined by Chiral HPLC analysis equipped with CHIRALPAK OD-H after benzoylation.
[b] Calculated based on the ee of 4 and the conversion.
[c] Determined by ^{1}H NMR spectra of the reaction mixture.
[d] Calculated based on ee of recovered (*S*)-4 and the conversion.
[e] Determined by Chiral HPLC analysis.
[f] Calculated by the ee of ester and the conversion.

(*S*)-**4** was converted to the corresponding benzoyl ester by benzoyl chloride and pyridine, the ee of **4** was determined as >99% ee by HPLC analysis equipped with chiralpak OD-H. $[\alpha]_D^{21}$ +23.7 (c 1.00, $CHCl_3$). Lit $[\alpha]_D^{21}$ +23.7 (c 0.31, $CHCl_3$)[7]. Propanoate of **4**: IR (neat) ν 1734 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.87 (3H, s), 0.98 (3H, s), 1.11 (1H, t, J = 7.6 Hz), 1.14–1.28 (4H, m), 2.01–2.12 (2H, m), 2.29 (2H, q, J = 7.6 Hz), 4.21 (1H, dd, J = 5.2, 10.8 Hz), 4.26 (1H, dd, J = 9.2, 10.8 Hz), 4.59 (1H, s), 4.81 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.2, 23.4, 25.2, 27.7, 28.7, 33.3, 34.3, 37.6, 52.2, 62.6, 109.7, 147.1, 174.6. EI-HRMS 210.1622 (Calced for $C_{13}H_{22}O_2$: 210.1620).

2.4. Synthesis of (S)-trixago1

2.4.1. (S)-1-[1′-Benzenesulfonyl]methyl]-2,2-dimehtyl-6-methylenecyclohexane (6)

To a solution of (S)-**4** (1.40 g, 9.09 mmol) in pyridine (5 mL) was added mesyl chloride (1.0 mL, 12 mmol) at 0 °C. The mixture was stirred at rt for 2 h, poured into water and extracted with a mixture of hexane and ethyl acetate (3/1). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was added to a mixture of sodium hydride (40% in mineral oil, 1.2 g) and thiophenol (2.2 g, 20 mmol) in DMF (100 mL) and stirred at 90 °C for 6 h. The mixture was cooled to rt, poured into water and extracted with a mixture of hexane and ethyl acetate (3/1). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (50 g), hexane/ethyl acetate (50/1)] to afford (*S*)-**5** (2.12 g, 8.62 mmol, 89% yield). To a mixture of (*S*)-**5** (2.12 g, 8.62 mmol) and $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (120 mg, 0.1 mmol) in ethanol (30 mL) was added aq. 30% hydrogen peroxide (30%, 15 mL) at 0 °C, the mixture was stirred at rt for 8 h and added ice-cold aq. sodium sulfite. The mixture was extracted with ethyl acetate, the organic layer was dried over sodium sulfate and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (30 g), hexane/ethyl acetate (10/1)] to afford (*S*)-**6** (2.39 g, 8.60 mmol, 99%). $[\alpha]_D$ +13.7 (c 1.0, $CHCl_3$). Lit[8]. $[\alpha]_D$ +10.8 (c 2.1, $CHCl_3$). ^{1}H and ^{13}C NMR spectra were identical with those of reported value [8].

2.4.2. 4-((1′S)-2′,2′-Dimethyl-6-methylenecyclohexyl) butan-2-ol (7)

To a solution of (*S*)-**6** (1.26 g, 4.53 mmol) in dry THF (30 mL) were added *n*-butyl lithium in hexane (2.6 M, 1.5 mL, 3.9 mmol) at 0 °C and then propylene oxide (1.0 mL, 16 mmol) were added to the mixture. The mixture was stirred at rt for 2 h, then poured into water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographed by silica gel [silica gel (50 g), hexane/AcOEt (10/1)] to afford crude product (1.5 g). To the mixture of the crude product, Na_2HPO_3 (1.50 g) and EtOH (2 mL) in THF (50 mL) was added Na (1.0 g) at 0 °C and the mixture was stirred at the same temperature for 12 h. The mixture was evaporated under reduced pressure and the residue was purified by column chromatography [silica gel (30 g), hexane/AcOEt (10/1)] to afford a diastereomeric mixture of (1′*S*)-**7** (639 mg, 3.26 mmol, 84%).

2.4.3. 5-[4-((1S)-2,2-Dimethyl-6-methylenecyclohexyl) butan-2-ylsulfanyl]-1-phenyltetrazole (**8a**)

To a solution of **7** (596 mg, 3.04 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (PTSH) (587 mg, 3.30 mmol) and triphenylphosphine (0.988 g, 3.77 mmol) in THF (10 mL) were added DEAD (2.2 M toluene solution, 1.5 mL, 3.3 mmol) in toluene at 0 °C. The mixture was stirred at 0 °C for 2 h, then poured into water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography [silica gel (15 g), hexane/AcOEt (10/1)] to afford **8a** (565 mg, 2.91 mmol, 96%) [8].

2.4.4. 5-[4-((1S)-2,2-Dimethyl-6-methylenecyclohexyl) butan-2-ylsulfonyl]-1-phenyltetrazole (**9a**)

To a mixture of **8a** (212 mg, 0.862 mmol) and $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (12 mg, 0.01 mmol) in EtOH (5 mL) was added aq. 30% hydrogen peroxide (30%, 1.5 mL) at 0 °C, the mixture was stirred at rt for 8 h and added ice-cold aq. sodium sulfite. The mixture was extracted with ethyl acetate, the organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (10 g), hexane/ethyl acetate (10/1)] to afford **9a** (239 mg, 0.86 mmol, 99% yield). Spectral date of **9a** were identical with those of reported value [8].

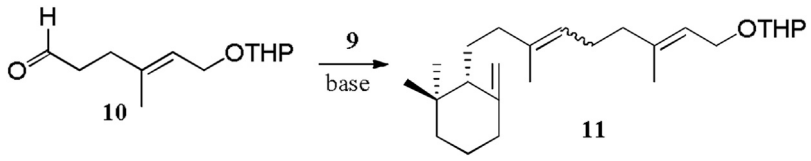
2.4.5. 2-[4-((1S)-2,2-Dimethyl-6-methylenecyclohexyl) butan-2-ylsulfonyl]benzothiazole (9b)

9b (441 mg, 1.17 mmol, 87% 2 steps) was prepared from **7** (290 mg, 1.48 mmol) by the same procedure described above using 2-benzothiazolethiol (BTSH) instead of PTSH. Spectral date of (*S*)-**9b** were identical with those of reported value [8].

2.4.6. (6E)-8-(2,4-Tetrahydropyranyloxy)-2,6-dimethyloct-6-ene-2,3-diol

To a solution of potassium carbonate (8.0 g, 62.5 mmol), $K_3[Fe(III)(CN)_6]$ (14.5 g, 0.044 mol), potassium osmate(IV) (15.4 mg, 41.8 mmol) and methanesulfonamide (2.0 g, 20 mmol) in water (100 mL) was added a solution of geraniol THP ether (5.0 g, 21 mmol) in *t*-BuOH (100 mL) at 0 °C, and the mixture was stirred at room temperature for 20 h. A saturated aqueous solution of sodium hydrogen sulfate (100 mL) was added to the reaction mixture and the resulting mixture was extracted by CH_2Cl_2 four times. The organic layers were collected, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (150 g), hexane/ethyl acetate (2/1)] to afford the diastereomeric mixture of 6,7-diol of THP-geraniol (4.14 g, 14.9 mmol, 71% yield). IR (neat) ν 3433, 1021 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.10 (3H, s), 1.15 (3H, s), 1.30–1.70 (7H, m) 1.64 (3H, s), 1.70–1.82 (1H, m), 1.99–2.09 (1H, m), 2.20–2.31 (1H, m), 2.60

Table 3
Reaction conditions for one-pot Julia coupling for the synthesis of **11**.



Entry	sulfone	Base	Temp	Yield(%)	*E*/*Z*
1	**9a**	LiHMDS	78	47	1.2/1
2	**9a**	NaHMDS	78	43	1.1/1
3	**9a**	KHMDS	78	30	3.3/1
4	**9a**	KHMDS	-90	46	5/1
5	**9b**	KHMDS	-78	23	1/1.1

Conditions: **9**, 200 mg, THF, 5 mL, Base, 1.1 eq.

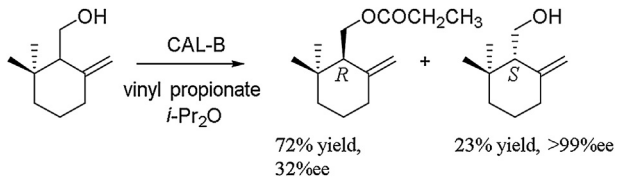
(2H, br) 3.29 (1H, d, *J* = 10.8 Hz), 3.41-3.51 (1H, m), 3.78-3.90 (1H, m), 3.91–3.42 (1H, m), 4.13–4.24 (1H, m), 4.57 (1H, t, *J* = 2.0 Hz), 5.30–5.54 (1H, m). ^{13}C NMR ($CDCl_3$) δ 16.3 (16.3 for the diastereomer), 19.5, 23.2, 25.4, 26.3, 29.4,30.6, 36.7, 62.3, 63.6 (63.7 for the diastereomer), 73.0, 78.0 (78.1 for the diastereomer), 98.0 (98.1 for the diastereomer), 121.0 (121.0 for the diastereomer), 139.9. EI HR-MS *m*/*z*: 272.1992 (Calced for $C_{15}H_{28}O_4$: 272.1988).

2.4.7. (4E)-6-(2,4-Tetrahydropyranyloxy)-4-methylhex-4-enal (**10**)

To a solution of sodium periodate (0.863 g, 4.05 mmol) in water (30 mL) was added a solution of the diol (1.00 g, 3.67 mmol) prepared in the Section 2.4.6. in EtOH (30 mL) at 0 °C, and the mixture was stirred at the same temperature for 2 h. A saturated aqueous solution of sodium hydrogen sulfate (100 mL) was added to the reaction mixture and the resulting mixture was extracted by CH_2Cl_2 three times. The organic layers were collected, dried over Na_2SO_4 and evaporated under reduced pressure to afford **10** (763 mg, 3.67 mmol, 100%), which was then used immediately for the next coupling reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.70 (3H, s), 1.45–1.88 (6H, m), 2.37 (2H, *t*, *J* = 7.2 Hz), 2.58 (2H, *t*, *J* = 7.2 Hz), 3.48–3.57 (1H, m), 3.90–3.97 (1H, m), 4.02 (1H, dd, *J* = 7.2, 12.0 Hz), 4.23 (1H, dd, *J* = 6.0, 12.0 Hz), 4.61 (1H, s), 5.39 (1H, t, *J* = 2.0 Hz), 9.78 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.8, 22.8, 28.7, 33.9, 34.8, 45.1, 65.6, 66.8, 101.3, 124.9, 141.1, 205.3.

2.4.8. General procedure of one pot Julia coupling of ***9*** and ***10***

To a solution of (1*S*)-**9a** (200 mg, 0.52 mmol) or (1*S*)-**9b** in dried THF (5 mL) was added base (1.1 eq.) indicated in Table 3 at −78 °C. The mixture was stirred for 1 h at the same temperature, then **10** was added to the mixture. The mixture was stirred for 3 h at the same temperature, and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (10 g), hexane/AcOEt (20/1)] afford **11** as stereoisomeric mixture. The results were listed in Table 3. The stereoisomeric ratio were determined by signals at 4.5 ppm on ^{1}H NMR. **11**: ^{1}H NMR: δ [0.75, 0.76] (3H, s each), [0.83, 0.84] (3H, s each), [1.51, 1.61] (3H, s each), 1.54 (3H, s), 3.36–3.48 (1H, m), 3.75–3.86 (1H, m), 3.90–3.99 (1H, m), 4.10–4.19 (1H, m) [4.46(*E*), 4.50 (*Z*)] (1H, s each), 4.56 (1H, *t*, *J* = 4.4 Hz), [4.67(*E*), 4.70 (*Z*)] (1H, s each), 5.01 (1H, *t*, *J* = 6.8 Hz), 5.20–5.32 (1H, m). EI HR-MS *m*/*z*: 374.3179 (Calced for $C_{25}H_{42}O_2$: 374.3185).



Scheme 2. Preparation of (*S*)-**4**.

2.4.9. (S)-Trixagol tetrahydropyranyl ether 11

0.5 M KHMDS in toluene (1.1 mL) was added to a solution of (*S*)-**9a** (198 mg, 0.529 mmol) in dry THF (5 mL) was added at −30 °C under Ar atmosphere and stirred for 1 h. The mixture was cooled at −90 °C and **10** (126 mg, 0.594 mmol) was added to the mixture and stirred for 3 h at the same temperature. The reaction mixture was warmed up to room temperature and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (10 g), hexane/AcOEt (20/1)] afford (*S*)-**11** (88.1 mg, 0234 mmol, 46%) as stereoisomeric mixture.

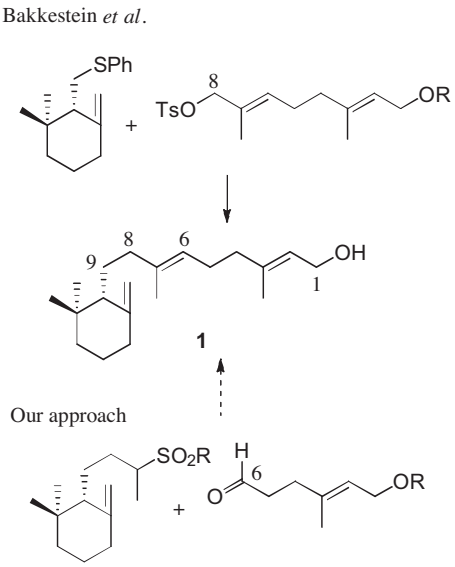
2.4.10. (S)-Trixagol (S)-1

To a solution of (*S*)-**11** (*E*/*Z* = 5/1) (63.4 mg, 0.169 mmol) in EtOH (2 mL) was added PPTS (2.57 mg, 0.015 mmol) and the mixture was stirred for 2 h under Ar atmosphere. The mixture was poured into sat. $NaHCO_3$ and extracted with AcOEt twice. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (60N) (5 g), hexane/AcOEt (15/1)] afford the stereoisomeric mixture at C6 position of (*S*)-**1** (*E*/*Z* = 5/1) (46.7 mg, 0.161 mmol, 95%). The mixture was further purified by silica gel column chromatography [silica gel (60N) (50 g), benzene/ether (10/1)] afford (*S*)-**1** (21.0 mg, 45%) and stereoisomeric mixture (25.7 mg, 55%) of (6*E*)-**1** and (6*Z*)-**1**. $[\alpha]_D^{21}$ = +14.1 (c 0.5, $CHCl_3$), lit [3]. $[\alpha]_D$ = +14 (c 2.6, $CHCl_3$). The spectral data was identical with those of reported value [1,3]. ^{1}H NMR ($CDCl_3$): δ 0.81 (3H, s), 0.88 (3H, s), 1.14–1.75 (10H, m), 1.57 (3H, s), 1.67 (3H, s), 1.85–2.13 (6H, m), 4.13 (2H, d, *J* = 6.8 Hz), 4.51 (1H, s), 4.73 (1H, s), 5.07 (1H, *t*, *J* = 6.4 Hz), 5.41 (1H, *t*, *J* = 6.4 Hz).

2.5. Synthesis of Luffarin-P

2.5.1. 1-Geranyl-3-hydroxyacetone.

To a solution of geranylacetone (15.7 g, 79 mmol) in THF (100 mL) was dropwise added LiHMDS (80 mL, 1 M solution in THF, 80 mmol) at −78 °C, the mixture was stirred for 30 min at −78 °C, then TMSCl (12 mL, 94 mmol) was added to the mixture at the same temperature and the resulting mixture was stirred for 30 min. The mixture was concentrated under reduced pressure, the residue was dissolved in hexane and the resulting participate was filtrated. The filtrate was concentrated under reduced pressure to afford the crude TMS-enolether of geranylacetone. The residue was added to a suspension of NBS (13.5 g, 75.8 mmol) in CH_2Cl_2 (300 mL) at −78 °C was stirred for 1 h at the same temperature. The mixture was concentrated under reduced pressure, the residue was dissolved in hexane and the resulting participate was removed by filtration. The filtrate was concentrated under reduced pressure



Scheme 3. Synthetic routes for synthesis of **1**.

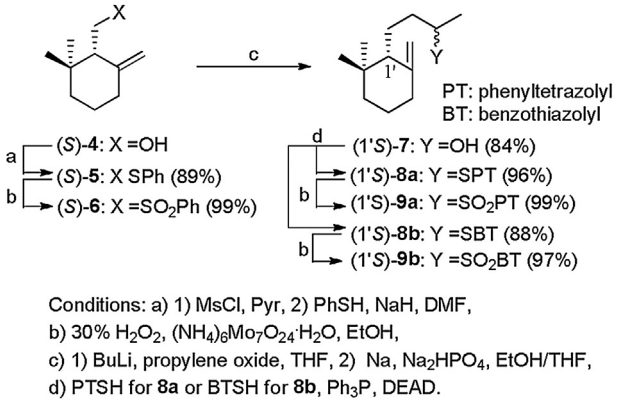
to afford the crude product of 1-bromo-3-geranylacetone. To the crude product of 1-bromo-3-geranylacetone in DMF (100 mL) was added KOAc (10 g, 0.10 mol) and stirred for 6 h at 50 °C. The mixture was poured into ice-cooled water (1 L) and was extracted with hexane/AcOEt (20/1), and the organic layer was concentrated in reduced pressure. The residue (**13** and **13′**) was added to a suspension of lipase PS-C (1.0 g) in 10% ethanol in hexane (300 mL), and was stirred for 6 h. The reaction mixture was filtrated by Celite 545, evaporated under reduced pressure, and purified by silica gel column chromatography [silica gel (100 g), hexane/AcOEt (12/1)] to afford 1-geranyl-3-hydroxyacetone **14** (9.53 g, 45.3 mmol, 60%) [9].

2.5.2. (3E,7E)-3-(4,8-Dimethylnon-3,7-dienyl) but-2-en-4-olide (**15**)

To a solution of 12 (333 mg, 0.169 mmol) in benzene (8 mL) was added (triphenylphosphoranylidene) ketene (485 mg, 0161 mmol) and the mixture was stirred for 5 h under Ar atmosphere at 60 °C. The mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (10 g), hexane/AcOEt (20/1)] to afford **15** (312 mg, 0.133 mmol, 83%). Spectral date of **15** were identical with those of reported value [10].

2.5.3. (3E)-3-(7,8-Dihydroxy-4,8-dimehtylnon-3-enyl) but-2-en-4-olide

To a solution of K_2CO_3 (800 mg, 6.25 mmol), $K_3[Fe(III)(CN)_6]$ (1.45 g, 4.4 mmol), potassium osmate(IV) (3.08 mg, 8.36 μmol) and methanesulfonamide (20 mg, 0.2 mmol) in water (10 mL) was added a solution of **15** (521 mg, 2.22 mmol) in *t*-BuOH (10 mL), and the mixture was stirred at room temperature for 20 h. A saturated aqueous solution of $NaHSO_3$ (100 mL) was added to the reaction mixture and the resulting mixture was extracted by CH_2Cl_2 four times. The organic layers were collected, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (10 g), hexane/ethyl acetate (2/1)] to afford (3*E*)-3-(7,8-dihydroxy-4,8-dimehtylnon-3-enyl) but-2-en-4-olide (435 mg, 1.60 mmol, 72% yield). IR (neat) ν 3420, 1780, 1742 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.11 (3H, s), 1.15 (3H, s), 1.29-1.60 (2H, m) 1.58 (3H, s), 1.98–2.48 (8H, m), 3.24 (1H, dd, *J* = 6.0, 8.8 Hz), 4.70 (2H, s), 5.11 (1H, t, *J* = 6.4 Hz), 5.80 (1H, s). ^{13}C NMR ($CDCl_3$): δ 16.0, 23.0, 25.6, 26.4, 28.5, 29.5, 36.5, 73.0, 73.2 77.8, 115.5, 122.4, 137.2, 170.4, 174.3. EI HR-MS *m*/*z*: 268.1669 (Calced for $C_{15}H_{24}O_4$: 268.1675).



Scheme 4. Synthesis of sulfone **9**.

2.5.4. (3E)-3-(7-Formyl-4-mehtylhex-3-enyl) but-2-en-4-olide (12)

To a solution of sodium periodate (0.231 g, 1.00 mmol) in water (30 mL) was added a solution of diol (0.232 g, 0.865 mmol) in EtOH (30 mL) at 0 °C, and the mixture was stirred at the same temperature for 2 h. A saturated aqueous solution of $NaHSO_3$ (10 mL) was added to the reaction mixture and the resulting mixture was extracted by CH_2Cl_2 four times. The organic layers were collected, dried over Na_2SO_4 and evaporated under reduced pressure to afford **12** (180 mg, 0.865 mmol, 100%), which was then used immediately for the next coupling reaction without further purification. ^1H NMR ($CDCl_3$) δ 1.64 (3H, s), 2.27–2.38 (2H, m), 2.45 (2H, *t*, *J* = 7.2 Hz), 2.54 (2H, td, *J* = 6.6, 1.6 Hz), 4.73 (2H, s), 5.40 (1H, t, J = 5.6 Hz), 5.84 (1H, s), 9.75 (1H, t, *J* = 1.6 Hz). ^{13}C NMR ($CDCl_3$) δ 16.1, 25.5, 28.4, 31.5, 41.9, 73.0, 115.5, 122.9, 135.5, 169.9, 173.9, 201.9.

2.5.5. Lufferin-P ((S)-2)

To a solution of (*S*)-**9a** (122 mg, 0.314 mmol) in dry THF (3 mL) was added 0.5 M KHMDS in toluene (0.6 mL, 0.3 mmol) at −30 °C, the mixture was cooled at −90 °C after stirring for 1 h at −30 °C, and 18 was added to the mixture. The mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [silica gel (10 g), hexane/AcOEt (50/1)] afford the stereoisomeric mixture at C3′ position of (*S*)-**2** (82.2 mg, 0.245 mmol, 74%). The mixture was further purified by silica gel column chromatography [silica gel (60N) (50 g), benzene/ether (10/1)] afford (*S*)-**2** (9.8 mg, 12%) and the stereoisomeric mixture (72.4 mg, 88%) of **2**. $[\alpha]_D^{20}$ +3.7 (c 0.13, $CHCl_3$). Lit [2]. $[\alpha]_D$ +5 (c 0.1, $CHCl_3$) Spectral data were identical with those of reported value [2]. ^1H NMR ($CDCl_3$) δ 0.81 (3H, s), 0.91 (3H, s), 1.17–1.27 (1H, m), 1.38-1.61 (4H, m), 1.59 (3H, s), 1.62 (3H, s) 1.65–2.11 (10H, m) 2.29 (2H, *t*, *J* = 7.2 Hz), 2.45 (2H, *t*, *J* = 6.6 Hz), 4.53 (1H, dd, *J* = 2.4, 15.2 Hz), 4.72 (2H, s), 4.77 (1H, d, *J* = 11.2 Hz), 5.02-5.12 (2H, m), 5.84 (1H, *t*, *J* = 1.6 Hz). EI HR-MS *m*/*z*: 370.2884 (Calced for $C_{25}H_{38}O_2$: 370.2872).

3. Results and discussion

3.1. Enantiomeric resolution of rac-4

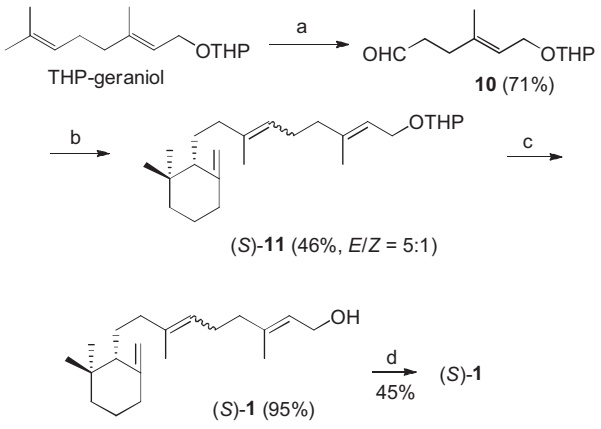
Because the chiral center of the primary alcohol **4** is distant from the reaction center, enzymatic resolution of *rac*-**4** is difficult [11]. In addition, as **4** does not contain a heteroatom, except for the hydroxyl group, the enzyme must recognize the small differences between the methylene and dimethyl groups, which are

separated from the hydroxyl group by three carbon atoms. Thus, enzymatic resolution of *rac*-**4** is challenging. *rac*-**4** was prepared from 6-methylhept-5-en-2-one in four steps to achieve 74% overall yield [6]. Several lipases, including pig porcine lipase (PPL), *C. antarctica* lipase B (CAL-B), Amano PS-C, Amano AK, Lipase QL, Lipase OF and Lipase MY were tested for their stereoselectivity of *rac*-**4** using a our conventional assay method [12]. The results are shown in Table 1. The quality of the reaction was determined by the *E* value [13] calculated on the basis of the conversion and the enantiomeric excess (ee) of recovered **4**. The reactions catalyzed by Amano PS, PPL, Amano AK, Lipase QL and CAL-B afforded (*R*)-ester whereas Lipase OF and Lipase MY afforded (*S*)-ester. Among them, CAL-B showed the best stereoselectivity as indicated by the *E* value (*E* = 7.1). It is known that changing the acyl donor affects the stereoselectivity and reactivity of lipase [12,14–16]. Several acyl donors in the transesterification of *rac*-**4** were tested, but their *E* values were lower than that of vinyl acetate (Table 2). However, elongation of the carbon chain of the acyl donor increased the reaction rate. Thus, to the reaction period for obtaining enantiomerically pure (*S*)-**4,** vinyl propionate was employed over vinyl acetate in the large-scale experiment. To achieve this, 3.0 g of *rac*-**4** was treated with CAL-B in vinyl propionate and *i*-Pr_2O for 14 days, propionate of (*R*)-**4** with a 32% ee was obtained in 72% yield, and enantiomerically pure (*S*)-**4** with >99% ee was recovered in 23% yield (700 mg) (Scheme 2). As CAL-B was deactivated in a week by acetaldehyde generated from vinyl propionate, fresh CAL-B, vinyl propionate, and diisopropyl ether were added after the evaporation of the reaction mixture to complete the reaction.

3.2. Synthesis of (S)-trixagol (1)

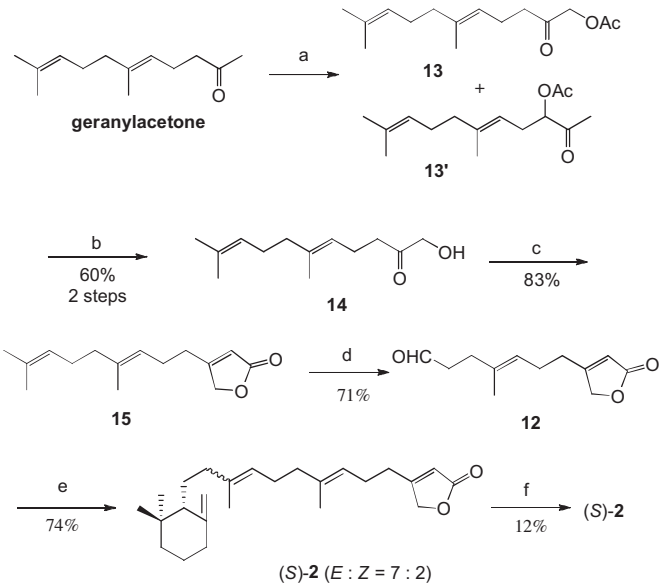
(*S*)-(+)-Trixagol **1** was isolated from *Bellardia trixago* [1]. Recently, several groups have achieved their total synthesis [3,17,18]. Bakkestein et al. reported the chiral synthesis of the (*S*)-**1** skeleton at the C8–C9 bond (Scheme 3) [3,19]. We attempted the skeleton construction of **1** at the C6–C7 double bond by one-pot Julia olefination [20] because it seemed difficult to apply their methods to the synthesis of **2**, which possesses the reactive α,β-unsaturated lactone moiety.

Mesylation of (*S*)-**4**, followed by treatment with sodium phenylthiolate at 80 °C, yielded 89% of sulfide **5** (Scheme 4). Oxidation by hydrogen peroxide in the presence of molybdenum complex afforded sulfone **6** in 99% yield. An anion generated from **6** and *n*-butyl lithium was reacted with racemic propylene oxide and then desulfonated by sodium in a mixture of THF and ethanol to give



Conditions: a) 1) K_2OsO_4, $K_3Fe(CN_6)$, K_2CO_3, $CH_3SO_2NH_2$, 2) KIO_4, b) **9a**, KHMDS, DMA, -90 °C, c) aq. HCl, d) silica-gel chromatography

Scheme 5. Synthesis of (*S*)-**1**.



Conditions: a) 1. LiHMDS, TMSCl, 2. NBS, 3. AcOK, b) Lipase PS-C, EtOH, c) (triphenylphosphoranylidene)ketene. d) 1) K_2OsO_4, $K_3Fe(CN_6)$, K_2CO_3, $CH_3SO_2NH_2$, 2) KIO_4, e) **9a**, KHMDS, DMA, -90 °C, f) silica gel chromatography

Scheme 6. Synthesis of Lufferin-P.

a diastereomer mixture of **7** in 84% yield in two steps. Treating **7** with PTSH under Mitsunobu reaction conditions gave **8a** in 96% yield, followed by oxidation by hydrogen peroxide and the addition of a catalytic amount of molybdenum complex afforded sulfone **9a** in 99% yield. Treating **7** with BTSH instead of PTSH using the same procedure afforded **8b** (88%) and then **9b** (97%)

Aldehyde **10** was prepared by selective oxidation from tetrahydropyranyl-protected geraniol (THP-geraniol) in 71% yield (Scheme 5). Several bases were tested for one-pot Julia coupling of **9a** or **9b** and aldehyde **10**; the results are listed in Table 3. The use of NaHMDS on the reaction with **9a** gave **11** in comparatively higher yield (43%) but the stereoselectivity of the reaction was low (*E*/*Z* = 1.1/1). One-pot Julia coupling of **9a** and aldehyde **10** using KHMDS yielded 30% of **11** with the best stereoselectivity. Based on the stereoselectivity of (*E*)-**11** and (*Z*)-**11**, the reaction of **9a** with KHMDS was chosen for the preparation of **11**. At −90 °C, the reaction yielded 46% of **11** with a stereoselectivity of 5/1. After removal of the THP group on **1** in 95% yield, silica gel column chromatography was applied to isolate 45% of (*S*)-**1** from the mixture of geometrical isomers of **1**. The spectral signature of synthesized **1**, including specific rotation, was superimposable with those of natural **1** [1,3].

3.3. Synthesis of (S)-lufferin-P (2)

(+)-Luffarin-P (**2**) was isolated from the Australian marine sponge, *Luffariella geometrica* [2]. While *S* was assumed to be the absolute configuration of (+)-**2** by comparison of its $[M]_D$ sign with that of (+)-luffarin W (**3**), this has not yet been determined [2,21]. (*S*)-**2** was synthesized from (1*S*)-**9a** and aldehyde **12** to determine the absolute configuration of (+)-**2** (Scheme 6). Aldehyde **12** was synthesized from geranylacetone. Geranylacetone was converted to TMS-enolether at −78 °C, and then treated with NBS. Following treatment with potassium acetate, crude 1-acetoxy-3-geranylacetone (**13**) contaminated with 1-acetoxy-1-geranylacetone (**13′**) (ca. 15%) was produced. On treating the mixture of **13** and **13′** with lipase Amano PS-C in hexane containing 10% ethanol, the lipase selectively reacted with **13** to afford

14 in 60% yield from geranylacetone. Treatment of **14** with Bestman's ketene [22] gave lactone **15** in 76% yield. The regioselective lipase-catalyzed reaction was necessary to obtain pure **15**, since **13**′ and its derivatives such as hydroxyketone and lactone were difficult to remove from **13** to **15**. Regioselective dihydroxylation of **15**, followed by oxidative cleavage of the diol by sodium periodate afforded **12** in 71% yield (two steps). One-pot Julia coupling between (*S*)-**9a** and **12** gave a mixture of geometrical isomers of (*S*)-**2** (*E*/*Z* = 7/2) in 74% yield. After separation by silica gel chromatography 12% of pure (*S*)-**2** could be isolated and 88% of the mixture of geometrical isomers could be recovered. The spectral signature of (*S*)-**2** was superimposable with those of natural (+)-**2**. Comparison of the specific rotation sign of synthetic (*S*)-**2** {+3.7 (c 0.13, $CHCl_3$)} with that of natural **2** {+5 (c 0.1, $CHCl_3$)} revealed that the absolute configuration of the natural (+)-**2** was *S* [2].

4. Conclusion

In this study, we attempted the lipase-catalyzed enantiomeric resolution of *rac*-**4**. Several reaserch groups have attempted the enzymatic enantiomeric resolution of *rac*-**4**, but with less than satisfactory results [8]. The lipase screening results showed *rac*-**4** undergoes a CAL-B catalyzed selective acylation. In addition, carbon chain elongation of the acyl donor accelerated the reaction rate to enhance the enantiomeric purity of the starting material. In case of the use of vinyl acetate, the reaction gave (*S*)-**4** with 96% ee at 3 weeks. Applying vinyl propionate to enhance enzymatic resolution results in >99% ee of (*S*)-**4** at 2 weeks. While the stereoselectivity and reactivity of the enzymatic enantiomeric resolution were not satisfied, enantiomerically pure (*S*)-**4** (23% isolated yield) was obtained by a simple procedure. Synthesized (*S*)-**4** was then applied for the first synthesis of (+)-lufferin-P. Thus, the absolute configuration of (+)-lufferin-P possesses an *S*-configuration.

Acknowledgements

The authors thank Ms. Yurie Kurimoto and Ms. Sumie Yasuhara for their supports.

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