Chemo-Enzymatic Syntheses of Both Enantiomers of Neodictyoprolenol and Neodictyoprolene; Possible Biosynthetic Intermediates of Sex Pheromones in Brown Algae

Yuuko Yamamoto, Yoshihiko Akakabe, Kenji Matsui, Hiroshi Shimidzu and Tadahiko Kajiwara*

Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753-8515, Japan. Fax: +81-839-33-5849. E-mail: kajiwara@ayu.agr.yamaguchi-u.ac.jp

* Author for correspondence and reprint requests

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Neodictyoprolenol [(−)-(*S*)-(1,5*Z*,8*Z*)-undecatrien-3-ol], dictyoprolenol [(−)-(*S*)-(1,5*Z*)-undecadien-3-ol] and their acetates neodictyoprolene [(+)-(*S*)-3-acetoxy-(1,5*Z*,8*Z*)-undecatriene] and dictyoprolene [(+)-(*S*)-3-acetoxy-(1,5*Z*)-undecadiene], which are interesting as possible biosynthetic intermediates of the sex pheromones (dictyopterene **B, C′** and **D′**) of brown algae, were synthesized by chemo-enzymatic methods through optical resolution of racemic neodictyoprolenol and dictyoprolenol using two lipases; Amano PS (*Pseudomonas* sp.) and Novozym 435® (*Candida* sp.). A combination of acylation of the alcohols and hydrolysis of the acetates by Novozym 435® produced neodictyoprolenol, neodictyoprolene, dictyoprolenol and dictyoprolene with high optical purities over 99% enantiomeric excess (*e.e.*).

This snythetic methods will make it easier to search these compounds in marine algae and to study their biosynthesis.

Introduction

It has been demonstrated that thalli of marine brown macroalgae produce some odoriferous compounds, which were secreted as physiologically active compounds such as sex pheromones and antifeedants in brown algae (Moor *et al.*, 1974; Wooland *et al.*, 1975; Boland *et al.*, 1987, 1989; Oldhaim *et al.*, 1996; Kajiwara *et al.*, 1993).

Recently, Yamada *et al.* (1979) have identified (+)-(*S*)-3-acetoxy-(1,5*Z*)-undecadiene [dictyoprolene **(+)-2a**] (Yamada *et al.*, 1979) and (+)-(*S*)-3-acetoxy-(1,5*Z*,8*Z*)-undecatriene [neodictyoprolene **(+)-1a**] (Yamada *et al.*, 1980, 1986) from the brown algae, *Dictyopteris prolifera.* These compounds are proposed as possible biosynthetic intermediates of dictyopterene **B, C′** and **D′**, the sex pheromones of the brown algae (Moore *et al.*, 1977). Kajiwara *et al.* (1982) have investigated a series of these compounds from thallus extracts of the species of *D. prolifera* and *D. undulata*, and have found out (1,5*Z*)-undecadien-3-ol (dictyoprolenol **2**), which is corresponded to the alcolhol form of **2a**. However, none of the enantionmers of these compounds have been prepared in a synthetic scale though they have been synthesized as racemates (Kajiwara *et al.*, 1993; Yamada *et al.*, 1986).

Thus, some convenient methods for enantioselective syntheses of both the enantiomers of **1, 2, 1a,** and **2a** are needed for identification of products and for elucidation of the biosynthetic pathway by incubation experiments with homogenates of brown thalli. Here, we describe a convenient enantioselective synthesis of **1, 2** and the corresponding acetates through lipase-catalyzed asymmetric acylation or hydrolysis of the racemates.

Materials and Methods

All air and moisture sensitive reactions were run ander N_2 atmosphere. All solvents were distilled before use. IR spectra were measured with a Hitachi 260–10 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ with TMS as the internal reference at 250 MHz with a Hitachi R-250 spectrometer. ^{13}C NMR spectra were measured in $CDCl_3$ at 62.5 MHz with the Hitachi R-250 spectrometer. Optical rotations were recorded by Jasco DIP-4 Digital Polarimeter with chloroform as the solvent. GC analyses were performed with a Shimadzu GLC-6A on a glass column of 2% OV-1 (column 3 mm×1 m; flow rate of N_2 30 ml/min; column temp. 50 to 210 °C at the rate of 2 °C/min),

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(column 3 mm×1 m; flow rate of N_2 50 ml/min; column temp. 70 to 210 °C at the rate of 3 °C/min), on a capillary column of SF-96 (column 0.25 mm× 50 m; flow rate of N_2 100 ml/min; column temp. 70 to 210 °C at the rate of 2 °C/min), on a capillary column of DB-WAX (column 0.53 mm×30 m; flow rate of N_2 80 ml/min made up by 40 ml/min; column temp. 50 to 180 °C at the rate of 2 °C/min) and on a capillary column of CP-Cyclodex 236 M (column 0.25 mm×50 m; flow rate of N_2 100 ml/min; column temp. 110 °C hold). Column chromatography war performed on Merck Kieselgel 60, No. 7734. Novozym 435 was purchased from Novo Nordisk.

Syntheses of (1,5Z,8Z)-undecatrien-3-ol [(±)-1] *and 3-acetoxy-1,5Z,8Z)-undecatriene* [(±)-1a]

The syntheses of **1** and **1a** is summarized in Fig. 1.

The base-promoted epoxy-opening reaction of ethylene oxide with acetylene, employing sodium amide in liq. ammonia, gave 3-butyn-1-ol (**5**) in 73% yield. Treatment of the alcohol **5** with 3,4-dihydro-2*H*-pyran (DHP) under a catalytic amount of conc. HCl gave tetrahydropyranyl ether (THP ether) **6** in 88% yield.

The hydroxy group of propargyl alcohol **7** was protected as a THP ether to give **8**, which was followed by the coupling reaction with ethyl bromide to afford compound **9** in 71% yield from **7**. The ether **9** was treated with *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) in methanol to give 2-pentynol (**10**). Resulting **10** was converted into 2-pentynyl bromide (**11**) with phosphorus tribromide (PBr_3) under a catalytic amount of pyridine in 74% yield from **9**.

Grignard coupling of THP ether **6** with the bromide **11** in tetrahydrofuran (THF) gave **12** in 75% yield. The tetrahydropyranyl protecting group of **12** was removed by treating with a catalytic amount of *p*-TsOH·H_2O in methanol to give (3,6)-nonadiynol (**13**) in 57% yield from **6**.

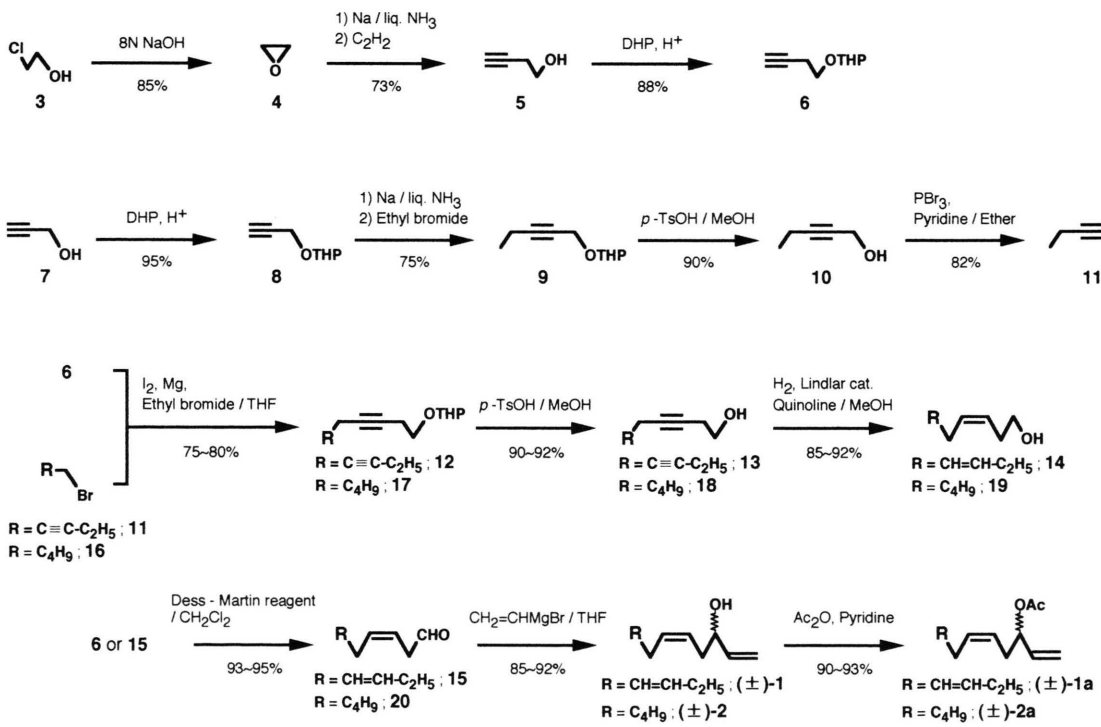


Fig. 1. Synthetic route of the racemic alcohols [**(±)-1** or **(±)-2**] and the acetates [**(±)-1a** or **(±)-2a**].

Catalytic hydrogenation of **13** under a Lindlar catalyst gave (3*Z*,6*Z*)-nonadienol (**14**, which was followed by oxidation with Dess-Martin reagent to give (3*Z*,6*Z*)-nonadienal (**15**) in 81% yield from **13**.

Finally, Grignard coupling of the aldehyde **15** with vinylmagnesium bromide in THF gave (1,5*Z*,8*Z*)-undecatrien-3-ol (neodictyoprolenol; **(±)-1**) in 85% yield, and **1** was treated with a mixture of acetic anhydride (Ac_2O) and pyridine to give the acetate derivative 3-acetoxy-(1,5*Z*,8*Z*)-undecatriene (neodictyoprolene; **(±)-1a**) in 90% yield (Fig. 1).

Neodictyoprolenol [**(±)-1**] IR (film) cm^{-1}: 3400 ~ 3300 (O–H), 3020, 2970, 2930, 2880 (C–H), 1679) (C=C), 1030 (C–O), 990, 920 (=C–H).

^{13}C NMR ($CDCl_3$) ppm: δ = 140.4, 132.2, 131.6, 126.6, 126.7, 124.6, 114.8, 72.4, 35.0, 25.7, 20.6, 14.2.

^1H NMR ($CDCl_3$) ppm: δ = 5.95 ~ 5.87 (m, 1H), 5.59 ~ 5.23 (m, 4H), 5.16 ~ 5.12 (d, 2H), 4.19 ~ 4.12 (m, 1H), 2.84 ~ 2.79 (t, 2H), 2.38 ~ 2.32 (t, 2H), 2.10 ~ 2.01 (m, 2H), 1.00 ~ 0.94 (t, 3H).

Neodictyoprolene [**(±)-1a**] IR (film) cm^{-1}: 3030, 2980, 2950, 2890 (C–H), 1740 (C=O), 1660 (C=C), 1230 (O–C=O), 990, 920 (=C–H).

^{13}C NMR ($CDCl_3$) ppm: δ = 169.9, 136.0, 132.0, 131.1, 126.7, 123.8, 116.5, 74.0, 32.1, 25.6, 21.0, 20.5, 14.1.

^1H NMR ($CDCl_3$) ppm: δ = 5.88 ~ 5.75 (m, 1H), 5.52 ~ 5.16 (m, 7H), 2.81 ~ 2.76 (t, 2H), 2.46 ~ 2.37 (m, 2H), 2.10 ~ 2.04 (m, 5H), 1.00 ~ 0.94 (t, 3H).

Syntheses of (1,5Z)-undecadien-3-ol [**(±)-2**] *and 3-acetoxy-(1,5Z)-undecadiene* [**(±)-2a**]

As shown in Fig. 1, C_{11}-dienol [(1,5*Z*)-undecadien-3-ol, **(±)-2**] and its acetate [3-acetoxy-(1,5*Z*)-undecadiene, **(±)-2a**] were prepared by Grignard coupling between C_9-THP ether and vinylmagnesium bromide.

Coupling of THP ether **6** with bromide **16**, employing sodium amide in liq. ammonia, afforded 2-(3-nonynyloxy)-tetrahydropyran (**17**) in 59.5% yield. According to the same way to the case of **(±)-1**, (1,5*Z*)-undecadien-3-ol (Dictyoprolenol; **(±)-2**) was obtained in 42.4% yield from **16**. The alcohol (**2**) was treated with Ac_2O in pyridine under mild condition to give 3-acetoxy-(1,5*Z*)-undecadiene (dictyoprolene; **(±)-2**) in 90.4% yield.

Dictyoprolenol [**(±)-2**] IR (film) cm^{-1}: 3370 (O–H), 2980, 2950, 2880 (C–H), 1050 (C–O), 1000, 925 (C=CH).

^{13}C NMR ($CDCl_3$) ppm: δ = 140.0, 132.8, 123.75, 114.0, 71.9, 34.5, 30.9, 28.7, 26.8, 22.0, 13.4.

^1H NMR ($CDCl_3$) ppm): δ = 5.11 ~ 5.97 (m, 5H), 4.15 (m, 1H), 2.32 (t, 2H), 2.04 (q, 2H), 1.68 (d, 1H), 1.23 ~ 1.43 (m, 6H), 0.89 (t, 3H).

Dictyoprolene [**(±)-2a**] IR (film) cm^{-1}: 3040 (C=C–H), 2980, 2950, 2880 (C–H), 1760 (C=O), 1250 (O–C=O), 1000, 940 (C=CH_2), 720 (*Z*, C=C).

^{13}C NMR ($CDCl_3$) ppm: δ = 169.5, 135.6, 132.5, 122.9, 116.0, 73.7, 31.7, 30.9, 28.6, 26.8, 22.0, 20.5, 13.4.

^1H NMR ($CDCl_3$) ppm: δ = 5.15 ~ 5.89 (m, 6H), 2.34 ~ 2.39 (m, 2H), 2.01 ~ 2.14 (m, 2H), 2.06 (s, 3H), 1.30 ~ 1.43 (m, 6H), 0.89 (t, 3H).

Screening of lipases for optical resolutions of **(±)-1, (±)-1a, (±)-2** *and* **(±)-2a**

Two lipases, Amano PS (*Pseudomonas cepacia*) and Novozym 435® (*Candida antarctica*), were examined to resolve both the enantiomers of **(±)-1a** *or* **(±)-2a** in varied conversion; the racemic acetate **(±)-1a** *or* **(±)-2a** prepared from **(±)-1** and **(±)-2a** respectively was testet in a phosphate buffer-acetone solution in the presence of both lipases at room temperature. The enantioselectivity of Novozym 435® was slightly superior to Amano PS although it required much more time than the latter. Novozym 435® retained (*S*)-selectivity for long reaction time may be due to immobilizing treatment while Amano PS tended to be losing the activity (Table I). Optical purities of **(*S*)-1a, (*R*)-1a, (*S*)-2a** and **(*R*)-2a** were determined by chiral GC analysis. Those of **(*S*)-1, (*R*)-1, (*S*)-2** and **(*R*)-2** were estimated as the corresponding acetate after treatment with Ac_2O in pyridine.

Preparations of (+)-(R)-alcohols [**(+)-1** *or* **(+)-2**] *and (−)-(R-acetates* [**(−)-1a** *or* **(−)-2a**] *by Novozym 435®-catalyzed acylation*

To a solution of **(±)-1** (0.5 g, 2.9 mmol) in vinyl acetate (15 ml) Novozym 435® (0.5 g) was added. After being stirred for 5 h at room temperature, the mixture was filtered and extracted with diethyl ether. The ethereal solution was washed with brine, and dried over anhydrous Na_2SO_4. The ex-

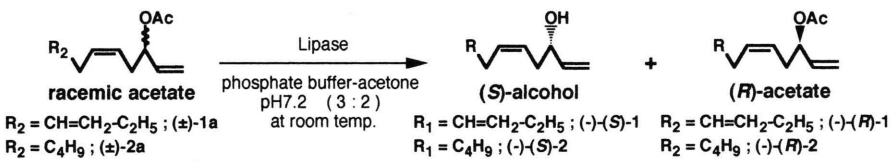
tract was concentrated *in vacuo*, and purified by silica gel column chromatography. Elution with *n*-hexane/diethyl ether (5:1 v/v) first furnished **(*S*)-1a** (0.4 g, 1.7 mmol) in 60% yield with 53% *e.e.* as estimated (as an acetate derivative) by GC. Subsequently, **(*R*)-1** (0.2 g, 0.9 mmol) in 32% yield, $[\alpha]_D^{23}$ +2.2° (*c* = 0.010, $CHCl_3$), was eluted, and its enantiomeric purity was estimated by GC as 99% *e.e.* Acetylation of **(*R*)-1** (99% *e.e.*, 0.1 g, 0.6 mmol) with Ac_2O (3 ml) in pyridine (2 ml) at 0 °C gave **(*R*)-1a** (0.1 g, 0.5 mmol) as a colorless oil in 90% yield, $[\alpha]_D^{23}$ −16.2° (*c* = 0.010, $CHCl_3$), with 99% *e.e.* as estimated by GC. The IR and ^1H NMR spectra **(*R*)-1** and the acetate **(*R*)-1a** were identical with those of the corresponding racemates.

(*R*)-2 and **(*R*)-2a** were prepared correspondingly to preparations of **(*R*)-1** and **(*R*)-1a**. The specific rotatory powers of **(*R*)-2** and **(*R*)-2a** were $[\alpha]_D^{23}$ +1.4° (*c* = 1.3, $CHCl_3$) and $[\alpha]_D^{23}$ −14.8° (*c* = 1.1, $CHCl_3$), respectively.

Preparations of (−)-(S)-alcohols **[(−)-1** *and* **(−)-2]** *and (+)-(S)-acetates* **[(+)-1a** *or* **(−)-2a]** *by Novozym 435®-catalyzed hydrolysis*

To a suspension of **(±)-1a** (0.5 g, 2.4 mmol) in phosphate buffer (pH 7.2, 15 ml) and acetone (10 ml) Novozym 435® (0.5 g) was added. After being stirred for 5 h at room temperature, the mixture was filtered and extracted with diethyl ether. The ethereal solution was washed with brine, and dried over anhydrous Na_2SO_4. The extract was concentrated *in vacuo*, and purified by silica gel column chromatography. Elution with *n*-hexane/diethyl ether (5:1 v/v) first furnished **(*R*)-1a** (0.3 g, 1.5 mmol) in 61% yield with 49% *e.e.* as estimated by GC. Subsequently, **(*S*)-1** (0.1 g, 0.7 mmol) in 31% yield, $[\alpha]_D^{23}$ −1.8° (*c* = 0.010, $CHCl_3$), was eluted and its enantiomeric purity was estimated (as an acetate derivative) by GC as 99% *e.e.* Acetylation of **(*S*)-1** (99% *e.e.*, 0.1 g, 0.6 mmol) with Ac_2O (3 ml) in pyridine (2 ml) at 0 °C gave **(*S*)-1a** (0.1 g, 0.5 mmol) as a colorless oil in 91% yield, $[\alpha]_D^{23}$

Table I. Enantioselectivities of Amano PS and Novozym 435 in hydrolysis.



Lipase	Substrate	Reaction time (hr)	Conv. (%)	(-)-(*S*)-1 or (-)-(*S*)-2 *e.e.* (%)	(-)-(*R*)-1a or (-)-(*R*)-1a *e.e.* (%)	*E***
Amano PS	**(±)-1a**	3	41.3	97.2	68.3	< 100
		5	50.5	88.5	87.9	
	(±)-2a	3	39.0	99.0	63.3	< 100
	(±)-16a*	5	13.2	97.4	14.7	< 100
Novozym 435	**(±)-1a**	5	33.2	99.0	49.1	> 300
		8	40.6	98.6	68.2	
	(±)-2a	3	30.2	99.3	43.0	> 300
	(±)-16a*	1	42.9	92.0	67.8	< 80

*3-acetoxy-(5*Z*,8*Z*)-undecadien-1-yn

**Enantiomeric ratio; a biochemical constant that is independent of substrate concentration and the extent of conversion (C. S. Chen and C. J. Shin, 1989).

+10.0° (c = 0.005, $CHCl_3$), with 99% *e.e.* as estimated by GC. The IR and ^1H NMR spectra of **(*S*)-1** and the acetate **(*S*)-1a** were identical with those of the corresponding racemates.

(*S*)-2 and **(*S*)-2a** were prepared correspondingly to preparations of **(*S*)-1** and **(*S*)-1a**. The specific rotatory powers of **(*S*)-2** and **(*S*)-2a** were $[\alpha]_D^{23}$ −2.1° (c = 1.1, $CHCl_3$) and $[\alpha]_D^{23}$ +17.4° (c = 1.2, $CHCl_3$), respectively.

Results and Discussion

Based on the characteristics of lipases summarized in Tables I and II, some optical resolution systems repeating or combining hydrolysis and/or acylation were attempted to obtain both enantiomers with high optical purity in good chemical yield.

The systems tried were:

(i) hydrolysis by Amano PS was repeated two times

(ii) hydrolysis by Amano PS was repeated three times

(iii) hydrolysis by Novozym 435® was repeated two times

(iv) one hydrolysis and one acylation by Novozym 435® were combined

(v) one acylation and one hydrolysis by Novozym 435® were combined

The best results were obtained by system (v) in which Novozym 435® was employed as a castalyst both in first acylation and in subsequent hydrolysis (Fig. 2). In the first acylation, **(+)-(*R*)-1** with 99.1% *e.e.* was obtained in 31.5% yield with an excess conversion of 65.2%. Then acetylated **(*S*)-1a** with 53.4% *e.e.* was hydrolyzed in 59.5% yield by the same lipase to afford **(*S*)-1** with 99.2% *e.e.* in 38.2% yield. System (iv) was also efficient and never inferior to (v) for preparation of the both enantiomers with high *e.e.* value. However, a preceding hydrolysis of the acetates by alkali treatment was required for the last asymmetric acylation and the overall yield was low.

Systems (i), (ii) and (iii) were able to afford only the (S)-enantiomer, since repeated hydrolysis by Amano PS or Novozym 435® never consumed the (S)-acetate completely and always left the (R)-fraction containing a small amount of (S)-enantiomer. Therefore, at least one acylation was necessary to obtain an enantiomerically pure (R)-form.

As seen in Tables I and II, the enantiomeric specificities of lipases to **(±)-2** and **(±)-2a** were very similar to those of **(±)-1** and **(±)-1a** although the reaction rate was slightly lower.

Acknowledgement

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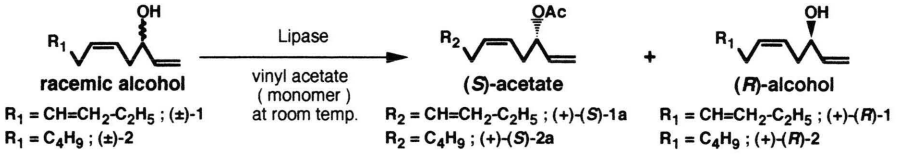
Table II. Enantioselectivities of Novozym 435 in acylation.



Lipase	Substrate	Reaction time (hr)	Conv. (%)	(+)-(*S*)-1a or (+)-(*S*)-2a *e.e.* (%)	(+)-(*R*)-1 or (+)-(*R*)-2 *e.e.* (%)	*E* **
Novozym 435	**(±)-1**	3	51.2	63.3	66.4	< 10
		4	62.2	55.2	91.0	
	(±)-2	4	61.1	57.1	89.7	< 10

**See Table I.

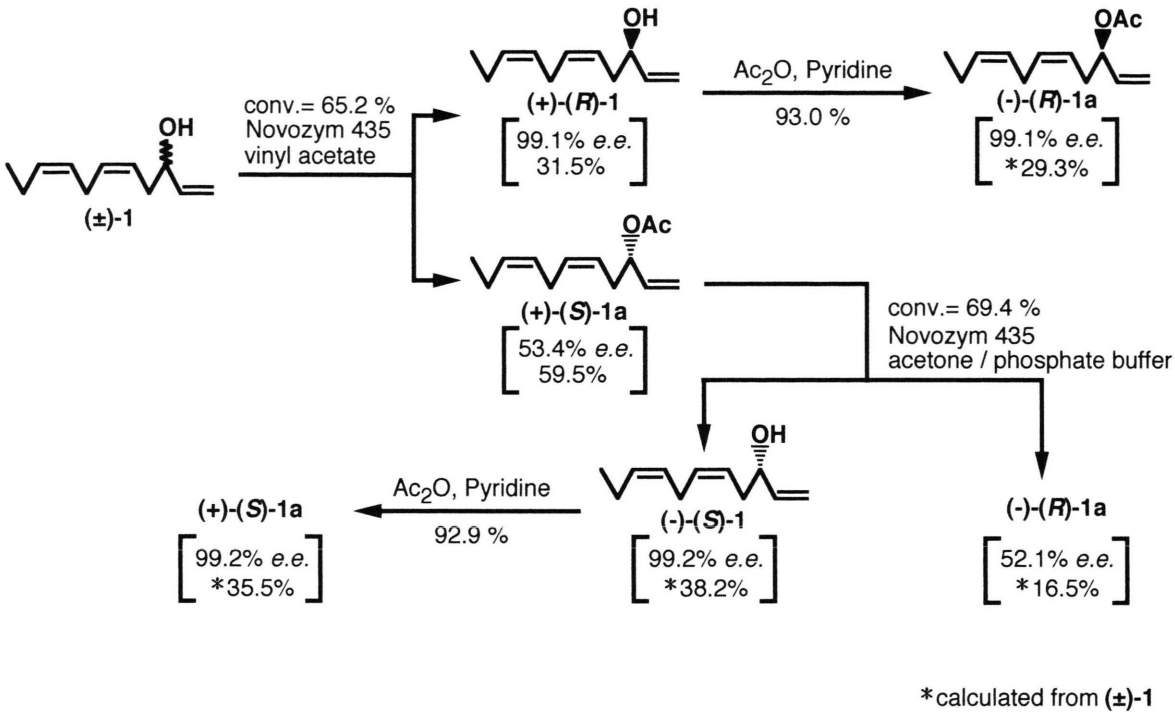


Fig. 2. Optical resolution of racemic alcohol [**(±)-1a**] by acylation and hydrolysis using Novozym 435.

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