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DEDIASTEREOMERIZATION OF DIBENZYLBUTANOLIDES BY PLANT CELL CULTURES

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Abstract – When a mixture of two diastereomers (4*R**-**3**) and (4*S**-**3**) was subjected to plant cell cultures, hydrolysis of acetate and dediastereomerization took place to give a single diastereomer (4*R**-**1**) with 100% diastereomeric excess and 0% enantiomeric excess.

INTRODUCTION

Lignans have attracted much attention for their variation of structures together with important pharmacological activities.[1] Podophyllotoxin and its analogues such as epipodophyllotoxin are naturally occurring or modified cytotoxic lignans, and can serve as precursors to clinical antitumor agents, etoposide and teniposide. Much effort has been devoted to developing an efficient method for the asymmetric synthesis of their lignans.[2, 3] Achiwa *et al.* reported efficient asymmetric synthesis of naturally occurring lignan lactones using asymmetric catalytic hydrogenation as a crucial step.[4] On the other hand, enzymes are known to be versatile and are widely used as catalyst in asymmetric syntheses. Natural (-)-deoxypodophyllotoxin is microbially convertible to (-)-epipodophyllotoxin.[5] We previously reported a novel deracemization method of racemic alcohols using plant cell cultures, i.e., 100% conversion of racemic alcohols to the corresponding optically active alcohols (100% ee).[6-8] Deracemization is the most promising method for the preparation of optically active alcohols from racemates, because it permits 100% conversion of the starting racemates to the corresponding chiral compound, whereas the theoretical maximum yield is 50% for enzymatic or chemical resolution. For the asymmetric synthesis of lignans, deracemization is the most promising method. Then, we tried deracemization of dibenzylbutanolides,

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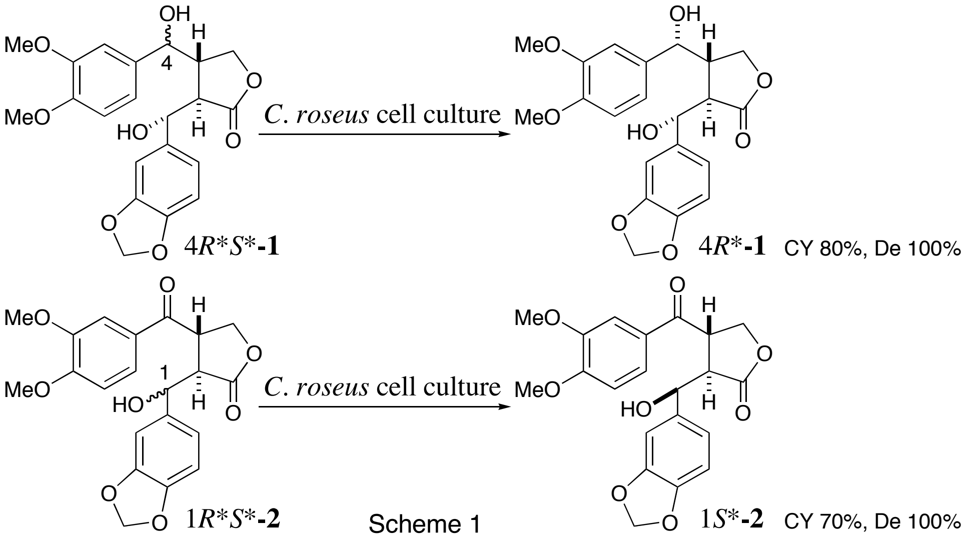
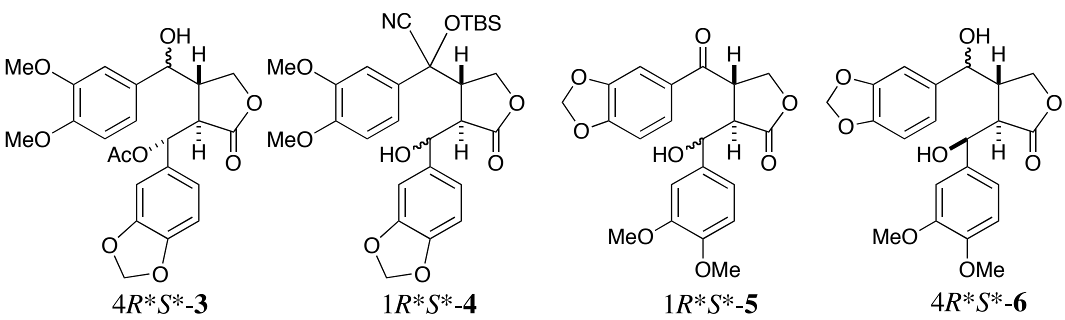
This paper is dedicated to Professor Steven M. Weinreb on the occasion of his 65th birthday.

which are the key intermediate for the synthesis of podophyllotoxin derivatives or furofuran series. But the deracemization of dibenzylbutanolides (4*R**-**1**, 1*S**-**2**) by plant cell culture failed to afford racemate (4*R**-**1**, 1*S**-**2**). But, in these experiment, we developed a novel dediastereomerization method of dibenzylbutanolides, i.e., reactions allowing the transformation of two diastereomers into one diastereomer in quantitative yield with plant cell cultures.[9,10] As shown in Scheme 1, the compound (4*R***S**-**1**) has two diastereomers; one is 4*R**-**1** and the other is 4*S**-**1**.



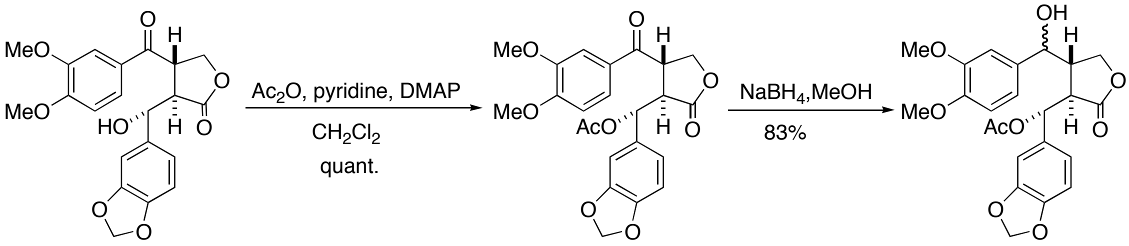
Scheme 1

When a mixture of the two diastereomers (4*R**-**1**) and (4*S**-**1**) (1:1 ratio) was subjected to *C. roseus* cells in B5[11] medium, only 4*R**-**1** was isolated in 80% chemical yield with 100% diastereomeric excess and 0% enantiomeric excess.[9] In the same way, a mixture of two diastereomers (1*R**-**2**) and (1*S**-**2**) (1:1 ratio) was converted to 1*S**-**2** in 70% chemical yield with 100% diastereomeric excess and 0% enantiomeric excess.[9] It is quite interesting that the plant cell culture can discriminate the two diastereomers. This new method is important for the diastereoselective synthesis of lignans. Then, we studied the biocatalytic dediastereomerization of substituted dibenzylbutanolide such as 4*R***S**-**3**, 1*R***S**-**4**, 1*R***S**-**5** and 4*R***S**-**6** to explore the catalytic ability of plant cell cultures.



RESULTS AND DISCUSSION

Dibenzylbutanolides (1*R***S**-**4**, 1*R***S**-**5**) were synthesized according to the reported procedure (1*R***S**-**4**[10],1*R***S**-**5**[12]). Dibenzylbutanolide (4*R***S**-**3**) was prepared as shown in Scheme 2. Compound (**7**) was synthesized from 3,4-dimethoxybenzaldehyde according to the reported procedure.[10] Compound (**7**) was acetylated with acetic anhydride, pyridine and dimethylaminopyridine to afford **8** quantitatively, which was reduced with $NaBH_4$ in MeOH to give 4*R***S**-**3** [the mixture of 4*R**-**3** and 4*S**-**3** (3:1 ratio)] in 83% yield. Compound (4*R***S**-**6**) was synthesized from 1*S**-**5**. Compound (1*S**-**5**) was reduced with $NaBH_4$ in MeOH to give 4*R***S**-**6** [the mixture of 4*R**-**6** and 4*S**-**6** (3:1 ratio)] in 71% yield.

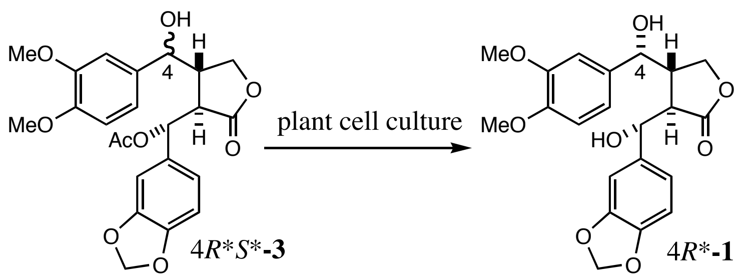


7 **8** 4*R***S**-**3**

Scheme 2. Synthesis of Dibenzylbutanolide 4*R***S**-**3**

We then proceeded to investigate dediastereomerization of dibenzylbutanolides (4*R***S**-**3**, 1*R***S**-**4**, 1*R***S**-**5** and 4*R***S**-**6**) with plant cell cultures. In this work, we used suspension-cultured cells which had originally been isolated from *Nicotiana* (*N.*) *tabacum* "Bright Yellow-2", *Daucus* (*D.*) carota, *Camellia* (*C.*) *sinensis* and *Catharanthus* (*C.*) *roseus*. These cell cultures (*N. tabacum*, *D. carota*, *C. roseus* and *C. sinensis*) were prepared as described in our previous papers.[13-16] The dediastereomerization of the compound 4*R***S**-**3** [the mixture of 4*R**-**3** and 4*S**-**3** (1:1 ratio)] was performed with freely suspended plant cell cultures in the stationary phase after incubation (*C. sinensis* 18 days, *N. tabacum* 22 days, *D. carota* 12 days, *C. roseus* 8 days). A substrate (50 mg) was added to the freely suspended *C. roseus* (B-5 medium, pH 5.5), *N. tabacum* "Bright Yellow-2" (MS[17] medium, pH 5.8), *D. carota* (MS medium, pH 5.8), and *C.sinensis* (B-5 medium, pH 5.8). The mixture was shaken at 25 °C in a rotary shaker (110 rpm) in the dark. Biotransformation of 4*R***S**-**3** was examined as shown in Table 1.

Table 1. Dediastereomerization of 4*R***S**-**3**



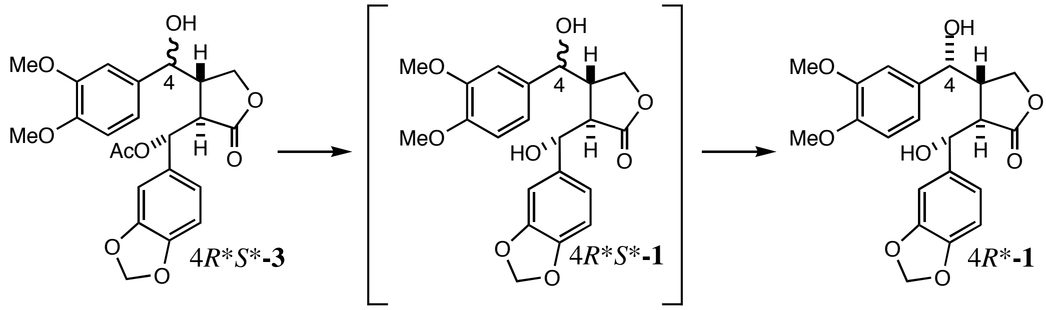
Entry	Plant cell culture	Time (days)	4*R**-**1** C.Y.(%)[a]	De(%)[b]	Ee(%)	Recovered (4*R***S**-**3**)
1	*C. roseus*	15	37	100	0	45
2	*C. sinensis*	15	11	100	0	74
3	*N. tabacum*	15	24	100	0	47
4	*D. carota*	15	0			88

[a] Isolated yield. [b] Both diastereomer ratio and optical yields were determined by HPLC analysis (chiralpak AD hexane / IPA = 5 / 1).

When racemic acetate (4*R***S**-**3**) [a mixture of two diastereomers (4*R**-**3**) and (4*S**-**3**) (1:1 ratio)] was subjected to *C. roseus* cell culture for 15 days, racemic acetate (4*R***S**-**3**) was converted to the desired single diastereomer butanolide (4*R**-**1**) with 37% chemical yield, 100% diastereomeric excess (De) and 0% enantiomeric excess (Ee) (Entry 1). The recovered acetate was a mixture of two diastereomers (4*R**-**3**) and (4*S**-**3**) (1:1 ratio). We next surveyed a variety of plant cell cultures (Entries 2-4). In the case of *C. sinensis* and *N. tabacum*, these yields of 4*R**-**1** were lower than that of *C. roseus*. But, De was 100% (Entries 2, 3). The recovered acetates (Entries 2,3) were a mixture of two diastereomers (4*R**-**3**) and (4*S**-**3**) (1:1 ratio). In the case of *D. carota*, the biotransformation of 4*R***S**-**3** was unsuccessful to afford recovered acetate [a mixture of two diastereomers (4*R**-**3**) and (4*S**-**3**) (1:1 ratio)] (Entry 4). The structure of product (4*R**-**1**) was confirmed by a comparison of the mp and ^{1}H-NMR data with that reported.[10] *C. roseus* cell culture is far superior for the present study.

Although the mechanism is not clear at present, it is reasonable to expect, from our results, that this reaction is performed by a two-step process as shown in Scheme 3. In this reaction, we presumed 4*R***S**-**1** to be the intermediate.

At first, a mixture of two diastereomers (4*R**-**3**) and (4*S**-**3**) (1:1 ratio) was hydrolyzed by plant cell culture-catalyzed hydrolysis of acetate to afford two diastereomers (4*R**-**1**) and (4*S**-**1**) (1:1 ratio). These results show that this hydrolysis is not enzyme-catalyzed diastereoselective hydrolysis based on remote recognition of the C_4-hydroxyl group away from the reaction site (acetyl group).[18] Next, a mixture of two



Scheme 3

diastereomers (4*R**-**1**) and (4*S**-**1**) was converted to a single diastereomer (4*R**-**1**) with 100% diastereomeric excess and 0% enantiomeric excess by the plant cell culture-catalyzed dediastereomerization method. The reaction time was 5 h in the case of dediastereomerization of 4*R***S**-**1** into 4*R**-**1** by *N. tabacum* cell culture.[9] But these reactions take a long time. These facts show that the hydrolysis of acetate requires a long time. In contrast, the following dediastereomerization proceeded relatively very quickly to afford 4*R**-**1** with 100% de.

We then proceeded to investigate the biotransformation of 1*R***S**-**4**, 1*R***S**-**5**, 4*R***S**-**6** by plant cell cultures, but biotransformation of these substrates was unsuccessful. No reaction products were given and the recovered material was afforded in 70-90 % yield. The reaction time and chemical yields are listed in the Experimental.

In conclusion, the process can be highly efficient if the substrate, as in the case of 4*R***S**-**3**, possesses an acetyl group at the benzyl position of the dibenzylbutanolides. We succeeded in a one-pot, two-step enzymatic bioprocess which includes: (1) hydrolysis of acetate, (2) dediastereomerization of two diastereomers into one diastereomer. Studies are now in progress to shorten the reaction time.

EXPERIMENTAL

General Experimental Procedures. ^{1}H-NMR spectra were measured at 270 MHz on a JEOL JNM-EX 270 FT NMR spectrometer. Chemical shifts are quoted in ppm with tetramethylsilane as an internal standard, and coupling constants (*J*) are given in Hz. FAB-MS was taken on a JEOL JMS-SX 102 mass spectrometer.

Preparation of (±)-*trans*-2-(α-acetoxy-3,4-methylenedioxybenzyl)-3-(3,4-dimethoxybenzoyl)-butanolide (8)

Racemic (±)-2-(α-hydroxy-3,4-methylenedioxybenzyl)-3-(3,4-dimethoxybenzoyl)butanolide (**7**) (100 mg, 0.25 mmol) was added to a stirred solution of acetic anhydride (0.5 mL), pyridine (1.0 mL), dimethylaminopyridine (DMAP, 20 mg) and CH_2Cl_2 (2 mL), and the mixture was stirred for 12 h at rt. The reaction solution was concentrated *in vacuo* and the residue was subjected to column chromatography on SiO_2 using hexane/AcOEt (1:1) as an eluent to give (±)-**8** in quantitative yield.

(±)-**8**: mp 151-153℃. FAB-MS *m/z* 442 (M^+).

^{1}H-NMR ($CDCl_3$) δ: 2.04 (3H, s, -OAc), 3.80 (1H, dd, *J*=4.6, 8.6 Hz), 3.91 (3H, s, -OMe), 3.97 (3H, s, -OMe), 4.13 (1H, m), 4.48-4.60 (2H, m), 5.80 (1H, d, *J*=1.4 Hz, -OCH_2O-), 5.87 (1H, d, *J*=1.4 Hz, -OCH_2O-), 6.22 (1H, d, *J*=4.6 Hz), 6.60-6.67 (3H, m, Ar-H), 6.87 (1H, d, *J*=8.3 Hz, Ar-H), 7.29 (1H, d, *J*=9.0 Hz, Ar-H), 7.35 (1H, dd, *J*=1.6, 8.4 Hz, Ar-H).

Preparation of (±)-*trans*-2-(α-acetoxy-3,4-methylenedioxybenzyl)-3-(3,4-dimethoxy-α-hydroxybenzyl) butanolide (4*R-3) and (±)-*trans*-2-(α-acetoxy-3,4-methylenedioxybenzyl)-3-(3,4-dimethoxy-β-hydroxybenzyl)butanolide (4*S**-3)**

To a solution of **8** (70 mg, 0.16 mmol) in MeOH (5 mL) was added in one portion $NaBH_4$ (8.6 mg, 0.23 mmol) at 0℃. The mixture was stirred for 1h at rt. The solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (20 mL) and the solution was washed with brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to preparative TLC using CH_2Cl_2/MeOH (50:1) as an eluent to give 4*R**-**3** (45 mg, 64.5 %) and 4*S**-**3** (15 mg, 21.5 %).

4*R**-**3**: FAB-MS *m/z* 444 (M^+).

^{1}H-NMR ($CDCl_3$) δ: 1.90 (1H, m), 2.12 (3H, s, -OAc), 2.76 (1H, m), 3.04 (1H, dd, *J*=3.7, 7.1 Hz), 3.78 (3H, s, -OMe), 3.87 (3H, s, -OMe), 4.27-4.38 (2H, m), 4.72 (1H, t, *J*=3.7 Hz), 5.94 (1H, d, *J*=1.7 Hz, -OCH_2O-), 5.96 (1H, d, *J*=1.7 Hz, -OCH_2O-), 6.10 (1H, d, *J*=3.6 Hz), 6.39-6.43 (2H, m, Ar-H), 6.53-6.60 (2H, m, Ar-H), 6.67-6.75 (2H, m, Ar-H)

4*S**-**3**: FAB-MS *m/z* 444 (M^+).

^{1}H-NMR ($CDCl_3$) δ: 1.93 (1H, br s, OH), 2.12 (3H, s, -OAc), 2.80 (1H, m), 2.82 (1H, dd, *J*=3.3, 7.9 Hz), 3.77 (3H, s, -OMe), 3.87 (3H, s, -OMe), 4.21-4.29 (2H, m), 4.58 (1H, dd, *J*=3.7, 9.0 Hz), 5.96 (1H, d, *J*=1.4Hz, -OCH_2O-), 5.96 (1H, d, *J*=1.4 Hz, -OCH_2O-), 6.10 (1H, d, *J*=3.0 Hz), 6.39 (1H, s, Ar-H), 6.48 (1H, d, *J*=1.6 Hz, Ar-H), 6.56-6.61 (2H, m, Ar-H), 6.67-6.74 (2H, m, Ar-H)

Preparation of (±)-*trans*-2-(β-hydroxy-3,4-dimethoxybenzyl)-3-(α-hydroxy-3,4-methylenedioxybenzyl)butanolide (4*R-6) and (±)-*trans*-2-(β-hydroxy-3,4-dimethoxybenzyl)-3-(β-hydroxy-3,4-methylenedioxybenzyl)butanolide (4*S**-6)**

To a solution of 1*S**-**5** (100 mg, 0.25 mmol) in MeOH (10 mL) was added in one portion $NaBH_4$ (19.5 mg, 0.52 mmol) at 0℃. The mixture was stirred for 1h at rt. The solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (40 mL) and the solution was washed with brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to preparative TLC using hexane/AcOEt (1:1) as an eluent to give 4*R**-**6** (53.2 mg, 64.5 %) and 4*S**-**6** (17.8 mg, 17.8 %).

4*R**-**6**: FAB-MS *m/z* 402 (M^+).

^{1}H-NMR ($CDCl_3$) δ: 2.71 (1H, t, *J*=8.9 Hz), 3.16 (1H, dd, *J*=4.0, 9.2 Hz), 3.66 (2H, m), 3.75 (1H, br s, OH), 3.89 (3H, s, -OMe), 3.90 (3H, s, -OMe), 3.96 (1H, br s, OH), 4.48 (1H, d, *J*=8.9 Hz), 5.42 (1H, d, *J*=4.0 Hz), 5.96 (2H, dd, -OCH_2O-), 6.69-6.77 (3H, m, Ar-H), 6.87 (1H, m, Ar-H), 6.98-7.01 (2H, m, Ar-H)

4*S**-**6**: FAB-MS *m/z* 402 (M^+).

^{1}H-NMR ($CDCl_3$) δ: 1.95 (1H, s, OH), 2.52 (1H, m), 2.97 (1H, t, *J*=8.2 Hz), 3.87 (3H, s, -OMe), 3.89 (3H, s, -OMe), 3.99 (2H, t), 4.08 (1H, s, OH), 4.35 (1H, dd, *J*=7.8, 9.0 Hz), 4.81 (1H, d, *J*=8.6 Hz), 5.95 (2H, dd, -OCH_2O-), 6.44-6.50 (3H, m, Ar-H), 6.69 (1H, m, Ar-H), 6.81-6.93 (2H, m, Ar-H)

Cultivation of *C. roseus* cells

Suspension cells of *C. roseus* were subcultured every 7 days by transferring a 1-week culture (8 mL) into B5 medium (80 mL) containing 2,4-dichlorophenoxyacetic acid (2,4-D) (1 mg/L) and 2% sucrose (pH 5.5) on a rotary shaker (110 rpm) at 25°C in the dark.

Cultivation of *D. carota* cells

Suspension cells of *D. carota* were subcultured every 7 days by transferring a 1-week culture (8 mL) into MS medium (80 mL) containing 2,4-D (2 mg/L) and 3% sucrose (pH 5.8) on a rotary shaker (110 rpm) at 25°C in the dark.

Cultivation of *N. tabacum* cells

Suspension cells of *N. tabacum* were subcultured every 7 days by transferring a 1-week culture (1.3 mL) into MS medium (80 mL) containing 2,4-D (2 mg/L) and 3% sucrose (pH 5.8) on a rotary shaker (110 rpm) at 25°C in the dark.

Cultivation of *C. sinensis* cells

Suspension cells of *C. sinensis* were subcultured every 10 days by transferring a 1-week culture (10 mL) into B5 medium (80 mL) containing 2,4-D (1.25 mg/L) and 5% sucrose (pH 5.8) on a rotary shaker (110 rpm) at 25°C in the dark.

Biotransformation of substrates (4*R*S**-3, 1*R***S**-4, 1*R***S**-5, 4*R***S**-6) with plant cell cultures**

A substrate (4*R***S**-**3**, 1*R***S**-**4**, 1*R***S**-**5**, 4*R***S**-**6**) (50 mg) was added to the freely suspended *C. roseus* (3 g of cells and 30 ml broth, B-5 medium, pH 5.5, 8 d old), *N. tabacum* ' Bright Yellow-2' (2.5 g of cells and 30 ml broth, MS medium, pH 5.8, 22 d old), *D. carota* (3 g of cells and 30 ml broth, MS medium, pH 5.8, 12 d old), and *C. sinensis* (3 g of cells and 30 ml broth, B-5 medium, pH 5.8, 18 d old). The mixture was shaken at 25°C on a rotary shaker (110 rpm) in the dark. At the termination of the reaction, the incubation mixture was filtered, and the filtered cells were washed with AcOEt. The filtrates and washings were combined and extracted with AcOEt. The AcOEt layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to silica gel column using CH_2Cl_2/MeOH (50:1) as an eluent. The reaction time and the chemical yield of 4*R***S**-**3** are listed in Table 1. The reaction time and the chemical yields of 1*R***S**-**4**, 1*R***S**-**5**, 4*R***S**-**6** are given below.

1*R***S**-**4** (*C. roseus* cell): reaction time 15 days, recovered 1*R***S**-**4** (70%). 1*R***S**-**4** (*N. tabacum* cell): reaction time 15 days, recovered 1*R***S**-**4** (70%). 1*R***S**-**4** (*D. carota* cell): reaction time 15 days, recovered 1*R***S**-**4** (90%).

1*R***S**-**5** (*C. roseus* cell): reaction time 30 days, recovered 1*R***S**-**5** (70%). 1*R***S**-**5** (*C. sinensis* cell): reaction time 18 days, recovered 1*R***S**-**5** (89%). 1*R***S**-**5** (*N. tabacum* cell): reaction time 18 days, recovered 1*R***S**-**5** (70%). 1*R***S**-**5** (*D. carota* cell): reaction time 18 days, recovered 1*R***S**-**5** (70%).

4*R***S**-**6** (*C. roseus* cell): reaction time 15 days, recovered 4*R***S**-**6** (72%). 4*R***S**-**6** (*C. sinensis* cell): reaction time 15 days, recovered 4*R***S**-**6** (89%). 4*R***S**-**6** (*N. tabacum* cell): reaction time 15 days, recovered 4*R***S**-**6** (70%). 4*R***S**-**6** (*D. carota* cell): reaction time 15 days, recovered 4*R***S**-**6** (90%).

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