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Pseudomonas fluorescens lipase-catalyzed asymmetric hydrolysis and transesterification of *meso*-2,5-bis(acetoxymethyl)- and bis(hydroxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran †

Kenji Matsuo,[a] Masakazu Tanaka,[a] Kiyoshi Sakai [b] and Hiroshi Suemune [a,*]

[a] Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812–82, Japan
[b] Kyushu Women's University, Jiyugaoka 1–1, Yahata-nishi, Kitakyushu 807, Japan

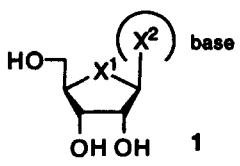
Abstract: *Pseudomonas fluorescens* lipase (PFL)-catalyzed asymmetric hydrolysis of *meso*-2,5-bis(acetoxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran **3** afforded the optically active monoacetate (−)-**7** of high enantiomeric excess (92% ee) in 94% yield. Transesterification of *meso*-bis(hydroxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran **6** using PFL in vinyl acetate gave the monoacetate (+)-**7** of 69% ee in low yield (15%). The absolute configuration of (−)-**7** was determined to be 2*S*,3*S*,4*R*,5*R*, by chemical correlation with D-allose **10**. © 1997 Published by Elsevier Science Ltd

Introduction

Metabolically stabilized nucleosides,[1] such as carbocyclic nucleoside, *C*-nucleoside, and *C*-carbocyclic nucleoside (**1a–c**, Figure 1), have attracted much interest among organic and medicinal chemists because some of them have strong biological activities as anti-viral and anti-tumor reagents. We have previously reported the effective synthesis of carbocyclic nucleosides, (−)-aristeromycin,[2] (−)-carbovir and (−)-BCA.[3] Therein, we reported that *meso*-1,3-bis(acetoxymethyl)cyclopentanes were hydrolyzed by *Rhizopus delemar* lipase (RDL)[4] or *Pseudomonas fluorescens* lipase (PFL)[5] into the monoacetates in an enantioselective manner. In this paper, we report the hydrolysis of *meso*-2,5-bis(acetoxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran **3** by RDL and PFL (Scheme 1). It was thought that the structure of *meso*-compound **3** was similar to that of *meso*-1,4-bis(acetoxymethyl)-2,3-(isopropylidenedioxy)cyclopentane **2** because only a cyclopentane carbon atom in compound **2** was replaced by an oxygen atom. Therefore, it was anticipated that the hydrolysis of **3** by PFL would proceed in an enantioselective manner to give the enantiomerically enriched monoacetate (−)-**7**. The chiral monoacetate **7** might be an important chiral building block in the synthesis of *C*-nucleosides such as showdomycin[6] and pseudouridine.[7]

Results and discussion

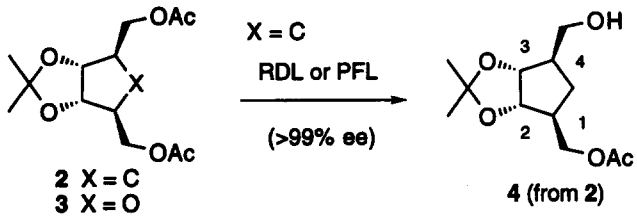
Substrates for enzymatic reaction could be prepared as follows. *cis*-Diol **5**, prepared according to the procedure by Addor,[8] was converted into the *meso*-diacetate **3** in 53% yield by a three-step sequence



1a X^1 = C, X^2 = N: Carbocyclic nucleoside
1b X^1 = O, X^2 = C: *C*-nucleoside
1c X^1 = C, X^2 = C: Carbocyclic *C*-nucleoside

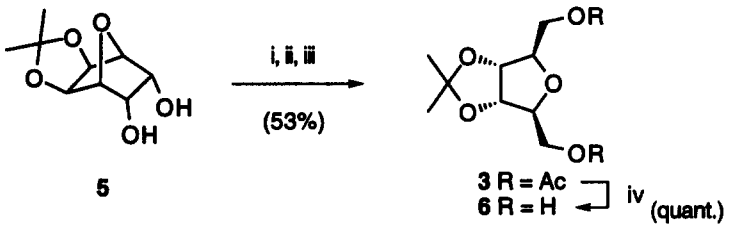
Figure 1. Carbocyclic nucleoside and *C*-nucleoside.

† Dedicated to Prof. Dr Dieter Seebach on the occasion of his 60th birthday.
* Corresponding author. Email: suemune@lyra.phar.kyushu-u.ac.jp



Scheme 1.

(Scheme 2): [(i) oxidation with $NaIO_4$; (ii) reduction with $NaBH_4$; (iii) acetylation with Ac_2O]. The spectroscopic data of **3** were identical with the reported values.[9] *meso*-Diol **6** was also prepared from **3** by solvolysis with K_2CO_3/MeOH in quantitative yield.



[a]*Reagents:* (i) $NaIO_4$; (ii) $NaBH_4$; (iii) Ac_2O, pyridine; (iv) K_2CO_3, MeOH

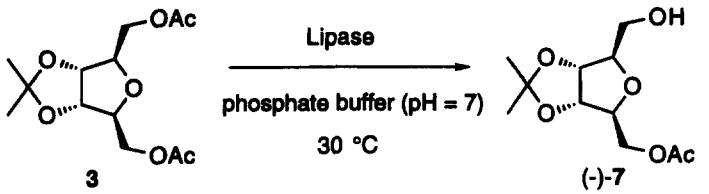
Scheme 2. [a]Preparation of *meso*-substrates.

Enzymatic hydrolysis

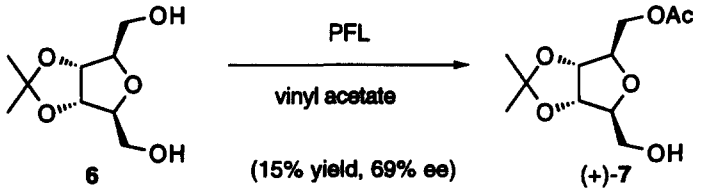
The results of enzymatic hydrolyses using RDL or PFL are summarized in Scheme 3. The specific rotations of both hydrolyzed products exhibited a minus sign. The enantiomeric excess (% ee) of the hydrolyzed products was determined by ^1H NMR spectra after conversion into the corresponding Mosher's esters [(+)-MTPA esters].[10] The ^1H NMR spectra of (+)-MTPA ester derived from racemic (±)-**7** showed the acetyl proton signals at δ 2.03 (s) and 2.07 (s) in the ratio of 1 to 1, while the ^1H NMR spectra of (+)-MTPA ester derived from the enzymatic hydrolyzed product (−)-**7** showed the corresponding signals at δ 2.03 (s) and 2.07 (s) in a different ratio. Unfortunately, the hydrolysis of **3** by RDL required prolonged reaction time (7 days), and the monoacetate (−)-**7** of low enantiomeric excess (13% ee) was obtained in poor yield. However, the hydrolysis by PFL afforded the monoacetate (−)-**7** of good enantiomeric excess (92% ee) in 94% yield. The enantiomeric excess was very high compared with the previously reported value. Jones *et al.*[9] reported that the enantiomeric excess of lipase-catalyzed hydrolysis of the same substrate **3** was 18% ee at the maximum, in the case that porcine pancreatic lipase was used as a catalyst.

PFL-catalyzed transesterification of *meso*-diol **6** was next attempted. In general, the transesterification of *meso*-diol and the hydrolysis of *meso*-diacetate by PFL are complementary.[4b,5,11] Therefore, it was expected that the transesterification of *meso*-diol **6** by PFL would afford monoacetate (+)-**7**, which is an enantiomer of hydrolyzed product (−)-**7**. As expected, PFL-catalyzed transesterification of **6** in vinyl acetate proceeded to give the monoacetate (+)-**7** of 69% ee, even though the yield of product and enantiomeric excess were not satisfactory.

We have previously reported that the hydrolysis of *meso*-diacetate **2** by PFL or RDL afforded the enantiomerically pure monoacetate **4**. The absolute configuration of 1-(acetoxymethyl)-4-(hydroxymethyl)-2,3-(isopropylidenedioxy)cyclopentane **4** was 1*S*,2*S*,3*R*,4*R*.[12] Therefore, we assumed the stereochemistry of (−)-**7** as 2*S*,3*S*,4*R*,5*R*, based on our three-site model of PFL[5] and box-type



Lipase	Monoacetate 7 (%)	(% ee)
RDL	20	13
PFL	94	92

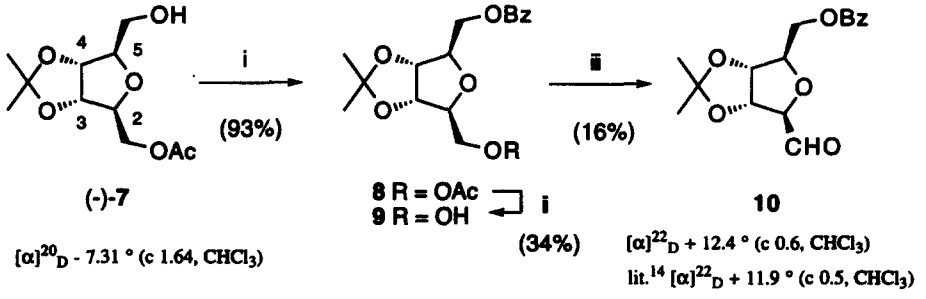


Scheme 3. Enzymatic reaction of *meso*-compounds.

model of RDL.[4] Our assumption of the absolute configuration of (−)-**7** was contrary to Jones' results.[9] Therefore, we tried to determine the absolute configuration of (−)-**7** by another route.[13]

Determination of absolute configuration

Monoacetate (−)-**7** was converted into benzoyloxy alcohol **9** by the protection of alcohol with benzoyl chloride (93%), and subsequent solvolysis with K_2CO_3/MeOH (34%). PCC oxidation of **9** afforded aldehyde **10** in 16% yield (Scheme 4). The specific rotation of this material showed $[\alpha]^{22}_D$ +12.4 (c 0.6, $CHCl_3$). Based on the comparison of specific rotation with the reported value ($[\alpha]^{22}_D$ +11.9, (c 0.5, $CHCl_3$)),[14] the absolute configuration of (−)-**7** was determined to be 2*S*,3*S*,4*R*,5*R*. Thus, the stereochemistry of (−)-**7** was in accordance with our lipase models.



[a]*Reagents*: (i) BzCl, pyridine; (ii) K_2CO_3, MeOH; (iii) PCC

Scheme 4. [a]Determination of absolute configuration.

Conclusion

It has become clear that the application of PFL to the hydrolysis of *meso*-2,5-bis(acetoxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran **3** would be very useful for the preparation of the optically active monoacetate (−)-**7** (92% ee), although a relatively long reaction time (5 days) was required.

The enantiomeric excess of (−)-**7** obtained by RDL-catalyzed hydrolysis was not satisfactory. The electrostatic effect of an oxygen in the tetrahydrofuran ring might affect the binding property of the

molecule to the active site of RDL. The absolute configuration of (−)-**7** was unambiguously determined by the chemical correlation with the aldehyde **10**, and found to be 2*S*,3*S*,4*R*,5*R*. The optically active monoacetate (−)-**7** might be an important chiral building block in the synthesis of metabolically stable *C*-nucleosides.

Experimental section

^{1}H NMR spectra were determined at 270 MHz. THF was distilled from Na/benzophenone before use, and CH_2Cl_2 was distilled from P_2O_5. RDL (EC 3.1.1.3) was purchased from Seikagaku Kogyo Corp. (Japan), and PFL (Amano PS) was supplied by courtesy of Amano Pharmaceutical Corp. (Japan), and were used as received.

anti,syn,anti-*2,5-Bis(acetoxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran* **3**

A suspension of diol **5** (280 mg, 1.39 mmol)[8] and $NaIO_4$ (1.2 g, 5.61 mmol) in THF (33 mL) and H_2O (12 mL) was stirred at room temperature for 2 h. The mixture was diluted with brine, and extracted with EtOAc, and then dried over $MgSO_4$. After removal of the solvent, the residue was dissolved in MeOH (35 mL). $NaBH_4$ (50 mg, 1.32 mmol) was added portionwise to the solution at 0°C, and the solution was stirred for 12 h. The reaction was quenched with acetone, and the solution was concentrated *in vacuo* to leave an oily residue. The residue was dissolved in pyridine (15 mL) and Ac_2O (2 mL), and the whole was stirred for 24 h. The solution was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel to give **3** (213 mg, 53%) as a colorless oil: IR (neat) 1750, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.54 (m, 2H), 4.26 (dd, *J*=3.6, 10.8 Hz, 2H),4.20 (m, 2H),4.12 (dd, *J*=5.0, 10.8 Hz, 2H), 2.11 (s, 6H), 1.55 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 170.7, 114.7, 82.4, 82.0, 64.4, 27.4, 25.5, 20.8; FAB(+)HRMS calcd for $C_{13}H_{21}O_7$ (M^++H) 289.1287, found 289.1299.

anti,syn,anti-*2,5-Bis(hydroxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran* **6**

A mixture of **3** (130 mg, 0.45 mmol) and K_2CO_3 (185 mg, 1.34 mmol) in MeOH (5 mL) was stirred at room temperature for 2 h. K_2CO_3 was filtered off, and the filtrate was concentrated *in vacuo* to leave an oily residue, which was purified by column chromatography on silica gel to give **6** (90 mg, quant.) as a yellowish oil: IR (neat) 3375 (br) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.75 (m, 2H), 4.13 (m, 2H), 3.90 (dd, *J*=2.5, 12.0 Hz, 2H), 3.71 (dd, *J*=3.1, 12.0 Hz, 2H), 3.40 (br, 2H), 1.54 (s, 3H), 1.35 (s, 3H); FABMS (*m/z*) 205 (M^++H).

(2S,3S,4R,5R)-2-(Acetoxymethyl)-5-(hydroxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran **7**

A mixture of **3** (2.30 g, 7.99 mmol) and PFL (250 mg) in a phosphate buffer (pH=7.0, 100 mL) and acetone (0.3 mL) was stirred at 30°C for 5 days. The solution was extracted with EtOAc, and dried over $MgSO_4$. Removal of the solvent afforded an oily residue, which was purified by column chromatography to give **7** (1.84 g, 94%) as a colorless oil: $[\alpha]^{20}_D$ −7.31 (*c* 1.64, $CHCl_3$); IR (neat) 3450, 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.70 (dd, *J*=3.6, 6.6 Hz, 1H), 4.52 (dd, *J*=4.3, 6.6 Hz, 1H), 4.18–4.32 (m, 3H), 4.13 (m, 1H), 3.83 (br d, *J*=12.0 Hz, 1H), 3.65 (br d, *J*=12.0, 1H), 2.34 (br, 1H), 2.11 (s, 3H), 1.55 (s, 3H), 1.35 (s, 3H); FAB(+)HRMS calcd for $C_{11}H_{19}O_6$ (M^++H) 247.1182, found 247.1187.

MTPA ester of **7**

The 270 MHz ^{1}H NMR spectrum of (+)-MTPA ester derived from the monoacetate (±)-**7** showed the acetyl proton signals at δ 2.03 (s) and 2.07 (s) in the ratio of 1 to 1, while the corresponding signal from (−)-**7** hydrolyzed by PFL was observed at δ 2.03 (s) and 2.07 (s) in the ratio of 200 to 9, and the corresponding signal from (+)-**7** transesterified by PFL was observed at δ 2.03 (s) and 2.07 (s) in the ratio of 18 to 100.

(2S,3S,4R,5R)-2-(Acetoxymethyl)-5-(benzoyloxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran 8

A solution of (−)-**7** (300 mg, 1.22 mmol) and benzoyl chloride (0.3 mL, 2.45 mmol) in pyridine (8 mL) was stirred at room temperature for 2 h. The solution was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel to give **8** (391 mg, 93%) as a colorless oil: $[\alpha]^{22}_D$ −5.87 (*c* 2.78, $CHCl_3$); IR (neat) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.04 (m, 2H), 7.60 (m, 1H), 7.45 (m, 2H), 4.67 (dd, *J*=4.0, 6.6 Hz, 1H), 4.58 (dd, *J*=4.0, 6.6 Hz, 1H), 4.52 (dd, *J*=4.0, 11.7 Hz, 1H), 4.42 (dd, *J*=4.8, 11.7 Hz, 1H), 4.35 (m, 1H), 4.29 (dd, *J*=3.3, 11.0 Hz, 1H), 4.22 (m, 1H), 4.16 (dd, *J*=4.8, 11.0 Hz), 2.05 (s, 3H), 1.57 (s, 3H), 1.36 (s, 3H); FABMS (*m/z*) 351 (M^++H).

(2S,3S,4R,5R)-5-(Benzoyloxymethyl)-2-(hydroxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran 9

A mixture of **8** (285 mg, 0.81 mmol) and K_2CO_3 (5 mg) in MeOH (8 mL) was stirred at room temperature for 3 h. After removal of the solvent, the residue was purified by column chromatography on silica gel to give **9** (84 mg, 34%) as a colorless oil: $[\alpha]^{22}_D$ +3.28 (*c* 2.78, $CHCl_3$); IR (neat) 3485, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.05 (m, 2H), 7.58 (m, 1H), 7.45 (m, 2H), 4.76 (dd, *J*=4.0, 6.6 Hz, 1H), 4.65 (dd, *J*=4.0, 6.6 Hz, 1H), 4.54 (dd, *J*=5.5 11.7 Hz, 1H), 4.47 (dd, *J*=3.3, 11.7 Hz, 1H), 4.35 (m, 1H), 4.15 (m, 1H), 3.83 (dd, *J*=2.9, 12.1 Hz, 1H), 3.67 (dd, *J*=3.7, 12.1 Hz, 1H), 2.20 (br, 1H), 1.57 (s, 3H), 1.37 (s, 3H); FABMS (*m/z*) 309 (M^++H).

*2,5-Anhydro-6-O-benzoyl-3,4-O-isopropylidene-*D*-allose 10*

A mixture of **9** (38 mg, 0.12 mmol), PCC (80 mg, 0.37 mmol), and NaOAc (100 mg, 1.22 mmol) in CH_2Cl_2 (4 mL) was stirred at 0°C for 3 h. After removal of the chromate by florisil short column, the residue was purified by column chromatography on silica gel to give **10** (6 mg, 16%) as a colorless oil: $[\alpha]^{22}_D$ +12.4 (*c* 0.6, $CHCl_3$); [lit.[12] $[\alpha]_D$ +11.9 (*c* 0.5, $CHCl_3$)].

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12. The numbering of hydrolyzed product **4** was assigned as shown in Scheme 1.
13. Jones *et al.* reported that the monoacetate **7** obtained by pig liver esterase (PLE)-catalyzed hydrolysis showed a plus sign of specific rotation, and they determined the absolute configuration of (+)-**7** as 2*S*,3*S*,4*R*,5*R* by converting it to (2*R*,5*S*)-2,5-anhydroallonic acid. We reviewed critically their evidence for the assignment of absolute configuration, and considered that the measured absolute value of specific rotation for the synthetic anhydroallonic acid {$[\alpha]^{25}_{D}$ −0.24 (*c* 4.51, water)} was too small to determine the absolute configuration accurately.
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