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TETRAHEDRON: *ASYMMETRY*

Chemoenzymatic synthesis of the C-13 side chain of paclitaxel (Taxol) and docetaxel (Taxotere)

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Abstract

Reduction of methyl 3-chloro-2-oxo-3-phenylpropanoate with various reducing agents gave *syn*- and *anti*-3-chloro-2-hydroxy-3-phenylpropanoates **3**, which underwent an efficient lipase-catalyzed resolution. All four diastereomers were subsequently converted to *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine methyl ester, C-13 side chain analogues of paclitaxel (Taxol). © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Paclitaxel **1a** (Taxol), an antimicrotubule agent isolated from the bark of *Taxus brevifolia*,[1] has recently attracted much attention because of its efficacy in the treatment of various types of cancer.[2] One major impediment to the wide use of Taxol in cancer chemotherapy is its extremely limited availability. Also, chemical complexity has prohibited the production of paclitaxel **1a** by total synthesis,[3] and the chemical complexity of paclitaxel dictates that its commercial production by total synthesis is not likely to be economical. The naturally derived 10-deacetylbaccatin **III** is obtained in fairly high yield (1 g/kg) from the leaves of the European yew (*Taxus baccata*).[4] It is important to recognize that the leaves are quickly regenerated and hence, a large amount of 10-deacetylbaccatin **III** can be supplied continuously without threatening the survival of the yew species. Thus, a viable approach for the preparation of paclitaxel **1a** is to utilize more accessible baccatin **III** or 10-deacetylbaccatin **III** as precursors via semi-synthetic routes.[5] The structures of compounds **1a**, **1b**, and **III** are described in Fig. 1.

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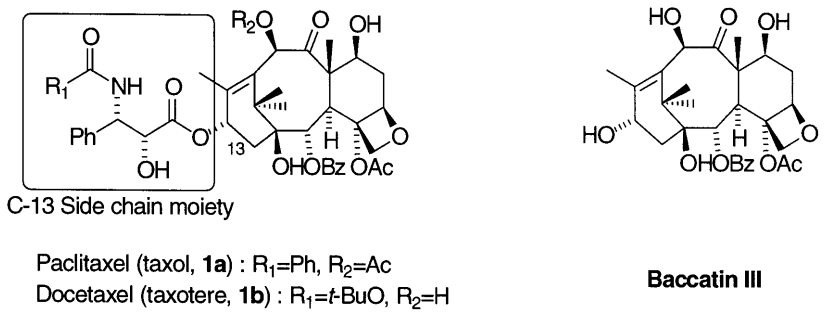


Figure 1.

As the role of the (2*R*,3*S*)-3-phenylisoserine moiety in the biological activity of paclitaxel and docetaxel became evident,[6] the development of short and practical synthetic routes for phenyl isoserine derivatives has become very important. Numerous papers concerning the preparation of the enantiomerically pure or enriched C-13 side chain of paclitaxel have been published.[7] Among these methods, a route employing enzyme is one important way. Although some papers describe lipase-catalyzed synthesis of the C-13 side chain of paclitaxel,[7d,g] it was difficult to obtain the compound with high enantiomeric excess. Here, we describe the preparation of 3-chloro-2-hydroxy-3-phenylpropanoate with high enantiomeric excess via lipase-catalyzed kinetic resolution of the reduction products of 3-chloro-3-phenylpyruvate obtained by Darzens-type reaction and its application to the synthesis of the C-13 side chain of paclitaxel.

The Darzens condensation has been widely used to prepare glycidic esters. The Darzens condensation of dichloroacetate with aromatic aldehydes gives 2-chloro-2,3-epoxypropanoate or its rearranged products, 3-chloro-2-oxopropanoate, which will become important key intermediates because it has reactive functional groups such as chlorine, carbonyl, and ester groups. In this paper, we describe a convenient preparation of *anti*-3-chloro-2-hydroxyester from Darzens reaction products and its lipase-catalyzed resolution, as summarized in Fig. 2. The C-13 side chain of paclitaxel was prepared from chiral *anti*-3-chloro-2-hydroxyester in a few steps.

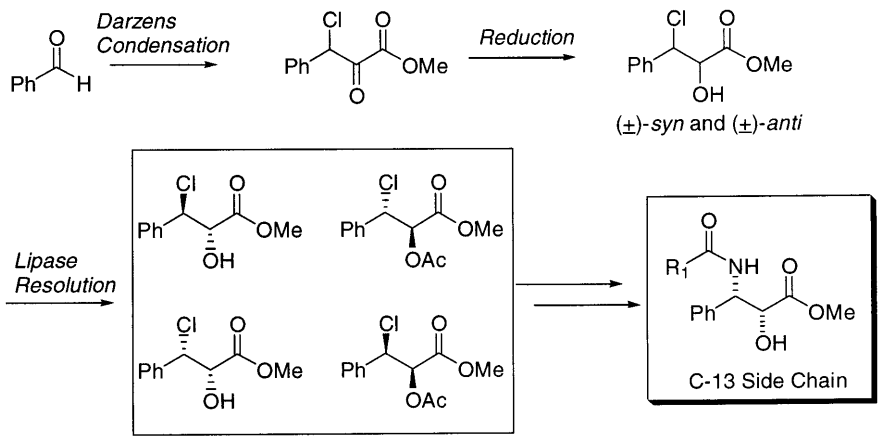
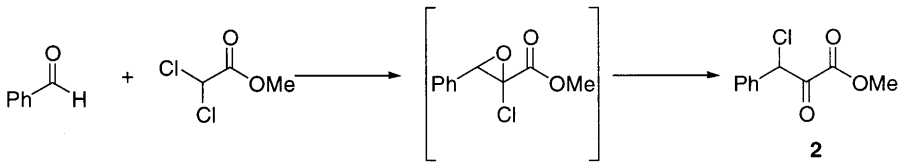


Figure 2.

This efficient synthesis can also be readily modified to give the side chain of docetaxel **1b**, a synthetic Taxol derivative, which was found to have significantly greater cancer chemotherapeutic potential than paclitaxel itself.[8]

2. Results and discussion

Base-catalyzed condensation of aldehydes with dichloroacetate has been previously reported.[9] This Darzens condensation reaction of dichloroacetates with aromatic aldehydes sometimes gives 3-chloro-2-oxoester as a rearrangement product of α-chloroglycidates.[9] In this report benzaldehyde was used as an aromatic aldehyde, and methyl 3-chloro-2-oxopropanoate **2** was obtained in high yield (Scheme 1).



Scheme 1. *Reagents and conditions*: NaOMe, THF, −50°C, 3 h, then rt overnight, 91%

Various reducing reagents were examined to obtain *anti*-3-chloro-2-hydroxyester **3**. Reduction with sodium borohydride gave a *syn*-selective product. On the other hand, L-Selectride®, K-Selectride®, KS-Selectride®, and lithium aluminum hydride in the presence of a small amount of silica gel[10] gave an *anti*-selective product predominantly. According to the dipole-type and Cram-type of the Felkin–Ahn model, the reduction with sodium borohydride is effective in the *syn*-selectivity of this reduction under influence of dipole, and the reduction with bulky reducing reagents is effective in the *anti*-selectivity under the influence of steric effect between the phenyl group and bulkiness of the reagent (Fig. 3). In this reduction, we found the best *anti*-selectivity can be obtained by KS-Selectride® in THF at −78°C (Table 1). These *syn*- and *anti*-isomers were separated by silica gel column chromatography.

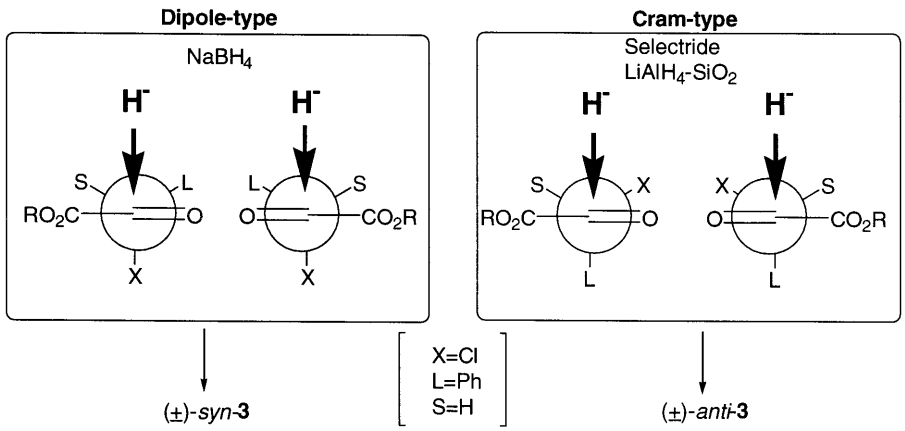
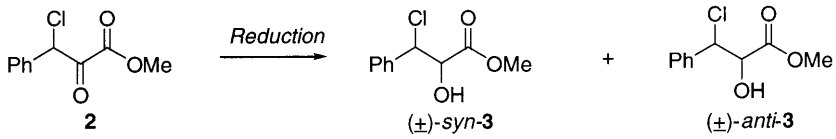


Figure 3. Felkin–Ahn model

Table 1
Reduction of methyl 3-chloro-2-oxo-3-phenylpropanoate



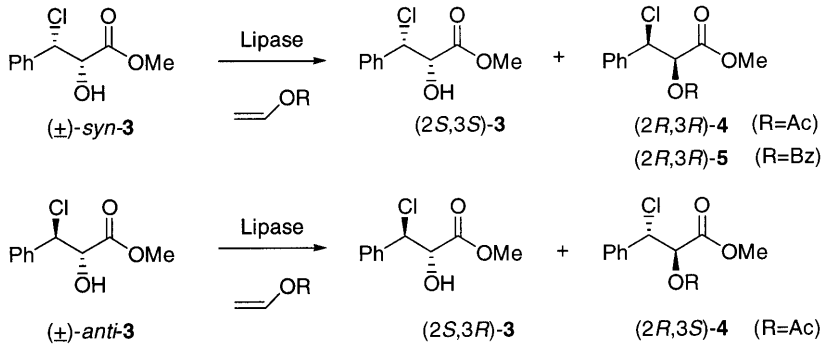
2 (±)-*syn*-**3** (±)-*anti*-**3**

Entry	Conditions				Product **3**	
	Reagent	Solvent	Temp. (°C)	Time (min)	Yield[a] (%)	*syn*/*anti*[b]
1	$NaBH_4$	MeOH/THF	0	10	88	76/24
2	$NaBH_4$	*iso*-PrOH	0	10	70	75/25
3	$LiAlH_4$–SiO_2	Et_2O	Rt	120	48	31/69
4	L-Selectride®	THF	−78	120	75	34/66
5	K-Selectride®	THF	−78	120	86	17/83
6	KS-Selectride®	THF	−78	120	81	13/87

[a] Isolated yield by SiO_2 column chromatography.
[b] Ratios of *syn* and *anti* were determined by analysis of GC–MS. Some of the products were analyzed by GC and the same results were obtained.

The kinetic resolution of racemic methyl *syn*- and *anti*-3-chloro-2-hydroxyester **3** was achieved by lipase-mediated enantioselective transesterification in organic media. The yield and enantiomeric excesses are listed in Table 2.

Table 2
The lipase catalyzed transesterifications of *syn*- and *anti*-**3**



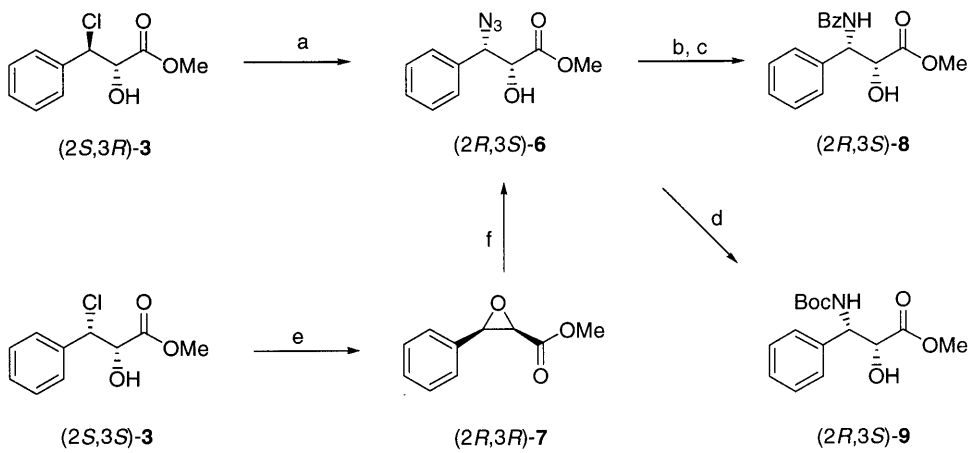
(±)-*syn*-**3** (2*S*,3*S*)-**3** (2*R*,3*R*)-**4** (R=Ac) (2*R*,3*R*)-**5** (R=Bz)

(±)-*anti*-**3** (2*S*,3*R*)-**3** (2*R*,3*S*)-**4** (R=Ac)

Entry	Conditions						Yield and ee[a]		E[b]
	3	R	Lipase	Solvent	Temp. (°C)	Time (h)	(2*S*)-**3**	(2*R*)-**4** or **-5**	
1	(±)-*syn*	Ac	Amano PS	$^iPr_2O$	35	25	50%, 95% ee	45%, 93% ee	>100
2	(±)-*syn*	Bz	Amano PS	$^iPr_2O$	35	240	32%, 18% ee	6%, 80% ee	11
3	(±)-*syn*	Bz	Meito SL	$^iPr_2O$	35	240	41%, 61% ee	12%, >99% ee	>100
4	(±)-*syn*	Bz	Meito SL	$^iPr_2O$	60	28	49%, 78% ee	31%, 80% ee	22
5	(±)-*anti*	Ac	Amano PS	$^iPr_2O$	35	27	46%, >99% ee	45%, 93% ee	>100
6	(±)-*anti*	Ac	Meito SL	$^iPr_2O$	35	46	42%, 86% ee	31%, 97% ee	>100
7	(±)-*anti*	Ac	TOYOBO	$^iPr_2O$	35	25	25%, 33% ee	8%, 96% ee	63

[a] Ee was determined by analysis of chiral GC.
[b] For E value, see Ref. 13.

Lipase Amano PS in the presence of vinyl acetate was suited for stereospecific transesterification of both (±)-*syn*- and (±)-*anti*-alcohol **3** with high enantiomeric excesses and chemical yields. Vinyl benzoate was also used to obtain benzoate product (2*R*,3*R*)-**5** from (±)-*syn*-**3** for the synthesis of C-13 side chains from (2*S*,3*S*)-**7**. Unfortunately we could not obtain (2*S*,3*S*)-methyl 3-azide-2-benzoyl-3-phenylpropanoate by reaction of (2*R*,3*R*)-**5** with sodium azide in DMF. The absolute configuration of the α-carbon of *anti*-**4** was determined to be (*S*) from that of known compounds **6** or **7**, which were prepared as shown in Scheme 2. On the other hand, the absolute configuration of the α-carbon of *anti*-**3** was also determined to be (*S*) after conversion to the epoxide. Lipase-catalyzed kinetic resolution of 3-hydroxy esters was reported by several groups. These reports show that the reaction of the (*S*)-isomer proceeds much faster than that of the (*R*)-isomer in the acylation of the hydroxy group as well as in the hydrolysis of its acetate. The present kinetic resolution also gives the same result as that of the literature.[11]



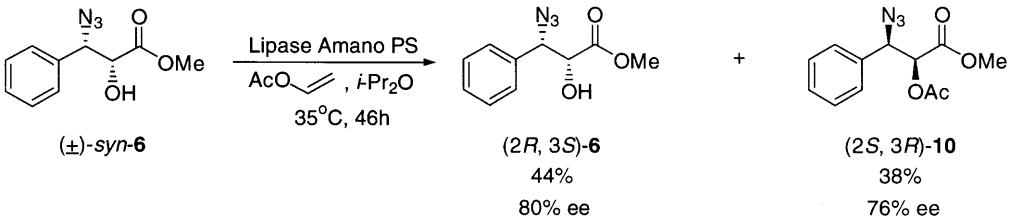
Scheme 2. *Reagents and conditions*: (a) NaN_3, DMF, 60°C, 42 h, 82%; (b) BzCl, DMAP, CH_2Cl_2, rt, 5 h, 100%; (c) H_2, Pd/C, MeOH, rt, 48 h, 71%; (d) $(Boc)_2O$, H_2, Pd/C, EtOAc, rt, 48 h, 70%; (e) NaOMe, MeOH, 0°C, 8 h, 27%; (f) NaN_3, NH_4Cl, acetone/water, reflux, 24 h, 75%

An alternative enantioselective synthesis of paclitaxel **1a** and docetaxel **1b** side chain from enzymatically resolved 3-chloro-2-hydroxyester **3** and 3-acetoxy-2-chloroester **4** was achieved as shown in Schemes 2 and 4.[7k]

Azide displacement of (2*S*,3*R*)-**3** with sodium azide in DMF at 60°C led to the C-3 inverted product (2*R*,3*S*)-**6** in 82% yield. The hydroxy azide (2*R*,3*S*)-**6** was also obtained from (2*S*,3*S*)-**3** via two steps, but epoxidation of (2*S*,3*S*)-**3** gave (2*R*,3*R*)-glycidate **7** in only 27% yield. The hydroxy azide (2*R*,3*S*)-**6** was then subjected to benzoylation, followed by hydrogenation with 10% Pd/C under 1 atm of hydrogen at room temperature in methanol,[7a] furnishing the paclitaxel side chain (methyl ester, (2*R*,3*S*)-**8**) in 71% yield (two steps) after recrystallization from methanol. Catalytic hydrogenation of the hydroxy azide **6** in ethyl acetate in the presence of 1.2 equivalent of di-*tert*-butyl dicarbonate furnished directly the docetaxel side chain (methyl ester, (2*R*,3*S*)-**9**) in 70% yield.

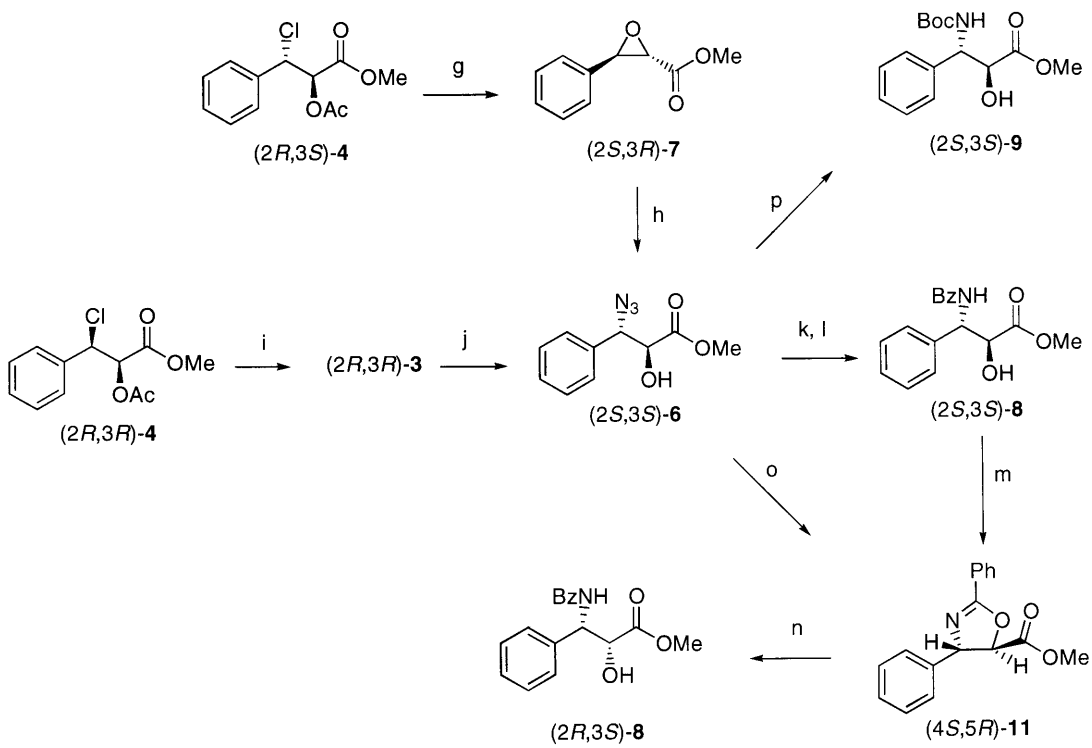
The resolution of (±)-*syn*-hydroxy azide **6** by lipase-mediated transesterification was also carried out by lipase Amano PS in the presence of vinyl acetate at 35°C, which gave the best selectivity for the resolution of *syn*- and *anti*-3-chloro-2-hydroxyester **3** (Scheme 3). The

enantiomeric excesses of the acetylated enantiomers showed 76% ee and that of unreacted enantiomers was 80% ee. However, this enantioselectivity is not as high as the resolution of *syn*- and *anti*-3-chloro-2-hydroxyester **3**. From this result, we can conclude that the resolution of (±)-*syn*-hydroxy azide **6** by lipase is not suitable for this side-chain synthesis. The absolute configuration of the α-carbon of acetate *syn*-**10** was determined to be (*S*), based on the sign of the optical rotation of unreacted (2*R*,3*S*)-**6**.



Scheme 3. The lipase catalyzed transesterification of **6**

On the other hand, enzymatically resolved 3-chloro-2-acetoxyester **4** was also applied to the synthesis of the paclitaxel side chain. (2*R*,3*R*)-Methyl 3-chloro-2-hydroxyester **3** was obtained in 90% yield from (2*R*,3*R*)-3-chloro-2-acetoxyester **4** by treatment of HCl. As shown in Scheme 4,



Scheme 4. *Reagents and conditions*: (g) K_2CO_3, MeOH, −20 to 0°C, 2 h, 93%; (h) NaN_3, NH_4Cl, acetone/H_2O, reflux, 23 h, 96%; (i) HCl, acetone/MeOH, rt, 24 h, 90%; (j) NaN_3, DMF, 60°C, 48 h, 83%; (k) BzCl, DMAP, CH_2Cl_2, rt, 4 h; (l) H_2, Pd/C, MeOH, rt, 48 h, 70% (over two steps); (m) PPh_3, DEAD, benzene, rt, 20 h, 90%; (n) 1N HCl, MeOH, reflux, 2 h, 90%; (o) PPh_3, DEAD, $PhCO_2H$, benzene, rt, 12 h, 66%; (p) $(Boc)_2O$, H_2, Pd/C, MeOH, rt, 72 h, 97%

(2*R*,3*R*)- and (2*R*,3*S*)-3-chloro-2-hydroxyester **3** were applied to the synthesis of (2*S*,3*S*)-*N*-benzoylphenylisoserine **8** and (2*S*,3*S*)-*N*-*tert*-butoxycarbonylphenylisoserine **9**, the side chain of *epi*-paclitaxel and *epi*-docetaxel, respectively, by the same method as shown in Scheme 2.

Treatment of (2*S*,3*S*)-**6**, which was prepared from (2*R*,3*R*)-**4**, under the Mitsunobu reaction conditions[12] gave (4*S*,5*R*)-**11** in 66% yield, which can also be obtained from (2*S*,3*S*)-**8** under the same conditions in 90% yield. Treatment of (4*S*,5*R*)-**11** with 1N HCl under reflux afforded the paclitaxel side chain (2*R*,3*S*)-**8** in 90% yield. The spectral (^{1}H and ^{13}C NMR) data of synthetic (2*R*,3*S*)-**8** were fully identical with those of the authentic paclitaxel side chain. Physical properties of synthetic **8** (mp 179–180°C) (lit.[7a] 183–184°C), and specific rotation {$[\alpha]_D^{20}$ −50.5 (*c* 1.00, MeOH)}{lit.[7a] $[\alpha]_D^{20}$ −49.6 (*c* 1.00, MeOH)} were also in good agreement with those reported previously.

Hydrogenation of (2*S*,3*S*)-**6** in methanol in the presence of 10% Pd/C and di-*tert*-butoxycarbonate gave (2*S*,3*S*)-**9** in 97% yield.

In conclusion, this work has demonstrated the preparation of optically active 3-chloro-2-hydroxy-3-phenylpropanoate via lipase-catalyzed kinetic resolution, whose starting material can be easily obtained by Darzens condensation with dichloroacetate in large scale. Two of the four diastereomers were obtained in high enantiomeric excess. Each diastereomer was applied to the synthesis of C-13 side chains of paclitaxel and docetaxel.

3. Experimental

3.1. General

Melting points were obtained in open capillary tubes on a Mel-Temp-II hot-stage microscope and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 200 spectrometer or Varian 500 spectrometer in $CDCl_3$ solution. ^{13}C NMR spectra (50 MHz) were recorded on a Varian Gemini 200 spectrometer in $CDCl_3$ solution. IR spectra were recorded on a JASCO FT/IR-5000. Optical rotations were recorded on a SEPA-300 HORIBA polarimeter (Lamp $\lambda = 589$ nm) in $CHCl_3$ or MeOH solutions. Enantiomeric excess was determined by Chiral-GC, GC-380 GL Science fitted with Chiral-DEXCB (0.25×25 mm). The ratio of *syn* and *anti* was determined by GC–MS, GC-17A SHIMADZU (sr. Phase TC-5, d$t = 0.25$ μm, 0.25×30 m), GC–MS-QP5000 SHIMADZU.

Reagents and solvents were purified by standard means. Tetrahydrofuran was distilled from sodium wire/benzophenone and stored under a nitrogen atmosphere. Dichloromethane, benzene, *N*,*N*-dimethylformamide, and methanol were distilled from calcium hydride. All other chemicals were used as received.

3.2. Preparation of methyl 3-chloro-2-oxo-3-phenylpropanoate **2**

Sodium (6.94 g, 302 mmol) was treated with anhydrous MeOH (100 ml). After evaporation of MeOH, the residue was cooled to −50°C in THF (400 ml), then methyl dichloroacetate (43.20 g, 302 mmol) and benzaldehyde (32.0 g, 302 mmol) were added under an atmosphere of nitrogen. Stirring was continued for 3 h and then allowed to stand overnight at room temperature. After evaporation of THF, the reaction mixture was extracted three times with EtOAc. The organic layer was dried over $MgSO_4$ and the solvent was evaporated. The crude

product was purified by vacuum distillation (bp 105°C/5 mmHg) to yield the ketoester **2**[9] (58.40 g, 91%) as a yellow oil: IR: 1740, 1458, 1247, 1064, 702 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.84 (s, 3H), 6.23 (s, 1H), 7.37 (m, 5H).

3.3. Reduction of methyl 3-chloro-2-oxo-3-phenylpropanoate ***2***

3.3.1. Reduction with sodium borohydride

To a stirred solution of $NaBH_4$ (1.00 g, 26 mmol) in THF (30 ml) was added dropwise MeOH (30 ml) solution of **2** (12.6 g, 60 mmol) at 0°C. Stirring was continued for 10 min at the same temperature, then the mixture was poured into ice water and neutralized with 1N HCl. The organic material was extracted three times with EtOAc and washed with brine (100 ml). The organic layer was dried over $MgSO_4$ and evaporated. The residual crude products were purified by column chromatography (eluent: hexane/EtOAc 8:1–5:1) to give (±)-**3** (11.1 g, 88%, *syn*/*anti* ratio = 76:24). Pure (±)-*syn*-**3** and (±)-*anti*-**3** were isolated by column chromatography (eluent: hexane/EtOAc 25:1–10:1). (±)-*syn*-**3** (4.05 g, 32%) as white crystals: mp 79–80°C (lit.[13] mp 77–79°C); R_f 0.17 (hexane/EtOAc 4:1). Spectral data were identical with those of an authentic sample. (±)-*anti*-**3** (2.34 g, 18%) as a colorless oil: R_f 0.15 (hexane/EtOAc 4:1); IR: 3426, 2958, 1744, 1495, 1456, 1286, 1120 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.93 (d, 1H, J = 7.20 Hz), 3.75 (s, 3H), 4.66 (dd, 1H, J = 4.00 and 7.20 Hz), 5.23 (d, 1H, J = 4.00 Hz), 7.3–7.4 (m, 5H). A mixture of (±)-*syn*-**3** and (±)-*anti*-**3** (4.83 g, 38%) was also obtained.

3.3.2. Reduction with lithium aluminum hydride–SiO_2

To silica gel (3.75 g, Merck 60, for column chromatography) dried at 150°C under vacuum (5 mmHg) for 3 h was added $LiAlH_4$ (266 mg, 7.00 mmol) and anhydrous ether (25 ml) under an atmosphere of nitrogen. After stirring for 2 h, **2** (1.06 g, 5.00 mmol) was added to this mixture and stirring was continued for another 3 h. The whole mixture was then heated to reflux temperature and kept for 15 min. After cooling and quenching with a few drops of aqueous ammonium chloride solution, the mixture was filtered and the silica gel was washed thoroughly with ether. The filtrate and washings were combined and evaporated. The residual crude mixture was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 20:1–5:1) to give **3** (514 mg, 48%, *syn*/*anti* ratio = 31:69).

3.3.3. Reduction with KS-Selectride®

To a stirred solution of **2** (212 mg, 1.00 mmol) in THF (5 ml) was added KS-Selectride® (1.0 M in THF, 0.710 ml, 0.710 mmol) under an atmosphere of nitrogen at −78°C. Stirring was continued for 120 min at the same temperature, and then 30% aqueous H_2O_2 (0.5 ml) was added dropwise. The reaction mixture was allowed to warm to room temperature, controlled to weak acidity by ammonium chloride, and extracted three times with EtOAc. The organic materials were washed with sodium bicarbonate and water, and dried over $MgSO_4$. The residual crude mixture was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 20:1–5:1) to give **3** (81.8 mg, 81%, *syn*/*anti* ratio = 13:87).

*3.4. Lipase-catalyzed kinetic resolution of methyl (±)-*syn*-3-chloro-2-hydroxy-3-phenylpropanoate* ***3***

A mixture of (±)-*syn*-**3** (3.21 g, 15.0 mmol), lipase (Amano PS, 3.21 g), vinyl acetate (3.86 g, 44.8 mmol), and diisopropyl ether (105 ml) was stirred for 25 h at 35°C. The reaction was

monitored by GC–MS and continued until half of the starting material was consumed. The reaction mixture was filtered and the solvent was evaporated. The residual crude mixture was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*S*,3*S*)-**3** (1.605 g, 50%, 95% ee by chiral GC) as white crystals: mp 79–80°C (lit.[14] mp 77–79°C); $[\alpha]_D^{17}$ +62 (*c* 1.4, $CHCl_3$). Spectral data were identical with those of an authentic sample.[14]

A mixture of (±)-*syn*-**3** (300 mg, 1.41 mmol), lipase (Meito SL, 600 mg), vinyl benzoate (966 mg, 6.52 mmol), and diisopropyl ether (15 ml) was stirred for 28 h at 60°C. The reaction was monitored by GC–MS and continued until half of the starting material was consumed. The reaction mixture was filtered and concentrated, and then the residual crude mixture was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*S*,3*S*)-**3** (147 mg, 49%, 78% ee by chiral GC) as white crystals: $[\alpha]_D^{17}$ +47 (*c* 1.4, $CHCl_3$) and (2*R*,3*R*)-**5** (136 mg, 30%, 80% ee by chiral GC) as white crystals: mp 86–87°C; $[\alpha]_D^{18}$ −131 (*c* 1.3, $CHCl_3$); IR: 2960, 2112, 1789, 1727, 1601, 1454, 1319, 1116, 1017, 704 cm^{-1}; ^1H NMR (200 MHz) δ 3.73 (s, 3H), 5.55 (d, 1H, $J=4.40$ Hz), 5.65 (d, 1H, $J=4.40$ Hz), 7.3–7.6 (m, 10H). Anal. calcd for $C_{17}H_{15}ClO_4$: C, 64.06; H, 4.74. Found: C, 63.99; H, 4.70.

*3.5. Lipase-catalyzed kinetic resolution of methyl (±)-*anti*-3-chloro-2-hydroxy-3-phenylpropanoate* ***3***

A mixture of (±)-*anti*-**3** (2.26 g, 10.5 mmol), lipase (Amano PS, 2.26 g), vinyl acetate (2.75 g, 31.9 mmol), and diisopropyl ether (75 ml) was stirred for 28 h at 35°C. The reaction was monitored by GC–MS and continued until half of the starting material was consumed. After the reaction mixture was filtered and the solvent was evaporated, the residual crude mixture was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 20:1–5:1) to give (2*S*,3*R*)-**3** (1.03 g, 46%, >99% ee) as a colorless oil: $[\alpha]_D^{19}$ −107 (*c* 1.5, $CHCl_3$); IR: 3426, 2958, 1744, 1495, 1456, 1286, 1120 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.93 (d, 1H, $J=7.20$ Hz), 3.75 (s, 3H), 4.66 (dd, 1H, $J=4.00$ and 7.40 Hz), 5.23 (d, 1H, $J=4.00$ Hz), 7.2–7.4 (m, 5H) and (2*R*,3*S*)-**4** (1.26 g, 47%, 93% ee by chiral GC) as white crystals; mp 87–88°C; $[\alpha]_D^{19}$ +77 (*c* 1.3, $CHCl_3$); IR: 1750, 1220, 1096, 772, 706, 532 cm^{-1}; ^1H NMR (200 MHz) δ 2.09 (s, 3H), 3.76 (s, 3H), 5.30 (d, 1H, $J=6.00$ Hz), 5.55 (d, 1H, $J=6.00$ Hz), 7.3–7.4 (m, 5H). Anal. calcd for $C_{10}H_{11}O_3Cl$: C, 56.16; H, 5.11. Found: C, 56.39; H, 5.11.

*3.6. Preparation of methyl (2*R*,3*S*)-3-azide-2-hydroxy-3-phenylpropanoate* ***6***

A mixture of (2*S*,3*R*)-**3** (921 mg, 4.29 mmol), sodium azide (1.395 g, 21.5 mmol), and DMF (30 ml) was stirred for 40 h at 60°C under an atmosphere of nitrogen. The solution was diluted with ethyl acetate (60 ml) and washed three times with water. The organic layer was dried over $MgSO_4$ and evaporated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*R*,3*S*)-**6** (782 mg, 82%) as white crystals: mp 74–75°C; $[\alpha]_D^{17}$ +92 (*c* 2.3, $CHCl_3$) [lit.[7a] $[\alpha]_D^{25}$ +105 (*c* 2.3, $CHCl_3$)]. Spectral data were identical with those of an authentic sample.[7a]

*3.7. Preparation of methyl (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine* ***8***

To a solution of (2*R*,3*S*)-**6** (700 mg, 3.17 mmol) and DMAP (387 mg, 3.17 mmol) in anhydrous CH_2Cl_2 (30 ml) was added dropwise benzoyl chloride (446 mg, 3.17 mmol) at 0°C.

Stirring was continued for 6 h at room temperature. The reaction mixture was extracted three times with ethyl acetate (50 ml), dried over $MgSO_4$ and evaporated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give benzoate of (2*R*,3*S*)-**6** (979 mg, 100%); $[\alpha]_D^{20}$ +110 (*c* 2.3, $CHCl_3$) [lit.[7k] $[\alpha]_D^{20}$ +98 (*c* 1.0, $CHCl_3$)]; IR: 2958, 2110, 1734, 1603, 1497, 1437, 1251, 1180, 754 cm^{-1}; ^1H NMR (200 MHz) δ 3.71 (s, 3H), 5.18 (d, 1H, $J=4.76$ Hz), 5.50 (d, 1H, $J=4.76$ Hz), 7.3–7.6 (m, 8H), 7.9–8.1 (m, 2H). Benzoate of (2*R*,3*S*)-**6** (950 mg, 3.07 mmol) was hydrogenated with 10% Pd/C (95 mg) in methanol (20 ml) under an atmosphere of hydrogen (1 atm) for 48 h at rt. The solution was filtered, and allowed to stand at 25°C for 48 h. The solution was concentrated and purified by recrystallization from MeOH to give (2*R*,3*S*)-**8** (652 mg, 71%) as colorless crystals: mp 179–180°C (lit.[7a] mp 183–184°C); $[\alpha]_D^{20}$ −50.2 (*c* 1.00, MeOH) [lit.[7a] $[\alpha]_D^{20}$ −49.6 (*c* 1.00, MeOH)]. Spectral data were identical with those of an authentic sample.[7a]

3.8. *Methyl (2*R,*3*S*)*-N-tert-*butoxycarbonyl-3-phenylisoserine* **9**

A suspension of 10% Pd/C (25 mg) in ethyl acetate (2 ml) was stirred at 20°C under an atmosphere of hydrogen for 10 min, whereupon a solution of di-*tert*-butyl dicarbonate (236 mg, 1.10 mmol) and (2*R*,3*S*)-**6** (200 mg, 0.905 mmol) in ethyl acetate (5 ml) was added. The stirring was continued for 48 h under an atmosphere of hydrogen (1 atm) at room temperature. The solution was filtered and evaporated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*R*,3*S*)-**9** (187 mg, 70%) as white crystals: mp 115°C [lit.[7n] mp 130.5–131.5°C]; $[\alpha]_D^{20}$ −6.8 (*c* 1.0, $CHCl_3$) [lit.[7n] $[\alpha]_D^{20}$ −7 (*c* 1.2, $CHCl_3$)]. Spectral data were identical with those of an authentic sample.[7n]

3.9. *Preparation of methyl (2*R,*3*R*)-3-phenylglycidate* **7**

Sodium (178 mg, 7.74 mmol) was treated with anhydrous MeOH (30 ml). After sodium dissolved in the solvent fully, the solution was cooled to 0°C, and then a solution of (2*S*,3*S*)-**3** (1.51 g, 7.03 mmol) in anhydrous MeOH was added under an atmosphere of nitrogen. After 10 h of stirring at 0°C, the reaction mixture was neutralized by 1N HCl and extracted three times with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*R*,3*R*)-**7** (335 mg, 27%) as a colorless oil; $[\alpha]_D^{17}$ +13 (*c* 1.1, $CHCl_3$) [lit.[7a] $[\alpha]_D^{26}$ +11 (*c* 4.4, $CHCl_3$)]. Spectral data were identical with those of an authentic sample.[7a]

3.10. *Preparation of methyl (2*R,*3*S*)-3-azide-2-hydroxy-3-phenylpropanoate* **6** *from (2*R,*3*R*)-***7***

A mixture of (2*R*,3*R*)-**7** (42.1 mg, 0.24 mmol), sodium azide (38.4 mg, 0.14 mmol), and NH_4Cl (47.4 mg, 0.89 mmol) in acetone/H_2O (4:1, 1 ml) was stirred for 24 h under reflux. The solution was concentrated and the residue was diluted with ethyl acetate (25 ml) and washed three times with water. The organic layer was dried over $MgSO_4$ and concentrated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*R*,3*S*)-**6** (39.3 mg, 75%) as white crystals. Spectral data were identical with those of an authentic sample.[7a]

3.11. Lipase-catalyzed kinetic resolution of methyl (2R,3S)-3-azide-2-hydroxy-3-phenylpropanoate **6**

A mixture of (±)-*syn*-**6** (200 mg, 0.905 mmol), lipase (Amano PS, 200 mg), vinyl acetate (390 g, 4.53 mmol), and diisopropyl ether (15 ml) was stirred for 46 h at 35°C. The reaction was monitored by GC–MS and continued until half of the starting material was consumed. After the reaction mixture was filtered and evaporated, the residual crude mixture was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 15:1–5:1) to give (2*R*,3*S*)-**6** (88 mg, 44%, 80% ee by chiral GC) as white crystals: $[\alpha]_D^{19}$ +97 (*c* 1.5, $CHCl_3$) and (2*S*,3*R*)-**10** (90.4 mg, 38%, 76% ee by chiral GC) was obtained as white crystals: mp 65°C; $[\alpha]_D^{19}$ −57 (*c* 1.3, $CHCl_3$) [lit.[7o] $[\alpha]_D^{20}$ −81.6 (*c* 2.0, $CHCl_3$)]; IR: 2110, 1752, 1510, 1377, 1222, 1102, 704 cm^{-1}; ^1H NMR (200 MHz) δ 2.14 (s, 3H), 3.69 (s, 3H), 5.07 (d, 1H, $J=4.82$ Hz), 5.23 (d, 1H, $J=4.82$ Hz), 7.3–7.4 (m, 5H).

3.12. Preparation of methyl (2S,3R)-3-phenylglycidate **7**

K_2CO_3 (23.2 mg, 0.167 mmol) was added to a solution of (2*R*,3*S*)-**4** (34.2 mg, 0.133 mmol) in MeOH (1.5 ml) at −20°C. The suspension was stirred vigorously between −20 and 0°C for 2 h, and then poured into saturated aqueous NH_4Cl. The mixture was extracted three times with EtOAc and the combined organic layers were washed with water, dried over $MgSO_4$, and concentrated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*S*,3*R*)-**7** (22.1 mg, 93%) as a colorless oil: $[\alpha]_D^{17}$ +148 (*c* 1.7, $CHCl_3$); IR: 2958, 1754, 1441, 1346, 1294, 1212, 1004, 897, 816, 696 cm^{-1}; ^1H NMR (200 MHz) δ 3.52 (d, 1H, $J=1.76$ Hz), 3.83 (s, 3H), 4.10 (d, 1H, $J=1.76$ Hz), 7.3–7.5 (m, 5H).

3.13. Preparation of methyl (2S,3S)-3-azide-2-hydroxy-3-phenylpropanoate **6**

A mixture of (2*S*,3*R*)-**7** (250 mg, 1.40 mmol), sodium azide (228 mg, 3.50 mmol), and NH_4Cl (224 mg, 4.20 mmol) in acetone/H_2O (4:1, 7 ml) was stirred for 23 h under reflux. The solution was concentrated, diluted with ethyl acetate (10 ml), and washed three times with water. The organic layer was dried over $MgSO_4$ and evaporated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*S*,3*S*)-**6** (296 mg, 96%) as a colorless oil: $[\alpha]_D^{20}$ +137 (*c* 2.3, $CHCl_3$) [lit.[7o] $[\alpha]_D^{25}$ +125 (*c* 1.0, $CHCl_3$)]. Spectral data were identical with those of an authentic sample.[7o]

(2*R*,3*R*)-**4** (210 mg, 0.82 mmol) was treated with conc. HCl (2 ml) in acetone/MeOH (3:4, 7 ml). The mixture was stirred for 23 h at room temperature and then diluted with water (15 ml). After repeating the extraction three times, the organic layer was dried over $MgSO_4$ and evaporated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*R*,3*R*)-**3** (158 mg, 90%) as white crystals: $[\alpha]_D^{20}$ −69 (*c* 1.3, $CHCl_3$). A mixture of (2*R*,3*R*)-**3** (158 mg, 0.74 mmol), sodium azide (143 mg, 2.21 mmol), and DMF (3.5 ml) was stirred for 48 h at 60°C under an atmosphere of nitrogen. The solution was diluted with ethyl acetate (10 ml) and washed three times with water. The organic layer was dried over $MgSO_4$ and evaporated. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1) to give (2*S*,3*S*)-**6** (136 mg, 83%) as a colorless oil.

3.14. Preparation of methyl (2S,3S)-N-benzoyl-3-phenylisoserine **8**

To a solution of (2*S*,3*S*)-**6** (177 mg, 0.80 mmol) and DMAP (4.9 mg, 0.04 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise benzoyl chloride (0.116, 1.00 mmol) at 0°C. Stirring was continued for 4 h at room temperature. The reaction mixture was extracted three times with ethyl acetate (50 ml), dried over $MgSO_4$ and evaporated. The residual crude product was hydrogenated with 10% Pd/C (18 mg) in methanol (1.6 ml) under an atmosphere of hydrogen (1 atm) for 48 h at room temperature. The solution was filtered and the residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1) to give (2*S*,3*S*)-**8** (167 mg, 70%) as white crystals: mp 138–140°C; $[\alpha]_D^{20}$ +16 (*c* 1.00, MeOH) [lit.[7k] $[\alpha]_D^{23}$ −23 (*c* 1, $CHCl_3$)]. Spectral data were identical with those of the authentic sample.

3.15. Preparation of (4S,5R)-2,4-diphenyl-5-methoxycarbonyl-2-oxazoline **11**

To a solution of (2*S*,3*S*)-**8** (60.0 mg, 0.20 mmol) in benzene (1.0 ml) were added PPh_3 (131 mg, 0.50 mmol) and DEAD (0.082 ml, 0.50 mmol) in 1.0 ml of benzene. The reaction mixture was stirred for 20 h at room temperature and then concentrated in vacuo. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 15:1–10:1) to give (4*S*,5*R*)-**11** (50.4 mg, 90%) as a colorless oil: $[\alpha]_D^{21}$ +15 (*c* 2.5, $CHCl_3$) [lit.[7k] $[\alpha]_D^{20}$ +13 (*c* 1, $CHCl_3$)]; IR: 3066, 3034, 2960, 2498, 1760, 1657, 1582, 1439, 1375, 1270, 1238, 1069, 911, 696 cm^{-1}. ^1H NMR (200 MHz) data were identical with those of an authentic sample.[7k]

To a solution of (2*S*,3*S*)-**6** (33.2 mg, 0.15 mmol) in benzene (0.5 ml) were added PPh_3 (197 mg, 0.75 mmol), DEAD (0.122 ml, 0.75 mmol), and benzoic acid (91.5 mg, 0.75 mmol) in 0.5 ml of benzene. The reaction mixture was stirred for 12 h at room temperature and then concentrated in vacuo. The residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 15:1–10:1) to give (4*S*,5*R*)-**11** (27.8 mg, 66%) as a colorless oil.

3.16. Hydrolysis of (4S,5R)-2,4-diphenyl-5-methoxycarbonyl-2-oxazoline **11**

A solution of (4*S*,5*R*)-**11** (50.4 mg, 0.18 mmol) in MeOH (3 ml) and 1N HCl (1.0 ml) was refluxed for 2 h. The solution was concentrated, and the organic layer was extracted with ethyl acetate. The combined extracts were washed with water, and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the residual crude product was purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 2:1) to give (2*R*,3*S*)-**8** (48.4 mg, 90%) as colorless crystals: $[\alpha]_D^{24}$ −49.9 (*c* 1.0, MeOH). Physical and spectral data were identical with those of the sample that was described in Section 3.14.

3.17. Methyl (2S,3S)-N-tert-butoxycarbonyl-3-phenylisoserine **9**

A suspension of 10% Pd/C (5 mg) in methanol (0.5 ml) was stirred at room temperature under an atmosphere of hydrogen (1 atm) for 10 min, whereupon a solution of di-*tert*-butyl dicarbonate (59.2 mg, 0.271 mmol) and (2*S*,3*S*)-**6** (50.0 mg, 0.226 mmol) in methanol (0.5 ml) was added. The stirring was continued for 72 h under an atmosphere of hydrogen (1 atm) at room temperature. The solution was filtered and evaporated. The residual crude product was

purified by column chromatography (silica gel Merck 60 with a mixture of hexane/EtOAc 10:1–5:1), giving (2*S*,3*S*)-**9** (64.8 mg, 97%) as white crystals: mp 120–121°C (lit.[14] mp 136–137°C); $[\alpha]_D^{23}$ +44 (*c* 1.2, $CHCl_3$) [lit.[14] $[\alpha]_D^{20}$ +30.1 (*c* 0.50, $CHCl_3$)]; IR: 3366, 1721, 1694, 1520, 1439, 1369, 1236, 1174, 1112, 1013, 866, 737, 518 cm^{-1}; ^1H NMR (200 MHz) δ 1.39 (s, 9H), 2.83 (d, 1H, $J=6.88$ Hz), 3.70 (s, 3H), 4.57 (dd, 1H, $J=3.38$, 6.88 Hz), 5.08 (dd, 1H, $J=3.38$, 8.06 Hz), 5.56 (d, 1H, $J=8.06$ Hz), 7.3–7.4 (m, 5H).

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References

1. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
2. For reviews, see: (a) Slichenmyer, W. J.; Van Hoff, D. D. *Anti-cancer Drugs* **1991**, *2*, 519. (b) Rowinskey, E. K.; Donehower, R. C. *J. Natl. Cancer Inst.* **1991**, *83*, 1778. (c) Schiff, P. B.; Fant, J.; Horwiz, S. B. *Nature* **1979**, *277*, 665.
3. For a review on the chemistry of Taxol, see: Kingston, D. G. I.; Samaranayake, G.; Ivey, C. A. *J. Nat. Prod.* **1990**, *53*, 1.
4. (a) Denis, J.-N.; Greene, A. E.; Guenard, D.; Gueritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917. (b) Chauviere, G.; Guenard, D.; Picot, F.; Senilh, V.; Potier, P. *C. R. Acad. Sci. Paris* **1981**, *293*, 501.
5. (a) Commercon, A.; Bezard, D.; Bernard, F.; Bourazat, J. D. *Tetrahedron Lett.* **1992**, *33*, 5185. (b) Ojima, I.; Sun, C. M.; Zucco, M.; Park, Y. H.; Duclos, O.; Kuduk, S. *Tetrahedron Lett.* **1993**, *34*, 4149.
6. Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. J. *J. Med. Chem.* **1992**, *35*, 145.
7. (a) Denis, J.-N.; Greene, A. E.; Serra, A. A.; Luche, M. J. *J. Org. Chem.* **1986**, *51*, 46. (b) Cardillo, G.; Gentilucci, L.; Tolomelli, A.; Tomasini, C. *J. Org. Chem.* **1998**, *63*, 2351. (c) Merino, P.; Castillo, E.; Franco, S.; Merchan, F. L.; Tejero, T. *Tetrahedron* **1998**, *54*, 12301. (d) Lee, D.; Kim, M.-J. *Tetrahedron Lett.* **1998**, *39*, 2163. (e) Kanazawa, A. M.; Denis, J.-N.; Greene, A. E. *J. Org. Chem.* **1994**, *59*, 1238. (f) Koskinen, A. M. P.; Karvinen, K. E.; Siirila, J. P. *J. Chem. Soc., Chem. Commun.* **1994**, 21. (g) Barco, A.; Benetti, S.; Risi, D. C.; Pollini, P. G.; Romagnoli, R.; Zanirato, V. *Tetrahedron Lett.* **1994**, *35*, 9289. (h) Bunnage, M. E.; Davies, S. G.; Goodwin, C. J. *J. Chem. Soc., Perkin Trans.* **1993**, 1375. (i) Righi, G.; Rumboldt, G. *J. Org. Chem.* **1996**, *61*, 3557. (j) Wang, Z.-M.; Kolb, H. C.; Sharpless, K. B. *J. Org. Chem.* **1994**, *59*, 5104. (k) Gou, D.-M.; Liu, Y.-C.; Chen, C.-S. *J. Org. Chem.* **1993**, *58*, 1287. (l) Deng, L.; Jacobsen, E. N. *J. Org. Chem.* **1992**, *57*, 4320. (m) Kearns, J.; Kayser, M. M. *Tetrahedron Lett.* **1994**, *35*, 2845. (n) Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* **1990**, *55*, 1957. (o) Honig, H.; Seufer-Wasserthal, P.; Weber, H. *Tetrahedron* **1990**, *46*, 3841. (p) Mamedov, V. A.; Hamamoto, H.; Tsuboi, S. Presented in Part at the 74th Annual Meeting of the Chemical Society of Japan, Kyotanabe, 30 March, 1998; Abstr. 2, pp. 1441.
8. Mangatal, L.; Adeline, M.-T.; Guenard, D.; Gueritte-Voegelein, F.; Potier, P. *Tetrahedron* **1989**, *45*, 4177.
9. (a) McDonald, R. N.; Schwab, P. A. *J. Org. Chem.* **1964**, *29*, 2459. (b) Takeda, A.; Tsuboi, S.; Nakashima, I. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 1067. (c) Tsuboi, S.; Furutani, H.; Takeda, A.; Kawazoe, K.; Sato, S. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2475. (d) Mamdeov, V. A.; Nuvetdinov, I. A. *Izv. Akad. Nauk. Ser. Khim.* **1992**, 2159.
10. Kamitori, Y.; Hojo, M.; Masuda, R.; Inoue, T.; Izumi, T. *Tetrahedron Lett.* **1982**, *23*, 4585.
11. (a) Akita, H.; Enoki, Y.; Yamada, H.; Oishi, T. *Chem. Pharm. Bull.* **1990**, *38*, 323. (b) Sugai, T.; Ohta, H. *Agric. Biol. Chem.* **1990**, *54*, 3337. (c) Tsuboi, S.; Yamafuji, N.; Utaka, M. *Tetrahedron: Asymmetry* **1997**, *8*, 375.
12. Pasto, M.; Moyano, A.; Pericas, M. A.; Riera, A. *Tetrahedron*: *Asymmetry* **1996**, *7*, 243.
13. Srivastava, R. P.; Zjawiony, J. K.; Peterson, J. R.; McChesney, J. D. *Tetrahedron*: *Asymmetry* **1994**, *5*, 1683.
14. Jost, S.; Gimbert, Y.; Greene, A. E.; Fotiadu, F. *J. Org. Chem.* **1997**, *62*, 6672.