A Practical Chemoenzymatic Synthesis of the Taxol C-13 Side Chain *N*-Benzoyl-(2*R*,3*S*)-3-phenylisoserine

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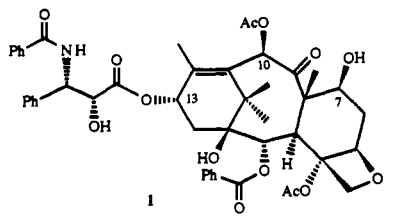
Introduction

Taxol (1), 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 commercial production of 1 by total synthesis.[3] Thus, a viable approach for the preparation of 1 is to utilize more accessible baccatin III (2) or 10-deacetylbaccatin III (3) as precursors via a semisynthetic route.[4]



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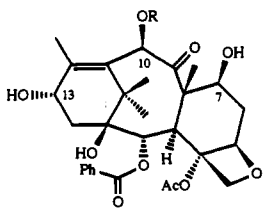
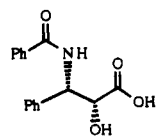


2 R = CH_3CO-
3 R = H



4

As the role of the *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine (4) moiety in the biological activity of 1 became evident,[5] enantioselective synthesis of the C-13 side chain has been the focus of many investigations.[6] Here, we describe a facile approach using enzymatically-prepared chiral *trans*-β-phenylglycidic esters as starting materials for the synthesis of 4.

Results and Discussion

Kinetic Resolution via Enantioselective Transesterification. The resolution of methyl *trans*-β-phenylglycidate ((±)-5) was achieved by lipase-mediated enantioselective transesterification in organic media.[7] The racemic methyl ester was first exposed to different lipases in hexane with *n*-butyl alcohol as the acyl acceptor. Although all the enzymes tested were capable of mediating the formation of 6, *Mucor miehei* lipase (Amano, MAP-10) was uniquely suited for this stereospecific transesterification with high optical and chemical yields (Table I). However, as noted in Table I, the enantioselectivity of this acyl-transfer reaction appeared to be complicated by the intrinsic thermodynamic equilibrium, of which the principle is well understood.[10] The overall selectivity gradually diminished as the reaction proceeded close to 50% conversion. Under these conditions, the kinetic and thermodynamic parameters were estimated to be $E = 33$ and $K = 0.11$.[11] To minimize the extent of reverse catalysis, i.e., to reduce the K value, two strategies were employed: selecting a sterically hindered alcohol as the acyl acceptor and increasing the alcohol concentrations.[7] It was found that with isobutyl alcohol–hexane (1:1; v/v) as the reaction medium, the backward reaction became virtually negligible. For example, when (±)-5 was incubated with Lipase MAP-10 in hexane–isobutyl alcohol (1:1), at 36% and 45% conversion, the enantiomeric excess (ee) values for the product fraction were 97% and 95%, respectively; those for the remaining substrate fraction were 56% and 77%, respectively. Thus, this enzymatic process coupled with substrate recycling afforded both fractions with high optical and chemical yields. In a typical experiment with 20 g of (±)-5 as substrate, the isolated yields for recovered (−)-5 (95% ee) and (+)-7 (95% ee) were 42% (8.5 g) and 43% (11.3 g), respectively. Other practical aspects of this enzymatic process include that (i) the product and substrate could be isolated by fractional distillation and (ii) the crude enzyme retained 85% of lipase activity after the reaction and could be easily recovered from the mixture by filtration. Even though the lipase is relatively inexpensive ($150/kg), recycle of the biocatalyst can reduce the operation cost.

Synthesis. As illustrated in Scheme I, both (−)-5 and (+)-7 can be efficiently utilized to prepare the desired C-13 side chain. Regioselective cleavage of the (2*R*,3*S*)-epoxide 5 with diethylamine hydrobromide[12] and dieth-

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(2) For reviews: (a) Slichenmyer, W. J.; Van Hoff, D. D. *Anti-Cancer Drugs* 1991, *2*, 519. (b) Rowinsky, E. K.; Donehower, R. C. *J. Natl. Cancer Inst.* 1991, *83*, 1778. (c) Holmes, F. A.; Walters, R. S.; Theriault, R. L.; Forman, A. D.; Newton, L. K.; Raber, M. N.; Buzdar, A. U.; Frye, D. K.; Hortobagyi, G. N. *Ibid.* 1991, *83*, 1797.

(3) For reviews on the chemistry of taxol: (a) Kingston, D. G. I.; Samaranayake, G.; Ivey, C. A. *J. Nat. Prod.* 1990, *53*, 1. (b) Kingston, D. G. I. *Pharmac. Ther.* 1991, *52*, 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) Holton, R. A. U.S. Patent 5,015,744; *Chem. Abst.* 1991, *115*, 159485.

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(6) A variety of strategies have been reported in the literature: Asymmetric epoxidation: (a) Denis, J.-N.; Greene, A. E.; Serra, A. A.; Luche, M.-J. *J. Org. Chem.* 1986, *51*, 46. (b) Deng, L.; Jacobsen, E. N. *J. Org. Chem.* 1992, *57*, 4320. Asymmetric dihydroxylation: (c) Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* 1990, *55*, 1957. Enzymatic resolution: (d) Honig, H.; Seufer-Wasserthal, P.; Weber, H. *Tetrahedron*, 1990, *46*, 3841. (e) Honig, H.; Seufer-Wasserthal; Weber, H. *Tetrahedron Lett.* 1990, *31*, 3011. Chiral precursor: (f) Denis, J.-N.; Correa, A.; Greene, A. E. *J. Org. Chem.* 1991, *56*, 6939. Asymmetric β-lactam formation: (g) Ojima, I.; Habus, I.; Zhao, M.; Georg. G. I.; Jayasinghe, L. R. *J. Org. Chem.* 1991, *56*, 1681. (h) Georg, G. I.; Mashava, P. M.; Akgun, E.; Milstead, M. W. *Tetrahedron Lett.* 1991, *32*, 3151.

(7) Chen, C. S.; Sih, C. J. *Angew. Chem., Int. Ed. Engl.* 1989, *28*, 596.

(8) Legters, J.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett.* 1989, *30*, 4881.

(9) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* 1982, *104*, 7294.

(10) Chen, C. S.; Wu, S. H.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* 1987, *109*, 2812.

(11) Considering reversible biocatalyzed kinetic resolution,

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P$$

$$B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} Q$$

$E = k_1/k_3$; $K = k_2/k_1$, where k_1, k_2, k_3, and k_4 are apparent rate constants. A full account of this quantitative expression is described in ref 10.

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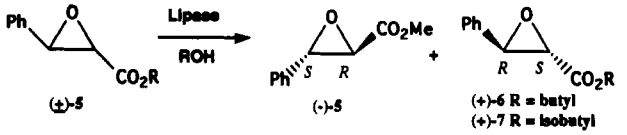
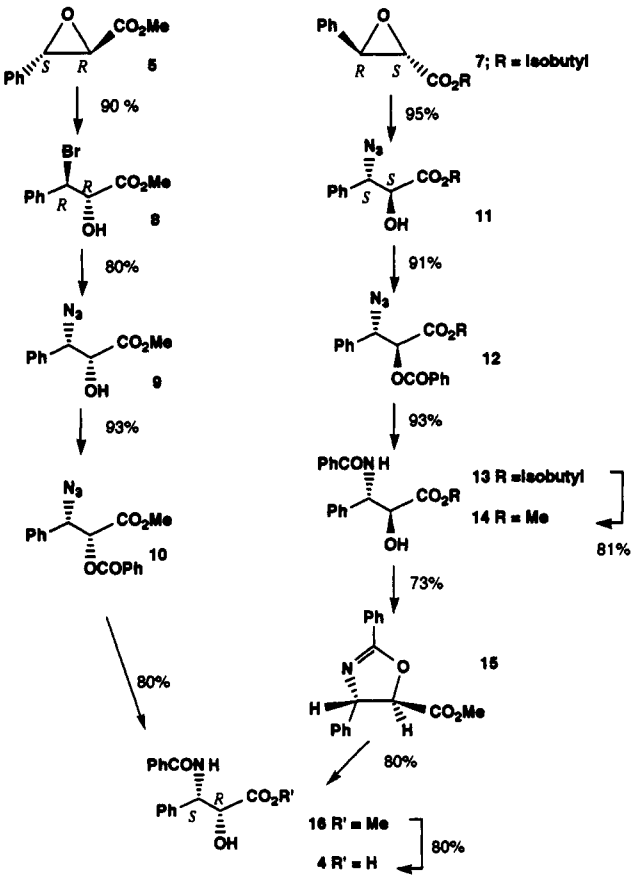
Table I. Enantioselective Transesterification of (±)-5 by Lipases[a]



lipase	stereopreference[b]	convn[c] % (time, h)	enantiomeric excess[d] substrate	enantiomeric excess[d] product
	ROH = Butyl Alcohol			
Pseudomonas sp. lipase AK	2*S*,3*R*	23 (12)	19	64
Pseudomonas sp. lipase P-30	2*S*,3*R*	41 (24)	35	50
porcine pancreas lipase	2*S*,3*R*	24 (48)	12	38
C. cylindracea lipase	2*S*,3*R*	37 (24)	26	44
Humicola lanuginisa lipase R-10	2*R*,3*S*	26 (36)	11	32
Mucor miehei lipase MAP-10	2*S*,3*R*	33 (23)	46	91
		41 (48)	59	85
		51 (82)	77	78
	ROH = Isobutyl Alcohol			
M. miehei lipase MAP-10	2*S*,3*R*	36 (24)	56	97
		45 (40)	77	95

[a] For the *n*-butyl alcohol experiments, all reactions were conducted with (±)-5 (1 g, 5.62 mmol), *n*-butyl alcohol (4.6 mL, 50 mmol), and the lipase (3 g) in water-saturated hexane (10 mL). The suspension was shaken (250 rpm) at 23 °C for the indicated time. The reaction was terminated by removing the enzyme powder through filtration. For the isobutyl alcohol experiments, the reaction was run with (±)-5 (20 g, 0.11 mol), isobutyl alcohol (80 mL), and lipase MAP-10 (60 g) in hexane (80 mL). The mixture was incubated at 30 °C on a rotary shaker (250 rpm). [b] Lit.[8] (2*R*,3*S*)-5, $[\alpha]^{20}_D = -173°$ ($CHCl_3$). [c] Conversion (*c*) was calculated from the equation, $c = ee(S)/[ee(S) + ee(P)]$, where ee(S) and ee(P) denote the values of enantiomeric excess of substrate and product fractions.[9] [d] The enantiomeric excess was determined by ^1H NMR analysis with a chiral shift reagent, Eu(hfc)$_3$.

Scheme I



ylaluminum chloride yielded the (2*R*,3*R*)-hydroxy bromide (−)-8 in 90% yield. No appreciable formation of the (2*R*,3*S*)-epimer could be detected. Azide displacement of (−)-8 led to the C-3 inverted product (+)-9 in 80% yield. The hydroxy azide 9 was then subjected to benzoylation, followed by hydrogenation, furnishing 16 in 80% yield.[13] Recrystallization of the resulting compound gave enantiomerically pure 16 that was then hydrolyzed by K_2CO_3/CH_3OH–H_2O to afford the C-13 side chain in 80% yield. The overall yield from (−)-5 was 40%.

On the other hand, the use of (+)-7 could be made of by refluxing with sodium azide and ammonium chloride in acetone–water to generate (+)-11. The (2*S*,3*S*)-hydroxy azide was subjected to benzoylation and then hydrogenation to yield the key intermediate (−)-13. To confirm the structure of the compound, the isobutyl ester was transformed to the corresponding methyl ester (−)-14 in 81% yield. The erythro isomer 14 was treated with thionyl chloride to give the oxazoline intermediate (+)-15, resulting in the inversion of configuration at the C-2.[14] Subsequent hydrolysis of (+)-15 with aqueous 1 N HCl in methanol, followed by recrystallization, yielded the enantiomerically pure threo isomer (−)-16 with an overall yield of 38% from (+)-7. The target molecule 4 could thus be afforded by alkaline hydrolysis of 16.

The synthetic approach described here provides an efficient route to the taxol C-13 side chain. Application of this strategy to the preparation of novel derivatives of 4 is currently in progress.

Experimental Section

Materials and Methods. ^1H NMR spectroscopy was carried out at 300 MHz in $CDCl_3$. Porcine pancreas lipase (Type II) and *Candida cylindracea* lipase were purchased from Sigma Chemical Co. (St. Louis, MO). *M. miehei* lipase and other microbial lipases used in this study were obtained from Amano International Enzyme Co. (Troy, VA). Elemental Analyses were performed by M-H-W Laboratories (Phoenix, AZ).

Enantioselective Transesterification of (±)-5 with Isobutyl Alcohol in Hexane by *M. miehei* Lipase MAP-10. Crude MAP-10 lipase (60 g of powder, 4 g of protein) was added to a mixture of (±)-5 (20 g, 0.11 mol) and isobutyl alcohol (80 mL) in hexane (80 mL). The resulting suspension was incubated at

(12) Gao, L.-X.; Saitoh, H.; Feng, F.; Murai, A. *Chem. Lett.* **1991**, 1787.

(13) 16: mp 181–183 °C; $[\alpha]^{20}_D = -49°$ (*c* 1, CH_3OH); lit.[6a] $[\alpha]^{20}_D = -49.6°$ (*c* 1, CH_3OH).

(14) (a) Johnson, W. S.; E. N. Schuber *J. Am. Chem. Soc.* **1950**, *72*, 2187. (b) McCasland, G. E.; Smith, D. A. *Ibid.* **1950**, *72*, 2190.

30 °C on a rotary shaker (250 rpm). After 40 h, the reaction was terminated by removing the crude enzyme powder through filtration. The organic solvent was taken to dryness under reduced pressure, and the residue was chromatographed over a silica gel column (hexane–ethyl ether, 20:1) to afford (+)-7 [11.3 g; $[\alpha]^{20}_D$ = +129° (c 1.5, $CHCl_3$); 95% ee] and (−)-5 [12.5 g; $[\alpha]^{20}_D$ = −133° (c 1.5, $CHCl_3$); 77% ee]. The remaining substrate (12.5 g) was reincubated with lipase MAP-10 (12 g) in hexane–isobutyl alcohol (1:1, 30 mL). The reaction was stopped after 48 h according to the procedure described above to furnish (−)-5 [8.5 g; $[\alpha]^{20}_D$ = −163° (c 1.5, $CHCl_3$); 95% ee]. Alternatively, 5 and 7 could be isolated by fractional distillation in nearly quantitative yields. The boiling points for the methyl and isobutyl esters were 85–95 °C (0.1–0.05 mmHg) and 117–119 °C (0.1 mmHg), respectively.

(2*R*,3*R*)-(−)-Methyl 3-Bromo-2-hydroxy-3-phenylpropionate (8). A solution of (−)-5 (8.5 g, 48 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a mixture of diethylamine hydrobromide (22.5 g, 71 mmol), diethylaluminum chloride (9 mL, 71 mmol), and CH_2Cl_2 (200 mL) at −41 °C. The mixture was stirred at −15 °C for 2.5 h, washed with 1 N HCl and water, dried, and concentrated. Column chromatography (hexane–ether, 20:1 → 5:1) of the residue gave 8 (11 g, 90%): $[\alpha]^{22}_D$ = −138° (c 1.5, $CHCl_3$); ^{1}H NMR δ 3.05 (d, J = 6.9 Hz, 1 H), 3.73 (s, 3 H), 4.70 (dd, J = 4.8, 6.8 Hz, 1 H), 5.26 (d, J = 4.5 Hz, 1 H), 7.29–7.46 (m, 5 H).

(2*R*,3*S*)-(+)-Methyl 3-Azido-2-hydroxy-3-phenylpropionate (9). A mixture of (−)-8 (10 g, 38 mmol), sodium azide (10 g, 155 mmol), and DMF (40 mL) was stirred at 65–75 °C for 40 h. The solution was diluted with ethyl acetate (200 mL), washed with water, dried, and concentrated. Column chromatography (hexane–ether, 25:1 → 5:1) of the residue afforded 9 (6.9 g, 80%): $[\alpha]^{20}_D$ = +107° (c 1.2, $CHCl_3$); ^{1}H NMR δ 3.09 (d, J = 6.6 Hz, 1 H), 3.79 (s, 3 H), 4.34 (dd, J = 4.8, 6.6 Hz, 1 H), 4.83 (d, J = 4.8 Hz, 1 H), 7.27–7.43 (m, 5 H).

(2*R*,3*S*)-(+)-Methyl 3-Azido-2-benzoxy-3-phenylpropionate (10). A solution of (+)-9 (6.9 g, 34 mmol) and DMAP (4.15 g, 34 mmol) in CH_2Cl_2 (50 mL) was treated with benzoyl chloride (4.2 mL, 34 mmol) at 23 °C for 1 h. The reaction mixture was processed in the usual manner. Column chromatography (hexane–ether, 25:1 → 5:1) of the residue furnished 10 (9.5 g, 93%): $[\alpha]^{20}_D$ = +98° (c 1, $CHCl_3$); ^{1}H NMR δ 3.69 (s, 3 H), 5.17 (d, J = 4.8 Hz, 1 H), 5.49 (d, J = 4.8 Hz, 1 H), 7.32–7.61 (m, 8 H), 7.98–8.11 (m, 2 H).

(2*R*,3*S*)-(−)-*N*-Benzoyl-3-phenylisoserine Methyl Ester (16). The azide 10 (8 g, 24 mmol) was hydrogenated with 10% Pd/C (2.5 g) in methanol (80 mL) under H_2 (50 psi) for 4 h. The solution was filtered, allowed to stand at 23 °C for 48 h, and concentrated. The solid was recrystallized over $CHCl_3$–methanol (50:1) to give 16 (5.5 g, 74%): $[\alpha]^{20}_D$ = −49° (c 1, CH_3OH), lit.[6c] $[\alpha]^{20}_D$ = −49.6° (c 1, CH_3OH); ^{1}H NMR (CD_3OD) δ 3.68 (s, 3 H), 4.60 (d, J = 3.8 Hz, 1 H), 5.58 (d, J = 3.8 Hz, 1 H), 7.32–7.61 (m, 8 H), 7.98–8.11 (m, 2 H).

Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.73; N, 4.68. Found: C, 68.42; H, 5.92; N, 4.76.

(2*R*,3*S*)-(−)-*N*-Benzoyl-3-phenylisoserine (4). To a solution of compound 16 (5 g, 17.4 mmol) in methanol (100 mL) was added K_2CO_3 (4.6 g, 87 mmol) in water (35 mL). After being stirred at 23 °C for 12 h, the solution was concentrated and extracted with CH_2Cl_2 (50 mL × 3). The aqueous layer was acidified to pH 1 and extracted with ethyl acetate (50 mL × 3). The combined ethyl acetate fractions were dried and concentrated. Recrystallization of the residue from $CHCl_3$ gave (−)-4 (3.8 g, 80%): mp 166–168 °C, lit.[6g] mp 167–169 °C; $[\alpha]^{20}_D$ = −37.8° (c 1, C_2H_5OH), lit.[6g] $[\alpha]^{20}_D$ = −37.8° (c 0.9, C_2H_5OH); ^{1}H NMR (CD_3OD) δ 4.55 (d, J = 3.3 Hz, 1 H), 5.62 (d, J = 3.3 Hz, 1 H), 7.23–7.55 (m, 7 H), 7.81–7.84 (m, 2 H).

(2*S*,3*S*)-(+)-Isobutyl 3-Azido-2-hydroxy-3-phenylpropionate (11). A solution of the (2*S*,3*R*)-epoxide 7 (11.2 g, 50 mmol), NaN_3 (8.13 g, 125 mmol), and NH_4Cl (10 g, 18.7 mmol) in acetone/water (4:1, 250 mL) was stirred under reflux for 20 h. The reaction mixture was concentrated and diluted with CH_2Cl_2 (100 mL). The solution was washed with water, dried, and concentrated. Column chromatography (25:1 → 5:1) of the residue afforded (+)-11 (12.7 g, 95%): $[\alpha]^{20}_D$ = +55° (c 1.1, $CHCl_3$); ^{1}H NMR δ 0.86 (d, J = 9.6 Hz, 6 H), 1.93–2.07 (m, 1 H), 3.15 (d, J = 5.7 Hz, 1 H), 3.79–4.01 (m, 2 H), 4.52–4.55 (m, 1 H), 4.86 (d, J = 5.7 Hz, 1 H), 7.27–7.41 (m, 5 H).

(2*S*,3*S*)-(+)-Isobutyl 3-Azido-2-benzoxy-3-phenylpropionate (12). Compound 11 (12.6 g, 47 mmol) was benzoylated, as described for 10, to yield (+)-12 (16 g, 91%): $[\alpha]^{20}_D$ = +54° (c 2, $CHCl_3$); ^{1}H NMR δ 0.81 (d, J = 6.7 Hz, 6 H), 1.75–1.91 (m, 1 H), 3.84–3.90 (m, 2 H), 5.15 (d, J = 5.3 Hz, 1 H), 5.60 (d, J = 5.3 Hz, 1 H), 7.34–7.61 (m, 8 H), 7.99–8.03 (m, 2 H).

(2*S*,3*S*)-(−)-Isobutyl *N*-Benzoyl-3-phenylisoserine (13). Conventional hydrogenation of 12 (15.5 g, 42 mmol), as described for 16, gave (−)-13 (13.5 g, 93%): $[\alpha]^{20}_D$ = −38° (c 1, $CHCl_3$); ^{1}H NMR δ 0.93 (d, J = 6.7 Hz, 6 H), 1.87–2.00 (m, 1 H), 3.13 (d, J = 6.3 Hz, 1 H), 3.86 (ABX, 2 H, J_{AB} = 10.5 Hz, J_{AX} = 6.6 Hz, J_{BX} = 6.7 Hz), 4.73 (dd, J = 3.5, 6.0 Hz, 1 H), 5.46 (dd, J = 3.5, 6.0 Hz, 1 H), 7.2 (d, J = 8.5 Hz, 1 H), 7.27–7.74 (m, 8 H), 7.81–7.84 (m, 2 H).

Anal. Calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10. Found: C, 70.40; H, 6.79; N, 4.05.

(2*S*,3*S*)-(−)-Methyl *N*-Benzoyl-3-phenylisoserine (14). A mixture of the isobutyl ester 13 (13.4 g, 39 mmol), Na_2CO_3 (6 g, 56 mmol), and 75% aqueous methanol (60 mL) was stirred at 23 °C for 4 h. The solution was concentrated, diluted with water (150 mL), and extracted with CH_2Cl_2. The aqueous layer was acidified to pH 1 and extracted with ethyl acetate (40 mL × 3). The combined ethyl acetate fractions were washed with water, dried, and treated with diazomethane. Recrystallization (from CH_2Cl_2–methanol, 50:1) of the residue afforded (−)-14 (9.6 g, 81%): $[\alpha]^{20}_D$ = −23° (c 1, $CHCl_3$); ^{1}H NMR δ 3.11 (d, J = 6.6 Hz, 1 H), 3.74 (s, 3 H), 4.71 (dd, J = 2.6, 6.6 Hz, 1 H), 5.66 (dd, J = 2.6, 6.6 Hz, 1 H), 7.18 (d, J = 6.6 Hz, 1 H), 7.27–7.55 (m, 8 H), 7.81–7.84 (m, 2 H).

Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.73; N, 4.68. Found: C, 68.21; H, 5.65; N, 4.69.

(4*S*,5*R*)-(+)-2,4-Diphenyl-5-(methoxycarbonyl)-2-oxazoline (15). A solution of (−)-14 (9.5 g, 31 mmol) and thionyl chloride (6 mL, 79 mmol) in $CHCl_3$ (40 mL) was stirred at 45 °C for 5 h. The solution was concentrated, dissolved in $CHCl_3$ (40 mL), and refluxed for 48 h. The solution was washed with water, dried, and concentrated. Column chromatography (hexane–ether, 25:1 → 5:1) of the residue furnished (+)-15 (6.5 g, 73%): $[\alpha]^{20}_D$ = +13° (c 1, $CHCl_3$); ^{1}H NMR δ 3.84 (s, 3 H), 4.91 (d, J = 6.5 Hz, 1 H), 5.45 (d, J = 6.5 Hz, 1 H), 7.29 (m, 8 H), 8.08 (m, 2 H).

(2*R*,3*S*)-(−)-*N*-Benzoyl-3-phenylisoserine Methyl Ester (16). The oxazoline (+)-15 (6.2 g, 22 mmol) was hydrolyzed by refluxing with 1 N HCl (22 mL) in methanol (65 mL) for 2 h. The solution was concentrated, diluted with CH_2Cl_2 (100 mL), washed with water, dried, and concentrated. Recrystallization (CH_2Cl_2–methanol, 50:1) of the residue gave (−)-16 (5.3 g, 80%). The physical data were identical to those aforementioned for the compound 16 derived from (+)-5.

Supplementary Material Available: ^{1}H NMR spectra of 4, 5, and 7–16 (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.