Total Synthesis of (−)-Bulgecinine

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A short synthetic route to (−)-bulgecinine (1), the amino acid moiety of bulgecins, was established by using 1,3-dipolar cycloaddition of *N*-benzyl-α-methoxycarbonylmethanimine *N*-oxide (6) to a chiral allylic alcohol (5) with moderate *threo* selectivity as the key reaction.

Key words (−)-bulgecinine; 1,3-dipolar cycloaddition; total synthesis; pyrrolidine alkaloid; chiral allylic alcohol

Bulgecins are antibiotic hydroxyproline glycosides originally isolated from cultures of *Pseudomonas acidophila* and *P. mesoacidophila* by Shinagawa *et al.*[1] They contain (−)-bulgecinine (**1**), which is a hydroxyproline derivative and has an antibiotic effect, as the common aglycon moiety (Fig. 1).

(−)-Bulgecinine (**1**) has been synthesized by several groups from chiral sources such as D-glucose,[2] D-glucuronolactone,[3] L-pyroglutamic acid,[4] L-2-amino-4-pentanoic acid[5] and (4*R*)-hydroxyproline[6] and recently, more efficiently, by Hirai *et al.*[7] *via* a highly stereoselective palladium-catalyzed intramolecular cyclization. In continuing our research on the synthesis of pyrrolidine antibiotics such as (+)-preussin using 1,3-dipolar cycloaddition reactions between nitrones and allylic alcohols,[8] we have developed a short synthetic route to the title antibiotic (**1**). The route is shown in Charts 1—3.

(*S*)-4-*tert*-Butyldiphenylsilyloxy-1-buten-3-ol (**5**)[9] was chosen as a dipolarophile to construct (*R*)-C-5 in compound **1** and prepared from compound **2**[10] as depicted in Chart 1. Namely, compound **2** was silylated to afford compound **3** in the usual way,[11] followed by Mitsunobu reaction of compound **3** with 3,5-dinitrobenzoic acid under the standard conditions to give the corresponding benzoate (**4**) in 58% yield.[12] Methanolysis of compound **4** gave the chiral allylic alcohol (**5**) in 83% yield. *N*-Benzyl-α-methoxycarbonylmethanimine *N*-oxide (**6**) was synthesized according to the method described previously.[13]

1,3-Dipolar cycloaddition reaction of the nitrone (**6**) with the chiral allylic alcohol (**5**) proceeded in refluxing toluene to give a mixture of stereoisomers of oxazolidines (**7**) in 85% yield. These isomers could not be separated from each other by silica gel column chromatography. Therefore, without further purification, the mixture was mesylated (96% yield) and the resultant mixture of mesylated products (**8**) was subjected to hydrogenolysis at room temperature using H_2–20%$Pd(OH)_2$ on carbon. The diastereomeric mixture of pyrrolidines obtained was separated by a combination of medium-pressure liquid chromatography (MPLC) and preparative thin layer chromatography (PTLC) to give compounds **9**, **10**, **11** and **12** in yields of 21, 9.1, 1.8, and 3.0% (production ratio = 60 : 26 : 5 : 9), respectively, based on the mesylated compounds (Chart 2).

The structures of the pyrrolidines (**9**, **10**, **11**, **12**) were determined on the basis of ^1H–^1H nuclear Overhauser effect (NOE) spectroscopy (NOESY) experiments. The observed ^1H–^1H NOEs are illustrated in Fig. 2.

Thus, it was demonstrated that the 1,3-dipolar cycloaddition of compound **6** with compound **5** proceeded with a moderate *threo* selectivity.[14] Among these pyrrolidines, compound **9** has the same stereochemistry as natural (−)-bulgecinine (**1**).

Compound **9** was then converted to compound (−)-**1** as shown in Chart 3. Briefly, compound **9** was treated with carbobenzoxy chloride (CbzCl) and sodium carbonate (Na_2CO_3) to give compound **13** in 74% yield,[15] and this, on desilylation in the usual manner, gave compound **14**. Finally, acid hydrolysis of compound **14** gave (−)- bulgecinine (**1**) in 67% yield. The ^1H- and ^{13}C-NMR

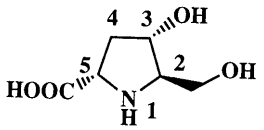
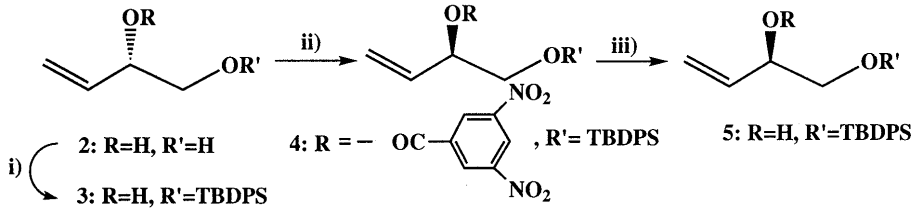


Fig. 1. (−)-Bulgecinine (**1**)



i) TBDPSCl, pyridine, room temperature ii) 3,5-dinitrobenzoic acid, DEAD, PPh_3, toluene iii) K_2CO_3, THF/MeOH

Chart 1. Preparation of Compound **5**

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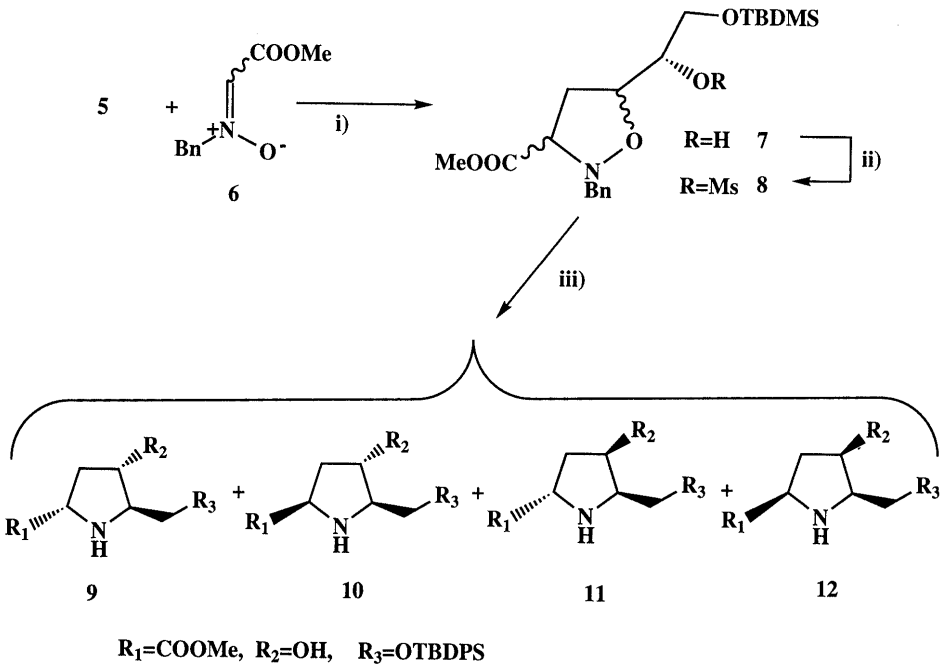


Chart 2. Construction of the Pyrrolidines **9**—**12**

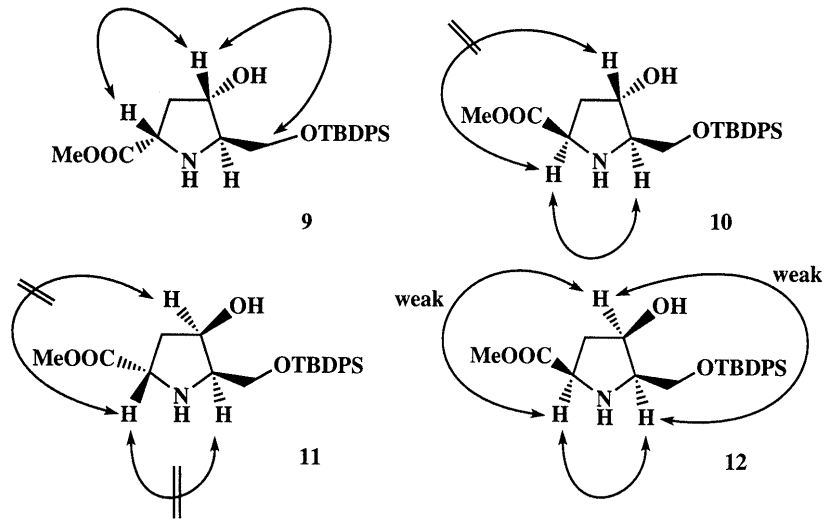


Fig. 2. ^1H–^1H NOESY of the Pyrrolidines **9**—**12**

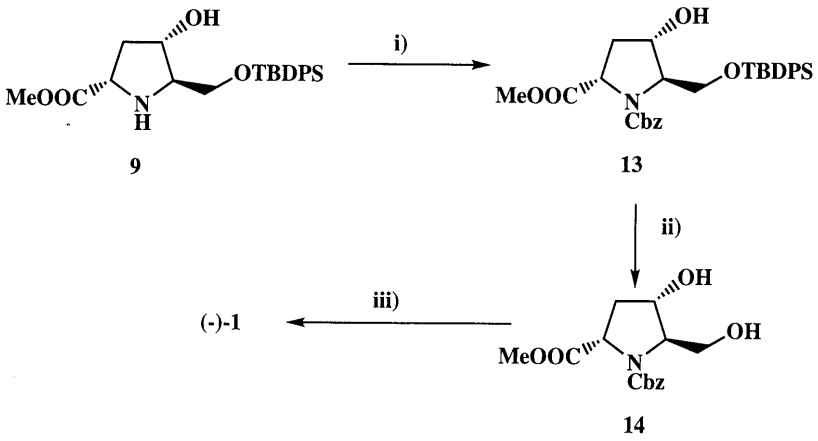
spectral data, and optical rotation of the synthetic (−)-bulgecinine ($[\alpha]_D = -13.9°$ ($c=0.36$, H_2O)) were identical with those of natural (−)-bulgecinine (**1**) ($[\alpha]_D = -13.1°$ ($c=0.95$, H_2O)[1]).

Experimental

The melting points were obtained using a Yanagimoto melting point apparatus and are uncorrected. ^1H-NMR spectra were recorded for solutions in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard on a Varian Gemini-300 or a Brucker AM-400 instrument. High-performance liquid chromatography (HPLC) was conducted using a PU-980 pump (Jasco Co., Ltd.), a UV-980 UV detector (Jasco Co., Ltd.), and a Chiralcel OD column (Daicel Co., Ltd.). MPLC was conducted using a UVILOG 5III UV detector (Oyo Bunko Kiki Co., Ltd., Tokyo) and Kiesel gel 60 (Merck AG, Darmstadt) as the packing material. PTLC was conducted using Merck TLC plates (Art. 1.05744). Optical rotations were measured with a DIP-360 (Japan Spectroscopic Co.) at 26 °C. Other spectral data were obtained by using the following instruments: IR spectra, Japan Spectroscopic Co. A-100; MS, Hitachi M-80B or Fisons Auto Spec instrument.

(*R*)-4-*tert*-Butyldiphenylsilyloxy-1-buten-3-ol (3) Compound **3** was prepared from (*R*)-3-hydroxy-1-buten-4-ol (**2**)[10] according to the method described previously for the silylation of the racemic **2**.[11] Colorless oil, $[\alpha]_D$ −3.4° ($c=3.23$, $CHCl_3$). The enantiomer excess (ee %) of this compound was 91% ee, as determined by HPLC (Chiralcel OD, Daicel Chemical Co., Ltd.).

(*S*)-4-*tert*-Butyldiphenylsilyloxy-3-(3,5-dinitrophenylcarbonyloxy)-1-butene (4) Diethyl azodicarboxylate (DEAD) (0.54 g, 3.12 mmol) was added to a dry toluene solution (20 ml) of compound **3** (0.86 g, 2.6 mmol), 3,5-dinitrobenzoic acid (0.662 g, 3.12 mmol), and PPh_3 (0.818 g, 3.12 mmol) at 0 °C and the mixture was stirred overnight at room temperature. The reaction mixture was washed successively with saturated $NaHCO_3$ (10 ml), H_2O (10 ml), and saturated NaCl (10 ml). The organic phase was dried with Na_2SO_4, then the solvent was evaporated under reduced pressure and the oily residue was purified by MPLC (hexane : AcOEt = 95 : 5) to give compound **4** (0.742 g, 58%). Colorless oil. $[\alpha]_D$ +17.5° ($c=1.03$, $CHCl_3$). IR (neat): 1735



i) CbzCl, Na_2CO_3, MeOH/H_2O, 0 °C ii) TBAF, THF, room temperature iii) 6N HCl, reflux

Chart 3. Synthesis of (−)-Bulgecinine (**1**)

(C=O) cm^{-1}. ^{1}H-NMR (300 MHz) δ: 1.02 (9H, s), 3.93 (2H, d, J=5.5 Hz), 5.34 (1H, dd, J=10.5, 1 Hz), 5.42 (1H, dt, J=17.2, 1 Hz), 5.77 (1H, m), 5.92 (1H, m), 7.35 (6H, m), 7.63 (4H, m), 9.11 (2H, d, J=2.1 Hz), 9.22 (1H, d, J=2.1 Hz). CI-MS m/z: 521 (M^++1). HR-MS Calcd for $C_{23}H_{19}N_2O_7Si$ (M^+−*tert*-Bu): 463.096115. Found: 463.096981.

(*R*)-4-*tert*-Butyldiphenylsilyloxy-1-butene-3-ol (5) Powdered K_2CO_3 (0.02 g) was added in one portion to a solution of compound **4** (0.728 g, 1.5 mmol) in MeOH (10 ml)–tetrahydrofuran (THF) (10 ml). The mixture was stirred at room temperature for 1 h. After addition of AcOH (5 ml), the solvent was evaporated *in vacuo*. The oily residue was purified by MPLC (hexane : AcOEt = 95 : 5) to give compound **5** (0.406 g, 83%). Colorless oil. The physical data of this compound were identical with those of racemic (**5**),[11] except for the specific rotation, $[\alpha]_D$ +5.05° (c=2.18, $CHCl_3$). The enantiomer excess (ee) of this compound is 91% ee, as determined by HPLC (Chiralcel OD, Daicel Chemical Co., Ltd.).

Isoxazolidine Mixture (7) A solution of *N*-benzyl-α-methoxycarbonylmethanimine *N*-oxide (**6**) (0.16 g, 0.8 mmol) and **5** (0.27 g, 0.8 mmol) in toluene (10 ml) was refluxed for 10 h. The solvent was evaporated off under reduced pressure, and the residue was purified by MPLC (hexane : AcOEt = 9 : 1) to give an isoxazolidine mixture (**7**) as a colorless oil (0.386 g, 85%).

Mesylate (8) MsCl (0.3 ml, 3.66 mmol) in CH_2Cl_2 (10 ml) was added dropwise to a solution of compound **7** (0.948 g, 1.83 mmol) and Et_3N (2.04 ml, 14.6 mmol) in CH_2Cl_2 (10 ml) at −78 °C, and the resulting mixture was stirred at 0 °C for 2 h, then poured into ice-water (30 ml). The organic phase was separated. The aqueous layer was extracted with CH_2Cl_2 (20 ml × 2). The combined organic phases were washed successively with 5% $NaHCO_3$ (20 ml × 3) and saturated NaCl (20 ml × 3). The organic phase was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by MPLC using CH_2Cl_2 to give a diastereomeric mixture of mesylated isoxazolidines (**8**) (1.05 g, 96%) as a colorless oil. Without further purification, the mesylated mixture was used in the subsequent reaction.

Pyrrolidines (9—12) A MeOH solution (15 ml) of compound **8** (0.237 g, 0.4 mmol) was subjected to hydrogenolysis with H_2–20% $Pd(OH)_2$ on carbon (400 mg) overnight. After filtration, the solvent was evaporated *in vacuo* and 10% $NaHCO_3$ (15 ml) was added. The aqueous phase was extracted with $CHCl_3$ (15 ml × 3), dried over Na_2SO_4 and filtered. The solvent was evaporated under reduced pressure to give an oily residue, which was purified by a combination of MPLC (hexane : AcOEt = 1 : 1) and PTLC (hexane : AcOEt = 1 : 1) to give four diastereomeric pyrrolidines, **9** (0.035 g, 21%), **10** (0.015 g, 9.1%), **11** (0.003 g, 1.8%), and **12** (0.005 g, 3.0%) in a ratio of 60 : 26 : 5 : 9, respectively.

(2*S*,4*S*,5*R*)-5-*tert*-Butyldiphenylsilyloxymethyl-4-hydroxy-2-methoxycarbonylpyrrolidine (**9**): Colorless viscous oil. $[\alpha]_D$ −7.00° (c=2.63, $CHCl_3$). IR (neat): 3350 (NH), 1730 (C=O) cm^{-1}. ^{1}H-NMR (400 MHz): 1.06 (9H, s), 2.00 (1H, m), 2.34 (1H, m), 3.32 (1H, m), 3.53 (1H, dd, J=10.0, 8.0 Hz), 3.66 (1H, dd, J=10.0, 5.4 Hz), 3.74 (1H, m), 3.75 (3H, s), 3.83 (1H, dd, J=12.0, 4.1 Hz), 4.21 (1H, m), 7.36—7.45 (6H, m), 7.63—7.67 (4H, m). MS m/z: 356 (M^+-*tert*-Bu). HR-MS Calcd for $C_{19}H_{22}NO_4Si$ (M^+−*tert*-Bu): 356.131812. Found: 356.131966.

(2*R*,4*S*,5*R*)-5-*tert*-Butyldiphenylsilyloxymethyl-4-hydroxy-2-methoxycarbonylpyrrolidine (**10**): Colorless viscous oil. $[\alpha]_D$ +8.7° (c=0.94, $CHCl_3$). IR (neat): 3400 (NH), 1740 (C=O) cm^{-1}. ^{1}H-NMR (400 MHz): 1.08 (9H, s), 2.11 (1H, ddd, J=14.0, 3.6, 1.5 Hz), 2.29 (1H, ddd, J=14.0, 9.7, 4.4 Hz), 3.06 (1H, m), 3.76 (3H, s), 3.84 (1H, dd, J=9.9, 3.6 Hz), 4.01 (2H, ddd, J=15.3, 10.6, 4.2 Hz), 4.30 (1H, br m), 7.37—7.47 (6H, m), 7.66—7.72 (4H, m). MS m/z: 356 (M^+−*tert*-Bu). HR-MS Calcd for $C_{19}H_{22}NO_4Si$ (M^+−*tert*-Bu): 356.131812. Found: 356.130959.

(2*S*,4*R*,5*R*)-5-*tert*-Butyldiphenylsilyloxymethyl-4-hydroxy-2-methoxycarbonylpyrrolidine (**11**): Colorless viscous oil. $[\alpha]_D$ +12.8° (c=0.28, $CHCl_3$). IR (neat): 3451 (NH), 1739 (C=O) cm^{-1}. ^{1}H-NMR (400 MHz): 1.08 (9H, s), 2.11 (1H, ddd, J=13.9, 3.6, 1.5 Hz), 2.29 (1H, ddd, J=14.0, 9.7, 4.5 Hz), 3.06 (1H, m), 3.76 (3H, s), 3.84 (1H, dd, J=9.9, 3.5 Hz), 4.01 (2H, ddd, J=15.4, 10.7, 4.2 Hz), 4.31 (1H, m), 7.38—7.46 (6H, m), 7.65—7.71 (4H, m). MS m/z: 356 (M^+−*tert*-Bu). HR-MS Calcd for $C_{19}H_{22}NO_4Si$ (M^+−*tert*-Bu): 356.131812. Found: 356.131905.

(2*R*,4*R*,5*R*)-5-*tert*-Butyldiphenylsilyloxymethyl-4-hydroxy-2-methoxycarbonylpyrrolidine (**12**): Colorless oil. $[\alpha]_D$ +13.2° (c=0.50, $CHCl_3$). IR (neat): 3400 (NH), 1745 (C=O) cm^{-1}. ^{1}H-NMR (400 MHz): 1.14 (9H, s), 2.10 (1H, dd, J=8.0, 5.5 Hz), 3.86 (1H, m), 3.66 (1H, dd, J=10.4, 6.3 Hz), 3.72 (3H, s), 3.76 (1H, dd, J=10.4, 4.7 Hz), 4.00 (1H, t, J=8.0 Hz), 4.23 (1H, dd, J=9.9, 5.3 Hz), 7.36—7.46 (6H, m), 7.61—7.67 (4H, m). MS m/z: 356 (M^+−*tert*-Bu). HR-MS Calcd for $C_{19}H_{22}NO_4Si$ (M^+−*tert*-Bu): 356.131812. Found: 356.132301.

(2*S*,4*S*,5*R*)-*N*-Benzyloxycarbonyl-5-*tert*-butyldiphenyloxymethyl-4-hydroxy-2-methoxycarbonylpyrrolidine (13) Na_2CO_3 (0.088 g, 0.83 mmol) in H_2O (12 ml) was added to a solution of compound **9** (0.263 g, 0.64 mmol) in MeOH (3 ml). CbzCl (0.12 ml, 0.83 mmol) was added dropwise to the mixture at 0 °C. The whole was stirred at 0 °C for 1 h, then extracted with Et_2O (20 ml × 3), and the organic extract was purified by MPLC (hexane : AcOEt = 4 : 1) to give compound **13** (0.291 g, 83%). Colorless crystals, mp 80—82 °C. $[\alpha]_D$ −20.5° (c=1.12, $CHCl_3$). IR (KBr): 3350 (NH), 1730 (C=O) cm^{-1}. ^{1}H-NMR (300 MHz): 1.02 (4.5H, s, *tert*-Bu), 1.04 (4.5H, s, *tert*-Bu), 1.99 (1H, dd, J=14.5, 4.0 Hz, pyrrolidine 3-H), 2.63 (1H, m, pyrrolidine 3H), 3.51—3.85 (6H, m), 4.07—4.16 (2H, m), 4.36—4.45 (2H, m), 4.96 (0.5H, d, J=12.3 Hz), 5.03 (1H, s), 5.16 (0.5H, d, J=12.3 Hz), 7.09—7.70 (15H, m). CI-MS m/z: 548 (M^++1). HR-MS Calcd for $C_{27}H_{28}NO_6Si$ (M^+−*tert*-Bu): 490.168591. Found: 490.168465.

(2*S*,4*S*,5*R*)-*N*-Benzyloxycarbonyl-5-hydroxymethyl-4-hydroxy-2-methoxycarbonylpyrrolidine (14) A 1.0 M solution of TBAF in THF (0.47 ml, 0.47 mmol) was added to a solution of compound **13** (0.236 g, 0.43 mmol) in THF (20 ml) at room temperature. The mixture was stirred overnight. Evaporation of the solvent *in vacuo* gave an oily residue, which was purified by MPLC (hexane : AcOEt = 1 : 1) to give a diol **14** (0.102 g, 77%). Colorless oil. $[\alpha]_D$ −21° (c=0.19, $CHCl_3$). IR (neat): 3450 (OH), 1740 (C=O), 1690 (C=O) cm^{-1}. ^{1}H-NMR (300 MHz): 1.98—2.06 (1H, m), 2.43—2.58 (1H, m), 3.39—3.81 (3H, m), 3.83 (3H, s), 4.07—4.47 (3H, m), 4.99—5.24 (3H, m), 7.26—7.35 (5H, m). MS m/z:

309 (M^+). HR-MS Calcd for $C_{15}H_{19}NO_6$ (M^+): 309.121238. Found: 309.121643.

(−)-Bulgecinine (1) A solution of compound **14** (0.02 g, 0.065 mmol) in MeOH (1 ml)–6 N HCl (1 ml) was refluxed for 5 h. Then, MeOH was evaporated off under reduced pressure. The aqueous residue was washed with *tert*-BuOH (5 ml × 3), and then subjected to ion exchange column chromatography (Dowex 50 × 8, eluted with 1 M aqueous pyridine). The solvent was evaporated under reduced pressure to give compound (−)-**1** as a colorless solid (0.007 g, 67%). The physical data of the synthetic (−)-bulgecinine ($[\alpha]_D = -13.9°$ ($c = 0.36$, H_2O)) were identical with those of natural (−)-bulgecinine (**1**) ($[\alpha]_D = -13.1°$ ($c = 0.95$, H_2O)[1]).

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9) Although compound (*R*)-**2** and racemic **5** are known compounds, the specific rotations of (*R*)-**2** and the enantiomers of compound **5** have not been reported.
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14) In reference 8, we have already reported that 1,3-dipolar cycloaddition of an allylic alcohol with a nitrone proceeds with moderate *threo* selectivity.
15) Because desilylation of **9** with tetrabutylammonium fluoride (TBAF) was unsuccessful, *N*-carbobenzoxylation of this compound was essential.