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Synthesis and Activities of Bactobolin Derivatives Having New Functionality at C-3

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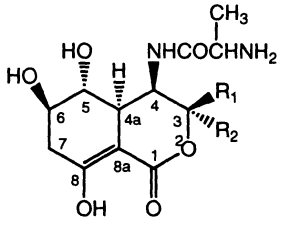
Some derivatives of bactobolin were prepared from bactobolin (**1**) by transformation of the dichloromethyl group at C-3 to the hydroxymethyl, carboxylic acid, methanesulfonyloxymethyl and aldehydeoxime groups. The derivatives proved to be less active than the parent antibiotic **1** against bacteria as well as cytotoxicity, indicating that the functionality at C-3 considerably influences the biological activity.

Bactobolin (**1**) produced by *Pseudomonas* sp. BMG13-A7[1] is of biological interest since it possesses potent antimicrobial and antitumor activities[1~5], suppressing effect on antibody production[6] and therapeutic effect on autoimmune encephalomyelitis[7]. However, the undesirable toxicities[1] have limited its pharmaceutical applications. The promising biological activity and the unique chemical structure of **1** have attracted interest in the total synthesis of **1**[8,9] and the new active analogues[10~12]. Until now, structural modification of **1** have been done in the side chain of amino acid[10,11] and the hydroxy groups of the skelton[12].

On the other hand, actinobolin (**8**)[13] from culture filtrate of *Streptomyces griseoviridus* var. *atrofaciens* is structurally identical to **1** except for the functionality at C-3. In spite of close structural similarity, the two antibiotics differ considerably in their biological activity and toxicity, **8** being less active than **1**. These facts suggest that the functionality at C-3 plays an important role on the biological activities.

In the course of our study of the structure-activity relationship of **1** and **8**, chloromethyl and dimethyl derivatives of **1** (**2** and **3**)[14] and 3-*epi*-actinobolin (**9**)[15] (Fig. 1) were proved to be less active than **1** against bacteria as well as cytotoxicity. These results prompted us to examine the effect of further modifying functionality at C-3 on biological activity.

Fig. 1. The structures of bactobolin (**1**), actinobolin (**8**) and their derivatives.



	R_1	R_2
Bactoboloin (**1**)	$CHCl_2$	CH_3
(**2**)	CH_2Cl	CH_3
(**3**)	CH_3	CH_3
(**4**)	CH_2OH	CH_3
(**5**)	COOH	CH_3
(**6**)	$CH_2OSO_2CH_3$	CH_3
(**7**)	CH=N-OH	CH_3
Actinobolin (**8**)	CH_3	H
(**9**)	H	CH_3

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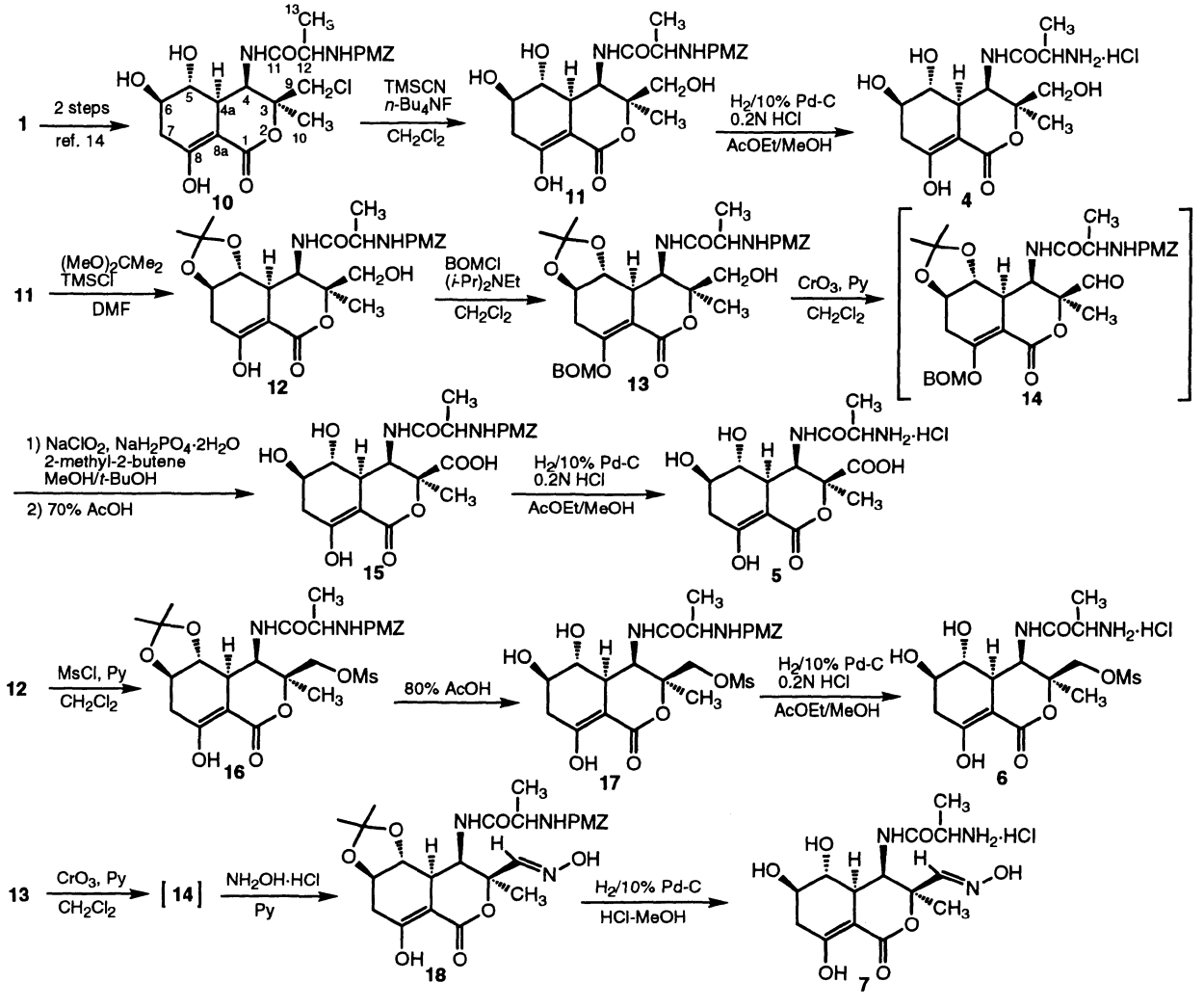
We here report the synthesis and biological activities of hydroxymethyl, carboxylic acid, methanesulfonyloxymethyl and aldehydeoxime derivtives of bactobolin (**4**, **5**, **6** and **7**, respectively) for studying an role of a sterically bulky and electronegative chlorine atom at C-9 in the biological activity of **1**.

Synthesis

The synthetic route of bactobolin derivatives is outlined in scheme **1**. The syntheses of bactobolin derivatives are began with the chloride **10** prepared from bactobolin (**1**) by our method[14]. Transformation of the chloromethyl group at C-3 has been proved to be quite difficult. After several unsuccessful attempts to substitute chlorine atom of chloromethyl group of **10**, we found that the chloride **10** was hydrolyzed in displacement with cyanide using hypervalent cyanosilicate derivatives[16], generated *in situ* by reaction of trimethylsilyl cyanide (TMSCN) with tetrabutylammonium fluoride (TBAF). The alcohol **11** was obtained even in low yield, while the expected cyano compound was not detected. It is not clear at this time why the chloride **10** was hydrolyzed to give an alcohol **11**. It is likely that hydrolysis of the chloride **10** may be carried out by water contaminated in commercial TBAF under this reaction system. This speculation may be supported by the fact of which this hydrolysis of **10** does not proceed under anhydrous condition in the presence of molecular sieve 4A. Removal of *p*-methoxybenzyloxycarbonyl (PMZ) group of **11** by hydrogenolysis gave the hydroxymethyl derivative **4** in 94% yield.

Protection of the 1,2-diol of **11** afforded to the acetonide **12** (75% yield), which was transformed into the enol ether **13** in 54% yield. Oxidation of **13** with CrO_3 gave the

Scheme 1. Syntheses of bactobolin derivatives.



corresponding aldehyde **14**, which was converted to the carboxylic acid **15** upon further oxidation with $NaClO_2$ and removal of the protecting groups in 64% yield. Hydrogenolysis of **15** with palladium on carbon afforded the free acid **5** in 75% yield.

Treatment of **12** with methanesulfonyl chloride in pyridine afforded the sulfonate **16** in 71% yield. Acid hydrolysis of an acetonide group of **16** followed by hydrogenolysis with palladium on carbon gave the mesylate **6** in 67% yield.

Oximation of **14** with $NH_2OH \cdot HCl$ in pyridine yielded the oxime **18** in 17% yield. Simultaneous removal of acetonide and PMZ groups of **18** by hydrogenolysis with palladium on carbon in an acidic condition afforded the oxime **7** in 69% yield. The well-known *syn-anti* isomerization of oxime[17] **18** is not observed in a solution of $CHCl_3$ or MeOH, while **7** isomerized in a solution of methanol at room temperature. The aldehydic protons of **7** are observed at δ 7.47 and 7.39 ppm in a ratio of 7 to 1, respectively, in ^1H NMR spectrum (CH_3OD), indicative of *syn* and *anti* isomeric mixture of **7** in a ratio of 7 to 1.

Biological Activities

Compounds **4**, **5**, **6** and **7** show less inhibitory activity than **1** against several microorganisms (Table 1) and cytotoxicity (Table 2). Especially, the carboxylic acid **5** loses antibacterial activity as well as cytotoxicity. Unexpectedly, electronegativity and sterically bulkiness of the functional groups at C-3 in this study are proved to be little effective for biological activity. These results indicate that the functionality at C-3 participates critically in the biological activity and that chlorinated functional group enhances the inhibitory activity against bacteria and the cytotoxicity.

Experimental

General Methods

Optical rotations were measured with Perkin-Elmer Model 241 polarimeter. ^1H NMR spectra were recorded with a Jeol GX-400 spectrometer. Chemical sifts are expressed in δ values (ppm) with teramethylsilane (δ 0.00)

Table 1. Antibacterial activities of bactobolin (**1**) and it's derivatives (**4**, **5**, **6**, **7**).

Test organism	MIC (μg/ml)				
	1	**4**	**5**	**6**	**7**
Staphylococcus aureus Smith	0.10	6.25	>100	12.5	25
E. coli K-12	1.56	>100	>100	>100	>100
Mycobacterium smegmatis ATCC607	0.78	>100	>100	25	50

MICs were determined by 2-fold broth dilution method at 37°C for 17 hours in nutrient medium.

Table 2. Cytotoxicity of bactobolin (**1**) and it's derivatives (**4**, **5**, **6**, **7**).

Cell	IC_{50} (μg/ml)				
	1	**4**	**5**	**6**	**7**
LB32T	0.17	39.68	>100	15.42	>100
L-1210	0.11	>100	>100	35.76	>100
EL-4	0.14	>100	>100	31.49	>100
$P388D_1$	0.07	16.01	>100	8.8	30
B16BL6	0.2	50.89	79.7	39.99	66.13
FS 3	0.71	69.67	>100	25.29	>100
Colon 26	0.23	46.54	>100	29.45	74.75

for $CDCl_3$, with (δ 3.30) for CD_3OD as an internal standard. The mass spectra were taken by Jeol SX 102 in the FAB mode.

3-Dedichloromethyl-3-hydroxymethyl-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (**11**)

To a solution of **10** (3.0 g, 5.8 mmol) in dichloromethane (150 ml) were added trimethylsilylcyanide (7.8 ml, 58.4 mmol) and 1.0 M solution of tetrabutylammonium fluoride in THF (58.4 ml, 58.4 mmol), and the reaction mixtrure was stirred at room temperature for 2 days. Evaporation of the solvent gave an oil, which was subjected several times to column chromatography on silica gel. Elution with a mixture of ethyl acetate-methanol (10 : 1) or chloroform-methanol (10 : 1) gave **11** (110 mg, 4%) as a colorless foam and the starting material **10** (450 mg): $[\alpha]_D^{25}=-23.1°$ (*c* 0.62, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 1.39 (3H, d, J=7.1 Hz, 13-CH_3), 1.40 (3H, s, 10-CH_3), 2.47 (1H, ddd, J=1.5, 9.5 and 18.9 Hz, 7-H_{ax}), 2.82 (1H, d with small coupling, J=9.5 Hz, 4a-H), 2.92 (1H, dd, J=7.1 and 18.9 Hz, 7-H_{eq}), 3.03 (1H, br s, OH), 3.19 (1H, t, J=9.5 Hz, 5-H), 3.29 (1H, br s, OH), 3.67 and 3.73 (2H, ABq, J=11.7 Hz, 9-CH_2), 3.81 (3H, s, OCH_3), 3.92 (1H, dt, J=7.1, 9.5 and 9.5 Hz, 6-H), 4.29 (1H, quintet, J=7.1 Hz, 12-H), 4.47 (1H, dd, J=3.4 and 9.5 Hz, 4-H), 4.63 (1H, br s, OH), 4.96 and 5.03 (2H, ABq, J=11.2 Hz, -CH_2Ph), 5.23 (1H, d, J=7.1 Hz, 12-NH), 6.88 (2H, d with small coupling, J=8.8 Hz, Ph), 7.06 (1H, d, J=9.5 Hz, 4-NH), 7.26 (2H, d with small coupling, J=8.8 Hz, Ph), 13.1 (1H, s, 8-OH); MS (FAB positive) *m/z* 495 $(M+H)^+$.

3-Dedichloromethyl-3-hydroxymethylbactobolin (**4**)

A solution of **11** (43 mg, 0.087 mmol) in a mixture of methanol (0.9 ml), ethyl acetate (0.1 ml) and 0.2 M HCl (0.3 ml) was stirred with 10% palladium on carbon (43 mg) under atmosphere of hydrogen at room temperature for 1 hour. After filtration, evaporation of the filtrate gave **4** as a colorless solid (30 mg, 94%); $[\alpha]_D^{25}=-4.7°$ (*c* 0.54, MeOH); ^1H NMR (CD_3OD, 400 MHz) δ 1.29 (3H, d, J=6.8 Hz, 13-CH_3), 1.35 (3H, s, 10-CH_3), 2.35 (1H, ddd, J=2.6, 9.7 and 18.7 Hz, 7-H_{ax}), 2.78 (1H, ddd, J=1.0, 6.8 and 18.7 Hz, 7-H_{eq}), 2.92 (1H, d with small coupling, J=9.7 Hz, 4a-H), 3.17 (1H, t, J=9.7 Hz, 5-H), 3.56 (1H, q, J=6.8 Hz, 12-H), 3.65 and 3.60 (2H, ABq, J=11.7 Hz, 9-CH_2), 3.79 (1H, dt, J=6.8, 9.7 and 9.7 Hz, 6-H), 4.62 (1H, d, J=3.9 Hz, 4-H); MS (FAB positive) *m/z* 331 $(M+H)^+$.

3-Dedichloromethyl-3-hydroxymethyl-5,6-*O*-isopropylidene-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (**12**)

To a solution of **11** (165 mg, 0.33 mmol) in *N*,*N*-dimethylformamide (1.2 ml) were added 2,2-dimethoxypropane (327 μl, 2.67 mmol) and chlorotrimethylsilane (21 μl, 0.17 mmol), and the reaction mixture was stirred at room temperature for 2 hours. After the reaction was quenched with pyridine (50 μl, 0.62 mmol), the solution was diluted with ethyl acetate. The solution was washed with water, dried over $MgSO_4$ and filtered. Evaporation of filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with chloroform-methanol (10 : 1) to give **12** (134 mg, 75%) as a colorless foam and the starting material **11** (17 mg). $[\alpha]_D^{23}=-27.3°$ (*c* 0.4, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 (3H, s, 10-CH_3), 1.37 (3H, d, J=7.5 Hz, 13-CH_3), 1.41 and 1.45 (3H each, s, isopropylidene), 2.62 (1H, ddd, J=2.0, 11.2 and 17.5 Hz, 7-H_{ax}), 2.95 (1H, dd, J=6.1 and 17.5 Hz, 7-H_{eq}), 3.14 (1H, d with small coupling, J=9.6 Hz, 4a-H), 3.24 (1H, br s, 9-OH), 3.34 (1H, t, J=9.6 Hz, 5-H), 3.62 (1H, dd, J=6.8 and 12.2 Hz, 9-CH), 3.70~3.83 (2H, m, 9-CH and 6-H), 3.81 (3H, s, OCH_3), 4.43 (1H, quintet, J=7.5 Hz, 12-H), 4.55 (1H, dd, J=3.9 and 10.0 Hz, 4-H), 5.04 and 4.96 (2H, ABq, J=12.0 Hz, CH_2Ph), 5.38 (1H, d, J=7.3 Hz, 12-NH), 6.88 (2H, d with small coupling, J=8.5 Hz, Ph), 7.10 (1H, d, J=10.0 Hz, 4-NH), 7.27 (2H, d with small coupling, J=8.5 Hz, Ph), 13.4 (1H, s, 8-OH); MS (FAB positive) *m/z* 535 $(M+H)^+$.

8-*O*-Benzyloxymethyl-3-dedichloromethyl-3-hydroxymethyl-5,6-*O*-isopropylidene-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (**13**)

To a solution of **12** (297 mg, 0.56 mmol) in dichloromethane (6 ml) were added benzylchloromethyl ether (115 μl, 0.83 mmol) and *N*,*N*-diisopropylethylamine (483 μl, 2.78 mmol), and the reaction mixture was stirred at room temperature for 2 hours. Evaporation of solvent gave an oil, which was subjected to preparative TLC on silica gel developed with toluene-acetone (2 : 1) to give **13** (195 mg, 54%) as a colorless foam and the starting material **12** (47 mg). $[\alpha]_D^{24}=-68.5°$ (*c* 0.44, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (3H, s, 10-CH_3), 1.30 (3H, d, J=6.9 Hz, 13-CH_3), 1.44 (6H, s, isopropylidene), 2.59 (1H, ddd, J=3.0, 10.5 and 16.6 Hz, 7-H_{ax}), 2.88 (1H, br s, 9-OH), 3.05 (1H, ddd, J=~1, 5.9 and 16.6 Hz, 7-H_{eq}), 3.30~3.45 (2H, m, 5-H and 4a-H), 3.67 (2H, m, 9-CH_2), 3.72 (1H, ddd, J=5.9, 8.8 and 10.5 Hz, 6-H), 4.14 (1H, quintet, J=6.9 Hz, 12-H), 4.59 (1H, dd, J=4.6 and 10.0 Hz, 4-H), 4.73 and 4.76 (2H, ABq, J=11.7 Hz, -CH_2Ph), 4.97 and 5.04 (2H, ABq, J=11.7 Hz, -CH_2Ph), 5.09 (1H, d, J=6.9 Hz, 12-NH), 5.17 and 5.20 (2H, ABq, J=7.1 Hz, –OCH_2O–), 6.48 (1H, br d, J=4.6 Hz, 4-NH), 6.87 (2H, d with small coupling, J=8.8 Hz, Ph(PMZ)), 7.27 (2H, d with small coupling, J=8.8 Hz,

Ph(PMZ)), 7.30~7.40 (5H, m, Ph(BOM)); MS (FAB positive) *m/z* 655 $(M+H)^+$.

3-Carboxy-3-dedichloromethyl-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (**15**)

To the mixture of CrO_3 (306 mg, 3.07 mmol) and pyridine (451 μl, 5.58 mmol) in dichloromethane (6 ml) was added a solution of **13** (189 mg, 0.279 mmol) in dichloromethane (6 ml), and the reaction mixture was stirred at room temperature for 1 hour. After filtration, the filtrate was evaporated to give an oil, which was dissolved in a mixture of *t*-butanol (4 ml) and water (1.4 ml). To the solution were added 2-methyl-2-butene (130 μl, 1.22 mmol), $NaH_2PO_4 \cdot 2H_2O$ (47.8 mg, 0.307 mmol) and $NaClO_2$ (85.7 mg, 0.948 mmol), and the reaction mixture was stirred at room temperature for 3 hours. After being quenched with isopropyl alcohol (100 μl), evaporation of the solvent gave an oil, which was subjected to flash column chromatography on silica gel. Elution with $CHCl_3$-MeOH (3 : 1) gave an oil. The oil was dissolved in 70% acetic acid (1 ml), and the solution was stirred at room temperature for 1 hour. Evaporation of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with chloroform - methanol (1 : 1) to give **15** as a colorless solid (9 mg, 6.4%, 3 steps from **13**): $[\alpha]_D^{22}=-33.4°$ (*c* 0.46, MeOH); ^1H NMR (CD_3OD, 400 MHz) δ 1.33 (3H, d, J=6.9 Hz, 13-CH_3), 1.48 (3H, s, 10-CH_3), 2.30 (1H, ddd, J=1.8, 9.7 and 18.4 Hz, 7-H_{ax}), 2.68 (1H, d with small coupling, J=9.7 Hz, 4a-H), 2.74 (1H, dd, J=6.8 and 18.4 Hz, 7-H_{eq}), 3.12 (1H, t, J=9.7 Hz, 5-H), 3.73 (1H, dt, J=6.8, 9.7 and 9.7 Hz, 6-H), 4.25 (1H, q, J=6.9 Hz, 12-H), 4.84 (1H overlapped with solvent, 4-H), 4.98 (2H, br s, CH_2Ph), 6.88 (2H, d with small coupling, J=8.8 Hz, Ph), 7.27 (2H, d with small coupling, J=8.8 Hz, Ph); MS (FAB negative) *m/z* 507 $(M-H)^-$.

3-Carboxy-3-dedichloromethylbactobolin (**5**)

Procedure used for the preparation of **5** from **15** was similar to those used for the preparation of **4** from **11**; the yield was 75%: $[\alpha]_D^{22}=-34.4°$ (*c* 0.21, MeOH); ^1H NMR (CD_3OD, 400 MHz) δ 1.40 (3H, d, J=6.9 Hz, 13-CH_3), 1.49 (3H, s, 10-CH_3), 2.29 (1H, ddd, J=2.6, 9.6 and 18.7 Hz, 7-H_{ax}), 2.70 (1H, d with small coupling, J=9.6 Hz, 4a-H), 2.76 (1H, dd, J=7.1 and 18.7 Hz, 7-H_{eq}), 3.06 (1H, t, J=9.6 Hz, 5-H), 3.74 (1H, dt, J=7.1, 9.6 and 9.6 Hz, 6-H), 3.81 (1H, q, J=6.9 Hz, 12-H); MS (FAB negative) *m/z* 343 $(M-H)^-$.

3-Dedichloromethyl-5,6-*O*-isopropylidene-3-methanesulfonyloxymethyl-*N*-(*p*-methoxybenzyloxycarbonyl)-bactobolin (**16**)

To a solution of **12** (11 mg, 0.021 mmol) in dichloromethane (0.5 ml) were added pyridine (16.7 μl, 0.21 mmol) and methanesulfonyl chloride (2.4 μl, 0.031 mmol) at room temperature, and the reaction mixture was stirred at room temperature overnight. Evaporation of the solvent gave an oil, which was dissolved in ethyl acetate. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with toluene - acetone (1 : 1) to give **16** as a colorless foam (9.0 mg, 71% yield): $[\alpha]_D^{22}=-43.6°$ (*c* 0.47, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.39 (3H, d, J=7.0 Hz, 13-CH_3), 1.40 (3H, s, 10-CH_3), 1.43 and 1.45 (3H each, s, isopropylidene), 2.63 (1H, ddd, J=~2, 10.7 and 17.9 Hz, 7-H_{ax}), 2.96 (1H, dd, J=5.7 and 17.9 Hz, 7-H_{eq}), 6.03 (1H, m, 4a-H), 3.12 (3H, s, SO_2CH_3), 3.36 (1H, t, J=8.9 Hz, 5-H), 3.76 (1H, ddd, J=5.7, 8.9 and 10.7 Hz, 6-H), 4.20 (1H, quintet, J=7.0 Hz, 12-H), 4.26 and 4.38 (2H, ABq, J=11.2 Hz, 9-CH_2), 4.63 (1H, dd, J=3.9 and 10.3 Hz, 4-H), 4.97 and 5.03 (2H, ABq, J=12.0 Hz, CH_2Ph), 5.09 (1H, d, J=7.0 Hz, 12-NH), 6.47 (1H, br s, 4-NH), 6.89 (2H, d with small coupling, J=8.8 Hz, Ph), 7.27 (2H, d with small coupling, J=8.8 Hz, Ph), 13.6 (1H, s, 8-OH); MS (FAB positive) *m/z* 613 $(M+H)^+$.

3-Dedichloromethyl-3-methanesulfonyloxymethyl-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (**17**)

A solution of **16** (10 mg, 0.016 mmol) in 80% aqueous acetic acid (0.5 ml) was stirred at room temperature overnight. Evaporation of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with toluene - acetone (1 : 1) to give **17** as a colorless foam (8.4 mg, 90% yield): $[\alpha]_D^{23}=-28.5°$ (*c* 0.34, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (3H, d, J=7.3 Hz, 13-CH_3), 1.44 (3H, s, 10-CH_3), 2.50 (1H, dd, J=9.9 and 18.7 Hz, 7-H_{ax}), 2.70 (1H, d with small coupling, J=9.2 Hz, 4a-H), 2.96 (1H, dd, J=7.2 and 18.7 Hz, 7-H_{eq}), 3.01 (1H, s, OH), 3.12 (3H, s, $-SO_2CH_3$), 3.19 (1H, m, 5-H), 3.81 (3H, s, OCH_3), 3.93 (1H, dt, J=7.2, 9.9 and 9.9 Hz, 6-H), 4.20 and 4.30 (2H, ABq, J=10.7 Hz, 9-CH_2), 4.22 (1H overlapped with 9-CH_2, 12-H), 4.36 (1H, d, J=3.9 Hz, OH), 4.43 (1H, dd, J=3.7 and 9.2 Hz, 4-H), 4.98 and 5.03 (2H, ABq, J=11.7 Hz, $-CH_2Ph$), 5.01~5.06 (1H, overlapped with CH_2Ph, 12-NH), 6.80 (1H, br s, 4-NH), 6.89 (2H, d with small coupling, J=8.8 Hz, Ph), 7.27 (2H, overlapped with $CHCl_3$, Ph), 13.09 (1H, s, 8-OH); MS (FAB positive) *m/z* 573 $(M+H)^+$.

3-Dedichloromethyl-3-methanesulfonyloxymethylbactobolin (**6**)

Procedure used for the preparation of **6** from **17** was similar to those used for the preparation of **4** from **11**; the yield was 74%: $[\alpha]_D^{21}=-28.4°$ (*c* 0.22, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.30 (3H, d, J=6.8 Hz, 13-CH_3), 1.43 (3H, s, 10-CH_3), 2.36 (1H, ddd, J=2.4, 9.8 and 18.7 Hz, 7-H_{ax}), 2.81 (1H, dd, J=6.7 and 18.7 Hz, 7-H_{eq}), 2.80~2.87 (1H, overlapped with 7-H_{eq}, 4a-H), 3.16 (3H, s, SO_2CH_3), 3.20 (1H, t, J=9.8 Hz, 5-H), 3.56 (1H, q, J=6.8 Hz, 12-H), 3.79 (1H, dt, J=6.7, 9.8 and 9.8 Hz, 6-H), 4.33 and 4.37 (2H, ABq, J=10.7 Hz, 9-CH_2), 4.62 (1H, d, J=3.9 Hz, 4-H); MS (FAB positive) *m/z* 409 $(M+H)^+$.

3-Dedichloromethyl-3-aldehydeoxime-5,6-*O*-isopropylidene-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (**18**)

To a solution of **14** prepared from **13** (36 mg, 0.055 mmol) in pyridine (1 ml) was added hydroxylamine hydrochloride (21 mg, 0.297 mmol), and the mixture was stirred at room temperature overnight. Evaporaiton of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with toluene - acetone (2 : 1) to give **18** as a colorless foam (5.0 mg, 17%, 2 steps from **13**): $[\alpha]_D^{24}=-55.7°$ (*c* 0.28, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (3H, d, J=6.4 Hz, 13-CH_3), 1.43 (3H, s, 10-CH_3), 1.45 and 1.46 (3H each, s, isopropylidene), 2.57 (1H, ddd, J=2.4, 11.0 and 17.5 Hz, 7-H_{ax}), 2.91 (1H, dd, J=5.8 and 17.5 Hz, 7-H_{eq}), 2.92~2.97 (1H, overlapped with 7-H_{eq}, 4a-H), 3.36 (1H, t, J=9.2 Hz, 5-H), 3.70 (1H, ddd, J=5.8, 9.2 and 11.0 Hz, 6-H), 3.81 (3H, s, OCH_3), 4.40 (1H, quintet, J=6.4 Hz, 12-H), 4.69 (1H, dd, J=3.7 and 10.0 Hz, 4-H), 4.96 and 5.08 (2H, ABq, J=11.7 Hz, $-CH_2$Ph), 5.54 (1H, d, J=6.4 Hz, 12-NH), 6.88 (2H, d with small coupling, J=8.3 Hz, Ph), 6.91 (1H, overlapped with Ph, 4-NH), 7.28 (2H, d with small coupling, J=8.3 Hz, Ph), 7.48 (1H, s, 9-H), 8.31 (1H, br s, oxime-OH), 13.2 (1H, s, 8-OH); MS (FAB positive) *m/z* 548 $(M+H)^+$.

3-Dedichloromethyl-3-aldehydeoximebactobolin (**7**)

A solution of **18** (14.8 mg, 0.027 mmol) in a mixture of methanol (0.5 ml) and 10% hydrogen chloride in methanol (0.5 ml) was stirred with 10% palladium on carbon (14 mg) under atmosphere of hydrogen for 2 hours. After filtration, evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - methanol (3 : 1) to give **7** as a colorless foam (7.0 mg, 69% yield): $[\alpha]_D^{24}=-11.9°$ (*c* 0.23, MeOH, isomer ratio: *syn*/*anti*=7 : 1); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.26 (3H, d, J=6.9 Hz, 13-CH_3 (*anti*)), 1.30 (3H, d, J=6.8 Hz, 13-CH_3 (*syn*)), 1.46 (3H, s, 10-CH_3 (*syn*)), 1.60 (3H, s, 10-CH_3 (*anti*)), 2.37 (1H, ddd, J=2.7, 9.7 and 18.6 Hz, 7-H_{ax} (*syn*)), 2.30~2.40 (1H, overlapped with 7-H_{ax} (*syn*), 7-H_{ax} (*anti*)), 2.65~2.85 (2H, m, 7-H_{eq} (*syn*)+4a-H (*syn*)), 2.78~2.87 (1H, overlapped with 7-H_{eq} (*syn*), 7-H_{eq} (*anti*)), 2.93 (1H, d with small coupling, J=9.3 Hz, 4a-H (*anti*)), 3.18 (1H, t, J=9.7 Hz, 5-H (*syn*)), 3.27~3.35 (1H, overlapped with solvent, 5-H (*anti*)), 3.50 (1H, q, J=6.9 Hz, 12-CH (*anti*)), 3.57 (1H, q, J=6.8 Hz, 12-CH (*syn*)), 3.73 (1H, dt, J=7.3, 9.7 and 9.7 Hz, 6-H (*syn*)), 3.83 (1H, dt, J=6.8, 9.6 and 9.6 Hz, 6-H (*anti*)), 4.53 (1H, d, J=3.9 Hz, 4-H (*anti*)), 4.64 (1H, d, J=3.9 Hz, 4-H (*syn*)), 7.39 (1H, s, 9-CH (*anti*)), 7.47 (1H, s, 9-CH (*syn*)); MS (FAB positive) *m/z* 344 $(M+H)^+$.

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