# Synthesis and Activities of Bactobolin Derivatives Based on the Alteration of the Functionality at C-3 Position

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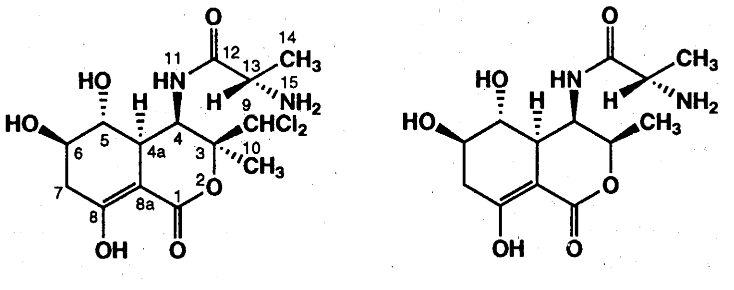
Some derivatives of bactobolin were prepared from bactobolin (**1**) by radical reduction and formation of the fused azetidine ring. The derivatives proved less active than the parent antibiotic **1** against bacteria, indicating that dichloromethyl group at C-3 position play an important role in biological activity.

Bactobolin (**1**) was isolated from the culture filtrate of *Pseudomonas* sp. BMG13-A7[1]. Compound **1** demonstrated various kinds of biological effects including antimicrobial and antitumor activities[1~5], suppressing effect on antibody production[6] and therapeutic effect on autoimmune encephalomyelitis[7]. Due to its fascinating biological activities, **1** have attracted interest in the synthesis of new active analogues. Until now, structural modifications of **1** have been done in the side chain of amino acid[8,9] and the hydroxyl groups of the skeltone[10]. On the other hand, actinobolin (**2**) structurally analogous to **1** from culture filtrate of *Streptomyces griseoviridis* var. *atrofaciens*[11] is considerably distinct from **1** in biological activity and toxicity, **2** being less active than **1**. Compound **2** bears a structural resemblance to **1** except for the functionality at C-3. These facts prompted us to examine the role of the dichloromethyl group at C-3 of **1** and of the conformation of **1** in biological activity and toxicity. We here report the synthesis of chloromethyl, dimethyl and azetidine-fused derivatives of bactobolin (**3**, **4** and **5**, respectively) by dechlorination of the dichloromethyl group and formation of the fused-azetidine ring.

## Synthesis

After several attempts, dechlorination of the dichloromethyl group of *p*-methoxybenzyloxycarbonylbactobolin (**6**) was best achieved by radical dehalogenation with tri-*n*-butyltin hydride and 2,2′-azobisisobutylonitril (AIBN) to give **7** (71% yield) and **8** (8% yield). Removal

Fig. 1. The structures of bactobolin and actinobolin.



**Bactobolin (1)** **Actinobolin (2)**

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Scheme 1. Reaction sequences.

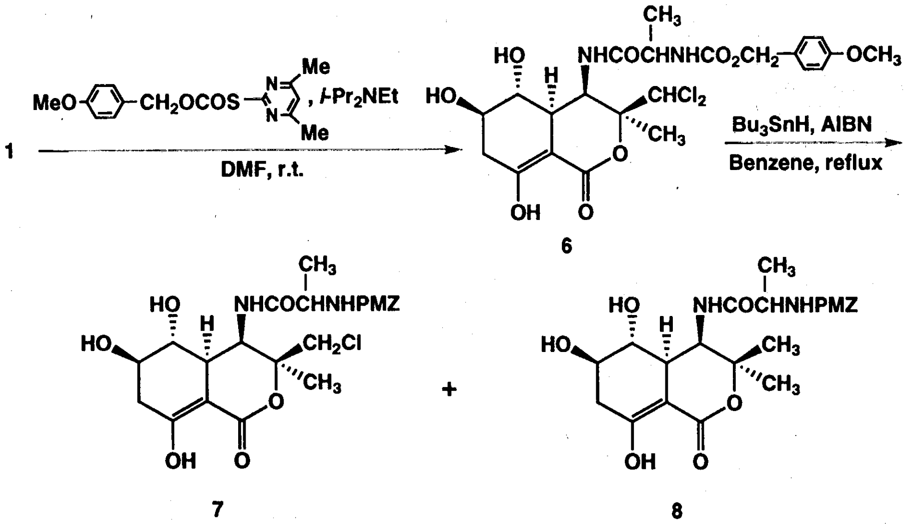
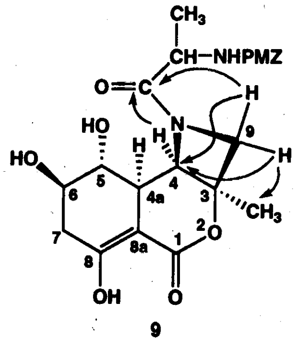


Fig. 2. HMBC correlations of 4-membered ring moiety of **9**.



of *p*-methoxybenzyloxycarbonyl groups of **7** and **8** by hydrogenolysis gave **3** and **4** in good yield, respectively.

Next, our attention was directed to the conformational change by formation of the fused tricyclic system utilizing the intermediate **7**. Treatment of **7** with lithium bis(trimethylsilylamide) at −20°C afforded the azetidine-fused derivative **9** (48% yield) *via* formation of the 4-membered ring fused to the lactone ring. The ring closure would be probably caused by nucleophilic attack of the amide to the monochloromethyl group. The HMBC experiments clarified the presence of the fused 4-membered ring system in **9** (Fig. 2). The HMBC correlation ($^3J$) between the carbonyl carbon of the amide moiety and one of methylene protons at C-9 shows C-N bond between the amide nitrogen and the carbon at 9 position. Hydrogenolysis of **9** afforded the azetidine-fused derivative **5**.

## Biological Activities

Compounds **3** and **4** as well as **2** showed less inhibitory activity than **1** against several microorganisms (Table 1) and cytotoxicity (Table 2). Interestingly, the intensity of inhibitory activity against bacteria as well as cytotoxicity of these analogues is proportional to the degree of substitution by chlorine atom at C-3. These results indicate that the functionality at C-3 considerably influences the biological activity and that the chlorinated functional group significantly enhances the inhibitory activity against bacteria and also the cytotoxicity.

Compound **5** having tricyclic skeltone weakly inhibited all microorganisms, suggesting that the conformation also play the major role to exhibit biological activity. The further structural modifications based on the alteration of the functionality at C-3 position are now in progress.

## Experimental

### General Methods

IR spectra were determined on a Hitachi Model 260-10 spectrometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. $^1$H NMR spectra were recorded with Jeol GX-400 spectrometer. Chemical shifts are expressed in $\delta$ values (ppm) with tetramethylsilane as an internal standard. The MS spectra were taken by Jeol SX102.

Scheme 2. Reaction sequences.

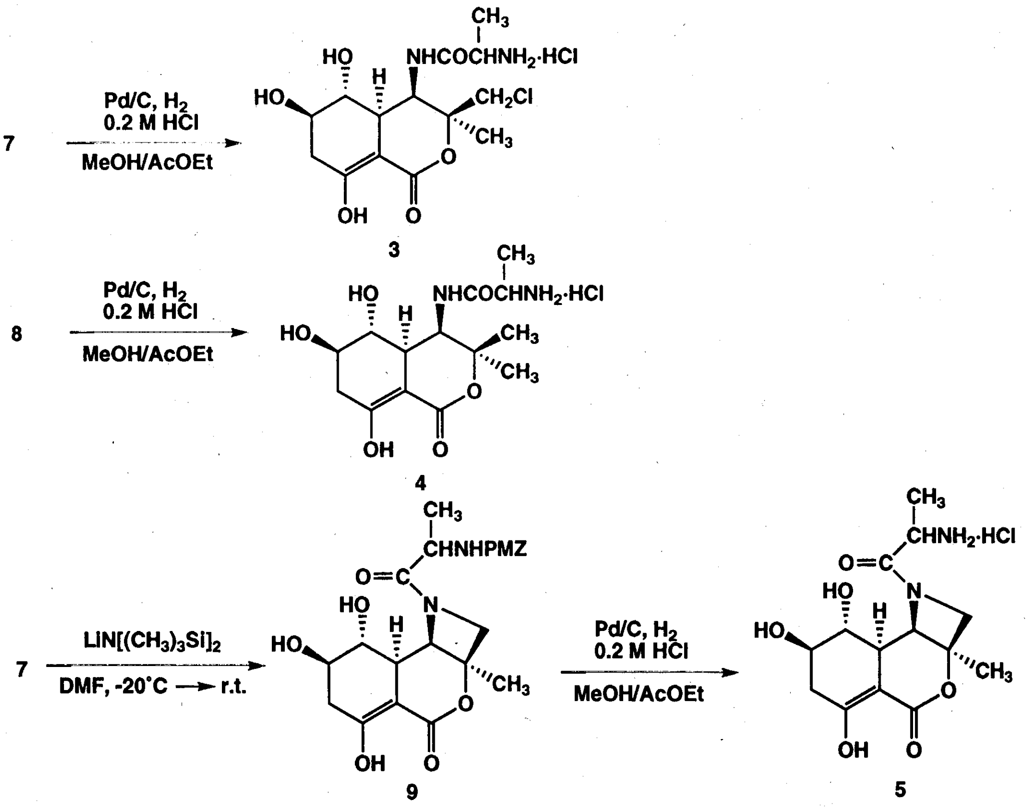


Table 1. Antibacterial activities of bactobolin (1), actinobolin (2) and bactobolin derivatives (3, 4, 5).

| Test organism | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| *Staphylococcus aureus* FDA209P | 0.20 | 3.13 | 0.78 | 3.13 | >50 |
| *Staphylococcus aureus* Smith | 0.20 | 6.25 | 0.78 | 3.13 | >50 |
| *Staphylococcus aureus* MRSA No. 5 | 0.39 | 12.5 | 0.78 | 6.25 | >50 |
| *Staphylococcus aureus* MS16526 (MRSA) | 0.39 | 6.25 | 1.56 | 6.25 | >50 |
| *Staphylococcus epidermidis* 109 | 0.20 | 6.25 | 0.78 | 6.25 | >50 |
| *Micrococcus luteus* FDA16 | 0.10 | 1.56 | 0.39 | 1.56 | >50 |
| *Micrococcus luteus* PCI1001 | 0.10 | 1.56 | 0.20 | 1.56 | >50 |
| *Bacillus anthracis* | 6.25 | 50 | 25 | >50 | >50 |
| *Bacillus subtilis* PCI219 | 0.39 | 25 | 3.13 | 12.5 | >50 |
| *Corynebacterium bovis* 1810 | 0.10 | 1.56 | 0.78 | 1.56 | >50 |
| *Escherichia coli* NIHJ | 0.20 | 1.56 | 0.78 | 3.13 | >50 |
| *Escherichia coli* K-12 ML1629 | 6.25 | 25 | 12.5 | 50 | >50 |
| *Shigella dysenteriae* JS11910 | 0.05 | 1.56 | 0.39 | 0.78 | >50 |
| *Salmonela typhi* T-63 | 6.25 | 25 | 12.5 | 50 | >50 |
| *Proteus vulgaris* OX19 | 0.39 | 3.13 | 0.78 | 6.25 | >50 |
| *Serratia marcescens* | 25 | >50 | >50 | >50 | >50 |
| *Pseudomonas aeruginosa* A3 | 25 | >50 | >50 | >50 | >50 |
| *Pseudomonas aeruginosa* GN315 | 50 | >50 | >50 | >50 | >50 |
| *Klebsiella pneumoniae* PCI602 | 3.13 | 25 | 12.5 | 50 | >50 |
| *Mycobacterium smegmatis* ATCC607* | 3.13 | 12.5 | 3.13 | 12.5 | >50 |

MICs were determined by 2-fold agar dilution streak method at 37°C for 18 and 42 hours*.

Table 2. Cytotoxicity of bactobolin (**1**), actinobolin (**2**) and bactobolin derivatives (**3**, **4**, **5**).

| Cell | $IC_{50}$ (μg/ml) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| L1210 | 0.11 | 48.1 | 0.60 | 9.33 | >100 |
| EL4 | 0.087 | 42.1 | 0.32 | 6.84 | >100 |
| P388 | 0.068 | 39.6 | 0.51 | 4.57 | >100 |
| IMC ca. | 0.078 | 40.8 | 0.26 | 5.71 | >100 |
| Colon 26 | 0.035 | 30.6 | 0.15 | 3.54 | >100 |
| HeLa | 0.34 | >100 | 5.44 | 97.8 | >100 |
| FS-3 | 0.28 | 99.6 | 0.92 | 47.5 | >100 |
| LB32T | 0.11 | 37.2 | 0.31 | 7.71 | >100 |
| Methyl green FS-3 | 0.71 | >100 | 10.4 | >100 | >100 |

The rate of survival cells was measured by MTT assay and $IC_{50}$ value was calculated.

### 9-Dechloro-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (7) and 9-Didechloro-*N*-(*p*-methoxybenzyloxycarbonyl)bactobolin (8)

To a solution of **6** (50 mg, 0.091 mmol) in benzene (1 ml) were added tri-*n*-butyltin hydride (44 μl, 0.24 mmol) and AIBN (4.5 mg, 0.027 mmol) at room temperature, and the reaction mixture was refluxed for 1 hour. 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. The filtrate was evaporated to give a crude oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - methanol (100 : 1) to give **7** (33 mg, 71% yield) and **8** (3.9 mg, 8% yield).

(**7**): $[\alpha]_D^{24}$ −2.9° (*c* 1.1, MeOH); IR ($CHCl_3$) 3426, 3000, 2949, 2910, 1710, 1660, 1615, 1515, 1260 (sh), 1245 (sh), 1235, 1075 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 1.38 (3H, d, $J=6.8$ Hz, 14-$CH_3$), 1.58 (3H, s, 10-$CH_3$), 2.49 (1H, dd, $J=9.8$ and 19.0 Hz, 7-Hax), 2.78 (1H, d with small couplings, $J=9.3$ Hz, 4a-H), 2.95 (1H, dd, $J=6.8$ and 19.0 Hz, 7-Heq), 3.10∼3.20 (2H, m, 5-H and 6-OH), 3.50 and 3.68 (2H, ABq, $J=11.2$ Hz, 9-$CH_2$), 3.80 (3H, s, −$OCH_3$), 3.94 (1H, dt, $J=7.3$ and 9.3 Hz, 6-H), 4.24 (1H, dq, $J=5.4$ and 6.8 Hz, 13-H), 4.56 (1H, dd, $J=3.4$ and 9.3 Hz, 4-H), 4.58 (1H, br s, 5-OH), 4.97 and 5.03 (2H, ABq, $J=11.5$ Hz, −$CH_2$−Ph), 5.20 (1H, br d, $J=5.4$ Hz, 15-NH), 6.88 (2H, d with small couplings, $J=8.8$ Hz, Ph), 6.97 (1H, d, $J=9.3$ Hz, 11-NH), 7.27 (2H, d with small couplings, $J=8.8$ Hz, Ph), 13.00 (1H, br s, 8-OH); MS (FAB positive) *m*/*z* 513 $(M+H)^+$.

(**8**): $[\alpha]_D^{24}$ −7.5° (*c* 0.89, MeOH); IR ($CHCl_3$) 3420, 2990, 2950 (sh), 2910 (sh), 1720, 1655, 1620, 1515, 1265 (sh), 1245 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) δ 1.39 (3H, d, $J=6.8$ Hz, 14-$CH_3$), 1.40 and 1.49 (each 3H, s, 9-$CH_3$ and 10-$CH_3$), 2.47 (1H, br dd, $J=9.6$ and 19.3 Hz, 7-Hax), 2.81 (1H, d with small couplings, $J=9.3$ Hz, 4a-H), 2.93 (1H, dd, $J=6.8$ and 19.1 Hz, 7-Heq), 3.05∼3.25 (2H, m, H-5 and 6-OH), 3.81 (3H, s, −$OCH_3$), 3.92 (1H, dt, $J=7.3$ and 9.8 Hz, 6-H), 4.20∼4.30 (2H, m, 4-H and 13-H), 4.54 (1H, br s, 5-OH), 5.04 and 4.96 (2H, ABq, $J=11.5$ Hz, −$CH_2$−Ph), 5.23 (1H, br d, $J=4.9$ Hz, 15-NH), 6.88 (2H, d with small couplings, $J=8.8$ Hz, Ph), 6.83∼6.93 (1H, 11-NH), 7.26 (2H, d with small couplings, $J=8.3$ Hz, Ph), 13.24 (1H, br s, 8-OH); MS (FAB positive) *m*/*z* 479 $(M+H)^+$.

### 9-Dechlorobactobolin (3)

A solution of **7** (14 mg, 0.027 mmol) in a mixture of methanol (2.5 ml), ethyl acetate (0.25 ml) and 0.2 M HCl (0.1 ml) was stirred with 10% Pd/C (14 mg) under atmosphere of hydrogen at room temperature for 1 hour. After filtration, evaporation of the filtrate gave **3** (8.5 mg, 82% yield): $[\alpha]_D^{24}$ +15.8° (*c* 0.50, MeOH); IR (KBr) 3400, 3050 (sh), 1680 (sh), 1650, 1600 (sh), 1560 (sh), 1230, 1120, 1070 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 400 MHz) δ 1.50 (3H, d, $J=7.3$ Hz, 14-$CH_3$), 1.59 (3H, s, 10-$CH_3$), 2.38 (1H, ddd, $J=2.4$, 9.8 and 18.6 Hz, 7-Hax), 2.85 (1H, ddd, $J=1.0$, 6.8 and ∼19 Hz, 7-Heq), 2.98 (1H, d with small couplings, $J=9.3$ Hz, 4a-H), 3.14 (1H, t, $J=9.3$ Hz, H-5), 3.70 and 3.74 (2H, ABq, $J=11.7$ Hz, $CH_2Cl$), 3.83 (1H, dt, $J=6.8$ and 9.8 Hz, 6-H), 3.99 (1H, q, $J=6.8$ Hz, 13-H), 4.63 (1H, d, $J=3.4$ Hz, 4-H); MS (FAB positive) *m*/*z* 349 $(M+H)^+$.

### 9-Didechlorobactobolin (4)

Compound **4** was obtained from **8** by the similar procedure for the preparation of **3** (yield 89%): $[\alpha]_D^{24}$ +10.1° (*c* 0.55, MeOH); IR (KBr) 3400, 3060 (sh), 2980 (sh), 1680 (sh), 1640, 1600 (sh), 1560 (sh), 1220, 1130 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 400 MHz) $\delta$ 1.39 and 1.49 (each 3H, s, 9-$CH_3$ and 10-$CH_3$), 1.50 (3H, d, $J=6.8$ Hz, 14-$CH_3$), 2.37 (1H, ddd, $J=2.4$, 9.8 and ~19 Hz, 7-Hax), 2.83 (1H, ddd, $J=1.0$, 6.8 and ~19 Hz, 7-Heq), 2.95 (1H, d with small couplings, $J=9.3$ Hz, 4a-H), 3.15 (1H, t, $J=9.6$ Hz, 5-H), 3.81 (1H, dt, $J=6.8$ and 9.8 Hz, 6-H), 3.99 (1H, q, $J=7.1$ Hz, 13-H), 4.45 (1H, d, $J=3.9$ Hz, 4-H); MS (FAB positive) *m/z* 315 $(M+H)^+$.

### 9-Didechloro-*N*-(*p*-methoxybenzyloxycarbonyl)-9,11-cyclobactobolin (**9**)

To a solution of **7** (0.220 g, 0.43 mmol) in *N*,*N*-dimethylformamide (13.2 ml) was added lithium bis(trimethylsilyl)amide (1.0 M hexane solution, 4.3 ml, 4.3 mmol) at −20°C, and the reaction mixture was stirred at −20°C for 30 minutes and was further stirred at room temperature for 3 hours. After dilution with ethyl acetate, the solution was washed with satd $NaHCO_3$ aq solution, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with chloroform - methanol (20 : 1) to give **9** (0.098 g, 48% yield) and the starting material **7** (0.037 g, conversion yield 58%). **9**: $[\alpha]_D^{24}$ −104.3° (*c* 0.25, MeOH); IR ($CHCl_3$) 3420, 2980, 2940, 2900 (sh), 1710, 1650, 1615, 1515, 1250 (sh), 1230, 1175, 1130, 1060 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 1.32 (3H, d, $J=6.8$ Hz, 14-$CH_3$), 1.70 (3H, s, 10-$CH_3$), 2.51 (1H, ddd, $J=2.4$, 10.3 and 18.6 Hz, 7-Hax), 2.68 (1H, m, 4a-H), 2.92 (1H, dd, $J=6.3$ and 18.6 Hz, 7-Heq), 3.14 (3H, br s, 6-OH), 3.18 (1H, dt, $J=4.4$ and ~10 Hz, 5-H), 3.81 (3H, s, $CH_3O$–), 3.93 (1H, dt, $J=6.4$ and 10.3 Hz, 6-H), 4.06 and 4.52 (2H, ABq, $J=10$ Hz, 9-$CH_2$–), 4.23 (1H, quintet, $J=$ ~7 Hz, 13-H), 4.69 (1H, d, $J=4.4$ Hz, 4-H), 4.99 and 5.06 (2H, ABq, $J=11.7$ Hz, –$CH_2$–Ph), 5.21 (1H, d, $J=4.4$ Hz, 5-OH), 5.24 (1H, d, $J=6.8$ Hz, 15-NH), 6.89 (2H, d with small couplings, $J=8.8$ Hz, Ph), 7.28 (2H, d with small couplings, $J=8.8$ Hz, Ph), 12.9 (1H, br s, 8-OH); MS (FAB positive) *m/z* 477 $(M+H)^+$.

### 9-Didechloro-9,11-cyclobactobolin (**5**)

Compound **5** was obtained from **9** by the similar procedure for the preparation of **3** (yield 84%): $[\alpha]_D^{24}$ −99° (*c* 0.09, MeOH); IR (KBr) 3400 (br), 2925 (sh), 1655, 1640 (sh), 1620 (sh), 1475, 1440, 1235, 1175, 1125 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 400 MHz) $\delta$ 1.42 (3H, d, $J=7.3$ Hz, 14-$CH_3$), 1.66 (3H, s, 10-$CH_3$), 2.39 (1H, ddd, $J=2.4$, 10.3 and 18.1 Hz, 7-Hax), 2.70 (1H, m, 4a-H), 2.76 (1H, dd, $J=6.6$ and 17.8 Hz, 7-Heq), 3.3~3.38 (1H, dd, $J=2.9$ and 9.8 Hz, 5-H), 3.83 (1H, dt, $J=6.4$ and ~10 Hz, 6-H), 4.08 and 4.32 (2H, ABq, $J=10.3$ Hz, 9-$CH_2$–), 4.13 (1H, q, $J=$ ~7.0 Hz, 13-H), 4.82 (1H, d, $J=5.4$ Hz, 4-H); MS (FAB positive) *m/z* 313 $(M+H)^+$.

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