Synthesis and Activity of 3-epi-Actinobolin

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3-epi-Actinobolin was synthesized by the chemical transformation of actinobolin involving a key step of the reconstruction of fused δ-lactone skeleton via intramolecular acylation reaction. The analogue with low toxicity weakly inhibits Gram-positive and Gram-negative bacteria.

Actinobolin (1) and its structurally related bactobolin (2) were isolated from the culture broths of Streptomyces and Pseudomonas respectively. They demonstrated various kinds of biological effects including antimicrobial and antitumor activities, suppressing effect on antibody production and therapeutic effect on autoimmune encephalomyelitis. Due to the unique structures and potent activities, these antibiotics have attracted intensive synthetic interest in the total synthesis and in the chemical modification. While 1 bears a structural resemblance to 2 except for the different substitution of C-3 (Fig. 1), its biological activity and cytotoxicity are considerably distinct from those of 2. The biological diversity stimulated our interest in the alteration of functionality at C-3 of 1. We considered the epimerization of the stereochemistry at C-3 as an initial probe of the structure-activity profile of this class of antibiotic. We here report the synthesis of 3-epi-actinobolin (17) by the chemical conversion of 1.

**Synthesis**

The synthetic route to 17 is outlined in Scheme 1. The synthesis of 17 began with the known carbamate 3. Stereoselective reduction of 3 with NaBH₄ under WARD’s conditions (50% MeOH in CH₂Cl₂, −78°C) gave the alcohol 4 in 98% yield. The large coupling constants (12 and 11.2 Hz) between H-5’ and Hax-6’ and between H-5’ and Hax-4’ in ¹H NMR spectrum of 4 are indicative of the equatorial hydroxyl group. Protection of the hydroxyl of 4 afforded the tert-butyldimethylsilyl (TBDMS) ether 5 (96% yield). The compound 5 has the different specific rotation and ¹H NMR spectrum from those of the starting 3, clearly indicating an epimerization at C-2’. This was also supported by the distinct physical data of the final 3-epi-actinobolin (17) from those of the natural actinobolin (1). Upon acetylation, 5 afforded the acetate 6. Removal of TBDMS group of 6 resulted in the alcohol 7 (92% yield), which was oxidized to the ketone 8 (94% yield). Hydrolysis of 8 gave the desired stereoisomer 9 as a sole product in 84% yield. The compound 9 has the different specific rotation and ¹H NMR spectrum from those of the starting 7, clearly indicating an epimerization at C-2’.

Fig. 1. The structures of actinobolin and bactobolin.

**Fig. 1.** The structures of actinobolin and bactobolin.

Actinobolin (1): R₁=H, R₂=CH₃

Bactobolin (2): R₁=CH₃, R₂=CHCl₂

dride\textsuperscript{14}) to afford the enol lactone 14 in 98\% yield. Simultaneous removal of both the PMS and the acetonide groups of 14 smoothly proceeded with HF to afford the 3-epi-actinobolamine as its HF salt 15. The synthesis of 3-epi-actinobolin (17) was completed by condensation of 15 with N-benzyloxycarbonylalanine followed by hydrogenolysis with H\textsubscript{2}/Pd-C.

Biological Activities

As shown in Table 1, 3-epi-actinobolin (17) showed less inhibitory activity than actinobolin (1) against several organisms. The analogue 17 was also found to have less cytotoxicity than 1 (Table 2). These results indicate that the stereochemistry at C-3, which would cause the conformational change, critically participates in the biological activity. Further chemical modification of 1 based on the alteration of functionality at C-3 are now in progress.

Experimental

General Methods

Melting points were determined with a Yanagimoto apparatus and were uncollected. IR spectra were determined on a Hitachi Model 260-10 spectrometer. Optical rotations were measured with a Perkin-Elmer
Table 1. Antibacterial activities of actinobolin (1), bactobolin (2) and 3-epi-actinobolin (17) by broth dilution method.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus aureus FDA209P</td>
<td>3.13</td>
</tr>
<tr>
<td>Staphylococcus aureus Smith</td>
<td>6.25</td>
</tr>
<tr>
<td>Staphylococcus aureus MRSA No. 5</td>
<td>12.5</td>
</tr>
<tr>
<td>Staphylococcus aureus MS16526 (MRSA)</td>
<td>6.25</td>
</tr>
<tr>
<td>Staphylococcus epidermidis 109</td>
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<tr>
<td>Micrococcus luteus FDA16</td>
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</tr>
<tr>
<td>Micrococcus luteus PCI11001</td>
<td>1.56</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>50</td>
</tr>
<tr>
<td>Bacillus subtilis PCI219</td>
<td>25</td>
</tr>
<tr>
<td>Corynebacterium bovis 1810</td>
<td>1.56</td>
</tr>
<tr>
<td>Escherichia coli NIHJ</td>
<td>1.56</td>
</tr>
<tr>
<td>Escherichia coli K-12 ML1629</td>
<td>25</td>
</tr>
<tr>
<td>Shigella dysenteriae JS11910</td>
<td>1.56</td>
</tr>
<tr>
<td>Salmonella typhi T-63</td>
<td>25</td>
</tr>
<tr>
<td>Proteus vulgaris OX19</td>
<td>3.13</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa A3</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa GN315</td>
<td>&gt;50</td>
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<tr>
<td>Klebsiella pneumoniae PCI602</td>
<td>25</td>
</tr>
<tr>
<td>Mycobacterium smegmatis ATCC607*</td>
<td>12.5</td>
</tr>
</tbody>
</table>

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

Table 2. Cytotoxicity of actinobolin (1), bactobolin (2) and 3-epi-actinobolin (17).

<table>
<thead>
<tr>
<th>Cell</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
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<tr>
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<td>1</td>
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<tr>
<td>L1210</td>
<td>48.1</td>
</tr>
<tr>
<td>ELA</td>
<td>42.1</td>
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<tr>
<td>P388</td>
<td>39.6</td>
</tr>
<tr>
<td>IMC ca.</td>
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<tr>
<td>Colon 26</td>
<td>30.6</td>
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<tr>
<td>HeLa</td>
<td>&gt;100</td>
</tr>
<tr>
<td>FS-3</td>
<td>99.6</td>
</tr>
<tr>
<td>LB32T</td>
<td>37.2</td>
</tr>
<tr>
<td>Methyl green FS-3</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

The rate of survival cells was measured by MTT assay and IC₅₀ value was calculated.
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 SX 102 in the FAB mode using 3-nitrobenzyl alcohol as a matrix.

\((1'R,2'R,3'R,4'R,5'R,5R)-4-[5'-Hydroxy-2',3'-(isopropylidenedioxy)cyclohexyl]-5-methyl-2-oxazolidinone\) (4)

To a solution of 3 (1.20 g, 4.5 mmol) in a mixture of dichloromethane (6 ml) and methanol (6 ml) was added sodium borohydride (0.510 g, 13.5 mmol) at -60° to 70°, and the reaction mixture was stirred for 30 minutes. After dilution with ethyl acetate, the solution was washed with water, dried over MgSO4 and filtered. The filtrate was evaporated to give an oil, which was subjected to column chromatography on silica gel. Elution with chloroform - methanol (10:1) gave a colorless solid of 4 (1.18 g, 98% yield), which was crystallized from a mixture of dichloromethane and hexane (10:1) to give colorless crystals; mp 106° to 108°; \([\alpha]_2^{D} +53.5° (c 0.72, CHCl3); IR (CHCl3) cm\(^{-1}\) 3450 (br), 2990, 2940, 2875, 1745, 1465, 1455, 1385, 1375, 1235, 1100, 1060, 1050 (sh), 1005, 840; \(^1\)H NMR (CDCl3, 400 MHz) \(\delta\) 1.18 (1H, q, \(J = 12\text{ Hz}\), 6'-Hax), 1.40 and 1.42 (each 3H, s, isopropylidene), 1.45 (3H, d, \(J = 5.9\text{ Hz}\), 5-CH3), 1.51 (1H, q, \(J = 11.2\text{ Hz}\), 4'-Hax), 1.77 (1H, m, 1'-H), 2.10 (1H, d with small couplings, \(J = 12\text{ Hz}, 6'\text{-Heq}\)), 2.44 (1H, d with small couplings, \(J = 11.2\text{ Hz}, 4'\text{-Heq}\)), 3.17 (1H, dd, \(J = 8.8\text{ and } 10.3\text{ Hz}\), 2'-H), 3.30 to 3.45 (3H, m, 3'-H and 4'-OH), 3.83 (1H, m, 5'-H), 4.67 (1H, dq, \(J = 6.1\text{ and } 7.1\text{ Hz}, 5'\text{-H})

\((1'R,2'R,3'R,4'R,5'R,5R)-5'-[(tert-Butyldimethylsilyl)oxy-2',3'-(isopropylidenedioxy)cyclohexyl]-5-methyl-2-oxazolidinone\) (5)

To a solution of 4 (0.685 g, 2.5 mmol) in dichloromethane (7 ml) were added tert-butyldimethylsilyl chloride (0.753 g, 5.0 mmol) and imidazole (0.510 g, 7.5 mmol) at room temperature, and the reaction mixture was stirred for 2 hours. After dilution with chloroform, the solution was washed with satd NaHCO3 aq solution and water, dried over MgSO4, and filtered. The filtrate was evaporated to give an oil, which was subjected to preparative TLC on silica gel developed with chloroform - methanol (30:1) to give 5 (0.388 g, 54% yield). \([\alpha]_2^{D} +35.4° (c 0.72, CHCl3); IR (CHCl3) cm\(^{-1}\) 2995, 2960, 2940, 2870, 1780, 1475, 1390 (sh), 1380, 1375 (sh), 1175, 1150, 1105, 845; \(^1\)H NMR (CDCl3, 400 MHz) \(\delta\) 0.06 (6H, s, -(CH3)2Si-), 0.88 (9H, s, (CH3)3C-), 1.13 (1H, dt, \(J = 10.7\text{ and } 12.7\text{ Hz}, 6\text{-Hax}\)), 1.23 (3H, d, \(J = 6.3\text{ Hz}, 5\text{-CH3}\)), 1.37 and 1.40 (each 3H, s, isopropylidene), 1.45 (1H, q, \(J = 11.2\text{ Hz}, 4'\text{-Hax}\)), 1.80 (1H, d with small couplings, \(J = 13\text{ Hz}, 6'\text{-Heq}\)), 2.19 (1H, m, 1'-H), 2.30 (1H, d with small couplings, \(J = 11.2\text{ Hz}, 4'\text{-Heq}\)), 2.36 (3H, s, CH3-Ph-), 3.14 (1H, dd, \(J = 8.8\text{ and } 10.7\text{ Hz}, 2'\text{-H}\)), 3.36 (1H, ddd, \(J = 8.3\text{ and } 11.7\text{ Hz}\)), 3.78 to 3.90 (2H, m, 4'-H and 5'-H), 4.57 to 4.64 (1H, d with aromatic proton), 4.64 and 5.00 (2H, ABq, \(J = 14.2\text{ Hz}, -CH2-Ph-\)), 7.21 (2H, d, \(J = 7.8\text{ Hz}, aromatic protons\)), 7.36 (2H, d, \(J = 8.3\text{ Hz}, aromatic protons\)); MS (FAB positive) m/z 554 (M + H)+.

\((1'R,2'R,3'R,4'R,5'R,5R)-1-[(tert-Butylidemethylsilyl)-oxy-2',3'-(isopropylidenedioxy)cyclohexyl]-3,4-(isopropylidenedioxy)cyclohexane\) (7)

To a solution of 6 (0.313 g, 0.57 mmol) in methanol (6
ml) was added sodium methoxide (0.305 g, 5.7 mmol) at 
0°C, and the reaction mixture was stirred for 3 hours. 
After dilution with ethyl acetate, the solution was washed 
with sat. NH₄Cl aq. solution and water, dried over 
MgSO₄, and filtered. Evaporation of the solvent gave an 
oil, which was subjected to preparative TLC on silica gel 
developed with ethyl acetate - hexane (1:2) to give 7 
(0.287 g, 96% yield) which was identical to the known 
compound reported by Weinreb et al.17)

\[
(1R,1'R,3R,4R,5R)-1-[(\text{tert-Butyldimethylsilyl})oxy]-
3,4-(\text{isopropylidenedioxy})-5-[(1'-\text{(4-methylphenyl)methanesulfonamide-2'}-\text{oxopropyl})\text{cyclohexane} (8)
\]

To a solution of 7 (0.170 g, 0.32 mmol) in dichloro-
methane (4 ml) was added pyridinium dichromate (1.2 g, 3.2 mmol) at room temperature, and the reaction mixture 
was stirred overnight. Filtration and evaporation of the 
filtrate gave an oil, which was subjected to preparative 
TLC on silica gel developed with ethyl acetate-hexane 
(1:2) to give 8 (0.106 g) and the starting material 5 
(0.034 g) (conversion yield 79%). \[\alpha_{D}^{24} +53.7° (c 0.91, \text{CHCl}_3)\); IR (CHCl₃) cm⁻¹ 3010 (sh), 2975, 2950, 2880, 
1730, 1395, 1385, 1350, 1270, 1240, 1165, 1140, 1120 (sh), 
1095, 1070, 845; ¹H NMR (CDCl₃, 400 MHz) \(\delta\) 0.034 
and 0.027 (each 3H, s, -(CH₃)₂Si-), 0.87 (9H, s, (CH₃)₃C-), 
1.21 (1H, dt, \(J=10.8\) and \(13.2\) Hz, 6-Hax), 1.41 and 1.42 
(each 3H, s, isopropylidene), 1.44-1.55 
(2H, 6-Heq and 2-Hax), 1.91 (1H, m, H-5), 2.20 (3H, s, 
CH₃CO-), 2.29 (1H, d with small couplings, \(J=11.7\) Hz, 
2-Heq), 2.36 (3H, s, CH₃-Ph-), 3.26 (1H, dd, \(J=8.8\) and 
10.7 Hz, 4-H), 3.35 (1H, ddd, \(J=3.4, 8.8\) and 11.7 Hz, 
3-H), 3.75 (1H, m, 1'-H), 4.06 (1H, dd, \(J=3.4\) and 9.3 Hz, 
1'-H), 4.19 and 4.28 (2H, ABq, \(J=13.7\) Hz, \(-\text{CH}_{2}-\text{Ph}-\)), 
5.27 (1H, d, \(J=9.3\) Hz, 1'-NH), 7.18 (2H, d, \(J=7.8\) Hz, 
aromatic protons), 7.28 (2H, d, \(J=8.3\) Hz, aromatic protons); MS (FAB positive) \(m/z\) 526 (M+H)+.

\[
(1R,1'R,2'S,3R,4R,5R)-5-\text{[2'-Acetoxy-1'-[(4-methyl-
phenyl)methanesulfonamide]propyl]}-\text{[(tert-butyl-
dimethylsilyl)oxy]-3,4-(isopropylidenedioxy)cyclohexane (10)
\]

A solution of 9 (0.465 g, 0.88 mmol) in pyridine (4.5 ml) 
was added acetic anhydride (1.1 ml), and the mixture was 
stirred at room temperature overnight. Evaporation of 
the solvent gave an oil, which was subjected to preparative 
TLC on silica gel developed with ethyl acetate - hexane 
(1:2) to give 10 (0.491 g, 98% yield). \[\alpha_{D}^{24} -34.1° (c 
0.36, \text{CHCl}_3)\); IR (CHCl₃) cm⁻¹ 2990 (sh), 2960, 2930, 
2870, 1735, 1435, 1385 (sh), 1375, 1335, 1260 (sh), 1235, 
1095, 1060, 845; ¹H NMR (CDCl₃, 400 MHz) \(\delta\) 0.054 
and 0.050 (each 3H, s, -(CH₃)₂Si-), 0.87 (9H, s, (CH₃)₃C-), 
1.27 (3H, d, \(J=6.3\) Hz, 2'-CH₃), 1.25-1.40 
(1H, m, 6-Hax), 1.38 (6H, s, isopropylidene), 1.51 (1H, q, 
\(J=11.5\) Hz, 2-Hax), 1.72 (1H, m, 5-H), 1.96 (1H, d 
with small couplings, \(J=13.2\) Hz, 6-Heq), 2.09 (3H, s, 
CH₃CO₂-), 2.30 (1H, d with small couplings, \(J=11.2\) Hz, 
2-Heq), 2.36 (3H, s, CH₃-Ph-), 3.24 (1H, dd, \(J=8.5\) and 
10.5 Hz, H-4), 3.31 (1H, ddd, \(J=3.4, 8.3\) and 11.7 Hz, 
3-H), 3.70-3.80 (2H, m, 1'-H and 1'-H), 4.30 (2H, s, 
\(-\text{CH}_2\text{-Ph}-\)), 4.61 (1H, d, \(J=10.3\) Hz, 1'-NH), 5.12 (1H, 
dq, \(J=3.4\) and 6.4 Hz, 2'-CH₃), 7.19 (2H, d, \(J=7.8\) Hz, 
aromatic protons), 7.33 (2H, d, \(J=8.3\) Hz, aromatic protons); MS (FAB positive) \(m/z\) 570 (M+H)+.
(1'R,1'R,2'S,3R,4R,5R)-5-[2'-Acetoxy-1'-[(4-methylphenyl)thiophenamidoyl]propyl]-1-hydroxy-3,4-(isopropylidenediacyl)cyclohexane (11)

To a solution of 10 (0.491 g, 0.86 mmol) in tetrahydrofuran (10 ml) was added 1M solution of tetrabutyrammonium fluoride in THE (1.05 ml, 1.1 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with satd NH₄Cl aq solution and extracted with ethyl acetate. The extract was dried over MgSO₄ and filtered. The filtrate was concentrated under reduced pressure. The residue was subjected to preparative TLC on silica gel developed with ethyl acetate-hexane (1:1) to give 11 (0.369 g, 94% yield). [α]D₂⁰ -36.3° (c 0.72, CHCl₃); IR (CHCl₃) cm⁻¹ 3375, 2990, 2940, 1735, 1390 (sh), 1385, 1340, 1245, 1230 (sh), 1165, 1145, 1095, 1065; ¹H NMR (CDCl₃, 400 MHz) δ 1.25 (3H, d, J=6.4 Hz, 2'-CH₃), 1.20-1.33 (1H, 6-Hax overlapped with 2'-CH₃), 1.38 and 1.41 (each 3H, s, isopropylidene), 1.47 (1H, q, J=11 Hz, 2-Hax), 1.65-1.75 (1H, m, 5-H), 2.09 (3H, s, -OCOCH₃), 2.14 (1H, d with small couplings, J=13.2 Hz, 6-Heq), 2.33 (3H, s, -CH₂-Ph-), 2.40 (1H, d with small couplings, J=9.8 Hz, 4-Heq), 2.01 (1H, ddd, J=4.9, 5.1, 10.0 and 12.2 Hz, 5-H), 2.10 (1H, d, J=9.8 Hz, 1'-NH), 2.09 (1H, dd, J=3.4 and 6.4 Hz, 2'-CH₃), 7.19 (2H, d, aromatic protons), 7.30 (2H, d, aromatic protons); MS (FAB positive) m/z 456 (M+H)+.

(1'R,2'S,3R,4R,5R)-5-[2'-Acetoxy-1'-[(4-methylphenyl)methanesulfonyl]propyl]-3,4-(isopropylidenediacyl)cyclohexanone (12)

To a solution of 11 (0.344 g, 0.76 mmol) in dichloromethane (4 ml) was added pyridinium dichromate (0.568 g, 1.5 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 1 hour. After filtration, the filtrate was evaporated to give an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - hexane (1:1) to give 12 (0.322 g, 94% yield). [α]D₂⁰ -53.5° (c 0.98, CHCl₃); IR (CHCl₃) cm⁻¹ 3025, 2990, 2940, 2875, 1740 (sh), 1725, 1395 (sh), 1385, 1340, 1230, 1215 (sh), 1115, 1135, 915; ¹H NMR (CDCl₃, 400 MHz) δ 1.26 (3H, d, J=6.4 Hz, 2'-CH₃), 1.44 and 1.47 (each 3H, s, isopropylidene), 1.88 (1H, d, J=9.8 Hz, 1'-NH), 2.03 (1H, dq, J=3.4 and 6.4 Hz, 2'-CH₃), 7.20 (2H, d, J=7.8 Hz, aromatic protons), 7.30 (2H, d, J=8.3 Hz, aromatic protons); MS (FAB positive) m/z 454 (M+H)+.

(1'R,2'S,3R,4R,5R)-5-[2'-Hydroxy-1'-[(4-methylphenyl)methanesulfonyl]propyl]-3,4-(isopropylidenediacyl)cyclohexanone (13)

To a solution of 12 (0.282 g, 0.62 mmol) in a mixture of methanol (4.8 ml) and water (3.8 ml) was added potassium carbonate (0.342 g, 2.5 mmol), and the reaction mixture was stirred at room temperature for 1 hour. After dilution with ethyl acetate, the solution was washed with water, dried over MgSO₄ and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate-hexane (1:1) to yield a colorless solid of 13 (0.236 g, 93% yield), which was crystallized from a mixture of dichloromethane and hexane (10:1) to give colorless crystals; mp 145-146°C; [α]D₂⁰ -34.3° (c 0.85, CHCl₃); IR (CHCl₃) cm⁻¹ 3525 (br), 3350 (br), 3025 (sh), 2980, 2930, 2875, 1721, 1390, 1335, 1230, 1215 (sh), 1115, 1135, 915; ¹H NMR (CDCl₃, 400 MHz) δ 1.27 (3H, d, J=6.4 Hz, 2'-CH₃), 1.43 and 1.45 (each 3H, s, isopropylidene), 1.88 (1H, d, J=9.8 Hz, 2'-OH), 2.03 (1H, m, 1-H), 2.31 (1H, dd, J=15.6 and 6.6 Hz, 6-Hax), 2.36 (3H, s, CH₃-Ph-), 2.53 (1H, t, J=14 Hz, 2-Hax), 2.50-2.60 (1H, m, 6-Heq), 2.88 (1H, ddd, J=2.0, 4.9 and 13.9 Hz, 2-Heq), 3.54 (1H, dq, J=6.3 and 10 Hz, 2'-H), 4.31 and 4.40 (2H, ABq, J=13.9 Hz, -CH₂-Ph-), 4.69 (1H, d, J=9.8 Hz, 1'-NH), 7.19 (2H, d, J=7.8 Hz, aromatic protons), 7.33 (2H, d, J=7.8 Hz, aromatic protons); MS (FAB positive) m/z 412 (M+H)+.

De-N-alanyl-5,6-O-(isopropylidene)-N-[(4-methylphenyl)methanesulfonyl]-3-epi-actinobolin (14)

To a solution of 13 (0.013 g, 0.032 mmol) and 1,1'-carbonyldiimidazole (0.025 g, 0.16 mmol) in anhydrous DMF (1.3 ml) was stirred at 70°C overnight, and to the mixture sodium hydride (7.6 mg, 60% oil dispersion) was added at 0°C. The reaction mixture was stirred at 20°C for 1 hour. After being quenched with satd NH₄Cl aq solution, the resulting suspension was diluted with ethyl acetate. The solution was washed with water, dried over...
MgSO₄, and filtered. Evaporation of the filtrate gave an oil, which was subjected to preparative TLC on silica gel developed with ethyl acetate - hexane (2:1) to give 14 (0.014g, 98% yield). [α]D 290 (c 0.46, CHCl₃); IR (CHCl₃) cm⁻¹ 3370, 3010, 2925, 1650, 1590, 1425, 1390, 1335, 1225, 1155, 1135, 1105; ¹H NMR (CDCl₃, 400 MHz) δ 1.26 (3H, Br s, J = 7.1 Hz, 3'-H); 2.65 (1H, d, J = 9.8 Hz, 2'-NH), 2.80 (2H, m, J = 8.6 Hz, 5'-H), 2.82 (1H, d, J = 6.8 Hz, 7'-Hax), 2.89 (1H, d, J = 7.8 Hz, aromatic protons), 3.71 (2H, d, J = 8.3 Hz, aromatic protons), 13.7 (1H, br s, 8-OH); MS (FAB positive) m/z 438 (M+H)+.

2'-N-(Benzylxocarbonyl)-3-epi-actinobolin (16)

Anhydrous hydrogen fluoride (HF) (20 ml) was condensed into a Teflon round-bottomed flask containing a solution of 14 (0.031g, 0.07mmol) in anisole (4ml) at -20°C. The reaction mixture was then stirred at 0°C overnight. Hydrogen fluoride and anisole were removed under reduced pressure to give the crude 15, which was subjected to the next step without purification. A solution of 14 (0.031g, 0.07mmol) in anisole (4ml) at 0°C was stirred overnight. Hydrogen fluoride and anisole were removed under reduced pressure to give the crude 15, which was subjected to the next step without purification. A solution of 15, N-benzylxocarbonyl-l-alanine (0.045g, 0.20 mmol), dicyclohexylcarbodiimide (0.049g, 0.24mmol) and triethylamine (57 µl) in dry dimethylformamide (0.4ml) was stirred overnight. Evaporation of the solvent gave an oil, which was subjected to preparative TLC on silica gel developed with chloroform - methanol (5:1) to give 16 (0.015g, 69% yield). [α]D 24 290 (c 0.22, CHCl₃); IR (CHCl₃) cm⁻¹ 3430, 3010, 2960 (sh), 1725, 1715, 1660, 1655, 1515, 1320, 1150; ¹H NMR (CDCl₃, 400 MHz) δ 1.43 (3H, d, J=6.8 Hz, 3-CH₃), 1.49 (3H, d, J = 6.8Hz, 2'-CH₃), 2.36 (3H, s, CH₃-Ph), 3.4 Hz, 4-H), 4.69 (1H, dq, J = 1.5 and 6.8Hz, 3-H); MS (FAB positive) m/z 301 (M+H)+.

3-epi-Actinobolin (17)

Compound 16 (0.0117g, 0.027mmol) in a mixture of ethyl acetate (0.3 ml), methanol (2ml) and 0.2 N HCl (0.098 ml) was stirred with 5% Pd/C (48 mg) under atmosphere of hydrogen at room temperature for 1 hour. After filtration, evaporation of the filtrate gave a hydrochloride of 3-epi-actinobolin 17 (0.008 mg, 88% yield). [α]D 24 24 290 (c 0.55, MeOH); IR (KBr) cm⁻¹ 3530, 2990 (sh), 1690, 1665 (sh), 1605, 1570, 1500, 1420, 1225, 1205, 1145, 1080; ¹H NMR (CD₂OD, 400 MHz) δ 1.43 (3H, d, J = 7.8 Hz, 3-CH₃), 1.49 (3H, d, J = 7.8 Hz, 2'-CH₃), 2.40 (1H, ddd, J = 2.4, 9.8 and 18.6 Hz, 7-Hax), 2.82 (1H, dd, J = 6.6 and 18.6 Hz, 7-Heq), 2.89 (1H, d, J = 7.8 Hz, aromatic protons), 7.31 (2H, d, J = 8.3 Hz, aromatic protons), 13.1 (1H, br s, 8-OH); MS (FAB positive) m/z 301 (M+H)+.

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References


