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Absolute Stereostructure and Total Synthesis of Leptomycin B

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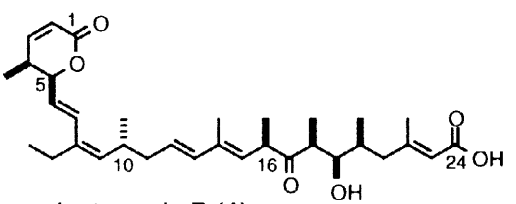
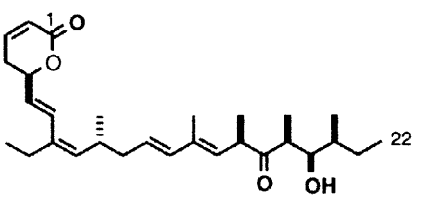
Abstract : The absolute stereostructure of leptomycin B, an antitumor antibiotic and inhibitor of nuclear protein export, was firstly presumed as **1** having 4*S*, 5*R*, 10*R*, 16*R*, 18*S*, 19*R*, 20*S* on the basis of NMR comparison with callystatin A (**2**) and then **1** was asymmetrically synthesized. The synthesized leptomycin B (**1**) was found identical with the authentic sample in HPLC and CD comparison as well as in other respects. This structural elucidation of the absolute stereostructure and total synthesis are the first example among the leptomycin family as *Streptomyces* metabolites.

Keywords: leptomycin B; callystatin A; antitumor polyketide; absolute stereostructure; total synthesis; Streptomyces

Leptomycin B (**1**) was first isolated as an antifungal antibiotic from *Streptomyces* sp. [1] and later found to inhibit an essential step for the initiation of DNA synthesis which occurrs at the end of the G1 and G2 phase [1c,1d]. Very recently, leptomycin B was recognized to specifically bind chromosome maintenance region 1 (CRM1) protein and inhibit nuclear export signal (NES)-mediated transport of Rev and U snRNA protein [2,3]. However, up to now, no study on the stereostructure of leptomycin B as well as other leptomycin/kazusamycin families has been reported. In the course of our study of bioactive substances from marine organisms, we have isolated a potent cytotoxic (IC_{50} 10 pg/ml against KB cell) polyketide named callystatin A (**2**) from the marine sponge *Callyspongia truncata* [4a]. In addition, we elucidated the absolute stereostructure of **2** [4b] and recently completed its first total synthesis [4c]. From the spectral similarities between **2** and leptomycin B, along with their attractive bioactivities, we were led to presume that leptomycin B has the same configuration as that of **2**; we then undertook asymmetric total synthesis to substantiate this postulation. Here, we describe the total synthesis of leptomycin B (**1**).



leptomycin B (**1**)



callystatin A (**2**)

The absolute stereostructure of leptomycin B was presumed as **1** having 4*S*, 5*R*, 10*R*, 16*R*, 18*S*, 19*R*, 20*S* on the basis of the following considerations. 1) The carbon framework of **1**

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was very similar to that of **2**, and the ^{1}H- and ^{13}C-NMR data ($\Delta\delta$ and $\Delta\delta c$ values for C7-C19 are <0.07 and <0.7 ppm in $CDCl_3$, respectively) as well as $[\alpha]_D$ value (in MeOH, **1**: -105.4° [5]; **2**: -107°) closely resembled each other. 2) The 4,5-*syn* orientation for **1** was clarified by comparison of H-3 chemical shift (δ 6.93) and $J_{3,4}$ coupling constant (5.6 Hz) with synthetic compounds [6].

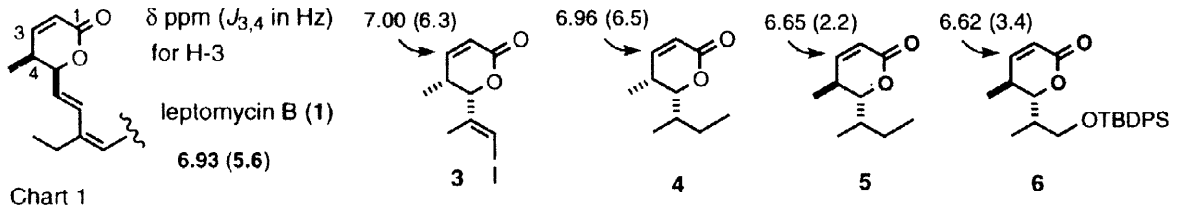

Chart 1

Chart 2 outlines our retrosynthetic analysis for **1**. The terminal carboxyl group could be introduced by oxidation of **7**, which could in turn be prepared *via* a similar route used for the total synthesis of **2** [4c].

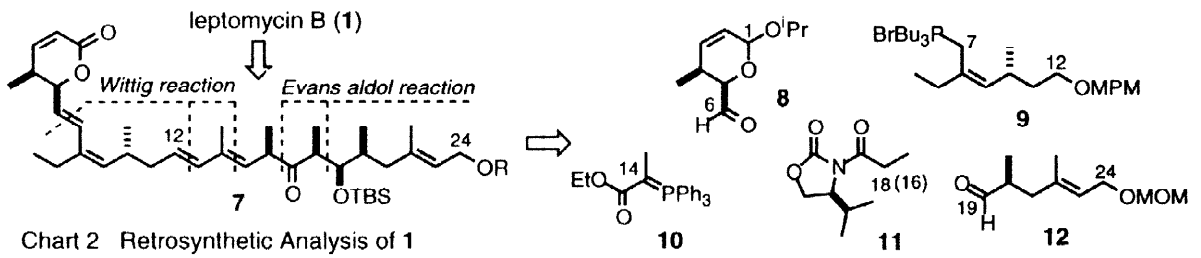

Chart 2 Retrosynthetic Analysis of 1

The synthesis of segment C_1-C_{12} (**19**) is depicted in Chart 3. The α,β-unsaturated δ-lactone **16** was synthesized by application of Willson's method [7] from the epoxide **14**, which was prepared from *trans*-crotyl alcohol (**13**) by Sharpless epoxidation [8]. Thus, *regio*-selective nucleophilic ring-opening followed by carboxylation, hydrogenation and thermal treatment furnished δ-lactone **16** in 70% total yield. After protection of δ-lactone as *O*-

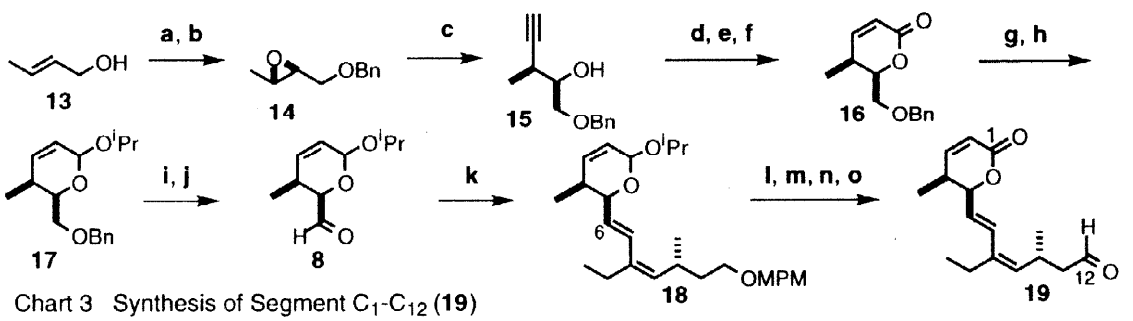

Chart 3 Synthesis of Segment C_1-C_{12} (**19**)

Reagents and conditions: **a**) TBHP, (+)-DIPT, $Ti(O^iPr)_4$, CH_2Cl_2, -20°C, 75% (96% ee), **b**) BnBr, NaH, TBAI, THF, 97%, **c**) Lithium acetylide·EDA complex, HMPA, 66% (and 27% regioisomer), **d**) ^nBuLi, $^iPr_2NH$, THF, -78 °C; CO_2, THF, -60°C, **e**) H_2, $Pd/BaSO_4$, quinoline, EtOH, **f**) reflux in PhH, 70% 3 steps, **g**) DIBAL-H, CH_2Cl_2, -78°C, **h**) ^iPrOH, PPTS, PhH, 55% 2 steps, **i**) Lithium di-*tert*-butylbiphenyl, THF, -78°C, 89%, **j**) $(COCl)_2$, DMSO, CH_2Cl_2; Et_3N, -78°C, 99%, **k**) **9**, $LiCH_2S(O)CH_3$, toluene, -78°C to 5°C, 59%, **l**) Dowex HCR-W2, acetone-H_2O, 40°C, **m**) Ag_2CO_3-Celite, PhH, 50°C, 94%, 2 steps, **n**) DDQ, CH_2Cl_2-^tBuOH-buffer (90:1:9), 89%, **o**) Dess-Martin periodinane, CH_2Cl_2, 99%.

isopropyl acetal, deprotection of the benzyl group and subsequent Swern oxidation afforded aldehyde **8**, which was further subjected to Wittig coupling with the segment C7-C12 (**9**) [4c] providing 6*E*-conjugated diene **18** selectively. Finally, in order to shorten the reaction steps at the later stage of the total synthesis, **18** was returned to its lactone form, which was further subjected to subsequent oxidation to furnish segment C1-C12 (**19**).

Next, the segment C13-C24 (**28**) was synthesized as depicted in Chart 4. The acid **20** prepared by ozonolysis of geraniol was condensed with lithium (*S*)-(-)-4-isopropyl-2-oxazo-

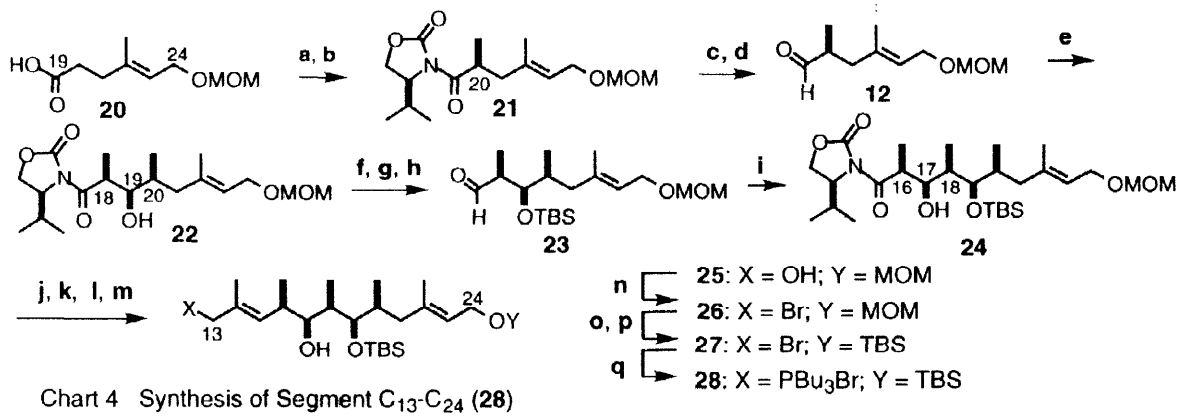

Chart 4 Synthesis of Segment C_{13}-C_{24} (**28**)

Reagents and conditions: **a**) PivCl, Et_3N; XvLi, 76%, **b**) LHMDS, THF, -78°C; MeI, -78~0°C, 80%, **c**) $AlMe_3$, MeONHMe•HCl, CH_2Cl_2, -20~0°C, quant., **d**) DIBAL-H, CH_2Cl_2, -78°C, 98%, **e**) **11**, $^nBu_2BOTf$, Et_3N, THF, -78~0°C, 82%, **f**) $AlMe_3$, MeONHMe•HCl, CH_2Cl_2, -20~0°C, 95%, **g**) TBSOTf, 2,6-lutidine, CH_2Cl_2, -20°C, 85%, **h**) DIBAL-H, THF, -78°C, 85%, **i**) **11**, $^nBu_2BOTf$, Et_3N, THF, -78~0°C, 94%, **j**) $AlMe_3$, MeONHMe•HCl, CH_2Cl_2, -78°C~r.t., 99% **k**) $LiAlH_4$, Et_2O, 0°C, 90%, **l**) **10**, toluene, 83%, **m**) DIBAL-H, CH_2Cl_2, -78°C, quant., **n**) CBr_4, Ph_3P, 2,6-lutidine, CH_3CN, quant., **o**) Me_2BBr, CH_2Cl_2, -78°C, 98%, **p**) TBSCl, imidazole, CH_2Cl_2, quant., **q**) $^nBu_3P$, CH_3CN, 93%.

lidinone (XvLi) and subsequent methylation [9] gave **21** and its diastereomer in 11:1 selectivity. After removal of the chiral auxiliary group, the aldehyde **12** was subjected to the first Evans aldol condensation with **11** under standard conditions [10] to give C18,19-*syn*, C19,20-*syn* adduct **22** (δ 3.67 (t, *J*=5.5 Hz, H-19)) in 82% yield. After removal of the chiral auxiliary group of **22** once again, the aldehyde **23** protected by *tert*-butyldimethylsilyl (TBS) was subjected to the second aldol condensation with **11** to give **24** (δ 3.57 (t, *J*=5.5 Hz, H-17)). In order to avoid removal of the robust methoxymethyl (MOM) group at the final stage of the total synthesis, the MOM group in **26** was changed with the TBS group to give **27**, which was further converted to segment C13-C24 (**28**).

The final stage of the total synthesis of **1** was carried out as summarized in Chart 5. The two segments **19** and **28** were condensed under mild conditions [4c] to provide 12*E*-diene **29** as the sole product. Oxidation of 17-OH in **29** followed by removal of the TBS groups furnished diol **30** [11]. Finally, successive oxidation of **30** with MnO_2 and $NaClO_2$ [12] afforded **1**. The synthesized leptomycin B (**1**) was identical with the authentic sample in all respects (HPLC, CD, UV, FAB-MS, ^1H- and ^{13}C-NMR, IR) [13].

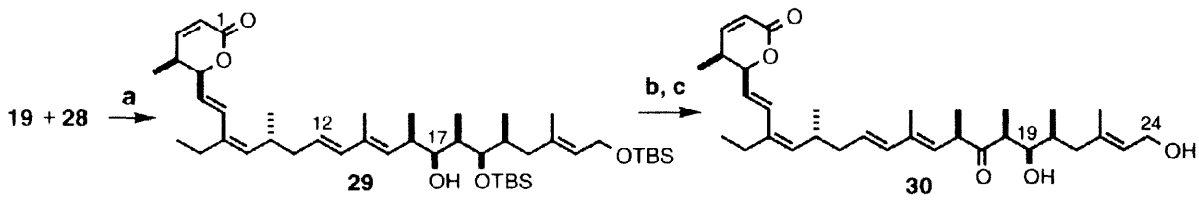

Chart 5 Total Synthesis of leptomycin B (**1**)
Reagents and conditions: **a)** $LiCH_2S(O)CH_3$, toluene, -78°C to 5°C, 90%, **b)** Dess-Martin periodinane, CH_2Cl_2, 71%, **c)** HF-pyridine/pyridine, THF, 89%, **d)** MnO_2, PhH, **e)** $NaClO_2$, NaH_2PO_4, H_2O_2, CH_3CN, 73%, 2 steps.

In conclusion , we did elucidate the absolute stereostructure of leptomycin B as **1** having 4*S*, 5*R*, 10*R*, 16*R*, 18*S*, 19*R*, 20*S* configurations and also synthesized asymmetrically. This absolute stereostructure elucidation and total synthesis are the first example among the leptomycin family as *Streptomyces* metabolites. We hope that this information may contribute to the understanding of the bioactivities of leptomycin B (**1**).

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11. **30**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.94 (dd, *J*=9.7, 5.6 Hz, H-3), 6.65 (d, *J*=15.7 Hz, H-7), 6.01 (d, *J*=16.0 Hz, H-13), 6.00 (d, *J*=9.7 Hz, H-2), 5.71 (dd, *J*=15.7, 6.7 Hz, H-6), 5.59 (dt, *J*=16.0, 6.6 Hz, H-12), 5.41 (t, *J*=7.3 Hz, H-23), 5.23 (d, *J*=9.7 Hz, H-9), 5.09 (d, *J*=10.2 Hz, H-15), 5.00 (dd, *J*=7.0, 4.0 Hz, H-5), 4.15 (d, *J*=7.3 Hz, H-24), 3.64 (m, H-16), 3.58 (t-like, J =*ca.* 5, H-19).
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13. Synthetic **1**: CD (MeOH) λmax nm (Δε): 300 (-22.4), 268 (0), 249 (+35.0), 229 (0), 222 (-3.4). Authentic leptomycin B: CD (MeOH): 300 (-22.4), 268 (0), 249 (+37.6), 229 (0), 222 (-3.4).