Total Synthesis and Inhibitory Activity against Gelatinase B of YL-01869P

KAZUHIKO TAMAKI[a], AKI MATSUBARA[b], KAZUHIKO TANZAWA[b] and YUKIO SUGIMURA[a,*]

[a]Exploratory Chemistry Research Laboratories, [b]Biological Research Laboratories, Sankyo Co., Ltd., 1-2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan

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During the course of our studies on low molecular inhibitors of matrix metalloproteinases (MMPs)[1], matlystatins were isolated from *Actinomadura atramentaria* as inhibitors of gelatinases[2]. One of these, matlystatin B (**1**), has been synthesized and its absolute configuration was determined as shown in Fig. 1[3]. The active site of **1** is the peripherally-located hydroxamic acid, which is thought to bind to Zn^{2+} at the active site of gelatinases to inhibit the enzymes[4].

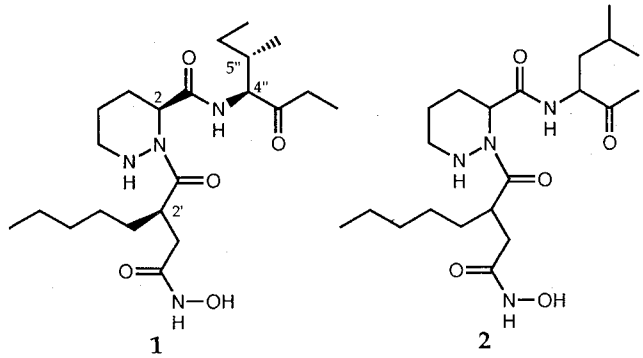
Another naturally occurring hydroxamic acid, YL-01869P (**2**)[5], was isolated from the culture broth of *Streptomyces* sp. by the Yamanouchi group. It is an antimicrobial agent and has also been shown to inhibit collagenase ($IC_{50}=0.9\,\mu M$), one of the MMPs. In the patent of this compound, the absolute and relative configurations remained to be ascertained and the inhibitory activity of **2** against gelatinase B was not described. However, **2** which has a close structural relationship to **1**, suggests to us an inhibitory activity of **2** against gelatinase B. Thus, in this report we describe the synthesis, determination of the absolute configuration, and inhibitory activity of **2** against gelatinase B.

Prior to the synthetic study on **2**, we assumed the absolute configuration of **2** to be the same as that of **1**. YL-01869P (**2**) was dissected at one of the amide bonds into two units (**3**, **4**) (Fig. 2). As for the left hand unit, the carboxylic acid **3** was synthesized according to the method already described in our total synthesis of **1**[6,7]. Following the route as depicted in scheme 1, the right hand unit was synthesized. Reaction of *Z*-Leu (**5**) with *N,O*-dimethylhydroxylamine in the presence of DCC as a coupling reagent afforded amide **6** in 89% yield. Compound **6** was converted to methylketone **7** by reacting with methylmagnesium bromide according to the Weinreb method[8] with an 82% yield (92% based on the recovered amide). Compound **7** was planned to lead to the right hand unit **4** by hydrogenation (10% Pd-C, MeOH). Unfortunately, because of the intermolecular reaction between the carbonyl group and the free amino group derived from removal of the *Z* group in **7**, the desired amine **4** was not isolated.

Thus, the carbonyl group in **7** was converted quantitatively into the acetal to afford **8** by reacting with ethylene glycol in the presence of *p*-toluenesulfonic acid (benzene, reflux). At this stage, the optical purity of **8** was determined to be >99% ee by HPLC analysis using a chiral stationary phase column (DAICEL CHIRALPAK AD hexane/isopropanol=20/1, flow rate 1.5 ml/minute, detection UV at 210 nm, elution time (*S*)-**8**: 42.31 minutes, (*R*)-**8**: 30.91 minutes). As shown in Scheme 2, the coupling of **3** and the amine, prepared by catalytic hydrogenation of **8**, was accomplished with diethylphosphoryl cyanide (DEPC)[9] as a coupling reagent in the presence of triethylamine to afford **9** in 67% yield.

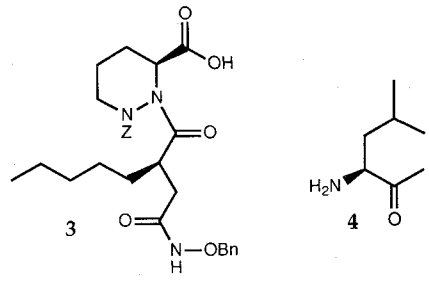
Having all of the carbon framework of **2** in **9**, the remaining steps were removal of all the protective groups. Removal of the acetal in **9** using *p*-toluenesulfonic acid in acetone at 35°C in 58% yield (64% based on the recovered **9**) followed by catalytic hydrogenation (10% Pd-C, MeOH) provided **2*** in 72% yield. The specific rotation ($[\alpha]_D^{25}$ −23.4° (*c* 0.92, $CHCl_3$)) and mp

Fig. 1.



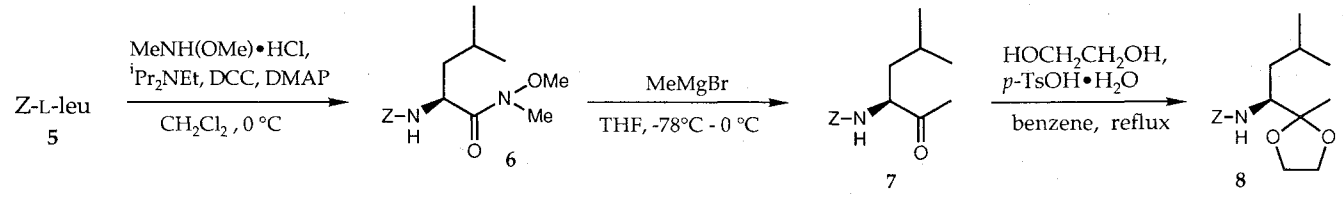
1 Matlystatin B **2** YL-01869P

Fig. 2.

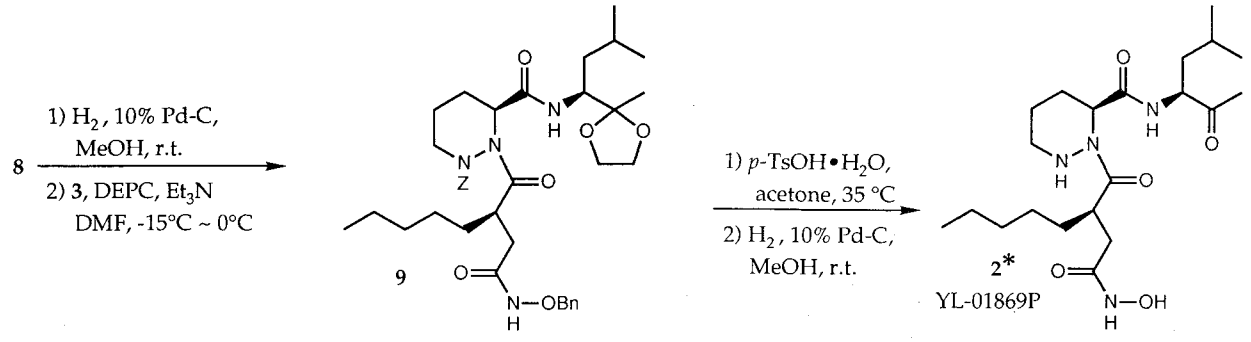


3 **4**

Scheme 1.



Scheme 2.



(52~54°C) of synthetic **2*** showed good agreement with those of YL-01869P (**2**) ($[\alpha]_D$ −22.8° (*c* 1.0, $CHCl_3$), mp 51.6~53.8°C). Thus, the absolute configuration of YL-01869P (**2**) was revealed to be as shown in Scheme 2. At this stage, the inhibitory activity of **2** against gelatinase B was examined. With an IC_{50} value of 1.6 μM, YL-01869P (**2**) turned out not to be as potent an inhibitor as matlystatin B (**1**) (IC_{50} = 0.57 μM) against gelatinase B. Further studies on the structure-activity relationships of gelatinase inhibitors are now in progress.

Experimental

All compounds were characterized by NMR spectra on a JEOL GSX 400 or a JEOL GX 270 spectrometer in $CDCl_3$ with tetramethylsilane as internal reference, by mass spectra on a JEOL JMS-AX505H • model or a JEOL JMS-SX/SX 102A and by IR spectra on a JASCO FT/IR-830 and were in full agreement with the assigned structures. Melting points were obtained on Yanagimoto micro melting point apparatus and are not corrected. Spectral properties of key intermediates (**8**, **9**) and synthetic YL-01869P (**2**) are as follows: compound **8**: colorless crystals, mp 52~53°C (recrystallized from petroleum ether), IR (KBr) 3344, 1719 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 0.92, 0.93 each (3H, d, *J*=6.5 Hz), 1.25 (1H, m), 1.30 (3H, s), 1.40, 1.68 each (1H, m), 3.73~4.05 (5H, complex), 4.65 (1H, br. d, *J*=10.2 Hz), 5.09, 5.14 each (1H, d, *J*=12.3), 7.25~7.45 (5H, complex), MS (EI) *m/z* $[M^+]$=307, *Anal* Calcd for $C_{17}H_{25}NO_4$: C, 66.43; H, 8.20; N, 4.56. Found: C, 66.29; H, 8.21; N, 4.67., $[\alpha]_D^{25}$ −39.3° (*c* 1.0, $CHCl_3$).; compound **9**: pale yellow oil, IR (film) 3311, 1707, 1678 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 0.70~2.50 (29H, complex), 3.08, 3.70 each (1H, m), 3.80~4.30 (6H, m), 4.84 (2H, br. s), 5.10 (1H, d, *J*=12.5 Hz), 5.03~5.20 (1H, m, overlapped with δ 5.10), 5.30 (1H, d, *J*=12.5 Hz), 7.23~7.50 (10H, m), 7.78 (1H, br. d, *J*=9.2 Hz), 8.23 (1H, m), HR-MS (FAB) *m/z* calcd for $[M+H]^+$ $C_{38}H_{54}N_4O_8$ 695.4020, found 695.4013, $[\alpha]_D^{25}$ −41.8° (*c* 1.2, $CHCl_3$).; synthetic YL-01869P (**2**): white powder, mp 52~54°C, IR (film) 3292, 1718, 1669, 1626 cm^{-1}, ^1H NMR (270 MHz, $CDCl_3$) δ 0.50~2.50 (29H, complex), 2.55~3.20 (2H, complex), 4.10, 4.63, 5.08, 5.41 each (1H, m), 7.65~8.90, 9.40~10.50 each (1H, br.), MS (FAB) *m/z* $[M+H]^+$=427, *Anal* Calcd for $C_{21}H_{38}N_4O_5 \cdot 0.1H_2O$: C, 58.88; H, 8.99; N, 13.08. Found: C, 58.71; H, 8.77; N, 12.90., $[\alpha]_D^{25}$ −23.4° (*c* 0.92, $CHCl_3$).

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