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Synthesis and determination of the absolute configuration of matlystatin B

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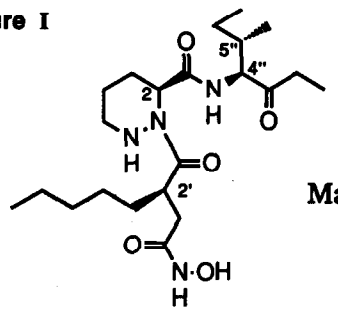
Abstract: The title compound (**1**) was first synthesized and its absolute configuration was determined as shown in figure I.

Type IV collagenases[1] are metalloproteinases which degrade type IV collagen, one of the major components of basement membranes. Many studies have recently indicated that metastatic tumor cells secrete larger amounts of type IV collagenases than nonmetastatic cells.[2] These findings suggest type IV collagenases to be potential targets of antimetastatic agents.

Matlystatins form a new class of inhibitors of type IV collagenases. All five congeners which comprise this class have been isolated from *Actinomadura atramentaria*[3]. The structure of matlystatin B had been determined previously. Prior to study, however, the stereochemistry of only a single chiral center, 2'*R*, has been elucidated[4]. Herein we describe a complete synthesis of matlystatin B and reveal that the 4 asymmetric centers have 2'*R*, 2*S*, 4"*S*, and 5"*S* configuration as shown in Figure I.

Figure I



Matlystatin B
1

We planned to synthesize matlystatin B by coupling of the 4 protected subunits, **2**, **5**, **9**, and *O*-benzylhydroxylamine.

The first subunit, protected piperazic acid (**2**), was prepared as follows. The synthesized racemic N^1-Z-piperazic acid[5] was resolved with l-ephedrine[6] then esterified with 2-methylpropene in the presence of sulfuric acid to give **2** (Figure II)[7].

The second subunit, *N*-Z-ethylketone (**5**), was prepared along the route depicted in scheme I. Treatment of a dichloromethane solution of Z-L-Ile(**3**) with DCC and *N*,*O*-dimethyl-hydroxylamine hydrochloride in the presence of $^{i}Pr_2NEt$ and DMAP resulted in **4** with 97% yield[8]. Addition of EtMgBr to the amide **4** by the method

of Weinreb[9] provided **5** in 80% converting yield. Although 0.7% of diastereomer was observed in **5**, optically pure **5** was obtained by recrystallization from H_2O-MeOH.

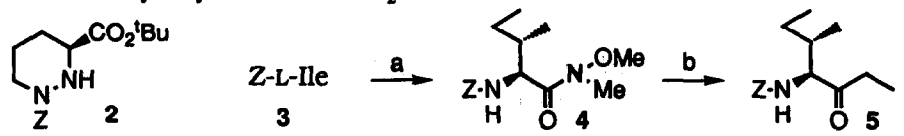
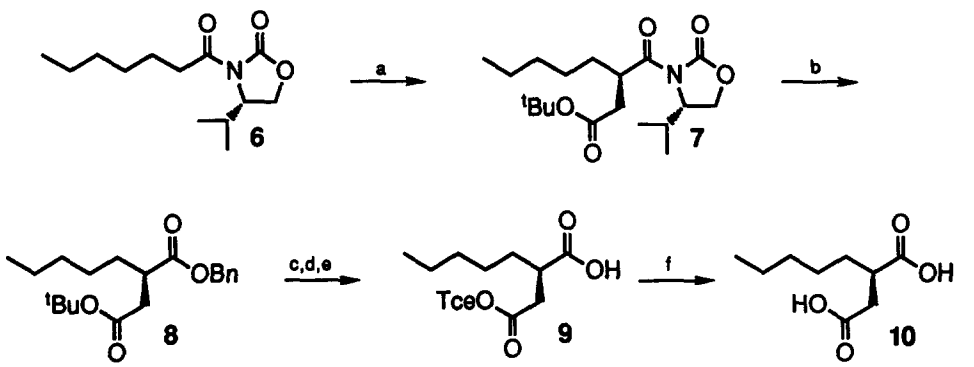


Figure II

Scheme I Reagents and conditions
(a) $MeN^+H_2(OMe)Cl^-$, $^iPr_2NEt$, DCC, DMAP, CH_2Cl_2, 0°C, 97%
(b) EtMgBr, THF, -15 °C - 0 °C, 56%(c.y. 80%)

The last carboxylic acid subunit (**9**) was synthesized by an Evans diastereoselective alkylation[10] as follows (scheme II). Alkylation of the lithium enolate derived from imide **6** with tert-butyl bromoacetate provided the alkylated product **7**(91%) after recrystallization(H_2O-MeOH). Removal of the oxazolidinone with lithium benzyloxide[10] gave **8** in quantitative yield. After hydrolysis , the carboxyl group was esterified with 2,2,2-trichloroethanol using an acid chloride method. The esterification was followed by hydrogenolysis of the benzyl ester with 10% Pd-C which provided 2*R*-trichloroethoxycarbonyl-heptanoic acid (**9**) . The overall yield from **8** to **9** was 94%. The stereochemistry of **9** was unambiguously determined by conversion to known *R*-n-pentyl-succinic acid (**10**) in 47% yield using a zinc reductive removal of the Tce ester[11].
($[\alpha]_D^{26}$ = +27.1° (c: 1.00, EtOH) , lit. $[\alpha]_D^{26}$ = +26.7° (c: 4.77, EtOH)[12])

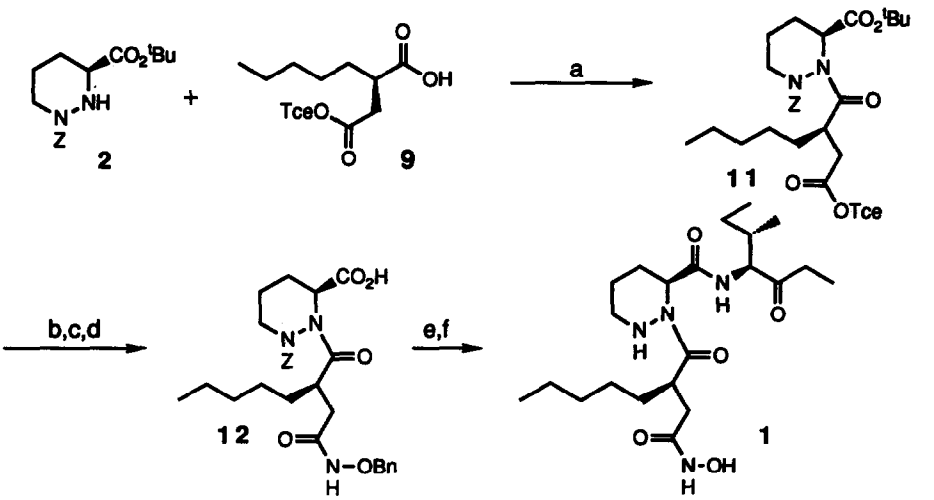


Scheme II Reagents and conditions
(a) (i) LDA, THF, -78 °C (ii) $BrCH_2CO_2tBu$, THF, -78 °C, 91% (b) BnOLi - BnOH, THF, 0 °C,quant.
(c) 4N HCl-1,4 -dioxane, r.t. (d) (i) $(COCl)_2$, benzene, 60 °C then 2,2,2,-TceOH, pyridine, THF, -15 °C
(e) H_2, 10%Pd-C, MeOH, r.t., 94% in 3 steps (f) Zn, 1M NH_4OAc aq., THF, r.t., 47%

The subunits **2**, **5**, and **9** which were prepared by the methods described above were coupled as shown in scheme III to afford **1**.

Protected piperazic acid (**2**) did not react with **9** using DCC or DEPC[13] method, nonetheless coupling of these segments was accomplished using an acid chloride method in the presence of *N*-ethylmorpholine to give coupling product (**11**) in 90% yield. At this stage 3.7% of the diastereomer of **11** was observed, but the diastereomer was separated easily by silica gel chromatography (Hexane : EtOAc = 6 : 1). After separation, the Tce group of **11** was removed using zinc in the presence of ammonium acetate as a buffer[11] to give carboxylic acid in 96 % yield. This compound was treated with *O*-benzylhydroxylamine in the presence of DEPC[13] as a

coupling reagent. This reaction gave tert-butyl ester of **12** in 77% yield, and the tert-butyl ester of the product was hydrolyzed to afford **12** in 96% yield. The obtained carboxylic acid (**12**) and the aminoketone, prepared by catalytic hydrogenation of **5**, were treated with DEPC[13] to afford *N-Z-O*-benzyl-matlystatin B in 80% converting yield. Succeeding hydrogenation in the presence of 10%Pd-C gave **1** [14] in 83% yield.



Scheme III Reagents and conditions
(a) **9**, $(COCl)_2$, benzene, 50 °C then **2**, *N*-ethylmorpholine, THF, -15 °C - r.t., 90% (b) Zn, 1M NH_4OAc aq., THF, r.t., 96%, (c) $H_3N^+OBnCl^-$, DEPC, Et_3N, THF:DMF (10:3), -15 °C, 77% (d) TFA, CH_2Cl_2, r.t., 96% (e) **5**, H_2, 10% Pd-C, MeOH, r.t. then **12**, DEPC, THF, -15 °C - r.t., 60%(c.y. 80%)(f) H_2, 10% Pd-C, MeOH, 83%

Since spectral properties (^{1}H-NMR, IR, MS, $[\alpha]_D$) of synthetic **1** were identical with those of natural compound, the stereochemistly of matlystatin B was unambiguously determined as shown in figure I. Furthermore, synthetic **1** inhibited both the 72 kDa and 92 kDa type IV collagenases and 50% inhibition of the 92 kDa type IV collagenase was achieved at a concentration of 1.43 μM. This value is also identical to that of natural matlystatin B[15].

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References and Notes

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14. **Synthetic matlystatin B:** colorless crystals mp 69-72 °C; $[\alpha]_D^{26}$ = -30.7° (c: 1.01, EtOH); IR (film) 3303, 2932, 1714, 1667, 1626, 1544 cm^{-1}; ^{1}H-NMR (CDCl3, 500 MHz) δ 0.85 (3H, t, J = 6.7 Hz), 0.87 (3H, t, J = 6.1 Hz), 0.92 (3H, d, J = 6.7 Hz), 1.00-2.20(15H, complex), 1.09 (3H, t, J = 7.3 Hz), 2.31 (1H, dd, J = 12.0, 4.4 Hz), 2.49(1H, br t, J = 12.0 Hz), 2.55 (2H, q, J = 7.3 Hz), 2.83 (1H, m), 3.01 (1H, br d, J = 12.8 Hz), 3.95 (1H, m), 4.64.(1H, dd, J = 8.5, 4.9 Hz), 4.75 (1H, br d, J = 12.8 Hz), 5.31 (1H, br s), 7.38 (1H,br s); MS (FAB) m/z $[M+H]^+$ = 441; HRMS (FAB) m/z calcd for $C_{22}H_{41}N_4O_5$ $[M+H]^+$ 441.3077, found 441.3096
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