

Total Syntheses of Miraziridine A and Its Analogues

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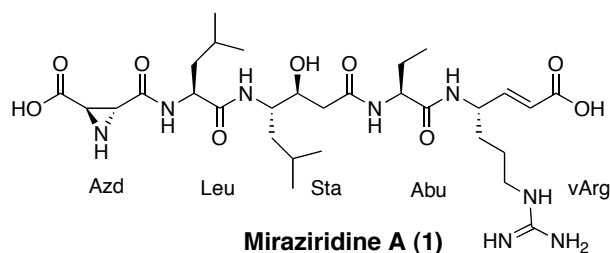
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Introduction

Miraziridine A (**1**) is a pentapeptide isolated from the marine sponge *Theonella aff. mirabilis* by Fusetani *et al.* in 2000 [1]. This natural peptide is composed of (2*R*, 3*R*)-aziridine-2,3-dicarboxylic acid (Azd), L-leucine (Leu), (3*S*, 4*S*)-statine (Sta), (*S*)- α -aminobutyric acid (Abu), and (*S*)-vinyllogous arginine (vArg). Miraziridine A (**1**) is reported to inhibit the action of cathepsin B with a IC₅₀ value of 1.4 mg/mL (2.1 μ M). The first total synthesis of miraziridine A (**1**) was described by N. Schascke in 2004 [2].

In the course of our recent research regarding cysteine protease inhibitors, we have conducted studies on the synthesis of miraziridine A (**1**), since its peptidyl unsaturated carboxylic acid and an aziridine structure [3], supposed to be efficient reactive groups for the thiol functional group of cysteine protease. In the present study, we report syntheses of miraziridine A (**1**) and its analogues. Toward the total synthesis of miraziridine A (**1**), we adopted a route introducing a side chain unprotected vArg-OEt at the late stage of the backbone construction, which makes it possible to adopt a convenient solid phase procedure for the fragment preparation. Truncated three analogues (Table 1) were also synthesized to estimate the major reactive site of miraziridine A (**1**) for cathepsin B.

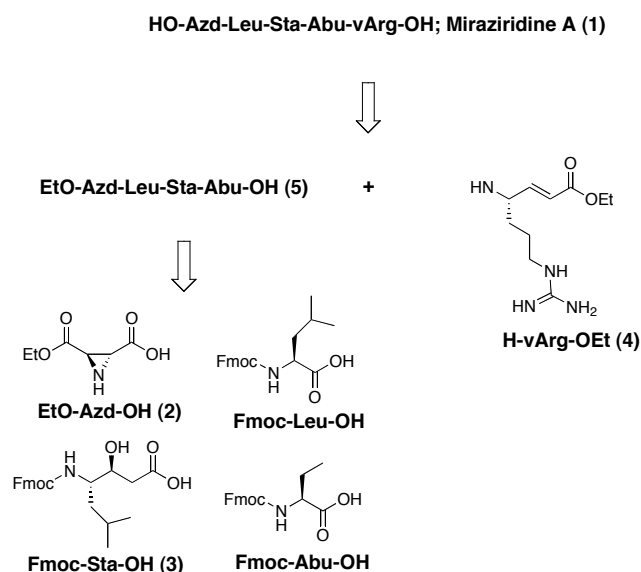


Results and Discussion

The synthetic route for miraziridine A (**1**) is shown in Scheme 1. The backbone structure is constructed by a condensation of N-terminal tetra-peptide derivative (**5**) and C-terminal H-vArg-OEt (**4**). N-terminal tetra-peptide containing EtO-Azd moiety is prepared by conventional Fmoc-based solid phase peptide synthesis (SPPS).

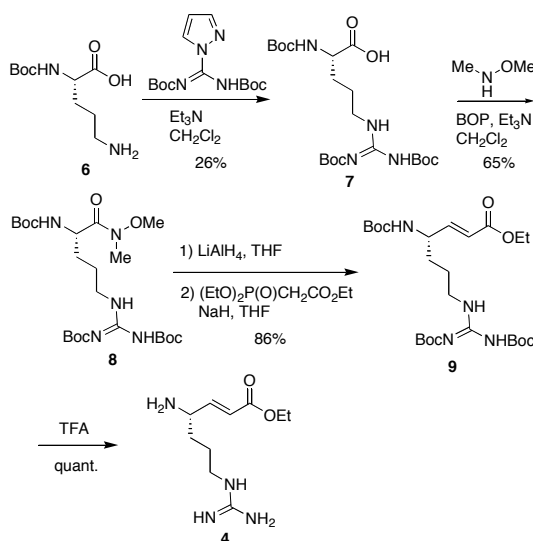
Prior to the total synthesis of miraziridine A (**1**), the necessary three unusual amino acids were synthesized. EtO-Azd-OH (**2**) and Fmoc-Sta-OH (**3**) were prepared as described in our literature [4]. H-vArg-OEt (**4**) was obtained in 5 steps sequence shown in Scheme 2. Boc-Arg(Boc)₂-OH (**7**) prepared from Boc-Orn-OH (**6**)

was converted to Boc-Arg(Boc)₂-N(OMe)Me (**8**) by Weinreb method. The aldehyde obtained by the reduction of **8** was converted to a desired Boc-vArg(Boc)₂-OEt (**9**) by Horner-Emmons-Wadsworth olefination.



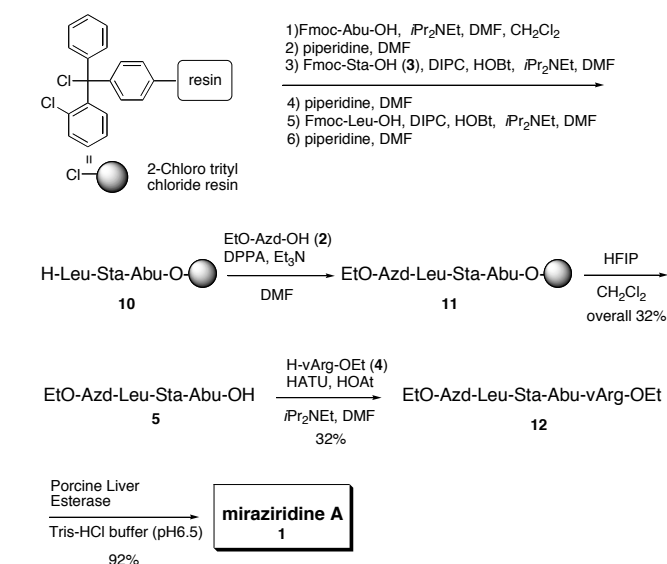
Scheme 1. Synthetic plan for miraziridine A (**1**).

TFA-mediated deprotection of **9** gave H-vArg-OEt (**4**).



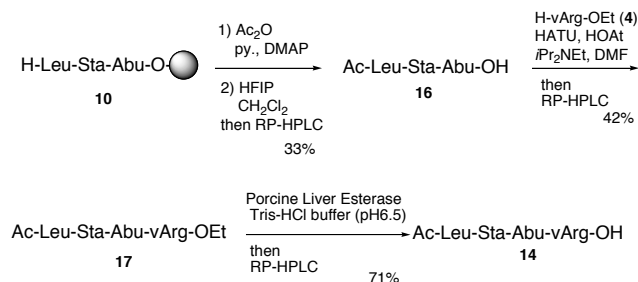
Scheme 2. Synthesis of H-vArg-OEt (**4**)

The synthesis of miraziridine A (**1**) was conducted according to the route shown in Scheme 3. The N-terminal tetrapeptide derivative, EtO-Azd-Leu-Sta-Abu-OH (**5**), was prepared by Fmoc-based SPPS. As a solid support, 2-chlorotrityl chloride resin was selected, because azidiridine dicarboxylic acid is unstable in the presence of strong acid. The deprotection/condensation procedure was repeated for the introduction of protected amino acids to give the tetrapeptide resin (**11**). The resin was treated with HFIP to cleave EtO-Azd-Leu-Sta-Abu-OH (**5**) in 32% overall yield. Coupling of the tetrapeptide (**5**) with H-vArg-OEt (**4**) was achieved by HATU/HOAt to give miraziridine A diethyl ester (**12**) in 32% yield. For the final deprotection, enzyme-assisted hydrolysis of the ester (**12**) in the presence of porcine liver esterase in tris-HCl buffer was conducted to afford miraziridine A (**1**) in 92% yield [5].



Scheme 3. Total synthesis of miraziridine A (1)

Three truncated analogues (**13**), (**14**), (**15**) were also synthesized to estimate the major reactive site of miraziridine A (**1**) for cathepsin B. H-Leu-Sta-Abu-OH (**15**) and Ac-Leu-Sta-Abu-vArg-OH (**14**) were prepared from intermediate tripeptide resin (**10**). Analogue **15** was prepared by the cleavage of **10** with HFIP as above in 45% overall yield. Analogue **14** was prepared according to the route shown in Scheme 4. Condensation of Ac-Leu-Sta-Abu-OH (**16**) and H-vArg-OH (**4**) with



Scheme 4. Synthesis of Ac-Leu-Sta-Abu-vArg-OH (13).

HATU/HOAt afforded tetrapeptide (**17**) in 42% yield. Saponification of the ester in the presence of porcine liver esterase gave Ac-Leu-Sta-Abu-vArg-OH (**14**) in 71% yield. Truncated analogue **13** was also prepared by saponification of **5**.

The inhibitory activity toward cathepsin B was determined with an assay using a Z-Arg-Arg-MCA substrate developed by Hiwasa *et. al.* [6] The inhibitory activities of miraziridine A (**1**) and its truncated analogues, (**13**), (**14**), and (**15**), for cathepsin B were evaluated using the corresponding IC₅₀ and Ki values (Table 1). Comparing IC₅₀ and Ki values of HO-Azd-Leu-Sta-Abu-OH (**13**) and Ac-Leu-Sta-Abu-vArg-OH (**14**), it was strongly suggested that the inhibitory activity is attributable mainly to the aziridine site of miraziridine A (**1**). Though the vinylogous arginine site had a rather weak effect compared with the aziridine site, the inhibitory activity of Ac-Leu-Sta-Abu-vArg-OH (**14**) was about 10 times that of H-Leu-Sta-Abu-OH (**15**) [5].

Table 1. Inhibitory activity for cathepsin B

inhibitor	IC ₅₀	Ki
HO-Azd-Leu-Sta-Abu-vArg-OH (1)	2 μM	3 μM
HO-Azd-Leu-Sta-Abu-OH (13)	9 μM	6.5 μM
Ac-Leu-Sta-Abu-vArg-OH (14)	100 μM	83 μM
H-Leu-Sta-Abu-OH (15)	950 μM	1000 μM

In conclusion, we have achieved a total synthesis of miraziridine A (**1**) via the coupling of a side-chain unprotected H-vArg-OEt (**4**) and Azd-containing tetrapeptide (**5**). The major reaction site of miraziridine A (**1**) for cathepsin B was estimated to be the N-terminal aziridine site. The structure-activity relationship of cathepsin B inhibitors will be discussed in detail.

Acknowledgments

We thank Dr. Nobutaka Fujii and Dr. Shinya Oishi (Kyoto University) for the measurement of mass spectra. This work was supported in part by a grant from Chisso, Co. Ltd., Nissui Research Foundation and a Grant-in-aid for Scientific research from the Japan Society for the Promotion of Science (Grant 16790084 to H.K.).

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