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Miraziridine A: natures blueprint towards protease class-spanning inhibitors

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Abstract—The natural product miraziridine A isolated from the marine sponge *Theonella* aff. *mirabilis* unifies within one molecule three structurally privileged elements: (i) (2*R*,3*R*)-aziridine-2,3-dicarboxylic acid, (ii) (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine), and (iii) (E)-(*S*)-4-amino-7-guanidino-hept-2-enoic acid (vinylogous arginine). The alignment of them realized in the tetrapetide allows for a simultaneous inhibition of the proteolytic activity of trypsin-like serine proteases, papain-like cysteine proteases, and pepsin-like aspartyl proteases. Therefore, this unique compound represents a blueprint for the design of protease class-spanning inhibitors.

Miraziridine A (**1**) (Fig. 1) is a natural product that has been isolated from the marine sponge *Theonella* aff. *mirabilis*.[1] Its structure was elucidated by a combination of chemical and 2D NMR techniques.[1] Among the great variety of protease inhibitors from natural sources, this tetrapeptide seems to be unique because it unifies within one molecule three inhibitory elements: (i) (2*R*,3*R*)-aziridine-2,3-dicarboxylic acid, (ii) (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine), and (iii) (E)-(*S*)-4-amino-7-guanidino-hept-2-enoic acid (vinylogous arginine). Whereas the former ones constitute known cysteine and aspartyl protease inhibitors,[2,3] the latter one possibly inhibits trypsin-like serine proteases. To investigate this hypothesis whether the alignment of the inhibitory elements present in **1** yields an inhibitor blocking proteases belonging to three different classes in a simultaneous fashion, the compound **1** was synthesized and the inhibitory properties assessed.

The retrosynthetic analysis (Fig. 1) was guided by the idea to introduce **2** at a late stage of the synthesis before the final deprotection step since the aziridine moiety is known to be sensitive to nucleophilic ring opening. To realize this approach, the regime of protecting groups within tetrapeptide **3** has to allow for a selective unmasking of the N-terminus. Thus, the Bpoc/Boc-combination[4] was selected that is also compatible with the double bond present in the vinylogous arginine. Correspondingly, three building blocks, i.e., (i) (2*R*,3*R*)-aziridine-2,3-dicarboxylic acid mono ethyl ester (**2**), (ii) Bpoc-Leu-(3*S*,4*S*)-Sta-Abu-OH (**4**), and (iii) H-vArg(Boc)$_2$-OEt (**5**) were selected for the total synthesis of **1**. The half ester **2** was obtained in a six-step sequence

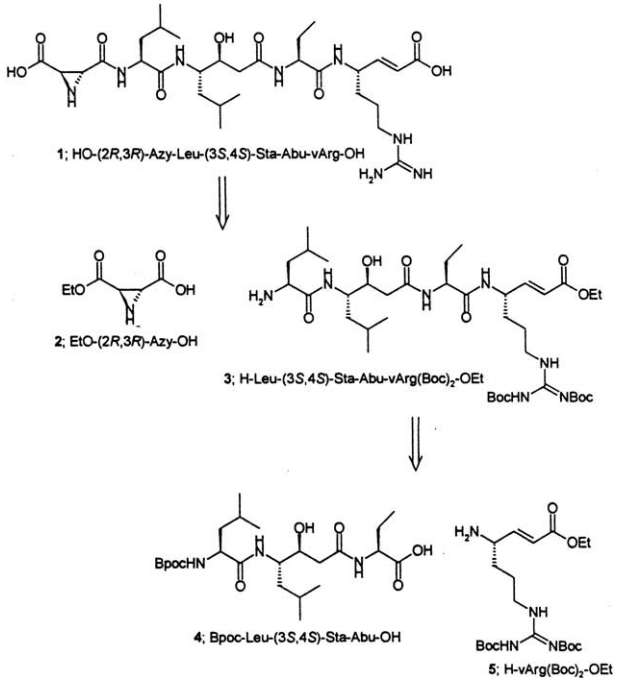

Figure 1. Disconnetion of miraziridine A (**1**) into subunits. The amino acid code for each compound is given below the formula respectively.

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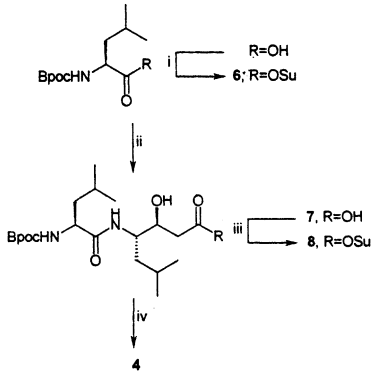
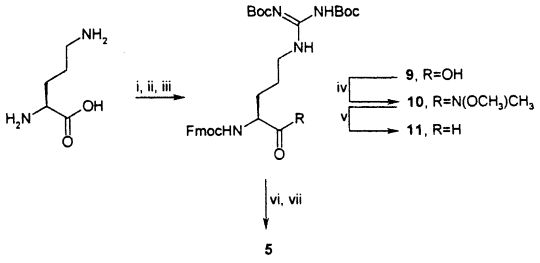
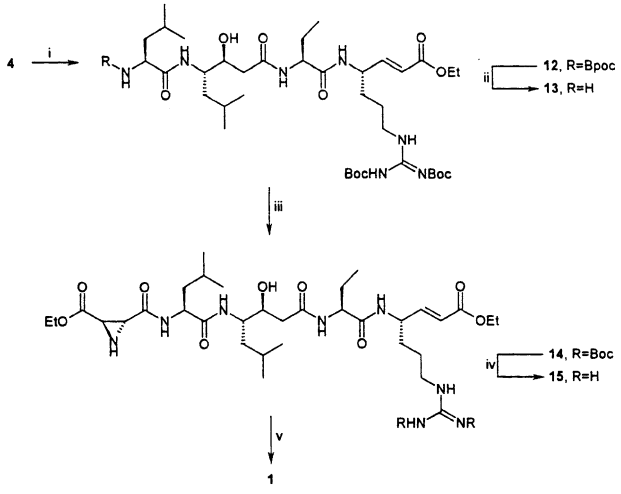


Scheme 1. Synthesis of tripeptide **4**. Reaction conditions: (i) HOSu, DCC, acetonitrile, 0 °C→rt; (ii) H-(3*S*,4*S*)-Sta-OH·HCl, DIEA, $CHCl_3$, rt (77% over two steps); (iii) HOSu, DCC, acetonitrile, 0 °C→rt; (iv) H-Abu-OH, 1N NaOH, dioxane/H_2O (1:1, v/v), rt (79% over two steps); Bpoc = 2-(biphenyl-4-yl)prop-2-yloxycarbonyl; HOSu = *N*-hydroxy-succinimide; Sta = statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; DIEA = *N*-ethyldiisopropylamine; DCC = *N*,*N*′-dicyclohexyl-carbodiimide; Abu = (*S*)-2-aminobutyric acid.



Scheme 2. Synthesis of vinylogous arginine **5**. Reaction conditions: (i) $CuCO_3{\cdot}Cu(OH)_2$, H_2O, reflux (88%); (ii) *N*,*N*′-bis-Boc-1-guanylpyrazole, DIEA, formamide, rt (78%); (iii) EDTA, $NaHCO_3$, Fmoc-OSu, H_2O/acteone (1:1, v/v), rt (84%); (iv) TBTU, HOBt, $HN(CH_3)OCH_3{\cdot}HCl$, DIEA, $CHCl_3$, 0 °C→rt (80%); (v) $LiAlH_4$, Et_2O, 0 °C→rt (67%); (vi) triethyl phosphonoacetate, NaH, THF, −50 °C→rt; (vii) $HNEt_2$/DMF (1:4, v/v) (42% over two steps); Fmoc = 9-fluorenylmethoxycarbonyl; EDTA = ethylenediaminetetraacetic acid; Fmoc-OSu = *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide; TBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; HOBt = 1-hydroxy-1*H*-benzotriazole; THF = tetrahydrofurane; DMF = *N*,*N*-dimethylformamide.

according to the literature starting from (2*S*,3*S*)-tartaric acid diethyl ester.[2,5] The tripeptide **4** was synthesized from Bpoc-Leu-OH[6] using common hydroxysuccinimide ester activation for each coupling step (Scheme 1). The orthogonally protected amino acid aldehyde **11**, selected as starting material for the synthesis of the vinylogous arginine **5**, was obtained via the corresponding Weinreb amide **10** in five steps from L-ornithine (Scheme 2).[7,8] According to a procedure of Bastiaans,[9] the crude aldehyde **11** was immediately converted by a Horner–Emmons–Wadsworth olefination using triethyl phosphonoacetate/NaH at −50 °C→rt to give a mixture of **5** and Fmoc-protected **5**. The latter one is readily deprotected using diethylamine/DMF (1:4, v/v). Scheme 3 shows the assembly of the building blocks. Using EDC/HOAt, the tripeptide **4** was coupled with **5** followed by the cleavage of the Bpoc-group with TFA/$CHCl_3$ (0.5:95.5, v/v). Then, the N-terminus was acylated with **2** by the DPPA-method according to Martichonok et al.[2] yielding protected miraziridine A (**14**). Finally, a two-step deprotection



Scheme 3. Assembly of the building blocks. Reaction conditions: (i) **5**·HCl, DIEA, EDC, HOAt, $CHCl_3$, 0 °C→rt (86%); (ii) TFA/$CHCl_3$ (0.5:99.5, v/v), 0 °C→rt (71%); (iii) **2**, DPPA, NEt_3, DMF (86%); (iv) TFA/$CHCl_3$ (5:95, v/v), 0 °C→rt (46%); (v) Porcine liver esterase, Tris·HCl buffer (50 mM, pH 8.0) (65%); EDC = *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOAt = 1-hydroxy-7-aza-1*H*-benzotriazole; TFA = trifluoroacetic acid; DPPA = diphenyl phosphorazidate; Tris = tris(hydroxymethyl)aminomethane.

Table 1. Inhibitory properties of Miraziridine A

Protease class	Protease	Affinity
Serine protease	Trypsin	6×10^{-5} M
Cysteine protease	Cathepsin L	1.0×10^{6} $M^{-1}s^{-1}$
	Cathepsin B	1.5×10^{4} $M^{-1}s^{-1}$
Aspartyl protease	Pepsin	1.4×10^{-8} M

procedure was performed. First attempts to saponify the ester functions by aqueous LiOH failed. The 'N-terminal' ester function could be removed smoothly with one equivalent LiOH at 0 °C, whereas saponification of the 'C-terminal' one was possible only by prolonged exposure to an excess of base which was accompanied by the nucleophilic ring opening of the aziridine moiety. To circumvent this problem, a enzyme-assisted ester hydrolysis using pig liver esterase was applied to give smoothly **1**. Starting from **4**, miraziridine A (**1**) was obtained in five steps in an overall yield of 16%. The ^1H NMR data of synthetic **1** are in good agreement with those reported for the natural product.[1] The optical rotation however, differs slightly between synthetic ($[\alpha]_D^{20}$ −89.9° (*c* 0.087, MeOH))[10] and natural ($[\alpha]_D^{20}$ −74° (*c* 0.085, MeOH))[1] **1**.

The capability of **1** to inhibit proteases belonging to different classes was assassed using trypsin, cathepsin B, cathepsin L, as well as papain (Table 1). The inhibitory potency of **1** against the serine protease trypsin is in the same order of magnitude as that of benzamidine (60 verus 18 μM, respectively) suggesting that the inhibition is mainly due to an P1/S1 interaction. Moreover, **1** is a potent and irreversible inhibitor of the papain-like cysteine proteases cathepsin B and L. Comparing the second order rate constants with those of HO-(2*R*,3*R*)-Azy-Leu-HN-CH_2-CH_2-$CH(CH_3)_2$ (cathepsin B: $k_2/K_i = 1.7\times10^4$ $M^{-1}s^{-1}$; cathepsin L: $k_2/K_i = 1.2\times10^5$ $M^{-1}s^{-1}$)[2] that structurally resembles the N-terminal

part of **1**, it becomes evident that the inhibitory activity can be attributed mainly to this part of the molecule. Additionally, this is supported from the structural point of view. An inspection of the X-ray structure of E-64 in complex with cathepsin L[11] shows that the N-terminal part of **1** can easily adopt an conformation that is similar to the binding mode of E-64, where the C-terminal part of **1** is fully solvent exposed. Finally, also the aspartyl protease activity of the pepsin is efficiently inhibited by **1** ($K_i = 14$ nM). Interestingly, the central part of the well known aspartyl protease inhibitor pepstatin (Iva-Val-Val-(3*S*,4*S*)-Sta-Ala-(3*S*,4*S*)-Sta-OH; $K_i = 0.046$ nM),[3] that is, -Val-(3*S*,4*S*)-Sta-Ala- is structurally mimicked by **1**. An inspection of the X-ray structure of pepsin in complex with pepstatin[12] revealed that particularly this structural motif is essential for the inhibitory properties of pepstatin. Furthermore, a comparison of the K_i-value of a truncated form of pepstatin (Iva-Val-(3*S*,4*S*)-Sta-Ala-Iaa; $K_i = 3.0$ nM)[13] with that of **1** suggests that the inhibitory activity originates form the tripeptide part-Leu-(3*S*,4*S*)-Sta-Abu-.

In conclusion, aligning three privileged inhibitory elements within one molecule, nature provides us with **1** a blueprint how to design efficiently protease inhibitors of the small molecule type capable to block simultaneously serine, cysteine, and aspartyl protease activity. In the light of pathopysiological conditions like tumor metastasis and invasion that is mediated in a cooperative manner by an ensemble of proteases belonging to different classes, protease class-spanning inhibitors appear as an interesting new approach to possibly interfere in such processes.

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