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Total synthesis of miraziridine A and identification of its major reaction site for cathepsin B

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Abstract—The synthesis of miraziridine A, a pentapeptide derivative isolated from marine sponge, and its truncated analogs has been achieved. To construct the backbone of miraziridine A, a side-chain-unprotected vinylogous arginine was condensed with an aziridine-containing fragment prepared by a conventional solid-phase procedure. An analog lacking the vinylogous arginine site showed comparable inhibitory activity with miraziridine A, whereas an analog lacking the aziridine site showed remarkably weak inhibitory activity for cathepsin B.

1. Introduction

Miraziridine A (**1**)[1] is a pentapeptide isolated from the marine sponge *Theonella aff. mirabilis* by Fusetani et al. in 2000. 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*)-vinylogous arginine (vArg). Miraziridine A (**1**) is reported to inhibit the action of cathepsin B with an IC_{50} value of 1.4 mg/ml (2.1 μM). The first total synthesis of miraziridine A (**1**) was described by Schaschke in 2004.[2] All condensation reactions for the backbone construction were conducted by the classical solution procedure, which is little applicable to the construction of libraries. N-terminal Azd was introduced at the final stage of the backbone construction, since the aziridine moiety is known to be sensitive to nucleophilic ring opening. Thus, a rather laborious process of combining protecting groups, Bpoc/Boc combination, was selected since it is compatible with the double bond present at the C-terminal vArg(Boc)$_2$ moiety. In addition, two deprotection procedures were necessary at the final stage to remove Boc from vArg(Boc)$_2$ using acid and ethyl esters by enzymatic hydrolysis.

In the course of our recent research regarding cysteine protease inhibitors, we have conducted studies on the synthesis of miraziridine A (**1**), which contains a peptidyl unsaturated carboxylic acid and an aziridine structure,[3,4] supposed to be efficient reactive groups for the thiol functional group of cysteine protease. In this paper, we report procedures for synthesizing miraziridine A (**1**) and its analogs in detail. 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. Three truncated analogs (**2**–**4**) (Fig. 1) were also synthesized to estimate the major reactive site of miraziridine A (**1**) for cathepsin B.

2. Results and discussion

The route for the synthesis of miraziridine A (**1**) is shown in Scheme 1. The backbone is constructed by the condensation of a N-terminal tetrapeptide derivative (**5**) and C-terminal H-vArg-OEt (**6**). An N-terminal tetrapeptide containing EtO-Azd moiety is prepared by conventional Fmoc-based solid-phase peptide synthesis (SPPS).

The necessary EtO-Azd-OH (**7**) was prepared starting with (2*S*,3*S*)-tartaric acid diethyl ester.[5,6] To conduct the final deprotection by single-step enzymatic hydrolysis, H-vArg-OEt

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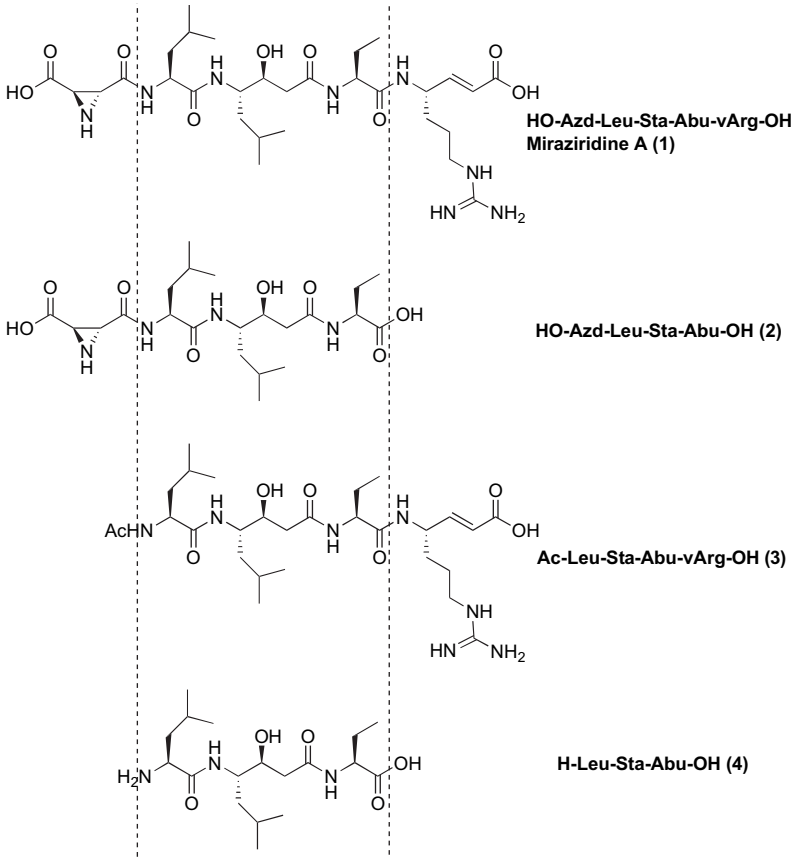
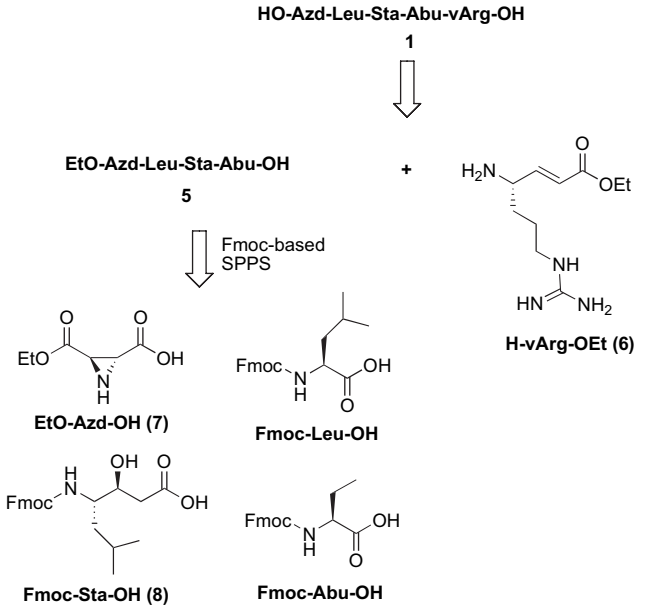


Figure 1. Miraziridine A (**1**) and its analogs (**2**–**4**).

(**6**) containing no protecting groups at the side-chain is employed.

We first tried to synthesize (*S*)-vinylogous arginine starting with Fmoc-Arg(4-methyl-2,3,6-trimethyl-benzenesulfonyl)-OH (Fmoc-Arg(Mtr)-OH, **9a**) or Boc-Arg(Mtr)-OH

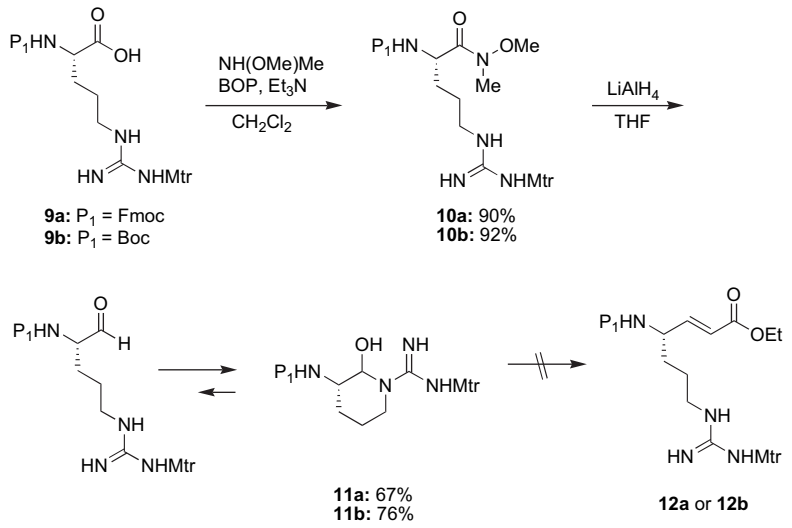


Scheme 1. Synthetic plan for miraziridine A (**1**).

(**9b**). Weinreb amidation of **9a** and **9b** gave **10a** and **10b** in 90 and 92% yield, respectively, without difficulty. Treatment of each product with $LiAlH_4$ afforded a cyclic hemiaminal **11a** or **11b** via an aldehyde in moderate yield. However, the succeeding Horner–Emmons reaction of **11a** and **11b** did not give the desired protected (*S*)-vinylogous arginines **12a** and **12b**, probably due to the unusual stability of the cyclic hemiaminals **11a** and **11b** (Scheme 2).[7]

To suppress the hemiaminal formation, we examined the effects of various protecting groups for the guanidine functional group (Table 1), i.e., Mtr, Boc, Cbz, and Alloc groups. Boc-Arg(Mtr,Boc)-OH (**15a**) was prepared by the *N*-ω-Boc protection of Boc-Arg(Mtr)-OH (**9b**) using $(Boc)_2O$/NaH. Weinreb amidation of **15a** gave Boc-Arg(Mtr,Boc)-N(OMe)Me (**16a**) with 48% yield. Boc-Arg (diCbz)-N(OMe)Me (**16b**) was similarly prepared from commercially available Boc-Arg(diCbz)-OH (**15b**) with 80% yield. Boc-Arg(diAlloc)-N(OMe)Me (**16c**) was obtained with 50% yield from Boc-Arg(diAlloc)-OH (**15c**), which was synthesized by Goodmann's guanidination[8,9] of Boc-Orn-OH (**13**) in the presence of Et_3N (Scheme 3). Other *N*-ω,*N*-ω′-di-protected arginine Weinreb amides, **16d** and **16e**, were similarly prepared using the corresponding Goodmann's reagent, **14d** and **14e**.

Each protected arginine Weinreb amide was then converted to the corresponding vinylogous arginine by treatment with $LiAlH_4$ followed by triethenyl phosphonoacetate/NaH (Table 1). Boc-Arg(Mtr,Boc)-N(OMe)Me (**16a**) gave the



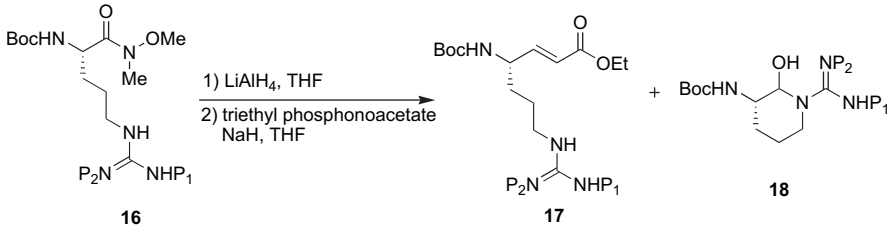
Scheme 2.

desired compound Boc-vArg(Mtr,Boc)-OEt (**17a**) with only 14% yield in two steps (entry 1). Though the Cbz-protected guanidine derivative (**16b**) was converted to Boc-vArg (diCbz)-OEt (**17b**) in good yield (entry 2), these Cbz groups were found to be little deprotected. *N*-ω-Alloc-protected arginine derivatives **16c** and **16d** gave the desired product **17c** and **17d** in low yield due to the instability of the guanidine Alloc group (entry 3 and 4). Boc-Arg(diBoc)-N(OMe)Me (**16e**) gave the corresponding aldehyde derivative as a main product, which was converted to Boc-vArg(diBoc)-OEt (**17e**) with 86% yield (entry 5). TFA-mediated deprotection of Boc groups in **17e** gave the desired H-vArg-OEt (**6**) quantitatively.

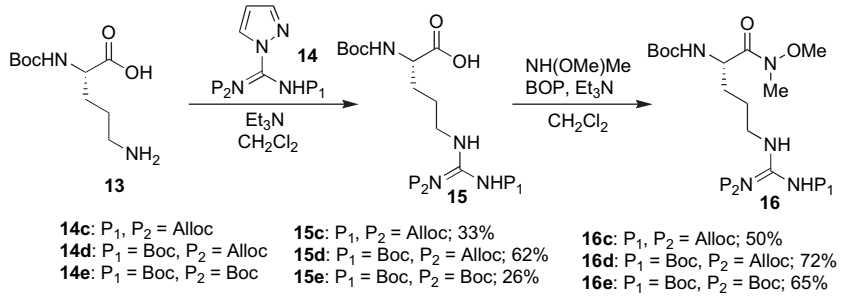
(3*S*,4*S*)-Fmoc-statine (**8**)[10] was prepared by a stereo-inversion of the β-hydroxy group of (3*R*,4*S*)-Boc-statine-OEt (**19**), which was easily prepared by diastereoselective reduction of the corresponding β-keto ester.[11,12] Diastereoselective reduction of the β-keto ester to yield the desired 3*S*-hydroxy group is known to be hard to achieve.[12] We found that a stereo-inversion of the 3*R*-alcohol of **19** can be achieved by using a mesyl leaving group to give the desired oxazolidinone (**20**) in 68% yield. Hydrolysis and Fmoc protection afforded the desired Fmoc-statine (**8**) in 75% yield (Scheme 4).

The synthesis of miraziridine A (**1**) was conducted according to the route shown in Scheme 5. 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 (3*R*,4*R*)-aziridine dicarboxylic acid is unstable in the presence of strong acid. Fmoc-Abu-OH was reacted with the 2-chlorotrityl chloride resin in DMF/CH_2Cl_2 in the presence of $^{i}Pr_2NEt$. The Fmoc group of resulting resin was removed with 20% piperidine/DMF, and Fmoc-Sta-OH (**8**) was condensed by DIPC/HOBt in the presence of $^{i}Pr_2NEt$. The same deprotection/condensation procedure was repeated for the introduction of Fmoc-Leu-OH to afford tripeptide resin (**21**). EtO-Azd-OH (**7**) was then condensed by diphenylphosphoryl azide (DPPA)[13]/Et_3N to give the tetrapeptide resin (**22**). The resin was treated with HFIP (hexafluoroisopropanol)[14]/CH_2Cl_2 (1:4) to cleave the tetrapeptide derivative from the resin. The product showed a single major peak on HPLC and was purified by preparative HPLC to afford a homogeneous tetrapeptide, EtO-Azd-Leu-Sta-Abu-OH (**5**), in 32% overall

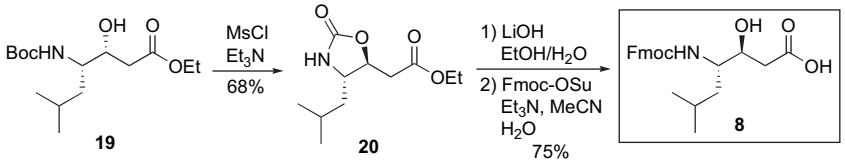
Table 1. Synthesis of vinylogous arginine



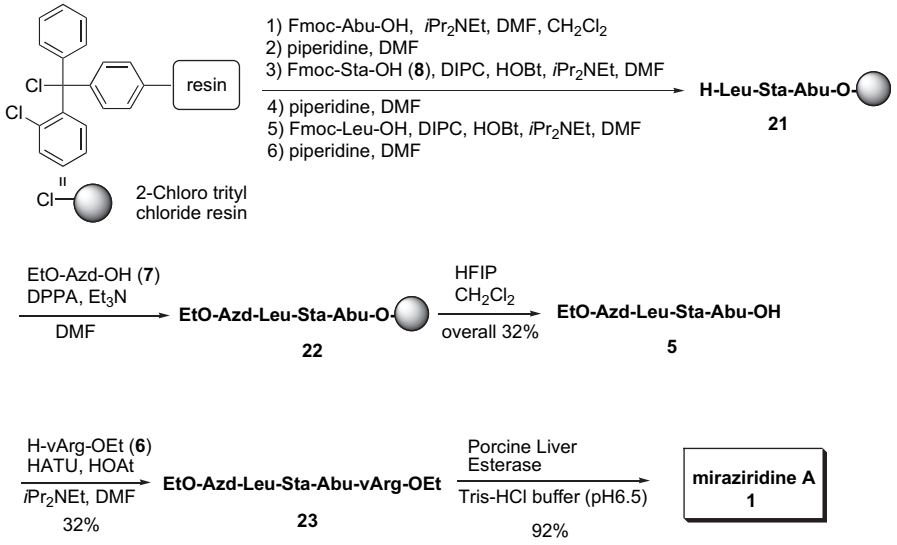
Entry	Substrate	P_1	P_2	Vinyl arginine (%)		Hemiaminal	
				Product	Yield (%)	Product	Yield (%)
1	**16a**	Mtr	Boc	**17a**	14	**18a**	ND
2	**16b**	Cbz	Cbz	**17b**	76	**18b**	ND
3	**16c**	Alloc	Alloc	**17c**	10	**18c**	75
4	**16d**	Boc	Alloc	**17d**	21	**18d**	0
5	**16e**	Boc	Boc	**17e**	86	**18e**	0



Scheme 3. Synthesis of protected arginine Weinreb amides **16c**, **16d**, and **16e**.



Scheme 4. Synthesis of (3*S*,4*S*)-Fmoc-statine (**8**).



Scheme 5. Total synthesis of miraziridine A (**1**).

yield. Coupling of the tetrapeptide (**5**) with H-vArg-OEt (**6**) was achieved by a 5 min reaction using *O*-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)[15]/1-hydroxy-7-azabenzotriazole (HOAt)[16] (Fig. 2a). Subsequent HPLC-based purification afforded the desired pentapeptide derivative, miraziridine A diethyl ester (**23**), in 32% yield. For final deprotection, enzyme-assisted hydrolysis of the ester (**23**) in the presence of porcine liver esterase in Tris/HCl buffer (pH 6.5) was conducted according to a published procedure.[2] By monitoring with HPLC, it was found that the N-terminal ester function could be removed within 15 h, whereas 132 h was necessary for the saponification of C-terminal ethyl ester (Fig. 2b). The crude product was purified by preparative HPLC to afford miraziridine A (**1**) in 92% yield. The spectroscopic data (^{1}H NMR, ^{13}C NMR, IR, FABMS, and TOFMS) on synthetic **1** were identical to those of the natural product reported by Fusetani et al., within the normal error limits.

Using the synthetic intermediates described above, truncated analogs of miraziridine A, i.e., compounds **2**–**4**, were prepared. H-Leu-Sta-Abu-OH (**4**) and Ac-Leu-Sta-Abu-vArg-OH (**3**) were prepared from intermediate tripeptide resin (**21**). Analog **4** was prepared by the cleavage of **21** with HFIP as above in 45% overall yield. Analog **3** was prepared according to the route shown in Scheme 6. After acetylation of **21** by Ac_2O/pyridine/DMAP, Ac-Leu-Sta-Abu-OH (**24**) was cleaved from the resin by HFIP with 33% overall yield. Coupling of the tripeptide (**24**) and H-vArg-OEt (**6**) with HATU/HOAt proceeded within 10 min. Subsequent purification by HPLC afforded a tetrapeptide derivative (**25**) in 42% yield. Enzymatic hydrolysis of the ester (**25**) in the presence of porcine liver esterase in Tris/HCl buffer (pH 6.5) gave Ac-Leu-Sta-Abu-vArg-OH (**3**) in 71% yield. The truncated analog **2**, HO-Azd-Leu-Sta-Abu-OH, was prepared by enzymatic hydrolysis of EtO-Azd-Leu-Sta-Abu-OH (**5**) using porcine liver esterase in 79% yield.

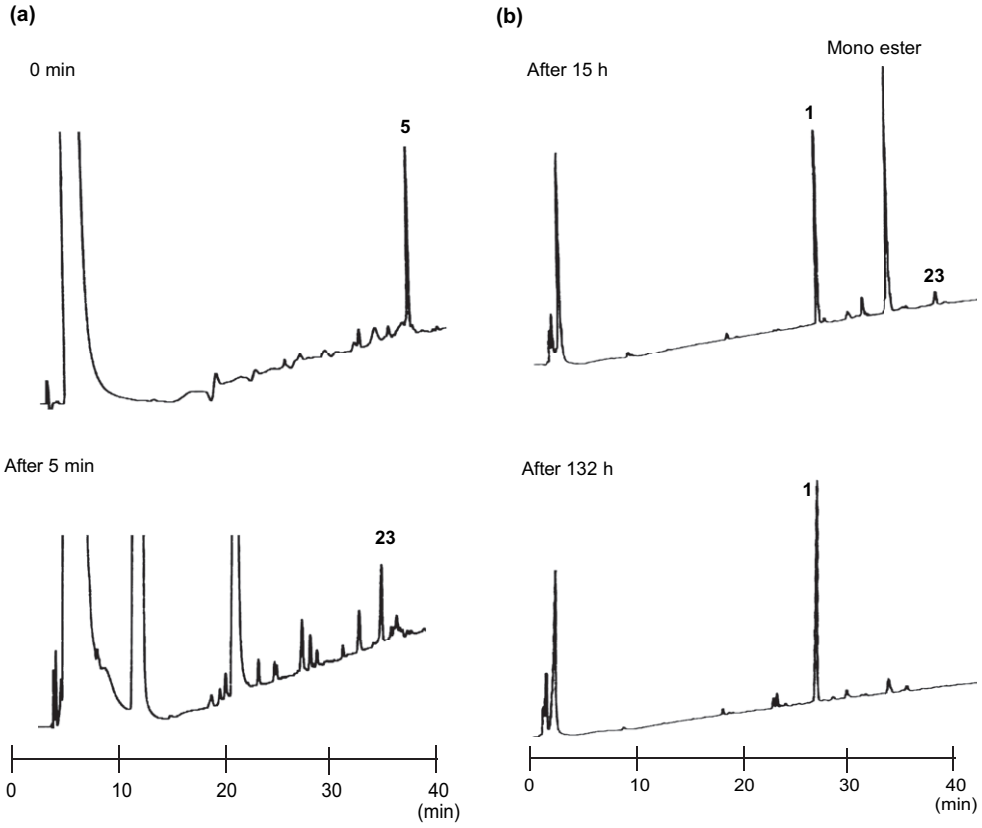
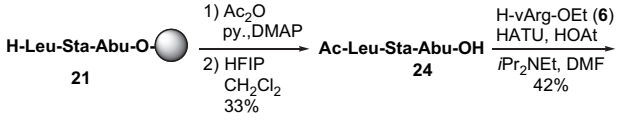
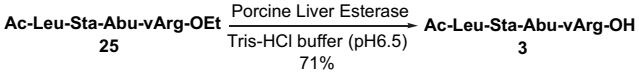


Figure 2. HPLC profiles for the total synthesis of miraziridine A (**1**): (a) coupling of **5** and **6**; (b) saponification of **23** to give miraziridine A (**1**) via mono ester.

The inhibitory activity toward cathepsin B was determined with an assay using a Z-Arg-Arg-MCA substrate developed by Hiwasa et al.[17,18] The inhibitory activities of miraziridine A (**1**) and its truncated analogs (**2**–**4**), were evaluated using





Scheme 6. Synthesis of Ac-Leu-Sta-Abu-vArg-OH (**3**).

the corresponding IC_{50} and Ki[19] values (Table 2). The IC_{50} value of the synthetic miraziridine A (2 μM) was very similar to that reported for the natural product (2.1 μM). Comparing IC_{50} and Ki values of HO-Azd-Leu-Sta-Abu-OH (**2**) and Ac-Leu-Sta-Abu-vArg-OH (**3**), it was strongly suggested that the inhibitory activity is attributable mainly to the aziridine site of miraziridine A (**1**). The results are consistent with the structural data on CA-074 in a complex with cathepsin B, which suggests that the N-terminal part of miraziridine A (**1**) can easily adopt a conformation that is similar to the binding mode of CA-074.[20] 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 (**3**) was about 10 times that of H-Leu-Sta-Abu-OH (**4**). Further investigations are now underway to clarify the inhibitory mechanism.

Table 2. Inhibitory activity of cathepsin B

Inhibitor	IC_{50} (μM)	Ki (μM)
HO-Azd-Leu-Sta-Abu-vArg-OH (**1**)	2	3
HO-Azd-Leu-Sta-Abu-OH (**2**)	9	6.5
H-Leu-Sta-Abu-vArg-OH (**3**)	100	83
H-Leu-Sta-Abu-OH (**4**)	950	1000

In conclusion, we achieved the total synthesis of miraziridine A (**1**) via the coupling of a side-chain-unprotected vArg-OEt (**6**) 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 reported in due course.

3. Experimental

3.1. General

Amino acids and coupling reagents were purchased from Novabiochem or Watanabe Chemical Industries. All manipulations were conducted under an inert atmosphere (N_2). All solvents were of reagent grade. THF was distilled from sodium and benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. All commercial reagents were of the highest purity

available. Analytical TLC was performed on silica gel (60 F-254, plates 0.25 mm). Column chromatography was carried out on Wakogel 60 (particle size, 0.063–0.200 mm). Analytical and preparative HPLC was performed on a HITACHI ELITE LaChrom system (OD, 220 nm) equipped with the Nacalai tesque COSMOSIL 5C18-AR-II (4.6×150 mm or 10×250 mm). ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectograms were recorded on a Bruker AM-300. Chemical shifts are expressed in parts per million relative to TMS (0 ppm) or $CHCl_3$ (7.28 ppm for ^{1}H and 77.0 ppm for ^{13}C) or MeOH (3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C). IR spectograms were obtained on a HORIBA FREEXACT-II FT-710 spectrometer. Optical rotations were recorded on a HORIBA SEPA-200 or SEPA-300 polarimeter at the sodium D line. Low-resolution mass spectra (LRMS) and high-resolution mass spectra (HRMS) were obtained on either a JOEL JMS-HX-211A or a JMS-HX-110A (EI or FAB) and Bruker Autoflex-II (MALDI-TOF). Fluorescent intensity was obtained on a Shimadzu RF-1500 under a xenon lamp (Ex 380 nm, Em 460 nm).

3.2. Synthetic chemistry

3.2.1. *N*-α-Fmoc-*N*′-ω′-Mtr-arginine-*N*,*O*-dimethylhydroxyamine (10a). To a solution of Fmoc-Arg(Mtr)-OH (5.00 g, 8.21 mmol) in CH_2Cl_2 (50 ml) were added NH(OMe)Me-HCl (1.20 g, 12.3 mmol), BOP (5.44 g, 12.3 mmol), HOBt (1.66 g, 12.3 mmol), and *N*,*N*-diisopropylethyl amine (4.29 ml, 24.6 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was poured into H_2O and extracted with ethyl acetate. Drying over $MgSO_4$ and subsequent evaporating gave crude Weinreb amide **10a**, which was chromatographed over silica gel (CH_3Cl/MeOH=9:1) to give **10a** (4.81 g, 7.39 mmol, 90%) as a colorless oil. $[\alpha]_D^{22}$ −1.8 (*c* 4.0, $CHCl_3$). IR (film) ν max cm^{-1}: 3421, 2942, 1716, 1637, 1120, 838, 755. ^{1}H NMR ($CDCl_3$) δ: 1.61 (3H, m), 1.74 (2H, m), 2.10 (3H, s), 2.59 (1H, s), 2.61 (3H, s), 2.69 (3H, s), 3.18 (3H, s), 3.30 (1H, m), 3.71 (2.5H, s), 3.77 (0.5H, s), 3.80 (3H, s), 4.17 (1H, t, *J*=6.8 Hz), 4.37 (2H, m), 4.70 (1H, br s), 5.77 (1H, d, *J*=8.1 Hz), 6.03 (2H, s), 6.50 (1H, s), 7.28 (2H, t, *J*=6.1 Hz), 7.36 (2H, t, *J*=7.6 Hz), 7.56 (2H, t, *J*=6.1 Hz), 7.76 (2H, d, *J*=7.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 11.9, 18.3, 24.1, 36.6, 36.7, 40.9, 47.1, 55.4, 67.1, 77.2, 111.7, 119.9, 124.7, 125.1, 127.1, 127.69, 127.73, 133.7, 136.5, 138.5, 141.2, 141.2, 143.6, 143.8, 156.6, 158.3, 183.9, 220.2. MALDI-TOFMS (M+H)$^+$ calcd for $C_{33}H_{42}N_5O_7S_1$: 652.273, found: 652.342.

N-α-Boc-*N*′-ω′-Mtr-arginine-*N*,*O*-dimethylhydroxyamine (**10b**): $[\alpha]_D^{28}$ −3.9 (*c* 0.3, $CHCl_3$). IR (film) ν max cm^{-1}: 3431, 3321, 2929, 1709, 1655, 1558, 1464, 1300, 985, 748. ^{1}H NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49–1.69 (4H, m), 2.12 (3H, s), 2.64 (3H, s), 2.70 (3H, s), 3.16 (3H, s), 3.19 (2H, m), 3.72 (3H, s), 3.82 (3H, s), 4.59 (1H, br s), 5.27 (1H, m), 6.52 (1H, s), 6.62 (2H, br s), 6.72 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 11.5, 17.9, 23.7, 25.2, 27.9, 36.4, 40.1, 55.0, 61.1, 79.1, 111.2, 124.0, 134.1, 135.9, 138.1, 155.2, 156.5, 157.7, 172.4. MALDI-TOFMS (M+H)$^+$ calcd for $C_{23}H_{40}N_5O_7S_1$: 529.257, found: 529.296.

3.2.2. *N*-α-Fmoc-*N*-ω-Mtr-hemiaminal (11a). To a solution of amide (**10a**) (52 mg, 80 mmol) in THF (3 ml) was added $LiAlH_4$ (15 mg, 392 mmol) at 0 °C and the mixture was stirred for 30 min at room temperature. To the mixture were added H_2O (25 ml) and 1 M NaOH (50 ml), and filtered on a Celite pad. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The product was purified with silica gel column chromatography ($CHCl_3$/MeOH=98:2) to give hemiaminal **11a** (32 mg, 54 mmol, 67%) as a colorless oil. $[\alpha]_D^{27}$ −1.8 (*c* 0.4, $CHCl_3$). IR (film) ν max cm^{-1}: 3423, 3338, 3016, 2939, 2854, 1716, 1635, 1252, 1120, 756. ^{1}H NMR ($CDCl_3$) δ: 1.60–1.85 (4H, m), 2.11 (3H, s), 2.54 (2.1H, s), 2.58 (0.4H, s), 2.60 (0.5H, s), 2.63 (2.1H, s), 2.67 (0.9H, s), 3.13–3.31 (1.4H, m), 3.48–3.58 (0.6H, m), 3.81 (3H, s), 3.63–3.76 (2H, m), 4.17 (1H, m), 4.33 (0.3H, m), 4.42 (1H, m), 4.76 (0.7H, m), 5.17 (0.3H, d, *J*=9.3 Hz), 5.55 (0.7H, d, *J*=9.9 Hz), 5.66 (0.3H, br s), 5.71 (0.7H, br s), 6.47 (1H, s), 6.55 (2H, br s), 7.28 (2H, m), 7.35 (2H, m), 7.52–7.76 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 11.9, 18.3, 23.5, 24.0, 24.4, 29.6, 50.7, 55.4, 70.0, 75.6, 80.8, 93.6, 111.8, 120.0, 120.1, 124.4, 125.0, 127.1, 127.7, 128.0, 129.6, 132.9, 136.8, 138.6, 139.7, 141.3, 143.9, 145.9, 156.1, 158.7. MALDI-TOFMS (M+H)$^+$ calcd for $C_{31}H_{37}N_4O_6S_1$: 593.243, found: 593.083.

N-α-Boc-*N*-ω-Mtr-hemiaminal (**11b**): $[\alpha]_D^{28}$ +13.4 (*c* 0.4, $CHCl_3$). IR (film) ν max cm^{-1}: 3423, 3338, 3006, 2976, 2939, 1697, 1635, 1518, 1122, 987, 756. ^{1}H NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.51–1.77 (4H, m), 2.14 (3H, m), 2.58 (3H, s), 2.67 (3H, s), 3.13 (1H, m), 3.45–3.72 (2H, m), 3.83 (3H, s), 4.67 (1H, br s), 4.98 (1H, d, *J*=9.3 Hz), 5.58 (1H, t, *J*=3.6 Hz), 6.54 (1H, s), 6.63 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 11.9, 18.2, 23.6, 24.0, 24.5, 28.3, 36.7, 39.2, 50.2, 55.4, 58.3, 75.9, 79.6, 111.8, 124.9, 133.1, 136.7, 138.5, 155.2, 156.0, 158.6. MALDI-TOFMS (M+H)$^+$ calcd for $C_{21}H_{34}N_4O_6S_1Na_1$: 493.210, found: 493.192.

3.2.3. 1*H*-Pyrazole-*N*-Alloc-1-carboxamidine. To a solution of 1*H*-pyrazole-1-carboxamidine hydrochloride (7.80 g, 53.2 mmol) in CH_2Cl_2 (100 ml) and DMF (170 ml) were added DIEA (11.0 ml, 63.8 mmol) and AllocCl (6.80 ml, 63.8 mmol) and the mixture was stirred for 4 h at room temperature. To the mixture were added satd aq NaCl and ether, and the organic layer was evaporated in vacuo to give 1*H*-pyrazole-*N*-Alloc-1-carboxamidine (10.3 g, 53.1 mmol, 99%) as a colorless oil. IR (film) ν max cm^{-1}: 3448, 3307, 3153, 3136, 2956, 2921, 1774, 1755, 1668, 1635, 1533, 1508, 1429, 1419, 1392, 1371, 1338, 1284, 1265, 1213, 1188, 1178, 1036, 937, 764. ^{1}H NMR ($CDCl_3$) δ: 4.96 (2H, tt, *J*=6.0, 1.2 Hz), 5.39 (1H, dq, *J*=10.5, 6.0 Hz), 5.50 (1H, dq, *J*=17.1, 1.2 Hz), 6.10 (1H, ddt, *J*=17.1, 10.5, 6.0 Hz), 6.45 (1H, dd, *J*=2.7, 1.5 Hz), 7.77 (1H, d, *J*=0.9 Hz), 8.19 (1H, dd, *J*=2.7, 0.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 65.5, 68.8, 109.2, 120.4, 130.7, 130.9, 144.5, 162.3. HRFABMS (M+H)$^+$ calcd for $C_{31}H_{15}N_6O_4$: 195.0882, found: 195.0886.

3.2.4. 1*H*-Pyrazole-*N*,*N*′-di-Alloc-1-carboxamidine (14c). To a solution of 1*H*-pyrazole-*N*-Alloc-1-carboxamidine (10.3 g, 53.2 mmol) in THF (160 ml) were added NaH (4.50 g, 186 mmol) and AllocCl (19.8 ml, 186 mmol) at 0 °C. The mixture was stirred for 6 h at room temperature. To the mixture were added H_2O and ether. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product

was purified with silica gel column chromatography (hexane/AcOEt=2:1) to give 1*H*-pyrazole-*N*,*N*′-diAlloc-1-carboxamidine (**14c**) (8.90 g, 32.0 mmol, 60%) as a colorless oil. IR (film) ν max cm^{-1}: 3153, 3136, 3089, 2951, 1819, 1784, 1747, 1685, 1649, 1435, 1398, 1356, 1261, 1205, 1180, 1115, 935, 766. ^1H NMR ($CDCl_3$) δ: 4.69–4.75 (4H, m), 5.19–5.44 (4H, m), 5.75–6.03 (2H, m), 6.51 (1H, dd, *J*=1.8, 1.2 Hz), 7.54 (1H, dd, *J*=1.5, 0.6 Hz), 8.30 (1H, d, *J*=3.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 67.9, 68.3, 110.9, 119.1, 119.6, 129.6, 130.5, 131.2, 144.7, 148.9, 157.2. HRFABMS $(M+H)^+$ calcd for $C_{12}H_{15}N_4O_4$: 279.1093, found: 279.1089.

3.2.5. 1*H*-Pyrazole-*N*-Boc-*N*′-Alloc-1-carboxamidine (14d). To a solution of 1*H*-pyrazole-1-carboxamidine (666 mg, 4.54 mmol) in CH_2Cl_2 (8.50 ml) and DMF (14.2 ml) were added DIEA (949 µl, 5.45 mmol) and Boc_2O (1.19 g, 5.45 mmol) at 0 °C. The mixture was stirred for 5 h at room temperature. To a mixture were added satd aq NaCl and ether. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified with column chromatography (hexane/AcOEt=4:1) to give 1*H*-pyrazole-*N*-Boc-1-carboxamidine (895 mg, 4.26 mmol) as a colorless oil. IR (film) ν max cm^{-1}: 3436, 3319, 3145, 3126, 2966, 1658, 1610, 1363, 1313, 1173, 980, 760. To a solution of 1*H*-pyrazole-*N*-Boc-1-carboxamidine (895 mg, 4.26 mmol) in THF (21.0 ml) were added NaH (358 mg, 14.9 mmol) and AllocCl (1.58 ml, 14.9 mmol) at 0 °C. The mixture was stirred for 11 h at room temperature. To the mixture were added satd aq NH_4Cl and AcOEt. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified with silica gel column chromatography (hexane/AcOEt=4:1) to give 1*H*-pyrazole-*N*-Boc-*N*′-Alloc-1-carboxamidine (**14d**) (1.10 g, 3.73 mmol, 82%) as a colorless oil. IR (film) ν max cm^{-1}: 3149, 3140, 2981, 2945, 1817, 1780, 1751, 1685, 1431, 1392, 1371, 1338, 1265, 1180, 1149, 1117, 1036, 937, 766. ^1H NMR ($CDCl_3$) δ: 1.40 (4H, s), 1.53 (4H, s), 1.60 (1H, s), 4.68–4.75 (1.75H, m), 4.95–4.98 (0.25H, m), 5.20–5.50 (2H, m), 5.75–6.13 (1H, m), 6.45–6.51 (1H, m), 7.73 (0.75H, dd, *J*=8.7, 0.9 Hz), 7.76 (0.25H, dd, *J*=9.3, 0.9 Hz), 8.19 (0.25H, d, *J*=3.0 Hz), 8.28 (0.75H, d, *J*=2.4 Hz). ^{13}C NMR ($CDCl_3$) δ: 27.5, 27.8, 27.9, 68.8, 109.2, 118.9, 120.3, 129.3, 130.7, 130.9, 144.5, 149.1, 157.4. HRFABMS $(M+H)^+$ calcd for $C_{13}H_{19}N_4O_4$: 295.1406, found: 295.1413.

3.2.6. *N*-α,*N*′-ω′-di-Boc-*N*-ω-Mtr-arginine (15a). To a solution of Boc-Arg(Mtr)-OH (256 mg, 437 µmol) in CH_2Cl_2 (5 ml) were added NaH (88 mg, 2.19 mmol) and $(Boc)_2O$ (191 mg, 874 µmol) and the mixture was stirred for 18 h at room temperature. To the mixture were added 1 M HCl and ether. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified with silica gel column chromatography ($CHCl_3$) to give *N*-α-*N*′-ω′-di-Boc-*N*-ω-Mtr-arginine (**15a**) (154 mg, 262 µmol, 66%) as a colorless oil. $[\alpha]_D^{27}$ +13.1 (*c* 1.4, $CHCl_3$). IR (film) ν max cm^{-1}: 3394, 3329, 3286, 2927, 2937, 1716, 1620, 1564, 1523, 1369, 1275, 1151, 1122, 912, 808, 733. ^1H NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.48 (9H, s), 1.55–1.95 (4H, m), 2.12 (3H, s), 2.60 (1H, s), 2.61 (2H, s), 2.68 (3H, s), 3.29 (1.32H m), 3.74 (0.68H, m), 3.81 (3H, s), 4.16 (0.68H, m), 4.26 (1.32H, m), 5.24 (0.68H, m), 6.40 (0.32H, m), 6.54 (1H, s), 7.56 (1H, br s), 7.83 (0.32H, br s), 8.34 (0.68H, br s), 9.13 (0.32H, s), 9.78 (0.68H, s). ^{13}C NMR ($CDCl_3$) δ: 11.8, 14.9, 18.0, 18.1, 18.2, 23.77, 23.83, 24.4, 24.7, 27.4, 27.8, 28.1, 29.4, 29.6, 30.7, 40.6, 44.9, 52.8, 55.3, 60.9, 65.7, 80.0, 83.9, 84.7, 111.6, 120.0, 124.8, 132.2, 132.5, 136.6, 136.8, 138.5, 150.3, 152.4, 154.4, 154.9, 155.5, 158.6, 175.4. HRFABMS $(M+H)^+$ calcd for $C_{26}H_{43}N_9O_9S$: 587.2751, found: 587.2721.

3.2.7. *N*-α-Boc-*N*-ω,*N*′-ω′-di-Alloc-arginine (15c). To a solution of Boc-Orn-OH (2.70 g, 11.6 mmol) in CH_2Cl_2 (58.0 ml) were added Et_3N (1.60 ml, 11.6 mmol) and 1*H*-pyrazole-*N*,*N*′-di-Alloc-1-carboxamidine (**14c**) (3.20 g, 11.6 mmol) and the mixture was stirred for 18 h at room temperature. To the mixture were added 1 M HCl and ether. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified with silica gel column chromatography ($CHCl_3$) to give *N*-α-Boc-*N*-ω,*N*′-ω′-di-Alloc-arginine (**15c**) (1.70 g, 3.80 mmol, 33%) as a colorless oil. $[\alpha]_D^{20}$ +15.0 (*c* 1.1, $CHCl_3$). IR (film) ν max cm^{-1}: 3336, 3140, 2978, 2935, 1732, 1716, 1637, 1626, 1417, 1369, 1323, 1259, 1211, 1144, 1051, 769. ^1H NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.71 (3H, s), 1.94 (1H, s), 3.47 (2H, d, *J*=3.3 Hz), 4.35 (1H, s), 4.62 (4H, d, *J*=5.7 Hz), 5.18–5.39 (4H, m), 5.83–6.03 (2H, m), 7.62 (1H, s), 8.36 (1H, s), 11.20 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 25.0, 28.3, 29.7, 40.5, 53.0, 66.2, 66.9, 80.0, 105.5, 117.8, 119.4, 130.9, 132.9, 132.9, 153.7, 156.0, 163.5, 175.8. HRFABMS $(M+H)^+$ calcd for $C_{19}H_{31}N_4O_8$: 443.2142, found: 443.2148.

3.2.8. *N*-α,*N*-ω-di-Boc-*N*′-ω′-Alloc-arginine (15d). To a solution of Boc-Orn-OH (1.92 g, 8.29 mmol) in CH_2Cl_2 (41.4 ml) were added Et_3N (1.16 mmol) and 1*H*-pyrazole-*N*-Boc-*N*′-Alloc-1-carboxamidine (**14d**) (2.44 g, 8.29 mmol) and the mixture was stirred for 15 h at room temperature. To the mixture was added 1 M HCl and ether. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified with silica gel column chromatography ($CHCl_3$) to give diBoc-Alloc-arginine (**15d**) (2.36 g, 5.16 mmol, 62%) as a colorless oil. $[\alpha]_D^{20}$ +17.0 (*c* 1.2, $CHCl_3$). IR (film) ν max cm^{-1}: 3329, 3145, 2979, 2935, 1718, 1639, 1621, 1417, 1369, 1269, 1255, 1157, 1142, 1053, 754. ^1H NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.48 (9H, s), 1.71 (3H, s), 1.93 (1H, s), 3.45 (2H, d, *J*=4.8 Hz), 4.34 (1H, s), 4.59 (2H, dd, *J*=5.7, 1.2 Hz), 5.19–5.39 (2H, m), 5.90–6.03 (1H, m), 8.48 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 25.1, 28.0, 28.1, 28.3, 29.7, 40.4, 53.0, 66.1, 66.9, 117.6, 131.0, 132.9, 153.0, 153.7, 155.7, 156.6, 163.4, 175.8. HRFABMS $(M+H)^+$ calcd for $C_{20}H_{35}N_4O_8$: 459.2455, found: 459.2462.

3.2.9. *N*-α,*N*-ω,*N*′-ω′-tri-Boc-L-arginine (15e). Procedure A: to a solution of Boc-Arg-OH (12.0 g, 36.7 mmol) in 2 N NaOH (210 ml) and 1,4-dioxane (180 ml) at 0 °C was added Boc_2O (16.5 g, 75.6 mmol) and the mixture was stirred for 3 h. To the mixture were added Boc_2O (8.20 g, 37.6 mmol) and 1,4-dioxane (90 ml). After 39 h, 3 M HCl (pH=7) and AcOEt were added. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified with silica gel column chromatography ($CHCl_3$/MeOH=99:1) to give *N*-α,*N*-ω,*N*′-ω′-tri-Boc-L-arginine (**15e**) (4.99 g, 10.5 mmol, 28%) as a colorless oil.

Procedure B: to a solution of Boc-Orn-OH (2.00 g, 8.81 mmol) in CH_2Cl_2 (20.0 ml) were added Et_3N

(1.20 mmol, 8.80 mmol) and 1*H*-pyrazole-*N*,*N*′-di-Boc-1-carboxamidine (**14e**)[8] (2.73 g, 8.81 mmol) and the mixture was stirred for 20 h at room temperature. To the mixture were added 1 M HCl and ether. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The product was purified with silica gel column chromatography ($CHCl_3$/MeOH=100:1) to give *N*-α,*N*-ω,*N*′-ω′-tri-Boc-L-arginine (**15e**) (1.08 g, 2.29 mmol, 26%) as a colorless oil. $[\alpha]_D^{27}$ +18.0 (*c* 1.06, $CHCl_3$). IR (film) n max cm^{-1}: 3327, 3286, 3153, 2979, 2933, 1720, 1643, 1616, 1415, 1367, 1333, 1159, 1138, 1053, 1026, 750. ^1H NMR ($CDCl_3$) *δ*: 1.45 (9H, s), 1.48 (9H, s), 1.49 (9H, s), 1.69 (3H, s), 1.90 (1H, s), 3.48 (2H, s), 4.32 (1H, s), 5.33 (1H, d, *J*=7.2 Hz). ^{13}C NMR ($CDCl_3$) *δ*: 25.3, 28.1, 28.3, 28.4, 29.9, 40.5, 53.2, 79.7, 80.0, 83.4, 153.3, 156.0, 156.4, 163.1, 175.3. HRFABMS $(M+H)^+$ calcd for $C_{21}H_{39}N_4O_8$: 475.2768, found: 475.2775.

3.2.10. *N*-α,*N*′-ω′-di-Boc-*N*-ω-Mtr-arginine-*N*,*O*-dimethylhydroxyamine (16a). To a solution of *N*-α-Boc-*N*-ω,*N*′-ω′-diCbz-arginine (**15a**) (154 mg, 262 mmol) in CH_2Cl_2 (5 ml) were added Et_3N (68 ml, 524 mmol), BOP (151 mg, 341 mmol), and NH(OMe)Me-HCl (33 mg, 341 mmol) and then the mixture was stirred for 3 h at room temperature. To the mixture were added 1 M HCl (pH=5) and ether. The organic layer was washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and evaporated in vacuo. The product was purified with silica gel column chromatography (hexane/AcOEt=2:1) to give amide (**16a**) (109 mg, 173 mmol, 66%) as a colorless oil. $[\alpha]_D^{27}$ +5.8 (*c* 0.85, $CHCl_3$). IR (film) *ν* max cm^{-1}: 3405, 3317, 3294, 1712, 1660, 1618, 1460, 1369, 1279, 1151, 1122, 916, 732, 675. ^1H NMR ($CDCl_3$) *δ*: 1.42 (3.6H, s), 1.43 (5.4H, s), 1.49 (5.4H, s), 1.50 (3.6H, s), 1.52–1.80 (4H, m), 2.14 (3H, s), 2.62 (1.2H, s), 2.63 (1.8H, s), 2.70 (3H, s), 3.16 (1.2H, s), 3.18 (1.8H, s), 3.29 (1H, q, *J*=6.6 Hz), 3.72 (1.2H, s), 3.74 (1.8H, s), 3.84 (3H, s), 4.62 (1H, m), 5.18 (1H, t, *J*=9.0 Hz), 6.55 (1H, s), 7.85 (0.4H, s), 8.31 (0.6H, s), 9.10 (0.4H, s), 9.80 (0.6H, s). ^{13}C NMR ($CDCl_3$) *δ*: 11.9, 18.3, 23.9, 24.5, 24.8, 27.88, 27.93, 28.3, 30.1, 30.2, 32.0, 40.1, 45.2, 49.9, 50.2, 55.4, 61.5, 79.5, 79.6, 83.9, 84.5, 111.6, 124.8, 132.9, 133.1, 136.6, 136.9, 138.7, 150.3, 152.5, 154.6, 154.9, 155.4, 158.6, 158.6, 172.5. HRFABMS $(M+H)^+$ calcd for $C_{28}H_{48}N_5O_9S_1$: 630.3173, found: 690.3204.

The following compounds (**16b–16e**) were prepared in a manner similar to **16a**.

N-α-Boc-*N*-ω,*N*′-ω′-di-Cbz-arginine-*N*,*O*-dimethylhydroxyamine (**16b**): $[\alpha]_D^{24}$ +0.95 (*c* 0.55, $CHCl_3$). IR (film) *ν* max cm^{-1}: 3389, 3292, 3059, 3032, 2972, 2934, 2370, 2322, 1716, 1662, 1610, 1510, 1456, 1379, 1255, 1199, 1170, 1097, 1055, 1005. ^1H NMR ($CDCl_3$) *δ*: 1.42 (9H, s), 1.52 (2H, s), 1.68 (2H, s), 3.13 (3H, s), 3.67 (3H, s), 3.98 (2H, t, *J*=6.6 Hz), 4.64 (1H, s), 5.14 (2H, s), 5.23 (2H, s), 7.29–7.41 (10H, m), 9.29 (1H, br s), 9.46 (1H, s). ^{13}C NMR ($CDCl_3$) *δ*: 24.8, 28.3, 30.0, 32.0, 44.4, 50.2, 61.4, 66.9, 68.8, 79.4, 127.7, 127.8, 128.1, 128.2, 128.6, 134.6, 136.8, 155.3, 155.6, 160.3, 163.7, 172.5. HRFABMS $(M+H)^+$ calcd for $C_{29}H_{40}N_5O_8$: 586.2879, found: 586.2877. Anal. Calcd $C_{29}H_{39}N_5O_8$: C, 59.47; H, 6.71; N, 11.96. Found: C, 59.62; H, 6.64; N, 11.99.

N-α-Boc-*N*-ω,*N*′-ω′-di-Alloc-arginine-*N*,*O*-dimethylhydroxyamine (**16c**): $[\alpha]_D^{20}$ −2.7 (*c* 0.69, $CHCl_3$). IR (film) *ν* max cm^{-1}: 3336, 2981, 2943, 1728, 1712, 1639, 1367, 1323, 1255, 1211, 1169, 1140, 1049, 995. ^1H NMR ($CDCl_3$) *δ*: 1.42 (9H, s), 1.67 (4H, s), 3.20 (3H, s), 3.45 (2H, q, *J*=6.3 Hz), 3.76 (3H, s), 4.61 (4H, dd, *J*=16.5, 5.7 Hz), 4.68 (1H, d, *J*=1.2 Hz), 5.18–5.38 (4H, m), 5.83–6.05 (2H, m), 8.30 (1H, s). ^{13}C NMR ($CDCl_3$) *δ*: 25.0, 28.3, 30.1, 32.0, 40.5, 49.9, 61.6, 66.2, 66.9, 79.6, 117.7, 119.3, 131.0, 133.0, 153.7, 156.0, 163.5, 172.6. HRFABMS $(M+H)^+$ calcd for $C_{21}H_{36}N_5O_8$: 486.2564, found: 486.2566.

N-α,*N*-ω-di-Boc-*N*′-ω′-Alloc-(4*R*)-arginine-*N*,*O*-dimethylhydroxyamine (**16d**): $[\alpha]_D^{20}$ −0.41 (*c* 0.67, $CHCl_3$). IR (film) *ν* max cm^{-1}: 3336, 2978, 2937, 1720, 1639, 1369, 1269, 1255, 1159, 1138, 1051. ^1H NMR ($CDCl_3$) *δ*: 1.45 (9H, s), 1.51 (9H, s), 1.53–1.79 (4H, m), 3.21 (3H, s), 3.45 (2H, q, *J*=6.2 Hz), 3.77 (3H, s), 4.59 (2H, dd, *J*=5.7, 1.5 Hz), 4.63–4.69 (1H, m), 5.21 (1H, dd, *J*=10.5, 1.4 Hz), 5.32 (1H, dd, *J*=17.1, 1.4 Hz), 5.92–6.05 (1H, m), 8.42 (1H, s), 11.36 (1H, s). ^{13}C NMR ($CDCl_3$) *δ*: 25.0, 28.0, 28.2, 28.3, 30.1, 32.0, 40.4, 50.0, 66.1, 66.2, 66.8, 79.6, 83.3, 117.6, 119.3, 133.1, 153.1, 155.5, 156.5, 162.3, 163.5, 172.6. HRFABMS $(M+H)^+$ calcd for $C_{22}H_{40}N_5O_8$: 502.2877, found: 502.2871.

N-α,*N*-ω,*N*′-ω′-tri-Boc-L-arginine-(4*R*)-arginine-*O*,*N*-dimethylhydroxyamine (**16e**): $[\alpha]_D^{20}$ +0.64 (*c* 1.5, $CHCl_3$). IR (film) *ν* max cm^{-1}: 3329, 2978, 2935, 1718, 1641, 1618, 1367, 1331, 1169, 1134, 1051, 756. ^1H NMR ($CDCl_3$) *δ*: 1.37 (9H, s), 1.43 (18H, s), 1.51–1.71 (4H, m), 3.14 (3H, s), 3.37 (2H, d, *J*=5.7 Hz), 3.70 (3H, s), 4.62 (1H, s), 5.19 (1H, d, *J*=9.0 Hz), 8.26 (1H, s), 11.43 (1H, s). ^{13}C NMR ($CDCl_3$) *δ*: 25.0, 27.9, 28.2, 28.2, 29.9, 31.9, 40.2, 50.0, 61.4, 79.0, 79.4, 82.9, 153.1, 155.4, 156.6, 162.3, 163.4, 172.6. HRFABMS $(M+H)^+$ calcd for $C_{23}H_{44}N_5O_8$: 518.3193, found: 518.3193.

3.2.11. *N*-α,*N*′-ω′-di-Boc-*N*-ω-Mtr-vinylogous arginine ethyl ester (17a). To a solution of amide (**16a**) (109 mg, 173 μmol) in THF (5 ml) was added $LiAlH_4$ (25 mg, 658 μmol) at 0 °C and the mixture was stirred for 30 min at room temperature. To the mixture were added H_2O (25 μl) and 1 M NaOH (50 μl), and filtered on a Celite pad. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude product was used for the next reaction without further purification. To a solution of triethyl phosphonoacetate (250 mg, 661 μmol) in THF (5 ml) was added NaH (25 mg, 735 μmol) at 0 °C. The mixture was stirred for 1 h and the crude product was added dropwise into it. After 20 min, satd aq NH_4Cl and AcOEt were added, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The product was purified with silica gel column chromatography (hexane/AcOEt=3:1) to give ethyl ester (**17a**) (16 mg, 24 μmol, 14%) as a colorless oil. $[\alpha]_D^{20}$ +5.3 (*c* 0.65, $CHCl_3$). IR (film) *ν* max cm^{-1}: 3329, 3286, 2979, 2870, 1720, 1618, 1369, 1279, 1244, 1153, 1122, 916, 810, 733, 677. ^1H NMR ($CDCl_3$) *δ*: 1.29 (3H, s, *J*=7.2 Hz), 1.44 (9H, s), 1.50 (9H, s), 1.55–1.78 (4H, m), 2.15 (3H, s), 2.63 (3H, s), 2.70 (3H, s), 3.30 (2H, q, *J*=6.0 Hz), 3.84 (3H, s), 4.18 (2H, q, *J*=7.2 Hz), 4.21 (1H, m), 4.45 (1H, m), 5.88 (1H, dd, *J*=15.6, 1.5 Hz), 6.55 (1H, s), 6.77 (1H, dd, *J*=15.6,

5.4 Hz), 8.32 (1H, t, J=5.1 Hz), 9.81 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 11.9, 14.2, 18.3, 23.9, 25.4, 28.0, 28.3, 32.0, 55.5, 60.5, 84.1, 111.7, 121.2, 124.9, 133.0, 136.8, 138.7, 147.6, 150.4, 152.7, 155.0, 158.8, 166.1. HRFABMS $(M+H)^+$ calcd for $C_{30}H_{49}N_4O_9S$: 641.3220, found: 641.3218.

The following compounds (**17b**–**17e**) were prepared in a manner similar to **17a**.

N-α-Boc-*N*-ω,*N*′-ω′-di-Cbz-vinylogous arginine ethyl ester (**17b**): mp 91–92 °C. $[\alpha]_D^{20}$ +14.5 (*c* 0.19, $CHCl_3$). IR (KBr) ν max cm^{-1}: 3404, 3346, 3277, 3088, 3067, 3030, 2982, 2937, 2874, 2361, 2343, 1724, 1693, 1655, 1612, 1520, 1450, 1381, 1302, 1251, 1174, 1101, 1010. ^{1}H NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.2 Hz), 1.41 (9H, s), 1.43–1.62 (2H, m), 1.66 (2H, d, J=7.2 Hz), 3.93–4.12 (2H, m), 4.18 (2H, q, J=7.1 Hz), 4.28 (1H, br s), 4.92 (1H, d, J=7.8 Hz), 5.15 (2H, s), 5.24 (2H, s), 5.88 (1H, dt, J=15.6, 1.5 Hz), 6.77 (1H, dt, J=15.6, 4.9 Hz), 7.27–7.43 (10H, m), 9.26 (1H, br s), 9.45 (1H, br s). ^{13}C NMR ($CDCl_3$): 14.3, 25.2, 28.4, 30.8, 44.3, 51.4, 60.4, 67.0, 68.9, 79.5, 120.7, 127.7, 127.8, 128.3, 128.7, 128.8, 134.5, 136.8, 148.1, 155.1, 155.7, 160.4, 163.7, 166.1. HRFABMS $(M+H)^+$ calcd for $C_{31}H_{41}N_4O_8$: 597.2926, found: 597.2924. Anal. Calcd $C_{31}H_{40}N_4O_8$: C, 62.40; H, 6.76; N, 9.39. Found: C, 62.35; H, 6.71; N, 9.32.

N-α-Boc-*N*-ω,*N*′-ω′-di-Alloc-(4*R*)-vinylogous arginine ethyl ester (**17c**) and hemiaminal (**18c**): Compound **17c**: $[\alpha]_D^{20}$ +14.5 (*c* 0.19, $CHCl_3$). IR (film) ν max cm^{-1}: 3336, 2976, 2929, 2850, 1718, 1639, 1367, 1259, 1213, 1167, 1051. ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, m), 1.46 (9H, s), 1.68 (4H, d, J=5.1 Hz), 3.51 (2H, s), 4.21 (3H, q, J=6.9 Hz), 4.34 (1H, s), 4.53–4.69 (4H, m), 5.22–5.42 (4H, m), 5.87–6.07 (3H, m), 6.83 (1H, dd, J=15.8, 5.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.2, 25.6, 28.4, 29.7, 31.6, 40.5, 41.3, 41.9, 55.5, 60.5, 66.2, 67.0, 81.2, 117.8, 119.5, 121.2, 131.3, 133.0, 147.8, 155.3, 162.8, 166.5, 190.5. HRFABMS $(M+H)^+$ calcd for $C_{23}H_{37}N_4O_8$: 497.2611, found: 497.2606. Compound **18c**: $[\alpha]_D^{20}$ +4.4 (*c* 0.95, $CHCl_3$). IR (film) ν max cm^{-1}: 3342, 2976, 2943, 2873, 1772, 1716, 1618, 1522, 1508, 1456, 1363, 1327, 1246, 1167, 1120, 756. ^{1}H NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.67–1.86 (4H, m), 2.16–2.27 (1H, m), 2.79–2.89 (1H, m), 3.61–3.68 (1H, m), 4.49 (1H, d, J=13.8 Hz), 4.61 (2H, d, J=8.4 Hz), 4.83 (1H, s), 5.26 (1H, dd, J=10.5, 0.9 Hz), 5.36 (1H, dd, J=17.1, 0.9 Hz), 5.92–6.03 (1H, m), 11.33 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 14.2, 22.1, 28.2, 28.3, 28.5, 43.9, 52.3, 60.4, 66.8, 118.6, 132.3, 145.6, 154.8, 155.0, 162.9. HRFABMS $(M+H)^+$ calcd for $C_{15}H_{27}N_4O_5$: 343.1981, found: 343.1982.

N-α,*N*-ω-di-Boc-*N*′-ω′-Alloc-(4*R*)-vinylogous arginine ethyl ester (**17d**): $[\alpha]_D^{20}$ +7.80 (*c* 0.92, $CHCl_3$). IR (film) ν max cm^{-1}: 3329, 3153, 2979, 1720, 1639, 1576, 1518, 1456, 1419, 1369, 1306, 1271, 1255, 1161, 1140, 1053, 756. ^{1}H NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz), 1.44 (9H, s), 1.49 (9H, s), 1.63–1.67 (4H, br s), 3.45 (2H, m), 4.19 (2H, q, J=7.1 Hz), 4.32 (1H, s), 4.60 (2H, dt, J=5.7, 1.4 Hz), 5.19–5.36 (2H, m), 5.81–6.06 (2H, m), 6.82 (1H, dd, J=15.6, 5.4 Hz), 8.43 (1H, s), 11.36 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 14.2, 25.6, 28.0, 28.2, 28.4, 29.5, 31.7, 40.5, 51.5, 55.5, 60.5, 66.1, 83.5, 117.7, 119.5, 121.1, 133.1, 147.9, 153.2, 155.1, 156.6, 157.5, 163.5, 166.2. HRFABMS $(M+H)^+$ calcd for $C_{24}H_{41}N_4O_8$: 513.2924, found: 513.2933.

N-α,*N*-ω,*N*′-ω′-tri-Boc-(4*R*)-vinylogous arginine ethyl ester (**17e**): $[\alpha]_D^{20}$ +2.4 (*c* 1.1, $CHCl_3$). IR (film) ν max cm^{-1}: 3384, 2979, 2933, 1716, 1610, 1512, 1367, 1275, 1254, 1165, 1149, 982, 756. ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.1 Hz), 1.44 (9H, s), 1.51 (18H, s), 1.62 (4H, br s), 3.80 (1H, s), 3.91 (1H, s), 4.16 (2H, q, J=3.4 Hz), 4.43 (1H, s), 5.70 (1H, d, J=8.4 Hz), 6.01 (1H, dd, J=15.9, 1.4 Hz), 6.86 (1H, dd, J=15.6, 5.0 Hz), 9.20 (1H, s), 9.36 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 14.2, 24.7, 28.0, 28.2, 28.4, 29.9, 44.4, 51.5, 60.3, 78.9, 79.3, 83.8, 118.0, 120.7, 148.7, 150.0, 154.9, 155.4, 160.7, 162.3, 163.6, 166.4. HRFABMS $(M+H)^+$ calcd for $C_{25}H_{45}N_4O_8$: 529.3237, found: 529.3228.

3.2.12. (4*R*)-Vinylogous arginine ethyl ester (6). To a solution of *N*-α,*N*-ω,*N*′-ω′-tri-Boc-(4*R*)-vinylogous arginine ethyl ester (**17e**) in CH_2Cl_2 (500 μl) was added TFA (500 μl). The mixture was stirred for 2 h at 0 °C. After evaporation of the TFA, the resulting precipitate was concentrated in vacuo to give (4*R*)-vinylogous arginine ethyl ester TFA salt (**6**) (4.5 mg, 10.4 μmol, 100%) as a colorless oil. $[\alpha]_D^{20}$ +5.2 (*c* 1.3, $CHCl_3$), IR (film) ν max cm^{-1}: 3363, 3188, 2922, 1670, 1203, 1138, 839, 800, 723. ^{1}H NMR (CD_3OD) δ: 1.30 (3H, t, J=6.9 Hz), 1.55–1.93 (4H, m), 3.24 (2H, t, J=6.6 Hz), 3.33 (1H, s), 3.95–4.02 (1H, m), 4.23 (2H, q, J=7.1 Hz), 6.19 (1H, d, J=15.6 Hz), 6.83 (1H, dd, J=15.8, 8.0 Hz), 7.88 (2H, s). ^{13}C NMR (CD_3OD) δ: 14.4, 25.8, 30.8, 41.7, 52.9, 62.1, 127.1, 142.5, 158.8, 166.6. HRFABMS $(M+H)^+$ calcd for $C_{10}H_{21}N_4O_2$: 229.1665, found: 229.1662.

3.2.13. (4*S*,5*S*)-Ethyl (*sec*-butyl-2-oxo-ozazolidin-5-yl)-acetate (20). To a solution of **19** (376 mg, 1.24 mmol) in CH_2Cl_2 were added MsCl (96 μl, 1.24 mmol) and Et_3N (259 ml, 1.86 mmol) at −20 °C and the mixture was stirred for 3 h at room temperature. To the mixture were added H_2O and ether, and the organic layer was washed with satd aq $NaHCO_3$, dried over $MgSO_4$, and evaporated in vacuo. The product was purified with silica gel column chromatography (hexane/AcOEt=2:1) to give oxazolidinone (**20**) (184 mg, 792 mmol, 68%) as a colorless oil. $[\alpha]_D^{20}$ −42.0 (*c* 0.68, $CHCl_3$), IR (film) ν max cm^{-1}: 3217, 3104, 2958, 2871, 1749, 1468, 1444, 1367, 1273, 1003, 968, 793. ^{1}H NMR ($CDCl_3$) δ: 0.93–0.96 (6H, m), 1.25–1.30 (3H, m), 1.41–1.67 (3H, m), 2.41 (1H, dd, J=17.7, 4.6 Hz), 2.81 (1H, dd, J=17.7, 6.9 Hz), 3.62 (1H, dt, J=9.2, 4.6 Hz), 4.19 (2H, t, J=7.1 Hz), 4.57 (1H, q, J=4.6 Hz), 5.30 (1H, s). ^{13}C NMR (CD_3OD) δ: 21.7, 23.1, 24.9, 36.1, 43.3, 55.0, 58.3, 155.1, 174.2. HRFABMS $(M+H)^+$ calcd for $C_{11}H_{19}N_1O_5$: 299.1300, found: 299.1299.

3.2.14. Fmoc-Sta-OH (8). To a solution of **20** (182 mg, 794 μmol) in MeOH was added aq LiOH. The mixture was stirred for 1.5 h at room temperature. Et_3N (152 μl, 1.09 mmol) and Fmoc-OSu (350 mg, 1.04 mmol) were added and then the mixture was stirred for 15 min at room temperature (pH=8.5–9.0). The mixture was evaporated in vacuo. The crude product was added to 1.5 N HCl and the resulting precipitate was filtrated to give Fmoc-Sta-OH (**8**) (316 mg, 794 μmol, 75%) as a white powder. All spectra

for the synthetic Fmoc-Sta-OH (**8**) were well consistent with those of the authentic standard (purchased from Novabiochem). ^{1}H NMR ($CDCl_3$) δ: 0.58 (0.68H, d, J=6.3 Hz), 0.73 (0.68H, d, J=6.3 Hz), 0.88 (4.64H, d, J=5.4 Hz), 1.16–1.32 (1H, m), 1.42–1.59 (2H, m), 1.86 (0.2H, d, J=5.4 Hz), 2.21 (0.2H, dd, J=15.6, 9.9 Hz), 2.47–2.54 (1.6H, m), 2.98 (0.75H, d, J=6.6 Hz), 3.15 (0.25H, br s), 3.64 (0.75H, m), 3.77 (0.25H, m), 3.98 (1H, m), 4.20 (1H, m), 4.37 (2H, m), 4.51 (1H, d, J=7.5 Hz), 5.36 (0.75H, d, J=9.3 Hz), 5.72 (0.25H, d, J=9.3 Hz), 6.83 (1H, br s), 7.22–7.43 (4H, m), 7.53 (2H, m), 7.70 (2H, m).

3.2.15. EtO-Azd-Leu-Sta-Abu-OH (5). To a suspension of 2-Cl-trityl chloride resin (192 mg, 1.31 mmol/g) in DMF (1 ml) and CH_2Cl_2 (500 µl) were added Fmoc-Abu-OH (245 mg, 755 µmol) and DIEA (132 µl, 755 µmol). The mixture was stirred for 2.5 h at 25 °C. To the resultant resin was added 20% piperidine in DMF and the mixture was stirred for 30 min at 25 °C. Fmoc-Sta-OH (**8**) (300 mg, 755 µmol) and Fmoc-Leu-OH (267 mg, 755 µmol) were successively condensed to this resin using DIPC (117 µl, 755 µmol)/HOBt (116 mg, 755 µmol) for 3 h. EtO-Azd-OH (**7**) (120 mg, 755 µmol) was condensed with DPPA (163 µl, 755 µmol) and Et_3N (105 µl, 755 µmol) for 4 h. The resulting resin was treated with HFIP/CH_2Cl_2 (1:3, 2 ml) for 2 h and the mixture was filtrated. After evaporation of the HFIP, ether was added, and the resulting precipitate was purified by preparative HPLC (CH_3CN/H_2O=43:57) to yield a tetrapeptide (**5**) (42 mg, 81.6 µmol, 32%) as a white powder. $[\alpha]_D^{28}$ −40.9 (*c* 0.3, MeOH). HPLC: t_R=13.76 min (CH_3CN, 30–40%/30 min). IR (film) ν max cm^{-1}: 3278, 3062, 2958, 2871, 1732, 1653, 1539, 1201, 1142, 723. ^{1}H NMR (CD_3OD) δ: 0.94–1.03 (15H, m), 1.31 (4H, t, J=7.1 Hz), 1.33–1.38 (1H, m), 1.57–1.67 (4H, m), 1.69–1.81 (2H, m), 1.86–1.96 (1H, m), 2.33 (2H, d, J=6.6 Hz), 2.80 (1H, d, J=2.7 Hz), 2.93 (1H, d, J=2.4 Hz), 3.98 (1H, m), 4.00 (1H, m), 4.19–4.29 (2H, m), 4.33 (1H, dd, J=8.1, 5.1 Hz), 4.42 (1H, t, J=7.4 Hz). ^{13}C NMR (CD_3OD) δ: 9.1, 13.0, 20.5, 20.8, 21.9, 22.4, 24.4, 24.6, 24.6, 34.6, 36.2, 39.9, 40.3, 40.5, 51.4, 52.5, 53.7, 61.5, 70.1, 169.0, 169.8, 172.7, 173.2, 174.0. MALDI-TOFMS (M+H)$^+$ calcd for $C_{43}H_{27}N_4O_8$: 515.308, found: 515.238. HRFABMS (M+H)$^+$ calcd for $C_{24}H_{43}N_4O_8$: 515.3081, found: 515.3077.

3.2.16. EtO-Azd-Leu-Sta-Abu-vArg-OEt (23). To a solution of H-vArg-OEt (**6**) (1.1 mg, 4.70 mmol) and EtO-Azd-Leu-Sta-Abu-OH (**5**) (2.4 mg, 4.70 mmol) in DMF (20.0 ml) were added DIEA (0.8 ml, 4.70 mmol), HATU (1.8 mg, 4.70 mmol), and HOAt (0.6 mg, 4.70 mmol) and the mixture was stirred for 5 min at room temperature. AcOH (0.8 ml) was added and the mixture was evaporated in vacuo. The residue was purified with preparative HPLC (CH_3CN/H_2O=38:62) to give EtO-Azd-Leu-Sta-Abu-vArg-OEt (**23**) (1.1 mg, 15.0 mmol, 32%) as a white powder. $[\alpha]_D^{30}$ −33.9 (*c* 0.01, MeOH). HPLC: t_R=37.15 min (CH_3CN, 2–40%/40 min). IR (film) ν max cm^{-1}: 3342, 2960, 1653, 1541, 1458, 1203, 1182, 1138, 1036, 800, 721. ^{1}H NMR (CD_3OD) δ: 0.88–1.06 (15H, m), 1.31 (6H, q, J=7.0 Hz), 1.34–1.38 (2H, m), 1.58–1.97 (10H, m), 2.27–2.49 (2H, m), 2.83 (1H, d, J=2.4 Hz), 2.96 (1H, d, J=2.1 Hz), 3.17–3.29 (2H, m), 3.90–3.99 (1H, m), 4.02–4.44 (1H, m), 4.12–4.44 (6H, m), 4.57–4.63 (1H, m), 5.99 (1H, dd, J=15.8, 1.5 Hz), 6.90 (1H, dd, J=15.8, 5.6 Hz), 7.50 (1H, d, J=9.6 Hz), 8.42 (1H, d, J=8.4 Hz). ^{13}C NMR (CD_3OD) δ: 10.9, 14.4, 14.6, 22.0, 22.5, 23.3, 23.7, 26.1, 26.2, 26.3, 31.8, 36.0, 37.7, 41.5, 41.5, 41.8, 41.9, 50.9, 52.2, 54.6, 57.0, 61.7, 63.0, 71.3, 122.3, 148.7, 158.6, 165.2, 167.8, 170.8, 171.3, 174.1, 174.5, 174.6. MALDI-TOFMS (M+H)$^+$ calcd for $C_{34}H_{61}N_8O_9$: 725.456, found: 725.344. HRFABMS (M+H)$^+$ calcd for $C_{34}H_{61}N_8O_9$: 725.4562, found: 725.4556.

3.2.17. Miraziridine A (1). To a solution of **23** (1.0 mg, 1.38 µmol) in DMSO (2.0 ml) were added H_2O (8 ml) and porcine liver esterase in Tris/HCl buffer solution (2.0 mg/80 ml) and the mixture was allowed to stand for 132 h at 30 °C. The mixture was filtered and the filtrate was purified by preparative HPLC (CH_3CN/H_2O=26:73) to give miraziridine A (**1**) (0.85 mg, 12.7 mmol, 92%) as a white powder. $[\alpha]_D^{30}$ −61.0 (*c* 0.10, MeOH). HPLC: t_R=25.52 min (CH_3CN, 2–40%/40 min). IR (film) ν max cm^{-1}: 3359, 3195, 2922, 2852, 1657, 1635, 1541, 1203, 1136, 721. ^{1}H NMR (CD_3OH) δ: 0.86 (3H, d, J=6.3 Hz), 0.90 (3H, d, J=6.3 Hz), 0.92 (3H, d, J=6.0 Hz), 0.97 (3H, d, J=6.3 Hz), 0.99 (3H, t, J=7.2 Hz), 1.32 (1H, m), 1.55–1.75 (10H, m), 1.81–1.92 (1H, m), 2.22–2.43 (2H, m), 2.68 (1H, d, J=2.1 Hz), 2.84 (1H, d, J=2.1 Hz), 3.11–3.24 (2H, m), 3.85–3.92 (1H, m), 3.98–4.03 (1H, m), 4.12–4.19 (1H, m), 4.26–4.33 (1H, m), 4.58 (1H, br s), 5.90 (1H, dd, J=15.5, 2.0 Hz), 6.83 (1H, dd, J=15.5, 5.9 Hz), 7.43 (2H, d, J=8.4 Hz), 8.07 (1H, d, J=6.9 Hz), 8.39 (1H, d, J=9.9 Hz), 8.46 (1H, d, J=8.4 Hz). ^{13}C NMR (CD_3OH) δ: 10.9, 21.9, 22.5, 23.3, 23.6, 26.0, 26.2, 26.4, 32.2, 37.2, 37.5, 41.5, 41.8, 42.1, 50.9, 52.2, 55.2, 57.5, 71.3, 82.6, 122.8, 148.9, 159.0, 170.0, 171.6, 174.0, 174.3. MALDI-TOFMS (M+H)$^+$ calcd for $C_{30}H_{53}N_8O_9$: 669.394, found: 669.268. HRFABMS (M+H)$^+$ calcd for $C_{30}H_{53}N_9$: 669.3936, found: 669.3945.

3.2.18. HO-Azd-Leu-Sta-Abu-OH (2). To a solution of **5** (2.0 mg, 3.9 mmol) in DMSO (5 ml) were added H_2O (20 ml) and porcine liver esterase in Tris/HCl buffer solution (4.0 mg/80 ml) and the mixture was allowed to stand for 132 h at 30 °C. The mixture was filtered and the filtrate was purified by preparative HPLC (CH_3CN/H_2O=30:70) to give HO-Azd-Leu-Sta-Abu-OH (**2**) (1.5 mg, 3.1 mmol, 79%) as a white powder. $[\alpha]_D^{28}$ −16.5 (*c* 0.02, MeOH). HPLC: t_R=24.08 min (CH_3CN, 2–40%/30 min). IR (film) ν max cm^{-1}: 3278, 3062, 2958, 2871, 1732, 1653, 1539, 1201, 1142, 723. ^{1}H NMR (CD_3OD) δ: 0.94–1.03 (15H, m), 1.25–1.38 (1H, m), 1.55–1.68 (4H, m), 1.69–1.81 (2H, m), 1.86–1.95 (1H, m), 2.32 (2H, d, J=6.6 Hz), 2.70 (1H, br s), 2.94 (1H, br s), 3.96 (2H, m), 4.32 (1H, dd, J=8.1, 5.1 Hz), 4.41 (1H, t, J=7.5 Hz), 7.58 (1H, d, J=9.0 Hz). ^{13}C NMR (CD_3OD) δ: 10.6, 21.9, 22.2, 23.4, 23.8, 25.9, 26.0, 26.1, 33.2, 37.1, 41.3, 41.7, 52.8, 53.9, 55.2, 71.5, 167.5, 169.0, 172.0, 174.7. MALDI-TOFMS (M+H)$^+$ calcd for $C_{22}H_{39}N_4O_8$: 487.276, found: 487.199.

3.2.19. Ac-Leu-Sta-Abu-OH (24). The resin (**21**) (50 mg) was stirred with Ac_2O (50 µl)/pyridine (200 µl) in the presence of a catalytic amount of DMAP. The resulting resin was treated with HFIP/CH_2Cl_2 (1:3, 1 ml) for 2 h. After evaporation of the HFIP, ether was added, and the resulting precipitate was purified with preparative HPLC (CH_3CN/H_2O=32:68) to yield tripeptide (**24**) (9.2 mg, 22.1 µmol,

33%) as a white powder. $[\alpha]_D^{25}$ −11.2 (*c* 0.42, MeOH). HPLC: t_R=28.31 min (CH_3CN, 30–40%/40 min). IR (film) ν max cm^{-1}: 3286, 2956, 2871, 1720, 1643, 1541, 1369, 1043, 754. ^1H NMR (300 MHz, CD_3OD) δ: 0.91 (3H, d, *J*=6.3 Hz), 0.94 (3H, d, *J*=6.3 Hz), 0.95 (3H, d, *J*=6.3 Hz), 0.99 (3H, d, *J*=6.3 Hz), 1.01 (3H, t, *J*=7.5 Hz), 1.33 (1H, m), 1.56–1.81 (6H, m), 1.89 (1H, m), 2.01 (3H, s), 2.34 (2H, d, *J*=6.6 Hz), 3.98 (2H, m), 4.32 (1H, dd, *J*=8.1, 5.1 Hz), 4.37 (1H, t, *J*=7.5 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ: 10.6, 21.9, 22.3, 22.4, 23.4, 23.8, 25.79, 25.83, 25.94, 26.0, 41.4, 41.57, 41.65, 41.71, 41.8, 52.6, 52.7, 53.8, 55.2, 55.3, 71.4, 71.5, 173.6, 174.1, 175.2, 175.4. MALDI-TOFMS $(M+H)^+$ calcd for $C_{20}H_{38}N_3O_6$: 416.276, found: 416.267.

3.2.20. Ac-Leu-Sta-Abu-vArg-OEt (25). To a solution of H-vArg-OEt (**6**) (3.8 mg, 16.1 μmol) and Ac-Leu-Sta-Abu-OH (**24**) (6.7 mg, 16.1 μmol) in DMF (20.0 μl) were added DIEA (2.7 μl, 16.1 μmol), HATU (6.2 mg, 16.1 μmol), and HOAt (2.1 mg, 16.1 μmol) and the mixture was stirred for 10 min at room temperature. AcOH (2 μl) was added and the mixture was evaporated in vacuo. The residue was purified with preparative HPLC (CH_3CN/H_2O=39:61) to give Ac-Leu-Sta-Abu-vArg-OEt (**25**) (4.2 mg, 6.7 μmol, 42%) as a white powder. $[\alpha]_D^{20}$ −5.9 (*c* 0.01, MeOH). HPLC: t_R=38.35 min (CH_3CN, 2–40%/30 min). IR (film) ν max cm^{-1}: 3307, 3195, 2958, 2873, 1653, 1541, 1448, 1369, 1284, 1203, 1182, 1095, 1041, 839, 802, 721. ^1H NMR (CD_3OD) δ: 0.86 (3H, d, *J*=6.3 Hz), 0.91 (3H, d, *J*=6.0 Hz), 0.93 (3H, d, *J*=6.3 Hz), 0.98 (3H, d, *J*=6.3 Hz), 1.01 (3H, d, *J*=7.5 Hz), 1.27 (3H, t, *J*=7.2 Hz), 1.31 (1H, m), 1.54–1.77 (10H, m), 1.89 (1H, m), 2.03 (3H, s), 2.31 (1H, dd, *J*=14.4, 6.9 Hz), 2.37 (1H, dd, *J*=14.4, 7.2 Hz), 3.21 (2H, m), 3.90 (1H, m), 4.03 (1H, td, *J*=7.2, 2.1 Hz), 4.13–4.24 (3H, m), 4.30 (1H, m), 4.59 (1H, m), 5.96 (1H, dd, *J*=15.9, 1.8 Hz), 6.88 (1H, dd, *J*=15.6, 5.4 Hz), 7.31 (1H, d, *J*=9.6 Hz), 8.37 (1H, d, *J*=8.7 Hz). ^{13}C NMR (CD_3OD) δ: 10.9, 13.6, 21.9, 22.5, 23.4, 23.7, 26.0, 26.2, 26.3, 31.8, 41.4, 41.7, 41.8, 41.9, 50.8, 57.1, 61.7, 71.3, 122.2, 148.8, 167.8, 173.9, 174.1, 174.6. MALDI-TOFMS $(M+H)^+$ calcd for $C_{30}H_{56}N_7O_7$: 626.424, found: 626.334.

3.2.21. Ac-Leu-Sta-Abu-vArg-OH (3). To a solution of **25** (2.1 mg, 3.35 μmol) in DMSO (4.0 μl) was added H_2O (16 μl) and porcine liver esterase in Tris/HCl buffer solution (4.0 mg/160 μl) and the mixture was allowed to stand for 72 h at 30 °C. The mixture was filtered and the filtrate was purified by preparative HPLC (CH_3CN/H_2O=23:77) to give Ac-Leu-Sta-Abu-vArg-OH (**3**) (1.5 mg, 2.39 μmol, 71%) as a white powder. $[\alpha]_D^{28}$ −18.3 (*c* 0.02, MeOH). HPLC: t_R=22.62 min (CH_3CN, 2–40%/40 min). IR (film) ν max cm^{-1}: 3329, 2958, 2866, 1653, 1639, 1541, 1458, 1373, 1201, 1184, 1142, 1043, 800. ^1H NMR (CD_3OD) δ: 0.87 (3H, d, *J*=6.3 Hz), 0.92 (3H, d, *J*=6.3 Hz), 0.93 (3H, d, *J*=6.3 Hz), 0.98 (3H, d, *J*=6.3 Hz), 1.01 (3H, t, *J*=7.5 Hz), 1.31 (1H, m), 1.55–1.80 (10H, m), 1.87 (1H, m), 2.03 (3H, s), 2.31 (1H, dd, *J*=14.4, 6.9 Hz), 2.37 (1H, dd, *J*=14.4, 7.2 Hz), 3.18 (2H, m), 3.92 (1H, m), 4.00 (1H, td, *J*=7.2, 2.1 Hz), 4.17 (1H, m), 4.29 (1H, m), 4.60 (1H, m), 5.92 (1H, dd, *J*=15.6, 1.8 Hz), 6.87 (dd, 1H, *J*=15.6, 5.4 Hz), 7.33 (1H, d, *J*=9.6 Hz), 8.09 (0.7H, d, *J*=6.6 Hz), 8.25 (0.3H, d, *J*=6.9 Hz), 8.21 (1H, d, *J*=9.0 Hz). ^{13}C NMR (CD_3OD) δ: 10.9, 21.9, 22.5, 23.4, 23.7, 26.0, 26.3, 31.9, 41.5, 41.7, 42.0, 50.8, 52.1, 54.5, 57.0, 71.4, 79.2, 122.5, 148.9, 163.8, 174.3, 174.6, 175.1. MALDI-TOFMS $(M+H)^+$ calcd for $C_{28}H_{52}N_7O_7$: 598.393, found: 598.361.

3.2.22. H-Leu-Sta-Abu-OH (4). To the resin (**21**) (59 mg) was added HFIP/CH_2Cl_2 (1:3, 1 ml) and the mixture was stirred for 2 h. After evaporation of the HFIP, ether was added, and the resulting precipitate was purified with preparative HPLC (CH_3CN/H_2O=35:65) to yield a tripeptide (**4**) (42 mg, 81.6 μmol, 32%) as a white powder. $[\alpha]_D^{20}$ −7.5 (*c* 0.04, MeOH), HPLC: t_R=32.00 min (CH_3CN, 2–40%/40 min). IR (film) ν max cm^{-1}: 3278, 2956, 2929, 2870, 1716, 1651, 1522, 1458, 1201, 1140, 837, 800. ^1H NMR (CD_3OD) δ: 0.94 (3H, d, *J*=6.6 Hz), 0.96 (3H, d, *J*=6.9 Hz), 1.00 (3H, d, *J*=7.8 Hz), 1.02 (3H, d, *J*=7.8 Hz), 1.03 (3H, t, *J*=5.3 Hz), 1.39 (1H, m), 1.53–1.79 (1H, m), 1.90 (1H, m), 2.30 (1H, dd, *J*=14.1, 7.5 Hz), 2.36 (1H, dd, *J*=14.1, 5.7 Hz), 3.90 (1H, m), 3.99 (1H, ddd, *J*=7.5, 5.4, 2.4 Hz), 4.08 (1H, ddd, *J*=9.9, 4.2, 2.7 Hz), 4.33 (1H, dd, *J*=8.1, 5.1 Hz). ^{13}C NMR (CD_3OD) δ: 9.8, 21.6, 21.8, 22.5, 23.1, 24.5, 24.8, 25.0, 39.7, 40.5, 40.8, 52.2, 52.3, 53.8, 70.2, 169.9, 172.5, 174.6. MALDI-TOFMS $(M+H)^+$ calcd for $C_{18}H_{36}N_3O_{85}$: 374.266, found: 374.281.

3.3. Inhibitory activity

Bovine spleen cathepsin B was purchased from Sigma Chemical company (St. Louis, MO). Cathepsin B (10 mU) was preincubated at 40 °C for 10 min in 90 μl of 50 mM MES (pH 6.0), 2 mM DTT, and 0.1% Brij-35. The solution was then mixed with 20 mM Z-Arg-Arg-MCA and each inhibitor (1 nM to 1 mM) in DMSO (10 μl), and the mixture was incubated at 40 °C for 10 min. The reaction was stopped by adding 100 μl of 100 mM sodium monochloroacetate, 30 mM sodium acetate, and 70 mM acetic acid (pH 4.3). Ki values were determined according to the method of Dixon and Webb.[19]

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