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Solution-phase synthesis of 2-cyano and 2-amido aziridinyl peptides

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Abstract

Starting from a library of 2-L-α-amino acyl (*E*)-acrylonitriles, different short 2-cyano and 2-amido aziridinyl peptides, potential protease inhibitors, were obtained under parallel solution-phase conditions. The transformations include careful selection of conditions for aziridine deprotection and cyano group partial hydrolysis.
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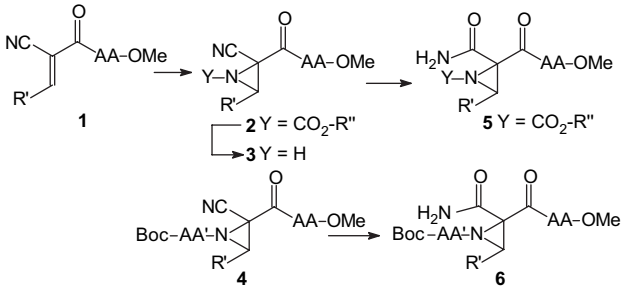
1. Introduction

The presence of small heterocycles, such as epoxides, aziridines, and thiiranes in peptide sequences is an important feature for the synthesis of electrophilic modified peptides, for their biological and pharmacological significance.[1] Among these small heterocycles, the aziridine functionality represents a valuable synthetic building block[2] due to its ability to undergo ring opening reactions with a wide range of nucleophiles.[3] We report here a solution-phase synthesis of 1-protected 2-cyano aziridinyl peptides **2**, versatile building blocks to obtain aziridinyl peptides **4**, through the free aziridines **3**, and of 2-amido aziridinyl peptides **5** and **6**, starting from (*E*)-acrylonitriles **1** bearing different L-α-amino ester residues (Scheme 1).



Scheme 1. Strategy for the synthesis of 2-cyano and 2-amido aziridinyl peptides.

The chemical behavior of aziridines has prompted the preparation of short peptides incorporating this subunit.[4] However, to date the reported procedures require many reaction steps and suffer from low yields[5] as well as from the incompatibility of the required protecting groups on the aziridine amine function, mainly with established solid-phase peptide synthesis protocols.[6] Versatile and rapid routes to synthesize modified peptides containing an aziridine ring would be welcomed.

Moreover, the aziridine ring substituents play an important role, modifying the reactivity and influencing the toxicity and above all the lipophilicity of these compounds. Studies on 2-cyano aziridines assigned an additional role to the cyano group for their pharmacological activity. In fact, the CN residue has been reported to react fast with cysteine at room temperature, behaving directly as the inhibitor of the active site of the cysteine proteases.[7]

We found that *N*-protected *O*-sulfonyl hydroxylamine derivatives[8] in the presence of inorganic bases can behave as good aziridinating agents of EWG (electron-withdrawing group) functionalized alkenes.[9] Therefore the latter were converted in high yields into their corresponding *N*-acyloxy aziridines bearing the most common EWG and different alkyl or aryl groups. These compounds can be regarded as interesting precursors of various natural or unnatural aminated compounds. Moreover,

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it is known that aziridines are activated toward nucleophilic ring opening not only through the protonation of the aziridine nitrogen but also by N-acylation.[10]

2. Results and discussion

Here we report a combinatorial ensemble of twenty-six *N*-functionalized aziridinyl peptides, 7 of which carry on the aziridine nitrogen another L-α-amino acidic residue and 2 malonic residues. Among them, 4 new amido aziridinyl peptides are synthesized by a highly selective hydrolysis of the cyano group.

2.1. Synthesis of 2-cyano aziridinyl peptides

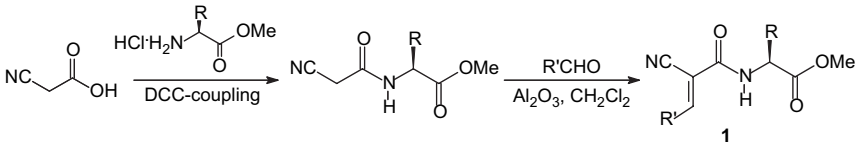
Very recently we prepared an array of peptides **1** starting from active methylene compounds, obtained from cyanoacetic acid and α-amino esters, and different aldehydes by Knoevenagel condensation reaction on Al_2O_3 (Scheme 2).[11]

Table 1
Aziridination of (*E*)-**1** with NsONH−Boc

Entry	Product	R	R′	Yield[a] (%)	dr[b]
1	**2a**	H	Et	82	—
2	**2b**	H	Pentyl	85	—
3	**2c**	H	^{i}Bu	91	—
4	**2d**	^{i}Pr	Et	81	2:1
5	**2e**	^{i}Pr	Pentyl	86	2:1
6	**2f**	^{i}Pr	^{i}Bu	91	2:1
7	**2g**	Bn	Et	90	2:1
8	**2h**	Bn	Pentyl	90	3:1
9	**2i**[c]	Bn	^{i}Bu	93	16:1

[a] After filtration through plugs filled with silica gel using a 9:1 hexane/ethyl acetate mixture.
[b] Determined by ^{1}H NMR spectroscopy and HPLC/UV analyses of the crude mixtures.
[c] Ref. 11.

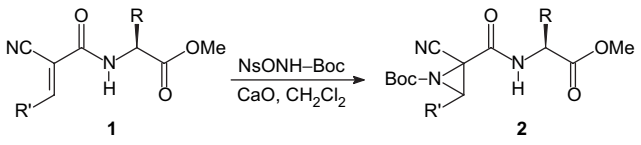
of applications. In fact, it is important to access each of the new diastereomeric products, because of the possible drastic difference of reactivity in biological matrices between stereo-



Scheme 2. Synthesis of (*E*)-acrylonitriles **1**.

Aziridine ring synthesis was tested using different nosyloxycarbamates (NsONH−Y, Ns=4-$NO_2C_6H_4SO_2$) in CH_2Cl_2 and in the presence of CaO as the base. The reactions were successfully carried out using aminating reagents with different Y groups (CO_2Et,[12] Boc,[8] Fmoc[13]) giving directly the *N*-acyloxy aziridines **2**. Full retention of the starting alkene configuration was always observed. The best results both for yields and diastereoselectivity were obtained by using *tert*-butyl nosyloxycarbamate (NsONH−Boc).

The aziridine library was constructed performing the amination reactions in a Carousel reaction station under heterogeneous phase conditions (CaO/CH_2Cl_2) at 0 °C (Scheme 3). For all reported reactions, substrate/NsONH−Boc/CaO molar ratio was 1:2:2.



Scheme 3. Synthesis of 1-Boc 2-cyano aziridinyl peptides.

N-Boc protected aziridinyl peptides **2** were obtained, after simple filtration of the crude reaction mixtures, in high yields and in the diastereomeric ratios as reported in Table 1.

A moderate diastereoselectivity was observed, except when phenylalanine methyl ester is used in combination with a hindered group on the carbon−carbon double bond (entry 9). Nevertheless, we underline that, at this stage of the research, a complete stereoselectivity can paradoxically result in a limit isomers. In all cases a separation of the diastereomeric mixture is desirable and actually performed by HPLC.

Since the discovery of epoxysuccinyl peptide E-64 in 1978 as a potent cysteine protease inhibitor,[14] a variety of inhibitors containing small rings, as electrophilic building blocks responsible for enzyme inhibition, have been developed.[15] The aziridinyl peptides **2** can be regarded as potential bioactive compounds, because the aziridine ring can be opened by nucleophiles not only through the cleavage of the C−N bond,[5a] but also through the cleavage of the C−C bond.[16] The advantage of an aziridine moiety compared to an epoxide ring is the option to lengthen the peptide chain via the aziridine N-atom. Addressing our interest in the synthesis of more complex peptidomimetic structures, the selective removal of the Boc protecting group results in a crucial step for obtaining a new functionalization site. Classic acidic conditions are not suitable for Boc removal, due to the presence of labile functional groups. The use of $BF_3\cdot Et_2O$[17] gave free aziridines, but in low yields. Finally, the deprotection reaction was successfully carried out using TBAF/THF[18] and the deprotected 2-cyano aziridinyl peptides **3** were obtained in the yields as reported in Table 2, after purification by flash chromatography.

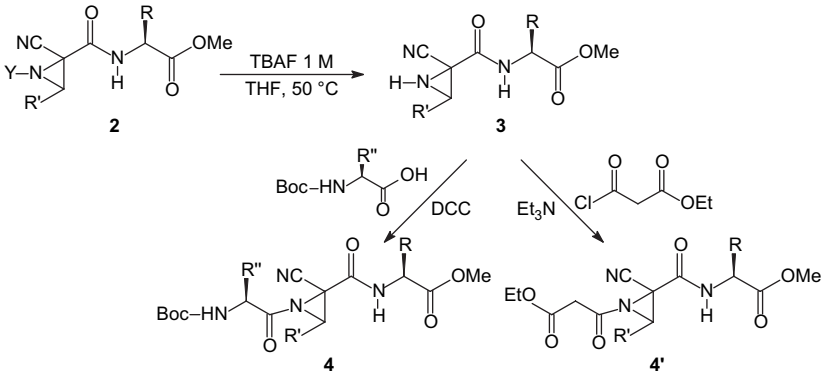
In order to introduce another α-amino acidic unit (entries 1−6) the DCC coupling procedure was chosen. Free cyano aziridines were made to react with commercially available *N*-Boc protected L-α-amino acids, namely Boc−L-Ala and Boc−L-Val, at room temperature. After work-up, 1-amino acyl 2-cyano aziridinyl peptides **4** carry the *N*-Boc that can be removed allowing a new possible site for peptide growth. As a different functionalization, a malonic unit was introduced on the aziridine

Table 2
Synthetic elaboration of *N*-Boc protected aziridinyl peptides

Entry	R	R′	Product	Yield[a] (%)	R″	Product	Yield[b] (%)
1	H	Et	**3a**	61	^{i}Pr	**4a**	91
2	H	^{i}Bu	**3c**	43	CH_3	**4c**	89
3	^{i}Pr	Et	**3d**	58	CH_3	**4d**	93
4	^{i}Pr	^{i}Bu	**3f**	51	CH_3	**4f**	88
5	Bn	Et	**3g**	65	CH_3	**4g**	95
6	Bn	^{i}Bu	**3i**	73	^{i}Pr	**4i**	90
7	Bn	^{i}Bu	**3i**	73	—	**4′i**	96

[a] After purification by flash chromatography on silica gel (eluent: 8:2 hexane/ethyl acetate).
[b] After purification by flash chromatography on silica gel (eluent: 7:3 hexane/ethyl acetate).

nitrogen by the reaction of **3i** with ethyl malonyl chloride leading to the *N*-malonyl 2-cyano aziridinyl peptide **4′i**, a methylene active compound suitable for further synthetic elaborations (Scheme 4).



Scheme 4. Deprotection and functionalization of aziridine nitrogen.

The results of synthetic elaborations of *N*-Boc protected aziridinyl peptides **2** were reported in Table 2.

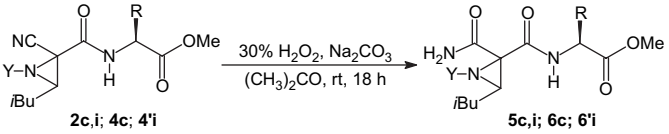
Recently, structures similar to aziridines **4**, also as a mixture of diastereomers, were tested like potential inhibitors of the M^{pro}, the main coronavirus protease.[19]

2.2. Synthesis of 2-amido aziridinyl peptides

The cyano group can be regarded as the precursor of other important functionalities, through selective chemical transformations. It is known that the presence of a C-terminal α-amido group on the peptide chain is essential for the biological activity of many peptide hormones.[20]

The hydrolysis of the cyano group can be achieved by means of several procedures, most of which are unsuitable for the preservation of aziridine rings and peptide bonds. In general the selective hydrolysis of nitriles to amides is not an easy goal, often suffering from low yields. In addition, tertiary nitriles are especially resistant toward hydrolysis and few successful examples are reported in the literature.[21]

This hydrolysis was attempted on representative members of 2-cyano aziridines, the *N*-Boc 2-cyano aziridines **2c** and **2i**,[11] the aziridinyl peptide **4c**, and the *N*-malonyl 2-cyano aziridinyl peptide **4′i** (Scheme 5).



Scheme 5. Selective hydrolysis of the cyano group.

C-Terminal modified amido peptides **5** and **6** were cleanly obtained in high yields and purity (HPLC/UV) for all selected aziridines without further purification. Remarkably, the tertiary CN groups carried on multifunctional heterocycles **2c**, **2i**, **4c**, and **4′i** were successfully converted into the desired amido function at room temperature, using an H_2O_2/Na_2CO_3 aqueous solution.[22] The results are reported in Table 3.

The selective hydrolysis led to construction of a malonic unit that is one of the most important structural modifications of the backbone of a natural peptide, allowing the reversal of the direction of the peptide bond and then the preparation of retro-peptides.[23]

Table 3
Synthesis of 2-amido aziridinyl peptides

Entry	Substrate	R	Y	Product	Yield (%)
1	**2c**	H	Boc	**5c**	81
2	**2i**	Bn	Boc	**5i**	93
3	**4c**	H	Boc–L-Ala	**6c**	91
4	**4′i**	Bn	$COCH_2CO_2Et$	**6′i**	90

3. Conclusions

A method for the preparation of cyano and amido peptides has been introduced. The strategy employs α-amino acidic functionalized (*E*)-acrylonitriles as building blocks to obtain libraries of different polyfunctionalized aziridines under parallel solution-phase conditions. The employment of L-α-amino acids for the construction of the starting acrylonitrile library can simplify the generation of biologically active compounds.

4. Experimental section

4.1. General methods

GC analyses were performed with an HP 5890 Series II gas chromatograph equipped with a capillary column (methyl

silicone, 12.5 m×0.2 mm) and a FID detector. IR spectra were recorded on a PERKIN ELMER 1600 FT/IR spectrophotometer in $CHCl_3$ as the solvent, and reported in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz or at 200 and 50 MHz with a Varian XL-300 or Gemini 200 NMR spectrometer, respectively, and reported in δ units. $CDCl_3$ was used as the solvent and $CHCl_3$ as the internal standard. ESI MS analyses were performed using a Micromass Q-TOF Micro quadrupole-time of flight (TOF) mass spectrometer equipped with an ESI source and a syringe pump. The experiments were conducted in the positive ion mode. HPLC analyses were performed with a VARIAN 9002 instrument using an analytical column (3.9×300 mm, flow rate 1.3 mL/min; detector: 254 nm) equipped with a Varian RI-4 differential refractometer, or a Varian 9050 UV/Vis detector. Eluents were HPLC grade. Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) silica gel plates. Silica gel 230–400 mesh was used for column chromatography. All solvents were dried following reported standard procedures.

4.2. Synthesis of 1-Boc 2-cyano aziridinyl peptides: general procedure

CaO (2 mmol) and NsONH–Boc (2 mmol) were added to a stirred solution of (*E*)-acrylonitriles **1a–i** (1 mmol) in CH_2Cl_2 at 0 °C. After the reaction was complete (TLC, 4 h), the crude aziridines were filtered through plugs filled with silica gel using a 9:1 hexane/ethyl acetate mixture, and the products **2a–i** were obtained as oils after solvent removal. The diastereomeric mixtures of 1-Boc 2-cyano aziridinyl peptides **2d–h** were separated by HPLC using an 8:2 hexane/ethyl acetate mixture (flow 1.3 mL/min) as the eluent.

4.2.1. tert-Butyl (2R,3R*)-2-cyano-3-ethyl-2-[(2-methoxy-2-oxoethyl)carbamoyl]aziridine-1-carboxylate (**2a**)*

Pale yellow oil, 82%. IR: 3402, 2250, 1736, 1697 cm^{-1} .^1H NMR: 1.14 (t, *J*=7.7 Hz, 3H), 1.42 (s, 9H), 1.63–1.82 (m, 2H), 3.07 (t, *J*=6.6 Hz, 1H), 3.77 (s, 3H), 4.08 (d, *J*=6.6 Hz, 2H), 7.04–7.18 (br, 1H). ^{13}C NMR: 10.9, 23.5, 28.1, 39.7, 42.2, 50.9, 52.9, 83.8, 114.5, 155.9, 161.2, 169.1. HRMS (ES Q-TOF) calcd for $C_{14}H_{22}N_3O_5$ $(M+H)^+$: 312.1554, found: 312.1563.

4.2.2. tert-Butyl (2R,3R*)-2-cyano-2-[(2-methoxy-2-oxoethyl)carbamoyl]-3-pentylaziridine-1-carboxylate (**2b**)*

Pale yellow oil, 85%. IR: 3404, 2252, 1738, 1694 cm^{-1}. ^1H NMR: 0.89 (t, *J*=6.6 Hz, 3H), 1.20–1.62 (m, 6H), 1.43 (s, 9H), 1.64–1.80 (m, 2H), 3.11 (t, *J*=6.6 Hz, 1H), 3.78 (s, 3H), 4.08 (d, *J*=6.6 Hz, 2H), 7.00–7.09 (br, 1H). ^{13}C NMR: 14.3, 22.7, 26.4, 28.1, 29.9, 31.4, 39.8, 42.2, 49.9, 53.0, 83.7, 114.6, 156.0, 161.2, 169.1. HRMS (ES Q-TOF) calcd for $C_{17}H_{28}N_3O_5$ $(M+H)^+$: 354.2023, found: 354.2030.

4.2.3. tert-Butyl (2R,3R*)-2-cyano-3-isobutyl-2-[(2-methoxy-2-oxoethyl)carbamoyl]aziridine-1-carboxylate (**2c**)*

Pale yellow oil, 91%. IR: 3404, 2249, 1735, 1689 cm^{-1}. ^1H NMR: 1.03 (d, *J*=6.6 Hz, 6H), 1.43 (s, 9H), 1.54–1.70 (m, 2H), 1.80–1.96 (m, 1H), 3.16 (t, *J*=6.6 Hz, 1H), 3.78 (s, 3H), 4.10 (d, *J*=6.6 Hz, 2H), 7.02–7.11 (br, 1H). ^{13}C NMR: 21.9, 22.4, 27.8, 38.0, 39.4, 41.9, 48.6, 52.6, 83.4, 114.6, 155.9, 161.0, 168.0. HRMS (ES Q-TOF) calcd for $C_{16}H_{26}N_3O_5$ $(M+H)^+$: 340.1867, found: 340.1881.

4.2.4. tert-Butyl (2R,3R*)-2-cyano-3-ethyl-2-{[(1S)-1-(methoxycarbonyl)-2-methylpropyl]carbamoyl}aziridine-1-carboxylate (**2d**)*

Pale yellow oil, 81%. IR: 3400, 2251, 1740, 1684 cm^{-1}. ^1H NMR (major isomer): 0.90–0.98 (m, 6H), 1.12 (t, *J*=6.6 Hz, 3H), 1.42 (s, 9H), 1.59–1.78 (m, 2H), 2.12–2.28 (m, 1H), 3.02 (t, *J*=6.6 Hz, 1H), 3.75 (s, 3H), 4.46–4.52 (m, 1H), 6.85–6.98 (br, 1H). ^1H NMR (minor isomer): 0.81–0.89 (m, 6H), 1.13 (t, *J*=6.6 Hz, 3H), 1.40 (s, 9H), 1.71–1.75 (m, 2H), 2.14–2.24 (m, 1H), 3.06 (t, *J*=6.6 Hz, 1H), 3.74 (s, 3H), 4.60–4.67 (m, 1H), 6.80–6.90 (br, 1H). ^{13}C NMR (major isomer): 10.5, 17.6, 18.8, 23.3, 27.8, 31.8, 39.6, 50.4, 52.4, 58.2, 83.3, 114.5, 155.9, 160.5, 171.1. ^{13}C NMR (minor isomer): 9.6, 17.7, 18.7, 23.1, 27.9, 31.8, 39.4, 50.4, 52.3, 58.1, 83.4, 114.2, 155.7, 160.7, 171.0. HRMS (ES Q-TOF) calcd for $C_{17}H_{28}N_3O_5$ $(M+H)^+$: 354.2023, found: 354.2039.

4.2.5. tert-Butyl (2R,3R*)-2-cyano-2-{[(1S)-1-(methoxycarbonyl)-2-methylpropyl]carbamoyl}-3-pentylaziridine-1-carboxylate (**2e**)*

Pale yellow oil, 86%. IR: 3402, 2254, 1738, 1679 cm^{-1}. ^1H NMR (major isomer): 0.92–0.99 (m, 9H), 1.23–1.64 (m, 6H), 1.43 (s, 9H), 1.66–1.77 (m, 2H), 2.13–2.28 (m, 1H), 3.07 (t, *J*=6.9 Hz, 1H), 3.76 (s, 3H), 4.46–4.54 (m, 1H), 6.94–6.97 (br, 1H). ^1H NMR (minor isomer): 0.85–0.93 (m, 9H), 1.24–1.61 (m, 6H), 1.41 (s, 9H), 1.62–1.75 (m, 2H), 2.13–2.28 (m, 1H), 3.10 (t, *J*=6.9 Hz, 1H), 3.76 (s, 3H), 4.47–4.53 (m, 1H), 6.95–7.01 (br, 1H). ^{13}C NMR (major isomer): 13.8, 17.6, 18.6, 22.3, 26.0, 27.7, 29.6, 31.0, 31.3, 39.7, 49.3, 52.5, 58.2, 83.2, 114.7, 156.0, 160.5, 171.1. ^{13}C NMR (minor isomer): 13.8, 17.7, 18.7, 22.3, 26.0, 27.7, 29.4, 31.0, 31.3, 39.6, 49.4, 52.4, 58.1, 83.4, 114.4, 155.8, 160.7, 171.0. HRMS (ES Q-TOF) calcd for $C_{20}H_{34}N_3O_5$ $(M+H)^+$: 396.2493, found: 396.2506.

4.2.6. tert-Butyl (2R,3R*)-2-cyano-3-isobutyl-2-{[(1S)-1-(methoxycarbonyl)-2-methylpropyl]carbamoyl}aziridine-1-carboxylate (**2f**)*

Pale yellow oil, 91%. IR: 3401, 2255, 1744, 1680 cm^{-1}. ^1H NMR (major isomer): 0.92–1.05 (m, 12H), 1.42 (s, 9H), 1.59–1.66 (m, 2H), 1.82–1.96 (m, 1H), 2.18–2.37 (m, 1H), 3.17 (t, *J*=6.6 Hz, 1H), 3.75 (s, 3H), 4.49–4.54 (m, 1H), 6.92–6.98 (br, 1H). ^1H NMR (minor isomer): 0.94–1.04 (m, 12H), 1.42 (s, 9H), 1.56–1.68 (m, 2H), 1.85–1.99 (m, 1H), 2.18–2.30 (m, 1H), 3.12 (t, *J*=6.6 Hz, 1H), 3.77 (s, 3H), 4.50–4.55 (m, 1H), 6.94–6.97 (br, 1H). ^{13}C NMR (major isomer): 17.6, 18.8, 21.9, 22.4, 26.6, 27.7, 31.1, 38.1, 39.6, 48.3, 52.4, 58.2, 83.2, 114.7, 156.0, 160.5, 171.1. ^{13}C NMR (minor isomer): 17.7, 18.7, 21.9, 22.5, 26.8, 27.6, 31.2, 37.8, 39.6, 48.3, 52.3, 58.1, 83.3, 114.6, 155.9, 160.6, 170.9. HRMS (ES Q-TOF) calcd for $C_{19}H_{32}N_3O_5$ $(M+H)^+$: 382.2386, found: 382.2401.

4.2.7. tert-Butyl (2R,3R*)-2-{[(1S)-1-benzyl-2-methoxy-2-oxoethyl]carbamoyl}-2-cyano-3-ethylaziridine-1-carboxylate (**2g**)*

Yellow oil, 90%. IR: 3405, 2250, 1742, 1682 cm^{-1}. ^1H NMR (major isomer): 1.11 (t, J=7.5 Hz, 3H), 1.44 (s, 9H), 1.48–1.61 (m, 2H), 2.91 (t, J=6.6 Hz, 1H), 3.13 (d, J=6.0 Hz, 2H), 3.72 (s, 3H), 4.76–4.86 (m, 1H), 6.88–6.96 (br, 1H), 7.07–7.18 (m, 2H), 7.24–7.35 (m, 3H). ^1H NMR (minor isomer): 1.24 (t, J=7.5 Hz, 3H), 1.42 (s, 9H), 1.58–1.84 (m, 2H), 2.97 (t, J=6.6 Hz, 1H), 3.16 (d, J=6.0 Hz, 2H), 3.73 (s, 3H), 4.76–4.86 (m, 1H), 6.88–6.96 (br, 1H), 7.08–7.15 (m, 2H), 7.23–7.36 (m, 3H). ^{13}C NMR (major isomer): 10.4, 23.1, 27.7, 37.6, 39.4, 50.5, 52.5, 54.0, 83.4, 114.1, 127.5, 128.8, 129.1, 134.9, 155.7, 160.4, 170.5. ^{13}C NMR (minor isomer): 10.4, 23.0, 27.6, 37.6, 39.3, 50.5, 52.5, 53.9, 83.4, 114.0, 127.4, 128.7, 129.1, 134.9, 155.6, 160.4, 170.6. HRMS (ES Q-TOF) calcd for $C_{21}H_{28}N_3O_5$ $(M+H)^+$: 402.2023, found: 402.2028.

4.2.8. tert-Butyl (2R,3R*)-2-{[(1S)-1-benzyl-2-methoxy-2-oxoethyl]carbamoyl}-2-cyano-3-pentylaziridine-1-carboxylate (**2h**)*

Yellow oil, 90%. IR: 3402, 2249, 1745, 1684 cm^{-1}. ^1H NMR (major isomer): 0.88 (t, J=6.6 Hz, 3H), 1.22–1.73 (m, 8H), 1.43 (s, 9H), 2.94 (t, J=6.6 Hz, 1H), 3.12 (d, J=6.0 Hz, 2H), 3.72 (s, 3H), 4.78–4.85 (m, 1H), 6.92–6.96 (br, 1H), 7.11–7.16 (m, 2H), 7.25–7.33 (m, 3H). ^1H NMR (minor isomer): 0.89 (t, J=6.6 Hz, 3H), 1.25–1.72 (m, 8H), 1.42 (s, 9H), 3.00 (t, J=6.6 Hz, 1H), 3.15 (d, J=6.0 Hz, 2H), 3.73 (s, 3H), 4.78–4.85 (m, 1H), 6.86–6.93 (br, 1H), 7.07–7.12 (m, 2H), 7.23–7.30 (m, 3H). ^{13}C NMR (major isomer): 13.8, 22.3, 25.9, 26.7, 27.6, 30.9, 37.5, 39.4, 49.5, 52.5, 54.0, 83.4, 114.1, 127.4, 128.8, 129.1, 134.9, 155.8, 160.5, 170.5. ^{13}C NMR (minor isomer): 13.8, 22.3, 25.9, 27.1, 27.7, 31.0, 37.6, 39.4, 49.5, 52.5, 53.9, 83.4, 114.1, 127.4, 128.7, 129.1, 134.9, 155.7, 160.4, 170.6. HRMS (ES Q-TOF) calcd for $C_{24}H_{34}N_3O_5$ $(M+H)^+$: 444.2493, found: 444.2512.

4.3. Deprotection of 1-Boc 2-cyano aziridinyl peptides: general procedure

To a THF solution of **2a**, **2c**, **2d**, **2f**, **2g**, and **2i** (0.2 mmol of pure major diastereomer) a solution of tetrabutylammonium fluoride (TBAF, 1 M in THF) in equimolar amount was added, and the mixture was stirred under reflux for 20 min. The crude mixture was then dissolved in ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$ in a separator funnel. The organic layer was dried over Na_2SO_4 and filtered. The deprotected 2-cyano aziridinyl peptides **3** were obtained as pure compounds after chromatographic purification on silica gel (eluent: 8:2 hexane/ethyl acetate mixture).

4.3.1. (2R,3R*)-2-Cyano-3-ethyl-2-[(2-methoxy-2-oxoethyl)carbamoyl]aziridine (**3a**)*

Pale yellow oil, 61%. IR: 3425, 3400, 2246, 1740, 1698 cm^{-1}. ^1H NMR: 1.11 (t, J=7.2 Hz, 3H), 1.58–1.76 (m, 2H), 2.26–2.35 (m, 1H), 2.48–2.60 (m, 1H), 3.78 (s, 3H), 4.06–4.17 (m, 2H), 6.85–7.90 (br, 1H). ^{13}C NMR: 10.8, 24.2, 34.7, 42.1, 47.5, 52.7, 114.0, 164.7, 170.5. HRMS (ES Q-TOF) calcd for $C_9H_{14}N_3O_3$ $(M+H)^+$: 212.1030, found: 212.1041.

4.3.2. (2R,3R*)-2-Cyano-3-isobutyl-2-[(2-methoxy-2-oxoethyl)carbamoyl]aziridine (**3c**)*

Pale yellow oil, 43%. IR: 3434, 3400, 2242, 1748, 1692 cm^{-1}. ^1H NMR: 0.97 (d, J=6.9 Hz, 6H), 1.45–1.55 (m, 2H), 1.77–1.86 (m, 1H), 2.24–2.42 (m, 1H), 2.53–2.60 (m, 1H), 3.75 (s, 3H), 4.02–4.18 (m, 2H), 6.91–7.10 (br, 1H). ^{13}C NMR: 22.4, 26.7, 34.6, 39.0, 42.0, 45.0, 52.5, 116.7, 164.7, 169.0. HRMS (ES Q-TOF) calcd for $C_{11}H_{18}N_3O_3$ $(M+H)^+$: 240.1343, found: 240.1347.

4.3.3. (2R,3R*)-2-Cyano-3-ethyl-2-{[(1S)-1-(methoxycarbonyl)-2-methylpropyl]carbamoyl}aziridine (**3d**)*

Pale yellow oil, 58%. IR: 3420, 3404, 2249, 1742, 1691 cm^{-1}. ^1H NMR: 0.86–1.16 (m, 9H), 1.43–1.60 (m, 2H), 1.74–1.82 (m, 1H), 2.18–2.29 (m, 1H), 2.46–2.60 (m, 1H), 3.76 (s, 3H), 4.36–4.52 (m, 1H), 6.86–7.07 (br, 1H). ^{13}C NMR: 11.0, 17.4, 18.2, 24.6, 30.3, 38.6, 48.9, 52.0, 57.7, 115.1, 160.5, 169.9. HRMS (ES Q-TOF) calcd for $C_{12}H_{20}N_3O_3$ $(M+H)^+$: 254.2976, found: 254.2984.

4.3.4. (2R,3R*)-2-Cyano-3-isobutyl-2-{[(1S)-1-(methoxycarbonyl)-2-methylpropyl]carbamoyl}aziridine (**3f**)*

Pale yellow oil, 51%. IR: 3425, 3404, 2250, 1741, 1688 cm^{-1}. ^1H NMR: 0.84–1.06 (m, 12H), 1.55–1.78 (m, 2H), 1.80–2.00 (m, 1H), 2.10–2.34 (m, 2H), 3.08–3.19 (m, 1H), 3.78 (s, 3H), 4.52–4.61 (m, 1H), 6.85–7.00 (br, 1H). ^{13}C NMR: 17.8, 18.6, 22.3, 22.9, 26.6, 30.4, 39.2, 49.7 52.4, 58.0, 115.2, 160.2, 171.0. HRMS (ES Q-TOF) calcd for $C_{14}H_{24}N_3O_3$ $(M+H)^+$: 282.1812, found: 282.1821.

4.3.5. (2R,3R*)-2-{[(1S)-1-Benzyl-2-methoxy-2-oxoethyl]carbamoyl}-2-cyano-3-ethylaziridine (**3g**)*

Yellow oil, 65%. IR: 3563, 3402, 2245, 1749, 1689 cm^{-1}. ^1H NMR: 1.15 (t, J=7.2 Hz, 3H), 1.42–1.59 (m, 2H), 2.25 (d, J=6.0 Hz, 1H), 2.43–2.57 (m, 1H), 3.18 (d, J=6.0 Hz, 2H), 3.74 (s, 3H), 4.80–4.85 (m, 1H), 6.70–6.92 (br, 1H), 7.11–7.16 (m, 2H), 7.24–7.36 (m, 3H). ^{13}C NMR: 10.09, 27.1, 37.7, 39.3, 45.0, 52.3, 54.8, 115.9, 127.3, 128.8, 129.2, 134.9, 164.1, 170.5. HRMS (ES Q-TOF) calcd for $C_{16}H_{20}N_3O_3$ $(M+H)^+$: 302.3477, found: 302.3462.

4.3.6. (2R,3R*)-2-{[(1S)-1-Benzyl-2-methoxy-2-oxoethyl]carbamoyl}-2-cyano-3-isobutylaziridine (**3i**)*

Yellow oil, 73%. IR: 3563, 3402, 2245, 1749, 1689 cm^{-1}. ^1H NMR: 0.95 (d, J=6.6 Hz, 6H), 1.39–1.59 (m, 2H), 1.75–1.85 (m, 1H), 2.27 (m, 1H), 2.44–2.52 (m, 1H), 3.20 (m, 2H), 3.74 (s, 3H), 4.73–4.90 (m, 1H), 6.82–6.96 (br, 1H), 7.09–7.18 (m, 2H), 7.24–7.35 (m, 3H). ^{13}C NMR: 22.4, 26.6, 34.6, 37.6, 39.0, 44.8, 52.5, 54.2, 116.4, 127.4, 128.6, 129.0, 135.1, 163.9, 170.6. HRMS (ES Q-TOF) calcd for $C_{18}H_{24}N_3O_3$ $(M+H)^+$: 330.1812, found: 330.1826.

4.4. Synthesis of 1-amino acyl 2-cyano aziridinyl peptides: general procedure

To a CH_2Cl_2 solution of the 2-cyano peptides **3a**, **3c**, **3d**, **3f**, **3g**, and **3i** (0.5 mmol of pure major diastereomers) commercial Boc–L-Ala or Boc–L-Val and DCC in equimolar amounts and DMAP in catalytic amounts were added at room temperature. The reactions were followed by TLC until completion (24 h). After filtration, aziridinyl peptides **4** were obtained as pure compounds after flash chromatography on silica gel (eluent: 7:3 hexane/ethyl acetate mixture).

4.4.1. Methyl ({[(2R*,3R*)-1-(L-N-[(tert-butoxycarbonyl)-valyl-2-cyano-3-ethylaziridin-2-yl]carbonyl}amino)acetate (**4a**)

Yellow oil, 89%. IR: 3679, 3435, 2251, 1747, 1698 cm^{-1}. ^1H NMR: 1.09 (t, J=7.2 Hz, 3H), 1.30–1.72 (m, 18H), 2.45–2.64 (br, 1H), 2.90–3.24 (m, 1H), 3.67–3.70 (m, 1H), 3.78 (s, 3H), 4.03–4.17 (m, 2H), 7.01–7.12 (br, 1H). ^{13}C NMR: 11.2, 22.3, 22.9, 25.7, 27.9, 34.2, 39.6, 41.8, 45.5, 53.0, 85.1, 115.8, 155.3, 155.5, 164.2, 170.1. HRMS (ES Q-TOF) calcd for $C_{19}H_{31}N_4O_6$ $(M+H)^+$: 411.2238, found: 411.2249.

4.4.2. Methyl [({(2R*,3R*)-1-[L-N-(tert-butoxycarbonyl)-alanyl]-2-cyano-3-isobutylaziridin-2-yl}carbonyl)amino]-acetate (**4c**)

Yellow oil, 91%. IR: 3683, 3436, 2249, 1749, 1698 cm^{-1}. ^1H NMR: 0.96–1.05 (m, 6H), 1.35–1.75 (m, 13H), 1.80–1.98 (m, 2H), 2.42–2.64 (br, 1H), 3.08–3.19 (br, 1H), 3.67–3.71 (m, 1H), 3.78 (s, 3H), 4.01–4.15 (m, 2H), 7.04–7.16 (br, 1H). ^{13}C NMR: 17.8, 18.6, 26.7, 27.6, 28.3, 34.6, 39.4, 42.0, 45.2, 52.6, 84.9, 116.5, 155.1, 155.4, 164.7, 169.1. HRMS (ES Q-TOF) calcd for $C_{19}H_{31}N_4O_6$ $(M+H)^+$: 411.2238, found: 411.2247.

4.4.3. Methyl (2S)-2-[({(2R*,3R*)-1-[L-N-(tert-butoxy-carbonyl)alanyl]-2-cyano-3-ethylaziridin-2-yl}carbonyl)-amino]-3-methylbutanoate (**4d**)

Yellow oil, 93%. IR: 3540, 3406, 2252, 1742, 1688 cm^{-1}. ^1H NMR: 0.79–1.03 (m, 6H), 1.11 (t, J=7.2 Hz, 3H), 1.20–1.24 (m, 3H), 1.42–1.64 (m, 11H), 1.71–1.89 (m, 1H), 2.10–2.40 (m, 1H), 2.41–2.58 (br, 1H), 3.60–3.89 (m, 1H), 3.76 (s, 3H), 4.46–4.55 (m, 1H), 6.78–7.00 (br, 1H). ^{13}C NMR: 11.6, 17.4, 18.5, 22.0, 22.7, 26.8, 27.8, 34.6, 39.4, 42.0, 45.2, 52.6, 84.9, 116.5, 155.1, 155.4, 164.7, 169.1. HRMS (ES Q-TOF) calcd for $C_{20}H_{33}N_4O_6$ $(M+H)^+$: 425.2395, found: 425.2403.

4.4.4. Methyl (2S)-2-[({(2R*,3R*)-1-[L-N-(tert-butoxy-carbonyl)alanyl]-2-cyano-3-isobutylaziridin-2-yl}carbonyl)amino]-3-methylbutanoate (**4f**)

Yellow oil, 88%. IR: 3540, 3406, 2252, 1742, 1688 cm^{-1}. ^1H NMR: 0.80–1.05 (m, 12H), 1.20–1.26 (m, 3H), 1.40–1.61 (m, 12H), 1.79–1.98 (m, 1H), 2.10–2.39 (m, 1H), 2.44–2.62 (br, 1H), 3.68–3.85 (m, 1H), 3.76 (s, 3H), 4.41–4.50 (m, 1H), 6.75–6.98 (br, 1H). ^{13}C NMR: 17.8, 18.6, 21.9, 22.3, 22.6, 27.0, 27.6, 34.5, 39.5, 42.3, 44.9, 52.7, 58.5, 84.9, 115.9, 155.2, 155.6, 165.0, 169.2. HRMS (ES Q-TOF) calcd for $C_{22}H_{37}N_4O_6$ $(M+H)^+$: 453.2708, found: 453.2708, 453.2692.

4.4.5. Methyl (2S)-2-[({(2R*,3R*)-1-[L-N-(tert-butoxy-carbonyl)alanyl]-2-cyano-3-ethylaziridin-2-yl}carbonyl)-amino]-3-phenylpropanoate (**4g**)

Pale orange oil, 95%. IR: 3502, 3400, 2244, 1746, 1692 cm^{-1}. ^1H NMR: 1.05 (t, J=7.2 Hz, 3H), 1.34–1.64 (m, 14H), 1.82–2.05 (m, 1H), 2.00–2.34 (br, 1H), 2.90–3.32 (m, 1H), 3.15 (d, J=6.0 Hz, 2H), 3.76 (s, 3H), 4.71–4.85 (m, 1H), 6.81–6.92 (br, 1H), 6.97–7.06 (m, 2H), 7.12–7.36 (m, 3H). ^{13}C NMR: 11.6, 23.8, 26.9, 27.8, 34.6, 39.4, 42.0, 45.2, 52.6, 54.2, 84.9, 116.5, 127.7, 128.6, 129.3, 135.1, 155.1, 155.4, 164.7, 169.1. HRMS (ES Q-TOF) calcd for $C_{24}H_{33}N_4O_6$ $(M+H)^+$: 473.2395, found: 473.2408.

4.4.6. Methyl (2S)-2-[({(2R*,3R*)-1-[L-N-(tert-butoxy-carbonyl)valyl]-2-cyano-3-isobutylaziridin-2-yl}carbonyl)-amino]-3-phenylpropanoate (**4i**)

Pale orange oil, 90%. IR: 3502, 3400, 2244, 1746, 1692 cm^{-1}. ^1H NMR: 0.76–1.02 (m, 12H), 1.34–1.46 (m, 2H), 1.41 (s, 9H), 1.48–1.66 (m, 1H), 1.69–1.92 (m, 1H), 2.00–2.34 (br, 1H), 2.90–3.32 (m, 3H), 3.67 (s, 3H), 3.98–4.20 (m, 1H), 4.71–4.85 (m, 1H), 6.92–7.05 (br, 1H), 7.12–7.36 (m, 5H). ^{13}C NMR: 17.5, 18.6, 21.9, 22.1, 22.6, 26.5, 27.8, 34.5, 37.4, 39.2, 44.4, 52.5, 53.9, 54.6, 85.0, 115.8, 127.3, 128.5, 129.0, 135.1, 155.0, 155.3, 164.7, 169.3. HRMS (ES Q-TOF) calcd for $C_{28}H_{41}N_4O_6$ $(M+H)^+$: 529.3021, found: 529.3038.

4.4.7. Synthesis of ethyl 3-[(2R*,3R*)-2-cyano-3-isobutyl-2-({[(1S)-1-benzyl-2-methoxy-2-oxoethyl]carbamoyl}aziridin-1-yl)]-3-oxopropanoate (**4′i**)

To a stirred solution of **3i** (1 mmol) in anhydrous CH_2Cl_2 ethyl malonyl chloride (1 mmol) was added dropwise at 0 °C. Then triethylamine (1 mmol) dissolved in anhydrous CH_2Cl_2 was gently added to the mixture. The solution was then stirred for additional 15 min and then filtered through a plug filled with a layer of silica gel. Compound **4′i** was obtained after solvent removal, as an orange oil, 96%. IR: 3563, 2245, 1742, 1694 cm^{-1}. ^1H NMR: 0.99 (d, J=6.0 Hz, 6H), 1.24–1.30 (m, 3H), 1.45–1.90 (m, 3H), 2.24–2.30 (m, 1H), 3.05–3.25 (m, 4H), 3.78 (s, 3H), 4.16–4.25 (m, 2H), 4.76–4.94 (m, 1H), 7.08–7.35 (m, 6H). ^{13}C NMR: 14.0, 21.9, 22.4, 26.6, 37.7, 38.1, 44.0, 48.3, 52.5, 54.2, 61.5, 114.1, 127.5, 128.7, 129.2, 134.9, 160.4, 166.1, 170.4, 172.6. HRMS (ES Q-TOF) calcd for $C_{23}H_{30}N_3O_6$ $(M+H)^+$: 444.2129, found: 444.2136.

4.5. Synthesis of 2-amido aziridinyl peptides: general procedure

To cyano aziridinyl peptides **2c**, **2i**, **4c**, and **4′i** (0.5 mmol) dissolved in acetone (4.0 mL), an 1 N aqueous solution of Na_2CO_3 (0.5 mL, 0.25 mmol) and 30% H_2O_2 (1.7 mL, 15.0 mmol) were added. The mixture was stirred for 18 h at

room temperature. After reaction completion (TLC), the solvent was removed under vacuum, and the resulting aqueous mixture was extracted with CH_2Cl_2. The products were obtained with high purity after solvent removal.

4.5.1. tert-Butyl (2R,3R*)-2-carbamoyl-3-isobutyl-2-[(2-methoxy-2-oxoethyl)carbamoyl]aziridine-1-carboxylate (**5c**)*

Viscous yellow oil, 81%. IR: 3511, 3392, 3324, 1797, 1732, 1698, 1667 cm^{-1}. ^1H NMR: 0.96 (d, J=6.0 Hz, 6H), 1.21–1.27 (m, 2H), 1.44 (s, 9H), 1.78–1.84 (m, 1H), 2.94–3.14 (br, 1H), 3.72 (s, 3H), 4.04–4.06 (m, 2H), 6.42–6.51 (br, 1H), 6.83–7.00 (br, 1H), 8.74–8.94 (br, 1H). ^{13}C NMR: 22.0, 22.4, 27.8, 36.3, 41.6, 49.2, 49.5, 52.3, 82.1, 157.8, 164.5, 169.8, 170.0. HRMS (ES Q-TOF) calcd for $C_{16}H_{28}N_3O_6$ $(M+H)^+$: 358.1973, found: 358.1984.

4.5.2. tert-Butyl (2R,3R*)-2-{[(1S)-1-benzyl-2-methoxy-2-oxoethyl]carbamoyl}-2-carbamoyl-3-isobutylaziridine-1-carboxylate (**5i**)*

Viscous orange oil, 93%. IR: 3511, 3394, 3322, 1734, 1697, 1665 cm^{-1}. ^1H NMR: 0.95 (d, J=6.0 Hz, 6H), 1.21–1.28 (m, 2H), 1.45 (s, 9H), 1.78–1.80 (m, 1H), 2.78–2.98 (br, 1H), 3.00–3.18 (m, 2H), 3.68 (s, 3H), 4.68–4.87 (m, 1H), 6.24–6.42 (br, 1H), 6.62–6.90 (br, 1H), 7.07–7.38 (m, 5H), 8.78–8.94 (br, 1H). ^{13}C NMR: 22.0, 22.4, 26.7, 27.9, 35.4, 36.2, 37.7, 49.3, 52.2, 54.1, 82.0, 127.0, 128.4, 129.2, 135.8, 157.8, 163.7, 169.9, 171.1. HRMS (ES Q-TOF) calcd for $C_{23}H_{34}N_3O_6$ $(M+H)^+$: 448.2442, found: 448.2456.

4.5.3. Methyl [({(2R,3R*)-1-[L-N-(tert-butoxycarbonyl)alanyl]-2-carbamoyl-3-isobutylaziridin-2-yl}carbonyl)amino]acetate (**6c**)*

Viscous yellow oil, 91%. IR: 3504, 3380, 3323, 1752, 1689, 1658 cm^{-1}. ^1H NMR: 0.91–1.01 (m, 9H), 1.23–1.57 (m, 10H), 1.72–1.86 (m, 2H), 2.38–2.55 (m, 2H), 3.72–3.80 (m, 1H), 3.75 (s, 3H), 4.01–4.15 (m, 2H), 5.70–5.90 (br, 1H), 6.82–7.01 (br, 1H), 8.95–9.16 (br, 1H). ^{13}C NMR: 17.6, 18.8, 26.7, 27.5, 28.3, 34.5, 39.8, 42.0, 45.2, 53.0, 84.9, 155.0, 155.4, 164.5, 169.3, 170.2. HRMS (ES Q-TOF) calcd for $C_{19}H_{33}N_4O_7$ $(M+H)^+$: 429.2344, found: 429.2352.

4.5.4. Ethyl 3-[(2R,3R*)-2-carbamoyl-3-isobutyl-2-({[(1S)-1-benzyl-2-methoxy-2-oxoethyl]carbamoyl}aziridin-1-yl)]-3-oxopropanoate (**6′i**)*

Viscous orange oil, 90%. IR: 3563, 2245, 1742, 1694 cm^{-1}. ^1H NMR: 0.99 (d, J=6.0 Hz, 6H), 1.24–1.30 (m, 3H), 1.45–1.90 (m, 3H), 2.24–2.30 (m, 1H), 3.05–3.25 (m, 4H), 3.78 (s, 3H), 4.16–4.25 (m, 2H), 4.80–4.89 (m, 1H), 6.20–6.28 (br, 1H), 6.60–6.81 (br, 1H), 7.08–7.35 (m, 5H). 8.84–9.03 (br, 1H). ^{13}C NMR: 14.1, 22.0, 22.4, 26.8, 37.5, 38.1, 44.4, 48.4, 52.8, 53.9, 61.8, 127.5, 128.7, 129.2, 134.9, 160.4, 166.1, 170.1, 170.6, 172.6. HRMS (ES Q-TOF) calcd for $C_{23}H_{32}N_3O_7$ $(M+H)^+$: 462.2235, found: 462.2247.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.01.098.

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