*Scientific paper*

# The Synthesis of (2*R*)-Aziridine-2-carboxylic Acid Containing Dipeptides

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*Received: 03-05-2021*

## Abstract

Optimized conditions for the synthesis of fully deprotected (2*R*)-aziridine containing dipeptides are described. Preparation of fully protected N- and C- terminal aziridine containing dipeptides was found to be straightforward and high yielding for the majority of compounds, whereas their full deprotection was possible only for C-terminal analogs. Deprotection of N-terminal derivatives using standard procedures of peptide chemistry was found difficult providing only mixtures of unidentifiable products. The described molecules have potential as building blocks in synthetic chemistry, in the chemical biology arena, as covalent modifiers, and as biomarkers.

**Keywords**: Dipeptides; aziridines; biomarkers; warheads; antibacterial agents

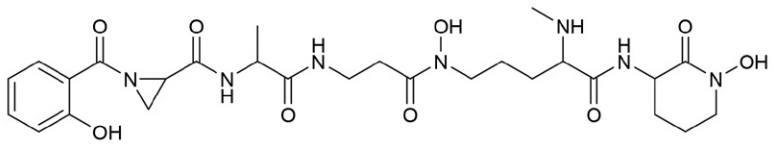
## 1. Introduction

Small molecules that mimic the structures of bioactive peptides are an important synthetic tool in organic chemistry. A special interest has been given to the synthesis of conformationally restricted β-turn dipeptide mimetics, peptide bond isosteres and nonproteinogenic derivatives to obtain drug-like target molecules.[1–3] A common strategy, that has been also widely applied, especially in the design of several protease inhibitors is to incorporate an electrophilic warhead that forms a covalent bond between amino acids in the active site and the inhibitor.[4,5]

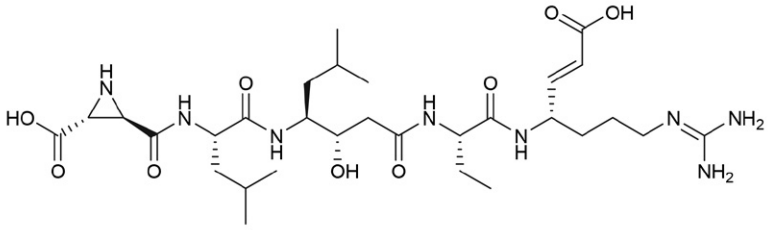
The importance of aziridine-2-carboxylic acid, as well as aziridine-containing peptides as useful intermediates in the synthesis of various amino acid and peptide derivatives, has been extensively recognized, both from a medicinal and a synthetic point of view.[6–10] Furthermore, despite their reactivity aziridine-containing peptides and related compounds may be found in numerous bioactive compounds of natural origin, such as madurastatin A1[11] and miraziridine A[12] (Figure 1). In addition, this heterocyclic fragment has also been incorporated in numerous synthetic peptides as reactive electrophilic warheads to obtain promising irreversible inhibitors of different proteases, such as cathepsins,[13,14] papain,[15] aspartate proteases,[16] HIV proteases[17] and SARS-CoV main protease.[18]

Their unusual reactivity due to ring strain renders aziridine-2-carboxylic acid derivatives important for the preparation of amino acids,[19–21] amino alcohols,[22,23] peptides and other peptide-like compounds.[17,24–33] In addition, they have also been used for labelling biomolecules with fluorine-18.[34,35]

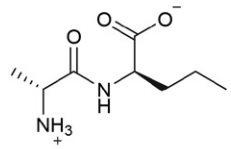
Consequently, the preparation of aziridine-containing dipeptides, which is a subject of this communication, is of great importance for medicinal as well as organic chemistry. There have been many reports in the literature describing their synthesis.[36,37] Nonetheless, up to now, literature reports on the preparation of partially or fully deprotected dipeptide derivatives have been rare. To the best of our knowledge, there has been only one report by Korn[36] describing the synthesis of fully deprotected H-Azy-Leu-OH and only a couple of papers describing the synthesis of N-deprotected[21,27,38,39] and C-deprotected dipeptides.[34,36] Most of them suffer from low isolated yields following work-up and chromatography which reflects the inherent instability of these compounds. At the same time, it is known that aziridine-containing peptides possess the potential antifungal,[16,40] antiviral[18] antiprotozoal[13,14] and cytostatic activity.[13–15,41] Clearly, a more versatile approach to deprotected aziridine-containing dipeptides is desired, given their potential to serve as electrophiles in reactions with appropriate nucleophiles, as biomarkers and bioactive compounds.



Madurastatin A1



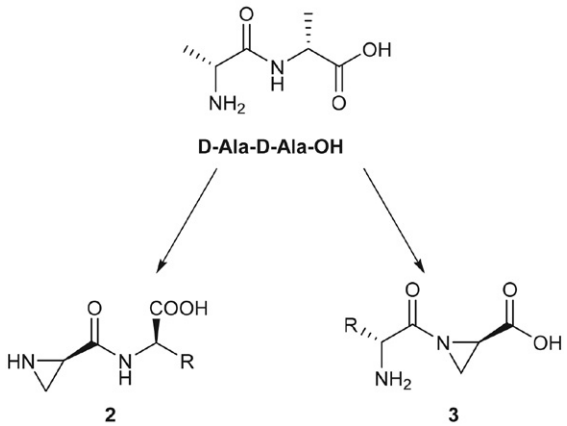
Miraziridine A



**1**, $IC_{50}$ (Ddl) = 0.55 mM

**Figure 1.** Natural products with aziridine ring and D-Ala:D-Ala ligase (Ddl) inhibitor **1**.

During our ongoing study concerning the dipeptide D-Ala-D-Ala, we were interested in preparing its electrophilic aziridine-containing analogs with potential biological activity. D-Ala-D-Ala sequence is found in the stem termini of peptidoglycan side-chain pentapeptide and is recognized by multiple essential bacterial enzymes. Any molecule that could mimic its structure thus offers an attractive potential toward the development of new inhibitors or as a false substrate for any of those enzymes.[42] Indeed, it was reported that dipeptides with D-Ala as a first amino acid inhibit bacterial enzyme D-Alanine:D-Alanine ligase (Ddl) in micromolar range (**1**, Figure 1).[43] (*R*)-aziridine-2-carboxylic acid was therefore chosen as a rigid surrogate of D-Ala because of its electrophilic character and close resemblance to D-Ala.



**2** **3**

**Figure 2**. N- and C-terminal aziridine containing dipeptides.

Herein, we report details of our efforts to develop a new approach to the preparation of a series of fully deprotected N-terminal aziridine containing dipeptides with general structure **2**. In addition, an attempt on the synthesis of C- terminal aziridine containing dipeptides with general structure **3** is also reported (Figure 2).

The designed molecules are very attractive not only because of their biological potential but also because aziridines have become important building blocks in synthetic chemistry. Such small dipeptides have a potential as covalent modifiers and are therefore useful in the chemical biology arena with different applications including bioconjugation, activity-based protein profiling and target identification.[6,8]

# 2. Experimental

## 2. 1. General Methods

The reactions were monitored by TLC carried out on Merck silica gel (60 F254) by using UV light as a visualizing agent, $KMnO_4$ in water, phosphomolybdic acid in ethanol and ninhydrin in ethanol and heat as developing agents. Column chromatography was performed using Merck Silica Gel 60. Proton nuclear magnetic resonance spectra ($^1$H NMR) were obtained at 400 MHz on Bruker Avance III 400 spectrometers. Spectra were recorded in $CDCl_3$, MeOD and DMSO-$d_6$ solutions. Chemical shifts are reported in ppm, referenced to tetramethylsilane (TMS) as the external reference. Carbon-13 nuclear magnetic resonance spectra ($^{13}$C NMR) were obtained at 100 MHz on Bruker 400 spectrometer. Chemical shifts are reported in ppm, referenced to the

solvent peak of $CDCl_3$. Low-resolution mass spectra were obtained with a Shimadzu GC-MS-QP2010 mass spectrometer. High resolution mass spectra (HRMS) were recorded on Q Executive Plus LC-MS/MS system (Thermo Scientific). Melting points were found on the Cambridge instruments melting point apparatus and are corrected. Optical rotation was found with Perkin Elmer 1241MC polarimeter at wavelength 589,3 nm (l = 10 cm). Ethyl acetate and methanol were used as solvents. Infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer. Melting points were determined using a Reichert hot-stage microscope and are corrected. HPLC analyses were performed on an HPLC Dionex UltiMate 3000 instrument with a UV-VIS detector. Three different analytical methods were used. Method 1: Column: Phenomenex Luna® 5 µm C18 100 Å; Injection volume: 10 µL; Flow rate: 1,5 mL/min; Detection wavelengths: λ = 210 nm, 220 nm, 254 nm and 280 nm; Column temperature: 25 °C; Mobile phase: 40% ACN in water to 100% ACN in 15 min. Method 2: Column: Supelco SUPELCOSIL™ LC-1 HPLC; Injection volume: 1 µL; Flow rate: 1 mL/min; Detection Wavelengths: λ = 195 nm, 210 nm, 220 nm and 254 nm; Column temperature: 25 °C; Mobile phase: 5% ACN in 20 mM phosphate buffer (pH = 2.10) to 60% ACN in 20 min. Method 3: Column: Agilent ZORBAX Extend-C18; Injection volume: 5 µL; Flow rate: 1 mL/min; Detection wavelengths λ = 210 nm, 220 nm, 254 nm and 280 nm; Column temperature25 °C; Mobile phase: 40% ACN in water to 100% ACN in 20 min.

## 2. 2. Synthesis and Characterization

**(R)-3-hydroxy-1-methoxy-1-oxopropan-2-aminium chloride (4).**

To a solution of D-serine (20.00 g, 190.3 mmol, 1.00 equiv.) in 500 mL MeOH, $SOCl_2$ (24.90 mL, 342.6 mmol, 1.80 equiv.) was added at 0 °C. The reaction mixture was stirred at 80 °C for 2 h and stirred at room temperature for an additional 20 h. The solution was concentrated under reduced pressure and diethyl ether (250 mL) was added to remove the excess of HCl. The solvent was evaporated under reduced pressure yielding 28.95 g (97.8%) of compound **2** as white crystals. mp 178.1–179.0 °C (lit. 175-176 °C[44]). $R_f$ 0.11 ($CH_2Cl_2$:MeOH = 7:1 + 3% $Et_3N$).

**Methyl trityl-D-serinate (5)**

To a cooled solution of **4** (29.27 g, 188.1 mmol, 1.00 equiv.) and $Et_3N$ (55.10 mL, 396.1 mmol, 2.11 equiv.) in $CH_2Cl_2$ (500 mL), trityl chloride (52.45 g, 188.1 mmol, 1.00 equiv.) was added and the mixture was stirred for 2 hours at 0 °C. The reaction mixture was washed with 10% citric acid solution (3 × 150 mL) and brine (150 mL). The organic phase was dried over anhydrous $Na_2SO_4$, the drying agent was filtered off and the solvent was evaporated under reduced pressure. The product was purified via flash column chromatography (hexane:EtOAc = 2:1) to obtain 55.70 g (81.9%) of **5** as white solid. mp 158.8-161.5 °C (lit. 152–154 °C[45]). $R_f$: 0.27 (hexane:EtOAc = 2:1). $^1$H NMR (400 MHz, $CDCl_3$): δ 2.29 (dd, *J* 6.2 Hz, 6.0 Hz, 1H, OH), 2.97 (bs, 1H, NH), 3.30 (s, 3H, $CH_3$), 3.50–3.61 (m, 2H, $CH_2$ and CH), 3.65–3.75 (m, 1H, $CH_2$), 7.16–7.32 (m, 9H, $CPh_3$), 7.43–7.51 (m, 6H, $CPh_3$) ppm.

**Methyl (R)-1-tritylaziridine-2-carboxylate (6)**

To a cooled solution of **5** (5.01 g, 13.9 mmol, 1.00 equiv.) and $Et_3N$ (4.25 mL, 30.5 mmol, 2.20 equiv.) in anhydrous THF (40 mL), methanesulfonyl chloride (1.08 mL, 14.0 mmol, 1.01 equiv.) was added dropwise. Reaction mixture was stirred for 30 minutes at room temperature. The temperature was then raised to 65 °C (reflux temperature) and reaction mixture was refluxed for 48 hours. The solvent was evaporated under reduced pressure, the solid residue was dissolved in EtOAc (40 mL) and washed with 10% citric acid solution (3 × 10 mL), saturated $NaHCO_3$ solution (3 × 10 mL) and brine (1 × 10 mL). The organic phase was dried over anhydrous $Na_2SO_4$, the drying agent was filtered off and the solvent was evaporated under reduced pressure. The product was purified via flash column chromatography (hexane:EtOAc = 4:1) to yield 3.42 g (71.9%) **6** as white crystals. mp 122.0–123.0 °C (lit. 122-123 °C[46]). $R_f$: 0.36 (Hexane:EtOAc = 4:1). $^1$H NMR (400 MHz, $CDCl_3$): δ1.41 (dd, *J* 6.3 Hz, 1.7 Hz, 1H, $CH_2$), 1.89 (dd, *J* 6.3 Hz, 2.7 Hz, 1H, CH), 2.26 (dd, *J* 2.7 Hz, 1.7 Hz, 1H, $CH_2$), 3.76 (s, 3H, $CH_3$), 7.19–7.31 (m, 9H, $CPh_3$), 7.47–7.52 (m, 6H, $CPh_3$) ppm. IR (ATR): ῡ 3705, 3467, 2973, 1742, 1596, 1489, 1445, 1394, 1328, 1234, 1181, 1011, 893, 843, 756, 697 $cm^{-1}$.

**Potassium (R)-1-tritylaziridine-2-carboxylate (7, $C_{22}H_{18}KNO_2$)**

To a cooled solution of **6** (5.78 g, 16.8 mmol, 1.00 equiv.) in THF (17 mL), 1M solution of KOH was added (16.84 mL, 16.84 mmol, 1.00 equiv.). Reaction mixture was stirred at room temperature for 24 hours. The solvent was removed under reduces pressure to obtain 6.15 g (99.4%) of **7** as lightly yellow solid. Reaction product was stable in salt form, but unstable in acid form. Yield: 99.4. mp Thermal $T_{dec}$ > 250.0 °C. $R_f$: 0.30 (hexane:EtOAc = 1:1 + 0.3% $CH_3COOH$). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.80–1.88 (m, 2H, $CH_2$), 3.71–3.78 (m, 1H, CH), 7.18–7.33 (m, 15H, $CPh_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 28.82, 34.95, 74.28, 126.67–128.36 (signals overlap), 129.51, 144.32, 178.15 ppm. MS *m/z* (relative intensity): 327.7 ($[M-H]^-$, 100%). HRMS-ESI: $[M-H]^-$ calcd for $C_{22}H_{18}NO_2$, 328.1343. found, 328.1341. IR (ATR): ῡ 3366, 3060, 2972, 2167, 1960, 1580, 1488, 1423, 1325, 1217, 1155, 1057, 1013, 900, 847, 750, 699 $cm^{-1}$. $[\alpha]_D^{20}$ +184.0 (*c* 0.31, EtOAc).

## 2. 3. General Procedure for Coupling reactions (Procedure A, Compounds 8a-8e)

To a cooled solution of compound **7** (1.00 equiv.) and protected D-amino acid (1.00–1.10 equiv.) in 50 mL dichloromethane, HOBt (1.10 equiv.), NMM (3.00 equiv.)

and EDC (1.10 equiv.) were added at 0 °C. The reaction mixture was stirred at room temperature for 24 h. The mixture was washed with 10% citric acid solution (3 × 50 mL), saturated $NaHCO_3$ solution (3 × 50 mL) and brine (1 × 50 mL). The organic phase was dried over anhydrous $Na_2SO_4$, the drying agent was filtered off and the solvent was evaporated under reduced pressure.

**Methyl ((R)-1-tritylaziridine-2-carbonyl)-D-alaninate (8a)**

The compound was synthesized according to the general procedure A. The product was purified via flash column chromatography. Initial mobile phase was $CH_2Cl_2$:hexane = 2:1, which was gradually replaced by hexane:EtOAc = 1:1. Yield: 1.198 g (53.1%) of **8a** as white crystals. Mp 161.0–165.2 °C. $R_f$: 0.48 (hexane:EtOAc = 1:1). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.44–1.51 (m, 4H, $CHCH_3$ and aziridine $CH_2$), 1.98–2.02 (m, 2H, aziridine CH and aziridine $CH_2$), 3.81 (s, 3H, $OCH_3$), 4.69 (qd, *J* 7.5 Hz, 7.2 Hz, 1H, $NHCHCH_3$), 7.20–7.34 (m, 10H, $CPh_3$ and CONH), 7.41–7.48 (m, 6H, $CPh_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 18.79, 30.12, 34.17, 47.61, 52.79, 74.82, 127.34, 128.00, 129.60, 143.45, 170.74, 173.46 ppm. MS (ESI$^+$): *m/z* = 436.85 ([M+Na]$^+$, 100%). HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_{26}H_{27}N_2O_3$, 415,2016. found, 415.2017. IR (ATR): ῡ 3705, 3293, 2972, 2869, 1746, 1645, 1532, 1491, 1448, 1359, 1276, 1206, 1165, 1056, 1009, 956, 905, 857, 771, 747, 703 cm$^{-1}$. HPLC: Method 1: $t_r$: 6.25 min (95.1% at 220 nm). $[\alpha]_D^{20}$ +132.1 (c 0.33, EtOAc).

**Methyl (R)-3-(1-tritylaziridine-2-carboxamido)propanoate (8b, $C_{26}H_{26}N_2O_3$)**

The compound was synthesized according to the general procedure A. The product was purified via flash column chromatography (hexane:EtOAc = 2:1), which was gradually replaced by hexane:EtOAc = 1:1. Yield: 1.24 g (62.9%) of **8b** as white crystals. mp 65.0-67.0 °C. $R_f$: 0.32 (hexane:EtOAc = 1:1). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.45 (dd, *J* 6.6 Hz, 0.7 Hz, 1H, aziridine $CH_2$), 1.96 (dd, *J* 2.7 Hz, 0.7 Hz, 1H, aziridine $CH_2$), 1.99 (dd, *J* 6.6 Hz, 2.7 Hz, 1H, aziridine CH), 2.57–2.71 (m, 2H, $CH_2COO$), 3.44–3.55 (m, 1H, $NHCH_2$), 3.65–3.79 (m, 4H, $NHCH_2$ and $OCH_3$), 7.20–7.30 (m, 9H, $CPh_3$), 7.33 (dd, *J* 6.2 Hz, 6.2 Hz, 1H, CONH), 7.38–7.44 (m, 6H, $CPh_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 29.94, 34.15, 34.29, 34.61, 52.10, 74.73, 127.30, 127.91, 129.48, 143.47, 171.12, 172.99 ppm. MS *m/z* (relative intensity): 436.7 ([M+Na]$^+$, 100%). *m/z* = 412.5 ([M-H]$^-$, 100%). HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_{26}H_{27}N_2O_3$, 415.2016. found, 415.2014. IR (ATR): ῡ 3706, 3333, 2949, 1733, 1663, 1522, 1442, 1365, 1256, 1180, 1059, 1009, 901, 858, 749, 702 cm$^{-1}$. HPLC: Method 1: $t_r$: 5.69 min (99.4% at 220 nm). $[\alpha]_D^{20}$ +109.4 (c 0.33, EtOAc).

***Methyl ((R)-1-tritylaziridine-2-carbonyl)-D-phenylalaninate* (8c)**

The compound was synthesized according to the general procedure A. The product was purified via flash column chromatography (hexane:EtOAc = 2:1), yielding 1.480 g (62.8%) of **8c** as white crystals. mp 120.5–122.0 °C. $R_f$: 0.23 (hexane:EtOAc = 2:1). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.37 (dd, *J* 6.7 Hz, 0.7 Hz, 1H, aziridine $CH_2$), 1.69 (dd, *J* 2.7 Hz, 0.7 Hz, 1H, aziridine $CH_2$), 1.95 (dd, *J* 6.7 Hz, 2.7 Hz, 1H, aziridine CH), 3.18 (dd, J 13.9 Hz, 6.2 Hz, 1H, $CH_2Ph$), 3.21 (dd, *J* 13.9 Hz, 6.0 Hz, 1H, $CH_2Ph$), 3.80 (s, 3H, $OCH_3$), 4.93 (ddd, *J* 8.7 Hz, 6.2 Hz, 6.0 Hz, 1H, $CH_2Ph$), 7.15–7.39 (m, 21H, $CPh_3$, Ph and CONH) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 30.04, 33.99, 38.06, 52.08, 52.66, 74.64, 127.25, 127.50, 127.90 (signals overlap), 128.91, 129.46, 135.98, 143.35, 170.54, 171.99 ppm. MS *m/z* (relative intensity): 488.6 ([M-H]$^-$, 100%). HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_{32}H_{31}N_2O_3$, 491.2329. found, 491.2326. IR (ATR): ῡ 3709, 3360, 3281, 2974, 2037, 1743, 1679, 1596, 1501, 1446, 1356, 1277, 1197, 1164, 1129, 1057, 1009, 903, 858, 808, 748, 703 cm$^{-1}$. HPLC: Method 1: $t_r$: 7.69 min (99.5% at 220 nm). $[\alpha]_D^{20}$ +74.9 (c 0.31, EtOAc).

***Methyl ((R)-1-tritylaziridine-2-carbonyl)-D-valinate* (8d)**

The compound was synthesized according to the general procedure B. The product was purified via flash column chromatography (hexane:EtOAc = 4:1), yielding 1.17 g (64.7%) of **8f** as white crystals. mp 111.0–113.0 °C. $R_f$: 0.34 (hexane:EtOAc = 3:1). $^1$H NMR (400 MHz, $CDCl_3$): δ0.97 (d, *J* 6.8 Hz, 3H, $CH(CH_3)_2$), 0.98 (d, *J* 6.8 Hz, 3H, $CH(CH_3)_2$), 1.51 (dd, *J* 6.2 Hz, 1.0 Hz, 1H, aziridine $CH_2$), 1.99–2.03 (m, 2H, aziridine $CH_2$ and aziridine CH), 2.25 (qqd, *J* 6.8 Hz, 6.8 Hz, 5.0 Hz, 1H, $CH(CH_3)_2$), 3.81 (s, 3H, $COOCH_3$), 4.59 (dd, *J* 9.3 Hz, 5.0 Hz, 1H, CHCOO), 7.21–7.32 (m, 9H, $CPh_3$), 7.34 (d, *J* 9.3 Hz, 1H, CONH), 7.42–7.47 (m, 6H, $CPh_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 17.95, 19.39, 30.33, 31.50, 34.28, 52.45, 56.53, 74.77, 127.32, 127.99, 129.54, 143.44, 170.93, 172.39 ppm. MS *m/z* (relative intensity):440.6 ([M-H]$^-$, 100%). HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_{29}H_{33}N_2O_3$, 457.2486. found, 457.2486. IR (ATR): ῡ 3273, 2965, 1742, 1649, 1554, 1490, 1444, 1314, 1264, 1210, 1150, 1011, 902, 749, 701, 634 cm$^{-1}$. HPLC: Method 1: $t_r$ 7.32 min (100.0% at 220 nm). $[\alpha]_D^{20}$ +145.0 (c 0.32, EtOAc).

**Dimethyl ((R)-1-tritylaziridine-2-carbonyl)-D-glutamate (8e)**

The compound was synthesized according to the general procedure A. The product was purified via flash column chromatography (hexane:EtOAc = 2:1), yielding 0.555 g (23.9%) of **8e** as white crystals. mp 151.5–152.5 °C. $R_f$: 0.23 (hexane:EtOAc = 2:1). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.49 (dd, *J* 6.0 Hz, 1.5 Hz, 1H, aziridine $CH_2$), 1.96–2.00 (m, 2H, aziridine $CH_2$ and aziridine CH), 2.05–2.16 (m, 1H, $CH_2COO$), 2.23–2.34 (m, 1H, $CH_2COO$), 2.36–2.54 (m, 2H, $CH_2$), 3.67 (s, 3H, $OCH_3$), 3.82 (s, 3H, $OCH_3$), 4.68 (ddd, *J* 8.3 Hz, 8.3 Hz, 5.1 Hz, 1H, CHCOO), 7.21–7.32 (m, 9H, $CPh_3$), 7.44–7.50 (m, 7H, $CPh_3$ and CONH) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 27.30, 30.05, 30.37, 34.13, 51.44, 52.13, 52.82, 74.84,

127.29, 127.95, 129.59, 143.44, 171.33, 172.23, 173.59 ppm. MS *m/z* (relative intensity):508.7 ([M+Na]$^+$, 80%). *m/z* = 484.5 ([M-H]$^-$, 70%). HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_{29}H_{31}N_2O_5$, 487.2227, found, 487.2228. IR (ATR): ῡ 3696, 3362, 3059, 2956, 2068, 1977, 1738, 1670, 1596, 1509, 1441, 1373, 1344, 1312, 1205, 1181, 1129, 1098, 1065, 1013, 904, 869, 820, 768, 747, 705 cm$^{-1}$. HPLC: Method 1: $t_r$: 6.33 min (99.9% at 220 nm). $[\alpha]_D^{20}$ +119.4 (c 0.32, EtOAc).

## 2. 4. General Procedure for Removal of Trityl Protection Group (Procedure B, Compounds 9a-9e)

To a cooled solution of the starting compound (**8a-8e**, 1.00 equiv.) and triethylsilane (1.75 equiv.) in 30 mL dichloromethane, trifluoroacetic acid (3.50 equiv.) was added drop-wise at 0 °C and stirred for another 30 min on ice bath. After the completion of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure and the solid residue was washed with 25 mL diethyl ether and water (25 mL). $NaHCO_3$ was added to the aqueous phase to give a solution with a pH = 10. To further reduce solubility of the product, NaCl was added and the aqueous phase was washed with ethyl acetate (6 × 25 mL). The collected organic phases were washed with brine (1×50 mL) and dried over anhydrous $Na_2SO_4$. The drying agent was filtered off and the solvent was evaporated under reduced pressure.

**Methyl ((R)-aziridine-2-carbonyl)-D-alaninate (9a, $C_7H_{12}N_2O_3$)**

The compound was synthesized according to the general procedure B. Yield: 0.140 mg (52.7%) as white crystals. mp 99.5-102.3 °C. *Rf*: 0.45 ($CH_2Cl_2$ +:MeOH = 9:1 + 3% $Et_3N$). $^1$H NMR (400 MHz, MeOD): δ 1.39 (d, I = 7.3 Hz, 3H, $CHCH_3$), 1.79–1.86 (m, 2H, aziridine $CH_2$), 2.55 (dd, *J* 5.7 Hz, 3.2 Hz, 1H, aziridine CH), 3.70 (s, 3H, $OCH_3$), 4.44 (q, *J* 7.3 Hz, 1H, $CHCH_3$) ppm. $^{13}$C NMR (100 MHz, MeOD): δ 17.65, 26.28, 30.42, 52.95, 172.73, 174.55 ppm, one signal covered by solvent. MS *m/z* (relative intensity):171,3 ([M-H]$^-$, 50%). HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_7H_{12}N_2O_3$, 172.0848. found, 173.0920. IR (ATR): ῡ 3701, 3286, 3196, 2973, 1732, 1665, 1560, 1451, 1405, 1374, 1344, 1211, 1137, 1058, 1011, 932, 826, 713 cm$^{-1}$. HPLC: Method 2: $t_r$: 4.29 min (94.6% at 195 nm). $[\alpha]_D^{20}$ +32.9 (c 0.33, MeOH).

**Methyl (R)-3-(aziridine-2-carboxamido)propanoate (9b)**

The compound was synthesized according to the general procedure B. The product was purified via flash column chromatography using $Al_2O_3$ ($CH_2Cl_2$:MeOH = 20:1), yielding 0.173 g (43.6%) of **9b** as white crystals. mp 93.8-95.8 °C. *Rf*: 0.26 ($CH_2Cl_2$:MeOH = 9:1 + 3% $Et_3N$). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.70–2.00 (m, 2H, aziridine $CH_AH_B$), 2.46 (bs, 1H, aziridine CH), 2.56 (t, *J* 6.1 Hz, 2H, $CH_2COO$), 3.49–3.60 (m, 2H, $NHCH_2CH_2$), 3.71 (s, 3H, $OCH_3$), 7.09 (bs, 1H, CONH) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 26.33, 30.34, 33.80, 34.98, 51.87, 171.11, 172.77 ppm. HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_7H_{12}N_2O_3$, 172.0848. found, 173.0924. IR (ATR): ῡ 3704, 3272, 3212, 2958, 2841, 1723, 1657, 1577, 1440, 1407, 1371, 1295, 1266, 1227, 1195, 1172, 1112, 1056, 1017, 978, 909, 884, 841, 740 cm$^{-1}$. HPLC: Method 2. $t_r$: 4.23 min (97.6% at 210 nm). $[\alpha]_D^{20}$ +32,9 (c 0,33, MeOH).

**Methyl ((R)-aziridine-2-carbonyl)-D-phenylalaninate (9c, $C_{13}H_{16}N_2O_3$)**

The compound was synthesized according to the general procedure B. The product was purified via flash column chromatography ($CH_2Cl_2$:MeOH = 20:1), yielding 0.450 g (67.9%) of **9c** as white crystals. mp 95.7–98.2 °C. *Rf*: 0.24 ($CH_2Cl_2$:MeOH = 20:1). $^1$H NMR (400 MHz, MeOD): δ 1.82 (bs, 2H, airidine $CH_2$), 2.53 (dd, *J* 5.5 Hz, 3.2 Hz, 1H, aziridine CH), 3.01 (dd, *J* 13.8 Hz, 8.8 Hz, 1H, $CH_2Ph$), 3.20 (dd, *J* 13.8 Hz, 5.6 Hz, 1H, $CH_2Ph$), 3.71 (s, 3H, $OCH_3$), 4.73 (dd, *J* 8.8 Hz, 5.6 Hz, 1H, $CHCH_2Ph$), 7.19–7.34 (m, 5H, Ph) ppm. $^{13}$C NMR (100 MHz, MeOD): δ 26.18, 30.24, 38.45, 52.77, 55.31, 127.98, 129.54, 130.23, 137.98, 172.72, 173.17 ppm. MS *m/z* (relative intensity): 271,24 ([M+Na]$^+$, 100%). HRMS-ESI (*m/z*): [M+H]$^+$ calcd for $C_{13}H_{16}N_2O_3$, 248.1161. found, 249.1230. IR (ATR): ῡ = 3703, 3208, 2945, 1736, 1661, 1543, 1446, 1401, 1362, 1219, 1164, 1110, 1055, 1012, 954, 914, 827, 699 cm$^{-1}$. HPLC: Method 2: $t_r$: 14.84 min (99.3% at 210 nm). $[\alpha]_D^{20}$ +18.7 (c 0.31, MeOH).

**Methyl ((R)-aziridine-2-carbonyl)-D-valinate (9d)**

The compound was synthesized according to the general procedure C, yielding 0.365 mg (75.5%) of **9d**. mp 93.5-94.8 °C. *Rf*: 0.42 ($CH_2Cl_2$:MeOH = 9:1 + 3% $Et_3N$). $^1$H NMR (400 MHz, MeOD): δ 0.96 (d, *J* 6.9 Hz, 3H, $CH(CH_3)_2$), 0.96 (d, *J* 6.9 Hz, 3H, $CH(CH_3)_2$), 1.85 (bs, 2H, aziridine $CH_2$), 2.16 (qqd, *J* 6.9 Hz, 6.9 Hz, 5.9 Hz, 1H, $CH(CH_3)_2$, 2.66 (dd, *J* 5.4 Hz, 3.1 Hz, 1H, aziridine CH), 3.72 (s, 3H, $COOCH_3$), 4.37 (d, *J* 5.9 Hz, 1H, $CHCOOCH_3$) ppm. $^{13}$C NMR (100 MHz, MeOD): δ 18.53, 19.57, 26.37, 30.33, 32.00, 52.67, 59.53, 173.17, 173.44 ppm. HRMS-ESI (*m/z*): [M-H]$^-$ calcd for $C_9H_{16}N_2O_3$, 200.1161. found, 199.1081. IR (ATR): ῡ 3704, 3278, 3202, 2967, 1731, 1666, 1558, 1463, 1439, 1391, 1346, 1318, 1285, 1235, 1203, 1160, 1069, 1005, 940, 891, 840, 822, 722 cm$^{-1}$. HPLC: Method 2. $t_r$: 6.98 min (95.0% at 195 nm). $[\alpha]_D^{20}$ +114.7 (c = 0.34, MeOH).

**Dimethyl ((R)-aziridine-2-carbonyl)-D-glutamate (9e, $C_{10}H_{16}N_2O_5$)**

The compound was synthesized according to the general procedure B. The product was purified via flash column chromatography using $Al_2O_3$ ($CH_2Cl_2$:MeOH = 50:1), which was gradually replaced by $CH_2Cl_2$:MeOH = 20:1, yielding 0.124 g (48.9%) of **9d** as colourless viscous oil. *Rf*: 0.38 ($CH_2Cl_2$:MeOH = 9:1). $^1$H NMR (400 MHz,

$CDCl_3$): δ 1.84 (bs, 1H, aziridine $CH_2$), 1.88–2.07 (m, 2H, aziridine $CH_2$ and $CH_2COO$), 2.16–2.28 (m, 1H, $CH_2$ COO), 2.31–2.48 (m, 2H, $CH_2$), 2.54 (bs, 1H, aziridine CH), 3.69 (s, 3H, $OCH_3$), 3.76 (s, 3H, $OCH_3$), 4.58–4.68 (m, 1H, CHCO), 7.16 (d, *J* 8.0 Hz, 1H, CONH) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): δ 26.73, 27.35, 30.13, 30.38, 51.64, 52.00, 52.69, 171.27, 172.13, 173.25 ppm. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{10}H_{16}N_2O_5$, 244.1059. found, 245.1131. IR (ATR): ῡ 3704, 3668, 2970, 2868, 1736, 1657, 1535, 1437, 1341, 1215, 1170, 1056, 1011, 833 $cm^{-1}$. HPLC: Method 2: $t_r$: 5.66 min (96.6% at 210 nm). $[\alpha]_D^{20}$ +54.0 (c 0.25, MeOH).

## 2. 5. General Procedure for Removal of Methyl protection group (procedure C, compounds 2a-2e)

To a cooled solution of **8a**-**8e** (1.00 equiv.) in MeOH (20 mL), 0.1 M LiOH solution (5.00 equiv.) was added drop-wise at 0 °C. After the completion of the reaction (monitored by TLC), the pH of the reaction mixture was adjusted to pH=7 with a 0.1 M HCl solution and the solvent was evaporated under reduced pressure.

**((R)-aziridine-2-carbonyl)-D-alanine (2a)**

The compound was synthesized according to the general procedure C. The product was purified via flash column chromatography (EtOAc:MeOH:$H_2O$ = 4:2:1), yielding 0.080 g (98%) of **2a** as lightly yellow crystals. mp thermal $T_{dec}$ > 250 °C. $R_f$: 0.26 (EtOAc:MeOH:$H_2O$ = 4:2:1). $^1$H NMR (400 MHz, MeOD): δ 1.36 (d, *J* 7.1 Hz, 3H, $CHCH_3$), 1.78–1.87 (m, 2H, aziridine $CH_2$), 2.58 (dd, *J* 5.8 Hz, 3.2 Hz, 1H, aziridine CH), 4.24 (q, *J* 7.1 Hz, 1H, $CHCH_3$) ppm. $^{13}$C NMR (100 MHz, MeOD): δ 19.48, 26.17, 30.85, 52.26, 171.82, 179.66 ppm. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_6H_{10}N_2O_3$:158.0691. found, 159.0764. IR (ATR): ῡ 3659, 3324, 3100, 2970, 2872, 1643, 1606, 1562, 1457, 1405, 1365, 1319, 1269, 1236, 1167, 1104, 1054, 1017, 981, 942, 919, 868, 837, 756, 685 $cm^{-1}$. HPLC: Method 2. $t_r$: 3.46 min (95.1% at 195 nm). $[\alpha]_D^{20}$ +66.7 (c 0.33, MeOH).

**(R)-3-(aziridine-2-carboxamido)propanoic acid (2b, $C_6H_{10}N_2O_3$)**

The compound was synthesized according to the general procedure C. The product was purified via flash column chromatography (EtOAc:MeOH:$H_2O$ = 4:2:1), yielding 0.096 g (65%) **2b** as white crystals. mp thermal $T_{dec}$ > 250 °C. $R_f$: 0.24 (EtOAc:MeOH:$H_2O$ = 4:2:1). $^1$H NMR (400 MHz, $D_2O$): δ 1.83 (d, *J* 3.1 Hz, 1H, aziridine $CH_2$), 1.87 (d, *J* 6.0 Hz, 1H, aziridine $CH_2$), 2.36 (t, *J* 6.9 Hz, 2H, $CH_2COO$), 2.56 (dd, *J* 6.0 Hz, 3.1 Hz, 1H, aziridine CH), 3.39 (t, *J* 6.9 Hz, 2H, $NHCH_2$) ppm. $^{13}$C NMR (100 MHz, $D_2O$): δ 24.82, 29.59, 33.32, 53.91, 172.50, 180.15 ppm. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_6H_{10}N_2O_3$, 158.0691. found, 159.0764. IR (ATR): ῡ 3702, 3659, 3258, 3086, 2975, 2871, 1646, 1556, 1404, 1312, 1259, 1162, 1126, 1061, 1016, 908, 832, 622 $cm^{-1}$. $[\alpha]_D^{20}$ +143.6 (c 0.30, MeOH).

**((R)-aziridine-2-carbonyl)-D-phenylalanine (2c)**

The compound was synthesized according to the general procedure C. The product was purified via flash column chromatography (EtOAc:MeOH:$H_2O$ = 4:1:1), yielding 0.163 g (71.4%) of **2c** as white crystals. mp thermal $T_{dec}$ > 180 °C. $R_f$: 0.37 (EtOAc:MeOH:$H_2O$ = 4:2:1). $^1$H NMR (400 MHz, $D_2O$): δ 1.75 (bs, 2H, aziridine $CH_AH_B$), 2.52 (bs, 1H, aziridine CH), 2.91 (dd, *J* 14.0 Hz, 8.8 Hz, 1H, $CH_2Ph$), 3.18 (dd, *J* 14.0 Hz, 4.9 Hz, 1H, $CH_2Ph$), 4.45 (dd, *J* 8.8 Hz, 4.9 Hz, 1H, CHCOO), 7.21–7.36 (m, 5H, Ph) ppm. $^{13}$C NMR (100 MHz, MeOH): δ 25.05, 29.48, 38.63, 54.38, 126.89, 128.61, 129.30, 137.59, 172.08, 178.00 ppm. HRMS-ESI: $[M-H]^-$ calc for $C_{12}H_{14}N_2O_3$, 234.1004. found, 233.0928. IR (ATR): ῡ 3371, 2976, 2117, 2005, 1595, 1418, 1273, 1162, 1106, 1056, 923, 696 $cm^{-1}$. HPLC: Method 2. $t_r$: 7.22 min (86.5% at 210 nm). $[\alpha]_D^{20}$ +25.7 (c 0.30, MeOH).

**((R)-aziridine-2-carbonyl)-D-valine (2d, $C_9H_{16}N_2O_3$)**

The compound was synthesized according to the general procedure C. The product was purified via flash column chromatography (EtOAc:MeOH:$H_2O$ = 4:2:1), yielding 0.109 g (63.7%) of **2e.** mp thermal $T_{dec}$ > 220 °C. $R_f$: 0.31 (EtOAc:MeOH:$H_2O$ = 4:2:1). $^1$H NMR (400 MHz, $D_2O$): δ 0.87 (d, *J* 7.0 Hz, 3H, $CH(CH_3)_2$), 0.90 (d, *J* 7.0 Hz, 3H, $CH(CH_3)_2$), 1.84 (d, *J* 3.4 Hz, 1H, aziridine $CH_2$), 1.90 (d, *J* 5.9 Hz, 1H, aziridine $CH_2$), 2.10 (qqd, *J* 7.0 Hz, 7.0 Hz, 5.6 Hz, 1H, $CH(CH_3)_2$, 2.68 (dd, *J* 5.9 Hz, 3.4 Hz, 1H, aziridine CH), 4.06 (d, *J* 5.6 Hz, 1H, CHCOO) ppm. $^{13}$C NMR (100 MHz, MeOD): δ18.36, 20.33, 26.30, 30.89, 32.67, 61.76, 172.45, 178.40 ppm. HRMS-ESI: $[M-H]^-$ calc for $C_8H_{13}N_2O_3$, 185.0932. found, 185.0933. IR (ATR): ): ῡ 3707, 3666, 3288, 2970, 2871, 1644, 1590, 1546, 1424, 1235, 1162, 1057, 1011, 939, 915, 835, 752, 669 $cm^{-1}$. HPLC: Method 2. $t_r$: 4.11 min (85.4% at 210 nm). $[\alpha]_D^{20}$ +52.6 (c 0.33, MeOH).

**Benzyl aziridine-2-carboxylate (10)**

To a cooled solution of **7** (15.01 g, 40.84 mmol, 1.00 equiv.) in acetonitrile (400 mL) benzyl bromide (4.85 mL, 40.8 mmol, 1equiv.) was added. After stirring at room temperature for 3 h, solvent was evaporated under reduced pressure. The oily residue was dissolved in $CH_2Cl_2$ (200 mL), and washed with water (200 mL), and brine (150 mL). The organic phase was dried over anhydrous $Na_2SO_4$, the drying agent was filtered off and the solvent was evaporated under reduced pressure to obtain 14.34 g (83.4%) viscous oil, which was used for further reaction without additional purification. The trityl group was removed following the standard procedure B, yielding 0.697 g (83.9%) of **10** as colourless oil. $R_f$: 0.32 (hexane:EtOAc = 1:2 + 3% $Et_3N$). $^1$H NMR (400 MHz, $CDCl_3$): δ 1.89 (dd, *J* 5.5 Hz, 1.4 Hz, 1H, $CH_2$), 2.04 (dd, *J* 2.9 Hz, 1.4 Hz, 1H, $CH_2$), 2.58

(dd, *J* 5.5 Hz, 2.9 Hz, 1H, CH), 5.18 (d, *J* 12.3 Hz, 1H, $CH_2Ph$), 5.22 (d, *J* 12.3 Hz, 1H, $CH_2Ph$), 7.32–7.42 (m, 5H, Ph) ppm. IR (ATR): ῡ 3285, 3229, 3065, 3037, 1725, 1564, 1456, 1402, 1360, 1187, 1113, 1007, 970, 871, 825, 744, 697 $cm^{-1}$. $^1H$ NMR spectra was found to be identical to the ones described in ref.[19]

**Benzyl (R)-1-((tert-butoxycarbonyl)-D-alanyl)aziridine -2- carboxylate (11)**

The compound was synthesized according to the general procedure A. The product was purified via flash column chromatography (hexane:EtOAc = 3:1), yielding 0.471 g (25.7%) of **11** as viscous oil. $R_f$: 0.23 (MF: hexane:EtOAc = 3:1). $^1H$ NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, $C(CH_3)_3$), 1.45 (d, *J* 7.1 Hz, 3H, $CHCH_3$), 2.63 (dd, *J* 3.1 Hz, 1.9 Hz, 1H, aziridine $CH_2$), 2.72 (dd, *J* 5.8 Hz, 1.9 Hz, 1H, aziridine $CH_2$), 3.28 (dd, *J* 5.8 Hz, 3.1 Hz, 1H, aziridine CH), 4.30 (qd, *J* 7.1 Hz, 7.1 Hz, 1H, $CHCH_3$), 5.01 (d, *J* 7.1 Hz, 1H, CONH), 5.20 (s, 2H, $CH_2Ph$), 7.32–7.42 (m, 5H, Ph) ppm. $^{13}C$ NMR (100 MHz, MeOD): δ18.98, 28.42, 30.96, 34.45, 51.30, 67.71, 80.02, 128.65, 128.73, 128.78 (overlapping of signals), 135.06, 155.33, 168.31, 184.23 ppm. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{24}N_2O_5$, 348.1685. found, 349.1753. IR (ATR): ῡ 3354, 3177, 3036, 1972, 2935, 2878, 1681, 1497, 1452, 1367, 1324, 1273, 1248, 1168, 1063, 1018, 944, 913, 854, 750, 699 $cm^{-1}$. HPLC: Method 3. $t_r$: 6.64 min (95.1% at 220 nm). $[\alpha]_D^{20}$ +84,4 (c = 0,27, EtOAc).

**Benzyl (R)-1-(((benzyloxy)carbonyl)-D-phenylalanyl) aziridine-2-carboxylate (13)**

The compound was synthesized according to the general procedure B. The product was purified via flash column chromatography (hexane:EtOAc = 3:1), yielding 1.67 g (92.4%) **11** as viscous oil. $R_f$: 0.62 (hexane:EtOAc = 1:1). $^1H$ NMR (400 MHz, $CDCl_3$): δ 2.57–2.63 (m, 2H, aziridine $CH_2$), 3.05–3.15 (m, 2H, $CH_2Ph$ and aziridine CH), 3.25 (dd, *J* 14.0 Hz, 5.8 Hz, 1H, $CH_2Ph$), 4.55–4.63 (m, 1H, CH), 5.0 (d, *J* 12.3 Hz, 1H, $OCH_2Ph$), 5.06 (d, *J* 12.3 Hz, 1H, $OCH_2Ph$), 5.17 (s, 2H, $OCH_2Ph$), 5.23 (d, *J* 8.1 Hz, 1H, OCONH), 7.12–7.42 (m, 15H, 3 × Ph) ppm. $^{13}C$ NMR (100 MHz, MeOD): δ 31.68, 35.63, 38.98, 58.87, 67.65, 68.65, 127.87, 128.75, 129.04, 129.57, 129.61, 129,76 (overlapping of 3 signals), 130.52, 136.95, 138.32, 138.70, 158.37, 169.74, 183.98 ppm. MS *m/z* (relative intensity): 480.6 ($[M+Na]^+$, 100%). HRMS-ESI: $[M-H]^+$ calcd for $C_{27}H_{26}N_2O_5$, 458.1842. found, 457.1773. IR (ATR): ῡ 3328, 3062, 3033, 2952, 1699, 1505, 1452, 1375, 1248, 1191, 1077, 1047, 1027, 910, 747, 696 $cm^{-1}$. HPLC: Method 3. $t_r$: 9.24 min (70.5% at 210 nm). $[\alpha]_D^{20}$ +45,6 (c = 0.43, EtOAc).

## 3. Results and Discussion

There are two common strategies concerning the synthesis of aziridine containing peptides. The first strategy starts from a partially protected serine dipeptides followed by cyclization. This approach is reportedly less efficient because several various by-products are formed in the cyclization step.[27,28,37,39,47] The second more efficient strategy, which starts from the aziridine-2-carboxylic acid, was therefore used.[34,39,48]

The synthesis of N-terminal aziridine containing dipeptides **2a-e** was straightforward and high yielding. It started with the introduction of a trityl protective group onto D-Ser-OMe (**4**)[49] in 82% yield followed by cyclization using methanesulfonyl chloride[50,51] to obtain the aziridine **6** in 72% yield. Next, the screening of different bases (NaOH, LiOH, KOH) and solvents (THF, $CH_3CN$ and EtOH) for the subsequent saponification was performed. 1M KOH in THF was found to be the most optimal in terms of yield (99%) and purity of the final carboxylate **7**. Our attempts to neutralize **7** with diluted HCl or acetic acid were not successful because of its decomposition upon neutralization. Potassium salt was therefore used in the coupling reactions between (*R*)-aziridine-2-carboxylate **7** and different amino acids to obtain the aziridine containing dipeptide products **8a-e** in 24–65% yield.[39] Acidolytic treatment of **8** following the literature reported procedures[37,39] using $CF_3COOH$ in $CH_2Cl_2$/MeOH or $CH_2Cl_2$ generated a mixture of degradation products and was therefore unsatisfactory for the synthesis of larger amounts of final products. The analysis of reaction mixtures by NMR confirmed that the products were present in very small quantities, which we were unable to purify. Difficulties in removing trityl as well as Boc protective group from aziridine peptides using $CF_3COOH$, formic acid or HCl have been previously reported.[7,36] Instead, optimization of reaction conditions following different reaction procedures demonstrated that the addition of $Et_3SiH$ to the reaction mixture was essential for the successful reaction.[52] Hence, reductive deprotection of N-tritylaziridines **8** and basification with triethylamine prior to isolation was successfully applied to obtain deprotected aziridines **9a-e** in 31–98% yield. Finally, basic hydrolysis with LiOH in a mixture of MeOH and $H_2O$ yielded compounds **2a–d** in 31–98% yield. A reaction with Glu derivative **9e** yielded **2e** as confirmed by MS and NMR analysis. However, a product was unstable on silica or $Al_2O_3$ and purification was therefore not possible. The majority of compounds were stable for up to 3 weeks if stored under argon and in the fridge. However, after prolonged storage, significant decomposition was observed, which is in agreement with literature data. It is not uncommon that many aziridine derivatives exhibit sequence-dependent instability in both reaction and purification steps, as well as on storage. This is caused by self-protonation to generate a reactive aziridinium species that subsequently decompose (Figure 3).[31,53,54]

The synthesis of compounds with general structure **3** was started from benzyl protected (*R*)-aziridine-2-carboxylic acid **10** which was then coupled with Boc-D-Ala and

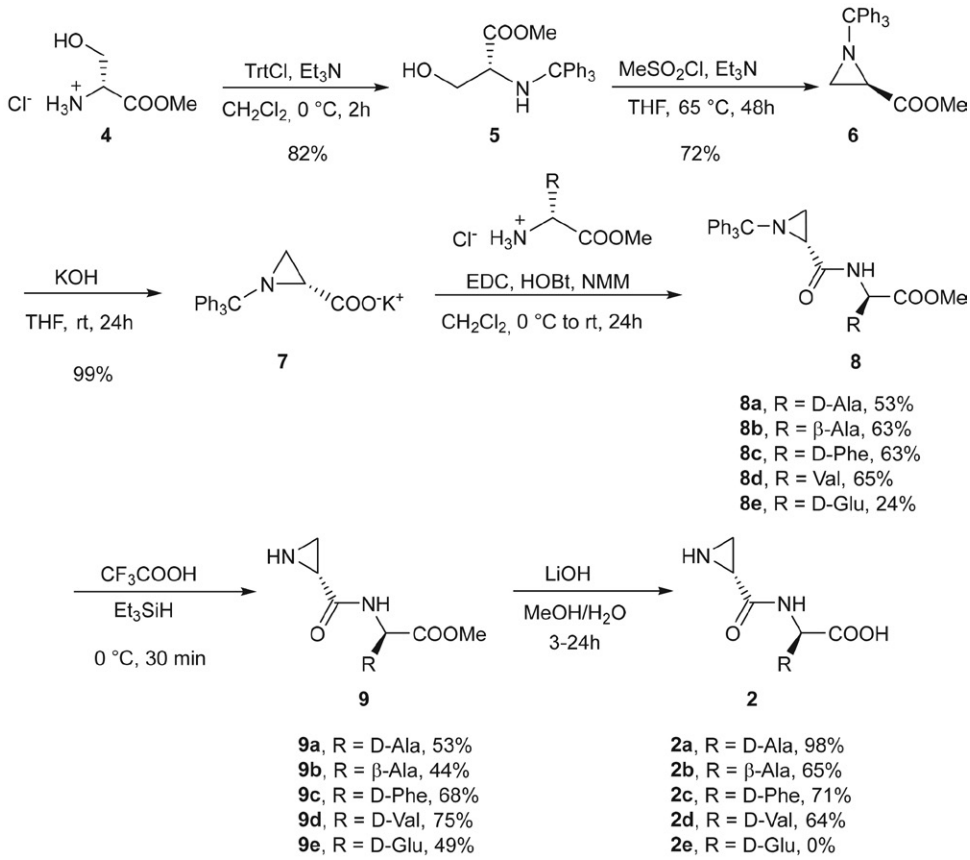



**Figure 3.** Synthesis of aziridine containing dipeptides.

Cbz-D-Phe to obtain *tert*-butoxycarbonyl and benzyloxycarbonyl protected dipeptides **11** and **13** in 26% and 32% yields, respectively. N-acylated aziridine derivatives have major issues with stability due to acylation and subsequent lower electronic density of the aziridine ring.[25] It was therefore expected that the removal of a protective group could potentially yield unstable compounds if free $NH_2$ group was present. Boc and Cbz protective groups were therefore chosen because they are easy to remove to hypothetical products **12** and **14** that have $NH_2$ groups in the form of a HCl salt or as a zwitterion, respectively. However, the deprotection of Boc to obtain **12** using $CF_3COOH$ or HCl in anhydrous THF or diethyl ether did not provide us with the final product and only a mixture of unidentifiable compounds could be isolated. NMR analysis indicated that the opening of the aziridine ring could be the cause. Interestingly, NMR analysis also indicated that the opening of the aziridine ring is favoured compared to the removal of Boc protective group at concentrations of HCl below 0.1 M. Deprotection under neutral conditions using catalytic hydrogenation was next applied to remove both benzyl protective groups from **13** and to yield **14** in ″zwitterionic″ form. However, a mixture of unidentifiable products was obtained, again (Figure 4).

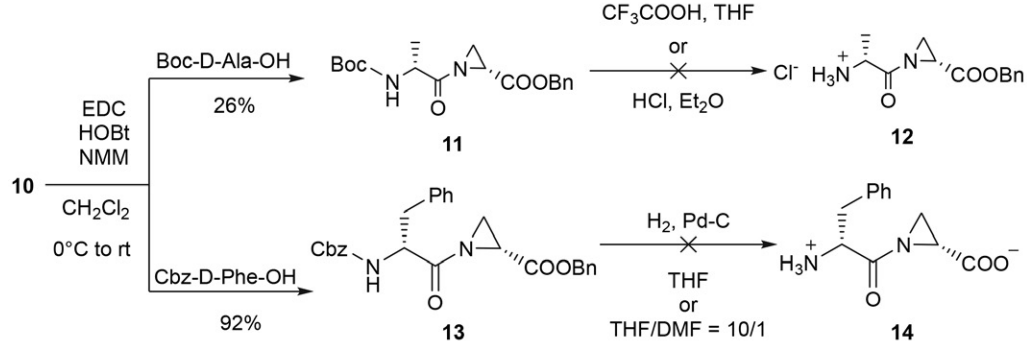



**Figure 4.** Study on the preparation of **12** and **14**.

## 4. Conclusions

In conclusion, fully deprotected aziridine-containing dipeptides can be easily synthesized from trityl protected aziridine-carboxylic acid. In most cases, the deprotection of the trityl group from the aziridine ring and final hydrolysis proceeds smoothly in moderate to high yields. However, significant decomposition of deprotected dipeptides was observed after 3 weeks even if stored under argon in the fridge. The synthesis of derivatives with aziridine-2-carboxylic acid in place of a second amino acid in the dipeptide sequence was not possible due to the instability of the acylated aziridine ring in the presence of a free amino group.

### Acknowledgement

This research was funded by Slovenian Research Agency (ARRS, research core funding no. P1-566 0208).

### Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Samo Kuzmič and Rok Frlan. The first draft of the manuscript was written by Rok Frlan and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The authors declare no competing financial interest.

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## Povzetek

V prispevku so opisani optimizirani pogoji za sintezo dipeptidov, ki vsebujejo popolnoma odščiten (2*R*)-aziridin. Priprava popolnoma zaščitenih N- in C- terminalnih dipeptidov, ki vsebujejo aziridin, je enostavna in poteka z visokim izkoristkom za večino spojin, medtem ko je njihova popolna odščita možna le za C-terminalne analoge. Odstranjevanje zaščite z N-terminalnih derivatov z uporabo standardnih postopkov peptidne kemije se je izkazalo kot težko, saj vodi do mešanice nedoločljivih produktov. Opisane molekule imajo velik potencial kot gradniki v sintezni kemiji, na področju kemijske biologije, kot kovalentni modifikatorji in kot biomarkerji.