Pergamon

Tetrahedron Vol. 50, No. 6, pp. 1717-1730, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4020/94 $6.00+0.00

0040-4020(93)E0068-Q

On The Synthesis of (2S)-Aziridine-2-Carboxylic Acid Containing Peptides

Andreas Korn, Sabine Rudolph-Böhner, Luis Moroder*

Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, D-82152 Martinsried

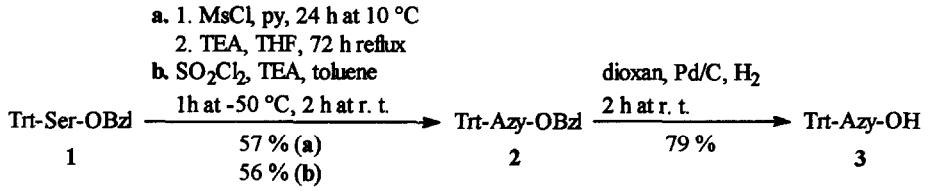
Abstract: Optimized conditions are described for the synthesis of 1-trityl-2-aziridine-carboxylic acid **3** (Trt-Azy-OH) and benzyl (2S)-aziridine-2-carboxylate **6** (H-Azy-OBzl) as useful derivatives for the synthesis of N- and C-terminal aziridine-containing peptides. Thereby, the use of the pentafluorophenyl ester of Trt-Azy-OH was found to be the method of choice in acylating steps, whereas acylation of H-Azy-OBzl several classical methods of peptide synthesis can be successfully used. The fully protected aziridine-2-carboxylic acid peptides are accessible in satisfactory yields as analytically defined products, but partial or total deprotection of these compounds again by standard procedures of peptide synthesis is surprisingly difficult in terms of satisfactory yields, whereby sequence-dependent unstability both in the reaction and purification steps as well as on storage was found to strongly limit the accessibility of these aziridine-containing peptides as promising active-site inactivators of cysteine-proteinases.

Introduction

Aziridine-2-carboxylic acid as well as aziridine-containing peptides have found useful application as intermediates in the synthesis of various amino acid[1] and peptide derivatives (for reviews see ref.[2]). Furthermore, aziridine-2-carboxylic acid and related compounds represent interesting enzyme substrates[3], but also promising new irreversible inhibitors of different proteases[4]. Therefore a versatile and efficient new synthetic route for aziridine-containing peptides would be recommendable, since the procedure most frequently used up to now, is based on partially protected serine peptides as starting compounds[5]. These have to be converted selectively to the corresponding O-tosyl or O-mesyl derivatives, which in turn are subjected to ring closure under anhydrous conditions in the presence of tertiary amines as auxiliary bases[5]. Cyclization of serine peptides can also be achieved by the use of the *Mitsunobu* reagent[6], although oxazoline and dehydroalanine derivatives have been reported as consistent side products. Besides formation of various byproducts to larger extents, the main drawback of these serine-peptide-based procedures is the severe limitations resulting from appropriate partial protection of the serine peptides. Correspondingly, more efficient appears to be a synthetic approach based on the direct use of suitable aziridine-2-carboxylic acid derivatives. In this context, so far, limited experiences have been gained in previous studies, e. g. on the acylation of benzyl aziridine-2-carboxylate with acyl chlorides[1c,7] or with urethane protected amino acids via DCC[4b,5,8]. In the present communication optimized conditions for the preparation of useful aziridine-2-carboxylic acid derivatives are described as well as their application in the synthesis of model peptides.

Results and Discussion

Various methods have been proposed for the synthesis of aziridine-2-carboxylic acid derivatives[8,9]. Among these we have compared the efficiency of the recently reported one-step preparation of optically active Trt-Azy-OBzl* (2) from Trt-Ser-OBzl (1)[9d] with that originally proposed by *Okawa*[5]. Both procedures, outlined in scheme 1, led to the desired aziridine compound as analytically well defined product in nearly identical yields of 50-60%. The absolute configuration could definitely be confirmed by X-ray analysis and NMR spectroscopy as benzyl (-)-(2S)-1-trityl-aziridine-2-carboxylate[10].



scheme 1

This compound **2** served then as starting material for producing suitable derivatives to be used as building blocks in the preparation of (2S)-aziridine-2-carbonyl-peptide and 1-peptidyl-(2S)-aziridine-2-carboxylic acid derivatives (Fig. 1).

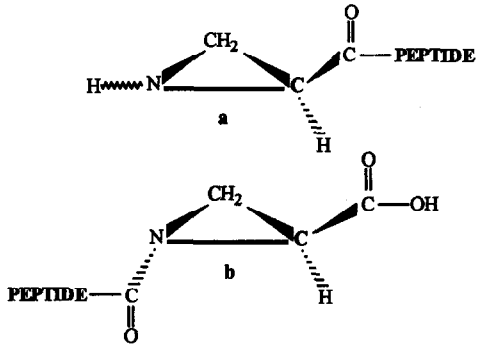
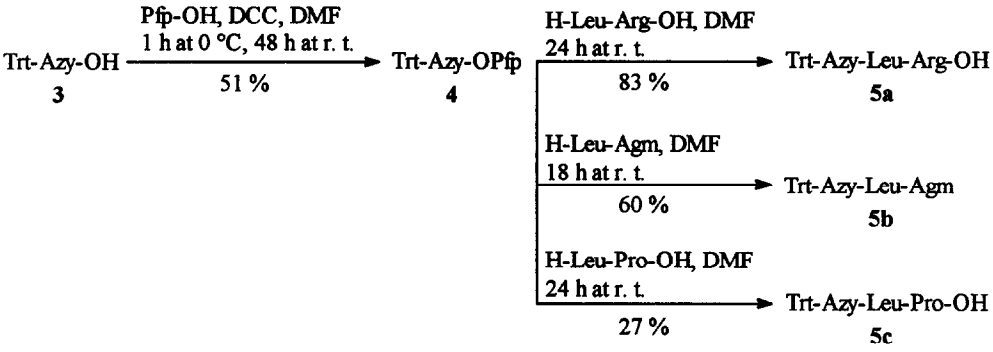


Fig. 1. (2S)-aziridine-2-carbonyl-peptide (**a**) and 1-peptidyl-(2S)-aziridine-2-carboxylic acid (**b**).

* *Abbreviations*: Standard abbreviations as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1972**, 247, 977) are used for amino acids and related derivatives; AcOEt, ethyl acetate; Agm, agmatine; Azy, (2S)-aziridine-2-carboxylic acid; DBSI, dibenzosulfimide; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DMF, dimethylformamide; DPPA, diphenyl phosphorazidate; EDIPA, ethyldiisopropylamine; HOAc, acetic acid; HOBt, 1-hydroxybenzotriazole; HOSu, N-hydroxysuccinimide; MeOH, methanol; MsCl, methanesulfonyl chloride; Mva, 4-methyl-valeroyl-; NMM, N-methylmorpholine; Pfp-OH, pentafluorophenol; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; TMS, tetramethylsilane; Tos-OH, p-toluenesulfonic acid.

Synthesis of (2S)-aziridine-2-carbonyl-peptide derivatives

As expected from previous studies of *Okawa et al.*[2b] on the hydrogenolytic N^{α}-deprotection of benzyloxycarbonyl-peptides containing the aziridine moiety, hydrogenation of compound **2** over palladized charcoal was found to proceed smoothly yielding Trt-Azy-OH (**3**) as crystalline material (scheme 1). Attempts to convert **3** into homogeneous and stable active esters as useful intermediates in peptide synthesis led to positive results only in the case of the pentafluorophenyl ester, whereas the related N-hydroxysuccinimide and N-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine ester could not be isolated despite all the precautions taken to operate under strictly anhydrous conditions both in the esterification and purification step. Formation of the latter two active esters, at least to some extents, occurs as well assessed by reacting the crude N-hydroxysuccinimide ester with H-Leu-OtBu and the crude N-3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine ester with H-Leu-Arg-OH to generate Trt-Azy-Leu-OtBu and Trt-Azy-Leu-Arg-OH, respectively. Although production of the di- and tripeptide could be confirmed by chromatographic comparison of the reaction mixtures with authentic samples of Trt-Azy-Leu-OtBu (**7**) and Trt-Azy-Leu-Arg-OH (**5a**), homogeneous products could not be isolated from the complex reaction mixtures. For this comparative analysis compound **7** was prepared according to the method of *Okawa*[5] from Trt-Ser-Leu-OtBu. Conversely, **5a** was obtained in good yields and as homogeneous product by acylating H-Leu-Arg-OH with Trt-Azy-OPfp (**4**). The latter reaction was found to proceed smoothly, a fact which confirms the usefulness of the newly developed pentafluorophenyl ester procedure for the synthesis of 1-trityl-(2S)-aziridine-2-carbonyl-peptides as outlined in Sheme 2. This is further supported by the successful synthesis of Trt-Azy-Leu-Agm·HCl (**5b**) and Trt-Azy-Leu-Pro-OH (**5c**).



scheme 2

Synthesis of 1-peptidyl-(2S)-aziridine-2-carboxylic acid derivatives

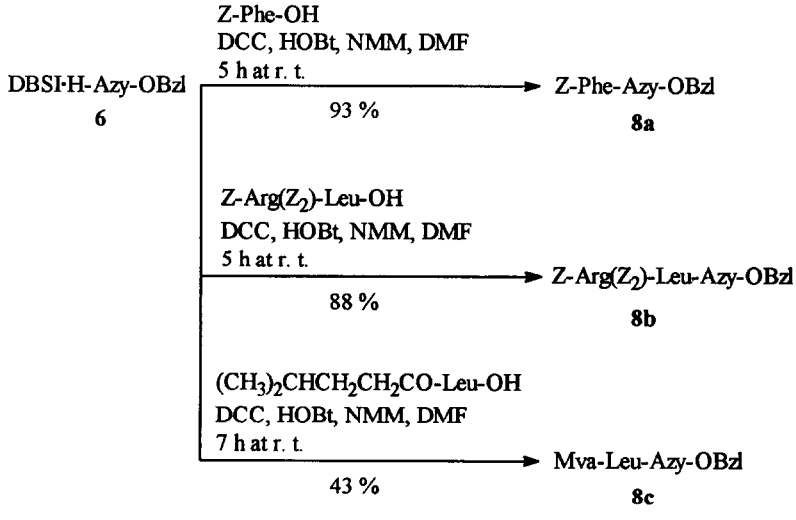
As mentioned above acylation of aziridine-2-carboxylic acid esters has been performed so far by the use of DCC or N-hydroxysuccinimide ester methods to produce acyl protected dipeptides[3,4b,5,8] or by benzyl chloroformate to generate the 1-benzyloxycarbonyl-(2S)-aziridine-2-carboxylic acid ester[1c,7]. In both cases benzyl (2S)-aziridine-2-carboxylate served as amino component which was obtained by acidolytic detritylation of **2**. As summarized in Table 1 acidolytic cleavage of the trityl group from Trt-Azy-OBzl (**2**) was attempted by applying various known procedures.

Table 1. Acidolytic Deprotection of Trt-Azy-OBzl (**2**) to Produce H-Azy-OBzl (**6**)

Cleavage Reagent	Equivalents	Reaction Time (h)	Temperature (°C)	Desired Product	Yield (%)
HCl/MeOH	10	3	r. t.	H-Azy-OBzl	no product; chloroalanine benzyl ester / 3-amino-2-chloro-propanoic acid benzyl ester
HCl/MeOH	1.1	18	r. t.	HCl·H-Azy-OBzl	no product; chloroalanine benzyl ester / 3-amino-2-chloro-propanoic acid benzyl ester
DBSI/MeOH/ $CHCl_3$	10	3	r. t.	H-Azy-OBzl	81 [a]
				DBSI·H-Azy-OBzl	50 [b]
DBSI/MeOH/ $CHCl_3$	1.1	18	r. t.	DBSI·H-Azy-OBzl	no product
TosOH/MeOH/ $CHCl_3$	1.1	6	r. t.	TosOH·H-Azy-OBzl	65
TosOH/MeOH/ $CHCl_3$	10	3	r. t.	H-Azy-OBzl	87 [a]
				TosOH·H-Azy-OBzl	51 [b]
TosOH/MeOH/ $CHCl_3$	10	2	r. t.	H-Azy-OBzl	98 [a]
				DBSI·H-Azy-OBzl	72 [b]

[a] in the crude product; [b] isolated

Exposure of compound **2** to trifluoroacetic acid as previously reported[1b,1c,2a,5] led to a mixture of products which according to HPLC was found to contain about 72% H-Azy-OBzl (**6**). The nature of the side products formed in this acid treatment was not determined. However, they should result at least partly from ring opening, since exposure of a Boc-3-methyl-aziridine derivative to trifluoroacetic acid has been reported to produce mainly the trifluoroacetylated threonine derivative[6a]. Alternatively, detritylation of **2** has been performed by treatment with formic acid as suggested by *Okawa et al.*[11] and *Kuyl-Yeheskiely et al.*[9d]. According to hplc, besides the desired compound **6** (73%) a set of side products was again formed. Moreover, detritylation of **2** with a small or larger excess of HCl led to almost quantitative decomposition with predominant formation of chloroalanine and 3-amino-2-chloropropanoic acid benzyl esters as well assessed by mass spectrometry of the isolated compounds. These findings fully agree with previous observations[4a,12]. More successful N^{α}-deprotection of **2** was achieved using milder acidolytic conditions, i. e. p-toluenesulfonic acid or dibenzosulfimide. In both cases the use of larger excesses proved to be advantageous, as the reaction time was found to affect the quality of the deprotection product more than the acidity of the reaction medium. Among the various salts prepared from **6** to facilitate its isolation as crystalline product, the dibenzosulfimide proved to be the most suited as it was obtained as chromatographically homogeneous and analytically well characterized compound without chromatographic purification steps. This salt was found to be perfectly stable on storage for longer periods of time. It decomposes, however, almost quantitatively within 18-24 h in CH_3CN/MeOH or DMF in absence of bases. Conversely, in the presence of 1 eq. auxiliary base, e. g. NMM or TEA, compound **6** is sufficiently stable to allow for acylation reactions to be performed. In fact, reaction of **6** as dibenzosulfimide with Z-Phe-OH via DCC/HOBt in presence of 1 eq. NMM led to the dipeptide **8a** in high yields as well characterized compound. Similarly successful were the synthesis of Z-Arg(Z_2)-Leu-Azy-OBzl (**8b**) and Mva-Leu-Azy-OBzl (**8c**) via acylation of **6** with Z-Arg(Z_2)-Leu-OH and Mva-Leu-OH, respectively, in presence of DCC/HOBt (scheme 3).



scheme 3

Attempts to acylate (2S)-aziridine-2-carboxylic acid as lithium salt in aqueous organic media with N-protected amino acid N-hydroxysuccinimide esters failed as well as the use of the *in situ* prepared trimethylsilyl ester of H-Azy-OH. Even by replacing the active esters with the much more reactive N-carbonic anhydrides of Fmoc-protected amino acids no dipeptides could be obtained in significant amounts. Conversely, the mixed anhydride procedure was found to be more promising particulary if iso-butylchloroformate was used to produce the mixed anhydride as demonstrated in the case of the synthesis of Z-Phe-Ala-Azy-OH (**9**) which could be obtained although in low yields. However, this compound was found to be surprisingly unstable both in solution or as solid according to HPLC.

Deprotection of (2S)-aziridine-2-carboxylic acid peptides

Since the hydrogenolytic removal of the benzyl group from Trt-Azy-OBzl was found to proceed quantitatively without formation of side products, it was surprising to note that hydrogenolysis of Z-Phe-Azy-OBzl (**8a**) and Z-Arg(Z_2)-Leu-Azy-OBzl (**8b**) over palladized charcoal in aqueous MeOH, n-BuOH or ethyl acetate/iso-propanol as well as in DMF with concomitant titration at pH 6.5 with $HClO_4$ (in the case of **8b**) in all cases led to complex mixtures of products from which the desired peptides could not be isolated. These findings contrast previous positive results obtained by *Okawa et al.*[2b] in similar synthetic steps. Unsuccessful were also the attempts to saponify the compounds **8a** and **8c** with NaOH or LiOH, although saponification of H-Azy-OBzl with LiOH is known to produce H-Azy-OLi as homogeneous crystalline product[5]. In view of the difficulties encountered above in the acidolytic deprotection of Trt-Azy-OBzl, exposure of Trt-Azy-Leu-OtBu (**7**), Trt-Azy-Leu-Arg-OH (**5a**) as well as Trt-Azy-Leu-Agm·HCl (**5b**) to trifluoroacetic acid treatment was again found to generate a mixture of products from which only chromatographic purification steps allowed to isolate the desired compounds H-Azy-Leu-OH (**10**), H-Azy-Leu-Arg-OH (**11**) and H-Azy-Leu-Agm (**12**), although in low yields. Even exposure of **5b** and Trt-Azy-Leu-Pro-OH (**5c**) to p-toluenesulfonic acid which allowed to produce **6** from **2** without any side products, did not generate the fully deprotected peptides as homogeneous compounds. In the case of **5c** the desired H-Azy-Leu-Pro-OH (**13**) was accompained by two side products from which one has been identified as ring-opened serine derivative as judged by mass spectrometry. Moreover, compounds **10**, **11** and **12** were found to be unstable on storage as lyophilized materials.

In conclusion, fully or partially protected aziridine-2-carboxylic acid containing peptides can be synthesized using both Trt-Azy-OPfp (**4**) and H-Azy-OBzl·DBSI (**6**) as intermediates. However, in all cases side products are formed leading to low yields due to the chromatographic steps needed for the isolation of the target compounds. Moreover, conversion of the peptide derivatives into the fully deprotected forms was successful only in selected cases strongly suggesting that the reactivity of the three membered aziridine ring is additionally affected by its topochemical environment. In this context it seems worthy to note that N-acylated aziridine-2-carboxylic acids are not stable at all, whereas N-alkylated or unprotected aziridine-2-carbonyl-peptides are significantly more stable.

Experimental Procedures

Melting points were determined on a Büchi apparatus and are uncorrected. All reagents and solvents were of the highest quality commercially available, and when necessary were further purified or dried by standard methods. One- and twodimensional ^{1}H-NMR spectra (internal standard TMS) were recorded on a Bruker AM 500 spectrometer at 500 MHz. Elemental analysis were obtained with a Heraeus CHN-O-Rapid apparatus. Amino acid analysis were performed on a Biotronic Analyzer (LC 6001). Acid hydrolysis was carried out with 6M HCl at 110 °C for 24 h and for 48 h, respectively. Optical rotations were measured in a 1 dm cell on a Perkin Elmer polarimeter (model 141). FAB-MS spectra were recorded on a Finnigan MAT 900. TLC was carried out on silica gel 60 plates (Merck) using the solvent systems: 1) n-heptane/tert.-butanol/pyridine (5:1:1); 2) n-butanol/HOAc/water/AcOEt (6:2:2:10). Compounds were visualized with the chlorine/o-tolidine, the fluorescamine and the aziridine[13] reagents. Analytical HPLC was performed on either a Macherey-Nagel Nucleosil C_8 or C_{18} column (5 µm particle size, 4 x 250 mm) with the following buffers: A) 5% CH_3CN/95% H_3PO_4 (2%); B) 80% CH_3CN/20% H_3PO_4 (2%); C) 15% CH_3CN/85% H_3PO_4 (2%); D) 50% CH_3CN/50% H_3PO_4 (2%); E) 30% CH_3CN/70% $NaClO_4$ (0.05 M, pH 6.5); F) 50% CH_3CN/50% $NaClO_4$ (0.05 M, pH 6.5) and G) 2% CH_3CN/98% H_3PO_4 (2%); flow rate: 1 mL/min. Reactions were performed at room temperature unless stated differently.

The following amino acid and peptide derivatives were obtained by standard procedures of peptide synthesis: **H-Ser-OBzl·TosOH**[14]; **H-Leu-Arg-OH** (mp 87-89 °C; $[\alpha]_D^{23}$ = -16.5 ° (c=1 MeOH); FAB-MS: m/e = 288 (100%), M + H^+ = 288.37 calcd. for $C_{12}H_{25}N_5O_3$); **H-Leu-Agm·HOAc·HCl** (FAB-MS: m/e = 244 (100%), M + H^+ = 244.36 calcd. for $C_{11}H_{25}N_5O$); **H-Leu-Pro-OH** (mp 60-62 °C; $[\alpha]_D^{23}$ = -64.7 ° (c=1 MeOH); FAB-MS: m/e = 229 (100%), M + H^+ = 229.30 calcd. for $C_{11}H_{20}N_2O_3$); **Trt-Ser-Leu-OtBu** (mp 63-65 °C; $[\alpha]_D^{23}$ = -68.7 ° (c=1 MeOH); anal. calcd. for $C_{32}H_{40}N_2O_4$: C, 74.39; H, 7.80; N, 5.42; found: C, 73.38; H, 7.98; N, 5.42); **Z-Phe-OH**[15]; **Z-Arg(Z_2)-Leu-OH**[16]; **Mva-Leu-OH** (mp 149-150 °C; FAB-MS: m/e = 230.5 (100%), M + H^+ = 230.33 calcd. for $C_{12}H_{23}NO_3$; anal. calcd. for $C_{12}H_{23}NO_3$: C, 62.85; H, 10.11; N, 6.11; found: C, 62.42; H, 10.13; N, 6.39); **Z-Phe-Ala-OH**[15].

Trt-Ser-OBzl (1)

The title compound **1** was prepared from H-Ser-OBzl·TosOH in CH_2Cl_2 according to *Nakajima et al.*[5]. The crude product was partitioned between AcOEt, 5% $KHSO_4$ and 5% $NaHCO_3$; the organic layer was washed with water and dried over Na_2SO_4. The solvent was evaporated and the oily residue chromatographed in aliquots on silica gel using petroleum ether/AcOEt (3:1) as eluent to give **1** as a syrup; yield: 54%; $[\alpha]_D^{23}$ = + 16.7 ° (c=1 MeOH) [Lit.[5]: $[\alpha]_D^{25}$ = + 64.8 ° (c=1 MeOH)].

Trt-Azy-OBzl (2)

Procedure a:

The title compound was synthesized by the method of *Okawa*[5] using methanesulfonyl chloride and then purified on silica gel using petroleum ether/AcOEt (10:1) as eluent; yield: 57%; mp 117-118 °C (Lit.[5]: 114-116 °C); $[\alpha]_D^{20}$ = -98.3 ° (c = 1 THF) [Lit.[5]: -95.5 ° (c=1 THF)]; FAB-MS: m/e = 420 (10%), 243 (100%), 91 (20%), M + H^+ = 420.53 calcd. for $C_{29}H_{25}NO_2$; ^{1}H-NMR ($CDCl_3$, 298 K): δ = 1.34 (dd, 1H, J_{cis} = 6.1 Hz, J_{gem} = 1.5 Hz, C3-H, aziridine); 1.85 (dd, 1H, J_{trans} = 2.6 Hz, J_{cis} = 6.1 Hz, C2-H, aziridine); 2.21 (dd, 1H, J_{trans} = 2.6 Hz, J_{gem} = 1.8 Hz, C3-H', aziridine); 5.14, 5.17 (2d, 2H, J_{gem} = 12.2 Hz, CH_2-O); 7.1 - 7.38 (m, 14H, aromatic H); 7.4 - 7.5 (m, 6H, aromatic H); anal. calcd. for $C_{29}H_{25}NO_2$: C, 83.03; H, 6.01; N, 3.34, found: C, 83.11; H, 5.92; N, 3.39.

Procedure b:

Following a modified version of the method described by *Kuyl-Yeheskiely et al.*[9d] crystalline compound **1** was obtained in 56% yield from n-hexane as reported previously[10].

Trt-Azy-OH (3)

Compound **2** (5.8 g, 13.8 mmol) was hydrogenated in dioxan (100 mL) over Pd/C. After 2 h the catalyst was filtered off and the solvent evaporated. The residue was chromatographed on a silica gel column (5 x 55 cm) using AcOEt as eluent to produce crystalline **3** (from n-hexane) in 79% yield (3.6 g); mp >175 °C decomp.; $[\alpha]_D^{23}$ = -59.6° (c = 1 DMF); FAB-MS: m/e = 330 (10%), 243 (100%), M + H^+ = 330.4 calcd. for $C_{22}H_{19}NO_2$; ^{1}H-NMR (DMSO-d_6/D_2O (5:1), 300 K): δ = 1.73 (dd, 1H, J_{cis} = 6.1 Hz, J_{gem} = 1.6 Hz, C3-H, aziridine); 2.15 (dd, 1H, J_{cis} = 6.1 Hz, J_{trans} = 2.8 Hz, C2-H, aziridine); 2.65 (dd, 1H, J_{trans} = 2.7 Hz, J_{gem} = 1.7 Hz, C3-H', aziridine); 7.9-8.2 (m, 15H, aromatic H).

Trt-Azy-OPfp (4)

To an ice-cold solution of **3** (1.0 g, 3.04 mmol) and HOPfp (0.62 g, 3.34 mmol) in DMF (5 mL) DCC (0.69 g, 3.34 mmol) was added. The mixture was then diluted with AcOEt (15 mL) and stirred for 1 h at 0 °C and for further 48 h at room temperature. Dicyclohexylurea was filtered off and the solvent was evaporated *in vacuo*. The residue was purified on a dried silica gel (120 °C, 24 h, reduced pressure) column (5 x 50 cm) using petroleum ether/AcOEt (11:1) as eluent. The solvent was evaporated and the solid lyophilized from dioxan to produce a colourless powder; yield: 770 mg (51%); mp 79-80 °C; $[\alpha]_D^{23}$ = -127.8 ° (c = 1 MeOH); HPLC (Nucleosil C_8): linear gradient from 100% A to 90% B in 25 min, t_R 28.46 min; FAB-MS: m/e = 495 (10%), 243.1 (100%), M^+ = 495.45 calcd. for $C_{28}H_{18}NO_2F_5$; ^{1}H-NMR ($CDCl_3$, 300 K): δ = 1.6 (dd, 1H, J_{gem} = 1.1 Hz, J_{cis} = 5.9 Hz, C3-H, aziridine); 2.16 (dd, 1H, J_{trans} = 2.4 Hz, J_{cis} = 5.9 Hz, C2-H, aziridine); 2.48 (dd, 1H,

J_{gem} = 1.1 Hz, J_{trans} = 2.4 Hz, C3-H', aziridine); 7.21-7.30 (m, 9H, aromatic H); 7.50 (d, 6H, J_{ortho} = 7.5 Hz, aromatic H).

Trt-Azy-Leu-Arg-OH (5a)

H-Leu-Arg-OH (0.26 g, 0.89 mmol) was treated with **4** (0.4 g, 0.81 mmol) in DMF (10 mL) for 24 h; then the solution was evaporated and the residue partitioned between AcOEt and water. The organic layers were combined, dried over Na_2SO_4 and concentrated, and the residue was precipitated with petroleum ether; yield: 401 mg (83%); mp >185 °C decomp.; amino acid analysis: Leu 1.01 (1), Arg 0.99 (1), Ser (from Azy)[1b] 0.47 (1); amino acid analysis with 20% $HClO_4$ according to *Korn et al.*[10]: Leu 1.00 (1), Arg 1.03 (1), Ser (from Azy) 0.83 (1), no significant racemization was observed[10]; FAB-MS: m/e = 599.3 (70%), 243.1 (100%), M + H^+ = 599.76 calcd. for $C_{34}H_{42}N_6O_4$; ^{1}H-NMR (CD_3OD, 300 K): δ = 0.96 (d, 3H, J = 6.3 Hz, Leu δ_2); 1.00 (d, 3H, J = 6.3 Hz, Leu δ_1); 1.37 (dd, 1H, J_{cis} = 6.2 Hz, J_{gem} = 1.2 Hz, C3-H, aziridine); 1.59-1.76 (m, 6H, Arg β_2, Arg γ, Leu β_1, β_2, Leu γ); 1.77-1.90 (m, 1H, Arg β_1); 2.00 (dd, 1H, J_{trans} = 2.7 Hz, J_{cis} = 6.2 Hz, C2-H, aziridine); 2.16 (dd, 1H, J_{trans} = 2.7 Hz, J_{gem} = 1.2 Hz, C3-H', aziridine); 3.13-3.20 (m, 2H, Arg δ); 4.27 (dd, 1H, J = 4.3 Hz, J = 5.1 Hz, Arg αCH); 4.53 (dd, 1H, J = 5.3 Hz, J = 9.7 Hz, Leu αCH); 7.23-7.30 (m, 9H, aromatic H); 7.48 (d, 6H, J_{ortho} = 7.4 Hz, aromatic H).

Trt-Azy-Leu-Agm·HCl (5b)

Compound **5b** was synthesized employing the procedure of **5a** and using 1.1 eq. of H-Leu-Agm·HOAc·HCl and 1.1 eq. of NMM. The product was lyophilized from tert.-BuOH; yield: 60%; mp 124-125 °C; FAB-MS: m/e = 555 (80%), 243 (100%), M + H^+ = 555.75 calcd. for $C_{33}H_{42}N_6O_2$; ^{1}H-NMR (CD_3OD, 300 K): δ = 0.95 (d, 3H, J = 6.3 Hz, Leu δ_2); 0.99 (d, 3H, J = 6.3 Hz, Leu δ_1); 1.36 (br d, 1H, J_{cis} = 6.2 Hz, J_{gem} not resolved, C3-H, aziridine); 1.52-1.65 (m, 7H, Leu γ, Leu β_2, β_1, Agm γ_2, γ_1, Agm β_2, β_1); 2.0 (dd, 1H, J_{cis} = 6.2 Hz, J_{trans} = 2.6 Hz, C2-H, aziridine); 2.15 (br d, 1H, J_{trans} = 2.5 Hz, J_{gem} not resolved, C3-H', aziridine); 3.17-3.27 (m, 4H, Agm δ_2, δ_1, Agm α_2, α_1); 4.49 (dd, 1H, J = 4.8 Hz, J = 9.8 Hz, Leu α); 7.22-7.30 (m. 9H, aromatic H); 7.47 (d, 6H, J_{ortho} = 7.5 Hz, aromatic H).

Trt-Azy-Leu-Pro-OH (5c)

The title compound was prepared as described for **5a** and using 1.1 eq. of H-Leu-Pro-OH and 1 eq. of TEA. The product was chromatographed on a LiChroprep RP18 column (2 x 130 cm) using 0.05 M ammonium acetate buffer (pH 7.0)/iso-propanol/n-BuOH (80/15/5, buffer A; 20/60/20, buffer B) as eluent; linear gradient from 20% B to 40% B in 8 h, in additional 5 h to 60% B and for further 5 h to 100% B, flow rate: 230 mL/h; yield: 27%; mp 176-177 °C; FAB-MS: m/e = 540 (20%), 243 (100%), M + H^+ = 540.69 calcd. for

$C_{33}H_{37}N_3O_4$; ^{1}H-NMR (DMSO-d_6, 300 K): δ = 0.92 (d, 3H, J = 6.6 Hz, Leu δ_2); 0.95 (d, 3H, J = 6.6 Hz, Leu δ_1); 1.11 (dd, 1H, J_{gem} = 1.2 Hz, J_{cis} = 6.2 Hz, C3-H, aziridine); 1.37-1.44 (m, 1H, Leu β_2); 1.48-1.55 (m, 1H, Leu β_1); 1.62-1.78 (m, 1H, Leu γ); 1.85 (dd, 1H, J_{trans} = 2.9 Hz, J_{cis} = 6.2 Hz, C2-H, aziridine); 1.81-1.86 (m, 1H, Pro β_2); 1.88-1.95 (m, 2H, Pro γCH_2); 2.05-2.12 (m, 1H, Pro β_1); 2.08 (dd, 1H, J_{gem} not resolved, J_{trans} = 2.9 Hz, C3-H', aziridine); 3.52 (m, 1H, Pro $\delta_2 CH_2$); 3.65 (m, 1H, Pro $\delta_1 CH_2$); 4.23 (dd, 1H, J = 4.1 Hz, J = 8.6 Hz, Pro α); 4.73 (ddd, 1H, J = 4.0 Hz, J = 8.7 Hz, J = 10.4 Hz, Leu α); 7.22-7.33 (m, 9H, aromatic H); 7.41 (d, 6H, J_{ortho} = 7.5 Hz, aromatic H); 8.29 (d, 1H, J = 8.7 Hz, Leu NH).

H-Azy-OBzl·DBSI (6)

To a stirred solution of **2** (2.98 g, 7.1 mmol) in chloroform (40 mL) a solution of Tos-OH·H_2O (13.5 g, 71 mmol) in MeOH (30 mL) was added. After 2 h the solvent was removed *in vacuo* and the residue partitioned between AcOEt and water. The aqueous phases were combined and neutralized with $NaHCO_3$. The product was reextracted with AcOEt, the organic phase dried over Na_2SO_4 and evaporated to oily residue. The product was dissolved in MeOH and upon addition of 1 eq. DBSI precipitated with ether; yield: 1.66 g (72%); mp 115-116 °C; $[\alpha]_D^{23}$ = -10.8 °; homogeneous on tlc (solvent system 1) and on HPLC (Nucleosil C_8; linear gradient from 100% A to 90% B in 25 min, t_R 8.40 min; FAB-MS: m/e = 178.0 (50%), 91 (40%), M + H^+ = 178.21 calcd. for $C_{10}H_{11}NO_2$; ^{1}H-NMR of free H-Azy-OBzl ($CDCl_3$, 300 K): δ = 1.83 (dd, 1H, J_{cis} = 5.4 Hz, J_{gem} = 1.3 Hz, C3-H, aziridine); 1.99 (dd, 1H, J_{trans} = 2.9 Hz, J_{gem} = 1.4 Hz, C3-H', aziridine); 2.53 (dd, 1H, J_{trans} = 2.9 Hz, J_{cis} = 5.4 Hz, C2-H, aziridine); 5.14 (d, 1H, J_{gem} = 12.2 Hz, CH_2-O); 5.17 (d, 1H, J_{gem} = 12.2 Hz, CH_2-O); 7.28-7.40 (m, 5H, aromatic H); anal. calcd. for $C_{22}H_{22}N_2O_6S_2$: C, 55.68; H, 4.67; N, 5.90, found: C, 55.61; H, 4.77; N, 6.10.

Trt-Azy-Leu-OtBu (7)

Following the procedure described for **2a** the title compound was prepared from Trt-Ser-Leu-OtBu; yield: 0.83 g (67%) of crystalline product after chromatography on a silica gel column (3 x 30 cm) using petroleum ether/AcOEt (6:1) as eluent; mp 66-67 °C; $[\alpha]_D^{23}$ = -101.3 ° (c = 1 MeOH); ^{1}H-NMR ($CDCl_3$, 300 K): δ= 0.94 (d, 3H, J = 5.9 Hz, Leu δ_2); 0.95 (d, 3H, J = 5.9 Hz, Leu δ_1); 1.44 (dd, 1H, J_{cis} = 5.5 Hz, J_{gem} = 2.1 Hz, C3-H, aziridine); 1.48 (s, 9H, tBu); 1.52-1.58 (m, 1H, Leu β_2); 1.61-1.66 (m, 2H, Leu β_1, Leu γ); 1.94 (br s, 1H, J not resolved, C3-H', aziridine); 1.95 (dd, 1H, J_{cis} = 5.3 Hz, J_{trans} = 2.7 Hz, C2-H, aziridine); 4.58 (t, d, 1H, $J_{\alpha,NH}$ = 8.8 Hz, $J_{\alpha,\beta}$ = 5.0 Hz, Leu α); 7.14 (d, 1H, $J_{\alpha,NH}$ = 9.0 Hz, Leu N-H); 7.13-7.28 (m, 9H, aromatic H); 7.43 (d, 6H, J_{ortho} = 7.7 Hz).

Z-Phe-Azy-OBzl (8a)

H-Azy-OBzl·DBSI (**6**) (0.3 g, 0.63 mmol) and Z-Phe-OH (0.21 g, 0.7 mmol) were treated in the presence of NMM (69.5 µl, 0.63 mmol) in dry DMF (4 mL) with HOBt (0.094 g, 0.7 mmol) and DCC (0.143 g, 0.7 mmol) for 5 h. The precipitate was filtered off and the filtrate was diluted with AcOEt (50 mL). The organic layer was washed with 5% $NaHCO_3$ (3 x 40 mL), water (3 x 40 mL) and dried over Na_2SO_4. The solvent was evaporated to an oily residue which was chromatographed on a silica gel column (3 x 40 cm) with petroleum ether/AcOEt (5:3) as eluent. The pure fractions were combined and then evaporated to oil; yield: 270 mg (93%); amino acid analysis (24 h): Phe 1.00 (1), Ser (from Azy)[1b] 0.43 (1); HPLC (Nucleosil C_{18}): linear gradient from 100% C to 90% B in 25 min, t_R 23.95 min.

Z-Arg(Z_2)-Leu-Azy-OBzl (8b)

The compound was prepared as described for **8a** from Z-Arg(Z_2)-Leu-OH and H-Azy-OBzl·DBSI and purified by chromatography on silica gel using petroleum ether/AcOEt (3:2) as eluent; yield: 88%; HPLC: (conditions as for **8a**, t_R 28.45 min).

Mva-Leu-Azy-OBzl (8c)

Prepared as described for **8a** from Mva-Leu-OH and H-Azy-OBzl·DBSI and after chromatographic purification on silica gel with petroleum ether/AcOEt (1:2) as eluent; **8c** was obtained in 43% yield; HPLC (Nucleosil C_8): linear gradient from 80% C to 100% D in 25 min, t_R 29.13 min.

Z-Phe-Ala-Azy-OH (9)

Z-Phe-Ala-OH (0.56 g, 1.5 mmol) was treated in dry THF (10 mL) with NMM (165 µL, 1.5 mmol) and iso-butylchloroformate (197 µL, 1.5 mmol); after 10 min at -15 °C a solution of H-Azy-OLi (0.14 g, 1.5 mmol) in H_2O / THF (2:1, 3 mL) was added and the mixture stirred for 2 h. The solvent was evaporated and the residue partitioned between AcOEt and 5% $KHSO_4$; the organic layer was washed with water, dried over Na_2SO_4 and concentrated to small volume. On addition of petroleum ether the precipitate was collected and chromatographed on a LiChroprep RP18 column (0.63 x 100 cm) using 0.05 M ammonium acetate buffer (pH 6.45)/iso-propanol/n-BuOH (92/6/2 buffer A; 70/20/10 buffer B) as eluent, linear gradient from 100% A to 100% B in 20 h, flow rate: 24 mL/h; yield: 257 mg (39%); FAB-MS: m/e = 440 (10%), 353 (40%), 91 (85%), M + H^+ = 440.48 calcd. for $C_{23}H_{25}N_3O_6$; amino acid analysis (24 h): Phe 1.00 (1), Ala 1.06 (1), Ser (from Azy)[1b] 0.37 (1); HPLC (Nucleosil C_8): from 80% C to 30% D in 20 min, t_R 21.79 min; due to the instability of **9** no reproducible NMR spectra were obtained.

H-Azy-Leu-OH (10)

To a stirred solution of 7 (0.48 g, 0.96 mmol) in CH_2Cl_2 (3.6 mL) TFA/H_2O (9:1, 2.5 mL) was added at -10 °C. After 6 h stirring at -10 °C the solvent was evaporated and the residue partitioned between ether and water. The aqueous layer was neutralized with DOWEX 44 (OH form), the resin was filtered off and the aqueous layer lyophilized. The crude pruduct was then purified on a silica gel column (2 x 42 cm) using $CHCl_3$/MeOH (12:1) as eluent. Rechromatography on a LiChroprep RP 18 column (0.63 x 100 cm) using 0.05 M ammonium acetate buffer (pH 6.2)/iso-propanol/n-BuOH (98/2/0 buffer A; 70/20/10 buffer B) as eluent (linear gradient from 100% A to 100% B in 12 h, flow rate: 24 mL/h) led to a yellow-green oil which was rechromatographed on silica gel using the same conditions as described above; yield: 26 mg oil (14%); FAB-MS: m/e = 201 (100%), M + H^+ = 201.12 calcd. for $C_9H_{16}N_2O_3$; HPLC (Nucleosil C_8): linear gradient from 100% E to 100% F in 20 min, t_R 15.10 min. The compound was found to be unstable on storage.

H-Azy-Leu-Arg-OH (11)

Following the procedure described for **10** the title compound was prepared from **5a** (0.3 g, 0.51 mmol); yield: 110 mg (61%) after chromatography on a LiChroprep RP18 column (0.63 x 100 cm) using 0.05 M ammonium acetate buffer (pH 5.1)/iso-propanol (95/5) as isocratic eluent, flow rate: 24 mL/h; FAB-MS: m/e = 357.1 (100%), M + H^+ = 357.43 calcd. for $C_{15}H_{28}N_6O_4$. The compound was found to be unstable during lyophilization as determined by HPLC (Nucleosil C_{18}, linear gradient from 100% G to 100% C in 30 min).

H-Azy-Leu-Agm (12)

Prepared as described for **10** from **5b** (0.19 g, 0.33 mmol) at room temperature for 3 h; yield: 36 mg (35%) after chromatography on a LiChroprep RP18 column (0.63 x 100 cm) using 0.05 M ammonium acetate buffer (pH 6.45)/iso-propanol/n-BuOH (98/2/0 buffer A; 92/6/2 buffer B) as eluent, linear gradient from 100% A to 100% B in 1 h, flow rate: 24 mL/h; FAB-MS: m/e = 313 (80%), M + H^+ = 313.42; 331 (80%), M + H_2O + H^+ = 331.43; 349 (100%), M + HCl + H^+ = 349.88; 427 (20%), M + TFA + H^+ = 427.45, calcd. for $C_{14}H_{28}N_6O_2$; HPLC (Nucleosil C_8): linear gradient from 100% E to 40% D in 25 min, t_R 13.33 min. The compound was found to be unstable on storage.

H-Azy-Leu-Pro-OH (13)

The title compound was prepared as described for **10** from **5c** (0.09 g, 0.17 mmol) using TosOH·H_2O (0.32 g, 1.7 mmol) instead of TFA in $CHCl_3$/MeOH (1:1) at room temperature for 2h; yield: 6.9 mg (14%) after chromatography on a LiChroprep RP18 column (1.27 x 100 cm) using 0.05 M ammonium acetate buffer (pH

6.4)/iso-propanol (98/2 buffer A; 50/50 buffer B) as eluent, linear gradient from 100% A to 100% B in 20 h, flow rate: 96 mL/h; FAB-MS: m/e = 298 (60%), M + H^+ = 298.36 calcd. for $C_{14}H_{23}N_3O_4$; the compound was found to be unstable during lyophilization as determined on HPLC (Nucleosil C_8; linear gradient from 100% G to 40% D in 25 min).

Acknowledgments

We thank the Bayerisches Staatsministerium für Unterricht und Kultus for partial financial support.

REFERENCES

1. (a) Okawa, K.; Yuki, M.; Tanaka, T.; *Chem. Lett.*, **1979**, 1085-1086. (b) Johnson, T.B.; Coward, J.K.; *J. Org. Chem.*, **1987**, *52*, 1771-1779. (c) Sato, K.; Kozikowski, A.P.; *Tetrahedron Lett.*, **1989**, *30*, 4073-4076. (d) Tanaka, T.; Nakajima, K.; Okawa, K.; *Bull. Chem. Soc. Jpn.*, **1980**, *53*, 1352-1355. (e) Nakajima, K.; Tanaka, T.; Neya, M.; Okawa, K.; in *Peptide Chemistry*, Shioiri, T. Ed.; Protein Research Foundation: Osaka, 1981; pp. 143-148.

2. (a) Okawa, K.; Nakajima, K.; *Biopolymers*, **1981**, *20*, 1811-1821. (b) Okawa, K.; Nakajima, K.; Tanaka, T.; *J. Synth. Org. Chem. Jpn.*, **1984**, *42*, 390-406.

3. Murphy, B.P.; Pratt, R.F.; *Biochemistry*, **1991**, *30*, 3640-3649.

4. (a) Gerhart, F.; Higgins, W.; Tardif, C.; Ducep, J.-B.; *J. Med. Chem.*, **1990**, *33*, 2157-2162. (b) Zhong, Z.; Bibbs, J.A.; Yuan, W.; Wong, C.-H.; *J. Am. Chem. Soc.*, **1991**, *113*, 2259-2263. (c) Moroder, L.; Musiol, H.-J.; Scharf, R.; *Febs Lett.*, **1992**, *299*, 51-53.

5. Nakajima, K.; Takai, F.; Tanaka, T.; Okawa, K.; *Bull. Chem. Soc. Jpn.*, **1978**, *51*, 1577-1578.

6. (a) Nakajima, K.; Sasaki, H.; Neya, M.; Morishita, M.; Sakai, S.; Okawa, K.; in *Peptide Chemistry*, Sakakibara, S. Ed.; Protein Research Foundation: Osaka, 1983; pp. 19-24. (b) Wipf, P.; Miller, C.P.; *Tetrahedron Lett.*, **1992**, *33*, 6267-6270.

7. (a) Nakajima, K.; Neya, M.; Yamada, S.; Okawa, K.; *Bull. Chem. Soc. Jpn.*, **1982**, *55*, 3049-3050. (b) Nakajima, K.; Oda, H.; Okawa, K.; *Bull. Chem. Soc. Jpn.*, **1983**, *56*, 520-522.

8. Kuyl-Yeheskiely, E.; Dreef-Tromp, C.M.; van der Marel, G.A.; van Bloom, J.H.; *Recl. Trav. Chim. Pays-Bas*, **1989**, *108*, 314-316.

9. (a) Legters, J.; Thijs, L.; Zwanenburg, B.; *Tetrahedron Lett.*, **1989**, *30*, 4881-4884. (b) Thijs, L.; Porskamp, J.J.M.; van Loon, A.A.W.M.; Derks, M.P.W.; Feenstra, R.W.; Legters, J.; Zwanenburg, B.; *Tetrahedron*, **1990**, *46*, 2611-2622. (c) Cainelli, G.; Panunzio, M.; *Tetrahedron Lett.*, **1991**, *32*, 121-124. (d) Kuyl-Yeheskiely, E.; Lodder, M.; van der Marel, G.A.; van Boom, J.H.; *Tetrahedron Lett.*, **1992**, *33*, 3013-3016.

10. Korn, A.; Rudolph-Böhner, S.; Moroder, L.; *Z. Naturforsch.*, **1993**, *48b*, 1146-1148.

11. Okawa, K.; Nakajima, K.; Tanaka, T.; Kawana, Y.; *Chem. Lett.*, **1975**, 591-594.

12. (a) Okawa, K.; Nakajima, K.; Tanaka, T.; Neya, M.; *Bull. Chem. Soc. Jpn.*, **1982**, *55*, 174-176. (b) Legters, J.; Willems, J.G.H.; Thijs, L.; Zwanenburg, B.; *Recl. Trav. Chim. Pays-Bas*, **1992**, *111*, 59-68.

13. Norpoth, K.; Schriewer, H.; Rauen, H.M.; *Arzneim.-Forsch. (Drug-Res.)*, **1971**, *21*, 1718-1721.

14. Voss, H.; *Z. Naturforsch.*, **1965**, *206*, 116.

15. Grassmann, W.; Wünsch, E.; Riedel, H.; *Chem. Ber.*, **1958**, *91*, 455.

16. Wünsch, E.; Wendelberger, G.; Thamm, P.; *Chem. Ber.*, **1971**, *104*, 2445-2453.

(*Received in Germany* 4 *October* 1993; *accepted* 26 *October* 1993)