Pergamon

Tetrahedron Vol. 50, No. 28, pp. 8381-8392, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4020/94 $7.00+0.00

0040-4020(94)00457-9

A Convenient Synthesis of Optically Pure (2*R*, 3*R*)-2, 3-Epoxysuccinyl - Dipeptides

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Abstract: (2*R*,3*R*)-*trans*-Epoxysuccinyl-dipeptides (**7a-7d**) were synthesized by acylation of dipeptides with the N-hydroxysuccinimde or pentafluorophenyl ester of monoethyl (2*R*,3*R*)-*trans*-epoxysuccinate (**6a-6b**). A nucleophilic oxirane ring opening by N-hydroxysuccinimide and pentafluorophenol during the preparation of the active esters could not be observed. Subsequent saponification of the monoethyl ester **7a-7d** with KOH in ethanolic solution allowed to produce the dipeptide derivatives as potassium salts (**8a-8d**) which were found to be stable on storage in the cold. The attempts to convert (2*R*,3*R*)-*trans*-epoxysuccinyl-glycyl-proline (**7a**) into the corresponding (2*S*,3*S*)-*trans*-epithiosuccinyl derivative via an oxygen sulfur exchange reaction with 3-methylbenzothiazole-2-thione failed completely as among the various products of unknown nature formed only the desulfurated fumaryl derivative could be isolated and characterized.

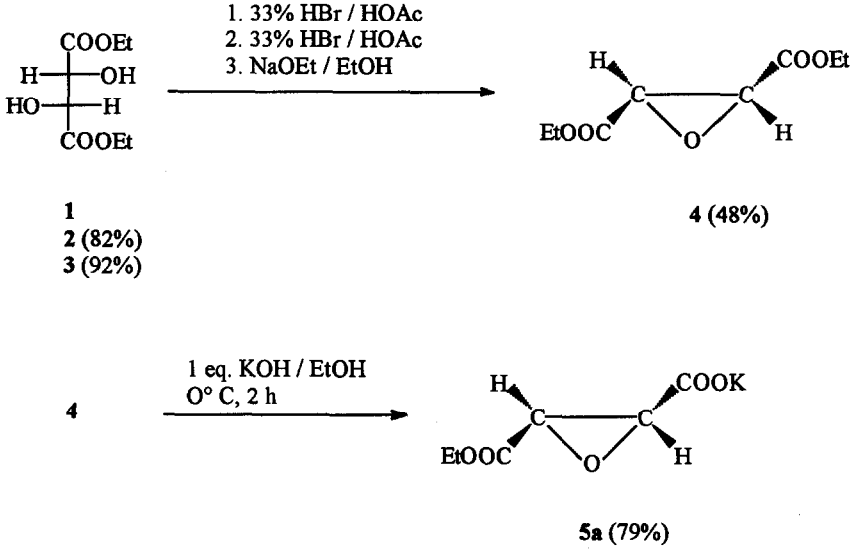
Introduction

Since the isolation of *L-trans*-epoxysuccinyl-leucylamido(4-guanidino)butane (E-64) from extracts of *Aspergillus Japonicus* TPR-46 and its discovery as a potent new inhibitor of cysteine-proteases[1], increasing interest has been paid to the epoxysuccinyl moiety as reactive handle for the design of specific inhibitors of this class of proteases[2]. Because of the fast reaction with the active-site thiol function[2c,2d,3], the high degree of specificity for cysteine proteases[1,2c] and the low toxicity[4] epoxysuccinyl-peptide derivatives represent a promising new type of therapeutic agents as cysteine proteases are known to be implicated in a variety of disease states. Nevertheless surprisingly little work has been reported on the optimization of synthetic protocols.

Although the *D-trans*-epoxysuccinyl-peptide derivatives are known to be weaker inhibitors than the related *L*-isomers we have focussed our attention on the synthesis of this isomer in view of a possible direct conversion of the epoxysuccinyl-peptides into the episulfides by oxygen sulfur exchange reactions[5] which occur with Walden inversion at both chiral C-atoms. This approach would allow us to investigate possible improvements in the synthetic accessibility of epoxysuccinyl-peptides independently of the *D*- or *L*-configuration and finally to attempt their conversion into the *L-trans*-epithiosuccinyl analogs as possibly interesting new inhibitors of cysteine-proteases. In fact, the thiirane moiety is expected to be at least as reactive as the oxirane ring and to form in analogy to the epoxy group a stable thioether bond with the active-site thiol function[6].

Results and Discussion

The key intermediate in the synthesis of *D*- (or *L*-) *trans*-epoxysuccinyl-peptides* is the related monoalkyl-epoxysuccinate. This derivative is available by resolution of racemic epoxysuccinic acid via diastereomeric salt formation[7] followed by esterification and partial saponification[8] or by stereoselective synthesis of the dialkyl ester of desired configuration[9] and partial hydrolysis to the monoalkyl ester. We have selected the second approach[9a] in a slightly modified version which allowed us to prepare from diethyl *L*-(+)-tartrate (**1**) following the route outlined in Sheme 1 the diethyl *D-trans*-epoxysuccinate (**4**) as optically homogeneous compound.



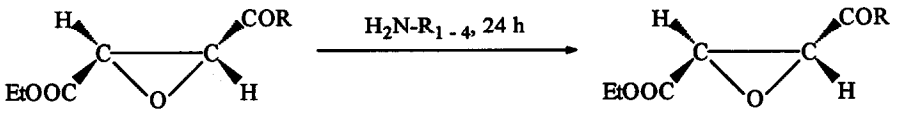
Scheme 1

Saponification of **4** with the stoichiometric amount of KOH produced the desired monoethyl *D-trans*-epoxysuccinate potassium salt (**5a**) in a satisfactory yield of about 40% over the three steps. This compound proved to be fully stable on storage in the cold over longer periods of time. A crucial step in this synthesis was found to be, upon fractional distillation of the diethylester **4**, a silica gel chromatography with

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; Agm, agmatine; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; DMSO; dimethyl sulfoxide; EtOAc, ethyl acetate; EtOH, ethanol; HOAc, acetic acid; HOBt, 1-hydroxybenzotriazole; MBTT, 3-methylbenzothiazole-2-thione; MeOH, methanol; NMM, N-methylmorpholine; tEps, (2*R*,3*R*) trans-epoxysuccinyl; TFA, trifluoroacetic acid.

petroleum ether/EtOAc as eluent. This allowed for separation of trace amounts of contaminating compound **2** and **3** as monitored by MS and NMR.

N^{α}–acylation of suitably protected amino acid or peptide derivatives with monoalkyl-epoxysuccinate has been performed, so far, using diethylphosphorylcyanide[1b] or DCC/HOBt[2e,2f,8] as coupling reagents as well as by the 4-nitrophenyl ester procedure[7a]. Thereby relatively low yields of epoxysuccinyl-peptides were obtained, a fact which could possibly be attributed to sterical hindrance from the oxirane ring or to side reactions involving nucleophilic ring opening, e.g. by HOBt. We have analyzed both aspects by the use of the N-hydroxysuccinimide (**6a**) and pentafluorophenyl ester (**6b**) which are known to be more reactive than the 4-nitrophenyl ester. Moreover N-hydroxysuccinimide and pentafluorophenol are expected to be weaker nucleophiles than HOBt and 4-nitrophenol, respectively. Although the epoxysuccinyl moiety is known to undergo facile attack by nucleophiles, this type of side reaction could not be observed during the preparation of the active esters using equimolar amounts of N-hydroxysuccinimide as a good nucleophile and pentafluorophenol as a weak nucleophile. With or without intermediate isolation of the active esters unprotected dipeptides were acylated with more or less success in terms of final yields as reported in Scheme 2.



6a-b

7a (55%)
7b (28%)
7c (16%)
7d (77%)

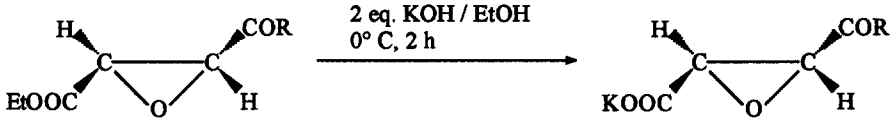
Comp.	R
6a	OSu
6b	OPfp

Comp.	R
7a	-Gly-Pro-OH
7b	-Leu-Pro-OH
7c	-Leu-Arg-OH
7d	-Leu-Agm·1/2H_2SO_4

Scheme 2

The N-hydroxysuccinimide ester procedure allowed to significantly enhance the yield of E-64 as *D*-isomer (**8d**), upon saponification of the monoethyl ester **7d** with KOH, from 8%[1b] to 23% in the overall reaction. Similarly, by the pentafluorophenyl ester method compound **8b** was obtained over the two steps in 26%

yield in comparison to the 9% yield reported for this epoxysuccinyl-peptide by the DCC/HOBt procedure[2e]. The monoethyl esters **7a-d** were converted to the free acids (**8a-d**) by saponification with KOH as enzymatic hydrolysis with pig liver esterase has been found to produce the free acids in relatively low yields[2e] (Scheme 3).



7a-d

8a (96%)
8b (92%)
8c (60%)
8d (30%)

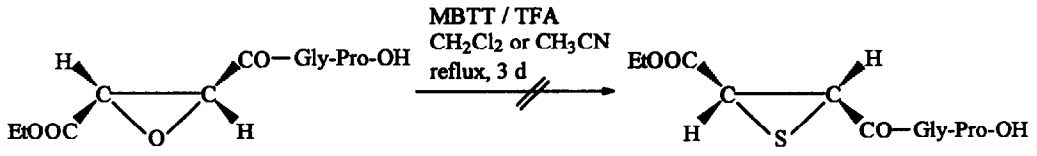
Comp.	R
7a	-Gly-Pro-OH
7b	-Leu-Pro-OH
7c	-Leu-Arg-OH
7d	-Leu-Agm·1/2H_2SO_4

Comp.	R
8a	-Gly-Pro-OK
8b	-Leu-Pro-OK
8c	-Leu-Arg-OK
8d	-Leu-Agm

Scheme 3

If needed, e.g. for **8c** and **8d,** preparative HPLC was used for the final purification whereby attention was paid to avoid the use of acids and buffer ingredients since nucleophilic oxirane ring opening was observed to occur particularly, in the lyophilization step.

Finally, the *D-trans*-epoxysuccinyl-glycyl-proline monoethyl ester (**7a**) was treated as model compound with 3-methylbenzothiazole-2-thione which has been proposed as the most efficient reagent for an oxygen sulfur exchange in the oxirane ring[5e]. Following the literature procedure, i.e. operating at 0 °C, as well as by refluxing the reaction mixture in CH_2Cl_2 as described for similar reactions with phosphine sulfides[5d] all attempts to produce the corresponding *L-trans*-epithiosuccinyl analog failed (Scheme 4).



7a

Scheme 4

A complex mixture of products was formed of which none of the components was found to exhibit the expected mass. Among the various products isolated one was identified as the desulfurated derivative according to the mass spectrum. Therefore alternative procedures are under investigation to possibly produce these epithiosuccinyl-peptides.

Experimental Procedures

Melting points were determined on a capillary melting point apparatus (Büchi) and are uncorrected. Elemental analysis were obtained with a Heraeus CHN-O-Rapid apparatus. Optical rotations were measured in a 1 dm cell on a Perkin Elmer polarimeter (model 241). 1D and 2D ^{1}H- as well as ^{13}C-NMR spectra were recorded at 300 K using a Bruker AM 500 spectrometer at 500 MHz for ^{1}H and a Bruker AM 400 spectrometer at 100.6 MHz for ^{13}C {^{1}H}, respectively. FAB-MS spectra were obtained on a Finnigan MAT 900 and EI-MS spectra on a Varian MAT 312, respectively. FT-IR data were determined with a Perkin Elmer FT-IR Spectrometer 1760 X. Analytical HPLC was performed on either Nucleosil C_8 (4 x 25 cm, 5 µm particle size, 300 Å) or C_{18} (4 x 25 cm, 5 µm particle size, 100 Å) columns (Macherey-Nagel, Düren, Germany) using the following buffers: A) 5% CH_3CN/95% H_3PO_4 (2%); B) 15% CH_3CN/85% H_3PO_4 (2%); C) 50% CH_3CN/50% H_3PO_4 (2%); flow rate: 1 mL/min. Preparative HPLC was performed on a Macherey-Nagel Nucleosil C_{18} (2 x 25 cm, 5 µm particle size, 300 Å) column using following buffers: I) 2% CH_3CN/98% H_2O; II) 5% CH_3CN/95% H_2O; III) 15% CH_3CN/85% H_2O; IV) 50% CH_3CN/50% H_2O; flow rate: 9 mL/min.

The following dipeptides were obtained by standard procedures of peptide synthesis: **H-Gly-Pro-OH**[10], **HCOOH·H-Leu-Agm·1/2 H_2SO_4** (semi solid; FAB-MS: m/e = 244.5 (100%), M+H$^+$ = 244.4 calcd. for $C_{11}H_{25}N_5O$); **H-Leu-Arg-OH**[11]; **H-Leu-Pro-OH**[11].

Diethyl (2*R*, 3*S*)-erythro-2-acetoxy-3-bromosuccinate (2)

Compound **2** was prepared from **1** (85.6 g, 0.415 mol) by treatment with 33% HBr/HOAc (292 mL) for 4h at room temperature; yield **2**: 106 g (82%); FT-IR: ν_{max} = 2985 (m), 1756 (s), 1374 (m), 1275 (m), 1212 (s), 1024 (m), 860 (m) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ = 1.30, 1.31 (2 x t, 6H, J = 7 Hz, 2 x $\underline{CH_3}$-CH_2-O-); 2.17 (s, 3H, CH_3-CO); 4.24, 4.28 (2 x q, 4H, 2 x CH_3-$\underline{CH_2}$-O-); 4.81 (d, 1H, J = 5 Hz, C3-H); 5.59 (d, 1H, J = 5 Hz, C2-H); anal. calcd. for $C_{10}H_{15}O_6Br$: C, 38.60; H, 4.86, found: C, 38.45; H, 5.02.

Diethyl (2*R*, 3*S*)-erythro-2-hydroxy-3-bromosuccinate (3)

The intermediate **2** (106 g, 0.339 mol) was treated with 32 mL of 33% HBr/HOAc in EtOH (962 mL) under reflux for 5h. Fractional distillation without further silica gel chromatography led to the homogeneous **3**; yield: 84.1 g (92%); bp 111-112 °C/~0.2 mm (Lit.[9a]: 123-125 °C/0.6 mm); n_D^{23} = 1.4635 (Lit.[9a]: n_D^{21} = 1.4628); $[\alpha]_D^{23}$ = -29.4 ° [neat] (Lit.[9a]: $[\alpha]_D^{21}$ = -28.9 °); FT-IR: ν_{max} = 3481 (m), 2984 (m), 1742 (s), 1468 (w), 1447 (w), 1371 (m), 1267 (s), 1222 (s), 1160 (s), 1114 (m), 1024 (s), 861 (m) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ = 1.01, 1.03 (2 x t, 6H, J = 7 Hz, 2 x $\underline{CH_3}$-CH_2-O-); 3.94-4.03 (m, 5H, 2 x CH_3-$\underline{CH_2}$-O-, -OH); 4.41, 4.43 (2 x d, 2H, J_1 = 5 Hz, C3-H, J_2 = 5 Hz, C2-H).

Diethyl (2*R*,3*R*)-*trans*-2,3-Epoxysuccinate (4)

Compound **4** was obtained from **3** (73.7 g, 0.274 mol) in EtOH (63 mL) by reaction with 7.56 g (0.329 mol) of sodium in EtOH (96 mL) for 1h at room temperature. After fractional distillation the product was chromatographed in aliquots of 1.5 g on a silica gel column (3 x 45 cm) using petroleum ether/EtOAc (4:1) as eluent. The product was isolated as colourless oil in 48% yield; bp 80-82 °C/~0.2 mm (Lit.[9a]: 100-104 °C/4 mm); n_D^{22} = 1.4384 (Lit.[9a]: n_D^{23} = 1.4354); $[\alpha]_D^{23}$ = -102.7 ° [c = 1 ether] (Lit.[9a]: $[\alpha]_D^{21.5}$ = -88.5 °); HPLC (Nucleosil C_8): linear gradient from 100% B to 90% C in 25 min, t_R 12.59 min; FT-IR: ν_{max} = 2986 (m), 1748 (s), 1469 (w), 1448 (w), 1372 (m), 1330 (m), 1280 (m), 1249 (m), 1231 (m), 1200 (s), 1096 (m), 1029 (s), 964 (w), 901 (m), 859 (w), 841 (w) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ = 1.26 (2 x t, 6H, J = 7.2 Hz, 2 x $\underline{CH_3}$-CH_2-O-); 3.61 (s, 2H, Eps C-H, C-H); 4.21 (q, 4H, J = 7.1 Hz, 2 x CH_3-$\underline{CH_2}$-O-); ^{1}H-NMR (DMSO): δ = 1.23 (2 x t, 6H, J = 7.2 Hz, 2 x $\underline{CH_3}$-CH_2-O-); 3.75 (s, 2H, Eps C-H, C-H); 4.19 (q, 4H, J = 7.1 Hz, 2 x CH_3-$\underline{CH_2}$-O-); ^{13}C-NMR (DMSO): δ = 13.8 (2 x $\underline{CH_3}$-CH_2-O-); 51.5 (Eps C-H, C-H); 61.6 (2 x CH_3-$\underline{CH_2}$-O-); 166.4 (2 x CO).

Ethyl (2*R*, 3*R*)-*trans*-2,3-Epoxysuccinate Potassium Salt (5a)

To an ice-cold solution of **4** (1.2 g, 6.4 mmol) in EtOH (5 mL) an ethanolic solution (11 mL) of KOH (359 mg, 6.4 mmol) was added dropwise. After additional 2h stirring at 0 °C the reaction mixture was diluted with ether, and the precipitate was collected by filtration and dried under reduced pressure at 30 °C to give

a colourless powder; yield **5a**: 1.0 g (79%); mp >159 °C decomp.; FAB-MS: m/e = 159.4 (100%), M^+ = 159.1 calcd. for $C_6H_7O_5$; HPLC (Nucleosil C_8): linear gradient from 100% B to 40% C in 25 min, t_R 4.65 min (98%); ^{1}H-NMR (D_2O): δ = 1.25 (t, 3H, J = 7.0 Hz, $\underline{CH_3}$-CH_2-O-); 3.49 (d, 1H, J = 2.0 Hz, Eps C-H); 3.58 (d, 1H, J = 2.0 Hz, Eps C-H); 4.25 (q, 2H, J = 7.0 Hz, CH_3-$\underline{CH_2}$-O-).

Ethyl (2*R*, 3*R*)-*trans*-2,3-Epoxysuccinate (5b)

To a solution of **5a** (512 mg; 2.58 mmol) in 5% $KHSO_4$ (50 mL) NaCl was added and the free acid was extracted with EtOAc (3x50 mL). The combined organic layers were washed with water (1x50 mL), dried over Na_2SO_4 and then evaporated to a colourless oil; yield **5b**: 320 mg (77%); EI-MS: m/e = 161 (2%), 87 (100%), 115 (50%), $M+H^+$ = 161.1 calcd. for $C_6H_8O_5$.

N-[[(2*R*,3*R*)-3-*trans*-(Ethoxycarbonyl)oxiran-2-yl]carbonyl]-glycyl-proline (7a)

5b (320 mg, 2.0 mmol) in DMF (2 mL) was reacted with pentafluorophenol (368 mg, 2.0 mmol) and DCC (412 mg, 2.0 mmol) in DMF (each in 4 mL) at 0 °C. The reaction mixture was allowed to reach room temperature and was then stirred for additional 24h. Dicyclohexylurea was filtered off and the filtrate was evaporated to dryness under reduced pressure. H-Gly-Pro-OH (344 mg, 2.0 mmol) in DMF (15 mL) was added to the residue and and the reaction was allowed to proceed for 24h at room temperature. Then the solvent was evaporated and the crude reaction mixture was chromatographed on a silica gel column (3 x 45 cm) using $CHCl_3$/MeOH (1:1) as eluent. Fractions containing homogeneous product were collected, concentrated to small volume and the product was precipitated with ether; yield **7a**: 346 mg; (55%); mp >195 °C decomp.; FAB-MS: m/e = 315.0 (50%), $M+H^+$ = 315.3 calcd. for $C_{13}H_{18}N_2O_7$; HPLC (Nucleosil C_{18}): linear gradient from 100% A to 40% C in 25 min, t_R 11.66 min (96%); ^{1}H-NMR (DMSO): δ = 1.23 (t, 3H, J = 7.1 Hz, $\underline{CH_3}$-CH_2-O-); 1.67-1.72 (m, 2H, β CH_2 Pro); 2.01-2.05 (m, 2H, γ CH_2 Pro); 3.29-3.37 (m, 4H, δ CH_2 Pro, α CH_2 Gly); 3.60 (d, 1H, J = 1.7 Hz, Eps C-H); 3.79 (d, 1H, J = 1.6 Hz, Eps C-H); 3.96 (dd, 1H, J = 5.8 Hz, J = 16.8 Hz, α CH Pro); 4.19 (q, 2H, J = 7.0 Hz, CH_3-$\underline{CH_2}$-O-); 8.52 (br s, 1H, NH Gly); ^{13}C-NMR (DMSO): δ = 13.9 ($\underline{CH_3}$-CH_2-O-); 22.1 (γ CH_2 Pro cis); 24.6 (γ CH_2 Pro trans); 28.9 (β CH_2 Pro trans); 31.2 (β CH_2 Pro cis); 41.3 (α CH_2 Gly); 45.5 (δ CH_2 Pro trans); 46.2 (δ CH_2 Pro cis); 51.2 (Eps C-H); 52.8 (Eps C-H); 60.8 (CH_3-$\underline{CH_2}$-O-); 61.0 (α CH Pro trans); 61.4 (α CH Pro cis); 164.9, 164.9, 166.4 (3 x CO); 167.1 (-COOH); ^{13}C-NMR (D_2O): δ = 16.0 ($\underline{CH_3}$-CH_2-O-); 25.1 (γ CH_2 Pro cis); 27.1 (γ CH_2 Pro trans); 32.3 (β CH_2 Pro trans); 34.5 (β CH_2 Pro cis); 44.3 (α CH_2 Gly); 49.6 (δ CH_2 Pro trans); 50.2 (δ CH_2 Pro cis); 55.4 (Eps C-H); 56.3 (Eps C-H); 64.7 (CH_3-$\underline{CH_2}$-O-); 64.9 (α CH Pro cis); 66.2 (α CH Pro trans); 170.6, 171.3, 171.7 (3 x CO); 182.5 (-COOH).

N-[[(2*R*,3*R*)-3-*trans*-(Ethoxycarbonyl)oxiran-2-yl]carbonyl]-leucyl-proline (7b)

Compound **7b** was synthesized as described for **7a** by acylation of H-Leu-Pro-OH with freshly prepared **6b**. The crude reaction product was partitioned between EtOAc and 5% $KHSO_4$ (3 x), washed with water (2 x) and dried over Na_2SO_4. The combined organic layers were evaporated and the residue was chromatographed on silica gel using $CHCl_3$/MeOH (2:1) as eluent and finally purified by preparative HPLC using an isocratic eluent of 90% III and 10% IV; fractions containing homogeneous material were combined and lyophilized twice from water; yield **7b**: 28%; mp >155-160 °C decomp.; FAB-MS: m/e = 370.7 (26%), M^+ = 370.4 calcd. for $C_{17}H_{26}N_2O_7$; m/e = 393.3 (30%), $M+Na^+$ = 393.4; m/e = 409.5 (26%), $M+K^+$ = 409.5; HPLC (Nucleosil C_8): linear gradient from 100% B to 40% C in 20 min, t_R 20.76 min (98%); ^{1}H-NMR (DMSO): δ = 0.85 (d, 3H, J = 6.6 Hz, δ_2 CH_3 Leu); 0.89 (d, 3H, J = 6.4 Hz, δ_1 CH_3 Leu); 1.22 (t, 3H, J = 6.9 Hz, $\underline{CH_3}$-CH_2-O-); 1.44-1.54 (m, 3H, β CH_2 Leu, γ CH Leu); 1.62-1.65 (m, 1H, β_2 CH_2 Pro); 1.83-1.95 (m, 3H, γ CH_2 Pro, β_1 CH_2 Pro); 3.47-3.49 (m, 1H, δ_2 CH_2 Pro); 3.57-3.62 (m, 1H, δ_1 CH_2 Pro); 3.59 (d, 1H, J = 1.8 Hz, Eps C-H); 3.74 (d, 1H, J = 1.8 Hz, Eps C-H); 4.18 (q, 2H, J = 6.9 Hz, CH_3-$\underline{CH_2}$-O-); 4.19-4.22 (m, 1H, α CH Pro); 4.57 (m, 1H, α CH Leu); 8.74 (d, 1H, J = 8.2 Hz, NH Leu); ^{13}C-NMR (DMSO): δ = 13.9 ($\underline{CH_3}$-CH_2-O-); 21.5 (δ CH_3 Leu); 21.9 (γ CH_2 Pro cis); 23.2 (δ CH_3 Leu); 24.0 (γ CH Leu); 24.2 (γ CH_2 Pro trans); 28.9 (β CH_2 Pro trans); 31.1 (β CH_2 Pro cis); 40.1 (β CH_2 Leu); 46.1 (δ CH_2 Pro); 48.8 (α CH Leu); 51.0 (Eps C-H); 52.7 (Eps C-H); 60.6 (CH_3-$\underline{CH_2}$-O-); 61.4 (α CH Pro); 164.5, 167.2, 168.7 (3 x CO); 169.7 (-COOH).

N-[[(2*R*,3*R*)-3-*trans*-(Ethoxycarbonyl)oxiran-2-yl]carbonyl]-leucyl-arginine (7c)

The N-hydroxysuccinimide ester **6a** was prepared as in DMF from **5b**, N-hydroxysuccinimide and DCC at 1:1:1 molar ratio and then reacted with H-Leu-Arg-OH as described for **7a**. The resulting crude reaction product was chromatographed on silica gel with MeOH/$CHCl_3$ (2:1) as eluent; yield **7c**: 16%; mp > 139 °C decomp.; FAB-MS: m/e = 430.5 (100%), $M+H^+$ = 430.5 calcd. for $C_{18}H_{31}N_5O_7$; HPLC (Nucleosil C_{18}): linear gradient from 100% A to 40% B in 25 min, t_R 20.80 min (80%); ^{1}H-NMR (DMSO): δ = 0.84 (d, 3H, J = 6.0 Hz, δ_2 CH_3 Leu); 0.88 (d, 3H, J = 6.1 Hz, δ_1 CH_3 Leu); 1.22 (t, 3H, J = 7.0 Hz, $\underline{CH_3}$-CH_2-O-); 1.36-1.68 (m, 7H, β CH_2 Leu, γ CH Leu, γ CH_2 Arg, β CH_2 Arg); 2.98-3.10 (m, 2H, δ CH_2 Arg); 3.71 (br s, 2H, 2 x Eps C-H); 3.84 (m, 1H, α CH Leu); 4.09 (q, 2H, J = 7.0 Hz, CH_3-$\underline{CH_2}$-O-); 4.26 (m, 1H, α CH Arg); 7.46 (d, 1H, J = 7.5 Hz, NH Leu); 7.60-8.11 (br, HNC-NH_2); 8.75 (d, 1H, J = 9.1 Hz, NH Arg); 9.41 (br s, 1H, ε NH Arg); ^{13}C-NMR (DMSO): δ = 13.8 ($\underline{CH_3}$-CH_2-O-); 21.4 (δ CH_3 Leu); 23.0 (δ CH_3 Leu); 24.2 (γ CH Leu); 25.2 (γ CH_2 Arg); 29.6 (β CH_2 Arg); 38.9 (δ CH_2 Arg); 40.4 (β CH_2 Leu); 51.1, 51.7, 53.0, 53.4 (2 x Eps C-H, 2 x α CH); 61.5 (CH_3-$\underline{CH_2}$-O-); 157.4 (HNC-NH_2); 165.0, 167.1, 170.0 (3 x CO); 174.6 (-COOH).

N-[[(2*R*,3*R*)-3-*trans*-(Ethoxycarbonyl)oxiran-2-yl]carbonyl]-leucyl-agmatine·1/2 H_2SO_4 (7d)

Compound **7d** was synthesized employing the procedure of **7c** by acylation of HCOOH·H-Leu-Agm·1/2 H_2SO_4 with freshly prepared **6a** in presence of 1 eq. of NMM. The crude product was purified on a silica gel column (3 x 46 cm) using $CHCl_3$/MeOH/H_2O (15:10:1) as eluent; the combined fractions were concentrated to a small volume, washed with toluene (5x50 mL) and then evaporated under reduced pressure to semi-solid material; yield **7d**: 77%; FAB-MS: m/e = 386 (100%), $M+H^+$ = 386.5 calcd. for $C_{17}H_{31}N_5O_5$; HPLC (Nucleosil C_{18}): linear gradient from 100% A to 40% B in 25 min, t_R 21.96 min (84%); ^{1}H-NMR (DMSO): δ = 0.83 (d, 3H, J = 6.1 Hz, δ_2 CH_3 Leu); 0.87 (d, 3H, J = 6.1 Hz, δ_1 CH_3 Leu); 1.21 (t, 3H, J = 7.0 Hz, $\underline{CH_3}$-CH_2-O-); 1.38-1.50 (m, 6H, β CH_2 Leu, β CH_2 Agm, γ CH_2 Agm); 1.55 (m, 1H, γ CH Leu); 3.00-3.06 (br m, 4H, α CH_2 Agm, δ CH_2 Agm); 3.07 (d, 1H, J = 1.8 Hz, Eps C-H); 3.30 (d, 1H, J = 1.8 Hz, Eps C-H); 4.12 (q, 2H, J = 7.0 Hz, CH_3-$\underline{CH_2}$-O-); 4.26 m, 1H, α CH Leu); 7.44-7.58 (br, 3H, HNC-NH_2); 8.12 (t, 1H, J = 5.5 Hz, NH Agm); 8.30 (br m, 1H, ε NH Agm); 8.70 (d, 1H, J = 8.2 Hz, NH Leu).

N-[[(2*R*,3*R*)-3-*trans*-(Oxycarbonyl)oxiran-2-yl]carbonyl]-glycyl-proline dipotassium salt (8a)

To an ice-cold solution of **7a** (204 mg, 0.65 mmol) in EtOH (3 mL) KOH (72.8 mg, 1.3 mmol) in EtOH (4 mL) was added. The reaction mixture was stirred for 2h at 0 °C and then diluted with ether. The precipitate was collected by filtration, washed extensively with ether, EtOH and again with ether; yield **8a**: 226 mg (96%); mp > 178-179 °C decomp.; FAB-MS: m/e = 363 (40%), 131 (100%), $M+2K^+$+H^+ = 363.4, calcd. for $C_{17}H_{31}N_5O_5$; HPLC (Nucleosil C_{18}): linear gradient from 100% A to 40% B in 25 min, t_R 5.80 min (98%); ^{1}H-NMR (DMSO): δ = 1.60-1.72 (m, 2H, β CH_2 Pro); 1.92-2.05 (m, 2H, γ CH_2 Pro); 2.92 (d, 1H, J = 2.2 Hz, Eps C-H); 3.19 (d, 1H, J = 2.2 Hz, Eps C-H); 3.38-3.40 (m, 2H, δ CH_2 Pro); 3.68 (dd, 1H, J = 5.0 Hz, J = 17.0 Hz, α_2 CH_2 Gly); 3.82 (dd, 1H, J = 5.6 Hz, J = 17.0 Hz, α_1 CH_2 Gly); 3.86 (dd, 1H, J = 8.2 Hz, J = 3.6 Hz, α CH Pro); 7.72 (t, 1H, J = 5.0 Hz, NH Gly); ^{13}C-NMR (D_2O): δ = 25.1 (γ CH_2 Pro cis); 27.1 (γ CH_2 Pro trans); 32.3 (β CH_2 Pro trans); 34.5 (β CH_2 Pro cis); 44.3 (α CH_2 Gly); 49.6 (δ CH_2 Pro trans); 50.2 (δ CH_2 Pro cis); 55.8 (Eps C-H); 57.3 (Eps C-H); 64.7 (α CH Pro cis); 64.9 (α CH Pro trans); 172.8, 176.6 (2 x CO, Gly, Eps); 181.9, 182.5 (2 x COO^-, Pro, Eps).

N-[[(2*R*,3*R*)-3-*trans*-(Oxycarbonyl)oxiran-2-yl]carbonyl]-leucyl-proline dipotassium salt (8b)

Compound **8b** was prepared from **7b** (100 mg, 0.27 mmol) as described for **8a** in 92% yield; mp > 223 °C decomp.; FAB-MS: m/e = 341.1 (30%), 183.4 (100%), M^+ = 341 .3 calcd. for $C_{15}H_{22}N_2O_7$; HPLC (Nucleosil C_8): linear gradient from 100% B to 100% C in 25 min, t_R 8.16 min (94%); ^{1}H-NMR (400 MHz, D_2O): δ = 0.94 (d, 3H, J = 6.0 Hz, δ_2 CH_3 Leu); 0.96 (d, 3H, J = 6.0 Hz, δ_1 CH_3 Leu); 1.64 (m, 2H, β CH_2 Leu); 1.67-1.76 (m, 1H, γ CH Leu); 1.85-1.95 (m, 1H, β_2 CH_2 Pro); 2.01 (m, 2H, γ CH_2 Pro); 2.19-2.27 (m, 1H, β_1 CH_2 Pro); 3.46 (d, 1H, J = 2.1 Hz, Eps C-H); 3.59 (d, 1H, J = 2.0 Hz, Eps C-H); 3.65 (m, 1H, δ_2 CH_2 Pro); 3.82 (m, 1H, δ_1 CH_2 Pro); 4.27 (dd, 1H, J = 5.1 Hz, J = 8.6 Hz, α CH Leu); 4.59 (dd,

1H, J = 3.3 Hz, J = 10.1 Hz, α CH Pro cis); 4.70 (dd, 1H, J = 5.5 Hz, J = 8.7 Hz, α CH Pro trans); ^{13}C-NMR (D_2O): δ = 23.4 (δ_2 CH_3 Leu); 24.9 (γ CH_2 Pro cis); 25.3 (δ_1 CH_3 Leu); 27.2 (γ CH Leu); 27.3 (γ CH_2 Pro trans); 32.2 (β CH_2 Pro trans); 34.4 (β CH_2 Pro cis); 41.7 (β CH_2 Leu); 50.1 (δ CH_2 Pro cis); 50.4 (δ CH_2 Pro trans); 53.0 (α CH Leu); 55.6 (Eps C-H); 56.9 (Eps C-H); 64.8 (α CH Pro cis); 64.9 (α CH Pro trans); 172.1, 174.5 (2 x CO, Leu, Eps); 176.6, 182.2 (2 x COO^-, Pro, Eps).

N-[[(2*R*,3*R*)-3-*trans*-(Oxycarbonyl)oxiran-2-yl]carbonyl]-leucyl-arginine dipotassium salt (8c)

Following the procedure of **8a** compound **8c** was obtained from **7c** (58.7 mg, 0.137 mmol) in 60% yield after chromatographic purification by preparative HPLC using as eluent a linear gradient from 100% I to 40% IV in 25 min; the product was lyophilized twice from water; FAB-MS: m/e = 402 (90%), 93 (100%), $M+H^+$ = 402.4 calcd. for $C_{16}H_{27}N_5O_7$; HPLC (Nucleosil C_{18}): linear gradient from 100% A to 40% C in 25 min, t_R 10.43 min (99%); ^{1}H-NMR (DMSO): δ = 0.80 (d, 3H, J = 5.2 Hz, δ_2 CH_3 Leu); 0.85 (d, 3H, J = 5.2 Hz, δ_1 CH_3 Leu); 1.26-1.68 (m, 7H, β CH_2 Leu, γ CH Leu, γ CH_2 Arg, β CH_2 Arg); 2.84 (m, 1H, δ CH_2 Arg); 3.15 (m, 1H, δ CH_2 Arg); 3.09 (br s, 1H, Eps C-H); 3.34 (br s, 1H, Eps C-H); 3.79 (m, 1H, α CH Leu); 4.23 (m, 1H, α CH Arg); 7.36 (d, 1H, J = 5.2 Hz, NH Leu); 8.3-8.9 (br, 4H, HNC-NH_2, NH Arg); 9.6-9.8 (br, 1H, ε NH Arg); ^{13}C-NMR (DMSO): δ = 21.1 (δ CH_3 Leu); 23.0 (δ CH_3 Leu); 24.2 (γ CH Leu); 25.2 (γ CH_2 Arg); 29.2 (β CH_2 Arg); 38.9 (δ CH_2 Arg); 40.1 (β CH_2 Leu); 51.6 (α CH Leu); 52.4 (Eps C-H); 53.2 (α CH Arg); 54.4 (Eps C-H); 157.8 (HNC-NH_2); 167.6, 170.3 (2 x CO, Leu, Eps); 174.5, 180.2 (2 x COO^-, Arg, Eps).

N-[[(2*R*,3*R*)-3-*trans*-(Oxycarbonyl)oxiran-2-yl]carbonyl]-leucyl-agmatine potassium salt (8d)

Compound **8d** was prepared from **7d** (228 mg, 0.525 mmol) as described for **8a**; the crude product was purified by preparative HPLC with a linear gradient from 100% II to 40% IV in 25 min; yield **8d**: 30%; mp 161-162 °C; FAB-MS: m/e = 358.3 (100%), $M+H^+$ = 358.4 calcd. for $C_{15}H_{27}N_5O_5$; HPLC (Nucleosil C_{18}): linear gradient from 100% A to 40% C in 25 min, t_R 17.33 min (99%); ^{1}H-NMR (DMSO): δ = 0.83 (d, 3H, J = 6.2 Hz, δ_2 CH_3 Leu); 0.87 (d, 3H, J = 6.2 Hz, δ_1 CH_3 Leu); 1.41-1.54 (m, 7H, β CH_2 Leu, γ CH Leu, β CH_2 Agm, γ CH_2 Agm); 3.01-3.09 (m, 4H, α CH_2 Agm, δ CH_2 Agm); 3.09 (d, 1H, J = 1.7 Hz, Eps C-H); 3.34 (d, 1H, J = 1.8 Hz, Eps C-H); 4.27 (m, 1H, α CH Leu); 7.42 (br s, 3H, HNC-NH_2); 8.04 (t, 1H, J = 5.5 Hz, NH Agm); 8.15 (d, 1H, J = 8.5 Hz, NH Leu); 8.72 (br s, 1H, ε NH Agm); ^{13}C-NMR (DMSO): δ = 21.7 (δ CH_3 Leu); 22.7 (δ CH_3 Leu); 24.3 (γ CH Leu); 25.7, 26.2 (β CH_2, γ CH_2 Agm); 37.8 (δ CH_2 Agm); 40.3 (α CH_2 Agm); 40.7 (β CH_2 Leu); 51.4 (α CH Leu); 52.4 (Eps C-H); 54.6 (Eps C-H); 157.1 (HNC-NH_2); 167.6, 170.1 (2 x CO, Leu, Agm); 171.6 (COO^-, Eps).

Reaction of 8a with 3-Methylbenzothiazole-2-thione

To an ice-cold solution of **7a** (45.9 mg, 0.146 mmol) in either CH_2Cl_2 or CH_3CN (3 mL) 3-methylbenzothiazole-2-thione (26.5 mg, 0.146 mmol) in the corresponding solvent (1.5 mL) and TFA (11.3 µL, 0.146 mmol) were added. No reaction was observed to occur within 3d at room temperature as judged by hplc even adding an additional equiv. of MBTT. After refluxing the solution for 3d, **7a** was quantitatively converted into a complex mixture of products. The solvent was then evaporated, the residue partitioned between water and EtOAc (3 x) to extract the MBTT and its corresponding oxide. The aquaeous layer was lyophilized and the residue chromatographed on a Sephadex RP 18 (25-40 µm) column (2 x 135 cm) using 5% isopropanol (95% water) and 15% isopropanol (85% water) as eluent; flow rate: 204 mL/h. The main product could be identified by MS as the desulfurated fumaryl compound: m/e = 299 (20%), $M+H^+$ = 299.3 calcd. for $C_{13}H_{18}N_2O_6$.

Acknowledgments

We thank Dr. Isolde Zetl for ^1H- and ^{13}C-NMR measurements and Prof. Dr. Wolfram Schäfer as well as Mrs. Ingrid Buhrow for mass spectra. We are grateful to the Bayerisches Staatsministerium für Unterricht, Kultur, Wissenschaft und Kunst for a Ph. D. grant for AK.

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(*Received in Germany* 25 *March* 1994; *accepted* 20 *May* 1994)