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TETRAHEDRON

Synthesis of the Endothiopeptide BOC-Trp-Ile-Ala-Aib-Ile-ValΨ[CSNH]Aib-Leu-Aib-Pro-OMe by a Variation of the 'Azirine/Oxazolone Method'

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Abstract: The synthesis of the decaendothiopeptide BOC-Trp-Ile-Ala-Aib-Ile-ValΨ[CSNH]Aib-Leu-Aib-Pro-OMe is described. The introduction of the thioamide group next to the bulky Aib occurred *via* a variation of the 'azirine/oxazolone method' without epimerisation. The structure of the decaendothiopeptide was etablished by single-crystal X-ray crystallography, thereby two types of helices could be observed.

INTRODUCTION

Peptides with backbone modifications have attracted considerable interest in recent years[1]. Among them, endothiopeptides with one or more thioamide groups instead of amide bonds play an important role for several reasons. Endothio-analogues of biologically active peptides can show protease resistance, thus allowing better bioavailability[2]. In addition, enhanced biological activity[3] and receptor selectivity[4] can be expected. Endothiopeptides have so far been prepared mainly by first converting a preformed dipeptide to the corresponding thiopeptide by the use of thionating reagents (e.g. Lawesson's reagent), followed by incorporation into the peptide sequence by segment coupling[5-8]. An alternative route, thioacylation, offers the advantage of selectivity in the introduction of thiocarbonyl groups in peptides. Several approaches have been reported using thioesters[9], dithioesters[10,11], aminothioacids in combination with PyBOP[12,13] and thioacylbenzimidazolines[14]. Unfortunately, these methods are accompanied by low yields or epimerisation. Recently, two efficent methods were described using thiacyl-*N*-phthalimides[15] and nitrobenzotriazoles[16] as thioacylating agents.

Other backbone modified peptides of considerable interest are those containing α-alkylated α-aminoacids. Two of these amino acids, Aib (α–aminoisobutyric acid) and Iva (isovaline) characterize the peptaibols, an important family of natural antibiotics, which alter the ionic permeability of biological membranes by forming channels[17,18]. The twofold substitution at the α-carbon atom in these amino acids restricts the conformational flexibility and so can stabilize or induce helices [19-21]. Due to the severe steric hindrance in these α-alkylated α-aminoacids, the synthesis of the related peptides is difficult[22-24]. With the 'azirine/oxazolone method' we

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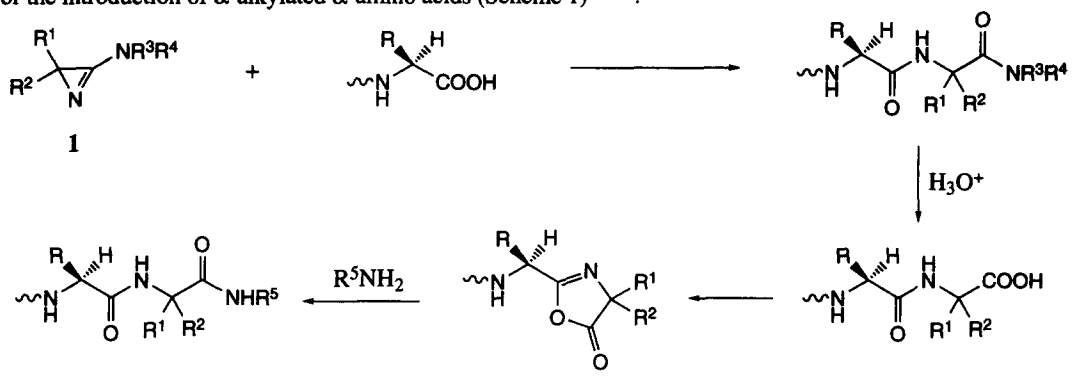
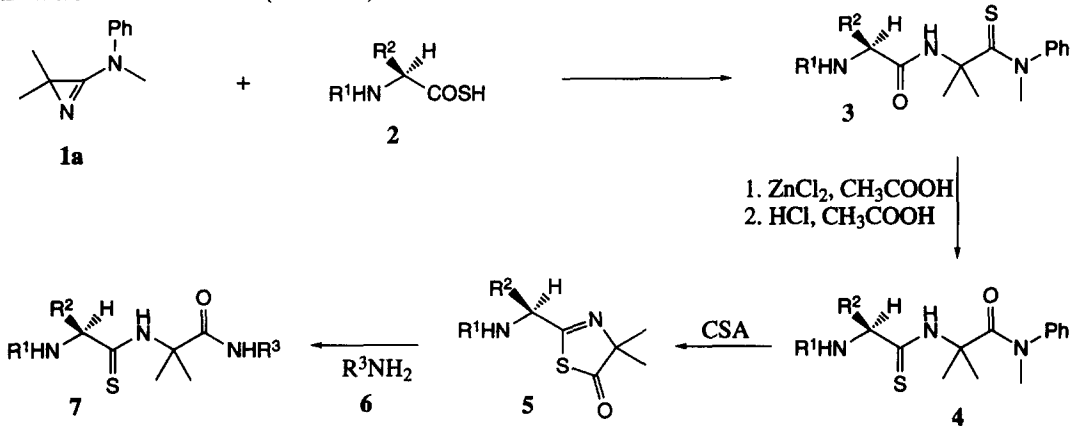
developed a convenient synthetic access to such peptides. 3-Amino-2*H*-azirines **1** proved to be useful synthons for the introduction of α-alkylated α-amino acids (Scheme 1)[25-27].



Scheme 1

On the other hand, FMOC amino acid fluorides turned out to be efficent reagents for the solid-phase synthesis of Aib containing peptides[28-30].

Recently, we succeeded in combining these two types of backbone modifications with a variation of the 'azirine/oxazolone method' (Scheme 2)[31].



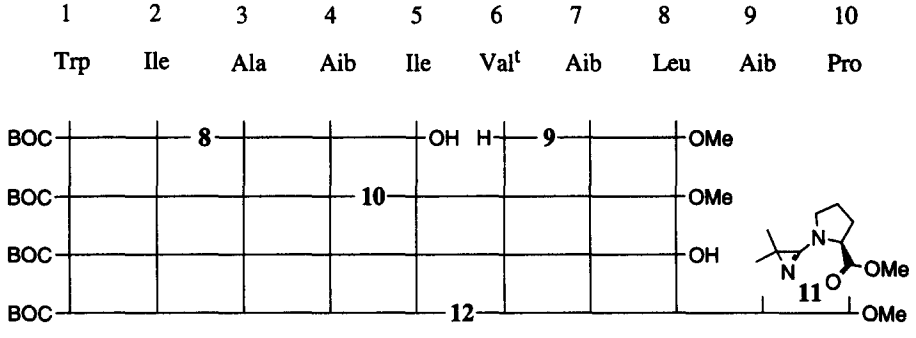
Scheme 2

Reaction of a N-protected α-amino thioacid **2** with 2,2-dimethyl-3-(*N*-methyl-*N*-phenylamino)-2*H*-azirine (**1a**) yields a dipeptide thioamide of type **3**. In contrast to the corresponding dipeptide amides, hydrolysis of the terminal thioamide group under the conditions of the 'azirine/oxazolone method' leads to extensive epimerisation. By using $ZnCl_2$ and HCl in acetic acid, conditions were established under which the transformation of **3** to **4** proceeds without epimerisation (ca. 90% yield). The acid catalysed conversion of endothiopeptide **4** into 1,3-thiazol-5(4*H*)-one **5** and direct coupling with a C-protected α-amino acid **6** gives endothiotripeptides of type **7** in high yields and without epimerisation.

To demonstrate the usefulness of this novel methodology we decided to synthesize, as a first example, the endothio derivative (-ValΨ(CSNH)Aib-) of the zervamicin IIA analogue, BOC-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Leu-Aib-Pro-OMe, whose X-ray crystal-structure was determined by *Karle* in 1986[32].

RESULTS AND DISCUSSION

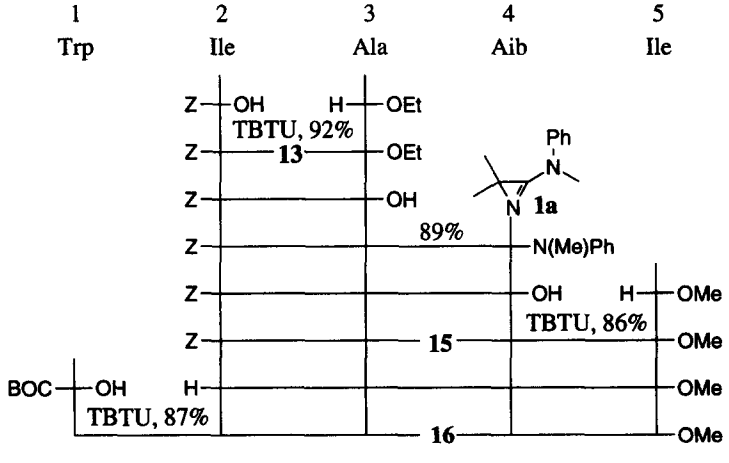
For the synthesis of the decaendothiopeptide **12** we chose the following strategy (Scheme 3): Coupling of the pentapeptide **8** (segment 1-5) with the endothiotripeptide **9** (segment 6-8) should yield the octaendothiopeptide **10**.



Scheme 3

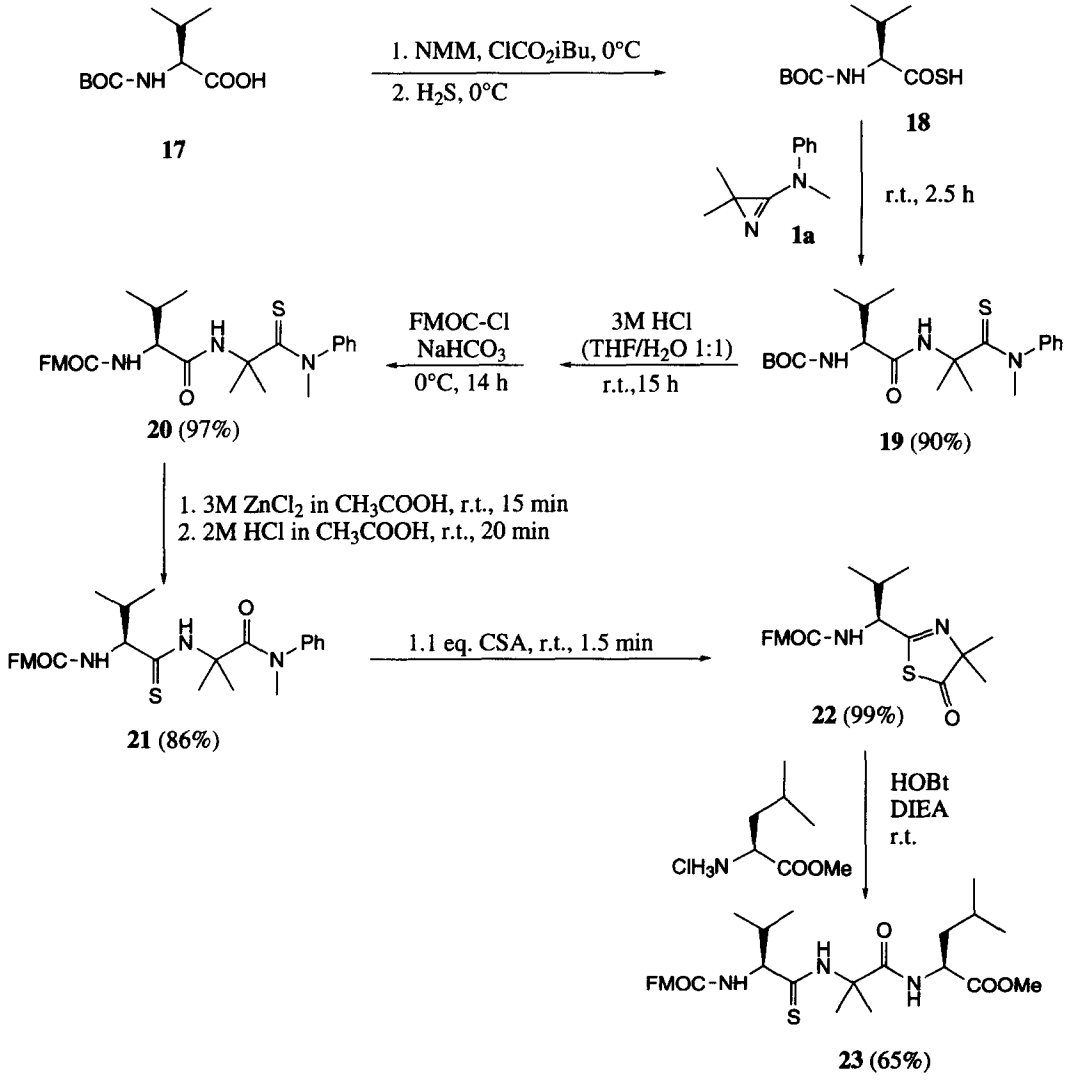
Base-catalyzed C-terminal deprotection of **10** and treatment with methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**11**), an Aib-Pro synthon, was expected to give the desired decaendothiopeptide **12** (segment 1-10).

Segment 1-5 was synthesized by standard solution peptide chemistry (Scheme 4), in which the Aib was



Scheme 4

introduced via the 'azirine/oxazolone method'. The reaction of Z-Ile-OH and NH_2-Ala-OEt with TBTU as coupling reagent in the presence of HOBt and DIEA produced dipeptide **13** in 92% yield. After C-terminal deprotection of **13** with LiOH (THF/MeOH/H_2O 3:1:1), treatment with 3-amino-2*H*-azirine **1a** gave tripeptide **14** in 89% yield, with respect to the consumed dipeptide **13**. Conversion of the terminal amide group in **14** into the acid functionality under the conditions of the 'azirine/oxazolone method' (3M HCl in THF/H_2O 1:1) and direct coupling with NH_2-Ile-OMe gave the segment 2-5 (**15**) in 86% yield. The Z-protecting group in the terapeptide **15** was cleaved hydrogenolytically and the crude product was reacted with BOC-Trp-OH and TBTU as coupling reagent in the presence of HOBt and DIEA, which led to segment 1-5 (**16**) in 87% yield.



Scheme 5

Segment 6-8 was prepared by a variation of the 'azirine/oxazolone method' (Scheme 5). First, the acid functionality of BOC-Val-OH (**17**) was transformated into the thioacid functionality via the reaction of the mixed anhydride of **17** with H_2S. The crude thioacid **18** was then treated with 3-amino-2*H*-azirine **1a** to afford thioamide **19** in 90% yield, with respect to **17**. Exchange of the N-terminal protecting group (from BOC in **19** to FMOC in **20**) occurred in 97% yield. The $ZnCl_2$/HCl-catalysed isomerisation from **20** to **21** proceeded with a yield of 86% without any epimerisation. Under acidic conditions, endothiopeptide **21** was converted quantitatively into the corresponding 1,3-thiazol-5(4*H*)-one **22**. The final coupling of NH_2-Leu-OMe with **22** in the presence of HOBt and DIEA gave the endothiotripeptide **23** in 65% yield. Base catalysed hydrolysis of segment 1-5 (**16**) gave the pentapeptide **8** and removal of the FMOC-protecting group in **23** by treatment with $NHEt_2$ yielded the endothiotripeptide **9** (segment 6-8) (Scheme 3). The reaction of the two deprotected segments **8** and **9** with TBTU as the coupling reagent in the presence of HOBt and DIEA gave after 4 h the octaendothiopeptide **10** (segment 1-8) in 72% yield, with respect to endothiotripeptide **23**. Saponification of segment 1-8 (**10**) with LiOH and direct treatment with azirine **11**[25] led to the endothiodecapeptide **12** (segment 1-10) in 72% yield with respect to **10**.

We succeeded in growing single crystals of the endothiodecapeptide **12** which were suitable for an X-ray diffraction analysis. The peptide forms a 1:1 solvate with ethanol. The molecule **12** forms two types of helices (Fig.1). N(7)-H, N(10)-H and N(22)-H interact intramolecularly with the amide O-atoms that are seven atoms along the peptide backbone, O(36), O(3) and O(15), respectively; graph set: S(10)[33] for each interaction. This is the normal intramolecular interaction found for peptides of this type and helps to form the 3_{10}-helical nature of the molecule. Interestingly, N(13)-H and N(16)-H form intramolecular hydrogen bonds with the amide O-atoms that are ten atoms along the peptide backbone, O(3) and O(6), respectively; graph set: S(13) for each interaction. Thus the molecule also contains an α-helix, which is normally not found in similar peptides. The amide O-atom, O(3), acts as an acceptor for two intramolecular hydrogen bonds.

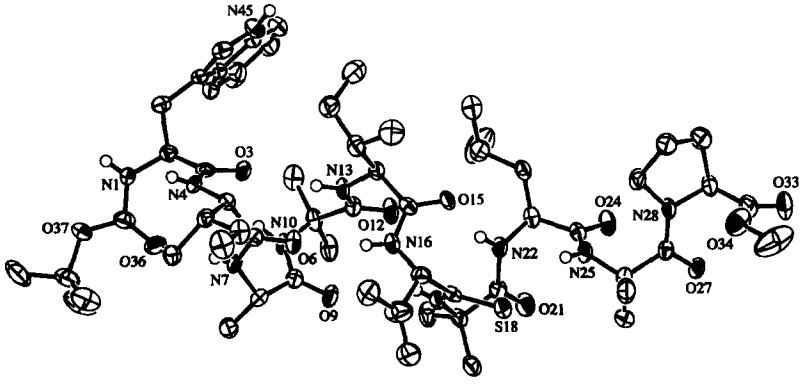


Fig.1. ORTEP Plot[34] *with 50% probability ellipsoids of the molecular structure of BOC-Trp-Ile-Ala-Aib-Ile-ValΨ[CSNH]Aib-Leu-Aib-Pro-OMe (12) (H-atoms omitted for clarity with the exception of NH atoms)*

N(1)-H forms an intermolecular hydrogen bond with the hydroxy O-atom of the ethanol molecule, which in turn is a donor for an intermolecular hydrogen bond with the amide O-atom, O(24), at the opposite end of a different peptide molecule. These two interactions combine to link the solvent and peptide molecules into infinite one-dimensional chains which run parallel to the z-axis; graph set: $C_2^2(28)$ N(4)-H forms an intermolecular hydrogen bond with the amide O-atom, O(27), at the opposite end of a neighbouring peptide molecule, thereby linking the peptide molecules into infinite one-dimensional chains which run parallel to the z-axis; graph set: C(26). N(45)-H of the side ring system forms an intermolecular hydrogen bond with the S-atom of a neighbouring peptide molecule, thus forming additional infinite one-dimensional chains which run parallel to the y-axis; graph set: C(23). The combination of the intermolecular interactions links the molecules into infinite 2-dimensional sheets which lie parallel to the yz-plane.

In conclusion, we described herein the synthesis and the X-ray structure of the Aib containing decaendothiopeptide **12**. The potential utility of the novel variation of the 'azirine/oxazolone method' has been illustrated with this example. In addition, we showed another application of the Aib-Pro synthon methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate **11** in peptide synthesis.

EXPERIMENTAL SECTION

Thin-layer chromatography (TLC): *Merck* 60 F_{254} silica gel-coated glass plates, 0.25 mm. Column chromatography: *Merck* 60 230-400 mesh silica gel. HPLC: *Bischoff* Nucleosil 100-5-C8 column, 5.0 μm, 250 x 4.6 mm, detection at 254 nm. NMR spectra: *Bruker*-ARX-300 and *Bruker*-AMX-600; chemical shifts δ (ppm) refer to residual $CHCl_3$ (7.27 ppm, ^1H) and to $CDCl_3$ (77.0 ppm, ^{13}C). Mass spectra: *Finnigan* MAT SSQ-700 (CI) and *Finnigan* MAT TSQ-700 (ESI). Optical rotations: *Perkin-Elmer* 241 polarimeter (c in g/100 ml $CHCl_3$, 18°C). IR: *Perkin-Elmer* 1600 Series FTIR, data in cm^{-1}. M.p.: *Mettler* FP5/FP52. Solvents were purified by standard procedures.

Z-Ile-Ala-OEt (**13**). 4.39 g (34.0 mmol) DIEA, 3.41 g (11.3 mmol) TBTU, 1.75 g (11.4 mmol) HOBt, and 1.741 g (11.34 mmol) H_2N-Ala-OEt·HCl were added to a solution of 3.006 g (11.33 mmol) Z-Ile-OH in 50 ml CH_3CN. After stirring the solution for a few min at room temperature a white solid began to precipitate. The suspension was stirred at room temperature for 21 h and then the solvent was evaporated. The residue was dissolved in CH_2Cl_2, extracted with $NaHCO_3$- and 2M HCl-solution, and dried over $MgSO_4$. After filtration, the filtrate was concentrated, and recrystallisation from ethyl acetate/CH_2Cl_2/hexane gave dipeptide **13** (3.791g, 92%) as a white solid; mp. 163.0-163.8°C. $[\alpha]_D$ = -9.7 (c = 1.04). IR ($CHCl_3$): 3430*m*, 3010*m*, 2970*s*, 2935*m*, 2880*m*, 2455*w*, 2385*w*, 1730*s*, 1675*s*, 1505*s*, 1455*s*, 1380*m*, 1340*m*, 1285*m*, 1260*s*, 1145*s*, 1090*m*, 1040*m*, 1025*m*, 980*w*, 915*w*, 860*m*, 695*m*. ^1H-NMR (300 MHz, $CDCl_3$): 7.35-7.30 (*m*, 5 arom. H); 6.56 (*d*, *J* = 6.8, NH); 5.47 (*d*, *J* = 8.5, NH); 5.14-5.10 (*m*, CH_2O); 4.58-4.53, 4.10-4.05 (2*m*, α-HC(Ile and Ala)); 4.18 (*q*, *J* = 7.1, CH_3CH_2O); 1.30-1.11 (*m*, β-HC(Ile) and γ1-H_2C(Ile)); 1.38 (*d*, *J* = 7.2, β-H_3C(Ala)); 1.27 (*t*, *J* = 7.1, CH_3CH_2O); 0.96-0.88 (*m*, γ2- and δ-H_3C(Ile)). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 172.5 (*s*, CO); 170.7 (*s*, CO); 156.2 (*s*, urethan-CO); 136.2, 128.4, 128.0, 127.9 (6 arom. C); 66.9 (*t*, CH_2O); 61.4 (*t*, CH_3CH_2O); 59.5, 48.0 (2*d*, α-HC(Ala and Ile)); 37.6 (*d*, β-HC(Ile)); 24.7 (*t*, γ1-H_2C(Ile)); 18.2, 15.3, 14.0, 11.3 (4*q*, γ2-H_3C(Ile), δ-H_3C(Ile), β-H_3C(Ala), and CH_3CH_2O). MS (CI): 383 (5), 382 (26,

$[M+NH_3]^+$), 366 (21), 365 (100, $[M+1]^+$), 310 (6), 309 (30), 274 (7), 257 (17). Anal. calc. for $C_{19}H_{28}N_2O_5$ (364.44): C 62.62, H 7.74, N 7.69; found: C 62.26, H 7.66, N 7.49.

Z-Ile-Ala-Aib-N(CH$_3$)Ph (**14**). A solution of 1.363 g (3.741 mmol) Z-Ile-Ala-OEt (**13**) and 0.323 g (7.70 mmol) LiOH·H_2O in 40 ml THF/MeOH/H_2O 3:1:1 was stirred at room temperature for 75 min, during which a white solid precipitated. The reaction mixture was diluted with CH_2Cl_2, washed with 2M HCl, and dried over $MgSO_4$. After filtration, the filtrate was concentrated in a rotary evaporator and dried under high vacuum. The produced crude Z-Ile-Ala-OH was dissolved in 75 ml CH_2Cl_2, cooled to 0°C, and 0.651 g (3.74 mmol) of 2,2-dimethyl-3-(*N*-methyl-*N*-phenylamino)-2*H*-azirine (**1a**) were slowly added. The mixture was warmed to room temperature and then stirred for 25 h. The solvent was removed *in vacuo*. The residue was chromatographed on silica gel (hexane/ethyl acetate 1:2) to give **14** (1.706 g, 89%) as a colorless viscous oil, which solidified. $[\alpha]_D$ = -33.9 (c = 1.07). IR ($CHCl_3$): 3680*w*, 3430*m*, 3005*m*, 2970*m*, 2935*w*, 2880*w*, 1720*s*, 1665*s*, 1595*m*, 1495*s*, 1455*m*, 1385*m*, 1365*m*, 1285*m*, 1250*m*, 1120*w*, 1090*w*, 1040*w*, 695*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.39-7.16 (*m*, 10 arom. H); 6.83 (br. *d*, *J* = 7.1, NH); 6.60 (*s*, NH); 5.53 (*d*, *J* = 8.5, NH); 5.08 (*m*, CH_2O); 4.10-4.04 (*m*, α-HC(Ile and Ala)); 3.23 (*s*, CH_3N); 1.92-1.05 (*m*, β-HC(Ile) and γ1-H_2C(Ile)); 1.47, 1.45 (2*s*, 2β-H_3C(Aib)); 1.25 (*d*, *J* = 10.3, β-H_3C(Ala)); 0.93-0.85 (*m*, γ2- and δ-H_3C(Ile)). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 172.2 (*s*, CO); 170.9 (*s*, CO); 170.4 (*s*, CO); 156.3 (*s*, urethan-CO); 144.6, 136.1, 129.3, 128.5, 128.1, 127.9, 127.8, 127.6 (12 arom. H); 66.9 (*t*, CH_2O); 59.6, 48.6 (2d, α-HC(Ile and Ala)); 57.9 (*s*, α-C(Aib)); 41.2 (*q*, CH_3N); 37.5 (*d*, β-HC(Ile); 26.4, 26.3 (2*q*, 2β-H_3C(Aib)); 24.5 (*t*, γ1-H_2C(Ile)); 17.9, 15.5, 11.3 (3*q*, γ2-H_3C(Ile), δ-H_3C(Ile) and β-H_3C(Ala)). MS (ESI): 533 ($[M+Na]^+$). Anal. calc. for $C_{28}H_{38}N_4O_5$ (510.63): C 65.86, H 7.50, N 10.97; found: C 65.39, H 7.50, N 10.50.

Z-Ile-Ala-Aib-Ile-OMe (**15**). A solution of 1.029 g (2.015 mmol) Z-Ile-Ala-Aib-N(CH_3)Ph (**14**) in 20 ml 3M HCl (THF/H_2O 1:1) was stirred at 35°C for 24 h. The reaction mixture was diluted with Et_2O and extracted with 2M HCl. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude Z-Ile-Ala-Aib-OH was dissolved in 20 ml CH_3CN, and 0.786 g (6.08 mmol) DIEA, 0.610 g (2.03 mmol) TBTU, 0.311 mg (2.03 mmol) HOBt, and 0.404 g (2.22 mmol) NH_2-Ile-OMe·HCl were added. After stirring for 18 h at room temperature, the cloudy, pale yellow solution was diluted with CH_2Cl_2 and washed with $NaHCO_3$- and 2M HCl-solutions. The organic phase was dried ($MgSO_4$), filtered and evaporated. The dark yellow residue was chromatographed on silica gel (ethyl acetate/hexane 2:1) to yield the tripeptide **15** (1.773 mmol, 86%) as a colorless thick oil, which slowly solidified. $[\alpha]_D$ = -21.6 (c = 1.04). IR ($CHCl_3$): 3430*w*, 3350*w*, 3030*w*, 3000*w*, 2965*w*, 2935*w*, 2880*w*, 1715*s*, 1675*s*, 1630*m*, 1505*s*, 1455*m*, 1440*m*, 1385*m*, 1265*m*, 1180*w*, 1145*m*, 985*w*, 910*w*, 840*w*, 695*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.33 (*s*, 5 arom. H); 7.08 (*d* br, *J* = 7.6, NH); 6.98 (*s*, NH); 5.58 (*d*, *J* = 7.9, NH); 5.14-5.04 (*m*, CH_2O); 4.54-4.06 (*m*, α-HC(Ile1/2 and Ala)); 3.70 (*s*, CH_3O); 1.96-1.08 (*m*, β-HC(Ile1/2) and γ1-H_2C(Ile1/2)); 1.54, 1.52 (2*s*, 2β-H_3C(Aib)); 1.36 (*d*, *J* = 6.9, β-H_3C(Ala)); 0.93-0.86 (*m*, γ2- and δ-H_3C(Ile1/2). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 173.9, 172.3, 171.7, 171.4 (4*s*, 4 CO); 156.4 (*s*, urethan-CO); 136.0, 128.4, 128.1, 128.0 (6 arom. C); 67.0 (*t*, CH_2O); 59.8, 56.6, 49.4 (3*d*, α-HC(Ile and Ala)); 57.3 (*s*, α-C(Aib)); 51.9 (*q*, CH_3O); 37.6, 37.2 (2*d*, β-HC(Ile1/2)); 25.1 (2q, 2β-H_3C(Aib)), 24.7 (2*t*, γ1-H_2C(Ile1/2)); 17.7 (*q*, β-H_3C(Ala)); 15.43, 15.38, 11.4, 11.2 (4*q*, γ2- and δ-H_3C(Ile1/2)). MS (ESI): 571 ($[M+Na]^+$). Anal. calc. for $C_{28}H_{44}N_4O_7$ (548.68): C 61.29, H 7.72, N 10.21; found: C 61.64, H 8.08, N 10.67.

BOC-Trp-Ile-Ala-Aib-Ile-OMe (**16**). In an atmosphere of hydrogen (balloon), a suspension of 0.410 g (0.747 mmol) Z-Ile-Ala-Aib-Ile-OMe (**15**) and 40 mg 10% Pd/C in 25 ml MeOH was stirred at room temperature for 3.5 h. The reaction mixture was filtered over cellite and the solvent was evaporated. The crude H_2N-Trp-Ile-Ala-Aib-Ile-OMe was added to a solution of 0.230 g (0.757 mmol) BOC-Trp-OH, 0.229 g (0.759 mmol) TBTU, 0.117 g (0.763 mmol) HOBt, and 0.098 g (0.759 mmol) DIEA in 5 ml CH_3CN. The solution was stirred for 80 min at room temperature and then diluted with Et_2O, extracted with $NaHCO_3$- and $KHSO_4$-solutions. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was chromatographed on silica gel (ethyl acetate/hexane 5:1) to give **16** as a viscous oil, which solidified. $[\alpha]_D$ = +3.45 (c = 1.05). IR ($CHCl_3$): 3670*w*, 3475*w*, 3430*m*, 3330*m*, 3005*m*, 2965*m*, 2935*m*, 2880*m*, 2460*w*, 2360*w*, 2360*w*, 1735*s*, 1675*s*, 1510*s*, 1460*s*, 1440*m*, 1385*m*, 1370*s*, 1275*m*, 1160*s*, 1095*w*, 1065*w*, 1010*w*, 915*w*, 855*w*, 690*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.23 (*s*, NH); 7.59-7.10 (*m*, 5 arom. H); 6.23 (*d*, *J* = 5.5, NH); 5.28 (*d*, *J* = 1.9, NH); 4.54-3.24 (*m*, α-HC(Trp, Ile1/2, Ala) and β-H_2C(Trp)); 3.65 (*s*, CH_3O); 2.81 (*s*, $(CH_3)_3C$); 1.98-1.12 (*m*, β-HC(Ile 1/2) and γ1-H_2C(Ile1/2); 1.41 (*s*, 2β-H_3C(Aib)); 1.37 (*d*, *J* = 7.4, β-H_3C(Ala)); 0.96-0.66 (*m*, γ2- and δ-H_3C(Ile1/2)). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 174.7, 173.3, 172.4, 172.2, 170.7 (5*s*, 5 CO); 156.6 (*s*, urethan-CO); 136.6, 127.0, 123.7, 122.3, 119.7, 118.4, 111.6, 108.8 (8 arom. C); 81.2 (*s*, *C*(CH_3)$_3$)); 59.2, 56.8, 56.3, 49.8 (4*d*, α-HC(Ile1/2, Trp, Ala)); 57.4 (*s*, α-C(Aib)); 51.7 (*q*, CH_3O); 37.4, 35.6 (2*d*, β-HC(Ile1/2)); 28.0 (*q*, C(*C*H_3)$_3$); 27.2 (*t*, β-H_2C(Trp)); 25.5, 25.3 (2*q*, 2β-H_3C(Aib)); 25.1, 24.4 (2*t* , γ1-H_2C(Ile1/2)); 17.0 (*q*, β-H_3C(Ala)); 15.5, 15.5, 11.7, 11.3 (4*q*, γ2- and δ-H_3C(Ile)). MS (ESI): 723 ([*M*+Na]$^+$). Anal. calc. for $C_{36}H_{56}N_6O_8$ (700.87): C 61.69, H 8.05, N 11.99; found: C 60.91, H 8.08, N 12.31.

BOC-Val-AibΨ[CS]-N(CH$_3$)Ph (**19**). A solution of 5.003 g (23.03 mmol) BOC-Val-OH in 50 ml THF was cooled to -10°C in an ice-salt bath. Then, 4.740 g (46.86 mmol) NMM was added followed by 3.480 g (25.48 mmol) isobutyl chloroformate. The mixture was stirred for 10 min, then a slow stream of H_2S was bubbled through the solution for 1 h. The suspension was stirred for another 2 h at -10°C. The reaction mixture was diluted with Et_2O and washed with 0.1 M H_3PO_4-solution. The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated. The formed BOC-Val-SH was dissolved in 100 ml CH_2Cl_2, cooled to 0°C, and 3.90 g (22.4 mmol) 2,2-dimethyl-3-(*N*-methyl-*N*-phenylamino)-2*H*-azirine (**1a**) were added slowly to the solution, which turned yellow. The reaction mixture was allowed to warm to room temperature and then stirred for 2 h. The solvent was evaporated and the intense yellow oil was chromatographed on silica gel (hexane/ethyl acetate 3:1) to give **19** (8.353 g, 90%) as a thick, yellow oil, which solidfied. $[\alpha]_D$ = -74.8 (c = 1.10). IR ($CHCl_3$): 3675*w*, 3430*m*, 3225*w*, 3005*m*, 2970*m*, 2935*m*, 2875*w*, 2455*w*, 2360*w*, 1705*s*, 1680*s*, 1595*w*, 1490*s*, 1465*w*, 1435*m*, 1370*s*, 1100*s*, 1050*w*, 1015*w*, 1005*w*, 970*w*, 925*w*, 870*w*, 835*w*, 694*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.43-7.18 (*m*, 5 arom. H); 7.07 (*s* br, NH); 5.16-5.13 (*d* br, NH); 3.74-3.69 (*m*, α-HC(Val)); 3.71 (*s*, CH_3N); 2.11 (*m*, β-HC(Val)); 1.64, 1.58 (2*s*, 2β-H_3C(Aib)); 1.47 (*s*, $(CH_3)_3C$); 0.92, 0.85 (2*d*, *J* = 6.8, γ1- and γ2-H_3C(Val)). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 208.5 (*s*, CS); 169.4 (*s*, CO(Val)); 155.7 (*s*, urethan-CO); 147.4 (*s*, arom. C(1)); 129.6, 128.6, 126.4 (3*d*, arom. C(2-5)); 79.5 (*s*, *C*(CH_3)$_3$); 62.7 (*s*, α-C(Aib)); 59.6 (*d*, α-HC(Val)); 51.1 (*q*, CH_3N); 31.4 (*d*, β-HC(Val)); 29.1, 28.9 (2*q*, 2β-H_3C(Aib)); 28.4 (*q*, $(CH_3)_3C$); 19.3, 17.4 (2*q*, γ1- and γ2-H_3C(Val)). MS (ESI): 430 ([*M*+Na]$^+$). Anal. calc. for $C_{21}H_{33}N_3O_3S$ (407.57): C 61.88, H 8.16, N 10.31; found: C 62.25, H 8.30, N 10.36.

FMOC-Val-AibΨ[CS]-N(CH$_3$)Ph (**20**). A solution of 2.036 g (4.996 mmol) BOC-Val-AibΨ[CS]-N(CH$_3$)Ph (**19**) in 60 ml 3M HCl (THF/H$_2$O) was stirred for 15 h at room temperature. The solution was cooled to 0°C, basicified with NaHCO$_3$, and diluted with 30 ml dioxane and 30 ml water. The yellow suspension was treated with 1.355 g (5.238 mmol) FMOC-Cl dissolved in 5 ml dioxane. After 1 h, the reaction mixture was warmed to room temperature and stirred for another 10 h. The suspension was diluted with water and extracted with Et$_2$O. The combined organic layers were dried (MgSO$_4$), filtered, evaporated, and the yellow residue was chromatographed on silica gel (ethyl acetate/hexane 3:1) to give dipeptide **20** (2.579, 97%) as a yellow viscous oil, which solidfied. $[\alpha]_D$ = -68.3 (c = 1.03). IR (CHCl$_3$): 3675*w*, 3430*m*, 3220*w*, 3070*w*, 3030*m*, 2970*m*, 2875*w*, 1790*s*, 1680*m*, 1595*w*, 1490*s*, 1465*s*, 1370*s*, 1320*m*, 1250*m*, 1165*w*, 1100*s*, 1045*m*, 1005*w*, 970*w*, 920*w*, 835*w*, 695*w*. ^{1}H-NMR (300 MHz, CDCl$_3$): 7.77-7.15 (*m*, 13 arom. H and 1 NH); 5.49 (*d* br, *J* = 7.3, CHC*H*$_2$O); 4.25 (*t*, *J* = 7.0, C*H*CH$_2$O); 3.81-3.80 (*m*, α-HC(Val)); 3.70 (*s*, CH$_3$N); 2.14-2.00 (*m*, β-HC(Val)); 1.65, 1.55 (2*s*, 2β-H$_3$C(Aib)); 0.93, 0.88 (2*d*, *J* = 6.7, γ1- and γ2-H$_3$C(Val)). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 208.4 (s, CS); 168.8 (*s*, CO(Val)); 156.2 (*s*, urethan-CO); 147.2, 143.9, 141.3, 129.6, 128.6, 127.7, 127.1, 126.4, 125.1, 120.0 (18 arom. C); 67.0 (*t*, CHCH$_2$O); 62.9 (*s*, α-C(Aib)); 60.1 (d, α-HC(Val)); 51.3 (*q*, CH$_3$N); 47.3 (*d*, C*H*CH$_2$O); 31.7 (*d*, β-HC(Val)); 28.9, 28.6 (2*q*, 2β-H$_3$C(Aib)); 19.1, 17.6 (2*q*, γ1- and γ2-H$_3$C(Val)). MS (ESI): 552 ([*M*+Na]$^+$), 530 ([*M*+1]$^+$). Anal. calc. for C$_{31}$H$_{35}$N$_3$O$_3$S (529.70): C 70.29, H 6.66, N 7.93; found: C 68.91, H 6.93, N 7.45.

FMOC-ValΨ[CSNH]Aib-N(CH$_3$)Ph (**21**). To a solution of 0.204 g (0.384 mmol) FMOC-Val-AibΨ[CS]N(CH$_3$)Ph (**20**) in 5 ml acetic acid, 2.05 g (15.0 mmol) ZnCl$_2$ were added. The reaction mixture was stirred at room temperature for 15 min; the colour of the solution turned from intense yellow to pale yellow. Then, 0.5 ml HCl-saturated acetic acid (ca. 2.1 M) was added and the solution was stirred for 20 min at room temperature. The mixture was basicified with NaHCO$_3$-solution and extracted with CH$_2$Cl$_2$. The combined organic phases were dried over MgSO$_4$, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane/ethyl acetate 1:1) to give **21** as a white solid (0.174 g, 86%). $[\alpha]_D$ = +27.3 (c =1.04). IR (CHCl$_3$): 3675*w*, 3375*w*, 3250*w*, 3005*m*, 2965*m*, 2875*w*, 1715*s*, 1640*s*, 1595*m*, 1505*s*, 1450*s*, 1420*m*, 1390*m*, 1365*m*, 1305*m*, 1250*m*, 1170*m*, 1120*m*, 1090*m*, 1035*m*, 1250*m*, 1170*m*, 1120*m*, 1090*m*, 1035*w*, 860*w*, 695*w*. ^{1}H-NMR (300 MHz, CDCl$_3$): 8.19 (*s*, NHCS); 7.77-7.22 (*m*, 13 arom. H); 5.81 (*d* br, NHCO); 4.47-4.37 (*m*, C*H*CH$_2$O); 4.25 (*t*, *J* = 7.1, CHC*H*$_2$O); 3.91-3.87 (*m*, α-HC(Valt); 3.25 (*s*, CH$_3$N); 2.18-2.07 (*m*, β-HC(Valt)); 1.71, 1.62 (2*s*, 2β-H$_3$C(Aib)); 0.96-0.86 (*m*, γ1- and γ2-H$_3$C(Valt)). ^{13}C-NMR (75.5, CDCl$_3$): 201.4 (*s*, CS); 171.8 (*s*, CO(Aib)); 155.9 (*s*, urethan-CO); 143.8, 141.3, 129.4, 128.3, 128.0, 127.7, 127.1, 125.1, 120.0 (18 arom. C); 67.0 (*t*, CHCH$_2$O); 66.9 (*d*, α-HC(Valt)); 61.9 (*s*, α-C(Aib)); 47.1 (*d*, CHCH$_2$O); 41.0 (*q*, CH$_3$N); 34.1 (*d*, β-HC(Valt)); 25.1, 23.7 (2*q*, 2β-H$_3$C(Aib)); 19.5, 18.0 (2*q*, γ1- and γ2-H$_3$C(Valt)). MS (CI): 424 (13), 423 (52), 201 (31), 179 (13), 109 (7), 108 (100). Anal. calc. for C$_{31}$H$_{35}$N$_3$O$_3$S (529.70): C 70.29, H 6.66, N 7.93; found: C 69.91, H 6.57, N 7.69.

FMOC-Val-thiazolone (**22**). To a solution of 0.174 g (0.328 mmol) FMOC-ValΨ[CSNH]Aib-N(CH$_3$)Ph (**21**) in 5 ml CH$_2$Cl$_2$, 84.0 mg (0.361 mmol) (±)-Camphor-10-sulfonic acid (β) (CSA) were added. After stirring for 1.5 min at room temperature the reaction mixture was quenched with NaHCO$_3$-solution and extracted with CH$_2$Cl$_2$. The organic layers were dried (MgSO$_4$), filtered, and the solvent evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate 6:1) to yield thiazolone **22** (0.137 g, 99 %) as a colorless, thick oil. $[\alpha]_D$ = -28.3 (c = 1.04). IR (CHCl$_3$): 3675*w*, 3435*m*, 3070*w*, 3030*w*, 2970*m*, 2935*m*, 2875*w*,

1720*s*, 1620*m*, 1505*s*, 1465*m*, 1450*s*, 1390*w*, 1380*w*, 1350*m*, 1305*m*, 1265*m*, 1110*m*, 1065*m*, 1030*m*, 990*m*, 920*m*, 865*w*, 690*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.77-7.25 (*m*, 8 arom. H); 5.42 (*d* br, *J* = 8.3, NH); 4.63-4.22 (*m*, *CH*CH_2O and α-HC(Val)); 2.21-2.17 (*m*, β-HC(Val)); 1.41, 1.39 (2*s*, 2β-H_3C(Aib)); 1.04, 0.92 (2*d*, *J* = 6.5 and 6.7, resp. γ1- and γ2-H_3C(Val)). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 210.5 (*s*, COS); 165.3 (*s*, CN); 156.1 (*s*, urethan-CO); 143.8, 143.6, 141.3, 127.7, 127.0, 125.0, 124.9, 119.9 (12 arom. C); 83.0 (*s*, α-C(Aib)); 66.9 (*t*, $CHCH_2O$); 59.9 (*d*, α-HC(Val)); 47.2 (*d*, $CHCH_2O$); 31.3 (*d*, β-HC(Val)); 24.5, 24.2 (2*q*, 2β-H_3C(Aib)); 19.3, 16.6 (2*q*, γ1- and γ2-H_3C(Val)). MS (CI): 425 (7), 424 (24), 423 (100, $[M+1]^+$), 320 (9), 227 (7), 201.2 (54), 179 (27), 178 (10).

FMOC-ValΨ[CSNH]Aib-Leu-OMe (**23**). To a solution of 0.235 g (0.555 mmol) thiazolone **22** in 5 ml CH_3CN, 0.144 g (1.11 mmol) DIEA, 0.170 g (1.11 mmol) HOBt, and 0.110 g (0.606 mmol) H_2N-Leu-OMe·HCl were added. The reaction mixture was stirred at room temperature for 7 d, during which the solution turned yellow. The mixture was diluted with CH_2Cl_2 and washed with $NaHCO_3$- and $KHSO_4$-solution. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate 2:1) to give the endothiotripeptide **23** as a viscous colorless oil, which solidified. The epimeric purity was shown by reversed-phase HPLC (CH_3CN/H_2O 1:1, 1 ml/min) to be higer than 99%. $[\alpha]_D$ = +4.6 (c = 1.03). IR ($CHCl_3$): 3670*w*, 3430*w*, 3375*m*, 3280*w*, 3005*m*, 2965*m*, 2875*w*, 1740*s*, 1710*s*, 1675*s*, 1505*s*, 1470*m*, 1450*s*, 1420*m*, 1390*m*, 1365*m*, 1310*m*, 1260*m*, 1125*m*, 1100*w*, 1030*m*, 980*w*, 860*w*, 690*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.35 (*s*, NH); 7.76-7.26 (*m*, 8 arom. H); 6.48 (*d* br, NH); 5.67 (*d*, *J* = 8.4, NH); 4.60-3.95 (*m*, $CHCH_2O$ and α-HC(Leu and Valt)); 3.68 (s, CH_3O); 2.20-1.45 (*m*, β-HC(Valt), β-H_2C(Leu) and γ-HC(Leu)); 1.76, 1.69 (2*s*, 2β-H_3C(Aib)), 0.99-0.82 (*m*, γ1- and γ2-H_3C(Valt), δ1- and δ2- H_3C(Leu)). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 203.1 (*s*, CS); 173.3 (*s*, CO); 172.4 (*s*, CO); 156.6 (*s*, urethan-CO); 143.7, 141.3, 127.8, 127.1, 125.1, 120.0 (12 arom. C); 68.3 (*d*, α-HC(Valt)); 67.3 (*t*, $CHCH_2O$); 60.8 (*s*, α-C(Aib)); 52.2 (*q*, CH_3O); 51.1 (*d*, α-HC(Leu)); 47.1 (*d*, $CHCH_2O$); 41.2 (*t*, β-H_2C(Leu)); 33.3 (*d*, β-HC(Val)); 25.7, 24.8, 22.8, 22.6, 21.8, 19.5, 18.5 (7*q*, 2β-H_3C(Aib), δ1- and δ2-H_3C(Leu), γ1- and γ2-H_3C(Valt), and γ-HC(Leu)). MS (ESI): 590 ($[M+Na]^+$). Anal. calc. for $C_{31}H_{41}N_3O_5S$ (567.74): C 65.58, H 7.28, N 7.40; found: C 65.70, H 7.39, N 7.12.

BOC-Trp-Ile-Ala-Aib-Ile-ValΨ[CSNH]Aib-Leu-OMe (**10**). To a solution of 74.2 mg (0.106 mmol) BOC-Trp-Ile-Ala-Aib-Ile-OMe (**23**) in 3 ml THF/MeOH/H_2O 3:1:1, 10.9 mg (0.260 mmol) LiOH·H_2O were added. The reaction mixture was stirred at room temperature for 4.5 h. The solution was diluted with Et_2O, extracted with $KHSO_4$-solution, the organic layers were dried ($MgSO_4$), filtered, evaporated, and dried under high vacuum to give crude BOC-Trp-Ile-Ala-Aib-Ile-OH. Then, 0.2 ml $NHEt_2$ was added to a solution of 57.0 mg (0.100 mmol) FMOC-ValΨ[CSNH]Aib-Leu-OMe (**23**) in 2 ml CH_3CN. The mixture was stirred at room temperature for 75 min, during which the color of the solution turned to intense yellow. The solution was concentrated in a rotary evaporator and the yellow residue was dried under high vacuum. The crude H_2N-ValΨ[CSNH]Aib-Leu-OMe, dissolved in 1 ml CH_3CN, was added to a solution of crude BOC-Trp-Ile-Ala-Aib-Ile-OH, 32.8 mg (0.109 mmol) TBTU, 16.9 mg (0.11 mmol) HOBt, and 13.9 mg (0.108 mmol) DIEA in 1 ml CH_3CN. The mixture was stirred at room temperature for 4 h, meanwhile a white solid precipitated. The suspension was diluted with Et_2O and washed with $NaHCO_3$- and $KHSO_4$-solution. The combined organic phases were dried over $MgSO_4$, filtered, and the filtrate was concentrated. The intense yellow residue was chromatographed on silica gel (ethyl acetate/hexane 3:1) to yield the octaendothiopeptide **10** (73.3 mg, 72%) as

a colorless viscous oil, which solidified under high vacuum. $[\alpha]_D$ = +7.8 (c = 1.05). IR ($CDCl_3$): 3475*w*, 3320*s*, 3000*w*, 2960*m*, 2935*m*, 2875*w*, 1735*m*, 1665*s*, 1525*s*, 1460*s*, 1440*m*, 1385*m*, 1370*m*, 1345*m*, 1275*m*, 1170*s*, 1125*w*, 1105*w*, 1075*w*, 1010*w*, 850*w*, 650*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.48 (*s*, NH); 8.48 (*s*, NH); 7.72 (*d*, *J* = 4.4, NH); 7.53-6.98 (*m*, 5 arom. H and 4 NH); 6.27 (*d*, *J* = 4.0, NH); 4.64-3.20 (*m*, α-HC(Trp, Ile1/2, Ala, Valt, Leu) and β-H_2C(Trp)); 3.62 (*s*, CH_3O); 2.19-0.83 (*m*, β-HC(Ile1/2, Valt), β-H_3C(Ala, β-H_2C(Leu), γ-HC(Leu), γ1-H_2C(Ile1/2), δ1- and δ2-H_3C(Leu)); 1.81, 1.74, 1.65, 1.58 (4*s*, 2β-H_3C(Aib1/2)); 1.42 (*s*, $(CH_3)_3C$). ^{13}C-NMR (74.8 MHz, $CDCl_3$): 201.8 (*s*, CS); 175.9, 174.0, 173.5, 173.2, 173.0, 171.8 (6*s*, CO); 157.1 (s, urethan-CO); 136.7, 126.9, 124.3, 122.2, 119.5, 118.1, 111.9, 108.1 (8 arom. C); 81.6 (*s*, $C(CH_3)_3$); 67.7, 60.5, 59.3, 56.7, 51.9, 51.3 (6*d*, α-HC(Trp, Ile1/2, Ala, Valt, Leu)); 67.7, 60.9 (2*s*, α-C(Aib1/2)); 51.7 (*q*, CH_3O); 40.6 (*t*, β-H_2C(Trp)); 38.5 (*q*, β-H_3C(Ala)); 36.0, 35.7 (2*d*, β-HC(Ile1/2)); 31.1, 24.5 (2*d*, β-HC(Val) and γ-HC(Leu)); 28.1 (*q*, $(CH_3)_3C$); 27.1, 25.2, 25.1 (3*t*, γ1-H_2C(Ile1/2) and β-H_2C(Leu)); 24.6, 24.5, 23.0, 22.9, 21.4, 19.6, 16.3, 16.1, 15.7, 15.4, 11.7, 11.5 (12*q*, γ2-H_3(Ile1/2), δ-H_3C(Ile1/2), δ1-H_3C(Leu), δ2-H_3C(Leu), γ1-H_3C(Valt), γ2-H_3C(Valt), 2β-H_3C(Aib1/2)). MS (ESI): 1036 ([*M*+Na]$^+$).

BOC-Trp-Ile1-Ala-Aib1-Ile2-ValΨ[CSNH]Aib2-Leu-Aib3-Pro-OMe (**12**). To a solution of 66.4 mg (0.065 mmol) BOC-Trp-Ile-Ala-Aib-Ile-ValΨ[CSNH)Aib-Leu-OMe (**10**) in 3 ml THF/H_2O/MeOH 3:1:1, 5.5 mg (0.013 mmol) LiOH·H_2O were added. After stirring for 3.5 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 and extracted with $KHSO_4$-solution. The organic layers were dried ($MgSO_4$), filtered, concentrated, and dried under high vacuum. The crude BOC-Trp-Ile-Ala-Aib-Ile-ValY[CSNH]Aib-Leu-OH was dissolved in 2 ml CH_2Cl_2 and treated with 14.5 mg (0.074 mmol) methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**11**) dissolved in 2 ml CH_2Cl_2. The solution was stirred at room temperature for 54 h, diluted with CH_2Cl_2, and washed with $KHSO_4$-solution. The combined organic phases were dried over $MgSO_4$, filtered, and the filtrate concentrated *in vacuo*. The residue was chromatographed on silica gel (ethyl acetate) to give the decaendothiopeptide **12** as a white solid. Mp. 207.6-208.6°C. $[\alpha]_D$ = -19.9 (c = 0.972). IR ($CHCl_3$): 3475*w*, 3310*s*, 3000*m*, 2965*m*, 2935*m*, 2875*w*, 2385*w*, 1740*m*, 1660*s*, 1530*s*, 1470*m*, 1440*m*, 1415*m*, 1385*w*, 1365*m*, 1280*m*, 1170*s*, 1095*w*, 1055*w*, 1030*w*, 1010*w*, 855*w*, 820*w*, 660*w*. ^{1}H-NMR (600 MHz, $CDCl_3$, cf. Fig. 2-5): 9.16 (*s*, NH); 8.63 (*s*, NH); 7.690 (*d*, *J* = 3.8, NH(Pro)); 7.660 (*d*, *J* = 4.6, NH(Valt)); 7.52 (*d*, *J* = 8.0, ε-HC(Trp)); 7.47 (*s*, NH(Aib3)); 7.46 (*d*, *J* = 8.9, ζ1-HC(Trp)); 7.40 (*s*, NH(Aib1)); 7.28 (*s*, δ1-HC(Trp)); 7.25 (*d*, *J* = 7.1, NH(Ile2)); 7.21 (*t*, *J* = 7.5, η-HC(Trp)); 7.11 (*t*, *J* = 7.5, ζ2-HC(Trp)); 7.01 (*d*, *J* = 8.2, NH(Leu)); 6.30 (*s*, NH); 5.42 (*s*, NH); 4.53-4.50 (*m*, α-HC(Pro)); 4.49-4.45 (*m*, α-HC(Leu)); 4.33 (*t*, *J* = 5.0, α-HC(Valt)); 4.25 (*s* br, α-HC(Trp)); 4.06-4.01 (*m*, α-HC(Ala)); 3.94 (*s* br, α-HC(Ile1)); 3.89-3.85 (*m*, 1δ-HC(Pro) and α-HC(Ile2)); 3.67 (*s*, CH_3O); 3.65-3.60 (*m*, 1δ-HC(Pro)); 3.34-3.21 (*m*, 2β-HC(Trp)); 2.53-2.50 (*m*, β-HC(Valt)); 2.16-1.94 (*m*, α–HC(Ile2), 1β-HC(Pro), and 1γ-HC(Pro)); 1.91 (*s*, 1CH_3(Aib2)); 1.89-1.74 (*m*, 1γ-HC(Pro), 1β-HC(Pro), 2β-HC(Leu), and γ-HC(Leu)); 1.71 (*s*, 1CH_3(Aib2)); 1.65-1.53 (*m*, β-HC(Ile1) and 1γ-HC(Ile2)); 1.60 (*s*, 1CH_3(Aib1) and 1CH_3(Aib3)); 1.57 (*s*, 1 CH_3 (Aib3)); 1.53 (*s*, 1 CH_3(Aib1)); 1.51-1.42 (*m*, γ-HC(Ile2)); 1.44 (*d*, *J* = 7.4, γ-HC(Ala)); 1.42 (*s*, $(CH_3)_3C$); 1.39-1.31 (*m*, 1γ-HC(Ile1)); 1.12 (*d*, *J* = 7.0, γ1-HC(Valt)); 1.08-1.02 (*m*, 1γ-HC(Ile1)); 1.05 (*d*, *J* = 6.9, γ2-HC(Val)); 1.00 (*d*, *J* = 6.9, 3γ-HC(Ile2)); 0.95-0.92 (*d* and *t*, 3δ1-HC(Leu) and 3δ-HC(Ile2)); 0.88 (*d*, *J* = 6.3, 3δ2-HC(Leu)); 0.85 (*t*, *J* = 7.5, 3δ-HC(Ile1)); 0.70 (*d*, *J* = 6.7, 3γ2-HC(Ile1)). ^{13}C-NMR (151 MHz, $CDCl_3$, cf. Fig 2-5): 203.3 (*s*, CS); 175.9 (*s*, CO(Aib1)); 174.0 (*s*, CO(Trp)); 173.7 (*s*, CO(Ile2)); 173.6 (*s*,

CO(Pro)); 173.31 (*s*, CO(Ala)); 173.27 (*s*, CO(Aib2)); 172.6 (*s*, CO(Aib3)); 171.95 (*s*, CO(Leu)); 171.92 (*s*, CO(Ile1)); 157.2 (*s*, O-CO-NH); 136.8 (*s*, δ2-C(Trp)); 127.0 (*s*, ε2-C(Trp)); 124.2 (*d*, δ1-HC(Trp)); 122.4 (*d*, η-CH(Trp)); 119.7 (*d*, ζ2-HC(Trp)); 118.3 (*d*, ε3-HC(Trp)); 112.0 (*d*, ζ1-HC(Trp)); 108.5 (*s*, γ-C(Trp); 81.7 (*s*, $C(CH_3)_3$); 70.8 (*d*, α-HC(Valt)); 61.7 (*s*, α-C(Aib2)); 61.2 (*d*, α-HC(Ile2)); 60.7 (*d*, α-HC(Pro)); 59.5 (*d*, α-CH(Ile1)); 56.9 (*d*, α-HC(Trp)); 56.8 (*s*, α-C(Aib1)); 52.8 (*d*, α-HC(Leu)); 52.0 (*d*, α-HC(Ala)); 51.8 (*s*, α-C(Aib3)); 48.4 (*t*, δ-H_2C(Pro)); 40.9 (*t*, β-H_2C(Leu)); 36.1 (*d*, β-HC(Ile2)); 35.8 (*d*, β-HC(Ile1)); 30.8 (*d*, β-HC(Valt)); 29.4 (*q*, β-H_3C(Aib2)); 28.2 (*q*, $(CH_3)_3$C); 28.1 (*t*, β-H_2C(Pro)); 27.5 (*q*, β-H_3C(Aib1)); 27.3 (*t*, β-H_2C(Trp)); 26.0 (*t*, γ-H_2C(Pro)); 25.9 (*t*, γ1-H_2C(Ile2)); 25.4 (*q*, β-H_3C(Aib3)); 25.3 (*t*, γ1-H_2C(Ile1)); 24.8 (*d*, γ-HC(Leu)); 24.8 (*q*, β-H_3C(Aib3)); 23.7 (*q*, δ1-H_3C(Leu)), 23.1 (*q*, β-H_3C(Aib1)); 22.0 (*q*, β-H_3C(Aib2)); 20.7 (*q*, δ2-H_3C(Leu)); 19.5 (*q*, γ1-H_3C(Valt)); 17.0 (*q*, γ2-H_3C(Valt)); 16.5 (*q*, β-H_3C(Ala)); 15.7 (*q*, γ2-H_3C(Ile2)); 15.6 (*q*, γ2-H_3C(Ile1)); 11.8 (*q*, δ-H_3C(Ile1)); 11.5 (*q*, δ-H_3C(Ile2)). MS (ESI): 1219 ($[M+Na]^+$), 621($[M+2Na]^{2+}$).

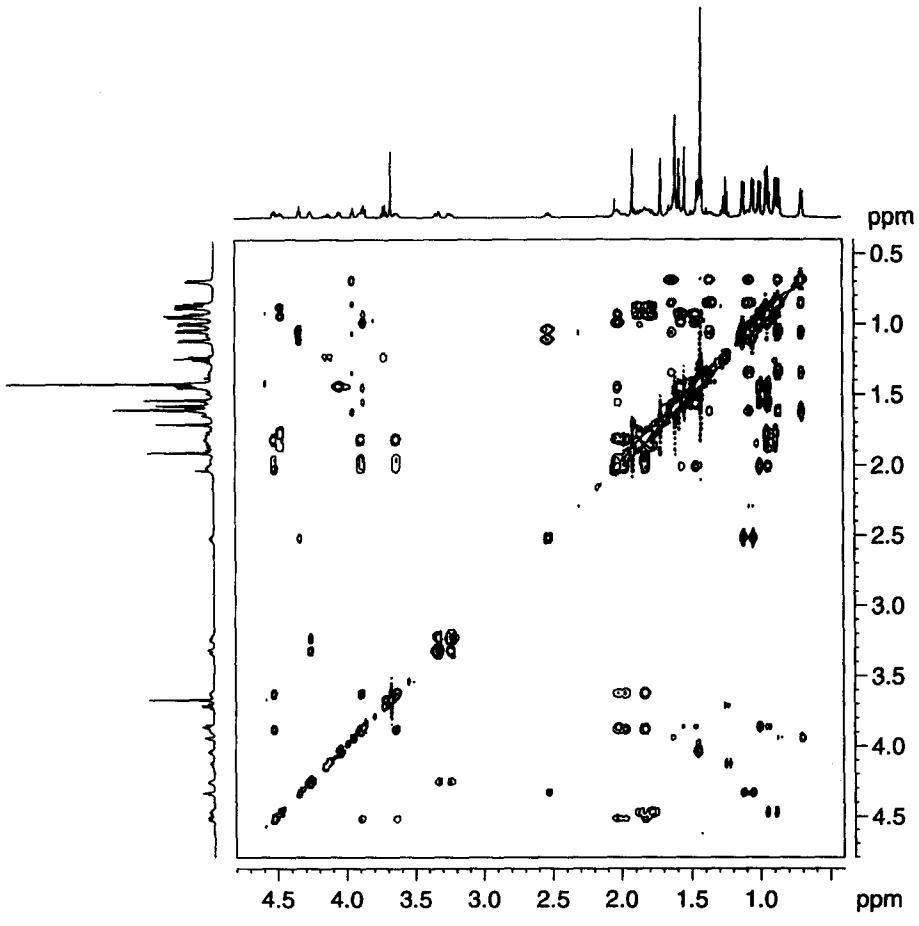


Fig. 2. *^{1}H-TOCSY-spectrum of 12* ($CDCl_3$, 600 MHz; 4.8-0.4 ppm)

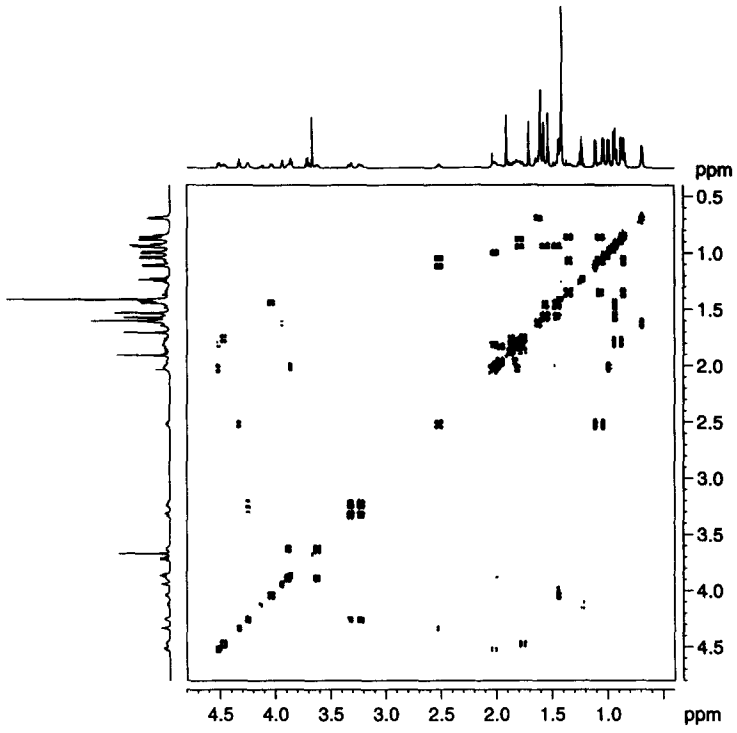


Fig.3. *^{1}H-DQF-COSY-spectrum of 12* ($CDCl_3$, 600 MHz; 4.8-0.4 ppm)

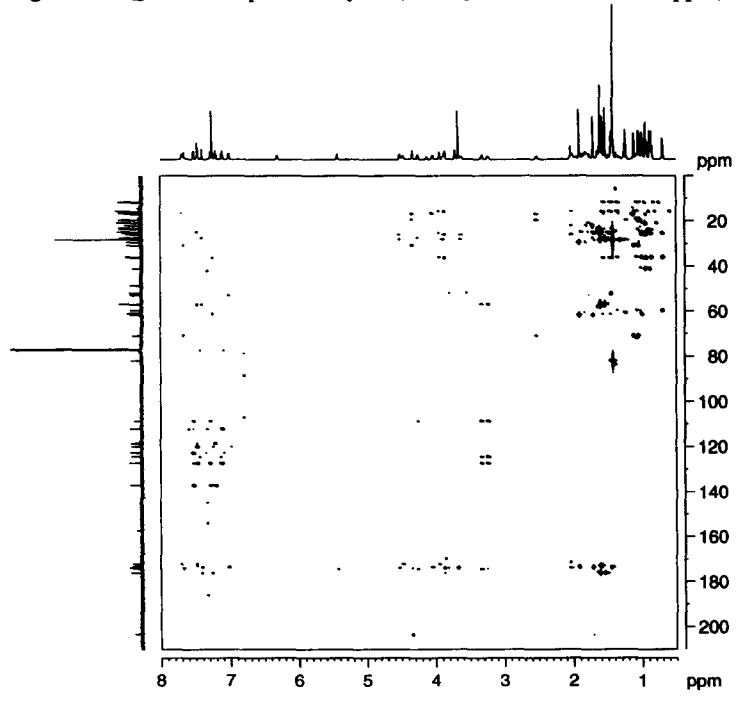


Fig. 4. ^{13}C, ^{1}H-(HMBC) long range-spectrum of 12 ($CDCl_3$, 600 MHz; 8.0-0.5 ppm and 210-0.0 ppm, respectively)

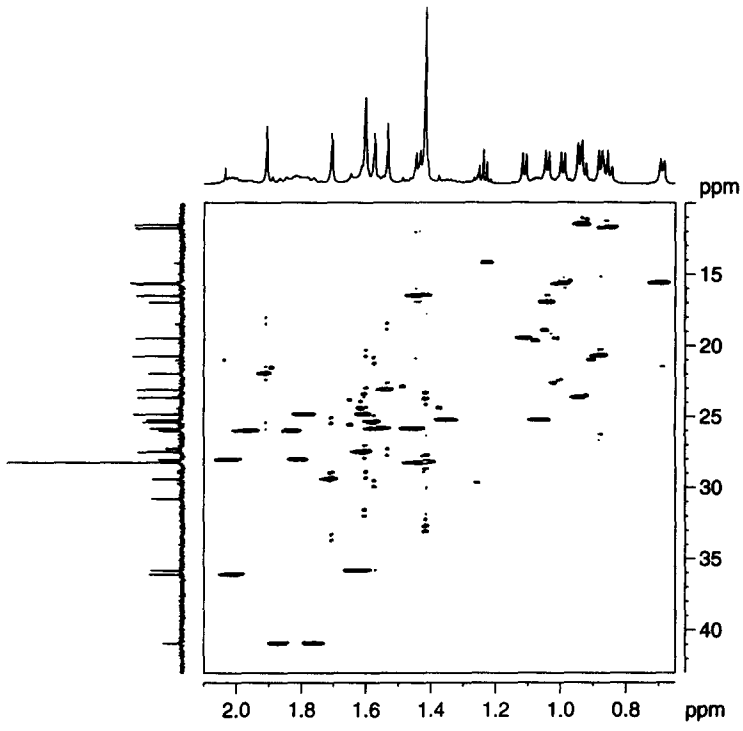


Fig. 5. *$^{13}C,^{1}H$-HSQC-spectrum of* **12** (CDCl$_3$, 600 MHz; 2.5-0.5 *and 43-10 ppm, respectively)*

Crystal Structure Determination of **12** (see Fig. 1)[35]. The intensities were collected on a Rigaku AFC5R diffractometer using graphite-monochromated MoK_α radiation and a 12 kW rotating anode generator. The intensities were corrected for *Lorentz* and polarization effects and an empirical absorption correction was applied[36]. The structure was solved by direct methods using SHELXS86[37], which revealed the positions of all non-H atoms. The asymmetric unit contains one peptide and one ethanol molecule. One of the methyl groups of the valine side chain is disordered over two positions which were refined with the relative site occupation factors of 0.7:0.3. The non-H atoms were refined anisotropically. All NH-atoms were placed in the positions indicated by a difference electron density map and their positions were allowed to refine together with individual isotropic displacement parameters. All of the remaining H-atoms were fixed in geometrically calculated positions [d(C-H) = 0.95 Å] and were assigned fixed isotropic displacement parameters with a value equal to 1.2 U_{eq} of the parent C-atom. Refinement of the structure was carried out on F using full-matrix least-squares procedures, which minimized the function $\Sigma w(|F_o|-|F_c|)^2$. A correction for secondary extinction was applied and two reflections were omitted from final refinement because of suspected extinction effects. Refinement[38] of the absolute structure parameter[39,40] yielded a value of 0.06(16), which confirms that the refined coordinates represent the true enantiomorph.

Neutral atom scattering factors for non-H atoms were taken from[41], and the scattering factors for H-atoms from[42]. Anomalons dispersion effects were included in F_{calc}[43], the values for f' and f'' were those of ref.[44]. All calculations were performed using the TEXSAN crystallographic software package[45].

Crystal data for **12**. A crystal of dimensions 0.23 x 0.33 x 0.43 mm was grown from a mixture of MeOH, EtOH, EtOAc, hexane and pentane. $C_{60}H_{97}N_{11}O_{12}S{\cdot}C_2H_6O$, M_r = 1242.6, monoclinic, space group $P2_1$, a = 12.923(4), b = 15.577(4), c = 18.449(4) Å, β = 109.01(2)°, V = 3511(2) Å^3, Z = 2, D_c 1.175 g cm^{-3}, μ(MoK_α) 0.111 mm^{-1}; T = 173(1), λ = 0.71069 Å. Cell dimensions from 25 reflections in the range 2Θ = 20–24°, $\omega/2\Theta$ scans, $2\Theta_{(max)}$ = 55°, 17458 reflections measured, including Friedel opposites of all unique reflections, 16127 symmetry-independent reflections, 9912 reflections with $I > 2\sigma(I)$ used in the refinement of 793 parameters. Final R = 0.0548, wR = 0.0406 ($w = [\sigma^2(F_o) + (0.005F_o)^2]^{-1}$), GoF = 1.610, secondary extinction coefficient = 1.3(2) x 10^{-7}, Δ_{max}/σ = 0.05, $\Delta\rho$(max; min) = 0.42; -0.30 eÅ^{-3}.

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REFERENCES AND NOTES

1. Spatola, A. *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Marcel Dekker: New York, 1983; Vol. 7.
2. Schutkowski, M.; Neubert, K.; Fischer, G. *Eur. J. Biochem.* **1994**, 455-461.
3. Hitotsuyanagi, Y.; Suzuki, J.; Takeya, K.; Itokawa, H. *Eur. J. Biochem.* **1994**, 1887-1889.
4. Lankiewicz, L.; Bowers, C. Y.; Reynolds, G. A.; Labroo, V.; Cohen, L. A.; Vonhof, S.; Siren, A. L.; Spatola, A. F. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 359-366.
5. Clausen, K.; Thorsen, M.; Lawesson, S.-O. *Tetrahedron* **1981**, *21*, 3635-3639.
6. Thorsen, M.; Yde, B.; Pederson, U.; Clausen, K.; Lawesson, S.-O. *Tetrahedron* **1983**, *39*, 3429-3435.
7. Seebach, D.; Ko, S. Y.; Kessler, H.; Köck, M.; Reggelin, M.; Schmider, P.; Walkinshaw, M. D.; Bölsterli, J. J.; Bevec, D. *Helv. Chim. Acta* **1991**, *74*, 1953-1990.
8. Jurayj, J.; Cushman, M. *Tetrahedron* **1992**, *48*, 8601-8614.
9. Elmore, D. T.; Guthrie, D. J. S.; Kay, G.; Williams, C. H. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1051-1055.
10. Le, H.-T.; Mayer, M.; Thoret, S.; Michelot, R. *Int. J. Peptide Protein Res.* **1995**, *45*, 138-144.
11. Hartke, K.; Barrmeyer, S. *J. Prakt. Chem.* **1996**, *338*, 251-256.
12. Hoeg-Jensen, T.; Spatola, A.; Holm, A. *Int. J. Peptide Protein Res.* **1996**, *47*, 190-200.
13. Hoeg-Jensen, T.; Olsen, C. E.; Holm, A. *J. Org. Chem.* **1994**, *59*, 1257-1263.
14. Zacharie, B.; Sauvé, G.; Penney, C. *Tetrahedron* **1993**, *49*, 10489-10500.
15. Brain, C. T.; Hallett, A.; Ko, S. Y. *J. Org. Chem.* **1997**, *62*, 3808-3809.
16. Shalaby, M. A.; Grote, C. W.; Rapoport, H. *J. Org. Chem.* **1996**, *61*, 9045-9048.
17. Ramakrishnan, N.; Balaram, P. *Acc. Chem. Res.* **1981**, *14*, 356-362.
18. Jung, G.; Brückner, H.; Schmitt, H. In *Structure and Activity of Natural Peptides;* Voelter, W.; Weitzel, G. Eds.; de Gruyter: Berlin, 1981; pp. 75-114.
19. Karle, I. L.; Flippen-Anderson, J. L.; Uma, K.; Balaram, P. *Biochemistry* **1989**, *28*, 6696-6701.
20. Altmann, K.-H.; Altmann, E.; Mutter, M. *Helv. Chim. Acta* **1992**, *75*, 1198-1210.

21. Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Precigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993,** *33*, 1061-1072.
22. Mayr, W.; Jung, G. *Liebigs Ann. Chem.* **1980,** 715-724.
23. Jakubke, H.-D.; Jeschkeit, H. *Aminosäuren, Peptide, Proteine*; Verlag Chemie: Weinheim, 1982.
24. Slomczynska, U.; Beusen, D. D.; Zabrocki, J.; Kociolek, K.; Redlinski, A.; Reusser, F.; Hutton, W. C.; Leplawy, M. T.; Marshall, G. R. *J. Am. Chem. Soc.* **1992,** *114*, 4095-4106.
25. Luykx, R.; Bucher, C. B.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **1996,** *79*, 527-540.
26. Bucher, C. B.; Heimgartner, H. *Helv. Chim. Acta* **1996,** *79*, 1903-1915.
27. Heimgartner, H. *Angew. Chem. Int. Ed. Engl.* **1991,** *30*, 238-264.
28. Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J. K.; Krause, E.; Bienert, M.; Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995,** *60*, 405-410.
29. Bertho, J.-N.; Loffet, A.; Pinel, C.; Reuther, F.; Sennyey, G. *Tetrahedron Lett.* **1991,** *32*, 1303-1306.
30. Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990,** *112*, 9651-9652.
31. Lehmann, J.; Wipf, P.; Bärtsch, A.; Heimgartner, H. *Helvetica Chimica Acta* , in preparation.
32. Karle, I. L.; Sukumar, M.; Balaram, P. *Proc. Natl. Acad. Sci.* **1986,** *83*, 9284-9288.
33. Bernstein, J.; Davis, R. E.; Shimoni, L.; Chang, N.-L. *Angew. Chem. Int. Ed. Engl.* **1995,** *34*, 1555-1573.
34. Johnson, C. K. ORTEP II, Report ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, Tennessee, 1976.
35. Crystallographic data (excluding structure factors) for the structure report in this paper have been deposited with the *Cambridge Crystallographic Data Center.* Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)-1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).
36. North, A. C. T.; Phillips, D. C.; Mathews, F. S. *Acta Crystallogr., Sect. A* **1968,** *24*, 351-359.
37. Sheldrick, G. M. *SHELXS86, Acta Crystallogr., Sect. A* **1990,** *46*, 467-473.
38. Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W. *Crystals User Guide*; Chemical Crystallography Laboratory: Oxford, England, 1985.
39. Flack, H. D. *Acta Crystallogr., Sect. A* **1983,** *39*, 876-881.
40. Bernardinelli, G.; Flack, H. D. *Acta Crystallogr., Sect. A* **1985,** *41*, 500-511.
41. Maslen, E. N.; Fox, A. G.; O'Keefe, M. A. In *International Tables for Crystallography*, Vol.C; Wilson, A. J. C. Ed.; Kluwer Academic Publishers: Dordrecht, 1992; Table 6.1.1.1, pp.477-486.
42. Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965,** *42*, 3175-3187.
43. Ibers, J. A.; Hamilton, W. C. *Acta Crystallogr.* **1964,** *17*, 781-782.
44. Creagh, D. C.; McAuley, W. J. In *International Tables for Crystallography*, Vol.C; Wilson, A. J. C. Ed.; Kluwer Academic Publishers: Dodrecht, 1992; Table 4.2.6.8, pp.219-222.
45. TEXSAN: Single Crystal Structure Analysis Software, Version 5.0; Molecular Structure Corporation, The Woodlands: Texas, 1989.