Small Cyclic Disulfide Peptides: Synthesis in Preparative Amounts and Characterization by Means of NMR and FT-IR Spectroscopy

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Keywords: Peptides / Disulfide bond formation / High dilution / Hydrogen bonds / ATR technique / Temperature-dependent IR spectroscopy

Two cyclic disulfide-bridged tetrapeptides [cyclo(Boc–Cys–Pro–Aib–Cys–OMe) and cyclo(Boc–Cys–Pro–Phe–Cys–OMe)] were prepared in high yield and purity. The use of double-walled reaction flasks, which were attached to an external cryostat, gave perfect temperature control of the reaction. The acetamidomethyl and trityl group were used for the protection of the thiol groups of Cys. Disulfide bond formation was obtained by cleavage of the protection groups and subsequent oxidation of the free thiols with iodine under high dilution conditions in one step. The obtained cyclic peptides were checked for purity by analytical HPLC, and identified by NMR spectroscopy and a variety of standard analytical methods (IR, m. p., FAB-MS, ELA, TLC). Conformational properties of the peptides were derived from one- and two-dimensional NMR experiments. Temperature-dependent NMR and FT-IR experiments allowed for the determination of the degree of inter- and intramolecular hydrogen bonding of the cyclic tetrapeptides. The results from the temperature-dependent NMR experiments are in good agreement with the observed dynamics of the peptides in the amide I and II region, which were determined by means of temperature-dependent FT-IR spectroscopy using the ATR technique.

Introduction

The dynamic behavior of BR (bacteriorhodopsin), which undergoes a reversible photocycle on irradiation,[1–3] is best known among all biomolecules. This protein has been extensively investigated by time-resolved infrared spectroscopy using the STEP/SCAN technique.[4] Up to now, no dynamic investigations have been carried out on much smaller biomolecules, which play an important role in the human organism. As many primary processes of small biomolecules take place on a time-scale faster than microseconds, real time dynamic investigations in the nanosecond time region provide an insight to the primary processes of protein folding in detail. Therefore, such results are well-suited to optimize algorithms of molecular modeling program packages. The hormone peptides oxytoxin and vasopressin are examples of small bioactive peptides. Such cyclic tetrapeptides containing a disulfide bridge and a folding motif have been of interest for a long time. A variety of peptides were synthesized and investigated by a variety of techniques to determine structural and conformational properties, binding affinity, catalytic properties and biological activity. To study protein- or peptide folding, a triggering event (that perturbs the protein/peptide from a "starting" conformation) is required to initiate the folding or unfolding process. In time-resolved spectroscopy, in general, the triggering event is a short laser pulse. Cyclic tetrapeptides containing a disulfide bridge are well-suited, since the disulfide bridge is a weak covalent bond (bond dissociation energy, 64.5 kcal mol^{-1}) and thus provides a predetermined breaking point. Thus, a disulfide bridge can easily and irreversibly be cleaved by UV light.

For the investigation of cyclic tetrapeptides containing a disulfide bridge with regard to the dynamic behavior (folding processes, formation of a motif or aggregation) using the STEP/SCAN technique, a few aspects have to be taken into consideration. Water is best suited for biological systems, but, due to the strong absorptions of water in the IR region, unsuitable for infrared spectroscopy. The problem can be avoided by a minimization of the spacer size of the spectroscopic cell or the use of the ATR technique. A good alternative is to keep a suitable protection group (photostable towards UV irradiation) at the N-terminus and the C-terminus of the peptides to make the compound soluble in solvents like tetrachloromethane, acetonitrile or cyclohexane, which are more suitable for IR spectroscopy. The investigation of folding processes by time-resolved IR spectroscopy, thus requires (i) that the peptides are available on a large scale (several grams); (ii) that the peptides are appropriately modified to ensure good solubility in the solvents used for the time-resolved investigations (C- and N-terminus); (iii) that no further photoactive or labile groups are present in the peptide.

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Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

SPPS (solid-phase peptide synthesis) is a well-established technique and offers numerous advantages over the classical method in solution, but, due to the limited loading capacity of the resin, it is less suitable for the synthesis of peptides on a large scale (several grams) in a research laboratory. A well-designed and optimized classical synthetic strategy under slightly modified and optimized reaction conditions leads more easily to the desired amount of peptides. In this paper we demonstrate the advantage of the classical solution method in combination with specially designed doubled-walled reaction flasks attached to an external cryostat to give perfect temperature control for the large scale synthesis of peptides. We focus on the synthesis of two cyclic tetrapeptides cyclo(Boc−Cys−Pro−Aib−Cys−OMe) (**1**) and cyclo(Boc−Cys−Pro−Phe−Cys−OMe) (**6**). Further, we characterized the structural properties of these peptides by NMR and temperature-dependent IR spectroscopy in view of time-resolved investigations, which are currently in progress.

Chemistry

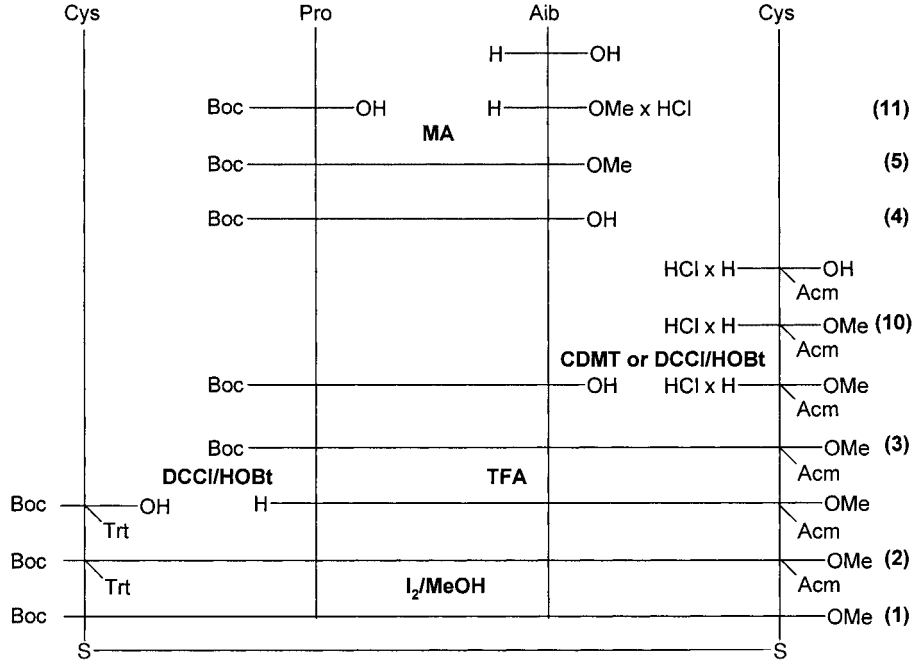
The cyclic tetrapeptide cyclo(Boc−Cys−Pro−Aib−Cys−OMe) (**1**) was prepared according to Scheme 1. The key intermediate, dipeptide Boc−Pro−Aib−OH (**4**), was synthesized in a slightly modified way according to the method published by Jung et al.[5] Tripeptide **3** was prepared by the elongation of the dipeptide **4** with CDMT as a suitable coupling reagent. Elongation to the linear tetrapeptide **2** was done with the use of DCCI/HOBt. Deprotection of the Boc-intermediate **3** was performed by treatment with TFA. The cyclic tetrapeptide **1** was obtained by oxidation of the linear form **2** with iodine in methanol under high dilution conditions.

The cyclic tetrapeptide cyclo(Boc−Cys−Pro−Phe−Cys−OMe) (**6**) was prepared according to Scheme 2. Linear tetrapeptide **7** was synthesized by a stepwise elongation using the mixed anhydride method with isobutyl chloroformate. Deprotection of the Boc-intermediates **8** and **9** was once again performed with the use of TFA. The cyclization that yields **6** was performed as described before.

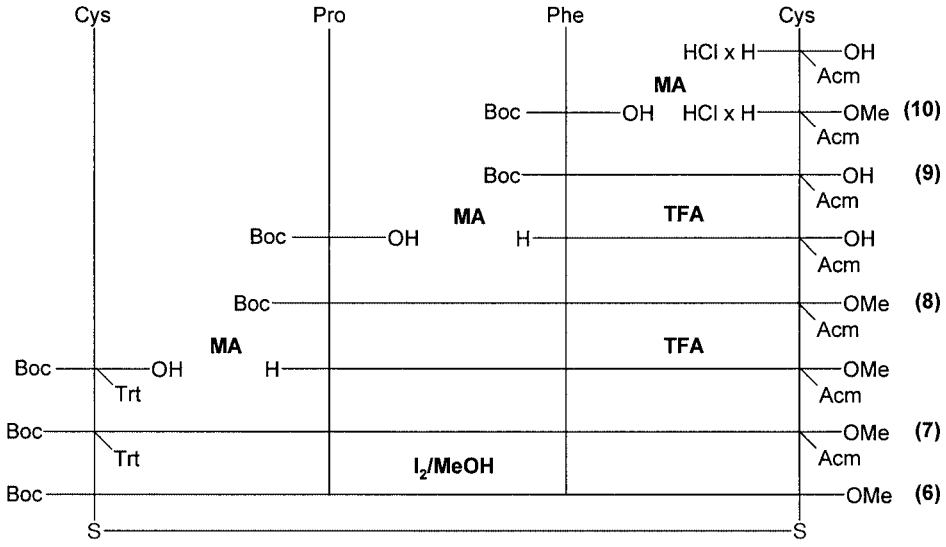
Discussion

Synthesis of Peptides

The strategy for the synthesis of peptide **1** is based on the fragment condensation of smaller peptides. In our case, dipeptide **4** was chosen as the starting material, as a good synthetic protocol for this compound was published by Jung et al.[5] several years ago. We increased the yield of this reaction by 5−10% with the use of double-walled reaction flasks in combination with an external cryostat, which gave perfect temperature control. This technique allowed the optimization of reaction temperatures and times for the coupling reactions. The coupling between cysteine derivative **10** and dipeptide **4** proved to be the most difficult step. The esterification of HCl*H−Cys(Acm)−OH was described to yield a colorless, hygroscopic foam,[6] which can be crystallized from isopropyl alcohol.[7] We obtained the resulting ester HCl*H−Cys(Acm)−OMe (**10**) as a colorless crystalline compound. After careful removal of the solvent and remaining thionyl chloride, the product from a large-scale preparation showed no tendency of forming a foam. Smaller quantities can be crystallized successively from dry ether and pentane.



Scheme 1



Scheme 2

Various coupling reagents and temperatures have been checked for the coupling reaction involving the a, a-dialkylated amino acid Aib (aminoisobutyric acid) in the case of peptide **3**. Although an excellent coupling method for Aib has been published by Carpino[8a] (using TFFH), we found two methods to be most suitable for attaching the cysteine derivative **10** to the dipeptide Boc−Pro−Aib−OH. The use of DCCI/HOBt as a coupling reagent gives the desired tripeptide **3** in fairly high yields; however, column chromatography is necessary to obtain the peptide in good purity. The second method uses CDMT[8b] as a coupling reagent giving the tripeptide in lower yields as in the method described before, but no further purification by column chromatography is necessary. The slightly basic properties of the triazine allows extraction under acidic conditions. We performed coupling reactions using the MA method for attaching Aib to the dipeptide **4**. However, an oily product was obtained, which gave only very low amounts of **3** after purification. Methods like preactivation or the use of very powerful coupling reagents like HATU, TBTU and HBTU showed no significant improvement over the coupling reaction. Deprotection was performed with TFA, which was diluted with hexane. The pure deprotected peptides were obtained after coevaporation with hexane and precipitation with dry ether in good yield. The deprotected peptides are stable at room temperature for about 3 days if stored in a desiccator under argon. Good coupling results can be obtained by using of a large excess of base, and dissolving the mixture by ultrasonication. The standard method DCCI/HOBt was used for coupling to the linear tetrapeptide **2**. The use of two different thiol protection groups in combination with a high dilution condition minimizes the amount of oligomers and polymers in the cyclization reaction. The yield of the desired cyclic tetrapeptide **1** after column chromatography is 60−65%.

The cyclic tetrapeptide **6** was obtained from cyclization of the key intermediate **7**. The linear tetrapeptide Boc−Cys(Trt)−Pro−Phe−Cys(Acm)−OMe (**7**) was synthesized by a stepwise elongation using the mixed anhydride method with isobutyl chloroformate, again in combination with temperature-controlled double-walled reaction flasks. Deprotection of the Boc protected intermediates was performed by treatment with TFA, as described before. Cyclization used I_2/methanol under high dilution conditions at ambient temperature. The yield of this oxidation reaction is much lower than in the case of cyclic tetrapeptide **1**. Column chromatography yields the desired cyclic tetrapeptide **6** in moderate yields (40−45%). The Aib/Phe exchange influences the conformation of the backbone. In the linear tetrapeptide **2**, the nonchiral amino acid Aib is used instead of the chiral amino acid Phe. This leads to a conformation of **2** that is less flexible than the conformation of **6**, resulting in a much closer arrangement of the Cys groups (see also NMR and IR investigations.), and thus higher yields of the cyclized product **1** relative to **6**.

NMR Studies

Well-resolved NMR spectra were recorded for the linear peptides **2** and **7**, and the cyclic peptides **1** and **6** in [D_6]DMSO at ambient temperature. ^{1}H NMR spectra for **1** and **2** are shown in Figure 1 (for data for **6** and **7**, see Supporting Information). The assignments are based on TOCSY, HMBC, HMQC and ROESY experiments. TOCSY and HMQC spectra for **1** and **2** are shown in the Supplementary Information.

The ^{1}H NMR spectra of the cyclic tetrapeptide cyclo[Boc−Cys−Pro−Phe−Cys−OMe] (**6**) in [D_6]DMSO shows a variety of complex signals, while one- and two-dimensional NMR investigations of **6** in $CDCl_3$ show two well-resolved sets of signals, which belong to two conformations. A similar influence of the solvent on peptide conformation has been observed by Balaram et al.[9] for the derivative cyclo[Boc−Cys−Pro−Phe−Cys−NHMe] (**12**). The complete assignment of the signals of **6** in $CDCl_3$ is

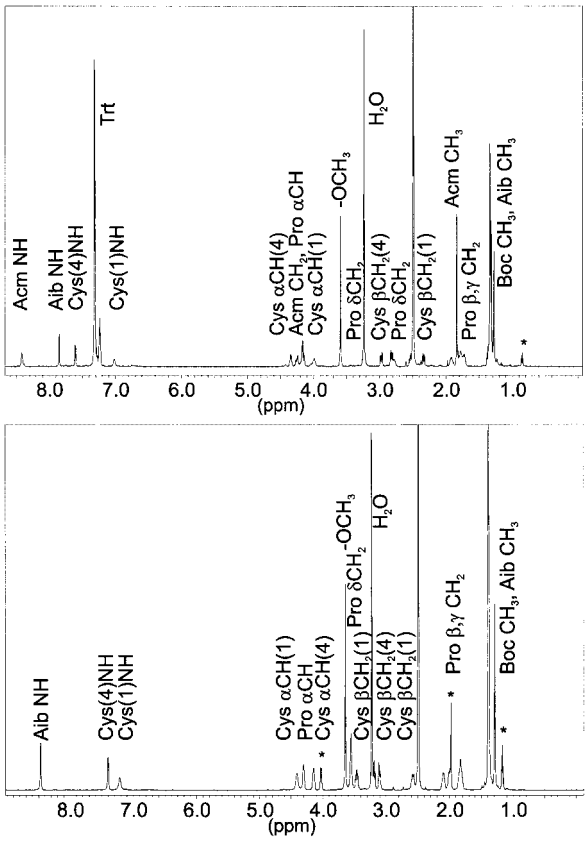


Figure 1. Top: ^{1}H spectra of **2**; bottom: ^{1}H spectra of **1**; both 600-MHz in [D_6]DMSO; signals marked with an asterisk belong to ethyl acetate (eluate)

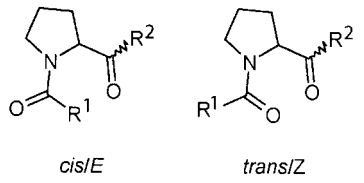


Figure 3. Schematic representation of the *cis*/*trans* conformers of a Xxx−Pro peptide bond

based on HMQC, HMBC, TOCSY and ROESY experiments. The two conformers were identified as the *trans* and *cis* species of **6** with respect to the Xxx−Pro amide bond. A distinction between the two conformers is based on the significant shifts of the β- and γ-carbon atom of the proline ring.[10,11] This information is easily extracted (^{13}C NMR, HMQC and HMBC). ^{13}C spectra of **1**, **2** and **7** show characteristic signals of Pro near δ = 29.5 ppm (Cβ) and 24.2 ppm (Cγ) indicating a *trans* Xxx−Pro peptide bond (Table 1).[10,11] As expected, **1**, **2** and **7** exist in the energetically favored *trans* conformation. In contrast, **6** shows a *trans* and a *cis* Xxx−Pro peptide bond in the major (**M**) and minor (**m**) forms, respectively. The ratio of the conformations (**M**:**m**) was estimated to approximately 2:1 by integration of the signals of the ^{1}H NMR spectra. This result is in agreement with the well-known activation barrier of 16.7−21.5 kcal mol^{-1} for the *cis*/*trans* isomerization along the Xxx−Pro peptide bond and the small energy difference of 0.5−1.4 kcal·mol^{-1} between *cis* Xxx−Pro and *trans* Xxx−Pro.[12−15] The complete data from the NMR experiments is summarized in Table 2

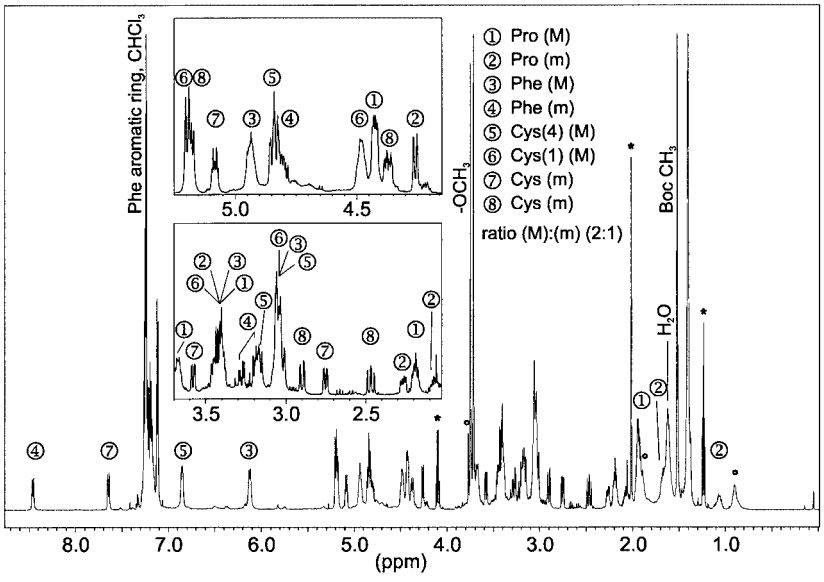


Figure 2. 600-MHz ^{1}H NMR spectrum of **6** in $CDCl_3$; (M) refers to the major, (m) to the minor conformation; signals marked with an asterisk or circle belong to the eluate; inset: top: magnification of the 4.2−5.5 ppm region; bottom: magnification of the 2.0−3.6 ppm region

Table 1. Characteristic shifts for Cβ and Cγ indicating a *trans* or *cis* Xxx−Pro peptide bond

Compound	Cβ [ppm]	Cγ [ppm]	Δδ(Cβ−Cγ) [ppm]
2	28.52	24.64	3.88
1	28.40	24.85	3.55
7	28.48	24.06	4.42
6	28.20 (**M**)	24.80 (**M**)	3.40 (**M**)
	32.00 (**m**)	21.60 (**m**)	10.40 (**m**)
trans	29.50[10]	24.20[10]	–
		25.0 ± 1.0[11]	–
		25.1 ± 0.5[11]	1.3 to 6.0[11]
cis	31.30[10]	22.50[10]	–
		22.4 ± 0.8[11]	–
		23.4 ± 0.3[11]	8.3 to 10.0[11]

Table 2. Chemical shifts of NH and CαH protons of **1**, **2**, **6**, and **7** relative to cyclo[Boc−Cys−Pro−Phe−Cys−NHMe] (**12**)[9]

		Exp. δ [ppm] Cys(1)	Pro	Aib	Phe	Cys(4)	NHMe
2	δ_{NH}[([D$_6$]DMSO)]	7.02	–	7.85	–	7.61	–
	$\delta_{C\alpha H}$[([D$_6$]DMSO)]	3.99	4.16	–	–	4.34	–
1	δ_{NH}[([D$_6$]DMSO)]	7.22	–	8.47	–	7.40	–
	$\delta_{C\alpha H}$[([D$_6$]DMSO)]	4.41	4.31	–	–	4.14	–
7	δ_{NH}[([D$_6$]DMSO)]	7.05	–	–	8.30	7.63	–
	$\delta_{C\alpha H}$[([D$_6$]DMSO)]	4.47	4.20	–	4.46	4.51	–
6	δ_{NH}[(CDCl$_3$)] (M)	5.19	–	–	6.13	6.85	–
	$\delta_{C\alpha H}$[(CDCl$_3$)] (M)	4.48	4.43	–	4.94	4.85	–
12	δ_{NH}[(CDCl$_3$)] (M)[9]	5.19	–	–	6.10	7.02	6.50
	$\delta_{C\alpha H}$[(CDCl$_3$)] (M)[9]	4.51	4.42	–	4.99	4.67	–

Information of relevance to conformational analysis can easily be taken from the ^{1}H NMR spectra. For example, the temperature-dependence of the chemical shift of the NH′s indicate hydrogen bonding, while the vicinal [^3J(NH,H−C(α)] coupling constants give information on the dihedral angles φ in the peptide part (Tables 3 and 4).

The data obtained by the temperature-dependent NMR experiments underline the influence of the sequence on the conformation. While **2** and **1** exist in only one rigid conformation and exhibit one intramolecular hydrogen bond between NH Cys(4) and CO Cys(1) (β-turn), which plays an important role in fixing this structure, **7** and **6** show no uniform conformation. Neither **6** nor **7** exhibit intramolecular hydrogen bonds. Rather, the characteristic Δδ values of **6** and **7** indicate solvent exposed NH groups, which cannot strengthen and fix the backbone. As the polarity of the solvents plays an important role, intramolecular hydrogen bonds in $CDCl_3$ are much stronger than in [D$_6$]DMSO, and it is not astonishing that **7** exists in a variety of conformations in [D$_6$]DMSO, but only in two in $CDCl_3$.

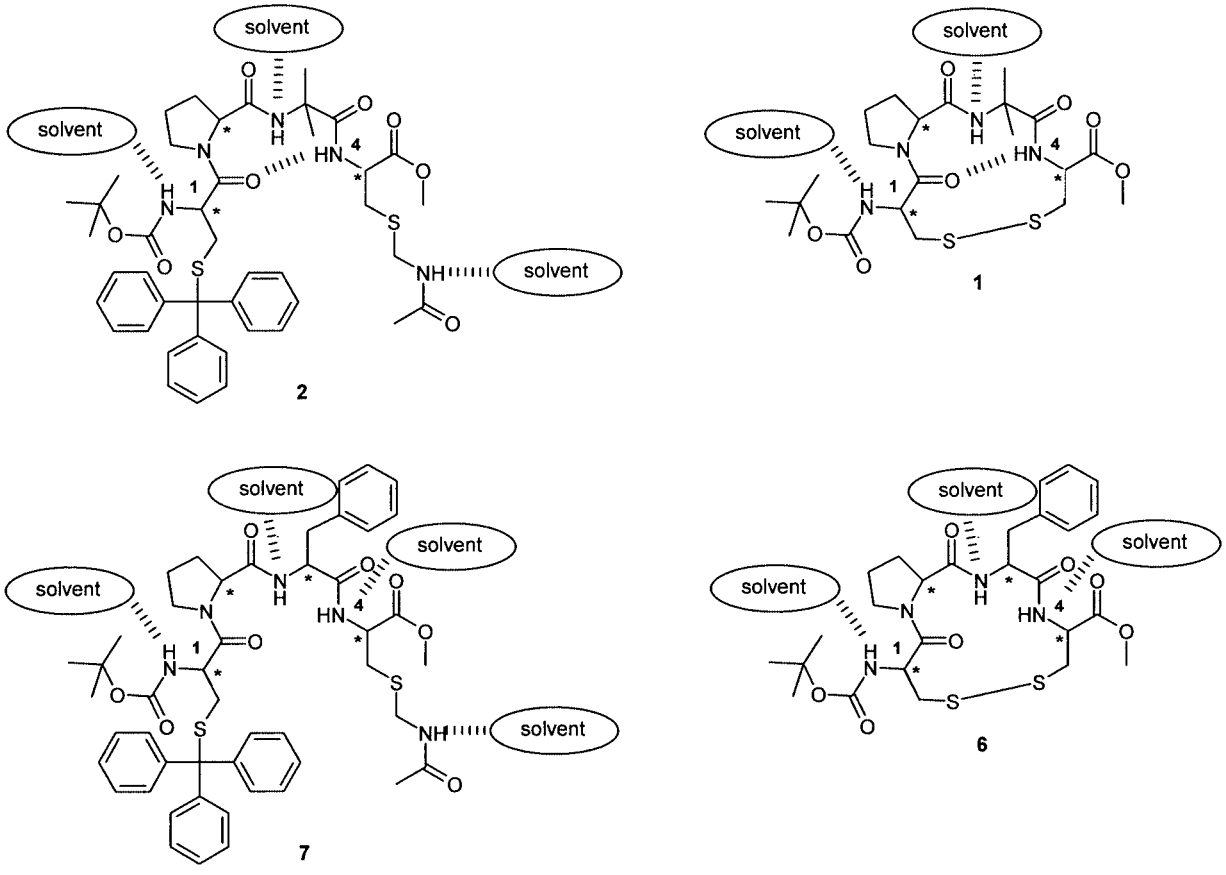


Figure 4. Linear (**2** and **7**) and cyclic tetrapeptides (**1** and **6**) investigated by temperature-dependent NMR and FTIR spectroscopy; dashed lines indicate inter- or intramolecular hydrogen bonding

Table 3. Vicinal ^3J[NH,H−C(α)] coupling constants

Compound	Amino acid	$^3J_{HNC\alpha H}$ [Hz]
2 ([D$_6$]DMSO)	Cys(1)	8.53
	Cys(4)	7.02
1 ([D$_6$]DMSO)	Cys(1)	8.03
	Cys(4)	6.52
7 ([D$_6$]DMSO)	Cys(1)	6.99
	Cys(4)	8.12
	Phe	7.75
6[a] ($CDCl_3$)	Cys(1) (**M**)	8.50
	Cys(4) (**M**)	7.36
	Phe (**M**)	9.44
	Phe (**m**)	8.88

[a] Cys(**m**): 8.68 and 5.67 Hz. Values may contain errors due to signal overlapping.

Table 4. *T*-gradients of the NH′s given in $\Delta\delta/T*10^{-3}$ ppm/K indicating intra- or intermolecular hydrogen bonding

Compound	Acm NH	Aib NH	Cys NH(1)	Cys NH(2)	Phe NH
2 ([D$_6$]DMSO)	5.5	5.8	9.2	2.4	–
1 ([D$_6$]DMSO)	–	6.0	10.3	1.7	–
7 ([D$_6$]DMSO)	5.5	–	9.5	5.2	6.3
6 ($CDCl_3$)	–	–	[a]	[a]	[a]

[a] No values determined due to signal overlapping.

Temperature-Dependent FTIR Studies

To support the results from the temperature-dependent NMR experiments, we carried out FTIR measurements with compounds **2** and **1** in trichloromethane in the temperature range from −10.0 to 50.0 °C with a spectral resolution of 4 cm^{-1} using an ATR unit and a thermojacket. All characteristic bands of the linear tetrapeptide **2** in the amide I and II regions showed a decrease in intensity on annealing (Figure 5).

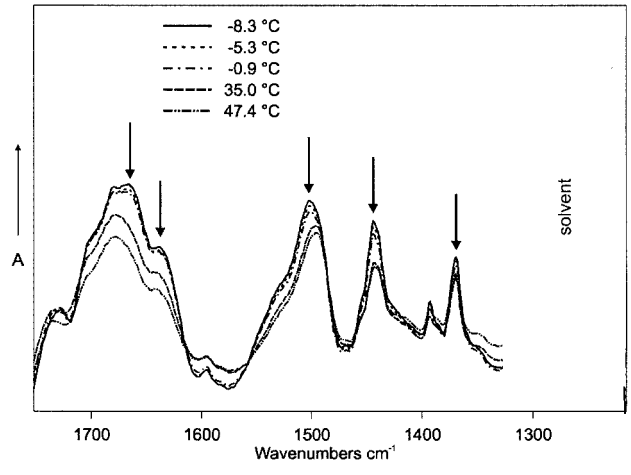


Figure 5. Temperature-dependent FTIR spectrum of **2** in $CHCl_3$; the spectral resolution is 4 cm^{-1}

This effect is in agreement with the results from the NMR experiments. If a hydrogen bond gets weaker, a new band at higher wavenumbers is expected to appear. Moreover, the band that is involved in the intramolecular hydrogen bond should show a less rapid decrease in intensity. However, none of these features has been observed for **2**. In contrast, cyclic tetrapeptide **1** showed a completely different behavior in the amide I and II regions (Figure 6).

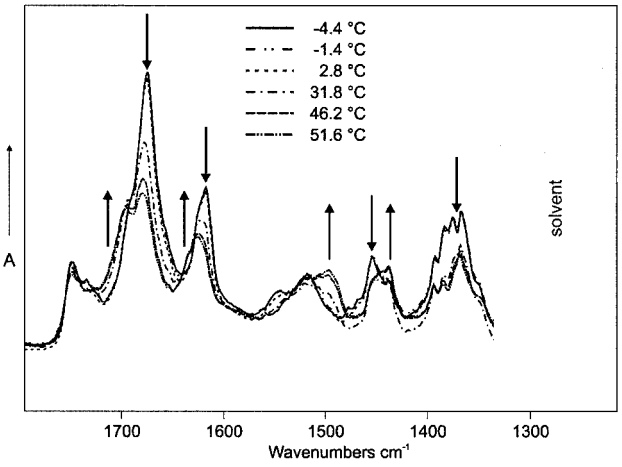


Figure 6. Temperature-dependent FTIR spectrum of **1** in $CHCl_3$; the spectral resolution is 4 cm^{-1}

Three bands (1749, 1675 and 1618 cm^{-1}) of the amide I region exhibit a decrease in intensity or the appearance of new bands on warming the solution. The decrease in intensity of the signal at 1749 cm^{-1} is very slow relative to the bands at 1675 and 1618 cm^{-1}. Both bands rapidly lose intensity on annealing and simultaneously form two new signals (shoulders) at 1695 and 1633 cm^{-1}. These observations are in good agreement with the results from the NMR experiments. This leads us to the conclusion that the band at 1749 cm^{-1} is assigned to the C=O stretching vibration of Cys(1) which, according to the NMR spectroscopic data, is involved in the formation of an intramolecular hydrogen bond with NH Cys(4) and the resulting β-turn. The other two bands can be assigned to the remaining C=O stretching vibrations of **1**, which are involved in intermolecular hydrogen bonds with the solvent or further molecules. On annealing, these intermolecular hydrogen bonds are rapidly cleaved. As this causes an increase in the bond order of the C=O stretching vibration, the new signals are observed at higher wavenumbers. On annealing, the bands of the amide II region react in a similar way (loss of intensity and simultaneous formation of new bands). However, a complete assignment of each band to a single amino acid is not possible without selective isotopic labeling or site-directed mutagenesis.

Conclusion

The selective use of temperature control in the classical peptide synthesis has been demonstrated to allow the large-scale synthesis of two cyclic tetrapeptides. These peptides are derivatives of the bioactive hormone peptides Oxytocine and Vassopressine. Double-walled reaction flasks attached to an external cryostat allowed perfect temperature control, and resulted in high yields of the two cyclic tetrapeptides and their precursors. We obtained information on the conformation of the peptides from one- and two-dimensional NMR experiments. Further, we investigated the dynamics of the peptides in the amide I and II region, by temperature-dependent FT-IR spectroscopy using the ATR technique. The results are in good agreement with the data obtained by temperature-dependent NMR spectroscopy.

Experimental Section

General: Melting points: Dr. Tottoli apparatus, uncorrected. IR spectra (KBr pellet): Perkin−Elmer 983G or 841 spectrometer. FAB mass: Varian MAT CH5, *meta*-nitrobenzyl alcohol matrix. Elementary analyses: Vario EL; averaged value of two measurements of the same sample. TLC: Macherey−Nagel SIL G/UV_{254} silica gel plates. Column chromatography: MATREX™ silica Si chromatography medium, Amicon Europe, CH-1001 Lausanne, pore diam. 60 Å, part size 020−45 my. ^{1}H NMR: Bruker DPX 200 (200.13 MHz), DRX 400 (400.13 MHz) and Bruker DRX 600 (600.13 MHz) instruments. ^{13}C NMR: Bruker DPX 200 (50.3 MHz), DRX 400 (100.4 MHz) and Bruker DRX 600 (150.9 MHz) instruments, δ rel. to Me_4Si. 2D NMR experiments (TOCSY, HMQC, HMBC, ROESY) on a Bruker DRX 400 or DRX 600 instrument. Drying agent: Na_2SO_4, if not mentioned otherwise. Boc protected amino acids were purchased from Calbiochem-Novabiochem. Double-walled reaction flasks (Supporting Information, see also the footnote on the first page of this article) were made at the glassblowing facility of our department. The temperatures given refer to the temperatures inside the reaction flask of the double-walled flasks. Temperature control was achieved by connecting an external cryostat with temperature control to the flasks. The cryostats can operate in a cooling or a heating mode (Haake Modell Fison CH or Lauda RC 20 CS).

Temperature-dependent FTIR measurements: For temperature-dependent FTIR measurements, a Bruker IFS66v/S (Bruker Optics, Ettlingen) was used in combination with an ATR unit (horizontal multireflection unit "Gateway", Specac, UK). The ATR unit was placed in the sample compartment of the FTIR spectrometer and equipped with a continuous-flow sample cell with a thermojacket (45°-ZnSe-crystal, 550 μL sample volume, ZnSe-crystal 6 reflections, temperature range −10 to 90 °C, Specac, UK). Temperature control was achieved by connecting the thermojacket to a cryostat (Haake Modell Fison CH or Lauda RC 20 CS). The temperature was also checked near the ZnSe-crystal by an electronic temperature sensor (Voltcraft 300 K Digital thermometer, Conrad Electronics, Germany). All measurements were carried out with 4 cm^{-1} resolution in the spectral range between 400 and 3000 cm^{-1}.

The abbreviations used are as follows: Acm = acetamidomethyl, Aib = aminoisobutyric acid, Boc = *tert*-butoxycarbonyl, CDMT = 2-chloro-4,6-dimethoxy-1,3,5-triazine, DCCI = dicyclohexylcarbodiimide, DCM = dichloromethane, DMF = dimethylformamide, HOBt = hydroxybenzotriazole, NMM = *N*-methylmorpholine, MA = mixed-anhydride method, TFA = trifluoroacetic acid, Trt = trityl, Cys(1) refers to the Boc-protected cysteine, Cys(4) refers to the cysteine carrying the methyl ester.

General Deprotection Procedure: The Boc-protected peptide or amino acid (10 mmol) was stirred in TFA (41 mL) in a flask sealed with a drying tube for 100 minutes at ambient temperature. Hexane (345 mL) was added, and the solution was stirred for a further 30 minutes. The solvent was evaporated, and the residue treated with a small portion of dry ether. Precipitated, deprotected peptide/amino acid was obtained as a colorless powder, which was checked for complete deprotection by ^{1}H NMR spectroscopy (removal of the Boc signal at about 1.35 ppm). The deprotected compound can be stored for about 3 days if placed in a desiccator under argon. Yield 95−98%.

Cyclization: Boc−Cys(Trt)−Pro−X−Cys(Acm)−OMe (2 mmol) was dissolved in freshly distilled methanol (400 mL). A solution of iodine (1.55 g, 6 mmol) in freshly distilled methanol (60 mL) was added over 10 minutes with vigorous mechanical stirring. Stirring was continued at ambient temperature for another 90 minutes. The reaction was quenched by the dropwise addition of a $Na_2S_2O_3$ solution (0.1 M) until the color of the methanolic solution had completely disappeared. The solution was evaporated to dryness, the residue taken up in water (50 mL) and extracted three times each with trichloromethane (150 mL). Drying of the combined organic phases, and removal of the solvent yielded an oil, which was treated with dry ether (few mL). This yielded a colorless precipitate, which was filtered off. The remaining solution was evaporated to dryness, and the foam obtained thus was purified by column chromatography. Elution with DCM/EtOAc (9:1 and 8:2) and ethyl acetate afforded pure colorless cyclic tetrapeptide. Yield 40−65%.

General Peptide Coupling Procedure, Elongation by Mixed Anhydride Method: Boc−X−OH (0.033 mol) and NMM (7.3 mL, 0.066 mol) were dissolved, whilst stirring, in DCM (170 mL) and cooled for 15 minutes at −15 °C. Isobutyl chloroformate (4.34 mL, 0.033 mol) was slowly added dropwise with continued stirring. The mixture was kept at −15 °C for 10 to 15 minutes. Thereafter, a well-dissolved solution of amino acid methyl ester hydrochloride or deprotected peptide (0.033 mol), NMM (7.3 mL, 0.066 mol), and DMF (15 mL) in DCM (120 mL) was added dropwise over 15 minutes to the stirred mixture at −15 °C. The mixture was stirred at −15 °C for another 15 minutes and then at room temperature overnight. The mixture was then taken up in EtOAc (800 mL). The organic layers were successively washed with 5% $KHSO_4$ (250 mL), water (250 mL) with sat. $NaHCO_3$ (260 mL), sat. $NaHCO_3$ (200 mL), and sat. NaCl (300 mL). The collected organic layers were dried, and the solvent was evaporated.

HCl*Aib−OMe (11): Aminoisobutyric acid (10.5 g, 0.1018 mol) was suspended in methanol (100 mL) and cooled by an ice bath. Whilst stirring, $SOCl_2$ (7.8 mL, 0.107 mol) was added dropwise to the solution. After the complete addition of thionyl chloride, the mixture was heated for four hours at 55 °C. The mixture was allowed to stand at room temperature whilst stirring overnight. The solvent was coevaporated five times each with methanol (50 mL). After complete removal of the solvent, the residue was recrystallized from methanol/ether (50 mL, 1:1) and placed in a desiccator with NaOH overnight. This yielded 15.36 g (98.3%) of a colorless compound. M.p. 180.0 °C (ref. 183−184 °C[16]). R_f = 0.59 in BuOH/glacial acetic acid/water/pyridine (15:3:12:10). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.95 (s, br., NH_3^+), 3.79 (s, OCH_3), 1.71

(s, CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.59 (CO), 57.48 (Cα), 53.33 (OCH_3), 23.93 (Cβ) ppm. IR: $\tilde{\nu}$ = 2960, 2658, 2585, 2036, 1748, 1596, 1523, 1469, 1319, 1237, 1196, 1086, 977, 877, 770 cm^{-1}. FAB-MS *m/z*: 118.1 (100) $[M - HCl + H]^+$. $C_5H_{12}ClNO_2$ (153.6): calcd. C 39.1, H 7.8, N 9.1; found C 38.46, H 7.54, N 8.82.

Boc-Pro-Aib−OMe (5): Dipeptide **5** was synthesized according to the procedure published by Jung et al.[5] Changes have been implemented that involve the temperatures, as the reaction was carried out in a double-walled flask under complete temperature control by an external cryostat.

The starting solution of Boc−Pro-OH in DCM/DMF was stirred for 30 minutes at −15 °C. Isobutyl chloroformate was added dropwise over 15−20 minutes without raising the temperature of the reaction mixture. The solution was then stirred for a further 10 minutes before adding a completely dissolved and precooled (−5 to 0 °C) solution of HCl*Aib-OMe (**11**) in NMM and DCM/DMF at −18 °C over a period of 15 minutes. The mixture was stirred for an additional hour at −15 °C. The temperature was then increased to −5 °C. After 90 minutes at −5 °C, the temperature was raised to room temperature and stirring was continued for an additional 90 minutes.

Another change was made during the workup procedure. The crude oil, which was obtained after drying and removal of the solvent, was crystallized from pentane/ethyl acetate (1:1) to yield an off-white compound (>82% yield). M.p. 79.5 °C (ref. 80 °C[5]). R_f = 0.83 in BuOH/acetic acid/water (3:1:1). ^1H NMR (200 MHz, [(D_6)]DMSO): δ = 8.16 (s, NH Aib), 4.05 (Pro α), 3.53 (s, OCH_3), 3.30 (br., Pro δ), 2.07−1.75 (m, br., Pro β,γ), 1.37−1.30 (m, Boc CH_3, Aib CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 174.76 (CO), 171.62 (CO), 156.69 (CO), 80.42 (qC Boc), 61.14 (Cα, Pro), 56.14 (Cα, Aib), 52.47 (OCH_3), 46.99 (Cδ, Pro), 30.78 (Cβ, Pro), 28.33 (Boc CH_3), 24.99 (Cβ, Aib), 24.66 (Cγ, Pro) ppm. IR: $\tilde{\nu}$ = 3319, 2990, 1741, 1672, 1533, 1407, 1288, 1153, 987, 775, 616 cm^{-1}. FAB-MS *m/z*: 337.2 (54) $[M + Na]^+$, 315.2 (96) $[M + H]^+$, 259.1 (47), 241.1 (7), 215.1 (100). $C_{15}H_{26}N_2O_5$ (314.4): calcd. C 57.3, H 8.3, N 8.9; found C 56.94, H 8.13, N 8.80.

Boc−Pro−Aib−OH (4): Saponification of dipeptide **5** was performed according to the literature procedure of Jung et al.[5] The reaction was performed in a double-walled reaction flask. The temperature inside the reaction flask was kept at 37.5 °C, controlled by an external cryostat. The reaction yielded 78.0% of an off white compound. Completeness of saponification was checked by NMR spectroscopy. No further purification was necessary. M.p. 159.0 °C (ref. 161 °C[5]). R_f = 0.78 in BuOH/acetic acid/water (3:1:1). ^1H NMR (200 MHz, [D_6]DMSO): δ = 12.15 (s, br., COOH), 7.96 (s, NH Aib), 4.02 (Pro α), 3.31 (br., Pro δ), 2.05−1.75 (k, br., Pro β,γ), 1.36−1.30 (m, Boc CH_3, Aib CH_3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 176.68 (CO), 173.14 (CO), 146.00 (CO), 81.00 (qC Boc), 61.36 (Cα, Pro), 56.80 (Cα, Aib), 47.10 (Cδ, Pro), 33.24 (Cβ, Pro), 28.31 (Boc CH_3), 25.74/25.07 (Cβ, Aib), 24.62 (Cγ, Pro) ppm. IR: $\tilde{\nu}$ = 3316, 2985, 2879, 1713, 1680, 1529, 1408, 1302, 1245, 1166, 1127, 943, 778, 615 cm^{-1}. FAB-MS *m/z*: 323.0 (100) $[M + Na]^+$, 301.1 (47) $[M + H]^+$, 245.0 (36), 223.0 (16), 201.0 (83). $C_{14}H_{24}N_2O_5$ (300.4): calcd. C 55.9, H 8.1, N 9.3; found C 55.39, H 8.79, N 9.05.

HCl*Cys(Acm)−OMe (10): Methanol (40 mL) was placed in the reaction flask at −15 °C. Whilst stirring, thionyl chloride (2.65 mL, 0.036 mol) was added dropwise to the methanol in a manner that the reaction temperature did not increase beyond −5 °C. HCl* Cys(Acm)−OH (7.5 g, 0.033 mol) was then added to the solution at once. Stirring was continued for another 30 minutes at −5 °C. The temperature was then raised to 45 °C. The clear solution was stirred for 4 hours at 45 °C, after which the solvent was removed carefully. During this process, the solution became oily, and then formed a colorless dry foam, which could be cracked by a spatula. The crude was placed overnight in a desiccator over KOH under dynamic vacuum of a membrane pump. This yielded 7.88 g (98.4%) of a colorless crystalline compound.

In the case of a smaller reaction scale, the foam sometimes became oily or assumed the consistency of chewing gum if exposed to air. If this occurred, one could purify the compound in the following manner (for example, 10 mmol scale): dry ether (20 mL) are added to the compound. The flask is then placed in a super sonic bath for 30 minutes. After removal of the solvent and repetition of this procedure with pentane (20 mL), the solvent is removed completely, yielding a colorless crystalline compound. M.p. 130.0−132.0 °C (ref. 132.0−134.0 °C[7]). R_f = 0.65 in methanol. ^1H NMR (400 MHz, (D_6)DMSO): δ = 8.81 (br., Acm NH), 4.27 (d, Acm β), 4.25 (Cys α), 4.12 (br., NH_3^+), 3.73 (s, OCH_3), 3.33 (Cys β), 3.11 (Cys β), 1.84 (s, Acm CH_3) ppm. ^{13}C NMR (100 MHz, (D_6)DMSO): δ = 169.84 (CO), 168.69 (CO), 53.05 (OCH_3), 52.21 (Cα, Cys), 40.70 (Cβ, Acm), 30.34 (Cβ, Cys), 22.70 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 2933, 1751, 1654, 1504, 1441, 1376, 1326, 1244, 1090, 1005, 851 cm^{-1}. FAB-MS *m/z*: 207.1 (100) $[M - HCl + H]^+$. $C_7H_{15}ClN_2O_3S$ (242.7): calcd. C 34.6, H 6.2, N 11.5, S 13.2; found C 34.64, H 6.13, N 11.59, S 13.11.

Boc−Pro−Aib−Cys(Acm)−OMe (3). Procedure I: Compound **4** (2.48 g, 0.00825 mol) and DCCI (1.87 g, 0.00908 mol) were dissolved whilst stirring in DMF (20 mL) and cooled at −2 °C for 15 minutes. A precooled (−5 to 0 °C) solution of **10** (2.0 g, 0.00825 mol) and triethylamine (2.30 mL, 0.0165 mol) in DMF (15 mL) were added dropwise. The mixture was stirred for 2 hours at −2 °C and for a further 18 hours at 20 °C, after which it was stirred for 18 hours at room temperature. Precipitated dicyclohexylurea was filtered off, and the solvent was removed in vacuo. The residue was dissolved in chloroform (15 mL) and successively washed with HCl (0.1 N, 60 mL), $NaHCO_3$ (0.5 N, 60 mL), and water (60 mL). The organic layer was dried and the solvent removed. The crude product was purified by column chromatography. Elution with DCM/Et_2O (7:3 and 1:1) and ethyl acetate afforded pure colorless tripeptide **3** (2.26 g, 56.6%).

Procedure II: CDMT (3.86 g, 0.022 mmol) and **4** (6.79 g, 0.02244 mol) were dissolved, whilst stirring, in DCM (31 mL), and cooled at −5 °C for 15 minutes. NMM (4.95 mL, 0.04488 mol) was slowly added dropwise to the solution in such a manner that the reaction temperature did not rise above 0 °C. The solution was stirred for a further 4 hours at −1 °C until nearly all CDMT was consumed. A mixture of **10** (5.34 g, 0.022 mol) and NMM (4.85 mL, 0.044 mol) in DCM/THF/CH_3CN (15:15:25 mL) was added dropwise to the solution whilst stirring at −5 °C. Stirring was continued for 2.5 hours at 0 °C. The temperature was then raised to room temperature, and the mixture was stirred overnight. The solvent was evaporated, and the residue was taken up in ethyl acetate (90 mL) and successively washed with water (24 mL), 10% citric acid (26 mL), water (26 mL), saturated $NaHCO_3$ solution (24 mL), and water (24 mL). The organic layer was dried, and the solvent evaporated. This yielded 5.62 g (52.3%) of pure tripeptide. No further purification was necessary. M.p. 120.0 °C (dec.). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.31 (br., Acm NH), 7.22 (br., NH Cys), 6.71 (br., NH Aib), 4.67 (Cys α), 4.44/4.19 (Acm β), 4.19 (Pro α), 3.71 (s, OCH_3), 3.43 (Pro δ), 2.96 (Cys β), 2.19−1.86 (m, br., Pro β, γ), 1.99 (s, Acm CH_3), 1.50 (Aib CH_3), 1.44 (s, Boc CH_3) ppm. ^{13}C NMR

(100 MHz, $CDCl_3$): 173.97 (CO), 172.22 (CO), 170.96 (CO), 170.69 (CO), 156.85 (CO), 80.83 (qC Boc), 60.43 (Cα, Pro), 57.10 (Cα, Aib), 52.93 (Cα, Cys), 52.61 (OCH_3), 47.20 (Cδ, Pro), 41.53 (Cβ, Acm), 33.91 (Cβ, Pro), 32.98 (Cβ, Cys), 28.34 (Boc CH_3), 26.16/25.60 (Cβ, Aib), 24.92 (Cγ, Pro), 23.00 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 3315, 2982, 2937, 1748, 1672, 1539, 1389, 1252, 1167, 1125, 1030, 755 cm^{-1}. FAB-MS *m/z*: 511.3 (100) $[M + Na]^+$, 489.3 (41) $[M + H]^+$, 389.2 (40). $C_{21}H_{36}N_4O_7S$ (488.6): calcd. C 51.6, H 7.4, N 11.5, S 6.6; found C 51.29, H 7.20, N 11.31, S 6.28.

Boc−Cys(Trt)−Pro−Aib−Cys(Acm)−OMe (2): Boc−Cys(Trt)−OH (5.0 g, 0.0108 mol) , DCCI (2.92 g, 0.014 mol), and HOBt (1.90 g, 0.014 mol) were dissolved, whilst stirring, in DMF (52 mL) and cooled for 30 minutes at −20 °C. A solution of equivalent amounts of deprotected **3** (0.0108 mol) [see general procedure for deprotection] and NMM (4.84 mL, 0.046 mol) in DMF (82 mL) was precooled for 15 minutes in an ice bath. After 30 minutes the precooled solution was added. The mixture was stirred for 2 hours at −20 °C. The temperature was then raised to 23 °C and stirring was continued at this temperature for a further 20 hours. The mixture was allowed to stand at room temperature for 1 hour. The precipitated dicyclohexylurea was filtered off, and the solvent was removed in vacuo. The residue was taken up in EtOAc/5% $KHCO_3$ (165 mL, 1:1). Again the solution was filtered to remove further precipitated dicyclohexylurea. The filtrate was successively washed three times each with 5% $KHCO_3$ (82 mL), three times each with 5% $KHSO_4$ (82 mL), and once with saturated NaCl solution (82 mL). Drying of the combined organic layers, and removal of the solvent yielded a colorless powder, which was further purified by column chromatography. Elution with DCM/Et_2O (1:1), DCM/EtOAc (4:6), and ethyl acetate afforded pure colorless linear tetrapeptide **2** (5.2 g, 57.8%). M.p. 102.0 °C (dec.). ^1H NMR (600 MHz, (D_6)DMSO): δ = 8.42 (t, Acm NH), 7.85 (s, NH Aib), 7.61 [d, NH Cys(4)], 7.32−7.21 (m, Trt), 7.02 [d, NH Cys(1)], 4.34 [Cys α(4)], 4.25/4.15 (Acm β), 4.16 (Pro α), 3.99 [Cys α(1)], 3.59 (s, OCH_3), 3.20/2.78 (Pro δ), 2.97/2.82 [Cys β(4)], 2.52/2.34 [Cys β(1)], 1.94−1.71 (m, br., Pro β, γ), 1.84 (s, Acm CH_3), 1.34−1.28 (m, Boc CH_3, Aib CH_3) ppm. ^{13}C NMR (100 MHz, $[D_6]$DMSO): δ = 174.07 (CO), 171.06 (CO), 170.71 (CO), 169.60 (CO), 169.08 (CO), 155.23 (CO), 144.47 (Trt), 129.34 (Trt), 128.14 (Trt), 126.88 (Trt), 78.33 (qC Boc), 66.58 (qC Trt), 60.04 (Cα, Pro), 56.02 (Cα, Aib), 52.62 [Cα, Cys(4)], 52.47 [Cα, Cys(1)], 52.00 (OCH_3), 46.64 (Cδ, Pro), 40.63 (Cβ, Acm), 32.52 [Cβ, Cys(1)], 31.77 [Cβ, Cys(4)], 28.52 (Cβ, Pro), 28.23 (Boc CH_3), 25.50/24.53 (Cβ, Aib), 24.64 (Cγ, Pro), 22.67 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 3314, 3062, 2982, 1743, 1684, 1659, 1522, 1444, 1369, 1248, 1169, 1045, 938, 863, 745, 702, 676, 619 cm^{-1}. FAB-MS *m/z*: 856.1 (92) $[M + Na]^+$, 834.1 (12) $[M + H]^+$. $C_{43}H_{55}N_5O_8S_2$ (834.07): calcd. C 61.9, H 6.6, N 8.4, S 7.6; found C 60.62, H 6.30, N 7.90, S 7.90.

Cyclo[Boc−Cys−Pro−Aib−Cys−OMe] (1): See general procedure for cyclization. M.p. 113.0−115.0 °C. ^1H NMR (600 MHz, $[D_6]$DMSO): δ = 8.47 (s, NH Aib), 7.40 [d, NH Cys(4)], 7.22 [d, NH Cys(1)], 4.41 [Cys α(1)], 4.31 (Pro α), 4.14 [Cys α(4)], 3.64 (s, OCH_3), 3.55 (Pro δ), 3.46/2.58 [Cys β(1)], 3.18/3.10 [Cys β(4)], 2.10−1.83 (m, br., Pro β, γ), 1.39−1.29 (m, Boc CH_3, Aib CH_3) ppm. ^{13}C NMR (150 MHz, $[D_6]$DMSO): δ = 174.42 (CO), 171.38 (CO), 170.51 (CO), 168.75 (CO), 154.85 (CO), 78.90 (qC Boc), 60.15 (Cα, Pro), 55.98 (Cα, Aib), 53.54 [Cα, Cys(1)], 52.23 (OCH_3), 51.95 [Cα, Cys(4)], 46.92 (Cδ, Pro), 37.23 [Cβ, Cys(4)], 34.70 [Cβ, Cys(1)], 28.40 (Cβ, Pro), 28.15 (Boc CH_3), 24.85 (Cγ, Pro), 22.89 (Cβ, Aib) ppm. IR: $\tilde{\nu}$ = 3323, 2983, 2882, 1752, 1684, 1641, 1519, 1444, 1367, 1305, 1244, 1168, 1044, 1016, 933, 858, 773 cm^{-1}. FAB-MS *m/z*: 541.0 (22) $[M + Na]^+$, 519.1 (42) $[M + H]^+$, 463.0 (14), 441.0 (4), 419.0 (52). $C_{21}H_{34}N_4O_7S_2$ (518.64): calcd. C 48.6, H 6.6, N 10.8, S 12.4; found C 48.21, H 7.11, N 10.03, S 12.50.

Boc−Phe−Cys(Acm)−OMe (9): Dipeptide **9** was synthesized as described above (MA method). No further purification was necessary. The reaction yielded a colorless powder (12.37 g, 82.2%). M.p. 99.0−100.0 °C (ref. 102.0−103.0 °C[17]). ^1H NMR (200 MHz, $CDCl_3$): δ = 7.32−7.19 (m, Phe), 7.04 (d, NH Cys), 6.75 (br., Acm NH), 5.06 (d, NH Phe), 4.74 (Cys α), 4.43 (Phe α), 4.30 (Acm β), 3.72 (s, OCH_3), 3.19−2.81 (Cys β, Phe β), 2.01 (s, Acm CH_3), 1.38 (Boc CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.73 (CO), 170.55 (CO), 170.41 (CO), 152.98 (CO), 136.43 (Phe), 129.33 (Phe), 128.68 (Phe), 126.99 (Phe), 80.38 (qC Boc), 55.96 (Cα, Phe), 53.01 (Cα, Cys), 52.73 (OCH_3), 42.28 (Cβ, Acm), 38.04 (Cβ, Phe), 33.88 (Cβ, Cys), 28.24 (Boc CH_3), 23.18 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 3334, 3060, 2975, 1729, 1691, 1648, 1527, 1437, 1415, 1249, 1167, 1049, 953, 859, 756, 712, 698, 634 cm^{-1}. FAB-MS *m/z*: 476.1 (52) $[M + Na]^+$, 454.2 (31) $[M + H]^+$. $C_{21}H_{31}N_3O_6S$ (453.5): calcd. C 55.6, H 6.8, N 9.3, S 7.1; found C 55.72, H 7.36, N 9.14, S 6.91.

Boc−Pro−Phe−Cys(Acm)−OMe (8): Tripeptide **8** was synthesized as described above (MA method). The residue was taken up in dry ether (approximately 250 mL) and refluxed for 20 minutes. This afforded a colorless powder, which was removed by suction filtration yielding 13.78 g (75.9%) of pure tripeptide. M.p. 113.0 °C (dec.). ^1H NMR (400 MHz, $[D_6]$DMSO): δ = 8.53 (d, Cys NH), 8.48 (t, Acm NH), 7.80 (d, Phe NH), 7.19 (m, Phe), 4.66 (Phe α), 4.50 (Cys α), 4.22 (Acm β), 4.04 (Pro α), 3.62 (s, OCH_3), 3.25 (Pro δ), 3.03−2.77 (Cys β, Phe β), 2.01−1.64 (m, br., Pro β, γ), 1.84 (s, Acm CH_3), 1.38−1.16 (Boc CH_3) ppm. ^{13}C NMR (100 MHz, $[D_6]$DMSO): δ = 172.26 (CO), 171.43 (CO), 170.90 (CO), 169.48 (CO), 153.38 (CO), 137.67 (Phe), 129.13 (Phe), 127.98 (Phe), 126.25 (Phe), 78.41 (qC Boc), 59.55 (Cα, Pro), 53.56 (Cα, Phe), 52.41 (Cα, Cys), 52.06 (OCH_3), 46.45 (Cδ, Pro), 40.51 (Cβ, Acm), 37.57 (Cβ, Cys), 30.77 (Cβ, Phe), 29.40 (Cβ, Pro), 27.85 (Boc CH_3), 22.88 (Cγ, Pro), 22.55 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 3296, 3064, 2974, 1742, 1655, 1540, 1395, 1306, 1256, 1162, 1123, 1029, 845, 749, 702 cm^{-1}. FAB-MS *m/z*: 573.2 (59) $[M + Na]^+$, 551.2 (30) $[M + H]^+$. $C_{26}H_{38}N_4O_7S$ (550.68): calcd. C 56.7, H 6.9, N 10.2, S 5.8; found C 56.41, H 6.54, N 10.00, S 5.43.

Boc−Cys(Trt)−Pro−Phe−Cys(Acm)−OMe (7): Linear tetrapeptide **7** was synthesized as described above (MA method). The residue was purified by column chromatography. Elution with DCM/Et_2O (1:1) and DCM/EtOAc (4:6) afforded pure colorless linear tetrapeptide **7** (20.70 g, 70.0%). M.p. 115.0 °C (dec.). ^1H NMR (600 MHz, $[D_6]$DMSO): δ = 8.42 (t, Acm NH), 8.30 (d, Phe NH), 7.63 [d, Cys NH(4)], 7.33−7.15 (m, Phe, Trt), 7.05 [d, Cys NH(1)], 4.51 [Cys α(4)], 4.47 [Cys α(1)], 4.46 (Phe α), 4.22 (Acm β), 4.20 (Pro α), 3.62 (s, OCH_3), 3.19/2.73 (Pro δ), 2.97/2.74 [Cys β(4)], 2.83 (Phe β), 2.52/2.34 [Cys β(1)], 1.93−1.59 (m, br., Pro β, γ), 1.83 (s, Acm CH_3), 1.35 (Boc CH_3) ppm. ^{13}C NMR (150 MHz, $[D_6]$DMSO): δ = 170.72 (CO), 170.70 (CO), 170.20 (CO), 169.40 (CO), 168.87 (CO), 155.05 (CO), 144.34 (Trt), 137.37 (Phe), 129.17 (Trt), 129.09 (Phe), 127.94 (Trt), 127.89 (Phe), 126.67 (Trt), 126.17 (Phe), 78.19 (qC Boc), 66.40 (qC Trt), 59.65 (Cα, Pro), 59.38 [Cα, Cys(4)], 53.49 (Cα, Phe), 52.36 [Cα, Cys(1)], 51.93 (OCH_3), 46.26 (Cδ, Pro), 40.56 (Cβ, Acm), 37.41 [Cβ, Cys(4)], 32.76 [Cβ, Cys(1)], 31.60 (Cβ, Phe), 28.48 (Cβ, Pro), 28.10 (Boc CH_3), 24.06 (Cγ, Pro), 22.49 (Acm CH_3) ppm. IR: $\tilde{\nu}$ = 3303, 3058, 2975, 1656, 1522, 1442, 1367, 1247, 1168, 1044, 856, 744, 702, 620 cm^{-1}. FAB-MS *m/z*: 918.3 (55) $[M + Na]^+$, 243.1 (100) $[Trt + H]^+$. $C_{48}H_{57}N_5O_8S_2$ (896.15): calcd. C 64.3, H 6.4, N 7.8, S 7.2; found C 63.65, H 6.75, N 7.34, S 6.73.

Cyclo[Boc−Cys−Pro−Phe−Cys−OMe] (6): See general procedure for cyclization. M.p. 85.0 °C (dec.). ^{1}H NMR (600 MHz, $CDCl_3$): δ = ca. 7.20 (m, Phe), 6.85 [d, Cys NH(4)], 6.13 (d, Phe NH), 5.19 [d, Cys NH(1)], 4.94 (Phe α), 4.85 [Cys α(4)], 4.48 [Cys α(1)], 4.43 (Pro α), 3.71 (s, OCH_3), 3.67/3.39 (Pro δ), 3.45/3.06 [Cys β(1)], 3.28/3.19 (Phe β), 3.16/3.02 [Cys β(4)] 2.19−1.94 (m, br., Pro β, γ), 1.40 (Boc CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 172.33 (CO), 170.10 (CO), 169.91 (CO), 169.73 (CO), 154.62 (CO), 137.89 (Phe), 129.66 (Phe), 128.69 (Phe), 127.29 (Phe), 80.93 (qC Boc), 61.96 (Cα, Pro), 55.66 [Cα, Cys(4)], 52.73 (OCH_3), 52.53 (Cα, Phe), 51.11 [Cα, Cys(1)], 47.77 (Cδ, Pro), about 40.00 [Cβ, Cys(1), Cys(4)], 36.17 (Cβ, Phe), 29.32 (Cβ, Pro), 28.26 (Boc CH_3), 24.86 (Cγ, Pro) ppm. IR: $\tilde{\nu}$ = 3325, 3067, 3034, 2982, 1747, 1676, 1520, 1439, 1369, 1304, 1248, 1167, 1046, 1024, 919, 866, 749, 702, 618 cm^{-1}. FAB-MS *m/z*: 603.2 (55) $[M + Na]^+$, 581.2 (30) $[M + H]^+$. $C_{26}H_{36}N_4O_7S_2$ (580.73): calcd. C 53.8, H 6.3, N 9.7, S 11.0; found C 51.46, H 6.27, N 8.46, S 11.11.

Acknowledgments

The authors thank Prof. M. Feigel for his valuable cooperation and fruitful discussions. This work was financially supported by the Deutsche Forschungsgemeinschaft (SFB 452) and the Fonds der Chemischen Industrie.

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Received March 16, 2004

