Highly Constrained Linear Oligopeptides Containing Heterocyclic α-Amino Carboxylic Acids

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Dedicated to Professor *John A. Robinson* on the occasion of his 60th birthday

Two spiroheterocyclic 2*H*-azirin-3-amines, **1f** and **1g**, were shown to be useful synthons for the dipeptides *N*-(4-aminotetrahydro-2*H*-pyran-4-yl)prolinate (Thp-Pro) and the corresponding thiopyran derivative, Tht-Pro, respectively. By coupling of 4-bromobenzoic acid with **1f** or **1g** and saponification, followed by repeating the coupling and saponification steps, oligopeptides of type 4-BrBz-(Thp-Pro)$_n$-OMe and 4-BrBz-(Tht-Pro)$_n$-OMe were prepared, and their conformations were evaluated in solution by NMR techniques and in the crystalline state by X-ray crystallography. All of these sterically highly congested oligopeptides adopt fairly rigid helical conformations. It is interesting to note that the hexapeptide with Thp forms a 3_{10}-helix, whereas the Tht analog has a β-bend ribbon spiral confirmation.

1. Introduction. – In the last few decades, it has been shown that 2*H*-azirin-3-amines (= 3-amino-2*H*-azirines) of type **1** (*Fig. 1*) can be successfully used in peptide synthesis as synthons for α,α-disubstituted α-amino acids (=2,2-disubstituted glycines) [2]. The reaction sequence for the introduction of such amino acids into peptides, *i.e.*, the azirine coupling with *N*-protected amino acids or peptides, followed by selective hydrolysis of the terminal amide function and coupling with the next amino component, is called the 'azirine/oxazolone method', and has been used for the preparation of peptaibols and peptaibol segments [3]. Thus, the reaction of a peptide acid with the Aib synthon **1a** leads to a peptide amide with a backbone extended by a 2-methylalanine (α-aminoisobutyric acid = 2-amino-2-methylpropanoic acid; Aib) moiety. After the selective acid-catalyzed hydrolysis of the terminal amide bond, the extended peptide acid is obtained, which can then be used for a further 'azirine coupling' or for segment condensation. This strategy has been employed extensively in the synthesis of linear oligopeptides [3][4], endothiopeptides [5], cyclic peptides [6], and cyclic depsipeptides [7] containing 2,2-disubstituted gycines, in particular Aib. It has also been shown that the 'azirine/oxazolone method' can be used under solid-phase conditions [8].

Furthermore, heterospirocyclic 2*H*-azirin-3-amines of type **1b** and **1c** were prepared and used as synthons for heterocyclic α-amino acids [9]. Incorporated in tripeptides of the type Z-Aib-Xaa-Aib-N(Ph)Me [9b], Z-Phe-Xaa-Val-OMe, and H-Asp-D-Ala-

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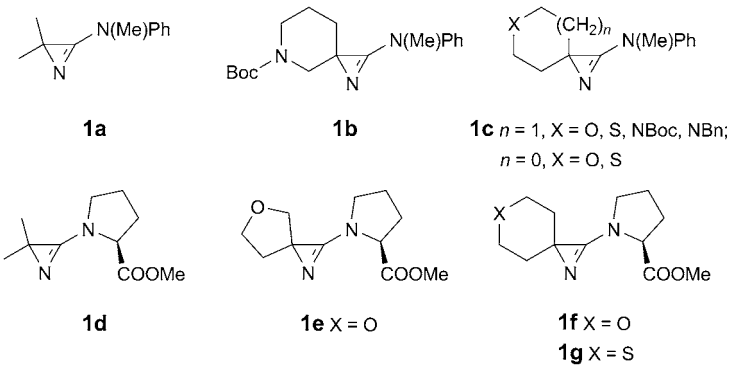


Fig. 1. *2H-Azirin-3-amines as synthons for Aib, heterocyclic α-amino carboxylic acids, Aib-Pro, and heterocyclic dipeptides*

Xaa-OMe [10], they behave like Aib and some other α,α-disubstituted α-amino acids that are known to stabilize secondary structures such as β-turns and helical conformations [11].

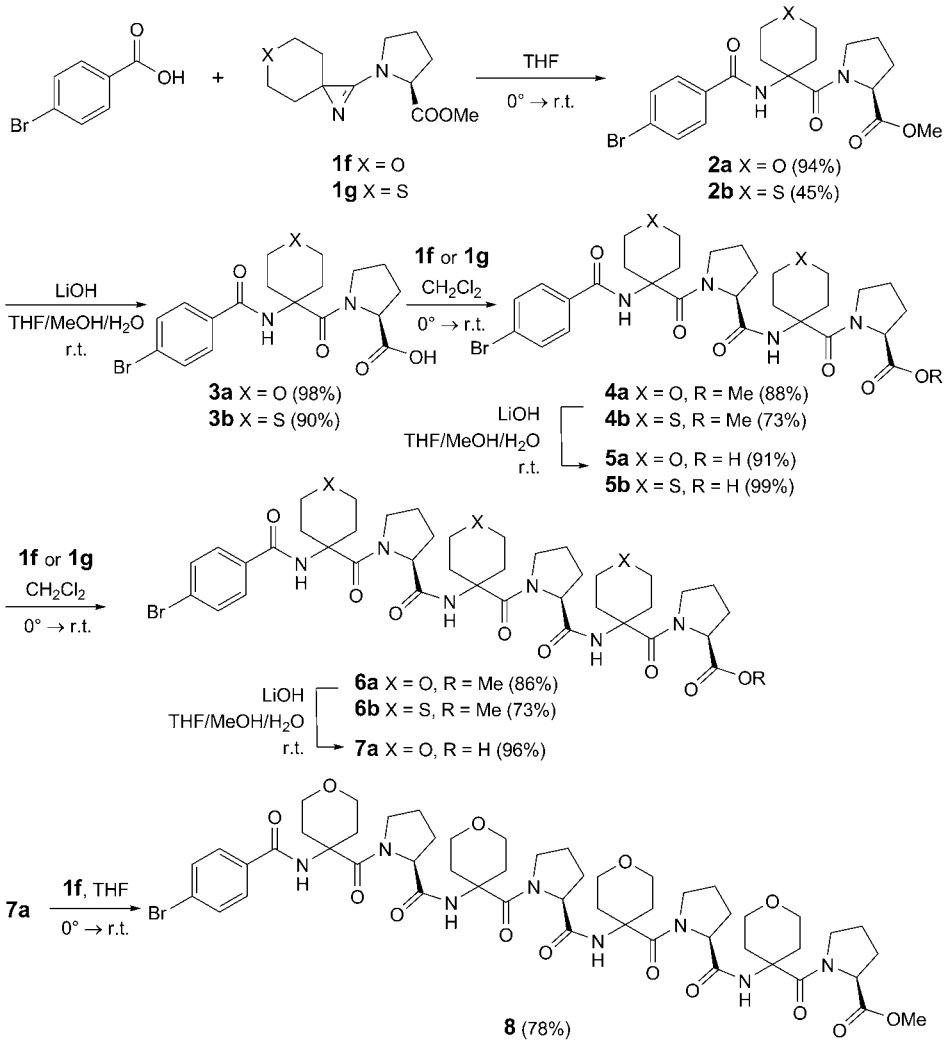
The first representative of a novel type of 2*H*-azirin-3-amines, namely methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**1d**), has been found to be suitable as a dipeptide synthon for the sequence Aib-Pro [3c][4b] (for analogs, see [12]). This building block has been used to prepare peptaibol antibiotics *Trichovirin I 1B* [3c], *Zervamicin II-2* [3d], and *Hypomurocins A1*, *A3*, and *A5* [3e][3g], as well as in the synthesis of endothiopeptides with a C-terminal Aib-Pro unit [5a–5c][5e]. Furthermore, repeated coupling of benzoic acids with **1d** led to Aib-Pro oligopeptides [13]. We also reported the synthesis of methyl *N*-(1-aza-5-oxaspiro[2.4]oct-1-en-2-yl)-L-prolinate (**1e**) [9c], its homolog **1f** [14], and the S-analog **1g** [15] as examples of heterospirocyclic *N*-(2*H*-azirin-3-yl)-L-prolinates. It has been shown that these novel amino azirines can be used as dipeptide synthons, *e.g.*, in the case of **1f**, for the dipeptide *N*-[(4-aminotetrahydro-2*H*-pyran-4-yl)carbonyl]-L-prolinate (Thp-Pro). As a model, the nonapeptide Z-Ser(ᵗBu)-Val-Thp-Pro-Aib-Leu-Thp-Pro-Leuol has been prepared by a combination of the 'azirine/oxazolone method' and segment coupling with HATU/HOBt [14].

Here, we report on the use of the amino-azirines **1f** and **1g** for the synthesis of the sterically constrained oligopeptides 4-Br-C_6H_4CO-(Thp-Pro)$_n$-OMe and 4-Br-C_6H_4CO-(Tht-Pro)$_n$-OMe by the 'azirine coupling', and the conformations of these oligopeptides in the solid state and in solution were determined. It is well-known [16] that peptides, which consist of consecutive (Aib-Pro) units, adopt a helical structure called a β-bend ribbon, similar to a sequence of type-III β-turn conformations. This finding was confirmed by our recently published results [13]. It was of interest to establish whether the heteroatoms O and S in the heterocyclic α-amino acids Thp (homoserine analog) and Tht (homocysteine analog), respectively, have an influence on the folding of the peptide backbone.

2. Results and Discussion. – 2.1. *Synthesis of Oligopeptides 4-Br–C_6H_4CO-(Thp-Pro)$_n$-OMe and 4-Br–C_6H_4CO-(Tht-Pro)$_n$-OMe.* The dipeptide synthon **1f** was

coupled with 4-bromobenzoic acid (4-Br-C_6H_4COOH) under standard conditions (*Scheme*): to a solution of 4-BrC_6H_4COOH (1 mol-equiv.) in THF at 0°, a solution of **1f** (1.1 mol-equiv.) was added. The mixture was stirred for 45 h at room temperature (TLC control). Chromatographic purification gave the dipeptide derivative 4-Br-C_6H_4CO-Thp-Pro-OMe (**2a**) in excellent yield (94%) as colorless crystals. Saponification of the ester was performed with $LiOH \cdot H_2O$ (4 mol-equiv.) in THF/MeOH/H_2O (3:1:1) at room temperature, which furnished the corresponding dipeptide acid **3a** in 98% yield. This product was sufficiently pure to be used in the next coupling reaction with the building block **1f**.

Scheme



After the addition of **1f** (1.1 mol-equiv.) to a solution of **3a** in THF at 0°, the mixture was stirred for 47 h at room temperature, and the chromatographic purification of the crude product gave 4-Br-C_6H_4CO-(Thp-Pro)$_2$-OMe (**4a**) in 88% yield as colorless crystals. Hydrolysis of the latter with LiOH · H_2O (4 mol-equiv.) in THF/MeOH/H_2O (3 : 1 : 1) led to the corresponding tetrapeptide acid **5a** in 91% yield, which was then used in a third coupling reaction with **1f** to afford the desired hexapeptide derivative **6a**. After column chromatography, 4-Br-C_6H_4CO-(Thp-Pro)$_3$-OMe (**6a**), containing three consecutive (Thp-Pro) units, was obtained in 86% yield. Its structure was established on the basis of the spectroscopic data and by an X-ray crystal-structure determination (*Fig. 2*, see *Chapt. 2.2.*). Saponification of **6a** with LiOH · H_2O (4 mol-equiv.) in THF/MeOH/H_2O (3 : 1 : 1) gave the corresponding hexapeptide acid **7a** in 96% yield, which was used directly in the next coupling reaction to give the octapeptide 4-Br-C_6H_4CO-(Thp-Pro)$_4$-OMe (**8**). The peptide methyl ester **8**, which consists of four consecutive Thp-Pro units, was obtained in 78% yield.

The synthesis of the hexapeptide 4-Br-C_6H_4CO-(Tht-Pro)$_3$-OMe (**6b**) was performed in analogy to that of **6a** (*Scheme*). The coupling of **1g** with 4-Br-C_6H_4COOH gave **2b** (45%), saponification of which with LiOH · H_2O (3 mol-equiv.) under the usual conditions led to the dipeptide acid **3b** in 90% yield, which was then coupled with 2 mol-equiv. of **1g** (120 h, room temperature) to give the tetrapeptide **4b** in 73% yield after chromatographic purification. Hydrolysis of **4b** with LiOH · H_2O (3 mol-equiv.) furnished the tetrapeptide acid **5b** in 99% yield. The last coupling reaction with **1g** was again carried out in THF (116 h, room temperature). The product **6b** gave, after column chromatography, the hexapeptide 4-Br-C_6H_4CO-(Ttp-Pro)$_3$-OMe in 73% yield. The structure of the latter was also established by X-ray crystallography (*Fig. 3*).

2.2. *Conformational Studies.* 2.2.1. *Hexapeptide Ester* **6a** *and Octapeptide Ester* **8**. Suitable crystals for the X-ray crystal-structure determination of 4-Br-C_6H_4CO-(Thp-Pro)$_3$-OMe (**6a**) were obtained from $CHCl_3$/hexane by slow evaporation of the solvent. The molecular structure is depicted in *Fig. 2*. There are two symmetry-independent molecules (A and B) of **6a**, and five $CHCl_3$ and three H_2O molecules in the asymmetric unit. Both peptide molecules are of the same stereoisomer and have similar helical conformations. The crystals are enantiomerically pure, and the absolute configuration of the molecule has been determined independently by the diffraction experiment as (3*S*,9*S*,15*S*). One five-membered ring in each of molecules A and B is disordered over two envelope conformations, where the atoms C(39) and C(91), respectively, occupy two alternate positions, which correspond to the envelope flap lying on opposite sides of the ring plane. The major conformation occurs in *ca.* 55% of the type A molecules and 53% of those of type B. Additionally, the Thp ring at C(78) in molecule B is disordered over two chair conformations, where the O-atom and its two neighboring C-atoms in the ring occupy two alternate positions, which correspond with inversion of the chair. The major conformation occurs in *ca.* 79% of the type B molecules. Three of the five $CHCl_3$ and all H_2O molecules are disordered over several orientations and were omitted from the model (see *Exper. Part*).

Amide groups in both symmetry-independent peptide molecules act as donors for H-bonds (*Table 1*). For each molecule, two of the interactions are intramolecular H-bonds, which form a regular pattern along the peptide chain and serve to maintain a fairly rigid helical conformation of the peptide. The most relevant torsion angles for the

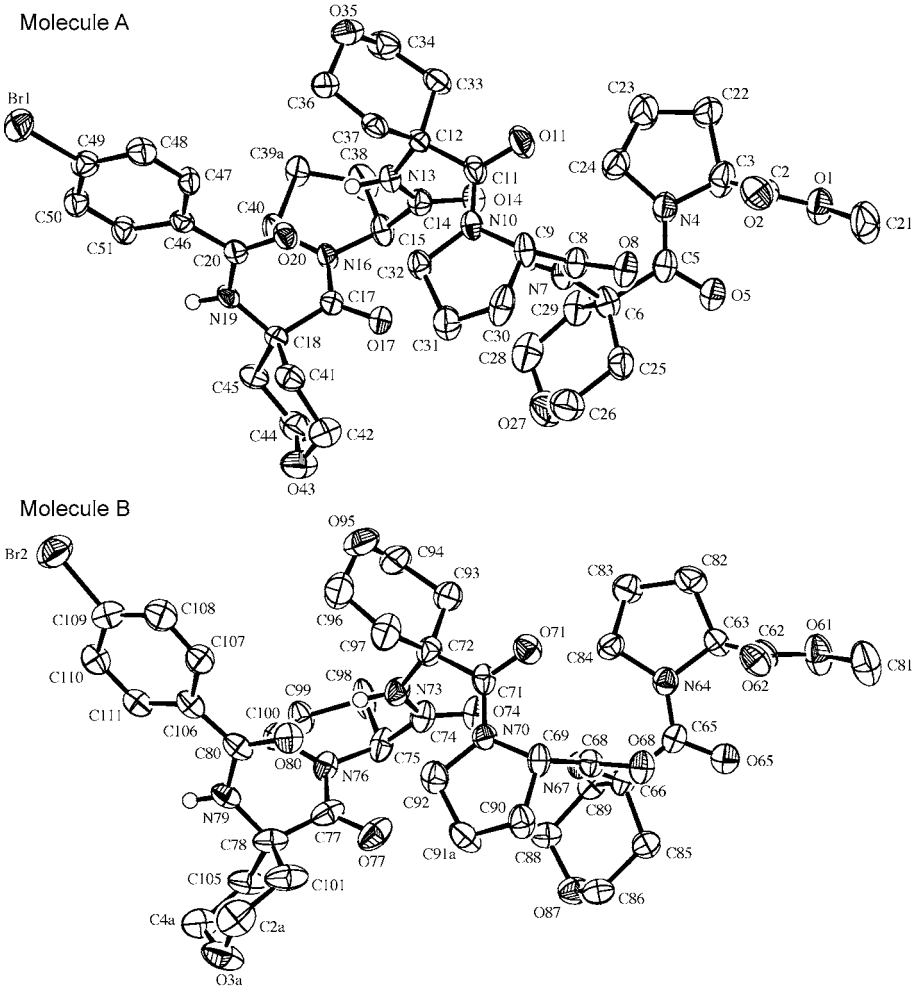


Fig. 2. *ORTEP Plot* [17] *of the molecular structures of the symmetry-independent molecules A and B of* **6a** (disorder conformations a; with 50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms and solvent molecules omitted for clarity)

peptide backbone of the two molecules A and B are compiled in *Table 2*. Structures of both molecules are stabilized by two $1 \leftarrow 4$ intramolecular H-bonds between the NH groups of the Thp^3 and Thp^5 residues, and the C=O groups of the 4-Br-C_6H_4CO group and of the Pro^2 residue, respectively. For instance, in molecule A, N(7)–H and N(13)–H interact with the amide O(14)- and O(20)-atoms, respectively, that are seven atoms further along the peptide backbone. Each of these interactions has the graph set motif [18] of S(10). Molecule B has an identical pattern of intramolecular interactions. In molecule A, N(19)–H, which is unable to form an intramolecular interaction because of its position in the backbone, forms bifurcated intermolecular H-bonds with

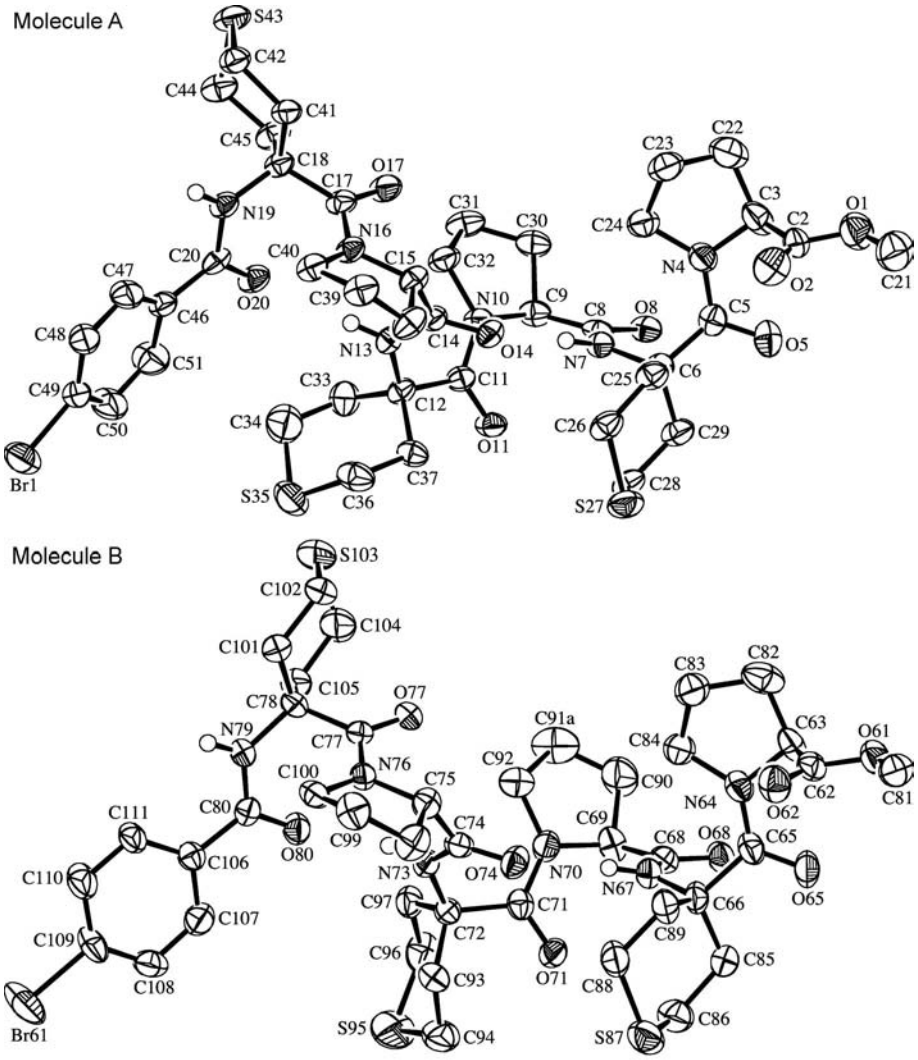


Fig. 3. *ORTEP Plot* [17] *of the molecular structures of the symmetry-independent molecules A and B of* **6b** (disorder conformation a of molecule *B*; with 50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms and solvent molecules omitted for clarity)

the ester carbonyl O(62)-atom and the amide O(68)-atom near the middle five-membered ring of an adjacent molecule B. In turn, molecule B forms the identical type of bifurcated intermolecular H-bonds with a further molecule A. These interactions link both of the symmetry-independent peptide molecules into extended $\cdots A \cdots B \cdots A \cdots B \cdots$ chains which run parallel to the [110] direction. The binary graph set motifs for the intermolecular H-bonds are $C_2^2(40)$ and $C_2^2(28)$ for the interaction with the ester and amide O-atoms, respectively. Due to the omission of the H_2O molecules from the

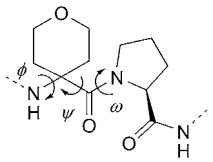
model, the involvement of these moieties in the H-bonding interactions could not be assessed.

Table 1. *Intra- and Intermolecular H-Bonds in* **6a**

D–H ··· A[a])	D–H [Å]	H ··· A [Å]	D ··· A [Å]	D–H ··· A [°]
N(7)–H(7) ··· O(14)	0.88	2.35	3.140(5)	149
N(13)–H(13) ··· O(20)	0.88	2.22	2.982(4)	145
N(19)–H(19) ··· O(68′)	0.88	2.34	3.090(4)	144
N(19)–H(19) ··· O(62′)	0.88	2.49	2.995(4)	117
N(67)–H(67) ··· O(74)	0.88	2.21	3.033(4)	156
N(73)–H(73) ··· O(80)	0.88	2.16	2.980(5)	156
N(79)–H(79) ··· O(8)	0.88	2.46	3.195(5)	142
N(79)–H(79) ··· O(2)	0.88	2.43	3.014(5)	124

[a]) Primed atoms refer to the molecule in the symmetry-related position $-1+x, -1+y, z$.

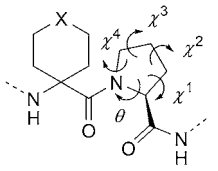
Table 2. *Selected Main-Chain Torsion Angles* [°] *of the Two Symmetry-Independent Molecules of* **6a**



Molecule **A**	Thp(1)	Pro(2)	Thp(3)	Pro(4)	Thp(5)	Pro(6)
ϕ	– 51.0(5)	– 64.0(5)	– 53.5(5)	– 71.3(5)	– 61.4(5)	– 55.2(7)
ψ	– 44.8(5)	– 31.8(5)	– 48.6(5)	– 27.8(6)	– 43.5(5)	– 45.0(7)
ω	– 168.5(3)	175.5(3)	– 167.5(4)	– 174.9(4)	– 162.7(4)	– 174.1(4)
Molecule **B**	Thp(1′)	Pro(2′)	Thp(3′)	Pro(4′)	Thp(5′)	Pro(6′)
ϕ	– 49.5(5)	– 69.3(5)	– 59.5(5)	– 68.7(5)	– 64.0(5)	– 54.8(5)
ψ	– 37.1(5)	– 26.7(6)	– 34.5(5)	– 23.0(6)	– 45.5(5)	– 44.9(6)
ω	179.5(3)	– 176.4(4)	– 166.0(4)	179.0(4)	– 166.2(4)	– 175.3(3)

The possibility of a relationship between the conformation adopted by a peptide molecule and the puckering of the pyrrolidine ring was also considered [19a]. The pyrrolidine ring of proline residues has two preferred conformations that are energetically equivalent [19b]. The four-atom fragment C(δ)–N–C(α)–C(β) is essentially planar, and the two conformations are distinguished by the direction in which the C(γ)-atom deviates from this plane. In the C(γ)-*exo* conformation, the C(γ)-atom and the C=O group are on opposite sides of the plane. The conformation is characterized by negative side-chain torsion angles, χ^1 and χ^3, of the pyrrolidine ring and positive ones for χ^2 and χ^4 (*Fig. 4*). In the C(γ)-*endo* conformation, the C(γ)-atom and the C=O group are on the same side of the above mentioned plane, and the signs of the side-chain torsion angles of the pyrrolidine ring are reversed. Intramolecularly H-

bonded conformations of $(Aib\text{-}Pro)_n$ sequences have been analyzed theoretically, and the obtained results revealed that the pyrrolidine ring puckering has a pronounced effect on the energies. Only C(γ)-*endo*-puckered Pro residues can be accommodated in $1 \leftarrow 4$ H-bonded regular structures [19c]. On the other hand, the experimental results obtained by *Toniolo et al.* have shown that both, C(γ)-*endo* and C(γ)-*exo* pyrrolidine ring puckerings, are observed in the heptapeptide 4-Br-C_6H_4CO-Aib-$(Aib\text{-}Pro)_3$-OMe and in the nonapeptide 4-Br-C_6H_4CO-Aib-$(Aib\text{-}Pro)_4$-OMe, which adopt a β-bend-ribbon structure [16e]. The same result was obtained in our recent study on ArCO-$(Aib\text{-}Pro)_n$-X peptides [13]. In the case of the hexapeptide ester 4-Br-C_6H_4CO-$(Thp\text{-}Pro)_3$-OMe (**6a**), C(γ)-*endo* and C(γ)-*exo* pyrrolidine ring conformations are observed in both molecules A and B.



6a X = O
6b X = S

Fig. 4. *Side-chain torsion angles of the Pro residue*

Unfortunately, no suitable crystals for X-ray crystallography could be obtained from the octapeptide **8**.

The conformations of the two peptides **6a** and **8** in solution were studied by NMR spectroscopy. An easily obtainable information is the dependence of the NH shifts on the polarity of the solvent or on the temperature: NH groups involved in intramolecular H-bonds show a very low dependence, whereas the chemical shifts of solvent-exposed NH groups are influenced more significantly [20] (see also [9b][13][21]). Therefore, for the two peptides, **6a** and **8**, the involvement of the NH groups in intramolecular H-bonds was evaluated on the basis of the solvent dependence of δ(NH) in $CDCl_3$/(D_6)DMSO (solvent-titration experiment [22]), and the temperature dependence was determined in the range of 265–314 K. The assignments of all ^{1}H- and ^{13}C-NMR signals were accomplished by means of HSQC and HMBC techniques. As shown in *Figs. 5* and *6* for the hexapeptide **6a** and the octapeptide **8**, two and three NH groups, respectively, are nearly unaffected by an increase of the proportion of (D_6)DMSO in the range of 0–12% and by varying the temperature. We presume that these signals emanate from two and three amide NH groups, respectively, which are involved in intramolecular H-bonds, forming two β-turns (as in the crystalline state of **6a**), and three β-turns in the case of **8**. In contrast, $\Delta\delta$ values for the benzamide NH groups in the solvent titration experiment are 0.85 and 0.689 ppm for **6a** and **8**, respectively, which are typical values for solvent-exposed NH groups. These values correspond to the temperature coefficients $\Delta\delta/\Delta T$, which are -12.79×10^{-3} and -9.06×10^{-3} ppm/K for **6a** and **8**, respectively (*Table 3*).

2.2.2. *Hexapeptide Ester* **6b**. Suitable crystals of 4-Br-C_6H_4CO-$(Tht\text{-}Pro)_3$-OMe (**6b**) for the X-ray crystal-structure determination were obtained from CH_2Cl_2/MeOH/hexane by slow evaporation of the solvent. The molecular structure is shown in *Fig. 3*. The compound in the crystal is enantiomerically pure, and the absolute configuration of

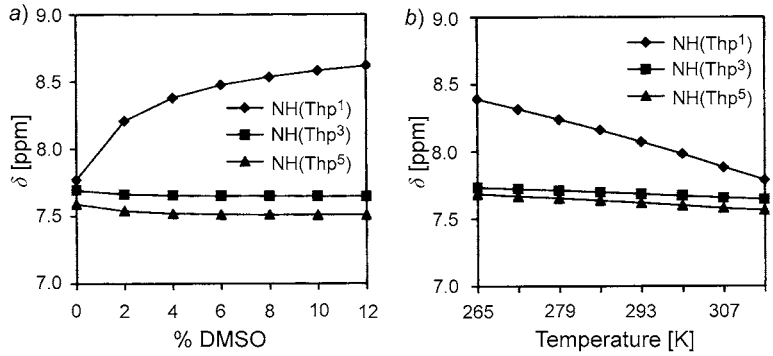


Fig. 5. *Dependence of the chemical shifts of the NH resonances of* **6a** *as a function of* a) *the* (D_6)*DMSO concentration* (% *v*/*v*) *in* $CDCl_3$ *and* b) *of the temperature in the range of 265–314 K*

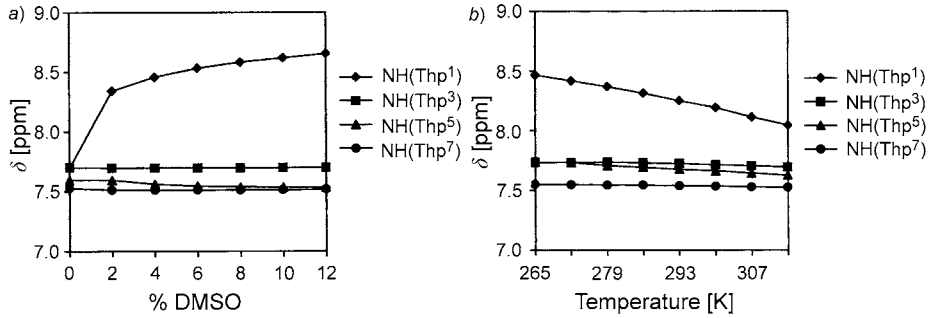


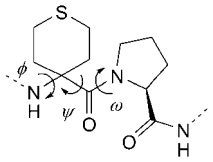
Fig. 6. *Dependence of the chemical shifts of the NH resonances of* **8** *as a function of* a) *the* (D_6)*DMSO concentration* (% *v*/*v*) *in* $CDCl_3$ *and* b) *of the temperature in the range of 265–314 K*

Table 3. *Temperature Coefficients of the Amide NH of the Peptides* **6a** *and* **8** *in the Range of 265–314 K*

Peptide	$-\Delta\delta/\Delta T$ [ppm/K]			
	NH(Thp(1))	NH(Thp(3))	NH(Thp(5))	NH(Thp(7))
6a	-12.79×10^{-3}	-1.92×10^{-3}	-2.62×10^{-3}	–
8	-9.06×10^{-3}	-1.13×10^{-3}	-2.31×10^{-3}	-0.6×10^{-3}

the molecule has been determined independently by the diffraction experiment. The molecule has the expected (3*S*,9*S*,15*S*)-configuration. The asymmetric unit contains two symmetry-independent peptide molecules, A and B, two fully occupied sites for MeOH molecules, and one additional MeOH site, which is only 43% occupied. The two independent peptide molecules have similar backbone conformations; the most relevant main-chain torsion angles for molecules A and B are compiled in *Table 4*. The main differences between the molecules are reversed chair puckering in two of the heterocyclic six-membered rings (rings containing S(35)/S(95) and S(43)/(S103)), plus

Table 4. *Selected Main-Chain Torsion Angles* [°] *of the Two Symmetry-Independent Molecules of* **6b**



Molecule A	Tht(1)	Pro(2)	Tht(3)	Pro(4)	Tht(5)	Pro(6)
ϕ	– 48.3(5)	– 67.3(5)	– 51.5(5)	– 69.0(5)	58.3(5)	– 60.2(6)
ψ	– 37.0(6)	– 20.4(6)	– 33.6(6)	– 4.5(6)	45.7(5)	148.7(4)
ω	+ 174.1(4)	– 173.7(4)	– 163.3(4)	– 177.7(4)	173.1(4)	161.8(4)
Molecule B	Tht(1′)	Pro(2′)	Tht(3′)	Pro(4′)	Tht(5′)	Pro(6′)
ϕ	– 51.9(6)	– 66.0(5)	– 40.5(6)	– 71.4(6)	61.7(6)	– 63.3(6)
ψ	– 35.3(6)	– 9.8(6)	– 47.0(6)	– 0.6(7)	43.3(6)	147.4(4)
ω	– 179.3(3)	178.0(4)	– 170.1(4)	– 171.5(4)	170.6(4)	167.8(4)

some small conformational differences in some of the five-membered rings and a slight pivot of the bromophenyl ring about its axis (23°). In peptide molecule A, both C(γ)-*endo* and C(γ)-*exo* pyrrolidine ring puckerings are observed (*Fig. 4*). The first five-membered ring has a half-chair conformation twisted on C(22)–C(23), the middle five-membered ring has an envelope conformation with C(31) as the envelope flap, and the third five-membered ring has a half-chair conformation twisted on C(38)–C(39), but distorted towards an envelope conformation. In peptide molecule B, the first five-membered ring has a similar half-chair conformation, twisted on C(82)–C(83), but distorted towards an envelope conformation. The middle five-membered ring is disordered over two conformations resulting from alternate flipping of the position of one C-atom in the ring. This ring has a half-chair conformation twisted on C(91a)–C(92) for the major conformation (60%) and twisted on C(90)–C(91b) for the minor conformation. The third five-membered ring in molecule B has an envelope conformation with C(99) as the envelope flap.

Each peptide molecule **6b** forms two intramolecular H-bonds, plus one intermolecular H-bond, which links the peptide molecules into extended chains (*Table 5*). Each peptide molecule also accepts one H-bond from a MeOH molecule. In peptide molecule A, the NH groups of the Tht^3 and Tht^5 residues, *i.e.*, N(7)–H and N(13)–H, form $1 \leftarrow 4$ intramolecular H-bonds with the amide O-atoms O(14) of Pro^2 and O(20) of the 4-Br-C_6H_4CO group, respectively, that are seven atoms further along the peptide backbone. Each of these interactions has the graph set motif of S(10). This serves to maintain a fairly rigid helical conformation of the peptide. The NH group of Tht^1, N(19)–H, which is unable to form an intramolecular interaction because of its position in the backbone, forms an intermolecular H-bond with the amide O(8′)-atom near the middle of a neighboring peptide molecule A. These interactions link the A molecules into extended chains which run parallel to the *y*-axis and have a graph set motif of C(14). Peptide molecule A also accepts an intermolecular H-bond from the OH group

Table 5. *Intra- and Intermolecular H-Bonds in* **6b**

D–H···A[a])	D–H [Å]	H···A [Å]	D···A [Å]	D–H···A [°]
N(7)–H(7)···O(14)	0.88	2.12	2.999(4)	173
N(13)–H(13)···O(20)	0.88	2.25	3.128(5)	173
N(19)–H(19)···O(8′)	0.88	2.21	3.056(4)	161
N(67)–H(67)···O(74)	0.88	2.11	2.988(5)	174
N(73)–H(73)···O(80)	0.88	2.03	2.889(5)	163
N(79)–H(79)···O(68′)	0.88	2.08	2.933(5)	165
O(112)–H(112)···O(5′)	0.84	2.10	2.834(7)	146
O(114)–H(114)···O(65)	0.84	1.99	2.734(7)	148

[a]) Primed atoms refer to the molecule in the symmetry-related position $x, -1+y, z$.

of one of the fully occupied MeOH molecules. Peptide molecule B is involved in an identical pattern of intra- and intermolecular H-bonds, so that, overall, two independent parallel chains of molecules run through the structure.

A hint about the conformation of **6b** in solution was obtained from ^{1}H-NMR spectroscopy. The involvement of the NH groups in intramolecular H-bonds was evaluated on the basis of the solvent dependence of δ(NH) values in $CDCl_3$/(D_6)DMSO (solvent-titration experiment); the assignments of all ^{1}H- and ^{13}C-NMR signals were carried out by using HSQC and HMBC techniques. As shown in *Fig. 7*, two NH groups of the hexapeptide **6a** are nearly unaffected by an increase of concentration of (D_6)DMSO. Therefore, we propose that these signals emanate from the two NH groups of Tht^3 and Tht^5, which are involved in two intramolecular H-bonds, forming two β-turns (as in the crystalline state). The $\Delta\delta$ value of the remaining NH group of Tht^1 is 0.85 ppm, which is typical for a solvent-exposed NH group.

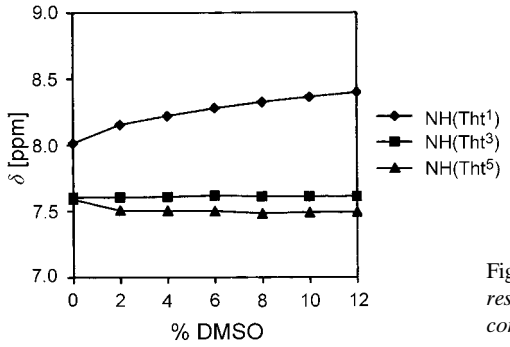


Fig. 7. *Dependence of the chemical shifts of the NH resonances of* **6b** *as a function of the (D_6)DMSO concentration* (% *v*/*v*) *in* $CDCl_3$

3. Conclusions. – The presented results show that the new spiroheterocyclic *N*-(2*H*-azirin-3-yl)prolinates **1f** and **1g** can be prepared according to the previously reported protocols. In the reactions with PhCOSH, 4-Br-C_6H_4COOH, and *N*-protected α-amino acids, both azirines behave like other 2*H*-azirin-3-amines (= 3-amino-2*H*-azirines) that have been extensively used as building blocks for α,α-disubstituted glycines in peptide

synthesis [14][15]. They have been shown to be useful synthons for the dipeptides *N*-[(4-aminotetrahydro-2*H*-pyran-4-yl)carbonyl]-L-proline (Thp-Pro) and *N*-[(4-aminotetrahydro-2*H*-thiopyran-4-yl)carbonyl]-L-proline (Tht-Pro), respectively. As models for sterically congested oligopeptides, 4-Br-C_6H_4CO-(Thp-Pro)$_n$-OMe and 4-Br-C_6H_4CO-(Tht-Pro)$_n$-OMe were prepared *via* repeated 'azirine coupling' analogous to the synthesis of ArCO-(Aib-Pro)$_n$-OMe peptides with the amino-azirine **1d** [13].

By X-ray crystallography and NMR techniques, it has been shown that the examined hexapeptides **6a** and **6b** adopt fairly rigid helical conformations with some significant differences, namely in the values of the ψ torsion angle for the Pro^2 and the Pro^4 residues in the peptide backbone. In the case of **6a**, containing consecutive (Thp-Pro) units, this torsion angle has the values of $-31.8(5)$ (Pro^2) and $-27.7(6)^\circ$ (Pro^4) (molecule A), and $-26.7(6)$ (Pro^2) and $-23.0(6)^\circ$ (Pro^4) (molecule B). The corresponding values in the case of **6b**, which consists of consecutive (Tht-Pro) units, are $-20.4(6)$ (Pro^2) and $-4.5(6)^\circ$ (Pro^4) (molecule A), and $-9.8(6)$ (Pro^2) and $-0.6(7)^\circ$ (Pro^4) (molecule B). It is worth mentioning that the values obtained in the case of **6a** could be compared with the values of a regular 3_{10}-helix ($\psi = -30^\circ$), whereas those obtained in the case of **6b** are much closer to the values obtained in the case of the β-bend ribbon spiral [16e].

We thank the analytical sections of our institute for spectra and elemental analyses, and the *Swiss National Science Foundation* and *F. Hoffmann-La Roche AG*, Basel, for financial support.

Experimental Part

1. *General.* Solvents were purified by standard procedures. TLC: *Merck* TLC aluminum sheets, silica gel *60 F_{254}*. Column chromatography (CC): *Uetikon-Chemie*, silica gel *C-560* (0.04–0.063 mm). M.p.: *Büchi Melting Point B-450* apparatus; uncorrected. IR Spectra: *Perkin-Elmer*, *Spectrum one* FT-IR spectrophotometer; in KBr unless otherwise stated; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AC-300* (^{1}H, ^{13}C, DEPT) at 300 and 75 MHz, resp., or *Bruker DRX-600* (^{1}H, ^{13}C, HSQC, HMBC, COSY) at 600 and 150 MHz, resp., in $CDCl_3$ at 300 K, unless otherwise stated; δ in ppm refer to residual C*H*Cl_3 (^{1}H, 7.27 ppm) and to *C*DCl_3 (^{13}C, 77.0 ppm); coupling constants *J* in Hz; ^{13}C-signal multiplicities from DEPT spectra. MS: *Finnigan SSQ-700* (CI with NH_3), or *Finningan TSQ-700* instrument (ESI); *m*/*z* (rel. %).

2. *Synthesis of Oligopeptides 4-Br-C_6H_4CO-(Thp-Pro)$_n$-OMe. General Procedure 1* (*GP 1*). To a soln. of 4-bromobenzoic acid or the corresponding peptide acid in dry THF was added the azirine **1f** [14] (1.1 equiv.), and the mixture was stirred at r.t. After completion of the reaction (TLC), the soln. was concentrated *in vacuo*, and the residue was purified by CC (SiO_2).

General Procedure 2 (*GP 2*). To a soln. of the corresponding peptide methyl ester in THF/MeOH/H_2O 3:1:1, LiOH·H_2O (4 mol-equiv.) was added. The mixture was stirred at r.t. After completion of the reaction (TLC), 1M HCl was added until pH 1 was reached, and the org. solvent was evaporated. The residue was extracted with CH_2Cl_2 or AcOEt, the combined org. phase was dried ($MgSO_4$), evaporated, and the residue was dried under h.v.

*Methyl 1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-L-prolinate* (4-Br-C_6H_4CO-Thp-Pro-OMe; **2a**). According to *GP 1*, with 4-Br-C_6H_4COOH (169 mg, 0.845 mmol) in dry THF (10 ml) and **1f** (220 mg, 0.925 mmol); stirring for 45 h. The obtained precipitate was collected by filtration, washed with AcOEt/hexane 1:1, and dried under h.v.: 348 mg (94%) of **2a**. Colorless crystals. M.p. 223°. IR: 3333*m*, 2956*m*, 2874*w*, 1743*s*, 1654*s*, 1616*s*, 1590*w*, 1528*m*, 1483*m*, 1421*w*, 1361*w*, 1301*w*, 1284*w*, 1208*w*, 1164*m*, 1106*m*, 1069*w*, 1011*m*, 939*w*, 847*m*, 758*s*, 614*w*. ^{1}H-NMR: 8.17 (*s*, NH); 7.78–7.75 (*m*, 2 arom. H); 7.63–7.59 (*m*, 2 arom. H); 4.59–4.56 (*m*, CH(α)(Pro)); 4.02–3.95 (*m*, 1 H of CH_2(δ)(Pro)); 3.83–3.81 (*m*, 2 H); 3.74–3.68 (*m*, 2 H of CH_2(2), CH_2(6)(Thp), MeO); 3.39–3.31 (*m*, 1 H of CH_2(δ)(Pro)); 2.63–2.55 (*m*, 1 H); 2.20–1.85 (*m*, 7 H). ^{13}C-NMR: 173.9, 171.6 (2*s*, 2 C=O); 166.8

(*s*, Ar*C*=O); 132.8 (*s*, 1 arom. C); 132.1, 129.4 (2*d*, 4 arom. CH); 126.9 (*s*, 1 arom. C); 64.9, 63.4 (2*t*, C(2), C(6)(Thp)); 61.0 (*d*, CH(α)(Pro)); 57.4 (*s*, C(4)(Thp)); 52.3 (*q*, MeO); 48.0 (*t*, CH_2(δ)(Pro)); 32.6, 32.4, 27.9, 26.0 (4*t*, 4 CH_2). ESI-MS: 463 (100, $[M(^{81}Br)+Na]^+$), 461 (96, $[M(^{79}Br)+Na]^+$), 383 (63).

*1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-*L*-proline* (4-Br-C_6H_4CO-Thp-Pro-OH; **3a**). According to *GP 2*, with **2a** (319 mg, 0.726 mmol) in 10 ml THF/MeOH/H_2O, LiOH·H_2O (122 mg, 2.904 mmol); stirring for 23 h: 302 mg (98%) of **3a**. The product was sufficiently pure to be used in the next step without further purification. M.p. 227°. IR: 3350*m*, 2977*m*, 2876*w*, 1736*s*, 1617*s*, 1592*m*, 1530*s*, 1482*m*, 1442*m*, 1422*s*, 1354*w*, 1311*m*, 1273*w*, 1250*w*, 1210*w*, 1165*s*, 1097*m*, 1071*m*, 1025*w*, 1013*m*, 972*w*, 943*m*, 895*w*, 850*m*, 777*m*, 757*m*, 710*w*, 651*w*, 605*m*. ^{1}H-NMR ((D$_6$)DMSO): 12.18 (*s*, OH); 8.59 (*s*, NH); 7.86–7.83 (*m*, 2 arom. H); 7.72–7.69 (*m*, 2 arom. H); 4.25–4.22 (*m*, CH(α)(Pro)); 3.83–3.65 (*m*, 4 H); 3.48–3.60 (*m*, 1 H); 3.20–3.15 (*m*, 1 H); 2.38–2.25 (*m*, 1 H); 2.10–1.69 (*m*, 7 H). ^{13}C-NMR ((D$_6$)DMSO): 173.8, 170.3 (2*s*, 2 C=O); 164.9 (*s*, Ar*C*=O); 133.2 (*s*, 1 arom. C); 131.5, 129.9 (2*d*, 4 arom. CH); 125.3 (*s*, 1 arom. C); 63.6, 62.5 (2*t*, C(2), C(6)(Thp)); 60.3 (*d*, CH(α)(Pro)); 56.6 (*s*, C(4)(Thp)); 47.3 (*t*, CH_2(δ)(Pro)); 31.8, 31.7, 27.5, 25.4 (4*t*, 4 CH_2). ESI-MS: 449 (86, $[M(^{81}Br)+Na]^+$), 447 (100, $[M(^{79}Br)+Na]^+$).

*Methyl 1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-*L*-prolyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]-*L*-prolinate* (4-Br-C_6H_4CO-(Thp-Pro)$_2$-OMe; **4a**). According to *GP 1*, with **3a** (250 mg, 0.588 mmol) in dry THF (12 ml) and **1f** (154 mg, 0.647 mmol), stirring for 47 h, CC (CH_2Cl_2/MeOH 100:1 → 20:1): 390 mg (88%) of **4a**. M.p. 194.2°. IR: 3319*m*, 2955*m*, 2870*w*, 1744*m*, 1646*s*, 1590*w*, 1530*m*, 1482*m*, 1442*w*, 1404*m*, 1301*w*, 1279*w*, 1247*w*, 1207*w*, 1162*m*, 1143*w*, 1107*m*, 1070*w*, 1030*w*, 938*w*, 845*m*, 804*w*, 760*m*. ^{1}H-NMR: 7.89 (*s*, NH); 7.73–7.70 (*m*, 2 arom. H); 7.62 (*s*, NH); 7.60–7.57 (*m*, 2 arom. H); 4.59–4.55 (*m*, CH(α)(Pro)); 4.19–4.18 (*m*, CH(α)(Pro)); 4.01–3.96 (*m*, 2 H of 2 CH_2(δ)(Pro)); 3.93–3.61 (*m*, 8 H); 3.56 (*s*, MeO); 3.32–3.29 (*m*, 1 H of CH_2(δ)(Pro)); 3.29–3.28 (*m*, 1 H of CH_2(δ)(Pro)); 2.70–2.64 (*m*, 1 H); 2.24–1.99 (*m*, 9 H); 1.98–1.71 (*m*, 6 H). ^{13}C-NMR: 173.0, 171.4, 171.3, 171.0 (4*s*, 4 C=O); 166.3 (*s*, Ar*C*=O); 132.2 (*d*, 2 arom. CH); 131.6 (*s*, 1 arom. C); 129.1 (*d*, 2 arom. CH); 127.2 (*s*, 1 arom. C); 64.4, 63.7, 63.2, 62.7 (4*t*, 2 C(2), 2 C(6)(Thp)); 62.6, 60.6 (2*d*, 2 CH(α)(Pro)); 57.7, 56.9 (2*s*, 2 C(4)(Thp)); 51.9 (*q*, MeO); 48.4, 47.5 (2*t*, 2 CH(δ)(Pro)); 32.5, 32.0, 31.5, 31.3 (4*t*, 2 C(3), 2 C(5)(Thp)); 28.4, 27.8 (2*t*, 2 CH(β)(Pro)); 26.0, 25.6 (2*t*, 2 CH(γ)(Pro)). ESI-MS: 703 (9, $[M(^{81}Br)+K]^+$), 701 (7, $[M(^{79}Br)+K]^+$), 687 (26, $[M(^{81}Br)+Na]^+$), 685 (25, $[M(^{79}Br)+Na]^+$), 665 (17, $[M(^{81}Br)+H]^+$), 663 (15, $[M(^{79}Br)+H]^+$), 536 (100, $[M(^{81}Br)-(Pro\text{-}OMe)+H]^+$), 534 (98, $[M(^{79}Br)-(Pro\text{-}OMe)+H]^+$), 409 (45, $[M(^{81}Br)-(Thp\text{-}Pro\text{-}OMe)+H]^+$), 407 (48, $[M(^{79}Br)-(Thp\text{-}Pro\text{-}OMe)+H]^+$), 312 (14, $[M(^{81}Br)-(Pro\text{-}Thp\text{-}Pro\text{-}OMe)+H]^+$), 310 (14, $[M(^{79}Br)-(Pro\text{-}Thp\text{-}Pro\text{-}OMe)+H]^+$), 284 (6, $[M(^{81}Br)-(Pro\text{-}Thp\text{-}Pro\text{-}OMe)-CO]^+$), 282 (6, $[M(^{79}Br)-(Pro\text{-}Thp\text{-}Pro\text{-}OMe)-CO]^+$), 130 (17, $[(Pro\text{-}OMe)+H]^+$).

*1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-*L*-prolyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]-*L*-proline* (4-Br-C_6H_4CO-(Thp-Pro)$_2$-OH; **5a**). According to *GP 2*, with **4a** (282 mg, 0.425 mmol) in 20 ml of THF/MeOH/H_2O and LiOH·H_2O (71.3 mg, 1.7 mmol), stirring for 25 h: 251 mg (91%) of **5a**. The isolated peptide acid was sufficiently pure to be used in the next step without further purification. M.p. 198°. IR: 3350*m*, 2955*m*, 2849*w*, 1746*s*, 1652*s*, 1617*s*, 1590*m*, 1525*m*, 1482*s*, 1411*m*, 1356*w*, 1299*w*, 1282*w*, 1248*w*, 1206*w*, 1162*s*, 1108*m*, 1070*m*, 1011*m*, 940*w*, 898*w*, 847*m*, 780*w*. ^{1}H-NMR ((D$_6$)DMSO): 12.14 (br. *s*, OH); 8.85 (*s*, NH); 7.88–7.85 (*m*, 2 arom. H); 7.79 (*s*, NH); 7.76–7.73 (*m*, 2 arom. H); 4.52–4.50 (*m*, CH(α)(Pro)); 4.18–4.14 (*m*, CH(α)(Pro)); 3.76–3.48 (*m*, 10 H); 3.42–3.24 (*m*, 2 H); 3.24–1.88 (*m*, 9 H); 1.82–1.65 (*m*, 6 H). ^{13}C-NMR ((D$_6$)DMSO): 173.8, 170.9, 170.6, 170.2 (4*s*, 4 C=O); 165.7 (*s*, Ar*C*=O); 137.7 (*s*, 1 arom. C); 131.6, 129.9 (2*d*, 4 arom. CH); 125.8 (*s*, 1 arom. C); 63.2, 63.1, 63.1, 62.2 (4*t*, 2 C(2), 2 C(6)(Thp)); 61.5, 60.3 (2*d*, 2 CH(α)(Pro)); 57.1, 55.9 (2*s*, 2 C(4)(Thp)); 47.9, 46.9 (2*t*, 2 CH_2(δ)(Pro)); 31.9, 31.8, 31.7, 31.0 (4*t*, 2 C(3), 2 C(5)(Thp)); 28.2, 27.5 (2*t*, 2 CH_2(β)(Pro)); 25.4, 25.4 (2*t*, 2 CH_2(γ)(Pro)). ESI-MS: 673 (96, $[M(^{81}Br)+Na]^+$), 671 (100, $[M(^{79}Br)+Na]^+$), 391 (20).

*Methyl 1-[(4-({1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-*L*-prolyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]-*L*-prolyl}amino)-3,4,5,6-tetrahydro-2*H*-pyran-4-yl]carbonyl}-*L*-prolinate* (4-Br-C_6H_4CO-(Thp-Pro)$_3$-OMe; **6a**). According to *GP 1*, with **5a** (205 mg, 0.316 mmol) in dry THF (12 ml) and **1f** (83 mg, 0.348 mmol), stirring for 25 h, CC (CH_2Cl_2/MeOH 80:1 → 20:1): 241 mg (86%) of **6a**. M.p. 334.5°. IR: 3274*m*, 2956*m*, 2875*w*, 1747*m*, 1637*s*, 1590*w*, 1536*s*,

1483*m*, 1441*w*, 1406*s*, 1356*w*, 1301*w*, 1282*w*, 1247*w*, 1208*w*, 1184*w*, 1161*m*, 1140*w*, 1107*m*, 1071*w*, 1032*w*, 1012*m*, 932*w*, 840*m*, 802*w*, 761*m*. ^{1}H-NMR: 8.41 (*s*, NH); 7.71 – 7.68 (*m*, 2 arom. H, NH); 7.59 – 7.57 (*m*, 2 arom. H, NH); 4.63 – 4.58 (*m*, CH(α)(Pro)); 4.41 – 4.31 (*m*, 2 CH(α)(Pro)); 3.98 – 3.67 (*m*, 12 H); 3.62 (*s*, MeO); 3.59 – 3.25 (*m*, 6 H); 2.58 – 2.43 (*m*, 2 H); 2.24 – 2.19 (*m*, 5 H); 2.18 – 1.75 (*m*, 17 H). ^{13}C-NMR: 173.3, 172.6, 171.9, 171.3, 171.2, 171.1 (6*s*, 6 C=O); 167.2 (*s*, Ar*C*=O); 131.8 (*d*, 2 arom. CH); 131.5 (*s*, 1 arom. C); 128.8 (*d*, 2 arom. CH); 127.0 (*s*, 1 arom. C); 64.2, 64.0, 63.6, 62.9, 62.9, 62.3 (6*t*, 3 C(2), 3 C(6)(Thp)); 62.9, 62.3, 60.6 (3*d*, 3 CH(α)(Pro)); 57.0, 56.7, 56.4 (3*s*, 3 C(4)(Thp)); 51.6 (*q*, MeO); 48.1, 47.7, 47.6 (3*t*, 3 CH$_2$(δ)(Pro)); 32.1, 31.4, 31.1, 31.0, 30.7, 30.6 (6*t*, 3 C(3), 3 C(5)(Thp)); 29.0, 27.8, 27.6 (3*t*, 3 CH$_2$(β)(Pro)); 25.7, 25.6, 23.4 (3*t*, 3 CH$_2$(γ)(Pro)). ESI-MS: 927 (22, [*M*(^{81}Br) + K]$^+$), 925 (18, [*M*(^{79}Br) + K]$^+$), 911 (100, [*M*(^{81}Br) + Na]$^+$), 909 (98, [*M*(^{79}Br) + Na]$^+$), 889 (21, [*M*(^{81}Br) + H]$^+$), 887 (21, [*M*(^{79}Br) + H]$^+$), 760 (16, [*M*(^{81}Br) – (Pro-OMe) + H]$^+$), 758 (13, [*M*(^{79}Br) – (Pro-OMe) + H]$^+$), 536 (12, [*M*(^{81}Br) – (Pro-Thp-Pro-OMe) + H]$^+$), 534 (12, [*M*(^{79}Br) – (Pro-Thp-Pro-OMe) + H]$^+$).

Suitable crystals for the X-ray crystal-structure determination were grown from $CHCl_3$/hexane by slow evaporation of the solvent at r.t.

*1-{[4-({1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-*L*-prolyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]-*L*-prolyl}amino)-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl}-*L*-proline* (4-Br-C$_6$H$_4$CO-(Thp-Pro)$_3$-OH; **7a**). According to *GP 2*, with **6a** (208 mg, 0.235 mmol) in 25 ml THF/MeOH/H_2O and LiOH · H_2O (39.4 mg, 0.939 mmol), stirring for 21 h: 198 mg (96%) of **7a**. The isolated peptide acid was sufficiently pure to be used in the next step without further purification. M.p. 338°. IR: 3313*m*, 2958*m*, 2875*w*, 1742*m*, 1643*s*, 1591*w*, 1531*s*, 1483*w*, 1443*w*, 1407*s*, 1387*s*, 1353*w*, 1303*w*, 1279*w*, 1246*w*, 1207*w*, 1141*m*, 1107*s*, 1073*w*, 1028*w*, 1011*m*, 937*w*, 843*m*, 803*w*, 760*m*, 605*w*. ^{1}H-NMR ((D$_6$)DMSO): 8.88 (*s*, NH); 7.89 – 7.86 (*m*, 2 arom. H); 7.83 (*s*, NH); 7.77 – 7.74 (*m*, 2 arom. H); 7.69 (*s*, NH); 4.48 – 4.45 (*m*, CH(α)(Pro)); 4.40 – 4.35 (*m*, CH(α)(Pro)); 4.21 – 4.17 (*m*, CH(α)(Pro)); 3.73 – 3.55 (*m*, 18 H); 2.27 – 1.56 (*m*, 24 H). ^{13}C-NMR ((D$_6$)DMSO): 173.7, 171.9, 170.8, 170.8, 170.4, 170.0 (6*s*, 6 C=O); 165.8 (*s*, Ar*C*=O); 132.3 (*s*, 1 arom. C); 131.5, 129.8 (2*d*, 4 arom. CH); 125.6 (*s*, 1 arom. C); 63.0, 63.0, 62.8, 62.6, 62.6, 62.1 (6*t*, 3 C(2), 3 C(6)(Thp)); 61.9, 61.2, 60.2 (3*d*, 3 CH(α)(Pro)); 56.8, 55.9, 55.8 (3*s*, 3 C(4)(Thp)); 47.8, 47.3, 47.0 (3*t*, 3 CH$_2$(δ)(Pro)); 31.7, 31.5, 31.4, 31.2, 31.1, 30.8 (6*t*, 3 C(3), 3 C(5)(Thp)); 28.5, 27.9, 27.4 (3*t*, 3 CH$_2$(β)(Pro)); 25.5, 25.4, 25.1 (3*t*, 3 CH$_2$(γ)(Pro)). ESI-MS: 897 (100, [*M*(^{81}Br) + Na]$^+$), 895 (91, [*M*(^{79}Br) + Na]$^+$).

*Methyl 1-({4-[(1-{[4-({1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-*L*-prolyl]amino}-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)carbonyl]-*L*-prolyl}amino)-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl}-*L*-prolyl)amino]-3,4,5,6-tetrahydro-2*H*-pyran-4-yl}carbonyl)-*L*-prolinate* (4-Br-C$_6$H$_4$CO-(Thp-Pro)$_4$-OMe; **8**). According to *GP 1*, with **7a** (143 mg, 0.164 mmol) in dry THF (15 ml) and **1f** (43 mg, 0.180 mmol), stirring for 72 h, CC (CH_2Cl_2/MeOH from 80 : 1 to 20 : 1): 143 mg (78%) of **8**. M.p. 334.5°. IR: 3491*m*, 3307*m*, 2958*m*, 2869*w*, 1744*m*, 1644*s*, 1530*s*, 1482*w*, 1443*w*, 1407*s*, 1386*w*, 1301*m*, 1280*w*, 1246*m*, 1207*w*, 1162*m*, 1141*w*, 1106*m*, 1029*w*, 1011*w*, 925*w*, 841*m*, 804*w*, 760*m*. ^{1}H-NMR: 8.13 (*s*, NH); 7.75 – 7.72 (*m*, 2 arom. H, NH); 7.65 (*s*, NH); 7.62 – 7.60 (*m*, 2 arom. H); 7.53 (*s*, NH); 4.61 – 4.56 (*m*, CH(α)(Pro)); 4.44 – 4.34 (*m*, 2 CH(α)(Pro)); 4.23 – 4.21 (*m*, CH(α)(Pro)); 3.88 – 3.72 (*m*, 13 H); 3.63 – 3.51 (*m*, 10 H); 3.33 – 3.26 (*m*, 1 H); 2.60 – 2.37 (*m*, 3 H); 2.26 – 2.14 (*m*, 8 H); 2.09 – 1.65 (*m*, 22 H). ^{13}C-NMR: 173.4, 173.3, 172.9, 172.1, 171.9, 171.7, 171.6, 171.5 (8*s*, 8 C=O); 167.2 (*s*, Ar*C*=O); 132.3 (*d*, 2 arom. CH); 131.6 (*s*, 1 arom. C); 129.2 (*d*, 2 arom. CH); 127.4 (*s*, 1 arom. C); 64.3, 64.2, 63.4, 63.0, 62.9, 62.9, 62.9, 62.4 (8*t*, 4 C(2), 4 C(6)(Thp)); 63.4, 62.7, 62.4, 60.8 (4*d*, 4 CH(α)(Pro)); 57.6, 57.0, 56.9, 56.5 (4*s*, 4 C(4)(Thp)); 51.9 (*q*, MeO); 48.6, 48.0, 48.0, 47.9 (4*t*, 4 CH$_2$(δ)(Pro)); 32.1, 32.0, 31.1, 31.0, 31.0, 30.9, 29.6, 29.4 (8*t*, 4 C(3), 4 C(5)(Thp)); 25.5, 28.5, 28.4, 28.4 (4*t*, 4 CH$_2$(β)(Pro)); 26.3, 26.0, 26.0, 25.9 (4*t*, 4 CH$_2$(γ)(Pro)). ESI-MS: 1135 (100, [*M*(^{81}Br) + Na]$^+$), 1133 (84, [*M*(^{79}Br) + Na]$^+$).

3. *Synthesis of Oligopeptides 4-Br-C$_6$H$_4$CO-(Tht-Pro)$_n$-OMe. General Procedure 3* (*GP 3*). To a soln. of the peptide methyl ester in THF/MeOH/H_2O 3 : 1 : 1, LiOH · H_2O (3 mol-equiv.) was added, and the mixture was stirred at r.t. After completion of the reaction (TLC), 10% $KHSO_4$ was added until pH 1 was reached, and the org. solvent was evaporated. The residue was extracted with AcOEt, the org. phases were dried ($MgSO_4$), the solvent was evaporated, and the residue was dried under h.v. and used without further purification.

*1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl}carbonyl)-*L*-proline* (4-Br-C$_6$H$_4$CO-Tht-Pro-OH; **3b**). According to *GP 3*, with **2b** [15] (190 mg, 0.42 mmol) and LiOH · H_2O

(52.5 mg, 1.252 mmol), 27 h: 167 mg (90%) of **3b**. White foam. M.p. 201°. IR: 3348*m*, 2952*m*, 1736*s*, 1640*s*, 1615*s*, 1590*w*, 1526*s*, 1481*s*, 1408*s*, 1310*m*, 1278*m*, 1254*w*, 1217*w*, 1180*m*, 1150*w*, 1070*m*, 1010*m*, 912*w*, 843*m*, 757*m*, 710*w*, 658*w*, 607*w*. ^{1}H-NMR ((D$_6$)DMSO): 12.18 (br. *s*, OH); 8.42 (*s*, NH); 7.85–7.82 (*m*, 2 arom. H); 7.71–7.68 (*m*, 2 arom. H); 4.24–4.22 (*m*, CH(α)(Pro)); 3.74–3.71, 3.39–3.35 (2*m*, CH$_2$(δ)(Pro)); 3.17–3.10 (*m*, 1 H); 2.99–2.91 (*m*, 1 H); 2.74–2.51 (*m*, 2 H); 2.43–2.25 (*m*, 3 H); 1.96–1.94 (*m*, 2 H); 1.92–1.76 (*m*, 3 H). ^{13}C-NMR ((D$_6$)DMSO): 173.5, 170.3 (2*s*, 2 C=O); 164.7 (*s*, Ar*C*=O); 133.0 (*s*, 1 arom. C); 131.2, 129.7 (2*d*, 4 arom. CH); 125.1 (*s*, 1 arom. C); 60.1 (*d*, CH(α)(Pro)); 57.8 (*s*, C(4)(Tht)); 47.1 (*t*, CH$_2$(δ)(Pro)); 32.6, 32.3 (2*t*, C(2), C(6)(Tht)); 27.2, 25.2 (2*t*, C(3), C(5)(Tht)); 23.3 (*t*, CH$_2$(β)(Pro)); 22.3 (*t*, CH$_2$(γ)(Pro)). ESI-MS: 481 (13, [*M*(^{81}Br) + K]$^+$), 479 (20, [*M*(^{79}Br) + K]$^+$), 465 (100, [*M*(^{81}Br) + Na]$^+$), 463 (86, [*M*(^{79}Br) + Na]$^+$).

*Methyl 1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl}carbonyl)-*L*-prolyl]amino}-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl)carbonyl]-*L*-prolinate* (4-Br-C$_6$H$_4$CO-(Tht-Pro)$_2$-OMe; **4b**). According to *GP 1*, with **3b** (167 mg, 0.378 mmol) and **1g** (192 mg, 0.756 mmol), stirring for 120 h, CC (CH$_2$Cl$_2$/MeOH 150:1 → 20:1): 191 mg (73%) of **4b**. White foam. M.p. 155°. IR: 3311*m*, 2950*m*, 2875*w*, 1743*s*, 1643*s*, 1531*s*, 1480*m*, 1402*m*, 1280*m*, 1255*w*, 1174*m*, 1103*w*, 1070*w*, 1040*w*, 1009*m*, 927*w*, 884*w*, 845*w*, 760*m*, 659*w*, 607*w*. ^{1}H-NMR: 7.82–7.87 (*m*, 2 arom. H); 7.69–7.66 (*m*, 2 arom. H); 7.52 (br. *s*, NH); 7.42 (*s*, NH); 4.59–4.51, 4.39–4.34 (2*m*, 2 CH(α)(Pro)); 4.09–4.08 (*m*, 1 H of CH$_2$(δ)(Pro)); 3.85–3.73 (*m*, 1 H of CH$_2$(δ)(Pro)); 3.69 (*s*, MeO); 3.23–3.09 (*m*, 2 H of 2 CH$_2$(δ)(Pro)); 2.83–2.51 (*m*, 12 H); 2.43–2.10 (*m*, 6 H); 1.87–1.74 (*m*, 6 H). ^{13}C-NMR: 173.2, 171.6, 171.3, 170.9 (4*s*, 4 C=O); 166.0 (*s*, Ar*C*=O); 132.2 (*d*, 2 arom. CH); 131.3 (*s*, 1 arom. C); 129.3 (*d*, 2 arom. CH); 127.4 (*s*, 1 arom. C); 62.7, 60.9 (2*d*, 2 CH(α)(Pro)); 59.1, 58.3 (2*s*, 2 C(4)(Tht)); 51.7 (*q*, MeO); 48.1, 47.6 (2*t*, 2 CH$_2$(δ)(Pro)); 33.2, 32.9, 32.6, 32.5 (4*t*, 2 C(2), 2 C(6)(Tht)); 28.4, 27.8 (2*t*, 2 CH$_2$(β)(Pro)); 26.1, 25.9 (2*t*, 2 CH$_2$(γ)(Pro)); 24.0, 23.7, 23.5, 23.1 (4*t*, 2 C(3), 2 C(5)(Tht)). ESI-MS: 719 (100, [*M*(^{81}Br) + Na]$^+$), 717 (51, [*M*(^{79}Br) + Na]$^+$).

*1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl}carbonyl)-*L*-prolyl]-amino}-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl)carbonyl]-*L*-proline* (4-Br-C$_6$H$_4$CO-(Tht-Pro)$_2$-OH; **5b**). According to *GP 3*, **4b** (191 mg, 0.275 mmol) and LiOH · H$_2$O (35 mg, 0.825 mmol), 24 h: 174 mg (99%) of **5b**. White foam. M.p. 239°. IR: 3305*m*, 2923*m*, 1735*m*, 1644*s*, 1528*s*, 1480*s*, 1400*m*, 1309*w*, 1279*w*, 1253*w*, 1179*m*, 1070*m*, 1010*m*, 910*w*, 844*w*, 758*w*. ^{1}H-NMR: 7.76–7.73 (*m*, 2 arom. H); 7.69 (br. *s*, NH); 7.62–7.59 (*m*, 2 arom. H); 7.44 (br. *s*, NH); 4.57–4.52, 4.48–4.44 (2*m*, 2 CH(α)(Pro)); 3.92–3.77 (*m*, 2 H of 2 CH$_2$(δ)(Pro)); 3.61–3.48 (*m*, 1 H of CH$_2$(δ)(Pro)); 3.21–3.13 (*m*, 1 H of CH$_2$(δ)(Pro)); 2.99–2.68 (*m*, 4 H); 2.61–2.22 (*m*, 9 H); 2.18–2.05 (*m*, 4 H); 1.99–1.74 (*m*, 7 H). ^{13}C-NMR: 173.5, 173.1, 172.0, 171.9 (4*s*, 4 C=O); 166.6 (*s*, Ar*C*=O); 132.3 (*d*, 2 arom. CH); 131.4 (*s*, 1 arom. C); 129.1 (*d*, 2 arom. CH); 127.5 (*s*, 1 arom. C); 62.9, 62.0 (2*d*, 2 CH(α)(Pro)); 59.0, 58.3 (2*s*, 2 C(4)(Tht)); 48.5, 48.3 (2*t*, 2 CH$_2$(δ)(Pro)); 34.0, 33.9, 32.7, 32.2 (4*t*, 2 C(2), 2 C(6)(Tht)); 28.8, 28.1 (2*t*, 2 CH$_2$(β)(Pro)); 26.2, 26.0 (2*t*, 2 CH$_2$(γ)(Pro)); 24.6, 24.0, 23.5, 23.0 (4*t*, 2 C(3), 2 C(5)(Tht)). ESI-MS: 705 (100, [*M*(^{81}Br) + Na]$^+$), 703 (85, [*M*(^{79}Br) + Na]$^+$).

*Methyl 1-{[4-({1-[(4-{[1-({4-[(4-Bromobenzoyl)amino]-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl}carbonyl)-*L*-prolyl]amino}-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl)carbonyl]-*L*-prolyl}amino)-3,4,5,6-tetrahydro-2*H*-thiopyran-4-yl]carbonyl}-*L*-prolinate* (4-Br-C$_6$H$_4$CO-(Tht-Pro)$_3$-OMe; **6b**). According to *GP 1*, with **5b** (137 mg, 0.201 mmol) and **1g** (102 mg, 0.402 mmol), stirring for 116 h, CC (CH$_2$Cl$_2$/MeOH 150:1 → 10:1): 191 mg (73%) of **6b**. White foam. M.p. 316° (dec.). IR: 3475*w*, 3390*w*, 3310*m*, 2948*m*, 2871*w*, 1757*s*, 1639*s*, 1591*m*, 1567*m*, 1522*s*, 1482*m*, 1402*s*, 1314*w*, 1278*m*, 1257*w*, 1209*w*, 1194*m*, 1172*m*, 1153*w*, 1098*w*, 1071*w*, 1041*w*, 1011*m*, 994*w*, 967*w*, 928*m*, 910*w*, 882*w*, 850*w*, 803*w*, 761*m*, 727*w*, 682*w*, 662*w*. ^{1}H-NMR ((D$_6$)DMSO): 8.61 (*s*, NH) 7.88–7.85 (*m*, 2 arom. H); 7.81 (*s*, NH); 7.75–7.72 (*m*, 2 arom. H, NH); 4.48–4.46 (*m*, 2 CH(α)(Pro)); 4.23–4.22 (*m*, CH(α)(Pro)); 3.59–3.56 (*m*, 2 H of 3 CH$_2$(δ)(Pro)); 3.59–3.51 (*m*, 4 H of 3 CH$_2$(δ)(Pro), MeO); 2.79–2.75 (*m*, 6 H); 2.73–2.70 (*m*, 4 H); 2.49–2.23 (*m*, 15 H); 2.19–2.02 (*m*, 11 H). ^{13}C-NMR ((D$_6$)DMSO): 172.5, 172.0, 171.0, 170.8, 170.7, 170.0 (6*s*, 6 C=O); 165.0 (*s*, Ar*C*=O); 132.6 (*s*, 1 arom. C); 131.4 (*d*, 2 arom. CH); 129.8 (*d*, 2 arom. CH); 126.9 (*s*, 1 arom. C); 61.4, 60.8, 60.1 (3*d*, 3 CH(α)(Pro)); 58.1, 57.3, 57.1 (3*s*, 3 C(4)(Tht)); 51.4 (*q*, MeO); 47.6, 47.2, 46.9 (3*t*, 3 CH$_2$(δ)(Pro)); 32.6, 32.5, 32.4, 32.3, 31.9, 31.8 (6*t*, 3 C(2), 3 C(6)(Tht)); 28.1, 27.9, 27.2 (3*t*, 3 CH$_2$(β)(Pro)); 25.4, 25.3, 25.2 (3*t*, 3 CH$_2$(γ)(Pro)); 23.0, 22.9, 22.8, 22.7, 22.6, 22.5 (6*t*, 3 C(3),

3 C(5)(Tht)). ESI-MS: 959 (100, $[M(^{81}Br)+Na]^+$), 957 (79, $[M(^{79}Br)+Na]^+$), 808 (4, $[M(^{81}Br)-(Pro\text{-}OMe)+H]^+$), 806 (4, $[M(^{79}Br)-(Pro\text{-}OMe)+H]^+$).

Suitable crystals for the X-ray crystal-structure determination were grown from CH_2Cl_2/MeOH/hexane by slow evaporation of the solvent.

4. *Solvent and Temp. Dependence of the Chemical Shifts of the NH Groups.* The peptides **6a**, **6b**, and **8** were dissolved in $CDCl_3$ (*ca.* 0.2M), and the chemical shifts of the NH groups were determined at *ca.* 30°. Then, using a syringe, 2, 4, 6, 8, 10, and 12% (*v*/*v*) of (D_6)DMSO were added, and, after each addition, the chemical shifts were determined again. For the determination of the temp. dependence, the NH absorption in $CDCl_3$ soln. (*ca.* 0.2M) was recorded between 265 and 314 K in 7-K intervals.

5. *X-Ray Crystal Structure Determinations of* **6a** *and* **6b** (see *Tables 1*, *2*, *4–6*, and *Figs. 2* and *3*)[2]). The determinations for **6a** and **6b** were accomplished on a *Nonius KappaCCD* area-detector diffractometer [23] using graphite-monochromated MoK_α radiation (λ 0.71073 Å) and an *Oxford Cryosystems Cryostream 700* cooler. Data reduction was performed with *HKL Denzo* and *Scalepack* [24]. The intensities were corrected for *Lorentz* and polarization effects. A numerical absorption correction [25] was applied in the case of **6a**, and an absorption correction based on the multi-scan method [26] was applied in the case of **6b**. In all cases, equivalent reflections, other than *Friedel* pairs, were merged. Data collection and refinement parameters are collected in *Table 6*. The structures were solved by direct methods using *SIR92* [27], which revealed the positions of all non-H atoms.

The asymmetric unit of **6a** contains two symmetry-independent peptide, five $CHCl_3$, and three H_2O molecules. The atomic coordinates were tested carefully for a relationship from a higher symmetry space group using the program PLATON [28], but none could be found. Three of the five $CHCl_3$ molecules and all H_2O molecules are disordered over several orientations, and attempts to model the disorder led to unsatisfactory results. Therefore, the SQUEEZE routine [29] of the program PLATON was employed. This procedure allowed omission of the disordered solvent molecules from the subsequent refinement model (the ordered $CHCl_3$ molecules were retained), and gave satisfactory refinement results, which were better than any attempt to include the disordered solvent atoms in the model. The voids left in the structure due to the omission of the disordered solvent molecules had a total volume of 1317 Å^3 per unit cell. Most of this volume comprised a single void of 1069 Å^3 centered at 0, 0, 0.5, and there were two smaller symmetry-related voids of 124 Å^3. There were no significant peaks of residual electron density to be found in these voids. The electron count in the voids was calculated to be *ca.* 415 e, which corresponds well with 408 e calculated for the six $CHCl_3$ and six H_2O molecules per unit cell that were omitted from the model. One five-membered ring in each of molecules A and B is disordered over two envelope conformations. Two positions were defined for the atoms C(39) and C(91), resp., and refinement of the site occupation factors yielded values of 0.55(2) and 0.53(2), resp., for the major conformations of molecules A and B. Additionally, the six-membered ring at C(78) in molecule B is disordered over two chair conformations. Two positions were defined for O(3), C(2), and C(4) in this ring, and refinement of the site occupation factor yielded a value of 0.79(1) for the major conformation. Pseudo-isotropic restraints were applied to the atomic displacement parameters of some of the disordered atoms in this ring. Bond-length restraints were applied to all bonds involving disordered atoms so as to maintain reasonable geometry.

The asymmetric unit of **6b** contains two symmetry-independent peptide molecules, two fully occupied sites for MeOH molecules, plus one partially occupied MeOH site. The site occupation factors for this latter molecule refined to a value of 0.43(1). The atomic coordinates of the molecules were tested carefully for a relationship from a higher symmetry space group using the program PLATON [28], but none could be found. One C-atom in the central five-membered ring of peptide molecule B is disordered over two positions, and refinement of the site occupation factors yielded a value of 0.60(2) for the major conformation. Bond-length restraints were applied to all bonds involving the disordered atom and to the C–O bonds of the MeOH molecules. The two positions for the disordered atom were also restrained to have similar pseudo-isotropic atomic displacement parameters.

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[2]) CCDC-890142 and -890144 contain the supplementary crystallographic data for this article. These data can be obtained free of charge from *The Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

Table 6. *Crystallographic Data for Compounds* **6a** *and* **6b**

	6a	**6b**
Crystallized from	hexane/$CHCl_3$	CH_2Cl_2/MeOH/hexane
Empirical formula	2 $C_{41}H_{55}BrN_6O_{11} \cdot 5\ CHCl_3 \cdot 3\ H_2O$	$C_{41}H_{55}BrN_6O_8S_3 \cdot 1.21$ MeOH
Formula weight	2426.57	974.78
Crystal color, habit	colorless, plate	colorless, prism
Crystal dimensions [mm]	$0.02 \times 0.22 \times 0.25$	$0.12 \times 0.15 \times 0.30$
Temp. [K]	160(1)	160(1)
Crystal system	monoclinic	triclinic
Space group	$P2_1$	$P1$
Z	2	2
Reflections for cell determination	284369	46166
2θ Range for cell determination [°]	4–50	4–50
Unit cell parameters a [Å]	18.2782(1)	9.3659(4)
b [Å]	12.9913(1)	12.9994(3)
c [Å]	24.1243(2)	19.2946(8)
α [°]	90	79.668(2)
β [°]	101.9790(2)	80.836(2)
γ [°]	90	87.877(2)
V [Å^3]	5603.75(7)	2281.5(2)
D_x [g cm^{-3}]	1.438	1.419
$\mu(\mathrm{Mo}K_\alpha)$ [mm^{-1}]	1.154	1.101
Scan type	ω	ϕ and ω
$2\theta_{(\max)}$ [°]	50	50
Transmission factors [min; max]	0.802; 0.962	0.832; 0.879
Total reflections measured	120234	32037
Symmetry-independent reflections	18888	15434
Reflections with $I > 2\sigma(I)$	16118	12109
Reflections used in refinement	18885	15433
Parameters refined; restraints	1187; 35	1142; 36
Final $R(F)$ [$I > 2\sigma(I)$ reflections]	0.0565	0.0496
$wR(F^2)$ (all data)	0.1550	0.1253
Weighting parameters [a; b][a])	0.1031; 1.3458	0.0616; 0.9994
Goodness of fit	1.052	1.015
Secondary extinction coefficient	0.0023(3)	0.0076(7)
Final $\Delta_{\max}/\sigma$	0.001	0.002
$\Delta\rho$(max; min) [e Å^{-3}]	0.59; −0.48	0.57; −0.33

[a]) $w^{-1} = \sigma^2(F_o^2) + (aP)^2 + bP$ where $P = (F_o^2 + 2F_c^2)/3$.

The non-H-atoms were refined anisotropically. All of the H-atoms were placed in geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2 U_{eq} of its parent atom (1.5 U_{eq} for the Me and OH groups). In the case of **6b**, the orientations of the O–H vectors were optimized to correspond with the direction indicated by residual electron-density peaks. The OH H-atom of the partially occupied MeOH molecule was not included in the model. The refinement of the structures was carried out on F^2 using full-matrix least-squares procedures [30], which minimized the function $\Sigma w(F_o^2 - F_c^2)^2$. A correction for secondary extinction was applied in both cases. Three and one reflection for **6a** and **6b**, resp., whose intensities were considered to be extreme outliers, were omitted from the final refinement of each structure. Refinement of the absolute structure parameter [31] yielded a value of 0.104(6) and 0.000(6), resp., which confidently confirm that the refined model represents the true enantiomorphs.

Neutral atom-scattering factors for non-H-atoms were taken from [32a], and the scattering factors for H-atoms were taken from [33]. Anomalous dispersion effects were included in F_c [34]; the values for f' and f'' were those of [32b]. The values of the mass-attenuation coefficients are those of [32c]. All calculations were performed using the SHELXL97 [30] program.

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