Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 6705

www.rsc.org/obc

PAPER

4-Methylpseudoproline derived from α-methylserine – synthesis and conformational studies†

Joanna Katarzyńska,[a] Adam Mazur,[a] Wojciech M. Wolf,[b] Simon J. Teat,‡[c] Stefan Jankowski,*[a] Mirosław T. Leplawy[a] and Janusz Zabrocki*[a]

Received 15th April 2012, Accepted 20th June 2012
DOI: 10.1039/c2ob25732g

This paper presents the synthesis and solution conformational studies of the tripeptides Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (**6a**) and Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (**6b**). Additionally, the X-ray structure of **6a** is given. NMR analysis corroborated by theoretical calculations (XPLOR) shows that in both peptides the amide bond between pseudoproline and the preceding amino acid is in the *trans* conformation. The same amide bond geometry was observed in the crystal state of **6a**. The latter is additionally influenced by the presence of two symmetrically independent molecules in an asymmetric unit. Both molecules adopt a conformation which resembles β-turn type II, stabilized by hydrogen bonding. The conformational preferences and prolyl *cis–trans* isomerization of Ac-(αMe)Ser($\Psi^{H,H}$Pro)-NHMe (**7**) were explored at the IEFPCM/B3LYP/6-31+G(d) level of theory in vacuum, water and chloroform. It has been shown that the *trans* isomer predominates in water solutions and the *cis* isomer is preferred in chloroform. The conformation of **7** is down-puckered independently of the geometry of the amide bonds, with lower puckering in the transition state of the *cis–trans* isomerization.

Introduction

Proline residues are widely recognized as the units controlling the dynamic behavior of peptides and proteins due to the inherently slow *cis–trans* isomerization of the Xaa–Pro bond. The side chain of proline is covalently bonded to a nitrogen atom and the N–C_α rotation (Φ angle) is restricted to about −60°. Proline residues are usually encountered in loops or turn motifs at the $i + 1$ position for β-turn type I or II when the Xaa–Pro amide bond is in the *trans* conformation, or at the $i + 2$ position of turn type VI in the *cis* form. What is characteristic of proline is a relatively low activation barrier for isomerization (20–22 kcal mol^{-1}) combined with a small difference in free energy between the isomers. As a consequence, the presence of proline residues multiplies the number of conformers and can complicate the characterization of a peptide structure by means of NMR. A range of approaches have been applied to study the relationship between the isomer geometry and biological activity of prolyl peptides. The Xaa–Pro amide bond was replaced by rigid *cis* or *trans* amide bond surrogates: substituted proline rings as in **I** or pseudoprolines (ΨPro) **II** (Scheme 1).[1]

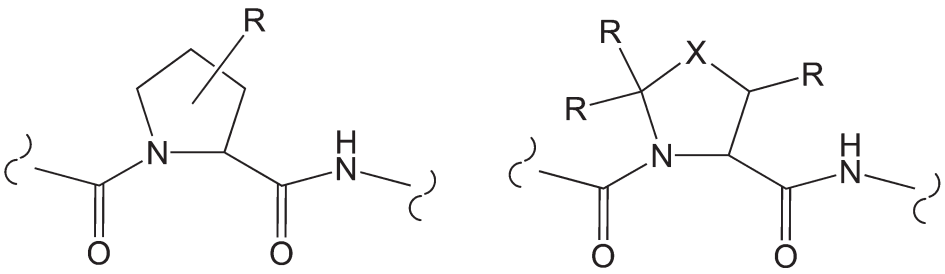
The effect of alkyl substitution on *cis–trans* isomerization was examined by introducing a methyl group at all prolyl carbons in Ac-(Me)Pro-NHMe. Only *trans* peptide bond isomers were observed in the crystal state.[2] The presence of methyl groups in positions 3 or 4 does not alter significantly the fraction of the *cis* isomer in comparison with native proline in water (about 25%) or in chloroform (15–20%).[3] The *R* or *S* configuration at carbon atom 4, as in Ac-(4-Me)Pro-NHMe, has a strong effect on the puckering of the proline ring. In water and in the crystal state, (4*S*-Me)Pro and (4*R*-Me)Pro residues prefer up- and down-puckering, respectively.[4] The introduction of a methyl group[5] in position 5 increases the population of the *cis*-isomer by about 5%, but 5,5-dimethyl[5] or bulky *tert*-butyl[6] substituents raise the *cis*-isomer population up to 66%. The methyl group in position 2 exclusively favors the *trans*-isomer in different solvents.[3] The presence of C^α-methyl-L-proline ((αMe)Pro) in N^α-acyl dipeptide *N*′-alkylamides of the RCO-(αMe)Pro-Xxx-NHR′ or RCO-Xxx-(αMe)Pro-NHR′ type (Xxx = L (or D)-Ala, Aib (α-aminoisobutyric acid) or L (or D)-(αMe)Pro) promotes the β-turn conformation and the *trans* conformation of the preceding tertiary peptide bond.[7]

Synthetic proline analogues **II**, known as pseudoprolines (ΨPro), readily obtained by cyclocondensation of the amino acids cysteine, threonine or serine with aldehydes or ketones are

[a]*Institute of Organic Chemistry, Lodz University of Technology, 90-924 Lodz, Poland. E-mail: stefan.jankowski@p.lodz.pl, janusz.zabrocki@p.lodz.pl*
[b]*Institute of General and Ecological Chemistry, Lodz University of Technology, 90-924 Lodz, Poland*
[c]*CLRC Daresbury Laboratory, Daresbury, Warrington, Cheshire WA4 4AD, UK*

†Electronic supplementary information (ESI) available. CCDC 870748. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob25732g

‡Now at: Advanced Light Source, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA, 94720, USA.



I: R = alkyl, OH, F

II: R_1, R_2, R_3 = H, Me, CF_3
X = O, S

Scheme 1 Modified proline rings.

the targets of interest as building blocks improving the efficiency of solid phase synthesis by preventing peptide aggregation and self-association.[8] To adjust the *cis–trans* isomer ratio, the pseudoproline ring was modified in position 2 by the incorporation of one or two methyl groups or an aryl substituent. Unsubstituted pseudoprolines are similar to proline in terms of their *trans* population,[9] whereas the 2,2-dimethyl derivatives adopt almost exclusively a *cis* amide conformation.[10,11] For 2-monosubstituted pseudoprolines containing a methyl or *p*-methoxyphenyl group, the *trans–cis* ratio depends on the stereochemistry at carbon 2: for the (2*S*)-diastereoisomer both forms are almost equally populated, whereas the (2*R*)-epimer preferentially adopts the *trans* form.[10] 2-Trifluoromethyl pseudoproline strongly influences the *cis–trans* rotational barriers with a moderate effect on the *cis–trans* population ratio.[12] For dipeptides containing threonine-derived pseudoprolines Xaa-Thr($\Psi^{Me,Me}$Pro) (Xaa = Gly, Phe, Val) the *cis* conformer is the predominant form in solution.[13] The oxazolidin-2-one ring (Oxd) can be regarded as a pseudoproline with an exclusively *trans* conformation of the preceding peptide bond. Oligomers containing Oxd exhibit a tendency to form intra- rather than intermolecular hydrogen bonds when the chain length increases.[14]

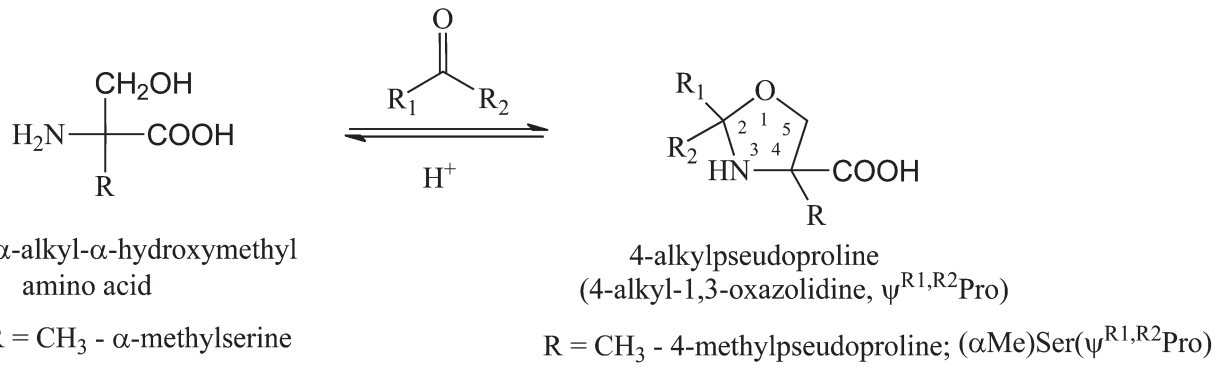
Substantial effort was put into theoretical calculations at different theory levels for unmodified proline[15] and substituted proline or pseudoproline[16] Ac-Xxx-NHMe in order to determine the substituent and solvent effects on the *cis–trans* isomerization and the ring puckering The calculated rotational barriers and populations of *cis* conformers in pseudoprolines **II** (O > S > CH_2) in water and chloroform are consistent with experimental data. The calculated energy barriers for ring flips show that down-to-up puckering transitions are dominant in the orders CH_2 < O < S *in vacuo* and CH_2 ≈ O < S in water.[17]

Ab initio calculations were carried out to analyze the effect of (4*R*)-substitution of the prolyl ring by electron-withdrawing groups (OH, F). Prolyl *trans–cis* isomerization is an entirely enthalpy driven process and the presence of electron-withdrawing substituents increases the population of the up puckered conformations. This factor is responsible for stabilization of triple helices of $(Pro\text{-}Xaa\text{-}Gly)_n$ sequences.[18,19]

Pseudoprolines have been introduced as proline analogues into molecules of the eleven-residue cyclopeptide cyclo(-Arg-His-Ile-Gly-Xaa-Gly-Arg-Ala-Phe-Cys-Tyr-) with a sequence based on the HIV-1_{MN} V3 variant.[20] Modification of cyclosporine C[21] and cyclosporine A[22] with ΨPro led to more constrained molecules retaining partial biological activity. The incorporation of pseudoproline at position 7 of the peptide hormone oxytocine led to a reduction in its agonistic activity, consistent with a decrease of the *trans* conformation.[23] The presence the Cys-derived pseudoproline Cys($\Psi^{Me,Me}$Pro) facilitated the synthesis of the cyclic octapeptide, cyclogossine B, as a traceless turn-inducer.[24] The introduction of Cys($\Psi^{Me,Me}$Pro) or Ser($\Psi^{Me,Me}$Pro) in position 3 of the insect kinin core pentapeptide led to a 100% prevalence of the *cis* isomer of Phe–Xxx-($\Psi^{Me,Me}$Pro) peptide bond.[25] When Cys($\Psi^{H,H}$Pro) was present isomers *cis* and *trans* were equally populated. The pseudoproline Thr($\Psi^{Me,Me}$Pro) was used in research into the structure and activity studies of sansalvamide A analogues.[26]

Research into the structure and activity studies of pseudoproline-containing analogues of opioid peptides, morphiceptin and endomorphin-2, showed the role of the *cis* conformation of the Tyr–Pro peptide bond in their bioactive conformations.[27] The endomorphin-2 analogue H-Tyr-Ser($\Psi^{H,H}$Pro)-Phe-Phe-NH_2, which exists in an conformational equilibrium around the Tyr–Ser($\Psi^{H,H}$Pro) bond with a *cis–trans* ratio of 45 : 55, displayed a 5-fold lower μ agonist potency in the GPI assay and a 10-fold lower δ potency in the MVD assay than the native endomorphin-2. Affinity towards both receptors decreased even more for the dimethylated endomorphin-2 analogue H-Tyr-Ser($\Psi^{Me,Me}$Pro)-Phe-Phe-NH_2, although the *cis–trans* isomerization was almost completely shifted towards the *cis* form. Preliminary studies of biological activity showed that the incorporation of (αMe)Ser-($\Psi^{H,H}$Pro) at position 2 in endomorphin-2 led to a new insight into the role of steric hindrance and configuration of carbon 4 in the pseudoproline moiety.[28] The (*R*)-Ser($\Psi^{H,H}$Pro) isomer interacts with the μ-receptor half as readily as the (*S*)-isomer and both isomers are much less active than the unmethylated analogue H-Tyr-Ser($\Psi^{H,H}$Pro)-Phe-Phe-NH_2. However, it should be noted that H-Tyr-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Phe-Phe-NH_2 possesses similar μ-receptor potency as [Leu^5]enkephalin. This observation suggests that the presence of a pseudoproline (*S*)-(αMe)-Ser($\Psi^{H,H}$Pro) moiety enables the adoption of the bioactive conformation, but makes the interaction with the μ-receptor more difficult.

In order to explore factors that determine the conformation of the pseudoproline ring derived from α-methylserine, two



Scheme 2 Transformation of α-alkyl-α-hydroxymethyl amino acids to 4-alkylpseudoproline.

tripeptides (**6a,b**) were synthesized and characterized by NMR spectroscopy.

Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t **6a**

Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t **6b**

Additionally X-ray analysis was performed for peptide **6a**.

Density functional computations were carried for Ac-(αMe)-Ser($\Psi^{H,H}$Pro)-NHMe **7** out to characterize its *cis–trans* isomerization process.

Results and discussion

Synthesis

Mutter and coworkers have been introduced pseudoprolines (ΨPro, 1,3-oxazolidines, Oxa and 1,3-thiazolidines, Thz), as a solubilizing protection technique for Ser, Thr and Cys in peptide synthesis.[8,29] We have used the pseudoproline concept for α-alkyl-α-hydroxymethyl amino acids, members of the family of nonproteinogenic, α,α-disubstituted amino acids with a hydrophilic side-chain.[30] α-Alkyl-α-hydroxymethyl amino acids, such as serine and threonine, can be transformed into a corresponding pseudoproline (oxazolidine) unit named 4-alkylpseudoproline[31] (Scheme 2).

For the synthesis of tripeptides **6a** and **6b** in solution two different coupling strategies were tested (Scheme 3). The first method was a "2 + 1" coupling strategy. Initially, dipeptides Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-OH **(3a)** and Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-OH **(3b)** were synthesized according to Mutter's procedure,[30] and oxazolidine, which was obtained *in situ*, was acylated by means of Fmoc alanine fluoride. (*R*)- and (*S*)-α-Methylserine (**1a** and **1b** respectively) were easily accessible by the Kaminski's method.[31] The introduction of pseudoproline as a dipeptide unit was a very significant advantage, because the problem of the secondary amine acylation was eliminated and the peptide chain was elongated by two residues in one step. However, this method gave dipeptides **3a** and **3b** in very low yield (15% and 14%, respectively). This was probably a consequence of decreased nucleophilicity of the nitrogen atom caused by the presence of an oxygen atom in the oxazolidine ring and the steric hindrance of the α-methyl group. Efforts to optimize the reaction conditions or use other acylating reagents were unsuccessful. Moreover, the isolation of the desired product from a heterogeneous mixture of components with very similar HPLC retention times became a very labor-intensive process. However, using TBTU as a coupling reagent[32] resulted in a 70% and 74% yield of the last step of synthesis of tripeptides **6a** and **6b**, respectively.

In contrast to the first method, second approach was accomplished in good yield when the stepwise method longer than Mutter's technique, was applied to the synthesis of peptides **6a** and **6b**. (*R*)- and (*S*)-4-Methylpseudoproline were used as the *N*-benzyloxycarbonyl derivatives **4a** and **4b** obtained in good yield (70%) by cyclocondensation of the corresponding (*R*)- and (*S*)-methylserine with formaldehyde in alkaline solution according to a known procedure.[33]

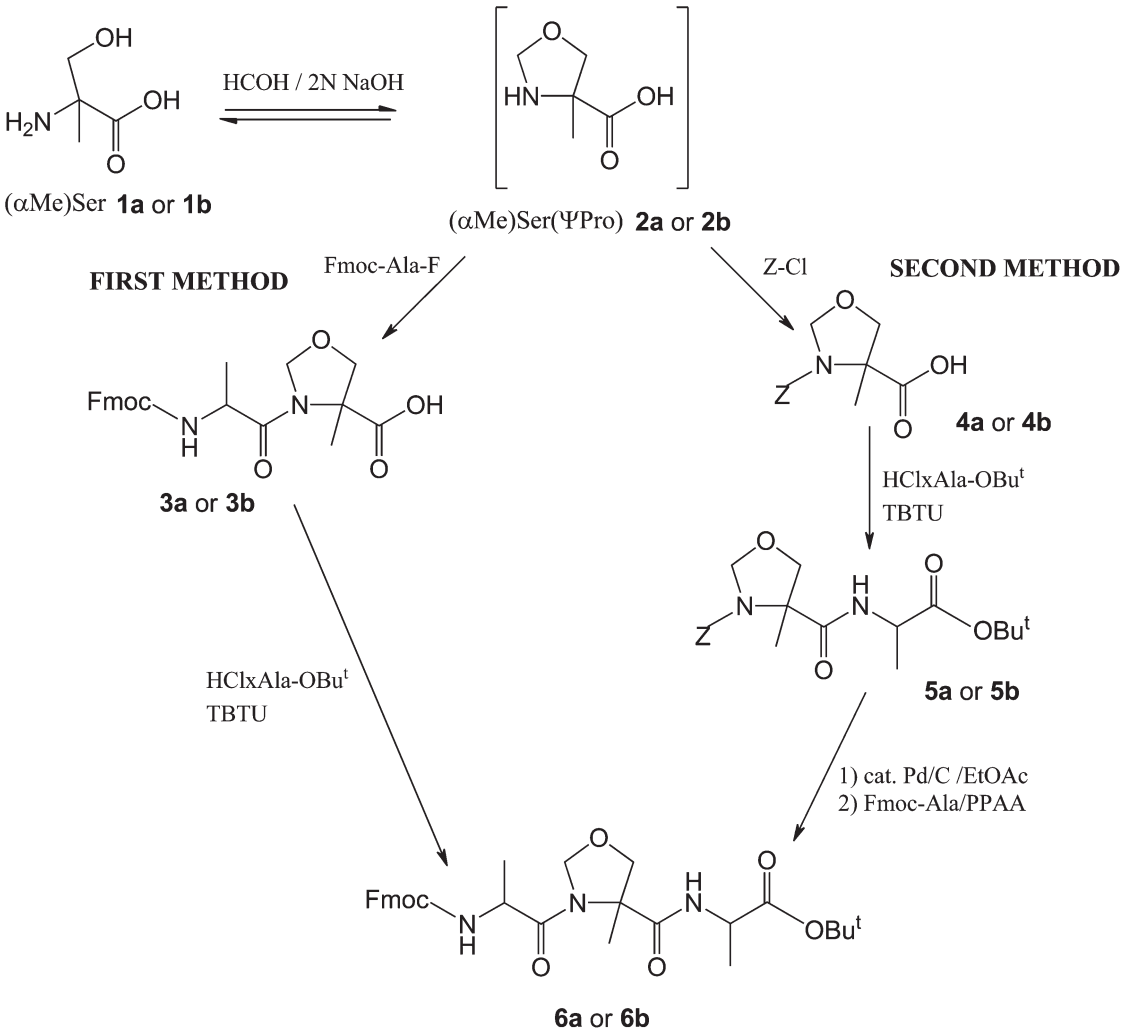
The dipeptides Z-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t **5a** and Z-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t **5b** with 4-methylpseudoproline as the N-terminus were synthesized conventionally in good yield using the TBTU carboxyl activation method. The Z-group was removed by catalytic hydrogenation.[34]

The NH-group in the 4-methylpseudoproline residue was acylated ("difficult coupling") by Fmoc-Ala C-activated by 1-propanephosphonic acid cyclic anhydride (PPAA),[35] the most efficient coupling reagent in our synthesis (with yields 38% and 34%, respectively). It is worth emphasizing that the additional advantage of this strategy is the fact that all by-products are easily accessible by commonly used purification techniques.

In summary based on our observations we can conclude that 4-methylpseudoproline derived from α-methylserine after protection of the NH-group is stable and can be isolated and stored for a long time. N-protected C-activated 4-methylpseudoproline is an efficient acylating component in the formation of peptide bonds. This result proves its usefulness in the synthesis of peptides with N-terminal 4-methylpseudoproline. On the other hand, acylation of the NH-group in 4-methylpseudoproline with C-activated amino acids gives the desired dipeptides with C-terminal pseudoproline in very low yield and this can be termed as "difficult coupling".

NMR analysis

^{1}H and ^{13}C NMR spectra recorded at 27 °C indicate the presence of one set of signals for dipeptides **3a** and **3b** as well as for tripeptides **6a** and **6b**, demonstrating that the peptides are rigid or undergo fast exchange, independently of the configuration at the C-4 carbon atom. Amide bond configuration was determined by analysis of cross-peak patterns in the corresponding ROESY



Scheme 3 Two strategies employed for the synthesis of tripeptides **6a** and **6b**.

spectra, namely the presence of strong correlation signals between Ala^1 H^{α} (**6a** and **6b**) or Ala^1 H^{β} (**3a** and **3b**) protons and methylene protons at the position 2 in the pseudoproline ring clearly identified the *trans* geometry of the Ala-(αMe)-Ser($\Psi^{H,H}$Pro) amide bond. Further inspection of the ROESY spectrum of tripeptide **6b** did not reveal any other signals. However, for **6a** additional ROE connectivities were detected and the interproton interactions were divided into three groups of distance restraints: strong (Ala^1H_{β}–(αMe)Ser^2H_{δ}), medium (Ala^1H_{β}–(αMe)Ser^2H_{β}, Ala^1H_{β}–(αMe)Ser^2H_{δ}, (αMe)Ser^2H_{β}–Ala^3H_{α}, (αMe)Ser^2H_{β}–Ala^3HN) and weak ((αMe)Ser^2H_{β}–Ala^1H_{α}, (αMe)Ser^2H_{β}–Ala^3H_{β}, (αMe)Ser^2H_{β}–Fmoc "ortho"), based on the peak integrals.

The temperature dependence of NH chemical shifts was analyzed in DMSO-d_6 within the range of 300–320 K. The temperature coefficients of NH^1 and NH^3 proton chemical shifts were found to be different and equal to −8.3/−0.50 and −4.8/−7.5 ppb K^{-1} for **6a** and **6b**, respectively. The observed temperature coefficients imply the presence of a strong hydrogen bonding interaction for **6a** and a weaker, if any, hydrogen bonding for **6b**.[36] The temperature effect on the ^{1}H NMR spectra of **6a** and **6b** in DMSO-d_6 is presented in Fig. S6 and S7 (ESI).† In addition in IR spectra of **6a** and **6b** recorded in $CDCl_3$ the additional broad signal below 3400 cm^{-1} (hydrogen-bonded NHs) was observed for **6a** only (Fig. S8, ESI).†

Crystal-state studies

It is remarkable that only a limited number of crystal structures of peptides containing a pseudoproline residue have been established so far. In particular, the dipeptides Cbz-Val-Thr($\Psi^{Me,Me}$Pro)-OMe and Cbz-Val-Thr($\Psi^{Me,Me}$Pro)-OH adopt a *trans*-conformation,[13] while a *cis*-amide bond conformation has been established for Fmoc-Ala-Cys($\Psi^{Me,Me}$Pro)-OH.[30b]

Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (tripeptide **6a**) crystals suitable for X-ray analysis were grown and used to determine its crystal-state structure. The peptide bond preceding $\Psi^{H,H}$Pro adopted the *trans* conformation as in solution. A view of the two symmetrically independent molecules **A** and **B** located in the asymmetric unit is given in Fig. 1 with atom labeling. Both symmetrically independent molecules are the same diastereoisomers with relative configurations (*S**,*R**,*S**) at atoms C16, C21, C24 and C46, C51, C54 in **A** and **B**, respectively.

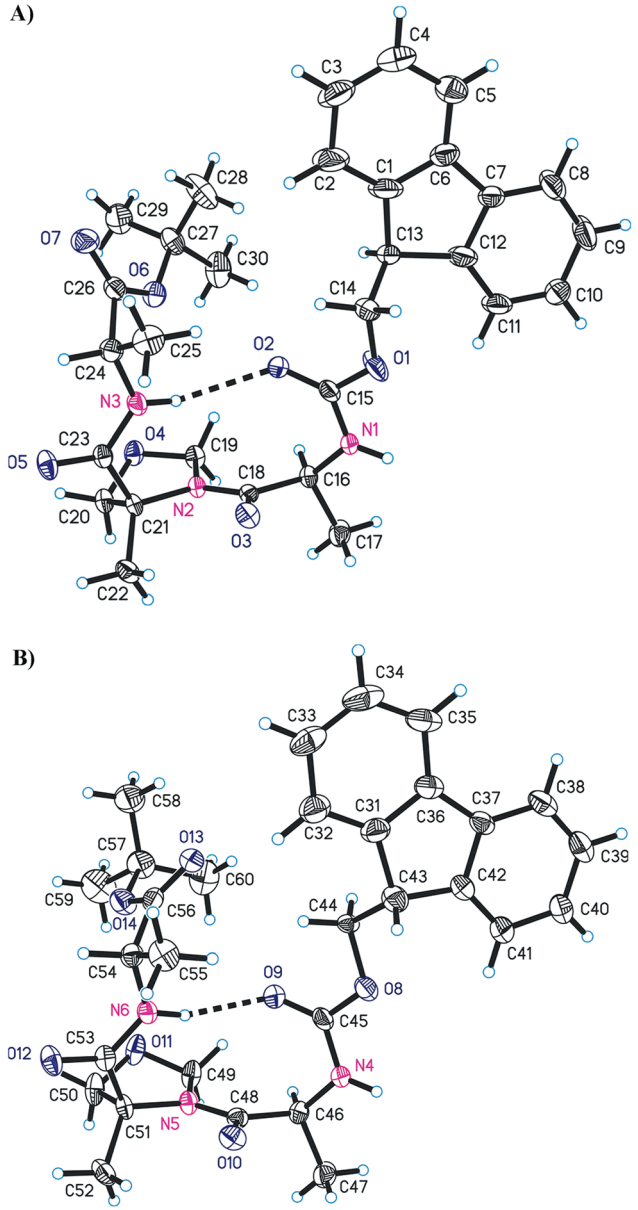


Fig. 1 Two symmetrically independent molecules **A** and **B** of Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (**6a**) with atom numbering. The intramolecular hydrogen bonding is indicated with a dashed line. Displacement ellipsoids are drawn at a 50% probability level.

The bond lengths and angles are quite close to those commonly encountered in related compounds.[37] The important feature influencing the peptide conformation, and especially crystal packing, is the existence of two symmetrically independent molecules in the asymmetric unit. For comparison, a superposition of the two independent molecules as determined by the least-squares fitting of the common fragments is presented in Fig. S9 (ESI).†

The observed backbone torsion angles, ψ, ψ, ω (°) for molecules **A** and **B** are: Ala[1], −57.4, 139.5, 179.0 (**A**), −58.8, 129.1, −179.6 (**B**); (αMe)Ser($\Psi^{H,H}$Pro), 78.8, 1.4, 176.0 (**A**), 69.6, 10.4, 178.2 (**B**); Ala[3], −124.5, −16.8, 177.6 (**A**), −131.9, 59.5, 171.9 (**B**). Both molecules adopt a conformation which resembles the β-turn type II[38] stabilized by a hydrogen bonding between the carbonyl group of the Fmoc protecting group first residue and the main chain nitrogen atom of the third residue (Fig. 1, ESI, Table S2†). The 1,3-oxazolidine rings adopt distorted envelope conformations with the 4-Ala substituent occupying axial positions. The main *pseudo*-symmetry elements are a mirror image passing through the C20 atom and a two fold axis passing through the N5 atom in the rings of molecules **A** and **B**, respectively. The endocyclic torsion angles and the three lowest asymmetry parameters[39] are summarized in Table S3 (ESI†). In the crystal, both symmetrically independent Fmoc residues are located almost perpendicular to one another. Their arrangement is stabilized by an unusual short intermolecular contact between the C5–H51 bond and the C31–C36 ring. The distance between the ring centroid Cg and the H51 atom is 2.76 Å, and the C5–H51···Cg angle is 169°. The environment of the molecules in the crystal with the hydrogen bond network is shown in Fig. S10 (ESI†). The hydrogen atoms bonded to atoms N1 and N4 are involved in intermolecular hydrogen bonds with carbonyl oxygen atoms. The hydrogen bonds form infinite chains, which extend along the *b* crystal axis.

Theoretical calculations

ROE cross-peaks and vicinal coupling constants were used as the geometrical restraints in theoretical calculations of **6a** and **6b** structures by means of the default simulated annealing protocol (*sa.inp*) implemented within the XPLOR-NIH program.[40] Structures of the main calculated clusters of peptides **6a** and **6b** are presented in Fig. 2 and 3.

Fig. 4 illustrates the good agreement between the structures determined in the crystal state and in solution for **6a**. The introduction of a methyl group at position 4 in the pseudoproline ring in tripeptides **6a** and **6b** caused a shift of *cis–trans* isomerization exclusively towards the *trans* isomer, as observed both in solution and in the crystal state.

We decided to investigate theoretically the influence of both modifications of the proline residue, the replacement of a carbon atom by an oxygen atom and the introduction of methyl group at the α-carbon atom on the puckering of the pseudoproline ring, *cis–trans* equilibrium, and rotational barriers of the amide bond *in vacuo*, in chloroform and in water. Calculations were carried out *in vacuo* for Ac-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-NHMe **7** (Fig. 5) at the B3LYP/6-31+G(d) level of theory and compared with literature data for proline (Pro), (*S*)-oxazolidine-4-carboxylic acid (Oxa) and Cys-derived (*R*)-thiazolidine-4-carboxylic acid (Thz), calculated at the HF/6-31+G(d) level.[14f]

The *trans* and *cis* isomer geometries were optimized for the *R* enantiomer. Transition states were located by calculating energy profiles along the ω' angle. The *trans* isomer was chosen as a starting point and the potential energy was scanned in two possible directions using 15° and −15° ω' steps. The resulting structures were further optimized as saddle points. The free energies of transition states differed by 2.5 kcal mol^{-1} in $CDCl_3$ and 4 kcal mol^{-1} in H_2O, depending on the direction of rotation. The lower reported values correspond to the transition state TS1 with the methyl and acetyl groups placed on the same side. The optimized *trans*, *cis* and TS structures of **7** are shown in Fig. 5. The

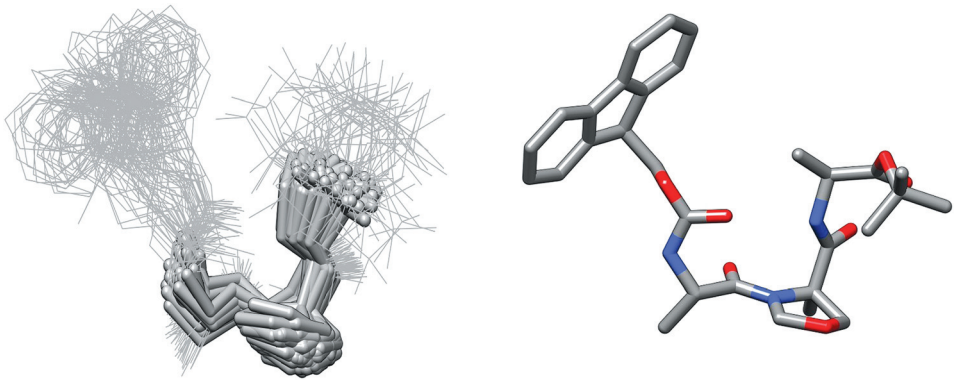


Fig. 2 Structures of the calculated main clusters (left) and the main central cluster structure (right) of Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (**6a**).

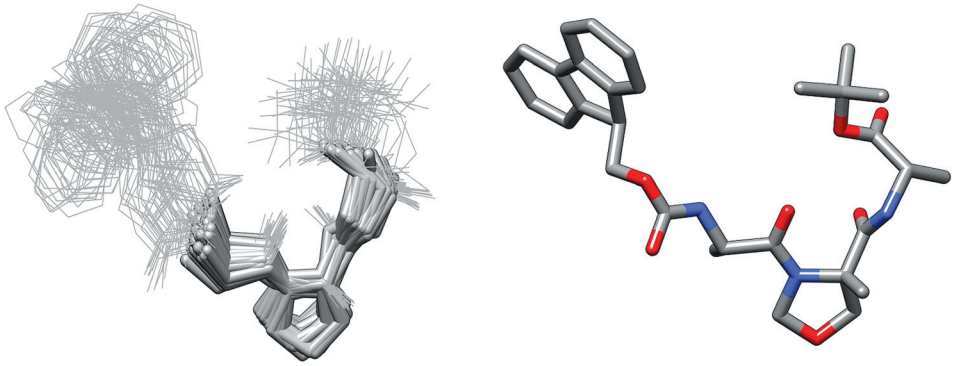


Fig. 3 Structures of the calculated main clusters (left) and the main central cluster structure (right) of Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (**6b**).

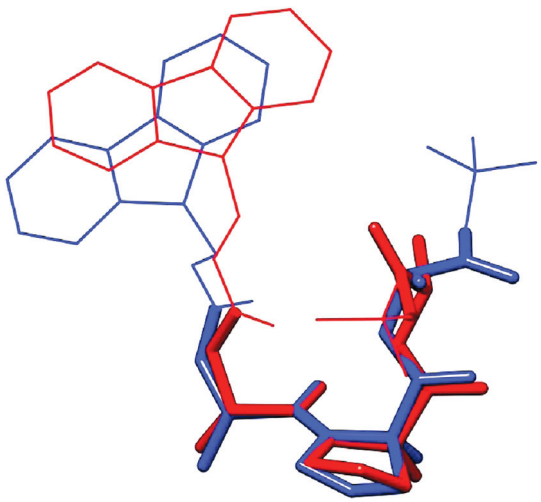


Fig. 4 Comparison of structures of **6a** determined in the crystal state by X-ray (red) and calculated from NMR data (blue).

conformation of **7** is down-puckered (C^{γ}-*endo*) independently of the geometry of amide bonds. The structures are stabilized by a weak hydrogen bond between NH (NHMe) and the nitrogen atom in the pseudoproline ring. The distance *d*(N–H···N) between the hydrogen atom and the acceptor nitrogen atom and angle N–H···N are 2.410 Å and 102.1° for the *trans* isomer, and 2.401 Å and 103.1° for the *cis* isomer, respectively. The stabilizing effect of the hydrogen bond was found for TS1 (2.257 Å and 105.6°), but not for TS2 (Fig. 5).

The calculated free energies of activation for the *trans*-to-*cis* rotation of Ac-Xxx-NHMe peptides are listed in Table 1. In chloroform and water the barriers of rotation for pseudoprolines are lower than for proline, except (2*R*)-CF_3-Oxa. The preferences of *cis* isomers in chloroform are in the order **7** > Oxa, CF_3-Oxa > Thz, Pro. In water with predominant *trans* isomers the highest populations of *cis* isomers are found for Oxa, CF_3-Oxa and Thz. The fractions of the *cis* isomer in pseudoproline **7** and proline are lower and comparable. This means that the effect of the presence of the oxygen atom was compensated by the steric effect of the methyl group.

The following parameters were used to characterize the pseudoproline ring: backbone torsion angles (ω, ϕ and ψ), endocyclic torsion angles χ_i (i = 0–4) (Fig. 6). and puckering amplitudes q_i (i = a, z or m). The parameter q_a of Han and Kang is the maximum angle between the mean plane and the line joining the geometrical center and each atom of the ring.[41] The parameter q_z of Cremer and Pople corresponds to the maximum z-displacement perpendicular to the mean plane of the ring.[42] The parameter χ_m of Altona and Sundaralingam is the maximum value attainable by the endocyclic torsion angles of the ring.[43] The *trans* ("t") and *cis* ("c") conformations of Ac–Xxx peptide

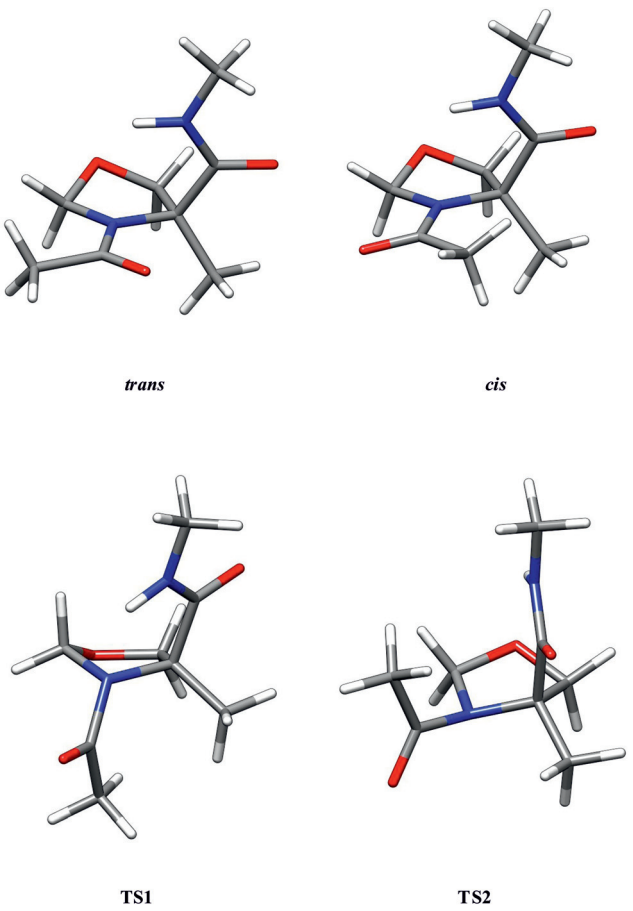


Fig. 5 Optimized structures of *trans* and *cis* isomers and transition states for *trans–cis* isomerization of Ac-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-NHMe **7**, optimized at the IEFPCM/B3LYP/6-31G(d) level.

bonds are defined by the orientation of the methyl carbon of the acetyl group and the C^{α} of the proline residue. If the γ-oxygen and the C═O group of the prolyl residue are on the same side of the plane defined by the three atoms C^{δ}, N and C^{α}, the conformation is defined as down-puckered. The opposite orientation of the oxygen atom corresponds to the up-puckered conformation.

The backbone conformations are described by capital letters in terms of the position on the $\phi - \psi$ map. Regions A and C are defined to lie within the following angles $-110° \leq \phi < -40°$ and $-90° \leq \psi < -10°$ (region A) or $50° \leq \psi < 130°$ (region C). Region B is defined as a bridge region between A and C.[44] The replacement of CH_2 in Pro by an oxygen atom, as in Oxa, or by a sulfur atom as in Thz, did not change significantly the backbone torsion angle values. Conformations of the *trans* and *cis* isomers of peptide **6** belong to regions B and A on the $\phi - \psi$ map, respectively. The ψ value of the *trans* isomer is very close to the boundary $\psi = 50°$ for the conformation C. For this reason conformation of the *trans* isomer of peptide **7** can be considered as C, equivalent to the γ-turn.

All puckering parameters q_i of the pseudoproline ring in **7** are lower in the transition state than in the *trans* and *cis* isomers (Table 2). The lower puckering in the transition state should facilitate *cis–trans* isomerization in a more crowded environment, in contrast to proline and unsubstituted pseudoprolines.[12,15f]

Table 1 Free energies of activation ($\Delta G^{\neq}$, kcal mol^{-1}) of *cis–trans* isomerization of Ac-Xxx-NHMe peptides and the fraction of the *cis* conformer in chloroform and water at IEFPCM/B3LYP/6-31+G(d) or determined experimentally

Peptide	*cis*, % Chloroform	$\Delta G^{\neq}$	*cis*, % Water	$\Delta G^{\neq}$
7	62	17.49	29	18.06
Pro[a]	9	19.32	23	21.61
Oxa[a]	18 (27[c])	16.85	39 (34[c])	18.45
Thz[a]	11	16.52	45	16.12
(2*S*)-CF_3-Oxa[b]	40[c]	18.47	43[c]	21.66
(2*R*)-CF_3-Oxa[b]	24[c]	14.22	45[c]	16.70

[a] From ref. 15*f*. [b] From ref. 12, calculations at B3LYP/6-311++G(d,p)//CPMC HF/6-31+G(d). [c] *Cis* content determined from NMR, ref. 12, in other cases calculated from $\Delta G = -RT \ln K$ where $K = [cis]/[trans]$.

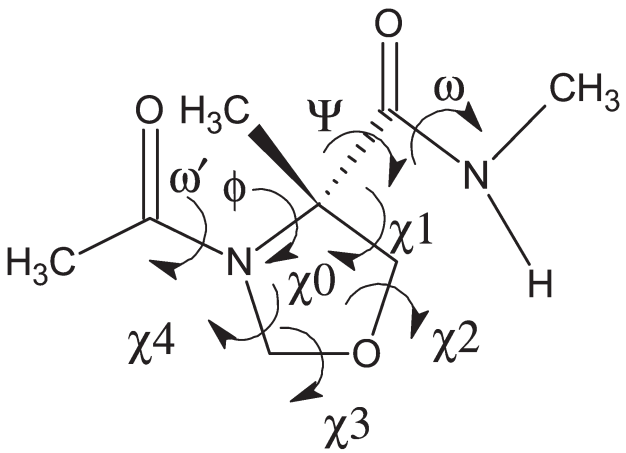


Fig. 6 Definition of torsion angles in peptide **7**.

Conclusions

4-Methylpseudoproline derived from α-methylserine after the protection of the NH-group is stable and can be isolated and stored for a long time. *N*-protected *C*-activated 4-methylpseudoproline is an efficient acylating component in the formation of peptide bonds. In the tripeptides Fmoc-Ala-(*R*)-(αMe)-Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (**6a**) and Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (**6b**) in solution, the amide bonds between Ala and (αMe)Ser($\Psi^{H,H}$Pro) adopt a *trans* geometry. The same geometry of this amide bond has been found for **6a** in the crystal state.

Theoretical calculations for the peptide Ac-(αMe)Ser($\Psi^{H,H}$Pro)-NHMe (**7**) have revealed that all the puckering parameters q_i of the pseudoproline ring are lower in the transition state than in the *trans* and *cis* isomers. The lower puckering in the transition state should facilitate *cis–trans* isomerization in a more crowded environment, in contrast to proline and unsubstituted pseudoprolines.

In chloroform, the *cis* isomer predominates for pseudoproline **7**, as opposed to proline existing almost exclusively as the *trans* isomer. In water, the fractions of the *cis* isomer are comparable for pseudoproline **7** and proline, suggesting that the effect of the presence of the oxygen atom in the proline ring is compensated by the steric effect of the methyl group.

Table 2 Backbone torsion angles, endocyclic torsion angles and puckering amplitudes of pseudoprolines **7** in Ac-Xxx-NHMe optimized at the B3LYP/6-31+G(d) level *in vacuo* and in solution

			Torsion angles (°)									Puckering amplitudes		
Peptide	Solvent	Isomer or TS	ω'	ϕ	ψ	ω	χ_0	χ_1	χ_2	χ_3	χ_4	q	q	χ_m
7[a]	*vacuo*	*trans*	−172.8	−75.2	47.3	175.9	−7.7	28.6	−40.9	35.1	−16.1	11.0	0.362	40.2
		cis	9.2	−75.3	−22.0	−174.1	−1.5	24.4	−39.8	37.9	−21.9	11.1	0.363	40.5
		TS	−50.5	−137.4	18.4	177.1	−22.5	0.6	22.5	−38.3	37.9	10.8	0.354	39.5
	H_2O	*trans*	−171.2	−74.5	−24.0	−176.8	−0.6	23.9	−40.0	38.7	−23.0	11.2	0.368	41.0
		cis	11.1	−79.2	−14.1	−177.0	−2.9	25.7	−40.7	38.0	−20.9	11.3	0.370	41.0
		TS	−50.3	−145.2	21.0	176.7	−27.9	9.0	14.2	−33.0	38.1	10.4	0.346	38.0
	$CHCl_3$	*trans*	−171.1	−75.7	−16.4	−178.2	−0.9	24.0	−39.9	38.4	−22.6	11.2	0.366	40.7
		cis	9.2	−75.6	−21.0	−175.2	−2.1	25.0	−40.4	38.1	−21.6	11.2	0.367	40.8
		TS	−50.7	−139.7	18.1	177.2	−23.8	2.6	20.6	−37.0	38.0	10.6	0.351	39.0

Experimental section

General remarks

All solvents were purified by conventional methods. Evaporations were carried out under reduced pressure. Optical rotations were measured in a 1 dm cell (1 ml) on a Horiba high-speed automatic polarimeter at 589 nm. For thin layer chromatography 250 nm silica gel GF precoated uniplates (Merck) were used with the following solvent systems: (A) $CHCl_3$–MeOH–AcOH 9 : 1 : 0.2, (B) EtOAc–hexane 9 : 1 : 0.1 (C) EtOAc–hexane 1 : 1, (D) $CHCl_3$–MeOH 19 : 1, (E) EtOAc–hexane 9 : 1. Chromatograms were visualized with chlorine followed by starch–KI and/or ninhydrin. Flash chromatography was performed with Merck silica gel 60 (230–400 mesh). HPLC was performed on a LDC/Milton-Roy analytical instrument using a Vydac C18 column (0.46 × 25 cm): flow 1.0 ml min^{-1}, detection at 220 nm with the following eluants (A) 0.05% trifluoroacetic acid in water and (B) 0.038% trifluoroacetic acid in acetonitrile–water 90 : 10 with a gradient application. IR spectra of solids were measured by using ATR method on FTIR spectrophotometer alpha (Bruker). FTIR absorption spectra were recorded using Nicolet 6700 FT-IR spectrophotometer. Solutions in deuterochloroform (99.8% d, Aldrich) (2 mM) were placed in a cell with KBr and path length of 1 mm. FAB-MS spectra were recorded on a Finnigan MAT 95 spectrometer. NMR spectra were recorded on a Bruker Avance II Plus spectrometer at 700 MHz (^{1}H) and 175 MHz (^{13}C), respectively. Measurements were carried out in deuterochloroform (99.96% d, Aldrich) at 25 °C with tetramethylsilane as the internal standard. ^{1}H and ^{13}C NMR resonances were assigned on the basis of COSY-DQF, ROESY, HMQC and HMBC experiments. ROESY spectra (recorded with 400 ms mixing time) were assigned and integrated and cross-peaks were classified cross as strong (1.8–2.5 Å), medium (1.8–3.5 Å) or weak (1.8–5.0 Å).

Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-OH (3a)

(*R*)-Methylserine **1a** (3.6 g, 30 mmol) was dissolved in 2 N NaOH (15 mL). Subsequently aqueous solution of formaldehyde (37%, 9 mL) was added dropwise under vigorous stirring and the resulting solution was stored for 48 h at room temperature, after which time the pH was 8–9. Then, THF (20 mL) was added to (*R*)-4-methylpseudoproline (**2a**) formed *in situ* and the solution was cooled to 0–5 °C. In order to perform acylation Fmoc-Ala-F (3.1 g, 10 mmol) solution in THF (10 mL) was added dropwise within 60 min, while pH was adjusted between 8 and 9 using saturated $NaHCO_3$ (~5 mL). The reaction mixture was stirred for another 30 min at 5 °C and then for 2 h at room temperature. After THF evaporation the reaction mixture was diluted with water (~30 mL) and extracted with diethyl ether (3 × 15 mL), and the diethyl ether extracts were discarded. After cooling to 0 °C, the solution was acidified with 6 N HCl (~15 mL) to pH 3–4. The suspension was extracted with ethyl acetate (3 × 30 mL). The organic phases were combined and washed with water (3 × 20 mL) and dried over $MgSO_4$. After evaporation the crude product was purified by flash chromatography (EtOAc–hexane–AcOH: 9 : 1 : 0.1) over silica gel to give 640 mg of **3a** in 15% yield; $[\alpha]_D$ = +4.59° (*c* 1, $CHCl_3$); R_f (A) = 0.53, R_f (B) = 0.22; HPLC (40–70% B, 25 min) t_R = 13.42 (100%); IR ν 3292, 2975, 2871, 1716, 1654, 1529, 1450, 1241, 1072, 962, 759, 740 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.37 (d, *J* = 6.5 Hz, 3H, C^β–H Ala), 1.66 (s, 3H, C^β–H (αMe) Ser), 3.83, (bd, *J* = 6.9 Hz, 1H, O–*CH*$_2$–C), 4.18 (bt, *J* = 6.7 Hz, 1H, *CH* Fmoc), 4.26–4.32 (m, 1H, C^α–H Ala), 4.32–4.42 (m, 3H, *CH*$_2$ Fmoc overlapped with O–C*H*$_2$–C), 5.08, (bs, 1H, N–C*H*$_2$–O), 5.22 (bs, 1H, N–C*H*$_2$–O), 5.78 (bs, 1H, NH), 7.29 (t, *J* = 7.4 Hz, 2H, C–H arom.), 7.37 (t, *J* = 7.4 Hz, 2H, C–H arom.), 7.57 (d, *J* = 7.4 Hz, 1H, C–H arom.), 7.59 (d, *J* = 7.4 Hz, 1H, C–H arom.), 7.76 (d, *J* = 7.4 Hz, 2H, C–H arom.); ^{13}C NMR (175 MHz, $CDCl_3$, TMS) δ 18.3 (C^β–H Ala), 19.5 (C^β–H (αMe) Ser), 47.1 (*CH* Fmoc), 49.2 (C^α–H Ala), 65.1 (C^{IV} (αMe)Ser), 67.2 (*CH*$_2$ Fmoc), 77.8 (O–*CH*$_2$–C), 80.2 (N–*CH*$_2$–O), 120.0, 125.1, 127.1, 127.8 (C–H arom.), 141.3, 143.7 (C arom.), 155.8 (C═O Fmoc), 170.2 (C═O (αMe)Ser), 173.7 (C═O Ala).

Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-OH (3b)

For the synthesis of **3b** the same procedure was applied as for **3a** using (*S*)-methylserine **1b** (3.6 g, 30 mmol). The pure product was obtained in 14% yield (600 mg); $[\alpha]_D$ = −27.09° (*c* 1, $CHCl_3$); HPLC (40–70% B, 25 min) t_R = 13.39 (100%); IR ν 3303, 2975, 2872, 1713, 1650, 1530, 1449, 1240, 1072, 946, 758, 739 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.36 (d, *J* = 6.8 Hz, 3H, C^β–H Ala), 1.69 (s, 3H, C^β–H (αMe)Ser), 3.91 (d, *J* = 8.9 Hz, 1H, O–C*H*$_2$–C), 4.26 (q, *J* = 6.8 Hz, 1H, C^α–H Ala), 4.28 (d, *J* = 8.9 Hz, 1H, O–C*H*$_2$–C), 4.21 (t, *J* = 6.9 Hz, 1H, *CH*

Fmoc), 4.36, 4.38 (dd, J = 1.8 Hz, J = 6.87 Hz, 2H, CH_2 Fmoc), 5.08 (d, J = 3.4 Hz, 1H, N–CH_2–O), 5.26 (d, J = 3.4 Hz, 1H, N–CH_2–O), 5.80 (bd, J = 7.2 Hz, 1H, NH), 7.29 (t, J = 7.4 Hz, 2H, C–H arom.), 7.37 (t, J = 7.4 Hz, 2H, C–H arom.), 7.58 (d, J = 7.4 Hz, 1H, C–H arom.), 7.60 (d, J = 7.4 Hz, 1H, C–H arom.), 7.76 (d, J = 7.4 Hz, 2H, C–H arom.); ^{13}C NMR (175 MHz, $CDCl_3$, TMS) δ 17.6 (C^{β}–H Ala), 19.7 (C^{β}–H (αMe) Ser), 47.1 (*CH* Fmoc), 49.2 (C^{α}–H Ala), 65.1 (C^{IV} (αMe)Ser), 67.2 (CH_2 Fmoc), 77.8 (O–CH_2–C), 80.2 (N–CH_2–O), 120.0, 125.1, 127.1, 127.8 (C–H arom.), 141.3, 143.7 (C arom.), 155.8 (C═O Fmoc), 170.1 (C═O (αMe)Ser), 173.8 (C═O Ala).

Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (6a from 3a) – method I

HCl·Ala-OBu^t (182 mg, 1 mmol) was added to the solution of **3a** (424 mg, 1 mmol), DIEA (0.35 mL, 2 mmol) and TBTU (321 mg, 1 mmol) in DCM (10 mL). After 12 h, the solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc (50 mL). The organic solution was washed with 5% $NaHCO_3$ (3 × 15 mL), water (3 × 15 mL), 10% $KHSO_4$ (3 × 15 mL) and water (3 × 15 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified on silica gel (EtOAc–hexane: 1 : 1) to yield 386 mg (70%) of **6a** as a foam. Crystallization from methanol–water gave the crystal form, mp. = 160–161 °C; $[\alpha]_D$ = +16.09° (c 1, $CHCl_3$); R_f (C) = 0.35, R_f(D) = 0.60; HPLC (50–80% B, 25 min) t_R = 15.36 (100%); IR ν 3326, 2977, 2871, 1703, 1660, 1530, 1478, 1251, 1152, 1071, 948, 759, 740 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.27 (d, J = 7.0 Hz, 3H, C^{β}–H Ala^3), 1.37 (d, J = 6.7 Hz, 3H, C^{β}–H Ala^1), 1.39 (s, 9H, Bu^t), 1.68 (s, 3H, C^{β}–H (αMe)Ser), 3.77 (d, J = 9.1 Hz, 1H, O–CH_2–C), 4.11 (q, J = 6.7 Hz, 1H, C^{α}–H Ala^1), 4.18 (t, J = 6.8 Hz, 1H, *CH* Fmoc), 4.38 (q, J = 7.0 Hz, 1H, C^{α}–H Ala^3), 4.28, 4.40 (dd, ABX, J = 6.8 Hz, J = 7.3 Hz, 2H, CH_2 Fmoc), 4.42 (d, J = 9.1 Hz, 1H, O–CH_2–C), 5.03 (d, J = 3.5 Hz, 1H, N–CH_2–O), 5.41 (d, J = 3.5 Hz, 1H, N–CH_2–O), 5.44 (bd, J = 6.7 Hz, 1H, NH^1), 7.17 (bd, J = 7.0 Hz, 1H, NH^3), 7.31 (t, J = 7.5 Hz, 2H, C–H arom.), 7.40 (t, J = 7.5 Hz, 2H, C–H arom.), 7.56 (d, J = 7.5 Hz, 1H, C–H arom.), 7.58 (d, J = 7.5 Hz, 1H, C–H arom.), 7.76 (d, J = 7.5 Hz, 2H, C–H arom.); ^{1}H NMR (700 MHz, DMSO-d$_6$) δ 1.12 (d, J = 7.3 Hz, 3H, C^{β}–H Ala^3), 1.18 (d, J = 6.9 Hz, 3H, C^{β}–H Ala^1), 1.30 (s, 9H, Bu^t), 1.46 (s, 3H, C^{β}–H (αMe)Ser), 3.79 (d, J = 8.8 Hz, 1H, O–CH_2–C), 4.11 (q, J = 6.7 Hz, 1H, C^{α}–H Ala^1), 4.18 (t, J = 6.8 Hz, 1H, *CH* Fmoc), 4.38 (q, J = 7.0 Hz, 1H, C^{α}–H Ala^3), 4.28, 4.40 (dd, ABX, J = 6.8 Hz, J = 7.3 Hz, 2H, CH_2 Fmoc), 4.36 (dd, J = 9.5, 6.0 Hz, 1H, O–CH_2–C), 5.03 (d, J = 3.5 Hz, 1H, N–CH_2–O), 5.06 (d, J = 3.7 Hz, 1H, N–CH_2–O), 5.28 (d, J = 3.7 Hz, 1H, NH^1), 7.18 (bd, J = 6.9 Hz, 1H, NH^3), 7.32 (t, J = 7.4 Hz, 2H, C–H arom.), 7.41 (t, J = 7.4 Hz, 2H, C–H arom.), 7.67 (dd, J = 7.2, 4.2 Hz, 2H, C–H arom.), 7.86 (d, J = 5.0 Hz, 1H, NH), 7.88 (d, J = 7.6 Hz, 2H, C–H arom.); ^{13}C NMR ($CDCl_3$, TMS) δ 16.8 (C^{β}–H Ala^3), 17.3 (C^{β}–H Ala^1), 18.7 (C^{β}–H (αMe)Ser), 27.5 (CH_3–C Bu^t), 46.6 (*CH* Fmoc), 48.6 (C^{α}–H Ala^3), 49.2 (C^{α}–H Ala^1), 65.2 (CH_3–*C* (αMe)Ser), 66.8 (CH_2 Fmoc), 79.6 (N–CH_2–O), 77.8 (O–CH_2–C), 81.0 (CH_3–*C* Bu^t), 119.5, 124.5, 126.5, 127.5 (C–H arom.), 140.8, 143.2 (C arom.), 155.8 (C═O Fmoc), 169.0 (C═O Ala^1), 170.2 (C═O (αMe)Ser), 171.3 (C═O Ala^3); HRFAB m/z calcd for $C_{30}H_{38}N_3O_7$ $[M + H]^+$ 552.2709, found 552.2704.

Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (6b from 3b) – method I

As reported for the synthesis of **6a** according to method **I**, the same procedure was applied to **6b**, starting from **3b**. The pure product was obtained in 73% yield (403 mg); $[\alpha]_D$ = −52.69° (c 1, $CHCl_3$); R_f (C) = 0.46, R_f(D) = 0.71; HPLC (50–80% B, 25 min) t_R = 14.18 (100%); IR ν 3303, 2977, 2887, 1719, 1657, 1527, 1450, 1248, 1152, 1071, 946, 759, 740 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.36 (d, J = 6.7 Hz, 3H, C^{β}–H Ala^3), 1.39 (d, J = 7.0 Hz, 3H, C^{β}–H Ala^1), 1.46 (s, 9H, *t*Bu), 1.72 (s, 3H, C^{β}–H (αMe)Ser), 3.77 (d, J = 8.9 Hz, 1H, O–CH_2–C), 4.28 (q, J = 7.0 Hz, 1H, C^{α}–H Ala^1), 4.24 (t, J = 6.8 Hz, 1H, *CH* Fmoc), 4.37–4.45 (m, 3H, C^{α}–H Ala^3 overlapped with CH_2 Fmoc), 4.56 (d, J = 8.9 Hz, 1H, O–CH_2–C), 5.14 (d, J = 2.8 Hz, 1H, N–CH_2–O), 5.20 (d, J = 2.8 Hz, 1H, N–CH_2–O), 5.62 (bd, J = 7.0 Hz, 1H, NH^1), 7.35 (t, J = 7.5 Hz, 2H, C–H arom.), 7.41–7.46 (m, 3H, NH^3 overlapped with C–H arom.), 7.61 (d, J = 7.5 Hz, 1H, C–H arom.), 7.62 (d, J = 7.5 Hz, 1H, C–H arom.), 7.79 (d, J = 7.5 Hz, 2H, C–H arom.); ^{13}C NMR (175 MHz, $CDCl_3$, TMS) δ 17.8 (C^{β}–H Ala^3), 17.9 (C^{β}–H Ala^1), 19.0 (C^{β}–H (αMe)Ser), 27.5 (CH_3–C Bu^t), 46.7 (*CH* Fmoc), 48.6 (C^{α}–H Ala^3), 49.0 (C^{α}–H Ala^1), 65.6 (CH_3–*C* (αMe)Ser), 66.6 (CH_2 Fmoc), 80.0 (N–CH_2–O), 77.2 (O–CH_2–C), 81.6 (CH_3–*C* Bu^t), 119.6, 124.7, 126.6, 127.3 (C–H arom.), 140.9, 143.4 (C arom.), 155.8 (C═O Fmoc), 169.5 (C═O Ala^1), 170.2 (C═O (αMe) Ser), 171.5 (C═O Ala^3); HRFAB m/z calcd for $C_{30}H_{38}N_3O_7$ $[M + H]^+$ 552.2709, found 552.2703.

Z-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-OH (4a)

(*R*)-Methylserine **1a** (1.2 g, 10 mmol) was dissolved in 2 N NaOH (5 mL). Then, an aqueous solution of formaldehyde (37%, 3 mL) was added dropwise under vigorous stirring and the resulting solution was stored for 48 h at room temperature, after which time the pH was 8–9. Then, $NaHCO_3$ (2 g, 24 mmol) and acetone (10 mL) were added to (*R*)-4-methylpseudoproline (**2a**) formed *in situ*, and the solution was cooled to 0–5 °C. Subsequently Cbz–Cl (3 mL, 15 mmol) was added dropwise under vigorous stirring, while the pH was adjusted to 9 with saturated $NaHCO_3$ (~1 mL). The reaction mixture was stirred for the next 30 min at 5 °C and for 2 h at room temperature. Then, acetone was evaporated, the reaction mixture was diluted with water (~30 mL) and extracted with diethyl ether (3 × 10 mL), the diethyl ether extracts were discarded. After cooling to 0 °C, the solution was acidified with 6 N HCl (~5 mL) to pH 3–4. The suspension was extracted with ethyl acetate (3 × 15 mL). The organic phases were combined and washed with water (3 × 10 mL) and dried over $MgSO_4$. After evaporation of the solvent the pure product was obtained in 72% yield (3.8 g) as a foam, $[\alpha]_D$ = +37.09° (c 1, $CHCl_3$); R_f (A) = 0.65, R_f (B) = 0.43; HPLC (10–90% B, 25 min) t_R = 15.15 (100%); IR ν 3317, 2971, 2871, 1704, 1408, 1352, 1240, 1064, 920,768, 716, 660 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.72 (s, 3H, C^{β}–H), 3.86 (d, J = 9.0 Hz, 1H, O–CH_2–C), 4.34

(d, J = 9.0 Hz, 1H, O–CH_2–C), 5.02 (d, J = 2.9 Hz, 1H, N–CH_2–O), 5.09 (d, J = 2.9 Hz, 1H, N–CH_2–O), 5.18 (bs, 2H, CH_2–Ph), 7.37 (bs, 5H, C–H arom.); ^{13}C NMR (175 MHz, $CDCl_3$, TMS) δ 19.5 (C^{β}–H (αMe)Ser), 63.9 (C^{IV} (αMe)Ser), 67.5 (CH_2–Ph), 77.9 (N–CH_2–O), 80.2 (O–CH_2–C), 127.9, 128.2, 128.5 (C–H arom.), 135.8 (C^{IV} Ph), 152.9 (C═O Z), 176.3 (C═O (αMe)Ser).

Z-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-OH (4b)

As reported for the synthesis of **4a**, the same procedure was applied to **4b**, starting from (*S*)-methylserine **1b** (1.2 g, 10 mmol). The pure product was obtained in 70% yield (3.7 g); $[\alpha]_D$ = −36.69° (*c* 1, $CHCl_3$); HPLC (10–90% B, 25 min) t_R = 15.13 (100%); IR ν 3339, 2978, 2871, 1705, 1411, 1358, 1241, 1066, 921,770, 713, 659 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.72 (s, 3H, C^{β}–H), 3.89 (d, J = 9.0 Hz, 1H, O–CH_2–C), 4.33 (d, J = 9.0 Hz, 1H, O–CH_2–C), 5.02 (d, J = 2.9 Hz, 1H, N–CH_2–O), 5.09 (d, J = 2.9 Hz, 1H, N–CH_2–O), 5.18 (bs, 2H, CH_2–Ph), 7.37 (bs, 5H, C–H arom.), C^{13} NMR ($CDCl_3$, TMS) 19.5 (C^{β}–H (αMe)Ser), 63.9 (C^{IV} (αMe)Ser), 67.5 (CH_2–Ph), 77.8 (N–CH_2–O), 80.2 (O–CH_2–C), 127.9, 128.2, 128.5 (C–H arom.), 135.8 (C^{IV} Ph), 152.9 (C═O Z), 176.4 (C═O (αMe) Ser).

Z-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (5a)

HCl × Ala-OBu^t (0.91 g, 5 mmol) was added to a solution of **4a** (1.3 g, 5 mmol), DIEA (1.7 mL, 10 mmol) and TBTU (1.6 g, 5 mmol) in DCM (30 mL). After 12 h, the solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc (100 mL). The organic solution was washed with 5% $NaHCO_3$ (3 × 30 mL), water (3 × 30 mL), 10% $KHSO_4$ (3 × 30 mL) and water (3 × 30 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified on silica gel (EtOAc–hexane: 1 : 1) to yield 1.5 g (76%) of **5a** as a foam; $[\alpha]_D$ = +70.49° (*c* 1, $CHCl_3$); R_f (C) = 0.35, R_f(D) = 0.60; HPLC (40–80% B, 25 min) t_R = 14.47 (100%); IR ν 3317, 2971, 2871, 1707, 1647, 1534, 1410, 1349, 1152, 1050, 946, 809, 733, 695 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.32 (d, J = 6.7 Hz, 3H, C^{β}–H Ala), 1.48 (s, 9H, Bu^t), 1.67 (s, 3H, C^{β}–H (αMe)Ser), 3.77–3.80 (m, 2H, O–CH_2–C), 4.39–4.40 (m, 1H, C^{α}–H Ala), 5.01–5.17 (m, 2H, N–CH_2–O), 5.22–5.26 (m, 2H, CH_2–Ph), 7.34–7.35 (bm, 1H, NH), 7.36–7.39 (m, 5H, C–H arom.); ^{13}C NMR (175 MHz, $CDCl_3$, TMS) δ 18.1 (C^{β}–H Ala), 19.7 (C^{β}–H (αMe)Ser), 28.0 (CH_3–C Bu^t), 49.0 (C^{α}–H Ala), 63.9 (C^{IV} (αMe)Ser), 67.4 (CH_2–Ph), 80.5 (N–CH_2–O), 81.9 (O–CH_2–C), 128.1, 128.3, 128.6 (C–H arom.), 135.9 (C^{IV} Ph), 152.8 (C═O Z), 171.4 (C═O Ala), 171.8 (C═O (αMe)Ser).

Z-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (5b)

As reported for the synthesis of **5a**, the same procedure was applied to **5b**, starting from **4b** (1.3 g, 5 mmol). The pure product was obtained in 82% yield (1.6 g), $[\alpha]_D$ = −36.39° (*c* 1, $CHCl_3$); R_f (C) = 0.35, R_f(D) = 0.60; HPLC (40–80% B, 25 min) t_R = 13.39 (100%); IR ν 3339, 2978, 2871, 1697, 1673, 1517, 1407, 1349, 1152, 1051, 953, 764, 745, 697 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$, TMS) δ 1.33 (d, J = 6.7 Hz, 3H, C^{β}–H Ala), 1.46 (s, 9H, Bu^t), 1.65 (s, 3H, C^{β}–H (αMe)Ser), 3.66–3.81 (bm, 2H, O–CH_2–C), 4.36 (q, J = 6.7 Hz, 1H, C^{α}–H Ala), 4.97 (bd, J = 3.0 Hz, 1H, N–CH_2–O), 5.13 (bd, J = 3.0 Hz, 1H, N–CH_2–O), 5.14 (d, J = 12.2 Hz, 1H, CH_2–Ph), 5.22 (d, J = 12.2 Hz, 1H, CH_2–Ph), 7.34–7.35 (bm, 1H, NH), 7.37–7.39 (m, 5H, C–H arom.); ^{13}C NMR (175 MHz, $CDCl_3$, TMS) δ 18.1 (C^{β}–H Ala), 19.8 (C^{β}–H (αMe)Ser), 28.0 (CH_3–C Bu^t), 49.0 (C^{α}–H Ala), 63.9 (C^{IV} (αMe)Ser), 67.5 (CH_2–Ph), 80.5 (N–CH_2–O), 81.9 (O–CH_2–C), 128.1, 128.3, 128.6 (C–H arom.), 135.9 (C^{IV} Ph), 153.0 (C═O Z), 171.4 (C═O Ala), 171.7 (C═O (αMe) Ser).

Fmoc-Ala-(*R*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (6a from 5a) – method II

A suspension of **5a** (432 mg, 1.1 mmol) and 10% cat. Pd/C (50 mg) in EtOAc (20 mL) was stirred for 2 h under H_2 (1 atm). The palladium carbon catalyst was filtered off over Celite, and the solution was concentrated *in vacuo*. The crude residue was dissolved in DCM (10 mL) and HCl × Ala-OBu^t (182 mg, 1 mmol), DIEA (0.52 mL, 3 mmol) and 50% PPAA in DMF (1.5 mL, 2.5 mmol) were added. After 12 h, the solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc (50 mL). The organic solution was washed with 5% $NaHCO_3$ (3 × 15 mL), water (3 × 15 mL), 10% $KHSO_4$ (3 × 15 mL), water (3 × 15 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified on silica gel (EtOAc–hexane: 1 : 1) to yield 210 mg (38%) of **6a** as a foam.

Fmoc-Ala-(*S*)-(αMe)Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (6b from 5b) – method II

As reported for the synthesis of **6a** by using method **II**, the same procedure was applied to the **6b**, starting from **5b** (1.1 mmol, 432 mg). The pure product was obtained in 34% yield (190 mg).

Molecular dynamics

All density functional calculations were carried out using the Gaussian 03 package.[45] Geometry optimizations employed the B3LYP/6-31+G(d) level of theory.[46] The solvation effects on conformational preferences were estimated with a polarizable continuum model (IEFPCM) in chloroform and water.[47] Vibrational frequencies were calculated for optimized structures at the same theory level in order to confirm that all of the optimized structures are true stationary points at the potential energy surface. Gibbs free energies, enthalpies and entropies were calculated at temperature of 298.15 K, 1 atm, with pressure and vibrational frequencies were not scaled.

X-ray single crystal structure analysis for Fmoc-Ala-(*R*)-(αMe)-Ser($\Psi^{H,H}$Pro)-Ala-OBu^t (6a)

Formula: $C_{30}H_{37}N_3O_7$, M_w = 551.63, colorless crystal 0.10 × 0.01 × 0.02 mm, a = 38.885(2), b = 7.501(1), c = 24.170(1) Å, V = 5906.7(5) Å^3, ρ_{calc} = 1.241 g cm^{-3}, μ = 1.03 cm^{-1}, semi-

empirical absorption correction based on multiple scanned equivalent reflections ($0.927 < T < 0.973$), $Z = 8$, crystal system: monoclinic, space group: $C2$, $\lambda = 0.68980$ Å, $T = 100$ K, ω scans, 28 384 reflections collected ($\pm h$, $\pm k$, $\pm l$), $2\theta_{max} = 55°$, 7930 unique reflections ($R_{int} = 0.076$) and 7318 observed reflections [$I \geq 2\sigma(I)$], 760 refined parameters, refinement in F^2, $R_{all} = 0.061$, $wR(F^2) = 0.1763$, max (min) residual electron density $\Delta\rho_{max} = 0.88$, $\Delta\rho_{min} = -0.54$) e Å^{-3}, hydrogen atoms were refined as riding on their parent carbon or nitrogen atoms. In the absence of significant anomalous scattering effects, Friedel pairs were merged for the final refinement cycles. Several crystallization attempts from water–methanol and water–ethanol mixtures yielded aggregates of small crystals, too weakly diffracting for analysis with standard laboratory X-ray sources. The final X-ray data were collected with a Bruker SMART APEX CCD area detector diffractometer mounted at station 9.8 of the Synchrotron Radiation Source at Daresbury Laboratory, Cheshire, England. Computer programs used: data collection SMART APEX,[48] data reduction SAINT-Plus,[49] absorption correction SADABS,[50] structure solution, refinement and molecular graphics SHELXTL.[51] CCDC 870748.

Acknowledgements

This work was partially supported by grant 4 T09A 065 22 from the State Committee for Scientific Research (Poland). Computer time was provided by the Interdisciplinary Center for Mathematical and Computational Modeling, Warsaw University (Poland). Authors are indebted to Dr Krzysztof Huben for final form of graphics preparation.

References

1 L. Moroder, C. Renner, J. J. Lopez, G. Tuchscherer and M. Mutter, Tailoring the cis–trans isomerization of amides, in *cis-trans Isomerization in Biochemistry*, ed. C. Dugave, Viley-VCH, Weinheim, 2006, ch. 11.
2 J. L. Flippen-Anderson, R. Gilardi, I. L. Karle, M. H. Frey, S. J. Opella, L. M. Gierasch, M. Goodman, V. Madison and N. G. Delaney, *J. Am. Chem. Soc.*, 1983, **105**, 6609–6614.
3 N. G. Delaney and V. Madison, *J. Am. Chem. Soc.*, 1982, **22**, 869–877.
4 Y. K. Kang, B. J. Byun and H. S. Park, *Biopolymers*, 2011, **95**, 51–61.
5 S. S. A. An, C. C. Lester, J. L. Peng, Y. L. Li, D. M. Tothwarf, E. Welker, T. W. Thannhauser, L. S. Zhang, J. P. Tam and H. A. Scheraga, *J. Am. Chem. Soc.*, 1999, **121**, 11558–11566.
6 E. Beausoleil and W. D. Lubell, *J. Am. Chem. Soc.*, 1996, **118**, 12902–12908.
7 (*a*) A. Moretto, F. Terrenzani, M. Crisma, F. Formaggio, B. Kaptein, Q. B. Broxterman and C. Toniolo, *Biopolymers*, 2008, **89**, 465–470; (*b*) M. De Poli, A. Moretto, M. Crisma, C. Peggion, F. Formagio, B. Kaptein, Q. B. Broxterman and C. Toniolo, *Chem.–Eur. J.*, 2009, **15**, 8015–8025.
8 T. Wöhr, F. Wah, A. Nefzi, B. Rohwedder, T. Sato, X. Sun and M. Mutter, *J. Am. Chem. Soc.*, 1996, **118**, 9218–9227.
9 D. Kern, M. Schutkowski and T. Drakenberg, *J. Am. Chem. Soc.*, 1997, **119**, 8403–8408.
10 P. Dumy, M. Keller, D. E. Ryan, B. Rohwedder, T. Wöhr and M. Mutter, *J. Am. Chem. Soc.*, 1997, **119**, 918–925.
11 M. Keller, C. Sager, P. Dumy, M. Schutkowski, G. S. Fischer and M. Mutter, *J. Am. Chem. Soc.*, 1998, **120**, 2714–2720.
12 D. Feytens, G. Chaume, G. Chassaing, S. Lavielle, T. Brigaud, B. J. Byun, Y. K. Kang and E. Miclet, *J. Phys. Chem. B*, 2012, **116**, 4069–4079.
13 J. K. Clegg, J. R. Cochrane, N. Sayyadi, D. Skropeta, P. Turner and K. A. Jolliffe, *Aust. J. Chem.*, 2009, **62**, 711–719.
14 (*a*) F. Bernardi, M. Garavelli, M. Scatizzi, C. Tomasini, V. Trigar, M. Crisma, F. Formaggio, C. Peggion and C. Toniolo, *Chem.–Eur. J.*, 2002, **8**, 2516–2525; (*b*) G. Angelici, G. Falini, H.-J. Hofmann, D. Huster, M. Monari and C. Tomasini, *Angew. Chem., Int. Ed.*, 2008, **47**, 8075–8078; (*c*) G. Angelici, G. Falini, H.-J. Hofmann, D. Huster, M. Monari and C. Tomasini, *Chem.–Eur. J.*, 2009, **15**, 8037–8048; (*d*) R. De Marco, A. Tolomelli, M. Campitiello, P. Rubini and L. Gentilucci, *Org. Biomol. Chem.*, 2012, **10**, 2307–2317.
15 (*a*) W. L. Jorgensen and J. Gao, *J. Am. Chem. Soc.*, 1988, **110**, 4212–4216; (*b*) S. Fischer, R. L. Dunbrack and M. Karplus, *J. Am. Chem. Soc.*, 1994, **116**, 11931–11937; (*c*) J. S. Jhon and Y. K. Kang, *J. Phys. Chem. A*, 1999, **103**, 5436–5439; (*d*) Y. K. Kang, *J. Mol. Struct.*, 2004, **675**, 37–45; (*e*) Y. K. Kang, *J. Phys. Chem. B*, 2004, **108**, 5463–5465; (*f*) Y. K. Kang and H. S. Park, *Biophys. Chem.*, 2005, **113**, 93–101; (*g*) Y. K. Kang, J. S. Jhon and H. S. Park, *J. Phys. Chem. B*, 2006, **110**, 17645–17655; (*h*) Y. K. Kang, *J. Phys. Chem. B*, 2007, **111**, 12551–12562.
16 (*a*) I. K. Song and Y. K. Kang, *J. Phys. Chem. B*, 2005, **109**, 16982–16987; (*b*) I. K. Song and Y. K. Kang, *J. Phys. Chem. B*, 2006, **110**, 1915–1927; (*c*) Y. K. Kang, *J. Phys. Chem. B*, 2002, **106**, 2074–2082; (*d*) Y. K. Kang and H. S. Park, *J. Phys. Chem. B*, 2007, **111**, 12551–12562; (*e*) A. E. Aliev and D. Courtier-Murias, *J. Phys. Chem. B*, 2007, **111**, 14034–14042.
17 Y. K. Kang, H. S. Park and B. J. Byun, *Biopolymers*, 2009, **91**, 445–455.
18 R. Berisio, L. Vitagliano, L. Mazzarella and A. Zagari, *Biopolymers*, 2000–2001, **56**, 8–13.
19 K. Okuyama, C. Hongo, R. Fukushima, G. Wu, H. Narita, K. Noguchi, Y. Tanaka and N. Nishino, *Biopolymers*, 2004, **76**, 367–377.
20 A. Wittelsberger, M. Keller, L. Scarpellino, L. Patiny, H. Acha-Orbea and M. Mutter, *Angew. Chem., Int. Ed.*, 2000, **39**, 1111–1115.
21 M. Keller, T. Wöhr, P. Dumy, L. Patiny and M. Mutter, *Chem.–Eur. J.*, 2000, **6**, 4358–4363.
22 L. Patiny, J.-F. Guichou, M. Keller, O. Turpin, T. Rückle, P. Lhote, T. M. Buetler, U. T. Ruegg, R. M. Wenger and M. Mutter, *Tetrahedron*, 2003, **59**, 5241–5249.
23 A. Wittelsberger, L. Patiny, J. Slaninova, C. Barberis and M. Mutter, *J. Med. Chem.*, 2005, **48**, 6553–6562.
24 M. S. Y. Wong and K. A. Jolliffe, *Aust. J. Chem.*, 2010, **63**, 797–801.
25 B. Zhang, J. Gong, Y. Yang and S. Dong, *J. Pept. Sci.*, 2011, **17**, 601–603.
26 M. R. Davies, E. K. Singh, H. Wahyudi, L. D. Alexander, J. B. Kunicki, L. A. Nazarowa, K. A. Fairweather, A. M. Giltrap, K. A. Jolliffe and S. R. McAlpine, *Tetrahedron*, 2012, **68**, 1029–1051.
27 M. Keller, C. Boissard, L. Patiny, N. N. Chung, C. Lemieux, M. Mutter and P. W. Schiller, *J. Med. Chem.*, 2001, **44**, 3896–3903.
28 J. Katarzyńska, N. N. Chung, P. W. Schiller, M. T. Leplawy and J. Zabrocki, Analogues of endomorphin-2 modified with 4-methylpseudoproline in position 2, in *Peptides 2004, Proc. 28th Eur. Pept. Symp.*, ed. M. Flegel, M. Fridkin, Ch. Gilon and J. Slaninova, KENES Int., Prague, 2004, pp. 982–983.
29 A. Katarzyńska, S. Jankowski, K. Huben, M. T. Leplawy and J. Zabrocki, Peptides of 4-methyl-pseudoproline derived from α-methylserine: synthetic approach and synthetic aspects, in *Peptides 2002, Proc. 27th Eur. Peptide Symp.*, ed. E. Benedetti and C. Pedone, Edizioni Ziino, Napoli, 2002, pp. 160–161.
30 (*a*) T. Haack and M. Mutter, *Tetrahedron Lett.*, 1992, **33**, 1589–1592; (*b*) A. Nefzi, K. Schenk and M. Mutter, *Protein Pept. Lett.*, 1994, **1**, 66–69; (*c*) T. Wöhr and M. Mutter, *Tetrahedron Lett.*, 1995, **36**, 3847–3848; (*d*) M. Mutter, A. Nefzi, T. Sato, X. Sun, F. Wahl and T. Wöhr, *Pept. Res.*, 1995, **8**, 145–153.
31 (*a*) Z. J. Kamiński, M. T. Leplawy and J. Zabrocki, *Synthesis*, 1973, 792–793; (*b*) Z. J. Kamiński and M. T. Leplawy, *Synthesis*, 1974, 292–293.
32 (*a*) V. Dourtoglou, J.-C. Ziegler and B. Gross, *Tetrahedron Lett.*, 1978, **19**, 1269–1272; (*b*) V. Dourtoglou, B. Gross, V. Lambropoulou and C. Ziodrou, *Synthesis*, 1984, 572–574.
33 M. Falorni, S. Conti, G. Giacomelli, S. Cossu and F. Soccolini, *Tetrahedron: Asymmetry*, 1995, **6**, 287–294.
34 D. Seebach, T. L. Sommerfeld, Q. Jiang and L. M. Venanzi, *Helv. Chim. Acta*, 1994, **77**, 1313–1330.
35 (*a*) H. Wissmann and H.-J. Kleiner, *Angew. Chem.*, 1980, **92**, 129–130; (*b*) J. Klose, M. Bienert, C. Mollenkopf, D. Wehle, C. Zhang, L. A. Carpino and P. Henklein, *Chem. Commun.*, 1999, 1847–1848.
36 H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 512–523.
37 F. H. Allen, D. G. Watson, L. Brammer, A. G. Orpen and R. Taylor, in *International Tables for Crystallography*, ed. E. Prince, Kluwer Academic Publishers, Dordrecht, 2004, vol. C, pp. 790–811.

38 C. M. Venkatachalan, *Biopolymers*, 1968, **6**, 1425–1436.
39 J. F. Griffin, W. L. Duax and C. M. Weeks, *Atlas of Steroid Structure*, IFI/Plenum, New York, 1984, vol. 2, p. 8.
40 C. D. Schwieters, J. J. Kuszewski, J. Tjandra and G. M. Clore, *J. Magn. Reson.*, 2003, **160**, 66–74.
41 S. J. Han and Y. K. Kang, *J. Mol. Struct. (THEOCHEM)*, 1996, **362**, 243–255.
42 D. Cremer and J. A. Pople, *J. Am. Chem. Soc.*, 1975, **97**, 1354–1358.
43 C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, 1972, **94**, 8205–8212.
44 S. S. Zimmerman, M. S. Pottle, G. Némethy and H. A. Scheraga, *Macromolecules*, 1977, **10**, 1–9.
45 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *Gaussian 03*, Gaussian Inc., Wallingford, CT, 2004.
46 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652.
47 S. Miertus, E. Scrocco and J. Tomasi, *Chem. Phys.*, 1981, **55**, 117–129.
48 *Bruker. Apex2, Version 2010/3; Bruker AXS*, Madison, Wisconsin, 2010.
49 *Bruker. Saint-plus, Version 7.68A*, Bruker AXS, Madison, Wisconsin, 2009.
50 *Bruker. Sadabs – Bruker Nonius Area Detector Scaling and Absorption Correction, Version 2008/1; Bruker AXS*, Madison, Wisconsin, 2008.
51 G. M. Sheldrick, *SHELXTL, version 2008/4*, Bruker AXS, Madison, Wisconsin, 2008.

