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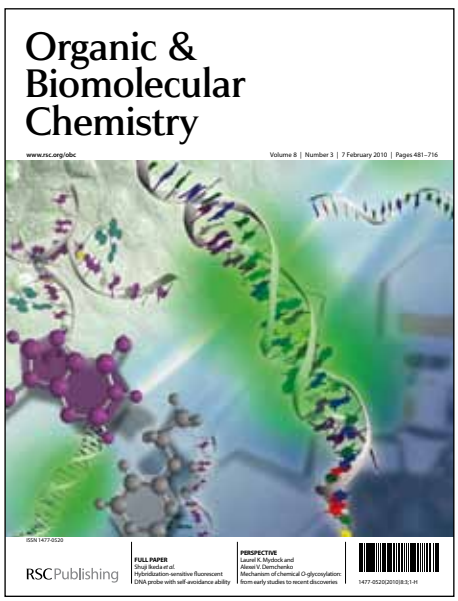

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Conformational behaviour of peptides containing 2-pyrrolidinemethanesulfonic acid residue (2PyMS)

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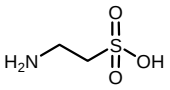
Conformational behaviour of model peptides containing 2-pyrrolidinemethanesulfonic acid residue (2PyMS) was studied in both crystalline state and in solution using X-Ray, NMR and IR experiments. It was found that in crystals dipeptide PhC(O)–2PyMS–Phe–NH*i*Pr adopted β-turn conformation, which was not stabilized by intramolecular hydrogen bond and could be classified as a type IV β-turn. In the crystalline state tripeptide PhC(O)–Ala–2PyMS–Phe–NH*i*Pr existed as an α-turn with uncommon *cis*-conformation of the amide bond formed by the pyrrolidine nitrogen atom of the 2PyMS residue, for which no close analogue can be envisaged among the tight turns identified so far. Although the tendency to adopt folded conformations was only partially retained in solution, 2-PyMS could be considered as a promising structural unit for the design of foldamers and peptidomimetics with unusual conformational properties.

Introduction

Structural modification of peptide backbones is a valuable approach to the design of peptidomimetics, peptide models and other molecules of both biological relevance and theoretical interest.[1] Sulfonamide is one of the possible surrogates used for the replacement of the peptide bond. It was introduced by Liskamp *et al.* as a transition-state analogue of the hydrolysis of the amide bond nearly 20 years ago.[2] A number of studies on the use of "sulfopeptides" in the design of biologically active compounds were reported since then. The nearest analogues of the peptides, sulfonamido-α-peptides, have found limited application due to the low stability of α-aminosulfonic acids and their derivatives.[3] On the contrary, sulfonamido-β-peptides have attracted much interest, in particular, for the design of potential tools for tumor imaging and/or radionuclide therapy,[4] PPI inhibitors,[5] chemotactic peptides,[6] HIV-1 non-nucleoside reverse transcriptase inhibitors,[7] factor Xa inhibitors,[8] inhibitors of leukocyte adhesion,[9] and scaffolds for MC4 pharmacophoric groups.[10]

It should be noted that most of these studies employed derivatives of the simplest β-aminosulfonic acid – taurine (**1**). It was shown, however, that peptides containing sulfonamide moiety demonstrated considerable flexibility,[11] whereas limitation of this property has been widely considered as desirable for both biological and conformational studies.[12] From this point of view, derivatives of the conformationally restricted β-aminosulfonic acids are much more promising. In particular, peptides derived from (*S*)-2-pyrrolidinemethanesulfonic acid (**2**) were evaluated as endomorphin-2 and morphiceptin analogues,[13] fibrillogenesis peptide inhibitors;[14] they were also used in the design of chiral ligands for enantioselective catalysis.[15]



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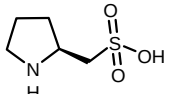

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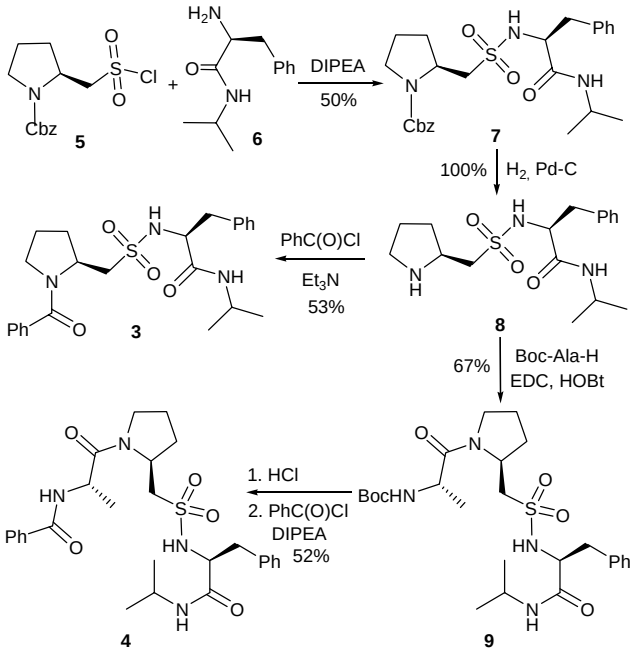
Whereas biological properties of sulfonamide peptides have aroused considerable interest, their conformational behavior has attracted much less attention. To the best of our knowledge, all of these studies dealt with the taurine-derived peptides.[16] Conformational preferences of peptides containing residue of **2** (for which we propose an abbreviation "2PyMS") were beyond the scope of investigation to date.

In this work, we report an experimental study on the conformational properties of the model peptides containing residues of (*S*)-2-pyrrolidinemethanesulfonic acid and phenylalanine. These peptides can be considered as analogues of the Pro–Phe sequence, which attracted much attention as a target for the replacement studies in the last decade.[17]

Results and discussion

Synthesis

Model peptides **3** (PhC(O)–2PyMS–Phe–NH*i*Pr) and **4** (PhC(O)–Ala–2PyMS–Phe–NH*i*Pr) were prepared from (*S*)-2-pyrrolidine-methanesulfonyl chloride derivative **5**[18] using standard coupling procedures (Scheme 1). Reaction of **5** and (*S*)-phenylalanine isopropylamide **6** in the presence of ethyl diisopropylamine (DIPEA) gave dipeptide **7** in 50% yield. Upon deprotection of **7** by catalytic hydrogenation, amine **8** was obtained quantitatively. Reaction of **8** with benzoyl chloride in the presence of triethylamine gave dipeptide **3** (53%). Coupling of **8** with (*S*)-*N*-benzoylalanine was accompanied by partial epimerisation. Therefore, reaction with the corresponding Boc derivative in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) was used instead, followed by the change of the protective group to give tripeptide **4**.



Scheme 1 Synthesis of model peptides **3** and **4**

Conformation: X-Ray diffraction data

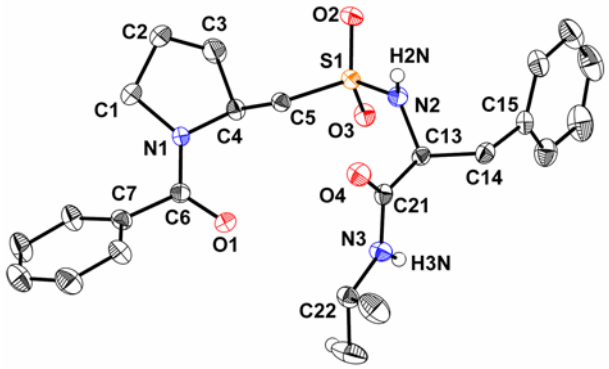


Fig. 1 ORTEP diagram of the compound **3**. Thermal ellipsoids are shown at 20% probability level

Crystals of **3** and **4** for the X-ray diffraction studies were obtained by slow evaporation of their solution in Hexanes – EtOAc. Both **3** and **4** crystallize in non-centrosymmetric space groups; that indicates presence of the single enantiomers in the crystals. Unit cell of **3** contains six equal molecules (Figure 1), whereas in the case of **4** two molecules with quite similar conformations are observed in a unit cell (the root-mean-square deviation (RMSD) value is 0.192 Å) (Figure 2). The lattice parameters of **3** and **4** are summarized in the Table 1.

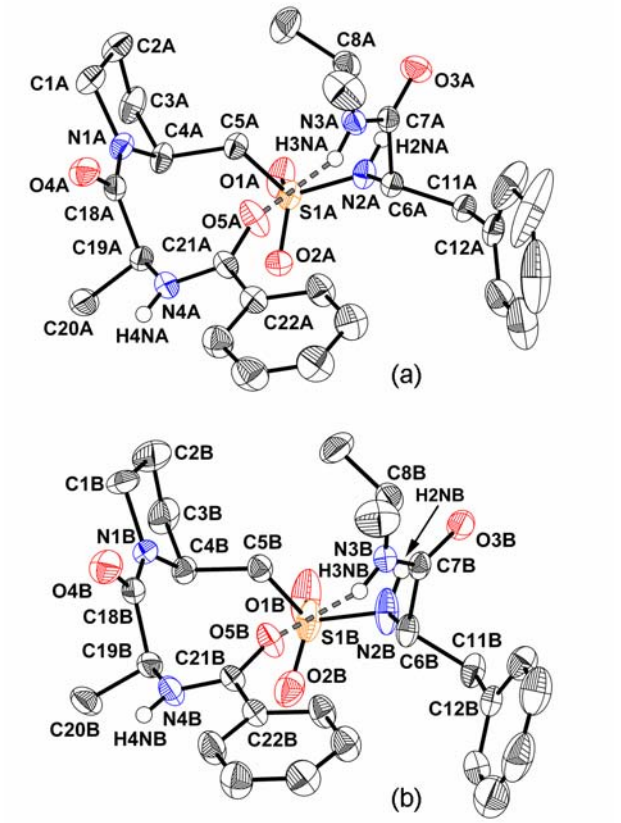


Fig. 2 ORTEP diagram of the compound **4**: (a) molecule A; (b) molecule B. Thermal ellipsoids are shown at 20% probability level

Conformation of the 2PyMS – Phe backbone in molecules **3** and **4** is rather similar (Table 1, see also Figure 3 for the definition of torsion angles). The pyrrolidine ring restricts φ_{i+1} value to –67.7(3) – –77.4(4)°, which corresponds to the folded *gauche*$^-$ (–*sc*) conformation. On the contrary, values of θ_{i+1} and ψ_{i+1} angles (150.0(3) – 172.1(2)° and –159.2(2) – –178.8(3)°, respectively) are characteristic for the extended *trans* (*ap*) conformation. Conformation of the Phe residues (as well as the Ala residue in the molecule of **4**) corresponds to the β region of the Ramachandran plot (φ = –75.3(4)° – –106.9(3)°; ψ = 122.0(3)° – 147.5(3)°).[19]

Normally, such combinations of ψ and θ angles allow one to expect the extended conformation of the peptide molecule,[20] taking into account that values close to 180° are more common for ω angles. In the case of **3** and **4**, situation is different due to the intrinsic properties of the sulfonamide bond.[11, 21] Values of ω_{i+2} are 63.0(4) – 80.0(2)° (*gauche*$^+$ (+*sc*) conformation). It reflects *anti* orientation of the nitrogen lone pair and the S–C bond. This situation is most frequently encountered in the crystal structures of sulfonamide derivatives.[16, 22] Moreover, the less common *cis* configuration[23] is observed for the amide bond formed by the pyrrolidine nitrogen atom in the molecule of **4** (ω_{i+1} = –11.2(6) – –12.0(6)°). This is the major difference in the

conformations of the peptide backbones of **3** and **4**: in the case of **3**, the corresponding amide bond (as well as all the other amide bonds in the molecules of **3** and **4**) has *trans* configuration (ω_i, ω_{i+1}, and ω_{i+3} = ±172.1(2)° – ±180.0(3)°).

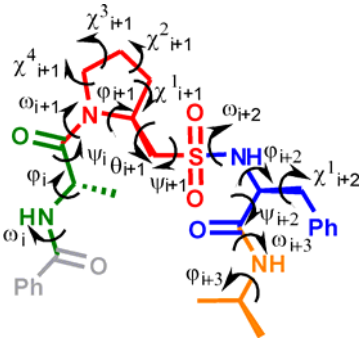


Fig. 3 Definition of the backbone and side chain torsion angles in the model dipeptides (compound **4** is given as an example)

Table 1 Backbone torsion angles (deg) in the molecules of **3** and **4**

Angle	Angle definition	**3**	**4** (A)	**4** (B)
ω_i	C19–N4–C21–C2	–	–172.2(3)	–173.2(3)
φ_i	C18–C19–N4–C21	–	–75.3(4)	–76.0(4)
ψ_i	N1–C18–C19–N4	–	147.5(3)	147.2(3)
ω_{i+1}	C4–N1–C6–C7 (**3**), C4–N1–C18–C19 (**4**)	–174.8(3)	–11.2(6)	–12.0(6)
φ_{i+1}	C6–N1–C4–C5 (**3**), C18–N1–C4–C5 (**4**)	–67.7(3)	–77.4(4)	–75.8(4)
θ_{i+1}	N1–C4–C5–S1	172.1(2)	150.0(3)	154.0(3)
ψ_{i+1}	C4–C5–S1–N2	–159.2(2)	177.6(3)	178.8(3)
ω_{i+2}	C5–S1–N2–C13 (**3**), C5–S1–N2–C6 (**4**)	80.0(2)	63.6(3)	63.0(4)
φ_{i+2}	S1–N2–C13–C21 (**3**), S1–N2–C6–C7 (**4**)	–88.8(3)	–99.3(3)	–106.9(3)
ψ_{t+2}	N2–C13–C21–N3 (**3**), N2–C6–C7–N3 (**4**)	144.8(2)	122.0(3)	124.2(4)
ω_{i+3}	C13–C21–N3–C22 (**3**), C6–C7–N3–C8 (**4**)	180.0(3)	178.2(3)	–177.1(3)
φ_{i+3}	C24–C22–N3–C21 (**3**), S1–N2–C6–C7 (**4**)	–66.9(4)	–146.3(4)	–149.9(4)

Combinations of the backbone torsion angles discussed above result in folded conformations of both **3** and **4**, which can be classified as tight turns. The key criteria for such an assignment is the distance between C^α atoms of the ending residues, which should be within 7 Å limit.[24] In the case of **3**, distance between the *ipso*-C of the phenyl ring (C^α_i) and the CH of the isopropyl fragment (C^α_{i+3}) is 5.706 Å, which clearly indicates presence of the β turn. Since no intramolecular hydrogen bond is present in the molecule, this conformation can be classified as type IV (miscellaneous) β turn.[24, 25] The pseudo dihedral angle formed by the four consecutive C^α atoms in the molecule of **3** is 9.7°; analogous geometry, *i. e.* linking protein chains approaching from almost anti-parallel directions, is found for the type II β turn.[26]

In the molecule of **4** distance between the C^α atoms of the ending residues ($C^\alpha_{i-1} - C^\alpha_{i+3}$) is 5.206 Å for the conformer A and 5.181 Å for the conformer B, which is characteristic for an α turn. In this case, no close analogue can be envisaged among the tight turns identified so far: the only reported α turn, which has *cis*-configuration of the central residue (type I-α_C turn) has too distinct values of other backbone torsion angles.[24, 27] The pseudotorsion angle $C^\alpha_{i-1} - C^\alpha_i - C^\alpha_{i+2} - C^\alpha_{i+3}$, formed by the C^α atoms of the benzoyl moiety, Ala and Phe residues, and the *i*Pr fragment in the molecule of **4** is –66.4° (conformer A) and –64.8° (conformer B). The turn conformation in the molecule of **4** is stabilized by the intramolecular hydrogen bond $C{=}O_{i-1} \leftarrow NH_{i+3}$ (H…O 2.01 Å; N–H…O 152° (conformer A) and H…O 2.03 Å; N–H…O 152° (conformer B)) (Figure 2). Additional stabilization is provided by the contact between C=O group of the benzoyl moiety and CH_2 unit of the 2PyMS residue (H…O 2.44 Å; C–H…O 160° Å (conformer A) and H…O 2.39 Å; C–H…O 165° (conformer B)).

The amide nitrogen atoms in the molecules of **3** and **4** have planar configuration (deviation from the planarity is less than 0.1°). In the case of the sulfonamide nitrogen atoms, a more pronounced pyramidalization is observed (sums of the angles centered at them are 342° – 355°), which is typical for sulfonamide derivatives.[11, 21]

Some remarkable differences are found in conformations of the side chains of the amino acid residues in the molecules of **3** and **4**. In particular, the pyrrolidine ring in the molecule of **3** adopts twisted conformation with deviations of C-3 and C-4 atoms from the mean-square plane formed by the rest of the ring of 0.33 Å and –0.26 Å, respectively. In the case of **4**, this five-membered ring is found in an envelope conformation; C-3 atom deviates from the mean-square plane formed by the other ring atoms by –0.53 Å (conformer A) and 0.59 Å (conformer B). In both cases, down puckering of the pyrrolidine ring is observed (Table 2).[28] Conformations of the side chain of the Phe residue are completely different for both investigated model peptides. In the case of **3**, the phenyl substituent adopts *gauche*$^-$ (*–sc*) orientation with respect to the C^α–N bond of the peptide backbone (χ^1_{i+2} = –62.8(3)°), whereas in the molecule of **4** the value of χ^1_{i+2} angle is –171.0(3)° (molecule A) or –170.9(3)° (molecule B), which corresponds to *trans* (*ap*) conformation.

Table 2 Pyrrolidine ring puckering in the molecules of **3** and **4**[28b]

Parameter	**3**	**4** (A)	**4** (B)
Degree of puckering	0.52	0.54	0.49
Polar angle, deg	92.6	78.5	76.6

It might be also noted that molecules of **3** and **4** differ in relative orientation of the isopropyl substituents. The extrapolated torsion angle φ_{i+3} is –66.9(4)° in the case of **3**, whereas for **4**, corresponding values are –146.3(4)° (conformer A) and –149.9(4)° (conformer B) (Table 1).

In the crystal lattice the molecules of **3** are linked by the intermolecular hydrogen bonds formed by Phe C=O group and *i*PrNH unit of the next molecule ((x–y+1, x+1, z–1/6); H…O 2.18 Å; N–H…O 164°), Phe NH unit and SO_2 fragment of the next molecule ((y–1, –x+y, z+1/6); H…O 2.22 Å; N–H…O 153°), as well as CH_2 unit of the 2PyMS residue and benzoyl C=O group of the next molecule ((y–1, –x+y, 1/6+z); H…O 2.39 Å; C–H…O 160°), which results in the formation of chains along the *c* axis (Figure 4a). Neighboring molecules in the chain are turned by 120°, so that their sulfur atoms form a spiral (Figure 4b).

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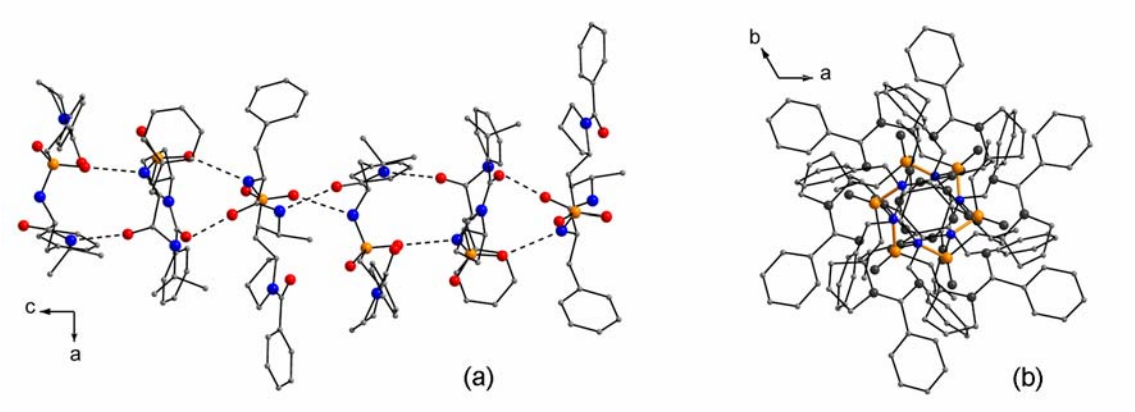

Fig. 4 Packing of the molecules of **3** in crystals viewed along: (a) *b* axis; (b) *c* axis

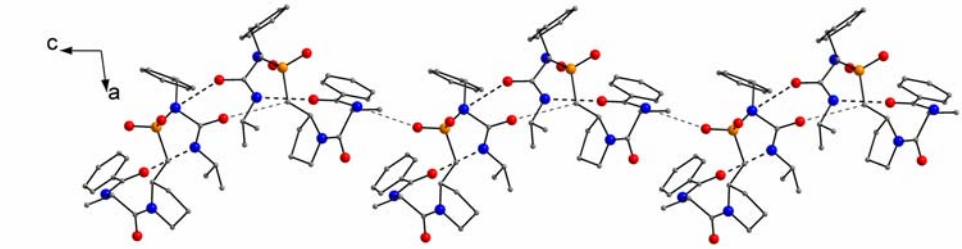

Fig. 5 Packing of the molecules of **4** in crystals viewed along *b* axis

In the crystals molecules A and B of **4** form dimers, connected by intermolecular hydrogen bonds formed by Phe NH unit of the molecule A and Phe C=O group of the molecule B (H…O 1.96 Å; N–H…O 163°), as well as CH_2 unit of the 2PyMS residue of the molecule B and Phe C=O group of the molecule A (H…O 2.38 Å; C–H…O 158°) (Figure 5). These dimers are linked by weak intermolecular hydrogen bonds formed by Ala NH unit of the molecule B and SO_2 fragment of the molecule A from the next dimer ((x, y, z–1); H…O 2.43 Å; N–H…O 163°) into chains running along the *c* axis, which are further held together *via* a number of weak intermolecular contacts.

Conformation: solution studies

Conformation of the peptides **3** and **4** in solution was studied using NMR and IR experiments. It was found that in $CDCl_3$ as a solvent both **3** and **4** existed as single rotamers around the amide bonds, showing only one set of signals in ^1H and ^{13}C NMR spectra.

Addition of DMSO-d^6 to the $CDCl_3$ solution of **3** resulted in the significant shift of both Phe and *i*Pr NH signals to the higher field (by 0.84 and 0.99 p. p. m. at 10% v/v DMSO-d^6, respectively) (Figure 6).

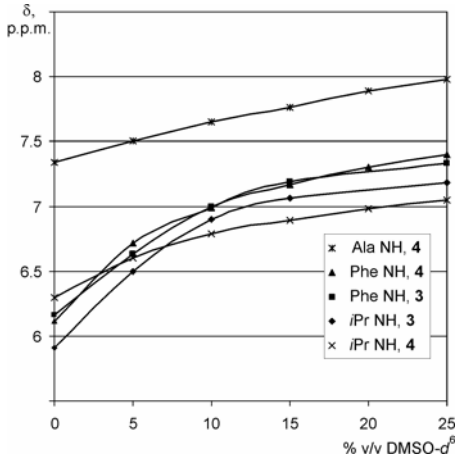

Fig. 6 Chemical shift dependence of the NH resonances of **3** and **4** as a function of DMSO-d^6 concentration (% v/v) in $CDCl_3$. Peptide concentrations 0.062 M (**3**) and 0.054 M (**4**)

In the case of **4**, accessibility of the NH protons to the solvent decreased in the series: Phe NH >> *i*Pr NH > Ala NH; at 10% v/v

DMSO-d^6 the signals were shifted by 0.87, 0.49 and 0.31, respectively. All these results show that the NH protons of **3** and **4** are not involved into strong intramolecular hydrogen bonding, and the conformation of **4**, observed in crystalline state, is not preserved in solution.

Analysis of the Phe NH – C_αH $^3J_{HH}$ coupling constants using modified Karplus equation shows that in the case of **3**, the Phe N–H and C_α–H bonds are almost antiperiplanar (estimated dihedral angle H–N–C_α–H value is 151°), which is consistent with the conformation observed in crystals (where the corresponding dihedral angle value is 174.5°). In the case of **4**, corresponding constant is less informative as the Karplus equation has two roots for the value of *J* observed (Table 3).

Table 3 Analysis of the Phe NH – C_αH coupling constants in **3** and **4**

Molecular fragment	$^3J_{HH}$, Hz	∠ H–N–C_α–H, °	
		Calculated[a]	X-Ray data
Phe NH – C_αH of **3**	8.3	151	174.5
Phe NH – C_αH of **4**	7.3	143 or 3	144.9

[a]Values of the dihedral angles were calculated using modified Karplus equation ($J = 6.98\cos^2\theta - 1.38\cos\theta + 1.72$) updated from[29]

The most significant correlations observed in the NOESY spectrum of **3** are shown in the Figure 7. Strong correlation between *ortho*-protons of the benzoyl substituent and 5-CH_2 group of 2PyMS, as well as *i*PrNH and protons of the Phe $CHCH_2$ fragment, characterize *trans* conformations of both amide bonds in the molecule of **3**. Correlations between C_αH of the Phe residue and the protons of the 2PyMS $CHCH_2$ fragment are characteristic for *gauche* (and not *trans*) conformation of the sulfonamide bond. Finally, a weak correlation between *ortho*-protons of the benzoyl substituent and methyl groups of the isopropyl substituent shows that at least some considerable population of the folded conformation is observed.

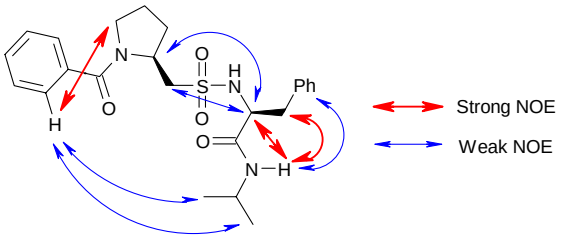

Fig. 7 Significant correlations observed in NOESY spectrum of **3** ($CDCl_3$ solution)

A similar pattern of the correlations is observed in the NOESY spectrum of **4** (Figure 8). In particular, correlations between *ortho*-protons of the benzoyl group and Ala NH,[30] Ala CH(CH_3) fragment and 5-CH_2 group of 2PyMS residue, as well as *i*PrNH and protons of the Phe $CHCH_2$ fragment are characteristic for *trans* configuration of the corresponding amide bonds. As in the case of **3**, correlations between Phe C_αH and NH with protons of the 2PyMS $CHCH_2$ fragment are characteristic for *gauche* (and not *trans*) conformation of the sulfonamide bond. Correlations between *ortho*-protons of the benzoyl substituent and Ala NH with protons of the isopropyl fragment show that at least some considerable population of the folded conformations (which are different from that found in the crystals) is observed. In our opinion, the main reason behind the differences in the conformational behaviour of **4** observed in solid state and in solution is that *cis* configuration of the amide bond, found in the crystals of **4**, is thermodynamically unfavourable; therefore, stabilisation provided by the intramolecular hydrogen bond is not enough to retain the α turn conformation in solution. This can be tuned by stabilisation of the conformer containing *cis* amide bond, in particular by introducing bulky substitutent at C-5 position of the 2PyMS residue, as it was described for proline derivatives.[32]

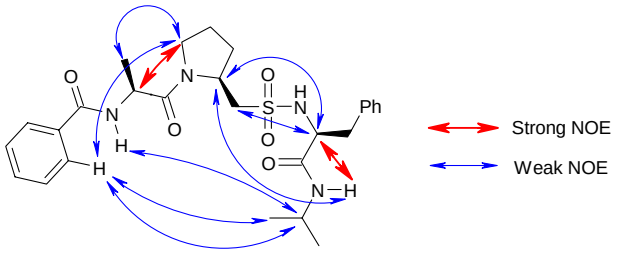

Fig. 8 Significant correlations observed in NOESY spectrum of **4** ($CDCl_3$ solution)

IR spectra of both **3** and **4** in $CHCl_3$ showed three bands in the NH stretching region, which can be assigned to free and hydrogen-bonded C(O)NH and SO_2NH (Table 4). Since relative intensities of the bands observed at ~3350 cm^{-1} and ~3190 cm^{-1} diminished upon dilution of the solutions (Figure 9), hydrogen bonds formed by the corresponding NH fragments were intermolecular. These data are in accordance with results of the NMR experiments discussed above. The bands at 1670 cm^{-1} (**3**) and 1662 cm^{-1} (**4**) in the Amide I region are characteristic for turn-type conformations,[32] which is also consistent with NMR data.

Table 3 Main absorption bands in IR spectra of **3** and **4** ($CHCl_3$, 30 mM (**3**) and 20 mM (**4**))

Bands, cm^{-1}		Assignment[a]
3	**4**	
3423	3419	ν(NH) free C(O)NH
3346	3365	ν(NH) hydrogen-bonded C(O)NH and free SO_2NH
3186	3186	ν(NH) hydrogen-bonded SO_2NH
1670	1662	Amide I
1622	1654	
1616	1637	
1338	1338	$\nu_{as}(SO_2)$
1327	1325	
1313	1313	
1151	1153	$\nu_s(SO_2)$
1134	1148	
	1136	

[a]The band assignment was done using the literature data for analogous systems.[31, 32]

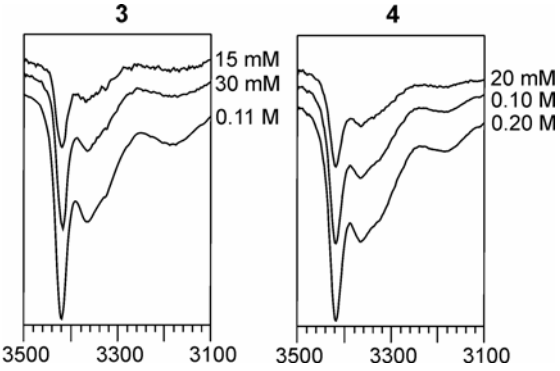

Fig. 9 NH stretching region of IR spectra of **3** and **4** ($CHCl_3$)

Analysis of ν(SO_2) regions (~1300 cm^{-1} and ~1150 cm^{-1}) of the IR spectra revealed that several conformational states are observed in solution for both **3** and **4**. This effect cannot be solely attributed to the formation of intermolecular hydrogen bonds, as the number of peaks in the above referenced regions is not affected by the dilution of the solutions. We assign existence of multiple ν(SO_2) bands to relatively high rotational barrier around the sulphonamide bonds (30–40 kJ/mol),[33] which is observable on IR spectroscopy timescale, but not on NMR.

Conclusions

The residue of (*S*)-2-pyrrolidinemethanesulfonic acid (2PyMS) is capable of inducing folded structures when introduced into model peptides. In particular, peptides PhC(O)–2PyMS–Phe–NH*i*Pr and PhC(O)–Ala–2PyMS–Phe–NH*i*Pr adopt β- and α-turn conformations in crystalline state, respectively. Folded conformations were only partially observed in solution, with no intramolecular hydrogen bonding retained. The intrinsic conformational properties of 2PyMS can be attributed to the conformational restriction provided by the pyrrolidine ring, as well as propensity of the sulphonamide bond to adopt *gauche* conformation. These features stabilize conformations with folded arrangement of those parts of the peptide backbone, which are defined by φ and ω torsion angles (φ ~ –70°, ω ~ 70°).

Experimental section

General

The solvents were purified according to the standard procedures. Compounds **5**[18] and **6**[34] were prepared according to the methods reported in literature. All other starting materials were purchased from Acros, Merck, Fluka, and UkrOrgSyntez. Analytical TLC was performed using Polychrom SI F254 plates. Column chromatography was performed using Kieselgel Merck 60 (230–400 mesh) as the stationary phase. ^1H, ^{13}C, and all 2D NMR spectra were recorded on a Bruker 170 Avance 500 spectrometer (at 499.9 MHz for Protons and 124.9 MHz for Carbon-13). Chemical shifts are reported in ppm downfield from TMS (^1H, ^{13}C) as an internal standard. IR spectra were obtained on Perkin Elmer BX II FT-IR spectrometer. ν_{max} (cm^{-1}) values in IR spectra are given for the main absorption bands. Elemental analyses were performed at the Laboratory of Organic Analysis, Department of Chemistry, Kyiv National Taras Shevchenko University. Mass spectra were recorded on an Agilent 1100 LCMSD SL instrument. HRMS were obtained using LTQ Orbitrap mass spectrometer.

N-((((2*S*)-1-benzyloxycarbonylpyrrolidin-2-yl)methyl)sulfonyl)-*L*-phenylalanine isopropylamide (7)

Amine **6** (11.0 g, 53 mmol) was dissolved in CH_2Cl_2 (200 mL), and DIPEA (12.9 g, 0.1 mol) was added. The mixture was cooled to 0 °C, and compound **5** (16.9 g, 53 mmol) in CH_2Cl_2 (100 mL) was added dropwise upon stirring. The resulting mixture was stirred at 0 °C for 30 min and at rt for 1 h, then washed with saturated aq $NaHCO_3$ (100 mL), 1 M aq $KHSO_4$ (100 mL), brine (100 mL), dried over Na_2SO_4 and evaporated in *vacuo*. The residue was purified by column chromatography (Hexanes – EtOAc (3 : 2) as eluent) to give **7** (7.13 g, 50%) as white amorphous solid. $[\alpha]_D$ –1.2 (*c* 1.42, MeOH). ^1H NMR ($CDCl_3$), δ (major rotamer) 7.22–7.39 (m, 10H), 5.92 (d, *J* = 6.0 Hz, 1H), 5.53 (d, *J* = 8.3 Hz, 1H), 5.13 (s, 2H), 5.04–5.22 (m, 1H), 4.30 (br s, 1H), 3.77–4.00 (m, 2H), 3.37–3.41 (m, 3H), 3.22 (dd, *J* = 12.7 Hz and 5.3 Hz, 1H), 3.11 (dd, *J* = 12.7 Hz and 7.2 Hz, 1H), 2.97 (br s, 1H), 2.71 (dd, *J* = 13.1 Hz and 8.7 Hz, 1H), 1.82–1.92 (m, 2H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ (major rotamer) 169.6, 154.9, 136.64, 136.55, 129.8, 128.8, 128.6, 128.2, 127.9, 127.2, 67.1, 58.8, 55.4, 53.8, 46.2, 41.7, 39.5, 30.4, 23.6, 22.4, 22.3. Anal. calcld. for $C_{25}H_{33}N_3O_5S$ C 61.58, H 6.82, N 8.62, S 6.58. Found C 61.75, H 6.57, N 8.64, S 6.31. MS (APCI) *m/z* 488 (MH^+); negative mode: 486 ($M–H^+$). HRMS (ESI) *m/z* calcld. for $C_{25}H_{33}N_3O_5SNa$ 510.2033. Found 510.2031.

N-(((2*S*)-pyrrolidin-2-ylmethyl)sulfonyl)-*L*-phenylalanine isopropylamide (8)

Compound **7** (13.0 g, 26.7 mmol) was dissolved in MeOH (200 mL), and 10% Pd-C (2 g) was added. A slow steam of hydrogen was bubbled through the mixture at rt upon stirring until the starting material disappeared (monitored by TLC). The catalyst was filtered off, and the filtrate was evaporated to dryness to give **8** (9.4 g, 100%) as white amorphous solid. The product was pure enough to be used in the next steps without any additional purification. An analytical sample was prepared by column chromatography (Hexanes – EtOAc (2 : 3) as eluent). $[\alpha]_D$ –6.4 (*c* 0.38, MeOH). ^1H NMR ($CDCl_3$), δ 7.25–7.31 (m, 4H), 7.20–7.24 (m, 1H), 6.47 (d, *J* = 7.8 Hz, 1H), 5.32 (br s, 2H), 4.18 (t, *J* = 7.2 Hz, 1H), 3.91–4.00 (m, 1H), 3.51–3.57 (m, 1H), 3.13 (dd, *J* = 14.0 Hz and 6.4 Hz, 1H), 2.99–3.05 (m, 2H), 2.97 (t, *J* = 7.0 Hz, 2H), 2.88 (dd, *J* = 14.0 Hz and 3.1 Hz, 1H), 1.89–1.96 (m, 1H), 1.73–1.82 (m, 1H), 1.64–1.74 (m, 1H), 1.35–1.42 (m, 1H), 1.09 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ 170.2, 136.8, 129.7, 128.7, 127.1, 58.7, 56.8, 53.9, 46.0, 41.7, 39.3, 31.2, 24.4, 22.5, 22.3. Anal. calcld. for $C_{17}H_{27}N_3O_3S$ C 57.76, H 7.70, N 11.89, S 9.07. Found C 57.63, H 7.96, N 12.02, S 8.80. MS (APCI) *m/z* 354 (MH^+); negative mode: 352 ($M–H^+$). HRMS (ESI) *m/z* calcld. for $C_{17}H_{27}N_3O_3SH$ 354.1846. Found 354.1848.

N-((((2*S*)-1-benzoylpyrrolidin-2-yl)methyl)sulfonyl)-*L*-phenylalanine isopropylamide (3)

8 (0.97 g, 2.7 mmol) was dissolved in CH_2Cl_2 (50 mL), and Et_3N (0.5 g, 4.9 mmol) was added. The mixture was cooled to 0 °C, and benzoyl chloride (0.38 g, 2.7 mmol) was added dropwise.

The resulting mixture was stirred at rt for 2 h, then washed with 1 M aq $KHSO_4$ (15 mL), brine (15 mL), dried over Na_2SO_4 and evaporated in *vacuo*. The residue was purified by column chromatography (Hexanes – EtOAc (1 : 1) as eluent) to give **3** (0.65 g, 53%) as white crystals. Mp 135–137 °C (Hexanes – EtOAc). $[\alpha]_D$ –37.7 (*c* 0.27, MeOH). IR ($CHCl_3$, cm^{-1}): 3423, 3346, 3186 (ν(NH)); 1670, 1622, 1616 (Amide I); 1338, 1327, 1313 ($\nu_{as}(SO_2)$); 1151, 1134 ($\nu_s(SO_2)$). ^1H NMR ($CDCl_3$), δ 7.42 (d, J = 7.1 Hz, 2H, 2′-C*H* of PhC(O)), 7.31–7.38 (m, 3H, 3′-and 4′-C*H* of PhC(O)), 7.18–7.22 (m, 4H, Phe 2′-and 3′-C_6H_5), 7.13 (t, J = 6.9 Hz, 1H, Phe 4′-C_6H_5), 6.19 (d, J = 6.8 Hz, 1H, *i*PrN*H*), 5.94 (d, J = 8.3 Hz, 1H, Phe N*H*), 4.58 (br s, 1H, 2PyMS $C^{\alpha}H$), 4.08 (q, J = 7.1 Hz, 1H, Phe $C^{\alpha}H$), 3.92 (oct, J = 6.6 Hz, 1H, C*H* of *i*Pr), 3.45 (dd, J = 13.6 Hz and 3.2 Hz, 1H, 2PyMS $C^{\alpha}HCHH$), 3.30–3.39 (m, 2H, 2PyMS 5-CH_2), 3.04–3.12 (m, 2H, Phe $C^{\alpha}HCH_2$), 2.66 (dd, J = 13.6 Hz and 8.4 Hz, 1H, 2PyMS $C^{\alpha}HCHH$), 2.12–2.20 (m, 1H, 2PyMS 3-C*H*H), 1.89–1.96 (m, 1H, 2PyMS 3-CH*H*), 1.77–1.84 (m, 1H, 2PyMS 4-C*H*H), 1.67–1.76 (m, 1H, 2PyMS 4-CH*H*), 0.98 (d, J = 6.6 Hz, 1H, CH_3 of *i*Pr), 0.91 (d, J = 6.6 Hz, 1H, CH_3 of *i*Pr). ^{13}C NMR ($CDCl_3$), δ 170.4 (*C*=O), 169.6 (*C*=O), 136.7 (1′-*C* of C_6H_5), 136.6 (1′-*C* of C_6H_5), 130.4 (4′-*C*H of PhC(O)), 130.0 (2′- or 3′-*C*H of Phe), 128.8 (2′- or 3′-*C*H of Phe), 128.5 (3′-*C*H of PhC(O)), 127.3 (2′-*C*H of PhC(O)), 127.1 (4′-*C*H of Phe), 59.1 (Phe $C^{\alpha}H$), 55.1 (2PyMS $C^{\alpha}HCH_2$), 53.4 (2PyMS $C^{\alpha}H$), 49.9 (2PyMS 5-CH_2), 41.8 (*C*H of *i*Pr), 39.4 (Phe $C^{\alpha}HCH_2$), 30.6 (2PyMS 3-CH_2), 24.9 (2PyMS 4-CH_2), 22.5 (CH_3 of *i*Pr), 22.3 (CH_3 of *i*Pr). Anal. calcld. for $C_{24}H_{31}N_3O_4S$ C 63.00, H 6.83, N 9.18, S 7.01. Found C 62.75, H 6.68, N 8.99, S 7.07. MS (APCI) *m/z* 458 (MH^+); negative mode: 456 ($M–H^+$). HRMS (ESI) *m/z* calcld. for $C_{24}H_{31}N_3O_4SNa$ 480.1927. Found 480.1928.

N-((((2*S*)-1-(*N*-*tert*-butyloxycarbonyl-*L*-alanyl)pyrrolidin-2-yl)methyl)sulfonyl)-*L*-phenylalanine isopropylamide (9)

Compound **8** (1.00 g, 2.8 mmol), HOBt (0.40 g, 3.0 mmol), and DIPEA (0.77 g, 6.0 mmol) were dissolved in DMF (100 mL). The mixture was cooled to –10 °C, and EDC (0.47 g, 3.0 mmol) was added. The resulting mixture was stirred at –10 °C for 1 h, then warmed to rt over 2 h, poured into H_2O (300 mL) and extracted with EtOAc (3×50 mL). The combined organic extracts were washed with 1 M aq $KHSO_4$ (50 mL), saturated aq $NaHCO_3$ (50 mL), brine (50 mL), dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by column chromatography (Hexanes – EtOAc (3 : 2) as eluent) to give **9** (1.00 g, 67%) as yellowish amorphous solid. $[\alpha]_D$ –26.3 (*c* 1.14, MeOH). ^1H NMR ($CDCl_3$), δ 7.28–7.31 (m, 4H), 7.22–7.25 (m, 1H), 6.33 (d, J = 7.3 Hz, 1H), 6.06 (d, J = 7.8 Hz, 1H), 5.52 (d, J = 7.5 Hz, 1H), 4.41 (quint, J = 6.6 Hz, 1H), 4.12–4.18 (m, 1H), 4.06–4.10 (m, 1H), 3.98–4.05 (m, 1H), 3.59–3.63 (m, 1H), 3.35–3.42 (m, 2H), 3.22 (dd, J = 13.2 Hz and 6.6 Hz, 1H), 3.10 (dd, J = 13.2 Hz and 7.0 Hz, 1H), 2.63 (dd, J = 13.5 Hz and 8.4 Hz, 1H), 1.91–2.00 (m, 4H), 1.46 (s, 9H), 1.28 (d, J = 6.6 Hz, 3H), 1.10 (d, J = 6.2 Hz, 3H), 1.04 (d, J = 6.2 Hz, 3H). ^{13}C NMR ($CDCl_3$), δ 171.8, 169.4, 155.2, 136.8, 129.8, 128.7, 127.0, 79.8, 59.0, 54.0, 53.4, 48.1, 46.3, 41.7, 39.4, 29.8, 28.5, 23.8, 22.4, 22.4, 18.3. Anal. calcld. for $C_{25}H_{40}N_4O_6S$ C 57.23, H 7.68, N 10.68, S 6.11. Found C 57.01, H 7.73, N 10.80, S 5.92. MS (APCI) *m/z* 425 ($MH^+ – CO_2 – C_4H_8$); negative mode: 523 ($M–H^+$). HRMS (ESI) *m/z* calcld. for $C_{25}H_{40}N_4O_6SNa$ 547.2561. Found 547.2560.

N-((((2*S*)-1-(*N*-benzoyl-*L*-alanyl)pyrrolidin-2-yl)methyl)sulfonyl)-*L*-phenylalanine isopropylamide (4)

Compound **9** (1.00 g, 1.9 mmol) was dissolved in 10% HCl in dioxane (10 mL). The resulting mixture was stirred for 2 h and evaporated to dryness. CH_2Cl_2 (50 mL) and DIPEA (0.65 g, 5.0 mmol) were added to the residue, and the mixture was stirred for 30 min, then cooled to –10 °C, and benzoyl chloride (0.27 g, 1.9 mmol) was added dropwise. The mixture was warmed to rt upon stirring over 1 h, then poured into H_2O (200 mL). The organic phase was separated, washed with 1 M $KHSO_4$ (50 mL), brine (50 mL), dried over Na_2SO_4 and evaporated in vacuo. The residue recrystallized from Hexanes – EtOAc to give 4 (0.52 g, 52%) as grayish crystals. Mp 148 – 149 °C (Hexanes – EtOAc). $[\alpha]_D$ –50.9 (*c* 0.62, MeOH). IR ($CHCl_3$, cm^{-1}): 3419, 3365, 3186 (ν(NH)); 1662, 1654, 1637 (Amide I); 1338, 1325, 1313 ($\nu_{as}(SO_2)$); 1153, 1148, 1136 ($\nu_s(SO_2)$). ^1H NMR ($CDCl_3$), δ 7.79 (d, J = 7.0 Hz, 2H, 2′-C_6H_5 of PhC(O)), 7.43 (t, J = 7.0 Hz, 1H, 4′-C_6H_5 of PhC(O)), 7.33–7.37 (m, 3H, 3′-C_6H_5 of PhC(O) and Ala N*H*), 7.18–7.20 (m, 4H, Phe 2′- and 3′-C_6H_5), 7.12–7.16 (m, 1H, Phe 4′-C_6H_5), 6.30 (d, J = 7.3 Hz, 1H, Phe N*H*), 6.13 (d, J = 6.0 Hz, 1H, *i*PrN*H*), 4.85 (quint, J = 6.0 Hz, 1H, Ala $C^{\alpha}H$), 4.18 (br s, 2PyMS $C^{\alpha}H$), 4.02 (q, J = 6.4 Hz, Phe $C^{\alpha}H$), 3.84–3.90 (m, 1H, $CH(CH_3)_2$), 3.50 (br s, 1H, 2PyMS 5-C*H*H), 3.38–3.43 (m, 1H, 2PyMS 5-CH*H*), 3.09–3.16 (m, 2H, 2PyMS $C^{\alpha}HCHH$ and Phe $C^{\alpha}HCHH$), 2.97 (dd, J = 13.1 Hz and 7.4 Hz, 1H, Phe $C^{\alpha}HCHH$), 2.60 (dd, J = 13.0 Hz and 6.0 Hz, 1H, 2PyMS $C^{\alpha}HCHH$), 1.77–1.88 (m, 4H, 2PyMS 3- and 4-CH_2), 1.34 (d, J = 5.7 Hz, 3H, Ala CH_3), 0.98 (d, J = 5.7 Hz, 3H, CH_3 of *i*Pr), 0.90 (d, J = 5.7 Hz, 3H, CH_3 of *i*Pr). ^{13}C NMR ($CDCl_3$), δ 171.7 (*C*=O), 169.6 (*C*=O), 166.5 (*C*=O), 136.8 (1′-*C* of C_6H_5), 133.9 (1′-*C* of C_6H_5), 131.9 (4′-*C*H of PhC(O)), 129.7 (2′- or 3′-*C*H of Phe C_6H_5), 128.8 (2′- or 3′-*C*H of Phe C_6H_5), 128.6 (3′-*C*H of PhC(O)), 127.3 (2′-*C*H of PhC(O)), 127.2 (4′-*C*H of Phe C_6H_5), 59.2 (Phe $C^{\alpha}H$), 55.0 (2PyMS $C^{\alpha}HCH_2$), 53.2 (2PyMS $C^{\alpha}HCH_2$), 47.5 (Ala $C^{\alpha}H$), 46.3 (2PyMS 5-CH_2), 41.7 (*C*H of *i*Pr), 39.4 (Phe $C^{\alpha}HCH_2$), 30.2 (2PyMS 3- or 4-CH_2), 23.8 (2PyMS 3- or 4-CH_2), 22.5 (CH_3 of *i*Pr), 22.4 (CH_3 of *i*Pr), 17.8 (Ala CH_3). Anal. calcld. for $C_{27}H_{36}N_4O_5S$ C 61.34, H 6.86, N 10.60, S 6.06. Found C 61.29, H 7.00, N 10.51, S 5.84. MS (APCI) *m/z* 529 (MH^+); negative mode: 527 ($M–H^+$). HRMS (ESI) *m/z* calcld. for $C_{27}H_{36}N_4O_5SNa$ 551.2299. Found 551.2299.

X-Ray diffraction studies

Crystals of **3** and **4** for X-ray diffraction studies were obtained by slow evaporation of their solution in Hexanes – EtOAc.

The colorless crystals of **3** ($C_{24}H_{31}N_3O_4S$) are hexagonal. At 293 K, $a = b$ = 12.342(1) Å, c = 28.361(5) Å, V = 3741.1(8) $Å^3$, M_r = 457.58, Z = 6, space group $P6_5$, d_{calc} = 1.219 g/cm^3, $\mu(MoK_\alpha)$ = 0.163 mm^{-1}, F(000) = 1464.

The colorless crystals of **4** ($C_{27}H_{36}N_4O_5S$) are triclinic. At 293 K, a = 8.9964(4) Å, b = 11.1739(4) Å, c = 14.3314(6) Å, α = 98.212(3)°, β = 97.800(4)°, γ = 91.098(4)°, V = 1411.6(1) $Å^3$, M_r = 528.66, Z = 2, space group P1, d_{calc} = 1.244 g/cm^3, $\mu(MoK_\alpha)$ = 0.157 mm^{-1}, F(000) = 564.

Intensities of 18252 reflections (7218 independent, R_{int}=0.072) for **3** and 13651 reflections (10282 independent, R_{int}=0.023) for **4** were measured on an Xcalibur 3 diffractometer (graphite monochromated MoK_α radiation, CCD-detector, ω scanning,

$2\Theta_{max} = 60°$).

The structure was solved by direct method using SHELXTL package.[35] Positions of hydrogen atoms were located from electron density difference maps and refined using riding model with $U_{iso} = nU_{eq}$ (n = 1.5 for methyl groups and 1.2 for other hydrogen atoms), except the atoms involved into the N–H...O hydrogen bonds which were refined using isotropic model. In the case of **4**, restriction was applied to the bond lengths of C(12)…C(17) aromatic ring (1.38 Å). Full-matrix least-squares refinement against F^2 in anisotropic approximation for non-hydrogen atoms was converged to wR_2=0.086 for 7189 reflections ($R_1 = 0.053$ for 3338 reflections with $F > 4\sigma(F)$, $S = 0.885$) in the case of **3**, and wR_2=0.138 for 10181 reflections ($R_1 = 0.053$ for 5727 reflections with $F > 4\sigma(F)$, $S = 0.932$) in the case of **4**.

Final atomic coordinates, geometrical parameters and crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, 11 Union Road, Cambridge, CB2 1EZ, UK (E-mail: deposit@ccdc.cam.ac.uk; fax: +44 1223 336033) and are available on request quoting the deposition numbers 901480 (**3**) and 901481 (**4**).

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1 (a) Grauer, A.; König. B. *Eur. J. Org. Chem.* **2009**, *30*, 5099–5111. (b) Vagner, J.; Qu, H.; Hruby, V. J. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292–296. (c) Ko, E.; Liu, J.; Burgess, K. *Chem. Soc. Rev.* **2011**, *40*, 4411–4421. (d) Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699–1720. (e) Giannis, A.; Rübsam, F. *Adv. Drug Res.* **1997**, *29*, 1–78.

2 (a) Moree, W. J.; van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron Lett.* **1991**, *32*, 409–412. (b) Moree, W. J.; van Gent, L. C.; van der Marel, G. A.; Liskamp, R. M. J. *Tetrahedron* **1993**, *49*, 1133–1150.

3 (a) Dujols, F. J. M.; Mulliez, M. E. *J. Org. Chem.* **1996**, *61*, 5648–5649. (b) Neelakantan, L.; Hartung, W. H. *J. Org. Chem.* **1959**, *24*, 1943–1948; (c) Frankel, M.; Moses, P. *Tetrahedron* **1960**, *9*, 289–294; (d) Gilmore, W. F.; Lin, H-J. *J. Org. Chem.* **1978**, *43*, 4535–4537.

4 Yim, C.-B.; Dijkgraaf, I.; Merkx, R.; Versluis, C.; Eek, A.; Mulder, G. E.; Rijkers, D. T. S.; Boerman, O. C.; Liskamp, R. M. J. *J. Med. Chem.* **2010**, *53*, 3944–3953.

5 Hara, T.; Durell, S. R.; Myers, M. C.; Appella, D. H. *J. Am. Chem. Soc.* **2006**, *128*, 1995–2004.

6 (a) Giordano, C.; Lucente, G.; Nalli, M.; Zecchini, G. P.; Paradisi, M. P.; Varani, K.; Spisani, S. *Farmaco* **2003**, *58*, 1121–1130. (b) Giordano, C.; Nalli, M.; Paradisi, M. P.; Sansone, A.; Lucente, G.; Spisani, S. *Farmaco* **2004**, *59*, 953–963. (c) Giordano, C.; Lucente, G.; Masi, A.; Paradisi, M. P.; Sansone, A.; Spisani, S. *Bioorg. Med. Chem.* **2006**, *14*, 2642–2652.

7 Piscitelli, F.; Coluccia, A.; Brancale, A.; La Regina, G.; Sansone A, Giordano, C; Balzarini, J.; Maga, G.; Zanoli, S.; Samuele, A.; Cirilli, R.; La Torre, F.; Lavecchia, A.; Novellino, E.; Silvestri, R. *J. Med. Chem.* **2009**, *52*, 1922–1934.

8 Young, R. J.; Campbell, M.; Borthwick, A. D.; Brown, D.; Burns-Kurtis, C. L.; Chan, C.; Convery, M. A.; Crowe, M. C.; Dayal, S.; Diallo, H.; Kelly, H. A.; King, N. P.; Kleanthous, S.; Mason, A. M.; Mordaunt, J. E.; Patel, C.; Pateman, A. J.; Senger, S.; Shah, G. P.; Smith, P. W.; Watson, N. S.; Weston, H. E.; Zhou, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5953–5957.

9 Carson, K. G.; Schwender, C. F.; Shroff, H. N.; Cochran, N. A.; Gallant, D. L.; Briskin, M. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 711–714.

10 Wels, B.; Kruijtzer, J. A.; Garner, K. M.; Adan, R. A.; Liskamp, R. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 287–290.

11 Baldauf, C.; Günther, R.; Hofmann, H.-J. *J. Mol. Struct.* **2004**, *675*, 19–28.

12 (a) Mann, A. In: *Practice of Medicinal Chemistry* (3rd Ed.); Wermuth, C. G., Ed.; Academic Press/Elsevier: Amsterdam, **2008**; pp. 363–379. (b) Grygorenko, O. O.; Radchenko, D. S.; Volochnyuk, D. M.; Tolmachev, A. A.; Komarov, I. V. *Chem. Rev.* **2011**, *111*, 5506–5568. (c) Komarov, I. V.; Grigorenko, A. O.; Turov, A. V.; Khilya, V. P. *Russ. Chem. Rev.* **2004,** *73*, 785–810.

13 Giordano, C.; Sansone, A.; Masi, A.; Lucente, G.; Punzi, P.; Mollica, A.; Pinnen, F.; Feliciani, F.; Cacciatore, I.; Davis, P.; Lai, J.; Ma, S. W.; Porreca, F.; Hruby, V. *Eur. J. Med. Chem.* **2010**, *45*, 4594–4600.

14 Giordano, C.; Masi, A.; Pizzini, A.; Sansone, A.; Consalvi, V.; Chiaraluce, R.; Lucente, G. *Eur. J. Med. Chem.* **2009**, *44*, 179–189.

15 Genari, C.; Ceccarelli, S.; Piarulli, U.; Montalbetti, C. A. G. N.; Jackson, R. F. W. *J. Org. Chem.* **1998**, *63*, 5312–5313.

16 (a) Calcagni, A.; Gavuzzo, E.; Lucente, G; Mazza, F; Morera, E.; Paradisi, M. P.; Rossi, D. *Biopolymers* **2000**, *54*, 379–387. (b) Calcagni, A.; Rossi, D.; Paradisi, M. P.; Lucente, G.; Luisi, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G.; Paci, M. *Biopolymers* **1997**, *41*, 555–567. (c) Calcagni, A.; Gavuzzo, E.; Lucente, G; Mazza, F.; Pinnen, F.; Pocheiti, G.; Rossi, D. *Int. J. Peptide Protein Res.* **1989**, *34*, 471–419. (d) Calcagni, A.; Gavuzzo, E.; Lucente, G; Mazza, F.; Pinnen, F.; Pocheiti, G.; Rossi, D. *Int. J. Peptide Protein Res.* **1989**, *34*, 319–324. (e) Moree, W. J., Schouten, A., Kroon, J.; Liskamp R. M. J. *Int. J. Peptide Protein Res.* **1995**, *45*, 501–507.

17 (a) Revilla-López, G.; Rodríguez-Ropero, F.; Curcó, D.; Torras, J.; Calaza, M. I.; Zanuy, D.; Jiménez, A. I.; Cativiela, C.; Nussinov, R.; Alemán, C. *Proteins: Struct., Funct., Bioinf.* **2011**, *79*, 1841–1852. (b) Revilla-López, G. ; Torras, J. ; Jiménez, A. I.; Cativiela, C.; Nussinov, R.; Alemán, C. *J. Org. Chem.* **2009**, *74*, 2403–2412. (c) Lasa, M.; Jiménez, A. I.; Zurbano, M. M. Cativiela, C. *Tetrahedron Lett.* **2005**, *46*, 8377–8380. (d) Cativiela, C.; Díaz-de-Villegas, M. D.; Gálveza, J. A.; Su, G. *Tetrahedron* **2004**, *60*, 11923–11932. (e) Gil, A. M.; Buñuel, E.; Jiménez, A. I.; Cativiela, C. *Tetrahedron Lett.* **2003**, *44*, 5999–6002. (f) Jiménez, A. I.; Cativiela, C.; Marraud, M. *Tetrahedron Lett*. **2000**, *41*, 5353–5356. (g) Jiménez, A. I.; Cativiela, C.; Gómez-Catalán, J.; Pérez, J. J.; Aubry, A.; París, M.; Marraud, M. *J. Am. Chem. Soc.* **2000,** *122,* 5811–5821.

18 Zhersh, S., Buryanov, V. V., Karpenko, O. V., Grygorenko, O. O., Tolmachev, A. A. *Synthesis* **2011**, 3669–3674.

19 Hollingsworth, S. A.; Karplus, P. A. *Biomol. Concepts* **2010**, *1*, 271–283.

20 (a) Vasudev, P. G.; Chatterjee, S.; Shamala, N.; Balaram, P. *Chem. Rev.* **2011,** *111,* 657–687. (b) Roy, R. S.; Balaram, P. *J. Peptide Res.* **2004**, *63*, 279–289.

21 Parkin, A.; Collins, A.; Gilmore, C. J.; Wilson, C. C. *Acta Cryst. B* **2008**, *64*, 66–71.

22 (a) Kalman, A; Parkanyi, L. Kucsman, Á. *Acta Cryst. B* **1980**, *36*, 1440–1443. (b) Brameld, K. A.; Kuhn, B.; Reuter, D. C.; Stahl, M. *J. Chem. Inf. Model.* **2008**, *48*, 1–24.

23 Pal, D.; Chakrabarti, P. *J. Mol. Biol.* **1999**, *294*, 271–288.

24 Chou, K.-C. *Anal. Biochem.* **2000**, *286***,** 1–16 and references therein.

25 (a) Hutchinson, E. G.; Thornton, J. M. *Peptide Sci.* **1994**, *3*, 2207–2216. (b) Sibanda, B. L.; Blundell, T. L.; Thornton, J. M. *J. Mol.*

Biol. **1989**, *206*, 759–777. (c) Wilmot, C. M.; Thornton, J. M. *Proteing Eng*. **1990**, *3*, 479–493.
26 Oldfield, T. J.; Hubbard, R. E. *Proteins* **1994**, 18324–18337.
27 (a) Chou, K. C. *Biopolymers* **1997**, *42*, 837–853. (b) Pavone, V., Gaeta, G., Lombardi, A., Nastri, F., Maglio, O. *Biopolymers* **1996**, *38*, 705–721.
28 (a) Milner-White, E. J.; Bell, L. H.; Maccallum, P. H. *J. Mol. Biol.* **1992**, *228*, 725–734. (b) Zefirov, N. S.; Palyulin, V. A.; Dashevskaya, E. E. *J. Phys. Org. Chem.* **1990**, *3*, 147–154.
29 Wüthrich, K. *NMR of proteins and nucleic acids*. John Wiley & Sons Inc., NY, **1986**, 320 pp.
30 Due to the signal overlap in the spectra obtained from $CDCl_3$ solution of **4**, NOESY experiment was also done in DMSO-d^6 – $CDCl_3$ (9 : 1) to get the NOEs for Ala NH.
31 (a) Gennari, C.; Gude, M.; Potenza, D.; Piarulli, U. *Chem. Eur. J.* **1998**, *4*, 1924–1931. (b) Langenhan, J. M.; Fisk, J. D.; Gellman, S. H. *Org. Lett.* **2001**, *3*, 2559–2562. (c) Schwenkkraus, P.; Otto, H.-H. Arch. Pharm. **1990**, *323*, 93–98.
32 Halab, L.; Lubell, W. D.; *J. Am. Chem. Soc.*, **2002**, *124*, 2474–2484.
33 (a) Haris, P. I.; Chapman, D. *Biopolymers (Peptide Sci.)* **1995**, *37*, 251–263. (b) Jackson, M.; Mantsch, H. H. *Crit. Rev. Biochem. Mol. Biol.* **1995**, *30*, 95–120. (c) Kong, J.; Yu, S. *Acta Biochim. Biophys. Sin.* **2007**, *39*, 549–559.
34 Baldauf, C.; Günther, R.; Hofmann, H.-J. *J. Mol. Struct. (Theochem)* **2004**, *675*, 19–28.
35 van Leeuwen, S. H.; Quaedflieg, P. J. L. M.; Broxterman, Q. B.; Liskamp, R. M. J. *Tetrahedron Lett.* **2002**, *43*, 9203–9208.
36 Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *A64*, 112–122.