Solid-state structure of cyclic dipeptides: an X-ray and computational study of *cis*- and *trans*-diketo-piperazines of *N*-methyl-phenylalanine with the thia-pipecolic acids and thia-prolines

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Ten new crystal structures of *cis* and *trans* bicyclic diketopiperazines (DKPs) of thia-pipecolic acid (with sulfur in the β , γ or δ position) or thia-proline (with sulfur in the β or γ position) and *N*-methyl phenylalanine [(NMe)Phe]: *cyclo*[(β -S)Pip-(NMe)Phe], *cyclo*[(γ -S)Pip-(NMe)Phe], *cyclo*[(δ -S)Pip-(NMe)Phe], *cyclo*[(β -S)Pro-(NMe)Phe] and *cyclo*[(γ -S)Pro-(NMe)Phe] were determined with X-ray crystallography. Density functional theory calculations of these molecules in the gas phase succeed in reproducing the observed molecular conformations in the crystal remarkably well. This illustrates the weak to moderate impact of intermolecular packing forces in the absence of classical N—H...O hydrogen bonds. The effect of sulfur on the geometry of the DKP ring and details of amide bond non-planarity are discussed. Molecular flexibility of the DKP ring, as estimated from the calculated deformation energies of its endocyclic ring torsion angles, is not in general the decisive factor for the occurrence of multiple symmetry independent molecules in the unit cell ($Z' > 1$), though in some cases a correlation is observed.

1. Introduction

As an important class of biologically active natural compounds (Sammers, 1975) and because they are unique and simple model systems for peptides and proteins, cyclic dipeptides (2,5-diketopiperazines or DKPs) have been the subject of several reviews (Anteunis, 1978; Rajappa & Natekar, 1993; Fischer, 2003). Recently, their role as model compounds became important in crystal engineering, *i.e.* the prediction of solid-state structure and the control over intermolecular forces that determine molecular packing patterns in organic crystals (MacDonald & Whitesides, 1994; Desiraju, 1995; Palacin *et al.*, 1997; Chin *et al.*, 1999).

The vast majority of experimental data on the conformation of cyclic dipeptides originates from X-ray studies on crystals and from NMR investigations in solution. Structural features in condensed phase are the result of the interplay of intra- and intermolecular factors; therefore, their interpretation is complicated. Separating these is facilitated when the gas-phase structure is available for these compounds. In the absence of such experimental information, modern quantum chemistry tools based on density functional theory (DFT) can be reliably used to provide a detailed description of the conformation of single gas-phase DKP molecules (Koch & Holthausen, 2001; Bouř *et al.*, 2002; Zhu *et al.*, 2007).

This report is part of a full characterization of the structural features of 28 cyclic dipeptides (Fig. 1) in the solid state, relying on X-ray analysis in combination with DFT calculations. This diverse set of model compounds offers unique insight into how molecular conformation and configuration (shape), as well as molecular flexibility control the crystallographic structure of cyclic dipeptides in particular, and peptides in general. Specifically, it concentrates on the interplay between ring size, CH₂/S substitution, methyl- and phenyl- side-chains and amide bonds, and their cumulative effect on the intra- and intermolecular forces constituting the solid-state structure.

In a previous paper, Budesinsky *et al.* (2010) discussed the solid-state structures of the eight non-sulfur analogs (1A), (1B), (2A), (2B), (9A), (9B), (10A) and (10B) (see Fig. 1) as a reference set for evaluation of the effect of sulfur on molecular structure and crystal packing in the 20 remaining sulfur analogs, in which a CH₂ group is substituted by sulfur at the β , γ or δ position of pipecolic acid [(3A)–(8B)] or the β or γ position of proline [(11A)–(14B)].

In this work, a detailed description is given of the molecular conformation and packing in the crystal structures of the ten *N*-methylated thia-analogs (4A), (4B), (6A), (6B), (8A), (8B), (12A), (12B), (14A) and (14B) (shown in red in Fig. 1). These *N*-methylated analogs all lack strong hydrogen bonds in their crystal structure, limiting the impact of packing on molecular structure, as opposed to the non-methylated sulfur analogs [(3A), (3B), (5A), (5B), 7(A), 7(B), (11A), (11B), (13A) and (13B)] which do have distinctive hydrogen bonding sub-motifs, and will be the subject of a forthcoming study. Here, the effect of CH₂/S substitution on the molecular structure of the *N*-methylated thia-analogs will be examined by comparing structural data in four sub-series: (2A), (4A), (6A), (8A) (*cis* and 6/6 ring fusion), (2B), (4B), (6B), (8B) (*trans* and 6/6 ring fusion), (10A), (12A), (14A) (*cis* and 5/6 ring fusion) and

(10B), (12B), (14B) (*trans* and 5/6 ring fusion). Furthermore, the correlation of molecular flexibility with the occurrence of multiple conformers in the crystallographic unit cell ($Z' > 1$) is verified.

2. Materials and methods

2.1. Synthesis and structure characterization

The title compounds were synthesized by coupling of *Z*-(NMe)Phe-OH to the thia-pipecolic acid methyl esters with EEDQ in dry THF as the coupling reagent, followed by removal of the *Z* protective group with HBr/AcOH and cyclization with saturated NaHCO₃, according to the procedure described by Anteunis *et al.* (1979) and Reyniers *et al.* (1985).

Melting points from chloroform/hexane: for (4A): 186–188°C; for (4B): 198–199°C; for (6A): 170.5–171°C; for (6B): 137–138°C; for (8A): 178–179°C; for (8B): 207–208°C; for (12A): 150°C; for (12B): 148.5–150°C; for (14A): 158–159°C and for (14B): 124°C.

The structure and purity of all described compounds was confirmed by ¹H and ¹³C NMR spectra (Dračinsky *et al.*, 2011a,b).

2.2. X-ray structure analysis

Up to now, crystal structures of the title compounds have remained largely unknown. In Tables 1 and 2 an overview is given of the relevant details with respect to the crystal structure analysis. Complete X-ray structure data files are deposited in the Cambridge Structural Database [(4A): 1400449, (4B): 1400450, (6A): 1400451, (6B): 1400452, (8A): 1400453, (8B): 1400454, (12A): 1400455, (12B): 1400456, (14A): 1400457, (14B): 1400458].

Figure 1

Structure of selected cyclic dipeptides (the title compounds are shown in red). Labels A and B refer to *cis* and *trans* isomers, respectively. Even and odd numbers refer to *N*-methylated and non-methylated compounds, respectively. c before ring name is used as an abbreviation for *cyclo*.

Table 1

Experimental details.

Experiments were carried out at 150 K using Mo $K\alpha$ radiation on a Nonius KappaCCD area detector. Radiation source was a fine-focus sealed tube. H-atom parameters were constrained.

	(4A)	(4B)	(6A)	(6B)
Crystal data				
Chemical formula	2(C ₁₅ H ₁₈ N ₂ O ₂ S)·H ₂ O	C ₁₅ H ₁₈ N ₂ O ₂ S	C ₁₅ H ₁₈ N ₂ O ₂ S	C ₁₅ H ₁₈ N ₂ O ₂ S
<i>M</i> _r	598.76	290.37	290.37	290.37
Crystal system, space group	Trigonal, <i>P</i> 3 ₁	Orthorhombic, <i>P</i> 2 ₁ 2 ₁ 2 ₁	Monoclinic, <i>P</i> 2 ₁	Monoclinic, <i>P</i> 2 ₁
<i>a</i> , <i>b</i> , <i>c</i> (Å)	8.4102 (2), 8.4102 (2), 36.562 (1)	6.2287 (1), 8.9737 (3), 25.9694 (7)	7.3126 (1), 27.1077 (5), 7.3745 (1)	9.8928 (2), 9.0312 (2), 16.4470 (5)
α , β , γ (°)	90, 90, 120	90, 90, 90	90, 106.0640 (11), 90	90, 97.8246 (15), 90
<i>V</i> (Å ³)	2239.61 (10)	1451.55 (7)	1404.75 (4)	1455.76 (6)
<i>Z</i>	3	4	4	4
<i>F</i> (000)	954	616	616	616
μ (mm ^{−1})	0.22	0.23	0.23	0.23
Crystal size (mm)	0.5 × 0.5 × 0.2	0.4 × 0.25 × 0.25	0.42 × 0.42 × 0.08	0.5 × 0.15 × 0.1
Data collection				
No. of measured, independent and observed [<i>I</i> > 2σ(<i>I</i>)] reflections	15456, 5110, 5051	11349, 3296, 2975	22183, 6211, 5572	23095, 6621, 5781
<i>R</i> _{int}	0.051	0.035	0.032	0.045
(sin θ /λ) _{max} (Å ^{−1})	0.617	0.649	0.648	0.650
Refinement				
<i>R</i> [<i>F</i> ² > 2σ(<i>F</i> ²)], <i>wR</i> (<i>F</i> ²), <i>S</i>	0.045, 0.113, 1.07	0.032, 0.073, 1.04	0.034, 0.083, 1.05	0.039, 0.082, 1.03
No. of reflections	5110	3296	6211	6621
No. of parameters	373	183	363	363
No. of restraints	1	0	1	1
Δρ _{max} , Δρ _{min} (e Å ^{−3})	0.37, −0.28	0.18, −0.23	0.16, −0.23	0.22, −0.22
Absolute structure	Flack (1983)	Flack (1983)	Flack (1983)	Flack (1983)
Absolute structure parameter	0.01 (12)	0.01 (6)	−0.03 (6)	−0.06 (5)
	(8A)	(8B)		
Crystal data				
Chemical formula	C ₁₅ H ₁₈ N ₂ O ₂ S		C ₁₅ H ₁₈ N ₂ O ₂ S	
<i>M</i> _r	290.37		290.37	
Crystal system, space group	Triclinic, <i>P</i> 1		Orthorhombic, <i>P</i> 2 ₁ 2 ₁ 2 ₁	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	7.4536 (1), 7.4603 (2), 13.6011 (3)		9.8689 (2), 10.6838 (2), 13.6421 (2)	
α , β , γ (°)	79.6877 (12), 89.2981 (14), 69.3688 (12)		90, 90, 90	
<i>V</i> (Å ³)	695.31 (3)		1438.39 (4)	
<i>Z</i>	2		4	
<i>F</i> (000)	308		616	
μ (mm ^{−1})	0.24		0.23	
Crystal size (mm)	0.45 × 0.32 × 0.2		0.4 × 0.25 × 0.25	
Data collection				
No. of measured, independent and observed [<i>I</i> > 2σ(<i>I</i>)] reflections	17028, 6242, 6027		25952, 3291, 3045	
<i>R</i> _{int}	0.031		0.035	
(sin θ /λ) _{max} (Å ^{−1})	0.648		0.650	
Refinement				
<i>R</i> [<i>F</i> ² > 2σ(<i>F</i> ²)], <i>wR</i> (<i>F</i> ²), <i>S</i>	0.029, 0.079, 1.04		0.031, 0.077, 1.06	
No. of reflections	6242		3291	
No. of parameters	363		183	
No. of restraints	3		0	
Δρ _{max} , Δρ _{min} (e Å ^{−3})	0.22, −0.20		0.20, −0.18	
Absolute structure	Flack <i>x</i> determined using 2865 quotients [(<i>I</i> +) − (<i>I</i> −)]/[(<i>I</i> +) + (<i>I</i> −)] (Parsons <i>et al.</i> , 2013).		Flack (1983)	
Absolute structure parameter	0.022 (19)		0.01 (7)	

Computer programs: *COLLECT* (Hooft, 1998) and *DENZO* (Otwinowski & Minor, 1997), *COLLECT* and *DENZO*, *SIR92* (Altomare *et al.*, 1994), *SHELXL2014/7* (Sheldrick, 2014).

Table 2

Experimental details.

Experiments were carried out at 150 K using Mo $K\alpha$ radiation on a Bruker APEX-II CCD area detector. H-atom parameters were constrained.

	(12A)	(12B)	(14A)	(14B)
Crystal data				
Chemical formula	C ₁₄ H ₁₆ N ₂ O ₂ S	C ₁₄ H ₁₆ N ₂ O ₂ S	C ₁₄ H ₁₆ N ₂ O ₂ S	C ₁₄ H ₁₆ N ₂ O ₂ S
M_r	276.35	276.35	276.35	276.35
Crystal system, space group	Tetragonal, $P4_32_12$	Orthorhombic, $P2_12_12_1$	Orthorhombic, $P2_12_12_1$	Orthorhombic, $P2_12_12_1$
a, b, c (Å)	7.7430 (2), 7.7430 (2), 43.5343 (14)	10.0987 (4), 11.0883 (4), 12.0825 (5)	7.7112 (7), 11.6576 (12), 14.8171 (14)	13.4996 (5), 14.5841 (5), 20.5461 (8)
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
V (Å ³)	2610.06 (13)	1352.97 (9)	1332.0 (2)	4045.1 (3)
Z	8	4	4	12
$F(000)$	1168	584	584	1752
μ (mm ⁻¹)	0.25	0.24	0.24	0.24
Crystal size (mm)	0.46 × 0.18 × 0.18	0.54 × 0.41 × 0.26	0.47 × 0.32 × 0.22	0.56 × 0.39 × 0.37
Data collection				
Absorption correction	Multi-scan SADABS2014/5	Multi-scan SADABS2014/5	Multi-scan SADABS2014/5	Multi-scan SADABS2014/5
$T_{\min}, T_{\max}$	0.894, 0.957	0.863, 0.941	0.895, 0.948	0.839, 0.917
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	15598, 2992, 2800	8542, 3096, 2931	8310, 3051, 2789	23583, 8811, 7668
R_{int}	0.026	0.020	0.047	0.030
$(\sin \theta/\lambda)_{\text{max}}$ (Å ⁻¹)	0.650	0.650	0.649	0.639
Refinement				
$R[F^2 > 2\sigma(F^2)], wR(F^2), S$	0.030, 0.075, 1.11	0.028, 0.069, 1.07	0.035, 0.082, 1.04	0.039, 0.102, 1.06
No. of reflections	2992	3096	3051	8811
No. of parameters	173	173	173	517
$\Delta\rho_{\text{max}}, \Delta\rho_{\text{min}}$ (e Å ⁻³)	0.21, -0.21	0.18, -0.23	0.28, -0.22	0.39, -0.26
Absolute structure	Flack (1983)	Flack (1983)	Flack x determined using 1085 quotients $[(I^+) - (I^-)]/[I^+ + (I^-)]$ (Parsons <i>et al.</i> (2013))	Flack (1983)
Absolute structure parameter	-0.06 (7)	-0.01 (6)	-0.03 (5)	-0.06 (5)

Computer programs: APEX2, SAINT, SADABS2015/5 (Bruker, 2015), SHELXS97 (Sheldrick, 2008), SHELXL2014/7 (Sheldrick, 2014).

2.3. Computational details

With the aid of the *Gaussian03* software package (Frisch *et al.*, 2004), the title compounds were subject to energy minimization in a single molecule approach. The B3LYP density functional (Becke, 1996) was applied and all atoms were described with the 6-311G(d,p) basis set (Krishnan *et al.*, 1980; McLean & Chandler, 1980). A similar approach was applied to

several model cyclic dipeptides (selected for the purpose of this discussion) and in our previous paper (Budesinsky *et al.*, 2010).

To probe the flexibility of the DKP ring in the title compounds, constrained energy minimizations were performed on all endocyclic torsion angles of the DKP ring $\varphi_1, \psi_1, \omega_1, \varphi_2, \psi_2, \omega_2$ (see Fig. 2). The fully optimized (gas phase)

Figure 2

Atom numbering scheme and definition of torsion angles in the diketopiperazine ring ($\varphi_1, \psi_1, \omega_1, \varphi_2, \psi_2, \omega_2$) and side-chains of Pip, Pro ($\chi_1, \chi_2, \dots$) and Phe ($\chi_{(\alpha)}, \chi_{(\beta)}$).

Table 3

Deformation energies of the endocyclic torsion angles φ , ψ and ω of the diketopiperazine ring.Energies (kcal mol⁻¹, 1 kcal mol⁻¹ = 4.184 kJ mol⁻¹). Column a: values at -12.5° and column b: values at +12.5° from the energy minimum.

Compound		φ_1		ψ_1		ω_1		φ_2		ψ_2		ω_2		Z'
		a	b	a	b	a	b	a	b	a	b	a	b	
(2A)	c[L-Pip-L-(NMe)Phe]	0.232	0.204	0.365	0.330	0.261	0.251	0.290	0.340	0.479	0.429	0.213	0.228	2
(4A)	c[L-(β -S)Pip-L-(NMe)Phe]	0.234	0.313	0.412	0.294	0.189	0.273	0.304	0.297	0.471	0.490	0.295	0.240	2
(6A)	c[L-(γ -S)Pip-L-(NMe)Phe]	0.192	0.188	0.333	0.341	0.255	0.188	0.236	0.354	0.503	0.390	0.169	0.194	2
(8A)	c[L-(δ -S)Pip-L-(NMe)Phe]	0.248	0.342	0.314	0.368	0.233	0.222	0.204	0.302	0.362	0.461	0.311	0.161	2
(2B)	c[D-Pip-L-(NMe)Phe]	0.133	0.170	0.172	0.209	0.248	0.189	0.192	0.282	0.418	0.336	0.250	0.209	1
(4B)	c[D-(β -S)Pip-L-(NMe)Phe]	0.117	0.117	0.199	0.238	0.261	0.185	0.150	0.212	0.435	0.380	0.185	0.204	1
(6B)	c[L-(γ -S)Pip-D-(NMe)Phe]	0.186	0.192	0.306	0.270	0.263	0.304	0.366	0.237	0.406	0.525	0.253	0.226	2
(8B)	c[D-(δ -S)Pip-L-(NMe)Phe]	0.205	0.167	0.274	0.352	0.258	0.199	0.209	0.236	0.445	0.423	0.198	0.238	1
(10A)	c[L-Pro-L-(NMe)Phe]	0.116	0.140	0.246	0.233	0.254	0.261	0.172	0.218	0.448	0.308	0.246	0.215	1
(12A)	c[L-(β -S)Pro-L-(NMe)Phe]	0.169	0.260	0.317	0.309	0.219	0.191	0.187	0.253	0.528	0.359	0.228	0.160	1
(14A)	c[L-(γ -S)Pro-L-(NMe)Phe]	0.173	0.360	0.344	0.321	0.246	0.263	0.315	0.348	0.485	0.549	0.335	0.194	1
(10B)	c[L-Pro-D-(NMe)Phe]	0.279	0.247	0.351	0.369	0.261	0.324	0.372	0.329	0.502	0.479	0.275	0.310	1
(12B)	c[D-(β -S)Pro-L-(NMe)Phe]	0.230	0.276	0.372	0.303	0.296	0.261	0.282	0.381	0.558	0.479	0.296	0.291	1
(14B)	c[L-(γ -S)Pro-D-(NMe)Phe]	0.256	0.161	0.228	0.288	0.119	0.133	0.449	0.269	0.427	0.572	0.237	0.275	3

structure was taken as a reference. Each individual torsion was constrained at a minus or plus 12.5° deviation from the optimal angular value in this reference structure, while all other conformational degrees of freedom were subject to energy minimization. Deformation energies (Table 3) are reported with respect to the energy of the reference structure.

Deformation energies of X-ray structures were calculated fixing all endocyclic torsion angles of the DKP ring simultaneously at their values observed in the solid state and optimizing remaining degrees of freedom.

2.4. Conformational description of cyclic dipeptides

2.4.1. Conformation of the DKP ring. The DKP ring can adopt four basic conformation types: planar, chair, boat or twist. The precise conformation of the ring can be determined from the torsion angles φ , ψ and ω which describe the peptide backbone (Fig. 2). Additional parameters are required for a detailed analysis of the amide bond geometry and its possible deviation from planarity. The parameters χ_C , χ_N and τ , introduced by Dunitz (Winkler & Dunitz, 1971), define the out-of-plane bending at the carbon and nitrogen atoms and the twisting of the amide bond, respectively.

To compare DKP ring geometries, Ciarkowski's formalism was used (Jankowska & Ciarkowski, 1987; Gdaniec *et al.*, 1987) describing any feasible conformation as a superposition of the three canonical forms – chair (C), twist (T) and boat (B):

$$T_i = x \cos [2\pi(i-1)/3] - y \sin [2\pi(i-1)/3] + z(-1)^{i-1},$$

where T_i are either endocyclic torsion angles α_i or the displacements of the ring atoms i (1, 2, 3, ... 6). x , y and z represent the contributions of the respective canonical forms: z is a measure of the chair character, whereas x and y describe

the twist-boat character in the pseudorotational plane. From the observation of a clustering of x and y coordinates in a set of 65 experimental DKP X-ray structures reviewed by Ciarkowski (Jankowska & Ciarkowski, 1987; Gdaniec *et al.*, 1987), it was proposed to change (specifically for the diketopiperazine) the x , y , z ring-puckering coordinate system to the l , t , z coordinate system, by performing a 23.3° counterclockwise rotation about the z axis. For reasons of comparability, this coordinate transformation was also applied to the data reported in the present study.

In semispherical coordinates ζ , λ and η (respectively latitude, longitude and amplitude) the endocyclic torsion angles α_i of the DKP ring are to a very good approximation generated by:

$$\alpha_i = \eta_{T/B} \cos [\lambda + 2\pi(i-1)/3] + (-1)^{i-1} \eta_C,$$

$$i = 1, 2, 3, \dots 6 (\varphi_1, \psi_1, \omega_1, \varphi_2, \psi_2, \omega_2),$$

where $\eta_{T/B} = \eta \cos \zeta$ and $\eta_C = \eta \sin \zeta$ are the contributions of the twist-boat and chair forms to the puckering amplitude η , and λ is the phase angle of pseudorotation (de Leeuw *et al.*, 1983). The relative contribution of the chair form X_C is expressed as $X_C = |\eta_C| / (|\eta_{T/B}| + |\eta_C|)$.

2.4.2. Conformation of the side-chains. Endocyclic torsion angles χ_1 to χ_6 (for Pip) and χ_1 to χ_5 (for Pro) are used to describe the conformation of the fused six- and five-membered rings (see Fig. 2). The side-chain orientation of (NMe)Phe is described by torsion angles $\chi_{(\alpha)} = \text{N}-\text{C}_\alpha-\text{C}_\beta-\text{C}_{\text{ipso}}$ and $\chi_{(\beta)} = \text{C}_\alpha-\text{C}_\beta-\text{C}_{\text{ipso}}-\text{C}_{\text{ortho}}$ shown in Fig. 2. The three energetically favored staggered rotamers for the orientation of the phenyl ring are denoted as F (folded), E_N (extended to amide nitrogen) and E_O (extended to amide oxygen), which is independent of the Phe configuration.

Table 4

Theoretical gas-phase geometries, as calculated with DFT (see §2.3), of the model compounds *cyclo*(Gly-Gly), *cyclo*(Phe-Gly), *cyclo*[(NMe)Phe-Gly], *cyclo*(Pip-Gly), *cyclo*[(S)Pip-Gly], *cyclo*(Pro-Gly) and *cyclo*[(S)Pro-Gly].

c is used as an abbreviation for *cyclo*.

Compound	DKP-ring						Peptide bond nonplanarity						Pip or Pro ring					
	φ_1	ψ_1	ω_1	φ_2	ψ_2	ω_2	$\chi_{(CS)}$	$\chi_{(N4)}$	$\tau_{(1)}$	$\chi_{(C2)}$	$\chi_{(N1)}$	$\tau_{(2)}$	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6
c(Gly-Gly)	28.1	-18.7	-8.5	28.2	-18.8	-8.4	0.4	11.1	-6.3	0.4	11.0	-6.3	—	—	—	—	—	—
c(L-Phe-Gly) [†]	22.3	-12.9	-11.3	27.4	-17.7	-6.4	-0.2	14.3	-8.1	0.2	7.4	-5.5	—	—	—	—	—	—
c[L-(NMe)Phe-Gly] [‡]	26.2	-17.2	-11.2	31.2	-21.5	-6.2	0.1	14.8	-7.7	0	4.2	-8.2	—	—	—	—	—	—
c[L-Pip-Gly]	5.0	-2.8	-3.25	7.1	-4.7	-1.1	-0.4	3.4	-2.8	0.4	2.0	-0.7	-54.3	53.5	-53.1	53.5	-57.0	57.1
c[L-(β -S)Pip-Gly]	35.0	-23.6	-7.7	28.9	-17.5	-13.5	-1.0	9.2	-5.1	0.6	23.8	-3.7	-55.1	52.1	-58.4	57.6	-63.6	66.8
c[L-(γ -S)Pip-Gly]	14.4	-7.5	-8.4	17.6	-10.3	-5.1	-0.6	9.2	-7.1	0.2	5.8	-4.6	-60.0	51.9	-51.8	59.7	-63.4	63.3
c[L-(δ -S)Pip-Gly]	19.4	-11.3	-8.8	21.4	-12.8	-6.9	-0.4	10.3	-6.9	0.2	11.7	-2.3	-58.2	59.6	-53.3	54.6	-64.3	61.6
c(L-Pro-Gly)	-32.8	29.6	2.4	-33.1	29.8	2.0	-1.1	-5.2	0.7	-0.3	2.9	7.2	-34.2	36.7	-24.6	2.8	20.0	—
c[L-(β -S)Pro-Gly]	-28.0	26.0	3.9	-33.4	29.9	-0.9	-1.6	-7.4	2.1	-1.0	6.6	5.8	-35.1	38.5	-31.0	4.6	23.7	—
c[L-(γ -S)Pro-Gly]	-31.2	25.6	4.0	-29.8	24.2	5.5	-1.0	-7.2	1.9	-0.7	-5.1	6.6	-41.5	34.9	-18.4	-4.2	30.5	—

[†] The orientation of phenyl group is defined by torsions: $\chi_{(\alpha 1)} = 65.1^\circ$; $\chi_{(\alpha 2)} = -62.2^\circ$; $\chi_{(\beta 1)} = 90.5^\circ$; $\chi_{(\beta 2)} = -88.9^\circ$. [‡] The orientation of phenyl group is defined by torsions: $\chi_{(\alpha 1)} = 65.9^\circ$; $\chi_{(\alpha 2)} = -63.7^\circ$; $\chi_{(\beta 1)} = 91.0^\circ$; $\chi_{(\beta 2)} = -87.9^\circ$.

3. Results and discussion

3.1. Structural analysis of simple gas-phase model compounds

In order to better understand the conformational features of the title compounds, it is instructive to first examine those features in a selected set of model cyclic dipeptide compounds in the gas phase, *i.e.* without any interference of intermolecular interactions as observed in a solid-state structure. Table 4 lists the structural characteristics that were determined using a single-molecule DFT approach for the compounds: *cyclo*(Gly-Gly) and *cyclo*(Xaa-Gly) with Xaa = Pip, (β -S)Pip, (γ -S)Pip, (δ -S)Pip, Pro, (β -S)Pro, (γ -S)Pro, Phe and (NMe)Phe.

3.1.1. The unsubstituted diketopiperazine ring. These DFT results suggest that the unsubstituted DKP ring in *cyclo*(Gly-Gly) prefers the twisted boat conformation $c_{\alpha}B-c_{\alpha}T^N$ ($z = -0.11$, $l = 27.9$ and $t = -5.5$), with amide bonds that deviate substantially from planarity ($\omega = -8.5$ and $\chi_{(N4)} = 11$). This is confirmed (Bettens *et al.*, 2000) by microwave measurements and *ab initio* calculations at the MP2 level. In contrast, X-ray crystallographic studies of *cyclo*(Gly-Gly) invariably find the planar ring structure in the solid state (Dorset, 2010). This not only demonstrates the important impact of crystal packing, but also points out the considerable conformational flexibility of the DKP ring. Similar to the iso-electronic 1,4-*cyclo*-hexadiene, a boat form minimizes Pitzer and Bayer strain in *cyclo*(Gly-Gly), and the low-energy cost for out-of-plane bending (rehybridization) of the amide nitrogen atoms renders φ more floppy than the ψ torsion angle. The twisted boat character ($c_{\alpha}B-c_{\alpha}T^N$) with $|\varphi| > |\psi|$, the non-planar amide bonds (ω same sign as ψ) and the pyramidal nitrogen (with $\chi_{(N)}$ of opposite sign of ω for optimal mesomeric stabilization) can therefore be understood as intrinsic characteristics of the DKP ring. These features are recognizable in the X-ray data of the title compounds (*see below*) and of many reported puckered substituted diketopiperazines (Jankowska & Ciarkowski, 1987).

3.1.2. The effect of ring fusion. The title cyclic dipeptide compounds are all bicyclic and the DKP and piperidine (or pyrrolidine) rings are fused, sharing the N and C α atoms. As such, the endocyclic torsion angles at the ring junction, φ_1 and χ_6 {or χ_5 in *cyclo*[(X)Pro-Phe]}], are interdependent. The ring size of the cyclic imino acid therefore limits the range of accessible φ_1 values for the DKP ring. Fusion to a six-membered ring imposes low φ_1 values, *i.e.* a flattening of the DKP ring, whereas fusion to a five-membered ring typically puckers the DKP ring by 30° to 40° with the C α -C β bond in pseudo-equatorial position. However, the approximate relation $|\chi - \varphi| \sim 60^\circ$ for a planar amide N is considerably relaxed for a pyramidal amide N typical of non-planar amide bonds.

For the non-sulfur analogs *cyclo*(Pro-Gly) and *cyclo*(Pip-Gly) single-molecule DFT calculations yield quasi-planar amide bonds and small N-pyramidalization $\chi_{(N)}$ (see Table 4), with $|\chi - \varphi| \sim 52^\circ$. Introduction of sulfur has large effects on $\chi_{(N)}$, and the relation $|\chi - \varphi + \chi_{(N)}| \sim 56^\circ$ is a more appropriate descriptor of torsion angle transmission at the ring junction. Apparently, two effects are at play. Firstly, as the result of the longer C-S bonds, a local flattening in the C-S-C region and an increased pucker at the ring junction lead to increased $|\chi_5|$ values [23.7° and 30.5° for the (β -S) and (γ -S)Pro analogs as compared to 20°] and $|\chi_6|$ values [66.8° , 63.3° and 61.6° for the (β -S), (γ -S) and (δ -S)Pip analogs as compared with 57.1°] in accordance with the *reflex effect* (Bucourt, 1974). Secondly, a generalized *anomeric effect*, *i.e.* the repulsion between the lone pair electron densities of sulfur and the amide nitrogen, affects the N-pyramidal character directly. The larger absolute values of $\chi_{(N1)}$ and related (negative) ω_2 in the (β -S) and (δ -S)Pip analogs lead to increased pucker. The extent of pucker of the DKP ring and the increased pseudo-axial (bowsprit) character of the C α -C β bond in the order β -S > δ -S > γ -S > CH $_2$ as determined for *cyclo*[(S)Pip-Gly] gas-phase models are largely preserved in similar calculations and the X-ray data of the *cyclo*[(S)Pip-(NMe)Phe] title compounds (*see below*).

Table 5

Comparison of experimental X-ray structures and theoretical gas-phase geometries ($^{\circ}$), as calculated with DFT (see §2.3), for *cis* [(2A), (4A), (6A) and (8A)] and *trans* [(2B), (4B), (6B) and (8B)] *cyclo*[(S)Pip-(NMe)Phe] isomers.

No.	DKP-ring						Peptide bond nonplanarity						Pipelic acid ring (C,C)						Phe (C,C)			
	φ_1	ψ_1	ω_1	φ_2	ψ_2	ω_2	$\chi(C5)$	$\chi(N4)$	$\tau_{(1)}$	$\chi(C2)$	$\chi(N1)$	$\tau_{(2)}$	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6	$\chi(\alpha_1)$	$\chi(\alpha_2)$	$\chi(\beta_1)$	$\chi(\beta_2)$
<i>cis</i> [(2A), (4A), (6A) and (8A)]																						
(2A) c[L-Pip-L-(NMe)-Phe]																						
m1	16.4	-3.4	-16.4	23.4	-10.0	-9.2	-3.8	13.3	-15.7	-0.1	11.2	-7.1	-54.0	52.6	-54.0	55.6	-59.2	58.7	66.4	-61.6	89.3	-89.7
m2	18.1	-3.3	-17.6	24.2	-9.2	-11.3	-4.4	13.2	-17.6	-0.8	12.1	-9.7	-54.2	52.9	-53.6	55.1	-59.6	59.0	65.2	-63.4	91.0	-88.6
Calc.	11.9	-5.8	-8.2	16.2	-9.8	-3.8	-2.6	1.7	-12.1	0.5	6.7	-1.4	-54.2	53.2	-53.1	53.5	-57.1	57.5	67.5	-63.9	92.9	-87.0
(4A) c[L-(β -S)Pip-L-(NMe)-Phe]																						
m1	21.2	-14.0	-0.9	9.9	-3.6	-12.2	0.9	-1.5	-4.2	-0.7	15.0	-8.7	-55.1	52.7	-61.3	61.7	-64.6	64.3	70.4	-57.7	92.2	-85.6
m2	24.2	-14.0	-2.4	10.7	-1.8	-16.3	-1.2	-0.1	-3.7	-3.6	17.1	-11.9	-54.9	54.0	-61.7	61.4	-64.3	65.1	69.9	-59.7	89.1	-87.5
Calc.	21.9	-12.8	-4.5	13.7	-5.2	-12.5	-2.2	-1.2	-8.0	-2.6	12.6	-9.8	-53.9	52.6	-59.4	58.1	-62.0	63.9	67.6	-63.9	92.9	-87.6
(6A) c[L-(γ -S)Pip-L-(NMe)-Phe]																						
m1	15.8	-3.1	-17.3	24.7	-11.3	-8.0	-4.2	12.9	-17.5	-0.8	5.6	-9.6	-60.8	53.1	-53.2	60.0	-63.3	63.7	65.4	-62.8	88.5	-91.1
m2	17.4	-5.9	-14.4	23.3	-11.4	-8.2	-3.4	10.9	-14.4	-0.5	7.4	-8.5	-60.0	52.5	-53.4	60.9	-64.1	63.8	67.2	-61.4	88.5	-91.0
Calc.	15.0	-7.4	-9.1	18.2	-10.4	-5.8	-2.3	2.6	-13.3	-0.3	5.5	-5.8	-60.2	52.5	-52.5	59.7	-62.8	62.9	67.0	-63.7	93.1	-86.8
(8A) c[L-(δ -S)Pip-L-(NMe)-Phe]																						
m1	22.5	-6.6	-16.5	24.6	-8.6	-14.4	-3.1	12.7	-17.2	-2.0	14.0	-12.8	-57.1	59.6	-55.4	57.5	-66.7	61.8	62.8	-66.0	92.4	-87.5
m2	22.2	-6.8	-16.0	24.2	-8.7	-14.0	-3.0	12.9	-16.2	-2.1	14.0	-11.8	-57.0	59.2	-55.1	57.7	-66.8	62.0	64.4	-64.2	90.9	-88.2
Calc.	19.0	-10.0	-8.4	18.5	-9.6	-8.9	-2.2	2.3	-12.3	-1.3	9.7	-6.8	-58.6	59.4	-52.6	53.7	-63.8	61.9	64.9	-66.0	90.3	-89.9
<i>trans</i> [(2B), (4B), (6B) and (8B)]																						
(2B) c[L-Pip-D-(NMe)Phe]†																						
	-17.7	10.7	10.7	-25.1	17.6	3.2	2.2	-9.4	9.6	-1.6	-15.4	-7.4	-53.7	56.1	-56.4	55.5	-56.2	54.2	-67.6	59.6	-89.3	86.5
Calc.	-18.4	8.7	12.4	-24.1	14.0	6.6	2.2	-7.9	14.7	-1.4	-15.2	-0.6	-52.7	54.8	-54.7	53.7	-54.7	53.3	-66.8	62.6	-91.9	87.2
(4B) c[L-(β -S)Pip-D-(NMe)-Phe]†																						
	11.3	-10.7	15.7	-18.8	17.2	-14.9	4.4	-8.2	18.8	-6.7	7.5	-15.6	-52.7	51.4	-58.6	60.2	-65.4	64.6	-69.2	57.5	-92.6	85.4
Calc.	10.0	-9.1	12.6	-14.9	14.0	-12.7	3.8	-4.6	17.0	-4.7	6.8	-13.9	-51.3	51.2	-59.1	59.6	-63.6	62.4	-69.1	60.0	-96.2	83.3
(6B) c[L-(γ -S)Pip-D-(NMe)-Phe]																						
m1	-22.9	7.7	18.7	-30.2	14.3	11.4	3.7	-12.5	21.2	-1.6	-20.8	3.3	-59.9	56.5	-55.5	60.2	-59.0	57.3	-61.2	65.5	88.6	-91.7
m2	-20.0	9.7	17.1	-33.5	22.3	3.5	3.6	-12.1	18.5	-3.0	-18.4	-8.5	-57.9	53.0	-53.6	61.1	-62.1	58.9	-69.5	57.2	86.8	-89.5
Calc.	-13.9	5.3	13.9	-24.2	14.9	3.4	3.1	-8.8	15.9	-2.2	-14.1	-5.1	-59.5	54.8	-54.3	60.5	-60.3	58.5	-67.1	62.1	85.0	-94.0
(8B) c[L-(δ -S)Pip-D-(NMe)-Phe]†																						
	-7.1	5.2	11.2	-24.5	21.4	-6.7	2.6	-7.3	12.5	-5.0	-8.1	-16.6	-57.3	61.6	-55.6	56.7	-64.2	58.4	-63.1	63.1	-86.2	87.9
Calc.	-4.9	-1.0	14.3	-20.7	13.7	-1.9	3.8	-7.5	17.3	-3.1	-5.7	-6.4	-56.3	60.6	-54.7	55.8	-63.6	58.0	-67.7	61.6	-94.0	85.5

† The signs of torsion angles are converted to those for c(L-,D-) enantiomer for direct comparison of *trans*-isomers.

The impact of sulfur substitution in *cyclo*[(S)Pro-Gly] remains mostly limited to conformational changes in the flexible five-membered ring (*see above*), with little effect on the conformation of the DKP ring and on the planarity of the amide bonds.

Energy differences (as obtained from the single-molecule DFT calculations) between the positional isomers of the thia-analogs indicate their relative stabilities. In the *cyclo*[(S)Pip-Gly] series, with the rigid six-membered piperidine ring, conformational effects are relatively small in the side-chain, but very substantial in the DKP ring. The *cyclo*[(β -S)Pip-Gly] and *cyclo*[(δ -S)Pip-Gly] compounds are respectively 1.8 kcal mol⁻¹ (1 kcal mol⁻¹ = 4.184 kJ mol⁻¹) and 0.5 kcal mol⁻¹ less stable than *cyclo*[(γ -S)Pip-Gly] conform a repulsive anomeric effect in the β and δ isomers. In the *cyclo*[(S)Pro-Gly] series it is the more flexible (five-membered) side-chain that deforms upon S-substitution, whereas the DKP ring remains quasi unchanged. Both *cyclo*[(β -S)Pro-Gly] and *cyclo*[(γ -S)Pro-Gly] exist in a North (N) and a South (S) form. The N form (characterized by $\chi_3 < 0$ for L-residues) is invariably observed (so far) in the solid state and is, as determined by solution ¹H NMR studies, also the more abundant conformer. Whereas for *cyclo*(Pro-Gly) and *cyclo*[(γ -S)Pro-Gly] the N form is favored by 0.30 kcal mol⁻¹

and 0.23 kcal mol⁻¹, for *cyclo*[(β -S)Pro-Gly] the S form is calculated to be 0.84 kcal mol⁻¹ more stable, consistent with a destabilization of the N form by a repulsive *rabbit-ear* effect (repulsion of nitrogen and sulfur lone pair electron densities).

3.1.3. Constraints imposed by the (NMe)Phe residue. To escape steric repulsion with the *N*-methyl and carbonyl groups (A_{1,2} strain or 1,2-allylic strain; Johnson & Malhotra, 1965) the benzyl group (as other amino acid side-chains) prefers the pseudoaxial (flagpole) orientation and is folded (F rotamer) over a twisted _{C α} B boat DKP ring, consistent with a presumed face-to-face attractive interaction between the phenyl and DKP rings. The single-molecule DFT calculations predict a local pucker for *cyclo*[L-(NMe)Phe-Gly] with $\varphi = 26.2^{\circ}$, $\psi = -17.2^{\circ}$ and $\omega = -11.2^{\circ}$ in good agreement with X-ray data of *cyclo*(L-Phe-Sar) (Cisarova, 2014, private communication), matching almost exactly the conformational preference of the unsubstituted *cyclo*(Gly-Gly).

3.2. Solid-state (X-ray) molecular structures of the title compounds

Torsion angles of the diketopiperazine ring and side-chains, and Dunitz-parameters describing the amide bond geometries as observed by X-ray diffraction on crystals of the ten title

Table 6

Comparison of experimental X-ray structures and theoretical gas-phase geometries ($^{\circ}$), as calculated with DFT (see §2.3), for *cis* [(10A), (12A) and (14A)] and *trans* [(10B), (12B) and (14B)] *cyclo*[(S)Pip-(NMe)Phe] isomers.

	DKP-ring						Peptide bond nonplanarity						Proline ring (C,C)					Phe (C,C)			
	φ_1	ψ_1	ω_1	φ_2	ψ_2	ω_2	$\chi(\text{C5})$	$\chi(\text{N4})$	$\tau(1)$	$\chi(\text{C2})$	$\chi(\text{N1})$	$\tau(2)$	χ_1	χ_2	χ_3	χ_4	χ_5	$\chi(\alpha 1)$	$\chi(\alpha 2)$	$\chi(\beta 1)$	$\chi(\beta 2)$
<i>cis</i> [(10A), (12A) and (14A)]																					
(10A) c[L-Pro-L-(NMe)Phe]	6.3	4.2	-19.4	23.5	-11.9	-1.8	-4.9	14.2	-19.8	2.9	23.2	16.6	-36.0	23.1	-1.4	-22.6	37.4	64.7	-62.6	90.2	-89.8
Calc.	-1.3	8.6	-14.9	12.9	-4.4	-0.6	-6.0	3.6	-20.2	3.0	17.8	13.6	-39.4	31.8	-12.3	-13.5	33.9	67.6	-61.4	94.9	-84.8
(12A) c[L-(β -S)Pro-L-(NMe)Phe]	14.4	-3.0	-13.7	19.2	-7.3	-8.6	-3.3	10.1	-14.0	-0.4	28.5	11.6	-24.1	-1.0	25.7	-46.4	44.7	63.3	-64.9	93.6	-88.4
Calc.	10.5	-0.9	-9.7	11.0	-1.2	-9.2	-4.5	0.3	-14.6	-0.4	26.8	8.8	-15.4	-10.9	34.4	-49.5	40.0	68.3	-61.4	97.2	-83.2
(14A) c[L-(γ -S)Pro-L-(NMe)-Phe]	12.0	2.3	-19.4	22.0	-7.0	-9.1	5.0	12.6	-21.1	0.1	19.3	1.0	-43.1	23.6	1.6	-29.1	48.4	67.1	-60.3	92.3	-88.7
Calc.	20.1	-6.1	-12.4	18.0	-4.2	-14.4	-3.6	4.4	-16.8	-1.1	25.2	-2.5	-38.9	17.2	8.4	-34.4	49.2	64.5	-65.6	88.7	-91.1
<i>trans</i> [(10B), (12B) and (14B)]																					
(10B) c[L-Pro-D-(NMe)Phe]	-27.0	25.5	1.2	-27.5	26.1	0.6	-1.1	-3.3	0.2	-0.4	4.0	5.6	-37.3	37.9	-23.2	-1.0	24.5	-67.5	61.1	87.8	-90.5
Calc.	-30.9	27.2	-0.3	-25.0	22.1	5.5	-1.3	0.2	0.9	0.4	0.0	10.6	-36.3	35.8	-21.1	-2.3	24.6	-67.1	63.2	89.1	-90.0
(12B) c[L-(β -S)Pro-D-(NMe)Phe] [†]	-20.9	19.1	5.8	-28.7	26.4	-2.6	0	-9.1	2.4	-0.9	7.6	3.3	-40.3	40.9	-29.6	-0.9	30.6	-67.9	59.8	94.8	-82.3
Calc.	-26.0	24.5	1.2	-25.9	24.5	0.8	-1.5	-1.0	2.8	-0.9	4.8	7.3	-37.0	38.0	-28.3	0.1	27.8	-66.1	63.7	87.0	-91.8
(14B) c[L-(γ -S)Pro-D-(NMe)-Phe]	-15.1	8.0	11.9	-24.9	17.2	2.0	2.8	-9.7	11.3	-1.8	0.2	5.8	-38.8	21.8	1.4	-26.8	43.9	-60.9	66.6	94.4	-81.8
m1	-18.5	13.9	6.3	-22.6	17.9	2.1	0.9	-6.4	5.2	-2.3	-3.5	2.9	-39.0	22.6	0.1	-25.2	42.9	-66.4	60.8	98.4	-80.7
m2	-28.5	24.3	2.1	-26.0	22.0	4.9	0	-4.3	-0.1	-1.0	-6.0	4.7	-42.8	33.4	-14.5	-9.8	35.1	-65.9	62.3	90.1	-87.7
Calc.	-28.8	22.7	3.7	-25.7	20.1	6.8	-0.3	-2.6	5.1	-0.6	-7.1	7.1	-41.6	31.4	-12.5	-11.6	35.6	-66.1	63.5	87.5	-91.4

[†] The signs of torsion angles are converted to those for c(L-D-) enantiomer for direct comparison of *trans*-isomers.

compounds are presented in Tables 5 and 6 for *cyclo*[(S)Pip-(NMe)Phe] and *cyclo*[(S)Pro-(NMe)Phe] analogs respectively. DFT calculations yield information on the conformation and torsion angles in the corresponding single gas-phase DKP molecules. Data for the corresponding non-sulfur 'reference' compounds published in our previous paper (Budesinsky *et al.*, 2010) is included in these tables for comparison. To facilitate their discussion, the data is grouped within each Table according to relative configuration: in *cis* isomers (2A), (4A), (6A) and (8A) and *trans* isomers (2B), (4B), (6B) and (8B) (Table 5) and in *cis* isomers (10A), (12A), and (14A) and *trans* isomers (10B), (12B) and (14B) (Table 6).

Corresponding ORTEP views of the title compounds are provided in Figs. 3 and 4 illustrating also that for compounds (4A), (6A), (8A) and (6B) there are two slightly different conformations present within the asymmetric unit cell ($Z' = 2$) and that for (14B) there are three ($Z' = 3$) different conformations, of which one is considerably different. The DKP ring flexibility for all compounds, as quantified by the deformation energies at -12.5° and $+12.5^{\circ}$ from the optimal endocyclic torsion angles, is also indicated in Table 3.

3.2.1. Side-chains. In all reported cyclic dipeptide crystal structures, the aromatic ring of (NMe)Phe is folded over the DKP-ring with $\chi(\alpha)$ absolute values between 61° and 70° (F-conformer, as illustrated in Figs. 3 and 4).

The relatively rigid six-membered rings of the thia-pipecolic acid residues retain a chair-form $\gamma\text{-C}^{\text{N}}$ with small but significant differences of the endocyclic torsion angles χ_i . The trends observed in the X-ray structures of the *cyclo*[(S)Pip-(NMe)Phe] series as the position of the S atom changes are reproduced by single-molecule DFT calculations (Table 5) and

parallel a similar trend of the *cyclo*[(S)Pip-Gly] model compounds (Table 4) that can be explained (*see above*) in relation to ring fusion at the χ_6 - φ_1 ring junction. *Cis* isomers have a 3 – 5° larger χ_6 value than the corresponding *trans* isomers in both the crystal and in DFT calculations, an effect

Figure 3
ORTEP views of molecules of cyclic dipeptides (4A), (4B), (6A), (6B), (8A) and (8B).

of the pseudoaxial preference of the benzyl group transmitted over the DKP ring.

The five-membered pyrrolidine and thiazolidine rings of the *cyclo*[(S)Pro-(NMe)Phe] series are relatively flexible. Major changes of endocyclic torsion angles are observed as sulfur is introduced at β or γ position and between *cis* and *trans* isomers. DFT single-molecule calculations reproduce the χ_i values for the most part, but significant deviations remain *e.g.* in (10A) and (14B). A dramatic illustration of crystal packing forces substantially altering the conformation of the thiazolidine ring is observed in the crystal of (14B) (Fig. 4) where three symmetry independent molecules occur in the unit cell, with molecule m3 very different from m1 and m2.

3.2.2. Geometry of the diketopiperazine ring. Depending on their relative configuration (*cis* or *trans*), the individual side-chains in *cyclo*[(S)Pip-(NMe)Phe] and *cyclo*[(S)Pro-(NMe)Phe] either displace C_α atoms to the same side or to the opposite sides of the average DKP ring plane. The title compounds are therefore discussed in four series, according to relative configuration: the *cis* (L,L) series [(2A), (4A), (6A) and (8A)] and the *trans* (L,D) series [(2B), (4B), (6B) and (8B)] of *cyclo*[(S)Pip-(NMe)Phe] isomers (Table 5) and the *cis* (L,L) series [(10A), (12A) and (14A)] and the *trans* (L,D) series [(10B), (12B) and (14B)] of *cyclo*[(S)Pro-(NMe)Phe] isomers (Table 6). In this way, within each series the impact of CH_2/S substitution is most explicitly revealed. A graphical overview of the conformations of the DKP ring is presented in Fig. 5 in terms of *l*, *t* and *z* coordinates (see *Materials and Methods*).

3.2.3. *Cis* [(2A), (4A), (6A) and (8A)] and *trans* [(2B), (4B), (6B) and (8B)] *cyclo*[(S)Pip-(NMe)Phe]. The average deviation Δ_{av} between the X-ray and calculated gas-phase torsion angles φ_1 , ψ_1 , ω_1 , φ_2 , ψ_2 , ω_2 of the diketopiperazine ring is in the range 2–6° both for the *cis* and the *trans* series. This signifies

Figure 4
ORTEP views of molecules of cyclic dipeptides (12A), (12B), (14A) and (14B).

that the solid-state molecular structure is essentially the same as in the gas phase for these compounds.

The differences of the X-ray structures between symmetry independent molecules m1 and m2 show that crystal packing effects can be substantial, *e.g.* for (6B) $\Delta\varphi_2$, $\Delta\psi_2$ and $\Delta\omega_2$ amount to 8°.

Major structural changes of the DKP ring occur upon CH_2/S substitution in the β -position of the pipecolic acid ring. Comparing X-ray torsion angle data of (2A) with (4A) the $\Delta\varphi_1$, $\Delta\psi_1$, $\Delta\omega_1$, $\Delta\varphi_2$, $\Delta\psi_2$, $\Delta\omega_2$ values shift respectively by +5.5, −10.6, +15.4, −13.5, +6.9 and −4.0°. For (2B) *versus* (4B) similar differences amount to +29, −21.4, +5.0, +6.3, −0.4 and −18.1°, respectively. The DFT gas phase calculations show corresponding values for (2A)/(4A): +10, −7, +3.7, −2.5, +4.6 and −8.7° and for (2B)/(4B): 28.4, −17.8, +0.2, +9.2, 0 and −19.3°. To the most part the effect of the introduction of the β -sulfur atom is similar in the gas phase and the solid state and, to that extent of a direct intramolecular origin.

The transmission of torsion angles at the χ_6/φ_1 ring junction as calculated and discussed in the *cyclo*[(S)Pip-Gly] series (see *above*) is operative, implying similar anomeric and reflex

Figure 5
Graphical representation of experimental X-ray (in black) and calculated gas-phase (in red) conformations of the DKP ring. (a) *l*, *t*-plot showing the twist-boat character in the pseudorotational plane. (b) *l*, *z*-plot showing the contribution of chair character expressed as the parameter *z*, perpendicular to the *l*, *t*-plane, and directly related to η_C . For (2B), (4B), (8B) and (12B) the signs of *l*, *t* and *z* parameters of D,L enantiomers were changed for direct comparison with L,D configuration of (6B), (10B) and (14B). In Table S2 in the supporting information lists numerical values of *l*, *t* and *z* of the title compounds and Fig. S1 illustrates the pseudorotation pathway of the various twist and boat forms of the DKP ring.

effects. For instance, by applying the approximate relation $\chi_6 - \varphi_1 + \chi_{(N1)} \sim 56.0^\circ$, φ_1 becomes:

$$\text{for (2B)} \quad \varphi_1 \sim 54.2 - 15.4 - 56.0 \sim -17.2$$

$$\text{for (4B)} \quad \varphi_1 \sim 64.6 - 7.4 - 56.0 \sim 16.1.$$

This example illustrates that the reflex effect [χ_6 from 54.2° in (2B) to 64.6° in (4B)] contributes to the increase of φ_1 , but transmission of this effect is blurred by changes in N1 hybridization. In this example the latter results from β -S/N1 lone pair repulsion (anomeric effect). Indeed, X-ray and calculated χ_6 values in both the *cis* and *trans* series of *cyclo*[(S)Pip-(NMe)Phe] consistently follow the trend β -S > δ -S $\sim$ γ -S > CH₂ of the Gly analogs (see Tables 5 and 6). The resulting effects on φ_1 , however, are quite different in both series.

In the *cis* series L-(S)Pip and L-(NMe)Phe fragments combine same signs of opposite φ and ψ angles, compatible with the preferred DKP twist-boat conformation. All *cis* analogs cluster in the right/low quadrant of the *l,t*-plot (Fig. 5) in the ${}^{\text{C}\alpha}\text{T}_{\text{N}-\text{N}}\text{B}$ region of the pseudorotation path. Although not fully understood, most structural features observed are reproduced in the single-molecule DFT calculations and are therefore of intramolecular origin. As χ_6 increases in the solid-state structures of (2A), (4A), (6A) and (8A), from 59° [(2A)] to 65° [(4A)] the pucker at the (S)Pip side of the DKP ring is enhanced roughly in line with the results of the single-molecule DFT calculations. In particular, the pronounced non-planarity of ω_2 (-12.2 and -16.3°) and related pyramidal N₁ ($\chi_{(N1)} = +15.0$ and $+17.1^\circ$) observed in (4A) is also present in the gas-phase calculations and can be interpreted to originate from sulfur/nitrogen lone pair interactions. Changes on both sides of the ring appear strongly correlated through mediation by the amide bonds. The larger φ_1 expected in (4A) seems tempered by the (β)S//phenyl contact, that apparently is lowering the pucker at the (NMe)Phe side of the DKP ring as well.

Distribution of the *trans* isomers in the *l,t*-plane (Fig. 5) is more diffuse and situated in the ${}^{\text{C}\alpha}\text{B}-{}^{\text{C}\alpha}\text{T}_{\text{N}}^{\text{N}}$ region. Compound (4B) is an eye-catching outlier in the *l,t* and *l,z* plots with a flattened ${}^{\text{N}}\text{T}_{\text{N}}^{\text{C}}-\text{N}\text{B}$ and relatively high chair character ($X_{\text{C}} = 0.88$, $z = 15^\circ$).

In the *trans* series individual preferences of L-(S)Pip and D-(NMe)Phe fragments tend to oppose one another: *e.g.* preferences from Table 4 for L-(β -S)Pip (φ , $\psi = +35.0$, -23.6°) and D-(NMe)Phe (φ , $\psi = -26.2$, $+17.2^\circ$) tend to impose opposite signs of φ_1 and φ_2 and of ψ_1 and ψ_2 in *cyclo*[L-(β -S)Pip-D-(NMe)Phe] [(4B)]. Also here most structural features observed are reproduced in the DFT calculations and have therefore an intramolecular origin. As in the non-sulfur analog (2B), also in (6B) and to a lesser extent in (8B) the preferred local pucker of the cyclic imino residue is inverted towards a pseudoequatorial $\text{C}_\alpha-\text{C}_\beta$ orientation with concomitant nitrogen inversion at low energy cost. Apparently, twist-boat DKP rings are favored. Only the (β -S)Pip fragment which has the highest preference for flagpole orientation (pseudoaxial character) and largest χ_6 , resists the N-inversion and keeps both $\text{C}_\alpha-\text{C}_\beta$ side-chains in their pseudoaxial positions in a

flattened chair DKP ring as observed in the solid state for *cyclo*[(β -S)Pip-(NMe)Phe] [(4B)] and as found in single-molecule DFT calculations. The anomeric effect provides an explanation for this behavior.

3.2.4. *Cis* [(10A), (12A) and (14A)] and *trans* [(10B), (12B) and (14B)] *cyclo*[(S)Pip-(NMe)Phe]. In the discussion of the effect of CH₂/S substitution in *cyclo*[(S)Pip-(NMe)Phe] isomers, again torsion angles at the χ_5/φ_1 ring junction as calculated and discussed in the *cyclo*[(S)Pip-(NMe)Phe] series (*see above*) are the starting point. The low χ_5 torsions (from 20° to 30°) for *cyclo*[L-(S)Pro-Gly] dictate a pseudoequatorial preference for the $\text{C}_\alpha-\text{C}_\beta$ bond in (S)Pro fragments.

Trans combinations of L-(S)Pro and D-(NMe)Phe fragments are compatible with the preferred DKP twist-boat DKP conformation, *i.e.* opposite torsion angles have equal signs. As χ_5 increases from 25° (10B) to 32° (12B) and $\sim 42^\circ$ [m1 and m2 in (14B)] the pucker at the (S)Pro side of the DKP ring is reduced: $|\varphi_1|$ diminishes (becomes less negative for L-residues) and even so does ψ_1 , the change being countered by increasingly positive ω_1 (for L) and related pronounced N₄ pyramidal character [$\chi_{(N4)}$ negative for L]. The (NMe)Phe fragment remains optimally puckered in its preferred F rotamer.

In the *cis* series of *cyclo*[(S)Pip-(NMe)Phe] individual preferences of L-(S)Pro and L-(NMe)Phe fragments strongly oppose one another. Nitrogen inversion at N₄ [with $\chi_{(N4)} = +10^\circ$ to $+15^\circ$ and related $\omega_1 = -15^\circ$ to -20°] and extremely pyramidal N₁ [$\chi_{(N1)} = +18^\circ$ to $+28^\circ$] accommodates a rather flattened (S)Pro side with the usually preferred pucker of the (NMe)Phe F rotamer. The largest conformational changes in (10A), (12A) and (14A) residue in the five-membered ring side-chains (*see above*).

3.3. Molecular packing

Intermolecular forces cause molecules, with characteristic shape and flexibility, to aggregate in a particular periodic pattern. In addition to molecular conformation (Tables 5 and 6), X-ray diffraction reveals all geometric details of molecular aggregation in the solid state.

Density functional theory optimizations of isolated molecules of the title compounds (Tables 5 and 6) succeed to reproduce the molecular conformations observed in the crystal remarkably well, with few but relevant exceptions. This illustrates the weak to moderate impact of intermolecular packing forces on the conformation of *N*-methylated DKPs, *i.e.* in the absence of classical NH...O hydrogen bonds. In this circumstance DFT can provide reliable estimates of molecular flexibility (*see Table 3*).

Rationalization of packing on the basis of intermolecular interaction energies has become increasingly feasible nowadays, but is considered beyond the scope of this paper.

The present crystal structures are discussed in terms of the packing of sub-motifs that embody the most strong and directional intermolecular interactions. Traditional hydrogen bonds ($\text{X}-\text{H}\cdots\text{Y}$, with X, Y = O or N) combine high strength and high directionality. Weaker non-classical hydrogen bonds ($\text{X}-\text{C}-\text{H}\cdots\text{Y}$, with X = N or S, Y = O, N or S) retain relevant

directionality. van der Waals dispersion interactions are the weakest but universal (*i.e.* irrespective of the element type) with little or no directionality (for review see Steiner, 2002).

3.3.1. *Cis* (S)Pip series: (2A), (4A), (6A) and (8A). The molecular shapes of (2A), (6A) and (8A) are nearly identical as can be concluded from experimental and calculated data (Table 5). Substitution of a CH₂ moiety with an S atom is close to an isosteric operation. It is not very surprising to observe that these molecules pack in an (almost) identical fashion. The three crystals have two symmetry-independent but only slightly different molecules m1 and m2 in their unit cell. These form m1-m2 dimers by aggregation of their (NMe)Phe moieties by two N—C α —H $\cdots$ O=C non-classical hydrogen bonds. This aggregation has a *pseudo* twofold rotation axis at the center of gravity of the dimer. Simple translations efficiently pack this m1-m2 dimeric sub-motif in two crystallographic dimensions [*a* and *b* for (2A) and (8A); *c* and *a* for (6A)] making penetrating polar contacts of the type Csp²—H $\cdots$ O=C(NMePhe), and (NMePhe)C β —H $\cdots$ O=C(Sip) between neighboring dimers. Thus bilayers are formed of *ca.* 13.5 Å thickness, with polar interior (with quasi perpendicular average DKP planes of m1 and m2) and both non-polar side-chains pointing outwards. These bilayers stack by simple translation along the *c* direction for (2A) and (8A) (space group *P*₁) and in combination with rotation over 180° along the *b* direction for (6A) (space group *P*₂₁). Non-polar interactions between bilayers are mostly of the van der Waals type (C—H $\cdots$ H—C and C—H $\cdots$ C) with contact distances in the range 2.4 Å (sum of van der Waals radii) to 2.8 Å and isotropic within a broad angular range. The sulfur atom in (6A) and (8A), residing in this non-polar zone with S—C—H $\cdots$ S contacts at van der Waals distance ± 0.15 Å, apparently has little effect on overall crystal packing, although it can not be excluded that in (6A) it contributes to preference of the *P*₂₁ over the *P*₁ space group (see Fig. 6).

Figure 6
Molecular packing of *cyclo*[L-(γ -S)Pip-L-(NMe)Phe] [(6A)] in the crystal viewed along the *c* axis (monoclinic, space group *P*₂₁). This packing is very similar to the triclinic *P*₁ crystal of (2A) and (8A).

Figure 7
Molecular packing of *cyclo*[L-(β -S)Pip-L-(NMe)Phe] [(4A)] in the crystal viewed along the *a* axis, showing three double layers parallel to the *a,b*-planes each rotated by 120° relative to its neighbor. Water molecules are involved in hydrogen bonds in the polar interior of the double layers.

In the hydrated crystal (4A) again aggregation of m1 and m2 molecules (that are only slightly different) by two highly directional O—H $\cdots$ O classical hydrogen bonds between one H₂O molecule and the two (β -S)Pip carbonyl O atoms brings the polar DKP rings oriented (quasi parallel) back to back. This sub-motif is amphiphilic and is closely packed in a two-dimensional network of mainly C—H $\cdots$ O short contacts to form a bilayer structure in the crystallographic *a,b* plane of *circa* 12.2 Å thickness (Fig. 7). Closest contacts involved are C—H_{meta} $\cdots$ O(NMePhe) (m1-m1 and m2-m2), C—H_{ortho} $\cdots$ O(β -S)Pip (m1-m2 and m2-m1) and (NMePhe)C α —H $\cdots$ O(water). Bilayers pack in the *c* direction conform the threefold screw axis, each bilayer rotated 120° relative to the next one. At the interface of the bilayer most interactions that are still in the attractive distance range (sum of van der Waals radii +0.4 Å) are between the non-polar side-chains and of C—H $\cdots$ H—C or C—H $\cdots$ C type, with the exception of two C—H $\cdots$ O contacts between the (NMePhe)C=O of m1 and H_{meta} of m2, and between the (NMePhe)C=O of m2 and H δ_{eq} of (β -S)Pip of m1. There are no short contacts with the β -S atom.

3.3.2. *Cis* (S)Pro series: (10A), (12A) and (14A). As molecules (10A), (12A) and (14A) have very similar molecular structures in the solid state (see Table 6), it is not surprising that two of them [(10A) and (12A)] crystallize in the same space group *P*₄₃₂₁₂, with nearly identical unit-cell parameters. Similar to their analogs in the *cis* (S)Pip series, the amphiphilic (10A) and (12A) molecules aggregate to form bilayers parallel to the crystallographic *ab*-plane, with a polar interior (with quasi parallel DKP rings, however) and the (NMe)Phe side-chains pointing outwards. Packing along the *c* direction rotates each bilayer relative to its neighbors by 90°. The lower pseudoaxial character of the C α —C β bond, the smaller size of the (S)Pro ring [as compared with the (S)Pip series] and the smaller inclination (25° as compared to 47° in the (S)Pip series) of the average plane of the DKP-rings relative to the *a,b*-plane create space to allow the intercalation of the phenyl groups of the bilayers (see Fig. 8). The C—H $\cdots$ O contacts within the bilayers in (10A) and (12A) are [compared to (2A), (6A) and (8A)] less numerous, weaker and presumably less directional as judged from their geometric parameters. In contrast, at the interface of the bilayer there is a larger number of van der Waals dispersive contacts (Σ van der Waals + 0.4 Å) mainly between the intercalating phenyl groups.

Unlike the parent compound (10A) and its β -sulfur analog (12A), the γ -sulfur analog (14A) does not crystallize in

Figure 8
Molecular packing of *cyclo*[L-(β -S)Pro-L-(NMe)Phe] [(12A)] in the crystal viewed along the *b* axis. Crystal system (tetragonal, space group *P*₄₃₂₁₂) and unit-cell parameters are very similar in (10A).

bilayers (space group $P4_32_12$), but prefers space group $P2_12_12_1$. This is noteworthy in view of their highly similar shapes (low mutual rmsd and isosteric CH_2/S substitution). In (12A) the β -sulfur 'activates' those H atoms (α and γ) that are already involved in $\text{C}-\text{H}\cdots\text{O}$ type contacts in the $P4_32_12$ crystal of (10A). In the γ -S analog (14A) the β and δ hydrogen atoms are activated by sulfur and appear to stabilize its $P2_12_12_1$ packing through non classical hydrogen bonds of the type $\text{S}-\text{C}-\text{H}\cdots\text{O}$ with $\text{H}_{\beta\text{t}}$ and $\text{H}_{\delta\text{t}}$ (Fig. 9).

3.3.3. $Z' = 2$ discussion of *cis* series. Why is it that similar above-molecular arrangements of bilayers lead to *pseudo* twofold rotation symmetry (of slightly different molecules) and low-symmetry space groups P_1 and $P2_1$ in the *cis* (S)Pip (with $Z' = 2$), but to *real* twofold rotation symmetry and high-symmetry space groups ($P4_32_12$) in the *cis* (S)Pro series (with $Z' = 1$)? Is side-chain ring size the crucial factor? Are DKP-rings fused to a five-membered ring as in the (S)Pro series less flexible (*i.e.* more rigid) than DKP rings fused to a six-membered ring as in the (S)Pip series? The DKP-ring flexibilities of the isolated molecules were estimated with the aid of DFT calculations and are also reported in Table 3. There is definitely no significantly positive correlation of multiple Z' behavior and flexibility of the diketopiperazine ring of (2A), (4A), (6A) and (8A) as opposed to (10A), (12A) and (14A). What then is the trigger for multiple Z' ? High *local* flexibilities in the crystal environment at the $m1|||m2$ interface of directional sub-motifs at the non-polar interface of bilayers could be a major cause of the observed disorder in the *cis* (S)Pip series.

3.3.4. *Trans* (S)Pip series: (2B), (4B), (6B), (8B) and *trans* (S)Pro series: (10B), (12B), (14B) (2B), (8B), (10B) and (12B). Four of the seven members in the *trans* *N*-methyl series crystallize in space group $P2_12_12_1$ with very similar unit-cell lengths a , b , c for (2B) (10.053, 10.837, 13.335 Å) and (8B) (9.869, 10.684, 13.642 Å) and for (10B) (10.135, 10.901, 12.160 Å) and (12B) (10.099, 11.088, 12.082 Å). The molecular packing of (8B) is shown in Fig. 10. At the level of inter-molecular contacts, the energetically important $\text{X}-\text{C}-\text{H}\cdots\text{O}$ ($\text{X} = \text{C}, \text{N}, \text{S}$) interactions are quasi identical. Penetration of

van der Waals spheres of representative contacts (distance – Σ van der Waals radii) common for (2B), (8B), (10B) and (12B), respectively are between H_{para} and the carbonyl oxygen of (NMe)Phe (–0.31, –0.31, –0.33 and –0.28 Å), between H_{α} of (NMe)Phe and the oxygen of the cyclic iminoacid [$\text{Xxx} = \text{Pip}, (\delta\text{-S})\text{Pip}, \text{Pro}$ and $(\beta\text{-S})\text{Pro}$] (–0.42, –0.42, –0.33 and –0.33 Å), between H_{α} of (NMe)Phe and the oxygen of (NMe)Phe (–0.14, –0.07, 0.13 and –0.04 Å) and between $\text{H}_{\gamma\text{c}}$ of Xxx and the oxygen of Xxx (–0.27, –0.21, –0.22 and –0.18 Å). Also, the large number of corresponding contacts between neighboring molecules at 'distance – Σ van de Waals radii' in the range 0 to 0.4 Å indicates similar contributions of dispersion forces to the stability of molecular packing in (2B), (8B), (10B) and (12B). These observations illustrate the remarkable tolerance of this molecular packing for CH_2/S substitution [(2B) *versus* (8B), (10B) *versus* (12B)] and even for deletion of a CH_2 group in the cyclic side-chain [(2B) *versus* (10B)].

3.3.5. *cyclo*[D-(β -S)Pip-L-(NMe)Phe] (4B). Also (4B) crystallizes in space group $P2_12_12_1$ but its unit-cell parameters are entirely different from those of the (2B), (8B), (10B), (12B) series. Close packing in the *a* direction ($a = 6.229$ Å) results from three non-classical hydrogen bonds: type (N)(S)– C_{α} – $\text{H}\cdots\text{O}=\text{C}$ between H_{α} [(β -S)Pip] and $\text{O}=\text{C}$ [(NMe)Phe], type (N)– C_{α} – $\text{H}\cdots\text{O}=\text{C}$ between H_{α} [(NMe)Phe] and $\text{O}=\text{C}$ [(β -S)Pip] and type (S)– C_{γ} – $\text{H}\cdots\text{O}=\text{C}$ between $\text{H}_{\gamma\text{t}}$ [(β -S)Pip] and $\text{O}=\text{C}$ [(NMe)Phe]. The contact area has 14 additional interatomic distances in the attractive range for dispersion forces. The presence of these multiple contacts at both sides of the DKP ring between translated molecules creates a 'tape' parallel to the *a* axis (see Fig. 11). The activation of H_{α} and $\text{H}_{\gamma\text{t}}$ by the geminal S-atom and the specific effect of β -sulfur

Figure 9

Molecular packing of *cyclo*[L-(γ -S)Pro-L-(NMe)Phe] [(14A)] in the orthorhombic crystal along *b* axis.

Figure 10

Molecular packing of *cyclo*[D-(β -S)Pip-L-(NMe)Phe] [(8B)] viewed along the *b* axis. The crystal system (orthorhombic), space group $P2_12_12_1$, cell parameters and intermolecular contacts are very similar in (2B), (8B), (10B) and (12B).

substitution on the orientation of the (β -S)Pip side-chain [as compared with (12*B*), (10*B*)/(12*B*) overlay rmsd of 0.31 Å] facilitate this tape motif characteristic for the (4*B*) crystal in the *trans* *N*-methyl series. The packing of these tapes is mediated by van der Waals dispersion forces with the exception of (again) β -S-related C—H...*X* hydrogen bond: (β -S)—C _{γ} —H_c...O=C[(NMe)Phe] (with H...O distance Σ van der Waals radii −0.2 Å).

3.3.6. *cyclo*[L-(γ -S)Pip-D-(NMe)Phe] (6*B*). The γ -sulfur analog (6*B*) crystallizes in space group *P*2₁ with two symmetry independent molecules (m1 and m2) in the unit cell (*Z*' = 2). A

Figure 11
Crystal packing of *cyclo*[D-(β -S)Pip-L-(NMe)Phe] [(4*B*)] viewed along the *a* axis. (a) The packing of the tapes (see text), that extend in the *a* direction into layered structures is shown and (b) normalized contact distances d_{norm} mapped on the Hirshfeld surface in color code (McKinnon *et al.*, 2004). The red spots are imprints of three non-classical hydrogen bonds (see text).

Figure 12
Crystal packing of *cyclo*[L-(γ -S)Pip-D-(NMe)Phe] [(6*B*)] viewed along the *b* axis, i.e. the direction of the twofold screw axis. Packing of m1 chains (green) and m2 chains (blue) is shown.

prominent feature of its packing is the two different chains generated by the twofold screw symmetry axis in the crystallographic *b* direction (Fig. 12). Within the m1 chain stabilizing contacts are: [(NMe)Phe]C _{α} —H...O=C[(γ -S)Pip] (−0.22 Å), C_{*meta*}—H...O=C[(γ -S)Pip] (−0.21 Å) and C_{*para*}—H...O=C[(γ -S)Pip] (−0.14 Å). The relative orientation of molecules within the m2 chain is very different with contacts: (N—Me)C—H...O=C[(γ -S)Pip] (−0.37 Å) and [(NMe)Phe]C _{β} —H...O=C[(γ -S)Pip] (−0.16 Å). The parallel packing of chains is tight between m1 and m2 chains (extending the crystal in the *c* direction) with (S)C _{δ} —H_{r(eq)}...O=C[(NMe)Phe] (−0.28 Å m1|||m2), (N)C _{ϵ} —H_{r(ax)}...S[(γ -S)Pip] (−0.21 Å m1|||m2), [(NMe)Phe]C _{α} —H...O=C[(NMe)Phe] (−0.13 Å m2|||m1; −0.18 Å m1|||m2) and a large number of van der Waals contacts in the attractive distance range. The parallel packing in the *a* direction between similar chains (m1|||m1 and m2|||m2) is loose with relatively few and weak contacts. Sulfur in γ -position considerably contributes to the stability of crystal packing through non-classical hydrogen bonds of the type N—C—H...S, S—C _{β} —H...S, S—C _{δ} —H...S, S—C—H...O and =C—H...S.

3.3.7. *cyclo*[L-(γ -S)Pro-D-(NMe)Phe] (14*B*). Compound (14*B*) crystallizes in the orthorhombic system and space group *P*2₁2₁2₁ with *Z*' = 3, i.e. three symmetry independent molecules in the crystal unit cell (Fig. 13). Unlike the other five multiple *Z*' crystals (*Z*' = 2) of (2*A*), (4*A*), (6*A*), (8*A*) and (6*B*) with very small structural differences between m1 and m2, (14*B*) is an interesting case because one of the three molecules (m3) is significantly different from the two others (Table 6). Although the overall shapes of compounds (10*B*), (12*B*) and (14*B*) of the *trans cyclo*[(S)Pip-(NMe)Phe] series are very similar, the γ -S isomer (14*B*) is the only member that has multiple *Z*'. Molecules m1 and m2 combine the α *E* envelope conformation of the thiazolidine ring with relatively low pucker of the DKP ring the φ_1 , ψ_1 = −15.1°, +8.0° (m1) and −18.5°, +13.9° (m2). In contrast, m3 has a α *T* ^{β} twist thiazolidine ring fused to a more puckered DKP ring with φ_1 , ψ_1 = −28.5°, +24.3°. Out-of-plane bending of the ω_1 amide bond is

Figure 13
Molecular packing of *cyclo*[L-(γ -S)Pro-D-(NMe)Phe] [(14*B*)] (orthorhombic, *P*2₁2₁2₁): (a) viewed along the *a* axis and (b) along the *b* axis with the three symmetry independent molecules colored green (m1), blue (m2) and red (m3).

substantial in m1 and m2, with ω_1 , $\chi_{(N4)}$ of 11.9° , -9.7° and 6.3° , -6.4° respectively. In m3 significant deviation from planarity occurs at the ω_2 amide bond: ω_2 , $\chi_{(N1)}$ of $+4.9^\circ$, -6.0° . These differences obviously must be attributed to intermolecular forces. Inspection of their crystal environments reveals that the (NMe)Phe carbonyl oxygen atoms (ω_2 amide bond) of both m1 and m2 are involved in strong non-classical hydrogen bonds with $O \cdots H$ distances lower than the sum of van der Waals radii by 0.41 Å and 0.39 Å respectively, whereas all contacts of the carbonyl oxygen of (NMe)Phe of m3 are at van der Waals distance or larger. Deformation energies of m1, m2 and m3 relative to the gas-phase full optimization are 0.344, 0.172 and 0.066 kcal mol⁻¹ respectively, *i.e.* in the range 0.01 to 0.40 kcal mol⁻¹ typical for crystals of *N*-methylated diketopiperazines (see Table S2 in the supporting information). The calculated gas-phase structure fits the observed crystal structure of m3 remarkably well suggesting that m1 and m2 are more deformed by crystal packing forces, *in casu* C=O $\cdots$ H—C bonds. The latter could increase resistance to out-of-plane bending of ω_2 and decrease nitrogen lone pair electron density, affecting transmission of torsion angles at the ring junction and anomeric interaction with sulfur lone pairs in the thiazolidine ring. Estimation of relative molecular flexibilities of (10*B*), (12*B*) and (14*B*) with the aid of DFT deformation energies of individual endocyclic torsion angles (Table 3) shows that (14*B*) requires less energy for deformation particularly in the region ω_2 – φ_1 – ψ_1 – ω_1 where structural differences are observed. Deformation of the DKP ring torsions in m1 and m2 (relative to m3) is consistently towards the ‘soft’ side of the one-dimensional DFT energy profiles except for ω_1 that is most flexible in both directions. Clearly the combination of packing requirements and molecular flexibility can lead to multiple Z' crystal structures.

4. Conclusions

This paper reports new X-ray crystal structures obtained at 150 K of ten sulfur analogs of *cis* and *trans* cyclo[Pip-(NMe)Phe] and cyclo[Pro-(NMe)Phe] with CH₂/S substitution at β , γ or δ position of the pipecolic acid and in β or γ position of proline residues and compares them with the previously published —CH₂— analogs. DFT single-molecule calculations of these compounds and of suitable model compounds improves insight in their molecular structures (both in the solid state and in the gas phase) and in their crystal packing.

As the phenylalanyl residue is *N*-methylated, classical NH $\cdots$ O hydrogen bonds are excluded and intermolecular packing forces are expected to have a weak to moderate impact on molecular structure. Not surprisingly therefore, the calculated gas-phase structures match the solid-state data to large extent. A rational interpretation of the conformational effects of CH₂/S substitution as observed in the solid state is given on the basis of DFT analysis of the requirements of the separate side-chains in the corresponding cyclo[(NMe)Phe-Gly], cyclo[(S)Pip-Gly] and cyclo[(S)Pro-Gly] models. In this way, conformational differences in side-chains and diketopi-

perazine rings and non-planarity of amide bonds between the title compounds, are partly explained.

Even in *N*-methylated DKPs crystal packing forces have substantial effects on the molecular structures as manifested most convincingly in the conformational differences of symmetry independent molecules of $Z' > 1$ crystals, especially in the case of (14*B*) with $Z' = 3$. *Cis* isomers have amphiphilic character: both apolar side-chains are oriented quasi parallel at one side of the polar diketopiperazine ring. With one exception [(14*A*)] they all aggregate to form bilayers in the solid state with alternating polar and apolar interfaces. Characteristic for the *cis* (S)Pip series (2*A*), (4*A*), (6*A*), (8*A*) is pseudo twofold rotation symmetry (of slightly different molecules) with $Z' = 2$ and low-symmetry space groups $P1$ and $P2_1$. In the *cis* (S)Pro series real twofold rotation symmetry and high-symmetry space groups ($P4_32_12$) with $Z' = 1$ are preferred. In the hydrated crystal (4*A*) the water molecules reinforce the amphiphilic submotif with classical OH $\cdots$ O hydrogen bonds.

Trans isomers with one exception [(6*B*)] all crystallize in the orthorhombic system and space group $P2_12_12_1$. The crystal structures of (2*B*), (8*B*), (10*B*) and (12*B*) show identical intermolecular interactions and very similar unit-cell parameters illustrating the tolerance of this common packing pattern to CH₂/S substitution and side-chain ring size. The β -S and γ -S *trans* isomers (4*B*) and (6*B*) have different packing interactions with relevant contributions of sulfur as acceptor in C—H $\cdots$ S or as activator in S—C—H $\cdots$ O in non-classical hydrogen bond contacts.

The correlation of flexibility of φ , ψ and ω torsion angles with the occurrence of multiple conformers in the unit cell ($Z' > 1$) was verified. Such correlation can be seen in the $Z' = 3$ case of (14*B*) (large perturbation of m1 and m2 *versus* m3) within the (10*B*), (12*B*), (14*B*) series, but not in the $Z' = 2$ cases with small structural perturbations only in (2*A*), (4*A*), (6*A*), (8*A*) and (6*B*).

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