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Effects of sulfoxide and sulfone sidechain–backbone hydrogen bonding on local conformations in peptide models†

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We examine peptide model systems designed to probe short-range N–H...O=S sidechain–backbone hydrogen bonding involving amino acid residues with sidechain sulfoxide or sulfone functional groups and its effects on local conformations. A strong 7-membered ring hydrogen bond of this type accompanies an intra-residue N–H...O=C interaction and stabilizes an extended backbone conformation in preference to classical folded structures.

Sulfoxides and sulfones are immensely important in chemistry.¹ They play useful roles as reagents or additives in organic synthesis,^{2a–e} and in the development of new materials,^{3a–c} and they appear ubiquitously in numerous small molecule therapeutics with wide-ranging activities against human pathologies such as cancer, diabetes, Alzheimer's disease and leprosy.^{4a–d}

Sulfoxide groups appear in an array of natural products, notably in the oxidized sidechains of residues present in some macrocyclic peptide families, including cyclolinopeptides,⁵ cycloleuoripeptides,⁶ axinellins,⁷ and phakellistatins.⁸ An oxidized tryptathionine bridge is a unique structural feature of the notorious amatoxin bicyclic octapeptides present in death cap mushrooms.⁹ While not part of the proteinogenic machinery, methionine sulfoxide is considered highly important in biological systems,¹⁰ and the redox system relating it with methionine may be involved in protein activity regulation and serve as a protective antioxidant.^{11,12} The related compound *S*-methylcysteine sulfoxide is a phytoalexin with beneficial effects on human health.¹³

The occurrence of sidechain sulfone moieties in native peptides is rarer, and may in some cases be an artefact of

isolation or storage.¹⁴ Nonetheless, such residues are important in synthetic peptides, for example in the activation of the anti-cancer prodrug canfosfamide,¹⁵ in the modulation of peptide secondary structure¹⁶ and bioactivity,^{17,18} or in substrate discrimination by the enzyme neutrophil elastase.¹⁹

Sidechain–backbone interactions, notably *via* hydrogen bonds (H-bonds), are of considerable importance in determining secondary and tertiary structures.^{20–23} Despite their prevalence in native and synthetic peptides, very little is known about the conformational effects imparted by sulfoxides or sulfones in peptides. In an isolated case, the oxidation of a macrocyclic thioether derived from a tripeptide was shown to induce a β -turn conformation stabilized by a transannular N–H...O=S H-bond implicating a sulfoxide or sulfone motif.²⁴ However, no studies have been designed to assess the intrinsic propensity of amino acid residues bearing sidechain sulfoxide or sulfone groups to form short-range sidechain–backbone interactions with local NH H-bond donors.

The peptide model Cbz-Attc-NHMe **1** (Attc = 3-aminothietane-3-carboxylic acid) featuring a 4-membered thiacycle appeared to be an attractive platform for the display of a sulfoxide group with a specifically *cis* or *trans* geometry (where these terms refer to the relative disposition of the nitrogen and the sulfoxide oxygen on the 4-membered ring) as well as a sulfone group. It was previously observed for compound **1** that a combination of C6 γ (sidechain) and C5 (backbone) H-bonds stabilized a predominant extended C5–C6 γ local conformation,²⁵ whereas the 5- or 6-membered thiacyclic ring homologs preferred folded δ conformations with π -amide interactions,²⁶ or C7 (backbone) H-bonds typical of γ -turns.²⁷

Compound **1** was subjected to mild oxidation using either hydrogen peroxide or *m*CPBA to provide a mixture of the *trans*-sulfoxide Cbz-Attc(*trans*-O)-NHMe **2** and the *cis*-sulfoxide Cbz-Attc(*cis*-O)-NHMe **3** in good yield (Scheme 1). The low diastereoselectivity was of no consequence for our purposes, since both **2** and **3** were required for study and were easily separated by chromatography. Sulfone Cbz-Attc(O,O)-NHMe **4** was prepared from **1** using 2 equiv. of *m*CPBA.

Quantum chemistry structural modelling studies of molecules **2–4** (Fig. 1) were carried out in the gas phase at DFT-D

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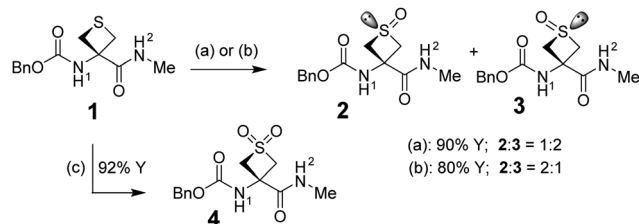
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Scheme 1 Compounds studied in this work and NH labels. Conditions: (a) H_2O_2 , AcOH, CH_2Cl_2 , 10 °C, 30 min. (b) mCPBA (1 equiv.), K_2CO_3 , CH_2Cl_2 , –78 °C, 3 h. (c) mCPBA (2 equiv.), K_2CO_3 , CH_2Cl_2 , 0 °C, 2 h.

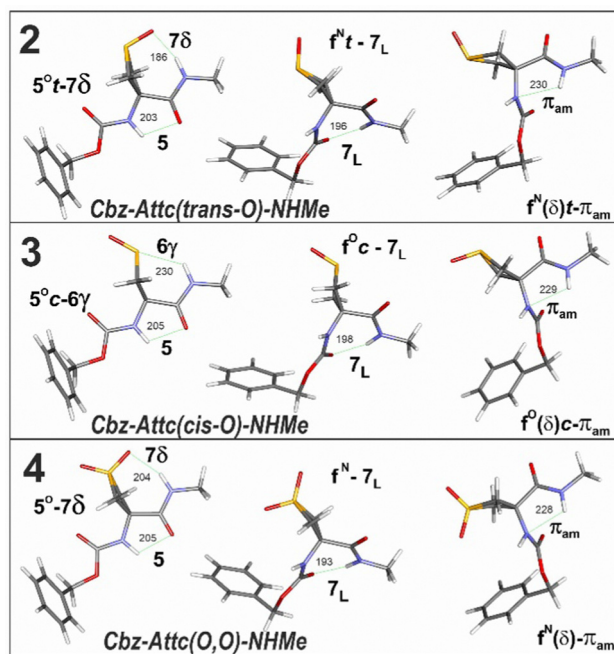


Fig. 1 Most stable theoretical structures of each backbone family of species 2–4 in chloroform solution, illustrated for the *gauche*-rotamer of the Cbz group with a *trans* geometry. Conformations are described according to the H-bonding status of their NH^1 and NH^2 moieties: type of H-bond (C5, C7, π_{am}) or f for free. The t and c labels refer to the *trans* and *cis* sulfoxide conformations respectively. N/O superscripts indicate the puckering of the thiacyclic ring, towards the closest backbone atom, N or O.

level and in chloroform solution accounted for by a polarizable continuum (for details see ESI,† Section S2.1). The structures were only marginally influenced by solvation (see Fig. S2.2 and S2.3 for comparisons, ESI†), although the energetics (Fig. 2) showed a significant dependence on the environment.

trans-Sulfoxide 2 showed one low-energy extended conformer, labelled C5/C7 δ , characterized by an intra-residue C5 backbone H-bond interaction for NH^1 and a sidechain C7 H-bond interaction between NH^2 and the sulfoxide oxygen, with H-bonding distances in the gas phase that are short for $\text{N-H}\cdots\text{O}=\text{C}$ ($d = 202$ pm) and remarkably short for $\text{N-H}\cdots\text{O}=\text{S}$ ($d = 183$ pm). Two alternative folded conformers, devoid of an H-bonding role for the sulfoxide and characterized by a free NH^1 and either a backbone C7 H-bond or a π -amide interaction for NH^2 , were higher (solution) or much higher (gas phase) in energy.

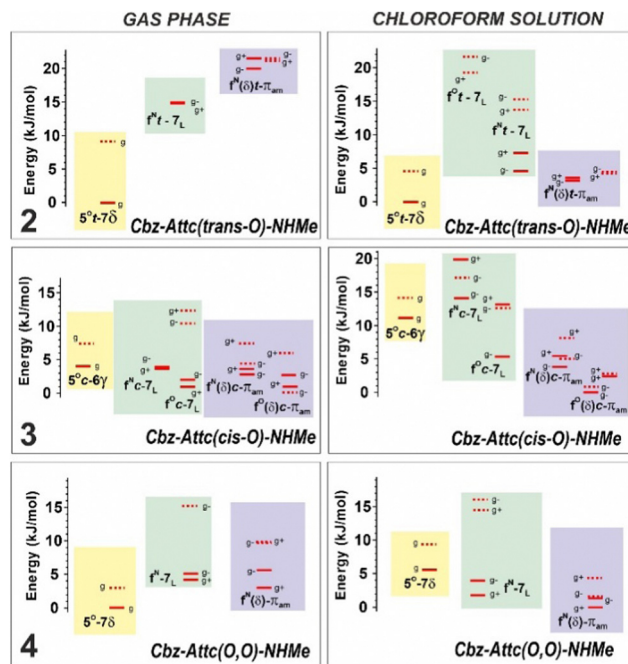


Fig. 2 Energetics of the significant conformations of 2–4 in the gas phase (left panels) and in chloroform solution (right panels) at 300 K. See Fig. 1 caption for conformational terminology and ESI,† Section S2.2 for details. Plain and dotted levels refer to *trans* and *cis* conformations of the carbamate respectively; labels $g^{+/-}$ stand for *gauche*^{+/–} orientations of the Cbz moiety.

In contrast, *cis*-sulfoxide 3 showed three stable conformer types. The first was extended, labelled C5/C6 γ , with an intra-residue C5 backbone H-bond for NH^1 and a sidechain C6 H-bond interaction between NH^2 and the sulfoxide sulfur. The gas phase H-bonding distances, $\text{N-H}\cdots\text{S}$ ($d = 240$ pm) and $\text{N-H}\cdots\text{O}=\text{C}$ ($d = 204$ pm), were very close to those found previously for the C5/C6 γ conformer of the non-oxygenated derivative 1.²⁵ The second and third conformers were folded, similarly to those detected for 2, with a C7 or a π -amide interaction for NH^2 and no H-bonding role for the sulfoxide. The folded conformers of 3 were slightly more stable than the extended conformer in the gas phase; the difference in energies was increased in solution with a stability order π -amide > C7 > C5/C6 γ .

Sulfone 4 showed three stable conformer types: C5/C7 δ , C7, and π -amide. In the C5/C7 δ conformer the $\text{N-H}\cdots\text{O}=\text{S}$ gas phase distance was perceptibly longer ($d = 196$ pm) than in the corresponding conformer of 2. Although it was the most stable form in the gas phase, this conformer was more energetic than the folded forms in solution.

It is striking that the oxygen atom of the sulfoxide or sulfone group is well inclined to form a C7 H-bond with NH^2 in 2 (particularly) and in 4, but not to form a C6 H-bond with NH^1 in 3 nor 4. Indeed, in compound 3 the only implication of the sulfoxide function involves the sulfur atom, akin to the behavior observed previously for 1. This behavior contrasts with that of the $\text{C}=\text{O}$ motif of asparagine side-chains, which is able to form both intra-residue C6 γ and inter-residue C7 δ H-bonds with neighboring backbone NH atoms.²⁸

Experimental assessment of the solution state behavior of 2–4 was carried out using IR spectroscopy in chloroform solutions (5 mM). The absorption bands in the amide I, II and A regions



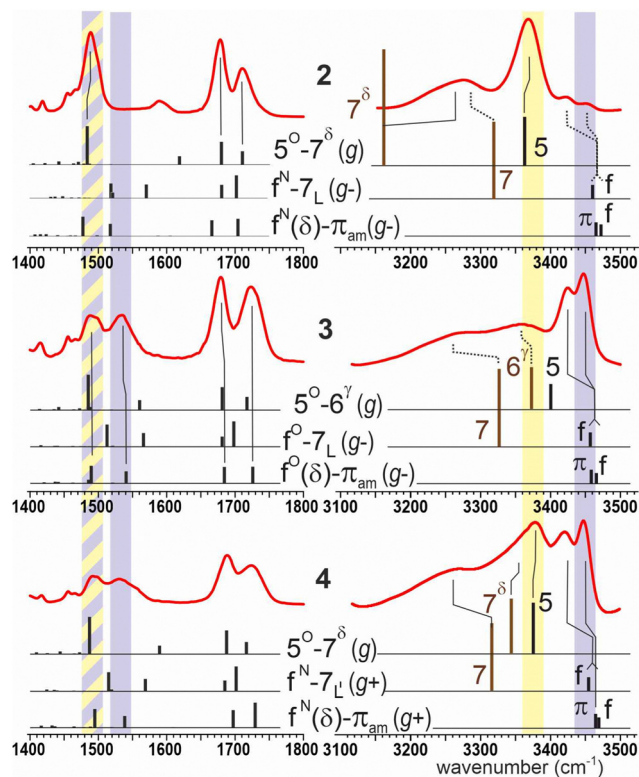


Fig. 3 Solution state IR spectra of compounds **2–4** in chloroform (5 mM) and the calculated IR spectral absorbances of the energetically relevant conformations. Diagnostic regions are color-coded for conformations featuring C5 (yellow) or π -amide (violet) interactions.

are shown in Fig. 3. No concentration effects were observed (see ESI,[†] Section S3), indicating the intramolecular nature of the non-covalent interactions. Interpretation of the data was in good agreement with the quantum chemistry calculations, which facilitated assignments, as previously demonstrated.²⁶

trans-Sulfoxide **2** showed two significant amide A (NH stretch) bands, at 3274 and 3368 cm^{-1} , indicating the presence of the dominant C5/C7 δ conformer. The latter band, well reproduced by theory, was assigned to NH¹; its C5 H-bond was perceptibly stronger than usual for an α,α -disubstituted amino acid, whose diagnostic C5 absorbance band (Fig. 3, yellow region) habitually appears around 3380 cm^{-1} .²⁹ The former band was remarkably red-shifted and was assigned to NH² engaged in a strong C7 δ N-H...O=S H-bond, despite its red-shift being seemingly overestimated by theory, presumably because its calibration was originally carried out on N-H...O=C H-bonds.²⁶ Small bands at 3421 and 3452 cm^{-1} were assigned to minor contributions from C7 and/or π -amide conformers. The amide I bands at 1678 and 1710 cm^{-1} were in good agreement with calculations for the C5/C7 δ conformer, while the amide II region showed a single intense band at 1488 cm^{-1} , considered to be a benchmark for a C5 interaction.³⁰

cis-Sulfoxide **3** showed a more complex set of amide A bands, best explained in terms of contributions from the three conformer families found by quantum chemistry. The most intense bands, at 3424 and 3447 cm^{-1} , were assigned to NH¹ and NH² respectively, in a dominant folded π -amide conformation (or a completely free

form, *i.e.* non H-bonded). The diagnostic π_{am} absorbance band for α,α -disubstituted amino acids (Fig. 3; violet region) was recently reported to appear around 3450 cm^{-1} .²⁶ A modest contribution from the extended C5/C6 γ conformer results in the plateau at 3350–3380 cm^{-1} , reminiscent of the C5/C6 γ conformer known for compound **1**. Similarly, the broad feature around 3280 cm^{-1} can be ascribed to a weakly populated C7 conformer. The amide I bands at 1679 and 1723 cm^{-1} of **3** were in good agreement with calculations for the π -amide conformer, as were the two bands in the amide II region at 1490 and 1534 cm^{-1} which are thus revealed as the spectroscopic signature in this region for a peptide that adopts the classical δ conformation featuring a π -amide interaction.²⁶

Sulfone **4** showed an IR absorption spectrum that suggested the presence of the three conformers found by quantum chemistry in balanced populations. As was the case for compound **3**, the high frequency amide A bands at 3418 and 3449 cm^{-1} arose from NH¹ and NH² respectively, in a π -amide (or a free) conformation, while the C7 conformer gave rise to the broad red band at *c.* 3260 cm^{-1} from H-bonded NH² and contributed to the 3418 cm^{-1} band from free NH¹. The remaining amide A feature, a band with a maximum at 3378 cm^{-1} (yellow region) and a broadened red-side shoulder around 3360 cm^{-1} was assigned to the C5/C7 δ conformer, by comparison with **2**. The appearance of broadened or overlapping peaks in the amide I and II regions likewise indicated that several conformations were present.

To complement solution phase assignments, a gas phase conformation-selective laser study was also conducted using UV and IR/UV double-resonance spectroscopies.³¹ The first study was carried out on the sulfoxide **2/3** mixture (ratio 3:2) obtained by synthesis (Scheme 1). The UV spectrum showed one main band and three minor bands that were all assigned to the same conformer A (see ESI,[†] Section S4). The conformer-selective IR absorption spectrum obtained (Fig. 4, center) showed two bands in the NH stretch region, at 3262 and 3377 cm^{-1} . Comparison with the calculated spectra for the stable forms of **2** and **3** led us to identify A as the C5/C7 δ conformer of **2**, with a red-shifted NH² in a strong N-H...O=S interaction and NH¹ in a C5 N-H...O=C interaction. The predominance of the C5/C7 δ conformer of **2** was in complete agreement with the calculations and the solution state studies.

The trend of theory to overestimate the red-shift of C6 γ or C7 δ bands was confirmed by the gas phase results, supporting the present solution assignment. In contrast, the absence of spectral data for **3** should be seen as a caveat for the gas phase technique (see ESI,[†] Section S4 for further details).

The gas phase study of sulfone **4** likewise revealed only one conformation type (Fig. 4, bottom). The conformer-selective IR absorption spectrum exhibited two intense bands in the 3350–3400 cm^{-1} region, a signature that was very similar to that of the principal C5/C6 γ conformer of compound **1** (Fig. 4, top). This similarity, together with the calculated spectra, indicated that this was the C5/C7 δ conformer of **4**. Its C5 interaction is slightly weaker than that observed for compound **2**, and more in line with the C5 band in other α,α -disubstituted amino acids such as **1** (Fig. 4, top). Its N-H...O=S C7 δ interaction, however, is much weaker than that of in *trans*-sulfoxide **2**, in qualitative agreement with the theoretical structures (Fig. 1) and the energetics (Fig. 2), leading to



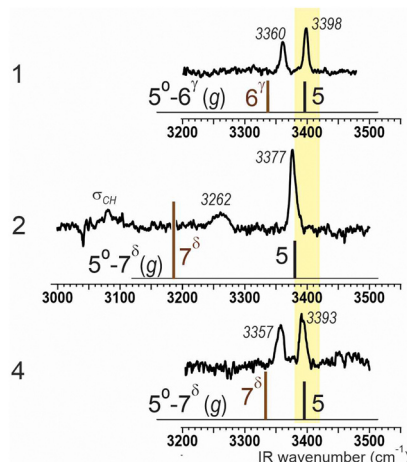


Fig. 4 Gas-phase conformation-selective NH stretch IR spectra for the main gas phase conformation of compounds **2** (center panel) and **4** (lower), together with the calculated spectra for the most stable C5 conformations. For comparison, the corresponding IR data for compound **1** are also given (top), adapted from ref. 25.

the C5/C7 δ conformer of **4** being challenged by the alternative folded forms in solution.

The solution-state behavior of compounds **2–4** in chloroform was examined by ^1H NMR spectroscopy (see ESI †). The NH chemical shifts and DMSO- d_6 titration results are in good agreement with a predominant C5/C7 δ conformer for compound **2** and a mixed conformational landscape for **3** and **4** in which folded C7 and π -amide interactions make significant contributions. Further supportive evidence was obtained from qualitative NOESY experiments. In compound **2**, a strong correlation was observed between NH 2 and the *syn* protons of the 4-membered ring, as expected for a C5/C7 δ conformer. In contrast, the corresponding correlation in compounds **3** and **4** was much weaker, whereas a strong correlation between NH 1 and the *syn* protons was in evidence, in agreement with folded conformations for these two compounds.

In summary, peptide model systems demonstrate that sulfonate and sulfone functional groups may form sidechain-backbone N-H $\cdots$ O=S hydrogen bonds with a neighboring NH amide atom, leading to a stabilized local geometry with an extended backbone and an intra-residue C5 H-bond. This type of secondary structure is quite rare,^{30,32} and has been proposed as a basis for molecular spacers.³³ Amino acids with oxidized thioether sidechains appear to be useful building blocks for the design and control of peptide architectures.

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Conflicts of interest

There are no conflicts to declare.

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